THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON ENERGY METABOLISM FOLLOWING THERMAL INJURY IN THE RAT AND SURGICAL TRAUMA IN MAN.

by

Christopher Charles Goll

A Thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Medicine.

May, 1981.

Institute of Physiology
and Department of Surgery,
The University,
Glasgow.

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Acknowledgements

I am grateful to Professors I.A. Boyd, J.M.A. Lenihan and D.C. Carter for their encouragement and active support which allowed the experimental work in the Institute of Physiology and Department of Surgery, Royal Infirmary, Glasgow University, described in this thesis to be carried out.

I am greatly indebted to Dr. R.G. Bessent for his helpful advice and suggestions throughout the experimental work and the preparation of the thesis. I am also indebted to Mr. J.R. Richards for his help and, with Professor A. Fleck, for the initiation of the projects. Dr. G. Al-Shamma and Mr. G. Gedeon kindly allowed my use of their results for the body composition and urinary nitrogen excretions of the animals in experiments 1 to 4.

The staff of the animal house, the Institute of Physiology under the supervision of Mr. F. Fleming and with the help of Mr. T. Baird gave much attention to the careful daily handling of the animals. The enthusiastic technical support of Mr. R. Wilson in the routine running of the animal calorimeter was also much appreciated.

The measurements on the patients in Study C would not have been possible without the active help and encouragement of Mr. A. Sim, Mr. N. Harris and the staff of Ward 33, Department of Surgery, Royal Infirmary. The help of the Department of Biochemistry, Royal Infirmary in

providing the analyses of urinary nitrogen in Study C and of Dr. Anderson, Department of Forensic Medicine, Glasgow University for analysing the butane gas used in calibrating the calorimeter are also gratefully acknowledged. I would like to thank Dr. J.W.L. Davies for reading the manuscript and Ms. Pat Kelly for her efficient typing of the same.

The animal work was supported by a grant from the M.R.C. and the human studies were supported by grant No. 7178/1.5 from the Wellcome Trust.

Declaration

This thesis has been written and composed entirely by myself. All the experiments were performed by myself, with technical sssistance where described. I benefitted from the previous establishment of the small animal calorimeter, particularly from the work of Mr. K. Carter, Mr. J.R. Richards and Mr. J.K. Drury. I also benefitted from the use of the animal body composition results from Dr. G. Al-Shamma and Mr. G. Gedeon's work.

Parts of the work described in this thesis have been reported in:-

Richards, J.R., Drury, J.K., Goll, C.C. & Bessent, R.G. (1978). Energy Exchanges and injury. Proceedings of the Nutrition Society, 37, 29-43.

Al-Shamma, G.A.A., Goll, C.C., Baird, T.B., Broom, J., Nicholas, G.A. & Richards, J.R. (1979). Changes in body composition following thermal injury in the rat. British Journal of Nutrition, 42, 367-375.

Gedeon, G., Shenkin, A., Fell, G.S., Al-Shamma, G., Goll, C. & Richards, J.R. (1981). Proceedings of the Nutrition Society in Press.

SUMMARY

Animal Studies

Energy metabolism following a standard thermal injury was studied using a purpose built small animal calorimeter. The effects of two environmental temperatures and two levels of dietary intake were investigated in three groups of rats over a period of approximately 50 days following injury.

(1) Injured animals kept at 20°C with a daily food intake of 15g showed a marked weight loss (30%) while pair fed uninjured animals grew 41%. The burn animals had no increase in sensible heat losses, though their insensible losses were 2.6 times those of the controls, giving them a hypermetabolism of 21%.

(2) Keeping the food intake at 15g per day but increasing the environmental temperature to 30°C, increased the insensible losses of the injured rats by 25%, but decreased their sensible losses by 55%. This meant that their total heat losses were reduced by 26%, but as the control animals also decreased their heat losses at the warmer temperature, the burn animals were still 21% hypermetabolic. The burn rats put on 25% in weight, almost as much as was lost by the similarly fed rats kept at 20°C.

(3) A final group of animals was kept at 20°C but the rats were allowed an increased food intake of 18g per day. The heat losses were very similar to those of the other group of rats kept at 20°C, though because of the higher

dietary intake, the injured animals showed a small weight gain over the experiment (4%).

Measurement of the thermogenic effect of the diet showed it to be significantly different in burn and control animals (2 & 7% of food energy respectively). Using this factor, plus measurements of the metabolisable food energy, allowed calculation of energy balance from the post-absorptive calorimetry results. This method gave a more positive energy balance for all groups of rats than the energy balance calculated from chemically measured body tissue changes. This was probably because the calorimetered heat losses, measured under quiet conditions , were less than the average daily energy expenditures which the body composition changes reflected. However, the difference in energy balance between burn and control animals was similar using either method of calculation. Increasing the environmental temperature from 20 to 30^{o}C has the same beneficial effect on energy balance as increasing the food intake by 5.1g (35%).

Human Studies

A continuous open circuit indirect calorimeter using a ventilated hood design was constructed to investigate the effect of environmental temperature on heat production in patients following surgery. The indirect calorimeter was calibrated and tested using nine volunteers, who showed a mean fasting metabolic rate of 99% of the value predicted from tables. After taking a mixed diet via a

nasogastric tube for 4.5 hours, their mean resting metabolic rate increased to 113% of the fasting value and their respiratory quotient increased to 0.88, in agreement with the values expected for this diet.

The heat production of 20 patients was measured pre-operatively and for the first 4 days following moderate severity elective surgery. Ten patients were nursed at 20°C and ten at 28°C. There were no significant differences between the heat productions and respiratory quotients of the groups at the two environmental temperatures. The heat production increased to 113% of the pre-operative value on the first post-operative day, reducing to 103% by the fourth day. Respiratory quotient decreased significantly over the post-operative period, indicating an increased utilisation of fat reserves. In contrast to the results of other studies, the mean increase of 8% in heat production over the four days following elective surgery was significant.

Abbreviations

BSA - Body surface area

DIT - Dietary induced thermogenesis

EBW - Empty body weight (i.e. weight of the cleansed animal carcase)

FI - Food intake

ME - Metabolisable energy of the diet (i.e. the gross food energy minus the urinary and faecal energy losses)

N - Nitrogen (usually urinary nitrogen)

NFE - Net food energy (i.e. the ME minus the energy increment associated with DIT)

RMR - Resting metabolic rate

RQ - Respiratory quotient

TNZ - Thermoneutral zone

WGM - Wet gas meter

In tables the following convention has been used to show statistical significance

ns - non-significant

* - $p < .05$

** - $p < .01$

*** - $p < .001$

CHAPTER 1 - INTRODUCTION

1.1 Introduction

Trauma in its many forms and degrees of severity is a common occurrence in our society. It ranges from the deliberate insult of surgery to the accidental injury associated with burning, road traffic accidents, sport etc. As the age range of people affected is wide, this can have an important effect on the community, as members with major economic as well as social roles are often involved. The consequences in terms of National Health Service bed costs are also important. Therefore investigation of the responses to injury and the factors affecting the time for recovery can be fully justified in both human and financial terms.

The study of the metabolic sequelae of trauma has been pursued for many years (Hunter, 1794). The description of the simultaneous changes in two of the most important indicators of general metabolic response, heat production and nitrogen excretion, began in the Royal Infirmary Glasgow with Sir David Cuthbertson's work on fracture patients (Cuthbertson, 1931). Despite the many differences, a common core of response is seen to all the various forms of injury (Tilstone and Cuthbertson, 1970). This has allowed progress on a common front rather than having to treat each type of trauma as an isolated area of study.

The response to injury can be affected by many variables, e.g. infection, immobilisation and quantity and quality of dietary intake (Whedon, Deitrick and Sharr, 1949; Clowes et al., 1976; Askanazi et al., 1980). Interest in

the possible beneficial effects of treatment at a high environmental temperature began with the work of Caldwell (Caldwell et al., 1959; Caldwell, 1962) using an animal model of thermal injury. This was extended to burn patients (Barr et al., 1968; Davies, Liljedahl and Birke, 1969) and to fracture, in both patients and animals (Campbell and Cuthbertson, 1967; Cuthbertson, Smith and Tilstone, 1968). The optimal environment and the degree of benefit derived from it are however not well established (Aulick et al., 1979; Arturson, 1978).

The object of this thesis is to report on the changes in energy metablism following two types of trauma and the effect that different environmental temperatures have on the response. Firstly, an animal model of thermal injury was studied and the effects of raised environmental temperatures and dietary intake compared. Secondly the effect of different ambient temperatures on the response to a moderate severity surgical procedure was studied in man.

1.2 Calorimetry

1.2.1 Historical Background

Measurements of heat associated with the metabolism of man and animals, have long been of scientific interest. Some of the first studies were carried out in Glasgow by Adair Crawford two centuries ago, contemporary with the first experiments of Lavoisier in France (Blaxter, 1978). Both measurements of heat loss (direct calorimetry) and estimates of heat production (indirect calorimetry) were made within the first few years of interest beginning in the field.

Although many experiments were carried out during the nineteenth century (Benedict and Carpenter, 1918), it was not until the beginning of the twentieth century that accurate studies with a sound theoretical base were performed (Passmore, 1967). Technological developments have not significantly increased the accuracy with which the determinations can be made but the range of subject activities that can be measured and the ease of making the measurements have improved (Garrow, 1978).

1.2.2 Direct Calorimetry

Man and animals are constantly producing heat from the chemical reactions in the body necessary to maintain a normal metabolism. As this energy is not stored in the body, where it would increase body temperature to unacceptable levels, it must be lost to the environment. Direct calorimetry, the measurement of this heat loss,

can be carried out in various ways, the accuracy of which depends only on the physical techniques used. No assumptions about the means of production need be made and the subject can be treated as a 'black box' producing heat.

Direct calorimeters initially were of the heat sink (Atwater and Benedict, 1905; Visser and Hodgson, 1960) or direct air (Mount et al., 1967) type where the heat produced by the subject in the calorimeter chamber was absorbed by the circulating fluid in the walls or by the ventilating air. The increase in energy content of the fluid or air could then be calculated from the temperature difference between the inlet and outlet ports. Corrections for changes in air pressure, enclosure temperature and the latent heat of vapourisation of any water (perspiration, respiratory water vapour) were also required.

Direct calorimeters having shorter response times than those mentioned above, have been designed using the gradient layer principle (Benzinger et al., 1958) and are now in common use (McLean, 1971; Spinnler et al., 1973; Carter, 1975). The majority of heat is lost through the walls of the gradient layer calorimeter and this is measured by multiple thermocouples which sense the temperature difference across the thickness of the wall. Heat lost to ventilating air and water vapour must also be measured.

As all direct calorimeters have to totally enclose the subject to measure heat loss, they are generally unsuitable for subjects who need attention (e.g. patients, children etc.). Though accurate and easily calibrated by electrical heating, they are usually bulky, expensive and lack versatility (Kinney, 1970).

1.2.3 Indirect Calorimetry.

Production of heat from food or tissue fuels in the body consumes oxygen and produces carbon dioxide and other excretory products. Measurement of gas exchange and urinary nitrogen losses provides an accurate method of indirect calorimetry when metabolic processes are in approximate equilibrium. Alternatively measurement of the metabolisable energy input in food together with the change in body tissue energy stores can provide a value for heat production.

Both of these methods require knowledge of the energy values of various foods, tissues and excretory products. Use of average energy values, for instance in the oxidation of fat and protein, can lead to a typical uncertainty of 2% in the result (Garrow, 1978), though theoretical errors up to 13% are possible if metabolism of a single molecular form of the substrate is compared with tissue of average composition (Blaxter, 1967).

The elimination of systematic errors depends on a complete collection of the metabolites. This applies particularly to the collection of all expired gas from a subject where routes of loss may be difficult to detect. Estimates

of food intake are also prone to errors where input can be overestimated because of unmeasured residues and the composition of the dietary intake may not be accurately known.

Measurement of the respiratory quotient (RQ), the ratio of carbon dioxide produced to oxygen consumed, is a useful indicator of what fuels are being processed or synthesised, and of any gas collection problems. However because of the ability of the body to accumulate stores and deficits, particularly of carbon dioxide (Thompson et al., 1979), and because of the use of average factors for tissue composition its usefulness is limited.

Though energy values derived from measurements on normal subjects have been used in injured patients (Kinney et al., 1968; Danielsson, Arturson and Wennberg, 1976a; Wilmore et al., 1977) this may be erroneous (Tilstone, 1972). However as long as routes of loss peculiar to the type of injury (e.g. exudate from a burn) are taken into account, the change in absorption of the diet is probably small (Cuthbertson, et al., 1972) and changes in the energy rich products in the urine (urea, ammonia, ketones etc.) can usually be monitored.

1.2.4. Energy Balance

Applying the laws of conservation of energy to the human or animal body, the difference between the energy input (E_{IN}) from the environment and the energy output (E_{OUT}) gives the change in body energy stores ΔE:-

$$\Delta E = E_{IN} - E_{OUT} \qquad 1.1$$

Energy input is usually in the form of thermochemical energy in the food, though under certain circumstances, e.g. warming a hypothermic patient, there can be a net input of energy via the physical routes of conduction, convection and radiation. The energy output is normally the net heat loss via the physical routes mentioned above plus evaporation and any thermochemical energy in excreta. Any net external work that is performed must also be included.

If the subject is in positive energy balance (i.e. ΔE positive) then in the short term this extra energy could be stored directly as heat. However in homeotherms the body temperature is closely controlled and if a progressive rise in body temperature of more than a few degrees is unavoidable, then life threatening consequences can ensue, e.g. heat stroke. Therefore energy excess is stored as extra body tissue, with fat being the most efficient on the basis of energy per unit weight. A negative energy balance induces the opposite effect. Any changes in body temperature are small and the required energy is produced from body tissue stores.

The ability to withstand a prolonged negative energy balance, whether caused by an increased energy output, a decreased energy input or both these factors, depends primarily on the tissue energy stores available. A typical 65 kg man has energy reserves of 280 MJ (Passmore and Robson, 1974) which with a normal energy expenditure

of 6.7 MJ per day represents 42 days of supply. A rat of 250g weight has energy stores of about 1600 kJ (Al- Shamma et al., 1979). With a metabolic rate of 100 kJ/day (Mitchell, 1962) this represents a 16 day reserve. As neither human nor rat can use more than about 40% of their tissue reserves without threatening life (Levenson, 1977; Kleiber, 1961), these calculations indicate that the rat is about 2.6 times more sensitive to energy deficit than man. This needs to be considered when using an animal model of human response.

While body weight loss of up to 10% has been shown not to affect the work capacity of normal man (Daws et al., 1972) a weight reduction of more than 10% in a patient should be avoided (Wilmore, 1977). As some patients are malnourished before entering hospital, a large energy deficit should be corrected as soon as possible. The negative energy balance can be caused both by the elevated heat production of the patient, e.g. after burning injury (Gump and Kinney, 1971), and a decrease in calorie intake (Clark, 1971).

1.3 Energy Metabolism

1.3.1 Measurement Conditions

Energy expenditure varies according to the activity state of the subject; being about 20% above resting levels when a human subject is sitting and 50% when standing (Durnin and Passmore, 1967). Therefore control and consistency in the physical conditions of calorimetric measurement are essential.

The term 'basal metabolic rate' has been used by many authors (DuBois, 1927; Keys et al., 1950) to describe energy expenditure measurement on a quiet, resting, post-absorptive subject. Even if the conditions are more strictly standardised to include a thermoneutral environmental temperature, complete mental and bodily rest and a measurement period just after waking, the metabolic rate is unlikely to be the constant irreducible value (i.e. 'basal') proposed by Mitchell (1962). Body temperature, previous nutrition and sleep patterns are at least three of the variables that can affect it (Grafe 1923, Buskirk et al., 1960).

Energy expenditure measured with the subject lying at rest and having had no recent large meals is termed resting metabolic rate (RMR). Measurements made in this manner are usually close to the basal values described above (Durnin and Passmore, 1967), while allowing a wider experimental scope. The respiratory quotient (RQ) in such circumstances is not necessarily well defined. However control of the nutritional input, particularly if it is small or constant, can allow useful comparisons.

To decrease the variability of RMR values environmental conditions should be as constant as possible. Psychological stress has been shown to have a large effect in humans (Kleitman, 1939; Arturson, 1977). This can be difficult to detect and impossible to quantify but can be mostly eliminated by use of a comfortable and

relatively isolated environment. Training of the human subject in the use of the calorimetry apparatus is also important (Robertson and Reid, 1952) particularly where a mask or mouthpiece is utilised (Kinney et al., 1964). Similar precautions apply with animals where regular and consistent handling are important factors (Richards et al., 1978).

1.3.2 The Effect of Environmental Temperature on Energy Metabolism

Homeotherms show marked changes in heat production with ambient temperature. The idealised response, shown in Figure 1 (from Precht et al., 1973), has a homeothermy range T_2 to T_5, where body temperature shows only a very small increase (Hardy and DuBois, 1940). Below and above this range, hypo- and hyperthermy set in with eventual cold and heat death at T_1 and T_6 respectively.

The region of minium heat production between T_3 and T_4 is termed the thermoneutral zone (TNZ) and T_3 the critical temperature. This zone can vary widely between mammals (Precht et al., 1973) because of their different insulative and heat loss mechanisms. Below the TNZ the body is utilising its maximum insulative properties but cannot keep heat losses below the TNZ minimum. Metabolic rate increases as extra heat is produced to maintain core temperature. Above the TNZ sensible heat losses (i.e. radiative, conductive and convective) decrease and insensible losses (i.e. evaporative) have to increase so that the heat produced by the chemical processes in the body can be dissipated. As sweat is hypotonic to blood

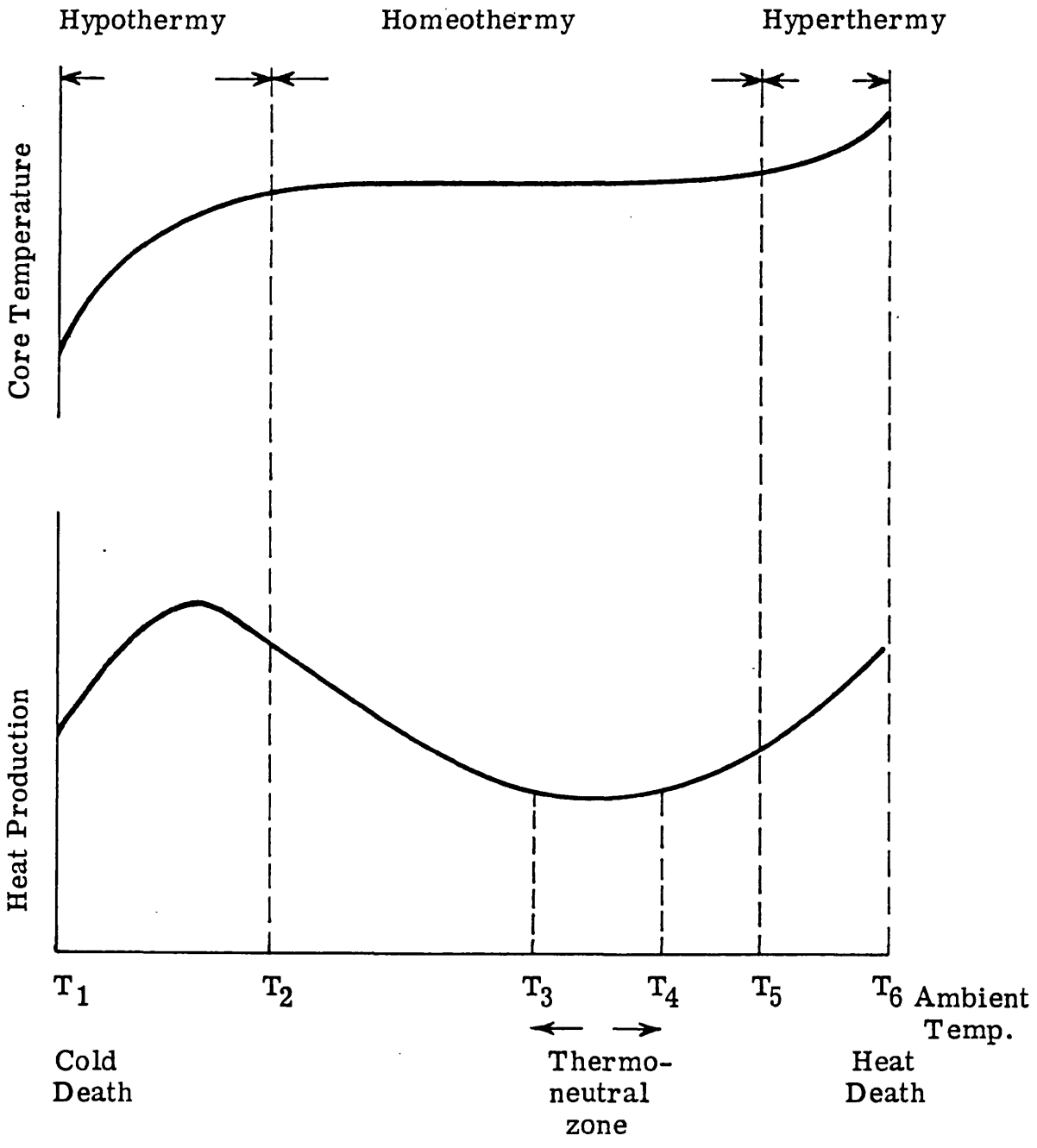


Figure 1. Idealised response of heat production and core temperature to different environmental temperatures in the homeotherm.

(Kuno, 1956) work must be performed in its secretion. This and the increased respiratory frequency are the main factors that increase the metabolic rate between temperatures T_4 and T_5. When the routes of heat loss are insufficient to maintain a constant body temperature, the rise in temperature itself increases the rate of the chemical reactions in the body. Metabolic rate increases by 10 to 13% per degree Centigrade elevation in core temperature (Wilmore, 1977). This exacerbates the problems of heat dissipation and leads quickly to heat death at temperature T_6.

Man has a TNZ extending from 26 to 35°C (Winslow, 1941; Hardy and DuBois, 1940) while the rat has a smaller zone covering 28 to 30°C (Benedict and MacLeod, 1929; Swift and Forbes, 1939). This is because compared with man, the rat has limited means of increasing its heat losses. Evaporative losses are restricted to the area around the testes and increasing skin blood flow, to decrease thermal resistance, is limited to the testes and tail. The rat can compensate to a limited extent behaviourally by putting saliva onto fur, stretching to increase body surface area and by decreasing activity (Folk, 1974).

The figures given for man refer to the nude state. Man, unlike the laboratory rat, can increase his insulation at low temperatures with appropriate housing and clothing. However, adaptive changes have been shown in subjects exposed to cold conditions in light clothing (Scholander et al, 1958; Davis, 1961). These changes reduced the

amount of shivering by either increasing metabolic rate, increasing insulation by decreasing peripheral blood flow or tolerating some drop in body temperature (Hammel, 1964). Though no gross physiological adapative changes were found in men living in an Antartic camp for several months (Edholm and Lewis, 1964), seasonal effects have been demonstrated on the rate of adaption to laboratory cold exposure (Davis, 1961).

1.3.3 The Effect of Food Intake on Energy Metabolism

Ingestion of significant quantities of food increases metabolic rate (Kleiber, 1961). This increase in heat production was termed specific dynamic action or effect (Rubner, 1902) though recently the more general term dietary induced thermogenesis (DIT) has been used.

Benedict and Carpenter (1918) measured that on average 12% of protein, 6% of carbohydrate and 2% of fat energy ingested were dissipated in an increased RMR following a meal. Accurate measurement of thermogenesis is difficult because of the many metabolic rate estimates required on a resting subject over a period of hours following the meal (Glickman et al., 1948). This may account for most of the variation between the results of various authors (Wachholder and Franz, 1944; Garrow and Hawes, 1972; Pittet, Gygax and Jequier, 1974).

The rise in metabolic rate following a meal starts almost immediately (Passmore and Richie, 1957) with the peak RMR usually within two hours (Rochelle and Horvath, 1969).

Sustained increases in oxygen consumption of up to 20% often occur (Gubner, Di Palma and Moore, 1947; Tuttle et al., 1953) so that control over dietary intake is necessary if small changes in RMR are to be measured.

Total or partial starvation commonly follows various trauma. Anorexia or injury to the gastro-intestinal tract are two of the common reasons. Total starvation in normal man has little immediate metabolic effect. Nitrogen excretion shows a small peak and then falls over 5 - 7 days to approximately half normal levels (Martin and Robison, 1922). Both body weight and RMR fall over this period. The decrease in RMR is at a faster rate than that of body weight so that heat production, expressed per kg, also falls (Consolazio et al., 1967). The body is therefore conserving protein and reducing the rate of use of tissue fuels.

Semistarvation shows a similar pattern to total starvation. Over 10 - 12 days normal subjects on 1.7 - 2.5 MJ of carbohydrate per day showed a small but non-significant decrease in their resting and exercise metabolic rates, when expressed per kg of body weight (Daws et al., 1972; Taylor et al., 1957). Absolute metabolic rates and body weights decreased by less than 10%. The subjects also showed significant decreases in respiratory quotient at rest and during working periods. Using the longer period of 24 weeks of semistarvation, Keys et al. (1950) described a weight loss of 24% in their subjects. The absolute metabolic rate decreased by 39%, so that metabolic rate per kg decreased by 19%. Even when the metabolic rate was

calculated per kg of active tissue (i.e. body weight minus the sum of fat plus thiocyanate space plus bone mineral) there was still a decline of 15%. The ability of the subjects to perform work was also greatly decreased.

The rehabilitation period, where weight loss was less than 10%, was under a week (Consolazio et al., 1967; Grande, Anderson and Keys, 1958). In Keys study (Keys et al., 1950) full rehabilitation to previous performance standards was slow covering 20 weeks. Increases in metabolic rate during refeeding are greater than expected from the weight increases measured (Grande et al., 1958; Benedict et al., 1919). This reflects the extra energy expenditure of regrowth, as body tissue stores are replenished.

1.4 Energy Metabolism after Trauma

The changes in energy metabolism following injury have been divided into two periods (Cuthbertson, 1942). The first, the 'ebb' phase, is a period of diminished heat production and thermoregulatory impairment (Stoner, 1970). The response of animals to standardised injuries during this shock phase has been well documented (Stoner, 1961; Jones, Crowell and Smith, 1968). The situation is more complex in human studies because of uncontrolable factors, e.g. the severity of trauma, time to hospital admission, effect of treatment etc. (Kinney, 1977).

Twenty four to forty eight hours after the injury, the

surviving patients enter a phase of increased metabolism, Cuthbertson's 'flow' phase. The increased heat production can continue for days, weeks or even months, depending on the severity of the trauma, and is usually accompanied by an increase in core termperature. This phase has been further sub-divided by Moore (1953) into an early catbolic phase turning later into an anabolic phase with restoration of body tissue stores. The main biochemical marker of the catabolic period is an increased nitrogen excretion (Kinney, Long and Duke, 1970) reflecting, in the absence of an increased input, a general depletion of body tissues. The increased heat production during the anabolic phase, when nitrogen excretion decreases is probably similar to the increased metabolic rates measured in uninjured subjects during 'catch'up' growth following a period of semistarvation (see section 1.3.3).

The magnitude of the increase in heat production during the flow phase depends on the severity of trauma. Figure 2, adapted from Wilmore (1977), shows the typical changes in RMR following different types of trauma. The nitrogen excretion and heat production of patients increase in parallel. Cuthbertson, originally studying patients with fractures (Cuthbertson, 1931, 1936) and later rats with fractures (Cairnie et al., 1957) concluded that the increase in metabolic rate resulted from the deamination of protein and the use of the residue as an energy source (Richards et al., 1978).

This work could not be confirmed by other authors who

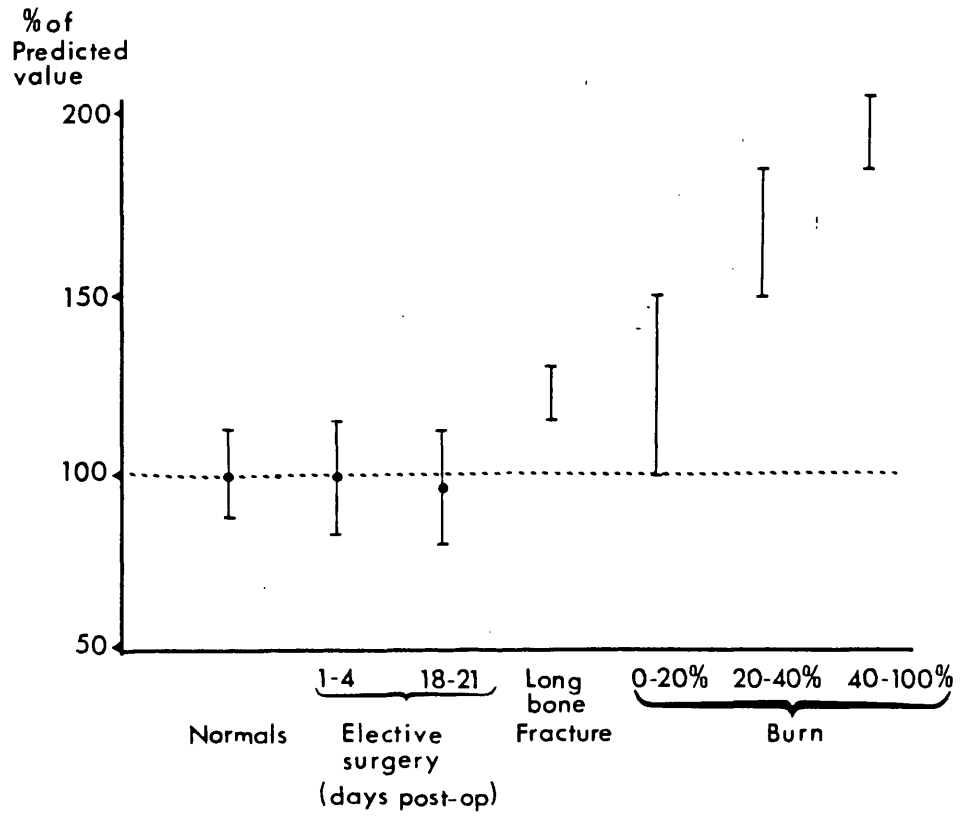


Figure 2. Resting metabolic rate after trauma.

studied rats with fractures or burns (Mitsche and Caldwell, 1967; Caldwell, 1970) and patients with a varietv of injuries (Duke et al., 1970). They showed that the percentage of energy derived from protein was approximately the same before and after injury and was not the main source of the energy increase. Other evidence suggests that fat mobilisation and oxidation are increased after injury (Birke, Carlson and Liljedahl, 1965; Robin et al., 1980). Changes in intermediary metabolism, particularly the use of amino acids for gluconeogenesis, have been advanced as the primary reason for the increased nitrogen excretion (Kinney, 1977). Other workers (Clowes, Randall and Cha, 1980) have suggested that increased proteolysis and gluconeogenesis takes place in the peripheral tissues because of a localised energy deficit.

Trauma and semistarvation ofter occur together and it is not clear how each affects the combined energy response. Some workers believe starvation is the major catabolic effect (Abbott and Albertson, 1963) while others have shown the effects to be additive (Clark, 1967). The differences in the complex endocrine changes following trauma (Johnston, 1972) and starvation (Levenson, 1977) are marked. The main energetic and nitrogen excretion differences between injury and starvation are:-

1) Trauma shows prolonged substantial increases in RMR while starvation shows a gradual decrease.

2) Nitrogen excretion is maintained at elevated levels following trauma while starvation quickly decreases nitrogen excretion to approximately half normal levels.

3) The percentage of energy produced from protein is maintained after trauma while it falls during starvation.

1.5 The Effect of Environment on Human Post-Traumatic Energy Metabolism

1.5.1 Burn Injuries

Early studies of thermal trauma revealed that it occupied an extreme position with regard to energy metabolism changes. Cope et al. (1953) studied 11 patients with burns involving 20 - 68% of their body surface area (BSA). Their metabolic rates were elevated 30 - 60% above normal until their wounds healed. Later studies (Harrison et al., 1964; Gump, Price and Kinney, 1970; Wilmore et al., 1974) showed that metabolic rate could increase up to 150% in the most severe burn cases. This compares with an upper limit of increase of 20 - 30% seen after long bone fracture or surgery (Cuthbertson, 1932; Kinney et al., 1968) and 50 - 60% with severe infection or multiple injury (DuBois, 1927; Duke et al., 1970).

Because the burn wound destroys the highly effective water vapour barrier of the skin (Moyer, 1962), evaporative water losses from the burn can be very large. Harrison et al. (1964) studying patients at ambient temperatures of 24 - 26^{o}C measured total insensible losses of up to 10 times normal rates, with strong correlations between the rate of evaporative water loss and both % BSA burn and

metabolic rate.

Because of the marked increases in heat production in burned patients, there can be great difficulty in maintaining energy balance. As the hypermetabolism can be prolonged, a negative balance leads to the use of limited tissue fuel stores and an undesirable loss of body mass (Cope et al., 1953; Davies, Liljedahl and Birke, 1969). Therefore either the food energy input must be increased or the raised post-injury metabolic rate decreased. The former course can be difficult and expensive because of the large caloric input required, while the latter, if possible by manipulating the environment, would be inexpensive and of low risk.

The large insensible loss from the burn and the decreased insulation over the vascular bed beneath the burn present uncontrolled routes of heat loss. Arturson (1977) proposed that this would produce surface cooling in the injured patient and so to maintain body temperature, metabolic rate must increase. Therefore to decrease the metabolic rate of a patient to 'normal' (i.e. a value commensurate with the body temperature of the patient) this heat loss must be nullified. The burn would then show a similar hypermetabolism to other closed injuries and disease.

However, Wilmore et al. (1974) and Aulick et al. (1979) suggested that though burn hypermetabolism is affected to a limited extent by the environment, the main reason for the increase in heat production is an internal reset in

metabolic activity. The pyrexia associated with burn injury in this theory only accounts for 20 - 30% of the increased metabolic rate when thermal drives have been satisfied (Aulick et al, 1979). Thermal injury must then have a different mechanism for control of metabolic rate than other trauma and disease.

The evidence for the latter theory comes from several sources. Zawacki et al. (1970) decreased the evaporative losses of 12 patients by covering their burn wounds with a water impermeable membrane for 12 hours. Their evaporative losses fell significantly over this period but their metabolic rate was practically unchanged. There are however, several objections to this study (Gump and Kinney, 1971). Only 2 short (5 minute) determinations of metabolic rate were made on each subject and no evidence was given that 12 hours was long enough for equilibrium to be attained. At the ambient temperature used (24 - 28^{o}C), any warming of the burn surface through reduced insensible losses would have increased radiative losses to the environment. In contrast, other studies have shown large decreases in metabolic rate after grafting (Moyer, 1962; Arturson 1977) and significant decreases in the metabolic rate of burned rats after wound covering with a similar impermeable membrane (Lieberman and Lansche, 1956).

Wilmore et al. (1974 and 1975) using a Douglas bag method studied the variation in metabolic rate of burn patients over a range of ambient temperatures from 19 to 33^{o}C. As the temperature increased insensible losses increased markedly while sensible losses decreased, although they

were both always above the losses of control subjects in the same environment. A patient with a 25% BSA burn even at 33°C would have a heat loss, predicted from Wilmore's data, 56% greater than a control.

Patients with burns of less than 50% BSA showed no reduction in metabolic rate above 25°C, while patients with large burns showed a small decrease (5 - 10%) when the temperature was increased from 25 to 33°C. Wilmore concluded that all burn patients were in their TNZ above approximately 30°C and hence the thermal drive associated with the burn had been met. As the rectal temperature was only 1.5 - 2.0°C above normal it could account for only 25% of the increase in metabolic rate. Hence the basic stimuli for the metabolic response appeared to be a central metabolic reset rather than extra heat loss occasioned by the burn.

Aulick, et al. (1979) confirmed some of Wilmore's work using a continuous indirect calorimetry system employing a ventilated hood rather than a Douglas bag technique. Seven patients with an average burn of 37% BSA (range 19 - 56%) when allowed to control their own room temperature, chose a mean value of 31.5°C, almost 3°C above that chosen by 5 control subjects. This contrasts with the earlier work (Wilmore, 1977) where the TNZ, and presumably the comfort zone, of patients with burns of less than 50% was similar to that of control subjects. Seven patients had their metabolic rate measured in a room at 30°C and then were warmed by one of two methods. Five were put

under uncontrolled radiant heaters while the room temperature was increased to 35 - 37°C for the other two patients. Only 4 of these appeared to reach stable thermal conditions (as measured by rectal temperature) probably because the heat input could not be adjusted. Over the short study period (0.5 - 3.0 hrs) there was only a mean fall of 3.5% in the metabolic rate of the seven patients. However in 3 of the 4 patients who reached stable conditwons, there was a mean decrease of 14%. All the patients increased their rectal temperatures, the average being 0.8°C. This suggests that the patients were not thermoregulating closely and that the 3 patients who decreased their heat production markedly had not been in their thermoneutral zone.

Workers in Sweden (Barr, Burke and Liljedahl, 1968) compared the metabolic rates of burn patients nursed in normal ward ambient conditions of 22°C and a relative humidity of 45% with patients nursed in a stream of warm dry air (WDA) which produced effective ambient conditions of 32°C and 20% relative humidity. Two patients with 20 and 30% BSA burns and a hypermetabolism of approximately 50% were treated sequentially in the two ambient conditions. There was a reproducable 5 - 11% decrease in metabolic rate when the patients were treated with WDA, and an increase (40 - 100%) in evaporative loss at the higher temperature similar to that reported by Wilmore (1975). Unlike Wilmore, who reported average skin temperatures of 35.9°C in his burn patients in an ambient temperature of 33°C, Barr reported burned skin temperatures

of 27 to 29°C and intact skin temperatures of 34 to 36°C in the WDA. Harrison et al (1964) had also reported skin temperatures lower than those measured by Wilmore.

Davies, Liljedahl and Birke (1969) using the same environmental conditions reported that patients with burns in the range 10 to 85% BSA and treated with WDA had metabolic rates 20 - 50% lower than a similar group treated at 22°C. The patients treated with WDA also had a significantly smaller weight loss. The patients in Wilmore's (1974) study had a similar 21% decrease in metabolic rate when the room temperature was increased from 21 to 33°C.

Birke et al. (1972) showed that patients in a severe injury group (45 - 100% BSA burn) had increases of over 100% in metabolic rate even when using the WDA treatment. The patients took 4 to 10 days to reach their peak metabolic rates. Two groups of 6 patients with burns in the range 20 to 30% BSA were compared under the two environmental conditions. Those treated with WDA had a heat production approximately 20% below the group treated at 22°C. As the wounds healed evaporative losses and metabolic rate declined in parallel in the two groups. Burn skin temperatures were again below ambient in the WDA group.

Other Swedish workers (Danielsson, Arturson and Wennberg, 1976a- 1976b) used a ventilated head canopy system to measure the oxygen consumption of thermally injured patients. They were nursed at an air temperature of 25 - 29°C and 40% relative humidity but also had 3 infrared

heaters above the bed which could be switched individually as the patient desired. The patients had their oxygen consumptions measured in various activity states including falling asleep and lying at ease. Falling asleep gave a metabolic rate 15 - 20% below the values measured when the patients were lying at ease. As measurements on normal subjects falling asleep (Grollman, 1930; Goll and Shapiro, 1981) have shown only a slow decline in metabolic rate over a period of several hours to approximately 10% below resting values, this appears to be rather a rapid and large decrease. Arturson (1978) proposed that the difference was 'tonic or rhythmic muscle activities' which would be present when lying at ease but not when asleep. However the subject must alter his means of thermoregulation at this point, probably by decreasing his core temperature and could not be considered to be in an equilibrium condition. A decrease in ventilation rate or depth, on falling asleep could also give an apparent decrease in oxygen consumption, though again this would not be an equilibrium situation. Therefore as other workers have quoted values of metabolic rate for patients resting (or lying at ease), Danielsson's measurements in this state will be used for comparison.

The 16 patients studied (13 to 66% BSA burn) had metabolic rates of up to 74 W / M^2 (64 kcal/M^2/hr). Though the tabulation of results makes comparison difficult, a patient with a burn of 60 - 65% BSA would have a metabolic rate of 64 - 70 W / M^2 (55 - 60 kcal/M^2/hr) while Wilmore's (1974) work would predict a metabolic rate 17 - 27% above this at 81 W / M^2 (70 kcal/M^2/hr) in an ambient temperature of 30 - 33^{o}C. Danielsson also showed a correlation of metabolic

rate against rectal temperature with an increase of 16% in heat production per degree centrigrade rise in core temperature. This is not much more than the average 13% per degree centigrade rate of increase in metabolic rate measured by DuBois (1927) in patients with various fevers. They are both much less than the increases per degree core temperature rise measured by Wilmore et al. (1974) and Aulick et al. (1979) who could only explain 20 - 30% of the observed increase in heat production using DuBois' figure. However the increase in core temperature and decrease in heat production observed in patients under infrared heaters (Aulick et al., 1979) would bring the rate of increase into closer agreement with Danielsson's figure.

In summary, the main points of agreement between the various authors are that:-

1) burn patients are hypermetabolic when compared directly with controls and this state continues until the wound heals or is grafted.
2) burn patients have an increased metabolic rate at temperatures below 25^{o}C compared with temperatures above it.
3) as ambient temperatures increase from around 20^{o}C to around 30^{o}C, insensible heat losses increase and sensible losses decrease.
4) in a given environment, the size of the burn correlates with evaporative water loss, and, though possibly less well, with metabolic rate. At an ambient temperature of 30 - 33^{o}C, a patient with a 25% BSA wound would have a metabolic rate increased between 37.5 to 55% (i.e. 1.5 to 2.1% per % BSA).

The main points of disagreement are whether

1) the uncontrolled heat losses from the wound, through the increased evaporative loss and decreased insulation, are the main stimuli for the burn hypermetabolism.
2) an ambient temperature of around 30°C meets the thermal drives of the burn or can infrared heating reduce heat losses further.
3) the effective burn skin temperature is close to ambient or close to the intact skin temperature.

1.5.2 Closed Injury

The reduction in metabolic response observed in patients with burn injury treated at higher ambient temperatures stimulated other studies of the effect of environmental temperature on closed injuries. However, because the response to this type of injury is smaller than for burns (Roe and Kinney, 1965) detection of an effect would be more difficult. Work on humans was preceded by animal studies. Campbell and Cuthbertson (1967) showed that with fracture of one femur in the rat, the small increases in heat production (7%) and nitrogen loss which were measured after injury at 20°C, were absent in animals kept at 30°C. The mechanism of this reduction was not apparent.

Cuthbertson, Smith and Tilstone (1968) observed that transfer from 20°C ambient conditions before fracture to 30°C conditions after fracture had the effect of abolishing the usual nitrogen response in rats and in four human fracture patients who were studied for up to 15 days after

injury. The oral temperatures of these patients stayed within normal values.

Cuthbertson et al. (1972) extended this work to two sets of patients with fractures, one group of 28 patients nursed at 20 - 22°C and the other group of 29 at 28 - 30°C. While there was no difference in the febrile response, nitrogen or energy input between the temperature groups, there appeared to be a systematic reduction in the nitrogen balances at the higher temperature. The reduction was larger for the more severely injured patients. There was a statistically significant pattern of reduction at the higher temperature only when the pooled results for all the patients at the two temperatures were compared. As energy expenditure measurements were not made, it could not be determined whether metabolic rate had been affected as in the animal experiments.

Spivey and Johnston (1972) studied 44 patients, the majority of whom had vagotomy and pyloroplasty operations, at two ambient temperatures. The first group (29 patients) was nursed at 24°C ($\pm$ 1.5°C) throughout the study while the second group (15 patients) was treated at 29°C($\pm$ 1°C) for the first three days after operation and then returned to 24°C. Over the first three days both groups of patients had a wide range of energy and nitrogen inputs. The nitrogen balances were similar at the two temperatures indicating no benefit of treatment at the higher temperature. The catabolic response to the surgery was relatively small. The results indicated that compared with an uninjured 70 kg man, the surgical patients would require only 350 mmol more

nitrogen and 2.5 MJ more energy to achieve nitrogen balance.

The initial observations (Cuthbertson, Smith and Tilstone, 1968; Campbell and Cuthbertson, 1967) that the metabolic response to a closed injury could be abolished by raising the environmental temperature to 30°C appear to be supported to a lesser extent by the later work of the Glasgow group (Cuthbertson et al., 1972) and not at all by Spivey and Johnston (1972), though the latter workers used a smaller temperature range. The observations in man are only of nitrogen metabolism and the extent to which heat production is affected has not been measured.

1.6 Animal Models of Burn Injury and the Effect of Environmental Temperature

1.6.1 Experimental Conditions

Apart from the work in the Department of Physiology Glasgow, several animal studies have been made using calorimetry to follow energy metabolism after thermal injury at different ambient temperatures. The major studies that will be described here are:-

Study 1 - Lieberman and Lansche (1956); Moyer (1962)

Study 2 - Caldwell et al. (1959), Moyer and Butcher (1967)

Study 3 - Caldwell, Hammel and Dolan (1966)

Study 4 - Herndon, Wilmore and Mason (1978)

The studies vary widely in the type of calorimeter, environmental and feeding conditions used. As these factors influence the comparison of one study with another and also to the human work they will be briefly outlined.

Studies 1 and 2 used the same apparatus, a multiple chamber indirect calorimeter (Benedict and MacLeod, 1929b) which measured water loss gravimetrically. Study 1 measured the effect of blocking water loss from the wound with an impermeable membrane while Study 2 examined the energy response to ambient temperatures between 24 and 32°C. Study 3 used a direct and indirect calorimeter similar to the one built in the Department of Physiology. Small changes in ambient temperature were made and the progress of the injured animals followed for 43 days. Rats were used in the first three studies but Study 4 used guinea pigs as well as young and adult rats. The oxygen consumption of these animals was measured at temperatures ranging from 19 to 33°C at the approximate 100% humidity prevailing in the calorimeter. The differences between the experiments are detailed in Table 1 and their consequences discussed below.

1) Only study 3 used direct and indirect calorimetry allowing comparison of heat production and heat loss. Study 4 measured oxygen consumption directly without a carbon dioxide measurement and so heat production could be incorrect by up to 5% because of the variation of the calorific equivalent of oxygen with respiratory quotient.

2) The animals in these studies were housed at one environmental temperature and calorimetered at another. Benedict and MacLeod (1929a) showed that rats kept at 21°C and calorimetered at 28°C had a heat production 4.5% higher than equivalent animals kept and calorimetered

Conditions	Study 1	Study 2	Study 3	Study 4
Calorimeter Type	Indirect plus Water loss	Indirect plus Water loss	Direct and Indirect	Oxygen Consumption
Housing Temperature (^{o}C)	-	28-30	20	26
Calorimeter Temperature (^{o}C)	24-26	24-28-32	27.5-30	19-33
Calorimeter Humidity	Dry	Dry	Dry	100%
Respiratory Quotient	Yes but not reported	Yes but not reported	0.725 Average	No
Duration in Calorimeter (min)	60	60	60 plus 20 before run	60 plus 30 before run
Type of Injury	Radiant burn	Radiant burn	Scald	Scald
% Body Surface Area	5-20	18	21.5	25 and 50
Body Weight (g)	250	250	300	180 and 500
Weight Graphs	No	No	No	Yes
Heat Production Standardisation	per m^2	per m^2	per kg	From best fit formula
Feeding	-	Ad Lib	Ad Lib	Ad Lib

Table 1 Comparison of the experimental conditions in the four animal model studies.

at 28°C.

3) The relative humidity in studies 1 to 3 was low (effectively zero) whereas the humidity in study 4 was quoted as approximately 100%. As the rat chamber was open to room air before the measurement began, presumably the humidity rose from room levels to 100% during the measurement. This would happen much more quickly for a burned animal than for a control. At 100% relative humidity, though water loss would be reduced markedly, it would not be zero. As long as respiratory tract and skin temperature were above the ambient, air saturated at the air temperature would not be saturated at the tract or skin temperature and so evaporative loss would still be possible (McLean, 1974).

For these reasons a control animal will be little affected by a one hour study at a high humidity but the response of a burned animal will depend on whether the hypermetabolism is caused mainly by evaporative losses or whether an internal metabolic reset predominates. If the former is true high humidity should benefit the rat bringing its metabolic rate closer to control values. If the latter is true the animal's metabolic rate should not decrease and at the higher environmental temperatures difficulties in losing all the heat produced would be found. At these higher temperatures core and skin temperatures would have to increase above the control values to increase sensible losses.

4) Feeding in all the studies was ad-lib. This makes comparison of control and burn weight changes unreliable. Studies 1 to 3 give little indication of weight changes which makes assessment of their heat production standardisation procedures (i.e. per m^2 and per kg) difficult when the animals grow apart.

1.6.2 Experimental Results

Study 1 (Lieberman and Lansche, 1956; Moyer, 1962).

Rats with a 15% BSA wound showed increases of 11% in metabolic rate while the eschar was intact and 21% with the eschar excised. Insensible heat loss, as a percentage of total heat loss, rose from 24% for the controls, to 39% for the animals with an eschar and 48% for those with the eschar excised. There was a gradual return to control levels as the wound healed. No significant difference was measured in the core temperatures of the burn and control animals though the eschar was 0.8°C and the open wound 1.0°C below the surrounding skin temperature.

One rat with a 26% increase in metabolic rate after a 15% BSA burn had the evaporative loss from its wound blocked using an impermeable membrane (Saran wrap). The insensible losses decreased by 9.7 W m^{-2} (200 kcal $m^{-2}d^{-1}$) while the total losses dropped by 7.7 W m^{-2} (160 kcal $m^{-2}d^{-1}$). The The animal's metabolic rate was then only 10% above the control value. The result is in contrast to similar work in humans (Zawacki et al., 1970) and would suggest that high humidity would have an effect on the animals in

Herndon's experiments (Herndon et al., 1978). Lieberman and Lansche (1956) reported simlar results on animals that had portions of skin excised. The increased metabolic rate and water loss returned close to control values on regrafting the excised area.

Study 2 (Caldwell et al., 1959; Moyer and Butcher, 1967).

As the working temperature of the calorimeter was increased from 24°C to 32°C the metabolic rate of rats with an 18% BSA burn steadily decreased. The control rats had a minimum metabolic rate at 28°C corresponding to their TNZ (see section 1.3.2). The burns were hypermetabolic by 22% at 24°C, 20% at 28°C and 6% at 32°C. This final value reflects the increase in the metabolic rate of the control animals at a temperature above their TNZ. The heat production of the burned animals at 32°C was 16% above the value for the controls at 28°C. Therefore the burns animals were hypermetabolic by 1 to 1.5% per % BSA burn.

