Genetic and Metabolic Aspects of Growth and its Components in Mice.

by

Stephen C. Bishop

Thesis presented for the degree of

Doctor of Philosophy
University of Edinburgh
1985.

ACKNOWLEDGEMENTS

There are many people who have contributed towards this thesis, both directly and indirectly, and in both in large and small capacities, and I can only hope to mention but a few of them.

Firstly, I must convey my deep gratitude to my supervisor, Prof. W.G. Hill, for his unerring advice and guidance throughout the course of my study, and for his good-natured supervision.

Equally valuable have been the staff of the mouse house, for the day to day management of the mice. I would especially like to thank Ann Walker and Frances Thompson for organisation of the facilities and occasional technical help, and also Frances for the brown adipose tissue and gonadal fat pad dissections. Dr. Gillian Sharp and Mr. S. King have also contributed greatly through the running of the selection lines, and by providing mice for my own experiments - whenever I demanded them.

For the calorimeter work I am totally indebted to Mr. J. Ireland, Dr. D. Wilson and Dr. M. Nielsen. Dr. Nielsen was invaluable in helping me to conceive of the idea of the calorimetry work, and he also provided much guidance in the initial stages of my research, and Dr. Wilson helped greatly in the design of the calorimeter. It is without the help of Mr. J. Ireland, however, that much of the work would not have been possible, as he patiently applied his considerable expertise to the frustrating and exacting job of constructing a calorimeter that worked, and then whole repeated the process when the second calorimeter was constructed.

Finally from a technical viewpoint, I must thank Mrs. Susan Brotherstone and Mr. J. Rasbash for help and advice with the inevitable computing problems.

Many thanks are also due to to my fellow Ph.D. students - Forbes Brien, Vijay Chauhan, Penelope Cook, Janet Williams and Karin Meyer to name but a few - for advice, encouragement and help during my three years of study. I am also indebted to my various personal friends for support during my term of study, and also for providing well needed and appreciated diversions from academic life at frequent intervals.

Penultimately I wish to thank the Association of Commonwealth

Universities for providing personal financial support by way of a Commonwealth Scholarship. Without this generous assistance it would not have been possible for me to study in Edinburgh. The Agriculture and Food Research Council is also acknowledged for funding the mouse research.

Finally, I would like to thank the countless mice, too many to be personally named, who unstintingly and unselfishly sacrificed themselves with sympathy and understanding for this Ph.D and, more importantly, for the advancement of science and the service of mankind.

CONTENTS

ABSTRACT

In an attempt to gain a greater understanding of growth and the genetic relationships between the components of growth (i.e. intake, maintenance requirements and fat and lean gain), correlated responses in lines of mice selected for 4 to 6 week intake (A or appetite lines), the ratio of gonadal fat pad weight (GFPW) to body weight (BW) at 10 weeks of age (F or fat lines) and estimated lean mass (BW-8*GFPW) at 10 weeks of age (P or protein lines) were studied.

In the A lines, increasing intake appears to have increased both maintenance requirements and intake in excess of maintenance proportionately. These changes are not related to either the quantity of brown adipose tissue present or temperature adaptation effects. Increasing intake has also reduced carcass fatness and this is probably due to a restriction placed on 4 week weight (carcass fatness and intake per se are positively correlated).

Increasing lean mass (P lines) has increased body size and efficiency but it has had only small effects on carcass composition, and intake and maintenance requirements in relation to metabolic bodyweight ($BW^{.75}$).

Increasing fat percentage (F lines) has resulted in large changes in fat mass, but little change in estimated lean mass at any age. Maintenance requirements appear to be related to lean mass rather than body weight, and the fatter lines are the more efficient lines. Fat percentage has been increased by changing the partition of net energy towards fat deposition as well as increasing total intake in excess of maintenance.

It was concluded that there is genetic variation in all of the components of growth; that many of the components can change independently of each other with selection; that maintenance is more closely related to lean mass than to body weight; and that for mice the ratio intake/maintenance is far more important in defining efficiency than is the type of tissue being deposited.

Attempts are made to extrapolate the results to domestic species, and the relevence of experiments using mice is discussed.

Section I INTRODUCTION AND REVIEW OF LITERATURE

1.1 INTRODUCTION

The value of an animal for meat production is determined by its rate of growth, efficiency of growth and the quality of meat it produces (N.B. quality may be thought of as being largely a function of carcass fatness, with the current consumer preference being for meat of a low fat content). It is desirable for both the consumer and the producer, therefore, that animals of a high quality should be produced as cheaply and as humanely as possible. These objectives can be met through both environmental means (e.g. improved health, nutrition and welfare) and genetic means, and it is to the genetic means of improving meat production that this thesis is addressed.

Genetic improvement of livestock is usually undertaken using selection programmes to improve the genetic merit of the animals being produced, or by using crossbreeding schemes to combine advantageous traits between lines of animals. Before undertaking these breeding schemes, however, it is necessary to have a detailed knowledge of the genetic components of the traits of interest so that selection strategies and indices can be designed which enable quick and efficient genetic progress, and also so that undesirable correlated responses to, or side effects of, selection can be predicted and avoided. Rate, efficiency and "quality" of growth are a complex interaction of traits, and thus considerable knowledge of the interactions of these traits is needed before suitable breeding strategies can be proposed. The aim of this thesis is to study the relationships between these traits, and thus to attempt to provide a greater understanding of their genetic inter-relationships.

Although the specific genetic parameters needed to derive selection indices (e.g. heritabilities and genetic correlations) must be derived separately for each species, the overall biological relationships between these traits, and the patterns of growth in general, have often been modelled using laboratory animals - for reasons of time, expense and experimental ease. In this thesis, therefore, the genetic aspects of growth will be studied using laboratory strains of mice. The relevance of using mice for modelling the growth of larger domestic animals will, however, be considered when the final conclusions are drawn.

1.2 GROWTH AND ITS COMPONENTS:GENERAL AND METABOLIC ASPECTS

This section gives a brief description of growth and its components, secondly considers the energy metabolism of a growing animal, and finally considers the outcome of these processes in terms of the efficiency of growth.

1.2.1 Growth

1.2.1.1 Definition and description

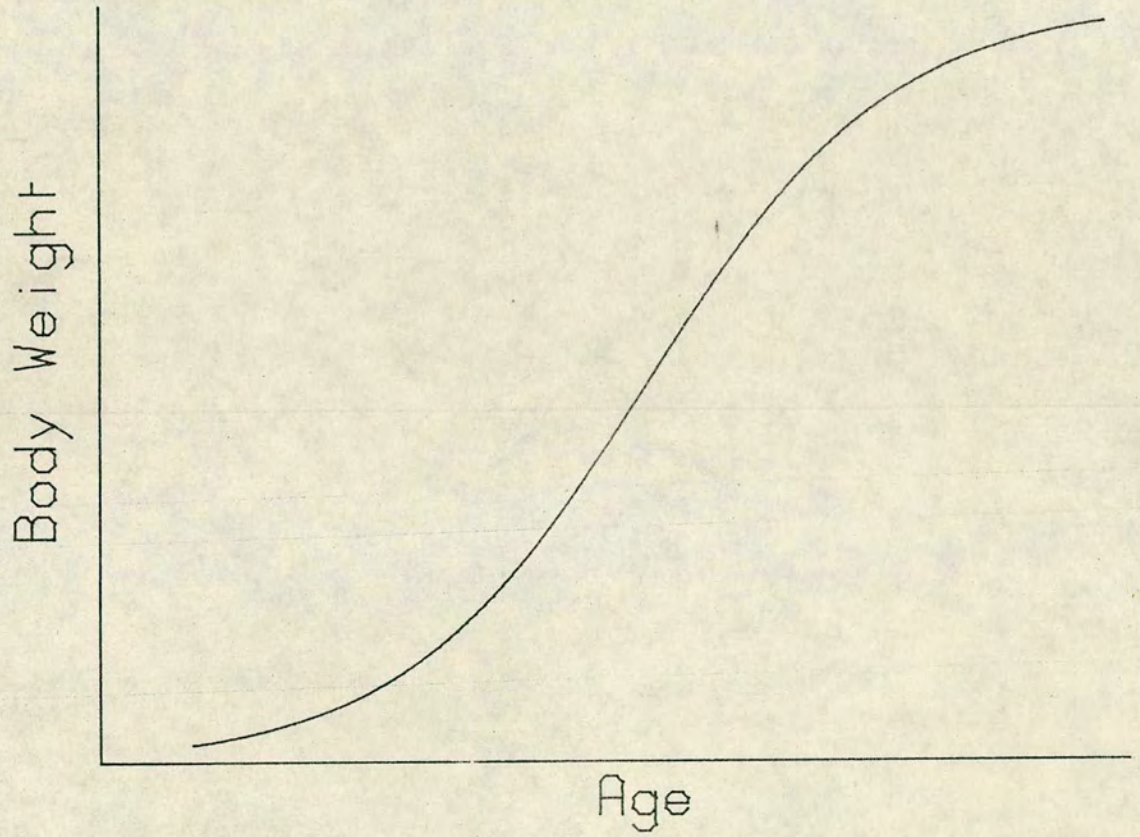
In its simplest form growth may be thought of as the synthesis and accretion of new biochemical units, from the time of conception until the animal reaches a relatively stable mature weight.

The growth of an animal is best described simply by considering it in the form of a growth curve, plotting body weight against either age (Eisen et al, 1969; Richards,1959) or cumulative food intake (Titus et al,1934; Parks,1970). The normal growth curve of body weight against time has a sigmoidal character which may be divided into an accelerating phase of growth, and a decelerating phase of growth as the animal approaches maturity. For mice, the maximum growth rate normally occurs between 4 and 5 weeks of age (Eisen et al,1969). Absolute growth rate (gain/time) usually increases with increasing mature body size, and in general, the time taken to reach mature weight is proportional to mature weight$^{.27}$ (Taylor,1965), for animals of a wide range of mature body size and species.

The general shape of an animal's growth curve is shown in fig. 1.1. This curve can be described mathematically by several empirically derived exponential equations relating body weight to time (Eisen et al,1969). The three most common such curves are the Bertalanffy, Gompertz and Logistic curves, all of which are merely special cases of a general family of curves - the Richards generalised curve (Richards,1959). The Richards curve is of the form:

$$Y=A(1-be^{-kt})^{1/(1-m)}$$

Fig. 1.1 Generalised Animal Growth Curve



where Y = body weight at time t

A = asymptote, or predicted final or mature weight

b = time scale parameter

k = rate of growth parameter

m = shape parameter

The Bertalannfy, Gompertz and Logistic curves vary by having different fixed m (shape parameter) values, i.e.2/3, lim. m→1 and 2, respectively. By fixing these m values, the weights at inflexion are fixed at 8/27, e^{-1} and 1/2 of final weight, respectively, for the Bertalannfy, Gompertz and Logistic curves. There are many instances of these curves having been fitted to growth data from many species (Eisen,1976), and all that need be said is that all of these curves have been shown to fit the data "well" under various circumstances, with the Richards curve allowing the greatest flexibility.

Titus et al (1934) derived curves relating body weights of chickens to cumulative food intake, whilst Parks (1970) succeeded in finding curves which fitted body weight to both cumulative food intake and age. Both methods were shown to give "adequate" descriptions of live weight.

When considering growth of an animal in terms of a growth curve, however, it is important to realise that these curves are purely mechanical or descriptive, and empirical in their derivation. Although approximate biological interpretations can be given to their parameters, the parameters have no relationship with the causal mechanisms of growth. Finally, these curves usually ignore the anatomical or carcass components of growth, as well as the efficiency of growth.

1.2.1.2 Carcass composition

The major components of the carcass are fat, protein, ash and water, of which water makes up by far the greatest proportion. The main output components of growth may be considered to be fat and lean (protein + water), as ash comprises only a small proportion of total body weight.

The relative proportions of the carcass components change throughout growth, and Clarke (1969) found the allometric equation:

$Y=aX^{b}$ where Y = carcass component weight

X = body weight

to be the most convenient method of relating an animals carcass composition to its body weight or growth. In general the exponent b is greater than, equal to and less than one for the fat, protein and ash components of the body, respectively. This means that animals tend to get relatively fatter as they grow, whereas their protein percentage remains almost constant.

Composition of the fat free tissue follows the pattern shown in fig. 1.2. The point at which the components reach a stable percentage is known as chemical maturity (Moulton,1923 in Sutherland et al,1974). It is generally accepted that at any given weight of fat free body, the ratio of water to fat free dry matter is relatively constant (Fowler et al,1976), thus overall dry matter% is a very good estimater of fat%.

Carcass composition and therefore the growth of the body components is important for two reasons. Firstly, fat and lean have different energetic costs of gain (section 1.2.2.3), and thus the relative proportions of each are important in defining the efficiency of growth. Secondly, the fat and lean contents of meat are important in terms of human preference and nutrition, and therefore help to determine the quality and saleability of meat.

1.2.2 Metabolism

1.2.2.1 Description

Metabolism in this thesis will refer to energy metabolism, which in its simplest form may be defined as the study of the processes of energy transfer in animals (Blaxter,1962). Furthermore, the method of analysing metabolism will be what Blaxter (1979) refers to as the "descriptive analysis", as it follows and describes the patterns of energy usage but makes little or no attempt to explain the causal mechanisms.

Stated in its simplest terms, metabolisable energy (ME) (food energy less excreta energy) is utilised firstly for the maintenance of life,

Fig. 1.2 Theoretical Composition of the Fat Free Body

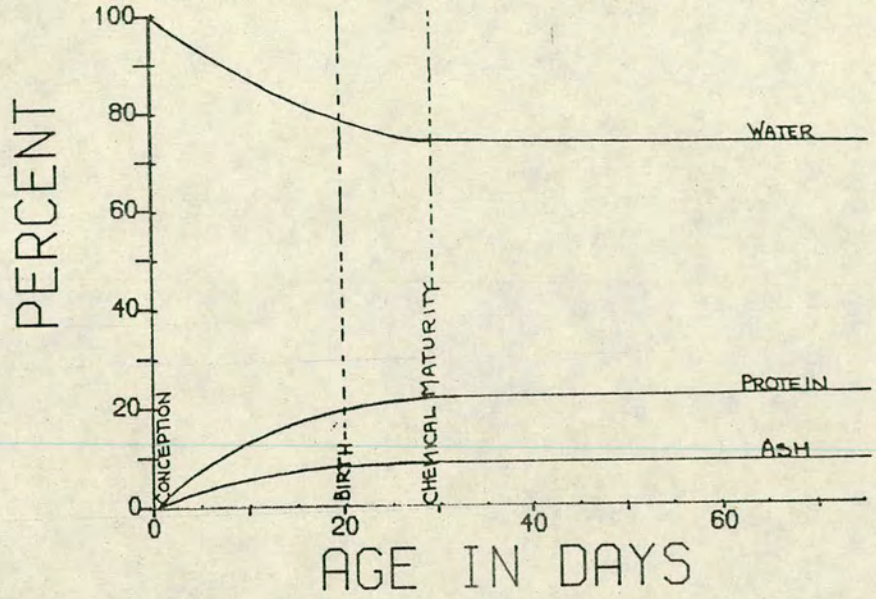
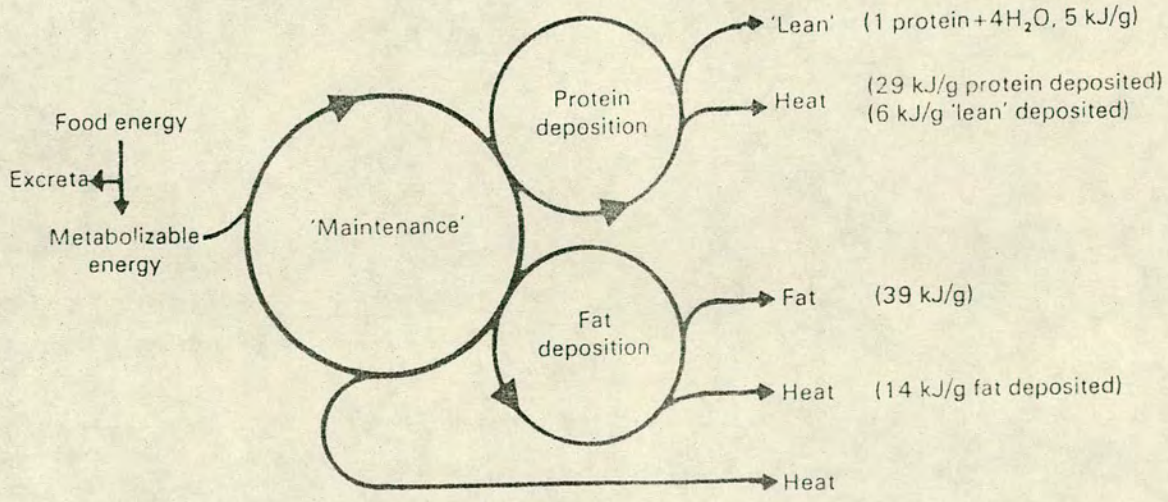


Fig. 1.3 Utilization of Food Energy for Growth. (Webster, 1977)



and energy surplus to this requirement (net energy) can be used to promote growth (Webster,1983). Energy for maintenance must of course be dissipated as heat, and since tissue will not be deposited with 100% efficiency, heat will also be produced by the growth processes (i.e. lean and fat deposition). A summary of these concepts is given in fig. 1.3.

Using this model as a framework, Kirkwood and Webster (1984) studied energy usage across a wide range of species of mammals and birds. They found that the patterns of energy usage for growth and heat production were similar over time, when age was expressed as a proportion of time taken to reach mature size, for the mammalian species studied. This model therefore appears to be quite adequate for describing and comparing mammals, although the study found considerable variation between the bird species studied. Birds, in general, appear to have somewhat different patterns of energy usage than mammals, so care may need to be taken when comparing birds and mammals.

The term "components of growth" will be used throughout this thesis to describe the separate parts of this model of energy usage. The "input component of growth" will refer to energy intake, whilst maintenance requirements and fat and lean deposition will be referred to as the "output components of growth".

1.2.2.2 Maintenance

A fasting animal uses most of its energy for protein turnover, maintenance of ion gradients and, in many environments, thermoregulation. This together with the work of digestion and general activity comprise the energy requirements for maintenance (Blaxter,1979). The energy given off by a fasted animal is known as fasting, or basal, heat production, and a measure of this is often used to help determine maintenance requirements (Blaxter,1962). For simple stomached (monogastric) animals, ME requirements for maintenance are approximately 1.3 times basal heat production, and in the ruminant this figure can vary from 1.35 to 1.5 (Webster,1981).

In a growing animal maintenance is best thought of as that metabolisable energy which is not used for growth, and in an adult animal of stable body size and normal physiological status (e.g. not

pregnant or lactating) maintenance requirements are simply the animals intake.

Fasting heat production of adult animals of differing mature body sizes, and different species, varies in proportion to body weight$^{.75}$ (metabolic body weight)(Webster,1981), and thus maintenance requirements are often expressed in relation to metabolic body weight. Some authors express caution in using this tool (i.e. metabolic body weight), however, as it is purely empirical in derivation. The Agricultural Research Council (1981;1982) prefer the use of the exponent .67 for growing animals, and Thonney et al (1976) suggest using body weight (BW)^{b} or ln BW as a covariable in the statistical model to correct for weight, instead of using a fixed exponent. Nevertheless metabolic body weight is useful for strictly comparative purposes, especially if used in conjunction with body weight per se.

In a non fasted animal heat output rises with increasing ME intake (Webster, 1981). This is known as the "heat increment of feeding" in ruminants, and "special dynamic effect" in monogastric animals, and is referred to above as the work of digestion. This heat output should not be confused with fasting heat production. Most attempts to explain the work of digestion in physiological terms are incomplete (Webster,1981).

There is much interest in the study of the factors affecting fasting heat production and maintenance requirements. It appears that tissues containing protein are much more metabolically active than tissues such as fat and wool, due to protein turnover. In a fasted sheep, the gut and liver alone may contribute up to 40% of the total heat production (Webster,1981). In the same paper, Webster reviews evidence from fat and lean animals over a range of species that maintenance may be more closely related to protein (or lean) mass, than body weight per se, and Fowler et al give similiar evidence in their 1976 paper. It appears, therefore, that protein turnover may be an important factor affecting maintenance. In this review no attempt will be made to discuss the complex relationships between protein turnover and protein deposition.

Another, albeit controversial,source of heat production in animals is brown adipose tissue (BAT). BAT is capable of producing large amounts of heat via the sympathetic nervous system, and whilst it is

present in most mammalian species early in life, its importance in adult animals is generally not known (Saxton and Eisen,1984). Cold-adapted animals show "non-shivering" thermogenesis, which increases heat production, and this phenomenon is related to the presence of BAT (Lindberg,1970).

Through encouraging rats to overeat, Rothwell and Stock (1979) observed an increase in heat production mediated through BAT, and they termed this "diet-induced thermogenesis". This phenomenon is considered to be an extension of the concept known as the "heat increment of feeding", or work of digestion, component of maintenance (A.J.F.Webster,1981), and if this were true then the amount or activity of BAT would be important in determining maintenance requirements. There have been reservations expressed about this experiment, however, in terms of the experimental technique, the relevence of the overeating factor in modelling normal metabolism, and lastly in the ability to extrapolate these results to other species where the quantity and importance of BAT is not known (Webster,1981; A.J.F.Webster,pers.comm.).

Although there have been many studies measuring the work of digestion component of maintenance using animals which have been given different diets, or different levels of intake on the same diet, there have been few studies looking at the differences between animals given the same feed (Webster,1981). The importance of the work of digestion component in causing differences between animals in heat output, or even maintenance requirements, is therefore not known.

Finally, thermal environment has a large effect on heat production (thermoregulation) and thus maintenance requirements, with heat production being minimal in a temperature range known as the thermoneutral zone. For mice this range is approximately 30-33 C (quoted by Ahmed,1982). Bateman and Slee (1979) found mouse intake to be three times greater at 1 C than 30 C, with growth rate being the same at both temperatures. Larger animals with ample coat cover (e.g. sheep and cattle) have wider thermoneutral zones than smaller laboratory animals, and do not expend as much energy maintaining body temperature (Ahmed,1982).

1.2.2.3 Fat and Protein Deposition

Two factors are of interest when considering fat and protein deposition, firstly the energetic costs of these processes, and secondly the patterns of partition between fat and protein deposition.

Firstly, consider deposition costs. In their landmark 1977 paper, Pullar and Webster were able to obtain accurate estimates of these costs. By studying the growth and carcass composition of obese and lean Zucker rats at differing levels of intake, they estimated the net efficiencies (kJ tissue/kJ energy) of fat and lean deposition to be .735 and .444, respectively. Assuming energy contents of 39.3 and 23.5 kJ/g for fat and protein, the requirements to deposit 1g of fat and protein, respectively, are then 53.4 and 52.9 kJ. These values are shown in fig. 1.3. Given that lean comprises approximately 4g of water to every gram of protein (Webster,1977), then lean deposition is five times more efficient, in energetic terms, than fat deposition. The figure for fat deposition is in agreement with the theoretical stoichiometric cost of fat synthesis, but protein deposition is less efficient than expected and this may be a reflection of protein turnover (Blaxter,1979).

One may question the validity of using physiologically aberrant animals (i.e. the obese rats) for such calculations, but these figures are in close agreement with those suggested by Kielanowski (1976) after an extensive literature review. The traditional approach used in the papers Kielanowski reviewed, has been to partition energy intake between maintenance, protein and fat deposition by mutliple regression analyses of intake and carcass composition data. This technique can be somewhat unreliable, however, as it is dependent on the assumptions used to relate maintenance to body weight. Maintenance is often the largest component of the analysis, and since it is usually described as aBW^{b}, small variations in the assumed values for a or b can lead to bizarre differences in the apparent costs of deposition (Pullar and Webster, 1977; M.K.Nielsen,pers.comm.).

Secondly, consider energy partition. It is generally accepted, and was mentioned in section 1.2.1.2, that animals get fatter as they age. In other words, the proportion of the animal's intake available for growth that is deposited as fat progressively increases as the animal

ages (and therefore proportionately less gets laid down as protein or lean).

Also of interest are the patterns of energy partition with increasing intake, at a given age. This is because recommended levels of feeding to avoid excessive fatness, and selection strategies to decrease fatness, depend on these patterns (Fowler et al,1976). The accepted (and intuitively correct) view is that as intake above maintenance increases, the proportion of energy being deposited as fat increases. This has been demonstrated, for example, in pigs by Davies and Lucas (1972b) and in birds - the Japanese Quail - by Farrell et al (1982). Whittemore and Fawcett (1976) believe that the ratio of energy deposited as fat to that as protein, is never less than one in the growing pig. This, however, is not true in the Japanese Quail (Farrell et al,1982), and carcass composition studies in the mouse would also indicate a ratio much less than one under most conditions (e.g. Clarke,1969). The exact patterns of partition of energy between fat and protein deposition, for different species, are not resolved, however, and this subject area attracts much debate (C.T.Whittemore, pers.comm.).

1.2.3 Efficiency of Growth

The concept of efficiency of growth, or efficiency, is generally discussed in terms of a few generally accepted definitions.

Gross efficiency is defined as gain(kg)/intake(kg),and the inverse of this ratio is known as the food conversion ratio. Energetic efficiency refers to cases when both gain and intake are expressed in energy units (kJ), and net efficiency refers to the efficiency of deposition of intake in excess of maintenance.

The (gross) efficiency of an animal will be affected by its ability to digest (and metabolise) food, its maintenance requirements and its intake surplus to these requirements, its partition of surplus energy between lean and fat, and the net efficiencies of lean and fat deposition.

For digestibility, Pym (1985) found that lines of chickens selected for food intake had a decreased digestibility compared to unselected chickens (62.9% vs 67.8%), however this appears to be the only

reported example of digestibility differences existing between animals. In general, digestibility differences, both within and between species, appear to be slight (Fowler et al, 1976; Sutherland et al,1974) and therefore digestibility will not be discussed further.

Net efficiencies may also be considered to be constant, due to the fact that the same biochemical pathways are always be used to synthesize a unit of fat or protein in a growing animal (A.J.F.Webster, pers.comm). The equivalence of the stoichiometric and estimated efficiencies of fat synthesis back this view. Problems of definition are encountered with protein synthesis and turnover, however, but assuming a constant net efficiency of .444 (Pullar and Webster, 1977) and assigning additional energy needed for protein turnover to maintenance, avoids this problem.

Efficiency, therefore, is simply a function of the amount of intake in excess of maintenance, and the partition of this energy between lean (efficient) and fat (inefficient) gain. Efficiency will therefore vary with an animal's intake, and also through its growth period.

Fig. 1.4 summarises the typical efficiency patterns of a growing animal, which can be derived from the above growth and carcass composition information.

Increasing intake and hence growth rate will improve efficiency, however the relationship is not linear due to the increasing fat deposition with increasing intake in excess of maintenance. The most extreme example of this declining increase in efficiency is seen in pigs. Pigs are capable of eating up to four times their maintenance requirements (Davies and Lucas,1972a), but because they deposit large amounts of fat at this feeding level they show an unexpected negative correlation between intake and efficiency (Fowler et al,1976). In a very elegant study, Davies and Lucas (1972a) found that an intake of approximately three times maintenance maximised efficiency for pigs of a wide range of body sizes. Mice appear to eat only 10 to 20% above maintenance during their fastest period of growth (Stephenson and Malik,1984).

Some mathematical properties of the definitions of efficiency should be mentioned. The correlation of efficieny and its inverse (food conversion ratio) is of course less than unity, with the departure from one being a function of the variance of efficiency (Timon and

Fig. 1.4 Factors affecting the efficiency of utilization of metabolizable energy (ME) for growth. RE is energy retention, H is heat production. W and M are weights at weaning and maturity, respectively. Em is maintenance requirement at mature body weight.

(Webster, 1980)

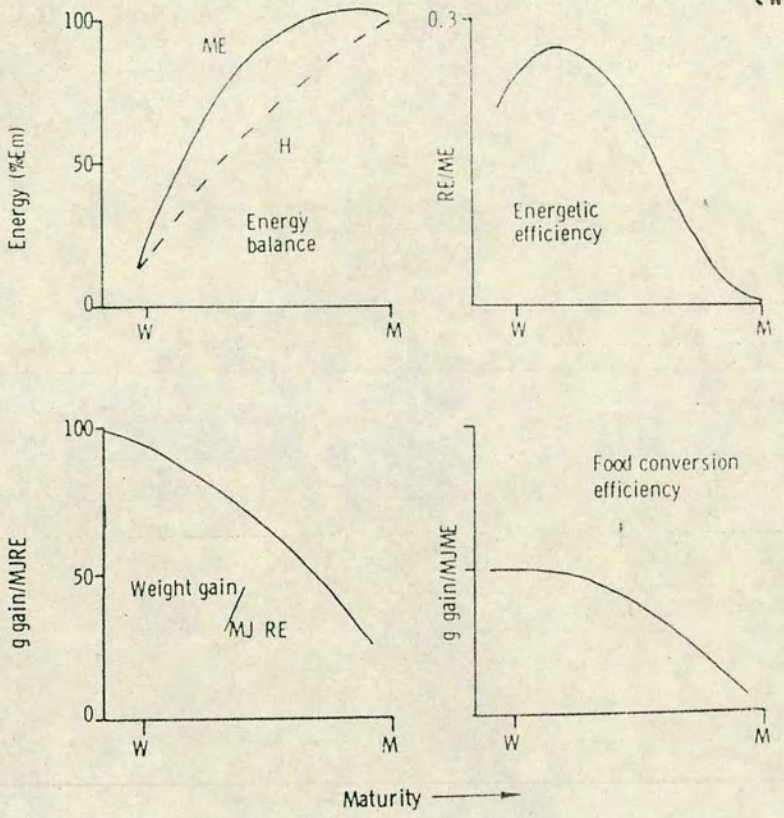
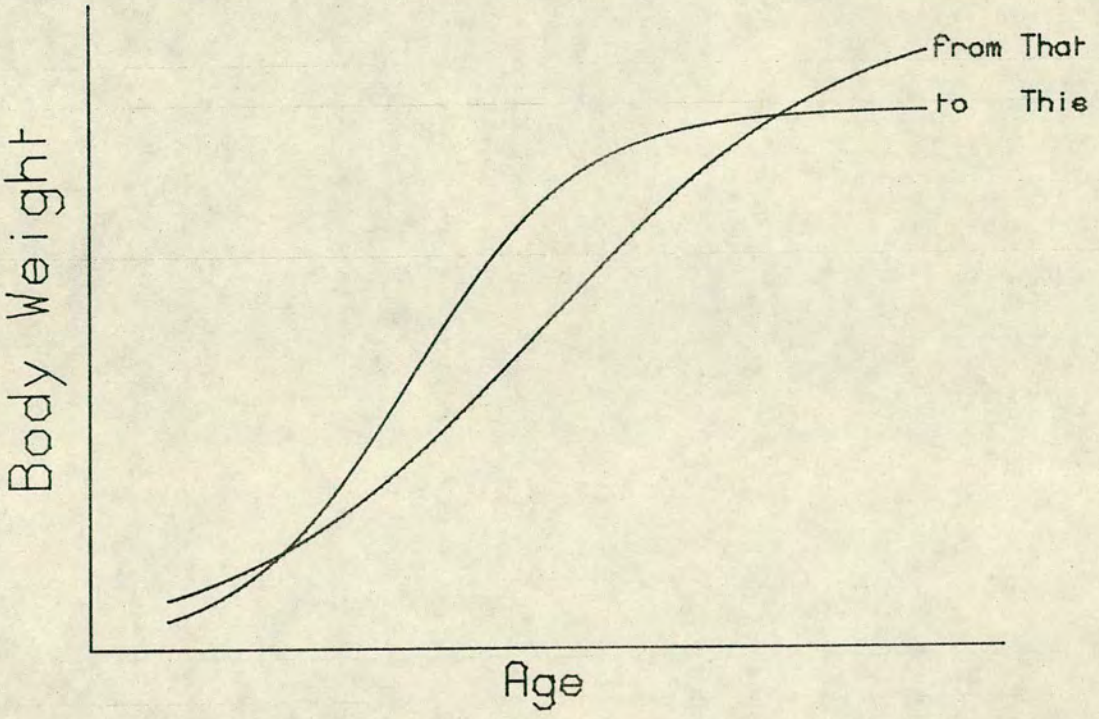


Fig. 1.5 Desired Changes in the Growth Curve



Eisen,1970). The same authors also point out that the coefficient of variation for intake/gain will be higher than that of gain/intake, and this may affect the choice of definition in a statistical analysis. Lastly, there is a degree of automatic correlation in a "part:whole" relationship, i.e. for the correlation of X1/X2 with either X1 (e.g. gain) or X2 (e.g. intake), and this will be a function of the coefficients of variation of X1 and X2, and the correlation between the numerator and the denominator (Sutherland,1965).

Finally, the design of experiments to compare the efficiencies of various types of animals can present a problem, due to the natural variation in efficiency during growth and with intake. The growth, intake and efficiency of animals are often compared in experiments with the measurements being taken over fixed time intervals, over fixed weight intervals, over fixed "maturity" (i.e. proportion of final weight) intervals, and even on a fixed total intake. Comparing such experiments, and drawing valid inferences, can present problems of almost intractable complexity - especially if the animals differ widely in size and growth rate.

1.3 GROWTH AND ITS COMPONENTS: GENETIC ASPECTS

Having described growth and the metabolic means by which it occurs, the questions of interest are whether or not there is genetic variation for growth and each of its components, and what are the genetic links between these various components.

Selection experiments are a powerful means of studying genetic characteristics because by creating lines of animals which differ greatly in some desired way genetic relationships often become apparent and analyses of genetic differences and variation become much easier. Thus, in dealing with the genetic aspects of growth and its components this section will concentrate mainly on the results of selection experiments, especially in mice.

1.3.1 Selection for Growth: Direct Responses

Selection for growth per se can be defined as selection for body weight at a given age, or selection for weight gain over a given age

period.

Growth has often been selected for in laboratory animals (but much less often in domestic animals) (i) as an easy means of testing or validating genetic theory, (ii) to quantify the genetic determination of growth (response to selection, in the simplest terms), and (iii) to study the means by which the changes in growth are mediated, i.e. the correlated responses to selection.

Reason (i) is of course still an area of much interest, but (ii) can now be considered to be answered, with the papers of Falconer (1953 and 1973) almost giving a complete answer in themselves. A comprehensive summary of published direct responses to selection in mice is given by McCarthy (1982). In summary, growth per se always appears to respond to selection, with realised heritabilies ranging from .15 to .5Ø, the usual range being .25 to .3Ø. Therefore, approximately 25% to 30% of the variation seen in growth is caused by additive genetic variation.

The question now to be addressed is by what means are the changes in body size and growth rate mediated.

1.3.2 Selection for Growth: Correlated Responses

1.3.2.1 Growth Curve

The ideal domestic animal is one that grows rapidly (and efficiently) to a relatively small mature size (so that the costs of maintaining the adult are lessened). It is desirable, therefore, to bend the growth curve as in fig. 1.5.

The use of mathematical growth curves will obviously be quite helpful in quantifying growth curve changes, as changes in the shape of the curves will be reflected by changes in their parameters. However, as the phenotypic and genetic correlations between weights at different ages always appear to exceed .5, and often approach unity in mice (Clarke,1969; LaSalle et al,1974; McCarthy and Bakker,1979), the effects of changing growth on the growth curve may not be great.

The results of the growth curve analyses of mice selected for growth are conflicting. Comparing the growth rates and mature sizes of mice selected for 3 week weight vs 3 to 6 week gain (Frahm and Brown,1977),

6 week body weight vs 6 week tail length (Eisen and Bandy,1977), and 6 week weight (Roberts,1961), some changes in the patterns of growth are apparent. In addition, comparing mice selected for 6 week weight with control line mice using growth curve analyses (Eisen et al,1969; Ahmed,1982), changes in the rate of growth parameter (k) have been observed, but Ahmed found replicate differences (i.e. genetic drift) to be more important than selection effects.

Several studies contradict these results, however. In comparing lines of mice selected for 3 to 6 week gain with control mice (Timon and Eisen,1969), and a variety of inbred and selected strains (Gall and Kyle,1969), no differences were found in the growth curve parameters defining the shape of the curve - using the Richards curve in the former study and the Bertalanffy curve in the latter. Finally, Baker and Chapman (1975) observed little change in the shape of the growth curves of rats selected for 3 to 9 week gain.

Eisen (1976) concluded that mere selection for body weight does not generally result in changes in the shape of the growth curve, but instances of changes occurring are described above. These changes do not appear to be consistent, however, and they vary between populations and replicates of mice selected for the same criteria. In conclusion, it may be said that selecting for growth at any one age can bend the growth curve, but is not a reliable or effective means of doing so.

1.3.2.2 Carcass Composition

Carcass composition will be discussed in terms of fat content only, as water, protein and ash percent are of necessity autocorrelated to fat percent, and expressed in terms of fat free carcass they merely indicate the animals degree of "chemical maturity" (section 1.2.1.2).

