STUDIES ON THE PHYSIOLOGY OF MICE SELECTED FOR LARGE OR SMALL BODY SIZE

by

Ruth E. Edwards, B.Sc. (Edin.)

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Tables and figures are appended to each chapter.

INTRODUCTION

Growth is extremely complex, and, being a continuous process from conception to maturity, will be influenced by genetic and environmental agencies acting at all levels of development and on many different physiological processes. The normal co-ordinated functioning of many physiological processes is necessary for the growth and development of an animal, and many physiologists have been concerned with the mechanisms through which growth is controlled. For example, post natal growth in mammals is dependent on the normal functioning of the endocrines, health and appetite and many other factors. Geneticists have studied growth from a different aspect. They have shown that many patterns of growth are gene-controlled and therefore heritable, and that heritable differences between animals can be used for the production of divergent strains by selection. As yet, however, few studies have been made of the effect of selection for particular characters on the basic physiology of an animal. The present study was undertaken with the intention of tracing differences in the physiology of strains of mice having large or small body size in response to previous selection.

Characters which are controlled by the action of a number of genes, such as body size, are known as quantitative characters. Our understanding of quantitative inheritance is based on the multiple gene hypothesis which was first postulated in 1910 by Nilsson-Ehle and by East, working independently. This hypothesis explains the normal continuous range of variation found in quantitative characters by proposing that the inheritance of these characters is accounted for by the action and segregation of a number of genes, each having a small effect but capable of

acting cumulatively. This hypothesis reconciled the continuous variation found in the inheritance of quantitative characters with the particulate nature of Mendelian segregations. The hypothesis has undergone many refinements and amplifications since it was first proposed, for example, the assumption of the additive action of genes conflicts with recent data which indicate that gene substitutions may have a geometric rather than an arithmetic effect (MacArthur 1944a).

The genetic variation arising from a large number of genes with small individual effects provides the basis for the production of divergent strains by means of selection. Goodale (1937, 1938, 1941) produced an increase of 70% in the adult body weight of mice as a result of selection for increased body size. More extensive selection experiments with mice have since been carried out by MacArthur (1944 a & b, 1949) and by Falconer (1948, 1953) and by Falconer and King (1953). MacArthur selected lines of mice for large and small body size at 60 days of age, and found an equal response to selection in the two lines, the changes in body size being correlated with changes in the relative proportions, time of onset of sexual maturity, and size of litter. He showed that as genetic variability in successive generations decreased, environmental variability increased, leaving phenotypic variability unchanged. Falconer's results, based on selection at six weeks of age, agreed in all important respects with those obtained by MacArthur. But in contrast to MacArthur, Falconer found that the rate of progress was greater in the small line, i.e. there was an asymmetry of response which could be entirely attributed to a maternal effect on weaning weight, as the weight gained from 3-6 weeks showed no asymmetry (Falconer 1955). Falconer estimated that many loci were concerned in determining body size.

A study of the physiological aspects of a quantitative character such as body size should help to elucidate the processes by which an organism acquires its adult form and function. Knowledge of the physiology of large and small selected lines of mice might lead to a better understanding of the growth process in these lines and of the physiological mechanisms through which this control was being exerted. The following work is therefore concerned largely with the effect of selection for large and small body size on growth, utilisation of foodstuffs, carcass composition and various aspects of metabolism. A few studies have been made by other workers of some aspects of the physiology of selected phenotypes. Strain differences in efficiency of food utilisation and body composition have been reported in two genetically distinct strains of rats by Palmer et al. (1946). They also investigated the effect of thyroid administration on growth, and suggested that the high efficiency strain of rats normally secreted less thyroxine than the low efficiency strain which had a higher basal metabolism. Falconer and Latyszewski (1952) selected for increased body size in mice both on high and low plane diets. After 8 generations of selection, groups from both planes of nutrition were reared on a full diet and analysed for abdominal fat. Offspring derived from parents on full diet had 24% more fat than those derived from parents on restricted diet; and whereas offspring of low diet parents maintained parental growth increases on a full diet, offspring of full diet parents did not maintain parental weight increases when reared on a restricted diet. Swine have also been selected for high or low feed requirements and Dickerson (1947) has found that low feed requirements tended to be positively correlated with rapid fat deposition and poor suckling ability. Baird et al. (1952) studied the causes of the different rates of growth in swine selected for rapid and

slow rates of gain and indicated that the rapid line consistently secreted larger amounts of growth hormone at all ages than the slow line, although in relation to body weight the pituitary weight was not significantly different in the two lines.

More is known of the manner in which single genes may affect normal growth. The action of a single mutant gene may have a considerable effect on a quantitative character such as body size. Some single gene substitutions such as 'pituitary dwarfism' (dw) and 'pigmy' (pg) reduce body size in mice. Pituitary dwarfism was first discovered by Snell in 1929. Animals homozygous for this gene show reduced body size from 12 days of age, and have shorter snouts, ears, and tails than their normal mates. The primary defect appeared to be an anomaly of the anterior pituitary in which the number of acidophilic cells was very much reduced and the gland contained very little, if any, growth-promoting hormone (Smith & MacDowell 1930, 1931; Francis 1944). The gene 'pigmy', which is not allelomorphic with 'pituitary dwarfism', also reduced body size and has a characteristic phenotype (King 1950). Greene (1940) found a simple recessive gene causing dwarfism in Polish rabbits.

Single gene substitutions can also cause an increase in body size in the mouse. Dickerson and Gowen (1947) showed that 'Yellow' (A^{y}) an autosomal dominant only found in the heterozygous condition produces obesity in mice by influencing food utilisation. Yellows were less active than normal litter mates, consequently energy expenditure per unit gain was reduced and the extra weight was entirely fat tissue. Benedict and Lee (1936) had previously demonstrated a lower basal metabolism in 'Yellow' mice than in normal litter mates. The reproductive period is also shortened in 'Yellow' mice, suggesting an endocrine imbalance in which the

normally operating balance of food intake to energy expenditure is impaired. A recessive gene 'obese' (ob) also causes obesity in mice whereby homozygous animals increase in weight very rapidly until they are about four times the weight of normal litter mates (Ingalls, Dickie & Snell 1950). Investigations into genetic-, traumatic-, and environmental-induced obesity have been carried out by Mayer (1953, 1955) who showed that the hyperglycemic syndrome associated with heriditary obesity was probably due to a disturbance in acetate metabolism. Until recently, obese animals were considered sterile but Lane and Dickie (1954) have shown that obese males will breed if, by restricting food intake, they were kept below 25-30 gms. weight. Wrenshall et al. (1955) have shown that the obese hyperglycemic syndrome of mice is characterised by an increase in the insulin content of the pancreas with associated hyperplasia and degranulation of the beta cells. Several coat colour genes are reported to influence body size in the mouse. The effect of some of these genes is undoubtedly due to a direct physiological action of the genes themselves. In other cases, size genes without known qualitative effects but which are closely linked genetically to the colour genes are probably responsible for influencing body size (see Gruneberg 1952).

Many factors are involved in the control of growth and will influence the rate of growth of an animal. In all adult animals there is generally a delicate balance between energy intake and total energy expenditure. This adjustment might be achieved in either of two ways; energy expenditure might be limited by food intake or alternatively might determine food intake. The latter type of adjustment is generally thought to occur. Growth will only occur, however, if total energy intake exceeds energy output. As far as is known, only when food intake is suboptimal does it

become a limiting factor in determining the rate of growth. In turn, little is known of the mechanisms which regulate food intake. Hunger may be associated with a lowering of the blood sugar by stimulation of the normal gastric hunger contractions (Carlson 1919) although Scott et al. (1938) have been unable to correlate spontaneous fluctuations of blood sugar with the desire for food. The hypothalamus is apparently concerned in the control of food intake; for example, disturbances of the mechanisms regulating food intake can be induced by hypothalamic lesions giving rise to 'hypothalamic obesity' (Hetherington & Ransom 1942, Brooks 1946, Mayer 1955).

Total energy expenditure can be subdivided into three main components, that energy essential for the maintenance of the animal, the energy required for body activity, and the energy cost associated with growth. The energy required for maintenance can be further subdivided into the energy expended in maintaining the basal metabolic rate and that energy expended due to the specific dynamic action of foodstuffs (SDA). The SDA of foodstuffs is the energy waste due to many intermediate reactions incident to food utilisation. Theoretically, by selection for a particular genotype, one could reach a stage where the maintenance requirements of the animal were so great that growth would be very much reduced. For example, it is known that large changes in the basal metabolic rate are associated with disorders of the thyroid gland, such that maintenance energy is increased or decreased twofold.

Some energy will be expended in body activity. While the activity of an animal need not be either a direct cause of, or in any way related to the size of the animal, changes in body size are sometimes associated with changes in levels of activity. The increased body weight of

hereditary obese mice, for example, has been found to be closely associated with differences in levels of activity; the gene 'obese' (ob) drastically reduced activity almost to nil (Mayer 1955), and Yellow mice (A^y) are heavier and less active than normal litter mates (Dickerson & Gowen 1947). On the other hand, reduced activity may also be associated with small body size, a depressed metabolic rate, and a low body temperature, as in 'pituitary' dwarfism (dw) (Benedict & Lee 1936; Schonholz & Osborn 1949). Behaviour patterns will therefore affect the energy spent in spontaneous activity, and these patterns may also be genetically determined and capable of modification by selection. Activity may also be influenced by the endocrines, e.g. the sex hormones (Slonaker 1924).

Many physiological processes are subject to hormonal control, and selection for large or small body size may exert its effect through the endocrines. Several hormones are known to be of fundamental importance in determining growth rates. The growth hormone secreted by the anterior pituitary is essential for normal development, and administration of growth hormone is generally accompanied by increased growth and nitrogen retention through its effects on protein and fat metabolism (Lee & Schaffer 1934; Li & Evans 1948; Greenbaum & MacLean 1953; and others). Thyroxin, which is essential for maintaining the normal basal metabolic rate, is also necessary for normal growth and development particularly in young animals, and in its absence, protein deposition is very much reduced (Leathem, quoted by Gaunt 1954). The androgenic steroids produced by the testis and to a lesser extent by the adrenal cortex exert a definite but limited effect on overall growth (Kochakian 1946; van Wagenen 1928, 1949; and others). Growth hormone, thyroxine, and the androgens all act as growth stimulants, and selection for large or small

body size might act directly on the levels of these or other endogenous hormones.

The present work is concerned with some aspects of the basic physiology of mice which have been selected for large or small body size, the intention being to investigate the mechanisms through which growth rates are being controlled. A study has been made of the effects of selection on total body weight during growth and maturity, and these effects have been related to the rate of deposition of fat and protein in the body. Nutrition experiments were undertaken to determine the quantity of food consumed and the efficiency of food utilisation and to discover whether larger animals consumed more food and utilised it more efficiently. The digestibility of foodstuffs, food balances, total energy expenditure, and the activity of large and small mice were studied as factors determining the efficiency with which foodstuffs are converted into body tissue.

MATERIAL

Large and small lines of mice from Falconer's selection programme were mainly used for the study, his construction of the foundation population and method of selection being as follows. The foundation population was derived from a four-way cross of inbred strains, CBA females were crossed to RIII males and C57 females to A males, and reciprocal crosses made of the F1's. This foundation population (generation 0) was regarded as random bred, and two lines were selected from it. Selection was based on body weight at six weeks of age, one line being selected for large size and the other for small. Individuals were selected on deviations from their litter means, selection therefore being entirely within litters. Litter size was standardised as far as possible to eight mice. Six pair matings within each line represented one generation, and the rate of inbreeding was kept to a minimum by the choice of least related pairs. The original four-way cross maintained without selection constituted the control line (for further details see Falconer 1953).

Mice not required for the continuance of Falconer's selection experiment were used for the physiological investigation. They were taken from generations 21-26 in the small line (RNS), 10-12 in the control line (RNC), and 25-30 in the large line (RNF). Over these generations the mean body weight of males at six weeks of age was 14.1, 21.2 and 30.0 gms., in small, control and large lines respectively. The progress that had already been made in response to selection is shown in Fig. 1. The large and small lines provided suitable material for the study, as not only were the differences in adult body weight already very marked, but both lines originated from the same foundation population. It was hoped that such an

investigation might indicate the underlying causes of the asymmetry of response observed by Falconer (1953). For comparison, the control line existed which had been constructed at a later date than the selected lines but which was nevertheless derived from the same four-way cross of inbred lines. During the course of selection the generation interval in the small line had increased as small line animals were slow to reach sexual maturity, hence comparisons between large and small lines were necessarily limited to the generations available during the course of the investigation.

Another strain of mice (CRL), selected for large body size and unrelated to Falconer's selection line, was also used. This strain provided a comparison with Falconer's large strain (RNF), as they were of comparable body weight and had been selected on similar criteria. Details of the derivation and maintenance of the CRL strain will be given later (see p. 19).

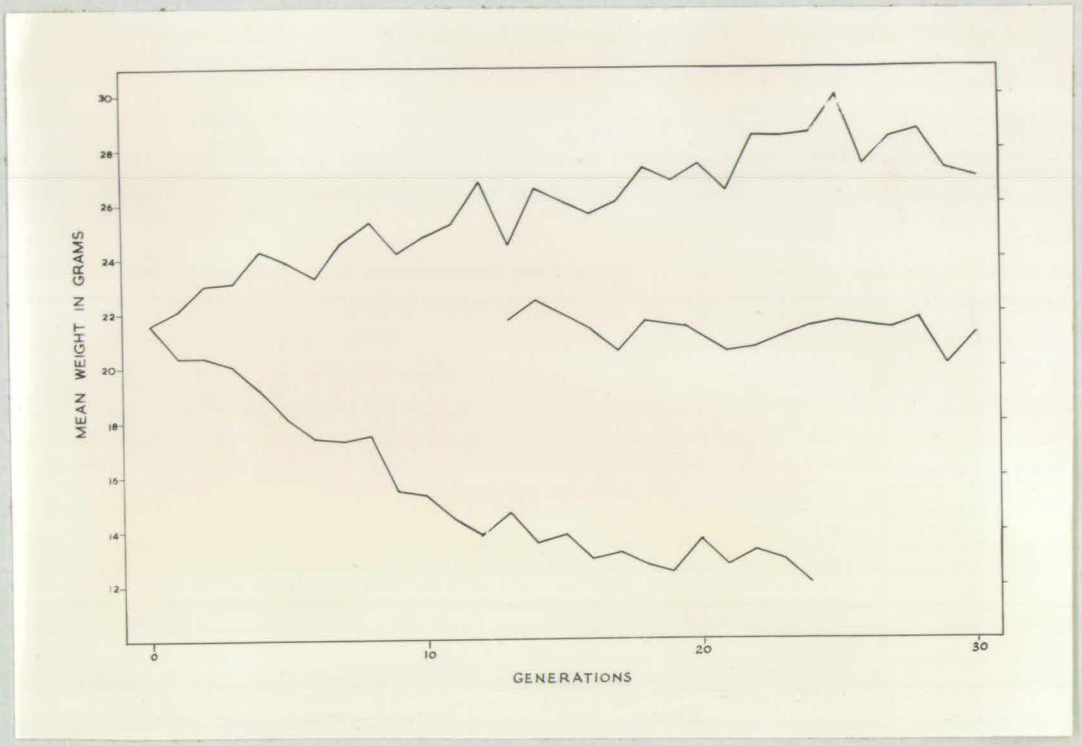

Figure 1. The progress already made in response to selection for large or small body size.
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CHAPTER 1

GROWTH STUDIES.

The growth of large, control and small lines was studied with two primary aims. First, a complete study was made of the growth patterns of the three lines from birth to maturity. Growth rates and body weights were compared to determine at what age differences in body weight between the three lines were first measurable, when the differences in growth rate were greatest, and whether differences in body size of the three lines were maintained throughout life. Secondly, relative growth rates were also compared in order to take into account the body weight at the beginning of the period of measurement as well as the absolute rate of gain.

Growth from birth to weaning is dependent both on the animal's own genotype and on the suckling ability of the mother, whereas subsequent growth is determined largely by the animal's own genotype. Comparisons that have been made will therefore be based on differences which may be a result of maternal influences or the animal's own genotype.

METHOD

Growth in large, control and small lines was studied by taking body weights from birth to 30 weeks. Matings of large, control and small lines were examined for births daily, and the error in age of offspring could therefore be up to 24 hours. Whole litters were weighed at birth, and the litter size was then reduced to eight where larger. Whole litters were weighed again at twelve days, and the mean weight of offspring at birth and at twelve days was calculated. Litters were weaned and sexed at 21 days and placed in storage cages (usually 6 to a cage).

Variations in body weight due to number, size and age of cage mates, and to temperature and diet, were minimised as far as possible. Individual weights were recorded from 21 days of age.

Three series of weights were taken of the three lines. In the first series, males and females were weighed at intervals from birth to twelve weeks. In the second, a group of males were weighed up to 30 weeks of age to obtain data on later growth (Series 2). Finally, in the third series, some males were weighed at two day intervals from 21 to 43 days for more precise information about the period of most rapid growth (Series 3). Weights were usually taken between 2.30 p.m. and 5.30 p.m. Taylor (unpublished) has shown that the body weight of a mouse generally falls during the morning, but remains relatively constant during the afternoon. Afternoon weights were therefore considered to be more reliable.

In the data on body weights, no correction has been made for litter size. The mean litter size of the large line (7.2) was higher than that of the small line (5.3), the controls being intermediate (6.8). Corrections made for differences in litter size would therefore exaggerate any differences in mean body weight between the three lines for litter size is negatively correlated with individual birth weights. Litter number was found to have no effect on mean three week weight of mice in the small line. The mean weight of males from first and second litters was 6.5 gms. This was not significantly different (t-test) from the mean weight of males from the fifth litter or more (6.3) when litter size was standardised to five or six for the comparison. Litter number has therefore also been ignored in data on mean body weights of all lines.

RESULTS

Mean birth weights and mean litter size (i.e. young born alive) of the three lines are given in Table 1. The differences in birth weights were significant ($P < .01$).

The mean body weights for the three lines from birth to twelve weeks (Series 1) are given in Table 1 and plotted in Fig. 2. The mean weight of females in any particular line was always higher than the mean weight of males in the lighter line (Fig. 2). At the time of the investigation, large differences existed in the weight of large, small and control lines. These differences expressed as a percentage of the control line are shown in Table 2:-

Table 2. Differences between the Mean Weights of Large and Small Lines.

Age / Difference as % of control weight	Birth	12 days	3 weeks	6 weeks	12 weeks
Males	23.2	38.4	49.5	75.0	70.4
Females			49.5	73.5	69.5

The mean body weights of males weighed up to 30 weeks of age (Series 2) are given in Table 3 and their growth curves are plotted in Fig. 3. During the investigation the small line was still responding to selection, consequently these males were lighter than those weighed up to 12 weeks which came from an earlier generation. The large line showed little or no response to selection during the course of these experiments, though a slight decrease in weaning weight was apparent. Differences in mean body weight between the three lines were fully maintained up to 30 weeks

of age, suggesting that the differences are permanent.

Both series showed that the growth curves (Figs. 2 and 3) of all three lines were similar in appearance when plotted arithmetically, but that the large line grew at a faster rate than the other two, the controls being intermediate. Differences in mean body weight at all stages of growth were highly significant (t-test). Selection had therefore markedly altered absolute growth rate and adult body weight, the differences obtained by selection persisting throughout life. Nevertheless, relative growth rates did not follow this pattern, as relative growth rates take into account the body weight at the beginning of the period of measurement.

Relative growth rates can be calculated as the instantaneous relative growth rate (k) from the equation derived by Brody (1945) who has criticised Minot's equation $\frac{(W_2 - W_1)}{W_1}$ for calculating percentage increases. k can be computed numerically as follows:-

$$k = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where $\ln W_2$ is the natural logarithm of the weight W_2 at time t_2, and $\ln W_1$ is the natural logarithm of weight W_1 at time t_1.

k is therefore the instantaneous relative rate of growth for a given unit of time. Values of k from birth to twelve weeks were calculated from data of Series 1 for large, small and control lines. Values from 12 to 30 weeks were calculated from Series 2. Values of k at various intervals between birth and 30 weeks are given in Table 4 for males and females separately, values for males from birth to 12 weeks being graphed in Fig. 4. Before 12 days, relative growth rates were similar for all three lines, but differences occurred from 21 to 35 days. From 21-28 days,

values of k for the large line were higher than those for the small line, those of controls being intermediate. Further, an increase in relative growth from 21 days which was found in large and control lines did not occur in the small line until 24 days and the relative growth in the small line was higher than in large and control lines from 28-35 days, indicating a retardation of growth in the small line. After 35 days, relative growth rate (though not absolute growth rate) was fairly similar in all three lines.

The time of maximum velocity of growth gives a measure of the equivalent physiological age of different lines (Brody 1945). An estimate of the time of maximum growth can be obtained by plotting absolute gain against time. Growth increments for two day intervals from 21 to 43 days are shown in Fig. 5. The pattern of absolute gain was similar in large and control lines, and the time of maximum growth was generally between 24 to 30 days. There was considerable variation, however, between animals within each line in time of maximum growth. After 30 days there was a rapid decrease in weight gained in each two day interval. In contrast, growth increments in the small line showed no definite peak; instead, the low level of gain was maintained up to 38 days after which time it began to decline.

DISCUSSION.

Both genetic and environmental factors would contribute to observed differences in birth weight, growth rate and adult body weights found between large, control and small lines of mice. However, Venge (1950) has shown that in reciprocal transplants of eggs between large and small strains of rabbits, both the genotype of the offspring and the uterine

environment influence birth weights. In the present experiment, inherited differences in mean birth weights of the three lines will therefore be influenced by factors such as maternal uterine environment, litter size, etc., which may be in turn dependent on the genotype of the mother. The differences in mean litter size of the three lines may be primarily genetic and largely dependent on the number of eggs shed. Venge (1953) has shown that in rabbits there is a relationship between inherited size and the mean number of ova produced per individual at each ovulation, but undoubtedly mean litter size will also be influenced by the capacity of the uterus to carry all the embryos.

Within each line of mice, litter size is probably the most important of the non-genetic factors contributing to differences in individual birth weights. A negative correlation between litter size and individual birth weights exists within offspring of a similar genotype (i.e. when genetic factors affecting mean litter size are excluded). The effect of litter size on birth weight (Bluhm 1929; Crozier & Enzmann 1935; and others) might be attributed to differences in gestation period, for Venge (1950) has shown that litter size influenced the period of gestation which in turn influences birth weight. Other non-genetic factors which may contribute to differences in birth weight include site of implantation, relative number of implants in each horn (Eckstein & McKeown 1955) and blood supply to the uterus.

Growth from birth to 14 days is to a large extent dependent on the suckling ability of the mother which in turn is partly under genetic control (Falconer 1947). Despite differences in birth weight, relative rates of growth are similar in the three lines of mice up to 12 days of age which indicates that the suckling ability of the small line mothers is not grossly

impaired, and that their young are as efficient in their growth as offspring from large and control lines. The effect on body weight of cross fostering large and small strains of mice was found to be maximal at 14 days (Butler and Metrakos 1950). Fostering altered growth rate but the effect was transitory and the adult body weight of fostered young was not significantly different from non-fostered litter mates. Butler and Metrakos also showed that weights between 14 and 20 days are determined as much by the genotype of the offspring as by the milk supply available. Falconer (1947) has shown that inbred lines suckle heavier litters when the offspring are hybrid and that the poor performance of inbred strains is in part due to the nature of the young. Venge (1953) was not able to show decisively any persistent effect of maternal influence on adult body weight after transplantation of ova between large and small strains of rabbits. Differences in weights between the three lines before weaning will therefore be a result of a joint effect between uterine and maternal influences on the one hand, and genetic potentialities of the individual on the other.

Differences in the absolute growth of the three lines are evident from birth onwards, and after 12 days differences also become evident in the relative growth rates of the three lines, the percentage increase in the small line being lowest. Both absolute and relative differences in growth rate reach a maximum from 3 to 5 weeks and decline thereafter. Also, the period of most rapid growth, which Brody (1945) has used as a measure of the equivalent physiological age, appears to be delayed in the small line when compared with large and control lines. The differences in absolute and relative growth rate which have been described are therefore primarily genetic and result from selection for large and small body

size. The power of selection to alter body size is therefore repeatable, as has been shown by the results of MacArthur (1944a) and Falconer (1953), in large and small mice, and by Goodale (1937) in large mice only.

The components of growth, i.e. the gain in body protein, water and fat, are obscured by measurements of total weight gained. The composition of the weight increases in the selected lines, and the proportions of gain in body weight which can be attributed to protein and to associated water (true growth), or to an increase in body fat, will be described in the next chapter.

Table 1. Mean Body Weight in Grams from Birth to 84 Days of Age.

Age in Days	Large Line (RNF) Males	Large Line (RNF) Females	Large Line (RNF) Mean Litter Size	Control Line (RNC) Males	Control Line (RNC) Females	Control Line (RNC) Mean Litter Size	Small Line (RNS) Males	Small Line (RNS) Females	Small Line (RNS) Mean Litter Size
0 (birth)	1.54±0.19		7.23	1.38±0.12		6.76±2.2	1.22±0.10		5.28
12	7.32		6.71	5.87		5.96	5.07		5.15
21	11.3±1.78	11.0		9.1±1.14	8.7		6.8±1.09	6.7	
24	14.6	14.1		11.3	10.5		7.5	7.2	
28	19.5	18.3		14.2	12.6		9.3	8.3	
35	27.0	23.6		18.9	16.3		12.2	10.4	
42	30.0±2.73	25.0		21.2±1.99	18.1		14.1±1.99	11.7	
49	32.2	26.0		23.0	19.0		15.4	12.3	
56	33.6	26.3		24.3	19.8		16.4	12.7	
63	34.6	27.7		25.3	20.5		17.6	13.3	
70	36.2	28.4		26.2	21.2		18.1	13.8	
77	37.8	29.4		26.8	21.7		19.0	14.4	
84	38.7±3.43	30.2		27.4±2.30	22.3		19.4±2.11	14.7	

Table 3. Mean Body Weight of Males from 21-210 Days of Age.

