https://theses.gla.ac.uk/

Theses Digitisation:

https://www.gla.ac.uk/myglasgow/research/enlighten/theses/digitisation/

This is a digitised version of the original print thesis.

Copyright and moral rights for this work are retained by the author

A copy can be downloaded for personal non-commercial research or study, without prior permission or charge

This work cannot be reproduced or quoted extensively from without first obtaining permission in writing from the author

The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the author

When referring to this work, full bibliographic details including the author, title, awarding institution and date of the thesis must be given

Enlighten: Theses
https://theses.gla.ac.uk/
research-enlighten@glasgow.ac.uk

STUDIES ON THE METABOLIC RESPONSE TO THERMAL INJURY IN THE RAT

A thesis submitted to the
University of Glasgow
for the degree of
Doctor of Philosophy
in the
Faculty of Medicine
by

JOHN ROONEY RICHARDS, M.B., Ch.B., F.R.C.S.(Ed.)

Institute of Physiology,
University of Glasgow,
Glasgow G12 8QQ.

October 1979.

ProQuest Number: 10753999

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

ProQuest 10753999

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Thesis
6268
Copy 1.
(Vol. 1.)

GLASGOW
UNIVERSITY
LIBRARY

SUMMARY

A gradient layer calorimetry system to hold one rat was designed and constructed by the author in collaboration with the Department of Clinical Physics and Bioengineering of the Western Regional Hospital Board. The calorimeter is capable of operating over a range of ambient temperature and humidity conditions between 20°C to 30°C and at 0.01 to 15g H_2O/m^3/dry air background humidity. This range is below that of thermally neutral conditions of 30-33°C for the laboratory rat.

By controlling the temperature of the gradient layer box water jacket, its surrounding insulated enclosure and the laboratory in which the calorimetry system was housed, a "no-load" thermopile output from the gradient layer box was achieved within ± 0.15mV of zero at operating temperatures of 20°C and 30°C. These conditions remained stable to within ± 0.02mV over 3 hours giving a ± 0.5% level of accuracy for thermopile measurement of heat loss for a 250g rat during this period.

Because of the close agreement between direct and indirect measurements of heat loss and production, with a difference of less than 2.4% at 20°C, and the stability of rat body heat content during calorimetry,

restraint of the rat and body thermometry proved unnecessary. This was an advance compared with previous rat calorimeter design and operation. Special animal facilities were designed and built by the author in the Institute of Physiology to house the experimental rats under study. These allowed close control of ambient temperature, noise, dust levels and spread of bacteria. Semi-automated microkjeldahl nitrogen assay methods were adapted for use in performing rat metabolic studies.

In thermoneutral environments ($30^{o}C$), calorimetry showed that normal male Wistar rats obeyed Voit's Surface Law with measured total heat loss between 1097-1039 $kcal/m^2/day$ or 5.42-5.13 W/kg. In a colder environment of $20^{o}C$, resting metabolic expenditure (RME) in normal rats was 16% greater than that found in thermoneutral conditions. An ambient temperature of $20^{o}C$ therefore represents a mild cold stress for the laboratory rat.

A reproducible burn injury model was developed by the author for use in the rat, in which the depth and extent of the burn could be precisely controlled. Calorimetry and metabolic studies were carried out on rats with 20% and 25% of body surface area full skin depth burns. This study constitutes the first gradient layer calorimeter measurement of partitioned heat losses after burn injury in rats to be made at the typical hospital ward temperature and humidity conditions of $20^{o}C$ with high relative humidity.

The 25% BSA burn proved to be the most satisfactory injury "model" which, when food intake was slightly restricted, resulted in severe weight loss in the rat comparable to that seen in extensively burned man. After a 25% BSA burn, RME increased steadily to a maximum of 15.7 W/kg by the 55th post burn day, 137% greater than control values. At the end of the study, on the 63rd post burn day, RME remained elevated at 15.2 W/kg, 121% greater than control values. Evaporative heat loss increased after injury to a maximum value of 6.4 W/kg on the 55th post burn day, 806% greater than controls. Dry or sensible heat loss also increased after a 25% BSA burn to a maximum of 10.2 W/kg on the 35th post burn day, 66% greater than controls. On the 63rd post burn day the rate of dry heat loss was still 54% greater than controls, at 9.5 W/kg.

Thermometry carried out in the 20% BSA burn study showed that there was a high rate of heat transfer from core to skin at 20^{o}C, comparable to that measured in uninjured control rats at 30^{o}C ambient temperature. This suggested that the burned rats' hypothalamic "set-point" for thermoregulation had been altered after injury as has been noted in man. In the burned rat at 20^{o}C ambient temperature, and with a loss of appropriate surface insulation, increased evaporative heat loss presented a major thermoregulatory challenge.

ACKNOWLEDGEMENTS

I am indebted to the staff of the Institute of Physiology, University of Glasgow, for the continued support and encouragement given to me during all phases of the work described in this thesis. Professor R. C. Garry, Professor I. A. Boyd, and Dr. Kathleen Ballard provided invaluable guidance and advice throughout. Mr. F. Fleming, Mr. J. J. Brown, and Mrs. C. Webb of the Institute technical staff deserve special mention. I am also indebted to Mr. J. K. Drury who carried out the majority of the daily calorimetry procedures for the later burn studies.

Construction of the gradient layer calorimeter was only made possible by the active support of Professor J. M. A. Lenihan, and Dr. J. S. Orr of the then W.R.H.B. Department of Clinical Physics and Bioengineering. I owe a particular debt to Mr. K. B. Carter of the D.C.P.B. staff for his collaboration in the design of the overall system, and also to Mr. A. Shaw, Mr. R. Donnet and many other members of the D.C.P.B. staff who provided the special technical skills without which the calorimeter could not have been created. Dr. R. G. Bessent and Mr. C. C. Goll have provided continued support in regard to experimental data analysis.

Dr. A. Fleck, Sir David Cuthbertson, and Dr. G. S. Fell provided guidance and practical help with the biochemical aspects of the work.

I am grateful also to Professor L. H. Blumgart and Professor D. C. Carter of the University Department of Surgery, Glasgow Royal Infirmary, for their help in enabling me to complete the studies described.

DECLARATION

This thesis has been entirely composed and written by myself. I was solely responsible for the physiological design features of the SEC - A - 04 L calorimetry system and for the initiation of its construction. I was jointly responsible for the technical aspects of its design. The rat environmental chamber, animal handling and preparation laboratory were designed and partly built by myself. I was solely responsible for the design of all experimental protocols and for the performance of all laboratory measurements including nitrogen determinations in the 5% BSA burn study. I benefitted from the work of Mr. J. K. Drury and Mr. J. J. Brown in the performance of technical procedures associated with calorimetry in the 25% BSA burn study. The staff of the Biochemistry Department of Glasgow Royal Infirmary carried out the nitrogen determinations in biological samples from the 20% and 25% BSA burn studies.

VOLUME 1

STUDIES ON THE METABOLIC RESPONSE TO THERMAL INJURY IN THE RAT

CONTENTS

VOLUME 1

CONTENTS PAGE NUMBER

VOLUME 1

CONTENTS PAGE NUMBER

VOLUME 1

CONTENTS PAGE NUMBER

VOLUME 1

CONTENTS PAGE NUMBER

VOLUME 1

CONTENTS PAGE NUMBER

VOLUME 2

STUDIES ON THE METABOLIC RESPONSE TO THERMAL INJURY IN THE RAT

CONTENTS PAGE NUMBER

VOLUME 2

CONTENTS PAGE NUMBER

VOLUME 2

CONTENTS PAGE NUMBER

CONTENTS PAGE NUMBER

VOLUME 2

CONTENTS PAGE NUMBER

FIGURES VOLUME 1

FIGURES VOLUME 1

FIGURES VOLUME 1

FIGURES VOLUME 1

FIGURES VOLUME 2

FIGURES VOLUME 2

TABLES VOLUME 1

TABLES VOLUME 2

"STUDIES ON THE METABOLIC RESPONSE TO THERMAL INJURY IN THE RAT"

PREAMBLE

> "The contemplation of those things which are normal is physiology, and it is the first thing to be learned by medical men. For that which is normal is right and serves as a criterion for both itself and the abnormal. By defining in its light departures from it and unnatural reactions, pathology becomes an art of therapeusis, and the opportunities for discovering multiple new remedies, derive."
>
> Harvey: De Circulatione Sanguinis 1660.

INTRODUCTION

I. THERMAL INJURY:

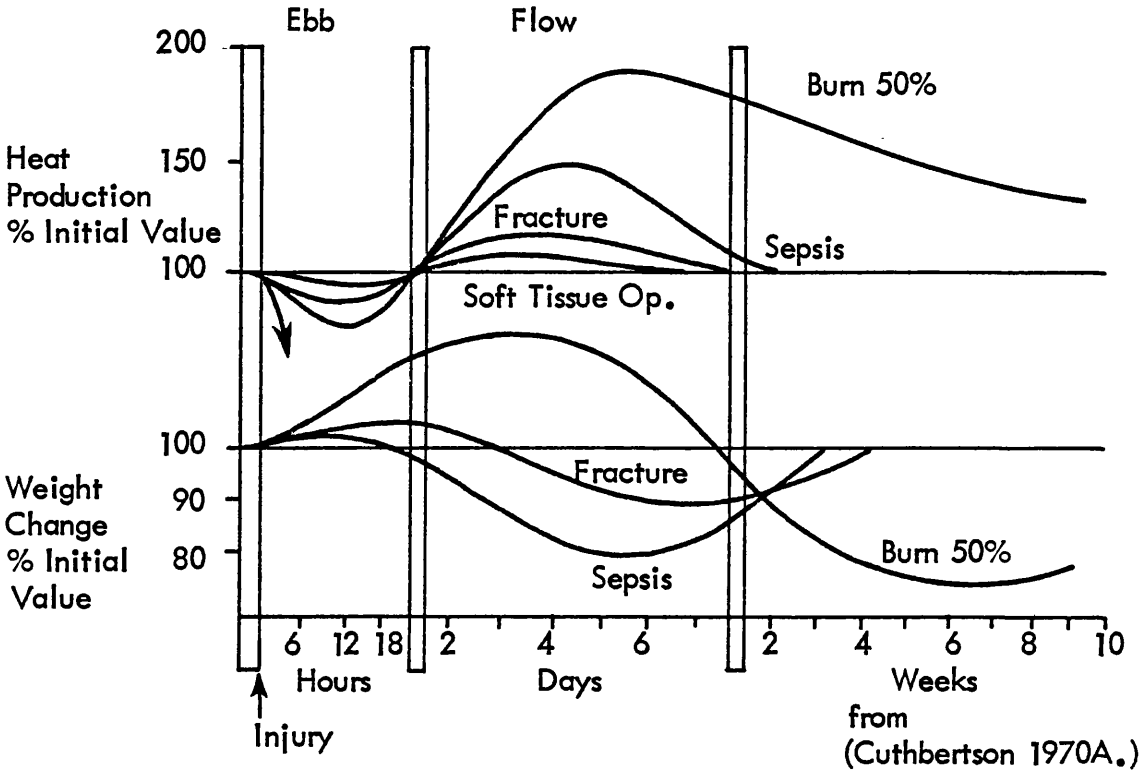
The extensive burn represents one of the most serious forms of injury known in man. Not only is there local tissue destruction at the site of injury, but multiple systemic responses are evoked. Gross physiological derangements occur, related to the damage to normal skin barriers which prevent loss of water, heat, protein, electrolytes and prevent invasion of bacteria. Smoke damage to the lungs, direct heat damage to red blood cells, indirect renal damage by hypovolaemia and shock, pain, immobilisation, infection and partial starvation, all contribute to the severity of the burn wound's assault upon the physiological defence mechanisms of the body. The unique position of the

burn wound in the spectrum of metabolic alteration following different forms of injury is illustrated in Figure 1 (Cuthbertson, 1970A).

Failure to achieve early wound closure and the presence of dead burn eschar jointly result in gross prolongation of the body's response to thermal injury beyond that seen even in multiple bone fractures or extensive abdominal surgery with sepsis (Moore and Brennan, 1975). The cause of the increased energy expenditure after burns remains obscure and disputed, mainly because methods of estimating energy expenditure in injured patients rely on indirect calorimetry (Kinney, 1975). No direct measurement of heat loss has been made in injured man, as no chamber calorimeters have been produced for the direct measurement of body heat loss which permit appropriate nursing and medical care of critically ill patients.

This thesis is concerned with defining the natural history of untreated post burn hypermetabolism and its relationship to post burn weight loss and energy balance. A burn injury in the rat was developed as an experimental model initially thought to represent burned man. Investigation of whole animal energy balance necessitated the design and construction of a rat gradient layer direct and indirect calorimeter with an associated metabolic study laboratory built to achieve controlled environmental conditions. Whole animal heat production and heat losses after a severe but non lethal burn were measured, as were

Fig. 1 THE METABOLIC RESPONSE TO TRAUMA (MAN)



urinary nitrogen and body weight losses. The relevance of these findings to current burn therapy is discussed.

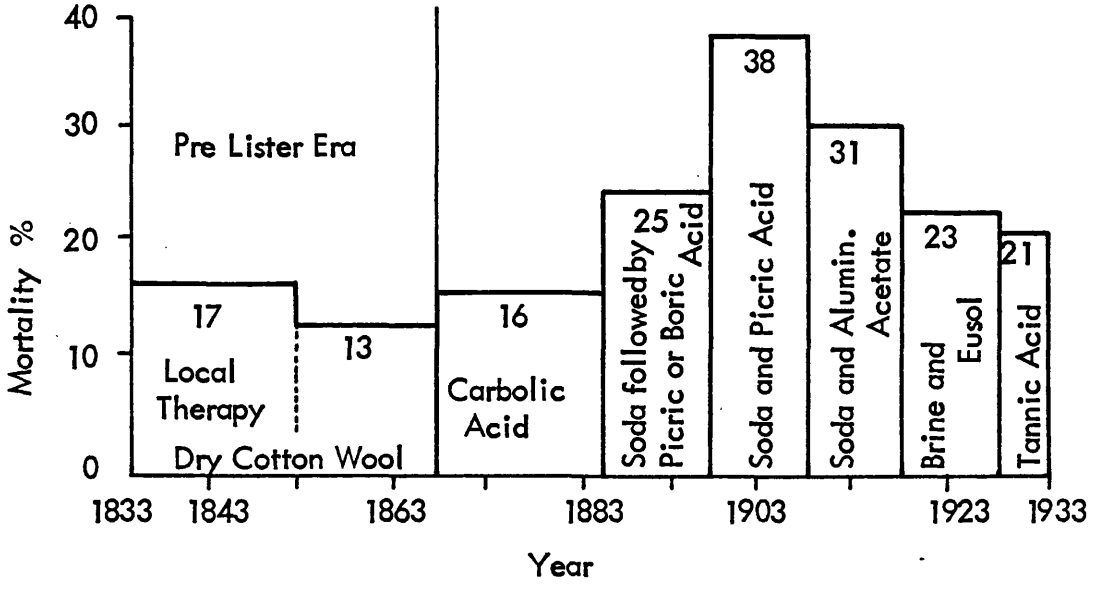
II. HISTORY OF THE THERAPY OF BURNS

A wide variety of therapies and local applications have been used since the beginning of recorded history in the treatment of extensive burns (Cockshott, 1956; Shedd, 1958; Harkins, 1942). Few, if any, of these regimens were effective. Significant advances in burn care began only during the last century (Moyer, 1954), and followed the rapid increases in knowledge of physiology and pathology made during that era (Moyer and Butcher, 1967). In 1833 Ballingal in Edinburgh accurately described and recognised three distinct ways of dying after thermal injury (Artz and Moncrief, 1969). His observations had a direct bearing on modern concepts of burn therapy. He noted: sudden unexplained death within 72 hours of injury, now known to be due primarily to hypovolaemic shock; death occuring at 10 - 15 days post burn associated with overwhelming sepsis; death late after burning, during the third to the sixth week, associated with extreme weight loss and malnutrition. The investigation and understanding of the deranged physiology causing death at each of these intervals led to the application of rational corrective therapeutic measures and to marked improvements in survival after burn injury, although the interval between gaining knowledge and its application has usually been prolonged. The extent of this delay is illustrated by the mortality figures for burns and scalds in over 10,000

cases treated in Glasgow Royal Infirmary from 1833 to 1933. This is shown in Figure 2. During this period, and particularly at the beginning of the 20th century, many advances in knowledge were made. The medical treatment offered to burn victims however remained conservative, dwelling mainly on specific problems of local care. In the order of their recognition, these were: the care of the wound; the easing of pain; the prevention of joint contractures; the treatment of wound infections. Development and use of topical wound applications reached its zenith, and the survival statistics shown in Figure 2 from 1870 onward are representative of the results obtained throughout the United Kingdom. All such applications were markedly inferior to burn dressings of dry cotton wool, and the high death rate with some of the topical agents used, was shown at the very time to be due to their toxic side effects once absorbed systemically from a raw burn surface (Elliot, 1906). Despite known toxicity, their use persisted until the middle of the 20th century.

Similar delay existed between the recognition of hypovolaemic shock as the main cause of Ballingal's "early burn death" (Tappeiner, 1881; Sneve, 1905; Copeland, 1887), and the widespread introduction of rational corrective measures (Underhill, 1930; Blalock, 1931). Although replacement fluid solutions were used successfully in the early treatment of burn shock (Underhill, 1923), it was not until the advent of mass burn casualties in World

Fig.2 MORTALITY FROM BURNS DURING A CENTURY



(adapted from Dunbar 1934 by Moyer 1954)

War II that research in depth was carried out into the optimal characteristics required for replacement solutions (Cope and Moore, 1944; Cope et al., 1948; Moyer et al., 1944). The use of fluid replacement regimes of mixtures of saline, plasma or blood has been so successful that few patients now die from the initial burn shock (Markley et al., 1959; Wilson and Stirman, 1960). The impact of generally improved wound care, and fluid management with the more widespread use of skin grafting techniques (Padgett, 1942) upon burn mortality has been profound. Since 1945 the availability of systemic antibiotics, routine anti-tetanus prophylaxis, improvements in anaesthesia and in blood transfusion, have further reduced mortality, influencing in particular deaths associated with sepsis. A better assessment of the effectiveness of these advances in treatment was made possible by the development of analytical statistical techniques which related patient age, burn depth, and area burned, to probable survival (Bliss, 1938; Berkson, 1953; Bull and Squire, 1949; Bull and Fisher, 1954).

Table 1 shows the reduction in mortality from burns, graded according to extent of burning, between 1927 and 1947-51. These dates represent time periods before and after the introduction and widespread use of rational treatment designed to prevent deaths from shock and infection. Although there was a sharp fall in the death rate following burns of up to 50 - 60% of the body surface area (BSA), large burns of more than 70 - 80% BSA continued

TABLE 1 BURN MORTALITY : 1927 AND 1947-51 ;

A COMPARISON

PERCENTAGE OF BODY SURFACE BURNED	MORTALITY %	MORTALITY %	PER CENT REDUCTION IN MORTALITY RATE
20	8	1	88%
30	38	6	84%
40	74	16	78%
50	95	34	64%
60	98	57	42%
70	99	78	21%
80	100	92	8%

(Table 1 adapted from Moyer et al., 1965)

to have virtually a 100% mortality. Extensively burned patients were able to survive the early shock stage, but could not ultimately survive because of extreme body weight loss and malnutrition. The effect of therapy in such individuals often was simply to prolong the time that it took for them to die.

This clinical situation lasted from 1950 to almost 1970. Research and clinical practice were concentrated on improving the treatment of the small to moderate size of burn wound to the relative neglect of extensive burn wounds. In particular, the prevention and treatment of infected slough remained a major clinical problem. Improved antibiotics were developed, along with a wide variety of escharotics and bactericidal surface application creams. Burn wound auto-toxins were isolated, but later found to be largely experimental artefacts (Moyer and Butcher, 1967; Artz and Moncrief, 1969). Host resistance to infection was strengthened by the introduction of techniques for conferring passive immunity, and wound care in general benefitted during this period by the re-introduction of the exposure method of treatment (Wallace, 1969).

With the problems of wound infection decreasing, attention was turned to the problem of death due to extreme cachexia and malnutrition in the more extensively burned patients. The direct relationship between weight loss and mortality was recognised (Studley, 1936; Keys et al., 1950), and the importance of overall energy balance in the

severely burned patient was reconsidered (Kinney et al., 1970). After an initial shock phase of short duration (Stoner, 1970; Fleck, 1976), increases in energy expenditure of up to 100% above normal values were recorded over a prolonged period after extensive burns (Gump and Kinney, 1971; Zawacki et al., 1970; Artz and Reiss, 1957; Moyer and Butcher, 1967), accompanied by a negative nitrogen balance and severe weight loss (Newsome et al., 1973; Soroff et al., 1961). With moderate sized burns, supplying a high calorie dietary intake greatly decreased post burn weight loss. But in the more extensively burned patient, even with the advent of techniques for complete parenteral feeding (Wretlind, 1972; Fischer, 1976), and the administration of 4000 - 8000 kcal per day by a combination of intravenous and oral routes, weight gain could not be achieved in the first few weeks after thermal injury, although it was possible to achieve weight stabilisation when the burn wound began to heal (Wilmore et al., 1971). Weight stabilisation at this point was considered to reflect a decrease in energy losses via the wound rather than metabolic adaptation leading to an increased assimilation and oxidation of the supplied nutrients, although in the absence of accurate partitioned heat loss studies, this remained speculative. It appeared that in the extensive burn, maintenance of body weight was essential for long term survival, but that burn wound energy losses often apparently exceeded the long term energy producing capacity of the body, even when excess nutrients were supplied. It seems likely that

this resulted in an unavoidable cumulative energy deficit which could only be met by breakdown of the burned individual's own tissue energy stores. Burns of over 70% BSA could not therefore be influenced by the conventional nutritional support therapy which had significantly reduced mortality following lesser burns.

By the late 1960's however it did become possible to reduce the grossly increased energy losses of the severely burned (Davies et al., 1969; Barr, 1968). This was achieved by keeping burned patients in a thermoneutral environment (Tilstone, 1972). Experimental evidence at the time suggested that this also decreased the protein catabolic response to burning and decreased weight loss post burn (Caldwell, 1970; Campbell and Cuthbertson, 1967). In specialised burns units, nursing patients at ambient conditions within their thermoneutral zone resulted in survival of very extensively burned patients. This alone was a significant result. But these patients, in addition, did not lose more than 10% of their pre-burn weight at any time during prolonged convalescence periods of many months (Davies, Lamke and Liljedahl, 1977). Despite these successes, the treatment of extensively burned patients remains empirical.

III. PROBLEMS IN CURRENT BURN THERAPY, AND RELATED RESEARCH QUESTIONS:

The relationship between weight loss after injury and whole body energy balance is poorly understood, mainly because of the lack of readily available methods for measuring energy expenditure in injured patients. Recently four possible explanations of the increased resting metabolic expenditure after severe injury have been suggested (Kinney, 1975). These are:

1. There may be an increase in cell and organ metabolism which requires an increase in energy (or A.T.P.) production, e.g. an increase in chemical work in the liver.

2. There may be a reduction in the amount of energy produced for a given amount of substrate oxidation and heat production, hence a given need for energy is met by a greater overall resting energy metabolism (Dolecek, 1969).

3. There may be an increase in the neuroendocrine stimuli for heat production, possibly from altered CNS responses (Wilmore, 1976).

4. There may be an increase in obligatory heat losses, with a corresponding increase in heat production to maintain body heat content and hence body temperature within homeostatic limits (Moyer and Butcher, 1967; Gump and Kinney, 1970; Harrison et al., 1964).

While the last hypothesis may seem particularly applicable in the case of post burn hypermetabolism, recent work has shown (Gump and Kinney, 1971; Zawacki et al., 1970) that this may not be the sole metabolic derangement occurring in the extensively burned patient. Burn wounds are a particularly complex form of injury in which one must separate the physiological effects of skin loss with its associated water (Moyer and Butcher, 1967) and protein losses (Davies, 1970), from the effects of tissue destruction, wound sepsis, and the possible sequential organ failures which stem from that (Sevitt, 1957), keeping in mind the additional effects of pain, partial starvation and immobilisation.

It is essential therefore to compare the burn wound with comparatively simpler forms of injury and to first re-examine in detail current knowledge of the factors which determine an individual organism's response to an injurious stimulus.

IV. REVIEW OF METABOLIC RESPONSES TO INJURY, INFECTION AND STARVATION

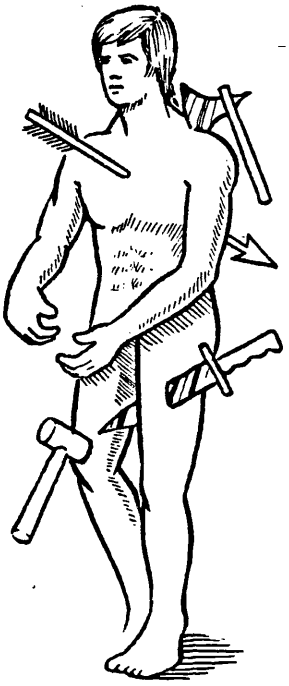
Injury in any form represents an extraordinary strain on the homeostatic mechanisms of an injured subject who responds with a series of typical reactions (Selye, 1937), concerned firstly with survival (Cannon, 1923), and then with healing (Schultis and Beisbarth, 1971, 1976).

The nature and severity of the injury conditions the character of both the immediate and delayed neurohumeral and biochemical changes which occur, not only locally but generally (Cuthbertson, 1976). Recovery from trauma, often complicated by bacterial infection and superadded starvation, requires the mobilisation and utilisation of endogenous fuel substrates (Clowes et al., 1976; Kinney, 1976). In a severe prolonged illness, the size of these body energy reserves may well prove to be the limiting factor in determining patient survival (Studley, 1936). This review concentrates on the history of our growing knowledge of the post shock recovery phase (Cuthbertson, 1942; Moore and Brennan, 1975), from the early descriptive studies, through the intensive biochemical and endocrine investigation of the last 35 years, to the current applied therapeutic phase in which manipulation of whole body (Blackburn and Bistrian, 1976) and even individual organ metabolic pathways (Bergstrom et al., 1972; Abel et al., 1973, 1976; Fischer et al., 1974; Aguirre et al., 1976) is becoming increasingly possible in injured or infected subjects. The work done during the early descriptive phase of trauma research was of fundamental importance and is outlined in Figure 3.

Nitrogen metabolism after injury.

In the 1930's Cuthbertson in Scotland first drew attention to the marked losses of nitrogen, sulphur and phosphorus which occurred in the urine of well nourished

Fig.3 OUTLINE OF EARLY RESEARCH FINDINGS



EARLY DESCRIPTIVE PHASE OF RESEARCH

Gross Whole Body Changes

* Weight loss
* Negative nitrogen balance
* Increased energy expenditure
* Hormonal derangements
* Fluid and electrolyte upset

MODIFYING FACTORS

Age, sex, body build, nutritional status, immobilization starvation, high energy feeding, blood loss, blood transfusion, anaesthesia, pain, sepsis.

individuals in good general health who sustained moderately severe traumatic injuries (Cuthbertson 1930, 1931, 1932, 1934, 1936). The timing, extent and duration of these increases in urinary nitrogen losses have been well described for a variety of injuries (Moore, 1952; Howard et al., 1944; Howard, 1945; Kreiger, 1954). In general these were related to the severity (Cuthbertson, 1942) and type of injury sustained (Davies et al., 1959), as much as 7% of the body nitrogen content being lost during the first 10 days following injury even on a normal food intake (Cuthbertson, 1932; Cairnie et al., 1957; Ministry of Health, 1964; Cuthbertson, 1972).

Cuthbertson found the nature of the disturbance following injury uncertain but considered that the increased urinary nitrogen losses which occurred probably represented a generalised protein disturbance originating in muscle (Cuthbertson, 1932, 1936; Cuthbertson et al., 1939), because the increase in urine nitrogen was mainly in the form of urea, with urinary N : S and N : K ratios highly suggestive of muscle catabolism. This finding was later supported by others (Frawley et al., 1955). Cuthbertson also investigated the relationship between weight loss after injury and the increased urinary nitrogen excreted, and found that he could account for most of the weight lost on the basis of loss of body protein (Cuthbertson, 1932; Cuthbertson et al., 1939). He reasoned that the breakdown of body protein stores after injury might be part of a primitive reflex to supply

energy for survival at a time when injury prevented the search for an adequate food supply.

Changes in energy expenditure after injury.

Cuthbertson's belief that protein was the main body tissue catabolised as a fuel after trauma was reinforced by his finding that patients who had suffered bony injury increased their oxygen consumption by 15 - 25%, and also had a rise in rectal temperature (Cuthbertson, 1932, 1970). These changes occurred in parallel with the post injury increased losses of urinary nitrogen (Cuthbertson, 1931, 1936). Cuthbertson calculated that the heat derived from the oxidation of protein corresponding to the extra nitrogen excreted in the urine after injury agreed very closely in magnitude and in timing with the increases in total metabolism. From this, and subsequent rat studies, Cuthbertson and his co-workers (Cuthbertson et al., 1939; Cairnie et al., 1957) concluded that the post injury increase in resting metabolism was due to "the heat resulting from the deamination process of protein breakdown and from the use of the non-nitrogenous residue as a source of energy."

Factors modifying the response to injury.

In a search for the cause of these injury responses, many factors were investigated, of these anaesthesia (Moore, 1957) and blood transfusion were found to play a minor role (Timoner et al., 1959) unlike haemorrhage, which

caused significant increases in urinary nitrogen losses (Flear and Clark, 1955). Prolonged immobilisation in healthy volunteers also increased urinary nitrogen excretion, but to a minor degree (Cuthbertson, 1929; Schonheyder et al., 1954; Deitrick et al., 1948), when compared with the overall injury response (Levenson et al., 1966). The effects of immobilisation could be prevented by increased activity (Whedon et al., 1949; Glickman et al., 1948). Other influences were recognised, which were capable of altering the degree, but not the character, of the protein changes after injury (Cuthbertson and Tilstone, 1968; Cuthbertson, 1975; Beatty, 1947; Beal et al., 1954; Calloway et al., 1955). For example, protein depleted man and experimental animals both failed to show the marked increase in nitrogen excretion characteristics of the injury response in normal subjects (Cuthbertson and Tilstone, 1969; Munro and Cuthbertson, 1943). In addition, rats fed different amounts of dietary protein showed net protein losses after injury which were directly proportional to the quantity of their pre-injury protein intakes (Munro and Chalmers, 1945). These observations reflected the importance of the body composition of the injured subject in determining the extent of the protein losses seen after injury (Cuthbertson, 1964; Moore and Ball, 1952; Border, 1970). The greatest losses occurred in well fed, heavily muscled young adult males, the smallest in females, the poorly nourished and the elderly. Some workers considered that partial starvation, which is a common occurrence after

severe injury, was the major cause of the marked post injury negative nitrogen balance (Abbott and Albertson, 1963; Dudley, 1959, 1968), but others found that injury and starvation occurring simultaneously resulted in an additive catabolic effect (Clark, 1967; Wilkinson, 1966). This sharply contrasted with the progressive decrease in both energy and nitrogen losses seen in simple starvation without injury (Keys et al., 1970; Lusk, 1931; Wilkinson, 1961; Levenson, 1975), and indicated that the relationship between injury and decreased food intake was complex. It was also known that it was possible to virtually eliminate the negative nitrogen balance after injury by feeding injured subjects high caloric supplements along with adequate amounts of protein (Cuthbertson, 1936; Co Tui et al., 1944; Riegel et al., 1947; Werner et al., 1949). This confusing situation was clarified by the work of Cuthbertson and Munro (Cuthbertson and Munro, 1937; Munro, 1964) who noted that on a fixed adequate protein intake, the energy level of the diet was the deciding factor in nitrogen balance, and with a fixed caloric intake, protein level was the determinant. Therefore at any fixed inadequate protein calorie intake there was an individual limiting energy level beyond which increasing calories without protein, or protein without calories, was ineffective in improving nitrogen balance. This relationship is shown in Figure 4. A similar relationship existed in the injured or septic subjects also, except that they required higher nitrogen intakes than the uninjured or the uninfected to achieve nitrogen balance (Kinney, 1976, 1975)

Fig. 4 RELATIONSHIP BETWEEN NITROGEN BALANCE PROTEIN INTAKE AND CALORIE INTAKE: NORMAL MAN. (Modified from Munro 1964)

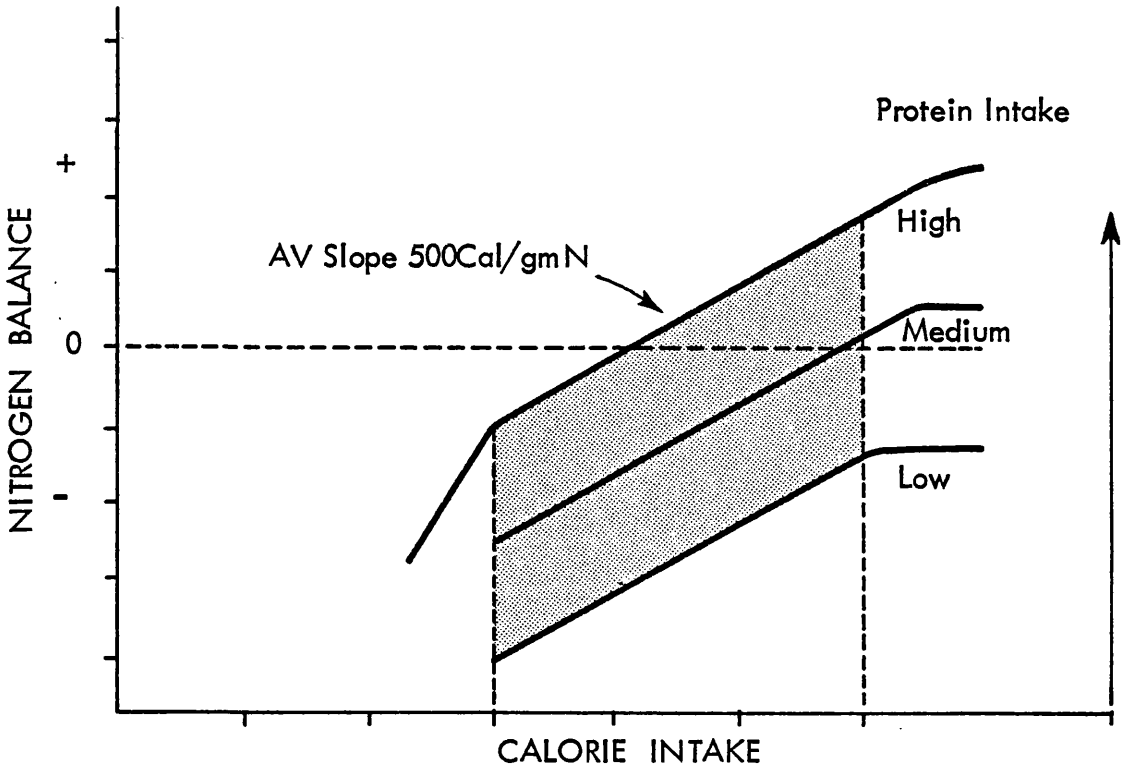


Fig. 5 THE INFLUENCE OF TRAUMA AND SEPSIS ON NITROGEN BALANCE

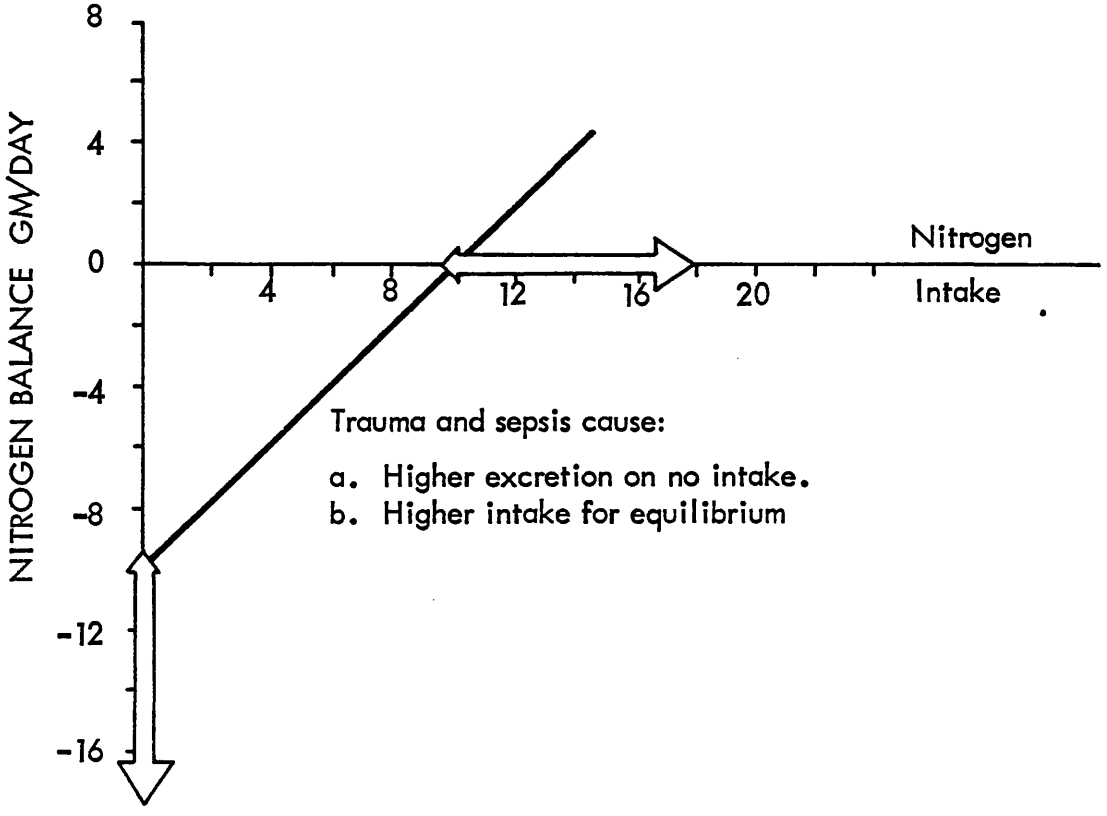
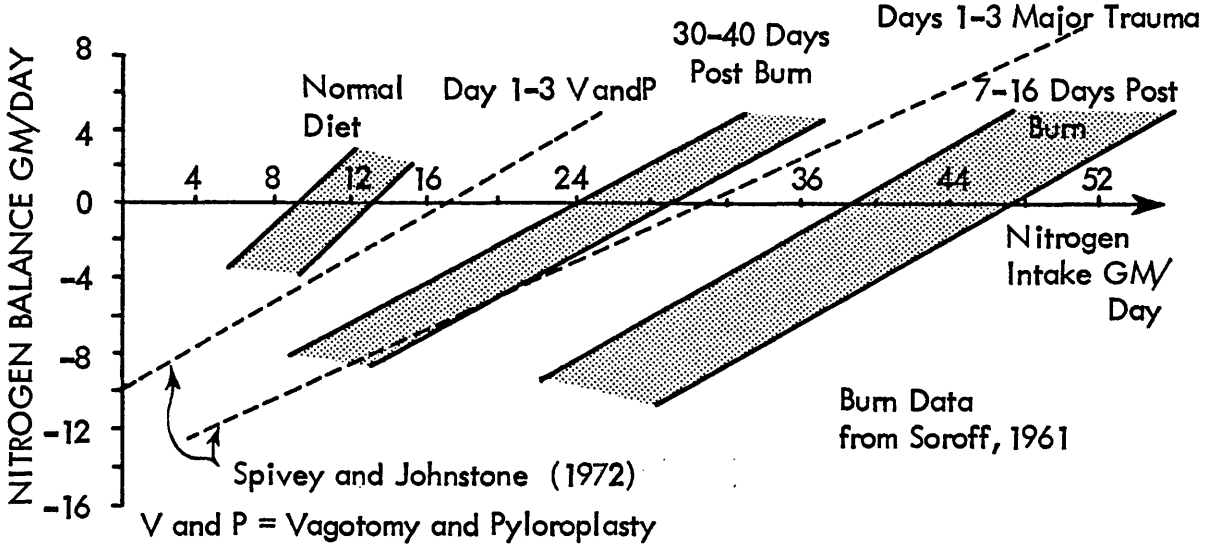


Figure 5. Diets offered to the injured therefore often lacked sufficient protein, or calories, or both, to achieve nitrogen equilibrium, and this factor, perhaps more than any other, explained the post injury negative nitrogen balance so often seen in patients (Moore and Brennan, 1975).

Hypercatabolic post injury states.

In severe injuries though, such as extensive full thickness burns (Wilmore et al., 1971), multiple fractures, or post operative peritonitis (Kinney, 1976), massive weight loss and very large increases in urinary nitrogen excretion occurred which could not be overcome, even by feeding excess amounts of protein and calories. Soroff found (Soroff et al., 1961) that in cases of major burns, at 7 - 16 days post burn, up to 47 g of nitrogen per day plus a very high caloric intake would be required to achieve balance, and at 30 - 40 days post burn up to 29 g nitrogen were required, compared with a nitrogen intake of only 9 - 13 g per day required to achieve balance in uninjured controls (Figure 6). Soroff's data not only illustrated the very high nitrogen intakes required in the catabolic post burn phase, but perhaps more importantly, showed that as the energy losses through the burn decreased, due to grafting and healing (Moyer and Butcher, 1967), the intake of nitrogen required to achieve equilibrium gradually became less until the normal range was reached at the end of convalescence. This coincided with the restoration of body weight. For comparison, data from 9 patients with multiple long bone fractures has been

Fig.6 INFLUENCE OF THE MAJOR BURN ON NITROGEN BALANCE



superimposed (Spivey and Johnston, 1972).

Hormonal changes after injury.

The endocrine changes following injury have been described in some detail (Moore, 1972; Johnstone, 1972) and represent an integrated neuroendocrine response to a noxious stimulus, characterised by sympatho-adrenal nervous system discharge - the "fright or flight" response of Cannon (Cannon, 1967) essential for survival during stress (Selye and Collip, 1936; Ingle et al., 1947; Ingle, 1951; Campbell et al., 1954). Hume, Stoner and others (Hume and Egdahl, 1959; Hume et al., 1962; Hume, 1974; Stoner, 1976) have studied the activation of the endocrine response, but it is the nature of the post injury endocrine changes which has attracted most attention (Cuthbertson and Tilstone, 1969; Hunt, 1972; Moore and Brennan, 1975). The phasic nature of these changes is well known (Hunt, 1972). There is an early catecholamine discharge which inhibits the release of insulin and also stimulates glucagon and ACTH production. The increasing cortico-steroid levels further inhibit the action of insulin on peripheral tissues. Salt and water metabolism is also disturbed with rises in aldosterone and ADH levels. In fact changes have been noted following severe injury in every endocrine system studied (Schultis and Beisbarth, 1976). It is however, only recently that the relationship between endocrine and metabolic changes after injury has become more fully understood. Moore now considers (Moore and Brennan, 1975) that most, if not all, of the post injury

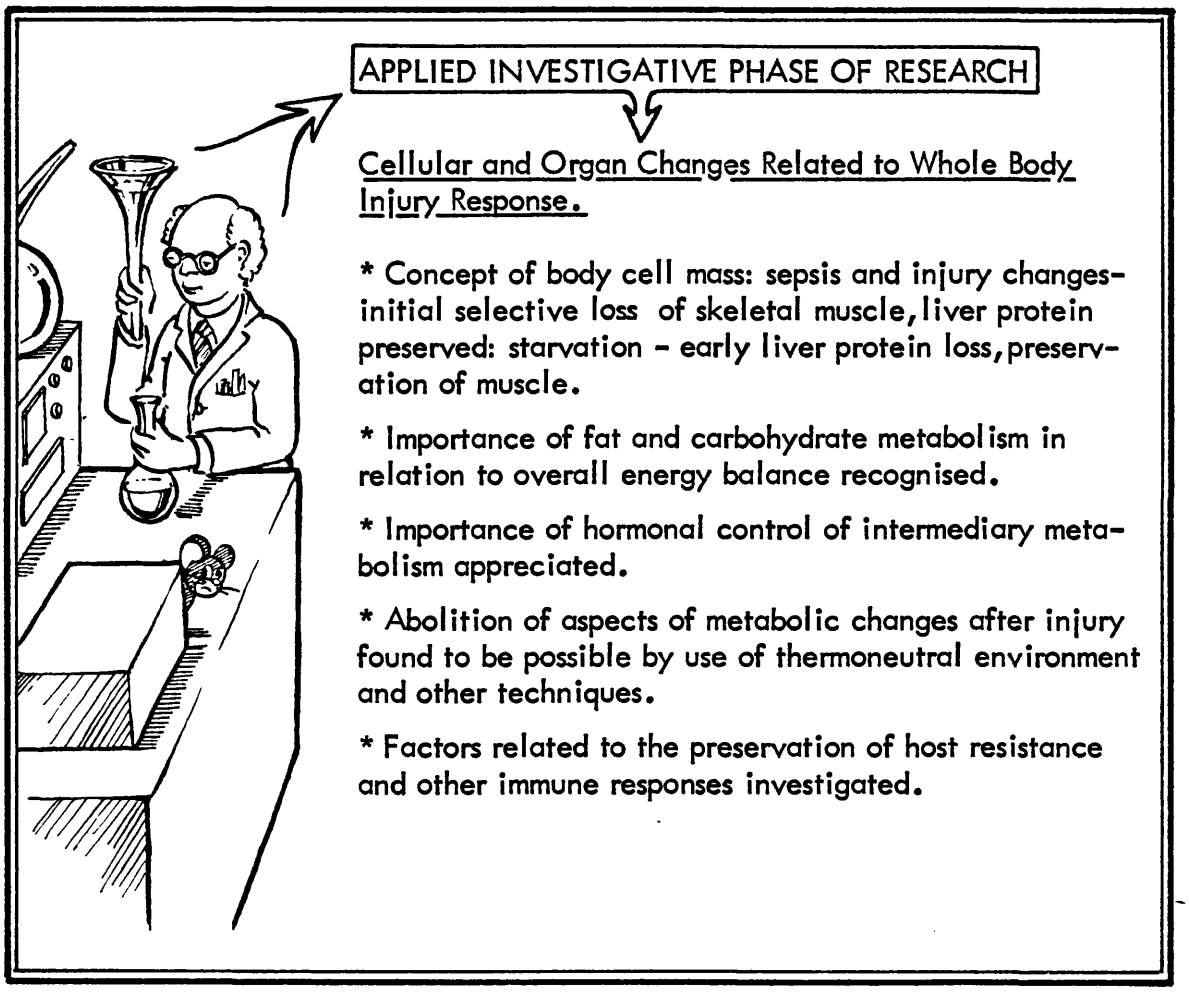
metabolic changes are due to the control exerted on intermediary metabolism by the endocrine system. This can influence the selection of tissue fuels for oxidation and the rate of their oxidation, and can also affect the body's ability to utilise administered nutrients as well as regulate salt and water balance.

This important conceptual advance has been due in large measure to the development of isotopic tracers, more refined biochemical techniques and basic studies which revealed the dynamic turnover rates of body tissues in normal and starved subjects (Cahill, 1970; Keys et al., 1970; Cahill et al., 1966). This phase of applied research began in the late 1940's and is outlined in Figure 7.

Body cell mass.

The foundations of these advances lay in the studies carried out by Moore and others (Moore, 1946; Moore et al., 1963; Pace and Rathburn, 1945) on the quantitative assessment of the total mass of body constituents and their individual flux rates. The concept of body cell mass emerged as that fraction of the body in which energy exchange exclusively occurred (Levenson et al., 1975). Levenson in New York recognised further specialised subdivisions of body cell mass, and carried out classic experimental studies on the rate of protein synthesis and catabolism in the tissues of the rat after extensive burns

Fig.7 OUTLINE OF APPLIED INVESTIGATIVE PHASE OF RESEARCH



(Levenson et al., 1959, 1976). Using ^{15}N labelled glycine as a tracer, he found that protein synthesis and catabolism were both greatly accelerated in the tissues of burned animals. This resulted in a net preservation of liver protein, but by contrast, the protein content of the carcass decreased, despite having a much slower turnover rate than liver. This decrease accounted mathematically for almost all of the increased urinary nitrogen losses caused by the injury. Reiss found similar liver and muscle protein changes in starved infected rats (Reiss, 1959) but noted a different response in starved control animals, in which there was marked loss of liver or visceral protein with little loss of muscle or peripheral protein stores. Injury and infection were therefore both characterised by net increased liver protein synthesis using amino acids released from net increased catabolism of muscle protein stores (Fleck and Munro, 1963), changes which could not be attributed to starvation alone (Table 2).

Starvation.

The importance of muscle amino acid metabolism and the adaptive changes which occur progressively in late starvation were subsequently studied in detail (Felig et al., 1967, 1969) and the role of alanine released from muscle and its importance as a glucose precursor in the liver was identified (Felig, 1973) along with more specific information on the factors regulating this process (Exton

TABLE 2. INJURY AND STARVATION CONTRASTED

	Injury (infection)	Starvation
PROTEIN METABOLISM	Prolonged increase in urinary nitrogen output, often 2-3 times greater than levels found in starvation alone. Gluconeogenesis from muscle amino acids increased. Relative preservation of liver protein content.	Brief small increase in urinary nitrogen output followed by a gradual decrease as adaptation to starvation occurs. Reduction of gluconeogenssis from muscle amino acids to conserve muscle protein. Early marked depletion of liver protein.
FAT METABOLISM	Fat oxidation rate greatly increased and exceeds that found in starvation. Free fatty acid levels elevated but ketonaemia not marked.	Fat oxidation rate increases slowly to supply majority of energy needs in the later stages of starvation. Marked ketonaemia occurs with ketoadaptation of brain and muscle metabolism.
CARBOHYDRATE METABOLISM	Tissue glucose oxidation increased and more rapid glucose turnover in the bloodstream in severe injury.	Tissue glucose utilisation is decreased.
EFFECT OF ADMINISTERED NUTRIENTS	Protein sparing action of administered carbohydrate or fat not marked.	Very pronounced protein sparing effect of administered carbohydrate and fat seen in starvation.

contd.

TABLE 2 contd.

	Injury (infection)	Starvation
HORMONE CHANGES	Elevations occur in the levels of adrenaline, noradrenaline, growth hormone, glucagon and corticosteroids. Insulin levels are increased but an insulin resistant type of glucose tolerance curve is found.	Small early increases are seen in adrenaline, glucagon and growth hormone. Insulin levels fall after a brief rise. An apparent deficiency of pancreatic secretion is seen in the starved subject associated with a decrease in pancreatic protein content.
ALTERATIONS IN ENERGY EXPENDITURE	Resting metabolic expenditure is increased after injury from +10% up to +100% from 10 days to 10 months after injury. The greatest increases being seen in major burns.	Resting metabolic expenditure falls progressively from normal values as starvation proceeds.
WATER AND SALT BALANCE	Water and salt conservation occurs immediately after injury and is prolonged. There is a relative insensitivity to sodium loading.	Water conservation begins gradually after 3 - 4 days starvation. Water overloading of tissues readily occurs. ECF volume increases.

et al., 1971; Felig and Wahren, 1971; Odessey et al., 1974; Fitzpatrick et al., 1975a).

In starvation, liver glucose production from amino acids was found to be progressively reduced, thereby conserving body protein, and favouring long term survival. After injury however it was noted that there was an increased flux of alanine and other amino acids from muscle which were converted by the liver into glucose (gluconeogenesis) (Krebs, 1964). This resulted in increased formation of urea in the liver as a by-product of this process.

Association of nitrogen metabolism and energy expenditure after injury.

During this period of very active research the relationship between changes in protein and amino acid metabolism and changes in energy expenditure was re-examined. Although the vague association of heat, fire and life dates back to antiquity, these relationships were not clearly understood and no quantitative measurement of the heat production of living animals was made until the late 18th century (Lavoisier, 1777; Crawford, 1788; Lavoisier and de La Place, 1780). Later still came the scientific proofs and comparisons of the different techniques of measurement of indirect and direct calorimetry (Rubner, 1894; Attwater and Rosa, 1899; Murlin and Lusk, 1915; Armsby and Moulton, 1925) and with this work came

the evolution of the law of conservation of energy (Glasstone, 1946) upon which modern animal energetic studies are based.

Our current knowledge of the body's response to injury can be traced through a similar historical development. Although many aspects of the effects of injury on the body had been studied, and changes in metabolism identified during the 18th and 19th centuries, the information obtained was fragmentary and unconnected (Cuthbertson, 1976). It was not until the early 1930's that Cuthbertson carried out his pioneering studies in Glasgow, which linked changes in body chemistry after bone fracture in man (Cuthbertson, 1930, 1931) with changes in energy expenditure (Cuthbertson, 1932) and formulated his unifying concept of a purposeful whole body response to injury which was directed towards the continuing provision of energy for survival even in the absence of an adequate food intake. These early studies of energy expenditure in patients were made using the crude indirect calorimetry techniques which had largely supplanted the direct calorimeters of the period, which were slow responding, massively constructed, and costly (Lefevre, 1911; Kleiber, 1950).

During the late recovery phase after bone fracture in man, Cuthbertson, as noted earlier, frequently found increases in oxygen consumption of 15 - 25% above resting

values, associated with a rise in rectal temperature. These increases in metabolic expenditure, which occurred in parallel with evidence of increased protein breakdown, were thought by Cuthbertson to originate in muscle, for the reasons previously given. The injured rat displayed similar changes (Cuthbertson, 1939). Despite these interesting early observations, research work into the metabolic responses to injury was mainly directed over the next two decades towards biochemical and hormonal events. Further studies of energy expenditure after injury did not take place until a major advance occurred in the technique of direct calorimetry.

In 1949 Benzinger and Kitzinger first described their design for a new type of calorimeter which used a special gradient layer for heat detection (Benzinger and Kitzinger, 1949). The adiabatic principles which had been responsible for the slow response time and relatively poor performance of earlier calorimeters were abandoned and replaced by direct measurement of the instantaneous flow of heat through the walls of the chamber. Not only the sensible heat loss (the sum of the radiative, convective and conductive components) but also the evaporative loss of the subject could be measured continuously and automatically. A calorimeter of the Benzinger type was built at the Rowett Research Institute in the early 1950's (Pullar, 1956, 1957, 1969) and this instrument was used by Cairnie and his co-workers to measure the increase in

metabolism after bone fracture in groups of rats (Cairnie et al., 1957). They found increases in metabolic rate of up to 7% above normal resting values in rats kept at an ambient temperature of 19°C after unilateral femur fracture. The average total metabolism of rats fed an 18.1% protein diet was 66.1 kcal/day or 145 kcal/$kg^{0.75}$ or 152 kcal/$kg^{0.75}$ on the third day after injury. This rise in metabolic rate was accompanied by an increase in urine nitrogen losses from 380 mg/day before injury to a maximum of 478 mg/day on the third day after fracture. The energy provided by the oxidation of this quantity of protein was 30 kcal/$kg^{0.75}$ before, and 37 kcal/$kg^{0.75}$ after injury, or 21% and 24% of the total metabolism respectively. The 7 kcal provided above basal by protein oxidation after injury was exactly sufficient to explain the 7 kcal increase in total metabolic expenditure measured by the calorimeter. The authors also noted very close agreement in timing between increased urinary nitrogen losses and increases in total metabolism after fracture. They found no evidence of an increase in fat or carbohydrate metabolism. Cairnie and his co-workers concluded that the post injury increase in resting metabolism was possibly the result of "heat from the process of deamination, and from the use of the non-nitrogenous residue as a source of energy," producing a form of endogenous specific dynamic action, as noted earlier.

Cairnie's views on the importance of post injury protein catabolism were challenged by Miksche and Caldwell (1967) who used a similar but more severe skeletal injury,

viz. bilateral femur fracture, and examined energy expenditure after injury in the rat using a sophisticated specially designed and constructed gradient layer calorimeter for a single rat (Caldwell et al., 1966). Miksche and his co-workers showed that fasted male albino rats had a significant increase of 14 - 20% in the rate of heat production, with increased sensible heat losses, and an increase in average body temperature (0.6 - 0.76^{o}C) following bilateral bone fracture. The major portion (40 - 70%) of the increment in the rate of heat production could they felt be accounted for by the Van't Hoff Q_{10} effect secondary to the elevation of the average body temperature. They calculated that the caloric equivalent of the increased urinary nitrogen excretion (using a value of 26.51 kcal per gm N; West, 1961) accounted for only 17% of the total increment in heat production on the first and third days, 35% on the second day and 26% on the fourth day post fracture. Taking the Van't Hoff effect and the caloric equivalent of the extra nitrogen excreted after injury together, these two factors accounted for 40% of the increased metabolic expenditure on the day of fracture, rising to 93% on the 2nd day post fracture. Respiratory quotient measurements indicated that control and injured rats were utilising fat as their main calorie source (RQ 0.7 - 0.73).

Direct comparison of Cairnie's and Miksche's apparently contradictory findings is however not possible because of significant differences in experimental

conditions. See Table 3. In particular, comparing fed with starving rats is misleading, as is the fact that urine was collected for nitrogen estimations over very different time intervals. Finally, one group of rats was mildly cold stressed, whereas the other group were near conditions of thermal neutrality. Because of these differences in experimental design, the significance of changes in protein metabolism after skeletal trauma in the rat and the relationship of these changes to energy expenditure remains disputed.

In other studies on rats with burn wounds, Caldwell and his co-workers had amassed considerable indirect evidence that increased protein catabolism by itself could not account for all of the increased energy expenditure seen after burning injury (Caldwell, 1961, 1962; Caldwell, et al., 1959, 1966). Caldwell's calorimetry studies on rats with 21% whole thickness dorsal burns (Caldwell et al., 1966) did establish that a different partitioning of heat losses occurred following burns compared with closed skeletal injuries. Experimental protocol, calorimetry and metabolic collection techniques were as outlined for the fracture studies. After burning, there was a significant increment in evaporative heat loss, up to five times the control values, but no increment in sensible heat losses. The increase in total heat loss was large, 47.6% above control values, and sustained, continuing up to and beyond the 43rd post burn study day. The caloric

TABLE 3. COMPARISON OF THE EXPERIMENTAL CONDITIONS OF RAT CALORIMETRY USED BY TWO SETS OF INVESTIGATORS (CAIRNIE ET AL., 1957; MIKSCHE & CALDWELL, 1967).

	Cairnie et al. (1957)	Miksche & Caldwell (1967)
Calorimeter	23 kg in adapted gradient layer	Single rat size, purpose-built gradient layer
Measurement period	22 out of every 24 h continuous measurement	3 periods of 20 min per 24 h
Rats, number in calorimeter	9 rats packed into boxes within calorimeter chamber	Single
Rats, number in study	9 in calorimetry group, 6 in separate metabolic cages. Urine nitrogen estimated on the latter	12 in calorimetry group. 12 in a separate metabolic group. Urine nitrogen estimated on the latter
Injury	Single femur fracture, open method	Bilateral femur fracture, closed method
Feeding regime	Rats restricted after fracture to mean pre-injury *ad lib.* intake	*Ad lib.* throughout
Duration of urine collections	24 h period	(14.01 - 23.59 hours) 10 h post-prandial period

contd.

TABLE 3 contd.

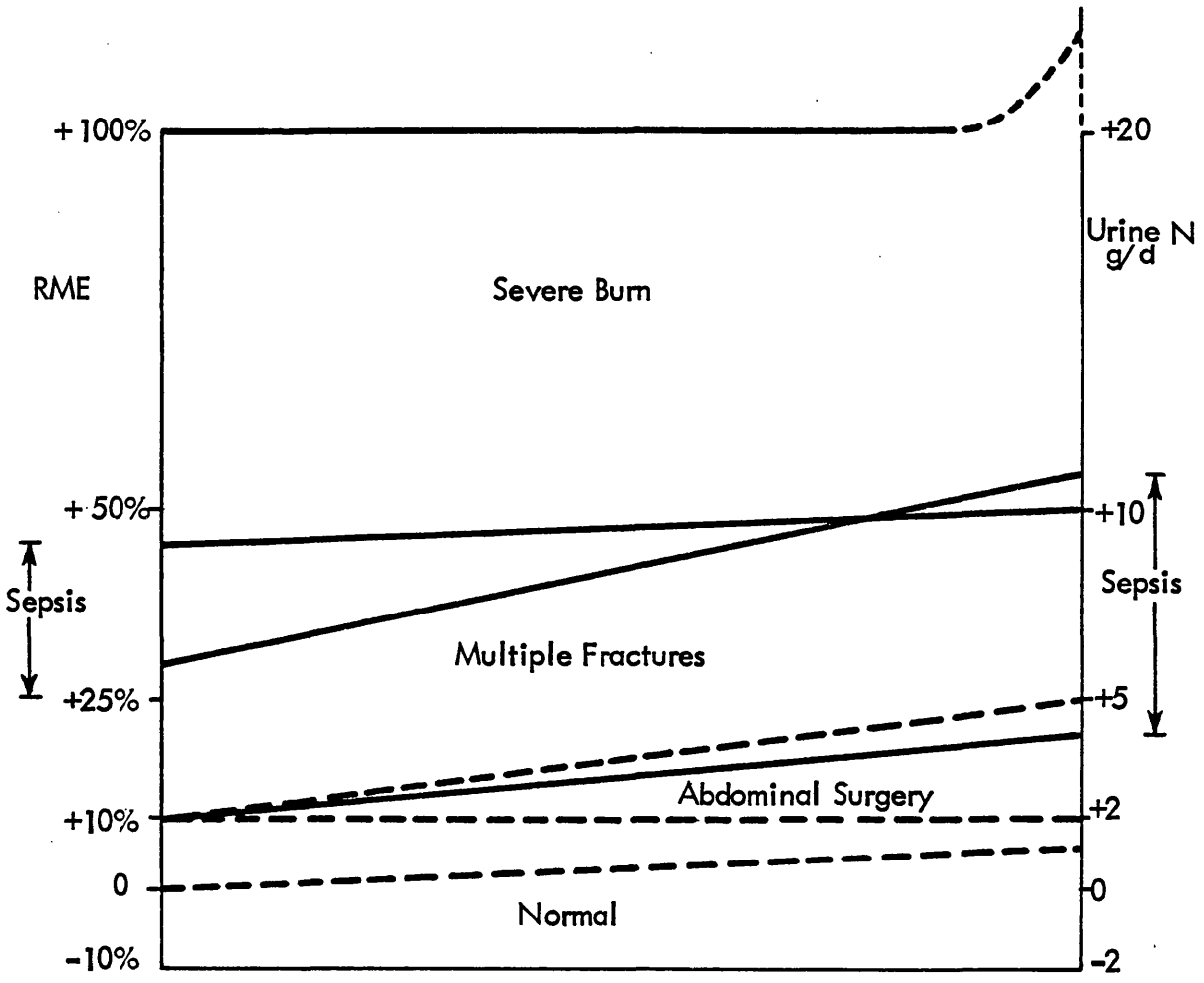
	Cairnie et al. (1957)	Miksche & Caldwell (1967)
Animal house temperature	19 ± 2°	25 ± 1°
Calorimeter operating temperature	19 ± 0.3°. Below rat thermoneutral zone	28.4 ± 0.23°. Within rat thermoneutral zone
Dietary status of rats during calorimetry	Non fasted, fed during calorimeter runs	18 h post-absorptive during calorimeter run

contribution made by the oxidation of the increased protein excreted remained around 20% of the total energy expenditure before and after injury (Caldwell, 1970).

The hypothesis advanced by Cuthbertson and his co-workers that the body used up its protein reserves (or protoplasmic mass) as a fuel substrate in order to meet the increased energy requirements after injury was therefore not supported by the available experimental evidence in man, or in animals.

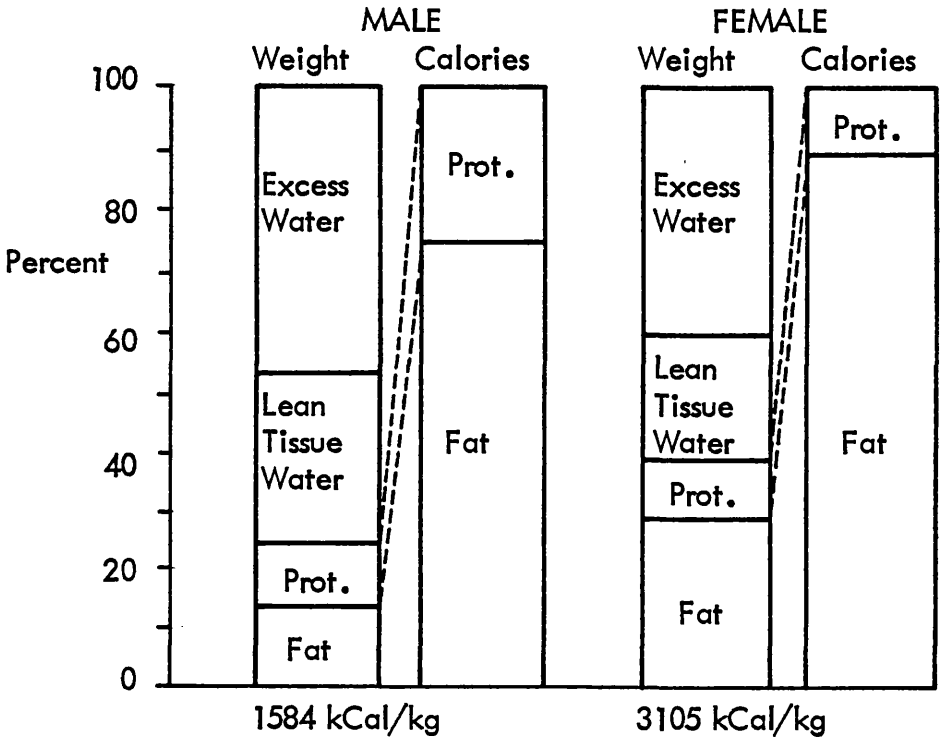
Further research was carried out by Kinney in New York who constructed the first practical indirect calorimetry system for use in a clinical situation (Kinney et al., 1964) and studied energy expenditure in relation to metabolic changes in a wide variety of surgical conditions (Kinney et al., 1968, 1970a, 1970b). These results are summarised in Figure 8. Very small increases in energy expenditure (less than 10%) were found after elective surgery. Multiple bone fractures were followed by increases in energy expenditure of up to 25% above normal. Sepsis, particularly peritonitis, resulted in larger increases in energy expenditure, which persisted for prolonged periods until the infection was overcome. Burn wounds produced the greatest increase in heat losses (from + 50% to + 125% above predicted normal values) due to destruction of the normal skin barriers preventing water and heat losses (Moyer and Butcher, 1967).

Fig. 8 RELATIONSHIP BETWEEN ENERGY CHANGES AND NITROGEN METABOLISM AFTER INJURY IN MAN.



In contrast with earlier estimated increases in energy losses after burning (Artz and Reiss, 1957; Moore, 1957), Kinney and his co-workers found that the measured increases in energy expenditure after burn injury were insufficient to explain the sustained weight loss of 0.3 - 0.6 kg/day which occurred in these patients. They therefore examined the balance between protein and non-protein tissue fuel during the period of weight loss. Protein provided only 12 - 22% of the total energy expenditure even in those forms of injury where the daily nitrogen excretion was significantly elevated. This was not inconsistent with the finding that the rate of weight loss after injury ran in parallel with the nitrogen losses, because body protein exists in a hydrated form (Kinney, 1959). Therefore the weight lost after major injury contains a high proportion of hydrated protein on a weight for weight basis, but when considered in terms of calorie contribution towards overall energy balance, the protein contribution formed only a small proportion of the total. The remainder (78 - 88%) was met by oxidation of catabolised body fat. But, even taking the hydration of protein into account, Kinney's group could not explain more than 60% of the weight loss found after severe injury (Figure 9). The remainder they suggested was due to the excretion of excess body water, but they were unable to corroborate this with experimental evidence. The accuracy of solely indirect calorimetry studies carried out in such circumstances therefore remains uncertain at present.

Fig.9 RELATIONSHIP BETWEEN POST OPERATIVE WEIGHT LOSS AND CALORIE BALANCE OVER 10 DAYS. (Redrawn from Kinney et al 1970)



To clarify this situation, an experiment was devised to measure whole body and splanchnic blood flow and oxygen consumption in patients with intraperitoneal infection (an accelerated nitrogen losing state with increased energy expenditure similar to injury) (Gump et al., 1970a) and in patients with burns (Gump et al., 1970b). The rationale being that if the hypermetabolism of injury was due solely to the deamination of labile body protein, this process would be likely to occur mainly in the liver. But in infection and after injury, the increased blood flow and oxygen consumption across the splanchnic viscera accounted for only 40 - 50% of the total increase, establishing that the hypermetabolism of this form of surgical fever involved tissues other than the liver, and chemical reactions other than deamination and urea synthesis.

Kinney and his co-workers therefore thought it incorrect to suggest that a specific protein catabolic effect took place after severe injury in order to provide the increased body energy needs. Rather it appeared that there was an increase in metabolism generally via the normal intermediary metabolic pathways. In keeping with this, Kinney presented evidence (Kinney et al., 1970a, 1970b) that protein degradation occurred after injury in order to meet the requirements for carbohydrate intermediates via hepatic gluconeogenesis, and not to meet whole body energy requirements. He pointed out that most amino acids yield carbohydrate intermediates or are

glucogenic on deamination, and, while fatty acids can provide two-carbon fragments readily for general tissue energy requirements, they cannot be used to provide a net gain of carbohydrate intermediates, glycogen, or circulating glucose (Coleman, 1969). Therefore while fat stores could be used to meet energy needs in the state of semi-starvation often associated with injury, protein represented the only sizeable reserve of carbohydrate intermediates and glucose percursors also essential for survival. These provided a source of carbon skeletons for the synthesis of non-essential amino acids to sustain the synthesis of body protein (Myers, 1950; Krebs, 1964), materials such as α-oxoglutamate for the Krebs' cycle, new glycerol for the synthesis of triglycerides, and glucose, an essential fuel for organs such as the brain in the absence of an adequate oral intake.

Carbohydrate metabolism after injury.

Kinney sought further evidence for his views in the existing literature on changes in carbohydrate metabolism after injury. Previous investigators had ascribed the initial hyperglycaemia following injury (Thompson, 1938; Hayes and Brandt, 1952; Drucker et al., 1953; Howard, 1955) to catecholamine mediated rapid mobilisation of body carbohydrate stores, together with a probable decrease in tissue utilisation of glucose due to the presence of a "peripheral resistance" to the action

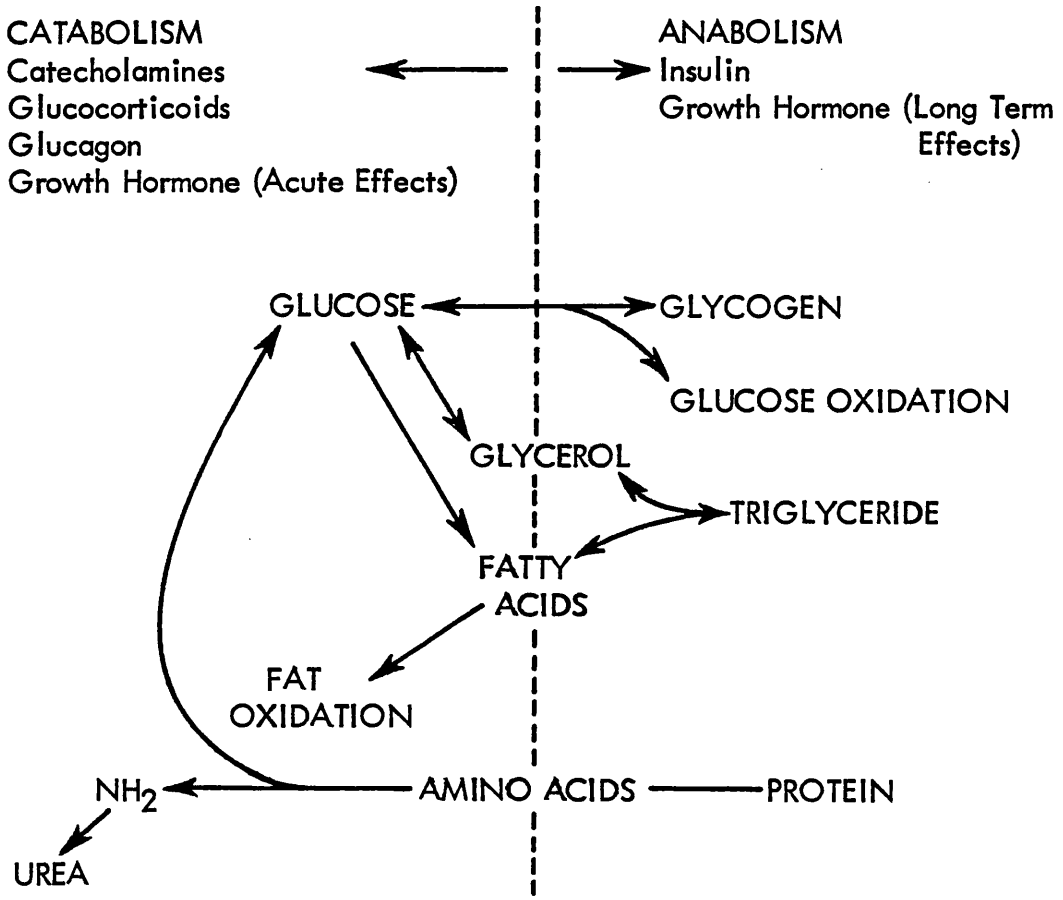
of insulin (Johnstone, 1968; Gump et al., 1974). However most of these observations were made during the early response to injury or so called "Ebb Phase". Carbohydrate stores within the body are insignificant (less than 0.6 Kg) and will last less than half a day unless replenished. Therefore after even relatively short periods, in the absence of food, body glucose requirements must be supplied by an increased hepatic production of glucose from protein precursors. Using an isotope tracer technique to follow dynamic changes in carbohydrate metabolism (Spencer et al., 1971), Kinney and his co-workers studied injured and septic patients after the initial shock or "Ebb Phase" and found in this situation that hyperglycaemia was the result of an increased glucose flow. That is, an increase in the synthesis of glucose relative to an increased turnover rate. Injury and sepsis apparently did not impair the ability of the body to oxidise glucose (Gump et al., 1974). In fact, in severely septic patients, glucose oxidation was increased (Long et al., 1971) and gluconeogenesis could not be suppressed, even by intravenous infusion of glucose (Long et al.,1976). This suggested that after injury the normal neurohumoral regulation of gluconeogenesis in muscle and liver had ceased to be effective, or had been over-ridden. The reason for this is unknown, but the explanation may be in the upset hormonal balance which follows severe injury.

Hormonal control of intermediary metabolism after injury.

Increases in the mainly "catabolic" hormones, adrenaline, noradrenaline, corticosteroids, glucagon and growth hormone predominate (Allison, 1974) (Figure 10). These changes can, of themselves, lead directly (Manchester, 1968) and indirectly (Himms-Hagen, 1967) to greatly enhanced gluconeogenesis from protein with concomitant depletion of glycogen stores, and accelerated mobilisation of fat reserves. Wilmore has recently stressed (Wilmore et al., 1976a) the central role of catecholamines as mediators of the metabolic response to severe thermal injury, regulating not only heat production but also tissue substrate flow, while Alberti has highlighted the need to review the role played by cortisol (Alberti, 1977). It may well prove, as Moore and Brennan have stated (1975) "that most, if not all, of the post traumatic metabolism is due to an interplay of endocrine forces on the selection of body fuels for oxidation, and the rate of their oxidation, on the ability to utilise extraneous nutrients, and on the conservation of water and salt."

However Clowes and his co-workers in Boston (Clowes et al., 1976) point out that this selection of tissue fuels by the endocrine system may not be quite so purposeful or pre-meditated as perhaps Moore and Brennan's statement implies. They demonstrated in patients and experimental animals that, in trauma or high flow septic

Fig. 10 HORMONAL CONTROL OF METABOLISM (Modified from Allison 1974)



states, there was a decreased glucose uptake by skeletal muscle, despite the presence of hyperglycaemia and hyperinsulinaemia. This "insulin resistant" state appeared to affect solely muscle, as the suppression of lipolysis and reduced levels of free fatty acids also seen in these subjects indicated that adipose tissue has responded normally to the presence of raised insulin levels (Ryan et al., 1974).

It was suggested that muscle "insulin resistance" led to a sequence of events culminating in a localised energy or tissue fuel deficit in muscle. Because of the altered hormonal climate, even those small amounts of glucose which were metabolised in muscle were not fully oxidised. Most of the glucose was anaerobically degraded to lactate even in the presence of an adequate tissue blood flow and normal oxygen consumption (Clowes et al., 1974). Because of the reduction in free fatty acid and ketone body availability, fat could not be utilised effectively as an energy source by "insulin resistant" muscle. This lack of locally available energy in muscle was compensated for by direct oxidation within muscle of the branch chain amino acids (leucine, isoleucine and valine) derived or cannibalised from its own protein structure. Subsequent transamination of these oxidised amino acids to pyruvate produced increased levels of alanine in the blood, which along with the excess lactate, glycerol, and certain other amino acids were cleared

from the blood by increased gluconeogenesis in the liver (Odessey et al., 1974). The increased glucose turnover after injury and sepsis could therefore be simply a by-product of a local energy deficiency in skeletal muscle. This could also explain why glucose infusions did not reduce gluconeogenesis after injury since it is only by the process of gluconeogenesis that excess blood lactate, alanine and certain amino acids can be metabolised. Clowes considers that changes in muscle metabolism alone can account for much of the metabolic sequelae to injury and sepsis, without the need to postulate an increased energy demand by all the tissues as a cause of wasteful protein degradation. Ultimately, selective oxidation of branch chain amino acids in muscle, irrespective of cause, would result in a failure of new protein synthesis in other parts of the body because synthesis of a specific protein in the wound or by the liver is blocked, if even one of the constituent amino acids of that protein is missing. Impaired protein synthesis has far reaching consequences. In particular, preservation of host resistance by an adequate production of specific immuno-protein in injury or in infection (Beisel, 1975; Wannemacher et al., 1975, 1977) may well determine ultimate survival in the nutritionally depleted septic postoperative patient or in the patient with extensive burns.

Endocrine alterations in relation to nutrition after injury.

Although controversy exists as to the detailed mechanisms of muscle proteolysis in severe injury or infection, there is little doubt regarding the effectiveness of current nutritional therapy in improving survival after extensive trauma. Moore in Boston has reviewed this topic at length (Moore and Brennan, 1975) and considers two main principles of nutritional management after injury to be important.

1. The abatement of those injury components which induce or prolong a "catabolic" neuroendocrine response in the body.

2. The provision of adequate amounts of appropriate nutrition, in a readily assimilable form, at the correct times after injury, in order to best maintain or restore body composition.

Taking the first of these themes, since it appears that many of the varying afferent inputs of injury such as psychic stimulation, pain, blood loss, hypoxaemia, tissue necrosis and sepsis, have as a common pathway, an increased discharge of sympatho-adrenal hormones, much of the standard treatment of the injured patient such as adequate analgesia, prompt replacement of blood and fluid losses, splinting of injured parts, thorough debridement

of wounds, oxygen therapy, and the use of antibiotics, already go a long way towards meeting Moore's first principle of nutritional management.

In addition to these direct methods of reducing sympatho-adrenal discharge, recent evidence suggests that additional homeostatic mechanisms may be deliberately invoked in order to reduce further the catecholamine regulated breakdown of body protein. Traditionally the hypercatabolism of thermal injury has been attributed mainly to accelerated water and heat losses from the wound surface which impose a powerful cooling load upon the thermo-regulating mechanisms of the body (Arturson et al., 1977). This leads to a compensatory catecholamine mediated increase in heat production, with rapid depletion of body energy stores. The expected protein and weight losses do not occur after severe burns when patients (Davies et al., 1969; Davies and Liljedahl, 1970) and experimental animals (Caldwell, 1970) are nursed at thermoneutral ambient temperatures (30 - 32^{o}C) (Tilstone, 1974). But Campbell and Cuthbertson (1967) found this also took place in rats with fracture of a femur where the cooling load is obviously much less. It was also found by Cuthbertson et al. (1968) that in patients with fractures treated after injury at 30^{o}C ambient temperature, the loss of urinary nitrogen was reduced. The mechanisms of this effect are not fully understood (Wilmore et al., 1975a, 1976b). Although some of the

reduction in weight loss seen at the higher ambient temperature could be related to reduced sensible heat losses (Caldwell, 1970) it is interesting that Liljedahl (1972) found a marked reduction in the expected excretion of catecholamines after thermal injury when patients were treated in thermoneutral environments compared with ordinary ward temperatures. As interpreted by Clowes and his co-workers earlier (Clowes et al., 1976) this would be likely to reduce or unblock the "insulin resistant" state of skeletal muscle thereby decreasing the need for self destructive oxidation of muscle branch chain amino acid pools. Much more experimental work is required to determine whether this hypothesis is valid, or whether it can be effectively applied to injuries other than burns, but the prospect of environmental control of body hormonal climate after injury is appealing.

It may be possible to overcome partially, muscle "insulin resistance" by giving excess amounts of the anabolic hormone insulin. Woolfson and Allison's recent studies (1977) indicate that insulin has a specific effect limiting proteolysis after injury, independent of the source or number of calories provided. Insulin therapy can also be effective after injury in enhancing active ion transport across cell membranes, bringing about a beneficial naturesis and diuresis (Flear et al., 1977). This seems to foster at least in a temporary way, an improvement in patients which is similar to that seen when recovery, or a return to anabolism (Moore and Brennan, 1975)

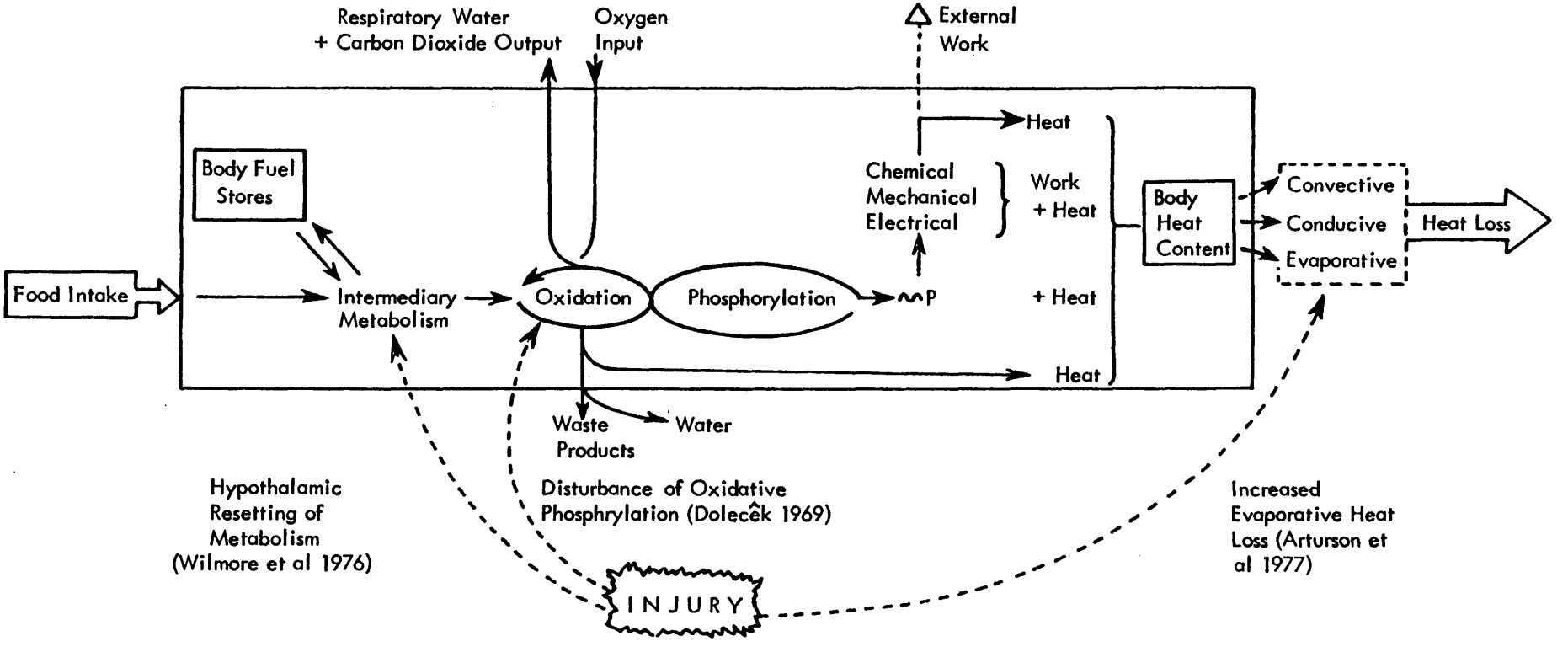
occurs naturally in the course of an illness. It is likely that adjunctive hormonal therapy of this type, used in selected circumstances, will form an increasing part of the nutritional care of the severely injured or burned.

With regard to the provision of adequate nutrition, Blackburn (Blackburn and Bistrian, 1976) has stated that recent developments in enteral and parenteral feeding rank in importance with the discovery of antibiotics in influencing the survival of many patients who would otherwise die. The price for this advance in therapeutic capability has been an increase in complexity, both of the knowledge expected of the physician, and also of the design of successful nutritional support programmes. The provision of protein and calories without regard to other factors influencing the rate of depletion of body stores is no longer sufficient. The objective must be to achieve a functional redistribution of protein in order to enhance those aspects of the metabolic response to injury which are beneficial, e.g. protein synthesis essential for repair, without at the same time, irreversibly and perhaps fatally depleting lean body mass. To this end, recent research work has involved (Blackburn and Bistrian, 1976) the development of nutritional techniques favouring rapid assimilation of those nutrients which best preserve body protein in a wide variety of differing clinical situations. Knowledge of food and whole body energetics thus assumes an increasing importance in the severely injured.

CONCLUSIONS

The paradox therefore exists that despite the discovery of an increasing amount of biochemical detail, energy balance after thermal injury remains neglected, and its key relationship to the nature of the tissue fuels utilised after injury uncertain. The complexity of the situation may be summarised diagramatically (Fig. 11).

Fig. 11 SCHEMATIC REPRESENTATION OF WHOLE BODY ENERGY EXCHANGE (Modified from Kinney 1976 - with permission)



DEVELOPMENT OF A GRADIENT LAYER CALORIMETRY SYSTEM

MATERIAL and METHODS

DESIGN CONSIDERATIONS

Although relatively inexpensive 'heat sink' or direct 'air' calorimeters have been used to study large animal energy expenditure (Visser and Hodgson, 1960; Kelly et al., 1963; Carlson et al., 1964; and Mount et al., 1967), these systems are slower to respond and less sensitive than the gradient layer (Benzinger and Kitzinger, 1949) calorimeters used to investigate the effects of injury on metabolism.

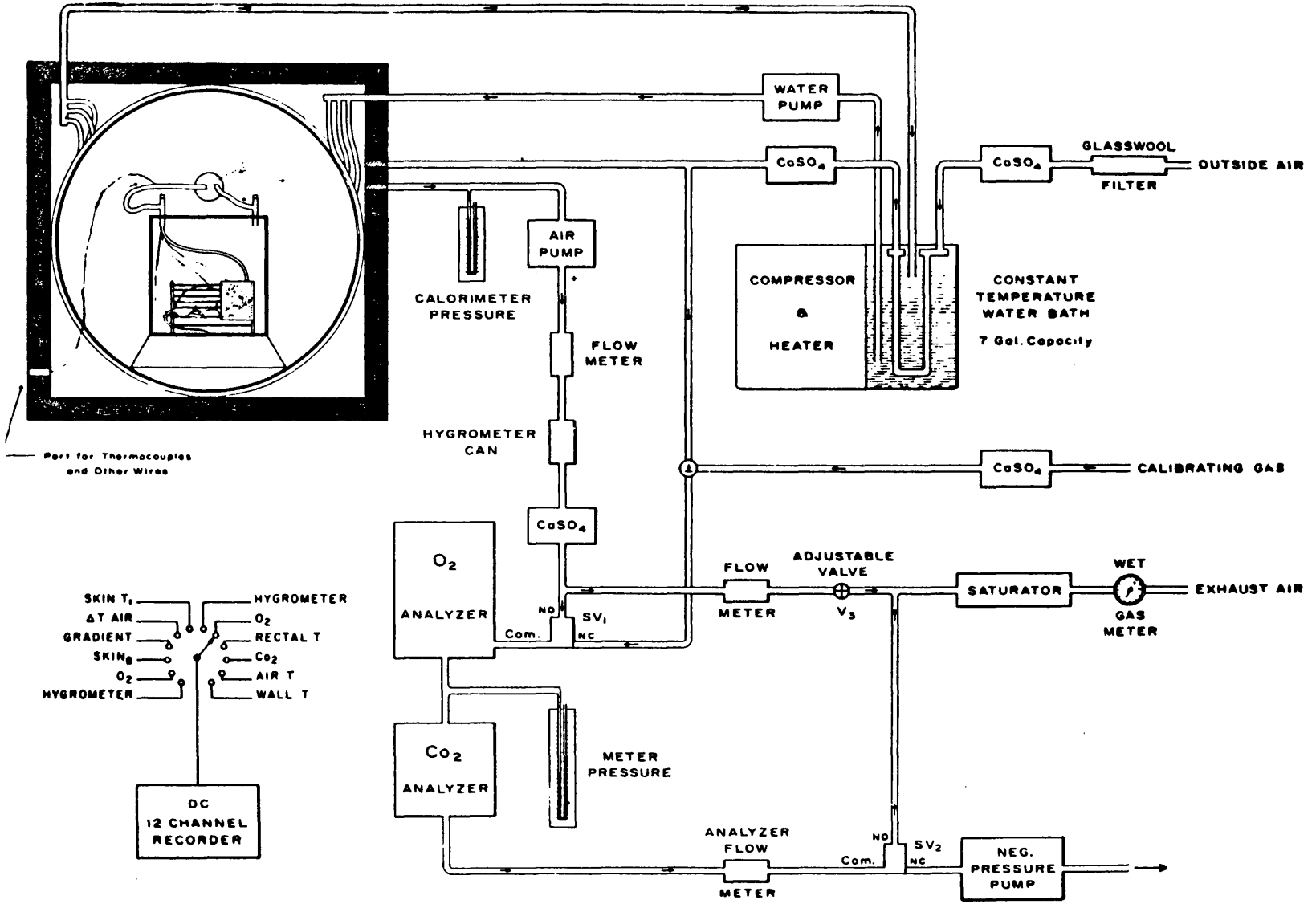
The single rat direct gradient layer and indirect calorimeter described by Caldwell et al. (1966) and used for burn studies in the rat, was selected as a prototype for the design of the Glasgow system. Accurate measurement of energy expenditure after thermal injury requires simultaneous measurement of heat production by indirect calorimetry (Flatt, 1969), together with determination of partitioned heat losses and change in body heat content by direct calorimetry (Puller, 1969). The following parameters must be measured: oxygen consumption, carbon dioxide production, evaporative

water loss, energy transfer across a gradient layer, temperature change of the air passing through the calorimeter, calorimeter average wall and air temperatures as well as rat wound, skin, and core temperatures.

The Caldwell calorimeter established that a small gradient layer box (500mm x 500mm x 300mm) containing 1638 copper-constant in thermo-couples producing 1.43 W per mV at 31^{o}C was, when linked to a ventilatory air circuit, sufficiently accurate and rapid in response to be satisfactory for studying energy expenditure in burned rats. This system is shown in Figure 12. Though the Caldwell calorimeter functioned reliably, several adverse features in its design restricted its usefulness for the type of burn studies proposed in Glasgow. The following points were considered:-

1. The volume of the Caldwell calorimeter box and its ventilating piping, when used with a Beckman F-3 oxygen analyser and type L/B 15A carbon dioxide analyser required air flow through the calorimeter to be regulated at between 1500 and 2000 ml per minute, depending upon oxygen depletion and water vapour loss by the rat under study. Severe burns completely destroy the normal skin evaporative water barrier (Moyer et al., 1966; Spruit and Malten, 1966) often leading to substantial evaporative water losses (Hardy et al., 1955; Roe, 1967; Roe and Kinney, 1964) which are

Fig.12 VENTILATION CIRCUIT OF GRADIENT LAYER CALORIMETER BUILT BY CALDWELL, HAMMEL & DOLAN IN 196



proportional to the extent of skin burned (Gump and Kinney, 1967). In the rat, studies have been made to measure the quantity of water loss following a standard burn wound (Morgan et al., 1955). A full thickness skin burn of 30% of body surface area increased the rate of insensible water loss by 108%, from 1.2g/kg/hr before burning, to 2.5g/kg/hr after burning. Measurement was made in still air at 26-28^{o}C. Rates of evaporative water loss from the burn area were similar to the rate of evaporation of water from an open beaker with a surface area equivalent to the burn (Moyer and Butcher, 1967; Caldwell et al., 1959; Fallon and Moyer, 1963).

In Glasgow, experiments with a 500 x 500 x 300 mm box indicated that if an air flow rate of 1 - 2 litres per minute is used with rats given a 20% body surface area burn, an uncontrolled accumulation of water vapour occurs around the rat and water precipitation takes place within the box at ambient temperatures of 20^{o}C. The humidity around burned rats in Caldwell's experiments must therefore have been uncontrolled and markedly different from the controls.

2. To prevent precipitation of water within the Caldwell calorimeter, first, only air dried to a dew point of -70^{o}C by passing it over calcium sulphate, could

be introduced into the calorimeter. Second, based upon the published data, it appeared necessary to maintain the mean calorimeter wall temperature between 27.4°C and 30.0°C, and the operating air temperature between 27.8°C and 30.6°C.

3. In addition to requiring air flow rates between 1500 - 2000 ml/min to ensure satisfactory oxygen depletion and carbon dioxide production for accurate measurement by the analysers selected, to achieve a rapid gas analyser response time (less than 1 min for the oxygen analyser), Caldwell opted to restrain the rat and place its head within a semi closed hood. This formed the air extraction point from the calorimeter chamber.

In summary, though capable of accurate measurement of rat heat exchanges, Caldwell's calorimeter had the following restrictions. Rigid immobilisation of the rat was essential to prevent it turning within the box during a measurement period. The calorimeter operating temperature could not be varied without risking water precipitation in the ventilation circuit. Humidity within the calorimeter was unknown and could not be controlled.

The study of thermal trauma within the Institute of Physiology at Glasgow University was a venture for which there was no precedent. All of the materials and basic apparatus used, including a special animal house,

and adjacent animal preparation and calorimeter laboratories, were designed and in most cases constructed by the author together with the individuals acknowledged in the preface of this thesis.

Financial support for development of the calorimetry system itself was provided by a grant from the Scottish Hospitals Endowment Research Trust. The Glasgow Calorimeter was therefore designed as one component of an integrated experimental system solely for the investigation of energy exchanges after burning injury. As it was anticipated that construction and calibration of the calorimeter system would require a minimum of 2 - 3 years, and that thereafter it would remain in use for a prolonged period, it was our objective to make the design flexible to permit investigation of foreseeable clinical advances in burn care. In particular the beneficial effect of treating burned patients at raised environmental temperatures was noted and it was thought that alteration of ambient humidity levels might also be important.

Consequently, in addition to performing the measurements made by the Caldwell calorimeter, the Glasgow Calorimeter was designed to

1. Operate accurately over a wide range of ambient temperature, from 20 - 30^{o}C.

2. Maintain controlled low or high relative humidity levels within the ventilatory system without risk of water precipitation.

3. Allow the rat freedom of movement within the calorimeter, yet retain rat instrument attachment leads over prolonged measurement periods.

4. Be readily calibrated and run by one person, by having a fully automatic data collection and handling system, and by simplifying the control system to a few essential switches, making other switches, valves, and necessary regulators automatic.

The author was solely responsible for the initiation and day to day direction of the calorimeter project, and jointly responsible for the final design. Close liaison was established with Mr. K. B. Carter of the then W.R.H.B. Department of Clinical Physics and Bioengineering who supervised detailed construction of the calorimeter and provided solutions to the myriad of electronic and mechanical engineering problems encountered. Assembly and where appropriate, manufacture of calorimeter components was performed by many members of the DCPB staff.

Wherever possible commercial components were used and assembly was adjusted to accommodate these ready made parts.

The calorimeter is housed in a specially built laboratory (Fig. 13) adjacent to the animal unit (Fig. 14).

DESIGN and CONSTRUCTION

Step 1. GRADIENT LAYER BOX

A gradient layer box suitable for heat emission studies in the rat was purchased from the Thermonetics Corporation, San Diego, U.S.A. (Poppendick and Hody, 1972). This is shown in Figure 15, and is listed as type SEC - A - 04 L by the manufacturer. This small box size (115mm x 115mm x 230mm) was selected because a relatively rapid calorimeter response time was required for physiological studies. It has been established that for gradient layers calorimeters, the total response time of the gradient layer is the sum of two factors (Bothe, 1958). First, the wall time constant, which is determined by the materials and type of construction in the gradient layer thermocouples, and by the techniques used for measuring the integrated temperature difference across the thermally insulating layer. These features are fixed by the manufacturer. Second, the convective heat transfer time. This is a function of the geometry and dimension of the gradient layer enclosure, the size of the animal within the enclosure, and the air flow rate around it. In the Glasgow system the convective heat transfer time has been decreased by closely matching the size of the

Fig. 13 PLAN OF CALORIMETER LABORATORY

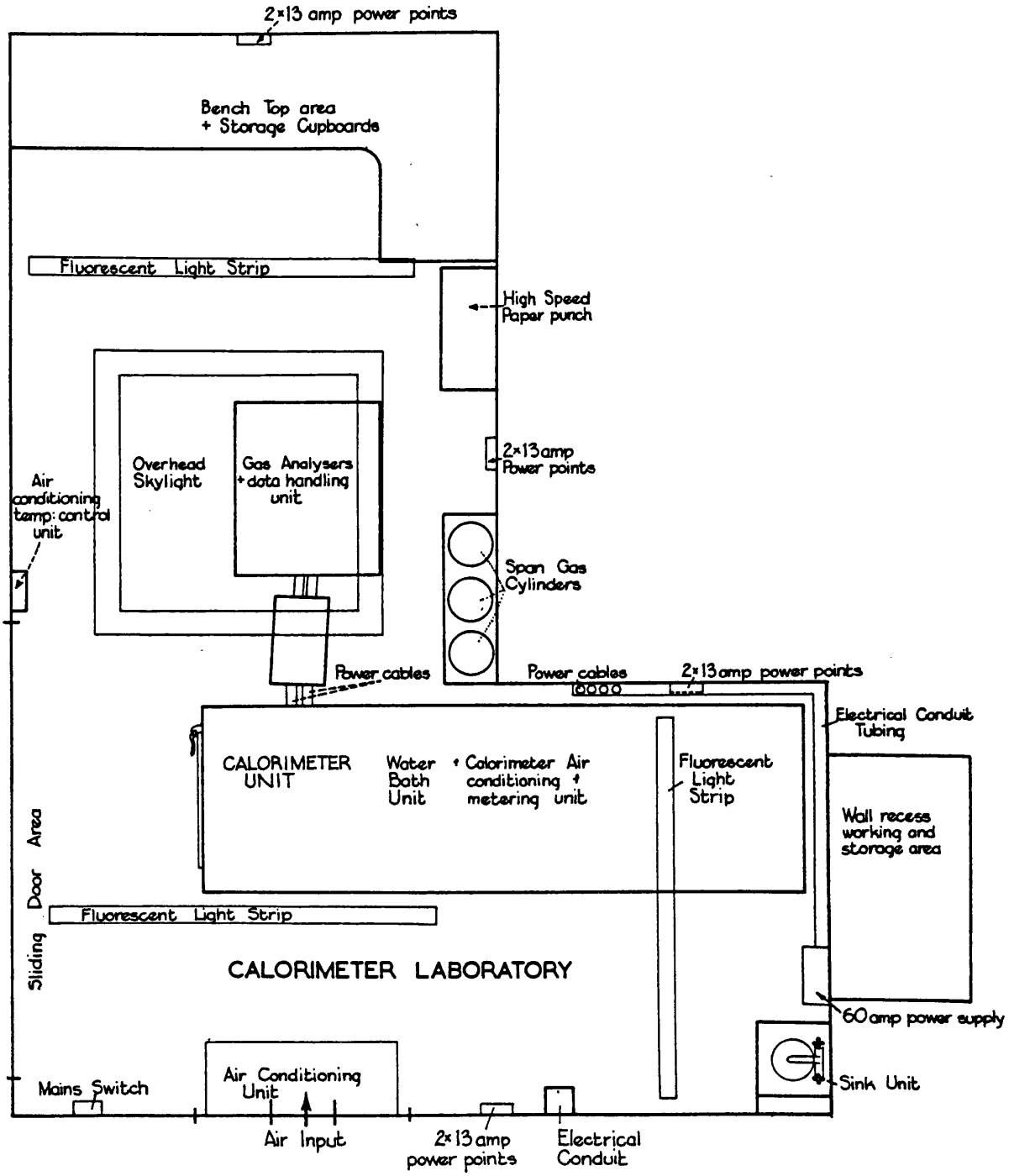


Fig. 14 PLAN OF ANIMAL HANDLING AND PREPARATION LABORATORY AND ENVIRONMENTAL CHAMBER

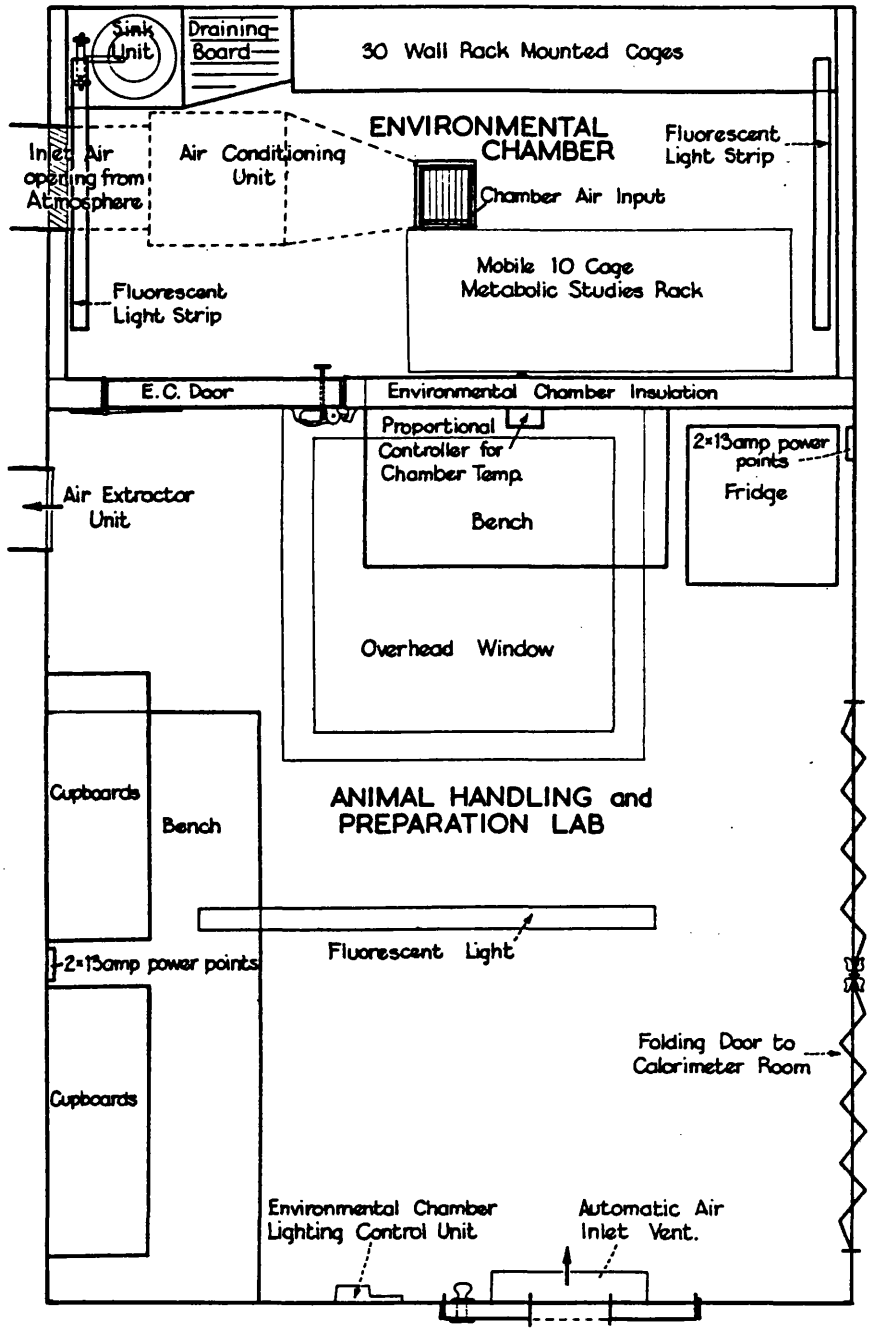
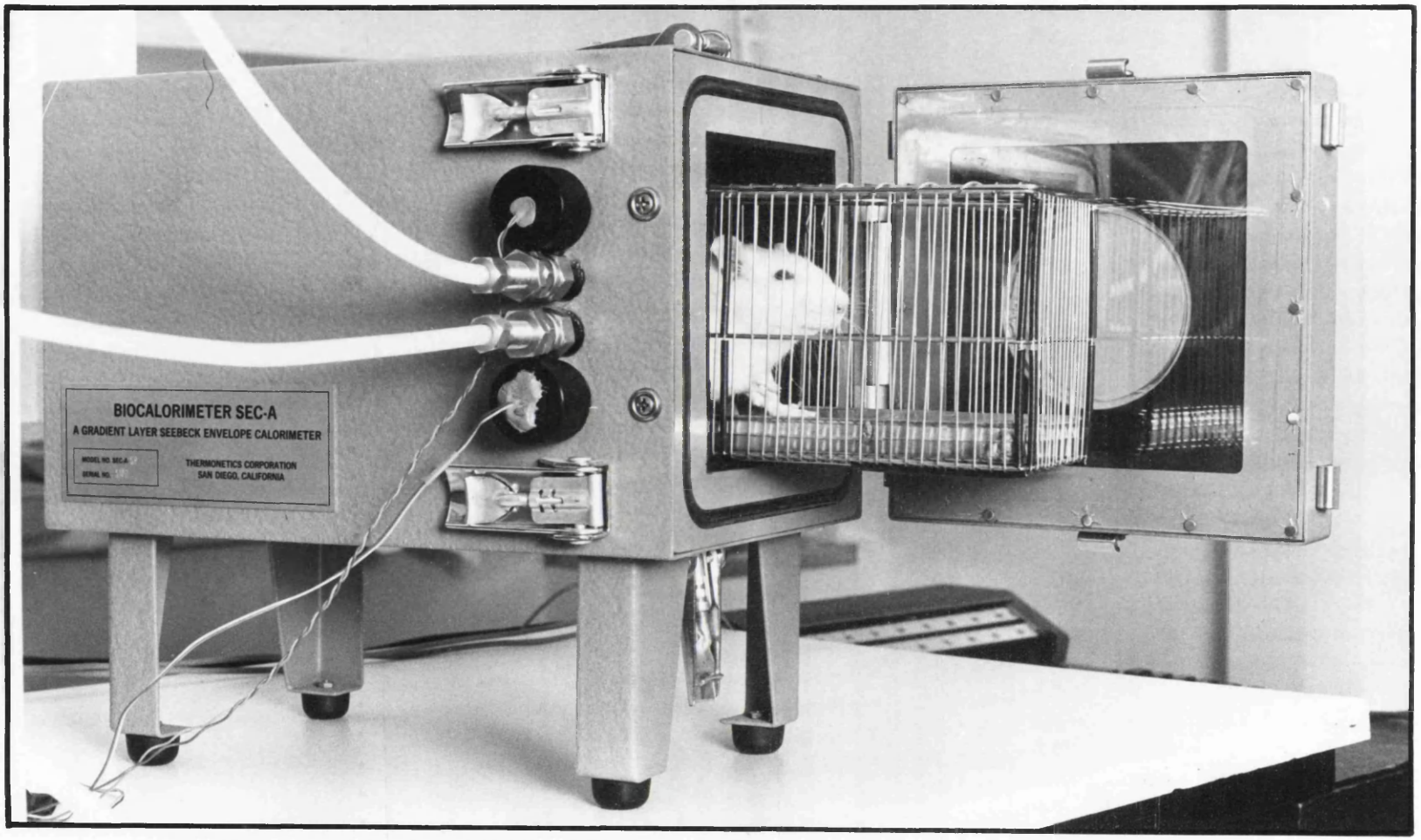


Fig. 15 THERMONETICS CORPORATION COMMERCIALLY BUILT GRADIENT LAYER BOX. MODEL SEC-A-04L



animal under study (150 - 300 g Wistar rats) with the internal dimensions of the gradient layer box, and by providing a rapid ventilating airflow within the box.

The walls of SEC - A - 04 L contain 3000 thermocouples linked in series which produce an output of 1 millivolt per 0.361 watt of heat production within the gradient layer enclosure. The thermopile response time to 90% is approximately 15 mins in zero airflow conditions. SEC - A - 04 L is unusual in that the heat detecting gradient layer is not carried into the front opening door of the box as is customary in other animal calorimeter installations (Hammel and Hardy, 1963; Quattrone, 1965; Dale et al., 1967; McLean, 1971). Instead the door is fitted with a circular transparent glass vacuum cartridge mounted between polycarbonate plastic shields to improve thermal insulation. This provides the rat with a window and source of illumination. Observation of the rat within SEC - A - 04 L indicated that the rat tended to sit facing the door window for long periods. This feature, therefore, effectively restrained the rat without the need to resort to physical means of preventing it from turning such as the restraining cage used by Caldwell in his calorimeter. The penalty of this design is that the door is an imperfect thermal insulator, and the manufacturer recommends that calorimeter operation be restricted to ambient temperatures. The effect of varying ambient temperature on thermopile response was examined. The results are

shown: Figure 16. An ambient temperature variation of 0.35°C alters thermopile output by 0.07 mV. This is due to heat leakage through the partially glass door.

The temperature of the internal environment of SEC - A - 04 L is set by the temperature of water circulating through its inbuilt water jacket. To achieve constant heat transfer through the gradient layer it is essential to precisely control the rate of change of water jacket temperature (Benzinger et al., 1958). The response of SEC - A - 04 L to linear changes in its water jacket temperature is shown in Figure 17. Thermopile output changed by 0.1 mV when the rate of change of the water jacket temperature was 0.007°C per minute.

OPERATION OF SEC - A - 04 L AT AMBIENT TEMPERATURES BETWEEN 20°C - 30°C.

As it was essential that the Glasgow calorimeter be able to function accurately over a range of environmental temperatures between 20°C - 30°C, substantial modification became necessary to enable SEC - A - 04 L to meet these design requirements.

Preliminary experiments with a 250g rat subjected to a 20% body surface area burn established that a thermopile

Fig. 16 EFFECT OF AMBIENT TEMPERATURE CHANGE ON SEC-A-04L THERMOPILE OUTPUT

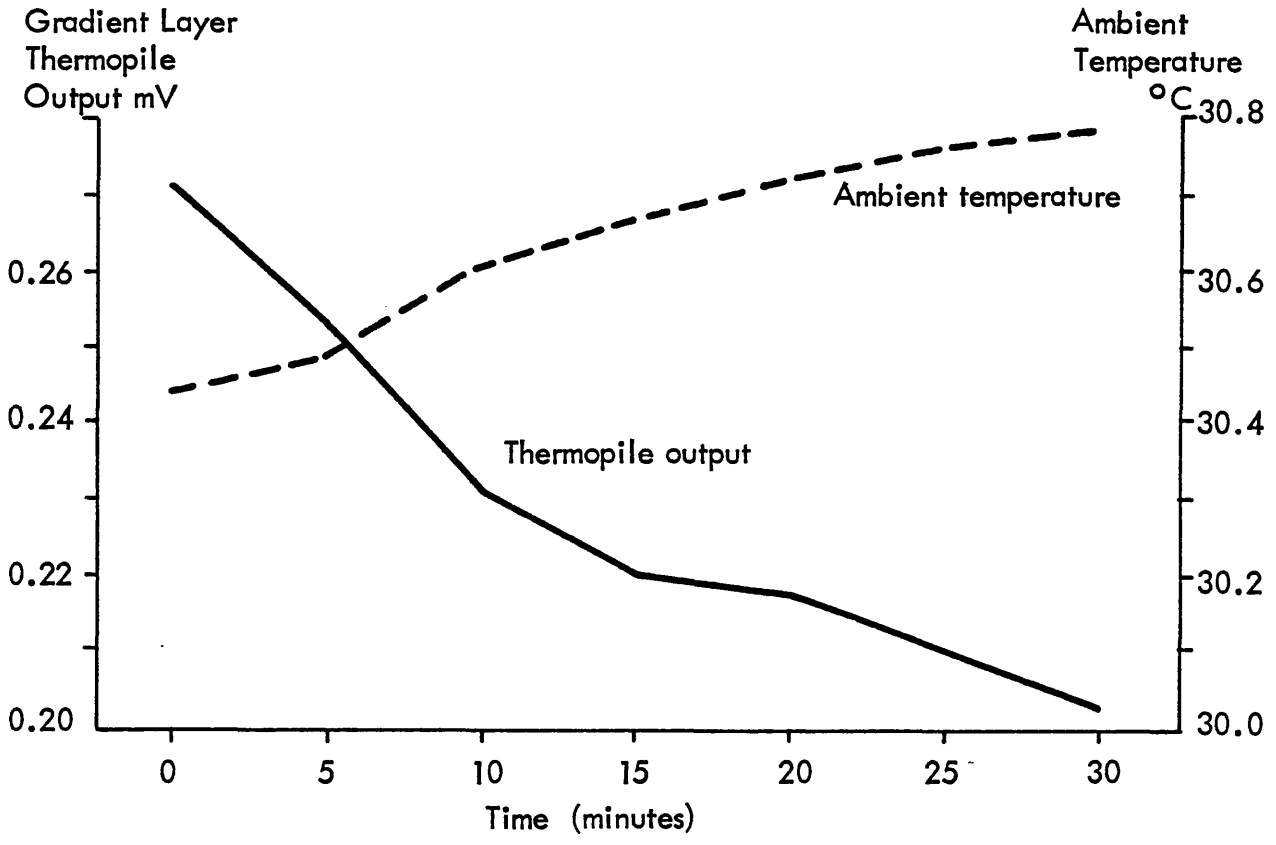
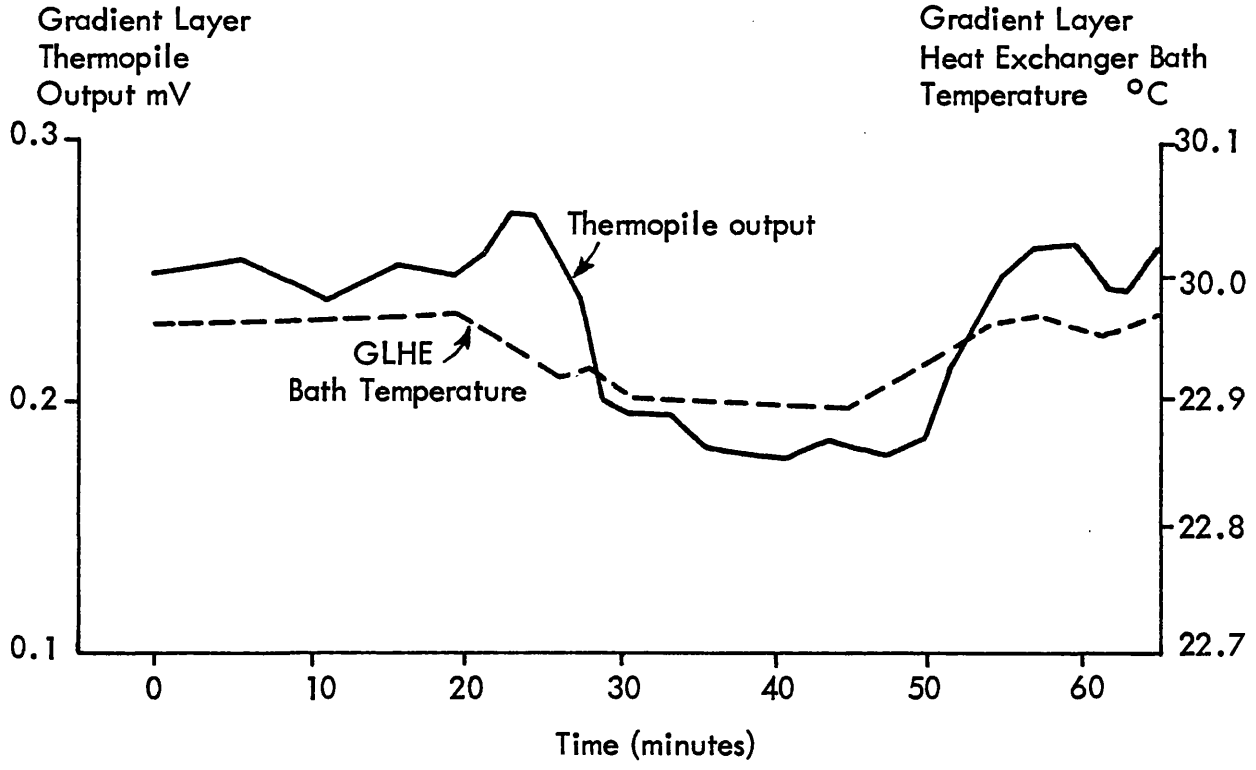


Fig. 17 EFFECT OF GRADIENT LAYER HEAT EXCHANGER WATER BATH TEMPERATURE CHANGE ON SEC-A-04L THERMOPILE OUTPUT



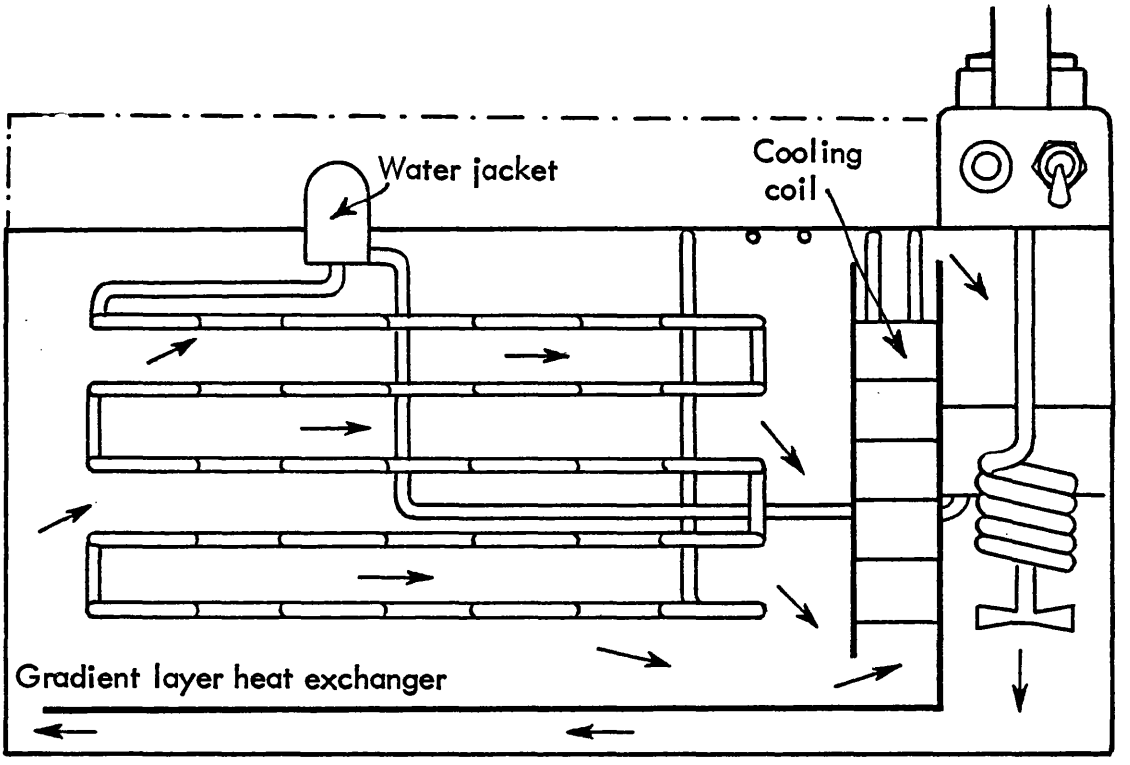
output of around 4mV was produced by SEC - A - 04 L. To measure this to the specified level of accuracy, i.e. $\pm$ 2%, a "no load" stability of 0.08mV must be achieved. Two conditions are therefore necessary and must be met over the entire operational temperature range of the calorimeter.

1. The rate of change of water jacket temperature must vary less than 0.002^{o}C per minute.

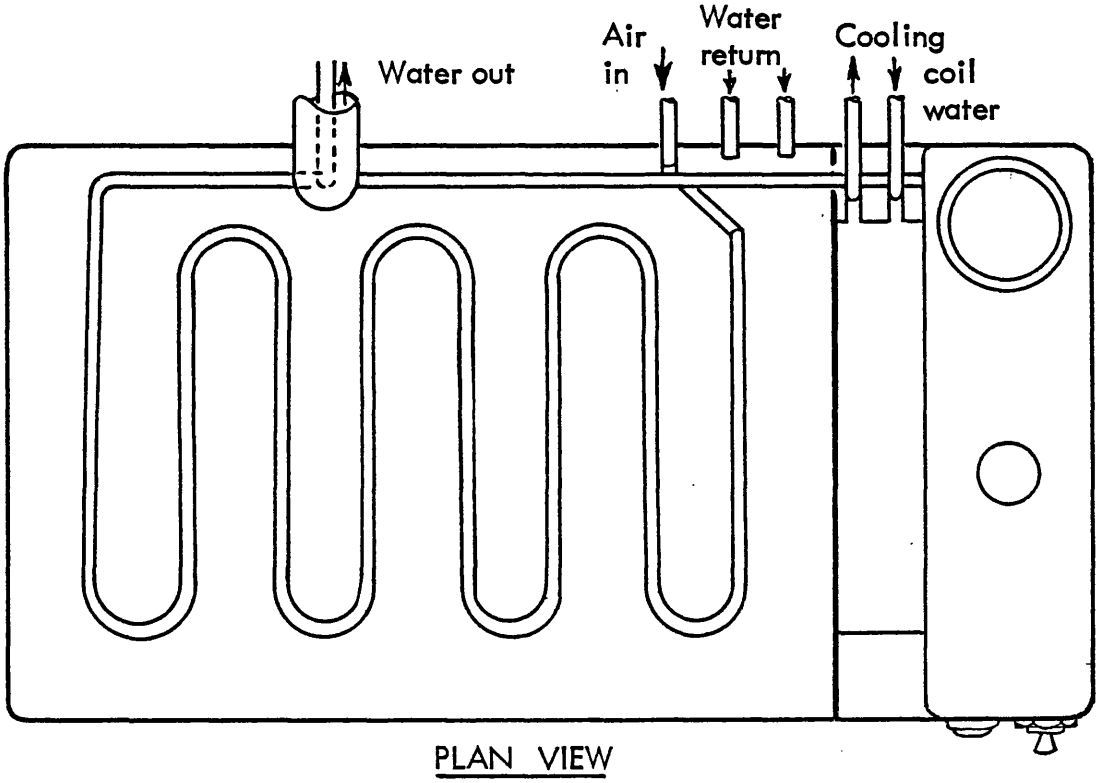
2. The ambient air temperature around SEC - A - 04 L must be controlled to $\pm$ 0.2^{o}C.

To fulfill condition 1, the temperature stability of a commercial large volume water bath was established. Grant water bath type SB 3X has its temperature controlled to within $\pm$ 0.01^{o}C by a mercury contact thermometer linked to a solid state switch arrangement. Heater wattage in this unit may be varied (10% - 100%) to ensure minimum fluctuations of temperature around the set point. To provide a full range of temperature operation, a cooling coil supplied by an external water circulating unit (Grant type LCH10) was added, shown in Figure 18. Figure 19 shows the water circulation circuits to the gradient layer box water jacket and the thermally insulated outer enclosure found necessary to fulfill condition 2. Twin impellers in the Grant bath circulate water over the mercury contact thermometer. Water then passes beneath the false bottom to the back, flows around the gradient layer heat exchanger

SB3X WATER BATH WITH COOLING COIL

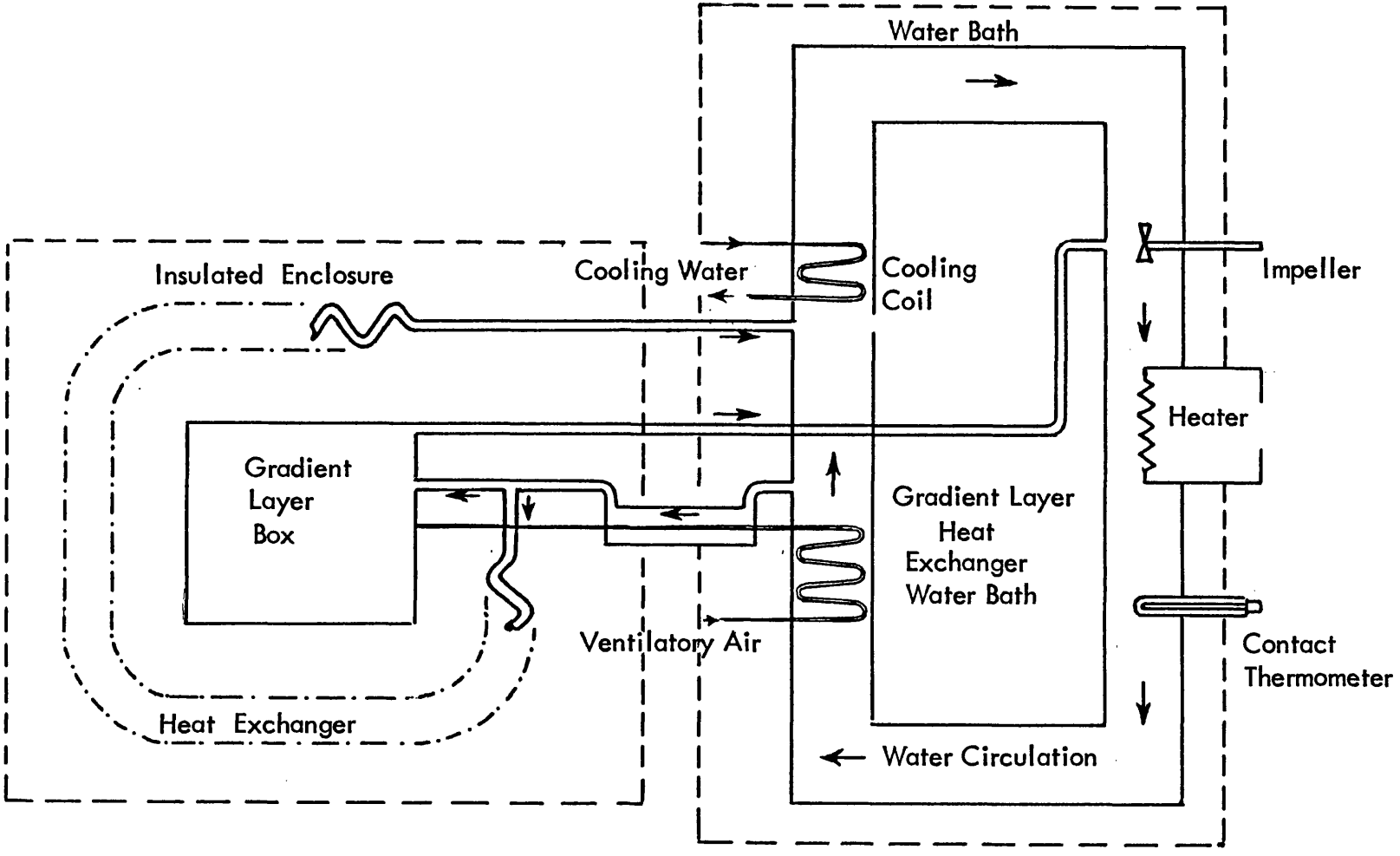


ELEVATION



PLAN VIEW

Fig.19 SCHEMATIC DIAGRAM SHOWING WATER CIRCULATION CIRCUITS TO THE GRADIENT LAYER BOX WATER JACKET AND THE INSULATED ENCLOSURE HEAT EXCHANGER.



and is channelled over the cooling coil. A submersed pump supplies the gradient layer box heat exchanger unit with water from the bath at a flow rate of 120ml per minute on a continuously recycling circuit. The cooling coil is made from 10 mm O.D. 1.5 mm thickness copper tubing 8 m long, coiled between two thin insulated sheets.

In order to stabilise ambient temperatures around the Grant water bath, the calorimetry laboratory was fitted with air conditioning. In this air conditioned temperature regulated laboratory, at an ambient temperature between 25 - 26°C, the rate of temperature change in the Grant water bath was less than 0.001°C per minute over 15 hours, with the mercury contact thermometer set to 20°C and the water circulating unit supplying water at an indicated 19°C. The maximum drift in water temperature was 0.03°C.

To fulfill condition 2, and regulate air temperature around SEC - A - 04 L to within $\pm$ 0.2°C at a range of temperature between 20°C and 30°C, it was found necessary to construct a small thermally insulated housing or enclosure around the gradient layer box itself. A standard laboratory oven was purchased and modified by stripping out the electrical heater unit and replacing this with a coiled copper tube heat exchanger supplied with water at 75 ml/min from the same submersed pump which circulates water around the gradient layer box off water jacket of

SEC - A - 04 L (Fig. 20). The copper tubing is 10 mm OD, 1.5 mm wall thickness and 7 m long.

During a typical 3 hour measurement of heat loss from a rat kept in SEC - A - 04 L, the inner wall temperature may rise by 1°C. The insulated housing temperature however rises at a slower rate and only infrequently exceeds 0.3°C above its starting value. Measurements are regularly made of changes in the insulated housing and SEC - A - 04 L inner wall temperatures, and if necessary appropriate correction factors applied to take account of variations in heat transfer between the inner wall and the outer housing.

To improve "no-load" stability when SEC - A - 04 L is operated at 30°C, a thin gauge aluminium baffle sheet was fitted within the front part of the insulated housing (Fig. 21) to prevent rapid air movement around the gradient layer water jacket of SEC - A - 04 L when the insulated housing door is opened. 'No load' stability of SEC - A - 04 L is further improved by closely controlling environmental temperature in the calorimeter laboratory itself (Fig. 22). This area is fully air conditioned by a Rootes Tempair installation which can maintain ambient temperature around the calorimetry unit and the data handling cabinet to within $\pm$ 1°C of the set temperature, when the sliding doors of the laboratory are kept closed. The net result of closely controlling the temperature of

Fig.20 SEC-A-04L INSTALLED WITHIN THE INSULATED HOUSING AND SURROUNDED BY THE COILED COPPER TUBE HEAT EXCHANGER

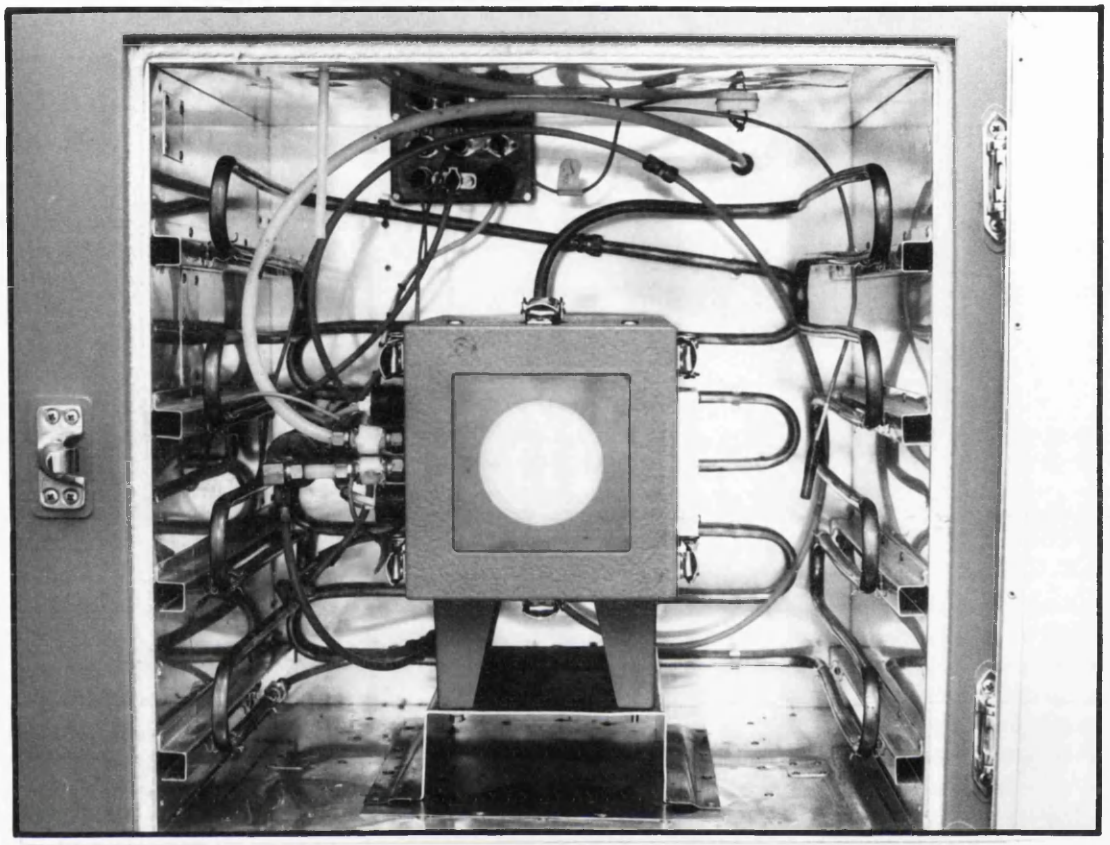
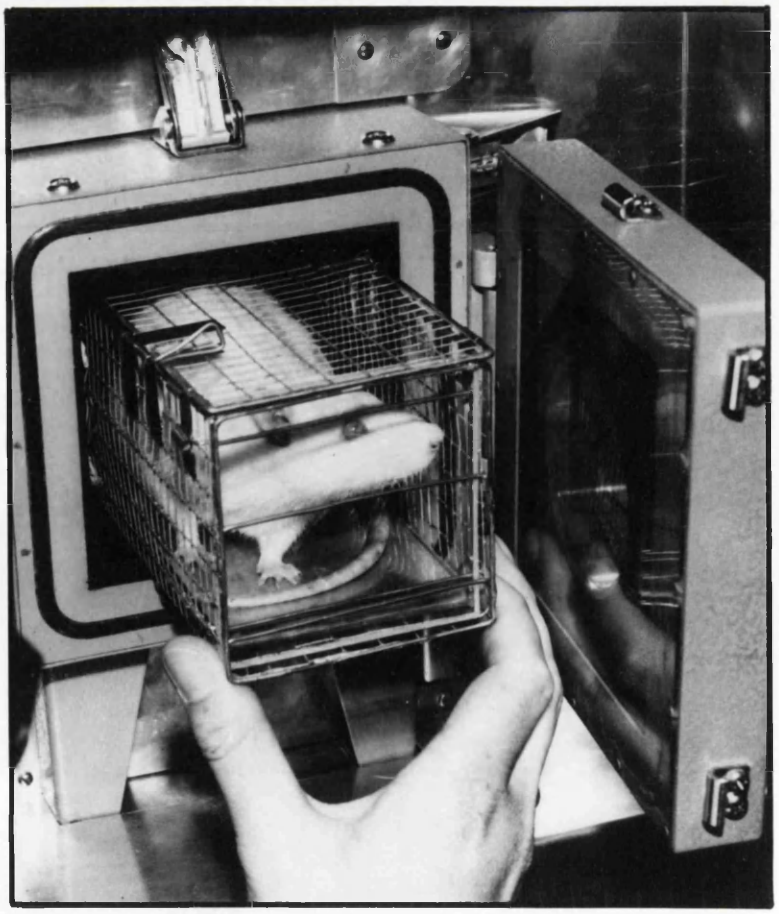


Fig.21 ALUMINIUM BAFFLE SHEET IN PLACE AROUND SEC-A-04L IN THE INSULATED HOUSING



the gradient layer water jacket, insulated housing, and calorimeter unit laboratory, is a 'no-load' thermopile output from SEC - A - 04 L which is within $\pm$ 0.15 mV of zero at operating temperatures of 20°C and 30°C, and which remains stable to within $\pm$ 0.02 mV over 3 hours.

CONTROL OF HUMIDITY AND VENTILATION WITHIN SEC - A - 04 L.

As it was uncertain which humidity levels are best in the treatment of severe burns a ventilation circuit was designed for SEC - A - 04 L in which high or low humidity levels could be created and accurately controlled over a wide range of operating ambient temperatures. The type of hygrometer selected gave the best results at humidity levels of 2g water vapour/m^3 dry air. In uninjured rats, flow rates of 1 - 1.5l/min satisfy these humidity requirements. Rats with 30% surface area burns have evaporative water losses of 108% greater than unburned controls (Morgan et al., 1955). In these animals, the optimal air flow rate for accurate humidity measurement is 4 - 8l/min. If lower flow rates are used, at 20°C even with 'dry' inlet air, precipitation of water vapours occurs within SEC - A - 04 L and its associated pipes and tubes. However, with 150 - 300g rats, air flow rates must be less than 2l/min to accurately measure oxygen consumption and carbon dioxide production. The conflicting air flow requirements for humidity measurement and gas

analysis were reconciled by creating a partial recirculation of air within the ventilating air circuit. Thus total air inlet/outlet flow can be restricted to less than 2l/min for optimal gas analysis, while at the same time, by limited recycling of air within one part of the ventilatory circuit, the internal flow rate through SEC - A - 04 L can be increased to 4 - 8l/min to suit the rate of evaporative water loss from burned rats.

VENTILATORY AIR CIRCUIT DESIGN

Large animal gradient layer calorimeter (GLC) installations in Britain were examined in detail. The best design features were noted from the GLC at the Rowatt Research Institute, Bucksburn, Aberdeen (Puller, 1969) and also from the GLC at the Hannah Dairy Research Institute, Ayrshire (McLean, 1971). The 'heat sink' calorimeter at the Institute of Animal Physiology in Babraham, Cambridge (Mount et al., 1966) was also studied. These calorimetry systems all required elaborate setting up and checking procedures by skilled and numerous technical staff. Data analysis contained many time consuming and labour intensive steps before useful physiological information could be obtained from an experimental study. In creating the rat calorimeter it was my intention that the system be capable of single handed foolproof operation by a user with minimum prior training. Rapid automated

data handling and rapid computer analysis of results was also an essential improvement required over existing designs. The final design of ventilatory air circuit for SEC - A - 04 L is shown in Figure 22. Despite the apparent complexity of the circuit, all of the air ventilating and conditioning equipment is mounted directly beside SEC - A - 04 L in the single module shown in Figure 23. Setting up, checking and running the system can be simply performed by a single operator, as all the control valves and switches function directly from a self explanatory mimic diagram of the air circuit engraved on the side of the module (Fig. 24). By adjustment of the appropriate valves and rotameters, inlet/outlet air flow rates can be selected at will and the rate of partial internal air recirculation varied independantly.

The air circuit functions as follows: Atmospheric air taken from a pipe on the roof of the laboratory is drawn by a small inlet pump through a fibre card filter and pumped into the ventilation circuit, where it is fully saturated with water vapour in a specially made humidifier. The ventilating air next passes into a purpose built heat exchange unit (dew point setting heat exchanger unit) which is run at a pre-selected temperature, within which the air is cooled to below its dew point. This temperature setting is measured by thermistor T_1 and controls the absolute humidity of the air which ventilates SEC - A - 04 L. Water vapour condensing within this heat exchanger is removed by a small water trap attached to the

Fig. 22 GRADIENT LAYER BOX - VENTILATORY CIRCUIT

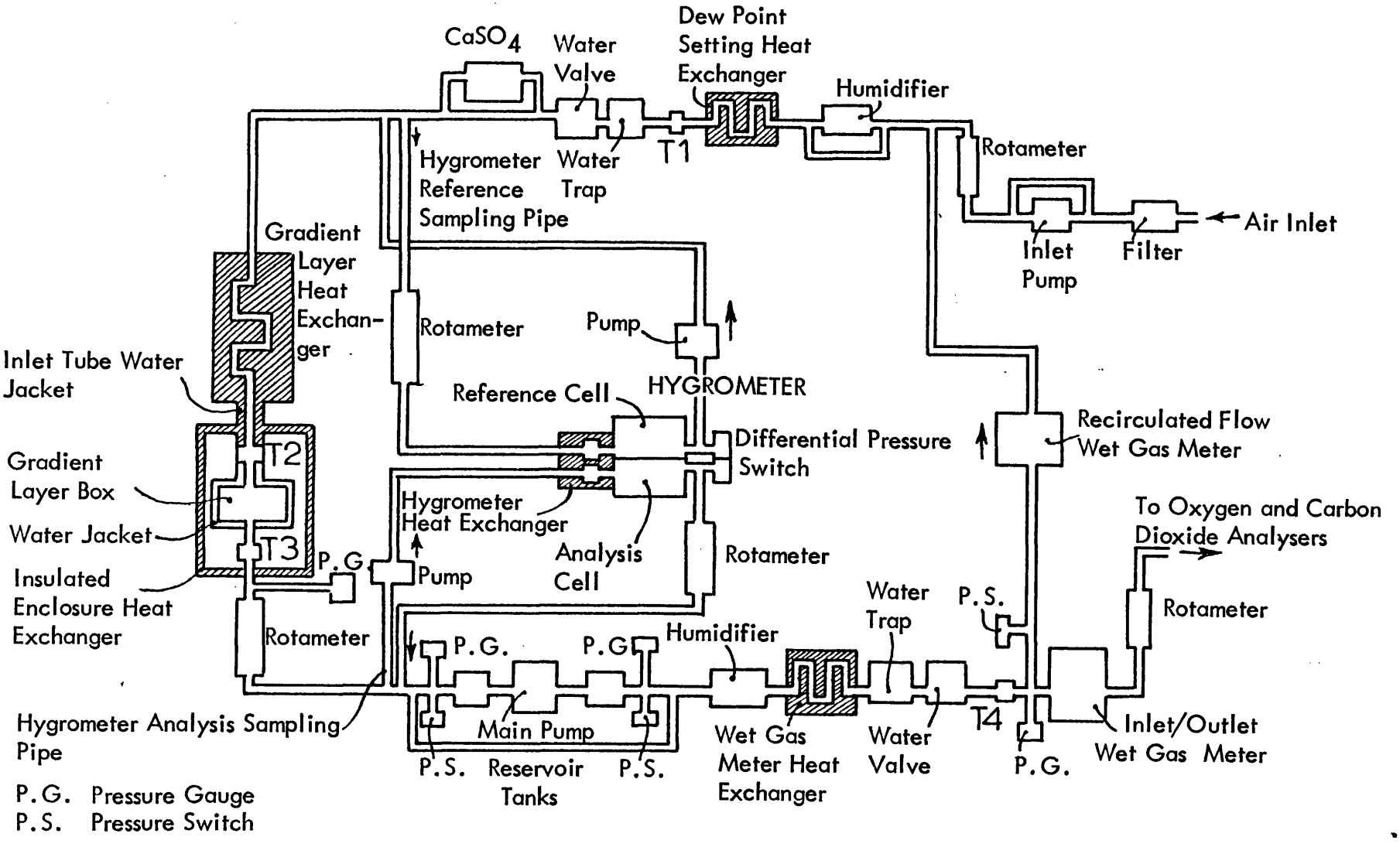
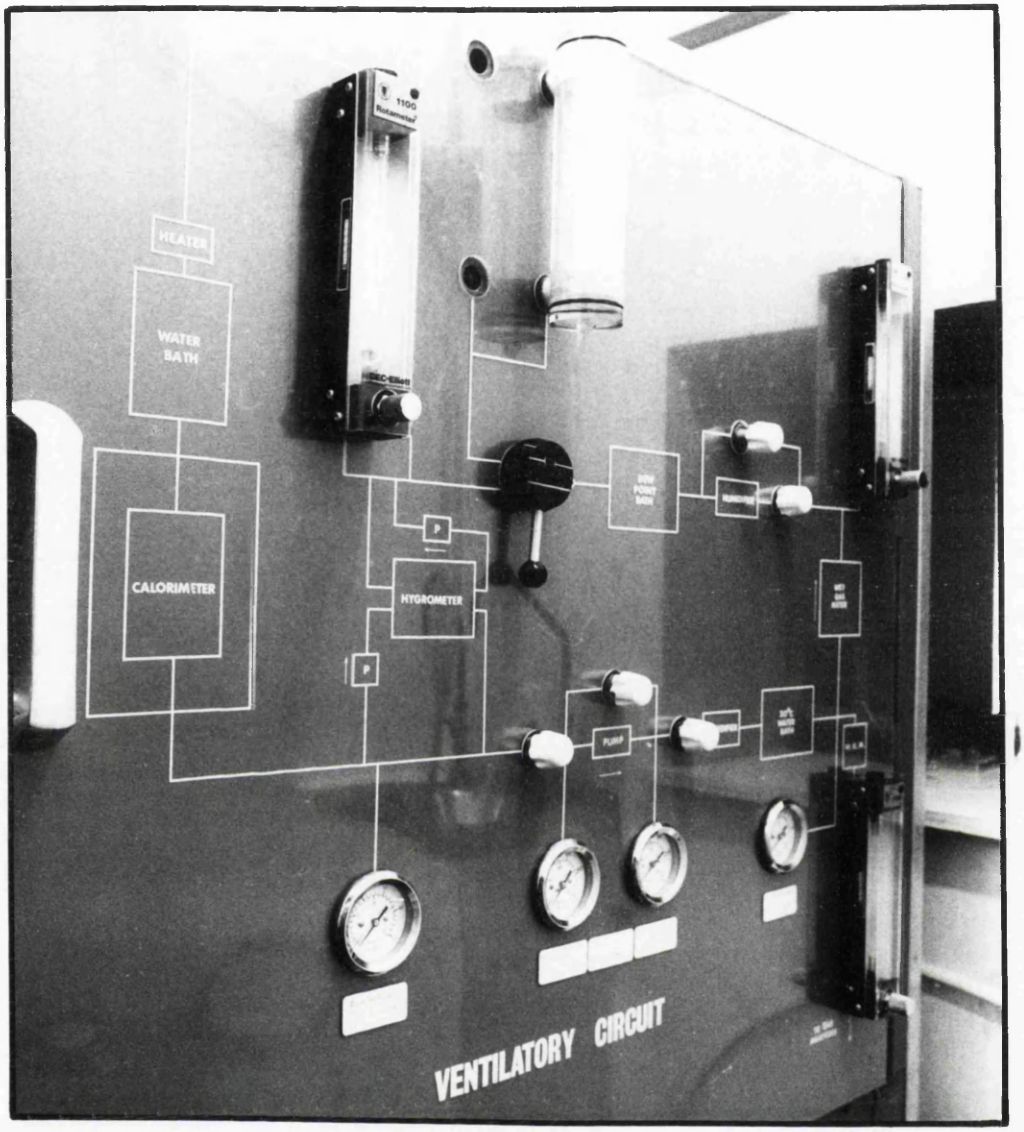


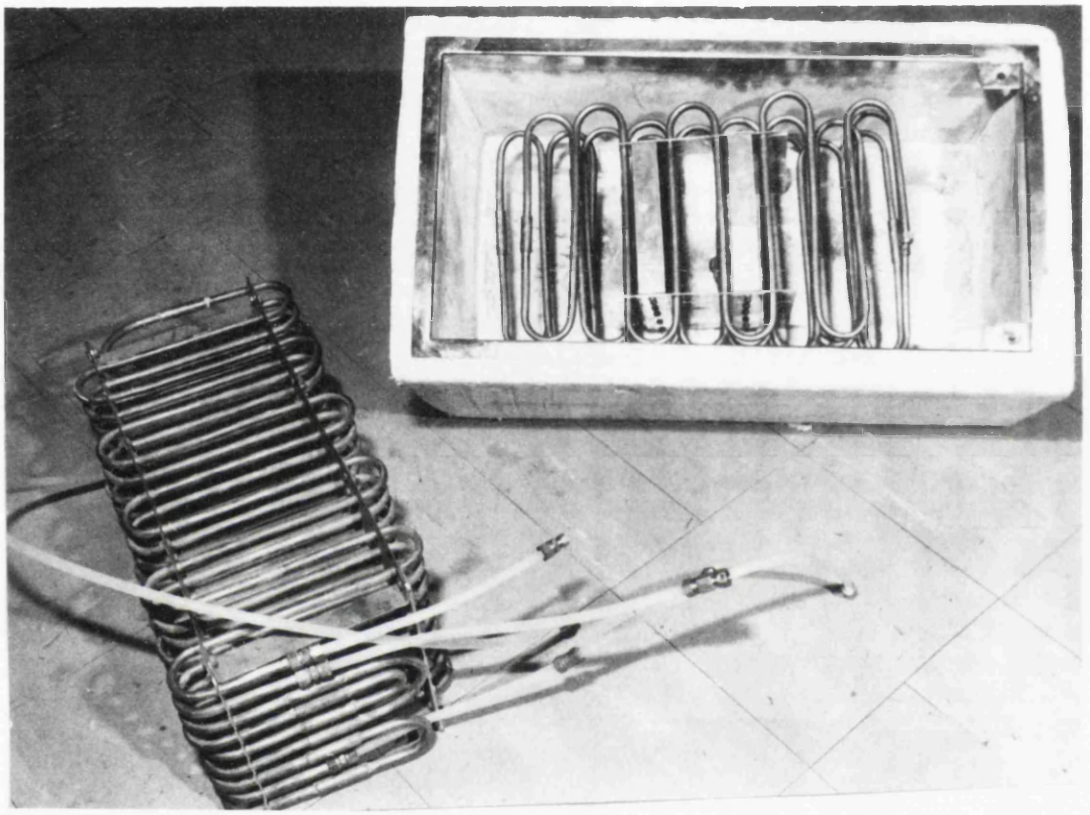
Fig.23 SEC-A-04L MOUNTED IN THE AIR VENTILATING AND CONDITIONING MODULE

Fig.24 MIMIC DIAGRAM CONTROL PANEL ON CALORIMETER AIR VENTILATING AND CONDITIONING MODULE



unit. At this point in the circuit air may either pass directly to the gradient layer heat exchanger unit if humid conditions are desired, or by turning a rotary valve, be diverted via twin calcium sulphate drying tubes to give very dry air conditions if a low dew point setting has been used. This produces an effective dew point of $-70^{o}C$. Whatever humidity value is chosen, ventilating air is brought to the operating temperature of SEC - A - 04 L in the gradient layer heat exchanger (Fig. 25) and its temperature measured by thermistor T_2. Changes in the ventilating air temperature, after its passage through the gradient layer box are measured at the outlet pipe by thermistor T_3. Air is drawn through SEC - A - 04 L by the main pump of the system which is sited beyond the hygrometer sampling pipe. It proved essential to smooth the rhythmic air pressure oscillations produced by this pump by adding twin air expansion chambers. Ventilating air is next fully saturated with water vapour after leaving the main pump, and then cooled to $20^{o}C$ in the Wet Gas Meter Heat exchanger in order to standardise its humidity and temperature (thermistor T_4) prior to accurate measurement of airflows within the ventilating system. Overall inlet/outlet flow rates and recirculated flow rates are separately measured by individual Wet Gas Meters. Gas analysis is performed on the air leaving the inlet/outlet Wet Gas Meter after passing through a cold trap and calcium sulphate drying tube. Recirculated air passes back into the inlet pipe of the ventilation system and the cycle is repeated.