Clarke (1969) in his extensive literature survey and allometric analysis of Falconer's (1973) Q strain came to the general conclusion that selection for increased growth results in mice which are leaner until the age of selection (as demonstrated by Lang and Legates,1969 and McPhee and Neill,1976), show little difference at selection, but subsequently become much fatter than their unselected controls (demonstrated by Biondini et al,1968; Clarke,1969; Eisen and

Bandy,1977; Hayes and McCarthy,1976; Hull,196Ø; McPhee and Neill,1976; and Timon et al,197Ø). The opposite applies to downwards selected mice. This phenomenon will be referred as the "Clarke effect".

The results of Hull (196Ø), who selected mice for body weight at 3, 4 1/2 and 6 weeks of age, and Hayes and McCarthy (1976) who did likewise at 5 and 1Ø weeks, suggest that the earlier in life mice are selected for body weight the greater is the manifestation of this Clarke effect.

Proudman et al (197Ø) and Pym and Solvyns (1979) when comparing selected lines of chickens also noted an increased fat content in the fasting growing lines, and in general, selection for growth per se has increased fatness in commercial strains of broilers and turkeys (H.Griffen,pers.comm.).

Hayes and McCarthy (1976) proposed a general explanation for these effects, suggesting that selection for body weight acts by (i) increasing the rate of food consumption and (ii) altering the partition of energy between lean and fat deposition. By this hypothesis, the selected mice will initially have both an increased quantity of energy available for growth and an increased partition of this energy towards (more efficient) lean deposition. As these animals mature and the rate of lean deposition decreases, however, there will be an excess of energy available for growth, and this will be deposited as fat. These two mechanisms are contradictory in the effects they have on fatness, but since the rate of fat deposition is relatively low early in life (increasing with age, section 1.2.1.2), the younger the animal is at selection the less important is the effect of partitioning energy away from fat towards lean deposition. Thus the younger the animal is at selection the greater the subsequent increase in fatness.

Only Ahmed (1982), Biondini et al (1968) and Lang and Legates (1969) have produced results in conflict with the Clarke effect. In the first two studies only individual replicates do not agree, however, and in the third very small sample sizes have produced inconsistent and fluctuating results.

Selection for growth affects the distribution of fat accretion, as well as its rate (Allen and McCarthy,198Ø). These authors found that the gonadal and kidney depots contributed disproportionately to the

increase in fat, and they suggested that it may be possible to select for a change in the distribution of fat -presumably away from the parts of the body important for meat production.

1.3.2.3 Intake and Efficiency

Selection for growth in mice always appears to increase both intake and efficiency, and lines of chickens analysed by Proudman et al (1970) and Wilson (1969) also show this result. Typical figures for mice are those of Roberts (1981) who found that mice selected for 6 week weight, which were 35% larger than their controls at this age, ate 22% more and were 35% more efficient between 3 and 6 weeks of age. From the results of Ahmed (1982), Brown and Frahm (1975), Eisen (1977) and Stephenson and Malik (1984) it can be deduced that although lines selected for growth always have an increased intake per se, they always have a decreased intake/body weight (metabolic body weight rule) and usually show little change in intake/$BW^{.75}$. Thus increasing body size or growth rate has only small, or insignificant, effects on intake in relation to body size.

Efficiency, as mentioned above, is a function of intake in relation to maintenance, as well as the partition of energy between fat and protein deposition. A question of interest, therefore, is whether or not energy available for growth has been increased, in part, by decreasing the maintenance requirements of these selected mice. Several studies have approached this question, and it appears that increasing growth rate may slightly reduce basal heat production/$BW^{.75}$ (Kownacki et al,1975; Kownacki and Keller,1978) and maintenance requirements/$BW^{.75}$ (Ahmed,1982; Stanier and Mount,1972; Stephenson and Malik,1984). Canolty and Koong (1976) could find no differences, however. Decreased fasting heat production has also been observed in cattle selected for growth rate (Frisch and Vercoe,1980). These studies tend to show variable results, however, and they are also dependent on the exponent used to define metabolic body weight, so they should be interpreted with caution.

Lifetime efficiency is also of interest. Timon and Eisen (1970) and Roberts (1981) studying lines of mice selected for 3 to 6 week gain and 6 week weight, respectively, found that selected lines were only

more efficient until 8 weeks of age, whereupon they become slightly less efficient than their controls. This is probably due to the greatly increased rate of fat deposition by selected lines after the age of selection (i.e. the Clarke effect). In terms of gross energetic efficiency, this increased fat gain increases the efficiency changes after the age of selection (Fowler,1962), but in general, however, energetic efficiency changes tend to mirror simple efficiency changes (Ahmed,1982; Timon et al,1970).

For net efficiency, only Canolty and Koong(1976) claim to have found changes - for the efficiency of fat deposition. They obtained this result by comparing mice under varying nutritional restrictions, but as mobilisation of fat appears to have occurred at their low feeding levels, their estimates of net efficiency may well be biased.

Finally, care must be taken if attempting to account for the responses in growth solely in terms intake and efficiency. This is because when growth is selected for, it appears to resemble a fixed and variable cost system. If one considers maintenance as a fixed cost (because increasing growth rate does not appear to cause large changes in maintenance requirements) and intake above maintenance as a variable cost (because this component must change, by definition), then the increase in intake will result in a disproportionate increase in efficiency - because this extra intake will be used for gain, rather than maintenance. Therefore, the increases in efficiency with selection for growth are, to a large extent, a function the increases in intake. An example of where this approach towards describing the efficiency of large and small mice may not have been appreciated can be seen in the paper of Roberts (1981), where it was concluded that appetite and efficiency contributed more or less equally to the response in growth.

1.3.3 Selection to Bend the Growth Curve

The desirability of changing the shape of the growth curve was mentioned in section 1.3.2.1, and experimental attempts to do this have been made with mice, chickens and turkeys.

McCarthy and Doolittle (1977) selected mice for combinations of increased, decreased and constant 5 and 10 week weights, however

selection was only partially successful. Gompertz curve analyses of these lines (McCarthy and Bakker,1979) revealed only small changes in the curve parameters, with a great deal of asymmetry apparent. Changing 5 week weight did appear to have a greater bending effect than changing 1Ø week weight, however. By selecting for the ratio (3 to 6)/(3 to 9) week gain Wilson (1973) only acheived a realised h^2 of .1, and a contemporary line selected for 3 to 6 week gain showed no change in the ratio. Correlated responses were not reported in either of these experiments.

Williams (1984) tried an alternative approach by selecting for combinations of 5 week body weight and 5 week testis weight - testis weight being an indicator of maturity at this age. This technique initially appeared to result in distinctly different growth curve shapes for the different lines, with mice selected for increased values of both traits initially growing faster and then reaching mature weight more quickly (Williams,1984) (i.e. the desired changes), and also being leaner at maturity (P.J.Cook, pers.comm.). After several further generations of selection these differences became less pronounced, however, with large differences existing between replicates (P.J.Cook, pers. comm.). From the results of these three experiments it appears that it is difficult to bend the growth of the mouse.

The growth curves of birds appear to be somewhat easier to bend, as is shown by the results of Ricard (1975) - for chickens, and Abplanalp et al (1963) - for turkeys. Ricard bent the growth curves of chickens by selecting for combinations of high and low weights at 8 and 36 weeks of age, and Abplanalp likewise succeded by selecting for 8 week weight, 24 week weight and an index designed to increase 8 week weight but hold 24 week weight constant. Correlated responses were not reported in either of these papers.

1.3.4 Selection for Food Intake

After considering the effects of selection for growth as a whole, it is necessary to study the effects of selection for each of the components of growth. Firstly consider the input component, food intake. Food intake has been selected for in mice by Sutherland et al

(1970) and Sharp et al (1984), and in chickens by Pym and Nicholls (1979).

Sutherland et al used mice already selected 9 generations for gain, and proceeded to select them a further 11 generations for 4 to 11 week food intake. A h^2 of .2 was realised, and appetite increased at twice the rate of a contemporary line selected for growth. Surprisingly, the response in growth continued at the same rate as in the line selected for growth, and thus efficiency continued to show a small increase. After changing the selection criteria to appetite, these mice subsequently showed a large correlated response in fat deposition (Biondini et al,1968).

Sharp et al (1984) selected mice for 4 to 6 week intake corrected for the starting weight by the phenotypic regression of food intake on 4 week weight. The intention was thus to increase intake but not body weight. This experiment realised a h^2 of .14, with the high-low divergence being 16% of the control mean after 11 generations. Although by this stage 4 week weights were still similiar, 4 to 6 week gain had changed, with the high-low divergence being 40% of the control mean (Sharp et al,1984). Efficiency from 4 to 6 weeks had also changed, with the high lines being slightly more efficient than the control lines, and vice versa for the low lines. The most surprising result, however, was for the high intake lines to become slightly leaner than the control and low line mice (Sharp et al,1984; S.Copland,unpublished; M.K.Nielsen,unpublished).

The study by Nielsen also gave indications of changes in maintenance, with the high lines appearing to have requirements 10% greater per unit metabolic body weight than the low selected lines (M.K.Nielsen,pers.comm.).

Selection for 5 to 9 week food intake in chickens (Pym and Nicholls,1979) was also successful, the realised h^2 being .44. The selected lines became larger, however they also became much fatter (Pym and Solvyns,1979) and their heat production and maintenance requirements increased (Pym and Farrell,1977; Pym 1985), so their efficiency actually decreased. Although it was not obvious in the earlier generations, it became apparent after 10 generations of selection that digestability had also decreased, compared to the control line chickens (Pym,1985). This appears to be the only reported

example of digestability differences between animals.

Selection for food intake therefore appears to give a slightly confusing picture. There is evidence that increases in maintenance requirements may occur, but only in two of the experiments have increases in fatness (which are an indicator of increases intake above maintenance) also occurred. In addition, the efficiency changes are inconsistent. The question of why selection for intake should result in leaner mice remains unresolved.

1.3.5 Selection for Carcass Components

As it is the carcass components, i.e. lean and fat, which are of interest in meat production, there have been several experiments in mice, poultry and pigs looking purely at the effects of selection on these traits. Most commercial pig and poultry breeding schemes concentrate selection on carcass components, as well as efficiency and, perhaps, growth.

Firstly consider the experiments with laboratory animals. McLellan and Frahm (1973) selected mice for hindleg muscle weight, and observed a realised h^2 of .44. Selection for rate and efficiency of protein gain in rats realised h^2's of .20 and .24, respectively (Notter et al, 1976). Finally, Sharp et al (1984) selected for estimated lean mass and estimated fat percentage in mice, and the realised h^2's from this experiment were .54 and .43, respectively.

McLellan and Frahm did not report correlated responses to selection in detail. The rate of protein gain lines (Notter et al,1976) became fatter than their controls, and the protein efficiency lines leaner, with overall increases in efficiency for the two lines being equal. These two criteria showed genetic correlations of almost one with overall gain and efficiency, however. In their careful study of these lines, Wang et al (1980) suggest small decreases (e.g.5%) in the maintenance requirements/$BW^{.75}$ for both criteria, although Notter et al (1976) were unable to find corresponding decreases in heat production.

The lines selected for estimated lean mass (Sharp et al,1984) show large increases in body size, intake and efficiency, but carcass composition changes are slight, whereas the lines selected for

increased and decreased fatness show only small body weight, food intake and efficiency changes from 4 to 6 weeks of age. The decreased fatness (leaner) lines are a little smaller, eat slightly less and are less efficient than the increased fatness lines - these results are probably opposite to what one would expect. M.K.Nielsen's unpublished study shows only slight decreases in estimated maintenance requirements for the increased lean mass and increased fatness lines.

Leclerq et al (1980) selected broilers for increased and decreased abdominal fat/body weight at 9 weeks of age. Although the responses were large (realised h^2 =.5), the fatness divergences did not increase after 9 weeks of age (Simon and Leclerq,1982) - despite the increasing relative rate of fat deposition as animals age. In general,the changes in food consumption were small, but the leaner lines became more efficient. Selection for leanness in broilers at the Poultry Research Centre, Roslin, Scotland, has also resulted in insignificant changes in food intake, heat production and energetic efficiency, but significant decreases in fatness and increases in (gross) efficiency (H.Griffen,pers.comm.).

Henderson et al (1983), and Ellis et al (1983a and b) have compared pigs selected for an index of gain, efficiency and decreased fatness with control line pigs. Selection has both decreased voluntary food intake (Henderson et al,1983), as may be expected from the results of Davies and Lucas (1972a), and caused a change in the partition of energy from fat towards lean deposition - at all intake levels (Ellis et al,1983b). Reducing intake appears to have had a much greater effect in increasing efficiency, than the partitioning changes (Ellis et al,1983a). The selected pigs have a lower energetic efficiency and appear to have higher maintenance requirements (Henderson et al,1983), despite their increased efficiency. In studying pigs selected for an index of growth and reduced fatness, Sundstol et al (1979) also appear to have found higher maintenance requirements for the leaner lines - with these lines also being more efficient with a reduced appetite. These authors, however, also quote previous evidence from these lines indicating lower maintenance needs, so the overall trends are unclear.

In conclusion, carcass components are very amenable to selection, although the correlated responses are slightly confusing. Selection for lean mass, or traits similar to lean mass, appears to have

similiar effects to selection for body weight, with the correlations between lean mass and body weight being high. The effects of selection designed to change carcass composition (e.g. reduce fatness), however, appear to depend on the species studied. In mice, reducing fatness slightly reduced body size, intake and efficiency; in poultry reducing fatness had a negligible effect on intake and heat production, but increased efficiency; and in pigs it reduced intake, increased efficiency and may also have increased heat production and maintenance. These differences may be a function of differences between species in intake relative to maintenance, as well as the actual selection criteria used. Further study is needed to resolve and account for them.

1.3.6 Selection for Efficiency

Finally the effects of selection for efficiency need to be considered. As efficiency is a composite trait, being affected by intake, maintenance and lean and fat deposition, there is much interest in how improvements in efficiency are mediated.

Efficiency has been selected for in mice using the following criteria: (i) the ratio gain/intake (Sutherland et al,1970; Yuksel et al,1981), (ii) gain on a fixed intake (Eisen,1977; Gunsett et al,1981; Hetzel and Nicholas,1982; McPhee et al,1982 and Yuksel et al,1981) and (iii) intake required to gain a set weight (Gunsett et al,1981). Eisen used an index of gain with a restriction on food intake, to achieve his objective.

Selecting simply for the ratio (i) (realised h^2's were .17 and .16, respectively) has resulted in larger body sizes and greater food intakes when the mice were selected during the decelerating growth phase, i.e. 4 to 11 weeks for Sutherland et al and 5 to 7 weeks for Yuksel et al, but a slightly decreased starting body weight and intake when selection was during the accelerating growth period, i.e. 3 to 5 weeks, Yuksel et al (1981). In both experiments, the selected lines tended to become slightly fatter than their controls, and reduced maintenance requirements are implied.

A fatness increase, as well as a small intake increase, was also observed by McPhee et al (1980), selecting for 5 to 9 week gain on a

fixed intake. Reductions in maintenance, and therefore a much increased intake in excess of maintenance, are once again implied. The authors had anticipated energy to be partitioned towards lean deposition, away from fat, but by 9 weeks of age the intake above maintenance is probably too small for most of the selection pressure to have been placed on partition. Only Hetzel and Nicholas (1978) have found lines of mice selected for efficiency to become leaner. In this experiment the age of selection was quite young - 3 to 6 weeks of age - and thus more pressure was able to be put on the partitioning effects. The realised h^2 was .19 (Hetzel and Nicholas,1982).

Eisen's lines realised a h^2 of .19, but after several generations voluntary intake did increase (and decrease in the low lines), although no change in intake/$BW^{.75}$ occurred. This is a good example of biological changes leading to changes in the genetic parameters of his index. By selecting for gain on a restricted index, Yuksel et al obtained similiar responses in efficiency as they did by selecting on an ad lib regime, and moreover, both types of lines were equally efficient on any given intake. Thus intake differences were not important in determining efficiency - once again implying maintenance changes.

Finally, in their interesting and original paper, Gunsett et al (1981) obtained very high h^2's of .56 and.73 for gain on a fixed food quota (100g of food) and food required for a fixed gain (17g for females, 20g for males), respectively. Through growth curve fitting procedures, see Parks (1970), increases in body size and food intake were described, but no changes in net efficiency could be found. The effects of selection on carcass composition or maintenance requirements are not reported in this paper.

In addition to selecting for gain and food intake, Pym and Nicholls (1979) also selected a line of broilers for 5 to 9 week efficiency. These lines responded with a h^2 of .21, showing an increase in body weight but no change in food consumption. In addition, the efficiency line birds became much leaner (Pym and Solvyns,1979) and had reduced fasting heat production and maintenance requirements (Pym and Farrell,1977; Pym,1985).

An interesting result from this experiment is the marked asymmetry of response between the intake and efficiency lines - the increased

intake lines showed a significant decrease in efficiency, whereas the efficiency lines showed no change in intake. This is contrary to genetic expectations, but can be explained metabolically: food intake has been increased by increasing both maintenance and intake above maintenance (hence increasing fatness and reducing efficiency), whereas efficiency has been increased by increasing intake above maintenance (as well as changing energy partition towards lean from fat), but reducing maintenance requirements. This is a good example of the need for knowlege of both metabolic and genetic (i.e. heritabilities and correlations) information to accurately predict the effects of selection.

Lines of pigs selected for indices incorporating efficiency have been described above. Increases in efficiency appear to be caused mainly by reducing intake, and hence fatness, and also by causing a slight partition of energy away from fat towards lean deposition.

In conclusion, efficiency is also amenable to selection, however realised h^2's rarely exceed .2. The means by which efficiency is increased are species dependent. In mice, the surprising result of an increase in fatness is often seen, as well as increased intake, and reductions in maintenance are usually implied. Chickens appear to show both decreased maintenance and decreased fatness, but no intake changes. Pigs become more efficient by reducing intake and fatness, however maintenance requirements may even increase. These species differences are probably a function of their respective intakes in relation to maintenance, but they still have to be fully quantified and explained. In particular, the mouse results have yet to be adequately explained.

1.4 DISCUSSION

This section will firstly summarise and discuss some of the more important parts of the review, considering areas where there is still some confusion, and secondly propose a line of research to approach these problems.

1.4.1 General

This review has considered genetic and metabolic aspects of growth within somewhat narrow definitions. Metabolism has been discussed purely in energetic and descriptive terms - largely ignoring the underlying biochemical processes, which have been arbitrarily defined as being outside the range of this work. Blaxter (1979) claimed that this purely descriptive approach generated a need to explain these causal mechanisms, but although this need is recognised and accepted, it would constitute a separate study in its own right.

Likewise, detailed descriptions of responses to selection have not been given, e.g. sex and replicate (genetic drift) differences have rarely been discussed, nor have the modes of gene action which allow direct and correlated responses to selection to occur been described (see e.g. Falconer,1981). A qualitative rather than a quantitative description of selection effects has been used - primarily for brevity, but also to avoid the cluttering and distracting effect of too many numbers.

In summary, the main factors or components affecting an animal's growth are its intake, maintenance requirements, and relative quantities of lean and fat growth. Selection experiments have demonstrated additive genetic variation for all of these components, as well as for overall growth itself. Some care should be taken when considering the genetic variation that maintenance exhibits, however, as it has yet to be quantified - e.g. by means of a selection experiment. Changes brought about in maintenance are often only implied by the results and in general are somewhat inconsistent, but nevertheless claims as recently as 1978 (Dickerson,1978) that there is "very limited" variation in this trait appear to be incorrect.

Some of the components of growth appear to be able to change independently of each other, and therefore may be uncorrelated. For example, consider maintenance and intake in excess of maintenance, at any given age. Selection for food intake appears to increase both components, whereas selection for increased efficiency (in mice and poultry) increases intake in excess of maintenance, but may actually decrease maintenance requirements themselves. An estimate of the genetic correlation of these traits would be of interest.

Some of the relationships expected from the metabolic studies have not demonstrated themselves in the genetic studies. This is especially true for the relationships between lean mass and maintenance requirements. Although there are several experiments in which these two traits have both moved in the same direction, there are also several studies in which they appear to have changed in opposite directions - especially with poultry.

There have been some slightly unexpected correlated responses to selection (especially in mice), mainly relating to energy partitioning, which have yet to be resolved. These include the phenomena of many lines of mice selected for efficiency becoming fatter, and lines selected for intake (Sharp et al,1984) becoming leaner. In general, there has been little documentation on how selection (for any of the components) affects the patterns of energy partition -e.g. for these specific problems areas, and also at various intake levels for any given animal.

Finally and perhaps most importantly, there are marked differences between species in correlated responses to selection which have yet to be fully explained. These differences tend to occur not when growth per se is selected for, but when the components of growth, or efficiency, are selected. For examples, see sections 1.3.5 and 1.3.6. It is important to resolve these differences so that valid extrapolation of results can be made between species -and this in turn will justify the experiments with laboratory animals.

1.4.2 Research Proposal

The metabolic framework within which the genetic aspects of growth have been discussed has been found to be very useful, so it proposed to study the genetic differences in the growth (and its components) of selected lines of mice, using this metabolic approach.

In the discussion some of the existing gaps in knowlege are described, and the aim of this study is to try to resolve some of these unanswered questions. It is proposed to use the lines of mice described by Sharp et al (1984) as the experimental units - these lines being particularly useful for this approach as they consist of lines differing widely in (i) appetite, (ii) lean mass and (iii)

carcass composition, and thus show variation in nearly all the components of growth.

Outstanding questions of particular interest which will be studied include the variation exhibited by maintenance requirements, the relationships between maintenance and carcass composition, and the inter-relationships of intake, carcass composition and efficiency. Finally, attempts will be made to devise means of extrapolating results across species, and thus explain the apparently different relationships shown by different species.

Section II GENERAL STUDY

2.1 INTRODUCTION

The aims of this research study as a whole are specified in section 1.4.2, and the aim of this first, general, study is simply to generate the basic data necessary to study the growth of the lines of mice selected for intake, lean mass and carcass composition (Sharp et al,1984), at the simplest level within the given metabolic framework. It is hoped that from the results the patterns of growth, and the components of growth, of the selected lines will become apparent, and that the areas requiring further study will be revealed.

The minimum required required measurements for this study are weights at various ages, food intake over the same age periods, and carcass composition determinations. Both weight and intake will be measured from very young ages (birth and weaning, respectively) until adulthood, so that complete pictures of growth and metabolism can be obtained - a general weakness in the discussed literature has been the limited age periods over which many of the measurements have been made. Finally, growth curves will be fitted and examined to allow a comparison of the metabolic approach with curve fitting approaches to describing growth.

2.2 MATERIALS AND METHODS

2.2.1 Selection Lines

The lines of mice studied comprise three distinct (i.e. different selection criteria) but contemporaneous sets of lines. They are:

i/ A or appetite lines - selected for 4 to 6 week intake, corrected for 4 week weight by the phenotypic regression of intake on 4 week weight (the aim being to change intake, but not 4 week weight).

ii/ F or fat lines - selected for the ratio of gonadal fat pad weight (GFPW) to body weight (BW), in 1Ø week old males (GFPW being an indicator of fat content, comprising approximately 1/8 of total body fat).

iii/ P or protein lines - selected for the index: BW-8xGFPW, or estimated lean mass, in 1Ø week old males.

For each selection criterion there were three contemporaneous lines, one selected for high(H) performance, one for low(L) performance, and one unselected control(C). These lines were replicated three times for each selection criteria, so 27 lines were maintained in total (3 selection criteria x 3 replicates x 3 directions of selection). Sixteen pair matings were made in each line up to generation 8; subsequently 8 pair matings were used. Selection was practised within litters

Fig. 2.1 shows the direct response to selection (pooled across replicates) for the A, P and F lines, until generation 16. The drop in food intake at generation 2 was associated with a change of diet, the new (current) diet being energetically more dense than the old diet. The (H-L)/C divergences at generation 16 were 23.1%, 44.5% and 151.Ø% for the A, P and F lines, respectively.

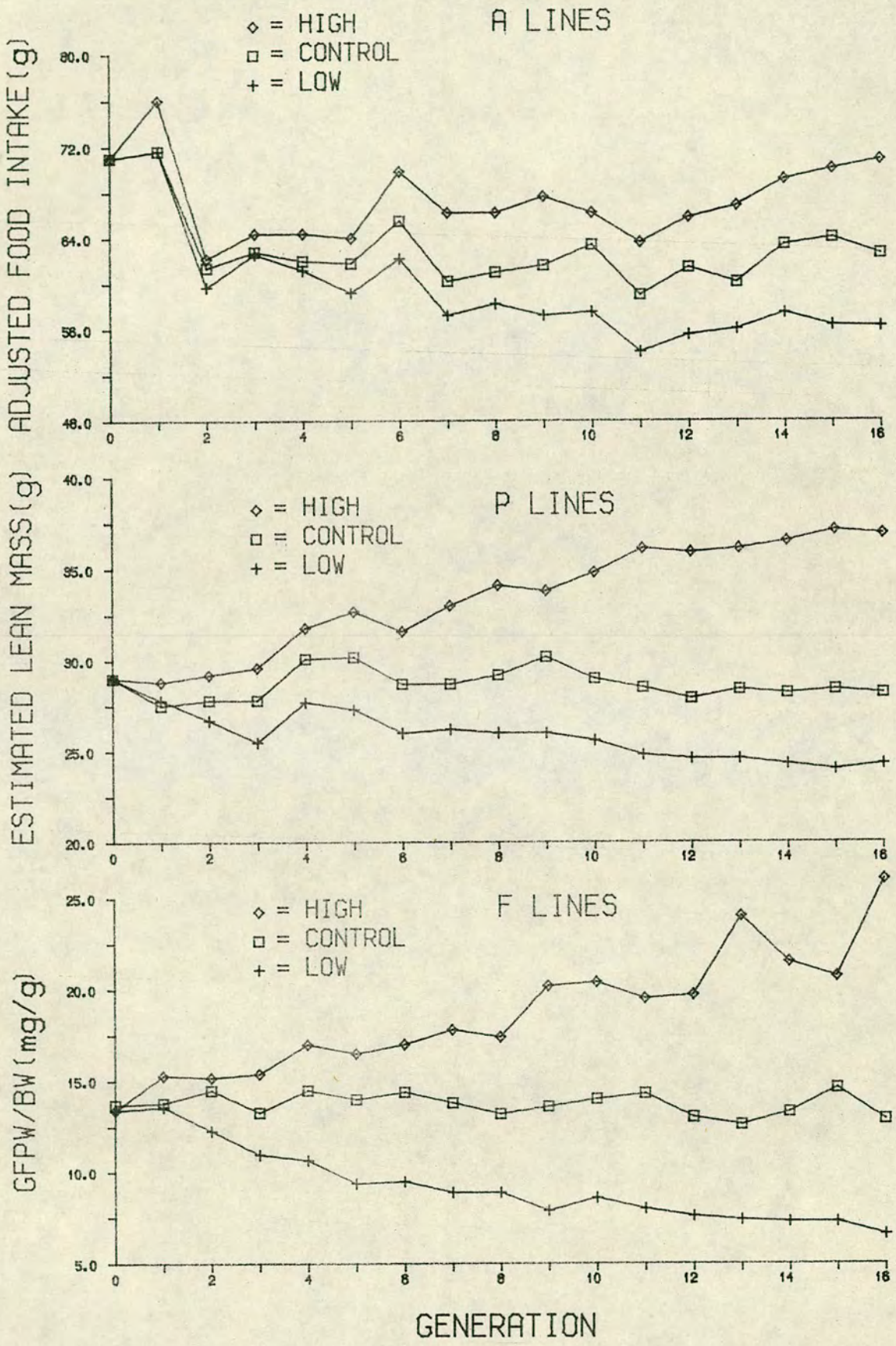
The realised divergent heritabilities after 11 generations of selection were .14+.Ø27, .54+.Ø12 and .43+.Ø59 for the A, P and F lines (Sharp et al,1984). The sampling variances were estimated empirically from the observed variance of the regression coefficients across lines.

A full account of the origins of the mice, selection procedures, and responses in the traits under selection (for the first 11 generations) is given by Sharp et al (1984).

2.2.2 Collection of Data

The mice used in this study were sampled from generation 14 of the selection experiment. From each of the 27 lines 4 full sib families were chosen at random, and from within each of these families 2 male and 2 female mice were sampled, giving a total of 432 mice. Each mouse was weighed weekly from birth until 17 weeks of age, whereupon it was slaughtered for carcass analyses. In addition, weekly food intake was measured on half of these mice after weaning at 3 weeks of age. These measurements were made on pairs of mice of the same sex and line, rather than individual mice, to reduce the feeding cage requirements.

Fig. 2.1 DIRECT RESPONSES TO SELECTION



The remainder of the mice were housed in stock cages. The diet, offered ad libitum, was Beta Diets Rat and Mouse No.1 Expanded Maintenance Diet (crude protein = 14.8%).

Fat, protein and water percentages were measured on batch samples of the 17 week old mice. Each sample comprised 4 mice of the same sex and line, with constraints on laboratory facilities imposing the limit of one batch per sex per line, hence 54 samples in total. The determinations were performed by the Edinburgh School of Agriculture.

Water content was estimated by freeze drying the samples. The samples were then minced, and nitrogen was digested and extracted using a modified Kjeldahl technique (Crooke and Simpson,1971), with protein being estimated as 6.25 times nitrogen content. Fat was extracted using standard soxhlet extraction techniques.

Also presented in this study are summary carcass composition data at three other ages, viz 26 and 44 days of age - on mice from generations 11,12 and 13 (M. K.Nielsen,unpublished), and 10 weeks of age - on mice from generation 7 (Sharp et al,1984). These analyses were carried out in part by the Edinburgh School of Agriculture and in part by the Rowett Research Institute, Aberdeen. The analytical techniques were as above, except that fat% was determined by the chloroform-methanol method (Atkinson et al,1972) and protein% estimated by difference from the fat and ash determinations, at the Rowett Research Institute.

To enable composition data of mice of different generations to be compared, all line means were adjusted, "standardised", to that expected after 14 generations of selection, assuming a linear regression of response in carcass composition on generation number.

2.2.3 Growth Curve Methodology

The mouse growth curves were considered using the 4 parameter Richards generalised growth function (Richards,1959). This function was chosen because it represents a general family of growth curves of which the three most common curves - the Logistic, Bertalanffy and Gompertz curves - are members, and thus the restricting effect of choosing any one curve is avoided. The Richards curve is of the form:

$$Wi = Si(1-bie^{-kit})^{1/(1-mi)}$$

for the ith individual,where-

Wi(t) = body weight at time t

bi = time scale parameter

ki = "rate of growth" parameter

mi = "shape" parameter, defining the proportion of mature weight at inflexion

Si = asymptote, or mature weight

The Bertalanffy, Gompertz and Logistic functions are derived by substituting m=2/3, lim. as m→1 and 2 into the Richards curve, respectively, thus fixing their weights at inflexion as 8/27, e^{-1} and 1/2 of mature weight, respectively. The parameter S is usually referred to as A, but because of the terminology "A lines" it has been renamed S for this study.

Prior to fitting the curve functions, the 18 observed weekly body weights were log transformed to take account of their increasing variance with increasing body weight. The logarithm of the Richards function was then fitted to each individual, using an iterative "hillclimbing" subroutine which minimised the sums of squared deviations between the logarithms of fitted weights and the log transformed observed weights.

Fitting the 4 parameter curve proved to be unsatisfactory, however, due to the very slow convergence of the parameters, and also invariable convergence to local rather than global maxima. This problem has been observed before (Eisen et al,1969; Timon and Eisen,1969), and the former authors suggest that it is due to the high correlations between estimates of some of the parameters in the function.

Rather than abandon this curve fitting technique, as was done by Eisen et al (1969), the curves were fitted by assuming (and fitting) the same numerical value of m for all individuals within lines of the same selection direction (i.e. the AH, AC, AL, PH, PC, PL, FH, FC and FL lines). This "constrained" Richards function was considered to have been fitted when the value of m giving the best fit for each of the 9 groups as a whole, was found. By using this technique, the Bertalanffy, Gompertz and Logistic curves were all compared, along with curves having values of m ranging from Ø to 1Ø.

It was also decided to attempt to describe growth in terms of the

fat and lean components of growth. Lean mass at each age was estimated as body weight less fat mass, and the fat masses were estimated from linear regressions of fat percent on age. Each selection direction shows almost linear increases in fat percentage with age (see results), and thus separate regressions were calculated for each of the nine sets of lines. No information on carcass composition prior to the age of weaning was available, so a constant 8% fat was assumed for all lines. The constrained Richards curves were then fitted to lean mass, as above, with the following function describing total body growth:

$$Wij(t) = Sij(1-bije^{-kijt})^{1/(1-mi)} + (Ui+Vit)Wij(t) + eij$$

where: Wij(t) is the body weight of the jth individual of the ith selection direction, at time t

: S,b,k and m are as defined above

: Ui+Vit is the linear regression of fat/body weight on time for the ith selection direction -post weaning

N.B. pre-weaning (i.e.Ø to 3 weeks), fat% assumed to be 8%, therefore: 1-Ui-Vit =.92, pre-weaning

The curve this equation describes will be referred to as the carcass components growth curve.

From the Richards curve several traits describing growth can be derived. The traits derived and examined in this study were:

(i) mean absolute growth rate: $Sk/(2(m+1))$

(ii) mean relative growth rate: which is the actual relative growth rate at inflexion: k/m

(iii) age at inflexion: $(\ln(b/(1-m)))/k$

and (iv) mass at inflexion: $m^{1/(1-m)}$

The derivations for these traits are given by Richards (1959) and Eisen et al (1969). Relative growth rate is growth rate in relation to body weight, at the time of measurement.

2.2.4 Derivation of Traits Related to the Components of Growth

From the data on growth, food intake and carcass composition, several traits pertinent to the study of the components of growth were derived. Firstly, in addition to considering food intake per se, an

attempt was made to remove body size effects by scaling intake by both body weight (BW) and metabolic body weight ($BW^{.75}$).

Maintenance requirements for all individuals on which intake was measured were estimated, and are defined here as catabolism. These estimates are presented scaled by both metabolic body weight and metabolic lean mass (lean $mass^{.75}$) in order to investigate the effects that carcass composition has on catabolism.

Lean mass was estimated as body weight minus fat mass (as in 2.2.3) and catabolism was estimated as metabolisable energy intake less the energy costs of fat and protein accretion. The estimation of fat accretion, by the regression of fat percent on age, is described in 2.2.3, and protein accretion was also estimated in the same manner. The metabolisable energy content of the diet was assumed to be the same for all lines, and was estimated from the manufacturers specifications as 10.636 kJ/g. The efficiencies of fat and protein deposition, derived by Pullar and Webster (1977), of 53.4 and 52.9 kJ/g, respectively, were assumed for all lines.

Doubts as to the constancy of digestability and metabolisability of food across the different lines ,especially the A lines, were raised, so a small digestability trial in the A lines was undertaken. The estimated digestabilities for the high and low appetite lines were 74.11±0.56% and 74.25±0.84%, and no food wastage was observed, so this assumption is considered to be valid. The validity of assuming constant efficiencies of fat and protein deposition was discussed in section 1.2.2.3.

The estimates of maintenance were defined as catabolism in recognition of the fact that they are somewhat indirect estimates, depending on the assumptions made and on the accuracy of the estimates of fat and protein accretion. The term "catabolism" is used as the trait it describes is the energy lost from the breakdown of ingested food units or body units.

The efficiency of growth, gain(g)/intake(g), was calculated and is presented as cumulative intake, i.e. total gain/intake from weaning onwards. To help explain the results, and also to extrapolate them to other species, an allied trait - the ratio intake(kJ)/maintenance(kJ) - was calculated. This trait will be called the "intake ratio".

2.2.5 Statistical Analyses

The estimates of the growth curve parameters S, b and k, along with mean absolute growth rate, mean relative growth rate and age at inflexion, were analysed assuming the following statistical model:

$$Yijklmn = U + Ti + Dij + Rik + Lijk + Sl + (DS)ijl + (RS)ikl + fijkm + Iijklmn$$

where: Ti = ith selection criterion (A,F or P)
Dij = jth direction of selection (H,C or L) in the ith criterion
Rik = kth replicate in the ith criterion
Lijk = ijkth line
Sl = lth sex
fijkm = mth family in the ijkth line
Iijklmn = nth individual in the mth family

For the analysis of the metabolic traits, the observed and estimated values for each trait were split into four time periods, viz 3-4, 4-6, 6-10 and 10-17 weeks. These correspond to an immediate post-weaning period, a period of rapid growth, a period of decelerating growth and a period approaching maturity, respectively, as well as being the ages at selection for the three criteria. The mean values from each of these periods were analysed assuming the following statistical model:

$$Yijklm = U + Ti + Dij + Rik + Lijk + Sl + (DS)ijl + (RS)ikl + Iijklm$$

where: Iijklm = mth feeding cage
and all other symbols are as above.

For both models linear contrasts were used to test the correlated responses to selection (H-L) and the symmetry of response ((H+L)/2-C) within each criterion, using the line (i.e. genetic drift) component of variance as the error term.