Age in Days	Large Line Weight in gms.	Control Line Weight in gms.	Small Line Weight in gms.
21	10.2	9.3	5.9
42	29.6	21.8	12.8
63	34.2	25.7	14.9
84	37.5	27.7	16.4
105	40.6	30.6	17.7
126	42.7	32.2	18.1
147	44.7	33.8	18.5
168	46.8	34.8	19.2
189	48.4	35.6	19.6
210	48.7	36.6	19.0

Table 4. Mean Percentage Increase Per Day (k).

Age in Days	Large Line (RMF)		Control Line (RNC)		Small Line (RNS)	
	Males	Females	Males	Females	Males	Females
0 (birth) -12	13.00		12.05		11.88	
12-21	4.82	4.48	5.13	4.38	3.26	3.01
21-24	8.45	7.36	6.43	6.26	3.44	2.34
24-28	7.21	7.97	5.70	4.48	5.33	3.55
28-35	4.64	3.62	4.07	3.59	5.76	3.14
35-42	1.50	0.82	1.64	1.50	2.15	1.75
42-49	1.01	0.53	1.18	0.70	1.27	0.64
49-56	0.64	0.19	0.79	0.58	0.91	0.46
56-63	0.42	0.72	0.58	0.48	0.99	0.70
63-70	0.64	0.37	0.50	0.52	0.39	0.51
70-77	0.62	0.48	0.32	0.37	0.65	0.58
77-84	0.34	0.36	0.32	0.36	0.36	0.33
84-105						
105-126	0.23		0.24		0.12	
126-147	0.22		0.22		0.10	
147-168	0.22		0.15		0.17	
168-189	0.16		0.10		0.11	
189-210	0.03		0.14		0.15	

Values of k from 0-84 days for all lines were calculated from Series 1.

Values of k from 84-210 days for males of all lines were calculated from Series 2.

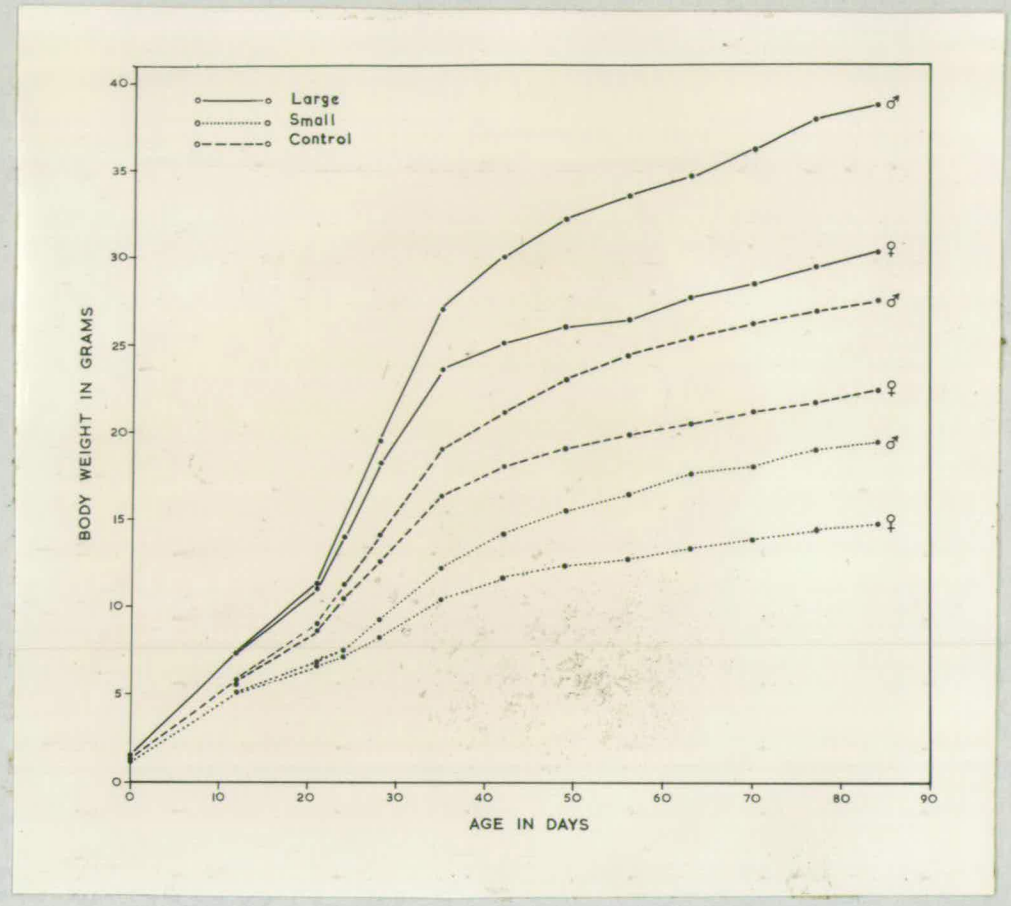

Figure 2. Growth curves of males and females of large, control and small lines from birth to 84 days of age.

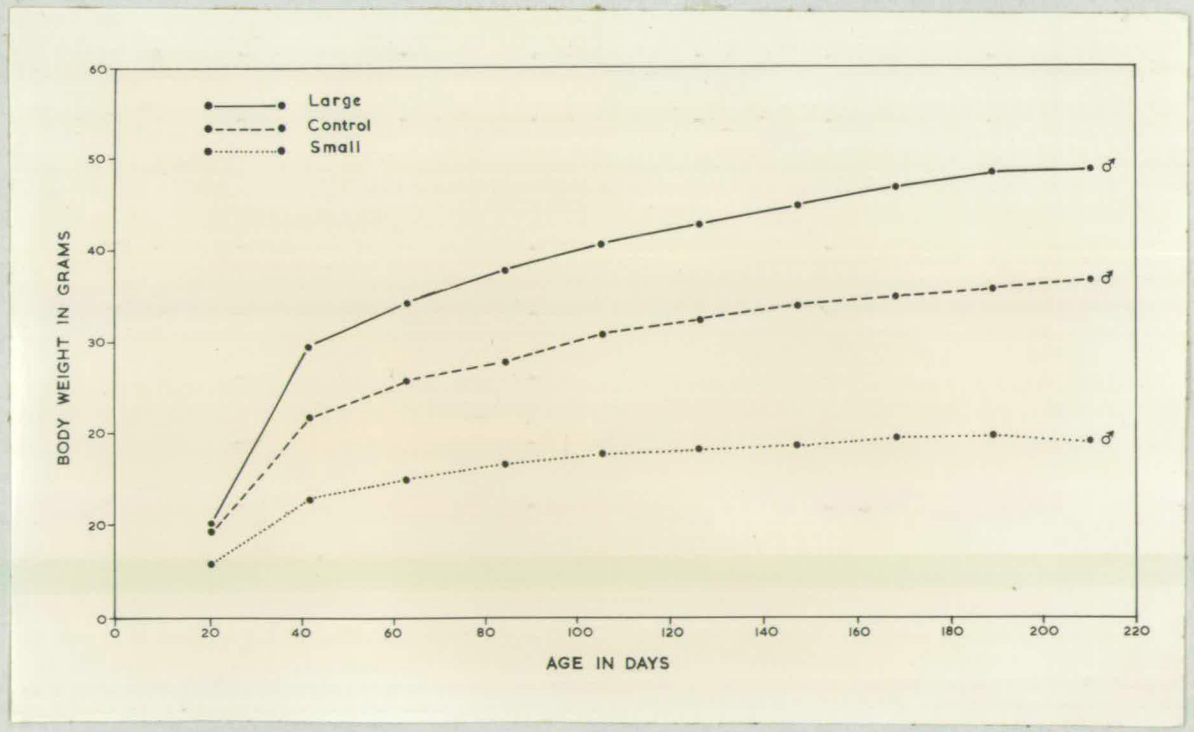

Figure 3. Growth of males of large, control and small lines from 3-30 weeks of age.

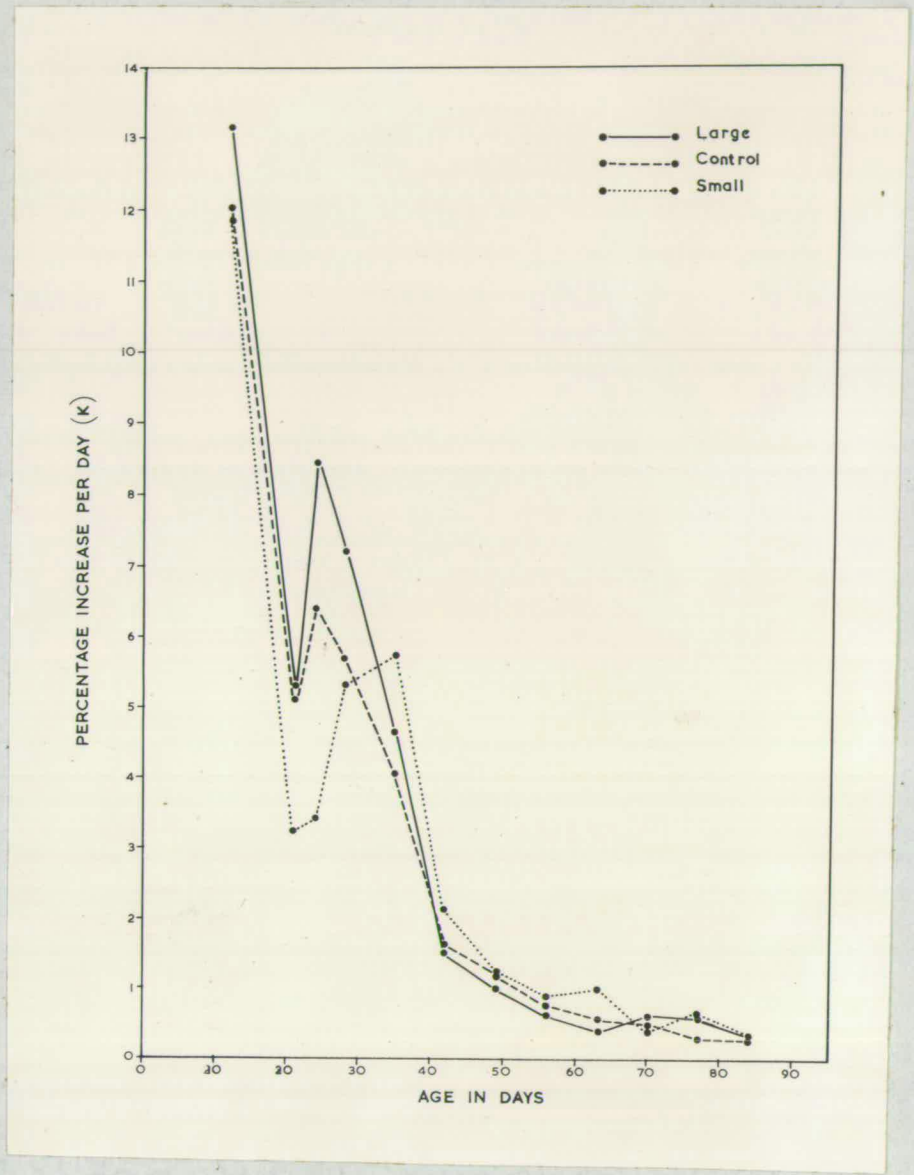

Figure 4. Percentage increase per day of males of large, control and small lines from birth to 84 days of age.

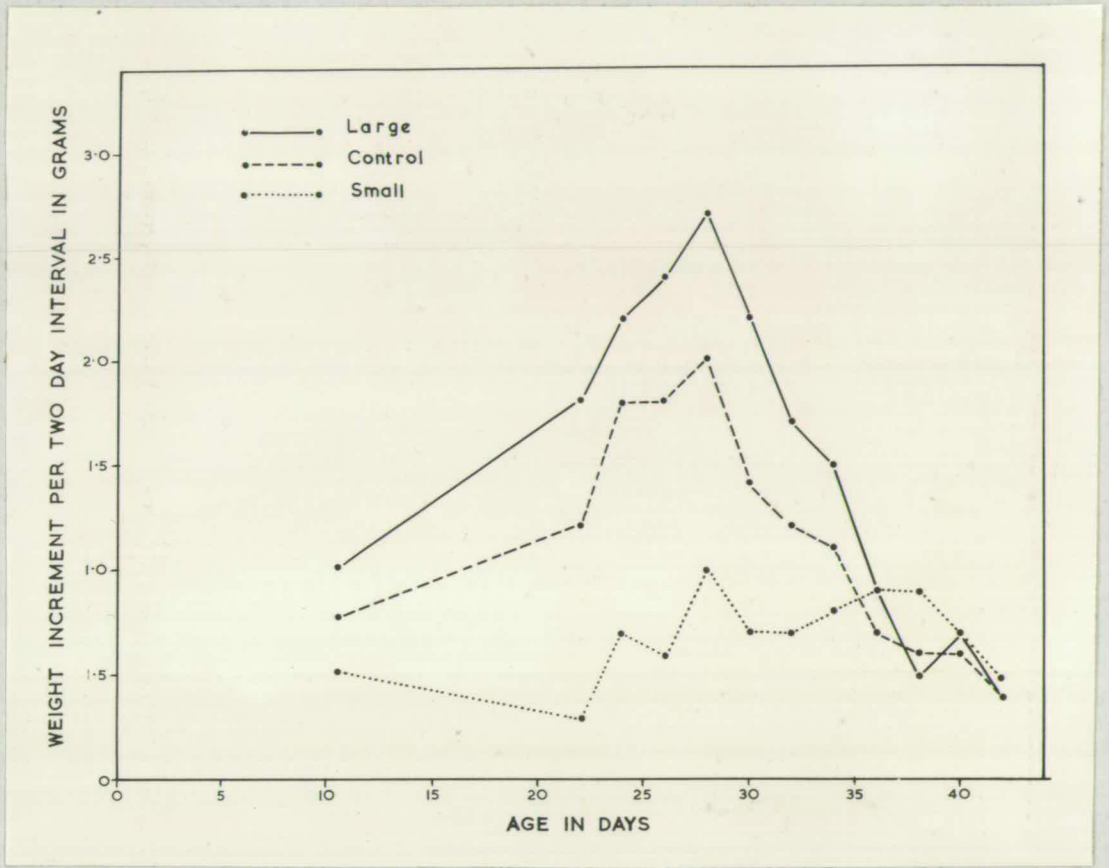

Figure 5. Growth increment for each two-day interval from 21-43 days of age.

CHAPTER 2

CARCASS COMPOSITION

Periodic measurements of change in body weight, though of considerable use for routine purposes, are not sufficient to measure true differences in growth between lines, for similar growth patterns may obscure differences that exist in body (carcass) composition. The following analysis was therefore undertaken to trace any differences in carcass composition between large, control and small lines at different ages, the observations being restricted to males.

Two distinct series of analysis were carried out. In the first series numbers of carcasses were analysed for fat and water content at three ages, i.e. 6, 9 and 12 weeks, to determine whether differences in carcass composition existed between these lines. In the second series, the analysis was concerned both with changes in carcass composition during growth within a line, and with the differences between large and small lines during the growing period.

Some mice from another strain selected for large body size were also analysed for nitrogen, fat and water. This strain (CRL) was completely unrelated to the RNF line and was derived as follows. Mice of four unrelated stocks, a crossbred derived from Goodale's and MacArthur's large strains (Falconer & King 1953), a stock selected for high lactation (Bateman 1954), and two mutant stocks, were crossed and selected for large body size. Selection, which was within families, was based on weight gained from 3 to 6 weeks of age. The purpose of the analysis was to compare carcass composition of two large strains which had been selected on similar criteria.

METHOD OF CARCASS ANALYSIS

The animals were killed and bled. The gut was excised without removing any abdominal fat or connective tissue from the carcass. The carcass was then weighed, cut into small pieces, and dried to a constant weight at 60°C to 80°C. Temperatures below 60°C were insufficient to dry the carcass before it decomposed; temperatures above 80°C were found to cause vaporisation of fat. The difference in weight before and after drying was regarded as the water content of the carcass. The ether soluble material of the whole carcass was then extracted in a Soxhlet Extractor using petroleum ether (B.P. 40°C to 60°C) as solvent. After evaporation of the petroleum ether the residue was weighed and this fraction was taken to represent carcass fat. Small quantities of non-fatty acids, e.g. cholesterol, which may be present in the residue as well as fat have been ignored in the analysis.

Total nitrogen was estimated in the same carcass by the Kjeldahl method, which depends on the conversion of various nitrogenous compounds into ammonium sulphate by boiling with concentrated nitrogen-free sulphuric acid. Subsequent addition of excess sodium hydroxide liberates ammonia which is collected in saturated boric acid solution and titrated as ammonium borate with acid of known strength. The estimated nitrogen, multiplied by the factor 6.25 gives the protein content of the carcass; this factor is based on the assumption that carcass protein contains on the average 16% of nitrogen. In the estimation of body nitrogen, sampling errors were reduced by digesting the entire carcass with sulphuric acid, and nitrogen determinations were made from samples of whole digest. The difficulty of obtaining a uniform sample of the carcass for analysis was therefore avoided.

RESULTS

Analysis 1. Fat and Water Content at 6, 9 and 12 weeks of Age in Large, Control and Small Lines of Mice.

The mean values for fat and water content of the carcass of mice from large, control and small lines at 6, 9 and 12 weeks of age are given in Table 5. Altogether, 92 mice were analysed (Table 5), and the data was treated statistically. A comparison of the proportion of fat and water in the carcass showed that animals in the large line consistently had more fat and less water than those of the small line at the three ages ($P < 0.05$). The percentage of fat increased with age in both lines but relatively faster in the large line. Control animals had a similar carcass composition to the small line at the three ages tested despite large differences in body weight.

Analysis 2. Protein, Fat and Water Content in the Carcass of Mice from Large and Small Lines from 13 to 107 Days of Age.

The quantity of carcass protein as well as grams of fat and water were determined in this analysis. The carcasses of 31 mice from the large line and 25 from the small line were analysed at irregular intervals from 13 to 107 days of age. The mean body composition, i.e. the mean amounts of protein, water and fat in the carcass of mice selected for large or small body size using the results obtained from analyses 1 and 2, are plotted against age in Figs. 6 and 7. Values for carcass protein, water and fat are plotted against carcass weight in Figs. 8 and 9. All data is given in Tables 6 and 7.

The total amounts of each body component will first be considered. Total body protein per carcass in mice of the large line showed an approximately linear increase up to 40 days of age after which time the rate of

protein deposition was reduced.

Protein formed a constant proportion of the carcass of animals of the large line up to a weight of approximately 22 grams, but above this weight the proportion of body protein appeared to decline (Fig. 8). The increase in carcass water in the large line was closely associated with carcass protein and showed a linear increase up to about 40 days of age, followed by a decrease after 40 days. The degree of hydration of body protein (i.e. the water/protein ratio) decreased with increasing age (Table 6), the decrease being most marked up to 46 days of age. This decrease in the water/protein ratio may be due to loss of extra-cellular water. In the large line fat was deposited at a slow rate before 35 days, so that prior to this age gain in weight was largely due to an increase in protein and associated water. From 35-60 days of age, however, the fat content of 'large mice' increased considerably (Fig. 6), and represented most of the total increase in body weight during this period. After 60 days there was a reduction in the rate of gain of body fat.

In the small line protein was deposited at a slower and more even rate over a longer period than in the large line and formed a constant proportion of the carcass at all carcass weights (Figs. 7 & 8). The water content of mice of the small line was closely correlated with body protein as in the large line, increased at an even rate over a longer period of time, and was fairly similar to, though slightly lower than, that of the large line for identical carcass weights despite large differences in age. The degree of hydration of body protein decreased with increasing age in a manner similar to that found in the large line. In contrast to mice of the large line there was no sudden increase in the small line in the rate of fat deposition at 35 days of age, the fat content of the carcass showing

a continuously slow but steady rate of increase. The amount of fat in the small line was approximately similar to, though slightly higher than, that of the large line for identical carcass weights, despite large age differences.

The percentage compositions of carcasses plotted against age for the large and small lines are shown in Figs. 10 and 11. The percentage of protein decreased slightly after approximately 40 days of age in the large line, whereas in the small line there was little, if any, change in the proportion of body protein over the whole age range (Fig. 10). The slight fall in % protein in the large line was largely due to a substantial increase in body fat (see below).

The percentage of fat was similar in the two lines up to 40 days of age (Fig. 11). The substantial increase in proportion of body fat, which had already been found in the first analysis from 6 weeks of age, actually began at about 38-40 days of age. In the small line, the increase in percentage fat was slight and gradual. The proportion of body fat and water after six weeks of age in mice of the large line agreed closely with results from the first analysis; in mice from the small line, however, the proportion of body fat was lower and the percentage of water was higher after 6 weeks of age than found in the first analysis. But, at the time of the second analysis, the mean carcass weight had also declined in the small line. The decrease in weight at 6 weeks of age between the time of the first and second analysis (1.4 gm) was probably largely due to a further decrease in fat in response to selection, for the amount of decrease in fat (0.51 gms.) was greater than the proportional decrease of any other component of the carcass, and represented approximately one-third of the total decrease in carcass weight.

The increase in percentage fat was in contrast to the decrease in percentage water with increasing age (Fig. 11). The percentage of water in the carcass was similar in the two lines until about 40 days, and thereafter was less in the large line. As was to be expected, the percentage water was negatively correlated with percentage fat (Fig. 11). The fall in percentage water found in the large line from 40 days was due not only to a decrease in the rate of water deposition associated with a reduced rate of protein deposition, but also to a substantial increase in fat deposition. To summarise the results of both analyses, growth in the large line from 14-40 days was largely due to increases in protein and associated water, whereas after this age increase in weight was caused mainly by fat deposition. In the small line, however, protein and associated water and fat were deposited at a more constant rate during the whole period of growth.

Analysis III. Comparisons of Carcass Composition of Two Strains of Mice Selected for Large Body Size.

Mice of two large strains (CRL and RNF) were analysed to trace differences in the composition of carcasses of similar weight and age, and to compare their carcass composition after selection for large size on slightly different criteria. (Strain CRL included mice of two sub-strains CFL and CRL. Both were derived from the same population and selected on weight gained from 3 to 6 weeks of age, but CRL mice were fed on a low plane diet, and CFL mice on a full diet between the ages of 3 to 6 weeks. No significant differences in carcass composition were found between the two sub-strains, and for the present purposes they will both be designated CRL). Data on RNF carcasses has been given previously in Analyses I and II. CRL mice were analysed at six and eight weeks of age only; details of

their carcass composition are given in Table 8 which also includes mean values for RNF mice at similar ages for comparison.

Although CRL mice were analysed at 6 and 8 weeks only, their carcass weights ranged from 19.2 to 32.5 gms. For similar carcass weights, the protein and water content was higher and the fat content lower in CRL than in RNF mice.

The mean percentages of fat and water for CRL mice of six weeks of age were compared with similar values found in the first analysis for RNF mice of the same age. The percentage of fat (9.0%) and water (67.0%) in CRL mice differed significantly ($P < 0.05$) from those in RNF mice (15.1% and 61.2% respectively).

It was concluded from the above data that for similar carcass weights or ages the percentage of protein and associated water was higher, and the proportion of fat lower in CRL than in RNF mice.

DISCUSSION

Induced Differences in Carcass Composition in Response to Selection for Body Size.

Falconer's selection programme for large and small body size, based on weight at six weeks of age, has altered both the total amounts of protein, water and fat in the carcass and also the percentages of these components in the carcass. The percentage composition of mice of the large and small lines was fairly similar during the period up to 35 days, i.e. when most of the increase in weight in both lines could be attributed to 'true growth' (protein deposition and bone growth). There was a considerable increase in the amount of carcass fat in mice of the large line from 35 days, however, which resulted in a lower percentage of protein

and water and a higher percentage of carcass fat in the large than in the small line after this age. Different rates of growth may account for some of the differences in percentage composition found between the large and small lines. In most animals a period of 'true growth' is followed by further increases in weight which can mainly be attributed to deposition of fat. Mice of the small line apparently fail to reach the stage of fat deposition, but continue 'true growth' to advanced ages. If the percentage composition of mice of the large and small lines are compared during the period of 'true growth', the proportion of body fat is higher and body protein and associated water is lower in the small than in the large line for similar carcass weights although the differences are slight. Some of the differences in carcass composition within a strain can therefore be attributed to different growth rates, so that at any absolute age each line, being at a relatively different stage in development (i.e. different physiological age), will have a different carcass composition.