The water losses of the injured rats were similar at 24 and 28°C but markedly increased at 32°C. As heat production was decreasing with increasing temperature, the sensible losses must have reduced at a greater rate than heat production as environmental temperature increased.

Study 3 (Caldwell, Hammel and Dolan, 1966).

Animals with a 21.5% BSA scald were always hypermetabolic when compared with control heat loss and production values. The energy values were calculated on a per kg

basis. As no weights were given and in a previous experiment (Caldwell, 1962) the weights of injured and control animals had been shown to diverge markedly, comparison of results between burn and control and with other studies is difficult. On a per kg basis the burns were hypermetabolic by 21% on the 15th day, 38% on the 36th day and 46% on the 43rd day, despite a 67% reduction in wound size over this period. The steadily increasing weight of the controls decreased their heat loss per kg by 15% over the 43 day period. Therefore a hypermetabolism of 20 - 30% between similarly sized injured and control animals was probable. A small increase (2.5°C) in calorimeter temperature on days 29 and 36 decreased the burn animals metabolic rate slightly. Throughout the study rectal and skin temperatures of the burn and controls were very similar.

Study 4. (Herndon, Wilmore and Mason, 1978)

This study investigated the changes in oxygen consumption in young and adult rats (approximate weights 200 and 500 g) and in guinea pigs following scalds of 25 and 50% BSA. The measurements on the injured animals were compared with estimates from a best fit regression line produced from measurements on control animals of a similar weight.

Both the adult and young rats with a 50% BSA scald showed a 31% increase in oxygen consumption at an ambient temperature of 31.5°C. The increase in consumption compared with the controls was only 22% at 26°C and 20% at 21.5°C in the adult rats though the absolute oxygen consumption increased for the injured animals. With a

25% BSA injury the adult rats kept at 31.5°C showed only a 9% increase in oxygen consumption compared with controls. The two severities of injury suggest an increase of 0.35 - 0.6% in metabolism per % BSA scald. This is low compared to the 1 - 1.5 % increase per % BSA injury seen in the other studies.

The hypermetabolism of the 50% BSA scald adult rats was observed to increase over the first 7 days after injury to a plateau which was then sustained for 38 days. Over this period the wound was steadily healing, decreasing to 19% of its original size (i.e. 9.5% BSA) after 45 days. There was no gradual decrease in metabolic rate with healing as seen in humans by Birke et al (1972).

Over the 30 day period following injury the animals with a 50% BSA scald lost weight while their controls grew. The approximate food energy input for the injured animals was 6.0 W (125 kcal d^{-1}) and for the controls 6.8 W(140 kcal d^{-1}). The animals were housed at 26°C and so their approximate heat productions (assuming the energy equivalent for one litre of oxygen consumed to be 20 kJ) were 3.9 and 3.3 W (81 and 69 kcal d^{-1}) for the injured and control animals respectively. Therefore both sets of rats were in large energy surpluses though the injured animals lost weight.

In summary the animal models show:-

1. that injured animals are always hypermetabolic compared with controls over the temperature range investigated and this continues until the wound heals or is grafted.

2. that injured animals steadily decrease their metabolic rates as the ambient temperature is increased from around 20°C to around 30°C.

3. that as the environmental temperature increases insensible heat losses increase and sensible losses decrease.

4. that in dry atmospheres the increase in metabolic rates of the injured animal when compared with a control is 1 - 1.5% per % BSA injury. At 100% relative humidity the increase is 0.35 - 0.6 per % BSA injury. Covering the wound to block water loss can produce a large decrease in insensible losses and heat production, while excising the eschar or skin has the opposite effect.

All the studies described have assumed that the 20 - 60 minute calorimetry periods used were representative of the heat losses outside the calorimeter i.e. that in the short time allowed the animals adapted to any temperature, air flow and humidity changes between their housing conditions and the calorimeter.

Chapter 2

Animal Experiments Materials and Methods

2.1 Small Animal Calorimeter

The direct and indirect calorimeter in the Department of Physiology, Glasgow University, which was used for all the small animal experiments in this thesis was originally constructed and calibrated by Carter (1975). Its performance and subsequent use have been described in various papers (Carter et al,1975, Carter, Drury & Richards, 1976) and theses (Drury, 1976; Gray, 1978; Richards, 1979). Therefore only a brief account of the calorimeter will be given.

A schematic diagram of the calorimeter is shown in Figure 3. Air is pumped into the calorimeter from outside the laboratory at 1 to 2 litres per minute, its oxygen concentration being monitored by a reference cell in the oxygen analyzer. The carbon dioxide concentration is not monitored but is assumed to remain at a constant low level (approximately 0.03%) during a calorimetry period. The air is brought to the required humidity by saturating it in a humidifier and then condensing the excess water vapour in a dew point (temperature T_1) heat exchanger. The air stream is warmed to the set ambient temperature (T_2) by the environmental temperature heat exchanger. This also acts as a heat sink, maintaining the walls of the gradient layer box at the environmental temperature.

The gradient layer box is large enough (115 mm x 115 mm x 230 mm) to take a single rat (Figure 4). Most of the

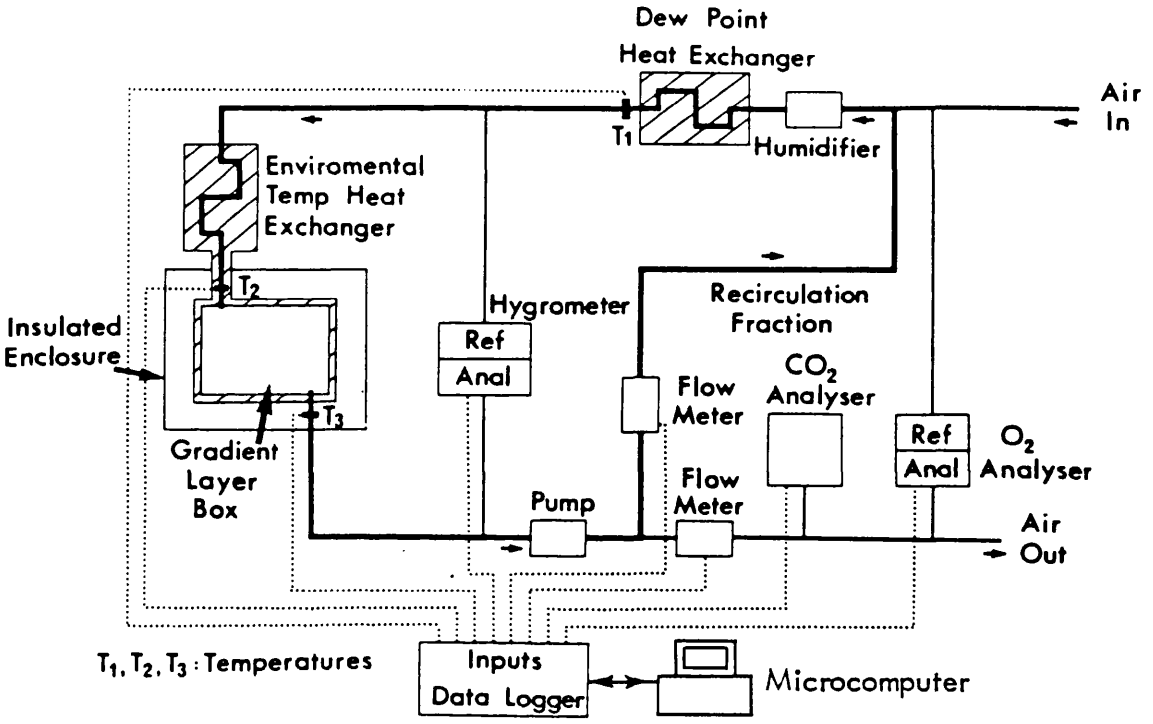


Figure 3. Schematic diagram of the small animal calorimeter.

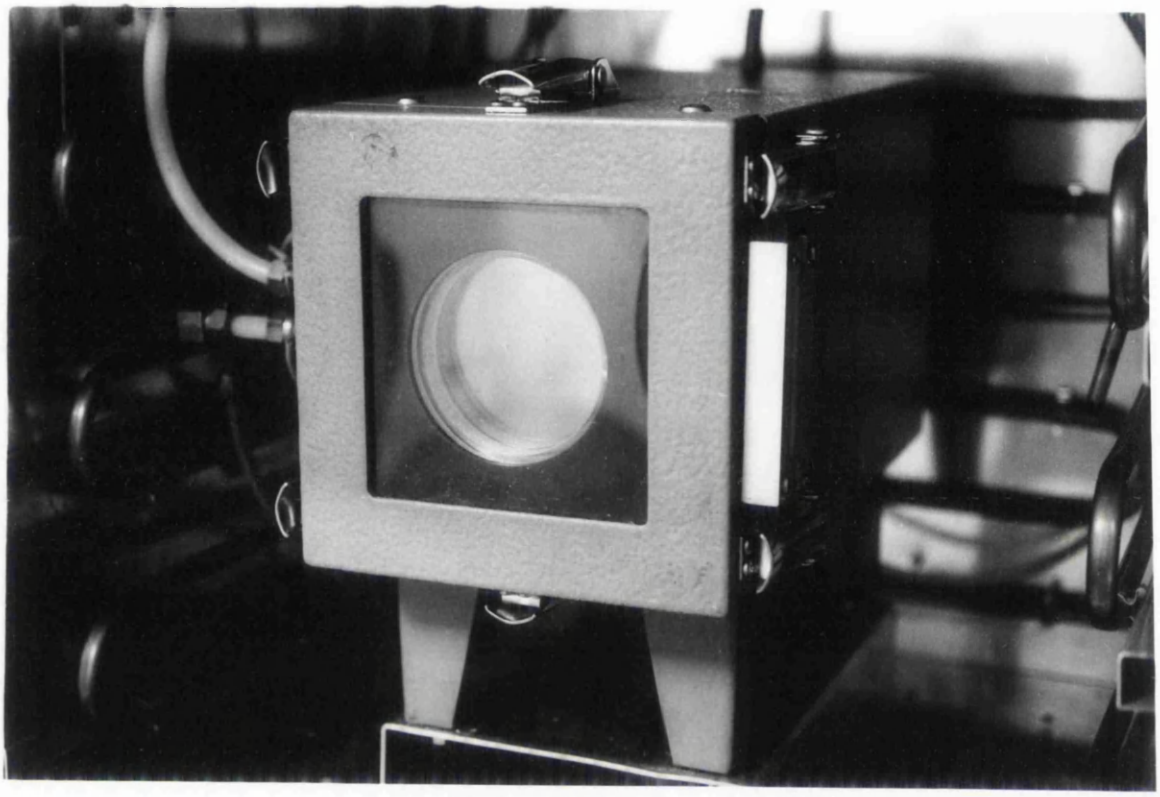


Figure 4. The gradient layer box.

sensible heat loss of the animal is conducted through the walls of the box to the surrounding heat sink. As the gradient layer has a thermal resistance, heat will only flow under a temperature gradient which, by Fourier's Law, is proportional to the rate of heat flow. With suitable calibration, measurement of the temperature drop across the gradient layer using thermocouples allows calculation of the rate of heat loss. Three thousand thermocouples give a high enough sensitivity (2.77 mV/W) to make it suitable for small animal energy determinations. Any heat loss to the ventilating air is calculated from the temperature difference across the box ($T_3 - T_2$) and the air flow.

The humidity increase produced by the animal is measured using a differential hygrometer, which samples the air stream before and after the gradient layer box, allowing insensible losses to be calculated. Because of the large water losses from a burned animal the total air flow through the box has to be between 6 and 8 litres per minute to stop condensation. A large proportion of this flow is recirculated and only 1 to 2 litres per minute is output, otherwise the changes in gas concentrations would be too small to measure accurately. The two flows are measured by separate wet gas flow meters.

The analogue outputs of the various instruments are scanned at 2 minute intervals by the data logger and also continuously recorded on a 12 channel pen recorder. For experiments 1 and 2 the data was punched onto paper tape

for off-line processing on a Varian 620-L minicomputer in the Department of Nuclear Medicine, Glasgow Royal Infirmary. This system was modified after experiment 2, as described in section 2.2. The calculation of the direct and indirect calorimetry values is shown in Appendix A.

2.2 Modification of the Calorimeter and Data Processing

Using the paper tape punch for data storage and off-line processing gave no accurate indication during a calorimetry run of heat loss, heat production and respiratory quotient. These indicators would allow a check on instrument errors, response to food intake etc. and hence appropriate action could be taken at the time of the experimental run, if they were available. Therefore when a microcomputer (PET 2001, CBM Ltd, figure 5) became available at the end of experiment 2, it was used to replace the paper punch. The computer initiated the data logging cycle, checked for data errors and relogged if necessary, and stored the data in an inbuilt cassette unit. The video display unit showed the current calorimetry values, though final processing and printing was completed off-line.

The data from the logger were input to and the command signals to the logger were output from the external user port of the microcomputer. The integrated circuit connected to this port within the microcomputer, a peripheral interface adapter (P.I.A., Mostek 6520) while using transistor - transistor logic (T.T.L.) compatible

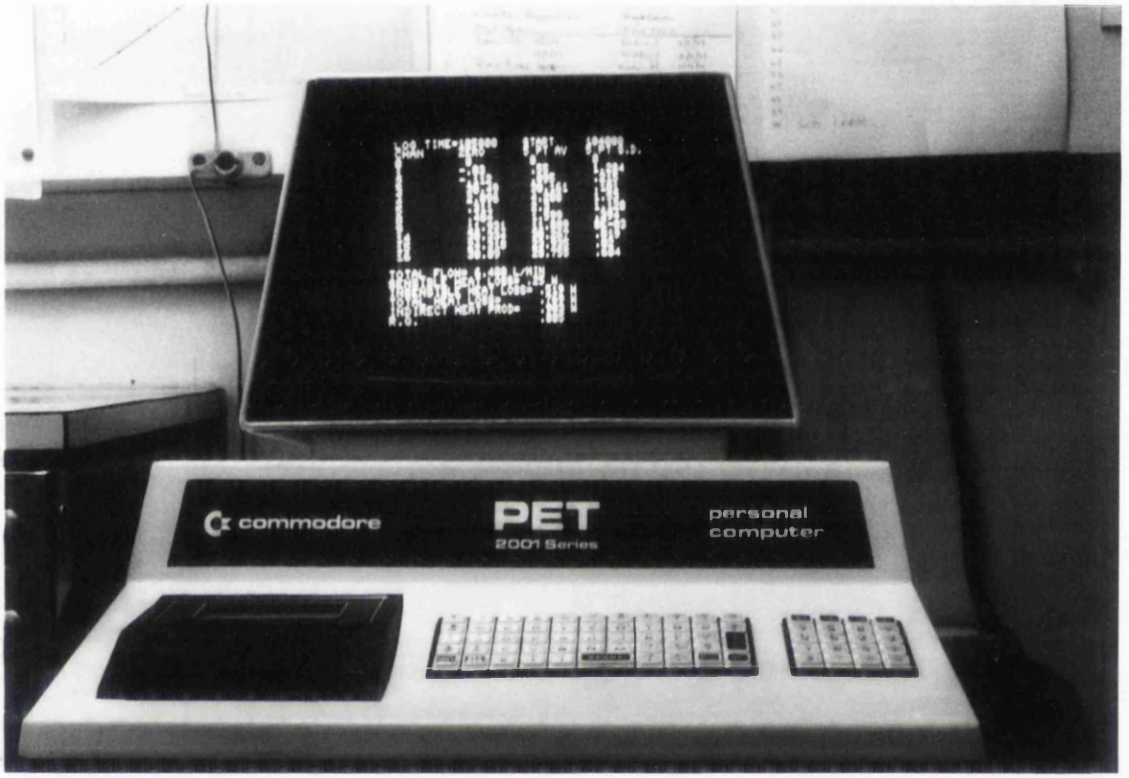


Figure 5. A typical microcomputer display during a calorimetry measurement in Experiments 3 and 4.

voltage levels was not able to sink or source the currents required by the 'reset' and 'initiate log' signal lines in the data logger. Therefore a buffer circuit of a single transistor (BC109, Mullard Ltd) emitter follower was used on each of these lines. The computer used the 'reset' function to clear the data logger electronics to a specified state after an error etc., and the 'initiate log' function to start the logging cycle. The computer detected a ' data ready ' signal input to the user port and then input the data in the same form, a parallel six bit code, as used with the paper punch. As the period between logging cycles was usually two minutes, the data input, storage and processing could all be controlled by programs written in BASIC programming language.

2.3 Body Composition and Metabolic Analyses

In order to estimate the use of body tissue through the experiments detailed in Chapter 3, rats were sacrificed at various times before and after injury and their body composition analysed in terms of fat, protein and water content. These measurements, as well as faecal and urinary nitrogen estimations, were performed by G. Al-Shamma in experiments 1 and 2, and by G. Gedeon in experiments 3 and 4 with assistance from the Departments of Biochemistry at the Royal Infirmary and the Western Infirmary Glasgow. Details of the techniques have been published (Al-Shamma, 1978; Al- Shamma et al., 1979; Gedeon, 1981) and only an outline will be given here of the body

composition analysis methods.

Technique A (Al- Shamma- Experiments 1 and 2)

a) Clean carcase gut of food and faeces and weigh to give the empty body weight (E.B.W.).

b) Dry the carcase in a hot air oven at 70°C until a constant weight is reached. The decrease in weight is taken as the total body water.

c) The dried carcase is digested under reflux in ethanolic sodium hydroxide.

d) Samples of the digest are taken for estimation of nitrogen by the micro-Kjeldahl method (Fleck, 1967) and for fat estimation by extraction of an acidified portion with light petroleum, purification and filtration with sodium hydroxide.

Technique B (Gedeon - Experiments 3 and 4)

a) The carcase is prepared as in step a) technique A.

b) The carcase is autoclaved at 150°C in a sealed vessel for 30 minutes to soften it so that the tissues can be homogenised in a commercial food blender.

c) Samples of the homogenate are taken for freeze drying to give the body water content and for digestion and measurement as in technique A for fat and protein.

d) A dried sample fo the homogenate can be used to measure tissue energy by bomb calorimetry as described in Section 2.4.

All the urinary and faecal nitrogen estimations were also performed by the micro-Kjeldahl technique (Fleck, 1967).

2.4 Bomb Calorimetry

2.4.1 Bomb Calorimetry Technique

For energy balance calculations, measurements of food, faecal and body tissue energy values were required and these were obtained using a Gallenkamp Model CB/110 adiabatic bomb calormeter. A known amount of dry sample was pelletised and sealed in a stainless steel bomb which was then charged to a pressure of 25 atmospheres with oxygen. The bomb was placed in an assembly of known thermal capacity surrounded by a heated water jacket. The water jacket temperature tracked that of the internal assembly so that no heat was lost through the jacket (i.e. adiabatic conditions). The bomb was fired electrically and measurement of the maximum temperature rise after firing allowed calculation of the energy of the pellet. Regular calibration checks were performed using benzoic acid.

2.4.2 Metabolisable Food Energy

The composition of the powdered, low residue diet used in all the experiments is recorded in Appendix B. When a diet batch was made, three 20g samples were taken at random from it. Three 1 to 2g pellets were made from each sample and their energy content measured in the bomb calorimeter. The results from the three pellets were

averaged for each sample and the results for the three diet batches are shown in Table 2. The nine sample results have a mean of 19.15 kJ/g and a coefficient of variation of 0.5%. Energy content differences between the diet lots were small enough for the mean value to be used in all the experiments.

An estimate of faecal losses was required so that the energy absorbed from the diet could be calculated. Faecal and urine collections were only possible from ten rats housed in metabolic cages (Figure 6) during any experiment. These cages had stainless steel mesh floors which allowed the excreta to pass into glass funnels. Below the funnels were glass separators which divided the urine and the faeces into different collecting flasks. Collections were made and the glassware replaced daily.

Because the diet was low residue, the faecal energy losses were expected to be a relatively small and constant fraction of the dietary intake. As routine daily collections of faeces were difficult because of diarrhoea or contamination with hair or food etc., 16 three day pooled collections of faeces were made over experiments 1 to 3 and an average value of faecal energy loss as a fraction of food intake calculated. Periods of almost constant food intake were chosen to reduce the error in associating a particular period of food intake with a later elimination of faeces.

The bomb calorimetry results are shown in Table 3. The calorific value per gram of dried faeces varied little

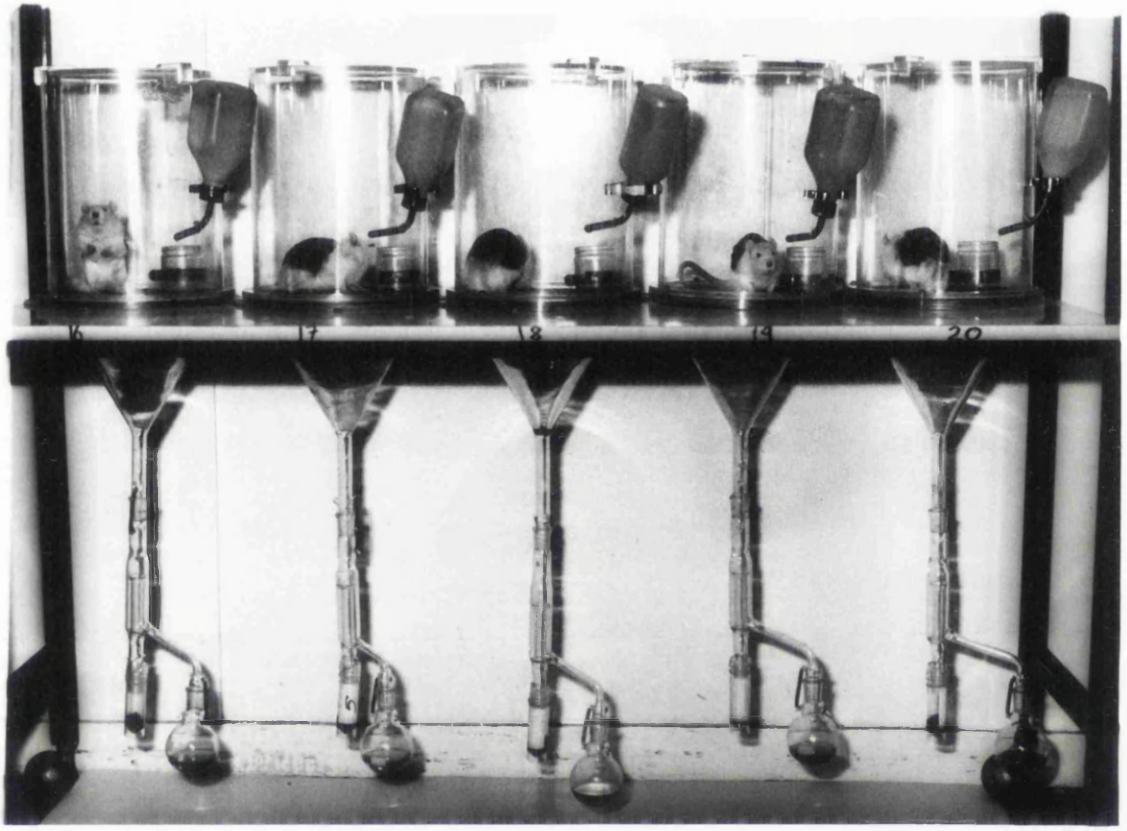


Figure 6. The metabolic cages and collection apparatus.

Diet lot	Sample 1 (kJ/g)	Sample 2 (kJ/g)	Sample 3 (kJ/g)	Mean (kJ/g)
I	19.05	19.08	19.10	19.08
II	19.10	19.05	19.11	19.09
III	19.28	19.31	19.22	19.27
Combined				19.15

Table 2. Gross food energy per gram of different diet lots.

	Faecal Calorific Value kJ/g	Faecal Total Calorific Value kJ	Food Gross Calorific Value kJ	Faecal Energy Food Energy %
mean	16.3	40.1	835	4.80
coefficient of variation (%)	5.4	20.4	4.2	19.0

n = 16

Table 3. Mean food energy input and faecal energy loss of the 16 three day faecal collections from rats in experiments 1, 2 and 3.

(mean 16.3 kJ/g, coefficient of variation 5.4%) suggesting that a relatively constant proportion of the dietary constituents was being eliminated. The total energy of the collections showed a bigger variation (coefficient of variation 20.4%) probably corresponding to the non-regularlity of elimination of faeces and the difficulty of collecting the small daily amounts involved. The faecal energy was 4.8% of the food energy input. Therefore it has been assumed that 95.2% of the food energy intake of each animal was absorbed.

Another route of energy loss was in the urine. Various authors (May and Nelson, 1972; Pullar and Webster, 1977) have shown that the energy per mmol of nitrogen in urine is constant over a range of dietary intakes in the un-injured animal. Though this has not been confirmed in injured animals, as the total correction was small (less than 5% of food energy) and the urea fraction in the urine was not significantly different for burn and control animals (Gedeon, 1981), the mean figure of 0.45 kJ/mmol (Pullar and Webster, 1977) was used. The urinary nitrogen was measured as indicated in Section 2.3, on the daily or three day pooled urine collections of the animals kept in the metabolic cages.

Therefore the metabolisable energy (ME) for any food intake (FI) in grams was:-

$$ME = 19.15 \times 0.952 \times FI - 0.448 \times N \qquad 2.1$$

where N was the urinary nitrogen in mmol.

2.4.3 Body Tissue Energy

Technique B of body composition analysis allowed samples of the homogenate to be taken for bomb calorimetry. The dried samples contained fat, protein and a mineral residue. Ashing tissue samples from experiment 3 gave an average ash content of 8%. Hence it was assumed that each gram of dry sample contained 0.92g of fat plus protein and the fractional fat in the sample was calculated as a fraction of the fat plus protein value. The five burned animals used from experiment 4 had their results corrected for the mineral residue in the same manner but using their individually measured ash residues.

A scatter diagram (Figure 7) of the energy per gram of the body composition sample versus the fractional fat in the sample (i.e. fat divided by fat plus protein) shows the best fit regression line plus the line calculated from literaure values of fat and protein energy (i.e. 39 and 23 kJ/g respectively (Brown, 1973)). The 5 burned animals from experiment 4 all have fractional fat values below 0.28. The other animals, both burn and control, from experiment 3 all have values above 0.28. The regression statistics are shown in Table 4. The gradients of the literature and best fit lines are significantly different, however, the gradient of the fitted line is heavily biassed by the 5 points from experiment 4. A regression analysis using only the results from experiment 3 gives a regression coefficient which is not significantly different from the literature value. It was felt that

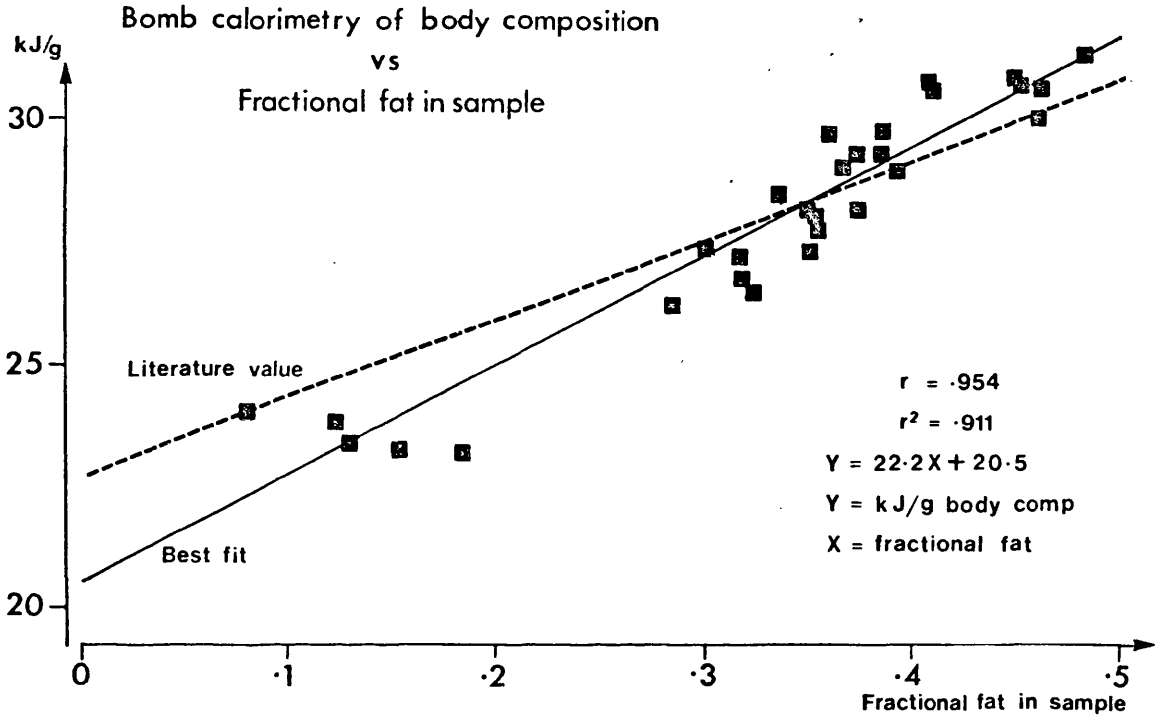


Figure 7. Energy content of body composition samples vs the fractional fat (corrected for mineral content) in the sample.

Best fit regression line $Y = bX + C$

where Y = body composition energy value (kJ/g)

X = fractional fat (fat ÷ (fat + protein))

Regression coefficient (b)	=	22.2
Standard deviation of b (S_b)	=	1.29
Intercept (c)	=	20.5
Correlation coefficient (r)	=	0.954
Coefficient of determination (r^2)	=	0.911
T value (H_o: p = 0)	=	17.23 (p - ***)
Degrees of freedom	=	27

The Literature value line gives $Y = BX + C$

where $B = 16$

and $C = 23$

The gradients (b & B) of the two lines are significantly different

$$T = \frac{22.2 - 16}{1.29} = 4.8 \quad (p - ***)$$

Table 4. The regression statistics for the body composition energy results.

more carcases with low fat contents would be required to establish the use of energy values other than the literature values. Comparison of the best fit line with the literature value line, shows the literature value to be 2.5% high at 0.5 fractional fat, 2.5% low at 0.3 and 8.0% low at 0.1. As most carcases have a fractional fat value between 0.3 and 0.5, the error involved in using the literature value was small.

2.5 Data Processing

2.5.1 Calculation of Energy Balances from Calorimetry

Calorimetry measurements were performed as frequently as possible on the ten animals (five burned and five control) kept in the metabolic cages during experiments 1 to 3. Because of the necessary calibration periods and the time required for stabilisation during calorimetry runs (Carter, 1975) only one or two animals could be measured each day. A separate rotation of burn and control groups was used so that burned animals were calorimetered more frequently than controls. Where necessary the energy values obtained for a burn or control rat were considered representative of the values for other animals on that day. Energy balance has been calculated in periods which coincide with the animal sacrifice points throughout the experiments. As these points were approximately 10 days apart, each animal was usually calorimetered during the period.

Energy balance was calculated from the mean heat loss, mean food intake and mean urinary nitrogen excretion of the burn and control groups over a period in between sacrifices.

The metabolisable energy of the food was calculated using equation 2.1. The difference between this and the mean heat loss could be used to give the energy balance over the period, except that the calorimetry was performed on post-absorptive rats and took no account of any dietary induced thermogenesis of the food intake (see Section 1.3.3). This was measured for burned and control animals at 20 and 30°C as part of experiments 3 and 4 (see Section 4.4) and expressed as a percentage of the gross food energy intake was:-

6.64% for the controls at 20°C
6.73% " " " " 30°C
1.40% " " burns " 20°C
2.53% " " " " 30°C

Therefore the net food energy assumed to be available to the rat was, using the variables ME and FI from equation 2.1:-

$$\text{Net Food energy (NFE)} = ME - \frac{DIT}{100} \times FI \times 19.15 \text{ (kJ)} \quad 2.2$$

where DIT represents one of the four percentage values given above. The energy blance was then calculated as the difference between the net food energy and the heat losses.

2.5.2 Calculation of Energy Balances from Body Tissue Changes

Energy balance could also be calculated from the changes in body tissues between sacrifice points. Sacrifice points were approximately ten days apart and animals kept in the same room as the metabolic caged animals, but in simple metal cages on spur racking were used during the

experiment. These animals were treated (i.e. fed, shaved, burned etc.) in exactly the same way as the metabolic animals but did not have calorimetry measurements. At the end of the experiment the metabolic caged animals were sacrificed and so this was the only point where the calorimetry and body composition energy balances can be exactly compared. However, the smoothness of the tissue energy balance curves show that the animals in the metal cages were undergoing similar tissue changes at the intermediate sacrifice points, to the metabolic caged rats.

Tissue changes were calculated by sacrificing a group of animals at the start and measuring their average body composition. Each animal sacrificed at a later point was then assumed to have had this average body composition at the start, and the absolute amounts of fat, protein and water were calculated from its live weight on the first day of the experiment. The difference between those estimates and the measured values on the day of sacrifice gave the individual tissue changes. A computer program was written to calculate the values. This procedure was similar to that used by Thomson (1965) and has been described in detail (Al-Shamma et al., 1979). The energy balance was then calculated using 39 and 23 kJ/g for fat and protein energy values respectively (see Section 2.4.3).

2.5.3 Statistical Analyses

All the calculations were performed on a microcomputer

(PET 2001, CEM Ltd) using specially prepared programs.

Unpaired Student T tests, Wilcoxon Rank Sum test and regression analyses (Snedecor and Cochran, 1967) were used where appropriate. Control and burned animals were compared on the basis that they had been randomly selected from a group of similar age and weight at the start of the experiment and that they were treated in an exactly similar manner (i.e. same food intake, environmental temperature, shaving, anaesthesia etc.) apart from the branding of the injured animals.

Chapter 3

Animal Experiments

3.1 Experiment 1: Environmental Temperature 20°C and Food Intake 15g

Forty male Wistar rats from the Department of Physiology, Glasgow University, semi-inbred closed colony were housed in the environmental chamber approximately two weeks before the production of the burn injury. The animals were between 10 and 12 weeks old and weighed 180 - 200g. During the two weeks any rats that failed to eat the 15g of diet offered daily or failed to grow at a steady rate were changed. Ten of the rats were housed in metabolic cages (see Section 2.4.2) the remainder being kept in metal cages fixed to the wall using spur racking. The temperature of the chamber was set to 20°C ± 1°C.

The backs of the rats were shaved using Aesculap Favorita V42947 hair clippers with 0.1 mm blades, 4 days prior to the injury to allow identification of the anagen areas of the skin. The thickness and blood supply of rat skin depend on its phase of growth (Zawacki and Jones, 1967). The anagen areas are the most suitable for production of a standard injury (Richards, 1979) and can be identified by their rapid hair regrowth. On the day of injury 20 animals with the largest areas of anagen skin on their dorsum were selected for branding. They received an intraperitoneal injection of vetinary nembutal (Abbott Laboratories) in a dose of 0.05 ml per 100g live weight, to achieve full anaesthesia. The total surface area of each

Experimental day	Expt 1	Expt 2	Expt 3	(sacrifice days)	No. metabolic cage rats (calorimetry group)	No. wall cage rats (non-calorimetry group)
-14					10	30
- 4 (shaving)					10	30
0 (selection)					5B 5C	15B 15C
0 (injury)					5B 5C	15B 12C
	13	7	10		5B 5C	12B 10C
	20	22	20		5B 5C	9B 8C
	31	44	30		5B 5C	6B 6C
	43	-	40		5B 5C	3B 4C
	57	51	50		0B 0C	3B 4C

B - Burned animals C - Control animals

The numbers of rats remaining after the common pre-injury procedures and after the different post-injury sacrifice points in experiments 1, 2 and 3.

animal was calculated using the formula validated on rats from the same colony (Richards, 1979):-

$$\text{Surface Area } (cm^2) = 10.1 \times (W)^{2/3} \qquad 3.1$$

where W is the weight in grams.

The backs of the animals were reshaved and the branding of the animals was performed by J.R. Richards, producing a 25% (± 0.5%) body surface area (BSA) injury over the dorsum. The flanks of the animals were avoided to reduce interference with their normal movements. The temperature and pressure of the branding head on the 'Eunyan Contact Burn Apparatus' (Wilkinson Sword (Research) Ltd.) were 300°C and 300g respectively and the head was held in contact for five seconds. The rats were given an intraperitoneal injection of 2 mls of glucose saline and allowed to recover. There was one death during the period of anaesthesia.

Three of the remaining animals were sacrificed to provide an estimate of initial body composition of the group and the rest were kept as controls. Two days later the controls underwent a mock procedure with the same anaesthesia, reshaving and intraperitoneal injection but no branding. The day of branding was considered to be the experimental start (day zero) for the burns and the mock branding was considered to be day zero for the controls. The gap of two days between the two experimental groups allowed the control animals to be offered the amount of diet the burned animals had eaten two days previously, for accurate pair feeding. All treatments of the

controls (e.g. sacrifice points etc.) were two calendar days after the burns.

All animals were weighed and had their food intake measured daily. The food was made into a paste form by mixing with water to reduce spillage, but all weighings were made when the diet was dry. Because of calibration periods only seven calorimetry runs could be performed in a week. As the burn response was of greater interest than the control changes, a greater proportion of injured animal measurements were performed. Though the amounts of faeces and urine lost in the calorimeter were recorded, the amounts were small (less than 2g) compared with the amounts excreted in the metabolic cages between runs. Therefore no correction to the metabolic collections were made. At least 50 minutes were allowed after the animal entered the calorimeter for the equipment to stabilise (Carter, 1975). The calorimetry values were calculated from a 60 minute period after stabilisation that showed no large activity peaks on the oxygen and hygrometer traces.

At approximately ten day intervals, three burned and two control animals from the metal wall cage group were sacrificed for body composition analysis. At the end of the experiment after 57 days the metabolic cage animals were also sacrificed.

3.2 Experiment 2: Environmental Temperature 20°C and Food Intake 18g

This experiment used the same protocol as experiment 1 except for three aspects. Firstly the burned rats were offered 18g of diet instead of 15g. Secondly the temperature and pressure of the branding head were slightly lower at 250°C and 250g. Thirdly the gap between the actual and mock branding was one day not two. The subsequent treatment of the control animals was staggered by one day throughout the experiment.

3.3 Experiment 3: Environmental Temperature 30°C and Food Intake 15g

This experiment used the same protocol as experiment 1 except that the environmental chamber and calorimeter working temperatures were raised from 20 to 30°C ($\pm$ 1°C) and some additional calorimetry experiments were performed to determine the thermogenic response to the oral diet.

The extra calorimetry was performed after the 60 minute period that provided the usual post-absorptive values had finished. A small metal tray had previously been filled with a measured amount of diet (approximately 3g) and kept at the calorimeter working temperature for at least 1.5 hours. The tray was quickly introduced into the front of the cage in the gradient layer box (Figure 8 shows an animal in the cage with the food tray). The animal was allowed half an hour to eat as much of the diet as it wished. Then the tray was quickly removed from the cage in the calorimeter and weighed to give the

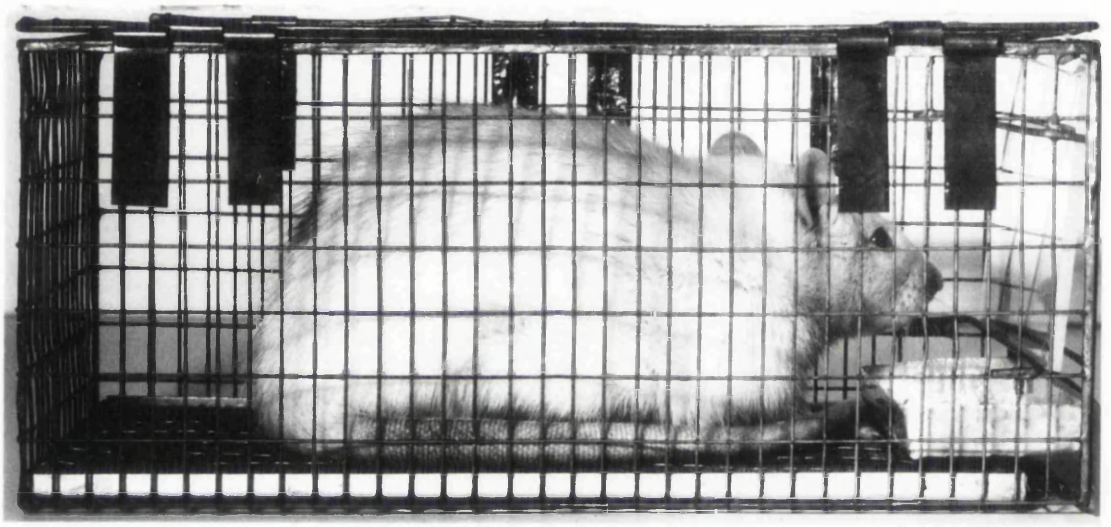


Figure 8. Rat in calorimetry cage with the food tray used in the thermogenesis experiments.

amount of diet consumed (usually 2 - 3g). The calorimetry was then continued for a further 2.5 hours. The results of these measurements of the thermogenesis of the diet have been reported under experiment 4.

3.4 Experiment 4: Dietary Thermogenesis Measurements at 20°C

This experiment measured the thermogenesis of the oral diet in burned and control rats as in experiment 3 but at the lower environmental temperature of 20°C. Five injured and three control rats were kept in metabolic cages and offered 15g of diet per day as in experiment 1. Because the calorimetry was solely to obtain data on thermogenesis, the rats were measured for up to six hours after feeding to ensure a return to pre-feeding metabolic rate. The experiment was terminated after 34 days and the burned animals sacrificed for body composition analysis.

Chapter 4

Experimental Results

4.1 Experiment 1: Environmental Temperature 20°C and Food Intake 15g.

The body weights and food intakes of the burned and control rats are shown in Figure 9. The food intake values were very similar with a mean of 14.41g for the burned and 14.08g for the control animals over the 57 days of the experiment. The control rats lost about 10g in weight immediately after the mock-burn because of the anaesthetic and low food intake because of pair feeding. From day 4 onwards they grew at 1.6g per day. The burned animals continually lost weight until after about 40 days they achieved a state of weight maintenance. By the end of the experiment they had lost 30% of their starting weight.

Figure 10 shows the partitioned heat loss and the net food energy intake (see section 2.5.1) for the burned animals. The insensible heat loss took 8 days to increase to its maximum. This was probably caused by an increase in the permeability of the eschar, though there was no visible change and the rats did not finally shed their eschars until 20 - 30 days after the burn. Insensible losses remained relatively constant from day 8. Total heat losses after reaching a maximum 8 - 20 days after the injury did decrease as sensible losses decreased. This reflected the reduction in size and therefore surface area in the burned animals as the experiment progressed. The

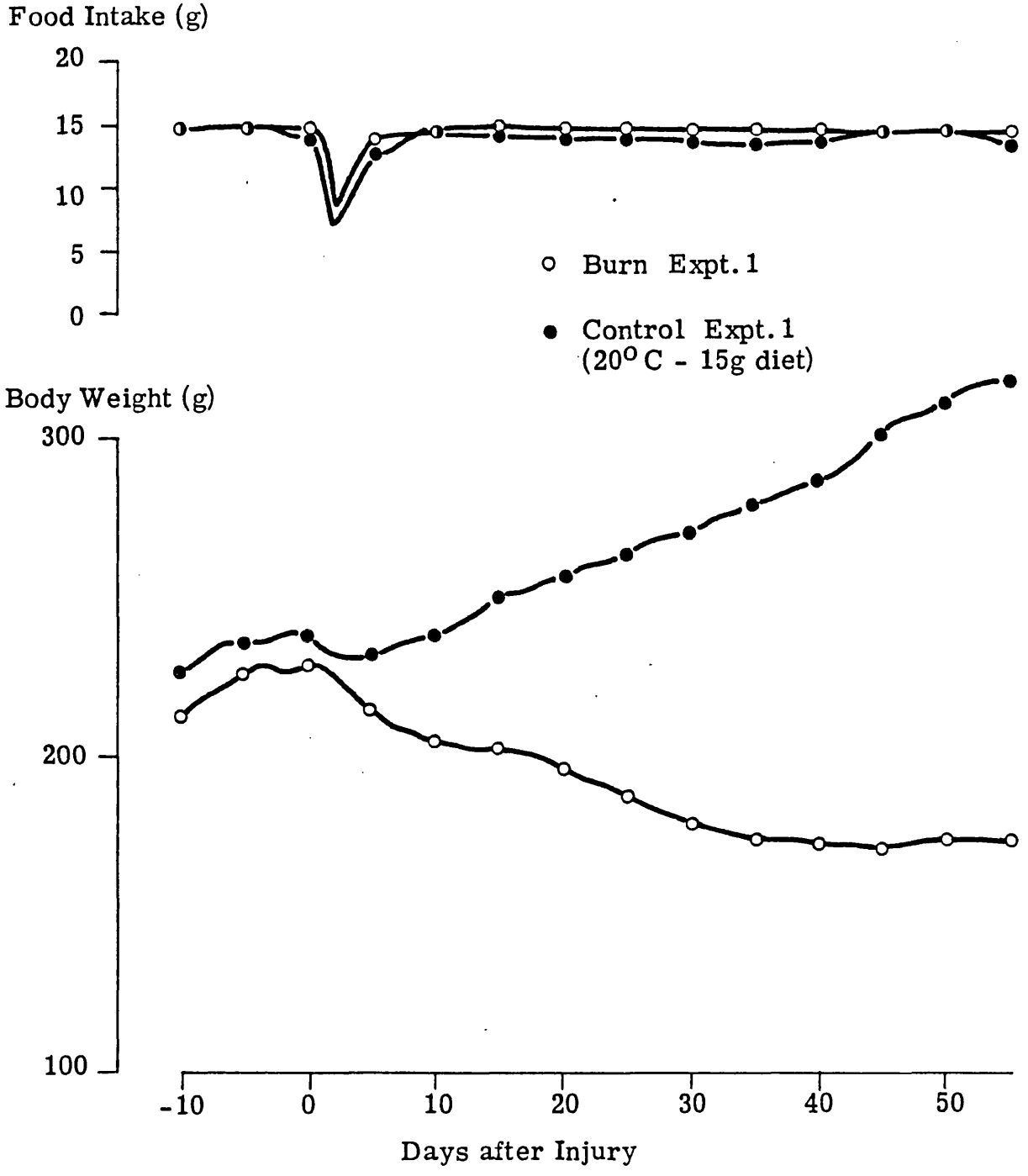


Figure 9. Body weights and food intakes of burned and control animals in Experiment 1.

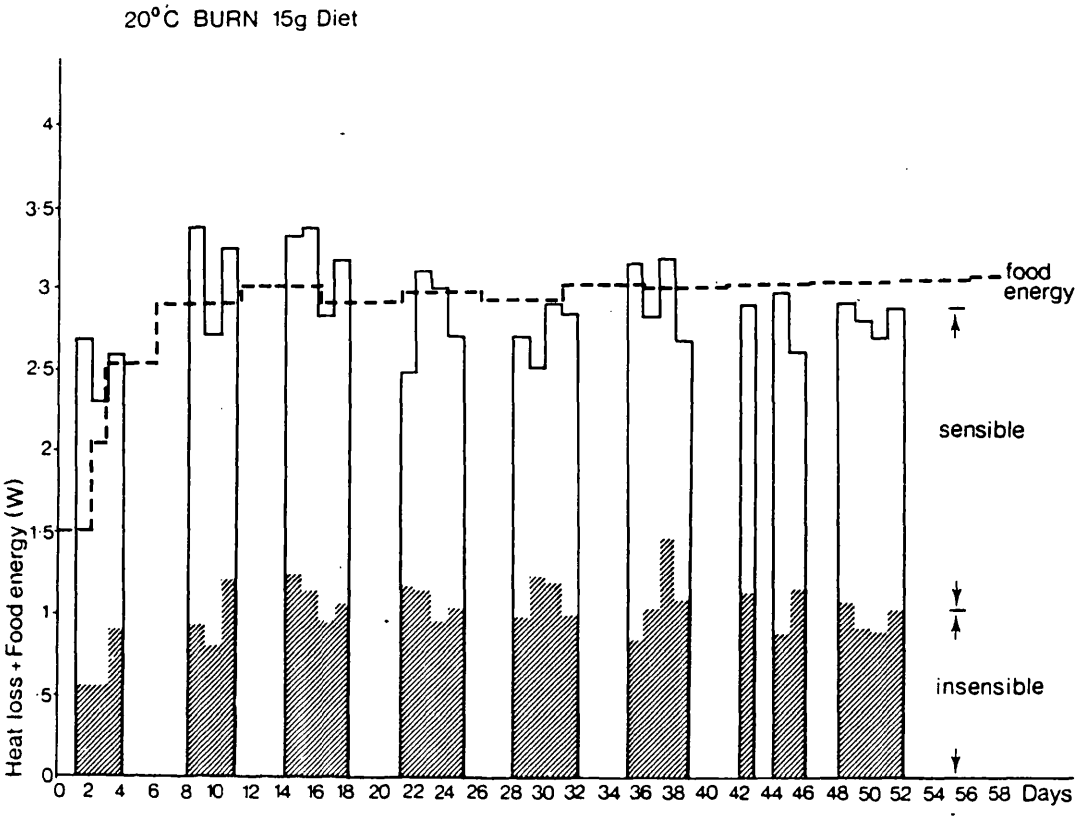


Figure 10. Partitioned heat loss and net food energy intake for the burned animals in Experiment 1.

net food energy line shows that there was a negative energy balance until approximately 40 days post-burn. This agreed with the weight changes, in that the burned animals continually lost weight up to this point. The individual calorimetry results are recorded in Table 5.

The control animals have a different response (Figure 11) with a much smaller insensible heat loss, coming mainly from respiratory water loss. They were in positive energy balance on all the days except 1, 7 and 26, which agreed with the early weight drop and continued growth (Figure 9). The calorimetry values for the individual control animals are recorded in Table 6.

The results of the energy balance calculations by the two techniques (see Sections 2.5.1 and 2.5.2) are shown numerically in Tables 7 and 8 for the burns and controls respectively and graphically in Figure 12. The balance calculated from the body composition changes was below that calculated from calorimetry for both burn and control. The graphs however have a similar shape. By the end of the experiment the difference between the two energy balance calculations was 981 kJ or 6.8% of the net food energy for the burns and 373 kJ or 2.8% of the net food energy for the controls.