2.3 RESULTS

2.3.1 Growth and Carcass Composition

This section will give a descriptive, or qualitative, summary of the growth curves and carcass composition of the selected lines. The results of the fitted growth curves will be discussed separately in 2.3.2.

The observed growth curves for the 9 selection directions are shown in fig. 2.2. All the A lines have similiar body weights until 4 weeks of age, as was the intention with the selection index used, whereupon they diverge - with the high intake (AH) lines becoming larger and the low intake (AL) lines becoming smaller than their controls. The P lines show much larger divergences in body weight than either the A or F lines, with the increased lean mass (PH) lines being larger, and the decreased lean mass (PL) lines being smaller throughout their entire growth period. The F lines show similar magnitudes of body weight change to the A lines, however by 17 weeks of age the increased fat (FH) lines still appear to be growing rapidly whilst the decreased fat (FL) lines have only very slow growth.

Shown in fig. 2.3 are water, fat and protein percentages for all selection directions, adjusted to that expected after 14 generations of selection. For all three criteria the differences in fat%, and their changes over time, are mirrored by the water% differences and changes. The protein% changes are also negatively related to fat% changes, however the magnitude of these changes are much smaller.

The A lines show distinct changes in fatness, with the AH lines being less fat than the AC or AL lines. These changes are apparent by 26 days of age, and possibly do not increase thereafter. Also of interest is the fact that the generation 7 determinations (10 weeks of age) shows the AL lines to be the fattest (as did another early study of carcass composition - S.Copland, unpublished), the determinations from generations 11,12 and 13 (26 and 44 days) find no difference between the AC and AL lines, and the generation 14 analyses show the AL lines to be slightly leaner than the AC lines. This will be discussed further in section 5.

In general, the P lines show little change in carcass composition,

Fig. 2.2

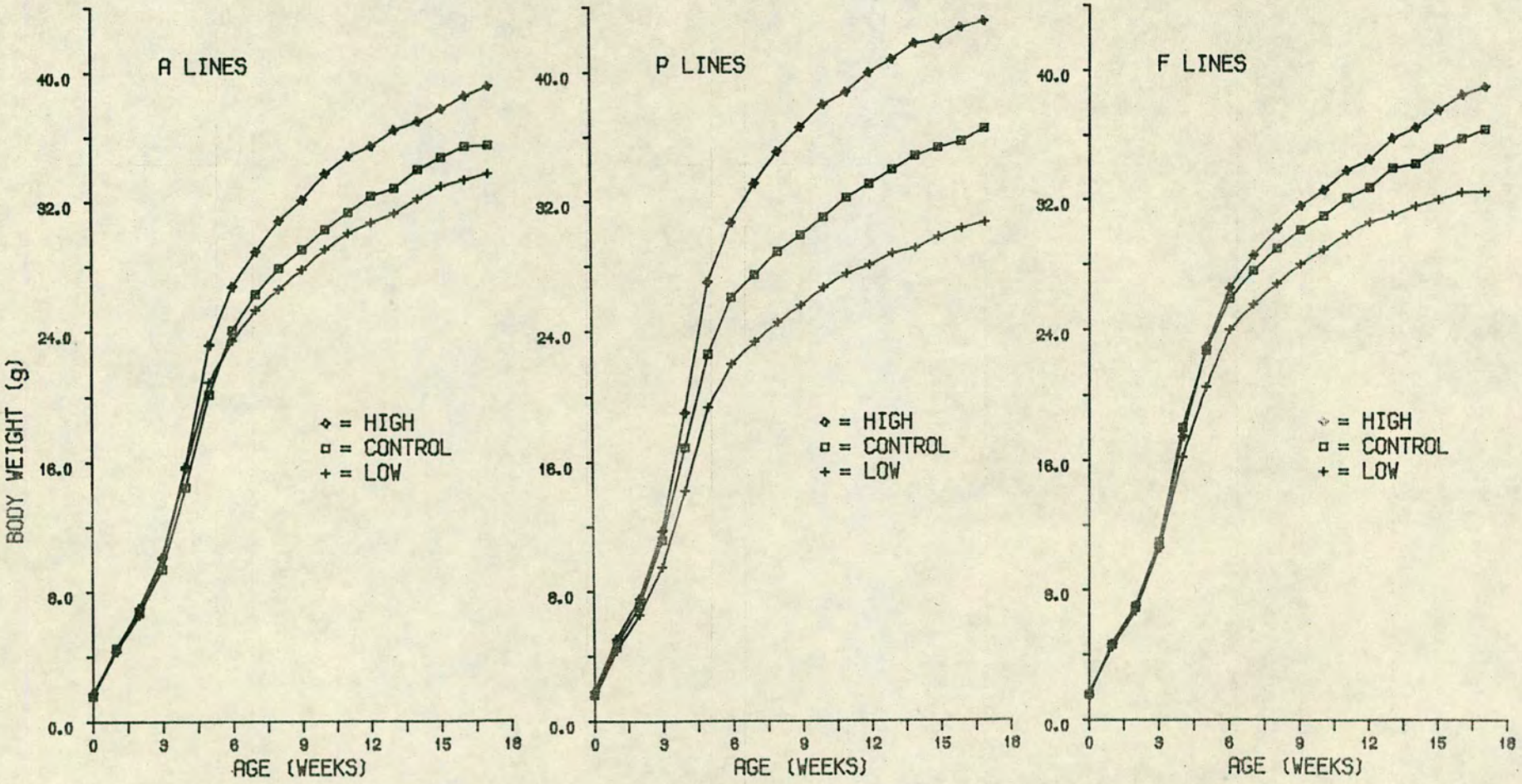
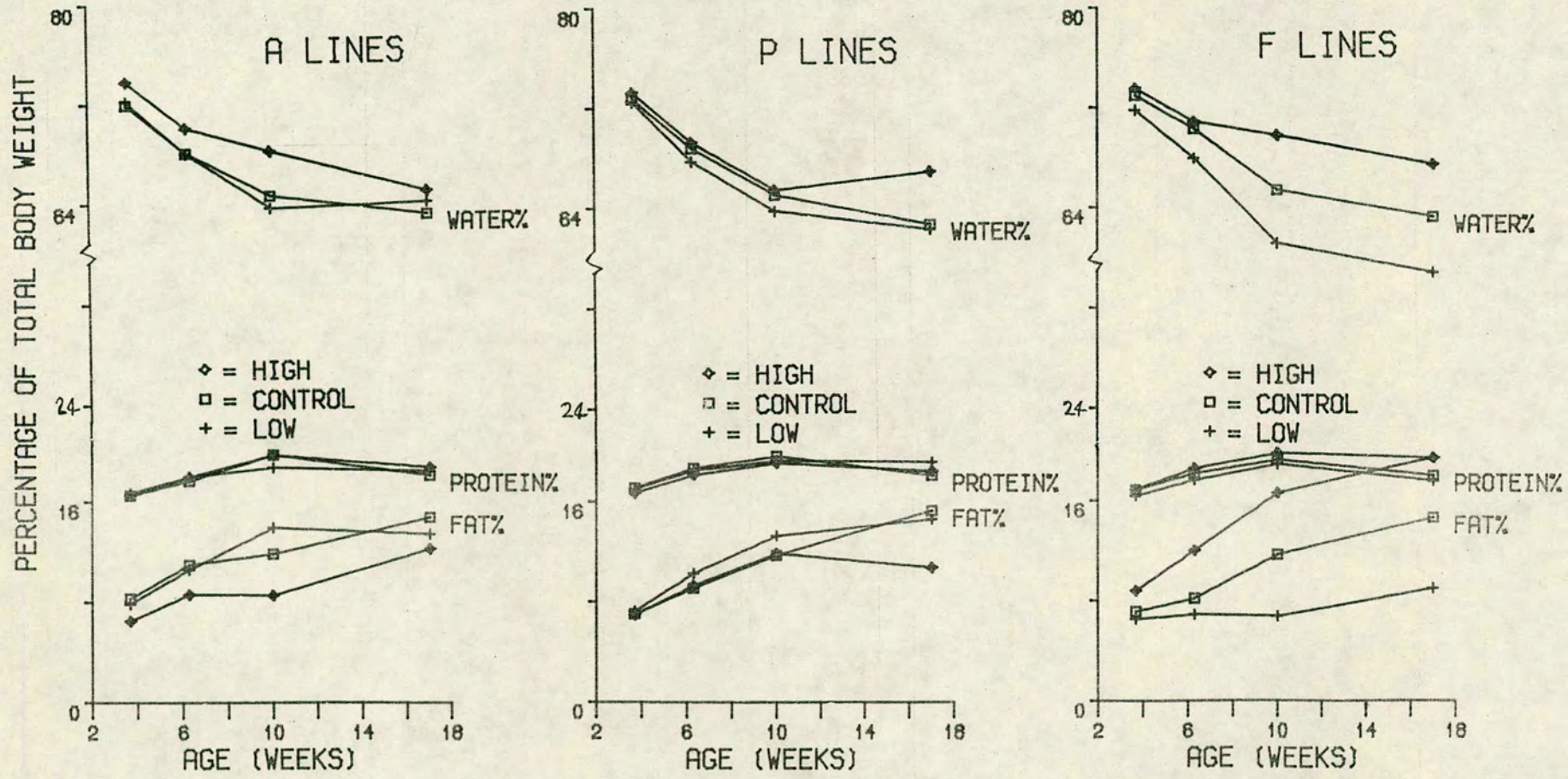

BODY WEIGHT VS AGE
A LINES
P LINES
F LINES
BODY WEIGHT (g)
AGE (WEEKS)
◇ = HIGH
□ = CONTROL
+ = LOW
0.0
8.0
16.0
24.0
32.0
40.0
0
3
6
9
12
15
18

Fig. 2.3

CARCASS COMPOSITION VS AGE



except for the decreased fatness of the PH lines at 17 weeks. There is some doubt as to the validity of this result, however, as in retrospect it was realised that some of the largest PH mice may not have been thoroughly dried. These were among the first samples to be processed, and no replication was possible. This point has not been used in the regression of fat% on age.

The F lines show large and consistent changes over age in all of the carcass components, with the FH lines becoming very fat and the FL lines remaining very lean throughout their lifetime. There are also small but consistent changes in protein%, with these divergences being in the opposite direction to the changes in fat%.

Growth curves for lean mass can also be derived from these results, using the regressions of fat% on time. The lean mass curves for the A and P lines are of course little different from the body weight curves, although the H-L divergence in the A lines is slightly increased. The F lines, however, appear to have equivalent lean masses throughout the entire measurement period.

2.3.2 Growth Curves

Table 2.1 shows the sums of squared deviations of the (logged) fitted curves from the (logged) observed weights, for each selection direction, for both the constrained Richards function and the carcass components function.

Table 2.1. Squared Deviations of Fitted Curve from Observed Weights

	Selection Direction								
	AH	AC	AL	PH	PC	PL	FH	FC	FL
Richards Function	.1151	.1484	.1016	.0971	.0901	.0882	.1160	.0941	.0743
Carcass Components Function	.1122	.1459	.0968	.0955	.0855	.0872	.1098	.0932	.0770

Fig. 2.4 FITTED CARCASS COMPONENTS GROWTH CURVES

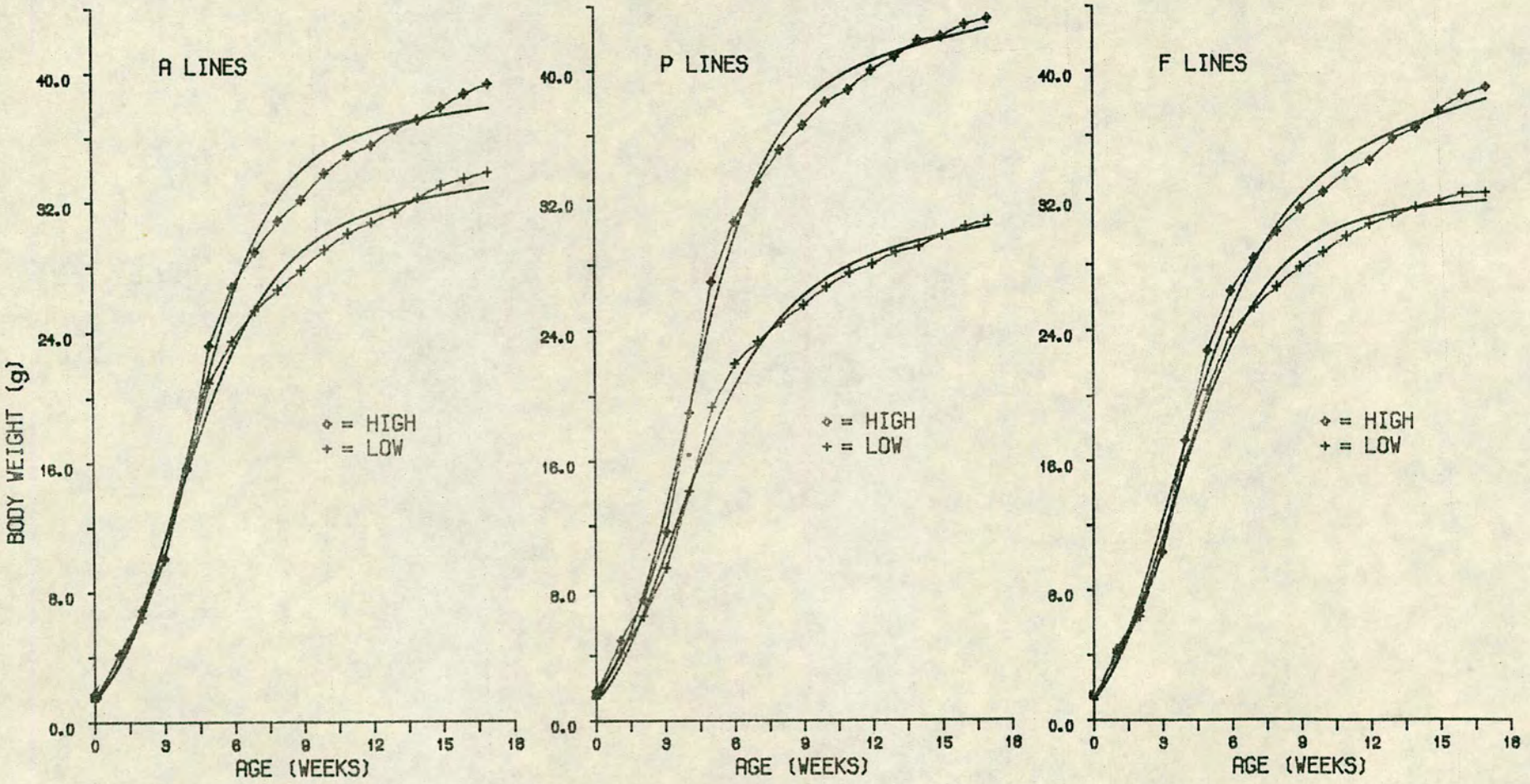


Table 2.2a. Parameters and Derived Traits from the Richards Curve Fitted to Lean Mass.

Selection Direction	(Mature weight) S	(Time scale) b	(Rate of growth) K	(Shape) M	[1]Lean mass at inflexion(g)	[2]Proportion of maturity at inflexion	[3]Age at inflexion (weeks)	[4]Mean absolute growth rate (g/week)	[5]Mean relative growth rate (g/g/week)
AH	33.17	-4.68	.521	1.59	15.12	.456	4.03	3.31	.328
AC	30.33	-0.51	.374	1.14	11.90	.392	3.58	2.61	.328
AL	28.13	-0.78	.413	1.20	11.31	.402	3.32	2.63	.344
PH	35.99	-3.04	.510	1.47	15.85	.441	3.69	3.70	.347
PC	30.15	-0.75	.435	1.19	12.07	.400	3.20	2.97	.365
PL	28.51	-0.03	.371	1.01	9.54	.370	2.91	2.37	.367
FH	30.63	-0.20	.392	1.06	11.61	.379	3.11	2.90	.370
FC	30.17	-0.78	.444	1.19	12.08	.400	3.15	3.04	.373
FL	29.35	-0.79	.422	1.20	11.80	.402	3.28	2.80	.352

Table 2.2b. Significant Effects

A:H-L	**	**	**	-	**	-	**	**	N.S.
A:symmetry[6]	N.S.	**	**	-	**	-	N.S.	*	N.S.
P:H-L	**	**	**	-	**	-	**	**	N.S.
P:symmetry	N.S.	**	N.S.	-	N.S.	-	N.S.	N.S.	N.S.
F:H-L	N.S.	**	N.S.	-	N.S.	-	N.S.	N.S.	N.S.
F:symmetry	N.S.	**	N.S.	-	N.S.	-	N.S.	N.S.	N.S.
Sex	**	**	N.S.	-	**	-	**	**	**
Family	**	**	**	-	N.S.	-	N.S.	**	**

No significant replicate or line effects were observed.

Tests: Main effects and (pooled) replicates against (pooled) lines, lines against families, sex against (pooled) replicate by sex interaction, and replicate by sex interaction and families against individuals.

1. $M^{(1/(1-m))}.S$ 2. $M^{(1/(1-m))}$ 3. $(\ln(b/(1-m)))/K$ 4. $SK/(2(1+m))$ 5. K/m 6. Contrast for symmetry = $((H+L)/2)-C$

*P < .05, **P < 01, otherwise P < .05

With the exception of the very lean Fl lines, the carcass components function always give a slightly closer fit to the observed body weights than the Richards function does. The following results, therefore, refer to the carcass components function, i.e. the Richards curve fitted to lean mass, with the fat increment being subsequently added on. The FL lines do give a poorer fit using this technique, but being the leanest lines, they are the lines with the least potential for improvement when fat accretion is taken account of.

Fig. 2.4 shows fitted carcass components curves for the High and Low selected lines of each criterion, and table 2.2 shows the values of the analysed traits and the statistical significance of the important effects.

Consider firstly the parameter and trait values. These traits refer to lean mass, not body weight, but for considering traits early in life - e.g. age at inflexion - the results for lean mass are very similar to those of body weight, as the amount and rate of fat accretion is quite small early in life. From the values of the m (shape) parameter, it is apparent that the optimal curve is close to the Gompertz curve ($m=1$, $m^{1/(1-m)} = e^{-1}$). The exceptions are the AH and PH lines which, from their m values, take slightly longer to reach their period of maximum growth (inflexion). Also revealed is a marked asymmetry in the response of the A lines growth curves to selection. A close inspection of fig. 2.2 confirms this with the AH and AL lines initially exceeding their control lines, but after 5 weeks of age the AL lines' growth rate drops below their controls whilst the AH-AC divergence increases. The equivalence of the FH, FC and FL lean mass growth curves is confirmed.

Inspection of the fitted curves (fig. 2.4) raises doubts as to usefulness of these techniques, however. For all lines the fitted curves err in the same way -by underestimating weight from 4 to 7 weeks of age, overestimating weight from 7 until 14 weeks and underestimating mature weight - quite severely so in the A lines. Close inspection of fig. 2.2 also reveals that age at inflexion is also always underestimated, by up to 4 to 5 days, and hence weight and proportion of maturity at inflexion are also underestimated. The rankings of the selection directions within each criterion do,

nevertheless, appear to be correct.

Statistical "inadequacies" of this section are outlined in the discussion.

2.3.3 Food Intake Traits

Fig. 2.5 shows unadjusted intake, and figs. 2.6 and 2.7 show the ratio of food intake to body weight and metabolic body weight respectively. Linear contrasts and components of the analyses of variance are shown in tables 2.3 and 2.4. Not shown are the components due to selection criterion and the sex by replicate and sex by direction interactions, as they are nearly always non-significant and are not important in the development of the arguments.

For all lines food intake per se increases rapidly until 6 weeks of age, i.e. through the period of rapid growth, but shows only a very small increase thereafter. There are large H-L divergences in the A and P lines at all ages, with the magnitude of the divergences being slightly larger in the P lines. There is considerable variation between weeks in the AH lines in the 10-17 week period, however the large "line" component of variation (table 2.3) suggest measurement error. The F lines show significant divergences during the fast growing period, with the FH lines eating more, however as the lines approach maturity the differences in food intake disappear.

For all lines, intake in relation to body weight declines throughout life, however intake/metabolic body weight tends to stabilise towards 17 weeks of age. In addition to having a larger intake per se, the AH lines also eat more in relation to body weight (fig. 2.6) and metabolic body weight (fig. 2.7) than the AL lines, until 10 weeks of age - after which time the trends become less clear. The A line H-L divergence is larger when scaled by $BW^{.75}$ than when scaled by BW. The PH lines eat less in relation to their body weight than the PL lines, and this divergence appears to increase with age, and hence, increasing divergence in body weight. When scaled by metabolic body weight, however, the H-L divergences become very small throughout the entire measurement period. For the F lines, the choice of either body weight or metabolic body weight makes little difference to the trends. During the fastest growth period (4-6 weeks) the FH lines eat slightly

Fig. 2.5 FOOD INTAKE VS AGE

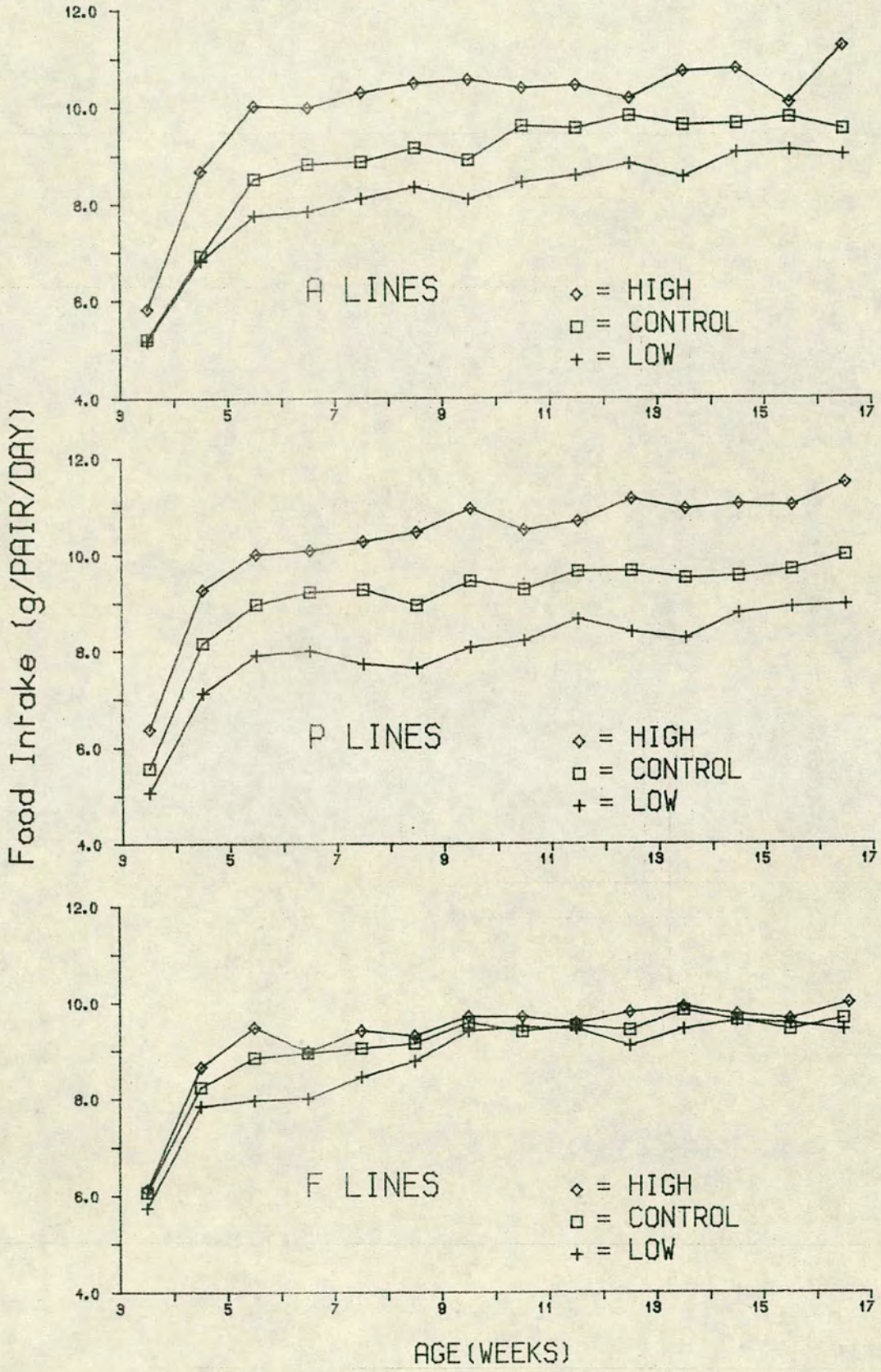


Fig. 2.6 FOOD INTAKE/BW VS AGE

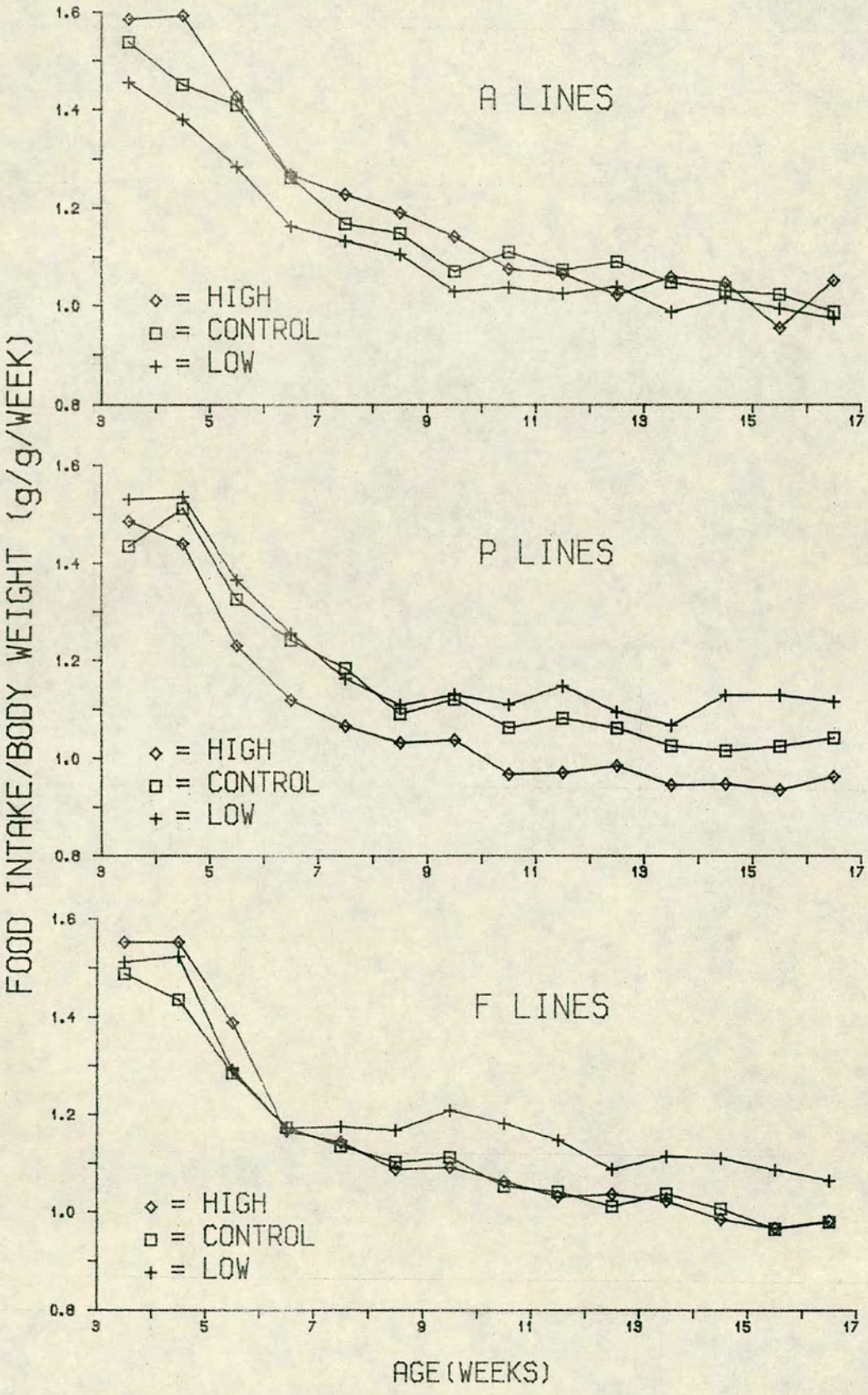


Fig. 2.7 F.I./METABOLIC BW VS AGE

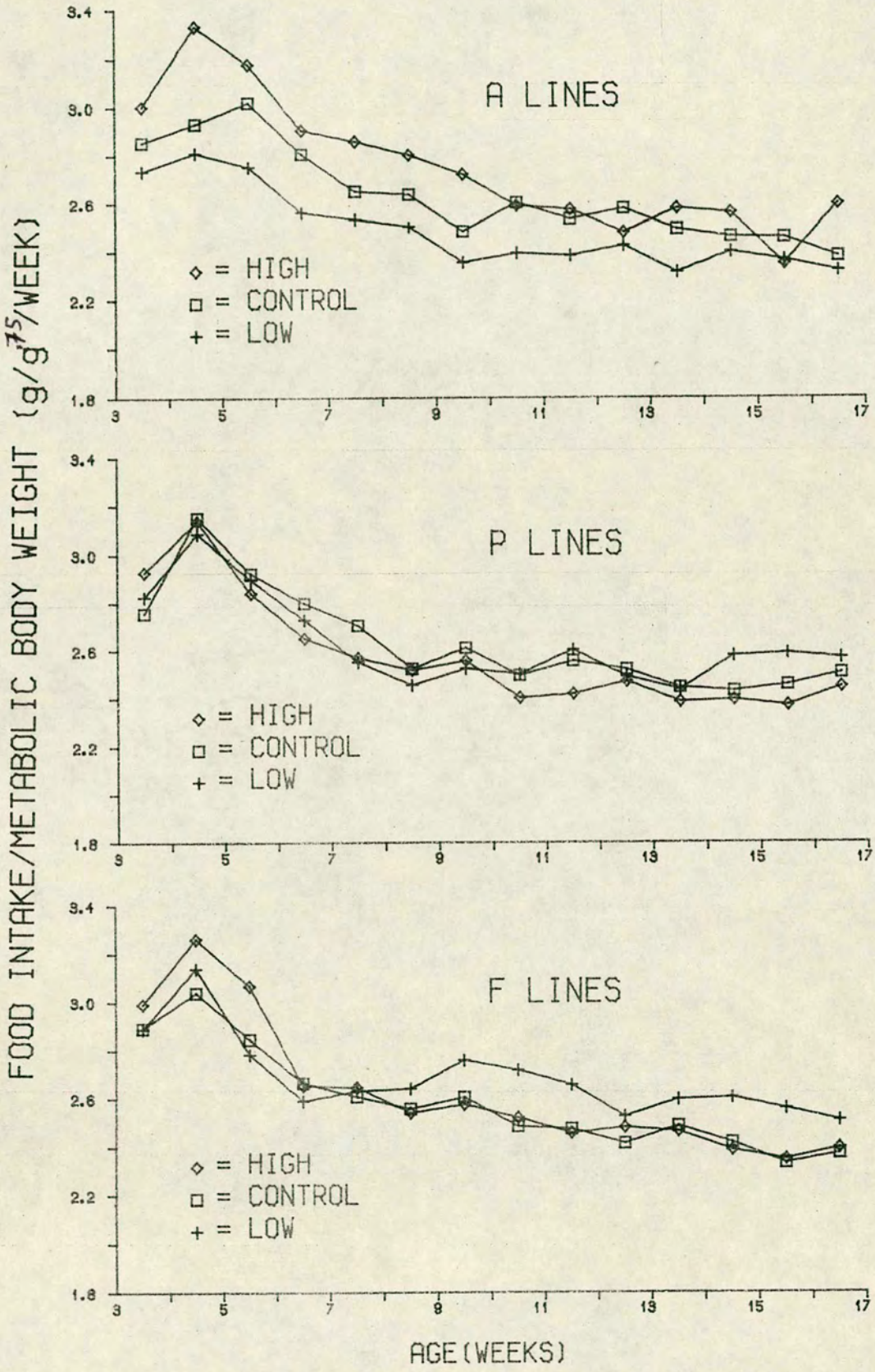


TABLE 2·3

TOTAL FOOD INTAKE

(g/pair/week)

Contrast	df	PERIOD 1	2	3	4
A:H-L	1	0.66*	1.97**	2.40**	1.70*
A:Symmetry	1	0.26	0.58	0.15	0.05
P:H-L	1	1.47**	2.25**	2.57**	2.35**
P:Symmetry	1	0.16	0.01	0.07	0.16
F:H-l	1	0.34	1.19*	0.69	0.32
F:Symmetry	1	-0.05	-0.05	-0.17	-0.03
Sex:M-F	1	0.04	0.62*	0.60*	0.14
Mean Squares					
Replicates	6	1.348+	2.746+	5.035	2.319
Lines	12	0.520	1.055*	2.270**	4.146**
Residual	58	0.383	0.462	0.531	0.546

+P ⩽ .1, *P ⩽ 0.5, **P ⩽ 0.1,

otherwise P > .1

Tests are: Contrasts and (pooled)

replicates against (pooled) lines

: Sex against (pooled)

replicate by sex interaction

: Lines against residual

Period 1 = 3 to 4 weeks

2 = 4 to 6 weeks

3 = 6 to 10 weeks

4 = 10 to 17 weeks

Symmetry Contrast is (H+L)/2 - C

Table 2.4

		Food Intake/Bodyweight (BW) (g/g/day)				Food Intake/BW$^{.75}$ (g/g$^{.75}$/day)			
		PERIOD				PERIOD			
Contrast	df	1	2	3	4	1	2	3	4
A:H-L	1	104*	.162**	.125*	.020	.228**	.463**	.389**	.145
A:Symmetry	1	.014	.008	.020	.018	.016	.044	.024	.031
P:H-L	1	-.031	-.111**	-.105*	-.156**	.140+	.017	-.001	-.131
P:Symmetry	1	.078+	.027	.043	.009	.127+	.049	.088	.014
F:H-L	1	.040	.070*	-.060	-.103+	.099	.218*	-.052	-.162
F:Symmetry	1	.055	.073*	.021	.048	.074	.120+	.023	.085
Sex:M-F	1	-.040*	-.037**	-.103**	0.158**	-.052	-.005	-.134**	-.274**
Mean Squares									
Replicate	6	.0266+	.0364**	.0314	.0355+	.1269*	.1660*	.2086+	.2185+
Line	12	.0110	.0062	.0117**	.0140*	.0280	.0363	.0766**	.0812**
Residual	58	.0085	.0050	.0033	.0044	.0336	.0200	.0183	.0225

more, however as the rate of growth slows, the FL lines have the higher relative intake.

2.3.4 Catabolism

Catabolism is shown relative to metabolic body weight in fig. 2.8 and metabolic lean mass in fig. 2.9. Linear contrasts and the analyses of variance are shown in table 2.5.

For all lines, catabolism rises quickly until about 5 weeks of age, whereafter it stays very constant throughout life. Large H-L divergences exist in the A lines for catabolism/$BW^{.75}$, until 10 weeks of age. When scaled by lean mass$^{.75}$ the trends are very similar, but the divergences are slightly smaller. The P lines show little differentiation between selection directions for catabolism/$BW^{.75}$, and since their carcass composition changes are slight, they also show little differentiation in catabolism/lean mass$^{.75}$. The F lines show no divergence in catabolism/$BW^{.75}$ over the fast growing periods, but have an increasing divergence with age as the lines become more differentiated in degree of fatness - with the FL lines having the higher catabolism. When scaled by lean mass, however, the H-L divergences disappear in the F lines, at all ages. As the H, C and L F lines have equivalent lean masses at all ages, this result is true no matter what exponent is used to define "metabolic" lean mass.

2.3.5 Efficiency and the Intake Ratio

Cumulative efficiency up to each age and the intake ratio are shown in figs. 2.10 and 2.11, respectively. Linear contrasts together with the analyses of variance are presented in table 2.6.

Cumulative efficiency shows a steady decline from 4 weeks of age onwards for all lines, as growth slows. Actual efficiency at each age will show a much steeper decline, being very low as the animal matures, and will follow the patterns of the intake ratio in fig. 2.11.