Modification of the growth rate by selection has altered the specific body composition of the three lines, as well as adult body size. Increased body weight with rapid gains and increased efficiency of utilisation of foodstuffs has often been found to be associated with an increase in fatness of the carcass. Palmer et al. (1946) and Dickerson (1947) have studied carcass composition in rats and pigs in relation to inherited differences in efficiency of food utilisation and rate and economy of gain respectively. In two genetically distinct strains of rats which had been selected for high and low efficiency of food utilisation, the low efficiency strain had a higher percentage of water and a lower percentage of fat than the high efficiency strain (Palmer et al. 1946). Similarly, Dickerson (1947), working on hog carcasses found that increased rate and economy of gain

was more largely associated with increased fat deposition than in bone and muscle growth, and that low feed requirements and rapid fat deposition were positively correlated.

Growth data alone, based on total body weight, may therefore be misleading in that similar growth patterns may obscure real differences in the character of the weight gains. Falconer made his selection on body weight at six weeks of age; at this age, however, fat is being deposited rapidly and protein deposition is nearly completed so that differences between animals in protein content may be obscured by variations in the amount of fat already deposited. If, however, selection had been made at 5 weeks of age, when fat deposition has barely commenced, the differences in weight between animals would have been entirely attributable to differences in protein and associated water, and heritable differences in fatness might therefore have been excluded.

It may be, however, that body protein is highly correlated with body fat, i.e. an increase in protein content of the carcass would be associated with an increase in carcass fat. Selection against carcass fat might then be difficult. The correlations between the body components in the large line (RNF) were therefore calculated. Body protein was very highly correlated with body water over all ages ($r = 0.99$) which may be expected for water is bound to protein in the tissues to maintain osmotic equilibria. Body water has therefore been used as an index of the total protein content of the carcass. In contrast, fat deposition is known not to be associated with an increase in body water. The correlation coefficients between body fat and body water were found to be +0.04, +0.63 and +0.38 at six, nine and twelve weeks respectively. Body fat and body water are therefore positively correlated but as body water is almost entirely

associated with carcass protein it should be possible to select against carcass fat, thereby increasing improvement in carcass protein and associated water.

The changes in body components in response to selection for large and small body size are as follows:-

	SMALL LINE		LARGE LINE	
	Change in Body Weight from Control Mean	% Change	Change in Body Weight from Control Mean	% Change
Total change	-5.4 gms.		+6.5 gms.	
*Protein change	-1.36 gms.	25.2	+1.26 gms.	19.4
Water change	-3.90 gms.	72.2	+3.22 gms.	49.5
Fat change	-0.14 gms.	2.6	+2.02 gms.	31.1

*Also includes changes in mineral, e.g. bone growth (see Tables 6 and 7)

Selection for small size has mainly affected deposition of protein and associated water, whereas selection for increased size has had the effect of increasing fat deposition as well as protein deposition. This asymmetrical response may be a result of selection at six weeks of age, because selection for increased body size will be made not only for those animals with increased protein deposition but also for those animals in which fat deposition is accelerated and occurs at an earlier age. It is conceivable that acceleration of fat deposition prior to the time of selection may be made at the expense of body protein and water. Carcass fat in the control line ranged from 6.3% to 11% of the carcass weight at six weeks indicating that there was considerable variation in carcass fat in the control line upon which selection might operate. Analysis of the

carcass of control animals prior to six weeks would give the time of deposition of fat with respect to protein deposition, and it would then be possible to determine whether acceleration of fat deposition in addition to the amount deposited had actually occurred in the large line. In contrast to this, selection for decreased body size has retarded both protein and fat deposition with a result that percentage composition of the carcass of small line mice was similar to that of unselected controls.

Very little is at present known of the factors causing an asymmetry of response to selection. In the strains at present being analysed, Falconer (1953, 1955) suggested that most of the asymmetry in total body weight could be attributed to a maternal influence on weaning weight of the offspring, large mothers being of relatively lower suckling ability as there was little asymmetry in weight gained from three to six weeks. This implies that an increase in fat content of the carcass tends to be associated with relatively poor suckling ability which in turn may affect the weaning weight of the offspring, and is in agreement with Dickerson (1947) and Dickerson and Grimes (1947) who showed that in sows, suckling ability tended to be negatively correlated with rapid fat deposition and low feed requirements.

Strain Differences in Response to Selection for Large Body Size.

Strain differences in carcass composition were found between CRL and RNF mice, CRL mice having a higher percentage of protein and associated water and a lower percentage of fat than RNF mice of the same carcass weight or age. Specific body composition is therefore under genetic control and capable of modification by selection, although within a strain some of this modification may be directly related to changes in

the growth rate as described previously. As growth rates in the two large strains analysed were similar, the differences found in carcass components could not be attributed to differences in growth rate but may be due to a number of other factors. The additive genetic variance of the foundation population must influence the rate of progress in response to selection for any character such as body size and the genes present in the foundation population will affect the nature of the response. Falconer's large line (RWF) was derived from a foundation population obtained by crossing four highly inbred lines, two of which were related in origin. He estimated that the additive genetic variance not available because the foundation population was constructed from four inbred lines would be half of the total variation in a random-bred population (Falconer 1953). In the formation of inbred lines, loci that are inviable in the homozygote will have been lost; certain genes that are present in an outbred stock may therefore have been lost in the formation of inbred lines. As outbred stocks were used in the construction of the foundation population from which the CRL strain was derived, it contained twice the additive genetic variance of the foundation population from which the RWF strain was derived. A large number of genes for increased body size must also have been present in this foundation population for one of the outbred strains was a cross-bred derived from Goodale's and MacArthur's large strains in which selection for genes increasing body size had already been made. The mean body weight in the earlier generations of selection was therefore considerably higher in the CRL strain than in the RWF strain.

Moreover, the criteria of selection, although similar, were not identical. In both cases selection was made within families but whereas RWF mice were selected on body weight at six weeks of age, CRL mice were

selected on weight gained between three and six weeks of age. Animals with the greatest weight gain from three to six weeks are not always the heaviest in the litter. When selection is made at six weeks of age, a considerable proportion of the variation within families may be due to fat already deposited, for fat deposition is very rapid from 38 to 46 days. The different criteria of selection may therefore have caused differences in the carcass compositions of the two large lines.

If two lines derived from a foundation population were selected on these two criteria, it would be of interest to compare carcass composition of the two lines after some generations of selection. If selection on total body weight is identical to selection on weight gained from three to six weeks, the rate of progress in response to selection and the nature of the response should be similar in the two lines. If this were not so, a comparison with the foundation population would show which line had deviated most during selection.

Physiological Analysis in Relation to Selection.

Although mean body weight and therefore total amounts of carcass components have been considerably altered by selection for particular genotypes, no substantial increases have been made in the percentage of protein in the carcass. Selection for increased body size has in one case maintained the proportion of protein in the carcass (CRL) but in the RNF line fat deposition accounts for a substantial part of the increase in total weight and percentage protein is even slightly decreased.

If 'true growth' is required, that is, a further increase in body protein as opposed to fat deposition, some other criterion than body weight is necessary for the basis of further selection programmes. For a selection programme in which increase in body protein is the primary

aim, the rate of improvement is unlikely to be maximum if selection is made on total body weight and the character of weight gains are not known, as the present work has shown that the relative amounts of fat, protein and water contributing to total carcass weight may differ in selection programmes in which the absolute rate of increase in weight is the same. In the application of selection programmes to domestic livestock improvement, increase in body protein ('true growth') is obviously desirable not only for production purposes but because increased fatness may lead to a decline in fertility (Lane & Dickie 1954).

Fat and water content can now be estimated in living tissues by measurement of density (Behnke 1942) and by tracer dilution techniques (Soberman et al. 1949; Mayer & Hagman 1953). The use of such techniques in a selection programme might increase the rate of improvement for carcass protein as the variation in weight due to carcass fat will have been excluded. It is evident that a method of assessing carcass composition on the live animal would be of practical value and might improve the efficiency of selection for true growth.

Artificial selection for a quantitative character such as body size reveals genetic variability and alters the mean body size far beyond the limits of variation found in the original population. A more rapid advance in our understanding of quantitative characters might be made if selection for particular genes or gene complexes affecting a quantitative character was related to basic physiological processes which affect growth and through which the 'growth genes' must act. If, prior to selection, an investigation was made into some of the more basic physiology associated with growth such as time and onset of fat and protein deposition and rate and economy of gain, selection might then be made with reference to at

least some of the physiological aspects concerned with growth. A character such as increased body size might then be subdivided into various physiological units which are directly related to increased body size, any one of which might aid further selection.

The present physiological investigation has been made at a time when differences in body weight between selected lines were already extreme as considerable selection had already taken place. There is, unfortunately, no data on the physiological make-up of the foundation population and knowledge of this population can only be derived from the control line which was constructed at a later date than the selected lines and which, although not deliberately selected, may differ from the original foundation population from which it was formed. Some information concerning the physiological differences occurring in response to selection for body size has already been presented and further aspects relating body size to other physiological processes will be discussed in later chapters.

Table 5. Mean Composition of the Carcass of Large, Control and Small Lines.

Age in Weeks	No. of Animals	Line	Weight (gms)	Carcass Weight (gms.)	Carcass Components Fat gms.	Water gms.	Dry Non Fat gms.	% Composition of Carcass % Fat	% Water	% Dry Non Fat
6	16	Large	29.8	23.9	3.6	14.8	5.5	15.1±3.16	61.2±3.12	23.7
6	6	Control	-	17.4	1.6	11.6	4.2	9.1	66.4	24.5
6	11	Small	14.6	12.0	1.5	7.7	2.8	11.9±1.86	64.1±2.65	24.0
8	6	Control	26.7	22.5	3.2	12.9	6.4	13.8	57.8	23.4
9	10	Large	34.0	28.3	5.8	16.0	6.5	20.4±3.43	55.9±2.49	23.7
9	17	Small	16.8	14.3	2.4	8.6	3.3	16.5±4.62	60.3±4.29	23.2
12	14	Large	39.4	33.4	9.3	17.1	7.0	27.6±2.71	50.8±2.36	21.6
12	5	Control	31.6	26.6	4.7	15.5	6.4	17.7	58.4	23.9
12	13	Small	19.7	16.9	2.8	10.4	3.7	16.3±4.81	61.6±4.01	22.1

Table 6. Carcass Composition of the Large Line (RNF).

Age (days)	Weight (gms.)	Carcass Weight (gms.)	Carcass Composition in gms. Protein	Fat	Water	Residue (i.e. minerals, etc.)	% Composition of Carcass Protein	Fat	Water	Water / Protein Ratio
13	6.8	6.04	1.12	.32	4.54	0.06	18.6	5.3	75.2	4.1
13	6.6	5.89	1.08	.25	4.42	0.14	18.3	4.3	75.0	4.1
19	8.9	7.46	1.49	.39	5.41	0.17	19.9	5.2	72.5	3.6
19	8.8	7.39	1.46	.49	5.28	0.16	19.7	6.6	71.4	3.6
21	10.0	8.00	1.60	.36	5.78	0.26	20.0	4.5	72.3	3.6
24	12.8	10.33	1.93	.65	7.44	0.31	18.7	6.3	72.0	3.9
28	17.5	13.51	2.56	.97	9.62	0.36	19.0	7.2	71.2	3.8
31	20.6	15.04	2.75	.86	10.87	0.56	18.3	5.7	72.3	4.0
31	21.5	16.61	3.10	1.27	11.77	0.47	18.7	7.6	70.9	3.8
34	16.6	11.29	2.10	.69	8.03	0.47	18.6	6.1	71.1	3.8
38	27.7	21.09	3.79	2.38	13.83	1.09	18.0	11.3	65.6	3.7
38	26.2	19.61	3.63	1.06	13.16	1.76	18.5	5.4	67.1	3.6
39	30.4	23.11	4.47	2.33	15.43	0.88	19.3	10.1	66.8	3.5
39	29.6	22.83	4.35	2.07	15.33	1.08	19.1	9.1	67.1	3.5
42	28.7	23.28	4.12	3.42	14.86	0.88	17.7	14.7	63.8	3.6
42	30.7	24.75	4.86	3.56	15.44	0.89	19.6	14.4	62.4	3.2
42	30.3	24.01	4.38	3.63	14.90	1.10	18.3	15.1	62.1	3.4
46	32.8	26.22	4.73	5.09	15.44	0.96	18.0	19.4	58.9	3.3
46	30.9	25.38	4.60	5.11	15.08	0.59	18.1	20.1	59.4	3.3
52	32.1	25.57	4.58	5.37	14.64	0.98	17.9	21.0	57.3	3.2
63	-	32.11	5.68	7.75	17.72	0.96	17.8	24.1	55.2	3.1
63	34.0	28.44	4.80	6.72	15.79	1.13	17.3	23.6	55.5	3.3
63	33.7	28.14	4.55	5.49	16.46	1.64	16.2	19.5	58.5	3.6
66	35.7	30.14	5.32	6.74	17.02	1.06	17.7	22.4	56.5	3.2
72	36.9	31.93	-	7.02	17.85	-	-	22.0	55.9	
76	39.9	32.95	-	6.22	18.58	-	-	18.8	56.4	
81	35.0	29.20	5.23	6.04	16.71	1.22	17.9	20.7	57.2	3.2
84	36.6	31.06	5.25	7.78	16.60	1.43	16.9	25.0	53.4	3.2
93	39.0	31.24	5.92	5.41	18.43	1.48	18.9	17.4	59.0	3.1

Table 7. Carcass Composition of the Small Line (RNS).

Age (days)	Weight (gms.)	Carcass Weight (gms.)	Carcass Components in Gms. Protein (N x 6.25)	Fat	Water	Residue (i.e. minerals)	% Composition of Carcass Protein	Fat	Water	Water / Protein Ratio
14	5.0	4.42	0.79	0.35	3.30		17.9	8.0	74.7	4.2
14	4.7	4.27	0.79	0.30	3.10	0.08	18.5	7.1	72.7	3.9
17	4.2	3.65	0.66	0.27	2.78	0.04	18.1	7.4	76.2	4.2
17	5.1	4.34	0.79	0.42	3.09	0.04	18.2	8.7	71.2	3.9
24	5.8	4.84	0.96	0.12	3.57	0.19	19.8	2.5	76.5	3.7
24	8.3	6.35	1.18	0.30	4.67	0.20	18.6	4.7	73.5	4.0
27	8.3	6.44	1.36	0.28	4.66	0.14	21.1	4.4	72.4	3.4
27	10.3	8.16	1.53	0.47	5.87	0.29	18.8	5.8	71.8	3.8
30	8.1	6.36	1.23	0.40	4.58	0.15	19.4	6.3	72.2	3.7
33	9.7	7.92	1.58	0.44	5.71	0.19	20.0	5.6	72.0	3.6
33	11.8	9.76	1.84	0.86	6.76	0.30	18.9	8.8	70.0	3.7
35	11.1	8.97	1.89	0.63	6.28	0.17	21.1	7.0	70.0	3.3
42	13.4	11.23	2.15	0.91	7.70	0.47	19.2	8.1	69.0	3.6
42	13.8	11.48	2.28	0.90	7.83	0.47	19.8	7.8	68.0	3.4
42	11.2	9.23	1.80	1.02	6.11	0.30	19.6	11.1	66.4	3.4
53	14.2	12.11	2.50	0.91	8.16	0.54	20.7	7.5	67.4	3.3
58	17.0	14.77	3.18	1.30	9.88	0.41	21.4	8.8	66.7	3.1
63	14.5	12.12	2.32	1.37	7.92	0.51	19.2	11.3	65.3	3.4
66	15.3	12.62	2.54	1.40	8.11	0.57	20.2	11.1	64.3	3.2
69	13.2	10.84	2.07	1.10	7.18	0.49	19.1	10.2	66.5	3.5
74	14.3	11.80	2.50	1.29	7.83	0.18	21.2	10.9	66.3	3.1
84	19.1	16.67	3.50	1.36	11.10	0.71	21.0	8.1	66.3	3.2
107	15.3	13.63	2.58	2.00	8.42	0.63	19.0	14.7	62.0	3.3
107	19.7	16.69	3.51	1.48	10.82	0.88	21.0	8.9	64.7	3.1
127	17.0	14.10	2.82	1.52	9.17	0.59	20.0	10.8	65.0	3.3

Table 8. Mean Carcass Composition of Two Large Strains (CRL and RNF).

Age in Weeks	Strain	Weight in gms.	Carcass Weight in gms.	Carcass Components in gms. Water	Fat	Protein	% Composition of Carcass % Water	% Fat	% Protein	Water/Protein Ratio
6	CRL	30.3±3.6	23.9±3.1	16.0±2.1	2.2±0.8	4.63±0.8	67.0±0.9	9.0±1.3	19.61±0.9	3.46
	RNF	29.8	24.0	14.8	3.6	4.4	61.2±3.12	15.1±3.16	18.5	3.32
8	CRL	36.8±2.5	29.8±2.1	18.9±1.1	3.8±0.7	5.9 ±0.5	63.5±1.7	12.6±1.8	19.7 ±0.5	3.19
	RNF	-	29.1	16.7	6.0	5.0	57.5	20.9	17.3	3.32

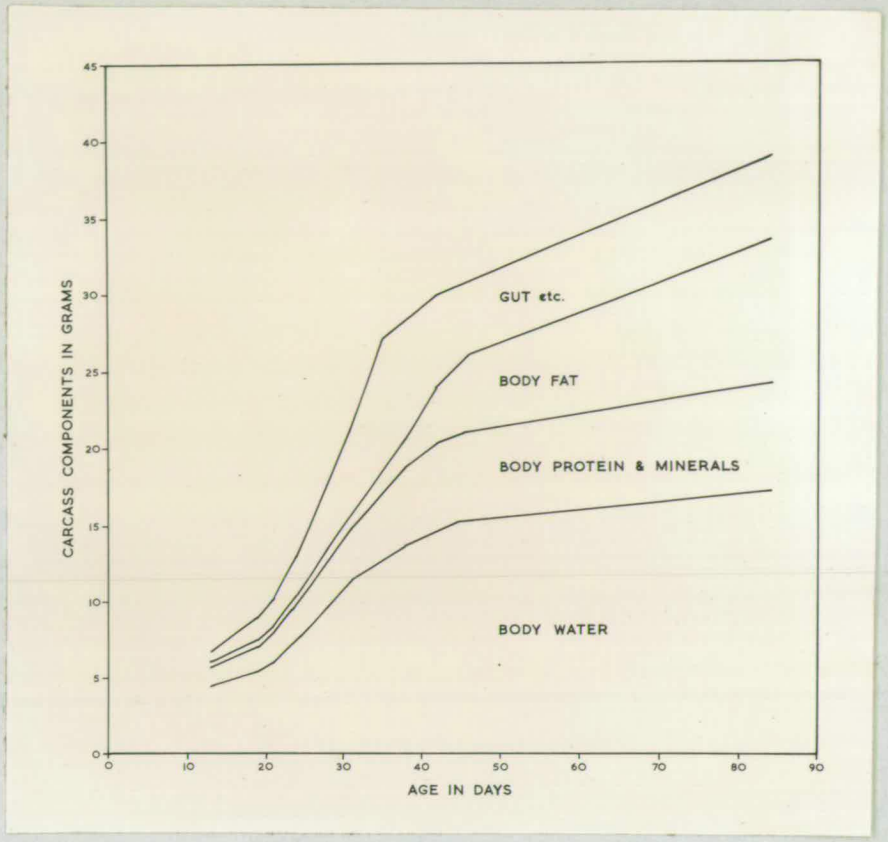

Figure 6. The relation between carcass composition and age, in mice of the large line (RNF).

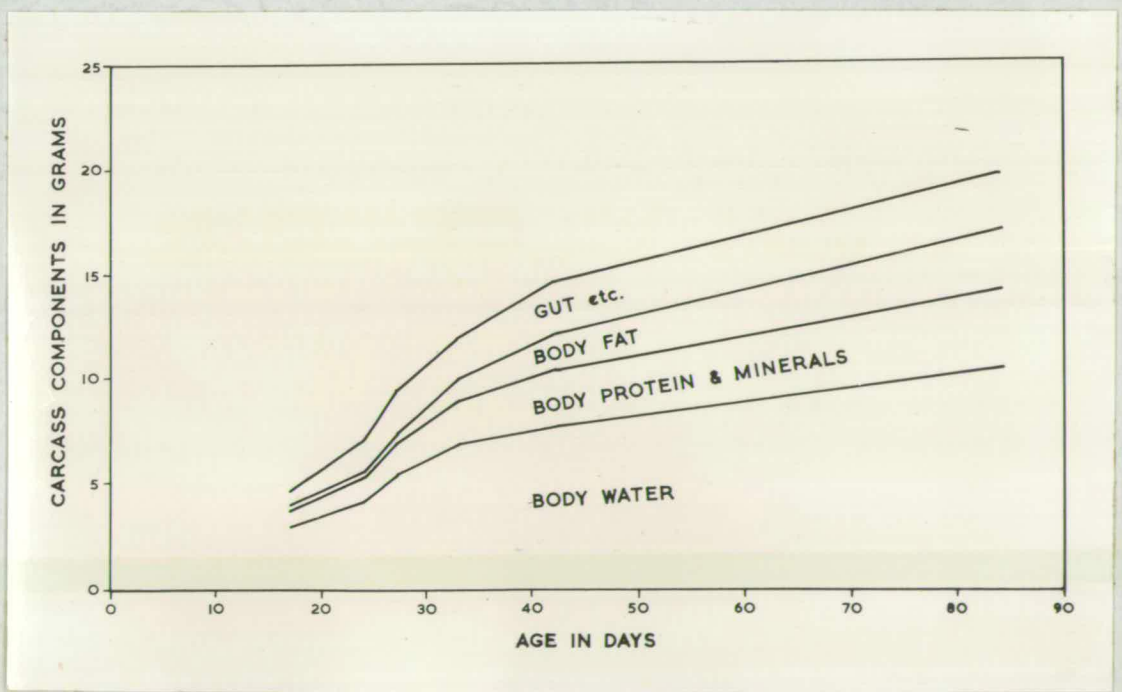

Figure 7. The relation between carcass composition and age, in mice of the small line (RNS).

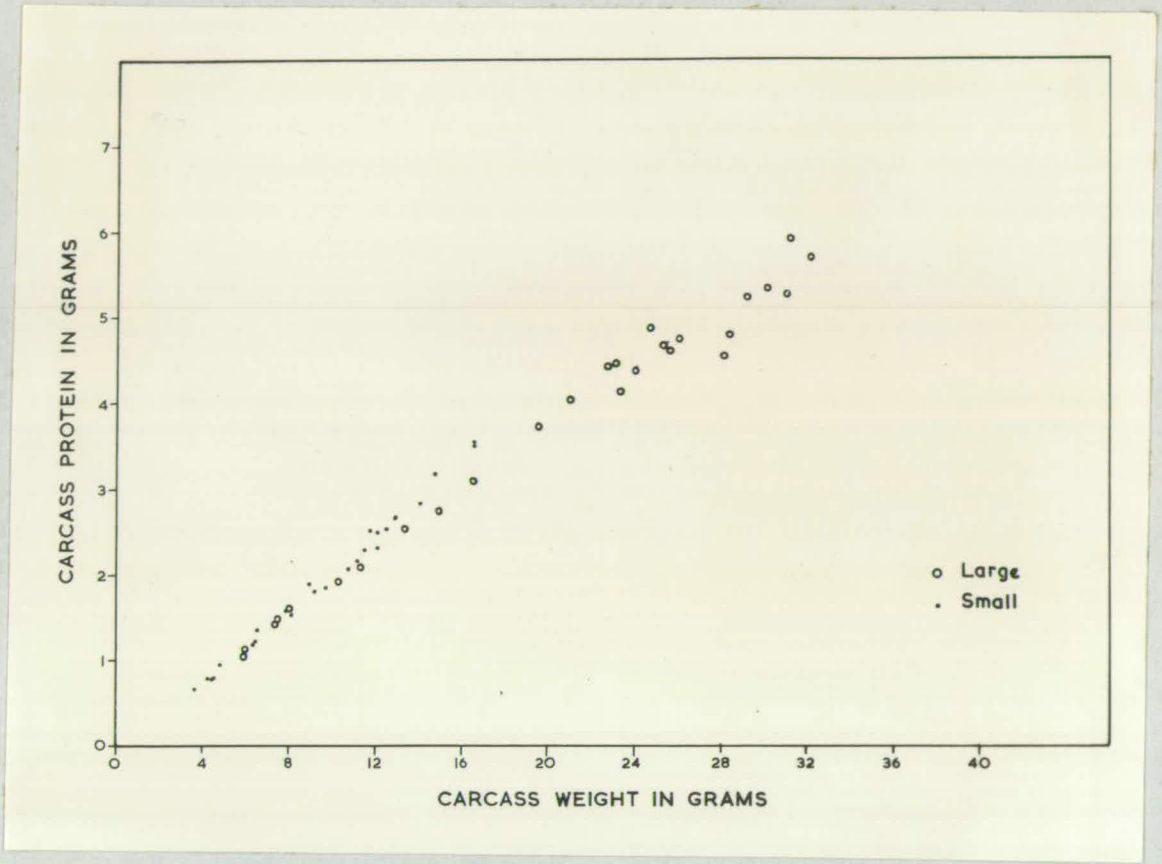

Figure 8. The relation between carcass protein and carcass weight in large and small lines.