Fig.25 HEAT EXCHANGER WATER BATH SHOWING COILED COPPER AIR TUBING



A detailed description of the components of the ventilating system follows.

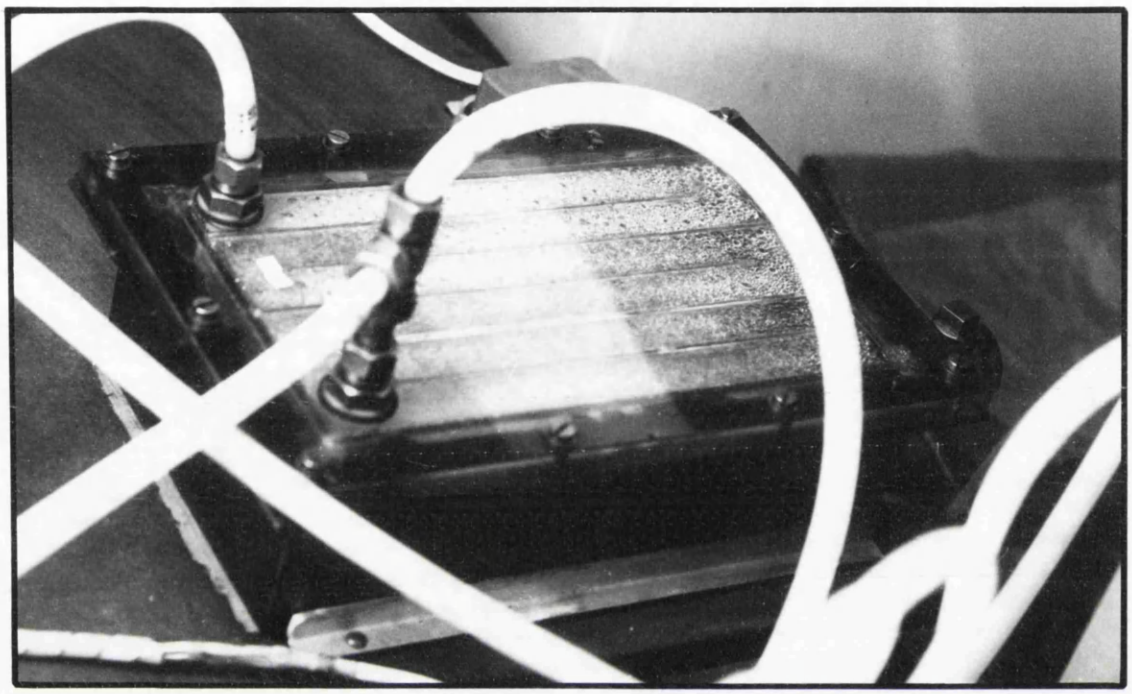
HUMIDIFIERS

The two humidifiers used in the ventilatory system are identical. A copper water bath 230mm x 150mm x 70mm was fitted with an airtight perspex lid constructed with parallel perspex vanes on its undersurface. This is shown in Figure 26. The bath is filled with water via a side mounted perspex tube which indicates water level to within 2.5 cm of the lid so that air flowing into the lid at one corner is directed by the vanes back and forth over the surface of the water to exit at the opposite corner of the lid. This gives an airpath length of 114 mm with very little airflow resistance. To achieve the most efficient humidification the water had to be heated to $30^{o}C$. This was done by using a thermostatically controlled 100W aquarium heater unit fitted to the bottom of the bath.

DEW POINT SETTING - HEAT EXCHANGER UNIT

Air temperature is controlled in this unit by passing along a 15 m length of copper tubing (10 mm OD and 1.5 mm wall thickness) coiled within a 50 gallon capacity Grant water bath insulated by 5 cm deep expanded polystyrene cladding. Water temperature in the bath is controlled by a Grant LCH10 standard water circulating

Fig.26 COPPER WATER BATH HUMIDIFIER UNIT



unit within the range 0 - 40°C ± 0.1°C. Cooling fully saturated air to a lower set temperature results in precipitation of water within the copper tubing. Drainage of this water is achieved by downward slanting of the tubing so that the water runs into a special water trap fixed to the underside of the insulated water bath. As an additional safety measure to detect excess water build up within the system, the outlet from the water trap leads to a specially developed water valve (Fig. 27). A floating ball valve seals at its inlet port as the water level rises within it, and as a result causes pressure build up within the ventilating air circuit. At a preset pressure the automatic pressure trip switches (Fig. 22) shut off both inlet and main air pumps and activate a warning light and alarm buzzer. With maximum cooling, the dew point heat exchanger dries the ventilating air to a dew point of 4°C. When dew point settings below this are required, then air leaving the heat exchanger is made to pass through twin tubes of granulated calcium sulphate (Dririte) mounted on the fascia of the mimic diagram (Fig. 24). Each tube (containing 200g Dririte) is sufficient to dry ventilating air to a dew point of -70°C for at least 5 hours at an airflow rate of 8l/min.

GRADIENT LAYER - HEAT EXCHANGER UNIT

Ventilating air is brought to the calorimeter operating temperature by passing along a 22 m length of

Fig.27a DEW POINT SETTING HEAT EXCHANGER UNIT WATER TRAP IN PLACE BENEATH INSULATED WATER BATH

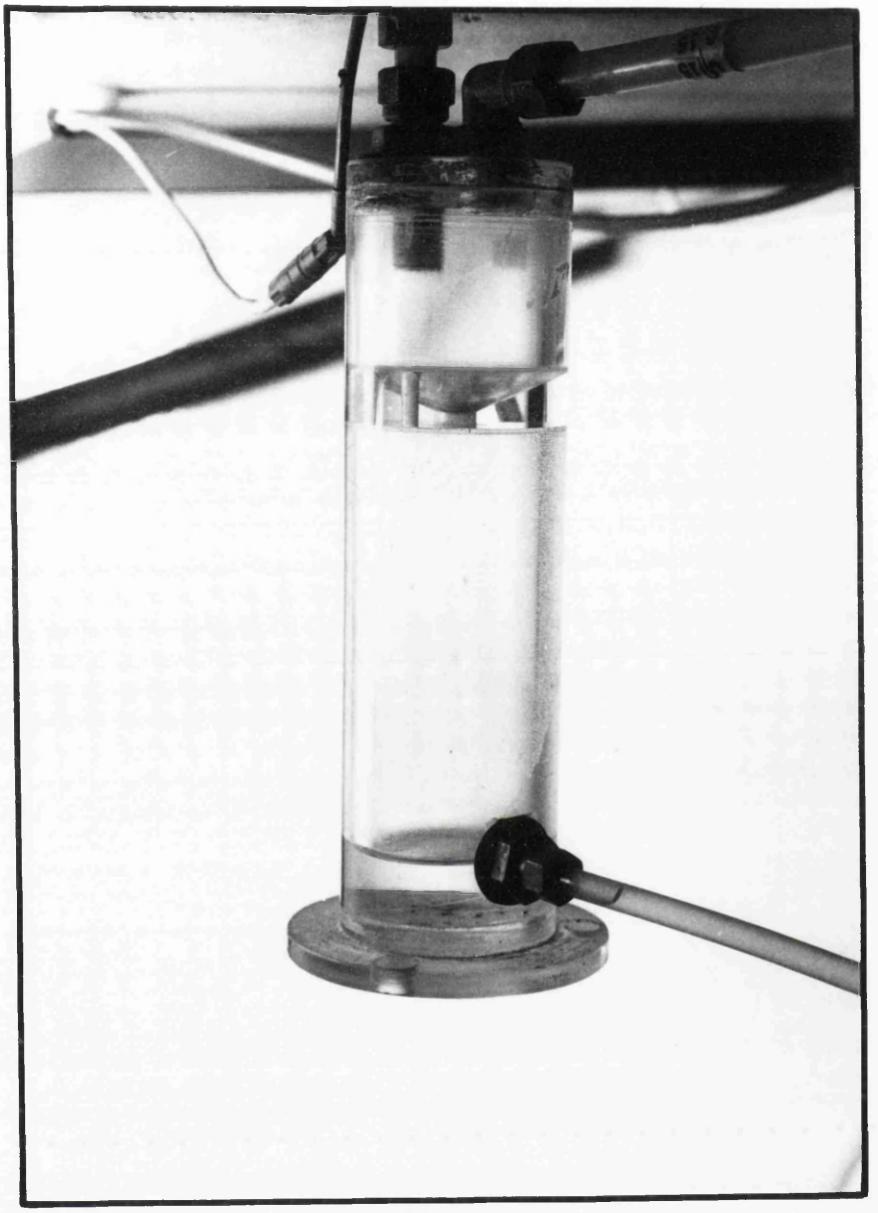
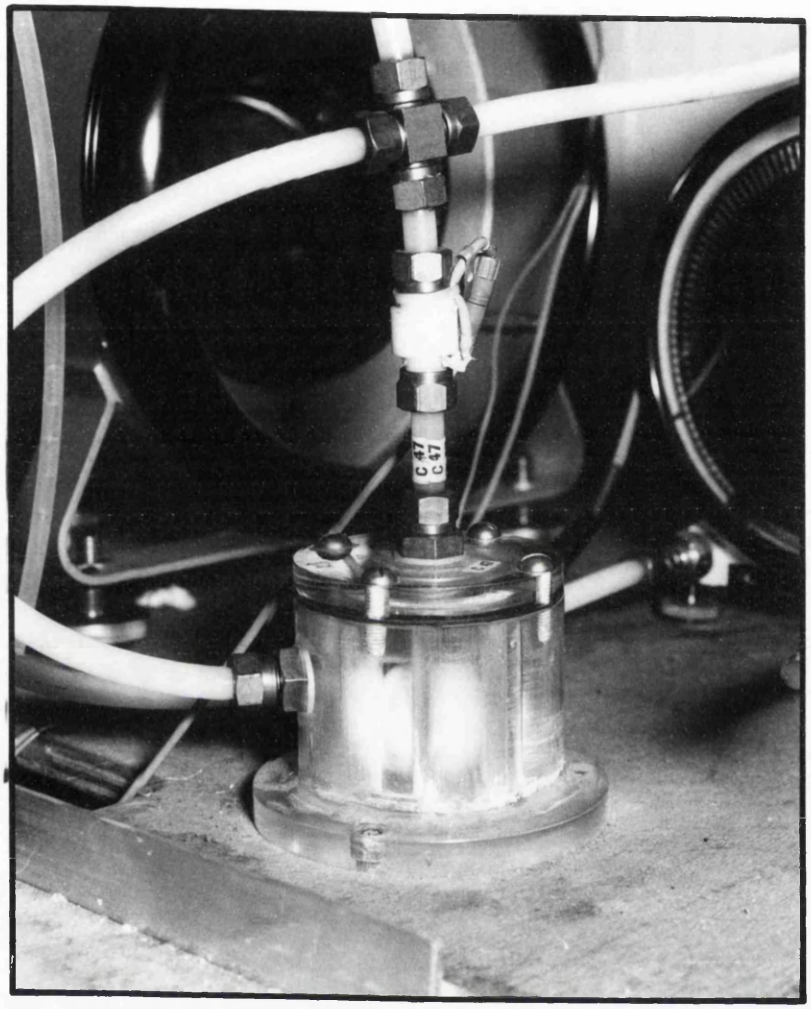


Fig.27b WATER TRAP FLOATING BALL VALVE



copper tubing (10mm OD and 1.5mm wall thickness) coiled within the water bath which supplies the gradient layer water jacket and the insulated housing heat exchanger (Fig. 18). A marked temperature change was found initially in air passing from the gradient layer heat exchanger copper tubing along the exposed short length (45 cm) of nylon tubing (10mm OD, 1.5 mm wall thickness) which carries air to SEC - A - 04 L. Even insulating the nylon tubing with 2 layers of 12 mm "Armaflex" insulating material failed to prevent significant cooling, 0.04^{o}C per cm. Therefore a special air pipe insulation unit (made of a 10 mm diameter copper pipe with surrounding 40 mm diameter copper water jacket) was made extending from the gradient layer water bath through the side of the housing (44 cm), ending 8 cm from the air inlet part of SEC - A - 04 L. This is shown in Figures 19 and 28.

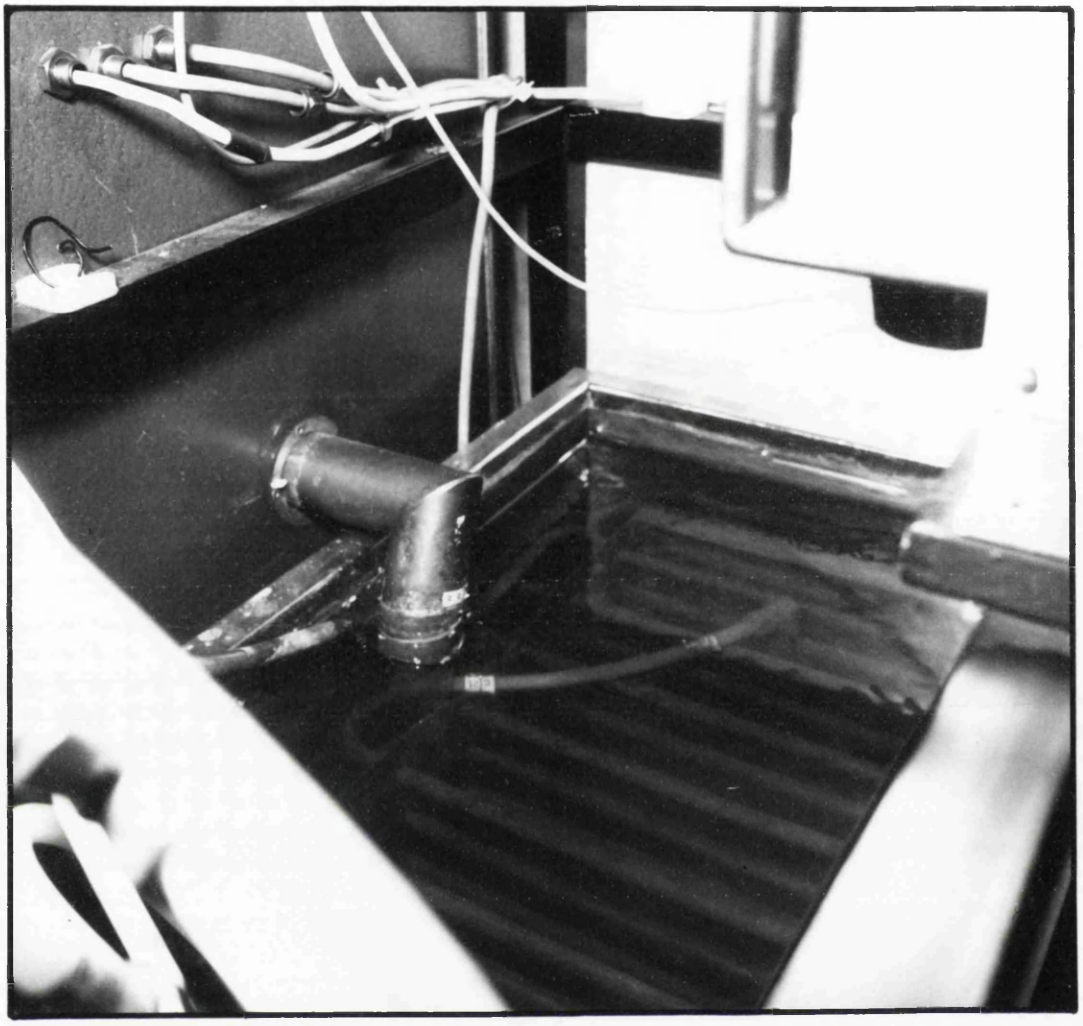
WET GAS METER - HEAT EXCHANGER UNIT

This is identical to the dew point setting - heat exchanger unit except that a simpler design of water trap is used, where the internal shelf is eliminated and ventilatory air passes directly over the collecting condensate.

WET GAS METER - AIR FLOW MEASUREMENT

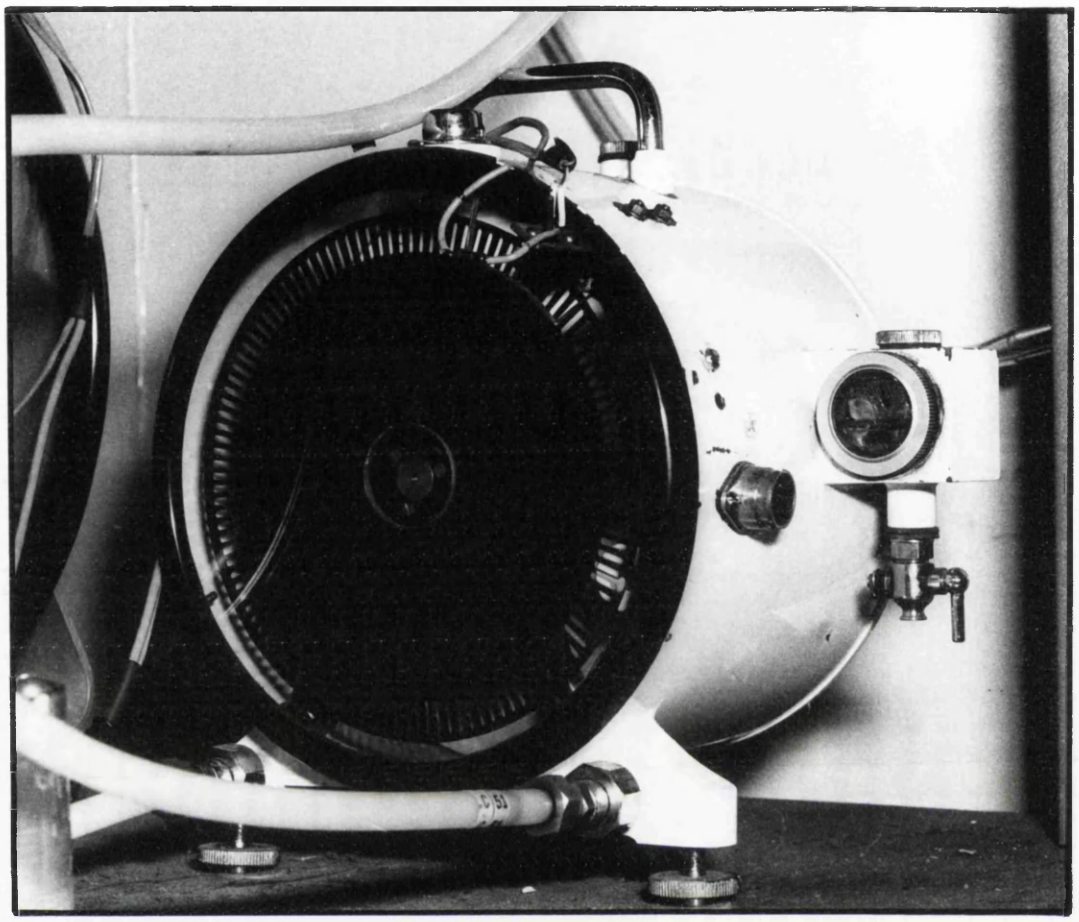
Volumetric displacement wet gas meters were selected as being the most accurate means available of

Fig.28 GRADIENT LAYER HEAT EXCHANGER AIR PIPE INSULATION WATER JACKET IN SITU BETWEEN G.L.H.E. WATER BATH AND REAR OF THE INSULATED HOUSING



measuring inlet/outlet air flows and recirculated air flow. Wright's Wet Gas Meter type M809LT, with measuring drum capacity of 0.5l is used to measure inlet/outlet flows (range 0.5 - 2l/min). Type M809NA with a measuring drum capacity of 15l is used to measure the recirculated and hence give the total air flow within the ventilating system, i.e. inlet/outlet air flow and recirculated air flow (range 0 - 6l/min). For the purposes of calculation, only outlet and total air flows need be measured. As manufactured, the wet gas meters record total air volume by moving gearing which turns indicator wheels seen as a set of numbers inset into the meter face. All the existing large animal calorimeters have similar systems and information regarding air flows has to be read off from the instrument and entered by hand into the subsequent calculations to determine energy expenditure. It was my intention that all such manual steps be eliminated from the Glasgow calorimeter and that all electrical systems information be 'digested' either at source or via a digital voltmeter and punched onto paper tape under the control of a commercial data logging system. In order to produce an electrical output, both wet gas meters were modified by the method of Boocock et al. (1971), where the face dial of each meter was replaced by a disc with circumferential slots. This disc rotates in front of a small light source (Fig. 29) and the resulting light pulses are detected by a photo cell. The photo cell

Fig.29 "DIGITISED" WRIGHT'S WET GAS METER TYPE M809LT SHOWING PHOTOELECTRIC CELL WITH SLOTTED DISC.



signals are then processed to give a digital output of integrated flow over predetermined time intervals and an analogue output of flow rate. The inlet/outlet flow measuring wet gas meter (M809LT) with a 0.5l drum was fitted with a disc with 120 peripheral slots producing 8 pulses per second at a flow rate of 1 l/min. The recirculation flow meter (M809NA) with a 15l drum has a disc with 360 slots, which gives 0.4 pulses/sec at the same air flow rate. The air inlet pipes of both wet gas meters are connected as shown in Figure 22. This reduces the equipment required to standardise the temperature (measured by thermistor T_4) and humidity (100% at 20°C) of the ventilating air prior to measurement of the flow rate. Similarly only one pressure gauge (PG) is required for both wet gas meters. The volume readings are therefore readily converted to STP by application of the appropriate correction factors. The method of data processing is described elsewhere. A pressure switch (PS) is also provided to limit pressure to 40 cm of water, preventing damage to the wet gas meters. The accuracy of wet gas meter type M809LT (inlet/outlet flow) was found to be $\pm$ 0.3% and type M809NA (recirculated flow) was $\pm$ 0.5%. This small error is acceptable, and within design specifications.

VENTILATORY AIR TEMPERATURE MEASUREMENT

YSI Thermilinear Thermistors type 44018 were used to measure air temperature at 4 points within the

ventilatory air system. These are shown as T_1, T_2, T_3 and T_4 in Figure 8. The thermistors were each mounted in mid air stream in specially made "Delrin" couplings. This material combines good thermal insulation with mechanical strength. The ends of the couplings were machined to lock directly to the 10 mm OD air piping used to form the ventilation circuit.

THERMISTOR T_1 measures the temperature of humidified inlet air after passing through the dew point setting heat exchanger unit. A bridge circuit is used to obtain the absolute temperature measurement in ^{o}C, Figure 30. The "Delrin" coupling containing thermistor T_1 lies directly between the piping of the heat exchanger unit and its water trap. As this heat exchanger unit is not 100% efficient, the temperature of air exiting from it varies with flow rate. At any given air flow rate therefore the required dew point temperature T_1 must be set by adjusting the temperature of the heat exchanger unit water bath. The following humidity test was used to check the accuracy of temperature measurement by Thermistor T_1 and the efficiency of the water trap. The heat exchanger unit water bath temperature was adjusted until T_1 indicated an outlet air temperature of $17.8^{o}C$, equivalent to a dew point humidity of 15g water vapour/m^3 dry air. A measured quantity of humidified air was then passed through the dew point setting heat exchanger unit and its water trap. The moisture content of the outlet air was measured by

Fig.31 BRIDGE CIRCUIT USING 2 MATCHED THERMISTORS YSI 44018 TO MEASURE TEMPERATURE DIFFERENCE

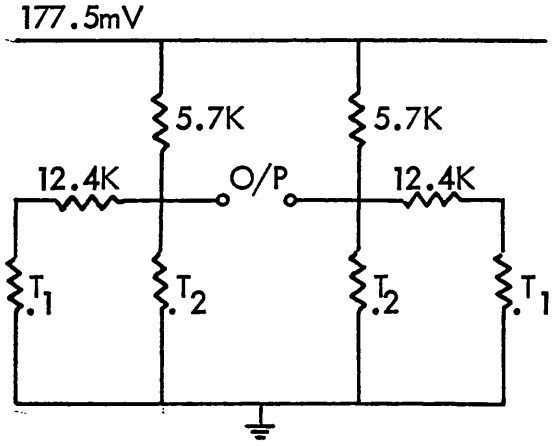
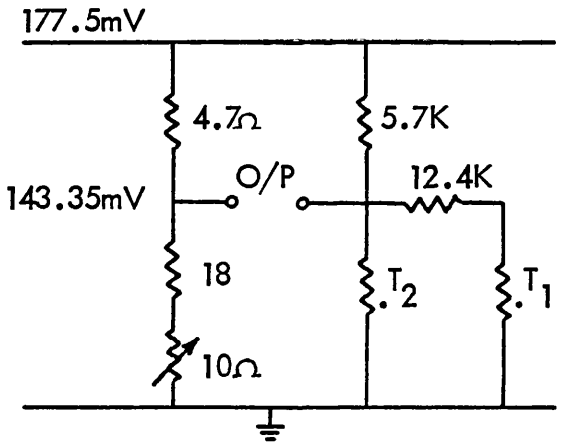


Fig.30 BRIDGE CIRCUIT USING THERMISTOR YSI 44018 TO MEASURE AIR TEMPERATURE



chemical absorption. Comparison of the dew point setting by both methods indicated agreement to within $\pm$ 0.1°C.

THERMISTORS T_2 and T_3 measure the difference in temperature between ventilating air entering and leaving SEC - A - 04 L. The thermistor "Delrin" couplings are mounted directly onto the inlet and exit air ports and lie within the insulated housing (Fig. 22). Two matched thermistors are used in a bridge circuit shown in Figure 31. For a temperature difference between the two thermistors of 3°C the maximum error due to non-linearity is $\pm$ 0.02°C. The thermistor time constant is 10 seconds with a sensitivity of 1 mV per °C.

THERMISTOR T_4 measures ventilating air temperature via a coupling mounted in the shared wet gas meter inlet pipe (Fig. 22).

The electrical signals from these thermistors are fed to the separately housed data logging unit.

EVAPORATIVE HEAT LOSS MEASUREMENT

The optimal background humidity for the treatment of burns is unknown. For the initial series of burn experiments in Glasgow two levels of background humidity in the ventilatory air circuit of SEC - A - 04 L were selected for evaluation, dry 0g water vapour/m^3 dry air, and wet 15 g water vapour/m^3 dry air. By varying the

rate of partial recirculation of air within SEC - A - 04 L the expected increases in the rate of evaporative water loss from the rat after thermal injury (Morgan et al., 1955) could be kept within the range 0 - 5g water vapour/m^3 dry air, depending upon the area of the burn.

A variety of methods for measuring water vapour were considered. Gradient layer platemeter systems as used in the large animal calorimeters (Hammel and Hardy, 1963; Puller, 1969; McLean, 1971) were too complex and costly to manufacture.

Chemical absorbtion methods were also unsuitable as the ventilating air sampled would be altered by the process of measurement, and the system could not be fully automated.

Because the ability to vary background humidity was an essential feature of our design, the hygrometer chosen had to be capable of accurately measuring both inlet and outlet air water content with a high sensitivity at low humidity levels, and a fast response time. A hygrometer system was chosen which operates an infra-red absorbtion principle. A type 200 industrial infra-red differential gas analyser was purchased from Analytical Developments Ltd. (Fig. 32). This instrument contains twin cells, one a reference cell which was connected to the calorimeter air inlet, the other an analysis cell

Fig.32 INFRA RED DIFFERENTIAL HYGROMETER (ANALYTICAL DEVELOPMENTS TYPE 200) MOUNTED WITHIN CALORIMETER AIR VENTILATING AND CONDITIONING MODULE

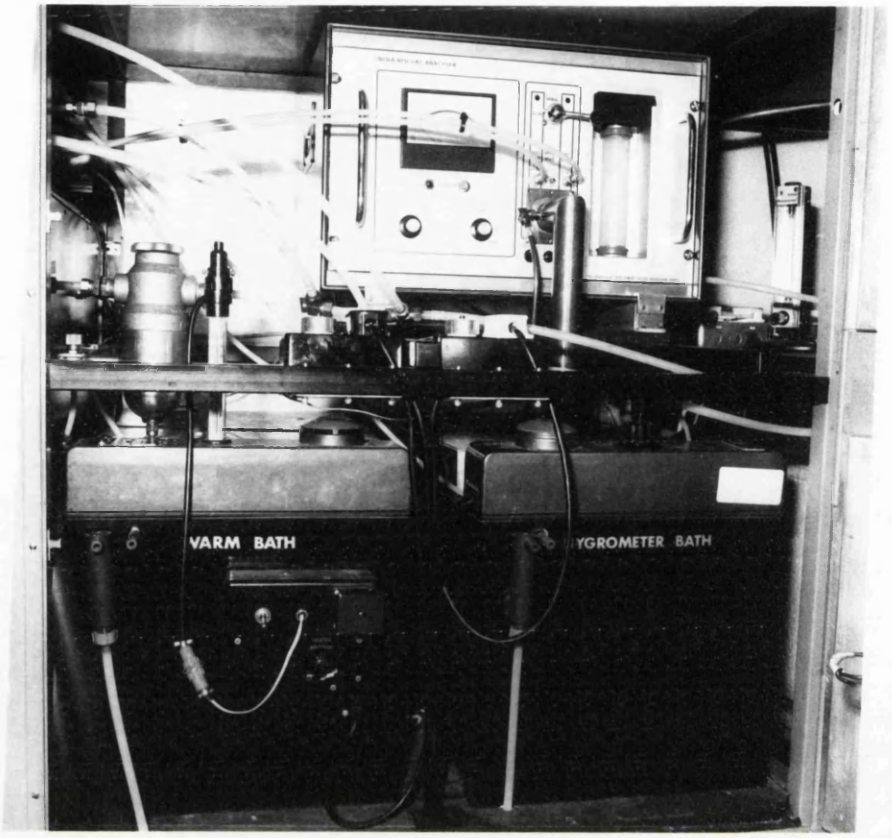
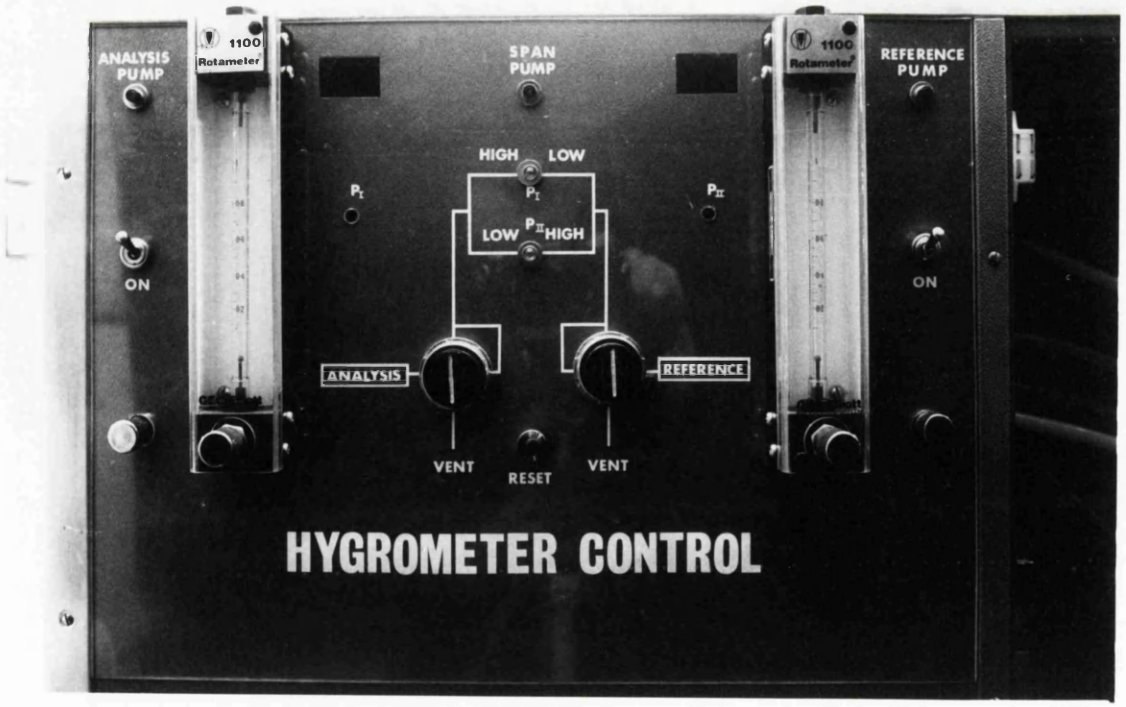


Fig.33 HYGROMETER CONTROL PANEL ON THE SIDE OF THE CALORIMETER AIR VENTILATING AND CONDITIONING MODULE



linked to the air outlet from SEC - A - 04 L (Fig. 22). Infra-red energy from a source at one end of the cells is absorbed by the air within each cell in proportion to its water vapour content. A "Luft" detector at the opposite end of the cells measures the difference in infra-red absorbtion between the two cells. Hence the amount of water vapour added to the ventilatory airstream through SEC - A - 04 L by the rat can be continuously monitored by differential comparison of inlet and outlet air water content by a single analyser.

During construction, the infra-red gas analyser was modified by the addition of a special set of optical filters to reduce the sensitivity of the analyser to the presence of carbon dioxide which also absorbs infra-red radiation. After modification, 100% carbon dioxide in the analysis cell gave only a 22% of full scale reading. As the air flow rate through SEC - A - 04 L was selected to keep the maximum carbon dioxide build up in the air circuit to less than 1%, the optical filters virtually eliminate the problem of cross sensitivity of the hygrometer to carbon dioxide.

The hygrometer is sensitive to both pressure (15 mm H_2O difference between reference and analysis cells produces 1% of full scale deflection) and temperature (the maker recommends that background temperature around the hygrometer be maintained within $\pm$ 1^{o}C of the set

temperature during an experimental run, and that the temperature of air entering reference and analysis cells be known). The hygrometer is mounted inside the calorimeter ventilatory air "conditioning" module (Fig. 23) close to SEC - A - 04 L, thus reducing sample tube lengths to the minimum and enhancing thermal stability of the instrument. The method of attachment of the hygrometer to the ventilatory air circuit of the calorimeter is shown in Figures 21 and 22. In operation, the reference cell which is provided with its own small air pump samples 400ml/min from the calorimeter ventilatory air circuit at a point proximal to the chamber of SEC - A - 04 L. Air pressure at this point is $\pm$ 100 mm H_2O when SEC - A - 04 L is at atmospheric pressure. The function of the analysis cell is similar except that it is sited down stream from the chamber of SEC - A - 04 L where air pressure is -50mm H_2O.

In order to regulate the pressure accurately to atmosphere within each cell of the hygrometer, an elaborate control system was created which included audible and visual alarm systems to detect pressure variations. This required a separate control panel (Fig. 33) mounted alongside the mimic diagram of the calorimeter ventilatory air circuit. Two valves mounted on the hygrometer control panel enable two sensitive differential pressure switches P_I and P_{II} (Actuated Controls Ltd., type LDH/0) to be linked in three ways, as shown in Figure 33.

1. One side to reference cell, other side to vent

2. One side to analysis cell, other side to vent

3. One side to analysis cell, other side to reference cell (as shown).

Each switch can be adjusted from the front of the control panel with a screwdriver to activate within the range 2.5 - 6.4 mm H_2O. The air flow rates in the hygrometer cells are indicated by rotameters mounted on the control panel. Working the control valves in the sequence 1, 2, 3 and adjusting the rotameter and pump valves as necessary, both hygrometer cells can be made to sample continuously at atmospheric pressure. During experimental runs the valves are set to position 3 (audible and visual alarms are activated when pressure fluctuations exceed $\pm$ 2.5 mm H_2O).

To ensure accurate temperature control of the air entering the hygrometer cells it was necessary to pre-heat the sampled ventilatory air to 33^{o}C in a separately constructed hygrometer heat exchanger unit.

HYGROMETER HEAT EXCHANGER UNIT

This was made up from two 10 mm OD (1.5 mm wall thickness) 40 cm long copper pipes fitted inside a 40 mm OD copper water jacket which is supplied with water at 33 $\pm$ 0.1^{o}C by a submersed pump in a specially thermostated

Grant water bath - type SB3, sited underneath the hygrometer. This layout is generally similar to that used to regulate SEC - A - 04 L inlet air temperature by the gradient layer heat exchanger unit.

After preheating to 33°C, air entering the hygrometer is further heated to 50°C ± 0.1°C by direct heat transfer from a thermostated heater block built into the analyser. Two thermistors record reference and analysis cell temperatures. These values are recorded on channel numbers 21 and 22, and are checked at intervals during each experiment.

One special safeguard was built into the hygrometer. The cells are readily damaged by precipitation of water, therefore a pair of electrodes was fitted to the sample pipe of each cell which detect the pressure of water droplets by a change in their electrical resistance. This automatically shuts off reference and analysis cell air pumps via a relay and a transistorised switch arrangement.

RESPIRATORY GAS ANALYSIS

Space restriction within the calorimetry laboratory (Fig. 13) prevented inclusion of the oxygen and carbon dioxide analysers in the ventilatory air "conditioning" module beside SEC - A - 04 L. The analysers were built

into the upper half of a separate instrument cabinet (Fig. 34) containing the digital volt meter, data logging system and automatic alarms. The air circuit supplying the analysers is shown in Figure 35. Both analysers are supplied in parallel at a slight positive pressure with exhaust gas from the ventilatory air circuit, and are vented directly to atmosphere. A 5 metre length of unlagged 10 mm OD nylon tubing links the analysers to the ventilatory air circuit. The air is thoroughly dried by first passing through a "cold trap" consisting of a wide neck glass 500 ml round bottom flask submerged in solid carbon dioxide then, after warming to room temperature, final drying is carried out by passing through a calcium sulphate filled tube on the rear of the instrument cabinet (Fig. 36). A four position 'selector' valve was installed which allows calibration of both gas analysers with zero and span gas mixtures without disturbing air flow during an experimental run. The operational positions of this valve are shown in Figure 35.

MEASUREMENT OF OXYGEN CONSUMPTION

To minimise potential errors due to atmospheric partial pressure variations, a differential system of oxygen measurement was selected based on the design of the Servomex SB 184 twin cell instrument. At the time of construction of the oxygen analyser, twelve surplus Servomex OA 150 analysers were available to us for use

Fig.34 DATA OUTPUT INSTRUMENT CABINET

Fig.35 GAS ANALYSERS' PLUMBING SHOWING SAMPLE AND CALIBRATION CIRCUITS

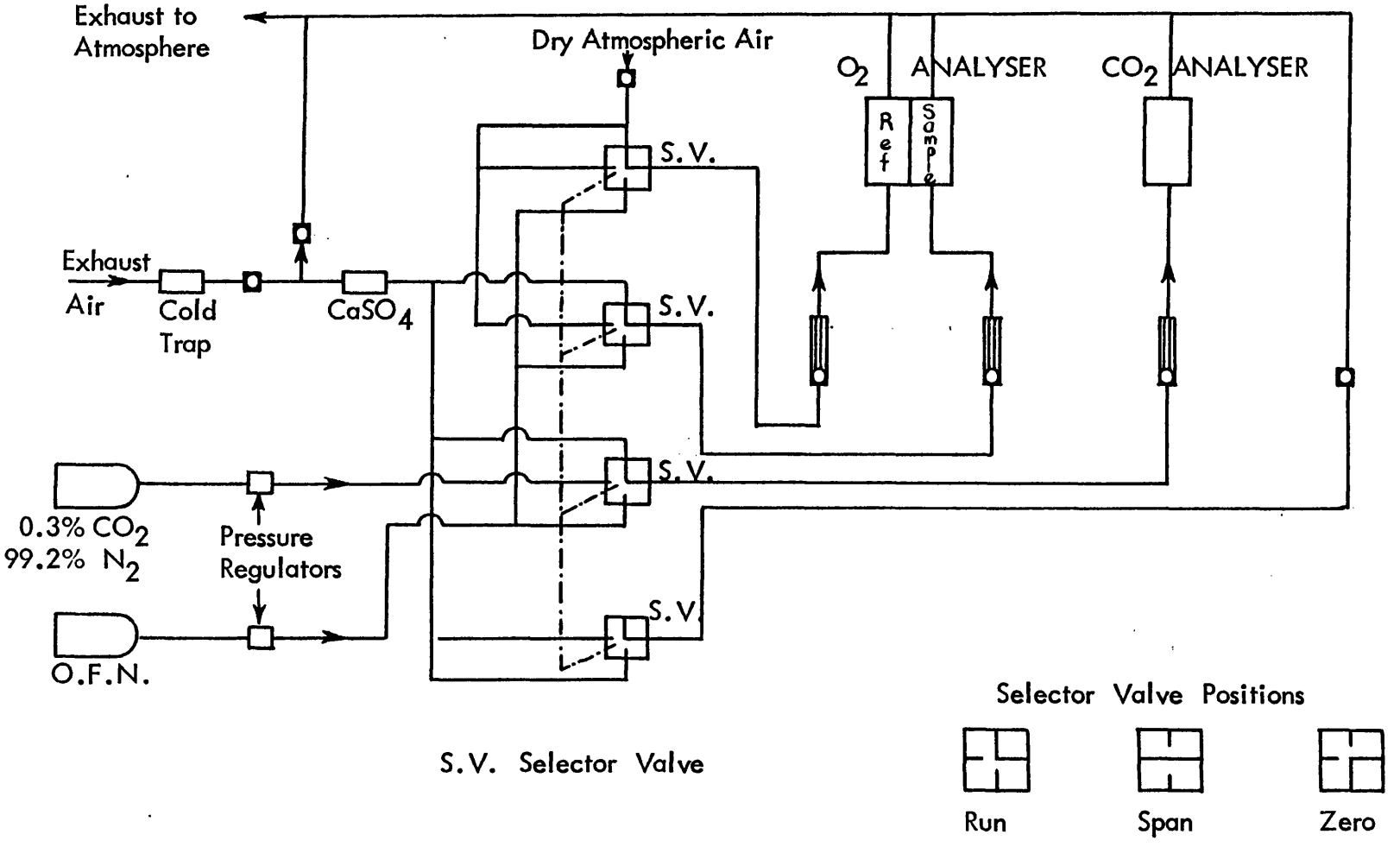
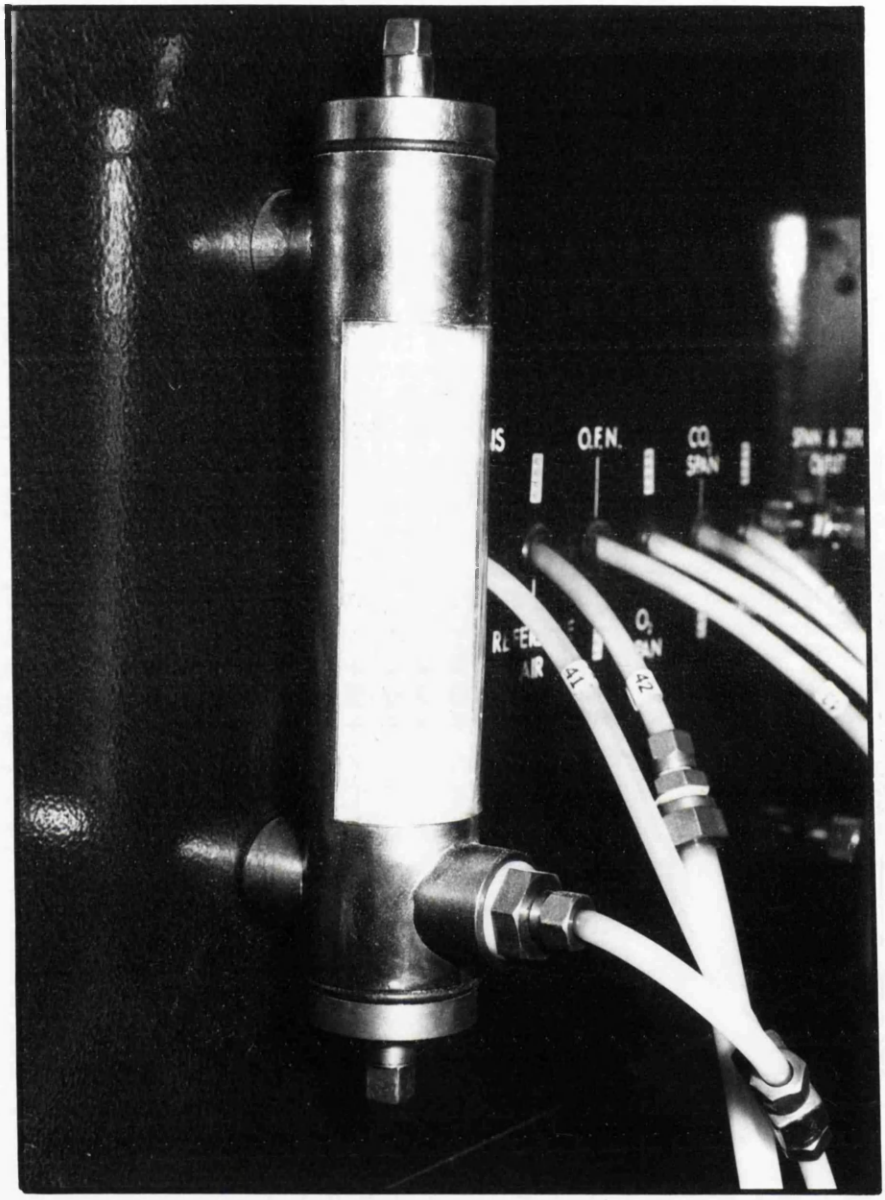


Fig.36 CALCIUM SULPHATE FILLED DRYING TUBE MOUNTED ON REAR OF INSTRUMENT CABINET



as components. The best of these instruments were 'cannibalised' and a differential paramagnetic oxygen analyser constructed from two flow matched Servomex OA 150 cells built into a thermostated enclosure (Fig. 37). This analyser produces 10 mV for a 1% difference in oxygen concentration and was constructed by the Instrument Section of the then WRHB Department of Clinical Physics and Bioengineering. The electrical output from this analyser was 'digitised' and fed into the data logging system in the same way as the other system signals.

MEASUREMENT OF CARBON DIOXIDE PRODUCTION

A simpler system was chosen to measure carbon dioxide concentration. A single cell industrial, gas infra-red analyser, type Lira 300 manufactured by Mine Safety Appliances in Glasgow was installed, sampling only exhaust air from SEC - A - 04 L. This gave a 10 mV output for a 1% concentration of carbon dioxide and had the virtue of proven stability and reliability in operation plus readily available factory servicing. The concentration of inlet carbon dioxide was assumed constant at 0.03% for the experimental runs.

GAS ANALYSER RESPONSE TIME

Without recirculation the response time of both gas analysers is 5 mins to 90% of the final value at an air flow rate of 2l/min in the ventilatory air circuit.

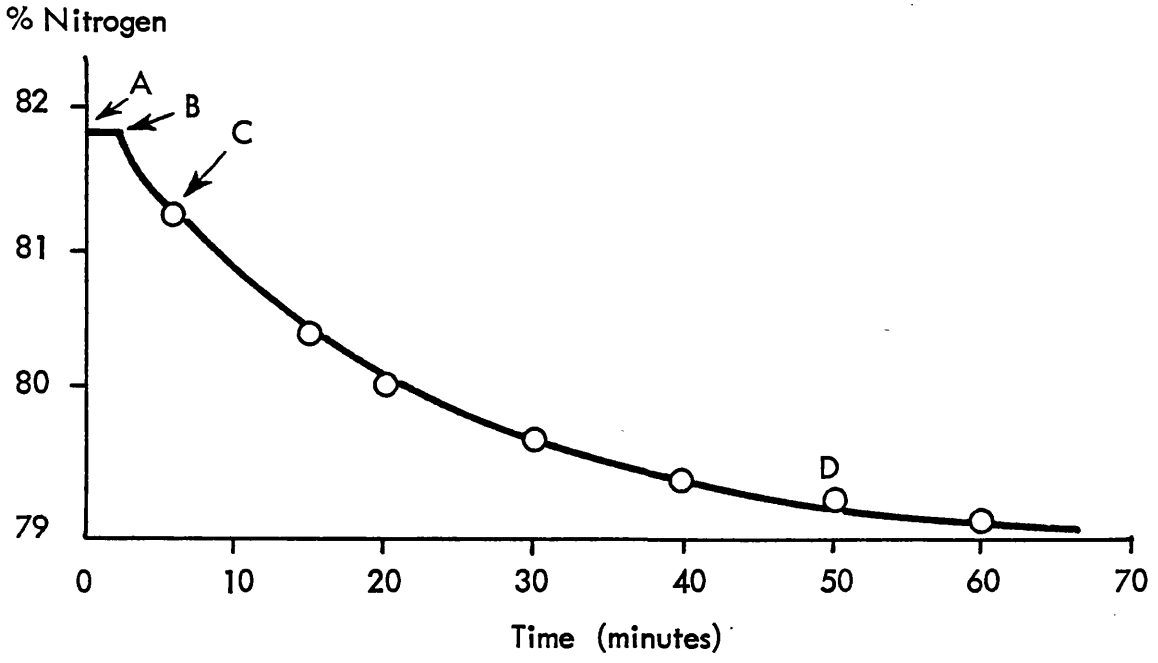
Fig.37 TWIN CELL OXYGEN ANALYSER IN INSULATED ENCLOSURE

The use of a partial air recirculation through SEC - A - 04 L to regulate humidity increases the gas analyser response times as the total ventilatory system air volume is involved in the gas exchanges. To measure the extent of the delay an experiment was carried out where the ventilating air stream was filled with nitrogen from a connector at the inlet air port of SEC - A - 04 L to a constant level of 81.8% at typical ventilating air flow rates of 5.55 l/min total flow and 1.021 l/min inlet/outlet flow. The nitrogen inflow was then shut off and the ventilatory system allowed to return to atmospheric nitrogen values keeping the ventilatory flow rates as above. Figure 38 shows the oxygen analyser output after time A when the inflow of nitrogen was shut off. Three phases are apparent.

1. A-B, duration 2 minutes, in which there is virtually no change. This represents the time taken for ventilatory air to pass from SEC - A - 04 L over a distance of 5 metres to the analyser.

2. B-C, duration 4 minutes. This is an exponential curve representing the time taken for ventilating air to travel once around the main air circuit, i.e. the recirculation time. By calculation from the rate of change of nitrogen concentration during this phase, 5.7 l of the ventilatory air circuit volume were involved in gas mixing.

Fig.38 STEP RESPONSE CHARACTERISTIC FOR GAS ANALYSIS. INLET AIR FLOW 1.2 l/min. TOTAL AIR FLOW 5.55 l/min.



3. C-D, duration 43 minutes (to 90% of the final value) is the final exponential curve involving the total ventilatory air system volume of 18 litres.

These response characteristics were acceptable as the primary function of the calorimetry system is to compare daily direct and indirect calorimetry measurements of RME in burned rats for several months after injury. Short term, i.e. minute to minute comparisons are unimportant in this regard. Therefore a prolonged overall response time due to the need for partial ventilatory air recirculation is not a handicap, bearing in mind the purpose for which the system was designed. Though it was not anticipated initially when selecting a small size of gradient layer enclosure that the effective air circuit volume would require to be as high as 18 litres when studying burned rats.

RAT TEMPERATURE MEASUREMENT.

Comparison of direct with indirect calorimetry over extended periods of up to six hours requires knowledge of changes in body heat content in the subject (Minard, 1970). To measure this, mean surface temperature and core temperature must be continuously determined (Caldwell et al., 1966). Small thermistors Y51 type 44006 were used to determine surface temperatures at various points on the rat, viz. back skin temperature,

burn wound temperature and base of tail temperature. The bridge circuit used is shown in Figure 39. The surface thermistors were embedded in 1.5 mm thin perspex discs which were glued to the rat with skin cement. Fixing multiple leads to the unanaesthetised rat proved initially unrewarding until a small simple velcro harness was devised (Fig. 40) which held all the surface thermistor leads brought together to a central point high on the rats' shoulder. This allowed the rat to move freely within the space of the holding cage for SEC - A - 04 L. Extensive training over a period of weeks of the rats selected for calorimetry and body temperature measurement proved an essential preliminary. Core temperature studies were made by inserting a micro-miniature thermistor, type 6C 32JS (Fenwell Electronics Inc), 1.5 mm tip diameter, covered by a flexible rubber sheath, into the rats' external auditory meatus to lie abutting the tympanic membrane. This was inserted with the rat under light ether anaesthesia and simply taped to the rat's ear to retain it. The linearising bridge circuit used for this temperature measurement is shown in Figure 41. The leads from all four rat thermistors are joined to form a thin multi-core cable at their junction with the velcro harness, connecting each thermistor to its individual bridge circuit via a small multi point plug. This permits the rat to be linked or withdrawn quickly from the electrical recording system of SEC - A - 04 L. This technique of rat thermometry

Fig. 39 BRIDGE CIRCUIT USING THERMISTOR YSI 44006 TO MEASURE SKIN TEMPERATURE

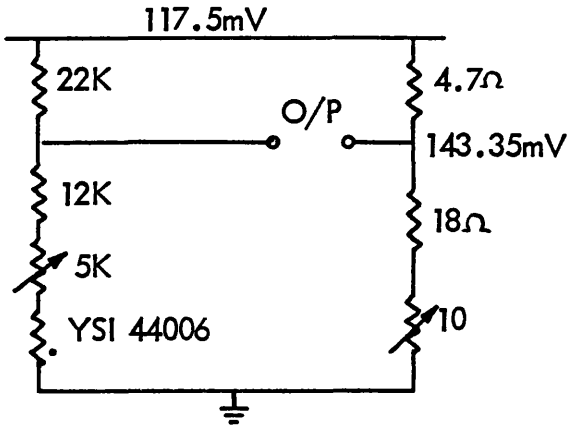


Fig. 41 BRIDGE CIRCUIT USING THERMISTOR GC 32J3 TO MEASURE TYMPANIC TEMPERATURE

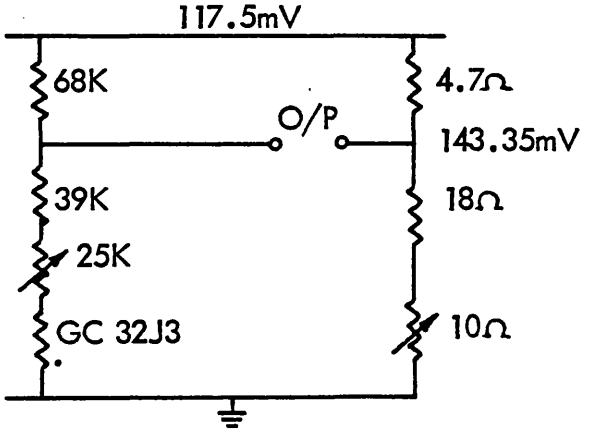
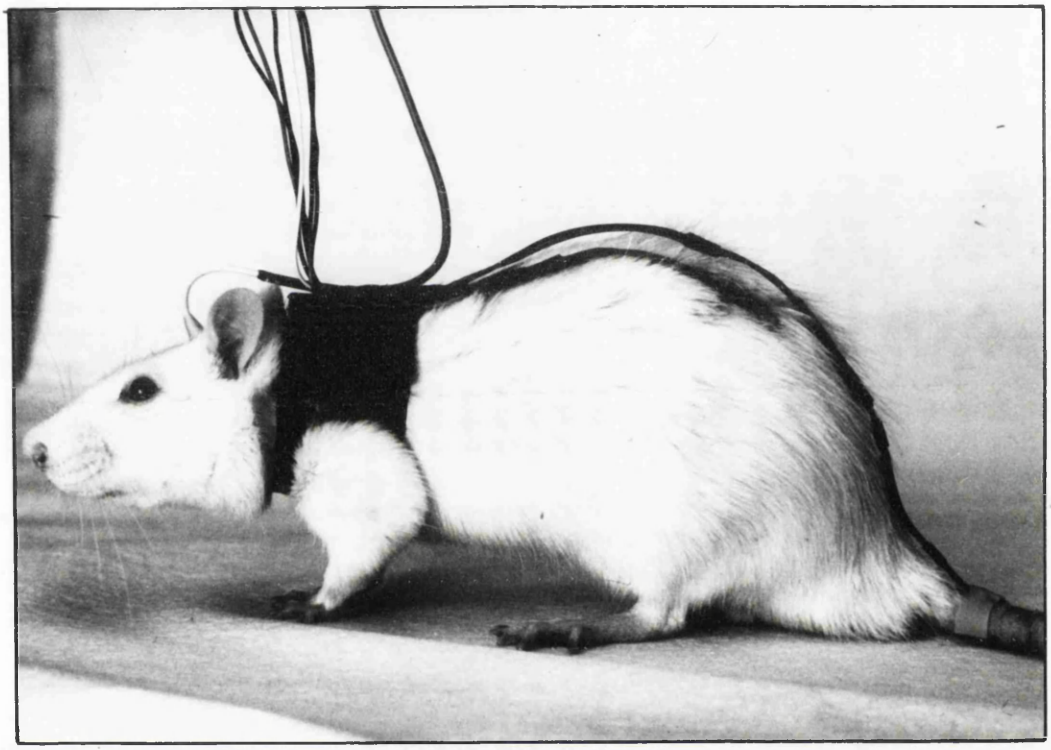


Fig. 40 RAT VELCRO THERMISTOR HARNESS SHOWING EXTERNAL AUDITORY MEATUS, BACK AND TAIL THERMISTORS IN PLACE

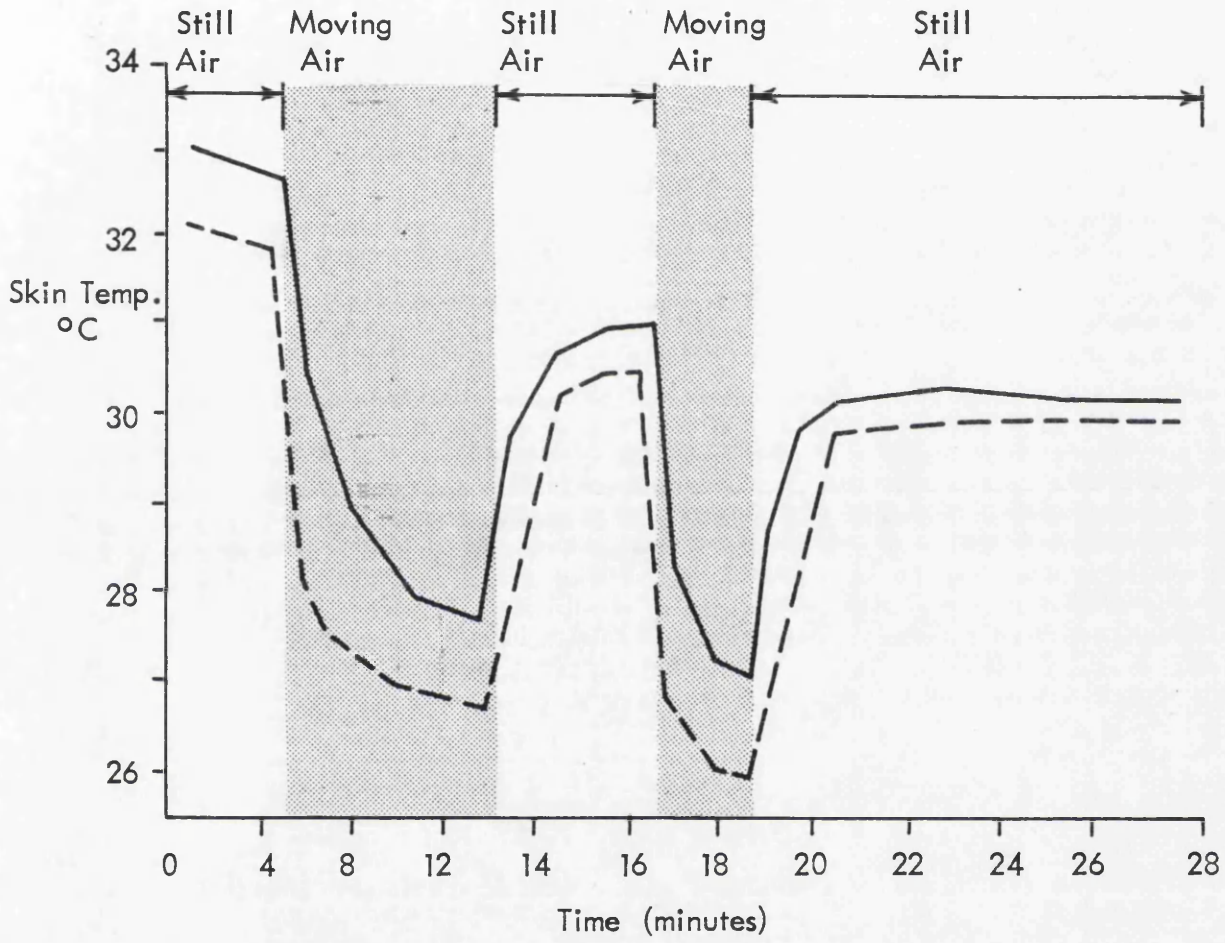


never proved entirely successful except with anaesthetised rats or with specially trained and tame control animals. Burned rats were so restless with the thermistor leads in place that their constant activity gave a misleading estimate of resting metabolic expenditure. An alternative method of surface temperature measurement which did not require body contact was evaluated (Fig. 42). An infra red radiometer was mounted 2 cm above the flank of an anaesthetised rat. The other flank had a V51 44006 thermistor disc cemented to it. Room air (20°C) was blown over the rat using a hair dryer with the heater element switched off. In still air, the thermistor recorded a mean temperature 0.41°C greater than the radiometer. In moving air conditions the discrepancy increased to 1.27°C. Thermistor measurement of skin and core temperatures in the calorimeter may overestimate the true value by these amounts. The implications of and need for measurement of body heat content changes in the rat during calorimetry are discussed later.

CALORIMETER DATA HANDLING UNIT

The large animal calorimeters studied when designing the Glasgow calorimetry system (Mount et al., 1966; Pillar, 1969; McLean, 1971) share very labour intensive data collecting methods, relying heavily on repeated visual inspection of instrument dials and chart recorders, with written notes, and subsequent manual computation of results.

Fig. 42 RAT FLANK SKIN TEMPERATURE MEASUREMENT THERMISTOR DISC vs RADIOMETER: AMBIENT TEMPERATURE 20° C



When planning the Glasgow calorimeter, advantage was taken of the expertise in the design of automated data handling systems which existed in the WRHB Department of Clinical Physics and Bioengineering. All measurement of information from the calorimeter such as flow rates, temperatures, heat outputs and so on, is derived as an electrical signal which is selected in a pre-determined sequence by an M.B. Metals Mini Logger, digitised by an S.E. Laboratories Digital Voltmeter type 213 EB, and then recorded on punched paper tape for later "off-line" analysis of experimental results by computer.

It was my original intention to have "on-line" computation of the data logger output with immediate graphical representation of results using a remote teletype linked to an existing PDP 8 computer on the ground floor of the Institute of Physiology. Regrettably, cost precluded this, so the alternative system of off-line computation had to be accepted. Some form of immediate visual monitoring of the most important physiological changes during a calorimetry run was however essential to determine the state of well-being of the rat under study. It was also important to have an immediate check on air flow rates and other parameters to determine correct function of the many calorimeter components so that in the event of a failure the experiment might be aborted at once. The cheapest practical solution

was the purchase of a twelve channel Foster Cambridge Multipoint pen recorder with a 10 mV full scale deflection, on which was continuously displayed the most important parameters for the conduct of an experiment. These, plus an additional 7 channels shown in Appendix I, are logged on punched paper tape. Channels 20 - 35 are checked but not recorded. All the data handling equipment is built into the lower half of the instrument cabinet containing the gas analysers (Fig. 34) together with the audio visual alarms from the ventilatory air circuits. In keeping with the original aim of simple operation of the calorimeter by one person, non essential switches and controls have been eliminated. It was however necessary to include range and polarity switches to cover the range of proposed operating conditions without reducing the sensitivity. These controls are located below the digital volt meter.

DCPB MODIFICATION OF DATA LOGGER

1. The data logging system can be adjusted to record on punched paper tape the output on channels 1 - 19, at any desired interval or frequency, using the inbuilt digital clock. The time of sampling is also recorded on the tape. This time signal identifies each set of results, see Figure 43. The logger was modified so that the accumulated counts over one sampling period from the inlet/

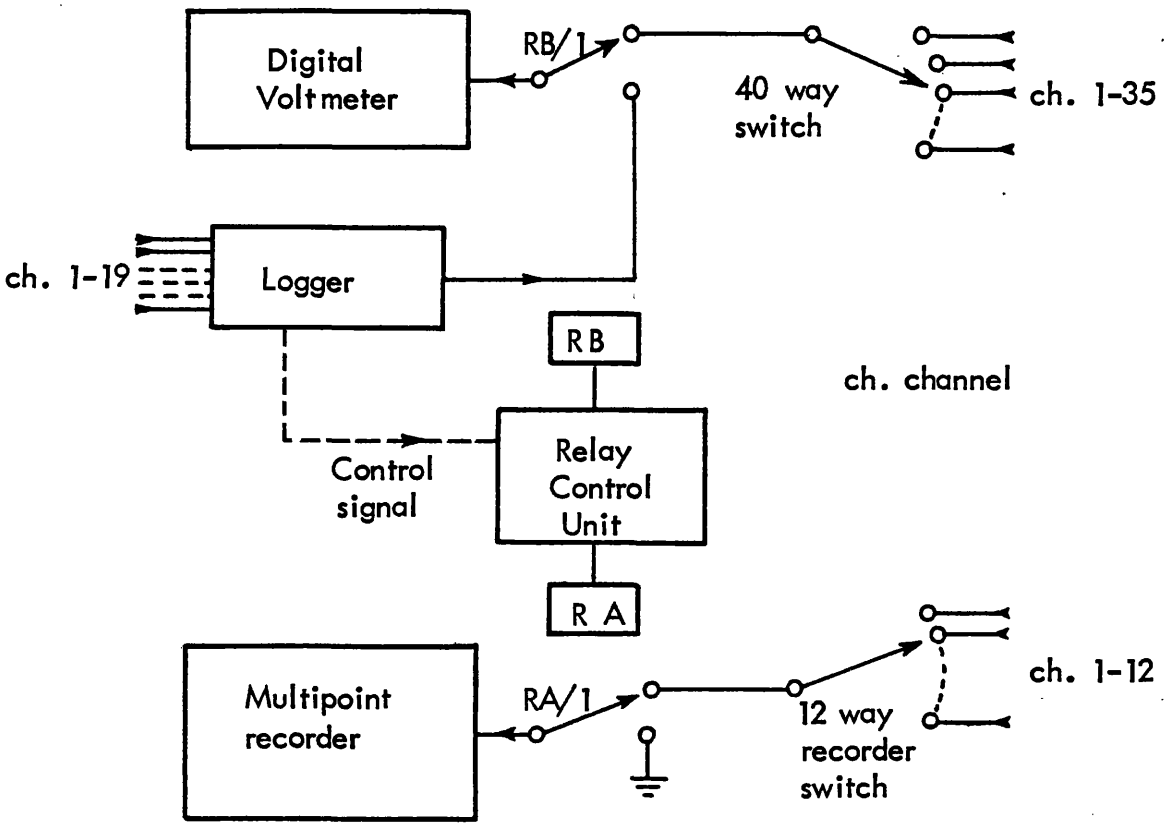
outlet wet gas meter were added to the heading data identifying a set of results. Over the next time period the accumulated counts from the recirculation wet gas meter were added to the heading data. Therefore each wet gas meter flow is recorded at alternate time periods.

2. During initial evaluation, apparently random, falsely high values were noted on the digital volt meter during the time when the pen recorder effected a channel change. After extensive laboratory testing, it was found that the source of error was the voltage generated at six second intervals by the pen recorder as it changed channels. Whenever the data logger selected channels 1 - 12, this electrical surge affected the digital volt meter reading. The instrument section of DCPB resolved the difficulty by disconnecting the channel change switch output from the rest of the pen recorder when the data logger was selecting channels 1 - 12. A schematic diagram of this modification is shown in Figure 44. A signal is sent from the data logger to the relay control unit energising relay RA to initiate a channel scan. RA/1 isolates the pen recorder by earthing its input until the data logger completes its channel selection when RA is de-energised. The voltage spike generated by the pen recorder

Fig. 43 DATA LOGGER OUTPUT SHOWING CHANNEL IDENTIFICATION

CHANNEL NUMBER	TIME	INLET/OUTLET WGM FLOW	TIME	RECIRCULATION WGM FLOW
	850	16725	9	1279
0	0	19	0	19
1	1	-103	1	2678
2	2	424	2	822
3	3	19930	3	19941
4	4	349	4	1872
5	5	660	5	2063
8	8	490	8	2713
9	9	15565	9	15544
10	10	36686	10	36680
11	11	30615	11	30616
12	12	30693	12	30699
13	13	32136	13	32140
14	14	20178	14	20294
15	15	19413	15	19456
16	16	20565	16	20571

Fig.44 DATA LOGGER AND PEN RECORDER SIGNAL SWITCHING SCHEMATIC DIAGRAM.



when changing channels is not recorded by the digital volt meter.

3. In a typical animal experiment, the data logger was set to scan information on channels 1 - 19, every 10 minutes. Scanning was completed in 20 seconds. For the remainder of each 10 minute period the digital volt meter was not used. A forty way two pole selector switch was therefore added to the circuit as shown in Figure 44 so that, when the data logger is not functioning, relay RB/1 links the digital volt meter to this switch, allowing any of the 35 channel outputs to be directly viewed on the digital volt meter. This additional facility is specially useful where, e.g. a prolonged check on the oxygen analyser output for 'baseline' drift (Ch.4) is required during routine maintenance testing. The digital volt meter output can also be linked to a separate chart recorder via two external electrical connectors where a continuous tracing is needed.

CALIBRATION PROCEDURES

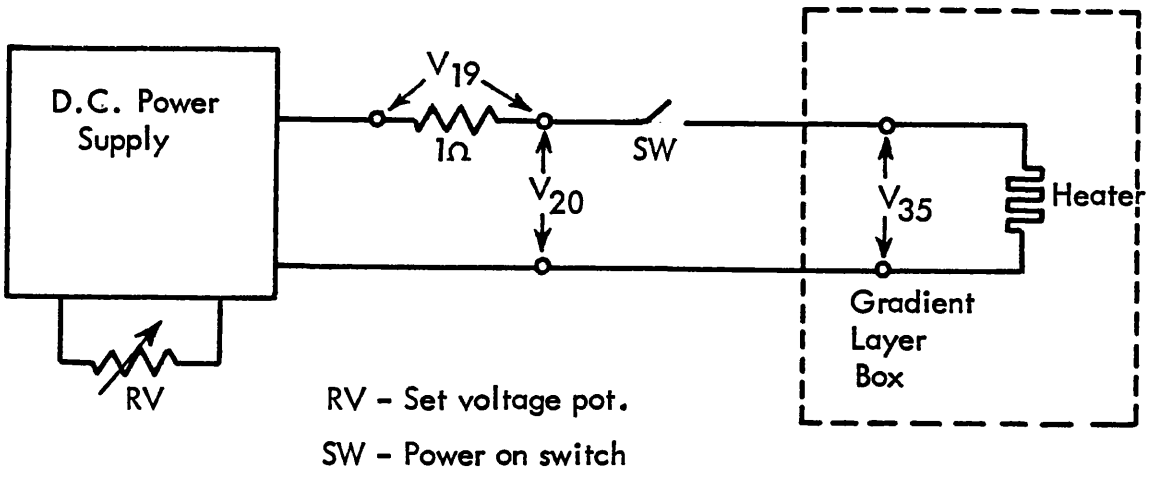
1. Gradient Layer Chamber of SEC - A - 04 L.

The initial checking and calibration of the thermopile output of SEC - A - 04 L was carried out using a

cylindrical electrical heater. This is supplied by the Thermonetics Corporation, and consists of a hollow tube 160 mm long by 30 mm diameter, wound with resistance wire, which is placed centrally in the gradient layer chamber during the calibration procedure. A special power supply, separate wiring, and plug was built into our calorimetry system to enable the manufacturers' calibration heater to be used routinely. The heater current and thermopile output from SEC - A - 04 L are readily monitored using the appropriate channels (20, 25 and 1) selected by the 40 position rotary selector switch, and displayed on the SE Labs digital volt meter.

A Kingshill S Series stabilised power supply provides a constant voltage which may be set from 2 to 15 volts by adjusting a resistance potentiometer (RV) mounted on the data handling instrument cabinet control panel (Fig. 45). The voltage supplied by this DC power unit (V20) may be checked on channel 20 using the digital voltmeter (accurate to $\pm$ 10). This is carried out with the calibration heater on/off switch SW open circuit. When SW is closed, the voltage passing across the calibration heater terminals (V55) can be displayed on channel 35. The current flowing through the heater wire is measured as a voltage drop (V19) across standard 1 ohm resistance (Cam Metric catalogue number 7500) in series with the heater. Heater power is thus

Fig. 45 GRADIENT LAYER BOX CALIBRATION HEATER POWER MEASUREMENT SCHEMATIC DIAGRAM



$V_{19} \times V_{35}$, measured with an accuracy of $\pm$ 0.1% between 1 - 10 W. Stability tests with the manufacturer's heater also proved satisfactory, i.e. $\pm$ 0.5% of a set value of 1.94 W after allowing a 20 minute 'warm-up' period (Fig. 46). 90% of the final value is reached in 15 minutes. Part of this time lag is due to the mass of the heater plastic cylinder causing a relative 'thermal inertia'. When a new heater was made from a 300 mm roll of 36 swg resistance wire wound without a supporting structure and placed centrally within the gradient layer chamber of SEC - A - 04 L, thermal inertia was removed, and 90% of the set value was reached in 3 minutes. This approximates to the time constant of the gradient layer itself. The gradient layer thermopile output was determined using this heater at a number of heater power settings (Fig. 47). A linear relationship was found with a constant sensitivity of 2.65 mV/W when the operating temperature of the calorimeter was set at 20°C. At 30°C, the sensitivity was increased to 2.80 mV/W due to a rise in thermal conductivity of the gradient layer (See similar effects shown in calibration graph of Benzinger and Kitzinger, 1949).

SEC - A - 04 L differs considerably from the 'ideal' six sided gradient layer system described by the latter authors in which all internal surfaces are equally heat sensitive and where the response to a

Fig.46 TYPICAL CALIBRATION TRACING OF THERMOPILE OUTPUT IN RESPONSE TO 1.94W DISSIPATED WITHIN THE GRADIENT LAYER BOX AT 20°C. NO AIR FLOW

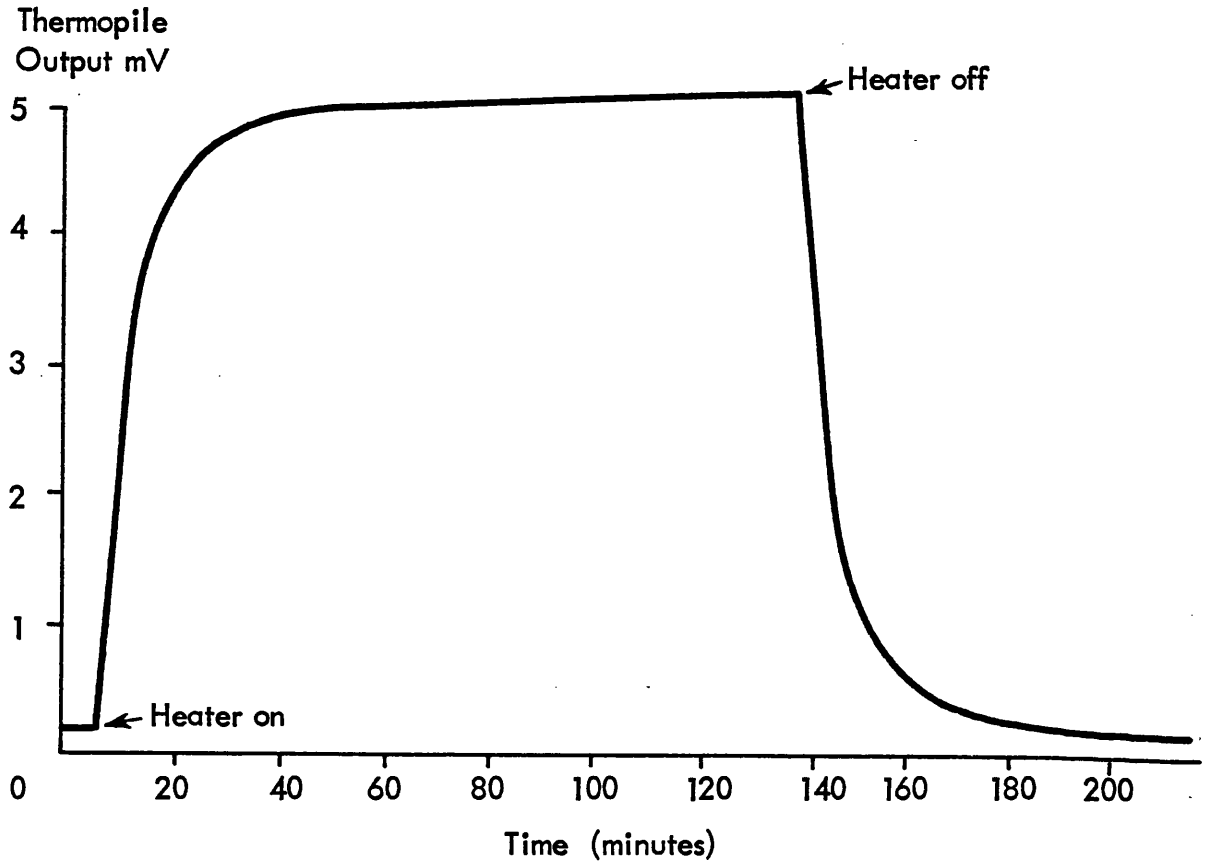
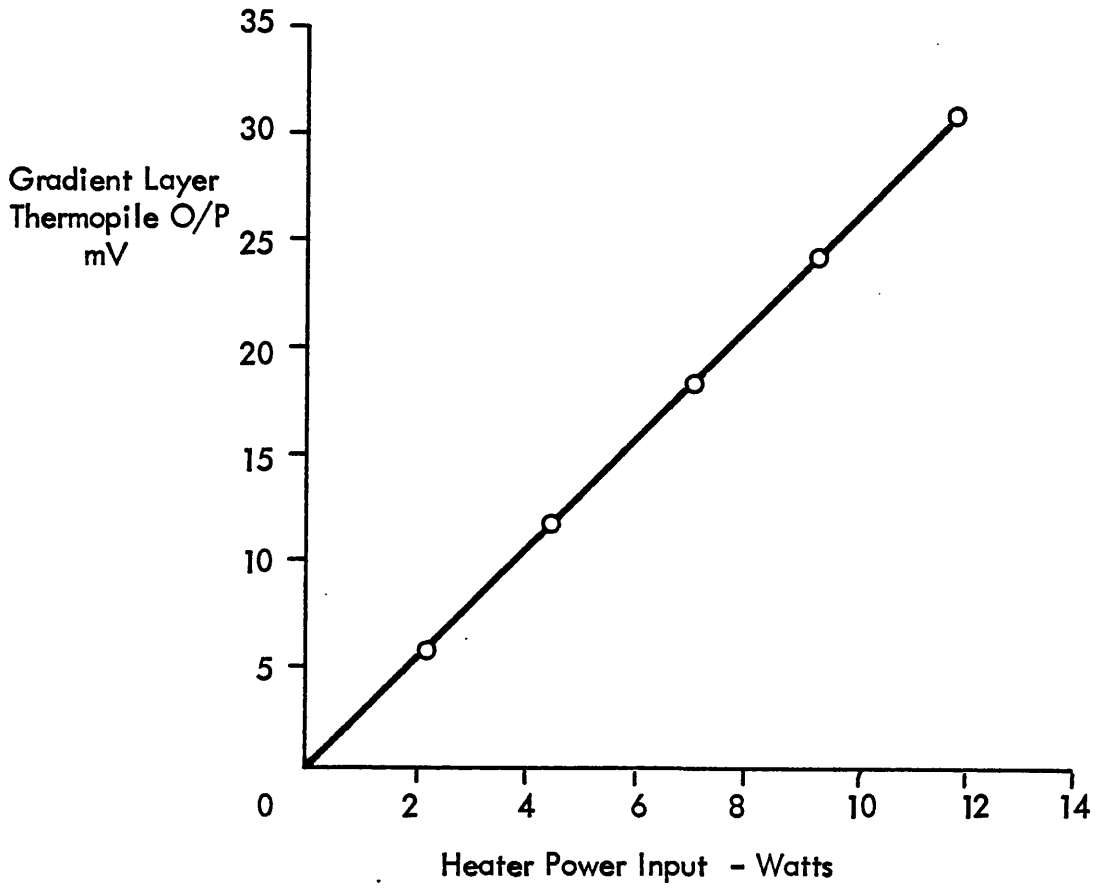
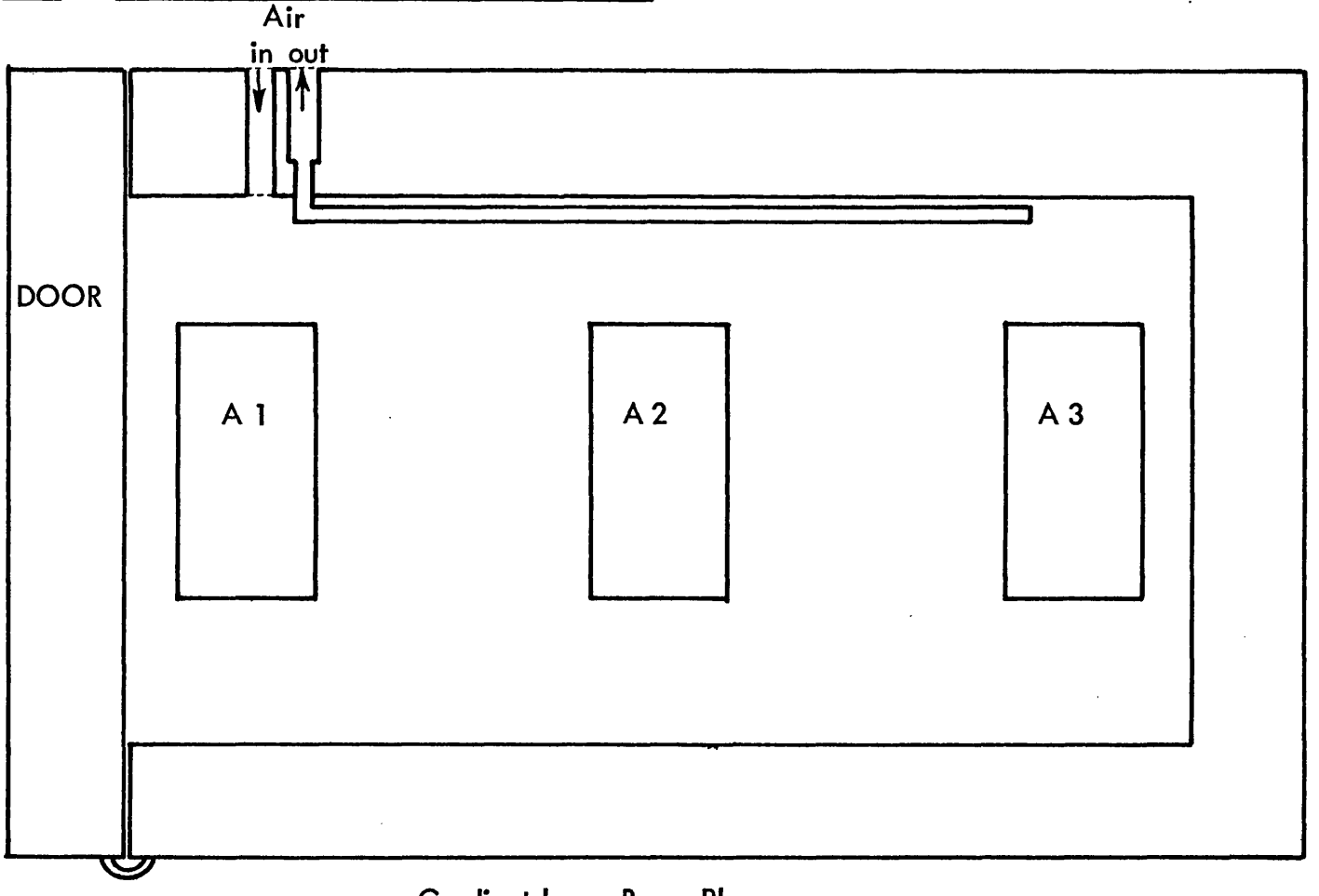


Fig. 47 VARIATION IN GRADIENT LAYER THERMOPILE OUTPUT WITH INCREASE IN HEATER POWER INPUT:AMBIENT TEMP. 20° C : ZERO AIR FLOW CONDITIONS



point heat source is independent of its position within the gradient layer box SEC - A - 04 L contains no thermocouples on the glass fronted door. It therefore seemed likely that lack of heat sensitivity on this surface might lead to inaccuracy if, e.g. the rat sat huddled near the door throughout a calorimetry experiment. To investigate just this possibility, another calibration heater was made by winding 450 mm of 32 swg copper wire around a shorter (50 mm), thinner (25 mm diameter) plastic tube than that supplied by Thermonetics Corporation. Black P.V.C. was wrapped over the wire and the ends of the wire were fitted to a square shaped connector block which also served as a stand for the completed calibration heater. Calorimeter operating temperature was set at 20°C, and the gradient layer thermopile output was determined with the heater in each of three positions with its longitudinal axis set parallel to the glass covered door as shown in Figure 48. The heater was supplied with approximately 2W at each position and the thermopile output recorded with no airflow through SEC - A - 04 L. The experiment was repeated with ventilatory air flow rates set at 2, 4, 6 and 7 litres per min. Air flow rates, air inlet and outlet temperatures (T_3 and T_4, Fig. 22), thermopile output, and heater power supplied were again measured (Table 4).

Fig. 48 POSITION OF CALIBRATION HEATER A.

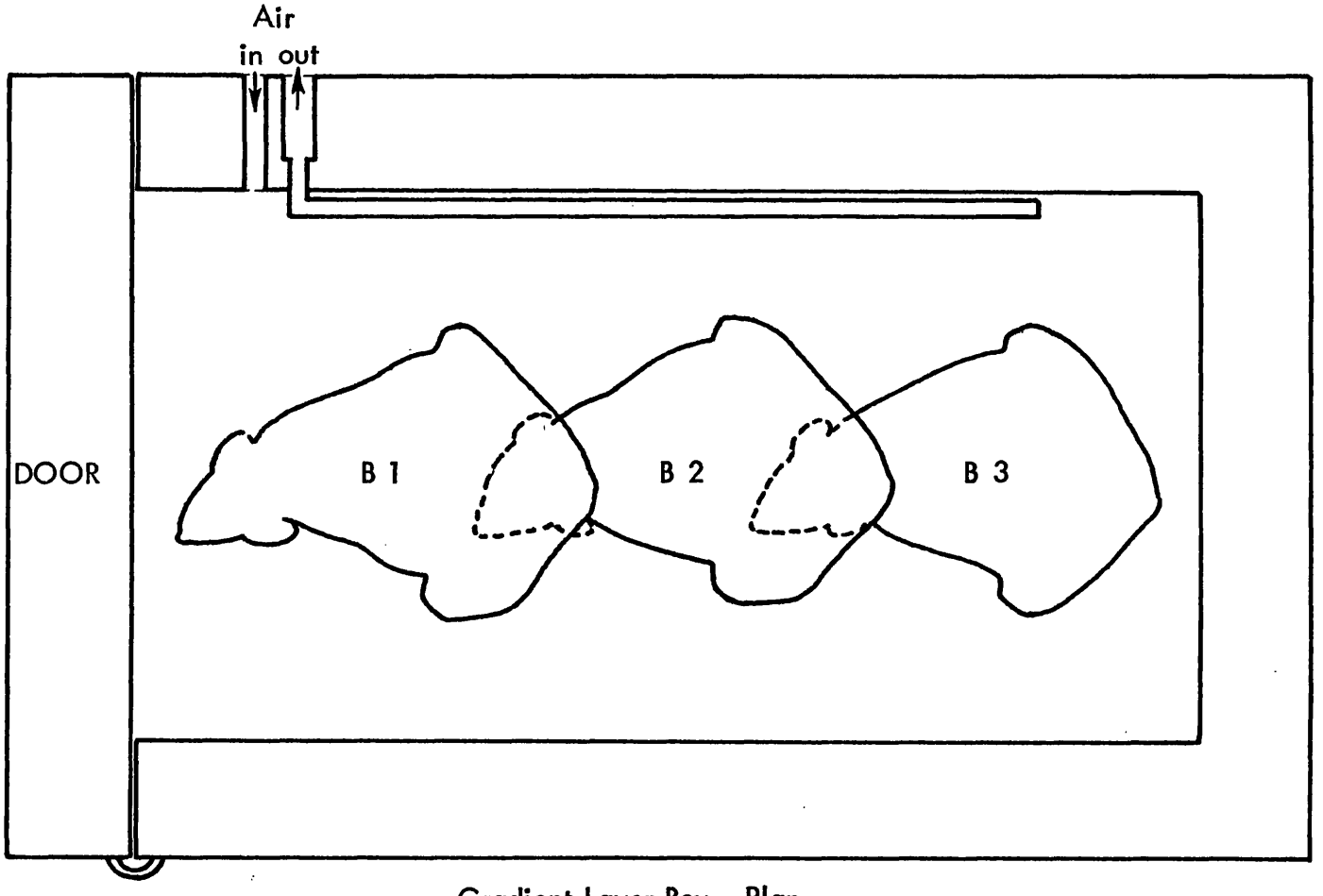


Gradient Layer Box - Plan

During the course of the preliminary calorimetry experiments it was noted that the rat under study frequently adopted a crouching hunched position alternating with an elongated stretching position when exploring the cage. These manoeuvres markedly altered the effective surface area of the rat, hence its radiant heat surface. The effect of differing rat posture upon thermopile output was also investigated by constructing calibration heaters shaped like hunched and stretching rats, heaters B and C respectively. These were made from plaster of Paris moulds of dead rats solidly frozen into the required postures. Each of these plaster rat models was wound spirally with 2 m of 32 swg copper resistance wire, wrapped with a single layer of black PVC tape, and fitted with electrical connectors as for heater A. Heater mould B (huddled rat) was placed in the wire holding cage supplied by Thermonetics Corporation, which fitted inside SEC - A - 04 L. The cage dimensions are 215 mm x 95 mm x 95 mm, and heater B (125 mm long) was sited at 3 points as shown in Figure 49, at the front, middle and back of SEC - A - 04 L.

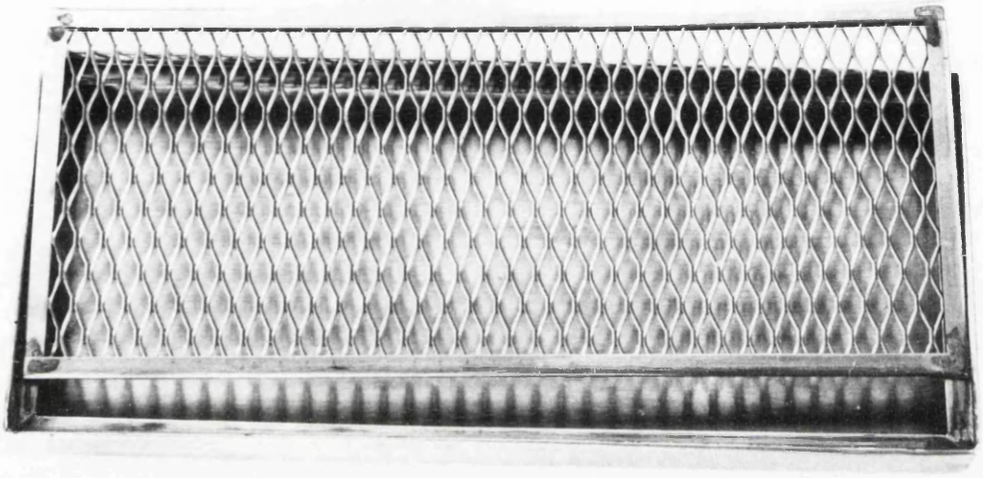
Heater C (195 mm long) was sited in the centre of the cage. Note the wire cage had been modified by the addition of a built up, wire mesh topped floor tray (Fig. 50). This is normally filled with a 5 mm

Fig.49 POSITION OF CALIBRATION HEATER B (HUDDLED RAT MOULD)



Gradient Layer Box - Plan

Fig. 50 CALORIMETER CAGE FLOOR TRAY SHOWING WIRE MESH FLOOR AND OIL PAN



depth mineral oil in order to trap rat urine during a run under the oil, preventing evaporation of urine.

CALIBRATION VALUES FOR GRADIENT LAYER CHAMBER OF SEC - A - 04 L.

The calibration value of the heater supplied by Thermometics Corporation was 0.377 W per mV at 20°C operating temperature. The total heat detected by the gradient layer of SEC - A - 04 L is calculated as follows:

Total sensible heat detected (W)

$$= K (V_1 - V_o) + F_T^1 (T_{out} - T_{in}) (DCp + Z\ Cw/1000)$$

where

- K = calibration factor at 20°C (0.377 W/mV)
- V_1 = Final thermopile output (mV)
- V_o = Initial thermopile output with no airflow (mV)
- F_T^1 = Airflow at NTP (l/sec)
- T_{in} = Temperature at SEC - A - 04 L air inlet port (°C)
- T_{out} = Temperature at SEC - A - 04 L air outlet port (°C)
- Cp = Specific heat at constant pressure for air (1.004J/g/°C)
- D = Density of dry air at NTP (1.293 g/l)
- Z = Absolute humidity of air (g H_2O/m^3 dry air at NTP)
- Cw = Specific heat of water vapour at 20°C (1.922J/g/°C)

Using this calculation, the percentage of heat supplied which was detected is shown in Tables 4, 5 and 6.

HEATER A (short tubular heater)

Without ventilatory air flow, the heat detected is approximately 9% higher with heater A in the centre position, than at either the glass fronted door or at the rear wall. The reduction in sensitivity at the front of SEC - A - 04 L, is due to heat loss via the glass door. Decreased sensitivity at the rear indicated, unexpectedly, the likelihood that the back wall contained no functioning thermocouples. Thermonetics Corporation failed to provide information regarding this in subsequent correspondence. Dismantling the gradient layer walls to confirm this was deemed unnecessary on the basis of subsequent findings.

HEATER B (huddled rat mould)

Of more practical significance, very little positional variation in the sensitivity of heat detection was noted when using the huddled rat shaped heater.

HEATER C (stretched rat mould)

A higher level of heat detection was found with the stretched out rat mould (90%) though this was lower than that found with the manufacturer's heater in the same central position (102%). The reduced overall sensitivity found using heater C is due to the partial insulation of the heat source from the floor of SEC - A - 04 L by the oil filled tray of the wire cage. In tests with heater A, the wire cage was not used.

Also shown in Tables 4A, 4B and 4C, the sensitivity of heat detection with all heaters is altered by air flow rate. From results obtained with heaters B and C the gradient layer calibration factor at 20^{o}C was taken as 0.388 W/mV at air flow rates up to 2 l/min and found to increase linearly to 0.395 W/mV at 7 l/min. Depending upon the movement and behaviour of the rat, these values may be inaccurate by $\pm$ 1.5% of the total heat detected. Based on the calibration data, an empirical relationship between the sensitivity of the gradient layer chamber, air flow rate, and rat weight was derived by Mr. K. B. Carter. This was subsequently used in the overall calculation of sensible heat loss.

TABLE 4A. Percentage of heat detected - Heater A

Airflow l/min.	A1 (front)	A2 (centre)	A3 (rear)
0	93.4	102	92.2
2	92.5 (1.0)	97.5 (1.3)	91.1 (1.3)
4	91.4 (2.7)	95.7 (3.1)	89.4 (4.5)
6	91.8 (3.9)	94.2 (4.4)	87.9 (8.6)
7	91.1 (4.1)	92.0 (5.0)	91.2 (11.5)

TABLE 4B. Percentage of heat detected - Heater B

Airflow l/min.	B1 (front)	B2 (centre)	B3 (rear)
0	95.0	96.5	96
2	95.0 (2.5)	96.9 (3.2)	95.7 (3.3)
4	94.5 (6.5)	96.4 (8.1)	95.0 (9.4)
6	94.1 (12.8)	95.9 (14.5)	94.4 (16.3)
7	93.9 (16.4)	95.8 (17.6)	94.7 (20.4)

TABLE 4C. Percentage of heat detected - Heater C

Airflow l/min.	
0	98.0
2	98.2 (2.7)
4	97.7 (7.2)
6	97.2 (13.4)
7	96.5 (17.0)

Note: Figures in parenthesis are the percentages of heat supplied to ventilating air.

At $20^{o}C$

$$K_{TH_{20}} = \frac{0.377}{(0.965 - 0.002F_T^1)(1 + 0.00015\ (W_r - 250)}$$

where $K_{TH_{20}}$ = Gradient layer calibration factor at $20^{o}C$.

F_T^1 = Total airflow rate at NTP (l/min)

W_r = Weight of rat (g)

Similarly at $30^{o}C$

$$K_{TH_{30}} = \frac{0.357}{(0.065 - 0.002F_T^1)(1 + 0.00015\ (W_r - 250)}$$

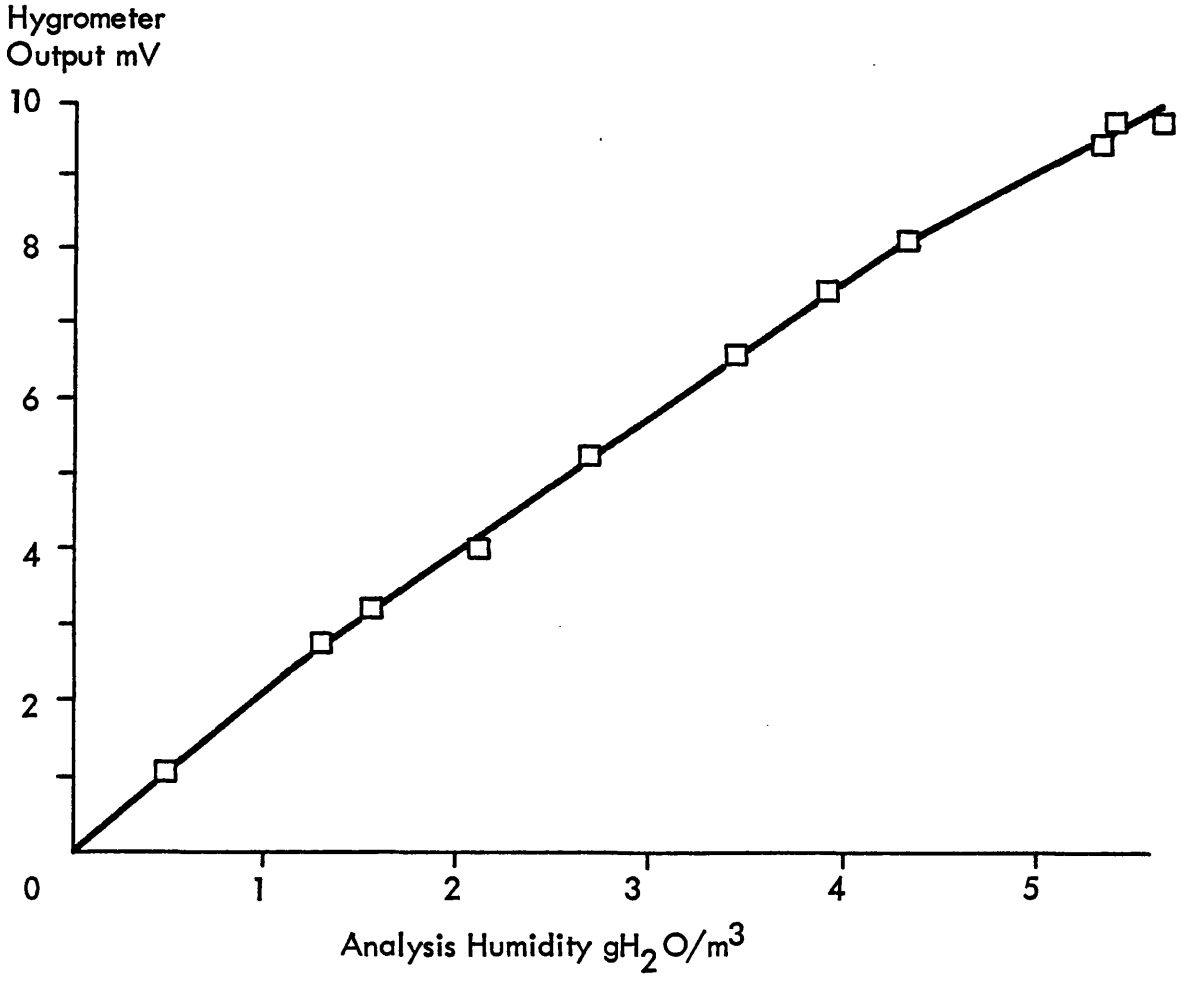
CALIBRATION PROCEDURES

2. Hygrometer - Analytical Developments Infra Red Gas Analyser.

For reasons discussed earlier, our hygrometer was calibrated at two background humidities, dry and wet, i.e. 0 and 15 g water vapour $/m^3$ dry air. The instrument was manufactured to give a linear response to humidity over the differential range of 0 - 5 g water vapour/m^3 dry air in either dry or wet humidity conditions. A range changeover switch is provided to keep instrument sensitivity identical at either humidity. Calibration at dry background humidity was performed as follows.

The reference cell of the hygrometer was filled with air chemically dried by passing through tubes of calcium sulphate (effective dew point -70°C) thus providing a 0.01 g water vapour /m^3 dry air background level. The hygrometer analysis cell was supplied with air humidified by passing over a saturated solution of lithium chloride kept at a set temperature between 20°C - 35°C. Various humidity values from 2.5 to 5 g water vapour /m^3 dry air were thus provided. Humidity values in the range 0 - 2.5 g water vapour /m^3 dry air were produced by diluting air at a known humidity of 2.5 g H_2O/m^3 with chemically dried air in varying proportions. Each humidity value was checked gravimetrically by passing air from the analysis cell over weighed amounts of magnesium perchlorate and then phorphorus pentoxide. By reweighing, the weight of water added was determined. The total air flow was measured by wet gas meter. The hygrometer calibration curve thus obtained is shown in Figure 51. The instrument response is non linear below 1 g H_2O/m^3 and above 4.5 g H_2O/m^3. Linearity is however acceptable within these values, which in practice represent the range in which most physiological measurements will occur. The constancy of the dry background humidity value is dependent upon the continued effectiveness of the calcium sulphate (Dririte) drying agent. Should this become ineffective or used up,

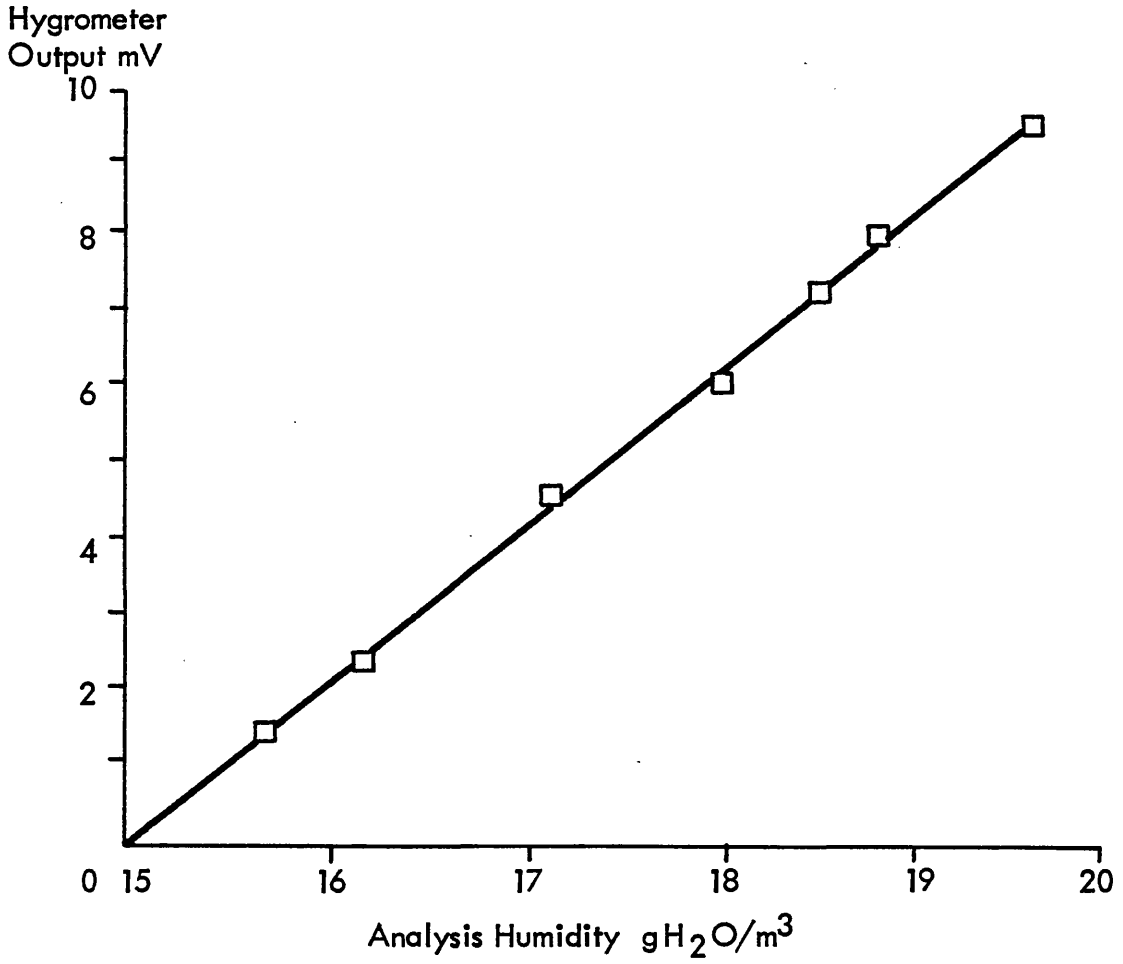
Fig.51 HYGROMETER CALIBRATION CURVE. BACKGROUND HUMIDITY 0g H_2O/m^3 DRY AIR AT N.T.P.



increases in background humidity up to 9.1 g H_2O/m^3 can be tolerated with little effect upon the hygrometer calibration curve.

To calibrate the hygrometer at the wet background humidity of 15 g H_2O/m^3, the reference cell was filled with air continuously recycled over a saturated solution of sodium chloride kept at 22.5°C ± 0.01°C. The analysis cell was filled with air humidified in the range 15 g H_2O/m^3 to 20 g H_2O/m^3 produced by fully saturating and then colling the air to a known dew point. The water vapour content of the air at each calibration point was again checked gravimetrically. Figure 52 shows the calibration curve of the hygrometer to be linear at background humidity values from 15 g H_2O/m^3 to 19.86 g H_2O/m^3. In the design of the calorimetry system (Fig. 22) background humidity values of 15 g H_2O/m^3 are dependent upon the temperature stability of the water bath of the dew point setting - heat exchanger unit. A fluctuation of ± 0.1°C in dew point produces a humidity change of ± 0.1 g H_2O/m^3, though this will not affect the calibration curve at the wet background humidity setting. The calibration characteristics of our instrument were therefore generally satisfactory and similar to those described by Parkinson and Legg (1971) for this type of instrument. Routine calibration of the hygrometer is carried out by placing a water soaked paper tissue in the chamber of

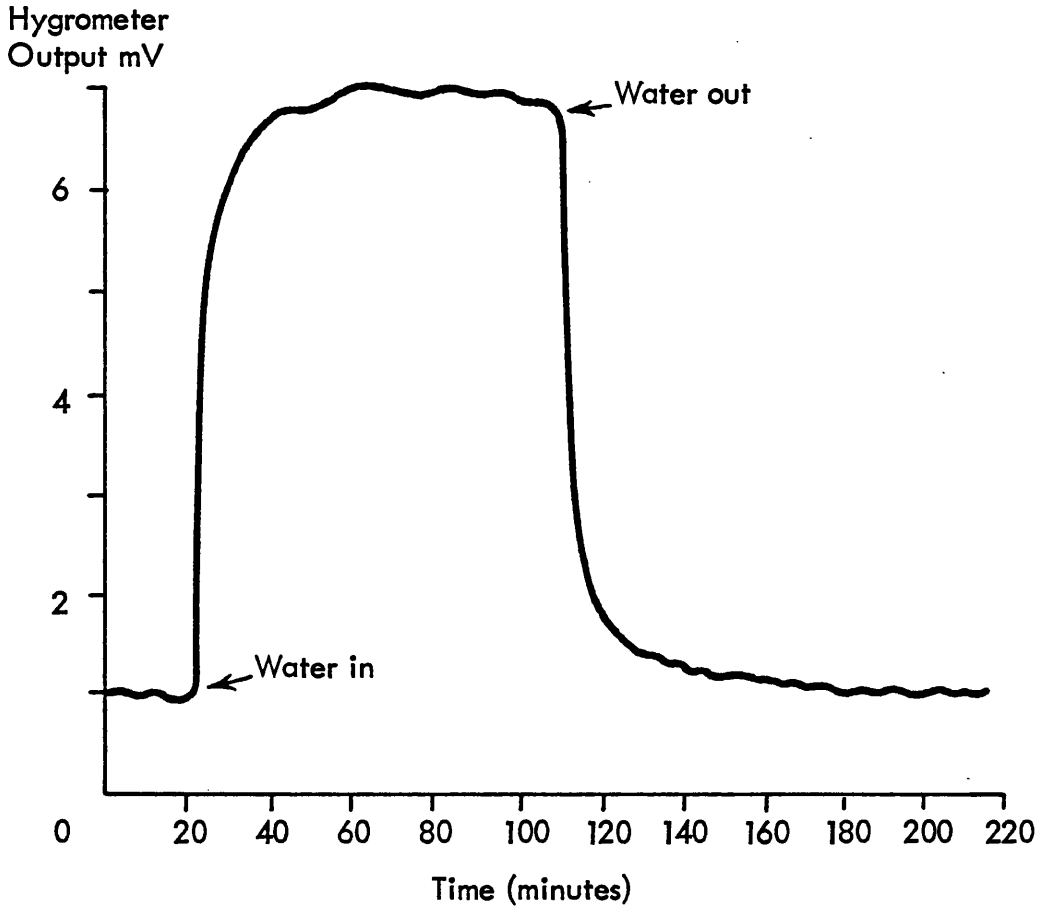
Fig. 52 HYGROMETER CALIBRATION CURVE. BACKGROUND HUMIDITY 15 g. H_2O/m^3 DRY AIR AT N.T.P.



SEC - A - 04 L on the floor tray of the wire cage which is carefully weighed. The ventilating airflow rate is adjusted until a relatively constant rate of water evaporation is seen. The hygrometer output on Channel 8 is continuously recorded on the Foster chart recorder and water is allowed to evaporate for a period of up to 120 minutes. The airflow through the chanber of SEC - A - 04 L is shown by the recirculation wet gas meter. The weight of water evaporated in the test is determined by reweighing the tray plus paper tissue. Figure 53 shows a typical hygrometer response to water soaked tissue in the gradient layer chamber. The slight fluctuations on the tracing are due to temperature variations of $\pm$ 0.1^{o}C in the dew point - heat exchanger water bath. By adjusting the valves on the hygrometer control panel (Fig. 33), the inlet ports of reference and analysis cells can be linked during a run thus allowing the hygrometer zero to be set up and checked at will.

Note - the hygrometer calibration run will also provide a check on the gradient layer chamber calibration at high airflow rates. As water evaporates within the chamber of SEC - A - 04 L, the latent heat required is drawn from the ventilatory air and from the chamber walls. This heat is measured, and using the latent heat of vapourisation of water at the chamber operating temperature the gradient layer chamber can be calibrated.

Fig. 53 TYPICAL HYGROMETER RESPONSE TO WATER SOAKED TISSUE WITHIN THE GRADIENT LAYER BOX. BACKGROUND HUMIDITY 15 g H_2O/m^3 DRY AIR AT N.T.P. TOTAL AIR FLOW 6 l/min.



CALIBRATION PROCEDURES

3. Wet Gas Meters - Volumetric Displacement Type.

The wet gas meters used, Wrights' type M809NA were supplied by Alexander Wright and Co., London, with certificates of calibration and accuracy. The accuracy of both meters was stated to be $\pm$ 0.5% throughout their measurement range. This was confirmed in Glasgow by the use of mercury piston air volume displacement cylinder.

CALIBRATION PROCEDURES

4. Oxygen Analyser - Paramagnetic Differential Cell Type.

The operating range of this analyser is a 1% span between 20.95% which is the percentage of oxygen in the atmosphere - Smithsonian tables, and 19.95%. Calorimeter flows are normally adjusted so that the rat oxygen consumption depletes atmosphere air by 0.4 - 0.7%. Reference cell and analysis cell are calibrated with oxygen free nitrogen (certificated gas from British Oxygen Company, Polmadie Works, Glasgow) as the zero setting gas. Atmosphere air is used in both cells as the span gas. A pressure calibrating method (Hammel et al., 1958) was used by Mr. K. B. Carter, which, after taking account of the diamagnetic properties of nitrogen, established the linearity of

the oxygen analyser response from 0 to 20.95%. This confirmed the suitability of routine calibration of the oxygen analyser cells with oxygen free nitrogen and air.

CALIBRATION PROCEDURES

5. Carbon Dioxide Analyser - Infra Red Single Cell Type.

The MSA type Lira 300 carbon dioxide analyser was calibrated initially using a gas mixture containing 1% CO_2, 99% oxygen free nitrogen, accurately analysed by the Lloyd-Haldane method. This gas was then diluted to produce a range of concentrations using Wostoff pumps. With these gases, the carbon dioxide analyser was shown to have a response which was linear to within $\pm$ 1 for carbon dioxide values between 0 and 1%.

Routine calibration is carried out using oxygen free nitrogen for setting zero. The span gas used is a certificated gas gravimetrically analysed, of around 0.8% carbon dioxide in nitrogen. The switching arrangements of calibration are shown in Figure 35.

CALORIMETER LABORATORY

The arrangement of calorimeter components shown in Fig.13 was chosen to provide the most efficient use of the available floor space and to provide ready access for routine servicing and occasional repair of major components. The gas analysers and data handling unit had to be placed at right angles to the calorimeter unit, thus preventing the operator from simultaneously observing the chart recorder output of essential physiological data, the rat within SEC - A - 04 L, and the valves, pressure and flow settings at the mimic diagram control panel where running adjustments to calorimeter settings are made. This was overcome by extensive use of automatic audio-visual alarms where flows or pressure settings are critical to the performance of the calorimeter as indicated in the preceding description of the individual components.

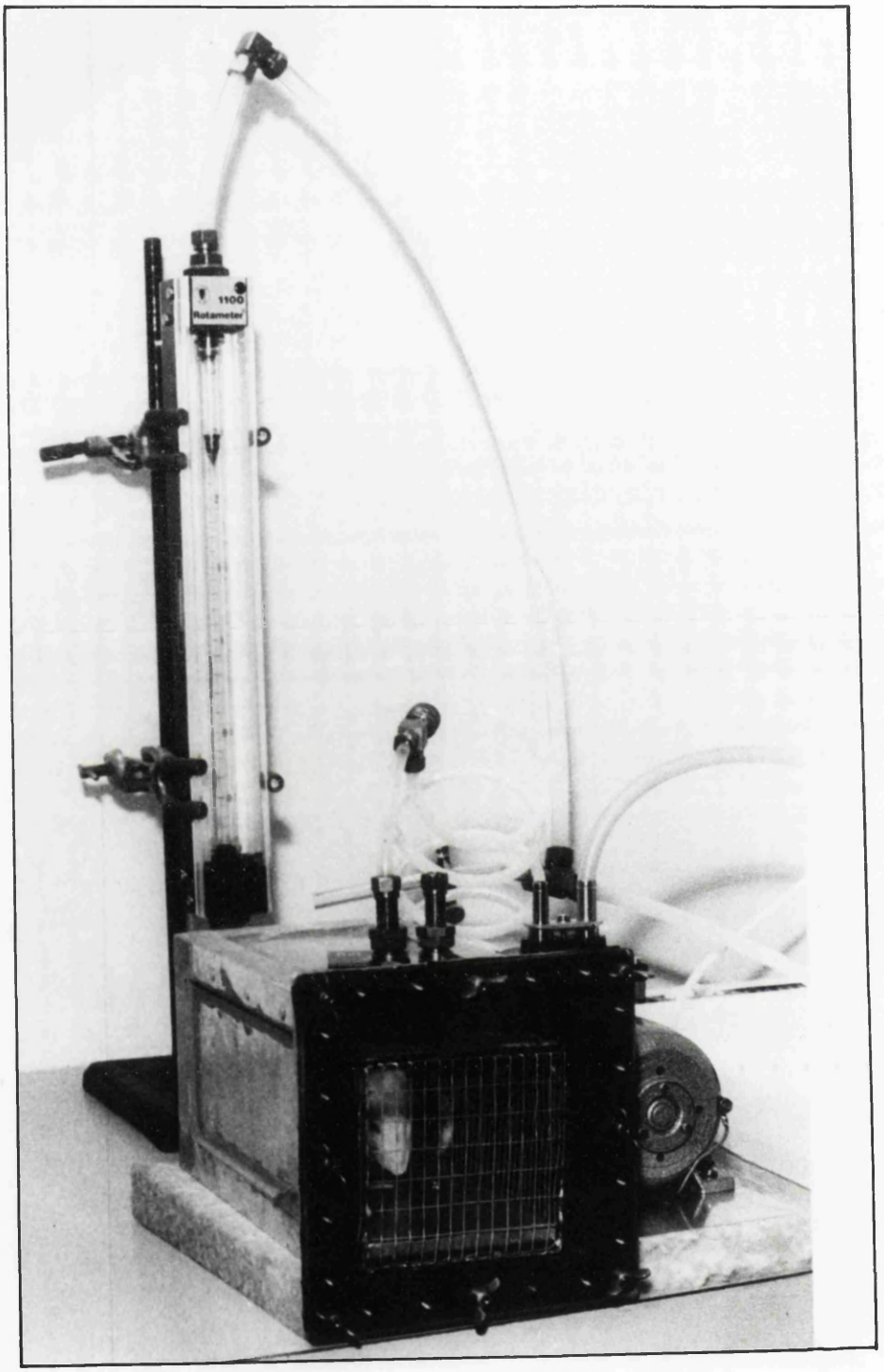
Access to the front loading glass door of SEC - A - 04 L (Fig. 23) is adjacent to the folding wall partition which links the calorimeter laboratory to the animal handling and preparation laboratory and rat environmental chamber (Fig. 13) described later. The calorimetry system was designed to be an integral part of an overall laboratory unit to study normal and injured rats over prolonged periods under closely controlled environmental conditions.

RAT CONDITIONING PROCEDURE FOR CALORIMETRY

All rats studied were allowed to acclimatise over a minimum period of 7 - 10 days in the Environmental Chamber at either 20°C ± 0.5°C or 30°C ± 0.5°C and were accustomed to calorimetry by placing them in a dummy calorimeter identical in size and internal appearance to SEC - A - 04 L. This dummy calorimeter was complete with its own air pump and ventilation system so that calorimeter pump noise and airflow conditions were also simulated (Fig. 54). Rats were trained in this dummy box for periods of 120 - 180 minutes per day and dummy thermistor leads and harness attached as shown in Fig. 40. The initial rat response was to chew off or otherwise vigorously remove all external leads within reach of teeth or paws. But within a relatively short time (5 - 10 days) a high proportion of trained rats were able to tolerate thermistor leads during actual calorimetry runs. Extensive rat training proved essential to the performance of thermometry (Drury et al., 1977), the results of which are presented.

All calorimetry runs were performed with rats in a post-absorbtive state. Rats were trained to eat the whole of their normal daily food intake within a 4 hour period in order to be 16 hours post-absorbtive by the next morning. Rats were fed Oxoid Diet 41B

Fig.54 "DUMMY" CALORIMETER WITH RAT TRAINING UNDER SIMULATED CALORIMETER NOISE AND AIRFLOW CONDITIONS



during the initial experiments to assess calorimeter performance. In all subsequent experiments rats were fed Nutritional Biochemicals Low Iodine Test Diet with supplementary iodide in their drinking water. This latter diet was used because of its high nutritional value (20% protein) and very low faecal residue. Figure 99 compares the faecal residues of rats eating L.I.T. vs. 41B diet. The use of a low residue diet resulted in rats defaecating less often, with more chance of acclimatised trained rats not defaecating at all during a calorimetry run of up to 200 minutes (See Table 11). Though allowance can be made for faecal heat losses during calorimetry, accurate comparison of direct and indirect calorimetry ultimately suffers. Micturition by the rat during a calorimeter run has similar consequences. The base of the wire inner cage used to place the rat into SEC - A - 04 L (Fig. 50) contains a small oil filled tray. The rat is supported 15 mm from the bottom of this tray by a wire mesh sheet which forms the cage floor. Rat urine falls through the wire mesh forming a pool on the base of the tray. The oil floats on the surface of the urine preventing further evaporation during the run.

PROCEDURE FOR CALORIMETRY

(1) The calibration and setting up sequences outlined in appendices 2 - 5 were completed.

(2) The urine collecting tray was filled to a depth of 2 mm with light mineral oil, carefully weighed on a Mettlar P.1200 top pan loading balance, and placed in the calorimeter wire cage.

(3) The rat was weighed (Figs. 93 and 94), (Richards et al., 1977) and its Velcro harness

(4) with thermistor leads attached. Skin cement was used to apply the thermistor discs to back skin and base of tail skin (Fig. 40). The external auditory meatal thermistor was inserted into the ear canal using a very brief ether anaesthetic and the thermistor lead taped to the rat's ear with Sleek tape cut to size. The rat was allowed to recover fully within the wire cage before being placed into SEC - A - 04 L and its thermistors connected to the appropriate bridge circuits (Figs. 39 and 41) via a multi pin plug. (Step 4 was omitted if thermometry was not carried out).

(5) The rat was sealed into the calorimeter by closing the front loading glass door of SEC - A - 04 L and the outer insulated enclosure door, and the time was noted on the data logger clock.

(6) A visual check on the Foster 12 channel chart recorder was made shortly after inserting the rat to ensure that all measuring and recording systems were functioning satisfactorily.

(7) If necessary, inlet air flow rate and recirculated flow rate was adjusted to keep carbon dioxide levels within SEC - A - 04 L around 0.5 - 0.6% and pressure adjustments made to the hygrometer cells.

(8) Throughout the run a close inspection of the rat was made via the double glass doors and the data output on the pen recorder regularly checked.

(9) On completion of calorimetry, the rat was removed from SEC - A - 04 L, re-weighed after detachment of leads and harness, and the weight of urine or faeces passed determined by difference after re-weighing the tray and cage.

If no urine or faeces had been passed during a run, then the weight lost by the rat was principally that due to evaporative water loss. Otherwise evaporative water loss was that weight lost by the rat after subtraction of the weight of urine and faeces excreted.

(10) After removal of the rat the calorimeter was re-sealed and the system allowed to continue running until all parameters had returned to baseline values. This allowed instrument drift to be determined and the appropriate baseline drift corrections applied when calculating the results of the run.

(11) Atmospheric pressure, room temperature and wet gas meter pressure were noted during the run and the values entered on a cyclostyled experimental record sheet together with the rat data. Flow settings were noted and comments recorded about the run.

(12) Initially the hygrometer was calibrated at the end of each run.

EXPERIMENTAL OBJECTIVES IN EVALUATION OF CALORIMETER PERFORMANCE

(1) To assess gradient layer function in direct calorimetry partitioning of heat losses - TOTAL, SENSIBLE, INSENSIBLE in normal rats subjected to various ambient temperature and humidity conditions,
viz. 20°C low RH, 20°C high RH, 30°C low RH, 30°C high RH.
To determine rat responses during calorimetry and note the effect of movement and posture on partitioned heat loss measurement and to compare these with heat loss measurements in an anaesthetised rat during deep anaesthesia and during recovery.

(2) To assess gas analyser function and to determine the indirect calorimetry system response to rat movement and to relate overall measurement of heat production by indirect calorimetry to measurement of heat loss by direct calorimetry in normal rats. To determine moment to moment variation in heat production and loss in the rat and to thereby establish the most suitable duration of calorimeter run which will establish a true measurement of RME in the rat.

(3) To assess changes in rat body heat content and tissue heat flux during calorimetry in normal rats at 20°C and 30°C. To establish the need for thermometry during calorimetry in long term studies of RME.

(4) To assess the direct calorimetry partitioning of heat losses after a standard burn injury (20% of body surface area full skin thickness dorsal burn) before and up to 40 days after burning.

(5) To assess changes in rat body heat content and tissue heat flux during calorimetry in a burned rat.

(6) To critically assess calorimeter performance during a long term study in a burned rat.

RESULTS

The computations used to determine heat production and heat loss are given in appendix 6. The time intervals during which total heat production or loss values were calculated are indicated where appropriate by vertical lines on the following graphs.

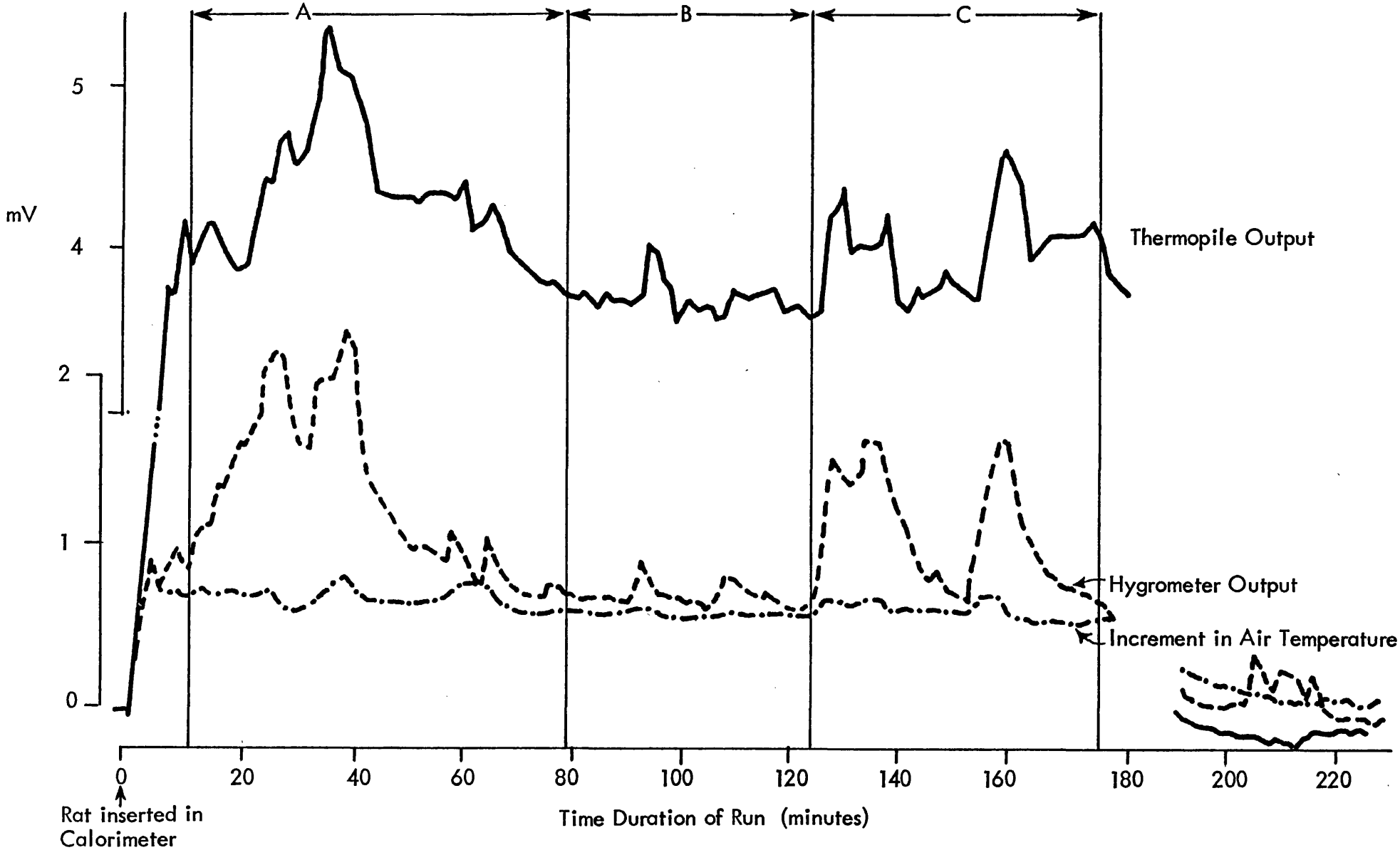
In the first set of graphical results which show direct calorimetry partitioning of heat losses, the thermopile output from SEC - A - 04 L's gradient layer wall, the increment in ventilating air temperature, and the hygrometer output of the increment in evaporative water loss have been traced directly from the Foster 12 channel chart recorder. The scale is given in millivolts as it appears on the chart recorder. This presentation was chosen to illustrate the responsiveness of the direct calorimetry measuring instruments and the relationship between sensible and insensible heat losses. The remainder of the flow rates, temperatures and other data required for the computation of partitioned heat losses are listed with each graph.

FIG. 55. CALORIMETRY CONDITIONS 20.1°C : 0% RH

Thermopile output represents the sum of radiated, convected and conducted heat loss detected by the gradient layer. The heat added to the ventilating air, i.e. the difference in temperature between inlet (T_2) and outlet (T_3) air is shown by the increment in air temperature trace. This value added to the thermopile output gives very closely the sensible or dry heat loss of the rat. The hygrometer output is the difference between inlet air moisture content and that of outlet. The hygrometer trace therefore represents the water added to the ventilating airstream by the rat under study irrespective of the background humidity, which in this case is 0% RH.

There is a sharp rise in thermopile output shortly after placing the rat within the calorimeter which reaches a maximum value 34 minutes after the start and returns to a baseline value 79 minutes after the start (Period A). A similar increase is seen in the hygrometer output which peaks at 38 minutes and returns to a baseline level 73 minutes after the start of the run. These increases corresponded to a period of vigorous exploratory movement by the rat on entering the calorimeter box. When activity ceased

Fig.55 NORMAL RAT : PARTITIONED HEAT LOSSES. 20.1° C AMBIENT TEMPERATURE LOW HUMIDITY CONDITIONS



the thermopile and hygrometer traces returned to a baseline value (Period B). This lack of movement continued from the 80th minute of the run up to the 124th minute when the rat was noted to stand up and start moving around the box. There was an immediate rise in thermopile and hygrometer outputs corresponding to this activity. A further period of rest and movement occurred with similar thermopile and hygrometer output changes to those seen in the first part of the run (Period C). Small fluctuations in the movement in air temperature trace occurred throughout the 180 minutes of the run without any definite pattern being observed.

Subsequent computation revealed that the total heat loss during Period A while the rat was active was 6.875 W/kg and during Period B when the rat was still, heat loss was less at 6.025 W/kg. Heat loss was at an intermediate value when the measurement Period C contained active and resting times, 6.63 W/kg. See Table 5. The partitioning of heat losses between sensible and insensible was affected by movement. Evaporative loss was slightly increased, by 0.22 W/kg when the rat was active. The heat losses during Period B represent the best approximation to measurement of true RME (resting metabolic energy expenditure) which it is possible to make in the

unanaesthetised rat. Rat training for calorimetry greatly reduces movement and exploratory behaviour during a run.

Nevertheless, it is essential to calculate heat loss (or later, heat production) from a visually selected time period when rat movement is least, preferably starting and ending the measurement when the traces indicate stable baseline values.

TABLE 5 (1) (See Fig. 55)

DATE 14/6/A	PERIOD A	PERIOD B	PERIOD C
RAT WEIGHT g		340.1	
ATMOSPHERIC PRESSURE mm Hg		767	
W.G.M. TEMPERATURE ^{o}C	23.434 $\pm$ 0.087	23.262 $\pm$ 0.120	23.067 $\pm$ 0.019
INLET AIR TEMPERATURE ^{o}C	20.123 $\pm$ 0.017	20.153 $\pm$ 0.002	20.148 $\pm$ 0.002
CALORIMETER WALL TEMPERATURE ^{o}C	21.262 $\pm$ 0.039	21.204 $\pm$ 0.062	21.173 $\pm$ 0.002
INLET/OUTLET AIRFLOW RATE L/min	0.686	0.694	0.697
TOTAL AIRFLOW RATE L/min	4.301	4.307	4.299
RECIRCULATED AIRFLOW RATE L/min	3.614	3.614	3.602
DEW POINT TEMPERATURE ^{o}C	6.481 $\pm$ 0.044	6.613 $\pm$ 0.059	6.667 $\pm$ 0.037
INCREMENT IN AIR TEMPERATURE ^{o}C	0.997 $\pm$ 0.066	0.918 $\pm$ 0.023	0.913 $\pm$ 0.004
URINE/FAECAL WEIGHT g	Nil	Nil	Nil
GROSS HEAT LOSS THROUGH WALLS W.	2.025	1.826	1.966
URINE HEAT LOSS W.	0	0	0
FAECAL HEAT LOSS W.	0	0	0

TABLE 5. (2) (See Fig. 55)

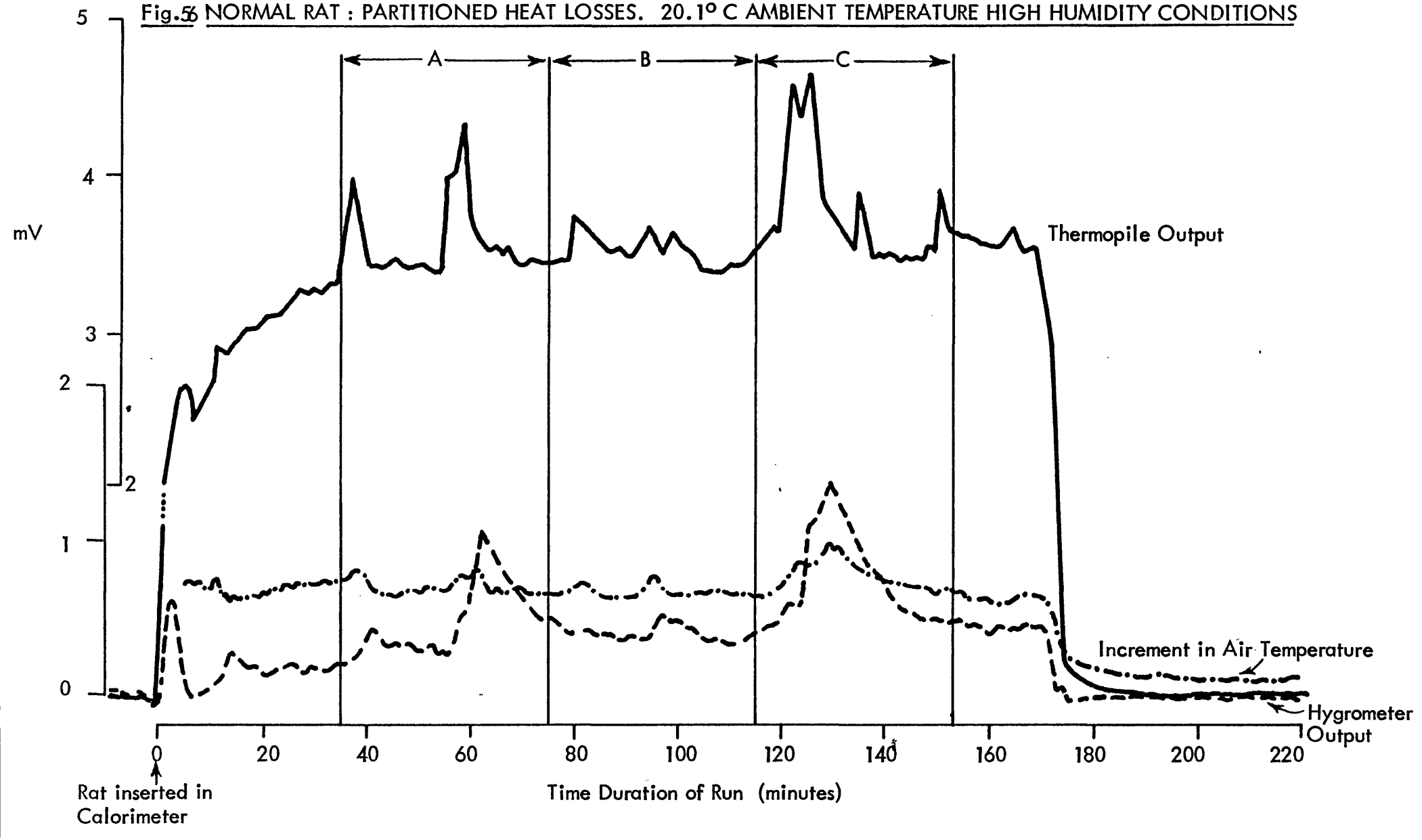
DATE 14/6/A	PERIOD A	PERIOD B	PERIOD C
SPEC. HEAT LOSS BY EXPIRED H_2O W.	2.527 $^{-03}$	1.575 $^{-03}$	2.342 $^{-03}$
NET HEAT LOSS THROUGH WALLS W.	2.022	1.824	1.964
HEAT LOSS TO AIRSTREAM W.	0.094	0.086	0.086
TOTAL DIRECT HEAT LOSS W.	2.338	2.049	2.255
PARTITIONED HEAT LOSS W/kg.			
SENSIBLE	6.221	5.619	6.027
INSENSIBLE	0.654	0.406	0.603
TOTAL	6.875	6.025	6.630
PARTITIONED HEAT LOSS % OF TOTAL			
SENSIBLE	90.5	93.3	90.9
INSENSIBLE	9.5	6.7	9.1

FIG. 56. CALORIMETRY CONDITIONS 20.1°C : 83% RH

In a more humid environment there was a slight decrease in the proportion of evaporative heat loss compared with that seen in a dry environment. (See Table 6). Rat movement in Period A did not alter the partitioning of heat losses from that seen in the 'quiet' Period B. Though slightly greater rat activity in Period C did increase the proportion of evaporative heat loss. The same close relationship was noted between increments in thermopile output and hygrometer output. In this case it is also possible to discern small rises in the increment in air temperature trace which corresponds to the increases in the other traces.

Thermopile output took more than 35 minutes from the start to reach a steady baseline value. It is possible that the occurrence of the first rat movement peak in thermopile output (between 36 - 42 minutes) interrupted this, and it may be that more than 50 minutes from the start is required before satisfactory baseline stability is seen on the thermopile output. Here the measurement of rat RME would be based only on the heat loss measured during Period B, 6.242 W/kg, which begins 75 minutes from the start during a period of baseline stability in the thermopile output. It may be noted that the

Fig.56 NORMAL RAT : PARTITIONED HEAT LOSSES. 20.1° C AMBIENT TEMPERATURE HIGH HUMIDITY CONDITIONS



hygrometer output trace is still falling when compared to its basal value in Period A. However when compared to the basal value seen in Periods B and C it has reached a minimum value at the 75th minute. For the most accurate results it is important to measure heat losses (and production later) when all traces are at a steady preferably basal value.

TABLE 6. (1) (See Fig. 56)

DATE 11/10/A	PERIOD A	PERIOD B	PERIOD C
RAT WEIGHT		272.9	
ATMOSPHERIC PRESSURE mm Hg		756	
W.G.M. TEMPERATURE ^{o}C	23.287 $\pm$ 0.033	23.430 $\pm$ 0.145	23.739 $\pm$ 0.104
INLET AIR TEMPERATURE ^{o}C	20.052 $\pm$ 0.002	20.064 $\pm$ 0.015	20.098 $\pm$ 0.023
CALORIMETER WALL TEMPERATURE ^{o}C	20.400 $\pm$ 0.049	20.428 $\pm$ 0.037	20.463 $\pm$ 0.065
INLET/OUTLET AIRFLOW RATE L/min	1.688	1.691	1.686
TOTAL AIRFLOW RATE L/min	5.040	4.902	4.854
RECIRCULATED AIRFLOW RATE L/min	3.352	3.211	3.168
DEW POINT TEMPERATURE ^{o}C	17.210 $\pm$ 0.012	17.216 $\pm$ 0.019	17.210 $\pm$ 0.016
INCREMENT IN AIR TEMPERATURE ^{o}C	0.896 $\pm$ 0.028	0.876 $\pm$ 0.027	1.026 $\pm$ 0.109
URINE/FAECAL WEIGHT g	0/0	0/0	0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.578	1.530	1.637
URINE HEAT LOSS W.	0	0	0
FAECAL HEAT LOSS W.	0	0	0

TABLE 6. (2) (See Fig. 56)

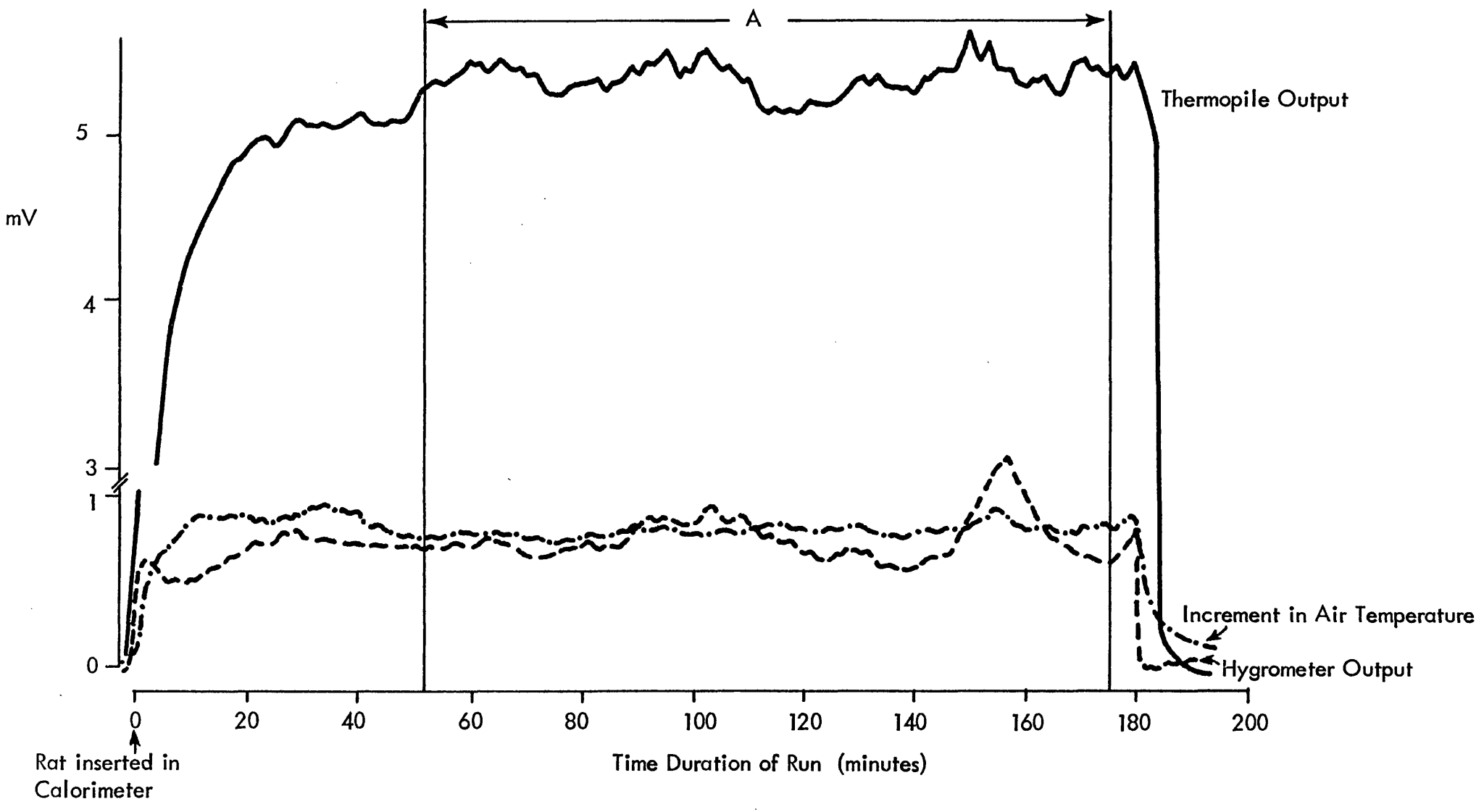
DATE 11/10/A	PERIOD A	PERIOD B	PERIOD C
SPEC. HEAT LOSS BY EXPIRED H_2O W.	0.943 -03	0.918 -03	1.538 -03
NET HEAT LOSS THROUGH WALLS W.	1.577	1.529	1.635
HEAT LOSS TO AIRSTREAM W.	0.099	0.095	0.110
TOTAL DIRECT HEAT LOSS W.	1.759	1.703	1.881
PARTITIONED HEAT LOSS W/kg			
SENSIBLE	6.144	5.950	6.396
INSENSIBLE	0.301	0.292	0.496
TOTAL	6.445	6.242	6.892
PARTITIONED HEAT LOSS % OF TOTAL			
SENSIBLE	95.3	95.3	92.8
INSENSIBLE	4.7	4.7	7.2

FIG. 57. CALORIMETRY CONDITIONS 20.1°C : 83% RH

The environmental conditions in this run are identical to those in Fig. 56, Table 6. The total heat loss measured over Period A was 7.23 W/kg. This is a higher value than that found in the previous run when the rat was inactive, 6.242 W/kg, and is comparable to an activity period in the previous run, 6.892 W/kg. The thermopile output in Fig. 57 does not display the clear rat activity peaks with corresponding changes in hygrometer output such as those in Figs. 55 and 56. Instead smaller 'flattened' peaks occur, at around 65 minutes, 94 minutes, 103 minutes and 152 minutes. Hygrometer output is similar but with a single larger peak around the 155th minute, like those associated with rat activity in the previous runs. There was no urination or defaecation by the rat under study during this or any of the preceding calorimeter runs.

The rat had a full thermistor harness in place (Fig. 40) during the run shown in Fig. 57 and its activity was therefore restricted. The rats studied in Figs. 55 and 56 were unfettered by thermistor attachments. In Fig. 57, at the 155th minute, the rat was noted to shift its position from the front to the rear of the cage. Relative lack of activity by the rat because of restraint by the thermistor harness does not result in a measurement of heat

Fig.57 NORMAL RAT : PARTITIONED HEAT LOSSES. 20.1° C AMBIENT TEMPERATURE HIGH HUMIDITY CONDITIONS



loss which reflects RME. The additional stress of the harness itself appears to cause an increased metabolic rate even in a fully trained tame rat which was accustomed to the calorimeter.

TABLE 7. (See Fig. 57)

DATE 10/10/A	PERIOD A
RAT WEIGHT g	309.7
ATMOSPHERIC PRESSURE mm Hg	760
W.G.M. TEMPERATURE ^{o}C	23.696 ± 0.118
INLET AIR TEMPERATURE ^{o}C	20.127 ± 0.023
CALORIMETER WALL TEMPERATURE ^{o}C	21.188 ± 0.065
INLET/OUTLET AIRFLOW RATE L/min	1.744
TOTAL AIRFLOW RATE L/min	5.266
RECIRCULATED AIRFLOW RATE L/min	3.522
DEW POINT TEMPERATURE ^{o}C	17.179 ± 0.010
INCREMENT IN AIR TEMPERATURE ^{o}C	0.845
URINE/FAECAL WEIGHT g	0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.997
URINE HEAT LOSS W.	0
FAECAL HEAT LOSS W.	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	1.667^{-03}
NET HEAT LOSS THROUGH WALLS W.	1.996
HEAT LOSS TO AIRSTREAM W.	0.098
TOTAL DIRECT HEAT LOSS W.	2.239
PARTITIONED HEAT LOSS W/kg	
SENSIBLE	6.761
INSENSIBLE	0.469
TOTAL	7.230
PARTITIONED HEAT LOSS % OF TOTAL	
SENSIBLE	93.5
INSENSIBLE	6.5

FIG. 58. CALORIMETRY CONDITIONS 30.1°C : 0% RH

Thermopile output is lower at 30°C than at 20°C. Heat loss during Period B, 5.175 W/kg (See Table 8) is 14% lower than the heat loss seen during an equivalent period (B) at 20.1°C in low relative humidity conditions, 6.025 W/kg (See Table 5). The increment in air temperature is also lower at 30°C. The hygrometer output was noted to be high from the moment the rat was sealed into the calorimeter. This is clearly different from the response of the hygrometer output seen in the earlier runs. The hygrometer output slowly fell from its initial peak to more normal basal values by the 50th minute. Thereafter the hygrometer output reflected two brief periods of rat movement during Period A and appeared to function normally during the period of rat inactivity, Period B. At the completion of the run it was found that the rat had passed 0.25 g of urine and 1.45 g of faeces. If this occurred immediately on the rat entering the calorimeter, it would explain the initial rise in hygrometer output. The total heat loss during Period A, 5.427 W/kg, was slightly higher than during Period B, 5.175 W/kg, largely due to the higher percentage of insensible heat loss (See Table 8) during Period A.