4.2 Experiment 2: Environmental Temperature 20°C and Food Intake 18g

The body weights and food intakes of the animals are shown in Figure 13. The average daily food intake over the 51 days was 16.88g for the burned animals and 16.09g

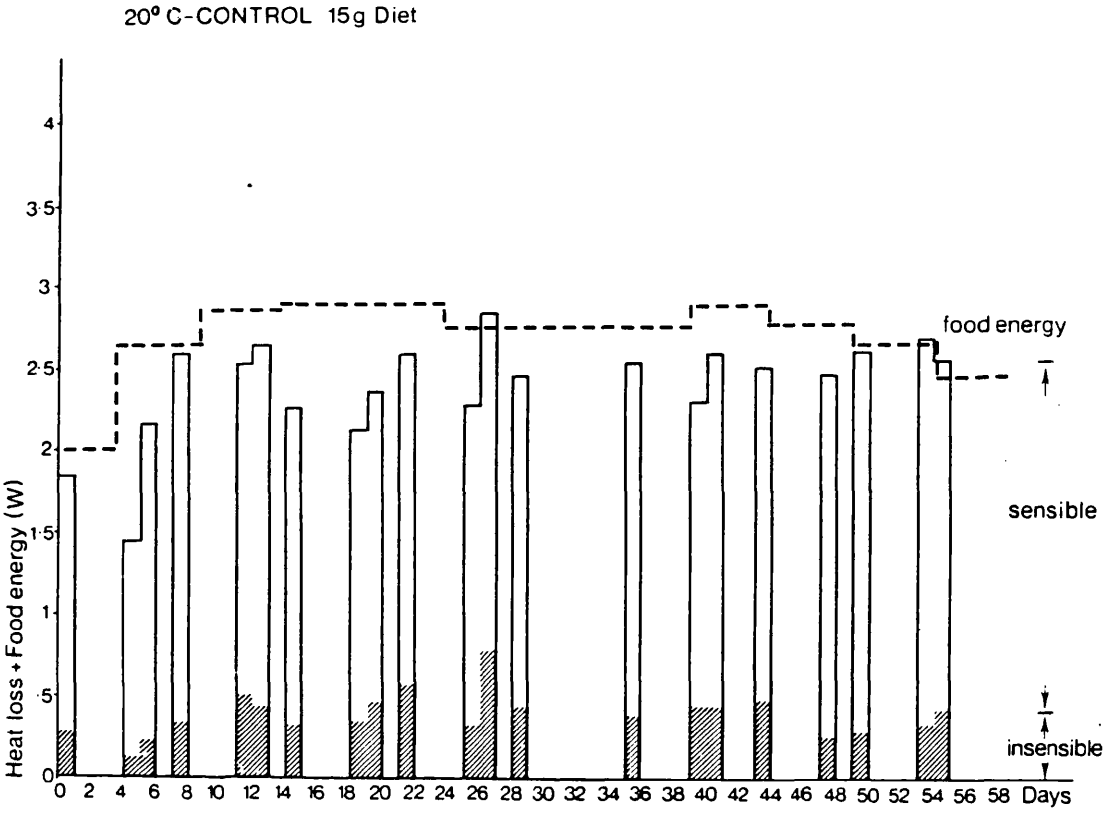


Figure 11. Partitioned heat loss and net food energy intake for the control animals in Experiment 1.

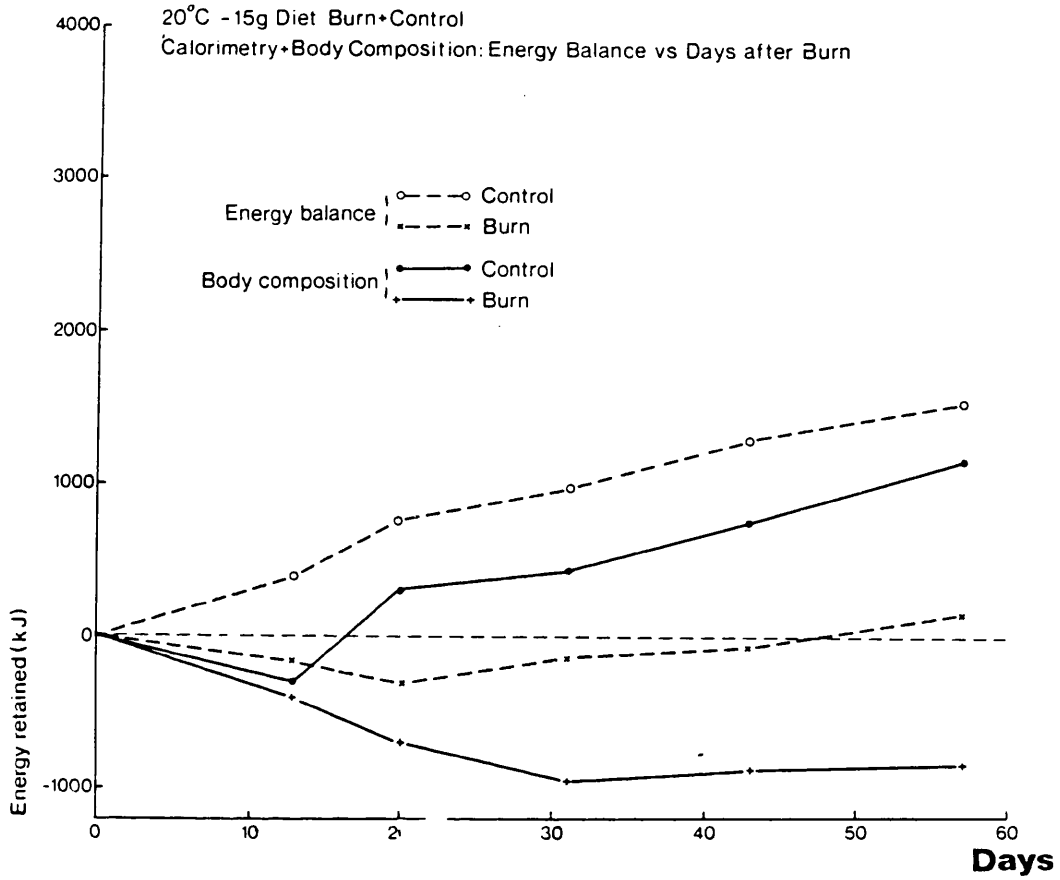


Figure 12. Calorimetry and body composition energy balances for burned and control animals in Experiment 1.

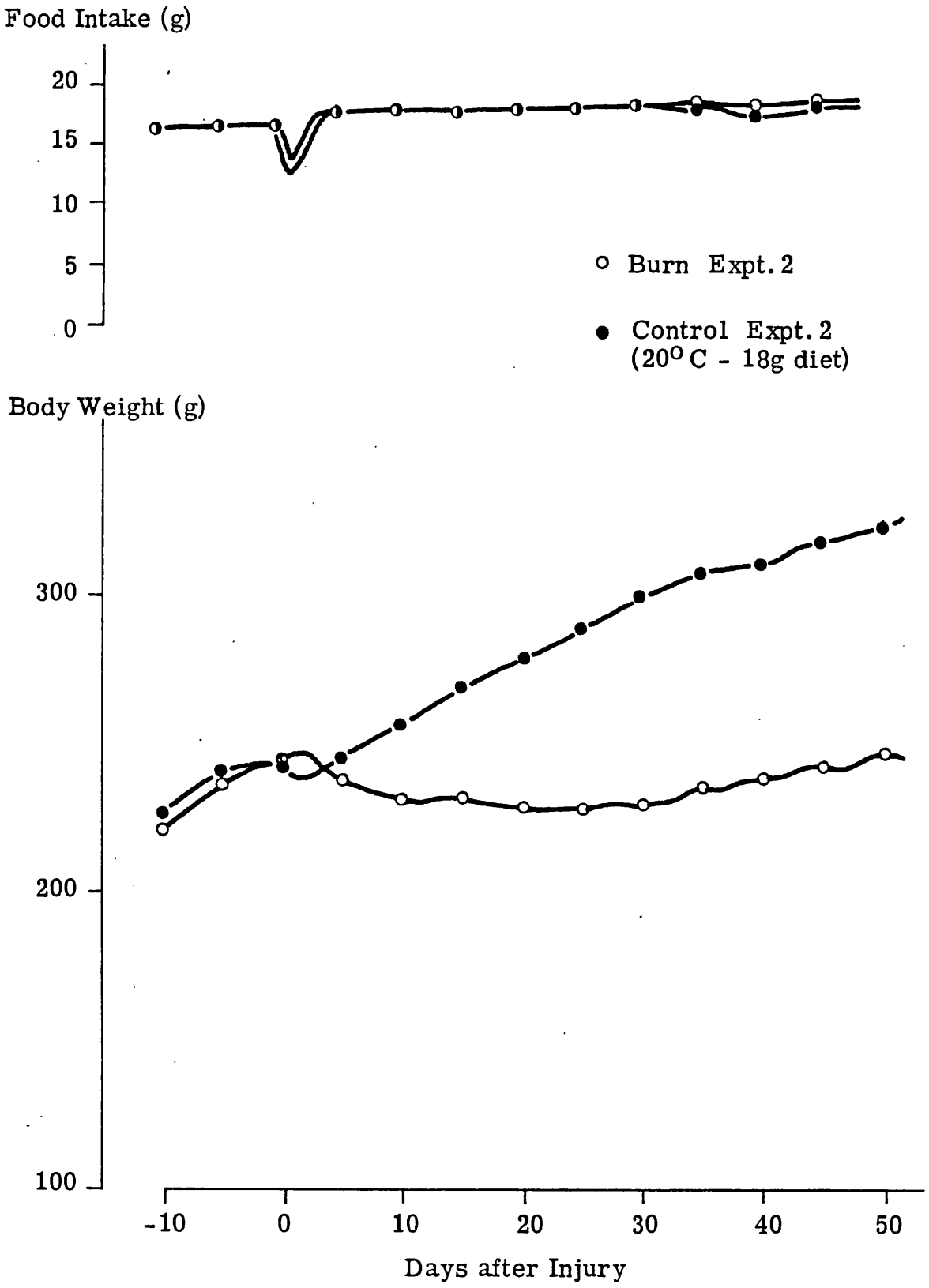


Figure 13. Body weights and food intakes of burned and control animals in Experiment 2.

Days After Burn	Rat Wt.	Insensible Heat Loss	Sensible Heat Loss	Total Heat Loss	Heat Production	RQ
	(g)	(W)	(W)	(W)	(W)	
1	219	0.57	2.12	2.69	2.59	.720
2	227	0.56	1.74	2.30	2.28	.702
3	230	0.92	1.67	2.59	2.34	.722
8	231	0.94	2.43	3.37	3.37	.757
9	182	0.81	1.90	2.71	2.56	.738
10	191	1.20	2.04	3.24	3.04	.756
14	207	1.24	2.07	3.31	2.99	.706
15	214	1.15	2.21	3.36	3.13	.759
16	198	0.97	1.86	2.83	2.57	.733
17	182	1.08	2.08	3.16	3.06	.730
21	162	1.17	1.30	2.47	2.04	.799
22	193	1.15	1.95	3.10	3.00	.729
23	208	0.96	2.04	3.00	2.91	.689
24	187	1.04	1.66	2.70	2.63	.731
28	178	0.99	1.69	2.68	2.61	.694
29	151	1.23	1.27	2.50	2.46	.809
30	178	1.20	1.70	2.90	2.80	.745
31	198	1.00	1.83	2.83	2.74	.717
35	162	0.85	2.29	3.14	2.96	.740
36	169	1.04	1.77	2.81	2.70	.788
37	146	1.46	1.71	3.17	3.02	.787
38	175	1.10	1.56	2.66	2.43	.756
42	191	1.13	1.75	2.88	2.66	.747
44	175	0.88	2.08	2.96	2.87	.703
45	140	1.15	1.45	2.60	2.30	.760
48	188	1.09	1.80	2.89	2.60	.779
49	187	0.92	1.86	2.78	2.61	.733
50	189	0.90	1.78	2.68	2.75	.740
51	190	1.03	1.83	2.86	2.89	.719
n = 29						
mean	187.9	1.03	1.84	2.87	2.72	.741
sem	4.38	0.035	0.050	0.051	0.055	.006

Table 5 Calorimetry results for the burned rats in Experiment 1.

Days After Burn	Rat Weight (g)	Insensible Heat Loss (W)	Sensible Heat Loss (W)	Total Heat Loss (W)	Heat Production (W)	R.Q.
1	236	0.29	1.56	1.85	1.71	.733
4	181	0.13	1.32	1.45	1.11	.686
5	258	0.24	1.93	2.17	2.07	.760
7	231	0.35	2.24	2.59	2.56	.707
11	232	0.51	2.02	2.53	2.39	.689
12	255	0.45	2.20	2.65	2.52	.718
14	200	0.33	1.93	2.26	2.14	.741
18	281	0.35	1.78	2.13	1.84	.751
19	264	0.47	1.89	2.36	2.23	.737
21	257	0.58	2.01	2.59	2.66	.741
25	287	0.33	1.94	2.27	2.29	.709
26	305	0.78	2.05	2.83	2.78	.757
28	275	0.43	2.02	2.45	2.28	.729
35	324	0.38	2.15	2.53	2.31	.772
39	280	0.45	1.84	2.29	2.10	.772
40	295	0.45	2.13	2.58	2.54	.742
43	327	0.49	2.01	2.50	2.33	.721
47	335	0.25	2.20	2.45	2.37	.736
49	336	0.29	2.31	2.60	2.63	.734
n = 19						
mean	271.5	0.40	1.98	2.37	2.26	.733
SEM	10.1	0.033	0.055	0.073	0.089	.006

Table 6 Calorimetry results for the control rats in Experiment 1.

Period (d)	Net Food Energy (kJ)	Calorimetry Heat Loss (kJ)	Calorimetry Energy Balance (kJ)	Body Fat Change (g)	Body Protein Change (g)	Body Tissue Energy Balance (kJ)
1 - 13	2999	3167	-168	-4.84	-9.28	-402
1 - 20	4777	5084	-307	-14.26	-6.65	-709
1 - 31	7571	7717	-145	-17.19	-11.89	-944
1 - 43	10681	10755	-72	-14.26	-13.92	-876
1 - 57	14331	14183	148	-14.70	-11.30	-833

Table 7 Calorimetry and body tissue energy balances for the burned animals in Experiment 1.

Period	Net Food Energy	Calorimetry Heat Loss	Calorimetry Energy Balance	Body Fat Change	Body Protein Change	Body Tissue Energy Balance
(d)	(kJ)	(kJ)	(kJ)	(g)	(g)	(kJ)
1 - 13	2875	2482	393	-7.34	-0.38	-295
1 - 20	4605	3843	762	8.32	-0.62	310
1 - 31	7228	6257	971	8.86	4.02	438
1 - 43	10100	8808	1292	16.05	5.76	759
1 - 57	13297	11770	1527	17.20	21.00	1154

Table 8 Calorimetry and body tissue energy balances for the control animals in Experiment 1.

for the controls. This was an increase of 17.1% (2.47g) over experiment 1 for the burns and 14.3% (2.01g) for the controls. This increase in diet allowed the burned animals to show a small weight gain (10g) over the experiment. The injured animals had a weight loss of 1.0g per day from day 1 to day 20 (8% of initial weight) and a slow weight gain, 0.9 per day, for the remaining period. The controls grew at a faster rate, 2.1g per day, than they did in experiment 1.

The heat loss, and net food energy graph for the burns (Figure 14) shows the animals to be in a positive energy balance from day 7. This was before their return to growth (Figure 13). After day 25 evidence of early marginal wound healing was noticed and this coincided with a gradual decrease in the insensible losses (Table 9). The small reduction in temperature and pressure of the branding head appeared to have permitted early reepithelialisation of the wound. There was no decrease in sensible heat losses. The controls (Figure 15) demonstrated a large positive energy balance after day 1, paralleling their rate of growth. Their individual calorimetry results are recorded in Table 10.

The change in the burn surface area of the injured animals can be estimated from the change in insensible losses from the burn. The measured total insensible losses however include the respiratory water loss and a small amount of passive diffusion of water vapour through the skin. These routes represent the total insensible loss from a control rat and for the control animals in this

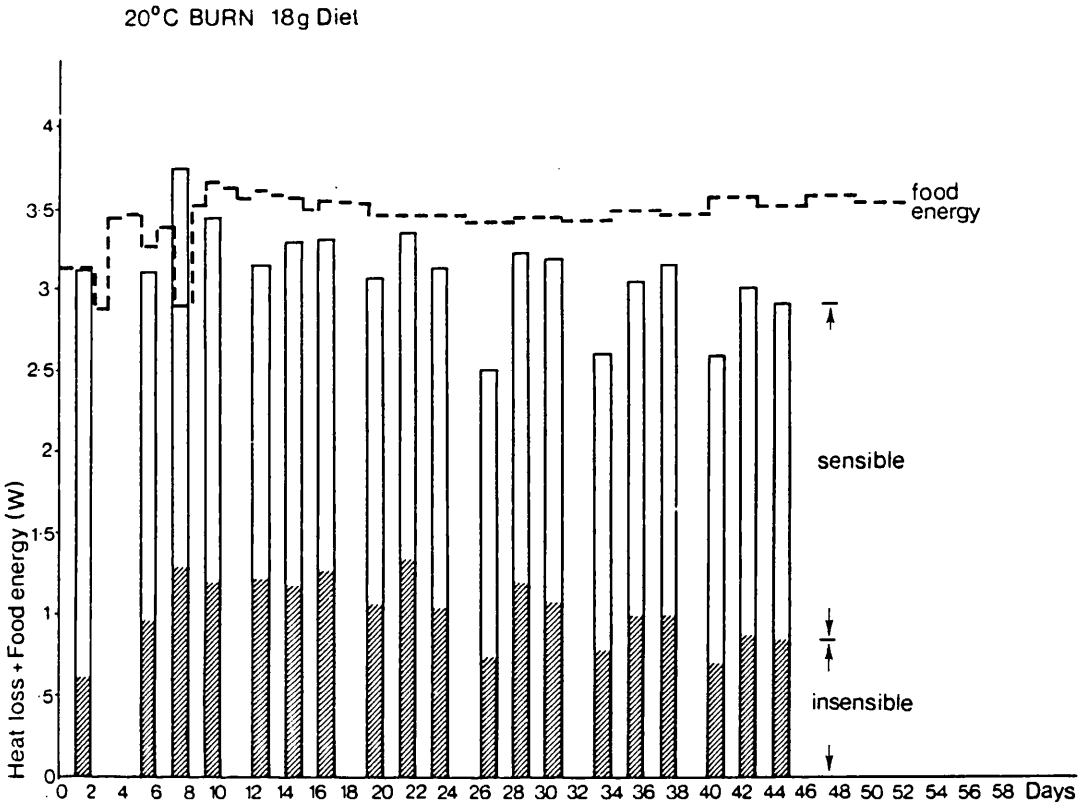


Figure 14. Partitioned heat loss and net food energy intake for the burned animals in Experiment 2.

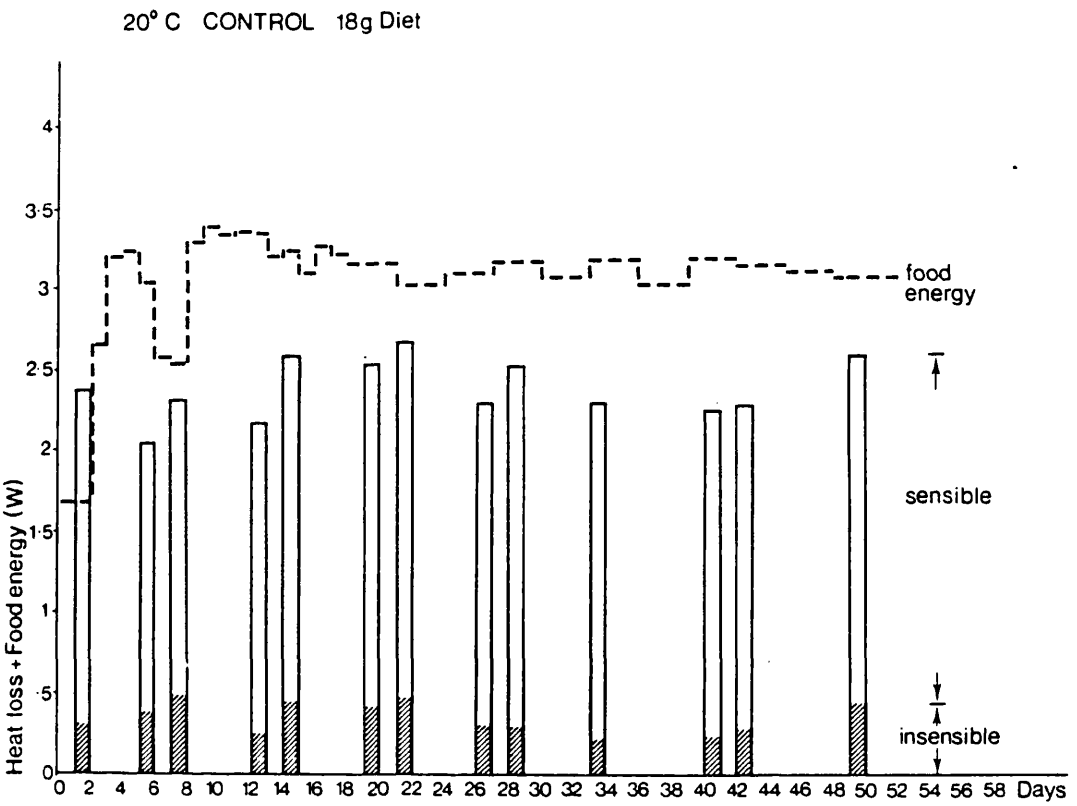


Figure 15. Partitioned heat loss and net food energy intake for the control animals in Experiment 2.

Days After Burn	Rat Weight	Insensible Heat Loss	Sensible Heat Loss	Total Heat Loss	Heat Production	RQ
	(g)	(W)	(W)	(W)	(W)	
1	268	0.60	2.50	3.10	3.22	.713
5	220	0.95	2.14	3.09	2.94	.689
7	239	1.28	2.45	3.73	3.33	.750
9	243	1.18	2.24	3.42	3.34	.735
12	218	1.20	1.93	3.13	3.08	.788
14	235	1.16	2.11	3.27	3.18	.732
16	247	1.25	2.04	3.29	3.06	.745
19	214	1.05	2.00	3.05	2.90	.756
21	229	1.33	1.99	3.32	3.13	.729
23	248	1.02	2.09	3.11	2.95	.737
26	202	0.74	1.75	2.49	2.49	.688
28	233	1.18	2.02	3.20	3.16	.730
30	245	1.06	2.10	3.16	3.02	.708
33	211	0.77	2.21	2.58	2.56	.680
35	233	0.98	2.04	3.02	3.08	.765
37	243	0.98	2.15	3.13	3.03	.702
40	224	0.69	1.88	2.57	2.61	.649
42	254	0.86	2.12	2.98	3.27	.699
44	248	0.83	2.05	2.88	2.65	.696
n = 19						
mean	234.4	1.01	2.10	3.08	3.00	.721
sem	3.8	0.049	0.040	0.069	0.059	.008

Table 9 Calorimetry results for the burned rats in Experiment 2.

Days After Burn	Rat Weight	Insensible Heat Loss	Sensible Heat Loss	Total Heat Loss	Heat Production	RQ
	(g)	(W)	(W)	(W)	(W)	
1	254	0.31	2.06	2.37	2.29	.785
5	232	0.38	1.66	2.04	1.95	.702
7	270	0.48	1.83	2.31	2.38	.707
12	245	0.25	1.95	2.17	2.20	.715
14	299	0.45	2.13	2.58	2.52	.765
19	266	0.42	2.10	2.52	2.59	.733
21	328	0.47	2.19	2.66	2.64	.739
26	272	0.30	1.99	2.29	2.38	.717
28	340	0.30	2.21	2.51	2.56	.725
33	284	0.22	2.05	2.27	2.33	.751
40	299	0.23	2.01	2.24	2.32	.703
42	356	0.28	1.99	2.27	2.06	.681
49	379	0.44	2.14	2.58	2.47	.815
n = 13						
mean	294.0	0.35	2.02	2.37	2.36	.734
sem	12.4	0.026	0.042	0.051	0.057	.010

Table 10 Calorimetry results for the control rats in Experiment 2.

experiment insensible losses were on average 14.8% of total heat loss. Assuming that the burned animals were ventilating normally, their respiratory water loss was calculated as 14.8% of their total heat loss. The water loss from the burn was then the difference between the measured loss and this estimate of respiratory loss. The average values of burn wound insensible loss are shown in Table 11. Assuming the wound to be unhealed for days 7 - 21, the average burn wound insensible loss (0.72W) over over this period has been taken to represent the loss from a 25% BSA burn. The effective burn area by the end of the experiment was only 14% of BSA.

The calorimetry and body tissue energy balance calculations (Tables 12 and 13) showed both burns and controls retaining more energy when the calculation used the calorimetry results rather than the body composition results. The difference between the two methods by the end of the experiment was 10.3% and 13.2% of food energy for the burns and controls respectively. The shapes of the energy balance curves (Figure 16) calculated by the two methods, were similar.

4.3 Experiment 3: Environmental Temperature 30°C and Food Intake 15g

Both burned and control animals put on more weight (Figure 17) than the animals in experiments 1 and 2. The mean food intakes were 13.93g for the burns and 13.65g for the controls, both being 97% of the burn and control intakes in experiment 1.

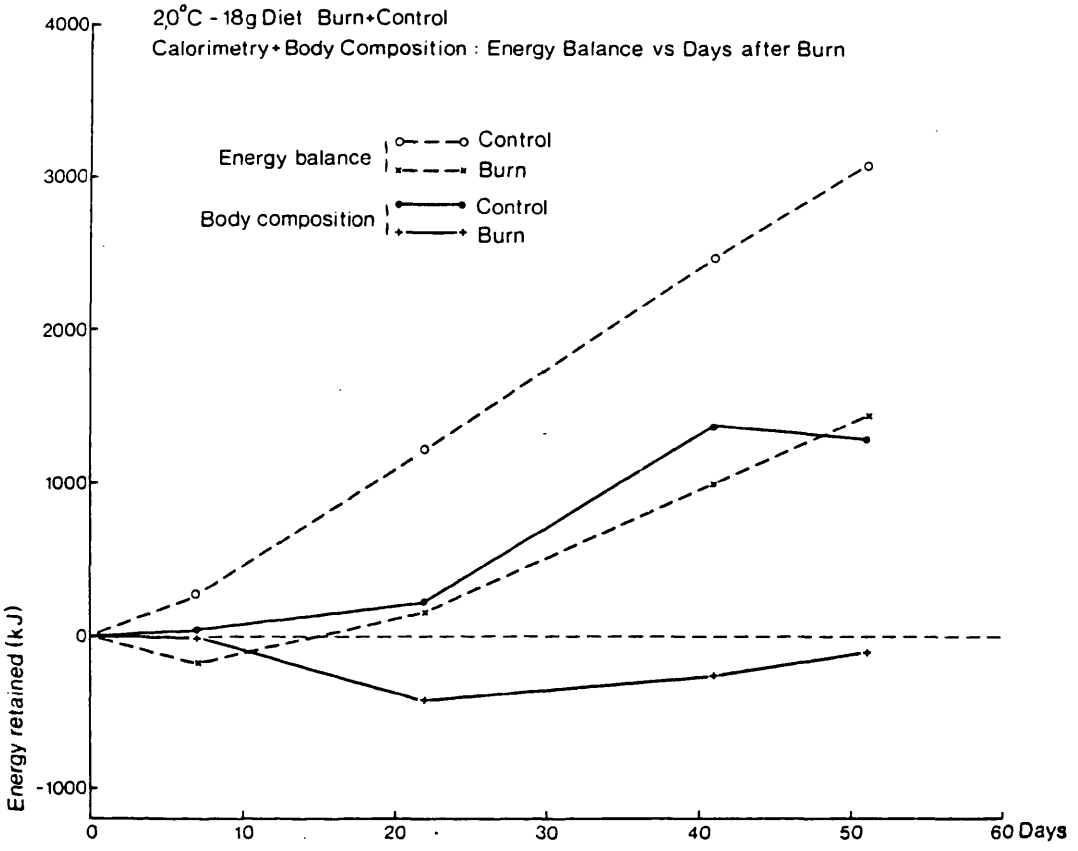


Figure 16. Calorimetry and body composition energy balances for burned and control animals in Experiment 2.

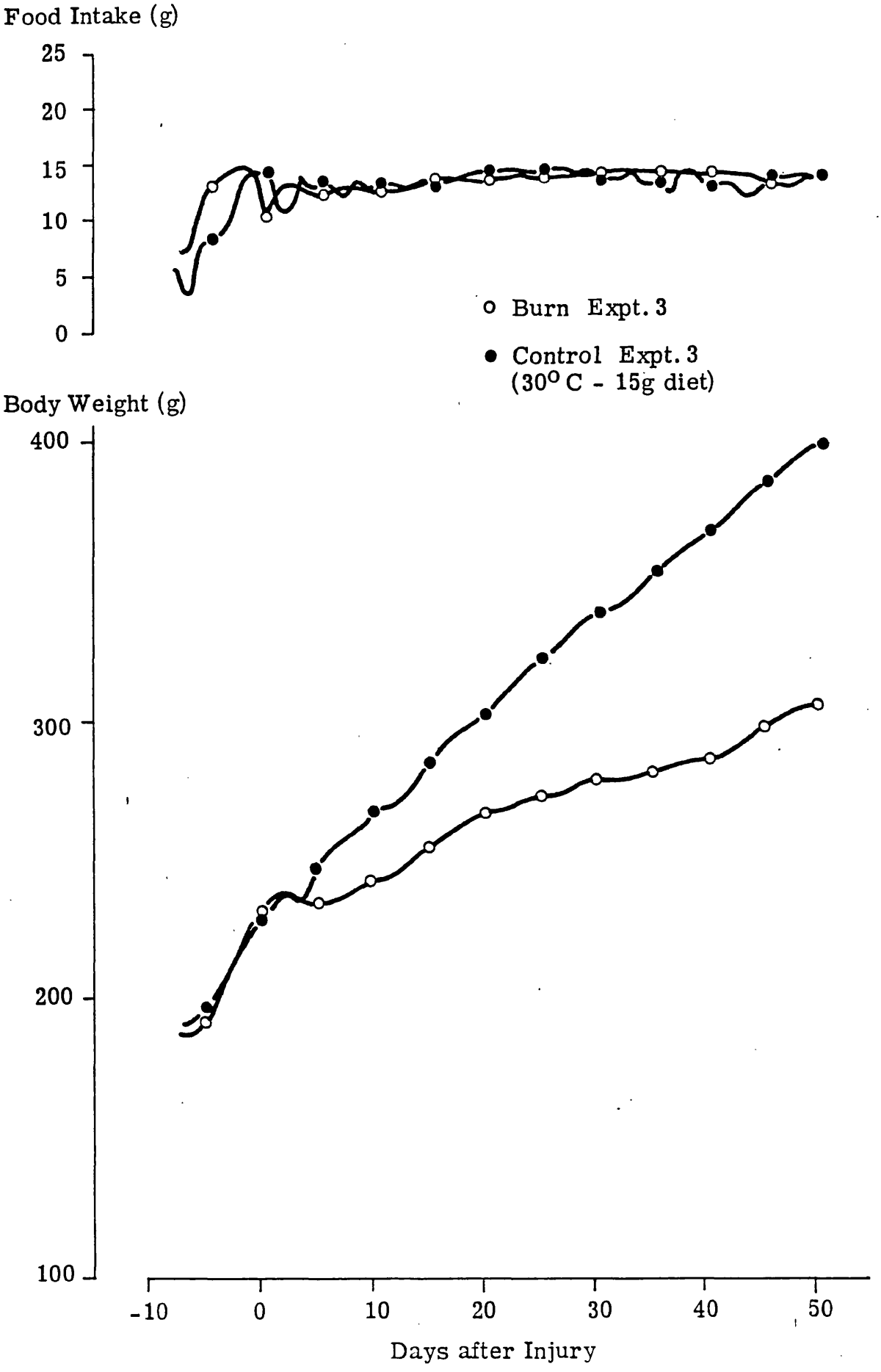


Figure 17. Body weights and food intakes of burned and control animals in Experiment 3.

Days After Burn (d)	Total Heat Loss (W)	Estimated Respiratory Loss + (W)	Total Insensible Loss (W)	Burn Wound Insensible Loss (W)	Effective BSA % * (%)
1	3.10	0.46	0.60	0.14	4.9
5	3.09	0.46	0.95	0.49	17.0
7	3.73	0.55	1.28	0.73	25.3
9	3.42	0.51	1.18	0.67	23.3
12	3.13	0.46	1.20	0.74	25.7
14	3.27	0.48	1.16	0.68	23.6
16	3.29	0.49	1.25	0.76	26.4
19	3.05	0.45	1.05	0.60	20.8
21	3.32	0.49	1.33	0.84	29.2
23	3.11	0.46	1.02	0.56	19.4
26	2.49	0.37	0.74	0.37	12.8
28	3.20	0.47	1.18	0.71	24.7
30	3.16	0.47	1.06	0.59	20.5
33	2.58	0.38	0.77	0.39	13.5
35	3.02	0.45	0.98	0.53	18.4
37	3.13	0.46	0.98	0.52	18.1
40	2.57	0.38	0.69	0.31	10.8
42	2.98	0.44	0.86	0.42	14.6
44	2.88	0.43	0.83	0.40	13.9

+ - 14.8% of total heat loss

* - the average insensible loss (0.72W) over days 7 - 21 inclusive has been taken as the loss of a 25% BSA burn.

Table 11 The effective burn wound % BSA in Experiment 2.

Period	Net Food Energy	Calorimetry Heat Loss	Calorimetry Energy Balance	Body Fat Change	Body Protein Change	Body Tissue Energy Balance
(d)	(kJ)	(kJ)	(kJ)	(g)	(g)	(kJ)
1 - 7	1814	2002	-187	1.83	-3.12	0
1 - 22	6363	6214	150	-7.34	-5.55	-414
1 - 41	11994	10991	1004	-3.66	-4.69	-251
1 - 51	15026	13572	1454	-2.80	-0.80	-91

Table 12 Calorimetry and body tissue energy balances for the burned animals in Experiment 2.

Period	Net Food Energy	Calorimetry Heat Loss	Calorimetry Energy Balance	Body Fat Change	Body Protein Change	Body Tissue Energy Balance
(d)	(kJ)	(kJ)	(kJ)	(g)	(g)	(kJ)
1 - 7	1633	1355	278	1.03	-0.08	38
1 - 22	5793	4569	1224	2.23	5.71	218
1 - 44	10882	8394	2488	27.58	13.53	1387
1 - 51	13528	10443	3084	21.70	19.60	1297

Table 13 Calorimetry and body tissue energy balances for the control animals in Experiment 2.

The burned animals were in positive energy balance (Figure 18) throughout the experiment, agreeing with the animals continuous weight growth of 1.8g per day. The mean total heat loss (Table 14) increased slightly (0.07 W) in the second half of the experiment as the rats grew. The almost constant burn insensible losses, at 2.5 times control losses, indicated a non-healing wound. The controls were in a greater positive energy balance (Figure 19) than the burns and maintained a growth rate of 3.7g per day. Their individual calorimetry results are recorded in Table 15.

The energy retention calculated from calorimetry values (Tables 16 and 17) again was greater than that calculated from body tissue changes. The difference between the two methods of calculation was 20.4 and 12.3% of the net food energy intake for burns and controls. Allowing for this systematic error, the shapes of the curves (Figure 20) were similar.

4.4 Experiment 4: Dietary Thermogenesis at 20°C and 30°C

The measurements of dietary induced thermogenesis (DIT) at 20°C were on animals treated in a similar manner to experiment 1 (i.e. 25% BSA injury, 15g diet offered etc.). The weight graphs for the two sets of burned animals from experiment 1 and 4 are compared in Figure 21. The animals had similar food intakes and weight losses (49g in experiment 1 and 46g in experiment 4) demonstrating the reproducibility of the experimental injury and environmental conditions. The DIT measurements at 30°C were made

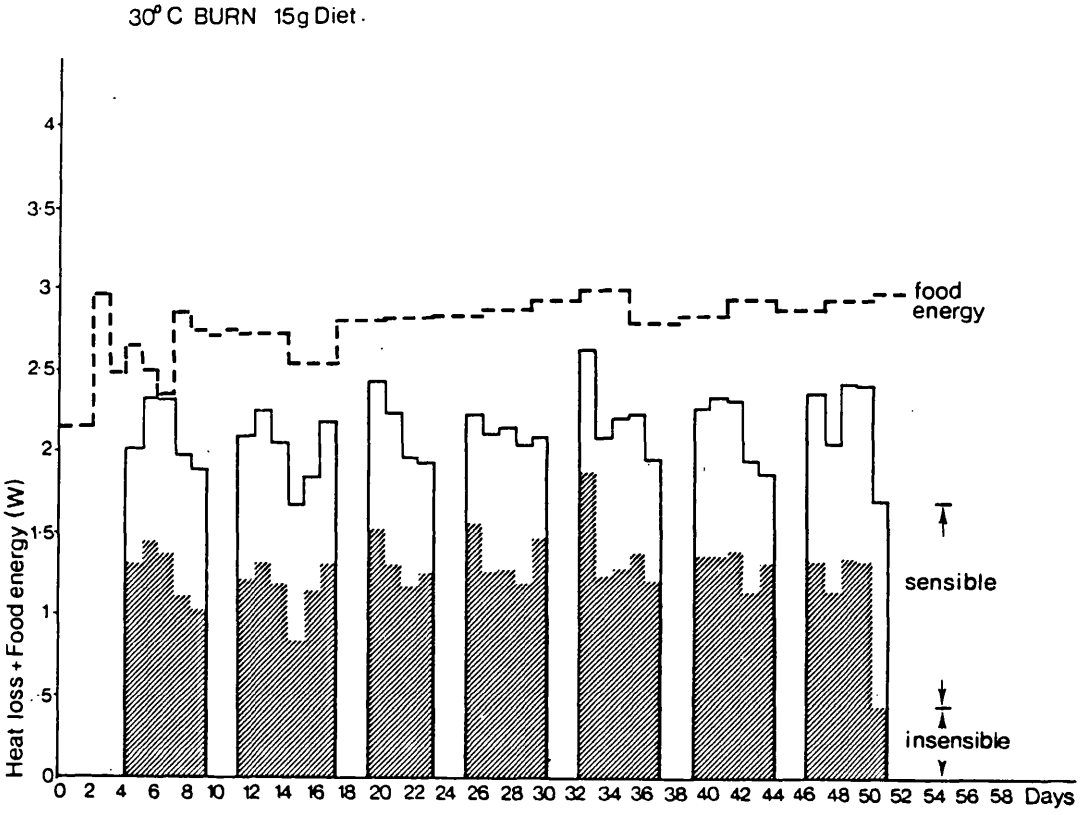


Figure 18. Partitioned heat loss and net food energy intake for the burned animals in Experiment 3.

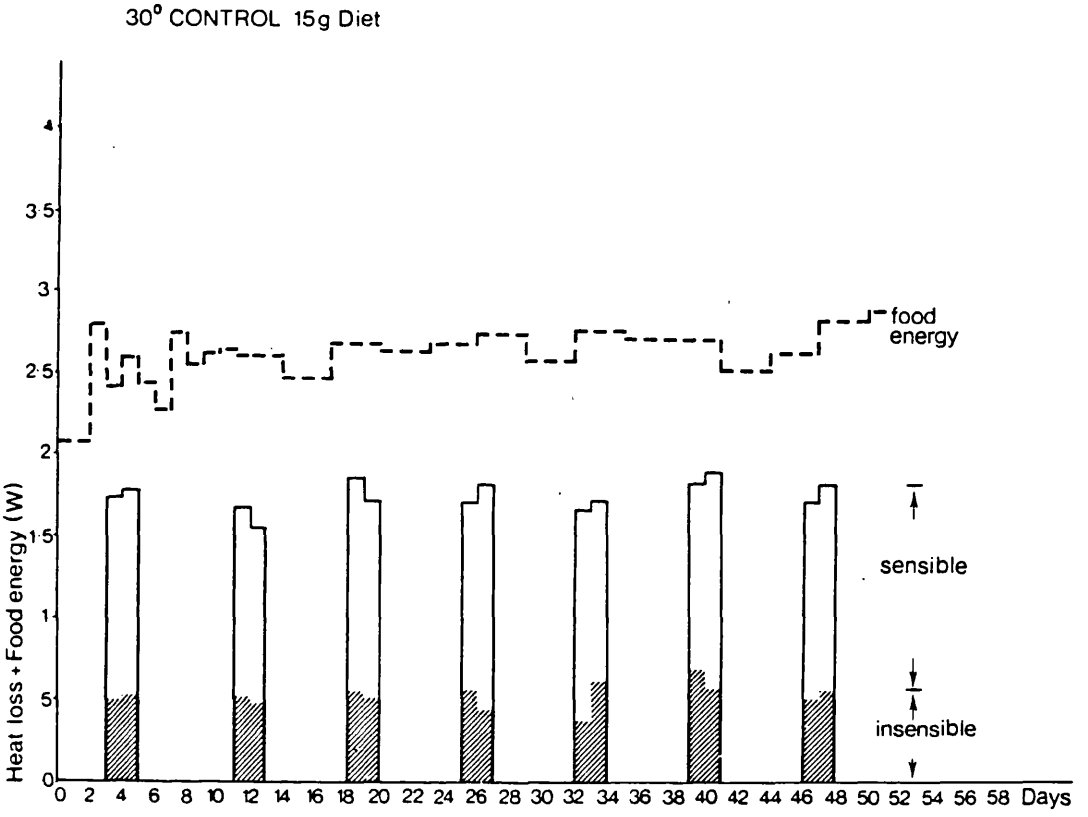


Figure 19. Partitioned heat loss and net food energy intake for the control animals in Experiment 3.

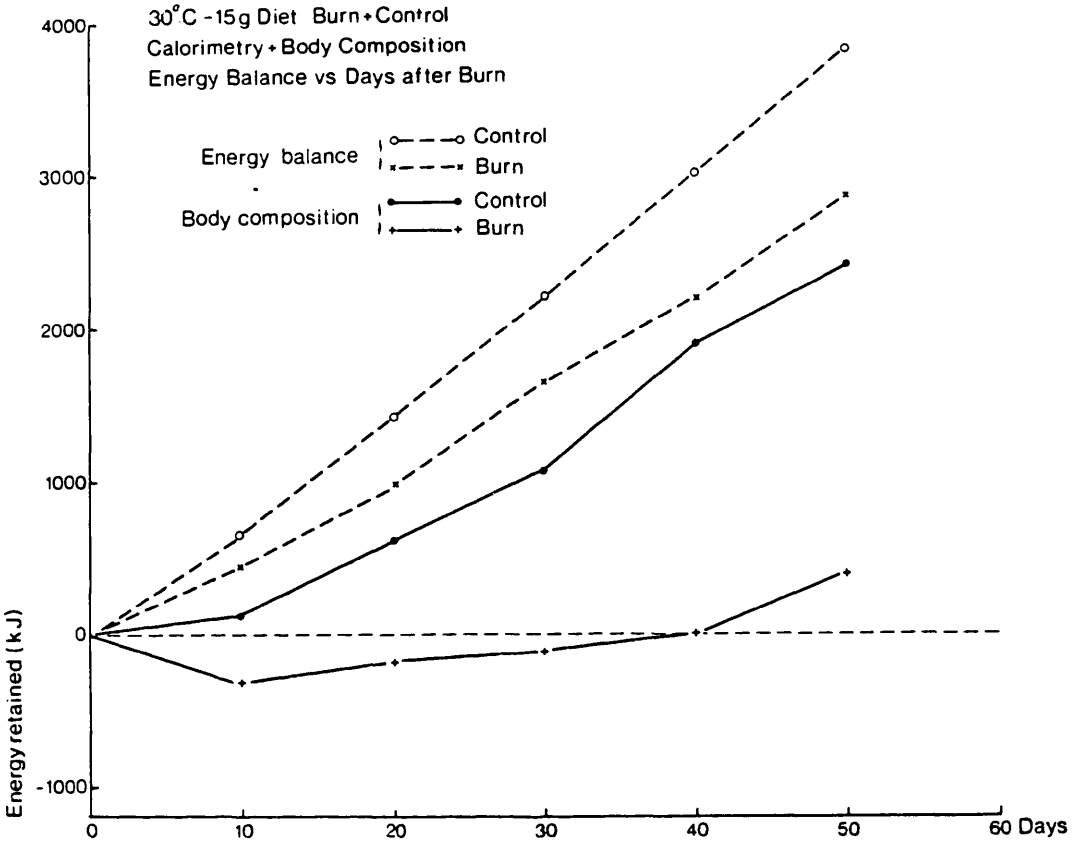


Figure 20. Calorimetry and body composition energy balances for burned and control animals in Experiment 3.

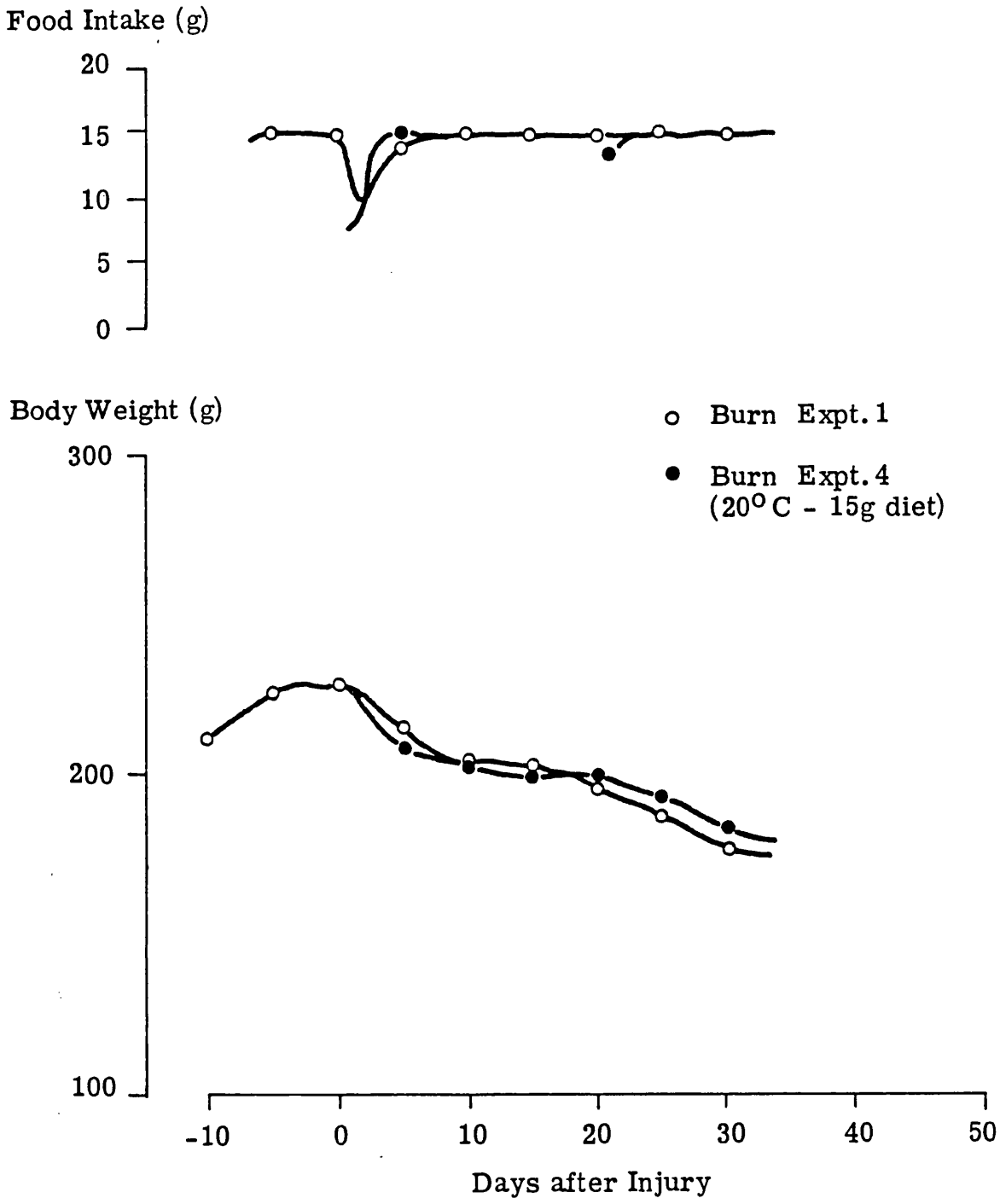


Figure 21. Comparison of the body weights and food intakes of the burned animals from Experiments 1 and 4.

Days After Burn	Rat Wt.	Insensible Heat Loss	Sensible Heat Loss	Total Heat Loss	Heat Production	RQ
	(g)	(W)	(W)	(W)	(W)	
4	251	1.32	0.68	2.00	1.83	.716
5	228	1.45	0.87	2.32	2.20	.775
7	254	1.36	0.95	2.31	2.34	.753
7	234	1.11	0.86	1.96	1.95	.726
8	217	1.02	0.86	1.88	1.79	.702
11	249	1.22	0.86	2.07	2.11	.746
12	267	1.32	0.91	2.23	2.33	.741
12	237	1.18	0.85	2.03	2.00	.736
14	231	0.84	0.82	1.66	1.71	.746
15	260	1.14	0.69	1.83	1.91	.770
16	259	1.31	0.85	2.16	2.27	.745
19	283	1.52	0.89	2.41	2.41	.722
19	264	1.30	0.92	2.22	2.10	.878
21	258	1.17	0.77	1.94	2.69	.800
22	272	1.26	0.66	1.92	1.90	.770
25	277	1.57	0.61	2.18	2.18	.791
26	295	1.26	0.82	2.09	2.26	.770
26	266	1.27	0.86	2.13	2.25	.768
28	267	1.19	0.83	2.02	2.11	.749
29	278	1.47	0.59	2.06	2.05	.781
32	278	1.87	0.73	2.60	2.66	.760
33	305	1.23	0.84	2.07	2.25	.766
33	275	1.28	0.91	2.19	2.13	.778
35	271	1.38	0.84	2.22	2.11	.809
36	289	1.20	0.73	1.93	1.60	.771
39	292	1.35	0.90	2.24	2.14	.779
40	322	1.35	0.96	2.31	2.01	.793
40	288	1.38	0.91	2.29	2.24	.729
42	279	1.14	0.79	1.92	1.86	.798
43	277	1.32	0.53	1.85	1.79	.821
46	312	1.32	0.01	2.34	2.21	.736
47	337	1.14	0.89	2.03	2.07	.775
47	308	1.34	1.06	2.40	2.46	.715
49	290	1.33	1.06	2.39	2.04	.758
50	373	1.25	0.44	1.68	1.68	.808
n = 35						
mean	276	1.29	0.82	2.11	2.09	.765
sem	5.3	0.029	0.023	0.036	0.039	.006

Table 14 Calorimetry results for the burned rats in Experiment 3.

