Studies of the Metabolic pattern of Pregnancy in the Rat.

by

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"It is important to discriminate the bodily pattern, which endures, from the bodily event, which is pervaded by the enduring pattern, and from the parts of the bodily event. The parts of the bodily event are themselves pervaded by their own enduring patterns, which form elements in the bodily pattern. The parts of the body are really portions of the environment of the total bodily event, but so related that their mutual aspects, each in the other, are peculiarly effective in modifying the pattern of either. This arises from the intimate character of the relation of whole to part. Thus the body is a portion of the environment for the part, and the part is a portion of the environment for the body; only they are peculiarly sensitive, each to the modifications of the other. This sensitiveness is so arranged that the part adjusts itself to preserve the stability of the pattern of the body."

A.N. Whitehead. Science and the Modern World, 1926.

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INTRODUCTION

In the last two or three decades studies of the gross or end product, metabolism of mammals under normal physiological conditions have been conspicuous only by their infrequency. The careful and precise methods and techniques developed by the giants of the physiology of metabolic processes, such as Voit, Rubner and Atwater, have become, to a great extent, swamped by half a century of possibly more sophisticated but frequently less precise techniques. The tradition of this type of work has been continued by particular schools of investigators, such as those of Benedict and of Brody, but otherwise only occasional investigations of this type have been done on animals and then, usually, with some pharmacological end in view.

Much information can still be obtained by these methods; much which has either not before been examined or examined only cursorily, or, further, has been examined under conditions which allow little information to be gleaned as to the normal processes of life. For the establishment of the so-called basal condition as a _sine qua non_ of reliability of metabolic data, largely under the insistence of Benedict, has prevented any but a few of the modern investigations from presenting an adequate and realistic picture of metabolic processes. It is not denied that a basal standard is of great value, and has been of great value in simplifying many of the metabolic problems which require consideration. It cannot be expected, however, that the results found under such "basal" conditions will have anything more than an academic application to normal physiological processes. Such standard or basal conditions are ideal for the analytic investigation of the components of metabolic exchanges. They are quite inapplicable to investigations involving the synthesis of a normal bodily activity. Application of such standard conditions to investigations of the latter type may give the appearance of simplifying the problem but, in the event, complicate the interpretation of the findings.

One of the aspects of energetic and general metabolic exchange which received only brief study from the early workers, and little since, is the event of pregnancy. Such work as has been done on the gross pattern of pregnancy and the studies of particular details of the changes during pregnancy present a number of curious anomalies which, it seemed, might be elucidated by a study of the total metabolic exchanges during pregnancy. Such, for example, are the slight increse which has been observed in food intake on the one hand, and, on the other, the increase in the basal metabolic rate with simultaneous formation of a considerable mass of new tissue. There are several theoretically possible ways of reconciling these observations, but a first, necessary step towards defining the most likely metabolic pattern is the measurement of the total energy metabolism as distinct from the basal metabolism. A parallel problem exists in the nitrogen retention known to occur in pregnancy and whether it can be held entirely accountable to such increase in food intake as occurs.

Less directly associated with the gross metabolic changes is the heterogonal growth relation of foetus and placenta, and the declining curve of reproductive efficiency with age and parity. Since, however, such changes must necessarily be a consequence of alteration in the metabolic pattern of the maternal organism, it seemed possible that their reflections might be observable in that pattern.

The following pages represent the preliminary stages of an attempt to find what associations exist between these and other phenomena of pregnancy in the rat, mainly by a study of the total energy, nitrogen and water exchanges. The experimental work has been largely confined to the establishment of a base-line which can be taken as the normal pattern of pregnancy and much work must yet be done before a generally representative picture even of this can be obtained. The extension of the component features of the normal into a unified pattern, in which the growth and metabolism of the conceptus and of the maternal organism are mutually linked and fitted into the broad gradients of parity and age, is indicated by only the most shadowy of pointers.

To do this work it has been necessary to devise and construct an instrument capable of measuring the total metabolism of an animal over long periods. The instrument which I constructed was based, in general conception, on an instrument devised for work on mice but had to be greatly modified for my purposes. The instrument had to be constructed *ab initio*, and subsequently much time and work had to be devoted to tests, modifications and the development of working techniques. Consequently, a large part of the following report is devoted to constructional and methodological detail.

REVIEW OF THE LITERATURE

As the work in this thesis falls under the three general heads of energy of nitrogen and of water metabolism, the literature of these three aspects is, for convenience, treated separately in the following review. To avoid the discontinuity introduced by the citation of tedious lists of original papers reference has been made, wherever the point at issue is adequately served by this procedure, to reviews or representative papers.

The small amount of previous work on the growth of the rat foetus and placenta is considered at the beginning of that section of the thesis, p.(244). Work on methods and technique, specialised aspects of the theory of interpretation of metabolic studies, and singular problems arising out of my experimental work but without general application to the theme of this investigation are briefly discussed at appropriate points in the text and are not included in this general review.

Energy Metabolism.

By far the greatest body of data on energy metabolism in pregnancy is that from clinical investigations of the basal metabolic rate in women (Magnus-Levy, 1904; Carpenter and Murlin, 1911; Root and Root, 1923;

Sandiford and Wheeler, 1924; Sandiford et al, 1931; Rowe ét al, 1925; Rowe and Boyd 1932; Johnston et al, 1938.) Most of these investigations have been made on a few women at irregular intervals throughout pregnancy, and, with very few exceptions, have been made using one of the standard clinical types of recording respirometer, such as the Benedict-Roth instrument, for 10 or 15 min. periods. The difficulties of achieving basal conditions in untrained subjects (cf Robertson and Reid, 1952), the patent inadequacy of one 15 min. period as representative of a one- or three-month period of pregnancy, let alone the inherent weakness of the apparatus used (Willard and Wolf, 1951), make much of this work suspect and the findings erratic. The great weight of the evidence from these findings (particularly those contributed by Rowe et al) does, however, indicate a pronounced rise in the total basal rate to an average maximum value of about +15 per cent. on any of the accepted standards, the maximum occurring just prior to parturition.

The significance of this work has resolved itself into a controversy between two views. The one view, crystallised by the arguments of Sandiford and her colleagues is that the rise in the total basal metabolism can be entirely accounted for by the metabolism of the increased metabolising mass formed by the conceptus and the increase in maternal tissue. Rowe and his colleagues, on the other hand, claim a true rise in maternal B.M.R. (The basic problem was posed by earlier workers, notably Rubner, but received its best expression in the above investigations. The former workers demonstrated that when their own results were expressed in terms of the sum of the foetal and maternal surfaces the metabolic rate per unit surface area was within the normal range. Rowe found a fall in B.M.R. to sub-normal levels during the third or fourth month of pregnancy, followed by a rise at a rate 13 per cent greater than could be accounted for by the increased tissue mass. Also, all workers have found that the B.M.R. rises at a time when the weight of the conceptus is negligible.

It is difficult to accept the validity of the device of expression of the B.M.R. as a function of the combined surface area. Nevertheless, although the "surface law" as a theoretical concept is susceptible of much criticism (Kleiber, 1947), and in any case does not permit direct comparison between infant and adult, it is quite probable that the embryo metabolises energy at a higher rate than the maternal tissue. Granting this on Carpenter and Murlin's (1911) evidence of the smooth transition in total basal rate from the ante- to the post-parturient state, it cannot further be assumed that the excess rate of the embryo is in even approximate correspondence with the relation between maternal and foetal surface. Nor can it be assumed that the embryonic relation of surface, or, if it be preferred, metabolic size, to heat production is linear; that is, the exponent of weight from which the estimate of metabolic size is derived, is not necessarily constant. This last suggestion is supported by the fact that the offspring does not reach its peak metabolism per unit surface area until some time after birth, in man about one year, and it is reasonable to suppose that a continued deceleration in heat production would be observed by extrapolating back into intra-uterine life.

In a review of the earlier work on the metabolism of pregnancy, Harding (1925) accepted this problem as still open and unsolved. Johnston *et al* (1938), on the basis of one case studied more intensively through the last six months of pregnancy along with previous work, consider that there is a fraction of the rise in B.M.R. truly accountable to increase in maternal energy production aside from the increase due to the increment of protoplasmic mass. Newton (1952) presents the problem as still unsolved but provisionally accepts the evidence of Rowe and his protagonists as strong indication that the changes in heat production carry an element of true energy change independent of protoplasmic mass.

Indirect evidence for a true increase in maternal B.M.R. is given by the pronounced hypertrophy of the anterior pituitary with, presumably, an increase in thyrotropin production, by the less pronounced hypertrophy of the thyroid itself and by the increase in blood iodine during pregnancy (Schwarz and Drabkin, 1931). Evidence on the response to thyroid administration during pregnancy is contradictory and inconclusive (Wilhelmj _et al_, 1933; Danforth and Loumos, 1936; Phatek _et al_, 1940), but there appears to be little doubt that there is some degree of physiological hyperthyroidism in normal pregnancy (Anselmino and Hoffmann, 1935; Neuweiler, 1935). Similarly there is doubt as to the extent of the effects on B.M.R. of ovarian hormones; claims have been made that they produce a rise in the metabolic rate of the reproductive tissues only, Verzár and Arvay (1931) that a general increase is apparent or that no effect is found, Kaufmann _et al_, (1932) and Brouha and Chevillard, (1932). This work is distributed unsystematically over many species including man and many of the discrepancies observed must certainly be due to this. The discrepancies are also, probably, due in part to the variability of the preparations used.

Valid direct evidence on basal rates in the rat and other laboratory animals has the advantage that absolutely longer periods of study are more easily possible, and that these periods are very much longer relative to the total duration of pregnancy. Such estimations suffer from the disadvantage that a basal state is much more difficult to achieve, the best approximation being a resting, post-absorptive state in which the state of rest is very unreliable and the post absorptive state may readily border on starvation. The main body of data on the resting metabolism of the pregnant rat, uncomplicated by hormone administration or other treatment, comes from Brody and his co-workers (Brody, 1945). They have found, in general, that the absolute total resting metabolism increases during pregnancy but, expressed per unit bodyweight, there is no pronounced increase. Pommerenke _et al_ (1930) found a 15 - 20 per cent. increase in the metabolism of pregnant rabbits but no increase when the results were expressed per unit of summed foetal and maternal surface.

Brody has established a relation between the birthweight of the young and the "energy increment of gestation", that is, the additional resting energy used apart from that stored, representable by the equation:-

$$\text{Total Cal. used} = 4400\ (\text{Birthweight in kg.})^{1.2}$$

Since the energy cost of gestation according to this equation increases with increasing birthweight (exponent 1.2), instead of decreasing as would be expected from the metabolic size relation (exponent 0.75), the existence of "maternal overheads" can be inferred (Newton, 1952). It is possible that the cost of generation of embryonic tissue, apart from the cost of its maintenance, may go some way towards explaining this theoretical discrepancy. In view, however, of the high energetic efficiencies observed in tissue formation (Needham, 1931) it is unlikely that this accounts for much energy in absolute terms.

In support of the proposition suggested above, that in the embryo the weight exponent defining metabolic size may not be constant, the work of Kleiber *et al* (1943) on the respiration of rat foetuses *in vitro* may be cited. The naked 13-day rat foetus, when slit to allow adequate oxygen penetration, had a metabolic rate only one tenth that expected if it behaved as an independent homeotherm (exponent 0.75) and one twentieth that expected from the surface law (exponent 0.67). Since the metabolic rate of the newborn is 2.5 times that of the normal adult rat on a surface basis, the bodyweight exponent for metabolic size must increase in the interim. On the other hand, the work of Bohr and Hasselbalch and of Hasselbalch (1900), on the carbon dioxide production of the embryo chick, indicated that energy production per unit weight is a maximum when tissue differentiation is most active. If the rat is comparable in this respect, maximum metabolic rate of the embryo should occur at about 10-13 days gestation.

The metabolic rate of the feotal membranes as measured *in vitro* is small relative to that of the embryo, Kleiber found it to be one third of the foetal rate at 13 days gestation in the rat. The growth rate of the mass of the membranes is very much lower than that of the embryo, and even assuming an approximate correspondence between metabolic rate and a function of weight, the maintenance energetic cost of the membranes should not greatly affect the picture. Since the metabolic rate of the membranes is so much lower than that of the foetus, such effect as this would have on the total metabolism would be to reduce the apparent total rate per unit weight of the pregnant organism.

To this basal rate of the membranes in vitrò must, of course, be added the cost of transport of materials from the maternal to the foetal bloodstreams, this being additional to the cost of organisation of the foetal tissues. The gaseous exchanges of the embryo appear to be explicable by simple diffusion mechanisms across the placental barrier (Barcroft, 1946). There are objections to accepting a simple diffusion process for oxygen transfer, but the reversibility of carbon dioxide transfer (Huggett, 1927) indicates a diffusion process for that at least.

The important series of researches by Flexner and his colleagues (Flexner and Gellhorn, 1942 a,b; Flexner and Pohl, 1941 a,b,c; Flexner et al, 1948; Gellhorn and Flexner, 1942; Gellhorn et àl, 1941; Pohl and Flexner, 1941; Pohl ët àl, 1941) using isotope techniques to study the transport of sodium, phosphorus and water across the placenta have established the major variables associated with transfer rate. This work gives no direct evidence on the energy cost of transfer but the form of variation shown, notably with placental type (Mossman, 1937), the transfer rate rising with the reduction in the number of placental layers, implies a simple diffusion mechanism for these simple substances. The difficulty of experimentally establishing a gradient foetus-wards for some substances, notably water (Barcroft, 1946) might suggest that a measure of local placental work is required for transfer, that is that there is secretion. Flexner, rather misleadingly, has defined the ratio of transfer rate to retention rate as a "safety factor". This factor, in the case of water, is of the order of 500:1, but this means that the relative foetus-wards flow of water, or true safety factor, is 500:499 which would be extremely difficult to demonstrate experimentally.

It is possible that more complex molecules do not cross the placenta by simple diffusion. It is unlikely that molecules of the order of magnitude of proteins cross the placenta as proteins, and the transfer of hormones and antibodies (Needham, 1931, 1945) could possible be explained by the transfer of component amino-acids and prosthetic groups with re-assembly on the foetal side, as appears to occur in the mechanism of lipid transfer (Popják, 1947). That relatively small molecules suffer transformations in the placenta, as in the formation of placental glycogen (the possibility that this may be entirely a decidual function is irrelevant to the present point), and the conversion of glucose to fructose (Huggett _et_ _al_, 1951) is presumptive evidence that any molecule above the simple ion may not pass by simple diffusion. The detailed kinetics of carbohydrate transfer has recently been investigated by Widdas (1952). Diffusion has, however, been claimed to explain all transfer phenomena in the placenta (Schlossmann, 1932), and this may be true of the end products after the breakdown and reorganisation activities of the placenta have been completed. All transformations, breakdowns and reassemblies which may be accomplished by the placenta *in vivo* will have a certain energy cost which may not be adequately represented in *in vitro* experiments. The decline of the metabolic rate of the human placenta with stage of gestation found by Wang and Hellman (1943), and the interpretation of placental function as altering from an anabolic to a purely membrane function (Reynolds, 1949) may not give a true indication of the contribution of this structure to the total energy release of the pregnant organism.

Earlier attempts to examine the metabolic rate of the mammalian embryo were fundamentally qualitative. Cohnstein and Zuntz (1884) estimated the oxygen and carbon dioxide contents of the blood in the umbilical artery and vein in the sheep embryo, and also the blood flow rates in these vessels. From their figures an estimate of oxygen consumption of 1.5 to 4.5 ml. O_2 per min. for a foetus of about 1300 g. can be obtained, or about 80 to 250 ml. O_2 per kg. foetal weight per hr. Comparison of the difference in blood gas concentrations between artery and vein indicate a respiratory quotient greater than 1 (1.01 and 1.6). Bohr (1900) investigated the subject by measuring the total metabolism of the laparotomised anaesthetised guinea-pig before and after clamping the cord of one foetus; from the two sets of observations a measure of the metabolism of the embryo could be found. Again the foetal R.Q. was always in the neighbourhood of unity. These experimental findings are unlikely to present a normal picture of the foetal metabolic process, as the operative procedures must certainly have a great effect (Barcroft, 1946). The oxygen consumption of the conceptus appears low, but a similar calculation from the data of Barcroft et al, (1939) gives a commensurate value of 228 ml. per kg. foetal weight per hr. for a sheep foetus of 1200 g. The high R.Q.'s observed, even if true for the species studied, are not necessarily true for the rat, which lays down very little fat in the intrauterine period (Widdowson, 1950). Unnatural fats are known to cross the rat's placenta (Sinclair, 1933; McConnell and Sinclair, 1937) so it is probable that such fat as is laid down in the foetal rat is derived from maternal fat. The findings do indicate, however, that carbohydrate is probably the main energy source for the foetus. Studies on the sugar and lactic acid concentrations in uterine venous, arterial and intervillous bloods indicate that also the placenta metabolises carbohydrate predominantly

(Loeser, 1932, a,b; Franken and Krebs, 1933), although the parallel finding that the metabolism is predominantly anaerobic is not necessarily true *in vivo* (Huggett and Hammond, 1952).

The only study that has been found of the total physiological energy requirement in pregnancy, as distinct from the basal metabolism, is that of Murlin (1910a) on two successive pregnancies in a bitch. The energy production in this work was calculated from the carbon dioxide and urinary nitrogen production using the Pettenkoffer-Voit apparatus. Although 24 hr. periods were studied, only two days study per week were made. No alteration in metabolic rate was found until the sixth week of gestation, but between the sixth and eighth weeks the M.R. rose by 9 per cent. Assuming Rubner's surface relation and Meeh's formula for surface area Murlin calculated that the excess energy usage at 3 days *ante partum* was almost exactly that attributable to the pups, The slight additional excess (less than 5 per cent. of the total excess) he attributed to the raised metabolising mass of the uterus.

A measure of the non-basal energy production in pregnancy in the rat was made by Danforth and Loumos (1936) in a study of the effect of thyroid administration, but the length of the estimation periods is not stated. These authors found that desiccated thyroid given orally had a much lower calorigenic effect in the pregnant than in the non-pregnant rat, but they also found no appreciable increase in the metabolism of untreated pregnant controls.

For a measure of the physiological energy requirement in pregnancy, therefore, we must depend largely on the less direct methods of measurement of food intake. A few studies of this sort have been made both on man and on animals. The human studies, which have been reviewed and reluctantly accepted by Garry and Stiven (1935) and Garry and Wood (1945) have indicated that there is little extra energy requirement during pregnancy. Although the basal energy expenditure rises towards the end of pregnancy the increase appears to be offset, at least in part, by a reduction in muscular activity. It is interesting to observe that the U.S. National Research Council tables of dietary standards (1945) make an allowance of 400 Cal. for the latter half of pregnancy, but the League of Nations Technical Commission (1935) tables make no allowance. The former standards are, however, uniformly more generous than others.

Food intake studies on experimental animals during pregnancy have been made by Wang (1925), Slonaker (1927), Cole and Hart (1938) and Murray (1941). Wang claimed an increase in food intake of the pregnant rat of 0-14 per cent., with a mean reduction in activity, as measured by revolving cages, of up to 96 per cent. Slonaker, in a technically more satisfactory investigation, found an almost immediate increase in food consumption of about 10 per cent. which remained substantially constant until the eighteenth day of gestation when there was a rapid fall in intake to about 60 per cent. of the non-pregnant level. Activity, again measured by revolving cages, was found to drop immediately after coitus to 17 per cent., rising later to 21 per cent. of the non-pregnant level. This fall in activity, Slonaker claims, is one of the most reliable criteria of successful mating. Oestrus, although in general suppressed during pregnancy and lactation, tended to recur at various stages of the gravid period. The most critical point was at about 14 days gestation and abortion and resorption were most liable to occur at this time. The findings of Cole and Hart (1938) are substantially the same as those of Slonaker, but they found an increase in food intake of about 30 per cent. within 48 hr. of coitus. Murray (1941) found a total increase in food consumption in rats of about 30 per cent. but in this case the increase was gradual and progressive throughout almost the whole of pregnancy. Murray also recorded the rapid decline in food consumption in the last four days of pregnancy. Although these investigations show great variations in the extent of the changes observed there is general agreement as to the form, namely an appreciable increase in food intake (but small compared to that observed in lactation) and a great reduction in activity.

Slonaker, in the same study, and more recently Brobeck _et al_ (1947), observed a pronounced cyclical variation in food intake and activity with the oestrous cycle. Both investigations showed a fall in food intake and bodyweight with a rise in activity during oestrus with the reverse tendencies during dioestrus. Brobeck _et al_ found a hyper- and hypothermia during dioestrus and oestrus respectively, which is in agreement with Tompkins' (1945) findings for the corresponding stages of the menstrual cycle. Brobeck's results show these cyclical changes to be very pronounced not only for the group but for individual animals also. They also found that in pseudopregnancy the pattern is similar to that in pregnancy up to 14-16 days (the normal period of termination of pseudopregnancy in the rat) with increased appetite and reduced activity.

If we take Slonaker's average value of 2 g. additional food per day during pregnancy this gives a total excess intake of the order of 40 g. during pregnancy. Even with a weight increase of about 60-70 g. in this time, corresponding to a litter of about 10 pups, the tissue deposition as dry matter would not be expected to amount to more than about 20 g. which would suggest an increased energy usage of about 4 Cal. or 10 per cent. per diem. There is, however, no evidence to show how much of this additional ingested food is absorbed, and it would require only a small reduction in absorption efficiency, 5 per cent., completely to offset the increased intake.

By and large, then, the studies on the rat give much more definite evidence of increased food consumption and inferentially of higher energy usage during pregnancy than do the corresponding human studies. The increase is, nevertheless, smaller than might be expected and relatively very much smaller than is found to occur in lactation. It must be recalled, however, that these studies are necessarily confined to animals, and to some degree to women, in a restricted and largely artificial environment. It may be that, under less synthetic conditions, the struggle for survival and quest for food would not permit such a great reduction in activity. When such increase in food intake as does exist is considered along with the very great reduction in activity it would appear that the increase in the physiological non-active energy expenditure may be at the upper limit of or rather greater than the increases recorded in the non-physiological "basal" energy expenditure.

Very little work has been done to give any indication of the existence or direction of any preferential combustion of fat or carbohydrate during pregnancy. Reprew, (1889), found a decline in the respiratory quotient as pregnancy advanced in a dog, a guinea-pig and three rabbits; he also found, however, a fall in the total metabolism of these animals which is not in agreement with the eight of the evidence. Oddi and Vicarelli (1891) found an increase in the R.Q. of pregnant mice. Most of the later work measures only oxygen or carbon dioxide production which gives no partition of energy source, but Spiga-Clerici (1937) found no change in R.Q. during human pregnancy while Johnston _et al_ (1938) found a steady rise in the fraction of the energy derived from fat in human pregnancy without any explanatory fall in food intake. As Cathcart and Markowitz (1927) have pointed out the simultaneous operation of processes of combustion and interconversion of proximate principles and combustion to and of intermediary metabolic products make it doubtful if the R.Q. derived from short period studies has any interpretable significance.

Nitrogen Metabolism.

There have been many studies of the nitrogen metabolism in pregnancy, but most of these have been on the nitrogen balance during relatively small periods of time towards the end of human pregnancies. Such clinical or para-clinical investigations are of interest but of very restricted utility as the period of study frequently forms such a small fraction of the total gestation, many of the subjects considered are available for study because of some coincident pathological condition and the method of assessment of the dietary intake of nitrogen is often unreliable.

Early work on nitrogen balance, by Hagemann (1891) and Jågeroos (1903) on the bitch and Ver Eecke (1901) on the rabbit, was unanimous in its evidence that, finally, pregnancy is a period of maternal sacrifice. Although nitrogen is gained during gestation the event of parturition, with its associated losses, and the high urinary nitrogen loss occurring for the first few days <u>post partum</u> entailed a nett nitrogen loss for the mother. Bar (1907) in a critical examination of this work corrected some of the values assumed by these authors for the nitrogen content of foetus and membranes and showed that some of the apparent nitrogen losses recorded were, in fact, either non-existent or were over-estimated. Even with Bar's modifications a number of the gestations did show very large nitrogen losses. Ver Eecke, working on rabbits, also found this nett nitrogen loss, and in this work some of the losses were extremely high amounting to as much as one sixth of the total non-pregnant maternal nitrogen.

Bar has suggested that the diet of the bitches in the former studies was inadequate. As a rule the nitrogen intake was about 0.8 g. per kg. bodyweight per diem. In one of Jägeroos' bitches, when the nitrogen intake was about 1.3 g., the energy intake was probably low, while in another the nitrogen intake was only 0.2 g. per kg. and the animal aborted at the eighth week of gestation. Also the animals in these studies showed pronounced anorexia from time to time which may have been of dietary origin although it was regarded as analogous to the "normal" morning sickness of human pregnancy. Ver Eecke's rabbits were allowed carrots ad libitum but their food utilisation decreased progressively with the course of pregnancy.

Bar and Daunay (1905) and Bar (1907) record extended and beautifully thorough work on bitches and women. Their investigations on bitches covered a total of 8 pregnancies in six animals, but in only three of these was the total excreted N measured. In all cases they found a positive nitrogen balance through almost the whole of gestation, and when a negative balance occurred it was confined to a few days at about the fourth or fifth week. The total maternal nitrogen balance over the whole of pregnancy, taking into account the parturient and post partum losses, was either positive or very slightly negative in each animal.

In the studies both of Jägeroos and of Bar the daily urinary nitrogen either fell uniformly throughout gestation, or rose to about mid-gestation and then fell more rapidly. The result of this was an increasing daily retention of nitrogen at least in the second half of gestation. Neither Ver Eecke's nor the two rabbits studied by Bar and Daunay showed this tendency very definitely.

Murlin (1910b), also working with bitches, found, in general, a negative nitrogen balance during the first half of gestation. He followed two animals through the whole of gestation and in one the total excreted nitrogen (separate figures for urine and faeces are not recorded) fell in the ninth week, and in the other fell progressively from the fifth week. He also found nitrogen retention occurring during the period of heat and ovulation.

Murlin has gathered his own findings and those of the work discussed above into a table according to the occurrence of positive or negative nitrogen balances at each week of gestation. In the following table I have expressed the number of positive balances for each week as a percentage of the total number recorded for that week:-

Week of Gestation	1	2	3	4	5	6	7	8	9
Per cent. +ve	62	54	33	29	46	75	90	100	100

This demonstrates, in a rather crude fashion, a further basis for the suggestion made above that negative balances tend to occur about the middle of gestation. This tendency has not, however, received any serious comment from any of the above workers.

From a consideration of the extended form of the above table which he constructed Murlin concluded that, in the bitch, maternal nitrogen loss is a characteristic of the first half of pregnancy and maternal nitrogen gain a characteristic of the second half. Since the embryo has not reached any appreciable size by the fourth or fifth week this statement effectively implies that the first half of pregnancy is characterised by a gross maternal nitrogen loss, a conclusion which Bar's work at least does not support.

Of the human studies, a considerable number, Slemons (1904), Hahl (1905), Bar (1907), Rowe et al (1934), Thompson and Pommerenke (1939), show a pronounced fall in the urinary nitrogen in the last days of pregnancy, while Bar's work shows a consistent decline in urinary nitrogen for some weeks ante partum. This fall appears largely to occur in urea nitrogen. Other work, however, does not show this fall so pronouncedly, and then only in the last two or three days, Hoffström (1910), Wilson (1916), Coons and Blunt (1930), Sandiford et al (1931), Coons and Marshall (1934), Oberst and Plass (1940). Rowe et al found that the diminution in urinary nitrogen was accentuated in toxaemic pregnancies and that the time taken by the mother's urine to return to normal levels post partum was lengthened in these cases. These workers also found that, in normal pregnancy, the total blood N.P.N. was low but uric acid was high, while toxaemia tended to reverse this relation.

Morris (1923), working on goats, found a rise in the total urine nitrogen in the last days of pregnancy.

The early tenet that the first half of pregnancy is a period of nitrogen loss has found mixed support from workers who have investigated the nitrogen balance in the early weeks of human pregnancy. Wilson (1916) and Coons and Blunt (1930) found a positive balance from the tenth or twelfth week onwards, while Sandiford et al (1931) and Thompson and Pommerenke (1939) found a negative balance in the early months. Brühl (1932) concluded from a review of the evidence that nitrogen balance is negative about mid-gestation.

In all studies covering the puerperal period in women and other animals, a period of negative nitrogen balance is found lasting for four or five days. Grammatikati (1884) suggested that this was due to nitrogen loss entailed in the process of initiation of lactation; Zacharjewski (1894), however, suggested that it was due to the nitrogen loss in involution of the uterus. The latter view was generally held until the work of Mellanby (1911) and Harding and Montgomery (1927), examining the nitrogen partition in the urine, renewed the belief that the nitrogen loss and creatinuria were referable to the onset of lactation. Harding and Montgomery, however, stated that uterine involution as a contributory factor was "not necessarily excluded".

The mechanism of nitrogen retention in pregnancy is not at all clear. Bokelmann and Scheringer (1933) found no evidence for increased nitrogen absorption in pregnancy and Coons and Marshall (1934) found that digestive disturbances may actively reduce absorption. There is not normally any evidence of high blood N.P.N. The hypertrophy of the anterior pituitary and the increase in numbers of acidophils during pregnancy (Severinghaus, 1937), which acidophils have been associated with the production of growth hormone, and the nitrogen retention which occurs after administration of growth hormone (Li and Evans, 1947) suggest that this may be involved in the nitrogen retention of pregnancy. This is, to some extent, supported by the similarity of the form of tissue deposition in pregnancy (Poo _et al_, 1939, 1940) to that sometimes found after administration of growth hormone (Lee and Schaffer, 1933, 1934). Also the acromegalic tendencies often observed during human pregnancy, with coarsening of the features, have been attributed to anterior pituitary activity, Dreyfuss (1922). There are, however, many objections to stepping from a statement of such similarities to the postulate that the anterior pituitary activity is responsible for the nitrogen retention in pregnancy. Although both the acidophils and the basophils of the adenohypophysis appear to be increased in human pregnancy there is evidence that, in rats (Herring, 1920) at least, the eosinophil count may fall. Herring also failed to find any hypertrophy of the anterior pituitary of the rat during pregnancy. Kleiber and Cole (1939) found no significant change in body composition due to the administration of growth hormone, but variations in the findings in this respect are to be expected due to the different initial stages of growth of the animals used, which may take the form of predominantly fat or protein synthesis (Mayer, 1949). It can probably be accepted that the induced growth takes the form of a continuation of the motion of growth operative at the beginning of treatment with somatotrophin (Lee and Schaffer, 1933, 1934). During the latter part of pregnancy in the rat and some other species ablation of the pituitary does not terminate pregnancy (Allen et al 1939) although it prevents the initiation of lactation; this is not to say, of course, that its absence does not affect the normal course of pregnancy, but it does suggest that if the control of nitrogen balance in pregnancy is hormonal the source of the control may be some organ other than the pituitary. A further complication of the problem must be noted in the rise in urinary corticoids during the period of maximum nitrogen retention (Venning, 1946) while adrenal cortex activity promotes protein catabolism and nitrogen loss (Tepperman et al, 1943).

Chalmers and Munro (1952)have shown that the nitrogen retention produced by the growth hormone occurs even with a dietary protein of inadequate amino acid composition. This indicates that the retained nitrogen is not necessarily assembled as true tissue protein. If the nitrogen retention in pregnancy is mediated by the same or a similar hormonal mechanism, then it is possible that here also the maternal deposits may not be present as true protein and may be lost in the same way. Poo ét ál have shown that the greater part of the nitrogen gained during pregnancy goes to the maternal tissues and carcass, so it is not unreasonable to suppose that the nitrogen lost in the puerperium may arise predominantly from these general deposits rather than from the reproductive tissues.

The importance of the placenta in maintaining the phenomena of pregnancy (Newton, 1935; Pritchard and Huggett, 1947) suggest that this structure may be instrumental in controlling nitrogen retention. It has been shown that many steroid hormones, including oestrogen and androgens, but excluding progesterone, are capable of stimulating nitrogen retention (Kochakian, 1946) and these hormones are found in the placenta although there is little incontrovertible evidence that they are produced there (Hammond and Huggett, 1952).

Further, the feeding of dried placenta (Hammett and McNeill, 1917) and of a protein-free placental extract (Arthus, 1934) to nursing mothers has been shown to increase the growth rate of the offspring. This effect is probably due to stimulation of the milk yield, but may also entail an increased nitrogen retention to allow of the increased milk yield. The findings of Cambell and Kosterlitz (1949) of a rise in the ribonucleic acid of the liver during pregnancy, independent of any change in the protein or phospholipid content, even on a protein-free diet, may, eventually, throw some light on this matter.

Water Metabolism

No record has been found of a satisfactory direct study of water balance in pregnancy. Attempts have been made to examine the water balance of pregnant women by Pommerenke and Thompson (1941) and Freyberg et al (1938), but only the fluid water intake and the urinary and faecal water output were directly measured, the other water sources and losses being represented by dubious estimates. Also the periods of study were short. These findings are quite irregular, and impossible to interpret with any degree of confidence.

It is common assumption, on the basis of indirect evidence, that water retention is high during pregnancy, and excessively high in incipient pre-eclampsia and pregnancy toxaemia. The number of studies on pregnant women which indicate a weight gain during pregnancy greatly in excess of the mass of the conceptus is legion. These studies have been made to establish the normals (Chesley, 1944), to attempt to establish criteria for the prediction of eclamptic tendencies

(McIlroy and Rodway, 1937) and for a multitude of associated reasons. Similar work has been done on rats, mice and guinea-pigs (Chesley, 1944) and on cows (Morgan and Davis, 1936). The immediate inference from this excessive weight gain, amounting in women to about 10 per cent. and in rats to about 12 per cent. of the non-pregnant bodyweight, is that it is largely composed of water. This inference is supported by the common occurrence of oedema in human pregnancy, accentuated in pre-eclampsia, and parallel dropsical conditions in other animals. The occurrence also of the 20-25 per cent. hydraemic increase in blood volume in human pregnancy (Keith _et al_, 1915, and, more recently, Dieckmann and Wagner, 1934, and also in rats, Bond, (1948), suggests a high water retention during pregnancy. The diuresis which occurs during the puerperium may result from the loss of this retained water. On the other hand the weight gain could be, and to some extent undoubtedly is a true tissue solids gain (_cf_ the work on nitrogen and mineral retentions). The hydraemia and the oedema could be due to shifts in tissue water distribution, and the puerperal diuresis could be a urea diuresis accompanying the puerperal nitrogen loss. Albers (1939) has, in fact, shown that the normal pregnancy increase in plasma volume is very much reduced in pregnancy with oedema, with a corresponding fall in the colloid osmotic pressure of the plasma. The significance of this indirect evidence is discussed by Chesley (1944), and Newton (1949, 1952).

Assuming this water retention, several attempts have been made to define the site or sites of water storage. Estimations of water content in various tissues (eg Hewitt and Van Liere, 1941) have not, in general, shown any very striking change. The increase in size of uterus and mammae will account for a considerable part of the weight increase, but this still leaves, after taking into account the weights of offspring, membranes and amniotic fluid, about one third of the total weight increase to be disposed of. General tissue and organ hypertrophy could, however, account for a considerable mass of water without any apparent change in concentration; thus the increase in total nitrogen of the liver, found by Poo et al (1939), without a corresponding increase in nitrogen concentration indicates the liver as a storage place for water also.

No adequate explanation of this water retention has, so far, been forthcoming. It is reasonable to suppose that if there is extra-cellular water retention then there will be a corresponding retention of sodium ions. The evidence found of a high sodium retention during pregnancy (Coons et al, 1935; Hummel et al, 1937; Freyberg et al, 1938), along with the evidence of clinical practice of salt restriction dehydration in pre-eclampsia (Chesley and Annitto 1943) duplicates the problem without appreciably furthering the answer. Indeed, the total sodium retained during pregnancy, if all ionised, would entail a much greater water retention than is indicated from the weight gain (Chesley, 1944).

An interesting facet of this aspect of the problem, however, is presented by the finding of Barelare and Richter (1938) that, in diet self-selection studies, the appetite for NaCl of the pregnant rat rises by a factor of 3 in the last week of gestation. This suggests that the sodium retention may normally be largely achieved by an increase in intake and may not entail any great alteration in excretion. Some support for this is found in the large body of evidence demonstrating the lack of any substantial change in renal function during normal pregnancy (Smith, 1951). Although the associated retention of water and sodium are complementary questions rather than question and answer, the association does serve as a reminder that whatever the normal degree and mechanism of water retention, this is liable to alteration by alteration in Na retention and, in particular, by restricted or excessive salt intake.

The tacit assumption that any water gained will be extra-cellular is contra-indicated by the work of Heppel and Schmidt (1938). They found that in rats, excess retention of potassium above foetal requirements is correlated with the maternal weight increase above foetal weight. In potassium deficiency, up to 0.14 per cent. in the diet, the mothers lost weight during pregnancy although they produced litters. Newton (1935) found that the weight increase in pregnant mice was maintained after destruction of the foetuses and was lost when the placentae were delivered at the normal time. Newton and Van Wagenen (1943) found, in similar work on the monkey, that in this species the weight gain was continued after destruction of the foetuses until delivery of the placentae at term. A preliminary report by Dewar and Newton (1950) of the results of direct water balance studies, states that the <u>post partum</u> weight loss in the "pseudo-parturient" mouse is 80 per cent. water and that water loss can occur at this time along with nitrogen retention. Newcomer (1947) found in the rat, by removal of various combinations of hypophysis, ovary, placenta and foetus, that only the placenta was capable by itself of producing the characteristic hydraemia of pregnancy. These findings indicate that the placenta is the dominant factor in water retention in pregnancy.

Several workers have observed the presence of an antidiuretic principle in pregnancy urine (Teel and Reid, 1939; Krieger and Kilvington, 1940), and in serum (Griffith _et al_, 1942), but there is doubt as to whether this is present in significant excess over non-pregnant levels. There is no indication that this particular anti-diuretic principle is of posterior pituitary origin, nor that the placenta elaborates a specific anti-diuretic.

Zuckerman _et al_ (1939) have shown that treatment with oestradiol increases the water and salt contents of all tissues in the rat. They also found evidence that progesterone and testosterone stimulate water and salt retention but with a delay of some days. Taylor _et al_ (1943) found that administration of oestrogens or progesterone reduced the loss of Na and of water in the puerperal period. The increase in urinary corticoid excretion during pregnancy suggests the mediation of the adrenal cortex in water retention; the expected coincident effect of nitrogen catabolism has already been pointed out (p. 29).

It seems possible, from the above evidence, that one or more of the placental hormones, oestrogen, progesterone, androgens (Hammond and Huggett, 1952), may affect water balance non-specifically, or that the placenta may act through the pituitary or adrenal cortex to produce water retention. Newcomer's evidence, however, argues against the pituitary being an essential link in the scheme. If the somatotrophin of the anterior pituitary is, in fact, concerned with the nitrogen retention, then the degree of polydipsia which occurs with its administration to non-pregnant animals would, presumably, also occur naturally in pregnancy. But in the non-pregnant organism this polydipsia appears to be balanced, if not over-balanced, by a polyuria (eg Gaebler and Bartlett, 1939) and so it is unlikely to account for any great degree of water retention. Also the post partum loss of water with simultaneous retention of nitrogen (vide supra) presents an obstacle to the conception of one hormone stimulating both retentions.

There is no evidence to indicate whether the assumed water retention is achieved by an increase in intake or a reduction in elimination or both. Dewar and Newton's (1950) finding that the puerperal water loss is achieved by a reduction in intake without a corresponding fall in excretion would suggest an increase in intake during retention.

Effects of Age and Parity.

The numerical assessment of reproductive efficiency within species has, in general, shown that this efficiency is at a maximum at a certain age group and at a certain parity. On either side of this age group and parity the reproductive efficiency falls away. The criteria of reproductive efficiency used are relatively simple and gross, such as stillbirth and neonatal death rates, total weight and numbers in a litter, number of young reared to weaning and so on. Such criteria have no validity for inter-species comparisons but have a broad value in intra-species comparisons. Baird (1945, 1947) has shown minimum stillbirth and neonatal death rates (for Scotland) to occur in the age group 20-24 years, and for the second and third child in women. Age and parity both appear to be significantly associated with these rates apart from their own association. Less clearly, from the hospital records of Aberdeen, he has shown a relation between prematurity and toxaemia and these factors. Strandskov and Einhorn (1948) have found substantially the same relation with age of mother in U.S. populations with an additional separation into white and coloured mothers, the latter showinghigher rates.

King (1924), Cox and Imboden (1936), Asdell et al (1941) and Murray (1941) have all shown in rats, a decline in litter size and in percentage of young weaned with increasing parity, the maximum lying at about the first or second litter. Their data on birth weights and weaning weights show no consistent change with parity. Mehner (1940), however, found no consistent change in litter size of guinea pigs with succeeding pregnancies. Korkman (1947) and Olsen (1939) have found substantially similar changes with parity in pigs, although the maximum efficiency occurred at a later point.

Rowe et al (1931c) found a lower maternal bodyweight gain in multiparae than in primiparae, but it may be noted that the former were subject to a rather different environment and degree of work. Robinson et al (1943) found a higher weight gain in primiparae, especially at the end of pregnancy, but they also found that the greater weight gains were made by the initially lighter women. McIlroy and Rodway (1937) found a lower gain, unassociated with infant weight, in older women and these workers also found that the greater gains were made by the initially lighter women. Bray (1938), on the other hand, claimed that neither parity, age nor body build of mothers is a factor determining changes in bodyweight. Morgan and Davis (1936) found that the monthly weight gains of dairy cows were, in the first gestation, progressively greater after the fourth month, but that in subsequent gestations the monthly increments were equal and progressively smaller with each successive gestation. The weights of the calves also declined with parity but this did not entirely account for the total decline in weight increment.

These data on declining weight gain could be interpreted as a decline in water retention with each succeeding pregnancy with as much validity as the assumption of water retention from the evidence of weight gain. Again, of course, there is no direct evidence to relate the two phenomena. It is also possible that the changes in weight gain may be due to conceptus weight even if independent of foetal weight. It is interesting, in this respect, to note the finding of Zacharjewski (1894) that the weight of amniotic fluid is about one third lower in multiparae than in primiparae, but this is based on only 5 and 4 cases respectively.

The evidence for changes in retention of individual substances is sparse and quite inconclusive.

Hahl (1905), Bar (1907) and Wilson (1916) all found evidence of a higher nitrogen retention in primiparae but the values showed a great scatter and the difference is not statistically significant. Hummel _et al_ (1937) in studies of one primipara and one quadripara found the nitrogen retention of the former greatly in excess of that of the latter while neither Zacharjewski (1894) nor Coons and Blunt (1930) show any tendency to difference. Similar inconclusive and statistically insignificant evidence is available for mineral retentions.

It is of interest that the first pregnancy in women seldon gives rise to _icterus gravis_ of the newborn in incompatable Rh matings while subsequent pregnancies show increasing liability to iso-immunisation of the new-born. (Mollison _et al_, 1948). The same effect appears to operate in animals in which a parallel condition is found (Bessis, 1947), and may also operate in the apparent sterility due to ABO incompatability (Waterhouse and Hogben, 1947). There is, at present, no conclusive evidence to show whether this increased sensitivity of the foetus to maternal antibodies or the increased liability of the maternal Rh negative blood to sensitisation by the foetal antigen is due to increasing maternal antibody titre, to physiologically increased permeability or to increased fragility of the placenta on successive gestations, or to some unknown factor.

MATERIALS

Experimental Animals

All the work reported here has been done using female rats of the hooded Wistar strain. These have been bred in this Institute as a closed colony, excluding brother-sister mating, from a nucleus of 10 females and 3 males obtained in 1948 from the stock of the Rowett Research Institute.

Details of the characteristics of the animals used in the metabolic work and of those used in the foetal and placental growth study are presented in table (28).

Diet

The use of a semi-synthetic diet, although very much more convenient for metabolic work, in that it can be chemically and energetically more rigidly defined (vide p.141), carries with it always the risk of nutritional deficiency. This is especially true when the requirement is for continued reproductive activity, and the many complex problems involved here have recently been reviewed by Russell (1948). The addition to a semi-synthetic diet of natural foods such as greens and fresh milk largely eliminates this risk, but such supplementation is not practicable in closed metabolic studies.

To avoid the possibility of nutritional deficiency at the cost of losing rigid definition of the diet, I decided to use a stock cube diet.

For the study on female 377 the Rowett Institute stock rat cube diet was used (Thomson, 1936; modified, Russell, 1948). This diet is adequate for reproduction but improved performance is obtained if it is supplemented by fresh milk and greens during pregnancy and lactation. The composition of this diet is as follows:-

Wheat Offal (bran)	17.7	per	cent.
Ground wheat	17.7	"	"
Sussex ground oats	17.7	"	"
Ground maize	8.8	"	"
Ground barley	8.8	"	"
White fish meal (60 per cent. protein)	4.5	"	"
Meat and bone meal (45 per cent. protein)	8.8	"	"
Dried skimmed milk	14.0	"	"
Dried yeast	1.2	"	"
NaCl	0.4	"	"
Cod-liver-oil	0.4	"	"

All the other studies were made using the Diet 41 (Bruce and Parkes, 1949) as prepared commercially by Associated Flour Millers (London). This diet is stated to be adequate, without supplement, for reproduction in the rat and mouse. The composition of this diet is as follows:-

Wholemeal flour	46.0	per	cent.
Sussex ground oats.	40.0	"	"
Fish-meal	8.0	"	"
Dried yeast	1.0	"	"
Dried skimmed milk	3.0	"	"
NaCl	1.0	"	"
Cod-liver-oil.	1.0	"	"

Digestible protein	13.6	"	"
Soluble carbohydrate	48.4	"	"
Fat	4.5	"	"
Fibre	1.4	"	"

It has become apparent both in this work and from the general breeding performance of stock rats receiving this diet in this Institute, that Diet 41 is not adequate without supplement for continued reproduction. On an exclusive regime of this diet weaning weights of stock rats have dropped from 45 to 30 g. I have been unable to get any third or further litter from rats on this regime and no satisfactory reproductive performance after the first litter.

The source of the deficiency in this diet is not known, but possible causes are discussed later (p.266) The protein content is given by Bruce and Parkes as 13.6 per cent. and my own analyses of food batches used show crude protein (total N x 6.25) to be 15-16 per cent.

of the fresh weight, or 17-18 per cent. of the air-dried weight, which should be adequate. As can be seen from the above table the protein is not first class, but, in any case, a relatively mild degree of protein deficiency should not have such a drastic effect on reproductive performance, (cf Russell, 1948).

THE METABOLISM APPARATUS

DISCUSSION OF DESIGN

Of the two methods of animal calorimetry, direct and indirect, or respiration, calorimetry the latter entails the construction of simpler and very much less expensive apparatus, and if either is to be used alone the indirect method is more informative, giving a chemical as well as a physical picture of the energy metabolism. For the present work, also, it is important that whatever type of instrument be adopted it must be adaptable to use for 24 hour periods of study.

Direct Calorimetry.

The modern water bio-calorimeter of the type constructed by Rubner for the dog, and Atwater and Rosa for man, is a complicated piece of machinery and its construction and operation is beset with technical difficulties. The more recent types of compensation (Benedict and Lee, 1937) and differential (Murlin and Burton, 1935) direct calorimeter are technically simpler instruments if used for purely direct calorimetry, but adaptation to the simultaneous recording of respiratory exchange greatly increases their complexity. A more recent development in direct calorimetry uses thermal gradient methods (Benzinger & Kitzinger, 1949). This method is more easily adaptable to simultaneous measurement of respiratory exchange and may also be capable of quantitatively partitioning the heat disposal by various routes. I did not consider, however, that the great additional technical difficulty and expense of direct calorimetry was justified in the present work. Once the basic concept of the application of the law of the conservation of energy to vital processes had been established a great part of the necessity for direct bio-calorimetry was eliminated. There is, undoubtedly, much still to be done by parallel direct and indirect methods in improving our knowledge of the energetic equivalence of respiratory exchange in different species under different dietary conditions, but this work has not been undertaken here.

Indirect Calorimetry.

I decided to use the method of indirect calorimetry, partly because of its greater simplicity, but largely to obtain this fuller chemical picture of the metabolic process. The design of a suitable apparatus, however, presented some difficulty.

The advance in the precision of indirect calorimeters from the pioneer experiments of Crawford and of Lavoisier and Laplace to the instruments constructed by Regnault and Reiset (1849) and by Haldane (1892) was great, and these latter workers set the type for all subsequent calorimeters of the closed or open circuit types respectively. A further major development in design produced the Benedict-Roth-Collins recording spirometer type of calorimeter. Otherwise development in indirect calorimetry lay largely in adapting these three types of instrument to the special size and habit requirements of different species.

Most of the closed circuit modifications are of a type not easily adaptable to the requirements of long-period trials. In the case of the Regnault-Reiset type of instrument the animal is subjected to an atmosphere almost fully saturated with water vapour, while in the case of the Benedict-Roth type of instrument the humidity of the inspired air, although reduced to some extent by the soda-lime absorbing tower, is still much higher than normal. In neither instrument can there be any measure of the water balance of the animal. The Benedict-Roth instrument makes no measurement of the CO_2 production and uses the oxygen consumption and an assumedly constant R.Q. as a valid measure of the energy expenditure. This instrument also depends for its operation on the ability of the respiratory pressure changes to operate the valve mechanisms; obviously such an arrangement is not feasible for very small animals. Both of these types of instrument are also limited by the relatively short metabolism period for which they can be operated. Although the Regnault-Reiset instrument could be modified to cover 24 hour periods it is unlikely that subjection of an animal to 100 per cent humidity for such periods would give a very normal picture of its energy metabolism. This criticism of limited time of study can be levelled with even greater force at the manometric type of apparatus in which the oxygen consumption is recorded by the fall in pressure within an air-tight animal chamber containing a CO_2 absorbent. This method of oxygen recording is not satisfactory unless very accurate records of atmospheric pressure are kept (Balogh, 1951), and the dependence of CO_2 absorption on simple diffusion is not a happy arrangement.

Haldane's open circuit method and its subsequent modifications (e.g. Benedict, 1938), eliminates most of the disadvantages of the standard closed circuit methods. The animal can be supplied with air of a normal humidity and the total water balance can be estimated. The ventilation rate of the instrument can be varied at will to reduce the CO_2 concentration to a very low value. The metabolism period can be of any length; with small animals all the CO_2 and water produced in one day can be easily absorbed, but with large animals the period of study can easily be maintained at one day by continuously aspirating a small sample from the main emerging air stream through an absorbing train (Ritzman and Benedict, 1929). Such an arrangement has, however, its own disadvantages. For small animals the oxygen consumption by this method is found as a weight difference involving the weight change in the animal which is extremely difficult to obtain with any degree of precision. Over short periods of study it is possible that the carbon dioxide production alone is a better measure of the energy expenditure than is the oxygen consumption alone, or even the oxygen consumption with the R.Q. (Poulton, 1938). For long periods of study, however, or any study which does not involve the post-absorptive condition the Adams and Poulton (1932, 1934) concept of the 'constant combustion ratio' cannot be accepted. For large animals, the air sampling mechanism, although simple in theory, is technically difficult if the records obtained are to be representative of the total gaseous exchange; it is also necessary, with large animals to meter the total airflow and to perform frequent gas analyses of the chamber air. These sources of error would not have been of such great importance if the open-circuit method had been used over long metabolism periods, but although capable of being so used it seldom has been. The end in view has usually been to obtain the metabolic rate of an animal under a constant state of stress or rest, either of which can only be maintained reliably for a short time. The emphasis laid on the constancy of the animal's state of activity, particularly the emphasis laid on the importance of the basal state, (Benedict, 1938), has, I believe, been largely responsible for the restriction of calorimeter design to the measurement of short period trials.

The methods of calorimetry based on the analysis of the expired air (Speck, 1892; Douglas, 1911,) are essentially open circuit methods since the inspired air is always atmospheric air. These methods could be adapted to longer periods of observation but, again, they require the actuation of a valve mechanism.

A combination of the open and closed types of circuit was used in the indirect calorimetry component of the Atwater-Rosa-Benedict calorimeter. A modification of this, to measure the purely pulmonary respiratory exchange in man was developed by Benedict (1912), and used by Benedict and Cathcart (1913), in their study of muscular work. This depends on the circulation of an isolated atmosphere, which is respired by the subject, through a water and carbon dioxide absorbing train by means of a rotary blower pump. As the gaseous products of metabolism are absorbed oxygen is passed in, to maintain the oxygen tension of the atmosphere, either directly from a cylinder (when the oxygen usage is measured gravimetrically), or from a spirometer (when it is measured volumetrically). The fundamental principle of this method was incorporated, by Knipping, in a small portable apparatus for the clinical estimation of the basal metabolic rate.

Used in its original form this method combines the best of both worlds, enabling direct measurement of all the components of the respiratory metabolism and easily adaptable, when used for small animals, to long period studies. The major technical difficulty in the construction of such a machine lies in devising a pumping device which will not be liable to leaks to the outside atmosphere.

When the present work was under consideration an adaptation of the above method for measuring the total metabolism of mice over 24 hour periods was published by Dewar and Newton (1948). This instrument uses a Dale-Schuster pump with bunsen valves for direction of the air-flow. The valve mechanism used by this pump in its intended function of fluid circulation is unsuitable for air. The use of a rubber membrane pump effectively eliminated the leak hazard from this part of the instrument; other objections to the use of this type of pump are discussed later (p. 80). The oxygen used is measured volumetrically being supplied continuously from a spirometer to maintain the pressure of the circulated atmosphere. This instrument is obviously the best small animal respiration calorimeter so far produced, and I decided to use the design, modified for use with rats. A fairly detailed description of the principles of design of the instrument is given in their published work, but I am indebted to the late Professor Newton and to Dr. Dewar for much valuable additional information on technical points, and for allowing me to inspect their apparatus.

The primary modification to be made in the instrument as designed by Dewar and Newton, was to increase its size to accommodate rats. Other minor modifications were made and these will be discussed at appropriate points in the following description of the apparatus.

Diagrams of the original design by Dewar and Newton, and of the final design adopted by me are given as figs. (1) and (2). Photographs of the apparatus which I constructed are given in figs. (3) and (4). Although the instrument described here is based on that of Dewar and Newton, considerable modifications had to be made to the original form in siting and construction of the components to adapt the apparatus for use with rats. As only a few of the accessory and none of the primary components were available commercially, the form of the apparatus as constructed by me is given in detail.

CONSTRUCTION OF THE METABOLISM APPARATUS

Constant Temperature Cabinet.

The entire apparatus must be maintained at a uniform temperature, not only to reduce the corrections necessary in volumetric measurements, but also to render the metabolism of the animal as far as possible independent of response to environmental temperature change. It is necessary, also to protect the animal from extreme temperature fluctuations which, in the rat, are very favourable to the development of respiratory infections.

A heat insulated cabinet which had been built in this department for another purpose, but long disused, was utilised. This is a double walled box, the outside wall of plasterboard, strengthened with ¼ in. x 2 in. wood straps, of dimensions 47 in. x 29 in. x 29 in., and the inside wall of plasterboard. A 2 in. interwall space is filled with granulated cork. The roof and floor are of ½ in. wood. There is no false floor. This gives inside dimensions of 43 in. x 25 in. x 27 in., and the design of the component parts and the assembly of the apparatus had to be considered in relation to the size of this cabinet.

The front of the cabinet is also double-walled, the outer wall of ½ in. wood, and is hinged on the upper edge, that is, it must be lifted up to allow free access to the interior, (cf. fig. 4). In its original form this door had a central 9 in. square double glass window and two 3 in. square openings to allow manual access to the contained apparatus; for these were substituted one double glass window 32 in. x 15 in. To the flying edge of the door was bolted a heavy steel eye so that the door could be held open by a chain and hook suspended from a ceiling joist.

The box was gutted of the remnants of its original contents. A toluene thermoregulator, a mercury and coil relay and an air fan were retained.

On top of the main cabinet there is a subsidiary box, fig. (3), not heat insulated, which had originally been used to house the motor which drove the air fan by a belt drive. I decided to mount the air pump and its driving motor here, the latter having a through shaft from which the fan could be operated by a pulley and belt drive as in the old system. This arrangement was altered at an early stage; details of the final powering arrangement are given on p.79 .

The plasterboard walls of the cabinet can only carry relatively light loads, so racks were made to carry the animal chambers. These chambers, with their accessories and the animal weigh about 8 kg. Three sets of racks were built; one on the back inside wall to carry the loaded chamber actually on experiment; one on the front left inside wall to carry the replacement chamber so that it could be kept at the cabinet temperature; and one on the front left outside wall to carry the chambers during the changeover period and during the preparation of a chamber for use. The racks were of vertical 1½ in. x 1¼ in. section wooden batons, set 6¼ in. apart, and each carrying 8 in. iron angle brackets. The batons are screwed to the heavy joists which form the frame of the cabinet. The angle brackets are set 18 in. above the floor of the cabinet, allowing sufficient clearance for the assembled chamber, and on their projecting ends are mounted small wooden stops to guard against the chamber being accidentally pulled off or shaken by vibration, figs. (3) and (4).

Two openings already existed in the roof of the cabinet. These are lined by 1¼ in. brass bushes to prevent scattering of the granulated cork. One, in the back left corner of the roof had been used for the thermoregulator, the bulb being inside and the adjustment outside the cabinet. I decided to continue to use it for this purpose. Although a thermoregulator might have been better installed in a more central position it would then have interfered with the free movement of other components of the apparatus, and I relied on the fan maintaining a substantially uniform temperature throughout the box. The other opening runs, in the front corner of the roof, from the main cabinet to the pump housing and was suitable for carrying the air ducts to and from the pump, fig. (14.)

Animal Chambers.

Two animal chambers were made to allow conveniently rapid procedure in the daily changeover, and also to avoid subjecting the animal to stale odours.

The design of these was based on that of Dewar and Newton, modified to accommodate an adult rat. Since an adult rat is of the order of 10 times greater in weight than an adult mouse the chamber size could not be increased to a corresponding degree or it would have become quite unmanageable. The chamber was, therefore, made of a size which appeared manageable but which would, I believed, adequately accommodate an adult rat. Later experience has indicated that this chamber is on the small side, allowing the animal very little freedom of movement, and in a new instrument now under construction the design has been largely altered.

A photograph and diagram of the assembled chamber, as constructed in the workshop here, are shown in figs. (5) and (6) respectively. The body is of 1/32 in. brass, brazed at the junction of the edges to form a 6 in. diameter cylinder. The base, of the same material, is conical, the angle of axial section being 120°; this base is brazed, at a ¼ in. vertical overlap, to the cylindrical body. At the apex of this cone is brazed a ½ in. brass tube 1¼ in. long, through which can pass freely the stem of a metal collecting funnel for urine. To the upper end of the cylinder is brazed a heavy flange, of ½ in. brass, 1.4 in. wide. This flange is tapped at eight points in its circumference and carries 1½x¼ in. brass bolts.

The lid of the chamber is of the same material as the body and carries a similar heavy brass flange. The roof of the lid is flat with a central ¾ in. tube outlet to carry the chamber thermometer. The flange of the lid has 5/16 in. holes bored to correspond to the bolts tapped into the body flange.

In the chamber originally designed by Dewar and Newton the air inlet and outlet tubes both passed through the lid of the chamber. Ventilation is, however, better achieved by bringing the air inlet to the bottom of the chamber with the outlet at the top. In my design the inlet tube passes through the wall of the body of the chamber, just below the flange, and is there connected by an angle block junction, to a vertical 5/16 in. diameter brass pipe 8 in. long, which passes down the wall of the chamber almost to the beginning of the conical base. The air outlet is a similar tube tapped into a block bush in the wall of the lid in a corresponding position. This achieves the bottom to top airflow but avoids the awkwardness of having a pipe descending from the lid to the bottom of the chamber with the possibility of its fouling the chamber contents during the removal or replacing of the lid. The close proximity of the external connections makes for ease of manipulation in the setting up and dismantling of the apparatus. The positioning of the connections near the flanges reduces the possibility of flexion loosening the joints and giving rise to leaks. The screw joints into the wall and block bushes are reinforced by locking nuts and all the threads were packed with "Bostik" cement when the chamber was finally assembled.

In the front of the chamber wall is a rectangular window, 6 in. x 6 in. Through this, light can reach the chamber and the animal can be viewed. The window substance is 1/16 in. perspex 7 in. x 7 in., bent in hot water along its long axis to the curvature of the chamber wall. Facilities for moulding the perspex at a more suitable temperature, 110°C., were not available when these chambers were being made. The perspex is fixed to the outside of the chamber wall by screws passing through drilled holes in the perspex and tapped into the chamber wall. The overlapping adjacent surfaces of perspex and brass ($\frac{1}{2}$ in. all round) were coated with "Bostik" and the window screwed tightly into place. This window was a major source of leaks due to the springing of the perspex. The number of binding screws was doubled and their threads were packed with "Bostik". This improved the gas-tightness of the chamber but did not completely solve the problem. The perspex-metal borders, both inside and out, were filled with "Durofix". These measures were effective in reducing the tendency to leaks. It was found later that the perspex was liable to minute fractures, caused by successive slight blows against the chamber racks; the leaks which developed in this way were cured by smearing perspex cement over the region.

The tube outlet from the base of the chamber is covered with $\frac{3}{4}$ in. rubber tubing sealed to the brass tube with "Bostik". The rubber projects $\frac{1}{2}$ in. beyond the end of the brass tube and the outside surface of its lower end was buffed to a slight taper, sufficient to allow it to act as an airtight stopper for the 100 ml. Erlenmeyer flasks used as urine collection vessels.

Gaskets for sealing the flange joint between the lid and body of the chambers were cut from an old motor-car inner tube. The washer lies over the whole breadth of the flange with slight projections inside and outside the chamber. Holes were cut in it at corresponding points to the bolts in the lower flange. Dewar and Newton found difficulty in getting a gas-tight seal with a rubber washer and suggested the use of plasticine. I found no difficulty in ensuring a gas-tight seal with a rubber gasket. Wing nuts were used to clamp the flanges but finger tightness of these was not adequate to ensure a tight seal. The wing nuts were retained in spite of this, as they are very much more convenient, and a spanner was made, fig. (7), with which they could be tightened. The use of a spanner on wing nuts enables a greater force to be applied than the bolts are capable of withstanding, and several bolts sheared in this way during the work; however an ample supply of spares was kept and the shearing presented no serious interruption. A photograph of the component parts of the chamber is shown in fig. (7).

All the metal parts of the chambers were chromium plated to prevent corrosion, to make for easier cleaning and, in general, to give the chambers a better appearance.

Animal Frames.

Frames were made, shown in fig. (8), which would fit into the chambers and which would carry the rat, food box and water bottle, and would collect scattered food and faeces so that all could be removed from the chamber simultaneously. The skeleton of the frame is of 10 I.W.G. gauge brass wire. The base is a circle of wire 6 in. diameter and this has rising from it four uprights brazed to the circular base. Diametrically opposite uprights are made of the one piece of wire 25 in. long, giving a height of 9½ in. and 6 in. orthogonal cross bars across the top. The cross bars are morticed and brazed at the point of crossing. Two grids of ⅜ in. 18 gauge brass mesh were soldered to this skeleton; one, 1 in. above the base ring serves as the floor and the other, 8½ in. above the base ring, as the roof of the effective living space of the animal. The whole structure is chromium plated.

The base circle has a re-entrant portion 1 cm. deep between two of the uprights, and recesses are cut out of both grids vertically above the re-entrant. These allow the frame to slide into the chamber, clearing the vertical inlet tube. The placing of the recesses is such that the forward uprights of the frame lie on either side of the window and do not obstruct the field of view through the window, (cf fig. 5).

The mesh of the floor grid allows faeces and scattered food to pass through. A lower grid of 0.02 in. mesh stainless steel covers the whole of the base ring and is attached to it by crimping the edges of the gauze round the wire. This mesh retains faeces and scattered food but allows the urine to pass through. Stainless steel was used to prevent corrosion and so obviate periodic replacement of the mesh. It was considered that a brass mesh of this gauge could not be plated satisfactorily. The use of stainless steel, however, necessitated attachment of the mesh by the crimping mentioned above, which has not proved a very secure method and has sometimes caused slight inconvenience.

The 1 in. space between the floor and faeces grids is adequate to prevent coprophagy. The space between the ceiling grid and the chamber roof allows a water bottle to sit on the ceiling with the delivery tube passing down into the living space.

Collection of scattered food by the steel grid is not perfect, an appreciable amount falling down the sides of the chamber to the urine funnel. It would be an improvement to have the whole lower third of the frame enclosed by this fine gauze.

Food Boxes.

Two food boxes were made of 1/64 in. brass after the design of Dewar and Newton, and one is shown in fig. (8). The plan section is 2 in. x 1½ in., with the posterior and anterior walls 4½ in. and 1¾ in. high respectively. The steeply sloping roof was adopted to prevent any smaller animals, which I might wish to examine in the instrument, from sitting on the top and contaminating the food. The sloping roof is hinged to the back wall and is held in the closed position by spring clips attached to the sloping edges of the side walls. The space for access to the food by the animal is 2 in. x 1½ in.

At the top and bottom of the outside of the back wall of the box are brazed brass blocks with recesses and screw bits so that the box can be firmly clamped to one of the uprights of the frame at any desired height. I consider a screw-bit fitting to be preferable to a spring clip to ensure that the box is not lowered or detached by the animal.

The lip of the trough of the box is recurved as a ¼ in. diameter cylinder to prevent any chafing of the animal's neck or paws while feeding, and also to reduce, as far as possible, the amount of food scattered. It is doubtful if this type of lip has done anything to achieve the latter end; experience has shown that some animals scatter food badly even with this design of box, while others are very clean eaters. (Compare serial numbers 78-128 and 129-173 in table (34) appendix (3).

The capacity of the trough of a box is about 45 g. of food, which is quite adequate for the daily requirements of any animal which was studied, even with considerable scattering of food. The boxes are chromium plated.

Urine Funnels.

These (fig. (8), were made to fit the lower conical part of the chamber with as little clearance as was convenient for inserting and removing them. The cone of the funnel is of 1/64 in. brass with an indent in the lip to allow clearance of the air inlet pipe. The shaft of the funnel is of $\frac{3}{8}$ in. brass pipe ($\frac{1}{4}$ in. bore) which passes freely through the lower exit tube of the chamber. The funnels are chromium plated.

The angle of the funnel cone is the same as that of the chamber, 120°. It was thought that it might be necessary to make new funnels with a more acute angle if these originals showed too great a tendency to retain the urine. This has not been necessary, as the flatter funnel not only allows adequate urine flow into the collecting flasks but also retains scattered food which escapes over the edge of the collecting grid. With a steeper funnel it is probable that a greater proportion of this widely scattered food might run into the urine flask.

The funnel shafts were pegged and soldered to the cones with soft solder. The constant wear caused by inserting and removing the funnels from the chamber removed the plating from the funnels at the junction of shaft and cone, and also from the exit tube of the chamber with the result that considerable corrosion took place at these points. This wear also weakened the shaft-cone joints of the funnels and they had to be re-bonded with silver solder.

Soldered on the inside of the cone of each funnel is a wire handle, fig. (8). This enables the funnel to be easily inserted and removed from the chamber.

Water Bottles.

I originally intended to use water bottles of the all glass globe type, but these are fragile, awkward to fill and inconvenient to weigh without losing a drop or two of water. In the event water bottles were made of 4 ounce cylindrical glass bottles, fitted with 5 mm.

bore glass teats in rubber bungs. The glass teat just passes through the roof grid and is of such a length, 3 in., that the nozzle is at a convenient height for the animal to drink. The bung was cut to such a depth that the top of the chamber lid, when in position, just touches the 'base' of the bottle. This prevents the bottle from being shaken too vigourously by the animal, with the attendant risk of the bung being loosened and the water escaping.

The diameter of the teat orifice presented some difficulty. If made of the customary diameter the pressure variations in the chamber due to the pump action tended to pump the water out of the bottle. A smaller orifice, however, is very liable to air-locks. Finally a 1.5 mm. orifice was used which effectively offset the pumping action, and, if the teat is filled with water before the water bottle is weighed, and the bottle is inserted in the chamber with the teat still free of air-locks, the occurrence of air-locks during the run is largely prevented. This orifice size is not completely satisfactory from the latter point of view, and on several occasions the animal's water intake has been artificially restricted.by the development of such air-locks during the day's run.

Chamber Thermometer.

It was found necessary to use an angle thermometer to measure chamber temperature, in order to clear the roof of the cabinet, fig. (4). The thermometer is mounted in a rubber bung inserted in the central exit tube on the roof of the chamber. The angle thermometers obtainable, were, however, rather larger than was convenient and had to be set so deeply in the bung to clear the roof that the mercury bulb fouled the junction of the cross-wires of the frame. The junction was, therefore, forced down towards the roof grid so that the cross wires formed an inverted pyramid. This did not adversely affect the rigidity of the frame and allowed the thermometer to be set 1 in. deeper in the chamber and so clear the roof of the cabinet. The thermometer covers the range 0-50°C. in one degree intervals, and can be read to an accuracy of about 1 /3°C. A similar thermometer, matched with the chamber thermometer in a water bath, is mounted on the outside of the spirometer vessel to record the general air temperature of the cabinet, *cf* fig. (4).

Spirometers.

Three spirometers were constructed, each of about 4½ litres capacity, so that the daily oxygen requirement of a medium sized rat, about 300 g., would be adequately supplied without refilling in the course of the day. When the apparatus was being assembled, however, it was decided to include only two spirometers to leave greater room in the cabinet for manipulation in the change-over process. Also, it seemed possible that I might want to include some form of continuous oxygen recording, and some space had to be left for this.

For reasons which will later be described the greater part of the experimental work using this calorimeter has been done using only one of these spirometers which was recharged with oxygen at intervals throughout the 24 hr.

Each spirometer consists of a perspex bell suspended in a water seal, figs. (9) and (10). The container or jacket of the water seal, modified from the containers of three old Benedict-Roth spirometers, consists of a cylindrical copper tank, 17 cm. diameter and 30 cm. in height, mounted on a flat brass base. This base has tapped into it three levelling screws so that the whole is mounted on an adjustable screw tripod. A central brass tube, 2 cm. bore, rises from the base to a height of 27 cm., and at the base is connected through a brass angle block junction to an outlet tube 4 mm. bore. This is the route for the entrance and exit of oxygen to and from the spirometer. The central duct is soldered to the angle junction and the lead-off tube is threaded into the block, the thread being packed with a little cotton waste and 'Bostik'.

A packed brass gland, set in the wall of the tank 7 cm. above the base, carries a 5 mm. bore vertical glass tube, which indicates the water level in the vessel. The water levelling tube is held at its upper end by a light brass loop soldered to the top of the tank wall.

The bell is a welded tube of 1/8 in. perspex, 15 cm. in diameter and 28 cm. in height. A flat roof of 1/8 in. perspex is cemented at one end. At the point in the roof where exactly plumb suspension was obtained is cemented a small perspex eye.

An angle bracket of 3/8 in. brass rod was erected in tubular guides soldered to the side of the spirometer vessel. This has two small brass pulleys, (1 in. diameter, 1/8 in. gauge), one at the angle of the bracket and the other at the end of the gallows arm, fig. (10). The length of the short arm and the diameter of the pulleys was calculated so that (a) the vertical tangent to the terminal pulley coincided with the axis of the spirometer vessel and (b) the outer vertical tangent of the angle pulley passed sufficiently clear of the upright of the bracket to allow a measuring scale and counterpoise to be set between the upright and the tangent.

The first 30 cm. of a half-meter stick was used for the measuring scale of the spirometer. The breadth of the scale was reduced, by removal of the blank half, to economise in space. The scale had screwed to each end of the blank edge a Terry clip by which it was attached to the gallows' upright. This enabled an adjustment of the scale to the optimum position while calibrating the apparatus; the scale position was later fixed by setting brass stops under each clip.

The counterpoise to the bell is of brass tube 2 cm. in diameter and 9-10 cm. long, filled with lead. The suspension used is a length of heavy nylon suture; this is attached to the perspex eye of the bell, while the other end passes through a small steel eye screwed into the centre of the head of the counterpoise. Also screwed into the edge of the head of the counterpoise is a perspex cursor with a central hair-line on the inside of the front face. In the first instance the cursor was made with only one face, riding in front of the scale; but the whole counterpoise was very liable to rotate on the suspension and carry the cursor away from the scale. A U-section cursor was, therefore, substituted which rides freely on the scale, but prevents the radial drifting of the counterpoise.

As the spirometer bell is suspended in a water seal and as the perspex is of considerable thickness, the buoyancy of the bell substance is of appreciable magnitude. The volume of the wall of the bell is 165 ml., and the total weight of the bell in air is 650 g. Thus when the bell has only its lower open rim in the water seal it requires a counterpoise of 650 g., while when immersed in the seal to the lower surface of the roof it only requires a counterpoise of 485 g. This difficulty is avoided in the comercial Benedict-Roth clinical respirometer by the use of a chain suspension accurately constructed so that as the bell moves lower in the water the difference in weight between the two vertical sections of chain exactly compensates for the change in apparent weight of the bell. Other methods of compensating the counterpoise are possible but all either increase the frictional resistance to the movement of the bell or require the use of a single large pulley which decreases the vertical clearance of the spirometer or, since the height of the cabinet is fixed, reduces the effective volume of the bell.

As the arbitrary centimeter scale had, in any case, to be calibrated for gas volume it was decided to suffer the uncompensated counterpoise and the probable non-linear calibration curve. The pressure changes in the bell produced by an uncompensated counterpoise are taken into account in the calibration curve, but these pressure changes are also transmitted to the main air circulating system through the oxygen intake valve. To reduce this effect to a minimum the counterpoise was made just to balance the bell at half immersion. Simple calculation from the cross-sectional area of the bell (180 sq. cm.) and the data on weight and displacement given above, show that this should produce a pressure variation on either side of atmospheric pressure for the full excursion of the bell of ±0.5 cm. water. In practice, the pressure range as measured is ±0.5 cm. water.

The tank of the spirometer contains a 0.02 per cent. solution of mercuric chloride to prevent growth of aerobic organisms (Dewar and Newton). A 5 litre aspirator bottle, set on a stool at the side of the apparatus, serves as a water reservoir from which the water level in the spirometers is adjust, figs. (2) and (3). A single duct with a through tap in its course, runs from the reservoir into the cabinet, where it branches by a T-piece to supply both spirometers. From a T-piece junction at the point of entry to the cabinet of the water duct a draining tube is led off. Taps are set in the individual spirometer branches so that they can be levelled independently. The details of this water supply circuit are shown in fig. (2).

It was found, after a short time of use, that moisture condensing on the inside roof of the spirometer bell dropped down the central spirometer duct and sometimes produced a waterlock in the junction block at the base. Small 'umbrellas' were made of thin copper sheet, fig. (10), which allowed free flow of air but protected the duct from this intermittent 'rain'.

Calibration of Spirometers.

The two spirometers which it was decided to use in the apparatus were calibrated by use of a 100 ml. burette, with two-way delivery tap, modified for use as a gas burette. A Hempel burette was not available. Fig. (11)shows the modifications made and the method of use. The water levels in the burette and in the spirometer levelling tube were adjusted at each calibration interval.

Before the actual calibration the amount of water in the spirometer vessel was adjusted so that when the bell was fully immersed, that is with the inside roof of the bell just clear of the central duct, the water level in the tank was some millimetres below the open top of the duct. The clearances of the duct by the bell and the water level were decided arbitrarily by inspection and no measurement was made. It may be noted here that, with the bell in this position, the water level within the bell is about 5 mm. above the surrounding level; this difference is due to the uncompensated counterpoise. With this amount of water in the tank the bell was allowed to find its own level, the air duct being left open, and the water level in the levelling tube marked by a ring of red glass ink. The spirometer scale was then fixed on its pillar at a point where it was known, by trial and error, to be clearly observable when the apparatus was assembled. The length of the suspending cord was adjusted so that when the bell was fully immersed the cursor coincided with the 1 cm. mark on the scale. Setting the cursor at this point rather than at zero ensured a margin over which the oxygen volume could still be estimated if, by accident, the bell were allowed to overshoot its calibrated excursion.

The calibration intervals were 20 ml. Duplicate calibrations were made for each spirometer and no detectable difference was observed between them. The mean readings for the 100 ml. intervals are given in tables, (24a) and (24b), appendix 2. The curves constructed for practical scale reading volume conversion were made from the 200 ml. intervals; this removed the slight irregularities, shown by the narrower intervals, which, it is believed, are due to subjective errors of visual interpolation of fractions of a millimetre on the spirometer scale. Reduced photographic reproductions of these curves are given as figs. (12) and (13).

Thermostatic Control.

The heating elements, to maintain the temperature of the cabinet, are two 100 watt electric lamps. These are covered with ventilated cans, painted matt black, to reduce any possible irritation to the experimental animal by their intermittent light and to ensure the maximum radiative efficiency. These heaters are inparallel with the main electricity supply and are controlled by a thermoregulator actuating a relay. The heater circuit of the apparatus is shown in fig. (2), and the position of the heaters in the cabinet in fig. (4).

The original design of the electrical equipment was complicated by the fact that when this apparatus was being built this Institute was still receiving a predominantly D.C. electrical supply. Shortly after the completion of the apparatus the conversion to an A.C. supply was also completed.

Initially the thermostat control was by a toluene thermoregulator and a primitive form of relay, already mounted on the cabinet, of the electro-magnet-mercury switch type. As this relay drew a heavy current constant trouble was experienced with oxidation of the mercury contact surfaces, both in the relay and in the thermoregulator. With the change in the electrical supply an electronic type of relay was substituted which draws only about 2mA through the thermoregulator. At the same time the toluene thermoregulator was replaced by a mercury variable contact thermometer; this has, in fact, a greater sensitivity for this purpose, having a lower heat capacity, and has the added advantage of being more easily adjusted than the toluene regulator.

For reasons of space conservation and accessibility of the other parts of the apparatus, the heaters were sited in the back left floor of the cabinet. This position is immediately below the thermoregulator and is very close to the animal chamber, and so raises the possibility of pronounced radiative effects on regulator and chamber. The chromium plating of the chamber and the nature of the surface of the mercury bulb of the contact thermometer reduce this effect. I have assumed that such radiative influence as may remain on the thermometer would simply alter the required setting of the thermometer to maintain a given mean cabinet temperature. Trials of the apparatus with no animal in the chamber showed no perceptible temperature difference between the inside of the chamber and the air at the right-hand side of the box, that is furthest away from the heaters.

The thermostat is set to maintain a cabinet air temperature of about 23°C. This is rather lower than the usual environmental temperature at which laboratory rats are maintained, but it was expected that the temperature in the animal chamber would tend to be slightly higher due to the heat production of the animal. In fact, the temperature in the chamber when containing an animal is fairly uniformly 3°C. higher than the air temperature of the cabinet.

In addition to the heaters, a 60 watt unshielded lamp is mounted on the centre front inside roof of the cabinet, fits, (2) and (4). This is under separate switch control and is independent of the thermostat. Its primary purpose is for observation of the apparatus, but in addition it serves as an auxiliary heater for rapidly raising the cabinet to its normal working temperature after the door has been open or the apparatus completely out of use for any considerable time.

The thermostat system is left to operate almost continuously, even when the apparatus is not actually in use. Starting from cold the cabinet takes from two to three days to settle to a constant and uniform temperature. If the heating system is left in operation the apparatus can be brought into use any any time and takes only about three hours after the fan has been turned on to attain a uniform temperature.

Very warm weather allowed over-heating of the cabinet. The temperature could usually be reduced to normal by omitting to latch the door of the cabinet, when it swings slightly outwards allowing some draught between room and cabinet. On a few occasions the door of the cabinet had to be kept fully open during the day to keep the temperature down to 23°C.

The intermittent 200 watt load of the heaters is rather heavy for the relay contacts which have had to be replaced once during the work. It would probably be advantageous to introduce a micro-switch to carry the heater load, the primary relay operating the switch.

Power.

Initially one D.C. motor powered both pump and fan. The 1/8 H.P. motor was coupled by a direct shaft drive to the pump, the shaft continuing through to drive the fan by a magnifying belt drive. The motor was old and showed signs of fatigue after a few day's continuous running. To relieve the load on the pump motor, and also to avoid the risk of failure of the driving belt, an independant universal motor, adapted from a pedal generator, was mounted on the side of the cabinet directly coupled to the fan shaft, fig. (15). On the D.C. supply this fan motor had to be run with a resistance in series, but is now run directly across the main A.C. supply. When the conversion to A.C. was complete a new 1/4 H.P. motor geared to 160 rev. per min. was installed to drive the pump, (fig. (3). The fan motor has now been running almost continuously for two years, and the pump motor for over 200 days without any sign of failure.

Pump and Valves.

The pump used to circulate the air is the standard commercial form of Dale-Schnuster membrane pump of continuously variable stroke, originally devised as a perfusion pump, (Dale and Schnuster, 1928). Its use for the present purpose was suggested by Dewar and Newton, and has the great advantage of, ideally, having no source of leaks. As explained later (p.100) the ventilation rate necessary to maintain a suitable atmosphere for an animal as large as a rat forced me to operate this pump at almost its maximum stroke. With almost continuous running for days at a time this creates the risk of development of small leaks or frank rupture of the rubber membrane. As far as could be detected such faults never developed, but to reduce the risk, during the period when the pump was running at or near full capacity, the membrane was replaced after every ten days running time.

This pump produces pressure fluctuations amounting, at full stroke, to about ±2 cm. water. The effect of this on the design of the water bottles has already been mentioned. It has been assumed that this pressure fluctuation has a negligible effect on the experimental animal. When taking a reading on the spirometer it is necessary to ensure that the pump is always at the same phase of its stroke, otherwise the pressure fluctuations can introduce an error of 30 ml.

Only the pump proper of the Dale-Schuster apparatus was utilised, the standard valve system was discarded. The valve system consists of a pair of Bunsen valves made from bicycle valve tubing mounted in a glass Y-piece as shown in fig. (14). This Y-piece is connected by glass and rubber tubing, sealed with 'Bostik', to the head of the pump. A further valve of the same type is set in the duct system on the outflow side of the chamber, after the entrance of the oxygen inlet from the spirometer, fig. (2), to prevent backflow of air contaminated with carbon dioxide into the spirometer in the event of a pressure build-up on the proximal side of the absorbing train. The constriction of the glass ducts to the 2 mm. orifice necessary to attach the valve tubing seriously obstructs the air flow. The use of wider bore rubber and/or heavier tubing makes the Bunsen valve too insensitive or, by its liability to distort, decreases its competence.

This whole pump and valve arrangement, although it has served the present purpose, is not completely satisfactory and will have to be re-designed before further work is done with this apparatus.

Air Ducts.

The air ducts are of 7 mm. bore hard glass tubing. The instrument was designed to have as much of the duct system as possible of glass tubing and as few as possible rubber joints. The rubber joints uniting separate lengths of glass tubing are of heavy wall 6 mm. bore rubber rubing cemented to the glass with "Bostik" and tightly wired. The connections between the tubes of the absorbing train are of heavy walled 4 mm. bore rubber tubing making push friction joints with side arms of the U-tubes; repeated tests under pressure under water have shown these push friction joints to be gas-tight. The connections to each end of the absorbing train and to the inlet and outlet pipes of the animal chamber are of heavy wall 6 mm. bore rubber tube cemented and wired to the glass ducts. These also are push friction joints to the absorption train and animal chamber, but as the rubber tube is of a rather wide bore the push joints are reinforced with tight rubber bands. In the glass-rubber-glass connections the two ends of glass almost touch within the rubber sheath, so that a very small area of rubber is left exposed to diffusive losses; there was still enough flexibility for the manipulations necessary in assembly of the apparatus. The possibility of diffusive losses of carbon dioxide through rubber surfaces has frequently given concern to those engaged in the measurement of respiratory exchange. On the basis of Haldane gas analyses of the air in different parts of the system (p. 119) I hold that any such effect in this instrument is imperceptible, and the major part of any such loss would occur through the pump diaphragm. I considered the possibility of using butyl rubber (butadiene-iso butylene co-polymer) membranes and tubing, but although this elastomer has a much lower gas diffusion constant than natural rubber, it is now difficult to obtain and has a lower elasticity which would reduce the efficiency of the push friction joints.

The whole of the glass duct system is mounted along the walls of the cabinet in "Terry" clips. Buffers of rubber tubing between the glass and the clips prevent any vibration noise from this source and also protect the glass from the possibility of vibration fracture.

The ducts from each spirometer pass to a three-way glass tap, T_1, from which the remaining branch passes to the oxygen drying train. By this tap either or both of the spirometers can be brought into the circuit at any time. The duct from the drying tubes passes to two three-way taps set in series. Of the three branches of the first or inlet tap, T_2, one passes from the drying tubes, one is free and through it oxygen from a cylinder can be passed to fill the spirometers, and one passes directly to a branch of the second tap, T_3. Of the ducts from the remaining branches of T_3, the manometer tap, one passes to a water manometer of 7 mm. bore glass tube, and the other to the oxygen inlet to the chamber circuit. These taps are all set on the outside of the cabinet with their branches passing through the cork-filled double wall in bushes of rubber tubing. In this way a change in the spirometer in circuit or refilling of a spirometer can be completed without opening the cabinet and so disturbing the temperature. The tap circuit can be seen in fig. (2) and the constructional arrangement in fig. (15).

Absorbing Trains.

Set in the air outflow from the animal chamber is a series of five 6 in. x $\frac{3}{4}$ in. U-tubes with side-arms charged with absorbent materials to absorb the water and carbon dioxide from the expired air of the animal. Also, set in the air duct between the spirometer and the oxygen inlet valve, is a series of two similar U-tubes carrying a water absorbent so that the oxygen passing into the main system is completely dry, fig. (2).