The AL lines are slightly more efficient than the AC and AH lines during period 1, due to a much higher intake ratio, however during period 2 (4-6 weeks, the period of selection), they are slightly less

Fig. 2.8 CATABOLISM/BW$^{.75}$ VS AGE

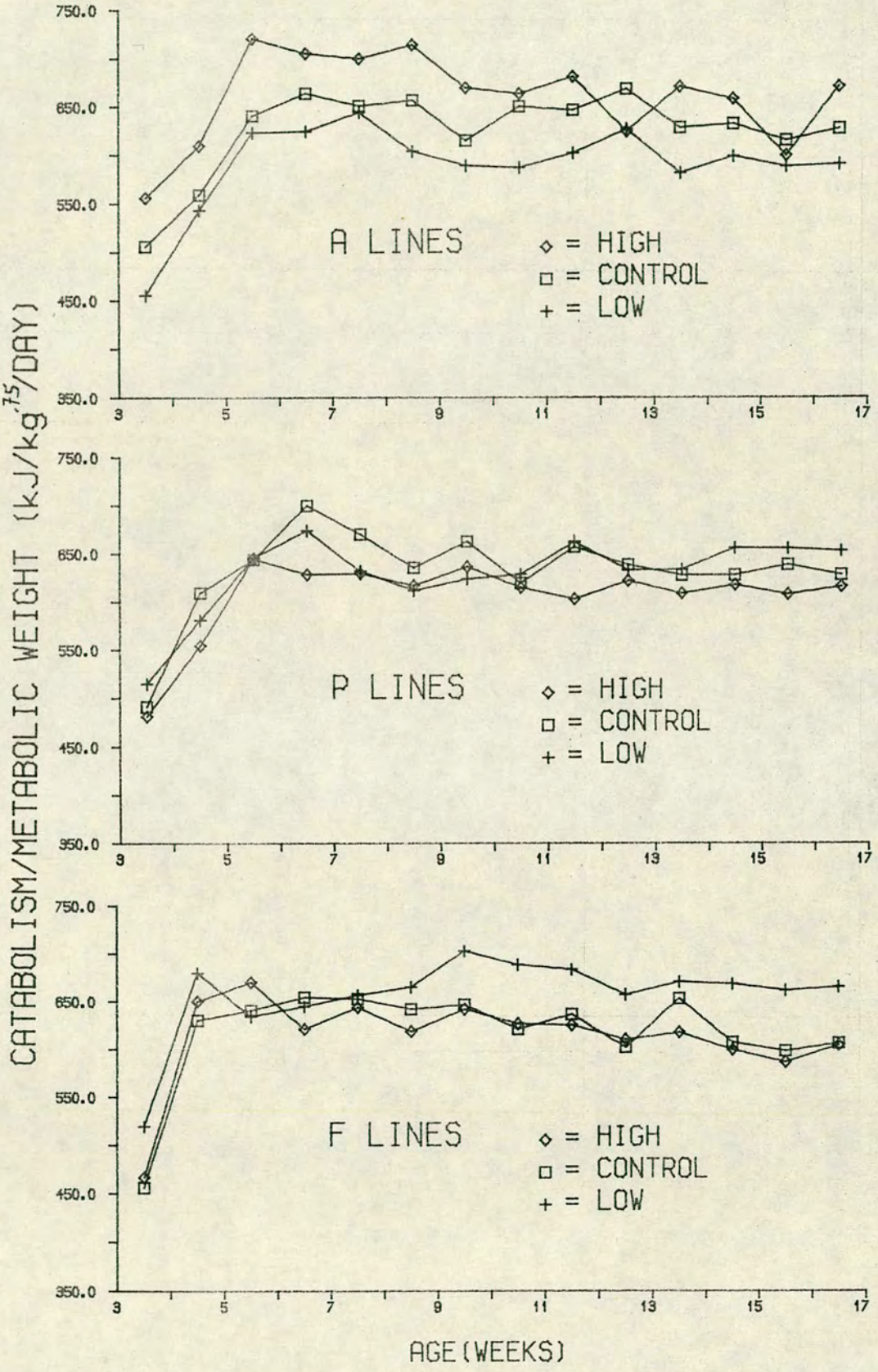


Fig. 2.9 CATABOLISM/LEAN MASS$^{.75}$ VS AGE

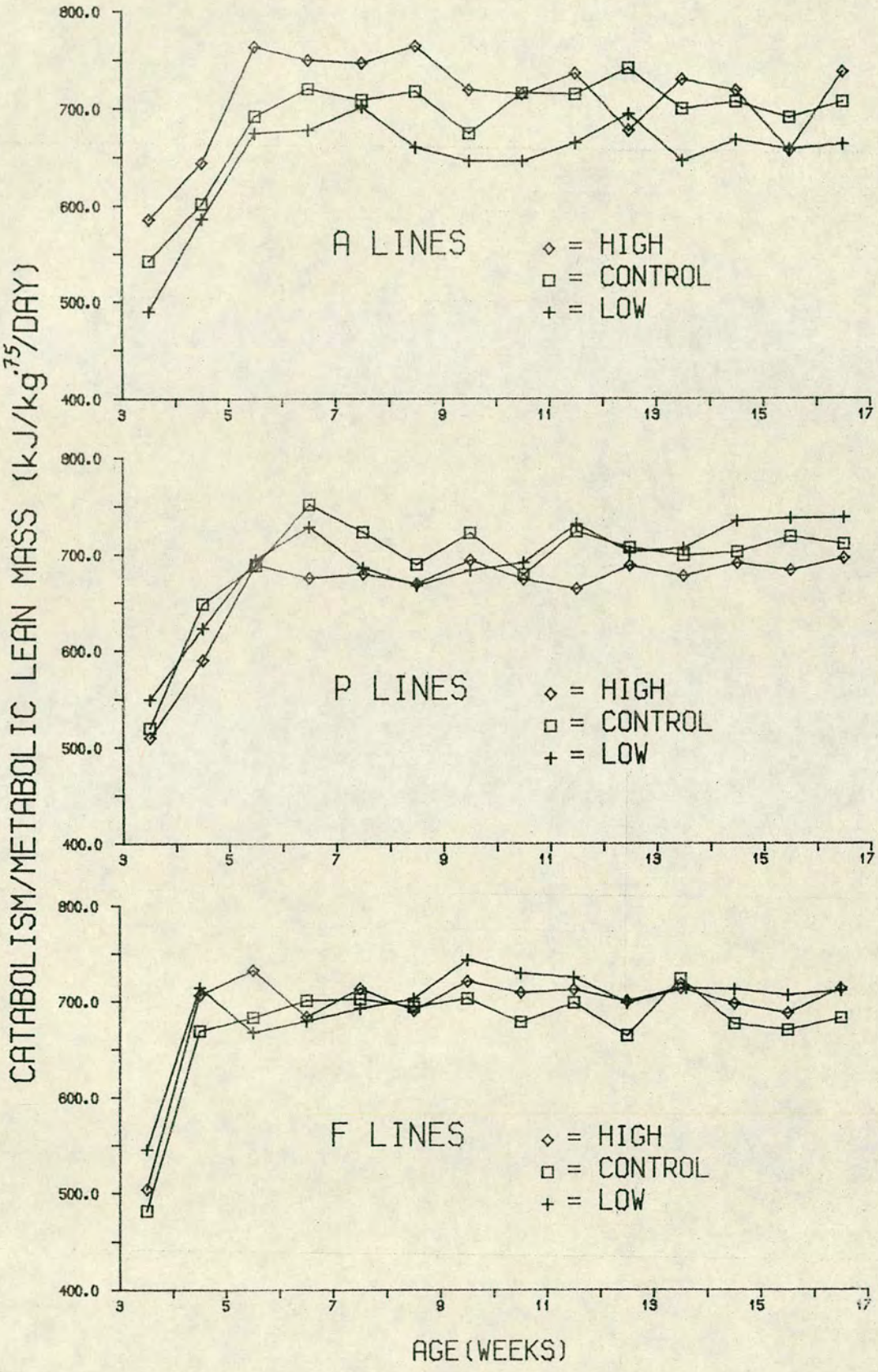


Table 2.5

		Catabolism/BW$^{.75}$ (kJ/kg$^{.75}$/day)				Catabolism/Lean Mass$^{.75}$ (kJ/kg$^{.75}$/day)			
		PERIOD				PERIOD			
Contrast	df	1	2	3	4	1	2	3	4
A:H-L	1	86.31**	93.11**	94.72**	41.13	84.92**	86.51**	87.35*	30.54
A:Symmetry	1	6.57	21.32	2.48	9.25	4.21	16.92	4.62	19.04
P:H-L	1	-30.46	-13.36	-15.84	-41.28	-34.93	-18.74	-20.75	-47.60
P:Symmetry	1	5.08	19.45	31.14	0.91	6.85	17.96	31.79	0.62
F:H-L	1	-55.30*	1.43	-41.86	-67.92^{+}	-48.91^{+}	24.01	-8.68	-16.74
F:Symmetry	1	37.24^{+}	30.17	5.86	25.61	41.61^{+}	36.76	9.10	27.74
Sex:M-F	1	-40.71**	-37.36**	-45.46**	-74.70**	-43.24**	-39.85**	-49.21**	-87.75**
Mean Squares									
Replicates	6	13809.7**	12834.3**	13142.7	16719.1^{+}	15652.8*	14693.6**	15424.4^{+}	20583.1^{+}
Lines	12	2840.8	2053.6	48240.0**	6340.2**	3247.6	2386.2	5694.4**	7851.5**
Residual	58	2953.5	1676.6	1377.7	1614.3	3358.9	1912.7	1616.8	1974.0

Fig. 2.10 CUM. FOOD EFFIC. VS AGE

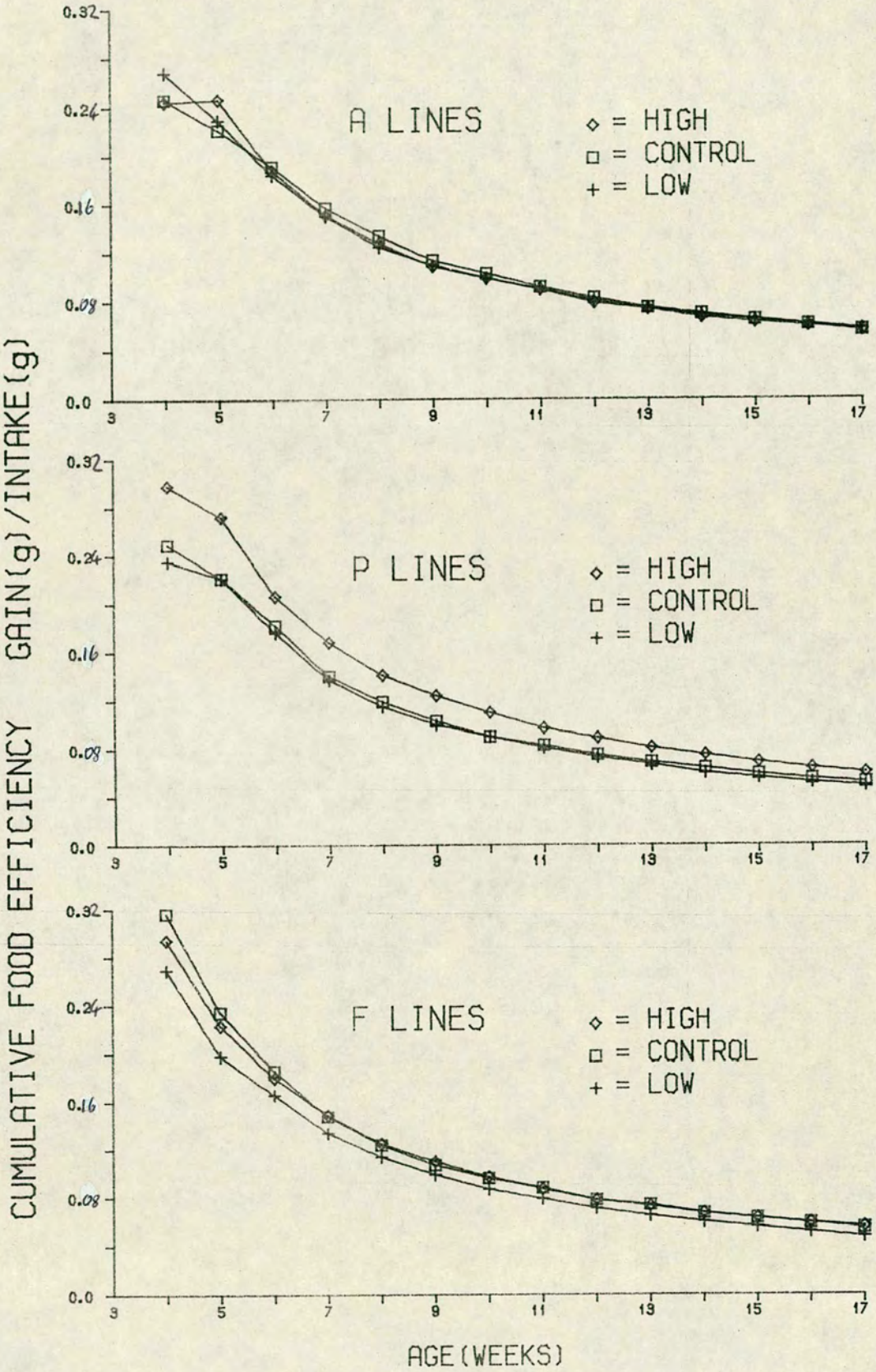


Fig. 2.11 F.I./CATABOLISM VS AGE

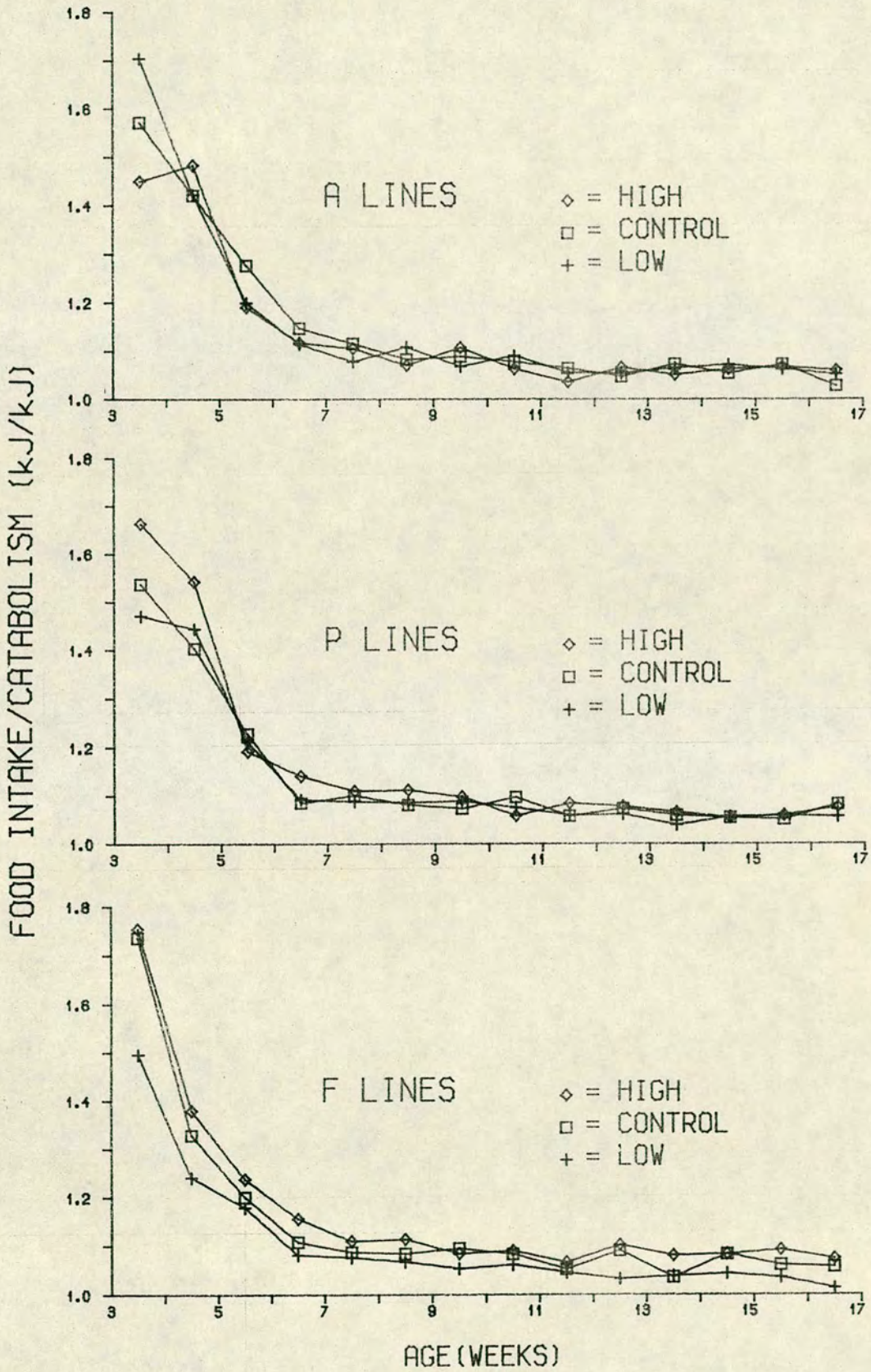


Table 2.6

		Cumulative Efficiency (g. gain/g. intake)				[1] Intake Ratio (kJ. intake/kJ. maintenance)			
		Age (week)				Period			
Contrast	df	4	6	10	17	1	2	3	4
A:H-L	1	-.0229	.0002	.0018	.0005	-.2008*	.0031	.0026	-.0055
Symmetry	1	.0038	.0028	.0029	.0009	.0054	.0123	.0161^{+}	.0021
P:H-L	1	.0674*	.0302*	.0194**	.0112**	.1784^{+}	.0381	.0265*	.0116
P:Symmetry	1	.0197	.0094	.0090	.0018	.0481	.0309	.0157	.0055
F:H-L	1	.0249	.0135^{+}	.0094^{+}	.0073^{+}	.2541*	.1033**	.0470**	.0460**
F:Symmetry	1	.0223	.0127*	.0046	.0031	.0912	.0166	.0019	.0053
Sex:M-F	1	.0321**	.0305**	.0179**	.0101**	.1089**	.0768**	.0212**	.0068*
Mean Squares									
Replicates	6	.01576**	.00022	.00013	.00006	.1856*	.0075	.0017^{+}	.0005
Lines	12	.00307	.00025	.00011	.00006*	.0426	.0044	.0006	.0003*
Residual	58	.00286	.00033	.00008	.00002	.0335	.0046	.0004	.0001

[1] Intake/Maintenance

efficient. These two effects cancel each other out, and after 5 weeks of age the A lines show almost identical efficiency until maturity. The A lines also display no H-L divergence in the intake ratio after 5 weeks. The PH lines always have a much higher cumulative efficiency than the PC and PL lines, as well as having a greater intake ratio in periods 1 and 3. In the F lines there are large and significant divergences in both cumulative efficiency and the intake ratio throughout life, with the fatter FH lines always being more efficient and having a greater intake ratio than the leaner FL lines. For all lines there appears to be a small amount of asymmetry in the responses to selection in efficiency and the intake ratio, with the C line values not being intermediate to the H and L line values, however this asymmetry is rarely significant.

2.4 DISCUSSION

This section will discuss the A, P and F line results separately, before considering the fitted growth curves. It will conclude with a general discussion of the results.

2.4.1 A Lines

Changing the input component of growth over and above that explicable by subsequent body weight changes has been successfully achieved, as scaling intake by $BW^{.75}$ instead of BW normally reduces rather than increases intake differences.

The index used, 4 to 6 week intake corrected for 4 week weight, was intended to restrict 4 week body weight change and fig. 2.2 indicates that this intention has been realised. Subsequently, however, there are divergences in body weight, but this is the expectation with selection for food intake (Sutherland et al, 1970; Pym and Nicholls,1979). The decreasing divergences in intake/BW and intake/$BW^{.75}$ as the mice mature are due to these body weight changes.

Increasing intake relative to metabolic weight has also increased catabolism, thus implying increases in maintenance - in agreement with M.K.Nielsen's (unpublished) results with the A line mice and also with Pym and Farrell (1979) in chickens. The magnitude of the catabolism

changes are in close agreement with Nielsen, who found a (H-L)/C divergence of 10% in generation 11 mice at 26 to 44 days of age. An independent verification of these results using calorimetry, is described in section 3.1.

The A lines show no divergence in efficiency after 5 weeks of age, in contrast with the high appetite lines of Sutherland et al (1970), where efficiency did increase. The A line result can be seen to be due to the lack of divergence in the intake ratio, as efficiency is a function of the proportion of an animals intake available for growth - i.e. the intake ratio. The maintenance requirements of the A lines therefore appear to have changed proportionately to their intakes. Prior to this age, however, there are efficiency changes in the A lines. The AL lines have a greater intake ratio and efficiency in period 1 than the AH lines, yet lesser values in period 2. These effects are simply due to both groups of lines having similar body weights at 4 weeks of age, despite having different levels of intake and catabolism. This finding underlines the need to conduct experiments over a range of ages, as in the review of literature the (correct) observation was made that the AH mice were more efficient from 4 to 6 weeks of age than the AL mice, but the incorrect conclusion was then drawn that the AH mice were therefore generally the more efficient mice.

The 17 week carcass composition results simply confirm the previous findings of Sharp et al (1984) and Nielsen (unpublished) of a decreased fatness in the AH lines. This general result does, of course, conflict with the increased fatness found by Sutherland et al (1970) and Pym and Solvyns (1979), in mice and chickens respectively, and with general expectations (see review of literature). The mice were not selected for food intake per se, however, but intake corrected for 4 week weight. This, together with the fact that the composition differences are apparent by 4 weeks of age but do not increase greatly subsequently, implies that the 4 week restriction has caused the composition changes. If maintenance requirements are a function of lean mass (see F line discussion) rather than body weight, then these changes are explicable: maintenance forms the greater part of the mouse's intake (fig. 2.11), therefore those mice with the greater intake and hence maintenance requirements, at the same body

weight, will tend to be the leaner mice. These will be the mice selected using the AH lines' criterion. Section 5 includes an experimental attempt to verify this hypothesis.

2.4.2 P Lines

The P lines appear to represent lines of mice which differ only in size, and therefore efficiency. They also appear to adhere closely to the metabolic body weight rule, with large differences in intake and catabolism almost disappearing when scaled by metabolic body weight.

Their size and hence efficiency changes result almost entirely from the intake ratio changes in periods 1 and 3, as there is little difference in carcass composition at these ages. It is not possible to ascribe these changes definitely to either increased (or decreased for the PL lines) intake, or decreased (or increased) catabolism, as these observed changes are very small. Most probably both factors contribute. Rats selected for increased protein gain showed small nonsignificant decreases in heat production (Notter et al, 1976; Wang et al,1980), in agreement with the P line results.

Selection for body weight usually results in increased fatness, although this effect decreases with increasing age at selection (Clarke,1969). Therefore, if changes in fatness are to be avoided, selection for lean mass rather than body weight may be appropriate.

In summary, the observed P line differences appear to be solely a function of body size, with all the components of growth changing correspondingly.

2.4.3 F Lines

The outstanding feature of the F lines are the large differences in fatness, which increase with age, yet an equivalence of lean mass at all ages. Their body weight differences are therefore caused totally by their differences in fatness. This indicates that lean mass and fat% are uncorrelated, and this is backed by the P line finding of a large change in lean mass but little change in fatness.

The similar total food intakes of the lines as they approach maturity indicate similar total maintenance requirements, but the

trends for intake scaled by body weight and metabolic body weight are somewhat confusing. These confusing trends are resolved, however, when the energy used for growth is accounted for, and catabolism is calculated. Catabolism differs between lines when scaled by $BW^{.75}$, but when scaled by lean mass these differences disappear. This provides evidence that maintenance requirements are more closely related to the lean portion of body mass than to body mass per se, in agreement with the conjectures of Webster (1981) and Fowler et al (1976). According to this hypothesis, therefore, fatter animals have lower maintenance requirements than leaner animals of the same body weight.

The FL lines have become less efficient than their FC and FH counterparts, despite the fact that it is less efficient to deposit fat than lean. This result is explicable, however, by their much reduced intake ratio as compared to their contemporary lines. In this instance, the decreased efficiency of fat deposition as compared to lean deposition has been outweighed by the increased weight gain and intake ratio of the FH lines, and vice versa for the FL lines.

In summary, selection designed to change fat% has resulted in lines of mice with the same "lean mass frame", but varying fat adjuncts on this frame. Metabolism appears to be a function of this lean mass frame rather than total body weight.

2.4.4 Growth Curves

The carcass components function often appears to fit the data quite poorly, with the same patterns of ill-fit being apparent for all lines. This curve is therefore rejected as a description of growth in this study. These problems of ill-fit are not specific to the carcass components curve, however, as it visually gives a better fit than any of the other curves tried, including of course the Bertalannfy, Gompertz and Logistic curves (hence its lower sums of squared deviations). These other curves all tend to err in the same manner, and by not taking account of fat deposition towards maturity they often underestimate mature weight even more severely.

Several statistical questions were left unanswered when this curve was rejected, the most important being the comparison of the Richards and carcass components curves. Although the carcass components curve

does usually give a slightly better fit, an extra parameter is in effect being fitted, and it is not known whether or not this extra "parameter" is removing enough variation for it to be statistically significant. It is unclear, therefore, whether or not the carcass components curve, as used in this study, is a useful improvement on the Richards curve.

Two aspects of the growth curve of the greatest interest are firstly weight and age at inflexion, and secondly mature weight. With this data, however, the Richards curve methology appears to always underestimate both traits. The curves studied fail in that by being of a sigmoidal nature they give curves that tail off towards an asymptote, whereas for all these lines growth is steady and almost linear from about 8 weeks of age. A very small m value would be required to account for this continual increase in weight, but this in turn would result in an even greater underestimate of inflexion.

The second unanswered statistical question is whether or not the different m values accepted for each group are actually "different" or not. Comparisons of within group versus between group variation in m values, and whether or not a global value of m would be acceptable, have not been done. Another question may perhaps be whether or not the m values used differ significantly from those of either the Bertalannfy, Gompertz or Logistic curves.

Finally, two somewhat more positive points. Although the carcass components curve does not appear to give satisfactory descriptions of growth, it has not been entirely insensitive to changes ("bending") in the shapes of the growth curves. This is illustrated by changes in the shapes of the AH and AL curves relative to the AC curve, which were revealed by the significant asymmetry effects in the analyses of the parameters and derived traits of these curves. Lastly and most importantly, although curve fitting has not been successful in this study, the results do indicate that derivation of curves that separately fit lean and fat mass may result in more powerful curve fitting techniques, especially for describing and comparing animals that differ greatly in carcass composition.

2.4.5 General Discussion

Several conclusions, or implications, about the relationships between the components of growth may be drawn from this study.

Firstly, there appears to be variation in maintenance requirements as well as intake eaten in excess of maintenance, the latter being a necessity to get variation in body size and growth rate. Maintenance and intake above maintenance also appear to be uncorrelated, and are capable of changing independently of each other, as was indicated in the review of literature. For example, the F line criterion has changed intake above maintenance, but not maintenance itself, whereas simply placing pressure on intake (as in the A lines) changes both components proportionately.

Secondly, the results from this study support the hypothesis of maintenance being related to lean mass rather than body weight itself: the F lines supply direct evidence, the A lines show reduced catabolism differences when scaled by lean mass instead of body weight, the P lines show little divergence in either catabolism or carcass composition, and the decreased fatness of the AH lines can be explained if this hypothesis is correct.

The results from conclusions 1 and 2 imply that lean mass (of which maintenance is a function) and carcass composition (a general indicator of intake above maintenance) are uncorrelated at any given age. The P and F line results do demonstrate this.

Thirdly, the A, P and F lines all support the importance of the intake ratio in defining efficiency. The contributions of the intake ratio and the type of tissue being deposited in affecting overall efficiency, for different species, will be discussed in section 6, but it can be shown that for any animal whose gross efficiency is less than 1g(tissue)/53kJ (the cost of depositing fat), depositing one further increment of fat will always improve efficiency. Efficiency for a mouse always appears to be less than this value, after 4 weeks of age, hence the FH lines which only differ from the FL lines in the amount of fat deposited, are the more efficient of the two groups of lines.

Growth curves were fitted as an alternative approach to describing growth. In this study the curves used were unable to describe growth well, and thus have not contributed to understanding of growth. Even

if they had been successful, however, they still would not have revealed the changes in the components of growth in the way that the metabolic approach has, and thus would have given fewer clues as to important areas for further research. A description using, for example, the metabolic approach would still have been necessary.

More elaborate curves using both food intake and age as descriptors may be derived (Parks,1970), but the "goodness of fit" problem still exists when interpreting traits derived from the function. The cruder and much simpler metabolic approach does not have these problems, and it is thus much more powerful.

2.4.6 Areas of Further Study

Firstly, the catabolism results only imply changes in maintenance, so the possible maintenance differences have to be studied in greater detail. They need to be verified, and possible causes of the changes studied. This work will be presented in section 3.

Secondly, little has been deduced so far about the changes in the patterns of energy partition. It is not known to what degree the composition changes are merely a function of intake available for growth, and to what extent the patterns of energy partition have been changed. A study of intake in excess of maintenance is given in section 4.

Thirdly, an experimental verification of the hypothesis explaining the decreased fatness of the AH lines is required, and is described in section 5. This study, at the phenotypic level, also provides a general verification of many of the results gained and conclusions drawn about growth and its components.

The general discussion of results comprises section 6.

Section III MAINTENANCE STUDIES

3.1 FASTING HEAT PRODUCTION

3.1.1 Introduction

In section 2 maintenance requirements for the A, P and F lines were estimated, and these estimates were used along with the growth and efficiency results to help to explain the effects of selection on the components of growth. These estimates were made somewhat indirectly, however, using food intake, growth and carcass composition data, and verification of these results using an independent method of calculation is required.

Maintenance, as discussed in the review, comprises the energy used for protein turnover, maintenance of ion gradients and bodily functions, and thermoregulation, as well as energy expended in digestion and general activity. The first group of requirements is known as fasting, or basal, heat production, and can be measured on fasted animals. Total maintenance energy requirements are approximately 1.3 times fasting heat (or energy) production in monogastric animals, (Webster,1981), and as this relationship is quite constant or predictable, fasting heat production can be used to calculate maintenance requirements. This study attempts to measure the fasting heat of mice from all the selected lines, as an alternative means of estimating their maintenance requirements and the changes which have occurred with selection. The results from this study are independent of those in section 2, as it is energy output that is being measured rather than energy input.

Heat production is usually measured by techniques known as indirect or respiration calorimetry, as respiration calorimeters are more precise and cheaper to run than the direct calorimeters which directly measure actual heat output (Blaxter,1962). Respiration calorimetry estimates heat production by measuring the compounds consumed and produced from the oxidation of food and body tissues. Oxygen and carbon dioxide measures can estimate heat production from fat and carbohydrate oxidation very precisely, however incomplete oxidation of proteins yield organic compounds containing nitrogen in the urine, and anaerobic fermentation of carbohydrates yields methane (Miller et al,1981). These authors consider that ignoring urinary nitrogen will

only overestimate heat production by 1% in mice, however, and as methane production will not be important, carbon dioxide and oxygen measurements are sufficient for mice.

Respiration calorimeters are of two types, (i) open circuit - which measure changes in the concentration of O2 and CO2 in a precisely measured airstream passing the animal, and (ii) closed circiut - which gravimetrically or volumetrically measure the O2 consumption and CO2 production of the animals in an air tight chamber. Miller et al (1981) consider closed circuit calorimeters to be inherently more accurate, due to the technical difficulties of precisely measuring gas concentrations (to an absolute accuracy of 5Ø ppm), as is required by open circuit calorimetry. Verification of these technical difficulties, and also the greater ease and accuracy of volumetrically and gravimetrically measuring O2 and CO2 is given by Boshowers and Nicaise (1981), measuring heat production in fasted and fully fed fowls. The fasting heat production of the A, P and F lines will therefore be measured using closed circuit respiration calorimetry techniques.

3.1.2 Materials and Methods

3.1.2.1 Source of Mice

The mice used in this study were sampled from generations 16 and 17 of the selection experiment. The calorimeter imposed the constraint of one heat determination per day, so only the H and L selected lines within each selection criterion were sampled, and within each line only male mice were used. Heat production was measured on pairs of mice at a young fast growing age (5-6 weeks) and again at adulthood (17 to 18 weeks of age). Between 7 and 8 pairs were sampled per line, and the total number of valid determinations was 256.

Heat production was measured on pairs of mice, rather than single mice, to avoid stress induced thermogenesis. Ahmed (1982) found that mice placed individually in a calorimeter show greater signs of stress than pairs of mice, and this increased heat production by up to 15%. Heat production was measured at two ages firstly so that the age effects on heat production could be compared with the age effects on

catabolism, and secondly so that an estimate of the repeatibility of separate determinations on the same mice could be made.

3.1.2.2 Design of Calorimeter

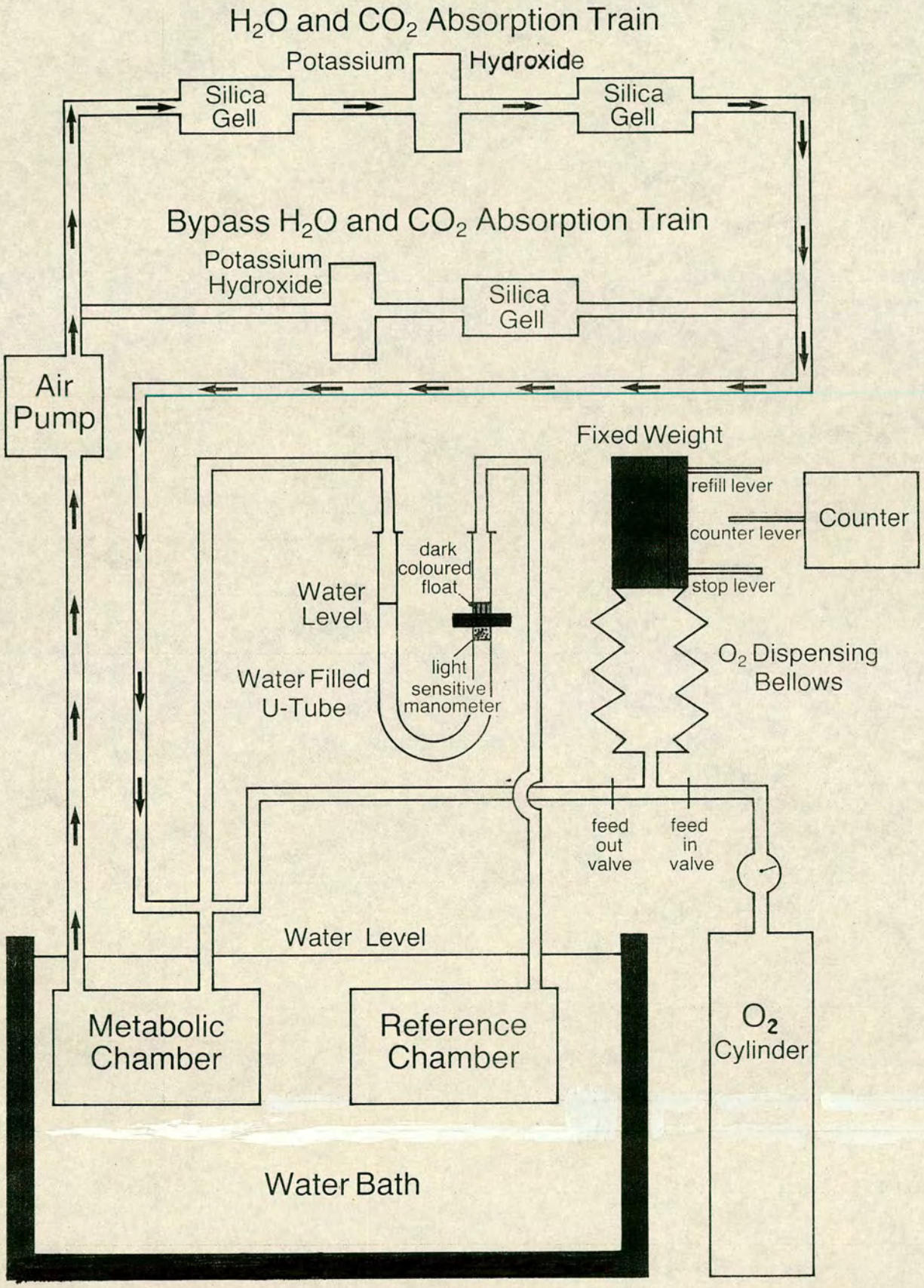
The calorimeter constructed was based on the design of the calorimeter described by Miller et al (1981). Fig. 3.1.1 shows the general design of the one used in this study, which was constructed by Mr.J.Ireland, with valuable help and advice also being given by Dr.M.K.Nielsen and Dr.D.Wilson.

Mice are placed in the metabolic chamber (a standard dessication bowl), which itself is placed in a water bath at a controlled temperature. Air is continuously pumped through the chamber and past the trains for absorption of CO2 and water. The bypass absorption train is used prior to the start of the measurement period, and during the measurement period the air is pumped through the main absorption train only. The first silica gel container, in the main absorption train, absorbs water produced by the mice, and the CO2 produced is trapped by bubbling the air through the potassium hydroxide. The second silica gell container absorbs water evaporated from the potassium hydroxide. This air is then returned to the metabolic chamber. Carbon dioxide output from the mice is measured by the weight gain of the potassium hydroxide and the second silica gell container.