Days After Injury	Weight	Insensible Heat Loss	Sensible Heat Loss	Total Heat Loss	Heat Production	Respiratory Quotient
(d)	(g)	(W)	(W)	(W)	(W)	
2	250	0.50	1.23	1.72	1.72	0.756
4	253	0.51	1.25	1.77	1.56	0.764
11	284	0.51	1.16	1.67	1.67	0.776
12	289	0.47	1.07	1.54	1.60	0.760
18	300	0.54	1.30	1.84	1.80	0.711
19	320	0.51	1.19	1.70	1.67	0.745
25	328	0.55	1.14	1.69	1.73	0.747
26	344	0.44	1.37	1.81	1.86	0.801
32	337	0.38	1.28	1.66	1.65	0.887
33	360	0.61	1.11	1.72	1.60	0.788
39	353	0.68	1.14	1.82	1.65	0.855
40	381	0.57	1.32	1.89	1.72	0.734
46	368	0.50	1.20	1.70	1.69	0.722
47	406	0.56	1.25	1.81	1.85	0.885
n = 14						
mean	327	0.52	1.22	1.74	1.70	0.781
sem	12.5	0.019	0.023	0.024	0.024	0.015

Table 15 Individual calorimetry results for the control animals in Experiment 3.

Period	Net Food Energy	Calorimetry Heat Loss	Calorimetry Energy Balance	Body Fat Change	Body Protein Change	Body Tissue Energy Balance
(d)	(kJ)	(kJ)	(kJ)	(g)	(g)	(kJ)
1 - 10	2246	1806	440	-6.28	-3.18	-318
1 - 20	4570	3603	967	-4.27	-0.85	-186
1 - 30	7024	5374	1650	-2.27	-1.06	-113
1 - 40	9486	7301	2185	0.83	-1.36	1
1 - 50	11966	9115	2851	6.30	7.20	411

Table 16 Calorimetry and body tissue energy balances for the burned animals in Experiment 3.

Period	Net Food Energy	Calorimetry Heat Loss	Calorimetry Energy Balance	Body Fat Change	Body Protein Change	Body Tissue Energy Balance
(d)	(kJ)	(kJ)	(kJ)	(g)	(g)	(kJ)
1 - 10	2160	1503	657	1.81	2.54	129
1 - 20	4389	2963	1426	10.41	9.07	615
1 - 30	6679	4475	2204	16.00	19.72	1078
1 - 40	9012	6004	3008	35.25	22.46	1892
1 - 50	11318	7517	3802	45.20	28.16	2410

Table 17 Calorimetry and body tissue energy balances for the control animals in Experiment 3.

on animals during experiment 3 but are reported here for ease of comparison.

The calculation of DIT for the animals at 20°C used the 60 minute period before the food was introduced (i.e. the post-absorptive period used in experiments 1 to 3) as a baseline for metabolic rate and respiration quotient. The food was introduced and any increase in heat production over the baseline integrated until the animal returned close to the initial values. This usually took 5 to 6 hours. The total energy increment was expressed per gram of food eaten to eliminate the effect of the different meal sizes which ranged from 1.2 to 4.8g.

The situation was slightly different for the animals at 30°C from experiment 3, where because of the other demands on the calorimeter the response to the food could only be followed for three hours after its introduction. Therefore the heat increment per gram of diet three hours after the meal was compared at 20 and 30°C (Tables 18 and 19). Neither the burns (0.154 and 0.370 kJ/g) nor the controls (0.732 and 0.748 kJ/g) at 20 and 30°C respectively showed any significant differences, To estimate the total heat increment of the animals at 30°C, the difference between the heat increment at three hours and the return to baseline for the animals at 20°C (i.e. 0.115 and 0.540 kJ/g for burn and control respectively) was added to the results at three hours for the rats at 30°C.

Expressing the DIT as a percentage of gross dietary energy:-

Control Run No.	Food Eaten (g)	3 Hrs Increase in RQ	3 Hrs Increase in Heat Production (kJ/g)	Total Increase in Heat Production (kJ/g)	Increase in Heat Production after 3 Hrs (kJ/g)
1	1.60	0.160	0.332		
2	2.45	0.219	0.886		
3	2.15	0.256	- 0.212		
4	2.25	0.265	0.718	1.045	0.327
5	2.90	0.251	0.936	1.072	0.136
6	3.07	0.246	1.230	1.629	0.399
7	3.27	0.165	1.094	1.454	0.360
8	2.20	0.241	1.172	2.028	0.856
9	3.25	0.193	0.543	1.607	0.064
10	3.30	0.208	0.617	1.252	0.635
mean	2.64	0.220	0.732	1.441	0.540
s.d.	0.59	0.038	0.439	0.350	0.328
Burn Run No.	(g)				
1	3.30	0.169	0.290	0.583	0.293
2	3.30	0.172	0.374	0.371	- 0.003
3	3.35	0.161	0.078	0.476	0.398
4	3.30	0.136	- 0.072	- 0.009	0.063
5	3.30	0.148	0.143	0.148	0.005
6	3.30	0.130	0.139	0.164	0.025
7	3.30	0.148	0.123	0.148	0.025
mean	3.31	0.152	0.154	0.269	0.115
s.d.	0.02	0.016	0.144	0.212	0.162

Table 18 Thermogenic response to oral food in burned and control animals at 20°C in Experiment 4.

Control Run No.	Food Eaten (g)	3 Hr Increase in RQ	3 Hr Increase in Heat Production (kJ/g)
4	4.04	0.100	0.843
11	4.82	0.218	0.942
18	3.24	0.206	0.674
25	3.00	0.137	0.650
32	3.00	0.091	0.452
46	3.00	0.085	0.946
mean	3.52	0.140	0.748
s.d.	0.75	0.059	0.191

Burn Run No.	Food Eaten (g)	3 Hr Increase in RQ	3 Hr Increase in Heat Production (kJ/g)
3	1.75	0.068	0.058
7	2.13	0.083	0.374
8	2.19	0.105	0.172
14	2.00	0.081	0.140
15	1.21	0.103	0.401
21	1.56	0.071	0.479
22	1.56	0.049	0.782
36	2.40	0.066	0.459
42	1.65	0.117	0.707
43	2.30	0.138	- 0.063
49	3.00	0.078	0.560
mean	1.98	0.087	0.370
s.d.	0.50	0.026	0.268

Table 19 Thermogenic response to oral food in burned and control animals at 30°C in Experiment 3.

	Weight (g)	Insensible Heat Loss (W)	Sensible Heat Loss (W)	Total Heat Loss (W)	Heat Production (W)
Expt. 1					
20°C/15g					
Burn	187.9	1.03	1.84	2.87	2.72
Control	271.5	0.40	1.98	2.37	2.26
Expt. 2					
20°C/18g					
Burn	234.4	1.01	2.10	3.08	3.00
Control	294.0	0.35	2.02	2.37	2.36
Expt. 3					
30°C/15g					
Burn	276.0	1.29	0.82	2.11	2.09
Control	327.0	0.52	1.22	1.74	1.70

Mean absolute calorimetry values from experiments 1, 2 and 3 extracted from Tables 5, 6, 9, 10, 14 and 15 to allow comparison of the results.

$20^{\circ}C$

Burns Heat Increment = 0.154 + 0.115 = 0.269 kJ/g = 1.40% 4.1

Controls Heat " = 0.732 + 0.540 = 1.272 kJ/g = 6.64% 4.2

$30^{\circ}C$

Burns Heat Increment = 0.370 + 0.115 = 0.485 kJ/g = 2.53% 4.3

Controls Heat " = 0.748 + 0.540 = 1.288 kJ/g = 6.73% 4.4

The DIT results for burns and controls were significantly different ($p < .01$) at both ambient temperatures. The increase in RQ over the first three hours was also significantly greater for the controls at 20 and $30^{\circ}C$ ($p < .001$ and $p < .05$ respectively) than the burns. These values of DIT have been used in calculating the net food energy available (see Section 2.5.1).

The mean weights and absolute calorimetry values from experiments 1 to 3 have been collected together and presented on the facing page to facilitate comparison of the results during the discussion (Chapter 5). The burn and control results are respectively from Tables 5 and 6 for experiment 1 ($20^{\circ}C$/15g diet), tables 9 and 10 for experiment 2 ($20^{\circ}C$/18g diet) and tables 14 and 15 for experiment 3 ($30^{\circ}C$/15g diet).

Chapter 5

Animal Experiments Discussion

5.1 Direct and Indirect Calorimetry Results

The mean heat production in experiments 1 to 3 varied between 94.8% and 99.6% of heat loss. This is a similar finding to Caldwell et al. (1966) who found heat production to be 97.7% to 100% of heat loss. These results are generally inferior to those of Carter (1975), though his experiments were on normal rats with careful selection of the calorimetry period to exclude micturation etc. This was not possible in an experiment requiring regular calorimetry measurements.

If the percentage of total energy being produced from protein was markedly different from the 12.5% assumed in the heat production formula used (Weir, 1949), this could cause a systematic error. Each extra 12.5% of heat produced from protein would decrease the calculated heat production by 1%. The values in Table 20 show the percentage to vary from 9.6 to 13.3% producing a negligible error. The most probable explanation of the discrepancy is that there was a small body heat content loss during the 60 minute calorimetry period. Using the worst case, (i.e. the burned animals in experiment 1) this would mean a decrease in mean body temperature of $0.6^{o}C$. For the burned animals in the less stressful $30^{o}C$ environment of experiment 3 the body temperature would only have needed to drop $0.06^{o}C$.

The RQs measured range from 0.721 to 0.781 and were within the expected post-absorptive range for the rat. Caldwell et al. (1966) reported a similar range (0.704 - 0.794) for post-absorptive burn and control rats. The RQ in experiment 3 was significantly higher, for both burn and control groups, than in experiment 1 ($p < .01$). This agreed with the higher percentage of protein being used to provide energy in experiment 3 (Table 20).

Comparison of heat loss in animals that differ in weight requires the use of an appropriate correction term. Energy expenditure increases as the weight of an animal increases but at a lower rate than the increase in weight (Kleiber, 1961). Caldwell et al. (1966) used a per kg correction and as the controls grew their heat losses per kg declined making comparison with the burned animals difficult. Caldwell et al. (1959), Lieberman and Lansche (1956) and Moyer (1962) used a surface area correction based on the formula of Lee (1929):-

$$\text{Surface Area } (cm^2) = 12.54 \times (W)^{0.6} \qquad 5.1$$

where W is the weight in grams. The surface area correction term applied to some of the current experimental results was established on rats from the Department of Physiology colony by Richards (1979) using a technique similar to that of Lee (1929). The formula is:-

$$\text{Surface Area } (cm^2) = 10.1 \times (W)^{0.667} \qquad 5.2$$

where W is the weight in grams.

The use of these surface area formulae is a convenient and effective mathematical tool to aid comparison of normal

Burn	Expt. 1 20°C - 15g	Expt. 2 20°C - 18g	Expt. 3 30°C - 15g
Av. Nitrogen (g d^{-1})	0.223	0.273	0.220
*Av. Protein Equivalent (g d^{-1})	1.39	1.71	1.38
+Energy Equivalent of Protein (kJ d^{-1})	23.6	29.1	23.5
Av. Heat Losses (kJ d^{-1})	248	266	182
$\therefore$% Protein Energy/Heat Losses	9.5	10.9	12.9
Control			
Av. Nitrogen (g d^{-1})	0.179	0.225	0.172
*Av. Protein Equivalent (g d^{-1})	1.12	1.41	1.08
+Energy Equivalent of Protein (kJ d^{-1})	19.0	24.0	18.4
Av. Heat Losses (kJ d^{-1})	205	205	150
$\therefore$% Protein Energy/Heat Losses	9.3	11.7	12.3

* 1g Nitrogen $\equiv$ 6.25 g Protein

\+ Energy content of protein allowing for urine energy loss = 17 kJ/g

Table 20 Protein energy as a percentage of total heat loss for burned and control animals in Experiments 1, 2 and 3.

animals, though it should not be taken to imply a causal link between surface area and metabolic rate (Kleiber, 1961; Mitchell, 1962). None of these formulae has been shown to be valid for burn rather than control animals.

Herndon et al. (1978) obtained a large number of oxygen consumption measurements on control animals over a wide weight range. With these results a best fit curvilinear regression was established that allowed prediction of the metabolic rate of a control animal at any weight. This was the term with which the heat production of a burned animal of known weight was compared.

The performance of the three correction terms can be assessed by illustrating their variation over the weight range of interest in the current and previous animal studies (i.e. approximately 200 - 500g). The various correction factors, as a percentage of their value at 200g body weight, are shown in Figure 22. Growth produces little difference between the factors (10%) up to 500g body weight. For comparison of heat losses of differently sized animals within a study, the three terms are almost equivalent.

Use of these correction factors does not take into account the fact that burned and control animals of the same weight can have radically different body compositions. A burned rat can have as little as 2 to 3% body fat while a control can have up to 20% (Al-Shamma et al., 1979). Therefore part of the hypermetabolism of an injured animal could be due to a greater active cellular mass than its

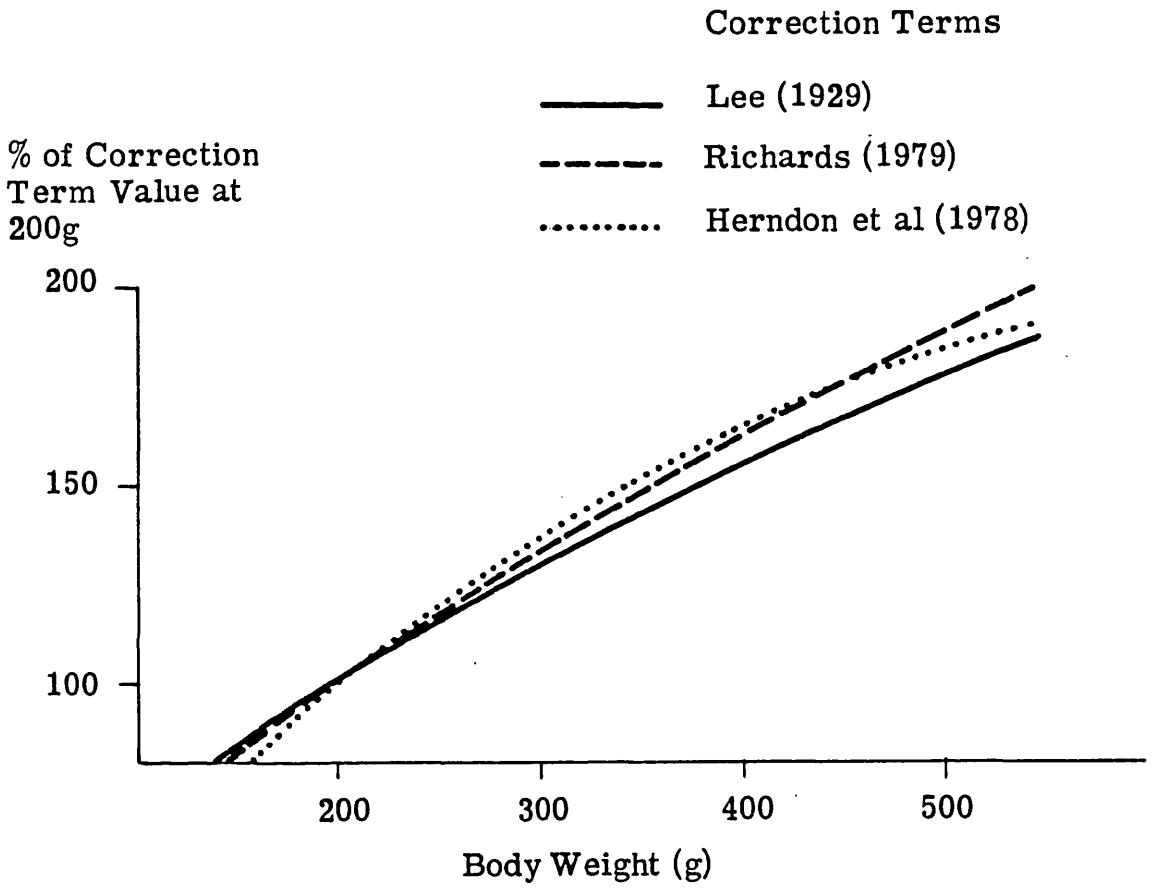


Figure 22. The variation with rat body weight of three terms used to standardise metabolic rate.

weight paired control. Comparison of burned animal results with a control animal with same fat free mass would be desirable but difficult practically.

Another difficulty arises if a correction based on weight is used. The burn wound is fixed in absolute size at the time of injury and until healing occurs will remain constant in area, particularly while the eschar is present. Therefore any losses that are related to the absolute burn area (e.g. evaporative water loss) will decrease in relative proportion to the total losses as the animal grows and increase if the animal loses weight.

Therefore in the current experiments, the control animals have been paired as closely as possible to the burns in terms of their starting age and weight, environment, feeding, shaving, anaesthesia etc. The controls represent burned animals without the injury. The energetic effect of the burn has been followed using the difference in absolute energy values between the groups of animals. Where appropriate, the calorimetry results have been calculated on a per unit surface area basis, particularly for comparison with other workers results.

5.2 The Effect of an Increased Environmental Temperature on Heat Losses following Thermal Injury

The effect on the absolute calorimetry values of increasing the ambient temperature is shown in Table 21. The burned animals had a 25% increase in insensible losses and a 55% decrease in sensible losses between experiments 1 and 3. The total heat losses were reduced by 26% at the warmer temperature despite the superior growth of the

	Burn Expt. 3 ÷Burn Expt. 1 (%)	Control Expt. 3 ÷Control Expt. 1 (%)	Burn Expt. 1 ÷Control Expt. 1 (%)	Burn Expt. 3 ÷Control Expt. 3 (%)
Insensible	125.2	130.0	257.5	248.1
Sensible	44.6	61.6	92.9	67.2
Total Heat	73.5	73.4	121.1	121.3

Table 21 Comparison of mean absolute calorimetry results in Experiments 1 and 3.

animals at 30°C which would tend to reduce the difference. The insensible losses were 2.6 and 2.5 times the control values at 20 and 30°C respectively. The burned animals were 21% hypermetabolic in both experiments.

The calorimetry values per unit surface area are shown for experiments 1 and 3 in Table 22. The insensible losses were less for the burn at the higher temperature but this reflected the much larger weights of the burned animals in that experiment. The insensible losses in both experiments were greatly increased over the control values. Even with the correcting effect of the surface area factor, the sensible losses of the burned animals at 30°C were only 76% of the controls and only 35% of the sensible losses of the burned animals at 20°C. The hypermetabolism decreased from 55% at 20°C to 35% at 30°C.

The absolute changes in sensible and insensible losses were in the direction that would be predicted theoretically. As the environmental temperature increases so the temperature difference between the intact and burned skin, and the environment decreases. The radiative, convective and conductive routes of heat loss decrease in close agreement with Newton's Law of Cooling until the TNZ is reached. (Hardy and Soderstrom, 1938).

The insensible losses are mainly passive diffusion losses until the animal reaches the top of its TNZ. As the ambient and therefore skin temperature rise so the saturated water vapour pressure beaneath the skin barrier rises. As long as the ambient air has a reasonably constant

	Burn Expt. 3 ÷ Burn Expt.1 (%)	Control Expt. 3 ÷ Control Expt. 1 (%)	Burn Expt. 1 ÷ Control Expt. 1 (%)	Burn Expt. 3 ÷ Control Expt.3 (%)
Insensible*	96.2	117.1	335.5	275.5
Sensible*	34.8	54.6	118.8	75.8
Total Heat*	56.9	65.0	154.9	135.5

*All heat losses calculated per unit surface area

Table 22 Comparison of mean calorimetry results, expressed per unit surface area, in Experiments 1 and 3.

water vapour pressure (i.e. a constant dew point temperature as in experiments 1 and 3) more water vapour must diffuse under the increased pressure gradient (McLean, 1974), and insensible losses therefore increase.

Reports of burn wound temperature in rats (Lieberman and Lansche, 1956; Richards, 1979) suggest that it is well above ambient temperature and close to that of the intact skin. Some measurements of wound temperatures close to ambient have been made in man (Harrison et al., 1964; Birke et al., 1972). The sensible losses of the burned animals in experiment 3 were only 76% (per unit surface area) of the control animals losses , suggesting that average skin temperature might have been low in the burns. Caldwell et al., (1959) and Barret al. (1968) have suggested that the environment becomes a direct source for some of the energy necessary to vaporise the water from the wound at temperatures around 30°C. This would allow the burn wound temperature to remain relatively high (i.e. close to the intact temperature) while the temperature of the ventilating air would be cooled.

The heat losses to the walls of the calorimeter (mainly radiative and conductive losses) and to the ventilating air (mainly convective loss) can be calculated separately (Table 23). The ratios of the losses were not significantly different for the burns and controls at 9.3 and 8.8% respectively. As there is no evidence for a general decrease in skin temperature after a burn, the most likely explanation of the decreased sensible loss was a wound temperature close to ambient. The reports cited

	1	2	
	Heat loss to ventilating air ($mW\ cm^{-2}$)	Heat loss through calorimeter gradient layer ($mW\ cm^{-2}$)	Ratio of 1 ÷ 2 %
Control (n = 14)			
mean	0.205	2.342	8.79
s.d.	0.021	0.284	0.54
Burn (n = 35)			
mean	0.159	1.729	9.27
s.d.	0.026	0.282	1.20
Unpaired T test	5.89	6.86	1.42
p	***	***	ns

Table 23 Heat loss through the calorimeter gradient layer and heat loss to ventilating air for burn and control animals at 30°C in Experiment 3 calculated per unit surface area.

earlier all used contact methods of taking the burn temperature. As there could be problems of good thermal contact and local disturbance of evaporative loss a non-contact method (e.g. thermography) is required to measure the effective wound temperature.

5.3 The Effect of Increased Caloric Intake on the Calorimetric Response to Thermal Injury

The absolute calorimetry values from experiments 1 and 2 (Table 24) showed the burn animals to have similar insensible losses. The increased respiratory water losses from the heavier animals in experiment 2 must have compensated for the decrease in burn size towards the end of the experiment. Sensible and total heat losses were greater in experiment 2 again because of the increased weight of the burned animals in that experiment. Comparison of burns with controls demonstrated a 2.5 to 3 times increase in insensible losses and a hypermetabolism of 20 - 30%.

A comparison of the results expressed per unit surface area (Table 25) demonstrated the similar responses in the two experiments. The insensible losses were 3.4 and 3.3 times the control values for the burns in experiments 1 and 2 respectively. The sensible losses of both sets of burned animals were 19 to 20% higher than the controls. This suggested that the burn wound temperature was above the surrounding skin temperature (i.e. the opposite to 30^{o}C ambient condition). Presumably this was because of the loss of physical insulation over

	Burn Expt. 2 ÷ Burn Expt. 1 (%)	Control Expt. 2 ÷ Control Expt. 1 (%)	Burn Expt. 1 ÷ Control Expt. 1 (%)	Burn Expt. 2 ÷ Control Expt. 2 (%)
Insensible	98.1	87.5	257.5	288.6
Sensible	114.1	102.0	92.9	104.0
Total Heat	107.3	100.0	121.1	130.0

Table 24 Comparison of mean absolute calorimetry results in Experiments 1 and 2.

	Burn Expt. 2 ÷ Burn Expt. 1 (%)	Control Expt. 2 ÷ Control Expt. 1 (%)	Burn Expt. 1 ÷ Control Expt. 1 (%)	Burn Expt. 2 ÷ Control Expt. 2 (%)
Insensible*	83.5	83.9	335.5	333.8
Sensible*	98.0	97.2	118.8	119.7
Total Heat*	92.7	94.8	154.9	151.3

*All heat losses calculated per unit surface area

Table 25 Comparison of mean calorimetry results, expressed per unit surface area, in Experiments 1 and 2.

the wound (i.e. skin and subcutaneous fat). The surrounding skin could increase its insulation to some extent by reducing its blood supply. The burn hypermetabolism was 50 - 55% in both the experiments.

Though there was no obvious benefit in calorimetric terms with the increased food substrates available in experiment 2, the greatly improved growth of the rats on 18g diet per day was apparent (Figures 9 and 13). The ability of burned rats to eat their way out of a significant burn injury has been reported before (Caldwell, 1962; Richards, 1979). This demonstrates the importance of pair feeding of controls to allow useful comparisons.

Using the calorimetry energy balances of the burn rats (Tables 7, 12 and 16) at the end of the three experiments, the comparative effects of food and temperature increase can be approximately assessed. The difference in energy balance at the end of experiments 1 and 2 is:-

Energy Balance difference = 1454 - 148 = 1306 kJ 5.3

and for experiments 1 and 3 is:-

Energy Balance difference = 2851 - 148 = 2703 kJ 5.4

The mean difference in food input between experiments 1 and 2 is:-

Difference in food input = 16.88 - 14.41 = 2.47g 5.5

Therefore the approximate increase in diet required to produce the same increase in energy balance in a burned rat as raising the environmental temperature from 20 to

$30^{o}C$ is:-

$$\text{Increase in diet} = \frac{2.47 \times 2703}{1306} = 5.1g \qquad 5.6$$

This is a 35% increase in the diet offered in experiment 1. Caldwell (1962) found that rats required an approximate increase of 52% in their dietary input in order to make burns at $20^{o}C$ grow at the same rate as burns at $30^{o}C$.

5.4 Energy Balance

In all the animal burn studies cited in the introduction, the animals were in the calorimeter for only a limited part of the post-injury period. This usually reflects the calorimeter design and the requirement to examine the response of more than one animal. Therefore it is important to know how representative of the average metabolism is the short period in the calorimeter.

If the calorimetry values were the same as the average daily heat loss and there were no appreciable measurement errors, then energy balance calculated using heat loss and food energy would equal the energy balance calculated from body tissue changes (see Sections 2.5.1 and 2.5.2). However, in all the experiments both burn and control animals have a consistently more positive calorimetry energy balance than body composition energy balance.

The calorimeter has been shown to be capable of an agreement between direct and indirect values of $\pm$ 1% under carefully controlled conditions (Carter, 1975). This limit is not routinely achievable with daily use of the calorimeter during a two month experiment. However the natural

variation in the heat loss of animals day to day because of different activity states, body temperatures etc., causes measurement variations much larger than the equipment errors. The coefficients of variation for total heat loss per unit surface area in the control rats was 12% in experiment 1, 8% in experiment 2 and 10% in experiment 3. These are similar to the 7 - 8% variation seen in repeated heat production determinations on man (Du Bois, 1927). However as multiple measurements (14 to 35) of the heat losses are made during an experiment, the standard error of the estimate of the mean should only be about 2.5%. As the differences between the two methods of energy balance measurement varies from 3 to 27% of the measured heat losses, this random error cannot explain it.

Several possible sources of systematic error exist:-

1) Food input is always overestimated because of spillage. This has been minimised by making the diet into paste form. Observation of the amount of food left in the cages and in the metabolic collection apparatus would suggest only 0.2g of diet is unmeasured. This is approximately 2% of the net food energy intake.

2) Some of the urinary nitrogen is lost on glassware and through slight decomposition before collection. However, if 10% of the collection were lost and Richards (1979) has shown the collection procedure to be better than this, there would only be an 0.4% error in the net food energy calculation.

3) Values of DIT tend to be underestimated because of difficulty in deciding when the animal has returned to baseline conditions. The measurements at 30°C followed the response of the animal for only three hours and depended on the 20°C results for extrapolation to the final value. However as most measurements of DIT diet in man lie between 6 and 10% (Benedict and Carpenter, 1918; Glickman et al., 1948), the underestimate is unlikely to be more than 3 to 4%.

The sum of these errors is only 6 to 7%. Errors in the body composition analyses are also unlikely to provide the large systematic shift required.

(i) The errors in the individual protein and fat determinations are less than 5% (Al-Shamma, 1978; Gedeon, 1981). Mean values of several animals are used at each point, so that errors are likely to have a 2 to 3% maximum.

(ii) As the sum of protein, fat and water contents for the groups of animals analysed lies within the range 96 to 100% and the mineral residue has been measured to be 2 to 3%, there appears to be no systematic over or underestimate of body components.

(iii) Bomb calorimetry measurements of body tissue energy agree closely with the literature values over the range of compositions where fat represents 25% to 50% of the dried carcase. The only animals to fall outside this range are the burns in experiment 1 and 4, and the measurements on the five animals in experiment 4 only showed an average 4% overestimate of body energy by the

literature values. Taking the worst case errors decribed above does not explain the large discrepancy in experiment 3.

The calorimeter cage is more confining than the cage in which the animal is normally kept. This could lead to a decrease in relative activity in the calorimeter especially as the calorimetry period is chosen from the part of the run showing the least change in the analyser outputs. The rat at 20°C however is rarely still and movement forms part of its behavioural thermoregulation. At 30°C the control rat is near the upper limit of its TNZ and the burned animal has much reduced heat losses. Under these circumstances the rat often rests.

Support for these observations comes from analysis of calorimetry runs at 20 and 30°C in experiments 3 and 4. The calorimetry period was divided into 12 five minute sections and the standard deviation calculated for the 12 heat loss results. More movement should produce a larger deviation. As a larger rat would produce larger increments in heat loss on movement, the standard deviation was divided by the weight to try to allow for this. The animals at 30°C showed significantly less activity (Wilcoxon Rank Sum test) than their counterparts at 20°C (Table 26).

Observations of the animals in their metabolic cages suggested that the control animals were as active as the burned at 20°C. However, at 30°C the burned animals appeared to be much more active than the controls. As 30°C

	Control - Standard Deviation of Heat Loss ÷ Rat Weight	Burn - Standard Deviation of Heat Loss ÷ Rat Weight
	0.413	0.565
	0.454	0.584
	0.427	0.406
	0.296	0.248
	0.341	0.485
A	0.244	0.411
	0.278	0.171
Experiment 3	0.185	0.207
	0.106	0.471
$30^{o}C$	0.484	0.262
	0.282	0.296
	0.191	0.305
	0.483	0.212
	0.069	
	0.300	0.479
	0.647	0.457
	0.848	0.481
B	0.321	0.445
	1.195	0.568
Experiment 4	0.825	0.580
	0.427	0.412
$20^{o}C$	0.641	
	0.722	
	0.612	
	0.595	
Rank A	120.5	111
Rank B	204.5	99
Wilcoxon Test p	**	*

Table 26 Standard deviation of heat loss per unit weight at 20 and $30^{o}C$.

represents the upper limit of the TNZ for an uninjured animal, heat produced by activity at this temperature would be a thermoregulatory embarrassment. Burn animals appear to have a TNZ that extends beyond 30°C (Caldwell et al., 1959) which agrees with the observation of higher than normal comfort temperatures for burned patients (Aulick et al., 1979). Therefore uninjured rats at 30°C may not be appropriate controls for the burned animals for behavioural reasons. Because of the general inactivity outside the calorimeter, the control animals are likely to deposit more body tissue than if they were as active as the burns, hence increasing the difference between the energy balance calculated for the burned and control animals from body composition results (Figure 20). More accurate monitoring of animal activity in and out of the calorimeter is required to solve this problem.

Most of the systematic errors can be eliminated by calculating the difference in calorimetric energy balance between the burned and control animals in the same experiment. This can also be applied to the body composition values. The results are plotted in Figure 23 as the energy cost of the burn. The lines for experiments 1 and 2 at 20°C for both calorimetry and body composition calculations lie within a narrow band. The calorimetry results at 30°C show a smaller energetic effect of the burn at that temperature. The body composition results show no decrease in the effect of the burn. As discussed above, this probably reflects the unsuitability of the control animals at this temperature, their lack of activity leading to an excessive deposition

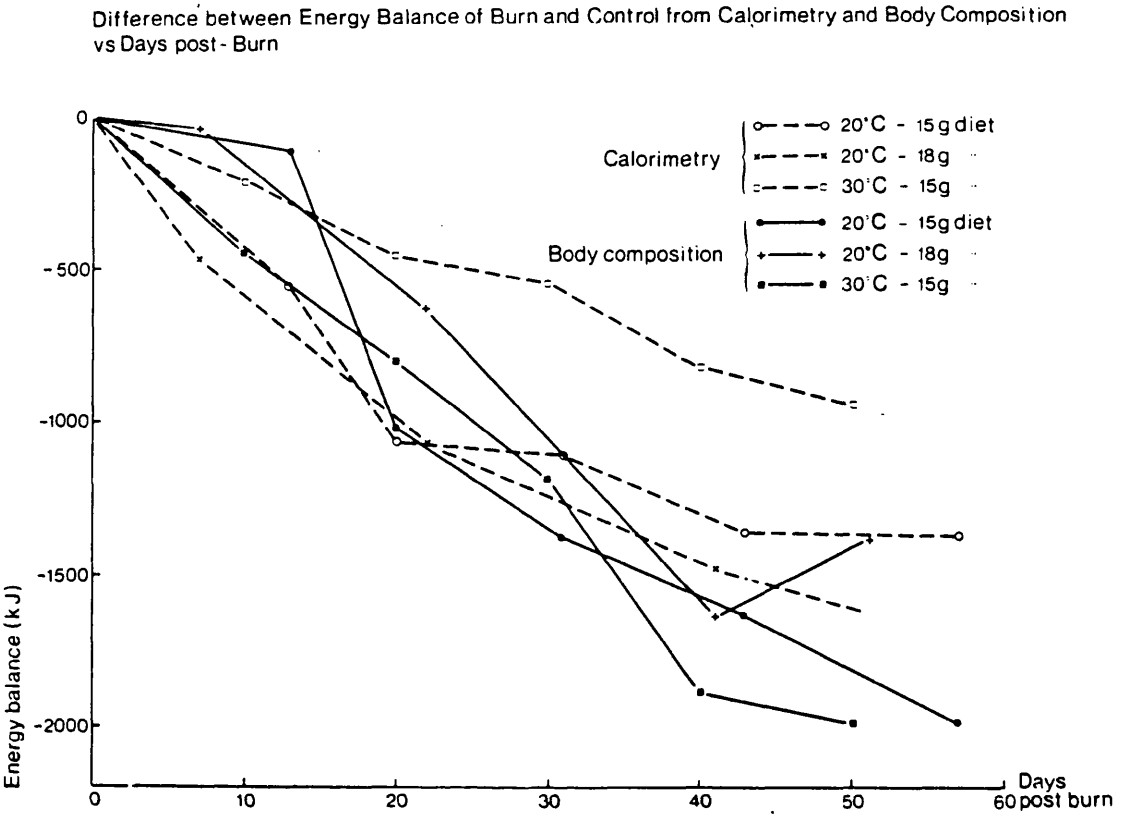


Figure 23. The difference between the energy balance of burned and control rats for Experiments 1, 2 and 3 calculated both from calorimetry and body composition results.

of body tissue energy.

The energy balance data can be summarised:-

(i) There are systematic differences between energy balance calculated from short term heat loss measurements and changes in body composition.

(ii) The majority of the difference can be ascribed to behavioural changes in and out of the calorimeter. These are particularly important at 30°C.

(iii) Comparing the differences between burned and control energy balances eliminates most of the systematic errors. At 20°C all the results show a similar energetic effect of the burn. The calorimetry results at 30°C, with the burned and control animals in similar quiet states during the calorimetry period show the energetic effect of the burn to be approximately halved. The body composition at 30°C appear to show a greater energetic effect of the injury, but this is probably caused by differences in the levels of activity of the burned and control rats outside the calorimeter.

5.5 Thermogenesis after Thermal Trauma

The thermogenesis of the oral diet was significantly different between burned and control animals at both ambient temperatures. There appears to be very few reports in the literature of measurements of DIT on diseased or traumatised animal or human subjects. Little effect on DIT was measured in patients with liver disease or hyperthyroidism (Aub and Means, 1921; DuBois, 1927),

however, during fever DIT was practically absent (Coleman and DuBois, 1914; DuBois, 1921). Recent texts (Wilmore, 1977; Kinney, 1977) state that DIT should be expected to be reduced in critically ill and febrile patients.

Administration of total parenteral nutrition with energy inputs in excess of the calculated energy requirements (Askanazi et al., 1980) has very different effects on depleted and traumatised groups of patients. Depleted patients showed a small (3%) increase in oxygen consumption while septic and injured patients increased their oxygen consumption 29%. The excess energy input appeared to be a stress to the second group, as these patients trebled their nor-adrenaline output over 4 days of feeding. The response was not rapid as seen in oral feeding experiments; the increase in oxygen consumption continuing over several days. It was also possible that the septic and injured patients were not stable and their heat production would have increased without feeding. Arturson (1978) stated that in burn patients ingestion of carbohydrate and fat increased metabolic rate by 5 to 30% for up to 9 hours and ingestion of protein produced an increase of 30 to 70% for as long as 12 hours. Similar effects of intravenous and orally administered amino acids were seen. Unfortunately no systematic presentation of the results was given.

The causes of DIT have not been established (Garrow, 1978) though they could be linked to the processes of fat and protein synthesis and deposition. It is known that depositing extra fat and protein is energy intensive

(McCracken and Weatherup, 1973). Measurements on rats kept at 22°C (Pullar and Webster, 1977) show that 2.25 kJ of metabolisable food energy are required to deposit 1 kJ of protein while 1.36 kJ are required to deposit 1 kJ of fat. The values of DIT measured in the current experiments increase in the same order as the deposition of fat and protein increased. Also during the feeding experiments a greater increase in RQ was measured in the control animals than in the burns, suggesting more lipogenesis in the controls.

To test whether DIT and fat and protein deposition were possibly associated in the continually growing control animals, the food energy used in DIT was compared with the calculated amounts of metabolisable food energy required in extra heat production to deposit fat and protein in the animals. From Pullar and Webster's figures the energy increment for 1 gram of protein is:-

$$\text{Energy increment for 1 g of Protein} = (2.25 - 1) \times 23 = 28.7 \text{ kJ} \qquad 5.7$$

and for Fat:-

$$\text{Energy increment for 1 g of Fat} = (1.36 - 1) \times 39 = 14.0 \text{ kJ} \qquad 5.8$$

The results of the comparison have been calculated using Tables 8, 13 and 17 and are shown in Table 27. The experiments at 20°C show reasonable agreement between DIT and the energy increment, with a much poorer agreement at 30°C. The explanation could be an underestimate of DIT

	Fat Deposited	Protein Deposited	Energy Increment of Deposition	Energy of DIT	Energy Increment DIT
	g	g	kJ	kJ	%
Experiment 1	17.2	21.0	1087	946	87
Experiment 2	21.7	19.6	1173	962	82
Experiment 3	45.2	28.2	1445	817	57

Table 27 The energy increment of fat and protein deposition vs the energy associated with dietary induced thermogenesis.

at 30°C though it would have to increase from 6.7% to 11.7% of food energy to bring the figures to agreement. Further work would be necessary to establish a causal link between deposition of fat and protein and DIT.

5.6 Comparison of Current and Previous Studies of Thermal Injury in the Rat.

5.6.1 Reproducibility of Results

One advantage of an animal model is that injury and environmental conditions can be standardised and a similar response produced if the experiment is repeated. In experiments 1 and 4 where the food input and ambient temperature were the same, the weight changes of the injured animals have been compared (Figure 21) and shown to be similar. The calorimetry results in experiments 1 and 2 were also closely comparable (Tables 24 and 25). Drury (1976), using the same calorimeter, performed an experiment over 63 days at 20°C with a 25% BSA burn and demonstrated a weight loss similar to experiment 1. The absolute calorimetry results are also in close agreement. (Table 28).

The weight changes in experiments 1 to 3 are also similar to those of Caldwell (1962) who performed nutritional studies at 20 and 30°C with burns of between 28 to 30% BSA. Animals on restricted feeding at 20°C lost approximately 30% of their initial weight in 60 days. Animals that ate 55% more, gained weight while another group of animals ate 18% less but were able to maintain their weight because the ambient temperature was increased

	Average Sensible Losses(W)		Average Insensible Losses (W)		Average Total Heat Losses(W)	
	Burn	Control	Burn	Control	Burn	Control
Drury (1976)						
20°C 25% BSA	2.01	2.09	1.18	0.28	3.19	2.37
Experiment 1						
20°C 25% BSA	1.84	1.98	1.03	0.40	2.87	2.37

Table 28 Comparison of calorimetry results from an experiment performed by Drury (1976) and Experiment 1.

to 30°C.

5.6.2 Hypermetabolism

The current study agrees with all the previous studies in finding the burned animals hypermetabolic at all the temperatures tested. On a per unit surface area basis the injured animals had an increase of 1 to 1.5% in metabolic rate per% BSA burn at 30°C. This is similar to the increase measured in other studies (Lieberman and Lansche, 1956; Caldwell et al., 1959; Caldwell et al., 1966) though much higher than that seen by Herndon et al. (1978). However, as his experiments were performed at 100% humidity the evaporative losses should be greatly reduced, although not completely stopped because skin and respiratory tract temperatures will be above ambient. Therefore Herndon's results can be interpreted as demonstrating that the metabolic rate is not minimised by raising the environmental temperature to 30°C at ordinary humidities. Further manipulation of the environment by increasing humidity (impractical in human work) or by providing infra-red heating, as suggested by Danielsson et al., (1976a), could decrease metabolic rate further. Herndon's 25% BSA burn animals had a similar degree of hypermetabolism (approximately 10%) to animals measured (Lieberman and Lansche, 1956) with their wounds covered by an impermeable membrane.

5.6.3 Insensible Losses

The burned animals showed huge increases (2 - 3.5 times) in insensible losses when compared with controls, in agreement with other workers' findings (Caldwell et al., 1959; Moyer, 1962; Caldwell et al., 1966). The absolute value of insensible loss increased 35% in burned animals in experiment 3 at the higher temperature when compared with experiment 1. Drury (1976) found a decrease of 35% in absolute insensible losses when comparing burned animals at 20 and 30°C. The control animals in the same experiments demonstrated no increase in insensible losses, contrary to most workers' findings in normal rats (Benedict and MacLeod, 1929a; Swift and Forbes, 1939). There was no theoretical argument to support these results. However Caldwell et al. (1959) found that burned animals calorimetered at both 24 and 32°C on the same day showed an increase in insensible losses of 21%. This avoided any difficulty in comparison of animals of different weights and confirms the values from the present study.

Insensible water loss is an important indicator of size and state of the wound. In experiments 1 and 3 the evaporative losses were sustained through the 50 to 60 days of the experiment. Drury (1976) using the same apparatus demonstrated similarly sustained losses. In experiment 2 using the lower branding temperature and pressure, healing and a reduction in metabolic rate were seen after 30 days. Caldwell et al (1966) and Herndon et

al. (1978), using a scald injury, observed fairly rapid re-epithelialisation in their animals, the wound size halving in 30 and 18 days respectively. Shangraw and Turinski (1981) using a very similar procedure showed no healing in their animals until 11 weeks following injury. While Caldwell et al. (1966) showed a decrease in the burn hypermetabolism during the healing process, in a similar way to experiment 2, Herndon et al. (1978) measured a constant hypermetabolism in their animals until 45 days after injury when their wound was only 19% of its original size. This again suggests that the high humidity in the calorimeter may be reducing the burned animals metabolic rate.

In both experiments 1 and 2 there was a delay of 7 to 10 days before the injured animals reached their maximum heat production. Drury (1976) and Herndon et al (1978) observed similar delays in attaining the maximum values of heat production. This could be caused by gradual revascularisation and an increased surface blood flow to the wound (Aulick et al., 1979). As the eschar did not separate until 20 to 30 days, in agreement with Shangraw and Turinski's (1981) observations, an alteration in the eschar is a less likely cause.

In summary, the current study agrees with previous animal studies in that:-

i) The injured animals are always hypermetabolic compared with controls at 20 and 30^{o}C.

ii) The metabolic rate of a burned animal decreases

when the ambient temperature is increased from 20 to 30°C.

iii) Raising the environmental temperature from 20 to 30°C increases insensible losses and decreases sensible losses.

iv) At 30°C, the metabolic rate of an injured animal calculated on a surface area basis, is increased 1 to 1.5% per % BSA injury above that of a control animal, at medium to low humidities.

v) In terms of weight, injured animals on similar dietary amounts grow better at 30 than at 20°C.

It has also been shown that:-

vi) Calorimetry values of heat loss and average daily energy expenditure values calculated from body tissue changes can differ by as much as 20%. Most of the difference can be ascribed to behavioural differences in and out of the calorimeter.

vii) The studies are reproducible nutritionally and calorimetrically.

viii) Increasing the environmental temperature from 20 to 30°C has a similar effect on energy balance to increasing food intake by 35%.

5.7 Comparison of the current Rat Burn Studies with Human Burn Studies.

If the summaries at the end of section 5.6.3 and 1.5.1 are compared, close agreement between the rat and human situation is seen. The current animal studies are in close parallel to those in humans by Barr etal. (1968), Birke et al. (1972), Wilmore et al. (1974 and 1975) and Aulick et al. (1979) as they cover the temperature range 20 - 30°C at medium to low humidities.

The injured animals with a 25% BSA burn are 35% hyper-metabolic compared with their controls at 30°C. For 25% BSA burn patients at similar temperatures Wilmore et al. (1974) measured an increase of 55%, Aulick et al. (1979) 50% and Birke et al (1972) 35 to 40% above control values. At 30°C the burned rats lost 61% of their heat through insensible losses. Wilmore et al. (1975) showed that for a similar patient (i.e. 25% BSA burn injury kept at 33°C) the insensible losses accounted for 65% of total losses. Birke et al. (1972) did not calculate the evaporative losses on a per unit surface area basis as for metabolic rate. However, if an average surface area of 1.75 m^2 for the patients is assumed, the evaporative losses represented approximately 50% of the total heat losses at 32°C.

The current studies showthat a gradually healing wound is attended by gradual decreases in insensible and total heat losses. This has been observed in patients by Birke et al. (1972) as second degree burns healed over a three week period. In the rat experiments at 20°C, a delay in

reaching maximum heat loss was observed. Birke et al. (1972) also observed a delay of 4 - 7 days in reaching a peak in metabolic rate in patients with a wide range of severities of burn.

These examples demonstrate that the animal model is a reasonably close quantitive parallel to human burn injury. It has advantages over the human situation in that the injury responses can be standardised and accurately reproduced. The ability to use a direct calorimeter allows accurate partitioning of heat losses. Body composition techniques can also give useful information on long-term energy metabolism changes. The degree of hypermetabolism measured in a rat with a 25% BSA burn is adequate for accurate calorimetry and produces a rapid enough tissue utilisation for the reduction in body weight observed in patients to be achieved.

CHAPTER 6.

Human Studies Materials and Methods

6.1 Choice of Calorimetry Technique

Many different methods of calorimetry can be used on human subjects. If RMR measurements are to be made on ill patients, access to the subject must be possible at all times, so calorimeters that totally enclose the subject (Bradham and Charleston, 1972; Dauncey and James, 1979) are not suitable. Relatively simple techniqes such as the Douglas bag (Barr et al., 1968, Wilmore et al., 1975), closed circuit spirometer (Harrison, Seaton and Feller, 1967; Zawacki et al., 1970) etc. only collect patient expired air for a short period of time (usually 5 to 10 minutes). This can introduce important sampling errors should the patient hypo - or hyperventilate during this period. No indication of changes in the heat production of the subject during the collection period are available, only a final average figure.

Since continuous gas flow measurement of oxygen and carbon dioxide concentrations became available in the 1950's (Garrow, 1974), continuous calorimetry has been possible. Using a suitable mouthpiece or mask and one way valve expired air can be analysed, breath by breath or after mixing (Wilmore, Davis and Norton, 1976). This technique has the advantage of relatively high (approximately 5%) changes in gas concentrations produced by the subject allowing accurate analysis with the possibility of also

measuring other respiratory parameters. However rapid sampling or effective mixing of the gas flow is required and the relatively low (approximately 5 l min^{-1}) pulsatile flow can be difficult to measure accurately. The valving provides some resistance to air flow and patients can also find mouthpieces and facemasks unacceptable (Spencer et al., 1972).

Use of an open or closed canopy system (ventilated hood system) instead of a mouthpiece or mask requires a relatively high air flow (25 - 50 l min^{-1}) to be drawn through the canopy to keep the carbon dioxide concentration around the patient to within acceptable physiological limits (i.e. less than 1%). The diluting effect of the increased air flow requires high accuracy of the gas analysers. However, the subject in the canopy notices no effect on his breathing and the constant air flow through the system can be measured with a simple gas meter. Adaptations of the canopy system have been used by a number of clinical investigators (Gump, Price and Kinney, 1970; Danielsson et al., 1976b; Aulick et al., 1979; Long et al., 1979b) and its use in this study allowed comparison with these reports.

6.2 Calorimetry Design

6.2.1 Canopy and Temperature Controlled Rooms

A closed rather than open canopy design has been used so that gaseous losses, particularly those of carbon dioxide, could be avoided and changes in the gaseous concentrations of the ventilating air, when staff were close to the canopy, could also be eliminated.