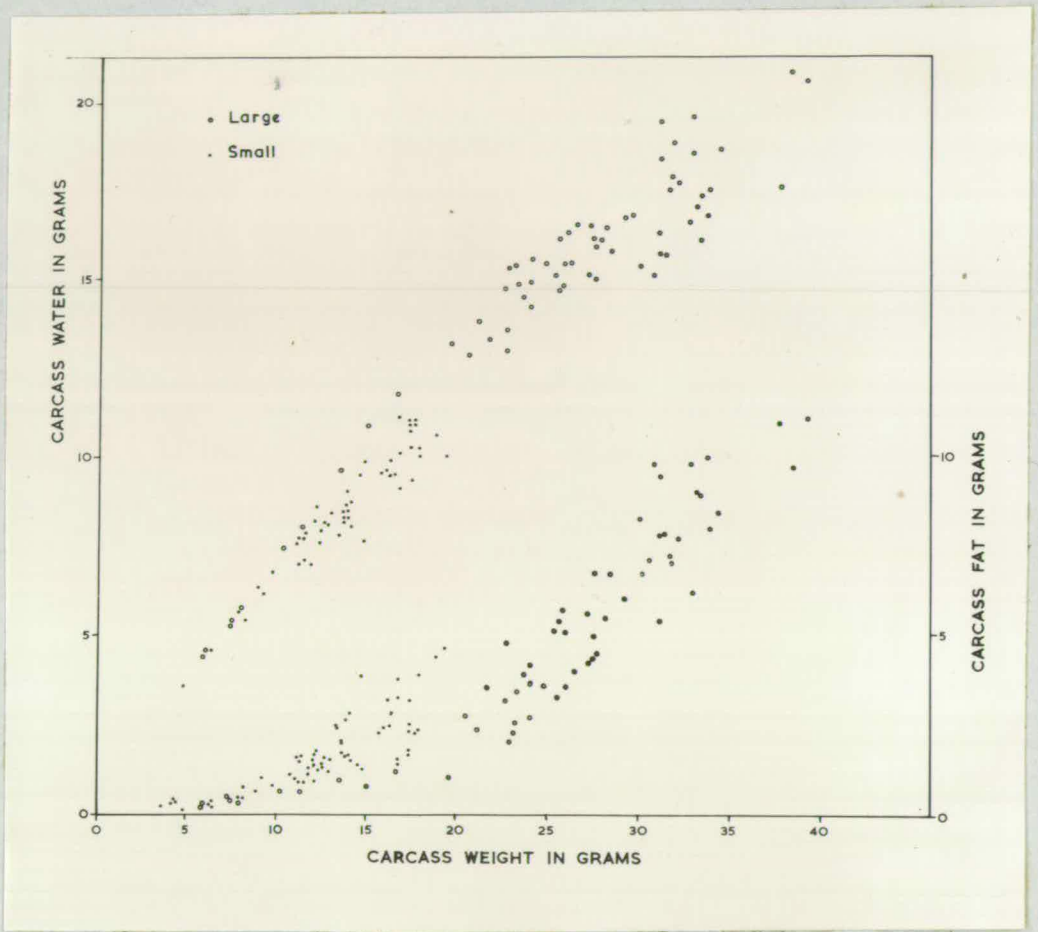

Figure 9. The fat and water content of the carcass of large and small mice in relation to carcass weight.

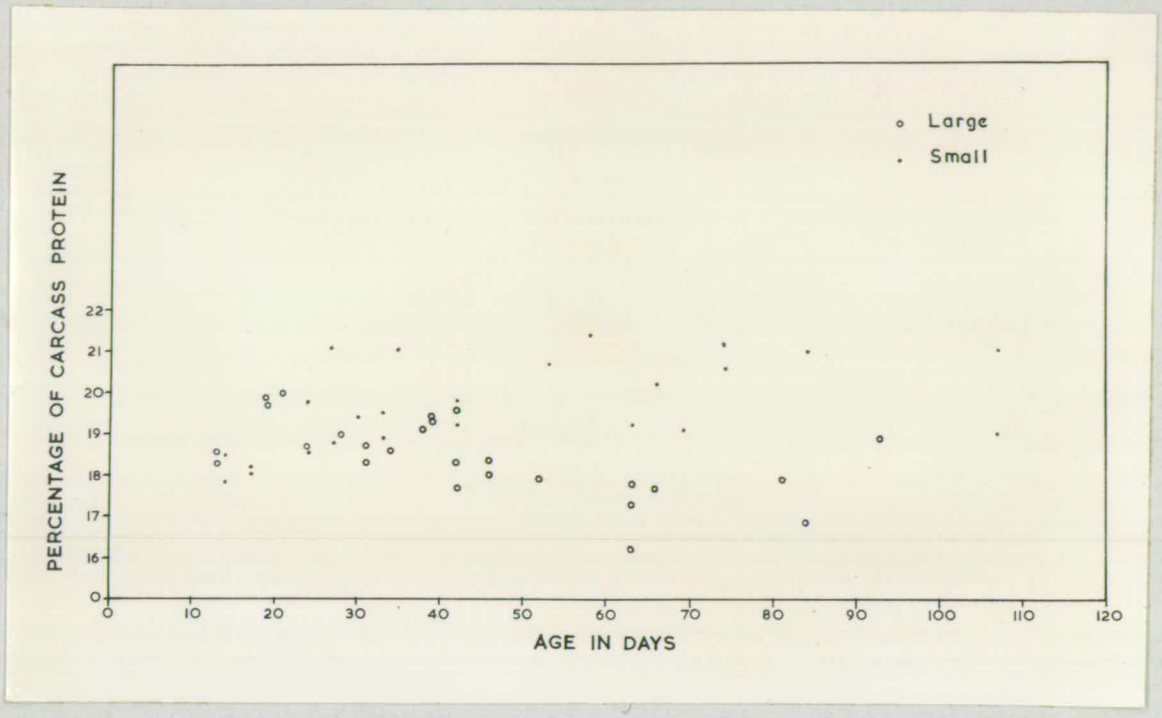

Figure 10. The percentage of carcass protein in large and small lines from 12-107 days of age.

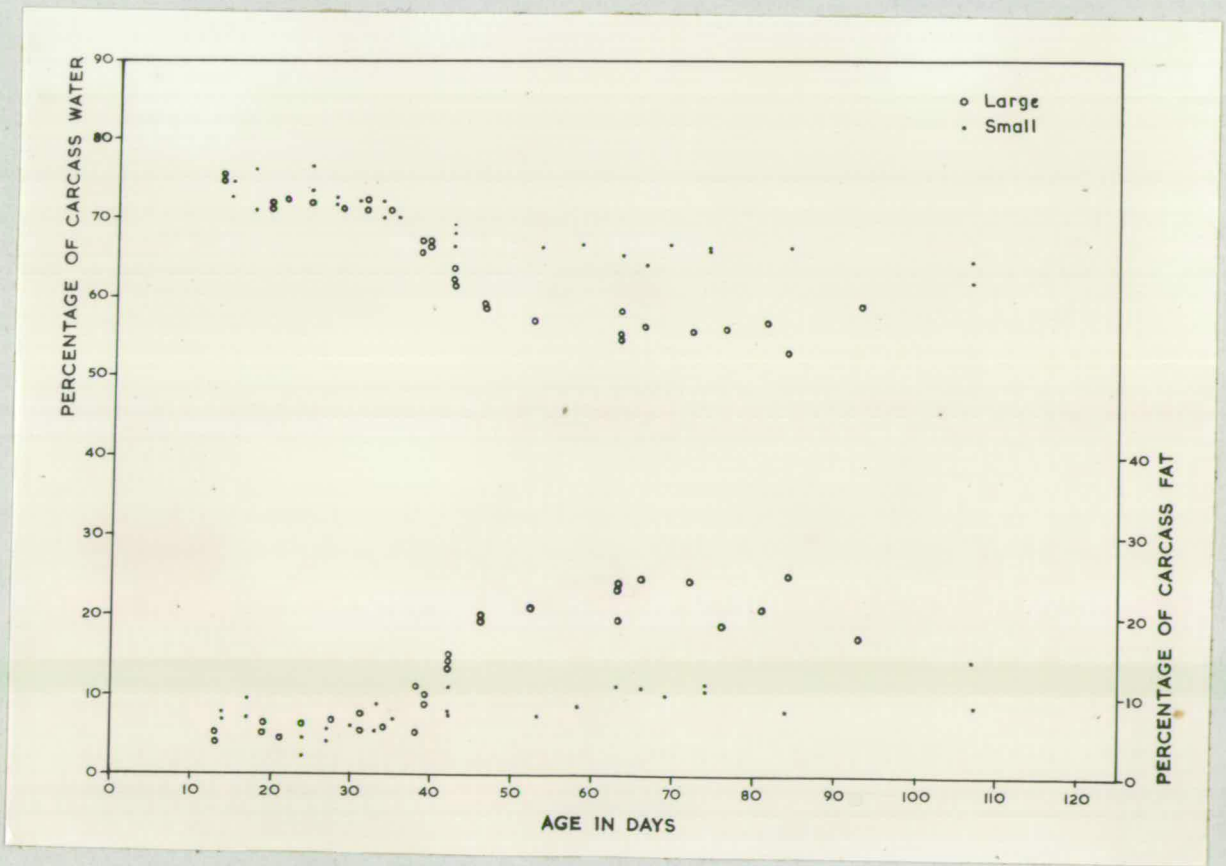

Figure 11. The percentage of carcass fat and water in large and small lines from 12-107 days of age.

CHAPTER 3

THE EFFICIENCY OF UTILISATION OF FOODSTUFFS

One of the problems in livestock improvement is the development of strains of livestock with increased efficiency of utilisation of food into meat or other products. Several workers have shown that the efficiency of food utilisation may be heritable. The gene 'Yellow' (A^{Y}) in mice causes heriditary obesity; these animals require less food per unit of gain, weight gains being largely fat tissue (Dickerson & Gowen 1947). Strain differences in gross efficiency have been reported in rats (Morris et al. 1933; Palmer et al. 1946) who selected two strains, one for high and the other for low efficiency of food utilisation, and in swine (Dickerson 1947; Dickerson & Grimes 1947). Efficiency of utilisation of foodstuffs may be partly hormone controlled, for in rats it can be related to the amount of growth hormone administered, the hormone stimulating growth and increasing efficiency within limits set by the genotype and sex of the animal (Nilson, Palmer & Kennedy 1935).

Several criteria have been used to measure the efficiency of utilisation of ingested food. The increase in weight in unit time per unit of food consumed will be called gross efficiency (W/F) which is a measure of the ability of an animal to convert foodstuffs into body tissues. Included in this expression and in other measures of efficiency is the food which is excreted in the faeces, as for practical purposes it is desirable that in selection for strains with increased body size, rate and economy of gain should be positively correlated. Other factors being equal, gross efficiency will greatly increase with increase in body

protein as protein is always associated with an increase in body water whereas fat deposition is not. It has been shown in the previous chapter that a considerable amount of the increase in weight in the large line from 38 days was in fat tissue, whereas in the small line fat was deposited at a more even rate. As the composition of the weight gains in the two lines are dissimilar, an alternative measure of efficiency in terms of energy units (Brody 1945) has been used. This measure takes into account the composition of the weight gains. The energy increase in the carcass in unit time is calculated as a fraction of the total energy content of the food consumed, energy being measured in calories. This fraction gives the total energetic efficiency. Values of the total energetic efficiency can be derived directly by measuring the energy content of the food consumed and the energy content of the body tissues. These expressions of efficiency therefore use the increase in weight or the increase in energy content of the animal over a given period after all other requirements included in maintenance costs have been satisfied, as a means of estimating the value extracted from the food by the animal. Only a small proportion of the food energy is stored in the body (2-11%) as against 70-80% used in maintenance and body work (Dickerson & Gowen 1947).

The efficiency with which food is converted into body tissue depends therefore on the ratio of the energy gain to the energy intake. Many of the factors influencing the efficiency of food utilisation will be those affecting total energy expenditure such as the energy required for maintenance and the energy utilised for spontaneous activity. The maintenance energy requirements include the energy necessary for maintaining the basal metabolic rate and the energy expended due to the specific dynamic

action of foodstuffs. If maintenance energy is very high as in thyrotoxicosis, weight gains will fall unless compensated by an equivalent adjustment of intake. Similarly, an increase in the energy required for body activity will leave less food available for growth unless compensated by adjustment in intake. The building up of excess food into body protein or into storage as body fat must also depend on other factors such as the efficiency of the digestive process and the hormonal control of growth. If little of the excess food is built into body tissue (true growth) most of the excess will be stored as body fat. Growth inevitably can only take place if the food substances necessary for building new tissues are present in the diet.

Differences in efficiency of food utilisation may exist between lines of mice which have been selected for large or small body size. Animals consuming more food are generally larger than those which eat less, for with increased consumption the amount of food required for maintenance becomes proportionately less and more can be utilised for growth. Differences in growth of large and small selected lines and their controls could be due either to increased food consumption or to differences in utilisation of food. The following investigation was carried out to determine the relative importance of these two factors.

METHOD

Two series of experiments were carried out. In the first, measurements of food intake and weight of animals were obtained concurrently at weekly intervals from 3-12 weeks of age in males and females of large, control and small lines. Two or three mice were housed per cage in clean cages containing no bedding material. The mean values for food intake and weight of animal were calculated for each line, though

variation within each line was obscured by this method. The food spilt had to be weighed at two-day intervals because it was found that a large error (10%) would have been introduced had this been ignored. All mice were fed ad libitum on stock diet from normal food hoppers; this food was analysed for protein, fat and water using the methods for the analysis of carcass components described in the previous chapter. From the mean values of food consumption and associated weight gains, values for total and energetic efficiency were calculated.

In the second series, a more detailed study of food consumption and associated weight changes was carried out over an age range of 3-6 weeks. At this time differences in growth rate between selected lines are at a maximum (Chapter 1). In contrast to the first experiment, mice were housed singly on wood-wool bedding and food consumption, body weights and food spilt were recorded at two-day intervals. Genetic differences in appetite or food consumption both within and between lines could be measured in this experiment. As before, mice were fed ad libitum on stock diet.

RESULTS

Mean values for gross efficiency (W/F) for males and females of large, control and small lines at weekly intervals from 3-12 weeks are plotted in Fig. 12, and given in Table 9. Gross efficiency was generally higher in males than in females of the same line, the sex difference being most marked from weaning to eight weeks of age. Gross efficiency decreased markedly from weaning to eight weeks of age in large and control lines but showed very little change after this age. This decrease must be a result of the increasing maintenance energy required as the animal grows larger coupled with a decreasing growth rate as the animal reaches

maturity. In contrast to this, gross efficiency in the small line remained constant or actually increased in males up to five weeks of age and decreased after this age. The difference between lines prior to 5 weeks will be discussed when gross efficiency from 3-6 weeks is dealt with in more detail in the second analysis. Gross efficiency in the small line was significantly higher from 5-6 weeks of age than that found in control and large lines during this period. Several factors may explain this difference. Firstly, the body weight of the small line is considerably less than that of large and control lines at six weeks of age and therefore the energy required for maintenance (i.e. maintenance of the basal metabolic rate) in large and control lines, will be very much higher than the maintenance requirements of the small line. Secondly, the composition of weight gains during this period are very different; in the large line weight gained from 35 days is largely fat tissue (see Chapter 2) which has a much higher calorific value than protein (9.45 as against 5.65). These differences in total energetic balances will be obscured when calculations are based on total weight gained.

Analysis of food intake and weight increases of males from all three lines from 21-43 days at two-day intervals gave more detailed information concerning changes in efficiency of food utilisation prior to six weeks, that is, during the period at which growth was occurring most rapidly. In this second analysis gain in body weight expressed both cumulatively and per two-day intervals are given in Table 10 together with values of efficiency derived from these measurements. Food intake was highest in the large line, intermediate in the control line and lowest in the small line at all ages from 21-43 days (Fig. 13). Results obtained in this experiment were similar to those obtained from the first experiment.

Up to 33 days of age gross efficiency was higher in the large line than the small line, controls being intermediate (Fig. 14). The increase in gross efficiency from 23 days in both small and control lines may be due to compensatory growth after an initial set-back caused by weaning, for factors affecting heat loss such as individual housing of experimental animals will have a greater effect on small animals. The rate of heat loss may affect growth and therefore efficiency measurements. As found in the first experiment, gross efficiency decreased with increasing age and the greater gross efficiency of the small line from 35-43 days, previously discussed, again occurred.

Because gross efficiency obscured real differences in composition of weight gain total energetic efficiency was also calculated. The food was analysed for fat, protein and water using the methods described in Chapter 2, and the percentage composition of each component in the food was obtained directly except the percentage of carbohydrate which was obtained by subtraction. The composition of the food (dry weight) used in these experiments is given in the following table.

Table 11. Percentage Composition of the Food

COMPONENTS	% DRY WEIGHT
Ash	8.24
Protein	20.95
Fat	4.98
Carbohydrate	65.83

The calorific values of the food (dry weight) was calculated from the percentage composition given above using the conversion factors 4.10, 9.45 and 5.65 Calories per gram, which are the average heats of oxidation

obtained by combustion in a bomb calorimeter of carbohydrate, fat and protein respectively (Widdowson 1955). The calorific value of the food was calculated to be 4.35 Calories per gram.

The calorific value of the carcass of large and small lines at different ages was derived from the figures for carcass fat and protein in the second analysis of carcass components given in Chapter 2, and converted into energy units using the conversion factors for fat and protein quoted above. Carbohydrate in the form of glycogen is stored in small quantities in the liver and muscle cells, but can be ignored for the purposes of calculating the energy content of the carcass due to the relatively large quantities of protein and fat. Calorific values of the carcass of large and small line plotted against weight are shown in Fig. 15 and Table 12. In the small line there was a steady increase in calorific value of the carcass whereas in the large line the calorific value of the carcass showed a more rapid increase during fat deposition, i.e. above a weight of 25 grams.

Total energetic efficiency in large and small lines was calculated for two-day intervals from 21-43 days as follows. Gain in weight for each two-day interval was translated into energy changes from the mean curves plotted in Fig. 15. The calorific value of the food per gram of dry weight was multiplied by the mean amount of food consumed per two-day interval to calculate the total food consumed in energy units. Values of total energetic efficiency, i.e. the ratio of the energy gain in the carcass divided by the food consumed in energy units, are given in Table 13. Total energetic efficiency was also calculated from 6-12 weeks using the data from Series 1 and these results are also included in Table 13. Total energetic efficiency was higher in the large line than in the small

line up to approximately four weeks of age. From four to just under six weeks of age, energetic efficiency in both lines was very similar which may be due to increased maintenance costs of the large animals as discussed previously. From about 39-84 days of age, energetic efficiency was again generally higher in the large than the small line. This difference, which was obscured in measurements of gross efficiency, may be attributable to differences in the composition of weight gains and the higher calorific value of deposited fat. Differences in energetic efficiency between the two lines were apparently controlled by two factors: in young animals by differences in growth rate and in older animals by differences in fat deposition.

DISCUSSION

Selection for body size has altered both the total quantity of food consumed and also the efficiency of utilisation of foodstuffs. Mice of the large line consume a greater quantity of food and use it more efficiently for growth purposes than those of the small line, particularly from 3-4 weeks of age. A greater gross efficiency in the large line is to be expected on the assumption that the energy required for maintenance will be reduced per unit of food consumed when the growth rate is high. But as animals of the large line increase in size, maintenance requirements will also be increased so that their gross efficiency declines and is similar to that of the small line from approximately five weeks of age. Mice of the small line, with a lower growth rate, eat less food and have a lower gross efficiency. Selection for small body size is therefore correlated with a decrease in total food intake and a lowering of the efficiency with which foodstuffs are converted into body tissue. When selection is applied to

livestock improvement, both increased size and economy of gain are desirable, as in the large line. Morris et al. (1933) and Palmer et al. (1946) selected for high or low efficiency of food utilisation in rats and found them to be associated with an increased or decreased body weight respectively. Efficiency of food utilisation and appetite therefore appear to be gene controlled and capable of modification by selection, changes in efficiency and appetite being positively correlated with changes in growth rate.

Energetic efficiency is a truer measure than gross efficiency of the ability of an animal to convert foodstuffs into body fat. To measure energetic efficiency, the total quantity of each component of the weight gained is estimated and multiplied by its calorific value per gram (i.e. fat x 9.45, protein x 5.65) which stresses the weight increments due to fat. In contrast, the qualitative differences in weight gains are not reflected in measurements of gross efficiency because the measure used is total body weight. Gross efficiency is a truer measure of the efficiency of animals to convert foodstuffs into body protein, for body protein is very highly correlated with body water which in turn is the major component of the carcass (see Chapter 2). An increase of one gram of body protein will therefore be associated with an increase of 3-4 grams of body water. Gross and energetic efficiency are therefore indications of two different aspects of growth.

In terms of energetic efficiency, the large line is generally more efficient than the small line. Up to four weeks of age the greater efficiency of the large line can be attributed to differences in growth rate, as was shown in measurements of gross efficiency. Between 4 and 6 weeks, however, energetic efficiency is similar in both lines; the relative

growth of the large line begins to decline during this period and the maintenance requirements due to increased body size will be higher than those of the small line. Although relative growth rates are fairly similar after approximately six weeks of age, fat, with a higher calorific value than protein, accounts for a large proportion of the weight increases in the large line and this is reflected in a higher energetic efficiency in the large than in the small line after this age.

Comparisons of gross and energetic efficiency are complicated by the fact that physiological age must influence efficiency measurements. Differences in efficiency between species and between individuals of one species at different ages will depend to some extent on physiological age, as the older the animal, the greater the maintenance cost in comparison with the total weight gained per unit time.

Some information on the various factors relating to efficiency of food utilisation in large and small lines can be obtained, and some of these factors such as digestibility of foodstuffs, protein balances, total energy expenditure and body activity will be discussed in the next chapter.

Table 9. Gross Efficiency of Large, Control and Small Lines from 3-12 Weeks of Age.

Line	Sex	No. in group	Age in Weeks 3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
Large	Male	15	.262	.127	.051	.044	.031	.013	.025	.021	.020
	Female	19	.194	.108	.029	.021	.010	.035	.008	.025	.024
Control	Male	18	.165	.118	.059	.048	.036	.020	.020	.013	.019
	Female	24	.142	.102	.050	.027	.022	.023	.018	.013	.016
Small	Male	26	.098	.122	.091	.049	.032	.035	.024	.022	.029
	Female	17	.088	.090	.061	.031	.010	.023	.018	.022	.022

Table 10. Utilisation of Food from 21-43 Days.

		Age in Days											
		21	23	25	27	29	31	33	35	37	39	41	43
Large	Body Weight in gms.	12.1	13.9	16.1	18.5	21.2	23.4	25.1	26.6	27.5	28.4	29.1	29.6
	Food Intake in gms. at each 2-day interval		7.6	8.8	10.2	11.7	12.8	13.3	13.6	13.6	13.5	13.3	13.2
	Food Intake (cumulative)		7.6	16.4	26.6	38.3	51.1	64.4	78.0	91.6	105.1	118.4	131.6
	Gross Efficiency		.237	.250	.235	.231	.172	.129	.110	.066	.067	.053	.039
Control	Body Weight in gms.	9.6	10.8	12.6	14.4	16.4	17.8	19.0	20.1	20.8	21.4	22.0	22.4
	Food Intake in gms. at each 2-day Interval		6.7	7.4	8.8	9.3	9.8	10.4	11.6	11.2	10.7	10.8	11.3
	Food Intake (cumulative)		6.7	14.1	22.9	32.2	42.0	52.4	64.0	75.2	85.9	96.7	108.0
	Gross Efficiency		.179	.243.	.205	.215	.143	.115	.095	.063	.056	.056	.035
Small	Body Weight in gms.	6.7	7.0	7.7	8.3	9.3	10.0	10.7	11.5	12.4	12.9	13.6	14.0
	Food Intake in gms. at each 2-day Interval		4.9	4.6	4.5	5.5	6.3	6.2	6.8	6.7	7.2	7.5	7.8
	Food Intake (cumulative)		4.9	9.5	14.0	19.5	25.8	32.0	38.8	45.5	52.7	60.2	68.0
	Gross Efficiency		.061	.152	.133	.182	.111	.113	.118	.134	.069	.093	.077

Table 12. Calorific Value of the Carcass of Large and Small Lines.

Large			Small		
Age (days)	Weight (gms.)	Calorific Value (Calories)	Age (days)	Weight (gms.)	Calorific Value (Calories)
13	6.8	9.34	14	5.0	7.77
	6.8	8.46		4.7	7.30
19	8.9	12.08	17	4.2	6.28
	8.8	12.86		5.1	8.44
21	10.0	12.44	24	5.8	6.57
24	12.8	17.05		8.3	9.49
28	17.5	23.61	27	8.3	10.32
31	20.6	23.67		10.3	12.98
	21.5	29.50	30	8.1	10.71
34	16.6	17.37	33	9.7	13.06
38	27.7	43.90		11.8	18.50
	26.2	30.53	35	11.1	16.61
39	30.4	47.31			
	29.6	44.14	42	13.4	20.75
42	28.7	55.60		13.8	21.35
	30.7	61.10		11.2	19.80
	30.3	59.05	53	14.2	22.70
46	32.8	74.82	58	17.0	30.25
	30.9	74.28	63	14.5	26.05
52	32.1	76.63	66	15.3	27.85
63	-	105.33	69	13.2	22.10
	34.0	90.62	74	14.3	26.30
	33.7	77.59		15.6	29.35
66	35.7	93.75	84	19.1	32.65
81	35.0	86.63	107	15.3	33.50
84	36.6	103.18		19.7	33.80
93	39.0	84.57	127	17.0	30.30

Table 13. Total Energetic Efficiency of Large and Small Lines from 21-84 Days of Age.