As the mice consume oxygen inside the chamber, the barometric pressure of the metabolic chamber falls relative to that of the reference chamber, which is also submerged in the water bath. This pressure gradient forces the float in the U tube downwards, and thus the corresponding water level rises. Once the float drops below the level of the light sensitive trigger, the feedout valve(1) opens and oxygen is dispensed into the metabolic chamber until the pressures become balanced, at which stage the float will have risen again, deactivating the trigger and closing the valve.

As the bellows become emptied the refill lever depresses the counter lever , which closes the feedout valve and opens the feedin valve and thus allows the bellows to be refilled, by the pressure within the O2 cylinder. When the bellows are full the stop lever pushes the counter lever upwards again, closing the feedin

CALORIMETER DESIGN



valve . The counter records the number of refills of the bellows, and therefore the total volume of oxygen consumed can be estimated.

Traditionally, closed circuit respiration calorimeters have compared the barometric pressure of the metabolic chamber to ambient pressure, however this makes the system (and results) very sensitive to sudden changes in ambient temperature and pressure (Blaxter,1962). By incorporating a constant reference chamber, the calorimeter used in this study is independent of such effects.

3.1.2.3 Experimental Procedure

Heat production was measured for a period of 5 to 6 hours on the fasted mice, these mice having been without food for 24 hours by the end of the measurement period. H and L line pairs were tested on alternate days. The temperature of the water bath was set at 29 C, which is the same as or similar to the temperatures used by Miller et al (1981) and Ahmed (1982).

At the start of the experimental period the air was pumped through the bypass absorption train rather than the main train. The bypass train was used for approximately one hour, by which time the pressures and temperatures within the system were usually equalised, the mice resting and the system functioning smoothly. If this was the case, then the air flow was switched to the main absorption train and the oxygen counter set to zero.

Heat production was estimated using the equation recommended by Miller et al (1981) and Ahmed (1982) of:

$$\text{Heat(kJ)} = 16.17*VO2 + 5.\emptyset2*VCO2$$

where V is the volume of the gases in litres at standard temperature and pressure (Temp. = 25 C, Press. = 1 atmosphere).

Volume of O2 was estimated directly from the number of times the bellows were emptied. Volume of CO2 was estimated using the gas equation: PV = nRT, using the standard values, i.e. T = 25 C, P = 1 and R = .Ø82Ø5, and n was estimated from the CO2 weight gain in the absorption train.

After several weeks of use, however, the O2 dispensing system became unreliable, with malfunctions being common, although the CO2 absorption was not affected. Even after these technical problems were

rectified, however, the RQ values (VCO2/V O2) showed a slow systematic decline, indicating a very slow progressive leak in the O2 metering system. As a result of these factors, heat production was estimated from CO2 production alone, assuming a constant RQ of .72 for all mice. This figure was the mean RQ for both the AH and AL lines before the malfunctions started, and is in approximate agreement with the RQ (.70) suggested by Blaxter (1962) to represent a fasting state in an animal, i.e. digestion of ingested food completed, glycogen reserves exhausted, and all energy derived from triglyceride and protein stores within the body.

The constant RQ of .72 means that heat = 27.47*VCO2, and using the gas equation constants:

Heat= 15.2698*Weight of CO2.

Heat production was scaled by both metabolic body weight ($BW^{.75}$) and metabolic lean mass (lean mass$^{.75}$), so that comparisons could be made with the catabolism results in section 2. Lean mass (body weight - fat mass) was calculated by extrapolating the estimates of fat% made in section 2 to that expected after 16 and 17 generations of selection, respectively.

3.1.2.4 Statistical Analysis

Heat production per se, heat/$BW^{.75}$, heat/lean mass$^{.75}$, body weight and lean mass were analysed assuming the following statistical model:

$$Yijklmn = U + Ti + Dij + Rik + Al + Lijk + (DA)ijl + (RA)ikl + pijkm + eijklmn$$

where: Ti = ith selection criterion (A,F or P)
Dij = jth direction of selection (H,C or L) in the ith criterion
Rik = kth replicate in the ith criterion
Lijk = ijkth line
Al = lthage (young or old)
pijkm = mth pair in the ijkth line (random)
eijklmn= random error with the nth reading

3.1.3 Results

Table 3.1.1 shows individual line means for body weight, total heat production and heat scaled by metabolic body weight ($BW^{.75}$), for the sampled mice. Table 3.1.2 gives the overall means (pooled across replicates) for body weight, lean mass, total heat output, heat/$BW^{.75}$ and heat/metabolic lean mass (lean $mass^{.75}$), and the analyses of variance for these traits are presented in table 3.1.3.

Table 3.1.1 is presented so that the degree of line and replicate variation can be appreciated. The (across replicate) means, for each selection direction, calculated from table 3.1.1 are not always equivalent to those given in table 3.1.2, as these means were calculated assuming two different statistical models. It was necessary to analyse the data using two models, so that all the effects in table 3.1.3 could be calculated. The degree of discrepency between tables 3.1.1 and 3.1.2 will reflect the imbalance of the data.

The body weights of the sampled mice in general are in close agreement with the weights shown in section 1, the only exception being the adult F line mice. Many of these particular mice were 19 or 20 weeks old before the determinations could be made, however, and this was taken account of when lean masses were estimated. The replicate differences are not large, therefore these mice appear to be representative of their various lines. The H and L F line mice have very similar predicted lean masses both when young and old, after 17 generations of selection, thus indicating that their body weight differences may be caused by fatness differences alone.

The A and P lines show large and consistent H-L divergences in total heat production, although the A line divergence is reduced at 17 weeks of age. The F lines, however, have almost identical total heat production from their H and L lines, at both ages. The repeatability of the heat measurements is .50. This is the repeatability of the measurements at 5-6 weeks and 17-18 weeks of age, on each pair of mice.

For heat/$BW^{.75}$ the A lines show large and significant divergences at the young age (5 to 6 weeks), with the high intake mice producing more heat, but no difference at maturity. They therefore have a significant age by direction of selection effect, and it is also the A lines'

TABLE 3.1.1. Individual Line Means for Bodyweight, Total Heat Output and Heat/[1]Metabolic Bodyweight

Line select.dir.	repl.	[2]Young Bodyweight (g)	[2]Young Total Heat (kJ/pair/day)	[2]Young Heat/B.W.$^{.75}$ (kJ/kg$^{.75}$/day)	[3]Old Bodyweight (g)	[3]Old Total Heat (kJ/pair/day)	[3]Old Heat/B.W.$^{.75}$ (kJ/kg$^{.75}$/day)
AH	1	22.97	63.42	538.8	39.59	76.30	430.3
	2	23.60	57.76	480.3	43.20	74.57	392.2
	3	23.22	62.59	530.7	40.51	74.51	412.6
AL	1	20.37	47.59	441.2	34.30	64.24	403.1
	2	22.81	55.85	477.4	37.22	72.93	431.9
	3	19.01	46.69	455.4	33.34	58.90	376.8
PH	1	26.77	60.61	460.4	44.64	73.86	382.1
	2	27.01	62.18	464.3	42.12	67.34	363.6
	3	27.85	63.67	465.0	39.61	68.96	385.9
PL	1	18.88	46.73	458.5	26.71	59.29	448.0
	2	17.92	50.38	514.8	31.52	55.68	372.0
	3	20.39	49.35	455.7	32.01	56.32	371.6
FH	1	25.52	55.77	436.4	40.56	65.60	363.1
	2	26.21	56.94	437.3	44.12	74.78	388.8
	3	27.11	56.73	424.9	39.95	69.16	388.9
FL	1	24.61	57.86	469.7	34.28	68.69	432.8
	2	23.09	50.22	424.5	34.43	73.29	462.9
	3	25.71	55.16	432.1	35.40	68.60	419.2

1 Bodyweight$^{.75}$

2 5 to 6 weeks of age

3 17 to 18 weeks of age (up to 19 and 20 weeks in F replicate 1 and 2 respectively).

TABLE 3.1.2. Means and [1]Divergences of Bodyweight, Lean Mass, Total Heat Output and Heat scaled by [2]Metabolic Bodyweight and [3]Metabolic Lean Mass

		Lines					
	Age	AH	AL	PH	PL	FH	FL
Bodyweight (g)	[4]Young	24.22	21.81	26.86	18.40	25.72	23.81
	div.(%)	10.47		37.38		7.71	
	[5]Old	40.14	33.87	42.48	30.75	42.10	35.41
	div.(%)	16.94		32.04		17.26	
Lean Mass (g)	Young	22.69	19.98	24.50	16.63	22.27	22.13
	div.(%)	12.70		38.27		0.63	
	Old	35.00	28.57	35.97	26.06	32.15	33.16
	div.(%)	20.23		31.95		-3.09	
Total Heat Output (kJ/pair/day)	Young	62.70	50.77	59.83	46.72	57.49	55.54
	div.(%)	21.04		24.60		3.45	
	Old	73.66	64.62	72.18	59.18	68.82	69.02
	div.(%)	13.09		19.79		-0.30	
Heat/B.W.$^{.75}$ ($kJ/kg^{.75}$/ day)	Young	512.25	447.23	451.83	469.01	449.39	461.43
	div.(%)	13.55		-3.73		-2.64	
	Old	415.95	414.66	387.50	404.47	363.70	418.25
	div.(%)	0.31		-4.29		-13.95	
Heat/Lean Mass$^{.75}$ ($kJ/kg^{.75}$/ day)	Young	541.31	482.09	484.44	506.38	497.64	485.97
	div.(%)	11.57		-4.43		2.37	
	Old	459.96	468.47	438.35	457.51	449.27	439.37
	div.(%)	-1.83		-4.28		2.23	

[1] 2(H-L)/(H+L) * 100%; [2] Bodyweight$^{.75}$; [3] Lean mass$^{.75}$; [4] 5 to 6 weeks of age

[5] 17 to 18 weeks of age (up to 19 and 20 weeks, in F replicates 1 and 2).

TABLE 3.1.3. Analyses of [1]Variance for Bodyweight, Lean Mass, Total Heat Output and Heat scaled by Metabolic Bodyweight and Metabolic Lean Mass.

		Mean Squares				
Source	d.f.	Bodyweight	Lean Mass	Total Heat	Heat/B.W $^{.75}$	Heat/lean mass$^{.75}$
Criterion	2	114.00**	57.44*	308.87	14311*	9724*
A: H-L	1	401.01**	444.53**	2344.50**	23410*	13689*
P: H-L	1	2304.49**	1576.09**	3401.00**	5822	8434
F: H-L	1	423.78**	4.35	17.43	25420**	2665
Replicate	6	23.31	18.39	62.56	1196	1508
Line	6	27.64	19.35	[2]168.07(i)	[2]6804(ii)**	[2]8001(ii)**
Age	1	12602.14**	7035.04**	9918.42**	267575**	145294**
Age x A: H-L	1	79.79	74.04*	44.71	21717**	24532**
Age x P: H-L	1	54.13	21.04	0.06	0	39
Age x F: H-L	1	131.35*	7.63	24.60	103645*	18
Age x Repl.	6	12.74	8.44	131.14	5988*	7245*
Age x Line	6	28.95	34.87	64.02	4924*	7427**
Pairs	119	22.44**	25.43**	107.78**	2077*	2494*
Residual	105	4.75	3.34	37.53	1363	1636

[1] Pairs tested against residual, all other effects against pairs

** (Prob. < .01), * (Prob. < .05) otherwise Prob. > .05

[2] A line (Repl. x Dir.) comprises (i) 80% and (ii) 70% of total repeatability (σ^2 pair/σ^2 pair + σ^2 residual of measurements at young and old age.

[3] r(total heat) = .50 r(heat/BW$^{.75}$) = .22 r(heat/L.M.$^{.75}$) = .22

large replicate variation that causes the overall replicate by direction interaction to be significant. From table 3.1.1 it can be seen that in replicates 1 and 3 the A lines have large divergences when young, but in replicate 2 there is almost no divergence. This pattern is similar at maturity, but all divergences are reduced (hence a negative divergence for replicate 2). As would be expected from the heat and lean mass results, the A lines have a slightly reduced H-L divergence when heat is scaled by lean mass, and this results in the divergence at maturity actually being negative. The overall trends are the same as for heat/$BW^{.75}$, however.

To find an explanation for the A line replicate differences, the carcass composition data (section 2) were re-examined. No replicate data were accessible for the analyses at 26 and 44 days, and at 10 weeks of age (on generation 7 mice) the replicate differences were small, however at 17 weeks of age (generation 14 mice) there were large replicate differences. The AH replicate 2 mice were much fatter than the AH mean, and the AL replicate 2 mice were much leaner, with the H line mice being fatter than the L line mice. Heat/lean $mass^{.75}$ was then calculated individually for each of the A lines at 17 weeks, using body weight less fat mass, estimated from generation 14 fat%'s, as a very crude estimate of lean mass. These individual line fat%'s and heat/lean $mass^{.75}$ estimates, along with the overall estimates of heat/lean $mass^{.75}$, are shown in table 3.1.4.

The mean H-L divergence for heat/lean $mass^{.75}$ is not affected (the slight discrepency being due to the different methods of estimating lean mass), however the large line variation is reduced by using individual line estimates of lean mass. About half of the negative H-L divergence in replicate 2 has been removed.

The P lines show small divergences at both ages for heat scaled by $BW^{.75}$ and lean $mass^{.75}$, with the PH lines always having the lower values. However, although these trends are similar and very consistent for both traits, they are always nonsignificant.

The F lines show little divergence in heat production/$BW^{.75}$ at the young age, although the FH lines do have slightly lower values. At the older age, however, the fatter FH lines have a much lower heat/$BW^{.75}$ than the leaner FL lines. This can be seen to be due to the equal total heat production of the FH and FL lines, but the larger bodysizes

TABLE 3.1.4. Fat percentage (generation 14 mice) and Heat/Lean Mass$^{.75}$ for 17 Week Mice, A Lines

Replicate	Fat %		[1]Heat/individual line lean mass$^{.75}$		[2]Heat/overall lean mass$^{.75}$	
	AH	AL	AH	AL	AH	AL
1	9.93	16.36	465.69	460.82	473.39	453.28
2	14.88	10.13	442.56	467.93	431.69	485.72
3	10.62	12.23	448.83	415.53	454.08	423.74
Mean	11.81	12.91	452.36	448.09	453.05	454.25

[1] Lean mass calculated using the fat %s of generation 14 mice at 17 weeks of age, as shown in the table, for each line separately.

[2] Lean mass calculated from the overall regression of fat % on age.

TABLE 3.1.5. [1]Divergence in Catabolism and Fasting Heat Production in the Selected Lines

		[2]Catabolism (kJ/kg$^{.75}$/day)	Fasting Heat Production (kJ/kg$^{.75}$/day)
A lines	5 weeks	11 to 14%	13.55%
	17 weeks	3 to 13%	0.31%
P lines	5	0 to -7%	-3.73%
	17	-6 to -7%	-4.29%
F lines	5	-5 to 5%	-2.64%
	17	-9 to -12%	-13.95%

[1] Divergence = 2(H-L)/(H+L)

[2] Given is the approximate range of divergences (i.e. lowest and highest) in the period of one week surrounding the time of measurement.

of the FH line mice. When heat is scaled by lean mass, however, all H-L divergences disappear at both ages. In other words, mice from the fat (FH) and lean (FL) lines produce the same heat in relation to their lean mass.

In general, the P and F line and replicate effects are not large, or consistent with age (table 3.1.4).

Both heat/$BW^{.75}$ and heat/lean $mass^{.75}$ show an over all repeatability of .22.

3.1.4 Discussion

The major concern with these results is that they are based on carbon dioxide measurements only, and also the fact that the same RQ was assumed for all lines, at both ages. It is quite common, however, for metabolic rate to be calculated from carbon dioxide or oxygen measurements alone (e.g. Meltzer et al,1982; Pennycuik,1967); and when comparing the fasting heat production of chickens from lines selected for body size, intake and efficiency, Pym and Farrell (1977) found all lines to have the same mean RQ. Carbon dioxide production alone, therefore, most probably is sufficient for finding differences between lines in heat production (although it would obviously be preferable to have oxygen measurements as well). In addition, the range of values obtained for heat production were within the range of published estimates of heat production for mice (Blaxter,1962; Kownacki et al 1975; Miller et al,1981), with the overall mean being 432.97kJ/$kg^{.75}$/day. Finally, the repeatability of the heat measurements (.50 for heat output per se, and .22 for heat/$BW^{.75}$) indicate that the calorimeter is able to distinguish between mice, especially as these repeat measurements were 12 weeks apart.

The outstanding feature of the results as a whole is the closeness with which they agree with the catabolism results. Table 3.1.5 gives a comparison of the catabolism and fasting heat production results, and it can be seen that the same trends with direction of selection and age are apparent, and moreover often the divergences are of a similar magnitude.

The A lines' large and significant divergence in heat production at 5 to 6 weeks of age agree closely with catabolism results, and thus

provide further evidence of changes in their maintenance requirements. The A lines do show considerable replicate variation, however, and it is this variation that is the cause of the significant (pooled) line effects. A lot of this variation at 17 weeks may be simply be due to carcass composition differences, but the data are not sufficient to make inferences about the variation at 5 to 6 weeks of age. Even after taking account of carcass composition, however, the replicate 2 results still differ from the replicate 1 and 3 results.

Intake/$BW^{.75}$ and catabolism/$BW^{.75}$, for the A lines (section 2), do not show these replicate differences. However, as catabolism was calculated assuming the same regressions of fat% on age for all three lines within each selection direction, divergences in catabolism may have been artificially created in replicate 2 - by undercorrecting for fat deposition towards maturity in the AH line, and overcorrecting in the AL line. The overall divergences would not have been affected, however, as the replicate 1 and 3 divergences would have been reduced to the same extent as the replicate 2 divergence was increased. It appears, therefore, that selection in replicate 2 has had a lesser effect on maintenance, but a greater effect on intake above maintenance (especially energy used for fat deposition) than selection in replicates 1 and 3. Reasons why the A lines show this replicate variation will be explored in section 5.

As observed for intake and catabolism, the P lines show no significant divergence in heat production scaled by either metabolic body weight or metabolic lean mass, although once again the PH lines always have slightly lower values. These small differences are consistent with age, however. The P line results support the proposal in section 2 that the changes in the intake ratios of the PH and PL lines may be caused in part by small changes in maintenance, despite the fact that the estimates of these changes in maintenance requirements can not be shown to be statistically significant.

The heat production results for the F lines are in almost complete agreement with the catabolism results, insofar as the large heat production differences which exist between lines when heat is scaled by metabolic body weight disappear almost entirely when expressed in relation to estimated lean mass. These results rule out the possibility of the F line differences being due to differences in

activity (i.e. the fatter mice being less active), as all mice tended to spend the whole measurement period sleeping. The method of estimating lean mass in section 2 indicated equal lean masses for the FH and FL lines in generation 14, and after extrapolating this to 17 generations of selection, equal lean masses are still predicted for the two groups of lines. Although there is no experimental verification of this prediction, the equal total heat output, and heat/lean mass$^{.75}$, of the FH and FL lines do indicate that maintenance is a function of lean mass in the F lines, as suggested in section 2.

In summary, this experiment appears to have given reliable estimates of heat production, and the changes in heat production caused by selection are, in general, consistent with the changes in catabolism. The conclusions made about maintenance requirements in section 2 therefore appear to be correct, as the heat production results verify the catabolism results. These results also indicate that changes in factors such as the work of digestion component of maintenance, do not need to be invoked to explain the observed changes in maintenance requirements.

The hypothesis about maintenance, and heat production, being proportional to lean mass is adequate to explain the F and P line results, but only part of the changes observed in the A lines. In the review of literature it was suggested that factors affecting maintenance requirements may include the rate of protein turnover and amount and activity of brown adipose tissue, with an environmental factor of importance being the thermal environment and the animals' adaptability to it (i.e. temperature by heat production by direction of selection interactions in this example). Although brown adipose tissue differences may affect both fasting heat production and the work of digestion, it is the effects it may have on fasting heat production that may be more relevant in this study. Brown adipose tissue and temperature adaptation effects will be investigated in sections 3.2 and 3.3, respectively, as possible causes of the still unexplained changes in the maintenance requirements of the A lines. Protein turnover in these lines is currently being studied by other workers. Activity differences have been ruled out as a major contributer to these heat production differences, as all mice appeared to sleep for most of the duration of their measurement period.

3.2 BROWN ADIPOSE TISSUE IN THE A LINES

3.2.1 Introduction

Differences between lines in estimated maintenance requirements were found in section 2, and in section 3.1 verification of these findings was given by the fasting heat production results. The trends observed in the P and F lines appear to be satisfactorily explained by the correlated changes in carcass composition, however the A lines show H-L divergences in both estimated maintenance requirements and fasting heat production which are not accounted for by carcass composition changes.

This section investigates the hypothesis that these observed metabolic differences in the A lines may be associated with changes in the quantity of the thermogenically active tissue - brown adipose tissue (BAT), and its lipid free dry matter active component (LFD). BAT is an important thermogenic tissue in young rodents, and in a study of cold adapted mice Sulzbach and Lynch (1984) found an additive genetic correlation of .73 $\pm$ .30 between LFD/body weight (BW) and O2 consumption/BW, on 7 week old mice - where O2 consumption is a measure of basal metabolic rate. The same authors also found correlations of 1.00 $\pm$ .41 and .63 $\pm$ .56 between LFD/BW and food consumption, and in a study of mice selected for increased and decreased 6 week weight, Lynch and Roberts (1984) found that differences in food consumption/BW were closely paralleled by differences in LFD/BW.

BAT differs from white adipose tissue by its larger number of mitochondria and lower lipid content. The mitochondria of BAT have a unique protein that enables large amounts of heat production (Cannon et al, 1982, in Saxton and Eisen,1984), and this is important in the "non-shivering" thermogenesis of animals adapted to cold environments (Lindberg, 1970). Rothwell and Stock (1979) claim that the quantity or activity of BAT is also important in a phenomenon known as "diet-induced thermogenesis", which is simply a large increase in heat output observed in animals (rats in this example) with excessive energy intakes. Diet induced thermogenesis may be thought of as an extension of the concept known as the "heat increment of feeding" or work of digestion component of heat production (A.J.F.Webster,1983).

As mentioned in section 3.1, however, this role for BAT may not be relevent in this study, as the fasting heat production results agree quite well with the catabolism results and thus there is no discrepancy to be explained.

It can be seen that the hypothesis used in this study considers BAT in a different role from that usually given to it, in that BAT is being investigated as a component of normal metabolism, and not as a mechanism responding to external stimuli. In section 3.3 temperature adaptation effects are studied, and if BAT diffences are found their importance may be for these adaptive purposes, however, rather than merely being a means of burning off excess intake.

A small scale study of the BAT and LFD contents of 6 week old mice from the A lines (replicates 1 and 3, only) has already indicated that small H-L divergences in LFD/BW may exist, with the AH lines having the greater quantities (N.M.Shukri, unpublished). Caution may need to be expressed when interpreting these results, however, as the mice studied had been under a prolong ed period of restricted feeding. A larger study under ad libitum feeding conditions was therefore felt to be necessary.

This study will look at the BAT and LFD contents of the A line mice at both a young fast growing age (4 weeks), and at an older age (13 weeks), so that comparisons can be made with the catabolism and fasting heat production results. Although caution should be used when considering the weight of a tissue (i.e. LFD) as a measure of its ability to perform a biochemical function, the use of LFD as an estimate of thermogenic ability is supported by several authors (e.g. Chaffee and Roberts,1971; Rothwell et al,1982 and Lynch and Sulzbach, 1984).

3.2.2 Materials and Methods

The mice used in this study were sampled from generation 20 of the selection experiment. Between 3 and 6 full sib families were chosen from each of the 9 A lines, giving a total of 40 families and 385 individuals (111 H, 126 C and 148 L line mice). From each family one half of the individuals were slaughtered and dissected at 4 weeks of age, with the remainder being slaughtered and dissected at 13 weeks of

age.

The BAT depot studied was the interscapular depot, which was dissected after the mice had been weighed and then killed by ether. The LFD content of the BAT was estimated after extracting the lipid content of the BAT by ether for 72 hours, and then drying the samples at 90 C for 6 hours. This technique was recommended by Carol B. Lynch (pers. comm.). The gonadal fat pad (GFP) was also dissected in the 13 week old mice, simply to monitor the carcass composition changes in the A lines. All the dissections were performed by Miss Frances Thompson.

Body weight (BW), BAT, LFD, BAT/BW and LFD/BW were analysed assuming the following statistical model:

$$Yijklmn = u + Ri + Dj + Ak + Sl + Lij + (RA)ik + (RS)il + (DA)jk + (DS)jl + (AS)kl + (LA)ijk + (LS)ijl + fijm + eijklmn$$

where: Ri = ith replicate
Dj = jth direction of selection (H, C or L)
Ak = kth age (4 or 13 weeks)
Sl = lth sex
Lij = ijth line
fijm = mth family in the ijth line
eijklmn = nth individual

The GFP data was analysed using the same model, except that the age effect and age interactions were not fitted. Only the data collected on 13 week old mice for the other traits were analysed simultaneously with the GFP data.

3.2.3 Results

The means of the analysed traits are shown in table 3.2.1 and the corresponding analyses of variance are shown in table 3.2.2.

The body weight means are in agreement with the A line body weight means in section 2, with the exception of the slightly larger (H-L) divergence at 4 weeks of age. The A lines as a whole show small divergences in 4 week weight in some of the later generations of selection (i.e. after about generation 13) (S. King,pers. comm.), so

Table 3.2.1 Line Direction of Selection and Sex Means for the Analysed Traits.

	Body Weight (BW) (g)					
	4 Weeks			13 Weeks		
replicate	H	C	L	H	C	L
1	17.00	16.92	13.25	36.38	34.05	30.33
2	17.15	15.58	15.83	36.31	31.69	32.55
3	16.26	12.89	13.29	39.42	31.86	26.92
μ	16.49	15.13	14.04	37.78	32.62	29.93
	M = 15.37	F = 15.07		M = 36.81	F = 30.07	
	Brown Adipose Tissue (BAT) (mg)					
1	71.74	60.74	59.96	117.07	92.20	110.63
2	67.82	66.42	63.62	104.69	81.37	104.11
3	58.11	56.85	55.61	110.31	94.22	81.42
μ	64.94	61.41	59.15	112.48	90.03	99.13
	M = 60.72	F = 62.94		M = 117.02	F = 84.07	
	Lipid Free Dry Content of the BAT (LFD) (mg)					
1	9.76	7.96	7.97	15.15	11.99	13.99
2	9.29	8.98	8.94	14.48	11.23	13.28
3	8.99	7.66	7.05	15.06	10.76	10.90
μ	9.34	8.19	7.95	15.07	11.30	12.81
	M = 8.32	F = 8.66		M = 13.98	F = 12.15	
	BAT/BW (mg/g)					
1	4.25	3.58	4.50	3.28	2.72	3.60
2	4.01	4.31	4.03	2.88	2.53	3.18
3	3.62	4.49	4.12	2.77	2.93	3.01
μ	3.96	4.11	4.22	2.99	2.76	3.27
	M = 4.00	F = 4.19		M = 3.20	F = 2.81	

(Continued)

M = Males F = Females

Table 3.2.1 (Continued)

LFD/BW (mg/g)

	4 Weeks			13 Weeks		
replicate	H	C	L	H	C	L
1	.585	.477	.600	.428	.354	.464
2	.562	.582	.568	.398	.352	.413
3	.568	.611	.534	.386	.340	.406
μ	.574	.554	.569	.403	.350	.429
	M = .553	F = .578		M = .382	F = .406	

Gonadal Fat Pad Weight (GFPW) (mg)

GFPW/BW (mg/g)

	13 Weeks			13 Weeks		
replicate	H	C	L	H	C	L
1	573.1	534.9	542.8	15.41	15.85	17.68
2	547.6	431.2	430.5	14.87	13.49	13.21
3	711.5	654.9	443.8	17.57	20.30	16.53
μ	610.7	540.3	472.4	15.95	16.54	15.80
	M = 610.6	F = 471.7		M = 16.51	F = 15.69	

M = Males F = Females

Table 3.2.2 Analyses of Variance for the Analysed Traits

		Mean Squares			
Component	d.f.	BW	BAT	LFD	BAT/BW
H-L(4 weeks)	1	178.93	999.3	57.60*	2.015
Symmetry	1	.68	16.8	8.60	.017
Line	4	38.58	154.4	4.26	4.259
H-L(13 weeks)	1	1802.54*	5213.2	149.40*	2.293
Symmetry	1	55.64	9077.3	245.23*	4.994
Line	4	107.36	2215.5	15.25	1.239
Replicate(overall)	2	74.80	3019.1	46.19	1.057
Age	1	30073.23**	135747.7**	1889.51**	107.261**
Sex	1	1082.78*	20658.2**	48.56*	.809
Line	4	109.42†	1384.1	15.19	4.775**
R x A	2	5.87	727.4	19.06*	.941
R x S	2	32.16*	42.2	1.24	.210
D x A	2	228.04*	2651.5	52.70**	1.621
D x S	2	16.46	848.7	8.83	.950
A x S	1	965.38**	28728.1**	109.41**	7.775**
L x A	4	29.26**	827.7**	0.00	.691
L x S	4	3.88	210.5	14.60*	0.00
Family	39	49.98**	1072.1**	13.78**	.758**
Residual	318	6.15	242.2	5.04	.362

Tests are: H-L and Symmetry contrasts against Line (at same age), Replicate against (overall) Line, Age against Replicate by Age, Sex against Replicate by Sex, (overall) Line against Family, Replicate by Age and Direction by Age against (overall) Line by Age, Replicate by Sex and Direction by Sex against (overall) Line by Sex, and Sex by Age, (overall) Line by Age, (overall) Line by Sex and Family against Residual

Symmetry = (H+L)/2-C

** P < .01, * P < .05, † P < .1, otherwise P > .1.

(Continued)

Table 3.2.2 (Continued)

Component	d.f.	Mean Squares		
		LFD/BW	GFPW	GFPW/BW
H-L (4 weeks)	1	.00075	-	-
Symmetry	1	.01272	-	-
Line	4	.06165	-	-
H-L (17 weeks)	1	[1].01977	559486	.69
Symmetry	1	.15889**	57	16.13
Line	4	.00555	114356	64.35
Replicate (overall)	2	.00385	228239	234.50
Age	1	2.6780*	-	-
Sex	1	.0537†	829481**	28.68
Line	4	.0499*	114336	64.35
R x A	2	.0419	-	-
R x S	2	.0045	2116	16.01
D x A	2	.0338	-	-
D x S	2	.0098	85393†	56.17†
A x S	1	.0000	-	-
L x A	4	.0144	-	-
L x S	4	.0082	0	0.00
Family	39	.0157**	89091.4**	55.38**
Residual	318	.00728	28938 (df=140)	20.53 (df=140)

[1]Both the H-C ($P < .05$) and L-C ($P < .01$) contrasts are significant.

these mice are representative of the selected lines.

The BAT weights have not changed greatly with selection, although the larger AH lines do have slightly larger quantities. The (H-L) divergences in LFD are relatively slightly larger, however, with the AH lines once again having the greater quantities. There is also significant asymmetry of the response in LFD to selection for food intake, at 13 weeks, with both the AH and AL lines having greater quantities of LFD than the AC lines.

The large DxA, SxA and LxA effects (table 3.2.2) for BAT and LFD tend to reduce or disappear when BAT and LFD are expressed in relation to BW. In addition, neither BAT/BW nor LFD/BW show any (H-L) divergence at either age, although there is considerable asymmetry of response in LFD/BW at 13 weeks, with the AC lines again having much lower values than the H and L selected lines. Both BAT/BW and LFD/BW show a reduction with age, and there is a tendency for females to have higher values than males.

The gonadal fat pad results are also shown in table 3.2.2. When expressed in relation to BW (i.e. GFPW/BW), there are no large or significant differences between the H, C and L A lines.

The phenotypic correlations between each of the traits measured, along with (within family) full sib correlations for each trait, are shown in table 3.2.3. Two times this full sib correlation gives an upward biased estimate of heritability (h^2). This bias consists of common environment effects post-weaning, as well as maternal effects, because mice of the same family and sex were housed in the same cages.

From the full sib correlations BW, BAT, GFPW, and GFPW/BW appear to have a much larger genetic component than BAT/BW, LFD and LFD/BW. In terms of correlations between traits, the more highly inherited traits are in general quite strongly correlated with each other, whereas the correlations of these traits with LFD and LFD/BW are generally much smaller. Most notable is the zero correlation between LFD/BW and the GFPW traits.

Finally, two aspects of the statistical analysis of this data are important. Firstly, traits such as BW and BAT often show increasing variation as their mean increases, and therefore the data must be transformed to give homogeneity of variance, so as to ensure a valid analysis of variance. For example, Lynch and Roberts (1984) log

Table 3.2.3 Phenotypic Correlations (off diagonal) and Full Sib [1] Correlations (on diagonal) of the Analysed Traits

	BW	BAT	LFD	BAT/BW	LFD/BW	GFPW	GFPW/BW
BW	.482 ±.069	.465	.247	-.074	-.275	.488	.263
BAT		.309 ±.067	.597	.752	.386	.526	.459
LFD			.184 ±.058	.477	.751	.131	.143
BAT/BW				.125 ±.051	.701	.334	.370
LFD/BW					.131 ±.052	-.006	.070
GFPW						.348 ±.069	.954
GFPW/BW							.303 ±.067

[1] Full sib correlation = $\frac{1}{2} h^2$ + maternal and common environment bias + dominance effects

transformed their BW and LFD data prior to analysing it. In this study, however, residual standard errors were homogenous at 4 and 13 weeks of age for all traits, so no transformation was necessary. Secondly, the replicate and direction of selection effects have been tested against the line effect in this analysis. Although this gives a correct test against genetic drift effects, it is a very weak test as there are only 4 df in the denominator. Ignoring genetic drift, these effects may have been tested against family, which with 39 d.f. enables a much more powerful test. When this was done, however, there were no important changes in the significance levels, and the conclusions made from this data set remained unchanged. In this example, therefore, the weakness of the test against the line effect does not matter.

3.2.4 Discussion

The GFPW results will be dealt with first, prior to discussing the BAT results. Estimates of the relative fatness of the A lines at 13 weeks of age, after 20 generations of selection, were made by way of the GFP dissections. The AH, AC and AL lines do not differ significantly from each other in GFPW, and thus differences in fatness do not appear to exist. This is contrary to the findings in section 2 of the AH mice being leaner than the AC and AL lines, but it does fit in with the observation that the carcass composition changes have not increased greatly as the generations of selection have proceded. This will be discussed further in section 5.

Interestingly the replicate differences which were thought to exist (section 3.1), with the replicate 2 AH mice being considerably fatter than the replicate 2 AL mice, are not apparent in these results. This discrepency may be due to either sampling effects (in either study) or the possibility that GFPW/BW is not indicative of overall fatness. It is not possible to tell which factor is responsible.

Finally, from the full sib correlations, GFPW and GFPW/BW both appear to have large genetic components (as has already been demonstrated in the P and F lines), and as expected, they are quite strongly correlated with BW.

The BAT and LFD results will now be discussed. For this study the

hypothesis was made that the observed H-L divergences in maintenance requirements and fasting heat production may be associated with corresponding changes in the quantity or activity of BAT. The results, however, do not support this proposition. LFD/BW is used as the indicator of the relative ability of an animal to dissipate heat through BAT activity, and the differences between the AH and AL lines in this trait are small and non-significant at both ages. BAT does not therefore appear to be an important factor in the metabolic differences between the AH and AL lines.

This conclusion is confounded somewhat, however, by the significant asymmetry shown by LFD/BW at 13 weeks age, where the AC lines have significantly lower LFD/BW than both the AH and AL lines. The reasons for this result are not clear, although they would not appear to be related to metabolic differences as (i) relationships between LFD/BW and metabolism were not demonstrated for the AH and AL lines, and (ii) no indications of asmmetry of this direction and magnitude were observed in the catabolism results.

The possibility of these results being explicable by abnormal BAT activity in the AL lines alone, with a true relationship existing with the AH and AC lines, can not yet be ruled out, however the apparent lack of relationship between LFD/BW and metabolism is also reflected in the observed replicate differences. As discussed in section 3.1, replicate 2 appears to differ from replicates 1 and 3 in that its H-L divergences in metabolism are much smaller, perhaps even being negative as the animals mature. This is not true for the results given in this section, where the LFD trends seen in replicate 2 are no different from those of replicates 1 and 3. The conclusion made above is therefore most probably correct.

These results do not confirm the suggestion from N.M.Shukri's (unpublished) study that the AH lines may have a greater LFD/BW than the AL lines. The pooled means for LFD/BW in Shukri's study were .76, .63 and .64 (mg/g), respectively, for the AH, AC and AL lines. It is not clear, however, whether these H-L or H-C differences are significant or not. The mice dissected in this study had undergone 3 weeks of restricted feeding immediately prior to their slaughter at 6 weeks of age, and as quantity and activity of BAT appears to be very responsive to external stimuli such as restricted or induced

overfeeding (Rothwell and Stock,1979), these results may have been affected by the restriction on food intake. Interestingly a small, albeit lesser, degree of asymmetry is also apparent in these results.