The canopy (Figure 24) was a

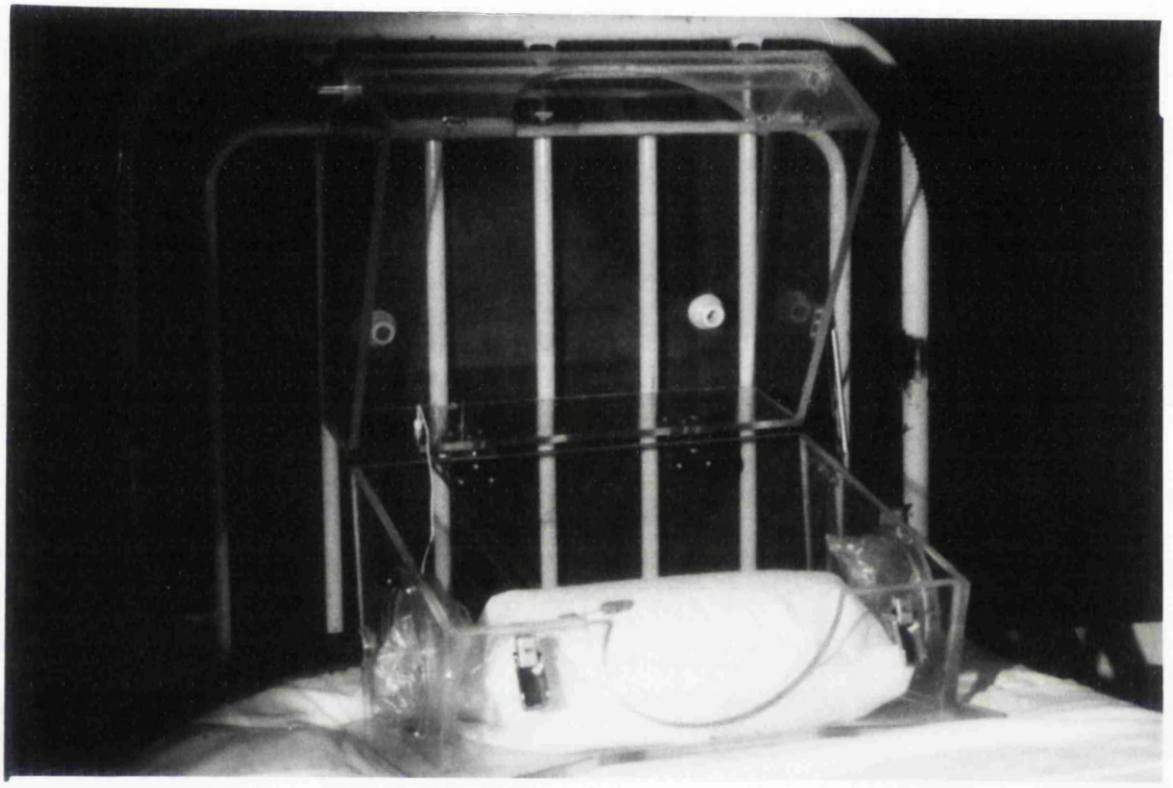


Figure 24. The perspex head canopy used in Studies A, B and C.

300 x 200 x 500 mm perspex box constructed by the Department of Clinical Physics and Bioengineering, Glasgow, to the design of Kinney et al. (1964).

A hinged top allowed access for the head of the subject which rested on a pillow while in the canopy. A silicone rubber sealing strip was let into the base to provide an airtight seal when the top was closed and secured with two quick release fasteners mounted on the box front. Two 25 mm diameter ports allowed naso-gastric or other tubing to be passed out of the canopy while retaining a seal. Two ports on the canopy sides, sealed with plastic bags, allowed the subject access to his face should he have an itch etc. during a calorimetry period. These bags were also sensitive indicators of any imbalance in air pressure between the canopy and the room.

A seal was required to stop any air leaks around the neck. A semi-rigid plastic ring was made to fit the 250 mm diameter hole in the canopy front. For each calorimetry run a clear polythene bag of approximately 250 mm diameter was attached to the ring using two elastic bands. The other end of the polythene bag was cut to fit between the base of the neck and the shoulders and around the top of the chest and back. This was attached to the skin of the subject using a non-allergic aerosol adhesive (Medical adhesive B, Dow Corning Ltd.) sprayed onto the plastic. It was found to give a reliable seal with no problems of tightness around the neck

(Spencer et al, 1972).

The canopy was used by Kinney et al. (1968) on patients lying flat on top of a bed. This was found to be unacceptable to some post-operative patients. Any collection of phlegm could produce coughing and breathing difficulties and the movement of the patient from his normal resting position on pillows to a flat position could be painful. Therefore slots were let into the canopy base to allow the canopy to be hung from the bed back using 12 mm wide strapping with adjustable buckles. The canopy could then be placed around the head of the patient with little disturbance. Figure 25 shows a patient in Study C, towards the end of a morning calorimetry period. Benedict and Bendict (1924) have reported no differences between the metabolic rate of subjects lying and reclining in a position similar to that used with the patients in Study C. Therefore measurements in either position on a quiet subject have been used as the resting metabolic rate.

The calorimeter studies were carried out in one of two single bedded metabolic rooms at the side of ward 33, Glasgow Royal Infirmary. Appropriate piping and cabling allowed collection of gases and electrical signals from either room, the analysis and processing being performed in a separate laboratory 20 m away. The two single bedded rooms shared an air conditioning system capable of maintaining the air temperature to within $\pm$ 1^{o}C of a preset value. The monitored relative humidity was below 55%,

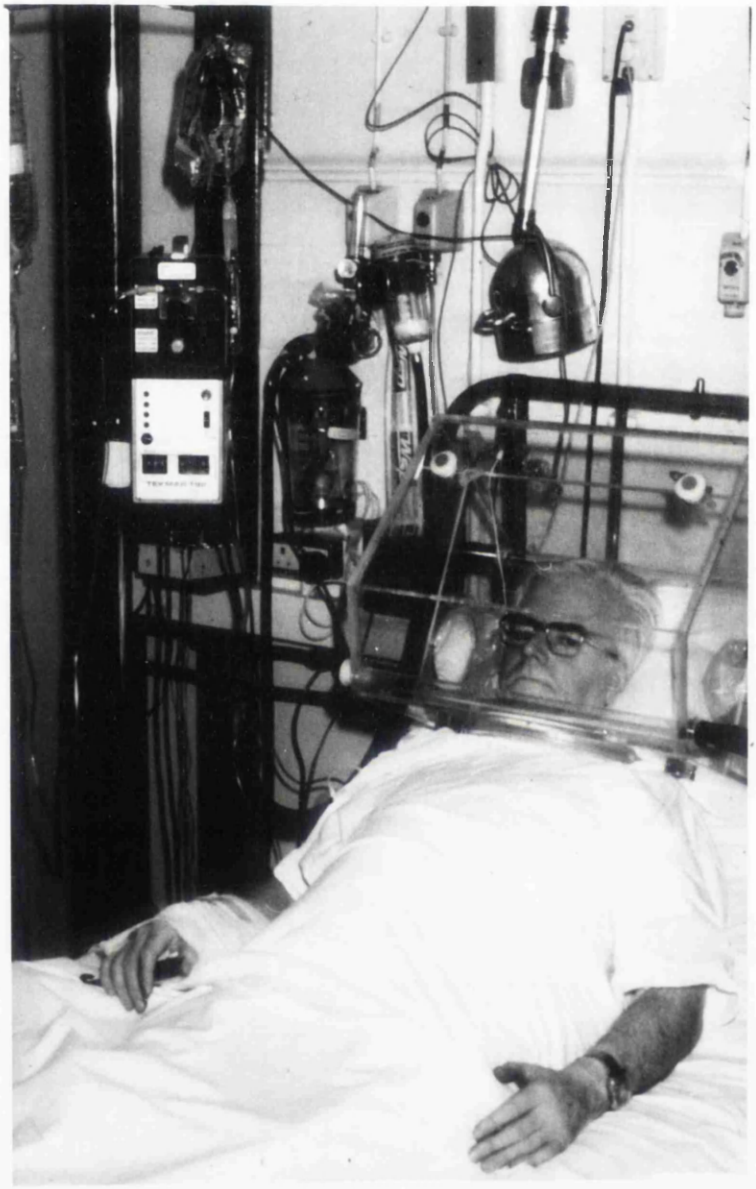


Figure 25. A patient during a calorimetry measurement in Study C.

with typical values being 35 - 45%. Each room had a colour television which appeared to greatly increase subject acceptance of the semi-isolation of these rooms compared with the open ward. Control of variables such as nursing procedure, noise etc. was facilitated by the use of these rooms.

6.2.2 Gas Collection and Analysis Circuit

The gas circuit layout is shown in Figure 26. Air was drawn from outside the building so that oxygen and carbon dioxide background concentrations were constant. Most of the air (approximately 35 l min^{-1}) was pumped down 25 mm stainless steel tubing to the patient rooms using an oil free pump (type 2D-416-2-2e, Compton Ltd) A small amount of air was diverted for analysis by the reference cell of the oxygen analyser. The air was returned from the canopy using a diapragm pump (type DAA-120, Gast Pumps Ltd) at a rate set to produce a pressure close to atmospheric in the canopy. Usually the canopy was at a slight negative pressure (about 25 mm of water) so that any leaks would be inward.

The total flow of gas coming from the canopy was measured with a wet gas meter (type M809ra, A. Wright & Co., London). Any leaching of water from the meter, which would affect its calibration accuracy, was prevented by using a humidifier to saturate the air before it reached the meter. The temperature and pressure of the gas at the meter was monitored using a thermistor (YSI 44011, Yellow Springs Instruments Ltd.) and pressure guage (R52764, Blease Medical Equipment Ltd.) to allow the correction of the

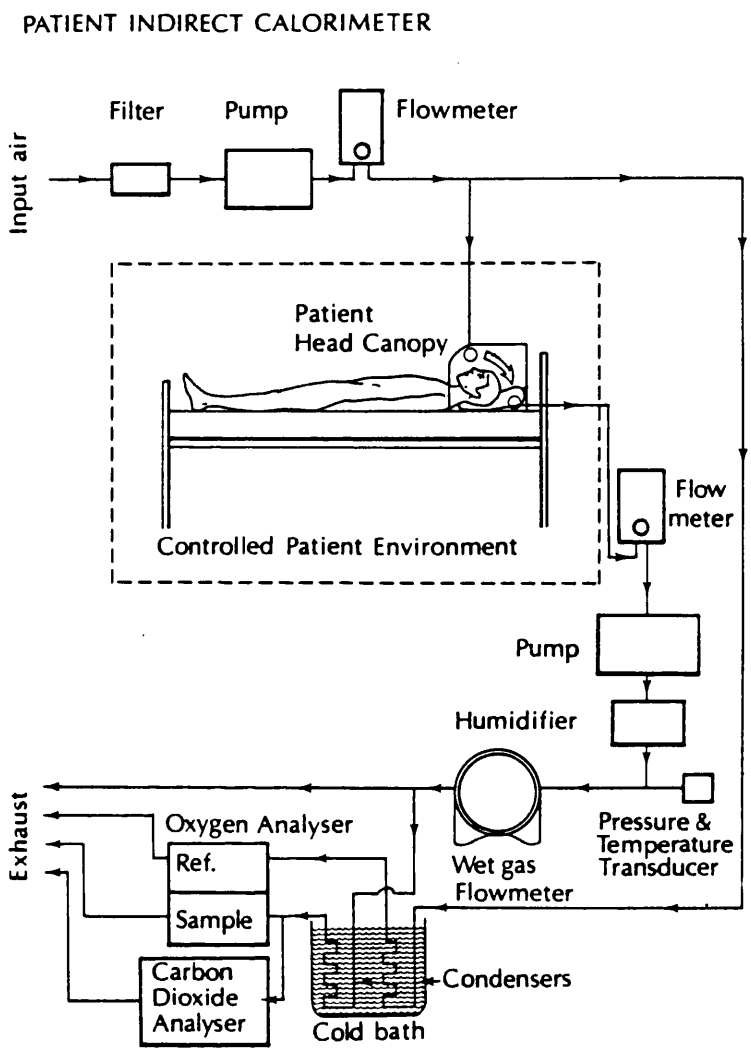


Figure 26. Patient indirect calorimeter gas circuit.

flow rate to dry gas conditions at standard temperature and pressure. The output of the wet gas meter was obtained in the manner described by Carter (1975). A disc with 360 holes at one degree intervals around its circumference was attached to the main rotor of the meter. The holes were detected as they passed between a fixed light emitting diode and photo-transistor (type 306-061, Radiospares Ltd.). The pulses of light switched the phototransistor into conduction which triggered a monostable (SN74121, Texas Instruments Ltd.). The logic pulses from the monostable were converted to an analogue output by a ratemeter (N.E. 4607, Nuclear Enterprises Ltd.), the output of which was regularly recorded by a computer controlled data logging system.

After flow measurement most of the gas was exhausted. Two to three litres per minute were taken for gas analysis. This gas must be desaturated to stop any condensation in the analyser cells. The external air taken for reference oxygen analysis must also have its water vapour pressure reduced to a known level. Initially drying towers containing calcium sulphate (Drierite, Hammond Ltd.) were used. The dew point of the air was reduced to -40^{o}C but the towers were quickly exhausted and required regular filling. An ice trap employing solid carbon dioxide (Drikold, DCL Ltd.) was also tried. However, occasional complete occlusion of the gas tubing by ice and the expense of regular supplies of solid carbon dioxide made it unsuitable.

Both these methods reduced the background humidity to

negligible levels. However a moderate humidity would be acceptable if it were accurately measurable and did not produce condensation in the analyser. Therefore an insulated cool bath, operating at a temperature of 4 to 5^{o}C maintained by a circulator (LC 10, Grants Instruments Ltd) with refrigerating capacity, was used. A heat exchanger made from 8mm O.D. copper tubing for the sample air flow and another made from 6mm O.D. copper tubing for the reference air flow, were immersed in the bath. Suitable drain points were included to allow the drainage of condensed water. To allow calculation of the water vapour partial pressure, a thermistor (YSI 44011, Yellow Springs Instruments Ltd.) was inserted in the exit air stream from the sample circuit heat exchanger. The use of the cold bath removed any requirement for consumables and avoided any occlusion of the gas piping.

The gas, of known dew point, was analysed for oxygen concentration using a dual cell paramagnetic oxygen analyser (OA184, Taylor Servonex Ltd.) and for carbon dioxide concentration using a single channel infra-red gas analyser (IRGA 20, Grubb Parsons Ltd). The oxygen analyser (Figure 27) was wall mounted to reduce any effects of vibration. The flow into the oxygen analyser cells was set at 75 ml min^{-1} with approximately 0.5 l min^{-1} being taken via a bypass flowmeter. The bypass air ensured that flow and pressure conditions in the measuring cell were stable. The flow through the carbon dioxide

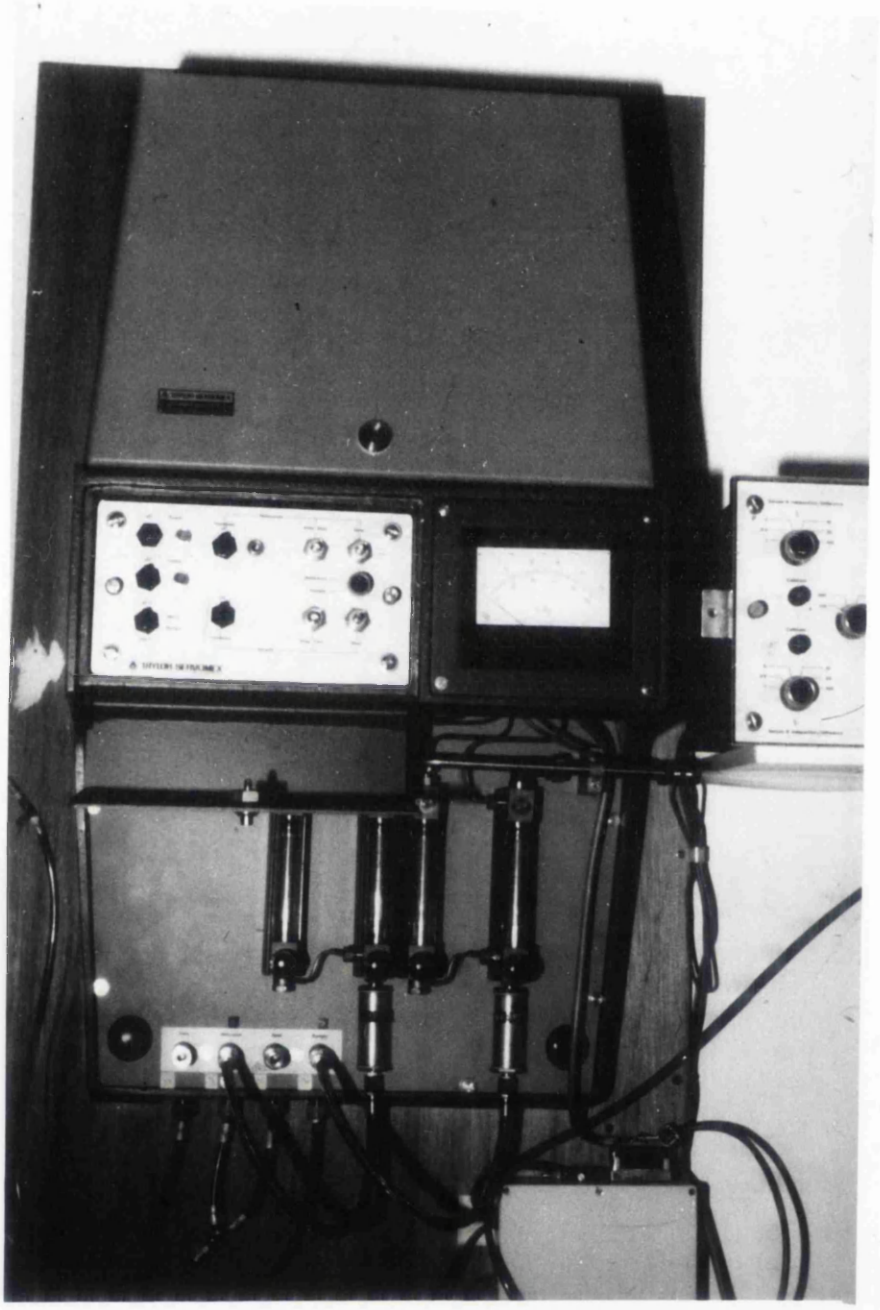


Figure 27. The oxygen analyser.

analyser (Figure 28) was set at the 1.67 l min^{-1} recommended by the manufacturer.

6.2.3 Additional Signal Sources

Monitoring of patient and room temperatures was required during calorimetry. An estimate of core temperature was also required but it was felt that the use of a rectal probe could be a stress big enough to cause a rise in the RMR of a subject. Though there is a temperature gradient along the auditory canal (Cooper, Cranston and Snell, 1964; Greenleaf and Castle, 1972), the amount of the underestimate of core temperature by a probe in the canal should be the same for a given patient on successive days in a constant environmental temperature. Therefore a silicone rubber earpiece, originally designed as a sound deadener in noisy situations, was fitted with a thermistor (YSI 44011, Yellow Springs Instruments Ltd.), the tip of the thermistor just emerging from the rubber moulding (Figure 29). The rubber ridges around the moulding allowed an easy but comfortable placement in the auditory canal. A similar thermistor was set permanently into the canopy near the air inlet to monitor the air temperature.

Room temperature was monitored using a caged thermistor (YSI 405, Yellow Springs Instruments Ltd.). This was hung at bed level behind the patient, the metal cage protecting it from any physical damage during the normal ward routine. Thermistors (YSI 409, Yellow Springs Instruments Ltd) with a 9mm diameter flat metal surface were taped

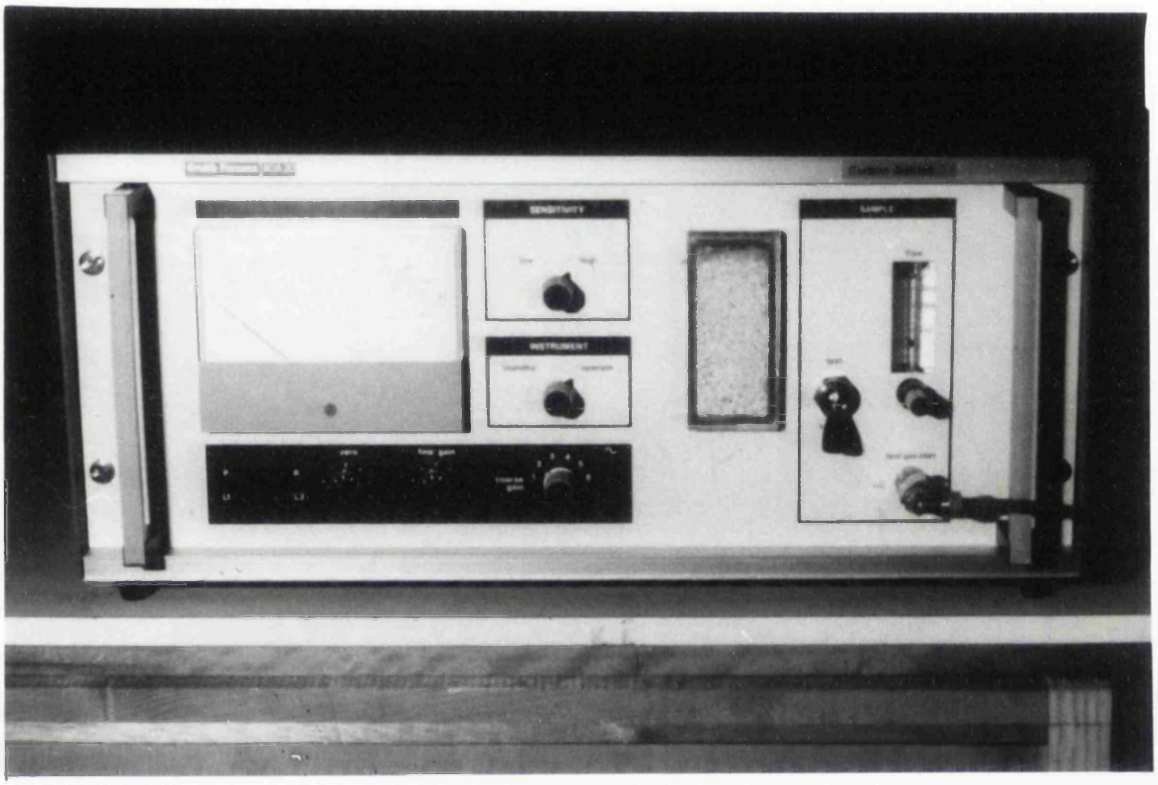


Figure 28. The carbon dioxide analyser.

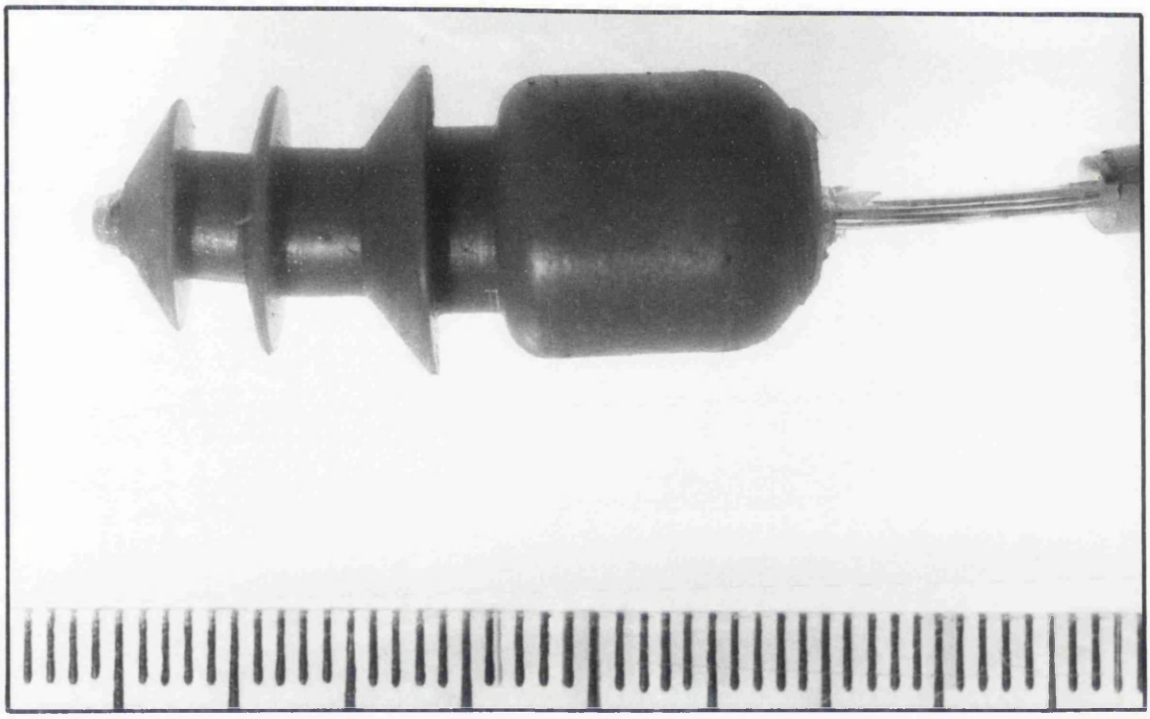


Figure 29. External auditory canal temperature probe.

on to the patient to measure skin temperatures when required.

Because of the importance of measuring metabolic rate during a resting period where movement was at a mimumum, an activity monitor was constructed. It allowed continuous monitoring of the subject, and exclusion of any portions of the calorimetry period that were affected by restlessness.

The detector used was a microwave Doppler module (CL 8960 Mullard Ltd.) that contained both a microwave generator (Gunn Diode) and a mixing diode. The frequency of the microwave beam (10.7 GH_z) from the Gunn diode was altered by 69 Hz for each 1 ms^{-1} of relative axial movement between the target and the module. A fraction of the original beam was coupled to the mixer diode so that difference frequency was produced on reception of the reflected beam (Fig. 30). This audio frequency was then amplified with a voltage gain of approximately 10000 (80 dB) using three operational amplifiers (SN72741, Texas Instruments Ltd). Because of the susceptibility of the circuit to mains frequency interference, the amplifier output was modified using a twin-tee 50 Hz bandstop filter. This did not restrict the detection of activity because movements which produce a 50 Hz signal have axial velocity of 0.7 ms^{-1} and these are unusual in a subject on a bed. Also a large number of movements occur obliquely to the beam and all have significant low velocity components because they start and finish at rest.

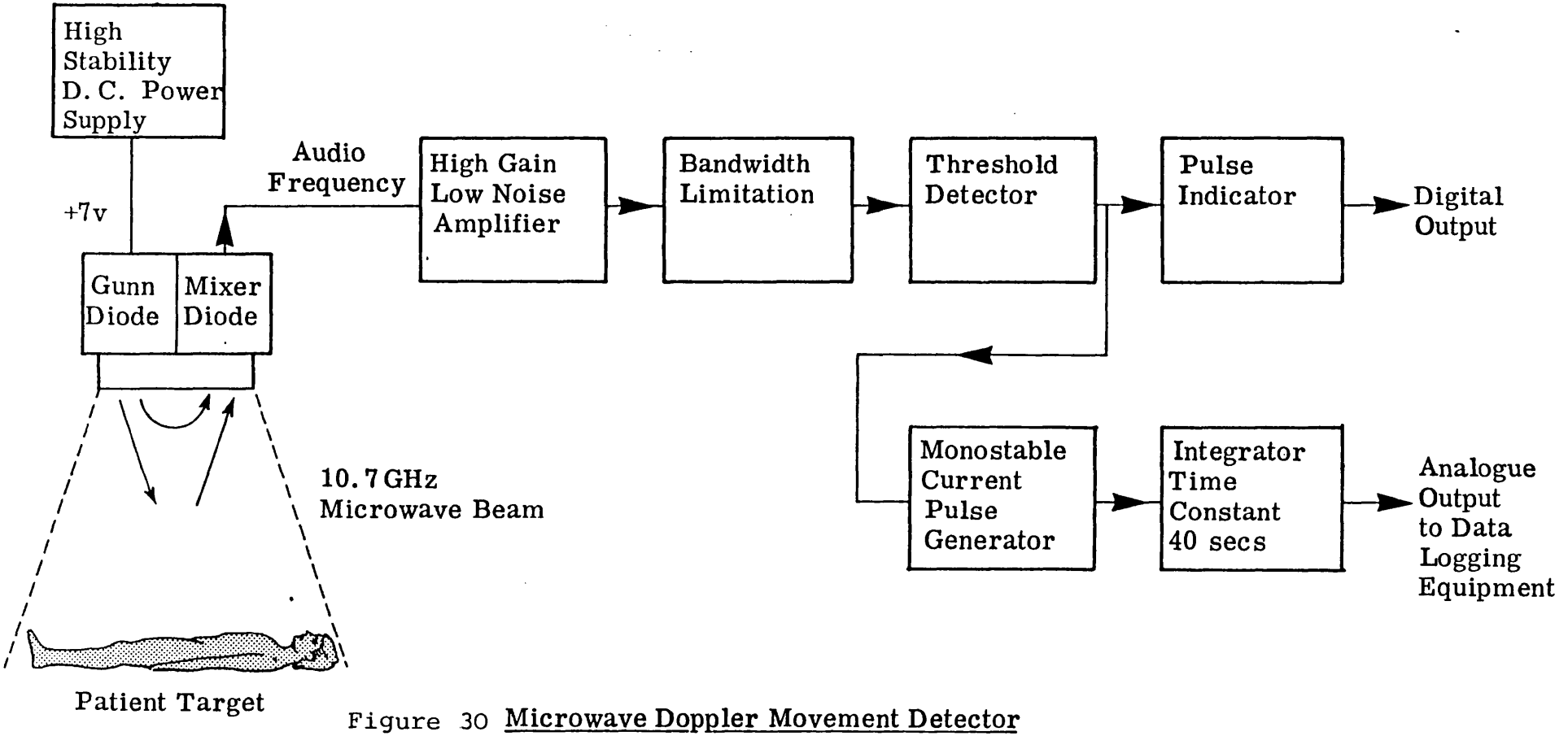


Figure 30 Microwave Doppler Movement Detector

The filtered signal was then detected using a threshold sufficiently high to ensure that there was no false triggering when there was no target movement. The number of pulses produced by the detector was approximately proportional to the distance moved by the target in the beam. The pulses were used to drive a light emitting diode to give a visual indication of the amount of movement detected. The leading edge of the pulses was also used to trigger a monostable (CD4098B, RCA Ltd.) which input a fixed charge per pulse into an integrator, built using an M.O.S.F.E.T. input operational amplifier (CA3130, RCA Ltd.) with a low leakage polycarbonate capacitor as feedback. The time constant of the integrator was reduced to 40 seconds to be compatible with the sampling frequency of the data logging equipment, by connecting a high value resistor across the capacitor. The movement detector was used in a qualitative role to exclude periods of restlessness rather than in a quantitative role to allow correction of values affected by movement. It also provided a useful record of any interruptions by staff during a calorimetry period.

6.2.4 Data Logging and Computer Equipment

The electronic signal sources were all either low D.C. voltages or pure resistances. A four and a half digit voltage and resistance meter (DMM 1041, Datron Ltd.) giving a precision of $\pm$ 1 in 20,000 and a calibrated accuracy of $\pm$ in 10,000 on the ranges required was utilised to convert the analogue input signals to digital form. The signal sources were switched through low thermal e.m.f

relays in a scanner unit (1200 Scanner, Datron Ltd) to the DMM (Figure 31). The appropriate relay to be closed was selected by a command transmitted over the I.E.E.E. - 488 communications bus from the microcomputer (PET 2001, C.B.M. Ltd.) to the scanner. The DMM range and mode (i.e. voltage or resistance) for that particular signal was selected and the analogue to digital conversion started by another command from the microcomputer.

On completion of the conversion, the digital representation of the measurement was transmitted back to the computer. The signal sources could be measured at a rate and in an order determined by the programming of the computer.

The results of the measurements were stored by the computer on mini-flexible discs for later reprocessing. The current values of the instrument outputs plus the calculated heat production and RQ were displayed on the computer screen and the calculated values recorded in graphical form on a matrix printer (Figure 32). An overall view of the instrumentation in the laboratory is shown in Figure 33.

6.3 Development and Testing

6.3.1 The Gas Circuit

Having established the gas circuit as shown in Figure 26, it was necessary to leak test the length of stainless steel pipe (approximately 25 m) bringing the gases back from the patient rooms. As the pump acted as a block to

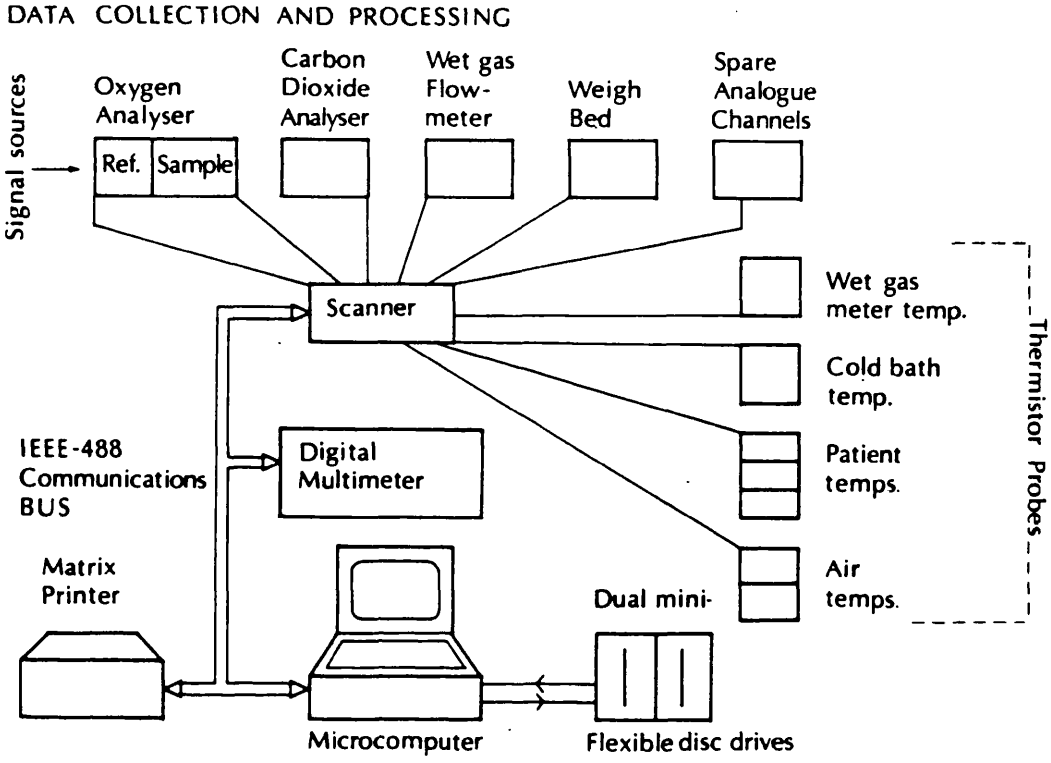


Figure 31. Data collection and processing equipment for the indirect calorimeter.

Figure 32. The microcomputer, disc drives and matrix printer.

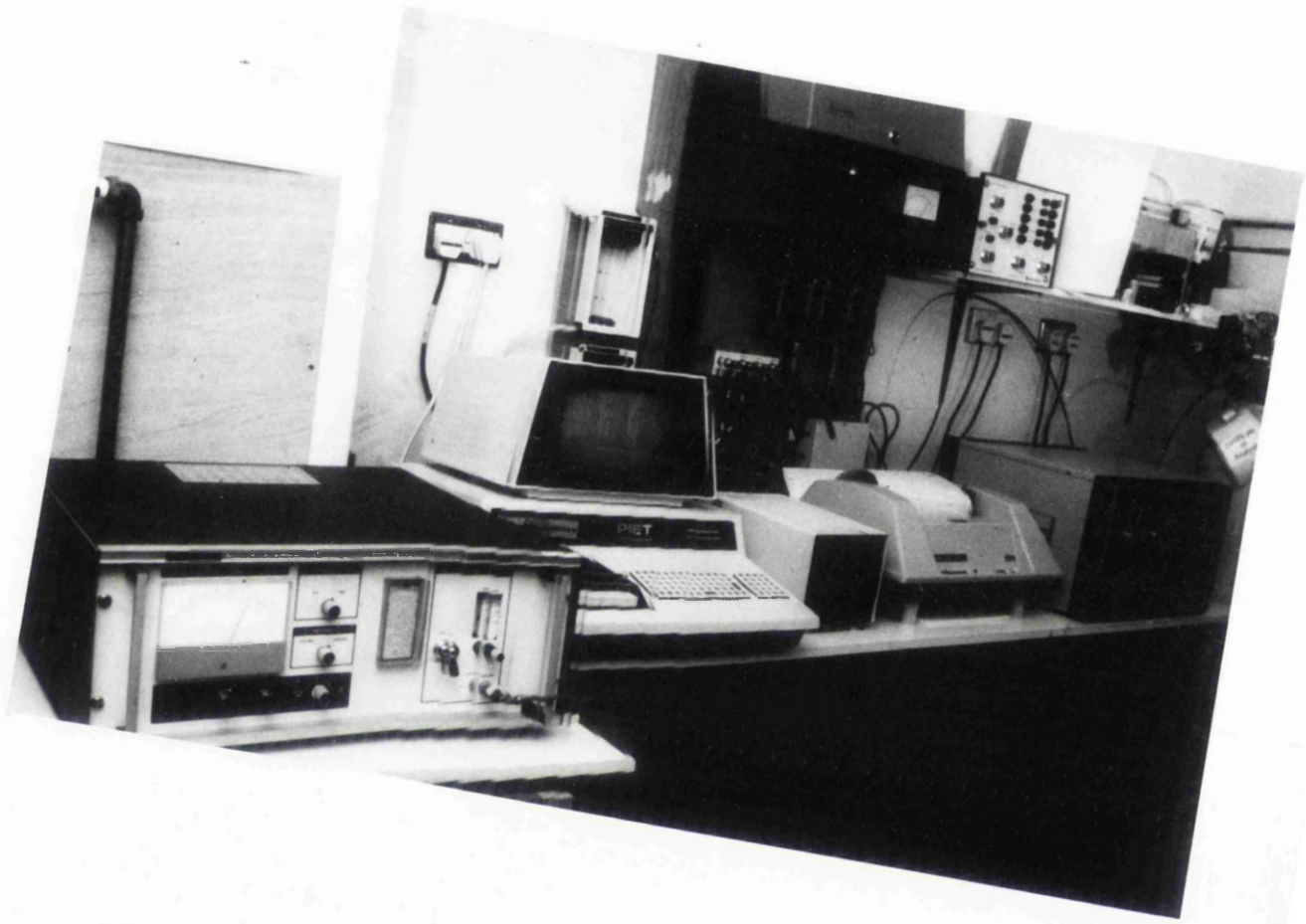


Figure 33. General view of the laboratory.

static air pressure tests, the piping between the pump and the wet gas meter could not be tested at the same time. The piping was occluded in the patient room and a manometer (Airflow Developments Ltd.) and a three way tap were attached to the piping instead of the pump in the laboratory.

Evacuating the pipe to its approximate working pressure of - 300 mm of water, pressure returned to atmospheric at a rate of 2.5 mm of water per minute. As the pipe with an internal diameter of 20 mm had a volume of 7.9 l, there was a leak of about 70 mls of air per minute. As this represented less than 0.2% of the total air flow and could only constitute an error if its composition varied markedly from the air input to the canopy, the effect of the leak was negligible. Pressure testing the short length of pipe from the pump to the wet gas meter gave a rate of pressure drop of less than 1 mm of water per minute at a pressure of 300 mm of water. This was similarly a negligible error.

The mixing of the expired gases before analysis needed to be enough to eliminate breath to breath variation but still allow general changes in gaseous production to be detected within about one minute. As the gases were collected in the canopy from a perforated perspex tube in front of the mouth of the subject, the canopy only contributed a small amount to the mixing. The response of the circuit from the outlet of the canopy onwards was checked by introducing a gas of known concentration (0.8% carbon dioxide and 99.2% nitrogen i.e. similar to

the carbon dioxide concentration during a calorimetry run) at two points in the circuit. Firstly the gas was rapidly introduced (i.e. in as close an approximation to a step function as possible) directly into the carbon dioxide analyser in the laboratory. The analyser response to the gas was output on a chart recorder. Secondly the gas was introduced at 35 to 40 litres per minute into the piping from the outlet port of the canopy in the patient room. The carbon dioxide analyser responses in these two situations are shown in Figure 34.

When the gas was introduced directly into the analyser, the output increased rapidly to reach 90% of its final level in six seconds. When the gas was introduced in the patient room there was a 36 second delay before any response was seen at the analyser. This transit delay was consistent with 20 to 25 litres of dead space between the canopy outlet port and the analyser. As the volume of the stainless steel tubing and wet gas meter totalled approximately 15 litres, the transit delay was close to the expected value. The analyser took another 37.5 seconds (i.e. 73.5 seconds in total) to reach 90% of its final output. Assuming that the response was a single exponential:-

$$\text{Concentration at analyser} = (1 - e^{-t/T}) \times C_F \qquad 6.1$$

where t is the time in seconds after the transit delay and C_F is the final concentration, then the time constant (T) is 16.3 seconds. A perfectly mixed volume V(l) having

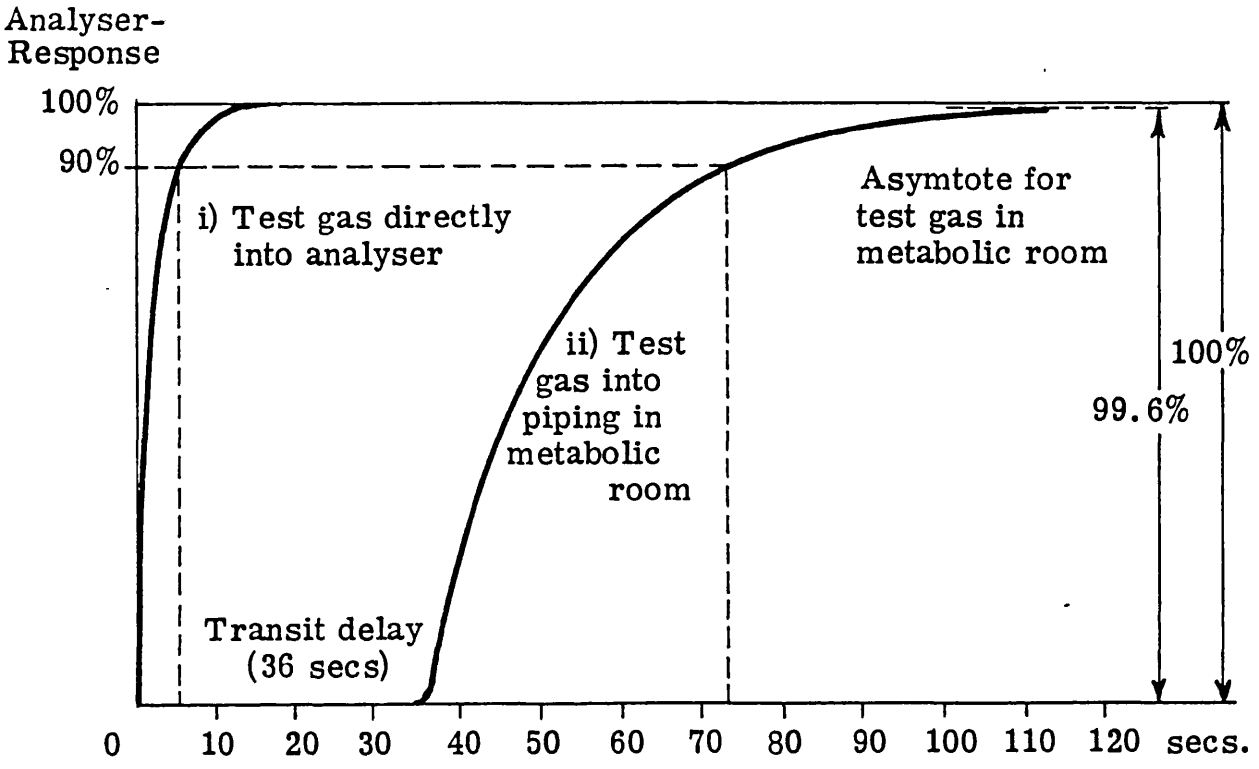


Figure 34. Carbon dioxide analyser response to test gas introduced i) directly into the analyser and ii) into the piping in the patient room.

an input flow rate F (ls^{-1}) would have a time constant (T) defined by equation 6.2 (Sorkin et al., 1980).

$$T = V/F \qquad 6.2$$

The effective mixing volume of the gas circuit can be calculated to be about 10 to 11 litres. As laminar (i.e. non-mixing) flow could be expected along most of the stainless steel piping, this value agreed with the volume of the pump and wet gas meter. The time constant (16.3 seconds) was sufficient to average breath to breath variations as it would cover a minimum of three breaths in the normal patient. The response however was fast enough to allow minute to minute variations in metabolism. to be measured.

When the test gas was introduced into the piping in the patient metabolic room, the final concentration at the analyser was 99.6% of the value obtained with direct input to the analyser. This demonstrated that there was negligible entrainment of air into the portion of piping operating below atmospheric pressure. Any outward leaks between the return pump and the analyser would not be detected, though leaks in this direction were not important once the total flow had been measured at the wet gas meter.

6.3.2 Oxygen and Carbon Dioxide Analyser Calibration

The linearity of both oxygen analyser cells was checked using the pressure calibration method of Hammel, Wyndham and Hardy (1958). The oxygen analyser measures a quantity

related to the number of oxygen molecules in the cell, so that reducing the cell pressure reduces the effective oxygen concentration. Using this method the concentration could be varied over the range 19 - 22% which exceeds the normal operating range (20 - 21%).

A manometer (Airflow Development Ltd.) was connected to one side of the analyser and a needle valve and 60 ml syringe were connected to the other. The syringe allowed the cell pressure to be varied over the range required and the cell pressure with respect to atmospheric pressure was read in mm of water from the manometer. The linear regressions performed on the results for the reference and sample cell are shown in Table 29. The calculations show very high correlation coefficients and a sample standard deviation from regression (i.e. the standard deviation of the differences between the measured oxygen concentrations and those values from the fitted regression line) of 0.01% of oxygen for both analysers. This last value represented about 1% of the change in oxygen concentration produced by a patient during a calorimetry run so that the analysers were sufficiently linear for use on the calorimeter.

Routine calibration of the gain of the oxygen analyser required a procedure simpler than the pressure test and a two point calibration using a span gas (atmospheric air with an oxygen concentration of 20.95% (Thompson et al., 1979)) and zero gas (oxygen free nitrogen) was employed. When comparing the sensitivity of the

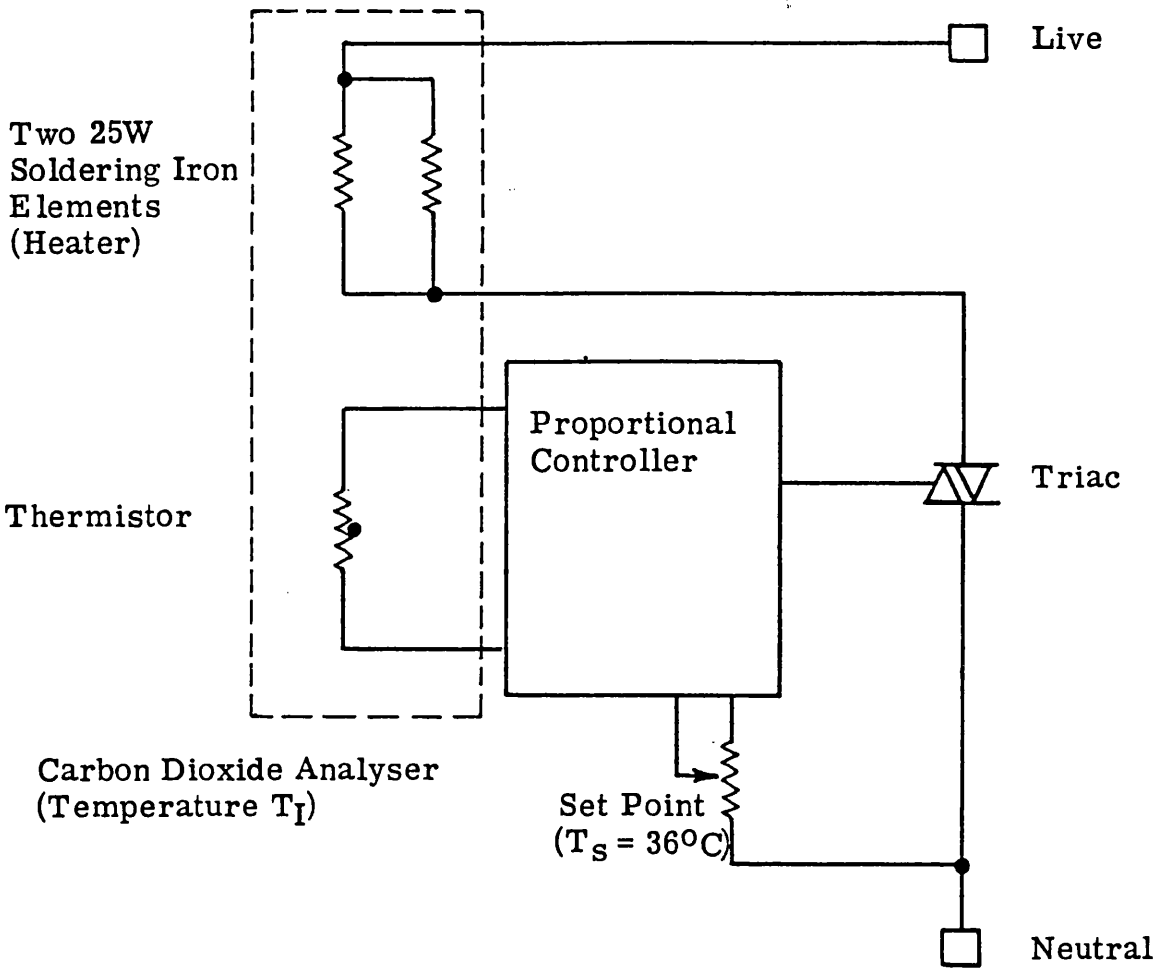
		Reference	Sample
Correlation coefficient	(r)	0.99998	0.99999
T value of correlation	(T)	591.6	479.6
p value	(p)	***	***
Degrees of freedom	(d.f.)	35	23
Regression coefficient (b) in % O_2 per mm of water		1.997×10^{-3}	2.036×10^{-3}
Standard deviation of the regression coefficient	(S_b)	3.38×10^{-6}	4.25×10^{-6}
Sample standard deviation from regression % O_2	($S_{y.x}$)	9.57×10^{-3}	9.92×10^{-3}

(see Snedecor and Cochran (1967) p. 138-)

Table 29: Linear regression calculations on data from the linearity calibration of reference and sample oxygen cells.

analysers by the two calibration methods account has to be taken of the diagmagnetic property of nitrogen. It has a magnetic susceptibility of -0.3% of that of oxygen. As pressure was reduced in the analyser cell the amount of nitrogen in the cell was also reduced and the change in analyser output was less than if just the oxygen had been removed (Carter, 1975). Also the replacement of 21% of oxygen with nitrogen when the zero gas (oxygen free nitrogen) was used to calibrate the analyser has to be corrected for the diagmagnetism of nitrogen. After these corrections had been performed the sensitivities of the reference and sample cells by the pressure calibration were 99.6 and 100.2% respectively of the sensitivities measured by the two point calibration technique. Therefore the two point calibration technique was adopted as the routine calibration procedure as the average sensitivity over the 0 to 21% range was within $\pm$ 0.5% of the sensitivity over the operating range 20 - 21%.