	2-Day Intervals										Weekly Intervals					
Age / Energetic Efficiency	21-23	23-25	25-27	27-29	29-31	31-33	33-35	35-37	37-39	39-41	41-43	42-49	49-56	56-63	63-70	70-77	77-84
Large	.065	.074	.081	.078	.085	.070	.079	.061	.073	.062	.065	.049	.076	.030	.046	.025	.01
Small	.011	.045	.066	.103	.066	.078	.073	.035	.046	.069	.037	.038	.023	.022	.015	.009	.00

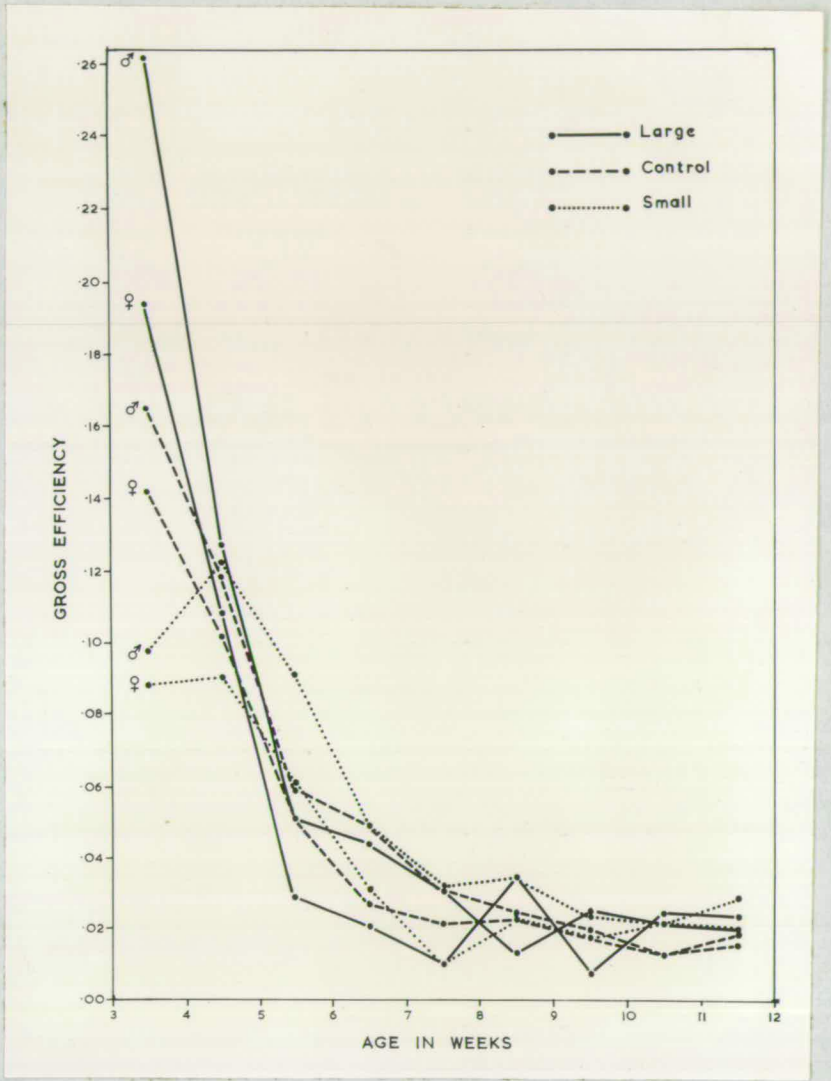

Figure 12. Gross efficiency of large, control and small lines calculated for weekly intervals from 3-12 weeks of age.

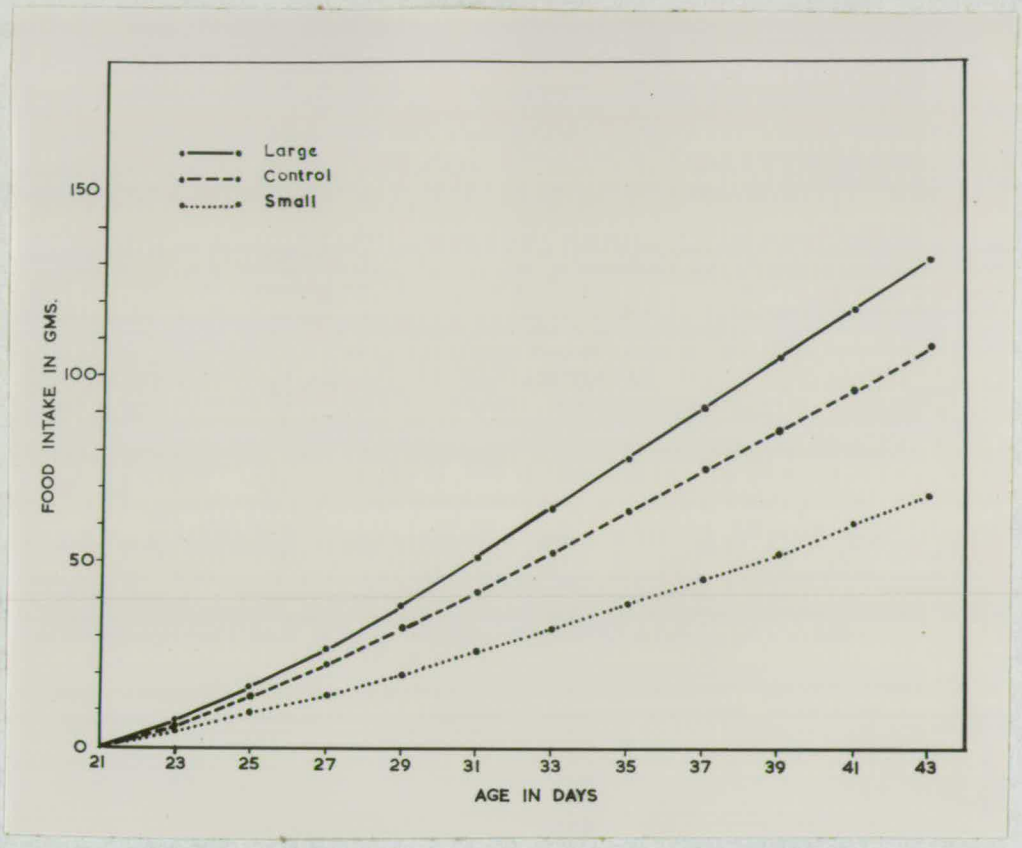

Figure 13. Cumulative food intake in large, control and small lines from 21-43 days of age.

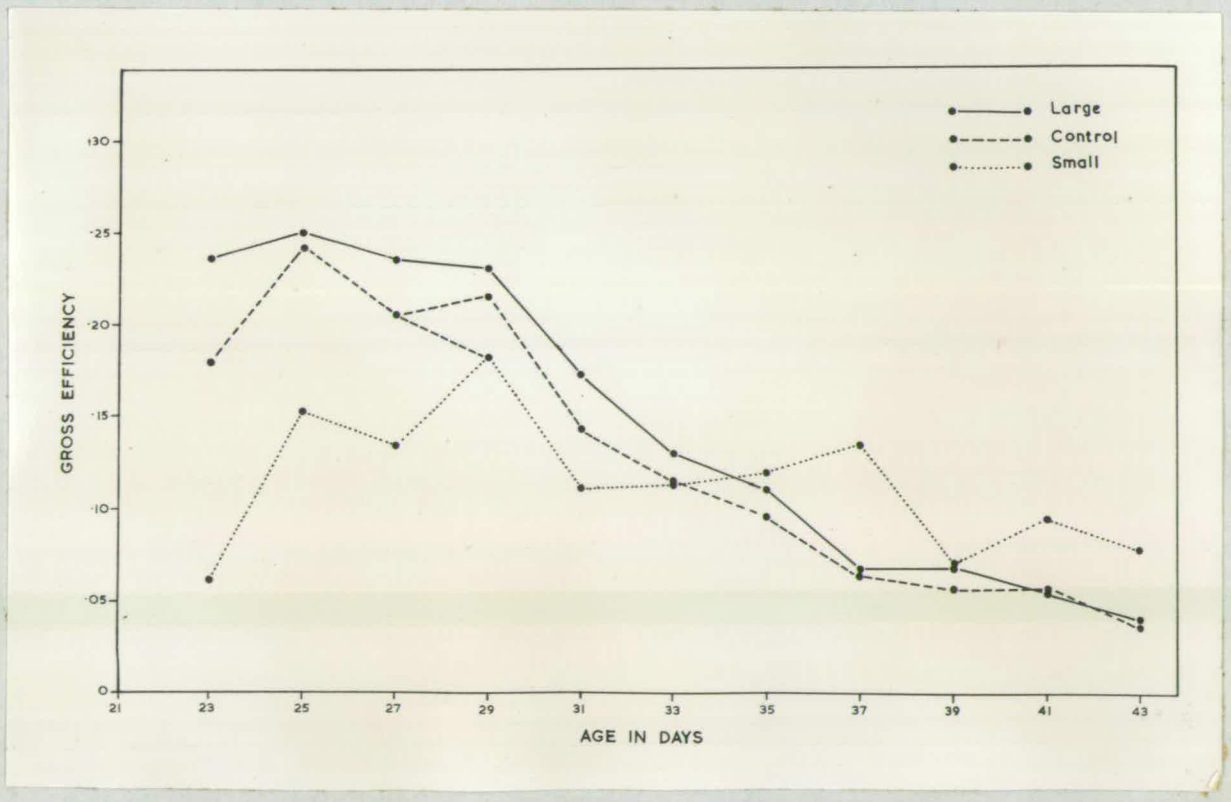

Figure 14. Gross efficiency of large, control and small lines calculated for each two-day interval from 21-43 days of age.

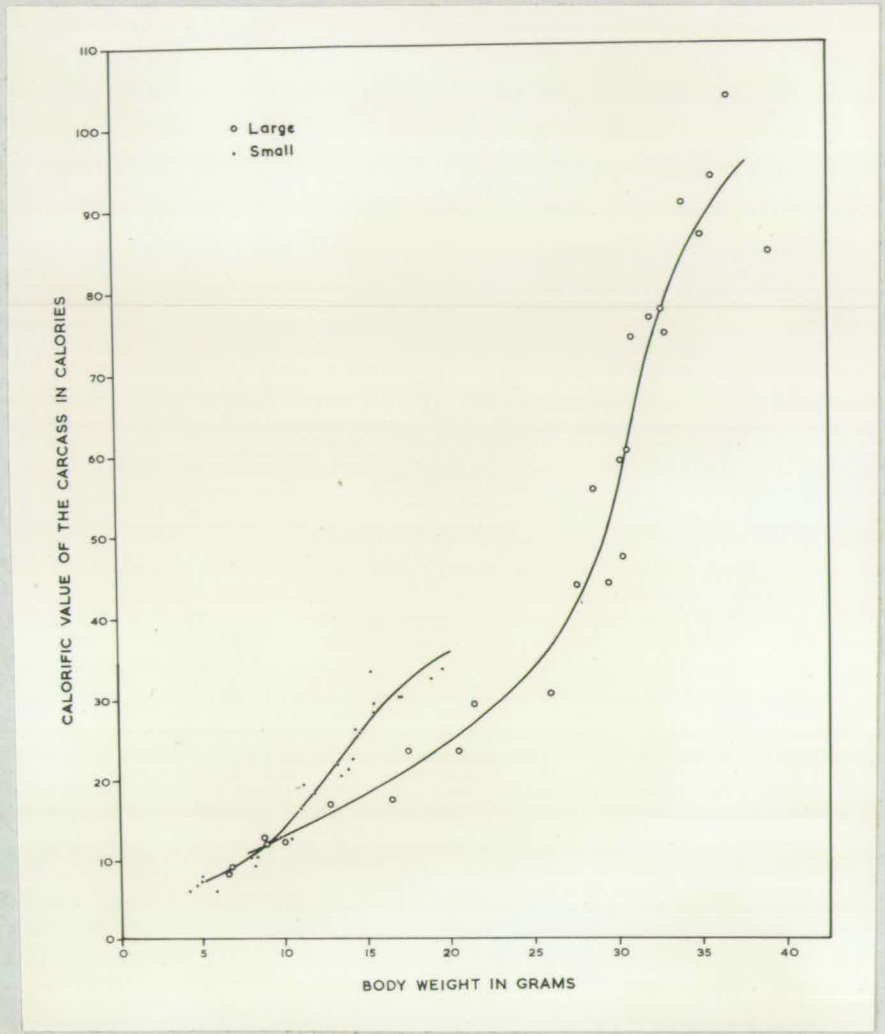

Figure 15. The relation between the calorific value of the carcass and total body weight. (Values were derived from data in Tables 6 and 7 in which the total amounts of protein, fat and water in the carcass were determined).

CHAPTER 4

ASPECTS OF THE METABOLISM OF MICE SELECTED FOR LARGE OR SMALL BODY SIZE

Many factors will influence the efficiency of food utilisation (i.e. weight gained per gram of food consumed) in large and small lines, and data on three of these factors will be presented in this chapter. First, experiments to determine the total proportions of fat, protein and carbohydrate absorbed from the gut, i.e. the digestibility of foodstuffs, will be described. Secondly, the total energy expenditure has been determined. Finally, the activity of mice of the two lines was measured to estimate whether the energy spent in activity was the same in both large and small lines. These three factors will affect the efficiency with which an animal con verts foodstuffs into body tissue.

The digestibility of foodstuffs was found by determining the total quantities of the fat, protein and carbohydrate consumed in the food and subtracting from these the total quantities excreted in the faeces. The studies were made to compare the percentage of the energy in the food available for metabolism in large and small lines. The amount of urinary nitrogen was also determined so that the total protein metabolised, and therefore the nitrogen retention in the body, could be estimated.

The relation between energy expenditure (energy metabolism) and body size has been studied by a number of workers. An extensive survey of comparative basal metabolism in mammals was made by Benedict (1938) who showed that heat production was positively correlated with weight within a species, but that differences existed between animals of the same weight in different species. Benedict and his co-workers also showed that there

were considerable deviations from the surface law (i.e. heat production is proportional to the two-thirds power of body weight) in warm-blooded species. A more recent survey has been made by Zeunthen (1947, 1953) relating oxygen uptake to body size in invertebrates. Expressing his results as respiration per unit nitrogen and respiration per unit weight, he confirmed Benedict's work and found that the percentage decrease in metabolic rate for a certain increase in body size was not a constant value. While much work has been done on interspecific comparisons, relatively few intraspecific comparisons have been made. Energy expenditure within a species can be influenced by size genes. Hereditary obese mice have an increased efficiency of food utilisation over normal litter mates which is partly due to a decreased energy expenditure (Dickerson & Gowen, 1947). In a strain of rats selected for a low efficiency of food utilisation, the basal metabolism was higher and the body temperature lower than in a strain selected for high efficiency (Palmer et al. 1946). The large difference in body size between Falconer's selected lines provided excellent material for a study of the energy expenditure of large and small lines within a species.

The measurement of the metabolism of mice under 'basal' conditions is difficult and unnecessary if replaced by measurements under 'standard' conditions, i.e. under conditions as normal as possible (Barbour & Trace 1937; Dewar & Newton 1948a). The total energy expenditure of mice from large and small lines was therefore determined under standard conditions to provide information of the relation between energy expenditure and the rate of growth.

I. The Digestibility of Foodstuffs and Total Energy Expenditure of Large and Small Lines.

The total food consumption and the excreta, and the carbon dioxide and oxygen exchange of mice were measured over periods of approximately 24 hours and under conditions as normal as possible. Measurements were made using mice from large and small lines at various ages from weaning to maturity. The open circuit apparatus used (designed by Dewar) was a modified version of that of Haldane (1892), though including many of the modifications of Dewar and Newton (1948a). The open circuit design has the disadvantage that oxygen consumption is determined indirectly by subtraction, but has the advantages of greater simplicity of design and construction. A general plan of the apparatus is given in Plate 1. The mouse was housed in a cylindrical chamber (M) which was enclosed in a wooden box maintained at a constant temperature (25°C) by means of a thermostat and an electric heating bulb. The cylindrical mouse chamber and its screw top were both made of perspex. The lower end was funnel shaped and was fitted with a urine collection tube (T). To ensure that the chamber was completely airtight, a thick rubber band was stretched tightly round the screw joint. The internal construction of the mouse chamber is shown in Plate 2. The food box was designed to admit only the head of the mouse so as to reduce spilling to a minimum. Powdered diet was fed to mice of the large line for it was found that these mice pulled whole cubes out of the food box, the food becoming contaminated with faeces and urine. The design had to be modified for small mice, however, for they could not be prevented from entering the food box and nesting in it, even though the entrance was reduced in size. They were

therefore fed with stock diet cubes in the food box illustrated in Plate 3, any food spilt being collected in the retaining trough.

Air was drawn through the apparatus by the filter pump (P); the rate of flow, which was recorded by the flow metre (F) and controlled by a screw clip on the outlet valve (O), was adjusted to between 300-400 mls. per minute. Carbon dioxide and water were removed from the incoming air before entering the mouse chamber (M) by the absorption tubes 1 and 2. To prevent the air passing through the mouse chamber being completely dry and so possibly injurious to the health of the mouse, a known quantity of water was added to the incoming air by bubbling it through tube X. Air leaving the chamber was passed through absorption tubes 3, 4 and 5 to absorb the carbon dioxide and water given out by the mouse.

The absorption tubes used were glass U-tubes fitted with ground glass taps which were made up as follows.

TUBE	PACKING	PURPOSE
Tube 1	½ Soda Asbestos* 4-10 Mesh (Carbosorb Brand) ½ Soda Asbestos 10-14 Mesh	To remove carbon dioxide from the incoming air.
Tube 2	Magnesium Perchlorate (Anhydrone)	To remove water from the incoming air.
Tube 3	Magnesium Perchlorate (Anhydrone)	To remove water from the air leaving the chamber. 1 absorption tube was found to be sufficient.
Tube 4	Soda Asbestos 4-10 Mesh	To remove carbon dioxide from the air leaving the chamber.
Tube 5	½ Soda Asbestos 10-14 Mesh	To remove any CO_2 not absorbed by Tube 4.
	½ Magnesium Perchlorate	To absorb any water lost from Tube 4.

*Soda asbestos has been shown to be the most efficient reagent for the absorption of CO_2.

A tendency for the proximal ends of the carbon dioxide and water absorption tubes 3 and 4 to become partially blocked was overcome by packing soda asbestos or magnesium perchlorate around fine glass tubes of varying lengths.

The faeces were removed from the grid and dried to a constant weight, the faecal nitrogen being determined by the micro-Kjeldahl method. Faecal nitrogen was converted into faecal protein by multiplying by the factor 6.25. The floor of the funnel and grid were carefully washed with water and these washings added to the urine which was then made up to a definite volume and filtered. The nitrogen content of the urine was determined after filtration by the micro-Kjeldahl method. The filtrate which contained the food spilt was weighed after drying.

When possible, the mouse was kept in a similar chamber to the experimental chamber for the day previous to the experiment in order to become accustomed to the apparatus. Before all determinations the apparatus was checked to ensure that it was completely airtight. All weights recorded were made to the nearest milligram; details of the weights recorded are given on pages 49-51, together with data calculated for a complete 24 hour experiment.

The accuracy of the apparatus can best be assessed under normal working conditions by the equation given below, although this equation includes oxygen uptake which is determined indirectly.

Sum of ingesta (Oxygen + water + food consumed) - sum of the excreta (carbon dioxide + water + faeces + urine) - weight change = 0.

The experimental error, (i.e. the discrepancy between the observed and calculated weight change) was calculated in 28 experiments. The error was then expressed as a percentage of the total ingesta. The mean percentage error was found to be 1.56%.

RECORDED DATA FOR ONE COMPLETE EXPERIMENT

	Initial Observation	Final Observation	Result
Age of mouse	29 days	30 days	
Water tube X	48.028 gms.	45.150 gms.	Water entering chamber = 2.878 gms.
Absorption tube 1	116.070 gms.	124.581 gms.	Water absorbed from chamber = 8.511 - 2.878 = 5.633 gms.
Absorption tube 2	122.551 gms.	125.842 gms.	3.291 } Total CO_2 = 3.76
Absorption tube 3	126.061 gms.	126.531 gms.	.470 } gms. = 1.915 litres
Food box + Food	56.686 gms.	51.441 gms.	5.245 gms. food eaten
Food spilt			.110 gms. food spilt Total food ingested = 5.135 gms. = 4.527 gms. dry weight
Water bottle	15.163 gms.	10.102 gms.	Water consumed = 5.061 gms.
Urine tube	7.899 gms.	8.438 gms.	+ 0.539 gms. urine
Mouse in holder	54.720 gms.	55.530 gms.	
Holder	37.263 gms.	37.228 gms.	Initial wt. mouse = 17.457 gms. Average wt.
Mouse	17.457 gms.	18.302 gms.	Final wt. mouse = 18.302 gms. 17.879 gms. Change in wt. = +0.945 gms.
Total weight of chamber including Mouse, Food & Water supplies & Urine tube.	336.309 gms.	329.750 gms.	6.559 gms. weight loss
Temperature	25° C		
Time	10.45 a.m.	11.02 a.m.	Duration 24.28 hours
Flow/minute	350 mls.		
Oxygen consumed	= (5.633 + 3.761) - 6.559 = 2.835 gms. = 1.984 litres.		

---oOo---

Total weight of faeces = 2.150 gms.

Dry weight of faeces = 1.811 gms.

Faecal nitrogen = 0.077 gms.

Urinary nitrogen = 0.064 gms.

Protein metabolism

Calories from protein = 0.064 x 30.59 Cals./g. = 1.957

CO_2 from protein = 0.064 x 5.548 l./g. = 0.355 l.

O_2 from protein = 0.064 x 6.639 l./g. = 0.425 l.

Total R.Q. = $= \frac{1.915}{1.984} = .965$

Non-protein R.Q. $= \frac{1.915 - .355}{1.984 - .425} = \frac{1.560}{1.559} = 1.000^*$

Calorific value O_2 at this R.Q. = 5.047 Cals./l.

Total heat expenditure = (1.559 l. x 5.047 Cals/l.) + 1.957 Cal.
= 9.824 Cals.

Metabolic rate = 9.712 Cals./24 hrs.

Average metabolic rate $= \frac{9.80 \times 24.0}{17.879 \times 24.28} = .543$ Cals/gm/24 hrs.

Metabolic rate = 1.183 Cals./$_W$0.73/24 hrs.

Foodstuffs ingested

Dry food eaten = 4.527 gms.

Nitrogen ingested = 4.527 x 0.034 = 0.154 gms.

Fat ingested = 4.527 x 0.050 = 0.266 gms.

Carbohydrate ingested = 4.527 x 0.658 = 2.979 gms.

Foodstuffs lost in faeces

Weight of dry faeces = 1.811 gms.

Faecal nitrogen = 0.077 gms.

Faecal fat = 1.811 x 0.024 = 0.043 gms.

Faecal carbohydrate = 1.811 x 0.578 = 1.046 gms.

*The high R.Q. is probably due to conversion of carbohydrate into fat.

Foodstuffs absorbed from gut		% of total absorbed from gut
Nitrogen absorbed	= 0.154-0.077 = 0.077 gms.	50.00
Fat absorbed	= 0.226-0.043 = 0.183 gms.	80.99
Carbohydrate absorbed	= 2.979-1.046 = 1.933 gms.	64.90
Nitrogen balance	= 0.154-0.077-0.064 = + 0.013 gms.	

Weight balance = (O_2 + food + water consumed) - (water from mouse chamber + CO_2 + moist faeces + urine) - Δ wt.

= (2.835 + 5.135 + 5.061) - (5.633 + 3.761 + 2.150 + 0.539) - 0.945

= 0.948 - 0.945 = +0.003

Error = .023%

Calculations. The following calculations were based on those used by Dewar & Newton (1948a). The energy associated with the metabolism of protein, together with the associated carbon dioxide and oxygen equivalents, were calculated from the urinary nitrogen. The equivalents used were those calculated by Dewar and Newton (1948a) in which the metabolism resulting in 1 gm. of urinary nitrogen was considered to require 6.639 gm. oxygen with the output of 5.548 gm. of carbon dioxide. The oxidation equation from which the above equivalents are obtained are necessarily approximations. The energy equivalent of one gram of urinary nitrogen was taken to be 30.59 Cals./gm. (Kriss & Miller 1934). The total respiratory quotient, or R.Q., $\left(\frac{\text{litres } CO_2}{\text{litres } O_2}\right)$ was determined; and by subtracting the oxygen and carbon dioxide of protein metabolism from the total oxygen and carbon dioxide exchange, the non-protein R.Q. could be calculated. From the non-protein R.Q., which is calculated to the third decimal place, the calorific value of the non-protein oxygen was derived using an extended form of the Lusk-Zuntz and Schumberg Tables (Lusk 1924).

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The total energy expenditure, and therefore the metabolic rate per unit weight, can then be calculated, using the average weight of the mouse, from the initial and final weights.

Food intake was calculated on the basis of dry material. The proportion of the total foodstuffs digested (total digestibility) was determined in each experiment as well as the digestibility of the constituent foodstuffs fat, protein and carbohydrate. Digestibility was estimated by determining the difference between the intake level and faecal loss and expressing this difference as a percentage of the intake level.

Faecal protein was determined concurrently with all measurements of energy expenditure. Due to the small quantities of fat in the faeces, faecal fat was obtained from an analysis of the faeces collected from a group of mice of the same line fed on the same diet. The main disadvantage of such a method is that the composition of the faeces may vary between different mice. Dewar and Newton (1948a) have estimated, however, that the error involved in the estimations of digestibility would be small.