Fig. 58 NORMAL RAT : PARTITIONED HEAT LOSSES. 30.1° C AMBIENT TEMPERATURE LOW HUMIDITY CONDITIONS

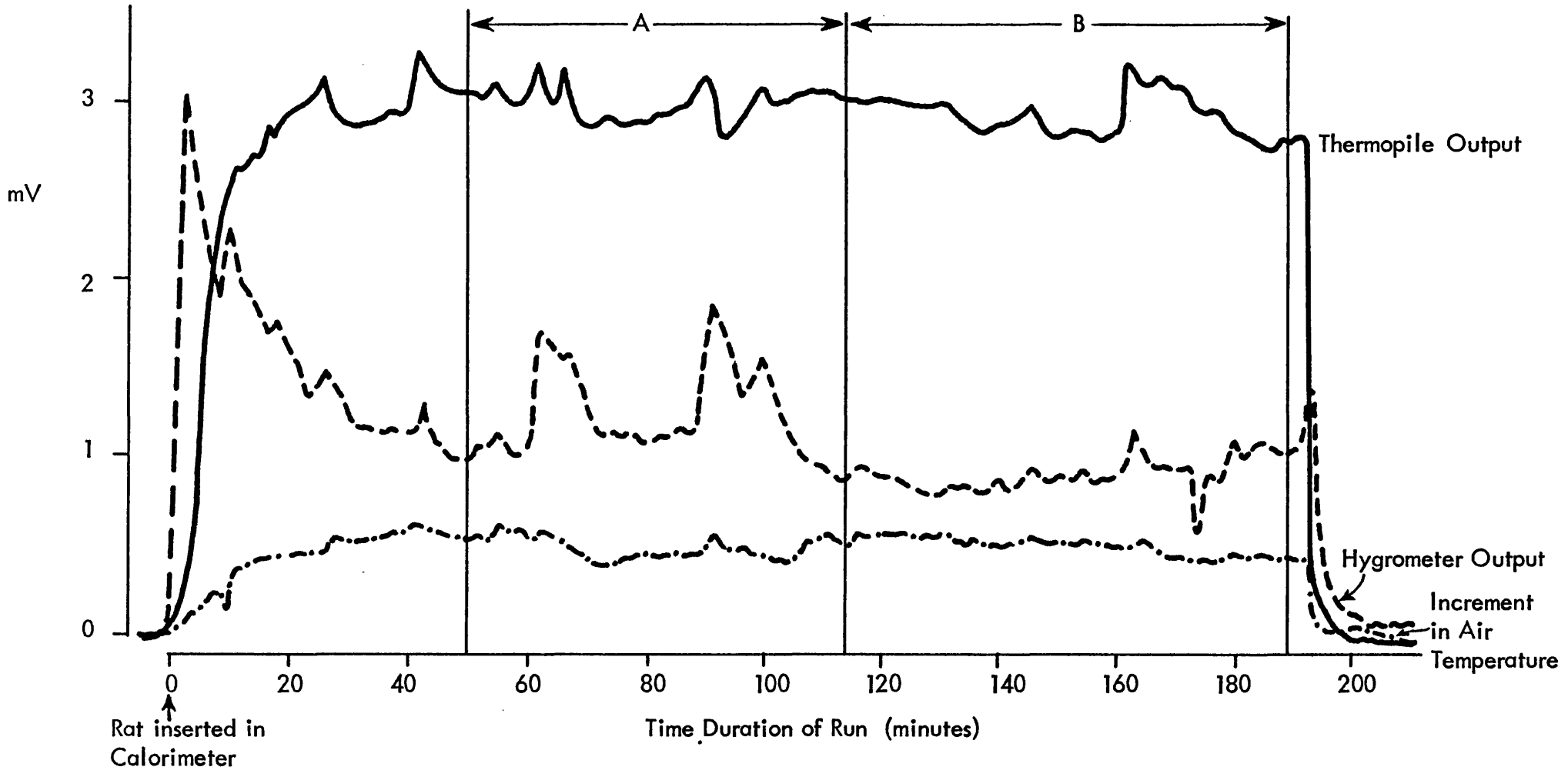


TABLE 8. (1)(See Fig. 58)

DATE 16/8/A	PERIOD A	PERIOD B
RAT WEIGHT g	323.75	
ATMOSPHERIC PRESSURE mm Hg	758	
W.G.M. TEMPERATURE ^{o}C	29.563 $\pm$ 0.060	29.543 $\pm$ 0.037
INLET AIR TEMPERATURE ^{o}C	30.107 $\pm$ 0.007	30.110 $\pm$ 0.010
CALORIMETER WALL TEMPERATURE ^{o}C	30.349 $\pm$ 0.040	30.390 $\pm$ 0.126
INLET/OUTLET AIRFLOW RATE L/min	1.648	1.650
TOTAL AIRFLOW RATE L/min	5.815	5.858
RECIRCULATED AIRFLOW RATE L/min	4.167	4.208
DEW POINT TEMPERATURE ^{o}C	6.994 $\pm$ 0.016	6.978 $\pm$ 0.031
INCREMENT IN AIR TEMPERATURE ^{o}C	0.654 $\pm$ 0.075	0.659 $\pm$ 0.062
URINE/FAECAL WEIGHT g	0.25/1.45	0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.410	1.393
URINE HEAT LOSS W.	1.471^{-03}	0
FAECAL HEAT LOSS W.	8.531^{-03}	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	1.100^{-03}	0.792^{-03}

TABLE 8. (2) (See Fig. 58)

DATE 16/8/A	PERIOD A	PERIOD B
NET HEAT LOSS THROUGH WALLS W.	1.399	1.392
HEAT LOSS TO AIRSTREAM W.	0.083	0.084
TOTAL DIRECT HEAT LOSS W.	1.757	1.675
PARTITIONED HEAT LOSS W/kg		
SENSIBLE	4.578	4.562
INSENSIBLE	0.849	0.612
TOTAL	5.427	5.175
PARTITIONED HEAT LOSS % OF TOTAL		
SENSIBLE	84.4	88.2
INSENSIBLE	15.6	11.8

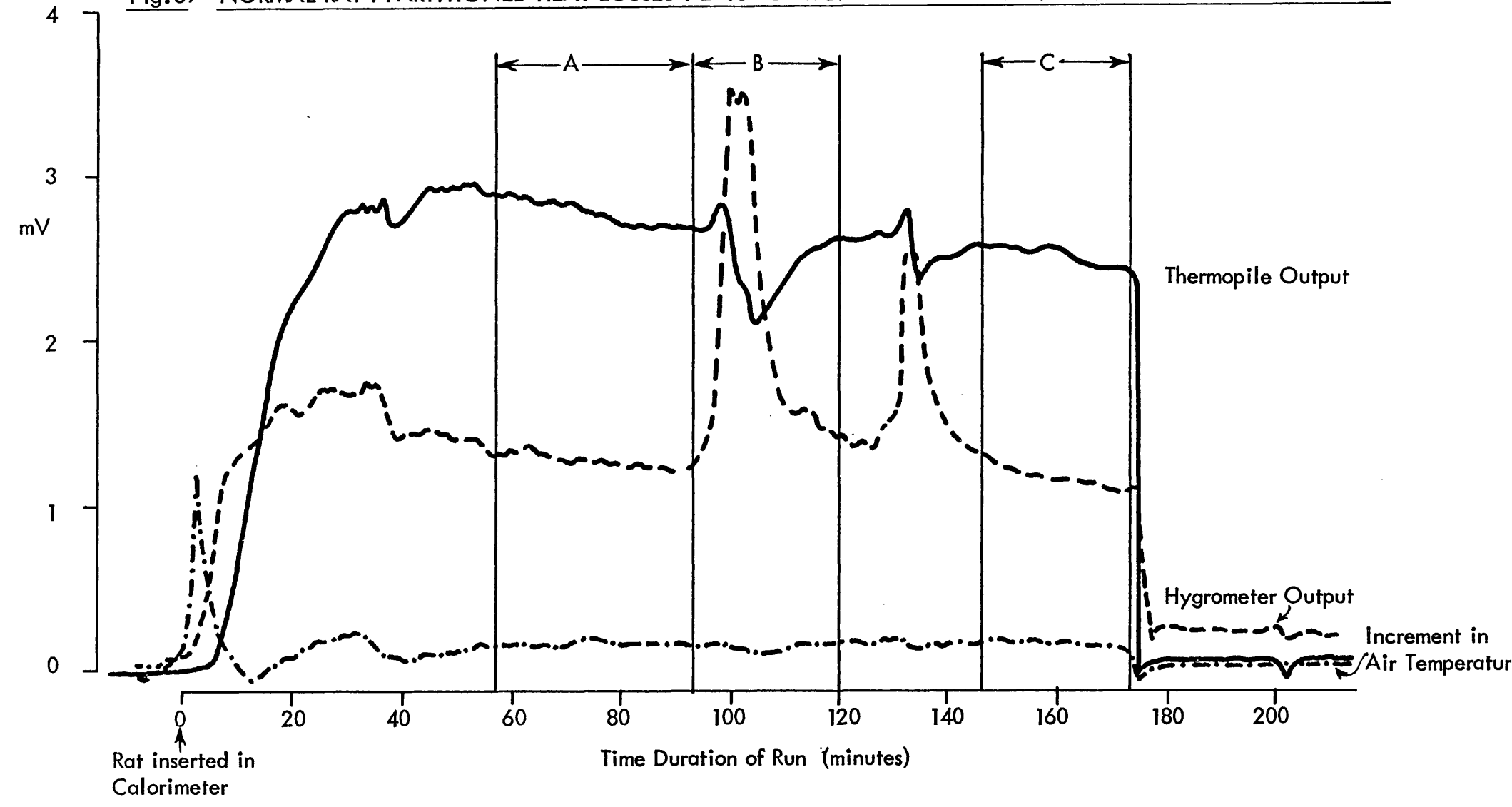
FIG. 59. CALORIMETRY CONDITIONS 29.6°C : 46% RH

In humid conditions at 30°C, sensible heat loss, 4.598 W/kg, is similar to that found in dry ambient conditions at 30°C, 4.562 W/kg (See Period A, Table 9 , and Period B, Table 8).

Insensible heat loss however is slightly increased and forms a greater proportion of the total heat loss (See Period A, Table 9) in humid conditions. There is also a slight reduction in the increment in air temperature (See Tables 8 and 9). It must be noted that during Period A virtually no rat movement occurs resulting in very little fluctuation in the thermopile output, hygrometer output, and heat added to the ventilating air.

There is a noticeable and progressive decrease in heat loss during the run between Period A, 5.42 W/kg, and Period C, 5.125 W/kg. As there was no change in the weight of the urine collecting tray between the start and finish of the calorimetry run it appeared that the rat had not passed urine or faeces during the run. However there was a marked increase in the hygrometer output during Period B associated with an initial small rise then larger fall in thermopile output. This pattern was repeated at 130 minutes,

Fig. 59 NORMAL RAT : PARTITIONED HEAT LOSSES . 29.6°C AMBIENT TEMPERATURE . HIGH HUMIDITY CONDITIONS



and is characteristic of rat urination during the run. Urine at rat core temperature causes the small initial rise in thermopile output as it is passed into the collecting tray, then as it evaporates in the high airflow conditions around the rat it takes heat from its surroundings producing the characteristic fall in thermopile output with an increase in hygrometer output at the same time. When the evaporative heat loss increases are due to rat activity as in Period A, Table 8, these are accompanied by a rise in thermopile output, not a fall.

This calorimetry run and the preceding run were carried out prior to the modification of the rat urine collecting tray as shown in Fig. 50. The addition of the oil filled floor pan eliminated this type of urine evaporation during calorimetry.

Thermometry was carried out on the rat studied in Fig.59 but not in the rat studied in Fig. 58.

TABLE 9. (1)(See Fig. 59)

DATE 3/9/A	PERIOD A	PERIOD B	PERIOD C
RAT WEIGHT g		291.5	
ATMOSPHERIC PRESSURE mm Hg		757	
W.G.M. TEMPERATURE ^{o}C	23.989 ± 0.049	24.042 ± 0.077	24.147 ± 0.043
INLET AIR TEMPERATURE ^{o}C	29.584 ± 0.008	29.591 ± 0.006	29.620 ± 0.004
CALORIMETER WALL TEMPERATURE ^{o}C	30.109 ± 0.030	30.111 ± 0.019	30.113 ± 0.011
INLET/OUTLET AIRFLOW RATE L/min	1.035	1.036	1.042
TOTAL AIRFLOW RATE L/min	5.894	5.905	5.932
RECIRCULATED AIRFLOW RATE L/min	4.859	4.869	4.889
DEW POINT TEMPERATURE ^{o}C	17.152 ± 0.007	17.166 ± 0.006	17.186 ± 0.007
INCREMENT IN AIR TEMPERATURE ^{o}C	0.361 ± 0.019	0.333 ± 0.031	0.364 ± 0.023
URINE/FAECAL WEIGHT g	0/0	0/0	0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.294	1.190	1.228
URINE HEAT LOSS W.	0	0	0
FAECAL HEAT LOSS W.	0	0	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	1.109^{-03}	1.817^{-03}	1.008^{-03}

TABLE 9. (2) (See Fig. 59)

DATE 3/9/A	PERIOD A	PERIOD B	PERIOD C
NET HEAT LOSS THROUGH WALLS W.	1.293	1.188	1.227
HEAT LOSS TO AIRSTREAM W.	0.047	0.043	0.048
TOTAL DIRECT HEAT LOSS W.	1.580	1.623	1.494
PARTITIONED HEAT LOSS W/kg			
SENSIBLE	4.598	4.225	4.373
INSENSIBLE	0.822	1.343	0.752
TOTAL	5.420	5.568	5.125
PARTITIONED HEAT LOSS % OF TOTAL			
SENSIBLE	84.8	75.9	85.3
INSENSIBLE	15.2	24.1	14.7

FIG. 60. CALORIMETRY CONDITIONS 20.1°C : 82% RH

This run was carried out to correlate by continuous direct observation, movement of the rat within SEC - A - 04 L, with thermopile output and hygrometer output. There appeared to be a 1 - 2 minute delay between movement and response in thermopile output and an approximately 4 minute delay in response in the hygrometer output.

The rat studied in this run was completely untrained and the movement pattern of this animal as indicated by the thermopile output during this run may be compared with that seen with a trained and completely tame rat in Fig. 56.

Fig.60 NORMAL RAT : PARTITIONED HEAT LOSSES. 20.1° C AMBIENT TEMPERATURE HIGH HUMIDITY CONDITIONS
THE EFFECT OF MOVEMENT ON THERMOPILE AND HYGROMETER OUTPUT

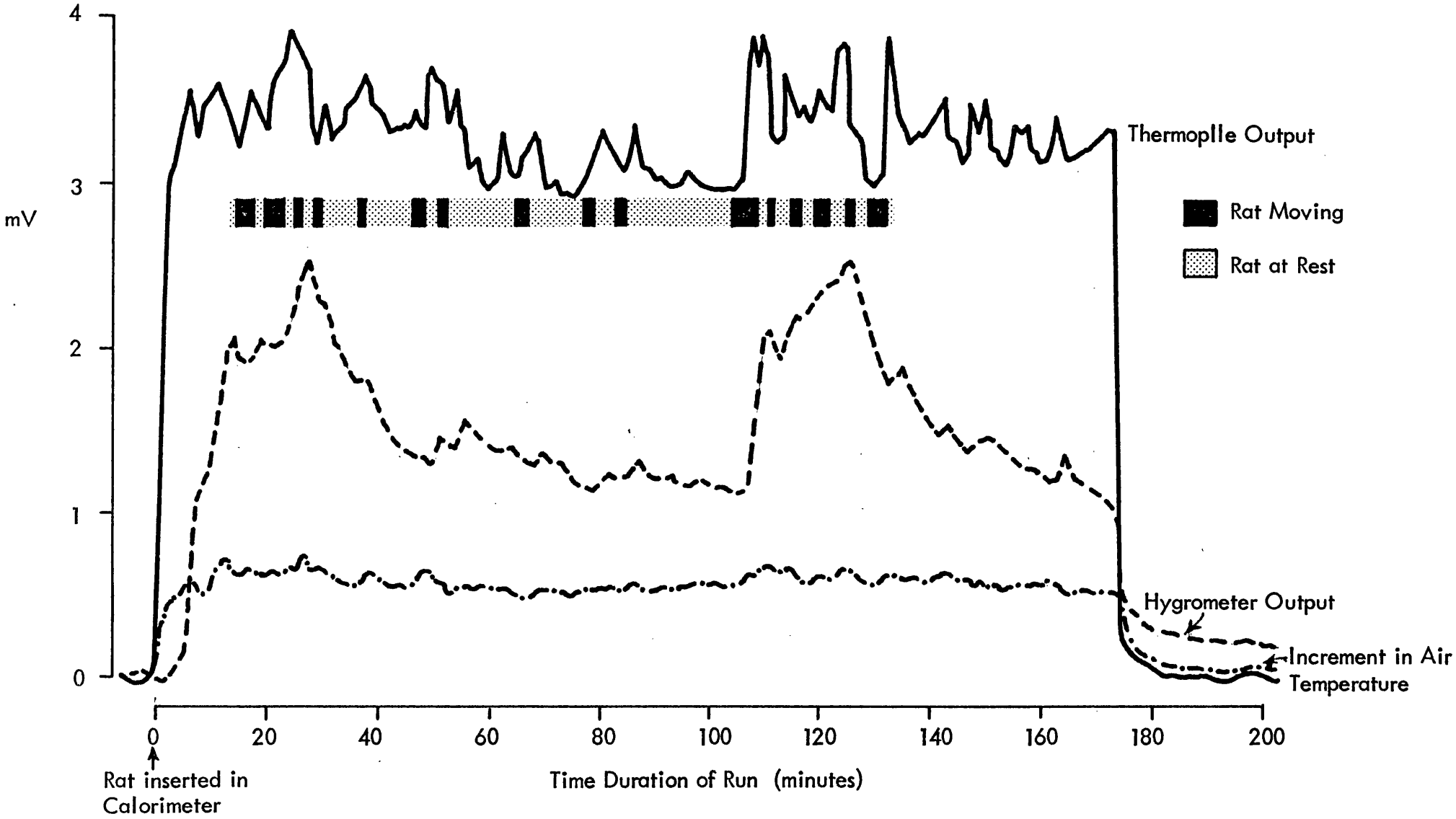
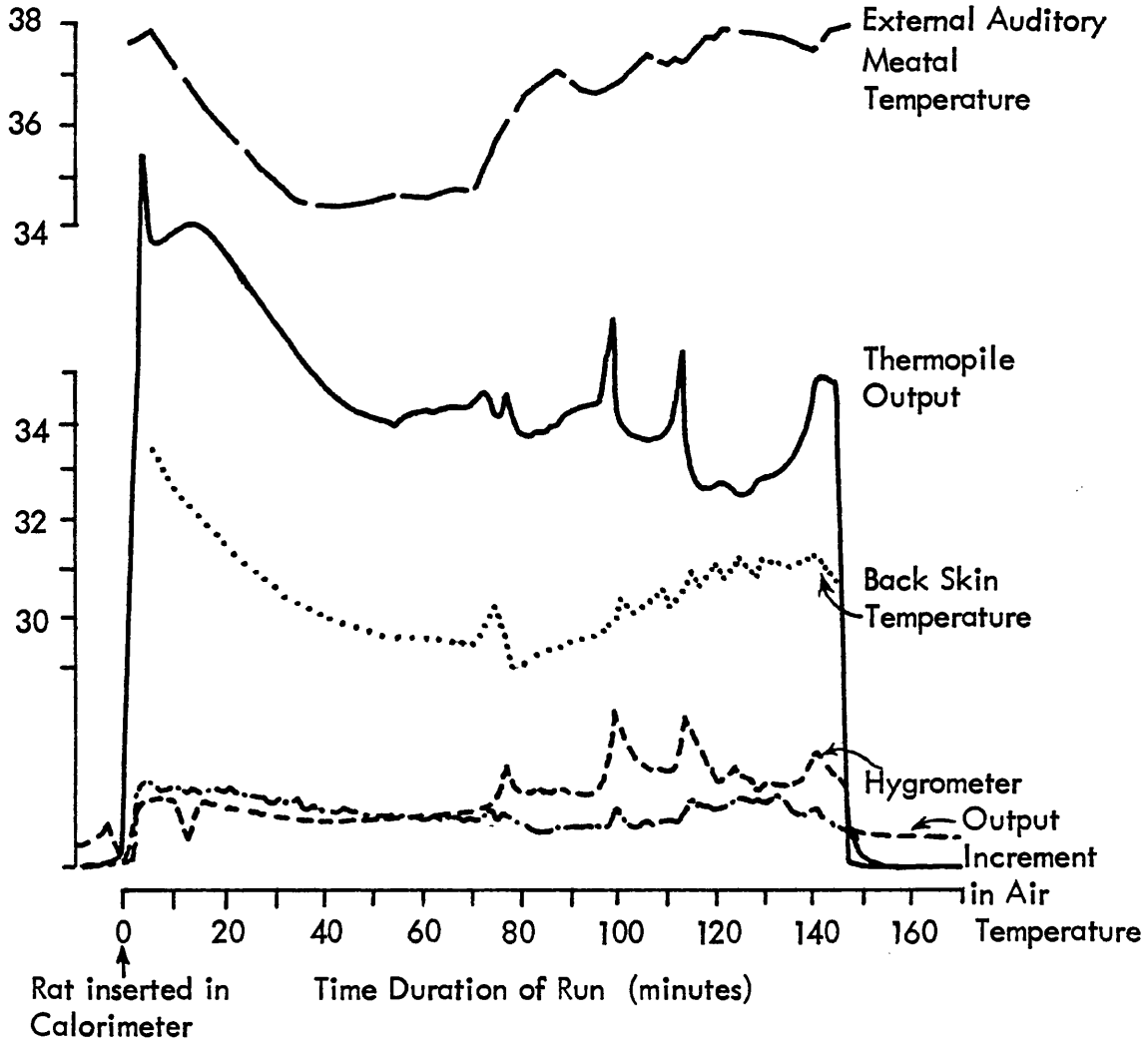


FIG. 61. CALORIMETRY CONDITIONS 20.1°C : 0% RH

0.15 mg of Nembutal was administered intraperitoneally to a study rat. This is sufficient to cause deep anaesthesia within 10 minutes. When the rat was unconscious, it was inserted into SEC - A - 04 L, and calorimetry and thermometry carried out until the rat recovered from the anaesthetic. Total ventilatory air flow rate was 6.80 l/min, inlet/outlet flow rate 0.95 l/min. During deep anaesthesia thermoregulation is impaired and the rat showed marked body cooling and fall in body heat content over the first 50 - 60 minutes of the run. The rat lay inert in one position and did not move until the 72nd minute. The first indication of recovery of thermoregulation was seen at 35 minutes after the start when the fall in external auditory meatus temperature halted and an increase in ear temperature occurred. This was slow at first (between 35 minutes and 70 minutes), but later rose sharply as movement occurred (between 70 minutes and 120 minutes). Back skin temperature was slower to respond, the first change from a steady fall in temperature not occurring until 70 minutes.

Hygrometer output and the increment in air temperature did not increase till the 72nd minute

Fig.61 ANAESTHETISED RAT : PARTITIONED HEAT LOSSES. 20.1° C AMBIENT TEMPERATURE . LOW HUMIDITY CONDITIONS



of the run when the first rat movement occurred.

At 47 minutes there was a change in thermopile output, 12 minutes after the first rise in external ear temperature. Though a downward drift in baseline occurred in thermopile output until the 125th minute, typical activity related peaks in the tracing were seen during this period as the rat recovered consciousness and began moving and turning.

The rat appeared uncoordinated and disorientated upon removal from the calorimeter. Though fully awake it moved jerkily and occasionally fell over onto its side. The effect of the Nembutal clearly persisted.

FIG. 62. CALORIMETRY CONDITIONS 20°C : 87% RH

Oxygen and carbon dioxide analyer function was assessed in this run. Heat production was computed using a modified form of Weir's equation (See appendix 6) but the oxygen and carbon dioxide analyser outputs are presented as they were charted on the Foster 12 channel recorder to illustrate their response characteristics in relation to body temperature measurements, thermopile and hygrometer outputs, and increments in air temperature (Fig. 63) The first 70 minutes of this run are not shown. This was regarded as an unstable settling in period prior to starting measurement of oxygen consumption, carbon dioxide production and thermometry. Despite prior training the rat never ceased moving around the cage at regular intervals of 20 - 30 minutes throughout the run. Calorimetry was continued for 250 minutes. Because of the 5 metre length of tubing between SEC - A - 04 L and the gas analyser, with the ventilatory air flow rates used in this and subsequent runs, there is a delay of approximately 1.75 minutes between changes in rat body temperatures and thermopile output, and the gas analyser's responses. When this delay is taken into account, there is a close correlation between changes in external ear temperature and heat production as indicated by the oxygen consumption trace. Base of tail temperature and

Fig. 62 20°C CONTROL RAT CALORIMETRY 320 MINUTE DURATION RUN : HIGH HUMIDITY: EFFECT OF CONTINUOUS MOVEMENT ON THERMOMETRY AND HEAT PRODUCTION

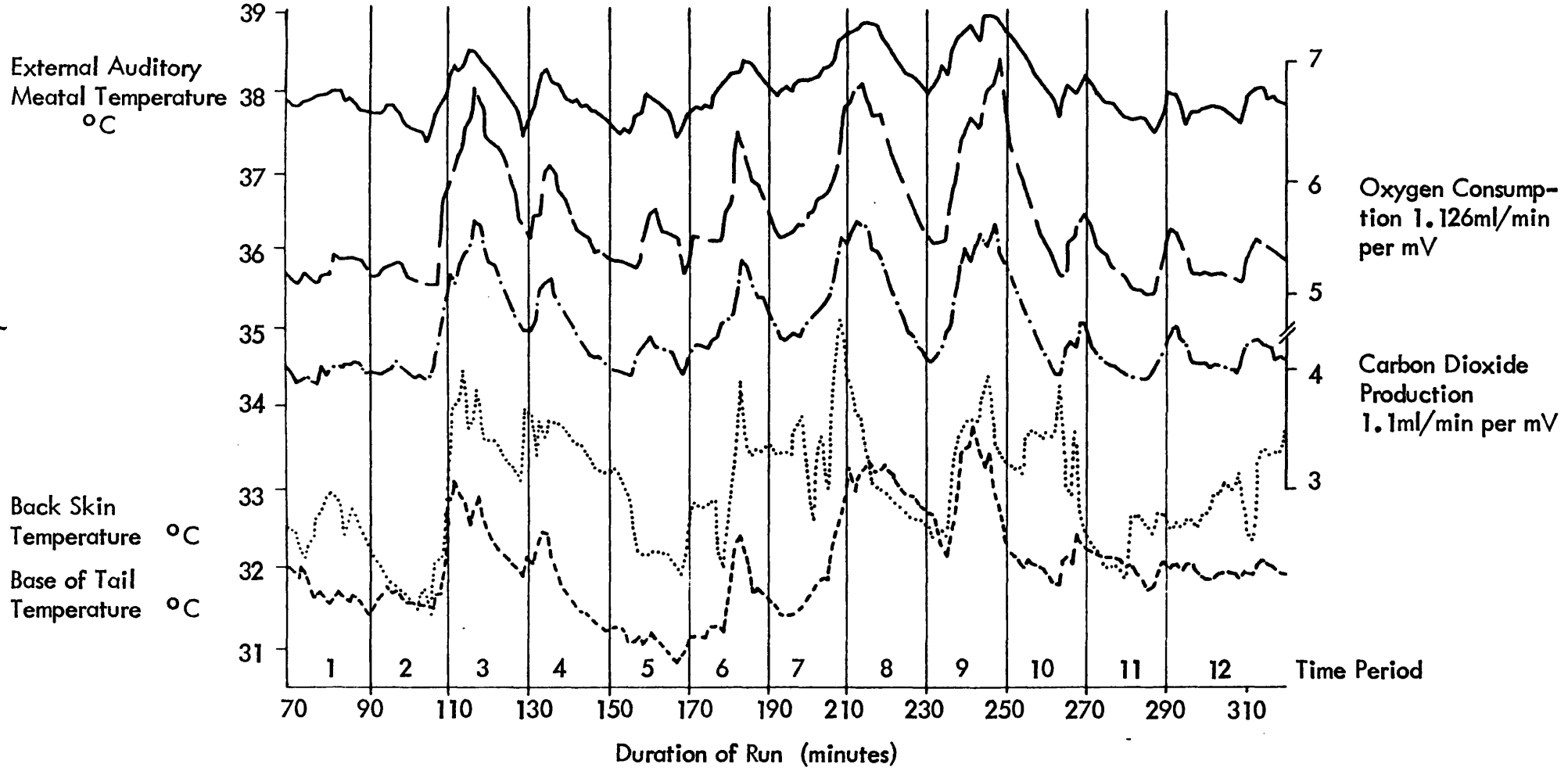
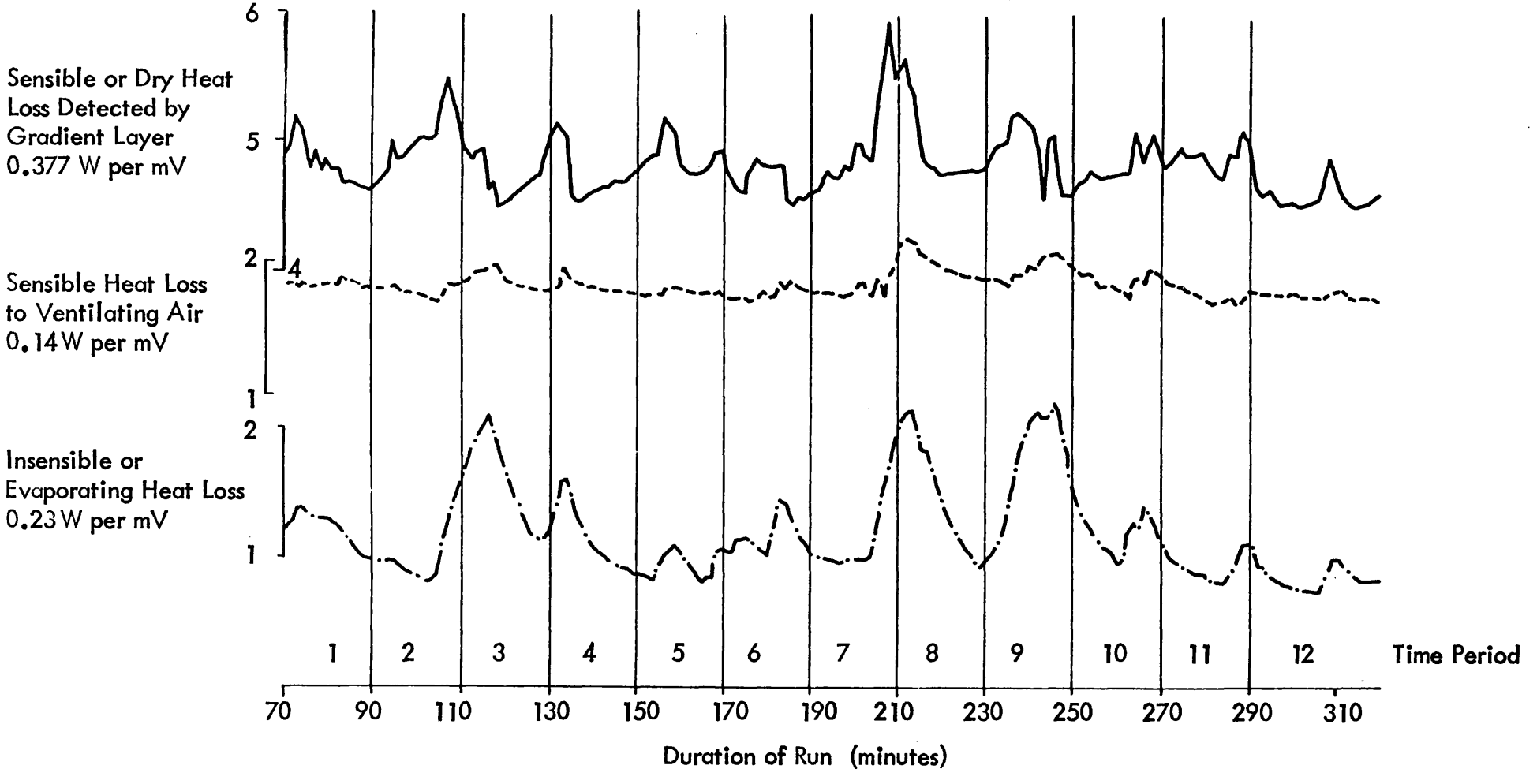


Fig.63 20° C CONTROL RAT CALORIMETRY 320 MINUTE DURATION RUN : HIGH HUMIDITY : EFFECT OF CONTINUOUS MOVEMENT ON HEAT LOSS



external ear temperature show very similar patterns throughout all of the run. Back skin temperature does not follow changes in heat production so closely. The 250 minute duration of the run was divided into 11 periods of 20 minutes and a final one of 30 minutes. Figure 63 shows the heat loss partitioning and the time divisions.

TABLE 10 gives the values for sensible and insensible heat loss and heat production for each period. The difference between heat production and heat loss for each time period is shown in Fig. 64 together with the calculated respiratory quotient (RQ) for each period. When the rat is intermittently moving, as in this run, then cyclic changes in heat production and heat loss take place. Changes in body heat content reflect the differences in heat storage or release which occur during each time period. Any single measurement period of 20 minutes may give a poor correlation between heat production and loss. When the animal is still during a run then a 40 minute measurement period would be a minimum time to assess RME. Where rat movement is continuous, a much longer time period would be required. With this run, it would not be possible to determine true RME even over the 250 minute time period. Agreement between direct and indirect calorimetry over the entire run, despite a persistently active rat, is better than 5% difference, with heat loss exceeding heat production. This also takes into account 0.6 g of urine and 2.0 g of faecal material passed by the rat during the run. Respiratory quotient does not vary significantly from period to period and is consistent with a post-absorbtive rat at 0.73 overall.

Fig.64 20 °C CONTROL RAT CALORIMETRY 320min. DURATION RUN. % DIFFERENCE BETWEEN DIRECT-INDIRECT CALORIMETRY AND R.Q. THROUGHOUT THE RUN

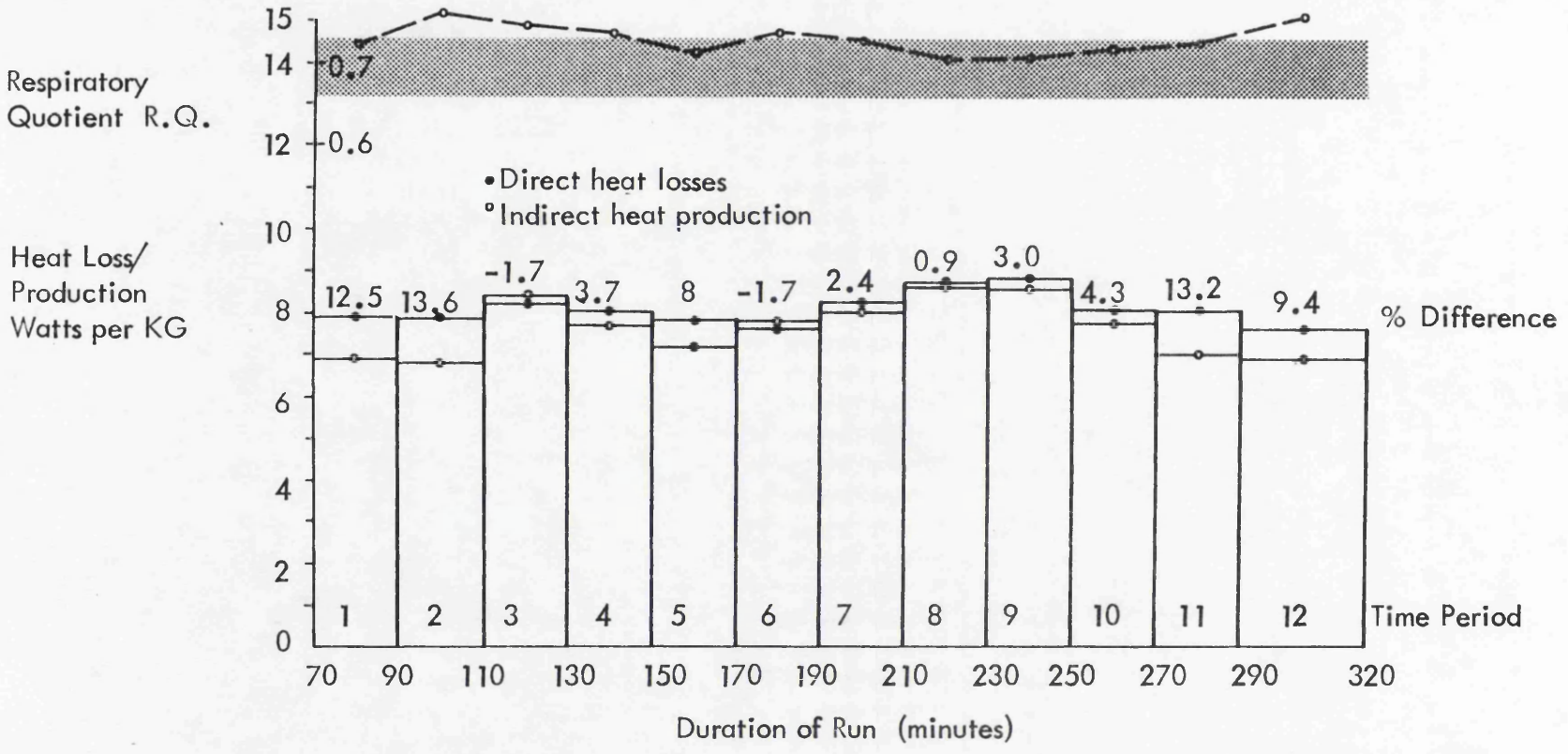


TABLE 10 A (1) (See Figs. 62, 63, 64).

DATE 27/8/A	ENTIRE RUN	PERIOD 1	PERIOD 2	PERIOD 3
RAT WEIGHT g.	289.5	292	291.5	290.0
ATMOSPHERIC PRESSURE mm Hg	759	759	759	759
W.G.M. TEMPERATURE ^{o}C	25.411 $\pm$ 0.232	25.135	25.187	25.186
INLET AIR TEMPERATURE ^{o}C	20.442 $\pm$ 0.018	20.429	20.424	20.445
CALORIMETER WALL TEMPERATURE ^{o}C	20.825 $\pm$ 0.040	20.826	20.842	20.787
INLET/OUTLET AIRFLOW RATE L/min	1.060	1.062	1.058	1.059
TOTAL AIRFLOW RATE L/min	5.687	5.646	5.655	5.657
RECIRCULATED AIRFLOW RATE L/min	4.626	4.584	4.598	4.598
DEW POINT TEMPERATURE ^{o}C	17.617 $\pm$ 0.034	17.550	17.570	17.601
INCREMENT IN AIR TEMPERATURE ^{o}C	1.797 $\pm$ 0.11	1.82	1.78	1.96
URINE/FAECAL WEIGHT g.	0.6/2.0	0.038/0.125	0.038/0.125	0.038/0.125
GROSS HEAT LOSS THROUGH WALLS W.	1.821	1.799	1.866	1.785
URINE HEAT LOSS W.	2.048 $^{-03}$	0.512 $^{-03}$	0.513 $^{-03}$	0.509 $^{-03}$
FAECAL HEAT LOSS W.	6.826 $^{-03}$	1.707 $^{-03}$	1.709 $^{-03}$	1.698 $^{-03}$
SPEC. HEAT LOSS BY EXPIRED H_2O.W.	3.027 $^{-03}$	3.254 $^{-03}$	2.404 $^{-03}$	4.222 $^{-03}$

TABLE 10 A (2) (See Figs. 62, 63, 64).

DATE 27/8/A	ENTIRE RUN	PERIOD 1	PERIOD 2	PERIOD 3
NET HEAT LOSS THROUGH WALLS W.	1.809	1.793	1.862	1.779
HEAT LOSS TO AIRSTREAM W.	0.226	0.227	0.223	0.245
TOTAL DIRECT HEAT LOSS W.	2.304	2.310	2.297	2.403
PARTITIONED HEAD LOSS W/kg. SENSIBLE	7.029	6.921	7.151	6.980
INSENSIBLE	0.927	0.989	0.730	1.306
TOTAL	7.957	7.910	7.880	8.286
PARTITIONED HEAT LOSS % OF TOTAL SENSIBLE	88.3	87.5	90.7	84.2
INSENSIBLE	11.7	12.5	9.3	15.8
INDIRECT HEAT PRODUCTION CO_2 PRODUCTION ml/min	4.801	4.399	4.500	5.450
O_2 CONSUMPTION ml/min	6.605	6.099	5.934	7.330
TOTAL HEAT PRODUCTION W.	2.192	2.021	1.985	2.443
HEAT PRODUCTION W/kg	7.572	6.922	6.810	8.424
∴ RESPIRATORY QUOTIENT	0.727	0.721	0.758	0.744
% DIFFERENCE : HEAT LOSS - HEAT PRODUCTION	+ 4.841	+ 12.485	+ 13.578	- 1.660

TABLE 10 B (1) (See Figs. 62, 63, 64).

DATE 27/8/A	PERIOD 4	PERIOD 5	PERIOD 6	PERIOD 7	PERIOD 8
RAT WEIGHT g.	289.5	289.0	288.5	288.0	287.5
ATMOSPHERIC PRESSURE mm Hg.	759	759	759	759	759
W.G.M. TEMPERATURE ^{o}C	25.183	25.254	25.295	25.360	25.455
INLET AIR TEMPERATURE ^{o}C	20.430	20.425	20.435	20.410	20.440
CALORIMETER WALL TEMPERATURE ^{o}C	20.777	20.862	20.823	20.850	20.840
INLET/OUTLET AIRFLOW RATE L/min	1.059	1.059	1.058	1.060	1.062
TOTAL AIRFLOW RATE L/min	5.657	5.689	5.676	5.665	5.680
RECIRCULATED AIRFLOW RATE L/min	4.598	4.630	4.618	4.605	4.618
DEW POINT TEMPERATURE ^{o}C	17.622	17.643	17.624	17.623	17.622
INCREMENT IN AIR TEMPERATURE ^{o}C	1.80	1.72	1.74	1.80	1.98
URINE/FAECAL WEIGHT g.	0.038/0.125	0.038/0.125	0.038/0.125	0.038/0.125	0.038/0.125
GROSS HEAT LOSS THROUGH WALLS W.	1.829	1.844	1.755	1.905	1.891
URINE HEAT LOSS W.	0.512^{-03}	2.056^{-03}	2.053^{-03}	2.052^{-03}	2.037^{-03}
FAECAL HEAT LOSS W.	1.708^{-03}	6.852^{-03}	6.844^{-03}	6.841^{-03}	6.789^{-03}
SPEC. HEAT LOSS BY EXPIRED H_2O.W.	3.072^{-03}	2.476^{-03}	2.804^{-03}	2.985^{-03}	4.306^{-03}

TABLE 10 B (2) (See Figs. 62, 63, 64).

DATE 27/8/A	PERIOD 4	PERIOD 5	PERIOD 6	PERIOD 7	PERIOD 8
NET HEAT LOSS THROUGH WALLS W.	1.824	1.833	1.743	1.893	1.878
HEAT LOSS TO AIRSTREAM W.	0.225	0.217	0.219	0.226	0.249
TOTAL DIRECT HEAT LOSS W.	2.322	2.268	2.209	2.383	2.514
PARTITIONED HEAT LOSS W/kg. SENSIBLE	7.080	7.092	6.799	7.356	7.398
INSENSIBLE	0.941	0.755	0.858	0.917	1.345
TOTAL	8.020	7.847	7.657	8.273	8.743
PARTITIONED HEAT LOSS % OF TOTAL SENSIBLE	88.3	90.4	88.8	88.9	84.6
INSENSIBLE	11.7	9.6	11.2	11.1	15.4
INDIRECT HEAT PRODUCTION CO_2 PRODUCTION ml/min	4.934	4.494	4.974	5.094	5.321
O_2 CONSUMPTION ml/min	6.727	6.306	6.751	7.005	7.547
TOTAL HEAT PRODUCTION W.	2.236	2.085	2.246	2.325	2.491
HEAT PRODUCTION W/kg.	7.725	7.215	7.785	8.073	8.664
∴ RESPIRATORY QUOTIENT	0.733	0.713	0.737	0.727	0.705
% DIFFERENCE : HEAT LOSS - HEAT PRODUCTION	+ 3.684	+ 8.046	- 1.673	+ 2.423	+ 0.899

TABLE 10 C (1) (See Figs. 62, 63, 64).

DATE 27/8/A	PERIOD 9	PERIOD 10	PERIOD 11	PERIOD 12
RAT WEIGHT g.	287.0	286.5	286.0	285.5
ATMOSPHERIC PRESSURE mm Hg	729	759	759	759
W.G.M. TEMPERATURE ^{o}C	25.550	25.658	25.718	25.765
INLET AIR TEMPERATURE ^{o}C	20.455	20.459	20.459	20.469
CALORIMETER WALL TEMPERATURE ^{o}C	20.799	20.835	20.864	20.806
INLET/OUTLET AIRFLOW RATE L/min	1.062	1.063	1.062	1.063
TOTAL AIRFLOW RATE L/min	5.707	5.695	5.734	5.730
RECIRCULATED AIRFLOW RATE L/min	4.646	4.632	4.672	4.667
DEW POINT TEMPERATURE ^{o}C	17.600	17.640	17.672	17.622
INCREMENT IN AIR TEMPERATURE ^{o}C	1.94	1.780	1.660	1.613
URINE/FAECAL WEIGHT g.	0.038/0.125	0.038/0.125	0.038/0.125	0.038/0.125
GROSS HEAT LOSS THROUGH WALLS W.	1.899	1.808	1.883	1.780
URINE HEAT LOSS W.	2.037^{-03}	2.047^{-03}	2.055^{-03}	2.057^{-03}
FAECAL HEAT LOSS W.	6.791^{-03}	6.824^{-03}	6.851^{-03}	6.857^{-03}
SPEC. HEAT LOSS BY EXPIRED H_2O.W.	4.694^{-03}	3.429^{-03}	2.696^{-03}	2.488^{-03}

TABLE 10 C (2) (See Figs. 62, 63, 64).

DATE 27/8/A	PERIOD 9	PERIOD 10	PERIOD 11	PERIOD 12
NET HEAT LOSS THROUGH WALLS W.	1.885	1.796	1.871	1.769
HEAT LOSS TO AIRSTREAM W.	0.245	0.224	0.211	0.205
TOTAL DIRECT HEAT LOSS W.	2.551	2.324	2.319	2.192
PARTITIONED HEAT LOSS W/kg. SENSIBLE	7.423	7.051	7.279	6.913
INSENSIBLE	1.466	1.061	0.829	0.765
TOTAL	8.889	8.113	8.109	7.678
PARTITIONED HEAT LOSS % OF TOTAL SENSIBLE	83.5	86.9	89.8	90
INSENSIBLE	16.5	13.1	10.2	10
INDIRECT HEAT PRODUCTION CO_2 PRODUCTION ml/min	5.298	4.809	4.398	4.522
O_2 CONSUMPTION ml/min	7.498	6.723	6.071	5.928
TOTAL HEAT PRODUCTION W.	2.476	2.225	2.014	1.985
HEAT PRODUCTION W/kg.	8.626	7.765	7.040	6.954
RESPIRATORY QUOTIENT	0.707	0.715	0.724	0.763
% DIFFERENCE : HEAT LOSS - HEAT PRODUCTION	+ 2.953	+ 4.283	+ 13.177	+ 9.430

TABLE 11 shows data from a further 16 calorimetry runs on 2 normal rats carried out without instrument leads attachments. Both rats were tame and fully adapted to frequent calorimetry runs. There was little rat movement while in the calorimeter.

The mean difference between heat loss and heat production in these runs was 2.4 $\pm$ 1.8 (SD) %. This lies within the expected accuracy limits of the calorimeter design and the assumptions made in the calculation of heat production. Further details of the runs described in Table 11 are given in Figs. 148 and 154.

This was regarded as satisfactory calorimeter performance.

TABLE 11 (1) CONTROL RAT CALORIMETRY : NON INSTRUMENTED RATS

RAT NO. 1.

RUN NO.	PERCENT DIFFERENCE DIRECT - INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION/ DEFAECATION	TOTAL DURATION CALORIMETRY (min)
3	2.573	0.683	80	-	160
7	4.917	0.704	90	-	155
11	1.535	0.713	100	-	170
18	1.461	0.659	90	-	140
24	0.318	0.681	50	+	140
32	1.427	0.681	90	-	150
37	2.669	0.667	90	+	170
43	5.181	0.683	80	+	145
46	0.543	0.706	90	-	145
48	3.453	0.703	90	+	170

TABLE 11 (2) CONTROL RAT CALORIMETRY : NON INSTRUMENTED RATS

RAT. NO. 8.

RUN NO.	PERCENT DIFFERENCE DIRECT - INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION/ DEFAECATION	TOTAL DURATION CALORIMETRY (min)
5	4.630	0.690	80	-	170
14	3.625	9.697	80	-	170
20	3.057	0.677	60	-	140
27	0.561	0.675	100	-	150
34	- 0.863	0.676	100	-	160
44	3.314	0.736	100	-	180

Mean difference DIRECT - INDIRECT ..... + 2.399 $\pm$ 1.776 (SD) %

Mean Respiratory Quotient ..... 0.689 $\pm$ 0.20 (SD)

Average Measurement Period ..... 86 minutes

Average Total Duration Calorimetry .... 157 minutes

Rat thermometry studies to determine changes in body heat content and tissue heat flux were made in a number of rats subjected to calorimetry at 20°C and 30°C ambient temperature conditions.

FIGURE 65 shows the variation in rat external auditory meatus, back skin and base of tail temperatures during calorimetry carried out under standard conditions. Outlet air flows were in the range 1 - 1.5 l/min with total airflows of 6 - 6.5 l/min. These settings are similar to those used in the control and burned rat calorimetry runs which are described subsequently. The inlet air temperatures and calorimeter wall temperatures are also shown. During the first 50 minutes auditory meatus temperature fell by approximately 1°C. The changes in back skin temperature and tail temperature are less marked. There is no further decrease in auditory meatus temperature after the first 50 minutes of the run. It is likely that core temperature was slightly elevated at the start as a result of rat movement at the time of insertion of the external ear thermistor, rather than core temperature falling due to rat cooling during calorimetry. Indeed after 50 minutes there was a slight increase in skin and tail temperature during the run. There was no evidence therefore of a significant fall in body heat content caused by the airflow conditions within the calorimeter at 20°C ambient temperature in normal rats. This was also true for control rats studied in the calorimeter at 30°C ambient conditions (Fig. 66).

Fig. 65 20°C CONTROL RAT CALORIMETRY : BODY AND CALORIMETER TEMPERATURE CHANGES WITH TIME DURING CALORIMETRY

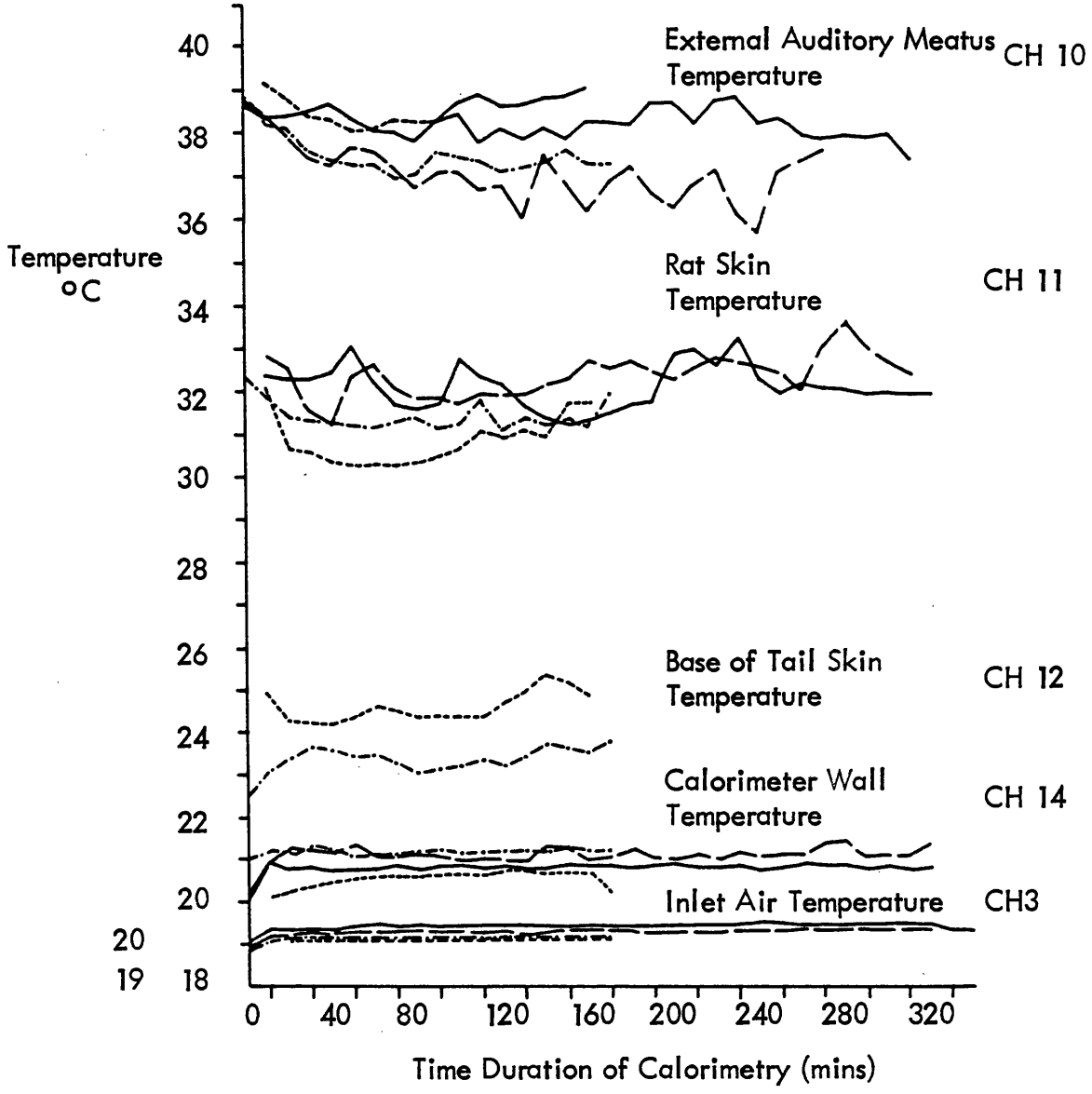
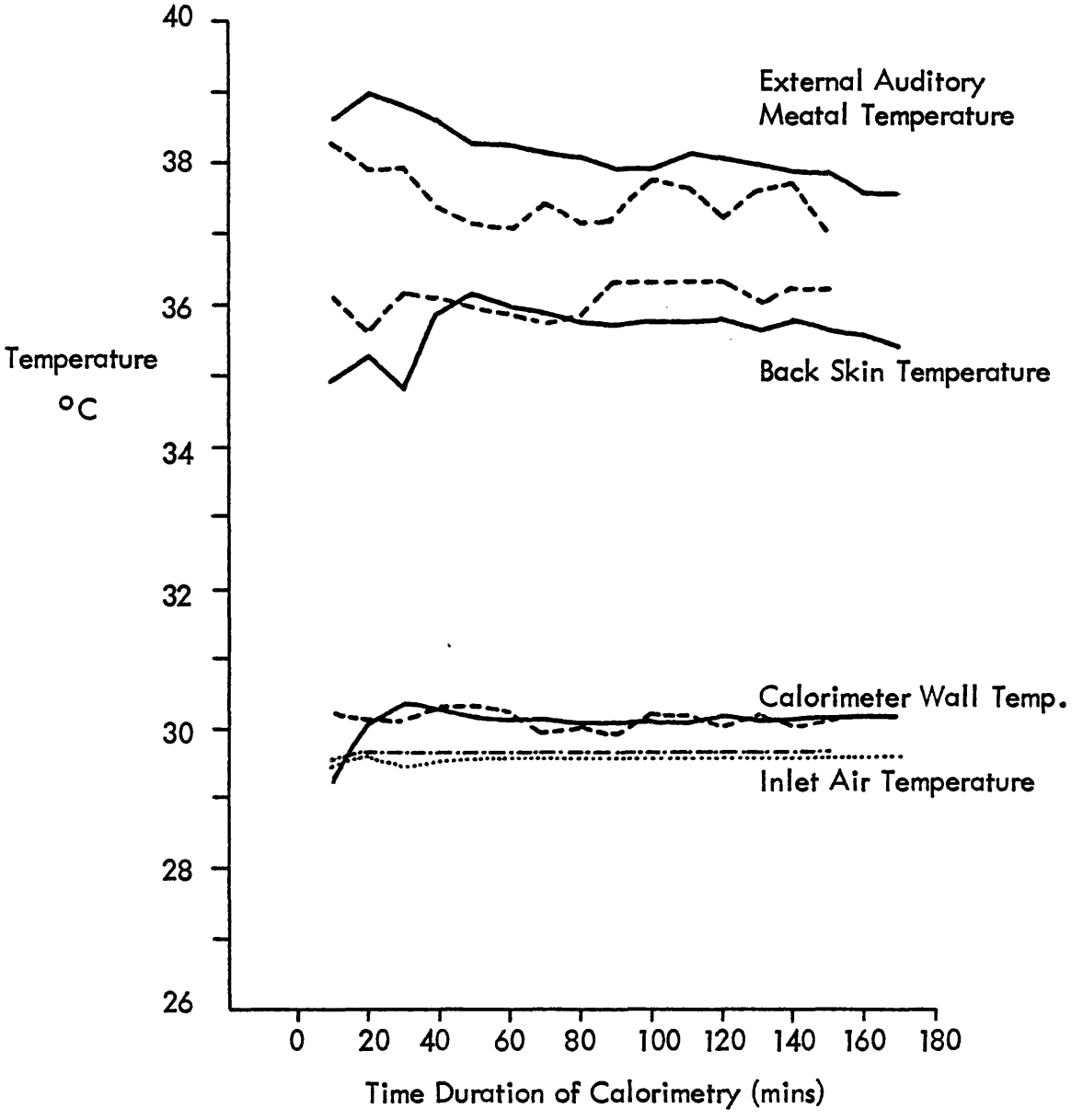


Fig.66 30°C CONTROL RAT CALORIMETRY. CALORIMETER AND BODY TEMPERATURE CHANGES WITH TIME.



One rat was subjected to a full skin depth dorsal burn of 20% of its body surface area by techniques fully described in a later section. It was my intention to evaluate not only the partitioning of heat losses which occurred after this injury but also to test calorimeter function over a prolonged experimental period of 40 - 50 days to establish the reliability of the system.

The results are shown graphically in Fig. 67. The control values given are those from the burned rat measured shortly before injury. Only heat loss is shown, as the temperature stability of our D.C.P.B. manufactured twin cell oxygen analyser proved unsatisfactory with prolonged use. Modification by thermostatting and insulation of the analyser was carried out subsequently. Tables 12A,12B,12C,12D give the results of this first burn study in detail. The percentage increase in sensible, insensible and total heat losses after burning compared with the pre-burn values (W/kg) is shown in Fig. 68. Destruction of the skin evaporative water barrier by thermal injury resulted in very large increases in evaporative water loss in the burned rat. These reached a maximum increase of 812 - 816% by the 26th - 28th day post-burn. Increased sensible heat losses were seen closely related to the increased evaporative water loss, with a maximum sensible heat loss of 152.5% on the 26th post-burn day. In absolute amounts, the daily

Fig.67 20% BSA BURN: DAILY PARTITIONED HEAT LOSS
RAT No 1

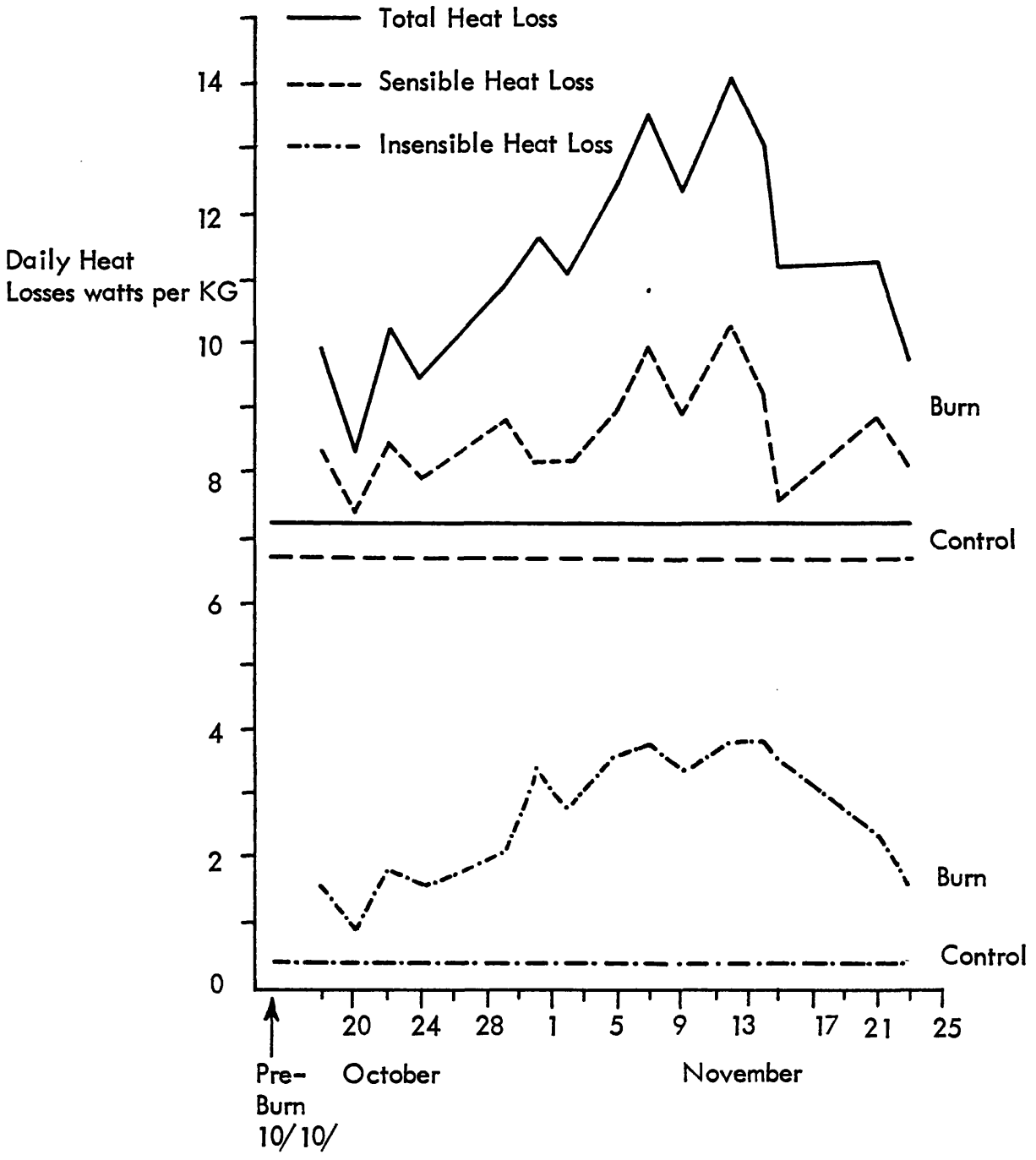
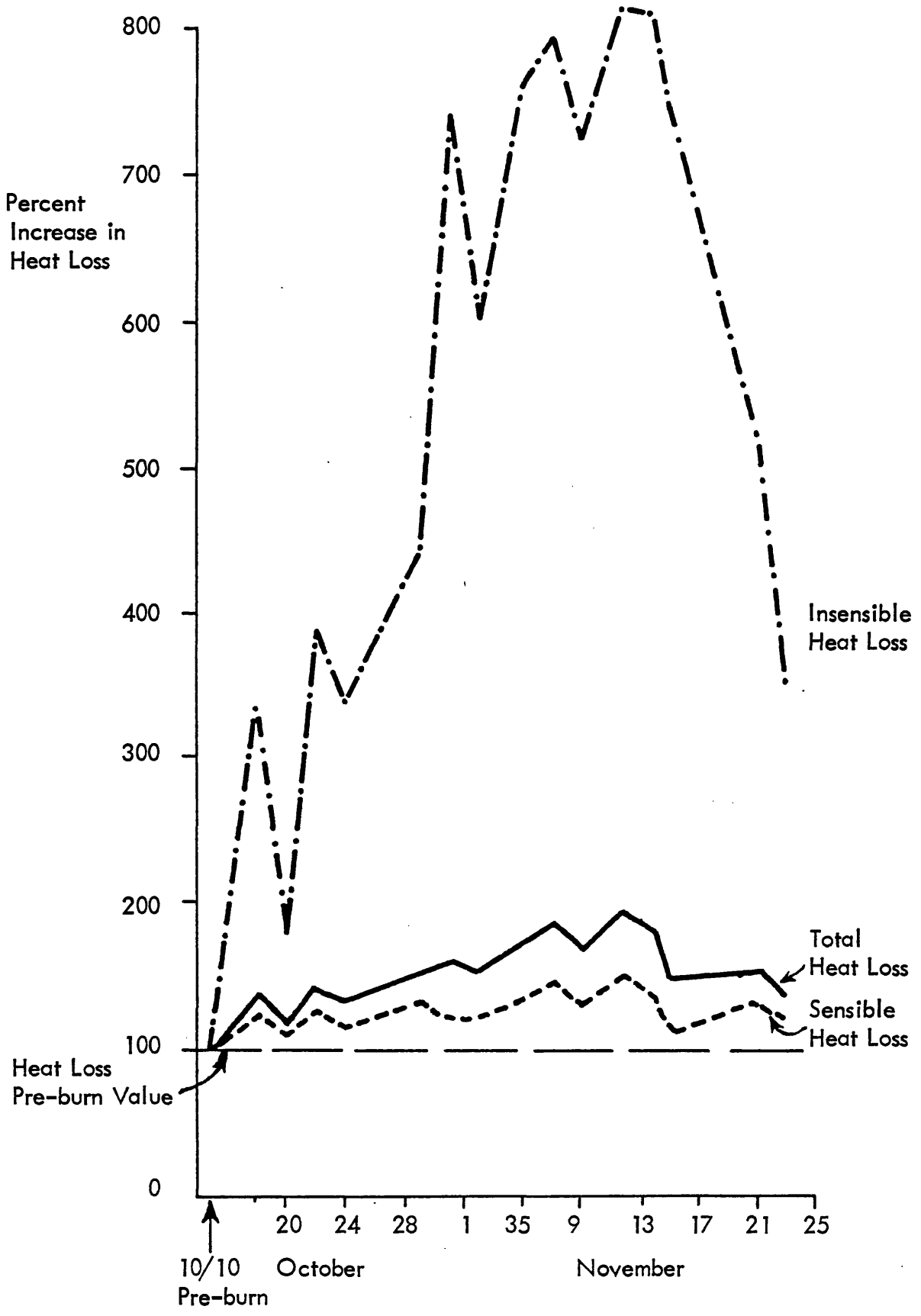


Fig.68 20% BSA BURN PERCENT INCREASE (W/kg) OVER PRE-BURN PARTITIONED HEAT LOSSES : RAT No.1



increase in evaporative heat loss (W/kg) expressed as a percentage of the daily increase sensible heat loss (W/kg) in the burned rat is shown in Table 13.

TABLE 12. A (1) (See Figs. 67, 68).

DATE	10/10 CONTROL	18/10/A	20/10/A	22/10/A
DAYS AFTER BURN	PRE-BURN	1	3	5
RAT WEIGHT g.	309.7	308.2	291.1	275.2
ATMOSPHERIC PRESSURE mm Hg.	760	760	754	758
W.G.M. TEMPERATURE ^{o}C	23.696	24.115	24.652	24.883
INLET AIR TEMPERATURE ^{o}C	20.127	20.190	20.043	20.129
CALORIMETER WALL TEMPERATURE ^{o}C	21.188	20.669	-	20.608
INLET/OUTLET AIRFLOW RATE L/min	1.744	1.773	1.401	1.339
TOTAL AIRFLOW RATE L/min	5.266	5.037	4.938	5.115
RECIRCULATED AIRFLOW RATE L/min	3.522	3.265	3.537	3.776
DEW POINT TEMPERATURE DEG. C.	17.179	17.222	15.228	15.311
URINE/FAECAL WT. g.	0/0	0/0	0/0	0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.997	2.395	2.065	2.174
URINE HEAT LOSS W.	0	0	0	0
FAECAL HEAT LOSS W.	0	0	0	0
SPEC. HEAT LOSS BY EXPIRED H_2O	1.667^{-03}	5.257^{-03}	2.766^{-03}	5.561^{-03}
NET HEAT LOSS THROUGH WALLS W.	1.996	2.390	2.062	2.168
HEAT LOSS TO AIRSTREAM W.	0.098	0.201	0.120	0.154

TABLE 12. A (2) (See Figs. 67, 68).

DATE	10/10 CONTROL	18/10/A	20/10/A	22/10/A
INCREMENT IN AIR TEMP. ^{o}C	0.845	1.800	1.102	1.360
TOTAL DIRECT HEAT LOSS W.	2.239	3.081	2.426	2.823
HEAT LOSS W/kg BODY WT.				
SENSIBLE	6.761	8.404	7.496	8.436
INSENSIBLE	0.469	1.594	0.837	1.823
TOTAL	7.230	9.998	8.334	10.259
HEAT LOSS % OF TOTAL				
SENSIBLE	93.5	84.1	89.9	82.2
INSENSIBLE	6.5	15.9	10.1	17.8
HEAT LOSS % OF CONTROL				
SENSIBLE	100	124.3	110.9	124.8
INSENSIBLE	100	339.8	178.5	388.7
TOTAL	100	138.3	115.3	141.9

TABLE 12. B (1) (See Figs. 67, 68).

DATE	24/10/A	29/10/A	31/10/A	2/11/A	5/11/A
DAYS AFTER BURN	7	12	14	16	19
RAT WEIGHT g.	274.2	267.4	274.7	273.1	258.5
ATMOSPHERIC PRESSURE mm Hg.	769	769	765	760	746
W.G.M. TEMPERATURE ^{o}C	25.077	24.599	24.827	24.958	23.995
INLET AIR TEMPERATURE ^{o}C	20.090	20.027	20.066	20.042	19.977
CALORIMETER WALL TEMPERATURE ^{o}C	20.689	20.688	20.428	20.608	20.661
INLET/OUTLET AIRFLOW RATE L/min	1.274	1.292	1.723	1.714	1.694
TOTAL AIRFLOW RATE L/min	4.902	4.960	6.615	6.588	6.512
RECIRCULATED AIRFLOW RATE L/min	3.629	3.668	4.892	4.874	4.818
DEW POINT TEMPERATURE DEG. C	15.261	15.257	15.102	15.124	15.301
URINE/FAECAL WT. g.	0/2.04	2.6/4.22	0/4.46	2.3/0	0/3.6
GROSS HEAT LOSS THROUGH WALLS W.	2.073	2.294	2.091	2.100	2.174
URINE HEAT LOSS W.	0	0.016	0	0.017	0
FAECAL HEAT LOSS W.	0.012	0.025	0.028	0	0.022
SPEC. HEAT LOSS BY EXPIRED H_2O	4.955^{-03}	6.274^{-03}	10.615^{-03}	8.794^{-03}	10.467^{-03}
NET HEAT LOSS THROUGH WALLS W.	2.056	2.247	2.052	2.074	2.142
HEAT LOSS TO AIRSTREAM W.	0.111	0.123	0.200	0.162	0.166

TABLE 12. C (1) (See Figs. 67, 68).

DATE	7/11/A	9/11/A	12/11/A	14/11/A	15/11/A
DAYS AFTER BURN	21	23	26	28	29
RAT WEIGHT g.	262.9	262.1	251.1	257.4	263.1
ATMOSPHERIC PRESSURE mm Hg.	764	759	746	754	760
W.G.M. TEMPERATURE ^{o}C	24.387	24.492	24.652	22.541	22.541 *
INLET AIR TEMPERATURE ^{o}C	20.011	20.005	20.043	20.087	20.087 *
CALORIMETER WALL TEMPERATURE ^{o}C	20.612	20.541	-	20.391	-
INLET/OUTLET AIRFLOW RATE L/min	1.731	1.717	1.685	1.722	1.736
TOTAL AIRFLOW RATE L/min	6.655	6.602	6.477	6.619	6.672
RECIRCULATED AIRFLOW RATE L/min	4.924	4.884	4.792	4.897	4.936
DEW POINT TEMPERATURE DEG. C	15.289	15.180	15.228	15.330	15.330
URINE/FAECAL WT. g.	1.22/2.42	0/2.8	0/1.56	0/3.27	0/2.78
GROSS HEAT LOSS THROUGH WALLS W.	2.427	2.169	2.398	2.206	1.873
URINE HEAT LOSS W.	0.006	0	0	0	0
FAECAL HEAT LOSS W.	0.012	0.018	0.009	0.017	0.019
SPEC. HEAT LOSS BY EXPIRED H_2O	10.938^{-03}	9.928^{-03}	10.628^{-03}	10.938^{-03}	10.483^{-03}

* Estimated value, appropriate channels not logged.

TABLE 12. C (2) (See Figs. 67, 68).

DATE	7/11/A	9/11/A	12/11/A	14/11/A	15/11/A
NET HEAT LOSS THROUGH WALLS W.	2.398	2.141	2.378	2.178	1.844
HEAT LOSS TO AIRSTREAM W.	0.196	0.206	0.211	0.190	0.174
INCREMENT IN AIR TEMPERATURE ^{o}C	1.333	1.415	1.473	1.303	1.178
TOTAL DIRECT HEAT LOSS W.	3.571	3.239	3.549	3.349	2.949
HEAT LOSS W/kg BODY WT.					
SENSIBLE	9.868	8.955	10.310	9.204	7.670
INSENSIBLE	3.717	3.400	3.825	3.808	3.540
TOTAL	13.585	12.355	14.135	13.012	11.209
HEAT LOSS % OF TOTAL					
SENSIBLE	72.6	72.5	72.9	70.7	68.4
INSENSIBLE	27.4	27.5	27.1	29.3	31.6
HEAT LOSS % OF CONTROL					
SENSIBLE	146.0	132.5	152.5	136.1	113.4
INSENSIBLE	792.6	724.9	815.6	811.9	754.8
TOTAL	187.9	170.9	195.5	180.0	155.0

TABLE 12.D (1) (See Figs. 67, 68).

DATE	21/11/A	23/11/A
DAYS AFTER BURN	35	37
RAT WEIGHT g.	272.2	268.7
ATMOSPHERIC PRESSURE mm Hg.	768	764
INLET AIR TEMPERATURE ^{0}C	20.087 *	20.087 *
CALORIMETER WALL TEMPERATURE ^{o}C	-	-
INLET/OUTLET AIRFLOW RATE L/min	1.759	1.746
TOTAL AIRFLOW RATE L/min	6.761	6.713
RECIRCULATED AIRFLOW RATE L/min	5.002	4.967
DEW POINT TEMP. DEG. C.	15.330	15.330
URINE/FAECAL WT. g.	0/3.45	0/0
GROSS HEAT LOSS THROUGH WALLS W.	2.275	2.037
URINE HEAT LOSS W.	0	0
FAECAL HEAT LOSS W.	0.019	0
SPEC. HEAT LOSS BY EXPIRED H_2O	7.497^{-03}	4.931^{-03}
NET HEAT LOSS THROUGH WALLS W.	2.249	2.032
HEAT LOSS TO AIRSTREAM W.	0.169	0.159

* Estimated value, appropriate channels not logged.

TABLE 12. D (2) (See Figs. 67, 68).

DATE	21/11/A	23/11/A
INCREMENT IN AIR TEMPERATURE ^{o}C	1.132	1.071
TOTAL DIRECT HEAT LOSS W.	3.082	2.626
HEAT LOSS W/kg BODY WT.		
SENSIBLE	8.882	8.155
INSENSIBLE	2.439	1.619
TOTAL	11.321	9.773
HEAT LOSS % OF TOTAL		
SENSIBLE	78.5	83.4
INSENSIBLE	21.5	16.6
HEAT LOSS % OF CONTROL		
SENSIBLE	131.4	120.6
INSENSIBLE	520.0	345.2
TOTAL	156.6	135.2

TABLE 13 A (See Figs. 67, 68).

DATE	18/10	20/10	22/10	24/10	29/10	31/10	2/11
POST BURN DAY	1	3	5	7	12	14	16
SENSIBLE W/kg BURN - CONTROL	1.643	0.735	1.675	1.144	2.102	1.438	1.429
INSENSIBLE W/kg BURN - CONTROL	1.08	0.368	1.354	1.121	1.599	3.004	2.371
% DIFFERENCE INSENSIBLE/ SENSIBLE	66	51	81	98	76	209	166

TABLE 13. B (See Figs. 67, 68).

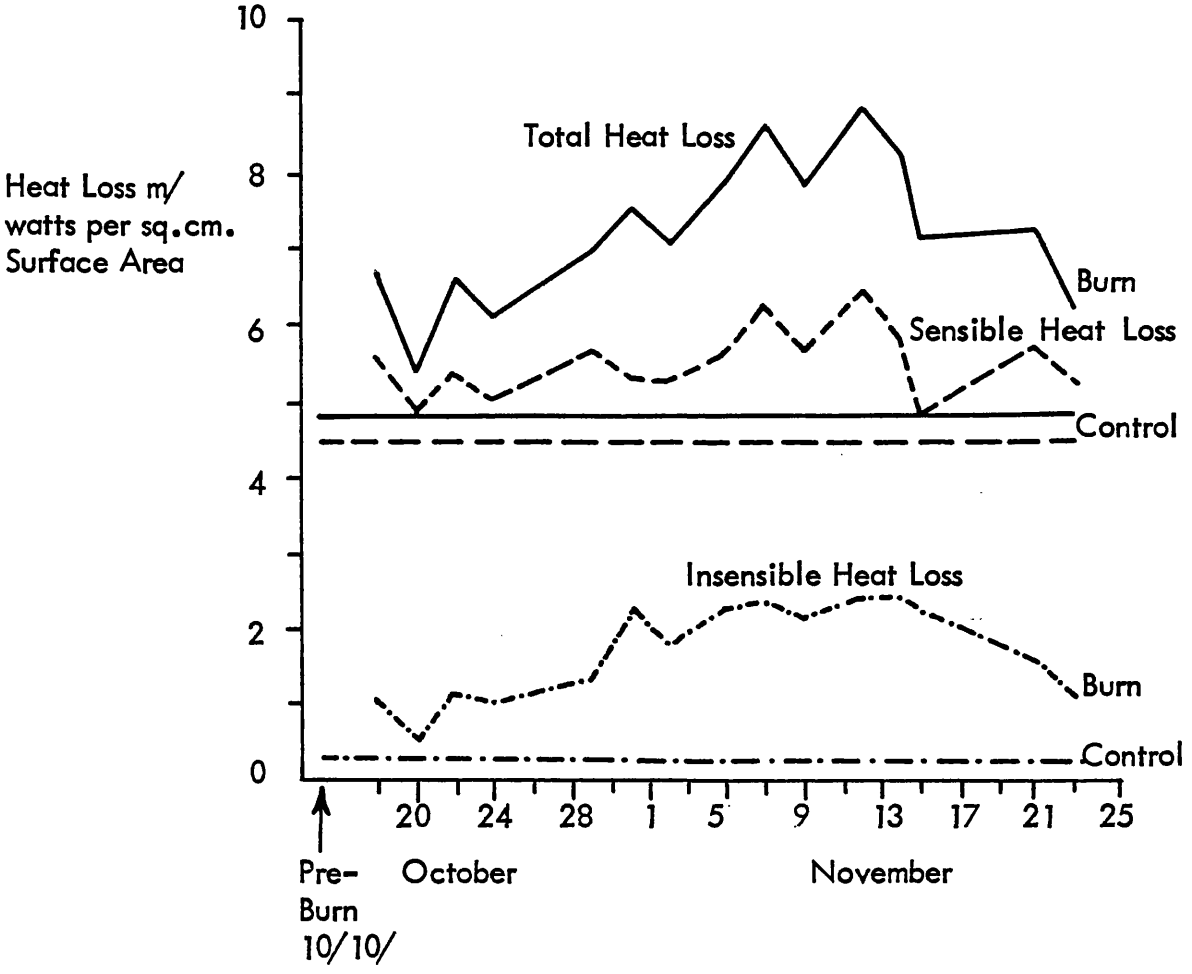
DATE	5/11	7/11	9/11	12/11	14/11	15/11	21/11	23/11
POST BURN DAY	19	21	23	26	28	29	35	37
SENSIBLE W/kg BURN - CONTROL	2.167	3.107	2.194	3.549	2.443	0.909	2.121	1.394
INSENSIBLE W/kg BURN - CONTROL	3.096	3.248	2.931	3.356	3.339	3.071	1.970	1.150
% DIFFERENCE INSENSIBLE/ SENSIBLE	143	105	134	95	137	338	93	83

Increased evaporative heat loss has a mean of 74% of the increased sensible heat loss up to the 12th post-burn day (29/10). Increases in evaporative or insensible heat loss exceed increases in sensible heat loss by 209% on the 14th post-burn day (14/10) and from then till the 29th post-burn day (15/11) the increase in evaporative heat loss exceeds the increase in sensible heat loss by a mean of 166%. It must be noted that the mean total airflow rate in the calorimeter for the runs between the 1st and the 12th post-burn day was 4.990 L/min while that for the later runs where evaporative heat loss increase exceeded the increase in sensible heat loss was 6.593 L/min. It must also be noted that during the runs on the 35th and 37th post-burn days when insensible heat loss was greatly reduced and returning towards normal values the increased insensible heat loss was less than the increase in sensible heat loss, 93% and 83% respectively on each day. The flow rate during each of these latter runs was 6.761 and 6.713 L/min.

The same relationships between sensible and insensible heat loss increases after burning hold true if expressed as milli Watts per square centimetre of body surface area. See Fig. 69.

One explanation for the increase in evaporative heat loss exceeding sensible heat loss might be that total

Fig. 69 20% BSA BURN: PARTITIONED HEAT LOSS AND SURFACE AREA RELATIONSHIP. RAT No 1



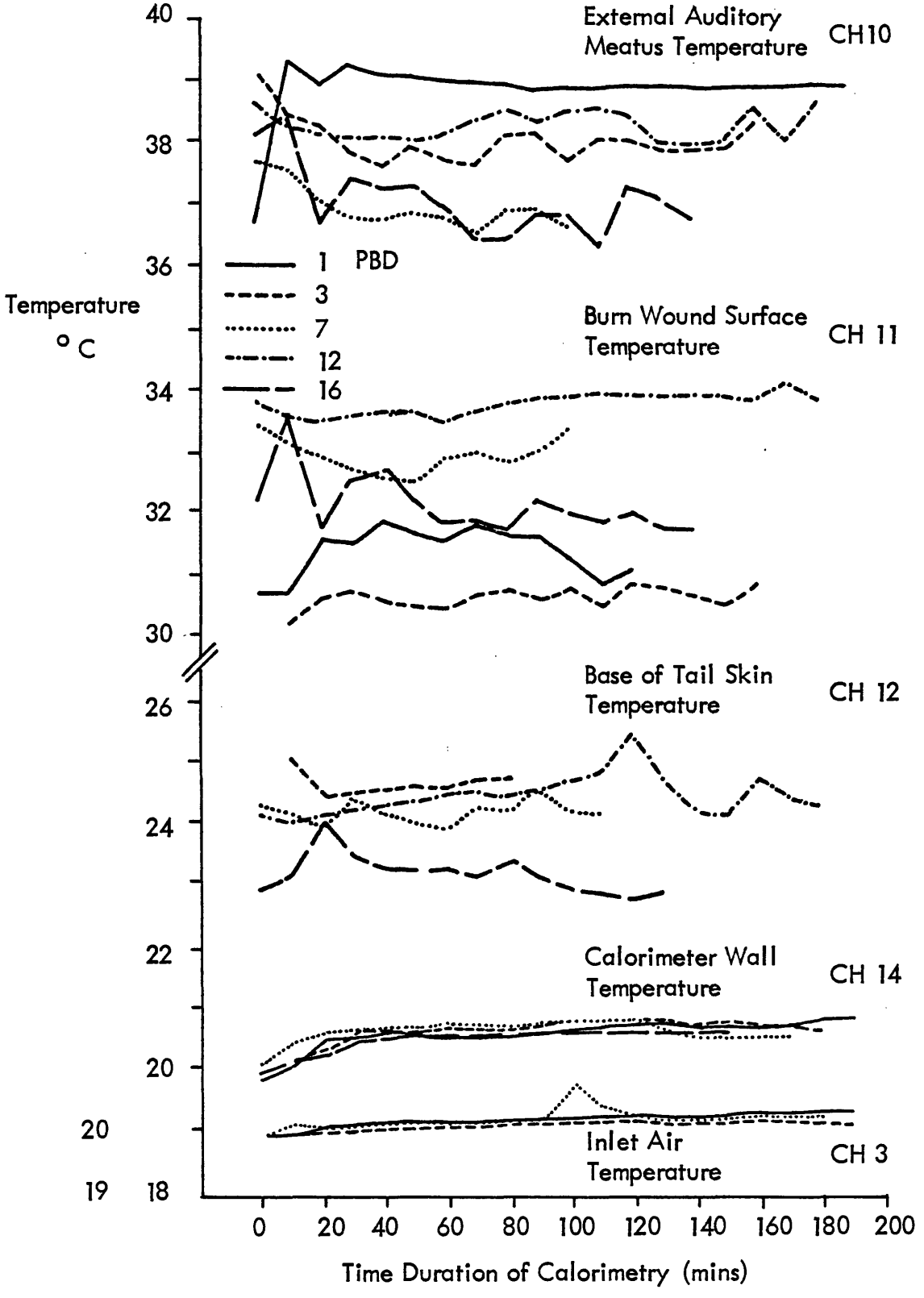
calorimeter airflow rates above 5 L/min created a high rate of convected heat loss from the burn wound surface with cooling of the rat during a run and decrease in body heat content.

FIGURE 70 shows the changes in rat external ear, burn wound surface, and base of tail temperatures during calorimetry. It takes 50 minutes from the start of the run for calorimeter wall temperature to stabilise. Though there is some fluctuation, after 50 minutes, the various rat temperatures measured show no evidence of a net decrease in body heat content. In particular, there is no fall in burn wound temperature with time during calorimetry.

The results presented in this figure are however derived from runs carried out between the 1st and the 16th post-burn day. The calorimeter runs between the 1st and the 12th post-burn day were those in which the total calorimeter airflow rates were less than 5 L/min, therefore good agreement between rat thermometry and partitioned heat loss measurement would be expected for runs, since it has been confirmed by rat body temperature measurement that no change in body heat content occurred in calorimeter runs for the 1st to the 12th post-burn day.

If Fig. 70 is examined closely, there is some evidence of a progressive slight fall in burn wound temperature and base of tail temperature in the data measured on the 16th post-burn day. Rat thermometry during calorimetry was discontinued after the 16th post-burn day because thermistors could not be attached to the burned

Fig.70 20% BSA BURN: RAT 1 BODY TEMPERATURES AND CALORIMETER TEMPERATURES: CHANGES WITH TIME DURING CALORIMETRY



surface due to breaking up of the burn wound eschar. The rat became increasingly agitated when further attempts were made in later calorimeter runs to measure external ear temperature and tail temperature. Further thermometry could not therefore be carried out in the burned rat. The implications of these findings in regard to the accuracy of burned rat calorimetry are fully discussed later.

FIGURES 71, 72 and 73 are shown to place the energy changes after burning in the rat in perspective with the energetic changes and nutritional requirements after burning injury described in man. Much of this literature is American where S.I. units are not used. I have therefore expressed the results here in kcal per 24 hours without weight correction. Rat body weight loss and changes in food intake after a 20% surface area full skin depth burn are presented and discussed elsewhere. See Figs. 124 and 125.

Fig.71 20% BSA BURN: DAILY PARTITIONED HEAT LOSS
RAT No 1

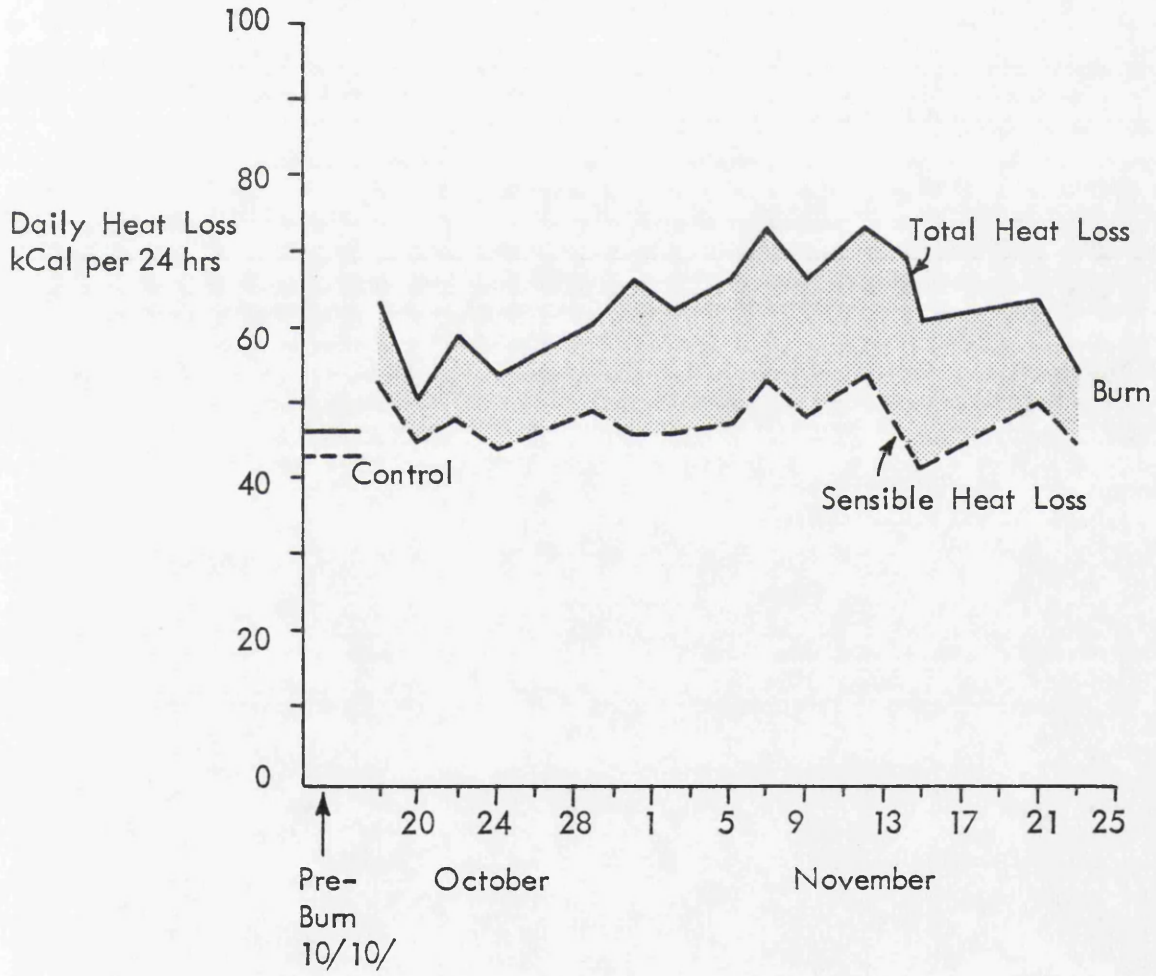


Fig. 72 20% BSA BURN: DRY OR SENSIBLE HEAT LOSS
RAT No 1

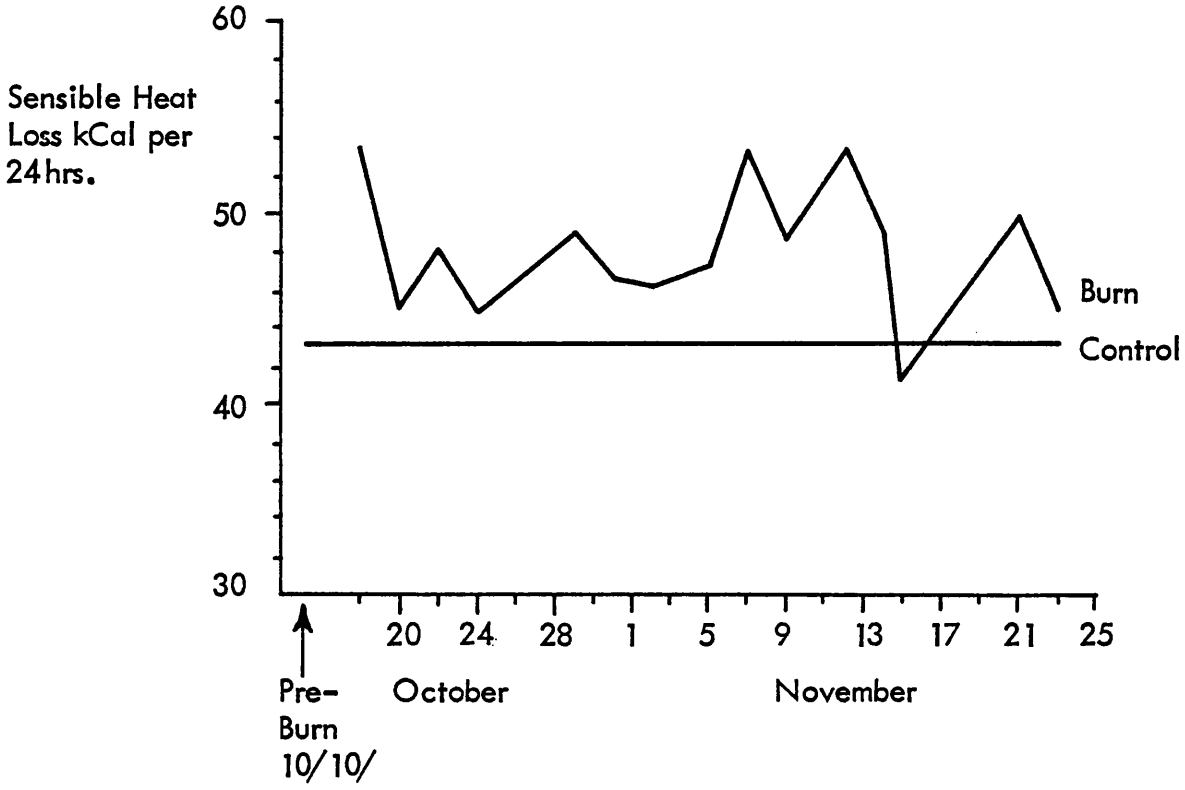
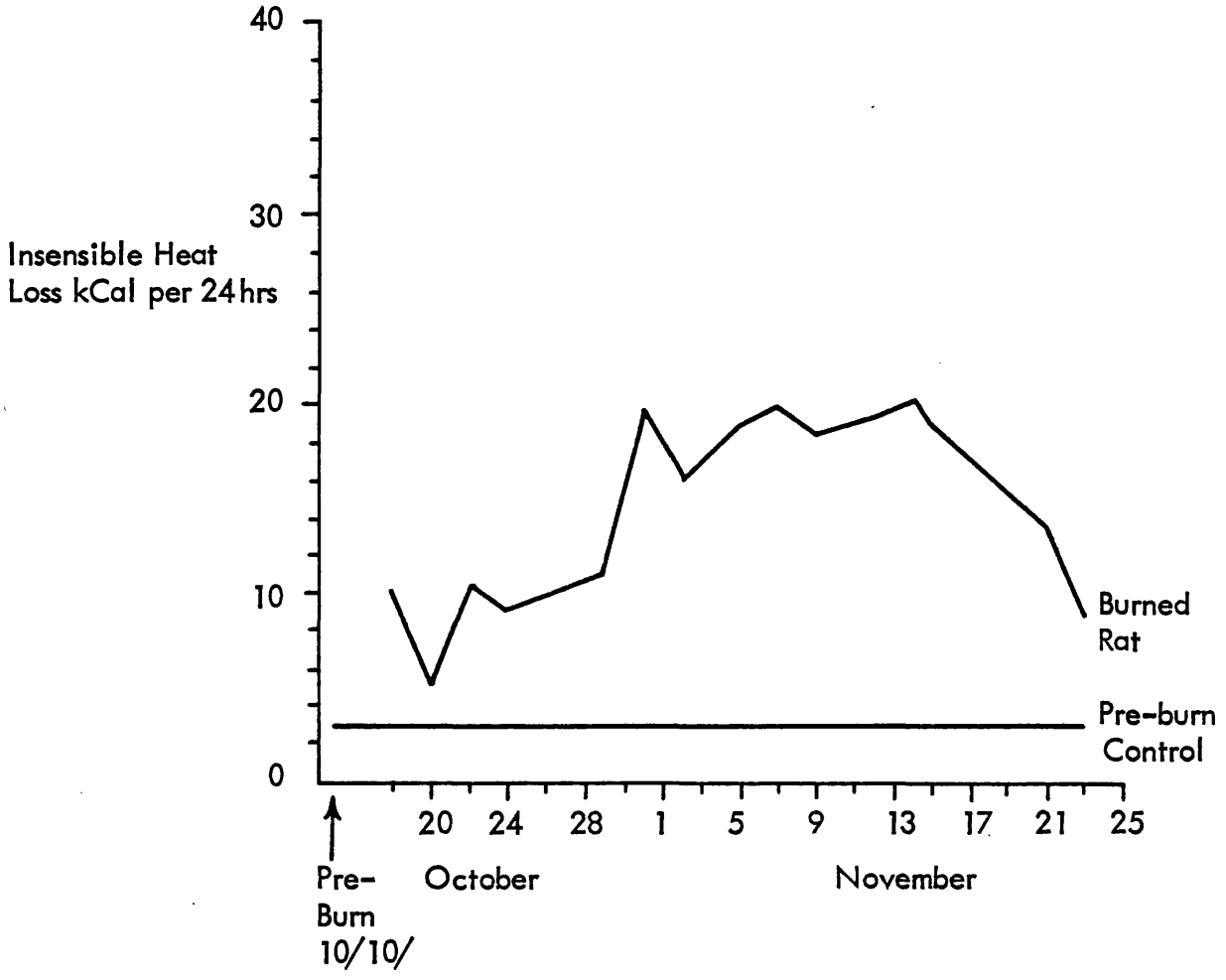


Fig. 73 20% BSA BURN: EVAPORATIVE OR INSENSIBLE HEAT LOSS.
RAT No 1



DEVELOPMENT OF A GRADIENT LAYER CALORIMETRY SYSTEM

DISCUSSION

DIRECT CALORIMETRY - NORMAL RATS

The rate of total heat loss, mean value 7.23 W/kg, detected by the SEC - A - 04 L gradient layer calorimetry system in a post absorbtive rat wearing a thermistor harness (Fig. 40) at an ambient temperature of 20°C (Fig. 57) is comparable with the value of 7.3 W/kg found by Swift and Forbes (1939) using indirect methods. In post absorbtive rats in my study, without thermistor lead attachments, the total heat losses measured were lower at 6.03 W/kg and 6.24 W/kg (Figs. 55 and 56). The proportion of evaporative or insensible heat loss was also lower than that found by Swift and Forbes (1939), 4.7 to 9.5% of total heat loss in my studies compared with 15.07% evaporative loss in that of Swift and Forbes.

In man, in conditions of thermal comfort, Hardy (1968) gives the evaporative heat loss of the average man as 25% of the total heat loss, with a skin to air evaporative loss of 21% and respiratory tract to air loss of 4% of the total loss.

The rat does not possess cutaneous sweat glands. Therefore it must lose most of its evaporative water loss via the respiratory tract like the dog (Kleiber, 1961), though male rats do have primitive scrotal sweat glands capable of increasing scrotal skin water loss which can play a role in rat thermoregulation.

The effect of rat movement on both sensible (dry) heat loss and insensible (evaporative) heat loss is marked. This is shown in Fig. 55 , during periods A and C. Similar increases in sensible heat loss and insensible heat loss may be seen as a form of 'step change' in the data chart recorder outputs of large animal gradient layer calorimeters, e.g. between recumbent and standing positions in the cow (McLean, 1971). In the rat it is probable that increased sensible heat loss on standing or with movement can be attributed to the alterations in exposed body surface area. Increased surface area increases radiant and convective heat losses (Spinnler et al., 1973). This is in agreement with Fourier's Law (Kleiber, 1961) that "for any given difference between internal temperature and surface temperature, the rate of heat transfer is proportional to the surface area when specific insulation (in the rat, depth of fur and skin covering) remain the same." The increased evaporative heat loss when the rat is active must be mainly due to an increased rate of evaporative loss from its respiratory

tract. This amountsto a 55% increase in insensible heat loss in Fig. 55 during Periods A and C compared with Period B.

It is of interest to note that in my studies, a typical value for resting metabolic energy expenditure (RME) in a 309 g male rat is 46 kcal per day. This total heat loss is 53% greater than the mean value of 30 kcal per day for total heat loss given by Kleiber et al. (1956) in 308 g female rats for basal metabolic rate (BMR). Sex difference not withstanding, RME in the present studies may therefore be considerably higher than "true" BMR.

Sensible (dry) heat loss in the unrestrained rat, 5.62 W/kg in Fig. 55, 5.95 W/kg in Fig. 56, was consistently lower than sensible heat loss in rats wearing thermistor lead harnesses (Fig.40), 6.76 W/kg, which were slightly restrained within SEC - A - 04 L on account of this (Fig. 57). Bartlett et al. (1958) postulated that restraint in rats subjected to calorimetry interfered with the heat producing and heat flow mechanisms of the animal by lessening the normal subcutaneous to skin surface temperature gradient, causing in effect a loss of normal skin insulation.

In the examples shown in Fig. 58 during Period C and in Fig. 59 during period C, exposure of rats to 30°C ambient temperature conditions reduced total heat loss by approximately 16% (to 5.15 W/kg) from that seen at 20°C ambient temperature. The thermopile output trace indicated less rat activity during calorimetry at 30°C than at 20°C ambient temperature. These findings are consistent with published reports of temperature acclimation in the rat.

The rat lacks cutaneous sweat glands, but despite less movement it has an increased insensible (evaporative) heat loss at an ambient temperature of 30°C in low relative humidity conditions, 11.8% at 30°C (Fig. 58 in Period B) compared with 6.7% at 20°C (Fig. 56 in Period B). Similar findings have been described in man (Dubois, 1937 - quoted in Kleiber, 1961). In the dog, which like the rat lacks cutaneous sweat glands, increased respiratory tract water loss occurs in response to high ambient temperatures, rather than sweating at the body surface (Dill et al., 1933). When the rat is active at 30°C ambient temperature as in Fig. 58 during Period A, the same increment in evaporative heat loss is noted (11.8% rising to 15.6%) due to the activity, as is seen at 20°C ambient temperature.

Caldwell et al. (1966) carried out studies on uninjured rats which showed that post absorbtive animals

at a calorimeter operating temperature of 28.26 ± 0.23°C had an average heat loss of 5.67 ± 0.18 kcal/kg/hr. Sensible (dry) heat loss was 4.65 ± 0.15 kcal/kg/hr or 82% of the total heat loss. Increasing the calorimeter operating temperature to 30.5°C decreased sensible (dry) heat loss to 3.65 kcal/kg/hr, but did not alter insensible (evaporative) heat loss at 1.08 kcal/kg/hr. The total heat loss was 4.72 kcal/kg/hr.

In the present studies, conditions in Fig. 59 (Table 9) were similar in regard to calorimeter operating temperature (29.54°C) and humidity to those in Caldwell et al.'s (1966) later calorimetry runs. Total heat loss in Fig. 59 during Period A was 4.66 kcal/kg/hr (5.42 W/kg). Sensible (dry) heat loss was 3.95 kcal/kg/hr (4.60 W/kg), and insensible (evaporative) heat loss was 0.71 kcal/kg/hr (0.82 W/kg). Sensible (dry) heat loss accounted for 84.8% of the total heat loss and insensible (evaporative) heat loss 15.2%. Providing runs with similar calorimeter operating temperatures are compared, there is agreement between the present studies and those of Caldwell et al. (1966) in regard to uninjured control rats.

In the calorimetry run shown in Fig. 59, an internal ventilatory air recirculation rate of 4.6 l/min through SEC - A - 04 L at an inlet air temperature of 29.6°C, even with moderately high humidity levels (46% RH), proved capable of very rapidly evaporating small quantities

of rat urine. Without the oil filled floor tray, complete evaporation of urine occurred during the run so that urination could not be detected by measuring a weight increase in the cage floor tray. If unrecognised such an event would give low "apparent" sensible heat loss values with disproportionately high evaporative heat losses. However evaporation of urine within the gradient layer box causes a characteristic fall or "blip" in thermopile output which is readily recognised. This effect is not seen in other runs presented (except Fig. 58) as the problem of urine evaporation during calorimetry runs was virtually completely eliminated by the use of the oil filled floor tray described previously.

Thermoregulation in the rat may be physical or metabolic (chemical), and the progressive fall in total and partitioned heat loss seen during and between Periods A and C in Fig. 59 probably represents metabolic adaptation to heat and humidity in addition to the physical adaptation to heat in the rat of lying still (Gelineo, 1964). The rat in Fig. 59 was kept at between 26 - 27^{o}C prior to the calorimetry measurements being carried out, and would not therefore have been fully heat adapted at the start of the run.

Figure 60 shows the typical response of an untrained rat placed in SEC - A - 04 L. Continuous thermopile "activity" peaks occurred with corresponding peaks in

hygrometer output and increments in air temperature. Slight delays were noted between the periods of activity and the instrument responses to it of between one and four minutes. The SEC - A - 04 L calorimetry system is not suitable for following very short periods of rapidly changing heat production or loss. Great care must be taken in making allowance for these known instrument delays when beginning and ending measurements, if data is taken from the Foster 12 channel shart recorder output.

Where no rat movement takes place as in an anaesthetised rat (Fig. 61) a better appreciation of the SEC - A - 04 L calorimetry system performance can be obtained as the animal begins to recover from the anaesthetic agent. The gradient layer detects an increased sensible (dry) heat loss as thermoregulation returns in the rat. This happens 12 minutes after the first noticeable change in external ear temperature, but 25 minutes before the first obvious change in rat posture. Though SEC - A - 04 L is handicapped by an internal ventilatory air recirculation which is essential for controlling high relative humidity conditions around burned rats, nonetheless in Fig. 61 the system displays sufficient sensitivity and rapidity of response in the measurement of partitioned heat losses to be satisfactory even in this type of drug effect study.

INDIRECT CALORIMETRY - NORMAL RATS

The indirect calorimetry function of the SEC - A - 04 L system was evaluated in the run shown in Fig. 62 (Tables 10A, 10B, 10C).

The average oxygen consumption of the 289.5 g rat in Fig. 62 was 6.61 ml/0_2/min over a 250 minute measurement period at a calorimeter operating temperature of 20.4°C. Values ranged from 5.93 ml/0_2/min to 7.55 ml/0_2/min. The highest corresponded with periods of high activity, the lowest with the least activity. These results are comparable with those of Bramante (1959) of oxygen consumption by a very active 290 g male rat at 26°C ambient temperature of 8.29 ml/0_2/min, and oxygen consumption ranging from 3.25 ml/0_2/min to 4.5 ml/0_2/min by a 250 g rat at 28°C ambient temperature. Further studies published later by the same author (Bramante, 1961) gave similar values.

Whole animal oxygen consumption represents the sum of the oxygen consumed by the individual organs. Under basal conditions, liver, skeletal, muscle and brain utilise the greatest quantities of oxygen (Brozek and Grande, 1955). During exercise the oxygen consumption and hence heat production of skeletal muscle can increase manyfold. Evidence of this increased oxygen consumption due to exercise in the rat and its effect on heat production is shown in Fig. 62 where the relationships

between oxygen and carbon dioxide analyser outputs and external ear, back and tail temperature measurements may be examined.

It has to be accepted that the gaseous mixing in the calorimeter has the effect of "dampening" or smoothing out the height of oxygen and carbon dioxide analyser responses and prolongs the apparent duration of the measured response where sudden bursts of activity and heat production take place. The only way to overcome this is to start and stop all measurement periods when instrument traces are at baseline values. It is therefore not possible to measure RME at any point during the run shown in Fig. 62 as the rat is persistently active throughout.

A simplified form of Weir's (1949) equation was used to calculate heat production from oxygen consumption and carbon dioxide production, as follows

Heat production (W) = 274 x O_2 consumption l/min at STP + 70 x CO_2 production l/min at STP.

The inclusion of urinary nitrogen excretion in the calculation of total heat production was considered but discarded for two reasons:

1. Weir in his equation corrected for the nitrogen contribution to total heat production by assuming

that 12.5% of the total oxidised energy came from protein breakdown. Kinney (1976) noted that protein breakdown accounted for between 12 - 22% of total energy expenditure before injury, and that this proportion did not change significantly after injury. The likely error created by not including urinary nitrogen excretion in the calculation of heat production in this simplified form of Weir's equation is less than 0.3%.

2. Accurate urine collections cannot be made (because of spillage and losses) on rats subjected to calorimetry in our laboratory. It is unlikely that the use of urinary nitrogen excretion values determined on other rats kept in metabolic cages in similar conditions to the calorimeter rats would significantly reduce the small potential error inherent in the form of Weir's equation used in the present studies. Marked differences were observed in the metabolic response to burns between rats subjected to calorimetry and rats similarly injured but simply kept in metabolic cages. This topic is considered in a later chapter.

The respiratory quotient (RQ) (Kleiber, 1961) is the ratio of volume of CO_2 produced divided by volume of O_2 consumed. These volumes must be corrected because of the small difference which occurs between the volume

of inspired and expired gas, accounted for by the discrepancy of volumes between oxygen consumed and carbon dioxide produced. The following correction factors were used in the calculation of respiratory quotient, hereafter R.Q.

$$RQ = \frac{\text{CO_2 concentration difference between measured CO_2 in SEC - A - 04 L outlet air and atmospheric air}}{\dfrac{\text{Apparent } O_2 \text{ concentration difference between measured } O_2 \text{ in atmosphere and SEC-A-04 L outlet air}}{0.791} - 0.265 \times \text{CO_2 concentration difference between measured CO_2 in SEC-A-04 L outlet air and atmospheric air.}}$$

During calibration of the carbon dioxide analyser with span gas passed through the calorimeter ventilatory air circuit it was noted that only 96.6% of the reading found by passing span gas direct to the analyser from the gas cylinder could be obtained. It was subsequently established that continual permeation of CO_2 took place through the plastic tubing material from which the ventilation air circuit was made. If sufficient time is allowed from the start of a run (15 - 20 minutes), then equilibrium appeared to be reached in regard to ventilatory air plastic tubing CO_2 permeation. It was necessary to introduce a small correction factor to take account of this. All carbon dioxide readings were

increased by 3.4%.

Kleiber (1961) made an extensive review of the range of RQ values found in differing species and noted the effect of age, diet and other factors. He stated that RQ was not a precise index of the nature of intermediary metabolism, but was merely an indicator of general trends. He considered that RQ measurement could be utilised in calorimetry studies as a guide to the "normality" of the basal metabolism of the animal under study. RQ ratios of less than 0.6 indicated a measurement error. RQ results greater than 1 led to the suspicion that the animal had not been fasted. Kleiber et al. (1956) in a study on female rats regarded RQ values outwith 0.69 to 0.75 (during 3 hour measurement periods to determine basal metabolism) as being unphysiological or indicative of laboratory error. Data from such runs was not used in their subsequent calculations.

The average RQ value in Fig. 62 for a post absorbtive active rat is 0.73, ranging from 0.705 to 0.763 in the twelve 20 minute measurement periods of the run. Table 11 gives the RQ values found in 16 post absorbtive calorimetry runs made using two tame trained rats under (basal) resting conditions at 20^{o}C ambient temperature. The average measurement period was 86 minutes and the average total duration of calorimetry 157 minutes. RQ was 0.69 $\pm$ 0.02 (standard deviation). Assuming a normal distribution

of data, 95% of our expected normal results with SEC - A - 04 L should fall within 2 standard deviations of this mean value. Therefore RQ ratios between 0.65 - 0.73 have been considered "physiological" for post absorbtive Wister rats in our calorimetry studies. Evidence from Swift and Forbes (1939), Cumming and Morrison (1960) and Caldwell et al. (1966) suggests that RQ values up to and including 0.75 must be considered within normal limits for post absorbtive rats.

COMPARISON BETWEEN DIRECT AND INDIRECT CALORIMETRY

In the run shown in Figs. 62, 63 and 64, total heat loss (7.96 W/kg) exceeds total heat production (7.57 W/kg) by 4.84% over the 250 minute measurement period. Fig. 64 shows the range of values for indirectly measured heat production and directly measured heat loss during each of the 12 time periods. Figs. 62 and 63 illustrate within a single run all the problems associated with relating heat production to heat loss over short (20 minute) time periods where the rat is active throughout. The oxygen and carbon dioxide analyser traces were offset by 1.75 minutes from the start and end of the measurement period to take account of the time taken for the gas sample to travel 5 metres from SEC - A - 04 L to the instrument cabinet. During periods 1 and 2 (total time 40 minutes) the rats' average net heat loss was 13% greater

than its heat production. The rat therefore lost body heat during these periods. This was confirmed by the decrease in external ear, back skin and base of tail temperature. Lost body heat was restored during period 3 when heat production rose and sensible heat loss continued to fall. This resulted in a small surplus in the rate of heat production over heat loss of 1.7% and a rise in body temperature.

This pattern of heat loss exceeding heat production, followed by a period of recovery in heat production with restoration of lost body heat content, recurred later in the run shown in Figs. 62, 63 and 64. The time intervals between these cycles was approximately 40 minutes. However, even allowing for possible error in the offsetting of traces when calculating results, continuous rat activity, and arbitrary selection of 20 minute measurement periods, it did appear that heat production and loss cycles were not always synchronised. This phenomenon has been observed in the cow by McLean (1975 - personal communication) where heat production and loss cycles are of 4 hours duration.

Pullar et al. (1967), Dale et al. (1967), compared heat production and heat loss in man and in apes, using gradient layer calorimeters at various ambient temperatures. They found that heat production exceeded heat loss by up

to 8%. They felt that an increase in stored body heat accounted for the difference, but made no measurements to confirm or refute this view during their experiments. Caldwell et al. (1966) measured changes in body heat stores in the rat by thermometry and also estimated heat flow from the "core" to the surface tissues during calorimetry runs. This latter approach was adopted to evaluate data from Figs. 62 and 63 in order to measure changes in body heat content during each time period in the rat under study and to calculate "tissue" and "air" heat conductance as defined by Caldwell and his colleagues. These calculations are presented in Tables 14A, B, C and D.

According to Fourier's Law (Kleiber, 1961) any alteration in the insulating properties of the skin alters the rate of heat transfer from body core to body surface. When an animal attempts to conserve heat, skin vaso-constriction occurs. Decreased circulation to the surface (peripheral) tissues increases the effective layer of body insulation, decreasing body heat loss and favouring body heat storage (Richards, 1973). The converse is true when an animal attempts to lose heat. Tissue conductance which is a measure of actual heat flow from the central core to the surface can be calculated from sensible heat loss, core temperature and skin temperature as follows:

TABLE 14. (A) THERMOMETRY & TISSUE CONDUCTANCE (Figs. 62,63)

NORMAL RAT 20°C AMBIENT TEMP. HIGH HUMIDITY CONDITIONS

TIME PERIOD	ENTIRE RUN (250 min)		1		2	
CORE TEMPERATURE (AUDITORY MEATUS) °C	39.95 ± 0.25		37.90 ± 0.08		37.65 ± 0.19	
DORSUM SKIN TEMPERATURE °C: UPPER BACK	32.94 ± 0.57		32.53 ± 0.23		31.91 ± 0.49	
BASE OF TAIL SKIN TEMPERATURE °C: LOWER BACK	31.93 ± 0.53		31.71 ± 0.19		31.70 ± 0.28	
MEAN SKIN TEMPERATURE °C	32.43 ± 0.45		32.12		31.81	
DIFFERENCE (CORE - MEAN SKIN) TEMPERATURE °C	5.49 ± 0.26		5.78		5.84	
SENSIBLE HEAT LOSS (W/m^2) & ($kcal/m^2/hr$)	46.04	39.59	45.46	39.09	46.94	40.37
TISSUE CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	8.39	7.21	7.87	6.76	8.04	6.91
INLET AIR TEMPERATURE °C	20.44 ± 0.02		20.43		20.42	
AIR CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	3.84	3.30	3.89	3.34	4.12	3.54
NET RATE OF CHANGE IN BODY HEAT CONTENT IN EACH PERIOD. + = GAIN, - = LOSS (W/m^2) & ($kcal/m^2$)	-0.84	-0.72	-2.16	-1.86	-2.34	-2.01

TABLE 14. (B) THERMOMETRY & TISSUE CONDUCTANCE (Figs. 62,63)

NORMAL RAT 20°C AMBIENT TEMP. HIGH HUMIDITY CONDITIONS

TIME PERIOD	3		4		5		6	
CORE TEMPERATURE (AUDITORY MEATUS) °C	38.13 ± 0.31		37.90 ± 0.26		37.64 ± 0.18		38.00 ± 0.25	
DORSUM SKIN TEMPERATURE °C: UPPER BACK	33.68 ± 0.38		33.51 ± 0.23		32.37 ± 0.42		33.05 ± 0.54	
BASE OF TAIL SKIN TEMPERATURE °C: LOWER BACK	32.18 ± 0.50		31.71 ± 0.41		31.03 ± 0.13		31.51 ± 0.42	
MEAN SKIN TEMPERATURE °C	32.93		32.61		31.70		32.28	
DIFFERENCE (CORE - MEAN SKIN) TEMPERATURE °C	5.20		5.29		5.94		5.72	
SENSIBLE HEAT LOSS (W/m^2) & ($kcal/m^2/hr$)	45.74	39.34	46.37	39.88	46.42	39.92	44.48	38.
TISSUE CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	8.80	7.57	8.77	7.54	7.81	6.72	7.78	6.69
INLET AIR TEMPERATURE °C	20.45		20.43		20.43		20.44	
AIR CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	3.67	3.15	3.81	3.27	4.12	3.54	3.76	3.23
NET RATE OF CHANGE IN BODY HEAT CONTENT IN EACH PERIOD. + = GAIN, - = LOSS (W/m^2) & ($kcal/m^2$)	+0.3	+0.26	-0.65	-0.56	-1.38	-1.18	+0.28	+0.

TABLE 14. (C) THERMOMETRY & TISSUE CONDUCTANCE (Figs. 62,63)

NORMAL RAT 20°C AMBIENT TEMP. HIGH HUMIDITY CONDITIONS

TIME PERIOD	7		8		9		10	
CORE TEMPERATURE (AUDITORY MEATUS) °C	38.12 ± 0.19		37.99 ± 0.28		38.48 ± 0.32		38.21 ± 0.37	
DORSUM SKIN TEMPERATURE °C: UPPER BACK	33.50 ± 0.49		32.93 ± 0.48		33.34 ± 0.68		33.33 ± 0.45	
BASE OF TAIL SKIN TEMPERATURE °C: LOWER BACK	31.68 ± 0.40		32.93 ± 0.40		32.86 ± 0.50		32.02 ± 0.17	
MEAN SKIN TEMPERATURE °C	32.59		32.93		33.10		32.68	
DIFFERENCE (CORE - MEAN SKIN) TEMPERATURE °C	5.53		5.06		5.38		5.53	
SENSIBLE HEAT LOSS (W/m^2) & ($kcal/m^2/hr$)	48.10	41.36	48.34	41.58	48.48	41.69	46.02	39.58
TISSUE CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	8.70	7.48	9.55	8.22	9.01	7.75	8.32	7.16
INLET AIR TEMPERATURE °C	20.41		20.44		20.46		20.46	
AIR CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	3.95	3.40	3.87	3.33	3.84	3.30	3.77	3.24
NET RATE OF CHANGE IN BODY HEAT CONTENT IN EACH PERIOD. + = GAIN, - = LOSS (W/m^2) & ($kcal/m^2$)	-0.44	-0.38	-0.17	-0.15	-0.57	-0.49	-0.76	-0.65

TABLE 14. (D) THERMOMETRY & TISSUE CONDUCTANCE (Figs. 62,63)

NORMAL RAT 20°C AMBIENT TEMP. HIGH HUMIDITY CONDITIONS

TIME PERIOD	11		12	
CORE TEMPERATURE (AUDITORY MEATUS) °C	37.71 ± 0.20		37.74 ± 0.20	
DORSUM SKIN TEMPERATURE °C: UPPER BACK	32.23 ± 0.30		32.86 ± 0.42	
BASE OF TAIL SKIN TEMPERATURE °C LOWER BACK	31.91 ± 0.16		31.89 ± 0.06	
MEAN SKIN TEMPERATURE °C	32.07		32.38	
DIFFERENCE (CORE - MEAN SKIN) TEMPERATURE °C	5.64		5.36	
SENSIBLE HEAT LOSS (W/m^2) & ($kcal/m^2/hr$)	47.49	40.84	45.07	38.
TISSUE CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	8.42	7.24	8.41	7.23
INLET AIR TEMPERATURE °C	20.46		20.47	
AIR CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	4.09	3.52	3.78	3.25
NET RATE OF CHANGE IN BODY HEAT CONTENT IN EACH PERIOD. + = GAIN, - = LOSS. (W/m^2) & ($kcal/m^2$)	-2.32	-2.00	-2.36	-2.03

Tissue conductance in ($W/m^2/^oC$) or ($kcal/m^2/hr/^oC$)

$$= \frac{\text{Sensible heat loss } (W/m^2) \text{ or } (kcal/m^2/hr)}{\text{Difference in core - mean skin temperature } ^oC}$$

Air conductance can be likewise calculated by dividing sensible heat loss by the difference between skin temperature and calorimeter ventilating air (operating) temperature. Conductance varies inversely with insulation and reflects peripheral blood flow. It will therefore have a minimum value in order to maximally conserve heat.

Consideration of tissue conductance in the rat under study in Figs. 62, 63 and 64 permits a numerical evaluation of the rate of cooling imposed on the rat by the internal recirculation of ventilating air within SEC - A - 04 L. During periods 1 and 2 the cooling effect is pronounced. The net rate of loss of body heat is 2.16 W and 2.34 W in each 20 minute period. In response, the rate of tissue heat conductance in the rat is at a low value, 7.9 $W/m^2/^oC$ and 8.04 $W/m^2/^oC$ respectively, to conserve body heat. By period 3 this process has been successful and the rate of heat gain exceeds loss by 0.3 W. Tissue heat conductance has increased to 8.8 $W/m^2/^oC$ and the rat no longer requires to conserve body heat quite so actively as in periods 1 and 2. In period 4 the increased rate of tissue conductance persists at

8.77 $W/m^2/^oC$, and the rate of whole body heat loss again slightly exceeds heat production by 0.65 W. By period 5 net body heat content has decreased still further with a net rate of heat loss of 1.38 W. But during this period the rat attempted to conserve body heat by lowering the rate of tissue heat conductance to 7.81 $W/m^2/^oC$. In period 6 this thermoregulation correction has been successful resulting in a net rate of gain in body heat content of 0.28 W, though the process of overall body heat conservation persisted judging by the continued low rate of tissue heat conductance at 7.78 $W/m^2/^oC$. During period 7 the rat did not attempt to conserve body heat as the rate of tissue heat conductance had again increased, to 8.7 $W/m^2/^oC$. This resulted in a net rate of loss of body heat content of 0.44 W. In periods 8 and 9 the rate of tissue heat conductance continued to remain high at 9.55 $W/m^2/^oC$ and 9.01 $W/m^2/^oC$ and the previous pattern was repeated with small net rates of loss of body heat content of 0.17 W and 0.49 W. Calorimetry conditions at this point in the experiment did not appear to be unduly cooling the rat as it failed to respond by decreasing its rate of tissue heat conductance. During successive time periods however (10, 11 and 12) the net rate of loss of body heat content continued to increase progressively in each period by 0.76 W, 2.32 W and 2.36 W respectively. The rates of tissue heat conductance again fell to counteract this, to 8.32, 8.42 and 8.41 $W/m^2/^oC$ respectively.

The calorimetry measurement was terminated at the end of period 12, the pattern of rate of change in body heat content and rates of tissue heat conductance in periods 11 and 12 being similar to those noted in periods 1 and 2. The net rate of loss of body heat content was small over the entire run, at 0.84 W/m^2, but the average rate of tissue heat conductance did reflect this slight cooling effect, at 8.39 $W/m^2/^oC$ indicating vasoconstriction in the rat.

It is possible that the use of a thermistor harness and leads to measure body temperature in the rat may increase the rate of cooling of the rat while in SEC - A - 04 L. Bartlett et al. (1958) postulated that even the slight restraint of a shoulder harness (Fig. 40) could result in a lowering of the skin "insulation" properties in the rat. The only way of determining the accuracy of this statement would have been to carry out surface temperature measurement in the rat using a radiometer with the insertion of some form of implant capable of telemetry for core temperature measurement. The latter was beyond our technical resources, though skin radiometry measurements were carried out. These are shown in Fig. 42 and indicated that thermistor skin measurements may over-estimate the skin temperature as determined by radiometer by up to 1.3^oC in moving air conditions. This did not apply to core temperature measurement. Radiometer measurement therefore increased the "apparent" core - skin

temperature difference. Thus in Figs. 62 and 63 based on radiometer measurement, the rate of tissue conductance for the entire run would have been 6.78 $W/m^2/^oC$ rather than the thermistor measured 8.39 $W/m^2/^oC$, a reduction of 19.2%. Tissue conductance values given elsewhere (Caldwell et al., 1966) are derived from thermistor or thermocouple leads. The value of rates of tissue heat conductance measurements lie in their relative change between experiments or during the course of a single experiment. In the SEC - A - 04 L calorimetry system, resting rat rates of tissue heat conductance seem reproducible and comparable with published values. Figures given in this thesis therefore are based exclusively on thermistor temperature measurement. Table 15A, calorimetry run 10/10/A (Fig. 57) at 20^oC ambient temperature with the rat under resting (non active) conditions, gives an indication of the agreement or similarity in tissue heat conductance values found at identical ambient temperatures in normal rats. See also Tables 14A, B, C and D.

Figure 65 shows data from rat body and calorimeter wall and air temperatures during calorimetry runs at 20^oC ambient temperature. The rate of tissue heat conductance is based on the temperature difference between core and skin temperatures. The mean difference diminishes after the 50th to the 60th minute. The rate of tissue heat flow therefore slightly increases during the course of the

TABLE 15A THERMOMETRY & TISSUE CONDUCTANCE

CONTROL RATS AT 20°C & 30°C : AND 20% BSA BURN

	PERIOD A		PERIOD A							
DATE	10/10/A (Fig.)		3/9/A (Fig.)		18/10/A (Table)		22/10/A (Table)		24/10/A (Table)	
POST BURN DAY	Control 20°C		Control 30°C		1		5		7	
CORE TEMPERATURE (AUDITORY MEATUS) °C.	37.26 ± 0.20		38.10 ± 0.13		38.54 ± 0.22		38.38 ± 0.15		36.75 ± 0.18	
SKIN TEMPERATURE °C DORSUM	31.27 ± 0.18		35.84 ± 0.10		-		-		-	
BURN WOUND TEMP. °C.	-		-		29.56 ± 1.2		30.14 ± 0.02		32.80 ± 0.20	
DIFFERENCE (CORE-SKIN/BURN) TEMP. °C.	5.99		2.26		8.98		8.24		3.95	
SENSIBLE HEAT LOSS (W/m^2) & ($kcal/m^2/hr$)	45.29	38.95	30.18	25.96	56.20	48.34	54.33	46.72	50.85	43.73
TISSUE CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	7.56	6.50	13.35	11.49	6.26	5.38	6.59	5.67	12.87	11.07
INLET AIR TEMPERATURE °C.	20.13		29.58		20.19		20.13		20.09	
AIR CONDUCTANCE ($W/m^2/°C$) & ($kcal/m^2/hr/°C$)	4.07	3.50	4.82	4.15	6.00	5.16	5.43	4.67	4.00	3.44

TABLE 15B THERMOMETRY & TISSUE CONDUCTANCE

20% BSA BURN

DATE	29/10/A (Table)		31/10/A (Table)		2/11/A (Table)	
POST BURN DAY	12		14		16	
CORE TEMPERATURE (AUDITORY MEATUS) ^{o}C.	38.38 $\pm$ 0.09		37.60 $\pm$ -		36.90 $\pm$ 0.30	
BURN WOUND TEMPERATURE ^{o}C.	33.81 $\pm$ 0.10		34.16 $\pm$ 1.47		31.87 $\pm$ 0.17	
DIFFERENCE (CORE - BURN) TEMP. ^{o}C.	4.57		3.44		5.03	
SENSIBLE HEAT LOSS (W/m^2) & ($kcal/m^2/hr$)	56.54	48.62	52.77	45.38	52.60	45.24
TISSUE CONDUCTANCE ($W/m^2/^{o}C$) & ($kcal/m^2/hr/^{o}C$.	12.37	10.64	15.34	13.19	10.46	8.99
INLET AIR TEMPERATURE ^{o}C.	20.03		20.07		20.04	
AIR CONDUCTANCE ($W/m^2/^{o}C$) & ($kcal/m^2/hr/^{o}C$)	4.10	3.53	3.75	3.22	4.45	3.82

calorimetry run. This does not indicate vasoconstriction or a response to an environment or airflow conditions which significantly "cool" the rat under study.

Similar temperature measurements were carried out during calorimetry runs at 30°C ambient temperature. These are shown in Figure 66. There is a progressive fall in core temperature and a rise in skin temperature up to the 60th minute. The rate of tissue heat flow therefore continues to increase up to the 60th minute, but thereafter remains stable. Table 15A gives the rate of tissue heat conductance for a normal rat undergoing calorimetry at 30°C ambient temperature, in calorimetry run 3/9/A (Fig. 59). The measurement period A began 57 minutes after the run started and ended at the 93rd minute. The mean skin temperature was approximately 4.5°C greater than that found at 20°C ambient temperature. The difference between core and skin temperature was 2.26°C. The rate of tissue heat (flow) conductance was increased at 13.35 $W/m^2/°C$. This calorimetry run at 30°C ambient temperature can be favourably compared with the control rat values found by Caldwell et al. (1966) during calorimetry runs at the same ambient temperature on the 36th post-burn day of his study. The rate of tissue heat conductance in Caldwell's rats was 12.17 kcal/kg/hr/°C. In Figure 59, the value found was 11.49 kcal/kg/hr/°C (expressed in the same units). Air conductance rate in Caldwell's study was 5.45 kcal/kg/hr/°C versus 4.15 kcal/kg/hr/°C in Figure 59. The core and skin temperatures

found by Caldwell in rats at 30°C ambient temperature were 38.3°C and 35.8°C respectively. In Figure 59 (Table 15A, run 3/9/A), rat mean core temperature was 38.1°C, mean skin temperature 35.84°C.

On the basis of the results presented in this thesis, I agree with the views expressed by Pullar et al. (1967) and Dale et al. (1967), that differences between direct and indirect calorimetry indicate the change in body heat content which occurs, providing that the measurement period is sufficiently long. At ambient temperatures within their calorimeters of 22 - 23.5°C, both authors found that heat production exceeded heat loss by 4 - 8%, with a net gain in body heat storage.

At 20°C ambient temperature in the rat, in higher airflow conditions than those used by Pullar et al. or Dale et al., heat loss exceeded heat production by a mean of 2.4 $\pm$ 1.78% (SD) in 16 calorimetry runs performed using 2 tame and fully trained rats under resting conditions. The results are given in Table 11. The average measurement period was 86 minutes and the rats did not wear thermistor shoulder harnesses. They were completely unrestrained within SEC - A - 04 L. The lack of stress in these rats may be inferred from the observation that rat number 8 did not urinate or defaecate at all during prolonged calorimetry runs, and that rat 1 urinated/defaecated on only 4 out of 10 runs, again of

long duration. Untrained rats always urinated or defaecated when being handled and placed in SEC - A - 04 L.

R.Q. measurements were "physiological" for post-absorbtive resting rats during these runs. Further details of these runs are given in Figures 148, 149, 153 and 154.

By allowing the rats under study to settle undisturbed in SEC - A - 04 L, core and skin temperatures "equilibrate" with calorimeter conditions during the first 50 minutes at 20°C and at 30°C ambient temperatures. This is also the time necessary for calorimeter wall temperature to stabilise. It can be inferred from the data shown in Figures 62 and 63 that 40 - 60 minutes is the minimum measurement period which will give a representative value for heat exchange when the rat under study is active. Time periods where the rat is at rest, and all instrument recordings are stable, are the only intervals which may be used for accurate comparison of direct and indirect calorimetry. When these criteria are all met, as is the case in each of the calorimetry runs described in Table 11 then the SEC - A - 04 L calorimetry system in the Institute of Physiology is capable of a degree of accuracy in the measurement of heat production and partitioned heat loss which is superior to that published for other gradient layer calorimetry systems. The ability to compare direct heat loss and indirect heat production to better than 2.4% mean difference (where 68% of the results could be

expected to lie within the range 0.6 to 4.2%, heat loss exceeding production), means that the SEC - A - 04 L calorimetry system is functioning within the limits of its design specification.

Where short term studies of changes in rat energy metabolism in response to drugs or ambient temperature changes are the objective, then body temperature measurements (thermometry) during the calorimetry run is essential in the proper evaluation of the results.

In the rat, wearing a shoulder Velcro harness with attached thermistors in order to measure body temperatures may of itself alter the resting steady state metabolism of the rat by "lowering" skin insulation value. The maximum change in body heat content possible in the calorimetry runs detailed in Table 11 in rats not wearing shoulder harnesses was less than the likely error or overestimation in sensible heat loss imposed by wearing them. Figures 55 and 56 indicate a mean sensible heat loss of 5.79 W/kg in rats at 20^{o}C ambient temperature without harnesses versus a sensible heat loss of 6.76 W/kg in a harnessed rat. The 18% increase in sensible heat loss associated with the harness is a typical figure though only 3 calorimetry runs are cited here to illustrate the point.

In uninjured rats at 20°C ambient temperature, thermometry did not contribute materially to the determination of resting metabolic energy expenditure. The process of calorimetry itself with high airflow conditions around the rat did impose a very small cooling effect upon the rat which it compensated for by adjustments in the rate of tissue heat conductance within 50 minutes from the start of exposure to calorimetry conditions.

At 30°C ambient temperature, exposure to calorimeter airflow conditions was also compensated for in the rat by an increment in the rate of tissue heat conductance which reached a stable value within 50 - 60 minutes from the start of the calorimetry run.

It must be accepted on the basis of other workers' findings (Kleiber et al., 1956; Kleiber, 1961) that values for RME in the uninjured rat in the present studies may exceed BMR by up to 50%.

DIRECT CALORIMETRY - BURNED RATS

The 20% of body surface area (BSA) full skin thickness dorsal burn inflicted on rat number 1, using techniques described elsewhere, proved a significant injury which caused weight loss after burning in the rat (Figs.124 and 125) comparable to that seen after an extensive burn in a human patient (Cuthbertson, 1976; Davies et al.,

1977(1).) Rat 1 lost 18% of its initial weight by the 4th week after burning. In this section I was primarily concerned with assessing the technical performance of the calorimetry system itself in relation to the problems encountered with a burned rat over a prolonged post-burn period, rather than forming views on the nature of the metabolic response to burn injury on the basis of changes in a single animal. However, it was difficult to separate these concepts. Technical and biological effects have inevitably become intermingled. The results of a detailed study of the daily changes in energy expenditure in a single rat are however of general relevance to later burn studies where the animals' injury, dietary intake, and environmental conditions are known and controlled.

Figures 67 and 68 demonstrate that a 20% BSA burn in the rat causes large increases in insensible (evaporative) heat loss. This finding is well recognised in the rat and in man (Moyer and Butcher, 1967). The absolute amounts of evaporative heat loss measured after the burn are given in Table 13. It is evident that a marked rise in evaporative heat loss occurred between the 12th and 14th post-burn day, from 2.07 W/kg, up to 3.47 W/kg respectively, increasing the proportion of evaporative heat loss from 19% to 30% of the total. Total airflow rate in the calorimeter was increased by 32% from 4.99 l/min to 6.59 l/min between the runs on the 12th and 14th post-burn days. It was important therefore to determine whether the

increase in evaporative heat loss measured at this time represented an increment due to change in the rat's skin, as for example, the burn eschar separating and the burn wound becoming more permeable to water loss, or whether the rise in evaporative heat loss was due to more rapid airflow around the rat convecting additional heat from the burn wound surface, irrespective of whether this was more water permeable or not.

Table 13 shows that increased evaporative heat loss made up on average 74% of the increased sensible heat loss on the runs between the 1st and the 12th post-burn day. This finding is consistent with the view that increased evaporative water loss is a major cause of post-burn hypermetabolism (Arturson et al., 1977). The remaining 26% increase in sensible heat loss could be attributed to the general effects of tissue damage found with any non burn soft tissue injury (Fig.1).

From the 16th to the 29th post-burn day, increased evaporative heat loss exceeds increased sensible heat loss by 66%, either indicating that airflow rate within the calorimeter is so high that the burned rat was rapidly losing stored body heat during the calorimetry run, or that Wilmore's theory regarding the aetiology of post-burn hypermetabolism is correct and that evaporative heat loss is not the cause of the increased metabolic rate after burning (Wilmore, 1977; Wilmore et al., 1976). Wilmore

believes that the post-burn increase in metabolism is due to hypothalamic "resetting" of thermoregulation.

In the calorimetry runs on the 35th and 37th post-burn days, total airflow rate is still high at 6.73 l/min (the same level as the runs between the 16th and 29th post-burn day), but the increase in evaporative water loss no longer exceeds the increased sensible heat losses. It seems most likely that the burn wound did become more water permeable (due to eschar separation) around the same time as the total calorimeter airflow was reset to a higher rate. The burn wound began to heal around the 35th to the 37th post-burn day, shrinking in size and becoming less porous.

The question of loss of body heat content during calorimetry runs by increased convection due to the rapid airflow conditions around the rat when its burn eschar separates is a complex problem which raises many issues. These are further discussed in regard to the 25% BSA burn results.

Measurements of body temperature changes during calorimetry runs were made on the burned rat. The results are shown in Figure 70. Rates of tissue heat conductance during these calorimetry runs were calculated for rat number 1 before and after injury. These values are given in Tables 15A and 15B.

Tissue heat conductance rate in the burned rat is less than the pre-burn value of 7.56 $W/m^2/^oC$, at 6.26 and 6.59 $W/m^2/^oC$ on the 1st and 5th post-burn days respectively. This indicates that the burned rat retains the ability to vasoconstrict appropriately in the face of increased evaporative heat losses. This ability was lost by the 7th post-burn day and remained absent during the rest of the post-burn runs in which thermometry could be carried out (till the 16th post-burn day). The average rate of tissue heat conductance over this period was 12.76 $W/m^2/^oC$, which would be an inappropriate thermoregulatory response in face of a cooling load if the rat were normal, and was not far short of the tissue conductance value of 13.18 $W/m^2/^oC$ found in an uninjured rat at 30^oC ambient temperature (Fig. 59).

Burn wound surface temperature was lower during the calorimetry runs on post-burn days 1 and 5 at 29.6^oC and 30.1^oC, compared with the average value on post-burn days 7, 12, 14 and 16 of 33.2^oC, reflecting the degree of increased sensible heat production.

Figure 70 indicated that a progressive loss of rat body heat content did not occur during the calorimetry runs on post-burn days 1, 3, 7 and 12. In the calorimetry run on post-burn day 16 there was evidence of a rapid loss of body heat content over the first 60 minutes of the run with relative stability for the remainder of the run.

There was no fluctuation in the rate of tissue heat conductance during this latter portion of the run. This did suggest that a total airflow rate through SEC - A - 04 L of 6.59 l/min decreased body stored heat in the burned rat. But, if the same criteria are applied to this run as for calorimetry runs on normal rats, and a 50 - 60 minute period is allowed to elapse at the start of the run before making measurements, then no net change in rat body heat content should occur during the latter portion of the calorimetry run when heat loss is measured. This is not to say that the rat under study did not increase its rate of heat production slightly during the early part of the calorimetry run to compensate for the increased cooling due to a high rate of airflow around it. This would make the measurement of RME higher than the true value by a small amount (around 7 - 10% in Fig. 70 post-burn day 16).

It is of interest to note that the rat tissue heat conductance rates given in Tables 15A and 15B are similar to the rates of heat conductance in man before and after burning injury, even allowing for differences in ambient temperature and in methods of calculation. Wilmore (1978) and Wilmore et al. (1976) gives a tissue conductance value of 6.64 kcal/m^2/hr/^{o}C for normal man at 25^{o}C and a value of 14.21 kcal/m^2/hr/^{o}C at 33^{o}C ambient temperature. This may be compared with uninjured rats at 20^{o}C and 30^{o}C ambient temperatures which have tissue conductance values

of 6.5 kcal/m^2/hr/^oC and 11.49 kcal/m^2/hr/^oC respectively. Note: Wilmore calculates tissue conductance using heat production values. Sensible heat loss values are used in the rat to make the same calculation.

Burned man nursed at 25^oC ambient temperature has a tissue conductance of 12.7 kcal/m^2/hr/^oC versus an average value of 11.0 kcal/m^2/hr/^oC in the rat at 20^oC ambient temperature measured at a similar time after injury as in man. Caldwell et al. (1966) gives a value of 11.0 kcal/m^2/hr/^oC for burned rats at 27.9^oC ambient temperature.

Therefore burned rat and burned man have similar characteristics, despite the differences between rat and human skin, in that both show evidence of increased peripheral blood flow after burn injury and cannot adequately reduce skin blood flow and effectively insulate when exposed to a cold environment. On these grounds the rat is a suitable subject in which a burn injury model relevant to human patients might be created.

The use of body temperature measurement in burned rats during calorimetry runs was not proved necessary where the objective was to study long term changes in energy expenditure after burns. Instead, the alternative technique of combined direct and indirect calorimetry proved to be an adequate check on the accuracy of each

calorimetry run, provided that the difference during the measurement period (minimum time 50 minutes) between heat loss and heat production was less than 5% and that RQ values were acceptable as outlined previously.

When used thus, the SEC - A - 04 L calorimetry system built in the Institute of Physiology, University of Glasgow, was found to be capable of following changes in partitioned heat losses in burned rats accurately. Because of the apparent similarity in thermoregulation after burn injury in rat and man, this calorimetry system was capable of providing information from studies on burned rats of real relevance in improving the care offered to burned patients.

DEVELOPMENT OF CONTROLLED ENVIRONMENT CONDITIONS

MATERIALS and METHODS

RAT CONTROLLED ENVIRONMENT CHAMBER: (MACROENVIRONMENT)

DESIGN CONSIDERATIONS AND USE

A statement made by Sargent (1957) defines the importance of environmental influences upon experimental animal laboratory observations. "The organism must be thought of as a whole, not the sum of separate parts. The microenvironment contains the immediate effector system, but this environment is in equilibrium with or affected by an environment extending to the sun and perhaps further." Therefore in order to study the metabolic consequences of burn injury in the rat free from uncontrolled influences of a disturbing nature (Lane-Petter, 1973), special animal housing facilities were designed and constructed which permitted control of the macroenvironment in regard to ambient temperature, ventilation rate, dark-light cycle, background sound level, odour, and psychological stress.

The rat living area and the experimental working area had also to:

1. Conform to the biological needs of the animal

2. Be convenient for access by the staff

3. Incorporate all the provisions for animal care required under the Home Office Animal Act

4. Meet the University of Glasgow regulations for the use of radioactive substances

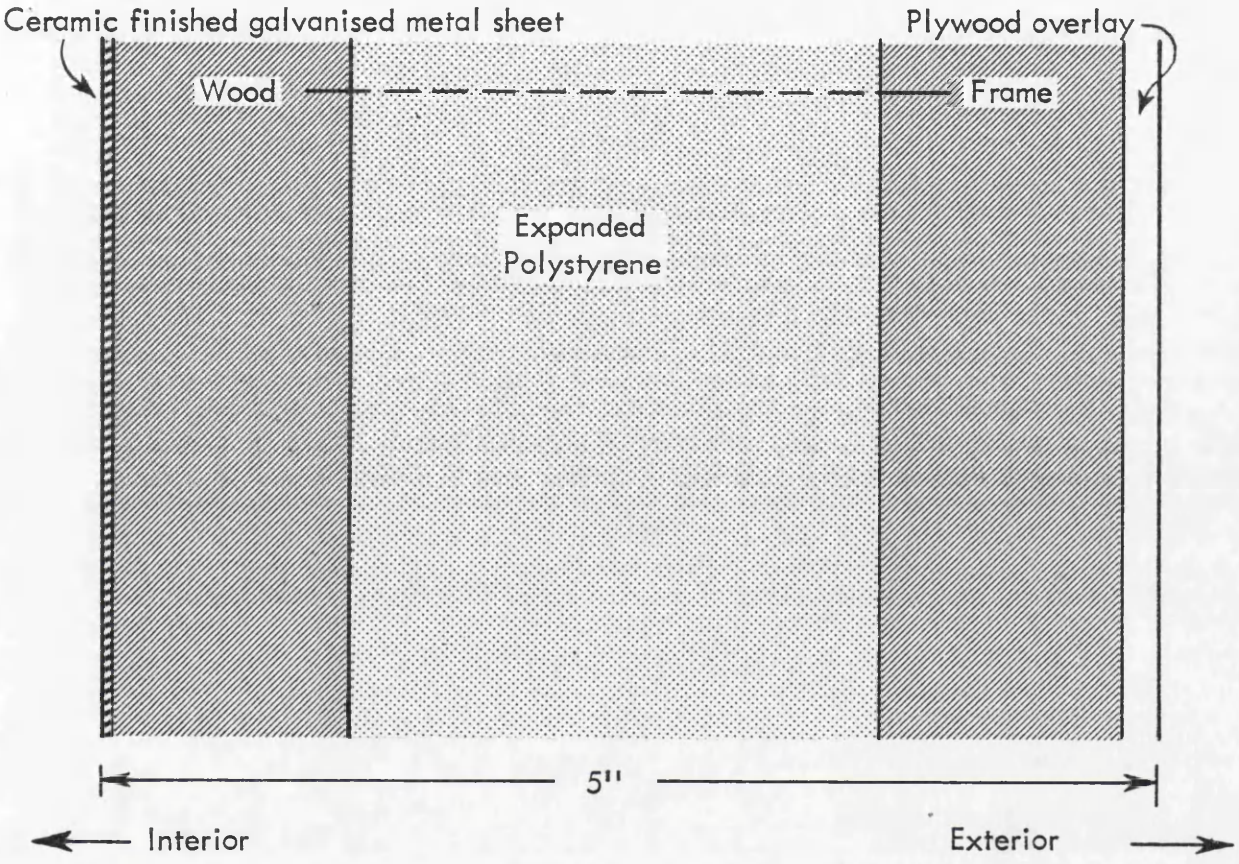
5. Be adjacent to the calorimeter laboratory.

The plan view of this construction is shown in Figure 14.

Environmental chamber specification:

A chamber measuring 9' x 5' x 6'2" (length, breadth, height) was constructed in the corner of an existing empty laboratory. The walls were framed in timber, and built with square cavities within the depth of the wall. Into these cavities expanded plastic foam blocks were fitted and cemented into place (Fig. 74). Wooden planking formed the roof with expanded plastic blocks glued to its under surface. Galvanised mild steel sheets with a white ceramic finish were then overlaid throughout the interior of the chamber and the edges flush fitted so that the surface was free of dust traps. This design of wall acted as a very efficient insulator against the transmission of noise

Fig.74 SECTION OF ENVIRONMENTAL CHAMBER - SHOWING INSULATED CONSTRUCTION



and heat. The chamber floor was formed of wooden planking covered by thick soft vinyl sound deadening material fitted to the walls by a system of bevelling and coving (Fig. 75), enhancing the effectiveness of chamber washing down procedures. The construction work of this chamber was carried out by a contract joiner and the author. The outer wall of the environmental chamber facing the animal handling laboratory (Fig. 76) was finished with an overlay of plywood which extended from floor to ceiling. This false front above the chamber proved necessary to reduce the noise from the air conditioning fan unit (Figs. 76 and 77). Initially a sliding door was considered, but later excluded because of cost and poor air sealing. A commercial 'meat safe' door was purchased as a cheaper alternative with a very efficient rubber gasket air seal and an internal construction similar to that of the chamber walls.

Temperature variation caused by opening the chamber door for access is minimised by the use of a large capacity (3 kW) heating system incorporating a special sensor which responds quickly to heat losses from the chamber. This heating system is shown in Figures 77, 78 and 79.

Fig.75 WALL-FLOOR JUNCTION IN ENVIRONMENTAL CHAMBER

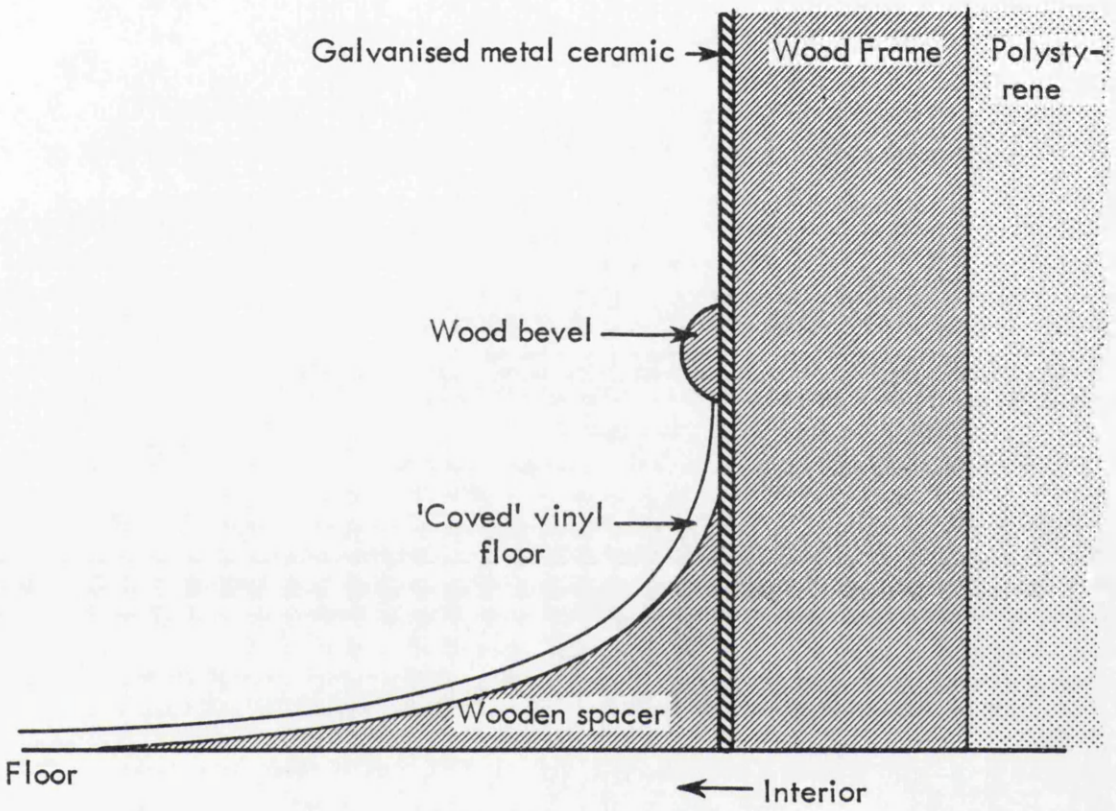


Fig76 EXTERIOR OF ENVIRONMENTAL CHAMBER VIEWED FROM ANIMAL HANDLING LABORATORY (photograph of chamber exterior)

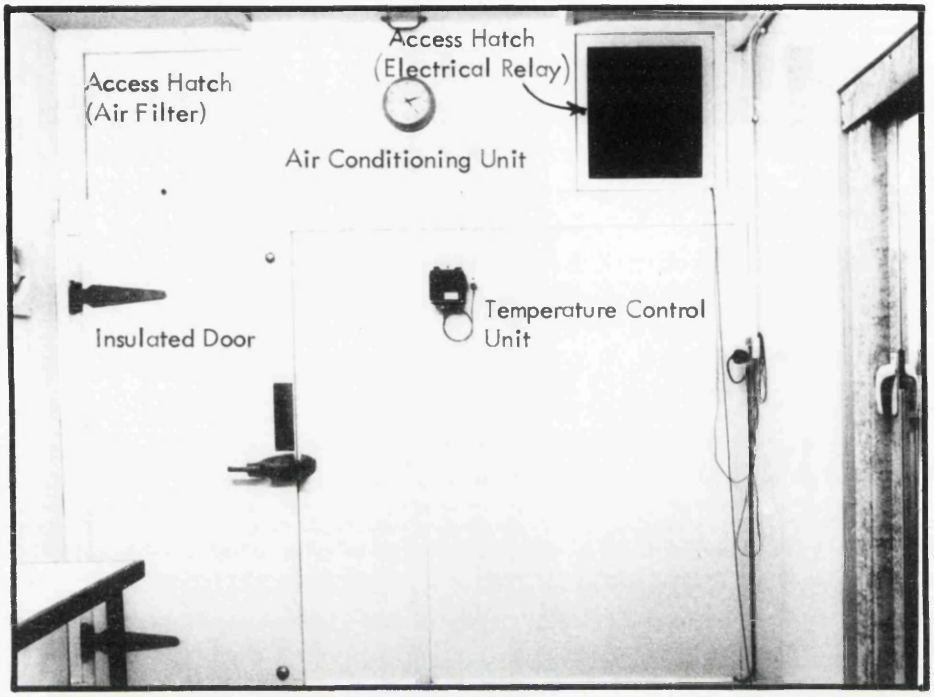


Fig.77 SCHEMATIC DIAGRAM OF ENVIRONMENTAL CHAMBER- SIDE ELEVATION

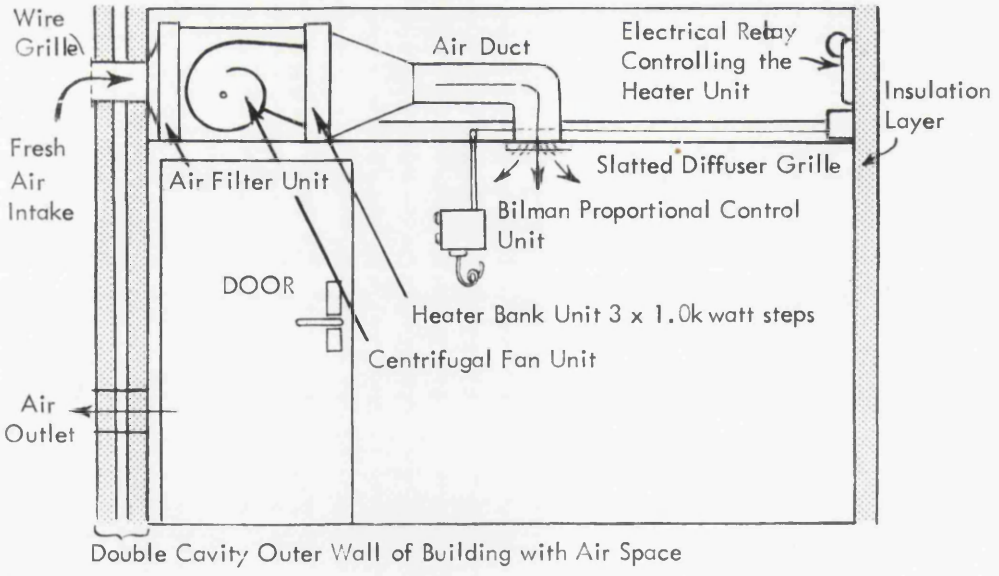


Fig.78 PLAN VIEW OF AIR CONDITIONING/HEATING UNIT AND VENTILATION CIRCUIT OF ENVIRONMENTAL CHAMBER.