Initial use of the carbon dioxide analyser showed that it was prone to short term drift due to temperature changes in the gas cells following small room temperature alterations. As this instrument did not have a thermostatically controlled heater for maintaining gas cell temperature as did the oxygen analyser and the infra-red gas analysers used in the small animal calorimeter (LIRA 100, M.S.A.; IRGA, Analytical Development Ltd.), a proportional temperature controller was built (Figure 35)



CARBON DIOXIDE ANALYSER HEATER and CONTROLLER

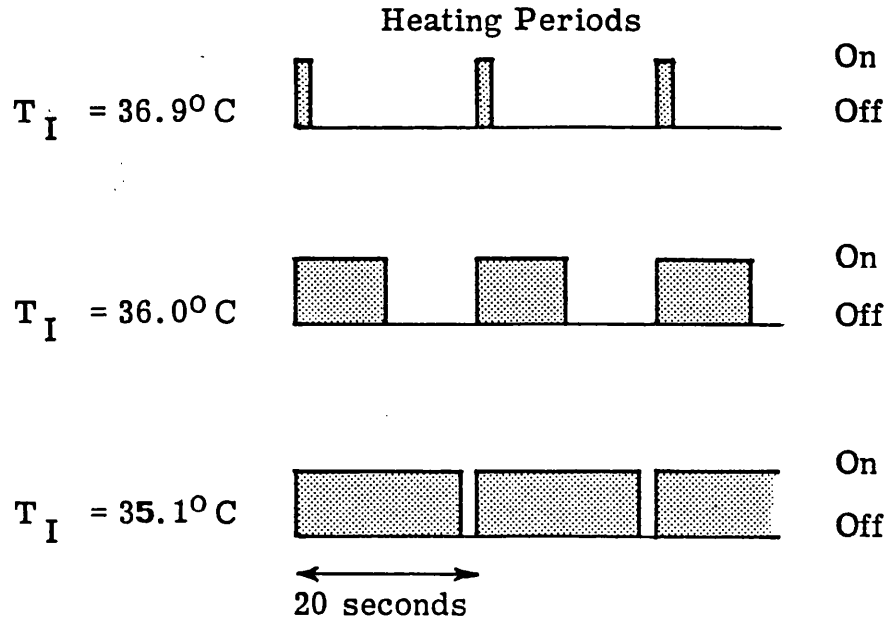


Figure 35. Proportional temperature controller for the carbon dioxide analyser.

to keep the internal temperature of the instrument at a nominal 36°C. Proportional control was used to minimise temperature variation and the proportional range was set to cover 35 to 37°C. A thermistor (YSI 44011, Yellow Springs Instruments Ltd.) sensed the instrument temperature and a controller integrated circuit (305 - 800, Radiospares Ltd.) supplied triggering pulses at points of zero mains voltage to switch on a triac (TRI 400-8, Radiospares Ltd.) for the required proportion of the heating period. When the triac was on, power was supplied to two 25W soldering iron elements mounted inside a small metal box in the analyser. The analyser still required 1 to 1.5 hours to reach temperature equilibrium from cold, so a 24 hour time switch was used to switch the heater on a few hours prior to use.

The linearity of the carbon dioxide analyser was checked by mixing air in different proportions with a bottled gas of composition 0.8% carbon dioxide and 99.2% nitrogen to produce a range of carbon dioxide concentrations between 0.03% and 0.80%. The concentration of carbon dioxide was measured indirectly by the oxygen analyser because as the percentage of carbon dioxide introduced from the bottled gas rose so the percentage of oxygen in the mixture fell in direct proportion. A high correlation ($r = 0.99985$, $p < 0.001$) and a sample standard deviation from regression of 0.005% carbon dioxide demonstrated that the analyser was linear to within $\pm$ 1% over its normal operating range (i.e. 0 to 1% carbon dioxide).

The analyser was routinely calibrated using the same zero gas as the oxygen analyser and a span gas of about 0.8% carbon dioxide (balance nitrogen) which was produced gravimetrically (Gold Star, B.O.C. Ltd.) and checked by the Lloyd Haldane method by the Department of Physiology, Glasgow University.

Routine calibration of both analysers was carried out using the zero and span gases described above. All the measurements were made under computer control. The readings were corrected for atmospheric pressure and in the case of the air used as a span gas for the oxygen analyser for its moisture content. The computer calculated correction factors, close to unity, to account for the long term gain drift in the analysers. Gain and zero drifts of the instruments were not corrected using the various mechanical and electronic adjustments available, but the correction factor was applied by the computer during the calculation of results. The long term stability of the analysers was improved with less interference and interdependent effects which made accurate setting difficult (e.g. a change in gain on adjustment of the zero point) were avoided.

As the analysers were measuring small concentration differences despite the precautions taken above the analyser output was subject to short term drift during a calorimetry period. The main reasons were:-

i) Room and analysis gas temperature changes

ii) Atmospheric pressure changes

iii) Air flow alterations in the analysis cell.

To correct for this drift, measurements using external air were routinely made at the beginning and end of a calorimetry period. Any change in the readings was assumed to have occured at a linear rate through the run. The measured gas changes during calorimetry were then recalculated by the computer using this linear baseline. The effect of this procedure on a calorimetry run with no subject in the canopy, can be seen in Figure 36. Over the one hour period (the normal period used for calorimetry) atmospheric pressure dropped by 1mm of mercury and the oxygen analyser output fell by 0.02% of oxygen. Both these changes are larger than normal, the average change in oxygen analyser output during the routine runs in Study C being half that value. The apparent heat production dropped to -1.4 W because of the analyser drift. Applying the linear baseline correction, the maximum error was -0.5 W and the average -0.2 W. With a typical subject with an RMR of 75 W, this represents a maximum error of 0.7% and an average error of 0.3%.

6.3.3 Wet Gas Meter Electronics

The conversion of the pulse output from the wet gas meter to analogue form by the ratemeter (see Section 6.2.2) was calibrated using a pulse generator (PG 2B, C.Lyons Ltd.) and a pulse counter and clock (Nim Range, Nuclear Enterprises Ltd.). The stability and exact frequency of the pulse generator were checked using the counter and clock.

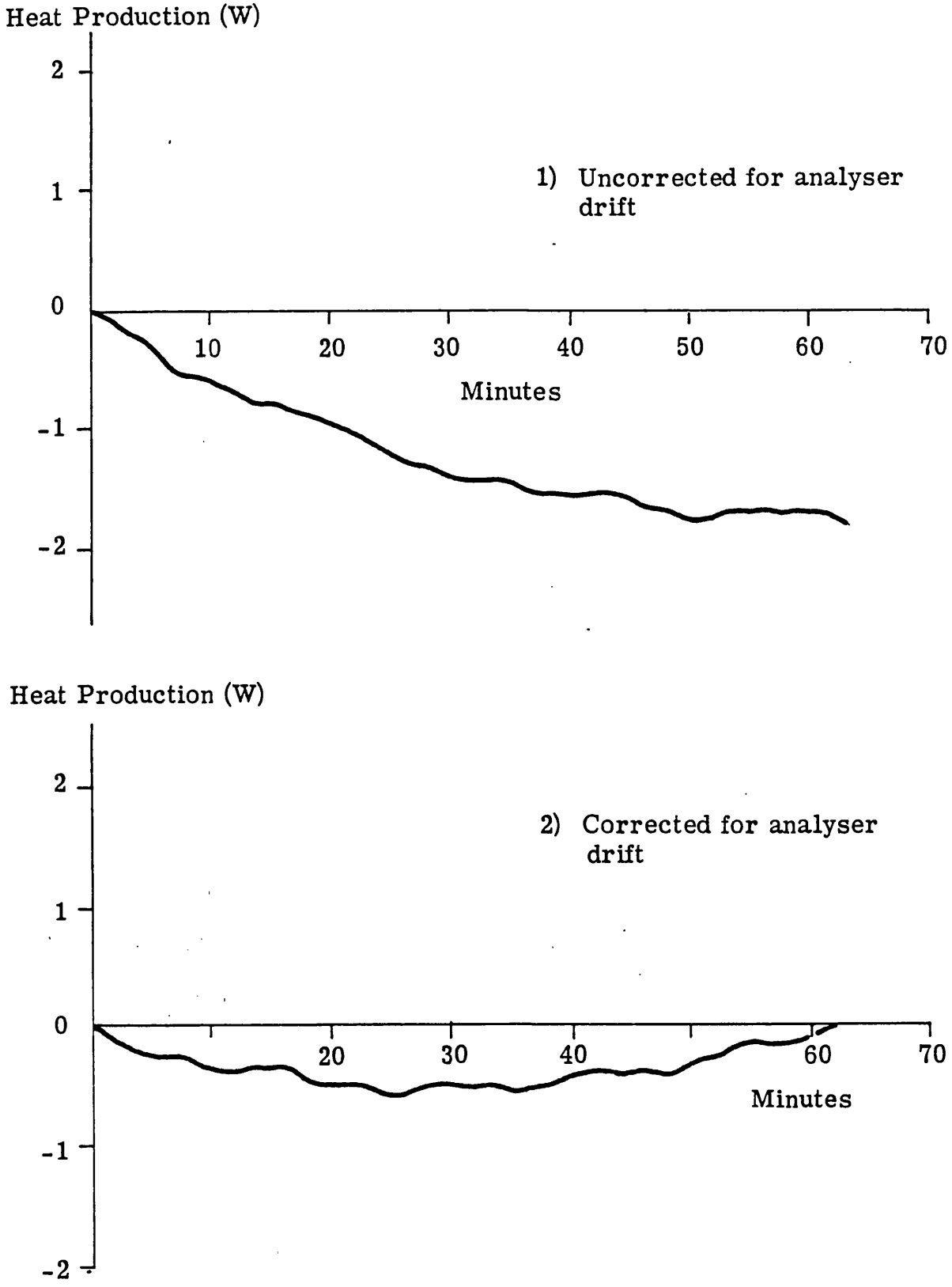


Figure 36. A calorimetry measurement with no subject in the head canopy showing the correction for drift in the gas analysers.

The generator frequency was varied to cover the normal input range. The manufacturer's calibration for the meter (i.e. that one revolution producing 360 pulses from the phototransistor was equivalent to 15 l of gas) was used to convert the pulse output to a rate of gas flow in litres per minute. (F).

The fitted regression line was:-

$$F = 75.9 \times V - 0.059 \quad (l\ min^{-1}) \qquad 6.3$$

where F was in litres per minute and V in volts was the ratemeter output. The correlation coefficient was high ($r = 0.99999$, $p < 0.001$) and the sample standard deviation from regression was 0.08 l min^{-1}. The small negative intercept reflected the voltage offset of the ratemeter at zero frequency input.

6.3.4 Gas Circuit Calibration

The overall accuracy of the indirect calorimeter can be checked in several ways. Though calibrated gases can be bled into the system at known flow rates (Kinney et al., 1964; Thompson et al., 1979), this depends for its accuracy on the flow meter and gas specifications. A simpler technique of burning a known weight of a fuel to simulate a subject (Caldwell et al., 1966; Kinney et al., 1968) was adopted to avoid further dependency on gas and flow meter calibrations.

Ethanol and butane were selected as appropriate fuels with 'respiratory quotients' of 0.667 and 0.615 respectively.

Initially ethanol (Analar grade, 99.7% pure) was burnt using a small lamp placed in the canopy. The lamp was weighed immediately before and after use and the total oxygen consumption and carbon dioxide production for that period calculated by the computer program normally used for processing calorimetry runs. If the ethanol was assumed to have been completely oxidised as in equation 6.4, then the recoveries of oxygen and carbon dioxide were 91.4 and 88.9% respectively of the calculated amounts (Table 30).

$$C_2H_5OH + 3O_2 \longrightarrow 2CO_2 + H_2O \qquad 6.4$$

It was noted that during the test the normally blue burning flame occasionally became partly yellow and some carbon deposits were also seen. On partial oxidation of ethanol carbon, being in a solid phase and unable to be oxidised further at the flame temperature, would condense out. Some carbon monoxide would also be formed but as it is a gas and could be oxidised further in the flame it would contribute a smaller error than carbon production. The conversion of ethanol to carbon is shown in equation 6.5

$$C_2H_5OH + O_2 \longrightarrow 2C + 3H_2O \qquad 6.5$$

If a percentage of the ethanol was burnt in this manner then as observed the 'RQ' would be lower than that calculated from equation 6.4. Assuming no error in the separately calibrated gas analysers, the measured 'RQ' of 0.649 can be used to estimate the proportionate contribution of each reaction. If Z mols of ethanol were used in reaction 6.4 and y mols in 6.5 then:-

Initial weight of lamp + ethanol = 525.5 g

Final weight of lamp + ethanol = 515.2 g

Ethanol used = 10.3 g

Assuming oxidation as in equation 6.4

	Measured		Calculated		Measured ÷ Calculated (%)
	mol	l	mol	l	
Oxygen consumed	0.613	13.73	0.671	15.03	91.4
Carbon Dioxide produced	0.398	8.91	0.447	10.02	88.9
RQ	0.649		0.667		97.3

Assuming oxidation as in equations 6.4 to 6.9

	Measured		Calculated		Measured ÷ Calculated (%)
	mol	l	mol	l	
Oxygen consumed	0.613	13.73	0.637	14.28	96.2
Carbon dioxide produced	0.398	8.91	0.413	9.26	96.2

Table 30: Calibration of the calorimeter using ethanol assuming either complete or partial oxidation as detailed in equations 6.4 to 6.9

Total ethanol used = z + y mols 6.6

Total oxygen consumed = 3Z + y mols 6.7

Total carbon dioxide produced = 2Z mols 6.8

and therefore the 'RQ' $= \frac{2Z}{(3Z + y)}$ 6.9

The solutions for Z and y are:-

Z = 0.2066 and y = 0.0169 6.10

indicating that 8.2% of the ethanol burnt to carbon. The percentage recoveries of oxygen and carbon dioxide increased to 96.2% of that calculated.

However, because of the varying reaction products and the possibility of ethanol evaporation at the edges of the low temperature flame, calibration with butane gas was tested. Commercial butane gas (Camping Gaz Ltd.) in a cartridge was burnt in a small luminaire designed for auxillary lighting purposes (Lumogaz, Camping Gaz Ltd.). The apparatus was self-contained and could fit inside the head canopy. The manufacturers stated that the gas composition should be between 100% butane and 90% butane with 10% propane added. Analysis using gas chromatography by Dr. Anderson, Department of Forensic Medicine, Glasgow University showed the mixture to be 93.30% butane, 0.34% propane and 6.38% butene. Comparison of the amount of oxygen consumed and carbon dioxide produced per unit weight of gas (Table 31) for the three mixtures of gas mentioned shows the relative insensitivity of the calibration procedure to differences in the gas composition.

	Consumption of Oxygen (mmol)	Production of Carbon Dioxide (mmol)	'RQ'
per g Butane	111.8	68.8	0.615
per g of 90% Butane + 10% Propane	112.0	68.7	0.614
per g of fuel as analysed	111.5	69.0	0.619

Table 31: The consumption of oxygen, production of carbon dioxide and 'RQ' of various fuels per g burned.

The result of burning the gas mixture in the canopy is given in Table 32. The high temperature flame produced (approximately 700 ^{o}C) should give complete oxidation of the gases as in equations 6.10 to 6.12.

$$\text{Butane} - 2\,C_4H_{10} + 13O_2 \longrightarrow 8CO_2 + 10H_2O \qquad 6.10$$

$$\text{Propane} - C_3H_8 + 5O_2 \longrightarrow 3CO_2 + 4H_2O \qquad 6.11$$

$$\text{Butene} - C_4H_8 + 6O_2 \longrightarrow 4CO_2 + 4H_2O \qquad 6.12$$

The recovery of oxygen was 97.2 and of carbon dioxide was 97.4% of the calculated value. These results were similar to the corrected results obtained when burning ethanol. As there was an almost equal error for oxygen and carbon dioxide, a flow measurement or gas leakage error affecting both gases was indicated. As earlier experiments have shown gas leakage from the circuit to be negligible, an undermeasurement of flow by the wet gas meter was the most probable source of error. The test described above was used as the system calibration and both oxygen and carbon dioxide measurements were increased by a factor of 1.028 to account for the underestimate of flow.

6.3.5 Calibration of Thermistor Temperature Probes

Two types of precision thermistor from one manufacturing source (Yellow Springs Instruments Ltd.) were used. A simple thermistor bead (YSI 44011) with a resistance of 100 kΩ at 25^{o}C was used for instrument temperature measurement. Room and patient temperatures were measured with series 400 thermistors (resistance 2kΩ at 25^{o}C) which used a similar thermistor bead but with different physical exteriors to give protection during use. All

Initial wt of lamp + butane fuel = 852.70g

Final wt of lamp + butane fuel = 830.64g

Weight of fuel used = 22.06g

	Measured		Calculated		Measured ÷ Calculated (%)
	mol	l	mol	l	
Oxygen consumed	2.391	53.59	2.460	55.14	97.2
Carbon dioxide produced	1.482	33.22	1.521	34.09	97.4

Table 32: Calibration of the calorimeter using the composition of the commercial butane fuel as analysed in Section 6.3.4

the thermistors were manufactured to a replacement tolerance of $\pm$ 0.1^{o}C.

Using a linearising bridge and stable excitation voltage, a thermistor can produce a direct voltage reading of temperature (Carter, 1975). However, as the digital meter employed (1041, Datron Ltd.) had resistance measurement capabilities, the thermistor was read directly. The test currents and duty cycle under normal logging conditions were below the level that would cause self heating in the thermistors.

The conversion of resistance to temperature was performed in the computer. Conversion using a formula rather than a 'look-up' table would save computer memory space and conversion time so as the theoretical resistivity of a pure semiconductor (Millman and Halkias, 1972) varies as shown in equation 6.13, a multiple regression fit using a logarithmic term was performed.

$$\text{Resistivity} \propto T^{-3/2} \times e^{E/2kT} \qquad 6.13$$

where T is the absolute temperature, E the semiconductor energy gap at 0^{o}K and k the Boltzman constant.

The regression fit was not to the required accuracy ($\pm$ 0.1^{o}C) however graphical inspection of the error terms showed that extra terms in resistance might reduce the errors. Addition of two extra terms brought the formula and manufacturers values into $\pm$ 0.1^{o}C agreement over the range used (5 to 40^{o}C). The formula (equation 6.14) could be used with either type of thermistor with a suitable choice of constants (see Appendix C for A_0 to A_3).

$$\text{Temperature } (^{o}C) = A_o + A_1 R + A_2R^2 + A_3 \log (R) \qquad 6.14$$

where R is the resistance in k and A_o to A_3 are constants.

As resistance measurements were two wire rather than four wire, the measured resistance in the leads between the meter and the thermistor was subtracted before conversion. An overall check of the procedure was performed by comparing the measured temperature of a water bath, using a partial immersion mercury thermometer accurate to $0.1^{o}C$ (Gallenkamp Ltd.), with the computer readout of the temperatures of thermistors completely immersed in the bath. All the thermistors were within $\pm$ $0.1^{o}C$ of the thermometer temperature over the required range (5 to $40^{o}C$).

6.3.6 Data Processing

The microcomputer (PET 2001, C.B.M. Ltd.) had control over the data logging equipment via the IEEE 488 communications bus (see Section 6.2.4). This meant that most of the operational checks and analyser calibrations as well as calorimetry data processing could be controlled by software in the computer. A number of programs were written to cover the main areas of calorimetry operation. These areas were:-

i) Routine switch on and equipment checks

ii) Manual input to the computer of subject details (Name, weight, etc.) plus any other values (atmospheric pressure etc.) not available electronically through the logging equipment.

iii) Checks on the stability of the equipment before the subject entered the head canopy.

iv) Logging of all instrument outputs at the required repetition rate and recording of the data.

v) Display of the current instrument values and the calculation of heat production and RQ during the calorimetry period.

vi) After the subject leaves the canopy, checks on the drift of the gas analysers since iii).

vii) Security storage of data in case of later equipment or operator error.

viii) Calculation of calorimetry results over any required period, including corrections for atmospheric pressure, pressure and temperature at the gas flow meter, water vapour pressure correction, gain calibration factors for the gas analysers and analyser baseline drift.

ix) Numerical and graphical printout of the results.

x) Control of the procedure and calculation of the results of the routine calibrations of the gas analysers.

The calculations used in these programmes are detailed in Appendix C. Heat production was calculated from oxygen consumption and carbon dioxide production using the same equation (Weir, 1949) as used in the indirect calorimetry calculation for the small animal calorimeter (Carter, 1975). No correction for variation of the fraction of protein calories from the 12.5% of total heat production assumed in this equation, has been made. In elective surgery patients (Duke et al., 1970) this fraction varied from 12 to 18% before and over the 10 day period following

operation. Neglecting this order of variation introduces an error of less than 0.5% in heat production calculations.

Heat production has been expressed in Watts where appropriate. However unlike RQ, heat production is known to vary with the size of individual (Mitchell, 1962). Therefore some form of standardisation procedure was necessary to compare results from subjects of different height and weight. The most widely used method is to express the results per unit surface area, usually using the formula of DuBois and DuBois (1916):-

$$\text{Surface area } (m^2) = 0.2025 \times (W)^{0.425} \times (H)^{0.725} \quad 6.15$$

where W is the weight in kg and H is the height in m.

This calculation has the disadvantage of underestimating surface area by 7% and showing a coefficient of variation of 11% when compared against an accurate geometric technique (Van Graan and Wyndham, 1964). The causal connection between surface area and metabolic rate is tenuous and the association is more likely to be between lean body mass and metabolic rate (Halliday et al., 1979). Durnin (1959) suggested that use of weight alone was a good enough reference standard. This neglects differences in build and Durnin and Passmore (1967) produced a table of normal resting energy expenditures for men at different weights with four degrees of fatness. At 70 kg going from fat to thin involved an increase of 15% in RMR as lean body mass increased. This type of variation is implicit in equation 6.15, because at a constant weight, as height and therefore leanness increase, so does surface area. As no

direct estimate of lean body mass was available and all correction procedures derived from simple measurements are to some extent arbitrary, the formula of DuBois and DuBois was used to allow easier comparison with previous workers' results. None of the subjects were considered to have a grossly abnormal build which might put them beyond the established range of the formula.

Predicted values of RMR show some variation with age. Where predicted values have been quoted they have been derived using surface area and age from the table of Fleisch (1951). This table was compiled from 24 sets of published data from other authors but it shows close agreement with the table of Robertson and Reid (1952) who measured the RMR of over 2000 British men and women. However the standards of Fleisch are more commonly quoted than those of Robertson and Reid (Wilmore, 1977).

Statistical analysis of the results was performed using specially written programs on the microcomputer used to control the calorimetry. Student T tests, and linear regression (Snedecor and Cochran, 1967) were used. In Study A an analysis of linear trend (McNemar, 1969) was used rather than a linear regression procedure because the trend was based on absolute values of heat production that varied from subject to subject. The analysis of the pattern of responses to operation in Study C was carried out using a pseudo-three way analysis of variance

(McNemar, 1969). This allowed analysis for the two variables (room temperature and time from operation) and their interaction. Comparisons between the daily post-operative mean values and the control day mean values were performed using Dunnett's (1955) procedure, using the estimate of variance from the pooled data. As other workers (Duke et al., 1970) had performed T-tests between the control day values and the average 4 day post-operative values for their patients, this was also carried out where appropriate in Study C.

CHAPTER 7

Human Studies

7.1 Outline of Studies

In study A nine healthy volunteers were used to establish the use of the calorimetry system and to provide RMR values which could be compared against standard tables. The thermogenic effect of a continuously administered enteric feed was also measured as it was hoped to use this feed in later patient studies (i.e. Study B) to provide a standardised oral feeding regime.

In Study B two patients who had undergone moderate severity surgery had regular calorimetry measurements for up to seven days following operation. For a number of reasons the study protocol was unsuitable and so for the next study it was modified and shortened, to cover only the four days after surgery. In Study C the changes in energy metabolism were measured in 20 patients following moderate severity surgery at one of the two ward temperatures (20 and 28^{o}C).

7.2 Study A

The subjects were 9 male volunteers (age range 18 - 29 years) with no known gastro intestinal problems and in good general health. Each subject fasted from 22.00 hours the previous evening and at 09.00 changed into light cotton clothing and entered the temperature controlled (28^{o}C $\pm$ 1^{o}C) ward room. As soon as possible a fine bore (1 mm internal diameter) P.V.C. nasogastric tube (Roussel

Laboratories Ltd.) was passed. The tube position was checked by auscultation over the epigastrum during rapid instillation of 10 ml of air. The subject then rested on the bed. At 10.00 the perspex canopy was placed over the head of the subject and an hour long calorimetry period commenced. The RMR was calculated from a 40 minute section of the run, starting after the subject became settled and comfortable in the canopy.

At 11.00 the canopy was removed and a continuous infusion of the diet at a rate of 194 ml per hour commenced. The food used was a proprietary whole protein diet (Isocal, Mead Johnston Ltd.), the composition of which is shown in Table 33. The rate was determined by filling a graduated burette (Travenol Ltd.) with 97 mls of the diet every 3o minutes. At the rate chosen, food energy was input at a rate of 232 W (200 kcal hr^{-1}). The subject was allowed to read or watch television while resting. At 15.00 the canopy was replaced for a second hour long calorimetry period while the subject continued to receive the infusion. This meant that the heat production of the subject was remeasured on average 4.5 hours after the start of the infusion.

7.3 Study B

Two male patients aged 50 and 68 years were allocated to the metabolic room on the ward before their operation. They were given a practice calorimetry run to familiarise themselves with the equipment and general procedure. That evening the patients were established on a

Constituents	energy %	amount per 100 ml (g)
Protein:		
soy protein isolate and caseinate solids	13	3.4
Fat:		
soy oil	30	3.6
Medium chain trigylceride oil	7	0.9
Carbohydrate:		
glucose oligosaccharides	50	13.3

Energy per 100 ml of the diet is 431 kJ.

Table 33: Constituents of the continuously administered diet (Isocal) in Study A.

continuous feed of Isocal (Mead Johnston Ltd.) via a fine bore nasogastric tube at a rate of 91 ml hr^{-1}. The next morning two more calorimetry measurements were made, the enteric feeding being discontinued after the second. The patients had an elective cholecystectomy operation the following day.

A calorimetry measurement was made on each post-operative day that the patient could tolerate it. The canopy had not been modified (see Section 6.2.1) and the patients had to lie flat for a calorimetry measurement. The only energy intake following surgery was the two litres per day of 5% Dextrose given intravenously until the patients were established either on enteric feeding using Isocal or a light oral diet approximately four days after the operation. The changes in heat production and RQ were measured for up to seven days following the operation.

7.4 Study C

Twenty male patients who were due to undergo elective cholecystectomy or vagotomy and drainage were studied. Ten were allocated to be nursed in the metabolic room with the environmental temperature at $20^{o}C \pm 1^{o}C$ and ten were allocated to be nursed at $28^{o}C \pm 1^{o}C$. Each patient was allowed the bed coverings he desired throughout the study. The weight and height of each patient was taken just before the operation to allow the

calculation of predicted metabolic rates.

One to seven days pre-operatively the patient was given a practice calorimetry run and then that evening allowed no further oral diet and established on an intravenous infusion of 5% Dextrose at a rate of two litres per day. The next (control) day, two more calorimetry measurements were performed, the infusion being discontinued after the second. A 24 hour urine collection was made to assess urinary nitrogen excretion. All the urinary nitrogen analyses were performed by the Department of Biochemistry, Royal Infirmary, Glasgow.

The patients underwent elective surgery and were returned to the metabolic room and established on an intravenous infusion of 5% Dextrose at a rate of two litres per day plus any zero calorie fluids (saline etc.) as necessary. This feeding regime was maintained for four days until the end of the study. Complete urine collections were also maintained over this period. A calorimetry measurement was performed each morning, starting on the morning after operation. No patient results were included where satisfactory data was not available on each of the four post-operative days. The modification of the head canopy (see Section 6.2.1) so that it could be attached to the back of the bed made the calorimetry procedure acceptable even on the first day after operation. The general experimental layout is shown in Table 34.

Both study B and study C were approved by the Ethical

	Pre-op			Operation	Post-op			
Day in Temperature Controlled Room	C-1	C	O-6 days gap	O	1	2	3	4
No of calorimetry measurements	1	2	O	O	1	1	1	1
Feeding	Oral	Dex	Oral	Dex	Dex	Dex	Dex	Dex

C - Control day - 1 - 7 days before operation

Oral - normal ward oral diet, established on I.V. Dextrose in the evening of control day minus one.

Dex - 2 Litres of 5% dextrose I.V. per day plus zero calorie fluids as necessary

Table 34: Study C: Calorimetry measurements and feeding.

Committee of the Royal Infirmary, Glasgow. The voluntary nature of the studies and their requirements were explained to each patient before inclusion in a study and the patient's signed consent was obtained in each case.

CHAPTER 8

Human Study Results

8.1 Study A

The subjects all completed the study without incident. Though the test required the consumption of less than one litre of Isocal over the five hours and the feed is approximately iso-osmolar (390m osm), all the subjects were asked if they experienced any diarrhoea or other problems after the study. None were reported. The age, height and weight of each subject are recorded in Table 35. The measured post-absorptive RMR values have a mean of 99.4% (± 1.9% sem) of their predicted levels, the range covering 90.4% to 106.8%.

The effect of continuous feeding on the RMR and RQ is shown in Table 36. Both RMR and RQ had significant increases ($p < 0.001$, one tail paired T test) over the 4.5 hours of feeding. The RMR rose an average of 10.7 W (range 8.6 to 13.2 W) which represented 13.4% of the post-absorptive value. The standard error of the mean of the increase in RMR, at 0.58 W was much less than the standard error of either the post-absorptive or fed RMRs, at 1.50 and 1.52 W respectively. This demonstrated that the increase in heat production produced by the continuous feed was relatively independent of the initial metabolic rate of the subject.

The RQ increased from a mean of 0.799 to 0.879 on feeding, reflecting the high carohydrate content of the diet. If

Subject No.	Age	Ht.	Weight	Surface Area	Predicted Heat Production	Measured Post-abs. Heat Production	Measured ÷ Predicted Heat Production
	yr	m	kg	m^2	W	W	%
1	27	1.83	68.0	1.89	81.8	84.7	103.5
2	18	1.88	61.0	1.84	85.4	80.6	94.4
3	29	1.60	60.3	1.63	70.0	72.0	102.9
4	24	1.83	71.4	1.93	84.5	83.5	98.8
5	25	1.68	70.5	1.80	78.3	74.5	95.1
6	28	1.73	62.0	1.74	74.8	79.2	105.9
7	25	1.77	74.5	1.91	83.1	80.3	96.6
8	18	1.87	63.5	1.86	86.3	78.0	90.4
9	28	1.78	69.3	1.86	80.0	85.4	106.8
mean	24.7	1.77	66.7	1.83	80.5	79.8	99.4
sem	1.4	0.03	1.7	0.03	1.8	1.5	1.9

Table 35 Details of subjects in Study A.

Subject	post-absorptive RMR (W)	FED RMR	Increase in RMR (W)	post-absorptive RQ	FED RQ
1	84.7	95.6	10.9	0.792	0.864
2	80.6	89.4	8.8	0.809	0.872
3	72.0	84.9	12.9	0.817	0.887
4	83.5	93.9	10.4	0.821	0.880
5	74.5	83.7	9.2	0.814	0.919
6	79.2	92.4	13.2	0.755	0.863
7	80.3	92.5	12.2	0.823	0.858
8	78.0	86.6	8.6	0.774	0.908
9	85.4	95.8	10.4	0.784	0.856
n = 9					
Mean	79.8	90.5	10.7	0.799	0.879
SE	1.50	1.52	0.58	0.008	0.007

Table 36: Post-absorptive resting metabolic rate and respiratory quotient and their values after an average of 4.5 hours of continuous feeding.

the diet were being metabolised directly to produce energy then it would have a calculated RQ of approximately 0.88, close to the observed value.

RMR increased at an average rate of 2.4 W per hour. To test whether there was still a significant trend of increase in heat production during the last calorimetry measurement, this period was divided into four sections of 10 minutes for each subject. The increase in RMR (i.e. the thermogenesis) was calculated for each 10 minute section. An analysis of variance for linear trend (see Section 6.3.6) showed no significant trend over the 40 minute period (Table 37). The best estimate of the rate of increase in heat production from regression analysis was 0.6 W per hour. This was only 25% of the average rate of increase in heat production that must have occurred. If no allowance for any further increase in heat production was made, the thermogenic effect of the diet, as a percentage of the food energy ingested, was 4.6% ($\pm$ 0.25% sem).

8.2 Study B

The calorimetry results for the two patients studied are shown in Table 38. The pre-operative control runs demonstrated reproducible results though the RQ of patient J.W. was lower than expected on the Isocal feed. The peak in heat production was measured on the first day after surgery in both cases. The heat production then decreased until refeeding or the oral or enteric diet was started.

time (minutes)	0 - 10	10 - 20	20 - 30	30 - 40	:	0 - 40
Thermogenesis (W) mean	10.7	10.5	10.9	10.9		10.7
s.d.	1.2	2.0	3.0	2.2		1.8

Test for linear trend F = 0.20 Degrees of Freedom 1,24 n.s.

Test for departure from linearity F = 0.29 Degrees of Freedom 2,24 n.s.

from McNemar (1969) page 394

Table 37: Analysis of trend in thermogenesis during the second RMR measurement in Study A using four consecutive 10 minute periods from each subject.

Patient H McP

Study Day*	-2	-1	1	3	5	6	7	29
RMR (W)	71.8	70.9	80.2	77.4	77.6	76.2	76.0	72.5
RQ	0.854	0.854	0.823	0.772	0.751	0.761	0.761	0.803
Feeding	Lo	Iso	Dex	Dex	Lo	Lo	Lo	Lo

Patient J.W.

Study Day*	-1	-1	1	1	2	4	5	6
RMR (W)	72.3	71.8	85.5	90.2	81.6	74.6	81.9	82.5
RQ	0.792	0.803	0.741	0.710	0.782	0.761	0.772	0.844
Feeding	Iso	Iso	Dex	Dex	Dex	Dex	Iso	Iso

*The day of operation was study day 0

Lo - light oral diet

Dex - 5% Dextrose intravenous infusion - 2 l d^{-1}

Iso - Isocal enteric feed - 91 ml hr^{-1}

Table 38: Heat Production and RQ results in Study B

The first patient (H.McP) could not tolerate the passing of a fine bore nasogastric tube 4 days after operation and was therefore put onto a light oral diet. Calorimetry was performed at least two hours after breakfast, thus heat production was approximately constant from day 3 to day 7. This patient returned as an out-patient for a calorimetry measurement on the 29th day after his operation. He had had a light breakfast before the study and so was in a comparable dietary state to day 5 to 7. His heat production had decreased to close to his pre-operative values.

The second patient (J.W.) decreased his heat production until refeeding on Isocal was started. His RMR increased by 7 W while the pro-rata increase in heat production expected from Study A would be about 5 W. This patient was not available for out-patient follow up.

The results of the measurements on these two patients indicated that the response was over a few days after operation, and hence this would be the period of most interest and that a constant nutritional input was required to allow comparisons between patients. These changes were incorporated in the design of Study C.

8.3 Study C

The details of the patients studied at 20°C and 28°C are listed in Tables 39 and 40. There were the same number of cholecystectomy and vagotomy and drainage operations at each temperature. The two groups of patients did not

No.	Op.	Age	Height	Weight	Surface Area	Predicted Heat Production	Control Heat Production	Control÷ Predicted
		yr	m	kg	m^2	W	W	%
1	C	45	1.70	74.0	1.84	77.9	79.9	102.6
2	V	58	1.60	61.0	1.62	66.3	72.9	110.0
3	C	68	1.69	59.0	1.67	65.8	60.2	91.5
4	V	25	1.70	72.0	1.82	79.5	79.0	99.3
5	C	35	1.71	77.5	1.89	80.4	68.8	85.6
6	V	20	1.75	85.0	2.00	90.0	91.0	101.1
7	V	63	1.68	56.5	1.63	65.4	76.6	117.1
8	V	56	1.62	62.0	1.65	67.6	71.5	105.8
9	C	71	1.59	63.0	1.64	64.0	71.4	111.6
10	V	33	1.78	68.0	1.84	78.3	93.8	119.9
mean		47	1.68	67.8	1.76	73.5	76.5	104.4
sem		5.8	.02	2.90	.04	2.79	3.19	3.41

Operations: C - Cholecystectomy
V - Vagotomy and Drainage

Table 39 Details of patients nursed at 20°C in Study C.

No.	Op.	Age	Height	Weight	Surface Area	Predicted Heat Production	Control Heat Production	Control÷ Predicted
		yr	m	kg	m^2	W	W	%
11	V	25	1.74	78.0	1.92	83.6	86.4	103.3
12	C	58	1.68	65.5	1.73	70.7	65.3	92.4
13	V	52	1.77	70.5	1.86	76.9	81.8	106.4
14	V	40	1.70	82.5	1.93	81.6	90.6	111.0
15	V	43	1.63	57.5	1.60	67.7	59.9	88.5
16	C	69	1.85	78.5	2.01	79.2	89.5	113.1
17	V	29	1.83	85.5	2.07	88.8	90.1	101.4
18	V	42	1.57	56.0	1.55	65.5	78.8	120.3
19	C	53	1.69	72.0	1.82	75.2	84.0	111.7
20	C	66	1.64	69.0	1.75	69.5	60.1	86.4
mean		48	1.71	71.5	1.82	75.9	78.6	103.4
sem		4.6	.03	3.14	.05	2.39	3.89	3.57

Operations: C - Cholecystectomy
V - Vagotomy and Drainage

Table 40 Details of patients nursed at 28°C in Study C.

differ significantly in any of the items listed in the tables. The measured heat productions have a mean of 104.4 and 103.4% of their predicted values for the patients at 20 and 28°C respectively.

The values of heat production per unit surface area (W m^{-2}) for the pre-operative control day and the first four days after operation are recorded in tables 41 and 42. The means showed a definite increase (Figure 37) on the day after surgery followed by a slow decline and return to almost the pre-operative control values by day 4. Every patient, except number 7, increased his heat production on the first day after surgery. To test for the effect of the time after operation and the ambient temperature on heat production a three way analysis of variance (see Section 6.3.6) was performed and the results set out in Table 43. There was no ambient temperature or interaction effect, but the changes in heat production following operation were highly significant. As the effect of environmental temperature was far from being significant the results at 20 and 28°C were combined. Comparing the post-operative days individually with the control measurement day, only the 4th day after operation did not demonstrate a significant increase in heat production (Table 44). The average increase over the 4 post-operative days was also significant.

As there were pre-operative control RMR measurements on each patient and the values for the two groups had been shown to have a similar mean and degree of variation

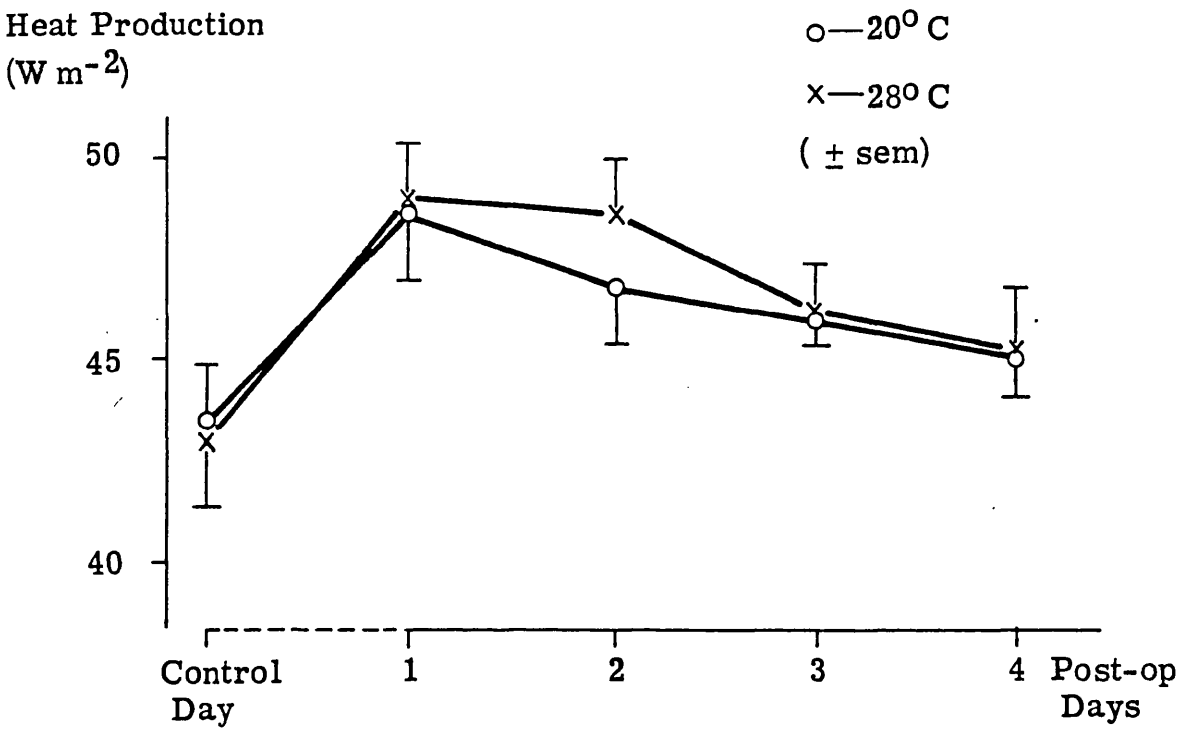


Figure 37. Heat Production (W m^{-2}) for the two groups of patients nursed at 20 and 28°C in Study C.

20°C Patients	Control Day (Wm^{-2})	Post-op. Day 1 (Wm^{-2})	Post-op. Day 2 (Wm^{-2})	Post-op. Day 3 (Wm^{-2})	Post-op. Day 4 (Wm^{-2})
1	43.3	53.6	45.9	46.5	41.6
2	44.9	50.1	49.8	47.3	48.3
3	36.1	39.8	36.5	42.7	43.0
4	43.3	51.6	47.4	45.8	44.1
5	36.4	49.2	47.1	43.4	42.5
6	45.5	50.7	50.9	48.3	43.0
7	47.1	42.3	42.9	44.5	43.6
8	43.4	45.8	44.8	45.8	45.6
9	43.6	44.8	50.1	46.8	45.8
10	51.1	58.1	51.4	46.2	49.9
mean	43.5	48.6	46.7	45.7	44.7
sem	1.42	1.73	1.43	0.55	0.84

Table 41 Resting metabolic rates of patients nursed at 20°C during Study C.

28°C Patients	Control Day (Wm^{-2})	Post-op. Day 1 (Wm^{-2})	Post-op. Day 2 (Wm^{-2})	Post-op. Day 3 (Wm^{-2})	Post-op. Day 4 (Wm^{-2})
11	45.0	46.7	47.7	45.3	47.4
12	37.7	48.9	49.9	48.3	46.4
13	44.1	51.0	54.8	44.4	41.2
14	46.9	52.9	50.3	52.2	51.5
15	37.4	47.5	44.3	42.4	42.5
16	44.5	46.8	43.3	40.6	41.9
17	43.6	47.9	49.2	44.3	42.7
18	50.8	57.7	54.6	52.2	55.2
19	46.2	49.6	49.1	48.9	42.8
20	34.4	40.6	41.1	40.8	37.8
mean	43.0	48.9	48.4	45.9	44.9
sem	1.59	1.41	1.43	1.35	1.65

Table 42 Resting metabolic rates of patients nursed at 28°C during Study C.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCE ESTIMATE	F RATIO	p VALUE
Individual	1215.08	18	67.50		
Temperature	4.48	1	4.48	0.07	ns
Day	380.34	4	95.09	13.41	***
Temperature x Day	12.93	4	3.23	0.46	ns
Remainder	510.39	72	7.09		

(from McNemar, 1969, p379)

Table 43: Pseudo 3-way analysis of variance of the RMR (Wm^{-2}) values from Study C.

20 and 28°C Patients Combined (n = 20)	Control Day (Wm^{-2})	Post-op. Day 1 (Wm^{-2})	Post-op. Day 2 (Wm^{-2})	Post-op. Day 3 (Wm^{-2})	Post-op. Day 4 (Wm^{-2})	Average of 4 Post-op. Days (Wm^{-2})
mean	43.3	48.8	47.6	45.8	44.8	46.8
Difference between control & post-op. day	-	5.5	4.3	2.5	1.5	3.5
95% confidence limits (†)	-	±2.1	±2.1	±2.1	±2.1	-
p value of difference(†)	-	**	**	*	ns	***(§)

(† - using Dunnett's (1955) procedure for multiple comparisons with a control value)

(§ - T test)

Table 44 Combined resting metabolic rates of patients nursed at 20 and 28°C during Study C.

(Tables 39 and 40), the post-operative RMR values were also calculated as a percentage of the control day measurement. This standardisation procedure did not reduce the variation of the heat productions in the two groups (Figure 38). The 20 patients demonstrated a 13.3% mean increase in heat production on the first post-operative day, which declined to a 4.3% increase by the fourth day. The average increase over the four days was 8.7%. Expressing the RMR values as a percentage of the predicted heat productions (Figure 39) also had no effect on the significance of the results.

The individual respiratory quotients are recorded for the two groups of patients in Tables 45 and 46. The RQ declined rapidly over the first one to two days after operation (Figure 40) with a slower rate of decrease up to the end of the study. The analysis of variance (Table 47) demonstrated the same pattern as heat production there being no environmental temperature or interaction effect but a very significant effect of the time from operation. Combining the results at both temperatures showed that each post-operative day RQ was significantly reduced when compared with the control day value (Table 48).

The values of external auditory canal temperature are recorded in Tables 49 and 50 and shown graphically in Figure 41. The mean values at 20^{o}C were almost a degree below the values recorded at 28^{o}C. This must reflect the change in temperature gradient along the auditory canal with alteration of the environmental temperature.

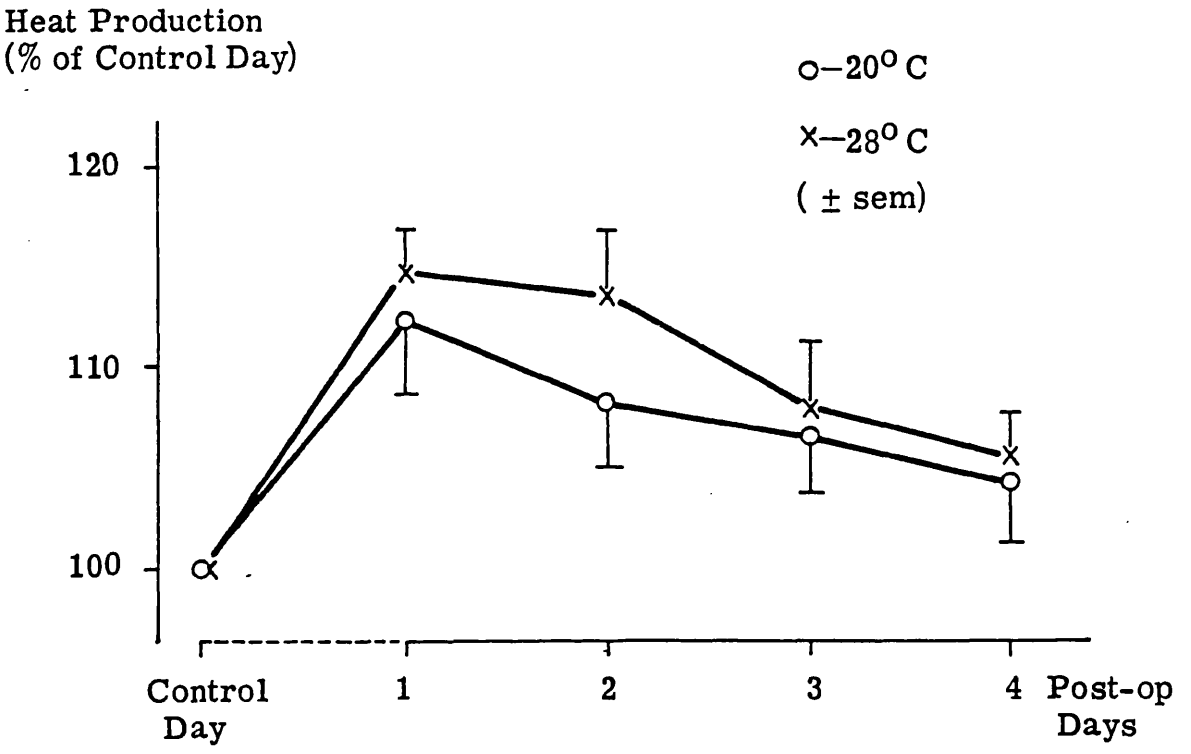


Figure 38. Heat production (% of control day value) for the two groups of patients nursed at 20 and 28°C in Study C.

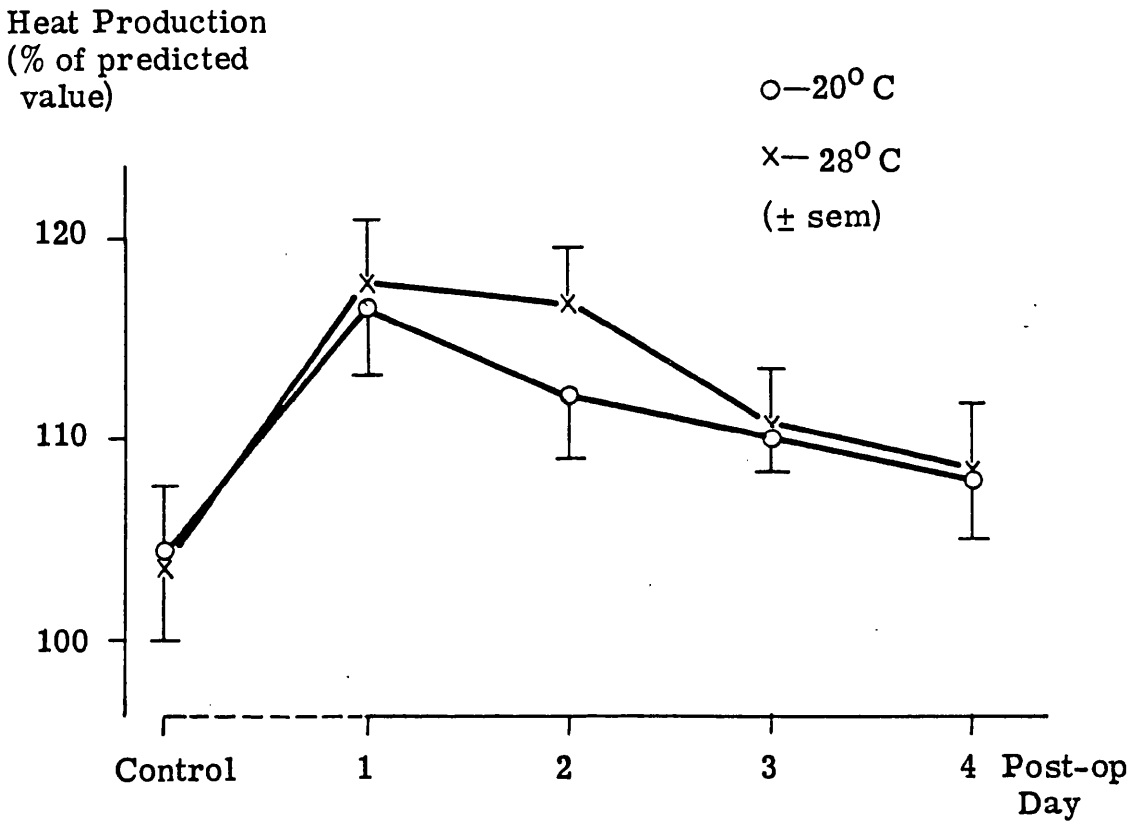


Figure 39. Heat production (% of predicted value) for the two groups of patients nursed at 20 and 28°C in Study C.