In some experiments, non-protein R.Q.s of greater than 1 were observed, indicating conversion of carbohydrate to fat. The additional energy expended in this conversion was estimated from the 'excess CO_2' using the factors derived by Williams, Riche and Lusk (1912) from the hypothetical equation of Bleibtreu (1901).

RESULTS

Analysis of food and faeces.

The food used for these experiments was stock diet, details of the method of analysis having been given in Chapter 2. The composition of the food, estimated as a percentage of the dry weight, was as follows:-

Protein 20.95% Fat 4.98% Carbohydrate 65.83% Ash 8.24%

The calorific value of the food was 4.35 Calories per gram (for further details see Chapter 3).

The composition of the faecal fat and carbohydrate was determined on faeces collected from a group of mice fed with this stock diet; the faecal protein was determined separately in each 24 hour experiment. The average composition of the faeces was determined assuming that nitrogen represented protein, and the total ether-soluble material represented fat; the faecal ash was also determined. Faecal carbohydrate was then obtained by subtraction. The faeces of mice of both large and small lines of different ages were analysed, protein in animals from weaning to 12 weeks, and fat and carbohydrate at three and six weeks only. It was found that the percentage of the various components of the faeces were similar at all ages within each line; all determinations of percentage composition of the faeces within a line were therefore combined. The composition of faeces expressed as percentages of the faecal dry weight were as follows:

Table 14. Composition of the Faeces

	% Ash	% Fat	% Protein	% Carbohydrate	Calorific value of faeces
Small line	16.56±1.02	3.43±1.55	27.98±3.89	53.03	4.08
Large line	16.25±2.28	2.39±0.22	23.57±2.96	57.79	3.93

The percentage of protein was significantly higher ($P < .01$) in the small than in the large line. The percentage of fat in the faeces was higher and the percentage of carbohydrate lower in the small than in the large line though differences in the percentage fat were not significant. The calorific value of the faeces (Table 14) was calculated using the factors

4.10, 9.45 and 5.65 Calories per gram for carbohydrate, fat and protein respectively. These factors are the average heats of oxidation of the three foodstuffs in a bomb calorimeter and do not take into account any foodstuffs absorbed from the gut which are excreted unchanged in the urine.

Digestibility of foodstuffs.

The total digestibility of food for each twenty-four hour experiment was determined from the measurements of the food consumed and the total faeces excreted. From the determinations of the calorific value of the food consumed and the calorific value of the faeces, the energy intake and the energy loss in the faeces could also be found for each 24 hour experiment and the calorific digestibility calculated. Total and calorific digestibility appeared to be unaffected by the age of the animal and a mean value for the two digestibilities has therefore been calculated for each line. The results are given in Table 15:

Table 15. Total and Calorific Digestibility

	No. of determinations	Total digestibility	Calorific digestibility
Small line	26	64.42 ± 1.46	67.63 ± 6.39
Large line	21	62.82 ± 0.83	66.97 ± 2.70

Despite large differences in food intake, and therefore in calorie intake (see Chapter 3), the total and calorific digestibility were not significantly different in large and small selected lines, i.e. the total proportion of foodstuffs absorbed, even if converted into energy units, was similar in both lines. However, there was some variation in digestibility between the different experiments. Some of this variation may be

due to the error in assuming that the amount of food in the gut is the same at the beginning as it is at the end of the experiment. In all estimates of digestibility, unabsorbed food in the alimentary canal at the end of the experiment will necessarily have been included in that digested. Greater variation was found in calorific than in total digestibility. This may have been due to variation in faecal composition between mice while the proportion of food excreted remained fairly constant. Variation in digestibility was higher in the small than in the large line (see Table 15).

The total amount of protein, fat and carbohydrate in the food consumed and in the faeces were determined and the differences between the amount consumed and that excreted was found. The differences were expressed as percentages of the total protein, fat and carbohydrate intake, i.e. digestibility of protein, fat and carbohydrate. Digestibility of protein was found to be similar at all ages, digestibility of fat and carbohydrate being similar at three and six weeks of age (i.e. the two ages tested). All data was therefore combined and mean values for the digestibility of protein, fat and carbohydrate of both large and small lines were calculated and are given in the following table:

Table 16. Digestibility of Protein, Fat and Carbohydrate.

	No. of determinations	Digestibility of Protein	Fat	Carbohydrate
Small line	26	53.31 ± 8.31	76.09	72.05
Large line	21	58.09 ± 6.58	82.42	67.47

A greater proportion of protein was absorbed in the large line ($P < 0.05$)

despite very large variations in digestibility of protein in different animals. Digestibility of fat was slightly greater in the large line whereas digestibility of carbohydrate was higher in the small line. Tests of significance were not made on fat and carbohydrate because these values for faeces were calculated from groups of animals not used in the 24 hour experiment (see Method). Differences in the digestibility of each food constituent are to be expected from the results of the analysis of faeces in the two lines.

Nitrogen Balances.

Nitrogen balances were obtained directly by subtracting the nitrogen loss in the urine from the digestible nitrogen of the food. Nitrogen balances in the large line were generally positive until about six weeks of age. After this age, nitrogen retention was reduced almost to nil except in animals where there was a considerable weight gain during the twenty-four hour experimental period. Negative nitrogen balances were infrequent in animals of the large line except in cases of weight loss. In contrast, positive nitrogen balances were relatively infrequent in experiments with mice of the small line, which may be attributed to their extremely slow rate of growth. In the small line, weight losses during the experimental period, though small, were frequent and nearly always associated with negative nitrogen balances. The nervous disposition of the small mice may have adversely affected their appetite in strange surroundings.

Respiratory Quotients.

Determinations of oxygen intake and carbon dioxide output were made concurrently with measurements of food intake and faecal loss. Measurements

of the oxygen consumption and the carbon dioxide output were used to calculate the respiratory quotient (R.Q.) and the total energy expenditure (see Method). The R.Q. for the combustion of carbohydrate and fat are known to be 1.00 and 0.72 respectively.

Twenty-one determinations were made using animals from the large line and twenty-six from the small. Values of the R.Q. were variable in both large and small lines though slightly higher in the large line. All R.Q.s exceeded 0.8 and more than one-quarter of the animals of both lines had an R.Q. exceeding one. In both lines, R.Q.s of greater than one were generally associated with positive weight increases. Dewar and Newton (1948b) have shown that if food intake is sufficient only for maintenance requirements, foodstuffs are burnt in the same proportions as that of the diet. Above maintenance levels, the R.Q. increases in proportion to the food intake, i.e. either carbohydrate is being preferred as a source of energy and fat is stored, or the same proportions are burnt and the excess carbohydrate is converted into fat and stored along with the corresponding excess of ingested fat. In growing animals, in which food intake far exceeds maintenance requirements, both of these processes may occur. Growing animals might therefore be expected to have higher R.Q.s than mature animals of the same line.

Energy Expenditure.

The same experiments to determine R.Q.s also gave information on energy expenditure. These experiments included a series of 24-hour determinations made on four mice at ages between 22 and 72 days in the large line, and between 22 and 40 days in the small line (Tables 17 and 18). The four animals so tested were two males from each of the large

and small lines. Other determinations were made on mice of ages between 23 and 50 days in the large line, and between 22 and 93 days in the small line. The metabolic rate of a few animals of the control line was also estimated and results are shown in Figs. 16 and 17. The metabolic rate of some mice of the large line between ages 14-17 days are also included in Fig. 17. Oxygen uptake and carbon dioxide output were estimated over a period of two hours only in these animals as the measurements were made prior to weaning. Values for their metabolic rates are therefore necessarily an approximation as the oxygen requirements due to protein metabolism could not be determined, but they did afford a comparison with small mice of comparable weight.

The total energy expenditure for each 24 hour period was higher in the large than in the small line; this is to be expected for increased body size will be associated with increased maintenance costs. Energy expenditure when expressed as per unit of body weight was higher in the small than in the large line for comparable ages (Fig. 16). These differences in energy expenditure per unit weight become negligible if animals of the same weight are compared (see Fig. 17). Energy expenditure in animals of the same weight in large and small lines was then fairly similar or slightly higher in the large line. Weight losses during the experiment may have artificially lowered the energy expenditure of the small line, for Dewar and Newton (1948a) have shown that loss of weight is generally associated with a low metabolic rate, and weight losses in the small line exceeded 3% in 5 out of a total of 27 determinations. However, the conclusions drawn from the comparison of the energy expenditure of mice from large and small lines for comparable body weights were unaffected if these animals were excluded. As was to be expected, there was some

variation in both lines when metabolic rate was plotted against age (Fig. 16); when metabolic rate was plotted against weight, variation was considerable in the small line and comparatively small in the large line (Fig. 17).

Divergences from the surface law in mature animals (energy expenditure proportional to $w^{0.66}$) has been observed by Benedict (1938), and Brody (1945) has shown that metabolic rates are more nearly proportional to $w^{0.73}$. Energy expenditure per 24 hour period per $w^{0.73}$ has therefore been plotted in Figs. 18 and 19. In animals from the large line, energy expenditure was relatively constant during the period of most rapid growth (7 to 25 gms.) but decreased as weight increments became progressively less (Fig. 18). Growing animals therefore had a higher metabolic rate per $w^{0.73}$ than mature animals of the same line. Metabolism per $w^{0.73}$ was extremely variable in the small line (Fig. 19) and in many growing animals it was as low as that found in adult mice of the large line. With two exceptions values in the small line were dependent on the change in weight during the experimental period, i.e. in animals in which weight loss occurred, metabolism per $w^{0.73}$ was lower than in animals with weight gains during the experimental period.

DISCUSSION

Although the total proportion of foodstuffs absorbed from the gut is similar in large and small lines, small differences occur in the proportion of fat, protein and carbohydrate absorbed by the two lines. In terms of calories, the amount digested was also similar. Mice selected for increased body size absorb a greater proportion of fat and protein, and a lower proportion of carbohydrate, than mice selected for small body size. If the percentage of foodstuffs absorbed (i.e. the digestible food) was

an important factor in limiting growth, much greater differences than those observed in the proportion of foodstuffs absorbed from the gut would be expected in view of the very large differences in growth rate. It is also evident that the increased gross and energetic efficiency of food utilisation in the large line, which has been discussed in the previous chapter, is not a result of a greater proportion of the food consumed being absorbed in the large line, for total and calorific digestibility have been shown to be similar in both selected lines. The differences in efficiency of food utilisation between large and small lines must have been due to other causes.

During growth, nitrogen balances were usually positive in the large line except in cases of weight loss during the experimental period, i.e. a certain proportion of the digestible protein was retained in the body. From about six weeks of age nitrogen retention was reduced almost to nil, which is in agreement with previous work (see Chapter 2) showing that increases in weight after this age are largely fat tissue and that protein deposition is nearly completed. The adult animal is therefore in a state of nitrogen equilibrium (the nitrogen absorbed is equal to the nitrogen lost in the urine) provided that the food consumed is sufficient for maintenance purposes. The small line absorbed a slightly lower proportion of protein than mice of the large line, but in contrast to the large line most of the digestible protein was metabolised and the nitrogen retention was either small or, in many cases, negative, particularly in those animals in which small weight losses occurred during the experiment. The proportion of protein absorbed to that metabolised appears to be higher in mice selected for large body size so that more protein is available for the laying down of body tissue.

Body size directly affects total metabolism and therefore energy expenditure as follows. First, increased size is associated with increased maintenance requirements, and secondly, energy expenditure per unit weight decreases with increasing size which is probably due to reduced heat loss per unit body weight. The total energy expenditure is therefore higher in the large than in the small line due to higher maintenance requirements, and the decrease in energy expenditure per unit weight found within large and small lines with increasing weight or age is attributable to reduced heat loss as a result of a relatively smaller surface area. In the present work, the metabolic rate per unit weight for 24-hour periods was generally similar in mice of large and small lines of comparable weights. Differences existing in the energy expenditure per unit weight for similar ages must therefore be due to weight differences. However, mice of large and small lines of the same weight are growing at quite different rates (see Chapter 1), and an increased growth rate has been shown to be associated with a rise in metabolism per $w^{0.73}$ (Brody 1945). This association was also found in the present experiment. A certain proportion of the energy expended is therefore directly concerned with the growth process, i.e. there is an energy cost associated with growth. Some of the energy expended by animals of the large line, which are growing at a faster rate than the small line, was therefore due to growth. This extra energy expenditure, which would be largely absent in the small line, may have obscured real differences in the energy required for maintenance between the two lines.

MacArthur (1944) has suggested that decreased body size as a result of selection may be due to increased energy expenditure in the small line so that less food is available for storage. There was no evidence in the

present analysis that this is true. Firstly, animals in the large and small lines of the same weight will have approximately the same surface area and therefore heat loss will be similar. Secondly, there is no evidence to support the theory that the metabolic rate is higher in the small strain than that which would be expected on the basis of their body size. It seems unlikely therefore that a high level of energy expenditure is directly responsible for limiting the rate of growth in the small line, for energy expenditure is generally higher in the large line as shown above.

The level of energy expenditure will directly affect the efficiency with which animals convert foodstuffs into body tissue. If maintenance requirements are low, more food energy will be available for storage, whereas if maintenance requirements are high, most of the digestible food will be metabolised and practically no food will be available for growth. The differences in energy expenditure in animals of large and small lines of the same age will therefore contribute to the differences found in the efficiency of food utilisation found between the two lines (in Chapter 3). The increased efficiency of the large line may therefore be due to the reduction in total energy expenditure per unit weight which in turn may be a result of the relatively smaller surface of large mice.

The differences observed in energy metabolism of the selected lines appear to be largely due to differences in growth rates so that at any absolute age, total energy expenditure in the two lines will be dissimilar as they will be at a relatively different stage in their development (i.e. in their physiological age). In turn, growth rates are probably controlled by other factors, e.g. the endocrines which may be under direct genetic control and therefore capable of modification by selection.

II. Activity Levels in Large and Small Lines of Mice.

The method of measuring activity was similar to that used by Bateman (unpublished). It consisted of an aluminium cage suspended as in Plate 4. The nest of wood wool was always arranged to be at the opposite end of the case to the water bottle and food basket. The water bottle and food basket were counterbalanced with weights to maintain the cage horizontal, and one end of the cage was replaced by perspex in order to see the mouse. All movements were recorded on a smoked drum by means of a pointer attached to one end of the cage. The amplitude of the movements recorded on the drum, i.e. the sensitivity of the cage to movements by the mouse, was adjusted by means of the supports fixed in the clamps A and B. The speed of the drum was adjusted to 12.6 cm. per hour in order that all movement of the mouse from one end of the cage to the other would be recorded. Records therefore only included one type of activity (i.e. movement) out of a whole range of activity patterns, e.g. cleaning, etc. The activity of each mouse was measured over approximately 18-23 hours (one recording), which should reduce error in the measurements due to diurnal rhythms of activity. Activity was particularly studied during the period when the differences in growth rate were maximum, i.e. from 3 - 6 weeks of age. Results were expressed in units of activity per day, a unit of activity being defined as the movement of a mouse from one end of the cage to the other.

RESULTS AND DISCUSSION

Activity levels were studied in males of large and small lines from 21-51 days of age. Results expressed as units of activity per day and units of activity per day per gram of body weight are given in Tables 19

20. The total number of activity units of 18 separate recordings were counted on four different occasions to determine the error in counting. From the variation in the counts of the total activity of one recording, the coefficient of variation could be calculated. The mean coefficient of variation in counts for the 18 recordings was 4.73. In a number of recordings from each line, the percentage of the total time spent at the nest end was calculated and these figures are included in Tables 19 and 20.

The large amount of variation in activity in both the large and small lines from 21-23 days may have been due to a weaning effect associated with loss of litter-mates. From 23 days of age, however, increase in weight in both lines was associated with increased total activity (Fig. 20). The increase in total activity with age appeared to be greater in the large line. But data on activity was difficult to interpret due to the large variation between mice of the same line, the same age, and the same weight.

In order to relate total activity with total energy expenditure, activity units were expressed per gram of body weight and plotted against age in Figure 21. From 23-40 days of age activity was higher in the small than in the large line. The increased energy expenditure found in the small line when expressed per gram of body weight may therefore be due to increased maintenance as a result of increased activity, which in turn may be due to a greater heat loss per unit weight in smaller animals. The increased maintenance requirements as a result of greater activity per unit weight might therefore be responsible for the decreased efficiency of food utilisation found in the small line during this period.

When animals of the same weight were compared, activity per unit body weight was not dissimilar in the two lines though few comparisons were available.

To summarise, total activity after 23 days of age increased in both lines with increasing age or weight, but more rapidly in the large line. The level of activity in mice of the large line was comparatively high at weaning, i.e. when growth is rapid. Although activity per gram of body weight was higher in the small than in the large line, these differences become negligible when animals of similar weights were compared. Activity was therefore similar in animals with different rates of growth. The level of activity, though contributing to maintenance requirements, therefore appeared not to be an important factor limiting growth in the small line and there was no evidence to support the suggestion that activity made a substantially greater contribution to total energy expenditure in the small than in the large line.

The reduced growth rate in the small line does not appear to be a result either of selection for mice with high levels of activity and therefore with abnormally high maintenance requirements, or of selection for high maintenance requirements through increased oxidation rates in the body tissues such as that which is associated with hyperthyroidism. The hypothesis that food which is usually converted into body tissue is needed for abnormally high maintenance requirements can therefore be excluded. Neither is the increased growth rate of the large line apparently associated with lower maintenance requirements or with marked reductions in total activity. Other factors are probably responsible for the changes in growth rate resulting from selection for large or small body size. A study of the endocrines which are directly concerned with the

control of growth may give more fundamental data on the determinants of the rates of growth in the selected lines than measurements of energy expenditure or body activity.

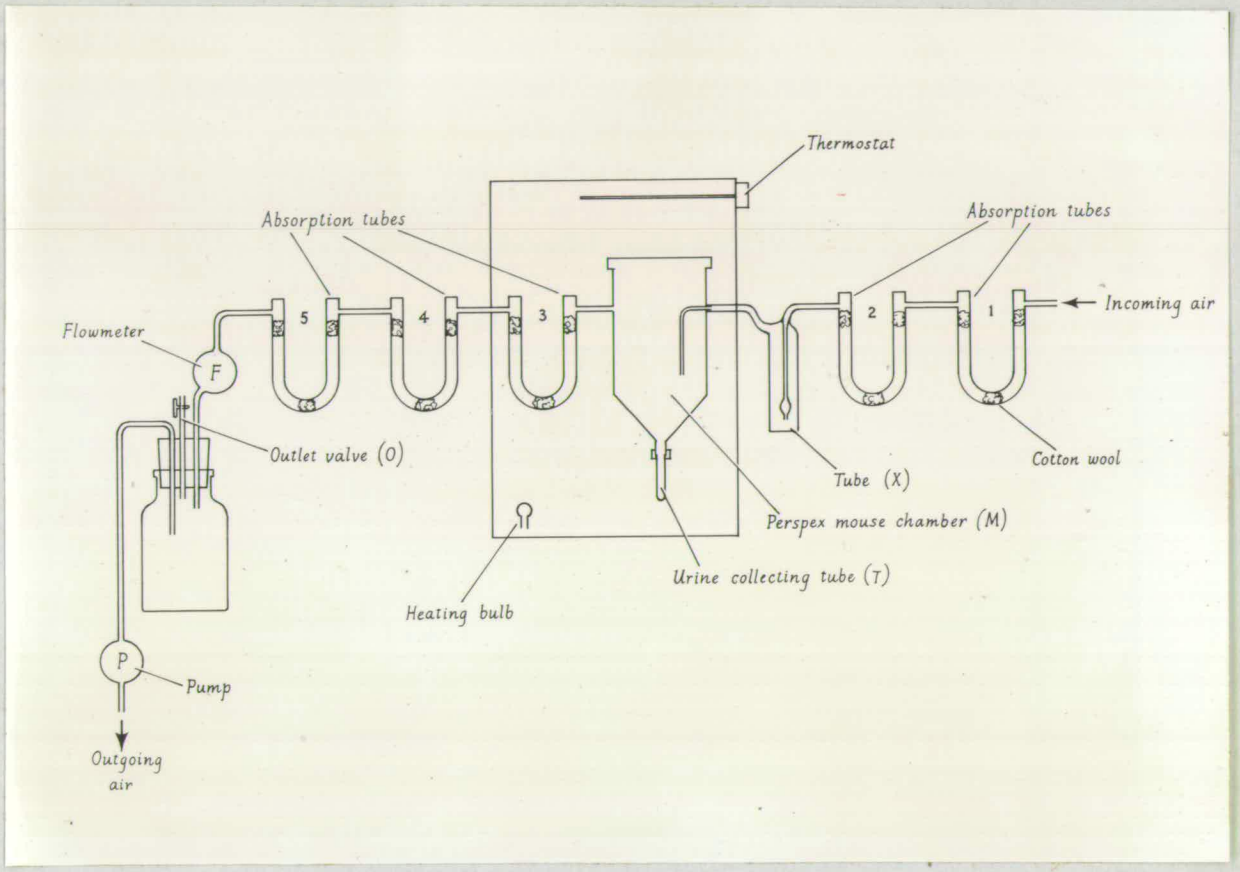

Plate 1. A plan of the open-circuit apparatus used to measure the total food consumption, the excreta, and the carbon dioxide and oxygen exchange of mice over a 24 hour period.

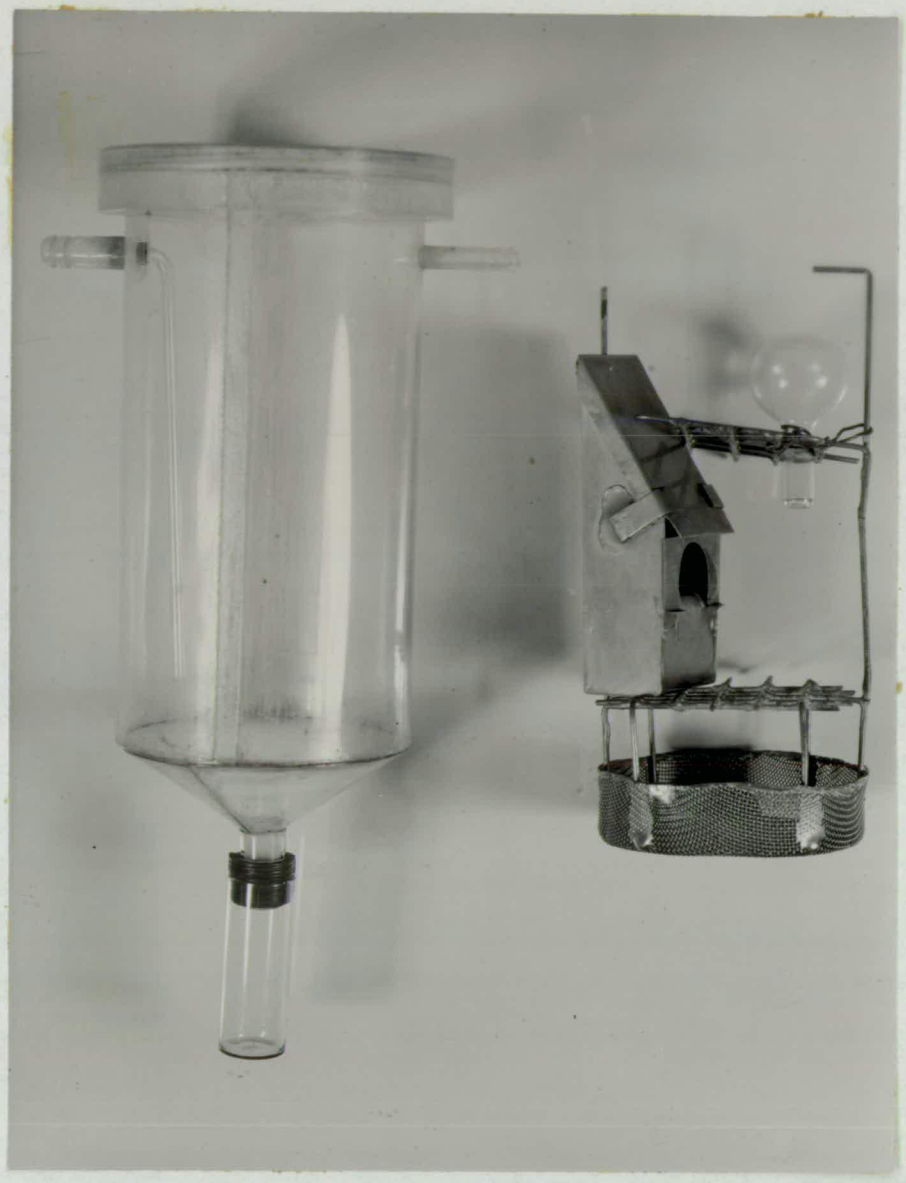

Plate 2.

A

Perspex mouse chamber (M) with urine collection tube (T)

B

The internal construction of the mouse chamber showing food box and water bottle.