These absorbing trains are mounted in wooden boxes 9 in. x $5\frac{1}{2}$ in. x 5 in. and $4\frac{1}{2}$ in. x $5\frac{1}{2}$ in. x 5 in. respectively. The U-tubes are held in the boxes by large "Terry" clips, one on the bottom and one on the side of the box. Both boxes are screwed to the front floor of the cabinet, the larger on the extreme left and the smaller on the extreme right as shown in fig. (4). The frontal position allows easy access to the tubes for removal and replacement, both at the end of the day's run and during the course of a run. These positions also leave the greater part of the front of the cabinet unobstructed for manipulation of the chambers and recording apparatus.

In the main absorbing train the first pair of tubes carry the water absorbing material, the second pair the carbon dioxide absorbent, and the last tube more water absorbent to pick up any water carried from the carbon dioxide absorbers. Since the air is being continuously circulated it is not of great importance that all the carbon dioxide be picked up from the air in one passage through the absorbers; in fact, Haldane gas analysis of theemerging air shows that all the carbon dioxide is absorbed in one passage. It is of great importance, however, that the air be completely dried before reaching the carbon dioxide absorbing tubes, and that no water be carried beyond the terminal guard tube; any such transposition of water would completely invalidate the determination of the weight of water and of carbon dioxide absorbed. I satisfied myself, by running trials with three water absorbing tubes in series, that no water normally passed the second tube, the third tube showed no weight change, and the water absorbing train was limited to the two tubes. Similarly an additional water absorption tube inserted after the guard tube showed no weight change.

The water absorbent used exclusively in the earlier part of the work was granulated anhydrous calcium chloride, 10-14 mesh. Throughout the entire work this material was used in the first tube to absorb the greater part of the water vapour, but it was found sometimes to be inefficient in holding water when used in the second tube and in the guard tube. It is believed that magnesium perchlorate is a more efficient absorber and holder of water, but I found great difficulty in obtaining this material. When, eventually, some magnesium perchlorate was obtain (Anhydrone - Hopkins and Williams) it was used always for the second and fifth tubes of the train and in the spirometer oxygen drying tubes. As, however, Anhydrone is a very expensive material I continued to use calcium chloride in the first tube of the train for gross water absorption. At the end of a day's run the first tube usually carries between 90 and 95 per cent. of the total water absorbed.

The second tube and the guard tube were each required to absorb about 1 g. of water per diem. To economise in material a pair of each of these tubes was kept charged and were used on alternate days, four or five times before emptying and recharging them. This procedure uses only one third of the absorbing power of a charged tube, so leaving an adequate safety margin. The validity of this procedure was tested by inserting additional freshly charged tubes after the part-used tubes, and proved to be perfectly safe as long as the part-used tubes are always inserted with the same pole towards the air stream. If the orientation is reversed as much as 0.2 g. of water can be carried out of the used tubes by the very dry air stream passing through the partly exhausted absorbent. One side arm of each tube used in these positions was carefully marked with glass ink so that a consistent orientation could be maintained. In the early part of the work the necessity for this precaution was not appreciated, and on a few occasions the weight of the terminal guard tube was less at the end of a run than at the beginning. At this stage of the work, however, the guard tube was the only part of the train being used repeatedly. Where this water transposition has occurred the relevant parts of the tables have been marked to indicate that a correction, based on the average weight of water retained by the guard tube, has been applied to the measured weights of water and carbon dioxide absorbed.

A major problem encountered with the water absorbing train was the tendency of the contents of the first tube to clog. The calcium chloride at the proximal pole of the train becomes liquid on full saturation with water and may effectively seal the train, stopping any further air-flow. Dispersion of short capillary tubes throughout the calcium chloride, as suggested by Dewar and Newton, did not, I found, give effective protection against this interruption of the air flow as they also became clogged with the semi-fluid mass. This hazard never makes its appearance until the second half of a 24 hr. run, so that with each run beginning at about 10.00 or 11.00 a.m. blockages tended to occur in the early morning. Three experimental animals were asphyxiated in this way due to blockage occurring at about 3.00 a.m., and on several occasions animals had to be hastily rescued from asphyxia after blockages occurring at about 6.00 a.m. On all these occasions, even when the animal was rescued in time, that day's run was vitiated and so, frequently, was the greater part of a series, as the animal took one or two days to recover from the high carbon dioxide tension to which it had been subjected.

For a short period the problem was circumvented by replacing the first water absorbing tube after 12 hr. This method doubles the consumption of calcium chloride and over any considerable period would have greatly increased the monetary cost of the work.

I then decided that the use of fairly wide bore tubes (2.5 mm. internal diamter) about 1-2 in. in length, dispersed regularly throughout the absorbent, each one overlapping the next by about half its length, might ensure a continuous free passage for the air-stream and still allow efficient absorption. This was found to be a highly successful method; the wider bore of the tubes renders them less liable than the capillary tubes to clogging, and also the greater glass surface presented allows the semi-fluid calcium chloride to form air channels down the sides of the tubes. The presence of these glass air-ways in the absorbent does not interfere with the efficiency of water absorption and no obstruction to the air circulation has occurred since this method was adopted, a total running time of about 100 days. It is unnecessary to use such precautions for the other water absorption tubes whether the absorbent is calcium chloride or anhydrone, as the amount of water absorbed by these tubes is never sufficient to cause clogging.

For the carbon dioxide absorption the absorbent of choice is soda asbestos (10-14 mesh). This has a carbon dioxide combining capacity of 44 g. per 100 g. absorbent compared with only 6 g. per 100 g. for soda lime. This enables the whole daily carbon dioxide production by a 250-300 g. rat (16 g.) to be absorbed by two freshly charged U-tubes, if the material is fully utilised.

The soda asbestos must be slightly moistened before it is an effective absorbent for carbon dioxide. In the first trials a small nasal atomiser was used for this purpose, but it was found that breathing gently two or three times on the material spread on a sheet of paper supplied adequate moisture whereas the atomiser tended to over-saturate the material. The breathing method must be performed with care, and, indeed, it is wise to avoid inspiring in the immediate vicinity of dry uncovered soda asbestos, as some of the dry material is a very fine powder and its deposition on the oral and tracheal mucosa is extremely uncomfortable.

The presence of this water and the production of water of reaction during the absorption creates, in this section of the absorbing train also, a tendency to clog. I never found a complete sealing of the soda asbestos tubes in this way, but the resistance to air flow can be greatly increased, reducing the ventilation rate of the instrument drastically, and producing a great rise in pressure on the output side of the pump with increased risk of leaks. The carriage of water along the train, as the proximal regions are exhausted and dried out, steadily increases the water concentration in the central and distal regions, so aggravating the situation. An equally serious effect of clogging of the soda asbestos is the great reduction in the utilisation of the material, as considerable blocks of sodden unused soda asbestos become sealed off from the air flow. As soda asbestos is also quite an expensive material it was desirable that it be utilised as fully as possible.

It seemed likely that the difficulty would be overcome if the absorbent could be dispersed in some inert medium which would prevent large groups of granules from coalescing. Fine capillary tubes, although they reduced the resistance to air flow, were ineffective in increasing the utilisation of the soda asbestos. Glass beads were a slight, but not great improvement as they tended to imbed themselves in the wet material. I decided to try dispersing the soda asbestos with glass helices of the type used for packing refluxing columns. Pyrex helices of 5 mm. diameter were used. These were highly successful in reducing resistance, prevented coalescence of large groups of granules and ensured almost complete utilisation of the absorbent. This method has been used in all the work reported here. The helices are almost completely recoverable when the exhausted soda asbestos is washed out of the U-tubes with hot water. There is, on each recovery a certain small loss of the helices, but over some 150 runs, each using about 1 ounce of helices, I have had a wastage of less than 4 ounces. The helices are freely dispersed in the soda asbestos, before the mixture is poured into the U-tubes, in the proportion of about one third helices to two thirds soda asbestos. This proportion is not, of course, critical, but error should be on the side of an increase in the fraction of helices. Too great a proportion of helices, however, means that an exhausted soda asbestos tube will have to be replaced before the end of a 24 hr. run.

The washing out both of the calcium chloride and of the soda asbestos from the U-tubes has to be done with hot water. Cold water will wash out anhydrone. The soda asbestos tubes can safely be left to be washed when time permits, but the calcium chloride has to be removed as soon as is possible after the tubes have been weighed. If left, the semi-liquid calcium chloride crystallises, expands and breaks the U-tube.

In the part of the work where the water and carbon dioxide absorbed in a day's run amounted to 18 and 20 g. respectively, it was sometimes necessary to replace exhausted tubes during the run. For this procedure a fixed routine was observed which prevented the possibility of contamination of the private atmosphere with the outside air, loss of expired air and backflow into the spirometer while pressure relations in the chamber circuit were re-equilibrating. The entire process took less than 1 minute.

The detailed sequence of this routine is given in appendix (1).

On some occasions this replacement process was done while the pressure in the chamber circuit was reduced by as much as 15-20 cm. of water. On no occasion was there any evidence of change in this pressure during the replacement process; thus the precautions against leaks appear to be quite adequate.

This replacement of exhausted tubes during a run was largely precautionary, suggested by the appearance of the train. On only one occasion were the soda asbestos tubes actually exhausted, and that was due to inefficient utilisation of the absorbent. Replacement during the run also enabled me to utilise part-used tubes, where the soda asbestos would otherwise be wasted.

Oxygen Inlet Valve.

The oxygen from the spirometer enters the main air circuit through a Bunsen valve, similar to those described above, mounted in a glass T-piece. The original position of this valve was copied from the plan of Dewar and Newton. The oxygen inlet meets the return flow of main circuit between the absorbing train and the pump as shown in fig. (1). It was found, however, that an initial oxygen withdrawal from the spirometer occurred on starting the pump, before any oxygen was used by the animal. This oxygen withdrawal also occurred when no animal was present.

The cause of this spurious oxygen consumption lay in the resistance of the absorbing train which, when the pressure throughout the circulating system was uniform except for the pressure pulses from the pump, permitted a lower air flow than the pump is capable of producing. This resistance caused the pressure in the section of the circuit between absorbing train and pump tended to fall so that oxygen passed in from the spirometer to keep this section at atmospheric pressure. Simultaneously the pressure on the outflow or chamber side of the pump rose until the basic pressure gradient across the absorbing train reached the level at which the air flow through the train was equal to the volume expelled by the pump.

This pressure build-up in the main part of the circuit meant that any leak developing there became of great quantitative importance. It also increased the liability to leaks of the chamber and of the push friction joints. In compensation, this arrangement had the advantage that a leak in the high pressure part of the system was immediately evident by the great increase in the rate of apparent oxygen usage. Indeed all the leaks present in the apparatus on its assembly were detected in this way, (p.110).

It was obvious that if the true oxygen consumption were to be accurately measured, either the spurious oxygen consumption must be accurately predicted or the pressure effec must be eliminated. To keep the advantage of rapid recognition of leaks I tried to calibrate the spurious oxygen usage with the speed and stroke of the pump. I found that the spurious oxygen usages were independent of pump speed over the speed range available. (This work was done using the resistance controlled D.C. Motor). This lack of response to pump speed suggests incompetence of the Bunsen valves at these speeds, and a better performance with this pump and valve arrangement might have been obtainable with a much lower pump speed. The spurious oxygen usage varied with the stroke of the pump, but the values showed a wide scatter due to the resistance of the absorbing train which could not be controlled precisely. (It may be noted here that at this time no dispersing material was being used in the absorbents so that the resistance of the train and hence the pressure build-up effects were probably magnified.) It was then obvious that, because of this variable resistance of the train, it would not be possible to derive a true oxygen consumption from the apparent consumption with any degree of accuracy.

As the values found in the pressure-pump calibration were never utilised in the experimental work they are not given in detail here, but to illustrate the magnitude of this effect the mean values for 'spurious' volume of oxygen are plotted against pump-stroke in fig. (16). Each point plotted is the mean of 3 to 6 measurements.

The irregularity of the graph points is largely due to variation in the resistance of the absorbing train.

Since the oxygen error could not be predicted it had to be eliminated. This was done by moving the oxygen inlet valve to a point in the system between the pump and the animal chamber, that is on the outflow side of the pump as shown in fig. (2). The pressure gradient across the absorbing train is now achieved by lowering the pressure in a length of duct of very small volume (50 ml.). The air removed therefrom is transferred to the other side of the pump, but being buffered by the large volume of the animal chamber produces there a negligible pressure change. Any tendency for back flow of air into the oxygen inlet at the beginning of a run is avoided by the procedure described in appendix (1). This position of the oxygen inlet valve has been maintained through the whole of the experimental work reported here.

The observations made in the attempt to calibrate the pressure effect enabled the volume of the main air circuit, with the animal chamber in position, to be calculated. Other observations were made, before the valve position was changed, to utilise the pressure effect to find the "ventilation rate" of the apparatus.

All the work to calibrate the pressure effect was done with an aneroid barometer, divided in mm. Hg, In the otherwise empty animal chamber. In this way the spurious oxygen consumption could be related to the pressure rise in the system (excluding the ducts between absorbing train and pump). The initial pressure in the animal chamber and the initial spirometer reading were recorded; the pump was turned on until the pressure in the chamber was constant; the final pressure and the final spirometer reading were recorded. If V is the volume of the system, v the volume of oxygen used, P the initial pressure and p the pressure rise, then:-

$$V = \frac{P.v}{p}$$

From 14 such estimations the volume of the system was found to be 7.12 litres.

A similar estimation of the total volume of the system was repeated in a slightly different fashion during the experimental work. The change in pressure shown by the water manometer, fig. (2), during the first 15 minutes of a run, during temperature equilibration, was related to the change in the spirometer reading immediately the spirometer is tapped into the circuit. From 8 estimations a mean value for total volume of 6.6 litres was obtained. The apparently lower volume found in this way is explained by the chamber contents, animal, etc., which were not present during the earlier estimation.

To estimate the "ventilation rate" with the oxygen inlet valve in its original position, as in fig. (1), a Bunsen valve was fitted to the chamber supply duct and the air inlet to the chamber was sealed. In this way the pump delivered its air load, through a non-return valve, to the external air, while the only source of gas to the pump was the oxygen from the spirometer. The rate of removal of oxygen from the spirometer could thus be taken as a measure of the "ventilation rate" of the pump.

Pump speed, 160 or 240 r.p.m., appeared to have no effect at strokes below 5 mm.; at strokes of 5 mm. and above, the highest pump speed produced increases in "ventilation rate" of from 1 to 5 per cent. over the lowest speed. These effects of pump speed were so slight and so variable that all the "ventilation rates" estimated have been combined, irrespective of pump speed, and plotted against stroke in fig. (17). The lack of effect of pump speed is due to incompetence of the valves.

It can be seen from this graph that the maximum ventilation rate achieved, at a stroke of 15 mm., is about 1.2 litre per min., but that at a stroke of 10 mm. a ventilation rate of 0.9-1.0 litres per min. is achieved. The slight irregularities in the graph are probably due to incompetence of the Bunsen valves.

It has been one of my constant anxieties throughout this work that leaks might develop in the pump membrane, where they would be extremely difficult to detect in practice, or that the membrane might rupture. To minimise this risk it was decided to limit the stroke of the pump to 8 mm. and accept a "ventilation rate" of 0.8 litre per min. I later found (p.102) that this "ventilation rate" was inadequate to deal with water vapour production and increased the stroke to 10 mm; in the last stages of the work the pump stroke had to be increased to 13 mm. to cope with the increased carbon dioxide production during pregnancy.

It should be noted that the "ventilation rate" as measured above is the maximum ventilation ability of the pump and valve system. This takes no account of reduction in ventilation rate due to the resistance of the absorbing train. This reduction is probably variable, but that it is not great can be seen by estimation of the working ventilation rate by application of the Fick principle to the carbon dioxide flow in the apparatus. One analysis of the air entering and leaving the absorbing train, table (26), appendix (2) showed carbon dioxide concentrations of 0.6 and 0.0 per cent. This means that for every litre of carbon dioxide absorbed, 167 litres are passed through the absorbing train. Since the carbon dioxide picked up by the train during this run was 11 g. or 5.5 l., this implies a daily ventilation rate of about

900 litres. The ventilation rate estimated in this way is in substantial agreement with the value of 1100-1200 litres calculated from fig. (17) at a pump stroke of 8 mm.

It can be seen from comparison of the above calculation with the general weights of carbon dioxide produced, table (33) that the ventilation achieved in this instrument lies at about the minimum which can be tolerated. Weights of 20 g. of carbon dioxide per diem have been observed several times during pregnancy. When the weight of carbon dioxide was seen to rise above 16 g. the pump stroke was increased to 13 mm. giving a maximum ventilation rate of 1.2 litres per min. At the maximum metabolic rates observed with a ventilation rate of 1.2 litres per min. the expected carbon dioxide concentration in the outflow air from the chamber, which can be taken as equal to the average carbon dioxide concentration in the animal chamber, is 0.65 per cent.

Such a carbon dioxide concentration in the inspired air is still within the tolerance range for man (Haldane and Priestly, 1905), although Gray (1950) states that an appreciable increase in respiration rate is detectable at a carbon dioxide concentration of 1.0 per cent. It has been commonly assumed in animal metabolism investigations that a carbon dioxide concentration of up to 1.0 per cent. has no appreciable effect on respiration (Benedict, 1938: Brody, 1945) and Benedict even goes so far as to describe

0.7 per cent. as an optimum concentration. The reason for this description is not clear, unless it be that, as he was using an open circuit apparatus and deriving carbon dioxide and oxygen consumption from Haldane analysis of samples of the outflowing air, this concentration of carbon dioxide is suitable from the point of view of analytical precision. It is, of course, also true that since 0.7 per cent. is about the usual concentration in these investigations it may be regarded as being the optimum in the sense of being standard.

Although on grounds of precedent the carbon dioxide concentrations occurring in this work all lie within the accepted tolerance limits, I am not satisfied that the precedents are justified. Very little work has been done on the effect of concentration of carbon dioxide of the order of 0.1-1.0 per cent., largely due to the difficulties of maintaining such concentrations for any considerable period.

That the apparatus has been, in fact, operating rather below the lower limit of tolerance for ventilation rate can be seen, in spite of the acceptable carbon dioxide concentrations, in its inability to absorb water vapour at a sufficiently high rate when the pump was running at 8 mm. stroke. This problem of instrumental water loss is considered in greater detail elsewhere (p.158), but it can be noted here than an average water vapour pick-up of 16 g. per diem at 23°C. and with a ventilation rate of 1000 litres per diem implies a saturation of the air coming into the absorbing train of 0.016 g. water per litre or a relative humidity of 80 per cent. This humidity was confirmed by direct estimation from wet and dry bulb thermometer readings.

The possibility of the occurrence of such high carbon dioxide concentrations and humidities was not appreciated when the initial tests of the apparatus were made. When it came to light in the scrutiny of the early experimental records it was apparent that the situation could be radically improved only by complete re-design of the pumping system. Operation of two Dale-Schuster pumps in parallel would increase the pressure pulse to too great an extent, while their operation in opposed phase would, in any case, require improvement in the competence of the valves to be effective in increasing the "ventilation rate".

As the existing ventilation capacity of the pump does not allow any serious distortion of the normal atmosphere, according to accepted standards, I continued and completed the series of experiments reported here without altering the apparatus.

Continuous Oxygen Recording.

Early in the course of the work I decided to record the oxygen consumption continuously. This record is made by a tracing point, fixed to the roof of the spirometer bell, bearing on a revolving drum.

The revolving drum used consists of two standard 6 in. kymograph drums bolted together in alignment, and mounted on the spindle of a standard Palmer continuously-variable speed kymograph. The continuous variation control was set permanently at such a position that the drum rotated approximately once in 8 hr. Trials were made using an inked writing point to record the movement of the spirometer bell, but with the rotation speed used (1 mm. per min.) slow drying inks spread badly on standard kymograph paper. A stock of 12 in. kymograph paper was available in this department and I decided to use this, smoked in the normal way, rather than get special paper more suitable for an inked pen.

Each paper was prepared, before placing the kymograph in position, by graduating it with a series of lines at 1 cm. intervals. The graduations were made with a celluloid writing point in the form of a cursor which travels on a short vertical length of metre stick. The metre stick is clamped on a standard muscle lever stand so that the writing point can be easily apposed to the rotating drum. For this purpose the drum is set in top gear but the fine adjustment is not altered. A general view of the graduating apparatus is shown in fig. (18). These graduations are not, of course, very accurately made. Their primary purpose was to enable the trend of oxygen consumption to be plainly evident at any time during the course of a run so that any abnormality, such as might develop from a leak or a blockage, could be immediately seen.

The spirometer writing point is of celluloid, cemented to the end of a 25 cm. straw muscle-twitch lever. The other end of the lever is cemented to the central suspending eye of the spirometer bell and is anchored to the edge of the bell by Chatterton's compound.

The spirometer bell tended to rotate on its suspension, so that the writing point drifted off the revolving drum. This was overcome by suspending a plumb line from the roof of the cabinet, the line adjusted to bear the writing point gently against the drum. The plumb line hangs from a roller runner running on a 6 in. section of H piece curtain rail screwed to the roof of the cabinet, fig. (4). In this way the plumb line is easily adjustable to the slightly different positions of the drum each time it is installed, and is also easily removed when the drum is to be withdrawn.

The maximum volume of oxygen supplied by the fully charged spirometer is 4.8 litres. (See calibration curve of spirometer I, fig. 12). With the writing arm attached, the bell can only drop to a scale reading of 1.8 cm. and the maximum delivery by the spirometer is 4.6 litres. The daily oxygen requirement of the animals used in this investigation was 6 to 10 litres so that the bell had to be recharged one or more times during the 24 hr. One such recharging had to be made each night at such at time that a supply of oxygen was available, adequate to meet requirements until the next morning. The rate of oxygen consumption usually increased during the night, sometimes by more than 10 per cent., so allowance had to be made for this. For non-pregnant rats I found that, as a rule, the spirometer recharged at 11.00 p.m. would survive until 9.00 or 10.00 a.m. the following morning. During the latter days of some pregnancies, however, the spirometer had to be recharged at 12.00 p.m. and again by 8.30 a.m. When the spirometer was re-charged it had to be equilibrated to the cabinet temperature for 15 min., and then a further 15 min. observation period was required to ensure that all was running smoothly; I found a tendency for blockages to occur shortly after filling the spirometer, due to the fall in air pressure in the chamber circuit during temperature equilibration. Again a standard routine was adopted for re-charging the spirometer, to avoid leakage and to obtain valid and comparable measures of the volume of the empty and full spirometer. The routine adopted is presented in detail in appendix (1).

For the first 15 runs after first introducing this continuous recording system I omitted to record the temperature and pressure at the times of re-charging the spirometer. The error introduced by this omission into the estimations of oxygen consumption will depend on the deviations of the temperature and pressure at these times from those recorded at the beginning and end of the run. In these cases I have assumed the intermediate temperatures and pressures to be linearly interpolable, in time, between the initial and final readings. Estimation of the oxygen consumption in a series of runs in which these intermediate readings were made, both by using the intermediate readings and by interpolation showed the error involved to be less than 1 per cent., the mean difference being 0.3 per cent.

It can be seen from fig. (19), which shows the detail of a typical trace where the spirometer is newly re-charged, that the first response is a slight, fairly rapid fall of the spirometer followed by a slow rise which has effectively levelled off by the end of the equilibration period. The initial fall I believe to be due to the extension of the nylon suspension under the increased load of the bell, while the slow rise is due to the warming of the oxygen in the bell to cabinet temperature. It can also be seen that after 15 min. the equilibration was complete, the trace having straightened to a horizontal line. This was fortunate, since in the high oxygen consumption runs the manometer capacity (20 cm. H_2O) was completely absorbed by the end of the 15 min. period.

No direct time record was made upon the drum, the spiral nature of the trace made it difficult to introduce. The constancy of rotation speed of the drum was checked initially by manual time marking at 15 min. intervals over a period of 8 hr. A general time check was available for all runs by the recording of the time at the points of recharging the spirometer and replacing exhausted absorption tubes, and these times were included in the annotation of the traces. All times were read from my watch, which was not reset within the course of any consecutive series of runs. The time to distance interval relations were checked on all traces by direct measurement and no appreciable inconsistency was found.

The minor variations and irregularites which occur in the traces are due to the slight temperature changes in the cabinet permitted by the thermostatic control. It is a weakness of the apparatus, in its present form, that the spirometer bell is more sensitive to temperature change than the recording thermometer. A reduced photographic reproduction of the detail of a trace made when the cabinet had to be opened intermittently, so altering the cabinet temperature, is shown in fig. (20). Figs. (21a) and (21b) show traces illustrating the effect of blockages of the absorbing train and the subsequent recovery when the blocked tube is replaced.

METHODS.

Test Methods for Respiration Calorimeter.

The air duct system was assembled in sections. Before assembly each section was tested for leaks by raising the internal pressure under water with a hand bellows. The only leak observed in this way was a pin-hole in a glass T piece.

On one occasion, when the apparatus was completely assembled and the pump first started a major leak was immediately apparent from the fall in the spirometer scale reading (p. 95). This was eventually found to be due to a further pin-hole in the side arm of one of the U-tubes of the absorbing train. Thereafter all U-tubes were tested under water before first use.

The standard method oftesting indirect calorimeters is that of the alcohol check. This method has been used successfully in all the larger types of calorimeter but in a small instrument such as this the method is extremely difficult to apply with any success. The alcohol checks which I have made on this instrument were, at best, only partially successful and never satisfactory.

The main difficulty lies in the fact that even the smallest viable alcohol flame has a combustion rate very much higher than the metabolic rate of a rat. The smallest flame which I was able to maintain, which could only just be detected by the eye in the dark animal chamber, burned at two and a half times the maximum animal metabolic rate observed. Such a combustion rate demands a far greater ventilation rate than the apparatus is capable of (p. 99).

An associated obstacle is that with this high combustion rate the atmosphere in the animal chamber, which carries the alcohol burner, is raised to a temperature much higher than normal, (under the best conditions 32°C.). This raised temperature has a number of effects.

(a) The pressure in the chamber circuit is raised, and no oxygen can pass into the system until sufficient carbon dioxide has been produced and absorbed to lower the pressure below atmospheric. This takes about 20 min., at the end of which time the oxygen tension has dropped to such a low level that the flame is in danger of expiring, and usually does. If the flame survives it has to continue burning at the low oxygen tension and seldom survives for more than half an hour.

(b) The high temperature of the chamber atmosphere combined with the relatively low ventilation rate produces a high degree of water condensation on the relatively cool walls of the chamber and air ducts.

(c) A long time, 1 - 2 hr., has to be allowed after extinction of the flame for the chamber to regain temperature equilibrium. During this period the precipitated water is being picked up, but there is now the risk of super-drying, Dewar and Newton (1948a). More important, however, is the continuous evaporation of alcohol that proceeds after the flame is extinguished. I have never been able to get a flame to survive until all the alcohol in the burner was used. Also, the burner has invariably had to be re-ignited several times in the course of a check. The residue, or part of the residue of the alcohol evaporates rapidly in the raised temperature of the chamber. Some, but not all of the evaporated alcohol is picked up by the water absorbers and is inextricably confounded with the estimation of water of combustion. Some of the evaporated alcohol is condensed on the sides of the chamber and ducts and saturates the air.

Several types of burner were used but the most successful consisted of a small cylindrical can with a close fitting lid. The lid has a central 1 mm. hole through which is passed a fine cotton wick, and a peripheral ½ mm. hole to act as an air vent.

The burner was set on a wire gauze at the bottom of the cylindrical portion of the animal chamber. It was ignited by a platinum coil raised to red heat by an electric current supplied, in the first checks, by lead accumulators, but later by the ignition unit constructed for the bomb calorimeter (p. 126). Several attempts were made to make satisfactory gas-tight inlets for the ignition leads through the chamber thermometer opening, but finally two terminals, shown in fig. (4), were set permanently in the roof of one chamber. To these terminals could be attached heavy brass wires adjusted in length so that they held the platinum coil just above the burner wick. It was hoped that the platinum would help to catalyse the combustion of the alcohol as suggested by Goodman and Gustavson (1947). The heater coil could have been kept active during the whole check in an effort to ensure complete combustion of the alcohol, but this raised the internal temperature of the chamber to such an extent that the previously mentioned difficulties were aggravated and the perspex window of the chamber was in danger of being badly distorted.

The following measures were tried in an effort to overcome the obstacles to this type of check:-

(a) The atmosphere in the chamber was enriched with oxygen in an effort to raise the lower limit of oxygen tension. The effect of this is to make flame burn more fiercely at the beginning and so raise the internal temperature still higher. On two occasions when the oxygen enrichment was too great the apparatus blew up! This method was abandoned.

(b) An attempt was made to construct a curve of the evaporation rate of alcohol from the unlit burner at different temperatures, in the hope that it might be possible in this way, to correct for alcohol evaporation. No satisfactory curve could be obtained.

(c) The burner was ignited before the push junction on the chamber was closed. The interval before sealing the system was about 10 sec. which gave time for the greater part of the temperature rise without the risk of any great loss of carbon dioxide. This device enabled the flame to survive for a longer time. It meant, however, that the pump had to be stopped when the chamber temperature fell to the level at which the chamber had been sealed. This did not give time for all the water to be picked up. Also, the thermometer, over the short initial interval, cannot be considered to give a very reliable indication of the mean temperature in the animal chamber at the instant of sealing. This method also was abandoned.

(d) The empty urine flask attached to the chamber during the alcohol checks was surrounded by ice water to reduce the pressure rise when the flame was ignited, and so reduce the degree of 'anoxia' to which the flame was subjected. This produced some improvement and I was able in this way to keep a flame alive, with only one or two re-ignitions, for 1½ to 2 hr.

(e) Various procedures were attempted to attain the same degree of dehydration of the apparatus at the beginning and end of the check. A modification of the method recommended by Dewar and Newton was used finally. In this a piece of cobalt chloride paper was inserted in the outlet tube from the animal chamber. The apparatus was assembled with the burner and ignition coil in position, the pump was turned on and allowed to run until the cobalt chloride nearest the chamber turned bright blue. The pump was then stopped, a fresh weighed absorbing train was set in position, the flame ignited and the pump re-started. After the flame was extinguished the pump was allowed to run until the cobalt chloride paper regained its pre-ignition appearance. By that time the temperature of the chamber had returned to its pre-ignition level.

On no occasion, however, was I able to get satisfactory results for the water production and alcohol used in these alcohol checks. Great error was produced in both of these by the evaporation of the alcohol. The oxygen consumption and carbon dioxide production, however, matched to give a very close approximation to the "respiratory quotient" of the alcohol. This has been accepted, in conjunction with the other tests described below, as being a satisfactory measure of the accuracy of the apparatus. The oxygen consumption, carbon dioxide production and R.Q. for the alcohol checks done, using ethanol, before the instrument was used for animal experiments are given in table (25), appendix (2).

At a much later stage of the work a further attempt was made to do more satisfactory alcohol checks. In this case methanol was burned in the hope that its combustion would be more effectively catalysed by the platinum coil. Again accurate relation between oxygen consumption and carbon dioxide production was obtained, but methanol consumption and water production values were completely vitiated by the evaporation of unburned methanol. The results of these tests are also given in table (25).

After these methanol tests I omitted to sweep all the residual methanol vapour out of the apparatus before continuing with the animal experiments. This caused methanol poisoning of the rat being studied at the time (female 524). As soon as I realised the cause of the very abnormal metabolic pattern which appeared (described later p.241), I took the animal off experiment and left the pump running with the apparatus on open circuit, that is with no absorbing train, for 24 hr. This cleared the instrument of methanol vapour.

Alcohol checks were found so difficult to make and gave such unsatisfactory results that I abandoned the idea of using them as a routine method of test. In their place two other indirect methods of test were used.

The more important routine test, made before the beginning of any experimental series, was a simple pressure test. An aneroid barometer was placed in the animal chamber and the apparatus set up in the usual way. The oxygen intake was cut off by tap T_3, cf fig (2), and the chamber circuit was blown up by a hand bellows to a pressure of about 5 cm. Hg above atmospheric; this is a very much higher pressure than the apparatus has normally to withstand. The pump was then turned on and about 15 min. allowed for the internal pressure relations about the pump and absorbing train to become constant. The pressure on the aneroid barometer was then recorded and the apparatus left to run for 4 to 6 hr. The pump stroke produces a pressure fluctuation in the chamber of 1 - 2 mm. Hg, but even so it is possible to read the mean pressure to within 1 mm. Hg. A pressure fall over this period of more than 1 mm. Hg was regarded as presumptive evidence of a leak, provided that the cabinet temperature had not altered detectably. Any leak so discovered was then localised by blowing up individual sections of the duct and chamber system under water. This method of testing indicated leaks occasionally, in the early stages of the work, and then the leaks were always found to occur in the animal chamber. The reasons for these leaks and the methods of dealing with them have already been described (p. 60). All the experiments detailed in this work were bounded by pressure tests of this type, to prove a leak-free system.

The liability of the chambers to develop leaks was so great, initially, that the chambers were subjected to almost daily pressure tests under water. In this way a leak was prevented from vitiating more than one day's experimental results. No value for gaseous exchange has been included where there was any suggestion of a leak. Fortunately no leak appeared in the apparatus throughout the whole of the major experimental series (rat females 524, 525).

A further method of test, employed at less frequent intervals, was the analysis of the air in the chamber circuit by the Haldane method (Haldane, 1898). This test was used primarily to check on the carbon dioxide concentration in the circulating air at the end of a 24 hr. run. It is obvious, however, that a leak to the external atmosphere, either inwards or outwards, will produce an alteration in the percentage of oxygen in the closed atmosphere. To take a numerical example:-

When the normal oxygen consumption is 7 litres per diem a leak inwards or outwards of 70 ml. in the day would produce an error of 1 per cent. deficit or excess in the apparent oxygen consumption. A leak of 70 ml. inwards means that 70 ml. of oxygen in the original closed atmosphere has been replaced by 14 ml. oxygen and 56 ml. nitrogen. This means that the normal oxygen concentration of 20 per cent. in a total air volume of about 7 litres has been reduced to 19.2 per cent. The corresponding change due to a leak outwards, where 70 ml. of air in the normal closed atmosphere has been replaced by 70 ml. oxygen, is to an oxygen concentration of 20.8 per cent. Since the raised carbon dioxide concentration in the closed atmosphere reduces the oxygen concentration in any case, a change in atmospheric composition attributable to leaks is more easily seen in the inverse changes in the nitrogen concentration. Changes of the magnitude of 0.1 per cent. are very easily distinguished by the Haldane method of air analysis, and that would correspond to a leak error of less than 0.2 per cent. The results of a series of such analyses are given in table (26), appendix (2).

From these air analyses the oxygen concentration does appear to be about 0.5 per cent. too low. The simple analyses figures have, however, to be adjusted for the presence of inert gas impurities in the oxygen used. I have been given, by the British Oxygen Company, the average figure of 0.5 per cent. impurity, mainly argon, present in the oxygen supplied for medical purposes. When the oxygen volume taken from the spirometer is corrected for this impurity, my gas analyses all give agreement with expected concentrations within 0.1 per cent. (The recorded oxygen volume measures the true oxygen usage and is not, of course, to be corrected for impurities). I regard the agreement with the "ideal" of these air analyses as being a very satisfactory proof of the accuracy of this apparatus for the measurement of gaseous exchange.

Experimental Procedures

Respiration Calorimeter

Apart from the oxygen consumption which is measured volumetrically, all the other basic measurements in this work are gravimetric. As the most precise valid interval on the spirometer is 0.1 mm., corresponding to about 2 ml. or 3 mg., I wished, if possible to make this the upper limit of instrumental error. Loads of up to 500 g. had to be weighed rapidly but difficulty was experienced in obtaining a balance which combined adequate sensitivity with high capacity. Finally a low quality "school" balance was used with a stated sensitivity of 10 mg. and a capacity of 2 kg. The sensitivity was improved to 1 mg. by raising the centre of gravity, and the stability recovered and speed of weighing improved by use of an eddy current damping device. This instrument is shown in fig. (22).

In view of the slight variation liable to occur in the objects weighed, as a result of surface contamination and atmospheric humidity, the weighings are considered to have a precision limit of 5 mg. although for mental convenience they were recorded to 1 mg. Repeated daily weighings of an unused, filled absorbing tube showed a maximum weight variation of 4 mg.

The rat on experiment was weighed, at the beginning and end of each run, in a ventilated can. The movement of the animal made accurate weighing impossible, so that this weight is, instrumentally, the least precise of all the measurements made. By patience and experience in catching the animal in its rare periods of comparative stillness, I believe that these weighings can be considered accurate to within 20 mg.

The faeces, picked from the grid with forceps, were weighed in the same can, both fresh and dried. The dry, empty can was weighed once a day, after the removal of the dried faeces. Its day to day weight variation was never more than 2 - 3 mg.

The clean, dry animal frame and urine funnel were weighed before the beginning of each run and the day to day variations in weight were also within 2 - 3 mg. but the funnel weights showed a very gradual increase over the whole experiment, due to corrosion. This change amounted to less than 1 mg. per diem. Loose, dry, scattered food lying in the funnel and on the faeces grid of the frame was shaken off onto a clean sheet of paper before these components were weighed at the end of a run, and weighed separately as scattered food. The weight of the urine and urine contaminated food still adhering was derived from the initial and final weights of the funnel and frame.

With the exception of the dry scattered food, which was weighed directly, all the weight data from which the final results of these experiments have been computed are in terms of weight differences. Thus, the weight of carbon dioxide produced is found as the summed weights of the soda asbestos tubes and the guard tube at the end of the run less their summed weights at the beginning. This largely eliminates any error which might occur from any maladjustment of the balance. The balance was adjusted at the beginning of each series of runs, but not in the course of a series. No error of adjustment of more than 2 mg. was ever found.

Records

A uniform routine was evolved for performing the daily chores while the metabolism trials were running. This routine had to be adapted primarily to performing the daily change-over single-handed and as speedily as possible. With practice I was able to complete the actual change-over with a break of only 10 - 15 min. between stopping and starting the pump. The time required for preparing and weighing the replacement components before, and weighing the used components after the actual change-over was, of course, very much greater. During this 15 min, period the animal chamber is removed from the cabinet, opened, the rat removed weighed and replaced in a fresh chamber which is then sealed and replaced in the circuit. During this time also, the spirometer is re-filled, the exhausted absorbing train removed and fresh tubes fitted in the circuit. The change-over period was lengthened at times by any one of a number of mischances, such as the shearing of a binding bolt in clamping the lid onto the fresh chamber.

The assembly and mounting of the fresh animal chamber with animal was completed before the absorbing train was replaced. The time necessary for completion of the latter process then allowed the chamber to rise to something near its normal working temperature before it was finally sealed. This reduces the correction to the measured oxygen consumption necessary to take account of the expansion of the air in the animal chamber. After complete assembly of the apparatus the pump was started. While the apparatus was coming into temperature equilibrium, with tap T_3 fig (2), at the manometer position, the oxygen recording drum was graduated in the way already described. The drum was then set in position in the cabinet, turned on and allowed to run for about 10 min. until the trace became regularly horizontal. On the lowest gear the Palmer drum does not start to rotate until some minutes after switching on.

The details of the working routine, including the order of pre- and post-changeover work, are given in appendix (1). The order of weighing of the replacement components before, and of the used components after the changeover was designed so that those most likely to be affected by the atmosphere were weighed last or first respectively. This was especially important for the used components; the funnel and frame invariably carried some water which evaporated very rapidly when exposed to the atmosphere of the laboratory.

To simplify and accelerate the making of records a record sheet was designed and cyclostyled.

Experience showed that many events and measurements, not foreseen when the record sheet was designed, would have to be recorded. Many of the additional records were necessitated by the introduction of the oxygen recording drum. These additional records were added to the standard form with hand-written description. Reproductions of examples of the record sheet as originally designed and as finally used are given in appendix (1).

Every effort was made to perform the actual changeover at about the same time each day, and the process was timed to be subject to as little interference as possible from other calls upon my time. It was not always possible to achieve this, however, and so the time of changeover varied by as much as one hour from day to day. Pre-periods could be, and frequently were arranged to run largely during weekends to avoid interruption, but no such cut and dried arrangement could be adopted for the pregnancy periods which were entirely determined by the day of mating.

The usual time of changeover was at 11.00 am. which gave adequate time for the preparation and weighing of components. For the last 60 days of the experiment other commitments rendered a constant morning changeover very uncertain and so the entire timing was displaced 3 hr. to 2.00 p.m. This time was maintained with fair constancy throughout the remainder of the experiment. Since the period between successive changeovers was still 23 - 24 hr., and these were still performed at a constant time of the day, it is believed that the altered timing had no effect on the experimental results.

Heats of Combustion.

The heats of combustion of the food and faeces were measured by a Berthelot-Mahler bomb-calorimeter. The instrument used is of a standard type and photographs of the components of the apparatus and of the assembled instrument are given in figs. (23) and (24).

The stirring device is driven by a 1/30 H.P. electric motor. The motor drives the stirrer through a 1:10 reducing belt drive. A rheostat, supplied with the apparatus and purporting to control the motor to a suitable speed, has had to be reinforced by a 60 or 100 watt light bulb in series, (wattage according to the state of the electricity supply), as shown in fig. (24).

The sample to be burned is ignited from the main electrical supply. A simple ignition unit was made of a heater transformer, supplying 1 amp. at 12 volts. A spring return press-button switch is fitted in the primary circuit.

As a preliminary to the use of the apparatus a standard had to be fixed for the amount of water in the calorimeter vessel. The assembled bomb was placed in the calorimeter vessel. Enough tap water was added just to cover the body and cap of the bomb without over-topping the insulators of the ignition contacts. This amounted to 1886 g. water. The bomb was then removed and the calorimeter vessel with water was weighed. A lead-filled brass tube, part of an old clock weight, was adjusted exactly to balance this water filled vessel, 2573 g. In all estimations made with this instrument the calorimeter vessel with water was balanced against this standard weight on a bullion balance sensitive to 0.1 g.

The water equivalent of the whole apparatus was estimated by burning pure benzoic acid, supplied as a thermochemical standard with a heat of combustion of 6.318 Cal. per g. Enough of this material was used to give a temperature rise of about 3°C., that is, assuming a water equivalent of the apparatus of about 2000 g., about 1 g. of benzoic acid. Similarly the quantities of the food and faeces samples burned to find their heat of combustion were 1.5 to 2.0 g.

A major obstacle encountered in these estimations was due to the type of bomb, which is of the screw-cap type with sealing by a lead washer. This cap has to be screwed on very firmly or the bomb will leak to a greater or less extent when oxygen is passed in to 25 atmospheres. On the other hand, if the cap is screwed on too forcibly the lead washer is liable to have its surface torn with the development of a permanent leak. I found that by lightly smearing the outer rim of the bomb body with silicone grease, the assembled bomb was leak-free with fairly light pressure which laid no strain on the lead washer. This device did not appreciably affect the estimated heat of combustion (*vide* p. 146). All heats of combustion estimations made on the experimental material were made using this method of leak prevention.

The food samples for heat estimation were prepared satisfactorily by the standard pelleting process. The food was used in the fresh state as it was very difficult to form pellets from desiccated food. The pellets made of the dried faeces, however, were very friable and even those pellets which survived the various manipulations necessary before ignition, frequently scattered badly in the bomb during combustion. Whenever any sign of unburned material remained in the bomb at the end of an estimation the record was discarded.

Out of some 50 estimations of the heat of combustion of faeces only 10 were completely satisfactory. These gave a mean heat of combustion of 3.759 Cal. per g. with a standard deviation of 0.02 Cal., and this mean value has been applied to all measured faeces weights.

Iron fuse wire was used for firing the samples in preference to platinum, because of its cheapness. The springiness of the iron wire makes its manipulation, in preparing the pellet and threading the wire into the ignition contacts, rather more difficult, but this disadvantage is easily overcome by patience and practice. A theoretically more serious objection to iron wire lies in the fact that the iron is oxidised in the combustion with the release of heat. The iron is only partially burned, and the amount burned is variable, so strictly the iron remaining should be estimated in order to calculate exactly the correction to be applied. The error involved is so small, however, that the labour of such a correction is not justified. The heat of oxidation of iron is 1.2 Cal. per g.; the average weight of fuse wire used in one estimation is 6 mg. of which about half is burned. This gives an average excess heat per estimation due to the combustion of the iron, of 0.0012 x 3 Cal., that is about 0.003-0.004 Cal. or 0.05 per cent. of an average total heat production. By omitting this correction from the water equivalent estimations it was automatically applied to all subsequent estimations of experimental material.

The standard procedure was used in making these estimations. The details of this procedure are given in appendix (1).

Nitrogen Estimations.

The nitrogen of the urine, faeces and food was estimated by the micro-Kjehldal method. The modification of Ma and Zuazaga (1942) was used, in which the distillate is passed into a 2 per cent. solution of boric acid, and the ammoniacal boric acid solution then titrated directly with 0.01 N H_2SO_4 using methyl red-bromocresol green indicator.

Urine

The urine was collected in a 100 ml. Erlenmeyer flask containing about 10 ml. 1 N H_2SO_4. At the end of a day's run the loose uncontaminated food from the urine funnel and the faeces grid of the frame was shaken off onto a clean sheet of paper. After the funnel and frame, with adhering urine-contaminated food, had been weighed this was carefully washed into the urine flask with warm distilled water. The contents of the flask were then filtered through a rapid filter paper and the residue washed three or four times with distilled water. The filtrate was made up to 100 ml. with distilled water.

The nitrogen was estimated in duplicate in 1 ml. aliquots of this solution. Agreement between duplicates was within 0.5 per cent.

In 8 tests, made by pipetting 5 ml. of a urine of known nitrogen content into the animal chamber with food scattered on the faeces grid and funnel, 97±1 per cent. recovery was obtained. It is probable, however that the values for urinary nitrogen recorded in practice are rather low as it is to be expected that an appreciable amount of the urine is absorbed by contact with the faeces. I have assumed that the estimation of the faeces nitrogen substantially takes account of this urinary loss from the point of view of total nitrogen balance, and that the error is sufficiently small not seriously to affect the protein correction in the estimation of the energy metabolism.

Faeces

The faeces, after drying, were ground in a mortar to a fine powder. This was not perfectly homogeneous as the fibre residues retained a quite appreciable particle size. Two samples of about 50 mg. were weighed on a torsion balance accurate to 1 mg. and the nitrogen content of these was estimated. Agreement between duplicates was within 5 per cent. Variation in the nitrogen content of the faeces from day to day was as much as 10 per cent. This day to day variation I attribute largely to absorption of nitrogen from urine, but this amounts to less than 5 per cent. error in the urinary nitrogen.

Food

Food samples were ground to a fine powder in a mortar and the nitrogen content was estimated on 50 mg. samples, measured as described for the faeces. Estimations were made on three samples from each food batch, and were made on the undried food. The nitrogen content was expressed in terms of dry food by applying the correction for moisture content appropriate to the food batch. Agreement between duplicates for these estimations was again within 5 per cent.

For more accurate estimation of the nitrogen content of the food and faeces a macro- method should have been used but facilities were not available for digesting the large number of samples involved. The collection of these data was intended to detect any gross change in the pattern of the nitrogen balance, and I believe that the precision achieved is adequate for this end.

These nitrogen data have been reported only for the major series, females 524 and 525. In the earlier work the urinary nitrogen values were fallaciously low, due to imperfect recovery from funnel and frame, and have been discarded.

Computation of Data.

The extraction of useful data from the raw records of the metabolic experiments entailed some lengthy, although usually straightforward calculation. These processes are described in some detail as they are, in some cases, a little involved and implicate the assumption of basic data which have not been experimentally confirmed here.

Oxygen.

It can be seen, figs. (12) and (13), that the spirometer calibration curves are effectively linear. The curve for Spirometer 2 departs from linearity at the lower end of the scale, but, in practice that part of the scale of that spirometer was never used. The straight line of best fit was applied to the curves by inspection with a straight edge and the equations to these for Spirometers 1 and 2 respectively are:-

(1) Volume O_2 in litres $= \dfrac{(\text{Initial scale - final scale})}{5.111}$ cm.

(2) Volume O_2 in litres $= \dfrac{(\text{Initial scale - final scale})}{5.075}$ cm.

Comparison of a series of volumes obtained by direct reading from the curves with those from the same scale readings calculated from the above expressions gives a mean error for the latter of +2.3 ml. with a maximum error of 15 ml. This is a maximum error less than the graduated precision of the spirometer scale.

To reduce the oxygen volumes to S.T.P. the most convenient method is to utilise the nomogram given by Weir (1949). In a copy of this nomogram 0.002 divisions were inserted by linear interpolation in the correction factor scale, fig. (25). This ensures that the reading precision of the correction factor "contains" the reading precision of the cabinet thermometer, - 0.3°C. The error in reading the correction factor with these interpolated graduations implies a maximum error in oxygen volume of 10 ml. that is 0.2 per cent on a full spirometer scale change.

A difficulty in the reduction of the scale readings to oxygen volumes at S.T.P. is introduced by the uncalibrated residual volume of the spirometer, along with the volume of the ducts leading to the tap, T_3.

If p and q be taken as the correction factors to be applied at the beginning and end, respectively, of one spirometer delivery, then:-

$$\text{Vol.}_{\text{S.T.P.}} \text{ of } O_2 = p \times \text{total initial vol.} - q \times \text{total final vol.} \qquad (1)$$

Letting x_1 and x_2 be the initial and final scale readings, letting the unknown "dead-space" volume by y litres, taking the lower limit of the spirometer calibration curve as 1 cm. and assuming the linear relation discussed above, then equation (1) becomes:-

$$\text{Vol.}_{\text{S.T.P.}} = p\left(\frac{x_1 - 1}{5.11} + y\right) - q\left(\frac{x_2 - 1}{5.11} + y\right) \text{ litres.} \qquad (2)$$

This can be rearranged to give:-

$$\text{Vol.}_{\text{S.T.P.}} = \frac{px_1}{5.11} - \frac{qx_2}{5.11} + (p - q)\left(y - \frac{1}{5.11}\right) \text{ litres.} \qquad (3)$$

When the two correction factors are equal this reduces to:-

$$\text{Vol.}_{\text{S.T.P.}} = \frac{p}{5.11}(x_1 - x_2) \text{ litres.} \qquad (4)$$

Precise determination of the dead space was not feasible, since it includes a considerable length of glass piping. An adequate measure of this volume was, however, derived from direct measurement of the linear dimensions of the component volumes. This method gives a dead space volume of about 650 ml. for both spirometers. Substituting this value for y in equation (3) above gives the following working equation for Spirometer 1;-

$$\begin{aligned} \text{Vol.}_{\text{S.T.P.}} &= px_1/5.11 - qx_2/5.11 + 0.454\,(p - q) \\ &= 1/5.11\,(px_1 - qx_2) + 0.454(p - q) \qquad (5) \end{aligned}$$

A similar equation can be derived for Spirometer 2:-

$$\begin{aligned} \text{Vol.}_{\text{S.T.P.}} &= px_1/5.075 - qx_2/5.075 + 0.453\,(p - q) \\ &= 1/5.075(px_1 - qx_2) + 0.453\,(p - q) \qquad (5a) \end{aligned}$$

It is necessary to introduce this correction for the dead space volume of the spirometer, for a change in atmospheric pressure of 2 cm. Hg over the period of one spirometer delivery is quite common. Ignoring the above correction in such an instance would introduce an error of about 40 ml. into the estimate of oxygen volume.

To this basic measure of oxygen consumption several corrections have to be applied:-

(1) The expansion of air in the chamber between the time of sealing the circuit and the time when the chamber attains its final running temperature, has to be considered as oxygen usage since it would appear as such if the final chamber temperature were allowed to fall to the starting level. The temperature difference producing this expansion is the difference between the chamber temperature at the instant of sealing the circuit and the temperature at the instant of breaking the circuit at the end of the run. The initial volume which is expanded is the air volume in the chamber. This has been taken throughout as 6.5 litres, being the approximate volume of the chamber (p. 98) less the estimated volume of the rat, food, water bottle and other contents.
The correction to be applied to the oxygen volume as measured at cabinet temperature and pressure is therefore:-
(Final chamber temperature - Initial chamber temperature) x 6.5/273 litres.

$$= 0.0238(T_2 - T_1) \text{ litres.}$$

To reduce this volume to S.T.P. a standard correction factor has been used, 0.9. The use of a constant factor here instead of a more complicated expression involving the true initial and final correction factors allows a maximum error in this correction of 1-2 ml. Introducing the correction factor into the above expression gives a total expansion correction of:- $0.0214\ (T_2 - T_1)$ litres.

(2) The evaporation of water from the spirometer tank alters the water level in the spirometer and so reduces the zero of the spirometer. The spirometer readings were made without adjusting the water level. The spirometer was re-levelled, after the final reading of a day's run was made, every third day of use, and the spirometer reading recorded before and after levelling. The increase in spirometer reading at each three-day levelling was very constant at 0.5 cm. An estimate of the error introduced due to this water evaporation is therefore given as:-

$$(0.5 \times 0.9)/(3 \times 5.11) = 30 \text{ ml. (approx.)}$$

(3) A correction, entailed by the raised carbon dioxide concentration in the apparatus, has to be applied to both the oxygen and carbon dioxide estimates. I have assumed that the carbon dioxide concentration at the end of a run is constant at 0.7 per cent. (cf. p. 101). In a total volume of 7.1 litres this amounts to some 50 ml.

Complete absorption of this by the absorbing train would extract a further 50 ml. of oxygen from the spirometer. Again a constant correction factor of 0.9 has been applied to reduce this to S.T.P., giving a total carbon dioxide correction of 45 ml. (It may be noted here that the above correction could have been ignored if the starting time of a run had been taken as the time of sealing the circuit instead of as the time of clamping the chamber (cf. p. 123). The carbon dioxide produced to raise the carbon dioxide concentration in the circuit would then be outwith the experimental period. It must also be noted, however, that such a change in technique would not increase the accuracy of the total estimate of oxygen consumption.)

Incorporating these corrections in the basic oxygen volume equations (p.136), gives the final equation used for estimation of oxygen volume. The corrections are applied only once to a day's run.

$$\text{Vol.}_{\text{STP}} = \left[\left\{ \frac{1}{5.11}(px_1 - qx_2) + 0.454(p-q) \right. \right] + 0.0238(T_2 - T_1) + 0.015 \text{ litres} \qquad (6)$$

Where the second spirometer was used the summed term of the above equation becomes the sum of expressions (5) and (5a) on p. 136

The oxygen volume extracted in the above manner is converted to oxygen weight for use in the total weight balance, by multiplication by the factor 1.429.

Carbon Dioxide.

The weight of carbon dioxide is found as the summed final weights of the soda asbestos absorbing tubes and the anhydrone guard tube less the summed initial weights of these tubes. The correction of 45 ml. or 0.093 g. CO_2, as derived above for oxygen, is added to this measured weight. The weight of carbon dioxide was converted to a volume by division by the factor 1.977.

Energy Expenditure.

To derive the energy expenditure of the animal from the measurements of oxygen and carbon dioxide, I have utilised the method described by Weir (1949). Similar but more involved methods are given by Leegaard (1931) and Abramson (1943). Weir derives the equation for total energy expenditure:-

Total Cal. = 3.941 x litres O_2 used + 1.106 x litres CO_2 produced - 2.17 x g. urinary N.

The numerical factors in this equation assume the following basic data.

	Carbohydrate	Protein	Fat
R.Q.	1.0	0.802	0.718
Cal./litre O_2	5.047	4.463	4.735

and 5.941 litres O_2 per g. urinary N.

The energy value for carbohydrate is that found by Zuntz (1897), and corresponds to a heat of combustion of 4.187 Cal. per g. which is the heat of combustion of glycogen. The energy value and R.Q. of fat are those found for mixed human fat by Cathcart and Cuthbertson (1931) modifying Zuntz and Schumberg's (1901) original values of 4.686 and 0.707 for animal fat. The energy value and R.Q. of protein are Lusk's (1928) modification of Loewy's (1911) computation of Rubner's (1894) findings for the metabolism of meat protein by the dog. The energy equivalence of urinary nitrogen is from Lusk (1928).

It is noteworthy that this particular association of numerical values involves a theoretical confusion. The values for carbohydrate and fat are based on the metabolism of body substance in the post-absorptive condition, whereas those for protein are based on the metabolism of a dietary protein. The practical error implied by this for the post-absorptive state is probably insignificant, but in the conditions of the present work, where total metabolism is measured, all energy values should be referred to the dietary component and not to the body substance.

I have not made any direct estimation of the carbohydrate distribution in the diets used, but the approximate distribution of carbohydrate estimated from the published raw material composition of the diets (pp. 42-44) is di-saccharide 10 and starch 90 per cent. The heats of combustion of these carbohydrates are 1350.8 Cal./g. Mol. and 4.1788 Cal. per g. respectively. Using the equations:-

$$\underset{\text{Lactose}}{C_{12}H_{22}O_{11}} + 12\ O_2 \longrightarrow 12\ CO_2 + 10\ H_2O + 1350.8\ \text{Cal.}$$

and

$$\underset{\text{Starch}}{\underset{100\text{ g.}}{(C_6H_{10}O_5)x}} + (6\ O_2)x \longrightarrow (6\ CO_2)_x + (5\ H_2O)_x + 417.88\ \text{Cal.}$$

it can readily be calculated that the energy equivalents of a litre of oxygen used to metabolise these carbohydrates are 5.009 Cal. and 5.037 Cal. respectively. Weighting these values according to the carbohydrate distribution in the diet gives a mean energy equivalent of oxygen for the metabolism of carbohydrate of 5.035 Cal. per litre of oxygen.

The standard constants used in the computation of the energy of metabolism of protein are those of Loewy as used by Weir. There are other estimates available for protein metabolism, Kriss and Miller (1934), Kriss and Voris (1937) for the albino rat, Rapport, (1924) for the dog, and a computation by Loewy (1911) on data recorded by Rubner (1898) and Rubner and Huebner (1898) for the metabolism of milk protein by man. They are not, however, any more valid for the present investigation, which does not use a pure protein, than are the more commonly used values.

It has been shown by Abramson and by Weir that the total energy estimate is insensitive to the protein metabolism term of the energy equation. I have therefore continued to use Loewy's constants for meat protein as being the best available for a mixed protein; these are perfectly satisfactory for comparative values within the present work, but it should be noted that the resultant absolute energy estimates may be in error. The maximum error involved, taking Kriss and Miller's casein values and Rapport's gelatin values as extremes is ± 0.5 per cent.

I made no elemental analysis of any of the dietary fats so any choice of constants for the metabolism of fat is necessarily arbitrary. There appeared, however, to be no argument for retaining the Cathcart and Cuthbertson figures for human fat, and so I have reverted to the Zuntz values for animal fat as given by Lusk.

Thus the constants finally utilised are as in the following table.

	Carbohydrate	Protein	Fat
R.Q.	1.0	0.802	0.707
Cal./1.O_2	5.035	4.463	4.686

The revised equation for total energy expenditure using these constants is:-

Total Cal. = 3.844 x litres O_2 used + 1.191 litres CO_2 produced - (1.996 x g. urinary N)

As noted elsewhere (p. 133) the urinary nitrogen was not measured in the earlier part of the work, and where it is measured is probably rather low due to absorption of urine by the faeces. For the purpose of energy estimation the measured urinary nitrogen has been taken as correct. For the part of the work where it was not measured it has been deduced from the food intake. A graph of measured urinary nitrogen plotted against food intake is given in fig. (48).

Heats of Combustion.

The principle of computing the water equivalent of the apparatus, from the combustion of a known weight of a substance of known heat of combustion, is elementary:-

$$\text{Total water equivalent} = \frac{\text{Heat of combustion per g. x g. burned}}{\text{Temperature rise}}$$

When the water equivalent is known, the heat of combustion of an unknown substance is simply the above equation rearranged to give:-

$$\text{Heat of combust. per g.} = \frac{\text{Total water equivalent x Temp. rise}}{\text{g. burned}}$$

A correction has to be applied to the recorded temperature rise to allow for the imperfect heat insulation of the apparatus. This correction is based on the rates of temperature change recorded before ignition and after the system has started to cool. It is assumed that Newton's Law of Cooling holds over the small temperature range involved. The Regnault-Pfaundler formula has been used for this correction:-

$$\text{Correction} = nv + \frac{v_1 - v}{t_i - t}\left[\Sigma(t) + \tfrac{1}{2}(t_o + t_n) - nt\right]$$

$$= nv + kP$$

Where n = number of half-minutes between firing and first reading after the maximum temperature.

v = rate of temperature fall per half-minute during the initial period.

v_i = rate of temperature fall per half-minute during the final period.

t & t_i = average temperatures during the initial and final periods respectively.

$\Sigma(t)$ = sum of the readings between firing and the start of cooling.

t_o = temperature at instant of firing.

t_n = first temperature during the cooling period.

$k = \frac{v_i - v}{t_i - t}$ = cooling constant. (If the insulation of the instrument is adequate this constant should not be greater than 0.0025).

This correction is added to the observed temperature rise to give the true temperature rise. An example of the complete computation for one estimation is given in appendix (1)

There are other graphical, and theoretically more simple, methods for computing the true temperature rise of the calorimeter, but the above method was used in all the estimations quoted here as the graphical work makes the simpler methods no more speedy.

For the estimation of the water equivalent of the apparatus a series of 10 estimations was made giving a mean of 2288 ± 7 g. This shows a rather wider scatter than should occur in these estimations. This calibration was repeated with 5 more estimations with benzoic acid by Mr. F.M. Simpson of the Department of Engineering of this University; his findings have a mean water equivalent of 2281 ± 5 g., not significantly different from mine and substantially the same scatter. Combination of the two sets of estimations gives a mean of 2286 ± 5 g. The inference from these figures is that, on this instrument, the expected error of a single observation is 0.85 per cent while that of the mean of duplicate observations is 0.6 per cent. I considered that, although this error is rather high, the mean of duplicate estimations has a precision sufficiently high for the present purpose. A further series of 5 estimations, made to test the effect of sealing with silicone grease, gave a mean water equivalent of 2287 ± 5 g.

About one-third to one-half of the error is attributable to the limiting accuracy of the thermometer.

The thermometer is divided in hundredths of a degree, and although, for mental convenience I interpolated to thousandths of a degree while recording the temperature rise, it was apparent from the fact that the mercury column moved in steps of from 0.003 to more than 0.01 degree, that the limit of precision of the thermometer was not better than the graduated interval and possibly rather less. The remainder of the error must be attributed to poor heat insulation of the apparatus giving rise to irregular cooling.

The heat of combustion measured by the bomb calorimeter is the heat at constant volume, corresponding to the thermodynamic equation:-

$$\Delta E = q - w$$

For the energy relations in biological systems the heat of combustion at constant pressure is a preferable measure, and is represented by the equation:-

$$\Delta H = q - w$$

where $\Delta H = \Delta E + RT\Delta n = E + 1.988T\Delta n$

in reactions involving gases. The term Δn is the change in the number of gram molecules of gas after combustion, and for the materials being considered here can be regarded effectively as the difference between the number of gram molecules of oxygen used and the number of gram molecules of carbon dioxide produced. The effects of the combustion of hydrogen, nitrogen and sulphur can be ignored as their oxidation products, under the conditions of the estimation, are non-gaseous,

The R.Q. for the total combustion of a material of the approximate composition of Diet 41 can be derived from the composition of the material as shown in table (27), appendix (2). From these data Δn can be expressed as:-

$$-\Delta n = \frac{1.12(1 - 0.91)}{32} = 0.00315$$

The value of the gas constant R is 1.988, and assuming an average final temperature of the bomb of 17°C., the heat of combustion of 1 g. food at constant pressure can be calculated:-

$$\Delta H = \Delta E - 1.988 \times 290 \times 0.00315 = \Delta E - 1.8 \text{ cal.}$$

It can be seen from table (27) that only a slight absolute change would be produced in this correction by a considerable change in R.Q. Thus I have substracted from each heat of combustion estimate, expressed per g. of food or faeces burned 2 cal., ie 0.002 Cal.

Energy Balance.

The energy balance of an animal can be expressed by the general equation:-

$$E_I = E_M + E_G + E_F + E_U$$

where E_I, E_G, E_F, E_U are respectively, the heats of combustion of the food consumed, the body substance gained, the faeces formed and the urine solids formed during a given period, and E_M is the energy expenditure as calculated from the respiratory exchange over the same period. It is necessary to assume that the faeces and urine voided are identical with the faeces and urine formed over that period. This assumption is not strictly accurate and especially may be liable to error in the case of faeces. The use of colouring matters to distinguish the faeces formed during the period of experiment, such as ferric oxide or chromium sesquioxide does not completely eliminate error since a certain amount of chyme mixing is bound to take place, although it would reduce the error considerably. I have not used such methods as they would have increased the necessary experimental time to cover a given period of study without, in my opinion, giving any commensurate increase in accuracy. From the relation found between the daily food intake and the corresponding faecal mass shown in fig. (49) and Table (9) I am satisfied that over a period of three or more days the faeces voided can be taken as a valid measure of the faeces formed during that time.

The heats of combustion of the food ingested and of the faeces voided have been measured directly with a bomb calorimeter as described on pp. 126-130 As the urine was passed into sulphuric acid to prevent the loss of nitrogen and the formation of noxious gaseous decomposition products, the energy of combustion of the urine solids was not measured directly. From data extracted from work by Forbes and co-workers (Forbes et al, 1939, 1946 a, b; Forbes and Swift, 1944; Black et al, 1950), in which the urinary energy and nitrogen in the albino rat are measured directly, a mean value of 8.6 Cal. per g. urinary nitrogen has been derived. All the urinary energy losses have been deduced from this figure applied to the urinary nitrogen. When the urinary nitrogen has not been measured directly I have applied the values found from fig. (48) as in the energy estimations.

The total period of each day's run varies from about 22 to 25 hr. The directly measured energy expenditure, therefore, does not bear a constant relation to the other components of the energy balance. On the other hand there is no way of telling whether the food, faecal and urinary energies, measured on the first and last days of a period, truly represent the whole day. Since the time interval represented by the measured energy expenditure is known precisely it seemed more rational to reduce this to a 24 hr. basis and assume the other components to represent a 24 hr. interval. This has been done, and the energy balances are computed on the basis of the 24 hr. energy expenditure. It is realised, however, that this is an arbitrary decision and there would be justification for assessing the energy balance entirely on the measured energy estimates.

Partition of Metabolised Energy.

The non-protein R.Q. can most conveniently be considered in the form:-

$$\text{Non-protein R.Q.} = \frac{(\text{Total } CO_2 - \text{Protein } CO_2) \text{ litres}}{(\text{Total } O_2 - \text{Protein } O_2) \text{ litres}}$$

Using Loewy's constants for protein metabolism, this gives:-

$$\text{Non-protein R.Q.} = \frac{\text{Total } CO_2 \text{ litres} - 4.765 \text{ urinary N g.}}{\text{Total } O_2 \text{ litres} - 5.941 \text{ urinary N g.}}$$

The energy derived from protein catabolism is:-

g. urinary N x litres O_2/g. urinary N x Cal./litre O_2 metabolising prot. = 5.941 x 4.463 x g. urinary N.

= 26.51 x g. urinary N

Subtraction of the energy derived from protein catabolism from the total energy production as given by the general energy equation (p.144) gives the non-protein energy production. From this could be derived the fraction of the non-protein energy attributable to the combustion of carbohydrate and of fat. If K be the energy equivalent of the 'non-protein oxygen' per litre, and x and y the fractions of that oxygen used to metabolise carbohydrate and fat respectively then:-

$$\text{Litres } O_2 \text{ used} = x + y = 1 \qquad (1)$$

$$\text{Cal. per litre} = K - 5.035x + 4.686y \qquad (2)$$

$$\text{From (1)} \qquad y = 1 - x$$

$$\text{Substituting in (2) } K = 5.035x + 4.686\,(1 - x)$$

$$= 0.349x + 4.686$$

$$\text{Therefore } 0.349x = K - 4.686$$

$$\text{Therefore } 5.035x = \frac{5.035}{0.349}\,(K - 4.686)$$

$$\text{Cal}_{CHO} = 14.43\,(K - 4.686) \qquad (3)$$

Expressing the energy from carbohydrate as a percentage of the total energy per litre of oxygen gives:-

$$\text{Cal}_{CHO} \text{ per cent.} = \frac{14.43\,(K - 4.686)100}{K}$$

$$= 1443 - \frac{6761.9}{K} \qquad (4)$$

The appearance of independence, in equations (3) and (4), of the non-protein R.Q. is spurious, in that the total R.Q. has already been introduced in deriving K.

Additionally and similarly the actual amounts of carbohydrate and fat can be derived.

Carbohydrate with an R.Q. of 1.0 requires 0.829 litres oxygen to metabolise 1 g. Fat, with an R.Q. of 0.707 requires 2.021 litres oxygen to metabolise 1 g. (vide p. 164).

Letting x be g. fat metabolised, y be g. carbohydrate metabolised, and V_{O_2} and V_{CO_2} the volumes of oxygen consumed and of carbon dioxide produced in the combustion of fat and carbohydrate, then:-

$$V_{O_2} = 2.021x + 0.829y \qquad (1)$$

$$V_{CO_2} = RV_{O_2} = 0.707 \cdot 2.021\,x + 0.829\,y \qquad (2)$$

Subtracting (2) from (1)

$$VO_2\,(1 - R) = 0.592x$$

$$\underline{\text{ie}} \quad x = \frac{V_{O_2}(1 - R)}{0.592} = \frac{V_{O_2} - V_{CO_2}}{0.592} \text{ g. fat.}$$

Dividing (2) by 0.707

$$\frac{RV_{O_2}}{0.707} = 2.021\,x + \frac{0.829}{0.707}\,y \qquad (3)$$

Subtracting (3) from (1)

$$V_{O_2} - \frac{RV_{O_2}}{0.707} = 0.829\,y - \frac{0.829}{0.707}\,y = 0.829\,y - 1.172\,y$$

Therefore:-

$$y = \frac{V_{O_2}\,(R - 0.707)}{0.243} = \frac{V_{CO_2} - 0.707V_{O_2}}{0.243} \text{g.CHO}$$

Putting the total non-protein oxygen consumed as 1 litre gives the expressions:-

$$\text{Fat metabolised in g.} = \frac{1 - R}{0.592}$$

$$\text{Carbohydrate metabolised in g.} = \frac{R - 0.707}{0.243}$$

As these values are used in the computation of the indirect water balance a nomogram has been constructed, shown in fig. (26), to give the amounts of fat and carbohydrate metabolised directly from the oxygen usage and carbon dioxide production from non-protein metabolism This is similar to a nomogram constructed by Forbech (1938), but covers a range more suitable for the present work.

When the non-protein R.Q. is greater than unity, or the non-protein energy value per litre of oxygen is greater than 5.035, the inference is that fat is being formed from carbohydrate (eg Pembrey, 1902). To find the equivalence of this increased energy value of oxygen, with R.Q.'s greater than unity, in terms of the amount of fat so formed the thermal relations of fat and carbohydrate must be examined. It is not proposed that the following equations bear any relation to the precise chemical processes involved in this conversion, but consideration of reactants and the final products of reaction is adequate to obtain the thermochemical relations, (Hess's Law).

The following reaction can be proposed for the conversion, using an empirical formula for fat which would give an R.Q. of about 0.71:-

$$\underset{1620\text{ g.}}{(C_6H_{10}O_5)10} \longrightarrow \underset{844\text{ g.}}{C_{54}H_{100}O_6} + \underset{264\text{ g.}}{6\ CO_2} + \underset{512\text{ g.}}{16\ O_2} \qquad (1)$$

For complete catabolism of this carbohydrate the equation is:-

$$(C_6H_{10}O_5)10 + 60\ O_2 \longrightarrow 60\ CO_2 + 50\ H_2O \qquad (2)$$

Wt. O_2 = 1920 g. ; Vol. O_2 = 1343.6 litres.

Energy value of this oxygen at 5.035 Cal./litre = 6765.026 Cal.

For complete combustion of the fat formed the equation is:

$$C_{54}H_{100}O_6 + 76\ O_2 \longrightarrow 54\ CO_2 + 50\ H_2O \qquad (3)$$

Wt. O_2 = 2432 g. ; Vol. O_2 = 1701.9 litres.

Energy value of this oxygen at 4.686 Cal./litre = 7975.103 Cal.

The apparent energy gain of this conversion is 1210.077 Cal.

The energy value of the 512 g. (358.3 litres) oxygen, released in equation (1), if used to metabolise carbohydrate is 1804.04 Cal. Thus the total energy loss of the system is 1804.04 - 1210.077 = 593.963 Cal. This energy is lost in the conversion of 1620 g. carbohydrate to 844 g. fat. There is therefore a gain in the energy value of the absorbed total oxygen of 593.963/844 = 0.7037 Cal. per g. fat deposited from carbohydrate.

Taking K as the non-protein energy value per litre of oxygen, and V as the total volume of oxygen used, the total amount of fat synthesised from carbohydrate can be found.

$$\text{Total energy gain of oxygen} = V(K - 5.035)\ \text{Cal.}$$

$$\text{Therefore Total fat synthesised} = \frac{V(K - 5.035)}{0.7037}\ \text{g.}$$

When K is less than 5.035 the above equation represents the amount of fat burned, whether as dietary fat or as body fat.

The total fat deposited is given as:-

Ingested fat + Synthesised fat - faecal fat
- Catabolised fat

Food Consumption.

The difference between the final and initial weights of the food box and food measures the true food consumption and the total scattered food. The weight of the uncontaminated scattered food was measured directly as described on p. (122). The dry weight of the urine contaminated scattered food on the urine funnel and faeces grid were estimated from the mean values given in table (30) by the process described on p. ( ). The dried weights were increased by 10 per cent. to restore the normal moisture content, giving working means of 37 and 21 per cent. for the food on funnel and grid respectively. These weights of scattered food are subtracted from the decrease in weight of food-box and food, to give the true food consumption. The total energy of combustion and the total nitrogen content of the ingested food are derived from this weight without adjustment for food moisture, these determinations having been made on the un-dried food.

Weight Balance.

If the apparatus is leak-free and if complete recovery of all components is obtainable then the summed weights of everything removed from the system at the end of a run should equal the summed weights of everything put into the system. This overall weight balance is most simply expressed in terms of the experimental data.

The direct weight balance equation, used in this work as a check on the accuracy of the apparatus is:-

$$W_o + W_f + W_w + Wt_i = W_{co} + W_e + W_u + Wt_f$$

where W_o to W_u, in order above, are weights of oxygen used, food ingested, fluid water ingested, carbon dioxide produced, faeces produced and urine voided; and Wt_i and Wt_f are the initial and final weights of the animal. The discrepancy between the two sides of the above equation as determined from the experimental data is a composite measure of inaccuracy of the apparatus and of the measuring methods. I have estimated that, apart from a gross error in recording of a weight or a volume, the instrumental errors of weight and volume measurement should never, even if consistently cumulative, amount to more than 0.15 g. It is to be expected that, in general, instrumental errors will be randomly distributed and will tend to zero. I have regarded any weight balance discrepancy in excess of 0.05 g., either positive or negative, as presumptive evidence of an error arising in the apparatus. This is, in fact, a very narrow tolerance limit, since the expression of the weight balance equation, in practice, entails the addition of two sets of 25 weights, each set amounting to a total of about 3 kg.

As can be seen from the weight balance data in table (40), appendix (3), most of the balances show a pronounced weight deficit of up to 1.0 g., although there are some in excess of this, on the right hand side of the equation; that is, there is a deficit in recovery of material from the apparatus. Experimental evidence is described on p. (170) for considering this weight deficit as water.

Water Balance.

The water balance of an animal over any period can be expressed by the general equation:-

$$W_i + W_m + W_f = W_u + W_r + W_t + W_e$$

where W_i to W_e in the above order are, fluid water intake, metabolic water of oxidation, food moisture, urine water, respiratory water, transpiratory water and water of solid excreta (<u>ie</u> faeces). It is not possible in this work to separate completely the different terms in this equation, since there is always a certain amount of water loss from the water bottle which is not consumed by the animal, there is contamination of faeces and scattered food by water bottle leaks and urine, while some of both of these fluid forms of water may evaporate and be picked up by the absorbing train. It is however, possible, within fairly narrow limits, to estimate the totals of both sides of the equation. The error of instrumental water loss which occurred in much of the experiment can be estimated by the discrepancy in total weight balance already described.

Due to the impossibility of measuring the exact components of the above theoretical water balance equation a working equation has to be substituted for it. The individual terms of the two equations differ, but the corresponding sides of the two equations are identical. The working equation used is:-

$$W_i + W_f + W_m = W_u + W_a + W_e + W_t + W_w + P$$

where W_i to W_w in the above order are fluid intake, moisture of ingested food, metabolic water, urinary water, water absorbed on scattered food, water of solid excreta, water in absorbing tubes and water increment of the animal. W_w may, of course, be positive or negative and is the unknown in the equation which is measured by difference. The final term P is the instrumental water loss, when it occurs, and is derived as the discrepancy in the weight balance.

Because of the doubt introduced into the direct water balance estimations by the instrumental water loss, the water balance was also estimated indirectly. Peter's et al (1933) give the equation for water balance:-

$$\Delta W = \Delta Wt + Se - Si + C + F + 0.49P$$

where ΔW is the water loss by the animal, ΔWt the bodyweight gain, Se and Si the weights of dry solid excreta and ingesta, and C, F. and P the weights of metabolised carbohydrate, fat and protein respectively. On the basis of Loewy's nitrogen metabolism data I have altered the factor 0.49 to 0.53; it is difficult to see how Peters et al derived the factor they give. C and F have been obtained from fig. (26). The water balance data used in this work have all been derived from this equation, and are the only water balance data reported as such. The direct water balances were, however, calculated, (and can be derived from the data given) and differed always from the indirect by the amount of the weight balance error. This correspondence of the direct and indirect balances is additional evidence that the weight error lies in water loss. It is not, of course, proof, since correspondence would have been obtained if the losses were due to gas leaks. (C and F are derived from the respiratory metabolism data.)

Fluid Water Intake.

The fundamental measure of the fluid intake is the difference in weight of the water bottle at the beginning and end of a run. This has been accepted, throughout, as a valid measure of the true fluid water intake. On a series of blank trials the water bottle was found to lose an average of 0.2 g. in the process of setting it in and removing it from the chamber, but it has not been considered profitable to apply any correction as the loss was small and very constant. The loss was always into the chamber and so is taken into account in the water balance.

Food Moisture./

Food Moisture.

The moisture content of the food used was very constant. It was estimated on samples of each batch of food by drying to constant weight (a total of 24 hr.) at 50°C. On one food batch it was estimated at intervals throughout the period for which that batch lasted and did not vary by more than 1 per cent. The food moistures found are given in table (29), appendix (2). The corresponding food moisture was applied to the measured food consumption on each day from each batch of food.

The possibility that the moisture content of the food might alter from beginning to end of a run was checked on three occasions. No appreciable difference was found in moisture content between the beginning and end of a run.

Metabolic Water.

The customary methods of estimating this source of water are cumbrous, if it be estimated from the true metabolism, or inaccurate if it be estimated from the food composition. In the first instance the calculation involves the detailed computation of the protein, carbohydrate and fat metabolised over each day of study. In the second instance standard values are used for the metabolic water per unit weight of dietary protein, carbohydrate and fat, eg 0.41, 0.60 and 1.07 g. water per g. dietary component respectively, (Brody 1945). This latter method assumes complete digestibility and energetic utilisation of all the ingested food; its accuracy could be improved by taking into account the intramolecular water of the faeces voided during the period, but as a rule this method is only used to give a rough estimate of metabolic water when the data on respiratory exchange, necessary for a precise estimate, are lacking.

This source of water is of great quantitative importance to the organism's water economy, frequently amounting to over one quarter of the total water supply (tables 39 and 40), and much work on water balance has been vitiated or greatly reduced in value by ignoring it (Du Bois, 1927).

To estimate metabolic water I have used a parallel method to that of Weir for the estimation of total energy expenditure. That is, I have derived the estimate from the respiratory exchange but have largely eliminated the usual cumbrousness of the individual calculations.

From the basic equations for the combustion of carbohydrate and fat can be derived constants for the equivalence of metabolising oxygen and metabolic water produced.

The carbohydrate equation is based on the combustion of poly-saccharide:-

$$C_6H_{10}O_5 + 6\ O_2 \longrightarrow 6\ CO_2 + 5\ H_2O$$

162 g. 192 g. 90 g.

From this can be derived the relations:-

0.555 g. H_2O || 1 g. CHO || 0.829 litres O_2

<u>ie</u> 1 litre O_2 || 0.669 g. H_2O

The fat equation is based on a hypothetical combustion of a mixture of equal parts of tripalmitin and triolein, suggested by Dewar and Newton (1948), which gives an R.Q. of 0.7081 which approximates closely to the R.Q. of 0.707 used in the derivation of the energy equation.

Tripalmitin: $C_{51}H_{98}O_6 + 72.5\ O_2 \longrightarrow 51\ CO_2 + 49\ H_2O$

Triolein: $C_{57}H_{104}O_6 + 80\ O_2 \longrightarrow 57\ CO_2 + 52\ H_2O$

Mixture: $C_{108}H_{202}O_{12} + 152.5\ O_2 \rightarrow 108\ CO_2 + 101\ H_2O$

1690 g. 4880 g. 1818 g.

From this can be derived the relations:-

1.076 g. H_2O || 1 g. Fat || 2.021 litres O_2

<u>ie</u> 1 litre O_2 || 0.532 g. H_2O

The equivalence of oxygen and water for the metabolism of protein has been derived from Loewy's data on protein metabolism, used for the energy equation. These data give, for the absorbed protein moiety which is completely metabolised (<u>ie</u> after removal of the material eliminated in the urine), the following elemental analysis:-

41.50 g. C + 4.40 g. H_2 + 7.69 g. O_2 = 53.59 g. protein

giving:- 4.40 g. H_2 + 35.20 g. O_2 = 39.60 g. water,

the protein requiring for metabolism a total of 145.87 g. O_2 or 138.18 g. respiratory O_2. This water is finally derived from the total absorbed and metabolised protein which can be derived from the data as:-

C	H	O	N	S
50.909 g.	7.058 g.	21.791 g.	16.280 g.	1.02 g.

Protein = 97.058

From this can be derived the relations:-

0.408 g. H_2O $\|$ 1 g. Protein $\|$ 0.995 litres O_2

ie 1 litre O_2 $\|$ 0.410 g. H_2O

From the foregoing calculations the following table of working values can be assembled:-

	Carbohydrate	Protein	Fat
R.Q.	1.0	0.802	0.707
g. H_2O per litre O_2	0.669	0.410	0.532
Vol. O_2 metabolising	x	y	z

From these values the following basic equations can be constructed:-

litres O_2 consumed $= x + y + z = V$ (1)

litres CO_2 produced $= RV = x + 0.802\,y + 0.707\,z$ (2)

Water produced $= W = 0.669\,x + 0.410\,y + 0.532\,z$ (3)

From (1) $x = V - y - z$ Substituting for x in (2)

$$RV = V - y - z + 0.802\ y + 0.707\ z$$

$$= V - 0.198\ y - 0.293\ z \qquad (4)$$

Substituting for x in (3)

$$W = 0.669\ (V - y - z) + 0.410\ y + 0.532\ z$$

$$W = 0.669\ V - 0.259\ y - 0.137\ z \qquad (5)$$

From (4) $z = (V - 0.198\ y - RV)/0.293$

Substituting for z in (5)

$$W = 0.669V - 0.259\ y - (V - 0.198\ y - RV)/0.293$$

$$= 0.669\ V - 0.259\ y - 0.4675\ V + 0.0926\ y + 0.4675\ RV$$

Collecting terms and re-arranging gives:-

$$W = 0.2015\ V + 0.4675\ RV - 0.1664\ y \qquad (6)$$

Using, as before (p. 141), Loewy's factor of 5.941 litres oxygen per g. urinary nitrogen, the last term of equation (6) can be expressed as 0.1664 x 5.941 x g. urinary N. This gives a final form for the water equation of:-

Metabolic water formed g. = 0.2015 x litres O_2 consumed
+ 0.4675 x litres CO_2 produced
- 0.989 x g. urinary N

Urinary Water.

The difference between the weights of the urine flask at the end and the beginning of a run gives the weight of urine. For the purpose of water balance estimation this weight has to be corrected for the urine solids. Because of the method of collecting the urine in sulphuric acid the urine solids were not routinely measured. On three occasions, during non-pregnant periods of experiment, the urine was collected in a dry flask and the urine solids measured directly by evaporation of the urine *in vacuo* over calcium chloride. The total solids found were 11.25, 11.96 and 13.51 per cent. and corresponded to total urine weights of 7.6, 7.1 and 5.5 g. This gives a mean solids content of 12.24 per cent. The number of observations is not sufficient to justify the calculation of any more precise relation, although the values found do indicate an inverse relation between the percentage of solids and the total urine weight. In all the calculations of urine water a working value for urine solids of 12 per cent. has been used, giving a corresponding value for urinary water of 88 per cent.

In addition to this directly measured urine, there is a certain amount of urine retained in the scattered food and faeces on the faeces grid and on the urine funnel. The water absorbed by the faeces is treated separately as true faeces moisture. The remainder of the water on grid and funnel is considered as a separate item in the general water balance estimation, and is not included in the urinary water in the detailed water partition. In the early part of the work this absorbed water was measured directly from the weights of the funnel and frame as removed from the chamber and their weights after drying in air at 50°C. This process was found seriously to interfere with the nitrogen recovery and direct estimation of the absorbed water was later sacrificed in favour of obtaining a more precise measure of the nitrogen balance (p. 133). In the major part of the work, therefore, this water has been estimated from the mean moisture content of the contamination on the funnel and frame in the runs where this was estimated directly. These values are given in table (30), appendix (2). A modification is necessary in order to take into account the original 10 per cent. moisture content of the food, so that the measured moisture in the contamination is reduced by 10 per cent. In this case it has not been considered worth while, when such small total quantities are involved, to use the precise moisture contents found for separate food batches.