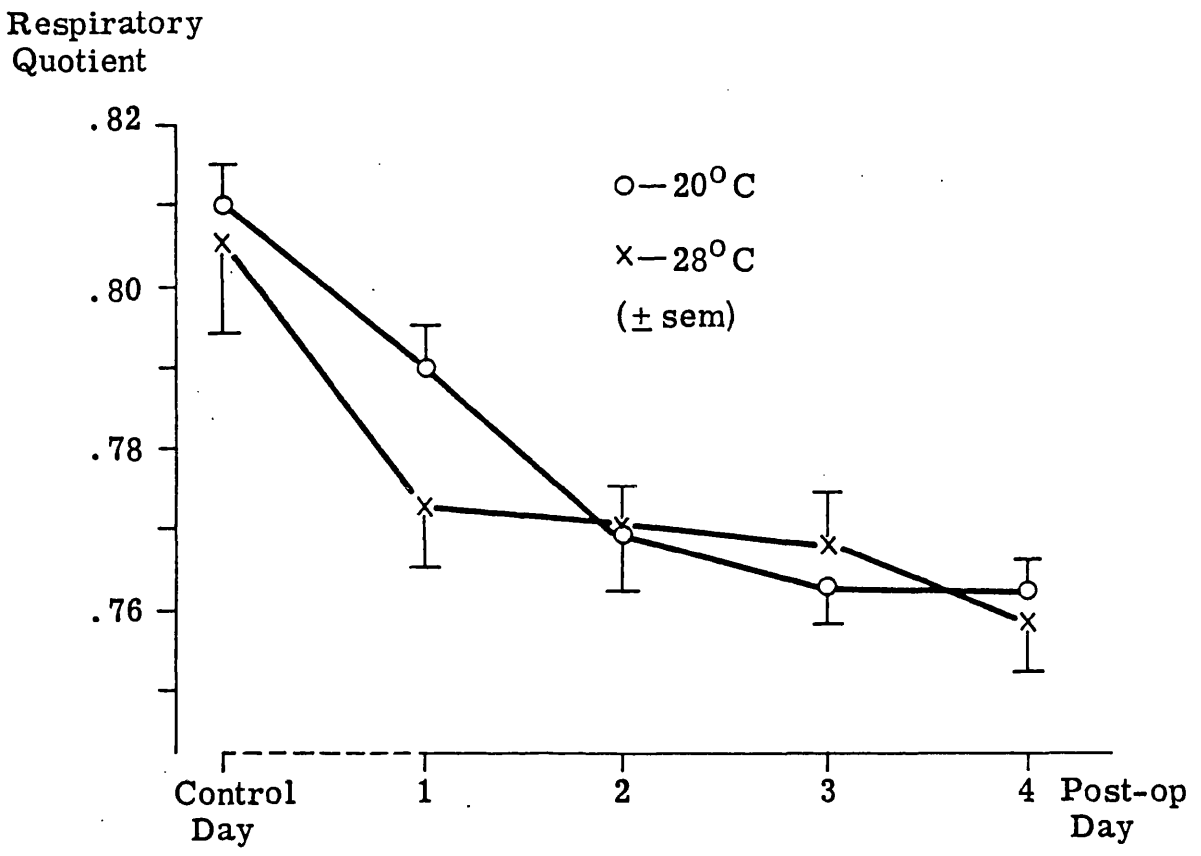


Figure 40. Respiratory quotient for the two groups of patients nursed at 20 and 28°C in Study C.

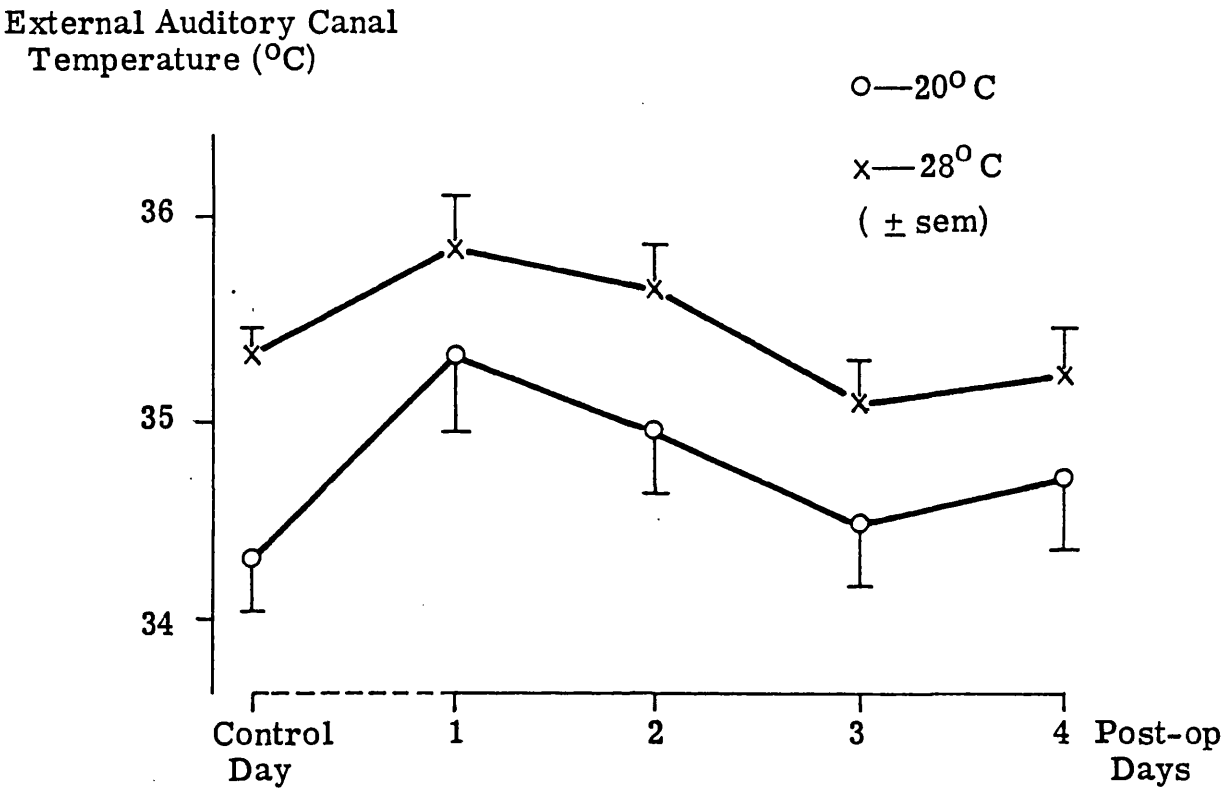


Figure 41. External auditory canal temperature (°C) for the two groups of patients nursed at 20 and 28°C in Study C.

20°C Patients	Control Day	Post-op. Day 1	Post-op. Day 2	Post-op. Day 3	Post-op. Day 4
1	.803	.800	.757	.756	.754
2	.799	.824	.791	.787	.751
3	.816	.798	.798	.762	.736
4	.794	.784	.747	.756	.773
5	.817	.776	.764	.760	.760
6	.803	.773	.787	.761	.774
7	.812	.761	.781	.744	.751
8	.851	.792	.781	.783	.778
9	.801	.792	.739	.759	.754
10	.806	.785	.735	.746	.765
mean	.810	.789	.768	.761	.760
sem	.005	.005	.007	.004	.004

Table 45 Respiratory quotients of patients nursed at 20°C during Study C.

28°C Patients	Control Day	Post-op. Day 1	Post-op. Day 2	Post-op. Day 3	Post-op. Day 4
11	.770	.740	.758	.744	.733
12	.814	.765	.730	.728	.740
13	.772	.775	.774	.751	.770
14	.793	.784	.779	.783	.802
15	.764	.777	.770	.761	.759
16	.810	.743	.786	.784	.754
17	.867	.799	.768	.798	.761
18	.808	.745	.771	.774	.741
19	.787	.783	.767	.751	.752
20	.870	.812	.788	.788	.752
mean	.806	.772	.769	.766	.756
sem	.012	.008	.005	.007	.006

Table 46 Respiratory quotients of patients nursed at 28°C during Study C.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCE ESTIMATE	F RATIO	p VALUE
Individual	179.83	18	9.991		
Temperature	3.31	1	3.312	0.33	ns
Day	312.35	4	78.087	24.17	***
Temperature x Day	12.64	4	3.160	0.98	ns
Remainder	232.57	72	3.230		

(from McNemar, 1969, p379)

Table 47: Pseudo 3 way analysis of variance of the RQ values from Study C.

20 and 28°C Patients Combined (n = 20)	Control Day	Post-op. Day 1	Post-op. Day 2	Post-op. Day 3	Post-op. Day 4
mean	.808	.780	.769	.764	.758
Difference between control & post-op. day	-	.028	·039	.044	.050
95% confidence limits (†)	-	±.014	±.014	±.014	±.014
p value of difference(†)	-	**	**	**	**

(† - using Dunnett's (1955) procedure for multiple comparisons with a control value)

Table 48 Combined respiratory quotients of patients nursed at 20 and 28°C during Study C.

20°C Patients	Control Day	Post-op. Day 1	Post-op. Day 2	Post-op. Day 3	Post-op. Day 4
	°C	°C	°C	°C	°C
1	34.2	34.9	35.0	34.7	35.2
2	35.7	37.2	35.8	35.1	36.6
3	33.9	36.2	35.8	34.9	34.4
4	34.8	36.4	36.1	35.4	34.7
5	35.0	36.4	36.4	35.8	35.2
6	35.0	34.9	34.9	34.8	35.6
7	33.9	34.0	33.9	33.9	34.2
8	32.9	33.3	33.3	32.6	32.8
9	34.0	34.1	34.1	33.4	33.7
10	33.6	35.2	34.2	33.6	33.5
mean	34.3	35.3	34.9	34.4	34.6
sem	0.26	0.39	0.33	0.32	0.35

Table 49 External auditory canal temperatures of patients nursed at 20°C during Study C.

28°C Patients	Control Day	Post-op. Day 1	Post-op. Day 2	Post-op. Day 3	Post-op. Day 4
	°C	°C	°C	°C	°C
11	35.2	35.3	35.9	34.8	35.1
12	36.2	36.0	35.6	34.7	35.8
13	35.1	35.9	36.4	35.2	35.5
14	36.2	37.0	36.7	36.4	36.4
15	35.2	36.5	35.8	35.0	35.4
16	35.0	34.5	34.5	34.6	34.1
17	35.2	36.0	36.0	35.5	35.4
18	35.0	37.0	34.8	33.9	35.1
19	34.9	34.5	34.7	34.7	33.8
20	34.9	34.9	35.4	35.1	34.4
mean	35.3	35.8	35.6	35.0	35.1
sem	0.15	0.29	0.23	0.21	0.25

Table 50 External auditory canal temperatures of patients nursed at 28°C during Study C.

A large proportion of the variation in temperature measurement must also be attributed to the difficulty in obtaining a reproducible placement in the ear day to day. However an increase in temperature was recorded in response to surgery and the analysis of variance (Table 51) shows the time from operation to be the most significant factor. The environmental temperature and interaction effects are just non-significant, both their p values lying between 0.1 and 0.05.

The urinary nitrogen response is recorded in Tables 52 and 53 and is shown graphically in Figure 42. As with heat production, there was an immediate rise after operation with a gradual decline over the next four days. This was except for post-operative day 3 for the patients nursed at 28^{o}C which showed a rise in nitrogen excretion. However there was a decrease to a level comparable to the 20^{o}C group on the next day. The analysis of variance (Table 54) demonstrated that the only significant effect was that of the operation. Combining the results at both temperatures (Table 55) showed that each post-operative day was elevated above the control nitrogen excretion value.

As there was no nitrogen input over the pre and post-operative periods, the urinary nitrogen excreted must have been derived from body tissue. On the assumption that 6.25 g of protein provided 1 g of nitrogen, the protein tissue catabolism was calculated. The energy equivalent of the catabolised protein (17 kJ per g - Brown, 1973) for both the control day and post-operative

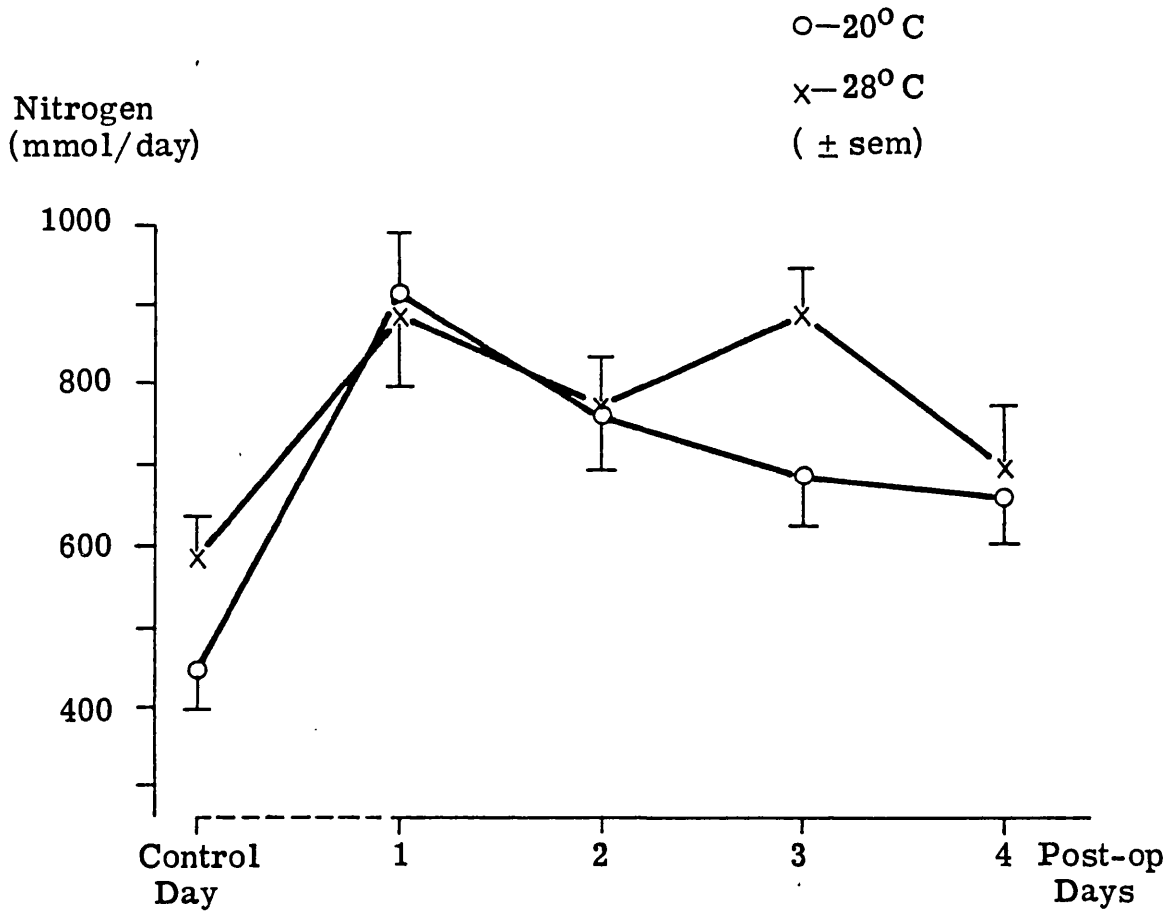


Figure 42. Urinary nitrogen excretion (mmol d^{-1}) for the two groups of patients nursed at 20 and 28°C in Study C.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCE ESTIMATE	F RATIO	P VALUE
Individual	51.97	18	2.887		
Temperature	12.67	1	12.666	4.39	ns(p<.1)
Day	11.70	4	2.925	10.49	***
Temperature x Day	2.57	4	0.642	2.30	ns(p<.1)
Remainder	20.08	72	0.279		

(from McNemar, 1969, p379)

Table 51: Pseudo 3 way analysis of variance of the external auditory canal temperature (^{o}C) from Study C.

20°C Patients	Control Day (mmol d^{-1})	Post-op. Day 1 (mmol d^{-1})	Post-op. Day 2 (mmol d^{-1})	Post-op. Day 3 (mmol d^{-1})	Post-op. Day 4 (mmol d^{-1})
1	566	693	917	753	612
2	618	966	959	868	979
3	356	1082	354	565	572
4	688	721	653	821	825
5	229	760	868	328	485
6	340	1213	811	874	755
7	400	784	397	461	455
8	347	1348	869	519	477
9	307	648	1032	641	491
10	590	842	701	928	740
mean	440	906	756	676	639
sem	49.5	75.3	72.7	64.3	56.3

Table 52 Urinary nitrogen excretion of patients nursed at 20°C during Study C.

28°C Patients	Control Day (mmol d^{-1})	Post-op. Day 1 (mmol d^{-1})	Post-op. Day 2 (mmol d^{-1})	Post-op. Day 3 (mmol d^{-1})	Post-op. Day 4 (mmol d^{-1})
11	690	1278	713	720	236
12	605	686	327	1174	405
13	585	792	720	942	808
14	804	846	707	734	676
15	403	1478	845	1189	660
16	504	944	662	734	635
17	861	871	932	956	896
18	352	661	685	760	1066
19	494	763	916	690	434
20	538	475	1113	830	923
mean	584	879	762	873	674
sem	51.6	93.8	66.3	58.9	82.1

Table 53 Urinary nitrogen excretion of patients nursed at 28°C during Study C.

SOURCE	SUM OF SQUARES	DEGREES OF FREEDOM	VARIANCE ESTIMATE	F RATIO	P VALUE
Individual	1042422	18	57912		
Temperature	123412	1	123412	2.13	ns
Day	1614361	4	403590	9.17	***
Temperature x Day	178238	4	44560	1.01	ns
Remainder	3167349	72	43991		

(from McNemar, 1969, p379)

Table 54: Pseudo 3-way analysis of variance of the urinary nitrogen excretion (mmol d^{-1}) from Study C.

20 and 28°C Patients Combined (n = 20)	Control Day ($mmol\ d^{-1}$)	Post-op. Day 1 ($mmol\ d^{-1}$)	Post-op. Day 2 ($mmol\ d^{-1}$)	Post-op. Day 3 $mmol\ d^{-1}$)	Post-op. Day 4 $mmol\ d^{-1}$)	Average of 4 Post-op. Days $mmol\ d^{-1}$)
mean	514	893	759	774	657	771
Difference between control & post-op. day	-	379	245	260	143	257
95% confidence limits (†)	-	±169	±169	±169	±169	-
p value of difference(†)	-	**	**	**	ns	***(§)

(† - using Dunnett's (1955) procedure for multiple comparisons with a control value)

(§ - T test)

Table 55 Combined urinary nitrogen excretions of patients nursed at 20 and 28°C during Study C.

period was expressed as a percentage of the total heat production (Tables 56 and 57).

After the operation the four day energy deficit was calculated from the total heat production minus the energy derived from the infused dextrose (1.76 MJ per day). No allowance was made for increased heat production above RMR levels through the activity of the patients. Measurements of this increment have shown it to be only of the order of 5% for a post-surgical patient (Kinney et al., 1968; Long et al., 1979). As glycogen stores are small (Levenson, 1977) the energy must have been derived from fat and protein. The protein energy (calculated as above) was substracted from the energy deficit to give the energy derived from fat. This latter was transformed into a weight of fat catabolised using a value of 39 kJ per g of fat (Brown, 1973) (Tables 56 and 57).

The percentage contribution of protein to total heat production rose from a mean for the 20 patients of 11.4% to 16.0% after surgery. The energy produced from fat stores was approximately four times that produced from protein. The mean amounts of fat and protein tissue lost during the post-operative period were 0.47 and 0.27 kg respectively.

Patients at 20°C	Control Protein % of Heat Production	Av. Post-op. Protein % of Heat Production	Total over 4 post-op. days: Energy Deficit	Protein Energy	Fat Energy	Protein Loss	Fat Loss
			MJ	MJ	MJ	kg	kg
1	12.2	14.8	23.7	4.4	19.2	0.26	0.49
2	14.6	20.5	21.2	5.6	15.6	0.33	0.40
3	10.2	16.4	17.1	3.8	13.3	0.23	0.34
4	15.0	15.1	23.5	4.5	19.0	0.26	0.49
5	5.7	12.2	23.6	3.6	19.9	0.21	0.51
6	6.4	16.3	27.1	5.4	21.6	0.32	0.55
7	9.0	12.8	18.1	3.1	15.0	0.18	0.38
8	8.4	18.4	19.7	4.8	14.9	0.28	0.38
9	7.4	15.8	20.3	4.2	16.1	0.25	0.41
10	10.8	14.6	26.4	4.8	21.6	0.28	0.55
mean	10.0	15.7	22.1	4.4	17.6	0.26	0.45
sem	1.02	0.78	1.06	0.25	0.95	0.08	0.14

Table 56 Protein fraction of heat production and 4 day energy balance, protein and fat utilisation for patients nursed at 20°C during Study C.

Patients at 28°C	Control Protein % of Heat Production	Av. Post-op. Protein of Heat Production	Total over 4 post-op. days: Energy Deficit	Protein Energy	Fat Energy	Protein Loss	Fat Loss
			MJ	MJ	MJ	kg	kg
11	13.8	14.2	24.7	4.4	20.3	0.26	0.52
12	16.0	13.3	22.7	3.9	18.8	0.23	0.48
13	12.3	15.8	24.4	4.9	19.6	0.29	0.50
14	15.3	12.8	28.3	4.4	23.9	0.26	0.61
15	11.6	25.3	18.3	6.2	12.1	0.37	0.31
16	9.7	14.7	23.8	4.4	19.4	0.26	0.50
17	16.5	16.5	26.7	5.4	21.2	0.32	0.54
18	7.7	16.0	23.2	4.7	18.5	0.28	0.47
19	10.2	13.9	23.7	4.2	19.5	0.25	0.50
20	15.4	20.6	17.9	5.0	12.9	0.29	0.33
mean	12.8	16.3	23.4	4.8	18.6	0.28	0.48
sem	0.95	1.22	1.03	0.21	1.13	0.09	0.15

Table 57 Protein fraction of heat production and 4 day energy balance, protein and fat utilisation for patients nursed at 28°C during Study C.

CHAPTER 9

Human Studies Discussion

9.1 Calorimetry

The nine subjects in Study A had a mean RMR of 43.6 W m^{-2} and a coefficient of variation of only 3.7%. These subjects were closely grouped in age, height and weight when compared with the patients in Study C and so might be expected to demonstrate a small variability. The 20 patients in Study C had a mean heat production on the control day of 43.3 W m^{-2} and a larger coefficient of variation of 9.3%. Berkson and Boothby (1938) measured a variation of the heat production of 332 male subjects of 6.7% and Harris and Benedict (1919) measured a similar figure of about 7.0%. The slightly higher variation than expected could be the effect of the stress of anticipation of their operation.

When age, as well as height and weight, was used in the standardisation procedure by expressing the measured values as a percentage of their predicted values from Fleisch's table then the post-absorptive values showed an increased coefficient of variation of 5.7% and 10.4% respectively. (Study A and C)

The distribution of the values of RMR for the subjects in Studies A and C are shown in Figure 43 as percentages of their predicted values. Part of the upward trend in the metabolic rates could be attributed to the continuous

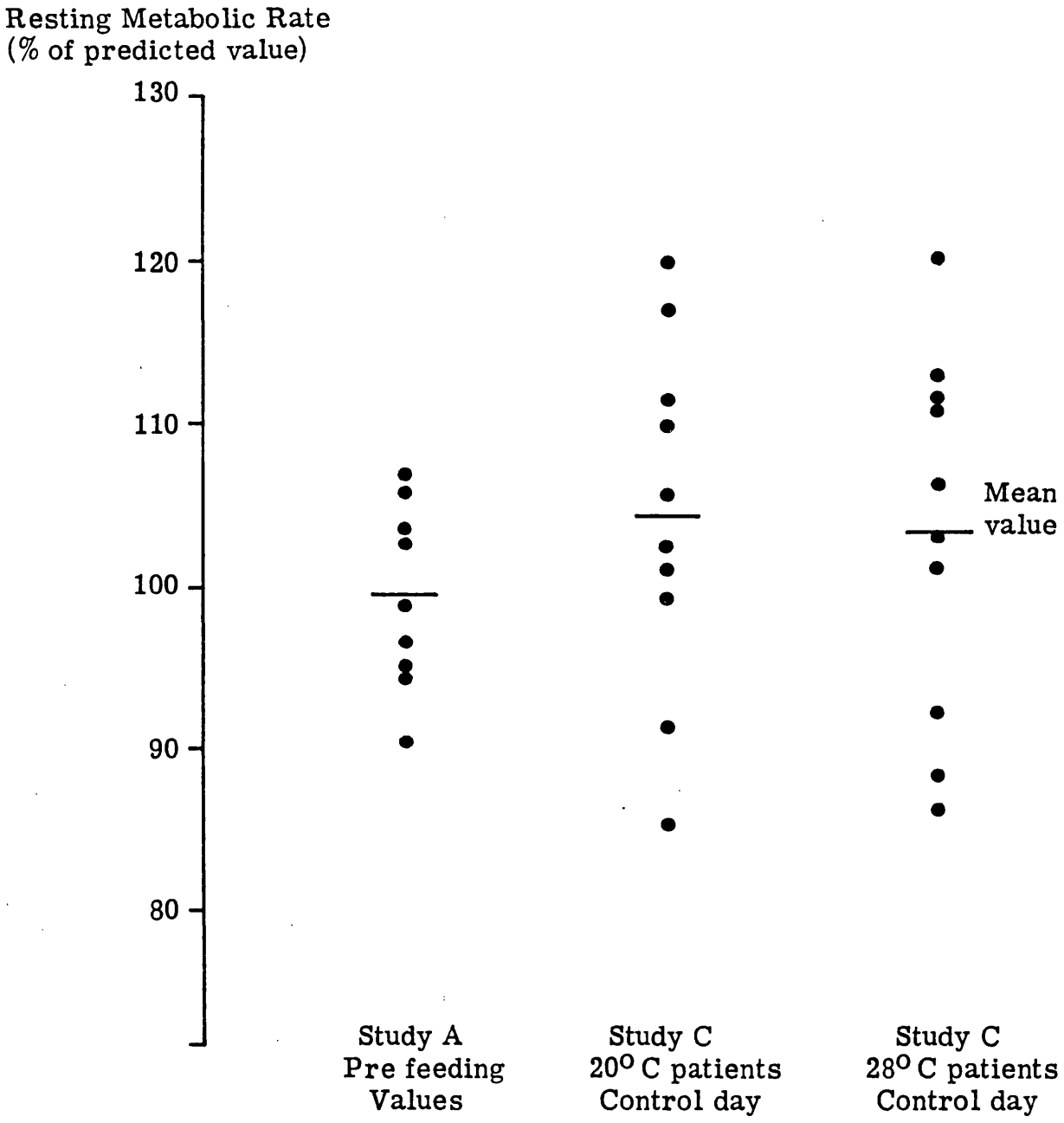


Figure 43. Resting Metabolic rate (% of predicted value) for the subjects in Study A before feeding and for the patients in Study C on the control day.

intake of dextrose. Assuming that intravenous carbohydrate has approximately the same thermogenic effect (6% of the dietary energy ingested) as oral carbohydrate (Wilmore, 1977), then the continuous administration of dextrose would have increased heat production by an average of 1.6%. Both study groups appear to have similar mean rates of heat production, close to their predicted values, although the less homogenous patient group demonstrated a larger variation.

The respiratory quotients in Study A varied between 0.75 and 0.83, which is within the accepted range for post-absorptive man (Kleiber, 1961). The patients in Study C had a wider range of RQs on the control day of 0.79 to 0.87 and a higher mean value because of their intake of carbohydrate. As Study C progressed the RQ dropped towards 0.76, the threshold of ketosis (Shaffer, 1921). However the intake of carbohydrate was enough to prevent significant ketosis. The RQ values at the end of the study were similar to the value of 0.75 quoted by Thompson et al. (1979) for a typical post-surgical patient.

9.2 Thermogenesis of a continuously administered diet

The thermogenic effect at 4.6% of the food energy ingested, is low compared with other measurements made on mixed diets. Benedict & Carpenter (1918) measured a mean value of 7.3% on a variety of mixed diets containing an average of 13% protein, 50% fat and 37% carohydrate energy approximately comparable to Isocal. This estimate must include a small

increment of heat production due to the mechanical work of eating (Benedict, Carpenter, 1918). Glickman et al. (1948) also used a mixed oral diet of an energy composition (7% protein, 39% fat, 54% carbohydrate) close to that of Isocal and showed a higher dietary induced thermogenesis (DIT) of 9.6%. More comparable with our experimental design, Garrow and Hawes (1972) gave Complan intragastrically over 30 minutes. Their DIT was 8.8%, but this could reflect the high protein energy content (27%) of Complan.

Low values for DIT could be obtained if equilibrium had not been attained by the second RMR measurement. Garrow and Hawes (1972) showed a return to baseline conditions within 4 hours of giving 4.52 MJ of Complan intragastrically. This was more than the 4.18 MJ of Isocal administered evenly over a 5 hour period. The analysis of linear trend performed in Section 8.1 showed no significant trend though the variability of the results would obscure a small rate of increase. The best estimate of the rate of increase during the second calorimetry period (0.6 W hr^{-1}) was well below the average rate of increase over the 4.5 hrs of the study (2.4 W hr^{-1}). Hence the subjects were probably close to equilibrium.

A low value for DIT may also be the result of larger meals of approximately the same composition producing a larger percentage thermogenesis (Wachholder and Franz, 1944; Miller, Mumford and Stock, 1967; Dauncey, 1980).

The non-linear response could be caused in many ways. For example, the larger meal could over stimulate enzyme production for its absorption or temporary storage of the surplus nutrients could require extra energy expenditure. Our subjects with an average fed RMR of 90.5 W would receive their daily energy requirement, assuming they continued to rest, in just over 9 hours. This is not a dissimilar period to that between breakfast (say 8 a.m.) and dinner (say 6 p.m.). Therefore our subjects could be considered to be consuming a normal amount of food energy over the usual period but in multiple small amounts. Garrow et al.(1981) has shown that multiple rather than single meals of the same total size and composition reduce weight loss and improve nitrogen balance in dieting patients. This would be consistent with the low value for DIT measured in Study A.

Another factor not present when a diet is administered intragastrically is the psychological stimulus of the sight and smell of the food. This could be an important factor when the DIT of an oral diet is measured, though the size of its effect is difficult to estimate.

Despite the limited thermogenic effect of the continuously administered diet, the coefficient of variation of the DIT was only 16.3%. This compares favourably with the coefficients measured by other workers using oral diets inducing much larger values of DIT. Glickman et al. (1948) reported a range of coefficients of variation of 16 to 19%, Wachholder and Franz (1944) 15 to 41% and Pittet et al. (1974) 21 to 41%.

The principal reason for this reduced variability is that only two equilibrium heat production measurements are required for the determination of the DIT, as against the multiple measurements required when following the bolus intake of food. The relative absence of the psychological stimuli of food when it is administered via a naso-gastric tube probably decreases both the absolute value and variability of the DIT measurement.

Further measurements would be desirable to confirm that the increased heat production is solely caused by the food intake. Heat production measurements before and after the second calorimetry period would be necessary to confirm that a plateau in heat production had definitely been attained. Also a number of measurements with the Isocal replaced by an acaloric fluid (e.g. saline) would show whether the fluid volume had any effect and whether there was an appreciable circadian rhythm effect on the result. Garrow and Hawes (1972) have shown little variation in the heat production of one subject given acaloric fluids intragastrically at a rate of 600 ml per hour over a period of over 6 hours. Measurements on a number of subjects would be required as a small variation in heat production hour to hour would be expected in any subject.

9.3 The Response to Moderate Severity Surgery at Different Environmental Temperatures

In Study C the heat production of the patients nursed at

20 and 28°C followed each other very closely no matter how the results were standardised to account for the varying size of the patients (Figures 37 to 39). The non-significant effect of the room temperature was also apparent in the RQ and nitrogen excretion results. The auditory canal temperature almost showed a significant response to ambient temperature but this was because of the known effect of the environment on the temperature gradient along the canal. An improved temperature probe that included a heater to reduce the temperature gradient to zero (Keatinge and Sloan, 1973; Little and Stoner, 1981) probably would not have shown any effect of temperature.

In burn patients a significant reduction in heat production is seen on changing the effective ambient temperature from 22 to 32°C (Davies and Liljedahl, 1970). In nude uninjured man metabolic rate is reduced by approximately 25% when the environmental temperature is increased from 20 to 28°C (Hardy, Stolwijk & Gagge, 1971) and female subjects in light cotton clothing show a 10% decrease in RMR at 28°C as opposed to 22°C (Dauncey, 1981). Unlike these examples the patients in Study C had a cotton bed gown and as much bed clothing as they required for comfort. The study was designed to observe any effect of the ambient temperature under clinically important conditions. Therefore it appears that the patients in Study C were able to adjust the microclimate around their body so that the environmental temperature had no detectable effect on their energy metabolism. It is also likely that the patients with more severe long bone fractures studied by Cuthbertson et al. (1972) were

less able to take full advantage of the insulating effect of bed clothing (Davies and Liljedahl, 1970, p 75) than were the surgical patients in Study C and in Spivey and Johnston's (1972) study.

There appears to be no benefit to energy metabolism of maintaining patients following a moderate severity surgical procedure in a warm environment (i.e. 28^{o}C). This agrees with previous work on the effect of environmental temperature on nitrogen excretion in patients with a similar severity of trauma (Cuthbertson et al., 1972; Spivey and Johnston, 1972).

9.4 Comparison of the Energy Metabolism Response to Moderate Severity Surgery with Previous Workers' Results

If the measurements on the two groups of patients in Study C are combined, they can be compared with the results of other studies of the response to surgical trauma. The patients in Study C had highly significant effects of surgery on RMR, RQ, nitrogen excretion and core temperature. This does not agree with all the previous reports of the effects of elective surgery.

Cope et al. (1953) reported that in six patients who had undergone surgical procedures ranging from herniorrhaphy to hysterectomy all the oxygen consumption determinations before and after surgery fell within the normally accepted range. A slight rise in oxygen consumption was measured immediately after operation though it returned to its pre-operative level 5 to 7 days after surgery.

Kinney et al. (1968) and Duke et al. (1970) measured a decrease in the average RMR over the first four days following elective surgery. The operative procedures were mixed (mainly gastrectomies and sub total colectomies) but they were no less severe than those of the present study. The conclusion of Duke et al. (1970) was that patients undergoing elective operation did not have a significant rise in RMR or nitrogen excretion. Danielsson et al. (1976a) supported these results when he reported that in 12 patients undergoing cholecystectomy, the metabolic rate on the day following the operation did not increase by more than 10% in any patient, the average increase being 7.1%.

The possible cause for this apparent disagreement could lie in the dramatic change in nutrition usually experienced by a surgical patient. The patients in Study C had the same nutritional input on their control measurement day and post-operatively, Duke's patients however were in positive energy and nitrogen balances pre-operatively and in negative balances over the first four post-operative days. Their limited metabolic response to surgery can not therefore be explained by a poor pre-injury protein intake (Munro and Chalmers, 1945; Fleck and Munro, 1965). However the positive energy balance before surgery could increase the control heat production values through thermogenesis, and the higher food intake and exercise component in the pre-operative patients could increase the urinary nitrogen excretion (Wilmore, 1977). Only when Duke's patients are referred to their pre-operative control

values are they significantly different from the patients in Study C (Table 58). The large spread of values in Duke's results means that the other comparisons (absolute values, urinary nitrogen excretion) are not significantly different.

Other studies have produced results that are in closer agreement to Study C. Clowes et al. (1976) described the maximal metabolic rates seen in their patients during an uneventful convalescence as only slightly above normal. The mean value given was 47.5 W m^{-2} (980 kcal m^{-2} d^{-1}) which is only just below the 48.8 W m^{-2} measured on the first post-operative day in the patients in Study C, and would represent a 10% increase on the control day value. Unfortunately there were no pre-operative values given in Clowes' study. Long et al. (1979a) measured the mean peak increase in RMR following surgery to be 23.9%. This is greater than measured in Study C, but as the surgical procedures used in Long's study are not detailed this could reflect the response to more severe surgical procedures.

The complicating effect of changes in nutrition associated with trauma have been noted before and it has been suggested (Abbott and Albertson, 1963) that the metabolic response to surgery, of the severity used in the present study, is simply a response to a reduced food intake. This conflicts with the results from Study C where the patients were on similar nutritional inputs pre- and post-operatively and yet still showed a significant metabolic response.

	RMR (Wm^{-2})	RMR % of control day value	Urinary Nitrogen ($mmol\ m^{-2}$)
Study C			
mean	46.8	108.7	433
Standard deviation	3.58	8.54	80.9
number	20	20	20
Duke et al (1970)			
mean	43.5	94.5	371
Standard deviation	6.54	14.88	136.0
number	15	11	15
Unpaired T	1.912	3.395	1.684
p	ns	**	ns

Table 58: Comparison of values averaged over the first four post-operative days, from Study C and Duke et al. (1970).

The percentage of energy derived from protein increased from 11.4% before to 16.0% after operation. This is similar to the findings of Duke et al. (1970) who measured the energy derived from protein as falling in the range 12 - 20% of heat production whatever the injury. The amounts of fat and protein lost over the last 4 post-operative days of 0.47 and 0.27 kg respectively (that is respectively 5.2 and 2.5% of the total body compartments of these tissues for a 65 kg man (Passmore and Robson, 1974). The absolute amounts are similar to those calculated by Kinney et al (1968) for his surgical patients. As can be seen from tables 56 and 57 fat provides approximately four times as much energy as protein post-operatively.

One point that has not been discussed with regard to the comparison of results in Study C with their pre-operative control values is the effect that the four day semi-starvation would have on a normal individual. Daws et al. (1972) studying uninjured subjects who had a similar carbohydrate oral diet to that administered intravenously to the patients in Study C, measured a decreased heat production of approximately 5% over the first four days. Also the nitrogen excretion would fall below normal levels in an uninjured subject in response to semi-starvation. Hence after four days, the comparisons should be being made against a decreasing control value for heat production and nitrogen (Long et al., 1979a). If this is taken into account, the patients have probably

not returned to a normal metabolism after four days despite their return to control heat production and nitrogen values and the metabolic response to surgery is still continuing.

In summary the results show:-

i) The calorimetry results were in agreement with predicted values.

ii) The thermogenic effect of a continuosly administered mixed diet was approximately 5% of the dietary energy input.

iii) With similar nutritional inputs before and after operation, the changes in heat production, urinary nitrogen, RQ and core temperature in response to a cholecystectomy or vagotomy and drainage were significant. The mean increase in RMR over the 4 day post-operative period was 8.7%.

iv) Treating a patient following moderate severity surgery in a room kept at 28°C rather than 20°C had no significant effect on the metabolic response measured in iii).

v) The heat production and nitrogen excretion returned close to their pre-operative values after four days though they were still probably higher than those of a control subject on the same dietary intake.

vi) Respiratory quotient decreased significantly through the post-operative period indicating an increased use of fat for energy.

vii) The energy metabolism demands of the response to moderate severity surgery though significant were minor compared to the body's total protein and fat reserves.

CHAPTER 10

Conclusions

The animal experiments indicate that the burn model is a close parallel to the human situation and that the results are reproducible. The hypermetabolism is significant and stable over an extended period. The results show that the calorimetry periods are representative of the average daily energy expenditures though the calorimetry values can underestimate the average figures by up to 20% at the higher temperature. The use of a control animal for comparison reduces the error involved in using the short calorimetry period but when the ambient temperature is into or above the control animals' TNZ, behavioural differences between the injured and uninjured animals become significant. The benefit for the injured animals of raising the environmental temperature by 10°C was confirmed though increasing the food intake by 35% would have a similar effect. This supports the practice of treating burned patients at a high ambient temperature especially where food intake is inadequate or being administered parenterally.

Though the metabolic rate is decreased at 30°C it is not certain whether this is the optimum environment for an injured rat. The results suggest that the burn is still a significant cooling load at 30°C and that the burn wound temperature is below that expected because of the evaporative losses and loss of insulation. If this cooling load could be further reduced so might heat

production and this would support Arturson's theory of the effect of burn injury in man. The two methods (high humidity and infra-red heating) of attempting to reduce hypermetabolism, which would allow comparison with previous animal and human work, are not practical on the current calorimeter. The high humidity causes condensation and loss of direct calorimetry results and the gradient layer box is not big enough to accept a heater. Also under these conditions use of an uninjured control animal for comparison might not be appropriate so that a 24 hour calorimetry measurement would be desirable. However as the hypermetabolism has been shown to be stable, a burned animal could be kept in several different environments for 2 to 3 day periods without expecting a significant change in the injury severity. Use of say a simple closed circuit apparatus for measuring oxygen consumption in which the rat could stay for most of a 24 hour period would be sufficient initially for determining the effect of high humidity or, the most practical treatment, use of infra-red heating.

In the human study though the effect of the operation was significant,no benefit of a reduction in energy expenditure was measured at the high treatment temperature. This is probably because of the relatively minor nature of the trauma and the fact that it is a closed injury. It is possible that a closed injury of a severity closer to that of a burn may show a benefit of treatment at a higher temperature and currently the magnitude of the energy response and the effect of temperature are being

studied in patients undergoing more severe forms of surgery.

The measurement of dietary induced thermogenesis on the burned animals showed that it was significantly reduced when compared with the uninjured controls. Though this agrees with earlier measurements on patients with disease, more recent work suggests an increased DIT in burned or multiply injured patients, particularly at high rates of intravenous feeding. The method of measurement of DIT described in the volunteer study could easily be applied to a range of enterally and parenterally fed patients. The possibility of a change in DIT with the rate of feeding could be investigated with particular interest in the possible stress caused by overfeeding after severe injury. The method is also suitable for investigating whether the thermogenic effects of injury, diet and cold are dependent or not. The results from the animal experiments suggest that injury and dietary thermogenesis are dependent while the cold induced thermogenesis is independent of both. More work is required to confirm this in the rat and to show whether the situation is similar in man.

Appendix A

Calibration Procedure and Calculation of Small Animal Calorimeter Results

The operation of the calorimeter and calculation of results has been presented in detail by other workers (Carter, 1975; Drury, 1976; Gray, 1978; Richards, 1979). The procedures will only be summarised, with any modifications to the previously established methods shown.

Calibration Procedures

Routine calibrations of the hygrometer, oxygen and carbon dioxide analysers were carried out twice each week. All the equipment was switched on as if for a calorimetry measurement and left for approximately one hour to reach normal operating temperatures.

The hygrometer was calibrated by measuring its output while a known amount of distilled water evaporated from a tissue in the bottom of a tray placed in the gradient layer box. The tray used was of flexible plastic rather than the previously used metal tray because:-

i) its lighter weight allowed the use of a more accurate balance for the determination of the 1 to 2g of water evaporated during a calibration.

ii) it could be folded in half to reduce any evaporative loss during the transfer from the balance to the gradient layer box.

The area under the hygrometer output curve was measured with the weight of water evaporated and the flow of dry gas through the box (see later in appendix) to give the sensitivity of the hygrometer. The area was measured by planimetry and the calculation performed manually for experiments 1 and 2. A program was written for the microcomputer in experiments 3 and 4 to measure the area under the hygrometer curve automatically and process the calibration data. The two methods of calculation were shown to agree to better than 1%.

The sensitivities of the oxygen analyser and carbon dioxide analyser were calibrated using a zero gas (oxygen free nitrogen) and a span gas (external air for the oxygen analyser and 0.8% carbon dioxide with the balance nitrogen for the carbon dioxide analyser). The electronics of each analyser was adjusted on calibration to give a 10mV deflection for each 1% concentration of the gas.

The baseline outputs of the hygrometer and gas analysers were routinely measured at the beginning and end of each calorimetry run when there was no animal in the gradient layer box. The value of the instrument outputs at any time during calorimetry was calculated as the deflection from the linearly interpolated baseline produced by joining the beginning and end baseline values.

Calculation of the Calorimetry Values

The total and outlet flows of gas in the calorimeter have to be reduced to dry gas values at standard temperature and pressure (STP), i.e. $0^{\circ}C$ and 760 mm Hg. As the gas in the wet gas meters (WGMs) is saturated with water vapour, the partial pressure of dry gas (PM) in the meters is:-

$$PM = \text{Pressure at the meters} - \text{Pressure of saturated water vapour at meter temperature (TW)}$$

$$= PA + \frac{PW}{1.357} - (1.43 \times TW - 11.5) \text{ mm Hg}$$

(For the key to the symbols used see the end of the appendix)

Therefore the factor (CF) to correct the flow measured under the prevailing conditions to the flow of dry gas at STP is:-

$$CF = \frac{PM}{760} \times \frac{273.2}{(273.2 + TW)}$$

The outlet WGM produces 480 pulses per litre of gas flow and the recirculation WGM 60 pulses per litre, so that the outlet flow corrected to STP is:-

$$FO = \frac{No \times CF}{480} \quad (l \text{ min}^{-1})$$

and the recirculated flow is:-

$$FR = \frac{NR \times CF}{60} \quad (l \text{ min}^{-1})$$

Hence the total flow of dry gas through the gradient layer box is:-

$$FT = FO + FR \qquad (l\ min^{-1})$$

Direct Calorimetry

Approximately 90% of the sensible heat loss is through the walls of the gradient layer box (Hl). This loss is:-

$$Hl = KTH \times VG \qquad (W)$$

The sensitivity ($KTH\ (WV^{-1})$) of the gradient layer box varies over the range 20 to 30°C. Its value can be approximated by the empirical formula (Carter, 1975):-

$$KTH = \frac{0.357 + 0.002 \times (T - 20)}{(0.965 - 0.002 \times FT) \times (1 + 0.00015 \times (WR-250))} \qquad (WV^{-1})$$

The majority of the remaining sensible heat loss is heat given to the ventilating gas. This has two components:-

i) the increase in the heat capacity of the dry air
ii) the increase in the heat capacity of the water vapour that constitutes the background humidity.

Therefore the heat given to the ventilating gas (H2) is:-

$$H2 = \frac{FT}{60} \times \left[D \times CA \times (TO - TI) + \frac{Z}{1000} \times CW \times (TO - TI)\right] \qquad (W)$$

There are three small corrections that need to be made to the sensible heat loss. The cooling of urine (H3) and faecal losses (H4) and the water vapour respired or

lost through the wound (H5) in the gradient layer box are not effectively heat losses from the animal. These corrections are approximated by:-

$$H3 = \frac{MU \times (TR - T) \times 4.18}{L \times 60} \quad (W)$$

$$H4 = \frac{MF \times (TR - T) \times 4.18}{L \times 60} \quad (W)$$

$$H5 = \frac{VH \times KH \times CW \times (TR - T)}{1000} \quad (W)$$

Assuming that most of this heat would have been lost through the gradient layer, the net heat loss (H6) through the walls is:-

$$H6 = H1 - H3 - H4 - H5 \quad (W)$$

and the total sensible heat loss (H7) is :-

$$H7 = H6 + H2 \quad (W)$$

The insensible heat loss (H8) associated with the evaporation of water vapour from the respiratory tract, burn wound and intact skin is calculated from:-

$$H8 = \frac{FT \times VH \times 760 \times KH \times 2420}{PA \times 60 \times 1000} \quad (W)$$

and the total direct losses (H9) are:-

$$H9 = H7 + H8 \quad (W)$$

Indirect Calorimetry

The carbon dioxide production (CP) is calculated from:-

$$CP = \frac{FO \times VC \times 760 \times 1.035}{PA} \quad (l\ min^{-1})$$

The oxygen consumption (OC) is calculated from the apparent change in oxygen concentration using a correction dependent on the amount of carbon dioxide produced:-

$$OC = \frac{FO \times VO \times 760}{0.7905 \times 1 \quad \times PA} - \frac{0.2095 \times CP}{0.7905} \quad (l\ min^{-1})$$

The heat production (HO) is calculated using Weir's (1949) simplified formula which assumes that 12.5% of the total heat production is produced from protein catabolism:-

$$HO = 274 \times OC + 70 \times CP \quad (W)$$

This introduces an error of less than 0.5% in the heat production over the range of urinary nitrogen excretions measured in experiments 1 to 4.

The results for experiments 1 and 2 were calculated on a minicomputer (620-L, Varian Ltd.) while the results for experiments 3 and 4 were calculated on a microcomputer (PET 2001, CBM Ltd.). Both programs integrated the voltage outputs of the instruments over the required period using a trapezoidal numerical approximation. This was of sufficient accuracy because the measurements

were of equilibrium states with no significance overall upward or downward trends. Manual integration (planimetry) and the computed integration agreed to within $\pm$ 1%. The program written for the microcomputer also allowed the usual 60 minute calorimetry period to be divided into smaller time units (say 5 minutes) and the variation of the various routes of heat loss and production to be estimated from these 5 minute blocks. This gave an indication of movement etc., during the calorimetry period. Examples of the two different result print-outs are shown after the end of the appendix.