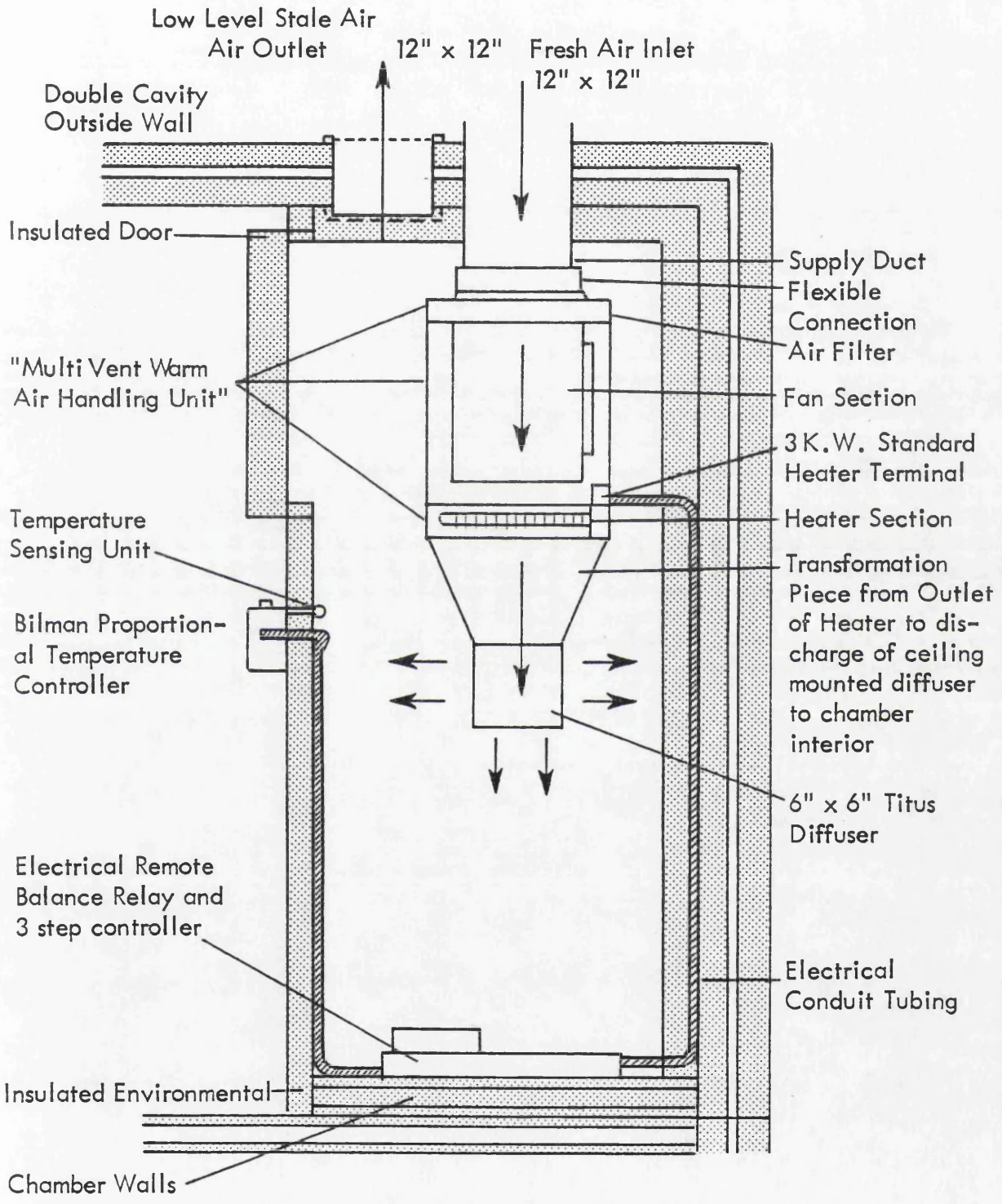
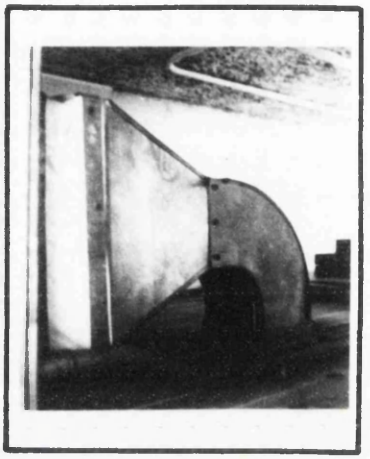


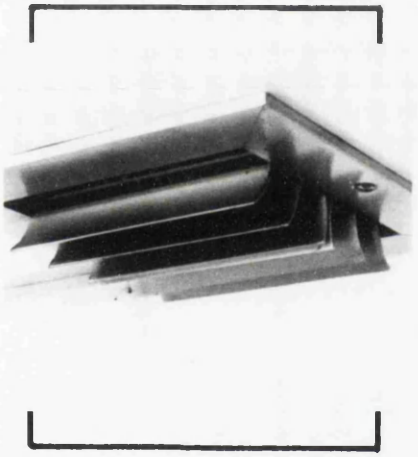
Fig.79 ENVIRONMENTAL CHAMBER AIR CONDITIONING UNIT

MULTIVENT AIR HANDLING UNIT
Showing

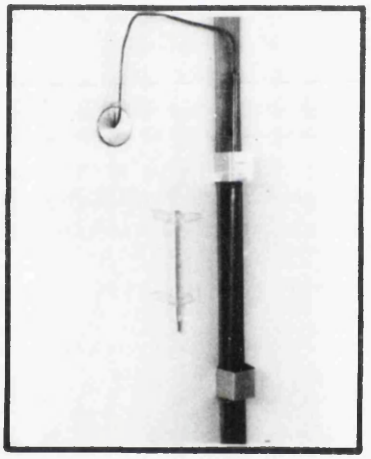
(A.) Filter and Fan Section and also Transformation Piece and Roof Inlet (B.)



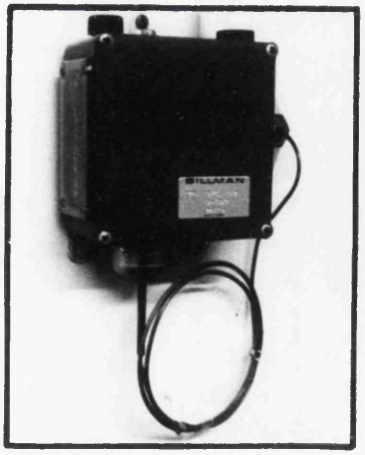
(C.) Titus Air Diffuser in Ceiling



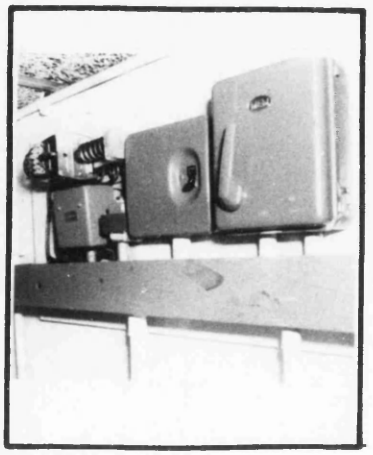
Temperature Sensing Unit (D.)



(E.) Bilman Type TD 7K controller



Electrical Remote Relay + 3step controller (F.)



Air conditioning and ventilation system specification:

Fresh air is drawn from outside the building, filtered, blown through a heater unit and distributed to the chamber interior by a Multivent Warm Air Handling Unit.

The Filter Section: consists of a 2" thick glass fibre air filter in an 18 G steel frame with a wide withdrawal point for easy access through the hatch above the chamber (checked monthly).

The Fan Section: comprises a centrifugal blower with a self-starting 250 v single phase motor resiliently mounted in the path of the fresh air inlet. This circulates 50 cub. ft. air/min at 0.4 standard water gauge achieving considerably in excess of the Home Office recommended 12 changes of air per hour, in approved animal units.

The Heater Section: has 3 pairs of 0.5 kW tubular black heat elements finned to avoid overheating, and fitted with an automatic high temperature safety cutout device in case of fan failure. The whole assembly is housed in a robust sheet steel chamber with removable side access panels and lined with a sound absorbing material. Discharge is via a flexible coupling to a 'transformation piece' and then to a ceiling mounted air diffuser. Air flow pattern within the chamber can therefore be altered by moving the angle of the vanes in the diffuser.

The chamber was designed to operate at 20 $\pm$ 1°C or at 30 $\pm$ 1°C. No provision was made for a refrigeration system as the air temperature in Scotland rarely rises above 20°C in the warmest part of the year. The decision to use a 'heating only' system was governed by cost. If refrigeration should be required for subsequent studies, the chamber design is such that a suitable air cooling unit can be mated to the 'transformation piece' on the roof with little modification to the existing structure.

A simplified circuit diagram of the heating system is shown in Figure 80. This is controlled by a Bilman TD7 temperature controller fitted with a bulb type K temperature sensing element. This works on a vapour pressure principle and consists of two main parts. The controller is shown in Figure 81.

The Temperature Sensing Unit: consists of a bulb with a capillary tube and bellows. This contains a fluid with a boiling point just below the lowest setting value of the temperature controller at atmospheric pressure. Ambient temperature determines the vapour pressure of the fluid which in turn determines the force in the bellows (Fig. 82).

The Contact Housing: contains a potentiometer which is operated by a balance arm arrangement, which compares

Fig.80 ENVIRONMENTAL CHAMBER. HEATING SYSTEM : CIRCUIT DIAGRAM

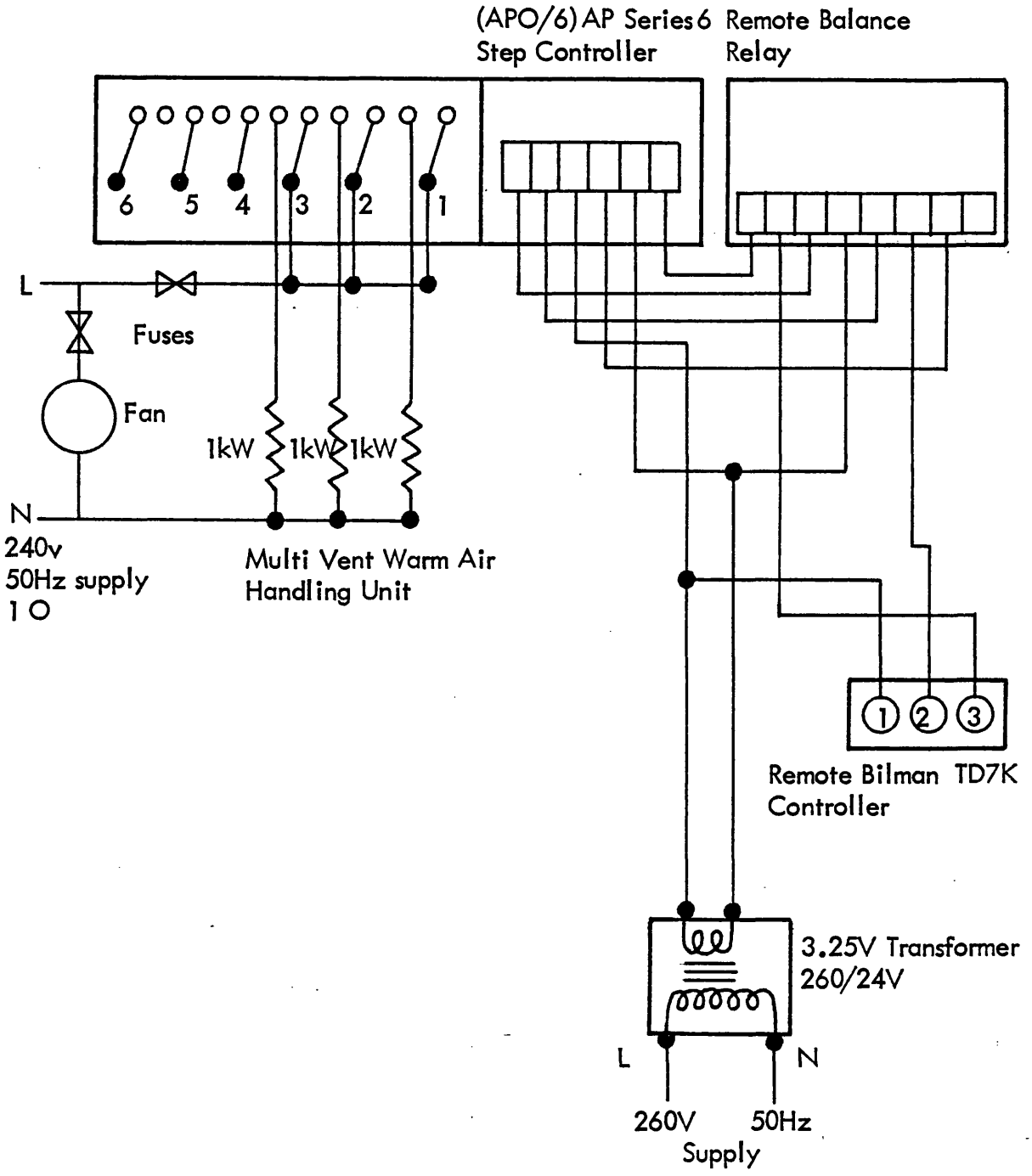
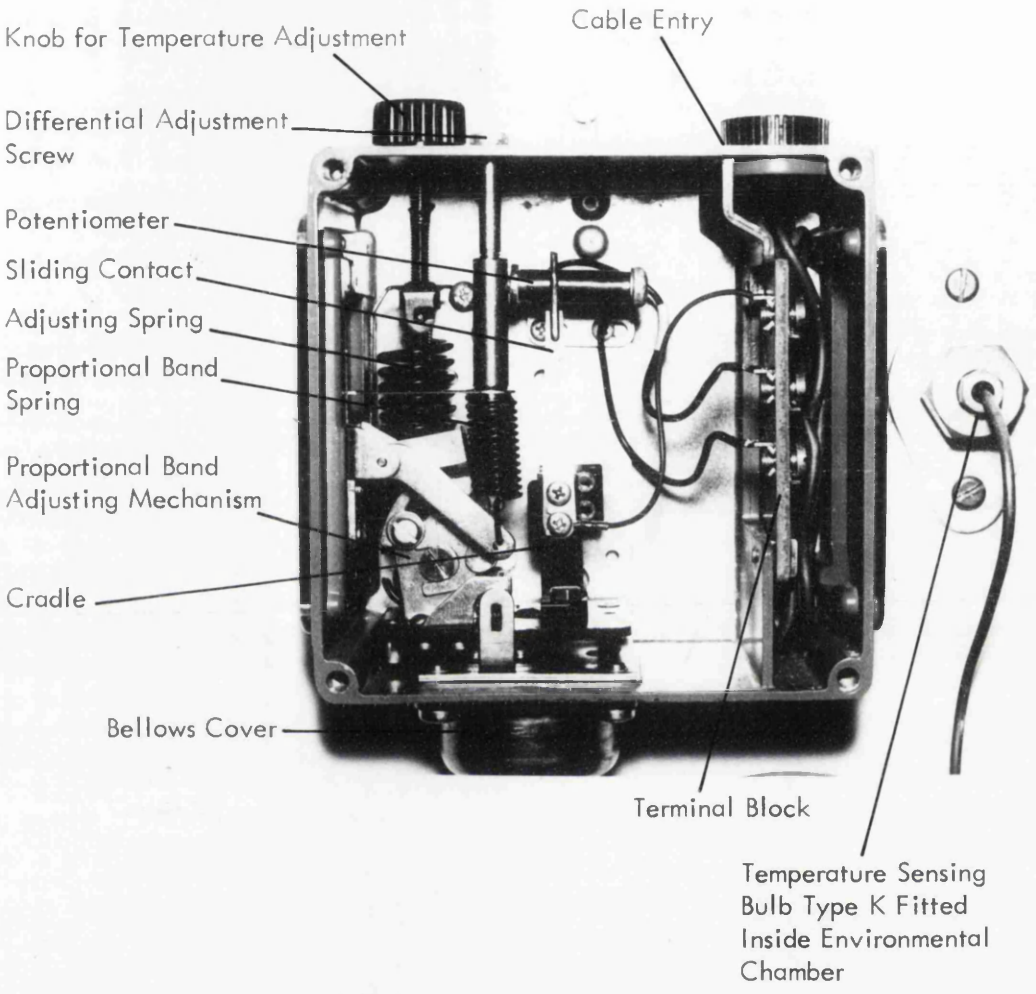
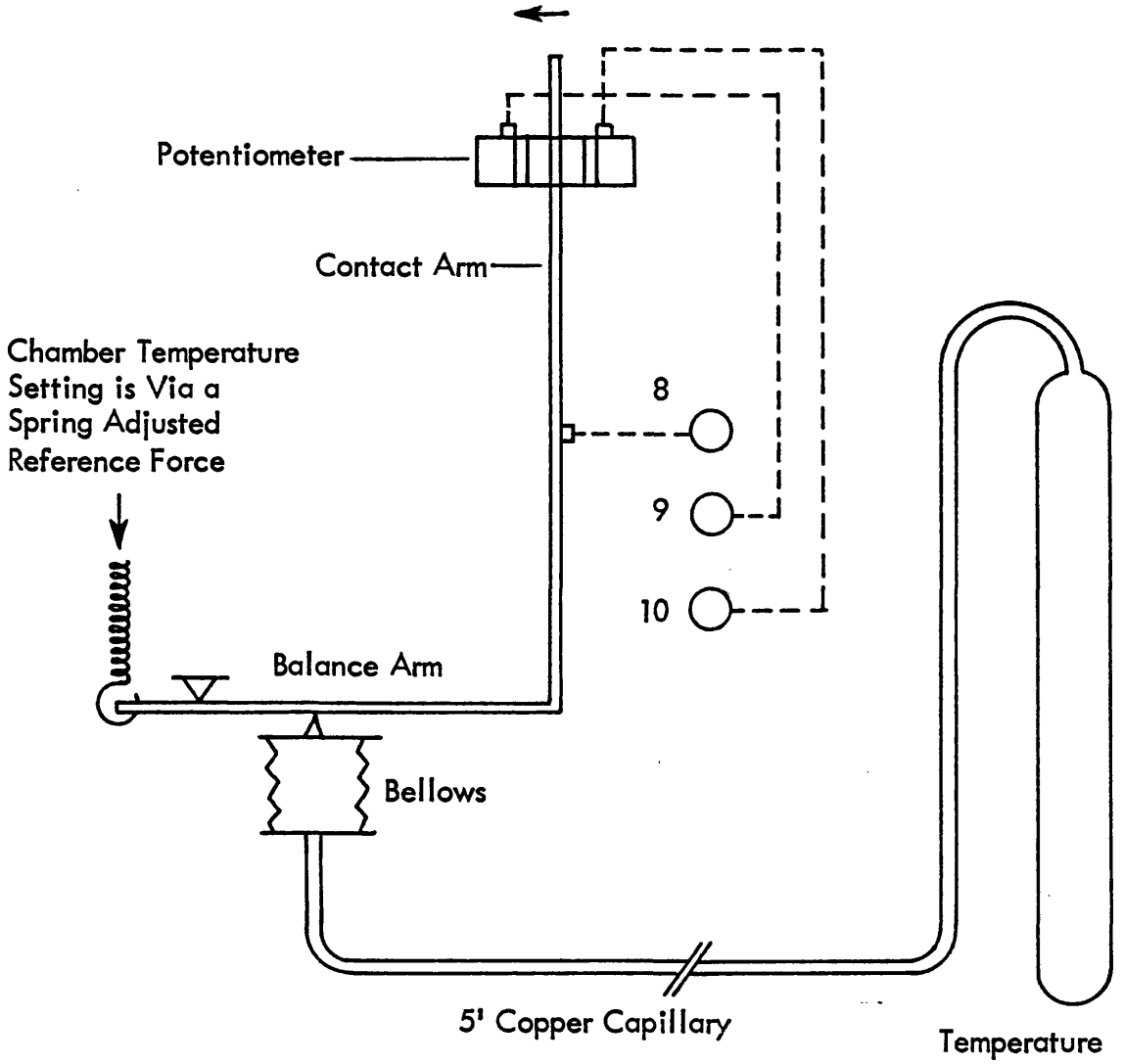


Fig.81 TEMPERATURE CONTROL UNIT FOR ENVIRONMENTAL CHAMBER



EXTERNALLY SITUATED UNIT FOR ENVIRONMENTAL CHAMBER

Fig.82 TD7 : BILMAN CONTROLLER : INTERNAL CONNECTIONS



The arrow shows the direction of movement of the contact arm on an increase in temperature

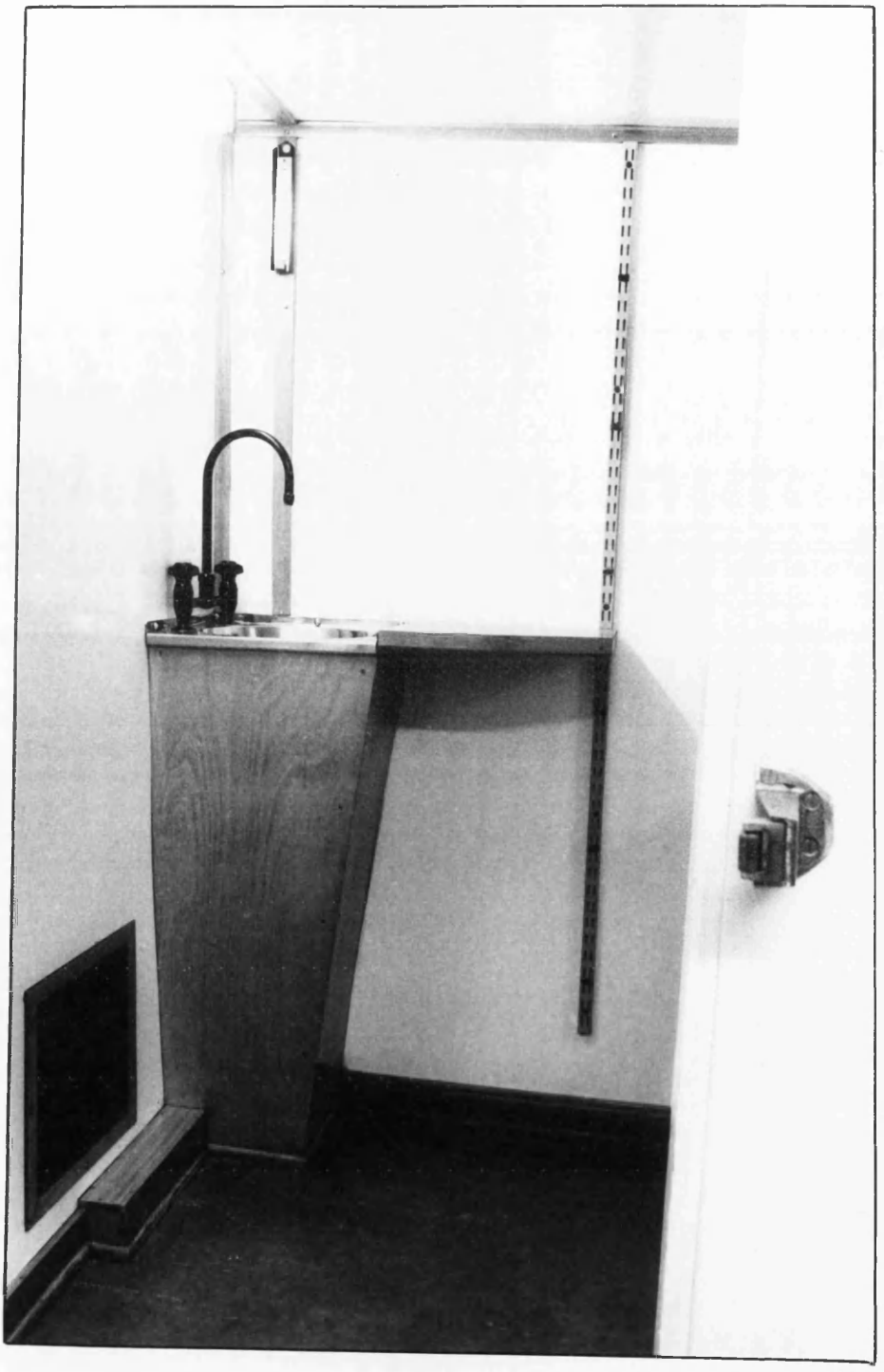
the bellows force with a spring adjusted reference force. The movement of the balance arm operates the potentiometer. The force of the reference spring is altered by the temperature adjustment knob. The proportional band of the temperature controller can be adjusted also by varying the tension in another spring (not shown in the diagram) by means of a screw located on top of the contact housing. The tension in this spring counteracts the movement of the balance arm. The amount of adjustment in this spring can be read on a graduated linear scale marked A to D. The minimum position at A gives the smallest proportional band. The same setting can give different values for different temperature set points, e.g. the proportional band at the lowest temperature setting for this control unit ($-10^{o}C$) would be 6 - $30^{o}C$ whereas at the highest temperature setting ($40^{o}C$) it would be 3 - $15^{o}C$.

Fitting of the Temperature Sensing Unit: Several positions within the chamber were tested to select the site which best indicated the average temperature of the chamber. The temperature sensing bulb was ultimately placed on the front wall of the chamber 5' from the floor, pointing downwards. The contact housing was fixed on the outer wall of the chamber 5'6" from the floor and connected via a 5' length of copper capillary tubing. Although intended for duct mounting, a type K sensor was found most suitable because of the high rate of air flow within the chamber.

Chamber Lighting: Two white fluorescent strip lights are fixed at ceiling level on each side wall (Fig. 14). A remotely sited time switch connected to mains electricity sets artificial daylight hours from 08.00 to 20.00 hours.

Chamber Washing Facilities: The injection and use of radioisotopes within the chamber required the provision of a small metal sink unit to satisfy safety regulations (Fig. 83). This was deliberately placed in the position of the greatest temperature variation in the chamber, i.e. facing the door. The rat wall cages and metabolic cage rack were positioned in the most temperature stable area. A small working surface was built beside the sink which was flush panelled in plywood.

Fig.83 ENVIRONMENTAL CHAMBER STAINLESS STEEL SINK UNIT WORK TOP



RAT CONTROLLED ENVIRONMENT CHAMBER: (MICROENVIRONMENT)

DESIGN AND CONSTRUCTION OF WALL MOUNTED CAGE RACKING SYSTEM:

Limited space and other factors made it necessary to design rat cages which offered low initial cost, flexibility in pattern of use, and which could be readily detached from the chamber and carried five floors down to the cage steriliser unit in the main Institute Animal House for periodic steam cleansing. The following system was adopted.

During construction of the environmental chamber, support brackets were built into the back wall to take vertically mounted 'Spur' racking strips. These were set along the back wall from floor to ceiling at 11" intervals (Fig. 84). From these strips specially adapted 'Spur' arms were inserted (Fig. 85), with edge welded pieces of angle iron projecting from the sides of the bracket. The rat cages are suspended between these projecting arms. Each cage is made from galvanised metal with an overhanging lip along the top of the cage to which a vibration and sound deadening foam rubber strip is glued. This rim engages the 'Spur' arms. Thirty cages are thus suspended in five rows (Fig. 86). The cages themselves are of a standard Home

Fig. 84 WALL MOUNTED CAGE RACKING

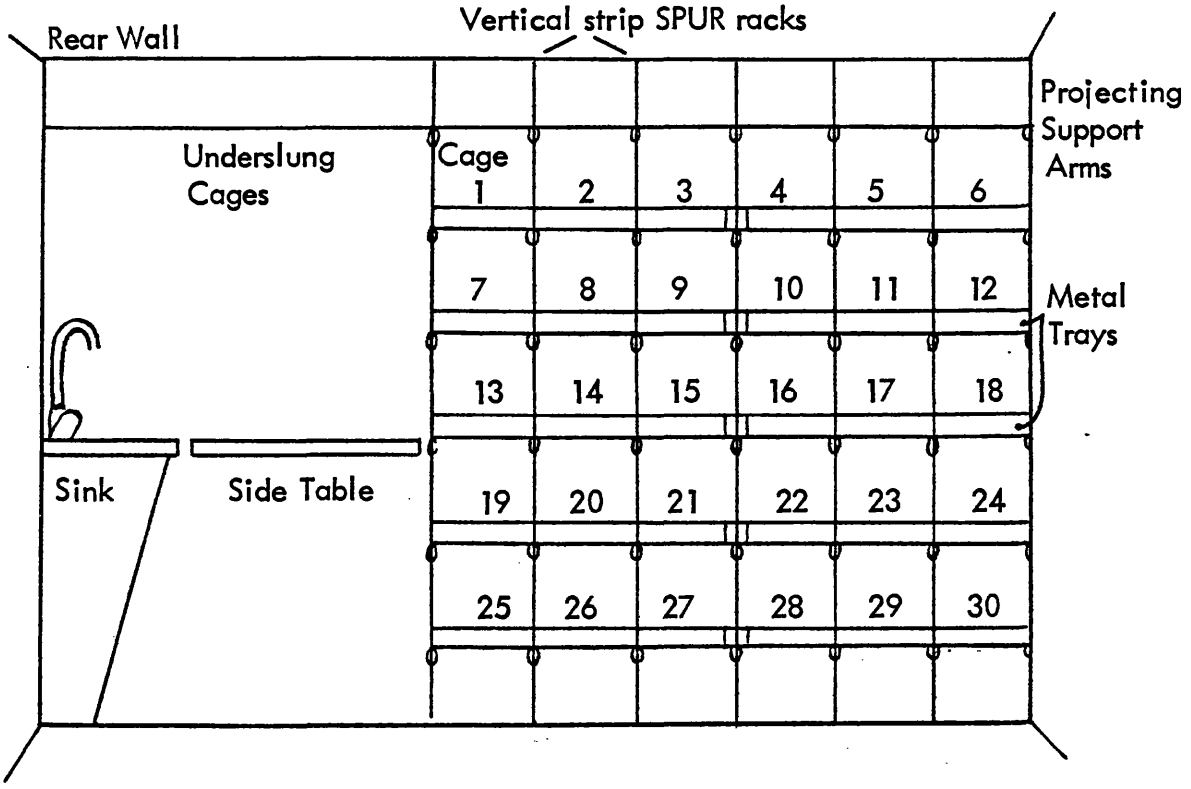


Fig. 85 CAGE DESIGN

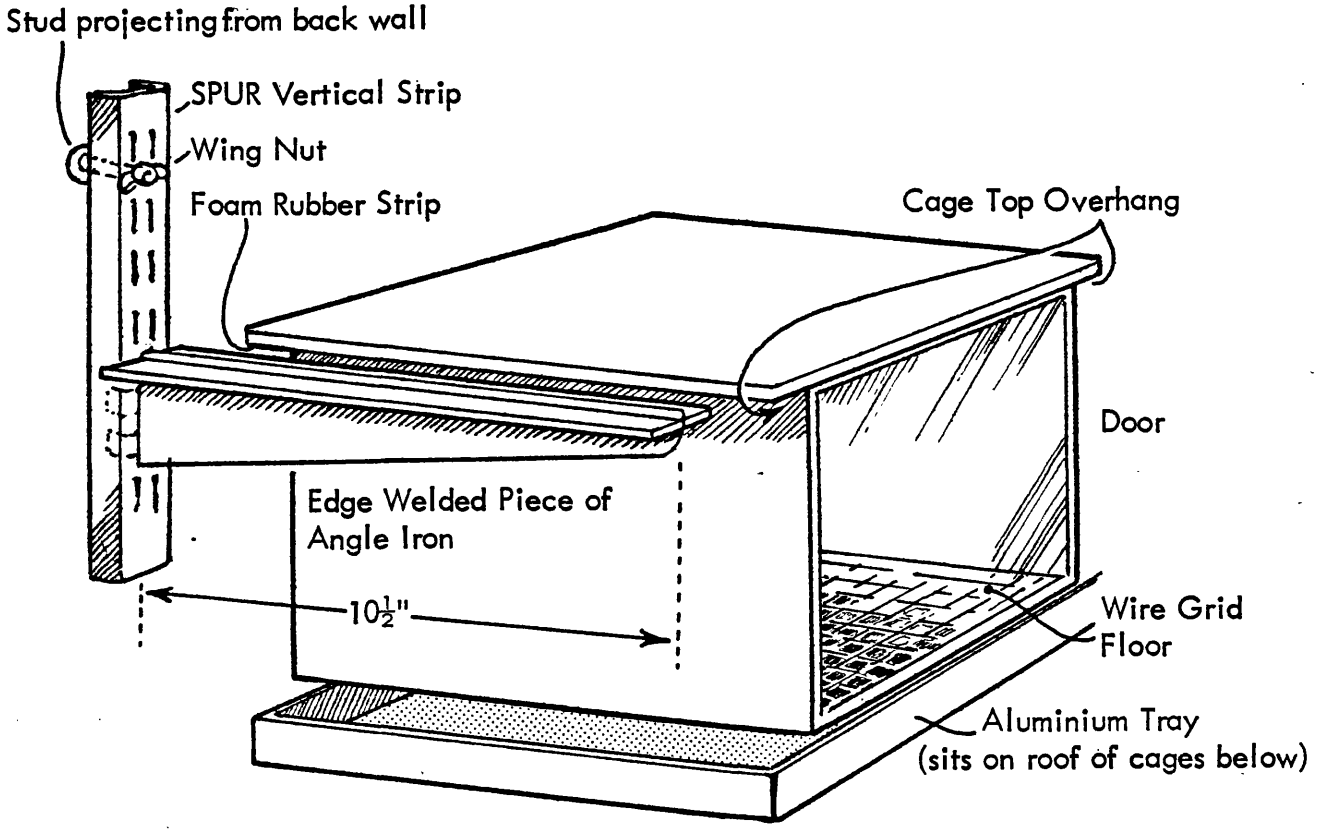
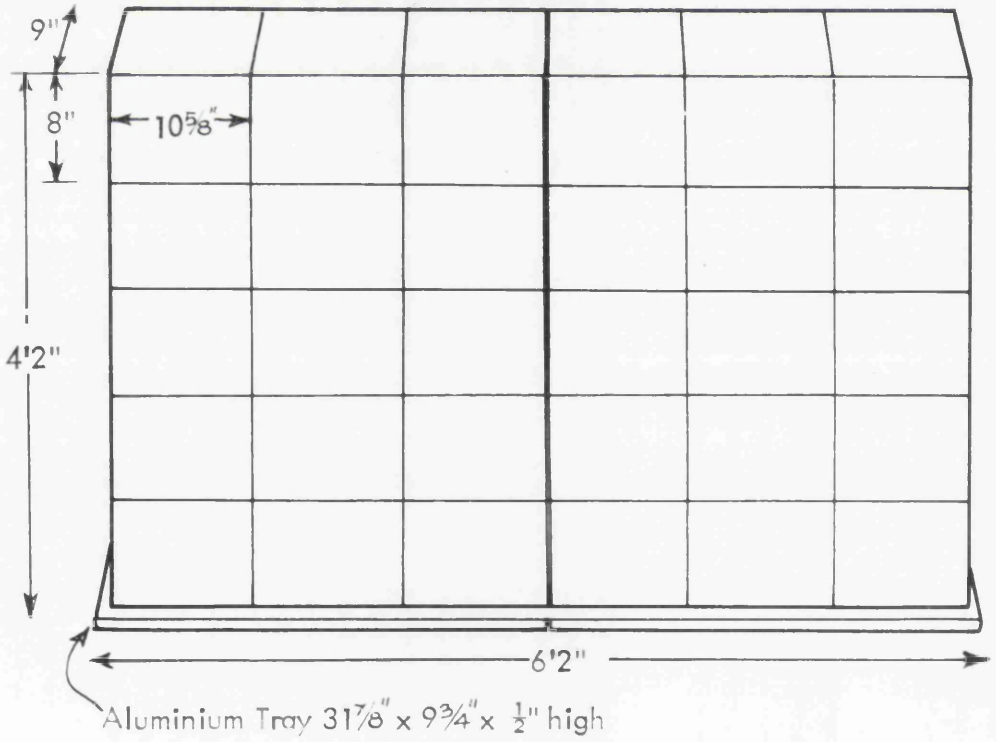


Fig.86 a WALL RACK CAGE ARRANGEMENT

Fig.86 b DIMENSIONS OF WALL RACK CAGES



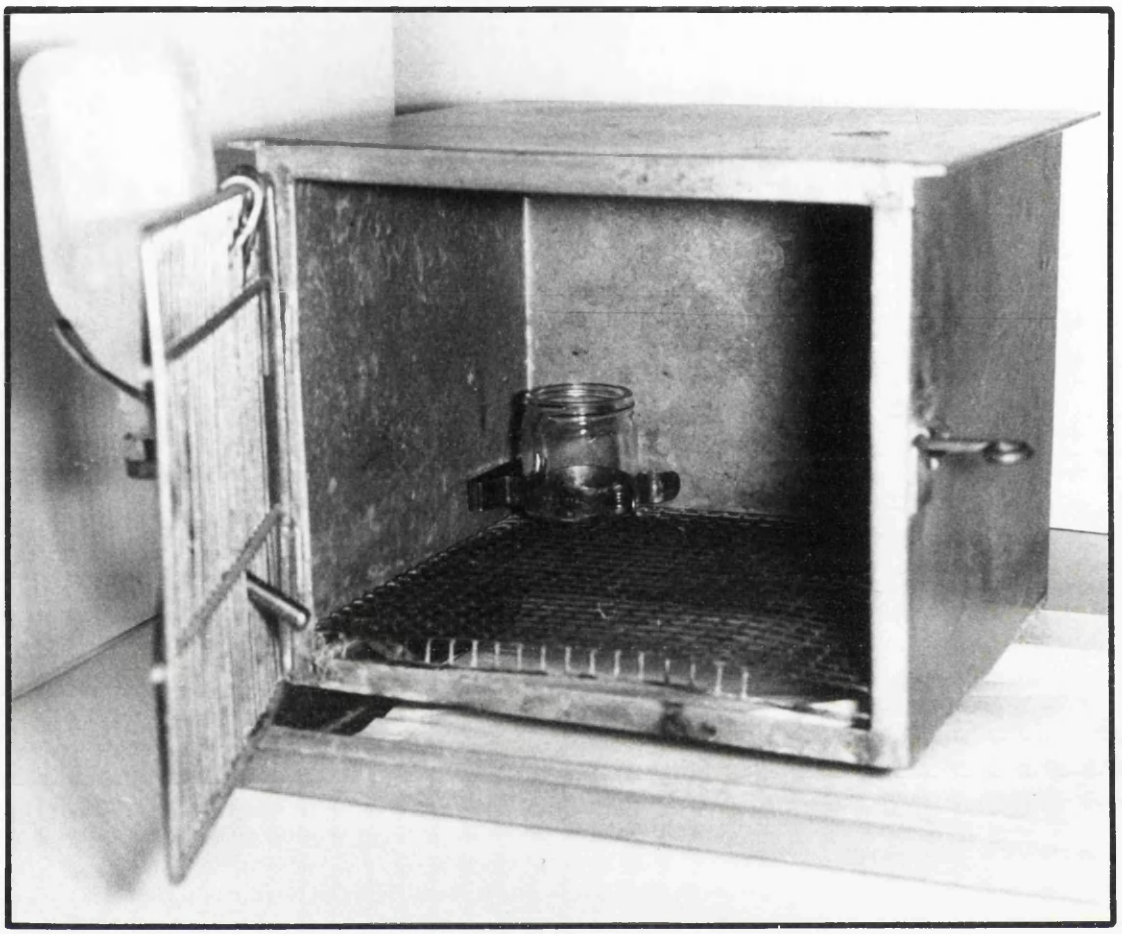
Office internal size 10⅝" x 9" x 8" with a wire grid floor. Rat excreta are collected on shallow aluminium trays suspended beneath the cage floors. These trays are strong, light, resistent to rat urine and are constructed to be exactly the same internal length and breadth as a double length of standard kitchen paper towelling which is used to absorb urine. Initial trials had quickly shown that in a high airflow system, the conventional animal house excreta absorbing materials, sawdust or wood chips, were widely scattered when the trays were lifted into the area under the roof air diffuser prior to removal for cleaning. Paper strips also decreased odour, dust, and allowed accurate estimates of diet spillage. Routine cleaning of cage floors consisted of disposing of soiled paper towels into a lidded plastic bin and laying down fresh paper strips. Cleaning of the cages themselves and filling of diet dishes and water bottles is carried out outwith the chamber in accord with the method of Tuffery (1958). After each experiment the cages are sterilised with TEGO. This chemical is particularly effective against Pseudomonas Aeruginosa (Perkins and Short, 1957), a very serious contaminant after burns (Dexter, 1971).

With this system of cage racking each animal is isolated from direct contact with its neighbours, minimising the possibility of cross infection from burn wounds, as the cages open only to the front into an

area of rapidly moving filtered fresh air. Psychological stimulation of group responses in the rat is also decreased by the individual housing of each rat. The small movement area available to each rat with the provision of food and water directly within the cage ensures that the burned rat is not disadvantaged after injury compared to the control as little movement or effort is required in either case to obtain nourishment.

Design of wall cage feeding dishes: Accurate measurement of daily food consumption by individual rats presented difficulty because of spillage of the powdered diet used. The rats readily overturned heavy flat bottomed glass food dishes and even proved adept at spilling diet from double glass dishes. Wetting powdered diet to form a mash was not helpful, nor was the use of a wall mounted hopper unit. The problem was resolved by attaching a standard glass diet jar to the left side wall at the rear of the cage by a 1½" diameter Terry Spring Clip. The rats cannot dislodge the dishes, yet the experimenter can remove them from the cage easily without disturbing the animals unduly (Fig. 87). Little food spillage occurs with the fixed dishes. The use of absorbent kitchen paper towel below the wire mesh cage floor ensures that urine quickly evaporates after excretion, leaving spilled diet on the surface of the paper from which it can be decanted directly into a balance pan for weighing.

Fig.87 WALL CAGE UNIT SHOWING FOOD DISH ATTACHMENT, WIRE GRID FLOOR AND PAPER LINED ALUMINIUM UNDER TRAY



Food spillage values are shown in the Results Section.

A practised operator can accurately weight individual food consumption for 30 rats within 60 minutes using a Mettler Pl200 top pan loading balance.

Water is fed into the cages via a vertically placed 100 ml plastic water bottle with ball value tip attached to the wire cage door with a single ¾" Terry Spring Clip. The value of this controlled environment upon rat growth and behaviour is shown in the Results Section.

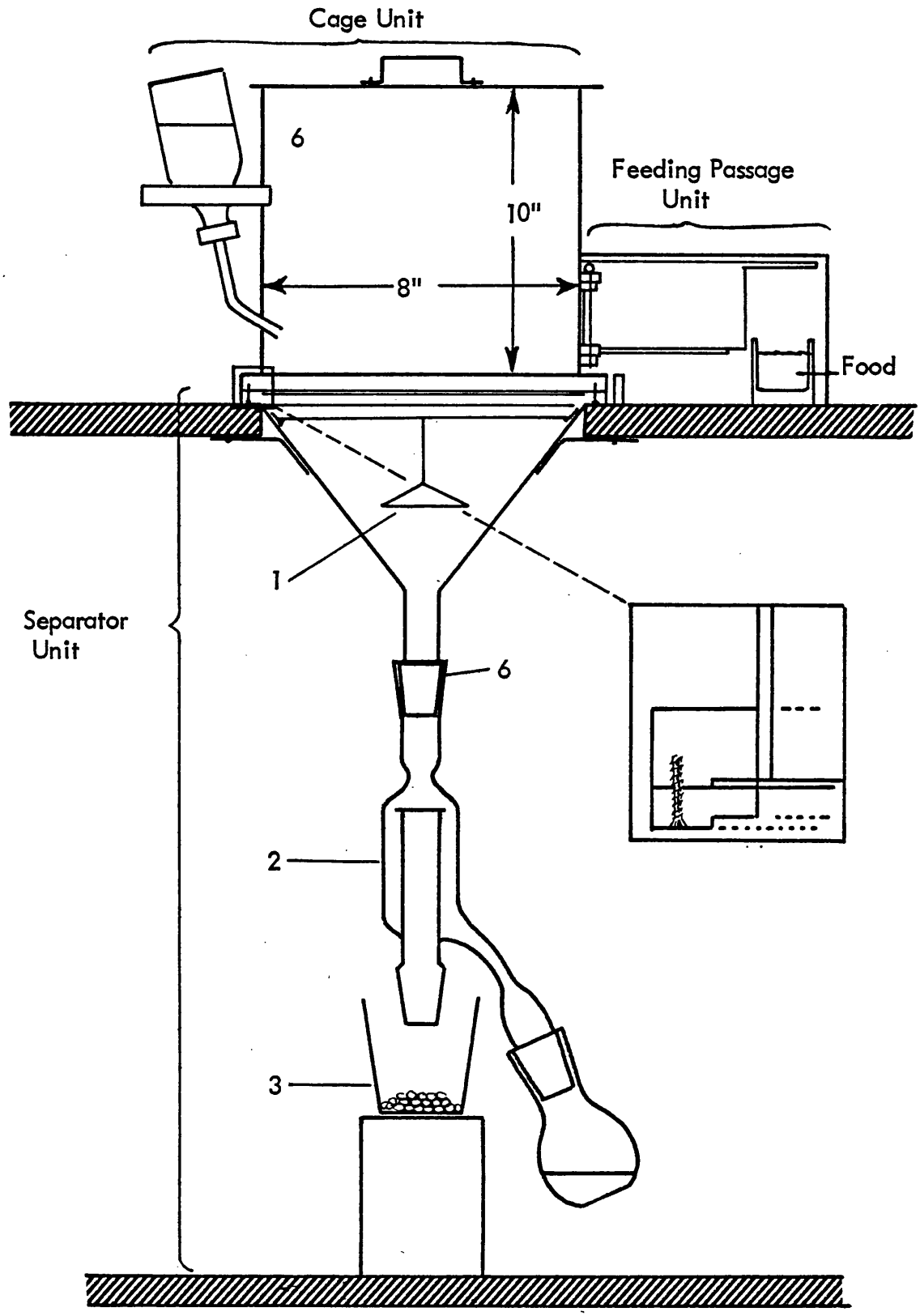
Design and construction of rat metabolism cages:

A cheap alternative to expensive stainless steel commercially manufactured metabolic cages was sought. The plastic and glass cage design (Fig. 88) of A. Thompson, Department of Agricultural Biochemistry, University of Newcastle upon Tyne was chosen as the most appropriate, and modified by the author in the following ways, viz:

1. The inverted cone (1) was replaced by an inverted glass bell resting in the base of the funnel.

2. The dimensions of the double tube section (2) of the separator unit were altered to achieve maximal urine and faecal separation with the type of low residue diet used.

Fig. 88 RAT METABOLISM CAGE DESIGN. ORIGINAL THOMPSON DESIGN



3. The faeces collecting pot (3) was replaced by a ground glass necked flask producing an airtight leakproof system, essential for radioisotope studies.

4. A special rack mounting system for the assembled cages was developed by the author.

5. The mounting of water bottles was altered and simplified.

6. Spring securing lugs were fitted to glassware parts to prevent accidental uncoupling of components while cages are being moved for sample collection or cleaning.

These modifications are shown in Figures 89, 90, 91. Glassware was made up by J. R. McCulloch & Son, Scientific Glass Blowers, 313 Shettleston Road, Glasgow G31. The plastic cages and food tunnels were fabricated by Bilman Plastic Industries Ltd., Hookergill, Co. Durham.

Design and construction of cage unit: This consists of an 8" diameter 8" high cylinder fabricated from ⅛" thick perspex. Cut into the wall of the cylinder there is a rectangular opening with rounded corners, 2¼" wide by 2⅛" high, the lower edge of which is 1" from the lower edge of the cylinder. This opening gives access to the feeding passage (Fig. 91). At a point some ¼ of the circumference from the feeding passage opening,

Fig.89 MODIFIED METABOLIC CAGE DESIGN

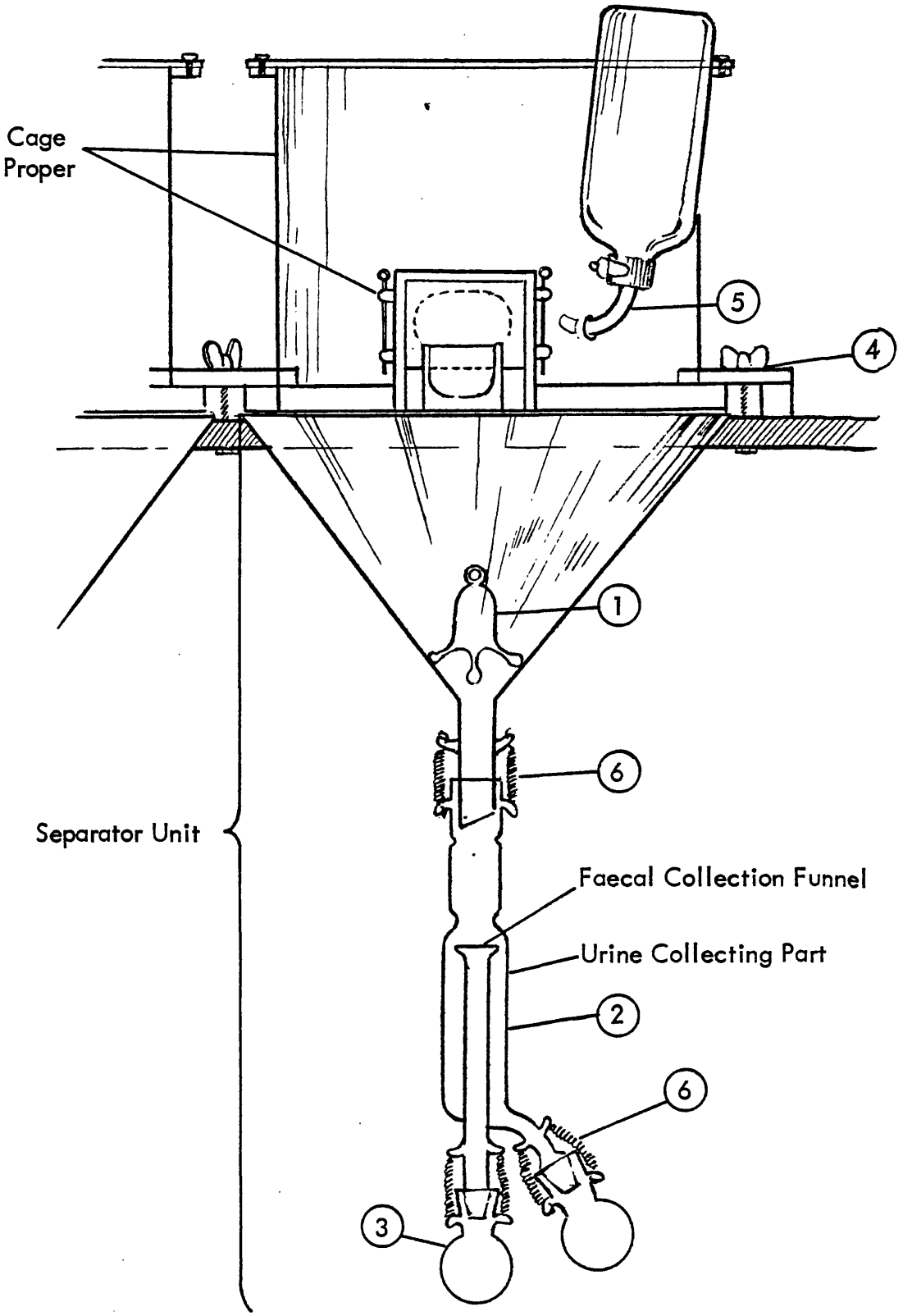
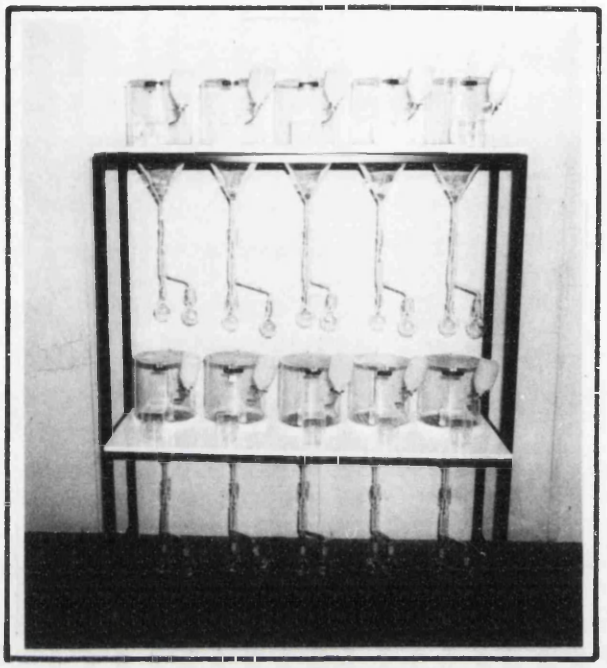


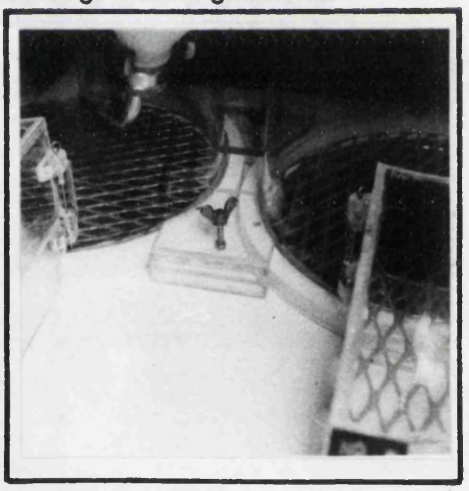
Fig. 90 METABOLIC CAGE DETAIL

A. Complete Unit: Mobile Rack with 10 Metabolic Cages



B. Glass Funnel Mounting

C. Cage Securing Brackets



D. Water Bottle Mounting

E. Separator Unit

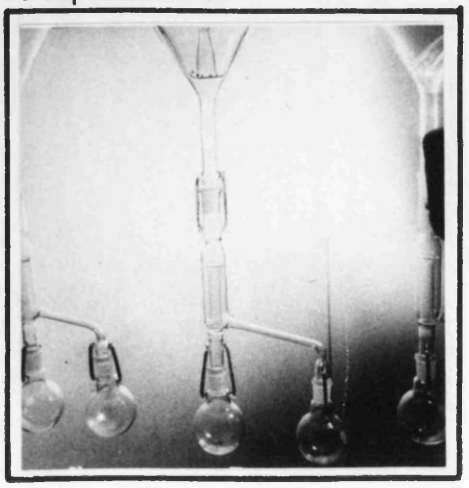
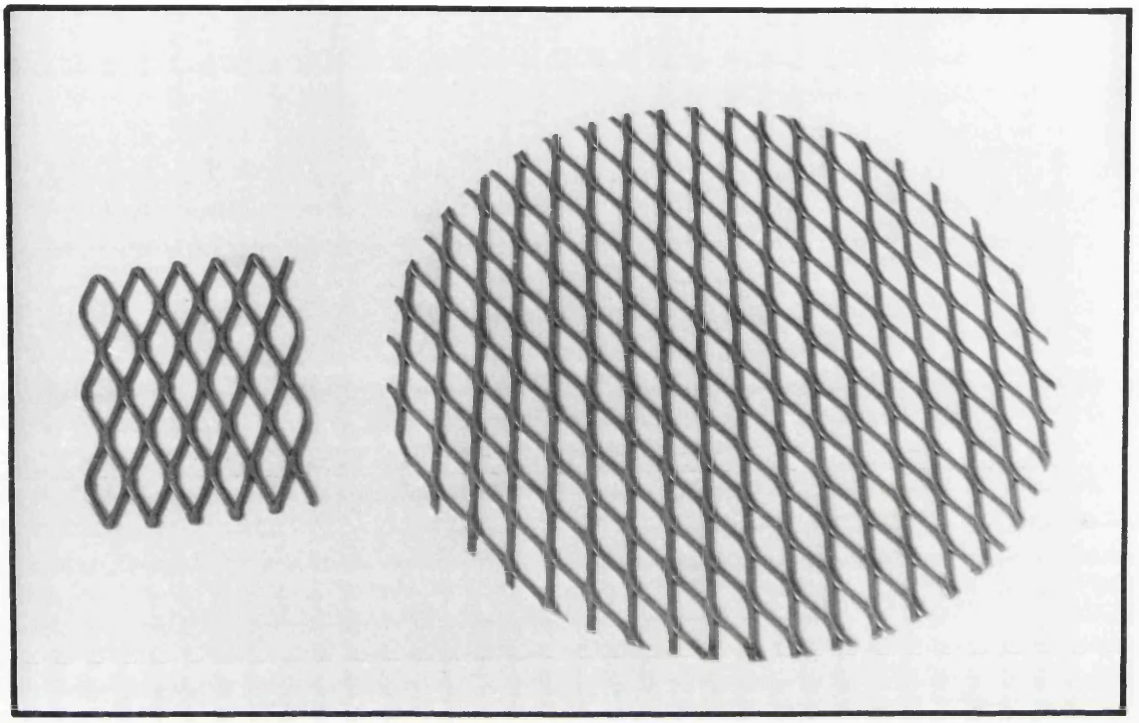
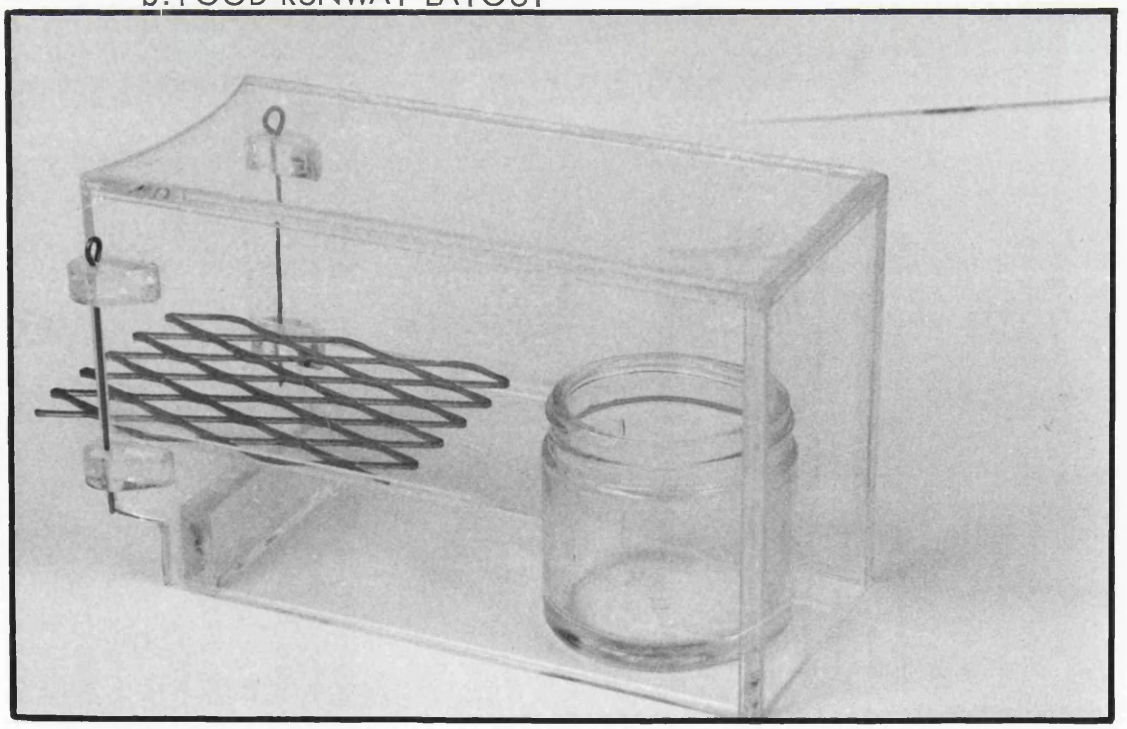


Fig.91 METABOLIC CAGE COMPONENTS

a. EXPANDED METAL FLOOR DESIGN



b. FOOD RUNWAY LAYOUT



is drilled a downward sloping hole ⅜" in diameter, centred approximately 2¼" from the lower edge of the cylinder. This accommodates the angled drinking tube. The polythene water bottle is supported by a Terry spring clip bolted to the cylinder wall some 2" above the sloping hole (Fig. 90).

Two ¾" x ¾" perspex lugs are cemented opposite to each other onto the outside of the cage cylinder, their tops level with the top of the cylinder; the lugs are drilled and tapped to take flat headed 2BA bolts (Fig. 89).

The latter locate with two 'key-hole' slots cut in the aluminium lids of the cages. This arrangement allows for locking of the lid with one hand, leaving the final screwing down to be carried out later.

The cage floor is an 8" circle of stainless steel, rolled, brushed, expanded metal (Expanded Metal Co. ref. 3318 SF) (Fig. 91). This material is said to offer foot comfort for the rats without obstructing the passage of faecal pellets. The pressing did have sharp edges which caused marked loss of belly hair. Filing down and sanding these edges reduced but did not eliminate this hair loss. The flow is sandwiched between 2 rebated perspex rings (internal diameter 8", external diameter 9") the upper of which is glued to

the lower edge of the cage cylinder. The lower perspex ring is attached to the first by 3 countersunk 2BA screws, and is rebated on its undersurface to engage the slightly projecting funnel rim of the separator unit (Figs. 89, 90B and 90C). This method of securing the floor produces no projections or obstruction to the passage of excreta.

Design and construction of feeding passage unit:

The separate feeding passage unit is attached to the cage by stainless steel pins passing through lugs attached to both it and to the cage cylinder. The passage is rectangular in shape, open at the bottom and floored with the same expanded metal as used for the cage floor. The feeding passage floor is set into a groove cut into the perspex side walls of the unit so that it slides out for cleaning. The feeding passage is 6" long, 2¾" wide and 3¾" high, the metal floor mesh being 3⅝" long and rebated 1⅜" from the lower edge. Glass feeding jars, either 2" high by 2" diameter, or 1¾" high by 2¼" diameter, are fitted into the end of the feeding passage and held in place by the passage metal floor. Diet spillage occurs therefore over the runway floor and not down into the collecting funnel of the cage proper. Spillage is swept off the cage rack top with a brush onto a filter paper and weighed. Sets of perspex liners were made to fit inside the feeding passage, reducing its height

and width. These were used occasionally where a rat persisted in attempting to sit in or defaecate within the feeding passage area. As with the wall cage units, food spillage of the powdered diet proved troublesome at first and a variety of feeding dish designs were tried. Ultimately the 2" x 2" glass jar was found to give the least spillage, and this was used throughout the subsequent burn studies.

Design and construction of metabolic cage urine/faecal separator units:

These were manufactured in 'Pyrex' glass and consist of

1. An 8" diameter glass funnel

2. A small glass bell with projecting feet which rests at the neck of the funnel, the function of which is to deflect urine to the side of the container preventing urine from falling directly into the faecal collection funnel (Fig. 89(1)).

3. The separator unit proper (Fig. 89(2)),made so that faecal pellets are deflected by the inner glass ridge into the faecal collection funnel. Urine on the other hand, on striking the inner glass ridge adheres to the glass and runs down the inner surface missing the edge of the free standing faecal collection funnel and

passes into the urine collecting flask.

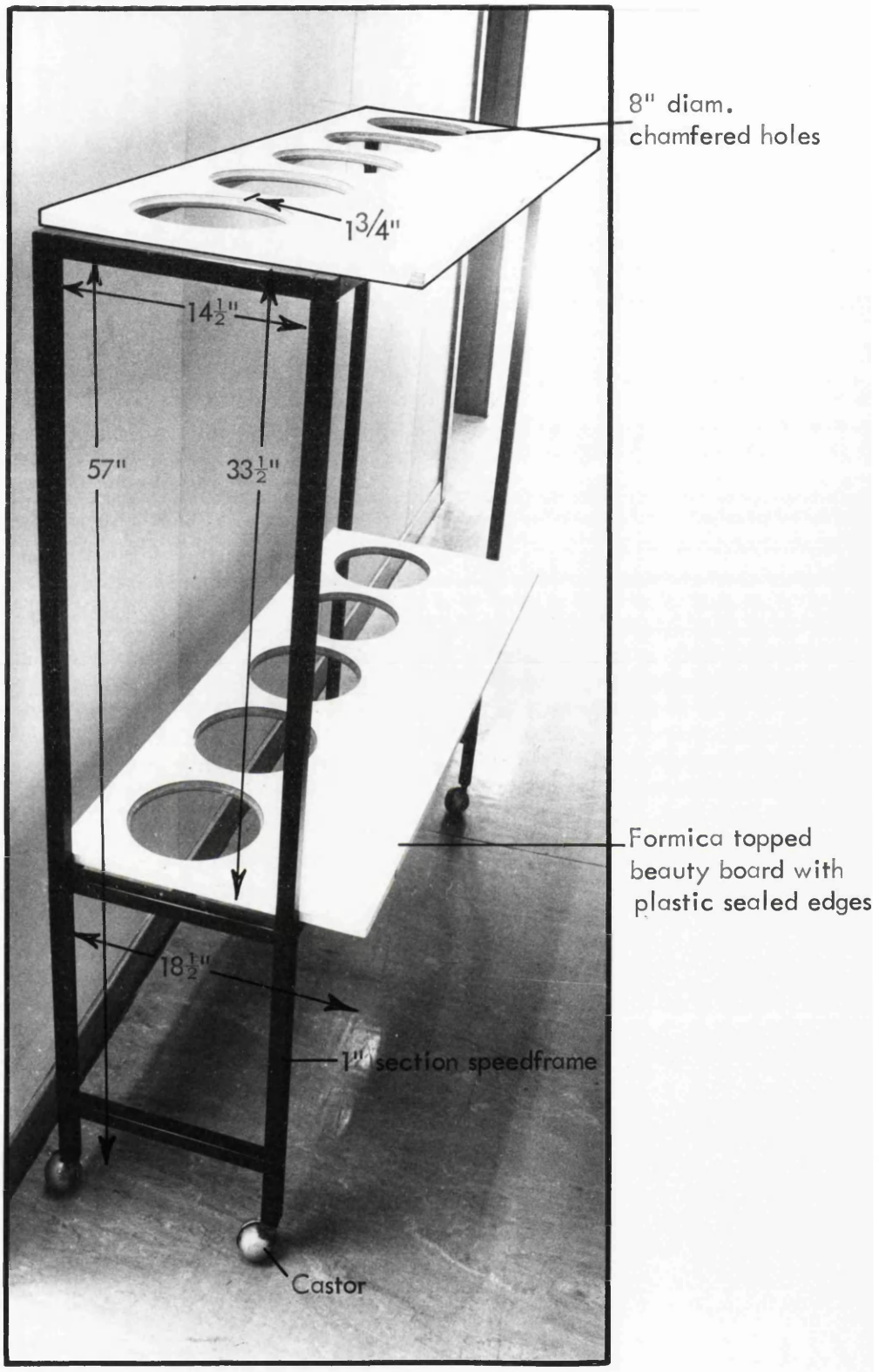
4. 50 ml or 100 ml 'quik-fit' flasks to collect the separated urine and faeces.

Design and construction of metabolic cage rack assembly:

Two 'Speedframe' racks were built to hold ten metabolic cages each in mobile units, the dimensions of which (Figs. 90A, C, 92) fitted within the narrow door and entrance way to the environmental chamber (Fig. 13). The advantages of this arrangement are that the rats do not require to be handled during routine cage servicing. Cages complete with animals, water bottles and feeding runways in situ can be lifted directly from a soiled rack and quickly transferred onto a fresh rack with preassembled glass separator units. The process takes less than 15 minutes for 10 rats. The faecal and urine collections in the soiled separators can be wheeled away to an adjacent laboratory for processing at leisure.

The decking of the racks are made from 'Beauty Board' with plastic tape sealed edges. The glass funnels are positively located in carefully chamfered holes in the board (Fig. 90). The cages are firmly pressed down onto the glassware by a system of perspex locking pieces (Fig. 90). A thin rubber gasket ring was used between cage lower surface and glass funnel

Fig. 92 "SPEEDFRAME" METABOLIC CAGE HOLDING RACK



but this was later discarded as unnecessary. The plastic cage units were arranged with the water bottles and the food runways facing into the environmental chamber.

General Comment: For most efficient metabolic collections and urine/faecal separation the following hold true:

1. A 10 day 'settling-in' period is essential for the rats to adjust to the new cage micro-environment. Rats often lose significant amounts of belly hair with this design of expanded metal cage floor even when its edges are smoothed down. These losses are greatest over the first 5 days, thereafter lessening appreciably.

   During the 'settling-in' period rats adjust from eating a pelleted commercial diet to eating a powdered diet. Spillage is high at first, but by 10 days, most rats learn to eat diet directly from the feeding dish with little resultant spillage.

   (See Results Section)

2. Rats occasionally have loose bowel motions for several days after transfer to the metabolic rack. If high bulk faecal residue diets such as Oxoid 41B are used this will result in persistent separator clogging with large amounts of heavy moist faecal material. The test diet used through-

out the subsequent injury studies was a 20% protein ultra low residue diet (L.I.T. diet from Nutritional Biochemicals Inc., U.S.A.) which produces small dry faecal pellets. (See Results Section) with good urine - faeces separation. The internal dimensions of the glass separator unit were matched to this latter type of faecal material.

Procedure for collection of samples from metabolic cage system:

URINE

1. At the end of each 24 hour collection period (10.00 hrs) the rack of 10 metabolic cages with soiled separator units and urine/faecal samples is wheeled from the environmental chamber into the animal handling and preparation laboratory.

2. Here the rack is disassembled. Perspex locking pieces are removed. The cylindrical cage units plus feeding passages are transferred to a fresh fully assembled rack with fitted food dishes and clean separator units. The locking pieces are replaced on the fresh rack which is wheeled back into the environmental chamber. Total time less than 15 minutes.

3. Samples are collected from the soiled separator units in the outer laboratory using the racking

frame as a stand. Using a Teflon squeeze bottle the walls of the glass collecting funnels are carefully irrigated with 0.1 N HCl (Analar Grade) using a circular motion sweeping down to the neck of the funnel, and taking care not to splash dilute acid directly into the faecal funnel. Five minutes later the urine collecting flask is detached from the separator unit and its contents poured into a 250 ml measuring cylinder. The urine flask is washed out a further four times into the measuring cylinder using the same 0.1 N HCl solution. If excess hair or food particles have been carried down into the urine flask the flask contents are first decanted through a filter funnel (Teflon) with high grade pure glass fibre wool. This does not affect nitrogen recovery. The 24 hour urine sample plus 0.1 N HCl wash is then made up to 250 ml with distilled water. The volumetric cylinder stoppered and thoroughly shaken. This is then 'digested' as outlined.

4. The soiled glassware is thoroughly washed in a detergent solution and rinsed in clean water. It is then dried in a heated cabinet prior to re-assembly for the next change-over.

FAECES

1. The initial steps are broadly similar to those for urine. Any dried faecal pellets adherent to the separator are shaken loose, otherwise the faecal collection flask is simply detached from the separator after the urine samples have been collected.

2. Faecal pellets are shaken or washed out of the collection flask with 0.1 N HCl as for urine, and for nitrogen analysis are poured into a 25 ml volume bottle of an MSE homogeniser (Type 7700). This mixture of dilute acid and faecal pellets is then homogenised for 5 minutes at 14,000 revs/min. This produces a fine suspension. The MSE universal bottle plus added contents are accurately weighed on a Mettler P1200 top pan loading balance. The suspension is then re-homogenised for a further two minutes and approximately $^{1}/_{4}$ to $^{1}/_{5}$ of the faecal suspension very quickly poured into a weighed micro-kjeldahl flask. The weight of faeces removed from the suspension is determined by re-weighing the kjeldahl flask after the addition of the faecal suspension. The percentage of the total faecal sample thus obtained is readily calculated.
The digestion stage is detailed later.

SINGLE HANDED RAT WEIGHING PROCEDURE

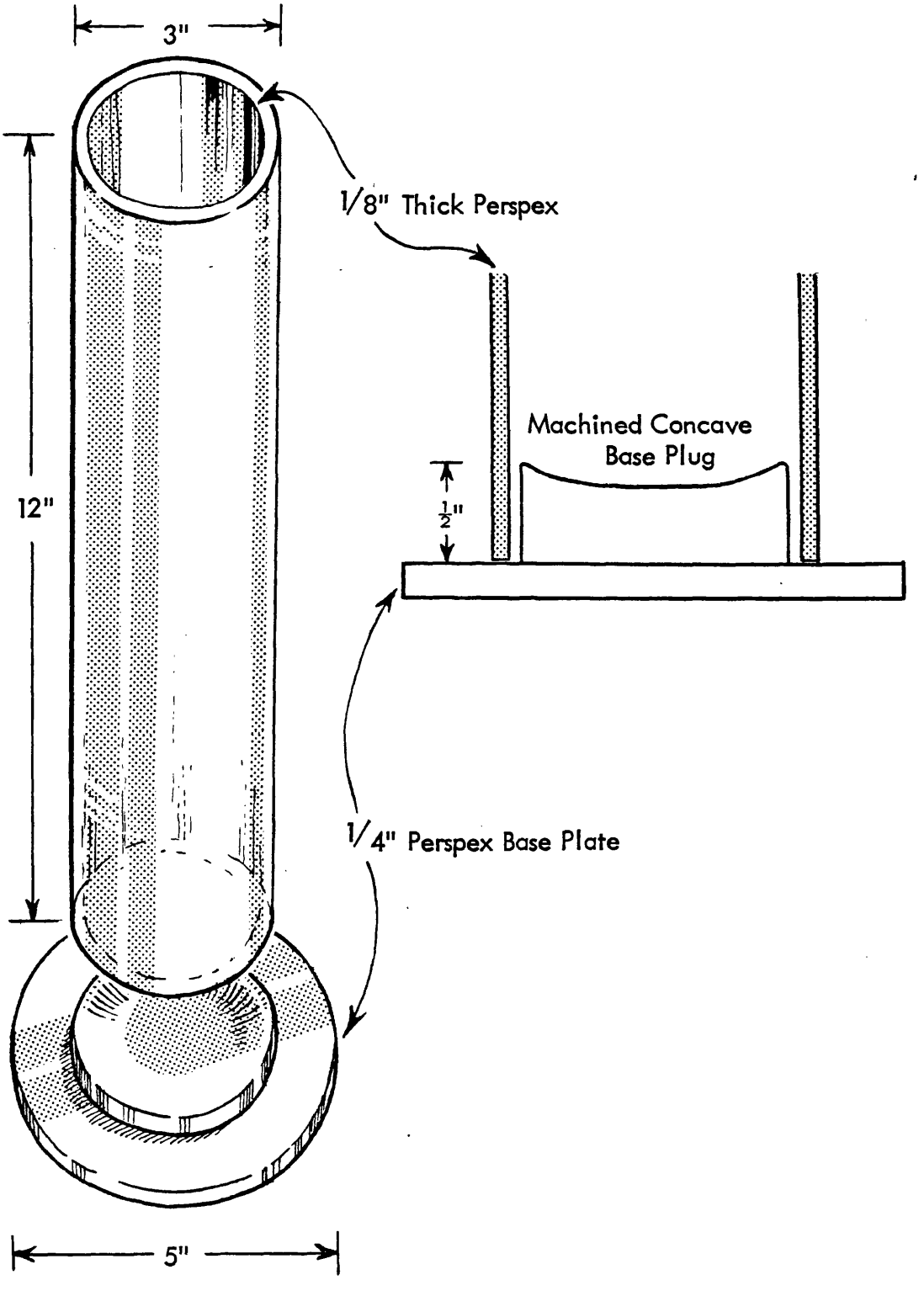
Rat metabolic studies demand accurate daily weight recordings for each animal. When up to 40 rats have to be weighed daily by one individual this task becomes tedious and time-consuming. It is difficult to avoid the tendency to rough handling of the rats in an endeavour to complete the measurement quickly. To minimise work for the handler, and reduce stress for the rat, the author devised the following equipment and simple single handed weighing technique.

Apparatus:

A 12" x 3" diameter clear perspex weighing cylinder was constructed with an accurately machined base plate which formed an easy push fit (Fig. 93). This base plate has a central concave depression to trap urine and faeces (if necessary) in the base when this is removed from the cylinder. The base plate is 5" in diameter to enable the cylinder to be placed securely in a vertical position for weighing.

The top of the cylinder is made from clear perspex so that when viewed from within the cylinder, the tube appears to be open-ended.

Fig.93 RAT WEIGHING CYLINDER (Dimensions)



Procedure:

Wall cages.

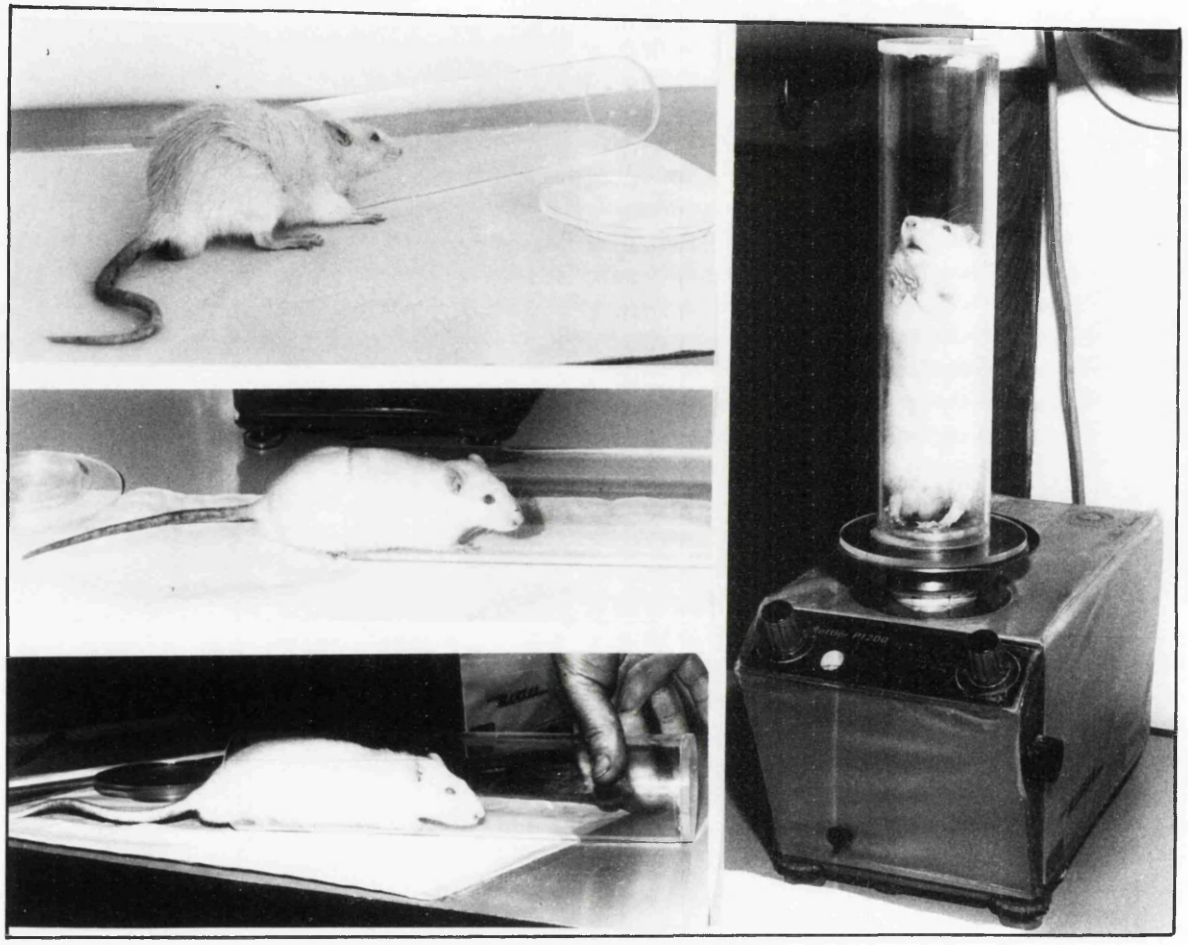
To weigh a rat, the base plate is removed and the open end of the perspex cylinder is held horizontal to the floor of the rat's cage after opening the wire cage door. Within days the rat learns to advance into the tube without handling.

Metabolic cages.

The two steel pins holding the feeding runway in place (Figs. 89, 90) are removed and the runway replaced by the weighing tube held horizontally with its open end over the cage opening. If the hand is then passed behind the metabolic cage, the animal passes into the weighing tube in order to avoid this potential danger.

Once in the tube, the rat's tail is gently tucked in and the base plate pushed securely home. The weighing cylinder complete with rat is gently placed vertically on the top loading pan of a Mettler P1200 balance, offset 330 g to compensate for the weight of the tube. The rat immediately throws out its forepaws and braces itself against the side of the tube and is momentarily motionless, awaiting the next movement. The weight is read during this brief period (3 - 5 secs) (Fig. 94. Without delay the weighing tube is held horizontal again and the rat returned to its cage. The

Fig.94 RAT WEIGHING CYLINDER SHOWING METHOD OF USE



sound of the base plate being removed stimulates the trained rat to back out of the tube into its cage.

The cage door is closed, the whole procedure requires less than 60 seconds and may be conducted without stress to operator or to the rat.

DEVELOPMENT OF CONTROLLED ENVIRONMENT CONDITIONS

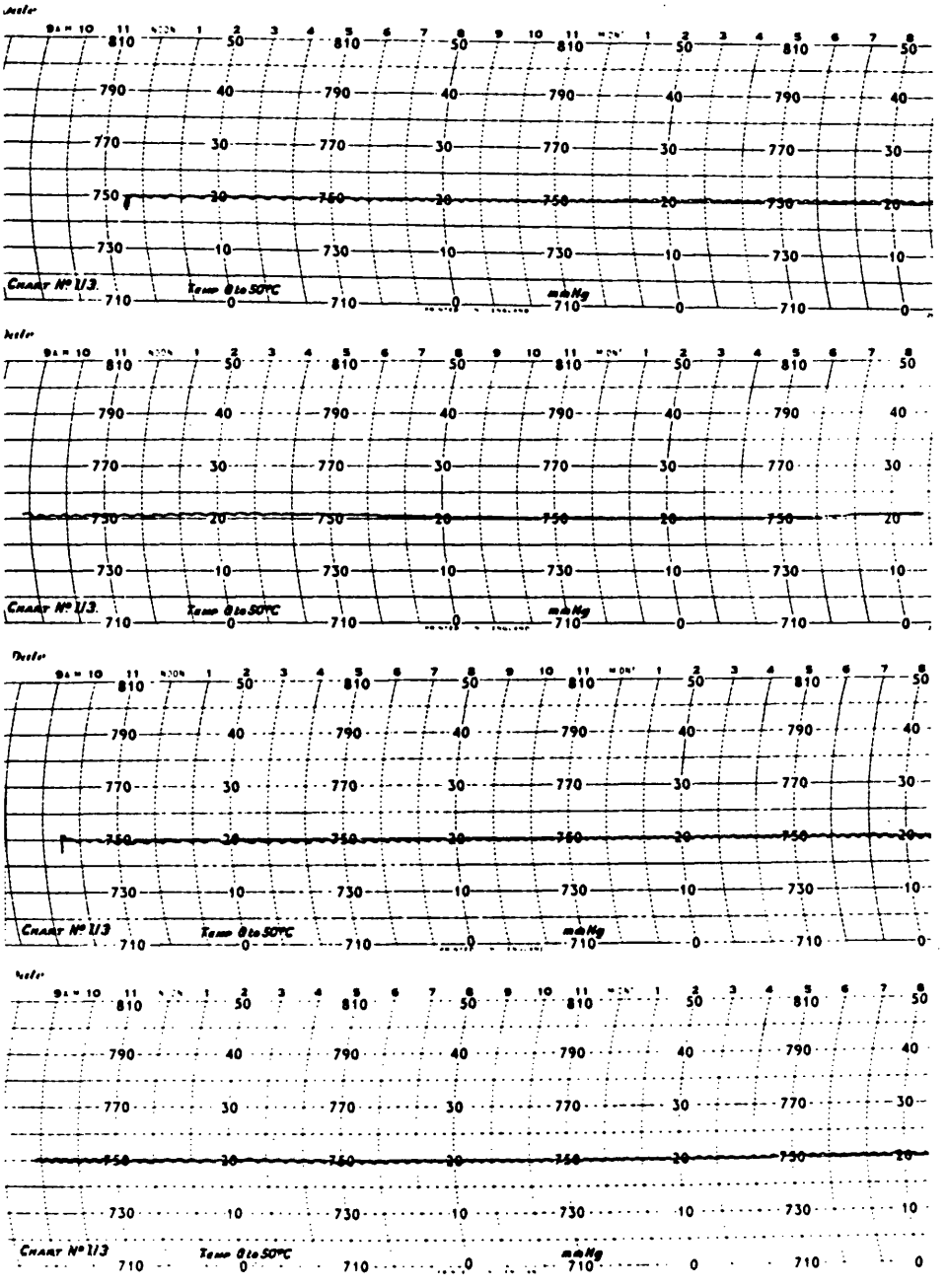
RESULTS

TEMPERATURE CONTROL WITHIN RAT ENVIRONMENTAL CHAMBER (MACROENVIRONMENT):

Studies on the metabolic response to injury were assessed in controlled conditions at 20°C and 30°C ambient temperatures. After initial construction the performance of the Multivent Warm Air Handling Unit was monitored to see the effectiveness of the proportional control unit in maintaining the environmental chamber temperature close to the required "set point" over long periods.

Figure 95 shows the daily traces from a calibrated thermograph chart recorder placed inside the environmental chamber over a 96 hour period. The ambient temperature setting used was 20°C, and the Bilman proportional controller was adjusted to give the narrowest temperature range possible around the set point. There was virtually no temperature drift during the 96 hours, and no difference was observed between day and night recordings, even when the interior temperature in the animal handling

Fig.95 24 HOUR TEMPERATURE RECORDINGS FROM THE RAT ENVIRONMENTAL CHAMBER



and preparation laboratory dropped more than 5°C below the daytime value. The chart recorder traces were confirmed by visual inspection of a max/min thermometer kept in the environmental chamber. The recorder was placed in the wall rack area in place of a cage unit which was removed for the purpose. Thermograph recordings made in the area of the sink unit showed greater deviation from the set point but did not exceed ± 1°C with normal usage of the environmental chamber. The thermograph recordings shown in Figure 95 were made with rats in the evnironmental chamber and normal rat servicing procedures in progress. The wall cages and metabolic cage rack were both in use.

The performance of the Multivent heating/ ventilation system is shown to best advantage in cold weather conditions as is the case in Figure 95. When outdoor air temperature exceeds 20°C then the accuracy of temperature control shown here is lost. In the northern hemisphere this is not a frequent occurrence even in summer. The experimental protocol was so arranged that studies which required a 30°C ambient temperature were performed during the summer months. The performance of the heating/ventilating system in controlling chamber temperature at a set point of 30°C was similar to that shown in Figure 95 though the variation was slightly greater at 30°C ± 1°C.

Occasional failure of the Multivent Warm Air Handling Unit to control ambient temperature within the range quoted did occur, but this was caused by, variously, dirt clogging the inlet air filter (This required changing 1 - 2 monthly), failure of a 1 kW heater bar, and visitors (unobserved) altering the proportional band settings on the Bilman control unit. Such events were rare.

Humidity within the chamber was not controlled, but intermittent measurement with a sling hygrometer indicated values between 35% and 55% R.H. at 20°C ambient temperature.

CONTROL OF NOISE WITHIN RAT ENVIRONMENTAL CHAMBER (MACROENVIRONMENT):

Though the environmental chamber wall construction proved an efficient sound insulator, greatly decreasing noise from the animal handling and preparation laboratory (loud shouts outwith the chamber are barely audible from within), the fan unit on the Multivent heating/ ventilating system was incurably noisy. Figures 96 and 97 indicate the sound levels perceived in the chamber with the ventilation system running. The noise produced by the fan unit takes the form of a low intensity continuous background hum at 50 decibels. This is not

Fig.96 NOISE LEVELS IN CONTROLLED ENVIRONMENT CHAMBER

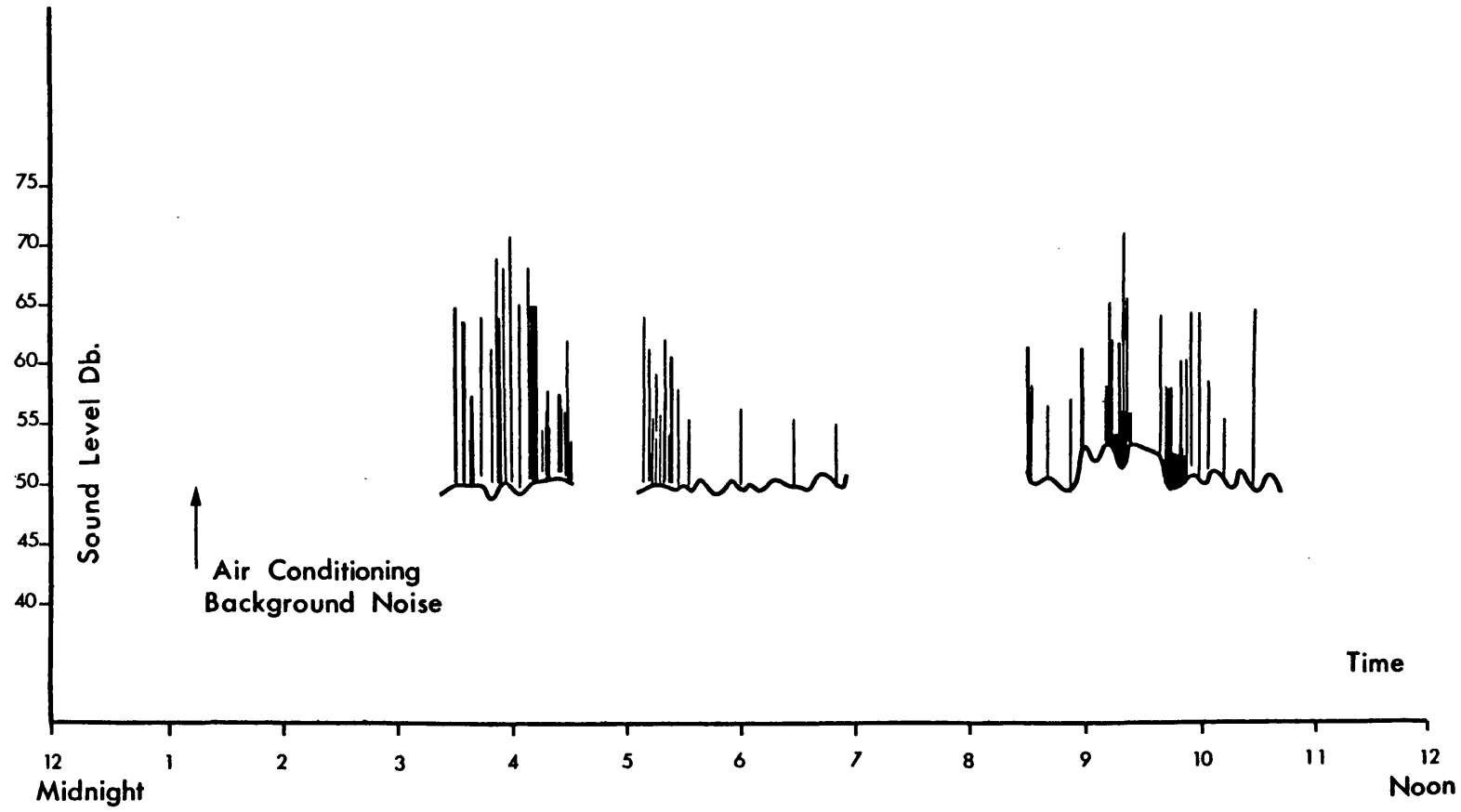
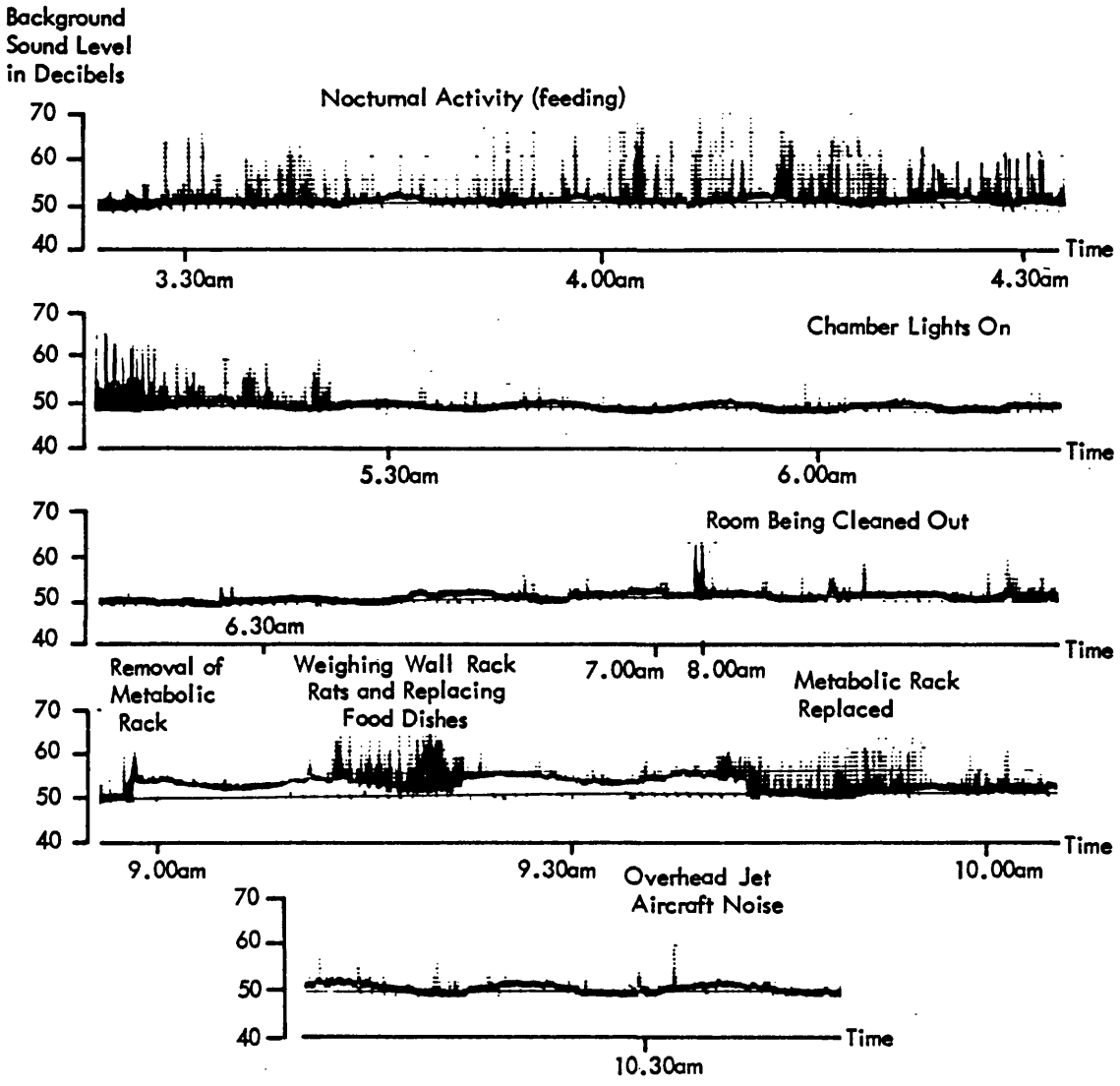


Fig. 97 BACKGROUND SOUND LEVELS WITHIN ENVIRONMENTAL CHAMBER



unpleasant to most humans and based (latterly) on rat urinary catecholamine excretion rate (Al-Shamma, Ph.D. Thesis, Glasgow University, 1978) did not appear to upset the rats in the least.

Figure 96 shows the periods of greatest noise within the chamber to be between 3.30 a.m. and 4.30 a.m., again at 5.00 a.m. - 5.30 a.m., between 8.30 - 10.30 a.m. and (not shown) another peak in noise level between 6.15 p.m. with fluctuating levels up till 12 midnight.

The chamber was being operated on a 12 hour light/dark cycle with lights on from 6.00 a.m. - 6.00 p.m. Sound recording was made by Dr. Nigel Orr of the Department of Clinical Physics and Bioengineering.

Figure 97 shows in more detail the daytime levels of noise associated with routine rat servicing procedures. The rat is nocturnal in habit. It is of interest to note that the noise during the hours of darkness (caused by the rats rattling their food dishes and cage doors) is of the same intensity as that which occurs during the day period due to rat weighing and food dish changing and so forth.

The sound recording indicates also the circadian rhythm of the rats in the environmental chamber.

PERFORMANCE OF RAT CAGE DESIGNS (MICROENVIRONMENT):

METABOLIC CAGE - MEASUREMENT OF RAT DAILY FOOD INTAKE.

In order to obtain the most efficient use of the metabolic cage design selected, some thought was given to the type of feeding dish used. In a metabolic balance study, accurate measurement of daily food intake is just as important as collection and measurement of excreta. If a single operator is responsible for weighing food intake in up to 40 rats daily over long periods of time, then a food container which results in no spillage of rat diet would be ideal. A variety of shapes and types of food dispenser were tried out. Food spillage occurred with all designs tested. Figure 98 shows a graphical comparison between a wide flat jaw 2" x 2" style and a taller, narrower design 1¾" x 2¼". Food spillage is expressed as a percentage of the daily food actually consumed by the rat (Table 16).

In the first weeks of use (days 3 - 8) there is no significant difference between the two designs ($P = 0.07$) in regard to spillage. But by the 3rd week, though the 2" x 2" jar design has significantly less spillage than the 1¾" x 2¼" design ($P = 0.001$), the most striking observation is that the quantity of food

Fig.98 A. METABOLIC CAGE FOOD SPILLAGE : COMPARISON OF GLASS FEEDING DISHES:

2" x 2" Glass dish vs. 1 3/4" x 2 1/4" Glass dish (food spillage as % of total daily food intake) 5 Rats in each group - mean shown
● 2" x 2" dish group o 1 3/4" x 2 1/4" dish group

Fig.98B. WALL CAGE FOOD SPILLAGE : COMPARISON OF SECURED vs UNSECURED FEEDING DISHES

2" x 2" Type: 5 Rats in each group - mean shown
x Unsecured feeding dish ····o··Secured feeding dish

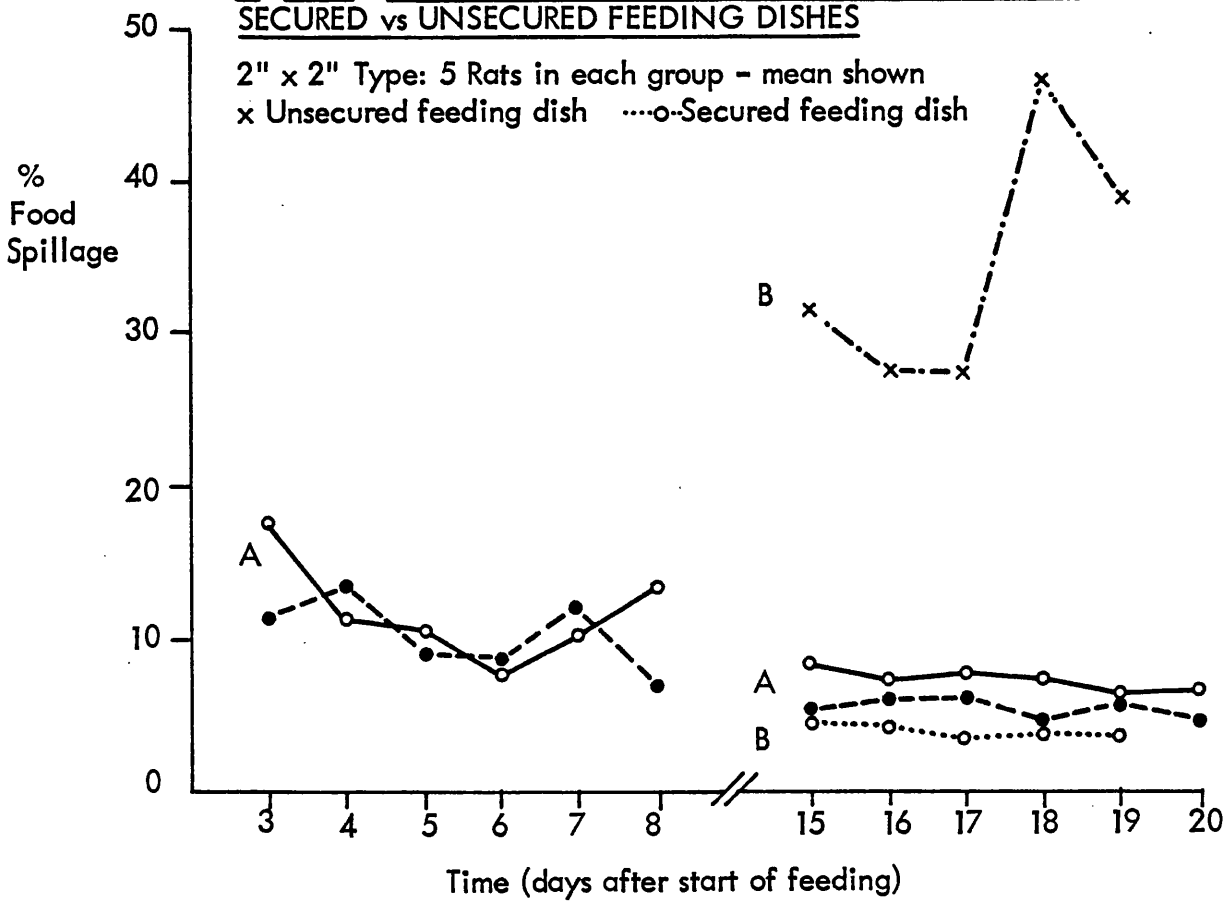


TABLE 16 (A)

METABOLIC CAGES : COMPARISON OF DAILY FOOD SPILLAGE WITH DIFFERENT FOOD CONTAINERS

(Food Spillage is expressed as a % of food consumption)

2" x 2" GLASS SCREW TOP JARS

DAY	3	4	5	6	7	8	15	16	17	18	19	20
RAT 1	9.4	14.3	6.2	12.5	19.0	4.5	2.6	3.9	5.8	2.6	9.1	3.7
RAT 2	3.7	16.5	8.3	8.9	12.4	8.1	6.4	6.3	2.9	4.2	6.7	4.9
RAT 3	14.7	8.6	5.3	5.3	9.2	5.8	8.7	8.2	9.1	6.0	5.4	5.0
RAT 4	10.7	11.6	8.4	3.9	3.7	5.1	3.9	4.8	6.8	4.7	4.0	3.5
RAT 5	17.5	16.8	17.3	12.1	16.4	11.2	4.5	6.8	6.0	6.7	3.9	6.9
MEAN $\pm$ SD	$\frac{11.2}{\pm 5.3}$	$\frac{13.6}{\pm 3.5}$	$\frac{9.1}{\pm 4.8}$	$\frac{8.5}{\pm 3.9}$	$\frac{12.1}{\pm 6.0}$	$\frac{6.9}{\pm 2.7}$	$\frac{5.2}{\pm 2.4}$	$\frac{6.0}{\pm 1.7}$	$\frac{6.1}{\pm 2.2}$	$\frac{4.8}{\pm 1.6}$	$\frac{5.8}{\pm 2.2}$	$\frac{4.8}{\pm 1.4}$

OVERALL MEAN DAYS 3 - 8: 10.2 $\pm$ 4.7%

OVERALL MEAN DAYS 15 - 20: 5.5 $\pm$ 1.8%

TABLE 16 (B)

METABOLIC CAGES : COMPARISON OF DAILY FOOD SPILLAGE WITH DIFFERENT FOOD CONTAINERS

(Food Spillage is expressed as a % of food consumption)

$1\frac{3}{4}$" x $2\frac{1}{4}$" GLASS JARS

DAY	3	4	5	6	7	8	15	16	17	18	19	20
RAT 6	22.7	19.4	8.7	9.9	16.4	27.0	8.9	9.1	7.0	10.4	6.8	7.2
RAT 7	9.4	9.8	10.6	4.9	11.8	12.5	3.9	5.7	6.4	6.0	4.5	4.8
RAT 8	14.7	11.9	8.2	7.5	7.9	13.5	10.4	6.1	8.9	10.2	8.7	8.2
RAT 9	15.1	2.8	16.4	8.7	5.8	5.9	11.1	6.8	5.0	5.9	5.8	6.2
RAT 10	26.4	12.6	8.9	8.1	9.4	8.8	7.8	7.9	12.2	4.8	6.8	6.5
MEAN $\pm$ SD	$\frac{17.7}{\pm 6.8}$	$\frac{11.3}{\pm 6.0}$	$\frac{10.6}{\pm 3.4}$	$\frac{7.8}{\pm 1.9}$	$\frac{10.3}{\pm 4.1}$	$\frac{13.5}{\pm 8.1}$	$\frac{8.4}{\pm 2.8}$	$\frac{7.1}{\pm 1.4}$	$\frac{7.9}{\pm 2.8}$	$\frac{7.5}{\pm 2.6}$	$\frac{6.5}{\pm 1.5}$	$\frac{6.6}{\pm 1.3}$

OVERALL MEAN DAYS 3 - 8 : 11.9 $\pm$ 5.9%

OVERALL MEAN DAYS 15 - 20: 7.3 $\pm$ 2.1%

The values given in this table were measured during the course of a single experiment. The two groups were mixed between upper and lower decks of the same metabolic Speedframe rack. The mean daily food intake for all 10 rats between Day 3 - Day 8 was 16.4 g $\pm$ 2.8 g, and between Day 15 and Day 20, 16.9 g $\pm$ 2.2 g.

spilled with both designs of jar has been almost halved. This same effect was noted with different shapes of food container tested. It was due to the rat changing its style of eating, rather than reflecting any quality inherent in the design of the food container itself. The rats used in these studies were accustomed (from weaning) to a form of pelletted diet which they ate by grasping with their forepaws after pulling a food pellet from a hopper at the side of the cage. They then retreated to a corner of the cage to eat the food avoiding the other rats in the same cage. In the metabolic cage food runway, the rat could be seen scooping up the powdered diet with its forepaws and liberally scattering it down into the food runway as it attempted to carry its food back into its solitary cage. With the passage of time, the rat could be seen eating its powdered diet directly from the food container in the same manner as a dog eating from a food dish. The rat no longer used its paws to carry food. This factor accounted for the reduction in food spillage seen.

The 2" x 2" glass jar was selected as it was marginally better than other designs and was available in quantity within the Institute. It could also be capped with a screw fitting lid enabling diet to be prepared in advance, in the precise quantity required, and stored without fear of spillage.

WALL RACK CAGE - MEASUREMENT OF RAT DAILY FOOD INTAKE.

The same problems with food spillage were found in the wall cage rats as with those in the metabolic cages. Reduction in food spillage was a matter of rat adaptation to the container used, and was unrelated to its shape or design. Many food dishes and dispensers were tested before this was appreciated. The rat will energetically move around or overturn any container that is not fixed within its cage. It was necessary therefore to secure the 2" x 2" glass food dish to the steel cage wall with a Terry Spring Clip as shown in Figure 87. This resolved the problem of food spillage as shown in Figure 98. The 15 - 19 day period is shown. The results are given in Table 17.

METABOLIC CAGE - URINE/FAECAL SEPARATOR UNIT FUNCTION.

The original Thompson urine/faecal separator unit dimensions (See detail 2, Fig. 88), proved too narrow to avoid rapid clogging with faecal matter. The inner glass tube was increased in diameter (Fig. 89) and the performance of the separator unit re-assessed. Oxoid 41B diet has a high faecal residue. The stools were moist and offensive and readily adhered to the side of

TABLE 17

WALL RACK CAGES : COMPARISON OF DAILY FOOD SPILLAGE WITH SECURED AND UNSECURED DISHES

(Food Spillage is expressed as a % of food consumption)

DAY	15	16	17	18	19	
RAT 1	37.5	14.9	28.4	91.0	85.2	
RAT 2	14.9	22.5	16.4	20.8	12.9	UNSECURED
RAT 3	18.7	16.5	22.9	36.4	30.3	GLASS DISH
RAT 4	82.5	67.8	65.9	87.8	69.4	
RAT 5	17.5	27.3	22.1	12.9	12.8	
RAT 6	17.7	16.4	8.9	31.3	22.2	
MEAN ± SD	31.5 ± 26.3	27.6 ± 20.3	27.4 ± 20.0	46.7 ± 34.1	38.8 ± 30.9	

OVERALL MEAN SPILLAGE 34.4 ± 36.1

DAY	15	16	17	18	19	
RAT 7	5.2	4.6	3.5	4.8	3.3	
RAT 8	6.7	3.9	2.5	6.4	5.0	
RAT 9	5.2	4.4	4.4	3.8	4.6	SECURED
RAT 10	2.5	2.9	2.7	3.8	2.2	GLASS DISH
RAT 11	6.8	6.5	4.3	2.0	4.5	
RAT 12	2.6	2.9	2.5	2.6	3.4	
MEAN ± SD	4.8 ± 1.9	4.2 ± 1.3	3.3 ± 0.9	3.9 ± 1.6	3.8 ± 1.1	

OVERALL MEAN SPILLAGE 4.0 ± 1.4

the inner glass tube of the separator. It was difficult to extract all the faecal matter from the system. Further, any urine passed over the adherent faecal matter could leach unknown quantities of nitrogen from the soft wet faeces. The procedure of daily urine and faecal collection was as distasteful and maloderous as the preceding description indicates.

The efficiency of the collecting system was improved by changing the diet eaten by the rats from one producing large amounts of faecal matter to one which had a low faecal residue, such as Low-Iodine Test Diet by Nutritional Biochemicals Inc. A comparison of food to faecal weights for both diets is shown in Fig. 99. Faecal material from rats eating L.I.T. diet was in the form of thin dried up pellets which were not adherent in the least to the collecting system glassware, resulting in very satisfactory and complete separation of urine and faeces. Tables 18 A and 18B give the values presented in Fig. 99. L.I.T. diet was used in all subsequent experiments except where specifically indicated otherwise. Rat drinking water was supplemented with potassium iodide to keep the rats euthyroid (5ug KI per 10 ml).

Fig.99 FOOD INTAKE VERSUS FAECAL WEIGHT: OXOID 41B DIET COMPARED WITH LOW IODINE TEST DIET

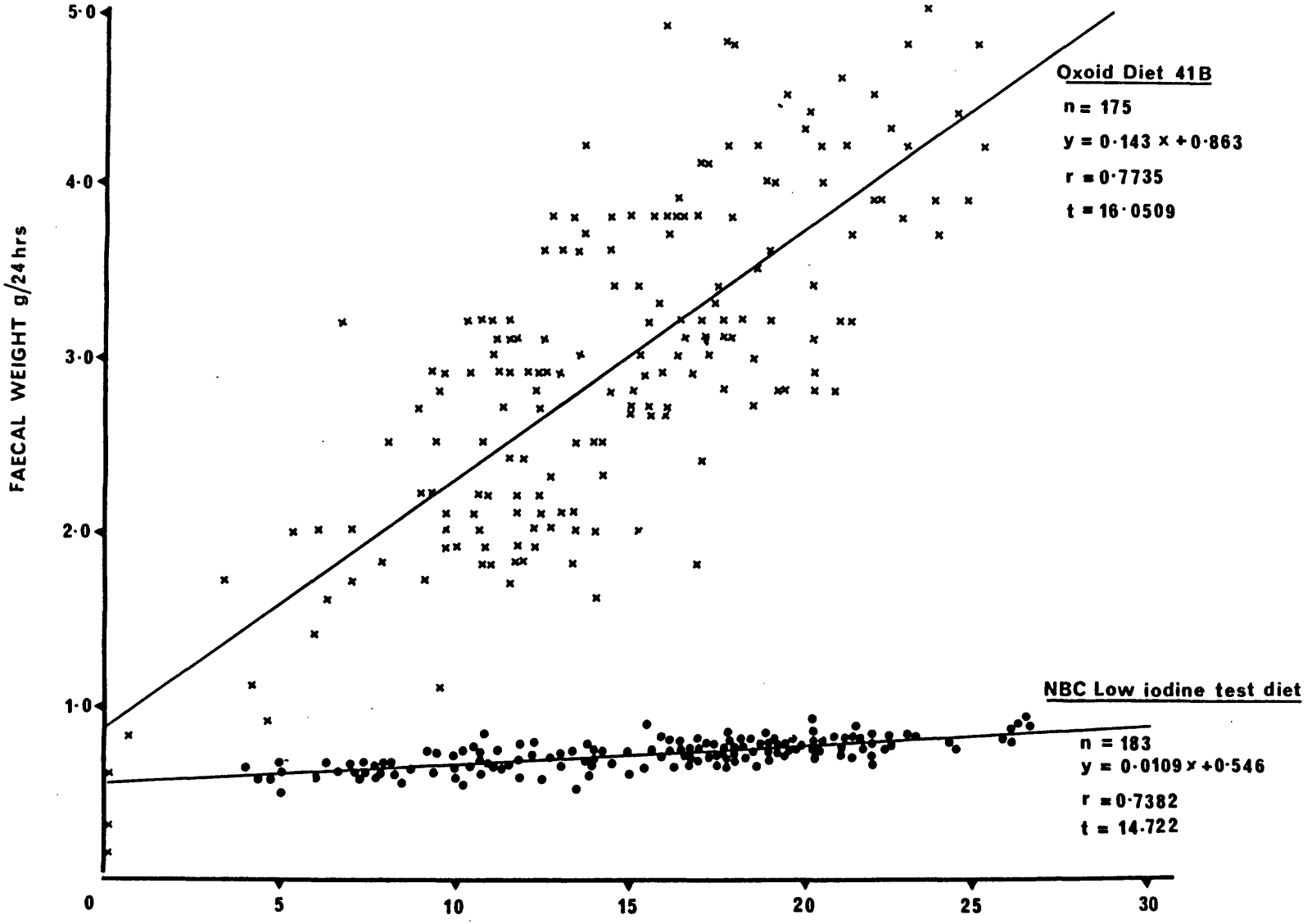


TABLE 18 A (1) COMPARISON OF FOOD VERSUS FAECAL WEIGHT

LOW IODINE TEST DIET (NBC Inc.)

183 observations were made on 20 rats. These were kept in the controlled environment chamber. Faecal weights and food weights are given in g.

FOOD	FAECES	FOOD	FAECES	FOOD	FAECES
4	0.61	10	0.70	13.5	0.52
4.4	0.58	10	0.63	13.8	0.77
4.7	0.57	10	0.58	13.8	0.70
5.0	0.65	10.2	0.73	13.8	0.68
5.0	0.62	10.2	0.66	13.8	0.60
5.0	0.49	10.2	0.55	13.9	0.65
6.0	0.57	10.4	0.64	13.9	0.68
6.3	0.66	10.5	0.76	14.0	0.70
6.7	0.61	10.6	0.75	14.0	0.73
7.0	0.65	10.7	0.73	14.2	0.73
7.2	0.63	10.7	0.69	14.5	0.67
7.1	0.60	10.7	0.60	14.9	0.72
7.3	0.58	10.9	0.68	15.0	0.61
7.4	0.60	10.9	0.84	15.4	0.63
7.4	0.66	10.9	0.66	15.4	0.71
7.5	0.63	11.1	0.65	15.5	0.88
7.7	0.65	11.2	0.73	15.6	0.74
7.7	0.63	11.3	0.63	15.9	0.70
7.8	0.58	11.5	0.65	15.9	0.82
7.9	0.61	11.6	0.65	16.2	0.73
8.0	0.66	11.7	0.68	16.2	0.80
8.0	0.64	11.9	0.78	16.3	0.64
8.2	0.65	11.9	0.59	16.5	0.78
8.3	0.60	12.2	0.70	16.5	0.75
8.4	0.57	12.3	0.78	16.6	0.70
8.7	0.63	12.5	0.58	16.7	0.72
9.2	0.74	12.7	0.69	16.7	0.75
9.3	0.61	13.0	0.70	16.7	0.65
9.5	0.65	13.0	0.65	16.8	0.76
9.5	0.72	13.3	0.72	16.8	0.70

$y = 0.0109x + 0.546$

$r = 0.7382$

$t = 14.722$

$n = 183$

TABLE 18 A (2) COMPARISON OF FOOD VERSUS FAECAL WEIGHT

LOW IODINE TEST DIET (NBC Inc.)

FOOD	FAECES	FOOD	FAECES	FOOD	FAECES
16.9	0.74	19.0	0.79	21.5	0.87
17.0	0.80	19.0	0.73	21.5	0.81
17.0	0.76	19.0	0.68	21.6	0.79
17.0	0.72	19.2	0.80	21.7	0.75
17.0	0.69	19.2	0.77	22.0	0.82
17.2	0.79	19.2	0.72	22.0	0.65
17.2	0.76	19.3	0.78	22.0	0.80
17.3	0.74	19.3	0.76	22.0	0.78
17.3	0.69	19.3	0.73	22.0	0.70
17.4	0.77	19.5	0.77	22.4	0.75
17.4	0.75	19.5	0.70	22.5	0.80
17.5	0.72	19.6	0.79	22.5	0.77
17.5	0.70	19.6	0.74	22.8	0.79
17.5	0.67	19.7	0.80	23.0	0.82
17.7	0.72	19.7	0.77	23.2	0.81
17.8	0.82	19.8	0.74	24.4	0.75
17.8	0.76	20.0	0.76	26.0	0.85
17.8	0.69	20.3	0.85	24.2	0.78
17.8	0.65	20.3	0.80	25.7	0.80
17.9	0.78	20.3	0.74	26.0	0.79
18.0	0.76	20.3	0.72	26.0	0.85
18.0	0.73	20.3	0.70	26.2	0.88
18.0	0.70	20.3	0.91	26.4	0.92
18.0	0.68	20.4	0.74	26.5	0.87
18.1	0.82	20.5	0.79		
18.2	0.80	20.5	0.77		
18.3	0.76	20.6	0.79		
18.4	0.69	20.7	0.78		
18.5	0.80	20.9	0.81		
18.5	0.74	21.0	0.77		
18.6	0.65	21.1	0.72		
18.8	0.78	21.1	0.74		
18.8	0.75	21.2	0.80		
18.9	0.84	21.4	0.70		

TABLE 18 B (1) COMPARISON OF FOOD VERSUS FAECAL WEIGHT

OXOID DIET 41B

175 observations were made on 20 rats, kept in the controlled environment chamber. Faecal weights and food weights are given in g.

FOOD	FAECES	FOOD	FAECES	FOOD	FAECES
0	0.3	10.3	3.2	10.8	1.8
0	0.17	10.7	3.2	10.9	1.8
0.1	0.6	11.0	3.2	11.5	1.7
0.6	0.9	11.4	3.2	11.3	2.7
3.3	1.7	11.1	3.1	11.5	2.4
4.1	1.1	10.4	2.9	11.9	2.4
4.6	0.9	11.2	2.9	12.2	2.0
6.7	3.2	11.5	2.9	12.2	1.9
5.9	1.4	12.0	2.9	12.4	2.2
5.3	1.9	12.5	2.9	12.4	2.1
6.0	2.0	13.0	2.9	12.7	2.3
6.3	1.6	11.0	3.0	13.3	2.3
7.0	2.0	11.5	3.1	12.7	2.0
7.0	1.7	11.7	3.1	13.0	2.1
7.8	1.8	12.5	3.1	13.3	1.8
8.0	2.5	12.4	2.9	13.4	2.0
9.5	1.1	12.3	2.8	14.0	2.0
9.1	1.7	12.4	2.7	15.2	2.0
9.0	2.2	11.7	2.2	16.9	1.8
8.9	2.7	11.7	2.1	12.5	3.6
9.3	2.9	11.7	1.9	13.0	3.6
9.6	2.9	11.7	1.8	13.5	3.6
9.5	2.8	11.7	1.8	12.7	3.8
9.4	2.5	10.5	2.1	13.3	3.8
9.3	2.2	10.6	2.0	14.0	1.6
9.7	2.1	10.6	2.2	13.7	4.2
9.7	2.0	10.7	2.5	13.5	3.0
9.7	1.9	10.9	2.2	13.6	3.7
10.0	1.9	10.8	1.9	13.4	2.5

TABLE 18 B (2) COMPARISON OF FOOD VERSUS FAECAL WEIGHT

OXOID DIET 41B

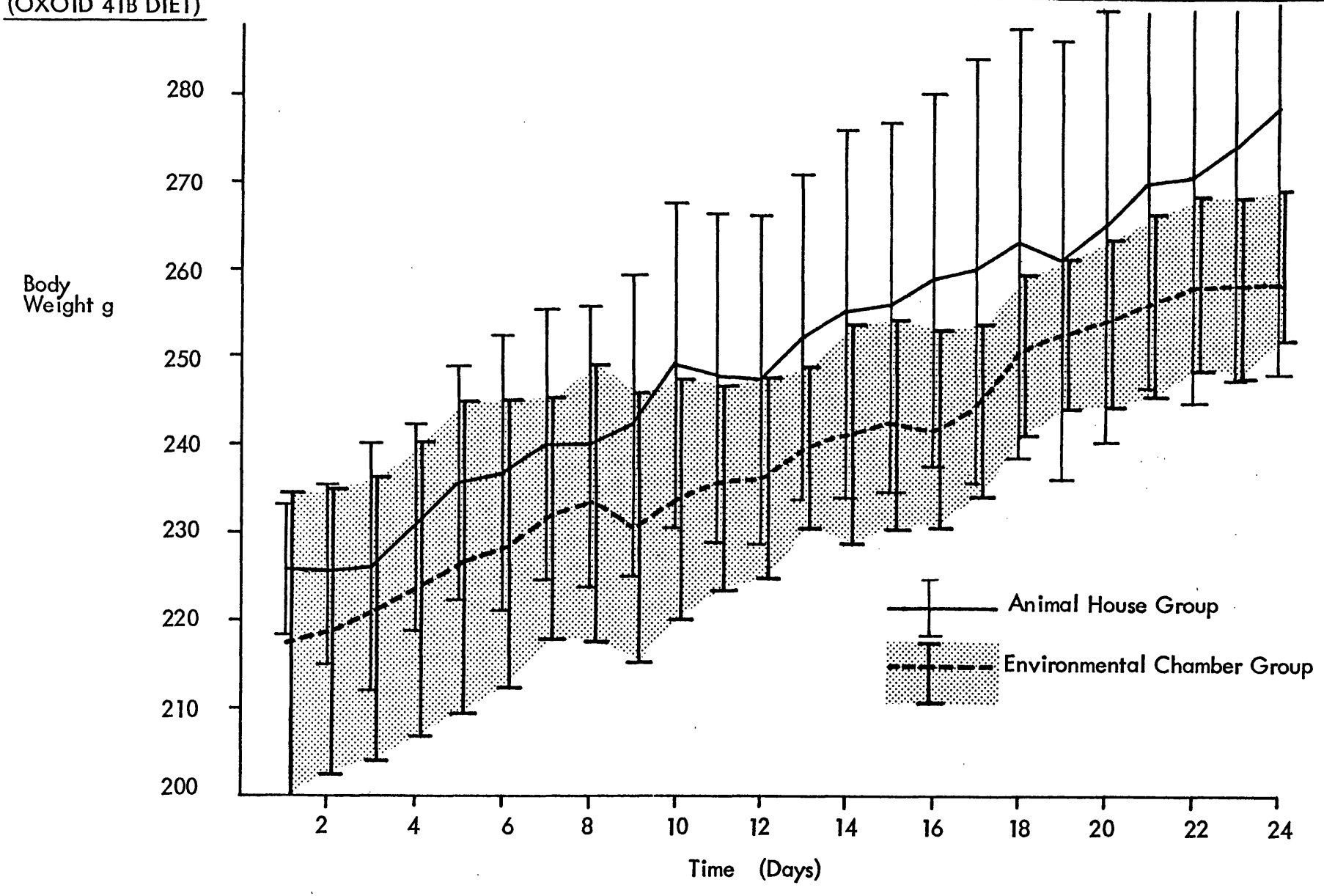
FOOD	FAECES	FOOD	FAECES	FOOD	FAECES
13.9	2.5	17.9	4.8	18.9	4.0
14.1	2.5	15.9	3.3	19.1	4.0
14.2	2.3	17.0	4.1	20.5	4.0
14.4	2.8	17.9	4.8	19.3	2.8
15.0	2.7	15.9	3.3	20.3	2.8
15.5	2.7	17.0	2.4	20.9	2.8
16.0	2.7	16.3	3.0	22.1	3.9
15.0	2.7	16.4	3.2	22.9	4.8
15.5	2.7	16.5	3.1	25.0	4.8
16.0	2.7	16.8	2.9	23.5	5.3
15.0	2.8	17.2	3.0	18.6	3.5
15.2	3.0	17.0	3.2	19.0	3.6
15.4	2.9	17.6	3.2	18.2	3.2
15.9	2.9	17.9	3.1	19.0	3.2
14.4	3.8	17.5	3.1	18.5	2.9
16.9	3.8	17.4	3.3	18.5	2.7
17.9	3.8	17.5	3.4	19.3	2.8
23.7	3.9	17.1	3.1	21.0	4.6
24.6	3.9	17.7	3.1	20.2	3.4
15.0	3.8	17.7	2.8	20.2	3.1
15.6	3.8	17.8	4.2	20.3	2.9
16.3	3.8	18.6	4.2	21.3	3.7
16.1	3.7	20.5	4.2	22.0	3.9
16.0	3.8	21.2	4.2	23.8	3.7
14.4	3.6	23.0	4.2	22.8	3.8
14.5	3.4	25.1	4.2	21.0	3.2
15.2	3.4	20.0	4.3	21.3	3.2
15.5	3.2	22.5	3.7		
16.0	4.9	20.1	4.4	$y = 0.143x + 0.863$	
16.3	3.9	24.4	4.4	$r = 0.7735$	
16.5	3.8	19.4	4.5	$t = 16.0509$	
17.0	4.1	21.9	4.5	$n = 175$	

EVALUATION OF RAT HANDLING AND WEIGHING TECHNIQUES IN RATS HOUSED IN THE ENVIRONMENTAL CHAMBER

The resultant effect of controlling rat environmental conditions in regard to background sound level, noise exposure, ambient temperature, light-dark cycle, special taming and handling, can be seen in Figure 100. The amount of diet was unrestricted. Oxoid diet 41B was used in both groups of rats. Male Wistar rats from the Institute of Physiology semi inbred closed colony were used in this and all other experiments in this thesis. 12 - 14 week old rats were randomly allocated to the environmental chamber or to the Institute general animal house. The growth rate in animal house (A.H.) rats was similar to that found in evnironmental chamber (E.C.) rats at 1.5 - 2.0 g body weight gain per day. The range of randomly selected rat weights was greater in the E.C. rats at the start. But by the end, the reverse was true. All rats were kept in wall rack or standing rack arrangements and were individually housed. E.C. rats were handled and tamed for a period each day and were carefully and gently weighed in the manner shown in Figure 94. A.H. rats were only handled in order to be weighed. This was done using a balance pan and counter weight system.

Greater variation within the group was found in the A.H. rats, whereas after the first 10 days there was relatively less change within the E.C. rat group.

Fig.100 RAT GROWTH : 10 RATS IN ANIMAL HOUSE vs. 10 RATS IN ENVIRONMENTAL CHAMBER : MEAN VALUES ± S.D.
(OXOID 41B DIET)



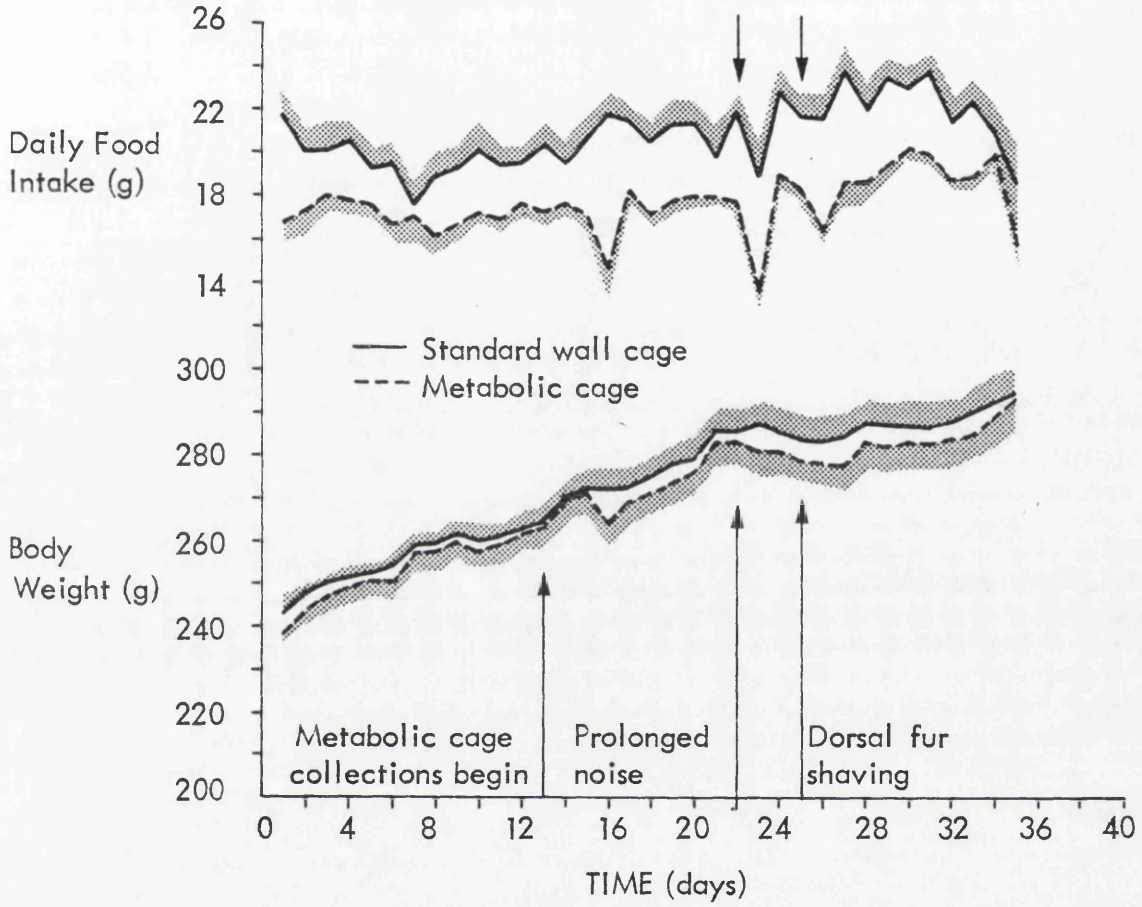
It appeared in subsequent studies that one cause for this similarity in growth rate found in many control and injured rat groups was the tendency for all rats within the environmental chamber to have a similar pattern of daily food intake.

COMPARISON OF FOOD INTAKE & GROWTH : METABOLIC CAGES vs WALL RACK CAGES

Figure 101 shows a comparison of food intake and growth between standard wall cage rats and metabolic cage rats within the environmental chamber. Both groups were randomly allocated at the start. Note the general similarity in daily changes in food intake between the groups during days 1 - 13. Growth in each group is also very similar over this period. When metabolic cage collection began on day 13, this affected only one group. The metabllic cage animals' food intake dropped sharply within 24 - 48 hours of the start of urine and faecal collections, then recovered. This was clearly reflected in the changes in their growth rate. Food intake and weight gain in the wall rack cage rats was unaffected.

However, both groups were equally affected when exposed to an 8 hour period of hammering and unusual human activity. A joiner performed repairs on day 22 in the Animal Handling and Preparation Laboratory adjacent to the rat environmental chamber. The reduction

Fig.101 COMPARISON OF FOOD INTAKE AND GROWTH BETWEEN STANDARD WALL CAGE RATS AND METABOLIC CAGE RATS Results
Mean ± Std.Error of Mean for 2 groups of 10 rats



in food intake and growth rate which followed this event affected both rat groups equally. An equal "compensatory" increase in food intake was noted in both groups the next day. Dorsal fur shaving in all rats was also followed by a decrease in food intake, then a more sustained rise in intake over the next few days. This affected both rat groups in the same way.

It appeared that there was no measurable difference between wall cage and metabolic cage rat growth and behaviour.

EFFECT OF LABORATORY "STRESS" ON THE RATE OF WEIGHT GAIN IN RATS KEPT IN THE ENVIRONMENTAL CHAMBER :
TWICE DAILY HEATING LAMP EXPOSURE

The effect of routine laboratory procedures was investigated on rat food intake and growth rate. Ten rats were subjected to twice daily 90 second periods of exposure to an infra red heating lamp held 18" above the rat, which was allowed to move freely on a bench beneath the lamp. This was sufficient to cause vaso-dilation in the rat and was used as a preliminary to taking a blood sample from the rat tail vein. The rats did not appear to be upset by this procedure and showed no undue urination or defaecation while being handled.

Fig. 102 EFFECT OF TWICE DAILY 90SECOND PERIODS OF HEATING LAMP EXPOSURE UPON GROWTH IN CONTROLLED ENVIRONMENT CONDITIONS Mean ± Std. Error of Mean Shown (10 rats)

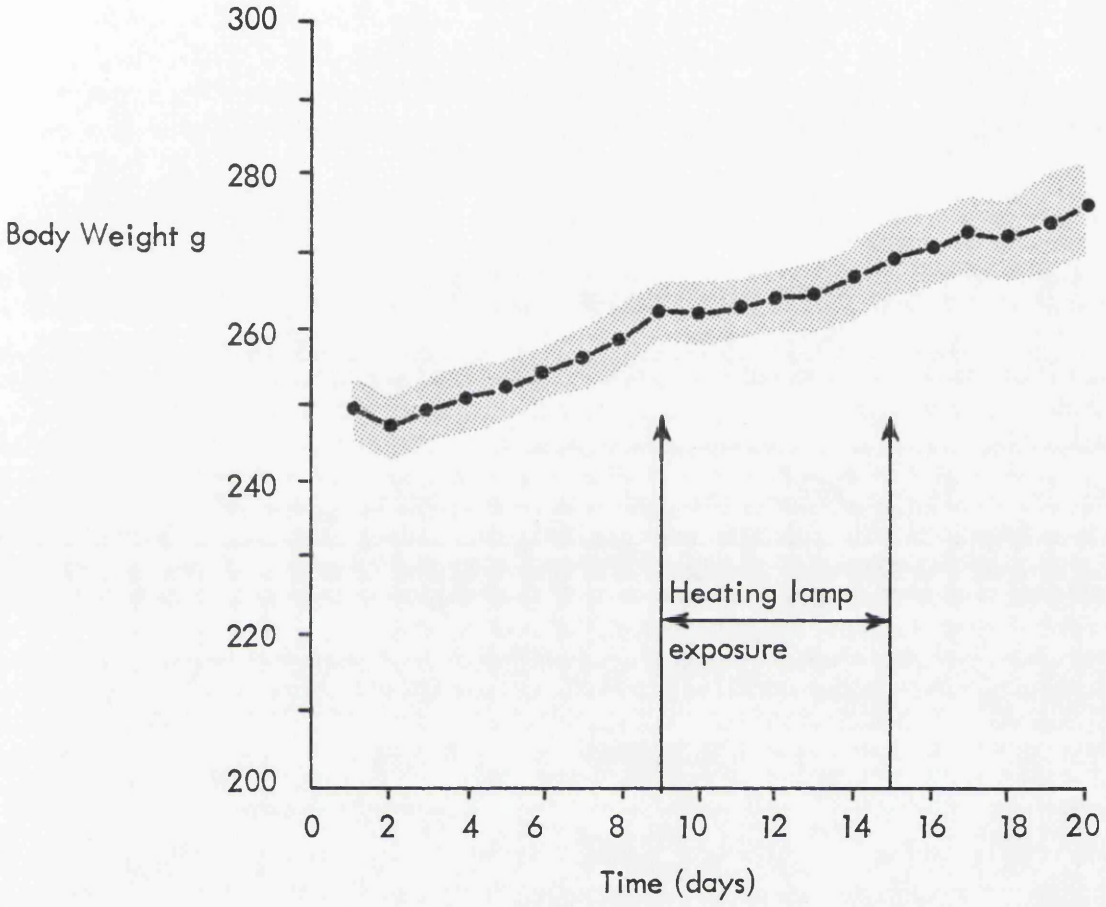


TABLE 19 RESPONSE TO REPEATED PERIODS OF 90 SECOND HEATING TWICE DAILY

Daily body weight is given in g.

DAY	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6	RAT 7	RAT 8	RAT 9	RAT 10
1	239.5	232.0	264.5	273.2	246.2	256.8	254.3	245.4	238.5	247.5
2	234.8	236.1	259.8	272.4	240.6	259.5	250.6	236.7	236.6	250.8
3	249.2	229.5	257.7	275.1	231.9	264.1	252.4	242.0	243.2	253.1
4	256.0	230.1	262.4	274.3	237.1	264.2	246.0	242.5	242.8	257.9
5	264.5	241.7	269.5	269.4	235.4	262.5	243.7	240.3	245.2	252.3
6	260.2	245.2	276.6	270.7	239.8	259.2	245.8	246.1	248.5	255.7
7	268.1	252.3	272.0	274.2	241.5	271.4	250.2	242.3	240.9	250.8
8	269.6	247.6	270.4	280.5	247.4	272.7	256.3	245.6	248.2	255.7
9	268.6	254.2	274.8	288.7	250.3	267.9	259.4	249.4	256.6	258.9
10	259.4	249.0	277.5	289.3	249.1	268.4	262.9	254.5	252.3	264.4
11	252.1	260.1	277.9	293.2	252.8	261.8	260.7	254.9	257.2	261.3
12	260.5	259.6	280.3	295.0	250.7	260.2	261.6	257.5	258.7	257.5
13	257.8	263.4	282.6	299.9	246.3	266.3	259.2	257.5	261.5	254.0
14	259.4	262.6	286.4	298.1	249.1	270.4	258.4	263.2	267.0	255.6
15	264.3	265.1	292.8	296.0	254.1	274.0	264.3	265.7	260.4	257.8
16	270.8	261.5	286.2	302.0	252.8	271.0	274.3	267.7	266.3	257.1
17	272.6	261.9	290.4	305.5	255.6	273.4	271.6	271.4	269.1	258.5
18	265.1	259.7	293.8	300.9	251.9	270.6	275.1	274.6	272.4	260.1
19	264.9	257.8	294.1	311.3	252.7	267.9	279.8	276.7	270.8	255.4
20	279.4	264.7	297.8	312.1	260.0	269.2	274.5	280.3	276.9	250.4

Heating period was from day 9 to day 15 inclusive.

Figure 102 indicated little mean change in growth rate as a result of heating lamp exposure, though individual rats did show some initial reaction (Table 19).

DAILY TAIL CLIPPING TO OBTAIN VENOUS BLOOD SAMPLE.

Repeated tail sectioning (less than $^{1}/_{32}$nd of an inch is removed to obtain blood samples) proved considerably more stressful to the rats under study (Fig. 103) than heating lamp exposure. This is reflected in the interruption of growth during the period of tail clipping. Individual animals showed a more marked response than the group mean indicated (Table 20).

Graded stress or mild injury responses were therefore readily shown in rats kept within the environmental chamber. Increasing the degree of "stress" produced increased depression of growth rate in response to it.

Fig. 103 EFFECT OF MULTIPLE TAIL AMPUTATION ON GROWTH IN CONTROLLED ENVIRONMENT CONDITIONS Mean ± Std. Error of Mean Shown (10 rats)

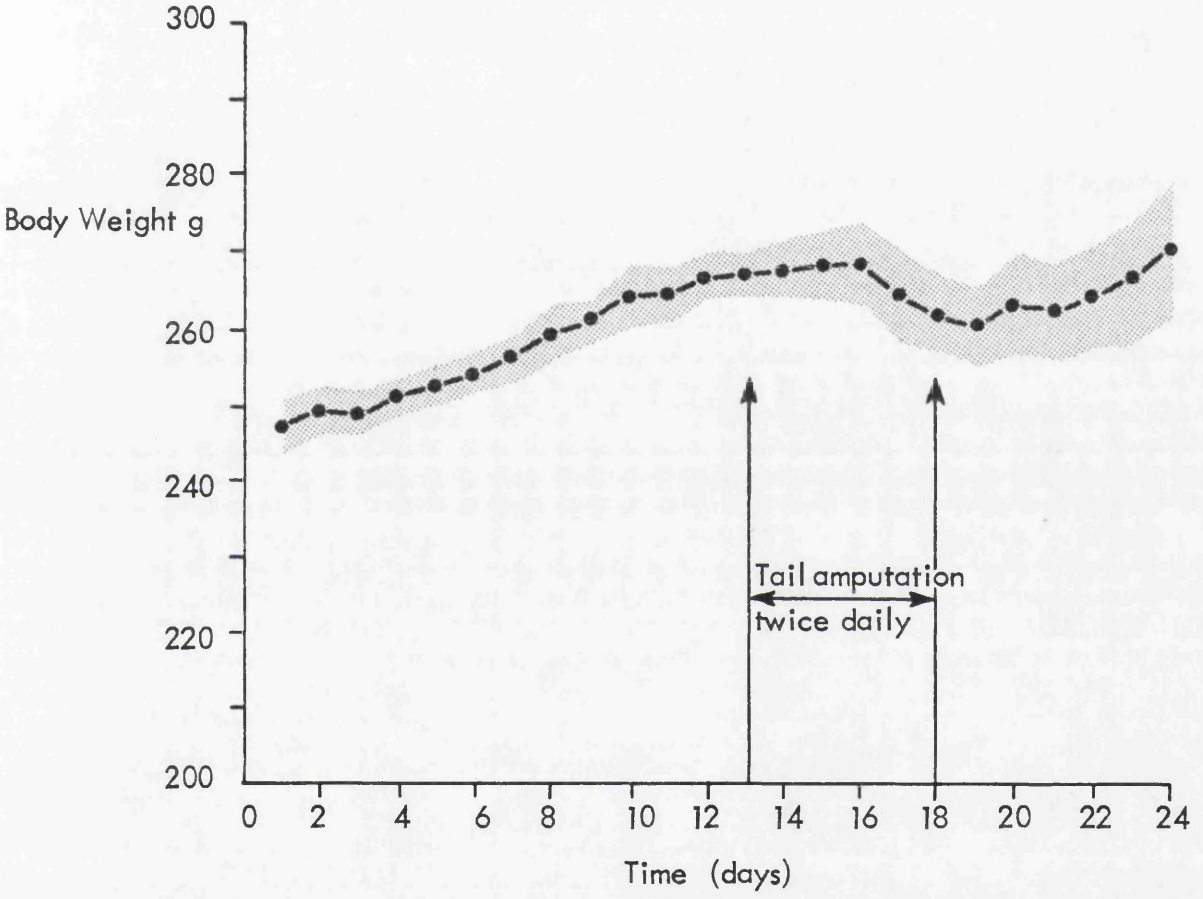


TABLE 20 EFFECT OF DAILY TAIL CLIPPING ON GROWTH RATE

Rat body weights are given in g.

DAY	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5	RAT 6	RAT 7	RAT 8	RAT 9	RAT 10
1	244.1	250.8	232.6	236.0	261.3	241.4	252.0	243.2	262.3	245.0
2	248.0	256.4	242.7	236.4	266.9	242.0	251.6	243.5	260.6	244.8
3	254.2	256.9	240.3	240.7	262.3	242.5	252.3	235.8	260.0	239.9
4	257.3	254.0	244.5	252.2	260.4	243.1	253.0	236.5	257.9	250.3
5	259.0	250.2	246.0	256.3	263.5	246.5	252.8	238.1	260.9	250.1
6	263.4	252.7	243.4	254.8	264.4	250.7	255.5	242.7	264.2	249.6
7	254.5	256.9	252.0	257.3	274.0	252.3	256.9	244.5	263.7	251.2
8	265.7	258.1	245.0	262.4	276.7	256.7	258.4	245.2	264.0	263.7
9	269.0	260.0	247.3	261.3	275.3	260.3	261.2	251.6	263.1	264.5
10	277.5	270.4	240.1	262.5	283.9	264.7	262.9	257.9	265.9	255.1
11	270.4	259.5	243.9	266.9	286.4	265.1	264.0	261.0	266.3	259.3
12	268.8	264.6	248.0	266.4	286.8	267,9	264.0	263.2	266.5	272.0
13	269.5	268.8	249.4	269.5	290.1	266.0	264.7	255.7	269.0	263.8
14	273.6	267.3	250.1	271.5	294.0	267.3	264.0	255.9	268.5	266.7
15	276.0	269.4	243.2	271.9	302.5	262.2	265.8	260.0	269.1	263.5
16	285.7	276.7	241.7	262.6	308.3	260.6	262.6	265.1	270.4	252.9
17	275.0	267.0	233.9	263.4	308.9	255.0	258.4	269.0	267.7	248.0
18	273.9	265.0	234.6	269.0	291.6	250.8	258.5	260.9	270.0	245.9
19	261.4	260.4	245.2	259.5	297.4	251.3	254.1	264.0	269.0	241.2
20	270.2	260.9	241.6	265.7	309.5	254.6	254.8	265.2	271.1	240.6
21	259.3	266.3	237.1	265.0	307.8	255.9	253.0	268.3	270.3	241.0
22	270.0	261.7	236.4	267.5	312.1	259.1	257.5	269.5	270.9	241.0
23	269.5	262.0	236.9	270.7	318.6	263.4	258.0	276.1	275.2	234.2
24	281.1	264.5	234.3	282.8	319.9	268.2	263.2	280.0	279.2	241.6

Tail clipping was carried out twice daily from day 13 to day 18 inclusive.

DEVELOPMENT OF CONTROLLED ENVIRONMENT CONDITIONS

DISCUSSION

Many diverse requirements had to be reconciled in order to create the completed small animal unit described in the preceding pages. Not the least of these was first, to obtain Home Office approval for the establishment of an additional separate animal facility within the Institute and second, approval for the detailed design of this unit.

My aim was to create an environment for the experimental animal free from uncontrolled extraneous influences in which a reproducible response to a controlled and specific injury or other stimulus could be measured precisely. This led me not only to a consideration of bricks, mortar and electrical components, but also to take account of the intellect and known behavioural characteristics of the laboratory rat.

For expert guidance I relied upon the text Animals for Research - Principles of Breeding and Management, edited by W. Lane-Petter (1963), in particular Chapter 1 - The Physical Environment of Rats and Mice,

and also on the advice of Mr. F. Fleming, head of the technical staff of the Institute of Physiology main animal house.

The first vital consideration before building the evnironmental chamber itself was to create around it an area in which all rat routine servicing procedures such as food dish weighing, water bottle changing, dirty cage sample removel, etc. could be performed without disturbing the rats under study. This area - designated the Animal Handling and Preparation Laboratory is shown in Figure 14.

The following points were considered in the design of the environmental chamber.

RAT MACROENVIRONMENT

The system of suspended wall rack cages was chosen for several reasons:

1. The all metal cage open only at the front gives the rat the greatest "feeling of security" and allows it to quickly define its own territory without unnecessary confrontation with its fellow rats. The rat cannot be overlooked from the top, sides, or back. Therefore potentially "threatening" situations for the rat are avoided.

2. The ventilating air does not cause any draughts across the cage, nor is dust or debris, which may be contaminated, likely to fall into the cage reducing potential cross contamination in groups of burned animals.

3. The wire floor allows urine and faeces to pass directly through onto the absorbent paper towelling beneath. The aluminium tray holding the paper is extremely light and rests freely on the roof of the cage beneath. No contaminated cage bedding or other material is therefore left in situ to create odours or spread infection. Food spillage can also be seen at a glance for each animal as a small pile of powder under the food dish.

4. Lighting within the chamber cannot fall directly on the rats in the wall rack cages. This is considered a desirable design feature for an animal which is principally nocturnal.

The metabolic cage rats do not have these advantages, as the cage is clear plastic on all sides with a solid metal top perforated by air holes. In the noisy conditions of a large busy animal unit such cages would seem most

unsuitable; however in the quiet undisturbed atmosphere with the environmental chamber, it is gratifying to note (Fig. 101) that there is no measurable difference in daily food intake and growth between wall rack cage rats and metabolic cage housed rats. This was fortunate rather than planned as the metabolic cage design was selected solely for its efficiency and its economy.

5. Noise was kept to a minimum in the chamber by fitting the wall rack cages with narrow rubber strips on the undersurface of the cage roof overhang, thus insulating them from air ventilation unit fan vibration which was occasionally transmitted down the spur arm upright supports.

The chamber floor itself was laid in thick sound deadening Vinyl material and all changing of glassware in the metabolic cages was performed outside the chamber in the Animal Handling and Preparation Laboratory. Inevitably some sound was generated during the cage changeover procedure. This is shown in Figure 97.

6. Hygiene within the chamber was catered for by making the entire contents readily removable for cleaning. The interior surfaces are washable and were cleaned with TEGO to act against endemic colonisation of the structure with Pseudomonas organisms.

Chamber ventilation with filtered (but not sterile) air also favoured hygienic conditions. The air was not recirculated but vented to atmosphere by a low level outlet. This system gives a slight positive pressure in the environmental chamber compared with the outer laboratory. All excreta was removed daily from the chamber with no changing of trays within the chamber itself, and therefore little dust scatter. Repeated wound swabbing of burned and control rats indicated no bacterial growth other than harmless commensals throughout the burn experiments described later. Pseudomonas was never isolated or identified in these culture swabs.

In the final analysis perhaps the most significant factor in producing controlled environmental conditions and an experimental population of normally eating, growing and living rats, lay within the animal itself and in its remarkable ability to adapt successfully to any living

conditions. An inkling of this adaptive behaviour is shown in Figure 98, A and B, in which the rats changed their eating habits very quickly so that they did not lose food by spillage. The rat's intelligence is often overlooked or underestimated. The following quotation was made by Lane-Petter (1963):

> "Animals are expensive, and their proper care entails a lot of labour. It is therefore only reasonable to design animal facilities so that, from the investigator's point of view, the nearest possible approach is made to the convenience of the chemical laboratory with its handy row of bottles."

This statement undoubtedly reflects the attitude of many research workers; it is essential however to emphasise the last part of Lane-Petter's remarks:

> "- Subject to the proviso that the biological needs of the animal are first assured."

The rat is often used as an analogue for the human in laboratory experimentation, but it is important to appreciate that some very significant behavioural differences exist between them.