Faeces Water.

The total faeces moisture was measured from the weight of the fresh faeces after removal from the grid, and their weight after drying in air for 24 hr. at 50°C. As mentioned above there is the possibility that some of the water so measured may be derived from the urine, some also may be derived from water spilt from the water bottle in inserting it into and removing it from the chamber. There is, in addition, the possibility that in some cases the faeces may be dried by the air flow through the chamber, though I do not consider that this would occur to any considerable extent in the high humidity which obtains in the animal chamber. No attempt has been made to correct for any of these errors. They do not, of course, appear as errors in the general water balance as the water is taken into account irrespective of its source. The total faeces water as measured directly has been regarded as being, in fact, voided in the faeces.

Vapourised Water.

The total vapourised water is measured as the difference between the final and initial weights of the water absorbing tubes of the absorbing train. The water loss found from the total weight balance to be occurring in the apparatus is due to inadequate ventilation by the pump. This discrepancy amounted to 0.0 - 2.0 g. per day, usually about 0.7 g. It was found that water was accumulating in the air ducts, and trials showed that the weight loss was almost quantitatively recoverable in the absorbing train by running the apparatus without animal or other water source. The apparatus was run empty for 24 hr., with an absorbing train in line, so that it was completely dried out. A normal three-day period was then started. At the end of the three-day live period the apparatus was again dried out in the same way. The summed weight loss over the three days, found from the weight balance, was 2.8 g., while the water picked up in the second drying period was 2.2 g. This is sufficiently close correspondence to show that by far the greater part of the weight balance discrepancy was due to instrumental water loss.

As mentioned on p. (100), the pump stroke was raised from 8 to 10 mm. This produced no detectable improvement in water recovery. No further action was taken at that time as further increase in the ventilation rate would have increased the strain on the pump. When the pump stroke had, later, to be further increased almost to its maximum to augment carbon dioxide removal the weight balance discrepancy was reduced.

Where this weight balance discrepancy exists it has been expressed as water, cf p. (160), and added to the vapourised water measured from the absorbing train weights. The total vapourised water found in this way includes the water of respiration, the water of transpiration, and any fraction of urinary, faecal or water bottle water which may be vapourised by the air flow through the chamber. In trials made to check on the water exchanges with water bottle and urine flask with water but no animals present in the chamber, the water absorbing tubes picked up an average of 1 g. water per diem. This may have come, however, from excessive drying of the apparatus and has not been taken into account in partitioning the water balance components.

As with the energy estimations, p. (150), the vapourised water loss has been corrected to a 24 hr. basis, but the fluid water intake and loss have been assumed to be valid estimates of the 24 hr. intake and loss as measured.

Plan of Experiment.

The prosecution of the experimental work was envisaged as falling into three main parts.

(1) To obtain an adequate series of measurements of the energy, nitrogen and water exchanges in the non-pregnant rat to serve as a basis for comparison with the measurements in pregnancy.

(2) To obtain one or two complete runs from beginning to end of pregnancy, so that any point during pregnancy at which a sudden or pronounced change occurred could be noted and incorporated in the later studies.

(3) On the basis of the findings in (2), to follow the exchanges of three litter-mate females as far as possible contemporaneously, over restricted and identical periods of pregnancy, through three successive pregnancies.

The basis of this plan has been followed, but unforeseen hazards and the unpredictable temperament of the metabolic apparatus restricted the scope of the experiment.

It was intended to fulfil the first part of the scheme, (1) above, from non-pregnant periods of the animals which would later be used for pregnancy studies. In this way the non-pregnant base-line would be directly comparable with the pregnancy findings and each animal would be its own control. This has, in fact, been done, but some additional non-pregnant measurements are available from animals which were asphyxiated by the apparatus before a pregnancy period could be studied, from animals in which other accidents prevented a pregnancy period, and from other animals studied incidentally during attempts to preclude asphyxiative breakdown of the instrument. Some early records showed definite evidence of leaks in the apparatus and have been discarded.

Only half of the second part of the scheme was fulfilled. The first rat of which records are reported female 377, was studied over three non-pregnant periods of 6, 4 and 5 days, and over one complete pregnancy from coitus to delivery. There were, however, two breaks each of one day, in the pregnancy study as a result of breakdown of the calorimeter. A further 3-day non-pregnant period using this animal, intended as a prelude to the second pregnancy was terminated by asphyxiation of the animal as a result of blockage of the absorbing train. The great difficulty, experienced in this part of the work, in maintaining the calorimeter as a reliable instrument for a period of 21 days without frequent inspection and overhaul, dissuaded me from attempting to follow another complete pregnancy. This, then, is the only study from which a total cumulative picture of the energy and water exchanges during pregnancy can be obtained. Nitrogen balances are not recorded for this study (vide p.133).

Females 407, 408 and 415, littermates, were studied over non-pregnant periods of 5, 3 and 5 days respectively. These studies were primarily undertaken to find methods of preventing blockage of the absorbing train and were not continued. The females were, however, younger and smaller than any of the others studied and so extend the range of information obtained in reproductive rest.

A study was started with littermate females 443, 444 and 445. This was intended to cover, in each animal, a 3-day non-pregnant period and the days 3-6, 11-14 and 17 to term, and was intended to be carried through three successive pregnancies. The full plan for the first pregnancy was achieved with female 443. This series was successively interrupted by a flood and later by illness of the author, so that all that could further be rescued was 4 days study during pregnancy with female 445 and 1 day of pregnancy with female 444. The study with these females was then abandoned. Apart from the one pregnancy which was successfully covered, this work did show that it would be impossible to keep three animals on experiment even approximately contemporaneously and still leave adequate time for maintenance of the apparatus.

It was decided, therefore, to confine part three of the experiment to two littermate females. It was also decided to extend the non-pregnant period to 5 days to cover a complete oestrous cycle. The final and major study of the work was therefore started on littermate females 522 and 524. Female 522 took very unkindly to the calorimeter and after a 5-day non-pregnant period, during which she voluntarily starved and dehydrated herself, was discarded. Female 525, of the same litter, was substituted and with 524 and 525 the experiment was continued on the general plan detailed below.

(1) A 5-day non-pregnant period.

(2) A pregnant period from the 9th to the 12th day of pregnancy.

(3) A pregnant period from the 15th day of the first pregnancy to term.

(4) A 5-day non-pregnant period after weaning of the previous litter.

(5) A pregnant period from the 9th to the 12th day of the second pregnancy.

(6) A pregnant period from the 15th day of the second pregnancy to term.

(7) A 5-day non-pregnant period after weaning of the second litter.

(8) A pregnant period from the 9th to the 12th day of the third pregnancy.

(9) A pregnant period from the 15th day of the third pregnancy to term.

(10) A 5-day non-pregnant period after weaning of the third litter.

This series was remarkably successful and showed the technique which I had developed to be capable of yielding fundamental information over long periods.

The first and second non-pregnant periods and the periods of the first pregnancy were completed according to the above plan. Thereafter, although the general scheme was maintained the plan broke down in detail. The points of breakdown and the reasons for them are as follows.

(a) The first pregnant period of the second pregnancy of female 525 covers only days 10-12. This was necessary as it mated a day earlier than expected and female 524 did not reach term until a day later than expected, so that the end of the second pregnancy of female 524 and the 10th day of the second pregnancy of female 525 overlapped. I arbitrarily decided it to be preferable to record the last day of a pregnancy at the expense of a 10th day.

(b) The 17th day of the second pregnancy of female 524 was missed. The thermostatic control of the calorimeter broke down.

(c) On the third non-pregnant period of female 524 this animal suffered from methanol poisoning (p. 116). The run was discontinued after three days, the animal allowed to rest for 2 weeks when its weight curve showed substantial recovery, and the third non-pregnant period was repeated.

(d) The third non-pregnant periods of both animals were initiated a week after the termination of the second pregnancy. In the second pregnancy only 4 young were born to each mother, and neither mother nursed her young.

(e) The third pregnancy of both animals was unsuccessful. Female 525 was continued on experiment until the 22nd day of pregnancy and killed a week later so that the uterus could be examined. Female 524 was taken off experiment at the 19th day of pregnancy, remated a week later, put on experiment again until the 19th day of pregnancy when she was killed for examination of the uterus. In consequence of this, section (10) of the experimental plan was not fulfilled for either animal.

Vaginal Smears.

Throughout most of the non-pregnant periods of the metabolic work vaginal smears were taken daily from the animal under experiment, to obtain an indication of the stage of oestrous, (Young *et al*, 1941). The sample of vaginal epithelium was taken by inserting a platinum loop, wrapped sparingly in dry cotton wool, into the vagina and withdrawing it without twisting or subjecting the vaginal wall to any unnecessary trauma. The use of a naked platinum loop was found to be too liable to upset the regularity of the cycle, and was found liable to precipitate a pseudo-pregnant condition. The use of cotton moistened with saline tended to obscure and distort the cellular picture during the evaporation of the saline before the fixation procedure. The sample was spread on a microscope slide, dried in air and stained with Leishman's blood stain (methylene blue eosinate in methanol) by the standard method used for blood films. The use of Leishman's stain for this purpose does not give as detailed a picture as that obtained by fixing the preparation in an ethanol-ether mixture and staining with methylene blue and eosin, but the cell types are adequately demonstrated and the method is much more convenient for routine work.

Initially I attempted to use the vaginal smear technique to predict the optimum time for putting the male to the female; but the actual time of mating, when the male is present continually, was found to bear little relation to the appearance of the immediately preceding smear.

The time of mating of the experimental animals was found by putting the male with the female and taking vaginal smears morning and evening until either a vaginal plug appeared or spermatozoa could be seen in the smear. The presence of spermatozoa in the smear is the more reliable sign of coitus as the vaginal plug is transient in the rat and usually drops out after a few hours. This procedure gives the time of coitus within about 12 hr. and the time of observation of plug or smears has been taken as the beginning of pregnancy. There is little point in attempting to fix the time of coitus more precisely as the interval between coitus and implantation probably varies from 6 to 14 hr. (Chang and Pincus, 1951).

Statistical Methods.

The methods used for summarising the data according to location and dispersion are those detailed in any standard text (*eg* Fisher, 1948). All regression equations computed have been assumed, from inspection of the graphical array, to be linear and no numerical test of linearity has been applied. The fraction of the total variance attributable, in each case, to regression indicates that this assumption is valid. The standard errors of coefficients of these equations have been derived from the analysis of variance used to test the significance of the coefficients.

The data from females 524 and 525, the major part of the work, have been analysed by the analysis of variance to test the significance of differences. This is the only part of the work, arranged in a factorial form, which is susceptible of this procedure. The failure of the third pregnancy in both animals has, from the point of view of the design, effectively limited the experiment to two pregnancies, and so only these two have been included in the analysis. The data have been taken in sets of three days, each set forming a period in the analysis. There are four periods in each pregnancy, one control and three pregnant. The variance between days within periods is left in the residual variance. This becomes, effectively, the analysis of three replicates, the inter-replicate variance being included in the residual.

The extension of the non-pregnant periods to five days to examine the effects of the oestrous cycle has made the non-pregnant or control periods unequal classes. The three days taken from each control period for the analysis are those three which end in oestrus as measured by the vaginal smear. This is quite an arbitrary choice and may not be ideal, but for valid comparison with the rest of the data it is, I believe, preferable to take, as far as possible, a series of consecutive days rather than choose three days out of five on a truly random basis.

One rather less common procedure used may deserve a brief explanation. The records for the 17th in female 524 and the 10th in female 525, day of the second pregnancy are lacking (vide p. 176). To avoid the great additional labour which would be entailed in an analysis of unequal classes the "missing plot" technique (Anderson, 1946) was used. The missing values were estimated, by algebraic analysis of the factorial scheme, so that the residual variance was minimised. The two missing values, in order as above, are given by the expressions:-

$$s = \frac{2(R_1 + 2G_3 - K + P_2)}{n - 6}$$

$$t = \frac{2(R_2 + 2G_2 - K + P_2)}{n - 6}$$

where K is the original incomplete total, R_1 and R_2 are the original totals for rats 524 and 525, P_2 the original total for the second pregnancy, G_2 and G_3 the original totals for the second and third periods, and n the total number of observations including the missing values.

The numerical estimates, computed from the above expressions, were inserted at the appropriate points in the scheme and the numerical analysis of variance computed in the normal way with a reduction of 2 in the degrees of freedom for the total and residual variance. Although the reduction in the degrees of freedom ensures that the error variance is a valid estimate, this procedure does magnify the weight to be allotted to the variance under examination. Where the number of values estimated in this way is small relative to the total number (2 in 48 in this instance) this magnification of the main effects is small and can probably be ignored (Kendall, 1946). I have, however, made an adjustment, by altering the effect variances in the ratio of the original: completed total variance for the same (reduced) number of degrees of freedom, when this ratio is appreciably different from 1.0. The detailed mechanism of this procedure should be readily apparent from the tables given of these analyses.

The interactions between main effects have been computed when the main effects were themselves significant. Where the interactions were significant they have been detailed in the tables of analyses, where they were insignificant they have not been quoted and the variance attributable to them has been included in the error variance.

Where probabilities of the significance of differences have fallen below the conventional 0.05 level, but are still sufficiently low to be suggestive I have given the probability rather than a simple statement of significance. Significance levels and the interpolation of probabilities have been derived from the tables of Fisher and Yates, (1948).

RESULTS

The full quantitative results of the experimental work on a *per diem* basis form a considerable body of data. These are presented *in extenso* in tables (31)-(41) in appendix (3), to which reference will be made in the text from time to time. It should not, however, be necessary to refer to these detailed tables for a general understanding of the text which, it is believed, is adequately illustrated by the figures and summary tables, derived from the primary data by summing and averaging over series of up to 5 days.

The data fall into three major groups, those concerned with energy balance, nitrogen balance and water balance and are considered in that order.

ENERGY METABOLISM

The full data on energy metabolism are given in tables (31)-(36) appendix (3). The primary data of the respiratory exchange, from which the energy expenditure is derived by the methods already described (p. 134) are available in tables (32) and (33) appendix (3).

Energy Metabolism in Reproductive Rest

Daily Energy Expenditure

The energy expenditure per 24 hr. in the non-pregnant condition is quite variable. This can be seen in fig. (27) in which the non-pregnant periods for females 524 and 525 are presented in histogram form, and in fig. (28) in the preliminary non-pregnant period for female 377. Within any given consecutive series of days the mean variation in energy expenditure amounts to 6.5 per cent. of the general mean.

Within broad limits the energy expenditure varied with the bodyweight of the animal. This weight variation is not always observable in the day-to-day weight changes but is apparent in the broad weight groups formed by the means of the different non-pregnant periods. In some cases the day-to-day energy expenditure does appear to follow the bodyweight changes, tables (31) and (35), but here the bodyweight changes are, it is believed, more truly associated with accidental restriction of food and water intake, while the corresponding changes in energy expenditure arise in great part from the fall in activity which normally accompanies such restriction. Such occasional detailed correspondence of metabolic rate and bodyweight does not fit the general M.R.-bodyweight line found for 'true' weight variation.

The average values for energy expenditure are plotted against the corresponding average bodyweights in fig. (29). Even for these average values it can be seen that the scatter is considerable. This is to be expected since the effect of individual variation is very prominent in such a small group of animals. In addition, it must be remembered that here total metabolism is being considered, and this consists of two major components each varying with bodyweight in a different way. One, the basal metabolism, varies with a fractional power of the bodyweight, say 0.75; the other, the metabolism of physical activity, probably varies more nearly directly with the bodyweight, that is with the mass moved. A further component of the metabolism, the specific dynamic action, will be more nearly related to the food intake than to the bodyweight as such.

The intercept on the energy axis given by the linear regression equation to these data, fig. (29) and table (1), is significantly greater than zero. The apparent implication of this, that the energy production at zero bodyweight is positive, is merely an indication that this linear regression line cannot, legitimately, be extrapolated back to zero. The intercept in this equation, however, (13.4 Cal.) is commensurate with that given by Benedict's (1938) linear relation for B.M.R. and bodyweight in the albino rat (7 Cal.).

A rise in the mean energy expenditure occurred from the first to the second non-pregnant periods, that is with the first pregnancy intervening. This can be seen for females 524 and 525 in fig. (27). This change is largely accountable to change in bodyweight; after the first pregnancy the bodyweight had reached a plateau, and in female 525, there was no further appreciable alteration in the mean non-pregnant energy expenditure. The pronounced drop in the expenditure of female 524 in the third non-pregnant period is associated with the fall and incomplete recovery in bodyweight of this animal after the methanol poisoning.

Vaginal smears were made daily for most of the non-pregnant periods but only with females 524 and 525 were these periods of sufficient length to cover a complete, or nearly complete oestrous cycle.

In fig. (30) the metabolic rates for each day of the non-pregnant periods of females 524 and 525 are plotted in order of recording. It can be seen that, within any one period for any one rat, the change in metabolic rate followed a fairly smooth course. An exception to this is the third period of female 524. Also given in these graphs is the position in time of the observed occurrence of vaginal oestrus. The one point of the vaginal cycle of the rat which I have found to be firmly identifiable as regularly recurrent is that of pro-oestrus (nucleated epithelial cells and cornified cells without leucocytes) and the time of oestrus has been fixed from this. It may be noted, however, that this vaginal oestrus does not necessarily coincide with the other observed criteria of the onset of heat determined by ovarian oestrus. This lack of correspondence between vaginal and ovarian oestrus has been observed by other workers (Freed et al, 1937, 1939), who have also observed that ovarian dysfunction of dietary origin may not be at all reflected by the vaginal cycle.

If the occurrence of this pro-oestrus stage of the vaginal cycle be taken as a fixed point in the oestrus cycle, whether or not corresponding to the onset of heat, and the metabolic rate curves of fig. (30) be brought together so that this point of the cycle coincides in each, fig. (31) is obtained. Here it can be seen that the metabolic rates do form a fairly well defined cycle about this "oestrus" point. The vertical displacement of the separate curves along the energy axis is largely due to bodyweight differences.

It can also be seen that the oestrus point lies in the ascending wave of the cycle in female 525, and on the descending wave in female 524. This forms additional evidence that, although the vaginal changes are cyclical, the stages are not necessarily a constant indication of the stage of the functional cycle. It appears possible that the peak metabolism period might be the period of heat as indicated by the increased activity of the animal, and that the cornified cell stage of the vaginal cycle may vary, at least between individuals, from trough to trough on either side of the metabolic peak.

A pronounced diurnal variation was observed in the rate of consumption of oxygen which was higher during the hours of darkness, 10.00 p.m. to 10.00 a.m., than during the day, 10.00 a.m. to 10.00 p.m. This diurnal change has not been analysed in detail but it can be seen in an example of the continuous oxygen record, fig. (32). In this figure the greatly increased slope of the oxygen consumption trace during the night can be clearly seen. The difference between day and night oxygen consumption rates was, in general, about 10 per cent. The reason for this diurnal variation lies in the greatly increased activity of the animals during the night. Herring and Brody (1938) have shown that it is extremely difficulty to suppress this diurnal variation, requiring both intermittent feeding and continuous light.

Daily Food Energy Intake

The food intake, expressed as total heat of combustion, in the non-pregnant condition, is shown in histogram form in fig. (33). A further example is shown in the preliminary period in fig. (28). Comparison of figs. (27) and (33) shows that the variation in food energy intake from day to day was very much greater than the variation in energy expenditure; in any given series of consecutive days the variation amounts to 18 per cent. of the general mean.

Oestrus Variation.

No consistent variation in food intake with stages of the oestrus cycle is discernible from the data, considered either in relation to the point of vaginal oestrus or the point of maximum metabolic rate.

Variation with Energy Expenditure.

Comparison of figs. (27) and (33) indicates an approximate correspondence between energy intake and expenditure. In fig. (34) the energy intake is plotted against the energy expenditure for all animals both non-pregnant and pregnant. That there was some association can be seen, but it was extremely slight; over the greater number of data there is no obvious association. From inspection of this scatter diagram it has not been considered profitable to fit a regression line. Both of these variables will, of course, show a reflected relation from their separate relations to bodyweight.

Relation to Non-protein R.Q.

In fig. (35) the total food energy has been plotted against the non-protein respiratory quotient. All the data for females 524 and 525 have been included in this diagram as no difference was found in this relation between the pregnant and the non-pregnant data. The regression equation for the combined data has been calculated and shows a significant relation between the two variables, table (2). This confirms the finding of Dewar and Newton (1948b), but the paramotors of the regression equation are different to those found by these authors. The different slope of the regression line found here is, of course, not comparable with the slopes found for mice, which consume only about 15 per cent. of the food used by a rat. Dewar and Newton found a difference in the slope of the regression line between the two mice which they studied, which may be due to a difference in efficiency of food utilisation. From inspection of the data plotted separately for females 524 and 525 I considered that in this case there was no appreciable difference between the slopes for the two animals.

The intercept of the regression line on the R.Q. axis is 0.767, and the one value on the graph corresponding to approximately zero food intake shows a corresponding R.Q. of 0.75. The theoretical intercept would, of course, be 0.707, and that is substantially the value found by Dewar and Newton for this constant of the equation for one animal. In fact the difference between the predicted R.Q.'s at zero food intake between the two investigations is significantly different statistically, but in any case it is not to be expected that the non-protein R.Q. for a whole day would drop to a minimum in one day's starvation. The carbohydrate stores in the liver and the carbohydrate residues in the gut from the previous day's feed have still to be utilised. The glycogen stores always normally existent must be expected to displace the physiological curve of this relation along the R.Q. axis so that the physiological intercept on the R.Q. axis would be greater than 0.707.

Daily Energy Balance

The energy balance in all the non-pregnant periods, fig. (36) was irregular, The mean value was not significantly different from zero in the first non-pregnant period but was significantly positive in the second non-pregnant period, table (6). This is unexpected since, in the first period growth was active and the stored energy of growth might be expected to appear, while in the second period the bodyweight had, substantially, reached a plateau.

These, and all other energy balances in this work, are subject to considerable but unknown distortion due to the amount, and hence energy equivalent, of the gut contents, especially at the beginning and end of a series. It must also be noticed that the energy balances given in figures and tables here are thermodynamic balances and include a moiety of unutilisable energy corresponding to the nitrogen increment of the organism. This moiety may be positive or negative according as the nitrogen increment is positive or negative.

Within individual series the energy balance corresponded directly to the bodyweight increment. This can best be seen in the preliminary period in fig. (38). This correspondence is probably largely a reflection of change in mass of gut contents.

Oestrus Variation

No consistent variation has been discerned with stage of the oestrus cycle.

Variation with Intake and Expenditure

The great variation in energy balance and in energy intake, from day to day, compared with the relatively small variation in the energy expenditure suggests that the magnitude and direction of the energy balance will be determined by the intake rather than by the expenditure. This is confirmed by comparison of figs. (27), (33) and (36) from which it can be seen that the energy balance variations correspond closely with the peaks and troughs of the energy intake histogram. This correspondence, again, is certainly partly due to the change in mass of gut contents from day to day, and the component of the association attributable to physiological energy balance is probably small.

Starvation

As already pointed out, p.174, female 522 voluntarily starved herself during the 5-day non-pregnant period for which she was on experiment. It must be noted that this is the only part of the work from which a picture of the metabolism during starvation can be obtained, and, being based on one animal for a relatively short period, may not be truly representative. Also, the cause of this voluntary starvation is quite unknown. It may be that some pathological or abnormal initial condition of the animal has distorted the simple starvation effect, but it may be remarked that the subsequent history of the animal showed no gross sign of abnormality.

Energy Expenditure.

The energy expenditure was low on the first day, 27 Cal., rose on the second day to 36 Cal. and then fell steadily to a final level of 25.2 Cal. per 24 hr., vide table (35). This final level is still considerably greater than the expected basal rate for this bodyweight, 15 Cal., as given by Benedict (1938).

Energy Intake.

The energy intake in this case, vide table (35), was closely associated with the energy expenditure, The intake on the first day was 13.6 Cal. with an increase on the second day to 39.1 Cal., corresponding with the increase in expenditure. Thereafter the fall in intake was rapid, being effectively zero on the fourth and fifth days.

Energy Balance.

The energy balance during this period of starvation was always negative, the magnitude of the loss closely following the changes in energy intake, *vide* table (35).

Non-protein R.Q.

The R.Q. had dropped to 0.73 by the fourth day, and on the fifth day fell to 0.54. Here, with the low food intakes over a period of 5 days, and effectively zero intake for 3 days the hepatic glycogen stores would certainly have been exhausted. Such low R.Q.'s have not infrequently been observed by other workers but have often been attributed to experimental error (Poulton, 1938).

The low R.Q.'s observed in hibernating animals may be due to a conversion of fat to carbohydrate, although Benedict and Lee (1938), on the basis of short period studies, hold them to be spurious and due to carbon dioxide retention in the tissues. This latter effect is unlikely to occur to any continued extent, as it is difficult to see how the animal could compensate such an acidosis as would occur if carbon dioxide were retained, at this rate, for a day or more. Neither is either suggestion likely to be the complete explanation, in the present case, when the energy expenditure is still so much above basal. It seems more likely that the low R.Q. may be the result of incomplete combustion of fats to intermediate metabolites.

Energy Metabolism in Pregnancy.

Daily Energy Expenditure.

Relation to Non-pregnant Levels.

It can be seen from figs. (27) and (28) that the daily expenditure did not show a great increase over non-pregnant levels. Fig. (28) shows only a slight increase over resting levels at the latter end of pregnancy with no apparent increase during the greater part of gestation.

In fig. (40) the mean energy expenditure for 3-day periods for females 524 and 525 are plotted. Again the absolute increase in expenditure is low, 9 per cent., but the change is, in fact, statistically significant, (P= 0.01), as can be seen from the analysis in table (3) the standard error of a 3-day period being 1.1 Cal.

Diurnal Variation.

This occurs during pregnancy but, in general, to a lesser extent than in reproductive rest. The direction is the same, a rise in oxygen consumption during the night. The difference between oxygen consumption during the night and the day was about 5-10 per cent., but during the second pregnancy this variation was frequently almost completely suppressed. An example of the oxygen consumption record found in pregnancy is given in fig. (37).

Variation with Stage of Gestation.

Fig. (28), from the data from female 377, shows an increase in metabolic rate in the last few days of gestation. Fig. (39), for female 443, shows a more pronounced and steadier increase, beginning at least by the 4th day, but still small. Fig. (40), however, shows a gradual increase throughout the whole of pregnancy, with a considerable increase between the non-pregnant period and the first pregnant period on the graph, days 10 to 12. The rise thereafter was not significant, but a significant fall in the mean energy expenditure is shown between the second and third pregnant periods, and this is also shown by the data for female 443.

The overall change between periods, in fig. (40) and table (3), was not great; the maximum mean pregnant level is 109 per cent. of the mean non-pregnant level. The changes during pregnancy, compared with each other and with the non-pregnant period are, however, significant ($P = 0.01$).

Effect of Parturition.

On the last day of study, during the first pregnancy of female 524, parturition commenced and the entire litter was delivered by the end of that period. For the 6 hr. prior to the beginning of parturition in this animal there was a great increase in metabolic rate, but there appeared to be no pronounced increase in activity during this period. This high metabolic rate was maintained throughout parturition, but after the birth of all the young the total metabolic rate of mother and young fell slightly although it was still well above the normal pregnant rate. These changes in the metabolic rate before, during and after parturition are responsible for the very high energy expenditure found in the last day of this pregnancy, _vide_ fig. (27).

In the corresponding day of the first pregnancy of female 525 parturition did not begin until the end of the 24 hr. period. The first pup was not delivered until about 2 hrs. after the animal was removed from the chamber. In this case, however, there was no increase in metabolic rate during the immediate ante-partum period. In the second pregnancy of these animals, parturition did not begin until some hours after the animals were taken off experiment, so that the immediate ante-partum period was not observed. During the last day of study during the second pregnancy no pronounced increase in metabolic rate was observed.

With female 377, also, the young were born while the animal was on experiment, but again no pronounced change in energy expenditure was observed, vide fig. (28).

Variation with Parity.

In fig. (40) the energy expenditure of females 524 and 525 are shown for the first and second pregnancies separately. The difference in energy expenditure between the two pregnancies is small but is statistically significant, table (3). The standard error of the means plotted in this graph is 1.1 Cal. from which it can be seen that the first and second non-pregnant periods are significantly different, but the curves come together during the pregnant periods suggesting that the rise in metabolic rate, relative to the non-pregnant level, is less in the second pregnancy. The interaction between period and parity, however, is not significant.

Daily Food Energy Intake.

Variation with Energy Expenditure.

This has already been described, p189.

Relation to Non-pregnant Levels.

Daily food energy intake rose immediately after coitus and continued to rise, although irregularly, until about the eighteenth day of pregnancy. Thereafter the food intake declined rapidly. These changes in food intake are best seen in fig. (28), showing the food intake of female 377. In fig. (41) the average intakes for female 443 are shown, and here the decline in the last days of pregnancy was not so pronounced.

As in the non-pregnant periods the day-to-day variation in energy intake during pregnancy was very much greater than the variation in expenditure, cf figs. (27) and (33), but the increase due to pregnancy was significantly greater than this variation.

In fig. (42) the mean intakes for females 524 and 525 are shown and the analysis of these data in table (4) show that the differences between the food intakes in different periods of pregnancy are significant. The maximum level of food intake during pregnancy shown by these data is 137 per cent. of the mean non-pregnant level.

Variation with Non-protein R.Q.

The food intake and the R.Q. vary together in pregnancy as in reproductive rest, vide fig. (35). In pregnancy, however, this is especially pronounced during the terminal decline in food intake. In fig. (28) can be seen how closely the R.Q. follows the food intake during this phase of pregnancy. The data from females 524 and 525, in fig. (43) and analysed in table (5), show that this change in R.Q. is real.

Variation with Parity.

The data from females 524 and 525 are plotted for the first and second pregnancies separately in fig. (42). Here it can be seen that the energy intake in the second pregnancy was greater, over the whole gestation period, than in the first. The intake in the first non-pregnant period was less than that in the second so that the mean rise from the non-pregnant level was less in the second pregnancy. Also the fall at the end of the first pregnancy was much greater than in the second. The difference in intake between the first and second pregnancies is statistically significant, table (4), and so, also, is the interaction between parity and period of pregnancy indicating that the difference is not a simple displacement. It would appear, therefore, that the differences between the two pregnancies, in rate of rise of food intake and in rate of fall in intake at the end of pregnancy are real.

Daily Energy Balance.

Relation to Non-pregnant Levels.

It can be seen from fig. (36) that the energy balance is very variable in pregnancy, but also the variation is rather less haphazard than in the non-pregnant condition. In figs. (44) and (45) and in table (6) it can be seen that the energy retention levels were significantly higher during the greater part of pregnancy.

The cumulative energy balance curve for female 377, fig. (38), follows closely the cumulative bodyweight curve, fig. (46), for that animal during pregnancy. In the last days of pregnancy, however, the cumulative balance fell, due to the great reduction in food intake over this period.

Fig. (44) for female 443 shows the energy balance to rise suddenly, within a few days of coitus, which confirms the appearance given in fig. (38), and then to remain constant until about the 18th day of gestation, when there was a considerable fall in balance to a level still above the non-pregnant. Fig. (45), for females 524 and 525, shows essentially the same form, but for both animals the fall during the last days was to a definitely negative value in the first pregnancy; this is shown also by inspection of fig. (36) in which the individual daily balances for these animals are presented.

The bodyweight was maintained or increased in the last days of pregnancy, in spite of the fall or reversal of the energy balance, so that over the whole of pregnancy the correspondence between bodyweight increment and energy increment is not so regular as was found for the non-pregnant condition. This can be seen by comparison of figs. (38) and (46).

Variation with Intake and Expenditure.

Fig. (36) and a comparison of figs. (27) and (33) shows that, as in reproductive rest, the direction and magnitude of the daily energy balance was almost entirely determined by the energy intake.

Variation with Parity.

The differences in the mean energy balances between the first and second pregnancies for females 524 and 525 are shown in fig. (45) and analysed in table (6). The initial non-pregnant energy balance was greater in the second than in the first period, the second pregnancy curve being displaced along the energy axis. It also appears, however, that the form of increase in energy balance is different in the second pregnancy, showing a continued increase throughout the greater part of gestation instead of remaining substantially constant after the initial post-coital rise. The fall in energy balance at the end of the second pregnancy was relatively small compared with that in the first pregnancy for these animals, but it is commensurate with that found for female 443, fig. (44). Again the pregnancy-period interaction component of the analysis, table (6), is significant, showing that these changes in form of the balance curves, as opposed to the simple displacement change, are real.

Partition of Energy Gain Between Mother and Conceptus.

It is only from the data on female 377 that a complete picture of this partition can be obtained. Approximations can, however, be made to the total energy gain of the maternal organism in the other animals by measurements of the contained areas in figs. (44) and (45).

These measured and estimated values for total energy gain are presented in table (7). Also given in this table are the total weights of young born in each case. Using the data on the composition of the new-born rat, given by Widdowson (1950), and the heats of combustion of the combustible components of the body, estimates of the heat of combustion of the new-born have been derived for each of the six pregnancies considered. It can be seen that in each case less than one third of the total energy gain was diverted to the foetus, a mean diversion of 20 per cent.

Strictly it is unnecessary to consider the energy diverted to the foetal membranes, in the rat, since these are completely eaten by the mother after parturition, and, therefore, contribute finally to the true maternal energy gain. In table (7), however, the total placental weight in each pregnancy has been computed from the number of young, assumed to be equal to the number of placentae, and the average weight of the placenta at term, found from the data presented later in this thesis. From these estimates and estimates of the total dry matter percentage of the placenta at term, table (3), and assuming that the heat of combustion of the placental dry matter is adequately represented if the dry matter is considered entirely as protein, estimates of the energy equivalent of the placentae have been derived. From these last it can be seen that, even regarding the placental energy as lost to the mother, there is still a gain to the maternal tissues of about 75 per cent. of the total energy retained during pregnancy. The assumption of the placental dry matter as being entirely protein is not, I believe, liable to introduce any gross error to the energy estimate, since the heat of combustion of protein, 5.65 Cal. per g., forms a rough average between that of fat and carbohydrate. Heat of combustion of protein was used here, in preference to the biological energy value of protein, 4.1 Cal. per g., to make the measure comparable with the energy balance data, _cf_ p. .

It may be noted that in females 524 and 525, the total energy gain in the second pregnancy is estimated to be almost twice as great as that in the first pregnancy, although the total weight of young born was only about half that in the first pregnancy. This produces a greatly inflated true maternal energy gain in the second pregnancy.

NITROGEN METABOLISM.

As already pointed out, p. 133, original data on nitrogen metabolism have only been recorded for females 522, 524 and 525. The nitrogen correction for the derivation of energy expenditure for the studies on other animals was found from fig. (48), relating nitrogen excretion to food intake.

The full data on nitrogen metabolism are given in table (38). The data on the nitrogen content of different batches of food, used in table (38), may be found in table (29).

Nitrogen Metabolism in Reproductive Rest.

Daily Nitrogen Intake.

This follows the course of food intake, with only slight deviation due to varying nitrogen contents of the food batches, so the variations in nitrogen intake are effectively identical with those in energy intake already discussed, pp. 189 and 200.

Daily Urinary Nitrogen Excretion

Variation with Food Intake.

Urinary nitrogen is plotted against food intake in fig. (48). The regression equations for this relation in the non-pregnant and pregnant condition have been computed separately and are given in fig. (48) and in tables (8a) and (8b). The two sets of data are significantly different but the regression equation to the combined data is also given, table (8c), as the correction in the energy metabolism equation for the other studies has been derived from it.

The intercept of the regression equation to the non-pregnant data on the nitrogen excretion axis is 90 mg., that is for zero food intake or the endogenous nitrogen excretion. This is significantly higher, cf table (8a), than the average value of 46 mg. per diem for endogenous nitrogen, derived from Brody's (1945) equation for rats of 200 g.

Oestrus Variation.

No association between urinary nitrogen and stage of the oestrus cycle has been found.

Daily Faecal Nitrogen Excretion.

The day-to-day variation in the nitrogen content of the faeces was not great and appeared random (*vide* p.   ). The total faecal nitrogen was largely determined by the total weight of the faeces, *cf* table (38).

In fig. (49) the dry faecal weight has been plotted against the food intake for all the data. It can be seen that there is a close correlation between the two variables with a relatively small residual variance. The intercept on the faeces axis, that is the faecal weight at zero food intake, is 0.75 g. which is significantly greater than zero, table (9). This "endogenous" faecal weight is about 25 per cent. of the average total faecal weight.

Daily Nitrogen Balance.

Difference from Zero.

The daily nitrogen balance for the normal non-pregnant periods studied was variable, as shown in fig. (51), but the mean balance is not significantly different from zero, *cf* table (11).

Relation to Bodyweight Increment.

Comparison of figs. (47) and (50) shows a general correspondence between nitrogen balance and bodyweight increment, but, as with the energy balance, it is probable that much of this apparent association is a reflection of variation in mass of gut contents.

Oestrus Variation.

No association between nitrogen balance and stage of the oestrus cycle has been found.

Variation with Intake and Loss.

Of the ingested nitrogen 80 per cent. was found to have been absorbed. This is about the expected absorption and indicates that the amount of urinary nitrogen taken up by the faeces is not large, cf p. (131).

Comparison of fig. (33), which parallels nitrogen intake, with figs. (50) and (51) for urinary nitrogen loss and nitrogen balance respectively show that the measured nitrogen balance was largely determined by the variations in intake. The urinary loss, although partly governed by the intake, was much more constant than the latter.

Starvation

The data for urinary nitrogen loss for the 5-day period on female 522 have been included in the non-pregnant regression line of fig. (48), and do not depart significantly from this general equation.

The nitrogen balance in this study was consistently and significantly negative throughout, falling to a minimum of -108 mg. on the fourth day. The nitrogen loss followed the bodyweight loss, cf serial nos. 73-77 in tables (38) and (41).

The urinary nitrogen loss fell to a minimum of 64 mg. on the fifth day. This was the lowest urinary nitrogen loss recorded in this work, but although nearer the value of 46 mg., found from Brody's (1945) equation for endogenous nitrogen excretion, is still considerably higher than any of the individual values for this bodyweight range from which Brody derived his equation.

Nitrogen Metabolism in Pregnancy.

Daily Nitrogen Intake.

As in the non-pregnant state, the nitrogen intake corresponded to the food intake, and the description of the changes in food energy intake, p. (200), apply also to nitrogen. That is, there was a 37 per cent. increase in daily nitrogen intake with great day-to-day variation. Also, correspondingly there was a rapid fall in nitrogen intake in the last days of pregnancy.

Daily Urinary Nitrogen Excretion.

Relation to Non-pregnant Levels.

Urinary nitrogen underwent no marked change during the greater part of pregnancy, as shown in figs. (50) and (52). This constancy occurred in spite of the alteration in food intake. The fall in urinary nitrogen which occurred in the last days of pregnancy, however, corresponds, in time, with the fall in food intake.

Variation with Food Intake.

The regression of urinary nitrogen on food intake in pregnancy is shown in fig. (48), and the analysis of its significance in table (8b). The slope of the regression line in pregnancy was steeper than in the non-pregnant state and the difference is significant. The intercept on the nitrogen axis, for zero food intake, is 31 mg. which is not significantly different from Brody's basal value or from zero, but is significantly different from the non-pregnant intercept.

As shown in fig. (52), the urinary nitrogen showed little change until a rapid fall began about the 16th or 17th day of gestation. This fall was statistically significant, table (10), and in both animals the urinary nitrogen was reduced, finally, to almost minimum levels, 150 mg. or 54 per cent. of non-pregnant levels.

Variation with Parity.

The analysis of the variation in urinary nitrogen, table (10), shows a highly significant difference between the first and second pregnancies. The period-pregnancy interaction is also significant. It can be seen from fig. (52) that these differences largely reside in the difference in initial, ie non-pregnant levels, and also in the great difference between the two pregnancies in rate of decline in the last days of gestation. The fall in urinary nitrogen was much more pronounced in the first pregnancy.

Daily Faecal Nitrogen Excretion.

The faecal mass, and hence the faecal nitrogen varied with the food intake in pregnancy as in the non-pregnant condition. The regression equation of faecal weight on food weight for the combined non-pregnant and pregnant datais given in fig. (49) and table (9). Inspection of the graphic arrays plotted separately showed no apparent difference between the sets of data during pregnancy and rest.

This association of faecal mass and food intake continued during the decline in food intake at the end of pregnancy. This is clearly shown by the mean food intakes and faecal weights for the last seven days of pregnancy plotted in fig. (53). The fall in faecal mass in the last days was so pronounced as to be apparent without any graphic construction, as shown by the photograph in fig. (54).

Daily Nitrogen Balance.

Relation to Non-pregnant Levels.

It can be seen from fig. (55) and table (11) that the nitrogen balances during pregnancy were significantly higher than those during rest at all periods of both pregnancies. Fig. (51), however, shows that the balances for individual days were very variable.

Relation to Bodyweight Increment.

Comparison of figs. (47) and (51) shows that the nitrogen balance paralleled the bodyweight increment of the animal, but again this was probably largely due to the variation in mass of gut contents.

Variation with Stage of Gestation.

Fig. (51) shows that, although the nitrogen balances were, in general, positive throughout pregnancy there was a pronounced fall in retention of nitrogen about the 17th to the 19th day of pregnancy with recovery thereafter.

In fig. (56) the nitrogen balances for the last seven days of pregnancy have been plotted separately for each pregnancy and each animal. Included in this figure are the balances for the third, unfruitful, pregnancies. It can be seen here, that,with one exception, the nitrogen balance had dropped to, or below zero on the 18th or 19th day of gestation. From this minimum level the balances rapidly recovered to significantly positive levels by the 20th or 21st day. Even in the exceptional instance, the second pregnancy of female 525, there is some suggestion of a decline with subsequent recovery.

In fig. (57) is shown the mean curve of nitrogen balance along with the scatter diagram of the individual balances. This shows a minimum mean nitrogen balance of 23 mg. occurring on the 18th day of gestation. The scatter, however, is very large, and the true scatter of balance values is exaggerated by the variation in the day of gestation at which the minimum was attained. The analysis of the data for the first two pregnancies, table (12), shows a very low level of significance for variation between days, $P = 0.2$. It may be noted, however, that this analysis includes the one exceptional series, and excludes the third, unfruitful pregnancies.

Variation with Intake and Loss.

Comparison of figs. (33), (50) and (51) shows that the nitrogen balance was paralleled in general trend by both intake and loss, but in detail only by intake. The general association with nitrogen loss is probably largely accountable to the association between intake and loss.

In fig. (53) the mean nitrogen balance, mean food intake and mean faecal loss are plotted for the last seven days of gestation. It can be seen that, whereas the nitrogen balance reached its minimum value at the 18th day, the food intake and faecal loss, and hence the nitrogen intake and faecal nitrogen loss continued to decline until the 20th day of gestation. This recovery of the nitrogen balance to positive levels must, therefore, be entirely achieved by the fall in urinary nitrogen during this period being at a greater rate than the fall in food intake. This is confirmed by the observed significant difference between the coefficients for the regression of urinary nitrogen on food intake in the pregnant and non-pregnant condition, p. (214).

Variation with Parity.

There was no significant difference between the nitrogen balances during the first and second pregnancies, vide fig. (55) and table (11).

Partition of Nitrogen Gain between Mother and Conceptus.

The same methods have been used to assess this partition as have been described for partitioning the energy gain. In this case, however, only four pregnancies are available for the estimate and all total nitrogen gains have had to be estimated. These estimates are presented in table (13).

It can be seen that here about 80 per cent. of the total nitrogen have been diverted to the embryo and associated structures in the first pregnancy. In the second pregnancy the fraction diverted to the embryo was only 40 per cent. These estimates exclude such nitrogen as may be present in the amniotic fluid at term. Due to the fall in total retention in the second pregnancy the actual amounts of nitrogen going to the maternal tissues were of the same order of magnitude in both pregnancies, about 400 and 700 mg. respectively. The energetic equivalent of this, expressed as protein, is 14 and 25 Cal. respectively, a small fraction of the true maternal energy gain.

WATER METABOLISM

The full data on water metabolism are given in tables (39), (40) and (41). In tables (39) and (40) are given the directly observed data and the corrections for instrumental water loss. In table (41) is given the water balance derived from the equation of Peters *et al*, *cf* p. (160), and the graphs and analyses of water balance have been constructed from this indirect balance.

Water Metabolism in Reproductive Rest.

Daily Water Intake.

Fluid Water.

The daily intake of fluid water showed great variation. This can be seen in figs. (58) and (59). Within any series of days the day-to-day variation amounted to 30 per cent. of the mean. Some of this variation has been caused by air locks developing in the water bottle with artificial restriction of water intake, but whenever this occurred the water intake on the subsequent day was greatly increased. However, even excluding these distortions due to technique the normal variation in water intake by the animals was large.

In fig. (60) the daily water intake has been plotted against the daily food intake. Inspection of the graphic array shows that the association is not close and no regression equation has been fitted to them. Such association as does exist is due to the extreme points in the array. When the food or water intake was extremely reduced the water or food intake dropped correspondingly.

No consistent variation was observed with stage of the oestrus cycle.

Metabolic Water.

The estimate of metabolic water, or water of oxidation, is derived according to a similar equation to that used for the derivation of energy expenditure, cf p. (166). Consequently, this water source shows the same variations as the energy expenditure, vide p. (184).

The metabolic water made up about 20 - 25 per cent. of the total water intake, table (39), but this fraction is very variable due to the variation in fluid water intake. The daily weight of metabolic water alone was relatively constant.

Food Moisture.

The moisture content of the food was fairly constant, cf table (29), at about 10 per cent. of the fresh food weight. The contribution of the food moisture to the total water intake was, therefore, very variable and the description of the variation in food intake, p. (189) can be taken to apply also to food moisture. Mean food moisture made up 6 per cent. of the mean total water intake

Daily Water Loss.

Vapourised Water.

As already stated, p. (171), the vapourised water loss has had to be corrected for the instrumental water loss which occurred. To this extent, therefore, these estimates may be considered as unproven. Also, p. (159), the measured partition of water loss into its various components can be accepted only with reservations.

The vapourised water loss (corrected and reduced to a per 24 hr. basis) was quite variable, as can be seen in fig. (58) for female 377, and in fig. (61) for females 524 and 525. The day-to-day variation amounted to 9.5 per cent. of the mean. The amount showed no pronounced association with bodyweight or with energy expenditure. The mean vapourised water in the non-pregnant condition for females 524 and 525 amounted to 15.0 g.

Urinary Water.

Urinary water, with a mean daily output of 6 g. in the non-pregnant condition, was variable and showed a tendency to follow the variations in fluid water intake, as can be seen from fig. (58) and from a comparison of figs. (59) and (62). The day-to-day variation within any given non-pregnant series was 25 per cent. of the mean.

Faecal Water.

The daily loss of water in faeces was also variable as can be seen in the preliminary period of fig. (58) for female 377. The amount of water by this route, however, contributed to the total loss was only 1.5 g. or about 5 per cent., and is of small relative importance.

Daily Water Balance.

Difference from Zero.

Fig. (63) shows that the daily water balance was very variable. The mean values, shown in figs. (71) and (72), were not large and, as can be seen from the analysis in table (17), are not significantly different from zero.

Relation to Bodyweight Increment.

Comparison of figs. (63) and (47) shows an approximate direct correspondence between water balance and bodyweight increment, and in figs. (70) and (46) the cumulative water balance and bodyweight increment curves can be seen to follow each other in form. Again, there is no way of telling how much of this correspondence is due to change in mass of gut contents and how much is due to true physiological gain of body water.

Oestrus Variation.

No consistent variation was observed with stage of the oestrous cycle.

Variation with Intake and Loss.

Comparison of figs. (59), (61) and (62) shows that both the total water intake and the total water loss were variable, and the correspondence between the intake and the balance variations is not any more apparent than that between loss and intake. The urinary water loss, of course, followed quite closely the fluid water intake so it is possible that the urinary water variations contributed substantially to the direction and magnitude of the water balance. It seems likely, however, from inspection of the figured representation of the variations, that the water balance has been predominantly determined by variations in water intake, although not necessarily entirely by variations in fluid water intake.

Starvation.

Water Intake.

The water intake of female 522 fell during the last 4 days of study, from a level of 18.4 to 6.8 g. The intake on the first day was only 6.3 g., as a result of a water bottle blockage. This fall corresponded to the fall in food intake.

Water Loss.

The total water loss fell from 17 to 13 g. by the third day of starvation. About 1 g. of this decline in water loss derived from a fall in urinary water, and the remainder from an immediate fall, by the second day, in vapourised water loss. Both intake and loss recovered slightly on the last day of the period, although the food intake continued to fall.

The full data on water intake and loss for this period are presented under serial numbers 73 - 77 in tables (39) and (40) respectively.

Water metabolism in Pregnancy.

Daily Water Intake.

Fluid Water.

It can be seen from figs. (64) and (65) and table (14) that the fluid water intake showed a considerable and significant increase, 56 per cent., during pregnancy. This is a rather greater increase than that found in food intake, 37 per cent., but as already pointed out, p.(222), and fig. (60) there appears to be no close association between the two increases.

As shown in fig. (58), the water intake of female 377 rose irregularly, but quite definitely, from the date of coitus until about the 15th day of pregnancy. Thereafter, there was a decline to approximately non-pregnant levels at term. In the case of female 445, fig. (64), the water intake had risen by 36 per cent. at the 3rd-6th days of gestation but showed no appreciable mean change thereafter and no terminal decline. In females 524 and 525, fig. (65), intake had attained almost its maximum level by the first pregnancy period, but it is not known whether this was achieved gradually or immediately after coitus. A significant decline in intake, almost to non-pregnant levels, occurred, at least in the first pregnancy, in the last days of gestation.

Although there was this terminal decline, the intake falling over the course of three or four days, there was no marked discontinuity in the intake corresponding with the onset of parturition.

The curves of intake in the first and second pregnancies of females 524 and 525 are also given in fig. (65), but it can be seen from table (14) that there was no significant difference between the two pregnancies. Fluid water intake was, however, one of the only three variables examined, all components of the water exchange, in which a suggestive difference was found between females 524 and 525 ($P = 0.1$).

Metabolic Water and Food Moisture.

The variations in these during pregnancy have not been graphed or analysed specifically, but it can be safely assumed that changes in metabolic water will parallel the changes in energy expenditure, and changes in food moisture will parallel the energy intake. In the former, which contributes the greater amount of water to the total intake, the increase due to pregnancy will not be large; in the latter the increase due to pregnancy will be larger but the amount of water contributed to the total intake is smaller, a maximum of about 2 g.

Daily Water Loss.

Vapourised Water.

Figs. (58) and (66) show the vapourised water loss during pregnancy for females 377 and 443 respectively. It can be seen that the change in this component of the water exchange was slight. There was, however, a tendency to increase, with a decline in the last days of gestation.

Substantially the same trend is shown in fig. (67) for females 524 and 525, but it can be seen from table (15) that, although the maximum increase over the non-pregnant level was only 4 per cent. the increase is significant.

Table (15) also shows that the difference between successive pregnancies was significant although, as seen in fig. (67), it was small absolutely. The decline in vapourised water loss in the last days of gestation was greater in the first than in the second pregnancy, and the significant pregnancy-period interaction indicates that the difference between pregnancies was not simply one of displacement.

Again, the diference between animals was significant to a suggestive level, P = 0.065.

Urinary Water.

In female 377 no appreciable alteration in urinary water loss was found during pregnancy, apart from day-to-day variation, fig. (58). In female 443, fig. (68), a mean increase of 3.5 g. was found in urinary water during the greater part of pregnancy, but a decline, almost to non-pregnant levels occurred in the last 3 days of gestation.

The urinary water losses of females 524 and 525, fig. (69), showed substantially the same trends as that of female 443, but the initial urine volumes were greater and the absolute increase during pregnancy rather smaller, 2.0-2.5 g. The mean increase above the non-pregnant level was 40 per cent., less than the increase in water intake relatively, and only one third of the increase in intake absolutely. It can be seen from table (16) that the increases during pregnancy were highly significant ($P < 0.001$).

The difference in amount of urinary water between rats was highly significant, and so also was that between successive pregnancies. The difference between rats is further confirmed by comparison of the urinary water losses shown in figs. (58), (62) and (68). The pregnancy-period interaction in the analysis is also significant, so the difference between pregnancies is unlikely to be entirely due to simple displacement. That displacement is a major component of the difference can be seen in fig. (69), but it can also be seen here that the decline in urinary water in the last three days of gestation was significantly greater in the first than in the second pregnancy (S.E. of period mean = 0.56 g.).

Faecal Water.

In all animals studied the faecal water showed a general assocation with the total mass of faecal material but the variation was great, cf table (40). The source of this variation probably lies, to a great extent, in normal variations in faecal consistency, but may also involve, to a variable degree, the absorption of urinary water by the faeces.

The faecal water, as measured, shown in fig. (58) for female 377, showed no consistent variation during pregnancy. Also, since this water accounts for only about 5 per cent. of the total water loss any variations have only a slight absolute effect on the water balance.

Daily Water Balance.

Relation to Non-pregnant Levels.

It can be seen from fig. (63) that the water balance over the last seven days of pregnancy is greater than the balance in the immediately preceding non-pregnant period, but it can also be seen that the balance during pregnancy is not necessarily noticeably positive. Thus, in the second pregnancy of female 524 the mean balance in the last seven days was only slightly above zero but the balance in the immediately preceding non-pregnant period was predominantly negative.

Table (17) shows that the probability of the increase in water balance during pregnancy being real is low, although suggestive ($P = 0.1$ for difference between periods).

Variation with Stage of Gestation.

In fig. (70) is plotted the cumulative water balance during pregnancy of female 377. Accumulation of water did not begin until the 15th day of pregnancy. Comparison of this figure with fig. (46) shows that water gain contributed nothing to the weight gain of the animal, 20 g., or 40 per cent of the final weight gain, which had occurred up to the 15th day.

In fig. (71) it can be seen that the water balance during the pregnancy of female 443 did not become positive or noticeably higher than the non-pregnant levels until a sudden increase between the 18th and 20th days of gestation. In females 524 and 525, however, the greater part of such increase in balance as occurred had appeared by the 9th day of gestation, at least in the first pregnancy, fig. (72).

A fall in daily water balance occurred at the end of all pregnancies except that of female 443. The extreme fall in balance which produced the fall in total organismic water shown in fig. (70), and the large negative balance shown in fig. (63) for the last day of the first pregnancy of female 524 was due to the great water loss occurring during parturition and probably largely due to the drying of foetal membranes before their ingestion by the mother. The negative balance which occurred on the 21st day of the first pregnancy of female 525, fig. (63), was due to an air lock in the water bottle on that day.

Variation with Intake and Loss.

The increase of over 50 per cent. in water intake during pregnancy, contrasted with increases of 4 and 40 per cent. in vapourised and urinary water respectively, tables (14), (15) and (16), shows that the water gain during pregnancy was achieved by an increase in water intake, without a corresponding rise in water losses. It is noteworthy, however, that, although the water intake increased, or started to increase immediately after coitus, this rise was offset by the increase in urinary water loss until about the last third of gestation. The increase in vapourised water during this period was so slight as to be negligible, cf fig. (67).

Variation with Parity.

The water balance was very much lower in the second than in the first pregnancy of females 524 and 525. It can be seen in fig. (72) that in the second pregnancy the water balance did not rise significantly above zero, but the final level of balance was significantly higher than the immediately preceding non-pregnant balance. It is shown in table (17) that the parity variation is barely significant, P = 0.03 for difference between pregnancies. Fig. (72) suggests that this difference may not be entirely one of displacement; the relative rise in balance by the first pregnant period is significant in the first pregnancy and negligible in the second. The pregnancy-period interaction component of the variance is, however, insignificant, <u>cf</u> table (17).

Partition of Water Gain between Mother and Conceptus.

The same methods have been used to assess this partition as have been described for partitioning the energy gain. The estimates of the partition are presented in table (18).

In no case is there any evidence that the pregnant organism makes a nett water gain, although there was, in all cases, a gross energy gain. That is, the total water gain during pregnancy either barely accounted for, or was inadequate to account for the total water required for the conceptus. Thus, in the study on female 377, the total water gain during pregnancy was 28 g. While the total weight of embryos and placentae at term was 38 g. This weight of embryonic material contains about 33 g. of water, and this is exclusive of the water of amniotic fluid and foetal membranes other than the placenta. Even taking into consideration the fact that the mother recovered some of this water, that from the fluid and membranes, the total water loss at birth entirely used up the water gained during pregnancy.

The estimates of the total water gain of the other incompletely covered pregnancies indicate that the gain was barely adequate to meet even the foetal requirements and this position is found in an exaggerated form in the second pregnancies, even although these yielded only four young. This means that, at best, the maternal organism post partum was in a state of precarious water equilibrium, and, at worst, was in a state of acute dehydration to the extent of a water loss of 23 g.

RELATION OF BODYWEIGHT INCREMENT TO OTHER BALANCES

It can be seen from fig. (46), showing the cumulative bodyweight for female 377, that the bodyweight increased fairly uniformly from the beginning to the end of gestation. The slight acceleration in rate of growth in weight, occurring at about the 16th day of gestation, can be related to a similar, though less pronounced, acceleration occurring at the same time in energy gain, fig. (38), but is more strikingly related to the initiation of water retention at this stage of gestation, fig. (70).

For the other animals bodyweights are not available throughout gestation so the points of initiation of increment are not known. It can be seen from figs. (73) and (74), however, that the bodyweights for these animals increased proportionally to the time interval since coitus. Comparison of figs. (73) and (71) shows that the bodyweight of female 443 increased, during the greater part of pregnancy, independently of water gain.

The bodyweight gain of females 524 and 525 was very much less in the second pregnancy than in the first, fig. (74). This is undoubtedly due, in part, to the smaller total weight of young in the second pregnancies, but comparison of fig. (74) with fig. (72) and table (18) suggests that a great part of this reduction in bodyweight gain in the second pregnancy was due to the absent or negative water gain which occurred.

In table (19) are assembled the energy, water and nitrogen balances and the bodyweight increments during the non-pregnant and pregnant periods of females 524 and 525. To reduce, as far as possible, the irregularity produced by the day-to-day variation the summed balances and increments over 3- to 5-day periods have been given. Subtracting the water balance from the bodyweight increment leaves that part of the bodyweight increment accountable to dry matter increment. A certain, but unknown fraction of the dry matter increment must be accountable to mineral increment, but it is unlikely that mineral gain throughout pregnancy amounts to more than 2 g., or about 0.25 g. over a 3-day period. (Cf Hummel et al, 1936). The remainder of the dry matter balance must be accountable to proximate principles.

It can be seen from table (19) that, in general, the energy balance agrees fairly closely with the dry matter component of the bodyweight increment. For several periods, however, a negative energy balance corresponds to a positive weight increment, and this occurs after removal from both energy and weight of the effect of nitrogen balance. Thus, in the third period the first pregnancy of female 524, the non-protein energy balance was found to be -30 Cal. and the protein-free dry bodyweight balance was found to be + 1.4 g. There appears to be only one way in which this could occur, apart from experimental error, and that is by the simultaneous combustion of fat and storage of carbohydrate. In this particular instance such a procedure, in its simplest form would entail the combustion of 6.9 g. fat and the storage of 8.3 g. carbohydrate, over the three day period, above the combustion of a weight and energy equilibrated mixture.

No data have been obtained which would enable a direct computation of the carbohydrate and fat stored. The amounts of these substances burned or synthesised have been computed, table (41), but the measurement of balances of these substances individually requires detailed knowledge of the fat and carbohydrate composition of the food and faeces, and this has not been obtained.

METHANOL POISONING.

The accidental 3-day period of methanol poisoning of female 524 has already been mentioned (p. 116 and 176). The data for the energy, nitrogen and water metabolism over this period can be found under serial numbers 107 - 109 in the detailed tables. The diagnosis of methanol poisoning was made, initially, on the circumstantial evidence that methanol checks had been made on the calorimeter immediately previous to this period. Additionally, at the end of the third day, however, a faint acrid smell of acetaldehyde could be detected in the urine and in the water absorbing tubes of the absorbing train. Also a faint smell of methanol could be detected in the animal chamber itself.

During this period the food intake fell to almost zero by the third day, table (34). The energy expenditure also fell rapidly, from 41 Cal. to 28 Cal. per 24 hr. while the energy balance fell to -30 Cal. on the third day. The non-protein R.Q. dropped to 0.75 on the third day, indicating that some carbohydrate was still being utilised. This is to be expected since the food intake on the second day was appreciable, 14.6 g.

The daily nitrogen intake during this period showed a rapid fall from 330 to 5 mg., corresponding to the fall in food intake. The daily nitrogen loss in the urine fell from 271 to 68 mg. This produced a fall in nitrogen balance, over the 3-day period, to -96 mg. per diem.

The water exchange followed a course rather different from that found during the 5-day period of "simple" starvation and dehydration in female 522. The water intake fell on the second day from 21.6 g. to 5.6 g. but recovered on the third day to normal. Corresponding to the fall in respiratory gaseous exchange the elimination of vapourised water also showed a marked fall, from 13.6 to 8.6 g., on the third day. The urinary water, however, was maintained at normal levels throughout. With the high urine volume the water balance remained negative on the third day in spite of the recovery in fluid water intake and the fall in vapourised water loss.

During this period the animal lost 27 g. or 12 per cent. of its initial bodyweight. In fig. (75) is shown the curve of bodyweight during and for 20 days after the poisoning incident. It can be seen that the animal continued to lose weight for 3 days after its removal from the calorimeter, entailing a total weight loss of 50 g. or 22 per cent. of its initial bodyweight. Recovery of this weight was slow and incomplete.

By the third day in the calorimeter the animal's appearance was unthrifty, its coat was staring, and its activity was negligible. Appearance, coat condition and activity appeared fully recovered within two days after its return to the animal house, although its bodyweight continued to decline.

After 10 days, when the bodyweight had recovered to 207 g. or 90 per cent. of the initial weight, the animal appeared perfectly healthy and normal, and was brought back into the experiment.

PLACENTAL GROWTH IN THE RAT.

Review of the Literature.

It has been well established for the rabbit, sheep, goat and man that the placenta stops growing a considerable time before term. The actual time of cessation or slowing of growth varies with the species. The major available data on placental growth have been summarised by Barcroft (1946). Also, Barcroft and Young (1945) showed that if the gestation of the rabbit be prolonged by the subcutaneous injection of chorionic gonadotropin and progesterone the foetuses continued to grow, but the placentae, although maintained without exceptional degenerative changes, showed no further increase in size.

It seemed possible that, if there is a cessation, or even a pronounced fall in the rate of growth of the placenta of the rat before the end of gestation, at the time when the growth rate of the foetus is at a maximum, the change might show some reflection in the general pattern of the maternal metabolism.

Claims have been made that the rat placenta reaches its maximum weight before term, but the evidence is slighter than for the species mentioned above, and is not completely in concord. Incidental data recorded by Flexner and Pohl (1941c) indicate that the maximum placental weight in the rat is achieved at about 19.5 days gestation; they found no weight increase between 18.7 and 20.9 days gestation. The measurement of a time scale in such a short gestation period is liable to error, and it seems unlikely that the gestation age can be fixed to within 0.1 of a day with any degree of reliability. However, referring the placental weight to the foetal weight with elimination of the time scale shows that the foetal weight increased from 1.4 to 3.6 g. without any corresponding increase in the placental weight. Flexner, indeed, suggests a fall in placental weight corresponding to the foetal weight increase from 2.5 to 5 g., with recovery of placental weight thereafter. Leonard (1945) found mean placental weights of 362 mg. at 19 days and 558 mg. at 22 days gestation; this does not indicate any cessation of growth during the 20th day, but no data are given for the intervening period. Pritchard and Huggett (1947) found that the surviving rat placenta, after experimental foetal death, stopped growing at about 16 days gestation. The size of the placenta was measured as the area of a central placental section. It must be noted here, however, that Leonard found that the placental weight at 22 days, when the foetus had been removed surgically was only 141 mg. compared with 558 mg. at normal term.

In view of the above disagreement concerning the form of placental growth in the rat, it was decided to investigate the matter further.

Animals.

Two series of Hooded Wistar rats were used. One, a general series of 13 females, was used to obtain a general picture of the form of placental growth. The other series consisted of 9 animals in three sets of three litter-mates from which it was hoped to obtain information on the effect of parity on placental growth. The characteristics of all the animals used are given in table (28), appendix (2).

Methods.

The animals were fed on the stock diet 41 used in the metabolic work. They were maintained in the animal house under the normal stock conditions. Dates and times of mating were determined by the appearance of vaginal smears, vide p. (177). The animals were killed at various times from the 12th to the 22nd day after mating, as shown in table (20). In the litter-mate series, each set of litter-mates was assigned, at random, to be killed on the 15th, 18th or 21st day of gestation. Animals within a litter-mate set were assigned, at random, to be killed on the appropriate day of the first, second or third pregnancy. In all cases the third pregnancy failed.

Of the general series, pregnancy failed in 5 animals, or 38 per cent. In one of these animals, female 431, killed on the 16th day of gestation, a full complement of resorption sites was visible to the naked eye, corresponding to the full number of corpora lutea present in the ovaries.

The animals were killed, on the appointed days, by coal gas. The abdomen was opened along the mid-line and the uterus removed, severing just below the cornual junction and at the points of emergence of the Fallopian tubes from the uterine horns. The mesentery and attached fat were carefully cut away. The full uterus was weighed rapidly, and the conceptuses were dissected out. Below the gestation age of 14 days it was not found possible to part the placenta cleanly from the uterine wall, and only one measurement is available for 12 days gestation. At gestation ages of 14 days and above, gentle blunt dissection was sufficient to ensure clean separation.

Each conceptus was dissected from the uterus and all weighings made of that embryo and placenta before the next conceptus was removed. This prevented any appreciable desiccation of the last embryos and placentae to be weighed

Whenever it could be dissected out without rupturing the amniotic sac, the entire conceptus was weighed on a beam balance. This was not always possible, especially in the later stages of gestation when the amniotic fluid has largely resorbed and has been replaced by a Wharton's jelly-like substance, and the amnion becomes very fragile with the first surface desiccation on being exposed to the air.

The amnion was then slit and the embryo removed, the surface of the latter dried with filter paper and the embryo then weighed on a 1000 mg. torsion balance. After 18 days gestation the embryos were too heavy for the capacity of this balance and were weighed on the beam balance. The umbilical cord and the remnants of the amnion were carefully dissected from the placenta which was then weighed on the torsion balance, again after blotting excess fluid off the surface.

After weighing the intact uterus, but before opening it, the ovaries were dissected out and the number of corpora lutea in each was counted, distinguishing right and left. The numbers of embryos in the right and left horns of the uterus were also recorded.

The entire process, from the removal of the uterus to completion of the weighings took from 10 to 20 min., depending on the number of embryos present. In all cases 2 to 4 embryos and placentae were not weighed, the conceptus being cut off, complete with its portion of uterine wall for immersion in fixative for histological examination.

Results.

The full detail of the original observations is presented in table (42), giving 84 placental and foetal weights from 14 animals. No difference was detected between the findings for the first and second pregnancies in the litter-mate series, and so all the results have been combined in one series. The mean values for weights of foetus, placenta and conceptus, with the total weight of the intact uterus, for each animal are presented in table (20).

The weights of foetuses and placentae, respectively, increased from about 140 and 115 mg. at 14 days to 5000 and 500 mg. at 22 days gestation. The weights of both embryos and placentae, within a given pregnancy and between different pregnancies of the same gestation age were very constant up to the 18th day of gestation. Thereafter the scatter of weights increased greatly. This can readily be seen from the table of original data, but is also apparent from the mean values plotted in figs. (76) and (78). Within any one pregnant uterus at any gestation age little consistent relation was found between the foetal weights and the corresponding placental weights.

In figs. (76), (77), (78), (80), (81) and (82) only mean values for foetal and placental weights from each animal have been plotted. The fitted curves in these figures, however, have been computed from the original observations. The analyses of the regressions are given in tables (21), (22) and (23).

The foetal weights show a bradyauxetic form of growth with time, as shown in fig. (76), which is to be expected from the generally known form of mammalian embryonic growth. The mean conceptus weight, the embryo with all membranes and waters, also plotted in fig. (76), follows a parallel course at least up to the 18th day of gestation which was the latest time at which these weights could reliably be measured.

In fig. (77) are plotted the values for the total weight of the pregnant uterus divided by the number of embryos present. Again the same form of growth is demonstrated, but displaced about 1 g. up the weight axis and showing a greater acceleration of growth.

The growth of the placenta can be seen, in fig. (78), to be isoauxetic, that is linear with time. The analysis of the regression equation to the data shows that regression accounts for 75 per cent. of the total variance. The plotted means in this figure show some slight indication of a discontinuity at about 18 days, but this may be merely a reflection of the greater scatter of individual weights beginning at this time.

There is no indication of any slowing of growth up to the 21st day of gestation. The mean placental weight for the one pregnant uterus examined on the 22nd day is inflated by the presence of one very heavy placenta, 908 mg., and so the appearance of continuation of placental growth up to the 22nd day may be spurious.

The equation found for the regression of placental weight on time is:-

$$P(\text{mg.}) = 46.3\ T(\text{days}) - 556$$

The constant term, 556, is obviously meaningless in this equation since placentation in the rat is not initiated until about the 9th day of gestation, Bridgeman (1948). The intercept of the line on the P axis at 12 days gestation, however, is not significantly different from the observed weight on that day, as shown in table (21).

In fig. (82) the placental weights have been plotted against the corresponding foetal weights. This device eliminates possible distortions introduced by inaccurate estimation of the equivalence of gestation ages. Again, the same bradyauxetic form of foetal growth is shown. If the placental growth be accepted as isoauxetic then the apparent slowing of placental relative to foetal growth can be taken as entirely due to the acceleration of foetal growth.

The choice of forms of equation which will most adequately represent the non-linear curves involving foetal growth can best be made by considering the logarithmic relation between foetal and placental weights. For simplicity some constants are omitted from the following theoretical equations.

If the placental growth with time be accepted as linear, and the foetal growth with time be assumed to be exponential the relations can be represented by:-

$$P = a + bT \qquad F = ce^{kT}$$

The joint relation, eliminating time, can then be represented in the form:-

$$P = r + s \log F$$

In fig. (79) absolute placental weight is plotted against log foetal weight, and it can be seen that the curve departs markedly from the linear form. It thus appears that an exponential equation will not adequately represent the data on foetal growth.

If, alternatively, Huxley's allometric equation be proposed for the representation of foetal growth, the relations become:-

$$P = a + bT \qquad F = cT^{k}$$

giving a joint relation of the form:-

$$\log P = r + s \log F$$

In fig. (80) log placental weight is plotted against log foetal weight and it can be seen that this relation is substantially linear. The analysis of the regression in table (22) shows that the regression accounts for 80 per cent. of the total variance. The equation found to this regression of log placental weight on log foetal weight is:-

$$\log P = 1.076 + 0.438 \log F$$

This can be converted to the absolute form giving:-

$$P(\text{mg.}) = 11.9\ F(\text{mg.})^{0.438}$$

In fig. (81) log foetal weight is plotted against log time and the regression equation for the logarithmic form of the allometric equation relating foetal weight and time is also given:-

$$\log F = 8.414 \log T - 7.565$$

Again the logarithmic relation is substantially linear, the regression removing over 90 per cent. of the total variance, <u>cf</u> table (23). This can be converted to the absolute form giving:-

$$F(\text{mg.}) = \frac{T(\text{days})^{8.414}}{36560000}$$

From the relations between placental weight and time, and foetal weight and time, another form of the relation between placental weight and foetal weight can be derived:-

$$P(\text{mg.}) = 367\,F(\text{mg.})^{0.119} - 556$$

In fig. (82) the line to this equation is drawn, along with the line to the equation derived from the directly computed logarithmic regression equation. It can be seen that both fit the data closely. The detailed difference in form is due, in great part, to the neglect of constants which would have made the analysis very unwieldy.

The weights of the empty pregnant uterus in each animal were estimated as the total weights of the pregnant uteri less the total conceptus weights contained by each. These empty weights are plotted against gestation age in fig. (83) along with five weights of non-pregnant uteri. It can be seen that there was little increase in uterine weight up to the 14th day of gestation. Thereafter there was a sharp increase in weight to the 15th or 16th day, followed by a slower rate of growth to term. Reynolds (1949) presents a curve of similar form for the growth of the pregnant uterus in the rat, but the increase shown there at about the 15th day of gestation is much less pronounced than that indicated by the present estimate. Also, Reynolds' curve shows only a slight tendency to deceleration of growth in the last days, and even this tendency is only apparent for small litters.

The point of rapid increase in weight of the empty uterus corresponds in time to the period of maximum acceleration of foetal and conceptus growth, <u>cf</u> fig. (76). The later inhibition of uterine growth occurs, however, when the rate of foetal growth in weight is almost at a maximum. It seems possible, therefore, that the rapid increase in uterine weight may be due to the stimulus to growth formed by the accelerating distention produced by the growth of the conceptus. The later inhibition of uterine growth, if this effect be real may be due to the growth inhibiting effect of oestrogens; or it may be, in part, a mechanical effect resulting from the release of tension after the conversion of the conceptus from a spherical to an ovoid form, Reynolds (1949).

It must be noted that many more measurements of uterine weight must be made before the detailed form of growth shown in fig. (85) can be confirmed, and the acceleration and inhibition phases shown to be real or spurious.

DISCUSSION AND INTEGRATION

The findings from this work, with respect to the energy exchanges of the rat, are in general agreement with expectation from previous work. The total energy expenditure, which has not previously been measured over such long periods, shows some increase, 10 per cent., in spite of the reduction in physical activity. It seems possible, therefore, as suggested earlier, p. (20), that the basal increase during pregnancy in the rat may be rather larger than the 15 per cent. reported for human pregnancies. On the other hand the diurnal variation in metabolic rate, presumably due to the very obvious, although unmeasured increase in activity during the night, covered a range of only about 10 per cent. of the mean expenditure. Thus it appears that activity in the rat may be performed at a high efficiency, that is with a small energy increment over basal, although this suggestion is at variance with accepted belief regarding muscular efficiency. Also, it must be considered that probably a considerable fraction of the increase in total energy expenditure is attributable to the increase in S.D.A. corresponding to the increased food intake.

The increase found in food intake is rather larger than that reported by previous workers, 37 as against 0 - 30 per cent. The present work, however, shows a substantial similarity in form of the increase in food intake, that is a rapid increase after coitus with a slower rise thereafter continuing until the ante-partum decline beginning about the 16th day.

The excess of the increase in intake over the increase in expenditure accounts for the continuously positive energy balance which was maintained during the greater part of pregnancy. The close parellelism observed in the form and magnitude of increment between energy balance and bodyweight is of particular interest in that almost the whole of the bodyweight increment up to about the 15th day of gestation can be accounted for as increment in proximate principles. Of the total bodyweight gain at term in the first pregnancy, including embryos and associated structures, of the order of half is accountable to energy-yielding dry matter gain.

If the embryonic structures alone be considered, only one fifth of their weight is dry matter, so that about two thirds of the total energy gain during pregnancy must go to maternal tissues.

The finding that an appreciable fraction of the total energy gain is retained by the maternal tissues is not unexpected from previous evidence. It is, perhaps, surprising that as little as one third or less of the total energy gain should be diverted to embryonic tissue. This finding, however, does go some way towards describing the composition of the permanent weight increment known to occur after the first pregnancy, "as many young husbands have reason to know" (Chesley, 1944).

The evidence given of an even larger energy increment during the second pregnancy when, in both cases studied, the number of young was less than half that in the first pregnancy, indicates that the stimulus to energy or proximate principle retention is not dependent on the mass of the embryonic tissues. This does not, of course, necessarily mean that the stimulus is not of embryonic origin. It is quite possible that if, for example, the stimulus arises from the placental hormones, one placenta can produce as adequate a stimulus as ten. On the other hand, the fact that the energy retention was continuous from the beginning of pregnancy argues in favour of the retention stimulus being of maternal origin.

The apparent parity effects found for energy intake, expenditure and balance cannot legitimately be extrapolated beyond the present series of experiments without further showing. This *caveat* holds also for all the other parity effects observed. Parity itself is associated with a number of other variables, and the effects apparently shown may easily be attributable to a reflected association with, for example, age or number of young. In the case of the energy exchanges, where invariably there was increase in the second pregnancy with a coincident drop in number of young, it seems unlikely that number of young is an important factor. The effect of age has yet to be examined, and Mayer's (1949) postulate of the change in direction of tissue synthesis with age suggests that the latter may play at least some part in altering the stimulus for energy retention during pregnancy. This possibility is further favoured by the fact that no parity effect was observed in nitrogen retention, with the corollary that the energy retention is largely in the form of fat storage. The high R.Q.'s which occurred during the greater part of pregnancy also confirm this.

The most striking aspect of the nitrogen metabolism data is the decline and recovery of the positive nitrogen balance. The balance reaches its lowest level at about the 19th day of gestation, and it appears that the decline is primarily due to the fall in food intake beginning about the 16th day. The source of the recovery in nitrogen balance lies in the fall in urinary nitrogen which occurs at a greater rate with fall in food intake during pregnancy than during reproductive rest.

Although the source of the recovery can be established from the data, the stimulus is unknown.

The possibilities of hormonal influences initiating and maintaining nitrogen retention during pregnancy have already been discussed, (p.p. 27-30). It is possible that a similar hormonal control may induce the fall in urinary nitrogen.

It must be stressed that this decline and recovery of the nitrogen balance in the last third of pregnancy has not been found to be statistically significant at conventional levels of probability. The great scatter observed is largely due, however, to the time relations of the decline, and not only to the existence or extent of the decline. This variation in the gestation age of the minimum nitrogen balance makes the data difficult to analyse adequately.

Because this change in nitrogen balance is not statistically significant, it cannot be accepted on the present evidence. The present findings, however, taken along with the indications from previous work of a minimum nitrogen balance in mid-pregnancy in the bitch, are sufficiently suggestive to encourage a further investigation of the nitrogen metabolism during pregnancy in the rat and other laboratory animals.

Apart from the one or two days of low or negative nitrogen balance about the 19th day of gestation, the balance was uniformly positive at all periods of pregnancy studied. The estimated total balance for the whole of pregnancy shows a great excess over that required for embryonic tissues. The excess was relatively greater in the second pregnancy where the number of young was less, but the total nitrogen retention in the second pregnancy was less. The summed result of these changes is that the total absolute amount of nitrogen retained by the maternal organism was approximately the same in each pregnancy, about 400 to 600 mg. Nitrogen retention can, therefore, only account for 2.5 to 4 g. of any excess weight gain of the mother over the weight of uterine contents. Similarly, it accounts for, at most, 30 per cent. of the total maternal energy gain.

The final maternal nitrogen gain is in agreement with the indications of previous work, especially that of Bar (1907). The appearance of a nitrogen gain makes it difficult to explain the reproductive failure of the experimental animals on the basis of any common nutritional deficiency since displacement to a negative nitrogen balance is commonly an early sign of such deficiency. Even although the particular endocrine relations existing during pregnancy might enable a nitrogen gain to occur in a condition of nutritional deficiency which would normally produce negative nitrogen balance, it is noteworthy that the balance was not significantly different from zero, or was positive during the non-pregnant periods.

Apart from the special changes in nitrogen balance occurring in the last days of gestation, the positive nitrogen balance which occurred over the greater part of gestation is entirely attributable to the increase in food intake during pregnancy. The increase in food intake of 37 per cent. amounts to an absolute increase in intake of about 5 g., or 125 mg. nitrogen. Taking account of the increase in faecal mass and nitrogen, cf p. (215), this gives an increased nitrogen absorption of about 80 to 90 mg. The increase in urinary nitrogen excretion, 5 per cent., reduces the nitrogen retention further, to about 70 mg., which figure completely covers the observed increase in nitrogen balance.

The findings on the pattern of water metabolism in pregnancy are very much less in accord, than those on energy and nitrogen metabolism, with a priori expectation from previous work. Expectation was necessarily from indirect evidence, and no other comparable direct data are, so far, available for any animal.

The water balance in reproductive rest was effectively zero. On the other hand all the components, both of intake and loss, contributing to the balance showed great variation. This was also true over the period of consistent water retention during pregnancy. It is apparent, therefore, that no reliable estimate of the direction or magnitude of the water balance can be obtained from measurement of intake or of output only, or merely of fluid water exchanges. A mean positive daily water balance of 4 g., the maximum observed amounts to only 13 per cent. of the total daily intake or output of water, and this could be completely masked by one component of gain or loss unless the balance be estimated from the total water or dry matter exchange.

The total heat eliminated by vapourisation of water, assuming all the water so measured to be vapourised from the animal, amounts to about 22.5 per cent. of the total energy expenditure. This is in close agreement with the finding of Greene and Luce (1931) who give an average value of 23.8 per cent.

There is no way of partitioning this vapourised water loss into the fractions arising from the lungs and from the skin. It is possible, however, on the basis of several assumptions, to derive an estimate of this fraction.

If the mean tidal air and respiration rate respectively, be taken as 1.5 ml. and 60 per min., as given by McCutcheon (1951), the total daily ventilation of the rat becomes:-

$(1.5 \times 60 \times 60 \times 24)/1000 = 130$ litres (approximately)

If it be further assumed that, under the conditions of the experiment, air was inspired at 25°C and 75 per cent.

relative humidity, and expired at 37°C and 100 per cent. relative humidity, then the loss of water by the rat per litre of ventilated air would be:-

$$0.045 - 0.018 = 0.027 \text{ g.}$$

This gives a total daily loss of water vapour in the expired air of about 3.4 g. or 23 per cent. of the total loss of vapourised water. Tennent (1946) found a loss via the lungs of 46 per cent. in an unanaesthetised and 26 per cent. in an anaesthetised animal.

There is evidence for man that the expired air is not fully saturated with water vapour at 37°C, but can be considered as equivalent to air fully saturated at 34°C (Galeotti, 1912; Osborne, 1913). If corresponding values for the rat be substituted in the above calculations, the respiratory water loss is lowered to 17 per cent. of the total vapourised water loss.

The great discrepancy between this estimate and that of Tennent is not necessarily real. McCutcheon's average for tidal air is possibly rather low, and he records a wide range, up to 6 ml. This range completely contains the discrepancy of the estimates.

No evidence was found in any study that the total cumulative water balance during pregnancy was in excess of the requirements of the conceptuses and, in general, it has been found that the gross water gain was less than that represented by the uterine contents. This finding is in direct contradiction to the water gain commonly assumed on the basis of bodyweight gain, and also on the basis of the haemodilution known to occur during pregnancy in the rat. The bodyweight gain was in excess of the accretion of uterine contents, but this excess is accountable to the maternal gain in proximate principles.

There is the possibility of species difference, for there is no reason to suppose that the rat is similar to man in this respect. There is wide variation in the indirect evidence for water gain during pregnancy even within one species, and it would be patently unjustifiable to attempt to refer these findings to mammalian pregnancy in general.

In view, also, of the abnormality of the reproductive process shown in this work by the failure of all third pregnancies and the rather unsatisfactory performance in the second pregnancies, it cannot be asserted that the form of water balance observed here is a normal or usual feature of pregnancy even in the rat. The form of water balance observed implies a considerable degree of dehydration of the maternal tissues. This is accentuated by the simultaneous accretion to the maternal organism of energy yielding dry matter to the extent of about 5 - 10 per cent. of the total initial bodyweight of the animals, or up to 25 per cent. of the initial body dry matter.

The evidences of zero or negative maternal water gain, co-existing with an excess maternal bodyweight gain which is substantially accountable to energetic dry matter gain, is however, suggestive. The animals showed no external sign that the dehydration to which they had been subjected was anything other than normal. The only evidence of a pathological condition was in female 524, in which there was found, on post mortem examination, heavy leucocytic invasion of the right ovary and tube with an ovarian cyst. This was evidently of recent origin and the last metabolic study to be considered in this work from this animal was made about a month before the animal was killed.

It has already been suggested that the failure in reproductive performance may have been of nutritional origin. The high incidence of failed gestations, especially in the later pregnancies, combined with the infrequency of observation of actual resorption sites suggest that if resorption was the cause of failed pregnancy then it occurred early in gestation or that the failure was one of implantation. These events could be caused by vitamin E deficiency or by deficiency of essential fatty acids. The presence in the diet of 1 per cent. cod liver oil makes the deficiency of essential fatty acids unlikely. Also none of the other lesions typical of this deficiency appeared. It is, on the other hand, noteworthy that the original form of Diet 41 contained 2 per cent. cod liver oil but that this was reduced to 1 per cent. because of the development of muscular dystrophy, due to vitamin E deficiency, developing in suckling young of mice receiving this diet, Bruce (1950). There is some evidence that the mouse may be more tolerant of vitamin E deficiency than the rat, since male mice do not develop testicular degeneration on an E deficient diet, Russell (1948), but no work has been done on the relative minimum protective levels of this vitamin for the two species. Bruce's (1950) revised Diet 41 was tested almost exclusively on mice and if these have a greater tolerance then the discrepancy, between her findings and the inadequacy of the diet for rats found by me might be explained.

The existence of maternal dehydration suggests a sodium or potassium deficiency and the work of Heppel and Schmidt (1938), <u>vide</u> p. 35, particularly indicates the latter. This question has not been investigated analytically. There is evidence that the stock rats used here show a preference for dilute saline as a drinking fluid. This is, however, very slight evidence of deficiency as it is a common preference of rat colonies and is rapidly lost if adequate supplies of dilute saline are made available along with fresh water.