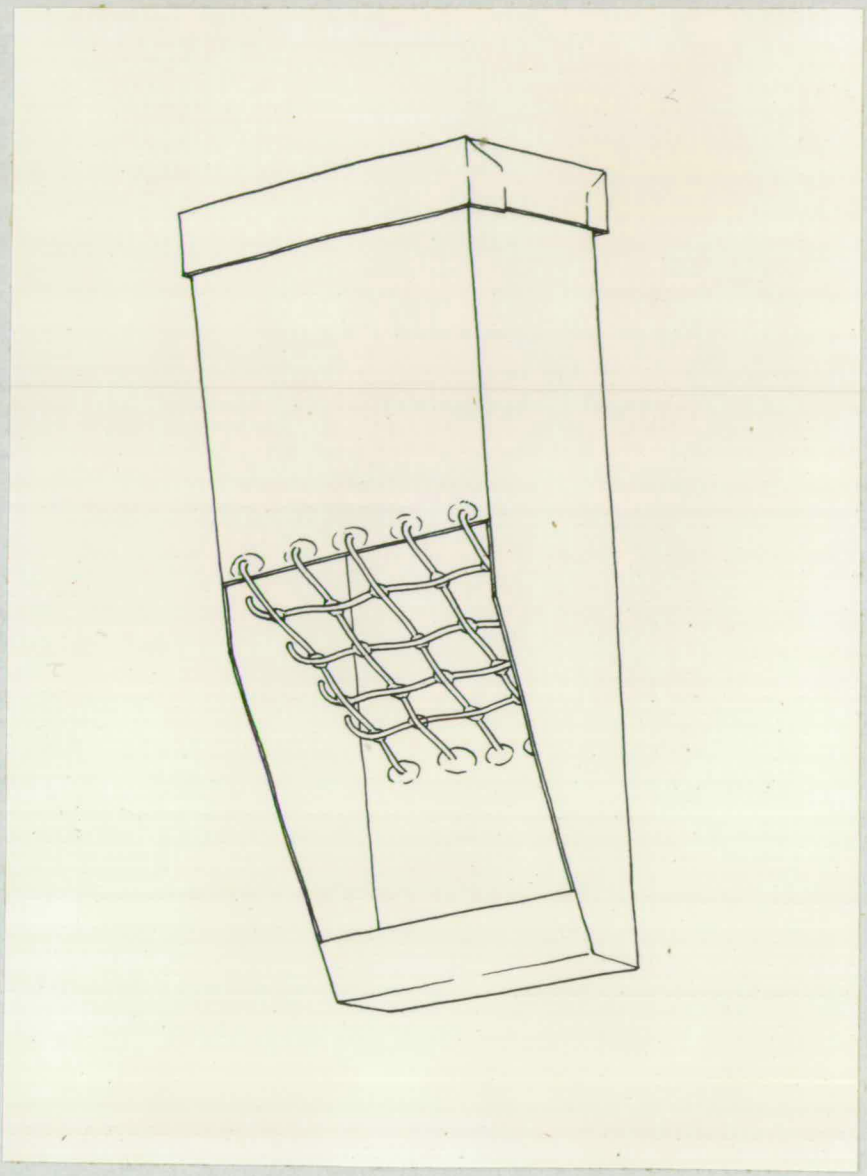

Plate 3. Modified food box used for small mice.

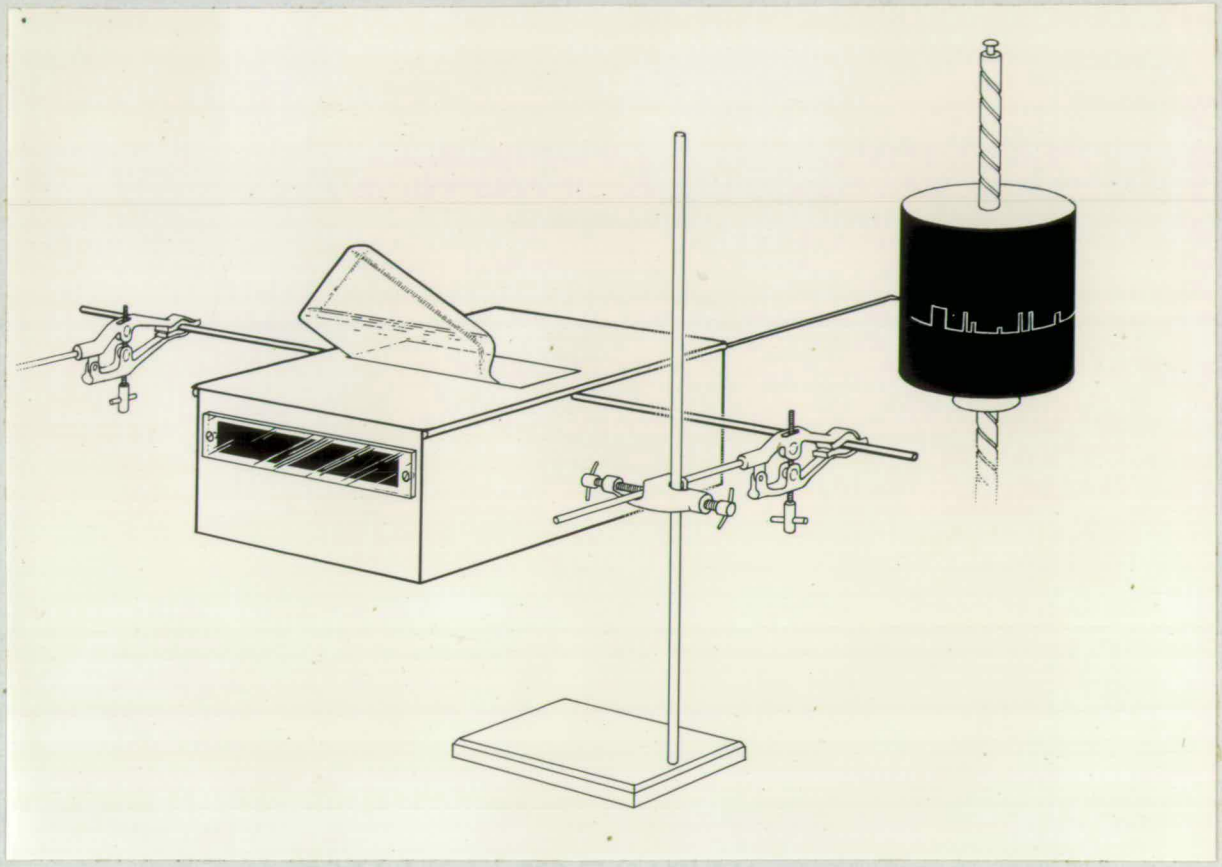

Plate 4. The construction of the apparatus used to measure body activity.

Table 17. Energy Expenditure of Two Male Mice of the Large Line.

Age in Days	Mouse	22-23	24-25	26-27	29-30	32-33	34-35	36-37	39-40	42-43	45-46	46-47	52-53	56-57	72-73
Weight in gms.	a			14.52	17.88	21.56		24.71	25.85		28.20				33.58
	b	13.20	14.85	17.04		22.93	24.52	26.67	27.92	28.97		29.82	31.80	32.16	
Energy Expenditure per Day in Calories	a			7.97	9.67	11.30		12.10	11.90		12.80				12.90
	b	7.55	8.47	9.86		11.95	12.05	11.60	12.65	11.70		11.20	13.61	12.00	
Energy Exp/Day/gm. in Calories	a			.55	.54	.52		.49	.46		.45				.38
	b	.57	.57	.58		.52	.49	.42	.45	.40		.38	.43	.37	
Energy Exp/Day/$W^{0.73}$ in Calories	a			1.13	1.18	1.20		1.15	1.11		1.12				.99
	b	1.15	1.18	1.25		1.21	1.19	1.03	1.12	1.00		.94	1.09	.95	

Table 18. Energy Expenditure of Two Male Mice of the Small Line From 22-40 Days of Age.

Age in Days		22-23	23-24	25-26	27-28	29-30	31-32	32-33	33-34	35-36	36-37	39-40
	Mouse											
Weight in gms.	a	7.42		8.66		10.40		11.83		12.70		13.35
	b		6.49	7.04	7.53	8.40	9.38		9.93		10.27	
Energy Expenditure per Day in Calories	a	5.50		5.57		6.44		6.60		7.30		6.36
	b		6.70	3.95	4.64	7.96	5.15		4.85		4.71	
Energy Expenditure /Day/gm. in Calories	a	.74		.64		.62		.55		.58		.48
	b		1.03	.56	.62	.95	.55		.49		.46	
Energy Exp/Day/$W^{0.73}$ in Calories	a	1.27		1.15		1.17		1.09		1.27		.96
	b		1.71	.95	1.04	1.68	1.00		.93		.86	

Table 19. Activity in the Large Line.

Age in Days	Weight in gms.	Units Activity Per Day	Units Activity Per Gram Per Day	% Time at nest end
21-22	10.6	525.6	49.4	45.5
	13.0	696.0	53.7	43.0
	12.9	324.0	25.2	43.2
	13.5	381.6	28.3	56.2
22-23	12.6	194.4	15.6	54.3
	12.7	340.8	26.9	55.3
	10.1	285.6	28.3	52.5
	14.5	547.2	37.7	52.5
23-24	13.1	170.4	13.0	-
	13.6	256.8	18.0	58.4
25-26	15.2	324.0	21.4	54.5
26-27	17.0	175.2	21.4	65.0
27-28	18.2	225.6	12.5	71.9
27-28	17.6	199.2	11.3	-
28-29	20.6	228.0	11.0	63.7
29-30	20.9	230.4	11.0	-
31-32	23.4	369.6	15.8	51.0
	21.8	369.6	17.0	59.4
32-33	21.4	405.6	19.0	42.7
33-34	24.8	494.4	19.9	53.0
34-35	28.3	271.2	9.6	61.5
	27.1	470.4	17.3	-
35-36	27.9	523.2	18.7	55.5
37-38	23.5	144.0	6.2	-
	29.6	506.4	18.0	57.0
	28.3	343.2	12.2	49.4
42-43	29.4	806.4	27.4	35.5
	30.5	840.0	27.6	-
45-46	34.2	535.2	15.6	44.1*
50-51	32.8	861.6	26.4	45.4*

* not included in Figs. 20.& 21.

Table 20. Activity in the Small Line.

Age in Days	Weight in gms.	Units Activity Per Day	Units Activity Per Gram Per Day	% Time at Nest End
21-22	7.3	314.4	43.0	43.0
	5.7	204.0	36.0	
	7.1	232.8	32.9	
	6.4	216.0	35.8	
22-23	7.7	336.0	43.7	
	7.5	398.4	53.0	
	5.8	132.0	21.3	
23-24	7.6	206.4	27.1	53.0
	6.1	144.0	23.5	
25-26	8.3	192.0	23.0	
	6.8	232.8	34.3	
	7.4	232.8	31.4	
26-27	7.2	124.8	17.3	
27-28	9.2	235.2	25.7	45.7
	7.4	194.4	26.4	
29-30	9.8	271.2	27.6	56.6
30-31	7.7	235.2	30.5	
31-32	10.3	307.2	29.8	64.5
32-33	8.2	170.4	20.9	
33-34	11.3	388.8	34.3	53.2
	9.9	297.6	30.0	56.5
34-35	8.9	196.8	22.1	
35-36	10.1	367.2	26.2	
	12.4	302.4	24.5	53.8
36-37	9.6	228.0	23.8	
37-38	12.7	271.2	21.6	50.7
	11.5	326.4	28.3	38.1
38-39	10.1	235.2	23.3	
39-40	11.4	244.8	21.4	58.3
	10.0	352.8	35.3	
41-42	12.0	405.6	21.8	
42-43	11.2	302.4	27.1	
44-45	11.9	381.6	32.2*	
46-47	12.2	321.6	26.4*	

* not included in Figs. 20 & 21.

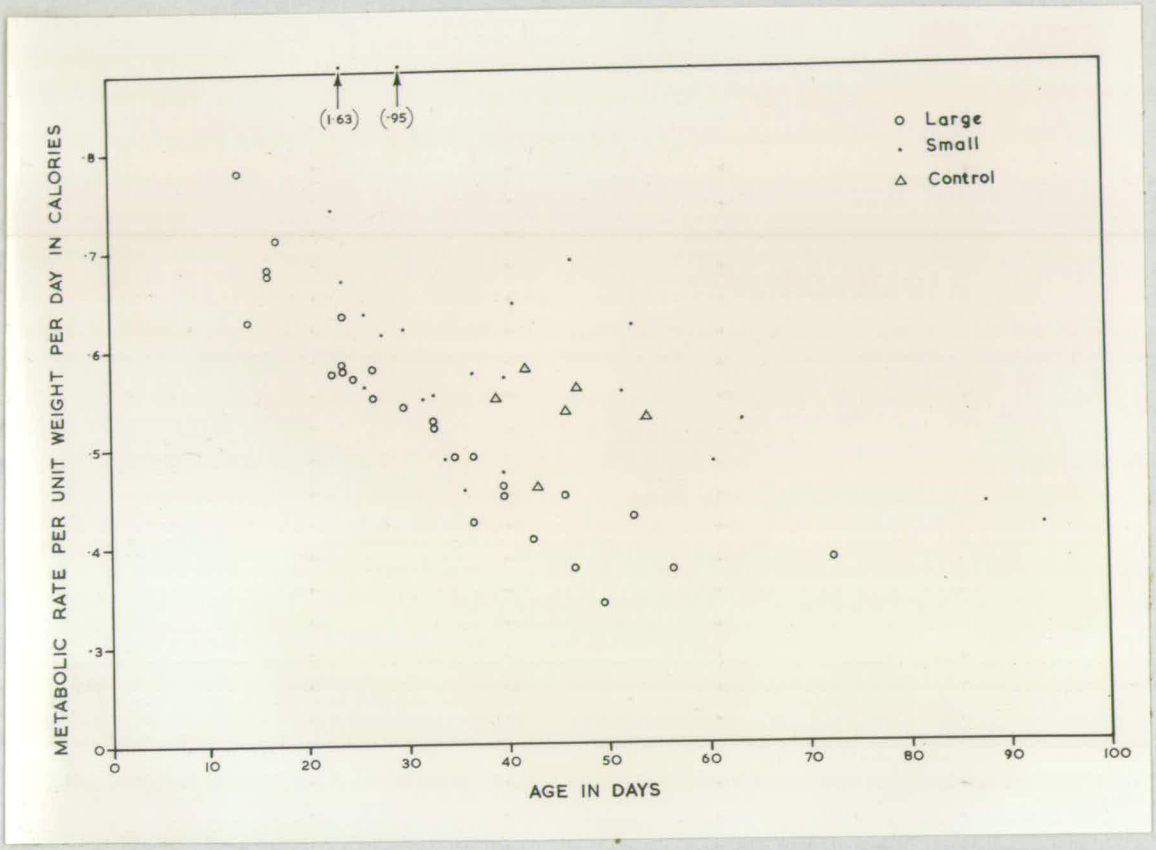

Figure 16. The relation between energy expenditure per unit weight per days and age in days in large, control and small lines.

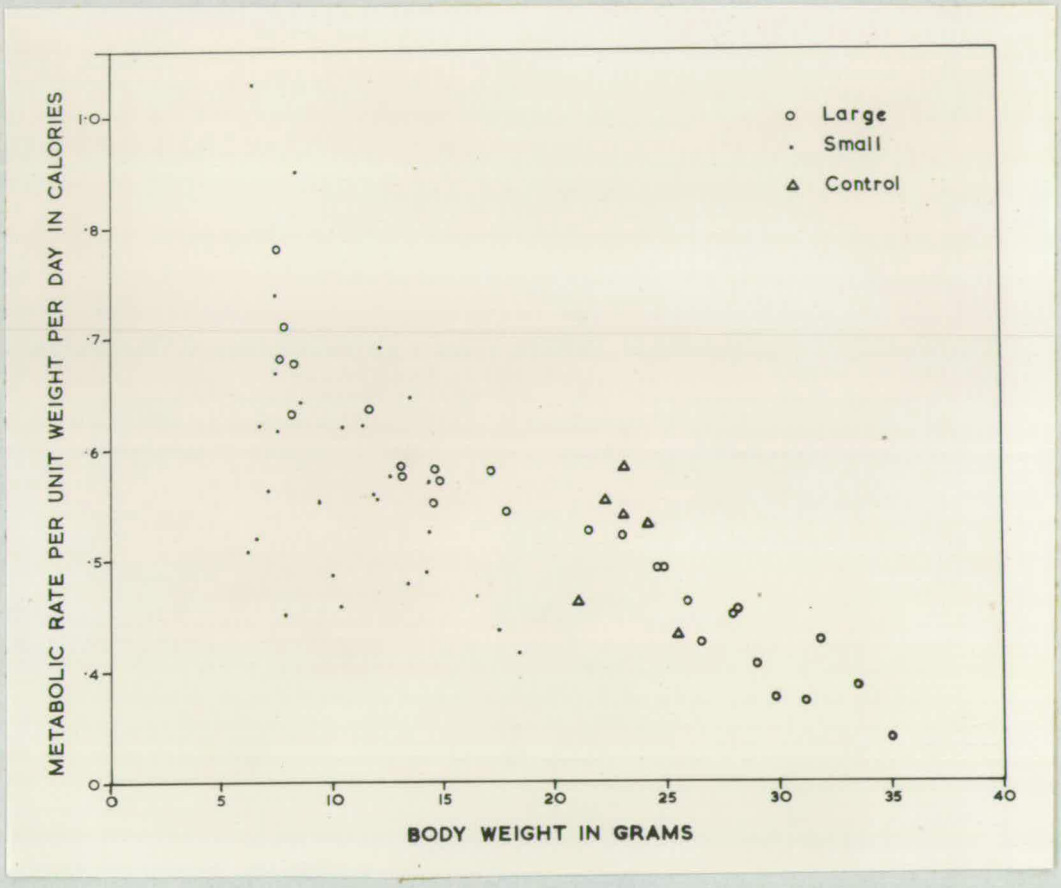

Figure 17. The relation between energy expenditure per unit weight per day and body weight in large, control and small lines.

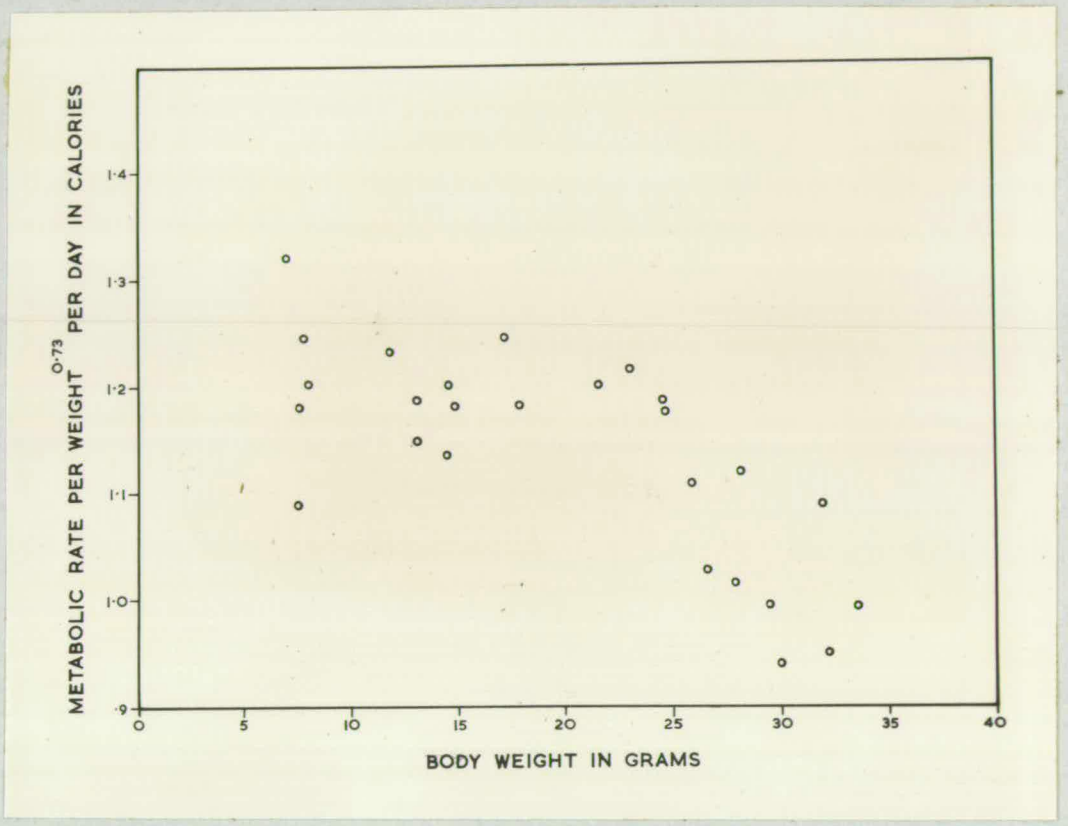

Figure 18. The relation between energy expenditure per $W^{0.73}$ per day and body weight in the large line (RNF).

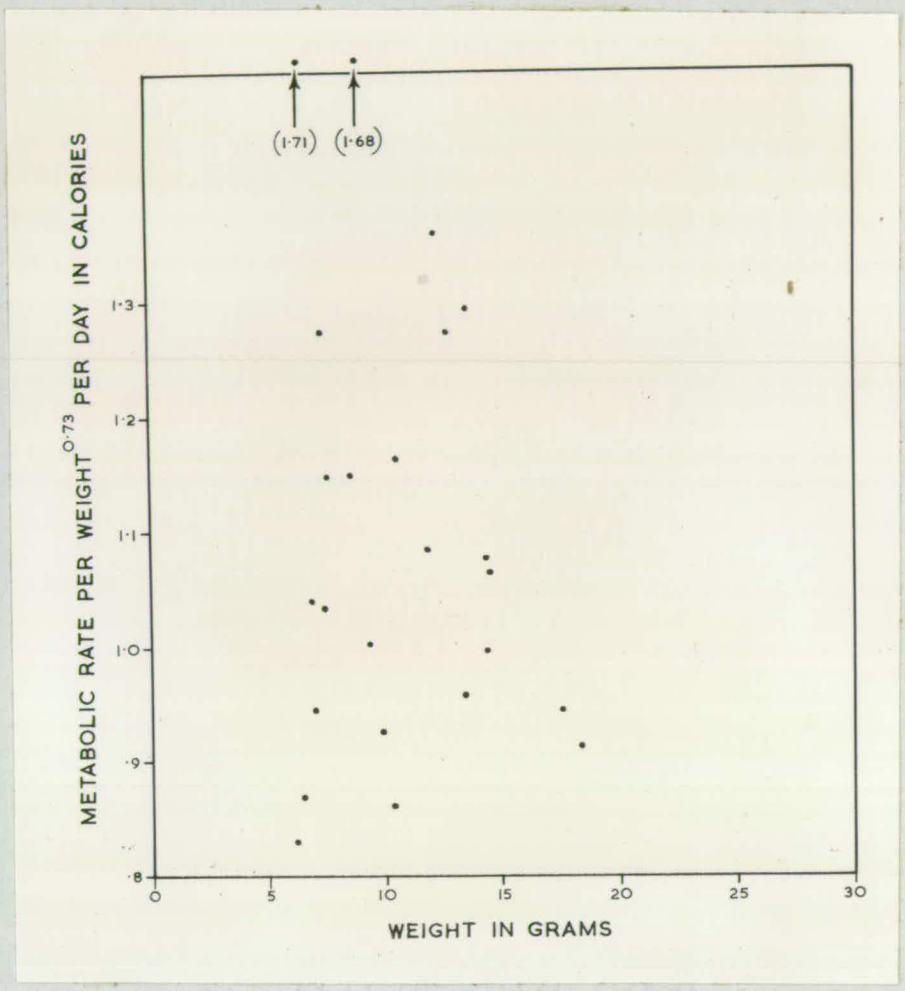

Figure 19. The relation between energy expenditure per $W^{0.73}$ per day and body weight in the small line (RNS).

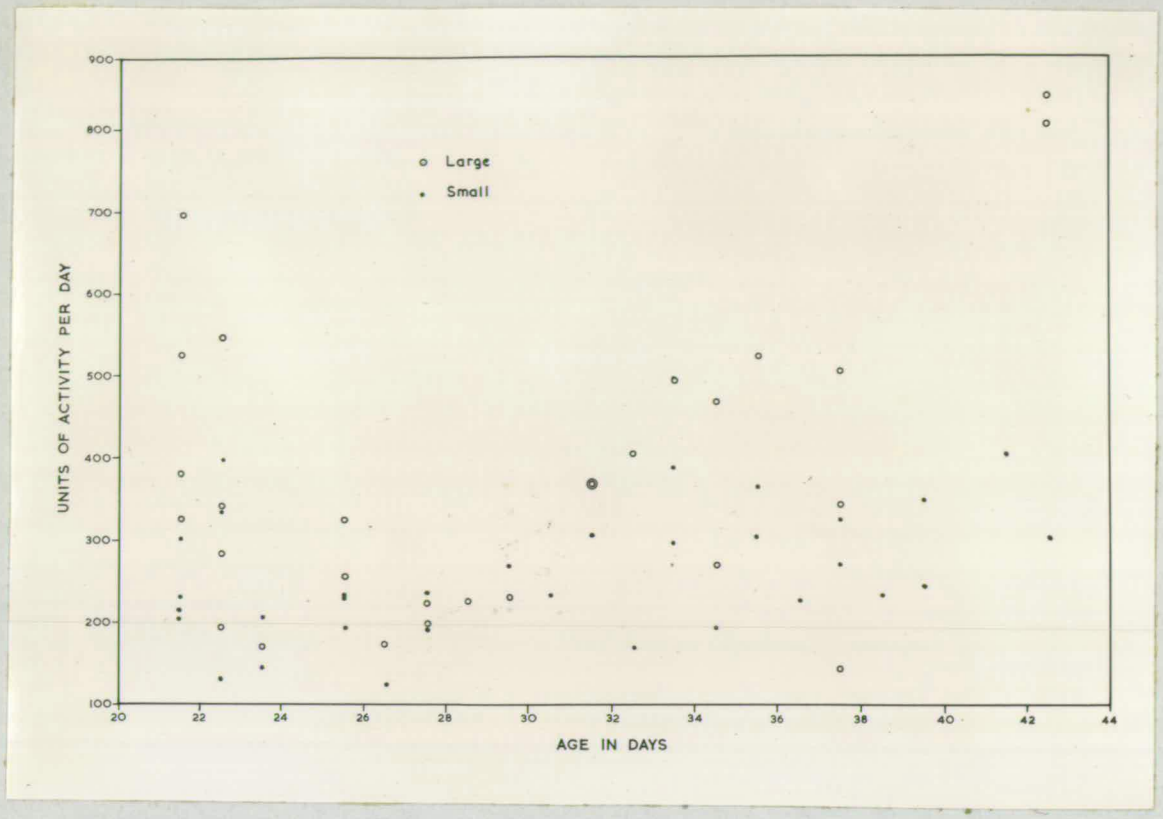

Figure 20. Body activity per day from 21-43 days of age in large and small lines.