CIRCADIAN RHYTHM

The sound recordings shown in Figures 96 and 97 indicate that the rat is most active during the dark portion of its light/dark cycle, i.e. it is a nocturnal animal. A large body of evidence exists confirming such circadian rhythms in a variety of systems, such as brain levels of biogenic amines (related to sleep and wakefulness, general metabolism and activity) (Friedman and Walker, 1968), plasma proteins (Schering et al., 1968) and in activity and subcutaneous body temperature (Bolles and Duncan, 1969; Hardy et al., 1970). The activity times of rats studied by Bolles and Duncan are similar to those shown in Figures 97 and 98, having regard to slight differences in timing of the light/dark cycle. The MRC view expressed by Lane-Petters (1963) is that a 14 hour light, 10 hour dark cycle is optimal for the rat. In the later burn experiments the light period for the rats was between 8.00 a.m. and 10.00 p.m. The dark period was from 10.00 p.m. to 8.00 a.m.

The significance of this is that in the preceding and subsequent experiments, calorimetry was performed on rats in their normal rest/sleep period. This may be considered to be beneficial if one takes the view that their body temperature and other functions, such as enzymic activity, should be at a minimum therefore giving a more accurate assessment of resting metabolic energy

expenditure in the rat. The rat, like the human, is capable of displaying signs of "apprehension" and "anticipation" such that repeated calorimetry measurements may prove to be a potent form of stress on the animal with all the hormonal consequences which that involves. In addition, the rat may be sleep deprived if the calorimetry is too frequent.

There were marked differences in rate of weight loss after injury between burned rats subjected to calorimetry and otherwise identically injured rats not so treated, both in the 20% BSA burn (Figs. 124, 126) and the 25% BSA burn (Fig. 145). There were even differences between control rats subjected to calorimetry and those which were not.

These observations support the view that repeated calorimetry on rats during their normal sleep period may invoke a form of stress response related in part to fear and sleep deprivation in the rat.

GROWTH RATE

Young adult rats in a state of health are continuously growing. Unlike man, their long bone epiphyses never fuse (Dr. H. Y. Elder - Zoologist, Institute of Physiology, personal communication).

It follows therefore that the effect of an injury on the rate of growth in the rat should be expressed in terms of the difference between an injured rat and a control rat, rather than describing the amount of weight lost by the injured animal from the day on which the injury was carried out. The latter method of calculation of weight loss would be appropriate in the case of man, in whom body weight is normally stable in health. Failure to appreciate this point led to apparent discrepancies between observations on the significance of the protein contribution of weight loss after injury between Cuthbertson et al. (1939) and Cairnie et al. (1957). This has been reported by the present author (Richards et al., 1976).

CONCLUSION

Though far from ideal, the environmental conditions created for the rat in the Institute of Physiology (within the imposed financial limits) provided a basis for the use of growth rate and food intake in the rat as a quantitative assessment of the effects of injury or stress on the animal. There was a significant reduction in uncontrolled stimuli affecting the rat, such as occur in any large conventional animal unit where conditions have to be a compromise in order to accommodate the diverse interests of the many legitimate users.

DEVELOPMENT OF NITROGEN ASSAY METHODS

MATERIALS and METHODS

NITROGEN ESTIMATION

In the rat and in man, nitrogen is excreted in urine and faeces principally in the form of urea. Estimation involves converting organic nitrogen to ammonia followed by measurement of the ammonia nitrogen. As several hundred samples accrue in the course of a single injury study, the semi-automated method of Fleck and Munro (1965) was selected for ammonia nitrogen analysis. This consists of a manual digestion step with completely automated ammonia analysis and is highly accurate.

Digestion Procedure:

This is a modified open tube micro-kjeldahl method with a sensitivity in the 2 - 12 mg N range.

Materials and Reagents:

1. Conc H_2SO_4 (Aristar)
2. "Special Kjeltabs" made by Thomson & Capper Ltd., Speke, Liverpool. From the review of Fleck and Munro (1965) it is clear that the catalyst

of choice is mercury, and that the ratio of salt (potassium sulphate) to conc. H_2SO_4 should be such as to give a digestion temperature of 370 to 410°C. For samples containing 2 - 12 mgm of Nitrogen this means using 2.4 g K_2SO_4, 100 mg HgO and 3 ml of 36N H_2SO_4. Fleck has had the potassium sulphate and mercuric oxide compounded into tablets each containing 1.2 K_2SO_4 and 50 mg HgO. These are the "Special Kjeltabs".

3. Long necked micro-kjeldahl flasks.

Standards:

1. Stock $(NH_4)_2\ SO_4$.
   23.596 g dry Analar $(NH_4)_2\ SO_4$ are weighed out accurately, dissolved in water and made up to 500 ml to give a stock solution containing 10 mg N/ml ((1g N = 4.7193g $(NH_4)_2\ SO_4$)).

2. For autoanalyser stock solution 20 ml of this solution are taken and made up to 1 litre (200 ugN/ml).

3. Urea.
   5 g of urea are weighed out accurately and made up to 1 litre with distilled water. This gives a solution for digestion containing 2.31 g N/ml such that aliquots from 1 - 5 ml give an

appropriate graded increase in N content. Several samples are included with each batch of urine or faecal samples.

4. Tryptophan.

From 20 to 83 mg of tryptophan are weighed out accurately into a micro-kjeldahl flask and digested (1 g N = 7.2857 g tryptophan). Several samples are included in each batch of urine or faecal samples.

Digestion Procedure: URINE

From the 250 ml volumetric cylinder containing a 24 hour collection of rat urine, a 2.5 ml aliquot is taken (containing approximately 2 - 12 mg N), and pipetted into a special long necked micro-kjeldahl flask (Fig.104) containing 2 glass beads, 2 'Special Kjeltabs' and 3 ml conc. H_2SO_4. One blank run containing 2.5 ml of water, 2 glass beads, 2 'Special Kjeltabs' plus 3 ml conc. H_2SO_4 in a micro-kjeldahl flask are also set up for every 5 (approximately) urine samples.

The walls of the kjeldahl flasks were washed down with distilled water - including the blank flask. The flasks are next transferred to a digestion rack (a Gallenkamp rack with close fitting glass vacuum hood was used. Fig.), and the excess water rapidly

Fig. 105 GALLENKAMP MICRO KJELDAHL DIGESTION RACK SHOWING VACUUM EXTRACTOR HOOD AND ALKALI SUCTION TRAP

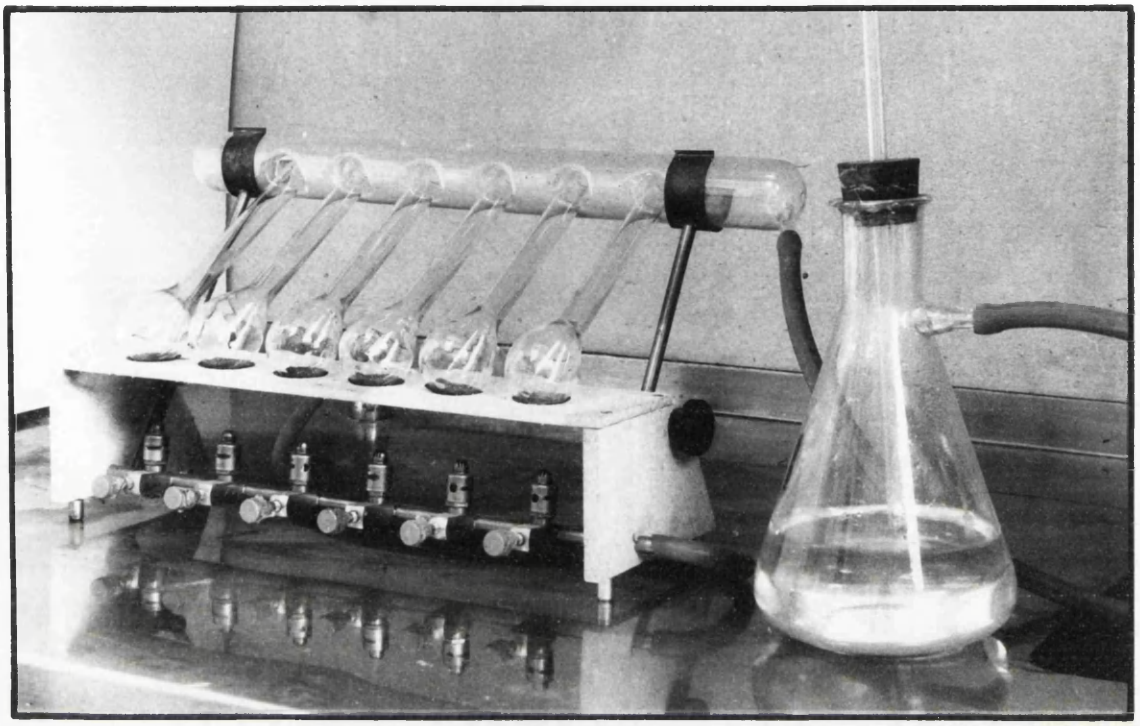
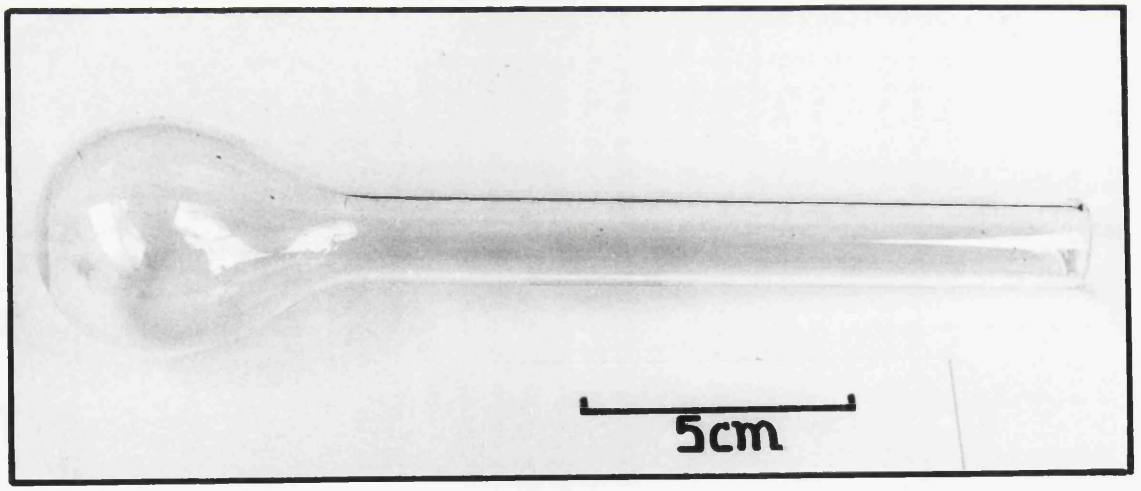


Fig. 104 LONG NECKED MICRO KJELDAHL FLASK



boiled off. The neck of the flask is kept at 35° to the horizontal. The constant boiling mixture is refluxed for approximately 20 minutes or until the digest has been clear for 10 minutes. Rarely is more than 30 minutes total heating required for this step. The onset of refluxing is easily identified by the appearance of a ring of acid at the neck of the flask. If the digest is not crystal clear on cooling, but becomes grey-white and solidifies, this indicates that the time of digestion has been excessive and the sample is discarded. The clear digest is allowed to cool, and its volume made up to the neck of the flask, and the total digest decanted into a 100 ml volumetric flask. After washing out the micro-kjeldahl flask four times into the volumetric flask the volume is made up to 100 ml and the diluted digest stored at 4°C until ammonia analysis can be made.

Digestion Procedure : FAECES

To the dilute suspension of faeces (prepared as described in the collection procedure), 2 glass beads, 2 'Special Kjeltabs' and 3 ml of conc. H_2SO_4 are added as for the urine sample, and digested. After boiling off the excess water, charring occasionally begins and the gas flame should be reduced slightly to avoid frothing which can occur with some faecal samples. Ten drops of 30 vol. H_2O_2 are added to defroth the

flasks if the frothing reaches the flask neck. It is often necessary to wash down the neck of the flask with water before completing digestion.

The remainder of the procedure is as for urine digestion except that with faeces the digest may remain yellow until it cools when the colouring fades leaving the digest clear. The digest is made up to 100 ml with distilled water in a volumetric flask and stored for ammonia analysis.

Digestion Procedure: FOOD

The diet used, i.e. LIT from Nutritional Biochemicals Inc., U.S.A., is a powder. 0.25g is taken and made into a suspension with a few ml of water. This mixture is then homogenised twice for 10 minutes each time at 14,000 revs/min. The whole of this homogenate is then washed into a micro-kjeldahl flask and the digestion procedure is carried out exactly as for faeces.

NITROGEN ESTIMATION : AMMONIA ANALYSIS

The method used in this study is described below and is that evolved by Fleck for use with a Technicon Sampler 2 and Tubular Flow Cell (Mann, 1963; Fleck, 1967). The rate of flow of sample and reagents through the cell is determined by suction via a line from the

pump manifold to the cell. This allows regulation of flow rate by selection of the appropriate internal diameter of tubing in the pump manifold.

The autoanalyser records the amount of light transmitted through the solution in the flow cell. As the solutions are being continuously pumped through the flow cell each bolus of colour is not uniform in intensity, but is less dense at the leading and trailing edges.

This characteristic of the system means that the flow rates must be carefully selected so that peak colour intensity is analysed. A sampling rate of 40 to 60 samples per hour was used in these studies.

Light transmission is plotted against ammonia content of each of a range of standard samples and the slope of the regression line calculated. This value is used in calculating all experimental results. A Wang programmable desk calculator was utilised for this step. The sensitivity of the system is 10 - 120 ug N as $(NH_4)_2\ SO_4$ per ml of solution.

Procedure: Reagents.

1. Sodium phenolate (Na $\bigcirc$Oh)

   Na_3PO_4 (Analar) 19 g (0.05M)

   NaOH (Analar) 14.5 g (0.365M)

   Phenol (Analar) 30 g (0.295M)

The reagents are added in the above order to approximately 800 ml of water, stirring to dissolve each one in turn, and made up to 1 litre. This is kept in the fridge and can be used for up to 2 weeks.

2. Sodium nitroprusside (NaNP)

A solution of NaNP 0.125 g/l (4.2×10^{-4}M) is made up fresh before each run and remains stable for at least four hours.

3. Sodium Hypochlorite (Na O Cl)

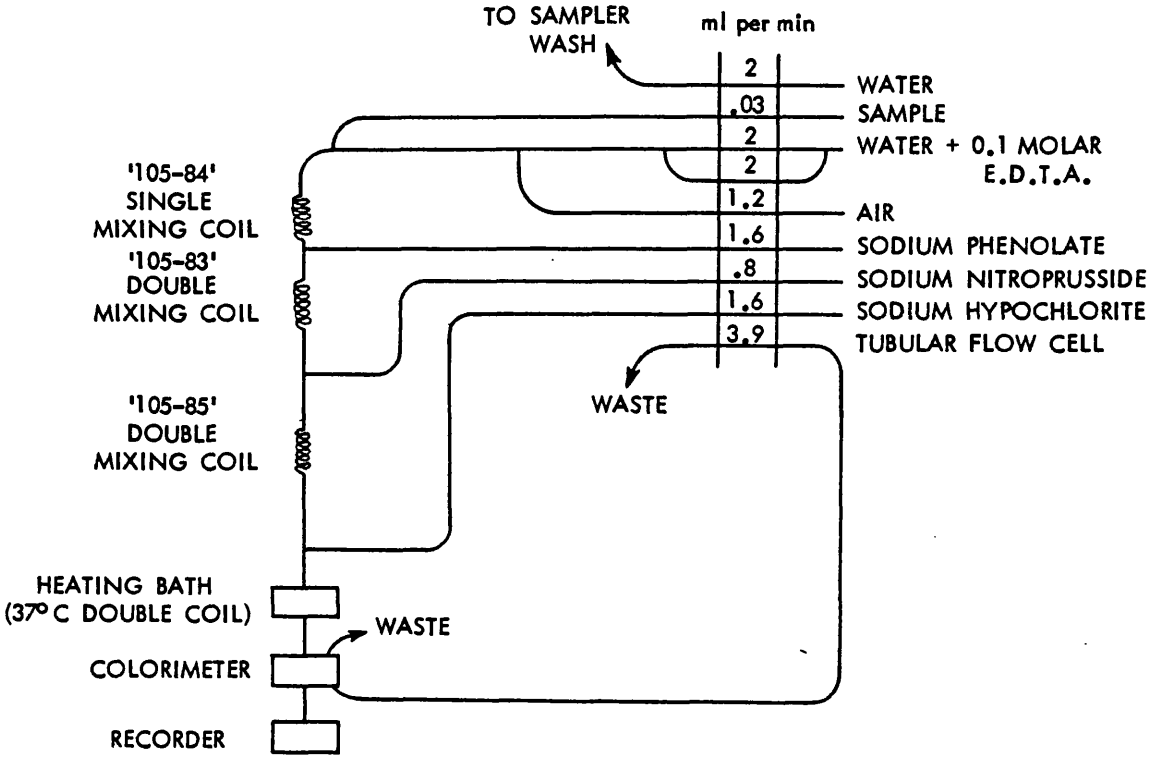
A $^{1}/50$th dilution of a 2.5% stock solution (0.015 M) is made up and stored in the fridge.

4. Standard Solutions.

Ammonium sulphate $(NH_4)_2\ SO_4$ Analar is used. A 200 ug N/ml stock solution is made up, and the autoanalyser standard curve prepared as follows. Into separate 100 ml volumetric flasks are pipetted 5 ml of stock $(NH_4)_2\ SO_4$ solution to give 10 ug N per ml, and 10, 20, 30, 40, 50, 60 ml are pipetted to give standards containing 20, 40, 60, 80, 100 and 120 ug N per ml of solution.

The scheme for mixing reagents and the sample by the tubular flow cell is shown in Figure 106. The sample is diluted with water, i.e. 0.05 ml/min of sample into air segmented (1.2 ml/min) water at 4 ml/min. Mixing occurs. Na phenolate is added

Fig. 106 FLOW DIAGRAM FOR AUTOANALYSER ESTIMATION OF NITROGEN



Sampling rate - 40/hour

Sensitivity - 10 to 120 µg N per ml

8mm Tubular Flow cell, 620 mµ Wavelength

Sample line meets the water line via an A6 platinum insert

(1.6 ml/min) and mixed. Na nitroprusside (0.8 ml/min) is also added and mixed. Finally Na hypochlorite (1.6 ml/min) is added and mixed before the overall mixture passes through a double heating bath regulated at 37°C. Approximately 8 minutes heating takes place. The solution is transferred to an open 6 mm flow cell and the transmission of light through it read with a 620 mu red filter.

Using the standard solution containing 10 - 120 ug N/ml and setting the water blank base line at 95% light transmission, a standard curve of percentage transmission against nitrogen concentration is constructed. This is related to optical density by calculating the linear regression line through these points and thus obtaining a straight line standard curve. This last step is carried out at the end of an experimental run. The samples to be measured are set up after the standard curve samples, each group of 5 samples being preceded by a duplicate digested blank. Following each group of samples, two water washes are set up. Two consecutive standards are inserted at intervals to check for drift during a run.

Notes on setting up the autoanalyser:

Prior to use the following are checked - that

1. the appropriate filters have been inserted

2. aperture No. 4 or 5 has been put in the reference side

3. the solution is passing through the flow cell

4. having placed the "O" aperture in front of the flow cell, the recorder is set to "O" % T with the "O" control

5. the "O" aperture is then removed

6. the helipot is set to 700 (100% T control)

7. the apertures are adjusted to obtain almost 100% T

8. the helipot is finally adjusted to obtain 100% T and locked.

The system described is in essence that originated by Dr. A. Fleck for the analysis of N in biological samples. The methods for preliminary preparation of food and faecal samples are those of the author. The autoanalyser equipment was housed in the Department of Pathological Biochemistry, Glasgow Royal Infirmary. The manual digestion step was carried out in the Institute of Physiology exclusively, for the preliminary evaluation of the system and for the 5% BSA burn experiment. In the later experiments, identical digestion equipment was used in the Biochemistry Department, Glasgow Royal Infirmary. The results obtained in the preliminary evaluation are presented.

NITROGEN ESTIMATION

RESULTS

OPTIMAL DIGESTION TIMES FOR STANDARDS

The recovery of nitrogen from standard samples of tryptophan and urea were estimated using the methods described. The initial work of Fleck in developing the method was repeated and digestion times between 5 and 45 minutes used. The percentage recovery of nitrogen with varying digestion times is shown for tryptophan in Figure 107 and for urea in Figure 108. Individual results are given in Tables 21 and 22 respectively.

The optimal digestion time for tryptophan and urea proved to be 20 minutes, though Fleck had earlier found digestion times of 10 minutes to be satisfactory for urea (Fleck, 1976, personal communication).

The percentage recovery of nitrogen from urea at 20 minutes was 98.1% $\pm$ 1.4% (SD) and from tryptophan 97.9% $\pm$ 2.6% (SD).

Fig. 107 TRYPTOPHAN STANDARDS : PERCENT NITROGEN RECOVERY WITH DIFFERING DIGESTION TIMES (8 samples at each point - mean ± SD shown)

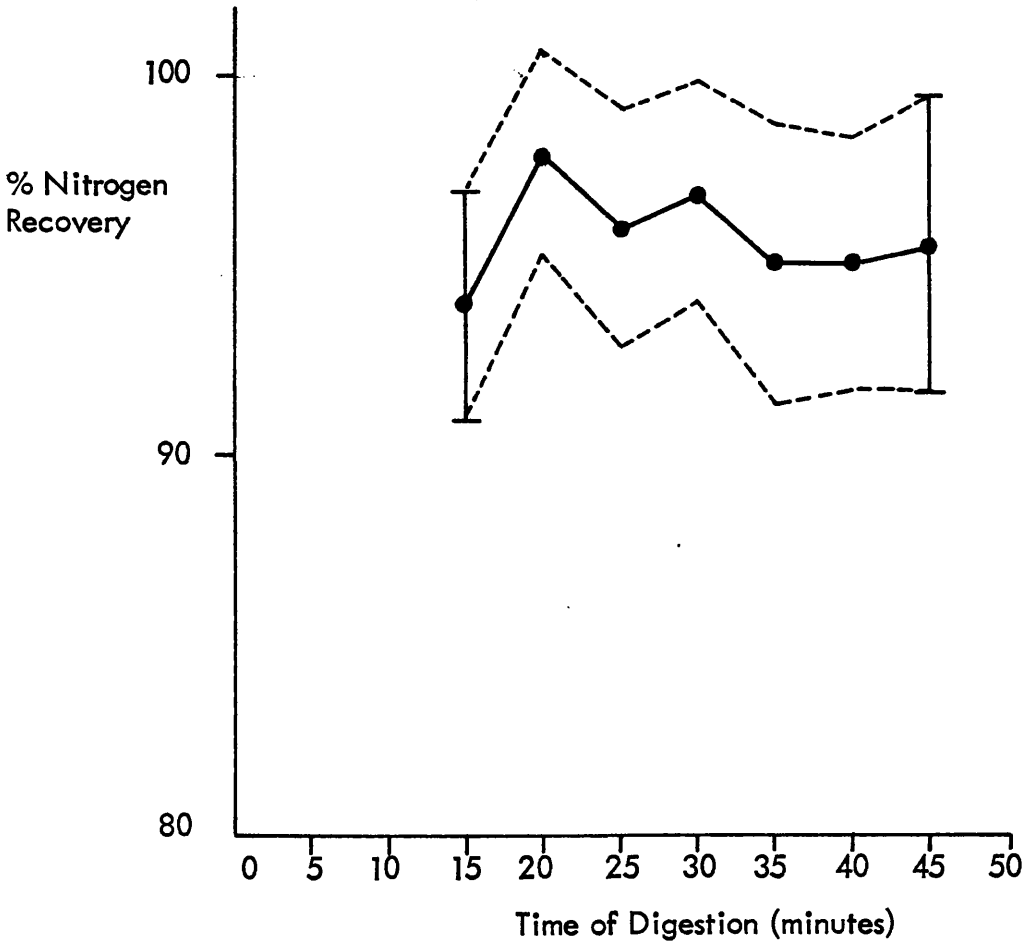


Fig. 108 UREA STANDARDS : PERCENT NITROGEN RECOVERY WITH DIFFERING DIGESTION TIMES. (6 samples at each point - mean ± SD shown)

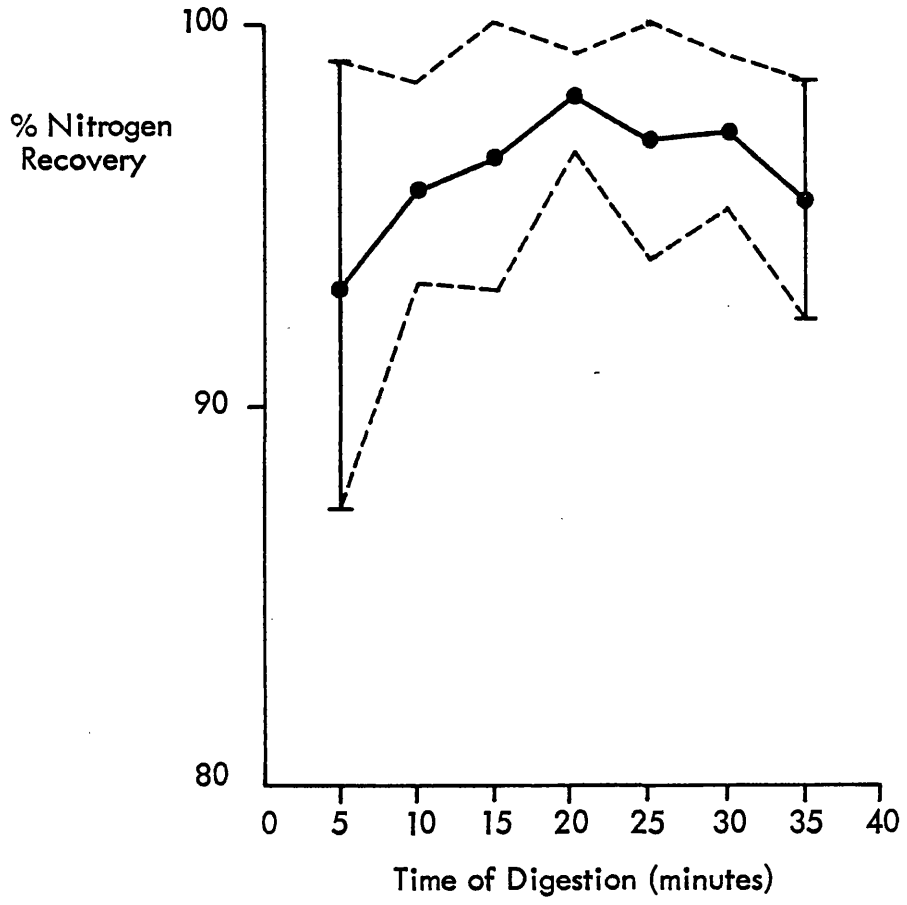


TABLE 21

TRYPTOPHAN STANDARDS : PERCENTAGE NITROGEN RECOVERY

TIME OF DIGESTION	% N. RECOVERIES												MEAN ± SD
10 min	* Solidified on cooling												
15 min	90.3	97.8	96.9	97.2	94.2	90.8	91.7	93.0					94.0 ± 3.0
20 min	99.5	93.4	91.7	97.8	97.9	99.8	98.9	99.7	97.8	98.6	99.8	99.7	97.9 ± 2.6
25 min	93.1	99.3	99.2	99.0	94.9	97.1	94.4	91.3					96.0 ± 3.1
30 min	96.8	99.3	96.4	99.1	98.5	98.3	96.2	90.5					96.9 ± 2.9
35 min	95.6	98.7	95.6	96.9	97.3	98.4	88.7	89.6					95.1 ± 3.8
40 min	96.2	94.9	97.5	98.6	94.1	98.1	92.6	88.5					95.1 ± 3.4
45 min	92.9	99.4	96.3	99.2	98.7	97.5	90.0	90.7					95.6 ± 3.9

TABLE 22

UREA STANDARDS : PERCENTAGE NITROGEN RECOVERY

TIME OF DIGESTION	% N. RECOVERIES												MEAN ± SD
5 min	84.8	86.3	95.4	96.6	97.3	97.9							93.1 ± 5.9
10 min	90.4	97.9	97.3	95.5	97.8	95.4							95.7 ± 2.8
15 min	95.1	90.2	99.1	99.2	98.1	97.3							96.5 ± 3.4
20 min	96.8	99.8	99.5	97.1	96.2	99.1	99.5	98.9	97.5	98.7	95.9	98.6	98.1 ± 1.4
25 min	91.0	99.1	98.7	97.9	98.6	96.9							97.0 ± 3.1
30 min	93.1	98.3	98.0	98.2	97.9	97.7							97.2 ± 2.0
35 min	90.9	92.7	96.0	95.9	98.9	98.1							95.4 ± 3.1

Using a 20 minute digestion time, in the 5% BSA burn study there appeared to be some variation in the percentage of nitrogen recovery between batches of standards, in that the nitrogen recovery from urea in the second batch was greater than that in Figure 108. The mean percentage nitrogen recovery for urea was 109.1% $\pm$ 4.7% (SD), and 94.5% $\pm$ 4% for tryptophan (Table 23). The only difference between the first and second batches lay in the freshness of the reagents used in the colorimetric stage of the ammonia analysis. For all studies thereafter, reagents were made up fresh on the day of use. The nitrogen values given for biological samples such as urine, faeces and food were corrected, where appropriate, by a very small percentage, to take account of small variations in nitrogen recoveries between batches of urea standards.

NITROGEN RECOVERIES FROM METABOLIC CAGE COLLECTING SYSTEM

Urea standards were used to estimate the likely losses of nitrogen which might be found in day to day practice due to adherence or spillage of rat urine around the cages or in the glass funnel and separator units.

A standard aliquot of urea was made up with water to 20 ml. This volume was approximately equivalent to the amount of urine passed in a day by the average 250 -

TABLE 23 A

UREA STANDARDS : PERCENTAGE NITROGEN RECOVERY

(20 min digestion time)

CALCULATED N. ug/ml	MEASURED N. ug/ml	% N. RECOVERY
41.4	45.1	108.9
82.9	88.4	106.7
41.4	43.7	105.5
41.4	44.4	107.2
41.4	43.4	104.8
51.8	56.6	109.3
72.5	77.2	106.5
62.2	65.7	105.7
62.2	65.7	105.7
62.2	70.5	113.4
41.4	42.9	103.6
41.4	42.3	102.2
41.4	43.2	104.3
41.4	41.6	100.5
41.4	48.1	117.0
62.6	74.0	119.0
82.9	96.5	116.4
103.6	111.3	107.4
51.8	58.3	112.5
93.0	102.8	110.5
62.2	71.4	114.9
72.5	79.3	109.4
41.4	45.8	110.6
41.4	46.0	111.1
41.4	46.4	112.1
93.0	102.8	110.5

MEAN N. RECOVERY

109.1%

$\pm$ 4.7% (SD)

TABLE 23 B

TRYPTOPHAN STANDARDS : PERCENTAGE NITROGEN RECOVERY

(20 min. digestion time)

CALCULATED N. ug/ml	MEASURED N. ug/ml	% N. RECOVERY
27.4	27.0	98.5
27.4	25.9	94.5
27.4	24.8	90.5

MEAN N. RECOVERY

94.5%

± 4% (SD)

300 g rat at 20°C ambient temperature. This solution was then poured into the metabolic cage glass collecting system in small driblets over the course of a morning in a manner similar to the way in which the rat normally urinated. The cages were then washed down and the sample collected in exactly the same manner as outlined previously for urine specimens. Table 24 gives the results of 20 such sample collections. Identical urea standard aliquots were analysed to give the expected or complete nitrogen recovery in the samples before these were poured into the cage glassware. These recoveries are shown in Table 22 at the 20 minute time period. The expected complete recovery for the standard aliquots was 98.1% ± 1.4% (SD). The mean recovery for identical aliquots after passage through the cage collecting system was 95.3% ± 3.5% (SD) (Table 24). The mean difference of 2.8% indicated the likely loss of nitrogen under idealised conditions due to spillage or other losses in the collecting system. Alternatively, the metabolic cage collecting system efficiency was 97.2%.

DETERMINATION OF NITROGEN CONTENT IN DUPLICATE SAMPLES OF RAT URINE

Duplicate samples of rat urine were taken during the initial 5% BSA burn experiment and their nitrogen content determined in order to assess the likely scatter in results due to variations in pipetting technique, difference in digestion and other factors.

TABLE 24

PERCENTAGE NITROGEN RECOVERY OF UREA ALIQUOT FROM METABOLIC CAGE COLLECTING SYSTEM / EFFICIENCY OF METABOLIC CAGE DESIGN

SAMPLE NUMBER	% NITROGEN RECOVERY	SAMPLE NUMBER	% NITROGEN RECOVERY
1	94.3	11	86.7
2	92.7	12	96.4
3	97.3	13	98.4
4	99.2	14	95.7
5	89.6	15	96.9
6	96.8	16	93.2
7	98.6	17	94.1
8	97.6	18	99.0
9	94.5	19	97.5
10	90.0	20	98.3

MEAN NITROGEN RECOVERY 95.3% $\pm$ 3.5% (SD)

The results are given in Table 25. 232 samples of rat urine were analysed in duplicate in 2 batches on the same day. Sample 1 was expressed as a percentage of Sample 2. Overall there was no significant mean difference between the two groups of samples, though there was considerable variation in occasional duplicate samples. The mean difference between sample groups 1 and 2 was 0.14% though the standard deviation was $\pm$ 6.4%.

The more gross differences are almost certainly due to small errors in volume measurement.

FOOD NITROGEN CONTENT

Low Iodine Test Diet was obtained direct from the Nutritional Biochemicals Corporation by air freight from the U.S.A. It was delivered in 100 lb weight drums. The nitrogen content varied slightly from drum to drum between 3.7%N and 3.9%N (by weight). The nitrogen content of diet samples within one batch were highly consistent, the scatter found in diet sample analysis being less than $\pm$ 3.5% (SD).

TABLE 25 A

NITROGEN CONTENT IN DUPLICATE SAMPLES OF RAT URINE

SAMPLE 1	: 2	% DIFF.	SAMPLE 1	: 2	% DIFF.	SAMPLE 1	: 2	% DIFF.
504	568	88.7	484	489	99.0	355	363	97.8
627	642	97.7	504	521	96.7	511	493	103.7
529	529	100	409	402	101.7	362	357	101.4
473	481	98.3	603	551	109.4	422	422	100
437	424	103.1	467	469	99.6	493	484	101.9
529	497	106.4	527	530	99.4	527	527	100
568	583	97.4	424	420	101.0	454	455	99.8
566	572	99.0	417	428	91.4	392	409	95.8
558	511	109.2	488	502	97.2	333	343	97.1
437	416	105.0	451	431	104.6	425	421	101.0
520	510	102.0	461	434	107.0	529	529	100
469	469	100	402	400	100.5	504	518	97.3
442	450	98.2	357	331	107.9	457	456	100.2
466	451	103.3	537	529	101.5	466	466	100
663	674	98.4	540	602	98.7	341	348	98.0
736	695	105.9	440	431	102.1	484	493	98.2
550	550	100	407	427	95.3	576	556	103.6
448	447	100.2	535	535	100	355	344	103.2
479	475	100.8	423	505	83.8	382	376	101.6
496	518	95.8	437	450	97.1	400	455	87.9
519	470	110.4	550	556	98.9	414	416	99.5
615	594	108.5	550	537	102.4	371	384	96.6
560	563	99.5	318	355	89.6	417	423	98.6
527	518	101.7	394	417	94.5	430	466	92.3
519	519	100	475	455	104.4	432	440	98.2
552	543	101.7	435	451	96.5	402	409	98.3
622	604	103.0	429	442	97.1	413	432	95.6
629	651	96.6	402	411	97.8	362	368	98.4
366	382	95.8	416	406	102.5	416	444	93.7
431	424	101.7	340	335	101.5	371	428	86.7
507	499	101.6	430	413	104.1	399	398	100.3
473	458	103.3	406	384	105.7	355	334	106.3
335	382	87.7	457	460	99.3	300	304	98.7
400	376	106.4	470	460	102.2	402	410	98.0

TABLE 25 B

NITROGEN CONTENT IN DUPLICATE SAMPLES OF RAT URINE

SAMPLE 1	SAMPLE 2	% DIFF.	SAMPLE 1	SAMPLE 2	% DIFF.	SAMPLE 1	SAMPLE 2	% DIFF.
372	359	103.6	521	538	96.8	464	493	93.9
411	425	96.7	554	544	101.8	366	357	102.5
552	529	104.3	514	523	103.4	564	448	125.9
470	488	96.3	410	437	93.8	401	430	93.3
402	409	98.3	493	546	90.3	464	548	84.7
553	510	108.4	554	453	122.3	512	530	96.6
420	410	102.4	617	610	101.1	497	497	100
542	543	99.8	506	493	102.6	305	259	117.8
312	312	100	514	514	100	494	578	85.5
500	488	102.5	456	462	98.7	453	473	95.8
583	594	98.1	474	470	100.9	437	479	91.2
321	354	90.7	395	421	93.8	348	385	90.4
592	535	110.7	410	418	98.1	389	450	86.4
1120	1120	100	450	444	101.4	436	484	90.1
1338	1347	99.3	603	554	108.8	404	404	100
1216	1205	100.9	607	614	98.9	319	320	99.7
1220	1186	102.9	508	489	103.9	458	486	94.2
1220	1235	98.8	479	486	98.6	436	422	103.3
1058	1046	101.1	486	456	106.6	380	333	114.1
1165	1168	99.7	508	441	115.2	422	433	97.5
979	976	100.3	442	430	102.8	410	418	98.1
1212	1230	90.5	444	390	113.8	408	424	96.2
431	416	103.6	349	357	97.8	530	554	95.7
563	560	100.5	515	437	117.8	453	453	100
555	555	100	442	432	102.3	537	476	112.8
481	476	101.1	493	480	102.7	172	175	98.3
513	535	95.9	441	434	101.6	486	430	113.0
451	469	96.2	464	450	103.1	379	372	101.9
543	538	100.9	466	471	98.9	427	458	93.2
428	420	101.9	529	520	101.7	450	459	98.0
512	484	105.8	354	349	101.4	358	395	90.6
511	508	100.6	515	421	122.3	253	280	90.4
582	629	92.5	420	445	94.4	429	398	107.8

TABLE 25 C

NITROGEN CONTENT IN DUPLICATE SAMPLES OF RAT URINE

SAMPLE 1	SAMPLE 2	% DIFF.
430	429	100.2
409	403	101.5
514	464	110.8
353	366	96.4
367	358	102.5
380	408	93.1
437	429	101.9
306	357	85.7
182	179	101.7
445	430	103.5
366	417	87.8
360	363	99.2
408	410	99.5
403	400	100.8
410	355	115.5
384	380	101.1
486	455	106.8
430	412	104.4
413	430	96.0
546	590	92.5
437	466	93.8
434	422	102.8
479	523	91.6
396	364	108.8
477	479	99.6
418	432	96.8
430	425	101.2
355	348	102.0
392	381	102.9
486	456	106.6
369	381	96.9

SAMPLE 1 ÷ 2
(Percent)

100.14%
± 6.4% SD

DETERMINATION OF FAECAL NITROGEN CONTENT

The measurement of faecal nitrogen content required preliminary preparation of samples prior to digestion as described previously. In uninjured normal rats eating L.I.T. Diet, which has a low faecal residue, compared with other standard rat diets (Fig. 99), faecal nitrogen content was measured and the results expressed as a percentage of the daily urinary nitrogen excretion. This is shown in Table 26. A mean value of 4.3 $\pm$ 2.5% (SD) was found in 75 observations made in 10 rats.

Faecal nitrogen content was also measured in 7 rats for 8 days after unilateral femur fracture under ether anaesthesia. The results are shown in Table 27. After bony injury the proportion of faecal nitrogen to urine nitrogen losses was 3.3 $\pm$ 1.9% (SD). The faecal/urine nitrogen values given in Table 26 for samples 23 - 75 are the pre-injury results (over a 9 day period before injury) for the 7 rats subjected to long bone fracture whose post-injury faecal/urine nitrogen values are given in Table 27. The mean value of faecal to urine nitrogen losses for this group of 7 rats before injury was 4.1 $\pm$ 2.4% (SD); this was just significantly different from the post-injury value of 3.3 $\pm$ 1.9% (SD) at the 5% confidence limit ($P = 0.04$).

TABLE 26 A FAECAL NITROGEN CONTENT - LOW IODINE TEST DIET (NBC) : NORMAL RATS

MEAN $\frac{\text{FAECAL NITROGEN}}{\text{URINE NITROGEN}}$ % = 4.3 $\pm$ 2.5% (SD)

	URINE N.	FAECAL N.	$\frac{\text{FAECAL N.}}{\text{URINE N.}}$ %
1	585	28	4.8
2	585	35	5.0
3	415	14	3.4
4	510	20	3.9
5	420	14	3.3
6	780	24	3.1
7	390	17	4.4
8	375	16	4.3
9	285	25	8.8
10	705	37	5.3
11	645	14	2.2
12	510	30	5.9
13	495	48	9.7
14	360	5	1.4
15	495	60	12.1
16	210	10	4.8
17	420	20	4.8
18	570	12	2.1
19	690	67	9.7
20	420	4	1.0
21	495	14	2.8
22	420	16	3.8
23	379	16	4.2
24	313	8	2.6
25	391	14	3.6
26	366	24	6.6
27	424	11	2.6
28	377	13	3.4
29	503	19	3.8
30	382	4	1.0

TABLE 26 B FAECAL NITROGEN CONTENT - LOW IODINE TEST DIET (NBC) : NORMAL RATS

	URINE N.	FAECAL N.	FAECAL N. / URINE N. %
31	522	20	3.8
32	431	19	4.4
33	409	29	7.1
34	537	11	2.0
35	476	16	3.4
36	395	4	1.0
37	403	44	10.9
38	492	11	2.2
39	366	8	2.2
40	292	13	4.5
41	347	16	4.6
42	278	14	5.0
43	336	15	4.5
44	391	15	3.8
45	435	9	2.1
46	352	11	3.1
47	387	29	7.5
48	354	9	2.5
49	340	13	3.8
50	391	21	7.2
51	336	14	4.2
52	209	26	12.4
53	467	9	1.9
54	372	14	3.8
55	413	6	1.5
56	391	16	4.1
57	400	11	2.8
58	400	10	2.5
59	317	20	6.3
60	358	9	2.5
61	341	16	4.7
62	391	12	3.1
63	377	7	1.9
64	358	14	3.9

TABLE 26 C FAECAL NITROGEN CONTENT - LOW IODINE TEST DIET (NBC) : NORMAL RATS

	URINE N.	FAECAL N.	$\frac{\text{FAECAL N.}}{\text{URINE N.}}$ %
65	367	14	3.8
66	282	13	4.6
67	396	10	2.5
68	390	7	1.8
69	401	12	3.0
70	348	9	2.6
71	362	23	6.4
72	390	6	1.5
73	443	17	3.8
74	290	31	10.7
75	404	22	5.4

TABLE 27 A FAECAL NITROGEN CONTENT - LOW IODINE TEST DIET (NBC) : IN RATS AFTER UNILATERAL FEMUR FRACTURE

MEAN $\frac{\text{FAECAL NITROGEN}}{\text{URINE NITROGEN}}$ % = 3.3 $\pm$ 1.9% (SD)

	URINE N.	FAECAL N.	$\frac{\text{FAECAL N.}}{\text{URINE N.}}$ %
1	479	13	2.7
2	502	17	3.4
3	491	3	0.6
4	348	7	2.0
5	472	9	1.9
6	511	5	1.0
7	388	4	1.0
8	385	14	3.6
9	564	14	2.5
10	362	17	4.7
11	439	9	2.1
12	444	6	1.4
13	398	19	4.8
14	521	13	2.5
15	437	7	1.6
16	437	21	4.8
17	503	24	4.8
18	478	3	0.6
19	410	17	4.1
20	481	21	4.4
21	364	4	1.1
22	346	24	6.9
23	385	18	4.7
24	585	15	2.6
25	415	4	1.0
26	510	20	3.9
27	420	14	3.3
28	367	5	1.4
29	443	25	5.6
30	378	9	2.4

TABLE 27 B FAECAL NITROGEN CONTENT - LOW IODINE TEST DIET (NBC) : IN RATS AFTER UNILATERAL FEMUR FRACTURE

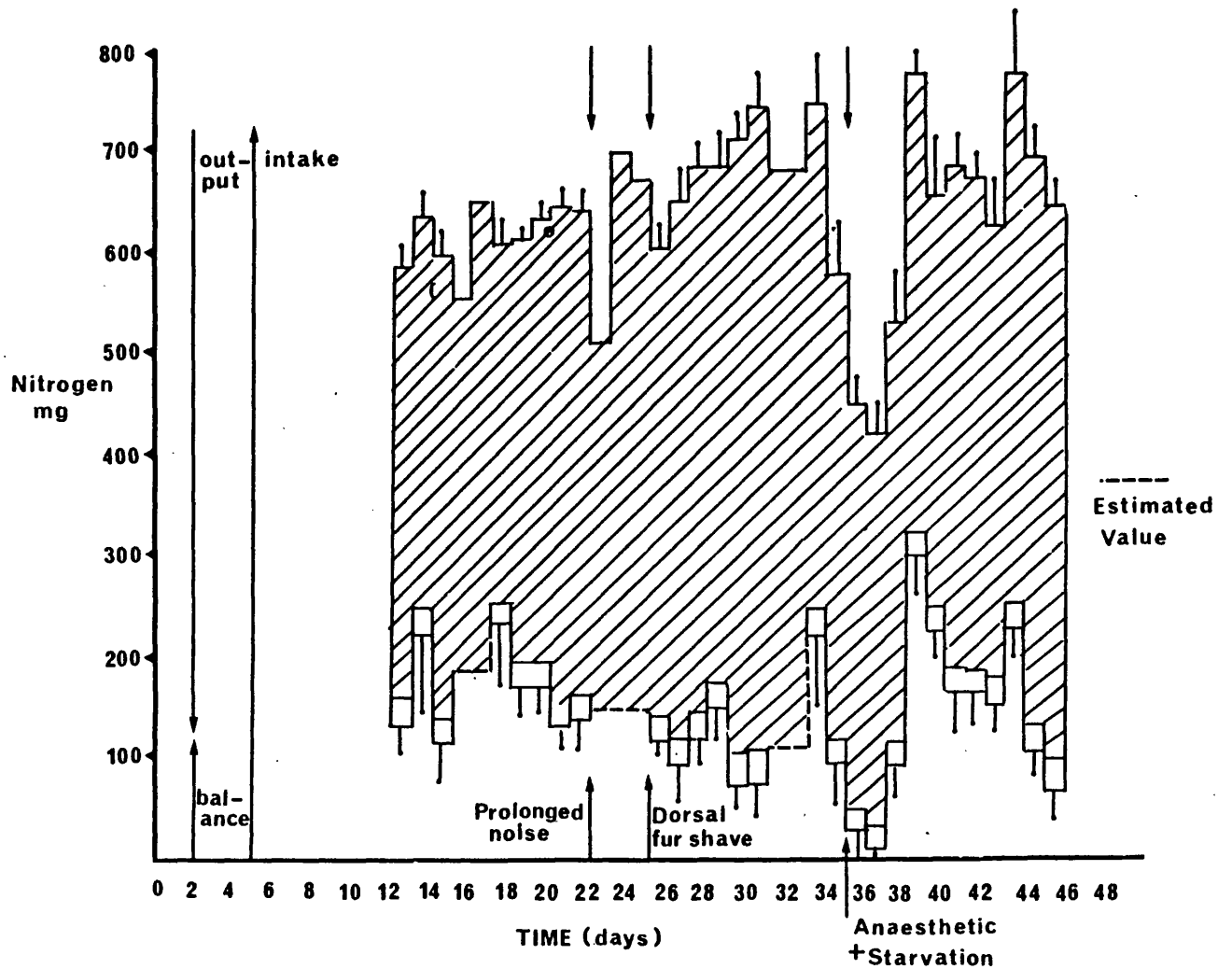
	URINE N.	FAECAL N.	$\frac{\text{FAECAL N.}}{\text{URINE N.}} \times 100$
31	458	27	5.9
32	412	11	2.7
33	379	17	4.5
34	460	10	2.2
35	494	16	3.2
36	391	25	6.4
37	407	17	4.2
38	381	6	1.6
39	310	24	7.7
40	445	21	4.7
41	378	9	2.4
42	395	9	2.3
43	344	10	2.9
44	300	20	6.7
45	368	15	4.1
46	480	5	1.0
47	405	34	8.4
48	479	7	1.5
49	423	13	3.1
50	461	13	2.8
51	374	10	2.7

It was felt that in most circumstances, when rats are fed L.I.T. Diet, faecal nitrogen content was unlikely to exceed 5% of the urinary nitrogen losses. Because of the very labour intensive nature of the faecal nitrogen measurements, no subsequent faecal nitrogen determinations were made, as the results did not appear to significantly affect the conclusions based on measurement of urine nitrogen losses alone.

A partial nitrogen balance study was carried out on uninjured control rats as part of the first 5% BSA burn experiment. Four uninjured rats were housed in the metabolic cages within the environmental chamber and food and urine nitrogen content measured for a period of 32 days. The results are presented in Figure 109. A 5% addition was made to the daily urinary nitrogen losses. The balance between nitrogen intake in food and nitrogen losses in excreta is also shown. Figure 119 gives an indication of the daily food intake and growth in these 4 control rats over the same time period as that shown in Figure 109.

From day (12-13) to day (20-21) the rats under study were in a positive nitrogen balance of 144 $\pm$ 66 (SD) mg N/day. The rate of body weight gain over the same period was 2.1 g/day. This indicated that for every gram of nitrogen retained 14.6 g of body weight

Fig. 109 5% BURN EXPERIMENT: THE EFFECT OF ANAESTHESIA +12 Hrs STARVATION ON NITROGEN (Control group-4 rats)
MEAN ± STD Error of mean shown.



gain took place in the control rats. Daily values for each rat are given in Tables 40 and 42.

If all the retained nitrogen were converted into muscle tissue, then between 25 - 30 g of live weight gain would be expected for each gram of nitrogen retained (D.P. Cuthbertson, 1976, personal communication). Since the rat is a continuously growing animal and growth includes bone as well as muscle, then a proportion of the nitrogen retained, perhaps up to 10% of the total, might be incorporated into the skeleton. Allowing for this factor the weight gain expected for each gram of nitrogen retained would still be approximately 25 g (rather than 30 g), as the growing rat also lays down body fat stores (with a very low nitrogen content) as well as muscle and bone. A breakdown of the values on which daily nitrogen (partial) balance was calculated over the 9 day study period is given in Table 28. The measured values found for mean nitrogen intake, mean nitrogen losses in urine and faeces (estimated) are given together with the body weight gain per gram of "retained" nitrogen. If one assumes that the daily rat weight measurement is accurate, then to achieve a body weight gain of 25 g for every gram of nitrogen retained (at a growth rate of 2.1 g/day), the average daily positive nitrogen balance must be 84 mg. This is 42% less than the daily nitrogen balance of +144 mg actually measured.

TABLE 28 DAILY NITROGEN BALANCE IN 4 CONTROL RATS : RANGE OF POSSIBLE METHODOLOGICAL COLLECTION ERRORS AND THEIR INFLUENCE ON THE CALCULATION OF DAILY NITROGEN BALANCE

9 DAY STUDY PERIOD	1 MEASURED VALUES	2 POSSIBLE % ERROR	3 + GLASS N. LOSSES	4 VALUES + % ERRORS
DAILY DIETARY NITROGEN mg	613	3.5	613	592
DAILY URINE NITROGEN mg	447	6.5	447	476
DAILY URINE N. LOSSES ON CAGE GLASSWARE mg	-	2.8	13	13
DAILY FAECAL NITROGEN mg	22*	5	22	23
NITROGEN BALANCE mg	144	-	131	80
DAILY WEIGHT GAIN PER g RETAINED N.	14.6	-	16.0	26.3

* Estimated value

As a basis for explaining this discrepancy, I took as a starting point the possible errors which might have occurred in the methods for estimating nitrogen in food, urine and faeces and ascribed to them the percentages shown in Column 2. Column 3 gives the daily nitrogen balance assuming a loss of 2.8% of the urinary nitrogen on the metabolic cage collecting system glassware (Table 28). This gives a nitrogen balance of +131 mg N daily which would result in a gain of 16.0 g body weight for each gram of nitrogen retained.

If the daily dietary nitrogen was over-estimated by 3.5% this would reduce the nitrogen intake to 592 mg. Similarly a small under-estimation of urine nitrogen losses together with a further small loss on the collecting system glassware would combine to reduce the daily nitrogen balance to +80 mg. Accepting these percentage error estimates would give a body weight gain of 26.3 g for each gram of nitrogen retained (Column 4).

In Figure 109, when the steady state situation in rat growth was disturbed, as happened on day 22 with a period of prolonged noise, day 25 with dorsal fur clipping, and again on day 35 by anaesthesia, the relationship between growth and nitrogen balance was altered. Periods of brief starvation and weight loss

made it difficult to compare these non steady state conditions with those found between days 12 - 21.

NITROGEN ESTIMATION

DISCUSSION

The method used for nitrogen analysis was a combination of a manual microkjeldahl digestion stage to convert organic nitrogen in the biological sample to ammonia, followed by measurement of the ammonia nitrogen by a colorimetric technique based on the indophenol-blue reaction (Russell, 1944). Fleck (1967) adapted the method of Mann (1963) to fully automate the colorimetric stage of ammonia analysis using a Technicon Auto-Analyser system. This method was used in all nitrogen analyses performed in this thesis. Fleck and Munro (1965) reviewed the methods used for nitrogen analysis and concluded that the kjeldahl digestion method when used with an appropriate catalyst and correct time of digestion was superior to other methods in measuring nitrogen in urine, and faecal material. Some of Fleck's original measurements were repeated to re-confirm the most appropriate digestion time for the urea and tryptophan standards. This was found to give satisfactory results. As a very large number of individual samples were generated by each

study, the use of a semi-automated method capable of handling 200 samples per day, proved to be the only practicable method. The rate of sampling during the colorimetric ammonia determination was however restricted to 40 samples per hour as it had been shown by Fleck (personal communication) that there was a slight loss of accuracy at sampling rates of over 60 per hour. The random error associated with the techniques of nitrogen measurement in the samples of food, urine and faeces which were analysed in this study are given in the preceding results section.

My objective was however to determine nitrogen balance accurately in normal (growing) and injured rats. Many factors had to be considered in order to attain this goal in addition to establishing a reproducible and accurate method of nitrogen estimation. The way in which, and the accuracy with which, biological samples can be collected from the rat represents a far greater source of potential error in metabolic balance studies than any likely random error in the nitrogen estimation itself. Isaksson and Sjögren (1967) critically reviewed the literature on mineral and nitrogen balance studies in man. Their remarks apply equally to the rat. They regarded the orthodox definition of "balance" as the difference between intake of a substance and its excretion with urine and faeces as being incomplete. They observed that where intake and output measurements

of a substance such as nitrogen were made, these had to be absolutely accurate as the values were usually very high compared with the balance figure. This meant that even slight errors in the intake or excretion could result in very large errors in the calculation of balance.

In the present rat studies the extent of possible methodological and collection errors and their influence on the calculation of daily nitrogen balance is shown in Table 28. This confirms the observations of Isaksson and Sjögren (1967), see Columns 2 and 4. Systematic over-estimation of dietary nitrogen intake by 3.5% daily is very probable if one considers the difficulty of collecting every particle of food spilled on the metabolic cage runway, from the glass funnel or cone, from the separator glassware, and from the glass fibre wool itself used as a final filter for the rat urine, and perhaps as food particles adherent to the rat's fur which are lost on the hands and coat of the handler when the rat is weighed. With a daily dietary intake of 16 g food per day, a 3.5% error would require loss to measurement of only 0.56 g.

Systematic underestimation of urinary nitrogen loss is also a well recognised hazard of nitrogen balance studies (Davidson and Williams, 1968; Owen, 1967). The 2.8% loss on the metabolic cage collecting

glassware in the present study (Table 28, Column 2) probably represents a drying effect though a small amount of spillage around the cage/funnel rim is also possible under some circumstances. The 6.5% error given here represents the sum of small errors associated with loss of nitrogen perhaps due to escape of gaseous ammonia during the digestion stage, and to pipetting errors, and spillage.

Faecal nitrogen losses may also be underestimated because of loss of volatile nitrogen during drying on the cage glassware (Davidson and Williams, 1968) or a high fibre content in faeces might result in incomplete digestion to ammonia. This latter point did not however apply in the present study as L.I.T. diet produced a very low residue faecal pellet which was readily homogenised and fully digested.

It is of interest to compare the results obtained for nitrogen retention (or balance) in the present study with other workers' findings. Beaton (1963) carried out nitrogen balance measurements on male Wistar rats of approximately the same body weight range as the rats described in Table 28 (260 - 274 g). Beaton's rats were fed a diet of similar type and protein source (20% casein) to that used in my own study, and were housed at 22^{o}C ambient temperature for part of his experiment. Over a four day period in steady state

conditions the mean increase in body weight found in a group of 10 rats was 6 g, or a growth rate of 1.5 g per day. Mean daily faecal nitrogen losses were 42 mg/rat. Mean daily urine nitrogen losses were 288 mg/rat. Daily nitrogen balance was + 241 mg. Therefore the rate of body weight gain per gram of retained nitrogen in Beaton's study was 6.2 g weight per gram retained nitrogen. This would indicate very significant errors in his measurements. Viewed in this light, our own attempt at measuring nitrogen balance in growing rats may be regarded as at least as good as measurements published in other studies. Beaton's study is representative of these and was selected, not because his results were particularly inaccurate, but because his rats, diet and conditions were so similar to those in the present study. I concur with the conclusions of Isaksson and Sjögren (1967) which I quote.

"There are many errors inherent in the metabolic balance technique, which limit the usefulness of the method and cast doubt on the validity of many studies published in the literature. Due account must be taken of these errors in all metabolic balance studies."

As a more direct measure of changes in body protein, water and fat content, chemical body composition analysis was carried out by Dr. G. A. A. Al-Shamma on rats

similar to those used here. These results are given later, and are compared with results obtained by conventional nitrogen balance measurements carried out as I have described.

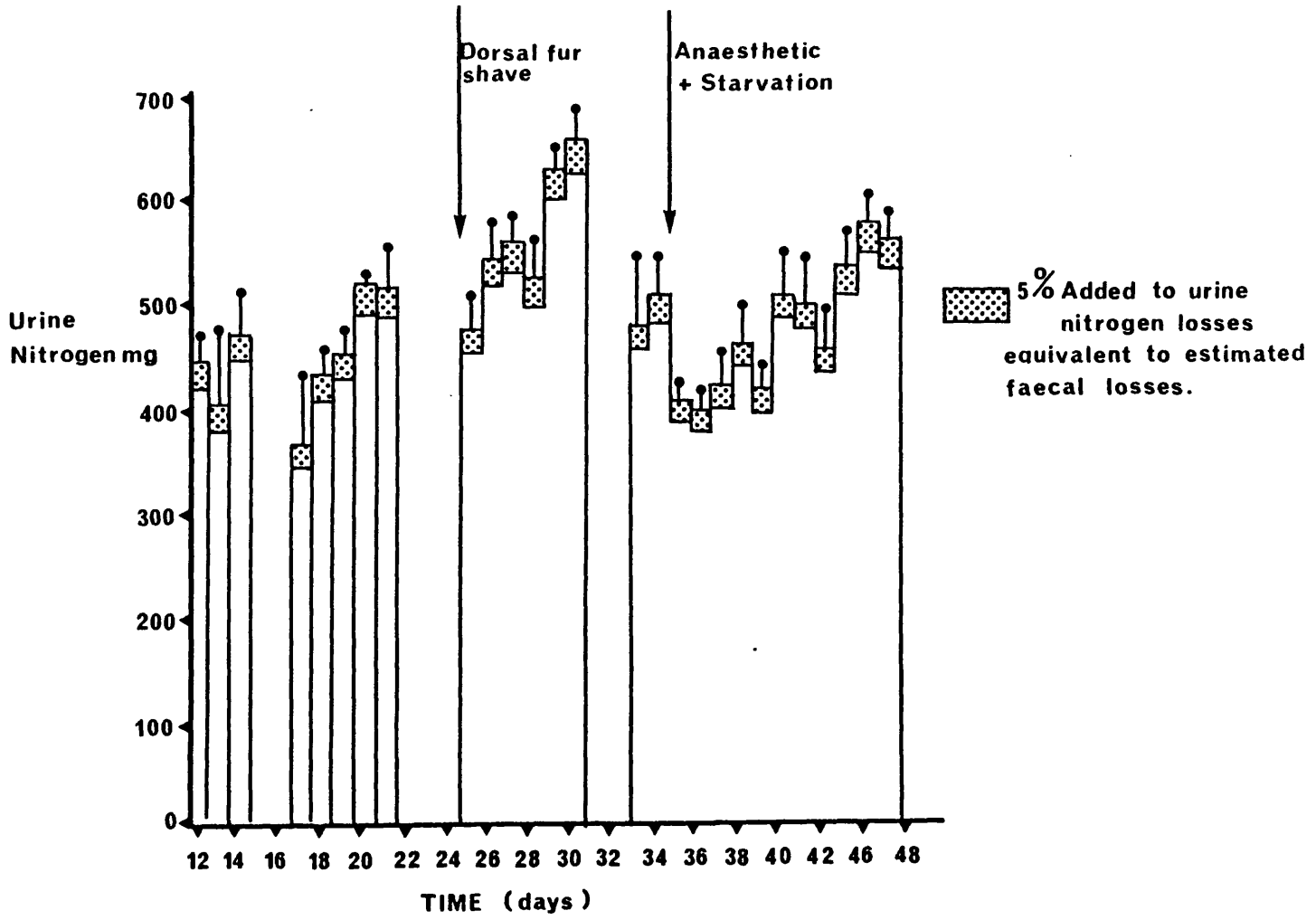
Many workers studying injury responses in man and experimental animals have used the excretion of urinary nitrogen alone as an index of protein catabolism in the body (Richards et al., 1976). Figure 110 shows the urinary nitrogen losses alone for the 4 control rats described also in Figure 109. Based solely on the increased excretion of nitrogen in the urine, it would appear that dorsal fur shaving in the rat has produced a greater mild injury or stress effect than Nembutal anaesthesia and starvation. This however is a very misleading interpretation of the data. Urine nitrogen reflects not only breakdown of protein in the body, but also protein synthesis and dietary protein intake thus:-

$$\begin{pmatrix}\text{URINE (N)}\\ \text{EXCRETION}\end{pmatrix} = \begin{pmatrix}\text{ENDOGENOUS}\\ \text{PROTEIN (N)}\\ \text{BREAKDOWN}\end{pmatrix} + \begin{pmatrix}\text{DIETARY}\\ \text{PROTEIN (N)}\\ \text{INTAKE}\end{pmatrix} - \begin{pmatrix}\text{TISSUE}\\ \text{PROTEIN (N)}\\ \text{SYNTHESIS}\end{pmatrix}$$

Consideration of Figure 109 in this light now indicates that the most likely origin of the increased urinary nitrogen excretion shown after dorsal fur shaving in the rat is an increased dietary nitrogen intake over

Fig. 110 5% BURN EXPERIMENT: URINARY NITROGEN LOSSES CONTROL GROUP (4 Rats)

Mean ± STD Error of mean shown



the same period. Therefore the increased nitrogen losses do not necessarily reflect increased protein breakdown.

Measurement of urinary nitrogen losses and nitrogen balance remain essential methods of comparison between groups of rats studied in identical conditions during the same experiment and may be used as a basis of comparison between groups regarding severity of injury and similarity to other studies in the published literature, known errors accepted and these notwithstanding.

Nitrogen metabolism, body weight gain and other factors are considered in relation to burn injury in subsequent sections of this thesis.

GLASGOW UNIVERSITY LIBRARY

STUDIES ON THE METABOLIC RESPONSE TO THERMAL INJURY

IN THE RAT

VOL 2

A thesis submitted to the
University of Glasgow
for the degree of
Doctor of Philosophy
in the
Faculty of Medicine
by

JOHN ROONEY RICHARDS, M.B., Ch.B., F.R.C.S.(Ed.)

Institute of Physiology,
University of Glasgow,
Glasgow G12 8QQ.

October 1979.

VOLUME 2

STUDIES ON THE METABOLIC RESPONSE TO THERMAL INJURY IN THE RAT

CONTENTS PAGE NUMBER

Thesis
6268
Copy 1.
(vol. 2.)

GLASGOW
UNIVERSITY
LIBRARY

VOLUME 2

CONTENTS PAGE NUMBER

VOLUME 2

CONTENTS PAGE NUMBER

VOLUME 2

CONTENTS PAGE NUMBER

VOLUME 2

CONTENTS PAGE NUMBER

FIGURES VOLUME 2

FIGURES VOLUME 2

TABLES VOLUME 2

TABLES VOLUME 2

DEVELOPMENT OF A MODEL BURN INJURY

In order to create a reproducible and precisely defined "painless" full thickness dorsal skin burn in the Wistar rat, a number of relevant and related issues were examined in detail. These were first, the measurement of surface area in the rat; second, the recognition of differences between rat and human skin and the need to identify the areas of skin which, in the rat, if burned, would heal at a similar rate to that found in man; third, the methods of producing sharply delineated burn wounds of known and controlled skin thickness in the rat; fourth, definition of the extent of burn area and level of dietary intake in the rat required to create the same pattern of weight loss seen in severely burned man.

MEASUREMENT OF BODY SURFACE AREA IN RATS

MATERIALS AND METHODS

Theoretical considerations and practical difficulties relating to the measurement of body surface area in animals are discussed fully by Kleiber (1961).

The method used here is a simplified version of that described first by Lee (1929).

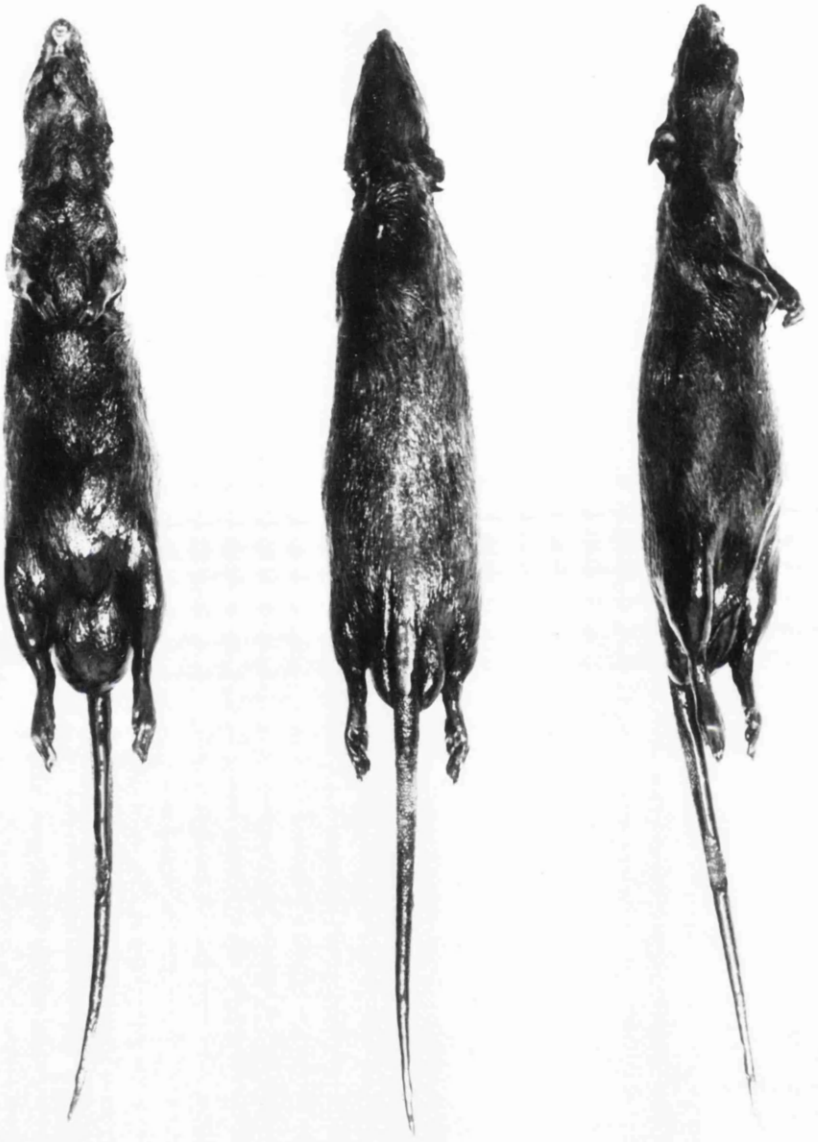
Procedure:

1. The rat under study is first killed by an overdose of Nembutal anaesthetic agent.

2. Immediately after death, the rat's pelt is thoroughly dried with a cloth and the entire surface of the animal thickly painted with HYMEG No. 1 Clear Baking Insulating Varnish (unthinned), allowing the varnish to soak well into the fur, thoroughly matting the hairs together (See Fig. 111).

3. While thevarnish is setting the rat is suspended from a frame by a length of fine wire looped around the rat's incisor teeth. The mouth is slightly open and the ears are made to stick out as in life. The rat's limbs are also slightly abducted from the trunk to re-create a life-like posture. If necessary, the legs are held away

Fig. 111 RAT CORPSE AFTER HYMEG APPLICATION : ANTERIOR, POSTERIOR AND LATERAL VIEWS



from the body by sticks placed between the paws.

4. The body is allowed to hang overnight in a warm room till the varnish is "set".

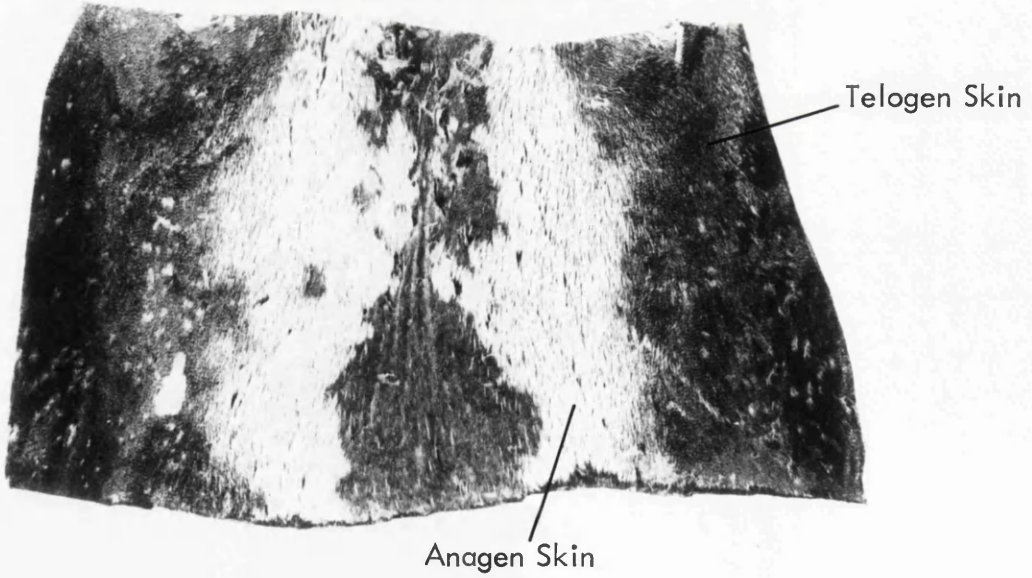
5. The rat is then carefully skinned, and the pelt is cut into several pieces such that each piece can lie flat if laid out on a sheet of paper (See Fig. 112). The removed skin, when coated with a rubbery plastic type of varnish such as HYMEG No. 1, forms a perfect mould of the rat's body contours. Every wrinkle is preserved on the animal as in life. An indication of the exactness of body moulding is given by the finding that it is necessary to cut the skin into between 12 and 25 separate pieces in order to make each lie flat. Unvarnished skin can be stretched flat in a single piece.

(In the varnished rats, the toes are allowed to stick together while the varnish "sets". When the rat is skinned, the whole foot is split parallel with the palm. This gives both surfaces of the entire foot, but does not quite give the area on the side of each toe. The error involved is small).

6. The surface area of the pieces of pelt thus removed is obtained by tracing the outline of the pieces onto high grade graph paper using a

Fig. 112 RAT BODY SURFACE AREA MEASUREMENT
a. VARNISHED RAT PELT AFTER REMOVAL

b. UNDERSURFACE OF DORSAL RAT PELT



fine ROTRING 0).35mm mapping pen. The outlines are next cut round with fine dissecting scissors, the paper shapes are put together and weighed to $\pm$ 0.1 mg.

7. Three additional areas of the graph paper of exactly 100 square centimetres are cut out, weighed, and the mean value taken. Next, the weight of the graph paper equivalent to the rat's surface area is compared with the mean weight of paper corresponding to 100 square centimetres, and the surface area of the rat thereby calculated.

8. The relationship between live body weight and surface area was determined. The results were compared with the equation devised by Sarrus and Rameaux (1839), as modified by Richet (1889), and Meeh (1879) - all quoted in Kleiber (1961) that $S = kW^{\frac{2}{3}}$. A further variation, $S = kW^{\frac{3}{4}}$ devised and advocated by Kleiber (1961) was also compared with the results in the present studies. Where S = surface area in square centimetres, k = a constant, determined by measurement, and W = body weight in grams.

MEASUREMENT OF BODY SURFACE AREA IN RATS

RESULTS

The results of rat skin surface area measurement in 10 rats (all male Wistar strain from the semi-inbred closed colony in the Institute of Physiology Animal Breeding Unit) are given in Table 29. The range of weights was 204 - 302 g. This is the range of weights used in the present studies. The Sarrus and Rameaux formula gave a Meeh constant value of k = 10.1, when compared with the measured skin surface areas in 10 rats. The Kleiber formula gave a k value of 6.4. For the calculation of body surface area from body weight, in a single species group of the same age, sex and approximate body weight, there is no significant difference between the formulae $S = 10.1\ W^{\frac{2}{3}}$ and $S = 6.4\ W^{\frac{3}{4}}$. Both give the correct answer. As the Sarrus and Rameaux formula $S = kW^{\frac{2}{3}}$ is the more established, and Kleiber's formula has no advantage over it, rat body surface area calculation in this thesis is based on $S = 10.1\ W^{\frac{2}{3}}$.

TABLE 29 A

BODY SURFACE AREA MEASUREMENT IN THE RAT

Sarrus & Rameaux formula ($S = kW^{\frac{2}{3}}$) $k = 10.1$

RAT NO.	MEASURED SURFACE AREA (sq.cm) S'	BODY WEIGHT (g) W	$\frac{2}{3}$ POWER OF BODY WEIGHT $W^{\frac{2}{3}}$	k $(= \frac{S}{W^{\frac{2}{3}}})$	CALCULATED SURFACE AREA (sq.cm) S
1	418	270	41.78	10.01	422
2	411	248	39.50	10.41	399
3	314	204	34.67	9.06	350
4	384	250	39.69	9.68	401
5	370	248	39.48	9.37	399
6	449	269	41.68	10.77	421
7	324	205	34.77	9.32	351
8	438	260	40.75	10.75	412
9	395	235	38.08	10.37	385
10	488	302	45.01	10.84	455
MEAN $\pm$ SD	399 ± 54	249 ± 30	39.54 ± 3.16	10.06 ± 0.66	400 ± 32

TABLE 29 B

BODY SURFACE AREA MEASUREMENT IN THE RAT

Kleiber formula ($S = kW^{\frac{3}{4}}$) $k = 6.4$

RAT NO.	MEASURED SURFACE AREA (sq.cm) S'	BODY WEIGHT (g) W	$\frac{3}{4}$ POWER OF BODY WEIGHT $W^{\frac{3}{4}}$	k $(= \frac{S}{W^{\frac{3}{4}}})$	CALCULATED SURFACE AREA (sq.cm) S
1	418	270	66.62	6.28	426
2	411	248	62.55	6.57	400
3	314	204	54.01	5.81	346
4	384	250	62.90	6.10	403
5	370	248	62.50	5.92	400
6	449	269	66.43	6.76	425
7	324	205	54.19	5.98	347
8	438	260	64.77	6.76	415
9	395	235	56.02	7.05	359
10	488	302	72.44	6.76	464
MEAN $\pm$ SD	399 $\pm$ 54	249 $\pm$ 30	62.24 $\pm$ 5.95	6.40 $\pm$ 0.43	398 $\pm$ 38

MEASUREMENT OF BODY SURFACE AREA IN RATS

DISCUSSION

Sarrus and Rameaux made the observation in 1839 that animal surface area was proportional to the two-third power of body weight. Later workers, in particular Meeh (1879) added a constant term "k" which enables the relationship between surface area and weight to be defined more precisely within any group of similarly shaped animals. "k" differs according to the shape of the animal.

Kleiber noted (1961) that the formulae used for calculating surface area in rats varied widely, with different authors giving "k" values of 7.42, 9.1, 10, 11.36 and 12.44. In these publications, surface area had to be determined by measurement to find a value for "k". The methods used to measure surface area differed. It is of interest to note that inclusion or exclusion of ears and tail in the rat can alter the area by 8%. Consequently it was not surprising that widely differing "k" values were found in different members of even a single species of animal.

Because of this lack of agreement I decided to carry out my own measurements of rat surface area using the long established method of Lee (1929), modernising

it slightly, by applying HYMEG No. 1 varnish and leaving the fur of the rat intact. The results of my own study indicated that the formula $S = 10.1 \times W^{\frac{2}{3}}$ gave satisfactory results, in agreement with the k value of 10.0 used recently by Walker and Mason (1968) in studies on burned rats, and also by Glasser in 1955.

A further independent confirmation of the probable accuracy of the formula $S = 10.1\ W^{\frac{2}{3}}$ for the calculation of surface area in rats in the present study was found when the calculated surface area and metabolic rate (determined by calorimetry) of my rats was compared with Voit's classic table on Surface Law (1901). This is shown in Table 30. The metabolic rates shown in this table were measured at "thermally neutral" ambient temperatures, therefore only those calorimetry measurements made at $30^{\circ}C$ ambient temperature are relevant in my own rats. The metabolic rates shown are those shown in the calorimetry run described in Figure 59, periods A and C, and in Table 15 , date 3/9/A. The values for heat loss in the fasting rat of 1097, and 1039 $kcal/m^2/day$ agree closely with the observation made by Rubner (quoted in Kleiber, 1961) "that fasting homeotherms produce daily 1,000 kcal of heat per square metre of body surface."

Rat surface area was therefore determined by the formula $S = 10.1 \times W^{\frac{2}{3}}$ in the present studies.

TABLE 30

VOIT'S TABLE ON SURFACE LAW (1901)

	ANIMAL	WEIGHT (kg)	kcal/kg/day	kcal/m^2/day	
	HORSE	441	11.3	948	
	PIG	128	19.1	1078	
	MAN	64.3	32.1	1042	
	DOG	15.2	51.5	1039	
	GOOSE	3.5	66.7	1018	
	RABBIT	2.3	75.1	776 - 917	
	HEN	2.0	71.0	1008	
*	RAT (A)	0.29	111.8	1097	*
*	RAT (C)	0.29	105.8	1039	*

* MEASUREMENTS MADE ON POST ABSORBTIVE RAT AT 30°C AMBIENT TEMPERATURE IN INSTITUTE OF PHYSIOLOGY, UNIVERSITY OF GLASGOW USING SURFACE AREA MEASUREMENT BASED ON THE FORMULA $S = 10.1 \times W^{\frac{2}{3}}$.

DEVELOPMENT OF A MODEL BURN INJURY

PREPARATION OF RAT SKIN

INTRODUCTION

IMPORTANCE OF THE RAT HAIR GROWTH CYCLE IN SKIN.

In man the influence of skin thickness on the severity of burn injury is widely recognised in medical practice (Jackson, 1953; Moncrief, 1965). According to Zawacki and Jones (1967) many experimental animals differ also in the expected skin thickness to be found at any given surface location. In rodents (Butcher, 1934, 1935; Montagna, 1962) skin thickness can also vary with the hair growth cycle. Skin with actively growing hair follicles (anagen skin) being markedly thicker than non-growing areas (telogen skin). Zawacki and Jones state that in rats, the physical and physiological contrast between anagen and telogen skin areas is so distinct that the healing of experimentally produced burns varies significantly with the phase of growth of the skin burned. They suggest that the same burn may result in partial thickness skin loss in anagen areas but full thickness destruction of skin in telogen areas. (This has been confirmed in the present studies).

This observation does not appear to be well known (Caldwell, 1961, 1962, 1970; Walker and Mason, 1968; Douglas and Jonsson, 1969), but clearly, for the production of a uniform burn wound, a constant skin depth at the area to be burned is at least as important as achieving a standard burning technique.

The following procedures were used to identify cases of 'uniform' skin thickness in the rats submitted to experimental burns by the author.

PREPARATION OF RAT SKIN

MATERIALS AND METHODS

Though not evident to simple visual inspection, the rapidly changing distribution of anagen and telogen skin areas can be revealed by noting the regrowth rate of hair after clipping and shaving. The following procedure was adopted.

Male Wistar rats aged 14 - 16 weeks of 250 $\pm$ 15 g body weight were used. Following the method of Zawacki and Jones (1967), the rats were anaesthetised by an intraperitoneal injection of Nembutal (2.5 mg/100 g body weight). The dorsal and dorso-lateral surfaces of the trunk fur were closely clipped with fine toothed electric clippers. Over the next 2 to 5 days, anagen skin areas exhibited rapid and obvious hair growth, whereas telogen skin areas remained bald. Mr. J. Brown of the animal unit staff devised and built a double mirror frame to photograph the back and side of each rat.

In an attempt to produce skin of a known growth phase, and hence uniform thickness and vascularity (Chase, 1958), a barium sulphide paste (Veet lanolin base hair removing cream) was used to further depilate

the skin of the shaved area (Miles and Miles, 1952), as recommended by Zawacki and Jones. The mortality from this technique was significant, more than 40% of the rats so treated dying within 12 hours; accordingly, an alternative, simplified method of identifying uniform depth skin was developed by the author. Anagen skin was selected in the rat as its healing rate following burn injury was comparable with that seen in humans. Rats similar to those previously used were tamed with special care and the dorsal and dorso-lateral fur closely clipped. The rats were not restrained and no anaesthetic was required to carry this out.

Next, the rats were shaved closely with a safety razor, soap and water, rinsed, dried and returned to their cages which were partially filled with crushed absorbent paper.

The purpose was to prevent excessive cooling of the rat due to sudden loss of fur insulation over the denuded area. The effect of this whole procedure on rat food intake and growth rate in controlled environmental conditions is shown in Figure 101.

The regrowth of hair was noted. Skin areas where obvious hair growth had occurred by 50 hours post-shaving were termed rapid anagen skin areas. These regions were outlined in indelible ink. Areas of skin

with emergent hair tips only were considered slow anagen areas, and where no growth occurred, telogen areas. Forty rats were studied and characterised patterns of hair regrowth identified. These are shown in Figure 113.

The hair regrowth was observed for a further 3 months in 6 rats and in another 6 rats the hair was re-clipped but not shaved at 3 week intervals for 3 months. This confirmed the earlier results of Haddow et al. (1945) indicating that 'rapid' anagen areas remained rapid growth areas for a minimum period of 7 - 8 weeks.

In 10 out of the 40 rats a histological study was made of the differences in vasculature between 'rapid' anagen and telogen skin areas. It was necessary to use a skin perfusion technique to carry this out.

RAT SKIN PERFUSION TECHNIQUE.

An attempt to reproduce Zawacki and Jones' (1967) method of demonstrating capillary blood vessels using Indian ink as the perfusing fluid proved unsatisfactory, and a Carmine-Gelatin preparation was adopted as an alternative. Preparation of a Carmine-Gelatin injection mass was carried out as indicated in Carleton's

Histological Techniques (4th Edition), using 0.25% added sodium nitrite to promote vascular dilatation. Injection technique was as follows:

1. The rat is anaesthetised with Nembutal (2.5 mg/ 100 g body weight by intraperitoneal injection.

2. The rat is placed supine on heated cotton wool under an infra red heating lamp.

3. The abdomen is opened and the abdominal aorta cannulated quickly. 1 ml of 1% sodium nitrite solution is injected to relax smooth muscle.

4. The entire circulating blood volume is removed by aspiration with a syringe via the aortic cannula thus killing the rat. The volume removed is then replaced by 15 - 20 ml of warm ($40^{o}C$) oxygenated Ringer solution containing 0.2% sodium nitrite and 0.25% sodium sulphate w/v.

5. The left external iliac vein is divided, tying a fine cannula with a syringe adaptor into the proximal end, and ligating the distal end.

6. Using a 50 ml syringe, warm Ringer solution (4) is forced into the aortic cannula until the perfusate from the external iliac cannula runs clear.

(The remainder of this method is a modification of Fischer's (1902) method.)

7. A 50 ml Carmine-Gelatin filled syringe is attached to the aortic cannula and the contents forced under pressure into the rat's vascular system. Adequate tissue perfusion is immediately evident by the development of bright red coloration. Perfusion pressure is maintained and the external iliac vein clamped. Further Carmine-Gelatin is forced into the rat and the aortic cannula stoppered.

8. The entire rat is immersed in 7.5% aqueous formalin plus 1.5% acetic acid for 48 hours.

9. The dorsal skin is separated from the rat by deep scalpel strokes. This tissue is 'fixed' as in (8), for a further 48 hours.

10. Histological sections of 15 um are made on a frozen section Cryostat and further sections were wax embedded. No further staining is required although sections may be stained with haemalum. The capillary network in these sections can be traced by the red colouring after "clearing" the section by the Spalteholz method (Institute of Physiology Histology Unit), modified for slide sections.

Further cryostat sections of rat anagen and telogen skin areas were made without prior perfusion of the rat. These were cut both vertically and obliquely

in the line of the hair follicles and stained with haemalum and eosin. Additional estimates of sub-surface depth of hair follicles were made using a standard micrometer eyepiece. The results are given in Table 31.

TABLE 31

RAT SKIN HISTOLOGY : SUB-SURFACE DEPTH OF HAIR FOLLICLES IN (RAPIDLY GROWING) ANAGEN SKIN, AND IN (SLOW GROWING) TELOGEN SKIN

RAT NUMBER	HAIR FOLLICLE DEPTH ANAGEN SKIN (mm)	HAIR FOLLICLE DEPTH TELOGEN SKIN (mm)
1	1.62	0.32
2	1.24	0.34
3	1.75	0.41
4	1.18	0.40
5	1.50	0.57
MEAN $\pm$ SD	1.46 ± 0.24	0.41 ± 0.10

H & E stained sections cut vertically to rat skin surface from areas where anagen and telogen growth was very distinct, in unperfused rats.

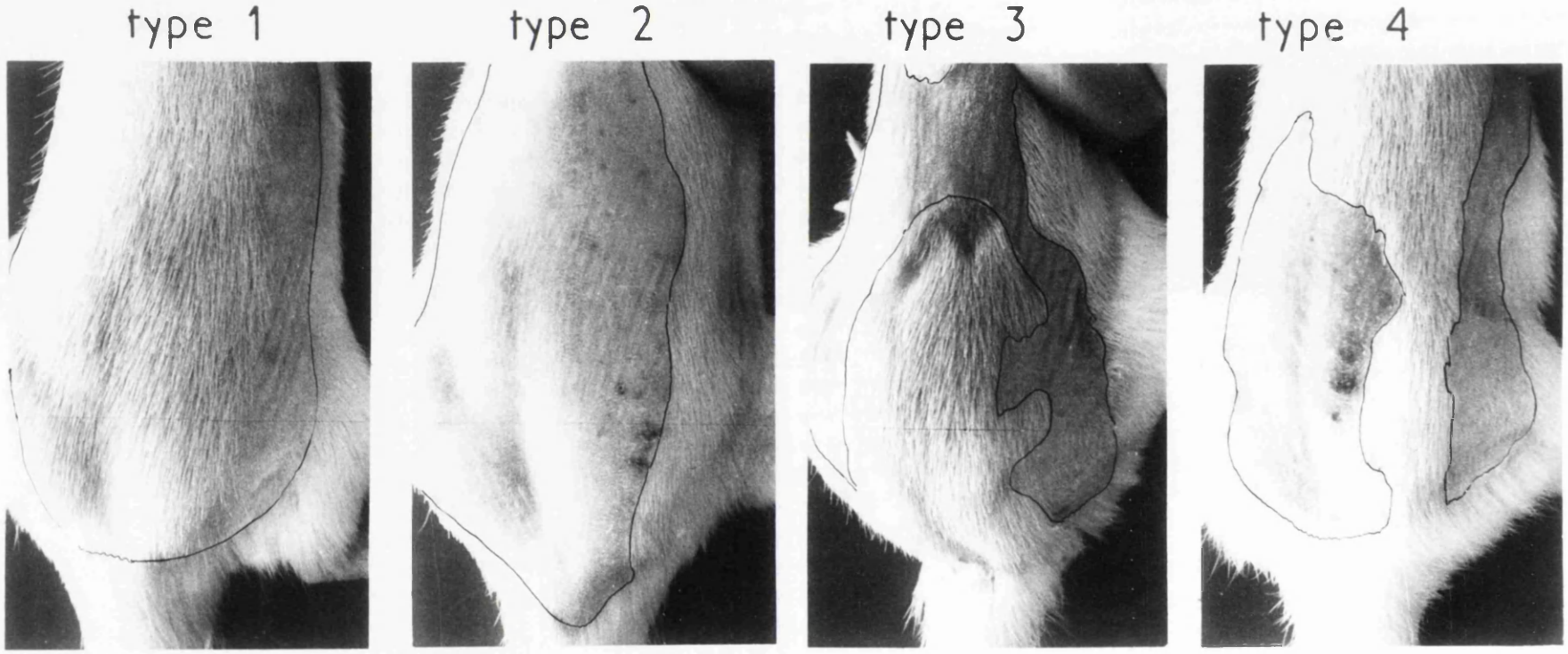
PREPARATION OF RAT SKIN

RESULTS

Figure 113 shows four characteristic patterns of "geographic" hair growth seen 5 days after dorsal fur clipping in 40 male Wistar rats. Areas of rapid skin hair growth (anagen skin) are obvious, and are sharply delineated from skin areas where no regrowth of hair has occurred (telogen skin). The type 1 pattern (entire back area anagen skin) occurred in 8 out of 40 rats (20%). The type 2 pattern (entire back area telogen skin) occurred in 7 out of 40 rats (17.5%). The type 3 pattern (peninsula of anagen skin within telogen area) occurred in 14 out of 40 rats (35%). The type 4 pattern (telogen islands within anagen area) occurred in 11 out of 40 rats (27.5%). Taken together the type 3 and 4 patterns of mixed hair growth areas accounted for 25 out of 40 rats (62.5%).

In those rats studies for a longer period (14 weeks) there was no change noted in the appearance or shape of the majority of rapid anagen skin areas (i.e. those areas with thick hair regrowth 3 days after dorsal fur close clipping).

Fig. 113 Hair growth patterns in ♂ Wistar rats. Anagen = growing areas: Telogen = resting areas



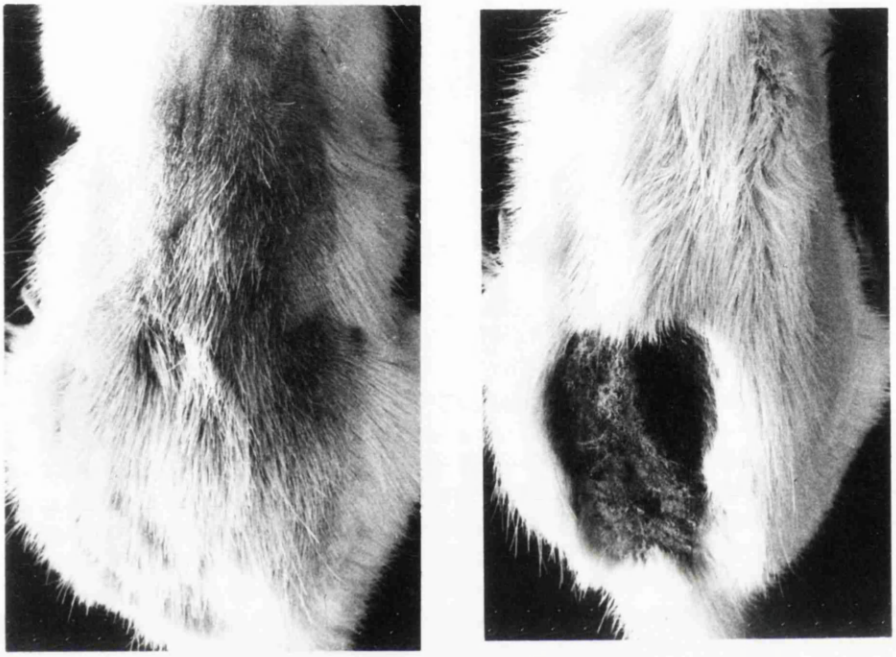
The histological study which was carried out on anagen and telogen skin areas in perfused and unperfused rats showed that anagen skin was characterised by large, deep, well vascularised, actively growing hair follicles with bulb like bases resting on (abutting) the panniculus carnosus. In telogen skin sections the hair follicles appeared smaller, more superficial, and much less well vascularised, their bases extending approximately halfway into the relatively shallow dermis.

Unexpected confirmation of the differences in thickness and depth of anagen skin hair follicles compared with those in telogen skin was found during the process of rat skin varnishing to determine skin surface area. When the varnished rats (Fig. 111) were left to dry in a warm room, the process for autolysis after death was rapid. One rat was left for 48 hours to dry thoroughly, and the pelt skin separated very readily from the subcutaneous fat which was autolysed. When the dorsal skin section was viewed from the underside (Fig. 112B), the thicker deeper hair follicle shafts could now be clearly seen standing out from the undersurface indicating an anagen skin area of characteristic pattern, while the telogen skin area follicles had largely autolysed.

Table 31 gives the results of a study in 5 rats with unperfused skin to determine the depth of anagen and telogen skin hair follicles. Telogen hair follicle depth was found to be only 28% of that found in anagen skin areas in the same animals.

Figure 114 shows unretouched photographs of identical burn wounds of 10 square centimetres surface area made using the Bunyan contact burn apparatus (described later). The temperature of the burning block ($300^{o}C$), the pressure with which it was applied (250 g), and the duration of its application (5 seconds), was the same for each rat. This produced a partial thickness burn on the anagen skin area, whereas on the telogen skin area, a full skin depth burn had been produced with obvious hair loss from the burned area and superficial blackening by the 4th post-burn day. (Note, the dorsal anagen and telogen skin areas had been identified by fur clipping 10 days prior to the burn injury, hence even the telogen skin area has a hair regrowth over it.)

Fig. 114 Burn appearances of identical wounds on Anagen and Telogen skin.



Anagen area Telogen area

Both wounds 4 days post burn

PREPARATION OF RAT SKIN

DISCUSSION

Zawacki and Jones (1967) emphasised the importance of the rat hair growth cycle in creating burn wounds of known depth on rat skin. They "prepared standard rat skin" in their study by applying (toxic) barium sulphide paste as an irritant to telogen skin areas, thus presumably stimulating a form of local inflammatory response in the skin area so treated. When these areas were examined histologically in specially prepared perfused skin specimens from their rats, these "activated telogen" skin areas had a uniform thickness and vascularity. The mortality from this procedure was unacceptably high and the simpler alternative method outlined previously was adopted by the author. This had no mortality, and appeared to upset the rats very little (Fig. 101). There was little difference between the uniformity of skin in regard to depth of hair follicles and overall vascularity when (rapid) anagen skin from the present study was compared with the findings of Zawacki and Jones for so-called "activated telogen" skin. Anagen skin areas also appear more comparable with human skin in terms of vascularity and potential healing rate after injury. In the present

study therefore only anagen skin areas were burned. It is essential to be fully cognisant of the precise nature of skin to be burned if an accurately controlled depth of burn injury is to be achieved. Figure 114 illustrates the dissimilarity in burn wound created by identical branding head applications in 2 rats.

Control of burn wound depth is important for two principal reasons. First, if a burn wound results in only partial skin thickness damage, then not all of the skin free nerve endings for pain receptors will have been destroyed. This type of burn injury may be extremely painful and hyperaesthetic, producing unnecessary stress and suffering in the experimental animal. If a burn wound can be made which totally destroys all the skin including the cutaneous pain receptors, then the wound will be numb and pain-free (in human patients at any rate - personal communication, Mr. W. H. Reid, Consultant in Charge of the Burns Unit, Glasgow Royal Infirmary), reducing suffering to an absolute minimum in the burned experimental animal. An anaesthetic burn wound surface also enables the injured rat to be handled normally after burning and no difficulty was found in persuading burned rats to enter the perspex weighing tube device daily (Fig. 94).

Second, if burn wound depth is too great, then underlying muscle tissue will also become heat damaged

(Fig. 117B). This will enhance the urinary excretion of muscle breakdown products out of all proportion to the predicted severity of the burn injury, normally directly related to the skin area destroyed (Moyer and Butcher, 1967). Muscle damage on the back will prevent normal feeding by the injured rat from its food container, compounding the severity of its metabolic response to injury with the additional effects of starvation. Such uncontrolled damage would negate the careful control of other aspects of the burned rat's environment in creating a uniform and reproducible burn injury.

DEVELOPMENT OF A MODEL BURN INJURY

PRODUCTION OF A CONTROLLED DEPTH BURN

MATERIALS

BUNYAN CONTACT BURN APPARATUS.

A special instrument was developed by Wilkinson Sword Ltd. Research Department for creating experimental burn wounds in animals. It uses an electrically heated, motor driven, plated metal block (of variable size) by which the temperature of the branding plate, the pressure with which it is applied, the time of its application and the area to be burned are all readily and independently controlled. For the present studies, settings were chosen for each of these parameters which ensured complete and uniform destruction of the whole skin depth, but which resulted in no damage whatever to the underlying musculature of the rat's back. The appearance of this apparatus and its component parts is shown in Figure 115. It is 10½" deep by 13½" wide, 15" in height and 40 lbs in weight. The temperature range of the burning head can be varied from 20°C up to 300°C and for any given setting should be within ± 0.25°C when stabilised at the operating temperature for 30 minutes prior to burning.

The drop in temperature during burning with the maximum load for the maximum time should not exceed $5^{o}C$.

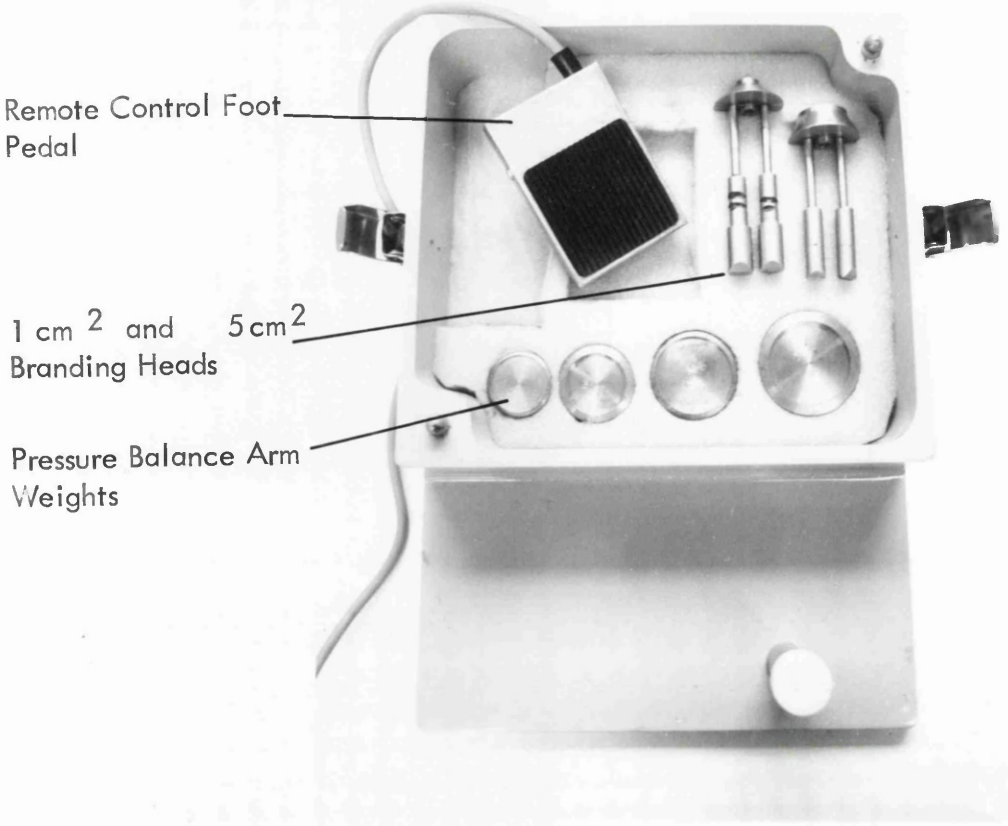
If the maximum temperature of $300^{o}C$ is used then the surface temperature of the branding head lies within $6^{o}C$ of the indicated temperature.

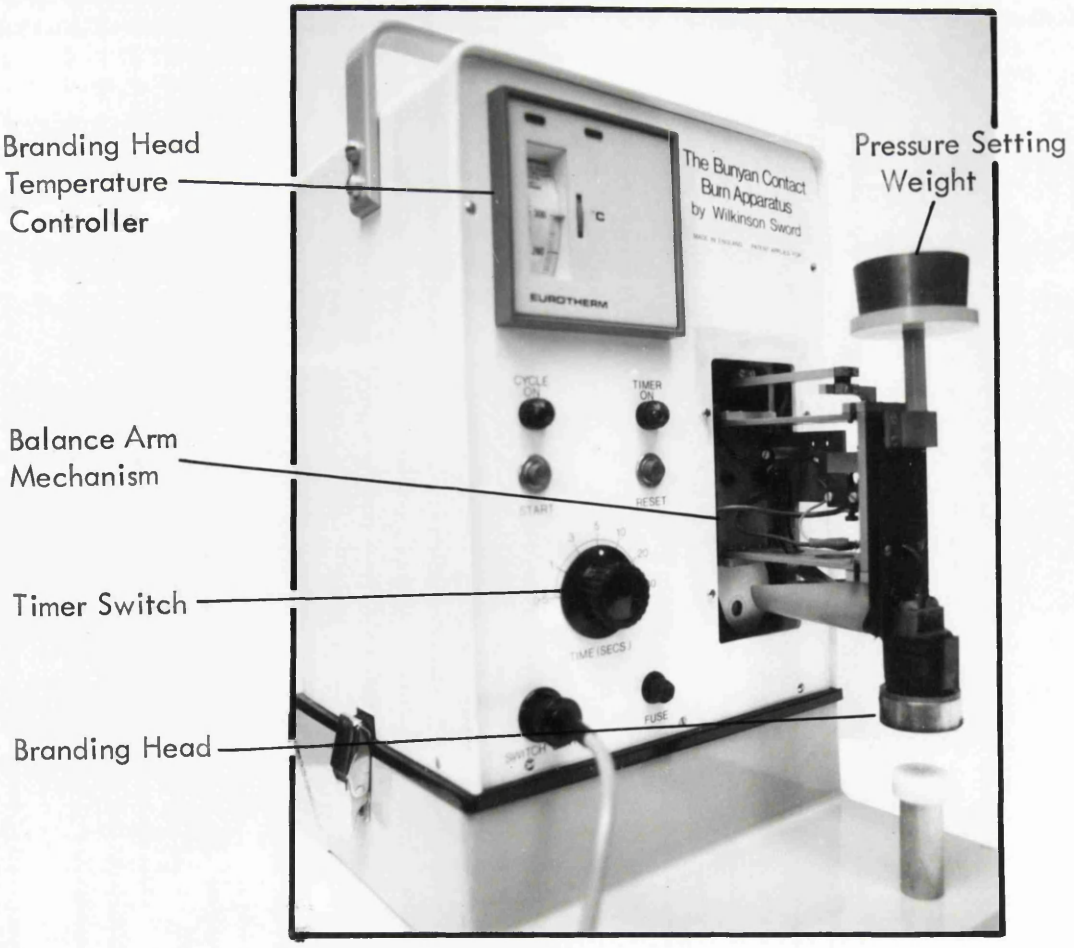
Six automatic time settings are provided, 1, 3, 5, 10, 20 and 30 seconds in addition to a manually operated time control switch.

The pressure of application of the branding head is varied by placing increasing weights (in the range 50 to 500 g, in 50 g increments) on the balance pan shown in Figure 115.

The area of the burn is adjusted by using one of the three branding heads provided, these being circular rods of chrome plated copper 1, 5, and 10 square centimetres in area. It is in practice not useful to have larger areas than this, as it becomes difficult to find suitably large areas of anagen skin, and if a 20% burn is used, e.g. in a 250 g rat with an approximate 400 sq. cm surface area, then 8 applications of the 10 sq. cm head are required in quick succession edge to edge to give an exact burn area. The small islands of healthy tissue at the sides are uniform

Fig. 115 BUNYAN CONTACT BURN APPARATUS : COMPONENTS





from burn to burn and although they result in different patterns of wound healing with individually shaped 20% area burns, if rats with similar anagen patterns in the same area of back are selected, then burns inter alia will be exactly the same area and comparable.

PRODUCTION OF A CONTROLLED DEPTH BURN INJURY

METHODS

With the required branding head fitted, the power is switched on. The indicator lamps on the temperature controller indicate when the apparatus is energised (Fig. 115). The burning head temperature is set by rotating the wheel to the right of the temperature scales until the required temperature setting is aligned with the right hand fixed pointer. The branding or burning head will then start heating and its temperature is indicated by the left hand pointer (Fig. 115).

When the burning head has stabilised at its set temperature, the fixed and moving pointers are aligned. This occurs within 15 minutes, but usually there is a slight overshoot of the set temperature and a further 45 minutes is required before the temperature drops to the fixed setting.

The burning interval is now set on the timer and the appropriate weights placed on the balance pan.

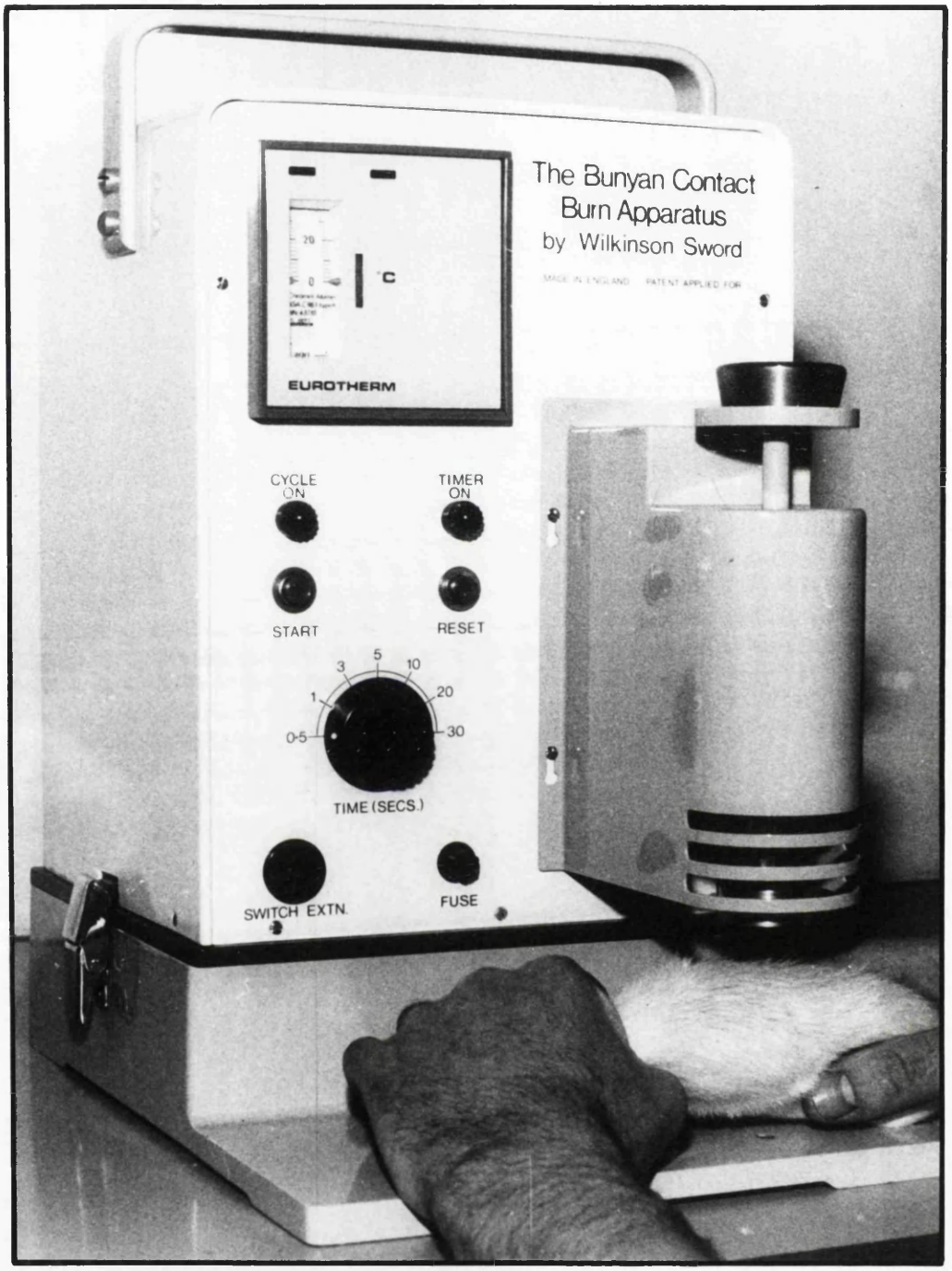
Keeping the plastic stop piece in place, the start button or foot switch is pressed and a burning sequence occurs. This is carried out prior to use

with an animal, as a check of the mechanism.

If this is satisfactory, the stop piece is unscrewed and the base plate reversed, the burning head now being placed over the edge of the bench as shown in Figure 116.

This ensures that the rat can now be swung up into position and held at bench top height by a seated operator. The skin area to be burned is lightly stretched between two fingers and the animal rotated or the skin area pulled so that the pressure will be evenly applied to the underlying tissues. When the anaesthetised rat is suitably positioned under the branding head, it is held steadily and the foot switch pressed. The remainder of the sequence of operation is automatic. The green warning light switches on and the branding head lowers. When the head makes contact with the rat, the red warning light comes on indicating activation of the timing device. When the set time has elapsed the warning lights go out and the branding head automatically retracts. The cycle of operation may be cancelled at any point by pressing the re-set button. The branding head returns at once to the start position. A short travel is necessary before the motorised branding head operates the timing sequence therefore a gap of 1.5 cm must be left between rat skin and the branding head at the start of the cycle.

Fig. 116 BUNYAN CONTACT BURN APPARATUS : METHOD OF USE



If no contact is made by the branding head before it reaches the bottom of its travel, it will automatically return to the start position without operating the timing sequence.

The application of the branding head is repeated a number of times, as required to produce 5%, 20%, 25% or 30% body surface area burns.

The calculation of rat surface area has been considered elsewhere.

PRODUCTION OF A CONTROLLED DEPTH BURN

RESULTS

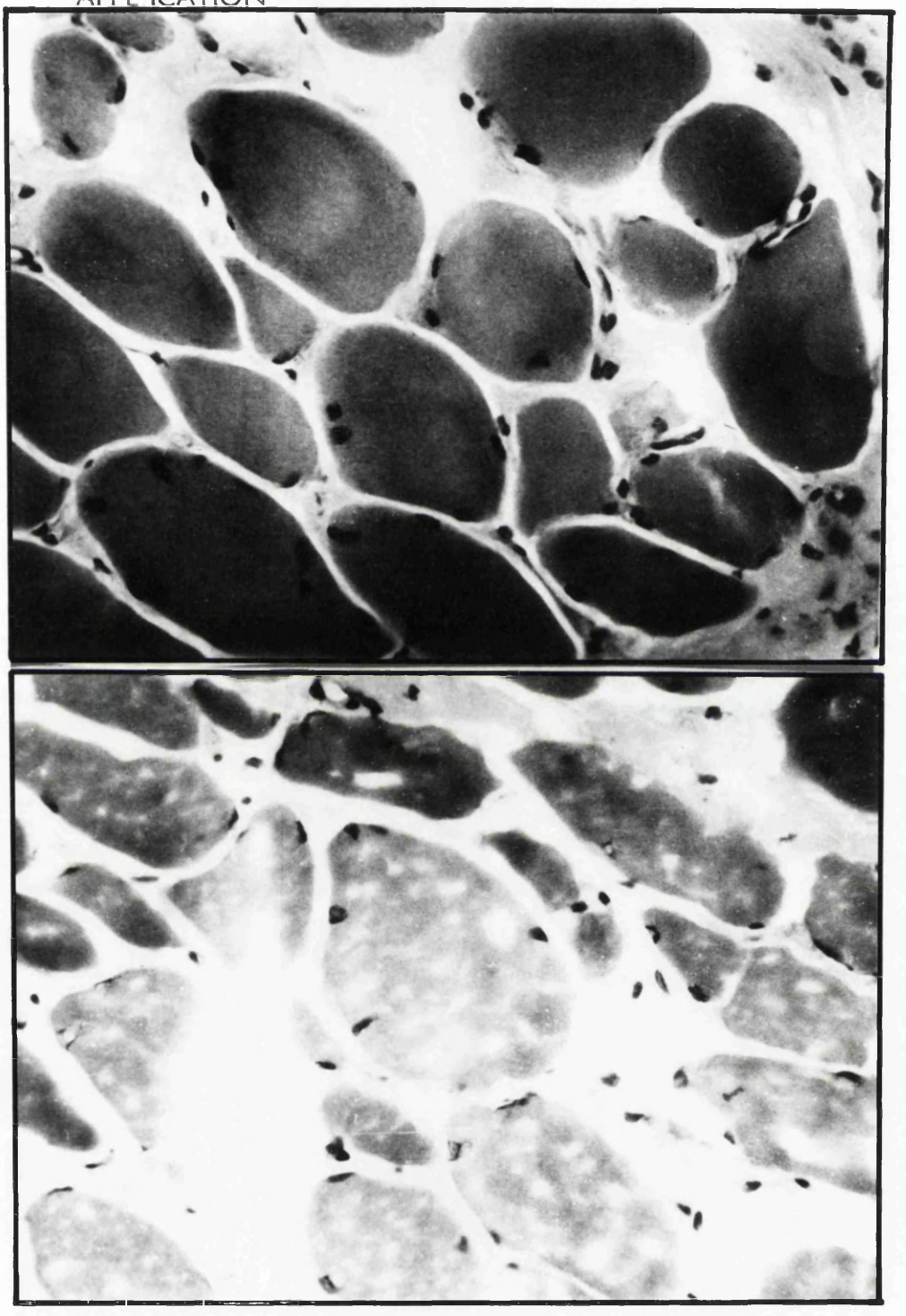
Initial testing of the branding head temperature, time of application, and pressure settings required to create consistent full skin thickness burns on anagen skin areas in the Wistar rat were carried out using anaesthetised rats which were sacrificed within 4 hours of burning. These animals were not permitted to recover from deep Nembutal anaesthesia throughout. Estimation of burn depth was made by conventional histological techniques of skin and deeper tissue sections taken from the site of branding head application. A pressure of application of 250 g with the branding head temperature set at 300^{o}C, applied for 10 seconds was found to produce full skin thickness destruction without underlying muscle damage.

Figure 117A shows muscle tissue underlying the site of the burn wound taken from a rat burned with these settings. Figure 117B shows muscle tissue with evidence of severe thermal damage (cytoplasmic vacuolation) from a rat where the pressure of branding head application was 500 g (300^{o}C, 20 seconds duration).

Fig. 117 CONTROLLED DEPTH BURN INJURY :

a. MUSCLE TISSUE UNDER BURN WOUND - CORRECT PRESSURE APPLICATION

b. MUSCLE TISSUE UNDER BURN WOUND - EXCESSIVE PRESSURE APPLICATION



PRODUCTION OF CONTROLLED DEPTH BURN

DISCUSSION

The techniques used to produce experimental burns in animals include flame burning of unanaesthetised rats (Dolacek, 1969), parabolic reflection of heat (Caldwell, 1966), hand application of heated brass blocks (Zawacki and Jones, 1967) and immersion in boiling water, the latter being the most widely used technique (Zitowitz and Hardy, 1958; Caldwell, 1961; Arturson, 1964; Walker and Mason, 1968; Douglas and Jonsson, 1969). Discounting, for the moment, ethical considerations, none of these methods produces reliable standard depth burn wounds with identical characteristics from animal to animal. Ideally six parameters require control in order to ensure reproducibility of a 'standard' injury.

These are:

1. Temperature of the burning fluid or metal plate
2. Time of application
3. Pressure or load with which the burning fluid/ metal plate is applied
4. Precise area to be burned
5. Selection of uniform known depth skin at area to be burned
6. Prevention of uncontrolled post burn wound pain.

The injury so produced should not affect the joint surfaces, limbs, throat or face, otherwise post injury movement and feeding ability will be restricted with adverse metabolic results due to starvation and immobilisation being superimposed upon the effects of the burn injury (Richards, 1977). The standard scald burn as developed by Walker and Mason was initially evaluated by the author. This type of burn is inflicted on anaesthetised rats by immersion of the area to be burned in boiling water while the animal is held in a protective template which limits the area of exposure. The depth of skin burning can be varied by altering the time of exposure while the pressure of water is relatively uniform.

1 mm thick aluminium sheet was cold rolled into a half cylinder, 3 cm radius by 30 cm length. Two half cylinders were filed to form a closely fitting complete cylinder around machined perspex end pieces. An aperture of calculated size was cut from the lower half cylinder and insulated. When the anaesthetised rat's back is pressed against the cut-out aperture it completes the cylinder hence representing a cylindrical surface of known area. In practice this apparatus is far from ideal for the following reasons:

1. The fixed template/aperture approach is difficult to reconcile with the random patterns of anagen/telogen hair growth (Fig. 113). To inflict a

25% of body surface area burn in 12 - 14 week male Wistar rats requires 80 - 120 sq. cm of skin entirely in an anagen phase. If only a rectangle aperture is used, 80% of the author's rats would be unsuitable.

2. With 250 - 320 g rats it is difficult to achieve greater than 15 - 18% body surface area (BSA) burns without water leakage at the edges of the aperture. Twelve rats were burned by this method and kept alive for up to 4 hours post injury, being deeply unconscious under Nembutal anaesthesia throughout. At death, skin histology (10 um frozen sections stained by haemalum and eosin) indicated consistently, areas of only partial thickness skin loss up to 1.2 cm wide at the wound margins (for an average burn size of 22% of body surface area). Variable areas of whole thickness skin loss and underlying muscle damage were found at the wound centre. Lack of accurate delineation of burn wound margins is regarded by the author as unethical in animal experiments of this nature, as partial thickness scalds may cause the animal considerable discomfort and pain, particularly when the animal is handled and weighed.

The Bunyan Contact Burn Apparatus was therefore selected as it proved capable of producing up to 30%

of body surface area burns with sharply demarcated margins. The burn wound caused by this apparatus was found to be uniform and reproducible from rat to rat ensuring complete full thickness thermal damage to skin without underlying tissue damage. The apparatus, by virtue of its interchangeable branding heads of small area, allowed anagen skin areas of differing shapes (Fig. 113) to be selectively burned.

DEVELOPMENT OF A MODEL BURN INJURY

METABOLIC RESPONSE TO INCREASING SEVERITY OF BURN INJURY

INTRODUCTION

It was my aim in this section to establish the extent of burn injury (of full skin thickness depth) in the rat, which would cause a metabolic response to burning comparable to that seen in severely burned man, in terms of body weight loss and urinary nitrogen excretion.

A series of graded burn injuries, of increasing severity (surface area), was carried out on male Wistar rats kept in the controlled environment conditions previously described. Burn injuries causing full thickness skin destruction of 5%, 20%, 25% and 30% of body surface area in the rat were studied. The 30% BSA burn resulted in an immediate post burn mortality of greater than 50% unless post burn fluid support therapy was given to the injured animal. The results of this study are not presented as my objective was to study the natural history of the "untreated burn" in the rat. There was no mortality from untreated 5%, 20% and 25% BSA full thickness burns in the rat. These results are presented.

METABOLIC RESPONSE TO 5% BODY SURFACE AREA FULL SKIN THICKNESS BURN

MATERIALS AND METHODS

Semi inbred male Wistar rats aged 12 - 14 weeks from the Institute of Physiology closed colony were kept in the controlled environment conditions described previously, at 20°C ambient temperature. They were fed ad libitum Low Iodine Test Diet from Nutritional Biochemicals Corporation, Inc. Their drinking water was supplemented with potassium iodide (5 ug/10 ml). They were weighed daily, food intake was measured, and metabolic collections made for urine and faeces as previously described. Ten rats were kept in metabolic cages (6 burned and 4 controls) and 10 rats were kept in wall rack cages (5 burned and 5 controls). These cages and the potential differences between them have been discussed (Fig. 101).

The control rats were treated identically with the burned rats, except that the burn itself was not carried out. Control rats received the same dose of Nembutal anaesthetic agent as the burned rats on a weight for weight basis. From the day of the burn/ anaesthetic, the control rats were pair fed with the burned rats (with a delay 24 hours) according to the

following pairings:

Rat 11 (burn) with rat 5 (control)

Rat 13 (burn) with rat 10 (control)

Rat 6 (burn) with rat 7 (control)

Rat 1 (burn) with rat 19 (control)

Rat 15 (burn) with rat 12 (control)

Rat 9 (burn) with rat 18 (control)

Rat 3 (burn) with rat 4 (control)

Rat 8 (burn) with rat 20 (control)

Rat 14 (burn) with rat 2 (control)

No control rats were pair fed with rats 16 and 17 which were both subjected to burn injury.

METABOLIC RESPONSE TO 5% BSA FULL SKIN THICKNESS BURN

RESULTS

Figure 118 shows the changes in body weight and food intake which follow a 5% BSA full thickness burn wound in the rat. The (11) burned and (9) control rat groups are almost equally divided between metabolic cages and wall rack cages. Note that although burned and control groups are not pair fed until after day 35, all the rats in the environmental chamber respond in a similar way to different forms of common "stress", such as prolonged noise on day 22, and dorsal fur clipping on day 25. Daily food intake and body weight values for each rat are given in Tables 32, 33, 34 and 35. Metabolic cage collections affect only half (approximately) of both burn and control rat groups (Fig. 101). The rate of daily growth was the same in burned and control groups between days 1 to 35, (1.6 g/day).

After day 35 the effectiveness of pair feeding can be seen in the similarity of food intake (with a 24 hour delay) between burn and control groups. The total diet eaten (mean for each rat) in the burn group was within 2% of the mean intake in the control group

Fig. 118 5% BURN FOOD INTAKE AND BODY WEIGHT vs TIME. COMPARISON OF BURN GROUP AND CONTROL GROUP Results Mean ± Std. Error of Mean for 11 Rats and 9 Rats Respectively.

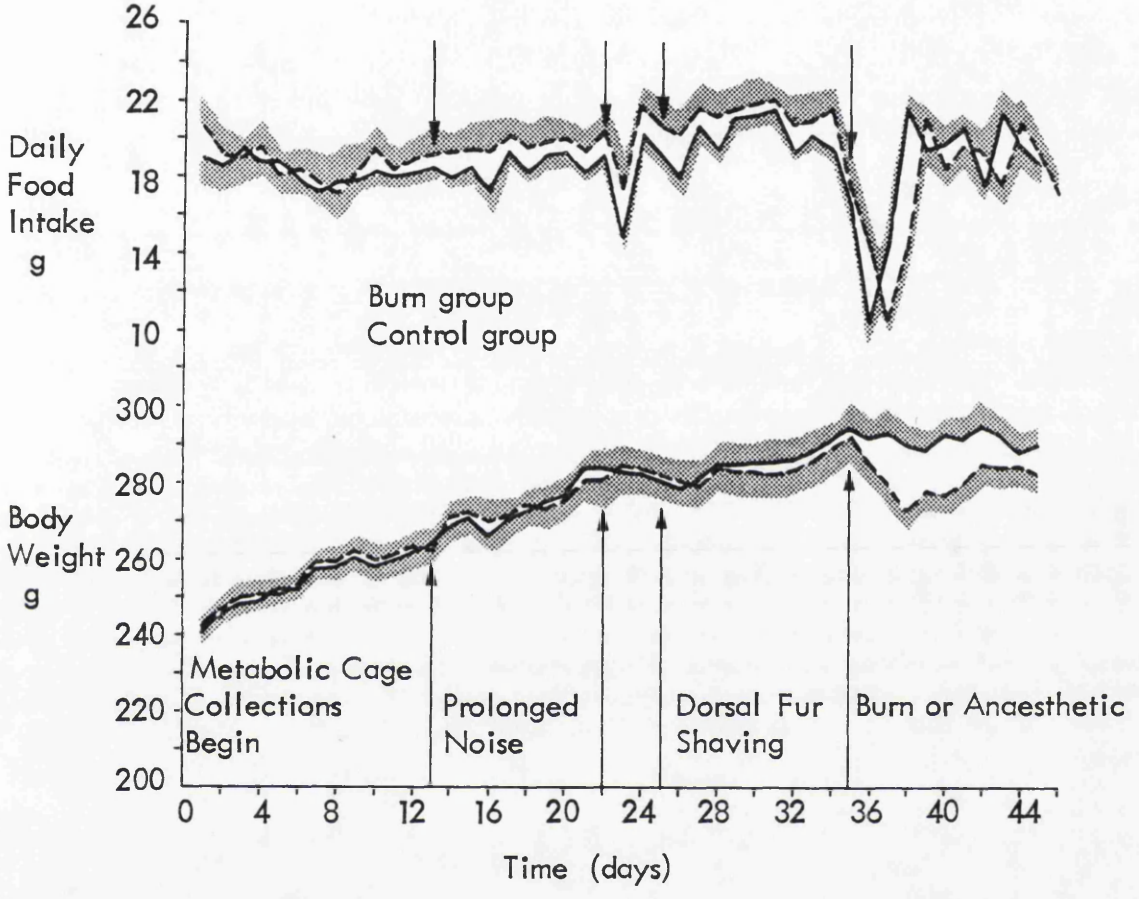


TABLE 32 A (1) BODY WEIGHT WALL RACK CAGES

BURN GROUP - RATS 1, 3, 6, 8, 9.

DAY	RAT 1	RAT 3	RAT 6	RAT 8	RAT 9
1	235.5	244.2	238.3	238.2	245.8
2	241.3	247.6	243.5	242.2	245.4
3	240.7	248.9	244.4	243	250.9
4	239.9	250.9	247.3	243	253.7
5	241.2	253.7	249.8	243.2	256
6	241.1	255.7	250.4	243.6	254.8
7	246.7	258	256.5	247.2	262.4
8	245.3	256.7	254.7	246.3	264.2
9	251.5	257.4	259.4	244.6	269.1
10	249.1	254	258	241.4	269.3
11	250.4	255.6	259	241.2	268.2
12	249.9	254.9	262.9	242.7	272.8
13*	252.1	256.1	266	243.4	273
14	257.9	260.4	272.4	247	281.5
15	257.1	262.4	272.2	249.1	287.1
16	258.8	262.5	273.5	248.8	285.5
17	260.1	263.6	273.9	248.4	286.3
18	261.1	264.2	276	251.8	291.4
19	262	265.9	279.8	252.3	293
20	263.6	269.4	282.6	254.3	294.4
21	270	273	289.8	259	303.2
22*	269.8	274.1	289.3	260.3	301.9
23	270.7	274.5	290.5	261.6	306.3
24	269.1	272.1	290.3	260.2	302.7
25*	265.9	273.3	287	256.4	303.6

TABLE 32 A (2) BODY WEIGHT WALL RACK CAGES

BURN GROUP - RATS 1, 3, 6, 8, 9.

DAY	RAT 1	RAT 3	RAT 6	RAT 8	RAT 9
26	267.4	273.5	288.9	250.6	302.4
27	268	274.1	288.5	253.5	303.9
28	270.6	276.9	294.7	254	308.9
29	270.8	277.2	292.8	255.3	308.5
30	267.8	276.6	293.8	257.7	310.5
31	270.1	275.8	290.6	256.7	312.7
32	270.1	278.9	293.3	258.8	313
33	273.8	275.8	296.5	262.2	314.1
34	275.2	279.9	295.1	264.7	320
35*	272	283.6	305	266	317
36	273.7	282.5	301.8	270.7	317.1
37	267.2	283.5	310.1	269.1	320.3
38	272.2	281.8	298.5	265.2	314
39	269.7	280.1	299.7	266.5	314.4
40	269.7	282.8	297.5	263.5	309.1
41	267.3	283.6	300.7	268.4	314.6
42	271.9	289.2	302.2	270.4	313.2
43	273.4	285.4	299.3	272.2	317
44	267.9	285.1	298.8	271.2	314.7
45	271.3	286	296.5	270.1	312

TABLE 32 B (1) BODY WEIGHT WALL RACK CAGES

CONTROL GROUP - RATS 2, 4, 5, 7, 10.

DAY	RAT 2	RAT 4	RAT 5	RAT 7	RAT 10
1	245.7	245.9	245.9	245.9	243.7
2	253.3	251.7	251.4	256.2	248.3
3	255.8	253.7	257.6	257.7	249.1
4	253.3	254.5	259.8	260	250.7
5	249.5	252	262	261.2	253.4
6	256.4	253	267.2	261.9	255.2
7	264	259.1	270.4	269.8	259.7
8	264.3	259.3	274.1	269.8	259.3
9	263.1	259.4	278.8	270.7	260.6
10	261.2	258.4	278	266.9	261.2
11	262.7	260.7	278.5	267.5	263.7
12	266	260.7	282.6	269.4	267.1
13	265.5	263.4	285	270.6	267.7
14	269.2	270.2	293	276.1	275.4
15	272.7	270.7	296.7	277	278.4
16	278.1	269.4	293.5	275.1	277.9
17	274.7	270.3	295.2	274.2	278.5
18	277.9	272.2	297.7	276.2	281.4
19	280.3	273.4	302.6	279.5	285.2
20	279.4	275.2	304	282.1	287.5
21	285.6	280.6	311	291.1	296.1
22	283	279.5	309	289.5	296.4
23	283.9	279.1	310.6	294.8	299.7
24	283.6	277.3	309.7	291.6	296.8
25	280.3	277.8	309.6	287.8	294.7

TABLE 32 B (2) BODY WEIGHT WALL RACK CAGES

CONTROL GROUP - RATS 2, 4, 5, 7, 10.

DAY	RAT 2	RAT 4	RAT 5	RAT 7	RAT 10
26	280.8	278.2	309.5	288.3	296.3
27	282	279.6	310.1	286.8	296.4
28	287.6	282.5	313.7	290.8	298.8
29	286.6	281.3	311.2	289.2	296
30	284	280.7	312	287.5	297.4
31	286.7	280.3	310.7	287.3	296.4
32	284.6	283.2	311.2	290.1	295.3
33	292	282.8	313.9	292.3	297.5
34	292.3	286.3	318	298.3	299.1
35	292.1	288.8	319.2	309.8	296.9
36	285.2	285.1	313.4	295.1	291.6
37	279.2	284.4	305.6	285.5	282.9
38	267.9	277.1	294.7	282.9	274
39	269.8	284.1	301.6	283.8	282.9
40	268.6	283.5	295.2	283.5	282.9
41	268.9	285.3	294.2	290.2	289.6
42	273.2	285.1	301	298.1	294.9
43	277.9	284.4	307.8	295.1	295
44	279.3	285.8	304.5	300.9	296.1
45	268.9	284.1	289.9	299.5	290.9

TABLE 33 A (1) DAILY FOOD INTAKE WALL RACK CAGES

BURN GROUP - RATS 1, 3, 6, 8, 9.

DAY	RAT 1	RAT 3	RAT 6	RAT 8	RAT 9
1	19.7	24.2	19.4	15.5	19.7
2	17.1	20.6	20	17.8	15.4
3	19.5	22.8	20	18.9	20.3
4	16.9	20.8	21.1	17.9	19
5	17.6	20.6	20.4	17.6	19.9
6	17.6	19.9	19.2	16.7	18.2
7	15.3	15.4	18.6	18.6	18.3
8	16.7	16.4	19.1	17.3	21.1
9	19.4	16.4	21.3	13.9	20.6
10	19.3	15.4	21.1	17	20
11	18.3	16.4	19.7	16.9	19.3
12	17.1	16.8	19.8	16.6	19.7
13*	18.9	18.2	20.9	16.7	19.4
14	17.4	16.5	20.3	16	19.2
15	17.9	18	20	18.6	22.2
16	21.6	20.8	21.8	19.3	19.3
17	21.2	20.5	21.8	18.7	20.3
18	19	16.6	20.1	17.1	21.5
19	19.3	18	22.6	19.7	20.3
20	19.6	20.3	22.2	20	19.4
21	17.8	17.3	21.3	16.4	20.1
22*	19.7	20.1	22.2	20.6	20.6
23	15.5	16.5	18.2	16.3	18.8
24	23.2	20.5	24.4	20	21.5
25*	19.1	22	22.7	17.1	21.2

TABLE 33 A (2) DAILY FOOD INTAKE WALL RACK CAGES

BURN GROUP - RATS 1, 3, 6, 8, 9.

DAY	RAT 1	RAT 3	RAT 6	RAT 8	RAT 9
26	21.4	19.6	23.2	13.6	21.3
27	20.4	22.2	24.4	21.5	23.7
28	17.7	19.6	23.1	19.3	22.5
29	22	20.4	23.1	22.8	25.3
30	18.7	21	23.1	22.4	23.7
31	23.5	23.8	24	27.5	20.9
32	19.1	22	21.3	17.5	19.4
33	21.4	19.4	24.8	20.5	21.4
34	16.3	19.2	20.2	18.2	22.4
35*	15.2	18.6	19.3	20.2	16.3
36	11.8	16.5	10.2	15.8	11.4
37	10.6	15	21.8	16.5	16.5
38	25	24.2	20.2	23.7	21.9
39	19.4	20	23.5	21	20.9
40	20.2	23.6	25.2	19.9	18.3
41	17.5	19.7	26.9	20.4	22.5
42	19.3	19.8	20.1	18.2	15.8
43	28.5	22.6	29.5	21.4	24.8
44	20.5	20.8	27.5	22.1	21.8
45	18.1	27.7	24.1	17.6	17

TABLE 33 B (1) DAILY FOOD INTAKE WALL RACK CAGES

CONTROL GROUP - RATS 2, 4, 5, 7, 10.

DAY	RAT 2	RAT 4	RAT 5	RAT 7	RAT 10
1	25.4	21.9	23.2	28.1	20.5
2	24.6	18.5	20.7	27.2	17.9
3	14.5	18.9	22.6	27.7	18.5
4	21.7	19.5	23.8	27.1	17.3
5	19.6	17.7	15	26.2	17.5
6	20.8	17.4	20	27	18
7	20.3	17.3	21.7	16.7	16.2
8	20.2	19.5	15	26.5	18
9	19	17.5	23.4	24	17
10	20.8	19.2	24.2	26.9	20.1
11	19.3	18.7	22	26	17.7
12	21.6	18.3	22.1	25	18
13	20.7	19.3	24.2	27	18.5
14	19.6	18.9	24	25	18.2
15	21.3	18.3	25.5	27	19.4
16	24.4	20.3	24.5	27	18.8
17	22.1	20.6	22	27	19.8
18	21.3	18.4	23.4	27.5	20.4
19	20.9	19.6	26.3	27	19.6
20	21	18.6	24.3	28	20.4
21	19.5	16.7	23	27	19.2
22	20.9	20.7	26.7	27	20.6
23	17.7	15.6	24.2	26.4	19
24	22.5	21.2	26.3	28	21.5
25	18.8	21	27.4	27.9	19.9

TABLE 33 B (2) DAILY FOOD INTAKE WALL RACK CAGES

CONTROL GROUP - RATS 2, 4, 5, 7, 10.

DAY	RAT 2	RAT 4	RAT 5	RAT 7	RAT 10
26	21.4	20.	24.4	29.1	21.8
27	22.7	21.6	29.5	29.7	22.5
28	21.6	20.9	24.9	29.9	20.2
29	22.2	22.9	26.1	30	20.4
30	22.8	21	24.1	30	23.4
31	24.8	22.5	26.5	23.8	21.6
32	20.3	21.6	24.2	29.9	18.6
33	22.4	19.5	23.9	30	20.4
34	20.4	20.2	23.8	29.8	20.4
35	12.8	19.9	28.8	22.5	12.1
36	14.4	17.2	17.9	18.1	13.2
37	12	12.3	9.9	10.2	7
38	12.9	15	12.4	21.8	12.7
39	18.2	24.2	22.8	20.2	20.9
40	17.3	20	16.2	23.5	20.3
41	17.5	23.6	16.5	25.2	22
42	19.5	17.2	20.5	26.9	17.8
43	18	19.8	12.8	20.1	21.2
44	16.8	22.6	14.2	29.5	21
45	18.7	18.5	16.2	27.5	16

TABLE 34 A (1) BODY WEIGHT METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

DAY	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17
1	248.6	250.1	241.8	229.5	239.7	231.9
2	259.9	259.7	244.7	233.5	243.1	242.6
3	262.8	262.5	246.1	234.8	249.7	246.9
4	264.4	263	247.9	234.5	253	248.9
5	266.9	266.1	246.6	236.3	256.3	252.5
6	269.1	268.7	246.9	233.8	255.6	252.5
7	274.2	275.6	252.7	241.2	262.2	261.4
8	276.2	276.3	252.9	242.7	261.8	260.8
9	277	279.4	255.5	246.3	266.1	262.9
10	274.7	278.6	253.1	242.2	260.5	261.4
11	277	280.2	253.9	245.4	264.4	264.1
12	279.7	282.5	257.4	246.9	267.9	266.1
13*	281.6	285	257.8	248.4	272	267.7
14	286.3	293.8	262	252.9	278.3	271.9
15	288.9	293.7	263	254.2	280.2	275.9
16	281.3	278.2	249.9	237.8	280.8	276
17	287.2	294.2	260.8	250.7	275.8	274.7
18	288.1	295.5	262.7	253.2	280.6	278.2
19	291.2	299	265.8	255.7	285.7	277.7
20	294.8	300.9	266.4	256.3	286.9	281.5
21	299.6	309.2	273.1	265	294.2	289.2
22*	298.5	311.1	273.7	264.8	299.1	286.4
23	295.6	306	273	260.4	294.2	285.6
24	298.9	308.6	272.2	261.8	290.1	286.3
25*	295.9	306.7	269.1	258.1	295.7	284.4

TABLE 34 A (2) BODY WEIGHT METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

DAY	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17
26	297.2	305.8	268.2	260.3	293.2	283.2
27	295.4	305.5	264.9	259.8	291.8	287
28	300.7	312.6	270.3	267	294.6	291.3
29	299.2	311.4	266.4	265.3	296.6	295.3
30	302	313	269.7	265.1	293.2	296.1
31	303.4	311	269	267.2	295.8	296.4
32	304.6	312.7	268.3	266.9	290.7	297.6
33	305.8	315.1	270.8	266.8	299	297.5
34	309.6	318.1	274.8	272.5	295.1	302.2
35*	311.2	323.6	278.8	274.7	310.7	308.3
36	306.7	315.2	276.1	274.8	296.2	301.9
37	309.6	316.7	275.6	277.4	301.	307
38	307.9	313.2	270.9	272.2	302.6	299.6
39	304.6	313.7	270.3	268.7	300.5	298
40	302.3	312.9	268.8	267.4	299	297.5
41	308.6	319.8	275.4	270.8	304.3	302.5
42	314.1	326.1	280	269.5	311	312
43	301	320.3	277.2	268.2	307.0	306.2
44	296.9	313.5	271.8	268.8	292.6	301.3
45	301.9	314.8	273.3	270.1	299.3	302.1
46			275.5			

TABLE 34 B (1) BODY WEIGHT METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

DAY	RAT 12	RAT 18	RAT 19	RAT 20
1	236.1	255	221.5	233.8
2	242.7	256.9	224.5	236.5
3	244.7	264.4	230.2	233.1
4	247.4	265.4	230.5	235.3
5	248	267.9	232.9	237.4
6	248.5	267.9	234.1	234.5
7	253.8	272.3	238.2	242.8
8	255.6	274.3	235.9	240.8
9	259.3	281.1	238.1	241.8
10	254.4	275.5	238	240
11	255.5	276.2	236.8	241.1
12	258.2	280.7	241.1	242.6
13	258.9	281.1	241	243.3
14	266.1	288.7	250.8	249.8
15	267.2	288.9	252.2	250.3
16	249.2	287.4	251	249.3
17	262.2	288	250.3	249.8
18	263.3	289.8	253.5	249.9
19	266.6	290.9	255.1	251.2
20	266.7	293	258.6	253.9
21	275.3	300.9	265.3	258.6
22	274.6	302.3	265.3	260
23	271.1	302.2	264.7	256.5
24	272.2	300.2	263.1	257.6
25	271	298.2	260.7	253

TABLE 34 B (2) BODY WEIGHT METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

DAY	RAT 12	RAT 18	RAT 19	RAT 20
26	268.1	296.5	258.4	253.1
27	269	296.7	254.4	253
28	275.5	305.7	256.3	258.8
29	274.5	305	254.4	256
30	272.4	308.4	254.1	257.9
31	271.6	302.9	255.7	257.9
32	276.2	306.9	256.6	258.5
33	274.4	307.9	260.3	258.4
34	278.5	314.9	262.2	262.5
35	286.1	314.9	262.2	267.1
36	280.2	304.2	258.6	262.1
37	272.5	298.9	257.2	261.3
38	264.4	292.6	249	259.9
39	272.1	299.7	256.4	264.4
40	268.1	300.2	256.2	260.4
41	270.5	302.3	257.6	265.6
42	274.5	310.2	258.9	271.9
43	276.6	302.1	258.7	268.7
44	271.9	304.4	263.5	266.8
45	278.8	303.3	265	270.1

TABLE 35 A (1) DAILY FOOD INTAKE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

DAY	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17
1	18.7	19.4	12.9	14.6	21.3	18
2	22.7	22.7	13.1	14.1	17.2	20.3
3	21.5	18.1	16.3	16.2	20	20
4	20.2	19.3	15.4	15	20.8	21
5	19	20	15.1	16.2	18	21
6	19.6	19.9	14.1	14.7	18.2	18.2
7	17.8	15.4	15.2	15.8	18.3	20.9
8	18.2	17.7	15.1	15.6	19.3	19.7
9	16.3	17.7	16.0	15.8	20.9	18.2
10	17.6	18.2	15.6	17.5	20.1	20
11	17.7	17.3	15.8	17.9	20.5	19.1
12	18.7	19	15.9	16.8	21.6	18.9
	METABOLIC COLLECTIONS BEGIN					
13	17.2	18.7	16.3	16.1	21.5	19.7
14	16.8	19.4	16	17.8	21.1	17.3
15	16.3	18.3	14.7	16.9	22	19.2
16	12	13.1	11.4	9.9	21.8	19.4
17	18.4	21.1	16.9	17.5	19	18.6
18	17.4	18.7	16.4	15.6	20.2	18.1
19	17.5	19.2	16.7	18.2	22.2	17.9
20	16.7	20	16.7	16.8	21.1	20.5
21	18	20.1	17.0	18.1	17.8	19.4
22*	16.1	20.1	16.6	17.4	21	17.7
23	14.5	14.5	14.3	11	12.1	14.7
24	19.2	20.6	17.7	18.5	19.2	19.7
25*	17.6	19.5	15.2	16.5	21.3	20.4

TABLE 35 A (2) DAILY FOOD INTAKE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

DAY	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17
26	18.5	17.1	14.6	16.4	15.1	16.6
27	20	20.9	16.3	17.2	18.9	23.7
28	18.7	21.2	17.7	18.4	17.5	19.2
29	18.8	20.5	17.4	17.4	21.6	24.9
30	22.1	22.2	21.1	17.8	19.9	22.7
31	19.4	19.6	19	18.5	20	23.2
32	18.6	20.3	18.7	18.3	17.8	21.2
33	18.2	19.4	18.8	16.9	21.7	21.4
34	19.2	22.5	19.2	19.4	17.6	21.6
35*	13.2	15.7	15.5	16	18.9	16.5
36	9.9	7.0	12	9.2	4.9	8
37	12.4	12.7	12.9	13.3	13.7	16.6
38	22.8	20.9	18.2	20.7	21.8	21.3
39	16.2	20.3	17.3	15.7	18.5	19.8
40	16.5	22	17.5	17.8	17.6	21.1
41	20.5	22.8	19.5	17.3	18.9	23.2
42	12.8	21.2	18	14.2	17.5	20.1
43	14.2	21	16.8	19.2	20.2	19.7
44	16.2	16	18.7	20.2	13.3	22.6
45	17.4	17.9	16.7	17.7	16.2	18.6
46		17.6	17.8			

TABLE 35 B (1) DAILY FOOD INTAKE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

DAY	RAT 12	RAT 18	RAT 19	RAT 20
1	15.9	21.1	13.3	13.4
2	16.3	18.8	13	14.8
3	17.2	22.7	15.6	14
4	16.9	19.9	14.9	15.3
5	17.3	20.8	14.2	15.2
6	15.9	17.9	14.1	14.1
7	25.9	17.1	10.1	14.6
8	16.1	17.8	8.9	14
9	16.5	19.1	12.4	14.1
10	16.7	17.8	15.5	14.1
11	14.1	16.7	15.1	14.9
12	16.9	18.7	16	14.4
METABOLIC COLLECTIONS BEGIN				
13	15.5	16.1	17.2	14.6
14	17.6	17.5	18.3	15.3
15	15.5	17.8	16.3	14.7
16	10.4	16.8	17.7	15.1
17	19.3	18.1	17.4	15.7
18	16.6	16.2	17.5	15.3
19	16.7	17.1	16.3	16.1
20	17.0	16.5	18.6	16.2
21	16.6	18.1	18.3	16.4
22	15.9	18.9	17.7	16.7
23	12.9	15.3	13.9	12.8
24	19	20.9	17.3	18.3
25	18	20.9	17.2	15.9

TABLE 35 B (2) DAILY FOOD INTAKE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

DAY	RAT 12	RAT 18	RAT 19	RAT 20
26	15.9	18	14.1	17.4
27	17.3	19.3	15.7	17.9
28	17.9	21.2	15.8	19.2
29	18.5	20.4	18	17.1
30	17.3	21.3	20.8	17.6
31	22.3	18.7	20.5	18.6
32	18.1	19.3	18.7	17.4
33	14.3	20.3	20.2	18.4
34	16.2	20.4	22	22.1
35	19.3	13.7	14	15.5
36	14.3	11.1	13.4	10
37	9.2	11.4	10.9	13.9
38	13.3	16.5	10.6	16.5
39	20.7	21.9	21.6	19.6
40	15.7	20.9	19.4	14.7
41	17.8	18.3	20.2	17.1
42	17.3	20.3	17.3	18.1
43	14.2	15.8	19.3	18.2
44	19.2	21.8	26.1	16.8
45	20.2	17.6	20	17.4
46		16.7	18.1	

between days 36 to 45 (173 g vs. 170 g respectively).

There was no significant difference in mean body weights between the burn group (295.5 $\pm$ 20.6 g SD) and the control group (293.0 $\pm$ 19.8 g SD) on the day of the burn, day 35. After a 5% BSA burn there was a cessation of growth rather than marked weight loss noted in the burned rats if the mean rat weight on day 35 is compared with that on day 45, 10 days after the burn (290.6 $\pm$ 17.2 g). This represents a reduction in weight of less than 2% from the mean weight of the burn group rats on the day of the burn compared with the mean weight 10 days after injury.

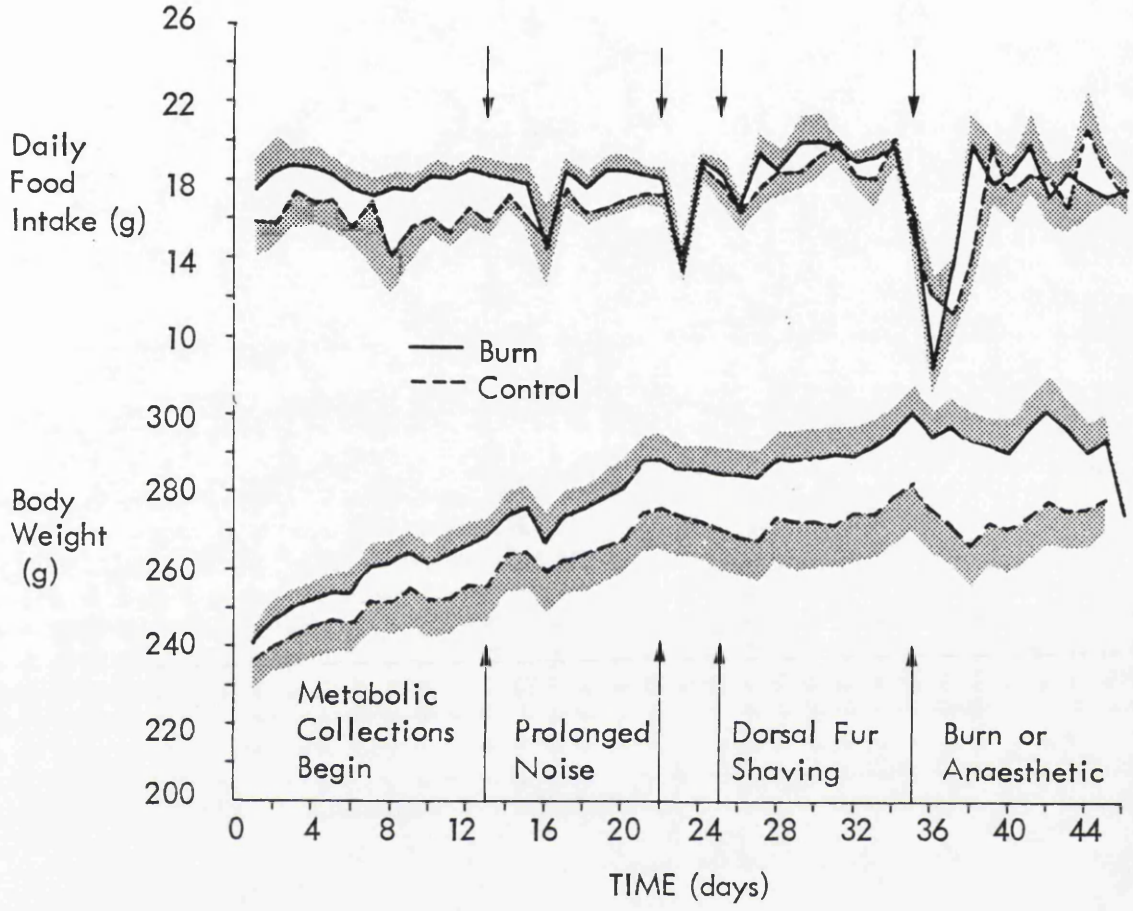
The control group rats suffered a marked drop in weight over the 3 day period after the anaesthetic administration due to food restriction with pair feeding (mean weight loss 19.4 g compared with 4.8 g weight loss in burn group), though they made up this weight loss by day 45. On day 45 there again was no significant difference between the mean weight of burned rats and control group rats (290.6 $\pm$ 17.2 vs. 283.8 $\pm$ 14.5 respectively). The burned rats' failure to lose weight initially was almost certainly due to post burn oedema and fluid retention. Though no parenteral fluid was given to any of these animals, the burned rats voluntarily increased their oral fluid intake considerably over this period.