It may be argued that if LFD is to be considered in a metabolic or heat production context, then it should perhaps be scaled by metabolic body weight ($BW^{.75}$) rather than BW. A regression of the logarithm of LFD on the logarithm of BW was undertaken to test this proposition, and the overall regression coefficient was .671 $\pm$ .047. This is not significantly different from .75 and thus $BW^{.75}$ may well be a more appropiate scaling factor than BW. The mean $LFD/BW^{.75}$ values were then calculated to be 1.141 and 1.094 $mg/g^{.75}$ for the AH and AL lines, respectively, at 4 weeks of age, and .989 and 1.001 $mg/g^{.75}$ for the AH and AL lines, respectively, at 17 weeks of age. Therefore, when LFD is considered in this "metabolic" context the AH and AL lines still do not appear to differ, although much of the age effect does disappear.

The traits of LFD, LFD/BW and BAT/BW all have much lower full sib correlations, and hence lower heritabilities, than BW, BAT and the GFPW traits (BAT per se is largely comprised of fat, as is demonstrated by its large correlation with GFPW, hence its higher heritability than LFD). In studies of random bred mice, Lacy and Lynch (1979) and Saxton and Eisen (1984) estimated the heritability of LFD/BW to be .08 and .06, respectively, and by using diallel crosses of mouse strains Lynch and Sulzbach (1984) also found LFD/BW to have a very small additive genetic variance. Lacy and Lynch also estimated a full sib correlation of .08 for LFD/BW, which is of a similar magnitude to the correlation of .13 estimated in this study. These results are all in agreement, and LFD/BW is therefore a trait which is probably only lowly heritable.

The phenotypic correlations show LFD and LFD/BW to be generally only weakly correlated with BW and the GFPW traits, and in particular, LFD/BW has a zero correlation with GFPW and GFPW/BW. This result is in agreement with the findings of Saxton and Eisen (1984) who studied fatness and LFD/BW in random bred mice. Saxton and Eisen were testing the hypothesis that LFD/BW (i.e. relative ability to dissipate energy through BAT) and fatness would be negatively correlated (after Rothwell and Stock,1979), however they concluded that this was not true. The results from this study simply confirm the conclusions of

Saxton and Eisen (1984).

The findings of high correlations between LFD/BW and O2 consumption, and LFD/BW and food intake (Sulzbach and Lynch,1984) appear to conflict with the findings of this study. The mice used in the Sulzbach and Lynch study had been acclimated to 4 C, however, and they were also tested at 4 C. This temperature effect may account for their result, as BAT appears to be a tissue which responds to such external stimuli as cold temperatures, due to its presumed role in non-shivering thermogenesis (i.e. heat production to maintain body temperature) in cold environments. In contrast to the Sulzbach and Lynch (1984) studies, Saxton and Eisen (1984) in their study of mice at 22 C found LFD/BW and food consumption to be almost totally uncorrelated at 6 weeks of age. All the mice studied in this thesis were maintained at 22C. The results described here therefore agree with the findings of Saxton and Eisen (1984).

In summary, the changes observed in the food intake and metabolism in the A lines are not associated with changes in the component of BAT which defines its relative thermogenic ability, i.e. LFD/BW, or even $LFD/BW^{.75}$. In addition, not only does LFD/BW not appear to be a very highly heritable trait, at the environmental temperatures in which the mice were studied, but LFD/BW also does not appear to be correlated with food intake.

3.3 TEMPERATURE EFFECTS ON FASTING HEAT PRODUCTION IN THE A LINES

3.3.1 Introduction

The catabolism and fasting heat production results for the A, P and F lines have been discussed in detail in sections 2 and 3.1, and the hypothesis that these two traits vary in proportion to lean mass is adequate to explain the P and F line results. The A lines, however, show H-L divergences in these traits over and above that accounted for by carcass composition changes, and the aim of this section is to test a hypothesis suggested in section 3.1 to explain this result, namely that the differences observed are a function of temperature adaptation effects.

Outside of a narrow temperature range known as the thermoneutral zone, 30 to 33 C for mice (quoted by Ahmed,1982), the heat output of an animal rises, for thermoregulatory purposes. Pennycuik (1967) found that the thermoneutral zone is quite clearly defined, and similar, for mice of a wide range of genetic backgrounds, and in her study heat production was minimum at 32 to 33 C. This increased heat production of course translates itself into increased food consumption at all temperatures below the thermoneutral zone, as demonstrated by Bateman and Slee (1979), for mice. The A, P and F line mice were all selected at 22 $\pm$ 2 C, and as this is considerably below the thermoneutral zone for mice it is possible that temperature adaptation effects have influenced selection, especially in the A lines. The question which may be asked, therefore, is whether or not selection for increased food intake has to some degree selected mice poorly adapted to this temperature (i.e. mice with increased heat production at 22 C compared to unselected mice), and conversely, has selection for decreased food intake selected mice which are well adapted to this relatively cool temperature.

Temperature adaptation effects, such as those proposed here, appear to have been observed in a study comparing mice selected for large (H) and small (L) body sizes (McCarthy,1984). The basal heat productions of mice from the two sets of lines were measured from 2 to 8 weeks of age, and large temperature by heat production interactions were observed. When measurements were taken at 15 C the L line mice had a

greater heat production in relation to bodyweight (i.e. heat/BW) than the H line mice, as may be expected, but at 32 C this difference disappeared. The hypothesis is therefore made that the H-L differences in heat production and maintenance requirements observed in the A lines, at temperatures below the thermoneutral zone, may similarly disappear or be reduced if the measurements are made within the thermoneutral zone. In other words, the heat production differences are hypothesized as being caused in part by the environmental stress of living at 22 C (with the AH lines producing more heat at this temperature). Therefore, with the removal of this stress it is possible that some of these heat production differences may disappear. This section aims to test this hypothesis, by measuring the fasting heat production of the A line mice at two temperatures, a "cool" ambient temperature (25 C) and a thermoneutral temperature (33 C).

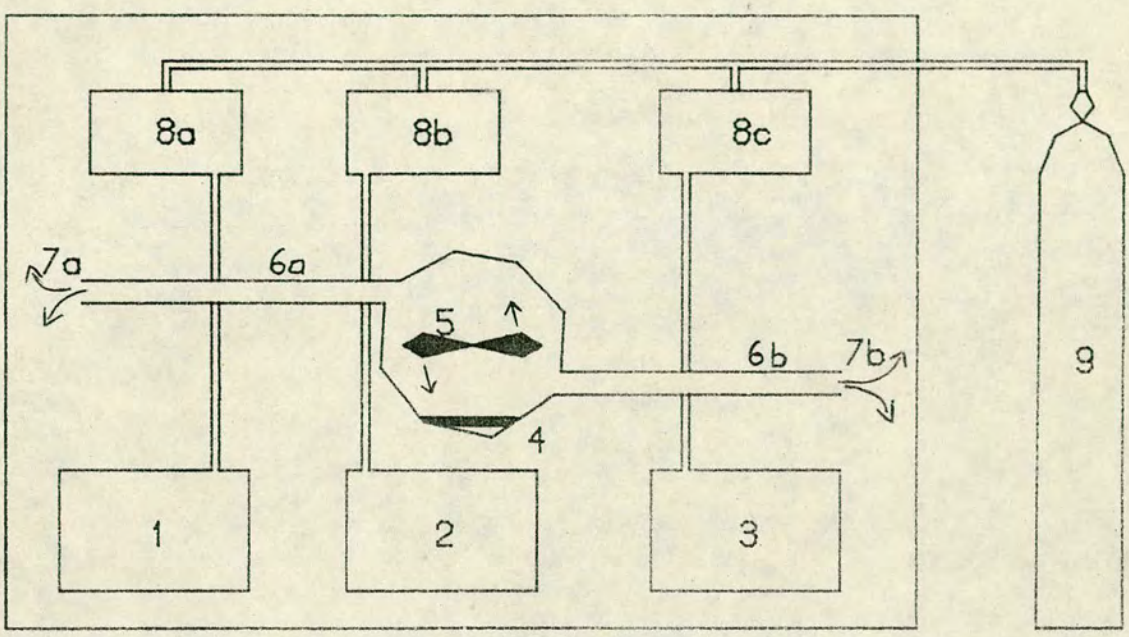
3.3.2 Materials and Methods

The mice used in this study were obtained from generation 21 of the selection experiment. From each of the 9 A lines, sixteen full sib pairs of mice (8 female pairs and 8 male pairs) were sampled for testing. Fasting heat production was then measured on each pair at 5 weeks of age and again at 17 weeks of age. Measurements were taken at both ages so that the results from this study could be compared with the results outlined in section 3.1. A total of 254 valid determinations were made.

Heat production was measured in the same way as was described in section 3.1, namely by using indirect calorimetry techniques. For this study, however, the calorimeter was modified such that the water bath was replaced by a wooden cabinet which enclosed the whole apparatus. Within this cabinet there were three metabolic chambers, as opposed to the single metabolic chamber of the previous study, and this enabled simultaneous comparisons of the H, C and L line mice. A simple diagram of the structure of the calorimeter is shown in fig. 3.3.1.

Each chamber functioned as an autonomous unit, and therefore was able to be run individually or along with the other two chambers. Situated in the centre of the cabinet was an air heater for temperature regulation and, as with the water bath calorimeter, quite

Fig. 3.3.1 Calorimeter Cabinet Layout



KEY

1, 2 and 3: Metabolic Chambers
4: Air Heater
5: Fan
6a and 6b: Air Conducting Unit (hollow piping)
7a and 7b: Air Flow
8a, 8b and 8c: Measurement Apparatuses (each unit is the same as that shown in Fig. 3.1.1)
9: Oxygen Source

precise temperature control was possible - to within ± Ø.25 C. The fan and air conducting unit (hollow piping) ensured that warm air was distributed to the extremeties of the cabinet, to avoid temperature gradients across the cabinet. The measurement apparatuses, i.e. the CO2 and water absorption trains, the reference chambers and the O2 feedin units, were the same as those used in the water bath calorimeter. In addition, the experimental procedures and the calculations used to estimate heat production in this study were the same as those outlined in section 3.1.

The ambient temperature of the room housing the calorimeter was 25 C, and thus this was the "cool" temperature in which the mice were tested. The thermoneutral temperature was 33 C, as mentioned above. At each age one half of the pairs of mice were tested at 25 C, and the other half at 33 C. It was not possible to give the mice time to acclimatise to the temperatures of 25 C and 33 C before recording their heat production. This should not matter, however, as Batemen and Slee (1979) studying mice at a variety of temperatures ranging from 1 C to 3Ø C, found the metabolic rate of mice was the same whether the animals had spent a few hours in the given environment, or up to 8Ø hours of continuous exposure.

The pairs tested at one temperature when young were then tested at the other temperature when old, to avoid a confounding of temperature and pair effects. The three selection directions (i.e. H, C and L) were rotated around the chambers, and pairs were never tested twice in the same chamber, to avoid confounding of the selection direction and chamber effects and the pair and chamber effects, respectively. Finally, sexes were also split equally between the treatments.

The fasting heat production estimates, expressed as heat/$BW^{.75}$, were analysed assuming the following statistical model:

$$Yijklmnop = U + Ri + Dj + Sk + Al + Tm + Cn + \text{all two way interactions between these main effects} + pijo + eijklmnop$$

where: Ri = ith replicate
Dj = jth direction of selection (H, C or L)
Sk = kth sex
Al = lth age (young or old)

Tm = mth temperature (25 or 33 C)
Cn = nth metabolic chamber
pijo = oth pair in the ijth line (random)
eijklmnop = random error with the pth reading

The DxT interaction is of course the effect of the greatest interest. If the hypothesis is correct, then this interaction should be significant.

3.3.3 Results

The mean fasting heat production values for each of the fixed effect classes and for the important two way interaction subclasses are shown in table 3.3.1. The corresponding analysis of variance is presented in table 3.3.2.

As expected, the A lines show a significant overall divergence ((H-L/C) in heat production, of 6.30%. Although the overall replicate means do not differ, there is considerable replicate variation in the observed divergences, i.e. replicates 1 and 3 appear to have clear H-L divergences in heat production, whereas no such divergence is apparent in replicate 2. This is in agreement with the results outlined in section 3.1. Although these replicate effects cause the line effect to approach significance at the 5% level, the overall H-L divergence is significant even when tested against the line effect (Prob. $\leq$.1).

The H-L divergence appears to be consistent with age, with no age by direction interactions being apparent. In addition, even though the overall mean heat production of the 17 week mice is less than that of the 5 week mice, this difference is neither large nor significant.

The temperature of the metabolic chamber in which the mice were tested has had a clear effect on heat production, with mice from all lines producing more heat at the "cool" temperature (25 C) than at the thermoneutral temperature (33 C). The aim of this experiment, however, was to test selection direction by temperature interactions for fasting heat production, with the hypothesis being that a larger H-L divergence would exist at 25 C than at 33 C. It appears that no such interactions exist, and the trends actually appear to be in the opposite direction - i.e. the AL lines show signs of being more responsive to temperature change than the AH lines.

Table 3.3.1 Fasting Heat Production Means ($kJ/kg^{.75}/day$) for the Fixed Effects and the Important Interactions

		Replicate 1	2	3	$\bar{x}$	Overall Divergence (100*(H-L)/C)
Direction	H	439.1	417.2	431.5	429.2	6.30%
	C	397.7	420.0	400.5	406.0	
	L	404.0	417.7	389.3	403.7	
	$\bar{x}$	413.6	418.3	407.1	413.0	

		Direction H	C	L	$\bar{x}$	Divergence
Age	5 weeks	428.1	413.0	411.2	417.6	4.11%
	17 weeks	429.5	398.8	395.8	408.4	8.46%

		H	C	L	$\bar{x}$	Divergence
Temperature	33°C	418.0	393.3	384.1	398.4	8.62%
	25°C	440.5	418.8	423.3	427.5	4.10%
	Difference (25°C-33°C)	22.54	25.48	39.27	29.09	

		H	C	L	$\bar{x}$	Divergence
Chamber	1	445.4	398.2	382.0	408.5	15.92%
	2	421.6	414.1	404.3	413.3	4.19%
	3	420.7	405.9	424.8	417.1	-1.00%

Temperature

		33°C	25°C	Difference (33°C-25°C)
Chamber	1	406.0	411.0	4.94
	2	388.2	438.5	50.31
	3	401.1	433.2	32.04

		33°C	25°C	Difference (33°C-25°C
Age	5 weeks	408.8	426.3	17.50
	17 weeks	388.0	428.7	40.68

Sex Males = 413.1
Females = 412.9

Table 3.3.2 [1,2]Analysis of Variance for Heat Production

Source	d.f.	MS	F
Replicate	2	2494.8	1.094
Dir.:H-L	1	26369.0	11.568**
Dir.:Symmetry	1	5982.3	2.625
Sex	1	2.5	.001
Age	1	5167.3	2.267
Temperature	1	51187.5	22.457**
Chamber	2	1512.1	.663
Line (Repl. x Dir.)	4	5031.8	2.208†
Repl. x Age	2	20845.6	9.145**
Dir. x Age	2	1741.0	.764
Dir. x Temp.	2	1603.7	.704
Dir. x Chamber	4	8103.7	3.555**
Age x Temp.	1	8032.7	3.524†
Temp. x Chamber	2	10414.4	4.569*
All other interactions	16	1915.1	.848
Pairs	129	2279.4	1.939**
Residual	82	1175.7	

[1] Pairs tested against residual, all other effects against pairs.
** (Prob. < .01), * (Prob. < .05), † (Prob. < .1) otherwise Prob. < .1.

[2] All the interactions not included had F values less than 1.0.

[3] Symmetry contrast = (H+L)/2-C

Repeatability (σ^2 pair/σ^2 pair + σ^2 residual) = .255

There is little difference between the overall chamber means, however the direction by chamber effect appears to be significant. It can be seen that the H line mice have their highest heat production in chamber 1, and their lowest in chamber 3, whereas the L line mice show the reverse trend. There are also interesting temperature by chamber effects, with mice housed in chamber 1 appearing to be less responsive to temperature change than those in chambers 2 and 3. A three way interaction of direction by temperature by chamber was suggested by these results, however after investigation it was found to be not significant. Finally, in terms of temperature effects, there are indications that the older mice are more responsive to temperature change than the younger mice, as they show the greater heat production differences between the two temperatures.

The last of the fixed effects studied, sex, appears to have no effect on heat production. When expressed in relation to metabolic body weight ($BW^{.75}$), the heat production means for males and females can be seen to be almost identical.

Finally, the repeatability of the heat output measurements of the mice was .255 in this study. This is the correlation between the determinations on the individual pairs at 5 and 17 weeks of age. All pairs were housed in different chambers and tested at different temperatures at the two ages.

3.3.4 Discussion

Before discussing the temperature effects it is necessary to ask whether or not these results, and the overall trends observed, are consistent and comparable with those of the previous calorimetry work.

Firstly, consider the absolute heat production values. The overall mean heat production obtained in this study, using the new calorimeter, is slightly lower than that obtained in the previous study (413.Ø vs 433.Ø $kJ/kg^{.75}$/day), however the range of values obtained in the two studies were similar. The repeatabilities of the measurements were also similar (.22 in the former study and .26 in this study), so it may be assumed that the results from the two studies are comparable. In addition, the fact that mice of both sexes were tested in this study whereas only male mice were tested in the

previous study can be seen to be of no importance, as both sexes have equal mean heat outputs and there were no important sex interactions.

Secondly, consider the direction of selection, and replicate effects. Although symmetric direction of selection effects have been found, the nature of the divergences differ from those of section 3.1. The divergence when young, 4.11%, is considerably smaller than that previously found, viz. 13.55%, and at 17 weeks of age a divergence of 8.46% is observed in this study whereas no such divergence was apparent in section 3.1. The 17 week divergence in this study actually agrees more closely with the catabolism results than the previous finding did, however, and therefore it probably is a true result. The discrepencies at 5 weeks of age may be due to sampling effects, or simply to the fact that 5 generations of selection separates the two studies - the nature of the H-L divergence may well have changed over this period. In contrast with the actual values of the divergences, however, the replicate trends are very similar to those previously observed. In both studies the largest divergence was in replicate 1, with replicate 3 having a marginally smaller divergence, whereas no divergence exists in either study for replicate 2. The results from the two studies do, therefore, appear to be comparable.

Thirdly, it can be seen that the overall age effect is not significant in this study, whereas in section 3.1 the older mice produced substancially less heat than the younger mice. Upon closer observation, however, the age by temperature interaction can be seen to be significant, and although there is no effect at 25 C, an age effect does appear to exist at 33 C. Thus at the warmer temperature age does appear to have had an effect on heat output, as was observed at 29 C in section 3.1, whereas at 25 C it does not.

The hypothesis tested was that temperature by direction of selection effects would exist, with the H-L divergences being smaller at 33 C than at 25 C. This proposed phenomenon has clearly not happened, and the hypothesis must therefore be rejected. The observed phenomenon of McCarthy's large and small mice responding differently to temperature changes therefore does not appear to apply to the A lines.

A procedural question to be answered is whether or not the difference between 25 C and 33 C was adequate to test the hypothesis, especially as McCarthy made his observations at 15 C and 32 C. It must

be remembered, however, that although cooler temperatures may have resulted in larger temperature effects on heat production, the hypothesis was specifically comparing 22 C to a thermoneutral temperature. Although the cool temperature was 25 C not 22 C in this experiment, the difference between these temperatures is not great and any interactions, if they exist, should still be apparent.

It may also be asked as to whether a comparison of the initial heat production results at 29 C with the catabolism results at 22 C could have answered the hypothesis, before undertaking the experiment. This comparison could not have distinguished temperature adaptation effects from general discrepencies between fasting heat production and catabolism, however, so the present experiment was necessary. In retrospect the results from this experiment may actually make the conclusions drawn in section 3.1 about the similarities between fasting heat production and catabolism stronger, as temperature effects can now be seen to not be important.

Finally, there are the direction by chamber, temperature by chamber and age by temperature effects to consider. The temperature by chamber effect, where mice in chamber 1 were less responsive to the different temperatures than mice in the other chambers, can most probably be explained by a temperature gradient within the calorimeter cabinet. In other words, this chamber probably did not experience the full 8 C difference between the temperatures, despite the efforts made in the design of the calorimeter to avoid such temperature gradient effects. The observation that the H, C and L line mice responded differently to the different chambers is curious, however, especially given the fact that there were no direction by temperature interactions. The distribution of the selection directions across the chambers was very well balanced, and no explanation can be given for this phenomenon. Lastly, older mice were slightly more responsive to temperature change than younger mice. This could perhaps be explained by a number of physiological or behavioural factors, e.g. lower activity, better huddling ability, etc.

In summary, the hypothesis was made that the H-L divergences in heat production in the A lines were temperature dependent, and that they would be smaller in the thermoneutral zone than at temperatures similar to those in which the selection was conducted. This was not

found to be true, however, with no temperature by heat production interactions occurring. This lack of temperature interaction may actually strengthen the comparisons between catabolism and fasting heat production made in section 3.1, as catabolism was measured at 22 C and fasting heat production at 29 C. In addition, the section 3.1 results were obtained on male mice only, and thus they are further strengthened by the finding that there are no sex differences in fasting heat production.

Section IV ENERGY PARTITION STUDY

4.1 INTRODUCTION

In the metabolic framework within which these studies have been made, an animal's metabolisable energy intake is defined as being used firstly to meet the animal's maintenance requirements, with the energy intake in excess of this requirement (net energy) being available for growth, i.e. fat and lean deposition. In section 2 a general study was undertaken on the A, P and F line mice to determine some of the inter-relationships of these components of growth, and the changes in these relationships with selection, and in section 3 aspects of the maintenance requirements of these mice were studied further. The effects of selection on the maintenance component of growth have therefore been studied, but relatively little is known yet about the effects of selection on the usage of energy in excess of maintenance in the A, P and F lines. In other words, little is known about the effects that selection has had on the partition of net energy between fat and lean deposition. The aim of this section is to investigate some of these effects. Aspects of energy usage for maintenance have been studied, and now aspects of the usage of energy in excess of maintenance will be studied.

In general, as intake in excess of maintenance increases, the proportion of this energy being deposited as fat also increases (section 1). Although the exact nature of this relationship is not known, if indeed an exact relationship exists (Blaxter,1962; C.T.Whittemore, pers. comm.), this relationship (i.e. the partition of energy between lean and fat deposition) is important for two reasons. Firstly, the means of improving lean growth or efficiency in domestic animals is dependent to a large extent on the nature of this partition, as well as the ways that these partition patterns may change with selection. Secondly, determining the optimum food allocations for animals such as pigs depends on the relative effects that restrictions on intake have on fat and lean deposition, i.e. once again the partition of energy.

At one extreme of the possible relationships, the proportions of each increment of net energy deposited as fat and lean may be constant at any level of intake, above the level at which at which fat deposition commences (young animals fed at or only slightly above

maintenance will mobilise fat in order to deposit lean tissue - Blaxter,1962). This relationship would be suggested from studies on Japanese Quail (Farrell et al,1982). With this model, selection to change carcass composition may simply change the relative proportions of energy deposited as fat and lean at any given level of intake, i.e. the partition of net energy between fat and lean above the level of intake at which fat deposition commences. Comparisons of genetically obese and lean rats (Pullar and Webster,1974 and 1977) show that these animals differ in their partition of energy between lean and fat deposition at all levels of intake, and thus their differences fit this model.

At the other extreme, the proportion of energy deposited as fat may remain relatively small with increasing intake, until a maximum rate of lean deposition is reached, after which stage all subsequent energy intake is deposited as fat, until ad libitum intake is reached. With this model, selection to change carcass composition may conceivably merely change the level of intake in excess of the level at which maximum lean deposition occurs, with no true partitioning changes occuring. Selection for decreased fatness (along with increased gain and efficiency) in pigs has has been effective more by reducing voluntary food intake than by changing energy partition (Henderson et al,1983), showing that this model may be partially correct for pigs.

For mice the patterns of energy partition, and how they respond to selection, are not generally known. The aim of this section is to attempt to provide some answers to this problem area. The A lines have been useful models for studying maintenance requirement differences, the P lines are good models for studying growth rate differences, and now the F lines are excellent models for studying energy partition.

The FH and FL lines are lines which differ greatly in carcass composition and fat content, yet they appear to have the same lean masses throughout life. Moreover, they appear to have the same total maintenance requirements (and hence the same maintenance requirements in relation to lean mass) at all ages, despite having different food intakes during their fast growing phases. The hypothesis can therefore be made that the FH and FL lines differ only in their energy intake in excess of that required for maintenance and total lean deposition - the FH lines are fat because they have large surplus intakes and the

FL lines are fat because they have a small surplus intake. In other words, their carcass composition differences are not caused by energy partition differences, but simply by differences in energy intake above maintenance. If this were true, small decreases in the intake of the FH lines would not affect their lean growth, it would merely decrease their rate of fat accretion.

This section investigates this hypothesis, i.e. that the differences between the FH and FL lines are due to differences in intake in excess of maintenance requirements, and that no true changes in energy partition have occurred. This hypothesis will be tested by means of a restricted feeding experiment in which mice from the FH and FL lines will be allocated rations such that both groups of mice have the same intake in excess of estimated maintenance.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Methodology

The hypothesis tested was that the differences in fatness between the FH and FL lines are due to intake differences alone. As mentioned above, this hypothesis was tested simply by feeding a sample of FL and FH line mice such that they had the same intake in excess of their estimated maintenance requirements. At the completion of the feeding period carcass fatness was determined on all mice, and the individual fat and lean gains during the experimental period were estimated.

If the findings in sections 2 and 3.1 were correct, then the FH and FL lines should start the experiment with equal lean masses and maintenance requirements. Moreover, if the hypothesis was correct, then the FH and FL lines should have gained equal amounts of fat and lean tissue during the course of the experiment, and they should have also have been allocated equal quantities of food. Since the FH and FL lines would start the experiment with differing body sizes and fat contents, however, they would be expected to finish the experiment with differing body sizes and fat contents.

Underlying this restricted feeding technique there is an alternative and perhaps more powerful means of analysing energy partition, as although this experiment aims to treat all mice identically,

individual mice will inevitably undergo differing degrees of restriction. If these individual levels of restriction can be estimated, then it is possible to compare the energy used to deposit fat with the total energy available for growth, and thus the patterns of energy partition can be seen. This experiment was designed such that these individual levels of restriction could be estimated, and thus regressions of energy used to deposit fat versus energy in excess of maintenance could be calculated, for the FH and FL lines.

4.2.2 Experimental Design

The period chosen for the experiment was 26 to 44 days of age. This period was chosen firstly because 26 and 44 days age were ages at which carcass composition determinations had already been made (section 2), and secondly because this time interval represents a period of fast growth. The experimental mice were sampled from generation 18 of the selection experiment. From each of the 3 FH and 3 FL lines 8 full sib families were sampled, and within each family 2 males and 2 females were chosen at random for the experiment, giving a total of 192 mice.

At 26 days of age each mouse was individually housed in a feeding cage where it remained for the duration of the experiment. Individual food rations for the mice were calculated from the data presented in section 2, according to individual estimated lean mass. The FL mice were fed 95% of their predicted ad libitum intake (to avoid food refusals), and each FH mouse was offered a ration equivalent to that which would have been given to an FL mouse of the same estimated lean mass. Each mouse was weighed on days 1, 7 and 13 of the experiment, and its subsequent 6 daily food allocations were estimated according to its line, sex and estimated lean mass. The mice were offered the same diet as in all the other studies described here, the Beta Diets Rat and Mouse No.1 Expanded Maintenance Diet (crude protein = 14.8%, estimated metabolisable energy content = 10.636 kJ/g).

The growth rates of the FL mice in the first replicate were slower than anticipated, indicating that the food requirements of the mice had been underestimated. For the second replicate, therefore, all rations were increased by 5%. Food requirements still appeared to be

underestimated, however, so all rations were increased by a further 5% for the third replicate.

At 44 days of age each mouse was slaughtered and freeze dried to estimate individual dry matter (DM) contents. Fat content, estimated by standard soxhlet extraction techniques, was determined on 36 bulked samples of minced freeze dried mice of the same line and sex.

4.2.3 Experimental Analyses

4.2.3.1 Dry matter % as a predictor of fat %

It was proposed to use DM% as an predictor of fat% for the individual mice, as (i) results already obtained indicated that DM% was a good predictor of fat%, and (ii) it was not feasible to determine the individual fat contents of all 192 mice. In order to determine the validity of DM% as a predictor of fat% under these experimental conditions, analyses of variance were undertaken on the bulked fat%'s and the mean DM%'s of the constituent mice within each sample. In addition, both within line and across line regressions of fat% on DM% were calculated. The analyses of variance are presented in table 4.1a and the regression coefficients, along with the corresponding correlations between fat% and DM%, are shown in table 4.1b. These results are presented here simply because they are necessary for the derivation of many of the traits of interest.

From the analyses in table 4.1a it can be seen that, with the exception of sex, both fat% and DM% are affected to a very similar degree by the factors included in the analysis, and the residual mean squares of these two traits are also of a similar magnitude. The regression coefficients and correlations presented in table 4.1b indicate a very close linear relationship between fat% and DM%. No significant direction of selection or line effects were observed in the within group regression analyses, i.e. the regression coefficients were homogeneous for all selection directions and lines. None of the regressions differed significantly from the overall regression. It was concluded, therefore, that DM% was a reliable predictor of fat% under these experimental conditions. The individual 44 day fat%'s required for the analyses of the results from this study were therefore

Table 4.1a Analyses of Variance of the Bulked Fat and Dry Matter Samples

		Fat %		DM %	
Component	d.f.	MS	F	MS	F
H-L	1	32.51	77.87**	23.10	77.16**
Replicate	2	7.37	17.65**	4.02	13.42**
Sex	1	6.39	15.31**	0.66	2.21
H-L x Repl.	2	3.69	8.84**	4.21	14.05**
H-L x Sex	1	8.40	20.12**	5.52	18.44**
Replic. x Sex	2	0.04	0.10	0.04	0.13
Residual	26	0.42		0.30	
	μ	6.28		31.11	
	C.V.	10.29%		1.76%	
	R^2	0.87		0.86	

** P < .01, otherwise P > .1. All effects tested against residual.

Table 4.1b Regression Coefficients of Fat % on Dry Matter %

	Regression	Correlation
Within selection direction	.929 ± .121	.898
Within line	.798 ± .169	.898
Across selection direction	1.188	
Across line	1.069 ± .242	.911
Overall	1.109 ± .090	.901

calculated using the overall regression, viz 1.109 x DM% - 28.232. This regression coefficient of 1.109 is in agreement with the generally accepted one to (negative) one relationship between fat% and water% in a carcass (Sutherland et al,1974).

4.2.3.2 Derivation and analyses of measured traits

As described above, the following traits were measured on each individual: weights at 26, 32, 38 and 44 days of age, food intake and DM% at 44 days of age. In addition, 44 day fat% for each mouse was estimated using the equation described in 4.2.3.1, viz 1.109xDM%-28.232, and thus individual 44 day lean mass (body weight - fat mass) could also be calculated.

To calculate overall fat and lean gain, individual 26 day fat mass and lean mass were also required, however. These two traits were estimated assuming mean fat%'s of 10.37% and 5.75% for the FH and FL lines, respectively. These fat%'s were obtained by extrapolating the figures obtained from the regressions of fat% on age for generation 14 mice (section 2) to those expected after 18 generations of selection, assuming a linear change in fat% with generation of selection. Lean mass was once again estimated as body weight less fat mass. This method realised estimates of 26 day lean mass of 13.094 and 12.964g for the FH and FL lines, respectively. These estimates are in line with the prediction of equivalent lean mass for the FH and FL line mice. From these results fat and lean gain over the duration of the experiment were estimated for each mouse.

In addition to these traits, the estimated maintenance requirements of each individual mouse were calculated, and these estimates are defined here as catabolism. Catabolism (metabolisable energy (ME) intake less the energy costs of fat and protein deposition) was calculated using the same assumptions as were made in section 2, i.e. the ME content of the diet = 10.636 kJ/g and the costs of fat and protein deposition are 53.4 and 52.9 kJ/g, respectively. Protein mass was estimated from lean mass assuming that protein comprised a constant 19% of lean mass at 26 days of age, and 19.5% at 44 days. These values were also estimated from the composition data presented in section 2.

This catabolism calculation was complicated, however, by the observation that many mice appeared to show net mobilisation of fat and/or protein over the duration of the experiment. The efficiency with which tissue is spared from mobilisation as energy intake below maintenance increases is slightly greater than the efficiency of tissue deposition above maintenance (Blaxter,1962), and from results presented by Blaxter (1962) these efficiencies (below maintenance) were estimated as .5 and .8 kJ/kJ of protein and fat tissue, respectively (compared to efficiencies .444 and .735 kJ/kJ tissue for the deposition of fat and protein). These efficiencies indicate that 47 and 49 kJ are required to spare 1g of protein and 1g of fat from degradation, respectively (at submaintenance intakes). These rather crude assumptions were tested by altering the assumed efficiencies in the calculations, and the assumptions were found to be very robust, with large variations in the assumed efficiencies not affecting the conclusions drawn from the study.

These measured and derived traits were analysed assuming the following statistical model:

$$Yijklm = U + Ri + Dj + Lij + Sk + (RS)ik + (DS)jk + fijkl + eijklm$$

where: Ri = ith replicate
Dj = jth direction of selection (H or L)
Lij = ijth line
Sk = kth sex
fijl = lth family in the ijth line
eijklm = mth individual in the lth family

4.2.3.3 Regression analyses

As outlined in 4.2.3.1 the experiment was also analysed using regression analyses. For each mouse an estimate of fat gain over the experimental period was available, as well as estimates of total maintenance requirements (or catabolism) and food intake. It was possible, therefore, to calculate individual intakes in excess of maintenance, and the fat deposited could thus be compared with and regressed upon energy intake above maintenance (i.e. net energy). Between replicate homogeneity (as had been observed for the F lines in

the study outlined in section 2) was assumed for this study, and the replicate effects were not included in the regression analyses. Because of the different levels of restriction employed for each of the replicates, not including the replicate effects obviously made the regression analyses much more powerful as it allowed far greater variation in the independent variable (intake in excess of maintenance).

For these analyses, the efficiencies of deposition of tissue and the efficiency with which tissue is spared from mobilisation were assumed to be equivalent (viz. 52.9 and 53.4 kJ/g of protein and fat, respectively), to make the FH and FL regression slopes comparable. This was necessary because the FH mice were more severely restricted than the FL mice, and many FH mice appeared to show a net mobilisation of fat over the course of the experiment. As mentioned above, however, it was found that assuming equivalent or slightly different efficiencies of tissue deposition above and below maintenance made little difference to the conclusions drawn from this study.

In addition, regressions of efficiency on intake in excess of maintenance were calculated in order to estimate the relative importance of intake in excess of maintenance versus type of tissue deposited, in determining efficiency.

The regression analyses was performed assuming the following statisticalmodel:

$$Yijkl = U + Di + bi(Xijkl - \overrightarrow{Xi}...) + Sj + (DS)ij + fik + eijkl$$

where: Yijkl = fat deposited by, or efficiency of, the lth individual
Xijkl = intake above maintenance for the lth individual (kJ)
Di = ith direction of selection (H or L)
Sj = jth sex
fik = kth family in the ith direction of selection
eijkl = lth individual in the family

4.3 RESULTS AND DISCUSSION

4.3.1 Measured and Derived Traits

The mean values of the measured and derived traits are shown in

table 4.2, and the corresponding analyses of variance are presented in table 4.3. Catabolism is presented firstly as a percentage of total intake (100 x catabolism/intake), this being 100 times the inverse of the intake ratio and, secondly, scaled by the average individual body weight (BW) and lean mass (LM) maintained over the experimental period, raised to the power .75.

The hypothesis tested predicted equal means for the FH and FL lines for the following traits: estimated inital lean mass, food intake, weight gain and estimated lean and fat gains, efficiency, catabolism/intake% and catabolism/$LM^{.75}$. Only the traits of initial BW, final BW, estimated final fat% and catabolism/$BW^{.75}$ would be expected to differ slightly (perhaps non-significantly) between the FH and FL lines. It can be seen, however, that with the exception of initial BW (and hence lean mass) all traits differ significantly between the FH and FL lines. This is in addition to the large replicate differences which exist for many of the traits because of the increasing food allocations with the second and third replicates.

The cause of these H-L differences can be seen in the catabolism results. The ability of this experiment to test the hypothesis is dependent on the catabolism/intake values being the same, or similar, for both the FH and the FL lines - as the aim was to give all mice equivalent intakes in excess of maintenance. This objective has not been realised, however, as the FH lines appear to have been subjected to much harsher dietary restrictions. This result suggests that the H-L differences observed in the experiment result from an inadequacy of the experimental design, i.e. miscalculated food allocations, rather than an inadequacy in the hypothesis. A close examination of the food allocated over the first 6 day period revealed that the FH mice were offered only 93% of the quantity that the FL mice were, despite the fact that they should have been allocated equal quantities due to their equal (estimated) initial lean mass. This initial underestimate of food requirements will have reduced subsequent weight gain in the FH lines and hence compounded the underfeeding.