(Randoin et al, 1950).

Against the possibility that the maternal dehydration may be the result of some nutritional or other abnormality associated with the reproductive failure, is the fact that only precarious maternal water equilibrium was maintained by female 377 although she received a stock diet known to be adequate for continued reproduction.

It is possible that the conditions of the experiment played some part in the production of dehydration. This is unlikely, however, as the high humidity in the animal chamber would not favour water loss.

The parity changes in vapourised and urinary water loss and in water balance, with no significant change in water intake show that the reduction of water gain in the second pregnancy resides in the increase in losses and not in a fall in intake. Much of this reduction in water gain is referable to the smaller number of young in the second pregnancies, and there may be no true parity change as such.

It is of interest to note that the appearance of positive water balances during pregnancy corresponds, in time to the period of accelerated growth of the embryos. It is also about this time that the placenta is attaining its mature form, so that on this evidence either foetus or placenta may supply the stimulus for water retention.

Newton's findings, described earlier, p. (35), suggest that the placenta is the active structure. It is also at this time that the uterus shows its accelerated growth period so that, whatever the stimulus producing the water retention it occurs at the time when water requirement by the uterus and contents becomes appreciable. It appears likely, therefore that, although the retention of energy finds its stimulus probably in the maternal organism, water retention is more probably under the control of embryonic activities. The term embryonic is used here to include all foetal membranes, although there is some evidence that the origin of some of the placental cells, which may be engaged in the endocrine activity of the placenta, may be maternal.

Apart from this association in time of water balance direction and embryonic growth, no relation has been observed between embryonic growth and maternal exchanges. The failure to show any slowing of placental growth in the rat means that if maternal exchanges are related to rate of placental growth the relation must be investigated in some other animal, such as the rabbit.

It is necessary to note here that the wet weight of the placenta is a relatively crude measure of placental size and may bear no very close relation to functional placental size. The latter will be determined by the total vascular area for physiological exchange between mother and foetus, and also by the permeability of the intervening tissue membranes. The total material exchanges between mother and foetus may also be, to a considerable extent, independent of even such a measure of physiological placental size, since they must be under the influence of the rates of maternal and foetal blood flow. Nevertheless, Flexner's demonstration of the transfer rate of ions as bearing a closer relation to the absolute growth rate of the placenta than to that of the foetus, indicates that the simple weight measure may not be very far removed from a truer physiological measure of placental size.

It is not possible, from the data available, to make any detailed assessment of the partition of the rise in energy expenditure between the maternal tissues and the embryonic tissues. The smaller increase in energy expenditure in the second pregnancies, 6 as compared with 14 per cent. in the first pregnancies suggests that the mass of embryonic tissue may play the dominant role. On the other hand, in all pregnancies there was a fall in metabolic rate in the last three days of pregnancy when the embryonic tissue is reaching its maximum mass, while the rise in metabolic rate occurs early in pregnancy, at least by the 9th day, when the mass of the embryonic tissue is negligible.

Even if the increase in metabolic rate were predominantly maternal an increase in rate per unit mass of tissue cannot be inferred when the great and continuous addition of tissue mass to the mother be considered. If, however, the greater part of the tissue mass accruing to the mother be fat, as the evidence indicates, then the metabolic rate of the additional tissue may be low.

SUMMARY

(1) The relevant literature on the energy, nitrogen and water metabolism during pregnancy is discussed.

(2) The methods employed in the present work, to investigate the metabolism during pregnancy in the rat are described in detail.

(3) The construction of a respiration calorimeter for the measurement of the total metabolism for continued successive periods of 24 hr. in the rat is described in detail.

(4) The operation of this instrument is described in detail.

(5) Theoretical considerations in the computation of energy exchanges from the form of data obtained are stated. The interpretation of these exchanges is discussed.

(6) The results, with illustrative diagrams, are presented of a total of 173 days' study of the metabolism of the hooded rat. These results cover six pregnancies and sixteen 3- to 5- day periods of reproductive rest. They include data on the components of energy, nitrogen and water exchange.

(7) Energy expenditure was found to rise by 10 per cent., food energy intake by 37 per cent. and energy balance was found to be consistently positive during pregnancy.

(8) Nitrogen balance was positive in all parts of pregnancy in which it was studied. Some evidence was found for a decline in nitrogen balance to near zero at about the 19th day of gestation, with subsequent recovery to positive levels.

(9) Water balance was not significantly different from zero until about the 15th day of gestation. Thereafter there was a consistent water gain by the pregnant organism. No evidence was found of a maternal water gain during pregnancy, and in five out of the six pregnancies there was evidence of dehydration of the maternal tissues. Water intake, vapourised water loss and urinary water loss rose during pregnancy by 56, 4 and 40 per cent. respectively

(10) Significant increases in energy expenditure, intake and balance; in respiratory quotient and in vapourised and urinary water losses, with decreases in fluid water intake, water balance and bodyweight increment were found between the first and second pregnancies. It is pointed out, however, that these changes may not be true parity effects, and may be due, in part, to associated change in litter size.

(11) Data are presented on the growth of the rat foetus and placenta. The growth of the foetus can be represented by an allometric equation. The growth of the placenta was found to be linear with time, and no evidence was found of any cessation or slowing of growth

before the end of gestation.

(12) The significance of these results is discussed, and an attempt is made to assess their general validity as representative of the pattern of pregnancy in the rat.

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Studies of the Metabolic Pattern of Pregnancy in the Rat

by

S.D. Morrison.

VOLUME 2

Figures and Tables

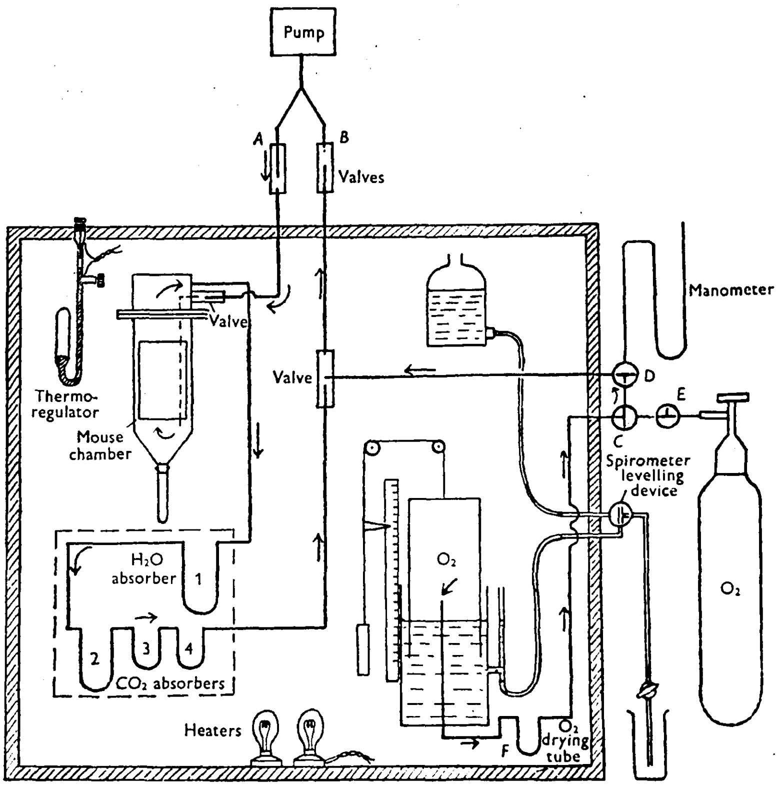


Fig. 1. General plan of mouse metabolism apparatus.

Diagram of respiration calorimeter as constructed by Dewar and Newton (1948).

Figure (1)

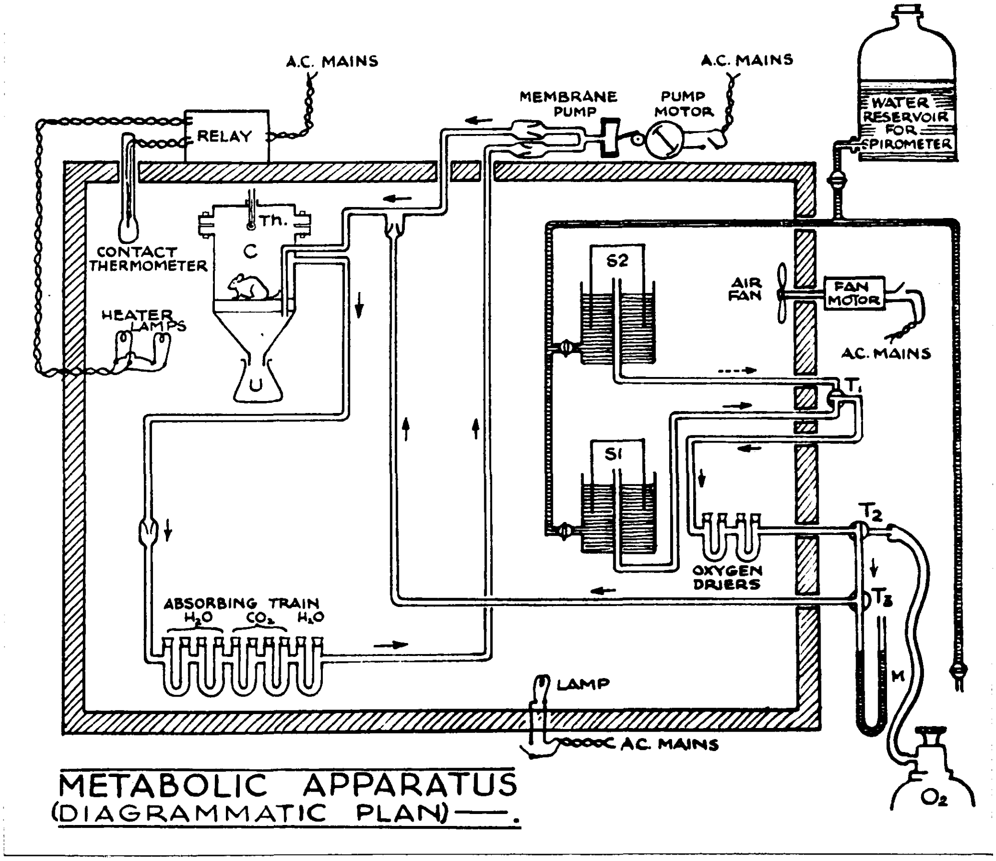
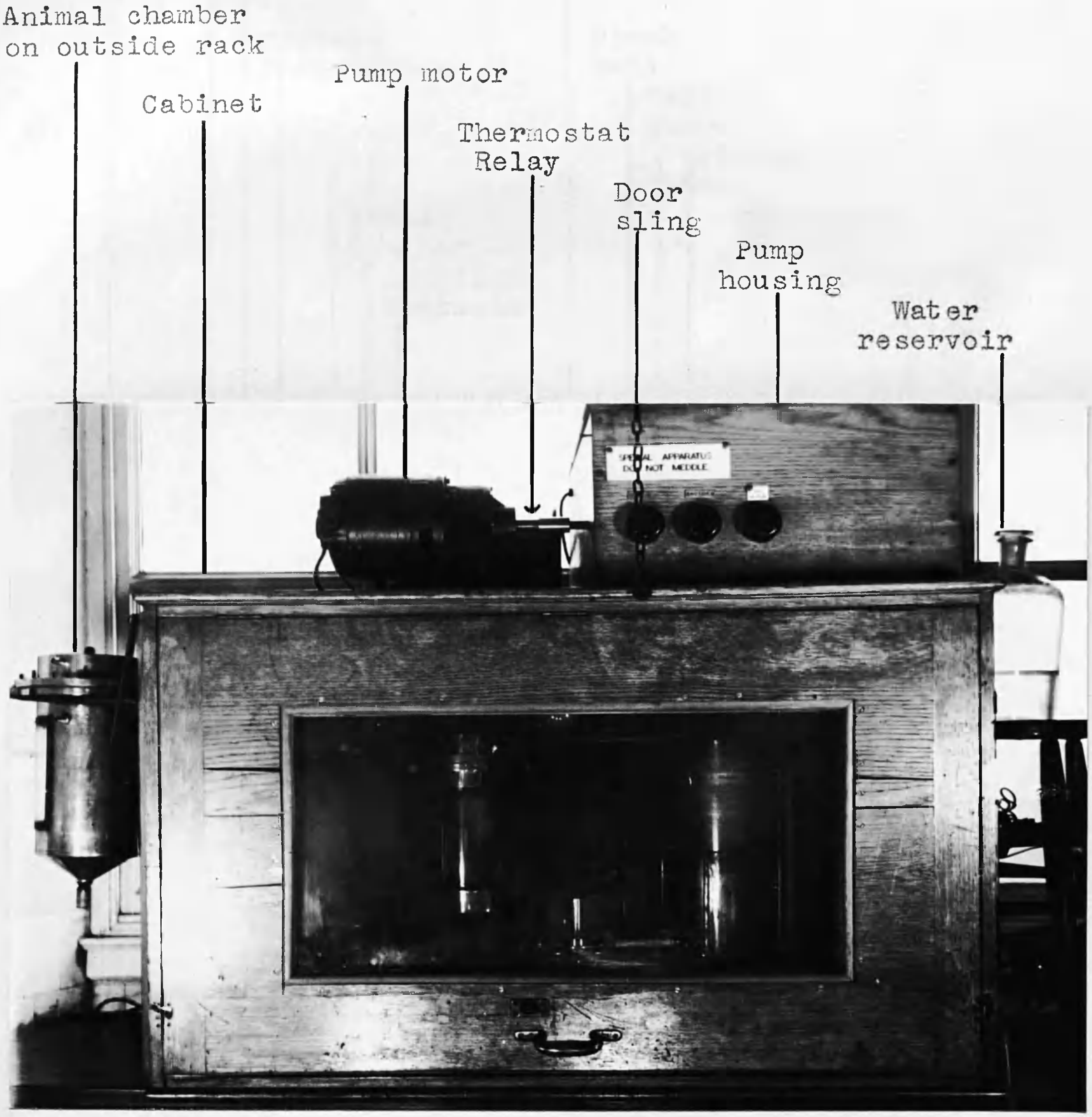


Diagram of respiration calorimeter used in this investigation.

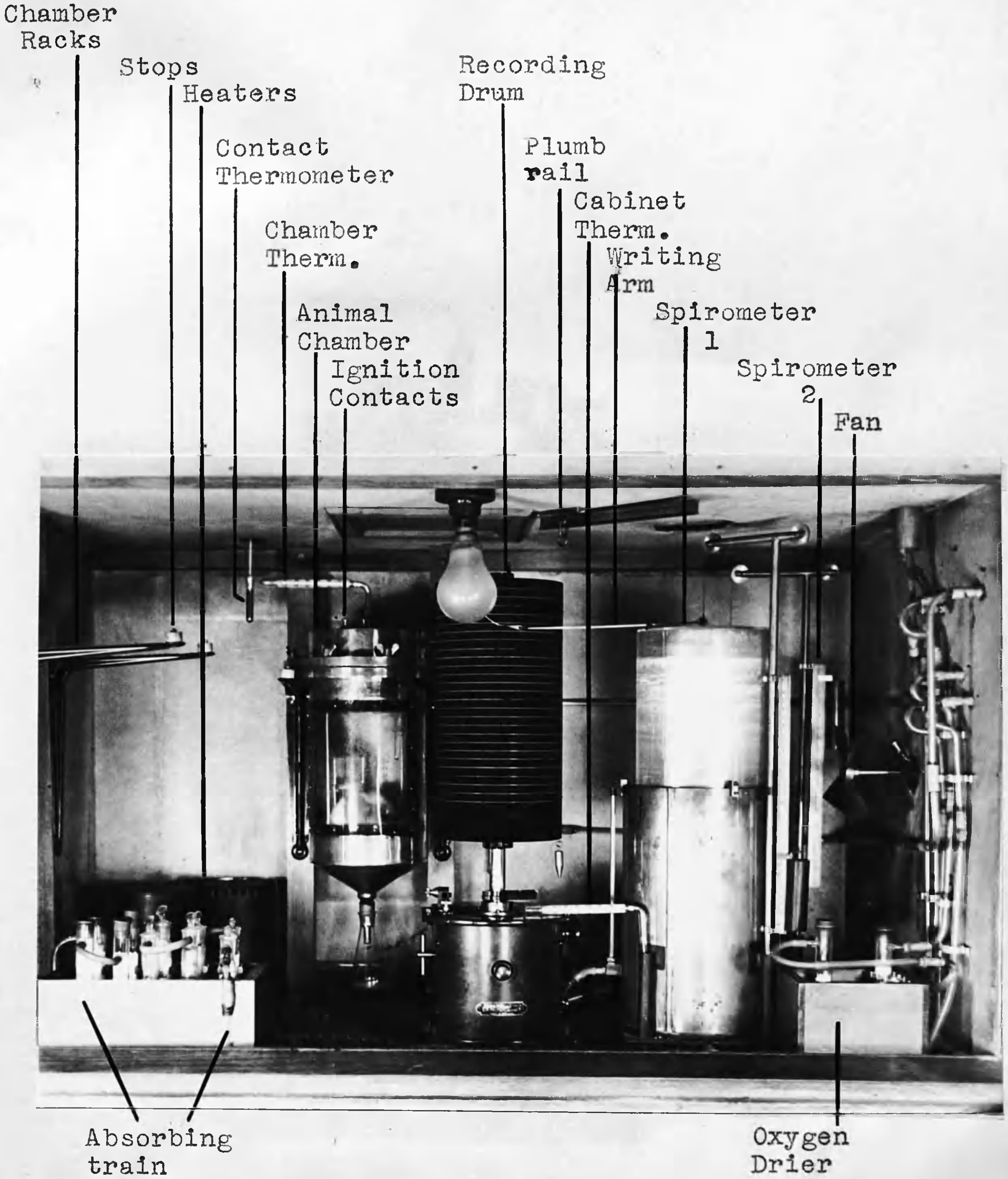
Figure (2)



General view of metabolic apparatus

(One tenth natural size)

<u>Figure</u> (3)



Inside view of cabinet
(one eighth natural size)

Figure (4)

Assembled Animal Chamber

(One quarter natural size)

Figure (5)

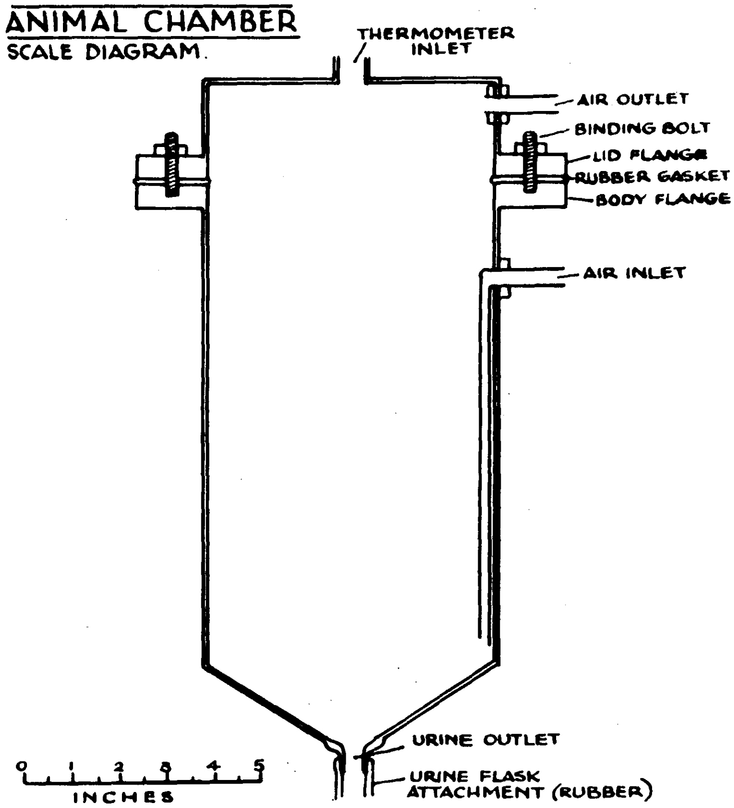
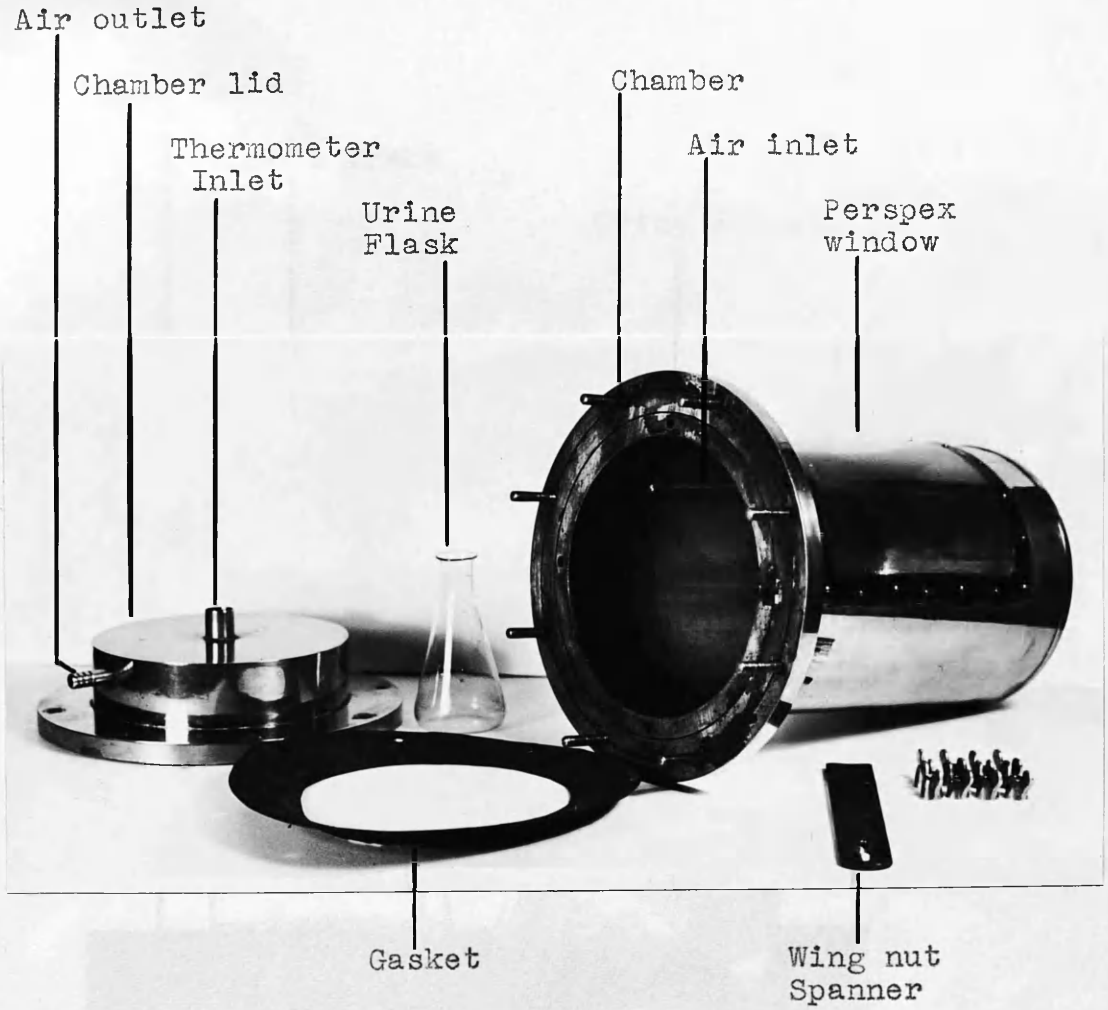

ANIMAL CHAMBER
SCALE DIAGRAM.
THERMOMETER INLET
AIR OUTLET
BINDING BOLT
LID FLANGE
RUBBER GASKET
BODY FLANGE
AIR INLET
URINE OUTLET
URINE FLASK ATTACHMENT (RUBBER)
0 1 2 3 4 5
INCHES

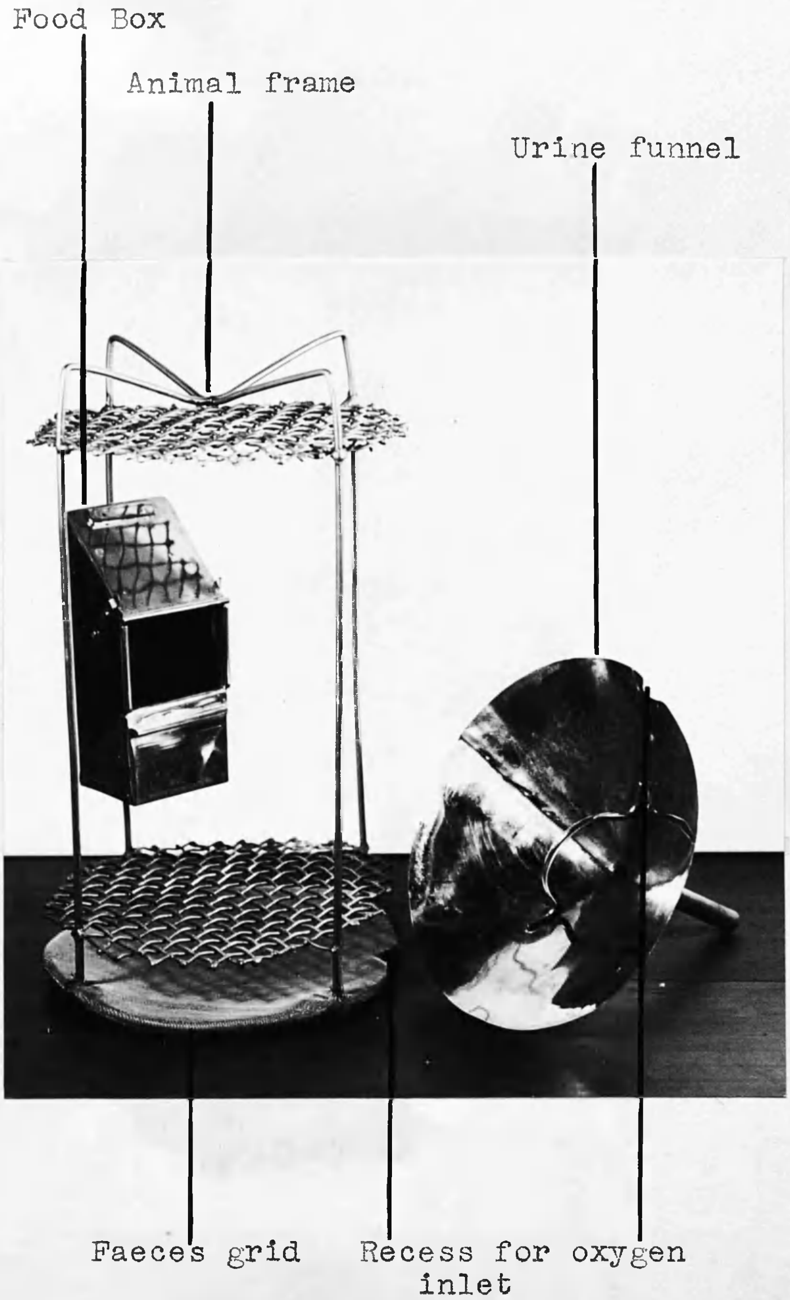
Figure (6)



Components of Animal Chamber

(One fifth natural size)

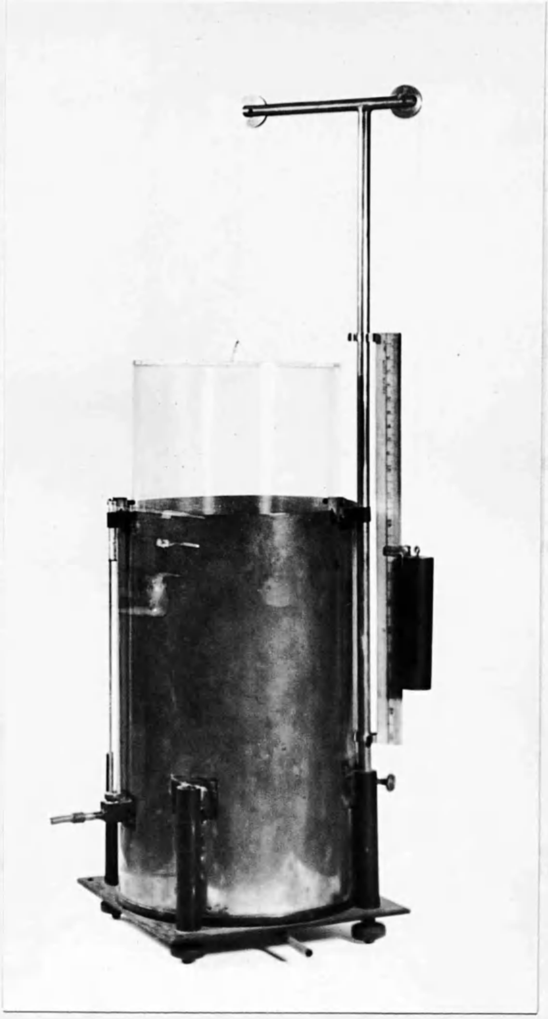
<u>Figure</u> (7)



Animal Frame and Urine Funnel

(One third natural size)

Figure (8)



Spirometer

(One sixth natural size)

Figure (9)

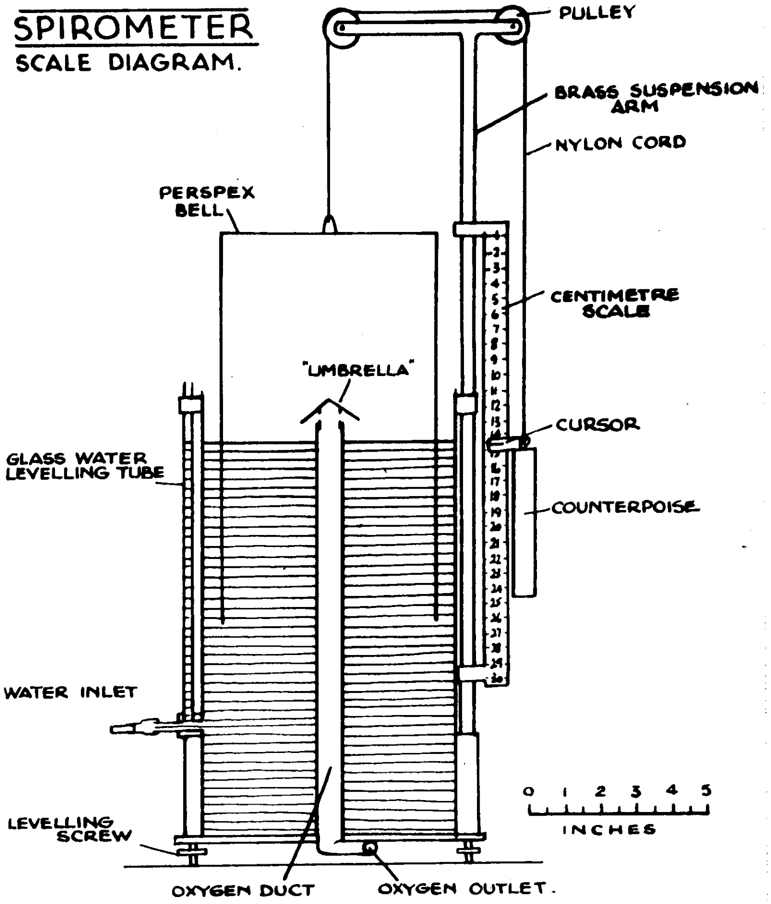

SPIROMETER
SCALE DIAGRAM.
PULLEY
BRASS SUSPENSION ARM
NYLON CORD
PERSPEX BELL
CENTIMETRE SCALE
"UMBRELLA"
CURSOR
GLASS WATER LEVELLING TUBE
COUNTERPOISE
WATER INLET
0 1 2 3 4 5
INCHES
LEVELLING SCREW
OXYGEN DUCT
OXYGEN OUTLET.

Figure (10)

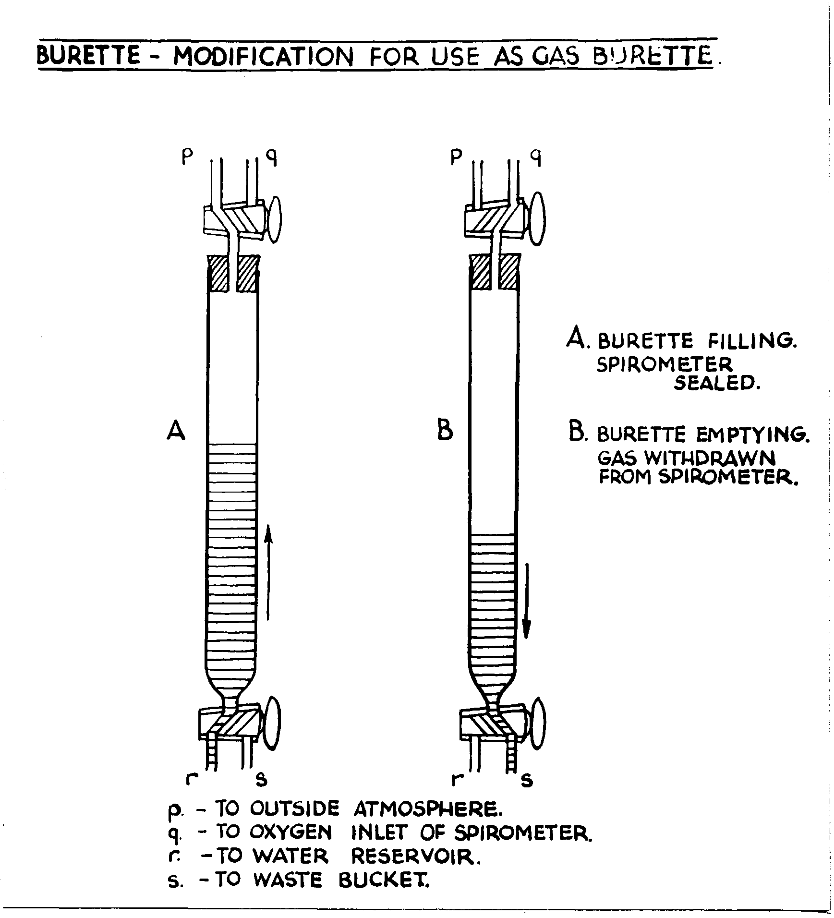
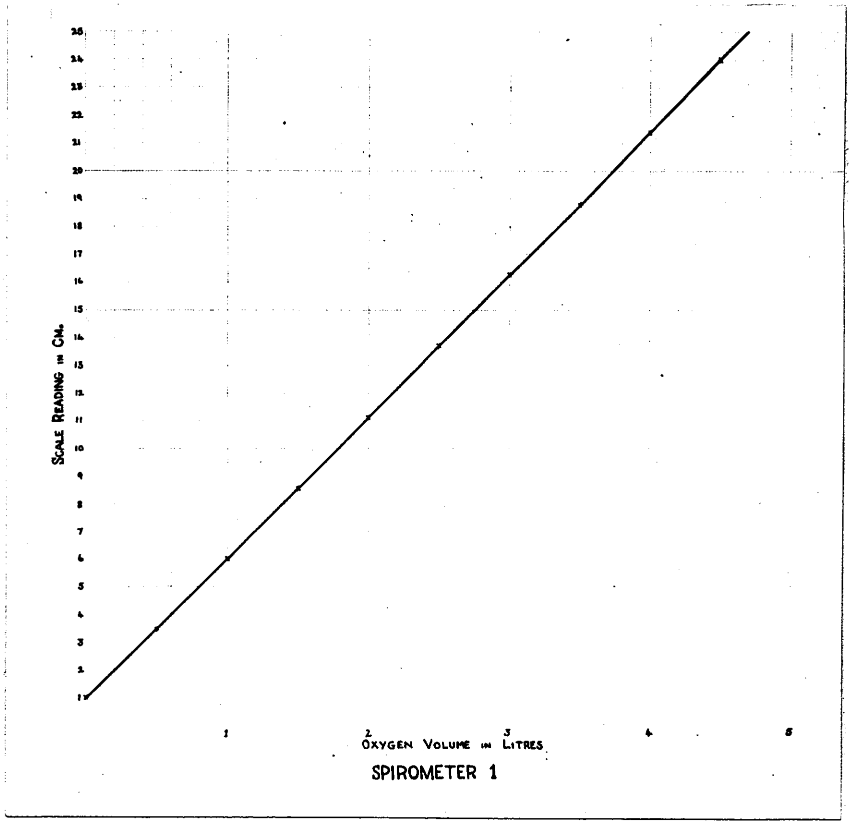

BURETTE - MODIFICATION FOR USE AS GAS BURETTE.
p
q
A
B
r
s
A. BURETTE FILLING. SPIROMETER SEALED.
B. BURETTE EMPTYING. GAS WITHDRAWN FROM SPIROMETER.
p. - TO OUTSIDE ATMOSPHERE.
q. - TO OXYGEN INLET OF SPIROMETER.
r. - TO WATER RESERVOIR.
s. - TO WASTE BUCKET.

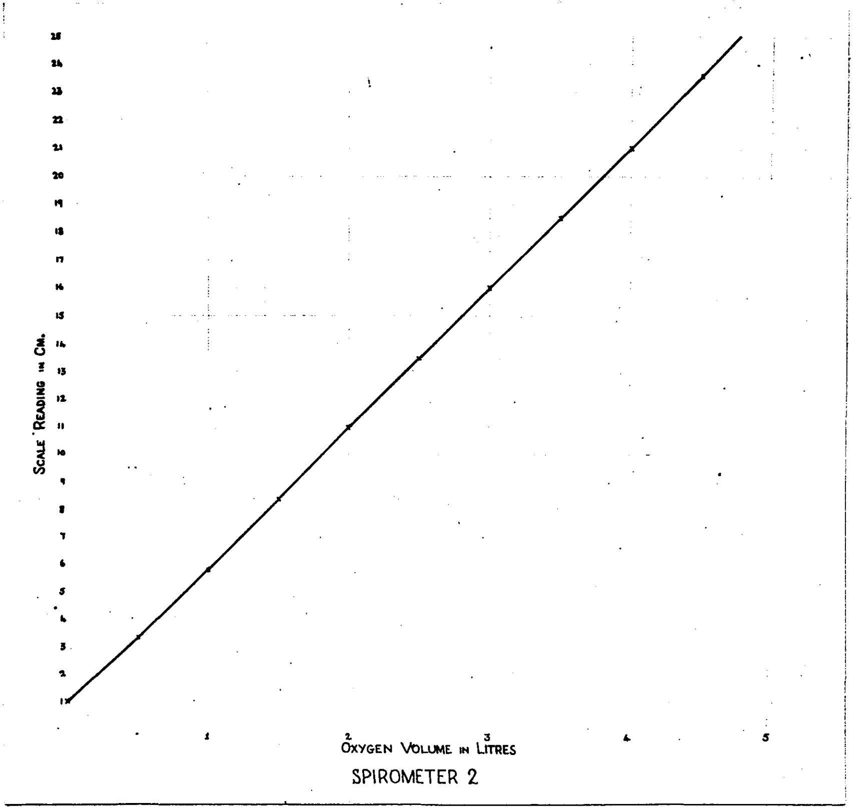
Figure (11)



Calibration curve of Spirometer 1.

Scale Two-fifths.

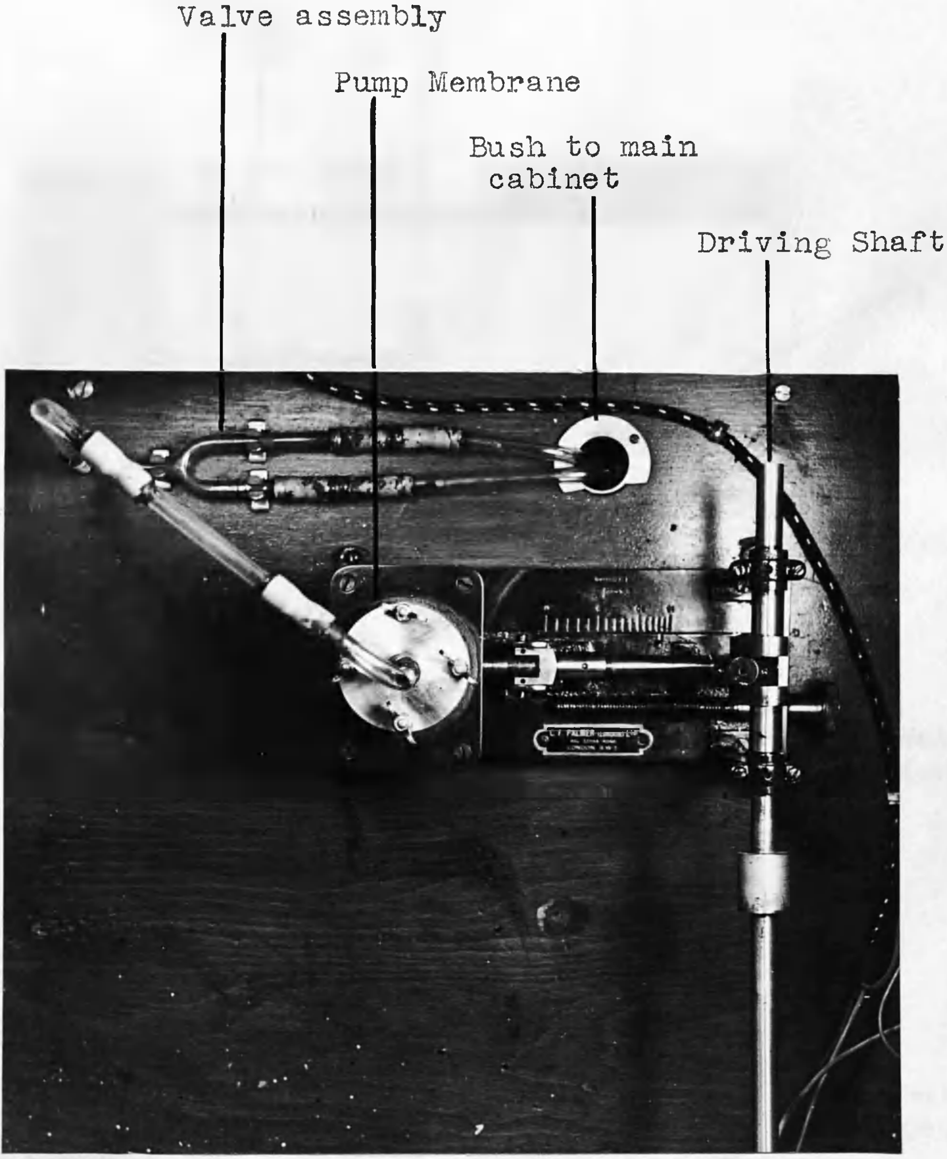
<u>Figure</u> (12)



Calibration curve of Spirometer 2.

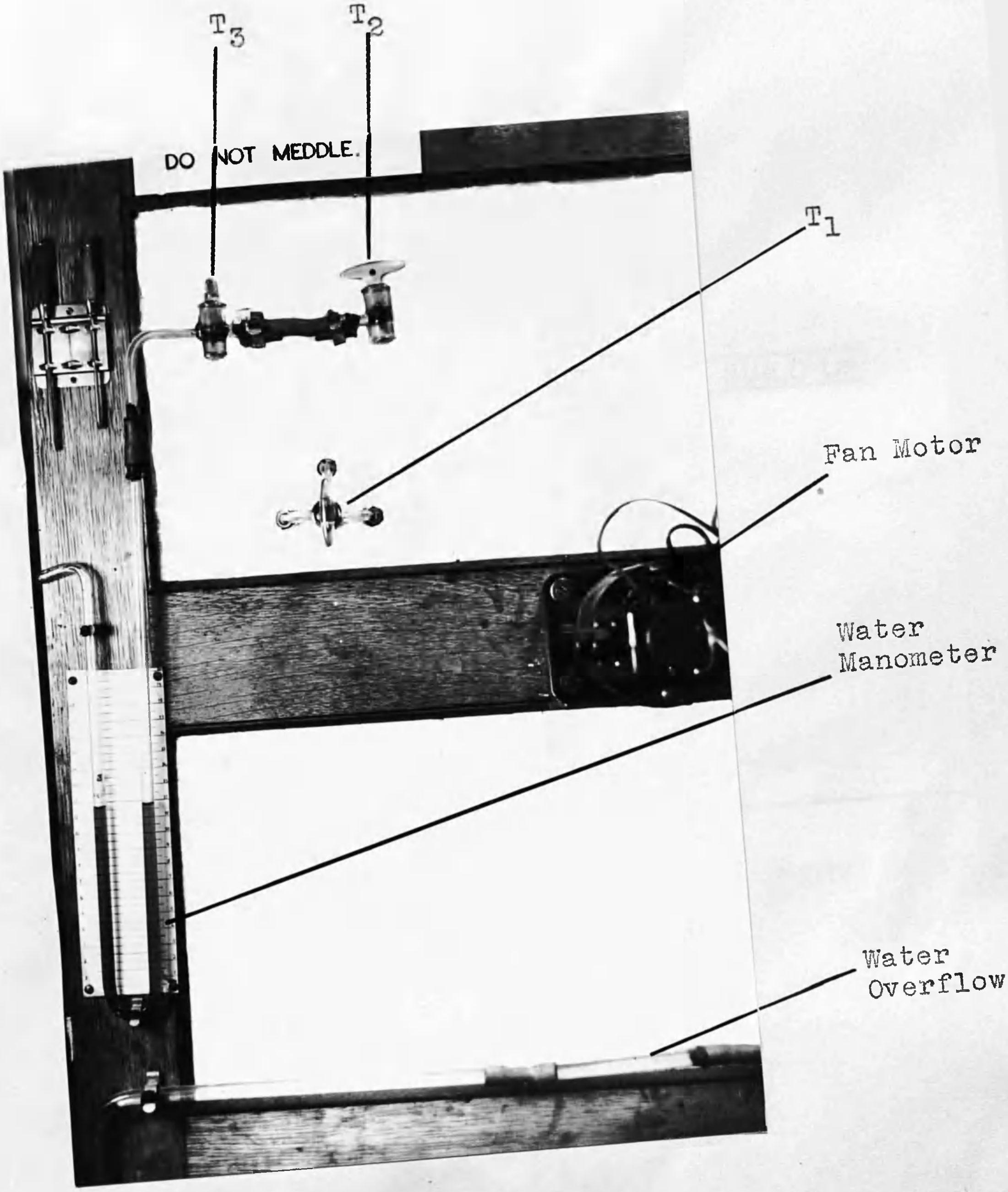
Scale two-fifths

<u>Figure</u> (13)



Pump and Valve Assembly

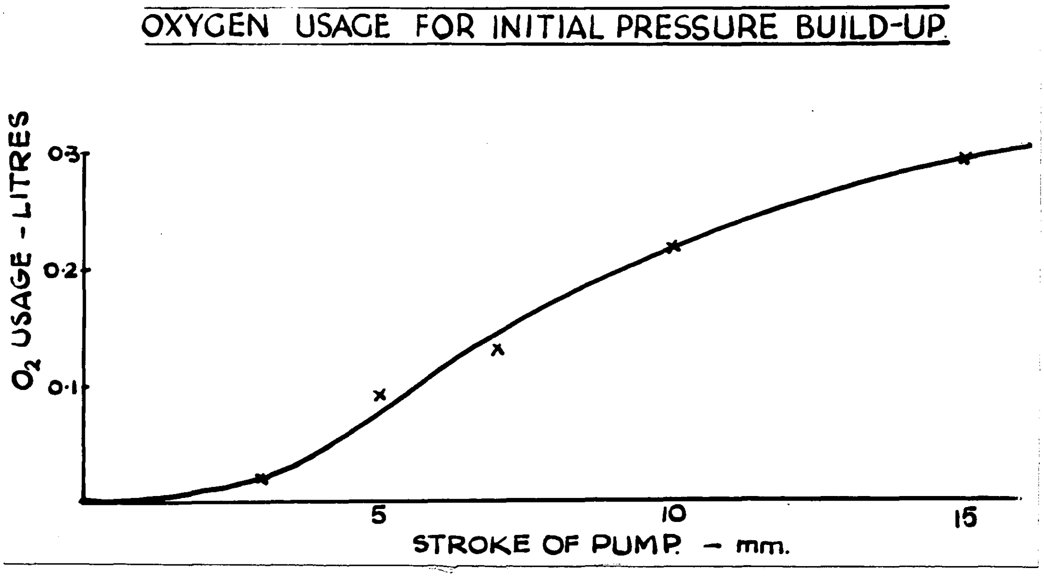
<u>Figure</u> (14)



Tap and Manometer Assembly

(One quarter natural size)

Figure (15)



cf. p. (96)

Figure (16)

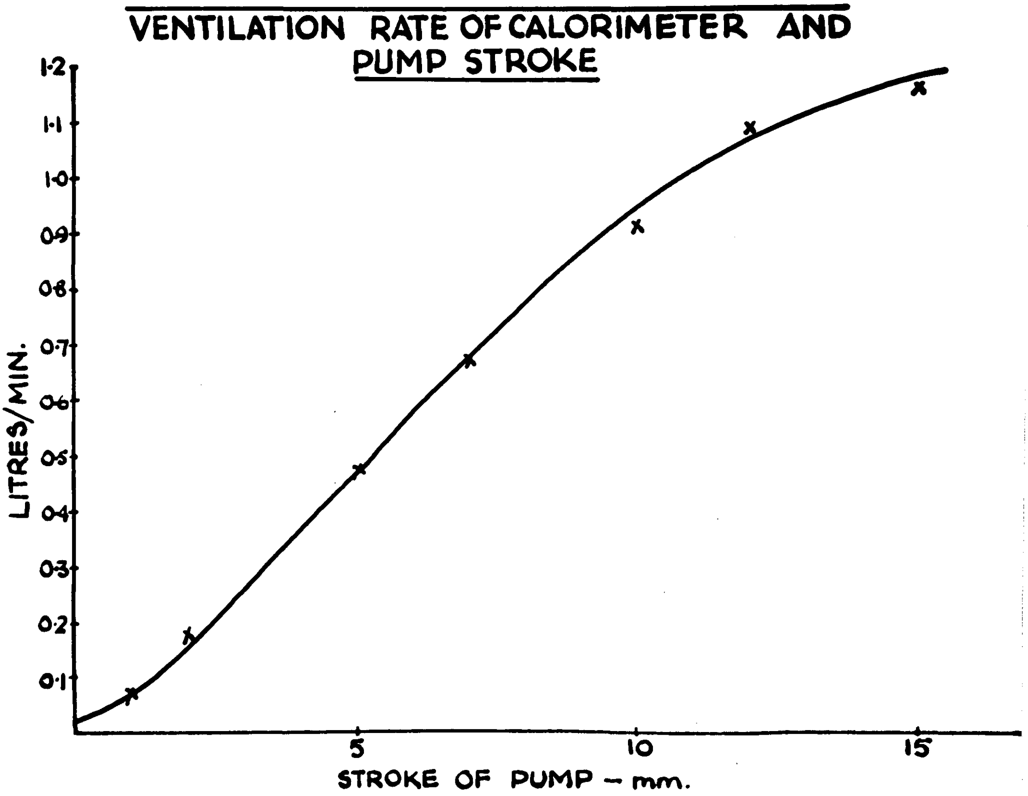
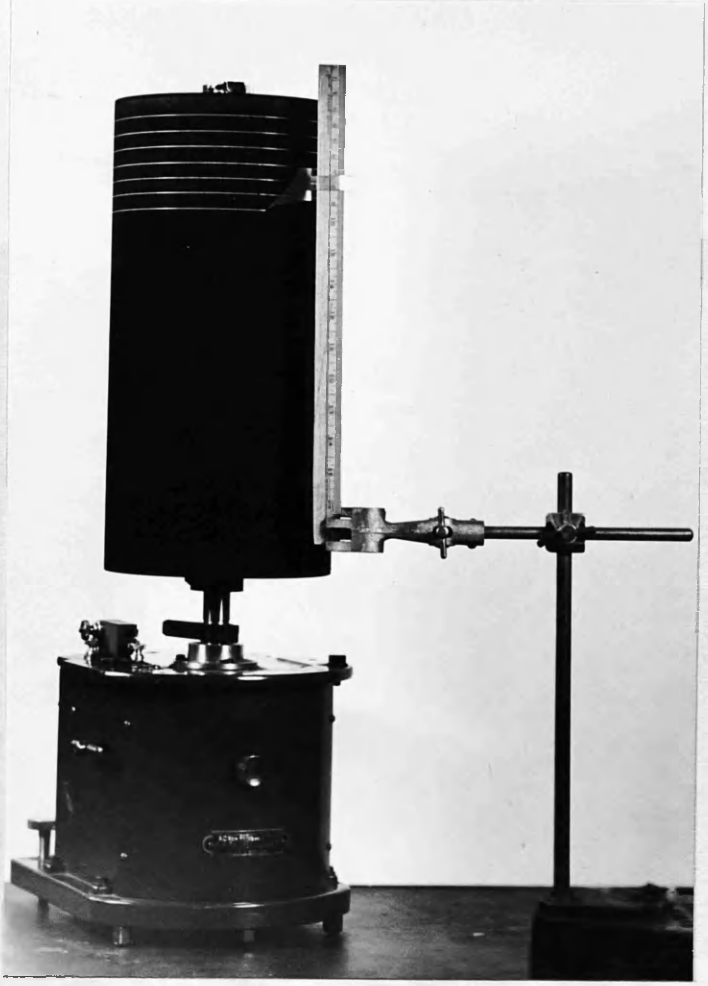

VENTILATION RATE OF CALORIMETER AND PUMP STROKE
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
LITRES/MIN.
5
10
15
STROKE OF PUMP – mm.

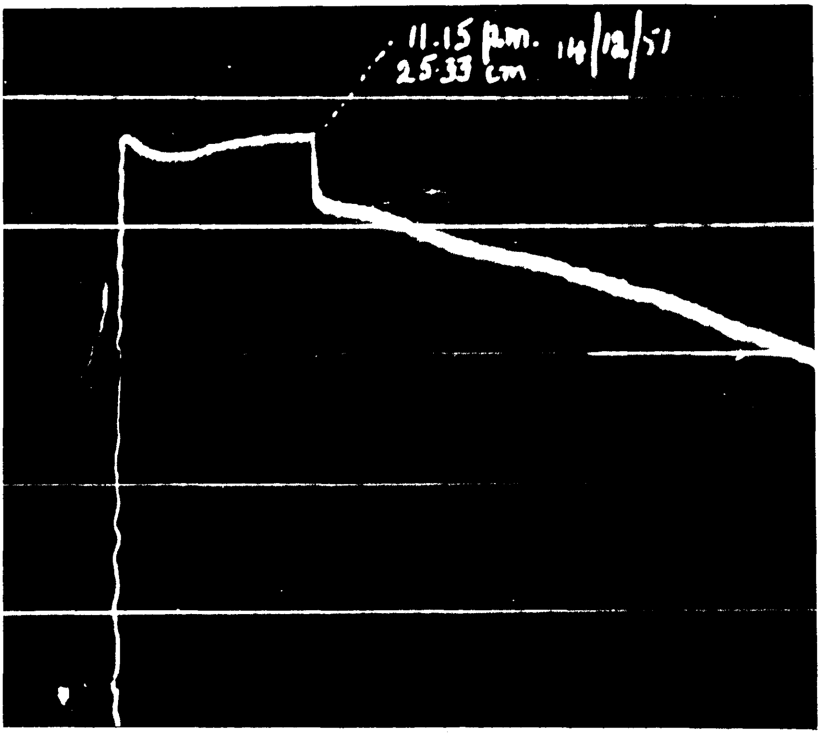
Figure (17)



Graduation of Oxygen Record Drum

Figure (18)

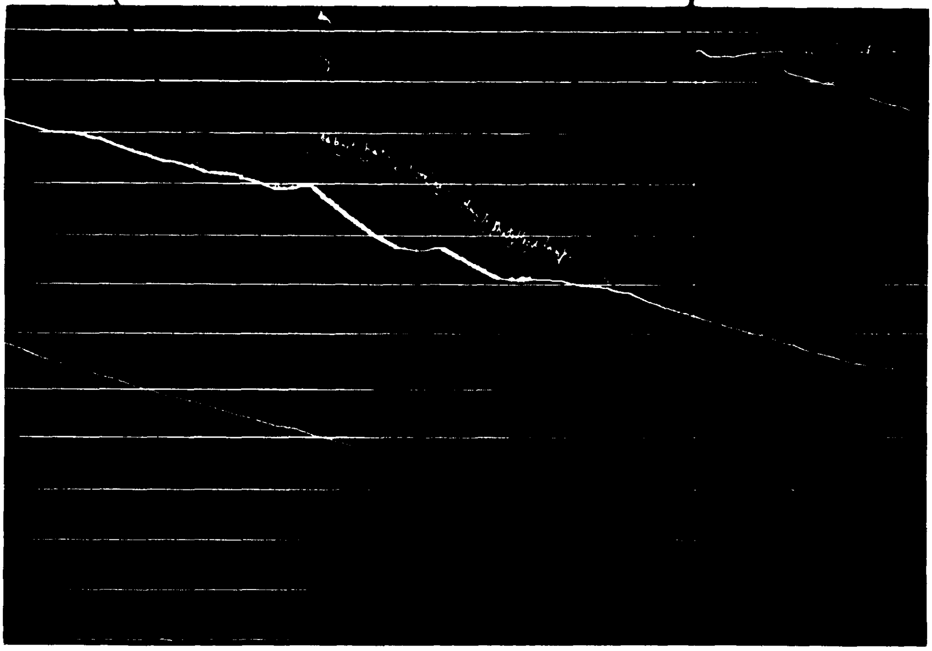
(Parallel lines 1 cm. apart)



Detail of Oxygen Trace on Re-filling Spirometer

Figure (19)

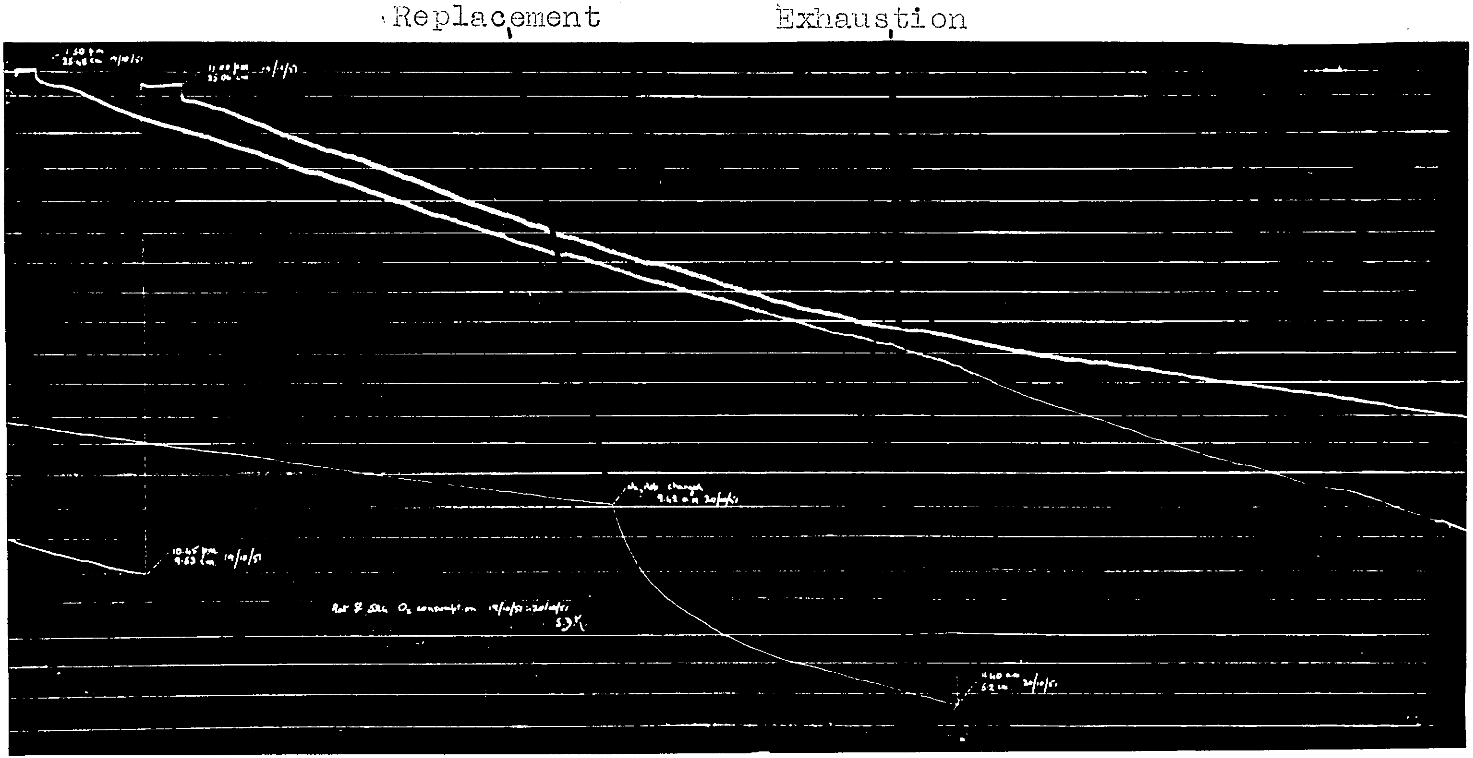
Temperature variation due to photo-flood lamp.



Detail of Effect of Temperature Variation

on Oxygen Trace

Figure (20)

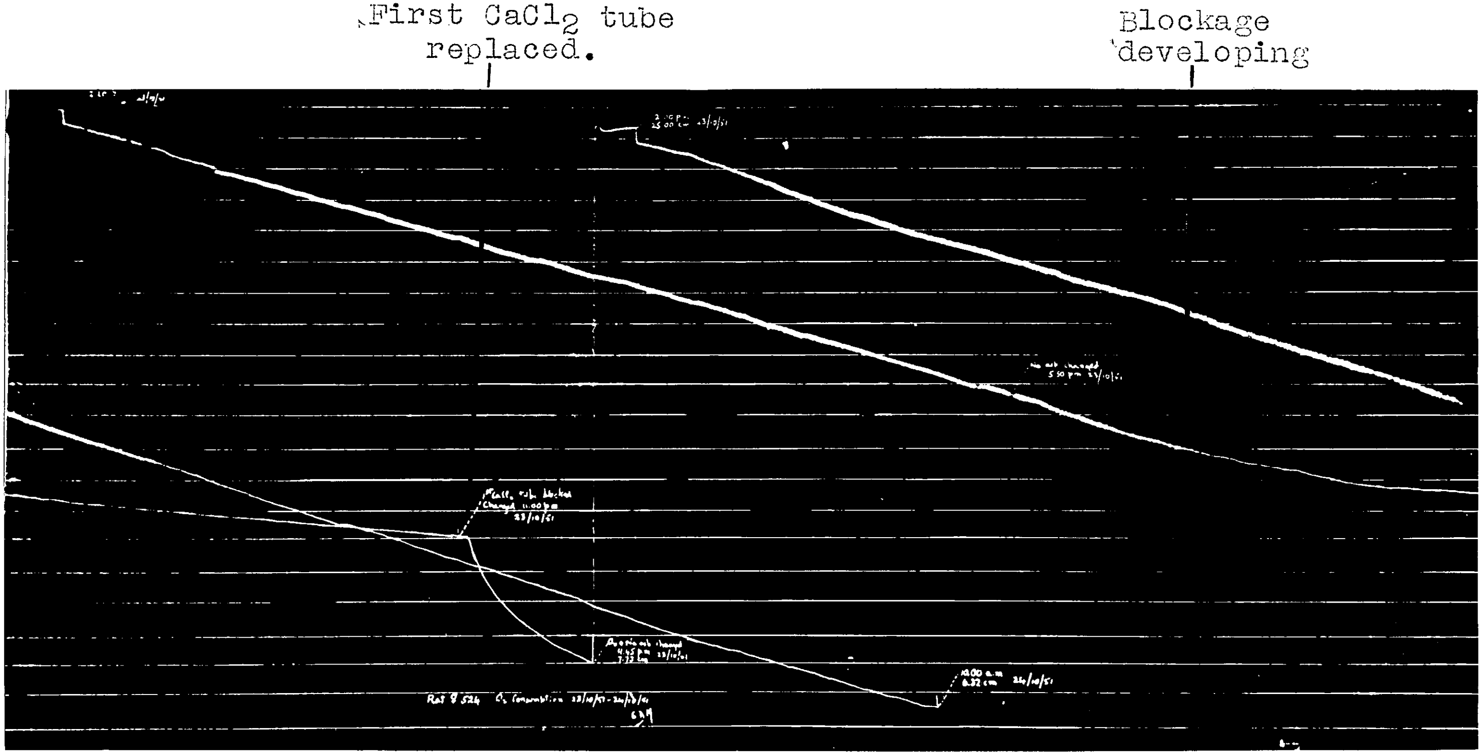


Specimen of oxygen consumption trace showing effect of exhaustion of absorbing train, and recovery after tube replacement.

Female 524 - non-pregnant.

Distance between parallel lines is 1 cm.

Figure (21a)

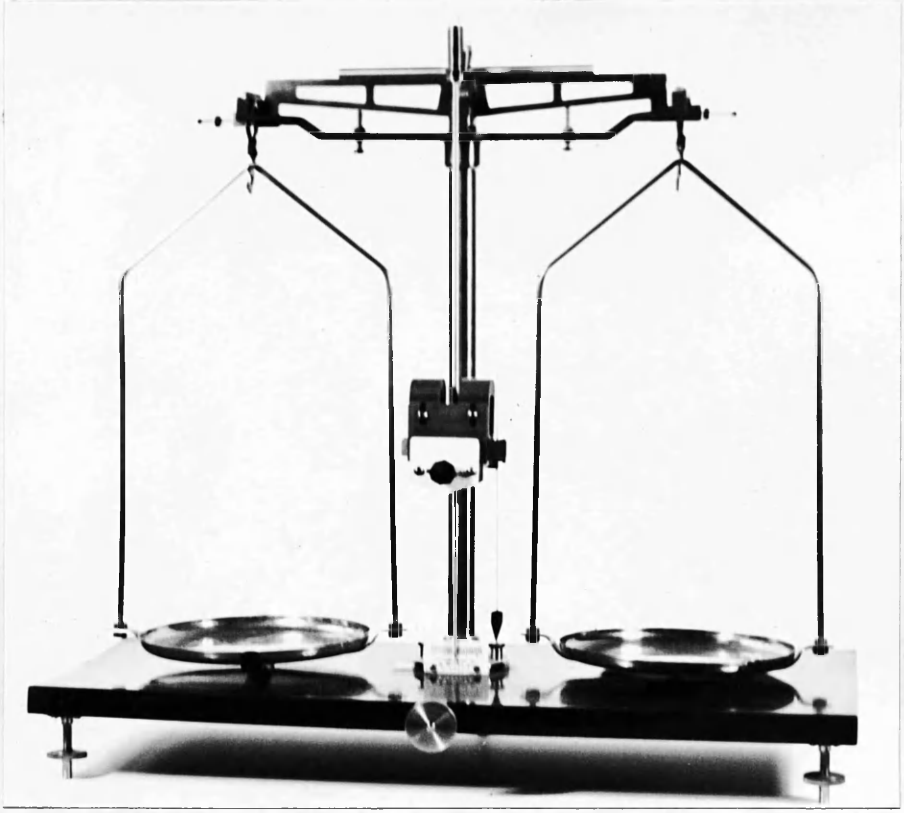


Specimen of oxygen consumption trace showing effect of blockage of absorbing train and recovery after tube replacement.

Female 524 - non-pregnant.

Distance between parallel lines is 1 cm.

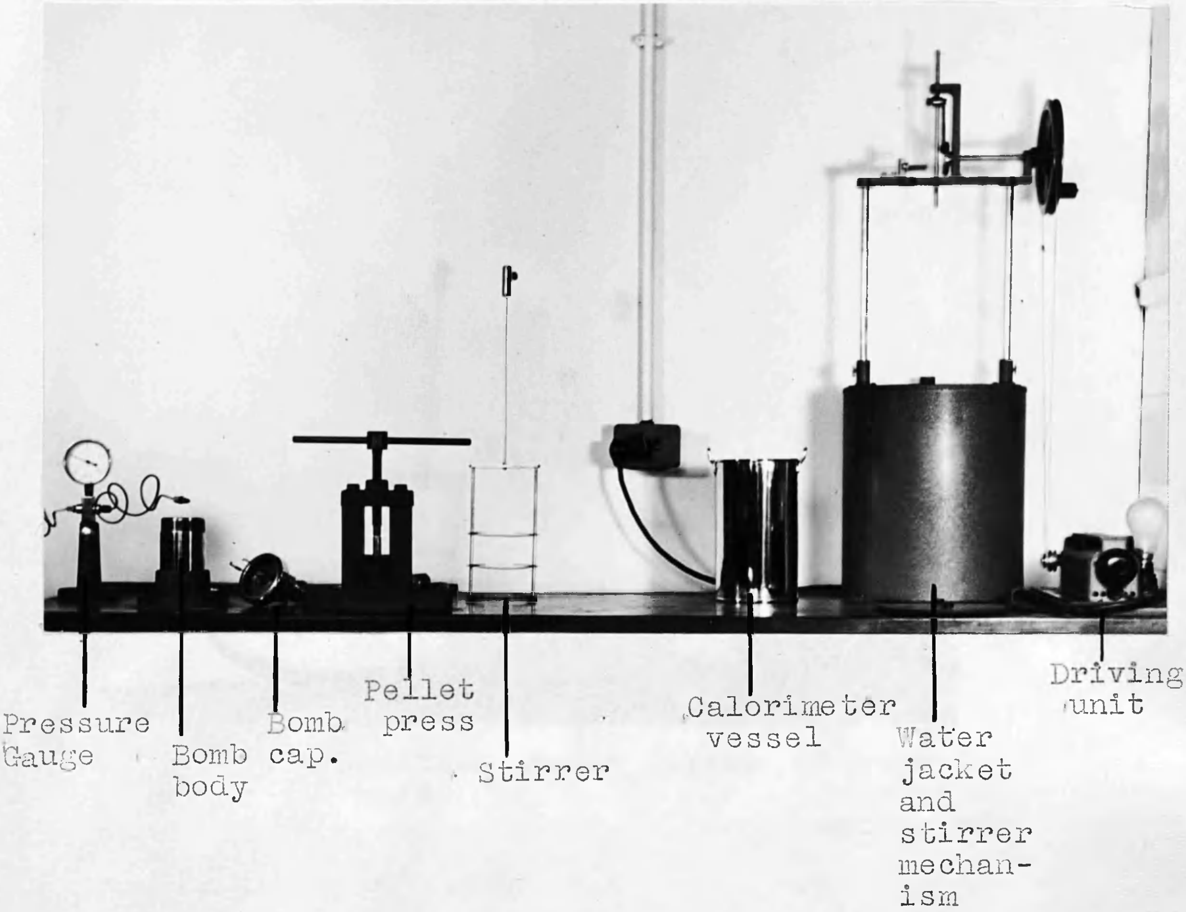
Figure (21b)



Balance with Magnetic Damping Attachment

and Sensitizing Bar

Figure (22)



Bomb Calorimeter Components

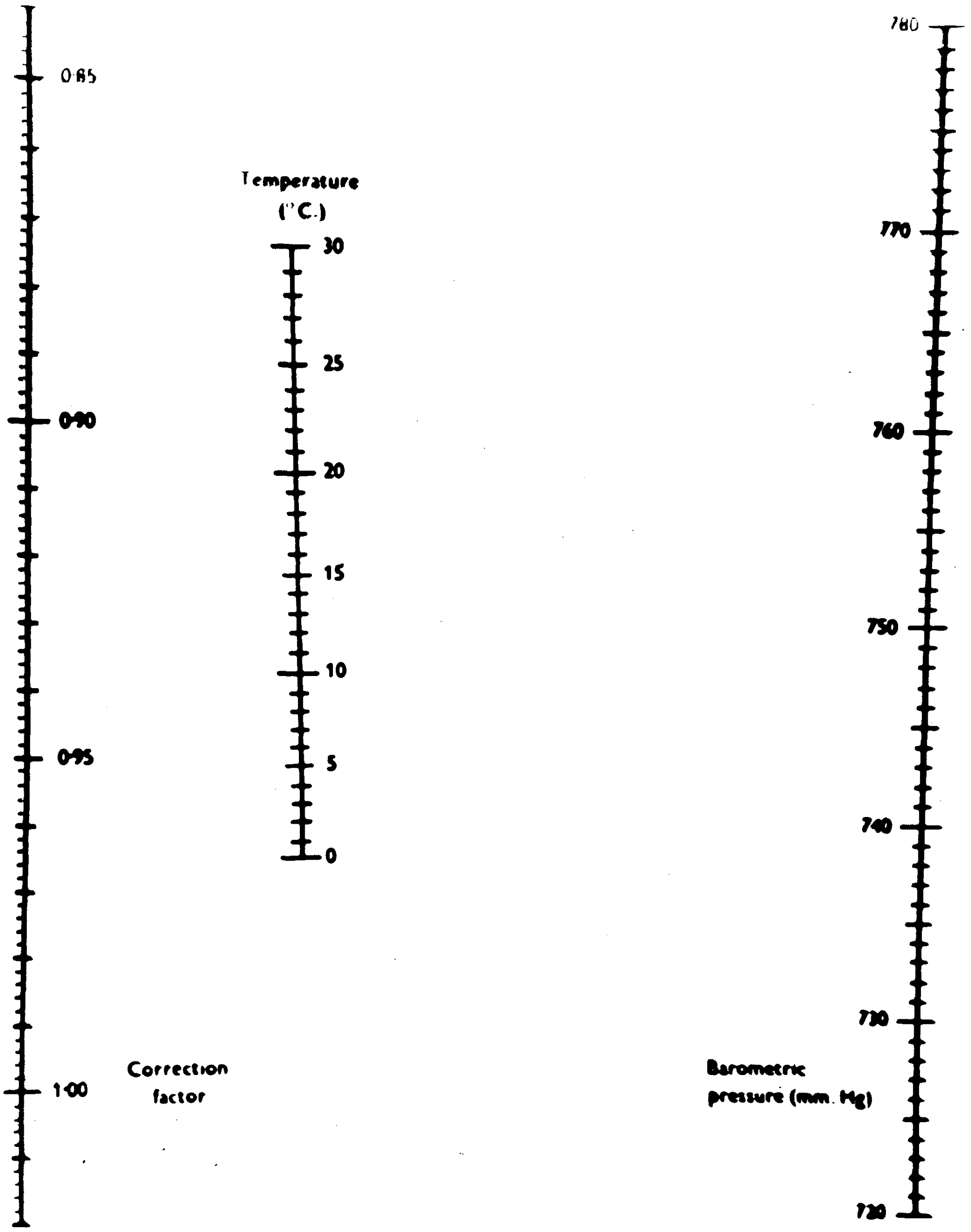
(One twelfth natural size).

<u>Figure</u> (23)

Assembled Bomb Calorimeter

(One tenth natural size).

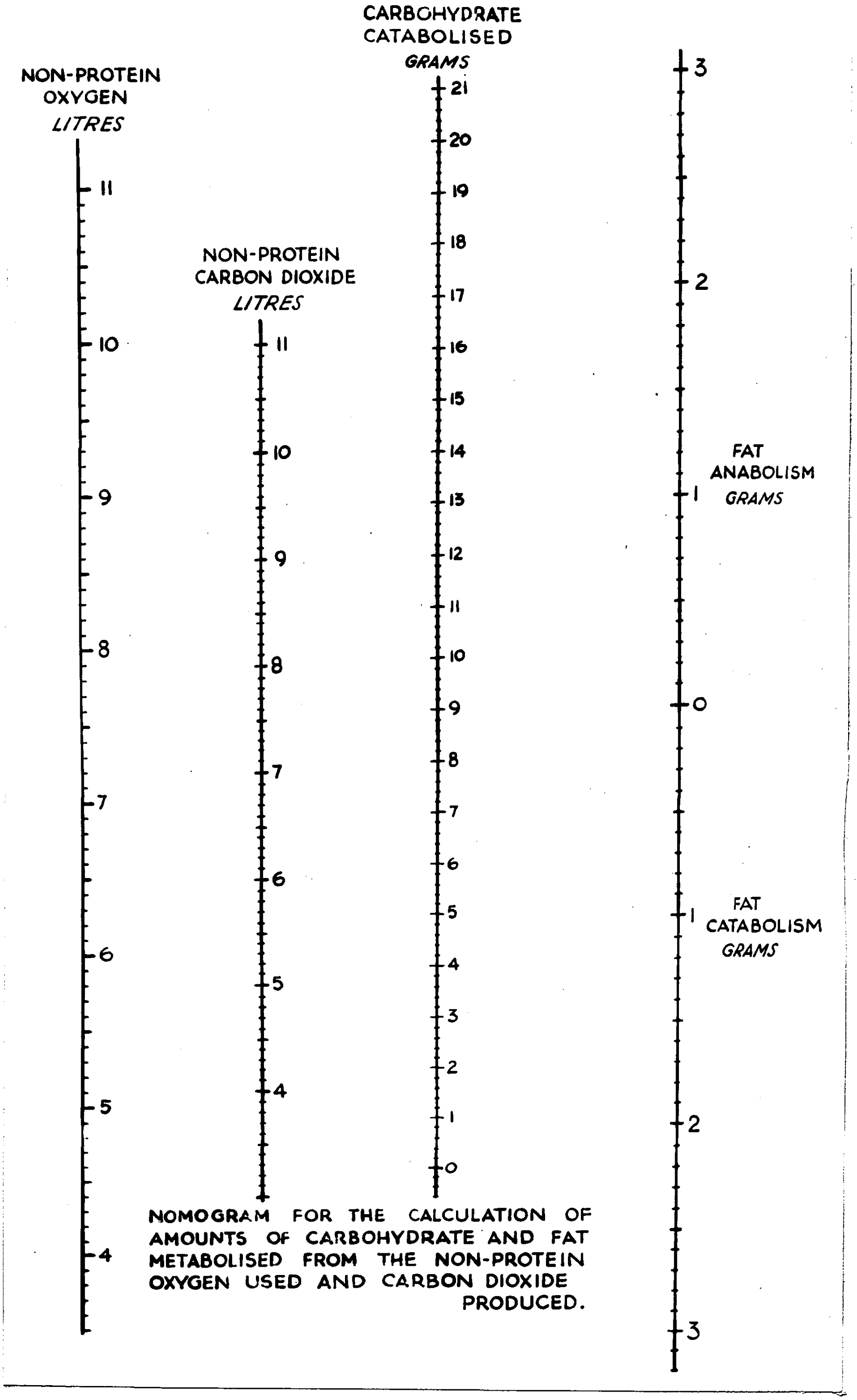
<u>Figure</u> (24)



Nomogram for derivation of correction factor for reduction of gas volumes to S.T.P.

(After Weir, 1949)

Figure (25)



NOMOGRAM FOR THE CALCULATION OF AMOUNTS OF CARBOHYDRATE AND FAT METABOLISED FROM THE NON-PROTEIN OXYGEN USED AND CARBON DIOXIDE PRODUCED.

Figure (26)

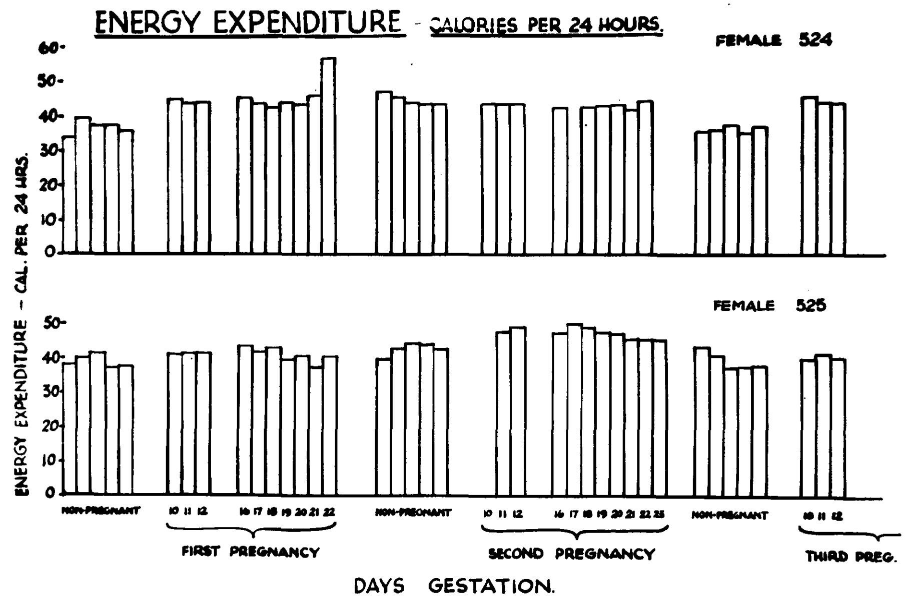

ENERGY EXPENDITURE - CALORIES PER 24 HOURS.
FEMALE 524
FEMALE 525
ENERGY EXPENDITURE - CAL. PER 24 HRS.
60
50
40
30
20
10
0
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON-PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION.

Figure (27)

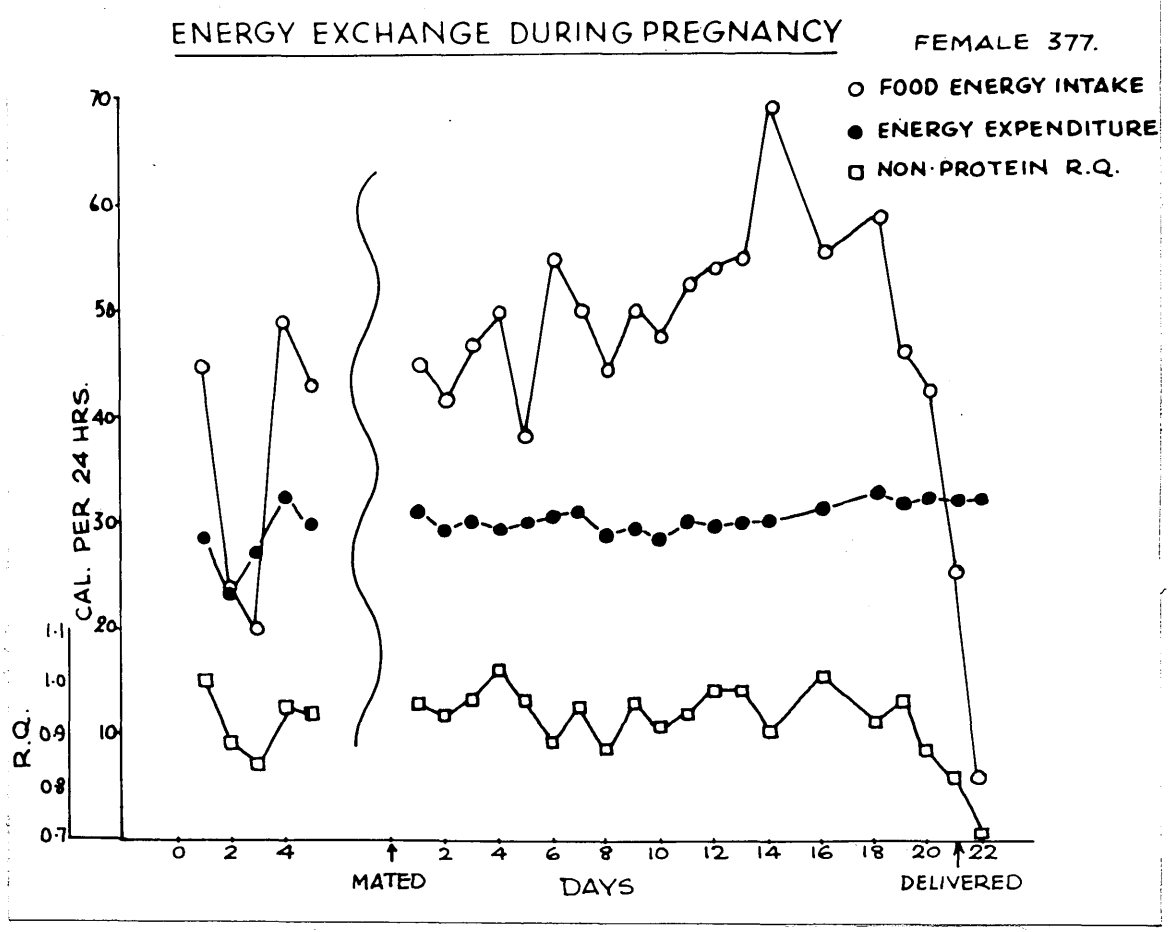

ENERGY EXCHANGE DURING PREGNANCY
FEMALE 377.
○ FOOD ENERGY INTAKE
● ENERGY EXPENDITURE
□ NON·PROTEIN R.Q.
CAL. PER 24 HRS.
70
60
50
40
30
20
10
R.Q.
1·1
1·0
0·9
0·8
0·7
0
2
4
MATED
2
4
6
8
10
12
14
16
18
20
22
DAYS
DELIVERED

Figure (28)

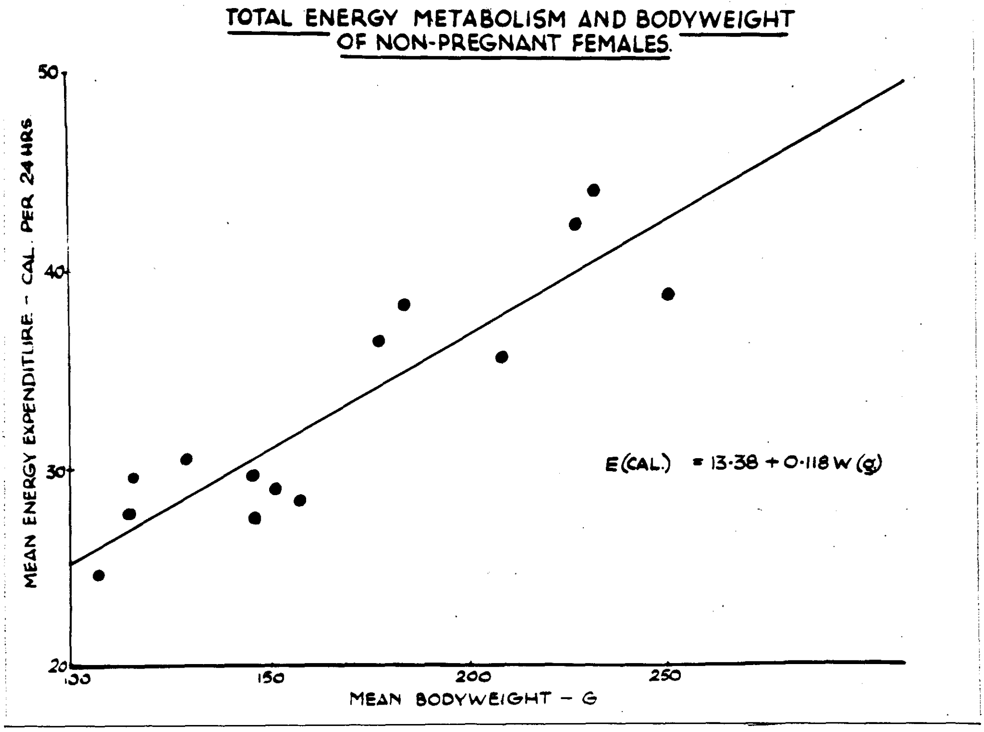

TOTAL ENERGY METABOLISM AND BODYWEIGHT OF NON-PREGNANT FEMALES.