A 5% BSA full thickness burn in these rats on ad libitum diet caused a trivial loss of body weight if the mean pre burn weight on day 35 is compared with the mean weight on day 45. However, the adult rat, unlike adult man, never reaches a plateau in body weight. Between day 1 and day 35, all the rats were growing at a mean rate of 1.6 g weight gain per day. If this had not been interrupted on day 35 in this study, the burned rats would have reached a mean body weight of 311.5 g on day 45 and the control rats 309 g.

Considered in this light, the burned rats on day 45 are only 93.3% of their expected mean body weight and control rats only 91.8% of predicted mean body weight, if normal growth had continued. When comparing weight loss after burn injury in adult man with that found after burn injury in the rat, it is essential to take into account the failure of the rat to grow in addition to measuring body weight actually lost from the date of injury.

Figure 119 shows the comparison in daily body weight and food intake between burned and control rats housed in the metabolic cages. Burned and control rats closely follow the patterns of food intake and body weight changes already described in Figure 118, though

Fig. 119 5% BURN FOOD INTAKE AND BODY WEIGHT vs TIME. COMPARISON OF BURN GROUP AND CONTROL GROUP. METABOLIC CAGES Results Mean ± Std. Error for 6 rats and 4 rats respectively



by chance there was a slight mean body weight difference between burn and control group at the start (240.3 $\pm$ 8.4 g SD vs. 236.6 $\pm$ 13.8 g SD respectively on day 1) and throughout the experiment (293.6 $\pm$ 17.8 g SD vs. 279.3 $\pm$ 17.0 g SD respectively on day 45).

Figure 120 shows the urinary nitrogen losses and estimated faecal nitrogen losses in metabolic cage burn group rats 11, 13, 14, 15, 16, 17. Figure 110 shows the urinary nitrogen losses and estimated faecal nitrogen losses in metabolic cage control group rats 12, 18, 19, 20.

Daily values for nitrogen intake, urine nitrogen losses and estimated nitrogen balance are given in Tables 36A, 36B, 36C and 37A, 37B, 37C, for individual rats.

The mean "basal" urinary nitrogen excretion in burn (Fig. 120, Table 36A) and in control (Fig. 110, Table 37A) rats is similar at 458 mg N per day and 456 mg N per day respectively between day (12-13) and day (21-22). See Table 38.

After dorsal fur shaving, burn (Table 36B) and control (Table 37B) rats show similar increases in urinary nitrogen excretion with means of 533 mg and 561 mg N per day between day (23-26) and day (34-35).

Fig. 120 **5 % BURN EXPERIMENT: URINARY NITROGEN LOSSES (Burn Group)**

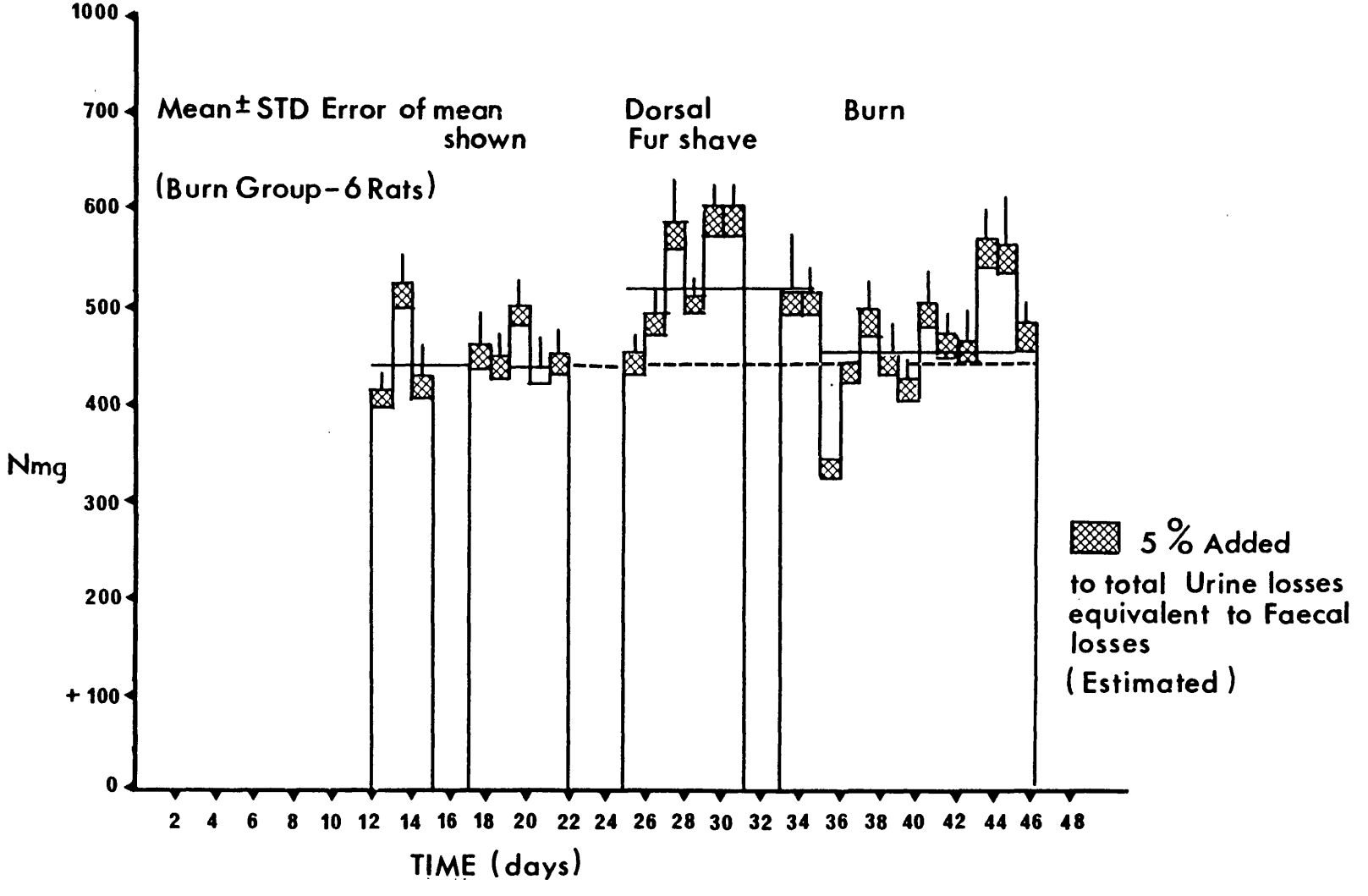


TABLE 36 A (1) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

PRE FUR SHAVING PERIOD

DAY 12-13	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
FOOD N. mg	636	692	603	596	796	729	675 ± 78
URINE N. (+ 5%) mg	486	385	441	430	424	336	417 ± 51
N. BALANCE mg	150	307	162	166	372	393	258 ± 112
DAY 13-14							
FOOD N. mg	622	718	592	659	781	640	669 ± 69
URINE N. (+ 5%) mg	578	438	659	437	569	476	526 ± 90
N. BALANCE mg	44	280	-67	222	212	164	143 ± 130
DAY 14-15							
FOOD N. mg	606	713	544	626	814	710	669 ± 96
URINE N. (+ 5%) mg	540	395	465	478	395	280	426 ± 90
N. BALANCE mg	66	318	79	148	419	430	243 ± 166

TABLE 36 A (2) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

PRE FUR SHAVING PERIOD

DAY 17-18	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
FOOD N. mg	643	692	607	577	747	670	656 ± 61
URINE N. (+ 5%) mg	507	513	378	381	414	560	459 ± 78
N. BALANCE mg	136	179	229	196	333	110	197 ± 79
DAY 18-19							
FOOD N. mg	648	710	618	673	821	662	689 ± 72
URINE N. (+ 5%) mg	459	542	460	412	380	429	447 ± 55
N. BALANCE mg	189	168	158	261	441	233	242 ± 105
DAY 19-20							
FOOD N. mg	618	740	618	622	781	759	690 ± 78
URINE N. (+ 5%) mg	495	443	596	475	449	526	497 ± 57
N. BALANCE mg	123	297	22	147	332	233	193 ± 117

TABLE 36 A (3) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

PRE FUR SHAVING PERIOD

DAY 20-21	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
FOOD N. mg	666	744	629	670	659	718	681 ± 42
URINE N. (+ 5%) mg	495	449	370	406	394	521	439 ± 60
N. BALANCE mg	171	295	259	264	265	197	242 ± 47
DAY 21-22							
FOOD N. mg	599	744	614	644	777	655	658 ± 70
URINE N. (+ 5%) mg	437	-	503	455	491	370	451 ± 53
N. BALANCE mg	162	-	111	189	286	285	207 ± 77

TABLE 36 B (1) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

PRE BURN PERIOD

DAY 25-26	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	685	692	540	607	559	614	616 ± 63
2	495	385	438	371	500	459	441 ± 54
3	190	307	102	236	59	155	175 ± 90
DAY 26-27							
1	740	773	603	636	699	877	721 ± 99
2	507	496	428	435	469	608	491 ± 66
3	233	277	175	201	230	269	230 ± 39
DAY 27-28							
1	692	784	655	552	648	710	674 ± 77
2	636	576	545	445	545	766	586 ± 108
3	56	208	110	107	103	-56	88 ± 86

TABLE 36 B (2) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

PRE BURN PERIOD

DAY 28-29	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	696	759	644	644	799	921	744 ± 27
2	535	445	568	483	550	503	514 ± 46
3	161	314	76	161	249	418	230 ± 123
DAY 29-30							
1	818	821	781	659	736	840	776 ± 68
2	640	513	613	552	645	632	599 ± 54
3	178	308	168	107	191	208	193 ± 66
DAY 30-31							
1	718	722	703	556	740	858	716 ± 96
2	642	513	613	552	645	632	600 ± 55
3	76	209	90	4	95	226	116 ± 85

TABLE 36 B (3) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

PRE BURN PERIOD

DAY 33-34	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	710	833	710	718	651	799	720 ± 61
2	592	-	-	519	328	618	514 ± 131
3	118	-	-	199	323	181	206 ± 86
DAY 34-35							
1	488	581	574	592	699	611	594 ± 75
2	436	567	-	531	503	530	513 ± 49
3	52	14	-	61	196	81	81 ± 69

1 = FOOD N. mg.

2 = URINE N. (+ 5%) mg.

3 = N. BALANCE mg.

TABLE 36 C (1) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

POST BURN PERIOD

DAY 35-36	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	366	259	444	340	181	296	318 ± 101
2	384	317	210	362	419	-	338 ± 81
3	-18	058	234	-22	-238	-	-20 ± 168
DAY 36-37							
1	459	470	477	492	507	614	503 ± 57
2	444	458	470	441	397	436	441 ± 25
3	15	12	7	51	110	178	62 ± 69
DAY 37-38							
1	844	773	673	766	807	788	775 ± 57
2	542	362	489	480	537	555	494 ± 71
3	302	411	184	286	270	233	281 ± 76

TABLE 36 C (2) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

POST BURN PERIOD

DAY 38-39	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	599	751	640	581	685	733	665 ± 70
2	478	513	443	355	378	527	449 ± 71
3	121	238	197	226	307	206	216 ± 61
DAY 39-40							
1	611	814	648	659	651	781	694 ± 83
2	415	355	432	427	458	465	425 ± 39
3	196	459	216	232	193	316	269 ± 104
DAY 40-41							
1	759	844	722	640	699	858	754 ± 85
2	571	562	487	466	361	560	501 ± 81
3	188	282	235	174	338	298	253 ± 65

TABLE 36 C (3) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

POST BURN PERIOD

DAY 41-42	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	474	784	666	525	648	744	673 ± 100
2	-	470	444	443	426	555	468 ± 51
3	-	314	222	82	222	189	205 ± 83
DAY 42 - 43							
1	525	777	622	710	748	729	717 ± 59
2	-	407	377	489	528	528	466 ± 70
3	-	370	245	221	220	201	251 ± 68
DAY 43-44							
1	599	592	692	747	492	836	660 ± 123
2	672	575	545	549	420	635	566 ± 87
3	-73	17	147	198	72	201	94 ± 109

TABLE 36 C (4) NITROGEN BALANCE METABOLIC CAGE RATS

BURN GROUP - RATS 11, 13, 14, 15, 16, 17.

POST BURN PERIOD

DAY 44-45	RAT 11	RAT 13	RAT 14	RAT 15	RAT 16	RAT 17	MEAN ± SD
1	644	662	618	655	599	688	644 ± 32
2	470	752	702	482	468	492	561 ± 130
3	174	-90	-84	173	131	196	83 ± 134

1 = FOOD N. mg.

2 = URINE N. (+ 5%) mg.

3 = N. BALANCE mg.

TABLE 37 A (1) NITROGEN BALANCE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

PRE FUR SHAVING PERIOD

DAY 12-13	RAT 12	RAT 18	RAT 19	RAT 20	MEAN ± SD
1	574	596	636	540	587 ± 40
2	481	496	450	399	457 ± 43
3	93	100	186	141	130 ± 43
DAY 13-14					
1	651	648	677	566	636 ± 48
2	449	532	183	481	411 ± 156
3	202	116	494	85	225 ± 187
DAY 14-15					
1	574	659	603	544	595 ± 49
2	606	439	451	426	481 ± 84
3	-32	220	152	118	114 ± 106
DAY 17-18					
1	614	599	648	566	607 ± 34
2	507	455	350	190	376 ± 140
3	107	144	298	376	231 ± 127
DAY 18-19					
1	618	633	603	596	613 ± 16
2	516	422	430	401	442 ± 51
3	102	211	173	195	171 ± 48
DAY 19-20					
1	629	611	688	599	632 ± 39
2	442	399	502	503	461 ± 50
3	187	212	186	97	171 ± 50

TABLE 37 A (2) NITROGEN BALANCE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

PRE FUR SHAVING PERIOD

DAY 20-21	RAT 12	RAT 18	RAT 19	RAT 20	MEAN ± SD
1	614	670	677	607	630 ± 35
2	531	522	-	531	528 ± 5
3	83	148	-	76	102 ± 40
DAY 21-22					
1	588	699	655	618	635 ± 37
2	531	551	-	492	524 ± 30
3	57	148	-	126	111 ± 47

1 = FOOD N. mg.

2 = URINE N. (+ 5%) mg.

3 = N. BALANCE mg.

TABLE 37 B (1) NITROGEN BALANCE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

PRE ANAESTHETIC PERIOD

DAY 25-26	RAT 12	RAT 18	RAT 19	RAT 20	MEAN ± SD
1	588	666	522	644	605 ± 64
2	499	511	460	480	488 ± 22
3	89	155	62	164	117 ± 50
DAY 26-27					
1	640	714	581	662	649 ± 55
2	482	642	524	576	556 ± 69
3	158	72	57	86	93 ± 45
DAY 27-28					
1	662	784	585	710	685 ± 84
2	556	645	525	540	567 ± 54
3	106	139	60	170	118 ± 47
DAY 28-29					
1	685	755	666	633	685 ± 52
2	523	583	590	445	535 ± 67
3	162	172	76	188	150 ± 50
DAY 29-30					
1	640	788	770	651	712 ± 78
2	687	635	652	588	641 ± 41
3	-47	153	118	63	71 ± 87
DAY 30-31					
1	828	692	759	688	742 ± 66
2	687	635	757	588	667 ± 72
3	141	57	2	100	75 ± 60

TABLE 37 B (2) NITROGEN BALANCE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

PRE ANAESTHETIC PERIOD

DAY 33-34	RAT 12	RAT 18	RAT 19	RAT 20	MEAN ± SD
1	599	755	814	818	744 ± 125
2	355	-	547	570	491 ± 118
3	244	-	267	248	253 ± 12
DAY 34-35					
1	714	507	518	574	602 ± 101
2	559	-	561	439	520 ± 70
3	156	-	-43	135	82 ± 109

1 = FOOD N. mg.

2 = URINE N. (+ 5%) mg.

3 = N. BALANCE mg.

TABLE 37 C (1) NITROGEN BALANCE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

POST ANAESTHETIC PERIOD

DAY 35-36	RAT 12	RAT 18	RAT 19	RAT 20	MEAN ± SD
1	525	411	496	370	451 ± 72
2	426	451	420	383	420 ± 28
3	99	-40	76	-13	31 ± 67
DAY 36-37					
1	340	428	403	514	421 ± 72
2	423	449	398	367	409 ± 35
3	-83	-21	5	147	42 ± 71
DAY 37-38					
1	492	611	392	611	527 ± 106
2	513	444	355	421	433 ± 65
3	-21	167	37	190	94 ± 102
DAY 38-39					
1	766	810	799	725	775 ± 38
2	554	377	488	482	475 ± 73
3	212	433	311	243	300 ± 98
DAY 39-40					
1	581	773	718	544	654 ± 109
2	443	488	426	354	428 ± 56
3	138	285	292	190	226 ± 75
DAY 40-41					
1	659	699	747	633	685 ± 50
2	581	606	458	438	521 ± 85
3	78	93	289	195	164 ± 98

TABLE 37 C (2) NITROGEN BALANCE METABOLIC CAGE RATS

CONTROL GROUP - RATS 12, 18, 19, 20.

POST ANAESTHETIC PERIOD

DAY 41-42	RAT 12	RAT 18	RAT 18	RAT 20	MEAN ± SD
1	640	752	640	670	676 ± 53
2	421	594	539	491	511 ± 73
3	219	158	101	179	165 ± 49
DAY 42-43					
1	525	585	714	673	624 ± 85
2	486	449	393	563	473 ± 71
3	39	136	321	110	151 ± 120
DAY 43-44					
1	710	807	966	622	776 ± 147
2	644	520	527	501	548 ± 65
3	66	287	439	121	228 ± 169
DAY 44-45					
1	747	651	740	644	696 ± 56
2	577	563	667	555	591 ± 52
3	170	88	73	89	105 ± 44

1 = FOOD N. mg.

2 = URINE N. (+ 5%) mg.

3 = N. BALANCE mg.

This represents a mean increase of 16.4% above basal urinary nitrogen excretion in the burn group rats and a 23.0% increase in the control group rats (Table 38).

A comparison of mean urinary nitrogen excretion between burn group rats and control group rats is shown in Figure 121. This indicates the very similar changes in the urinary excretion of nitrogen in burn group and control group rats. Even in the period after the burn between day (35-36) and day (44-45) when the control group was pair fed with the burn group, the similarity in response as judged by urinary nitrogen excretion continued.

The mean daily urinary nitrogen excretion in the burn group was 473 mg (Table 36C) between day (35-36) and day (44-45), compared with 481 mg (Table 37C) in the control group. This represented a 3.3% increase above basal urinary nitrogen excretion after thermal injury in the burn group and a 5.5% increase after anaesthesia in the control rats (Table 38).

Estimated nitrogen balance for the burn group rats is shown in Figure 122, and for the control group rats is shown in Fig. 109. Mean daily food nitrogen intake (L.I.T. Diet is 3.7% N.) was slightly less in the control group rats (616 mg) compared with the burn group rats (675 mg) between day (12-13) and day (21-22).

Fgi Fig. 12 URINARY NITROGEN LOSSES - COMPARISON OF BURN GROUP WITH CONTROL GROUP

MEANS SHOWN (Burn group - 6 Rats: Control Group 4 Rats)

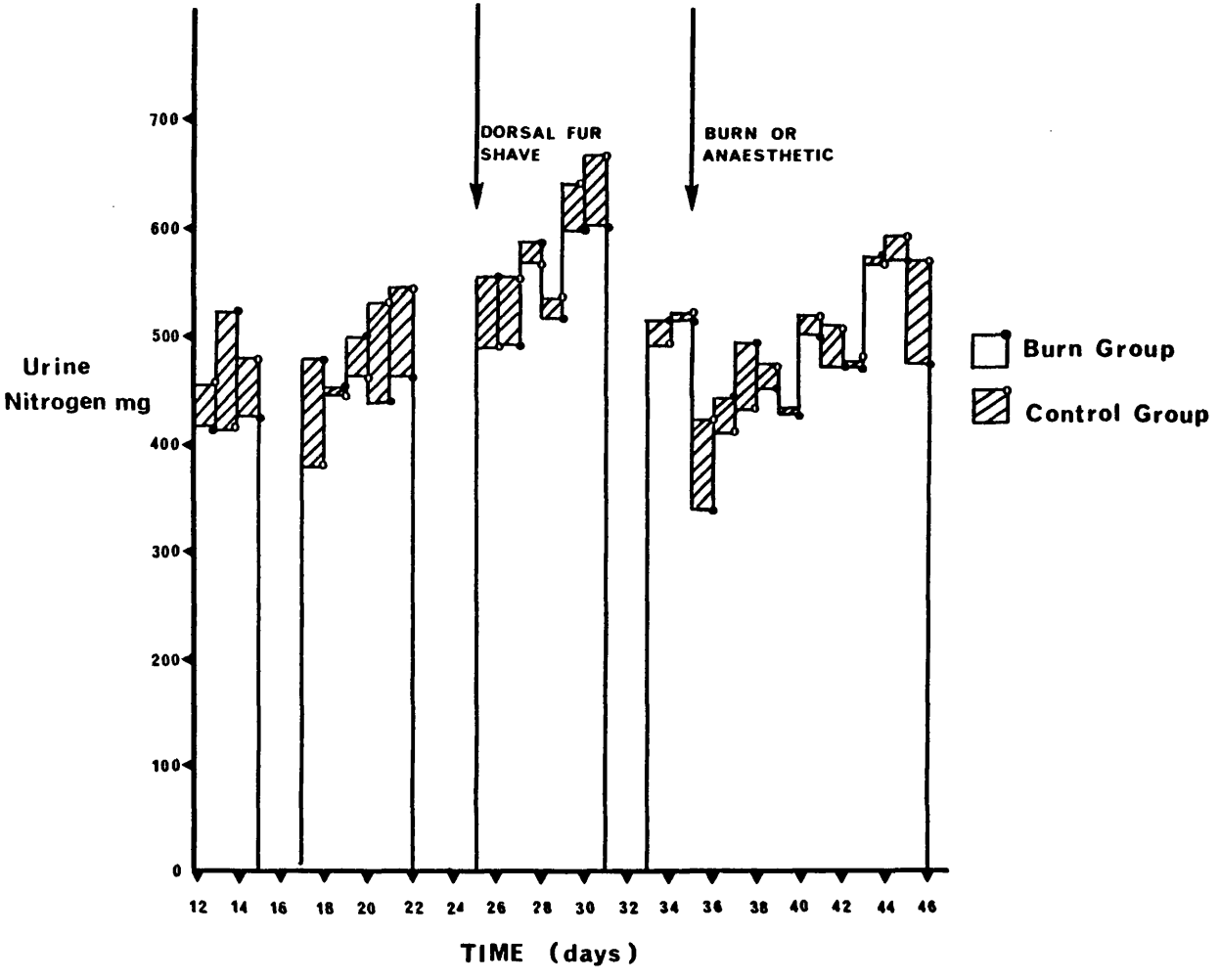
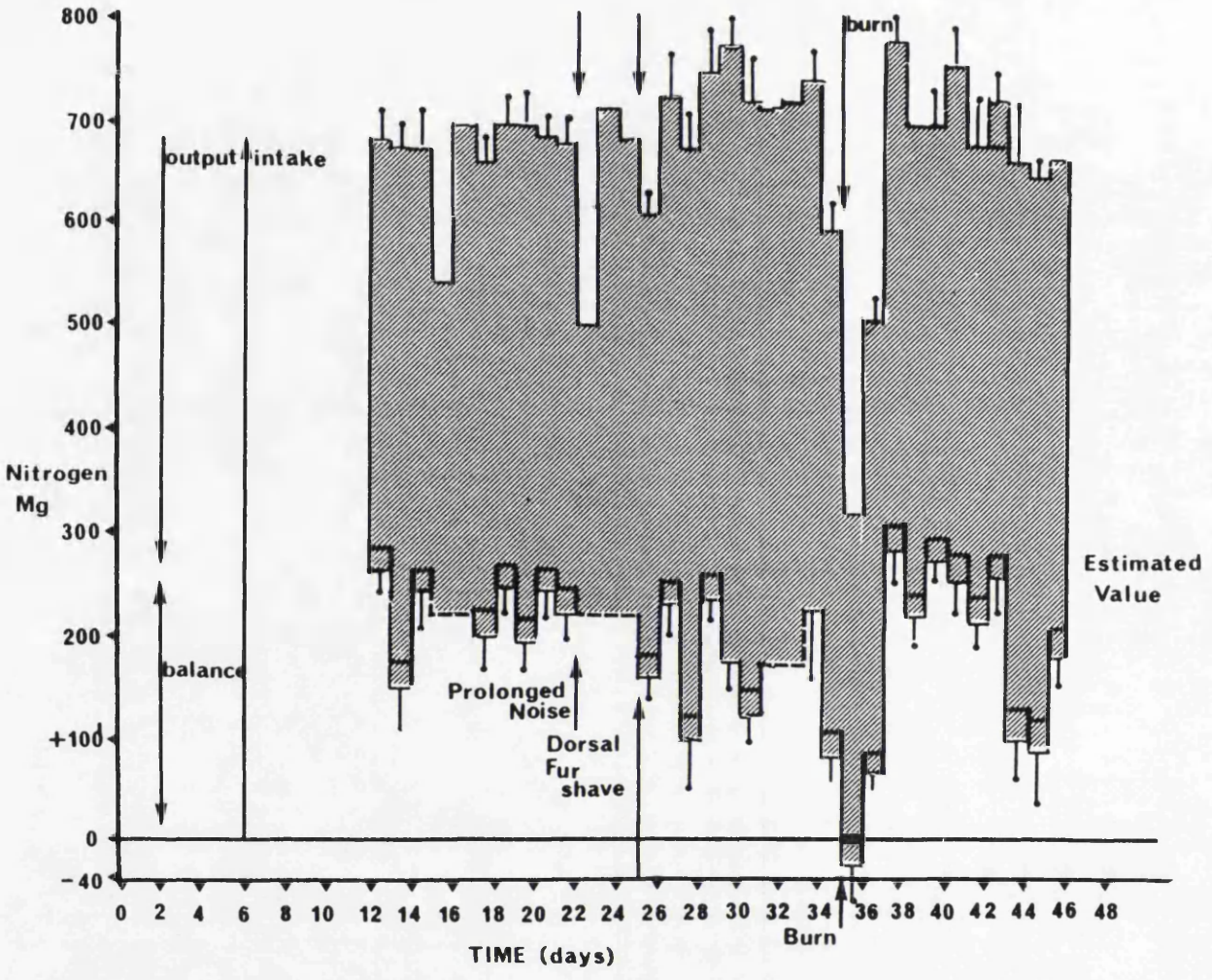


Fig. 122 5 % BURN EXPERIMENT: NITROGEN BALANCE (Burn Group) AFTER 5 % SURFACE AREA BURN



Both burn and control rats showed an increase in mean daily food nitrogen intake after dorsal fur shaving, a 3.1% increase above "basal" intake to 696 mg N in the burn group, and a 10.1% increase above previous "basal" intake to 678 mg N in the control group. After thermal injury, the mean daily food nitrogen intake, between day (35-36) and day (44-45) decreased in burn group rats by 4.8% below "basal" intake to 644 mg. In the control group rats, after anaesthesia, the mean daily food nitrogen intake was little changed from "basal" intake at 628 mg, an increase of 1.9% (Table 38).

Burn and control group rats showed similar reductions in mean daily nitrogen retention (+ve balance) after dorsal fur shaving, and after burn/anaesthesia.

The mean daily nitrogen balance was less in the control group than in the burn group and the control group mean daily food intake was slightly less.

While the changes described here are not statistically significant, they do represent trends which are identical in both groups of rats studied.

TABLE 38 A MEAN DAILY NITROGEN BALANCE (± SD) METABOLIC CAGE RATS

5% BSA BURN

BURN GROUP - RATS 11, 13, 15, 16, 17.

	MEAN DAILY FOOD N. mg.	% CHANGE FROM "BASAL"	MEAN DAILY N. EXCRETION mg.	% CHANGE FROM "BASAL"	MEAN DAILY BALANCE mg.	% CHANGE FROM "BASAL"
DAY (12-13) to DAY (21-22)	675 ± 68	"BASAL"	458 ± 72	"BASAL"	216 ± 108	"BASAL"
DAY (25-26) to DAY (34-35)	696 ± 97	+ 3.1%	533 ± 87	+ 16.4%	165 ± 96	- 23.6%
DAY (35-36) to DAY (44-45)	644 ± 145	- 4.8%	473 ± 94	+ 3.3%	171 ± 134	- 20.8%

TABLE 38 B MEAN DAILY NITROGEN BALANCE ($\pm$ SD) METABOLIC CAGE RATS

5% BSA BURN

CONTROL GROUP - RATS 12, 18, 19, 20.

	MEAN DAILY FOOD N. mg.	% CHANGE FROM "BASAL"	MEAN DAILY N. EXCRETION mg.	% CHANGE FROM "BASAL"	MEAN DAILY BALANCE mg.	% CHANGE FROM "BASAL"
DAY (12-13) to DAY (21-22)	616 $\pm$ 40	"BASAL"	456 $\pm$ 91	"BASAL"	160 $\pm$ 99	"BASAL"
DAY (25-26) to DAY (34-35)	678 $\pm$ 85	+ 10.1%	561 $\pm$ 85	+ 23.0%	117 $\pm$ 76	- 26.9%
DAY (35-36) to DAY (44-45)	628 $\pm$ 141	+ 1.9%	481 $\pm$ 80	+ 5.5%	147 $\pm$ 122	- 8.1%

METABOLIC RESPONSE TO 5% BODY SURFACE AREA FULL SKIN THICKNESS BURN

DISCUSSION

A 5% BSA full skin thickness burn caused a trivial but measurable change in growth rate in burned rats. Identical body weight changes were induced in control rats by (pair) feeding them the same food intake as eaten by the burned rats from day 35 to day 45. Urinary nitrogen excretion alone is not a good index of protein catabolism or injury response. The most striking increase in urinary nitrogen excretion seen in this particular study was after dorsal fur shaving. This was almost entirely due to an equivalent increase in food nitrogen intake over the same period. After burn injury/anaesthetic, there was no overall increase in food intake over a 10 day period. It was of note that control group rats in the metabolic cages were lighter than the burn group rats in metabolic cages. Metabolic cage control rats also ate less and were in less positive nitrogen balance than burn group rats throughout the entire period of study.

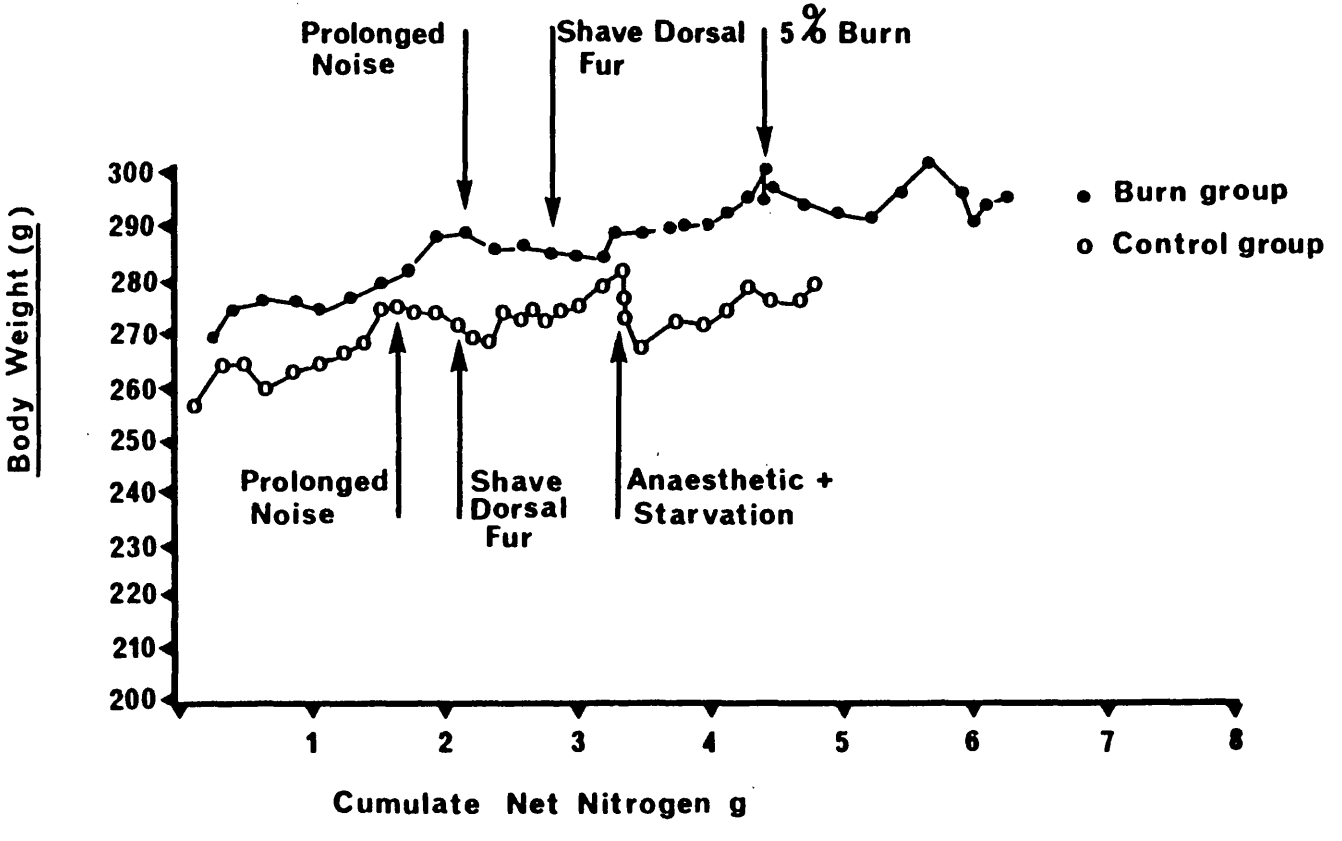
Conventional nitrogen balance graphs (Figs. 122 and 109) do not show these differences very clearly. Another form of data presentation was devised by the author together with Dr. R. G. Bessent in an endeavour

to show small differences in growth and nitrogen balance more clearly between injured and control rats. This is shown in Figure 123. Here mean daily body weight of the rat is plotted against the mean daily cumulative (total) amount of nitrogen retained (or lost). Figure 123 therefore contains the essence of the information in Figures 119, 122 and 109 combined, but without a time axis. The period of study represented in Figure 123 is from day 13 to day 45. Figure 123 shows the "efficiency" of conversion of retained body nitrogen to body weight gain. When the rat gains body weight and retains nitrogen daily, then the slope of the line will be upward going from left to right. When the rat loses body weight and loses body nitrogen then the slope of the line will be reversed, going downward from right to left.

This form of data presentation does appear to highlight small differences between burn and control rat groups, though with any form of cumulative data presentation small daily errors in measurements are also cumulated and these errors may be considerable at the end of a 32 day study period. In the controlled environment system devised and built by the author, it is likely that any measurement errors which exist will affect control rats and burn rats equally.

FgFig. 123 **THE EFFECT OF 5% BURN ON GROWTH EFFICIENCY BODY WEIGHT vs CUMULATIVE NITROGEN RETENTION.**

Mean values shown (Burn group 6 Rats - Control group 4 Rats)



The problems of data presentation in metabolic balance studies are discussed for the 20% BSA burn and the 25% BSA burn.

METABOLIC RESPONSE TO 20% BODY SURFACE AREA FULL SKIN THICKNESS BURN

MATERIALS AND METHODS

Two separate studies were made of the metabolic response to a 20% BSA full skin thickness burn. The first study was made on the single rat subjected to calorimetry. The changes in sensible and insensible heat loss have been presented and discussed earlier, for that animal (Figs. 67, 68 and 69). In this section, the changes in food intake and weight loss after burn injury are presented. This rat was allowed ad libitum diet, but was given only 4 hours per day in which to eat it. This was done to ensure that the rat was at least 12 hours post absorbtive at the start of calorimetry runs. This pattern of feeding was used in all rats subjected to calorimetry in the later 25% BSA burn studies.

In the second study of the metabolic response to a 20% BSA full skin thickness burn, rats were fed ad libitum, without the overnight food restriction used in rats subjected to calorimetry. 10 male semi inbred Wistar rats aged 12 - 14 weeks from the Institute of Physiology closed colony were kept in the environmental chamber at $20^{o}C \pm 0.5^{o}C$ ambient temperature. The diet used was Low Iodine Test Diet from N.B.C. Their

drinking water was supplemented with potassium iodide (5 ug KI per 10 ml). The rats were weighed daily, food intake was measured, and metabolic collections made for urine and faeces as previously described. All rats were kept in the metabolic cages (5 burned and 5 control). The control rats were treated identically with the burned rats, except for the burn injury itself.

The experiment began on February 27th, metabolic collections started on March 4th. Dorsal fur shaving was carried out on March 11th. Rats 1, 2, 3, 8 and 9 were burned on March 19th. Rats 4, 5, 6, 7, 10 were given the same dose of Nembutal anaesthetic (on a weight for weight basis) as the burned rats. The burn group rats were starved for 24 hours on April 2nd, control group rats on April 3rd. Burn group rats were sacrificed on April 17th. Control rats 4, 5, 7 and 10 were removed from the study on April 4th. Control rat 6 was studied until April 17th.

Control rats were fed the mean daily food intake (with a 24 hour delay) of the burned rats, from the day of the burn injury. They were not pair fed prior to that.

The results presented first are the weight changes and food intake of the single rat subjected to

calorimetry runs and overnight food deprivation after a 20% BSA burn injury.

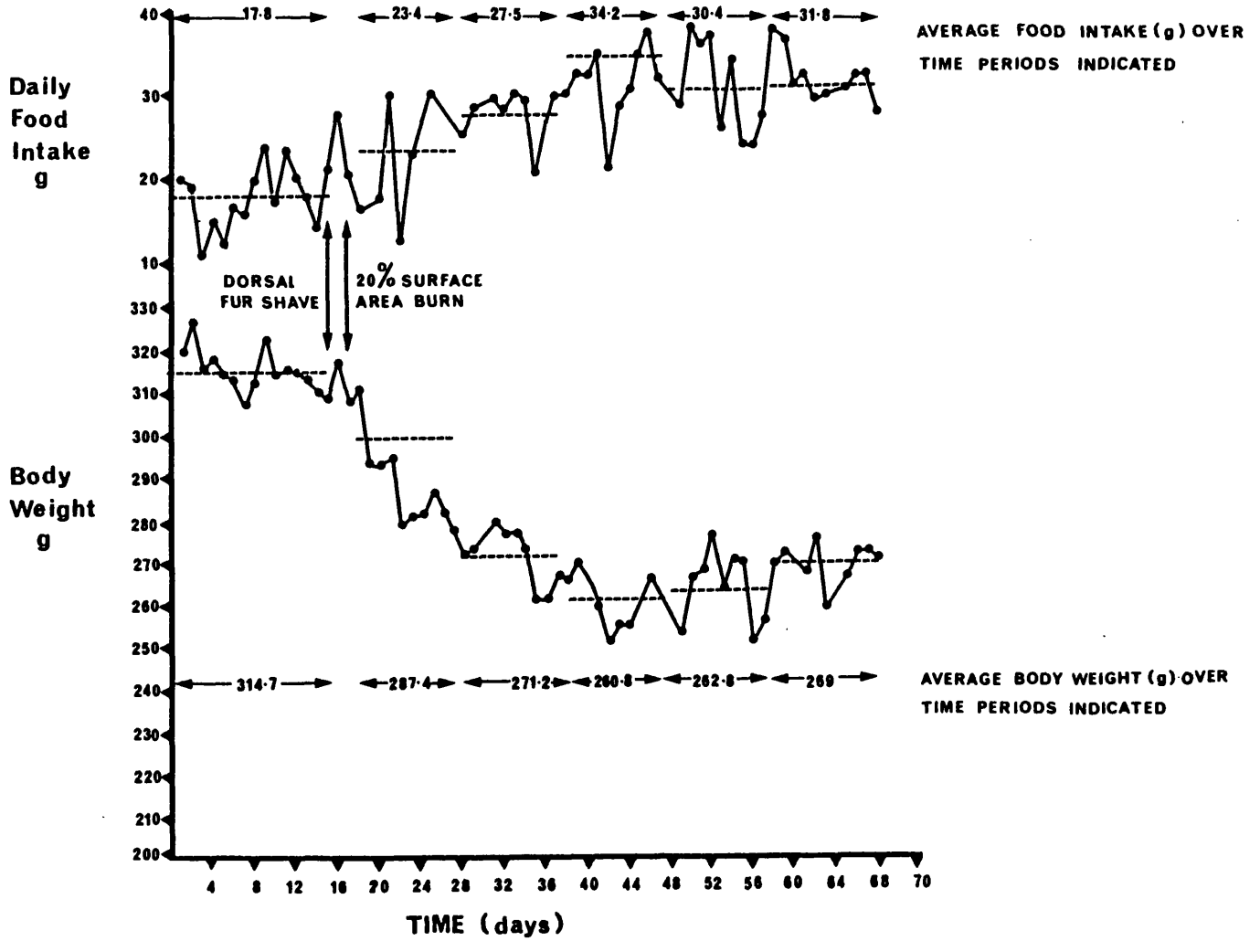
METABOLIC RESPONSE TO 20% BODY SURFACE AREA FULL SKIN THICKNESS BURN

RESULTS

Figure 124 shows the daily food intake and body weight of a single rat subjected to calorimetry and overnight food deprivation before and after a 20% of body surface area full skin thickness dorsal burn. The mean daily food intake in the 14 day period before burn injury was 17.8 g. The food intake was much less than this during the first week, as the food was removed from the cage 4 hours after being supplied. Within 4 days, the rat learned to adapt, eating his food within this period (between 2.00 p.m. to 6.00 p.m. approximately). By the second week, his daily food intake exceeded the mean intake.

Note that with calorimetry runs daily, and this feeding regimen, the rat steadily lost weight over the 14 day period prior to fur shaving and burn injury. This weight loss is in sharp contrast to the steady growth rate of 1.5 - 2 g/day expected in healthy uninjured rats kept in the same conditions and fed the same diet. The rate of weight loss was accelerated after the burn, and was accompanied by a progressive increase in daily food intake. It was noticeable in

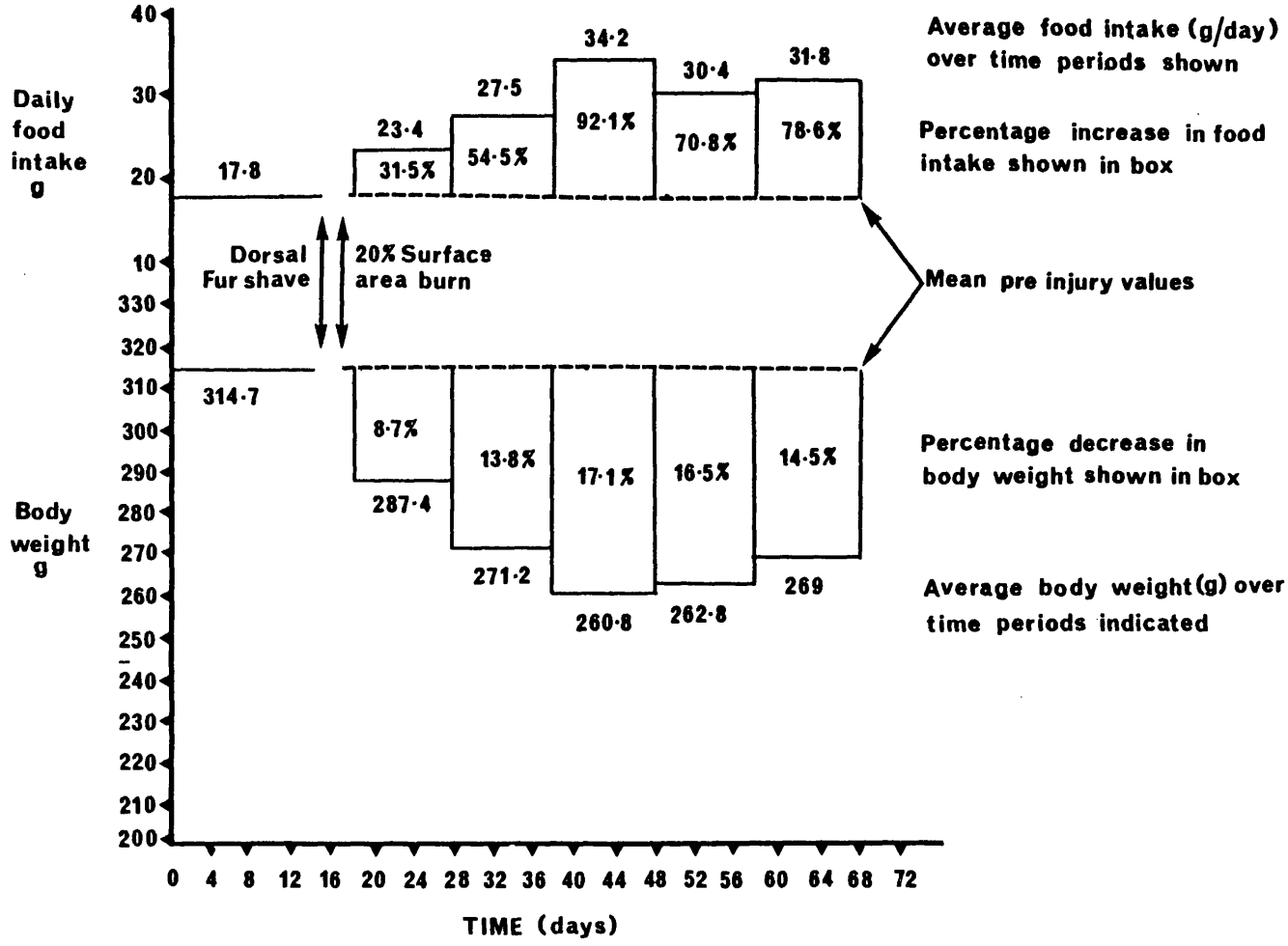
Fig. 124 20% SURFACE AREA BURN: SINGLE RAT SUBJECTED TO CALORIMETRY: FED AD LIBITUM BUT 4HR. PER DAY IN WHICH TO CONSUME FOOD: FOOD INTAKE SHOWN AGAINST BODY WEIGHT CHANGE



this rat that the pattern of weight loss was that of 3 - 4 days sharp drop in weight followed by 3 - 4 days of partial weight recovery. This pattern then repeated itself. The same observation was true, to a lesser extent, of daily food intake. This type of cyclical pattern in growth or weight loss was not an isolated observation peculiar to this rat, but could be seen in individual rat growth rates and food intakes in other studies in the same conditions. Figure 125 shows the same data as Figure 124, but presented semi-diagrammatically. The percentage decrease in body weight is shown over a 50 day period after burning. The percentage increase in food intake is also shown for comparison.

At first sight it appeared that a 20% BSA burn had caused a significant metabolic effect on the rat with up to 17% weight loss after injury. But it is incorrect to attribute all of this weight loss to the burn injury, as the rat was losing weight before the burn was carried out (Fig. 124). It is not possible even to work out a steady rate of weight loss and estimate the weight which the rat would have reached by, e.g. day 40, as conditions prior to the burn were not in a steady state as the rat was learning to adapt to overnight food deprivation and calorimetry simultaneously.

Fig. 125 20% SURFACE AREA BURN: SINGLE RAT SUBJECTED TO CALORIMETRY: FED AD LIBITUM BUT RESTRICTED TO 4 HOUR PERIOD PER DAY IN WHICH TO CONSUME FOOD: FOOD INTAKE SHOWN AGAINST BODY WEIGHT CHANGE – AVERAGE VALUES FOR 10 DAY INTERVALS POST BURN



It did seem that there was a relationship between the extent of the weight lost (whatever its cause) and the rat's attempt to compensate by voluntarily increasing its food intake. By the third 10 day period, the rat's average daily food intake had increased by 92% over the average pre injury intake. The rat ate 34.2 g of powdered diet in less than 4 hours. The injured rat is capable of adjusting its voluntary food intake to overcome the effects of serious burn injury to a far greater extent than is burned man (personal observation). This is true only providing that the rat remains able to feed normally and does not for example have restricted movement due to burns of joints or limbs.

A second 20% BSA burn study was carried out to separate the effects of the burn injury itself from those of the calorimetry heat loss measurements on the burned rat.

Figure 126 shows the individual daily body weights for rats in the burn group before and after thermal injury. The growth rate for each time period shown is given by the slope (m) of the linear regression line drawn through the weight values. The point of interception of the line on the body weight axis is given (c), as is the correlation coefficient of the line (r).

Fig. 126 20% BSA BURN : BURN GROUP : RAT Nos. 1,2,3,8,9

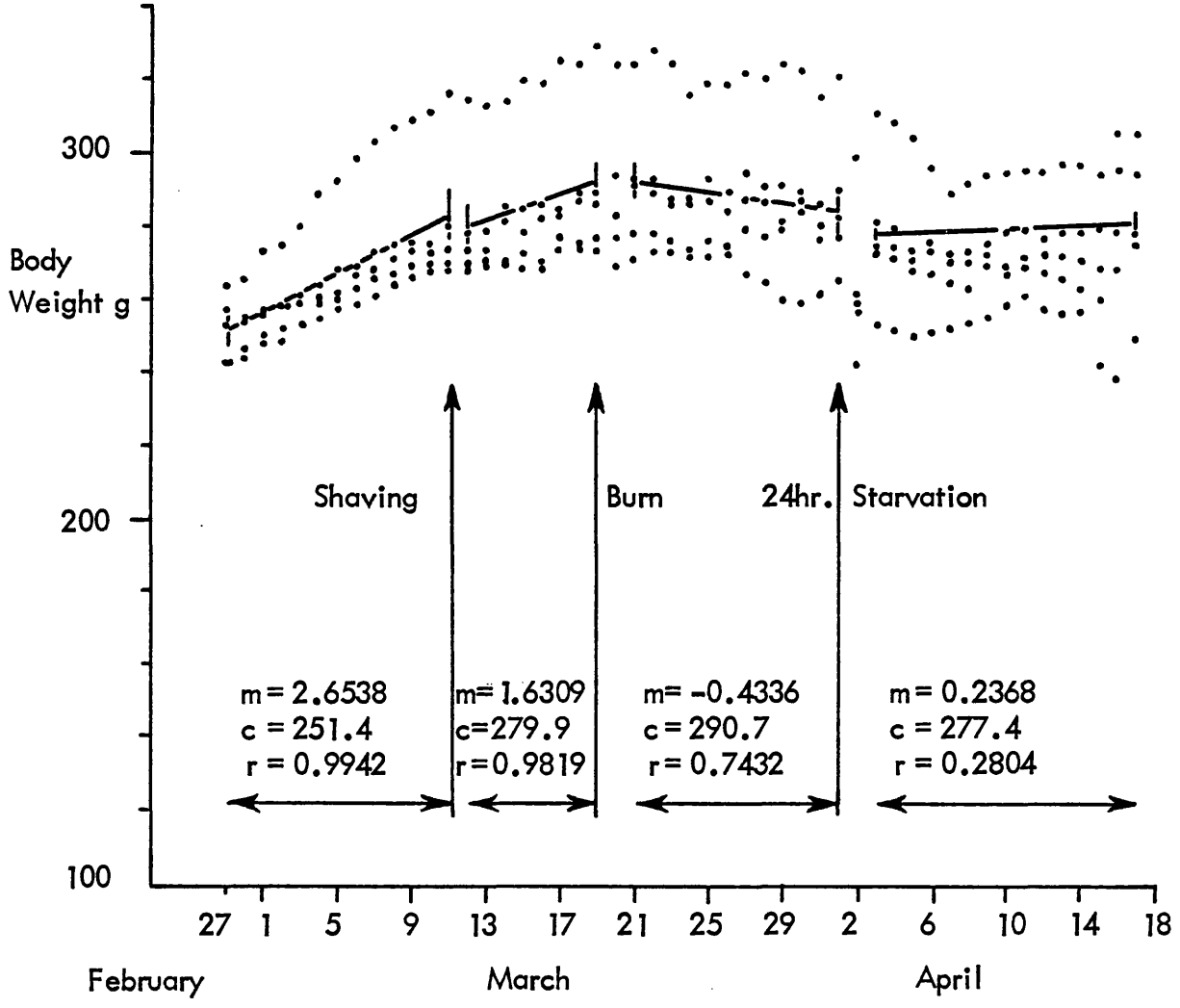


Figure 127 gives the same information as Figure 126 but for the control group rats. Burn and control group rats exhibited steady growth during the period prior to dorsal fur shaving, 2.6 g/day and 1.9 g/day respectively. Both groups suffered a slight decrease in the rate of growth as a result of dorsal fur shaving, to 1.6 g/day in the burn group and 1.4 g/day in the control rats.

Individual rat daily values are given in Tables 39 and 40.

A 20% body surface area full skin thickness burn in rats not subjected to calorimetry runs and fed ad libitum diet, caused only minor post burn weight loss. In the 12 days after burning the mean body weight fell only 7 g (from 291 $\pm$ 22.1 (SD) g to 284 $\pm$ 19.8 (SD) g), or 2.4% of the weight at the day of injury. The rate of weight gain in the control rats was accelerated to 2.7 g/day within a few days of the anaesthetic administration, but because of an initial 2 days weight loss after the anaesthesia and initially decreased food intake, the control rats gained only 8 g overall in the 12 day period after anaesthesia from 283 $\pm$ 24 g to 291 $\pm$ 27.3 g.

Comparing the weight gain in controls with the weight lost in the burn group at the 12th day after

Fig. 127 20% BSA BURN : CONTROL GROUP : RAT Nos. 4,5,6,7,10

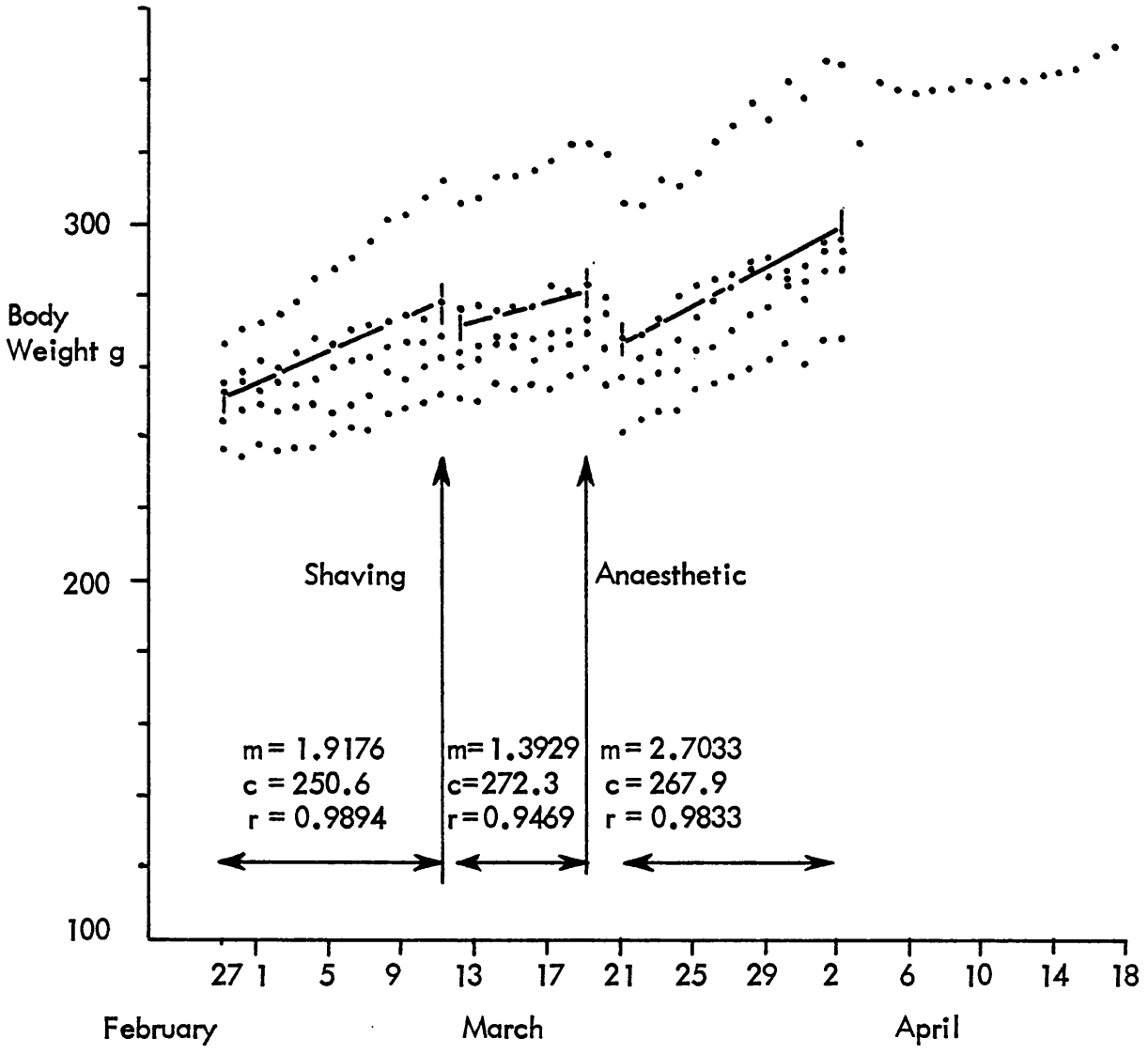


TABLE 39 (1) 20% BODY SURFACE AREA FULL SKIN THICKNESS BURN : DAILY BODY WEIGHT g.

BURN GROUP - RATS 1, 2, 3, 8, 9.

	DATE	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
	27 FEB	264	243	258	243	254	252 ± 9.3
	28	266	247	254	244	255	253 ± 8.5
	1 MARCH	273	250	257	248	256	257 ± 9.8
	2	275	252	258	249	258	258 ± 10.1
	3	280	253	261	253	259	261 ± 11.1
*	4 MC	289	259	260	255	264	265 ± 13.6
	5	293	260	261	258	268	268 ± 14.5
	6	299	263	267	259	269	271 ± 15.9
	7	303	266	268	261	273	274 ± 16.7
	8	307	267	271	264	275	277 ± 17.4
	9	309	266	273	269	275	278 ± 17.5
	10	311	269	273	269	275	279 ± 17.8
*	11 SH	316	270	274	269	280	282 ± 19.6
	12	314	268	273	269	278	280 ± 19.2
	13	313	270	274	270	279	281 ± 18.2
	14	314	270	281	270	286	284 ± 18.1
	15	320	273	278	268	285	285 ± 20.7
	16	319	270	282	269	286	285 ± 20.3
	17	325	277	283	274	284	289 ± 20.8
	18	324	275	287	274	289	290 ± 20.3
*	19 B	329	277	286	274	289	291 ± 22.1
	20	324	277	283	269	294	289 ± 21.4
	21	324	278	291	271	292	291 ± 20.4
	22	328	278	289	273	293	292 ± 21.6
	23	324	276	286	273	286	289 ± 20.4

TABLE 39 (2) 20% BODY SURFACE AREA FULL SKIN THICKNESS

BURN : DAILY BODY WEIGHT g.

BURN GROUP - RATS 1, 2, 3, 8, 9.

DATE	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
24	316	273	286	272	287	287 ± 17.8
25	319	276	293	272	287	289 ± 18.6
26	319	273	289	273	284	288 ± 18.9
27	322	279	295	267	287	290 ± 20.7
28	320	277	291	265	286	288 ± 20.5
29	324	281	280	260	291	287 ± 23.4
30	322	287	289	259	284	288 ± 22.4
31	315	276	280	261	286	284 ± 19.8
1 APRIL	321	282	290	265	277	287 ± 21.1
* 2 ST	299	257	261	242	259	264 ± 21.2
3	311	274	281	253	274	279 ± 21.0
4	308	271	279	252	274	277 ± 20.2
5	304	268	274	250	271	273 ± 19.5
6	296	267	275	251	274	273 ± 16.2
7	289	264	270	252	272	269 ± 13.4
8	292	263	270	254	273	270 ± 14.1
9	294	269	272	255	275	273 ± 14.0
10	295	267	278	259	268	273 ± 13.8
11	295	269	279	261	271	275 ± 12.9
12	295	267	276	257	272	273 ± 14.0
13	297	265	278	256	271	273 ± 15.5
14	297	263	278	257	270	273 ± 15.5
15	294	260	279	242	268	269 ± 19.6
16	305	268	295	238	278	277 ± 26.0
17	305	275	294	249	277	280 ± 21.3

TABLE 40 (1) 20% BODY SURFACE AREA BURN STUDY :

DAILY BODY WEIGHT g.

CONTROL GROUP RATS - 4, 5, 6, 7, 10.

	DATE	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
	27 FEB	245	253	267	237	256	252 ± 11.4
	28	248	256	271	235	259	254 ± 13.4
	1 MARCH	250	254	273	238	262	255 ± 13.1
	2	248	256	275	237	260	255 ± 14.1
	3	249	256	279	238	264	257 ± 15.5
*	4 MC	250	257	285	238	269	260 ± 18.0
	5	247	260	288	242	267	261 ± 18.1
	6	250	262	291	243	271	263 ± 18.8
	7	252	263	296	243	272	265 ± 20.4
	8	259	266	302	247	273	269 ± 20.6
	9	257	267	303	249	275	270 ± 21.0
	10	260	267	308	250	274	272 ± 22.1
*	11 SH	263	269	313	253	279	275 ± 23.0
	12	261	265	307	252	271	272 ± 21.3
	13	263	266	308	251	278	273 ± 21.7
	14	269	268	314	256	277	277 ± 22.1
	15	269	267	314	255	278	277 ± 22.5
	16	269	263	316	256	278	276 ± 23.6
	17	270	267	319	255	284	279 ± 24.6
	18	267	271	323	259	282	280 ± 25.2
*	19 AN	274	271	323	261	284	283 ± 24.0
	20	276	266	320	256	281	280 ± 24.4
	21	269	258	307	243	268	269 ± 23.7
	22	270	257	307	246	264	269 ± 23.1
	23	275	260	313	249	265	272 ± 24.6
	24	281	260	312	249	269	274 ± 24.2

TABLE 40 (2) 20% BODY SURFACE AREA BURN STUDY :

DAILY BODY WEIGHT g.

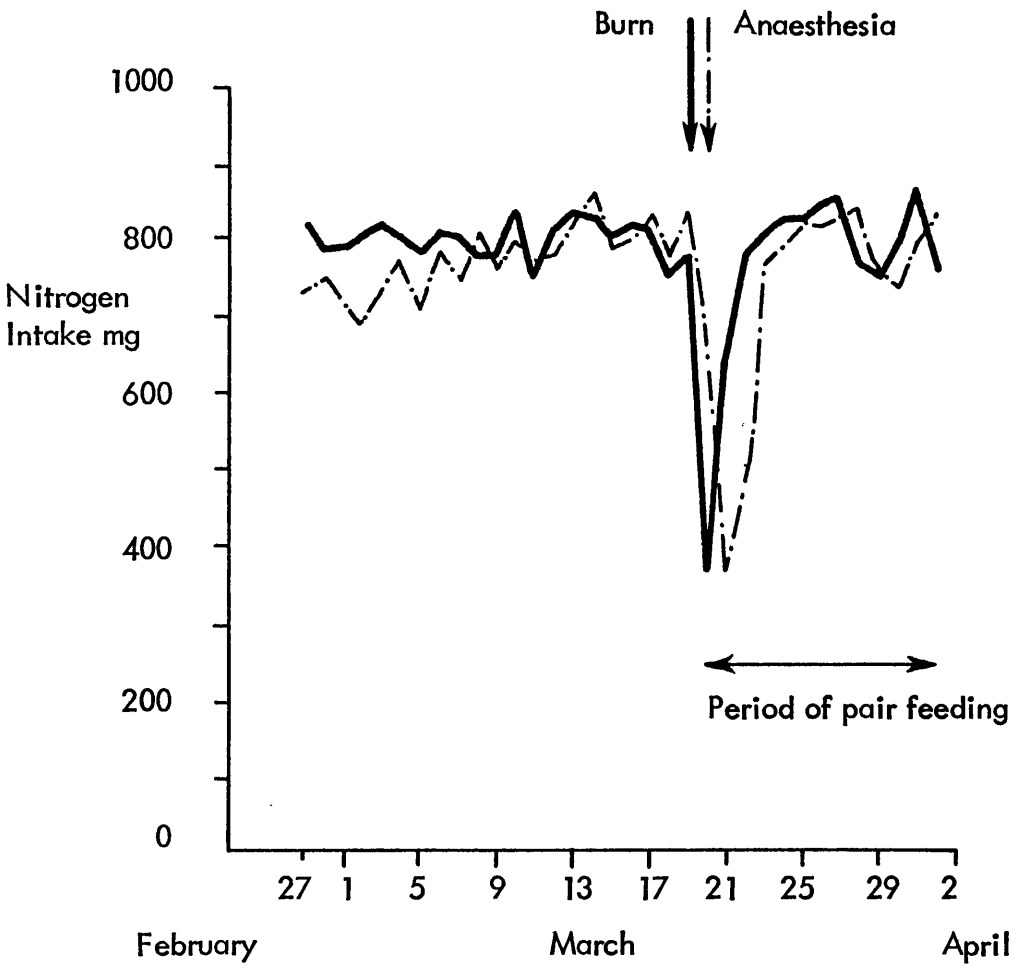
CONTROL GROUP RATS - 4, 5, 6, 7, 10.

DATE	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
25	284	266	316	255	275	279 ± 23.2
26	286	267	324	257	279	283 ± 25.7
27	287	271	329	259	284	286 ± 26.5
28	289	276	335	261	290	290 ± 27.7
29	287	278	330	264	292	290 ± 24.7
30	286	285	341	268	287	293 ± 27.7
31	285	280	336	263	289	291 ± 27.3
1 APRIL	296	288	347	269	295	299 ± 28.9
* 2 ST	294	289	346	269	297	299 ± 28.5
3			324			
4			341			
5			339			
6			338			
7			339			
8			339			
9			341			
10			340			
11			342			
12			342			
13			343			
14			344			
15			345			
16			349			
17			351			

injury, the mean difference was 5%. By any classification the 20% BSA full skin thickness dorsal burn produced only trivial weight loss after injury. Nor was there evidence of increased voluntary food intake in the burned rats after injury as was the case in the single rat subjected to calorimetry runs after an identical 20% BSA burn injury (Fig. 125). Figure 128 shows the mean daily dietary intake (as nitrogen) for burn and control rat groups. Individual daily values for nitrogen balance are given in Tables 41 and 42.

There was no significant difference in mean daily food nitrogen intake between before and after a 20% BSA burn, nor between before and after the control anaesthetic administration. Group pair feeding proved an effective means of ensuring similar dietary intakes in burn and control rat groups. Though not pair fed prior to the burn/anaesthetic, there is little difference in the pattern of food ingestion between burn and control group rats during the period March 12th to March 19th. During the period February 27th to March 11th control rats ate less than burn rats, were lighter in weight and grew at a slower rate than burn rats. Figure 129 shows the daily mean differences in nitrogen losses between burn and control group rats before and after a 20% BSA burn. (Nitrogen losses are given as urinary nitrogen excretion + 5%). This form of graphical representation was used as I believe it is

Fig.128 20% BSA BURN : EFFECT OF PAIR FEEDING : COMPARISON OF NITROGEN INTAKE BETWEEN BURN AND CONTROL RAT GROUPS



Burn Group
Nos. 1,2,3,8,9

Pre burn mean intake	Post burn intake
804.19 ± 23.04	794.33 ± 61.10
D 27/2 - 19/3 incl.	D 21/3 - 1/4 incl.

Control Group
Nos. 4,5,6,7,10

Pre anaes. mean intake	Post anaes. intake
777.81 ± 44.63	771.83 ± 90.52
D 27/2 - 19/3 incl.	D 22/3 - 2/4 incl.

TABLE 41 A (1) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

PRE FUR SHAVING PERIOD

DAY 27 FEB	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg.	1088	703	788	736	795	822 ± 154
URINE N. (+ 5%) mg.	619	445	436	443	523	501 ± 73
N. BALANCE mg.	469	258	312	293	272	321 ± 85
DAY 28 FEB						
FOOD N. mg.	847	766	718	781	855	793 ± 58
URINE N. (+ 5%) mg.	550	448	476	501	387	472 ± 61
N. BALANCE mg.	297	318	242	280	468	321 ± 87
DAY 1 MAR						
FOOD N. mg.	884	781	781	770	747	793 ± 53
URINE N. (+ 5%) mg.	580	499	538	479	539	527 ± 39
N. BALANCE mg.	304	282	243	291	208	266 ± 40
DAY 2 MAR						
FOOD N. mg.	903	747	825	747	803	805 ± 65
URINE N. (+ 5%) mg.	623	535	658	504	454	555 ± 84
N. BALANCE mg.	280	212	167	243	349	250 ± 69

TABLE 41 A (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

PRE FUR SHAVING PERIOD

DAY 3 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg.	895	784	785	814	773	811 ± 50
URINE N. (+ 5%) mg.	589	559	517	566	412	529 ± 70
N. BALANCE mg.	306	225	268	248	361	282 ± 50
DAY 4 MAR						
FOOD N. mg.	906	777	673	892	777	805 ± 96
URINE N. (+ 5%) mg.	563	459	525	392	470	482 ± 66
N. BALANCE mg.	343	318	148	500	307	323 ± 125
DAY 5 MAR						
FOOD N. mg.	910	758	725	762	777	786 ± 72
URINE N. (+ 5%) mg.	692	463	489	499	458	520 ± 98
N. BALANCE mg.	218	295	236	263	319	266 ± 41
DAY 6 MAR						
FOOD N. mg.	973	747	832	762	755	814 ± 95
URINE N. (+ 5%) mg.	617	426	455	469	477	489 ± 74
N. BALANCE mg.	356	321	377	293	278	325 ± 40

TABLE 41 A (3) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

PRE FUR SHAVING PERIOD

DAY 7 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	906	758	755	799	814	806 ± 61
URINE N. (+ 5%) mg	585	504	459	455	498	500 ± 52
N. BALANCE mg	321	254	296	344	316	306 ± 34
DAY 8 MAR						
FOOD N. mg	903	721	788	740	770	784 ± 71
URINE N. (+ 5%) mg	665	517	551	529	584	569 ± 59
N. BALANCE mg	238	204	237	211	186	215 ± 22
DAY 9 MAR						
FOOD N. mg	829	725	766	884	718	784 ± 71
URINE N. (+ 5%) mg	654	466	551	573	526	554 ± 69
N. BALANCE mg	175	259	215	311	192	230 ± 55
DAY 10 MAR						
FOOD N. mg.	992	821	747	795	832	837 ± 92
URINE N. (+ 5%) mg	635	612	605	603	587	608 ± 17
N. BALANCE mg	357	209	142	192	245	229 ± 80

TABLE 41 A (4) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

PRE FUR SHAVING PERIOD

DAY 11 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg.	844	740	681	744	814	765 ± 65
URINE N. (+ 5%) mg	509	428	581	457	505	496 ± 58
N. BALANCE mg	335	312	100	287	309	269 ± 96

The metabolic collections made on the morning of, e.g. day 28, are those collected over the preceding 24 hours, i.e. day 27-28. For convenience this collection period is labelled day 28.

TABLE 41 B (1) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

PRE BURN PERIOD

DAY 12 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	832	784	855	762	825	812 ± 38
URINE N. (+ 5%) mg	586	522	468	636	571	557 ± 64
N. BALANCE mg	246	262	387	126	254	255 ± 92
DAY 13 MAR						
FOOD N. mg	873	803	751	899	862	838 ± 60
URINE N. (+ 5%) mg	627	533	616	605	550	586 ± 42
N. BALANCE mg	246	270	135	294	312	252 ± 70
DAY 14 MAR						
FOOD N. mg	866	784	855	795	892	838 ± 47
URINE N. (+ 5%) mg	501	586	635	617	567	581 ± 52
N. BALANCE mg.	365	198	220	178	325	257 ± 83
DAY 15 MAR						
FOOD N. mg	955	836	729	770	729	804 ± 95
URINE N. (+ 5%) mg	514	467	570	518	564	527 ± 42
N. BALANCE mg	441	369	159	252	165	277 ± 124

TABLE 41 B (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

PRE BURN PERIOD

DAY 16 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	862	810	862	758	825	823 ± 43
URINE N. (+ 5%) mg	555	598	601	551	579	577 ± 23
N. BALANCE mg	307	212	261	207	246	246 ± 41
DAY 17 MAR						
FOOD N. mg	951	810	744	847	740	818 ± 87
URINE N. (+ 5%) mg	573	575	588	595	630	592 ± 23
N. BALANCE mg	378	235	156	252	110	226 ± 102
DAY 18 MAR						
FOOD N. mg	736	688	758	814	773	754 ± 47
URINE N. (+ 5%) mg	536	394	582	582	551	529 ± 78
N. BALANCE mg	200	294	176	232	222	225 ± 44
DAY 19 MAR						
FOOD N. mg	862	792	651	788	814	781 ± 79
URINE N. (+ 5%) mg	594	544	531	560	613	568 ± 34
N. BALANCE mg	268	248	120	228	201	213 ± 58

TABLE 41 C (1) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

POST BURN PERIOD

DAY 20 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	403	396	303	514	233	370 ± 107
URINE N. (+ 5%) mg	567	567	420	471	513	508 ± 63
N. BALANCE mg	-164	-171	-117	43	-270	-138 ± 114
DAY 21 MAR						
FOOD N. mg	670	599	566	666	677	636 ± 50
URINE N. (+ 5%) mg	578	596	520	646	647	597 ± 53
N. BALANCE mg	92	3	46	20	30	39 ± 33
DAY 22 MAR						
FOOD N. mg	858	784	622	884	784	786 ± 102
URINE N. (+ 5%) mg	706	731	703	761	661	712 ± 37
N. BALANCE mg	152	53	-81	123	123	74 ± 94
DAY 23 MAR						
FOOD N. mg	844	773	788	895	710	802 ± 71
URINE N. (+ 5%) mg	742	665	636	505	607	631 ± 87
N. BALANCE mg	102	108	152	390	103	171 ± 124

TABLE 41 C (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

POST BURN PERIOD

DAY 24 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	818	755	862	844	847	825 ± 42
URINE N. (+ 5%) mg	780	653	493	663	640	646 ± 102
N. BALANCE mg	38	102	369	181	207	179 ± 125
DAY 25 MAR						
FOOD N. mg	951	751	858	773	818	830 ± 79
URINE N. (+ 5%) mg	653	611	523	453	655	579 ± 88
N. BALANCE mg	298	140	335	320	163	251 ± 92
DAY 26 MAR						
FOOD N. mg	914	799	862	829	821	845 ± 45
URINE N. (+ 5%) mg	694	644	708	549	587	636 ± 68
N. BALANCE mg	220	155	154	280	234	209 ± 54
DAY 27 MAR						
FOOD N. mg	914	873	877	770	829	853 ± 55
URINE N. (+ 5%) mg	354	601	675	597	647	575 ± 128
N. BALANCE mg	560	272	202	173	182	278 ± 162

TABLE 41 C (3) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

POST BURN PERIOD

DAY 28 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	740	807	858	677	799	776 ± 70
URINE N. (+ 5%) mg	685	653	630	583	565	623 ± 49
N. BALANCE mg	55	154	228	94	234	153 ± 79
DAY 29 MAR						
FOOD N. mg	973	795	440	710	840	752 ± 199
URINE N. (+ 5%) mg	608	601	501	659	681	610 ± 70
N. BALANCE mg	365	194	-61	51	159	142 ± 160
DAY 30 MAR						
FOOD N. mg	803	825	855	810	696	798 ± 60
URINE N. (+ 5%) mg	824	561	737	653	628	681 ± 102
N. BALANCE mg	-21	264	118	157	68	117 ± 106

TABLE 41 C (4) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

POST BURN PERIOD

DAY 31 MAR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	1036	795	714	980	788	863 ± 138
URINE N. (+ 5%) mg	833	623	594	802	736	717 ± 106
N. BALANCE mg	203	172	120	178	52	146 ± 60
DAY 1 APRIL						
FOOD N. mg	965	729	832	744	659	766 ± 83
URINE N. (+ 5%) mg	670	488	504	587	475	545 ± 82
N. BALANCE mg	195	241	328	157	184	221 ± 67

TABLE 41 D (1) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

LATE POST BURN PERIOD

DAY 2 APR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	0	0	0	0	0	0
URINE N. (+ 5%) mg	246	372	489	351	367	365 ± 86
N. BALANCE mg	-246	-372	-489	-351	-367	-365 ± 86
DAY 3 APR						
FOOD N. mg	1110	1109	1100	1075	1002	1079 ± 45
URINE N. (+ 5%) mg	827	715	817	826	759	789 ± 50
N. BALANCE mg	283	394	283	249	243	290 ± 61
DAY 4 APR						
FOOD N. mg	807	633	800	875	745	772 ± 90
URINE N. (+ 5%) mg	704	554	567	596	652	615 ± 63
N. BALANCE mg	103	79	233	279	93	157 ± 92
DAY 5 APR						
FOOD N. mg	752	792	725	796	822	777 ± 39
URINE N. (+ 5%) mg	677	680	607	661	468	619 ± 89
N. BALANCE mg	75	112	118	135	354	158 ± 111

TABLE 41 D (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

LATE POST BURN PERIOD

DAY 6 APR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	564	733	706	866	830	740 ± 119
URINE N. (+ 5%) mg	583	665	572	713	492	645 ± 64
N. BALANCE mg	-19	68	134	153	138	95 ± 71
DAY 7 APR						
FOOD N. mg	548	704	790	679	760	696 ± 94
URINE N. (+ 5%) mg	438	544	657	666	688	599 ± 106
N. BALANCE mg	110	160	133	13	72	98 ± 57
DAY 8 APR						
FOOD N. mg	681	680	616	800	785	712 ± 78
URINE N. (+ 5%) mg	679	576	580	588	438	572 ± 86
N. BALANCE mg	2	104	36	212	347	140 ± 141
DAY 9 APR						
FOOD N. mg	790	794	704	796	750	767 ± 40
URINE N. (+ 5%) mg	679	462	696	566	514	583 ± 102
N. BALANCE mg	111	332	8	230	236	184 ± 126

TABLE 41 D (3) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

LATE POST BURN PERIOD

DAY 10 APR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	730	670	825	845	675	749 ± 82
URINE N. (+ 5%) mg	678	639	560	689	665	646 ± 52
N. BALANCE mg	52	31	265	156	10	103 ± 107
DAY 11 APR						
FOOD N. mg	740	785	765	809	760	772 ± 26
URINE N. (+ 5%) mg	542	647	598	618	677	616 ± 51
N. BALANCE mg	198	138	167	191	83	156 ± 47
DAY 12 APR						
FOOD N. mg	848	751	740	734	819	778 ± 52
URINE N. (+ 5%) mg	562	574	550	639	665	598 ± 51
N. BALANCE mg	286	177	190	95	154	180 ± 69
DAY 13 APR						
FOOD N. mg	763	674	892	660	785	755 ± 94
URINE N. (+ 5%) mg	682	463	630	573	615	593 ± 82
N. BALANCE mg	81	211	262	87	170	162 ± 79

TABLE 41 D (4) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

BURN GROUP - RATS 1, 2, 3, 8, 9.

LATE POST BURN PERIOD

DAY 14 APR	RAT 1	RAT 2	RAT 3	RAT 8	RAT 9	MEAN ± SD
FOOD N. mg	860	805	822	740	810	807 ± 43
URINE N. (+ 5%) mg	682	463	630	573	615	593 ± 82
N. BALANCE mg	178	342	192	167	195	214 ± 72
DAY 15 APR						
FOOD N. mg	819	618	819	248	736	648 ± 238
URINE N. (+ 5%) mg	536	412	600	436	317	460 ± 110
N. BALANCE mg	283	206	219	-188	419	188 ± 226
DAY 16 APR						
FOOD N. mg	800	815	885	332	736	714 ± 220
URINE N. (+ 5%) mg	536	412	600	436	317	460 ± 110
N. BALANCE mg	264	403	285	-104	419	254 ± 211

TABLE 42 A (1) NITROGEN BALANCE METABOLIC CAGE RATS ; 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

PRE FUR SHAVING PERIOD

DAY 27 FEB	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	636	836	803	677	751	741 ± 84
URINE N. (+ 5%) mg	422	526	419	390	307	413 ± 79
N. BALANCE mg	214	310	384	287	444	328 ± 89
DAY 28 FEB						
FOOD N. mg	666	818	836	603	855	755 ± 114
URINE N. (+ 5%) mg	400	450	562	390	241	409 ± 116
N. BALANCE mg	266	368	274	213	614	346 ± 159
DAY 1 MAR						
FOOD N. mg	699	710	755	670	718	710 ± 31
URINE N. (+ 5%) mg	405	520	539	387	188	408 ± 140
N. BALANCE mg	294	190	216	283	530	302 ± 135
DAY 2 MAR						
FOOD N. mg	677	766	799	566	670	696 ± 91
URINE N. (+ 5%) mg	556	563	592	457	419	517 ± 75
N. BALANCE mg	121	203	207	109	251	179 ± 61

TABLE 42 A (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

PRE FUR SHAVING PERIOD

DAY 3 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	673	744	803	692	755	733 ± 52
URINE N. (+ 5%) mg	546	532	591	422	365	491 ± 94
N. BALANCE mg	127	212	212	270	390	242 ± 97
DAY 4 MAR						
FOOD N. mg	659	729	925	692	851	771 ± 113
URINE N. (+ 5%) mg	461	249	583	397	382	414 ± 122
N. BALANCE mg	198	480	342	295	469	357 ± 119
DAY 5 MAR						
FOOD N. mg	581	799	807	703	670	712 ± 94
URINE N. (+ 5%) mg	499	331	560	486	352	446 ± 99
N. BALANCE mg	82	468	247	217	318	266 ± 142
DAY 6 MAR						
FOOD N. mg	818	773	844	710	803	790 ± 51
URINE N. (+ 5%) mg	535	517	430	400	278	432 ± 103
N. BALANCE mg	283	256	414	310	525	358 ± 111

TABLE 42 A (3) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

PRE FUR SHAVING PERIOD

DAY 7 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	585	762	910	681	807	749 ± 123
URINE N. (+ 5%) mg	447	438	566	415	323	438 ± 87
N. BALANCE mg	138	324	344	266	484	311 ± 126
DAY 8 MAR						
FOOD N. mg	807	795	899	751	807	812 ± 54
URINE N. (+ 5%) mg	567	346	469	422	453	451 ± 80
N. BALANCE mg	240	449	430	329	354	361 ± 84
DAY 9 MAR						
FOOD N. mg	659	784	862	777	762	769 ± 73
URINE N. (+ 5%) mg	518	489	638	558	365	514 ± 100
N. BALANCE mg	141	295	224	219	397	255 ± 96
DAY 10 MAR						
FOOD N. mg	777	829	840	777	784	801 ± 31
URINE N. (+ 5%) mg	601	450	652	404	485	518 ± 104
N. BALANCE mg	176	379	188	373	299	283 ± 97

TABLE 42 A (4) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

PRE FUR SHAVING PERIOD

DAY 11 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	655	803	918	718	770	773 ± 99
URINE N. (+ 5%) mg	475	492	574	538	331	482 ± 93
N. BALANCE mg	180	311	344	180	439	291 ± 112

TABLE 42 B (1) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUPS - RATS 4, 5, 6, 7, 10.

PRE ANAESTHETIC PERIOD

DAY 12 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	707	788	781	873	777	785 ± 59
URINE N. (+ 5%) mg	485	559	652	542	244	496 ± 153
N. BALANCE mg	222	229	129	331	533	289 ± 154
DAY 13 MAR						
FOOD N. mg	807	847	847	773	814	818 ± 31
URINE N. (+ 5%) mg	630	650	743	663	379	613 ± 138
N. BALANCE mg	177	197	104	110	435	205 ± 135
DAY 14 MAR						
FOOD N. mg	873	807	995	884	744	861 ± 94
URINE N. (+ 5%) mg	574	355	654	628	535	549 ± 118
BALANCE N. mg	299	452	341	256	209	312 ± 93
DAY 15 MAR						
FOOD N. mg	736	792	873	762	807	794 ± 52
URINE N. (+ 5%) mg	575	462	560	518	535	530 ± 44
N. BALANCE mg	161	330	313	244	272	264 ± 67

TABLE 42 B (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

PRE ANAESTHETIC PERIOD

DAY 16 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	784	781	914	758	795	806 ± 62
URINE N. (+ 5%) mg	601	543	689	533	246	522 ± 166
N. BALANCE mg	183	238	225	225	549	284 ± 150
DAY 17 MAR						
FOOD N. mg	740	844	973	744	783	835 ± 97
URINE N. (+ 5%) mg	570	474	595	571	365	515 ± 96
N. BALANCE mg	170	370	378	173	508	320 ± 146
DAY 18 MAR						
FOOD N. mg	614	844	925	773	733	779 ± 117
URINE N. (+ 5%) mg	542	426	660	554	235	483 ± 162
N. BALANCE mg	72	418	265	219	498	296 ± 168
DAY 19 MAR						
FOOD N. mg	814	825	921	814	851	845 ± 45
URINE N. (+ 5%) mg	589	482	728	625	275	540 ± 172
N. BALANCE mg	225	343	193	189	576	305 ± 164

TABLE 42 C (1) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

POST ANAESTHETIC PERIOD

DAY 20 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	747	640	770	548	607	662 ± 94
URINE N. (+ 5%) mg	566	547	573	645	359	538 ± 107
N. BALANCE mg	181	93	197	-97	248	124 ± 136
DAY 21 MAR						
FOOD N. mg	514	392	403	233	296	368 ± 108
URINE N. (+ 5%) mg	531	450	450	440	398	454 ± 48
N. BALANCE mg	-17	-58	-47	-207	-102	-86 ± 74
DAY 22 MAR						
FOOD N. mg	662	590	670	677	548	629 ± 57
URINE N. (+ 5%) mg	642	565	668	626	499	600 ± 68
N. BALANCE mg	20	25	2	51	49	29 ± 21
DAY 23 MAR						
FOOD N. mg	884	781	858	784	607	783 ± 108
URINE N. (+ 5%) mg	671	548	738	589	521	613 ± 90
N. BALANCE mg	213	233	120	195	86	170 ± 63

TABLE 42 C (2) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

POST ANAESTHETIC PERIOD

DAY 24 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	888	770	844	710	770	796 ± 70
URINE N. (+ 5%) mg	596	556	546	566	462	545 ± 50
N. BALANCE mg	292	214	298	144	308	251 ± 70
DAY 25 MAR						
FOOD N. mg	817	751	848	847	844	821 ± 41
URINE N. (+ 5%) mg	540	501	629	572	598	568 ± 50
N. BALANCE mg	277	250	219	275	246	253 ± 24
DAY 26 MAR						
FOOD N. mg	755	744	947	818	836	820 ± 81
URINE N. (+ 5%) mg	542	622	672	611	324	554 ± 137
N. BALANCE mg	213	122	275	207	512	266 ± 148
DAY 27 MAR						
FOOD N. mg	781	795	914	821	844	831 ± 52
URINE N. (+ 5%) mg	543	544	799	625	373	577 ± 154
N. BALANCE mg	238	251	115	196	471	254 ± 132

TABLE 42 C (3) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

POST ANAESTHETIC PERIOD

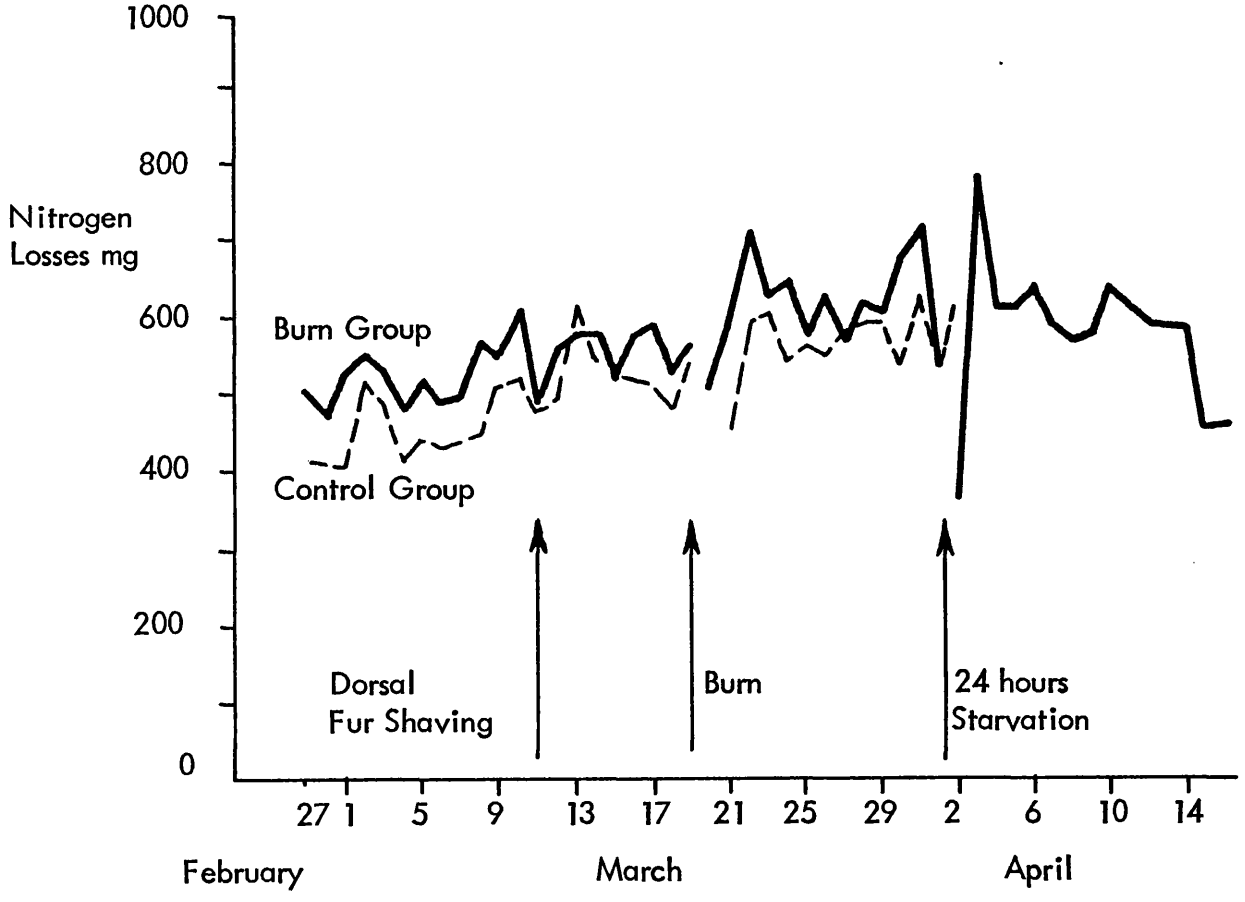
DAY 28 MAR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	755	869	910	829	851	843 ± 57
URINE N. (+ 5%) mg	614	572	709	546	546	597 ± 68
N. BALANCE mg	141	297	201	283	305	246 ± 72
DAY 29 MAR						
FOOD N. mg	673	803	740	799	840	771 ± 65
URINE N. (+ 5%) mg	604	690	495	671	503	593 ± 91
N. BALANCE mg	69	113	245	128	337	178 ± 110
DAY 30 MAR						
FOOD N. mg	710	777	973	799	433	738 ± 196
URINE N. (+ 5%) mg	593	565	655	561	311	537 ± 132
N. BALANCE mg	117	212	318	238	122	201 ± 84
DAY 31 MAR						
FOOD N. mg	807	814	795	710	830	791 ± 47
URINE N. (+ 5%) mg	776	684	613	682	406	632 ± 139
N. BALANCE mg	31	130	182	28	424	159 ± 162

TABLE 42 C (4) NITROGEN BALANCE METABOLIC CAGE RATS : 20% BODY SURFACE AREA BURN STUDY CONTROL GROUP - RATS 4, 5, 6, 7, 10.

POST ANAESTHETIC PERIOD

DAY 1 APR	RAT 4	RAT 5	RAT 6	RAT 7	RAT 10	MEAN ± SD
FOOD N. mg	847	795	999	788	707	827 ± 108
URINE N. (+ 5%) mg	592	560	580	552	429	543 ± 65
DAY 2 APR						
FOOD N. mg	655	733	840	630	812	734 ± 93
URINE N. (+ 5%) mg	610	625	669	618	571	619 ± 35
N. BALANCE mg	45	108	171	12	241	115 ± 93

Fig. 129 20% BSA BURN : NITROGEN LOSSES : BURN GROUP - RAT Nos. 1,2,3,8,9 vs. CONTROL GROUP - RATS Nos 4,5,6,7,10



Burn GP
Days 27/2 -11/3 inc. 528 ± 41
Days 12/3 - 19/3 inc. 565 ± 25
Days 21/3 - 1/4 inc. 629 ± 54

Control GP
Days 27/2 - 11/3 inc. 456 ± 43
Days 12/3 - 19/3 inc. 531 ± 40
Days 22/3 - 2/4 inc. 581 ± 32

easier to see differences between superimposed data than with the bar histogram presentation used in the description of 5% BSA burn results in the previous section.

Prior to dorsal fur shaving the control rats excreted less nitrogen than the burn group rats. This is consistent with the differences in weight and food intake between the groups, which have already been noted. Both groups showed an increase in nitrogen excretion after dorsal fur shaving (See Table 43).

In Figure 129, the burn group rats were deprived of food to assess the effect of 24 hours starvation between 1st and 2nd April. The mean body weight of the group on April 1st was 287 ± 21 (SD) g, and on April 2nd it was 264 ± 21 (SD) g. The mean difference of 13 g agrees well with the weight of food normally eaten by the rat, which would of course not be present in the starved rats' alimentary tract. 24 hours starvation had little appreciable effect, the rats compensated for it in a normal way by increasing their mean nitrogen food intake over the next 24 hours, to 1079 mg or 29.2 g of diet. Food intake was back to normal values (mean food nitrogen intake 772 mg ot 20.9 g diet) by the day after that.

TABLE 43 A MEAN DAILY NITROGEN BALANCE (± SD) METABOLIC CAGE RATS - 20% BODY SURFACE AREA BURN

BURN GROUP - RATS 1, 2, 3, 8, 9.

	MEAN DAILY FOOD N. mg	% CHANGE FROM "BASAL"	MEAN DAILY N. EXCRETION	% CHANGE FROM "BASAL"	MEAN DAILY BALANCE mg	% CHANGE FROM "BASAL"
DAY 27/2 to DAY 11/3	802 ± 76	"BASAL"	528 ± 41	"BASAL"	+277 ± 74	"BASAL"
DAY 12/3 to DAY 19/3	809 ± 65	+ 1%	565 ± 25	+ 7%	+244 ± 77	- 11.9%
DAY 21/3 to DAY 1/4	794 ± 103	- 1%	629 ± 54	+ 19.1%	+161 ± 112	- 42%

TABLE 43 B MEAN DAILY NITROGEN BALANCE (± SD) METABOLIC CAGE RATS - 20% BODY SURFACE AREA BURN

CONTROL GROUP - RATS 4, 5, 6, 7, 10.

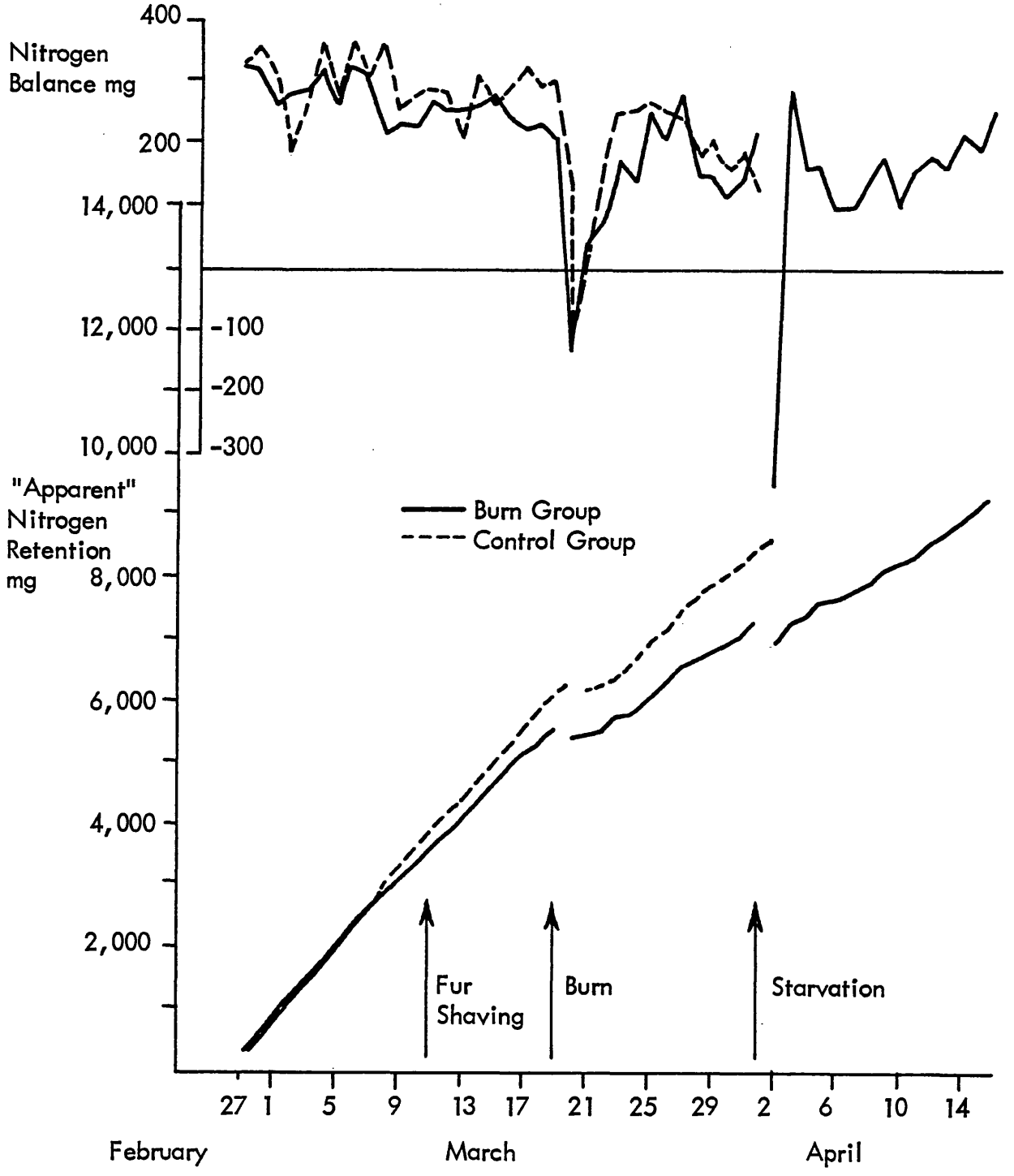
	MEAN DAILY FOOD N. mg.	% CHANGE FROM "BASAL"	MEAN DAILY N. EXCRETION	% CHANGE FROM "BASAL"	MEAN DAILY BALANCE mg.	% CHANGE FROM "BASAL"
DAY 27/2 to DAY 11/3	755 ± 83	"BASAL"	456 ± 43	"BASAL"	+299 ± 114	"BASAL"
DAY 12/3 to DAY 19/3	815 ± 74	+ 7.9%	531 ± 40	+ 16.4%	+284 ± 130	- 5%
DAY 22/3 to DAY 2/4	782 ± 100	+ 3.6%	581 ± 32	+ 27.4%	+201 ± 114	-33%

Nitrogen excretion fell to 365 $\pm$ 86 mg over the period of starvation and increased during the 24 hours after it to 789 mg. This showed that the relationship between food nitrogen intake and nitrogen excretion was a direct one.

Figure 130 shows the mean estimated or "apparent" nitrogen retention and nitrogen balance in the burned and control group rats. The overall mean values for each time period are also given in Table 43.

Burn and control groups show similar increases in the level of urinary nitrogen excretion above "basal" values, 19% and 27% respectively. But both groups showed increased nitrogen excretion after dorsal fur shaving of 7% and 16%. Therefore the increase in urinary nitrogen excretion attributed to the burn was 12%, and to the anaesthetic alone plus pair feeding 11%. There was no significant difference in metabolic response between rats with a 20% BSA burn and the pair fed control rats which were anaesthetised but not burned. As daily mean food nitrogen intake was virtually unchanged throughout the whole of the experimental period, changes in mean daily nitrogen balance reflected the increases in nitrogen excretion, nitrogen balance being reduced by the appropriate amount. See Table 43.

Fig. 130 20% BSA BURN : CUMULATIVE "APPARENT" NITROGEN RETENTION AND NITROGEN BALANCE

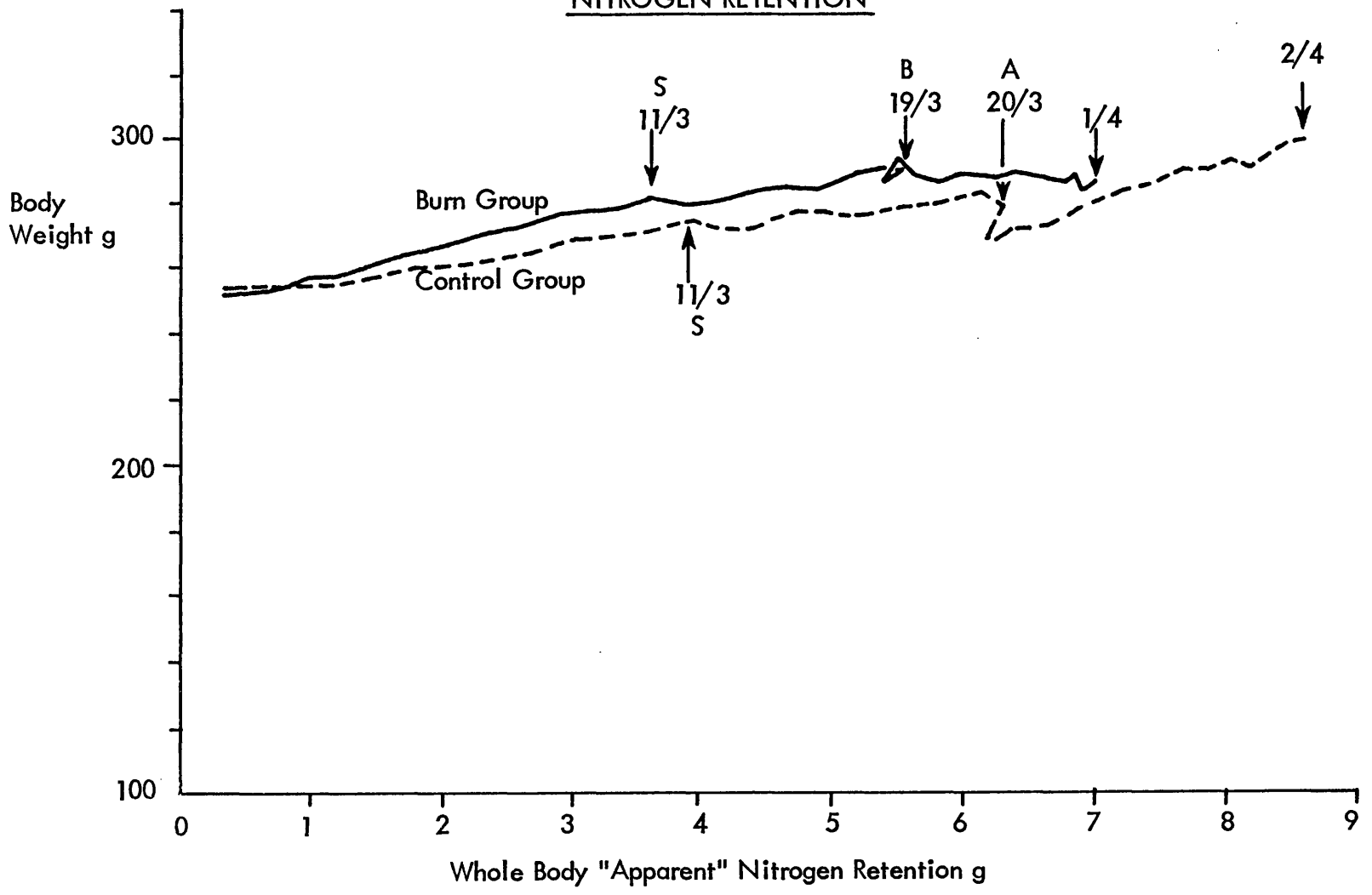


Presentation of the data in the form of mean daily estimated nitrogen retention in Figure 130 indicated a slightly slower rate of cumulative retention in the burn group rats in the period before injury. If the period 19/3 to 1/4 is examined, though the traces have separated, the rate of nitrogen retention in burn group and control group rats is similar.

A 24 hour period of starvation during the post burn period hardly affected the burned rats' retention rate of nitrogen at all. However, taken over the whole experimental study period, the cumulative nitrogen retention plot did indicate increasing differences in cumulative nitrogen retention between burn and control group rats which were not so readily appreciated by simply considering the mean daily nitrogen balance figures of each group.

Figure 131 shows the "efficiency" of conversion of retained nitrogen into body weight gain. This is the same form of plot shown in Figure 123 for the 5% BSA burn. The time scale for each common inflection point on the burn group and control group traces is arrowed. For example, S, 11/3 stands for dorsal fur shave on March 11th. This presentation also shows the similarity in pattern of metabolic response between burn and control groups and indicates the extent of the

Fig.131 20% BSA BURN : BURN GROUP RATS 1,2,3,8,9 vs. CONTROL GROUP RATS 4,5,6,7,10 BODY WEIGHT GAIN AND "APPARENT" NITROGEN RETENTION



reduction in overall nitrogen retention in the burned rats compared with control rats.

Figure 132 shows the same form of data plot as Figure 131, but only burn group rats are presented. The response to the 24 hour period of food deprivation can be clearly seen indicating the usefulness of this form of data presentation in revealing sudden changes during an experimental period. Figure 133 shows the linear regression of daily nitrogen intake on (estimated) nitrogen balance in the burn group rats before and after thermal injury. This was done to compare the effects of a 20% BSA burn injury in the rat with the patterns of response seen in man. See Figures 5 and 6 for comparison. The mathematical expressions which characterise each of the lines shown in Figure 133 are given in Table 44. Though the average nitrogen intake and balance based on line AA (March 11th - March 19th), 804 mg and 247 mg respectively, agrees closely with the mean values given for the same period in Table 43 of 809 mg and 244 mg, this form of calculation is not always reliable where all the food intake points lie within a very narrow range. This is the case with the lines BB, CC, DD and EE. The slope of the line is unduly influenced by the outlying points.

Fig. 132 20% BSA BURN : BURN GROUP RAT Nos. 1,2,3,8,9

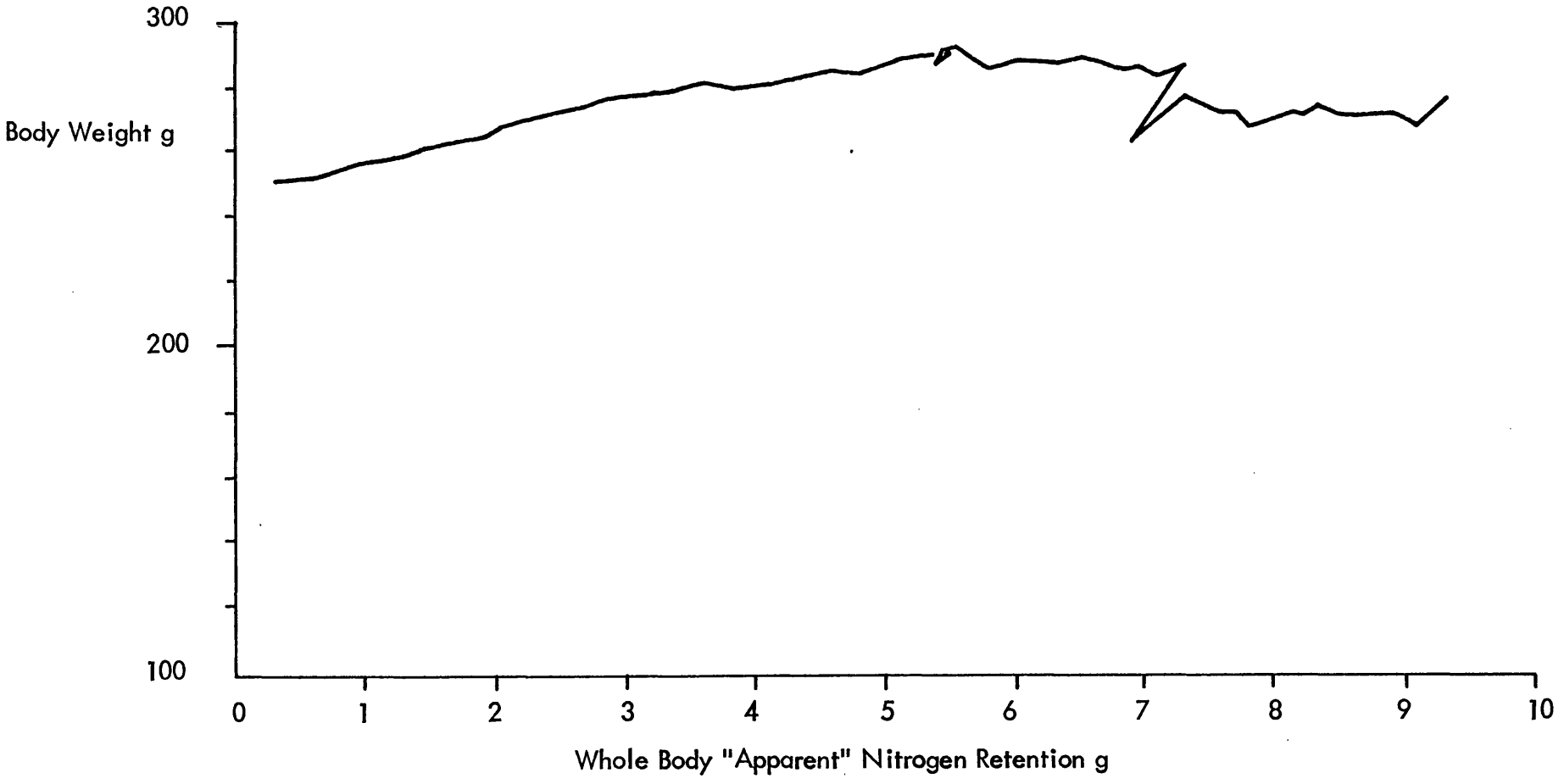


Fig.133 LINEAR REGRESSION : NITROGEN INTAKE ON NITROGEN BALANCE : 20% BSA BURN

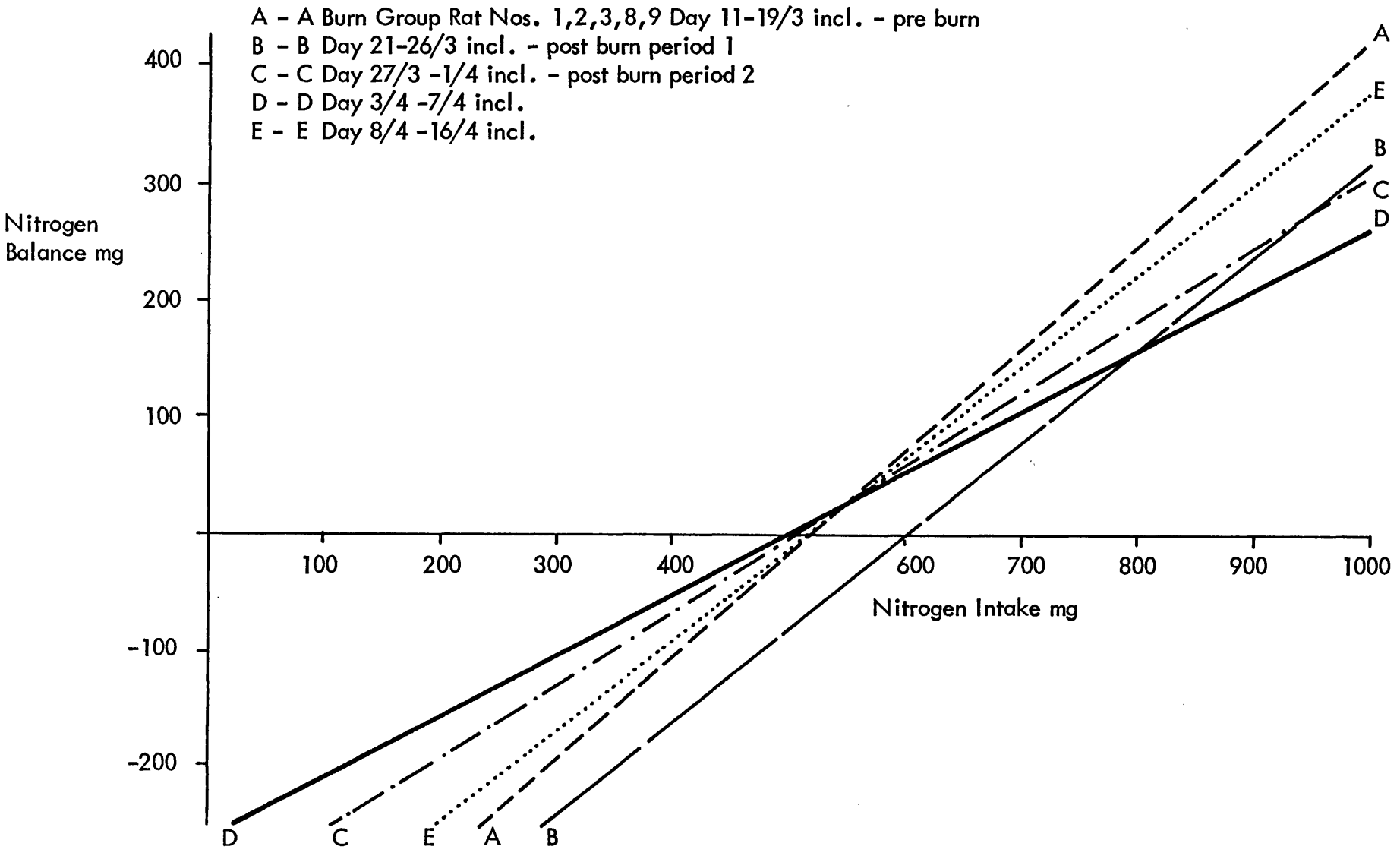


TABLE 44

LINE A - A DAY 11/3 to 19/3 inclusive	c = - 443.2 m = 0.858 r = 0.724	Average N. Balance 207 mg N. Intake 804 mg
LINE B - B DAY 21/3 to 26/3 inclusive	c = - 477 m = 0.792 r = 0.706	Average N. Balance 147 mg N. Intake 787 mg
LINE C - C DAY 27/3 to 1/4 inclusive	c = - 325 m = 0.625 r = 0.592	Average N. Balance 176 mg N. Intake 801 mg
LINE D - D DAY 3/4 to 7/4 inclusive	c = - 259 m = 0.515 r = 0.788	Average N. Balance 159 mg N. Intake 813 mg
LINE E - E DAY 8/4 to 16/4 inclusive	c = - 373 m = 0.736 r = 0.685	Average N. Balance 176 mg N. Intake 745 mg

Nevertheless, line BB shows that in the immediate post burn period the whole line shifts to the right compared to line AA. This agrees with the findings of Soroff (1961) shown in Figure 6, for the progress of human patients after burn injury. Lines CC, DD and EE have moved back to the left indicating recovery, though the slope of these lines cannot be regarded as reliable for the reason mentioned.

METABOLIC RESPONSE TO 20% BODY SURFACE AREA FULL SKIN THICKNESS BURN

DISCUSSION

There is a marked difference in the metabolic response to a 20% BSA dorsal burn, judged by post burn weight loss and food intake between a rat subjected to regular calorimetry runs over a 50 day period, and rats given an identical injury but not subjected to regular calorimetry runs. The rat subjected to calorimetry runs post burn had lost, by the 30th post burn day, 17% of its original weight on the day of injury. The rate of weight loss by this rat seemed related to the increments in heat losses measured after burning. Compare Figure 125 with Figure 67. The rat's increase in food intake after burning was remarkable, and seemed to be directly related to the increased heat losses. The animal only began to regain body weight when the evaporative heat losses from the burn wound surface diminished. See also Figure 68.

It would be difficult to find a better example of successful adaptive behaviour than that shown by this burned rat in "learning" to survive by increasing his food intake despite the handicap of having to eat all of his daily food ration within a 4 hour period when he would normally be asleep.

A 20% BSA dorsal burn in these circumstances appeared a severe injury in the rat resulting in weight loss similar in extent to that seen in man with burns of moderate severity. In a further experiment, an identical 20% BSA dorsal burn in 5 rats kept in metabolic cages proved a trivial injury. By the 12th post burn day they had lost only 2.4% of their original weight on the day of burning and were only 5 - 6% lighter than equivalent pair fed control rats. Additional confirmation that the thermal injury was not severe could be found in the fact that the burned rats did not increase their food intake at all after burning and yet by the 20th day or so post burn, they began to regain body weight. This was despite a 24 hour period of food deprivation a few days earlier. This experiment emphasised the importance of having comparable control group rats in assessing the effect of injury on nitrogen excretion and nitrogen balance. Other workers have regarded increased urinary nitrogen excretion levels of 19 - 20% above basal after an injury (Table 43) as being significant (Cuthbertson et al., 1939; Cairnie et al., 1957; Cuthbertson and Tilstone, 1970). It was therefore interesting to note that pair fed control rats which were uninjured, but subjected to identical conditions and treatment as given to the burned rats, including Nembutal anaesthetic administration, showed an even greater increase in

urinary nitrogen excretion above "basal" levels than burned rats (Table 43). Anaesthesia clearly proved a stressful experience for the control rats.

If the observations on these rats in the second 20% BSA burn study were valid, and I had no reason to suppose that they were not, then it had to be accepted that some aspect or component of performing calorimetry runs in the burned rat had been responsible for increasing the severity of a 20% BSA burn. The possibilities considered were:

1. that the practice of overnight food deprivation with a restricted feeding time and daily calorimetry runs induced a chronic anxiety or "stressed" state in the burned rat, leading to elevated catecholamine excretion and a generally more catabolic hormonal climate. See Fig. 10. This in turn accelerated the rate of metabolism and post burn weight loss.

2. that the use of instrument leads for rat temperature measurement with a Velcro harness was contributing to the stress of calorimetry runs. It was necessary to anaesthetise the rat briefly with ether in order to insert the external auditory meatus thermistor temperature probe before each run. Thermometry measurements were carried out on every calorimetry run on the burned rat up

to the 25th post burn day. The thermometry results presented in Figure 70 and Tables 15A and 15B only give data from those runs where temperature measurements were considered satisfactory, i.e. where thermistors remained correctly in place. Repeated brief ether anaesthesia and restraint during calorimetry might well be expected to adversely affect the rat under study by greatly increasing experimental "stress".

3. that the airflow rate over the burned rat within SEC - A - 04 L was excessive in the later runs after the 14th post burn day, and that this might have led to repeated cooling and cold stress of the burned rat during each calorimetry run.

The following conclusions were drawn from these considerations:

1. In subsequent calorimetry runs in burned rats, a single animal would not be put into the calorimeter more often than twice in any one week and that overnight dietary restriction would only be used in order to make the rat under study post absorbtive for calorimetry. For the remainder of the time the burned rats' food intake would not be interfered with.

2. Rat body temperature measurements would not be made during calorimetry runs in subsequent experiments. They had been found to be unnecessary and probably increased RME values, thereby decreasing rather than increasing the accuracy of long term calorimeter assessment of energy balance in the burned rat.

3. Total and recirculated airflow rates would be carefully regulated at the minimum necessary values to control humidity within SEC - A - 04 L.

4. Rats to be studied would be given extensive training to allow adaptation to regular calorimetry runs before burn injury.

In this way I hoped that the process of measurement itself would not alter the very thing which I was attempting to measure, viz. the metabolic response of the rat to graded severity of burn trauma.

METABOLIC RESPONSE TO A 25% OF BODY SURFACE AREA FULL SKIN THICKNESS BURN

MATERIALS AND METHODS

Separate but related studies were made of the metabolic response to a 25% BSA full skin thickness dorsal burn in 10 male Wistar rats kept in metabolic cages (5 burn and 5 control) and 5 male Wistar rats kept in wall rack cages (3 burn and 2 control). Metabolic cage and wall rack cage rats were burned on the same day using the method previously described. Control rats were given equivalent doses (on a weight for weight basis) of Nembutal anaesthetic agent 24 hours later. The control rats were then fed the mean of the burned rat group's food intake, with a delay of 24 hours.

The aim of this experiment was to establish post burn conditions for the rat which were comparable with those given to human hospital patients. Food intake after burn injury in the rat was restricted to 20 g of Low Iodine Test Diet per day (with 5 ug KI per 10 ml drinking water). The effect of this is discussed.

The metabolic cage rats were weighed daily, food intake was measured, and metabolic collections made for urine and faeces as previously described. The experiment began on 29th June, dorsal fur clipping was carried out on 24th July, metabolic collections were started on 7th August, and burn injury was carried out on 14th August. Control group Nembutal anaesthesia alone was carried out on 15th August. Food intake was ad libitum up till the 14th - 15th August, in both burn and control rat groups. The mean daily dietary intake in the burn group rats over the entire period, 29th June to 14th August (pre-burn) was 19.6 $\pm$ 2.4 (SD) g. The mean daily dietary intake in the control group rats over the period 29th June to 15th August (pre-anaesthesia alone) was 20.9 $\pm$ 3.5 (SD) g. Taken together, this gave a mean daily food intake of 20.3 g for all the rats over the time periods given. Restricting the rat daily food intake to 20 g diet did not appear to represent a significant reduction (less than 1.5%) in normal food intake. It was used as a device to ensure that burned rats' food intake could be kept as constant as possible in the post burn period, in the hope of eliminating those changes in urinary nitrogen excretion due solely to alterations in food nitrogen intake, such as were seen in the 5% BSA burn study.

The burn group rats were therefore fed 20 g diet daily from the day of burn injury, and the control rats were pair fed the mean daily intake of the burned rats, but with a 24 hour delay. Metabolic collections were made for 33 days after the burn, till the experiment ended on 16th September.

Wall rack cage rats were burned according to the foregoing schedule, but they were subjected to regular post burn calorimetry runs in addition, to determine daily resting energy expenditure. Details of the methods used and the results found are given separately in the final experimental section of this thesis.

METABOLIC RESPONSE TO 25% BODY SURFACE AREA FULL SKIN THICKNESS BURN

RESULTS

Figure 134 shows the individual daily body weight for 5 rats in the burn group before and after burn injury. The growth rate for each time period, indicated by the arrowed horizontal lines, is given by the slope (m) of the linear regression line drawn through the weight values. The point of intercept of this line on the body weight axis is given (c), as is the correlation coefficient of the line (r). Figure 135 gives the same information as Figure 134 but for 4 control group rats. The 5th control rat (No. 2) died from a salivary gland tumour on 8th August and is therefore not included.

Growth rate in the burn group rats was 2.2 g weight gain per day in the period before injury (29th June - 14th August), compared with a daily weight gain of 2.7 g in control group rats over the same period (29th June - 15th August). Dorsal fur shaving on 24th July produced slight interruptions in growth rate in both burn and control groups, as seen previously

Fig.134 25% BSA BURN : METABOLIC CAGE BURN GROUP - RAT Nos. 4,5,7,8,10

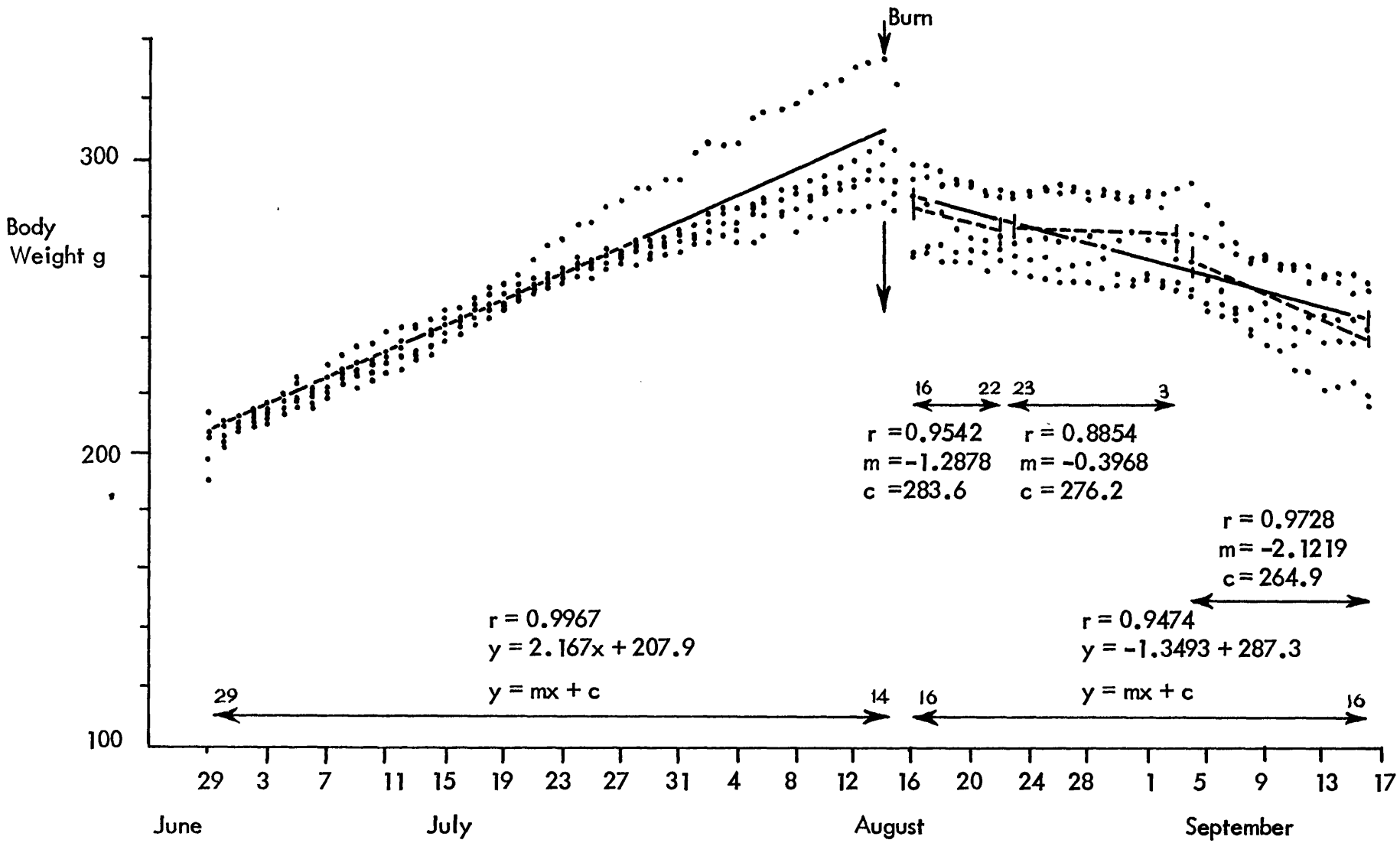
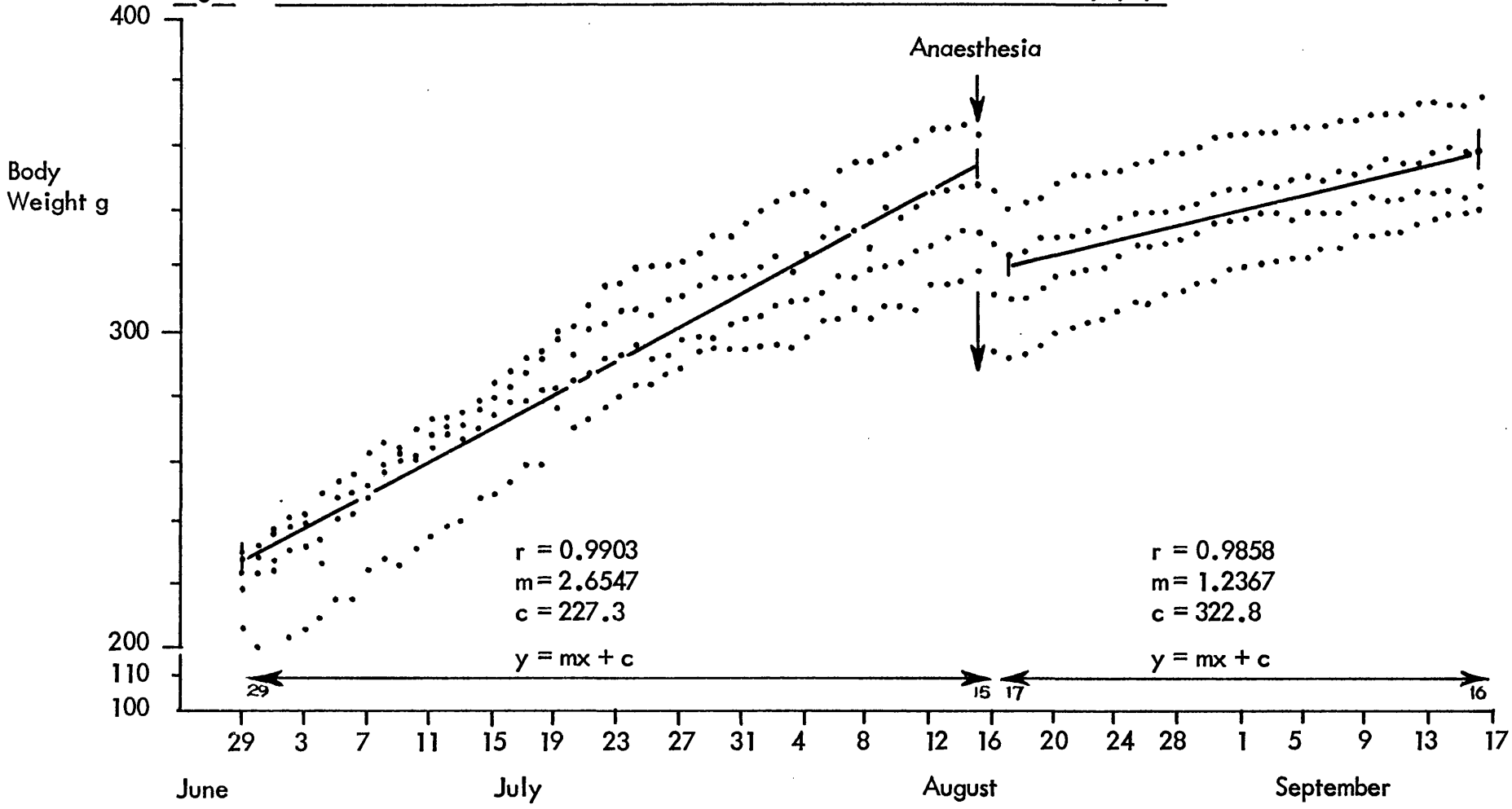


Fig.135 25% BSA BURN : METABOLIC CAGE CONTROL GROUP - RAT Nos. 1,3,6,9



in the 20% BSA burn study, Figures 126 and 127. Growth rate in the burn group rats during the period (25th July to 14th August) was 1.8 g per day and growth rate in control group rats over the same period after fur shaving (25th July to 15th August) was 2.0 g per day.

Individual rat daily body weights for burn group rats are given in Table 45, and for control group rats are given in Table 46.

After a 25% of body surface area full skin thickness burn, the burn group rats suffered a continuing weight loss of 1.35 g per day overall during the period (16th August to 16th September) while on a fixed dietary intake. The rate of weight loss appeared to vary. Three distinct periods were noted. There was an initial "step change" in body weight immediately after burning (due to decreased dietary intake) followed by the first period of steady weight loss. The rate of weight loss in this period (from 16th August to 22nd August) was 1.3 g per day. The rate of weight loss then decreased and remained slower for some time, at 0.4 g per day (from 23rd August to 3rd September). Thereafter, in the third phase of post burn weight loss observed, the rate of weight loss became more rapid at 2.1 g per day (from 4th September to 16th September).

In contrast, the pair fed control group rats continued to grow after the day of anaesthetic

TABLE 45 A 25% BSA BURN STUDY, DAILY BODY WEIGHT g.

BURN GROUP - RATS 4, 5, 7, 8, 10.

DATE	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
29 JUNE	207	214	191	207	198	203 ± 9.2
30	209	211	202	205	204	206 ± 3.7
1 JULY	209	213	210	209	210	210 ± 1.6
2	209	215	211	211	214	212 ± 2.6
3	210	217	211	212	215	213 ± 2.9
4	213	220	217	215	217	216 ± 2.5
5	215	224	219	218	225	220 ± 4.0
6	215	222	219	217	221	219 ± 2.7
7	219	226	221	221	231	224 ± 4.8
8	223	228	227	226	233	227 ± 3.7
9	222	230	228	228	237	229 ± 5.3
10	224	230	230	227	237	230 ± 5.0
11	227	235	232	231	241	233 ± 5.3
12	229	236	237	233	242	235 ± 4.9
13	232	235	235	234	242	235 ± 3.8
14	233	240	241	236	245	239 ± 4.5
15	238	244	245	241	248	243 ± 4.0
16	240	245	245	243	249	244 ± 3.3
17	244	249	250	247	252	248 ± 3.3
18	245	250	255	249	256	251 ± 4.4
19	249	250	254	250	258	252 ± 4.0
20	252	253	257	254	261	256 ± 3.9
21	255	256	260	256	265	258 ± 4.2
22	257	257	261	259	271	261 ± 5.8
23	262	259	264	260	273	263 ± 5.7

TABLE 45 B 25% BSA BURN STUDY, DAILY BODY WEIGHT g.

BURN GROUP - RATS 4, 5, 7, 8, 10.

DATE	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
24 JULY	264	260	267	263	278	266 ± 6.6
25	264	259	265	263	278	266 ± 7.3
26	268	263	270	265	284	270 ± 8.4
27	266	265	269	267	287	271 ± 9.0
28	270	263	271	269	291	273 ± 10.4
29	272	266	268	270	290	273 ± 9.7
30	275	267	272	270	293	276 ± 10.2
31	276	268	272	273	293	277 ± 9.7
1 AUG.	277	272	272	275	302	279 ± 12.7
2	281	275	271	278	305	282 ± 13.5
3	281	277	274	282	305	284 ± 12.2
4	281	272	276	278	306	283 ± 13.3
5	283	271	281	283	314	287 ± 16.1
6	286	274	280	285	316	288 ± 16.3
7	290	281	282	286	317	291 ± 15.0
8	290	275	283	288	319	291 ± 16.7
9	292	280	286	287	323	294 ± 17.0
10	294	279	288	289	325	295 ± 17.6
11	297	282	291	291	327	298 ± 17.2
12	300	282	291	294	331	299 ± 18.7
13	303	284	294	296	333	302 ± 18.7
14	306	285	292	299	334	304 ± 19.0
15	303	283	288	293	326	299 ± 17.1
16	294	268	269	288	298	283 ± 14.2
17	295	269	270	285	298	283 ± 13.6
18	291	266	271	282	296	281 ± 13.0
19	292	266	269	277	293	279 ± 12.7

TABLE 45 C 25% BSA BURN STUDY, DAILY BODY WEIGHT g.

BURN GROUP - RATS 4, 5, 7, 8, 10.

DATE	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
20 AUG.	292	265	269	274	291	278 ± 12.5
21	288	263	268	273	289	276 ± 12.0
22	287	265	269	274	290	277 ± 11.0
23	288	261	267	271	288	275 ± 12.3
24	289	260	267	273	288	275 ± 13.0
25	290	260	267	274	291	276 ± 13.9
26	288	259	264	272	291	275 ± 14.5
27	290	259	264	273	290	275 ± 14.5
28	288	259	265	274	287	274 ± 13.1
29	290	257	267	270	289	274 ± 14.4
30	288	258	261	273	288	273 ± 14.3
31	286	258	260	275	288	273 ± 14.1
1 SEPT.	286	260	261	274	289	274 ± 13.6
2	284	258	259	272	289	272 ± 14.4
3	279	259	256	267	291	270 ± 14.6
4	275	255	255	266	292	268 ± 15.7
5	274	249	251	259	286	264 ± 15.6
6	271	247	251	256	279	261 ± 13.8
7	269	249	245	249	273	257 ± 12.7
8	266	250	244	242	267	254 ± 11.9
9	267	251	238	246	268	254 ± 13.2
10	264	248	235	244	264	251 ± 12.8
11	264	243	230	238	265	248 ± 15.7
12	265	247	229	241	258	248 ± 14.1
13	260	247	223	239	261	246 ± 15.9
14	258	246	223	238	261	245 ± 15.5
15	255	246	224	238	261	245 ± 14.5
16	259	242	217	220	256	239 ± 19.7

TABLE 46 A 25% BSA BURN STUDY, DAILY BODY WEIGHT g.

CONTROL GROUP - RATS 1, 3, 6, 9.

DATE	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
29 JUNE	230	219	230	206	221 ± 11.4
30	233	224	230	200	222 ± 14.9
1 JULY	238	228	237	204	227 ± 15.5
2	240	231	240	204	229 ± 17.3
3	240	232	243	206	229 ± 15.8
4	247	237	250	210	236 ± 18.3
5	249	242	253	215	240 ± 16.7
6	249	243	256	216	241 ± 17.5
7	252	248	263	225	247 ± 15.8
8	259	257	266	228	252 ± 16.5
9	264	260	263	226	253 ± 18.0
10	261	261	271	232	256 ± 16.9
11	264	268	273	235	260 ± 17.0
12	269	270	274	239	263 ± 16.0
13	267	271	276	241	264 ± 15.8
14	270	276	280	248	269 ± 14.2
15	275	280	285	250	273 ± 15.7
16	279	283	289	253	276 ± 15.9
17	279	289	293	259	280 ± 15.2
18	282	295	293	259	282 ± 16.6
19	283	301	300	267	288 ± 16.1
20	286	304	294	270	288 ± 14.1
21	288	310	302	273	293 ± 16.4
22	293	316	304	277	297 ± 16.3
23	294	317	308	281	300 ± 16.2
24	297	322	309	284	303 ± 16.0

TABLE 46 B 25% BSA BURN STUDY, DAILY BODY WEIGHT g.

CONTROL GROUP - RATS 1, 3, 6, 9.

DATE	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
25 JULY	293	322	307	285	302 ± 16.5
26	294	323	312	288	304 ± 16.1
27	299	324	313	290	307 ± 15.3
28	299	327	317	295	310 ± 15.2
29	299	332	319	296	312 ± 16.9
30	304	332	320	296	313 ± 15.9
31	306	337	320	296	315 ± 17.7
1 AUG.	307	340	322	297	317 ± 18.7
2	310	343	326	298	319 ± 19.6
3	311	346	321	297	319 ± 20.6
4	313	347	327	300	322 ± 20.0
5	314	342	332	305	323 ± 16.7
6	320	353	335	306	329 ± 20.1
7	319	356	334	309	330 ± 20.5
8	322	356	339	306	331 ± 21.9
9	323	359	342	310	334 ± 21.3
10	325	361	339	310	334 ± 21.6
11	328	363	342	309	336 ± 22.7
12	330	367	346	317	340 ± 21.3
13	332	367	347	317	341 ± 21.1
14	335	368	348	318	343 ± 21.1
15	334	365	349	311	340 ± 22.8
16	314	347	330	296	322 ± 21.8
17	313	341	327	294	319 ± 20.1
18	313	344	328	295	320 ± 21.0
19	316	345	332	298	323 ± 20.3
20	320	350	332	302	326 ± 20.0

TABLE 46 C 25% BSA BURN STUDY, DAILY BODY WEIGHT g.

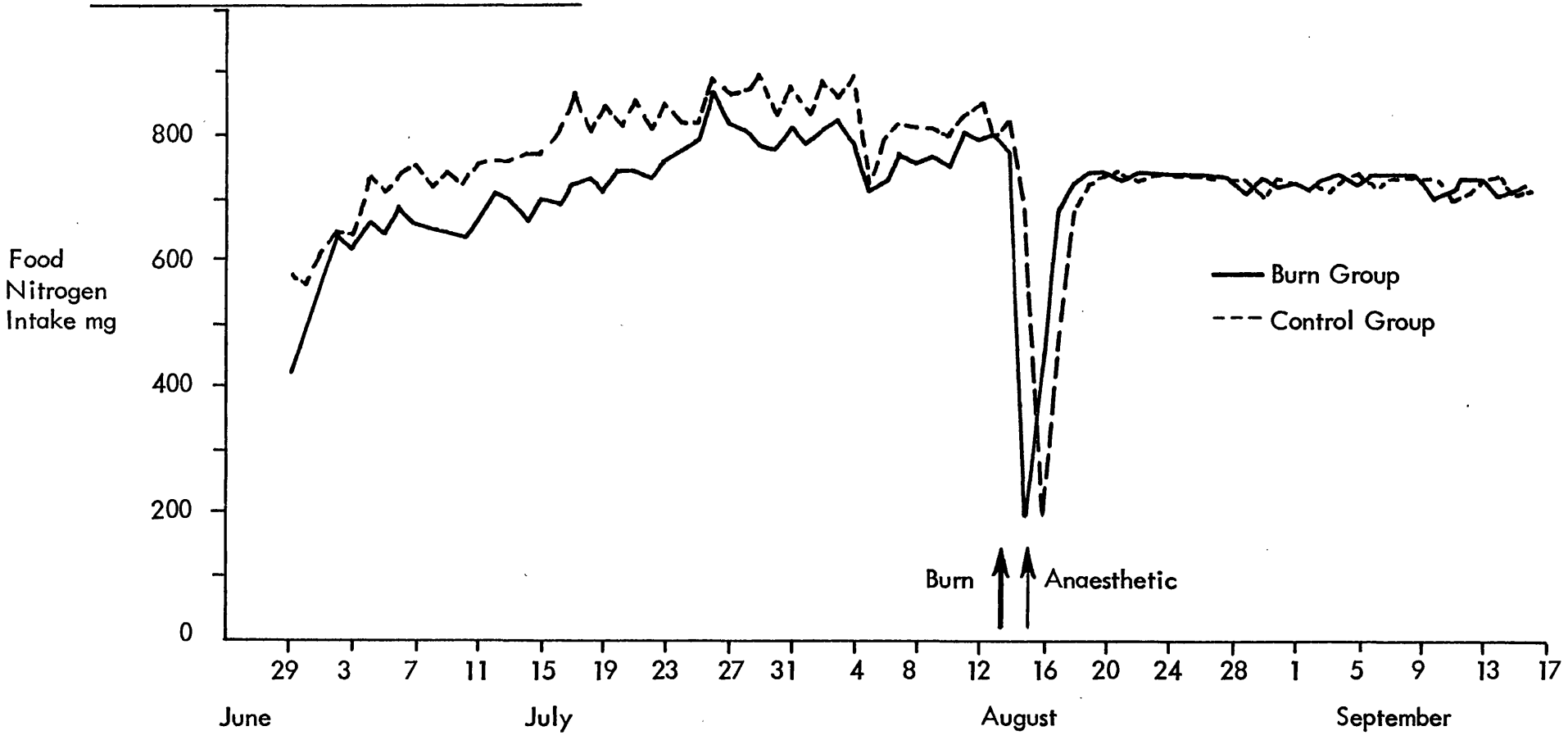
CONTROL GROUP - RATS 1, 3, 6, 9.

DATE	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
21 AUG.	321	352	333	304	327 ± 20.4
22	322	352	335	305	329 ± 20.0
23	323	353	336	306	330 ± 19.8
24	327	354	339	309	332 ± 19.2
25	330	357	340	312	335 ± 18.8
26	330	357	340	312	335 ± 19.2
27	331	360	341	315	337 ± 18.8
28	332	360	343	316	338 ± 18.2
29	335	362	344	318	340 ± 18.2
30	337	364	347	319	342 ± 18.5
31	339	365	348	323	344 ± 17.9
1 SEPT.	339	366	348	323	344 ± 17.9
2	340	367	350	325	346 ± 17.7
3	340	367	349	325	345 ± 17.6
4	339	369	351	326	346 ± 18.2
5	341	369	352	326	347 ± 17.9
6	340	369	351	329	347 ± 16.1
7	341	370	354	330	349 ± 17.3
8	344	370	353	333	350 ± 15.6
9	346	372	355	333	352 ± 16.5
10	345	372	358	334	352 ± 16.5
11	345	373	357	335	352 ± 16.1
12	348	375	357	337	354 ± 16.3
13	347	376	360	339	356 ± 16.0
14	348	375	362	340	356 ± 15.3
15	346	375	360	341	356 ± 15.3
16	350	378	360	342	357 ± 15.3

administration, at the rate of 1.24 g per day (from 17th August to 16th September). This was a 40% reduction from the pre-anaesthetic growth rate of 2.0 g per day, over the period (25th July to 15th August). The mean body weight of the control group rats on 14th August was 343 $\pm$ 21 (SD) g. Their mean body weight on 16th September at the end of the experiment was 357 $\pm$ 15 (SD) g, an increase of 14 g or 4.1%. The mean body weight of burn group rats on the day of burn injury was 304 $\pm$ 19 (SD) g. Their mean body weight at the end of the study, on 16th September, was 239 $\pm$ 20 (SD) g, an overall loss of 65 g weight. Burned rats therefore suffered a mean weight loss after injury of 21.4% and suffered an additional overall growth failure of 4.1% (based on control rat results over the same period), making a total weight deficit of 25.5% after injury, compared with equivalent control rats.

As stated previously, the post burn food intake had been fixed at 20 g diet daily (equal to 740 mg nitrogen). The average food intake for all rats between 29th June and 14th-15th August was 20.3 g diet per day. The reduction in food intake was less than 1.5%. If the data regarding food intake is plotted as shown in Figure 136 (in the form of diet nitrogen intake), then it becomes evident that the food nitrogen intake in the pre-burn period between 29th

Fig. 136 25% BSA BURN : BURN GROUP : RAT Nos. 4,5,7,8,10 vs CONTROL GROUP : RAT Nos. 1,3,6,9 NITROGEN INTAKES AD LIBITUM - 6% FEEDING POST- BURN



June and 14th - 15th August was not constant throughout. Individual rat daily nitrogen intakes are given in Tables 47 and 48 for burn group and control group rats respectively. The following points were considered.

In the burn group rats, mean daily food intake between 29th June and 24th July, the day of fur shaving, was 18.1 $\pm$ 2.2 (SD) g. In the period between fur shaving and burn injury (from 25th July to 14th August) mean food intake was 21.3 $\pm$ 0.1 (SD) g, and in the period between the start of metabolic cage excreta collections and the burn injury (from 8th August to 14th August), mean daily food intake was 21.1 $\pm$ 0.6 (SD) g. Based solely on this latter period, a fixed level of daily food intake of 20 g diet represents a 5.2% reduction from ad libitum values. Because of food spillage, burned rats only managed to actually consume 19.7 $\pm$ 0.3 g of diet daily. The reduction in food intake between pre- and post-burn was therefore 6.6%.

In the control group rats, mean daily food intake between 29th June and 24th July was 20.3 $\pm$ 2.2 (SD) g. In the period between fur shaving and anaesthesia (from 25th July to 15th August), mean daily food intake was 22.6 $\pm$ 1.2 (SD) g, and between the start of metabolic cage collections and anaesthesia (from

TABLE 47 A (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

PRE-BURN PERIOD

DAY 8 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	792	688	733	777	810	760 ± 49
URINE N. (+5%) mg.	437	587	462	458	608	510 ± 81
N. BALANCE mg.	355	101	271	319	202	250 ± 101
DAY 9 AUG.						
FOOD N. mg.	825	755	692	670	877	764 ± 88
URINE N. (+5%) mg.	459	261	430	333	584	414 ± 124
N. BALANCE mg.	366	494	262	337	293	350 ± 90
DAY 10 AUG.						
FOOD N. mg.	810	755	688	651	873	756 ± 90
URINE N. (+5%) mg.	468	209	500	333	430	389 ± 118
N. BALANCE mg.	342	546	188	318	443	367 ± 135

TABLE 47 A (2) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

PRE-BURN PERIOD

DAY 11 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	840	784	807	755	840	805 ± 37
URINE N. (+5%) mg.	459	353	363	306	517	399 ± 86
N. BALANCE mg.	381	431	444	449	323	406 ± 53
DAY 12 AUG.						
FOOD N. mg.	833	755	744	792	862	797 ± 50
URINE N. (+5%) mg.	517	182	509	446	531	437 ± 146
N. BALANCE mg.	316	573	235	346	331	360 ± 126
DAY 13 AUG.						
FOOD N. mg.	881	744	733	844	803	801 ± 64
URINE N. (+5%) mg.	325	324	487	468	591	401 ± 88
N. BALANCE mg.	556	420	246	376	212	362 ± 139
DAY 14 AUG.						
FOOD N. mg.	833	747	681	803	799	773 ± 60
URINE N. (+5%) mg.	411	205	445	499	496	412 ± 16
N. BALANCE mg.	422	542	236	304	303	361 ± 121

TABLE 47 B (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 1

DAY 16 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	662	492	348	696	93	458 ± 248
URINE N. (+5%) mg.	650	442	636	547	592	573 ± 84
N. BALANCE mg.	12	50	-288	149	-499	-115 ± 269
DAY 17 AUG.						
FOOD N. mg.	699	710	685	722	592	682 ± 52
URINE N. (+5%) mg.	467	238	485			443 ± 117
N. BALANCE mg.	232	472	200	218	72	239 ± 145
DAY 18 AUG.						
FOOD N. mg.	729	696	740	736	736	727 ± 18
URINE N. (+5%) mg.	510	357	601	547	623	527 ± 106
N. BALANCE mg.	219	339	139	189	113	200 ± 88

TABLE 47 B (2) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 1

DAY 19 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	736	736	740	740	740	739 ± 2
URINE N. (+5%) mg.	508	523	648	637	575	579 ± 64
N. BALANCE mg.	228	213	92	103	165	160 ± 62
DAY 20 AUG.						
FOOD N. mg.	733	740	740	740	740	739 ± 3
URINE N. (+5%) mg.	462	455	601	320	682	504 ± 141
N. BALANCE mg.	271	285	139	420	58	235 ± 140
DAY 21 AUG.						
FOOD N. mg.	736	714	736	740	740	733 ± 11
URINE N. (+5%) mg.	534	545	572	624	721	600 ± 77
N. BALANCE mg.	202	169	164	116	19	133 ± 71
DAY 22 AUG.						
FOOD N. mg.	736	740	736	740	740	739 ± 2
URINE N. (+5%) mg.	541	570	555	616	510	559 ± 39
N. BALANCE mg.	195	170	181	124	230	180 ± 39

TABLE 47 C (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 2

DAY 23 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	740	736	740	740	736	739 ± 2
URINE N. (+5%) mg.	604	730	703	689	626	670 ± 54
N. BALANCE mg.	136	6	37	51	110	69 ± 56
DAY 24 AUG.						
FOOD N. mg.	736	736	740	740	740	739 ± 2
URINE N. (+5%) mg.	370	611	638	576	598	559 ± 108
N. BALANCE mg.	366	125	102	164	142	180 ± 107
DAY 25 AUG.						
FOOD N. mg.	733	736	740	740	740	738 ± 2
URINE N. (+5%) mg.	542	560	661	139	648	591 ± 59
N. BALANCE mg.	191	176	79	201	92	147 ± 56
DAY 26 AUG.						
FOOD N. mg.	736	736	740	740	740	739 ± 2
URINE N. (+5%) mg.	467	553	537	590	605	550 ± 54
N. BALANCE mg.	269	183	203	150	135	189 ± 53

TABLE 47 C (2) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY
BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 2

DAY 27 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	736	736	740	740	740	739 ± 6
URINE N. (+5%) mg.	551	571	655	635	668	616 ± 51
N. BALANCE mg.	185	165	85	105	72	123 ± 50
DAY 28 AUG.						
FOOD N. mg.	736	707	740	740	740	733 ± 14
URINE N. (+5%) mg.	553	478	433	543	616	525 ± 71
N. BALANCE mg.	183	229	307	197	124	208 ± 67
DAY 29 AUG.						
FOOD N. mg.	736	662	666	740	740	709 ± 41
URINE N. (+5%) mg.	442	504	524	573	549	519 ± 50
N. BALANCE mg.	294	158	142	167	191	190 ± 61
DAY 30 AUG.						
FOOD N. mg.	733	714	736	736	740	732 ± 10
URINE N. (+5%) mg.	547	532	553	510	585	546 ± 27
N. BALANCE mg.	186	182	183	226	155	186 ± 21

TABLE 47 C (3) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 2

DAY 31 AUG.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	729	666	740	733	740	722 ± 32
URINE N. (+5%) mg.	461	510	533	476	495	495 ± 28
N. BALANCE mg.	268	156	207	257	245	227 ± 46
DAY 1 SEPT.						
FOOD N. mg.	740	692	740	703	740	723 ± 24
URINE N. (+5%) mg.	673	367	348	288	591	454 ± 168
N. BALANCE mg.	67	325	392	415	149	269 ± 154
DAY 2 SEPT.						
FOOD N. mg.	740	736	736	618	740	714 ± 54
URINE N. (+5%) mg.	479	774	939	782	487	692 ± 202
N. BALANCE mg.	261	-38	-203	-164	253	22 ± 223
DAY 3 SEPT.						
FOOD N. mg.	725	740	740	725	740	734 ± 8
URINE N. (+5%) mg.	582	574	517	516	532	544 ± 32
N. BALANCE. mg.	143	166	223	209	208	190 ± 34

TABLE 47 D (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 3

DAY 4 SEPT.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	736	736	740	740	740	739 ± 2
URINE N. (+5%) mg.	611	504	602	525	598	568 ± 49
N. BALANCE mg.	125	232	138	215	142	171 ± 49
DAY 5 SEPT.						
FOOD N. mg.	733	651	740	740	740	721 ± 39
URINE N. (+5%) mg.	627	542	484	664	675	599 ± 83
N. BALANCE mg.	106	109	256	76	65	122 ± 77
DAY 6 SEPT.						
FOOD N. mg.	736	733	736	733	740	735 ± 2
URINE N. (+5%) mg.	640	593	654	478	556	585 ± 71
N. BALANCE mg.	96	140	82	255	184	150 ± 70
DAY 7 SEPT.						
FOOD N. mg.	740	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	601	582	598	469	590	568 ± 56
N. BALANCE mg.	139	158	142	271	150	172 ± 56

TABLE 47 D (2) NITROGEN BALANCE METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 3

DAY 8 SEPT.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	736	736	740	740	740	739 ± 2
URINE N. (+5%) mg.	542	583	618	571	571	578 ± 27
N. BALANCE mg.	194	153	122	169	169	161 ± 26
DAY 9 SEPT.						
FOOD N. mg.	740	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	533	502	569	469	753	565 ± 128
N. BALANCE mg.	207	238	171	271	-13	175 ± 129
DAY 10 SEPT.						
FOOD N. mg.	736	555	740	725	740	699 ± 81
URINE N. (+5%) mg.	666	466	563	479	537	542 ± 80
N. BALANCE mg.	70	89	177	246	203	157 ± 75
DAY 11 SEPT.						
FOOD N. mg.	733	592	736	736	740	707 ± 65
URINE N. (+5%) mg.	462	217	464	344	448	387 ± 107
N. BALANCE mg.	271	375	272	392	292	320 ± 59

TABLE 47 D (3) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 3

DAY 12 SEPT.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	740	703	740	740	740	733 ± 17
URINE N. (+5%) mg.	508	613	546	480	656	561 ± 74
N. BALANCE mg.	232	90	194	250	84	172 ± 81
DAY 13 SEPT.						
FOOD N. mg.	740	703	740	736	740	732 ± 16
URINE N. (+5%) mg.	462	376	471	442	275	405 ± 82
N. BALANCE mg.	278	327	269	294	465	327 ± 80
DAY 14 SEPT.						
FOOD N. mg.	740	585	740	740	740	709 ± 70
URINE N. (+5%) mg.	493	529	519	504	491	507 ± 16
N. BALANCE mg.	247	56	221	236	249	202 ± 82

TABLE 47 D (4) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

BURN GROUP - RATS 4, 5, 7, 8, 10.