In terms of the actual values obtained for catabolism in the experiment, two points are of interest. Firstly, the FH lines have significantly lower values for both catabolism/$BW^{.75}$ and catabolism/$LM^{.75}$ than the FL lines, despite the fact that both groups

Table 4.2 Line and Overall Means of the Measured and Derived Traits

	Initial BW(g)		Food Intake (g)		Weight Gain (g)	
Replicate	H	L	H	L	H	L
1	14.38	12.88	51.93	58.97	1.809	3.038
2	14.43	13.97	54.52	65.71	2.369	5.727
3	15.01	14.41	61.36	72.68	4.732	7.991
mean	14.61	13.75	56.04	65.79	2.970	5.585
Estimated Initial Lean Mass =	13.09	12.94				

	Efficiency (g.gain/g.food)		Final BW(g)		Estimated Lean Gain (g)	
Replicate	H	L	H	L	H	L
1	.035	.050	16.19	15.92	2.267	2.936
2	.044	.086	16.80	19.70	2.396	5.449
3	.078	.110	19.75	22.40	4.957	7.553
mean	.052	.082	17.58	19.34	3.207	5.313

	Estimated Fat Gain (g)		Estimated Final Fat %		Catabolism/Intake % (kJ/kJ)	
Replicate	H	L	H	L	H	L
1	-0.458	0.102	6.191	5.230	99.32	93.94
2	-0.027	0.278	8.623	5.352	95.27	89.49
3	-0.226	0.437	6.651	5.539	93.25	86.46
mean	-0.237	0.272	7.155	5.374	95.95	90.00

	Catabolism/$BW^{.75}$ ($kJ/Kg^{.75}$/day)		Catabolism/$LM^{.75}$ ($kJ/Kg^{.75}$/day)	
Replicate	H	L	H	L
1	702.7	791.2	748.9	825.2
2	699.9	746.9	753.8	779.3
3	708.0	747.3	755.2	780.3
mean	703.6	761.8	752.7	795.0

Table 4.3 Analyses of Variance for the Measured and Derived Traits

Source	d.f.	Mean Squares			
		Initial BW	Food Intake	Weight Gain	Efficiency
H-L	1	33.86	4411.1**	317.24**	.0422**
Replicate	2	17.65	2059.6**	238.85**	.0403**
Line	2	4.84	84.1	21.95*	.0028
Sex	1	20.38*	1.1	33.92	.0058
(H-L) x Sex	1	3.31	110.0	49.05	.0078
Replicate x Sex	2	0.84	25.0	21.56**	.0042**
Family	41	17.54**	149.6**	4.44**	.0012**
Residual	135	1.28	13.7	2.08	.0004

		Final BW	Estimated Lean Gain	Estimated Fat Gain	Estimated Final Fat %
H-L	1	143.81*	205.75**	12.011**	119.66**
Replicate	2	385.18**	209.06**	1.758**	21.79**
Line	2	47.32	24.43**	.538†	21.32**
Sex	1	106.88	34.94	.008	3.64 (1)
(H-L) x Sex	1	77.83	29.90	2.348*	29.53**
Replicate x Sex	2	30.02**	19.22**	.076	.11
Family	41	21.56**	3.55**	.181**	3.75**
Residual	135	3.60	1.69	.085	1.34

(1) Replicate x Sex mean square is a poor test. Tested against residual sex is non-significant.

		100 x Catabolism/ Food Intake	Catabolism/ $BW^{.75}$	Catabolism/ $LM^{.75}$
H-L	1	1656.83**	157410**	82967**
Replicate	2	708.23**	9228*	8054†
Line	2	7.73	10646*	13158**
Sex	1	36.41	85303	97589
(H-L) x Sex	1	325.29†	38336	33334
Replicate x Sex	2	58.95**	10076**	11380**
Family	41	26.64**	2736**	2754**
Residual	135	10.72	1253	1251

Tests are: H-L, Replicate and Line against Family, Sex and (H-L) x Sex against Replicate x Sex, and Family and Replicate x Sex against residual.

** $P < .01$, * $P < .05$, † $P < .1$, otherwise $P > .1$

were expected to have equal mean values for catabolism/LM$^{.75}$ (sections 2 and 3.1). These observed H-L catabolism differences are probably merely a reflection of the relative levels of restriction, however, as the FH lines underwent much harsher restrictions than the FL lines, and continued dietary restrictions reduce basal heat production (Blaxter,1962). Secondly, the absolute catabolism values for these mice are approximately 10% greater than the catabolism values estimated in section 2. This increase may be due to the fact that the mice in this study were housed singly, whereas the mice in the study outlined in section 2 were housed in pairs. Mice housed singly can undergo stress induced thermogenesis (Ahmed,1982) which increases their maintenance requirements, and they also lack the opportunity to huddle for warmth. In both studies the mice were housed at 22 C. Since the food allocations were estimated assuming that maintenance requirements would be the same as the catabolism values calculated in section 2, these apparent increased maintenance requirements account for the underestimated food requirements in replicates 1 and 2.

As mentioned above, there are large H-L differences in food intake, weight gain (with the experimental design gain and intake are mutually dependent) and efficiency. In section 1.3.2.3 of the literature review an attempt was made to explain how an increase in intake may lead to a disproportionate increase in efficiency (in a mouse), as intake in excess of maintenance will be probably be increased to a far greater extent than will be the intake used for maintenance itself. These results demonstrate this phenomenon: food intake increased by 18% and 23% between replicates 1 and 3 in the FH and FL lines, respectively, and these increases in intake lead to respective increases in efficiency (gain/food) of 123% and 120%.

The last group of traits, the carcass composition traits, merely highlight the inadequacies described above. The FH lines were expected to deposit at least as much fat during the experimental period as the FL lines, however they show a net loss of fat in addition to their decreased lean gain. These results do, however, demonstrate the concept outlined in the introduction that fat will not be deposited until a certain level of intake in excess of maintenance is reached, and moreover, that under some circumstances fat may be mobilised in order to continue lean growth. Many mice gained weight even though

they were fed rations over the course of the experiment which, in retrospect, were estimated to be below their maintenance requirements. Despite their apparent fat loss, however, the FH lines remained fatter than the FL lines as a result of the large differences in initial fatness - estimated to be 1Ø.37% (FH) vs 5.75% (FL).

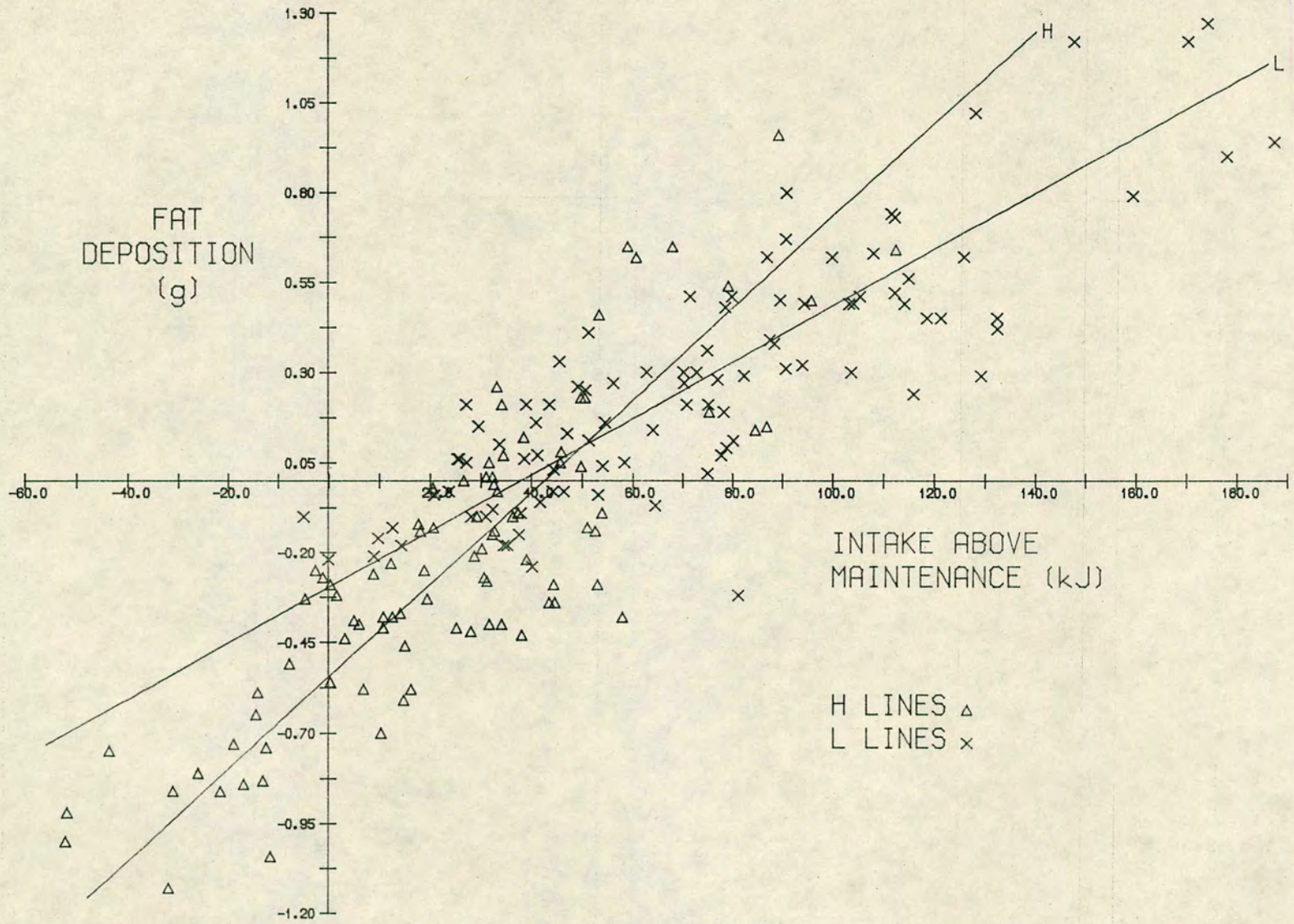
Finally, the method of calculating rations according to estimated lean mass can now be analysed and criticised. This method unfortunately appears to have made the design of the experiment somewhat inflexible, as once "unexpected" carcass composition changes occurred, food allocations would have become inconsistent with the aims of the experiment. This phenomenon will have occurred with the FH lines when the initial underestimate of food requirements caused the mice to become leaner than expected - whereupon subsequent food requirements would once again have been underestimated.

In summary, this experiment has been unable to address the specific hypothesis made, due to an inadequacy of the experimental design. Not only were the food rations underestimated for the experimental as a whole, and underestimated for the FH lines relative to the FL lines, but the method of allocating food rations according to estimated lean mass does not appear to have been flexible enough to take account of the observed changes in carcass composition. The data collected is, however, still suitable for a more general comparison of fat deposition with level of restriction, as is outlined in section 4.3.2. Despite these mentioned limitations, three important phenomena have been observed from these results, so far. Firstly, the mice appeared to grow even at submaintenance intake levels, by apparently mobilising fat and depositing lean. Secondly, overall maintenance requirements were higher than expected, and this may have been due to housing the mice singly, as opposed to pairs in the experiment where the previous estimates were made. Thirdly, the severe restrictions on the FH lines appear to have reduced their catabolism levels relative to those of the FL lines.

4.3.2 Regression Analyses

The regressions of fat gain on intake in excess of catabolism, or maintenance, are shown in fig. 4.1. These regressions are termed the

Fig. 4.1 FAT DEPOSITION vs INTAKE ABOVE MAINTENANCE



"observed" fat regressions, in order to distinguish them from theoretically derived "partition only" regressions described below (N.B. the fat gain values used for the "observed" fat regressions were themselves estimated, rather than "observed"). These "observed" regression coefficients, along with the values of intake in excess of maintenance at the X axis intercepts, the "partition only" regression coefficients and the regression coefficients of efficiency on intake in excess of maintenance are shown in table 4.4. The derivations and meanings of the "partition only" regression coefficients are described below.

With the observed fat regressions, both the slopes and the intercepts of the regression lines with the X axis are of interest. The X axis intercept indicates the estimated amount of energy deposited as lean before fat deposition occurs, and the regression slope then indicates the partition of energy between subsequent lean and fat growth. It can be seen from fig. 4.1 that both the FH and FL lines appear to have almost identical X axis intercepts, yet they have significantly different regression slopes. It was estimated that this "obligatory" lean deposition, before fat deposition commences, comprises approximately 40% of the estimated lean growth on ad libitum intake over this time period, for both the FH and FL lines. The regression slopes indicate that once this obligatory requirement for lean growth has been met, the FH and FL lines partition their remaining energy differently - with the FH lines diverting relatively more energy towards fat deposition than the FL lines. The hypothesis that the fatness differences between the FH and FL lines are solely a function of intake differences, must therefore be rejected.

A further question of interest relating to these energy partition models, however, is whether or not the differences in the regression slopes are sufficiently great to account for all of the fatness differences between the FH and FL lines, on ad libitum intake (i.e. are the fatness differences a result of partitioning differences alone, with each additional unit of net energy being partitioned identically between lean and fat deposition, until ad libitum intake is reached). If the differences between the regression slopes are not great enough, then a "food intake in excess of maintenance" effect, as proposed in the hypothesis, must be invoked to explain the

discrepency. The "partition only" regression coefficients were calculated to address this problem.

The "partition only" regression slopes were calculated from the F line growth data collected in the section 2 study. Fat and lean accretions from 28 to 44 days of age were estimated and then extrapolated to those expected after 18 generations of divergent selection, once again assuming a linear change in fat% with generation of selection. By using the X intercept values obtained in this experiment, theoretical regressions of fat gain on intake in excess of maintenance were calculated, assuming that the fatness differences between the FH and FL lines are wholely caused by partition differences. In other words, the regression coefficients were calculated assuming that every increment of net energy in excess of the value at the X intercept was partitioned identically between fat and lean, until ad libitum intake was reached, for both groups of lines. These "partition only" regressions were then scaled (increased by approximately 10%) to make the FL "partition only" regression coefficient equal to the FL observed regression coefficient. The observed fat and partition only regression coefficients are represented diagrammatically in fig. 4.2.

In fig. 4.2 the FH "observed" fat regression line is drawn only up to the level of FL ad libitum intake, as this was the maximum intake level reached in the experiment. The FH "observed" fat regression line would be expected to lie between the FL and FH "partition only" regression lines, as is shown in the diagram. If this line differs significantly from the FH "partition only" line, then an "intake in excess of maintenance" effect, as described above, must be invoked. This is because when extrapolated, the FH line must eventually reach the same point for fat gain on ad libitum intake that the "partition only" line does.

From table 4.4 it can be seen that the observed fat regression coefficient is less than the "partition only" coefficient, but not significantly so. It appears, therefore, that once fat deposition has commenced, the differences in fatness between the FH and FL lines are a function of energy partitioning differences alone. It is interesting that given these partitioning differences the FH line mice continue eating until they have the same rate of lean gain as the FL line mice,

Figure 4.2 Diagrammatic Representation of the "Observed fat" and "Partition Only" Regressions

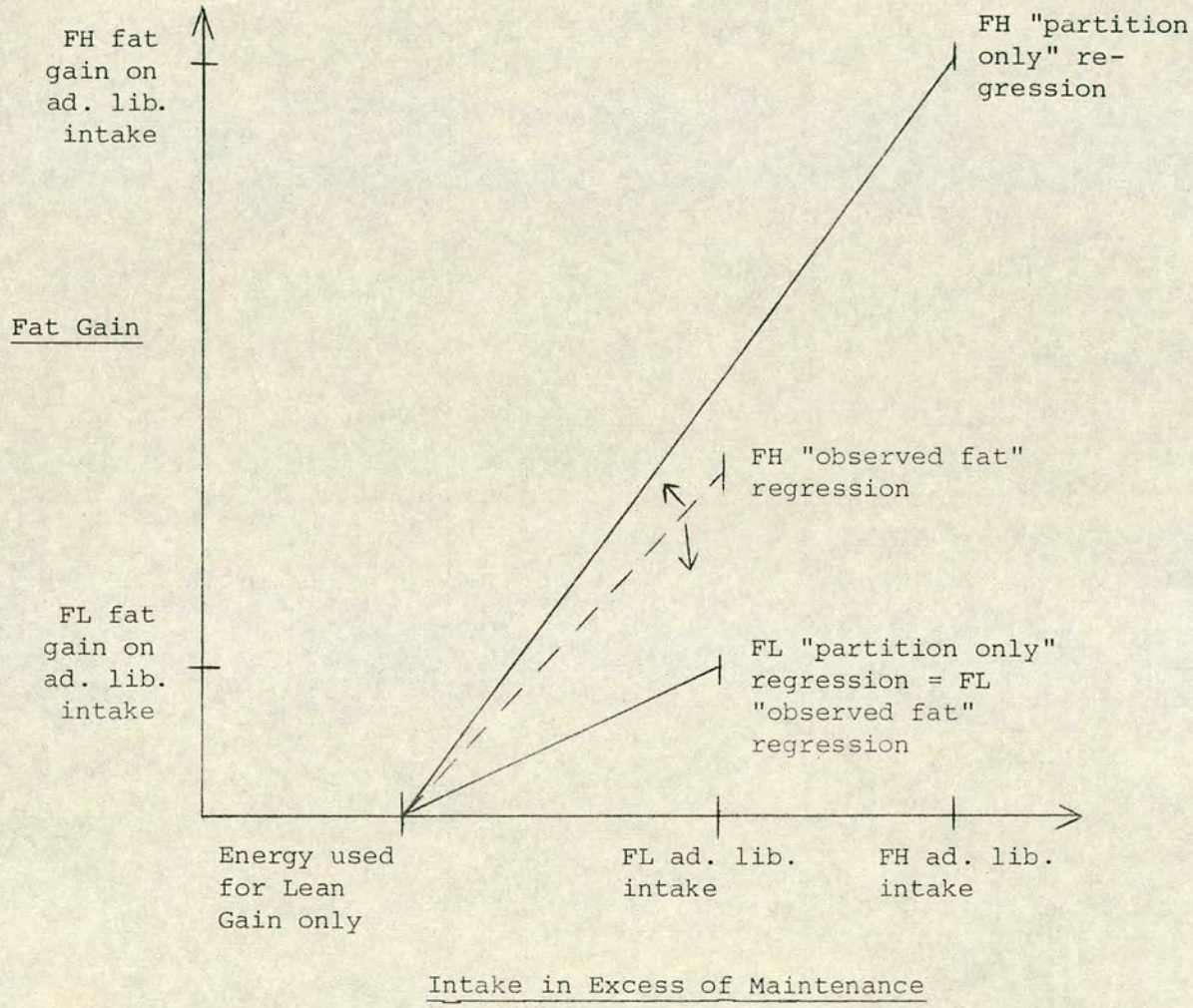


Table 4.4 Regressions on Intake in Excess of Maintenance

	FH Lines	FL Lines	[1] Significance
Observed Fat Regression Coefficients (g/kJ)	.01278 ± .00072	.00781 ± .00061	**
Intake at X Intercept (kJ)	42.45	37.94	N.S.
"Partition Only" Regression Coefficients (g/kJ)	.01369	.00781	-
Efficiency Regression Coefficients (g/g/kJ)	.00074 ± .00006	.00076 ± .00005	N.S.

[1] Shown is the probability of the coefficients being significantly different.

** P > .99, N.S. P < .90.

although this is consistent with the observation that the two sets of lines have the same rate of lean gain before fat deposition occurs.

Finally, regressions of efficiency on intake in excess of maintenance are also shown in table 4.4. Due to their greater relative rate of lean deposition the FL lines are of course more efficient than the FH lines, however the difference can be seen to be very small and non-significant. It can be concluded, therefore, that actual intake in excess of maintenance is a far more important determinant of efficiency than the type of tissue being deposited, in this study. This is the same general result as was implied by the F line results in section 2.

4.3.3 General Discussion

This study does appear to have been able to provide some clues as to the nature of energy partitioning in mice, although not by the means suggested by the experimental design. The results of the study are of course dependent on the assumptions made about the relative fatness and lean mass differences between the FH and FL lines, however these differences (and similarities) are very distinct and consistent, so even large errors in these assumptions should not affect the results greatly.

The hypothesis made that the fatness differences have been created merely by altering intake in excess of that required to achieve maximum lean deposition has been rejected. The F lines' fatness differences appear to have been created by changing the actual partition of energy between fat and lean, as is suggested in the alternative model given in the introduction. An effect of intake is still important, however, as the FH and FL lines continue to have ad libitum intakes that enable them to have equivalent or similar rates of lean gain. Thus, differences observed are a complex interaction of energy intake and energy partitioning differences. Rate of lean gain appears to be the feature that the F lines have in common, when considering both the intake level at which fat deposition commences and also ad libitum intake.

Detailed assumptions about the partition of energy between lean and fat gain have previously been made by Whittemore and Fawcett (1974 and

1976), in their computer modelling of the growth of young pigs. Although their model is interactive with the quality of the diet, it is static in terms of describing different genotypes, i.e. the effects of selection on energy partition can not be determined, and as such it is difficult to compare it with the F line results. There do, nevertheless, appear to be two important differences between their assumptions and these results. Firstly, they assume a minimum fat gain to protein gain ratio of 1, whereas the F line results suggest that this is incorrect, at least for mice, as below a certain level of intake no fat is deposited. Results quoted by Blaxter (1962), for a range of species, agree with the F line results. Secondly, a maximum rate of protein accretion is implicit for each diet in the Whittemore and Fawcett model, and above the corresponding intake level only fat will be deposited. This model was suggested by the hypothesis, but the F line results do not imply it. Pigs have much larger intakes in excess of maintenance than mice, however, and therefore a model such as this may be correct for pigs - especially if an environmental constraint such as dietary protein is imposed.

A model of energy partition equivalent to the general model implied by these results can be seen, however, in the results of a study on Japanese Quail (Farrell et al,1982). In this study a steady increase in fat gain as intake increased, above the base level at which no fat deposition occurred, was implied, in agreement with the F line results.

Unfortunately, there have been few studies which have detailed changes in energy partition as carcass composition has been genetically changed (i.e. by selection). Ellis et al (1983a and b) and Henderson et al (1983) have, however, studied the effects of selection in pigs for an index of decreased fatness and increased gain and efficiency. The selected pigs became leaner than their unselected controls, and they also had reduced voluntary food intakes. Although these changes were brought about to a small extent by changes in energy partition, the reductions in food intake were a much more important factor in decreasing fatness and increasing efficiency. The model of partition suggested by these pig results is different from the F lines, however it does have a parallel in that the FL lines have also reduced their intake in addition to changing their partition of

energy away from fat. It may be concluded, therefore, that both food intake and the patterns of energy partition may change when selection is employed to change carcass composition.

Finally, the complex changes in the usage of energy in excess of maintenance in the F lines appear to have occurred independently of maintenance, as their total maintenance requirements have not changed - in contrast to the A lines where changes in both maintenance and growth accurred. Although the hypothesis of maintenance being a function of lean mass does explain this F line phenomenon, it becomes obvious that it is very difficult to separate the effects of maintenance, growth and carcass composition.

Section V PHENOTYPIC RELATIONSHIPS BETWEEN FOOD INTAKE, CARCASS COMPOSITION AND GROWTH

5.1 INTRODUCTION

The experiment outlined in this section is designed to meet two specific objectives, and it will complete the experimental investigations in this thesis.

The first objective is to attempt to test experimentally the hypothesis made in section 2 concerning the carcass composition changes in the A lines. As has been shown, the lines selected for increased food intake, the AH lines, responded to selection by becoming leaner than their controls and the AL lines. This is of course contrary to the general positive relationship between intake and fatness, and it is also contrary to the previously observed changes in mice actually selected for food intake (Sutherland et al,197Ø). The A line mice were not selected for intake per se, however, but for intake corrected for body weight -again as outlined in section 2. The hypothesis was therefore made that it was the correction for body weight that caused the "unexpected" composition changes, and that if intake per se had been the selection criterion then the AH lines would indeed have become fatter instead of leaner.

The first aim of this section is to test this hypothesis, at the phenotypic level, by measuring individual 4 and 6 week body weights, 4 to 6 week food intake and carcass composition on unselected control line mice. It is hypothesized that carcass fatness will be negatively correlated with 4 to 6 week intake corrected for 4 week weight (the A line criterion), but positively correlated with 4 to 6 week intake per se. It would be impractical to test this hypothesis at the genetic level because of the large number of mice required.

This experimental technique also allows more general investigations into the possible effects of a restriction on body weight whilst selecting for food intake, and these investigations constitute a large part of the investigations for the second objective.

The second objective of this section is more general and concerns the relationships between the input (intake) and output (maintenance energy expenditure and fat and lean gain) components of growth. From the results outlined in the previous studies several implications have been drawn about the relationships between these components of growth, however often these implications have been drawn merely from between

line comparisons of the selected mice. The second aim of this section, therefore, is to study these relationships and to see whether or not they can be demonstrated at the phenotypic level within a population of unselected mice. In other words, the results that have been obtained are being re-evaluated at the phenotypic level. The simple measurements of body weights, intake, and individual carcass composition are sufficient for this investigation to be undertaken. In addition, from the results it may also be possible to suggest possible criteria for further selection experiments -in the problem areas warranting further detailed study by this means.

5.2 MATERIALS AND METHODS

5.2.1 Source of Data

From generations 17 and 18 of the selection experiment 31 full sib families were sampled, and from within each family 3 male and 3 female pups were chosen at random for the study. At 4 weeks of age each mouse was weighed and housed individually in a feeding cage. Ad libitum food intake (the same diet as for the previous studies) was measured until 6 weeks of age, at which stage each mouse was reweighed and slaughtered for carcass analyses. Dry matter% (DM%) was measured on each mouse, and on 120 mice individual fat% was determined. Fat was extracted from the freeze dried and minced samples using standard soxhlet extraction techniques, with the determinations being performed by the Edinburgh School of Agriculture. Constraints on laboratory facilities did not allow fat% to be determined on the remainder of the mice, and for these mice fat% was estimated from the regression of fat% on DM% derived from this data set.

5.2.2 Traits Considered

The following traits were measured or derived for both the first and second objectives of this study:

4 week weight		(4WW)
6 week weight		(6WW)
average weight	((4WW + 6WW))/2)	(AV.W)

weight gain	(GAIN)
food intake	(FI)
efficiency (gain/intake)	(EFF)
dry matter %	(DM%)
fat %	(FAT%)
average lean mass	(AV.LM)
total catabolism	(CATAB)
intake/catabolism	(CATRAT)
energy used for gain	(EFORGN)
catabolism/average weight$^{.75}$	(CAT/BW)
catabolism/average lean mass$^{.75}$	(CAT/LM)

All traits except for those describing changes in carcass composition (i.e. catabolism, lean mass and energy in excess of maintenance) were calculated directly from the data set.

Catabolism was calculated in the same way as in sections 2 and 4, i.e. energy intake less the costs of fat and protein gain. The same assumptions concerning the energy density of the diet (10.636 kJ/g) and the costs of fat and protein deposition (53.4 and 52.9 kJ/g, respectively) were also used. In order to estimate the individual fat and protein gains from 4 to 6 weeks of age, however, several assumptions about the changes in these components over this time period had to be made. Fat gain was estimated assuming a constant proportional increase in fat percentage over time, as the results outlined in section 2 indicate that this is probably the most realistic way of describing the increase in fat percentage, from 4 to 6 weeks of age, for mice of a wide range of fat contents. For the control lines in section 2, 4 week fat percentage was approximately .841 times 6 week fat percentage, and as this value was quite constant across the control lines individual 4 week fat content in this study was estimated as .841 times that at 6 weeks of age. For each mouse then, fat accretion from 4 to 6 weeks of age was calculated, as was average lean mass (body weight less fat mass). In the section 2 results it can be seen that protein content does not vary greatly between lines, so constant individual protein percentages were therefore assumed in this experiment. From the results outlined in section 2, protein contents were assumed to be 16.93% and 17.93% of body weight, at 4 and 6 weeks of age, respectively. Individual protein

accretions were estimated using these assumptions, and thus catabolism was calculated for each mouse.

Once the individual catabolism values were obtained individual intake ratios, i.e. intake/catabolism, could be calculated. The final traits calculated were simply catabolism scaled by average metabolic body weight and average metabolic lean mass. Energy used for gain (intake in excess of catabolism) was calculated in the derivation of the catabolism values.

5.2.3 Numerical and Statistical Analyses

The first objective of this study, as mentioned above, was to study the effect that pre-correcting intake for body weight has on the correlation between intake and carcass composition. Rather than simply pre-correcting the data, however, this problem can be approached with greater power and flexibility by considering the selection index used for the A lines. The A line selection criterion was $FI-b(4WW-\overline{4WW})$, where b is the regression of FI on 4WW, and of interest are the correlations of carcass composition with this criterion and carcass composition with intake per se. This selection criterion can be generalised to allow any degree of correction for 4WW, however, simply by multiplying the regression coefficient (b) by a constant (k) which can take any value. The correlation coefficient of interest can therefore be redefined as follows:

$$corr(Y,\ X+kb(Z-\overline{Z}))$$

where: Corr = correlation
Y = correlated trait of interest (e.g. fat%)
X = primary trait selected for (e.g. FI)
Z = trait corrected for (e.g. 4WW)
b = regression of X on Z
k = constant defining degree of correction used

By varying the value of k in the index, an index with any degree of correction on trait Z can be created, and this allows a powerful means of approaching the problem.

If: k = Ø then selection on the index will be for X alone

k = -1 then selection will be for X corrected for trait Z
(i.e. the A line criterion)

As k approaches negative infinity, selection will reduce Z

As k approaches positive infinity, selection will increase Z

This generalised correlation can be expressed in terms of its variance and covariance terms, for evaluation at any value of k, as follows:

$$\frac{Cov(Y,X) + kbCov(Y,Z)}{\sqrt{(Var(Y)*(Var(X)+2kbCov(X,Z)+k^2 b^2 Var(Z)))}}$$

where: Var = variance
Cov = covariance

Selection in the A lines was on a within family basis, so for consistency the correlations in this section were calculated on a within family, or residual, basis. The residual variance and covariance components required to calculate the correlation coefficients were estimated from the data set, assuming the following statistical model for each trait:

$$Yijk = U + Si + fj + eijk$$

where: Si = ith sex
fj = jth family (random)
eijk = kth individual of the jth family

Correlations of the index with FAT% and DM% were then calculated for values of k ranging from large negative to large positive.

The second objective of this study was to re-evaluate the relationships between the components of growth drawn from the previous investigations. This task was firstly approached at a very simple level, merely from a consideration of the correlations estimated between the measured and derived traits.

The problem was then further approached in the same manner as described above, by calculating the correlations between selection indices derived from the generalised index described above and various traits of interest. A very large number of possible indices could be calculated from these equations, however to keep the number of results to a manageable and meaningful level only the indices of food intake

corrected for (i) 4WW, (ii) AV.W, (iii) 6WW and (iv) GAIN were calculated. Index (i) was studied simply as a further means of investigating the A line criterion. The other three indices were studied firstly to approach the question of what would have happened if these alternative and equally viable indices had have been used, instead of the A line criterion, and secondly as a means of generally appraising the relationships between the input and output components of growth. The correlations calculated were then considered within the metabolic framework used in these studies.

When intake was corrected for 6WW, AV.W and GAIN the b values derived from the data set, i.e. 1.706, 2.051 and .934, were used. When intake was corrected for 4WW, however, a b value of 1.922 derived from that used by Sharp et al (1984) was used -to mimic the index used in the selection experiment. This value is also more similar to the b values for 6WW and AV.W than the value derived from the data set (1.541), and it thus allows easier comparisons between the three indices. The correlations were calculated with the value of k in the indices varying from large negative to large positive.

Finally, the regression of energy used to deposit fat on EFORGN was calculated in an attempt to re-evaluate the section 4 results.

5.2.4 Estimation of Standard Errors

The standard error of a correlation coefficient is normally estimated as $(1-r^2)/\sqrt{n-3}$. This formula only becomes accurate with large sample sizes, e.g. df greater than 500, however there are only 151 residual df in this study. In addition, in this study many of the correlations calculated were between highly derived traits, rather than simple measurements. Doubt was therefore expressed on two levels as to whether or not this theoretical equation would adequately describe the actual standard errors of these correlations. It was therefore decided to attempt to estimate these standard errors using an empirical "computer intensive" procedure known as bootstrapping (Efron,1982). This method is described below.

A second type of standard error, necessary for the testing of the hypothesis concerning the carcass composition changes in the A lines, is that of the value of k necessary to obtain a pre-determined

correlation between two traits. Specifically, one may ask what is the standard error of the k value which results in a correlation of zero between carcass fatness and the selection index, and therefore is this k value significantly different from a specified k value, e.g. -1. The estimation of this type of standard error is not easily tractable mathematically, and therefore an empirical approach must also be used. Bootstrapping techniques were therefore used to calculate this type of standard error as well.

Bootstrapping is a powerful empirical means estimating statistical parameters in which the restrictive Gaussian assumptions of traditional analyses (e.g. normal distribution of random effects) are replaced by large scale computer computations (Diaconis and Efron, 1983). Bootstrapping techniques therefore allow analyses of data whose properties do not conform to these assumptions, as well as providing a means of reliably analysing small data sets. These techniques also allow numerical exploration of statistical properties which are not mathematically tractable, or easily manipulated analytically. The mathematical and statistical properties of these and similar techniques are outlined in detail by Efron (1982), as are verifications of the accuracy of these techniques in a wide variety of situations.

Bootstrapping is based on the simple concept of repeated resampling of a data set. From a given data set of n independent observations a random sample of size n is drawn (with replacement), and from this sample a statistic t is calculated. This process is then repeated many times (e.g. 1000 times) until a whole distribution of t statistics are obtained. This distribution is then treated as if it represented the distribution of t statistics of real samples of size n, and thus estimates of the variability, or sampling properties, of t can be made. For example, from a given data set repeated estimates of a correlation coefficient between two variables can be made, and standard errors and empirical confidence intervals can then be calculated for this correlation coefficient.

The standard errors of the estimated correlation coefficients in this study were calculated in this way. The total data set consisted of 183 individual observations for each trait, however the residual degrees of freedom of 151 meant that there were, in reality, only 151

independent observations. Each individual trait was therefore pre-corrected for its sex effect, and then transformed and scaled using linear contrasts to standardise the mean and variance for each family, and to obtain to obtain n-1 independent observations within each family -where n is the number of individuals in each family. A set of 152 "independent observations" (the extra df corresponding to the sex effect) was therefore obtained.

From this set of independent observations, a subset of 152 observations was drawn at random, and the correlation coefficient between two traits of interest was calculated. This sampling procedure was repeated 1000 times, with the standard error of the obtained correlations being empirically calculated. This whole process was then repeated an arbitrary two to three times, and the standard errors were averaged to obtain the "bootstrapped" standard error for each correlation. These standard errors were calculated for the correlations of FI with all other traits, and also for the correlations of FI, corrected for 4WW and 6WW, with FI, GAIN, DM%, FAT%, EFFIC and CAT/BW, for the following values of k: -4, -3, -2.5, -2, -1.5, -1.25, -1, -.75, -.5, -.25, 0, 1, 2 and 4.

Bootstrapping the standard error of the value of k giving a pre-determined correlation required a slightly more elaborate algorithm. The procedure was as follows: (i) an initial set of 152 random integers between 1 and 152 was drawn; (ii) an initial "estimated" k value was specified, and the value of $FI-kb(Z-\overline{Z})$ was calculated for each individual; (iii) the data set was then transformed to obtain 152 "independent observations"; (iv) the set of random numbers was then used to draw a subset of 152 observations, and the correlation between the index and the correlated trait was calculated; (v) the program then entered a "number finding" algorithm which compared the estimated correlation with the desired correlation, and thus was able to make a better estimate of k. Using this new k value, and the set of random numbers already specified, steps (ii) to (iv) were repeated until the desired value of k was found. The whole process was then repeated 100 times, so that 100 k values were obtained, and thus the standard error of k was calculated. Only 100 samples were taken for these computations, as opposed to 1000 in the previous calculations, because of the extremely time consuming nature of the process.

From the results presented below it was decided that the following values of k were of interest: (i) the k value giving a correlation of zero between DM% and FI corrected for 4WW, AV.W and 6WW; (ii) the k value giving a correlation of zero between FAT% and FI corrected for 4WW, AV.W and 6WW; and (iii) the k value maximising the correlation between CAT/LM and FI corrected for 4WW, AV.W and 6WW.

5.3 RESULTS

5.3.1 Means, Standard Deviations and Simple Correlations

The means and standard deviations for each of the considered traits are shown in table 5.1 along with the phenotypic within family correlations between each of the traits. These correlations will be discussed below. In table 5.2 the correlations and "theoretical" standard errors of FI with each of the measured traits are shown again, along with the corresponding bootstrapped correlations and standard errors.