Figure (29)

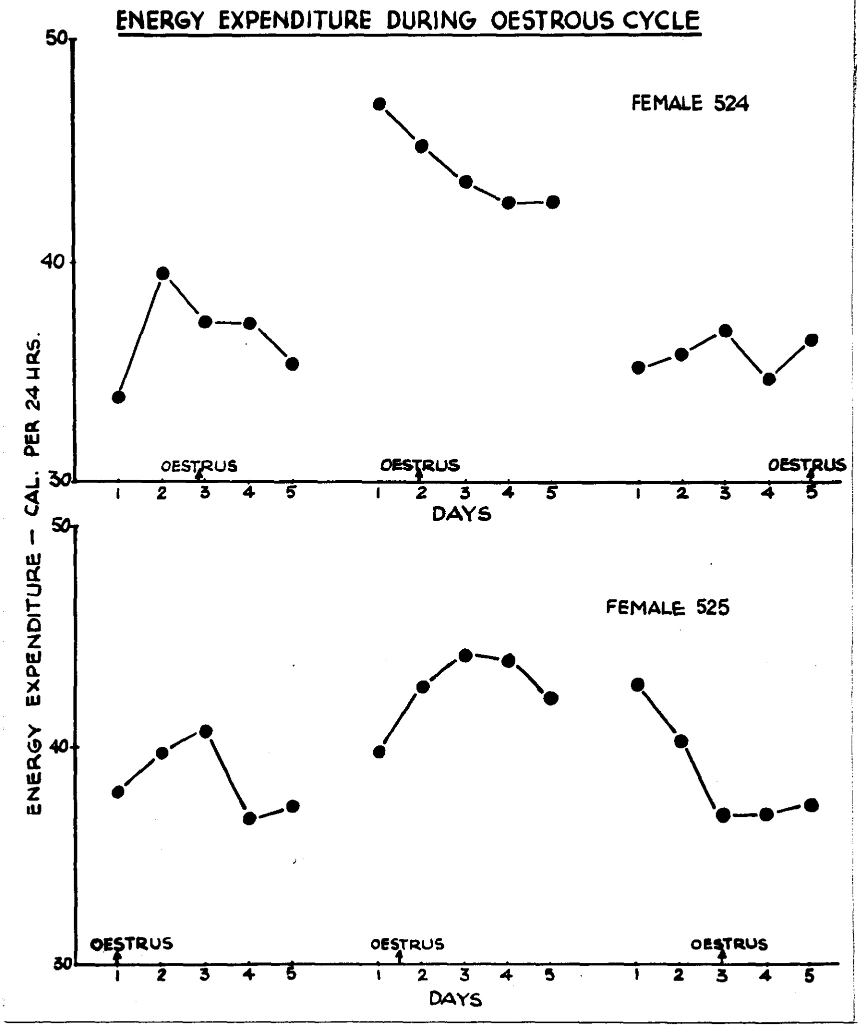
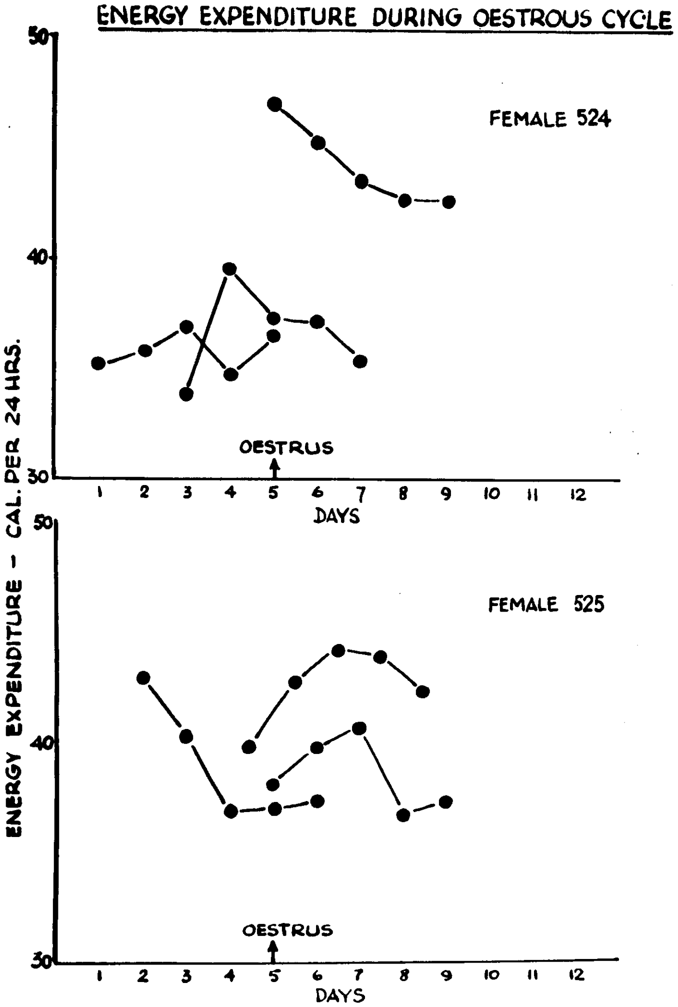
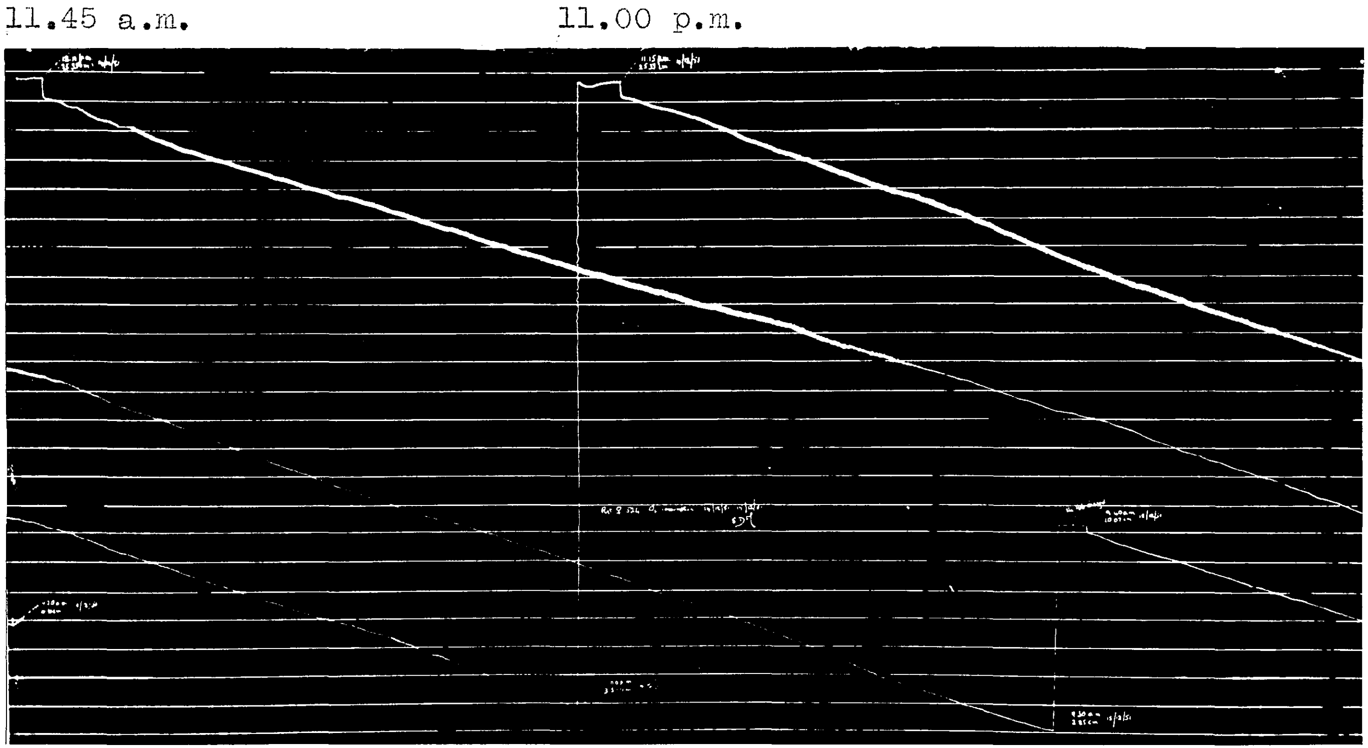

ENERGY EXPENDITURE DURING OESTROUS CYCLE
FEMALE 524
FEMALE 525
ENERGY EXPENDITURE - CAL. PER 24 HRS.
50
40
30
OESTRUS
1
2
3
4
5
DAYS

Figure (30)



ENERGY EXPENDITURE DURING OESTROUS CYCLE
FEMALE 524
FEMALE 525
OESTRUS
OESTRUS
DAYS
DAYS
ENERGY EXPENDITURE - CAL. PER 24 HRS.
50
40
30
50
40
30
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5 6 7 8 9 10 11 12

Figure (31)



Specimen of oxygen consumption trace showing increased oxygen consumption during the night.

Female 524 - non-pregnant.

Distance between parallel lines is 1 cm.

Figure (32)

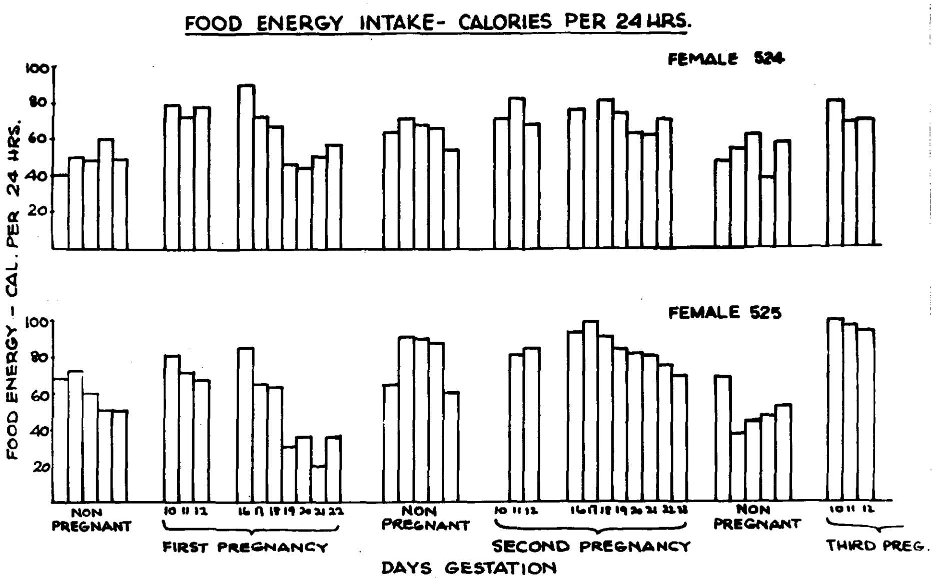

FOOD ENERGY INTAKE- CALORIES PER 24 HRS.
FEMALE 524
FEMALE 525
FOOD ENERGY - CAL. PER 24 HRS.
100
80
60
40
20
NON PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (33)

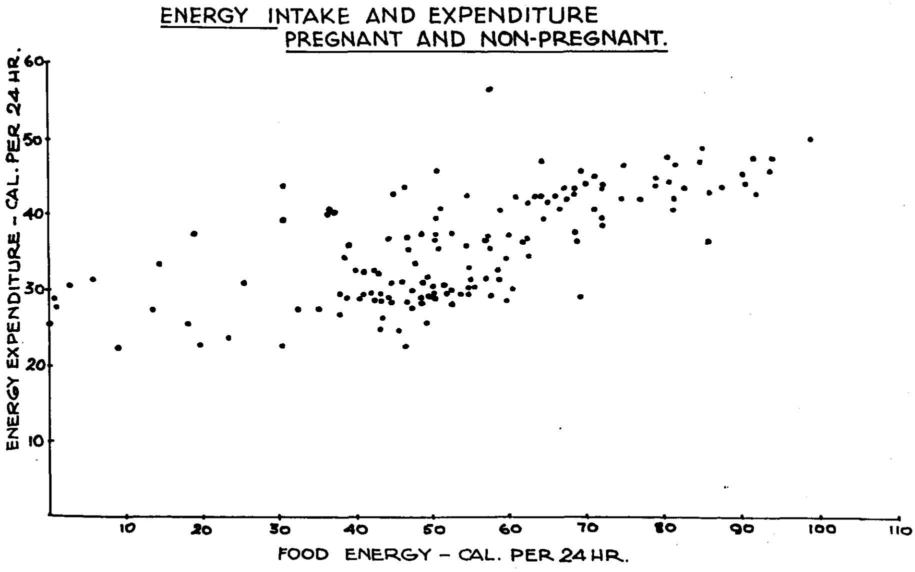

ENERGY INTAKE AND EXPENDITURE
PREGNANT AND NON-PREGNANT.
ENERGY EXPENDITURE - CAL. PER 24 HR.
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100
110
FOOD ENERGY - CAL. PER 24 HR.

Figure (34)

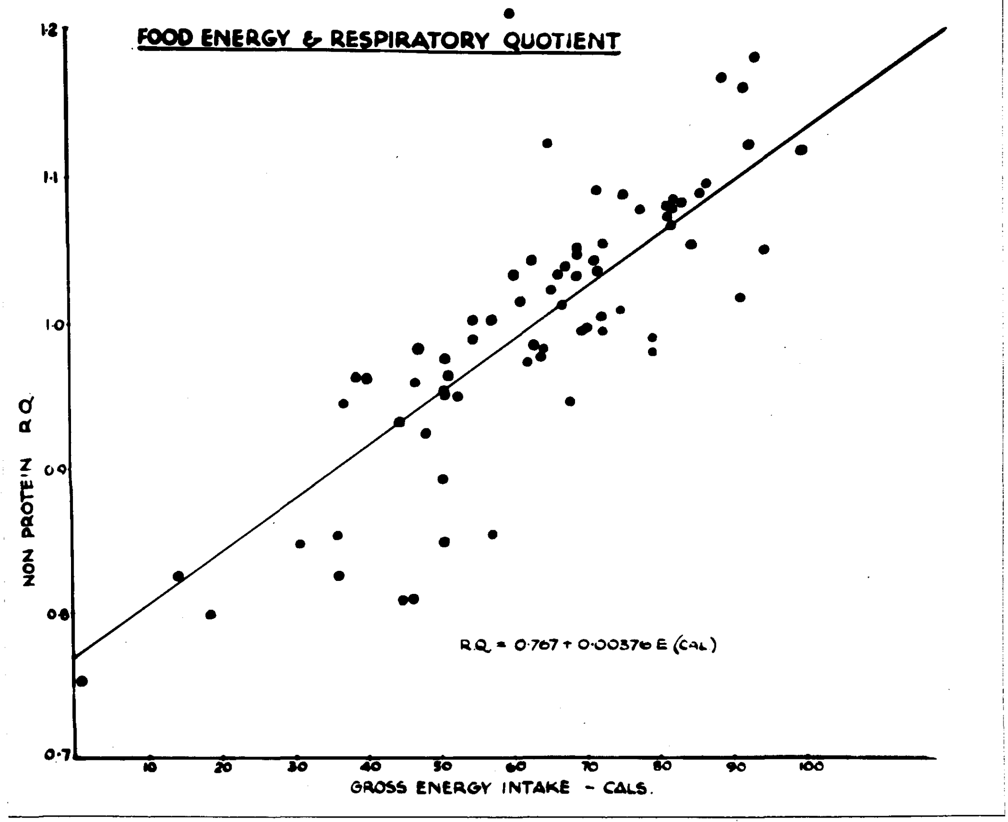

FOOD ENERGY & RESPIRATORY QUOTIENT
1·2
1·1
1·0
0·9
0·8
0·7
NON PROTEIN R.Q.
10
20
30
40
50
60
70
80
90
100
GROSS ENERGY INTAKE - CALS.
R.Q. = 0·767 + 0·00376 E (CAL)

Figure (35)

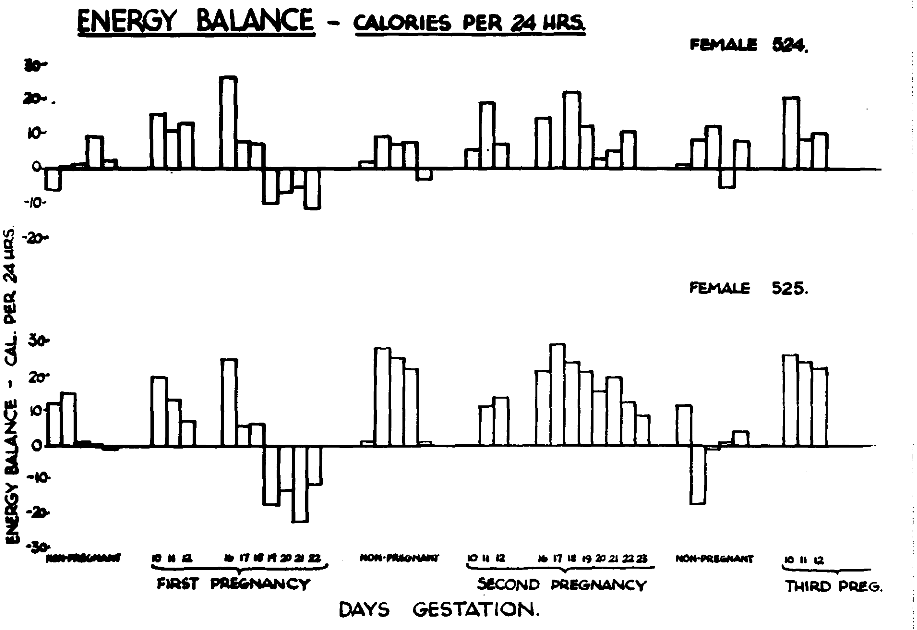
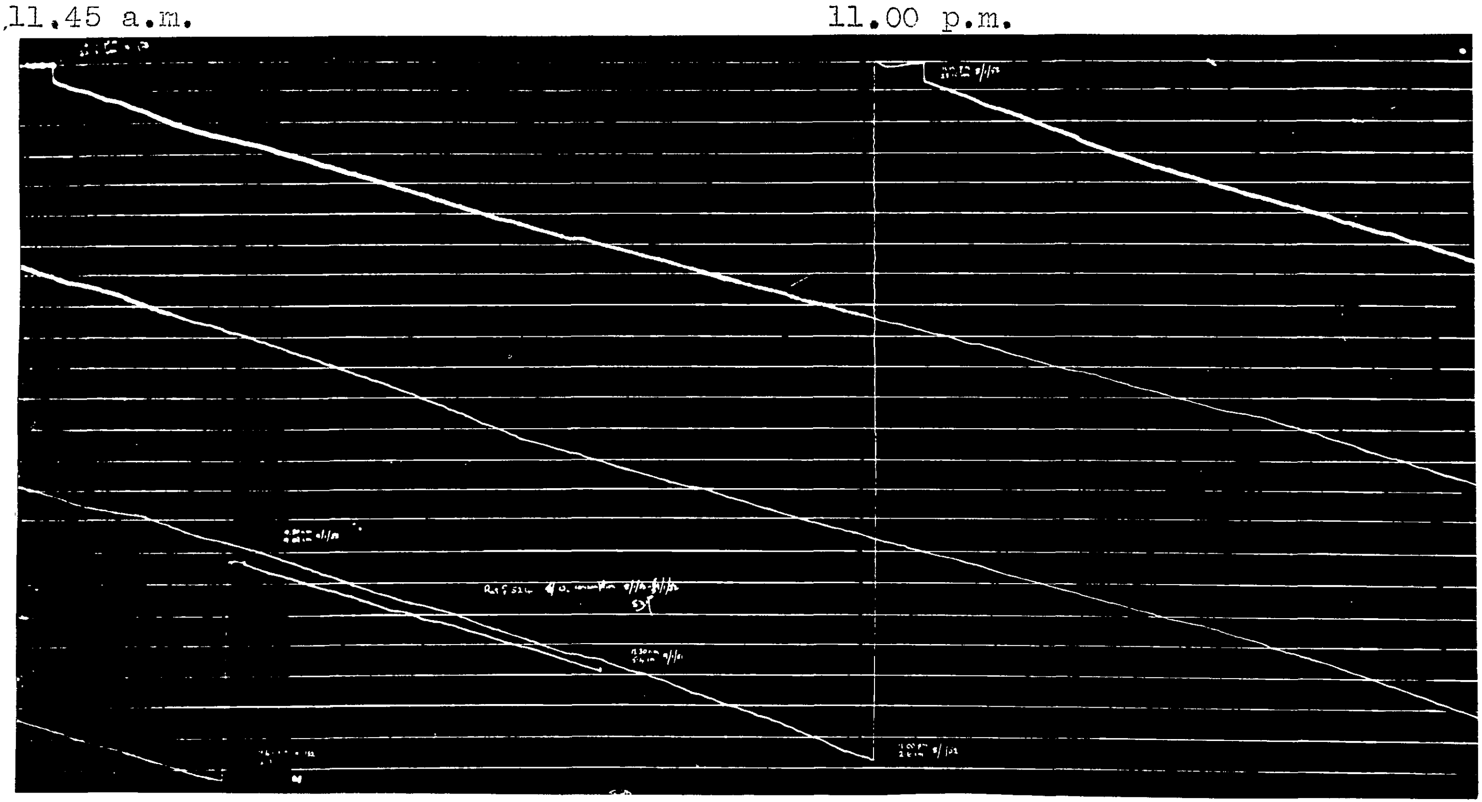

ENERGY BALANCE - CALORIES PER 24 HRS.
FEMALE 524.
30
20
10
0
-10
-20
FEMALE 525.
30
20
10
0
-10
-20
-30
ENERGY BALANCE - CAL. PER 24 HRS.
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON-PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION.

Figure (36)

Figure (37)



Specimen of oxygen consumption trace showing slightly increased oxygen consumption during the night.

Female 524 - 12th day, second pregnancy.

Distance between parallel lines is 1 cm.

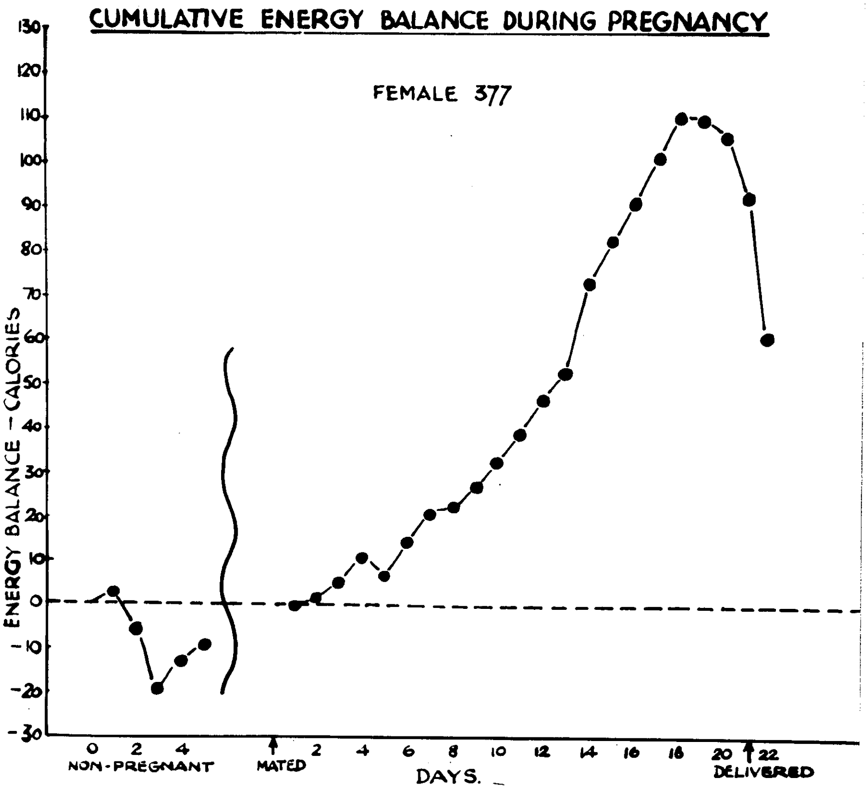

CUMULATIVE ENERGY BALANCE DURING PREGNANCY
FEMALE 377
ENERGY BALANCE - CALORIES
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
0
2
4
NON-PREGNANT
MATED
2
4
6
8
10
12
14
16
18
20
22
DELIVERED
DAYS.

Figure (38)

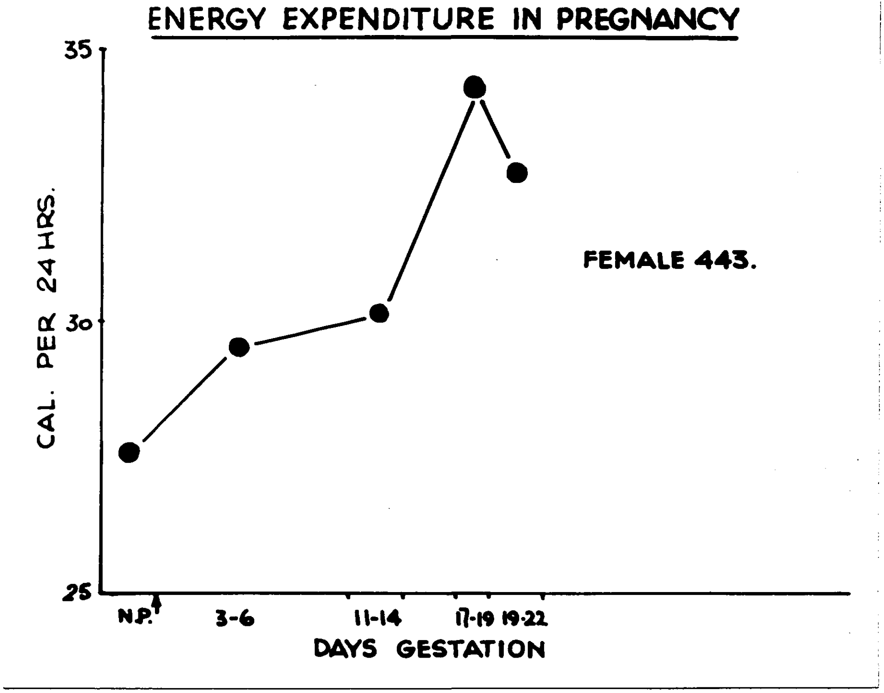

ENERGY EXPENDITURE IN PREGNANCY
35
30
25
CAL. PER 24 HRS.
FEMALE 443.
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (39)

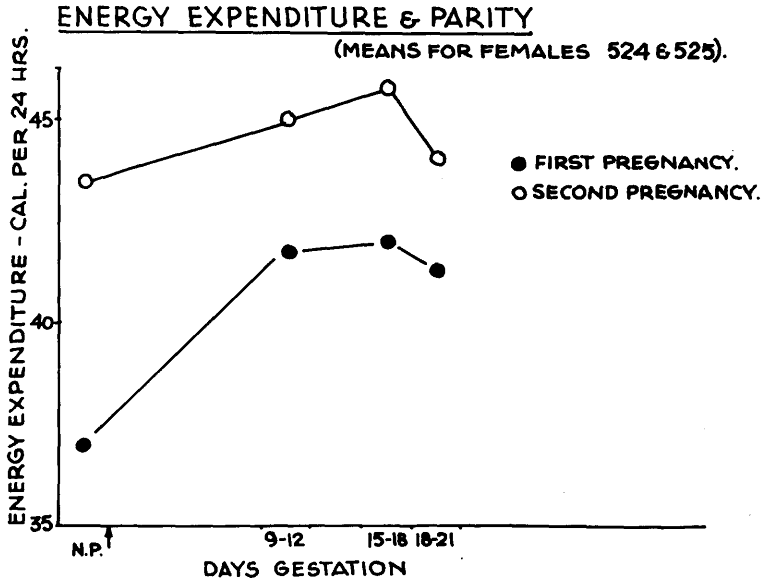

ENERGY EXPENDITURE & PARITY
(MEANS FOR FEMALES 524 & 525).
ENERGY EXPENDITURE - CAL. PER 24 HRS.
45
40
35
N.P.
9-12
15-18
18-21
DAYS GESTATION
● FIRST PREGNANCY.
○ SECOND PREGNANCY.

Figure (40)

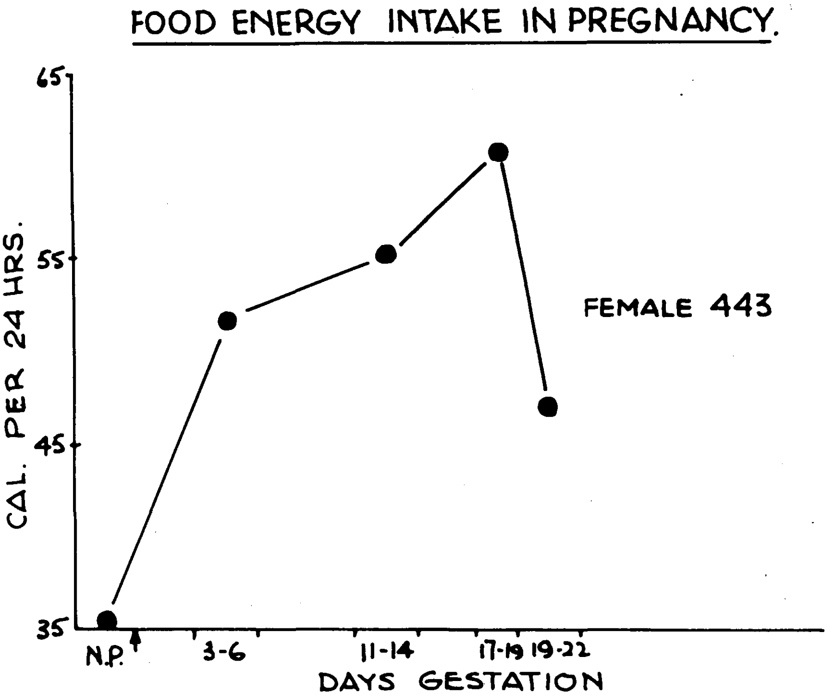

FOOD ENERGY INTAKE IN PREGNANCY.
65
55
45
35
CAL. PER 24 HRS.
FEMALE 443
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (41)

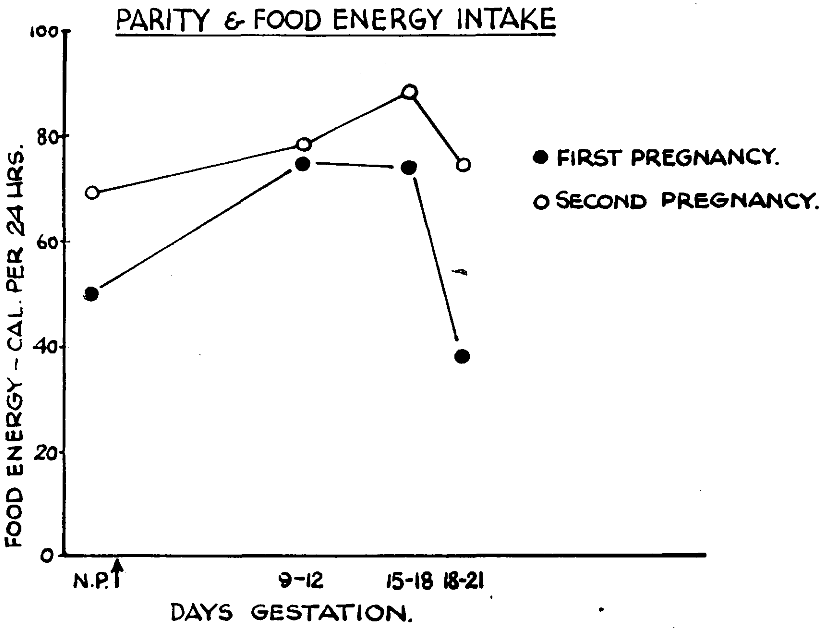

PARITY & FOOD ENERGY INTAKE
100
80
60
40
20
0
FOOD ENERGY - CAL. PER 24 HRS.
N.P.
9-12
15-18
18-21
DAYS GESTATION.
● FIRST PREGNANCY.
○ SECOND PREGNANCY.

Figure (42)

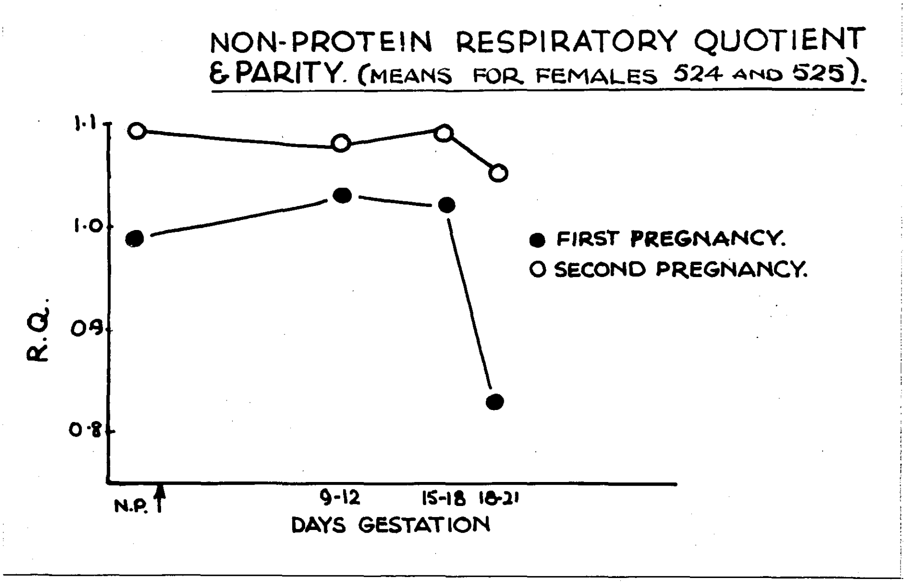

NON-PROTEIN RESPIRATORY QUOTIENT
& PARITY. (MEANS FOR FEMALES 524 AND 525).
1·1
1·0
0·9
0·8
R.Q.
N.P.
9-12
15-18
18-21
DAYS GESTATION
● FIRST PREGNANCY.
O SECOND PREGNANCY.

Figure (43)

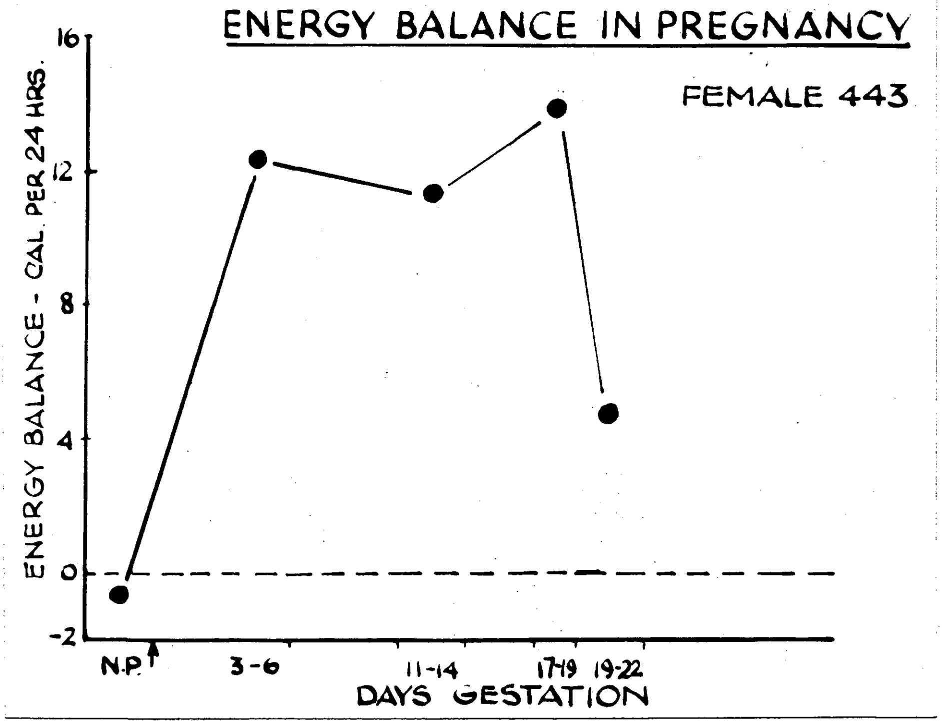

ENERGY BALANCE IN PREGNANCY
FEMALE 443
ENERGY BALANCE - CAL. PER 24 HRS.
16
12
8
4
0
-2
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (44)

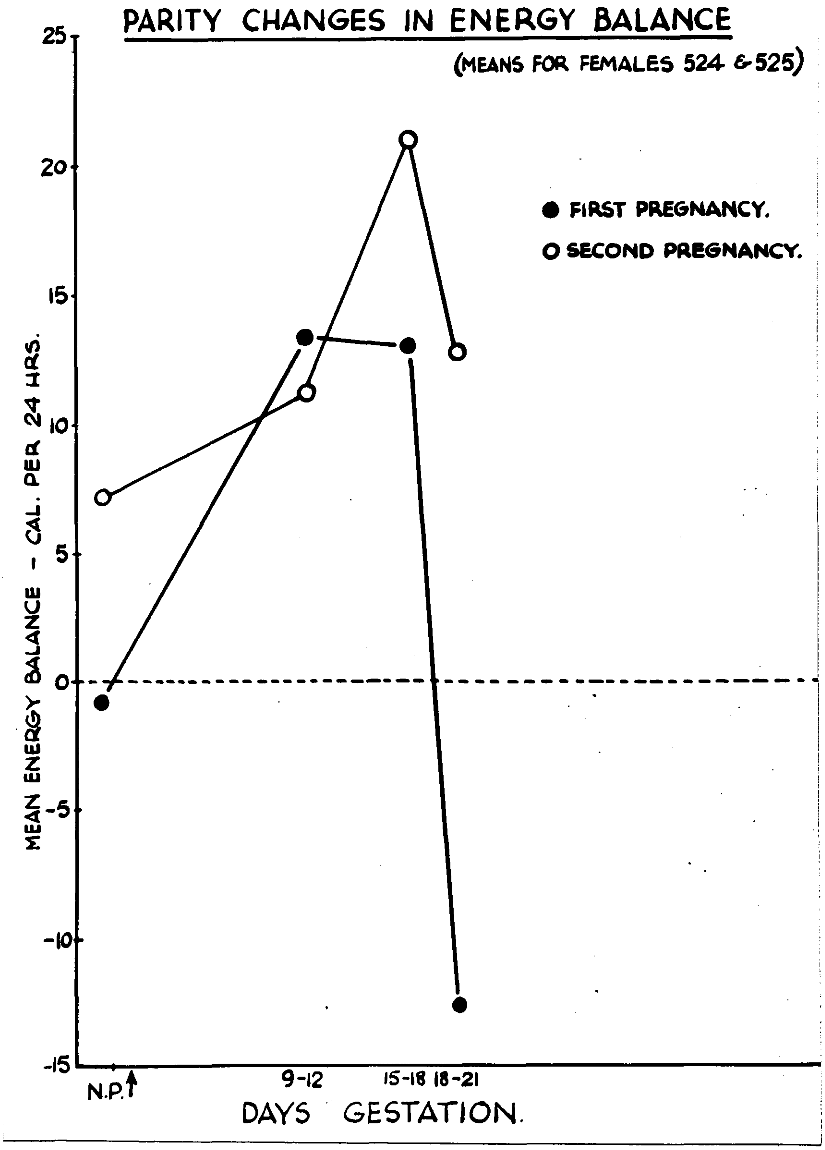

PARITY CHANGES IN ENERGY BALANCE
(MEANS FOR FEMALES 524 & 525)
● FIRST PREGNANCY.
○ SECOND PREGNANCY.
MEAN ENERGY BALANCE - CAL. PER 24 HRS.
25
20
15
10
5
0
-5
-10
-15
N.P.
9-12
15-18
18-21
DAYS GESTATION.

Figure (45)

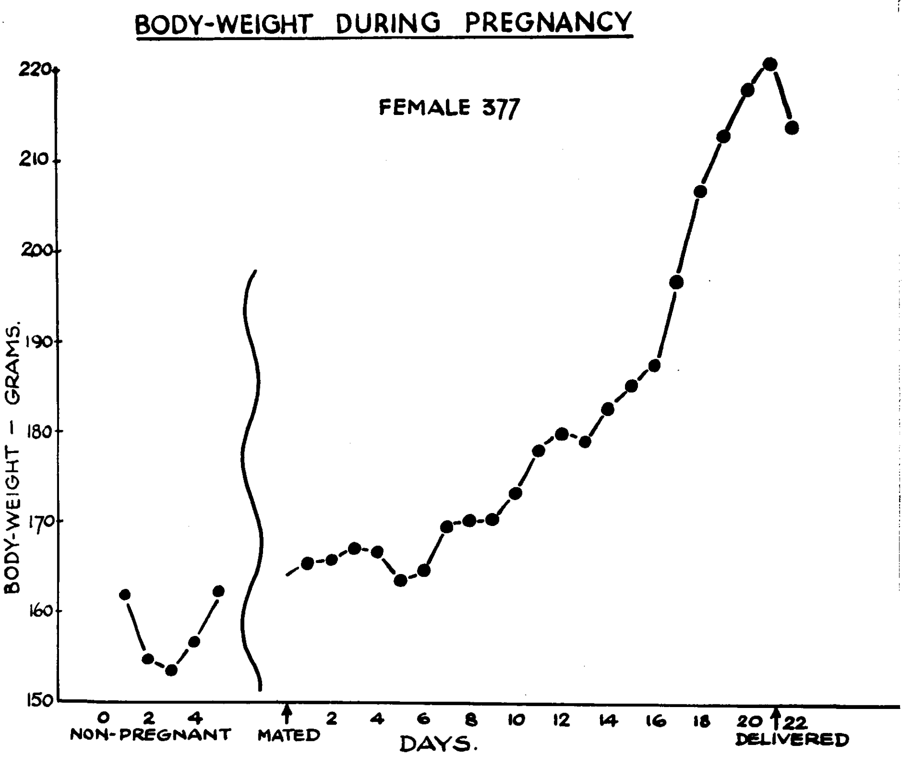

BODY-WEIGHT DURING PREGNANCY
FEMALE 377
BODY-WEIGHT - GRAMS.
220
210
200
190
180
170
160
150
0
2
4
NON-PREGNANT
MATED
2
4
6
8
10
12
14
16
18
20
22
DAYS.
DELIVERED

Figure (46)

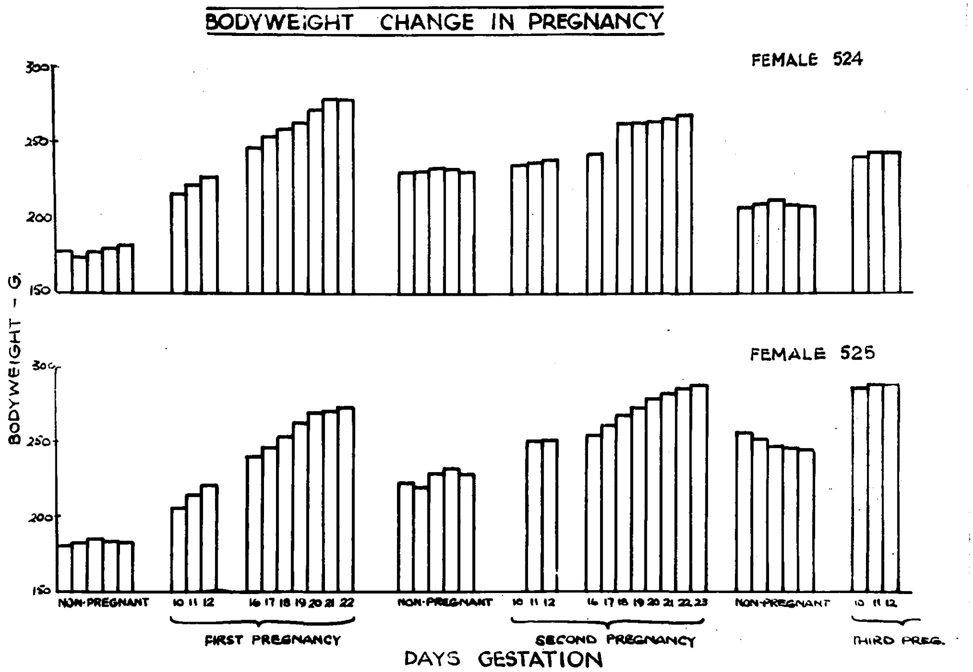

BODYWEIGHT CHANGE IN PREGNANCY
FEMALE 524
FEMALE 525
BODYWEIGHT - G.
300
250
200
150
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22 23
NON-PREGNANT
10 11 12
FIRST PREGNANCY
SECOND PREGNANCY
THIRD PREG.
DAYS GESTATION

Figure (47)

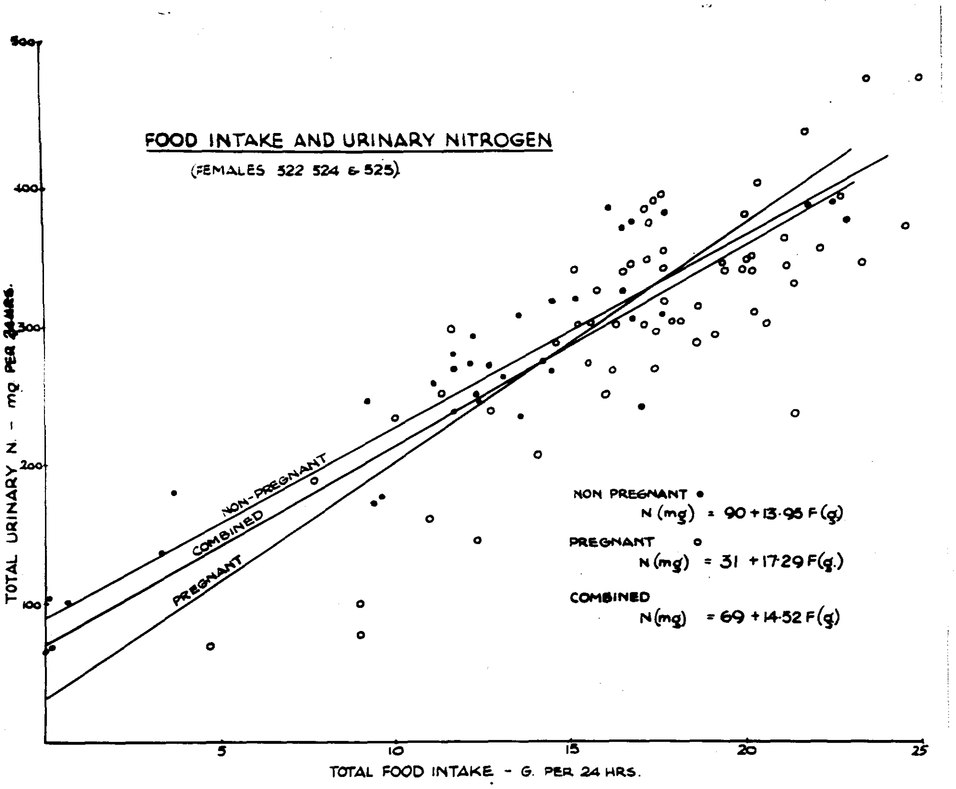

FOOD INTAKE AND URINARY NITROGEN
(FEMALES 522 524 & 525)
TOTAL URINARY N. - mg PER 24 HRS.
500
400
300
200
100
TOTAL FOOD INTAKE - G. PER 24 HRS.
5
10
15
20
25
NON-PREGNANT
COMBINED
PREGNANT
NON PREGNANT ●
N (mg) = 90 + 13·95 F (g)
PREGNANT ○
N (mg) = 31 + 17·29 F (g.)
COMBINED
N (mg) = 69 + 14·52 F (g)

Figure (48)

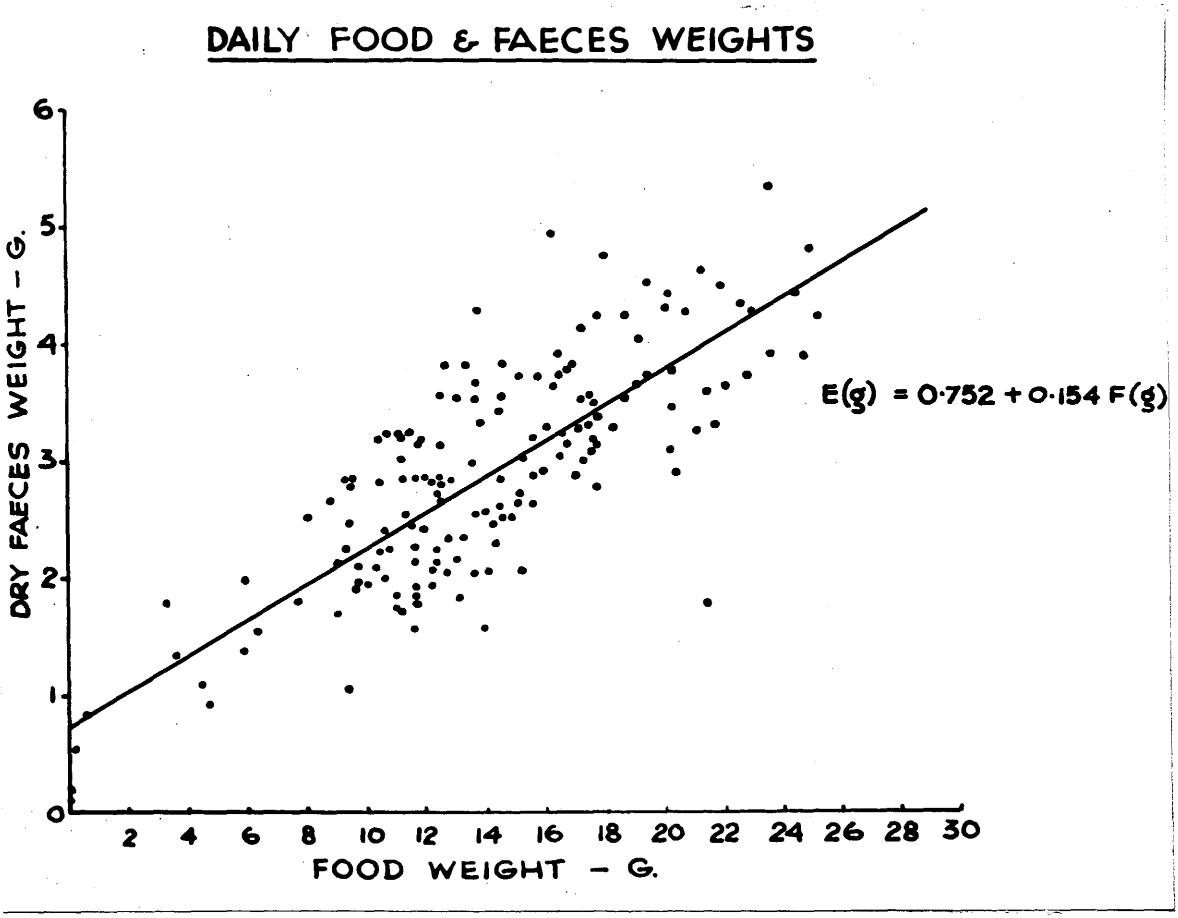

DAILY FOOD & FAECES WEIGHTS
DRY FAECES WEIGHT - G.
0
1
2
3
4
5
6
FOOD WEIGHT - G.
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
E(g) = 0·752 + 0·154 F(g)

Figure (49)

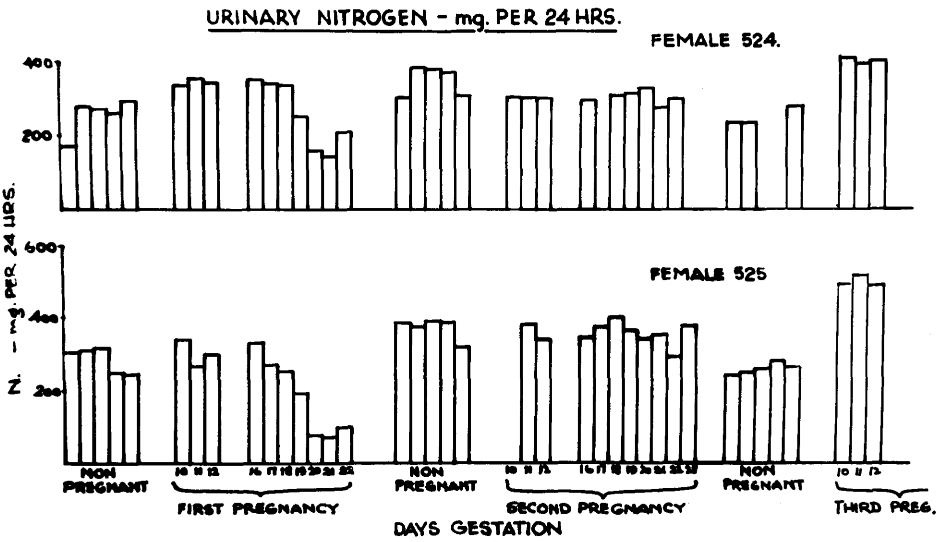

URINARY NITROGEN - mg. PER 24 HRS.
FEMALE 524.
400
200
N. - mg. PER 24 HRS.
600
FEMALE 525
400
200
NON PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (50)

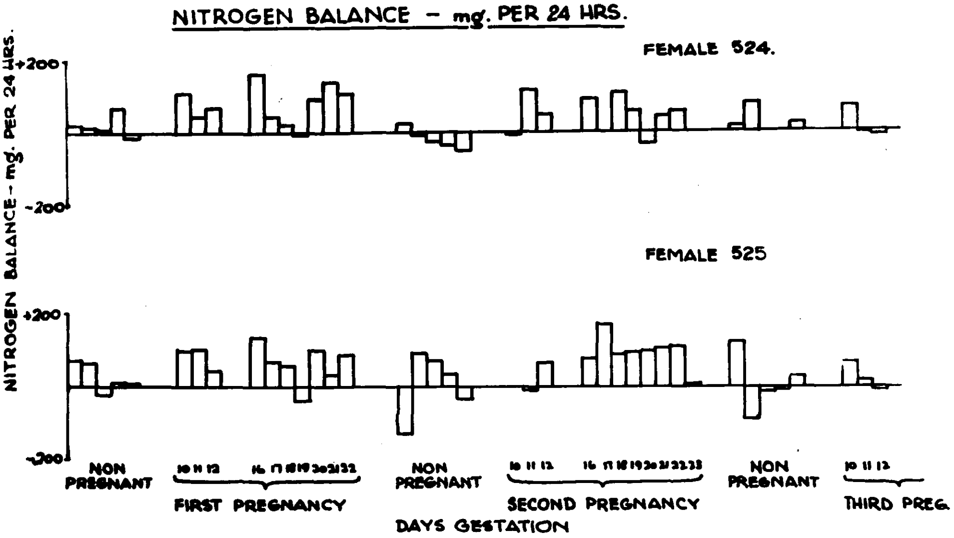

NITROGEN BALANCE – mg. PER 24 HRS.
FEMALE 524.
FEMALE 525
NITROGEN BALANCE – mg. PER 24 HRS.
+200
-200
+200
-200
NON PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (51)

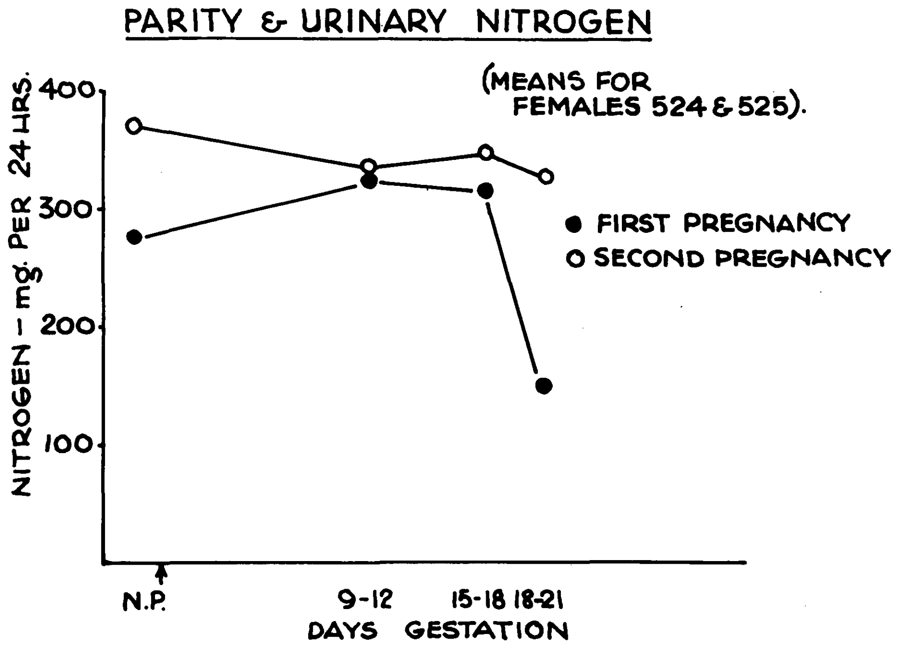

PARITY & URINARY NITROGEN
(MEANS FOR FEMALES 524 & 525).
NITROGEN – mg. PER 24 HRS.
400
300
200
100
● FIRST PREGNANCY
○ SECOND PREGNANCY
N.P.
9-12
15-18
18-21
DAYS GESTATION

Figure (52)

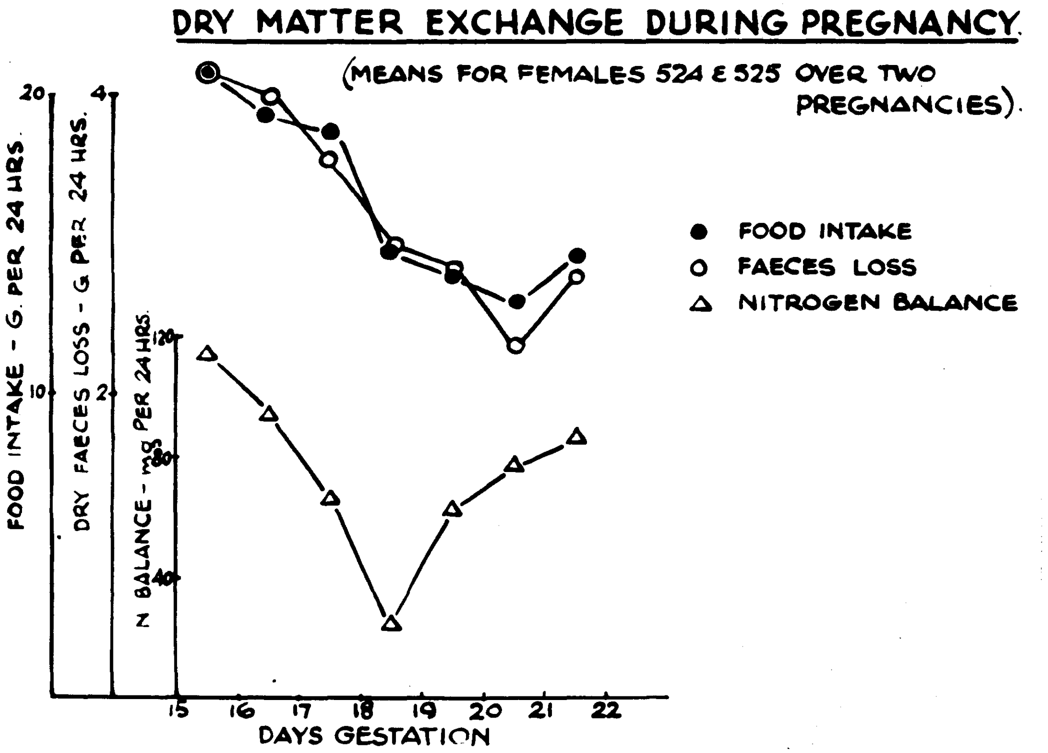
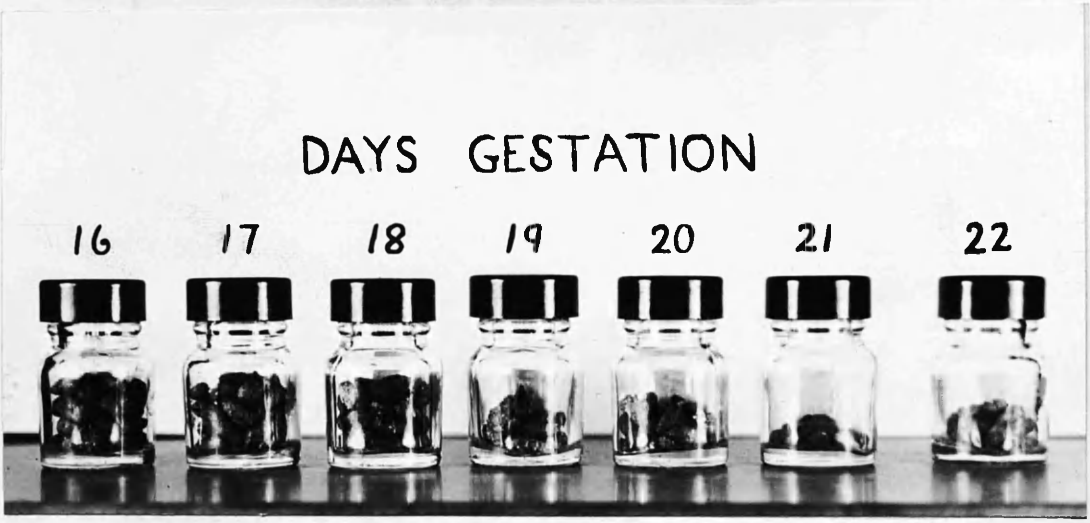

DRY MATTER EXCHANGE DURING PREGNANCY.
(MEANS FOR FEMALES 524 & 525 OVER TWO PREGNANCIES).
● FOOD INTAKE
O FAECES LOSS
Δ NITROGEN BALANCE
FOOD INTAKE - G. PER 24 HRS.
20
10
DRY FAECES LOSS - G PER 24 HRS.
4
2
N BALANCE - mg PER 24 HRS.
120
80
40
15
16
17
18
19
20
21
22
DAYS GESTATION

Figure (53)



Dried faeces during last days of pregnancy. Female 525 - first pregnancy.

Figure (54)

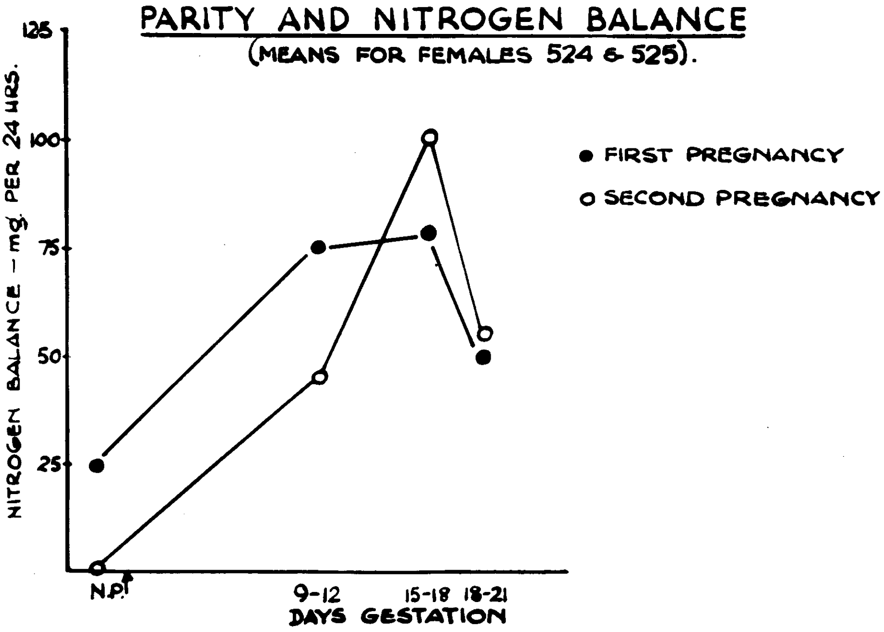

PARITY AND NITROGEN BALANCE
(MEANS FOR FEMALES 524 & 525).
NITROGEN BALANCE – mg. PER 24 HRS.
125
100
75
50
25
N.P.
9-12
15-18
18-21
DAYS GESTATION
● FIRST PREGNANCY
○ SECOND PREGNANCY

Figure (55)

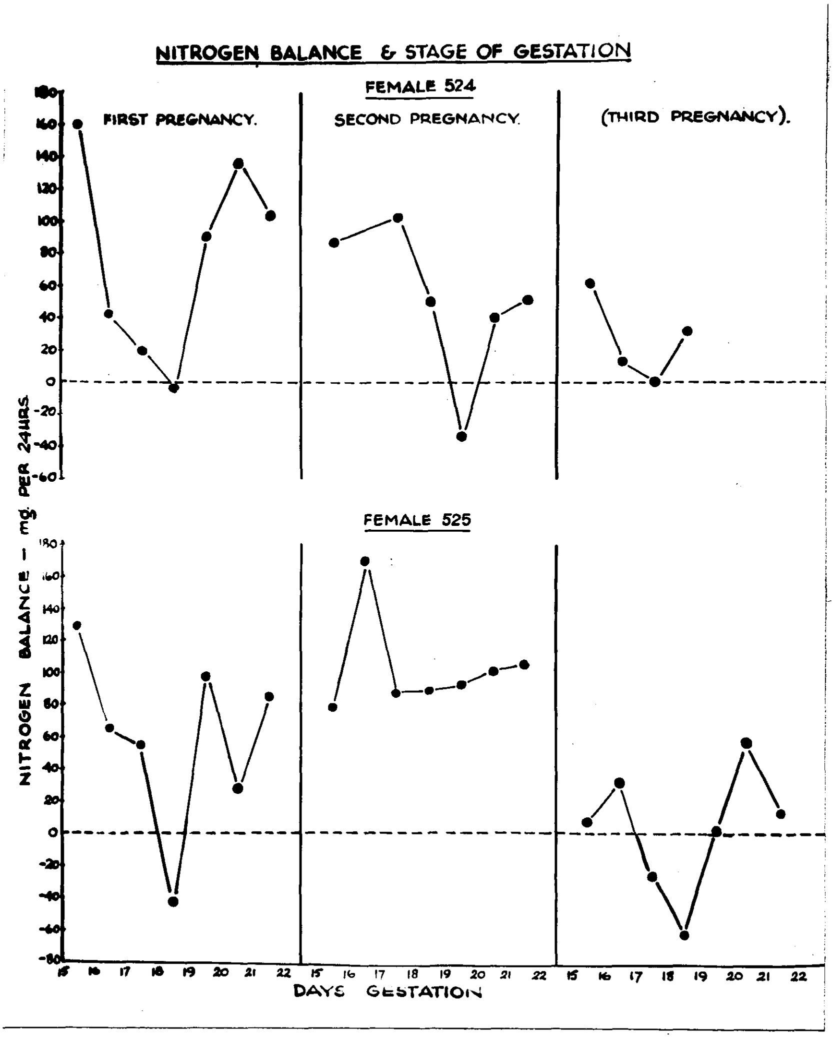
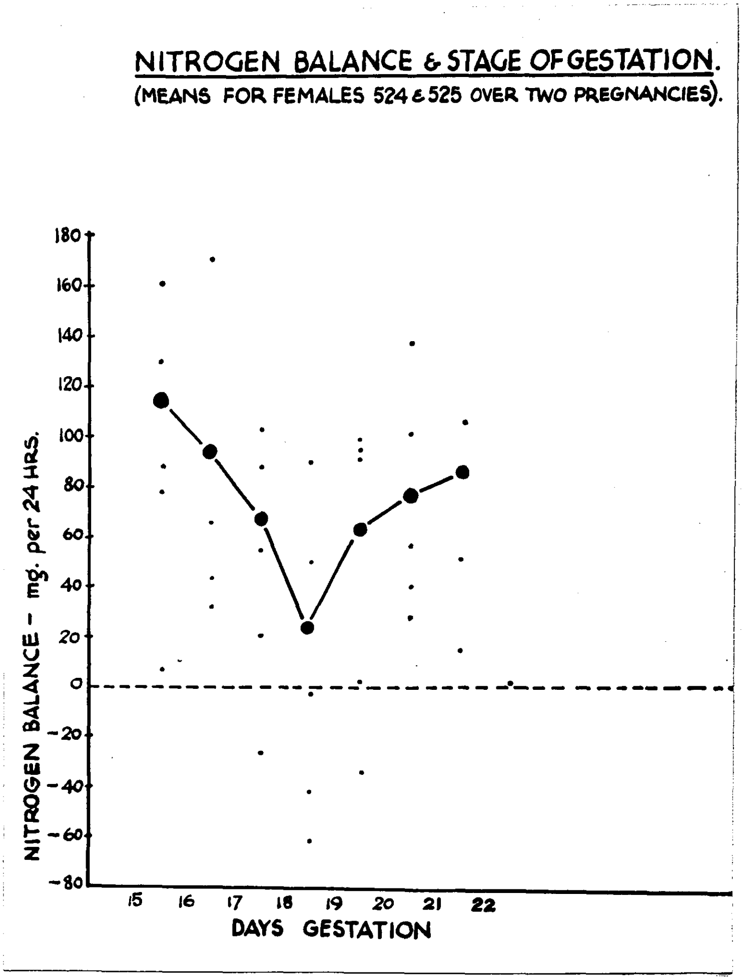

NITROGEN BALANCE & STAGE OF GESTATION
FEMALE 524
FIRST PREGNANCY.
SECOND PREGNANCY.
(THIRD PREGNANCY).
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
FEMALE 525
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
NITROGEN BALANCE — mg PER 24HRS.
15
16
17
18
19
20
21
22
DAYS GESTATION

Figure (56)



NITROGEN BALANCE & STAGE OF GESTATION.
(MEANS FOR FEMALES 524 & 525 OVER TWO PREGNANCIES).
NITROGEN BALANCE - mg. per 24 HRS.
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
15
16
17
18
19
20
21
22
DAYS GESTATION

Figure (57)

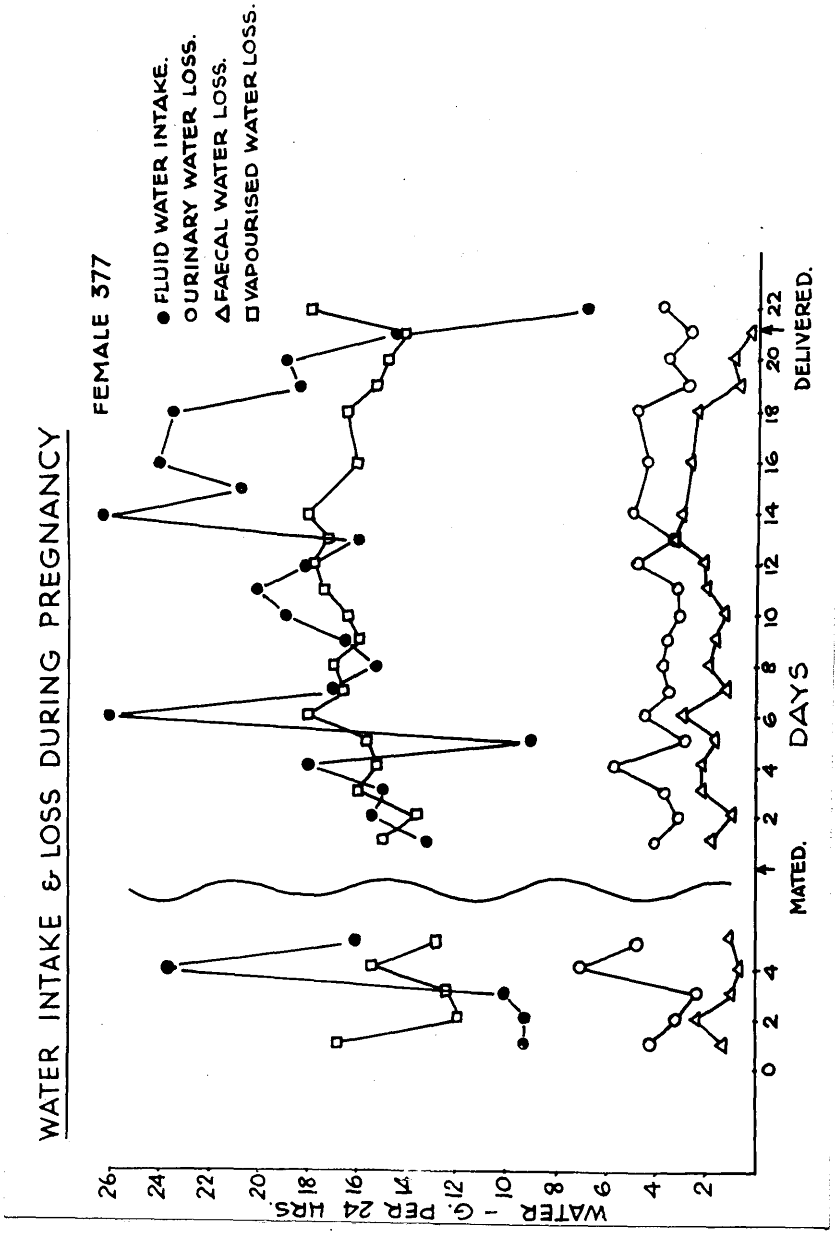

WATER INTAKE & LOSS DURING PREGNANCY
FEMALE 377
● FLUID WATER INTAKE.
○ URINARY WATER LOSS.
Δ FAECAL WATER LOSS.
□ VAPOURISED WATER LOSS.
WATER - G. PER 24 HRS.
26
24
22
20
18
16
14
12
10
8
6
4
2
0
2
4
2
4
6
8
10
12
14
16
18
20
22
DAYS
MATED.
DELIVERED.