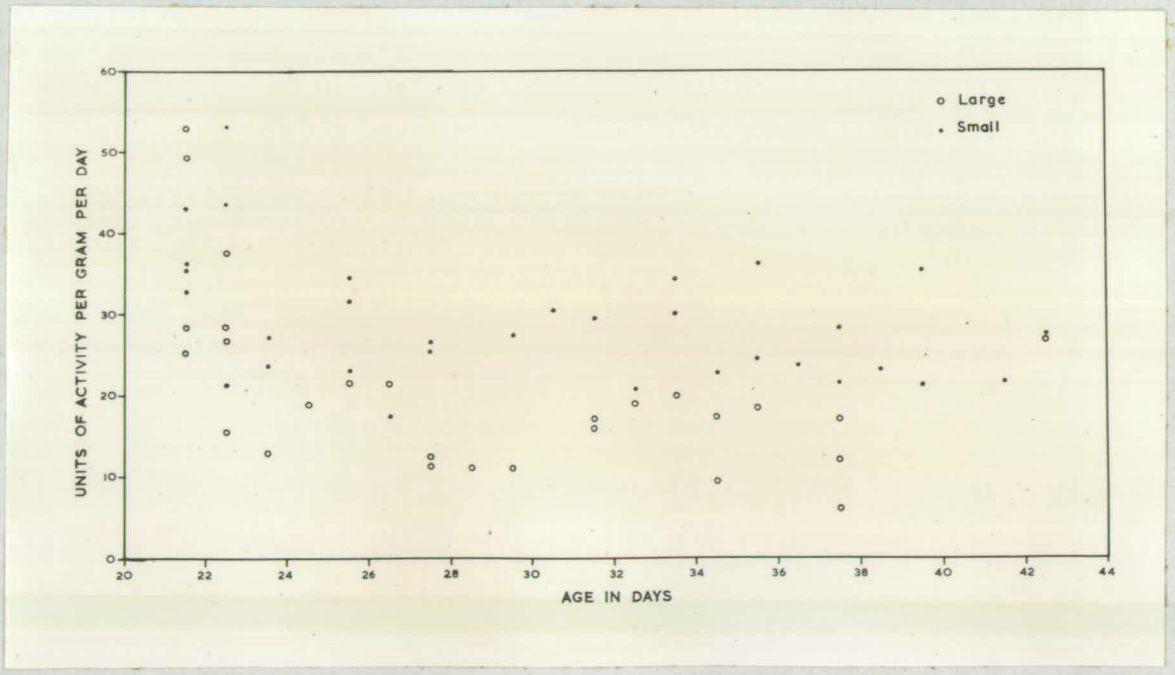

Figure 21. The relation between activity per unit body weight per day and age in large and small lines.

CHAPTER 5

CONCLUSIONS - THE ROLE OF THE ENDOCRINES

An increase in body weight is obviously desirable in the development of strains of livestock suitable for the production of meat. Various methods of selection have been used as a means to obtain an increase in weight far beyond the extremes found in the original population. But increased body size loses much of its value if other factors such as the composition of the carcass, the efficiency of food utilisation, or fertility are adversely affected. The present work has been concerned with some of the physiological consequences resulting from selection for large or small body size in mice. Some aspects of the physiology of mice selected for large and small body size, and of their unselected controls, have been studied in the previous chapters, and these data are co-ordinated in the present chapter.

Differences in body weight between mice of large, control and small lines were already apparent at birth. Absolute growth rates from birth until weaning were always highest in the large line, intermediate in the control line and lowest in the small line, though relative growth rates did not follow this pattern. The relative rate of gain was fairly similar in all three lines from birth to twelve days of age, but differed after twelve days of age. This age coincides with the time that maternal influence on body size will begin to decline as the young begin to wean themselves. After 12 days, the growth rate of the offspring will therefore become increasingly more dependent on their own genotype rather than dependent on the suckling ability of the mother (i.e. the milk supply available).

From twelve days until weaning at three weeks, the relative rate of growth was higher in the large than in the small line, the controls being intermediate. The weight gained in both large and small lines during this period was largely protein and associated water.

From weaning until five weeks of age, relative and absolute growth rates were consistently higher in the large than in the small line, controls being intermediate. The increase in weight in both large and small lines during this period was mainly protein and associated water, although small increases were made in body fat. The protein and fat were deposited at a much faster rate in the large than in the small line. Food intake was higher in the large than in the small line, controls again being intermediate; this is to be expected because in addition to the greater growth rate in the large line, maintenance costs increase with body weight. However, calculations made of the efficiency with which food was converted into body tissue show that despite higher maintenance costs, the efficiency of food utilisation was greater in the large than in the small line, the controls being intermediate. Differences in the efficiency of food utilisation were not caused by differences in the total digestibility of foodstuffs between the large and small lines, although the digestibility of protein and fat was higher, and the digestibility of carbohydrate lower, in the large than in the small line. Total energy expenditure (metabolic rate) per unit weight was higher in the small line during this period, which may be due to the relatively greater heat loss of smaller animals. The higher energy expenditure per unit weight due to greater heat loss in the small line may have been due to increased activity, as small mice were generally more active than large mice of the same age. The higher metabolic rates per unit weight of the small line therefore

contributed to the higher maintenance requirement per gram and this may account for the low efficiency with which food was utilised for less food would be available for growth.

From five to six weeks of age, growth increments began to decrease in the large line and relative growth rates were actually higher in the small line during this period. From 38 to 40 days of age fat deposition increased rapidly in the large line and this coincided with the time at which the rate of protein deposition began to decline. By contrast, fat and protein are deposited in the small line at the same rate as that prior to five weeks of age. Gross efficiency was higher in the small line during this period while energetic efficiency of food utilisation was similar in both lines; this may be attributed to the increased maintenance requirements and the decreasing growth increments of the large line. The metabolic rate per unit weight was higher in the small line and this may again be attributed to increased activity.

From six weeks of age, the relative rates of growth of the three lines were not very different, though usually slightly higher in the small than in the large line. In the large line, most of the weight increases could now be attributed to fat and relatively small increases were made in protein and associated water. In the small line, however, the rates of protein and fat deposition were similar to those found from three to six weeks of age. Gross efficiency of food utilisation was similar in both selected lines, but gross efficiency does not assess the composition of the weight gained. More calories will be required to store one gram of fat than one gram of protein as the energy content of stored fat is nearly twice that of protein. Calorific efficiency was therefore found to be higher in the large than in the small line. The metabolic

rate per unit weight was generally higher in the small line. It is interesting to note that the high metabolic rate of the small line probably reduced gross efficiency at this age, whereas the gross efficiency of the large line was probably determined by the higher food energy required to gain one gram of weight, i.e. because these gains were almost entirely fat tissue.

Several factors may cause the differences in growth rate found between the selected lines and energy expenditure could be one of these factors. The energy expenditure per unit weight is higher in the small than in the large line during the period of most rapid growth, i.e. from three to six weeks of age. But there is little or no evidence that the level of energy expenditure is responsible for the differences in growth rate between the large and small lines since metabolic rates per unit weight were similar or even slightly higher in the large line for comparable body weights. However, real differences in the basal metabolic rate may have been obscured in measurements of total energy expenditure. Moreover, the components contributing to maintenance requirements may not be the same in both lines though the proportion of maintenance energy spent in body activity seemed to be similar in both lines. More critical experiments would be required to investigate the relative importance of the various components contributing to energy metabolism.

Many workers have shown that the normal functioning of the endocrines is essential for growth. Further investigations have therefore been made to study the role of the endocrines in control of growth in the selected lines, for hormonal regulation of the rate of growth may indirectly affect the relative importance of the various components contributing to total energy metabolism. The pituitary, the thyroid gland and the adrenal

cortex are examples of endocrines which might have been affected during selection for large or small body size, the hormones released by these endocrines being essential for normal growth. The pituitary was selected for study.

If selection has reduced the levels of endogenous hormones in the small line, these animals may respond to administration of exogenous hormones. Some preliminary work has been carried out to study the response of the small line to several of the pituitary hormones. The slow rate of growth of small mice may be due to a deficiency in the production of growth hormone. Beef somatotrophin (Armour Laboratories Ltd.) was therefore injected subcutaneously daily for 12-14 days into small mice of between three and six weeks of age. Preliminary results are given in the following table:

Table 21. Response of the Small Line to Injections of 1 mg. Growth Hormone Daily.

	Treated	Untreated Litter Mates
Initial weight	6.2	6.3
Final weight	13.9	11.4
% Increase	124.2	80.9

The results so far indicate that the small line will respond to growth hormone if a sufficiently high dose (1 mg. per day) is given. No response was obtained with doses of less than 1 mg. High doses of the hormone may be required either because these small mice are relatively insensitive to growth hormone, or because there is some degree of species specificity in response to growth hormone. Wilhelmi (1955) has already

demonstrated differences among the growth hormones of different species that he has analysed. At cessation of treatment, the carcass fat and protein was determined in a few treated and untreated animals using the method described in Chapter 2. The carcasses of those animals treated with growth hormone contained a higher percentage of protein and a lower percentage of fat than untreated control litter mates. These effects of growth hormone on carcass composition are similar to those described by Lee and Schaffer (1934), Young (1945), Li and Evans (1948) and Greenbaum and MacLean (1953). The results of these preliminary experiments indicate that mice of the small line are deficient in their endogenous production of growth hormone and that they are not refractory to injections of somatotrophin. A deficiency in the production of growth hormone by mice of the small line would be similar to conditions found in the pig, for Baird et al. (1952) found significantly lower amounts of growth hormone per unit of pituitary tissue in pigs selected for low rates of gain than in those selected for high rates of gain.

Studies are also in progress to investigate the effect of selection on the gonatrophic activity of the anterior pituitary, and its relation to the infertility found in the small line of mice. A large percentage of the females of the small line are infertile until three to four months of age as judged by the arrival of their first litters. Several factors appear to be responsible for this infertility. Firstly, many small females paired with proven males fail to come into oestrus and mate, as judged by the presence of a vaginal plug. These females appear to be sexually immature, for treatment with the gonadotrophins pregnant mare serum and human chorionic gonadotrophin, using a method similar to that described by Runner and Gates (1954) for immature mice, will induce oestrus,

ovulation and mating in 89% of them. Secondly, approximately one third of all females which mated on their natural oestrus failed to ovulate. The eggs of some females which mated and ovulated were examined under the phase-contrast microscope and 95% of them were fertilised. Finally, only 44% of those females which had mated during natural oestrus had implanted embryos when killed at 12-16 days gestation. Many of the small females therefore have an oestrous cycle and ovulate but their embryos fail to implant. Lack of embryos may be due to improper uterine stimulation as a result of the failure of the corpus luteum of pregnancy to develop. Smithberg and Runner (1956) have maintained pregnancies in immature mice which had mated in response to treatment with gonadotrophins by daily injections of progesterone. Similar treatment of small mice which had mated on natural oestrus increased the number of females with implants from 44% to 82%. The lack of implants in the remaining females may have been partly due to a failure to ovulate. The infertility in many mice selected for small body size therefore appears to be due to a deficiency of the follicle-stimulating and luteinising hormones of the anterior pituitary, and some females also appear to be deficient in progesterone which again may be due to hypofunctioning of the anterior pituitary in failing to produce prolactin (luteotrophin). Treatment of pregnant females with luteotrophin should determine whether the corpora lutea are normal and capable of response by secreting progesterone. Mice of the small line therefore appear to be deficient in the production by the anterior pituitary of growth hormone, follicle stimulating hormone, luteinising hormone and probably luteotrophin.

These preliminary studies support the hypothesis that the primary effect of selection has been to alter the endogenous production of some

of the hormones secreted by the anterior pituitary. Selection for small body size appears to have reduced the levels of these hormones and selection for large body size may have increased them. Many of the differences in the large and small lines in carcass composition, efficiency of food utilisation and energy expenditure are explicable in terms of different growth rates, in that at any absolute age, animals of the two lines will be at relatively different stages in their development. In contrast, differences in the rate of growth appear to be controlled by the levels of circulating hormones, these levels being influenced by the genotype of the animal. The extension of these studies to other strains of mice selected for large or small body size may reveal whether the same physiological mechanisms are always affected by selection.

SUMMARY

Differences in body weight between mice selected for large and small body size and their controls were apparent at birth and were fully maintained up to 30 weeks of age. Differences in absolute and relative rates of growth between the three lines reached a maximum from 21-35 days of age and declined thereafter.

Growth in the large line from 14-40 days of age was largely due to an increase in body protein and water; after this age, increase in weight was caused mainly by fat deposition. In the small line, protein, water and fat were deposited at a more constant rate during the whole growing period. Different rates of growth could largely account for the differences in the percentage composition found between mice of the large and small lines of the same age. The percentage composition of two large strains which had been selected on similar criteria was analysed; one strain was found to have a higher percentage of protein and water and a lower percentage of fat than the other despite the similar criteria of selection. The role of physiology as an aid to further selection programmes is discussed.

Selection for increased body size was associated with increased food consumption and greater economy of gain. Mice of the small line ate less food and were not so efficient in converting foodstuffs into body tissue.

The digestibility of foodstuffs was similar in large and small lines. Though energy expenditure per unit weight was higher in small than in large mice of the same age, these differences became negligible when animals of similar weights were compared. Neither total energy expenditure nor body activity were apparently factcrs limiting growth in the small line.

Mice of the small line responded to treatment with certain exogenous hormones. The primary effect of selection for large or small body size may have been to increase or decrease the levels of some of the hormones secreted by the anterior pituitary, thereby altering growth rates. Changes in growth rates may have caused the differences between the large and small lines in carcass composition, efficiency of food utilisation and energy expenditure.

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REFERENCES

BAIRD D.M., NALBANDOR A.V. & NORTON H.W. 1952. Some physiological causes of genetically different rates of growth in swine. J. Animal Science, 11, 292-300.

BARBOUR H.G. & TRACE J. 1937. Standard metabolism in the white mouse. Amer. J. Physiol., 118, 77-86.

BATEMAN N. 1954. The measurement of milk production of mice through pre-weaning growth of suckling young. Physiol. Zool., 27, 163-173.

BEHNKE A.R. 1953. The relation of lean body weight to metabolism and some consequent systematizations. Ann. N.Y. Acad. Sci., 56, 1095-1142.

BENEDICT F.G. 1938. Vital Energetics. A study in comparative basal metabolism. Publ. Carnegie Instn. No. 503.

BENEDICT F.G. & LEE R.C. 1936. La production de chaleur de la souris. Etude de plusiers races de souris. Ann. Physiol. & Physicochim. biol., 12, 983-1064.

BLEIBTREU M. 1901. Fettmast und respiratorischer Quotient. Pflüger's Arch. gesam. Physiol., 85, 345-400.

BLUHM A. 1929. Ueber Einige das Geburtsgewicht der Säugetiere beeinflussende Faktoren. Arch. Entw. Mech. Org., 116, 348-381.

BRODY S. 1945. Bioenergetics and Growth. Reinhold Publishing Corporation, New York.

BROOKS C.H. & LAMBERT E.F. 1946. A study of the effect of limitation of food intake and the method of feeding on the rate of weight gain during hypothalamic obesity in the albino rat. Amer. J. Physiol., 147, 695-707.

BUTLER L. & METRAKOS J.D. 1950. A study of size inheritance in the house mouse. Canadian J. of Research, 28, Sect. C. & D.

CARLSON A.J. 1919. The Control of Hunger in Health and Disease. University of Chicago Press, Chicago.

CROZIER W.J. & ENZMANN E.V. 1935. On the relation between litter size, birth weight and rate of growth in mice. J. Gen. Physiol., 19, 249-263.

DEWAR A.D. & NEWTON W.H. 1948a. The determination of total metabolism in the mouse. Brit. J. Nutrit., 2, 123-141.

DEWAR A.D. & NEWTON W.H. 1948b. The relationship between food intake and respiratory quotient in mice, Brit. J. Nutrit., 2, 142-145.

DICKERSON G.E. 1947. Composition of hog carcasses as influenced by heritable differences in rate and economy of gain. Res. Bull. Ia. Agric. Exp. Sta., 354, 489-524.

DICKERSON G.E. & GOWEN J.W. 1947. Hereditary obesity and efficiency of food utilisation in mice. Science, 105, 496-498.

DICKERSON G.E. & GRIMES J.C. 1947. The effectiveness of selection for efficiency of gain in Duroc swine. J. Animal Sci., 6, 266-287.

ECKSTEIN P. & MCKEOWN T. 1955. The influence of maternal age, parity and weight on litter size in the guinea-pig. J. Endocrinology, 12, 115-119.

FALCONER D.S. 1947. Milk production in mice. J. Agr. Sci., 37, 224-235.

_____ 1948. Selection for size in mice. Heredity, 2, 403 (Abstr.)

_____ 1953. Selection for large and small size in mice. J. Genet., 51, 470-501.

_____ 1955. Patterns of response in selection experiments with mice. Cold Spr. Harb. Sym. quant. Biol., 20, 178-196.

FALCONER D.S. & KING J.W.B. 1953. A study of selection limits in the mouse. J. Genet., 51, 561-581.

FALCONER D.S. & LATYSZEWSKI M. 1952. The environment in relation to selection for size in mice. J. Genet., 51, 67-80.

FRANCIS T. 1944. Studies on hereditary dwarfism in mice. Acta. Path. et Microbiol. Scand., 21, 928-956.

GOODALE H.D. 1937. A study of the inheritance of body weight in the albino mouse by selection. Genetics, 22, 193-194 (Abstr.).

_____ 1938. A study of the inheritance of body weight in the albino mouse by selection. J. Heredity, 29, 101-112.

_____ 1941. Progress report on possibilities in progeny-test breeding. Science, 94, 442-443.

GREENBAUM A.L. & MCLEAN P. 1953. Changes in body composition and respiratory quotient of adult female rats treated with purified growth hormone. Biochem. J., 54, 400-407.

GREENE H.S. 1940. A dwarf mutation in the rabbit. J. Exp. Med., 71, 839-856.

GRUNEBERG H. 1952. The Genetics of the Mouse. 2nd edition. Martinus Nijhoff, The Hague, Netherlands.

HALDANE J.S. 1892. A new form of apparatus for measuring the respiratory exchange of animals. J. Physiol., 13, 419-430.

HETHERINGTON A.W. & RANSON S.W. 1942. The spontaneous activity and food intake of rats with hypothalamic lesions. Amer. J. Physiol., 136, 609-617.

INGALLS A.M., DICKIE MM. & SNELL G.D. 1950. Obese, a new mutation in the house mouse. J. Hered., 41, 317-318.

KING J.W.B. 1950. Pygmy, a dwarfing gene in the house mouse. J. Hered., 41, 249-252.

KOCHAKIAN C.D. 1946. The protein anabolic effects of steroid hormones. Vitamins and Hormones, 4, 255-310.

KRISS M. & MILLER R.C. 1934. The derivation of factors for computing the gaseous exchange and the heat production in the metabolism of casein by the albino rat. J. Nutrit., 8, 669-674.

LANE P.W. & DICKIE M.M. 1954. Fertile obese male mice. J. Heredity, 45, 56-58.

LEATHEM J.H. 1952. Quoted by Gaunt R. Dynamics of Growth Processes, 1954. Princeton Univ. Press, New Jersey.

LEE N.O. & SCHAFFER N.K. 1934. Anterior pituitary growth hormone and composition of growth. J. Nutrition, 7, 337-363.

LI C.H. & EVANS H.M. 1948. The biochemistry of pituitary growth hormone. Rec. Prog. Hormone Res., 3, 3-44.

LUSK G. 1924. Analysis of the oxidation of mixtures of carbohydrate and fat. J. Biol. Chem., 59, 41-42.

MACARTHUR J.N. 1944a. Genetics of body size and related characters. I. Selecting small and large races of the laboratory mouse. Amer. Nat., 78, 142-157.

_____ 1944b. Genetics of body size and related characters. II. Satellite characters associated with body size in mice. Amer. Nat., 78, 224-237.

_____ 1949. Selection for small and large body size in the house mouse. Genetics, 34, 194-209.

MAYER J. 1953. Genetic, traumatic and environmental factors in the etiology of obesity. Physiol. Rev., 33, 472-508.

_____ 1955. Mechanism of regulation of food intake and multiple etiology of obesity. Voeding, 16, 62-88.

MAYER J. & HAGMAN N. 1953. Total body water and blood volume in hereditary obese-hyperglycaemic syndrome of mice. Proc. Soc. Exp. Biol. Medicine, 82, 647-649.

MORRIS H.P., PALMER L.S. & KENNEDY C. 1933. An experimental study of inheritance as a factor influencing food utilisation in the rat. Minn. Agr. Exp. Sta. Tech. Bull., 92.

NILSON H.W., PALMER L.S. & KENNEDY C. 1935. Physiological effects of pituitary growth hormone: growth and efficiency of food utilisation. Amer. J. Physiol., 111, 341-351.

PALMER L.S., KENNEDY C., CALVERLEY C.E., LOHN C. & WESWIG P.H. 1946. Genetic differences in the biochemistry and physiology influencing food utilisation for growth in rats. Minn. Agr. Exp. Sta. Tech. Bull., 176.

RUNNER M.N. & GATES A. 1954 . Conception in prepuberal mice following artificially induced ovulation and mating. Nature, 174, 222-223.

SCHONHOLZ D.H. & OSBORN C.M. 1949. Temperature studies in dwarf mice. Anat. Rec., 105, 605 (Abstr.).

SCOTT W.N., SCOTT C.C. & LUCKHARDT A.B. 1938. Observations of the blood sugar level before, during and after hunger periods in humans. Amer. J. Physiol., 123, 243-247.

SLONAKER J.R. 1924. The effect of puberscence, oestruation and menopause on the voluntary activity in the albino rat. Amer. J. Physiol., 68, 294-315.

SMITH P.E. & MACDOWELL E.C. 1930. An hereditary anterior pituitary deficiency in the mouse. Anat. Rec., 46, 249-257.

_____ 1931. The differential effect of hereditary mouse dwarfism on anterior pituitary hormones. Anat. Rec., 50, 85-93.

SMITHBERG M. & RUNNER M.N. 1956. The induction and maintenance of pregnancy in prepuberal mice. J. Exp. Zool., 133, 441-457.

SNELL G.D. 1929. Dwarf, a new Mendelian recessive character in the house mouse. Proc. Nat. Acad. Sci. Wash., 15, 733-734.

SOBERMAN R.J., BRODIE B., LEVY B., AXELROD J., HOLLANDER V. & STEELE J. 1949. The use of antipyrine in the measurement of total body water in man. J. Biol. Chem., 179, 31-42.

VAN WAGENEN G. 1928. Some effects of early castration on the growth of the male rat. Amer. J. Physiol., 84, 461-467.

VAN WAGENEN G. 1949. Accelerated growth with sexual precocity in female monkeys receiving testosterone propionate. Endocrinology, 45, 544-546.

VEIGE O. 1950. Studies of the maternal influence on the birth weight in rabbits. Acta Zool., 31, 1-148.

_____ 1953. Studies of the maternal influence on the growth in rabbits. Acta ag. Scandinav., 3, 243-291.

WIDDOWSON E.M. 1955. Assessment of energy value of human foods. Proc. Nutrit. Soc., 14, 142-154.

WILHELMI A.E. 1955. Comparative biochemistry of growth hormone from ox, sheep, pig, horse and fish pituitaries. The Hypophysial Growth Hormone, Nature and Actions. McGraw-Hill Book Co., U.S.A.

WILLIAMS H.B., RICHE J.A. & LUSK G. 1912. Metabolism of the dog following the ingestion of meat in large quantity. J. Biol. Chem., 12, 349-376.

WRENSHALL G.A., ANDRUS S.B. & MAYER J. 1955. High levels of pancreatic insulin coexistent with hyperplasia and degranulation of beta cells in mice with the hereditary obese hyperglycemic syndrome. Endocrinology, 56, 335-340.

YOUNG F.G. 1945. Growth and diabetes in normal animals treated with anterior lobe diabetogenic extract. Biochem. J., 39, 515-536.

ZEUNTHEN E. 1947. Body size and metabolic rate in the animal kingdom. Compt. rend. trav. lab. Carlsberg, Ser. chim., 26.

_____ 1953. Oxygen uptake as related to body size in organisms. Quart. Rev. Biol., 28, 1-12.