Abbreviations used

CA - Specific heat of air at a constant pressure = 1.0045 ($Jg^{-1}\ ^{o}C^{-1}$)

CF - Correction factor to dry air at STP

CP - Carbon dioxide production ($l\ min^{-1}$)

CW - Specific heat of water vapour = 1.922 ($Jg^{-1}\ ^{o}C^{-1}$)

D - Density of dry air at STP = 1.293 ($g\ l^{-1}$)

FO - Outlet flow ($l\ min^{-1}$)

FR - Recirculated flow ($l\ min^{-1}$)

FT - Total flow ($l\ min^{-1}$)

HO - Heat production (W)

H1 - Gross heat loss through gradient layer (W)

H2 - Heat to ventilating air (W)

H3 - Heat loss of urine (W)

H4 - Heat loss of faeces (W)

H5 - Heat loss from animal water vapour (W)

H6 - Net heat loss through gradient layer (W)

H7 - Sensible heat loss (W)

H8 - Insensible heat loss (W)

H9 - Total heat loss (W)

KH - Hygrometer calibration factor ($g\ m^{-3}\ V^{-1}$)

KTH - Gradient layer calibration factor ($W\ V^{-1}$)

L - Duration of calorimetry (min)

MF - Mass of faeces (g)

MU - Mass of urine (g)

NO - Pulse rate from outlet WGM (pulses min^{-1})

NR - Pulse rate from recirculation WGM (pulses min^{-1})

OC - Oxygen consumption ($l\ min^{-1}$)

PA - Atmospheric pressure (mm Hg)

PM - Partial pressure of dry gas at WGM (mm Hg)

PW - Gauge pressure at WGM (cm H_2O)

T - Average calorimeter temperature = $\frac{TI + TO}{2}$ (^oC)

TI - Calorimeter inlet temperature (^oC)

TO - Calorimeter outlet temperature (^oC)

TR - Animal core temperature (usually assumed to be 36^oC) ^oC

TW - Temperature of WGM

VC - Carbon dioxide analyser output (V)

VG - Gradient layer output (V)

VH - Hygrometer output (V)

VO - Oxygen analyser output (V)

WGM - Wet gas meter

WR - Rat weight (g)

Z - Humidity of inlet air ($g\ m^{-3}$)

Typical small animal calorimeter print-out from the mini-computer used in Experiments 1 and 2.

```
DAY              MONTH            YEAR
 22               8                77
RUN NUMB  29
RAT NUMB  1
RAT WT - G                        ? 257
AT PRES - MM HG                   ? 757
W.G.M. TEMP - DEG C                23.9043
W.G.M. PRES - CMS H20             ? 23
OUT P.R.                           630.543
RECIRC P.R.                        299.539

STP CORR FACTOR                    .913553
OUTLET FLOW                        1.29524     L/MIN
RECIRC FLOW                        4.56074     L/MIN
TOTAL FLOW                         5.85599     L/MIN

CAL MV                             4.72915
DEW PT TEMP - DEG C                14.3331
IN TEMP - DEG C                    20.521
TEMP INC - DEG C                   1.44636
HYGR CAL FACTOR                   ? .776734
HYGR MV                            1.7767
02 MV                              5.01231
C02 MV                             3.66103
URINE WT - G                      ? 2.9
FCL WT - G                        ? 0
RUN TIME - MIN                    ? 230
RAT TEMP - DEG C                  ? 36

DIRECT CALORIMETRY

TOTAL WATER LOSS = .484332     GMS OVER PERIOD = 60    MINS

GROSS HEAT LOSS THROUGH WALLS   1.77353     W
URINE HT LOSS                   1.29302E-02    W
FAECAL HT LOSS                  0     W
SPEC HT LOSS BY EXPIRED H20     3.50476E-03    W

NET HEAT LOSS THROUGH WALLS      1.75705     W

HT LOSS TO AIR STREAM            .136732     W
```

Typical small animal calorimeter print-out from the microcomputer used in Experiments 3 and 4.

DATE 01 04 80 RUN 9 RAT 5 RAT WT 267
AT PRESS 750.5 mmHg WGM PRESS 29 cmW HYGR CAL 2.019 gm-3
TOT FLOW 5.967 l/M OUT FLOW 1.193 l/M
AIR IN 30.71 oC DEW PT 12.826 oC

CHANS	BEG ZEROES	END ZEROES	MEANS
0	0	0	0
1	-.011	1E-03	-5E-03
2	-.061	-5E-03	-.033
3	30.657	30.708	30.71
4	1.966	2.094	2.03
5	.525	.617	.571
6	.32	.409	.364
7	12.724	12.837	12.826
8	30.496	30.591	30.775
9	26.883	27.178	27.163
10	30.593	30.752	30.742

WT URINE .57 g WT FAECES 0 g WT INFUSED 0 g BURN
LOG START 105700 RAT IN 105900 INFUSE ON 000000
LOG END 142300 RAT OUT 133300 INFUSE OFF 000000

INTEGRAL START TIME 120000 INTEGRAL END TIME 130000

NUMB OF PERIODS= 12

No	TITLE	MEAN	S.D.	C.V.
0	HEAT PRODUCTION	2.33	.0841	3.61
1	HEAT THRO BOX	.847	.0392	4.63
2	HEAT TO AIR	.07	7.1E-03	10.2
3	HEAT TO URINE	1E-03	0	.54
4	HEAT TO FAECES	0	0	0
5	HEAT TO WATER	5E-03	2E-04	3.69
6	NET HEAT THRO BOX	.841	.0393	4.67
7	SENSIBLE	.91	.0347	3.81
8	INSENSIBLE	1.32	.0479	3.63
9	TOTAL LOSSES	2.23	.0457	2.05
10	R. Q	.741	.0421	5.68
11	% DIFF	-4.5	3.9467	-87.7
12	OXYGEN	7.078	.2457	3.47
13	CARBON DIOXIDE	5.250		

Appendix B

The Composition of the animal diet

	g/kg	
Dextrin	546.2	(1)
Glucose	100.0	
Arachis oil	100.0	
Lactalbumin	200.0	(2)
Minerals	51.1	(3)
Vitamins	2.7	(4)

(1) Dextrin type II, Sigma Chemical Co. Ltd. London.

(2) Lactalbumin, practical grade, Sigma Chemical Co. Ltd. London .

(3) Contained: Rodgers and Harper salt mixture (ICN Pharmaceuticals Ltd.), 50 g/kg; supplemented by ((in mg/kg) Cr K $(SO_4)_2$ $12H_2O$, 48; $MnSO_4$ $4H_2O$, 124; $ZnSO_4$ $7H_2O$,155; Ni Cl_2 $6H_2O$, 4.1; Sn Cl_2 $2H_2O$, 3.8; Co Cl_2 $6H_2O$, 0.81; NH_4VO_3; 0.46; NaF, 5.5; KIO_4, 1.5; Na_2SiO_3 $5H_2O$, 755.

(4) Contained (mg/kg): p-aminobenzoic acid 10, D-biotin 5, folic acid, 5; myo-inostil, 400; nicotinic acid, 30; Ca D - pantothenate, 20;pyridoxine, 10; riboflavin, 10; thiamine, 10; cyanocobalamin, 0.03; choline chloride, 200; retinyl acetate, 8; cholecalciferol, 0.25; α-tocopherol, 200; menadione 5.

(3 and 4 were prepared by the Department of Biochemistry, Western Infirmary, Glasgow)

Appendix C

Operation of the Human Indirect Calorimeter and Calculation of Heat Production Values.

The initial instrument checks, data collection and processing for a calorimetry measurement are all guided or performed by the microcomputer (PET 2001, CBM Ltd.) connected to the equipment. A sequence of programs, each loaded automatically into the computer by the preceding one, has been written to perform the necessary tasks. Once the computer has been switched on and the first program (named 'INIT') loaded, the computer prompts necessary operator actions by displaying appropriate messages on the screen. The sequence of operations requested by the 'INIT' program are as follows:-

1) Switch the carbon dioxide analyser from standby to operate.

2) Select sample gas flow into carbon dioxide analyser.

3) Switch oxygen analyser lamps and feedback on.

4) Switch data logging equipment and gas flow ratemeter on.

5) Connect the air supply to the correct room.

6) Connect the data cable to the correct room.

7) Fill humidifier.

8) Check water level in the wet gas meter.

9) Switch on pumps.

10) Check that both oxygen amalyser cells are monitoring the correct air streams.

11) Set the flow through the oxygen analyser cells to 75 ml min^{-1} with a flow of approximately 0.5 l min^{-1} through the bypass flowmeters.

12) Set the flow through the carbon dioxide analyser to 1.67 l min^{-1}.

13) Load disc drive number 3 with a blank, formatted minidisc.

The 'INIT' program also automatically loads a section of computer memory from location 28160 upwards with a machine code program that allows the transmission of a 'Group Executive Trigger' (G.E.T.) command over the IEEE-488 bus. This should be a standard bus command but it is not provided by the microcomputer operating software. The machine code program also allows direct input of data transmitted over the bus into a string variable specified in the program.

At the completion of the 'INIT" program, the operator is asked if a gas analyser calibration is required or whether a calorimetry measurement using a subject is to be performed. If a calibration is required the program 'CALIB' is automatically loaded. This program supervises the two point (zero and span gas) calibrations of the oxygen reference and sample cells and the carbon dioxide analyser as specified in Section 6.3.2. The computer

monitors the output of the analysers with the appropriate gases flowing through them and computes the gain calibration factor after appropriate correction for atmospheric pressure and the water vapour pressure of the external air used as the span gas for the oxygen analysers.

At the finish of the 'INIT' program if a calorimetry measurement is required the data logging program 'DLOG8' is loaded. This program requires the manual input of the following data:-

1) Subject name
2) Date
3) Atmospheric pressure (mmHg)
4) Wet gas meter pressure (cm H_2O)
5) Oxygen calibration factor
6) Carbon dioxide calibration factor
7) Patient weight (kg)
8) Data channels to be logged (see end of Appendix C)
9) Logging period (usually 15 or 30 seconds)

At the beginning and end of a calorimetry period the head canopy is bypassed so that all the gas analyser cells are measuring external air. These values (beginning and end zeroes) are recorded by the computer and used to correct for any drift in the analysers during the calorimetry measurement (see Section 6.3.2.). The head canopy, with the subject installed, is connected to the gas circuit once the beginning zeroes have been

measured and the desired data channels are scanned and recorded at the logging repetition rate (15 or 30 seconds). The voltage from the wet gas meter ratemeter (V(4)) is converted to a flow in 1 min^{-1} using:-

$$\text{Flow(VF)} = 757.9 \text{ X } V(4) - 0.059 \quad (1\ min^{-1})$$

The resistance measurements (in kΩ) from the YSI 44011 thermistors (Channels 6,8,9,14 and 15) and the YSI series 400 thermistors (Channels 10,11,12 and 13) are converted to temperature measurements using:-

$$\text{Temperature} = A_0 + A_1 \text{ X } R + A_2 \text{ X } R^2 + A_3 \text{ X } \log(R) \quad (^oC)$$

where R is the resistance measured and the values of the constants A_0 - A_3 are

Constant	YSI 44011	YSI Series 400
A_0	143.699	41.8004
A_1	0.05793	3.03863
A_2	$-6.0081 \text{ X } 10^{-5}$	-0.1420
A_3	-26.9245	-28.3414

The means and standard deviations of the channels over the last five logging periods are continually displayed on the computer screen, as are the calculated flow rate, heat production and respiratory quotient. These latter two values are also printed in graphical form during a calorimetry run. Provision has also been made in the program to restart a calorimetry measurement quickly and with minimal loss of data, after a temporary computer

malfunction or loss of mains power. After the end zeroes have been recorded, with the head canopy by-passed at the end of a calorimetry measurement, the next program 'CAL DISCKOPY' is loaded. This makes a security copy of the logged data on another mini-disc and loads the next program '2D COMP'. This compacts the logged data onto a storage mini-disc so that the data occupies minimal storage space and is also suitable for final processing by the subsequent program 'AUTO IND CAL'.

The final processing of the logged data allows account to be taken of the baseline drift of the analysers using the beginning and end zero measurements. A graphical output of heat production, respiratory quotient, movement and external auditory canal temperature is possible for any specified portion of the calorimetry run with any specified frequency of point plotting.

Numerical output of the various results over any desired portion (30 to 45 minutes usually) of the measurement is also available. This can be subdivided into smaller periods (say 5 minutes) and the standard deviation of these periods calculated to give an estimate of the stability of the run. An example of the graphical and numerical outputs for patient 6 in Study C on his second post-operative day is given on the following pages.

The changes in instrument outputs are calculated as differences from the linearly interpolated baseline

connecting the beginning and end zeroes. The values are integrated using Trapezoidal numerical integration which is of sufficient accuracy for the equilibrium situations being measured. The gas flow through the wet gas meter must be calculated as dry gas at standard temperature and pressure, i.e. $0^{o}C$ and 760 mm Hg. As the gas in the meter is saturated with water vapour, the partial pressure of dry gas (PM) is:-

PM = Pressure at the wet gas meter
- Pressure of the saturated water vapour at the meter temperature (TW)

$$PM = PA + \frac{PW}{1.3595} - (1.43 \times TW - 11.5) \text{ mm Hg}$$

(for the key to the symbols used, see the end of the appendix).

The correction factor (CF) to correct the flow to dry gas at STP is:-

$$CF = \frac{PM}{760} \times \frac{273.2}{(273.2+TW)}$$

Therefore the flow (F) is:-

$$F = VF \times CF \quad (l \text{ min}^{-1})$$

As the gas measured in the gas analysers contains water vapour at a humidity determined by the cold bath temperature (TB), its vapour pressure (VP) must be calculated. Over the temperature range ($2^{o}C$-$10^{o}C$) this

can be calculated from:-

$$VP = 4.5864 + 0.3186 \times TB + 0.0142 \times TB^2 \quad \text{mm Hg}$$

If the gas in the cells was dry the analyser outputs would be increased by a factor (SP):-

$$SP = \frac{PA}{PA-VP}$$

Therefore, using the gain calibration factors of the carbon dioxide and oxygen analysers (CC and OC respectively) the carbon dioxide production (CO) is:-

$$CO = SP \times CC \times F \times VC \times \frac{760}{(100 \times PA)} \quad (1\ min^{-1})$$

and oxygen consumptiom (O) is:-

$$O = SP \times OC \times \left(\frac{VO \times 760 \times F}{PA \times 79.054}\right) - \frac{0.20946 \times CO}{0.79054} \quad (1\ min^{-1})$$

The respiratory quotient (RQ) therefore is:-

$$RQ = \frac{CO}{O}$$

and the heat production (I) is:-

$$I = O \times (87 + 27 \times RQ) \times \frac{4186}{(60 \times 22.414)} \quad (W)$$

Key to Symbols

CC - Carbon dioxide analyser gain calibration factor

CF - Flow correction factor to dry gas at S.T.P.

CO - Carbon dioxide production ($1\ min^{-1}$)

F - Flow of dry gas ($1\ min^{-1}$)

I	-	Heat production (W)
OC	-	Oxygen analyser gain calibration factor
O	-	Oxygen consumption (l min^{-1})
PA	-	Atmospheric pressure (mm Hg)
PM	-	Partial pressure of dry gas in wet gas meter (mm Hg)
PW	-	Guage pressure of wet gas meter (cm H_2O)
RQ	-	Respiratory quotient
SP	-	Correction factor for water vapour pressure in analyser cells
TB	-	Temperature of cold bath (^oC)
TW	-	Temperature of wet gas meter (^oC)
VC	-	Percentage increase in carbon dioxide concentration (%)
VF	-	Uncorrected flow through wet gas meter (l min^{-1})
VO	-	Percentage decrease in oxygen concentration (%)
VP	-	Vapour pressure of water vapour at TB (mm Hg)

Data Logging Channels

Channel Number	Channel Identification
0	Oxygen analyser reference cell
1	Oxygen analyser sample cell
2	Differential oxygen analyser output
3	Carbon dioxide analyser
4	Gas flow ratemeter output
5	Movement detector output
6	Cold Bath temperature
8	Wet gas meter temperature
9	Carbon dioxide analyser temperature
10	Patient temperature No. 1
11	Patient temperature No. 2
12	Patient temperature No. 3
13	Room temperature
14	External auditory canal temperature
15	Head canopy air temperature

Typical graphical print-out from the human indirect calorimeter.

PATIENT = J CONAGHAN STUDY 3

DATE= 16 10 80
ATMOS PRESSURE= 755.5 MM HG
WGM PRESSURE= 27.5 CM H20
WEIGHT = 85 KG

CHAN	MEAN	BEG ZEROES	END ZEROES
0	204.96890	205	205
1	202.96470	209.2	209.1
2	1.9650504	-4.1802	-4.1938
3	5.3616386	.6016	.718
4	35.929855	35.979008	35.653969
5	15.028487	0	11.744
6	4.7193781	4.4996	4.827
8	22.835025	22.515	23.1604
9	35.579512	35.347	35.8042
10	28.742016	19.731	19.8212
11	28.338403	20.1362	19.8274
12	29.814764	19.8634	19.7854
13	19.272168	19.8158	19.6076
14	23.247941	21.79	0
15	21.300084	23.29	0

02 CALIBRATION FACTOR 1.000425 CO2 CALIBRATION FACTOR 1.05578

RUN START 092909
PATIENT IN I
PATIENT OUT 101857
RUN END 102903
START TIME FOR HEAT PROD CALCS= 093500
FINISH TIME FOR HEAT PROD CALCS= 100500
INTEGRATION TIME PERIOD= 5 MINS
OFFSET TO BE ADDED TO MOVEMENT= 0

PERIOD 1 FROM 093500 TO 094000
HEAT PRODUCTION= 101.6 WATTS R.Q. = .759
02 CONS = 304 mL/MIN CO2 PROD = 231 mL/MIN
MOVEMENT = 8
TEMP 1= 31.35 C TEMP 2= 31.43 C TEMP 3= 31.91 C TEMP 4= 19.07 C
TEMP 5= 22.31 C TEMP 6= 34.36 C

PERIOD 2 FROM 094000 TO 094500
HEAT PRODUCTION= 102.8 WATTS R.Q. = .764
02 CONS = 307 mL/MIN CO2 PROD = 235 mL/MIN
MOVEMENT = 3.7
TEMP 1= 31.44 C TEMP 2= 31.25 C TEMP 3= 32.5 C TEMP 4= 19.38 C
TEMP 5= 22.38 C TEMP 6= 34.69 C

PERIOD 3 FROM 094500 TO 095000
HEAT PRODUCTION= 101.2 WATTS R.Q. = .767
02 CONS = 302 mL/MIN CO2 PROD = 232 mL/MIN
MOVEMENT = 15.5
TEMP 1= 31.58 C TEMP 2= 31.04 C TEMP 3= 32.97 C TEMP 4= 19.15 C
TEMP 5= 26.41 C TEMP 6= 30.8 C

PERIOD 4 FROM 095000 TO 095500
HEAT PRODUCTION= 101.3 WATTS R.Q. = .767
02 CONS = 302 mL/MIN CO2 PROD = 232 mL/MIN
MOVEMENT = 7.3
TEMP 1= 31.67 C TEMP 2= 30.93 C TEMP 3= 33.3 C TEMP 4= 19.11 C
TEMP 5= 34.84 C TEMP 6= 22.46 C

PERIOD 5 FROM 095500 TO 100000
HEAT PRODUCTION= 101 WATTS R.Q. = .763
02 CONS = 301 mL/MIN CO2 PROD = 230 mL/MIN
MOVEMENT = 17.5

TEMP 1= 31.76 C TEMP 2= 30.93 C TEMP 3= 33.29 C TEMP 4= 19.45 C
TEMP 5= 34.86 C TEMP 6= 22.5 C

PERIOD 6 FROM 100000 TO 100500
HEAT PRODUCTION= 102.3 WATTS R.Q. = .777
02 CONS = 304 mL/MIN CO2 PROD = 236 mL/MIN
MOVEMENT = 34
TEMP 1= 31.37 C TEMP 2= 30.75 C TEMP 3= 33.41 C TEMP 4= 19.02 C
TEMP 5= 34.89 C TEMP 6= 22.58 C

STATISTICAL RESULTS

AVERAGES OF 6 PERIODS

PERIOD 0 FROM 093500 TO 100500
HEAT PRODUCTION= 101.7 WATTS R.Q. = .766
02 CONS = 303 mL/MIN CO2 PROD = 232 mL/MIN
MOVEMENT = 14.3
TEMP 1= 31.53 C TEMP 2= 31.05 C TEMP 3= 32.9 C TEMP 4= 19.2 C
TEMP 5= 29.28 C TEMP 6= 27.9 C

STANDARD DEVIATIONS

PERIOD 0 FROM 093500 TO 100500
HEAT PRODUCTION= .69 WATTS R.Q. = 5.9E-03
02 CONS = 2 mL/MIN CO2 PROD = 2.4 mL/MIN
MOVEMENT = 10.97
TEMP 1= .165 C TEMP 2= .245 C TEMP 3= .585 C TEMP 4= .177 C
TEMP 5= 6.298 C TEMP 6= 6.054 C

Typical numerical print-out from the human indirect calorimeter.

REFERENCES:

Abbott, W.E. & Albertsen, K. (1963)
The effect of starvation, infection, and injury on the metabolic processes and body composition. Annals New York Academy of Science, 110, 941 - 964.

Al-Shamma, G.A. (1978)
Lipid Metabolism after injury: effects of nutrition and environmental temperature. Ph.D. Thesis, Glasgow, University of Glasgow.

Al-Shamma, G.A.A., Goll, C.C., Baird, T.B., Broom, J., Nicholas, G.A. & Richards J.R. (1979)
Changes in body composition following thermal injury in the rat. British Journal of Nutrition, 42, 367-375.

Arturson, M.G.S. (1977)
Transport and demand of oxygen in severe burns, Journal of Trauma, 17, 179-198.

Arturson, M.G.S. (1978)
Metabolic Changes following thermal injury. World Journal of Surgery, 2, 203-214.

Askanazi, J., Carpentier, Y.A., Elwyn, D.H., Nordenstrom, J., Jeevanandam, M., Rosenbaum, S.H., Gump, F.E. & Kinney, J.M. (1980)
Influence of Total Parenteral Nutrition on Fuel Utilisation in injury and Sepsis. Annals of Surgery, 191, 40-46.

Atwater, W.O. & Benedict, F.G. (1905)
A Respiration Calorimeter with Appliances for the Direct Determination of Oxygen. Publication 42, Washington D.C., Carnegie Institute.

Aub, J.C. & Means, J.H. (1921)
The basal Metabolism and the specific dynamic action of protein in liver disease. Archives of Internal Medicine, 28, 173-191.

Aulick, L.H., Hander, E.H., Wilmore, D.W., Mason, A.D. & Pruitt, B.A. (1979).
The relative significance of thermal and metabolic demands on burn hypermetabolism. Journal of Trauma, 19(8), 559-566.

Barr, P.O., Birke, G., Liljedahl, S.O. & Plantin, L.O. (1968)
Oxygen Consumption & water loss during treatment of burns with warm dry air. Lancet, 1, 164 - 168.

Bendict, F.G. & Benedict, C.G. (1924)
Body Posture and minor muscular movements as affecting heat production. Proceedings of the National Academy of Science, U.S.A., 10, 498-500.

Benedict, F.G. & Carpenter, T.M. (1918)
Food ingestion and energy transformation with special reference to the stimulating effects of nutrients. Publication 261, Washington D.C: Carnegie Institute.

Benedict, F.G. & MacLeod, G. (1920a)
The heat production of the albino rat.
II Influence of environmental temperature, age and sex; comparison with the basal metabolism of man. Journal of Nutrition, 1, 367-398.

Benedict, F.G. & MacLeod, G. (1929a)
The heat production of the albino rat.
I Technique, activity control and influence of fasting. Journal of Nutrition, 1, 343-366.

Benedict, F.G., Miles, W.R., Roth, P., Smith, M. (1919)
Human Vitality and efficiency under prolonged restricted diet. Publication 280, Washington D.C. : Carnegie Institute.

Benzinger, T.H., Huebscher, R.G., Minard, D. & Kitzinger, C. (1958)
Human Calorimetry by means of the gradient principle. Journal of Applied Physiology, 12, Supplement 51, 524

Berkson, J. & Boothby, W.M. (1938)
Studies on the energy metabolism of normal individuals. The interindividual and the intraindividual variability of basal metabolism. American Journal of Physiology, 121, 669-683.

Birke, G., Carlson, L.A. & Liljedahl, S.O. (1965)
Lipid metabolism and trauma. III Plasma lipids and lipoproteins in burns. Acta Medica Scandinavica 178, 337-350.

Birke, G., Carlson, L.A., Von Evler, U.S., Liljedahl, S.O. & Plantin, L-O. (1972).
Studies on Burns XII, Lipid Metabolism, Catecholamine excretion, basal metabolic rate and water loss during treatment of burns with warm dry air. Acta Chirurgica Scandanavica 138, 321-333.

Blaxter, K.L. (1967)
Techniques in energy metabolism studies and their limitations. Proceedings of the Nutrition Society, 26, 86-96.

Blaxter, K.L. (1978)
Adair Crawford and Calorimetry. Proceedings of the Nutrition Society, 37, 1-3.

Bradham, G.B. & Charleston, S.C. (1972)
Direct Measurement of total metabolism of a burned patient. Archives of Surgery, 105, 410-415.

Brown, A.A. (1973)
Energy Metabolism. In Physiology and Biophysics. Digestion, Metabolism, Endocrine Function and Reproduction. ed. Ruch, T.C. & Patton, H.D., Vol. 3, 20th edition, Philadelphia: W.B. Saunders.

Buskirk, E.R., Thompson, R.H., Moore, R. & Whedon, G.D. (1960)
Human energy expenditure studies in the National Institute of Arthritis & Metabolic Diseases Metabolic Chamber.
I. Interaction of cold environments and specific dynamic effect.
II. Sleep. American Journal of Clinical Nutrition, 8, 602-613.

Cairnie, A.B., Campbell, R.M., Cuthbertson, D.P. & Pullar, J.D. (1957)
The heat production consequent upon injury. British Journal of Experimental Pathology, 38, 504-511.

Caldwell, F.T. (1962)
Metabolic Response to thermal trauma:
II Nutritional Studies with rats at two environmental temperatures. Annals of Surgery, 155, 119-126.

Caldwell, F.T. (1970)
Changes in Energy Metabolism during recovery from injury. In Energy Metabolism in Trauma, ed. Porter, R. & Knight, J., 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. Journal of Applied Physiology, 21, 1665-1671.

Caldwell, F.T., Osterholm, J.L., Sower, N.D. & Moyer, C.A. (1959)
Metabolic response to thermal trauma of normal and thyroprivic rats at three environmental temperatures. Annals of Surgery, 150, 976-988.

Campbell, R.M. & Cuthbertson, D.P. (1967)
Effect of environmental temperature on the metabolic response to injury. Quarterly Journal of Experimental Physiology, 52, 114-129.

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, Glasgow: University of Glasgow.

Carter, K.B., Drury, J.K. & Richards, J.R. (1976)
Calibration & performance of a small animal gradient layer calorimeter box. Medical and Biological Engineering, 14, 465-470.

Carter, K.B., Richards, J.R., Shaw, A., Boyd, I.A. & Harland, W.A. (1975)
Calorimetry System for Studying Small animals after injury. Medical & Biological Engineering, 13, 551-557.

Clark, R.G. (1967)
Surgical Nutrition. British Journal of Surgery, 54, 455-459.

Clark, R.G. (1971)
Caloric requirements after operation. Proceedings of the Nutrition Society, 30, 158-165.

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.
Surgical Clinics of North America, 56, 1169-1184.

Clowes, G.H.A., Randall, H.T. & Cha, C-J. (1980)
Amino acid and energy metabolism in septic and traumatized patients. Journal of Parenteral and Enteral Nutrition, 4, 195-205.

Coleman, W. & DuBois, E.F. (1915)
Calorimetric observations on the metabolism of typhoid patients with and without food. Archives of Internal Medicine, 15, 887-938.

Consolazio, C.F., Matoush, L.O., Johnson, H.L., Nelson, R.A. & Krzywicki, H.J. (1967a)
Metabolic aspects of acute starvation in normal humans (10 days). The American Journal of Clinical Nutrition, 20, 672-683.

Consolazio, C.F., Nelson, R.A., Johnson, H.L., Matoush, L.O., Krzywicki, H.J. & Isaac, G.J. (1976b)
Metabolic aspects of acute starvation in normal humans. Performance and cardiovascular evaluation. American Journal of Clinical Nutrition, 20, 684.

Cooper, K.E., Cranston, W.I. & Snell, E.S. (1964)
Temperature in the external auditory meatus as an index of central temperature change. Journal of Applied Physiology, 19, 1032-1035.

Cope, O., Nardi, G.L., Quijano, M., Rovit, R.L., Stanburg, J.B. & Wight, A. (1953)
Metabolic rate and thyroid function following acute thermal trauma in man. Annals of Surgery, 137, 165-174.

Cuthbertson, D.P. (1931a)
The distribution of nitrogen & sulphur in the urine during conditions of increased catabolism. Biochemical Journal, 25, 236-244.

Cuthbertson, D.P. (1932)
Observations on disturbance of metabolism produced by injury to the limbs. Quarterly Journal of Medicine, 25, 233-246.

Cuthbertson, D.P. (1936)
Further observations on the disturbance of metabolism caused by injury with particular reference to the dietary requirements of fracture cases. British Journal of Surgery, 23, 505-502.

Cuthbertson, D.P. (1942)
Post shock metabolic response. The Lancet, 1, 433-437.

Cuthbertson, D.P., Fell, G.S., Smith, C.M. & Tilstone, W.J. (1972)
Metabolism after injury. 1. Effects of severity, nutrition and environmental temperature on protein, potassium, zinc and creatine. British Journal of Surgery, 59, 925-931.

Cuthbertson, D.P., Smith, G.M. & Tilstone, W.J. (1968)
The effect of transfer to a warm environment (30°C) on the metabolic response to injury. British Journal of Surgery, 55, No.7, 513-516.

Danielsson, U., Arturson, G. & Wennberg, L. (1976a)
The elimination of hypermetabolism in burned patients. Burns, 2, 110-114.

Danielsson, U., Arturson, G. & Wennberg, L. (1976b)
A new technique for the long-term stable measurement of energy expenditure. Burns, 2, 107-109.

Dauncey, M.J. (1980)
Metabolic effects of altering 24 hour energy intake in man, using direct and indirect calorimetry. British Journal of Nutrition, 43, 257-269.

Dauncey, M.J. (1981)
Influence of mild cold on 24 hour energy expenditure, resting metabolism and diet induced thermogenesis. British Journal of Nutrition, 45, 257-267.

Dauncey, M.J. & James, W.P.T. (1979)
Assessment of the heart rate method for determining energy expenditure in man using a whole body calorimeter. British Journal of Nutrition, 42, 1-13.

Davies, J.W.L., Liljedahl, S.O. & Birke, G. (1969)
Protein metabolism in burned patients treated with 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 temperatures. In Energy Metabolism and Trauma, ed. Porter, R. & Knight, J., London: Churchill.

Davis, T.R.A. (1961)
Chamber cold acclimatization in man. Journal of Applied Physiology, 16, 1011-1015.

Daws, T.A., Consolazio, C.F., Hilty, S.L., Johnston, H.L., Krzywicki, H.J., Nelson, R.A. & Witt, N.F. (1972)
Evaluation of cardiopulmonary function and work performance in man during caloric restriction. Journal of Applied Physiology, 33, 211-217.

Drury, J.K. (1976)
The effect of a raised environmental temperature on the metabolic response to thermal injury in the rat. Ph.D Thesis, Glasgow: University of Glasgow.

DuBois, D. & DuBois, E.F. (1916)
Clinical Calorimetry: a formula to estimate the approximate surface area if height and weight be known. Archives of Internal Medicine, 17, 863-871.

DuBois, E.F. (1921)
The basal metabolism in fever. Journal of the American Medical Association, 77, 352-357.

DuBois, E.F. (1927)
Basal Metabolism in Health and Disease. Second Edition, Philadelphia: Lea and Febiger.

Duke, J.H., Jorgensen, S.B., Broell, J.R., Long, C.L. & Kinney, J.M. (1970)
Contribution of protein to calorie expenditure following injury. Surgery, 68, 168-174.

Dunnett, C.W. (1955)
A multiple comparisons procedure for comparing several treatments with a control. Journal of the American Statistical Association, 50, 1096-1121.

Durnin, J.V.G.A. (1959)
The use of surface area and of body weight as standards of reference in studies on human energy expenditure. British Journal of Nutrition, 13, 68-71.

Durnin, J.V.G.A. & Passmore, R. (1967)
Energy, Work and Leisure. London: Heinemann.

Edholm, O.G. & Lewis, H.E. (1964)
Terrestial animals in cold: man in polar regions. In Handbook of Physiology, Section 4, 435-446, Washington D.C. American Physiological Society.

Fleck, A. (1967)
The determination of organic nitrogen. Proceedings of the Association of Clinical Biochemistry. 4, 212-215.

Fleck, A. & Munro, H.N. (1965)
The determination of organic nitrogen in biological materials. A review. Clinica Chimica Acta. 11, 2-12.

Fleisch, A. (1951)
Le Metabolisme basal standard et sa determination au moyen du "Metacalculator" Helvetica Medica Acta, 18, 23-44.

Folk, G.E. (1974)
Textbook of Environmental Physiology, Ch.6, Philadelphia: Lea and Febiger.

Garrow, J.S. (1974)
Energy Balance and Obesity in Man (First Edition), Amsterdam: Elsevier/North Holland.

Garrow, J.S. (1978)
Energy Balance and Obesity in Man, (Second edition), Amsterdam: Elsevier/North Holland.

Garrow, J.S. Durrant, M., Blaza, S., Wilkins, D., Royston, P. & Sunkin, S. (1981)
The effect of meal frequency and protein concentration on the composition of the weight lost by obese subjects. British Journal of Nutrition, 45, 5-15.

Garrow, J.S. & Hawes, S.F. (1972)
The role of amino acid oxidation in causing specific dynamic action in man. British Journal of Nutrition, 27, 211-219.

Gedeon, G. (1981)
Protein metabolism and catecholamine response in trauma and injury. Submitted April 1981, Ph.D. Thesis, Glasgow: Glasgow University.

Glickman, N., Mitchell, H.H., Lambert, E.H. & Keeton, R.W. (1948)
The total specific dynamic action of high-protein and high carbohydrate diets on human subjects. Journal of Nutrition, 36, 41-57.

Goll, C.C. & Shapiro, C. (1981)
Heat production during sleep. Journal of Physiology, In Press.

Grafe, E. (1923)
Die pathologische Psyiologie des Gesamtstoff - und Kraftwechsels bei der Ernahrung des Menschen. Munchen: J.F. Bergmann.
Cited by Keys, A., Brozek, J., Henschel, A., Mickelsen, O. & Taylor, H.L. (1950) in The Biology of Human Starvation, Vol 1. Minneapolis: The University of Minnesota Press.

Grande, F., Anderson, J.T. & Keys, A. (1958)
Changes in basal metabolic rate in man in semistarvation and refeeding. Journal of Applied Physiology, 12, 230-238.

Gray, W.M. (1978)
The effects of whole body irradiation on thyroid hormones and metabolism in rats. Ph.D. Thesis. Glasgow: University of Glasgow.

Greeleaf, J.E. & Castle, B.L. (1972)
External auditory canal temperature as an estimate of core temperature. Journal of Applied Physiology, 32, 194-198.

Grollman, A. (1930)
Physiological variations in the cardiac output of man. The pulse rate, blood pressure, oxygen consumption, arterio-venous oxygen difference, and cardiac output of man during normal nocturnal sleep. American Journal of Physiology, 15, 274-284.

Gubner, R., Di Palma, J.R. & Moore, E. (1947)
Specific dynamic action as a means of augmenting peripheral blood flow. American Journal of Medical Science, 213, 46-52.

Gump, F.E. & Kinney, J.M. (1971)
Energy Balance and weight loss in burned patients. Archives of Surgery, 103, 442-448.

Gump, F.E., Price, J.B. & Kinney, J.M. (1970)
Blood flow and oxygen consumption in patients with severe burns. Surgery, Gynacology and Obstetrics, 130, 23-28.

Halliday, D., Hesp, R., Stalley, S.F., Warwick, P.M., Altman, D.G. & Garrow, J.S. (1979)
Resting metabolic rate, weight, surface area and body composition in obese women on a reducing diet. International Journal of Obesity, 3, 1-6.

Hamm el, H.T. (1964)
Terrestial animals in cold: recent studies of primitive man. In Handbook of Physiology, Section 4, 413-434, Washington D.C. : American Physiological Society.

Hammel, H.T. Wyndham, C.H. & Hardy, J.D. (1958)
Heat production and heat loss in the dog at 8 - 36°C environmental temperature. American Journal of Physiology, 194, 99-108.

Hardy, J.D. & DuBois, E.F. (1940)
Differences between men and women in their response to heat and cold. Proceedings of the National Academy of Sciences, U.S.A., 26, 389-398.

Hardy, J.D. & Soderstrom (1938)
Heat loss from the nude body and peripheral blood flow at temperatures of 22°C to 35°C. Journal of Nutrition, 16, 493-510.

Hardy, J.D., Stolwijk, J.A.J., Gagge, A.P. (1971)
In Comparative Physiology of Thermoregulation Ed Whitton, G.C., Vol. 2, Ch. 5, New York: Academic Press.

Harris, J.A. & Benedict, F.G. (1919)
A biometric study of basal metabolism in man, Publication No. 279, Washington D.C. : Carnegie Institute.

Harrison, H.N., Moncrieff, 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.

Harrison, T.S., Seaton. J.F. & Feller, I. (1967)
Relationship of increased oxygen consumption to catecholamine excretion in thermal burns, Annals of Surgery, 165, 169-172.

Herndon, D.N., Wilmore, D.W. & Mason, A.D. (1978)
Development and analysis of a small animal model simulating the human postburn hypermetabolic response. Journal of Surgical Research, 25, 394-403.

Hunter, J. (1794)
A treatise on the blood, Inflammation and Gunshot Wounds. London: Nicol.

Johnston, I.D.A. (1972)
The endocrine response to trauma. In Parenteral Nutrition ed. Wilkinson, A.W. Edinburgh: Churchill Livingstone.

Keatinge, W.R. & Sloan, R.E.G. (1973)
Measurement of deep body temperature from external auditory canal with servo-controlled heating around ear. Journal of Physiology, 234, 8-9P.

Keys, A., Brozek, J., Henschel, A., Mickelsen, O. & Taylor, H.L. (1950).
The Biology of Human Starvation. Vol. 1, Minneapolis: The University of Minnesota Press.

Kinney, J.M. (1967)
The effect of injury on metabolism. British Journal of Surgery, 54, 435-437.

Kinney, J.M. (1977)
The metabolic responses to injury. In Nutritional Aspects of Care in the Critically Ill. Ed. Richards, J.R. & Kinney, J.M. Edinburgh: Churchill Livingstone.

Kinney, J.M., Long, C.L., Gump, F.E. & Duke, J.H. (1968).
Tissue composition of weight loss in surgical patients. I. Elective operation. Annals of Surgery, 168, 459-474.

Kinney, J.M., Long, C.L. & Duke, J.H. (1970)
Carbohydrate and nitrogen metabolism after injury. In Energy Metabolism in Trauma, ed. Porter, R. & Knight, J. London: Churchill.

Kinney, J.M., Morgan, A.P., Domingues, F.G. & Gilner, K.J. (1964)
A method for continuous measurement of gas exchange and expired radioactivity in acutely ill patients. Metabolism, 13, 205-211.

Kleiber, M. (1961)
The Fire of Life: An Introduction to Animal Energetics. New York and London: John Wiley and Sons, Inc.

Kleitman, N. (1939)
Sleep and Wakefulness. Chicago: University of Chicago Press.

Kuno, Y. (1956)
Human Perspiration Springfield, Illinois: Thomas.

Lee, M.O. (1929)
Determination of surface area of white rats with its application to expression of metabolic results. American Journal of Physiology, 89, 24-33.

Levenson, S.M. (1977)
Starvation. In Nutritional aspects of care in the critically Ill. ed. Richards, J.R. & Kinney, J.M. Edinburgh: Churchill Livingstone.

Lieberman, Z.H. & Lansche, J.M. (1956)
Effects of thermal injury on metabolic rate and insensible water loss in the rat. Surgical Forum, 7, 83-88.

Little, R.A. & Stoner, H.B. (1981)
Body temperature after accidental injury. British Journal of Surgery, 68, 221-224.

Long, C.L., Schaffel, N., Geiger, J.W., Schiller, W.R. & Blakemore, W.S. (1979).
Metabolic response to injury and illness. Estimation of energy and protein needs from indirect calorimetry and nitrogen balance. Journal of Parenteral and Enteral Nutrition, 3, 452-456.

Long, C.L., Carlo, M.A., Schaffel, N., Schiller, W.S., Blakemore, W.S., Spencer, J.L. & Broell, J.R. (1979b).
A continuous analyzer for monitoring respiratory gases and expired radiactivity in clinical studies. Metabolism, 28, 320-332.

McCracken, K.J. & Weatherup, S.T.C. (1973)
Energy metabolism of growing rats, 2 Estimation of efficiency of deposition of protein and fat. Proceedings of the Nutrition Society, 32, 66A-67A.

McLean, J.A. (1971)
A gradient layer calorimeter for large animals. Journal of the Institute of 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.W., London: Butterworth.

McNemar, Q. (1969)
Psychological Statistics, Fourth Edition, New York: John Wiley & Sons.

Martin, C.H. & Robison, R. (1922)
The minimum nitrogen expenditure of man and the biological value of various proteins for human nutrition. Biochemistry Journal, 16, 404-447.

May, M.A. & Nelson, T.S. (1972)
Correlation of Nitrogen to the heat of combustion of rat urine. Journal of Anmial Science, 35, 38-40.

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.

Miller, D.S. Mumford, P. & Stock, M.J. (1967)
Gluttony. 2: Thermogenesis in overeating man. American Journal of Clinical Nutrition, 20, 1223-1229.

Millman, J. & Halkias, C.C. (1972)
Integrated Electronics: Analog and Digital Circuits and Systems. Tokyo: McGraw Hill Kogakusha, Ltd.

Mitchell, H.H. (1962)
Comparative Nutrition of Man and Domestic Animals. Vol. 1. New York: Academic Press.

Moore, F.D. (1953)
Bodily change in surgical convalescence. Annals of Surgery, 137, 289-315.

Mount, L.E. Holmes, C.W. Start, I.B. & Legge, A.J. (1967)
A direct calorimeter for the continuous recording of heat loss from groups of growing pigs over long periods. Journal of Agricultural Science, 68, 47-55.

Moyer, C.A. (1962)
Metabolic and endocrine changes. The metabolism of burned mammals and its relationship to vapourizational heat loss and other parameters. In Research in Burns, ed. Artz, C.P. Oxford: Blackwell.

Moyer, C.A. & Butcher, H.R. (1967)
In Burns, Shock and Plasma Volume Regulation, Ch. 5, pp 162-181. St. Louis: C.V. Mosby & Co.

Munro, H.N. & Chalmers, M.I. (1945)
Fracture metabolism at different levels of protein intake. British Journal of Experimental Pathology, 26, 396-404.

Passmore, R. (1967)
Energy Balances in man. Proceedings of the Nutrition Society, 26, 97-101.

Passmore, R. & Ritchie, F.J. (1957)
The specific dynamic action of food and the satiety mechanism. British Journal of Nutrition, 11, 79-85.

Passmore, R. & Robson, J.S. (1974)
A companion to Medical Studies. Vol. 3. Oxford: Blackwell Scientific Publications.

Pittet, P., Gygax, P.H. & Jequier, E. (1974)
Thermic effect of glucose anad amino acids in man studies by direct and indirect calorimetry. British Journal of Nutrition, 31, 343-349.

Precht, H., Christophersen, J., Hensel, H. & Larcher, W. (1973)
Temperature and Life. Berlin: Springer;Verlag.

Pullar, J.D. & Webster, A.J.F. (1977)
The energy cost of fat and protein deposition in the rat. British Journal of Nutrition, 37, 355-363.

Richards, J.R. (1979)
Studies on the metabolic response to thermal injury in the rat. Ph.D. Thesis. Glasgow: University of Glasgow.

Richards, J.R., Drury, J.K., Goll, C.C. & Bessent, R.G. (1978)
Energy exchanges and injury. Proceedings of the Nutrition Society, 37, 29-43.

Robertson, J.D. & Reid, D.D. (1952)
Standards for the basal metabolism of normal people in Britain. Lancet, 1, 940-943.

Robin, A.P., Nordenstrom, J., Askanazi, J., Elwyn, D.H., Carpentier, Y.A. & Kinney, J.M. (1980)
Plasma clearance of fat emulsion in trama and sepsis: use of a three stage lipid clearance test. Journal of Parenteral and Enteral Nutrition, 4, 505-510.

Rochelle, R.H. & Horvath, S.M. (1969)
Metabolic responses to food and acute cold stress. Journal of Applied Physiology, 27, 710-714.

Roe, C.F. & Kinney, J.M. (1965)
The caloric equivalent of fever.
II Influence of major trauma. Annals of Surgery, 161, 140-147.

Rubner, M. (1902)
Die gesetze des energieverbrauchs bei der ernahrung. Liepzig: Deutche.
Cited by Kleiber, M. (1961) in The Fire of Life: an introduction to animal energetics, p. 267. New York and London: John Wiley & Sons, Inc.

Scholander, P.F., Hammel, H.T., Lange Anderson, K. & Loyning, Y. (1958)
Metabolic acclimation to cold in man. Journal of Applied Physiology, 12, 1-8

Shangraw, R.E. & Turinski, J. (1981)
Chronic alterations in energy metabolism in the burned rat. Burns, 7, 261-266.

Smith, E.E. & Crowell, J.W. (1964)
Effects of hemorrhagic hypotension on oxygen consumption in dogs. American Journal of Physiology, 207, 647-649.

Snedecor, G.W. & Cochran, W.G. (1967)
Statistical Methods, 6th Edition. Iowa: Iowa State University Press.

Sorkin, B., Rapoport, D.M., Falk, D.B. & Goldring, R.M. (1980)
Canopy ventilation monitor for quantitive measurement of ventilation during sleep. Journal of Applied Phsyiology, 48, 724-730.

Spencer, J.L., Zikria, B.A., Kinney, J.M., Broell, J.R., Michailoff, T.M. & Lee, A.R. (1972)
A system of continuous measurement of gas exchange and respirator functions. Journal of Applied Physiology, 33 523-28.

Spinnler, G., Jequier, E., Favre, R., Dolivo, M. & Vannotti, A. (1973)
Human calorimeter with a new type of gradient layer. Journal of Applied Physiology, 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. British Journal of Surgery, 59, 93-97.

Stoner, H.B. (1961)
Critical analysis of traumatic shock models. Federation Proceedings, 20, Supplement 9, 38-48.

Stoner, H.B. (1970)
The acute effects of trauma on heat production in Energy Metabolism in Trauma, ed. Porter, R. & Knight, J. London: Churchill.

Swift, R.W. & Forbes, R.M. (1939)
The heat production of the fasting rat in relation to the environmental temperature. Journal of Nutrition, 18, 307-318.

Taylor, H.L. Buskirk, E.R., Brozek, J., Anderson, J.T. & Grande, F. (1957)
Performance capacity and effects of caloric restriction with hard physical work on young men. Journal of Applied Physiology, 10, 421-429.

Thompson, J., Brice, J.E.H., Kurdy, N.M.G. & Walker, C.H.M. (1979)
A modified open-circuit method for estimation of oxygen consumption and carbon dioxide excretion in adults, infants and small animals. Plfugers Archiv, European Journal of Physiology, 380, 197-202.

Thomson, D.J. (1965)
Energy retention in lambs as measured by the comparative slaughter technique. In Energy Metabolism, ed. Blaxter, K.L. London:Academic Press.

Tilstone, W.J. (1972)
The effects of environmental conditions on the requirements for energy after injury. In Parenteral Nutrition. ed. Wilkinson, A.W. Edinburgh: Churchill Livingstone.

Tilstone, W.J. & Cuthbertson, D.P. (1970)
The protein component of the disturbance of energy metabolism in trauma. In Energy Metabolism in Trauma, ed. Porter, R. & Knight, J. London: Churchill.

Tuttle, W.W., Horvath, S.M., Presson, L.F. & Daum, K. (1953)
Specific dynamic action of protein in men past 60 years of age. Journal of Applied Physiology, 5, 631-634.

Van Graan, C.H. & Wyndham, C.H. (1964)
Body surface area in human beings. Nature, 204, 998.

Visser, J. & Hodgson, T. (1960)
The design of a human calormeter for the determination of body heat storage. South African Mechanical Engineer, May, 234-268.

Wachholder, K. & Franz, H. (1944)
Die Spezifisch-dynamische Stoffwechselsteig-erung bei gemischter kost. Pflugers Archiv Gesammte Phsyiologie Menschen Tiere, 247, 632-648.

Weir, J.B. de V. (1949)
New Methods for calculating metabolic rate with special reference to protein metabolism. Journal of Physiology, 109, 1-9.

Whedon, D.G. Deitrick, J.E. & Sahrr, E. (1949)
Modification of the effects of immobilization upon metabolic and physiologic functions of normal men by the use of an oscillating bed. American Journal of Medicine, 6, 684-711.

Wilmore, D.W. (1977)
The Metabolic Management of the Critically Ill, New York: Plenum Publishing Co.

Wilmore, D.W., Aulick, L.H., Mason, A.D. & Pruitt, B.A. (1977)
Influence of the burn wound on local and systemic responses to injury. Annals of surgery, 186, 444-456.

Wilmore, D.W., Long, J.M., Mason, A.D. Skreen, R.W. & Pruitt, B.A. (1974)
Catecholamines: Mediator of the hypermetabolic response to thermal injury. Annals of Surgery, 180, 653-669.

Wilmore, D.W., Mason, A.D., Johnson, D.W. & Pruitt, B.A. (1975)
Effect of ambient temperature on heat production on heat loss in burn patients. Journal of Applied Phsyiology, 38, 593-597.

Wilmore, J.H., Davis, J.A. & Norton, A.C. (1976)
An automated system for assessing metabolic and respiratory function during exercise. Journal of Applied Physiology, 40, 619-624.

Winslow, C.E.A. (1941)
Temperature, it's measurement and control in science and industry. New York: Reinhold.

Zawacki, B.E., Spitzer, K.W., Mason, A.D. & Johns, L.A. (1970).
Does increased evaporative water loss cause hyper-metabolism in burned patients ? Annals of Surgery, 17, 236-240.

Zawacki, B.E. & Jones, R.J. (1967)
Standard depth burns in the rat: the importance of the hair growing cycle. British Journal of Plastic Surgery, 20, 347-354.