Figure (58)

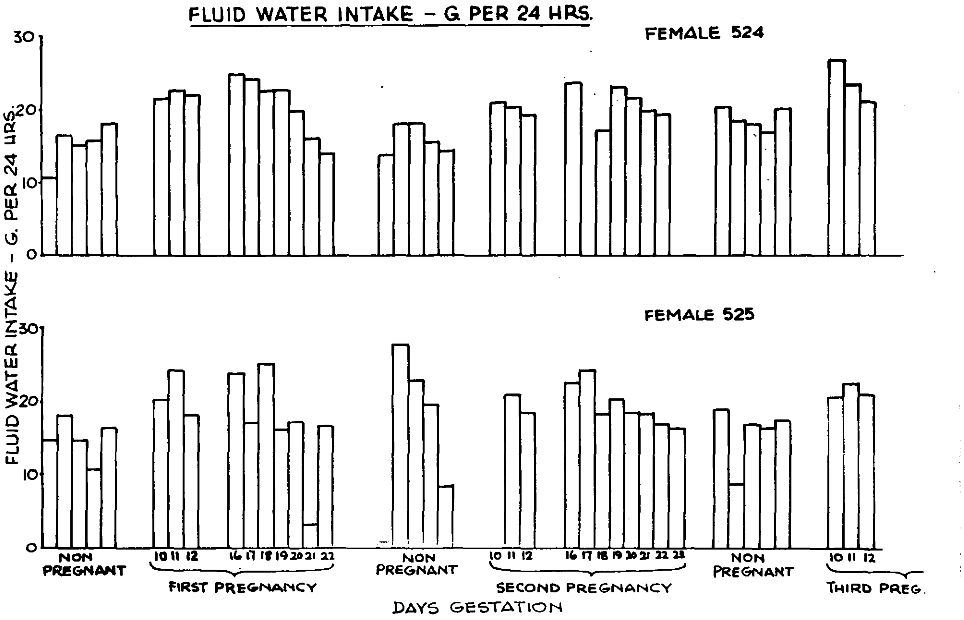

FLUID WATER INTAKE – G. PER 24 HRS.
FEMALE 524
FEMALE 525
FLUID WATER INTAKE – G. PER 24 HRS.
30
20
10
0
NON PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (59)

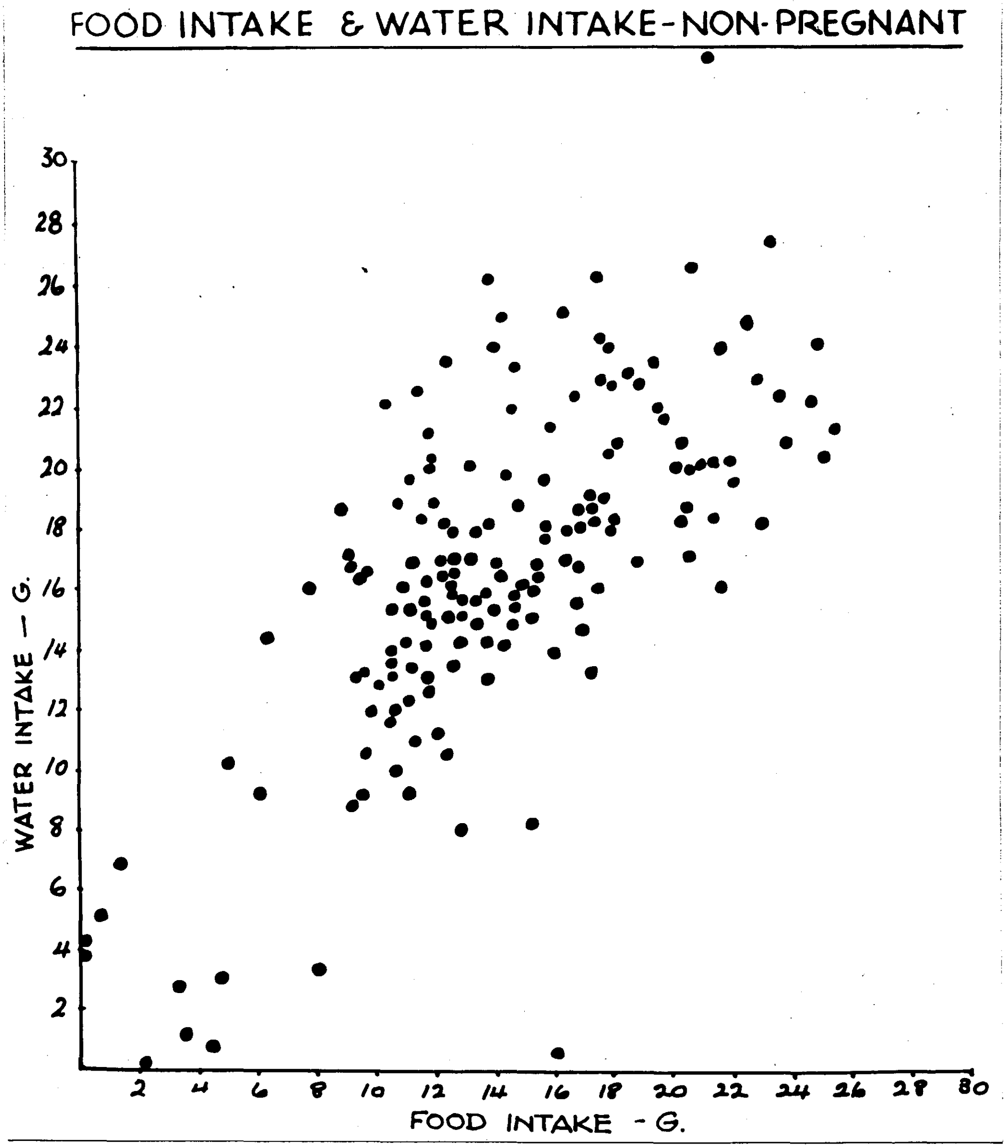

FOOD INTAKE & WATER INTAKE-NON-PREGNANT

Figure (60)

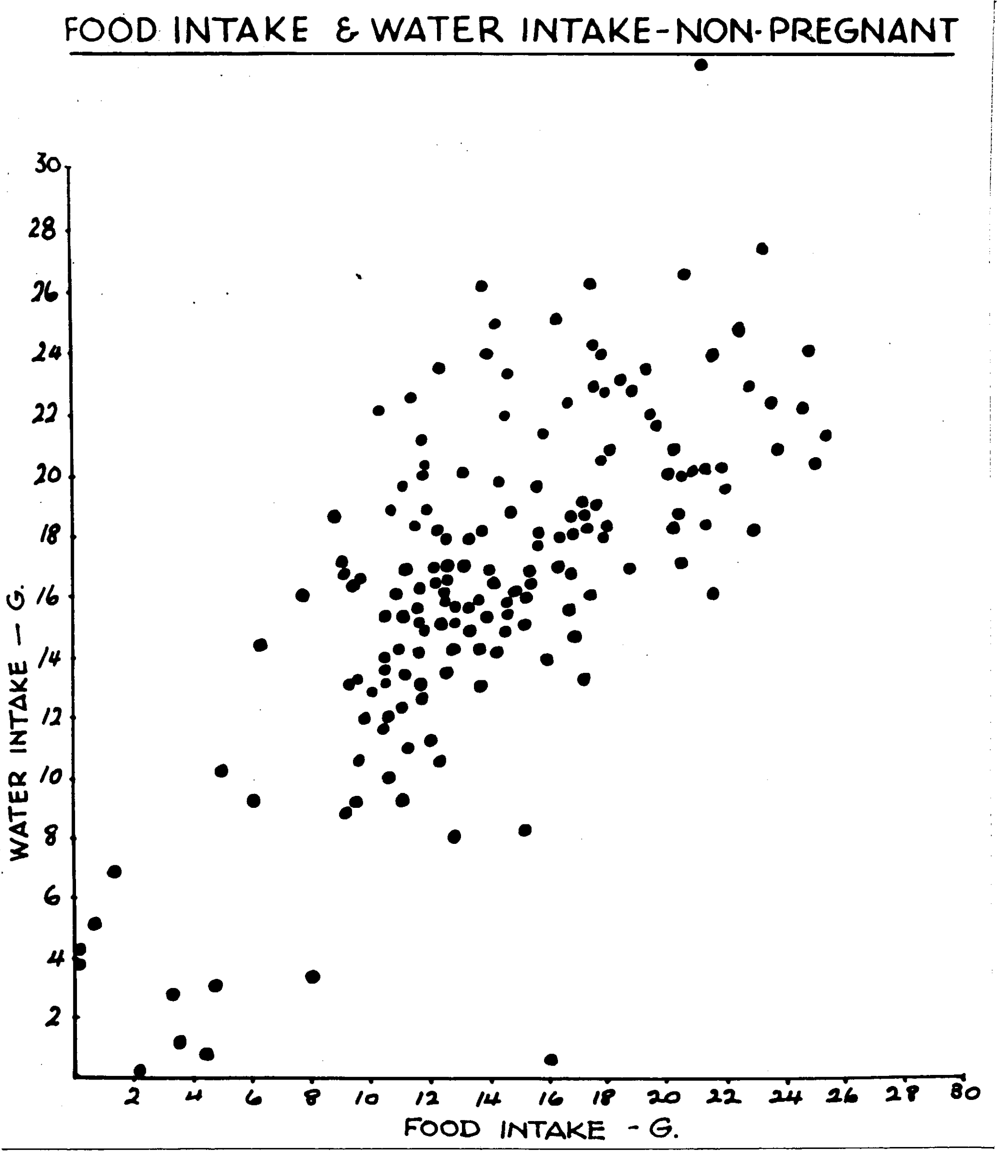

FOOD INTAKE & WATER INTAKE-NON-PREGNANT
WATER INTAKE - G.
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
FOOD INTAKE - G.
2
4
6
8
10
12
14
16
18
20
22
24
26
28
80

Figure (60)

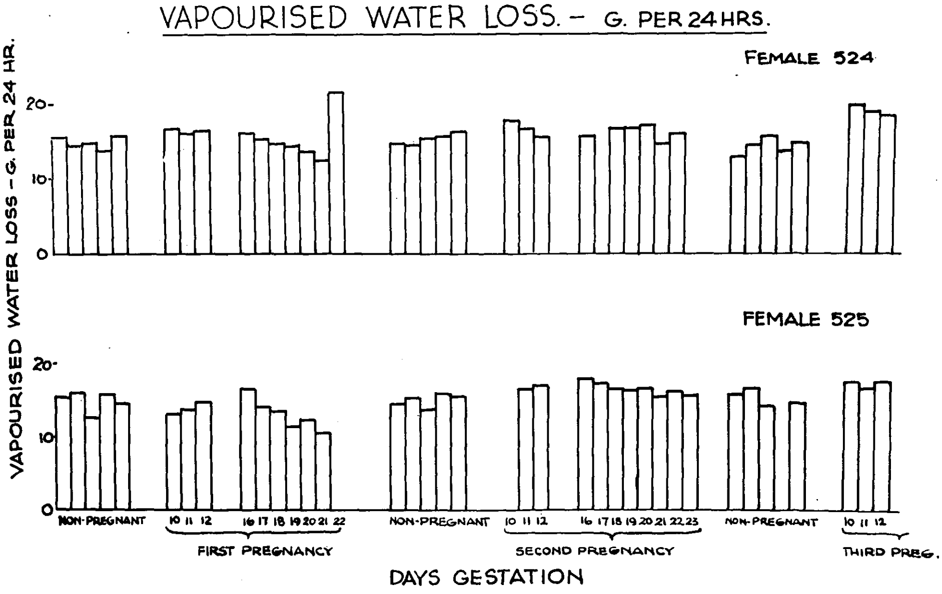

VAPOURISED WATER LOSS. - G. PER 24 HRS.
FEMALE 524
FEMALE 525
VAPOURISED WATER LOSS - G. PER 24 HR.
20-
10-
0
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON-PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (61)

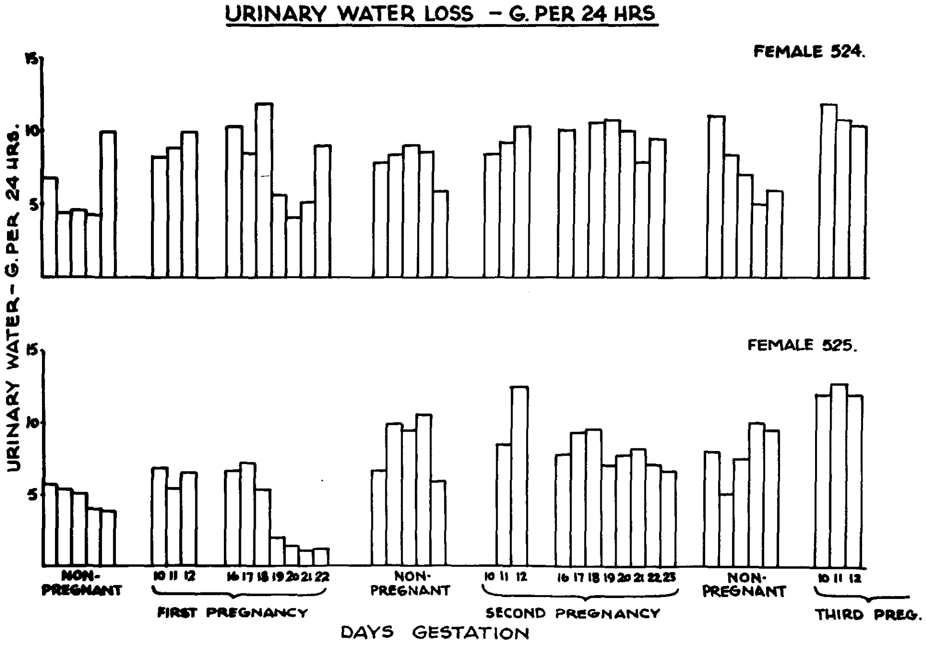

URINARY WATER LOSS - G. PER 24 HRS
FEMALE 524.
FEMALE 525.
URINARY WATER - G. PER 24 HRS.
15
10
5
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON-PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON-PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (62)

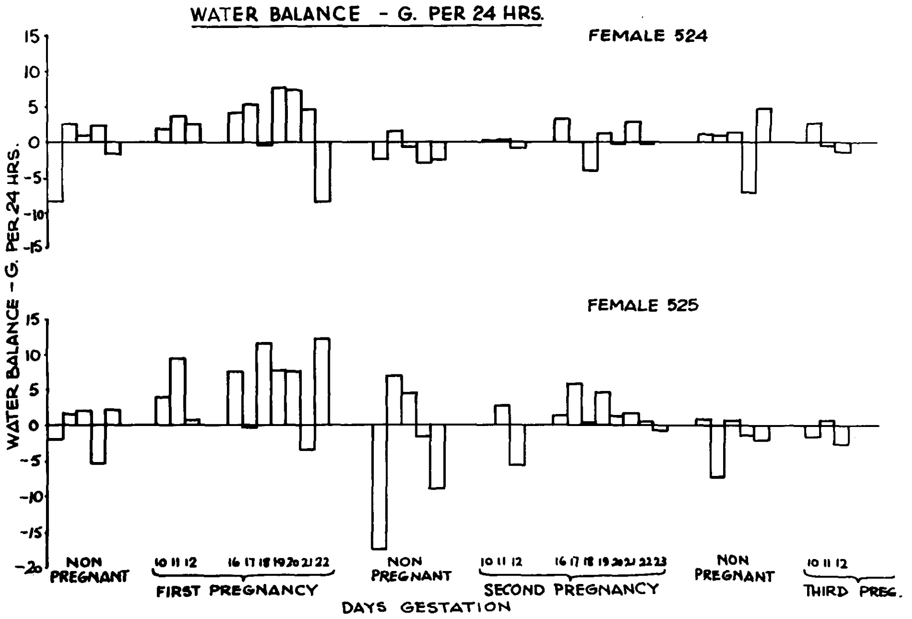

WATER BALANCE - G. PER 24 HRS.
FEMALE 524
FEMALE 525
WATER BALANCE - G. PER 24 HRS.
15
10
5
0
-5
-10
-15
-20
NON PREGNANT
10 11 12
16 17 18 19 20 21 22
FIRST PREGNANCY
NON PREGNANT
10 11 12
16 17 18 19 20 21 22 23
SECOND PREGNANCY
NON PREGNANT
10 11 12
THIRD PREG.
DAYS GESTATION

Figure (63)

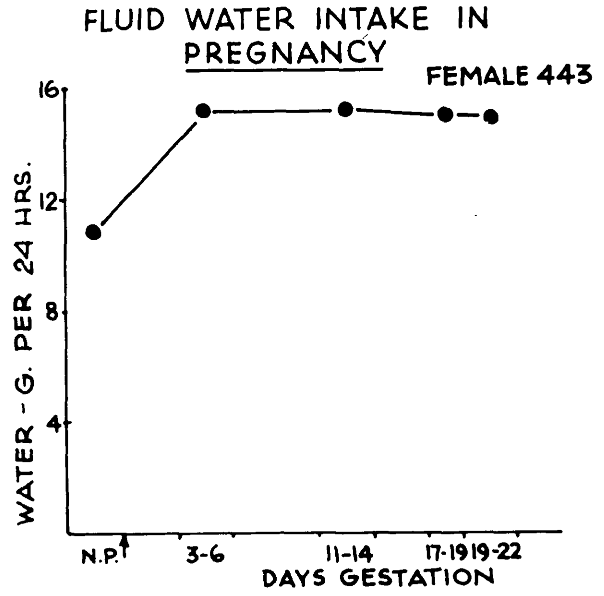

FLUID WATER INTAKE IN PREGNANCY
FEMALE 443
WATER - G. PER 24 HRS.
16
12
8
4
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (64)

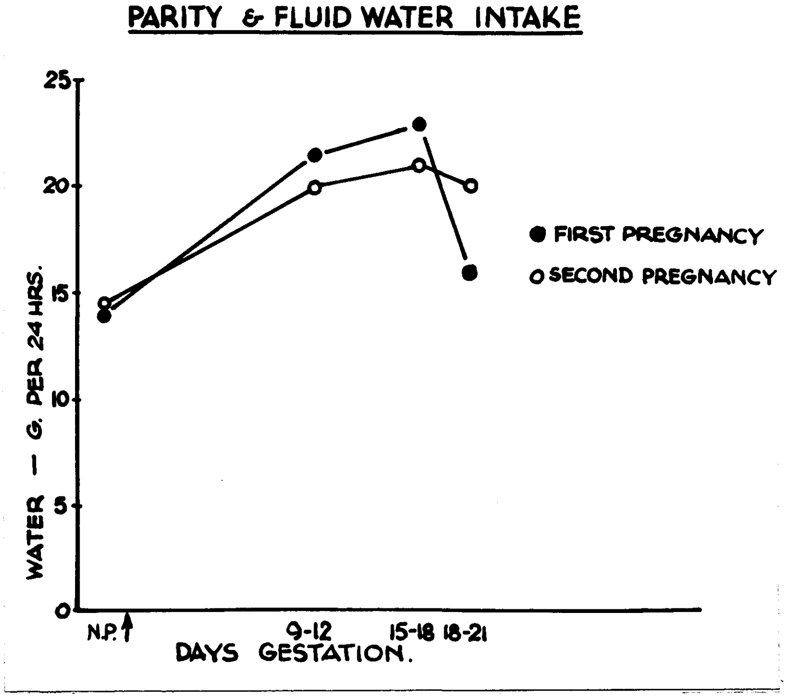

PARITY & FLUID WATER INTAKE
WATER — G. PER 24 HRS.
25
20
15
10
5
0
N.P.
9-12
15-18
18-21
DAYS GESTATION.
● FIRST PREGNANCY
○ SECOND PREGNANCY

Figure (65)

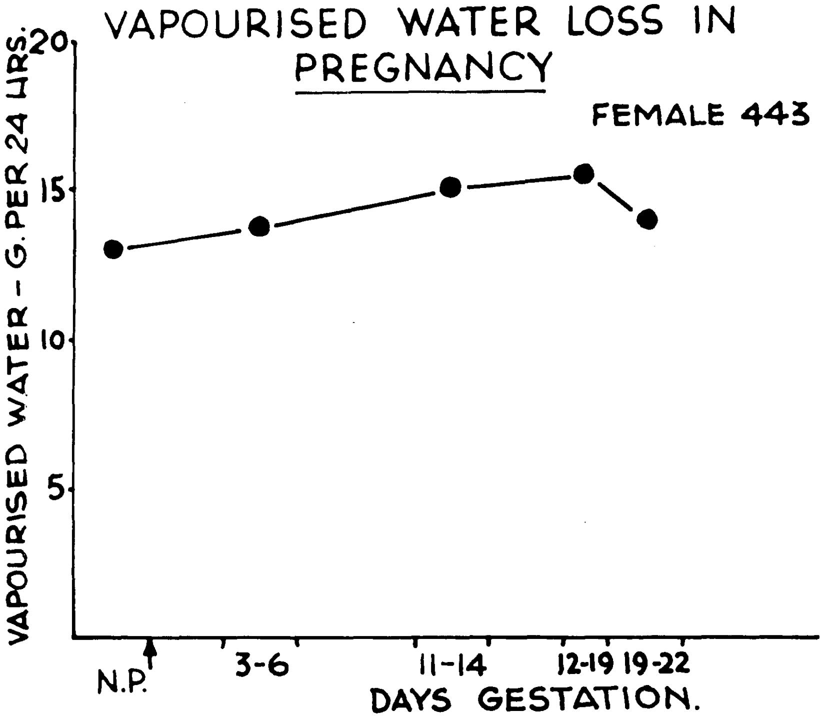

VAPOURISED WATER LOSS IN PREGNANCY
FEMALE 443
VAPOURISED WATER - G. PER 24 HRS.
20
15
10
5
N.P.
3-6
11-14
12-19
19-22
DAYS GESTATION.

Figure (66)

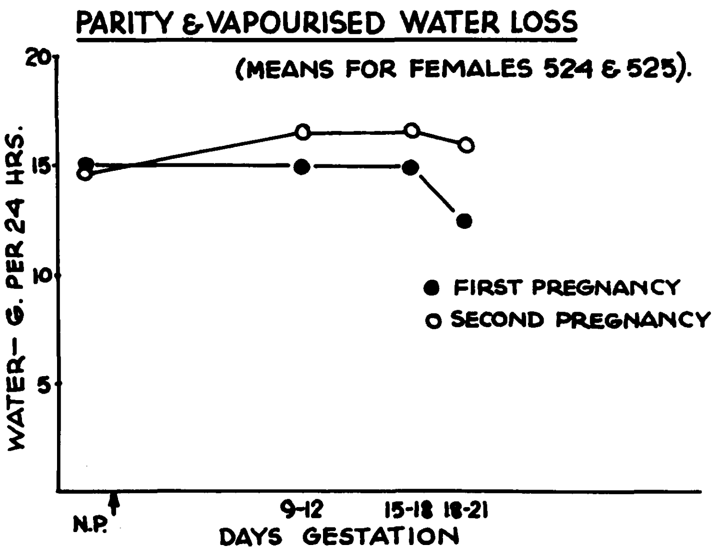

PARITY & VAPOURISED WATER LOSS
(MEANS FOR FEMALES 524 & 525).
WATER– G. PER 24 HRS.
20
15
10
5
N.P.
9-12
15-18
18-21
DAYS GESTATION
● FIRST PREGNANCY
○ SECOND PREGNANCY

Figure (67)

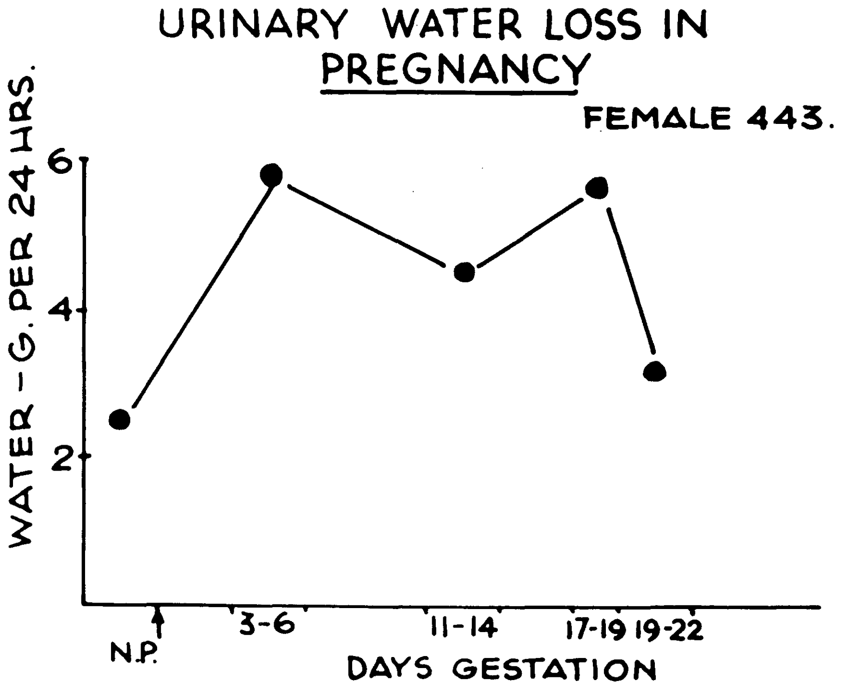

URINARY WATER LOSS IN
PREGNANCY
FEMALE 443.
WATER - G. PER 24 HRS.
6
4
2
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (68)

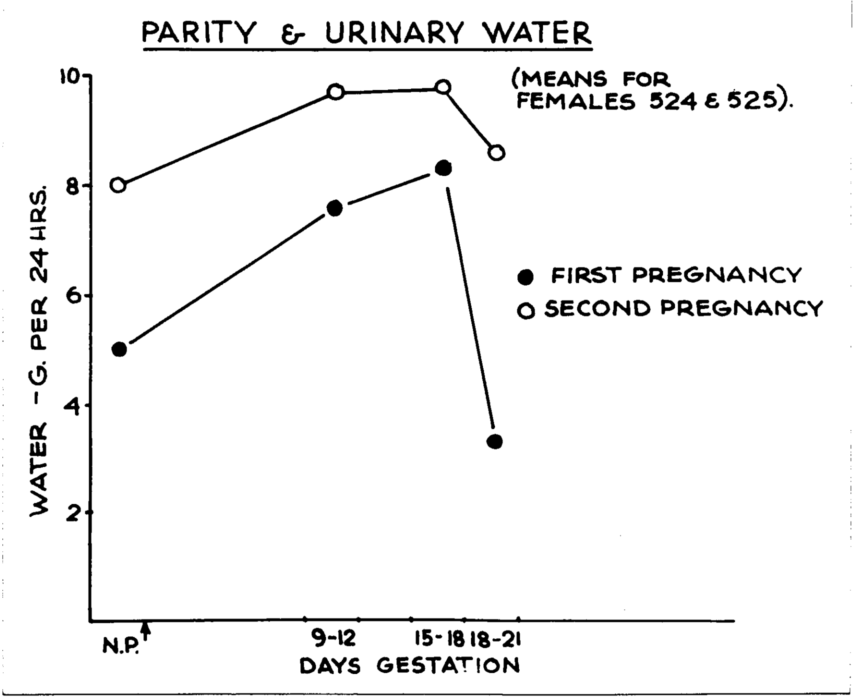

PARITY & URINARY WATER
(MEANS FOR FEMALES 524 & 525).
WATER - G. PER 24 HRS.
10
8
6
4
2
N.P.
9-12
15-18
18-21
DAYS GESTATION
● FIRST PREGNANCY
○ SECOND PREGNANCY

Figure (69)

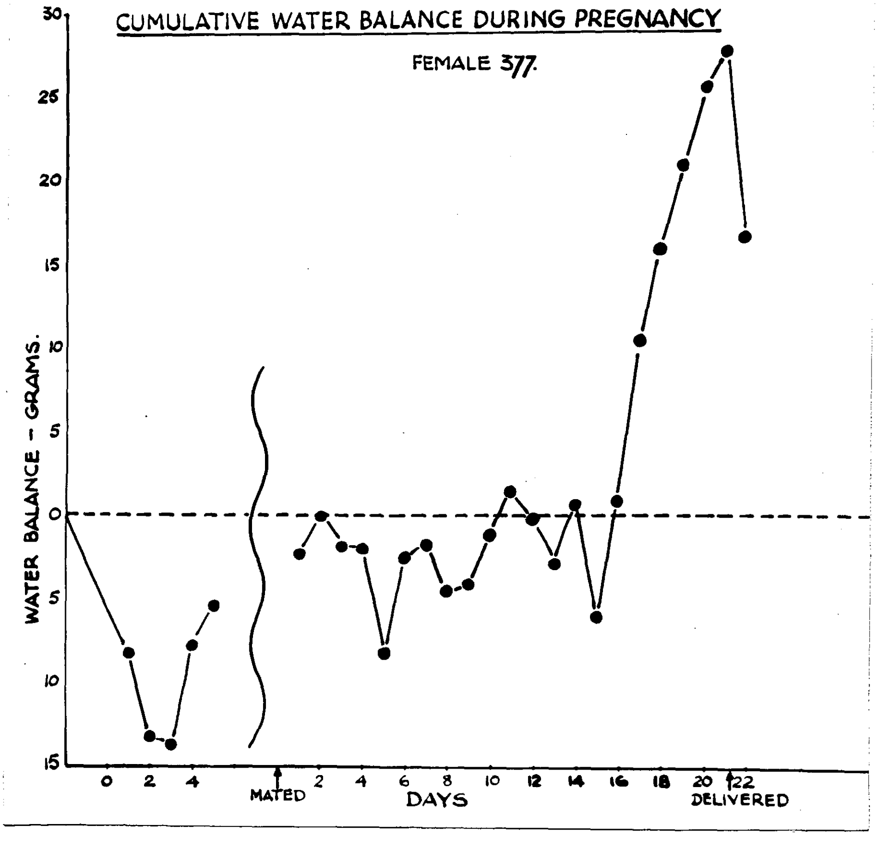

CUMULATIVE WATER BALANCE DURING PREGNANCY
FEMALE 377.
WATER BALANCE - GRAMS.
30
25
20
15
10
5
0
5
10
15
0
2
4
MATED
2
4
6
8
10
12
14
16
18
20
22
DAYS
DELIVERED

Figure (70)

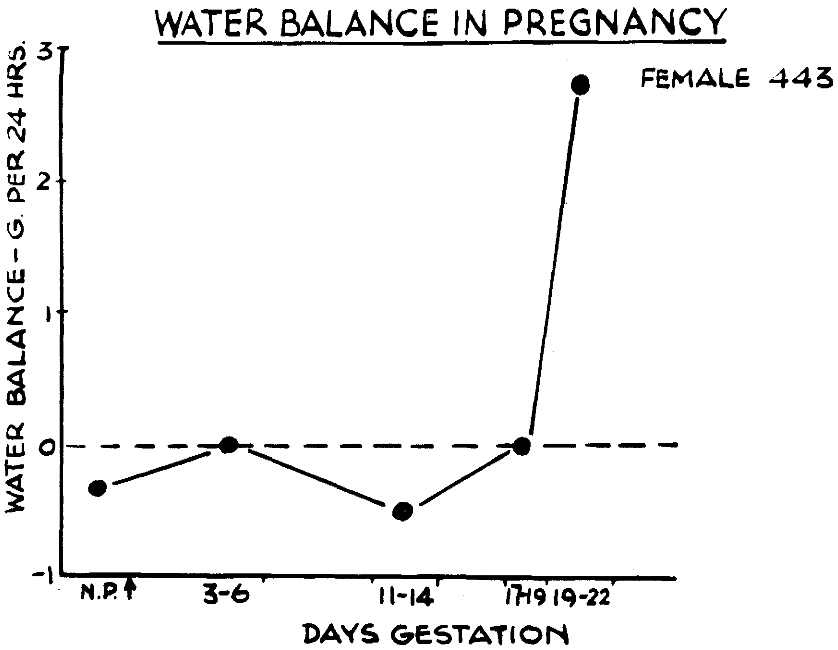

WATER BALANCE IN PREGNANCY
FEMALE 443
WATER BALANCE - G. PER 24 HRS.
3
2
1
0
-1
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (71)

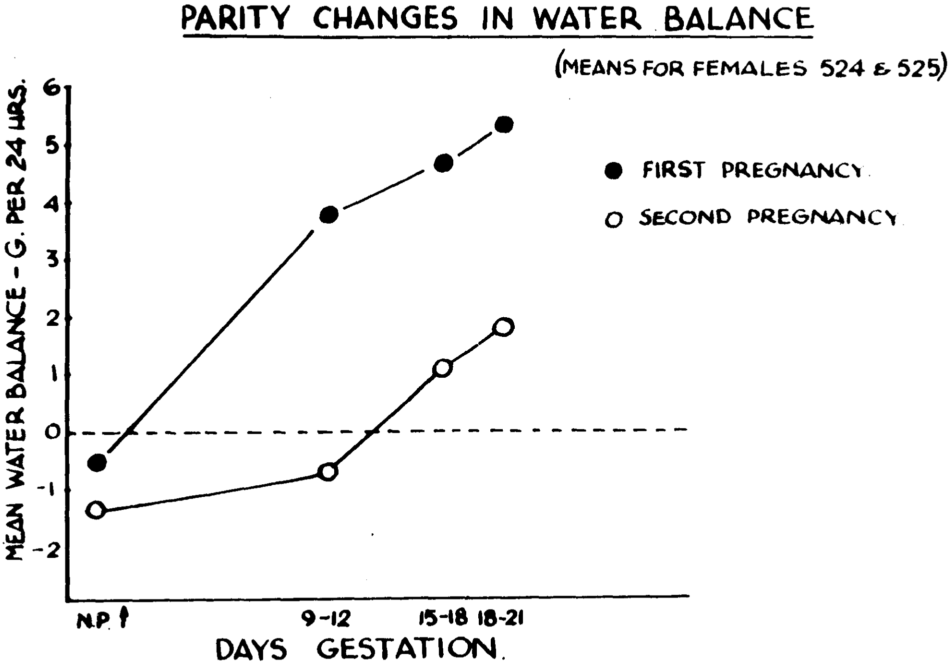

PARITY CHANGES IN WATER BALANCE
(MEANS FOR FEMALES 524 & 525)
● FIRST PREGNANCY
O SECOND PREGNANCY
MEAN WATER BALANCE - G. PER 24 HRS.
6
5
4
3
2
1
0
-1
-2
N.P.
9-12
15-18
18-21
DAYS GESTATION.

Figure (72)

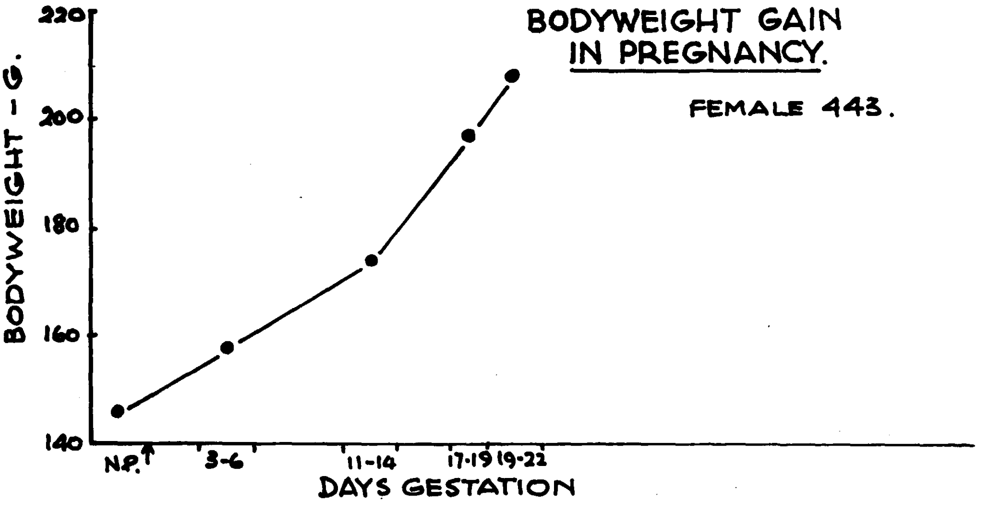

BODYWEIGHT GAIN
IN PREGNANCY.
FEMALE 443.
BODYWEIGHT - G.
220
200
180
160
140
N.P.
3-6
11-14
17-19
19-22
DAYS GESTATION

Figure (73)

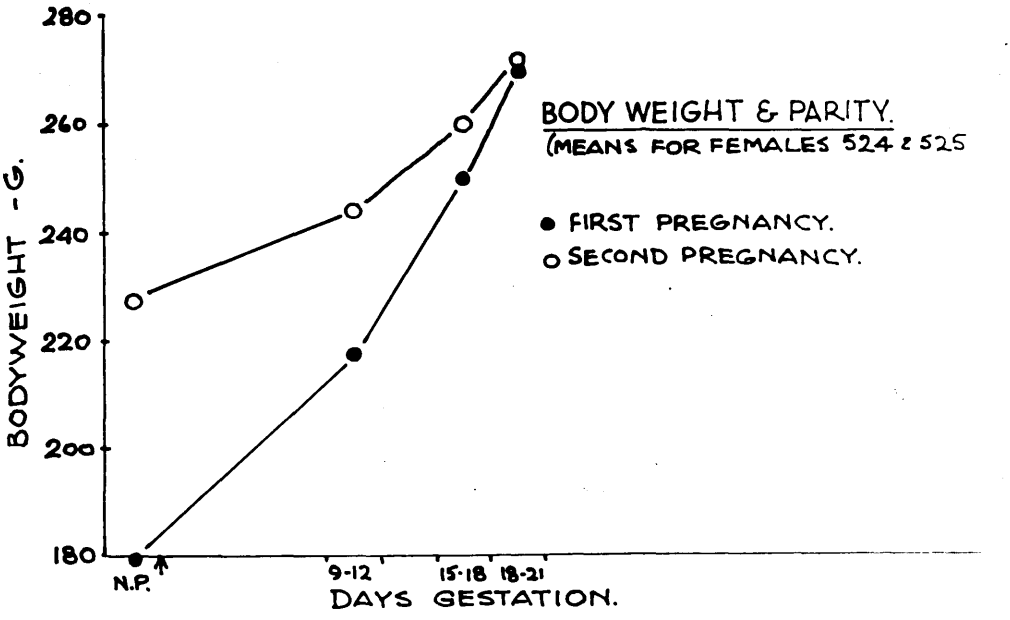

280
260
240
220
200
180
BODYWEIGHT - G.
N.P.
9-12
15-18
18-21
DAYS GESTATION.
BODY WEIGHT & PARITY.
(MEANS FOR FEMALES 524 & 525
● FIRST PREGNANCY.
○ SECOND PREGNANCY.

Figure (74)

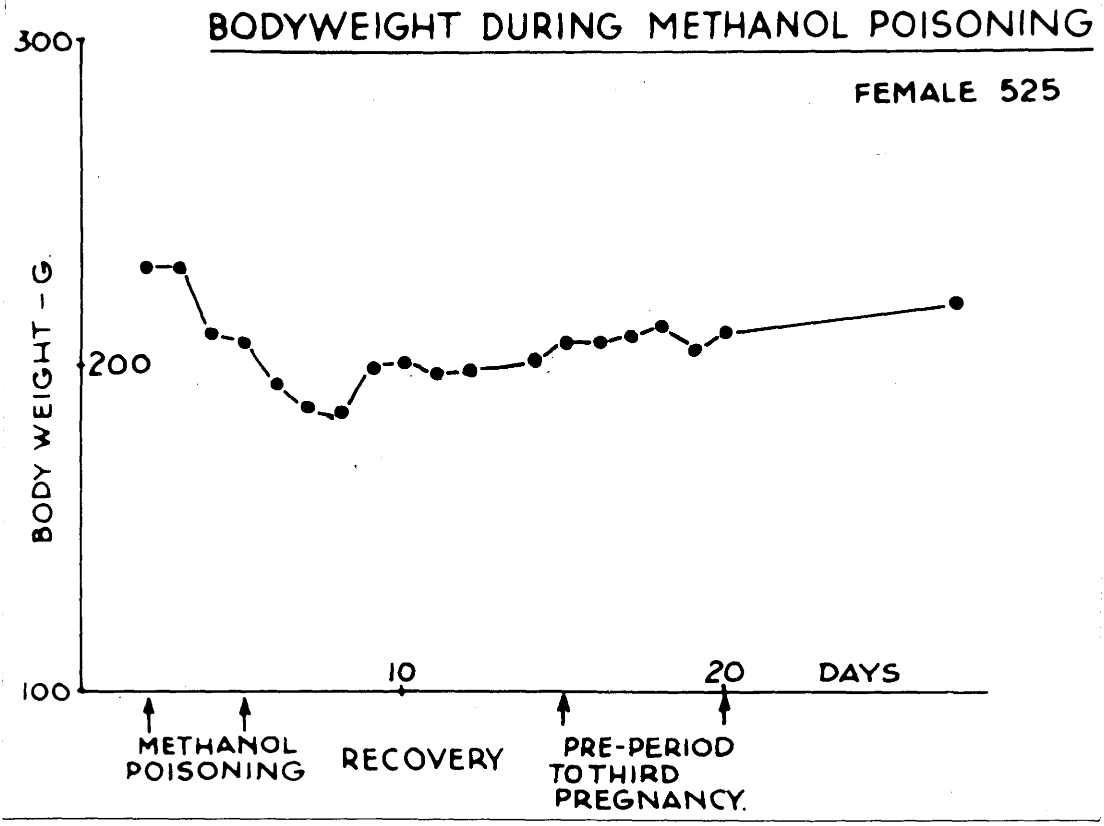

BODYWEIGHT DURING METHANOL POISONING
FEMALE 525
BODY WEIGHT - G.
300
200
100
10
20
DAYS
METHANOL POISONING
RECOVERY
PRE-PERIOD TO THIRD PREGNANCY.

Figure (75)

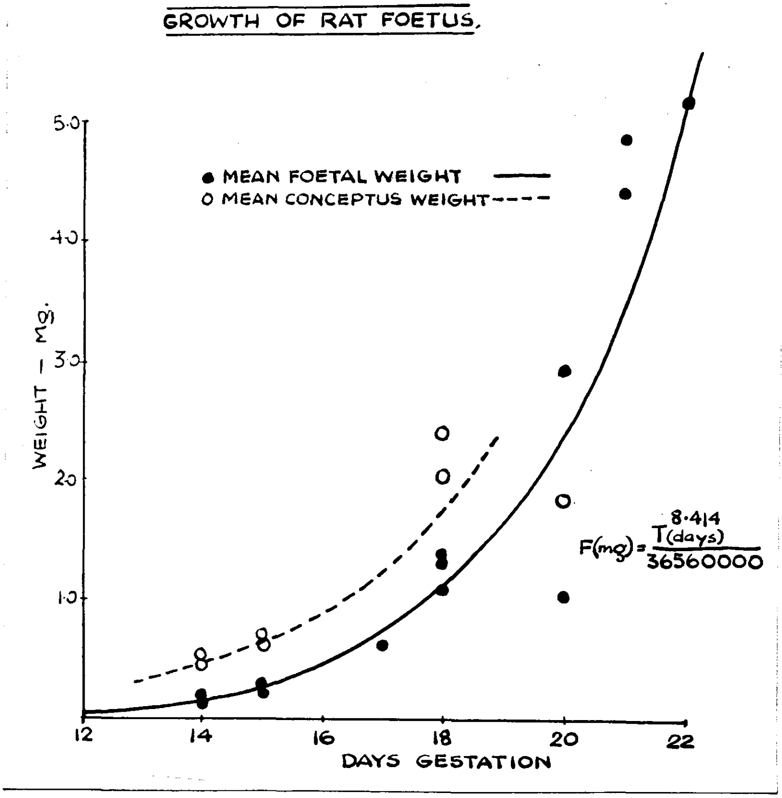

GROWTH OF RAT FOETUS.
● MEAN FOETAL WEIGHT
O MEAN CONCEPTUS WEIGHT
WEIGHT – Mg.
5.0
4.0
3.0
2.0
1.0
12
14
16
18
20
22
DAYS GESTATION
F(mg) = T(days)^8.414 / 36560000

Figure (76)

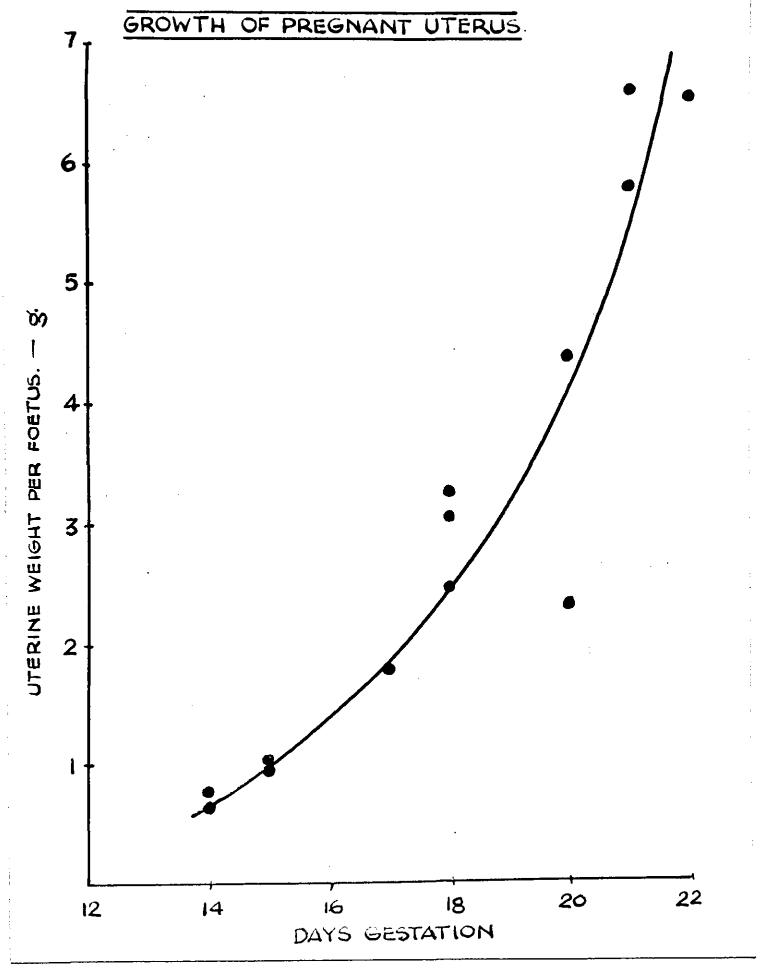

GROWTH OF PREGNANT UTERUS.
UTERINE WEIGHT PER FOETUS. — g.
1
2
3
4
5
6
7
12
14
16
18
20
22
DAYS GESTATION

Figure (77)

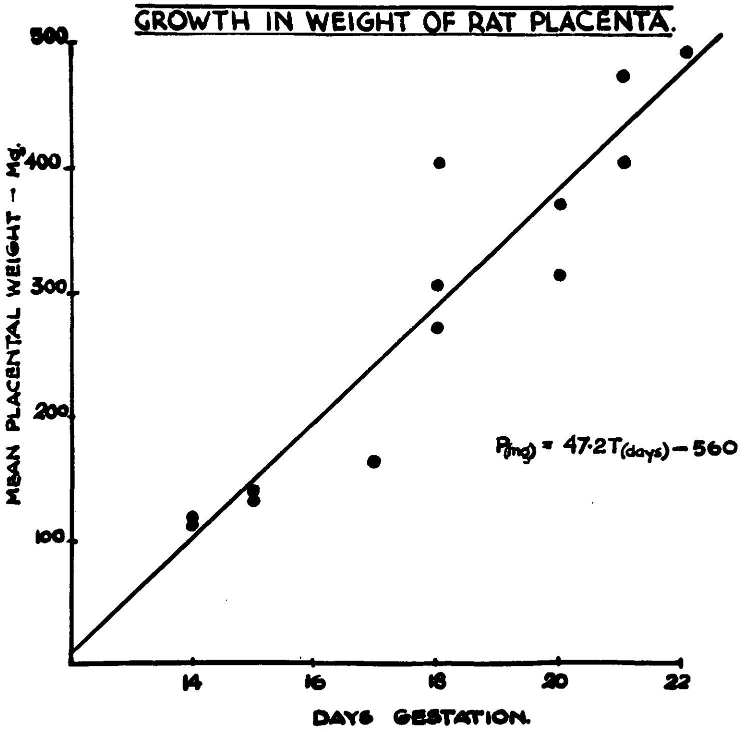

GROWTH IN WEIGHT OF RAT PLACENTA.
MEAN PLACENTAL WEIGHT – Mg.
500
400
300
200
100
14
16
18
20
22
DAYS GESTATION.
P(mg) = 47·2T(days) – 560

Figure (78)

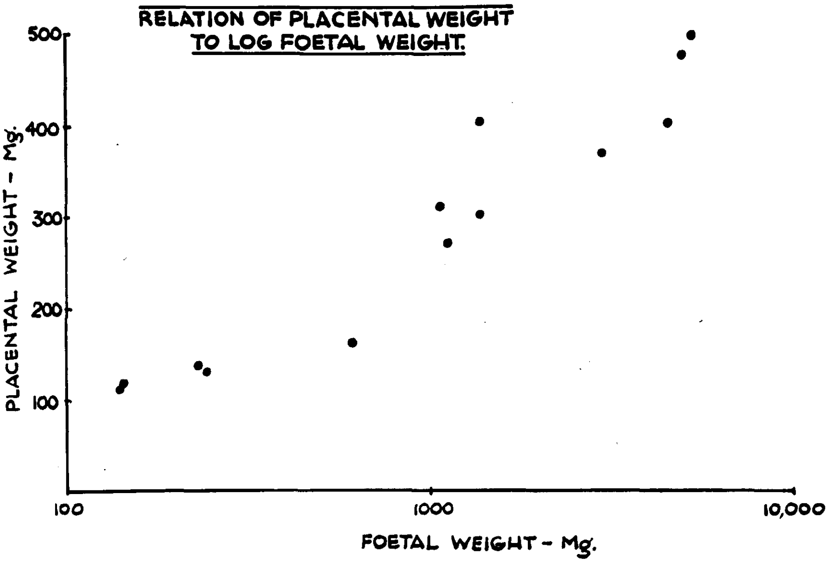

RELATION OF PLACENTAL WEIGHT TO LOG FOETAL WEIGHT.
PLACENTAL WEIGHT - Mg.
500
400
300
200
100
100
1000
10,000
FOETAL WEIGHT - Mg.

Figure (79)

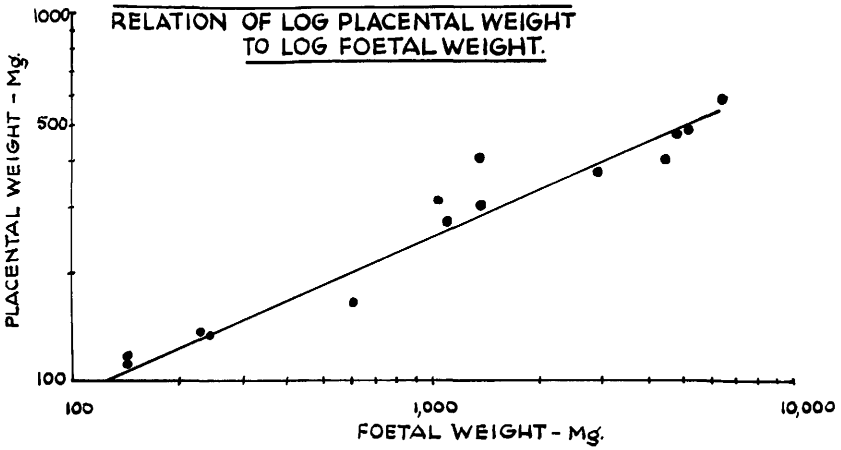

RELATION OF LOG PLACENTAL WEIGHT TO LOG FOETAL WEIGHT.
PLACENTAL WEIGHT - Mg.
1000
500
100
100
1,000
10,000
FOETAL WEIGHT - Mg.

Figure (80)

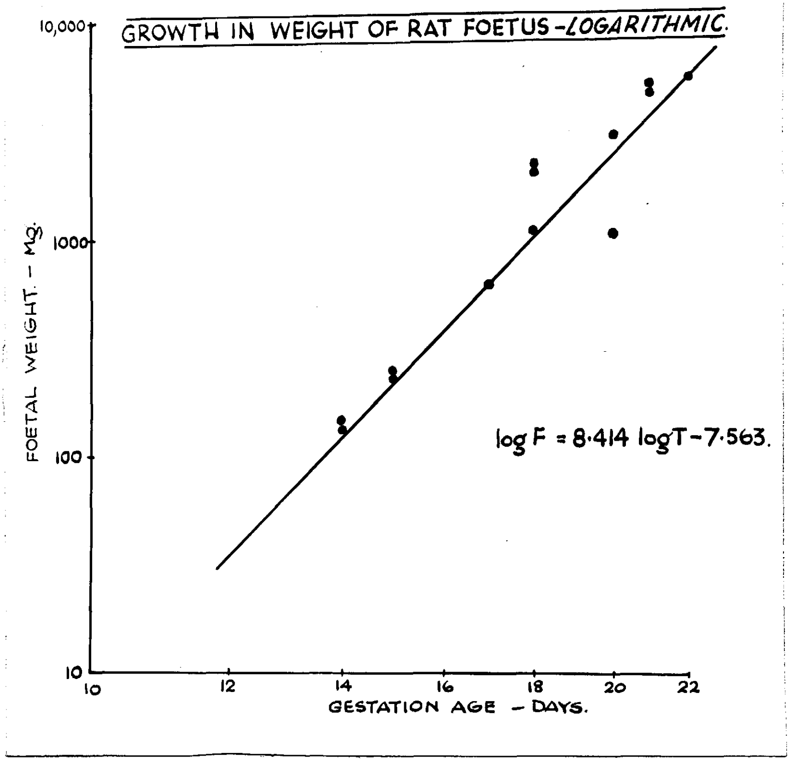

GROWTH IN WEIGHT OF RAT FOETUS -*LOGARITHMIC*.
10,000
1000
100
10
FOETAL WEIGHT. - Mg.
log F = 8·414 log T - 7·563.
10
12
14
16
18
20
22
GESTATION AGE - DAYS.

Figure (81)

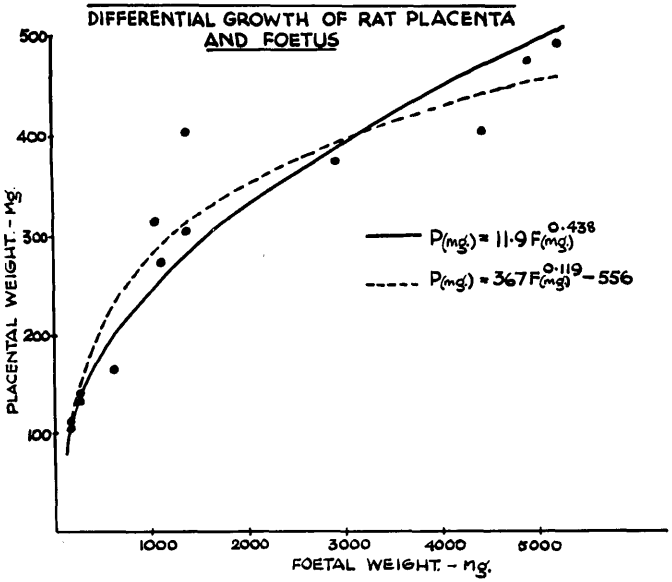

DIFFERENTIAL GROWTH OF RAT PLACENTA AND FOETUS
500
400
300
200
100
PLACENTAL WEIGHT - Mg.
1000
2000
3000
4000
5000
FOETAL WEIGHT - Mg.
$P_{(mg.)} = 11{\cdot}9\,F_{(mg.)}^{0.438}$
$P_{(mg.)} = 367\,F_{(mg.)}^{0.119} - 556$

Figure (82)

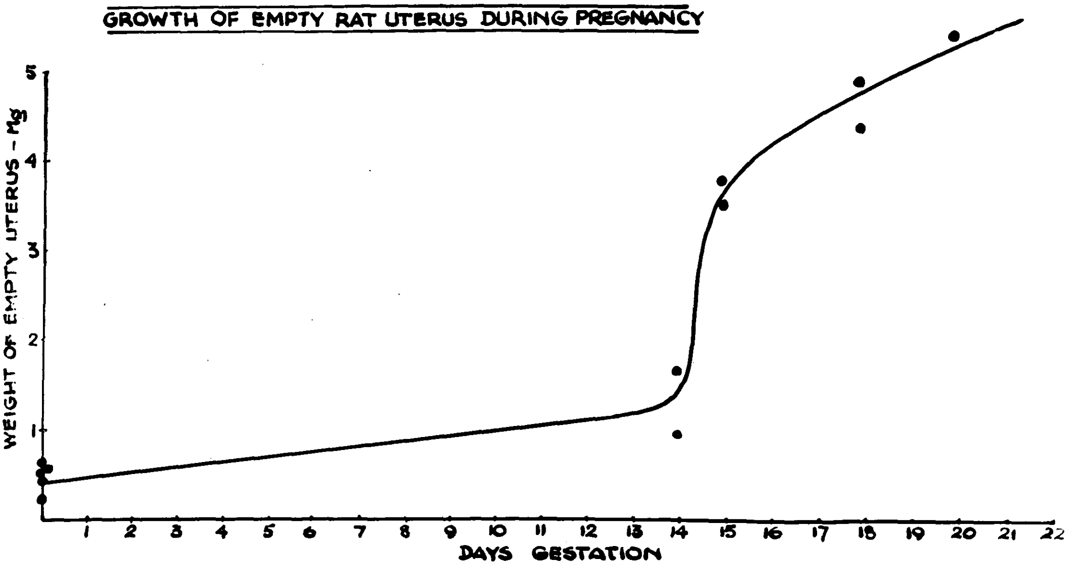

GROWTH OF EMPTY RAT UTERUS DURING PREGNANCY
WEIGHT OF EMPTY UTERUS - Mg
1
2
3
4
5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
DAYS GESTATION

Figure (83)

Table (1)

REGRESSION OF DAILY ENERGY EXPENDITURE ON BODYWEIGHT.

Reproductive Rest - cf Fig. (29). $N = 14$

Theoretical linear equation, where E is daily energy expenditure in Cal. and W is bodyweight in g. -

$$E = \underline{a} + \underline{b}\,(W - \overline{W})$$

Computed linear equation -

$$E = 33.15 + 0.118\,(W - 167.5)$$

<u>ie</u> $E = 13.38 + 0.118W$

Analysis of Regression. Difference of coefficient $\underline{b}$ from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	391.8894	1	391.8894	48.8	7.0	<0.001
Residual	96.3469	12	8.0289			
Total	488.2363	13				

Standard Error of $\underline{a} = 0.757$

Standard Error of $\underline{b} = 0.0169$

Standard Error of Intercept on E axis = 2.93

Table (2)

REGRESSION OF RESPIRATORY QUOTIENT ON FOOD ENERGY INTAKE

cf Fig. (35). N = 96

Theoretical linear equation, where R is respiratory quotient, and E is daily food energy intake in Cal. -

$$R = \underline{a} + \underline{b}(E - \bar{E})$$

Computed linear equation -

$$R = 1.012 + 0.00376(E - 65.07)$$

ie $$R \doteq 0.767 + 0.00376E$$

Analysis of regression. Difference of coefficient b from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	0.4831	1	0.4831	102.8	10.1	<0.001
Residual	0.4420	94	0.0047			
Total	0.9251	95				

Standard Error of a = 0.00699

Standard Error of b = 0.000371

Standard Error of Intercept on R axis = 0.0252

Difference of observed from theoretical intercept on R axis = 0.06

t of difference = 2.38 P = 0.02

Table (3)

ANALYSIS OF VARIANCE OF ENERGY EXPENDITURE - Females 524 and 525

N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P
Between Rats	5.13	1	5.13		
Between Pregnancies	194.81	1	194.81		
Between Periods	99.62	3	33.2		
Error	294.24	40	7.35		
Completed Total	593.80	45			
Original Total	572.89	45			
Between Pregs. (Corrected)	187.95	1	187.95	25.57	<0.001
Between Periods. (Corrected)	96.12	3	32.04	4.36	0.01

Overall Mean = 42.5 Cal.

Standard Error of Period Mean = 1.11 Cal.

Maximum Mean Pregnant Level is 109 per cent. of N.P.

Table (4)

ANALYSIS OF VARIANCE OF FOOD ENERGY INTAKE - Females 524 and 525 N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P.
Between Rats	82.95	1	82.95		
Between Pregnancies	4205.64	1	4205.64		
Between Periods	5626.33	3	1875.44		
Preg.-Period Interaction	1618.57	3	539.52		
Error	3131.43	37	84.63		
Completed Total	14664.92	45			
Original Total	13828.52	45			
Between Pregs.(Corr.)	3965.92	1	3965.92	46.86	<0.001
Between Periods(Corr.)	5305.63	3	1768.54	20.90	<0.001
Interaction (Corr.)	1526.31	3	508.77	6.01	0.002

Overall Mean = 68.4 Cal.

Standard Error of Period Mean = 3.75 Cal.

Maximum Mean Pregnant Level is 137 per cent. of N.P.

Table (5)

ANALYSIS OF VARIANCE OF NON-PROTEIN RESPIRATORY QUOTIENT

N = 48 - females 524 and 525

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P
Between Rats	0.0420	1	0.0420		
Between Pregnancies	0.1542	1	0.1542		
Between Periods	0.1158	3	0.0386		
Preg-Period Interaction	0.0482	3	0.0161		
Error	0.0571	37	0.0015		
Completed Total	0.4173	45			
Original Total	0.3992	45			
Between Rats (Corr.)	0.0402	1	0.0402	26.8	<0.001
Between Pregs. (Corr.)	0.1475	1	0.1475	98.3	<0.001
Between Periods (Corr.)	0.1108	3	0.0369	24.6	<0.001
Preg. Period Interaction (Corr.)	0.0461	3	0.0154	10.3	<0.001

Overall Mean = 1.02

Standard Error of Period Mean = 0.016

Maximum Mean Pregnant Level is 102 per cent. of N.P

Table (6)

ANALYSIS OF VARIANCE OF ENERGY BALANCE - Females 524 and 525

N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P
Between Rats	30.56	1	30.56		
Between Pregnancies	1311.48	1	1311.48		
Between Periods	2474.82	3	824.94		
Preg.-Period Interaction	1066.45	3	355.48		
Error	1229.10	37	33.22		
Completed Total	6081.85	45			
Original Total	5771.49	45			
Between Pregs. (Corr.)	1244.59	1	1244.59	37.46	< 0.001
Between Periods (Corr.)	2348.60	3	782.87	23.57	< 0.001
Preg.-Period (Corr.)	1012.06	3	337.35	10.15	< 0.001

Overall Mean = 8.5 Cal.

Standard Error of Period Mean = 2.35 Cal.

Maximum Mean Pregnant Level is 563 per cent. of N.P.

Table (7)

PARTITION OF ENERGY GAIN BETWEEN MOTHER AND EMBRYOS.

Pregnancy	Total Energy Gain Cal.	Total Weight Young g.	Energy Equiv. of Young Cal.	Per Cent. Total Cal. to Young	Total Weight Placentae g.	Energy Equiv. of Plac. Cal.	Per Cent. Total Cal. to Concept.
Female 377 First	93	38 (9)	27	29	4.5	4.5	34
Female 443 First	112	45(10)	32	28	5	5	33
Female 524 First	150	54(10)	39	26	5	5	29
Female 524 Second	275	22(4)	16	6	2	2	6.5
Female 525 First	150	45(10)	32	21	5	5	25
Female 525 Second	275	20(4)	14	5	2	2	6

Figures in brackets are numbers of young.

Table (8a)

REGRESSION OF URINARY NITROGEN ON INGESTED FOOD WEIGHT.

Non-pregnant Series - cf Fig. (48). N = 36

Theoretical linear equation, where N is urinary nitrogen in mg. and F is ingested food in g.

$$N = \underline{a} + \underline{b}(F - \bar{F})$$

Computed linear equation.

$$N = 262 + 13.95(F - 12.34)$$

ie $$N = 90 + 13.95F$$

Analysis of Regression. Difference of coefficient b from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	245949	1	245949	216	14.7	<0.001
Residual	38738	34	1139			
Total	284687	35				

Standard Error of a = 5.625

Standard Error of b = 0.949

Standard Error of Intercept on N axis = 12.99

Table (8b)

REGRESSION OF URINARY NITROGEN ON INGESTED FOOD WEIGHT.

Pregnant Series - *cf* Fig. (48). $N = 62$

Theoretical linear equation, where N is urinary nitrogen in mg. and F is ingested food in g. -

$$N = a + b\,(F - \bar{F})$$

Computed linear equation -

$$N = 332 + 17.29\,(F - 17.43)$$

ie $N = 31 + 17.29F$

Analysis of Regression. Difference of coefficient *b* from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	349349	1	349349	135	11.6	<0.001
Residual	155073	60	2585			
Total	504422	61				

Standard Error of *a* = 6.457

Standard Error of *b* = 1.49

Standard Error of Intercept on N axis = 26.70

Difference between *b* (non-pregnant) and *b* (pregnant) = 3.34

Standard error of difference = 1.28; $t = 2.6$; $0.02 > P > 0.01$

Table (8c)

REGRESSION OF URINARY NITROGEN ON INGESTED FOOD WEIGHT.

Combined Series - *cf* Fig. (48). $N = 98$

Theoretical linear equation, where N is urinary nitrogen in mg. and F is ingested food in g. -

$$N = \underline{a} + \underline{b}(F - \overline{F})$$

Computed linear equation -

$$N = 295 + 14.52(F - 15.56)$$

ie $$N = 69 + 14.52F$$

Analysis of Regression. Difference of coefficient *b* from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	636827	1	636827	286	16.9	<0.001
Residual	214037	96	2230			
Total	850864	97				

Standard Error of *a* = 4.77

Standard Error of *b* = 0.859

Standard Error of Intercept on N axis = 14.19

Table (9)

REGRESSION OF FAECES WEIGHT ON FOOD WEIGHT

cf Fig. (49). $N = 163$

Theoretical linear equation, where E is faecal excreta in g. and F is food intake in g. -

$$E = a + b(F - \overline{F})$$

Computed linear equation -

$$E = 2.912 + 0.154(F - 14.03)$$

ie $$E = 0.752 + 0.154F$$

Analysis of Regression. Difference of coefficient b from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	94.7683	1	94.7683	278.7	16.7	<0.001
Residual	54.9655	161	0.34			
Total	149.7338	162				

Standard Error of a = 0.0457

Standard Error of b = 0.00921

Standard Error of Intercept on E axis = 0.137

Table (10)

ANALYSIS OF VARIANCE OF TOTAL URINARY NITROGEN - Females 524 and 525 N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P.
Between Rats	1190	1	1190		
Between Pregnancies	73868	1	73868		
Between Periods	72393	3	24131		
Preg.-Period Interaction	52558	3	17519		
Error	76092	37	2057		
Completed Total	276101	45			
Original Total	267473	45			
Between Pregs. (Corr.)	71556	1	71556	34.79	<0.001
Between Periods (Corr.)	70127	3	23376	11.36	<0.001
Interaction (Corr.)	50913	3	16971	8.25	<0.001

Overall Mean = 305 mg.

Standard Error of Period Mean = 18.5 mg.

Maximum Mean Pregnant Level is 105 per cent. of N.P.

Table (11)

ANALYSIS OF VARIANCE OF NITROGEN BALANCE - Females 524 and 525

N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P
Between Rats	1430	1	1430		
Between Pregnancies	560	1	560		
Between Periods	36037	3	12012	4.09	0.012
Error	114124	40	2936		
Completed Total	152151	45			
Original Total	156813	45			

Overall Mean = 54.3 mg.

Standard Error of Period Mean = 21.7 mg.

Maximum Mean Pregnant Level is 690 per cent. of N.P.

Table (12)

ANALYSIS OF VARIANCE OF NITROGEN BALANCE FOR LAST SEVEN DAYS OF GESTATION. Females 524 and 525

N = 28

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P.
Between Days	18,880	6	3147	1.76	0.2
Preg-Period Interaction	24,698	6	4116	2.31	0.1
Error	24,987	14	1785		
Total	68,565	26			

Overall Mean = 74 mg.

Standard Error of Day Mean = 16 mg.

Table (13)

PARTITION OF NITROGEN GAIN BETWEEN MOTHER AND EMBRYOS

Pregnancy	Total N Gain mg.	Total Weight Young g.	Nitrogen Content of Young mg.	Per Cent. Total N to Young	Total Weight Placentae g.	Nitrogen Content of Plac. mg.	Per Cent. Total N to Conceptus
Female 524 First	1309	54(10)	940	72	5	140	82
Female 524 Second	1040	22(4)	384	37	2	55	42
Female 525 First	1309	25(10)	784	60	5	140	76
Female 525 Second	1040	20(4)	349	34	2	55	39

Figures in brackets are numbers of young.

Table (14)

ANALYSIS OF VARIANCE OF FLUID WATER INTAKE - Females 524 and 525. N = 48.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P.
Between Rats	57.20	1	57.20		
Between Pregnancies	2.61	1	2.61		
Between Periods	443.69	3	147.90		
Error	841.88	40	21.05		
Completed Total	1345.38	45			
Original Total	1321.03	45			
Between Rats (Corr.)	56.17	1	56.17	2.67	0.1
Between Periods (Corr.)	435.70	3	145.23	6.89	0.001

Overall Mean = 18.7 g.

Standard Error of Period Mean = 1.87 g.

Maximum Mean Pregnant Level is 156 per cent. of N.P.

Table (15)

ANALYSIS OF VARIANCE OF VAPOURISED WATER LOSS (Corrected)

Females 524 and 525

N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P
Between Rats	3.74	1	3.74		
Between Pregnancies	37.46	1	37.46		
Between Periods	21.49	3	7.16		
Preg-Period Interaction	24.53	3	8.18		
Error	37.31	37	1.01		
Completed Total	124.53	45			
Original Total	119.55	45			
Between Rats (Corr.)	3.59	1	3.59	3.55	0.065
Between Pregs.(Corr.)	35.96	1	35.96	35.60	<0.001
Between Periods (Corr.)	20.63	3	6.88	6.81	<0.001
Interaction (Corr.)	23.55	3	7.85	7.77	<0.001

Overall Mean = 15.2 g.

Standard Error of Period Mean = 0.41 g.

Maximum Mean Pregnant Level is 104 per cent. of N.P.

Table (16)

ANALYSIS OF VARIANCE OF URINARY WATER LOSS - Females

524 and 525

N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P.
Between Rats	43.70	1	43.70		
Between Pregnancies	104.43	1	104.43		
Between Periods	88.30	3	29.43		
Preg-Period Interaction	25.28	3	8.43		
Error	68.42	37	1.85		
Completed Total	330.13	45			
Original Total	310.83	45			
Between Rats(Corr.)	41.14	1	41.14	22.24	<0.001
Between Pregs.(Corr.)	98.32	1	98.32	53.15	<0.001
Between Periods (Corr.)	83.13	3	27.71	14.98	<0.001
Interaction (Corr.)	23.80	3	7.93	4.29	0.01

Overall Mean = 7.5 g.

Standard Error of Period Mean = 0.56 g.

Maximum Mean Pregnant Level is 140 per cent. of N.P.

Table (17)

ANALYSIS OF VARIANCE OF WATER BALANCE - Females 524 and 525

N = 48

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	P
Between Rats	7.52	1			
Between Pregnancies	110.41	1	110.41	5.34	0.03
Between Periods	140.25	3	46.75	2.26	0.10
Error	826.74	40	20.67		
Completed Total	1084.92	45			
Original Total	1082.47	45			

Overall Mean = +1.8 g.

Standard Error of Period Mean = 1.86 g.

Mean Non-pregnant Balance = -0.89 g.

Maximum Mean Pregnant Balance = + 3.58 g.

Table (18)

PARTITION OF WATER GAIN BETWEEN MOTHER AND EMBRYOS

Pregnancy	Total Water Gain g.	Total Weight Young g.	Water Content of Young g.	Total Water per cent. of water of Young.	Total Weight Placentae g.	Water Content of Plac. g.	Total Water as per cent. of Conceptus Water
Female 377 First	28	38(9)	33	85	4.5	3.7	76
Female 443 First	1.3	45(10)	39	3.3	5	4.2	3
Female 524 First	68	54(10)	47	144	5	4.2	133
Female 524 Second	-2.6	22(4)	19	-14	2	1.7	-13
Female 525 First	68	45(10)	39	151	5	4.2	157
Female 524 Second	-2.6	20(4)	17	-15	2	1.7	-14

Figures in brackets are numbers of young

Table (19)

RELATION BETWEEN BODYWEIGHT AND ENERGY BALANCES - Females 524 and 525

	Period	Bodyweight Increment g.	Water Increment g.	"Dry BW" Increment g.	Energy Increment Cal.	"Non-prot "Dry BW Incr" g.	"Non-prot Energy Increment" Cal.
				Female 524			
	5 days N.P.	-2.14	-4.33	+2.19	+6.54	+1.61	+3.26
First Pregnancy	Per. 1 (3 days)	+17.69	+8.33	+9.36	+40.22	+8.00	+32.52
	Per. 2 (3 days)	+17.49	+9.23	+8.26	+41.69	+6.87	+33.82
	Per. 3 (3 days)	+22.99	+20.18	+2.81	-22.11	+1.40	-30.06
	5 days N.P.	-2.38	-7.26	+4.88	+23.58	+5.52	+27.22
Second Pregnancy	Per. 1 (3 days)	+2.43	-0.62	+3.15	+31.85	+2.19	+26.45
	Per. 2 (2 days)	+4.66	-1.06	+5.72	+37.63	+4.54	+30.96
	Per. 3 (3 days)	+5.48	+3.53	+1.95	+20.23	+1.60	+18.25
	5 days N.P.	+2.60	+0.44	+2.16	+23.58	+1.00	+17.01

<u>Table</u> (19) (Contd.)

Female 525

	5 days N.P.	+4.53	-1.44	+5.97	+28.23	+5.23	+24.03
First Pregnancy	Per. 1 (3 days)	+20.95	+14.37	+6.58	+40.35	+5.08	+31.88
	Per. 2 (3 days)	+26.59	+19.00	+7.59	+37.15	+6.03	+28.36
	Per. 3 (3 days)	+7.38	+11.99	-4.61	-53.58	-5.13	-56.51
	5 days N.P.	-8.28	-16.64	+8.36	+76.89	+8.27	+76.36
Second Pregnancy	Per.1 (2 days)	-0.52	-3.04	+2.52	+24.92	+2.19	+23.05
	Per.2 (3 days)	+17.12	+6.94	+10.18	+74.76	+8.09	+62.97
	Per. 3 (3 days)	+15.39	+7.30	+8.09	+56.19	+6.33	+46.23
	5 days N.P.	-11.43	-9.69	-1.74	-2.31	-1.97	-3.58

Table (20)

FOETAL AND PLACENTAL GROWTH - MEAN VALUES

Rat No.	Gest. Age Days	Parity	Total No. Foet.	Total No. C.L.	Mean Foetal Weights mg.	Mean Placenta Weights mg.	Mean Concept. Weights mg.	Total Uterine Weight g.	Per cent. D.M. Foetus	Per cent. D.M. Placenta
561	12	1	17	17	18	14	33	4.22	-	-
532	14	1	7	12	142	116	504	5.15	-	-
466	14	1	5	13	141	114	451	3.19	-	-
461	15	1	13	14	243	131	687	12.67	-	-
462	15	2	11	11	230	138	686	11.05	8.8	17.3
490	17	1	12	17	605	163	-	21.28	-	-
454	18	1	11	14	1100	272	2015	26.99	-	-
434	18	1	12	13	1363	305	-	36.65	-	-
435	18	2	5	12	1360	406	2380	16.21	14.3	18.0
457	20	1	10	13	2913	372	-	44.08	-	-
444	20	2	12 *	18	1033	313	1885	29.87	12.4	19.4
439	21	1	15	16	4415	405	-	87.77	-	-
438	21	2	6	16	4855	478	-	39.86	17.3	17.5
479	22	1	7 *	15	5132	495	-	46.22	-	-

* +1 resorbing foetus

Table (21)

REGRESSION OF PLACENTAL WEIGHT ON GESTATION AGE.

cf Fig. (78). $N = 84$

Theoretical linear equation, where P is placental weight in mg. and T is gestation age in days:-

$$P = \underline{a} + \underline{b}\,(T - \bar{T})$$

Computed linear equation:-

$$P = 280 + 46.3\,(T - 18.06)$$

ie $P = 46.3T - 556$

Analysis of Regression. Difference of coefficient *b* from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	1219602	1	1219602	230	15.2	<0.001
Residual	435056	82	5306			
Total	1654658	83				

Standard Error of *a* = 7.95

Standard Error of *b* = 3.05

Standard Error of Intercept on P axis for $(T = 12)$ = 20.26

Table (22)

REGRESSION OF LOG. PLACENTAL WEIGHT ON LOG. FOETAL WEIGHT.

cf Fig. (80). $N = 84$

Theoretical linear equation, where f is log(foetal weight mg.) and p is log(placental weight mg.) -

$$p = \underline{a} + \underline{b}(f - \bar{f})$$

Computed linear equation -

$$p = 2.38 + 0.438(f - 2.98)$$

ie $$p = 1.076 + 0.438f$$

Analysis of Regression. Difference of coefficient b from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	4.8367	1	4.8367	322	17.19	<0.001
Residual	1.2339	82	0.0150			
Total	6.0706	83				

Standard Error of $\underline{a} = 0.0133$

Standard Error of $\underline{b} = 0.0244$

Taking F as foetal weight in mg. and P as placental weight in mg., the above logarithmic relation can be converted to the direct relation:-

$$P = 11.9\ F^{0.438}$$

Table (23)

REGRESSION OF LOG. FOETAL WEIGHT ON LOG. GESTATION AGE.

cf Fig. (81). $N = 84$

Theoretical linear equation, where f is log (foetal weight mg.) and t is log (gestation age days) -

$$f = \underline{a} + \underline{b}\,(t - \bar{t})$$

Computed linear equation -

$$f = 2.98 + 8.414\,(t - 1.253)$$

ie $$f = 8.414t - 7.563$$

Analysis of Regression. Difference of coefficient b from zero.

Source of Variance	Crude Squares	D.F.	Mean Squares	Variance Ratio	t	P
Regression	23.3719	1	23.3719	1021	31.95	<0.001
Residual	1.8759	82	0.0229			
Total	25.2478	83				

Standard Error of a = 0.0165

Standard Error of b = 0.263

Taking F as foetal weight in mg. and T as gestation age in days, the above logarithmic relation can be converted to the direct relation -

$$F = \frac{T^{8.414}}{36460000}$$

APPENDIX I

Experimental Procedures

Procedure for Daily Change-over in Operation of Respiration Calorimeter.

Order of Preliminary Work.

(1) Weigh charged food box.

(2) Weigh soda asbestos tubes.

(3) Weigh guard anhydrone tube.

(4) Weigh dry animal frame.

(5) Weigh dry urine funnel.

(6) Weigh dry animal cannister.

(7) Fill water bottle. Ensure delivery tube of bottle full of water. Dry outer surface of water bottle thoroughly. Weigh water bottle.

(8) Weigh water absorption tubes. (one $CaCl_2$ and one anhydrone).

(9) Put about 10 ml. 1 N H_2SO_4 into urine flask. Wash down neck of flask with distilled water. Dry inside neck of flask. Weigh urine flask.

Order of Events during Change-over.

(1) Switch off oxygen recording drum and record time and spirometer reading. Remove drum; remove trace; renew paper and smoke.

(2) Switch off pump and adjust to standard point of phase.

(3) Record time, spirometer reading, barometric pressure and cabinet temperature.

(4) Turn tap T_3 to manometer position.

(5) Switch off fan. Open door of cabinet, close U-tube stopper taps, record chamber temperature.

(6) Place fresh chamber on inside spare rack. Remove used animal chamber to outside rack.

(7) Turn tap T_2 to oxygen inlet position and let spirometer fill slowly from oxygen cylinder. Close cabinet. The spirometer is watched during the subsequent work andwhen full, T_2 is returned to original position and the cylinder valve closed.

(8) Open used animal chamber. Remove water bottle. Remove rat and place in weighing cannister. Replace lid of chamber but do not bolt down.

(9) Weigh rat in cannister.

(10) Transfer used animal chamber to inside rack and fresh chamber to outside rack.

(11) Attach urine funnel to fresh animal chamber and insert urine funnel and animal rack with attached food box. Transfer rat to fresh animal chamber.

(12) Place fresh water bottle in animal chamber. Bolt down lid. Record time.

(13) Place freshly loaded animal chamber in position. Remove used animal chamber to outside rack.

(14) Remove used absorbing train. Replace with fresh absorbing train.

(15) Attach ducts to animal chamber and to absorbing train.

(16) Record animal chamber temperature. Open stopper taps in absorbing train.

(17) Switch on pump and close door of cabinet.

(18) Graduate oxygen record paper.

(19) Place drum in cabinet. Adjust drum to suitable height and appose writing point to drum with plumb line.

(20) Switch on drum and leave until running smoothly. During this period watch the manometer level for any indications of leaks in the apparatus.

(21) Record time, spirometer reading, cabinet temperature and barometric pressure. Turn T_3 to spirometer position.

Order of Events after Change-over.

(1) Remove animal frame from used chamber and place the former on clean sheet of paper.

(2) Remove urine funnel. Shake dry food off funnel on to the same sheet of paper. Place urine funnel, inverted, on paper so that no fluid is lost from it.

(3) Remove urine flask.

(4) Transfer faeces from grid of frame to animal weighing cannister. Shake dry food from frame onto paper.

(5) Weigh urine flask.

(6) Weigh urine funnel and then place in mouth of flask.

(7) Weigh animal frame.

(8) Scrape contaminated food from animal frame into urine funnel. Wash frame and funnel residues into urine flask.

(9) Weigh water absorbing tubes.

(10) Weigh cannister with moist faeces. Put cannister and faeces in drying oven.

(11) Weigh water bottle.

(12) Weigh food box.

(13) Weigh soda asbestos and guard tubes.

(14) Brush dry scattered food residues from chamber onto paper. Weigh combined dry food residues.

(15) Wash out exhausted U-tubes.

(16) Annotate and varnish oxygen consumption trace.

(17) Prepare urine for nitrogen estimation. <u>cf</u> p. (130)

(18) Weigh dried faeces after 24 hr. drying.

Procedure for Recharging Spirometer.

(1) Turn the tap T_3 to connect oxygen inlet to spirometer, so disconnecting it from the spirometer.

(2) Record time and spirometer reading.

(3) Turn the tap T_2 to connect spirometer to oxygen cylinder inlet. Fill spirometer to the required level. Restore T_2 to normal position.

(4) Leave apparatus for 15 min. during which time the Oxygen usage can be seen by the fall in pressure in the chamber circuit shown by the water manometer.

(5) Record cabinet temperature and barometric pressure.

(6) Record spirometer scale reading and time after 15 min.

(7) Turn the tap T_3 first to "manometer-spirometer" position, to allow the manometer to return to its resting level. Then turn T_3 to "spirometer only" position.

(8) Keep apparatus under observation for a further 15 min. period.

Procedure for Replacing Exhausted or Blocked Absorbing Tubes.

(1) Switch off pump motor.

(2) Turn the tap T_3 to connect the oxygen inlet to the manometer and to disconnect it from the spirometer.

(3) Adjust the phase of the pump so that the pressure in the chamber circuit is equal to the atmospheric pressure.

(4) Seal the duct system on both sides of the absorbing train, or on both sides of the tube to be replaced, by use of the tap stoppers in suitable U-tubes or by clamping the rubber connections between the tubes.

(5) Replace the relevant tube by a weighed, unused tube.

(6) Unseal the absorbing train and re-start the pump.

(7) Allow T_3 to remain in its altered position until the manometer registers a slightly reduced pressure in the chamber circuit. Then tap the spirometer back into the circuit.

Procedure for Bomb Calorimetry.

(1) The silica cruicible, used to carry the pelleted material, is weighed with a 6 cm. length of fuse wire.

(2) The material to be burnt is pelleted in the press with the fuse wire embedded in it.

(3) The pelleted substance, with the fuse wire, is placed in the crucible and the whole weighed. The difference between the weights found in (1) and (3) gives the weight of substance used.

(4) The crucible with pellet is attached to the spring holder formed by the contacts in the bomb cap. The ends of the fuse wire are bound to the contacts.

(5) A light film of silicone grease is applied to the outside rim of the bomb body.

(6) The cap is screwed onto the body of the bomb and lightly tightened with a large spanner.

(7) The bomb is filled with oxygen to 25 atmospheres.

(8) The calorimeter vessel is filled to the approximate level with tap water at about 15°C. Water is added or withdrawn by a pippette until the vessel is exactly balanced by the standard weight. The vessel is then placed in the water-jacket of the apparatus.

(9) The leads from the ignition unit are bound to the external contacts on the bomb cap, and the bomb is carefully lowered into the calorimeter vessel, and arranged to sit in the bottom centre of the vessel.

(10) The stirrer is fitted to the driving mechanism and the position of the bomb adjusted so that the stirrer clears the bomb.

(11) The thermometer is carefully passed into the calorimeter vessel, through the spring clip on the stirrer superstructure, until it reaches the standard immersion mark. The thermometer is always the last part of the apparatus to be set in place and the first part to be removed at the end of an estimation.

(12) The stirrer motor is switched on and the stirrer is allowed to run, at about 1 cycle per sec., for about 5 min. This allows the temperature relations of the different parts of the apparatus to become steady.

(13) The thermometer reading is recorded at ½ min. intervals for three min., or until the rate of rise or fall of temperature has been substantially constant for three min.

(14) The ignition switch is depressed at the end of the three min. pre-period and the temperature is recorded at ½ min. intervals until the rate of temperature fall has been steady for three min. The stirrer motor is switched off.

(15) The apparatus is dismantled in the reverse order of assembly. On removing the cap from the bomb the inside of the bomb is closely inspected for signs of scattered material other than ash, or any other sign of incomplete combustion.

Example of Computation of Heat of Combustion

Food Sample - Batch A, Diet 41

Weight of food sample = 1.881 g.

Temperature readings at half-minute intervals.

	Pre-ignition Temperatures °C.		Heating Period Temperatures °C.		Cooling Period Temperatures °C.
	17.57		17.85	t_n	20.902
	17.568		18.90		20.896
	17.568		20.10		20.89
	17.568		20.53		20.881
	17.565		20.80		20.876
	17.56		20.88		20.87
	17.558		20.905		20.86
	17.556		20.91		20.853
t_o	17.554		20.91		
		t_{n-1}	20.91	t_1 =	20.879
t =	17.574				
		Σt =	202.695	v_1 =	0.007
v =	0.002	$\frac{1}{2}(t_o + t_n)$ =	19.228	k =	0.005
n =	11		221.923		3.305
nv =	0.022	$-nt$ =	-193.314	=	0.0015
		P =	28.609		

Observed temperature rise = $t_n - t_o$ = 3.348

Correction = $nv + kP$ = 0.022 + 0.0429

= 0.0649

Corrected temperature rise = 3.413

Water equivalent of calorimeter = 2285 g.

Total heat produced = 7.799 Cal.

Total heat per g. food = 4.146 Cal.

Heat of combustion per g. food at constant volume

= 4.143 Cal.

Example of Record Sheet as Originally Designed.

METABOLIC DATA

Rat No.　Pregnancy No.　Oestrus　Diet

		Initial	End
	Date		
	Time		
	Barometric Pressure		
	Chamber Temperature		
O_2 Consumption	Spirometer 1		
	Spirometer 2		
	Spirometer 1 A		
H_2O Absorption	Tube H 1		
	Tube H 2		
CO_2 Absorption	Tube C 1		
	Tube C 2		
Chemical H_2O Absorption	Tube CH 1		
	Tube CH 2		
Spirometers Driers	Tube D 1		
	Tube D 2		
Rat and Faeces wt.	Box No.		
	Box + Faeces		
Food	Box No.		
	Waste on Tray		
Urine	Flask No.		
Drinking Water	Bottle No.		

Notes

Example of Record Sheet as Finally Used.

METABOLIC DATA

Rat No. 525 **Pregnancy No.** 3 **Oestrus** **Diet** Stock

		Initial	Final
	Date	14/3/52	15/3/52
	Time	1.50 p.m.	2.15 p.m.
	Barometric Pressure	763 mm.	754 mm.
	Chamber Temperature	21.2°C.	26.0°C.
	(Cabinet Temperature)	22.1°C.	25.2°C.
O_2 Consumption	Spirometer 1	25.35	5.58
	Spirometer 2	25.21	3.93
	Spirometer 1 A	24.10	13.65
H_2O Absorption	Tube H 1	213.974	128.473
	Tube H 2	109.921	211.640
CO_2 Absorption	Tube C 1	211.484	227.395
	Tube C 2		
Chemical H_2O Absorption	Tube CH 1	100.727	101.921
	Tube CH 2		
Spirometer Driers	Tube D 1		
	Tube D 2		
Rat and Faeces Wt.	(Box + Rat)	455.46	456.85
	Box + Faeces		165.331
Food	(Box + Food)	186.070	163.296
	Waste on Tray		6.641
Urine	Flask No.	54.667	61.814
Drinking Water	Bottle No.	160.596	143.825

Notes/

Notes

B. Funnel	94.989	B. Frame	270.545
E. Funnel	95.358	E. Frame	271.463

Box + Dried Faeces	164.929
Dry weight of Box	162.847

T_3 Open	O_2 changed	O_2 changed	$CaCl_2$ changed	Drum off
2.10 pm.	11.30 pm.	9.00 am.	9.30 am.	2.00 pm.
	T_3 open	T_3 open		Sp. 14.08
	11.45 pm.	9.15 pm.		
	22.4° 760	22.3° 757		

APPENDIX 2

Tables of Technical Data

Table (24a)

CALIBRATION OF SPIROMETER I

cf. Fig (12)

Volume ml.	Scale Reading cm.	Volume ml.	Scale Reading cm.	Volume ml.	Scale Reading cm.
0	1.00	1600	9.05	3200	17.30
100	1.40	1700	9.60	3300	17.80
200	1.90	1800	10.10	3400	18.30
300	2.45	1900	10.60	3500	18.80
400	2.95	2000	11.10	3600	19.35
500	3.45	2100	11.60	3700	19.85
600	3.95	2200	12.15	3800	20.35
700	4.45	2300	12.65	3900	20.85
800	4.98	2400	13.15	4000	21.40
900	5.50	2500	13.70	4100	21.95
1000	6.00	2600	14.20	4200	22.45
1100	6.50	2700	14.70	4300	22.95
1200	7.00	2800	15.20	4400	23.45
1300	7.50	2900	15.75	4500	24.00
1400	8.05	3000	16.25		
1500	8.55	3100	16.80		

Table (24b)

CALIBRATION OF SPIROMETER II

cf. Fig. (13)

Volume Ml.	Scale Reading cm.	Volume ml.	Scale Reading cm.	Volume ml.	Scale Reading cm.
0	1.00	1600	8.85	3200	17.00
100	1.30	1700	9.40	3300	17.50
200	1.80	1800	9.90	3400	18.00
300	2.30	1900	10.40	3500	18.50
400	2.80	2000	10.95	3600	19.00
500	3.30	2100	11.45	3700	19.50
600	3.80	2200	11.95	3800	20.00
700	4.35	2300	12.45	3900	20.50
800	4.85	2400	12.95	4000	21.00
900	5.30	2500	13.45	4100	21.55
1000	5.80	2600	14.00	4200	22.05
1100	6.30	2700	14.50	4300	22.60
1200	6.85	2800	15.00	4400	23.10
1300	7.35	2900	15.50	4500	23.60
1400	7.85	3000	16.00	4600	24.15
1500	8.35	3100	16.50		

Table (25).

ALCOHOL CUMBUSTION CHECKS ON RESPIRATION CALORIMETER

	Total Vol. O_2 Litres	Total Wt. CO_2 g.	Total Vol. CO_2 Litres	Total R.Q.	Total Wt. H_2O g.	Total Wt. Alcohol g.
			Using Ethanol			
	0.328	0.422	0.214	0.652	0.352	0.318
	1.385	1.852	0.937	0.677	1.073	1.051
	1.484	1.971	0.997	0.672	1.119	1.034
	1.801	2.365	1.199	0.666	1.483	1.335
	1.985	2.659	1.345	0.678	1.638	-
			Using Methanol			
	2.892	3.805	1.925	0.666	3.735	4.094
	1.171	1.507	0.762	0.651	1.307	1.545
	0.766	0.994	0.503	0.657	1.733	1.119
TOTALS	11.812		7.882	0.667		

Table (26)

HALDANE GAS ANALYSES.

Per cent. O_2	Per cent. CO_2	Source of Sample
19.88	0.59	Intake of absorbing train
19.97	0.63	" " " "
19.73	0.75	" " " "
19.77	0.71	" " " "
18.92	1.61	Animal Chamber 3 min. after Pump stopped.
20.05	0.00	Outlet from absorbing train
20.24	0.00	" " " "

Table (27)

COMPUTATION OF Δn FROM APPROXIMATE FOOD COMPOSITION

Dietary Component	Amount Burned in g. per g. Food	Oxygen Used		Carbon Dioxide Prod.		R.Q.
		g.	litres	g.	litres	
Protein	0.16	0.177	0.124	0.168	0.085	0.69
Carbohydrate	0.68	0.80	0.56	1.11	0.56	1.00
Fat	0.05	0.143	0.10	0.14	0.071	0.707
Total	0.89	1.12	0.784	1.418	0.716	0.91

From these data n can be expressed as:-

$$\Delta n = \frac{1.12(0.91-1)}{32} = 0.00315$$

Table (28)

Animals Used in Metabolic Work.

Rat No.	Date of Birth	Age at First use days	Age at First Mating days	Age at End Experiment days
377	29/1/50	97	163	258
407	20/8/50	64	-	70
408	20/8/50	86	-	90
415	21/8/50	94	-	104
443	28/12/50)	150	154	176
444	28/12/50)	179	163	180
445	28/12/50)	169	165	178
522	2/7/51)	102	-	108
524	2/7/51)	109	120	295
525	2/7/51)	123	135	263

Animals Used in Growth Studies

356	27/1/50		626	638
366	29/1/50		677	689
408	20/8/50		418	432
431	28/9/50		368	384
434	31/12/50)		274	292
435	31/12/50)		280	355
436	31/12/50)		275	446
437	31/12/50		336	352
438	31/12/50)		283	356
439	31/12/50)		276	297
440	31/12/50)		287	449
444	28/12/50		337	356
454	24/1/51		260	278
457	24/1/51		259	279
460	26/2/51)		218	342
461	26/2/51)		227	242
462	26/2/51)		220	275
466	18/3/51		211	225
479	24/3/51		197	219
490	25/3/51		195	212
491	25/3/51		195	213
532	2/7/51		155	169
561	7/11/51		122	134

Bracketed groups are littermate groups.

Table (29)

COMPOSITION OF DIETS

	Rowett Inst.	Batches of Diet 41 A	B	C	D	E
Nitrogen mg./g.	29.3	27.6	26.8	24.8	26.1	26.4
Lipid g. per cent.	4.6	------------- 4.2 ------------				
Heat of Combustion Cal./g.	4.04	4.17	4.09	4.03	4.03	4.00
Water g. per cent.	12.0	9.7	9.4	10.0	12.1	10.2

Table (30)

MOISTURE CONTENT OF SCATTERED FOOD.