POST BURN PERIOD 3

DAY 15 SEPT.	RAT 4	RAT 5	RAT 7	RAT 8	RAT 10	MEAN ± SD
FOOD N. mg.	729	622	740	740	740	714 ± 52
URINE N. (+5%) mg.	491	466	595	511	577	528 ± 56
N. BALANCE mg.	238	156	145	229	163	186 ± 44
DAY 16 SEPT.						
FOOD N. mg.	729	666	740	740	740	723 ± 32
URINE N. (+5%) mg.	568	537	537	364	612	524 ± 95
N. BALANCE mg.	161	129	203	376	128	199 ± 103

TABLE 48 A (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

PRE-ANAESTHETIC PERIOD

DAY 8 AUG.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	829	903	870	666	817 ± 105
URINE N. (+5%) mg.	553	513	464	491	506 ± 38
N. BALANCE mg.	276	390	406	175	311 ± 108
DAY 9 AUG.					
FOOD N. mg.	762	899	810	773	811 ± 62
URINE N. (+5%) mg.	505	512	460	525	501 ± 28
N. BALANCE mg.	257	387	350	248	310 ± 69
DAY 10 AUG.					
FOOD N. mg.	818	862	792	722	799 ± 59
URINE N. (+5%) mg.	520	468	456	460	476 ± 29
N. BALANCE mg.	298	394	336	262	323 ± 56
DAY 11 AUG.					
FOOD N. mg.	821	921	866	733	836 ± 80
URINE N. (+ 5%) mg.	481				525 ± 37
N. BALANCE mg.	340	394	295	214	311 ± 76

TABLE 48 A (2) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

PRE-ANAESTHETIC PERIOD

DAY 12 AUG.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	810	892	862	847	855 ± 34
URINE N. (+5%) mg.	466	527	638	404	480 ± 109
N. BALANCE mg.	344	480	224	443	375 ± 115
DAY 13 AUG.					
FOOD N. mg.	836	799	810	736	796 ± 43
URINE N. (+5%) mg.	535	523	562	537	540 ± 17
N. BALANCE mg.	301	276	248	199	256 ± 44
DAY 14 AUG.					
FOOD N. mg.	784	847	870	781	821 ± 45
URINE N. (+5%) mg.	477	441	585	360	466 ± 93
N. BALANCE mg.	307	406	285	421	355 ± 69
DAY 15 AUG.					
FOOD N. mg.	710	725	736	555	682 ± 85
URINE N. (+5%) mg.	477	459	488	465	473 ± 13
N. BALANCE mg.	233	266	248	90	209 ± 81

TABLE 48 B (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 1

DAY 17 AUG.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	459	459	459	459	459 ± 0
URINE N. (+5%) mg.	375	379	281	329	341 ± 46
N. BALANCE mg.	84	80	178	130	118 ± 46
DAY 18 AUG.					
FOOD N. mg.	681	681	681	681	681 ± 0
URINE N. (+5%) mg.	389	504	417	457	442 ± 49
N. BALANCE mg.	292	177	264	224	239 ± 50
DAY 19 AUG.					
FOOD N. mg.	729	729	729	729	729 ± 0
URINE N. (+5%) mg.	454	469	446	569	485 ± 57
N. BALANCE mg.	275	260	283	160	244 ± 57
DAY 20 AUG.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	523	421	502	461	477 ± 45
N. BALANCE mg.	217	319	238	279	263 ± 45

TABLE 48 B (2) NIGROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 1

DAY 21 AUG.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+ 5%) mg.	480	586	493	442	501 ± 61
N. BALANCE mg.	260	154	247	298	239 ± 61
DAY 22 AUG.					
FOOD N. mg.	733	733	733	733	733 ± 0
URINE N. (+5%) mg.	539	566	551	467	552 ± 14
N. BALANCE mg.	194	167	182	266	181 ± 14
DAY 23 AUG.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	521	355	511	392	445 ± 84
N. BALANCE mg.	219	385	229	348	295 ± 84

TABLE 48 C (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 2

DAY 24 AUG.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	696	328	558	611	548 ± 158
N. BALANCE mg.	44	412	182	129	192 ± 157
DAY 25 AUG.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	475	366	428	576	462 ± 88
N. BALANCE mg.	265	374	312	164	278 ± 89
DAY 26 AUG.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	489	498	533	580	525 ± 41
N. BALANCE mg.	251	242	207	160	215 ± 41
DAY 27 AUG.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	573	529	393	483	495 ± 78
N. BALANCE mg.	167	211	347	257	245 ± 77

TABLE 48 C (2) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 2

DAY 28 AUG.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	736	736	736	736	736 ± 0
URINE N. (+5%) mg.	426	542	499	403	467 ± 64
N. BALANCE mg.	310	194	237	333	269 ± 64
DAY 29 AUG.					
FOOD N. mg.	733	733	733	733	733 ± 0
URINE N. (+5%) mg.	430	510	510	562	503 ± 55
N. BALANCE mg.	303	223	223	171	230 ± 55
DAY 30 AUG.					
FOOD N. mg.	710	710	710	710	710 ± 0
URINE N. (+5%) mg.	479	384	489	560	478 ± 72
N. BALANCE mg.	231	326	221	150	232 ± 72
DAY 31 AUG.					
FOOD N. mg.	733	733	733	733	733 ± 0
URINE N. (+5%) mg.	824	141	394	462	456 ± 282
N. BALANCE mg.	-91	592	339	271	277 ± 282

TABLE 48 C (3) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 2

DAY 1 SEPT.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	722	722	722	722	722 ± 0
URINE N. (+5%) mg.	588	518	355	340	450 ± 122
N. BALANCE mg.	134	204	367	382	272 ± 122
DAY 2 SEPT.					
FOOD N. mg.	722	722	722	722	722 ± 0
URINE N. (+5%) mg.	411	401	680	518	503 ± 130
N. BALANCE mg.	311	321	42	204	219 ± 130
DAY 3 SEPT.					
FOOD N. mg.	714	714	714	714	714 ± 0
URINE N. (+5%) mg.	587	519	468	530	526 ± 48
N. BALANCE mg.	127	195	246	184	188 ± 49
DAY 4 SEPT.					
FOOD N. mg.	733	733	733	733	733 ± 0
URINE N. (+5%) mg.	537	503	453	469	490 ± 38
N. BALANCE mg.	196	230	280	264	243 ± 37

TABLE 48 D (1) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 3

DAY 5 SEPT.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	602	504	547	480	532 ± 54
N. BALANCE mg.	138	236	196	260	208 ± 53
DAY 6 SEPT.					
FOOD N. mg.	722	722	722	722	722 ± 0
URINE N. (+5%) mg.	445	541	523	525	508 ± 43
N. BALANCE mg.	277	181	199	197	212 ± 43
DAY 7 SEPT.					
FOOD N. mg.	736	736	736	736	736 ± 0
URINE N. (+5%) mg.	504	505	506	505	505 ± 1
N. BALANCE mg.	232	231	230	231	231 ± 1
DAY 8 SEPT.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	524	534	442	544	511 ± 46
N. BALANCE mg.	216	206	298	196	229 ± 47

TABLE 48 D (2) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 3

DAY 9 SEPT.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	504	566	490	298	465 ± 116
N. BALANCE mg.	236	174	250	442	275 ± 116
DAY 10 SEPT.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	455	528	484	517	496 ± 34
N. BALANCE mg.	285	212	256	223	244 ± 33
DAY 11 SEPT.					
FOOD N. mg.	699	699	699	699	699 ± 0
URINE N. (+5%) mg.	453	453	465	458	457 ± 6
N. BALANCE mg.	246	246	234	241	242 ± 6
DAY 12 SEPT.					
FOOD N. mg.	707	707	707	707	707 ± 0
URINE N. (+5%) mg.	416	503	514	444	469 ± 47
N. BALANCE mg.	291	204	193	263	238 ± 47

TABLE 48 D (3) NITROGEN BALANCE, METABOLIC CAGE RATS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 3, 6, 9.

POST-ANAESTHETIC PERIOD 3

DAY 13 SEPT.	RAT 1	RAT 3	RAT 6	RAT 9	MEAN ± SD
FOOD N. mg.	733	733	733	733	733 ± 0
URINE N. (+5%) mg.	400	369	469	365	401 ± 48
N. BALANCE mg.	333	364	264	368	332 ± 48
DAY 14 SEPT.					
FOOD N. mg.	740	740	740	740	740 ± 0
URINE N. (+5%) mg.	408	457	453	471	447 ± 27
N. BALANCE mg.	332	283	287	269	293 ± 27
DAY 15 SEPT.					
FOOD N. mg.	710	710	710	710	710 ± 0
URINE N. (+5%) mg.	453	458	415	374	420 ± 35
N. BALANCE mg.	257	272	295	336	290 ± 34
DAY 16 SEPT.					
FOOD N. mg.	714	714	714	714	714 ± 0
URINE N. (+5%) mg.	458	510	524	516	502 ± 30
N. BALANCE mg.	256	204	190	198	212 ± 30

8th August to 15th August), mean daily food intake was 21.7 $\pm$ 1.4 (SD) g. Based again on this latter time period, a fixed level of daily food intake in the control rats of 20 g diet represents a 7.8% reduction from ad libitum values. But the control rats only actually ate 19.7 $\pm$ 0.3 (SD) g of diet daily, precisely the same intake as the burn group rats. Therefore the true reduction in daily food intake in the control rats after the day of anaesthesia (from 19th August to 16th September) was 9.2%.

Note in Figure 136, the post burn (and anaesthesia) food nitrogen intake was fixed throughout the post burn period, and was identical for burn and control group rats. This eliminated the effect of variation in dietary nitrogen intake alone causing changes in urinary nitrogen excretion, though it did not preclude the possibility of different handling of the fixed quantity of dietary nitrogen by the body after injury. The slight reduction (of less than 10%) in the normal rat ad libitum intake after injury is comparable with the inadvertent partial starvation to which many burned human patients are presently subjected in the U.K. hospitals (personal observation). Therefore I felt that this was a satisfactory level of food intake in the burned rat. It must be accepted however that it did result in a decrease in the overall rate of growth in control rats. This is discussed later.

Figure 137 shows the mean daily nitrogen losses in burn and control group rats before and after burn injury and control anaesthesic administration respectively. Individual rat daily nitrogen losses are given in Tables 47 and 48 for burn group and control group rats. Group mean values for separate pre- and post injury period (nitrogen balances) are given in Table 49.

In burn group rats, during the first phase of post burn weight loss (from 16th August to 22nd August), mean daily nitrogen excretion increased significantly by 27.9% above pre-injury values, while in the control group rats during the equivalent time period, nitrogen excretion decreased by 6.7%. These differences in nitrogen excretion between burn and control rat groups were not attributable to differences in dietary nitrogen intake as both groups showed identical nitrogen intakes and almost identical decreases from pre-burn/anaesthetic levels of intake. In the second period of post burn weight loss shown in Figure 134 (from 23rd August to 3rd September), daily nitrogen excretion rate remained elevated in the burn group rats at 563 $\pm$ 69 (SD) mg, an increase of 33.1% above pre-injury values. Daily nitrogen excretion rate apparently fell slightly in the third period post burn (between 4th September to 16th September) to 532 mg but, the weight loss after a 25% BSA burn on

Fig. 137 25% BSA BURN : RAT Nos. 4,5,7,8,10 - BURN GROUP vs. RAT Nos. 1,3,6,9 - CONTROL GROUP NITROGEN LOSSES

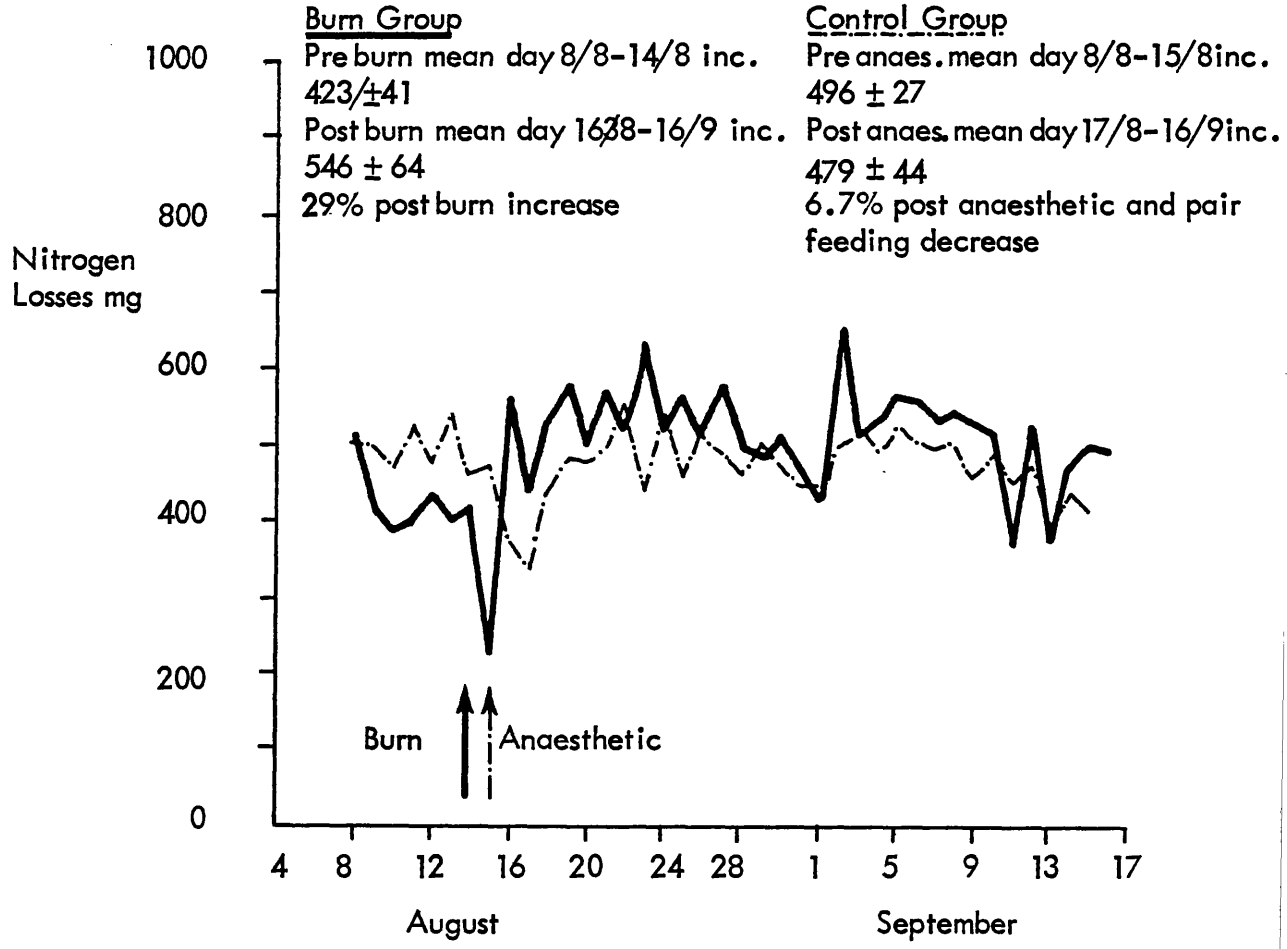


TABLE 49 A MEAN DAILY NITROGEN BALANCE (± SD) METABOLIC CAGE RATS

25% BSA BURN

BURN GROUP - RATS 4, 5, 7, 8, 10.

	MEAN DAILY FOOD N. mg.	% CHANGE FROM PREBURN	MEAN DAILY N.EXCRETION	% CHANGE FROM PREBURN	MEAN DAILY BALANCE	% CHANGE FROM PREBURN
DAY 8/8 - 14/8	779 ± 21	PREBURN	423 ± 41	PREBURN	356 ± 48	PREBURN
DAY 16/8 - 22/8	688 ± 130	- 11.7%	541 ± 54	+ 27.9%	147 ± 123	- 58.7%
DAY 23/8 - 3/9	730 ± 23	- 6.3%	563 ± 69	+ 33.1%	167 ± 70	- 53.1%
DAY 4/9 - 16/9	725 ± 40	- 6.9%	532 ± 66	+ 25.8%	193 ± 61	- 45.8%

TABLE 49 B MEAN DAILY NITROGEN BALANCE ($\pm$ SD) METABOLIC CAGE RATS

25% BSA BURN

CONTROL GROUP - RATS 1, 3, 6, 9.

	MEAN DAILY FOOD N. mg.	% CHANGE FROM NEMB.	MEAN DAILY N.EXCRETION	% CHANGE FROM NEMB.	MEAN DAILY BALANCE	% CHANGE FROM NEMB.
DAY 8/8 - 15/8	802 $\pm$ 52	PRE NEMB.	496 $\pm$ 27	PRE NEMB.	306 $\pm$ 52	PRE NEMB.
DAY 17/8 - 23/8	689 $\pm$ 104	- 14.1%	463 $\pm$ 65	- 6.7%	226 $\pm$ 59	- 26.1%
DAY 24/8 - 4/9	730 $\pm$ 11	- 9.0%	492 $\pm$ 31	- 0.8%	238 $\pm$ 32	- 22.2%
DAY 5/9 - 16/9	727 $\pm$ 15	- 9.4%	476 $\pm$ 40	- 4.0%	251 $\pm$ 39	- 18.0%

* PRENEMB. = PERIOD PRIOR TO NEMBUTAL ADMINISTRATION

fixed dietary intake was so marked in the rat, that the effect of this weight loss must be taken into account when commenting upon the rate of nitrogen excretion. Table 50 gives the daily nitrogen excretion rate, expressed per gram of mean rat body weight, for each of the time periods described. Nitrogen excretion rate progressively increased after burn injury, and was greatest at 2.11 g N per gram body weight during the 3rd period of post burn weight loss, when the rate of body weight loss was at its peak. In control rats, the daily nitrogen excreted per gram body weight was unchanged from the pre Nembutal level of 1.47 mg N/g body weight during periods 1 and 2, and fell, during period 3, to 1.35 g N/g body weight.

As dietary nitrogen intake was virtually constant in the post burn, post anaesthetic period, nitrogen balance reflected the changes in nitrogen excretion closely, and was reduced, appropriately, post burn. This is shown in Figure 138. The estimated cumulative nitrogen retention is also given in this figure. This form of data plotting does indicate clearly the increasing differences in rate of nitrogen retention between burn and control rat groups. The differences in rate between post burn periods 1, 2 and 3 can also be detected. At the end of the study, on 16th September, the mean cumulative nitrogen retained in the burn group

Fig. 138 25% BSA BURN: NITROGEN BALANCE AND CUMULATIVE "APPARENT" NITROGEN RETENTION

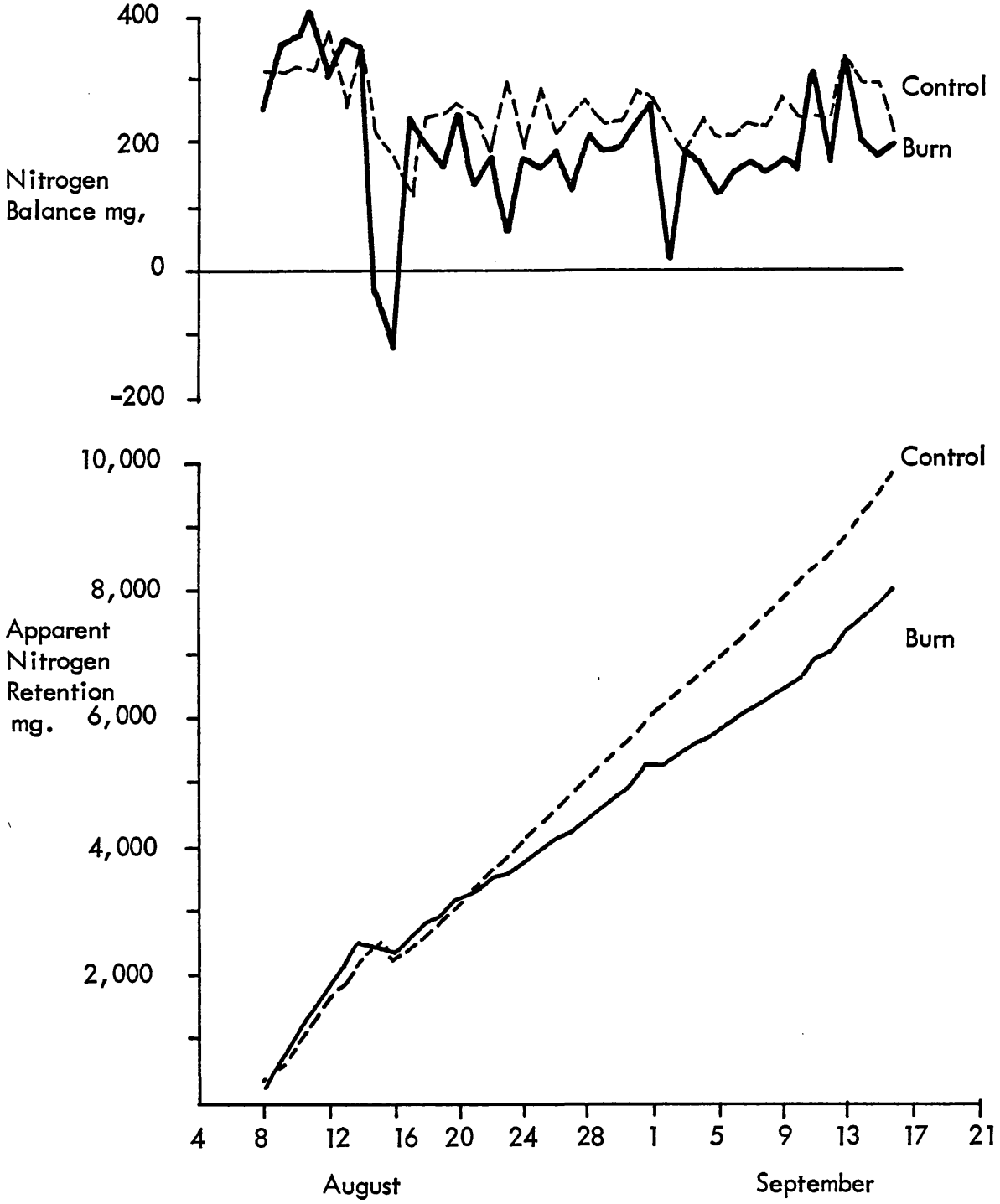


TABLE 50 A

25% BSA BURN STUDY : BURN GROUP RATS 4, 5, 7, 8, 10;

CONTROL GROUP RATS 1, 3, 6, 9 : NITROGEN EXCRETION

RATE EXPRESSED PER GRAM BODY WEIGHT

BURN GROUP

TIME PERIOD	MEAN BODY WEIGHT g.	MEAN DAILY N. EXCRETION mg.	DAILY N. EXCRETION RATE (mg/g WEIGHT)	% CHANGE FROM PRE BURN PERIOD
PRE BURN 8/8 - 14/8	297	423	1.42	PRE BURN
POST BURN 1 16/8 - 22/8	280	541	1.93	+ 35.9%
POST BURN 2 23/8 - 3/9	274	563	2.05	+ 44.4%
POST BURN 3 4/9 - 16/9	252	532	2.11	+ 48.6%

TABLE 50 B 25% BSA BURN STUDY : BURN GROUP RATS 4, 5, 7, 8, 10; CONTROL GROUP RATS 1, 3, 6, 9 : NITROGEN EXCRETION RATE EXPRESSED PER GRAM BODY WEIGHT

CONTROL GROUP

TIME PERIOD	MEAN BODY WEIGHT g.	MEAN DAILY N. EXCRETION mg	DAILY N. EXCRETION RATE (mg/g WEIGHT)	% CHANGE FROM PRE NEMB. PERIOD
PRE NEMBUTAL 8/8 - 15/8	337	496	1.47	PRE NEMBUTAL
POST NEMB. 1 17/8 - 23/8	325	463	1.42	- 3.4%
POST NEMB. 2 24/8 - 4/9	340	492	1.45	- 1.4%
POST NEMB. 3 5/9 - 16/9	352	476	1.35	- 8.2%

rats was 8007 $\pm$ 1266 (SD) mg, and in the control group rats was 9735 $\pm$ 638 (SD) mg. This is a difference of 1728 mg N overall.

For comparison, the data which is given in Figures 136, 137 and 138 is shown as conventional bar histograms of nitrogen balance before and after injury (or anaesthetic) for burned rats in Figure 139, and for control rats in Figure 140.

The significant differences between burn and control group rats are clearly shown in Figure 141 in which rat body weight is plotted against estimated cumulative nitrogen retention. Although no time markings are given, the phasic nature of the post burn weight loss is apparent. Note that the burned rat appears to continue to retain whole body nitrogen while losing body weight, an incongruous situation, which is discussed later. Compare Figure 141 with Figure 131 (20% BSA burn study).

Figure 142 shows the linear regression of nitrogen intake on nitrogen balance in the control group rats before and after anaesthesia. Where food intake nitrogen is fixed at one particular level, then the slope of any regression equation will be unduly influenced by the values of outlying points as

Fig.139 25% BSA BURN : NITROGEN BALANCE - BURN GROUP RAT Nos. 4,5,7,8,10

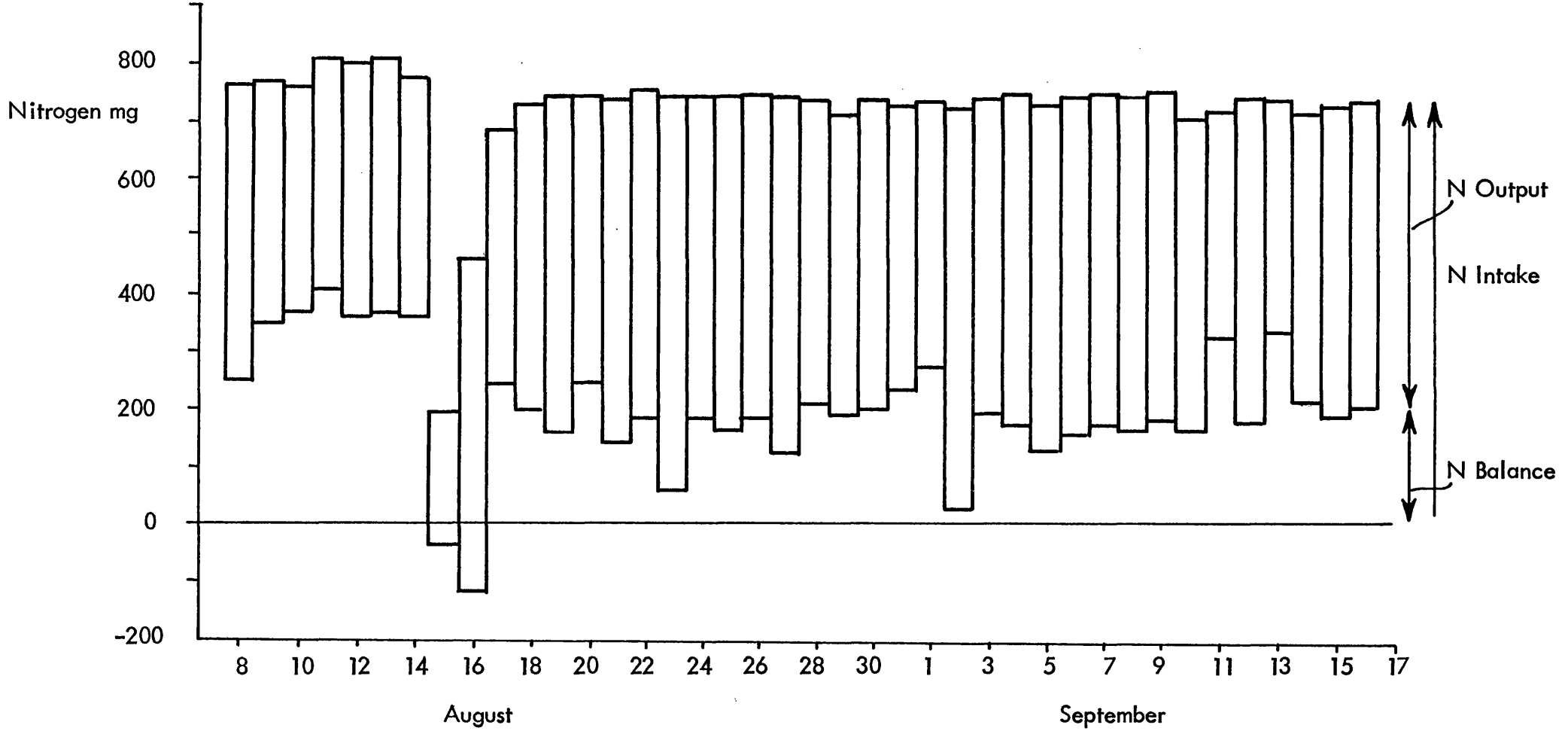


Fig.140 25% BSA BURN : NITROGEN BALANCE - CONTROL GROUP RAT Nos. 1,3,6,9

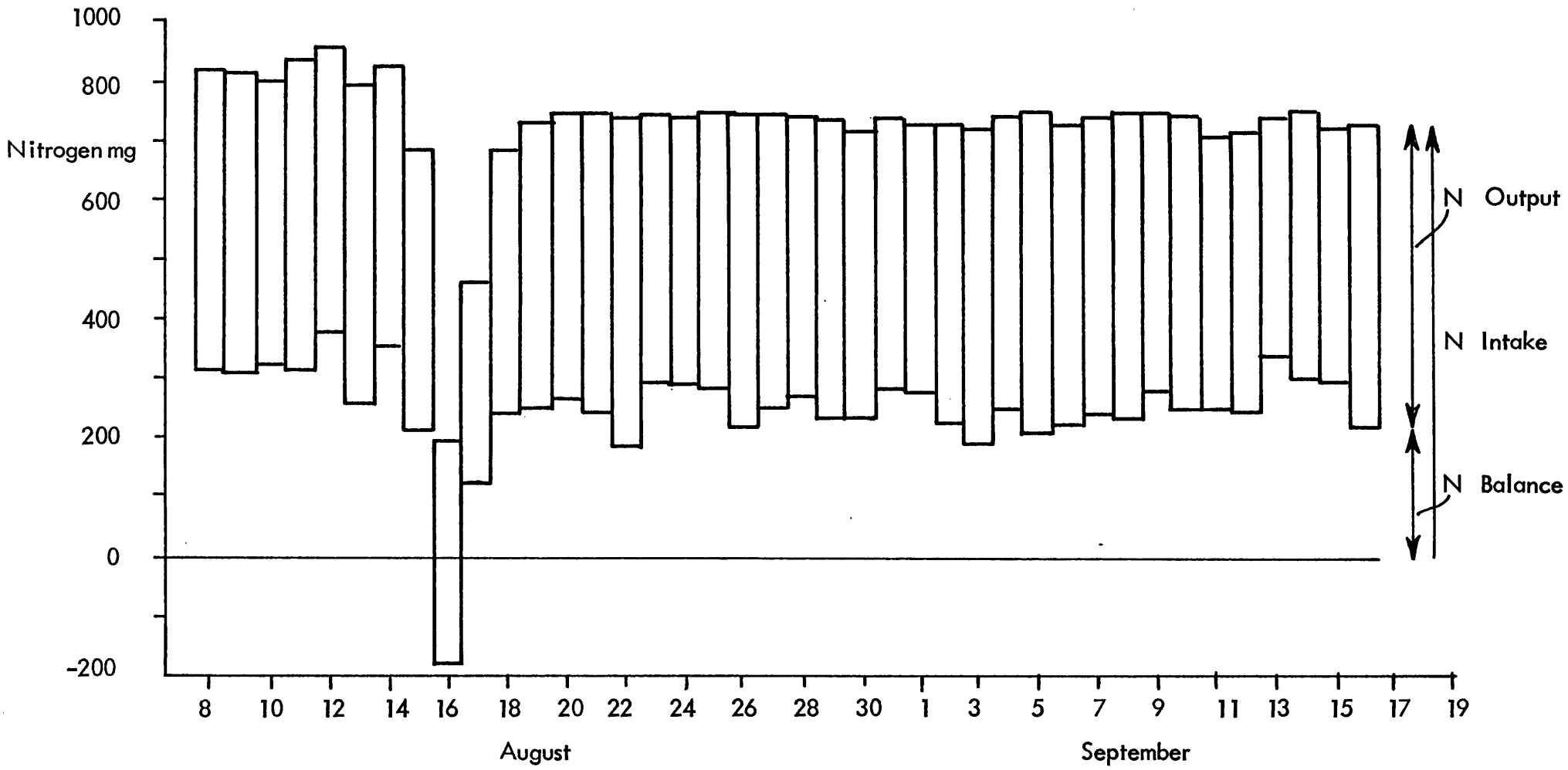


Fig. 141 25% BSA BURN : BURN GROUP RATS 4,5,7,8,10 vs. CONTROL GROUP RATS 1,3,6,9 BODY WEIGHT GAIN vs. "APPARENT" NITROGEN RETENTION

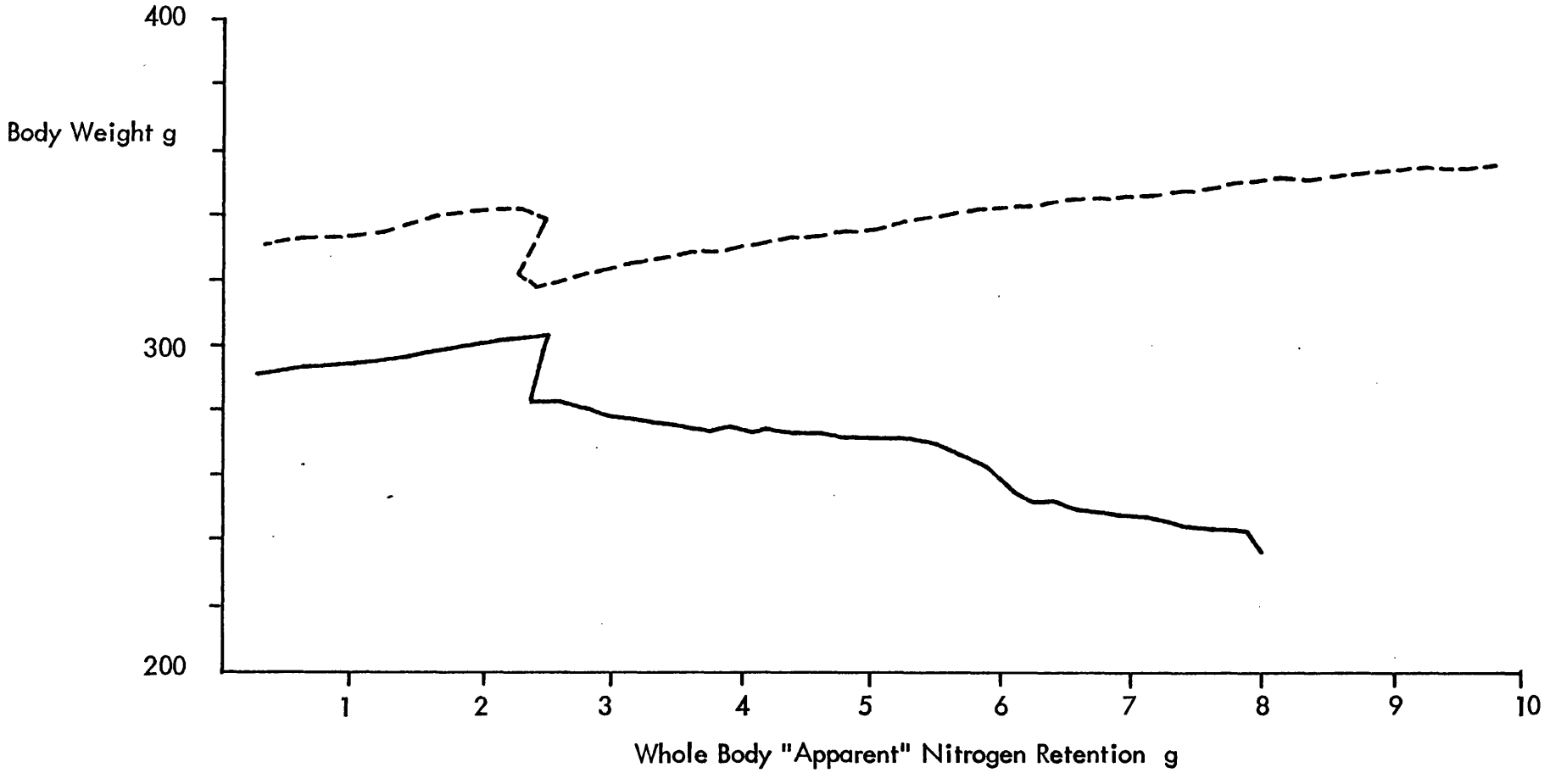
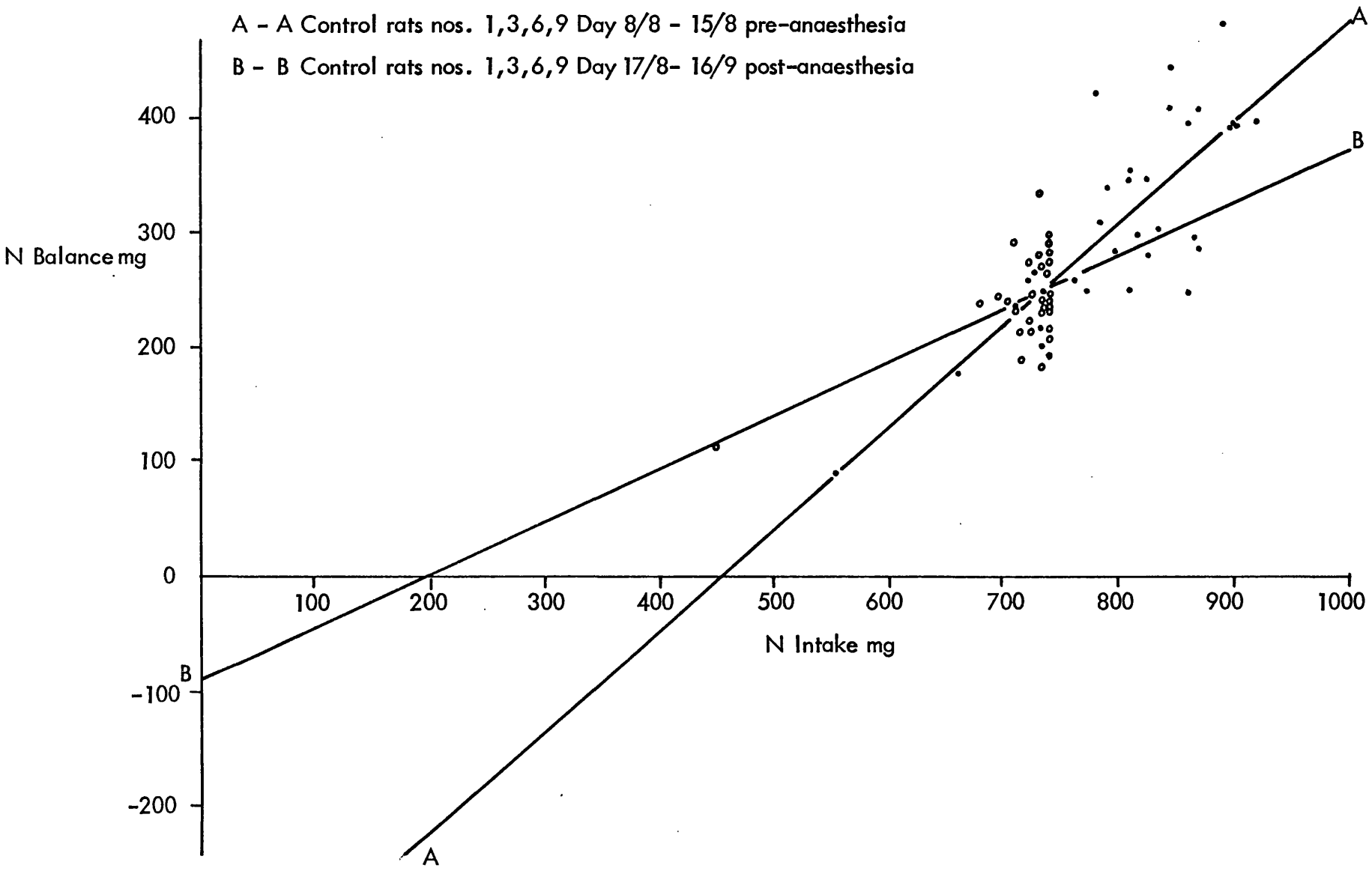


Fig. 142 LINEAR REGRESSION : NITROGEN INTAKE ON NITROGEN BALANCE : 25% BSA BURN



indicated in the 20% BSA burn study. Figure 142 illustrates this effect. This form of calculation of data did not give reliable results in the 25% BSA burn study, and is not presented; only the control rat results are shown.

METABOLIC RESPONSE TO A 25% OF BODY SURFACE AREA FULL SKIN THICKNESS BURN

DISCUSSION

A 25% of body surface area full skin thickness dorsal burn in the male Wistar rat produced significant post burn weight loss, at 20°C ambient temperature, when post injury dietary food intake was fixed at 20 g of Low Iodine Test Diet daily. This represented a 6.2% true reduction in food intake from pre injury ad libitum levels for the burned rats, and a 9.2% true reduction for pair fed control rats.

If the burned rat, kept at 20°C ambient temperature, is allowed free access to food after burn injury, the rat will try to compensate for the energy losses via the burn wound by increasing its food intake. This was shown in the 20% BSA burn study in the rat subjected to calorimetry runs (Figs. 67, 68 and 125), and has also been independently noted by Caldwell (1970, 1962) in a similar series of studies using burned rats. At 20°C ambient temperature, Caldwell's rats with a 30% BSA burn, on fixed diet, lost a mean of 1.67 g of weight per day over a 60 day

period after injury. His control rats gained 1 g in weight per day. Caldwell's results are similar to my own findings with comparable rats given a 25% BSA burn, kept at 20°C ambient temperature, where the daily mean weight loss was 1.35 g in the burned rats over a 33 day period after injury. Control rats gained 1.2 g body weight per day. The burned rats in my study lost 21.4% of their body weight, measured on the day of injury, over a 33 day post burn period. This degree of weight loss makes this model burn injury in the rat comparable in regard to weight loss after injury with the findings in extensively burned human patients. If one takes into account the "growth failure" also, shown by the burned rat, this increases the effect of the burn injury on the rat, making the total weight difference between burn and control rats 25.5% at the end of the study.

Because of differences in measured pre-burn levels of dietary nitrogen intake, the dietary restriction on control rats was slightly greater than that affecting burned rats, and greater than intended. This resulted in the growth rate of control group rats decreasing slightly as a result of restricted feeding. However, Table 50 shows that the daily nitrogen excretion rate expressed per gram of body weight was unaffected in the control group rats, until the 5th September to 16th September period, when it fell slightly from

1.45 mg N/g body weight to 1.35 mg N/g body weight. This probably represented an adaptative response to increasingly effective food deprivation (Levenson et al., 1975) as the control rats grew heavier on a fixed level of diet intake. In the burned rats, on the same level of dietary intake, there were progressive increases in the rate of nitrogen excretion, expressed per gram of body weight. By the third post burn period, the rate of nitrogen excretion was 2.11 mg N per gram of body weight. This was 48.6% greater than the pre burn level, and 56.3% greater than the control rats' rate of nitrogen excretion over the same period. These are significant metabolic changes in the burned rats, which cannot be attributed to changes in dietary intake (See Table 49).

In control and burned rats, the contrasting effects of adaptation to starvation and metabolic response to injury may be recognised.

The 25% BSA burn study revealed the inadequacy of estimating daily nitrogen balance or whole body nitrogen retention, using a fixed value of 5% of urinary nitrogen losses to account for the daily faecal nitrogen losses. In control rats, the 5% value gives reasonable results for nitrogen balance over short periods. But in burned rats, and over prolonged study periods of up to 40 days or more, small daily

errors become large cumulative discrepancies.

Figure 141 shows that, over a prolonged period of 33 days after burning, the burn group rats lost body weight at the rate of 1.35 g per day overall. Yet, despite this weight loss, the burned rats remained in positive daily nitrogen balance (See Table 49). These are incompatible observations. If one makes the assumption that the chemical composition of weight or tissue lost from the body is similar to the chemical composition of tissue gained or synthesised from retained dietary nutrients (Brozek, 1968), then the following calculations may be made.

Figure 138 shows that over the 33 day post burn period, burn group rats retained a mean of 5,506 mg of whole body nitrogen. Control group rats retained 7,496 mg of nitrogen over the same time period. Burn group rats therefore had an overall daily nitrogen balance of + 167 mg N/day. Their rate of weight loss over this period was 1.35 g/day. Taking these values as correct, for the moment, this means that for every gram of nitrogen retained in the burned rat, the rat <u>lost</u> 8.1 g body weight ! If one reverts to the assumption that lean body tissue contains approximately 1 gram of nitrogen for every 25 - 30 gram

weight of tissue, and that the weight lost also has these proportions, then for a daily weight loss of 1.35 g, the rat should have had a daily nitrogen balance of -45 to -54 mg N, to account for the weight loss, not the actual value of + 167 mg N daily which was measured, based on estimated faecal nitrogen values, and which did not take into account the likely evaporation of nitrogen, or other losses, from the glassware of the collecting system.

In the control rats, the overall daily nitrogen balance for the 33 day period after Nembutal anaesthesia was + 227 mg N/day. The rate of daily weight gain (Fig. 135) was 1.24 g/day. Therefore 5.46 g body weight were gained for every gram of nitrogen "apparently" retained.

It is of interest to note that since the same systematic errors in the measurement of nitrogen balance affect burned and control rats equally, even though the absolute values for cumulative nitrogen retention may be incorrect over a prolonged study period, the differences between burn and control groups should be valid, the errors cancelling out. Hence, the mean difference in nitrogen retention between burn group rats and control group rats was 1.99 g over the 33 day post burn period. Control rats gained a mean of 14 g body weight. Burned rats lost

a mean of 65 g body weight. Allowing for slight mean body weight differences between burn and control group rats on the day of injury, the mean weight difference between burn and control group rats was 77.5 g at the end of the study. Therefore 38.9 g body weight were lost from the burned rats for every gram of nitrogen lost, when compared with identically fed control rats. This value for nitrogen content of tissue is far more in keeping with the known proportions of nitrogen content in body tissue composed of muscle and adipose tissue (Brozek, 1968).

METABOLIC RESPONSE TO 25% BODY SURFACE AREA FULL SKIN THICKNESS BURN IN RATS UNDERGOING CALORIMETRY STUDIES OF POST BURN ENERGY EXPENDITURE

MATERIALS AND METHODS

Five male Wistar rats were kept in wall rack cages as a separate part of the 25% BSA burn study, described in the preceding section. These 5 rats (3 burn and 2 control) were subjected to exactly the same procedures as the metabolic cage rats in the previous experiment, but in addition underwent regular calorimetry studies to determine energy expenditure after 25% BSA burn injury.

The wall rack cage rats were weighed daily and food intake was measured as described previously. The rats were permitted ad libitum food prior to burn injury, but thereafter were restricted to a 20 g maximum daily intake of Low Iodine Test Diet, with added potassium iodide in their drinking water (5 ug/10 ml). In addition, control rats were fed the mean of the burn group daily food intake, from the day of injury, but with a 24 hour delay, as in the previous experiment.

The experiment began on 24th July for these 5 rats, with dorsal fur clipping. Records of daily body weight

and food intake are presented only from 9th August, which was the start of the metabolic cage collections in the previous experiment. Burn injury (25% BSA) was carried out on 14th August. Control rat Nembutal anaesthesia was administered on 15th August.

Burn and control group rats were then subjected to regular calorimetry runs, on a rotational basis, at intervals of 2 - 3 days during the post burn period. Food intake was restricted overnight only on days prior to the individual rat undergoing calorimetry runs. These 5 rats had been thoroughly tamed and handled, to accustom them fully to calorimeter conditions, prior to the recorded start of the experiment on 8th August. Calorimetry runs were carried out as described in a previous section of this thesis, but with the exception that these rats were completely unfettered within SEC - A - 04 L. No shoulder harnesses or instrument lead attachments were used, and rat body temperatures were not recorded during calorimetry runs for the reasons already given.

The study was ended on 16th October, 63 days after the burn injury.

METABOLIC RESPONSE TO 25% BODY SURFACE AREA FULL SKIN THICKNESS BURN IN RATS UNDERGOING CALORIMETRY STUDIES

RESULTS

Figure 143 shows the individual daily body weights for 3 rats in the burn (calorimetry) group, before and after injury. The rate of growth for each time period, indicated by the arrowed lines, is given by the slope (m) of the linear regression line drawn through the weight values. The intercept point of this line on the body weight axis (c) is given, as is the correlation coefficient (r) of the line. Figure 144 gives similar information for the 2 control (calorimetry) group rats.

Burn (calorimetry) group rats did not grow normally in the pre burn period (9/8 - 14/8). The mean group body weight on 9/8 was 370 $\pm$ 9 g and on 14/8 it was 369 $\pm$ 8 g. The control group rats however did grow during this period (9/8 - 15/8) by 1.16 g/day. Individual burn and control (calorimetry) group rat daily weights and food intakes are given in Tables 51, 52, 53 and 54.

After 25% BSA full skin thickness dorsal burn, the burn (calorimetry) group rats lost weight rapidly (- 3.15 g/day between 14/8 - 16/9). The rate of weight

Fig. 143 25% BSA BURN : WALL CAGE CALORIMETER BURN GROUP
RAT Nos. 3, 4, 18

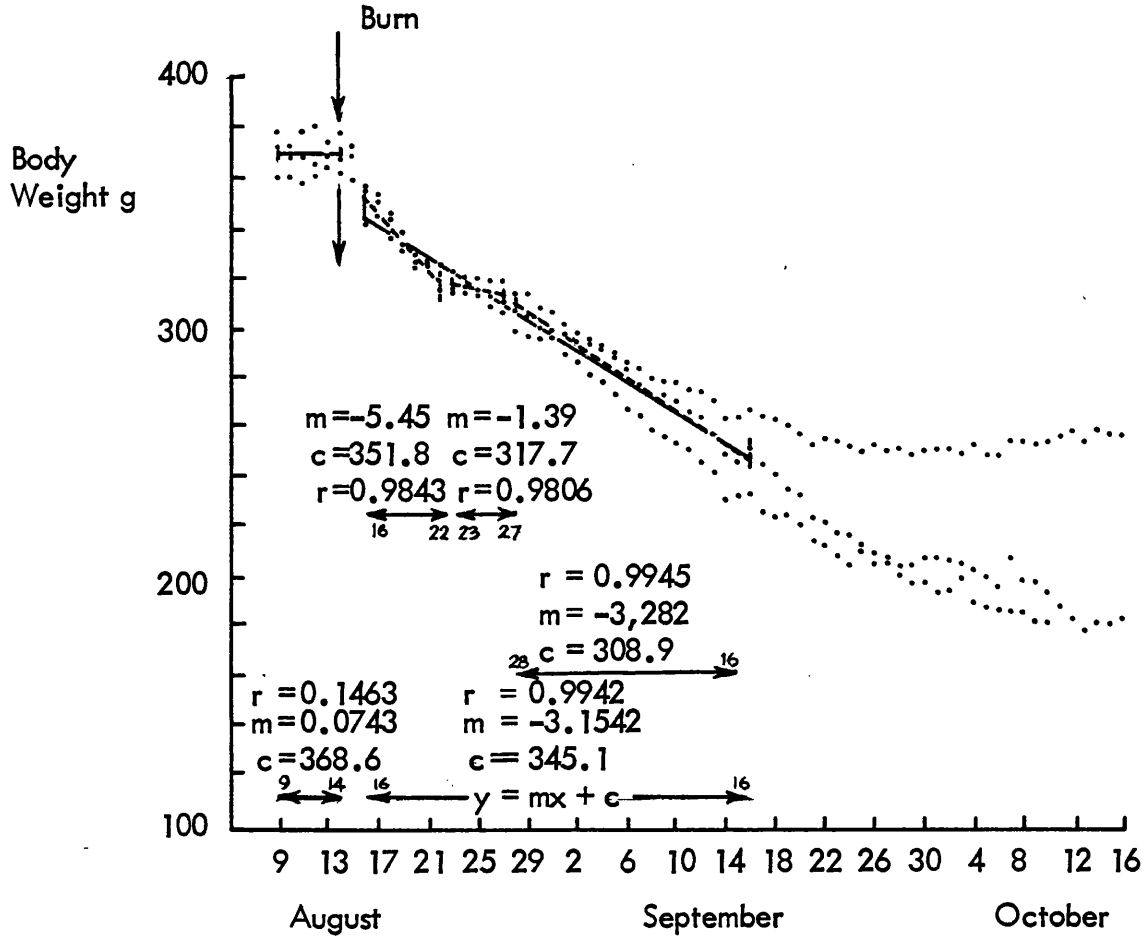
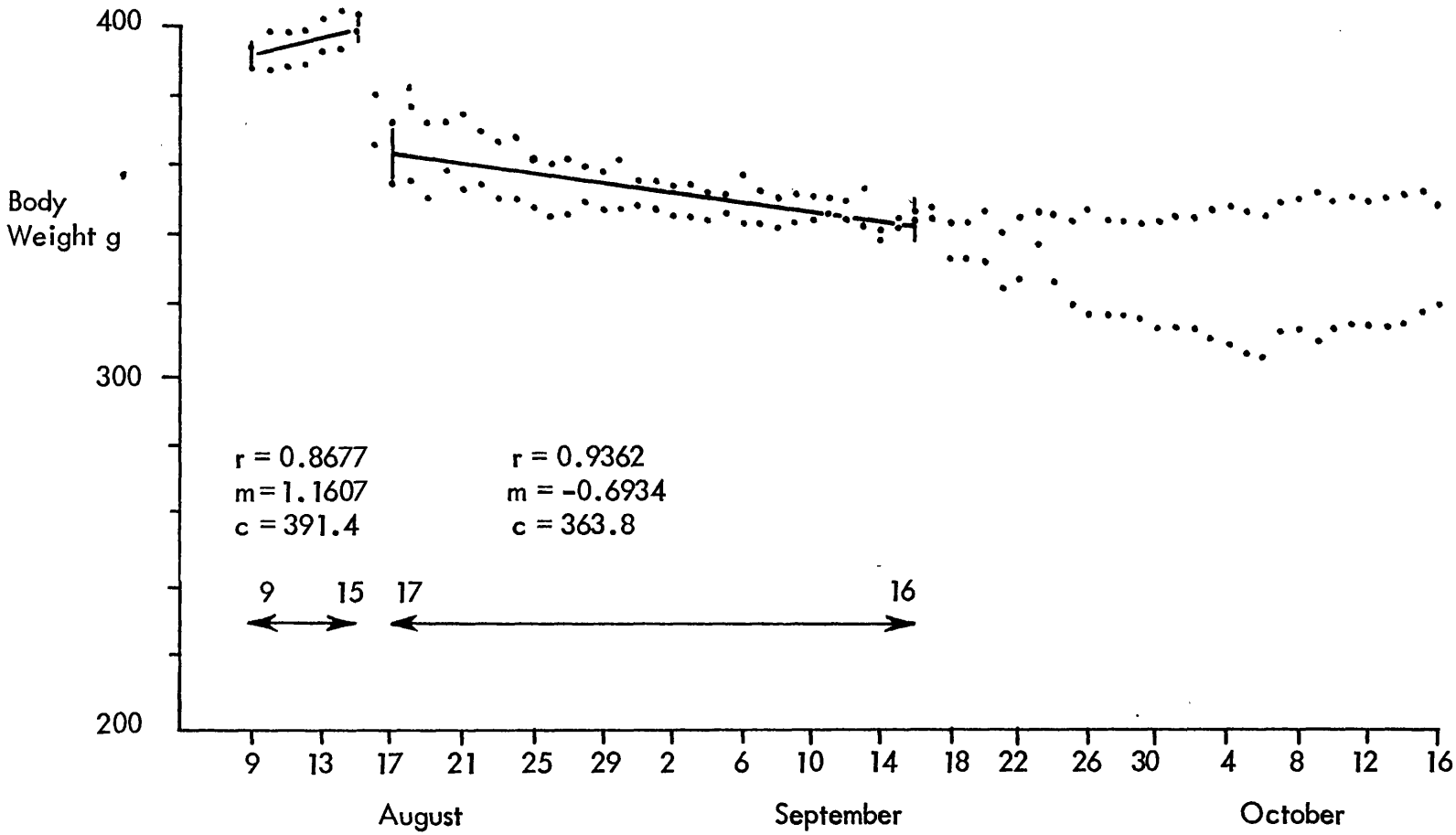


Fig. 144 25% BSA BURN: WALL CAGE CALORIMETER CONTROL GROUP RAT Nos. 1,8



loss was variable. There was an initial "step-change" downward in weight over the first 24 - 48 hours following burning. Thereafter, there was a period of steady, rapid weight loss (- 5.45 g/day between 16/8 - 22/8). A second period of less rapid weight loss then occurred (- 1.39 g/day between 23/8 - 27/8) which was of relatively short duration. A third phase of rapid weight loss followed this (- 3.28 g/day between 28/8 - 16/9), which remained steady, in 2 out of 3 rats, almost to the end of the study.

The pair fed control (calorimetry) group also lost weight over this same time period (a steady rate of weight loss of - 0.69 g/day between 17/8 - 16/9).

It would appear, at first sight, that the calorimetry runs adversely affected both burn and control group rats in their ability to grow and gain weight normally, as was the case in the 20% BSA burn study, even though the precautions outlined in the discussion section of that study had been acted upon in the calorimetry runs carried out on the rats in the 25% BSA burn study.

However, calculation of the food intake in burn and control (calorimetry) group rats indicated an alternative, more direct explanation. In the burn (calorimetry) group rats, the mean ad libitum food intake

TABLE 51 A

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY BODY WEIGHT g. BURN GROUP - RATS 3, 4, 18.

DATE	RAT 3	RAT 4	RAT 18	MEAN ± SD
9 AUGUST	378	372	360	370 ± 9
10	373	368	360	367 ± 7
11	379	368	358	368 ± 10
12	381	365	361	369 ± 10
13	375	369	364	369 ± 5
14	378	367	362	369 ± 8
15	373	369	359	367 ± 7
16	354	357	341	351 ± 8
17	350	353	345	349 ± 4
18	346	344	336	340 ± 8
19	334	339	332	335 ± 4
20	330	324	326	327 ± 3
21	327	325	327	326 ± 2
22	325	319	315	320 ± 5
23	323	314	315	317 ± 5
24	320	317	314	317 ± 3
25	320	313	314	315 ± 4
26	319	309	312	313 ± 5
27	319	307	310	312 ± 7
28	314	299	308	307 ± 8
29	313	297	305	305 ± 8
30	309	296	299	301 ± 7
31	306	296	299	300 ± 5

TABLE 51 B

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY BODY WEIGHT g. BURN GROUP - RATS 3, 4, 18.

DATE	RAT 3	RAT 4	RAT 18	MEAN ± SD
1 SEPT.	301	289	298	299 ± 2
2	298	286	296	293 ± 7
3	293	281	295	290 ± 7
4	291	278	291	287 ± 7
5	290	273	288	284 ± 9
6	283	267	286	279 ± 10
7	277	264	284	275 ± 10
8	273	259	280	271 ± 11
9	273	256	280	270 ± 12
10	270	254	279	268 ± 13
11	266	251	275	264 ± 12
12	264	246	275	262 ± 14
13	257	242	271	257 ± 15
14	249	231	264	248 ± 16
15	246	232	264	247 ± 16
16	251	233	267	250 ± 17
17	245	226	264	245 ± 19
18	240	224	263	242 ± 20
19	235	225	261	240 ± 19
20	233	221	258	237 ± 19
21	223	215	253	230 ± 20
22	221	212	255	229 ± 23
23	217	209	255	228 ± 21
24	216	205	252	224 ± 25

TABLE 51 C

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY BODY WEIGHT g. BURN GROUP - RATS 3, 4, 18.

DATE	RAT 3	RAT 4	RAT 18	MEAN ± SD
25 SEPT.	210	212	250	224 ± 23
26	205	210	253	223 ± 27
27	206	207	251	221 ± 26
28	201	204	251	219 ± 28
29	198	204	249	217 ± 28
30	197	207	250	218 ± 28
1 OCTOBER	194	207	250	217 ± 30
2	194	206	251	217 ± 30
3	189	205	249	215 ± 31
4	189	203	253	215 ± 34
5	187	199	248	211 ± 32
6	186	196	248	210 ± 34
7	186	207	255	216 ± 35
8	185	198	254	212 ± 37
9	181	197	254	211 ± 38
10	180	193	254	209 ± 40
11	187	-	256	
12	183	-	258	
13	178	-	256	
14	182	-	260	
15	181	-	257	
16	182	-	257	

TABLE 52 A

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY FOOD INTAKE g. BURN GROUP - RATS 3, 4, 18.

DATE	RAT 3	RAT 4	RAT 18	MEAN ± SD
9 AUGUST	19.4	23.6	27	23.3 ± 3.8
10	19.2	20.4	21.3	20.3 ± 1.1
11	23.3	23.3	22.3	23.0 ± 0.6
12	24.4	20.4	24.4	23.1 ± 2.3
13	18.2	22.8	24.9	22.0 ± 3.4
14	21.6	21.0	21.3	21.3 ± 0.3
15	2.2	6.6	4.3	4.4 ± 2.2
16	1.5	12.5	3.6	3.9 ± 5.8
17	19.0	17.3	17.9	18.1 ± 0.9
18	20.0	17.1	19.0	18.7 ± 1.5
19	8.7	17.6	19.0	15.1 ± 5.6
20	20.0	3.6	19.5	14.4 ± 9.3
21	18.7	20.0	19.5	19.4 ± 0.7
22	22.8	20.0	20.0	20.9 ± 1.6
23	20.0	20.0	20.0	20.0 ± 0
24	20.0	20.0	19.8	19.9 ± 0.1
25	20.0	20.0	19.8	19.9 ± 0.1
26	19.9	20.0	20.0	19.9 ± 0.1
27	20.0	20.0	19.8	19.9 ± 0.1
28	20.0	14.1	19.6	17.9 ± 3.3
29	20.0	20.0	20.0	20.0 ± 0
30	20.0	20.0	15.5	18.5 ± 2.6
31	20.0	20.0	20.0	20.0 ± 0

TABLE 52 B

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY FOOD INTAKE g. BURN GROUP - RATS 3, 4, 18.

DATE	RAT 3	RAT 4	RAT 18	MEAN ± SD
1 SEPT.	20.0	20.0	20.0	20.0 ± 0
2	20.0	20.0	20.0	20.0 ± 0
3	20.0	20.0	20.0	20.0 ± 0
4	19.8	20.0	19.8	19.9 ± 0.1
5	20.0	20.0	20.0	20.0 ± 0
6	20.0	20.0	19.7	19.9 ± 0.2
7	20.0	20.0	20.0	20.0 ± 0
8	20.0	20.0	20.0	20.0 ± 0
9	20.0	20.0	20.0	20.0 ± 0
10	20.0	20.0	20.0	20.0 ± 0
11	20.0	20.0	19.5	19.8 ± 0.3
12	20.0	20.0	20.0	20.0 ± 0
13	20.0	19.9	19.9	19.9 ± 0.1
14	20.0	20.0	20.0	20.0 ± 0
15	20.0	20.0	19.5	19.8 ± 0.3
16	20.0	20.0	20.0	20.0 ± 0
17	20.0	20.0	20.0	20.0 ± 0
18	20.0	20.0	20.0	20.0 ± 0
19	20.0	20.0	20.0	20.0 ± 0
20	20.0	20.0	20.0	20.0 ± 0
21	20.0	20.0	20.0	20.0 ± 0
22	20.0	20.0	20.0	20.0 ± 0
23	20.0	20.0	20.0	20.0 ± 0
24	20.0	20.0	20.0	20.0 ± 0

TABLE 52 C

WALL CAGE CALORIMETRY RATS ; 25% BSA BURN STUDY : DAILY FOOD INTAKE g. BURN GROUP - RATS 3, 4, 18.

DATE	RAT 3	RAT 4	RAT 18	MEAN ± SD
25 SEPT.	19.8	20.0	19.0	19.6 ± 0.5
26	20.0	20.0	20.0	20.0 ± 0
27	20.0	20.0	20.0	20.0 ± 0
28	20.0	20.0	20.0	20.0 ± 0
29	20.0	20.0	20.0	20.0 ± 0
30	20.0	20.0	20.0	20.0 ± 0
1 OCTOBER	20.0	20.0	20.0	20.0 ± 0
2	20.0	20.0	20.0	20.0 ± 0
3	20.0	20.0	20.0	20.0 ± 0
4	20.0	20.0	20.0	20.0 ± 0
5	20.0	20.0	20.0	20.0 ± 0
6	20.0	20.0	18.5	19.5 ± 0.9
7	20.0	20.0	20.0	20.0 ± 0
8	20.0	20.0	20.0	20.0 ± 0
9	19.7	20.0	20.0	19.9 ± 0.2
10	20.0	20.0	20.0	20.0 ± 0
11	19.0	-	20.0	
12	20.0	-	20.0	
13	20.0	-	20.0	
14	20.0	-	20.0	
15	20.0	-	20.0	
16	20.0	-	20.0	

TABLE 53 A

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY BODY WEIGHT g. CONTROL GROUP - RATS 1, 8.

DATE	RAT 1	RAT 8	MEAN	DATE	RAT 1	RAT 8	MEAN
9 AUG.	389	395	392	1 SEPT.	349	356	353
10	388	398	391	2	346	355	351
11	389	398	393	3	346	355	350
12	390	399	394	4	345	353	349
13	393	403	398	5	347	352	350
14	393	405	399	6	344	358	351
15	390	403	396	7	344	353	349
16	367	381	374	8	343	352	347
17	355	373	364	9	345	352	348
18	356	377	367	10	345	351	348
19	352	373	362	11	347	352	349
20	359	373	366	12	345	350	348
21	354	375	364	13	344	355	349
22	355	370	362	14	342	339	340
23	351	367	359	15	345	343	344
24	351	369	360	16	345	347	346
25	350	362	354	17	346	338	342
26	347	362	354	18	344	334	339
27	347	362	354	19	344	334	339
28	350	361	355	20	348	333	340
29	348	359	354	21	342	326	334
30	348	362	355	22	346	328	337
31	349	356	353	23	347	328	338

TABLE 53 B

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY BODY WEIGHT g. CONTROL GROUP - RATS 1, 8.

DATE	RAT 1	RAT 8	MEAN
24 SEPT.	347	327	337
25	345	329	333
26	348	319	334
27	345	318	332
28	345	318	331
29	344	317	331
30	345	314	329
1 OCT.	346	315	330
2	346	314	330
3	348	311	329
4	349	310	330
5	347	307	327
6	346	306	326
7	350	313	332
8	351	314	332
9	352	310	331
10	350	314	332
11	351	315	333
12	350	315	333
13	351	315	333
14	353	315	334
15	354	320	337
16	350	321	335

TABLE 54 A

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY FOOD INTAKE g. CONTROL GROUP - RATS 1, 8.

DATE	RAT 1	RAT 8	MEAN	DATE	RAT 1	RAT 8	MEAN
9 AUG.	26.5	20.0	23.3	1 SEPT.	20.0	19.5	19.8
10	23.4	27.8	25.6	2	20.0	19.7	19.9
11	24.0	25.8	24.9	3	20.0	19.8	19.9
12	25.3	27.0	26.2	4	20.0	19.8	19.9
13	26.5	29.0	27.8	5	19.8	19.3	19.6
14	26.3	28.6	27.5	6	20.0	20.0	20.0
15	20.0	19.0	19.5	7	20.0	19.7	19.9
16	4.4	4.4	4.4	8	20.0	19.8	19.9
17	8.0	8.0	8.0	9	20.0	20.0	20.0
18	18.1	18.1	18.1	10	20.0	20.0	20.0
19	18.7	18.6	18.7	11	20.0	20.0	20.0
20	18.3	18.2	18.3	12	19.8	19.8	19.8
21	19.8	19.5	19.7	13	20.0	19.8	19.9
22	19.8	18.3	19.1	14	20.0	18.0	19.0
23	20.0	19.7	19.9	15	20.0	19.0	19.5
24	20.0	19.7	19.9	16	20.0	19.7	19.9
25	20.0	14.0	17.0	17	20.0	14.5	17.3
26	20.0	20.0	20.0	18	20.0	15.0	17.5
27	20.0	19.7	19.9	19	20.0	18.0	19.0
28	20.0	19.7	19.9	20	20.0	18.0	19.0
29	19.8	19.8	19.8	21	20.0	16.0	18.0
30	20.0	18.5	19.3	22	20.0	18.0	19.0
31	20.0	19.7	19.9	23	20.0	18.0	19.0

TABLE 54 B

WALL CAGE CALORIMETRY RATS : 25% BSA BURN STUDY : DAILY FOOD INTAKE g. CONTROL GROUP - RATS 1, 8.

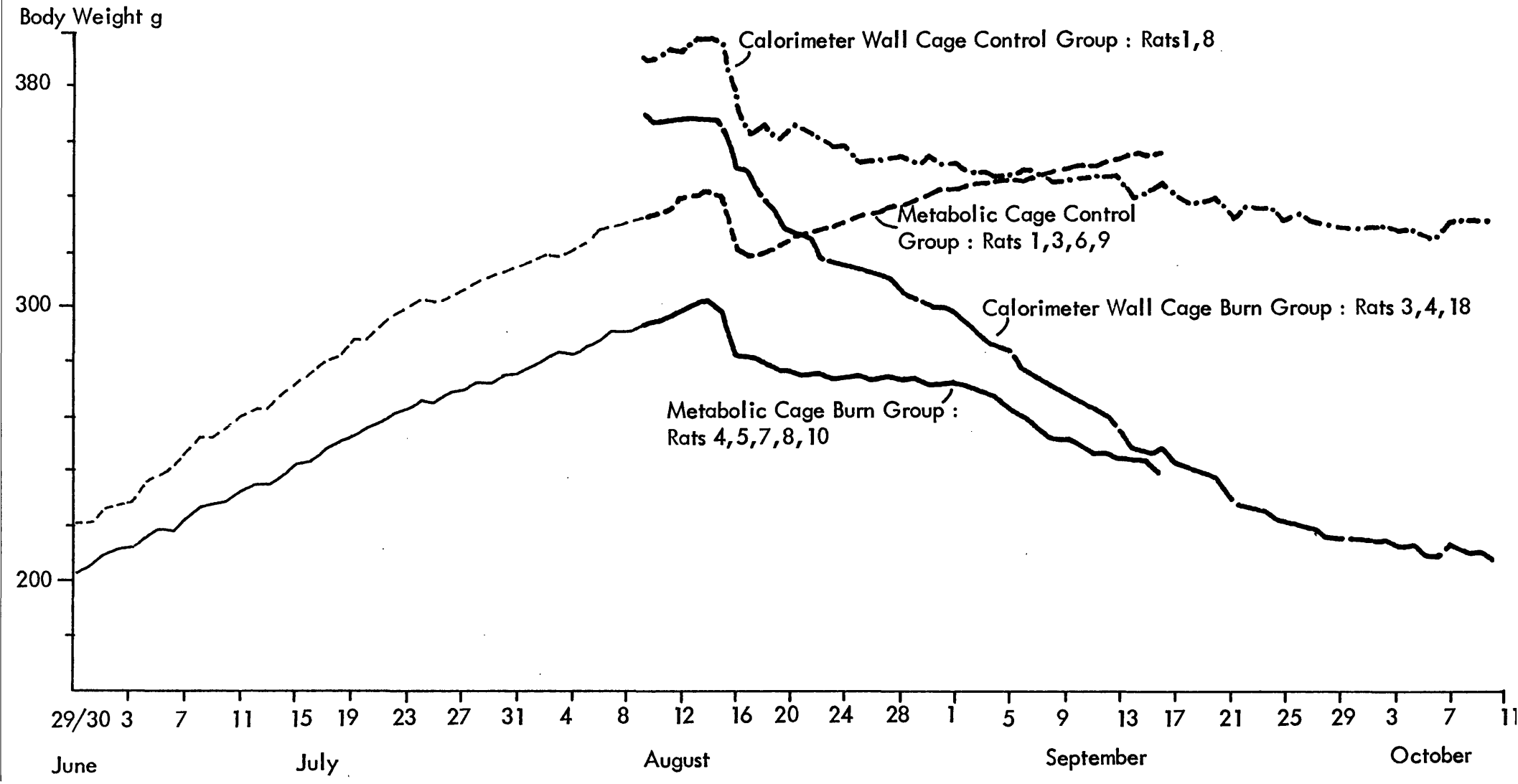
DATE	RAT 1	RAT 8	MEAN
24 SEPT.	20.0	18.0	19.0
25	20.0	18.0	19.0
26	19.4	17.9	18.7
27	20.0	18.5	19.3
28	20.0	20.0	20.0
29	20.0	18.5	19.3
30	20.0	17.0	18.5
1 OCT.	20.0	19.0	19.5
2	20.0	19.0	19.5
3	20.0	18.0	19.0
4	20.0	18.0	19.0
5	20.0	19.0	19.5
6	20.0	18.0	19.0
7	20.0	19.5	19.8
8	20.0	19.0	19.5
9	20.0	19.0	19.5
10	20.0	20.0	20.0
11	20.0	20.0	20.0
12	19.5	19.5	19.5
13	20.0	19.5	19.8
14	20.0	20.0	20.0
15	20.0	20.0	20.0
16	20.0	20.0	20.0

between 9/8 - 14/8 was 22.2 g Low Iodine Test Diet per day. After burn injury, actual dietary intake on a fixed 20 g/day allowance was 19.0 g/day, a reduction of 16.6%. In the control (calorimetry) group rats, the mean ad libitum dietary intake between 9/8 - 14/8 was 25.9 g/day, and between 17/8 - 16/9 was 10.2 g/day, a reduction of 25.9%.

These reductions in dietary intake in the calorimetry burn and control group rats were much greater than those in the metabolic cage rats of 6.6% and 9.2% in burn and control groups respectively, and were greater than intended. Calorimetry study rats grew more rapidly than anticipated during their intensive calorimetry training period in the early part of the experiment, so that on day 14/8 the mean body weight in the burn (calorimetry) group rats was 369 g, 24% greater than the 304 g mean body weight in the metabolic cage burn group rats on the same day. The same was true of the control (calorimetry) group rats with a mean body weight of 396 g, 15% greater than the mean body weight of the control (metabolic cage) group rats of 343 g on day 14/8.

These differences in mean growth rates between calorimeter wall cage rats and metabolic cage rats are shown in Figure 145. In control rats, calorimeter study rats lost weight after anaesthesia on 14/8, while metabolic cage rats gained weight. The effects of food

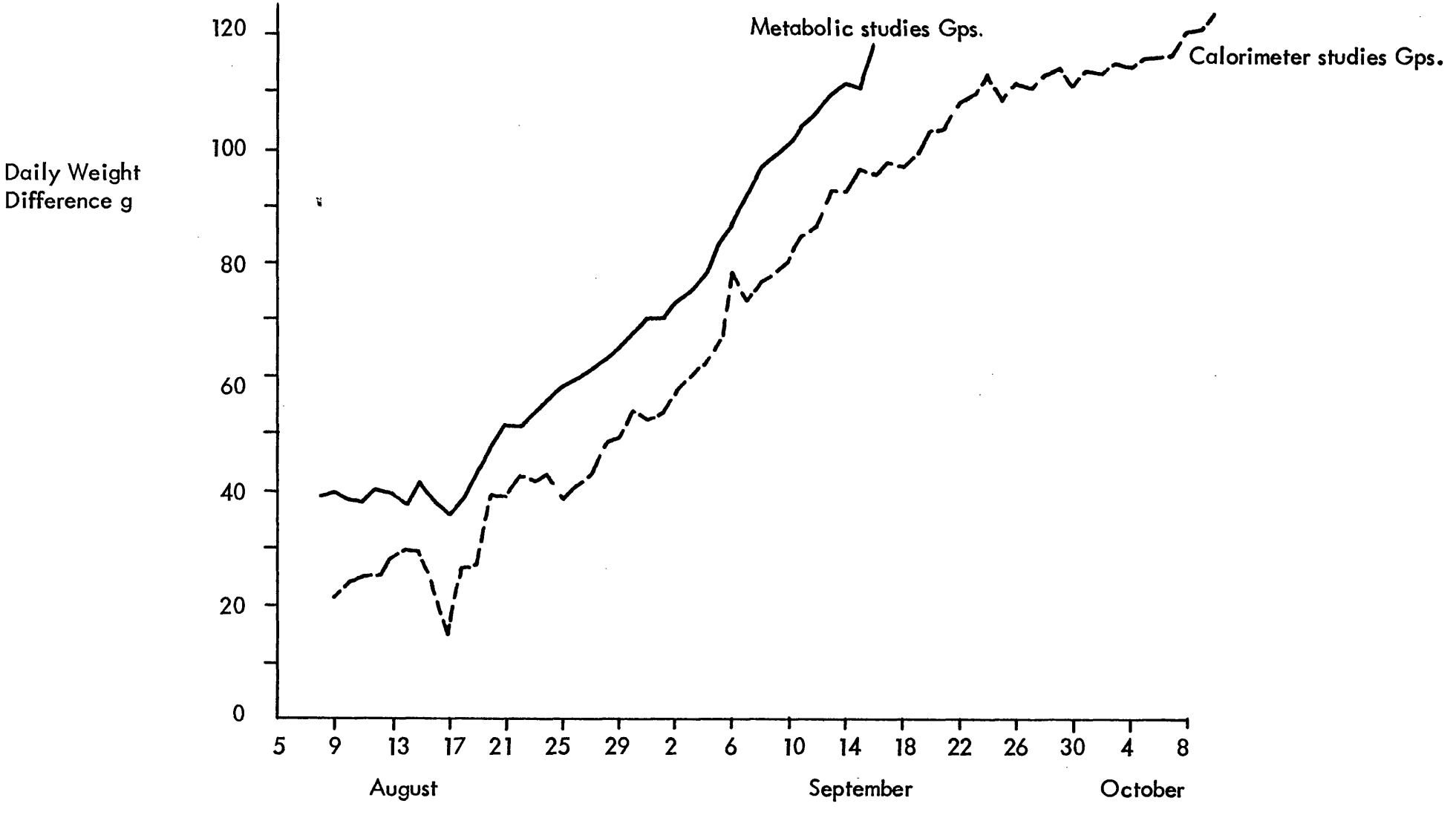
Fig.145 25% BSA BURN : EFFECT OF EXPERIMENTAL CONDITIONS AND BURN INJURY ON BODY WEIGHT CALORIMETER WALL CAGE RATS vs. METABOLIC CAGE RATS



deprivation being proportionately greater in the initially heavier rats. In burned rats, the rate of weight loss was greater in the calorimeter study group. To test whether the hypothesis that these differences between calorimeter and metabolic rats were due largely to the effects of greater food restriction in the calorimeter rats, the daily differences in mean body weights between burn and control groups were compared in calorimeter and metabolic cage rats. This is shown in Figure 146. When the effects of different dietary intakes are effectively removed, as in this form of comparison, it becomes evident that the 25% BSA burn has had an identical effect on both calorimeter rats and metabolic cage rats.

The changes in energy expenditure after a25% BSA burn (measured by gradient layer calorimetry), in one group of wall cage rats, are therefore directly relevant to the understanding of the metabolic consequences of an identical 25% BSA burn (as measured by conventional metabolic balance techniques) in another group of metabolic cage rats not subjected to calorimetry.

Fig.146 25% BSA BURN : MEAN WEIGHT DIFFERENCE CONTROL - BURN GROUP CALORIMETER vs. METABOLIC STUDIES RAT GROUPS



METABOLIC RESPONSE TO 25% BSA FULL SKIN THICKNESS BURN IN RATS UNDERGOING CALORIMETRY STUDIES

DISCUSSION

These body weight and food intake changes are discussed together with the results of the changes in energy expenditure at the end of the following section.

GRADIENT LAYER CALORIMETER MEASUREMENT OF PARTITIONED HEAT LOSSES AND HEAT PRODUCTION AFTER 25% BODY SURFACE AREA DORSAL BURN

MATERIALS AND METHODS

The procedure used for calorimetry runs in the 25% BSA burn study was as outlined in Appendices 2 - 5. Rat body thermometry was not used for the reasons previously given.

RESULTS

Figure 147 shows the mean total heat loss and heat production measured in the burn group rats over the 63 day post burn period between 14th August and 16th October. The values given for heat production and loss (in Watts) represent the rates of daily resting metabolic energy expenditure (RME) per rat. The values here are uncorrected for rat weight. The respiratory quotient values for each run are shown along with the percentage difference between directly measured total heat losses, and indirectly measured total heat production. Figure 148 shows the results for control rats presented in the same format over the 61 day period between control anaesthetic administration on 14th August and 14th October.

Fig. 147 25% BSA BURN : DIRECT-INDIRECT CALORIMETRY BURN GROUP RATS 3, 4, 18 % DIFFERENCE vs. HEAT PRODUCTION - HEAT LOSS vs RESPIRATORY QUOTIENT

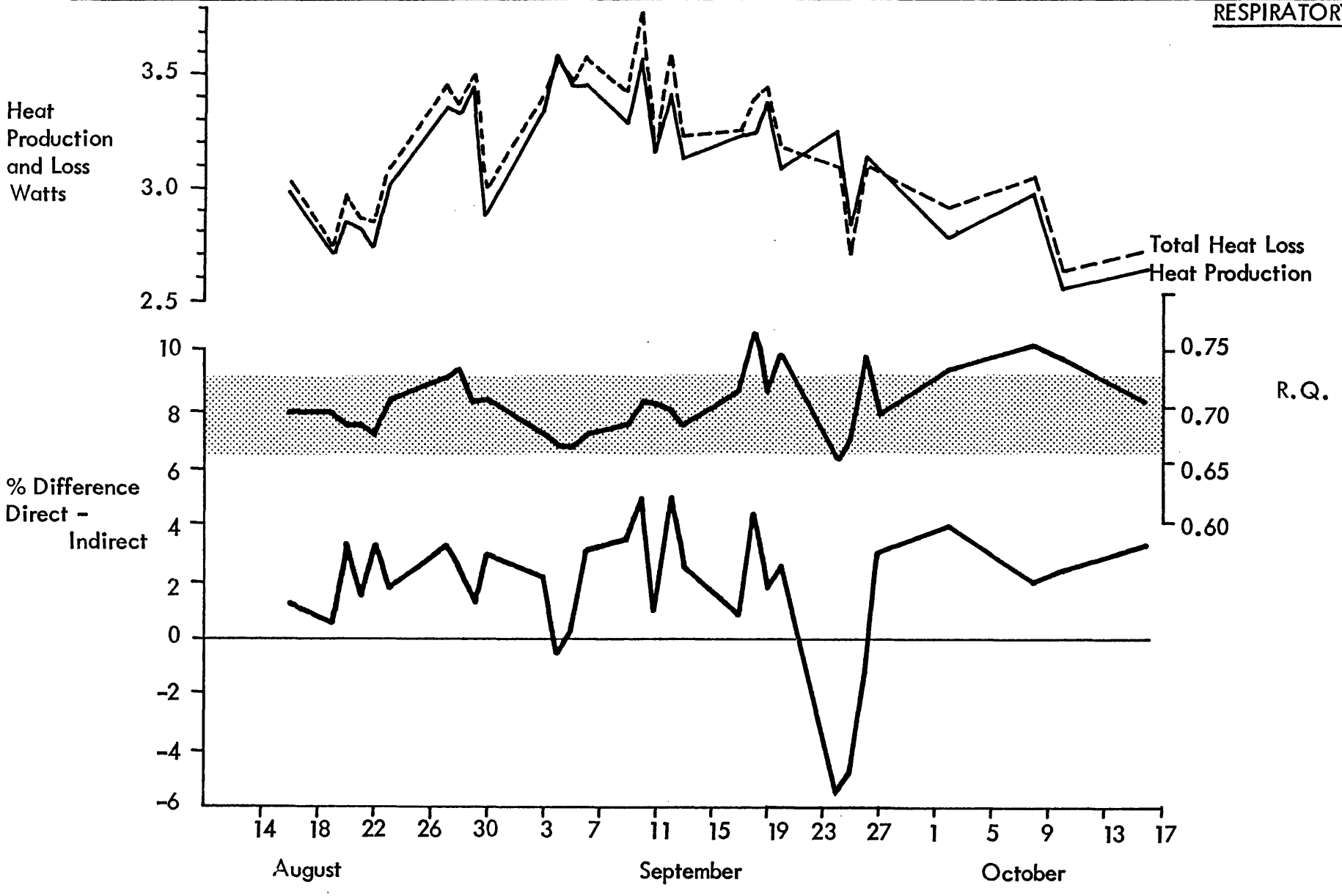
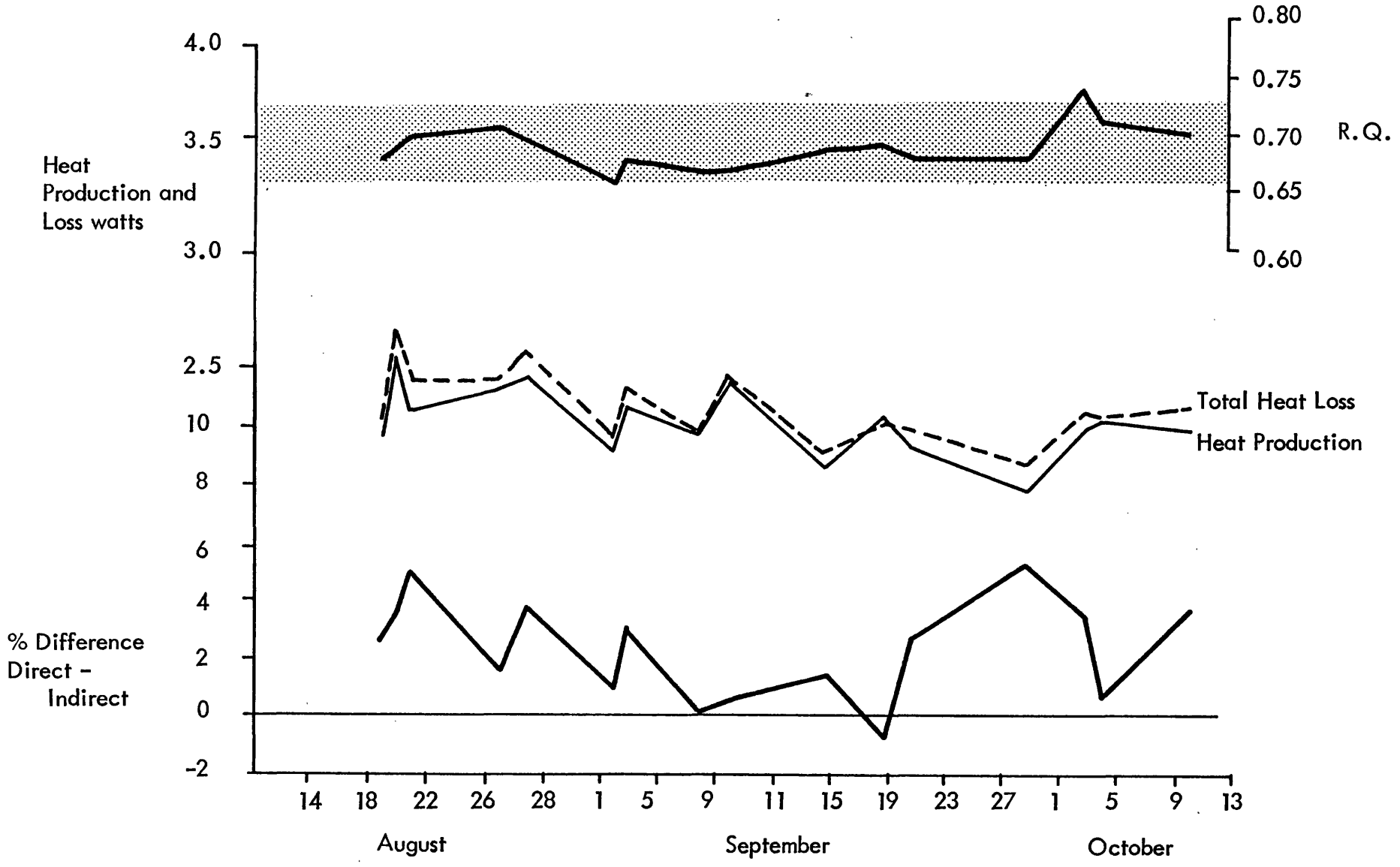


Fig. 148 25% BSA BURN : DIRECT - INDIRECT CALORIMETRY CONTROL GROUP RATS 1 and 8 % DIFFERENCE vs. HEAT PRODUCTION HEAT LOSS vs. RESPIRATORY QUOTIENT



TECHNICAL ASPECTS OF CALORIMETER PERFORMANCE DURING 25% BSA BURN STUDY:

Before describing the biological implications of the results shown in Figures 147 and 148, it is essential to consider first the performance of the calorimetry system from a technical point of view, in order to determine the degree of accuracy with which changes in energy expenditure in the rat may be measured in a long term experiment.

Details of calorimeter performance are given for each run in Tables 55 and 56, for the burn group rats and control group rats respectively. The important aspects of this information are summarised in Tables 57 and 58 for burn and control rat groups.

The first prerequisite for accepting the validity of calorimeter measurements of heat loss and heat production in rats without body temperature recordings was to establish that it was possible to obtain agreement between heat loss and heat production to better than $\pm$ 5% in burned and control rats. This would indicate that no significant change in body heat content had occurred. It has already been shown in the control group rats that the mean difference between heat loss and heat production was 2.4 $\pm$ 1.8 (SD) % (Heat loss - heat production) for this experiment. See Tables 11 and 58. In burn group rats,

TABLE 55 A (1) (See Figure 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	16/8/B	19/8/B	20/8/B	21/8/B	22/8/B
POST BURN DAY	2	5	6	7	8
RAT WEIGHT g.	352.3	332	321.9	324.7	314
ATMOSPHERIC PRESSURE mm Hg	752	770	766	761	762
W.G.M. TEMPERATURE ^{o}C	23.683	23.87	24.03	23.92	23.3
INLET AIR TEMPERATURE ^{o}C	20.20	20.56	20.45	20.35	20.28
CALORIMETER WALL TEMPERATURE ^{o}C	21.47	21.73	22.0	21.67	21.75
INLET/OUTLET AIRFLOW RATE L/min	1.258	1.319	1.287	1.268	1.258
TOTAL AIRFLOW RATE L/min	6.269	6.495	6.412	6.308	6.287
RECIRCULATED AIRFLOW RATE L/min	5.011	5.176	5.125	5.040	5.029
DEW POINT TEMPERATURE ^{o}C	15.47	16.10	15.54	15.54	15.54
INCREMENT IN AIR TEMPERATURE ^{o}C	1.97	1.74	1.74	1.70	1.80
URINE/FAECAL WEIGHT g.	1.72/0	0.57/0	0/0	0.89/0	0/0
GROSS HEAT LOSS THROUGH WALLS W.	2.148	1.785	1.816	1.773	1.861
URINE HEAT LOSS W.	0.010	3.219^{-03}	0	5.744^{-03}	0
FAECAL HEAT LOSS W.	0	0	0	0	0

TABLE 55 A (2) (See Figure 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	16/8/B	19/8/B	20/8/B	21/8/B	22/8/B
SPEC. HEAT LOSS BY EXPIRED H_2O. W.	6.622^{-03}	7.349^{-03}	9.572^{-03}	8.170^{-03}	7.803^{-03}
NET HEAT LOSS THROUGH WALLS W.	2.131	1.774	1.806	1.759	1.853
HEAT LOSS TO AIRSTREAM W.	0.273	0.250	0.246	0.237	0.250
TOTAL DIRECT HEAT LOSS W.	3.030	2.726	2.960	2.762	2.837
PARTITIONED HEAT LOSS W/kg.					
SENSIBLE	6.822	6.096	6.377	6.146	6.698
INSENSIBLE	1.799	2.115	2.819	2.361	2.337
TOTAL	8.601	8.212	9.196	8.507	9.035
PARTITIONED HEAT LOSS % OF TOTAL					
SENSIBLE	79.3	74.2	69.3	72.2	74.1
INSENSIBLE	10.7	25.8	30.7	27.8	25.9
OXYGEN CONSUMPTION ml/min	9.074	8.223	8.707	8.261	8.352
CARBON DIOXIDE PRODUCTION ml/min	6.363	5.762	5.978	5.695	5.700
RESPIRATORY QUOTIENT	0.701	0.701	0.687	0.689	0.682

TABLE 55 A (3) (See Figure 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	16/8/B	19/8/B	20/8/B	21/8/B	22/8/B
TOTAL INDIRECT HEAT PRODUCTION W.	2.992	2.711	2.960	2.716	2.741
HEAT PRODUCTION W/kg.	8.493	8.166	8.885	8.364	8.729
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	1.263	0.553	3.380	1.677	3.393
RAT NUMBER	3	3	4	3	18

TABLE 55 B (1) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	23/8/B	27/8/B	28/8/B	29/8/B	30/8/B
POST BURN DAY	9	13	14	15	16
RAT WEIGHT g.	321.5	317.1	298.5	310.7	295.9
ATMOSPHERIC PRESSURE mm/Hg.	755	760	760	758	759
W.G.M. TEMPERATURE ^{o}C	23.44	20.715	20.51	20.285	19.728
INLET AIR TEMPERATURE ^{o}C	20.30	20.052	20.109	20.121	20.049
CALORIMETER WALL TEMPERATURE ^{o}C	21.82	21.19	21.32	21.75	-
INLET/OUTLET AIRFLOW RATE L/min	1.284	1.303	1.287	1.233	1.276
TOTAL AIRFLOW RATE L/min	6.287	6.446	6.257	6.172	6.372
RECIRCULATED AIRFLOW RATE L/min	5.003	5.143	4.970	4.939	5.096
DEW POINT TEMPERATURE ^{o}C	15.59	15.656	15.742	15.711	15.62
INCREMENT IN AIR TEMPERATURE ^{o}C	1.97	1.85	1.91	2.01	1.58
URINE/FAECAL WEIGHT g	0/0	0/0	0/0	0/0	0/2.47
GROSS HEAT LOSS THROUGH WALLS W.	1.752	2.057	1.920	2.025	1.721
URINE HEAT LOSS W.	0	0	0	0	0
FAECAL HEAT LOSS W.	0	0	0	0	0.015

TABLE 55 B (2) (See Fig. 147). CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	23/8/B	27/8/B	28/8/B	29/8/B	30/8/B
SPEC. HEAT LOSS BY EXPIRED H_2O. W.	0.011	0.012	0.013	0.013	0.012
NET HEAT LOSS THROUGH WALLS W.	1.741	2.044	1.908	2.013	1.694
HEAT LOSS TO AIRSTREAM W.	0.274	0.263	0.264	0.274	0.222
TOTAL DIRECT HEAT LOSS W.	3.071	3.463	3.358	3.490	2.976
PARTITIONED HEAT LOSS W/kg.					
SENSIBLE	6.265	7.277	7.275	7.361	6.477
INSENSIBLE	3.286	3.645	3.976	3.874	3.584
TOTAL	9.551	10.922	11.251	11.236	10.061
PARTITIONED HEAT LOSS % OF TOTAL					
SENSIBLE	65.6	66.6	64.7	65.5	64.4
INSENSIBLE	34.4	33.4	35.3	34.5	35.6
OXYGEN CONSUMPTION ml/min	9.136	10.069	9.832	10.416	8.739
CARBON DIOXIDE PRODUCTION ml/min	6.444	7.356	7.252	7.410	6.184

TABLE 55 B (3) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	23/8/B	27/8/B	28/8/B	29/8/B	30/8/B
RESPIRATORY QUOTIENT	0.705	0.731	0.738	0.711	0.708
TOTAL INDIRECT HEAT PRODUCTION W.	3.016	3.345	3.272	3.443	2.886
HEAT PRODUCTION W/kg.	9.380	10.548	10.961	11.084	9.756
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	1.794	3.420	2.576	1.350	3.028
RAT NUMBER	3	3	4	3	18

TABLE 55 C (1) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18

DATE	3/9/B	4/9/B	5/9/B	6/9/B	9/9/B
POST BURN DAY	20	21	22	23	26
WAT WEIGHT g.	290.6	275.3	287.4	283.1	270.2
ATMOSPHERIC PRESSURE mm Hg.	736	743	739	740	751
W.G.M. TEMPERATURE ^{o}C	20.228	21.223	19.429	21.562	19.593
INLET AIR TEMPERATURE ^{o}C	20.125	20.199	20.045	20.212	20.052
CALORIMETER WALL TEMPERATURE ^{o}C	21.22	21.47	-	21.54	21.30
INLET/OUTLET AIRFLOW RATE L/min	1.256	1.221	1.258	1.201	1.263
TOTAL AIRFLOW RATE L/min	6.159	6.007	6.244	5.961	6.184
RECIRCULATED AIRFLOW RATE L/min	4.903	4.786	4.986	4.760	4.921
DEW POINT TEMPERATURE ^{o}C	15.653	15.78	15.632	15.781	15.606
INCREMENT IN AIR TEMPERATURE ^{o}C	1.92	2.01	2.01	1.90	2.0
URINE/FAECAL WEIGHT g.	0/0	0/0	0.47/0	0/1.04	0.82/0
GROSS HEAT LOSS THROUGH WALLS W.	2.009	1.882	2.017	2.060	1.954
URINE HEAT LOSS W.	0	0	2.723^{-03}	0	5.030^{-03}
FAECAL HEAT LOSS W.	0	0	0	6.333^{-03}	0

TABLE 55 C (2) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	3/9/B	4/9/B	5/9/B	6/9/B	9/9/B
SPEC. HEAT LOSS BY EXPIRED H_2O. W.	0.012	0.015	0.013	0.014	0.013
NET HEAT LOSS THROUGH WALLS W.	1.996	1.867	2.002	2.040	1.936
HEAT LOSS TO AIRSTREAM W.	0.261	0.267	0.277	0.250	0.273
TOTAL DIRECT HEAT LOSS W.	3.409	3.569	3.453	3.570	3.412
PARTITIONED HEAT LOSS W/kg.					
SENSIBLE	7.768	7.753	7.929	8.088	8.177
INSENSIBLE	3.962	5.214	4.084	4.521	4.449
TOTAL	11.730	12.966	12.013	12.609	12.626
PARTITIONED HEAT LOSS % OF TOTAL					
SENSIBLE	66.2	59.8	66.0	64.1	64.8
INSENSIBLE	33.8	40.2	44.0	35.9	35.2
OXYGEN CONSUMPTION ml/min	10.172	10.950	10.507	10.560	10.015
CARBON DIOXIDE PRODUCTION ml/min	6.881	7.387	7.088	7.128	6.873

TABLE 55 C (3) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	3/9/B	4/9/B	5/9/B	6/9/B	9/9/B
RESPIRATORY QUOTIENT	0.676	0.675	0.675	0.675	0.686
TOTAL INDIRECT HEAT PRODUCTION W.	3.333	3.586	3.441	3.459	3.290
HEAT PRODUCTION W/kg.	11.469	13.028	11.973	12.217	12.175
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	2.232	-0.480	0.335	3.108	3.571
RAT NUMBER	3	4	3	18	3

TABLE 55 D (1) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	10/9/B	11/9/B	12/9/B	13/9/B	17/9/B
POST BURN DAY	27	28	29	30	34
RAT WEIGHT g.	250.4	263.0	237.5	255.0	241.4
ATMOSPHERIC PRESSURE mm Hg	761	760	758	751	757
W.G.M. TEMPERATURE ^{o}C	19.798	19.821	19.879	20.214	19.391
INLET AIR TEMPERATURE ^{o}C	20.046	20.033	20.065	20.092	19.979
CALORIMETER WALL TEMPERATURE ^{o}C	-	21.29	-	-	-
INLET/OUTLET AIRFLOW RATE L/min	1.269	1.263	1.223	1.278	1.260
TOTAL AIRFLOW RATE L/min	6.180	6.193	6.056	6.246	6.309
RECIRCULATED AIRFLOW RATE L/min	4.911	4.930	4.833	4.968	5.049
DEW POINT TEMPERATURE ^{o}C	15.621	15.629	15.635	15.681	15.593
INCREMENT IN AIR TEMPERATURE ^{o}C	1.82	1.62	1.83	1.94	1.63
URINE/FAECAL WEIGHT g.	1.41/0.57	0.34/0	0.64/0.5	1.83/0	1.56/0
GROSS HEAT LOSS THROUGH WALLS W.	2.155	1.838	1.929	1.759	1.893
URINE HEAT LOSS W.	8.222^{-03}	1.997^{-03}	3.945^{-03}	11.920^{-03}	9.735^{-03}
FAECAL HEAT LOSS W,	3.324^{-03}	0	3.082^{-03}	0	0

TABLE 55 D (2) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	10/9/B	11/9/B	12/9/B	13/9/B	17/9/B
SPEC. HEAT LOSS BY EXPIRED H_2O. W.	0.015	0.013	0.016	0.013	0.013
NET HEAT LOSS THROUGH WALLS W.	2.128	1.823	1.906	1.735	1.870
HEAT LOSS TO AIRSTREAM W.	0.248	0.221	0.245	0.268	0.227
TOTAL DIRECT HEAT LOSS W.	3.776	3.198	3.592	3.212	3.269
PARTITIONED HEAT LOSS W/kg.					
SENSIBLE	9.493	7.775	9.058	7.853	8.689
INSENSIBLE	5.588	4.386	6.070	4.746	4.855
TOTAL	15.081	12.161	15.128	12.599	13.544
PARTITIONED HEAT LOSS % OF TOTAL					
SENSIBLE	62.9	63.9	59.9	62.3	64.2
INSENSIBLE	37.1	36.1	40.1	37.7	35.8
OXYGEN CONSUMPTION ml/min	10.850	9.573	10.355	9.527	9.790
CARBON DIOXIDE PRODUCTION ml/min	7.742	6.789	7.228	6.526	7.010

TABLE 55 E (1) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	18/9/B	19/9/B	20/9/B	24/9/B
POST BURN DAY	35	36	37	41
RAT WEIGHT g.	220.0	231.5	217.2	212.8
ATMOSPHERIC PRESSURE mm Hg	766	760	761	750
W.G.M. TEMPERATURE ^{o}C	19.196	19.34	19.01	18.404
INLET AIR TEMPERATURE ^{o}C	19.986	19.998	19.924	19.893
CALORIMETER WALL TEMPERATURE ^{o}C	-	-	-	-
INLET/OUTLET AIRFLOW RATE L/min	1.297	1.277	1.280	1.281
TOTAL AIRFLOW RATE L/min	6.287	6.142	6.269	6.217
RECIRCULATED AIRFLOW RATE L/min	4.990	4.865	4.989	4.936
DEW POINT TEMPERATURE ^{o}C	15.585	15.614	15.552	15.506
INCREMENT IN AIR TEMPERATURE ^{o}C	1.773	1.636	1.516	1.64
URINE/FAECAL WEIGHT g.	0.424/0	1.65/0.45	1.143/0	1.2/0.3
GROSS HEAT LOSS THROUGH WALLS W.	2.020	2.103	1.836	1.805
URINE HEAT LOSS W_u	7.458^{-03}	9.200^{-03}	11.105^{-03}	7.110^{-03}
FAECAL HEAT LOSS W.	0	2.509^{-03}	0	1.778^{-03}

TABLE 55 E (2) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	18/9/B	19/9/B	20/9/B	24/9/B
SPEC. HEAT LOSS BY EXPIRED H_2O W.	0.012	0.013	0.013	0.012
NET HEAT LOSS THROUGH WALLS W.	2.001	2.079	1.812	1.784
HEAT LOSS TO AIRSTREAM W.	0.246	0.222	0.210	0.225
TOTAL DIRECT HEAT LOSS W.	3.394	3.448	3.179	3.091
PARTITIONED HEAT LOSS W/kg.				
SENSIBLE	10.212	9.937	9.312	9.444
INSENSIBLE	5.215	4.958	5.329	5.083
TOTAL	15.427	14.895	14.641	14.527
PARTITIONED HEAT LOSS % OF TOTAL				
SENSIBLE	66.2	66.7	63.6	65.0
INSENSIBLE	33.8	33.3	36.4	35.0
OXYGEN CONSUMPTION ml/min	9.663	10.225	9.286	9.987
CARBON DIOXIDE PRODUCTION ml/min	7.454	7.329	6.948	6.633

TABLE 55 E (3) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	18/9/B	19/9/B	20/9/B	24/9/B
RESPIRATORY QUOTIENT	0.771	0.717	0.748	0.664
TOTAL INDIRECT HEAT PRODUCTION W.	3.243	3.385	3.099	3.262
HEAT PRODUCTION W/kg.	14.742	14.621	14.270	15.332
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	4.443	1.838	2.533	-5.544
RAT NUMBER	4	3	4	3

TABLE 55 F (1) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18

DATE	25/9/B	27/9/B	2/10/B	8/10/B	10/10/B
POST BURN DAY	42	44	49	55	57
RAT WEIGHT g.	209.0	203.9	203.1	194.3	177.5
ATMOSPHERIC PRESSURE mm Hg	738	747	759	758	762
W.G.M. TEMPERATURE ^{o}C	18.87	19.044	18.975	19.272	19.535
INLET AIR TEMPERATURE ^{o}C	19.896	20.16	20.062	20.09	20.058
CALORIMETER WALL TEMPERATURE ^{o}C	-	-	-	-	21.13
INLET/OUTLET AIRFLOW RATE L/min	1.261	1.255	1.279	1.304	1.301
TOTAL AIRFLOW RATE L/min	6.104	6.118	6.294	6.275	6.260
RECIRCULATED AIRFLOW RATE L/min	4.843	4.864	5.015	4.971	4.959
DEW POINT TEMPERATURE ^{o}C	15.54	15.572	15.532	15.566	15.586
INCREMENT IN AIR TEMPERATURE ^{o}C	1.67	1.36	1.133	1.29	0.984
URINE/FAECAL WEIGHT g.	1.22/0.5	1.06/0.4	1.096/0	0.67/0.77	0.154/0.143
GROSS HEAT LOSS THROUGH WALLS W.	1.590	1.634	1.738	1.655	1.566
URINE HEAT LOSS W.	8.123^{-03}	6.595^{-03}	9.795^{-03}	3.964^{-03}	1.660^{-03}
FAECAL HEAT LOSS W.	3.329^{-03}	2.489^{-03}	0	4.556^{-03}	1.541^{-03}

TABLE 55 F (2) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	25/9/B	27/9/B	2/10/B	8/10/B	10/10/B
SPEC. HEAT LOSS BY EXPIRED H_2O W.	0.010	0.012	0.012	0.014	0.011
NET HEAT LOSS THROUGH WALLS W.	1.568	1.613	1.716	1.632	1.552
HEAT LOSS TO AIRSTREAM W.	0.255	0.184	0.157	0.179	0.136
TOTAL DIRECT HEAT LOSS W.	2.705	2.879	2.904	3.061	2.627
PARTITIONED HEAT LOSS W/kg.					
SENSIBLE	8.581	8.813	9.228	9.323	9.513
INSENSIBLE	4.362	5.310	5.075	6.434	5.292
TOTAL	12.943	14.123	12.303	15.757	14.804
PARTITIONED HEAT LOSS % OF TOTAL					
SENSIBLE	66.3	62.4	64.5	59.2	64.3
INSENSIBLE	33.7	37.6	35.4	40.8	35.7
OXYGEN CONSUMPTION ml/min	8.642	8.470	8.380	8.965	7.670
CARBON DIOXIDE PRODUCTION ml/min	5.881	5.940	6.163	6.800	5.784

TABLE 55 F (3) (See Fig. 147) CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	25/9/B	27/9/B	2/10/B	8/10/B	10/10/B
RESPIRATORY QUOTIENT	0.680	0.701	0.735	0.759	0.754
TOTAL INDIRECT HEAT PRODUCTION W.	2.834	2.793	2.787	2.999	2.563
HEAT PRODUCTION W/kg	13.562	13.701	13.727	15.440	14.444
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	-4.781	2.989	4.026	2.015	2.434
RAT NUMBER	4	4	4	4	3

TABLE 55 G (1) (See Fig. 147)

CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	16/10/B
POST BURN DAY	63
RAT WEIGHT g.	179.5
ATMOSPHERIC PRESSURE mm Hg	758
W.G.M. TEMPERATURE ^{o}C	19.669
INLET AIR TEMPERATURE ^{o}C	20.12
CALORIMETER WALL TEMPERATURE ^{o}C	21.09
INLET/OUTLET AIRFLOW RATE L/min	1.292
TOTAL AIRFLOW RATE L/min	6.343
RECIRCULATED AIRFLOW RATE L/min	5.051
DEW POINT TEMPERATURE ^{o}C	15.566
INCREMENT IN AIR TEMPERATURE ^{o}C	1.12
URINE/FAECAL WEIGHT g.	0.76/0.46
GROSS HEAT LOSS THROUGH WALLS W.	1.563
URINE HEAT LOSS W.	5.079^{-03}
FAECAL HEAT LOSS W.	3.074^{-03}
SPEC. HEAT LOSS BY EXPIRED H_2O W.	0.012
NET HEAT LOSS THROUGH WALLS W.	1.543
HEAT LOSS TO AIRSTREAM W.	0.157
TOTAL DIRECT HEAT LOSS W.	2.725
PARTITIONED HEAT LOSS W/kg	
SENSIBLE	9.475
INSENSIBLE	5.713
TOTAL	15.187

TABLE 55 G (2) (See Fig. 147)

CALORIMETRY RESULTS : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18.

DATE	16/10/B
PARTITIONED HEAT LOSS % OF TOTAL	
SENSIBLE	62.4
INSENSIBLE	37.6
OXYGEN CONSUMPTION ml/min	7.966
CARBON DIOXIDE PRODUCTION ml/min	5.679
RESPIRATORY QUOTIENT	0.713
TOTAL INDIRECT HEAT PRODUCTION W.	2.634
HEAT PRODUCTION W/kg	14.680
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	3.341
RAT NUMBER	3

TABLE 56 A (1) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DAY	19/8/B	20/8/B	21/8/B	27/8/B
POST ANAESTHESIA DAY	5	6	7	13
RAT WEIGHT g.	348.0	365.2	349.6	343.5
ATMOSPHERIC PRESSURE mm Hg	770	765	761	761
W.G.M. TEMPERATURE ^{o}C	24.687	24.349	24.443	21.195
INLET AIR TEMPERATURE ^{o}C	20.499	20.554	20.429	20.635
CALORIMETER WALL TEMPERATURE ^{o}C	22.46	22.52	22.30	22.03
INLET OUTLET AIRFLOW RATE L/min	1.278	1.282	1.269	1.306
TOTAL AIRFLOW RATE L/min	6.433	6.341	6.329	6.406
RECIRCULATED AIRFLOW RATE L/min	5.155	5.059	5.060	5.100
DEW POINT TEMPERATURE ^{o}C	15.632	15.595	15.520	15.72
INCREMENT IN AIR TEMPERATURE ^{o}C	1.67	1.87	1.64	1.52
URINE/FAECAL WEIGHT g	0/0	0/0	0/0	0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.835	2.135	1.994	2.001
URINE HEAT LOSS W.	0	0	0	0

TABLE 56 A (2) (See Fig. 148 CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DAY	19/8/B	20/8/B	21/8/B	27/8/B
FAECAL HEAT LOSS W.	0	0	0	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	2.261^{-03}	3.120^{-03}	2.372^{-03}	2.510^{-03}
NET HEAT LOSS THROUGH WALLS W.	1.833	2.132	1.991	1.999
HEAT LOSS TO AIRSTREAM W.	0.237	0.262	0.229	0.215
TOTAL DIRECT HEAT LOSS W.	2.284	2.674	2.443	2.451
PARTITIONED HEAT LOSS W/kg				
SENSIBLE	5.949	6.554	6.351	6.444
INSENSIBLE	0.615	0.767	0.638	0.691
TOTAL	6.564	7.321	6.989	7.135
PARTITIONED HEAT LOSS % OF TOTAL				
SENSIBLE	90.6	89.5	90.9	90.3
INSENSIBLE	9.4	10.5	9.1	9.7
OXYGEN CONSUMPTION ml/min	6.782	7.755	7.041	7.296
CARBON DIOXIDE PRODUCTION ml/min	4.629	5.352	4.954	5.204

TABLE 56 A (3) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DAY	19/8/B	20/8/B	21/8/B	27/8/B
RESPIRATORY QUOTIENT	0.683	0.690	0.704	0.713
TOTAL INDIRECT HEAT PRODUCTION W.	2.226	2.550	2.323	2.413
HEAT PRODUCTION W/kg	6.395	6.982	6.645	7.025
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	2.573	4.630	4.917	1.535
RAT NUMBER	1	8	1	1

TABLE 56 B (1) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DAY	29/8/B	4/9/B	5/9/B	10/9/B
POST ANAESTHESIA DAY	15	21	22	27
RAT WEIGHT g.	350.3	339.7	345.1	339.1
ATMOSPHERIC PRESSURE mm Hg	758	740	739	762
W.G.M. TEMPERATURE ^{o}C	21.319	20.211	20.285	20.164
INLET AIR TEMPERATURE ^{o}C	20.256	20.066	20.116	20.09
CALORIMETER WALL TEMPERATURE ^{o}C	21.95	-	21.31	-
INLET/OUTLET AIRFLOW RATE L/min	1.284	1.239	1.254	1.288
TOTAL AIRFLOW RATE L/min	6.228	6.235	6.144	6.157
RECIRCULATED AIRFLOW RATE L/min	4.944	4.996	4.890	4.869
DEW POINT TEMPERATURE ^{o}C	15.817	15.642	15.685	15.656
INCREMENT IN AIR TEMPERATURE ^{o}C	2.00	1.49	1.54	1.76
URINE/FAECAL WEIGHT g.	0/0	0/0	0/0	1.0/0
GROSS HEAT LOSS THROUGH WALLS W.	1.986	1.779	1.994	1.837
URINE HEAT LOSS W.	0	0	0	7.490^{-03}

TABLE 56 B (2) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DAY	29/8/B	4/9/B	5/9/B	10/9/B
FAECAL HEAT LOSS W.	0	0	0	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	3.240^{-03}	2.344^{-03}	2.392^{-03}	1.651^{-03}
NET HEAT LOSS THROUGH WALLS W.	1.982	1.777	1.991	1.828
HEAT LOSS TO AIRSTREAM W.	0.275	0.205	0.209	0.239
TOTAL DIRECT HEAT LOSS W.	2.566	2.195	2.419	2.220
PARTITIONED HEAT LOSS W/kg				
SENSIBLE	6.445	5.835	6.376	6.095
INSENSIBLE	0.881	0.625	0.633	0.451
TOTAL	7.326	6.460	7.009	6.546
PARTITIONED HEAT LOSS % OF TOTAL				
SENSIBLE	88.0	90.3	91.0	92.6
INSENSIBLE	12.0	9.7	9.0	7.4
OXYGEN CONSUMPTION ml/min	7.508	6.631	7.155	6.744
CARBON DIOXIDE PRODUCTION ml/min	5.233	4.367	4.844	4.596

TABLE 56 B (3) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DAY	29/8/B	4/9/B	5/9/B	10/9/B
RESPIRATORY QUOTIENT	0.697	0.659	0.677	0.681
TOTAL INDIRECT HEAT PRODUCTION W.	2.473	2.163	2.345	2.212
HEAT PRODUCTION W/kg	7.061	6.366	6.794	6.525
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	3.625	1.461	3.057	0.318
RAT NUMBER	8	1	8	1

TABLE 56 C (1) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DATE	12/9/B	19/9/B	23/9/B	25/9/B
POST ANAESTHESIA DAY	29	36	40	42
RAT WEIGHT g.	344.5	339.7	318.6	343.0
ATMOSPHERIC PRESSURE mm Hg	756	760	739	739
W.G. M. TEMPERATURE ^{o}C	20.382	19.569	20.448	19.116
INLET AIR TEMPERATURE ^{o}C	20.122	20.046	19.983	20.029
CALORIMETER WALL TEMPERATURE ^{o}C	-	-	-	21.29
INLET/OUTLET AIRFLOW RATE L/min	1.292	1.274	1.256	1.266
TOTAL AIRFLOW RATE L/min	6.198	6.145	6.059	6.150
RECIRCULATED AIRFLOW RATE L/min	4.906	4.871	4.803	2.884
DEW POINT TEMPERATURE ^{o}C	15.695	15.618	15.53	15.575
INCREMENT IN AIR TEMPERATURE ^{o}C	1.75	1.56	1.70	1.80
URINE/FAECAL WEIGHT g.	0/0	0/0	0/0	1.95/0
GROSS HEAT LOSS THROUGH WALLS W.	1.906	1.773	1.738	1.783
URINE HEAT LOSS W.	0	0	0	12.061^{-03}

TABLE 56 C (2) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DATE	12/9/B	19/9/B	23/9/B	25/9/B
FAECAL HEAT LOSS W.	0	0	0	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	3.485^{-03}	1.494^{-03}	3.200^{-03}	2.259^{-03}
NET HEAT LOSS THROUGH WALLS W.	1.903	1.771	1.734	1.769
HEAT LOSS TO AIRSTREAM W.	0.240	0.212	0.227	0.244
TOTAL DIRECT HEAT LOSS W.	2.465	2.119	2.254	2.222
PARTITIONED HEAT LOSS W/kg				
SENSIBLE	6.219	5.839	6.159	5.870
INSENSIBLE	0.937	0.400	0.919	0.609
TOTAL	7.156	6.239	7.077	6.478
PARTITIONED HEAT LOSS % OF TOTAL				
SENSIBLE	86.9	93.6	87.0	90.6
INSENSIBLE	13.1	6.4	13.0	9.4
OXYGEN CONSUMPTION ml/min	7.485	6.368	6.941	6.616
CARBON DIOXIDE PRODUCTION ml/min	5.050	4.335	4.692	4.414

TABLE 56 C (3) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DATE	12/9/B	19/9/B	23/9/B	25/9/B
RESPIRATORY QUOTIENT	0.675	0.681	0.676	0.667
TOTAL INDIRECT HEAT PRODUCTION W.	2.451	2.089	2.274	2.163
HEAT PRODUCTION W/kg	7.116	6.150	7.138	6.305
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	0.561	1.427	-0.863	2.669
RAT NUMBER	8	1	8	1

TABLE 56 D (1) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DATE	3/10/B	7/10/B	8/10/B	14/10/B
POST ANAESTHESIA DAY	50	54	55	61
RAT WEIGHT g.	345.1	304.3	344.6	349.9
ATMOSPHERIC PRESSURE mm Hg	763	747	760	763
W.G.M. TEMPERATURE ^{o}C	19.819	19.495	20.328	19.621
INLET AIR TEMPERATURE ^{o}C	20.178	21.099	20.27	20.17
CALORIMETER WALL TEMPERATURE ^{o}C	-	-	-	21.33
INLET/OUTLET AIRFLOW RATE L/min	1.277	1.280	1.302	1.323
TOTAL AIRFLOW RATE L/min	6.216	6.094	6.250	6.270
RECIRCULATED AIRFLOW RATE L/min	4.939	4.814	4.948	4.947
DEW POINT TEMPERATURE ^{o}C	15.604	15.57	15.646	15.551
INCREMENT IN AIR TEMPERATURE ^{o}C	1.59	1.66	1.74	1.66
URINE/FAECAL WEIGHT g.	1.13/0	0/0	0/0	2.05/0
GROSS HEAT LOSS THROUGH WALLS W.	1.707	1.724	1.752	1.771
URINE HEAT LOSS W.	8.170^{-03}	0	0	12.620^{-03}

TABLE 56 D (2) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DATE	3/10/B	7/10/B	8/10/B	14/10/B
FAECAL HEAT LOSS W.	0	0	0	0
SPEC. HEAT LOSS BY EXPIRED H_2O W.	1.719^{-03}	3.642^{-03}	2.967^{-03}	3.454^{-03}
NET HEAT LOSS THROUGH WALLS W.	1.697	1.721	1.749	1.755
HEAT LOSS TO AIRSTREAM W.	0.218	0.223	0.240	0.230
TOTAL DIRECT HEAT LOSS W.	2.074	2.304	2.267	2.304
PARTITIONED HEAT LOSS W/kg				
SENSIBLE	5.551	6.389	5.772	5.673
INSENSIBLE	0.458	1.183	0.806	0.912
TOTAL	6.009	7.572	6.578	6.585
PARTITIONED HEAT LOSS % OF TOTAL				
SENSIBLE	92.4	84.4	87.7	86.2
INSENSIBLE	7.6	15.6	12.3	13.8
OXYGEN CONSUMPTION ml/min	5.991	6.698	6.828	6.744
CARBON DIOXIDE PRODUCTION ml/min	4.090	4.926	4.821	4.741

TABLE 56 D (3) (See Fig. 148) CALORIMETRY RESULTS : 25% BSA BURN STUDY

CONTROL GROUP - RATS 1, 8.

DATE	3/10/B	7/10/B	8/10/B	14/10/B
RESPIRATORY QUOTIENT	0.683	0.736	0.706	0.703
TOTAL INDIRECT HEAT PRODUCTION W.	1.966	2.228	2.254	2.225
HEAT PRODUCTION W/kg	5.697	7.321	6.542	6.358
% DIFFERENCE (HEAT LOSS - HEAT PRODUCTION)	5.181	3.314	0.543	3.453
RAT NUMBER	1	8	1	1

TABLE 57 A (1) 25% BSA BURN STUDY : CALORIMETRY RESULTS

BURN GROUP RATS 3, 4, 18.

* Days after burn injury on 14/8/B

DATE OF RUN	RAT NUMBER	PERCENT DIFFERENCE DIRECT-INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION DEFAECATION	TOTAL DURATION OF CALORIMETRY
16/8/B (2)*	3	1.263	0.701	90	+ (u)	170
19/8/B (5)*	3	0.553	0.701	80	+ (u)	180
20/8/B (6)*	4	3.380	0.687	70	-	168
21/8/B (7)*	3	1.677	0.689	70	+ (u)	160
22/8/B (8)*	18	3.393	0.682	100	-	175
23/8/B (9)*	3	1.794	0.705	60	-	140
27/8/B (13)*	3	3.420	0.731	90	-	170
28/8/B (14)*	4	2.576	0.738	70	-	160
29/8/B (15)*	3	1.350	0.711	100	-	170

TABLE 57 A (2) 25% BSA BURN STUDY : CALORIMETRY RESULTS

BURN GROUP RATS 3, 4, 18.

* Days after burn injury on 14/8/B

DATE OF RUN	RAT NUMBER	PERCENT DIFFERENCE DIRECT-INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION DEFAECATION	TOTAL DURATION OF CALORIMETRY
30/8/B (16)*	18	3.028	0.708	80	+ (f)	170
3/9/B (20)*	3	2.232	0.676	120	-	180
4/9/B (21)*	4	-0.480	0.675	100	-	170
5/9/B (22)*	3	0.335	0.675	110	+ (u)	180
6/9/B (23)*	18	3.108	0.675	80	+ (f)	170
9/9/B (26)*	3	3.571	0.686	80	+ (u)	170

TABLE 57 B (1) 25% BSA BURN STUDY : CALORIMETRY RESULTS

BURN GROUP - RATS 3, 4, 18.

* Days after burn injury on 14/8/B

DATE OF RUN	RAT NUMBER	PERCENT DIFFERENCE DIRECT-INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION DEFAECATION	TOTAL DURATION OF CALORIMETRY
10/9/B (27)*	4	4.946	0.714	120	+ (u) (f)	180
11/9/B (28)*	3	1.093	0.709	100	+ (u)	180
12/9/B (29)*	4	5.024	0.698	110	+ (u) (f)	170
13/9/B (30)*	3	2.610	0.685	90	+ (u)	160
17/9/B (34)*	3	0.874	0.716	70	+ (u)	170
18/9/B (35)*	4	4.443	0.771	60	+ (u)	160
19/9/B (36)*	3	1.838	0.717	120	+ (u)	190

TABLE 57 B (2) 25% BSA BURN STUDY : CALORIMETRY RESULTS * Days after burn injury

BURN GROUP - RATS 3, 4, 18.

DATE OF RUN	RAT NUMBER	PERCENT DIFFERENCE DIRECT-INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION DEFAECATION	TOTAL DURATION OF CALORIMETRY
20/9/B (37)*	4	2.533	0.748	50	+ (u)	110
24/9/B (41)*	3	-5.544	0.664	80	+ (u) (f)	180
25/9/B (42)*	4	-4.781	0.680	70	+ (u) (f)	160
27/9/B (44)*	4	2.989	0.701	110	+ (u) (f)	170
2/10/B (49)*	4	4.026	0.735	60	+ (u)	120
8/10/B (55)*	4	2.015	0.759	90	+ (u) (f)	180
10/10/B (57)*	3	2.434	0.754	50	+ (u) (f)	175
16/10/B (63)*	3	3.341	0.713	90	+ (u) (f)	160
MEAN VALUES ± SD		2.0 ± 2.3	0.71 ± 0.03	86 ± 20	73% +	167 ± 17

TABLE 58 A — 25% BSA BURN STUDY : CALORIMETRY RESULTS

CONTROL GROUP - RATS 1, 8.

* Control days after anaesthesia 14/8/B

DATE OF RUN	RAT NUMBER	PERCENT DIFFERENCE DIRECT-INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION DEFAECATION	TOTAL DURATION OF CALORIMETRY
19/8/B (5)*	1	2.573	0.683	80	-	160
20/8/B (6)*	8	4.630	0.690	80	-	170
21/8/B (7)*	1	4.917	0.704	90	-	155
27/8/B (13)*	1	1.535	0.713	100	-	170
29/8/B (15)*	8	3.625	0.697	80	-	170
4/9/B (21)*	1	1.461	0.659	90	-	140
5/9/B (22)*	8	3.057	0.677	60	-	140
10/9/B (27)*	1	0.318	0.681	50	+	140
12/9/B (29)*	8	0.561	0.675	100	-	150

TABLE 58 B — 25% BSA BURN STUDY : CALORIMETRY RESULTS

CONTROL GROUP - RATS 1, 8.

* Control days after anaesthesia 14/8/B

DATE OF RUN	RAT NUMBER	PERCENT DIFFERENCE DIRECT-INDIRECT	RESPIRATORY QUOTIENT	MEASUREMENT PERIOD (min)	URINATION DEFAECATION	TOTAL DURATION OF CALORIMETRY
19/9/B (36)*	1	1.427	0.681	90	-	150
23/9/B (40)*	8	-0.863	0.676	100	-	160
25/9/B (42)*	1	2.669	0.667	90	+	170
3/10/B (50)*	1	5.181	0.683	80	+	145
7/10/B (54)*	8	3.314	0.736	100	-	180
8/10/B (55)*	1	0.543	0.706	90	-	145
14/10/B (61)*	1	3.453	0.703	90	+	170
MEAN VALUES $\pm$ SD		2.4 $\pm$ 1.8	0.69 $\pm$ 0.02	86 $\pm$ 14	25% +	157 $\pm$ 13

there was no significant difference in calorimeter performance. Heat loss exceeded heat production by only 2.0 ± 2.3(SD)%. See Table 57. This level of agreement indicates that the previously described procedures developed for calorimetry runs were satisfactory. These were, (1) that a minimum period of 50 minutes was allowed to elapse from the start of a run before measurements of heat loss or production were regarded as accurate, (2) that comparison of heat loss with heat production was only made when, at the start and finish of a measurement period, the rat was inactive and neither gaining nor losing heat rapidly, (3) that the minimum time period for accurate measurement was 50 minutes, and (4) that the "lag time" in gas analyser response was taken into account. The mean measurement period on which heat loss/production values were based in the 25% BSA burn study was 86 ± 20 (SD) minutes in the burned rat group (Table 57). The range was from 50 - 120 minutes. The total duration of the calorimetry runs was 167 ± 17 (SD) minutes. In the control rat group, the mean measurement period was again 86 minutes ± 14 minutes (SD). The range was 59 - 100 minutes and the total duration of calorimetry runs was 157 ± 13 minutes.

Respiratory quotient measurement in the control rats indicated a mean RQ of 0.69 ± 0.02. This value

indicated that post absorbtive rats utilised principally body fat and was taken as a further indication of the "normality" of metabolism of the rat under study (Kleiber, 1961), and incidentally confirmed the accuracy of the oxygen consumption and carbon dioxide production values, measured separately by the gas analysers.

The accuracy of heat production and loss measurement in normal and burned rats using the SEC - A - 04 L calorimetry system lies within the design limits of its specifications. The ability to measure heat production and heat loss simultaneously with an agreement of better than 2.5% (heat loss exceeding heat production), exceeds the performance of similar gradient layer systems used for the study of large animals and man (Pullar et al., 1967; Dale et al., 1967; McLean, 1971; Spinnler et al., 1973).

I believe that the use of unharnessed rats, which were not subjected to body temperature measurement or restraint of any kind during calorimetry runs, was therefore justified in control and burned rats. These values for energy expenditure in the rat, obtained in this way, reflect true RME more closely than measurements made on restrained rats rigidly clamped within an enveloping head cover or hood, inside a gradient layer calorimeter (Caldwell et al., 1966;

Bartlett et al., 1958). The results obtained in the present 25% BSA burn study also justified the use of the simplified form of Weir's equation (1949) in the calculation of heat production. It is of interest that urination took place during calorimetry in only 25% of the control runs, but that in burned rats, urination and/or defaecation occurred in 73% of the runs. See Tables 57 and 58. In burned rats the mean difference between heat loss and heat production in those runs without urination or defaecation was $2.0 \pm 1.4\%$. In runs with urination alone it was $2.1 \pm 1.4\%$. In runs in which defaecation and urination took place it was $1.3 \pm 4.1\%$.

CHANGES IN ENERGY EXPENDITURE AFTER 25% BSA FULL SKIN THICKNESS DORSAL BURN IN THE RAT:

Figure 147 indicates a marked increase in energy expenditure in the burn group rats compared with uninjured control rats (Fig. 148) over the whole of the 63 days post burn period which is shown. Overall mean rate of heat loss (results expressed per rat and uncorrected for body weight) during the entire measured post burn period (30 runs) was 3.160 ± 0.313 W, compared with 2.329 ± 0.162 (SD) W mean rate of heat loss in uninjured control rats (16 runs). Table 59 gives the mean rate of heat loss per rat for calorimeter burn group and control group rats for each of the 3 post burn time periods shown in Tables 49 and 50, which

TABLE 59 CHANGES IN ENERGY EXPENDITURE AFTER 25% BSA BURN

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

(RESULTS EXPRESSED AS RATE OF HEAT LOSS (W) PER RAT)

TIME PERIOD	MEAN RATE OF HEAT LOSS PER RAT : W. BURN GROUP	MEAN RATE OF HEAT LOSS PER RAT : W. CONTROL GROUP	% INCREASE (BURN GROUP - CONTROL GROUP)
POST BURN PERIOD (1) 16/8 - 22/8	2.863 $\pm$ 0.13	2.467 $\pm$ 0.20	+ 16.1
POST BURN PERIOD (2) 23/8 - 3/9	3.295 $\pm$ 0.22	2.404 $\pm$ 0.19	+ 37.1
POST BURN PERIOD (3) 4/9 - 16/9	3.473 $\pm$ 0.20	2.368 $\pm$ 0.13	+ 46.7
POST BURN PERIOD (4) 17/9 - 16/10	3.026 $\pm$ 0.28	2.221 $\pm$ 0.09	+ 36.2

describe the metabolic cage rats' responses to a 25% BSA burn injury. Post burn period 4, also shown in Table 59, is the 30 days extra time for which the calorimeter rats were studied beyond the metabolic cage rat group.

Because of the differences in body weight between burn and control group rats, which increased during the experiment, presentation of heat loss values without taking this body weight change into account proved to be misleading. When weight loss was taken into account as in Figure 149, it became evident that the fall in the rate of heat loss per rat in the burn group rats during post burn period 4 (17/9 - 16/10) was entirely due to their rapid decrease in body mass. See Figure 143. Table 60 gives the rate of total heat loss in burn and control group rats expressed in Watts per kg body weight.

There is a significant and progressive increase in the rate of total heat loss (per kg body weight) in the burned rats compared with the control group rats. This progressive increase in total heat losses after burning in the calorimeter burn group rats (shown in Table 59) may be compared with the increased rate of daily nitrogen excretion found in the metabolic cage 25% BSA burn study rats, which is shown in Table 50.

Fig. 149 25% BSA BURN : TOTAL HEAT LOSS AND TOTAL HEAT PRODUCTION EXPRESSED PER UNIT BODY WEIGHT BURN RATS 3, 4, 18 vs. CONTROL RATS 1, 8

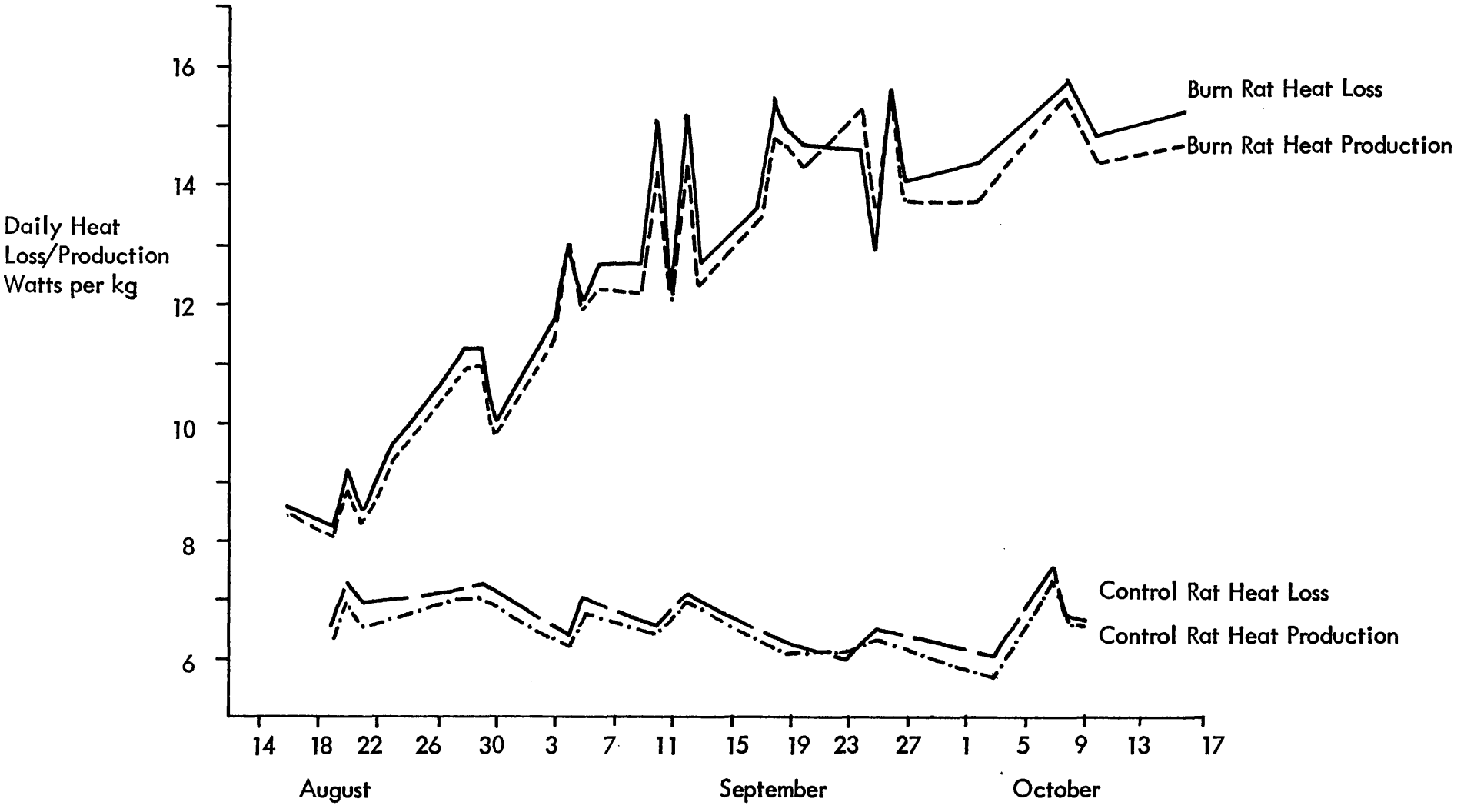


TABLE 60 CHANGES IN ENERGY EXPENDITURE AFTER 25% BSA BURN

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

(RESULTS EXPRESSED AS RATE OF HEAT LOSS (W) PER KG BODY WEIGHT)

TIME PERIOD	MEAN RATE OF HEAT LOSS PER KG : W. BURN GROUP	MEAN RATE OF HEAT LOSS PER KG : W. CONTROL GROUP	% INCREASE (BURN GROUP - CONTROL GROUP)
POST BURN PERIOD (1) 16/8 - 22/8	8.710 ± 0.40	6.958 ± 0.38	+ 25.2
POST BURN PERIOD (2) 23/8 - 3/9	10.792 ± 0.80	6.974 ± 0.45	+ 54.7
POST BURN PERIOD (3) 4/9 - 16/9	13.148 ± 1.24	6.904 ± 0.32	+ 90.4
POST BURN PERIOD (4) 17/9 - 16/10	14.559 ± 0.82	6.648 ± 0.52	+ 119.0

Although differences are shown between the rates of total heat loss and total heat production in Figure 149, these affect burn and control group rats equally (See Tables 57 and 58). Since the rat is a homeotherm, then its rate of total heat loss must equal its rate of heat production over a 24 hour period (Hardy et al., 1970). In this thesis I have therefore regarded daily rates of heat loss and heat production as being equivalent. Figures 150 and 151 show the partitioning of the measured rates of heat loss in burned and control rats into insensible (evaporative) heat loss, and sensible (dry) heat loss respectively. The top portion of each Figure gives the mean change in rat surface area after burn injury in burn group and control group rats.

Table 61 gives the partitioned heat losses for burn group and control group rats over the 4 post burn time periods shown in Tables 59 and 60.

The partitioning of heat losses into insensible (evaporative) and sensible (dry) components was relatively constant in the control rats over the entire post burn study period of 61 days. Insensible heat loss accounted for 10.5% $\pm$ 2.6(SD)% of the total measured heat losses in these animals. Whereas, over the 63 day post burn study period in the burn group rats, insensible or evaporative heat loss accounted for

Fig.150 25% BSA BURN : INSENSIBLE HEAT LOSS BURN GROUP RATS 3.4.18 : CONTROL GROUP RATS 1,8

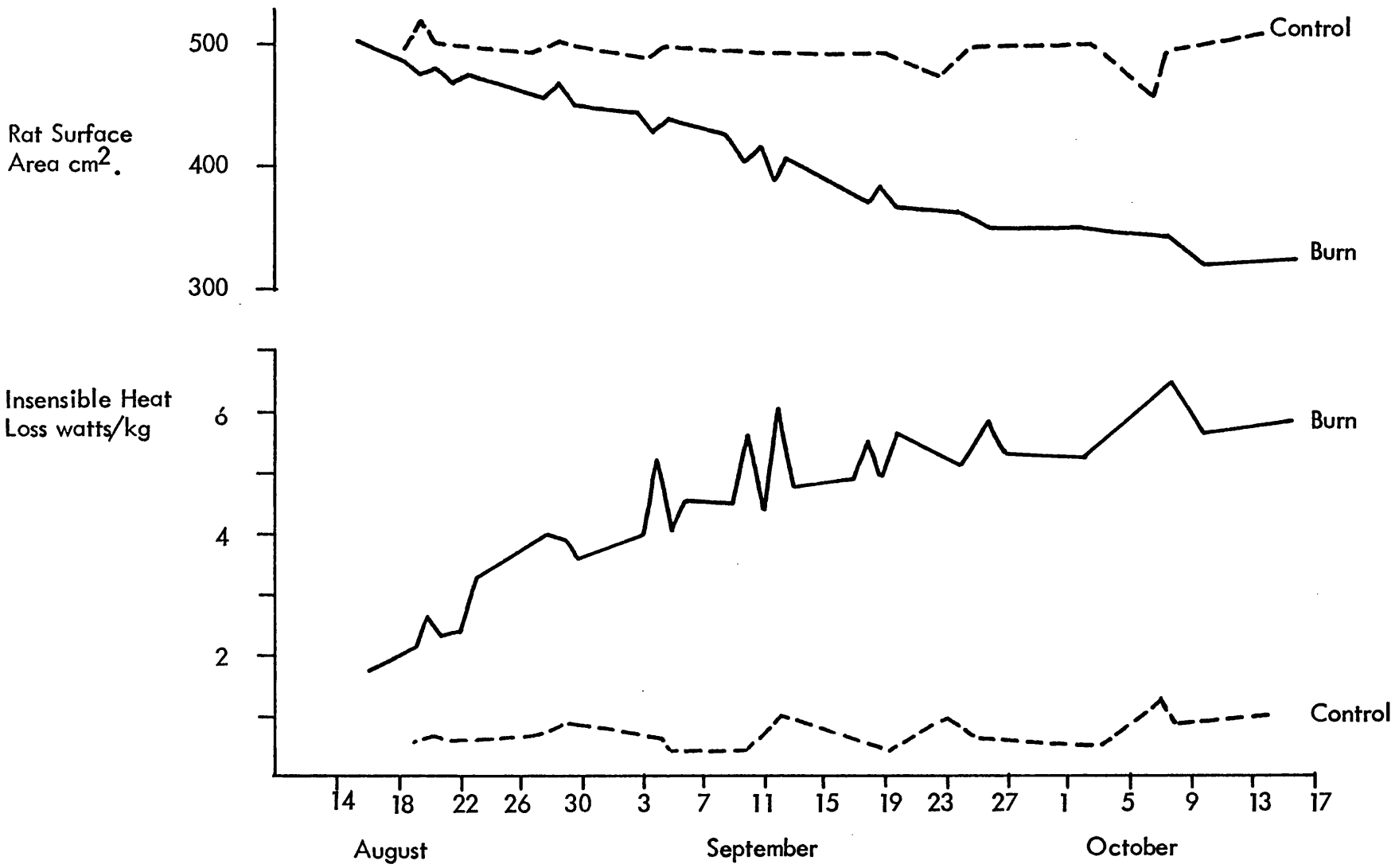


Fig. 151 25% BSA BURN : SENSIBLE HEAT LOSS BURN GROUP RATS 3, 4, 18 : CONTROL GROUP RATS 1, 8

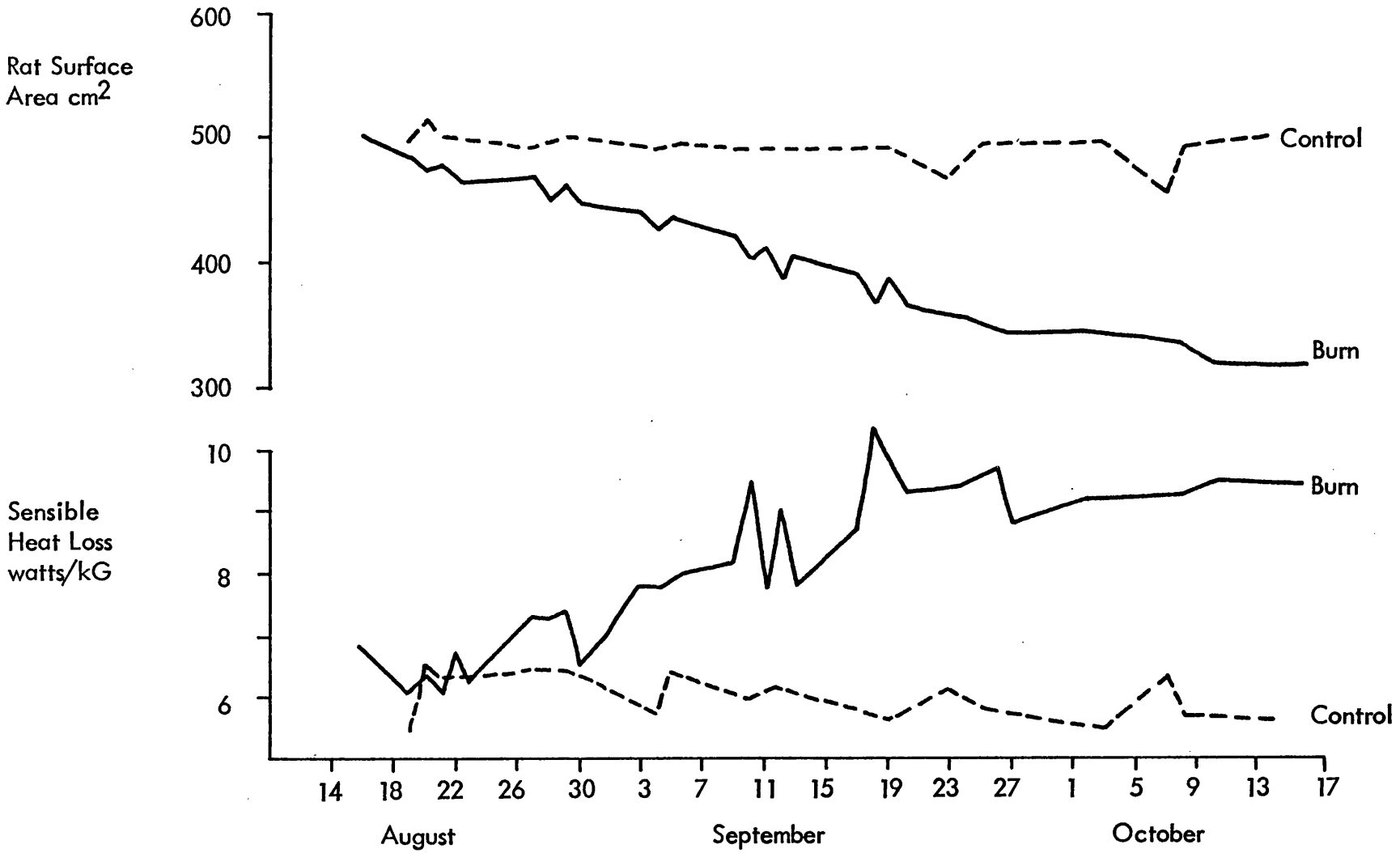


TABLE 61 A PARTITIONED HEAT LOSSES AFTER 25% BSA BURN INJURY

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

(RESULTS EXPRESSED AS RATE OF HEAT LOSS (W) PER KG BODY WEIGHT)

RATE OF INSENSIBLE (EVAPORATIVE) HEAT LOSS (W/KG)

TIME PERIOD	BURN GROUP	CONTROL GROUP	BURN - CONTROL DIFFERENCE (W/KG)	BURN - CONTROL % DIFFERENCE
POST BURN PERIOD (1) 16-17/8 to 22-23/8	2.286 $\pm$ 0.37	0.673 $\pm$ 0.08	1.61	+ 239.7
POST BURN PERIOD (2) 23-24/8 to 3-4/9	3.721 $\pm$ 0.27	0.732 $\pm$ 0.13	2.99	+ 408.3
POST BURN PERIOD (3) 4-5/9 to 16/9	4.882 $\pm$ 0.68	0.674 $\pm$ 0.25	4.21	+ 624.3
POST BURN PERIOD (4) 17/9 to 16/10	5.239 $\pm$ 0.52	0.755 $\pm$ 0.28	4.48	+ 693.9

TABLE 61 B PARTITIONED HEAT LOSSES AFTER 25% BSA BURN INJURY

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

(RESULTS EXPRESSED AS RATE OF HEAT LOSS (W) PER KG BODY WEIGHT)

RATE OF SENSIBLE (DRY) HEAT LOSS (W/KG)

TIME PERIOD	BURN GROUP	CONTROL GROUP	BURN - CONTROL DIFFERENCE (W/KG)	BURN - CONTROL % DIFFERENCE
POST BURN PERIOD (1) 16-17/8 to 22-23/8	6.428 $\pm$ 0.32	6.285 $\pm$ 0.31	0.14	+ 2.3
POST BURN PERIOD (2) 23-24/8 to 3-4/9	7.168 $\pm$ 0.59	6.241 $\pm$ 0.35	0.93	+ 14.9
POST BURN PERIOD (3) 4-5/9 to 16/9	8.339 $\pm$ 0.66	6.230 $\pm$ 0.14	2.11	+ 33.9
POST BURN PERIOD (4) 17/9 to 16/10	9.321 $\pm$ 0.50	5.893 $\pm$ 0.29	3.43	+ 58.2

a mean of 34.3% $\pm$ 5.9(SD)% of the total heat losses. The rate of insensible (evaporative) heat loss in the burned rats, expressed as Watts per kilogram, increased progressively during post burn periods 1 and 2 (See Table 61) and reached a high sustained level during post burn periods 3 and 4 (from the 21st to the 63rd post burn day), which was an average of 659% greater than that of control rats. These increases in the rate of evaporative heat loss after burn injury were consistent with the maturation of the burn eschar, which had completely disintegrated by the 21st post burn day, leaving a raw open wound surface which had no evidence of central re-epithelialisation (confirmed histologically in a separate study). The rates of insensible (evaporative) heat loss measured in the 25% BSA burn study were greater than those found after a 20% BSA burn. See Tables 12A, 12B, 13 and Figures 67, 68. This is consistent with the observation that the rate of evaporative water (and hence heat) loss from a burn wound is proportional to its surface area (Gump and Kinney, 1967). The close agreement between heat loss and heat production in the 25% BSA burn study shows that the calorimetry conditions of 6.1 - 6.4 l total flow/min did not cause significant cooling in burned rats due to increased convection of water from the burn wound surface. A 25% BSA burn made the injured rats markedly hypermetabolic over the whole of the 63 day post burn study period. There was a mean sustained increase in RME which was 105% greater than

that in control rats over post burn periods 3 and 4 (day 21 - day 63 post burn).See Table 60. The rate of sensible (dry) heat loss (W/kg) was also markedly increased compared to control rats, with a sustained average increase of 46% over post burn periods 3 and 4 (Table 61).