Wet Wt. Increase in Funnel g.	Dry Wt. Increase In Funnel g.	Dry Food per cent. of Funnel Increase	Wet Wt. Increase in Frame g.	Dry Wt. Increase in Frame g.	Dry Food per cent. of Frame Increase
0.489	0.221	45.2	1.192	0.752	63.1
0.462	0.308	66.7	1.382	1.066	77.1
0.322	0.186	57.8	0.842	0.693	82.3
0.326	0.197	60.4	0.476	0.307	64.5
0.326	0.193	59.2	0.431	0.278	64.4
0.260	0.173	66.5	0.697	0.464	66.5
0.279	0.145	52.0	0.502	0.386	76.8
0.360	0.231	64.2	0.652	0.371	56.9
0.560	0.342	61.1	3.404	2.587	76.0
0.374	0.215	57.5	0.488	0.394	80.7
0.700	0.448	64.0	1.899	1.336	70.7
0.313	0.138	44.1	0.566	0.362	64.0
0.435	0.221	50.8	0.718	0.429	59.8
0.438	0.231	52.7	0.749	0.633	84.5
0.194	0.092	47.4	0.368	0.276	75.0
0.187	0.119	63.6	0.548	0.388	70.8
Means		57.4			71.9
Means for conversion to fresh food.		63.0			79.0

APPENDIX 3

Detailed Tables of Experimental Results

TABLE (31)

SEQUENCE AND DATES OF STUDIES

(a) Serial No.	(b) Date	(c) Days Gestation	(d) Mean Bodyweight g.	(e) Notes
Female 377.				
1	6-7/5/50	N.P.	105.4	Water bottle blockage - dehydration
2	7-8	N.P.	110.7	Water bottle spilled.
3	8-9	N.P.	121.4	
4	9-10	N.P.	123.2	
5	10-11	N.P.	123.8	$CaCl_2$ tube broke on removal
6	11-12	N.P.	123.1	Spirometer seal sucked back.
7	15-16/5/50	N.P.	130.7	
8	16-17	N.P.	130.1	
9	17-18	N.P.	127.4	
10	18-19	N.P.	128.8	Thermostat erratic - Box temp. 17°C.
11	29-30/6/50	N.P. (O)	161.8	
12	30/6-1/7/50	N.P. (M)	154.8	

(a)	(b)	(c)	(d)	(e)
13	1-2	N.P. (D)	153.4	
14	2-3	N.P. (D)	156.6	
15	3-4	N.P. (P)	162.2	Oestrus at end of run
16	11-12/7/50	1	165.3	
17	12-13	2	165.8	
18	13-14	3	167.0	
19	14-15	4	166.9	
20	15-16	5	163.6	
21	16-17	6	164.4	
22	17-18	7	169.6	Fall in weight of last absorbing tube.
23	18-19	8	170.1	
24	19-20	9	170.0	
25	20-21	10	173.2	
26	21-22	11	177.8	
27	22-23	12	179.9	
28	23-24	13	178.9	

(a)	(b)	(c)	(d)	(e)
29	24-25	14	182.6	
30	25-26	15	185.0	Leak - other data discarded.
31	26-27	16	187.2	? Leak
32	27-28	17	196.8	No run. Apparatus tested.
33	28-29	18	206.	
34	29-30	19	212.5	
35	30-31	20	217.3	Fall in weight of last absorbing tube.
36	31/7-1/8/50	21	220.3	
37	1-2	22	213.4	Young born during night.
38	27-28/9/52	N.P.	170.7	
39	28-29	N.P.	168.1	Blockage in absorbing train.
40	29-30	N.P.	168.9	
Female 407.				
41	23-24/10/50	N.P.	102.9	
42	24-25	N.P.	103.7	
43	25-26	N.P.	106.4	

(a)	(b)	(c)	(d)	(e)
44	26-27	N.P.	109.8	
45	27-28	N.P.	112.7	
Female 408.				
46	14-15/11/50	N.P.	114.4	Initial leak - corrected
47	15-16	N.P.	115.4	
48	16-17	N.P.	118.1	
Female 415				
49	22-22/11/50	N.P.	144.6	Soda asbestos exhausted - tube replaced.
50	23-24	N.P.	147.0	
51	24-25	N.P.	150.0	Soda asbestos clogged - no other data.
52	29-30/11/50	N.P.	150.1	
53	30/11-1/12/50	N.P.	152.8	
Female 443.				
54	28-29/5/51	N.P.	144.9	
55	29-30	N.P.	144.2	

(a)	(b)	(c)	(d)	(e)
56	30-31	N.P.(P)	149.1	
57	4-5/6/51	4	156.1	
58	5-6	5	158.4	
59	6-7	6	158.9	
60	12-13/6/51	12	171.7	
61	13-14	13	175.9	
62	14-15	14	176.3	Partial failure of pump-motor union - anoxia
63	18-19/6/51	18	195.1	
64	19-20	19	198.5	Blockage in $CaCl_2$ tube.
65	20-21	20	202.8	Initial leak - corrected.
66	21-22	21	209.2	
67	22-23	22	213.2	
Female 445.				
68	15-16/6/51	4	222.7	
69	16-17	5	223.2	

(a)	(b)	(c)	(d)	(e)
70	17-18	6	222.7	
71	23-24	12	230.1	
Female 444.				
72	25-26/6/51	16	232.4	Rat dehydrated initially.
Female 522.				
73	12-13/10/51	N.P.(O)	182.5	
74	13-14	N.P.	177.7	
75	14-15	N.P.(D)	176.0	Starvation, dehydration.
76	15-16	N.P.	168.4	
77	16-17	N.P.	161.1	
Female 524.				
78	19-20/10/51	N.P.(D)	177.1	
79	20-21/10/51	N.P.(P)	173.5	
80	21-22/10/51	N.P.(O)	175.9	
81	22-23	N.P.(M)	179.0	

(a)	(b)	(c)	(d)	(e)
98	6-7/1/52	10	235.5	Pump stroke increased from 8 to 10 mm.
99	7-8	11	236.9	
100	8-9	12	237.9	
101	12-13/1/52	16	242.9	
102	15-16/1/52	18	262.4	
103	16-17	19	263.1	
104	17-18	20	264.0	
105	18-19	21	265.7	
106	19-20	22	268.4	Blood on tray. Young born 21/1/52.
107	11-12/2/52	N.P.	230.4	
108	12-13	N.P.	220.5	Methanol poisoning.
109	13-14	N.P.	208.8	
110	25-26/2/52	N.P. (M)	207.2	
111	26-27	N.P. (D)	208.3	
112	27-28	N.P. (D)	211.0	Urine total solids measured.

(a)	(b)	(c)	(d)	(e)
82	23-24	N.P.(D)	180.5	
83	9-10/11/51	10	215.8	
84	10-11	11	221.7	
85	11-12	12	227.8	
86	15-16/11/51	16	246.4	
87	16-17	17	254.4	
88	17-18	18	258.5	Blocked $CaCl_2$ tube
89	18-19	19	263.5	? Leak after replacement
90	19-20	20	272.1	
91	20-21	21	279.4	Parturition beginning at end of run.
92	21-22	22	278.0	Parturition - Young born for most of run.
93	11-12/12/51	N.P.(P)	230.0	
94	12-13	N.P.(O)	230.7	
95	13-14	N.P.(M)	232.5	
96	14-15	N.P.(D)	231.8	
97	15-16	N.P.(D)	229.7	

(a)	(b)	(c)	(d)	(e)
113	28-29	N.P. (P)	208.4	Urine total solids measured.
114	29/2-1/3/52	N.P. (O)	206.9	
115	20-21/3/52	10	240.0	
116	21-22	11	243.0	
117	22-23	12	243.3	
118	26-27/3/52	16	239.3	
119	27-28	17	240.3	
120	28-29	18	239.9	
121	29-30	19	237.4	
122	12-13/4/52	10	249.1	
123	13-14	11	247.3	
124	14-15	12	244.6	
125	18-19/4/52	16	234.8	
126	19-20	17	235.1	
127	20-21	18	234.6	
128	21-22	19	235.6	Killed 3.45p.m. Right ovarian cyst.

(a)	(b)	(c)	(d)	(e)
Female 525				
129	2-3/11/51	N.P. (O)	180.3	
130	3-4	N.P. (M)	182.5	
131	4-5	N.P. (D)	184.7	
132	5-6	N.P. (D)	183.4	
133	6-7	N.P. (P)	183.3	
134	23-24/11/51	10	204.6	
135	24-25	11	214.2	
136	25-26	12	220.7	
137	29-30/11/51	16	240.1	Rat left in open cage for 2 hr. while leak checks done. Data corrected for this.
138	30/11-1/12/51	17	246.8	
139	1-2	18	253.8	
140	2-3	19	263.5	? Slight leak.
141	3-4	20	270.0	
142	4-5	21	270.6	Water bottle blockage.
143	5-6	22	273.4	One young born at end of run.

(a)	(b)	(c)	(d)	(e)
144	31/12/51-1/1/52	N.P. (O)	223.2	Different diet accidentally fed for few days previously
145	1-2	N.P. (M)	219.4	
146	2-3	N.P. (D)	228.5	
147	3-4	N.P. (D)	232.7	
148	4-5	N.P. (P)	228.5	
149	20-21/1/52	11	251.4	
150	21-22	12	251.1	
151	25-26/1/52	16	254.4	Water seal sucked back.
152	26-27	17	261.4	
153	27-28	18	267.8	
154	28-29	19	273.3	
155	29-30	20	278.6	
156	30-31	21	282.4	
157	31/1-1/2/52	22	285.7	
158	1-2	23	287.2	Young born early morning 3/2/52.

(a)	(b)	(c)	(d)	(e)
159	18-19/2/52	N.P. (D)	257.1	
160	19-20/2/52	N.P. (P)	252.5	
161	20-21	N.P. (O)	247.5	
162	21-22	N.P. (D)	247.3	
163	22-23	N.P. (D)	245.8	
164	4-5/3/52	10	286.5	
165	5-6	11	288.0	
166	6-7	12	288.7	
167	10-11/3/52	16	301.6	Pump stroke increased from 11 - 15 mm.
168	11-12	17	303.0	
169	12-13	18	301.8	
170	13-14	19	295.7	
171	14-15	20	293.3	
172	15-16	21	295.4	
173	16-17	22	296.6	Transferred to animal house. Wt. maintained 295 g.

TABLE (32)

Spirometer Scale Readings - Original and Reduced to S.T.P.

(a) Serial No.	(b) Initial Spir. Reading cm.	(c) Temp. °C.	(d) Press mm.Hg.	(e) p	(f) Final Spir. Reading cm.	(g) Temp. °C.	(h) Press mm.Hg.	(i) q	(j) px_1	(k) qx_2
1.	23.20	22.0	764.5	0.907	9.85	22.0	765.5	0.908	21.04	8.94
	23.00	22.0	764.5	0.907	10.30	22.0	765.5	0.908	20.86	9.35
2.	23.15	19.5	765.5	0.919	10.20	20.0	768	0.920	21.27	9.38
	23.75	19.5	765.5	0.919	11.10	20.0	768	0.920	21.83	10.21
3.	24.40	20.0	768	0.920	8.30	22.5	767	0.908	22.45	7.54
	23.60	20.0	768	0.920	8.80	22.5	767	0.908	21.71	7.99
4.	24.60	22.0	767	0.910	8.10	22.0	770.5	0.914	22.39	7.40
	24.00	22.0	767	0.910	8.75	22.0	770.5	0.914	21.84	8.00
5.	25.20	21.0	770.5	0.919	10.45	22.0	770	0.913	23.16	9.54
	24.30	21.0	770.5	0.919	10.00	22.0	770	0.913	22.33	9.13
6.	24.40	21.0	770	0.918			768.5			
	24.70	21.0	770	0.918			768.5			
7.	24.95	21.0	768	0.916	6.10	21.0	764.5	0.911	22.85	5.56
	24.35	21.0	768	0.916	11.50	21.0	764.5	0.911	22.30	10.48
8.	24.70	21.0	764.5	0.911	7.45	21.0	755	0.899	22.50	6.70
	24.40	21.0	764.5	0.911	7.45	21.0	755	0.899	22.23	6.70
9.	24.95	21.0	755	0.900	8.10	19.0	753	0.905	22.46	7.33
	24.80	21.0	755	0.900	10.00	19.0	753	0.905	22.32	9.05

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
10.	25.25 24.7	20.5 20.5	753 753	0.899 0.899	6.75 7.25	20.5 20.5	752.5 752.5	0.898 0.898	22.70 22.21	6.06 6.51
11.	24.15 25.10	23.0 23.0	750 750	0.884 0.884	5.00 11.90	24.0 24.0	752.5 752.5	0.883 0.883	21.35 22.19	4.42 10.51
12.	24.65 24.30	23.0 23.0	752.5 752.5	0.887 0.887	7.90 13.50	23.5 23.5	760.5 760.5	0.895 0.895	21.86 21.55	7.07 12.08
13.	24.20 24.00	23.0 23.0	761 761	0.898 0.898	8.65 13.20	24.0 24.0	761 761	0.894 0.894	21.73 21.55	7.73 11.80
14.	24.55 24.50	23.0 23.0	761 761	0.898 0.898	5.70 7.30	24.0 24.0	761 761	0.894 0.894	22.05 22.00	5.10 6.53
15.	25.40 23.60	23.0 23.0	761 761	0.898 0.898	3.90 11.80	24.5 24.5	762.5 762.5	0.893 0.893	22.81 21.19	3.48 10.54
16.	25.10 24.95	23.5 23.5	748 748	0.880 0.880	6.10 8.00	23.5 23.5	756 756	0.890 0.890	22.09 21.96	5.43 7.12
17.	24.65 24.45	23.0 23.0	756 756	0.892 0.892	6.00 9.50	23.0 23.0	755.5 755.5	0.891 0.891	21.99 21.81	5.35 8.46
18.	24.65 25.05	23.0 23.0	755.5 755.5	0.891 0.891	6.45 9.40	24.0 24.0	748 748	0.877 0.877	21.96 22.32	5.66 8.24
19.	25.25 24.95	23.0 23.0	748 748	0.882 0.882	7.15 9.40	24.0 24.0	751.5 751.5	0.882 0.882	22.27 22.01	6.31 8.29

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
20.	24.90 25.00	23.0 23.0	751.5 751.5	0.886 0.886	6.80 9.40	23.0 23.0	742.5 742.5	0.875 0.875	22.06 22.15	5.95 8.23
21.	24.70 24.95	23.0 23.0	742.5 742.5	0.875 0.875	5.80 7.90	23.5 23.5	746.5 746.5	0.878 0.878	21.61 21.83	5.09 6.94
22.	25.00 25.05	23.0 23.0	746.5 746.5	0.880 0.880	5.70 8.50	23.0 23.0	755 755	0.891 0.891	22.00 22.04	5.08 7.57
23.	25.35 25.00	22.5 22.5	755 755	0.893 0.893	7.10 9.65	24.0 24.0	760 760	0.892 0.892	22.64 22.33	6.33 8.61
24.	25.00 25.00	23.5 23.5	760 760	0.895 0.895	7.35 9.55	24.0 24.0	754 754	0.884 0.884	22.38 22.38	6.50 8.44
25.	25.15 25.20	24.0 24.0	759 759	0.890 0.890	8.60 9.70	24.0 24.0	754 754	0.884 0.884	22.38 22.43	7.60 8.57
26.	25.00 25.50	23.0 23.0	754 754	0.889 0.889	6.55 9.50	23.5 23.5	752 752	0.885 0.885	22.23 22.67	5.80 8.41
27.	25.15 25.15	23.0 23.0	752 752	0.887 0.887	7.15 9.25	23.5 23.5	746 746	0.878 0.878	22.31 22.31	6.28 8.12
28.	25.05 25.00	23.0 23.0	746 746	0.880 0.880	6.95 8.60	23.5 23.5	754 754	0.887 0.887	22.04 22.00	6.16 7.63
29.	25.10 25.20	23.0 23.0	754 754	0.890 0.890	6.65 9.50	23.0 23.0	755 755	0.891 0.891	22.34 22.43	5.93 8.46
30.	25.20 25.40	21.5 21.5	755 755	0.897 0.897	12.30 13.30	23.0 23.0	757.5 757.5	0.894 0.894	22.60 22.78	11.00 11.89

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
31.	25.10	22.0	757.5	0.898	6.10	24.0	757.5	0.889	22.54	5.42
	25.15	22.0	757.5	0.898	10.15	24.0	757.5	0.889	22.58	9.02
32.										
33.	25.25	23.0	758.5	0.895	4.95	22.5	761	0.900	22.60	4.46
	24.90	23.0	758.5	0.895	7.30	22.5	761	0.900	22.29	6.57
34.	24.90	22.5	761	0.900	6.30	24.0	755	0.886	22.41	5.58
	25.00	22.5	761	0.900	8.55	24.0	755	0.886	22.50	7.58
35.	25.25	22.0	755	0.895	5.05	23.0	751.5	0.886	22.60	4.47
	23.35	22.0	755	0.895	7.70	23.0	751.5	0.886	22.69	6.82
36.	25.60	22.0	751.5	0.891	6.45	23.5	750	0.882	22.81	5.69
	25.30	22.0	751.5	0.891	8.40	23.5	750	0.882	22.54	7.41
37.	25.10	22.0	750	0.889	5.85	23.5	748.5	0.880	22.31	5.15
	25.05	22.0	750	0.889	7.05	23.5	748.5	0.880	22.27	6.20
38.	25.20	20.0	754	0.902	13.20	25.0	750	0.875	22.73	11.55
	25.15	20.0	754	0.902	7.65	25.0	750	0.875	22.69	6.69
39.	25.10	24.0	750	0.880	9.00	23.0	752.5	0.888	22.09	7.99
	25.10	24.0	750	0.880	10.05	23.0	752.5	0.888	22.09	8.92
40.	25.10	21.0	752.5	0.896	6.50	22.0	747	0.885	22.49	5.75
	25.10	21.0	752.5	0.896	6.85	22.0	747	0.885	22.49	6.06

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
41.	25.15	21.0	767.5	0.915	1.40	22.0	765.5	0.908	23.01	1.27
42.	25.10	22.0	765.5	0.908	19.30	22.5	764.5	0.904	22.79	17.45
	25.00	22.5	764.5	0.904	4.35	22.5	763.5	0.903	22.60	3.93
43.	25.05	22.0	763.5	0.905	16.40	23.0	762.2	0.899	22.67	14.74
	23.85	23.0	762.2	0.899	5.25	23.0	761	0.898	21.44	4.71
44.	25.20	22.0	761	0.902	18.55	23.5	760.2	0.895	22.73	16.60
	25.00	23.5	760.2	0.895	3.05	23.5	759.5	0.895	22.38	2.73
45.	24.95	22.0	759.5	0.900	17.90	23.5	761	0.896	22.46	16.04
	24.65	23.5	761	0.896	2.75	23.5	762.5	0.898	22.09	2.47
46.	25.05	21.0	742.5	0.884	10.85	23.5	747.8	0.880	22.14	9.55
	25.05	23.5	747.8	0.880	6.05	23.5	753	0.896	22.04	5.36
47.	24.65	23.0	753	0.888	11.5	22.0	749	0.887	21.89	10.20
	24.90	22.0	749	0.887	5.55	22.0	745	0.883	22.09	4.90
48.	25.25	21.0	745	0.887	9.60	23.0	748	0.882	22.40	8.47
	24.80	23.0	748	0.882	6.85	23.0	750.5	0.885	21.87	6.06
49.	24.80	20.0	747	0.894	9.85	23.0	749.2	0.884	22.17	8.71
	24.95	23.0	749.2	0.884	7.85	23.0	751.5	0.886	22.06	6.96
50.	25.30	23.0	751.5	0.886	11.20	23.0	751.3	0.886	22.42	9.92
	24.95	23.0	751.3	0.886	4.25	23.0	751	0.886	22.11	3.77

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
51.	25.40	22.0	751	0.890	9.35	-	-	-	22.61	-
	25.00	-	-	-	-	-	-	-	-	-
52.	25.15	24.0	748	0.878	0.75	24.0	746	0.875	22.08	8.53
	24.95	24.0	746	0.875	7.35	24.0	744	0.873	21.83	6.42
53.	25.20	23.0	744	0.877	11.00	24.0	738	0.865	22.10	9.52
	25.00	24.0	738	0.865	6.40	24.0	731	0.857	21.63	5.48
54.	24.00	21.0	755.8	0.900	9.45	23.0	759.4	0.896	21.60	8.47
	24.10	23.0	759.4	0.896	9.90	23.0	763	0.900	21.59	8.91
55.	24.00	22.0	763	0.905	8.83	23.5	764.3	0.900	21.72	7.95
	24.00	23.5	764.3	0.900	6.75	23.5	765.5	0.901	21.60	6.08
56.	24.10	24.0	765.5	0.899	10.50	22.5	766.2	0.907	21.67	9.52
	24.05	22.5	766.2	0.907	5.70	21.3	767	0.913	21.81	5.20
57.	23.82	24.0	761.5	0.894	9.17	25.0	761.8	0.890	21.30	8.16
	24.00	25.0	761.8	0.890	6.32	25.0	762.1	0.890	21.36	5.62
58.	24.08	25.2	761.8	0.888	7.88	25.2	761.2	0.888	21.38	7.00
	24.00	25.2	761.2	0.888	6.70	25.2	760.6	0.886	21.31	5.94
59.	24.05	25.0	760.6	0.888	8.10	24.0	761.4	0.894	21.36	7.24
	24.00	24.0	761.4	0.894	5.15	24.0	762.2	0.895	21.46	4.61
60.	24.70	22.5	751	0.888	8.70	23.2	752.7	0.886	21.93	7.71
	24.20	23.2	752.7	0.886	4.78	23.2	754.3	0.889	21.44	4.25

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
61.	24.05	23.0	754.3	0.890	5.90	23.0	753.1	0.888	21.40	5.24
	23.95	23.0	753.1	0.888	6.65	23.0	752	0.887	21.27	5.90
62.	24.03	23.0	754.3	0.890	5.10	23.0	756.2	0.892	21.39	4.55
	23.95	23.0	756.2	0.892	10.95	23.0	758	0.895	21.36	9.80
63.	24.45	23.5	757	0.891	6.12	23.5	758.1	0.892	21.78	5.46
	24.55	23.5	758.1	0.892	6.93	23.5	759.3	0.894	21.90	6.20
64.	24.50	23.5	759.3	0.894	6.37	23.5	759.1	0.894	21.90	5.69
	24.55	23.5	759.1	0.894	10.00	23.5	759	0.894	21.92	8.94
65.	24.50	23.5	758.5	0.893	13.82	23.3	759	0.894	21.88	12.36
	24.45	23.3	759	0.894	4.60	23.3	759.5	0.895	21.86	4.12
66.	24.60	22.5	759.5	0.898	6.09	24.5	759.4	0.889	22.09	5.41
	24.53	24.5	759.4	0.889	5.05	24.5	759.3	0.889	21.81	4.49
67.	24.73	24.5	759.3	0.889	6.63	24.5	758.2	0.888	21.98	5.89
	24.52	24.5	758.2	0.888	5.25	24.5	757.2	0.887	21.77	4.66
68.	24.35	23.5	758.5	0.893	6.80	24.2	758.6	0.890	21.74	6.05
	24.32	24.2	758.6	0.890	3.56	24.2	758.7	0.890	21.64	3.17
	10.11	24.2	758.7	0.890	7.60	24.2	758.8	0.890	9.00	6.76
69.	24.43	24.0	758.8	0.891	4.38	23.0	754.9	0.890	21.77	3.90
	24.70	23.0	754.9	0.890	3.93	23.0	751	0.885	21.98	3.48
70.	24.5	23.0	751	0.886	5.50	23.5	753	0.886	21.71	4.87
	24.53	23.5	753	0.886	2.85	23.5	755	0.888	21.73	2.53
	10.0	23.5	755	0.888	7.18	23.5	757	0.891	8.88	6.50

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
71.	24.55	24.5	757.2	0.886	6.75	24.5	756.2	0.885	21.75	5.97
	15.00	24.5	756.2	0.885	8.64	24.5	755.2	0.884	13.28	7.64
	24.20	24.5	755.2	0.884	6.63	24.5	754.2	0.883	21.39	5.85
72.	24.65	24.0	751.1	0.881	4.15	24.2	752	0.882	21.72	3.66
	24.57	24.2	752	0.882	4.60	24.2	753	0.883	21.67	4.06
73.	24.83	21.0	757.5	0.903	6.22	23.5	757.5	0.892	22.42	5.55
	24.97	23.5	757.5	0.892	10.40	23.5	757.5	0.892	22.27	9.28
74.	24.75	22.5	757.5	0.896	6.10	23.3	758.8	0.896	22.18	5.47
	25.00	23.3	758.8	0.896	3.78	23.3	760.12	0.896	22.40	3.39
75.	25.18	22.5	761.2	0.900	7.43	22.5	763.5	0.903	22.66	6.71
	24.93	22.5	763.5	0.903	5.63	22.5	765.7	0.906	22.51	5.10
76.	25.05	22.5	765.7	0.906	9.97	23.0	765.6	0.903	22.70	9.00
	24.97	23.0	765.6	0.903	6.33	23.0	765.5	0.903	22.55	5.72
77.	25.25	22.5	765.5	0.906	11.33	23.3	767.1	0.904	22.88	10.24
	24.98	23.3	767.1	0.904	8.10	23.3	768.7	0.906	22.58	7.34
78.	25.48	21	753.3	0.898	9.53	22.3	750.3	0.888	22.88	8.46
	25.06	22.3	750.3	0.888	5.08	22.3	747.2	0.884	22.25	4.49
79.	25.08	22.3	747	0.884	4.60	22.3	747.2	0.884	22.17	4.07
	25.08	22.3	747.2	0.884	2.22	22.3	747.5	0.884	22.17	1.96
	8.02	22.3	747.5	0.884	4.50	22.3	747.7	0.884	7.09	3.98

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
80.	25.35	22.3	747.8	0.884	6.35	23.3	753	0.886	22.41	5.63
	25.00	23.3	753	0.886	3.55	23.3	758.1	0.893	22.15	3.17
	10.02	23.3	758.1	0.893	7.15	23.3	763.2	0.899	8.95	6.43
81.	25.03	23.0	763.2	0.900	6.53	23.0	764	0.902	22.53	5.89
	24.95	23.0	764	0.902	3.55	23.0	765	0.903	22.50	3.21
	10.00	23.0	765	0.903	8.10	23.0	765.8	0.904	9.03	7.32
82.	25.32	21.5	765.8	0.910	7.73	22.5	766	0.906	23.04	7.00
	25.00	22.5	766	0.906	6.32	22.5	766.2	0.906	22.65	5.73
83.	25.33	21.3	736.7	0.874	2.47	22.5	740.6	0.875	22.14	2.16
	25.18	22.5	740.6	0.875	22.51	22.5	741	0.875	22.03	19.70
	25.33	22.5	741	0.875	3.95	22.5	741.5	0.876	22.16	3.46
	10.08	22.5	741.5	0.876	6.22	22.5	742	0.876	8.83	5.45
84.	25.43	22.3	741.7	0.877	2.45	22.3	746.6	0.883	22.30	2.16
	25.30	22.3	746.6	0.883	2.62	22.3	743	0.878	22.34	2.30
	10.06	22.3	743	0.878	5.83	22.3	742.2	0.877	8.83	5.11
85.	25.27	22.3	741.9	0.879	2.1	23.0	741.5	0.874	22.21	1.84
	25.17	23.0	741.5	0.874	22.78	23.0	741.3	0.874	22.00	19.91
	25.20	23.0	741.3	0.874	3.53	23.0	742.5	0.875	22.02	3.09
86.	25.47	21.0	742	0.883	3.00	22.3	745.3	0.881	22.49	2.64
	25.20	22.3	745.3	0.881	21.73	22.3	745.3	0.881	22.20	19.14
	25.75	22.3	745.3	0.881	3.65	22.3	742.3	0.878	22.25	3.20
	10.10	22.3	742.3	0.878	6.03	22.3	741.4	0.876	8.87	5.28

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
87.	25.32	22.0	741	0.877	2.18	22.5	736	0.869	22.21	1.89
	25.27	22.5	736	0.869	2.72	22.5	736.1	0.869	21.96	2.36
	10.05	22.5	736.1	0.869	5.18	22.5	736.3	0.870	8.73	4.51
88.	25.45	21.5	736	0.873	2.25	22.0	735.8	0.871	22.22	1.96
	25.30	22.0	735.8	0.871	23.30	22.0	735.8	0.871	22.04	20.29
	25.34	22.0	735.8	0.871	5.12	22.0	731.6	0.866	22.07	4.43
	10.11	22.0	731.6	0.866	5.55	22.0	731	0.865	8.76	4.80
89.	25.40	21.5	731	0.870	2.23	22.3	730.7	0.863	22.10	1.92
	25.33	22.3	730.7	0.863	22.60	22.3	731	0.863	21.86	19.50
	25.28	22.3	731	0.863	2.10	22.3	733.9	0.867	21.82	1.82
	10.13	22.3	733.9	0.867	6.89	22.3	734	0.868	8.78	5.98
90.	25.49	22.3	734	0.868	1.98	22.3	734	0.867	22.13	1.72
	25.25	22.3	734	0.867	22.10	22.3	734.5	0.868	21.89	19.18
	25.21	22.3	734.5	0.868	3.98	22.3	734.2	0.868	21.88	3.45
	10.06	22.3	734.2	0.868	6.25	22.3	734.5	0.868	8.73	5.43
91.	25.00	22.0	734.6	0.870	2.18	22.3	729.5	0.862	21.75	1.88
	25.23	22.3	729.5	0.862	22.45	22.3	729	0.861	21.74	19.33
	25.22	22.3	729	0.861	0.85	22.3	733	0.866	21.71	0.74
	10.03	22.3	733	0.866	5.15	22.3	734.5	0.868	8.69	4.47
92.	25.52	22.0	734.6	0.870	2.47	22.3	737	0.871	22.20	2.15
	25.23	22.3	737	0.871	5.10	22.3	742	0.877	21.98	4.47
	25.33	22.3	742	0.877	4.78	22.3	747	0.883	22.21	4.22
93.	25.38	19.3	766.8	0.881	3.15	22.0	765.5	0.908	22.36	2.86
	25.33	22.0	765.5	0.908	1.70	22.0	764	0.906	23.00	1.54
	10.11	22.0	764	0.906	4.67	22.0	763.3	0.905	9.16	4.23

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
94.	25.10	21.3	763.2	0.908	2.19	22.5	763	0.902	22.79	1.98
	25.26	22.5	763	0.902	24.17	22.5	763	0.902	22.78	21.80
	25.29	22.5	763	0.902	2.92	22.5	762.2	0.902	22.81	2.63
	10.15	22.5	762.2	0.902	6.37	22.5	761.9	0.901	9.16	5.74
95.	25.43	21.5	761.4	0.905	3.15	22.5	760.3	0.899	23.01	2.83
	25.18	22.5	760.3	0.899	2.46	22.5	755.8	0.894	22.64	2.20
	10.06	22.5	755.8	0.894	6.67	22.5	755	0.893	8.99	5.96
96.	25.35	22.5	755	0.893	3.55	23.0	753.7	0.889	22.64	3.16
	25.33	23.0	753.7	0.889	2.85	23.0	755.5	0.891	22.52	2.54
	10.07	23.0	755.5	0.891	6.30	23.0	755.2	0.891	8.97	5.61
97.	25.18	22.7	755.2	0.892	3.30	23.0	755	0.891	22.46	2.94
	25.23	23.0	755	0.891	2.00	23.0	756.6	0.892	22.48	1.78
98.	25.43	20.3	764.5	0.914	3.35	22.2	763.8	0.904	23.24	3.03
	25.23	22.2	763.8	0.904	2.83	22.2	765.8	0.907	22.81	2.57
	10.02	22.2	765.8	0.907	7.75	22.2	766	0.907	9.09	7.03
99.	25.32	22.0	766	0.908	2.86	23.0	765.6	0.904	22.99	2.59
	25.20	23.0	765.6	0.904	3.53	22.5	751.5	0.888	22.78	3.13
	9.00	22.5	751.5	0.888	5.65	22.5	748.5	0.884	7.99	4.99
100.	25.15	22.0	748	0.886	2.60	22.0	746.5	0.884	22.28	2.30
	25.15	22.0	746.5	0.884	2.03	22.0	743.1	0.880	22.23	1.79
	0.02	22.0	743.1	0.880	5.40	22.0	742.5	0.879	7.94	4.75
101.	25.37	20.0	752.7	0.901	2.87	21.5	758.7	0.902	22.86	2.59
	25.20	21.5	758.7	0.902	2.90	21.5	751	0.892	22.73	2.59

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
102.	25.38	21.7	746	0.885	7.10	23.0	749.3	0.884	22.46	6.28
	25.13	23.0	749.3	0.884	3.22	22.5	750.5	0.887	22.21	2.86
	20.02	22.5	750.5	0.887	13.25	22.5	749.2	0.886	17.76	11.74
103.	25.18	22.5	749	0.885	6.50	22.5	736.8	0.870	22.28	5.66
	25.23	22.5	736.8	0.870	2.86	22.5	732.7	0.865	21.95	2.47
	20.12	22.5	732.7	0.865	13.35	22.5	734.5	0.868	17.40	11.59
104.	24.92	22.3	734.6	0.868	5.66	23.0	745	0.878	21.63	4.97
	24.97	23.0	745	0.878	2.24	22.3	755.5	0.894	21.92	2.00
	20.01	22.3	755.5	0.894	11.73	22.3	757.8	0.897	17.89	10.52
105.	25.27	22.0	758	0.899	7.36	23.0	762.2	0.899	22.72	6.62
	25.21	23.0	762.2	0.899	3.63	22.7	766	0.905	22.66	3.29
	20.07	22.7	766	0.905	10.92	22.7	766.5	0.906	18.16	9.89
106.	25.33	22.5	766.5	0.907	8.67	23.0	766.8	0.905	22.97	7.84
	25.32	23.0	766.8	0.905	2.30	21.7	766.8	0.911	22.96	2.10
	25.23	21.7	766.8	0.911	15.86	22.5	767.8	0.908	22.98	14.40
107.	25.21	20.8	750.6	0.895	8.22	22.7	755	0.892	22.56	7.33
	25.22	22.7	755	0.892	2.17	22.9	755.8	0.892	22.50	1.94
	24.13	22.9	755.8	0.892	18.95	23.0	755	0.891	21.52	16.88
108.	25.24	22.7	755	0.892	9.15	23.4	755	0.889	22.51	8.13
	25.17	23.4	755	0.889	6.10	22.7	753.7	0.890	22.38	5.43
	17.99	22.7	753.7	0.890	14.08	22.6	752	0.889	16.01	12.52

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
109.	25.15	22.2	751.5	0.889	15.05	23.2	746.2	0.879	22.36	13.23
	27.99	23.2	746.2	0.879	2.00	23.00	749.7	0.884	19.33	1.77
110.	25.29	22.3	771.2	0.913	10.07	23.5	771.3	0.909	23.09	9.15
	25.21	23.5	771.3	0.909	7.20	22.5	770.7	0.912	22.92	6.57
	24.11	22.7	770.7	0.912	17.30	22.3	769.7	0.912	21.99	15.78
111.	25.28	22.2	769.4	0.912	10.13	22.6	769.3	0.910	23.06	9.22
	25.20	22.6	769.3	0.910	7.05	22.3	769.8	0.911	22.93	6.42
	24.11	22.3	769.8	0.911	17.15	22.3	769.1	0.911	21.96	15.62
112.	25.27	22.2	768.9	0.911	10.84	22.6	768.8	0.909	23.02	9.85
	25.13	22.6	768.8	0.909	6.07	22.3	767	0.908	22.84	5.51
	24.09	22.3	767	0.908	17.81	22.5	765.9	0.906	21.87	16.14
113.	25.45	22.2	765.5	0.907	9.95	22.7	762.6	0.901	23.08	8.96
	25.13	22.7	762.6	0.901	7.18	22.5	763.2	0.903	22.64	6.48
	24.06	22.5	763.2	0.903	18.30	22.7	763.8	0.903	21.73	16.52
114.	25.15	22.5	763.8	0.904	9.53	22.5	764.1	0.904	22.74	8.62
	25.09	22.5	764.1	0.904	4.90	22.4	759.3	0.898	22.68	4.40
	24.07	22.4	759.3	0.898	17.47	22.5	756.2	0.894	21.61	15.62
115.	25.11	23.1	752.3	0.887	3.00	22.7	755.8	0.893	22.27	2.68
	25.17	22.7	755.8	0.893	4.13	22.6	756	0.894	22.48	3.69
	24.13	22.6	756	0.894	14.78	22.2	752.8	0.891	21.57	13.17

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
116.	25.18	22.1	752	0.891	4.87	22.6	745.5	0.881	22.44	4.29
	25.21	22.6	745.5	0.881	4.27	22.5	746	0.882	22.21	3.77
	24.08	22.5	746	0.882	13.55	22.7	750	0.886	21.24	12.01
117.	25.40	22.2	750.3	0.888	6.01	23.1	753.3	0.888	22.56	5.34
	25.25	23.1	753.3	0.888	5.09	22.8	753.9	0.890	22.42	4.53
	24.10	22.8	753.9	0.890	14.00	22.2	753.6	0.892	21.45	12.49
118.	25.15	21.7	762	0.905	7.40	22.8	762	0.900	22.76	6.66
	25.03	22.8	762	0.900	6.52	22.6	762	0.901	22.53	5.87
	22.03	22.6	762	0.901	13.79	22.8	761.3	0.889	19.85	12.26
119.	25.03	22.7	761	0.899	6.51	23.0	760	0.897	22.50	5.84
	25.03	23.0	760	0.897	5.35	22.4	759.8	0.898	22.45	4.80
	22.08	22.4	759.8	0.898	14.05	22.8	758.2	0.896	19.83	12.59
120.	25.50	22.5	758	0.897	5.85	22.9	757.2	0.894	22.87	5.23
	25.11	22.9	757.2	0.894	2.84	22.8	755.5	0.892	22.45	2.53
	22.08	22.8	755.5	0.892	11.86	22.8	754	0.890	19.70	10.56
121.	25.10	22.3	753.9	0.892	7.99	22.7	753.1	0.890	22.39	7.11
	25.08	22.7	753.1	0.890	2.23	22.7	752.3	0.889	22.25	1.98
	24.07	22.7	752.3	0.889	14.23	23.3	751.7	0.885	21.40	12.59
122.	25.32	22.2	758.8	0.898	7.68	22.9	761	0.898	22.74	6.90
	25.1	22.9	761	0.898	7.73	23.0	761.5	0.898	22.54	6.94
	24.06	23.0	761.5	0.898	18.61	23.2	761.2	0.897	2161	16.69

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
123.	25.43	23.0	761.5	0.898	6.53	24.2	762.5	0.894	22.84	5.84
	10.05	24.2	762.5	0.894	3.7	23.2	763.5	0.900	8.98	3.33
	25.17	23.2	763.5	0.900	3.62	22.9	764.3	0.902	22.65	3.27
	14.27	22.9	764.3	0.902	8.12	22.8	764	0.902	12.87	7.32
124.	25.20	22.3	763.7	0.904	4.26	22.5	763.7	0.903	22.78	3.85
	25.12	22.5	763.7	0.903	1.90	23.2	764.8	0.902	22.68	1.71
	24.15	23.2	764.8	0.902	17.03	22.9	764.7	0.903	21.78	15.38
125.	25.22	22.5	763	0.902	5.31	23.2	760.2	0.896	22.75	4.76
	25.22	23.2	760.2	0.896	1.98	22.9	757.1	0.894	22.60	1.77
	24.23	22.9	757.1	0.894	14.37	23.1	754.9	0.890	21.66	12.79
126.	25.18	22.5	754.7	0.892	6.75	23.0	753.9	0.889	22.46	6.00
	25.20	23.0	753.9	0.889	8.35	23.0	751.4	0.886	22.40	7.40
	24.07	23.0	751.4	0.886	13.88	22.9	750.1	0.885	21.33	12.28
127.	25.17	22.4	750	0.886	7.57	22.8	745	0.879	22.30	6.65
	25.10	22.8	745	0.879	5.52	22.7	736.4	0.869	22.06	4.80
	20.15	22.7	736.4	0.869	10.25	22.7	735.4	0.868	17.51	8.90
128.	25.72	22.5	735.1	0.868	8.80	23.0	735	0.866	22.32	7.62
	25.17	23.0	735	0.866	4.25	23.0	739.7	0.872	21.80	3.71
	20.08	23.0	739.7	0.872	11.83	23.7	742.3	0.872	17.51	10.32
129.	24.5	19.5	748.3	0.898	4.43	21.3	746.2	0.887	22.00	3.93
	25.03	21.3	746.2	0.887	4.47	21.3	744.1	0.884	22.20	3.95
	10.05	21.3	744.1	0.884	7.35	21.3	742	0.881	8.88	6.48

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
130.	25.15	21.0	741.8	0.883	4.10	20.5	740.5	0.884	22.21	3.62
	25.10	20.5	740.5	0.884	3.25	20.5	739.2	0.882	22.19	2.87
	10.02	20.5	739.2	0.882	7.25	20.5	738	0.880	8.84	6.38
131.	25.25	20.0	737.5	0.882	4.13	21.3	740.1	0.879	22.27	3.63
	25.23	21.3	740.1	0.879	3.33	21.3	743.8	0.884	22.18	2.94
132.	25.23	21.3	744	0.884	5.20	21.5	741.4	0.880	22.30	4.58
	25.18	21.5	741.4	0.880	2.95	21.5	738.7	0.877	22.16	2.59
133.	25.20	21.3	738.5	0.877	4.45	22.5	740.7	0.875	22.10	3.89
	25.12	22.5	740.7	0.875	3.30	22.5	745	0.880	21.98	2.90
134.	25.53	20.0	746	0.893	9.15	22.5	741.7	0.876	22.80	8.02
	25.33	22.5	741.7	0.876	2.08	22.5	734.9	0.868	22.19	1.81
	10.05	22.5	734.9	0.868	6.57	22.5	735.3	0.868	8.72	5.70
135.	25.27	22.0	735.7	0.871	2.55	22.7	743.7	0.878	22.01	2.24
	23.36	22.7	743.7	0.878	2.71	22.7	754.5	0.892	20.51	2.42
	10.10	22.7	754.5	0.892	7.85	22.7	756	0.893	9.01	7.01
136.	25.21	22.5	756	0.894	3.87	22.5	762.8	0.902	22.54	3.49
	25.16	22.5	762.8	0.902	3.38	22.5	765.5	0.905	22.69	3.06
	10.09	22.5	765.5	0.905	6.7	22.5	765.5	0.905	9.13	6.06
137.	25.30	21.0	762.2	0.908	4.40	21.5	762.8	0.907	22.97	3.99
	25.33	21.5	762.8	0.907	2.64	21.5	759.5	0.902	22.97	2.38

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
138.	25.57	21.0	758.3	0.904	5.80	22.5	758	0.896	23.12	5.20
	25.46	22.5	758	0.896	2.43	22.5	757	0.896	22.81	2.18
	10.12	22.5	757	0.896	7.55	22.5	757.5	0.896	9.07	6.76
139.	25.37	21.7	757.5	0.899	13.83	22.5	757.6	0.896	22.81	12.39
	22.03	22.5	757.6	0.896	9.39	22.5	757.3	0.896	19.74	8.41
	25.25	22.5	757.3	0.896	1.95	22.5	758.6	0.897	22.62	1.75
140.	25.40	21.5	758.5	0.901	3.75	23.0	759	0.895	22.89	3.36
	25.37	23.0	759	0.895	1.92	23.0	753.2	0.888	22.71	1.70
141.	25.20	21.5	752.4	0.894	4.04	22.7	751	0.887	22.53	3.58
	25.3	22.7	751	0.887	3.23	22.7	752	0.888	22.44	2.87
	10.05	22.7	752	0.888	7.00	22.7	751.1	0.887	8.92	6.21
142.	25.55	22.3	751.4	0.889	5.50	23.0	750.5	0.885	22.71	4.87
	25.32	23.0	750.5	0.885	4.40	23.0	750	0.884	22.41	3.89
	10.09	23.0	750	0.884	7.63	23.0	749.4	0.884	8.92	6.74
143.	25.33	22.5	749.5	0.886	2.85	22.5	751	0.888	22.44	2.53
	25.27	22.5	751	0.888	22.96	22.5	751.2	0.888	22.44	20.39
	25.32	22.5	751.2	0.888	3.37	22.5	750.6	0.887	22.48	2.99
	9.05	22.5	750.6	0.887	2.25	22.5	749.1	0.885	8.03	1.99
144.	25.25	20.3	751.8	0.898	6.55	21.8	746.3	0.885	22.67	5.80
	25.07	21.8	746.3	0.885	1.42	21.8	745.8	0.884	22.19	1.26
	10.02	21.8	745.8	0.884	8.50	21.8	745.5	0.884	8.86	7.51

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
145.	24.98	21.3	745	0.885	3.01	21.3	740.1	0.880	22.11	2.65
	25.08	21.3	740.1	0.880	3.28	21.3	736.8	0.875	22.07	2.87
	10.03	21.3	736.8	0.875	6.05	21.3	737.2	0.876	8.78	5.30
146.	25.43	20.5	737.3	0.880	4.23	21.3	743.5	0.883	22.38	3.74
	25.10	21.3	743.5	0.883	0.9	21.3	750.5	0.892	22.16	0.8
	10.10	21.3	750.5	0.892	6.25	21.3	751.5	0.894	9.01	5.59
147.	25.28	21.3	751.6	0.894	3.82	21.3	757.8	0.901	22.60	3.44
	25.10	21.3	757.8	0.901	2.10	21.3	756.4	0.900	22.62	1.89
	10.03	21.3	756.4	0.900	6.07	21.3	755	0.898	9.03	5.45
148.	25.20	21.0	754	0.898	16.85	22.2	750	0.887	22.63	14.95
	25.15	22.2	750	0.887	7.87	22.2	755.7	0.895	22.31	7.04
	25.12	22.2	755.7	0.895	3.17	22.2	761	0.901	22.48	2.86
149.	25.10	22.3	768	0.909	8.36	21.6	772	0.918	22.82	7.67
	25.08	21.6	772	0.918	-0.05	21.2	773.3	0.926	23.02	-0.05
	25.00	21.2	773.3	0.921	16.52	21.6	772.4	0.918	23.03	15.17
150.	25.47	21.0	772.2	0.921	3.31	22.3	768.4	0.910	23.46	3.01
	25.25	22.3	768.4	0.910	3.03	22.3	763.5	0.904	22.98	2.74
	25.10	22.3	763.5	0.904	15.73	22.3	761.2	0.901	22.69	14.17
151.	25.37	20.0	746.3	0.893	6.23	22.2	746	0.883	22.66	5.50
	25.15	22.2	746	0.883	-0.43	21.5	745.5	0.885	22.21	-0.38
	20.07	21.5	745.5	0.885	10.17	21.0	746.3	0.889	17.76	9.04

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
152.	25.37	21.0	746.3	0.889	5.45	22.2	747.6	0.884	22.55	4.82
	25.2	22.2	747.6	0.884	1.50	21.3	748.2	0.889	22.28	1.33
	25.07	21.3	748.2	0.889	13.00	22.0	748.2	0.886	22.29	11.52
153.	25.42	22.2	748.2	0.885	6.15	22.0	748.3	0.886	22.50	5.45
	25.15	22.0	748.3	0.886	2.17	21.2	745.5	0.886	22.28	1.92
	25.20	21.2	745.5	0.886	13.02	22.0	743	0.880	22.33	12.25
154.	25.15	21.5	743	0.882	5.47	21.8	743.8	0.882	22.18	4.82
	25.17	21.8	743.8	0.882	2.13	21.7	745.7	0.884	22.20	1.88
	25.07	21.7	745.7	0.884	13.83	22.5	749	0.885	22.16	12.24
155.	25.47	22.5	749.5	0.886	5.98	22.5	754.3	0.892	22.57	5.33
	25.20	22.5	754.3	0.892	2.55	21.8	754.1	0.895	22.48	2.28
	25.17	21.8	754.1	0.895	14.36	22.5	750	0.886	22.53	12.72
156.	25.37	22.0	749.3	0.888	7.48	22.2	732	0.865	22.53	6.47
	25.22	22.2	732	0.865	2.87	22.0	730	0.864	21.82	2.48
	25.15	22.0	730	0.864	14.30	22.8	732	0.863	21.73	12.34
157.	25.13	23.2	732.2	0.862	5.03	22.8	733.3	0.865	21.66	4.35
	25.27	22.8	733.3	0.865	2.75	22.5	732.9	0.865	21.86	2.38
	25.17	22.5	732.9	0.865	12.86	23.3	735.2	0.864	21.77	11.11
158.	25.17	22.6	735.5	0.868	6.9	23.0	738.1	0.870	21.85	6.00
	24.91	23.0	738.1	0.870	2.94	22.6	739.6	0.874	21.67	2.57
	25.04	22.6	739.6	0.874	11.04	23.0	740.1	0.872	21.88	9.63

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
159.	25.27	22.3	766.8	0.908	8.28	23.6	768.5	0.905	22.95	7.49
	25.20	23.6	768.5	0.905	2.43	23.7	768.2	0.904	22.81	2.20
	24.10	23.7	768.2	0.904	16.20	23.8	767.3	0.902	21.79	14.61
160.	25.29	23.2	767	0.904	10.22	23.7	766.8	0.902	22.82	9.22
	25.20	23.7	766.8	0.902	3.23	23.5	763.9	0.899	22.73	2.90
	24.08	23.5	763.9	0.899	16.37	24.0	762.7	0.896	21.65	14.67
161.	25.45	23.9	762.7	0.897	10.66	24.0	763.3	0.887	22.83	9.56
	25.20	24.0	763.3	0.897	5.11	24.0	764	0.898	22.60	4.59
	20.10	24.0	764	0.898	13.21	23.9	764.3	0.898	18.05	11.86
162.	25.17	24.0	764.3	0.898	10.33	24.0	763.9	0.897	22.60	9.27
	25.14	24.0	763.9	0.897	4.79	24.0	763.1	0.896	22.55	4.29
	20.07	24.0	763.1	0.896	14.27	23.8	763.2	0.898	17.98	12.81
163.	25.57	23.3	763.1	0.899	9.57	24.0	763.5	0.897	22.99	8.58
	25.25	24.0	763.5	0.897	4.55	23.9	764	0.898	22.65	4.09
	24.17	23.9	764	0.898	16.07	24.0	763.7	0.897	21.70	14.41
164.	25.67	22.2	742	0.878	2.98	22.5	743	0.878	22.54	2.62
	25.27	22.5	743	0.878	2.62	22.1	743.1	0.879	22.19	2.30
	24.05	22.1	743.1	0.879	13.79	21.8	744	0.882	21.14	12.16
165.	25.10	21.2	744.5	0.885	2.45	21.5	750.4	0.892	22.21	2.19
	25.07	21.5	750.4	0.892	22.62	21.5	751	0.892	22.36	20.18
	25.23	21.5	751	0.892	2.92	21.2	754.7	0.898	22.51	2.62
	24.07	21.2	754.7	0.898	14.34	21.2	753.5	0.896	21.61	12.85

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
166.	25.41	21.5	753.5	0.896	2.35	21.2	748.3	0.890	22.77	2.09
	25.20	21.2	748.3	0.890	2.04	21.0	746.5	0.889	22.43	1.81
	24.10	21.0	746.5	0.889	13.48	21.0	744.6	0.886	21.42	11.94
167.	25.31	21.7	758.3	0.900	3.34	22.7	759.5	0.898	22.78	3.00
	25.30	22.7	759.5	0.898	2.73	22.4	759.8	0.898	22.72	2.45
	24.02	22.4	759.8	0.898	13.53	22.3	760	0.899	21.57	12.16
168.	25.21	22.1	760	0.900	4.10	22.3	759.5	0.899	22.64	3.69
	25.23	22.3	759.5	0.899	2.40	22.4	760	0.899	22.68	2.16
	24.07	22.4	760	0.899	13.03	22.3	759.6	0.899	21.64	11.71
169.	25.57	22.3	759.5	0.899	4.67	22.6	760	0.898	22.99	4.19
	25.27	22.6	760	0.898	2.47	22.5	761.8	0.901	22.69	2.23
	24.21	22.5	761.8	0.901	14.57	22.5	762.7	0.902	21.81	13.14
170.	25.20	22.3	762.6	0.902	3.28	22.5	764.3	0.904	22.73	2.97
	25.22	22.5	764.3	0.904	1.90	22.5	764.1	0.904	22.80	1.72
	24.16	22.5	764.1	0.904	14.54	22.3	763.5	0.904	21.84	13.14
171.	25.35	22.1	763.2	0.904	5.58	22.4	760.5	0.900	22.92	5.02
	25.21	22.4	760.5	0.900	3.93	22.3	757.2	0.896	22.69	3.52
	24.90	22.3	757.2	0.896	13.65	23.2	754.5	0.889	21.59	12.13
172.	25.17	22.6	754.2	0.892	2.83	22.7	753	0.890	22.45	2.52
	25.27	22.7	753	0.890	7.27	22.7	753	0.890	22.49	6.47
	24.10	22.7	753	0.890	12.55	23.2	753	0.887	21.45	11.13
173.	25.20	22.8	753.3	0.890	6.74	22.8	756	0.892	22.43	6.01
	24.72	22.8	756	0.892	2.72	22.8	756.8	0.894	22.05	2.43
	24.13	22.8	756.8	0.894	15.10	22.9	756.6	0.893	21.57	13.48

TABLE (33)

RESPIRATORY EXCHANGE

(a) Serial No.	(b) (px_1-qx_2)	(c) $1/5.11 \times (px_1-qx_2)$	(d) $0.454 \times (p-q)$	(e) $0.0238 \times (T_2-T_1)$	(f) 0.015	(g) Total Vol. O_2 Litres	(h) Weight O_2 Grams	(i) Weight CO_2 Grams	(j) Volume CO_2 Litres	(k) R.Q.
1.	12.10 11.51	2.367 2.268	0.0 0.0	0.131	0.015	4.781	6.832	7.087	3.584	0.749
2.	11.89 11.62	2.326 2.290	0.0 0.0	0.107	0.015	4.738	6.771	7.994	4.043	0.851
3.	14.91 13.72	2.917 2.703	0.005 0.005	0.143	0.015	5.788	8.271	10.829	5.477	0.944
4.	14.99 13.84	2.932 2.727	-0.002 -0.002	0.119	0.015	5.789	8.272	10.744	5.434	0.940
5.	13.62 13.20	2.664 2.601	0.003 0.003	0.167	0.015	5.453	7.792	10.630	5.377	0.985
6.	-	-	-	-	-	-	-	10.164	5.141	-
7.	17.29 11.82	3.382 2.329	0.002 0.002	0.119	0.015	5.849	8.358	10.834	5.479	0.936
8.	15.80 15.53	3.091 3.060	0.005 0.005	0.119	0.015	6.295	8.996	11.796	5.966	0.946
9.	15.13 13.27	2.960 2.615	-0.002 -0.002	0.048	0.015	5.634	8.051	10.144	5.130	0.911

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
10.	16.64 15.70	3.255 3.094	0.0 0.0	0.095	0.015	6.459	9.230	12.182	6.161	0.953
11.	16.93 11.68	3.312 2.301	0.0 0.0	0.143	0.015	5.771	8.247	10.828	5.476	0.948
12.	14.79 9.47	2.893 1.866	-0.004 -0.004	0.107	0.015	4.873	6.964	8.333	4.214	0.863
13.	14.00 9.75	2.739 1.921	0.002 0.002	0.119	0.015	4.798	6.856	7.880	3.986	0.829
14.	16.95 15.47	3.316 3.048	0.002 0.002	0.143	0.015	6.526	9.326	11.836	5.986	0.917
15.	19.33 10.65	3.781 2.099	0.002 0.002	0.155	0.015	6.054	8.651	10.910	5.518	0.911
16.	16.66 14.84	3.259 2.924	-0.005 -0.005	0.107	0.015	6.295	8.996	11.474	5.803	0.904
17.	16.64 13.35	3.255 2.631	0.0 0.0	0.119	0.015	6.020	8.603	10.780	5.452	0.904
18.	16.30 14.08	3.189 2.774	0.006 0.006	0.143	0.015	6.133	8.764	11.259	5.694	0.928
19.	15.96 13.72	3.122 2.703	0.0 0.0	0.095	0.015	5.935	8.481	11.325	5.728	0.964
20.	16.11 13.92	3.151 2.743	0.005 0.005	0.095	0.015	6.014	8.594	11.074	5.601	0.931

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
21.	16.52 14.89	3.232 2.934	-0.001 -0.001	0.119	0.015	6.298	9.000	10.701	5.412	0.857
22.	16.92 14.47	3.310 2.851	-0.005 -0.005	0.107	0.015	6.273	8.964	11.339	5.735	0.913
23.	16.31 13.72	3.191 2.703	0.0 0.0	0.119	0.015	6.028	8.614	10.190	5.154	0.854
24.	15.88 13.94	3.106 2.747	0.005 0.005	0.107	0.015	5.985	8.553	10.903	5.514	0.920
25.	14.78 13.86	2.891 2.731	0.003 0.003	0.119	0.015	5.762	8.234	10.057	5.086	0.883
26.	16.43 14.26	3.214 2.810	0.002 0.002	0.143	0.015	6.186	8.840	11.049	5.588	0.903
27.	16.03 14.19	3.136 2.796	0.004 0.004	0.107	0.015	6.062	8.663	11.225	5.678	0.935
28.	15.88 14.37	3.106 2.832	-0.003 -0.003	0.119	0.015	6.066	8.668	11.226	5.678	0.935
29.	16.41 13.97	3.210 2.753	0.0 0.0	0.095	0.015	6.073	8.678	10.459	5.290	0.870
30.	11.60 10.89	2.269 2.146	0.001 0.001	0.143	0.015	4.575	6.538	-	-	-
31.	17.12 13.56	3.349 2.672	0.004 0.004	0.143	0.015	6.187	8.841	11.662	5.898	0.952

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
32.	- -	- -	- -	-	-	-	-	-	-	-
33.	18.14 15.72	3.549 3.097	-0.002 -0.002	0.119	0.015	6.776	9.683	11.976	6.057	0.893
34.	16.83 14.92	3.292 2.940	0.002 0.002	0.143	0.015	6.394	9.137	11.731	5.933	0.928
35.	18.13 15.87	3.547 3.127	0.004 0.004	0.119	0.015	6.816	9.740	11.471	5.802	0.852
36.	17.12 15.13	3.349 2.981	0.004 0.004	0.152	0.015	6.501	9.290	10.486	5.303	0.814
37.	17.16 16.07	3.357 3.166	0.004 0.004	0.143	0.015	6.689	9.559	9.369	4.739	0.708
38.	27.18	5.318	0.013	0.190	0.015	5.536	7.911	10.125	5.121	0.923
39.	27.27	5.336	-0.004	0.155	0.015	5.502	7.862	9.658	4.885	0.888
40.	33.17	6.490	0.005	0.131	0.015	6.641	9.490	11.654	5.894	0.887
41.	21.74	4.253	0.003	0.131	0.015	4.402	6.290	9.044	4.575	1.039
42.	24.01	4.697	0.002	0.143	0.015	4.857	6.941	9.527	4.819	0.992
43.	24.66	4.824	0.003	0.167	0.015	5.009	7.158	9.614	4.863	0.971
44.	25.78	5.043	0.003	0.143	0.015	5.204	7.437	9.918	5.017	0.964
45.	26.04	5.094	0.001	0.155	0.015	5.265	7.524	10.506	5.314	1.009

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
46.	29.27	5.726	-0.001	0.167	0.015	5.907	8.441	10.888	5.507	0.932
47.	28.88	5.649	0.002	0.155	0.015	5.821	8.318	10.879	5.503	0.945
48.	29.74	5.818	0.001	0.167	0.015	6.001	8.575	10.938	5.533	0.922
49.	28.56	5.587	0.004	0.179	0.015	5.785	8.267	10.916	5.157	0.891
50.	30.84	6.033	0.0	0.143	0.015	6.191	8.847	11.405	5.769	0.932
51.	-	-	-	-	-	-	-	-	-	-
52.	28.96	5.665	0.002	0.131	0.015	5.813	8.307	11.428	5.780	0.994
53.	28.73	5.620	0.014	0.143	0.015	5.792	8.277	11.682	5.909	1.020
54.	25.81	5.049	0.0	0.119	0.015	5.183	7.407	8.775	4.439	0.856
55.	29.29	5.730	0.002	0.148	0.015	5.895	8.424	10.397	5.259	0.892
56.	28.76	5.626	-0.006	0.071	0.015	5.766	8.240	10.573	5.348	0.928
57.	28.88	5.649	0.002	0.119	0.015	5.785	8.267	10.952	5.540	0.958
58.	29.75	5.820	0.001	0.107	0.015	5.943	8.493	11.170	5.650	0.951
59.	30.97	6.058	-0.003	0.083	0.015	6.153	8.793	11.377	5.755	0.853
60.	31.41	6.144	0.0	0.119	0.015	6.278	8.971	12.552	6.349	1.011

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
61.	31.53	6.168	0.001	0.143	0.015	6.327	9.041	12.332	6.238	0.986
62.	28.40	5.556	-0.002	0.119	0.015	5.688	8.128	11.055	5.592	0.983
63.	32.02	6.264	-0.001	0.143	0.015	6.421	9.176	12.475	6.310	0.983
64.	29.19	5.710	0.0	0.131	0.015	5.856	8.368	11.294	5.713	0.976
65.	27.26	5.333	-0.001	0.143	0.015	5.490	7.845	10.091	5.104	0.930
66.	34.00	6.651	0.004	0.143	0.015	6.813	9.736	12.115	6.128	0.899
67.	33.20	6.495	0.001	0.131	0.015	6.642	9.491	11.080	5.604	0.844
68.	36.40	7.120	0.001	0.126	0.015	7.260	10.375	13.694	6.927	0.954
69.	36.37	7.115	0.003	0.095	0.015	7.228	10.329	13.694	6.927	0.958
70.	38.52	7.536	-0.002	0.143	0.015	7.692	10.992	14.322	7.244	0.942
71.	37.14	7.265	0.001	0.131	0.015	7.411	10.590	13.281	6.718	0.906
72.	35.67	6.978	-0.001	0.119	0.015	7.111	10.162	12.162	6.459	0.908
73.	29.86	5.841	0.005	0.119	0.015	5.980	8.545	8.840	4.471	0.748
74.	35.72	6.987	0.0	0.143	0.015	7.145	10.210	12.689	6.418	0.898
75.	33.36	6.526	-0.003	0.095	0.015	6.633	9.479	8.888	4.496	0.678

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
76.	30.53	5.972	0.001	0.071	0.015	6.059	8.658	8.874	4.489	0.741
77.	27.88	5.454	0.0	0.124	0.015	5.593	7.992	6.164	3.118	0.557
78.	32.18	6.295	0.006	0.043	0.015	6.359	9.087	11.790	5.964	0.938
79.	41.42	8.102	0.0	0.179	0.015	8.296	11.855	15.088	7.632	0.920
80.	38.28	7.489	-0.007	0.095	0.015	7.592	10.849	13.516	6.837	0.901
81.	37.64	7.363	-0.002	0.010	0.015	7.386	10.555	14.412	7.290	0.987
82.	32.96	6.448	0.002	0.179	0.015	6.644	9.494,	12.242	6.192	0.932
83.	44.39	8.683	-0.001	0.119	0.015	8.816	12.598	16.560	8.376	0.950
84.	43.90	8.588	0.0	0.076	0.015	8.679	12.402	16.304	8.247	0.950
85.	41.39	8.097	0.002	0.148	0.015	8.262	11.806	15.328	7.753	0.938
86.	45.55	8.910	0.003	0.083	0.015	9.011	12.877	17.276	8.738	0.970
87.	44.14	8.635	0.003	0.095	0.015	8.748	12.501	16.624	8.409	0.961
88.	43.61	8.531	0.004	0.107	0.015	8.657	12.371	15.654	7.918	0.915
89.	45.34	8.869	0.001	0.131	0.015	9.016	12.884	14.424	7.296	0.809
90.	44.85	8.773	0.0	0.107	0.015	8.895	12.711	14.241	7.203	0.810

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
91.	47.47	9.287	0.001	0.119	0.015	9.422	13.464	15.770	7.977	0.847
92.	55.55	10.867	-0.006	0.079	0.015	10.955	15.655	18.396	9.305	0.849
93.	45.89	8.978	-0.006	0.167	0.015	9.154	13.081	17.104	8.651	0.945
94.	45.39	8.879	0.003	0.150	0.015	9.047	12.928	17.526	8.865	0.980
95.	43.65	8.539	0.005	0.167	0.015	8.726	12.469	16.931	8.564	0.981
96.	42.82	8.376	0.001	0.171	0.015	8.563	12.237	16.542	8.367	0.977
97.	40.22	7.868	0.0	0.083	0.015	7.966	11.383	15.090	7.633	0.958
98.	42.51	8.316	0.003	0.124	0.015	8.458	12.086	16.623	8.408	0.994
99.	43.05	8.421	0.011	0.155	0.015	8.602	12.292	17.498	8.851	1.029
100.	43.61	8.531	0.003	0.114	0.015	8.663	12.379	17.137	8.668	1.001
101.	40.41	7.905	0.004	0.029	0.015	7.953	11.365	16.058	8.122	1.021
102.	41.55	8.128	0.0	0.095	0.015	8.238	11.772	16.491	8.341	1.013
103.	41.91	8.198	0.008	0.107	0.015	8.328	11.901	16.914	8.555	1.027
104.	43.95	8.597	-0.013	0.076	0.015	8.675	12.397	16.248	8.219	0.947
105.	43.74	8.556	-0.003	0.098	0.015	8.666	12.384	16.166	8.177	0.944
106.	44.52	8.709	0.0	0.107	0.015	8.831	12.619	16.755	8.475	0.960

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
107.	40.43	7.909	0.002	0.162	0.015	8.088	11.558	14.921	7.547	0.933
108.	34.82	6.811	0.001	0.098	0.015	6.925	9.896	11.287	5.709	0.824
109.	26.69	5.221	0.002	0.160	0.015	5.398	7.714	8.078	4.086	0.757
110.	36.50	7.140	0.0	0.017	0.015	7.172	10.249	13.483	6.820	0.951
111.	36.69	7.177	0.0	0.095	0.015	7.287	10.413	13.768	6.964	0.956
112.	36.23	7.087	0.002	0.086	0.015	7.190	10.275	14.023	7.093	0.987
113.	35.49	6.942	0.002	0.083	0.015	7.042	10.063	13.052	6.602	0.938
114.	38.39	7.510	0.005	0.079	0.015	7.609	10.873	14.453	7.311	0.961
115.	46.78	9.151	-0.002	0.040	0.015	9.204	13.153	18.047	9.128	0.992
116.	45.82	8.963	0.002	0.114	0.015	9.094	12.995	17.591	8.898	0.978
117.	44.07	8.621	-0.002	0.040	0.015	8.674	12.395	16.921	8.559	0.987
118.	40.35	7.893	0.007	0.074	0.015	7.989	11.416	14.312	7.239	0.906
119.	41.55	8.128	0.001	0.069	0.015	8.213	11.736	14.957	7.566	0.921
120.	46.70	9.135	0.003	0.081	0.015	9.234	13.195	17.172	8.686	0.941
121.	44.36	8.678	0.003	0.124	0.015	8.820	12.604	15.484	7.832	0.888

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
122.	36.36	7.113	0.0	0.090	0.015	7.218	10.315	14.566	7.368	1.021
123.	47.58	9.308	-0.002	0.050	0.015	9.371	13.391	19.406	9.816	1.047
124.	46.30	9.057	0.0	0.007	0.015	9.079	12.974	18.007	9.108	1.003
125.	47.69	9.329	0.005	0.055	0.015	9.404	13.438	18.080	9.145	0.972
126.	40.51	7.924	0.003	0.090	0.015	8.032	11.478	15.163	7.670	0.955
127.	41.52	8.122	0.008	0.102	0.015	8.247	11.785	15.850	8.017	0.972
128.	39.98	7.821	-0.002	0.112	0.015	7.946	11.355	15.362	7.770	0.978
129.	38.72	7.574	0.005	0.143	0.015	7.737	11.056	14.996	7.586	0.980
130.	40.37	7.897	0.001	0.114	0.015	8.027	11.471	15.873	8.029	1.000
131.	37.88	7.410	-0.001	0.174	0.015	7.598	10.858	16.751	8.473	1.115
132.	37.29	7.295	0.003	0.186	0.015	7.499	10.716	12.990	6.571	0.876
133.	37.29	7.295	-0.001	0.131	0.015	7.440	10.632	13.615	6.887	0.926
134.	38.18	7.469	0.011	0.167	0.015	7.662	10.949	15.349	7.764	1.013
135.	39.86	7.797	-0.010	0.136	0.015	7.938	11.343	16.258	8.224	1.036
136.	41.75	8.167	-0.005	0.095	0.015	8.272	11.821	15.872	8.028	0.971

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
137.	39.57	7.741	0.003	0.143	0.015	7.902	11.292	16.020	8.103	1.025
138.	40.86	7.993	0.004	0.155	0.015	8.167	11.671	15.863	8.024	0.982
139.	42.62	8.337	0.001	0.107	0.015	8.460	12.089	15.937	8.061	0.953
140.	40.54	7.930	0.001	0.095	0.015	8.041	11.491	13.380	6.768	0.842
141.	41.23	8.065	0.003	0.167	0.015	8.250	11.789	13.889	7.025	0.852
142.	38.54	7.539	0.002	0.155	0.015	7.711	11.019	12.177	6.159	0.799
143.	47.49	9.290	0.0	0.119	0.015	9.424	13.467	15.368	7.773	0.825
144.	39.15	7.658	0.006	0.155	0.015	7.834	11.195	15.966	8.076	1.031
145.	42.14	8.243	0.004	0.167	0.015	8.429	12.045	18.062	9.136	1.084
146.	43.42	8.494	-0.006	0.167	0.015	8.670	12.389	18.313	9.263	1.068
147.	43.47	8.504	-0.002	0.138	0.015	8.655	12.368	18.375	9.294	1.074
148.	42.57	8.327	-0.001	0.148	0.015	8.489	12.131	16.257	8.223	0.969
149.	46.08	9.014	-0.004	0.107	0.015	9.132	13.050	18.337	9.275	1.016
150.	49.21	9.626	0.009	0.124	0.015	9.774	13.967	19.957	10.095	1.033
151.	48.47	9.482	0.002	0.179	0.015	9.678	13.830	19.152	9.687	1.001

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
152.	49.45	9.673	0.001	0.199	0.015	9.888	14.130	20.551	10.395	1.051
153.	47.49	9.290	0.002	0.107	0.015	9.414	13.453	19.465	9.846	1.046
154.	47.60	9.311	-0.001	0.148	0.015	9.473	13.537	18.720	9.469	1.000
155.	47.25	9.243	0.0	0.088	0.015	9.346	13.355	18.902	9.561	1.023
156.	44.79	8.762	0.011	0.126	0.015	8.914	12.738	17.849	9.028	1.013
157.	47.45	9.282	-0.001	0.071	0.015	9.367	13.385	18.046	9.128	0.974
158.	47.20	9.233	-0.002	0.112	0.015	9.358	13.373	17.524	8.864	0.950
159.	43.25	8.460	0.003	0.079	0.015	8.557	12.228	17.127	8.663	1.012
160.	40.41	7.905	0.004	0.131	0.015	8.055	11.511	14.659	7.415	0.921
161.	37.47	7.330	0.0	0.102	0.015	7.447	10.642	13.344	6.750	0.906
162.	36.76	7.191	0.0	0.053	0.015	7.259	10.373	13.276	6.715	0.925
163.	40.26	7.876	0.001	0.105	0.015	7.997	11.428	14.606	7.388	0.924
164.	48.79	9.544	-0.002	0.074	0.015	9.631	13.763	20.945	10.594	1.100
165.	50.85	9.947	-0.005	0.057	0.015	10.014	14.310	21.235	10.741	1.073
166.	50.78	9.933	0.005	0.086	0.015	10.039	14.346	21.056	10.650	1.061

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)
167.	49.46	9.675	0.0	0.071	0.015	9.761	13.948	21.016	10.630	1.089
168.	49.40	9.664	0.0	0.067	0.015	9.746	13.927	19.548	9.888	1.015
169.	47.93	9.376	-0.001	0.052	0.015	9.442	13.493	18.169	9.190	0.973
170.	49.72	9.726	-0.001	0.083	0.015	9.823	14.037	17.138	8.669	0.883
171.	46.53	9.102	0.007	0.114	0.015	9.238	13.201	17.195	8.698	0.942
172.	46.27	9.051	0.002	0.088	0.015	9.156	13.084	17.499	8.851	0.967
173.	44.13	8.633	-0.001	0.095	0.015	8.742	12.492	16.784	8.490	0.971

TABLE (34)

FOOD CONSUMPTION

(a) Serial No.	(b) Food Box Wt.Diff. g.	(c) Dry Scattered Food g.	(d) Frame Wt. Increase Measured g.	(e) Frame Wt. Increase as Food. g.	(f) Funnel Wt. Increase Measured. g.	(g) Funnel Wt. Increase as Food. g.	(h) Total Wt. Food Consumed. g.
1.	7.638	2.972	2.971	2.347	0.190†	0.119	2.200
2.	12.114	2.493	2.493	1.969	0.190†	0.119	7.533
3.	12.489	0.173	0.172	0.136	0.190†	0.119	12.061
4.	11.935	0.128	0.127	0.100	0.190†	0.119	11.588
5.	14.930	1.442†	1.442†	1.140	0.133	0.119	12.229
6.	-	-	-	-	-	-	-
7.	10.884	0.118	0.117	0.092	0.159	0.100	10.574
8.	13.060	0.124	0.123	0.097	0.117	0.074	12.765
9.	8.298	0.116	0.116	0.092	0.143	0.090	8.000
10.	14.405	-	0.335	0.188*	0.127	0.080	14.137
11.	11.629	0.259	0.259	0.205	0.125	0.079	11.086
12.	6.511	0.287	0.287	0.227	0.139	0.088	5.909

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
13.	8.516	1.968	1.967	1.554	0.175	0.110	4.884
14.	14.554	1.254	1.253	0.990	0.113	0.071	12.239
15.	11.143	0.183	0.183	0.145	0.119	0.075	10.740
16.	11.395	0.114	0.114	0.090	0.086	0.054	11.137
17.	10.753	0.160	0.160	0.126	0.131	0.083	10.384
18.	12.023	0.144	0.144	0.114	0.108	0.068	11.697
19.	12.674	0.118	0.118	0.093	0.115	0.072	12.391
20.	10.005	0.286	0.285	0.225	0.182	0.115	9.379
21.	14.114	0.222	0.221	0.175	0.229	0.144	13.573
22.	15.024	1.363	1.362	1.076	0.225	0.142	12.443
23.	11.815	0.392	0.391	0.309	0.152	0.096	11.018
24.	12.956	0.248	0.248	0.196	0.167	0.105	12.407
25.	12.499	0.329	0.328	0.259	0.203	0.128	11.783
26.	13.526	0.222	0.221	0.175	0.175	0.110	13.019
27.	14.039	0.311	0.310	0.245	0.229	0.144	13.339
28.	14.019	0.181	0.180	0.142	0.177	0.112	13.584

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
29.	18.002	0.251	0.250	0.197	0.223	0.140	17.191
30.	11.652	-	-	-	-	-	-
31.	14.286	0.240	0.239	0.189	0.161	0.101	13.756
32.	-	-	-	-	-	-	-
33.	14.974	0.186	0.185	0.146	0.154	0.097	14.545
34.	12.066	0.282	0.281	0.222	0.214	0.135	11.427
35.	11.046	0.211	0.211	0.167	0.181	0.114	10.554
36.	10.193	2.116	2.116	1.672	0.207	0.130	6.275
37.	5.670	2.216	2.215	1.750	0.455	0.287	1.417
38.	10.298	0.437	0.437	0.345	0.201	0.127	9.389
39.	10.293	0.733	0.732	0.578	0.431	0.272	8.710
40.	11.953	0.827	0.828	0.654	0.502	0.316	10.156
41.	12.229	0.366	0.652	0.408*	0.360	0.254*	11.201
42.	15.582	1.395	3.404	2.846*	0.560	0.376*	10.965
43.	11.238	0.203	0.488	0.433*	0.374	0.237*	10.365
44.	15.686	1.858	1.899	1.470*	0.700	0.493*	11.865
45.	11.652	0.494	0.493	0.389	0.494	0.311	10.458

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
46.	10.238	0.359	0.566	0.398*	0.313	0.152*	9.329
47.	11.628	0.345	0.718	0.472*	0.435	0.243*	10.568
48.	13.954	0.507	0.749	0.696*	0.438	0.254*	12.497
49.	13.148	1.644	1.192	0.827*	0.489	0.243*	10.434
50.	15.377	2.116	1.382	1.173*	0.462	0.339*	11.749
51.	-	-	-	-	-	-	-
52.	16.083	0.728	0.842	0.762*	0.322	0.205*	14.388
53.	14.136	1.669	0.476	0.338*	0.326	0.217*	11.912
54.	5.024	0.147	0.431	0.306*	0.326	0.212*	4.359
55.	13.059	1.006	0.697	0.510*	0.260	0.190*	11.733
56.	10.681	0.390	0.502	0.425*	0.279	0.159*	9.707
57.	14.884	0.439	0.438	0.346	0.245	0.154	13.945
58.	10.441	0.341	0.341	0.269	0.191	0.120	9.711
59.	15.776	0.580	0.580	0.458	0.355	0.224	14.514

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
60.	15.644	1.183	1.183	0.935	0.458	0.289	13.237
61.	15.587	0.707	0.706	0.558	0.313	0.197	14.125
62.	14.429	0.867	0.866	0.684	0.377†	0.238	12.640
63.	17.217	1.510	1.509	1.192	0.254	0.160	14.355
64.	18.265	1.641	1.641	1.296	0.377†	0.238	15.090
65.	15.117	1.884	1.884	1.488	0.365	0.230	11.515
66.	18.119	2.648	2.648	2.092	0.291	0.183	13.196
67.	15.147	2.590	2.590	2.046	0.413	0.260	10.251
68.	19.771	1.727	1.726	1.364	0.183	0.115	16.565
69.	16.517	1.438	1.437	1.135	0.332	0.209	13.735
70.	16.905	1.028	1.026	0.811	0.230	0.145	14.921
71.	17.574	1.969	1.969	1.556	0.375	0.236	13.813
72.	32.440	6.473	6.472	5.113	0.322	0.203	20.651
73.	4.049	0.383	0.382	0.302	0.159	0.100	3.264
74.	11.231	0.921	0.921	0.728	0.268	0.169	9.413

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
75.	1.385	0.378	0.378	0.299	0.110	0.069	0.639
76.	0.473	0.131	0.130	0.103	0.200	0.126	0.113
77.	0.309	0.137	0.137	0.108	0.095	0.060	0.004
78.	13.014	1.229	2.667	2.080	0.163	0.103	9.602
79.	14.381	1.493	0.615	0.480	0.386	0.243	12.165
80.	15.191	2.685	0.832	0.649	0.222	0.140	11.717
81.	19.303	3.695	1.113	0.868	0.441	0.278	14.462
82.	16.924	2.957	2.056	1.604	0.195	0.123	12.240
83.	22.628	2.652	0.486	0.379	0.369	0.232	19.365
84.	20.874	2.266	0.987	0.770	0.286	0.180	17.658
85.	25.093	5.256	0.443	0.346	0.280	0.176	19.315
86.	28.407	5.347	1.004	0.783	0.335	0.211	22.066
87.	21.179	3.027	0.430	0.335	0.271	0.171	17.646
88.	19.747	0.030	5.348	2.723	0.731	0.461	16.533
89.	17.072	5.300	0.346	0.270	0.246	0.155	11.347
90.	17.798	6.029	0.758	0.591	0.252	0.159	11.019
91.	19.770	6.892	0.388	0.303	0.321	0.202	12.373

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
92.	18.261	1.208	1.904	1.485	3.017	1.517	14.051
93.	22.588	5.899	0.755	0.589	0.324	0.204	15.896
94.	23.359	5.130	0.457	0.356	0.217	0.137	17.736
95.	20.362	2.455	1.294	1.009	0.236	0.149	16.749
96.	18.547	1.200	0.878	0.685	0.266	0.168	16.494
97.	15.118	0.824	0.738	0.576	0.257	0.162	13.556
98.	18.683	0.437	0.335	0.261	0.192	0.121	17.864
99.	21.687	0.470	0.650	0.507	0.187	0.118	20.592
100.	17.736	0.371	0.223	0.174	0.166	0.105	17.086
101.	20.298	0.617	0.620	0.484	0.086	0.054	19.143
102.	20.629	0.178	0.159	0.124	0.150	0.094	20.233
103.	19.323	0.405	0.265	0.207	0.126	0.079	18.632
104.	16.477	0.176	0.661	0.516	0.072	0.045	15.740
105.	15.961	0.091	0.190	0.148	0.288	0.181	15.541
106.	17.837	0.089	0.269	0.210	0.141	0.089	17.449

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
107.	13.444	0.304	0.405	0.316	0.164	0.103	12.721
108.	4.781	0.802	0.336	0.262	0.122	0.077	3.640
109.	0.487	0.120	0.162	0.126	0.065	0.041	0.200
110.	13.759	1.527	0.469	0.366	0.235	0.148	11.718
111.	15.516	1.494	0.418	0.326	0.167	0.105	13.591
112.	16.908	0.996	0.368	0.304	0.194	0.101	15.507
113.	11.672	1.517	0.548	0.427	0.187	0.131	9.597
114.	16.533	1.677	0.550	0.429	0.332	0.209	14.218
115.	22.624	1.503	0.870	0.679	0.147	0.093	20.349
116.	18.927	1.245	0.262	0.204	0.153	0.096	17.382
117.	19.110	1.101	0.384	0.300	0.134	0.084	17.625
118.	14.120	2.231	0.494	0.385	0.198	0.125	11.379
119.	13.412	0.354	0.261	0.204	0.164	0.103	12.751
120.	16.675	0.955	0.445	0.347	0.356	0.224	15.149
121.	12.324	1.777	0.479	0.374	0.294	0.185	9.988

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
122.	18.318	0.984	0.558	0.435	0.250	0.157	16.742
123.	21.904	0.550	1.410	1.100	0.151	0.095	20.159
124.	18.069	0.101	0.794	0.619	0.197	0.124	17.225
125.	19.264	0.532	0.494	0.385	0.270	0.170	18.177
126.	13.620	0.831	0.572	0.446	0.113	0.071	12.272
127.	15.261	0.181	0.450	0.351	0.207	0.130	14.599
128.	16.011	0.102	0.329	0.257	0.089	0.056	15.596
129.	30.092	11.309	2.277	1.776	0.317	0.200	16.807
130.	26.190	7.167	1.323	1.032	0.550	0.346	17.645
131.	17.323	1.397	1.601	1.249	0.288	0.181	14.496
132.	15.943	2.814	0.825	0.643	0.394	0.248	12.338
133.	16.977	2.901	1.842	1.437	0.388	0.244	12.395
134.	25.470	3.841	1.901	1.483	0.389	0.245	19.901
135.	19.804	0.187	2.593	2.023	0.312	0.197	17.397
136.	19.247	0.457	2.894	2.257	0.317	0.200	16.333
137.	24.408	1.602	1.674	1.306	0.222	0.140	21.360

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
138.	17.750	0.768	0.769	0.600	0.285	0.180	16.202
139.	19.240	2.273	1.090	0.850	0.204	0.129	15.988
140.	9.447	1.437	0.338	0.264	0.195	0.123	7.653
141.	12.753	2.615	1.223	0.954	0.270	0.170	9.014
142.	13.221	7.875	0.560	0.437	0.320	0.202	4.707
143.	16.793	6.268	1.677	1.308	0.291	0.183	9.034
144.	26.397	7.444	3.330	2.597	0.452	0.285	16.071
145.	31.670	7.744	0.960	0.749	0.464	0.292	22.885
146.	30.247	3.245	5.422	4.229	0.443	0.279	22.494
147.	28.991	5.494	1.750	1.365	0.504	0.318	21.814
148.	24.263	6.218	3.379	2.636	0.372	0.234	15.175
149.	33.524	11.353	2.462	1.920	0.351	0.221	20.030
150.	30.314	5.649	5.563	3.332	0.293	0.185	21.148
151.	36.842	7.327	7.415	5.784	0.656	0.413	23.318
152.	31.079	3.257	3.871	3.019	0.362	0.228	24.575
153.	29.427	4.835	2.029	1.583	0.440	0.277	22.732

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
154.	26.209	3.271	1.941	1.514	0.547	0.345	21.079
155.	23.962	1.225	2.955	2.305	0.313	0.197	20.235
156.	24.240	3.355	0.738	0.576	0.329	0.207	20.102
157.	20.668	1.482	0.573	0.447	0.211	0.133	18.606
158.	18.751	0.826	0.689	0.537	0.210	0.132	17.256
159.	24.196	5.145	2.291	1.787	0.378	0.238	17.026
160.	13.393	3.392	0.672	0.524	0.399	0.251	9.226
161.	16.209	4.269	0.981	0.765	0.137	0.086	11.089
162.	16.750	4.234	0.963	0.751	0.202	0.127	11.638
163.	21.073	6.577	1.640	1.279	0.199	0.125	13.092
164.	34.059	4.354	6.105	4.762	0.293	0.185	24.758
165.	33.188	6.452	2.834	2.211	0.357	0.225	24.300
166.	33.166	5.484	5.127	3.999	0.319	0.201	23.482
167.	38.107	10.442	2.966	2.313	0.454	0.286	25.066
168.	29.810	6.234	2.224	1.735	0.247	0.156	21.685
169.	23.064	4.680	1.263	0.985	0.431	0.272	17.127

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
170.	16.906	4.163	1.269	0.990	0.193	0.122	11.631
171.	22.774	6.641	0.918	0.716	0.369	0.232	15.185
172.	21.233	2.919	0.555	0.433	0.265	0.167	17.714
173.	23.373	6.385	1.880	1.466	0.453	0.285	15.237

* Moisture measured directly cf Table (30)

† Weight not measured directly – mean values used.

TABLE (35)

ENERGY BALANCE

(a) Serial No.	(b) Gross Food Energy Cal.	(c) Gross Faecal Energy Cal.	(d) Urine Energy Loss Cal.	(e) Energy Expenditure 3.844 x Litres O_2 Cal.	(f) Energy Expenditure 1.191 x Litres CO_2 Cal.	(g) Energy Expenditure 1.996 x g.Urine N Cal.	(h) Total Energy Cal.	(i) Total Energy Expend /24 hr. Cal.	(j) Energy Balance Cal.
1.	8.877	-	0.826	18.378	4.269	0.192	22.455	23.016	-24.965
2.	30.396	-	1.514	18.213	4.816	0.351	22.678	29.686	-10.804
3.	48.666	10.735	2.081	22.249	6.524	0.483	28.290	28.601	+ 7.249
4.	46.758	10.849	2.021	22.253	6.472	0.469	28.256	29.810	+ 4.078
5.	29.344	-	-	20.961	-	-	-	-	-
6.	-	-	-	-	-	-	-	-	-
7.	42.666	9.110	1.892	22.484	6.526	0.439	28.571	29.914	+ 1.750
8.	51.507	10.849	2.176	24.198	7.106	0.505	30.799	31.692	+ 6.790
9.	32.280	9.488	1.565	21.657	6.110	0.363	27.404	27.979	+ 6.752
10.	57.043	-	2.348	24.828	7.338	0.545	31.621	32.412	+12.283
11.	44.732	11.416	1.961	22.184	6.522	0.455	28.251	28.844	+ 2.511
12.	23.843	7.560	1.299	18.732	5.019	0.301	23.450	23.450	- 8.466

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
13.	19.707	5.179	1.170	18.444	4.747	0.271	22.920	27.160	+13.802
14.	49.384	7.409	2.107	25.086	7.130	0.489	31.727	32.647	+ 7.221
15.	43.336	8.618	1.918	23.272	6.572	0.445	29.399	30.075	+ 2.725
16.	44.938	12.34	1.969	24.198	6.912	0.457	30.653	30.990	- 0.155
17.	41.899	8.392	1.875	23.141	6.494	0.435	29.200	29.521	+ 2.111
18.	47.197	11.983	2.038	23.575	6.782	0.473	29.884	30.213	+ 2.963
19.	49.998	11.907	2.124	22.814	6.822	0.493	29.143	29.464	+ 6.503
20.	37.844	10.811	1.746	23.118	6.671	0.405	29.384	29.913	- 4.626
21.	54.767	14.137	2.279	24.210	6.446	0.529	30.127	30.549	+ 7.802
22.	50.208	10.773	2.133	24.113	6.831	0.495	30.449	30.997	+ 6.305
23.	44.458	12.209	1.952	23.172	6.139	0.453	28.858	28.887	+ 1.410
24.	50.062	13.532	2.133	23.006	6.568	0.495	29.079	29.486	+ 4.911
25.	47.544	12.058	2.047	22.149	6.058	0.475	27.732	28.425	+ 5.014
26.	52.532	13.419	2.210	23.779	6.656	0.513	29.922	30.251	+ 6.652
27.	53.823	14.440	2.245	23.302	6.762	0.521	29.543	29.750	+ 7.388
28.	54.811	16.254	2.279	23.318	6.763	0.529	29.552	29.966	+ 6.312

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
29.	69.366	15.763	2.743	23.345	6.301	0.637	29.009	30.256	+20.604
30.									
31.	55.505	13.381	2.305	23.783	7.025	0.535	30.273	31.484	+ 8.335
32.									
33.	58.689	14.515	2.399	26.047	7.214	0.559	32.702	32.702	+ 9.073
34.	46.108	12.323	2.004	24.579	7.067	0.465	31.181	31.836	- 0.055
35.	42.585	12.247	1.892	26.201	6.910	0.439	32.672	32.345	- 3.899
36.	25.320	5.783	1.342	24.990	6.316	0.311	30.995	31.987	-13.792
37.	5.718	-	0.722	25.713	5.644	0.168	31.189	32.093	-31.097
38.	37.885	9.979	1.746	21.280	6.099	0.405	26.974	26.974	- 0.814
39.	35.145	9.412	1.659	21.150	5.818	0.385	26.583	27.540	- 3.466
40.	40.979	10.093	1.840	25.528	7.020	0.427	32.121	32.474	- 3.428
41.	46.574	11.866	1.978	16.921	5.449	0.459	21.911	22.437	+10.293
42.	45.592	7.704	1.944	18.670	5.739	0.451	23.958	24.725	+11.219
43.	43.098	13.184	1.866	19.255	5.792	0.433	24.614	24.836	+ 3.212
44.	49.335	9.970	2.064	20.004	5.975	0.479	25.500	25.730	+11.571

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
45.	43.484	9.723	1.883	20.239	6.329	0.437	26.131	26.131	+ 5.747
46.	38.790	9.352	1.737	22.707	6.559	0.403	28.863	29.065	- 1.364
47.	43.842	8.240	1.892	22.376	6.554	0.439	29.369	30.544	+ 3.266
48.	51.963	11.042	2.141	23.068	6.590	0.497	29.161	29.569	+ 9.211
49.	43.385	11.660	1.875	22.238	6.142	0.435	27.945	28.448	+ 1.402
50.	48.852	7.540	2.047	23.798	6.871	0.475	30.194	30.949	+ 8.316
51.	-	-	-	-	-	-	-	-	-
52.	49.825	14.173	2.382	22.345	6.884	0.553	28.676	28.991	+14.279
53.	49.530	11.907	2.064	22.264	7.038	0.479	28.823	29.227	+ 6.332
54.	18.125	4.532	1.101	19.923	5.287	0.255	24.955	25.230	-12.738
55.	48.786	7.416	2.047	22.660	6.263	0.475	28.448	28.647	+10.676
56.	40.362	8.652	1.780	22.165	6.369	0.413	28.121	28.937	- 0.007
57.	57.983	6.551	2.322	22.238	6.598	0.539	28.297	29.316	+19.794
58.	40.378	8.158	1.780	22.845	6.729	0.413	29.161	29.248	+ 1.192
59.	60.349	11.824	2.399	23.652	6.854	0.557	29.949	30.039	+16.087
60.	55.039	9.723	2.236	24.133	7.562	0.519	31.176	31.299	+16.557

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
61.	58.732	8.528	2.348	24.321	7.429	0.545	31.205	31.299	+16.557
62.	52.557	15.738	2.159	21.865	6.660	0.501	28.024	28.052	+ 6.608
63.	59.688	9.476	2.382	24.682	7.515	0.553	31.644	34.144	+13.686
64.	62.744	11.289	2.477	22.510	6.804	0.575	28.739	34.630	+14.348
65.	47.879	10.218	2.012	21.104	6.079	0.467	26.716	33.181	+ 2.468
66.	54.869	7.540	2.227	26.189	7.298	0.517	32.970	32.970	+12.132
67.	42.624	8.652	1.858	25.532	6.674	0.431	31.775	32.220	- 0.106
68.	68.877	13.390	2.657	27.907	8.250	0.617	35.540	36.500	+16.320
69.	57.110	10.506	2.296	27.784	8.250	0.533	35.501	37.028	+ 7.280
70	62.042	10.382	2.451	29.568	8.628	0.569	37.627	36.611	+12.598
71.	57.434	13.761	2.313	28.488	8.001	0.537	35.952	35.592	+ 5.768
72.	85.867	-	3.182	27.335	7.693	0.739	34.289	36.175	
73.	13.572	7.457	1.170	22.987	5.325	0.271	28.041	27.368	-22.423
74.	39.139	4.408	1.475	27.465	7.644	0.341	34.768	36.020	- 2.764
75.	2.657	3.502	0.869	25.497	5.355	0.202	30.650	30.650	-32.364

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
76.	0.470	1.112	0.890	23.291	5.346	0.207	28.430	28.743	-30.275
77.	0.017	0.494	0.550	21.499	3.714	0.128	25.085	25.160	-26.187
78.	39.925	11.618	1.514	24.444	7.103	0.351	31.196	33.785	- 6.992
79.	50.582	8.528	2.348	31.890	9.090	0.545	40.435	39.465	+ 0.241
80.	48.719	7.993	2.322	29.184	8.143	0.539	36.788	37.193	+ 1.211
81.	60.133	10.877	2.270	28.392	8.682	0.527	36.547	37.205	+ 9.781
82.	50.894	10.794	2.528	25.540	7.375	0.587	32.328	35.270	+ 2.302
83.	79.029	15.532	2.924	33.889	9.976	0.679	43.186	44.568	+16.005
84.	72.062	14.008	3.053	33.362	9.822	0.709	42.475	43.834	+11.167
85.	78.825	18.828	2.984	31.759	9.234	0.693	40.300	43.967	+13.046
86.	90.051	15.120	3.062	34.638	10.407	0.711	44.334	45.132	+26.737
87.	72.013	17.634	2.941	33.627	10.015	0.683	42.959	43.432	+ 8.006
88.	67.471	15.574	2.924	33.278	9.430	0.679	42.029	42.029	+ 6.944
89.	46.307	10.382	2.167	34.658	8.690	0.503	42.845	43.745	- 9.987
90.	44.969	7.292	1.376	34.192	8.579	0.319	42.452	42.919	- 6.618

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
91.	50.494	8.817	1.247	36.218	9.501	0.289	45.430	45.930	- 5.500
92.	57.342	10.671	1.789	42.111	11.082	0.415	52.778	56.314	-11.432
93.	63.822	12.113	2.623	35.188	10.303	0.609	44.882	46.991	+ 2.095
94.	71.210	13.060	3.294	34.777	10.558	0.764	44.571	45.195	+ 9.661
95.	67.247	13.102	3.225	33.543	10.200	0.749	42.994	43.596	+ 7.324
96.	66.223	12.607	3.191	32.916	9.965	0.741	42.140	42.604	+ 7.821
97.	54.427	12.442	2.657	30.621	9.091	0.617	39.095	42.653	- 3.325
98.	71.724	19.776	2.614	32.513	10.014	0.607	41.920	43.597	- 5.737
99.	82.677	17.798	2.597	33.066	10.542	0.603	43.005	43.478	+18.804
100.	68.600	15.203	2.587	33.301	10.324	0.601	43.024	43.497	+ 7.313
101.	76.859	16.892	2.528	30.571	9.673	0.587	39.657	41.997	+15.442
102.	81.235	14.379	2.666	31.667	9.934	0.619	40.982	42.007	+22.183
103.	74.807	17.716	2.709	32.013	10.189	0.629	41.573	42.155	+12.227
104.	63.196	15.368	2.804	33.347	9.789	0.651	42.485	42.358	+ 2.666
105.	62.397	13.225	2.348	33.312	9.739	0.545	42.506	41.486	+ 5.338

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
106.	70.006	12.813	2.554	33.946	10.094	0.593	43.447	44.055	+10.584
107.	51.138	8.487	2.331	31.090	8.988	0.541	39.537	40.960	- 0.640
108.	14.633	5.562	1.548	26.620	6.799	0.359	33.060	33.159	-25.636
109.	0.804	2.266	0.585	20.750	4.866	0.136	25.480	27.901	-29.948
110.	47.106	9.023	2.047	27.569	8.123	0.475	35.217	35.217	+ 0.819
111.	54.636	8.487	2.021	28.011	8.294	0.469	35.836	35.836	+ 8.292
112.	62.338	10.836	2.528	27.638	8.448	0.587	35.499	35.919	+12.055
113.	38.580	7.910	1.772	27.069	7.863	0.411	34.521	34.625	- 0.727
114.	57.156	10.135	2.374	29.249	8.707	0.551	37.405	36.507	+ 8.140
115.	80.704	12.030	3.466	35.380	10.871	0.804	45.447	44.993	+20.215
116.	68.937	13.678	3.371	34.957	10.598	0.782	44.773	43.698	+ 8.190
117.	69.901	13.184	3.406	33.343	10.194	0.790	42.747	43.345	+ 9.966
118.	53.232	7.540	2.030	30.710	8.622	0.471	38.861	38.861	- 4.801
119.	50.570	9.723	2.055	31.571	9.011	0.477	40.105	40.666	- 1.874
120.	60.081	11.042	2.477	35.495	10.345	0.575	42.265	43.907	+ 2.655

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
121.	39.612	8.075	2.012	33.904	9.328	0.467	42.765	44.005	-14.480
122.	66.399	15.697	2.976	27.746	8.775	0.691	35.830	43.354	+ 4.372
123.	79.951	15.780	3.019	36.022	11.691	0.701	47.012	43.533	+17.619
124.	68.314	14.544	2.993	34.900	10.848	0.695	45.053	46.360	+ 4.417
125.	72.090	13.678	2.606	36.149	10.892	0.605	46.436	45.786	+10.020
126.	48.671	9.270	2.141	30.875	9.135	0.497	39.513	41.528	- 4.268
127.	59.900	10.424	2.477	31.701	9.548	0.575	40.674	40.105	+ 4.894
128.	61.854	11.948	2.614	30.544	9.258	0.607	39.191	41.190	- 6.102
129.	68.589	15.780	2.632	29.741	9.035	0.611	38.165	37.898	+12.279
130.	72.009	14.420	2.657	30.856	9.563	0.617	39.802	39.683	+15.249
131.	59.158	14.750	2.743	29.207	10.091	0.637	38.661	40.633	+ 1.032
132.	50.351	11.289	2.159	28.826	7.826	0.501	36.151	36.657	+ 0.246
133.	50.584	11.824	2.124	28.599	8.202	0.493	36.308	37.216	- 0.580
134.	81.216	17.881	2.933	29.453	9.247	0.681	38.019	40.414	+19.988
135.	70.997	14.750	2.313	30.514	9.795	0.537	39.772	40.687	+13.247

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
136.	66.655	16.192	2.589	31.798	9.561	0.601	40.758	40.758	+ 7.116
137.	85.760	14.832	2.855	30.375	9.651	0.663	39.363	42.945	+25.128
138.	65.051	15.079	2.313	31.394	9.557	0.537	40.414	41.707	+ 5.952
139.	64.192	13.596	2.159	32.520	9.601	0.501	41.620	42.369	+ 6.068
140.	30.727	7.457	1.617	30.910	8.061	0.375	38.596	39.098	-17.445
141.	36.191	8.817	0.662	31.713	8.367	0.154	39.926	40.046	-13.334
142.	18.899	3.749	0.593	29.641	7.335	0.138	36.838	37.354	-22.797
143.	36.272	7.045	0.834	36.226	9.258	0.194	45.290	40.127	-11.734
144.	64.525	20.476	3.320	30.114	9.619	0.770	38.963	39.664	+ 1.065
145.	91.883	17.716	3.234	32.401	10.881	0.750	42.532	42.660	+28.273
146.	90.313	18.046	3.354	33.327	11.032	0.778	43.581	44.060	+24.853
147.	87.583	18.581	3.337	33.270	11.069	0.774	43.565	43.870	+22.100
148.	60.928	15.326	2.752	32.632	9.794	0.639	41.787	42.247	+ 0.603
149.	80.420	18.416	3.268	35.103	11.047	0.758	45.392	47.707	+11.029
150.	84.909	19.158	2.958	37.571	12.023	0.687	48.907	48.907	+12.886

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
151.	93.738	22.124	2.976	37.202	11.537	0.691	48.048	47.087	+21.551
152.	98.792	16.109	3.199	38.009	12.380	0.743	49.646	49.994	+29.490
153.	91.383	15.450	3.388	36.187	11.727	0.786	47.128	48.825	+23.720
154.	84.738	13.514	3.130	36.414	11.278	0.727	46.965	47.294	+20.800
155.	81.345	15.656	2.924	35.926	11.387	0.679	46.634	47.147	+15.618
156.	80.810	12.772	3.019	34.265	10.752	0.701	44.316	45.247	+19.772
157.	74.796	14.708	2.477	36.007	10.871	0.575	46.303	45.377	+12.234
158.	69.369	12.442	3.225	35.972	10.557	0.749	45.780	45.322	+ 8.380
159.	68.445	11.948	2.081	32.893	10.318	0.483	42.728	42.856	+11.560
160.	37.089	11.660	2.116	30.963	8.831	0.491	39.303	40.286	-16.973
161.	44.578	7.210	2.227	28.626	8.039	0.517	36.148	36.907	- 1.766
162.	46.785	6.551	2.408	27.904	7.998	0.559	35.343	37.004	+ 0.822
163.	52.530	8.982	2.270	30.740	8.799	0.527	39.012	37.334	+ 4.044
164.	99.527	19.817	4.197	37.022	12.617	0.974	48.665	49.346	+26.185
165.	97.686	18.499	4.412	38.494	12.793	1.024	50.263	50.816	+23.959

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
166.	94.398	18.293	4.180	38.590	12.684	0.970	50.304	49.952	+21.973
167.	100.765	18.634	4.515	37.521	12.660	1.048	49.133	48.445	+30.171
168.	87.174	13.678	3.801	37.464	11.777	0.882	48.359	48.698	+20.997
169.	68.851	13.596	3.311	36.295	10.945	0.768	46.472	47.820	+ 4.124
170.	46.757	9.435	2.563	37.760	10.325	0.595	47.490	47.490	-12.731
171.	61.044	8.570	2.933	35.511	10.359	0.681	45.189	44.421	+ 5.120
172.	71.210	11.660	2.726	35.196	10.542	0.633	45.105	44.970	+11.854
173.	61.253	12.484	2.589	33.604	10.112	0.601	43.115	45.831	+ 0.349

TABLES (36) AND (37)

TABLE (36)

RESPIRATORY QUOTIENT

TABLE (37)

TIMES OF RUN

(a) Serial No.	(b) O_2 for Protein Metab. Litres	(c) CO_2 for Protein Metab. Litres	(d) Non-Prot. O_2 Litres	(e) Non-Prot. CO_2 Litres	(f) Non-Prot. R.Q.	(g) Time of Run Hr.Min	(h) Time of Run Hr.	(i) Conversion Factor to per 24 hr.
1.	0.570	0.457	4.211	3.127	0.743	23.25	23.42	1.025
2.	1.046	0.839	3.692	3.204	0.868	18.20	18.33	1.309
3.	1.438	1.153	4.350	4.324	0.994	23.45	23.75	1.011
4.	1.396	1.120	4.393	4.314	0.982	22.45	22.75	1.055
5.	-	-	-	-	-	23.10	23.11	1.036
6.	-	-	-	-	-	23.00	23.00	1.043
7.	1.307	1.048	4.542	4.431	0.976	22.55	22.92	1.047
8.	1.503	1.206	4.792	4.760	0.993	23.20	23.33	1.029
9.	1.081	0.867	4.553	4.263	0.936	23.30	23.50	1.021
10.	1.622	1.301	4.837	4.860	1.005	23.25	23.42	1.025
11.	1.355	1.086	4.416	4.390	0.994	23.30	23.50	1.021

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
12.	0.897	0.720	3.976	3.494	0.879	24.00	24.00	1.000
13.	0.808	0.648	3.990	3.338	0.837	20.15	20.25	1.185
14.	1.455	1.167	5.071	4.819	0.950	23.20	23.33	1.029
15.	1.325	1.063	4.729	4.455	0.942	23.28	23.47	1.023
16.	1.360	1.091	4.935	4.712	0.955	23.45	23.75	1.011
17.	1.295	1.039	4.725	4.413	0.934	23.45	23.75	1.011
18.	1.408	1.129	4.725	4.565	0.966	23.45	23.75	1.011
19.	1.467	1.177	4.468	4.551	1.019	23.45	23.75	1.011
20.	1.206	0.967	4.808	4.634	0.964	23.35	23.58	1.018
21.	1.574	1.263	4.724	4.149	0.878	23.40	23.67	1.014
22.	1.473	1.182	4.800	4.553	0.949	23.35	23.58	1.018
23.	1.349	1.082	4.679	4.072	0.870	23.58	23.97	1.001
24.	1.473	1.182	4.512	4.332	0.960	23.40	23.67	1.014
25.	1.414	1.134	4.348	3.952	0.909	23.25	23.42	1.025
26.	1.527	1.225	4.659	4.363	0.936	23.45	23.75	1.011
27.	1.551	1.244	4.511	4.434	0.983	23.50	23.83	1.007
28.	1.574	1.263	4.492	4.415	0.983	23.40	23.67	1.014

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
29.	1.895	1.520	4.178	3.770	0.902	23.00	23.00	1.043
30.	-	-	-	-	-	19.25	19.42	1.236
31.	1.592	1.277	4.595	4.621	1.006	23.05	23.08	1.040
32.	-	-	-	-	-	-	-	-
33.	1.658	1.329	5.118	4.728	0.924	24.00	24.00	1.000
34.	1.384	1.110	5.010	4.823	0.963	23.30	23.50	1.021
35.	1.307	1.048	5.509	4.754	0.863	24.15	24.25	0.990
36.	0.927	0.743	5.574	4.560	0.818	23.15	23.25	1.032
37.	0.499	0.400	6.190	4.339	0.701	23.20	23.33	1.029
38.	1.206	0.967	4.330	4.154	0.959	24.00	24.00	1.000
39.	1.147	0.920	4.355	3.965	0.910	23.10	23.17	1.036
40.	1.271	1.020	5.370	4.874	0.908	23.45	23.75	1.011
41.	1.366	1.096	3.036	3.479	1.146	23.27	23.45	1.024
42.	1.343	1.077	3.514	3.742	1.065	23.15	25.25	1.032
43.	1.289	1.034	3.720	3.829	1.029	23.47	23.78	1.009
44.	1.426	1.144	3.778	3.873	1.025	23.47	23.78	1.009
45.	1.301	1.044	3.964	4.270	1.077	24.00	24.00	1.000

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
46.	1.200	0.963	4.707	4.544	0.965	23.50	23.83	1.007
47.	1.307	1.048	4.514	4.455	0.987	23.05	23.08	1.040
48.	1.479	1.186	4.522	4.347	0.961	23.40	23.67	1.014
49.	1.295	1.039	4.490	4.118	0.917	23.35	23.58	1.018
50.	1.414	1.134	4.777	4.635	0.970	23.25	23.42	1.025
51.	-	-	-	-	-	-	-	-
52.	1.646	1.320	4.167	4.460	1.070	23.45	23.75	1.011
53.	1.426	1.144	4.366	4.765	1.091	23.40	23.67	1.014
54.	0.760	0.610	4.423	3.829	0.866	23.45	23.75	1.011
55.	1.414	1.134	4.481	4.125	0.921	23.50	23.83	1.007
56.	1.230	0.986	4.536	4.362	0.962	23.20	23.33	1.029
57.	1.604	1.287	4.181	4.253	1.017	23.10	23.17	1.036
58.	1.230	0.986	4.713	4.664	0.989	23.55	23.92	1.003
59.	1.658	1.329	4.495	4.426	0.985	24.55	24.92	1.003

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
60.	1.545	1.239	4.733	5.110	1.080	22.55	22.92	1.003
61.	1.622	1.301	4.705	4.937	1.049	23.55	23.92	1.003
62.	1.491	1.196	4.197	4.396	1.047	23.58	23.97	1.001
63.	1.646	1.320	4.775	4.990	1.045	22.15	22.25	1.079
64.	1.711	1.372	4.145	4.341	1.047	19.55	19.92	1.205
65.	1.390	1.115	4.100	3.989	0.973	19.20	19.33	1.242
66.	1.539	1.234	5.274	4.894	0.928	24.00	24.00	1.000
67.	1.283	1.029	5.359	4.575	0.854	23.40	23.67	1.014
68.	1.836	1.472	5.424	5.455	1.006	23.22	23.37	1.027
69.	1.586	1.272	5.642	5.655	1.002	23.00	23.00	1.043
70.	1.693	1.358	5.999	5.886	0.981	24.40	24.67	0.973
71.	1.598	1.282	5.813	5.436	0.935	24.15	24.25	0.990
72.	2.198	1.763	4.913	4.696	0.956	22.45	22.75	1.055
73.	0.808	0.648	5.172	3.823	0.739	24.35	24.58	0.976
74.	1.016	0.815	6.129	5.603	0.914	23.10	23.17	1.036
75.	0.600	0.481	6.033	4.015	0.665	24.00	24.00	1.000

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
76.	0.614	0.494	5.445	3.995	0.734	23.45	23.75	1.011
77.	0.380	0.305	5.213	2.813	0.540	23.55	23.92	1.003
78.	1.046	0.839	5.313	5.125	0.965	22.10	22.17	1.083
79.	1.622	1.301	6.634	6.331	0.954	24.35	24.58	0.976
80.	1.604	1.287	5.988	5.550	0.927	23.45	23.75	1.011
81.	1.568	1.258	5.818	6.032	1.037	23.35	23.58	1.018
82.	1.747	1.401	4.897	4.791	0.978	22.00	22.00	1.091
83.	2.020	1.620	6.796	6.756	0.994	23.15	23.25	1.032
84.	2.109	1.692	6.570	6.555	0.998	23.15	23.25	1.032
85.	2.062	1.653	6.200	6.100	0.984	22.00	22.00	1.091
86.	2.115	1.696	6.896	7.042	1.021	23.35	23.58	1.018
87.	2.032	1.630	6.716	6.779	1.009	23.45	23.75	1.011
88.	2.020	1.620	6.637	6.298	0.949	24.00	24.00	1.000
89.	1.497	1.201	7.519	6.095	0.811	23.30	23.50	1.021
90.	0.951	0.762	7.944	6.441	0.811	23.45	23.75	1.011
91.	0.861	0.691	8.561	7.286	0.851	23.45	23.75	1.011
92.	1.236	0.991	9.719	8.314	0.855	22.30	22.50	1.067

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
93.	1.812	1.453	7.342	7.198	0.980	22.55	22.92	1.047
94.	2.275	1.825	6.772	7.040	1.040	23.40	23.67	1.014
95.	2.228	1.787	6.498	6.777	1.043	23.40	23.67	1.014
96.	2.204	1.768	6.359	6.599	1.038	23.45	23.75	1.011
97.	1.836	1.472	6.130	6.161	1.005	22.00	22.00	1.091
98.	1.806	1.449	6.652	6.959	1.046	23.05	23,08	1.040
99.	1.794	1.439	6.808	7.412	1.089	23.45	23.75	1.011
100.	1.788	1.434	6.875	7.234	1.052	23.45	23.75	1.011
101.	1.747	1.401	6.206	6.721	1.083	22.40	22.67	1.059
102.	1.842	1.477	6.396	6.864	1.073	23.25	23.42	1.025
103.	1.871	1.501	6.457	7.054	1.092	23.40	23.67	1.014
104.	1.937	1.553	6.738	6.666	0.989	24,05	24.08	0.997
105.	1.622	1.301	7.044	6.876	0.976	24.35	24.58	0.976
106.	1.764	1.415	7.067	7.060	0.999	23.40	23.67	1.014
107.	1.610	1.291	6.478	6.256	0.966	23.10	23.17	1.036

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
108.	1.069	0.858	5.856	4.851	0.828	23.55	23.92	1.003
109.	0.404	0.324	4.994	3.762	0.753	21.55	21.92	1.095
110.	1.414	1.134	5.758	5.686	0.987	24.00	24.00	1.000
111.	1.396	1.120	5.891	5.844	0.992	24.00	24.00	1.000
112.	1.747	1.401	5.443	5.692	1.046	23.05	23.08	1.040
113.	1.224	0.982	5.818	5.620	0.966	23.55	23.92	1.003
114.	1.640	1.315	5.969	5.996	1.005	24.35	24.58	0.976
115.	2.394	1.920	6.810	7.208	1.058	24.15	24.25	0.990
116.	2.329	1.868	6.765	7.030	1.039	24.35	24.58	0.976
117.	2.353	1.887	6.321	6.672	1.056	23.40	23.67	1.014
118.	1.402	1.125	6.587	6.114	0.928	24.00	24.00	1.000
119.	1.420	1.139	6.793	6.427	0.946	23.40	23.67	1.014
120.	1.711	1.372	7.523	7.314	0.972	24.45	24.75	0.970
121.	1.390	1.115	7.430	6.717	0.904	23.20	23.33	1.029
122.	2.056	1.649	5.162	5.719	1.108	19.50	19.83	1.210

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
123.	2.085	1.673	7.286	8.143	1.118	25.55	25.92	0.926
124.	2.067	1.658	7.012	7.450	1.062	23.20	23.33	1.029
125.	1.800	1.444	7.604	7.701	1.013	24.20	24.33	0.986
126.	1.479	1.186	6.553	6.484	0.989	22.50	22.83	1.051
127.	1.711	1.372	6.536	6.645	1.017	24.20	24.33	0.986
128.	1.806	1.449	6.140	6.321	1.029	22.50	22.83	1.051
129.	1.818	1.458	5.919	6.128	1.035	24.10	24.17	0.993
130.	1.836	1.472	6.191	6.557	1.059	24.05	24.08	0.997
131.	1.895	1.520	5.703	6.953	1.219	22.50	22.83	1.051
132.	1.491	1.196	6.008	5.375	0.895	23.40	23.67	1.014
133.	1.467	1.177	5.973	5.710	0.956	23.25	23.42	1.025
134.	2.026	1.625	5.636	6.139	1.089	22.35	22.58	1.063
135.	1.598	1.282	6.340	6.942	1.095	23.27	23.45	1.023
136.	1.788	1.434	6.484	6.594	1.017	24.00	24.00	1.000
137.	1.972	1.582	5.930	6.521	1.100	22.00	22.00	1.091

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
138.	1.598	1.282	6.569	6.742	1.026	23.15	23.25	1.032
139.	1.491	1.196	6.969	6.865	0.985	23.35	23.58	1.018
140.	1.117	0.896	6.924	5.872	0.848	23.42	23.70	1.013
141.	0.457	0.367	7.793	6.658	0.854	23.55	23.92	1.003
142.	0.410	0.329	7.301	5.830	0.799	23.40	23.67	1.014
143.	0.576	0.462	8.848	7.311	0.826	27.5	27.08	0.886
144.	2.293	1.839	5.541	6.237	1.126	23.35	23.58	1.018
145.	2.234	1.792	6.195	7.344	1.185	23.55	23.92	1.003
146.	2.317	1.858	6.353	7.405	1.166	23.45	23.75	1.011
147.	2.305	1.849	6.350	7.445	1.172	23.50	23.83	1.007
148.	1.901	1.525	6.588	6.698	1.017	23.45	23.75	1.011
149.	2.258	1.811	6.874	7.464	1.086	22.50	22.83	1.051
150.	2.044	1.639	7.730	8.456	1.094	24.00	24.00	1.000
151.	2.056	1.649	7.622	8.038	1.055	24.30	24.50	0.980
152.	2.210	1.773	7.678	8.622	1.123	23.50	23.83	1.007
153.	2.341	1.877	7.073	7.969	1.127	23.10	23.17	1.036

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
154.	2.163	1.734	7.310	7.735	1.058	23.50	23.83	1.007
155.	2.020	1.620	7.326	7.941	1.084	23.45	23.75	1.011
156.	2.085	1.673	6.829	7.355	1.077	23.30	23.50	1.021
157.	1.711	1.372	7.656	7.756	1.013	24.30	24.50	0.980
158.	2.228	1.787	7.130	7.077	0.993	24.15	24.25	0.990
159.	1.438	1.153	7.119	7.510	1.055	23.55	23.92	1.003
160.	1.461	1.172	6.594	6.243	0.947	23.25	23.42	1.025
161.	1.539	1.234	5.908	5.516	0.934	23.30	23.50	1.021
162.	1.663	1.334	5.596	5.381	0.962	22.55	22.92	1.047
163.	1.568	1.258	6.429	6.130	0.953	25.05	25.08	0.957
164.	2.899	2.325	6.732	8.269	1.228	23.40	23.67	1.014
165.	3.048	2.444	6.966	8.297	1.191	23.45	23.75	1.011
166.	2.887	2.316	7.127	8.334	1.169	24.10	24.17	0.993
167.	3.119	2.502	6.642	8.128	1.224	24.20	24.33	0.986
168.	2.626	2.106	7.120	7.782	1.093	23.50	23.83	1.007

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
169.	2.287	1.835	7.155	7.355	1.028	23.20	23.33	1.029
170.	1.770	1.420	8.053	7.249	0.900	24.00	24.00	1.000
171.	2.026	1.625	7.212	7.073	0.981	24.25	24.42	0.983
172.	1.883	1.511	7.273	7.340	1.009	24.05	24.08	0.997
173.	1.788	1.434	6.954	7.056	1.015	22.35	22.58	1.063

TABLE (38)

NITROGEN BALANCE

(a) Serial No.	(b) Total Urine N mg.	(c) Wt. Dry Faeces g.	(d) N/g. Faeces mg.	(e) Total Faeces N mg.	(f) Food Wt. g.	(g) N/G Food mg.	(h) Total Food N mg.	(i) N Balance mg.
73.	136	1.814	25.0	45	3.264	27.64	90	- 91
74.	171.5	1.066	25.0	27	9.413	27.64	260	+ 61
75.	101	0.845	25.0	21	0.639	27.64	18	-104
76.	103.5	0.270	25.0	7	0.113	27.64	3	-108
77.	64	0.120	25.0	3	0.004	27.64	0	- 67
78.	176	2.818	24.4	69	9.602	27.64	265	+ 20
79.	273	2.068	24.2	50	12.165	27.64	336	+ 13
80.	270	1.938	24.75	48	11.717	27.64	324	+ 6
81.	264	2.638	25.4	67	14.462	27.64	399	+68
82.	294	2.615	22.25	58	12.240	27.64	338	- 14
83.	340	3.770	18.9	71	19.365	26.76	517	+106
84.	355	3.404	22.4	76	17.658	26.76	472	+ 41

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
85.	347	4.573	21.3	98	19.315	26.76	516	+ 71
86.	356	3.668	20.1	74	22.066	26.76	590	+160
87.	342	4.281	20.0	86	17.646	26.76	471	+ 43
88.	340	3.778	21.85	82.5	16.533	26.76	442	+ 20
89.	252	2.524	21.2	54	11.347	26.76	303	- 3
90.	160	1.769	24.35	43	11.019	26.76	294	+ 91
91.	145	2.139	23.9	48	12.373	26.76	330	+137
92.	208	2.585	24.6	63.5	14.051	26.76	376	+104
93.	305	2.939	22.9	67	15.896	24.82	394	+ 22
94.	383	3.165	20.7	65.5	17.736	24.82	442	- 6
95.	375	3.178	21.8	69	16.749	24.82	415	- 29
96.	371	3.061	25.3	77	16.494	24.82	409	- 39
97.	309	3.015	25.9	78	13.556	24.82	336	- 51
98.	304	4.795	29.2	140	17.864	24.82	443	- 1
99.	302	4.317	22.8	98.5	20.592	24.82	511	+110
100.	301	3.689	21.4	79	17.086	24.82	424	+ 44

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
101.	294	4.097	23.1	94	19.143	24.82	475	+ 87
102.	310	3.485	26.0	90.5	20.233	24.82	502	+102
103.	315	4.296	22.6	97	18.632	24.82	462	+ 50
104.	326	3.725	26.4	98	15.740	24.82	390	- 34
105.	273	3.211	22.7	73	15.541	24.82	386	+ 40
106.	297	3.100	27.4	85	17.449	24.82	433	+ 51
107.	271	2.064	25.5	52.5	12.721	26.12	332	+ 8
108.	180	1.349	30.4	41	3.640	26.12	95	- 44
109.	68	0.554	60.0	33.2	0.200	26.12	5	- 96
110.	238	2.189	26.0	57	11.718	26.12	306	+ 11
111.	235	2.056	20.7	42.5	13.591	26.12	355	+ 77
112.	-	2.632	27.2	71.5	15.507	26.12	405	-
113.	-	1.924	35.2	68	9.597	26.12	250	-
114.	276	2.462	28.8	71	14.218	26.12	371	+ 24

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
115.	403	2.915	23.8	69	20.349	26.42	537	+ 65
116.	392	3.320	22.5	75	17.382	26.42	459	- 8
117	396	3.200	25.4	81	17.625	26.42	465	- 12
118.	236	1.829	22.1	40	21.379	26.42	564	+288
119.	239	2.364	25.6	61	12.751	26.42	337	+ 37
120.	-	2.679	26.7	72	15.149	26.42	400	-
121.	234	1.959	33.7	66	9.988	26.42	264	- 36
122.	346	3.812	29.9	114	16.742	26.42	442	- 18
123.	351	3.830	28.0	107	20.159	26.42	532	+ 74
124.	348	3.525	27.0	95	17.225	26.42	455	+ 12
125.	303	3.318	35.0	116	18.177	26.42	480	+ 61
126.	249	2.252	27.6	62	12.272	26.42	324	+ 13
127.	288	2.530	38.1	96	14.599	26.42	385	+ 1
128.	304	2.902	26.1	76	15.596	26.42	412	+ 32
129.	306	3.825	23.5	89	16.807	27.64	466	+ 71

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
130.	309	3.499	28.6	100	17.645	26.76	471	+ 62
131.	319	3.576	26.1	93.5	14.496	26.76	387	- 26
132.	251	2.743	26.4	72.5	12.338	26.76	330	+ 7
133.	247	2.870	27.5	79	12.395	26.76	331	+ 5
134.	341	4.341	21.8	94.5	19.901	26.76	532	+ 96
135.	269	3.580	26.7	95.5	17.397	26.76	465	+100
136.	301	3.929	23.1	91	16.333	26.76	436	+ 44
137.	332	3.596	19.1	68.5	21.360	24.82	530	+129
138.	269	3.662	18.6	68	16.202	24.82	402	+ 65
139.	251	3.302	27.4	90.5	15.988	24.82	397	+ 55
140.	188	1.807	24.2	44	7.653	24.82	190	- 42
141.	77	2.140	22.6	48.5	9.014	24.82	224	+ 98
142.	69	0.912	23.0	21	4.707	24.82	117	+ 27
143.	97	1.705	24.4	41.5	9.034	24.82	224	+ 85
144.	386	4.974	30.0	149	16.071	24.82	399	-136
145.	376	4.297	23.7	102	22.885	24.82	568	+ 90

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
146.	390	4.375	22.7	99.5	22.494	24.82	558	+ 68
147.	388	4.513	26.9	121.5	21.814	24.82	541	+ 31
148.	320	3.722	25.1	93.5	15.175	24.82	376	- 38
149.	380	4.467	28.4	127	20.030	24.82	497	- 10
150	344	4.652	25.3	117.5	21.148	24.82	525	+ 63
151.	346	5.373	28.7	154	23.318	24.82	578	+ 78
152.	372	3.911	25.4	99.5	24.575	26.12	641	+169
153.	394	3.752	29.8	112	22.732	26.12	593	+ 87
154.	364	3.281	29.6	97	21.079	26.12	550	+ 89
155.	340	3.796	25.5	97	20.235	26.12	529	+ 92
156.	351	3.102	23.6	73	20.102	26.12	525	+101
157.	288	3.569	26.0	93	18.606	26.12	486	+105
158.	375	3.020	24.1	73	17.256	26.12	450	+ 2
159.	242	2.899	28.3	82	17.026	26.12	444	+120
160.	246	2.834	29.2	83	9.226	26.12	241	- 88

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
161.	259	1.747	24.8	43	11.089	26.12	289	- 13
162.	280	1.589	22.9	36	11.638	26.12	304	- 12
163.	264	2.180	22.5	49	13.092	26.12	342	+ 29
164.	488	4.808	18.9	91	24.758	26.12	646	+ 67
165.	513	4.485	22.9	103	24.300	26.12	634	+ 18
166.	486	4.444	30.8	137	23.482	26.12	613	- 10
167.	525	4.275	28.8	123	25.066	26.12	655	+ 7
168.	442	3.318	27.6	91.5	21.685	26.12	566	+ 32
169.	385	3.300	26.6	87.8	17.127	26.12	447	- 26
170.	298	2.291	29.7	68	11.631	26.12	304	- 62
171.	341	2.082	25.3	52.7	15.185	26.12	396	+ 2
172.	317	2.832	30.9	87.4	17.716	26.12	442	+ 57
173.	301	3.026	27.1	82	15.237	26.12	398	+ 15

TABLE (39)

Components of Water Intake

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
			Metabolic Water				
Serial No.	Total Fluid Intake g.	Food Moisture g.	0.2015 x litres O_2 g.	0.4675 x litres CO_2 g.	0.989 x g. N g.	Total Metabolic Water g.	Total Water Intake g.
1.	0.191	0.220	0.963	1.654	0.095	2.522	2.933
2.	49.756	0.753	0.955	1.869	0.174	2.650	53.159
3.	17.020	1.206	1.166	2.539	0.240	3.465	21.691
4.	14.071	1.159	1.166	2.519	0.233	3.452	18.682
5.	-		1.099	2.514	-		
6.			-	-	-		
7.	10.116	1.057	1.179	2.540	0.218	3.501	14.674
8.	17.975	1.277	1.268	2.768	0.251	3.785	23.037
9.	3.416	0.800	1.135	2.377	0.180	3.332	7.548
10.	25.038	1.414	1.301	2.859	0.270	3.890	30.342
11.	9.315	1.109	1.163	2.539	0.226	3.476	13.900
12.	9.268	0.591	0.982	1.949	0.149	2.782	12.641
13.	10.186	0.488	0.967	1.863	0.135	2.695	13.369
14.	23.590	1.224	1.315	2.777	0.243	3.849	28.663
15.	16.057	1.074	1.220	2.559	0.221	3.558	20.689

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
16.	13.364	1.114	1.268	2.692	0.227	3.733	18.211
17.	15.426	1.038	1.213	2.528	0.216	3.525	19.989
18.	15.062	1.170	1.236	2.641	0.235	3.642	19.874
19.	18.000	1.239	1.196	2.657	0.245	3.608	22.847
20.	9.051	1.088	1.212	2.597	0.201	3.608	13.747
21.	26.187	1.574	1.269	2.509	0.263	3.515	31.276
22.	16.988	1.443	1.264	2.660	0.246	3.678	22.109
23.	15.256	1. 278	1.215	2.388	0.225	3.378	19.912
24.	16.478	1.439	1.206	2.557	0.246	3.517	21.434
25.	18.862	1.367	1.161	2.357	0.236	3.282	23.511
26.	20.146	1.510	1.246	2.591	0.254	3.583	25.239
27.	18.037	1.547	1.221	2.654	0.258	3.617	23.201
28.	15.932	1.576	1.222	2.633	0.262	3.593	21.101
29.	26.294	1.994	1.224	2.452	0.316	3.360	31.648
30.	20.779		0.922	-	-		
31.	24.022	1.596	1.247	2.736	0.265	3.718	29.336
32.							
33.	23.412	1.687	1.365	2.811	0.276	3.900	28.999

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
34.	18.277	1.326	1.288	2.753	0.231	3.810	23.413
35.	18.880	1.224	1.373	2.712	0.218	3.867	23.971
36.	14.453	0.728	1.310	2.458	0.154	3.614	18.795
37.	6.826	0.164	1.348	2.215	0.083	3.480	10.470
38.	16.518	1.089	1.116	2.394	0.201	3.309	20.916
39.	18.722	1.010	1.109	2.284	0.191	3.202	22.934
40.	22.228	1.178	1.338	2.755	0.212	3.881	27.287
41.	10.991	1.089	0.887	2.139	0.228	2.798	14.878
42.	12.326	1.066	0.979	2.253	0.224	3.008	16.400
43.	13.908	-	1.009	2.273	0.215	3.067	-
44.	14.322	-	1.049	2.345	0.238	3.156	-
45.	13.151	-	1.061	2.484	0.217	3.328	-
46.	12.920	0.907	1.190	2.575	0.200	3.565	17.392
47.	11.951	1.027	1.173	2.573	0.218	3.528	16.506
48.	13.543	1.215	1.209	2.587	0.247	3.549	18.307
49.	11.718	1.014	1.166	2.411	0.216	3.361	16.093
50.	21.341	1.143	1.247	2.697	0.236	3.708	26.191

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
51.	-	-				-	-
52.	22.041	1.399	1.171	2.702	0.274	3.599	27.039
53.	11.337	1.158	1.167	2.762	0.237	3.692	16.187
54.	0.695	0.424	1.044	2.075	0.127	2.992	4.111
55.	20.192	1.140	1.188	2.459	0.236	3.411	24.743
56.	11.875	0.944	1.162	2.500	0.205	3.457	16.276
57.	16.924	1.355	1.166	2.590	0.267	3.489	21.768
58.	13.043	0.944	1.198	2.641	0.205	3.634	17.621
59.	15.488	1.411	1.240	2.690	0.276	3.654	20.553
60.	14.912	1.287	1.265	2.968	0.257	3.976	20.175
61.	16.503	1.373	1.275	2.916	0.270	3.921	21.797
62.	14.230	1.229	1.146	2.614	0.249	3.511	18.970
63.	14.933	1.395	1.294	2.95 0	0.274	3.970	20.298
64.	15.062	1.467	1.180	2.671	0.285	3.566	20.095
65.	15.624	1.119	1.106	2.386	0.232	3.260	20.003
66.	15.675	1.283	1.373	2.865	0.256	3.982	20.940
67.	13.615	0.996	1.338	2.620	0.214	3.744	18.555

(a)	(b)	(c)	(d)	(c)	(f)	(g)	(h)
68.	16.831	1.610	1.463	3.238	0.306	4.595	22.836
69.	13.130	1.335	1.456	3.238	0.264	4.430	18.895
70.	16.057	1.450	1.550	3.389	0.282	4.657	22.164
71.	13.263	1.343	1.493	3.141	0.266	4.368	18.974
72.	33.658	2.007	1.433	3.020	0.366	4.087	39.752
73.	2.781	0.317	1.205	2.090	0.135	3.160	6.258
74.	13.225	0.915	1.440	3.000	0.170	4.270	18.410
75.	5.104	0.062	1.337	2.102	0.100	3.339	8.505
76.	3.898	0.011	1.221	2.099	0.102	3.218	7.127
78.	10.617	0.933	1.281	2.788	0.174	3.895	15.445
79.	16.528	1.182	1.672	3.568	0.270	4.970	22.680
80.	15.045	1.139	1.530	3.196	0.267	4.459	20.645
81.	15.846	1.406	1.488	3.408	0.261	4.635	21.887
82.	18.154	1.190	1.339	2.895	0.291	3.943	23.287
83.	21.696	1.826	1.776	3.916	0.337	5.355	28.877
84.	22.758	1.665	1.749	3.855	0.351	5.253	29.676
85.	22.045	1.821	1.665	3.625	0.344	4.946	28.812

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
86.	24.787	2.081	1.816	4.085	0.352	5.549	32.417
87.	24.026	1.664	1.763	3.931	0.339	5.355	31.045
88.	22.404	1.559	1.744	3.702	0.337	5.109	29.072
89.	22.646	1.070	1.817	3.411	0.249	4.979	28.695
90.	19.728	1.039	1.792	3.367	0.158	5.001	25.768
91.	16.017	1.167	1.899	3.729	0.144	5.484	22.668
92.	14.058	1.325	2.207	4.350	0.206	6.251	21.734
93.	13.873	1.590	1.845	4.044	0.302	5.587	21.050
94.	18.99	1.774	1.823	4.144	0.379	5.588	25.561
95.	18.124	1.675	1.758	4.004	0.371	5.391	25.190
96.	15.569	1.649	1.725	3.912	0.367	5.270	22.488
97.	14.251	1.356	1.605	3.568	0.306	4.867	20.474
98.	20.826	1.786	1.704	3.931	0.301	5.334	27.946
99.	20.111	2.059	1.733	4.138	0.299	5.572	27.742
100.	19.061	1.709	1.746	4.052	0.298	5.500	26.270
101.	23.509	1.914	1.603	3.797	0.291	5.109	30.532
102.	17.093	2.023	1.660	3.899	0.307	5.252	24.368

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
103.	22.784	1.863	1.678	3.999	0.312	5.365	30.012
104.	21.377	1.574	1.748	3.842	0.323	5.267	28.218
105.	19.663	1.554	1.746	3.823	0.270	5.299	26.516
106.	19.146	1.745	1.779	3.962	0.294	5.447	26.338
107.	15.223	1.533	1.630	3.528	0.268	4.890	21.646
108.	1.238	0.439	1.395	2.669	0.178	3.886	5.563
109.	15.430	0.024	1.088	1.910	0.067	2.931	18.385
110.	20.276	1.412	1.445	3.199	0.236	4.397	26.085
111.	18.180	1.638	1.468	3.256	0.233	4.491	24.309
112.	17.790	1.869	1.449	3.316	0.291	4.474	24.133
113.	16.642	1.156	1.419	3.086	0.204	4.301	22.099
114.	19.948	1.713	1.533	3.418	0.273	4.678	26.239
115.	26.595	2.069	1.855	4.267	0.399	5.723	34.387
116.	22.854	1.768	1.832	4.160	0.388	5.604	30.226
117.	20.625	1.792	1.748	4.001	0.392	5.357	27.774
118.	16.069	2.174	1.610	3.384	0.234	4.760	23.003
119.	15.741	1.297	1.655	3.537	0.237	4.955	21.995

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
120.	16.128	1.541	1.861	4.061	0.285	5.637	23.306
121.	12.780	1.016	1.777	3.661	0.232	5.206	19.002
122.	18.740	1.703	1.454	3.445	0.343	4.556	24.999
123.	19.974	2.050	1.888	4.589	0.348	6.129	28.153
124.	18.613	1.752	1.829	4.258	0.345	5.742	26.107
125.	23.218	1.849	1.895	4.275	0.300	5.870	30.937
126.	15.146	1.248	1.618	3.586	0.247	4.957	21.351
127.	18.764	1.485	1.662	3.748	0.285	5.125	25.374
128.	18.036	1.586	1.601	3.632	0.301	4.932	24.554
129.	14.689	1.585	1.559	3.546	0.303	4.802	21.076
130.	18.155	1.664	1.617	3.754	0.306	5.065	24.884
131.	14.782	1.367	1.531	3.961	0.316	5.176	21.325
132.	10.587	1.163	1.511	3.072	0.248	4.335	16.085
133.	16.204	1.169	1.499	3.220	0.245	4.474	21.847
134.	20.057	1.877	1.544	3.630	0.338	4.836	26.770
135.	24.341	1.641	1.600	3.845	0.266	5.179	31.161
136.	18.046	1.540	1.667	3.753	0.298	5.122	24.708

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
137.	23.997	2.136	1.592	3.788	0.329	5.051	31.184
138.	17.049	1.620	1.646	3.751	0.266	5.131	23.800
139.	25.183	1.599	1.705	3.769	0.249	5.225	32.007
140.	16.137	0.765	1.620	3.164	0.186	4.598	21.500
141.	17.081	0.901	1.662	3.284	0.076	4.870	22.852
142	3.012	0.471	1.554	2.879	0.068	4.365	7.848
143.	16.677	0.903	1.899	3.634	0.096	5.437	23.017
144.	0.648	1.607	1.579	3.776	0.382	4.973	7.228
145.	27.534	2.289	1.698	4.271	0.372	5.597	35.420
146.	22.960	2.249	1.747	4.330	0.386	5.691	30.900
147.	19.621	2.181	1.744	4.345	0.384	5.705	27.507
148.	8.162	1.518	1.711	3.844	0.317	5.238	14.918
149.	20.931	2.003	1.840	4.336	0.376	5.800	28.734
150.	18.412	2.115	1.969	4.719	0.341	6.347	26.874
151.	22.424	2.332	1.950	4.529	0.343	6.136	30.892
152.	24.233	2.961	1.992	4.860	0.368	6.484	33.678
153.	18.169	2.739	1.897	4.603	0.390	6.110	27.018

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
154.	20.343	2.540	1.909	4.427	0.360	5.976	28.859
155.	18.667	2.438	1.883	4.470	0.337	6.016	27.121
156.	18.322	2.422	1.796	4.221	0.348	5.669	26.413
157.	16.931	2.242	1.887	4.267	0.285	5.869	25.042
158.	16.104	2.079	1.886	4.144	0.371	5.659	23.842
159.	18.833	2.052	1.724	4.050	0.240	5.534	36.419
160.	8.903	1.112	1.623	3.467	0.244	4.846	14.861
161.	16.876	1.336	1.501	3.156	0.256	4.401	22.613
162.	16.288	1.402	1.463	3.139	0.277	4.325	22.015
163.	17.149	1. 578	1.611	3.454	0.261	4.804	23.531
164.	20.415	2.983	1.941	4.953	0.483	6.411	29.809
165.	22.245	2.928	2.018	5.021	0.508	6.534	31.707
166.	20.894	2.830	2.023	4.979	0.481	6.521	30.245
167.	21.412	3.020	1.967	4.970	0.520	6.426	30.858
168.	20.339	2.613	1.964	4.623	0.438	6.149	29.101
169.	13.306	2.064	1.903	4.296	0.381	5.818	21.188
170.	12.792	1.402	1.979	4.053	0.295	5.737	19.931

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
171.	16.771	1.830	1.861	4.066	0.338	5.589	24.190
172.	18.035	2.135	1.845	4.138	0.314	5.669	25.839
173.	16.364	1.836	1.762	3.969	0.298	5.433	23.633

TABLE (40)

Components of Water Loss

(a) Serial No.	(b) Urine Weight g.	(c) Urine Water g.	(d) Vapour -ised Water g.	(e) Weight Balance g.	(f) Vap. Water Corrected per 24 hr. g.	(g) Water on Funnel and Frame g.	(h) Faeces Water g.	(i) Total Water Loss g.
1.	0.230	0.202	8.451	-0.561	9.24	0.695	-	-
2.	15.652	15.000	10.839		(14.84)	0.603	-	-
3.	5.466	4.810	14.354	-0.236	14.59	0.107	1.313	20.82
4.	4.705	4.140	11.493	-1.454	13.66	0.098	1.250	19.15
5.	-	-	-	-	-	-	-	-
6.	-	-	13.445	-	(14.02)	-	-	-
7.	3.473	3.056	14.211	-1.665	16.62	0.084	1.478	21.24
8.	3.899	3.431	13.726	-1.111	15.27	0.069	1.687	20.46
9.	2.254	1.984	13.517	-0.573	14.39	0.077	0.409	16.86
10.	4.108	3.615	13.169	-1.161	14.69	0.194	-	-
11.	4.802	4.226	15.634	-0.801	16.78	0.100	1.404	22.51
12.	3.621	3.186	7.608	-6.291	11.90	0.111	2.415	17.61

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
13.	2.160	1.901	10.068	-0.316	12.31	0.478	1.078	15.77
14.	7.590	6.679	13.803	-1.186	15.42	0.305	0.880	23.28
15.	5.241	4.612	11.500	-0.916	12.70	0.082	1.170	18.56
16.	4.543	3.998	13.646	-1.151	14.96	0.056	1.810	20.82
17.	3.568	3.140	12.832	-0.680	13.66	0.082	0.990	17.87
18.	4.165	3.665	14.323	-1.354	15.85	0.070	2.237	21.82
19.	6.392	5.625	15.516	+0.453	15.23	0.068	2.211	23.75
20.	3.190	2.807	14.330	-1.096	15.70	0.127	1.730	20.36
21.	4.937	4.345	16.870	-1.055	18.18	0.131	3.016	25.67
22.	3.651	3.213	14.228	-2.242	16.77	0.369	1.322	21.67
23.	4.158	3.659	15.988	-0.989	16.99	0.138	1.878	22.67
24.	4.063	3.575	14.917	-0.795	15.93	0.114	1.700	21.32
25.	3.332	2.932	15.568	-0.528	16.50	0.144	1.349	20.93
26.	3.548	3.122	16.226	-1.075	17.49	0.111	2.036	22.76
27.	5.332	4.692	16.603	-1.247	17.97	0.150	2.154	24.97
28.	3.801	3.345	15.634	-1.365	17.24	0.103	3.442	24.13

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
29.	5.509	4.848	16.270	-3.700	(18.01)	0.136	3.133	26.13
30.	-	-	-	-	-	-	-	-
31.	4.831	4.251	14.184	-1.119	15.92	0.110	2.661	22.94
32.	-	-	-	-	-	-	-	-
33.	5.297	4.661	15.363	-0.998	16.361	0.096	2.414	23.53
34.	3.044	2.679	14.308	-0.643	15.26	0.138	0.731	18.81
35.	3.860	3.397	14.317	-0.550	14.72	0.111	1.013	19.24
36.	2.436	2.144	12.974	-0.709	14.12	0.521	0.300	17.09
37.	3.588	3.157	15.077	-2.231	17.81	0.633	-	-
38.	5.093	4.482	16.406	-1.598	18.00	0.166	2.028	24.68
39.	4.031	3.547	11.899	-	-	0.313	3.564	15.76
40.	3.967	3.491	16.063	-1.954	18.22	0.360	4.862	26.93
41.	0.669	0.589	14.451	-0.808	15.63	0.350	1.578	18.15
42.	1.663	1.463	11.433	-1.426	13.27	0.742	0.671	16.15
43.	1.670	1.470	11.592	-0.888	12.59	0.192	0.061	14.31
44.	1.086	0.956	12.335	-0.266	12.71	0.636	0.979	15.28

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
45.	1.865	1.641	11.782	-1.068	12.85	0.287	0.779	15.56
46.	3.410	3.000	12.854	-0.822	13.77	0.329	0.751	17.85
47.	2.785	2.451	11.494	-0.043	12.00	0.438	0.506	15.40
48.	2.496	2.196	14.164	-0.652	15.02	0.237	0.664	18.12
49.	2.864	2.520	16.309	-1.623	18.25	0.611	1.564	22.95
50.	2.170	1.910	13.630	-1.073	15.07	0.332	0.425	17.74
51.	-	-	-		-	-	-	-
52.	3.435	3.023	14.994	-1.119	16.29	0.197	1.777	21.29
53.	3.557	3.130	15.059	-0.946	16.23	0.247	0.956	20.56
54.	1.960	1.725	11.487	-0.409	12.03	0.239	0.306	14.30
55.	2.762	2.429	12.750	-0.631	13.47	0.257	0.902	17.06
56.	3.066	2.698	11.958	-1.476	13.82	0.197	1.227	17.94
57.	5.157	4.538	12.219	-2.060	14.79	0.183	0.440	19.95
58.	6.805	5.988	12.113	-0.595	12.75	0.143	0.421	19.30
59.	7.022	6.179	12.599	-0.890	13.53	0.253	0.697	20.68

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
60.	4.056	3.569	13.833	-0.575	14.45	0.417	0.860	19.30
61.	4.406	3.877	13.065	-1.039	14.15	0.264	0.343	18.63
62.	5.827	5.128	11.911	-4.234	16.16	0.321	3.493	25.10
63.	4.169	3.669	12.659	-1.343	15.11	0.411	0.575	19.77
64.	7.499	6.599	11.770	-	(15.39)	0.484	-	-
65.	2.963	2.607	10.824	-0.788	14.42	0.531	0.716	18.27
66.	3.390	2.983	13.888	-0.455	14.34	0.664	0.286	18.27
67.	2.310	2.033	10.315	-2.657	13.15	0.697	1.045	16.93
68.	6.126	5.391	14.905	-0.569	15.89	0.430	1.267	22.98
69.	5.054	4.448	15.648	-1.066	17.43	0.425	1.243	23.55
70.	5.026	4.423	12.843	-1.242	13.70	0.300	0.769	19.19
71.	5.402	4.754	16.197	-0.877	16.90	0.552	1.817	24.02
72.	4.187	3.685	15.701	-1.016	17.64	1.478	-	-
73.	2.863	2.519	13.840	-0.611	14.10	0.139	0.203	16.96
74.	2.948	2.594	10.816	-0.620	11.85	0.292	0.207	14.94
75.	1.546	1.360	11.144	-0.099	11.24	0.120	0.230	12.95

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
76.	0.425	0.374	9.561	-0.824	10.50	0.101	0.224	11.20
77.	1.211	1.066	11.218	-0.499	11.75	0.064	0.055	12.94
78.	8.116	7.142	13.252	-1.088	15.53	0.647	1.490	24.81
79.	4.803	4.227	13.721	-0.945	14.31	0.278	0.920	19.74
80.	4.900	4.312	13.364	-1.157	14.68	0.265	0.363	19.62
81.	4.449	3.915	13.092	-0.519	13.86	0.408	1.145	19.33
82.	10.722	9.435	13.379	-0.982	15.67	0.524	0.794	26.42
83.	9.145	8.048	15.180	-0.981	16.68	0.244	2.319	27.29
84.	9.622	8.467	15.155	-0.369	16.02	0.323	1.411	26.22
85.	11.000	9.680	13.796	-1.008	16.15	0.201	0.260	26.29
86.	11.272	9.919	14.595	-1.164	16.04	0.345	2.010	28.31
87.	9.418	8.288	14.652	-0.314	15.13	0.195	2.019	25.63
88.	10.215	8.989	12.252	-2.487	14.74	2.895	3.134	29.76
89.	6.129	5.394	13.658	-0.308	14.26	0.167	1.177	21.00
90.	4.401	3.873	13.356	-0.166	13.67	0.260	0.345	18.148
91.	5.548	4.882	11.549	-0.572	12.25	0.204	0.492	17.83

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
92.	8.029	7.066	15.756	-4.143	21.52	1.919	1.245	31.75
93.	8.653	7.615	13.013	-1.072	14.75	0.286	1.553	24.20
94.	9.293	8.178	13.847	-0.329	14.37	0.181	1.262	23.99
95.	9.893	8.706	13.997	-0.815	15.02	0.372	1.856	25.95
96.	9.390	8.263	14.182	-0.882	15.23	0.291	1.702	25.49
97.	6.456	5.681	13.816	-0.917	16.07	0.257	2.019	24.03
98.	9.534	8.390	15.379	-1.411	17.46	0.145	2.514	28.51
99.	10.180	8.958	16.649	+0.136	16.69	0.212	1.785	27.65
100.	11.588	10.197	14.778	-0.553	15.50	0.110	1.201	27.01
101.	11.292	9.937	12.598	-2.168	15.64	0.168	2.326	28.07
102.	11.990	10.551	16.072	-0.227	16.71	0.091	1.391	28.74
103.	12.019	10.577	16.028	-0.530	16.79	0.105	1.395	28.87
104.	11.162	9.823	16.006	-1.018	16.97	0.172	1.403	28.37
105.	8.675	7.634	14.653	-0.306	14.60	0.149	0.856	23.24
106.	10.620	9.346	14.984	-0.624	15.83	0.111	1.333	26.62

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
107.	6.990	6.151	13.564	-0.827	14.91	0.150	0.576	21.79
108.	8.731	7.683	12.596	-0.327	12.96	0.119	0.425	21.19
109.	8.202	7.218	8.634	-0.548	10.05	0.060	0.067	17.40
110.	12.402	10.914	14.395	+1.476	12.92	0.190	0.900	24.92
111.	9.320	8.202	13.402	-0.867	14.27	0.154	0.547	23.17
112.	7.790	6.855	14.181	-0.432	15.20	0.157	1.067	23.28
113.	5.456	4.801	13.121	-0.399	13.56	0.177	0.753	19.29
114.	6.465	5.689	14.412	-0.385	14.44	0.244	0.958	21.33
115.	13.224	11.637	19.017	-0.395	19.22	0.245	0.464	31.57
116.	12.063	10.615	19.025	-0.318	18.88	0.115	0.499	30.11
117.	11.608	10.215	17.637	-0.378	18.27	0.134	0.657	29.28
118.	5.585	4.915	15.125	-0.401	15.53	0.182	0.250	20.88
119.	5.738	5.049	15.389	-0.375	15.98	0.118	0.746	21.89
120.	6.796	5.980	17.176	-0.695	17.33	0.230	1.074	24.61
121.	4.458	3.923	15.603	-0.578	16.65	0.214	0.756	21.54
122.	9.224	8.117	16.053	-0.674	20.24	0.216	1.692	30.27

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
123.	10.178	8.957	19.596	-1.764	19.78	0.366	1.544	30.65
124.	7.097	6.245	19.626	-0.786	21.00	0.248	1.593	29.09
125.	6.860	6.037	21.169	-0.650	21.51	0.209	1.177	28.93
126.	6.596	5.804	16.260	-0.536	17.65	0.168	0.621	24.24
127.	7.837	6.897	16.963	+0.349	16.38	0.176	0.411	23.86
128.	8.968	7.892	15.982	-0.465	17.29	0.105	1.083	26.37
129.	5.733	5.045	14.412	-1.022	15.33	0.618	1.686	22.68
130.	5.557	4.890	14.893	-1.121	15.97	0.495	1.693	23.05
131.	5.283	4.649	12.882	+0.952	12.54	0.459	1.927	19.58
132.	4.171	3.670	14.756	-0.622	15.59	0.328	0.821	20.41
133.	3.800	3.344	13.837	-0.576	14.77	0.549	1.174	19.84
134.	7.116	6.262	10.911	-1.364	13.05	0.562	3.464	23.34
135.	5.338	4.697	12.791	-0.426	13.52	0.685	2.683	21.59
136.	6.475	5.698	13.680	-0.798	14.48	0.754	2.862	23.79
137.	7.093	6.242	11.820	-3.057	16.23	0.450	1.893	24.82
138.	7.909	6.960	10.628	-2.877	13.94	0.274	2.969	24.14

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
139.	5.744	5.055	12.701	-0.469	13.41	0.315	1.662	20.44
140.	2.238	1.969	10.952	-0.106	11.20	0.146	0.382	13.70
141.	1.295	1.140	11.591	-0.445	12.07	0.369	1.265	14.84
142.	1.089	0.958	9.752	-0.161	10.05	0.241	0.156	11.41
143.	0.981	0.863	12.976	+4.353	-	0.477	0.624	
144.	6.619	5.825	12.698	-1.424	14.38	0.900	3.361	24.47
145.	10.681	9.399	12.950	-2.167	15.16	0.383	3.434	28.38
146.	9.285	8.171	11.351	-2.239	13.74	1.357	3.133	26.40
147.	11.377	10.012	13.710	-1.607	15.42	0.571	3.140	29.14
148.	5.746	5.056	13.740	-1.321	15.23	0.881	2.861	24.03
149.	8.867	7.803	13.589	-4.379	(16.38)	0.672	1.660	26.52
150.	11.456	10.081	15. 184	-1.778	16.96	2.339	3.091	32.47
151.	6.691	5.888	15.492	-2.660	17.79	1.874	4.050	29.60
152.	9.395	8.268	14.831	-2.065	17.01	0.986	1.439	27.70
153.	10.147	8.929	14.993	-0.774	16.33	0.609	1.434	27.30

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
154.	7.401	6.513	15.732	-0.415	16.26	0.629	0.951	24.35
155.	7.600	6.688	14.808	-1.568	16.56	0.766	1.977	25.99
156.	9.043	7.958	14.871	-0.198	15.39	0.284	1.095	24.73
157.	7.757	6.914	15.641	-0.697	16.01	0.204	1.168	24.30
158.	7.235	6.367	14.862	-0.992	15.70	0.230	1.790	24.09
159.	8.415	7.405	15.153	-0.652	15.85	0.644	1.557	25.46
160.	5.408	4.759	15.193	-1.060	16.66	0.296	0.970	22.69
161.	8.224	7.237	13.544	-0.379	14.22	0.267	0.402	22.13
162.	11.044	9.719	12.511	-0.450	13.57	0.287	0.272	23.85
163.	10.302	9.066	14.606	-0.695	14.64	0.435	0.705	24.85
164.	11.875	10.450	16.730	-0.572	17.54	1.451	2.075	31.52
165.	13.553	11.927	15.372	-1.033	16.59	0.755	1.842	31.11
166.	12.152	10.694	16.309	-1.356	17.54	1.246	3.289	32.77
167.	14.715	12.949	16.528	-1.042	17.68	0.821	2.369	33.82
168.	10.588	9.317	17.371	-0.367	17.86	0.580	0.919	28.68

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
169.	9.182	8.080	17.716	-1.442	19.71	0.437	0.530	28.76
170.	5.945	5.232	18.011	-0.509	18.52	0.350	0.466	24.57
171.	7.147	6.289	16.218	-0.384	16.32	0.339	0.402	23.35
172.	7.446	6.552	16.912	-0.670	17.53	0.220	0.494	24.80
173.	5.520	4.858	17.105	-0.440	18.65	0.582	0.916	25.01

TABLE (41)

WATER BALANCE

(a) Serial No.	(b) Weight Incr. g.	(c) Faeces D.M. g.	(d) Urine D.M. g.	(e) CHO Metab. g.	(f) Fat Metab. g.	(g) Prot. Metab. Nx.3.29	(h) D.M. Ingest. g.	(i) Water Balance g.
1.	-8.5	-	0.03	0.5	1.8	0.32	1.98	-
2.	+19.1	-	0.65	2.2	0.9	0.58	6.78	-
3.	+2.29	2.84	0.66	5.0	0.06	0.80	10.85	+0.80
4.	+1.3	2.87	0.56	4.8	0.15	0.77	10.43	+0.02
5.	-0.1	-	-	-	-	-	-	-
6.	-	-	-	-	-	-	-	-
7.	-5.89	2.41	0.42	4.9	0.19	0.72	9.52	-6.77
8.	+4.67	2.87	0.47	5.5	0.07	0.83	11.49	+2.92
9.	-9.93	2.51	0.27	4.2	0.49	0.60	7.20	-9.06
10.	+12.65	-	0.49	5.8	-0.02	0.90	12.72	+9.60
11.	-7.85	3.02	0.58	5.1	0.05	0.75	9.98	-8.33
12.	-6.15	2.00	0.43	2.7	0.82	0.50	5.32	-5.02

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
13.	-1.33	1.37	0.26	2.05	1.09	0.45	4.40	-0.51
14.	+7.61	1.96	0.91	5.0	0.4	0.81	11.02	+5.67
15.	+3.44	2.28	0.63	4.4	0.49	0.73	9.67	+2.30
16.	-2.30	3.21	0.55	4.9	0.37	0.75	10.02	-2.54
17.	+3.35	2.22	0.43	4.25	0.54	0.72	9.35	+2.16
18.	-0.96	3.17	0.50	4.8	0.3	0.78	10.53	-1.94
19.	+0.75	3.15	0.77	5.65	-0.14	0.81	11.15	-0.16
20.	-7.29	2.86	0.38	4.9	0.31	0.67	8.29	-6.46
21.	+8.41	3.74	0.59	3.3	0.95	0.87	12.00	+5.86
22.	+2.48	2.85	0.44	4.6	0.33	0.82	11.00	+0.52
23.	-1.59	3.23	0.50	3.1	1.0	0.75	9.74	-2.75
24.	+1.40	3.58	0.49	4.6	0.31	0.82	10.97	+0.23
25.	+4.80	3.19	0.40	3.5	0.67	0.78	10.42	+2.92
26.	+4.50	3.55	0.43	4.25	0.5	0.85	11.51	+2.57
27.	-0.40	3.82	0.64	4.9	0.14	0.86	11.79	-1.83
28.	-1.60	4.30	0.46	4.9	0.13	0.87	12.01	-2.95
29.	+9.10	4.17	0.66	3.3	0.65	1.05	15.20	+3.73

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
30.	-4.3	-	-	-	-	-	-	-6.6
31.	+8.60	3.54	0.58	5.5	-0.04	0.88	12.16	+6.90
32.	+10.65	-	-	-	-	-	-	+9.5
33.	+7.75	3.84	0.64	4.45	0.65	0.92	12.86	+5.39
34.	+5.10	3.26	0.37	5.1	0.32	0.77	10.10	+4.82
35.	+4.70	3.24	0.46	3.4	1.28	0.72	9.33	+4.47
36.	+1.15	1.53	0.29	2.4	1.73	0.51	5.55	+2.06
37.	-14.93	-	0.43	-0.3	3.09	0.28	1.25	-11.0
38.	-3.95	2.64	0.61	4.5	0.2	0.67	8.30	-3.63
39.	-1.35	2.49	0.48	3.5	0.67	0.63	7.70	-1.28
40.	+3.10	2.67	0.48	4.45	0.8	0.70	8.98	+3.22
41.	-1.30	2.88	0.08	5.48	-0.75	0.76	10.11	-2.96
42.	+2.90	1.87	0.20	5.0	-0.4	0.74	9.90	+0.41
43.	+2.42	3.20	0.20	4.7	-0.15	0.71	9.36	+1.72
44.	+4.35	2.42	0.13	4.8	-0.16	0.79	10.71	+1.62
45.	+1.43	2.36	0.22	6.0	-0.56	0.72	9.44	+0.73

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
46.	-0.64	2.27	0.41	4.85	0.27	0.66	8.42	-0.60
47.	+2.70	2.00	0.33	5.0	0.13	0.72	9.54	+1.34
48.	+2.78	2.68	0.30	4.6	0.31	0.82	11.28	+0.21
49.	-6.30	2.83	0.34	3.85	0.59	0.72	9.42	-7.39
50.	+11.07	1.83	0.26	5.0	0.27	0.78	10.61	+8.60
51.	-	-	-	-	-	-	-	-
52.	-8.35	3.44	0.41	6.1	-0.5	0.91	12.99	-10.98
53.	-3.87	2.89	0.43	6.7	-0.63	0.79	10.75	-4.44
54.	-11.83	1.10	0.24	3.85	0.97	0.42	3.94	-9.19
55.	+10.29	1.80	0.33	3.9	0.57	0.78	10.59	+7.08
56.	-0.40	2.10	0.37	4.6	0.3	0.68	8.76	-1.11
57.	+6.54	1.59	0.62	5.2	-0.1	0.89	12.59	+2.15
58.	-1.98	1.98	0.82	5.35	0.08	0.68	8.77	-1.84
59.	+3.09	2.87	0.84	5.0	0.11	0.92	13.10	-0.27
60.	+2.47	2.36	0.49	7.2	-0.67	0.86	11.95	+0.76

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
61.	+6.15	2.07	0.53	6.45	-0.35	0.90	12.75	+3.70
62.	-5.58	3.82	0.70	5.8	-0.33	0.83	11.41	-6.17
63.	+4.53	2.30	0.50	6.6	-0.38	0.91	12.96	+1.50
64.	+2.20	2.74	0.90	5.75	-0.34	0.95	13.62	-1.42
65.	+6.59	2.48	0.36	4.4	0.18	0.77	10.40	+4.38
66.	+5.98	1.83	0.41	4.7	0.63	0.85	11.91	+2.49
67.	+3.15	2.10	0.28	3.1	1.34	0.71	9.25	+1.43
68.	+3.53	3.25	0.74	6.6	-0.05	1.02	14.95	+0.14
69.	-2.48	2.55	0.61	6.7	-0.01	0.88	12.40	-4.15
70.	+1.48	2.52	0.60	6.7	0.17	0.94	13.47	-1.06
71.	-3.95	3.34	0.65	5.4	0.62	0.89	12.47	-5.52
72.	+25.66	-	0.50	4.9	0.36	1.22	18.56	-
73.	-13.72	1.81	0.34	0.6	2.25	0.45	2.95	-11.22
74.	+ 4.19	1.07	0.35	5.1	0.9	0.56	8.50	+3.67
75.	-7.63	0.85	0.19	-1.0	3.41	0.33	0.58	-4.43

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
76.	-7.56	0.27	0.05	0.5	2.4	0.34	0.10	-4.10
77.	-7.01	0.12	0.15	-3.6	4.06	0.21	0.00	-6.07
78.	-9.90	2.82	0.97	5.6	0.29	0.58	8.67	-8.31
79.	+2.80	2.07	0.58	6.7	0.48	0.90	10.98	+2.55
80.	+2.05	1.94	0.59	5.35	0.71	0.89	10.58	+0.95
81.	+4.20	2.64	0.53	7.4	-0.22	0.87	13.06	+2.36
82.	-1.27	2.62	1.29	5.4	0.16	0.97	11.05	-1.88
83.	+5.46	3.77	1.10	8.0	0.03	1.12	17.54	+1.94
84.	+6.23	3.40	1.15	7.75	0.02	1.17	15.99	+3.73
85.	+6.00	4.57	1.32	7.00	0.12	1.14	17.49	+2.66
86.	+9.40	3.67	1.35	8.9	-0.29	1.17	19.99	+4.21
87.	+6.67	4.28	1.13	8.25	-0.10	1.13	15.98	+5.38
88.	+1.42	3.78	1.23	6.5	0.56	1.12	14.97	-0.36
89.	+8.49	2.52	0.74	3.2	2.34	0.83	10.28	+7.84
90.	+8.92	1.77	0.53	3.3	2.50	0.53	9.98	+7.57
91.	+5.57	2.14	0.67	5.0	2.12	0.48	11.21	+4.77

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
92.	-8.27	2.59	0.96	5.8	2.35	0.68	12.73	-8.62
93.	-1.77	2.94	1.04	8.2	0.22	1.00	14.31	-2.68
94.	+3.26	3.17	1.12	9.25	-0.5	1.26	15.96	+1.60
95.	+0.30	3.18	1.19	8.9	-0.5	1.23	15.07	-0.77
96.	-1.75	3.06	1.13	8.6	-0.41	1.22	14.84	-2.99
97.	-2.38	3.02	0.77	7.4	-0.05	1.02	12.20	-2.42
98.	+0.48	4.80	1.14	9.2	-0.52	1.00	16.08	+0.02
99.	+2.49	4.32	1.22	10.6	-1.02	0.99	18.53	+0.07
100.	-0.53	3.69	1.39	9.8	-0.67	0.99	15.38	-0.71
101.	+5.31	4.10	1.36	9.5	-0.88	0.97	17.23	+3.13
102.	-0.65	3.49	1.44	9.5	-0.78	1.02	18.21	-4.19
103.	+2.03	4.30	1.44	10.2	-1.05	1.04	16.77	+1.19
104.	-0.12	3.73	1.34	7.7	0.12	1.07	14.17	-0.33
105.	+3.57	3.21	1.04	7.65	0.29	0.90	13.99	+2.67
106.	+1.65	3.11	1.27	8.5	0.0	0.98	15.70	-0.19

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
107.	+0.41	2.06	0.84	6.8	0.35	0.89	11.19	+0.16
108.	-20.06	1.35	1.05	2.8	1.7	0.59	3.20	-15.77
109.	-3.40	0.55	0.98	0.85	2.04	0.22	0.18	+1.06
110.	+0.16	2.19	1.49	6.5	0.12	0.78	10.31	+0.93
111.	+2.07	2.06	1.12	6.8	0.06	0.77	11.95	+0.93
112.	+3.29	2.63	0.93	7.5	-0.45	0.97	13.64	+1.23
113.	-8.58	1.92	0.65	6.4	0.2	0.68	8.44	-7.17
114.	+5.66	2.46	0.78	7.3	-0.09	0.91	12.50	+4.52
115.	+5.79	2.92	1.59	9.8	-0.73	1.33	18.28	+2.42
116.	+0.30	3.32	1.45	9.2	-0.48	1.29	15.61	-0.53
117.	+0.11	3.20	1.39	8.9	-0.57	1.30	15.83	-1.50
118.	+1.18	1.83	0.67	6.0	0.75	0.78	10.22	+0.99
119.	+0.63	2.36	0.69	6.6	0.6	0.79	11.45	+0.22
120.	-1.35	2.68	0.82	8.1	0.35	0.95	13.61	-2.06
121.	-3.68	1.96	0.53	5.9	1.19	0.77	8.97	-2.30

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
122.	-0.44	3.81	1.11	8.5	-1.0	1.14	15.04	-1.92
123.	-3.16	3.83	1.22	12.3	-1.49	1.15	18.11	-4.26
124.	-2.07	3.53	0.85	10.1	-0.72	1.14	15.47	-2.64
125.	+3.37	3.32	0.82	9.5	-0.18	1.00	16.33	+1.50
126.	-2.70	2.25	0.79	7.5	0.12	0.82	11.02	-2.24
127.	+1.73	2.53	0.94	8.25	-0.19	0.95	13.11	+1.10
128.	+0.12	2.90	1.08	8.1	-0.31	1.00	14.01	-1.12
129.	+0.26	3.83	0.69	7.9	-0.37	1.01	15.22	-1.90
130.	+4.14	3.50	0.67	8.9	-0.64	1.02	15.98	+1.61
131.	+0.21	3.58	0.63	11.6	-1.92	1.05	13.13	+2.02
132.	-2.89	2.74	0.50	4.6	0.05	0.83	11.17	-5.34
133.	+2.81	2.87	0.46	6.0	0.45	0.81	11.23	+2.17
134.	+7.80	4.34	0.85	8.8	-0.88	1.12	18.02	+4.01
135.	+11.32	3.58	0.64	10.0	-1.02	0.86	15.76	+9.62
136.	+1.83	3.93	0.78	8.2	-0.2	0.99	14.79	+0.74
137.	+12.72	3.60	0.85	9.5	-1.0	1.09	19.22	+7.54

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
138.	+0.74	3.66	0.95	8.6	-0.33	0.89	14.58	-0.07
139.	+13.13	3.30	0.69	7.8	0.17	0.83	14.39	+11.53
140.	+6.24	1.81	0.27	3.9	1.78	0.62	6.89	+7.73
141.	+6.89	2.14	0.16	4.6	1.91	0.25	8.11	+7.84
142.	-5.75	0.91	0.13	2.7	2.44	0.23	4.24	-3.58
143.	+11.40	1.71	0.12	4.2	2.58	0.32	8.13	+12.200
144.	-18.03	4.97	0.79	9.5	-1.2	1.27	14.46	-17.16
145.	+10.50	4.30	1.28	12.2	-1.94	1.24	20.60	+6.98
146.	+7.80	4.38	1.11	11.9	-1.82	1.28	20.24	+4.41
147.	+0.51	4.51	1.37	12.1	-1.85	1.28	19.63	-1.71
148.	-9.06	3.72	0.69	8.3	-0.2	1.05	13.66	-9.16
149.	+4.41	4.47	1.06	10.6	-1.0	1.25	18.03	+2.76
150.	-4.93	4.65	1.38	12.2	-1.2	1.13	19.03	-5.80
151.	+4.28	5.37	0.80	10.8	-0.71	1.14	20.508	+1.17
152.	+9.66	3.91	1.13	13.0	-1.6	1.22	21.61	+5.71
153.	+3.18	3.75	1.22	12.1	-1.5	1.30	19.99	+0.06

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
154.	+7.83	3.28	0.89	10.5	-0.75	1.20	18.54	+4.41
155.	+2.84	3.80	0.91	11.3	-1.05	1.12	17.80	+1.12
156.	+4.72	3.10	1.09	10.3	-0.91	1.15	17.68	+1.77
157.	+1.84	3.57	0.93	9.5	-0.16	0.95	16.36	+0.27
158.	+1.08	3.02	0.87	8.3	0.08	1.23	15.18	-0.60
159.	+1.64	2.90	1.01	10.1	-0.67	0.80	14.97	+0.81
160.	-10.78	2.83	0.65	6.5	0.54	0.81	8.11	-7.56
161.	+0.70	1.75	0.99	5.4	0.65	0.85	9.75	+0.59
162.	-1.13	1.59	1.33	5.8	0.32	0.92	10.24	-1.41
163.	-1.86	2.18	1.24	6.5	0.46	0.87	11.51	-2.12
164.	+0.48	4.81	1.43	14.3	-2.61	1.61	21.77	-1.75
165.	+2.58	4.49	1.63	13.8	-2.3	1.69	21.37	+0.52
166.	-1.13	4.44	1.46	13.5	-2.06	1.60	20.65	-2.84
167.	-0.34	4.28	1.77	14.0	-2.51	1.73	21.99	-3.06
168.	+3.26	3.32	1.27	11.2	-1.12	1.45	19.07	+0.31

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)
169.	-5.85	3.30	1.10	9.4	-0.35	1.27	15.06	-6.19
170.	-6.25	2.29	0.71	6.4	1.32	0.98	10.23	-4.78
171.	+1.39	2.08	0.86	8.1	0.21	1.12	13.36	+0.40
172.	+2.76	2.83	0.89	9.0	-0.13	1.04	15.58	+0.81
173.	-0.28	3.03	0.66	8.75	-0.19	0.99	13.40	-0.44

Table (42)

FOETAL AND PLACENTAL GROWTH

(Individual Measurements)

Rat No. Gest. Age Days	Foetal Weights mg.	Placenta Weights mg.	Concept. Weights mg.
561 12 days	18	14	-
532 14 days	156	138	538
	136	119	483
	143	123	529
	150	111	484
	126	88	486
466 14 days	152	123	470
	131	105	432
461 15 days	249	92	628
	258	133	670
	244	146	678
	248	148	706
	283	158	749
	283	126	729
	249	128	660
	257	137	672
	112	113	-
462 15 days	236	148	721
	224	150	713
	230	130	698
	226	113	650
	229	164	675
	232	135	649
	230	127	-
490 17 days	569	155	-
	670	163	-
	687	167	-
	652	150	-
	446	160	-
	641	184	-
	569	163	-
454 18 days	1100	271	-
	960	182	1780
	1240	277	2100
	1210	316	2170
	1040	257	2030
	930	263	1950
	1220	338	-

Table (42) Cont.

Rat No. Gest. Age Days	Foetal Weights mg.	Placenta Weights mg.	Concept. Weights mg.
434	1390	284	-
18 days	1268	278	-
	1292	352	-
	1412	310	-
	1264	305	-
	1450	315	-
	1310	292	-
	1520	301	-
435	1260	435	-
18 days	1370	362	2380
	1450	422	-
457	3670	340	-
20 days	3150	414	-
	3420	402	-
	2530	371	-
	2600	323	-
	2650	381	-
	2370	373	-
444	908	337	-
20 days	926	348	-
	864	256	-
	1085	295	-
	1330	366	1670
	1100	297	-
	1080	265	2010
	992	362	1900
	1010	291	1960
439	4850	438	-
21 days	5050	364	-
	4750	498	-
	4500	380	-
	4525	375	-
	4050	393	-
	4650	395	-
	4600	407	-
	3500	412	-
	3675	392	-
438	4820	395	-
21 days	5070	549	-
	4580	462	-
	4950	508	-
479	5400	373	-
	5430	385	-
	4100	908	-
	5520	376	-
	5210	432	-