The 25% BSA full skin thickness burn injury in the rat is therefore comparable, in terms of the increases in metabolic rate which follow it, with the most major human burn cases (Cuthbertson, 1970A; Wilmore et al., 1976).

The change in rat surface area (calculated as previously discussed) after 25% BSA burn injury is shown in Figures 150 and 151 in relation to the changes in partitioned heat losses. This relationship is shown more clearly for the burn group rats in Figure 152 and for control group rats in Figure 153. There was a progressive increase in the rate of total sensible and insensible heat losses expressed as milli Watts per square centimetre of body surface area, over the first 30 days after injury in the burned rats (Fig. 152). From this time (around the 12 - 13 September), till the 63rd post burn day (16 October), the rate of insensible (evaporative) heat loss, expressed as mW/sq.cm BSA, appeared to be practically constant.

Fig. 152 25% BSA BURN : PARTITIONED HEAT LOSSES AND SURFACE AREA RELATIONSHIP BURN GROUP RATS 3, 4, 18

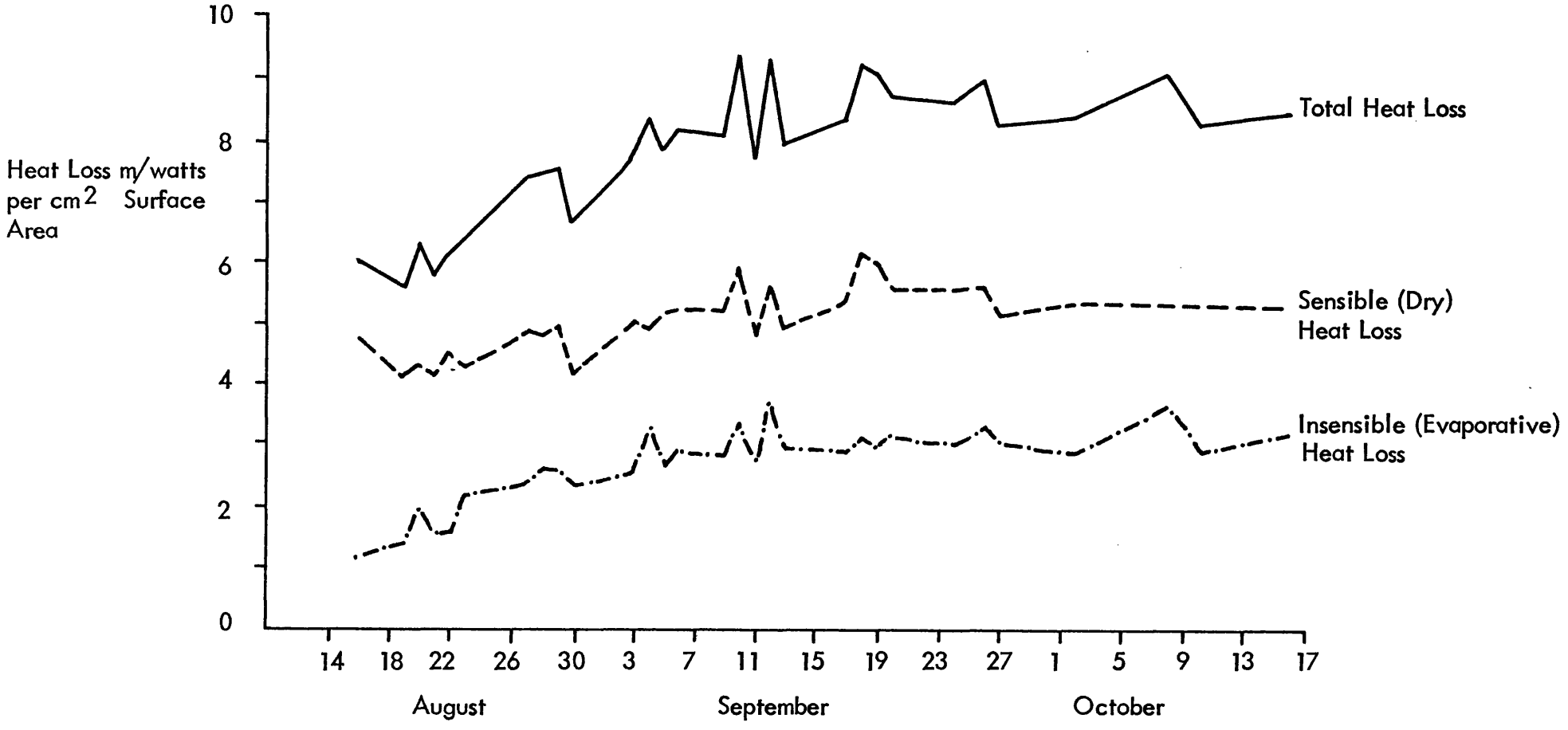
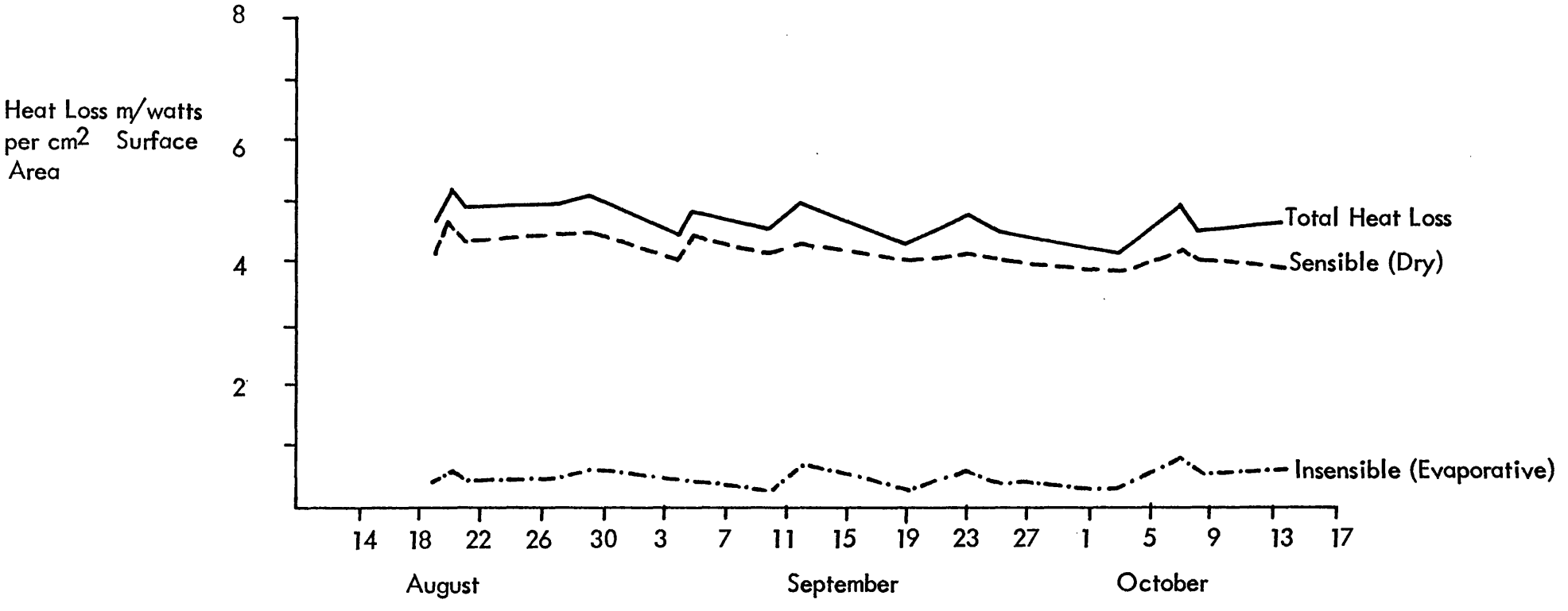


Fig. 153 25% BSA BURN : RELATIONSHIP BETWEEN BODY SURFACE AREA AND PARTITIONED HEAT LOSSES
CONTROL GROUP RATS 1,8



This indicated that the effective burn wound area decreased at approximately the same rate as the decrease in rat total body surface area. This was consistent with the observation that no healing of the full thickness burn wound occurred in the centre of the wound. Physical contracture of the wound was due to re-epithelialisation from the wound margins only. Figure 153 shows the constancy of the relationships between body surface area and the rates of sensible and insensible heat losses (mW/sq.cm BSA).

Daily partitioned heat losses for burn group rats and control group rats are compared in Figures 154, 155 and 156. The values given represent the total (estimated) resting heat losses over 24 hours and not the rates of heat loss as shown previously. The majority of the human nutritional literature in the U.K. and in the U.S.A., still present energy data in units of kilocalories. I have therefore used these units in Figures 154, 155 and 156 to enable ready comparison of the diminutive scale of changes in energy expenditure in the rat after 25% BSA burn with those which occur in burned man.

In Figure 154, it is evident that the increased insensible (evaporative) heat loss in the burned rats (shown by the stippled area between total and sensible heat losses) is the principal physical cause of the

Fig. 154 25% BSA BURN : DAILY PARTITIONED HEAT LOSSES BURN RATS 3,4,18 vs CONTROL RATS 1,8

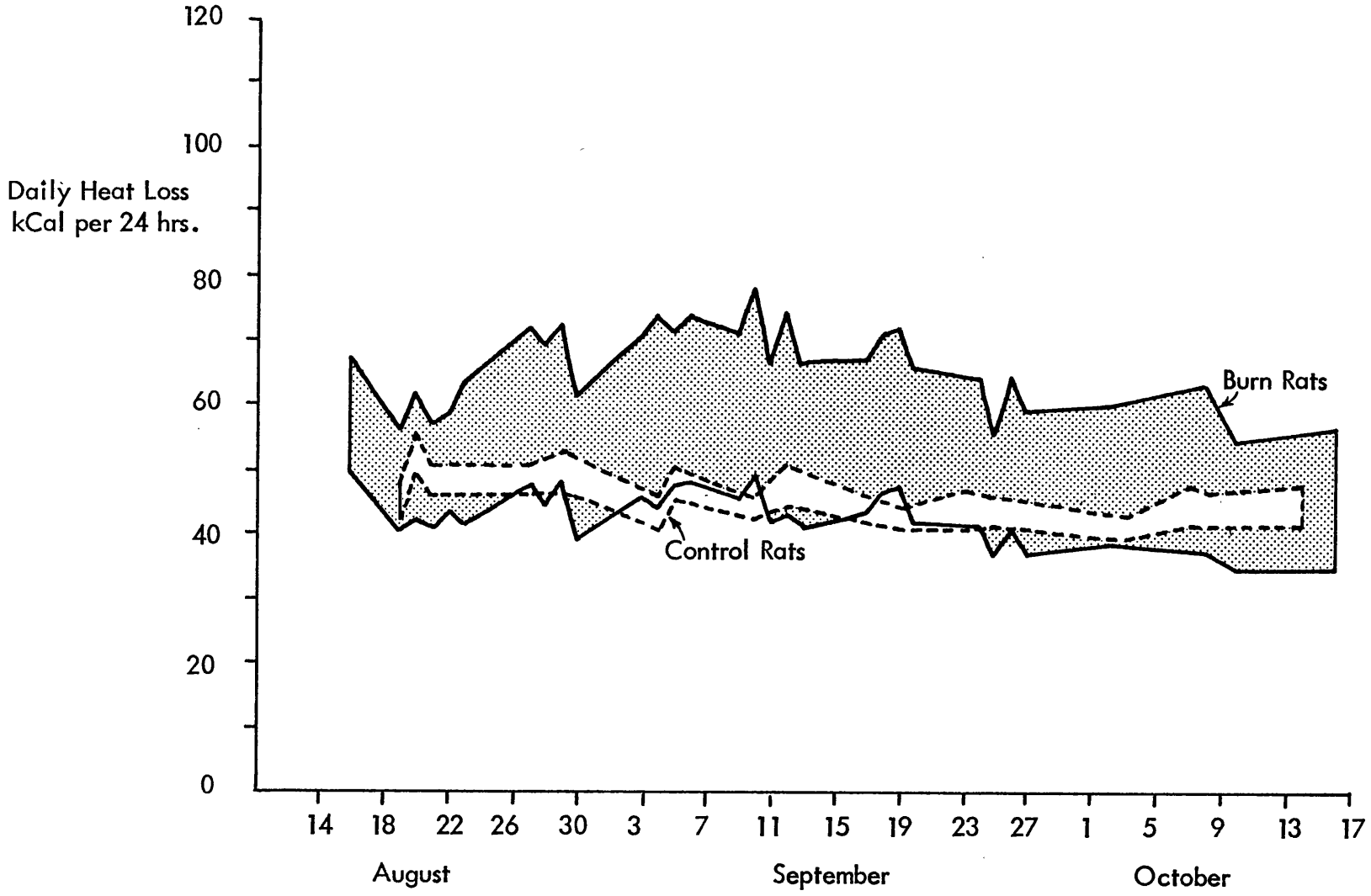


Fig. 155 25% BSA BURN: EVAPORATIVE OR INSENSIBLE HEAT LOSS BURN GROUP RAT Nos. 3, 4, 18 : CONTROL GROUP RAT Nos. 1, 8

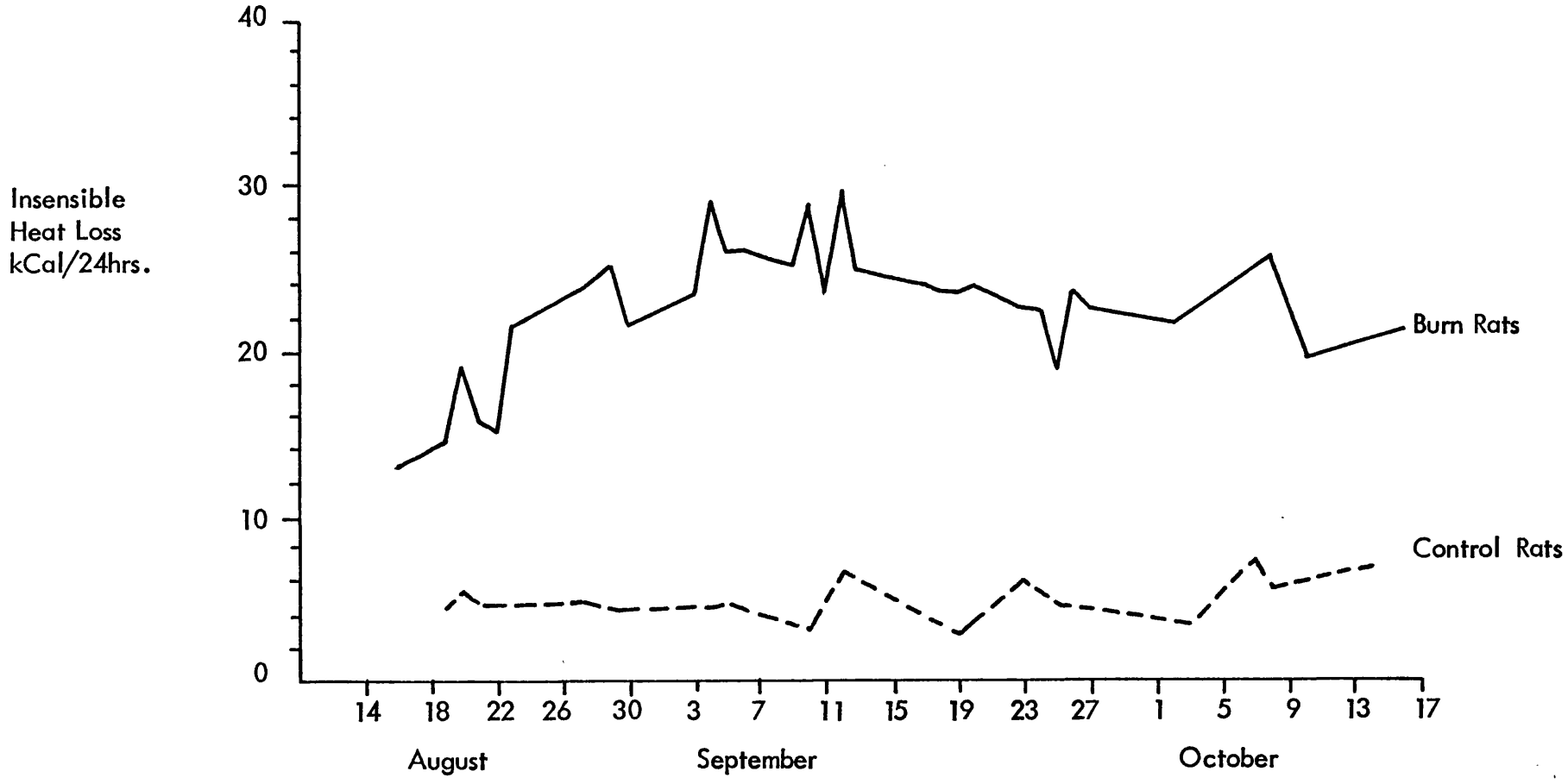
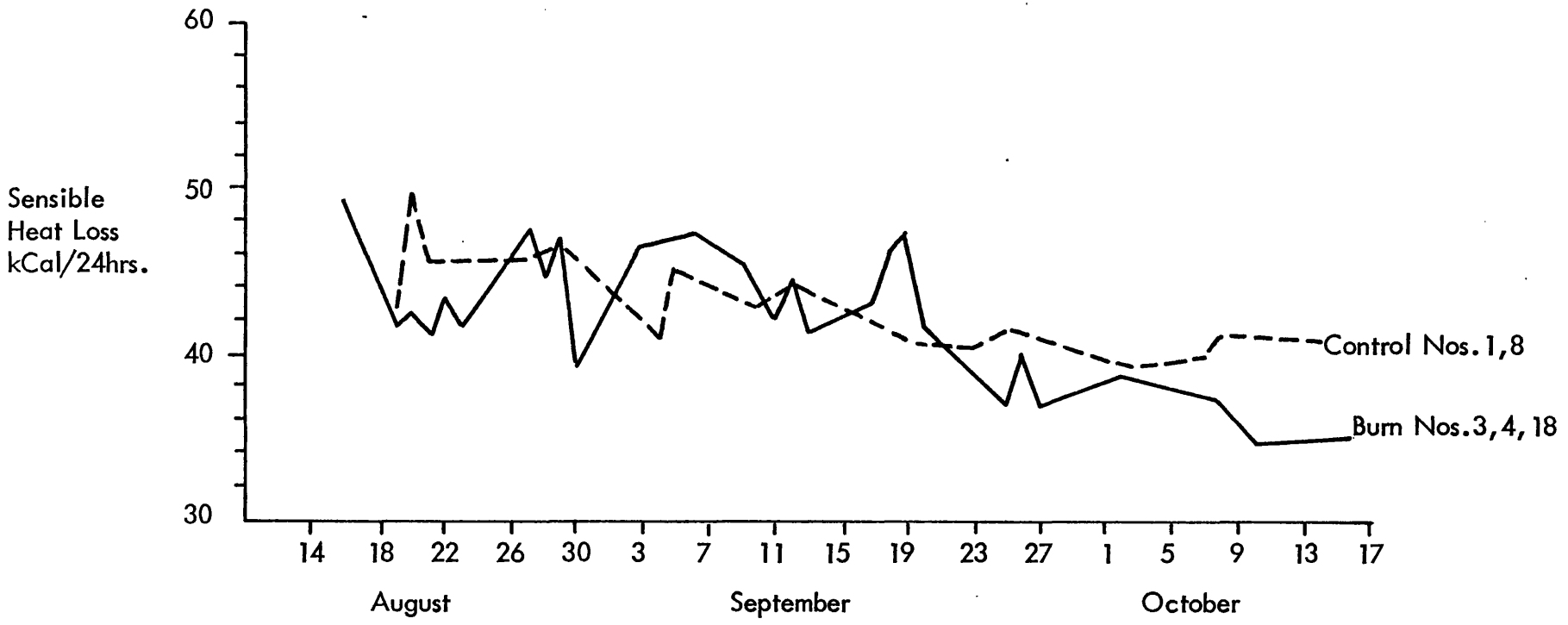


Fig. 156 25% BSA BURN: SENSIBLE OR DRY HEAT LOSS BURN GROUP - RAT Nos. 3,4,18 vs. CONTROL GROUP - RAT Nos. 1,8



hypermetabolism following thermal injury. Figure 155 gives the comparison between burn group and control group insensible (evaporative) heat losses (kcal/ 24 hours) over the 63 day post burn period.

The slight fall in total daily insensible (evaporative) heat loss over the period from 12 - 13 September till 16 October agreed with the observation that the burn wound margins contracted slightly as the burned rat lost body weight. The total daily insensible heat loss in control rats remained steady.

Figure 156 shows the total daily sensible (dry) heat losses in the burn group and control group rats. Note that total daily sensible heat loss in the burned rats fell progressively with the sharp drop in body weight which the rats suffered after 25% BSA burn injury. The extent of the decrease in total daily sensible heat loss in burned rats would have been even greater if the rate of sensible heat loss per unit weight of body tissue had not also progressively increased during the post burn period. See Figure 151 and Table 61.

NATURE OF TISSUE FUEL UTILISED AFTER 25% BSA BURN INJURY:

Figure 157 shows the respiratory quotients measured during calorimetry runs in burned and control group rats over the 63 day post burn period already described. The mean daily body weights for each of these groups are also shown (for the calorimetry rats). This enables a direct comparison to be made of RQ (which indicates the nature of the tissue fuel being utilised) and the extent of body weight loss in (calorimeter) burn and control group rats. Table 62 gives the RQ values for each of the previously described post burn periods. The small differences in RQ between burn group and control group rats are not statistically significant. Even though there is an upward trend in RQ values during post burn period 4, the mean value is still within the physiological range for normal post absorbtive rats which are utilising body fat as their principal energy source (Kleiber, 1961).

The contribution of protein to resting metabolic energy expenditure (RME) in control group and burn group rats was calculated by the method of Lusk (1928) as quoted by Kinney et al. (1970) (24 hour urine nitrogen x 6.25 x 4 = kacl protein energy utilised).

Fig. 157 25% BSA BURN : RESPIRATORY QUOTIENT AND WEIGHT LOSS BURN RATS 3,4,18 vs. CONTROL RATS 1,8

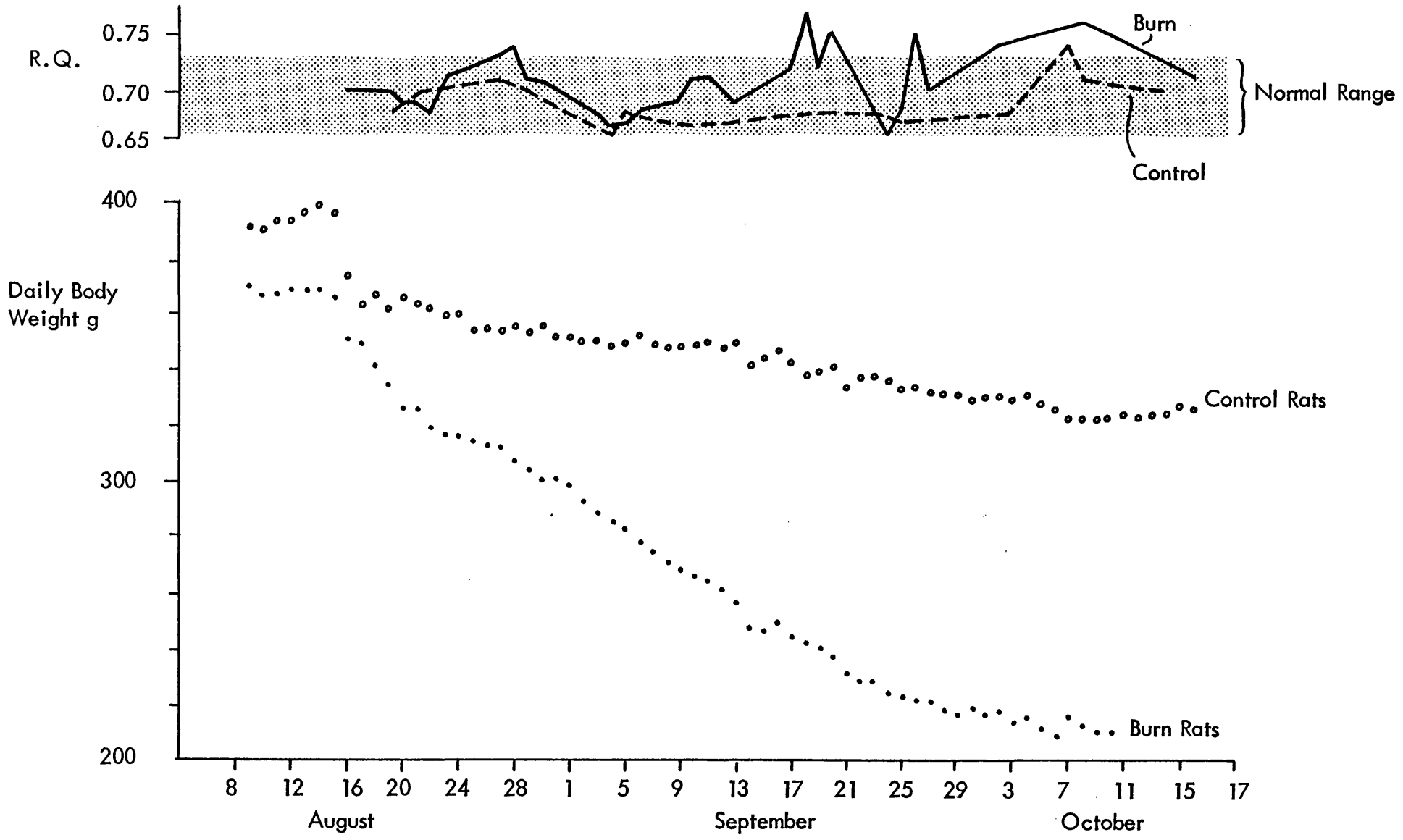


TABLE 62 RESPIRATORY QUOTIENT : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

TIME PERIOD	BURN GROUP	CONTROL GROUP
POST BURN PERIOD (1) 16-17/8 to 22-23/8	0.692 ± 0.01	0.692 ± 0.01
POST BURN PERIOD (2) 23-24/8 to 3-4/9	0.712 ± 0.02	0.690 ± 0.03
POST BURN PERIOD (3) 4-5/9 to 16/9	0.690 ± 0.02	0.678 ± 0.003
POST BURN PERIOD (4) 17/9 to 16/10	0.723 ± 0.03	0.693 ± 0.02

The results are given for post burn periods 1 - 3 in Table 63. The protein contribution to RME in the burn group rats (based solely on urinary nitrogen excretion) ranged from 17.6% to 21.7% of the total. There was no significant difference in the protein contribution to RME in the control group rats. Results here ranged from 21.6% to 23.1%. These findings in the burned rat are similar to the observations made by Duke et al. (1970) in patients with major trauma or sepsis, that protein contributed approximately 12% to 22% of the total RME calories.

This was a further indication of the similarity in the metabolic response to trauma between rat and man. Fat was the principal energy source utilised by burn group and control group rats alike during the calorimetry measurement periods.

TABLE 63 A URINARY PROTEIN CONTRIBUTION TO RME : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

BURN GROUP - RATS 3, 4, 18.

TIME PERIOD	URINE PROTEIN ENERGY UTILISATION (kcal/24 hours)	R.M.E. (kcal/24 hours)	% PROTEIN CONTRIBUTION TO R.M.E.
POST BURN PERIOD (1) 16/8 - 22/8	12.85	59.09	21.7
POST BURN PERIOD (2) 23/8 - 3/9	13.38	68.00	19.7
POST BURN PERIOD (3) 4/9 - 16/9	12.63	71.67	17.6

TABLE 63 B URINARY PROTEIN CONTRIBUTION TO RME : 25% BSA BURN STUDY

BURN GROUP - RATS 3, 4, 18 vs. CONTROL GROUP - RATS 1, 8.

CONTROL GROUP - RATS 1, 8.

TIME PERIOD	URINE PROTEIN ENERGY UTILISATION (kcal/24 hours)	R.M.E. (kcal/24 hours)	% PROTEIN CONTRIBUTION TO R.M.E.
POST ANAESTHESIA PERIOD (1) 17/8 to 23/8	11.00	50.92	21.6
POST ANAESTHESIA PERIOD (2) 24/8 to 4/9	11.69	49.61	23.6
POST ANAESTHESIA PERIOD (3) 5/9 to 16/9	11.30	48.87	23.1

GRADIENT LAYER CALORIMETER MEASUREMENT OF PARTITIONED HEAT LOSSES AND HEAT PRODUCTION AFTER 25% BSA DORSAL BURN

DISCUSSION

It was my aim to create an experimental burn injury in the rat which was comparable in as many respects as possible with a severe burn as it affects a typical hospital patient. It is possible only in the laboratory to select an homogenous subject group where the variation due to differing age, sex, nutritional state, presence of infection, or intercurrent disease can be eliminated. The need for treatment in the subject group may also be dispensed with, and the pathophysiology of the injury studied in an environment where ambient temperature, lighting and noise levels can be closely controlled.

The experimental rats in this study were kept at $20^{o}C \pm 0.5^{o}C$ ambient temperature and between 35 - 55% relative humidity, which was comparable to average ward temperature and humidity levels in Glasgow Royal Infirmary. Food intake after burn injury was slightly restricted in the rat in order to simulate the sub-optimal level of nutrition which is often an inadvertent accompaniment of present hospital care in the severely

ill (Davies, 1977). This food restriction was achieved in the rat by supplying a constant daily food intake of 20 g LIT diet. The arbitrary use of a fixed dietary intake ensured that burn and control group rats consumed virtually identical quantities of food during the study period (p.287). But, because of pre-injury differences in rates of growth and in levels of food intake, the effective reduction in food consumption after injury was greater than intended in the wall rack caged rats subjected to calorimetry (p.308).

Cairnie et al. (1957), Caldwell et al. (1966) and Miksche and Caldwell (1967) (Table 3) carried out calorimetry measurements on one group of rats while making metabolic collections on different rats kept in similar experimental conditions. Though the assumption was made by these workers that both groups of rats would undergo identical patterns of weight loss after injury, this cannot be accepted without experimental proof. In the present studies, the metabolic effect of the 25% BSA burn injury was shown to be similar in rats subjected to calorimetry compared with rats kept in metabolic cages (p.309, Fig. 146). In wall cage rats subjected to calorimetry, mean body weight on the day of burning was 370 $\pm$ 9 g, and by the 57th post-burn day, the mean weight was 209 $\pm$ 40 g, an overall reduction of 43.5%. This was a very severe and sustained weight loss pattern.

The rate of weight loss in the metabolic cage rats was similar, in that they had lost 24.1% of their initial weight by the 33rd day after burning, when they were sacrificed. This extended time course of post-burn weight loss in the rat is like that seen in severely burned man (Gump et al., 1973; Kinney, 1970).

The 25% body surface area full skin thickness burn proved the most suitable injury model in the rat for several reasons. This was the largest area of burn which could be created on the rat without the animal being able to reach the burned surface and remove or eat the eschar or deepen the wound by scratching it, with resultant infection or bleeding from the raw surface. Skin areas around hip and shoulder joints remained unburned, thus movement and the ability of the burned rat to feed normally were not affected by the injury. This was not the case with burn areas which exceeded 30% of the rat's body surface area. In rats subjected to 50% of body surface area burns, the effect of restricted movement on the rat's feeding ability may be inferred from data published by Herndon et al. (1978) in Figure 6 of their article. Food intake was less in rats with 50% BSA burns which were allowed unlimited access to food compared to control rats, over the initial 15 - 20 day post-burn period. Herndon et al. (1978) claimed that only burn wounds of greater than 50% of body surface

area in "mature" adult rats could simulate the pattern of weight loss observed in severely burned man. The results presented in this thesis are contrary to their findings. When full skin thickness burn wounds were created in the male Wistar young adult rat, a burn size of 25% of the rat's surface area was sufficient to cause severe weight loss provided that the rat was prevented from voluntarily increasing its post-burn food intake. In the present study this was achieved by restricting the daily food intake. In Herndon et al.'s (1978) study it is of interest to note that in what they considered to be their most satisfactory burn model (50% BSA burn area), they experienced an unplanned 10 - 15% reduction in food intake in their burned rats compared with control rats. This was probably due to the extensive burn eschar on these animals restricting joint movement and feeding ability. The partial starvation component is therefore similar in the present study and in Herndon et al.'s (1978) study, whether it was achieved by design or by accident. However, it has to be accepted that any food restriction whatever after burn injury will affect the experimental rat to a greater degree than it would burned man. The evidence suggests (Fig. 125, p. 273), that the injured rat differs from man in its ability to compensate for the energy losses through the burn wound by voluntarily increasing its food intake. If this mechanism is denied to the injured rat, as in the present study,

where food intake is inadequate to meet the increased energy demands of the burn injury, then the energy deficit can only be met by utilising the energy stores of the body. The principal energy source is fat (Kinney et al., 1970). There are significant differences in average body fat content between male Wistar rats in the present study and human patients of similar sex and age. The fat content of male Wistar rats is approximately 10% of their weight (Al-Shamma, 1978), whereas in man fat content is approximately 15 - 20% of weight, or even more in affluent Western cultures (Levenson et al., 1977). Thus faced with prolonged energy deficits, the burned rat will use up its fat stores within a shorter period than man. Therefore, the burned rat appears to be more dependent upon maintaining an adequate dietary intake in order to survive after injury than is man with his larger energy reserves. Despite this, the burned rats subjected to calorimetry and food restriction proved to be better survivors than expected. These rats lost 43.5% of their original weight by the 57th post-burn day. A similar rate and extent of body weight loss in man would usually be fatal (Levenson et al., 1977).

In rats subjected to calorimetry, it must be accepted that periods of overnight starvation prior to calorimetry may be detrimental to normal food

utilisation (Brozek, 1968), and may impose an additional form of post-injury "stress" (p.283). It is, however, of interest to note that burned patients are routinely submitted to equivalent periods of starvation prior to anaesthesia for skin grafting, a process which may be repeated many times in extensively burned patients. The selection of a 25% BSA burn size had additional advantages as an injury "model" in the rat. There is no mortality in the young male Wistar rat if the depth of burning is accurately restricted to the skin using the Wilkinson Sword Bunyan Contact Burn Apparatus. The rat is kept under deep Nembutal anaesthesia throughout. Post-burn intravenous fluid therapy is unnecessary, as the burned rat quickly replaces evaporative water losses from the burn surface by increasing its oral fluid intake. Cutaneous pain receptors are destroyed by a full skin thickness burn. The burn wound therefore lacks sensation, allowing the burned rat to be handled normally and weighed daily with little evidence of pain response when its burn eschar is touched.

CHANGES IN ENERGY EXPENDITURE AFTER 25% BSA BURN INJURY: DIRECT CALORIMETRY.

There was a highly significant and sustained rise in the rate of heat loss measured by direct calorimetry in rats after a 25% BSA burn compared with uninjured

controls. When the rate of total heat loss was expressed per unit of body weight, the rise was noted to be progressive (Fig. 149), from + 25% above control values during post-burn period 1, up to + 119% during post-burn period 4 (Table 60). This progressive rise in the rate of heat loss per unit weight is similar to but greater in magnitude than that seen after a 20% BSA burn in the rat. Compare Figure 69 with Figure 149. The increased rate of total heat loss after 25% BSA burn injury in the rat is similar to the increased rates of heat production measured by indirect calorimetry, reported in very extensively burned patients (Barr et al., 1968; Davies et al., 1977; Wilmore, 1977; Arturson et al., 1977). (Fig. 8).

It is important to note that the values for the rates of total heat loss and total heat production given in this section represent the rates of resting energy expenditure (RME) in the rat, and <u>not</u> basal metabolic rates (BMR) which may be up to 50% less. This is discussed elsewhere, p. 127. Measurement of total daily energy expenditure after burn injury in the rat would require continuous calorimetry throughout each 24 hour daily period and would have to take into account the overnight feeding and activity periods noted in Figures 96 and 97. Though this has been attempted by Cairnie et al. (1957) (Table 3), provision of food and water plus the need for regular excreta

removal, make such a study impractical with the SEC - A - 04 L calorimetry system described in this thesis. Only in Figures 154, 155 and 156 are estimates given for total daily rat RME and partitioned direct heat losses. The total daily heat loss estimated in Figure 154 for burn group rats is simply a 24 hour extrapolation from the calorimetry measurement periods shown in Table 57. The mean measurement period was 86 $\pm$ 20 minutes. The equivalent control group rat data is given in Table 58. The daily RME heat loss values shown in Figure 154 therefore represent a considerable underestimation of the total daily energy losses in both burned and control rats. Kinney et al. (1970) estimated that movement and normal exercise in ambulant injured man would add 22% to the daily RME values. As a rough approximation, this would probably represent a minimum value in burned rats where full mobility is retained after injury. A further increment in total daily energy production must be included to take account of the activity of feeding plus the specific dynamic effect of the food itself. It seems probable therefore that the values given for RME in Figure 154 underestimate the total daily energy expenditure by up to 30 - 50%.

INSENSIBLE (EVAPORATIVE) HEAT LOSS AFTER 25% BSA BURN INJURY

It has previously been noted (p. 40) that in the rat (Morgan et al., 1955; Moyer and Butcher, 1967), as in man (Gump and Kinney, 1970), complete thermal destruction of the normal skin water barrier leads to substantial evaporative water losses in the post-burn period. In the present 25% BSA burn study, the rate of evaporative (insensible) heat loss was increased by + 694% over the 33rd to the 63rd post-burn day (period 4, Table 61). (Figs. 150 and 154). There is no evidence therefore in the present study to suggest that a 25 - 30% BSA burn in the rat is a trivial injury in which the burned wound area shrinks to half its original size within 7 days, as is claimed by Herndon et al. (1978). Quite the reverse is true: there is little evidence of significant wound healing in the injury "model" created in the present study even by the 63rd post-burn day. As the burned rat loses body mass and shrinks in the physical size (see Caldwell, 1970, Fig. 5), around the unhealing raw burn surface, the continuing high rate of evaporative heat loss becomes a relatively greater stimulus to increase the rate of heat production in the remaining weight of lean body mass. Figures 150, 151 and Table 61 show the clear relationships between the rates of evaporative (insensible) heat loss and

the rates of dry (sensible) heat loss in burned and control group rats, over post-burn period 1 - 4. The partitioning of heat losses after 25% BSA burn injury shown in the present study differ from those observed by Caldwell et al. (1966) in rats with 20% BSA burns, where no increment in (dry) sensible heat loss was found after burn injury, despite large increases in (evaporative) insensible heat loss. According to McLean (1974), this relationship between insensible and sensible heat losses can only be clearly seen when as in the present rat study, food intake is constant. In situations where food intake varies and sweat evaporative water loss is an important route of heat transfer, as in human patient studies, then a direct relationship is lost (Zawacki et al., 1970; Wilmore et al., 1976; Wilmore, 1977). (Fig. 11)

In the present study, in rats with 25% BSA full depth skin burns, which receive a fixed post-burn energy intake, the evidence based on direct calorimetry measurements of partitioned heat losses is consistent with the view that increased evaporative heat loss is the principal physical cause of post-burn hyper-metabolism.

Fur covered rodents which lack conventional cutaneous sweat glands are clearly different from man in their mechanisms of body heat transfer. Detailed

thermometry studies of differences between skin, core and wound temperatures in the rat are not relevant to man except in a general way. Nevertheless provided partitioned heat exchange can be measured accurately, and it is known that no change in body heat content occurs during the course of a calorimetry measurement period, (p.153), then relevant information may be derived from the rat burn 'model' which is similar to published findings regarding tissue heat conductance in burned man. This has been previously considered (p. 153). General conclusions based upon partitioned heat loss measurements in the rat regarding the aetiology of post-burn hypermetabolism should therefore be valid in man. These observations in the rat which indicated that increased evaporative heat loss was the principal physical cause of post-burn hypermetabolism were in agreement with the findings and views of Arturson et al. (1977) regarding burned man. The view advanced by Wilmore et al. (1976B) that the principal cause of post-burn hypermetabolism is a central hypothalamic resetting of metabolic rate, in which the increased evaporative heat losses via the wound play only a passive role, simply providing a convenient route of loss for the extra heat production, could not be substantiated in the present study.

INDIRECT CALORIMETRY

The calculation of heat production has previously been considered in detail (p. 132). Because of potential

errors in 24 hour urine collections inherent in any metabolic collection system even under ideal conditions (p. 218) together with the fact that nitrogen losses had to be measured on a separate group of rats not subjected to calorimetry runs, a simplified form of Weir's (1949) equation was used to calculate heat production values from oxygen consumption and carbon dioxide production. Weir assumed that 12.5% of the total oxidised energy was derived from protein breakdown. In the present study, based on the metabolic cage urine collections, the protein oxidation contribution to RME was slightly greater than the value assumed by Weir at around 20%. (Table 63). There was no significant difference between percentage values before and after burn injury, nor between burn and control group rats. These values in the rat are in agreement with the findings of Kinney et al. (1970) in severely injured man, where protein oxidation, based on urinary nitrogen excretion, accounted for between 12 to 22% of daily RME.

In the present study, urinary nitrogen losses increased by up to 33% above pre-burn 'basal' levels (Table 49). Similar findings have been noted by Tilstone and Cuthbertson (1970) and Cuthbertson (1976). These increases in urinary nitrogen excretion did not materially alter the percentage contribution made by

protein oxidation to total heat production (RME), suggesting that there was no "selective" protein catabolic effect being caused by the burn injury. Rather it suggested that normal metabolic processes were being stimulated to a higher rate to meet the energy demands of the injury (p. 28). Though not presented in this thesis, the author, in conjunction with colleagues, has noted prolonged greatly elevated rats of urinary catecholamine excretion in rats with 25% BSA burn injuries (Richards et al., 1978; Al-Shamma, 1978). As in burned man, urinary catecholamine excretion remained elevated until the burn wound began to heal. This would be sufficient to maintain an increased metabolic rate over a prolonged period after burn injury. There appeared to be a direct relationship between the rate of evaporative heat loss and the level of catecholamine excretion, though it could not be adequately confirmed in the study cited because of the small number of rats studied.

The precise nature of tissue fuel utilised after severe injury in man has proved difficult to determine by indirect metabolic balance techniques (Moore and Brennan, 1975). Kinney and his co-workers (Kinney et al., 1970; Gump et al., 1973) developed and used indirect calorimetry techniques in man. Direct calorimetry has been attempted in burned patients,

but does not appear to be a practicable technique (Bradham, 1972).

The evidence produced by Kinney et al. (1970) indicated that body fat was the major calorie source after severe injury (Fig. 9). RQ measurements in the post-absorbtive rat in the present study suggest that fat is also the principal energy course utilised in both burned and uninjured control rats, during the calorimetry measurement periods (p. 322, Table 62). RQ measurements, however, represent at best only an indication of general trends in metabolism (p. 134) (Kleiber, 1961), and in addition only reflect happenings during the relatively short calorimetry measurement period in the post-absorbtive state. Figure 9 (Kinney et al., 1970) illustrates the problems in calculating data from indirect measurements of short duration, and extrapolating that data to a 24 hour value. The daily calorie balance must be particularly suspect because of the difficulty of "estimating" total daily energy expenditure from short term measurements of RME. For this reason, I have not produced a similar figure or graph based on the rat data in this thesis. An alternative method exists, in the experimental rat, by which the nature of the tissue fuel utilised after injury may be accurately measured.

For some time, the tissue composition of weight gained in relation to various trial diets has been

studied in livestock of agricultural importance (Brozek, 1968). Animals are slaughtered at intervals to provide accurate analysis of chemically determined body composition. This provides a check upon the accuracy of conventional indirect calorimetry techniques in farm animals (p. 52). Similar slaughter techniques may be used in experimental rats to provide accurate information of the absolute changes in body composition after experimental burn injury (Al-Shamma, 1978). Direct measurement of body composition at intervals also provides a check on the validity of the indirect nitrogen balance methods classically used in the rat to determine the severity of injury (Baird et al., 1979). This technique of direct body composition analysis has been developed by the author to complement the conventional indirect calorimetry techniques described in this thesis. As these studies form the basis of current and future work, details of the methods and results are not presented as a part of this thesis, though the knowledge gained from these studies (Al-Shamma, 1978) has been taken into account in formulating the following concluding statements.

CONCLUSIONS

The burn wound occupies a unique and extreme position in the spectrum of energetic, metabolic and weight changes after injury. The aetiology of hyper-

metabolism after severe burns in man remains disputed, the facts obscured in part by difficulties inherent in the interpretation of indirect methods of measuring energy expenditure in critically ill subjects. Though direct measurements of heat loss have been attempted in burned patients, the practical difficulties of maintaining appropriate medical and nursing care proved insurmountable in these studies.

The burn wound represents a complex and severe physiological assault upon the homeostatic mechanisms of the body with many specific derangements principally related to the destruction of the skin. In this thesis, in order to make a successful model of an injury where so many factors interact, it had to be viewed as a problem where individual components were first identified and then controlled. Therefore, by varying one factor, its effect upon the other relevant factors could be isolated. Such an approach appeared essential, as previous studies of burn injury varied in so many different aspects that the work of one group could not be directly compared with that of others, resulting in a loss of valuable scientific information and needless duplication.

The rat proved to be the most suitable animal in which to create a burn injury model applicable to man. First, more was known about the rat metabolic

response to injury than that of any other animal, including man. The known responses to burn injury in the rat in regard to nitrogen excretion and energy expenditure were similar to those in man. The cost and scale of facilities required, namely, controlled laboratory conditions and the construction of a direct gradient layer and indirect calorimetry system were within acceptable limits, and could be created within existing laboratory space and did not require special building construction or plumbing. The design, construction and evaluation of precisely controlled rat living conditions, with attention to ambient temperature, light dark cycle, noise and dust levels, bacterial spread, design of cages, food dishes, type of diet, metabolic collecting system glassware, special cage racks, careful handling and rat weighing techniques, though it consumed much of the author's time and initial effort, represented the absolutely essential basic laboratory groundwork for the metabolic and calorimetry studies presented in this thesis. By defining the laboratory conditions in such detail, other workers can reproduce the system which has proved successful in the Institute of Physiology. A similar approach was adopted in developing a burn injury "model" which could recreate the weight loss and other metabolic effects of severe burn injury in man. It proved possible to produce a 25% BSA burn wound which did not have any immediate post burn mortality when untreated, and which appeared to cause little pain

and suffering in the rat, nor interfere with its movement and feeding. Yet, by preventing the rat from voluntarily increasing its post burn food intake, this injury resulted in severe weight loss such as is seen in human burn cases, where partial starvation is frequently an inadvertent accompaniment to the injury.

Nitrogen balance techniques in the rat, as in man, despite particular attention to detail in the matter of excreta collection, resulted in small daily cumulative errors which were difficult to avoid and which produced a progressive inaccuracy which was directly in proportion to the duration of the study. Measurement of urinary nitrogen excretion nevertheless proved valuable in the burned rat in order that the severity of the burn wound could be judged both of itself, and in regard to nitrogen excretion measurements by others in similar studies in rat and man.

Construction of the SEC - A - 04 L calorimetry system as described in this thesis represented a major investment in time and effort by the then W.R.H.B. Clinical Physics and Bioengineering Department. The Glasgow calorimeter proved to be the most accurate installation of its type in current use, reflecting as much the skill and care with which it was assembled as any inherent superiority in its overall design. In general terms the selection of a single rat size gradient layer design of calorimeter has been justified

by the results obtained in this thesis. It proved to be entirely suitable for the measurement of the rate of resting metabolic energy expenditure, and for the partitioning of heat losses. It was less useful in its ability to measure the total daily energy expenditure where a slightly larger size of gradient layer box would have proved superior, in that the injured rat could have lived, eaten, and slept within the calorimeter. As it was, if individual rats were starved overnight prior to regular calorimetry runs, then their responses to injury were aggravated by the technique of calorimetry itself. This did prove a handicap in designing experiments, and it was ultimately necessary to abandon rat body temperature measurements during calorimetry runs on this account.

The results obtained in the present studies are in agreement with the view that increased evaporative heat loss is the principal physical cause of post burn hypermetabolism in the rat, and that these findings are applicable to man because of the many similarities noted between burned rat and burned man. RQ measurements indicated that tissue fat stores were the main body energy source in burned and control rats. This has been confirmed by subsequent direct chemical body composition analysis of burned rats in further studies carried out after the work described in this thesis was completed. On the basis of these results, the

following therapeutic measures are likely to be of particular benefit in reducing hypermetabolism and weight loss in burned patients.

First, the rapid reduction of evaporative water loss via the wound surface must be achieved. The most practicable approach is to cover the burn areas immediately with skin grafts.

Second, adequate nutrition must be continuously supplied in an assimilable form throughout the post burn phase in order to preserve body energy stores (in particular, patients should not be repeatedly starved prior to anaesthesia and skin grafting). Third, reduction of catecholamine secretion stimulated by the energy losses via the burn wound must be achieved. This involves the use of elevated environmental temperatures to reduce "cold stress" on the burned subject, and may require the use of selective adrenergic blocking agents in addition to other measures. Such studies form the basis of current and future work using the special facilities of the Institute of Physiology, University of Glasgow.

GENERAL DISCUSSION

TECHNICAL ACHIEVEMENTS

1. This is the first gradient layer calorimeter measurement of partitioned heat losses after burn injury in rats to be made at typical hospital ward ambient temperature and humidity conditions, 20°C with high humidity. This was made possible by the special design features of the new calorimeter. Previous measurements have been limited to conditions of near thermal neutrality with a completely dry atmosphere, 27.4-30°C at a dew point of -70°C (Caldwell et al., 1966).

2. A close correlation with less than 2.4% difference at 20°C ambient temperature was obtained between direct and indirect calorimetry measurements of energy expenditure in normal and in burned rats. This agreement between independently calculated values for heat loss and heat production in rats was achieved as a result of the design features of the Glasgow University calorimeter, and the values obtained are comparable with similar measurements made in large animal gradient layer calorimetry systems which have significantly greater thermal inertia (Dale et al., 1967; Pullar et al., 1967; Mount et al., 1967; McLean, 1971).

3. The gradient layer calorimetry system to hold one rat was designed and constructed in collaboration with the Department of Clinical Physics and Bioengineering of the Western Regional Hospital Board. The calorimeter is designed to operate over a range of ambient temperature ahd humidity conditions between 20-30^{o}C and at 0.01g to 15g H_2O/m^2/dry air background humidity. This range is below that of thermally neutral temperature conditions of 30-33^{o}C for the laboratory rat.

4. By controlling the temperature of the gradient layer box water jacket, its surrounding insulated enclosure, and the laboratory in which the calorimeter was housed, a "no-load" thermopile output from the gradient layer box was achieved within $\pm$ 0.15mV of zero at operating temperatures of 20^{o}C and 30^{o}C. These conditions remained stable to within $\pm$ 0.02mV over three hours, giving a $\pm$ 0.5% level of accuracy for thermopile measurement of heat loss for a 250g rat during this period.

5. Significant changes in rat body heat content were avoided by using an internal partial air recirculation to control humidity within the calorimeter gradient layer box. This was confirmed by skin and core temperature measurements at 20^{o}C and 30^{o}C ambient temperatures in normal rats and at 20^{o}C ambient temperature in burned rats.

6. Because of the close agreement between direct and indirect measurements of heat loss and heat production and the stability of body heat content, restraint of the rat and multiple body temperature measurements proved unnecessary. This was an advance compared with previous rat calorimeter design in which the rat under study was pinioned and harnessed (Caldwell et al., 1966).

7. At the time of installation the automated data handling system incorporated in the Glasgow University calorimeter was unique.

CALORIMETRY FINDINGS - NORMAL RATS

1. In a thermoneutral environment of 30°C, normal rats in the present study obeyed Voit's Table on Surface Law (Table 30), with measured total heat loss between 1097-1039 kcal/m^2/day or 5.4 W/kg - 5.1 W/kg.

2. In a colder environment of 20°C, resting metabolic expenditure (RME) in normal rats was 6.0 W/kg, 16% greater than that found at thermoneutral conditions. An ambient temperature of 20°C therefore represents a mild cold stress for the laboratory rat.

3. RME measured in rats at 30°C ambient temperature approximately equals published basal metabolic rate values (BMR) measured in thermoneutral conditions (Kleiber, 1956).

4. The velcro shoulder harness with thermistors, used to measure body temperatures, caused abnormally high and variable metabolic rates in normal rats due to struggling. RME measurement was therefore regarded as unreliable in harnessed rats.

5. In unrestrained normal rats completely accustomed to the calorimeter, occasional random activity occurred at 20^{o}C ambient temperature. For accurate comparison of direct measurement of heat loss with indirect measurement of heat production, measurement periods were selected which began and ended during periods of rat inactivity.

6. Inactive normal rats at 20^{o}C ambient temperature in high humidity conditions had an insensible heat loss of 0.3-0.6 W/kg, representing 4.7-9.5% of the total heat loss.

7. Small increases in the rate of sensible heat loss were found to accompany rats movement between recumbent and standing positions. This was attributed to the alteration in exposed surface area with increased radiant and convective heat loss.

8. During activity, normal rats increased the rate of insensible heat loss by up to 55% over the resting rate, associated with increased respiratory tract water loss. Increased sensible heat loss was also

noted during rat movement, associated with increased dorsal skin, tail, and external auditory meatus temperatures. Increased heat production matched increased heat losses during these periods of rat activity.

9. Respiratory quotient measurements in post absorbtive unrestrained rats at 20°C ambient temperature were within the established physiological range for such conditions (Kleiber, 1961). The mean RQ value was 0.69 $\pm$ 0.2 (SD).

10. Fat was therefore the principal metabolic fuel in post absorbtive normal rats.

CALORIMETRY FINDINGS - BURNED RATS

A summary of the results in the 20% BSA burn study is given in Table 64 A summary of the results in the 25% BSA burn study is given in Table 65.

TABLE 64 — 20% BSA BURN STUDY : SUMMARY OF RESULTS : 20° AMBIENT TEMPERATURE

Time Period: Days Post Burn	RME W/kg	% Incr. Above Pre-Burn	EVAP. Heat Loss W/kg	% Incr. Above Pre-Burn	DRY Heat Loss W/kg	% Incr. Above Pre-Burn	Core - Skin Heat Transfer Rate $W/m^2/^oC$
Pre-Burn	7.2	-	0.5	-	6.8	-	Pre-Burn 7.6
Maximum Value Reached	14.1	96	3.8	711	10.3	53	Post-Burn Days 1-16 10.7
Mean Value Over Days 1-37	11.3	56	2.7	470	8.6	28	Maximum Rate on Day 14 15.3
Value on Day 37	9.7	35	1.6	245	8.2	21	At 30°C Amb. Temp. 13.4

TABLE 65 25% BSA BURN STUDY : SUMMARY OF RESULTS : 20°C AMBIENT TEMPERATURE

Time Period: Days Post Burn	RME W/kg	% Incr. Above Control	EVAP. Heat Loss W/kg	% Incr. Above Control	DRY Heat Loss W/kg	% Incr. Above Control	NITROGEN EXCRETION mgN/g/wt/ day	% Incr. Above Control	RESPIRATORY QUOTIENT
2-8	8.7	25	2.3	240	6.4	2	1.9	36	0.69
9-20	10.8	55	3.7	408	7.2	15	2.0	44	0.71
21-33	13.1	90	4.9	624	8.3	34	2.1	49	0.69
34-63	14.6	119	5.2	694	9.3	58	-	-	0.72

METABOLIC RESPONSE TO BURN INJURY - EFFECT OF INCREASING BURN SIZE

5% BSA BURN

NITROGEN EXCRETION

There was no significant difference in nitrogen excretion between burn and control rat as a result of a 5% BSA burn. There was a significant increase in nitrogen excretion after fur shaving in burned and in control group rats, largely explained by the effect of increased food intake upon nitrogen excretion.

BODY WEIGHT CHANGE

There was no significant difference between burn and control groups rats' mean body weights as the result of a 5% BSA burn.

20% BSA BURN

NITROGEN EXCRETION

There was no significant difference in daily nitrogen excretion between rats with a 20% BSA burn and pair fed control rats which were anaesthetised but not burned.

BODY WEIGHT CHANGE

There was no significant difference between burned and pair fed control group rats as a result of a 20% BSA burn.

25% BSA BURN

Nitrogen excretion results are summarised in Table 65.

RESTING METABOLIC EXPENDITURE - RME

The term RME is used in this thesis to describe basal metabolic rate plus the energy required for minimal physical activity by the fasted rat during the calorimetry measurement period. This is the technique of classical calorimetry studies. RME is expressed as the rate of heat production per unit time and the value obtained applies only to the period of measurement. Intermittent RME measurements, as used in this thesis, provide an accurate method of assessing the level of basal metabolism, because the variations due to rat activity and food intake are excluded. Reliable measurement of evaporative and dry heat loss is only possible in resting, near basal conditions. Such measurements allow the importance of evaporative heat loss to be assessed in relation to changes in RME.

Rat body weight changes and daily nitrogen excretion were measured to provide an index of the severity of the burn injury. No quantitative correlation can be made between such measurements and daily RME values. To correlate rat tissue and metabolic changes after burns with changes in energy expenditure it would be necessary to measure the total daily energy balance continuously over the entire post burn period. The SEC-A-04L calorimetry system can not be adapted to carry out such a study. The modifications needed would entail an increase

in the size of the gradient layer box with access for food and water supply and cage cleaning to comply with the Home Office regulations. The interference to the calorimeter that would be caused by using these facilities would disturb the heat balance to a significant degree. This is because the rat calorimetry system has a small thermal mass and poor thermal inertia properties. Gradient layer calorimeters successfully used to study total daily energy balance in cows are massive in comparison to SEC-A-04L, and possess significantly greater thermal inertia (McLean, 1971). Such calorimeters can not satisfactorily be scaled down to suit a rat. Previous workers have attempted to overcome this problem by using groups of 9 rats within a larger gradient layer box, but this was not a successful solution (Cairnie et al., 1957). The only way to overcome this problem is to use a larger animal of at least 30kg such as the hairless mini-pig for long term energy balance studies. When a rapid response characteristic is not required, the gradient layer calorimeter has no advantage over the cheaper and more robust "heat-sink" design (Mount et al., 1967).

EVAPORATIVE WATER LOSS

The rate of evaporative water loss, expressed as W/kg, increased progressively after burn injury, in parallel with the gradual disintegration of the burn eschar, and the rate of body weight loss in burned rats.

The rate of evaporative heat loss was greater in the 25% BSA burn than in the 20% BSA burn, and was directly proportional to the surface area of the burn wound. In the 25% BSA burn, there was no evidence of central healing of the burn wound even by the 63rd day after burning. In absolute terms, evaporative heat loss remained elevated throughout the post burn period while at the same time the burned rat's body weight fell. This increased the rate of evaporative heat loss expressed per unit body weight.

At 20^{o}C ambient temperature, with control rats in resting post absorbtive state, evaporative heat loss accounted for about 10% of the total RME, while in rats with a 25% BSA burn, evaporative heat loss accounted for about 34% of total RME under the same conditions. Dry heat loss accounted for about 90% of total RME in the same control rats and about 66% of total RME in rats with a 25% BSA burn. Thermometry was carried out in the 20% BSA burn group only and indicated that rat core temperature can rise by 1.3^{o}C. If a similar increase in core temperature took place in the 25% BSA burn group and continued throughout the post burn period, then it would account for an increase of 17% in RME according to Wilmore's calculations (Wilmore, 1977). This increase is significantly less than the 34% increase in RME required to compensate for the increased evaporative heat loss observed in this group of rats. From these observations

it is concluded that at 20°C, 10°C below the rat thermoneutral zone, increased evaporative heat loss constitutes the principal physical demand for increased RME in a rat with a 25% BSA burn.

In the 20% BSA burn study, there was a high rate of heat transfer from core to skin. This indicates a loss of normal surface insulation in the burned rat. At 20°C ambient temperature, the rate of core to skin heat transfer in the burned rat was comparable to that measured in uninjured control rats at 30°C ambient temperature. This suggests that the burned rat's hypothalamic "set-point" for thermoregulation may be increased, as noted in burned man by Aulick et al. (1979).

RAT BODY COMPOSITION CHANGES AFTER INJURY

FAT

In further studies with Dr. G. A. A. Al-Shamma, the body composition of rats sacrificed at intervals after 25% BSA burn injury was determined by direct chemical analysis (Al-Shamma et al., 1979). Burned rats lost weight throughout the 60 day post burn period, and by the 56th day they were 30% less than their initial weight. No significant healing or reduction in the area of the burn wound was noted. Identically fed uninjured control rats gained weight steadily by 1.6g per day.

During the first 30 days after injury, body fat content in burned rats fell to 20-30% of the initial value, representing a reduction from 97g to 25g of fat per kilogram of body weight. This low fat content remained steady for the last 30 days of the experiment. A direct linear relationship was found between relative body fat and relative total body water for injured and for control rats.

PROTEIN

Body protein was also rapidly lost over the first 30 days after injury, falling to 75% of the initial value. However by the 60th post burn day, there was no significant

difference in the relative protein content of burned and uninjured rats. A direct linear relationship was demonstrated between the amounts of total body water and total body protein in both groups.

WATER

In man, Kinney et al. (1970) postulated a loss of body water in excess of that associated with loss of protein after injury. By direct chemical analysis of burned rats, this author and his colleagues (Al-Shamma et al., 1979) found no evidence for loss of this so-called "excess body water" as a consequence of injury. The relationships between fat and water, and protein and water were not significantly altered when a 25% BSA burn was accompanied by diet restriction, a severe enough challenge to inhibit rat growth and cause rapid weight loss.

CORRELATION OF FAT AND PROTEIN LOSSES WITH RME

Data from these body composition studies supported the calorimetric observations that body fat was the principal energy source in burned rats over the first 30 days after injury. This conclusion is consistent with the finding that mean RQ was 0.70 $\pm$ 0.02 (SD) during this period. However the significant protein losses which occurred at this time were not reflected in the post absorbtive respiratory quotient. This result was influenced

by the experimental procedure which involved fasting the rats for 12 hours prior to calorimetry. Because of this it can be argued that these post absorbtive calorimetry studies primarily determined the response to short periods of starvation in burned and in uninjured rats. The same criticism applies to the experiments of Caldwell et al. (1966) who employed an 18 hour fast period prior to calorimetry in rats.

In retrospect, a 4 hour food deprivation period would have been sufficient to render the rat post absorbtive in order to measure its "basal" metabolic status.

COMPARISON OF RESULTS WITH PREVIOUS RAT BURN INJURY STUDIES

Caldwell, Hammel and Dolan's experiments in 1966 are the only comparable gradient layer calorimeter studies of partitioned heat loss after burn injury in rats. Although they housed their rats at 20^{o}C, they were obliged to operate their calorimeter at 30^{o}C to avoid water condensation in the air circuit. This limitation was a consequence of the dry atmosphere, dew point -70^{o}C, within the calorimeter, necessitated by the single humidity detector. The steep water vapour partial pressure gradient between rat epidermis and air caused a high rate of evaporation and accompanying water condensation problems. Unfortunately these conditions prevented the operation of the calorimeter at 20^{o}C and also promoted evaporative

heat loss to the extent that no increase in dry heat loss was found in the burned rats. The finding that only evaporative heat loss increased after injury while rat core and skin temperatures remained normal differs from the present author's results, and is partly explained by these calorimeter design problems. Another contributory factor is the difference in severity of the burn. Whereas the 20% BSA full skin depth burn wound used in this thesis did not contract and heal after 60 days, the 20% BSA burn of Caldwell et al. contracted to 13% BSA after 22 days and 6% BSA after 43 days.

INFLUENCE OF THERMONEUTRAL ENVIRONMENT

Studies by the present author and colleagues on burned rats housed at 30°C ambient temperature (Drury, 1976; Richards et al., 1978), demonstrated that at thermoneutral conditions the injured rats remained slightly hypermetabolic compared to control rats. This is believed to be a direct consequence of the increased rate of evaporative heat loss from the burn wound. The rate of sensible dry heat loss was similar in burned and control rats at 30°C ambient temperature, and was 43% less than the value at 20°C ambient temperature before injury. Total RME was reduced by 30% in burned rats by increasing the ambient temperature from 20°C to 30°C. This reduction in RME was presumably related primarily to the reduction in sensible dry heat loss in thermoneutral

conditions. The rate of evaporative heat loss was similar at both ambient temperatures. In general these calorimeter studies in rats at 30°C agree with the findings of Aulick et al. (1979) in man. However the metabolic rates of burned rats did not return to normal when housed in thermo-neutral conditions, as suggested by Arturson and his co-workers in man (Arturson, 1978; Arturson et al., 1977; Danielsson et al., 1976). The evidence in this thesis suggests that post burn hypermetabolism is primarily the result of thermoregulatory drives in the rat at low ambient temperature of 20°C. An elevated metabolic state is produced with raised core and skin temperatures. Considered in terms of thermoregulation, the burned rat appears unable to compensate for the loss of normal surface insulation at the area of the burn wound by attempting to decrease heat losses elsewhere in the skin by vasoconstriction. An increase in its metabolic rate is essential to balance the increased cooling effect of evaporative water loss from the burn wound.

Wilmore et al. (1977) put forward an explanation for the increased skin bloow flow and heat loss after burns which is not related solely to heat exchange considerations. They found that glucose provides a specific fuel for the granulation tissue in the burn wound, and that it is metabolised to lactate by the "primitive" newly formed granulation tissue. The lactate is recycled by the liver to new glucose, the energy required to support this increased hepatic gluconeogenesis being derived principally

from fat. It was suggested that increased peripheral circulation occurs after burns to transport heat and glucose preferentially to the wound. The energy cost of the hyperdynamic circulation and reparative processes is reflected in the increased metabolism and breakdown of body tissues.

RQ measurements in burned patients in this study were between 0.70 and 0.73 as measured by post absorbtive calorimetry, similar to the RQ values found by the present author in burned rats housed at 20°C ambient temperature. Evidence of increased glucose flow has also been found in the injured rat (Allsop et al., 1978; Wolfe and Burke, 1978).

Although the increased demands of the burn for glucose and the associated metabolic adaptations may be the major determinants of events in thermoneutral environments, recent biochemical evidence (Aprille et al., 1979) suggests that prolonged increased catecholamine secretion caused by cold exposure or by increased evaporative heat loss may evoke regulatory mechanisms in "target cells" which directly contribute to hypermetabolism. The present author and his colleagues (Richards et al., 1978) showed that increased catecholamine excretion occurs up to 60 days after 25% BSA burn injury in the rat at 20°C ambient temperature. Furthermore the increase in catecholamine excretion was directly related to the rate

of increase in evaporative heat loss. Sustained elevation of metabolic rates after burns is also correlated with chronically elevated catecholamine levels in man and experimental animals (Wilmore et al., 1976). Aprille et al. (1979) have shown that in rats with 20% BSA burns kept at unspecified room temperatures, adipose tissue adenylate cyclase remained fully responsive to chronic catecholamine stimulation, suggesting that the regulation of hormone sensitivity in adipose tissue cells was altered by burn injury. Unlike control animals, burned rats were resistant to fat cell adenylate cyclase desensitisation by acute exposures to catecholamine administration. Chronically elevated catecholamine levels can therefore remain a powerful stimulus to the breakdown of fat stores and increased hepatic gluconeogenesis after burn injury.

RELEVANCE OF THESIS FINDINGS TO BURN INJURY

The results in this thesis indicate the significant thermal cooling effect of even moderate (25%BSA) full skin depth burns when the subject is kept at an environmental temperature 10^{o}C below thermoneutral ambient conditions. This effect is due to a combination of increased evaporative heat loss from the burn wound and a loss of normal surface insulation, with an increased rate of core to surface heat transfer.

When food energy intake is not increased beyond the level consumed before injury, then rapid depletion of body fat stores occurs, presumably associated with chronic elevated catecholamine excretion caused by the increased evaporative heat loss. Rapid covering of the burn wound with split skin allografts or homografts should confer significant advantage to the burned patient if they reduce the rate of evaporative heat loss from the wound surface and provide additional surface insulation. This would improve thermal energy balance and reduce catecholamine excretion and weight loss. The level of food energy intake should be calculated to exceed the likely energy losses through the burn wound and repeated episodes of even brief starvation prior to general anaesthesia for repeated or muptiple skin grafts should be avoided altogether in the burned subject.

Finally, the value of indirect calorimetry has been established. The usefulness of this measurement in burned patients is limited by the need for fasting which should be avoided.

FURTHER WORK IN RELATION TO "MODEL" BURNS IN ANIMALS

Three main areas for further research in burns using animal models appear important at this time.

1. Defining the role of catecholamines in post burn hypermetabolism.

Basic research into cellular mechanisms of catecholamine actions such as that carried out by Aprille et al. (1979) appears of fundamental importance to our present understanding of post burn hypermetabolism. Investigation of those factors which modify catecholamine excretion such as the use of adrenergic blocking drugs or cold exposure should be extended to the burned rat. Further work using impermeable coverings to block evaporative water loss from the burn coupled with measurement of catecholamine excretion would be valuable.

2. Relating energetic and body composition changes after burns to differing nutritional intakes.

The technique of direct body composition analysis gives a precise measurement of chemical changes in body tissues in experimental animals, changes in which can only be indirectly estimated in man. Measurement of changes in whole body and individual organ composition in response to different feeding regimes and diets coupled with the application of impermeable coverings and thermoneutral environment could provide information of considerable therapeutic importance. Long term studies of different diets and ambient temperatures can be assessed in burned

animals by the same techniques that farm animals of economic value are assessed in regard to the efficiency and cost effectiveness of agricultural feedstuffs and living conditions. Studies such as these carried out on the normal growing pig by Mount et al. (1967) using a "heat-sink" calorimeter provide a valuable model for similar studies on burned animals.

3. Determining tissue substrate flux and turnover after burns.

The work of Wolfe and Burke (1978) and Allsop et al. (1978) recently indicated the importance of glucose and lactate metabolism in burned animals. By combining body composition analysis with isotope infusion studies it is possible to use animal models to determine tissue specific activities. Further studies of substrate changes must be measured in relation to protein and fat synthetic and catabolic rates at the same time as measurements are made of whole body energy expenditure. Such information would be fundamental to our understanding of the mammalian response to injury.

FURTHER RESEARCH WORK IN RELATION TO BURNED PATIENTS

The following topics appear important at this time.

1. Determination of the importance of burn energy losses and body thermoregulation in relation to the enhanced circulatory and metabolic demands of the healing wound.

   Indirect calorimetry combined with core and skin temperature measurements has recently provided important information on heat and energy exchanges in burned patients nursed at various controlled ambient temperatures within the range of thermoneutrality (Aulick et al., 1979). These studies should be extended to include the role of the hypothalamus in thermoregulation after burn injury over a wider range of ambient temperatures.

   The application of radiant heat to burned patients as an alternative to elevation of the ambient temperature to the thermoneutral range remains controversial (Arturson, 1978; Aulick et al., 1979), and its merits need assessment.

   Wilmore's use of isolated limb plethsmography, indirect calorimetry and simultaneous measurement of substrate metabolism in burned and non burned limbs has already provided unique information on

the influence of the burn wound itself on the local and systemic responses to burn injury (Wilmore et al., 1977; Aulick et al., 1979). Such studies could be repeated with the addition of the splanchnic and central venous catheter methods of Gump et al. (1970), to provide more information of liver gluconeogenesis in relation to the increased glucose uptake and lactate formation of the burn wound.

2. Determination of whole body fat, protein, and carbohydrate changes in relation to the composition of tissue lost after burn injury.

The importance of body fat as an energy source and the factors regulating its rate of utilisation after burns have received relatively little attention in comparison with that paid to body protein as an energy store and the interpretation of nitrogen losses. Studies of fat synthetic and catabolic rates in relation to the rates of protein synthesis and catabolism are needed to understand the ways in which body fat may best be preserved and regained in burned patients.

THE ROLE OF THE EXPERIMENTAL ANIMAL IN BURN RESEARCH

In my view the experimental animal will remain important in future research in two ways:

1. Direct body composition analysis of animals sacrificed at intervals after burn injury will provide essential information in our understanding of the absolute nature of tissue changes after burns, and will complement indirect studies of tissue synthetic and catabolic rates made in man. Preliminary results by the present author and his colleagues indicate the importance of body composition analysis in understanding the relationship between whole body water, protein, and fat, as weight is progressively lost after burn injury (Al-Shamma et al., 1979).

2. Body composition analysis in animals can readily be used to assess the "efficiency" of conversion of various foodstuffs into body tissue and to assess the effectiveness of different diets after injury. Such studies are routine in the field of animal husbandry (Brozek, 1968). Animal studies alone can provide this direct information after injury.

In regard to studies on thermoregulation after burn injury, the use of animals for research has been largely superseded by the recent work of Wilmore and his co-workers in the Brooke Army Hospital Burns Unit in Texas on burned human patients (Aulick et al., 1979). Detailed information from indirect calorimetry and body

thermometry has become available in controlled thermal environments in burned man. The real value of indirect calorimetry has been demonstrated in this thesis. The laboratory rat differs from man in its response to cold exposure. In the fur covered rodent, skin vasoconstriction is less important than in man. The rat increases its rate of heat production rapidly in response to cold via brown fat and non shivering thermogenesis (Gelineo, 1964) in contrast to the heat conservation responses to cold in man (Richards, 1973).

In regard to metabolic studies on burn injury, the continually growing rat resembles the human infant more closely than adult man. Failure to grow must be considered as well as weight actually lost, a factor which makes the interpretation of nitrogen balance studies more difficult in the rat than those in adult man.

Finally the use of the Wilkinson Sword Bunyan Contact Burn Apparatus to produce experimental full skin thickness burns in the rat may have unforeseen disadvantages. The application of dry thermal energy to skin by a heated metal plate, with precise control of temperature, pressure and time of burning is similar to the method described by Stadtler et al. (1972). Kremer et al. (1979) subsequently noted that such methods cause complete loss of skin water content and can result in the production of a specific cutaneous burn toxin. They found a macro-

molecular lipid-protein toxin complex which caused intra-mitochondrial vacuolation of liver cells 5 to 7 days after injury. In vitro experiments indicated that purified toxin suppressed liver cell metabolism in isolated liver cells. This toxin was not formed in burns where complete loss of cutaneous water content did not occur.

"Model" burn injuries in animals may be useful to our understanding of the effects of burns on humans, but only in a general way for the reasons already mentioned. The animal "model" can be used to examine directly the relationships between energy expenditure, body composition, and the chemical composition of weight lost after burn injury, as well as to determine the most "cost effective" form of post burn nutritional support.

The rat is not useful as an analogue for man in studies of thermoregulation after burn injury in the light of the detailed information now available using indirect calorimetry and other techniques in burned man (Aulick et al., 1979).

APPENDIX 1

SEC - A - 04L CHANNEL IDENTIFICATION

CHANNEL NUMBER			
0	....	0 Volts	
1	....	Gradient layer thermopile output	Black dot
2	....	Temperature difference between outlet and inlet airflow ($T_3 - T_2$)	Green dot
3	....	Temperature of inlet airflow (T_2) ...	Purple dot
4	....	Oxygen analyser differential output	Yellow dot
5	....	Carbon dioxide analyser output	Blue dot
6	....	Inlet/outlet airflow	Red dot
7	....	Recirculated airflow	Black circle
8	....	Hygrometer differential output	Green circle
9	....	Dew point heat exchanger outlet air temperature (T_1)	Purple circle
10	....	Rat external auditory meatus temperature	Yellow circle
11	....	Rat dorsum temperature/wound surface	Blue circle
12	....	Rat base of tail temperature	Red circle

13	....	Rat dorsum temperature/skin
14	....	Gradient layer chamber wall temperature
15	....	Wet gas meter air temperature (T_4)
16	....	Insulated housing temperature
17	....	Set atmosphere pressure
18	....	Set Wet Gas Meter pressure
19	....	Calibration heater current

APPENDIX 1

SEC - A - 04L CHANNEL IDENTIFICATION

CHANNEL NUMBER		
20	....	Set calibration heater voltage
21	....	Hygrometer reference cell air temperature
22	....	Hygrometer analysis cell air temperature
23	....	Temperature of proportional heater
24	....	Room air temperature
25	....	Set 177.5 mV
26	....	Set 143.35 mV (0°C)
27	....	Set 138.35 mV (5°C)
28	....	Set 128.35 mV (15°C)
29	....	Set 125.85 mV (17.5°C)
30	....	Set 123.35 mV (20°C)
31	....	Set 118.35 mV (25°C)
32	....	Set 115.85 mV (27.5°C)
33	....	Set 113.35 mV (30°C)
34	....	Oxygen analyser block temperature
35	....	Calibration heater voltage

APPENDIX 2

SEC - A - 04L CALORIMETRY SYSTEM

OPERATING PROCEDURE

1. Ensure that all analysers and data recording equipment have been switched to 'on' position for a minimum period of 3 hours before commencing experimental run.

2. Ensure that calorimetry laboratory temperature is stable - check function of air conditioning/ ventilation equipment and observe wall thermostat setting and max/min thermometer.

3. Check the water levels in:

   i inlet air humidifier
   ii wet gas meter humidifier
   iii dew point setting heat exchanger water bath and its water trap
   iv wet gas meter heat exchanger water bath and its water trap
   v inlet/outlet flow wet gas meter
   vi recirculation flow wet gas meter
   vii gradient layer heat exchanger water bath
   viii hygrometer heat exchanger water bath

4. Ensure that correct operating temperature settings have been made and that heat exchanger water bath temperatures are correct and stable.

APPENDIX 2

SEC - A - 04L CALORIMETRY SYSTEM

OPERATING PROCEDURE

5. Check calibration of the Digital Voltmeter ($\pm$ 1.1913 and zero).

6. Adjust settings on Channels 25 - 33 to the required values.

7. Check temperature readings on all water bath circulators.

8. Check function of the temperature controller in gradient layer heat exchanger water bath.

9. Check that the rotary air valve on mimic diagram panel is in correct position for humidity setting required.

10. Check condition of the drying agents in:

    i Main ventilatory air circuit - twin cells on mimic diagram panel

    ii Hygrometer

    iii Gas analyser air circuit

    iv Cold trap

APPENDIX 2

SEC - A - 04L CALORIMETRY SYSTEM

OPERATING PROCEDURE

11. Ensure that gradient layer chamber door is closed, switch on main ventilatory air circuit pumps and hygrometer internal pumps. Regulate the inlet/outlet and recirculation air flow rates as required and adjust till pressures in gradient layer chamber and across main air pump are optimal.

12. Adjust inlet/outlet air flow and recirculation air flow on channels 6 and 7 at Zero and Span settings.

13. Set flow rates on gas analyser rotameters to 50 ml/minute.

14. Check oxygen analyser block temperature on Channel 34 at $32^{\circ}C$.

15. Calibrate oxygen and carbon dioxide analysers at zero and span settings as shown in separate appendices.

16. Turn gas analyser selector valve to 'run' position. See Fig. 35.

APPENDIX 2

SEC - A - 04L CALORIMETRY SYSTEM

OPERATING PROCEDURE

17. Adjust hygrometer control valves to give atmospheric pressure in Reference and Analysis cells at a flow rate of 5 ml/min.

18. Check hygrometer Reference and Analysis cell temperatures on Channels 21 and 22. Both temperatures should be $50^{\circ}C \pm 0.1^{\circ}C$.

19. Carry out hygrometer calibration as shown on separate appendix, at zero and span settings.

20. Turn gas analyser selector valve back to 'run' position.

21. Set Foster chart recorder range switches to required values, and switch on recorder.

22. Wait till baseline values are constant.

23. Re-check dew point heat exchanger water bath temperature on Channel 9 and adjust water circulator unit temperature if required.

APPENDIX 2

SEC - A - 04L CALORIMETRY SYSTEM

OPERATING PROCEDURE

24. Note room (calorimeter laboratory) temperature and atmospheric pressure. Record these values and all other relevant experimental information on the cyclostyled data sheets.

25. Set Channel 17 valve to that of atmospheric pressure reading.

26. Read Wet Gas Meter pressure and set Channel 18 to this value.

27. Calibrate rat temperature probes on Channels 10, 11, 12 and 13.

28. Set the number of channels to be recorded by data logging system.

29. Set the experimental time on the data logger clock and set the required time sampling interval using the 'Clock Call' control.

APPENDIX 2

SEC - A - 04L CALORIMETRY SYSTEM

OPERATING PROCEDURE

30. Switch on Facit high speed paper punch. Depress 'punch' key on the data logger to activate.

31. Leave entire system running for a minimum of 30 minutes prior to insertion of rat into chamber and note Foster chart recorder evidence of satisfactory equipment function.

APPENDIX 3

HYGROMETER - ANALYTICAL DEVELOPMENTS

OPERATING PROCEDURES

1. Switch on inlet air pump and main recirculation air pump. Adjust valves on mimic diagram control panel to set gradient layer chamber pressure to - 2 cm H_2O.

2. Switch on hygrometer air pump and adjust gain control. A20 for high background humidity, B24 for dry background humidity.

3. Switch on Analysis and Reference air pumps. Adjust control valves to give atmosphere pressure in each cell at air flow rate of 5 ml/min. The correct sequence of adjustment steps is shown for each cell on the instruction sheet attached adjacent to the mimic diagram.

4. Check hygrometer heat exchanger water bath temperature ($33^{\circ}C$).

5. Check hygrometer Analysis and Reference cell temperatures on Channels 21 and 22. Both temperatures should be $50^{\circ}C \pm 0.1^{\circ}C$.

APPENDIX 3

HYGROMETER - ANALYTICAL DEVELOPMENTS

OPERATING PROCEDURES

6. Allow system to function with ventilatory air flow through gradient layer chamber until hygrometer output stabilises (Channel 8).

7. When stability has been achieved adjust zero if required. Note: A zero reading can be obtained in two positions of the zero control; the only correct setting is that which gives an increase in the meter reading when the zero control is turned in a clockwise direction. When making a zero setting adjustment it should be possible for the meter needle to pass below the zero mark. If this is not possible then further adjustment is required and the instrument instruction manual must be consulted. If it is necessary to check zero during an experiment a selector switch mounted on the hygrometer allows Analysis and Reference cell air inlets to be linked.

8. Re-check hygrometer output on Channel 8. Note all air flows, temperatures and other parameters on Foster chart recorder.

APPENDIX 3

HYGROMETER - ANALYTICAL DEVELOPMENTS

OPERATING PROCEDURE

9. Weigh special alloy tray containing a water saturated (3 ml) square of absorbent tissue. Open gradient layer chamber door, place tray and contents on floor of the gradient layer chamber and quickly shut door.

10. Select Channel 8 and note hygrometer output on Foster chart recorder over next 60 - 100 minutes. Check that data logging system and 'Clock Call' in logging data at 10 minute intervals as described in Appendix 1.

11. Record all experimental details of calibration run on cyclostyled sheet as for Appendix 1, in particular noting entry and exit times for the tray.

12. Open gradient layer chamber door, remove tray and quickly shut door. Allow hygrometer to return to stable zero reading.

13. Re-weigh tray and record the weight of water evaporated during the calibration run.

APPENDIX 3

HYGROMETER - ANALYTICAL DEVELOPMENTS

OPERATING PROCEDURE

14. From the Foster chart recorder tracing, and from the data logger output, plus the written record of the run, compute the hygrometer calibration factor.

APPENDIX 4

OXYGEN ANALYSER

OPERATING PROCEDURE

1. Switch on analyser and allow to 'warm-up' for at least 3 hours prior to use.

2. If oscillation is noted on either Analysis or Reference cell meters, turn the appropriate meter control knob to 'Amp Zero' setting.

3. Using the cell selector switch on the instrument panel, the output from the Analysis cell or the Reference cell or their difference may be displayed at will on the Foster chart recorder and Channel 4 of the digital voltmeter. Each cell is calibrated separately as follows:

   i. Check cell temperature on Channel 34. This should be $32^{o}C \pm 0.5^{o}C$.

   ii. If not already so positioned, turn the meter control knob to 'Amp Zero' setting. The meter needle should now indicate 20 on the 100 scale. If it does not, the 'Amp Zero' control is turned very slowly (with a screwdriver) until the required value is shown.

APPENDIX 4

OXYGEN ANALYSER

OPERATING PROCEDURE

3. iii. Turn the meter control knob to '25%' setting. Set the gas selector valve to 'zero' thus passing oxygen free nitrogen into the cell. Adjust panel rotameter flow rate to 50 ml/min $\pm$ 2 ml/min. Leave nitrogen flowing for a minimum of 10 minutes, then adjust the 'zero' control to give a zero reading on the digital voltmeter (Channel 4). The digital voltmeter should be set to the 110 mV range.

iv. Turn the gas selector valve to 'Span' position. Switch on the ventilatory circuit inlet pump and flush the cell with atmospheric air. This is the span gas. Re-adjust the panel rotameter to give a flow rate of 50 ml/min $\pm$ 2 ml/min. Allow air to pass through the cell for a minimum period of 10 minutes. Adjust the 'Span a' control to give a 21% oxygen reading on the panel meter. Then adjust 'Span b' control to give 209.5 mVm on the digital voltmeter. The digital voltmeter span is dependent upon the meter span, but not vice-versa.

APPENDIX 4

OXYGEN ANALYSER

OPERATING PROCEDURE

4. When each cell has been separately calibrated, to record oxygen depletion in the air passing through the chamber of SEC - A - 04L, the cell selector switch is set at 'Differential', and the gas selector valve is turned to 'Sample'. The Reference cell then receives atmospheric air from the ventilatory circuit inlet pump and the Analysis cell receives outlet air flow from the gradient layer chamber of SEC - A - 04L (See Fig. 22). Ensure that the air flow rate through both cells is 50 ± 2 ml/min.

5. Set the digital voltmeter range to 110 mV. The oxygen analyser output on Channel 4 then represents atmospheric oxygen - gradient layer chamber outlet air oxygen. A 1% oxygen difference between 21% and 20% gives 10 mV.

APPENDIX 5

CARBON DIOXIDE ANALYSER

OPERATING PROCEDURE

1. Switch on analyser and allow to 'warm-up' for at least 3 hours prior to use.

2. Turn the gas selector valve to 'zero' position and allow oxygen free nitrogen to flush the analyser at 5 ml/min, for at least 10 minutes, then adjust the zero setting using the digital voltmeter (110 mV range) on Channel 5. The zero control has a considerable time delay.

3. Next turn the gas selector valve to the 'Span' position and allow the span gas to flow through the analyser at 5 ml/min for a minimum of 10 minutes. The span control is adjusted to bring the digital voltmeter reading to match the previously measured value of the span gas (approx. 0.8% CO_2 in nitrogen).

4. The zero and span controls interact. It is essential to repeat the zero and span procedure until neither requires further adjustment.

APPENDIX 6

CALCULATION OF CALORIMETRY RESULTS

The calculation of the calorimetry results has been presented in detail by other workers (Carter, K.B., M.Sc., 1975 and Gray, W.M., Ph.D., 1978) and only a summary is presented here. (The key to symbols used is at the end of the appendix).

The recirculated and outlet flows must be calculated under the standard conditions of dry air at standard temperature and pressure (STP), i.e. $0^{o}C$ and 760 mmHg. As the gas is saturated just before the wet gas meters the partial pressure of the dry gas in the meters (P) is :-

P = Pressure at the meters - Pressure of saturated water vapour at WGM temperature (T_w)

$$= P_A + \frac{P_w}{1.357} - (1.43 \times T_w - 11.5) \text{ mmHg.}$$

Therefore the correction factor to STP is

$$CF = \frac{P}{760} \times \frac{273.2}{(273.2 + T_w)}$$

The outlet WGM produces 480 counts at the logger per litre of gas flow through it and the recirculation meter produces 24 counts per litre. Therefore the outlet flow (F_o) corrected to STP is

$$F_o = \frac{N_o \times CF}{480} \quad (l.min^{-1})$$

The recirculated flow is :-

$$F_R = \frac{N_R \times CF}{24} \quad (l.min^{-1})$$

and the total flow F_T is

$$F_T = F_o + F_R = \left(\frac{N_o}{480} + \frac{N_R}{24} \right) \times CF \quad (l.min^{-1})$$

Direct calorimetry

The main route of sensible heat loss is through the walls of the calorimeter box (H_1)

$$H_1 = K_{TH} \times V_G \quad (W)$$

The K_{TH} valve over the range 20 - 30°C has been calibrated at

$$K_{TH} = \frac{0.357 + 0.002 \times (T_c - 20)}{(0.965 - 0.002 \times F_T) \times (1 + 0.00015 \times (W_R - 250))} \quad (WV^{-1})$$

Another route of heat loss is the heat given to the ventilating air in the calorimeter box (H_2). The gas ventilating the calorimeter box is dry air plus the background humidity water capour. Any water vapour respired or lost from the wound will also heat up the ventilating gases and must be subtracted to give the direct heat losses to the ventilating gases.

$$H_2 = \frac{F_T}{60} \left[D \times C_p \times (T_o - T_I) + \left(\frac{Z}{1000} \right) \times C_w \times (T_o - T_I) \right] - \left(\frac{V_H \times K_H}{1000} \right) \times C_w \times (36 - T_o) \quad (W)$$

The cooling of urine and faecal losses are another small correction to the direct losses that can be approximated by

$$H_3 = \frac{(M_U + M_F) \times (T_R - T_C) \times 4.18}{L \times 60} \quad (W)$$

Sensible direct heat losses (H_4) are therefore :-

$$H_4 = H_1 + H_2 - H_3 \quad (W)$$

The insensible heat loss (H_5) as the water vapour evaporates from the respiratory tract and wound area can be calculated as below

$$H_5 = \frac{F_T}{60} \times V_H \times \frac{760}{P_A} \times \frac{A}{1000} \times 2420$$

The total direct heat losses (H_6) are

$$H_6 = H_4 + H_5.$$

Indirect calorimetry

The carbon dioxide production (CP) is calculated using

$$CP = F_O \times \frac{V_C}{1000} \times \frac{760}{P_A} \times 1.035 \quad l.min^{-1}$$

The oxygen consumption (OC) is calculated with regard to the difference in volume between inspired and expired gas when RQ does not equal unity.

$$OC = \frac{F_O}{.7905} \times \frac{V_O}{1000} \times \frac{760}{P_A} - \frac{0.2095}{0.7905} \times CP \quad l.min^{-1}$$

Using Weir's simplified formula assuming 12.5% of heat is produced from protein metabolism (this approximation involved an error of C 0.3% over the protein metabolism range 10 - 15%) the heat production H_6 is

$$H_6 = 274 \times OC + 70 \times CP \quad (W)$$

GLOSSARY OF ABBREVIATIONS

- P - Partial pressure of dry gas at wet gas meters (mmHg)
- P_A - Ambient pressure (mmHg)
- P_w - Pressure over ambient at WGM (cms.H_2O)
- T_w - Temperature of WGM (^oC)
- CF - Correction factor to STP dry air
- F_o - Outlet flow (l min^{-1})
- N_o - Number of counts in outlet wet gas meter channel per min. (l min^{-1})
- F_R - Recirculated flow (l min^{-1})
- N_R - Number of counts in recirculation WGM channel per min (l min^{-1})
- F_T - Total flow (l min^{-1})
- H_l - Heat loss through gradient layer box walls (W)
- K_{TH} - Calibration value for GLB (WV^{-1})
- V_G - Output of GLB (mV)
- T_C - Average calorimeter temperature = $\frac{T_o + T_I}{2}$ (^oC)
- T_I - Calorimeter inlet temperature (^oC)
- T_o - Calorimeter outlet temperature (^oC)

W_R - Weight of rat (g)

D - Density of dry air at STP = 1.293 (g/l)

C_p - Specific heat at constant pressure for air = 1.0045 ($Jg^{-1}\ ^oC^{-1}$)

C_w - Specific heat of water vapour = 1.922 ($Jg^{-1}{}^oC^{-1}$)

Z - Humidity of inlet air ($g\ m^{-3}$)

V_H - Output of hygrometer (mV)

H_2 - Heat loss to ventilating air (W)

H_3 - Heat loss error due to urine and faecal cooling (W)

M_u - Mass of urine (g)

M_F - Mass of faeces (g)

T_R - Core temperature of rat (^oC)

L - Length of run (min)

H_4 - Sensible heat losses (W)

H_5 - Insensible heat losses (W)

A - Hygrometer calibration factor ($g\ m^{-3}\ mV^{-1}$)

H_6 - Total direct heat losses (W)

CP - Carbon dioxide production ($l\ min^{-1}$)

V_C - Carbon dioxide analyser output (mV)

OC - Oxygen consumption

H_6 - Indirect heat production (W)

APPENDIX 7

COMPOSITION OF LOW IODINE TEST DIET

(NUTRITIONAL BIOCHEMICALS CORPORATION, U.S.A.)

% BY WEIGHT

YELLOW CORN MEAL	78%
PROTEIN	19%
WHEAT GLUTEN	18%
BREWERS YEAST	2%
CALCIUM CARBONATE	1%
SODIUM CHLORIDE	1%

DRINKING WATER SUPPLEMENTED WITH POTASSIUM IODIDE

(5ug/10ml)

ABBOTT, W.E. & ALBERTSON, K. (1963) The effect of starvation, infection and injury on the metabolic processes and body composition. Ann. N.Y. Acad. Sci., 110, 941-964.

ABEL, R.M. (1976) Parenteral nutrition in the treatment of renal failure. In Total Parenteral Nutrition, ed. Fischer, J.E. pp. 143-170. Boston : Little Brown & Co.

ABEL, R.M., BECK, C.H., ABBOTT, W.M., RYAN, J.A., BARNETT, G.O. & FISCHER, J.E. (1973) Improved survival from acute renal failure after treatment with intravenous essential L-amino acids and glucose: Results of a prospective double blind study. New Engl. J. Med., 288, 695-699.

AGUIRRE, A., FUNOVICS, J., WESDORP, R.I.C. & FISCHER, J.E. (1976) Parenteral nutrition in hepatic failure. In Total Parenteral Nutrition, ed. Fischer, J.E. pp. 219-230. Boston : Little Brown & Co.

ALBERTI, K.G., BATSTONE, G.F. & JOHNSTON, D.G. (1977) Hormonal changes in trauma : role of cortisol. In Nutritional Aspects of Care in the Critically Ill, ed. Richards, J.R. & KINNEY, J.M. pp. 225-252. Edinburgh, London & New York : Churchill Livingstone.

ALLISON, S.P. (1974) Carbohydrate and fat metabolism - response to injury. In Parenteral Nutrition in Acute Metabolic Illness, ed. Lee, H.A. pp. 167-175. London & New York : Academic Press.

AL-SHAMMA, G.A.A. (1978) Lipid metabolism after injury : Effects of nutrition and environmental temperature. Ph.D. Thesis, University of Glasgow.

AL-SHAMMA, G.A.A., GOLL, C.C., BAIRD, T.B. & RICHARDS, J.R. (1979) Changes in body composition following thermal injury in the rat. Brit. J. Nutr. In press.

ARMSBY, H.P. & MOULTON, C.R. (1925) The Animal as a Converter of Matter and Energy. New York : Chemical Catalog. Co., Inc.

ARTURSON, G. (1964) The infliction and healing of a large standard burn in rats. Acta Path. Microbiol. Scand., 61, 353.

ARTZ, C.P. & MONCRIEF, J.A. (1969) In The Treatment of Burns, Ch. 1, pp. 1 - 21. Philadelphia & London : W. B. Saunders Co.

ARTURSON, G., DANIELSSON, U. & WENNBERG, L. (1977) The effect of infra-red radiation on the metabolic rate and nutrition of patients with severe burns. In Nutritional Aspects of Care in the Critically Ill, ed. Richards, J.R. & Kinney, J.M. Edinburgh, London & New York : Churchill Livingstone.

ARTZ, C.P. & REISS, E. (1957) The Treatment of Burns, Philadelphia : W. B. Saunders Co.

ATWATER, W.O. & ROSA, E.B. (1899) Description of new respiration calorimeter and experiments on the conservation of energy in the human body. U.S. Dept. Agr. Off. Exp. Sta. Bull., p. 63.

ATTWATER, W.D. & ROSA, E.B. (1899) A new respiration calorimeter. U.S. Dept. of Agriculture, Washington. Bull. 63.

BAIRD, T.B., BELL, J., BROOM, J., FLECK, A., GOLL, C.C., GORDON, S., NICHOLAS, G.A. & RICHARDS, J.R. (1979) Nitrogen retention in growing animals : discrepancy between direct determination of body composition and metabolic balance studies. Submitted to the British J. Nutr.

BALLINGALL, Sir.G. (1833) Outlines of Military Surgery, (2nd ed.) Edinburgh : Adam & Chas. Black.

BARR, P.O., BIRKE, G., LILJEDAHL, S.-O. & PLANTIN, L.-O. (1968) Oxygen consumption and water loss during treatment of burns with warm dry air. The Lancet, 1, 164-168.

BARTLETT, R.G., MANTEL, N., FOSTER, G.L. & BERNSTEIN, P. (1958) Core to surface thermal gradients in the rat at several environmental temperatures. Am. J. Physiol., 193(3), 541-546.

BEAL, J.M., CORNELL, G.N. & GILDER, H. (1954) Factors influencing nitrogen metabolism in surgical patients. Surgery, 36, 469-484.

BEATTY, J. (1947) Metabolic disturbances after injury. Brit. Med. J., 2, 813-817.

BEATON, J.R. (1963) Nitrogen metabolism in cold exposed rats. Canadian J. Biochem. & Physiology, 41, 1169-1179.

BEISEL, W.R. (1975) Metabolic response to infection. Annual Review of Medicine, 26, 9 - 20.

BENZINGER, T.H., HUEBSCHER, R.G., MINARD, D. & KITZINGER, C. (1958) Human calorimetry by means of the gradient principle. J. Appl. Physiol, 12, Supplement 51, 524.

BENZINGER, T.H. & KITZINGER, C. (1949) Direct calorimetry by means of the gradient principle. Rev. Sci. Instrum., 20, 849-860.

BERGSTROM, J., BUCHT, H., FURST, P., HULTMAN, E., JOSEPHSON, B., NOREE, L.O. & VINNARS, E. (1972) Intravenous nutrition with amino acid solutions in patients with chronic uraemia. Acta Med. Scand., 191, 359-367.

BERKSON, J. (1953) A statistically precise and relatively simple method of estimating the bioassay with quantal response based on the logistic function. J. Amer. Statis. Ass., 48, 565.

BLACKBURN, G.L. & BISTRIAN, B.R. (1976) Nutritional care of the injured and/or septic patient. Symposium on Response to Infection and Injury II. Surg. Clin. North America, 56, 1195-1224.

BLALOCK, A. (1931) Experimental shock. Importance of loss of fluid in production of blood pressure after burns. Arch. Surg., 22, 610-616.

BLISS, C.I. (1938) The determination of dosage mortality curve from small numbers. Q.J. Pharm. Pharmacol., 11, 192.

BOLLES, R.C. & DUNCAN, P.M. (1969) Daily course of activity and subcutaneous body temperature in hungry and thirsty rats. Physiol. Behav. 4(1), 87-89.

BOTHE, W. (1958) Some calculations concerning the gradient calorimeter. J. Appl. Physiol., 12, 525-528.

BOOCOCK, G., SWINGLER, M.R.M. & TIDSWELL, B.M. (1971) An automated gas meter. J. Sci. Instrum., 4, 611 - 613.

BORDER, J.R. (1970) Metabolic response to short term starvation, sepsis and trauma. In Surgery Annual, ed. Cooper, P. & Nyhus, L.M. pp. 11-33. New York : Appleton Century Crofts.

BRADHAM, G.B. & CHARLESTON, S.C. (1972) Direct measurement of total metabolism of a burned patient. Arch. Surg., 105, 410-413.

BRAMANTE, P.O. (1959) Apparatus for automatically recording oxygen consumption, respiratory movements and activity of small animals. J. Appl. Physiol., 14, 1063-1065.

BRAMANTE, P.O. (1961) Quantitation of oxygen consumption and spontaneous muscular activity of the rat. J. Appl. Physiol., 16(6), 982-990.

BROZEK, J. & GRANDE, F. (1955) Body composition and basal metabolism in man : correlation analysis versus physiological approach. Hum. Biol., 27, 22.

BROZEK, J. (ed.) (1968) Body composition in animals and man : Proceedings of a Symposium held May 4 - 6 (1967) at University of Missouri, Columbia. Washington, D.C. : National Academy of Sciences.

BULL, J.P. & FISHER, A.J. (1954) A study of mortality in a burn unit : a revised estimate. Ann. Surg., 139, 269-274.

BULL, J.P. & SQUIRE, J.R. (1949) A study of mortality in a burns unit : standards for the evaluation of alternative methods of treatment. Ann. Surg., 130, 160-173.

BUTCHER, E.O. (1934) The hair cycles in the albino rat. Anat. Record, 61, 5-19.

CAHILL, G.F. (1970) Starvation in man. New Engl. J. Med., 282, 668-675.

CAHILL, G.F., HERRERA, M.G., MORGAN, A.P., SOELDNER, J.S., STEINKE, J., LEVY, P.L., REICHARD, G.A. & KIPNIS, D.M. (1966) Hormone - fuel inter-relationships during fasting. J. clin. Invest., 45, 1751-1769.

CAIRNIE, A.B., CAMPBELL, R.M., CUTHBERTSON, D.P. & PULLAR, J.D. (1957) The heat production consequent upon injury. Brit. J. Exptl. Path., 38, 504-511.

CALDWELL, F.T. (1961) The effect of early eschar excision upon the nutritional state and rate of wound closure of rats with full thickness thermal burns. Surgery, 49, 454-460.

CALDWELL, F.T. (1962) Metabolic response to thermal trauma II. Nutritional studies with rats at two environmental temperatures. Ann. Surg., 155, 119-126.

CALDWELL, F.T. (1970) Changes in energy metabolism during recovery from injury. In Energy Metabolism in Trauma, Ciba Foundation Symposium, ed. Porter, R. & Knight, J. pp. 23-38. London : Churchill.

CALDWELL, F.T., HAMMEL, H.T. & DOLAN, F. (1966) A calorimeter for simultaneous determination of heat production and heat loss in the rat. J. Appl. Physiol., 21, 1665-1671.

CALDWELL, F.T., OSTERHOLM, J.L., SOWER, N.D. & MOYER, C.A. (1959) The metabolic response to thermal trauma of normal and thyroprivic rats at three environmental temperatures. Ann. Surg., 150, 976-988.

CALLOWAY, D.H., GROSSMAN, M.I., BOWMAN, J. & CALHOUN, W.K. (1955) The effect of previous level of protein feeding on wound healing and the metabolic response to injury. Surgery, 37, 935-946.

CAMPBELL, R.M. & CUTHBERTSON, D.P. (1967) The effect of environmental temperature on the metabolic response to injury. Quart. J. Exptl. Physiol., 52, 114-129.

CAMPBELL, R.H., SHARP, G., BRYNE, A.W. & CUTHBERTSON, D.P. (1954) Cortisone and the metabolic response to injury. Brit. J. exp. Path., 35, 566-576.

CANNON, W.B. (1923) in Traumatic Shock. New York : Appleton

CANNON, W.B. (1967) in The Wisdom of the Body. New York : W. W. Norton Co.

CARLSON, L.D., HONDA, N., SASAKI, T. & JUDY, W.V. (1964) A human calorimeter. Proc. Soc. Exp. Biol. Med., 117, 327-331.

CARTER, K.B. (1975) Development of a calorimetry system and its use in the comparison of direct and indirect calorimetry in small animals. M.Sc. Thesis, University of Glasgow.

CHASE, H.B. (1958) in The Biology of Hair Growth, ed. Montagna, W. & Ellis, R.A. New York : Academic Press, Inc.

CLARK, R.G. (1967) Surgical nutrition. Brit. J. Surg., 54, 445-459.

CLOWES, G.H.A., O'DONNELL, T.F., BLACKBURN, G.L. & MAKI, T.N. (1976) Energy metabolism and proteolysis in traumatized and septic man. Symposium on Response to Infection and Injury II. Surg. Clin. North America, 56, 1169-1184.

CLOWES, G.H.A., O'DONNELL, T.F., RYAN, N.T. & BLACKBURN, G.L. (1974) Energy metabolism in sepsis : Treatment based on different patterns of in shock and high output state. Ann. Surg., 179, 684-696.

COCKSHOTT, W.P. (1956) The history of the treatment of burns. Surg, Gynec. & Obstet., 102, 116-124.

COLEMAN, J.E. (1969) Metabolic inter-relationships between carbohydrates, lipids and proteins. In Duncan's Diseases of Metabolism, ed. Bondy, P.K. & Rosenberg, L.E. pp. 89-198. Philadelphia : W. B. Saunders Co.

COPE, O., GRAHAM, J.B., MOORE, F.D. & BALL, A.B. (1948) The nature of the shift of plasma protein to extra-vascular space following thermal trauma. Ann. Surg., 128, 1041-1055.

COPE, O. & MOORE, F.D. (1944) Study of capillary permeability in experimental burns and burn shock using radioactive dyes in blood and lymph. J. Clin. Invest., 23, 241-257.

COPELAND, W.P. (1887) The treatment of burns. Med. Rec. 31, 518.

COTUI, F.W., WRIGHT, A.M., MULHOLLAND, J.H., COWABBA, V., BARCHAM, I. & VINCI, V.J. (1944) Studies on surgical convalescence. I Sources of nitrogen loss post gastrectomy and effect of high amino acid and high calorie intake on convalescence. Ann. Surg., 120, 99-122.

CRAWFORD, A. (1788) Experiments and observations on animal heat and the inflammation of combustible bodies. (2nd ed.), London, cited by KLEIBER, M. (1961) in The Fire of Life - an introduction to animal energetics, p. 410. New York & London : John Wiley & Sons, Inc.

CUMMING, M.C. & MORRISON, S.D. (1960) The total metabolism of rats during fasting and refeeding. J. Physiol., 154, 219-243.

CUTHBERTSON, D.P. (1929) The influence of prolonged muscular rest on metabolism. Biochem. J., 23, 1328-1345.

CUTHBERTSON, D.P. (1930) The disturbance of metabolism produced by long bone and non bony injury, with notes on certain abnormal conditions of bone. Biochem. J., 24, 1244-1263.

CUTHBERTSON, D.P. (1931) The disturbance of nitrogen and sulphur in the urine during conditions of increased catabolism. Biochem. J., 25, 235-244.

CUTHBERTSON, D.P. (1932) Observations on disturbance of metabolism produced by injury to the limbs. Quart. J. Med., 25, 233-246.

CUTHBERTSON, D.P. (1934) Certain aspects of metabolic response to injury. Glas. Med. J., 121, 41-58.

CUTHBERTSON, D.P. (1936) Further observations on the disturbance of metabolism caused by injury with particular reference to the dietary requirements of fracture cases. Brit. J. Surg., 23, 505-520.

CUTHBERTSON, D.P. (1942) Post shock metabolic response. The Lancet, 1, 433-437.

CUTHBERTSON, D.P. (1964) Physical injury and its effects on protein metabolism. In Mammalian Protein Metabolism, Volume 2, ed. Munro, H.N. & Allison, J.B. pp. 373-414. New York : Academic Press.

CUTHBERTSON, D.P. (1970A) General discussions - protein metabolism after injury. In Energy Metabolism in Trauma - Ciba Foundation Symposium, ed. Porter, R. & Knight, J. p. 98. London : Churchill.

CUTHBERTSON, D.P. (1972) Protein requirements after injury - quality and quantity. In Parenteral Nutrition, pp. 4 - 23. Edinburgh & London : Churchill Livingstone.

CUTHBERTSON, D.P. (1975) Nutrition in relation to trauma and surgery. Progress in Food and Nutrition Science 1(4), 263-288.

CUTHBERTSON, D.P. (1976) Surgical metabolism : historical and evolutionary aspects. In Metabolism and the Response to Injury, pp. 1 - 34. ed. Williamson, A.W. & Cuthbertson, Sir David. Bath : Pitmen Medical Publishing Co.

CUTHBERTSON, D.P., McGIRR, J.L. & ROBERTSON, J.S.M. (1939) The effect of fracture of bone on the metabolism of the rat. Quart. J. Exptl. Physiol., 29, 18-25.

CUTHBERTSON, D.P. & MUNRO, H.N. (1937) Study of effect of over-feeding on protein metabolism of man : protein saving effect of carbohydrate and fat when superimposed on diet adequate for maintenance. Biochem. J., 31, 694-705.

CUTHBERTSON, D.P. & TILSTONE, W.J. (1968) Metabolic changes following different types of injuries and their relation to diet and evnironmental temperature. In Combined Injuries and Shock, ed. Schildt, B. & Thoren, L. pp. 99-114. Stockholm : Intermedes Proceedings, Swedish Res. Inst. Natl. Defense.

CUTHBERTSON, D.P. & TILSTONE, W.J. (1969) Metabolism in the post injury period. Adv. Clin. Chem, 12, 1-55.

DALE, H.E., SHANKLIN, M.D., JOHNSON, H.D. & BROWN, W.H. (1967) Energy metabolism of the chimpanzee : a comparison of direct and indirect calorimetry. J. Appl. Physiol., 22(4), 850-853.

DAVIDSON, J. & WILLIAMS, R.B. (1968) A note on the estimation of nitrogen retention in chicks by body analysis and balance methods. Brit. J. Nutr., 22, 291-295.

DAVIES, J.W.L. (1970) Protein metabolism following injury. J. Clin. Path., 23, Suppl. (Roy. Coll. Path.), 4, 56-64.

DAVIES, J.W.L., LAMKE, L.-O. & LILJEDAHL, S.-O. (1977) Treatment of severe burns. I. Pathophysiology and treatment of patients with burns covering 30-60% of the body surface. Acta Chirurgica Scandinavica, Suppl. 468, p. 5-23.

DAVIES, J.W.L., LAMKE, L.-O. & LILJEDAHL, S.-O. (1977) Treatment of severe burns. II. Metabolic studies during the successful treatment of three adult patients with burns covering 80-85% of the body surface. Acta. Chirurg. Scand., Suppl. 468. pp. 25-60.

DAVIES, J.W.L., LILJEDAHL, S.-O. & BIRKE, G. (1969) Protein metabolism in burned patients treated in a warm (32°C) or cool (22°C) environment. Injury, 1, 43-56.

DAVIES, J.W.L. & LILJEDAHL, S.-O. (1970) Protein catabolism and energy utilization in burned patients treated at different environmental temperature. In Energy Metabolism in Trauma, Ciba Foundation Symposium. ed. Porter, R. & Knight, J. pp. 59-71. London : Churchill.

DAVIES, J.W.L., RICKETTS, C.R. & BULL, J.P. (1959) Serum albumin catabolism and nitrogen excretion. The Lancet, 1, 346.

DEITRICK, J.E., WHEDON, G.D. & SHAW, E. (1948) Effects of immobilization on various metabolic and physiologic functions of normal men. Am. J. Med., 4, 3-36.

DEXTER, F. (1971) Pseudomonas Aeruginosa in a regional burns centre. J. Hyg. Camb., 69, 179-185.

DILL, D.B., BOCK, A.V. & EDWARDS, H.T. (1933) Mechanism for dissipating heat in man and dog. Am. J. Physiol., 104, 36-43.

DOLECEK, R. (1969) Metabolic Response of the Burned Organism, ed. Nowinski, W.W. Springfield, Illinois: Chas. C. Thomas.

DOUGLAS, L.G. & JONSSON, C.-E. (1969) Oxygen consumption in skin of experimentally burned rats. Scand. J. Plast. Reconstr. Surg., 3, 23-26.

DRUCHER, W.R., MILLER, M., CRAIG, J.W., JEFFRIES, W.M., LEVEY, S. & ABBOTT, W.E. (1953) A comparison of the effect of operation on glucose and fractose metabolism. Surg. Forum, 3, 548-555.

DRURY, J.K., RICHARDS, J.R. & BROWN, J.J. (1977) A rat training programme suitable for calorimetry. J.I.A.T., 28, 27.

DRURY, R.A.B. & WALLINGTON, E.A. (1967) ed. Carleton's Histological Techniques, 4th ed. Oxford University Press.

DUBOIS, E.F. (1937) The mechanism of heat loss and temperature regulation. Stanford University Press, Stanford, California, quoted in Kleiber (1961)

DUDLEY, H.A.F. (1959) The neuro-endocrine response to injury. J. Roy. Coll. Surg., Edinburgh 4, 137-146.

DUDLEY, H.A.F. (1968) Surgical convalescence. J. Roy. Coll. Surg. Edinburgh, 13, 1-11.

DUNBAR, J. (1934) Review of the burn cases treated in the Glasgow Royal Infirmary during the past 100 years (1833-1933), with some observations on the present day treatment. Glasgow Medical Journal, 122, 239-255.

ELLIOT, E.J. (1906) A case of Picric Acid poisoning followed by severe constitutional poisoning. The Lancet, 1, 1175.

EXTON, J.H., UI, M., LEWIS, S.B. & PARK, C.R. (1971) in Regulation of Gluconeogenesis, ed. Soling, H.D. & Willems, B. New York : Academic Press.

FALLON, R.H. & MOYER, C.A. (1963) Rates of insensible perspiration through normal burned tape stripped and epidermally denuded living human skin. Ann. Surg., 158, 915-923.

FELIG, P. (1973) The glucose-alanine cycle. Metabolism, 22, 179-207.

FELIG, P., MARLISS, E., OWEN, O.E. & CAHILL, G.F. (1969) Role of substrate in the regulation of hepatic gluconeogenesis in fasting man. Advances in Enzyme Regulation, 7, 41-46.

FELIG, P., OWEN, O.E., WAHREN, J. & CAHILL, G.F. (1967) Amino acid metabolism during prolonged starvation. Journal of Clinical Investigation, 48, 584-594.

FELIG, P. & WAHREN, J. (1971) Influence of endogenous insulin secretion on splanchnic glucose and amino acid metabolism in man. Journal of Clinical Investigation, 50, 1702-1711.

FISCHER, B. (1902) Zbl. allg. Path. path. Anat., 13, 977 as quoted from Institute of Physiology, Histology Unit, University of Glasgow.

FISCHER, J.E. (1976) ed. Total Parenteral Nutrition, Boston, Little Brown & Co.

FISCHER, J.E. YOSHIMURA, N., JAMES, H., AGUIRRE, A., CUMMINGS, M.G., ABEL, R.M. & DIENDOERFER, F. (1974) Plasma amino acids in patients with hepatic encephalopathy : Effect of amino acid infusions. Am. J. Surg., 127, 40-47.

FITZPATRICK, G.F., MEGUID, M.M., O'CONNELL, R.C., O'CONNOR, N.E., BALL, M.R. & BRENNAN, M.F. (1975) Nitrogen sparing by carbohydrate in man. Intermittent or continuous enteral compared with continuous parenteral glucose. Surgery, 78(1), 105-113.

FLATT, W.P. (1969) Methods of calorimetry (B) Indirect. In International Encyclopaedia of Food & Nutrition, ed. Cuthbertson, D.P., 17, 491-520. London : Pergamon Press.

FLATTERY, K.V. & SELLARS, E.A. (1971) Brown adipose tissue, catecholamines and survival in the cold In Bioenergetics - Proceedings of the International Symposium on Environmental Physiology, ed. Smith, R.E. pp. 141-147. Federation of American Societies for Experimental Biology.

FLEAR, C.T.G., BHATTACHARYA, S.S. & NANDRA, G.S. (1977) Cellular exchanges and happenings after injury and in the critically ill. In Nutritional Aspects of Care in the Critically Ill, ed. Richards, J.R. & Kinney, J.M. pp. 195-224. Edinburgh, London & New York : Churchill Livingstone.

FLEAR, C.T.G. & CLARK, R. (1955) The influence of blood loss and blood transfusion upon changes in metabolism of water, electrolytes and nitrogen following civilian trauma. Clin. Sci., 14, 575-599.

FLECK, A. (1967) The determination of organic nitrogen. Proc. Assn. Clin. Biochemists, 4, 212-215.

FLECK, A. (1976) The early metabolic response to injury. In Shock - Clinical and Experimental Aspects, ed. Ledingham, I. McA. Elsevire : North Holland Biomedical Press.

FLECK, A. & MUNRO, H.N. (1963) Protein metabolism after injury. Metab. Clin. Exptl., 12, 783-789.

FLECK, A. & MUNRO, H.N. (1965) The determination of organic nitrogen in biological materials. A review. Clinica Chim. Acta, 11, 2-12.

FRIEDMAN, A.H. & WALKER, C.A. (1968) Circadian rhythms in rat mid-brain and caudate nucleus biogenic amine levels. J. Physiol., 197, 77-85

FRAWLEY, J.P., ARTZ, C.P. & HOWARD, J.M. (1955) Muscle metabolism and catabolism in combat casualties. Arch. Surg, 71, 612-616.

GELINEO, S. (1964) Organ systems in adaptation : the temperature regulating system. In Handbook of Physiology (Section 4 : Adaptation to the Environment), ed. Dill, D.B., Adolph, E.F. & Wilber, C.G. pp. 259-282. Washington, D.C. : American Physiological Society.

GLASSTONE, S. (1946) Textbook of Physical Chemistry, 2nd ed. New York : D. Van Nostrand Co.

GLICKMAN, N., KEETON, R.W., COLE, W.H., CALLOWAY, N.O., MITCHELL, H.H., SAPIENZA, A.R., DYNIEWICZ, J. & HAWES, D. (1948) Early ambulation in surgical convalescence. Arch. Phys. Med. 29, 770-783.

GRAY, W.M. (1978) The effects of whole-body irradiation on thyroid hormones and metabolism in rats. Ph.D. Thesis, University of Glasgow.

GUMP, F.E. & KINNEY, J.M. (1970) Caloric and fluid losses through the burn wound. Surg. Clin. North. Am. 50, 1235-1248.

GUMP, F.E. & KINNEY, J.M. (1971) Energy balance and weight loss in burned patients. Arch. Surg., 103, 442-448.

GUMP, F.E., LONG, C.L., KILLIAN, P. & KINNEY, J.M. (1974) . Studies of glucose intolerance in septic injured patients. J. Trauma, 14, 378-388.

GUMP, F.E., PRICE, J.B., & KINNEY, J.M. (1970A) Whole body and splanchnic blood flow and oxygen consumption measurements in patients with intra-peritoneal infection. Ann. Surg., 171, 321-328.

GUMP, F.E., PRICE, J.B. & KINNEY, J.M. (1970B) Blood flow and oxygen consumption in patients with severe burns. Surg., Gynec. & Obstet., 130, 23-28.

HADDOW, A. & RUDALL, K.M. (1945) Artificial coat coloration and the growth of hair. Endeavour, 4, 141-147.

HAINSWORTH, F.R. (1968) Evaporative water loss from rats in the heat. Am. J. Physiol., 214(5), 979-982.

HAMMEL, H.T. & HARDY, J.D. (1963) A gradient type of calorimeter for measurement of thermoregulatory responses in the dog. In Temperature - Its Measurement and Control in Science and Industry, ed. Hardy, J.D. pp. 31-42. New York: Reinhold.

HAMMEL, H.T., WYNDHAM, C.H. & HARDY, J.D. (1958) Heat production and heat loss in the dog at 8 - 36^{o}C environmental temperature. Amer. J. Physiol., 194, 99-108.

HARDY, J.D. (1968) Heat transfer. In Physiology of Heat Regulation and the Science of Clothing. ed. Newburgh, L.H. New York : Hofner.

HARDY, J.D., GAGGE, A.P. & STOLWIZK, J.A.J. (1970) eds. Physiological and Behavioural Temperature Regulation. Springfield, Illinois : Chas. C. Thomas.

HARDY, J.D., NEELY, W.A. & WILSON, F.C. (1955) Thermal burns in man. VII. Insensible fluid loss. Surgery, 38, 692-695.

HARKINS, H.N. (1942) in The Treatment of Burns. Springfield, Illinois : Chas. C. Thomas.

HARRISON, H.N., MONCRIEF, J.E., DUCKETT, J.W. & MASON, A.D. (1964) The relationship between energy metabolism and water loss from vaporization of severely burned patients. Surgery, 56, 203-211.

HAYES, M.A. & BRANDT, R.L. (1952) Carbohydrate metabolism in the immediate postoperative period. Surgery, St. Louis, 32, 819-827.

HERNDON, D.N., WILMORE, D.W. & MASON, A.D. (1978) Development and analysis of a small animal model simulating the human post burn hypermetabolic response. J. Surg. Research, 25, 394-403.

HIMMS-HAGEN, J. (1967) Sympathetic regulation of metabolism. Pharm. Rev., 19, 367-461.

HIMMS-HAGEN, J., BUKOWIECKI, L., BEHRENS, W. & BONIN, M. (1971) Mechanism of non-shivering thermogenesis in rats. In Bioenergetics - Proceedings of the International Symposium on Environmental Physiology

contd...

ed. Smith, R.E. pp. 127-133. Federation of American Societies for Experimental Biology.

HORWITZ, B.A. & SMITH, R.E. (1971) Function and control of brown fat thermogenesis during cold exposure. In Bioenergetics - Proceedings of the International Symposium on Environmental Physiology, ed. Smith, R.E. pp. 134-140. Federation of American Societies for Experimental Biology.

HOROWITZ, J.M. (1971) Neural control of thermogenesis in brown adipose tissue. In Bioenergetics - Proceedings of the International Symposium on Environmental physiology, ed. Smith, R.E. pp. 115-121. Federation of American Societies for Experimental Biology.

HOWARD, J.E. (1945) Protein metabolism during convalescence from trauma. Arch. Surg., 50, 166-170.

HOWARD, J.E., PARSON, W., STEIN, K., EISENBERG, H. & REIDT, V. (1944) Studies on fracture convalescence. I. Nitrogen metabolism after fracture and skeletal operation on healthy males. Bull. Johns Hopkins Hosp., 75, 156-168.

HOWARD, J.M. (1955) Studies of the absorbtion and metabolism of glucose following injury. Ann. Surg., 141, 321-326.

HSIEH, A.C.L. & CARLSON, L.D. (1957) Role of the thyroid in metabolic response to low temperature. Am. J. Physiol., 188, 40-44.

HUNT, A.C. (1972) ed. Pathology of Injury. Current knowledge and future development. Report of a Working Party of the Royal College of Pathologists The Endocrine Response to Trauma, pp. 83-88.

HUME, D.M. (1974) Endocrine and metabolic response to surgery. In Principles of Surgery (2nd ed.) ed. Schwartz, S. pp. 1 - 43. New York : McGraw-Hill.

HUME, D.M., BELL, C. C. & BARTLER, F. (1962) Direct measurement of adrenal secretion during operative trauma and convalescence. Surgery, 52, 174-187.

HUME, D.M. & EGDAHL, R.H. (1959) The importance of the brain to injury in the endocrine response. Ann. Surg., 150, 697-712.

INGLE, D.J. (1951) Parameters of metabolic problems. Recent Progr. Hormone Res., 6, 159-194.

INGLE, D.J., WARD, E.O. & KUIZENGA, M.H. (1947) Relationship of adrenal glands to changes in urinary non protein nitrogen following multiple fractures in force-fed rat. Am. J. Physiol., 149, 510-515.

ISAKSSON, B. & SJÖGREN, B. (1967) A critical evaluation of the mineral and nitrogen balances in man. Nitrogen Balance Techniques and their Limitations, Symposium Proceedings. Proc. Nutr. Soc., 26, 106-116.

JACKSON, D.M. (1953) The diagnosis of the depth of burning. Brit. J. Surg., 40, 588-596.

JOHNSTON, I.D.A. (1968) The endocrine response to trauma. Scientific Basis of Medicine Annual Review, pp. 224-241.

JOHNSTONE, I.D.A. (1972) The endocrine response to injury. Adv. Clin. Chem., 15, 225-285.

KELLY, C.F., BOND, T.E. & HEITMAN, H. (1963) Direct "air" calorimetry for livestock. Trans. A.S.A.E., 6, 126-128.

KEYS, A., BROZEK, J., HENSCHEL, A., MICKELSEN, O. & TAYLOR, H.L. (1950) in The Biology of Human Starvation. Minneapolis : The University of Minnesota Press.

KINNEY, J.M. (1959) Influence of intermediary metabolism on nitrogen balance and weight loss. Metabolism, 8, 809-826.

KINNEY, J.M. (1975) Energy requirements of the surgical patient. In Manual of Surgical Nutrition, ed. Ballinger, W.F., Collins, J.A., Drucker, W.R. & Zeppa, R. pp. 223-235. Philadelphia, London : W. B. Saunders Co.

KINNEY, J.M. (1976) Energy requirements for parenteral nutrition. In Total Parenteral Nutrition, ed. Fischer, J.E. pp. 135-142. Boston : Little Brown & Co.

KINNEY, J.M. (1976) Surgical diagnosis, patterns of energy, weight and tissue change. In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. pp. 121-134. Bath : Pitman Medical Pub. Co.

KINNEY, J.M., DUKE, J.H., LONG, C.L. & GUMP, F.E. (1970) Tissue fuel and weight loss after injury. J. Clin. Path., 23, Suppl. (Roy. Coll. Path.), 4, 65-72.

KINNEY, J.M., LONG, C.L., & DUKE, J.H. (1970) Carbohydrate and nitrogen metabolism after injury. In Energy Metabolism in Trauma, ed. Porter, R. &
contd....

Knight, J. pp. 103-126. London : Churchill.

KINNEY, J.M., LONG, C.L., GUMP, F.E. & DUKE, J.H. (1968) Tissue composition of weight loss in surgical patients. I. Elective operation. Ann. Surg., 168, 459-474.

KINNEY, J.M., MORGAN, A.P., DOMINGUES, F.J. & GILDNER, K. (1964) A method for continuous measurement of gas exchange and expired radioactivity in acutely ill patients. Metabolism, 13, 205-211.

KLEIBER, M. (1932) Body size and metabolism. Hilgardia, 6, 315-353.

KLEIBER, M. (1950) in Biophysical Research Methods. New York : Interscience Publishers Inc.

KLEIBER, M. (1961) Development of calorimetry. In The Fire of Life. An introduction to animal energetics. pp. 116-122. New York & London: John Wiley & Sons, Inc.

KLEIBER, M., SMITH, A.H. & CHERNIKOFF, T.N. (1956) Metabolic rate of female rats as a function of age and body size. Am. J. Physiol., 186, 9-12.

KLEIBER, M. (1961) Body size and metabolic rate. In The Fire of Life, pp. 177-216. New York & London : John Wiley & Sons, Inc.

KREBS, H. (1964) Gluconeogenesis. The Croonian Lecture, 1963. Proceedings of the Royal Society, London, Series Biol. 159, 545-564.

KREIGER, H., ABBOTT, W.E., LEVEY, S., BABB, L.L. & HOLDEN, W.D. (1954) Metabolic alteration in surgical patients. III. The influence of peritonitis on nitrogen, carbohydrate, electrolyte and water balance. Surgery (St.Louis), 36, 580-597.

LANE-PETTER, W. (1973) The physical environment of rats and mice. In Animals for Research, ed. Lane-Petter, W. London : Academic Press.

LAVOISIER, A.L. (1777) Mem. de l'Academie des sciences, 1777, 185 (Oeuvres, 2, 174, 1862) cited by Kleiber, M. (1961) in The Fire of Life.

LAVOISIER, A.L., DE LA PLACE, P.S. (1780) Memoire sur la chaleur. In Memoires de l'Academie Royale, p. 355. Oeuvres de Lavoisier, Tome II, Paris. Imprimarie Imperiale (1862) p. 283, cited by Kleiber, M. (1961) in The Fire of Life.

LEE, M.O. (1929) Determination of surface area of white rats with its application to expression of metabolic results. Amer. J. Physiol., 89, 24-33.

LEFEVRE, J. (1911) In Chaleur Animale et Bioenergetique. Paris, Masson et cie.

LEVENSON, S.M., CROWLEY, L.V., OATES, J.F. & GLINOS, A.D. (1959) Proc. Second Army Sc. Conf., 2, 109. Quoted in Levenson et al. (1976) and results shown in that paper.

LEVENSON, S.M., CROWLEY, L.V. & SEIFTER, E. (1976) Effects of injury on wound healing and wound infection. In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. Bath: Pitman Press.

LEVENSON, S.M., CROWLEY, L.V. & SEIFTER, E. (1975) Starvation. In Manual of Surgical Nutrition , ed. Ballinger, W.F., Collins, J.A., Drucker, W.R., Dudrick, S.J. & Zeppa, R. pp. 236-264. Philadelphia & London : W. B. Saunders Co.

LEVENSON, S.M., PULASKI, E.J. & DEL GUERICO, L.R.M. (1966) Metabolic changes associated with injury. In Physiologic Principles of Surgery, pp. 1-43. Philadelphia : Saunders Co.

LILJEDAHL, S.-O. (1972) The nutrition of patients with extensive burns. In Parenteral Nutrition, ed. Wilkinson, A.W. pp. 208-212. Edinburgh, London & New York : Churchill Livingstone.

LONG, C.L., KINNEY, J.M. & GEIGER, J.W. (1976) Non suppressability of gluconeogenesis by glucose in septic patients. Metabolism, 25, 193-201.

LONG, C.L., SPENCER, J.L., KINNEY, J.M. & GEIGER, J.W. (1971) Carbohydrate metabolism in normal man and effect of glucose infusion. J. Appl. Physiol., 31, 102-109.

LUSK, G. (1928) The Elements of the Science of Nutrition, 4th Ed. London : W. B. Saunders Co.

LUSK, G. (1931) The Science of Nutrition, 4th ed. Philadelphia : W. C. Saunders Co.

MANCHESTER, K.L. (1968) Hormonal control of protein biosynthesis. In Biological Basis of Medicine, ed. Bittar, E.E. & Bittar, N., Volume 2, p. 221. London & New York : Academic Press.

MANN, L.T. (1963) Spectrophotometric determination of nitrogen in total micro kjeldahl digests. Anal. Chem. 35, 2179-2182.

MARKLEY, K., BOCANEGRA, M., BAZAN, A., TEMPLE, R., CHIAPPORI, M. & MORALES, G. (1959) Clinical evaluation of saline solution therapy in burn shock. Comparison of plasma therapy with saline solution therapy. J.A.M.A., 170, 1633-1640.

McLEAN, J.H. (1971) A gradient layer calorimeter for large animals. J. Inst. Heating and Ventilating Engineers, 39, 1-8.

McLEAN, J.A. (1974) Loss of heat by evaporation. In Heat Loss from Animals and Man, ed. Monteith, J.L. & Mount, L.E. pp. 19-31. London : Butterworth.

MIKSCHE, L.W. & CALDWELL, F.T. (1967) The influence of fever, protein metabolism and thyroid function on energy balance following bilateral fracture of the femur in the rat. Surgery, 62, 66-72.

MILES, A.A. & MILES, E.M. (1952) Vascular reactions to histamine, histamine-liberator and leukotaxine in skin of guinea pigs. J. Physiol., 118, 228-257.

MINARD, D. (1970) Body heat content. In. Physiological and Behavioural Temperature Regulation, ed. Hardy, J.D. Gagge, A.P. & Stolwijk, J.A.J. pp. 345-357. Springfield, Illinois : Chas. C. Thomas.

MINISTRY OF HEALTH (1964) Requirements of man for protein. Rep. publ. Hlth. med. Subj. London, No. 111. London : H.M.S.O.

MONCRIEF, J.A. (1965) Burns of specific areas. J. Trauma, 5, 278-291.

MONTAGNA, W. (1962) The structure and function of skin, 2nd ed. New York : Academic Press Inc.

MOORE, F.D. (1946) Determination of total body water and solids with isotopes. Science, 104, 157.

MOORE, F.D. (1957) Endocrine changes after anaesthesia, surgery and unanaesthetised trauma in man. Recent Progr. Hormone Res. 13, 511-582.

MOORE, F.D. (1972) In Homeostasis : Bodily Changes in Trauma and Surgery, ed. Sabiston. Philadelphia : W. B. Saunders Co.

MOORE, F.D. & BALL, M.R. (1952) In The Metabolic Response to Injury, Springfield, Illinois : Chas. C. Thomas.

MOORE, F.D. & BRENNAN, M.F. (1975) Surgical Injury : Body composition, protein metabolism and neuroendocrinology. In Manual of Surgical Nutrition, ed. Ballinger, W.F., Collins, J.A., Drucker, W.R. & Zeppa, R. pp. 169-222. Philadelphia & London : W. B. Saunders Co.

MOORE, F.D., OLESEN, K.H., McMURRAY, J.D., PARKER, H.V. BALL, M.R. & BOYDEN, C.M. (1963) In The Body Cell Mass and its Supporting Environment. Philadelphia & London : W. B. Saunders Co.

MORGAN, H.C., ANDREWS, R.P. & JURKIEWICZ, M.J. (1955) The effect of thermal injury on metabolic rate and insensible water loss in the rat. Surg. Forum, 6, 78-80.

MOYER, C.A. (1954) The treatment of severe thermal injury - its developments and accomplishments during the past century. Western J. Surg. Obstet. Gynec., 62, 39-51 (Div.I), 107-120 (Div.II).

MOYER, C.A. & BUTCHER, H.R. (1967) In Burns Shock and Plasma Volume Regulation, Chapter 8, pp. 247-257. St. Louis: C. V. Mosby Co.

MOYER, C.A., & BUTCHER, H.R. (1967) ed. In Burns Shock and Plasma Volume Regulation, Chapter 5, p. 152. Vaporisational heat loss. St. Louis : C. V. Mosby Co.

MOYER, C.A., BRENTANO, L., GRAVENS, D.L., MARGRAF, H.W. & MONAFO, W.W. (1965) Treatment of large human burns with 0.5% silver nitrate solution. Arch. Surg., 90, 812-867.

MOYER, C.A., COLLER, F.A., IOB, V., VAUGHAN, H.H. & MARTZ, D. (1944) A study of the inter-relationship of salt solutions, serum and defibrinated blood in the treatment of severely scalded anaesthetised dogs. Ann. Surg., 120, 367-376.

MOYER, C.A., DILLON, J.S. & BUTCHER, H.R. (1966) Function of human skin in relation to its macro-molecular structure. Arch. Surg., 92, 222-242.

MOUNT, L.E., HOLMES, C.W., START, I.B. & LEGGE, A.S. (1967) A direct calorimeter for the continuous recording of heat loss from groups of growing pigs over long periods. J. Agric. Sci. Camb. 68, 47-55.

MUNRO, H.N. (1964) General aspects of the regulation of protein metabolism by diet and by hormones. In Mammalian Protein Metabolism, ed. Munro, H.N. & Allison, J.B. (Vol. 1) pp. 381-481. New York : Academic Press.

MUNRO, H.N. & CHALMERS, M.I. (1945) Fracture metabolism at different levels of protein intake. Brit. J. Exptl. Path., 26, 396-404.

MUNRO, H.N. & CUTHBERTSON, D.P. (1943) Response of protein metabolism to injury. Biochem. J., XII, 37.

MURLIN, J.R. & LUSK, G. (1915) Animal Calorimetry. XII. The influence of the ingression of fat. J. Biol. Chem., 22, 15-41.

MYERS, J.D. (1950) Net splanchnic glucose production in normal men and in various disease states. J. Clin. Invest., 29, 1421-1429.

NEWSOME, T.W., MASON, A.D. & PRUITT, B.A. (1973) Weight loss following thermal injury. Ann. Surg., 178, 215-217.

ODESSEY, R., KHAIRALLAH, E.A. & GOLDBERG, A.L. (1974) Origin and possible significance of alanine production by skeletal muscle. J. Biol. Chem. 249, 7623-7629.

OWEN, E.C. (1967) Nutrition balance techniques and their limitations. Nitrogen balances. Proc. Nutr. Soc., 26, 116-124.

PACE, N. & RATHBURN, E.N. (1945) Studies on body composition. III. The body water and chemically combined nitrogen content in relation to fat content. J. Biol. Chem. 158, 685-691.

PADGETT, E.C. (1942) Care of military and civilian injuries : Care of injuries of the face and jaws. Am. J. Orthodontics, 28, 213-221.

PARKINSON, K.J. & LEGG, B.J. (1971) A new method for calibrating infra red gas analysers. J. Phys. E. Sci. Instrum., 4, 598-600.

PERKINS, F.T. & SHORT, D.J. (1957) A new technique in the sterilization of animal houses, racking and cages. Journal of Animal Technicians Assoc. 8(1).

POPPENDIEK, H.F. & HODY, G.L. (1972) Design considerations and applications of gradient layer calorimeters for use in biological heat production measurement. In Measurement and Control in Science and Industry, Volume 4 (3) pp. 2079-2088. ed. Plumb, H.H. Pittsburgh : Instrument Society of America.

PULLAR, J.D. (1956) Abst. Communications. 20th Internat. Physiol. Congress, Brussels, p. 741.

PULLAR, J.D. (1957) Energy expenditure in livestock. Advancement of Science, 13, 491-493.

PULLAR, J.D. (1969) Methods of Calorimetry. (A) Direct. In International Encyclopaedia of Food and Nutrition, ed. Cuthbertson, D.P. Ch. 17, pp. 471-490. London : Pergamon Press.

PULLAR, J.D., BROCKWAY, J.M. & McDONALD, J.D. (1967) A comparison of direct and indirect calorimetry. In Energy Metabolism of Farm Animals, ed. Blaxter, J.L., Kielanowski, J. & Thorbek, G. pp. 415-421. Newcastle : Griel Press.

QUATTRONE, P.D. (1965) Improved gradient layer animal calorimeter. Rev. Sci. Instrum. 36, 832-838.

REISS, E. (1959) Protein metabolism in Infection. I. Changes in certain visceral proteins studied with Glycine - N^{15}. Metabolism, 8, 151-159.

RICHARDS, J.R. (1977) Metabolic responses to injury, infection and starvation : An overview. In Nutritional Aspects of Care in the Critically Ill, ed. Richards, J.R. & Kinney, J.M. pp. 273-302. Edinburgh, London & New York : Churchill Livingstone.

RICHARDS, J.R., DRURY, J.K. & BROWN, J.J. (1976) A simple method for weighing rats. J.I.A.T., 27, 29-31.

RICHARDS, J.R., DRURY, J.K., GOLL, C. & BESSENT, R.G. (1978) Energy exchanges and injury. Proc. Nutr. Soc., 37, 29-43.

RICHARDS, S.A. (1973) Temperature regulation. London & Winchester : Wykeham Publications Ltd.

RIEGEL, C., KOOP, C.E., DREW, J., STEVENS, L.W. & RHOADS, J.E. (1947) The nutritional requirements for nitrogen balance in surgical patients during the early post operative period. J. clin. Invest., 26, 18-23.

ROE, C.F. (1967) Evaporative water and heat loss. In Symposium, Sixth National Burn Seminar. J. Trauma, 7, 147-152.

ROE, C.F. & KINNEY, J.M. (1964) Water and heat exchange in third degree burns. Surgery, 56, 212-220.

RUBNER, M. (1894) Ztschr. Biol. 30, 73, cited by Kleiber, M. (1961) in The Fire of Life.

RUSSEL, J.A. (1944) The calorimetric estimation of small amounts of ammonia by the phenol-hypochlorite reaction. J. Biol. Chem., 156, 457-461.

RYAN, N.T., BLACKBURN, G.L. & CLOWES, G.H.A. (1974) Differential tissue sensitivity of elevated endogenous insulin levels during experimental peritonitis in rats. Metabolism, 23, 1081-1089.

SARGENT, F. (1957) A prospectus in bioclimatology : Historical observations on its national and international growth. Fed. Proc. 16, 603.

SCHERING, L.E., PAULY, J.E. & TSAI, T.-H. (1968) Circadian fluctuation in plasma proteins of the rat. Am. J. Physiol. 215(5), 1096-1101.

SCHONHEYDER, F., HEILOKOV, N.S.C. & OLESEN, K. (1954) Isotopic studies on mechanism of negative nitrogen balance produced by immobilization. Scand. J. Clin. Lab. Invest. 6, 178-188.

SCHULTIS, K. & BEISBARTH, H. (1971) Post traumatic energy metabolism. In Parenteral Nutrition, ed. Wilkinson, A.W. pp. 255-265. Edinburgh : Churchill Livingstone.

SCHULTIS, K., BEISBARTH, H. (1976) Pathobiochemistry of post stress metabolism. In Parenteral Nutrition, ed. Ahnefeld, F.W., Burri, C., Dick, W. and Halmagyi, S.M. pp. 31-44. Berlin & New York : Springer-Verlag.

SELYE, H. (1937) Studies on adaptation. Endocrinology, 21, 169-188.

SELYE, H. & COLLIP, J.B. (1936) Fundamental factors in interpretation of stimuli influencing endocrine glands. Endocrinology, 20, 667-672.

SEVITT, S. (1957) In Burns - Pathology and Therapeutic Applications. London : Butterworth & Co.

SHEDD, D.P. (1958) Historical landmarks in the treatment of burns. Surgery, 43, 1024-1036.

SNEVE, H. (1905) The treatment of burns and skin grafting. J.A.M.A., 45, 1-8.

SOROFF, H.S., PEARSON, E. & ARTZ, C.P. (1961) An estimation of the nitrogen requirements for equilibrium in burned patients. Surg, Gynec. & Obstet., 112, 159-172.

SPENCER, J.L., LONG, C.L. & KINNEY, J.M. (1971) A model for glucose metabolism in man. Industrial & Engineering Chemical Fundamentals, 10, 1-12.

SPINNLER, G., JEQUIER, E., FAVRE, R., DOLVO, M. & VANNOTTI, A. (1973) Human calorimeter with a new type of gradient layer. J. Appl. Physiol., 35, 158-165.

SPIVEY, J. & JOHNSTON, I.D.A. (1972) The effect of environmental temperature and nutritional intake on the metabolic response to abdominal surgery. Brit. J. Surg., 59, 93-97.

SPRUIT, D. & MALTEN, K.E. (1966) The regeneration rate of the water loss of heavily damaged skin. Dermatologica, 182, 115-123.

STONER, H.B. (1970) Energy metabolism after injury. J. Clin. Path., 23 Suppl. (Roy. Coll. Path.), 4, 47-55.

STONER, H.B. (1976) Changes in the central nervous system and their role in the metabolic response to injury. In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. pp. 179-193. Bath : Pitman Medical Press.

STONER, H.B. (1976) An integrated neuro-endocrine response to injury. In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. pp. 194-201. Bath : Pitman Medical Press.

STONER, H.B. (1976) Causative factors and afferent stimuli involved in the metabolic response to injury, In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. pp. 202-214. Bath : Pitman Medical Press.

STUDLEY, H.O. (1936) Percentage of weight loss : A basic indicator of surgical risk in patients with chronic peptic ulcer. J.A.M.A., 106, 458-460.

SWIFT, R.W. & FORBES, R.M. (1939) The heat production of the fasting rat in relation to the environmental temperature. J. Nutr., 18, 307-318.

TAPPEINER, Von H. (1881) Ueber Veränderungen des Blutes und der Muskeln nach ausgedehnten Hautverbrennungen. Centralbl. Med. Wissenschaften. 31, 385.

TILSTONE, W.J. (1972) The effects of environmental conditions on the requirements for energy after injury. In Parenteral Nutrition, ed. Wilkinson, A.W. pp. 24-33. London : Churchill Livingstone.

TILSTONE, W.J. (1974) The effects of environmental conditions on the metabolic requirements after injury. In Parenteral Nutrition in Acute Metabolic Illness, ed. Lee, H.A. pp. 197-210. London & New York : Academic Press.

TIMONER, J., RIDDELL, A.G. & CARR, G.R. (1959) The effect of blood transfusion on the metabolic response to gastric surgery. Clin. Sci., 18, 561-564.

THOMPSON, V. (1938) Studies of trauma and carbohydrate metabolism with special reference to the existence of traumatic diabetes. Acta Medica Scandinavica, Suppl. 91.

TUFFERY, A.A. (1958) Animal house hygiene. Journal of Animal Technicians Association, 9(2), 33-39.

UNDERHILL, F.P. (1923) Blood concentration changes in extensive superficial burns and their significance for systemic treatment. Arch. Intern. Med., 32, 31-49.

UNDERHILL, F.P. (1930) The significance of anhydraemia in extensive surface burns. J.A.M.A., 95, 852-857.

VISSER, J. & HODGSON, T. (1960) The design of a human calorimeter for the determination of body heat storage. The South African Mech. Eng. May 243-268.

WALKER, H.L. & MASON, H.L. (1968) A standard animal burn. J. Trauma, 8, 1049-1051.

WALLACE, A.B. (1969) Personal communication. Ceres, Fife.

WANNEMACHER, R.W. & BEISEL, W.R. (1977) Metabolic response of the host to infectious disease. In Nutritional Aspects of Care in the Critically Ill, ed. Richards, J.R. & Kinney, J.M. pp. 135-160. Edinburgh, London &New York : Churchill Livingstone.

WANNEMACHER, R.W., PEKAREK, R.S., KLAINER, A.S., BARTELLONI, P.J., DU PONT, H.L., HORNICK, R.B. & BEISEL, W.R. (1975) Detection of a leukocytic endogenous mediator-like mediator of serum amino acid and zinc depression during various infectious illnesses. Infection & Immunity, 11(4), 873-875.

WEIR, J.B. de V. (1949) New methods for calculating metabolic rate with special reference to protein metabolism. J. Physiol., 109, 1-9.

WERNER, S.C., HABIT, D.U., RANDALL, H.T. & LOCKWOOD, J.S. (1949) Postoperative nitrogen loss - a comparison of the effects of trauma and caloric adjustment. Ann. Surg., 130, 688-694.

WEST, E.S., & TODD, W.R. (1961) Textbook of Biochemistry, p. 880. New York : Macmillan.

WHEDON, D.G., DEITRICK, J.E. & SHARR, E. (1949) Modification of the effects of immobilization upon metabolic and physiologic functions of normal men by the use of an oscillating bed. Am. J. Med., 6, 684-711.

WILLIAMS, H.B. (1912) A small respiration calorimeter. J. Biol. Chem., 12, 317-347.

WILKINSON, A.W. (1961) Starvation and operation. The Lancet ii, 783-784.

WILKINSON, A.W. (1966) In Wound Healing, ed. Illingworth, C. London : Churchill.

WILMORE, D.W. (1976) Hormonal responses and their effect on metabolism. In Symposium on Response to Infection and Injury. II. Surgical Clinics of North America, 56, 999-1018.

WILMORE, D.W. (1977) The Metabolic Management of the Critically Ill. New York : Plenum Publishing Co.

WILMORE, D.W., CURRERI, P.W., SPITZER, K.W., SPITZER, M.E. & PRUITT, B.A. (1971) Supranormal dietary intake in thermally injured hypermetabolic patients. Surg. Gynec. Obstet. 132, 881-886.

WILMORE, D.W., LONG, J.M., MASON, A.D. & PRUITT, B.A. (1976A) Catecholamines as mediators of the metabolic response to thermal injury. In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. pp. 287-299. Bath : Pitman Medical Press.

WILMORE, D.W., TAYLOR, J.W., HANDER, E.W., MASON, A.D. & PRUITT, B.A. (1976B) Central nervous system function following thermal injury. In Metabolism and the Response to Injury, ed. Wilkinson, A.W. & Cuthbertson, Sir David. pp. 274-286. Bath : Pitman Medical Press.

WILSON, B.J. & STIRMAN, J.A. (1960) Initial treatment of burns. J.A.M.A., 173, 509-516.

WOOLFSON, A.M.J., HEATLEY, R.V. & ALLISON, S.P. (1977) Significance of insulin in the metabolic response to injury. In Nutritional Aspects of Care in the Critically Ill, pp. 367-388. ed. Richards, J.R. & Kinney, J.M. Edinburgh, London & New York : Churchill Livingstone.

WRETLIND, A. (1972) Complete intravenous nutrition : Theoretical and experimental background. Nutr. Metabol. 14, Suppl. 1.

ZAWACKI, B.E. & JONES, R.J. (1967) Standard depth burns in the rat : The importance of the hair growing cycle. Brit. J. Plast. Surg., 20, 347-354.

ZAWACKI, B.E., SPITZER, K.W., MASON, A.D. & JOHNS, L.A. L970) Does increased evaporative water loss cause hypermetabolism in burned patients ? Ann. Surg., 171, 236-240.

ZITOWITZ, L. & HARDY, J.D. (1958) Influence of cold exposure on thermal burns in the rat. J. Appl. Physiol. 12(2), 147-154.

REFERENCES (SUPPLEMENT)

AULICK, L.H., HANDER, E.H., WILMORE, D.W., MASON, A.D., and PRUITT, B.A. (1979) The relative significance of thermal and metabolic demands on burn hypermetabolism. The Journal of Trauma, 19, 559-566.

KREMER, B., ALLGÖWER, M., SCHEIDEGGER, A.-M., SCHMIDT, K.H., SCHÖLMERICH, J., WÜST, B. and SCHOENENBERGER, G.A.(1979) Toxin-specific ultrastructural alterations of the mouse liver after burn injuries and the possibility of a specific antitoxic therapy. Scand J. Plast. Reconstr. Surg. 13, 217-222.

STÄDTLER, K., ALLGÖWER, M., CUENI, L.B. and SCHOENENBERGER, G.A. (1972) Pathophysiologische Untersuchungen an einem Verbrennungsmodell der Maus. Res. Exp. Med. 158, 23.

WILMORE, D.W., AULICK, L.H., MASON, A.D. and PRUITT, B.A.(1977) Influence of the burn wound on local and systemic responses to injury. Ann. Surg. 186(4), 444-458.

WOLFE, R.R. and BURKE, J.F. (1978) Effect of glucose infusion on glucose and lactate metabolism in normal and burned guinea pigs. The Journal of Trauma 18(12), 800-805.

GLASGOW UNIVERSITY LIBRARY