The comparison in table 5.2 serves mainly as a mutual check on the two methods of estimating standard errors. It can be seen, for these simple correlations between two "observed" traits, that the bootstrapped correlations are nearly always the same as the actual correlations, and that the bootstrapped standard errors are usually similar to those estimated by normal theory. From these results it would appear that both methods give similar estimates of standard errors, and therefore, the standard errors of the simple correlations will be assumed to be those estimated by $(1-r^2)/\sqrt{150}$. Thus, using standard t test procedures a correlation of .16 is significantly different from zero at the 5% level, and .18 at the 1% level.

5.3.2 Food Intake and Carcass Composition

In agreement with the section 4 results, FAT% and DM% are highly correlated (table 5.1), and the regression of FAT% on DM% is close to 1. These two traits also have similar standard deviations, despite their greatly different means, and FAT% therefore has a much higher coefficient of variation than DM%. It can also be seen that the

Table 5.1 Means, Standard Deviations and Correlations of the Considered Traits.

	4WW g	6WW g	AV.W g	GAIN g	FI g	EFF g/g	DM %	FAT %	AV.LM g	CATAB kJ	CATRAT kJ/kJ	EFORGN kJ	CAT/BW $kJ/Kg^{.75}/day$	CAT/LM $kJ/kg^{.75}/day$
Mean	16.96	24.81	20.89	7.85	64.55	.122	32.61	9.06	19.13	551.5	1.25	135.1	720.3	768.8
Standard deviation	1.56	1.87	1.54	1.54	4.26	.023	1.33	1.45	1.39	41.0	.05	23.6	42.0	44.7

Correlations

	6WW	AV.W	GAIN	FI	EFF	DM %	FAT %	AV.LM	CATAB	CATRAT	EFORGN	CAT/BW	CAT/LM
4WW	.61	.88	-.27	.56	-.50	.32	.14	.85	.69	-.40	-.12	.09	.11
6WW	.92	.60	.75	.35	.18	.08	.90	.45	.38	.66	-.26	-.25	
AV.W	.23	.74	-.04	.27	.12	.98	.62	.03	.34	-.11	-.09		
GAIN	.34	.93	-.11	-.04	.23	-.16	.86	.92	-.40	-.41			
FI	.00	.21	.14	.72	.85	.04	.43	.41	.44				
EFF	-.20	-.10	-.04	-.46	.90	.81	-.56	-.58					
DM %	.81	.11	.10	.14	.22	-.08	.07						
FAT %	-.08	-.04	.28	.32	-.15	.04							
AV.LM	.64	-.03	.28	-.08	-.10								
CATAB	-.48	-.10	.70	.70									
CATRAT	.90	-.62	-.58										
EFORGN	-.42	-.37											
CAT/BW	.98												

Table 5.2. "Theoretical" and Bootstrapped Standard Errors for the Correlations of FI with the other Considered Traits

Correlated Trait:	4WW	6WW	AV.W	GAIN	EFF	DM %
"Theoretical"	.56 ± .056	.75 ± .036	.74 ± .037	.34 ± .072	.00 ± .081	.21 ± .078
Bootstrapped	.56 ± .058	.75 ± .037	.74 ± .038	.34 ± .076	.00 ± .088	.21 ± .073

Correlated Trait:	FAT %	CATAB	CATRAT	EFORGN	CAT/BW	CAT/LM
"Theoretical"	.14 ± .080	.85 ± .022	.04 ± .081	.43 ± .066	.41 ± .068	.44 ± .066
Bootstrapped	.14 ± .080	.85 ± .027	.04 ± .091	.44 ± .068	.41 ± .067	.44 ± .065

correlations of DM% with each of the traits calculated independently of carcass composition (e.g. 4WW, EFF) are of the same sign as those of FAT%, but approximately twice the magnitude. These observations indicate that DM% is a very good predictor of FAT%, and in this study may in fact be a more reliable indicator of carcass composition and fatness than the fat determinations themselves. As a result, DM% and FAT% are discussed interchangeably below.

Figs. 5.1 (DM%) and 5.2 (FAT%) show the effects of the corrections for 4WW on the correlation between carcass composition and corrected FI (i.e. the index FI- $b(4WW-\overline{4WW})$. On the X axis is plotted the k value of the generalised index $X+kb(Z-\overline{Z})$, and on the Y axis is plotted the actual value of the correlation of this index with trait Y. The Y axis therefore has bounds of -1 to 1. Also shown in these two figures are the effects of using AV.W, 6WW and GAIN as the traits corrected for, instead of 4WW, and these results will be discussed below.

Consider firstly the correlation of DM% with corrected FI. The correlation coefficient on the vertical axis is of course .21 (the correlation between DM% and FI), and the plotted curve tends towards + or -.32 (the correlation of 4WW with DM%) as k gets very large or small. The point of greatest interest, however, is the correlation coefficient at k = -1, and for DM% it can indeed be seen to be slightly negative, as was expected from the A line results. For FAT%, at k= -1 the correlation is still positive, but as k decreases this correlation does become negative. The most important feature of these two graphs, however, is not so much the actual values of the correlations, but the fact that the correlation between FI and carcass fatness decreases quickly as the degree of correction of FI for 4WW increases.

Also shown in figs. 5.1 and 5.2 are the effects of placing restrictions on AV.W and 6WW. It can be seen that correcting for these traits, rather than 4WW, would also have had an effect on carcass composition, although not as great.

In terms of the standard errors of the correlation coefficients, the discrepancy between the "theoretical" and bootstrapped standard errors showed systematic trends as the value of k changed, although these trends differed between the 4WW and 6WW indices. The discrepancies for the correlations with DM% never exceeded + or -5%, however, and for

Fig. 5.1 CORRELATION OF DM% AND CORRECTED FI vs K

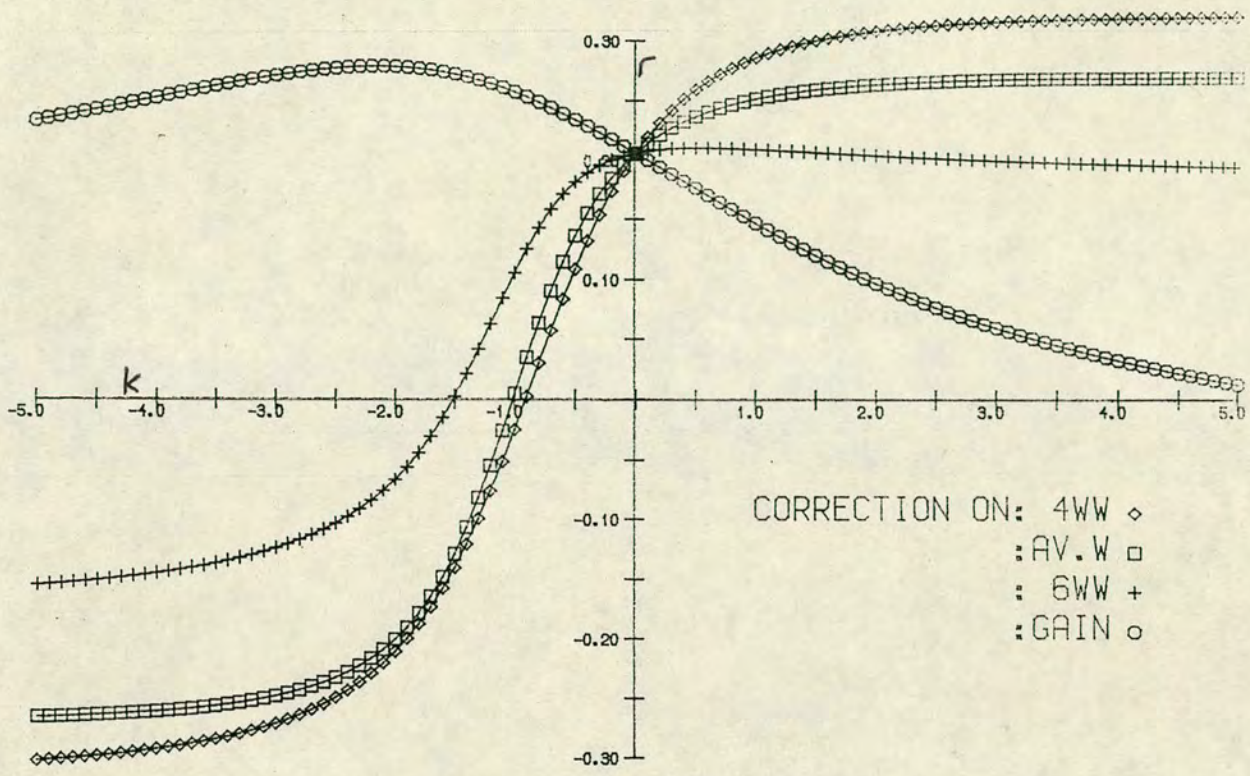
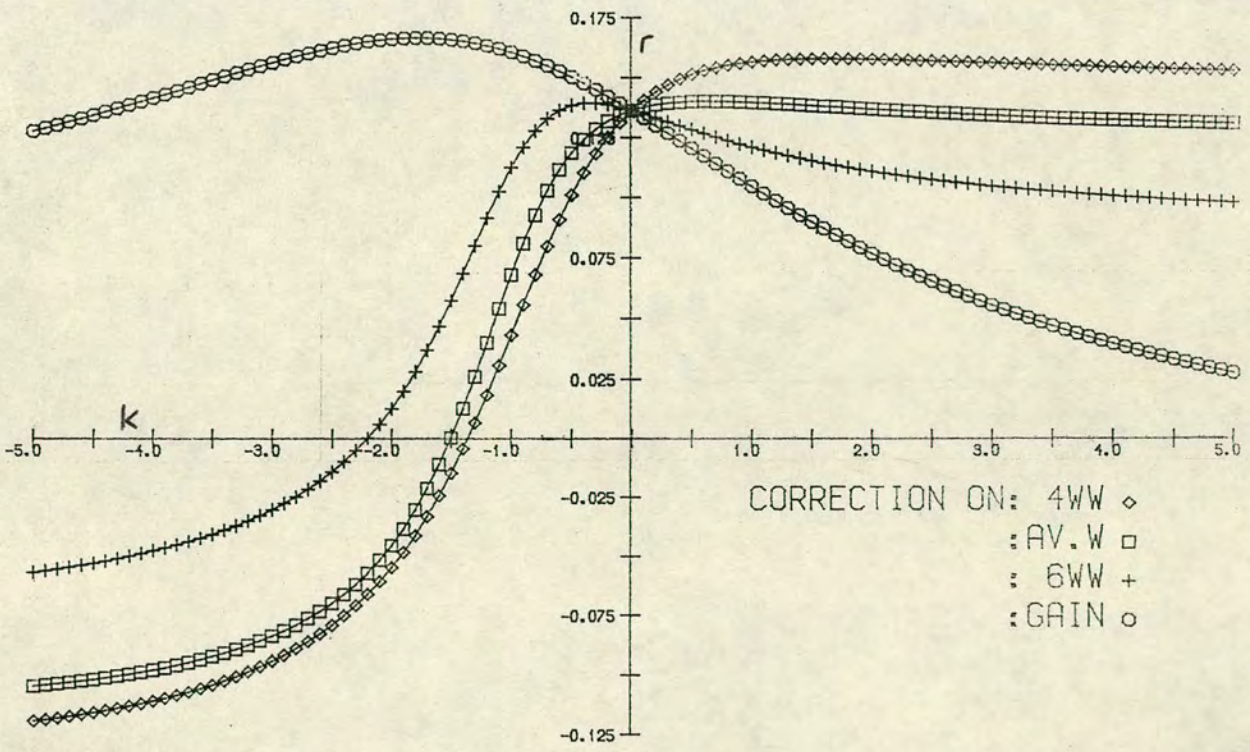


Fig. 5.2 CORRELATION OF FAT% AND CORRECTED FI vs K



FAT% they never exceded + or -10%. Moreover, the discrepancies were almost zero at the k values of interest, i.e. -1 and Ø. It is therefore assumed, once again, that the true standard errors of these correlations are those predicted by normal theory, i.e. the standard error of r=Ø is .Ø82. For DM% then, the correlation at k=Ø differs significantly from that at k = -1.

The alternative means of measuring the variability of these results was to test the sampling properties of the parameter k. The question asked was how variable are the k values which give correlations of zero for these indices (and therefore does the A line criterion actually predict a decrease in carcass fatness). As described above, the standard error of this k value was bootstrapped for the correlations of DM% and FAT% with indices applying corrections on 4WW, AV.W and 6WW. The results are as follows:

correction on 4WW, k = - .9Ø s.e. = .34
AV.W, k = -1.Ø1 s.e. = .4Ø
6WW, k = -1.52 s.e. = 1.Ø5

and for FAT% the results are as follows:

correction on 4WW, k = -1.35 s.e. = 1.Ø4
AV.W, k = -1.5Ø s.e. = .76
6WW, k = -2.21 s.e. = 1.72

These standard errors appear to have the same implications as the correlation standard errors, for both DM% and FAT%. It appears that the index of most interest, FI corrected for 4WW (k=-1), is not significantly different from the index with k = -.9Ø, in terms of the correlated response in DM% it predicts, however the sampling error (.34) indicates that it does differ from the index of FI per se (k=Ø). Therefore, although it is not possible to demonstrate that the A line criterion would select leaner mice, the hypothesis that the k value affects the expected change in carcass composition, for k ranging from -1 to Ø, is most probably correct. Finally, it can be seen that the standard errors for the considered k values vary in proportion to the gradients of the plotted curves, as may be intuitively expected.

5.3.3 Further Investigations

Figs. 5.3 to 5.10 show the correlations between the selection indices (FI corrected for 4WW, AV.W, 6WW or GAIN) and FI, GAIN, EFF, CATRAT, CATAB, EFORGN, CAT/BW and CAT/LM, respectively. Once again, k is plotted on the X axis and the value of the correlation on the Y axis. The important features of these graphs will be outlined in the discussion.

The standard errors of the correlation coefficients were bootstrapped for several traits. The correlations and standard errors are too numerous to list, so only the trends of the discrepancies between the two estimates of the standard errors are given. The actual values of r can be read from the figures, and the "theoretical" standard errors were once again estimated as $(1-r^2)/\sqrt{150}$. The following are the trends of the discrepancies between these bootstrapped and "theoretical" correlations, expressed as (bootstrapped -"theoretical")/"theoretical".

(1) correlated trait is FI, index correcting FI for 4WW:
bootstrapped correlations are a constant 8% greater,
correcting for 6WW: constant 5% greater.

(2) GAIN, correcting for 4WW: approximately equivalent
correcting for 6WW: 14% greater when k is large or small,
equivalent when k is from -1 to 0.

(3) EFF, correcting for 4WW: equivalent until k = -1, then gradual increase until 10% greater when k is large,
correcting for 6WW: 18% greater when k is large or small,
approximately equivalent at k = -1.

(4) CAT/BW, correcting for 4WW: constant 6 to 10% smaller,
correcting for 6WW: approximately equivalent.

It can be seen that for most traits the two estimates of the standard errors appear to be in approximate agreement, although for EFF the correlations may be 10 to 20% more variable than one would expect. With the exception of EFF, therefore, it may be assumed that the variability of the correlations is adequately described by the standard formula, i.e. $(1-r^2)/\sqrt{150}$.

It can be seen from figs. 5.9 and 5.10 that intake corrected for

Fig. 5.3 CORRELATION OF FI AND CORRECTED FI vs K

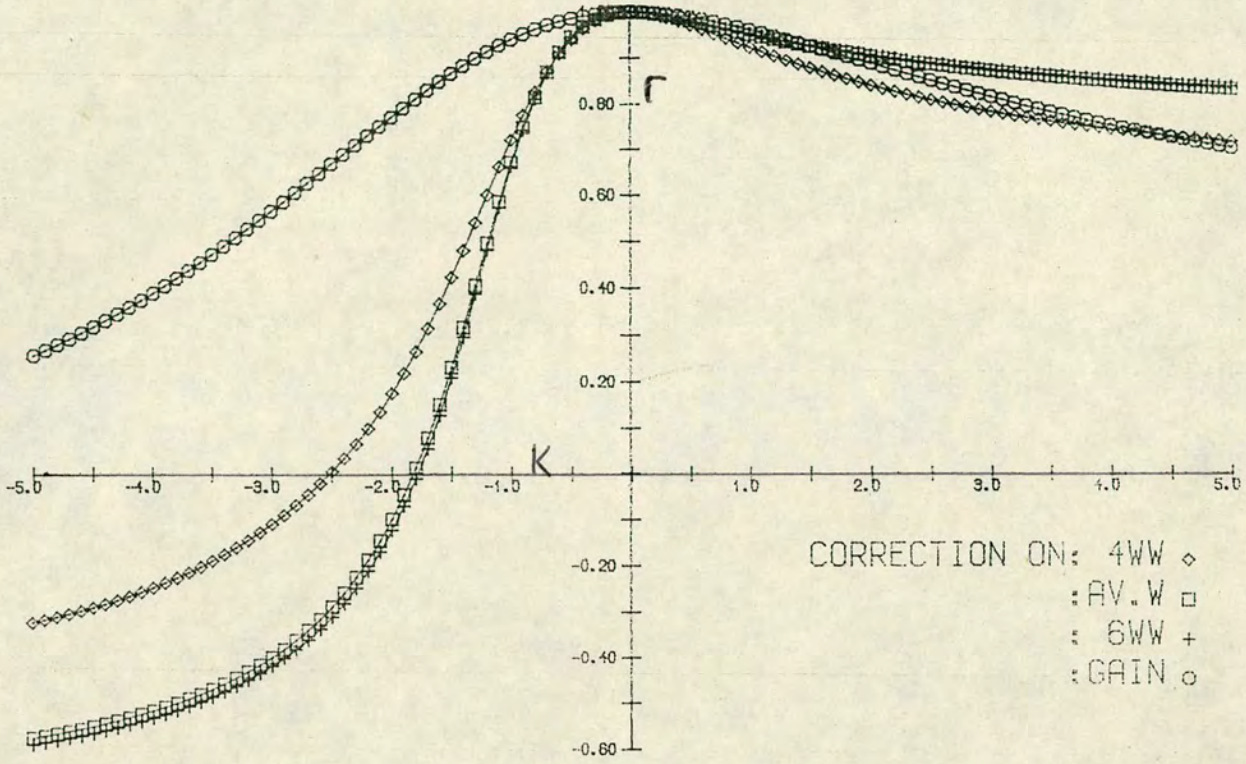


Fig. 5.4 CORRELATION OF GAIN AND CORRECTED FI vs K

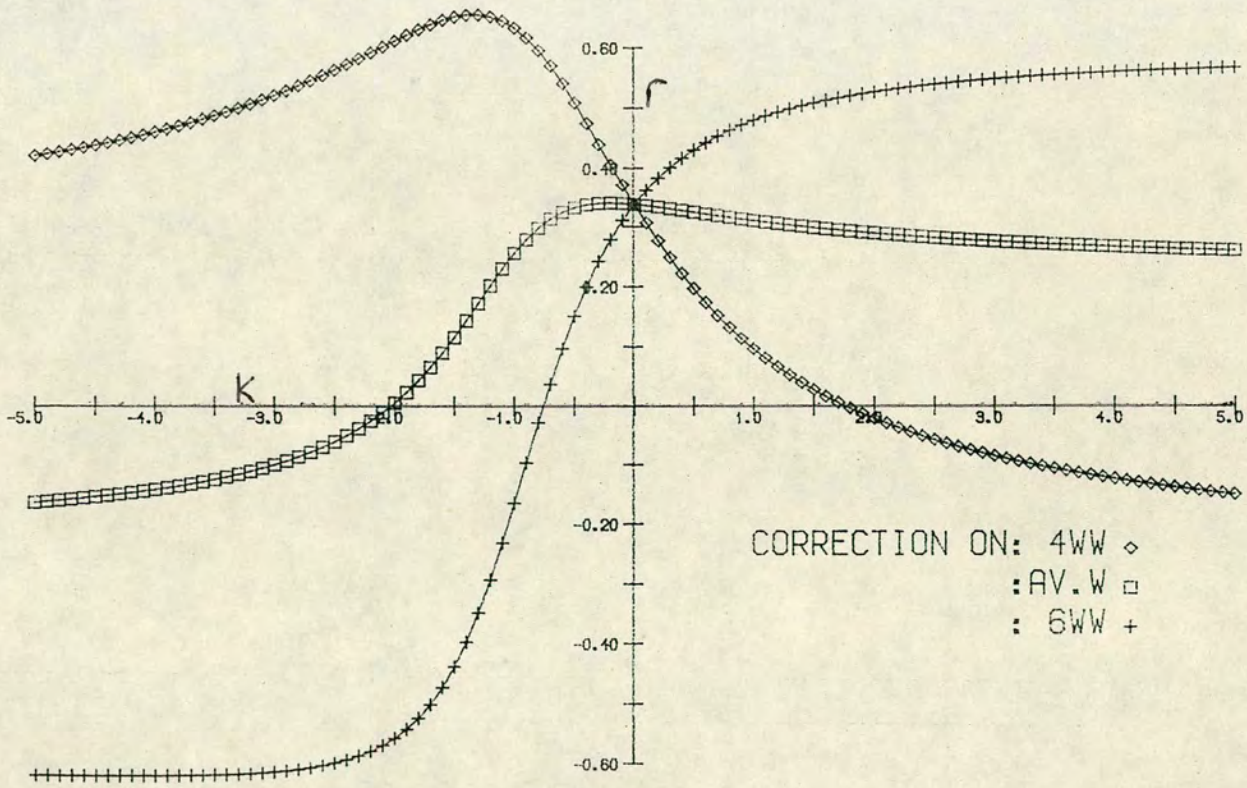


Fig. 5.5 CORRELATION OF EFF AND CORRECTED FI vs K

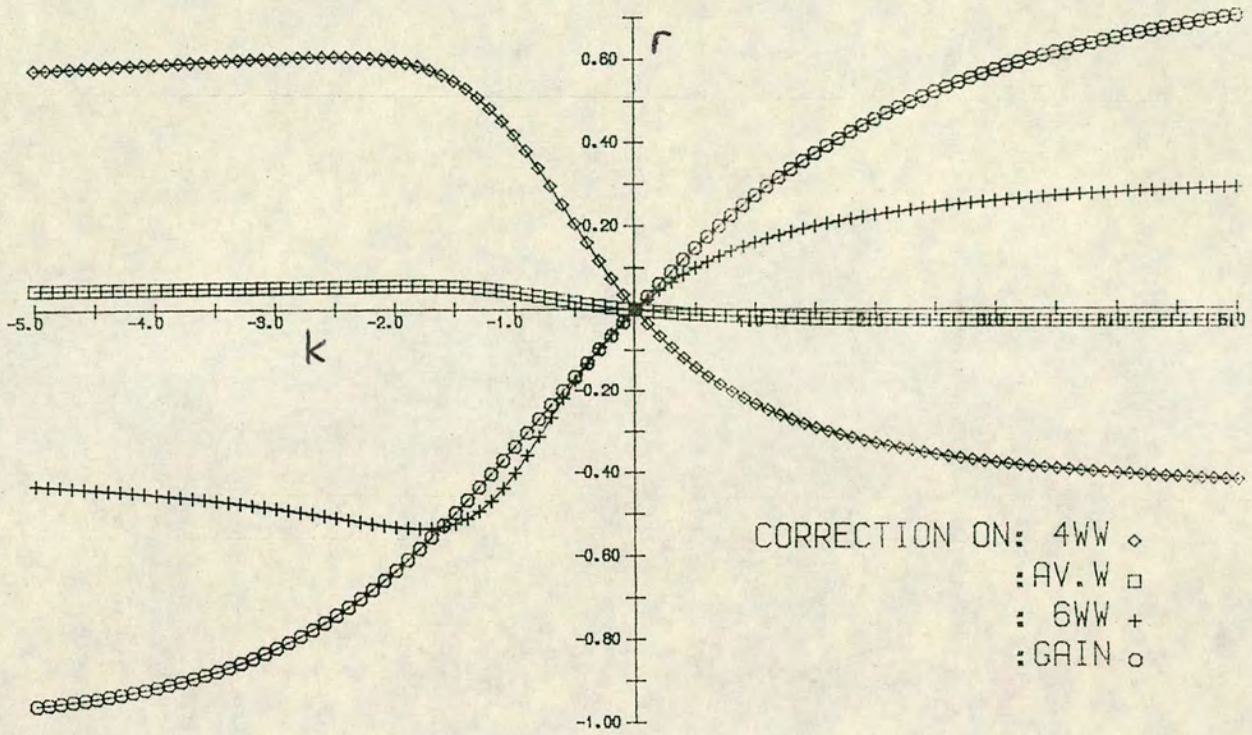


Fig. 5.6 CORRELATION OF CATRAT AND CORRECTED FI vs K

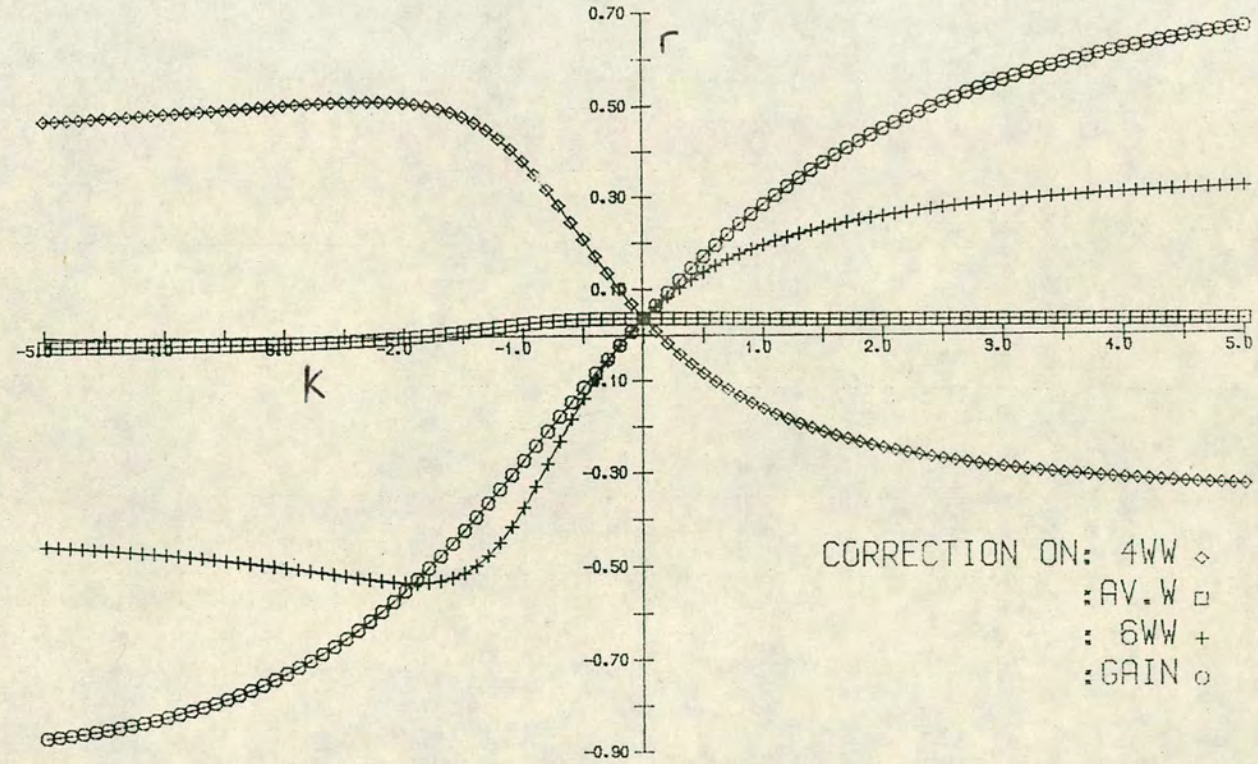


Fig. 5.7 CORRELATION OF CATAB AND CORRECTED FI vs K

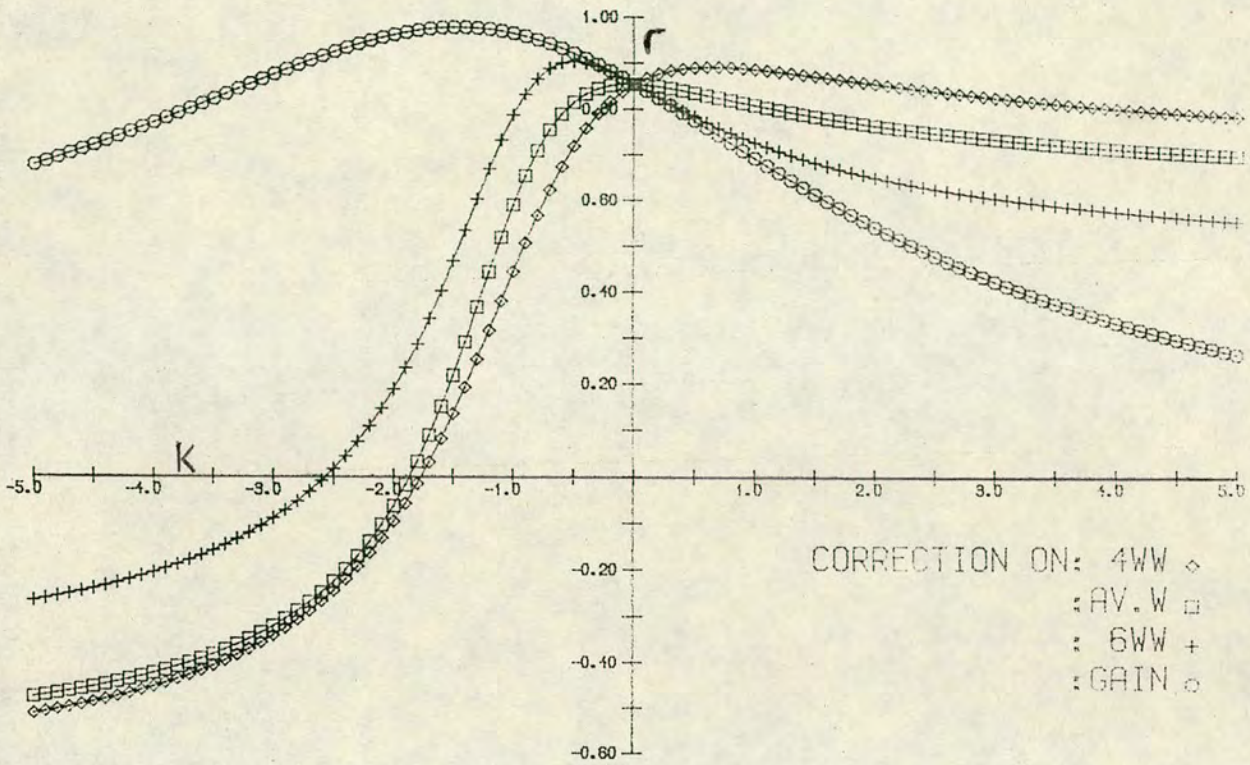


Fig. 5.8 CORRELATION OF EFORGN AND CORRECTED FI vs K

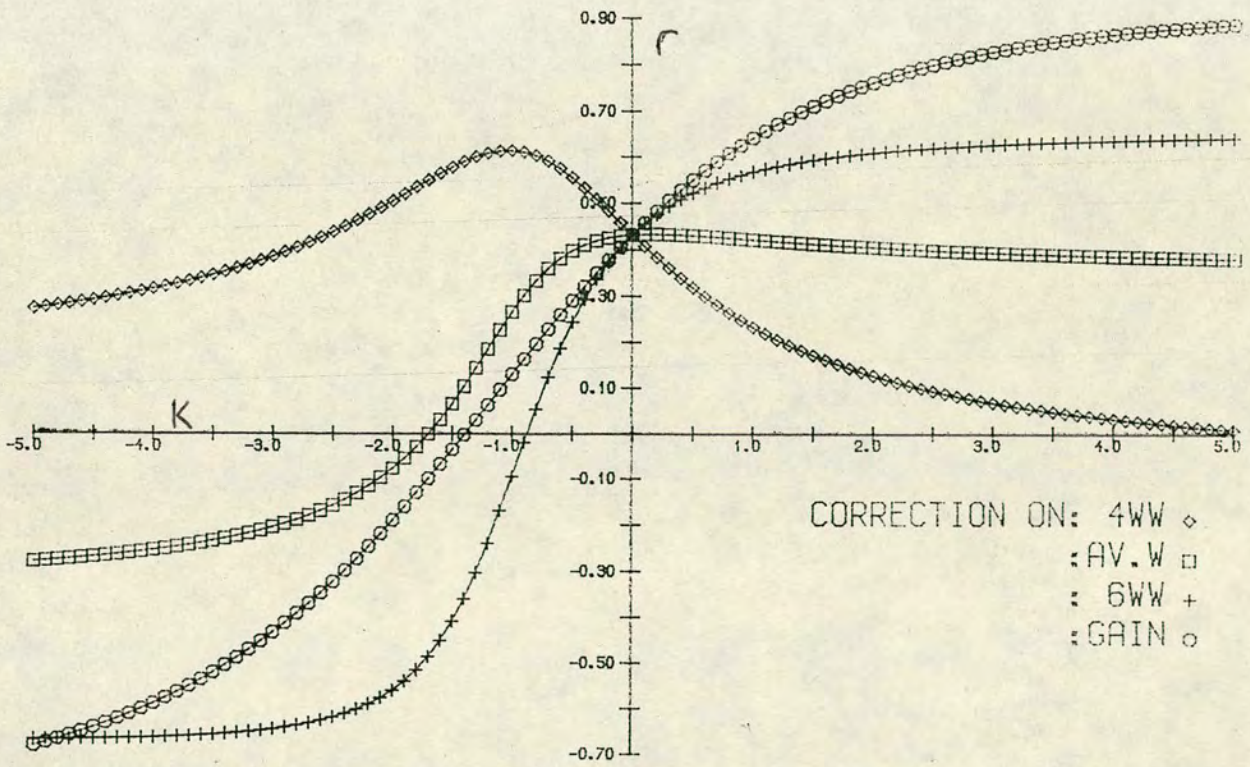


Fig. 5.9 CORRELATION OF CAT/BW AND CORRECTED FI vs K

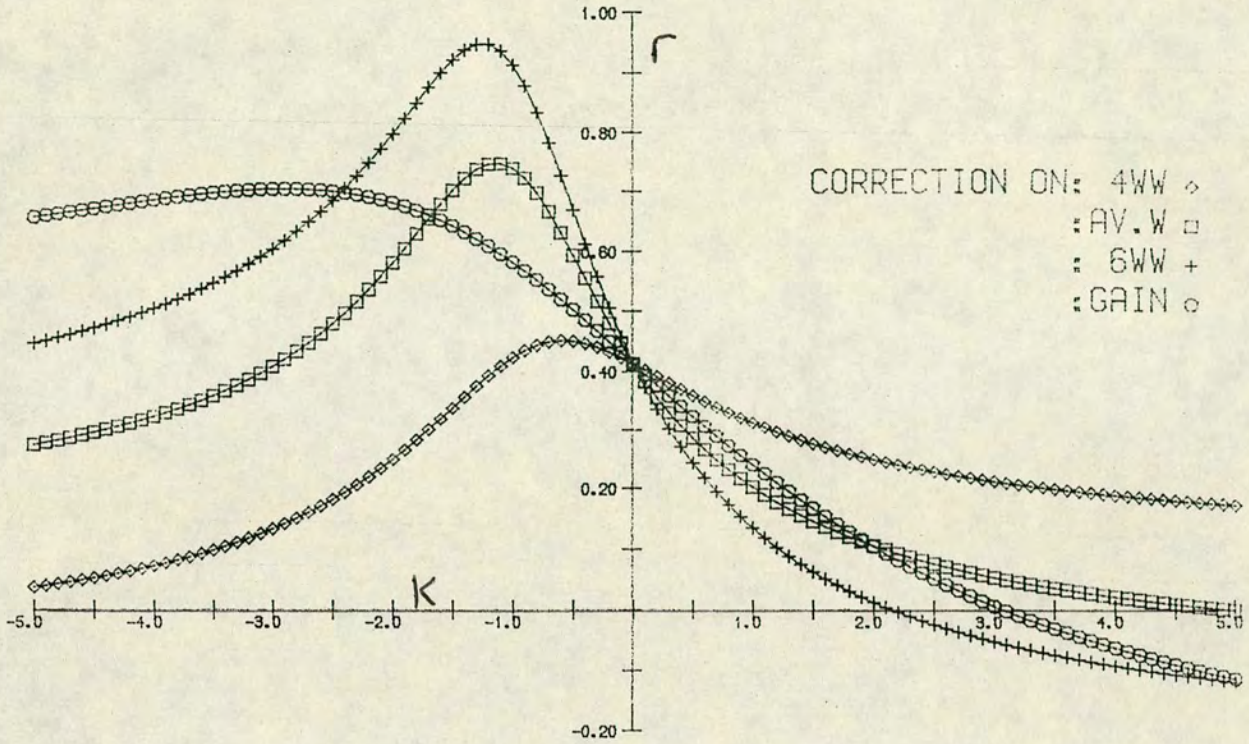
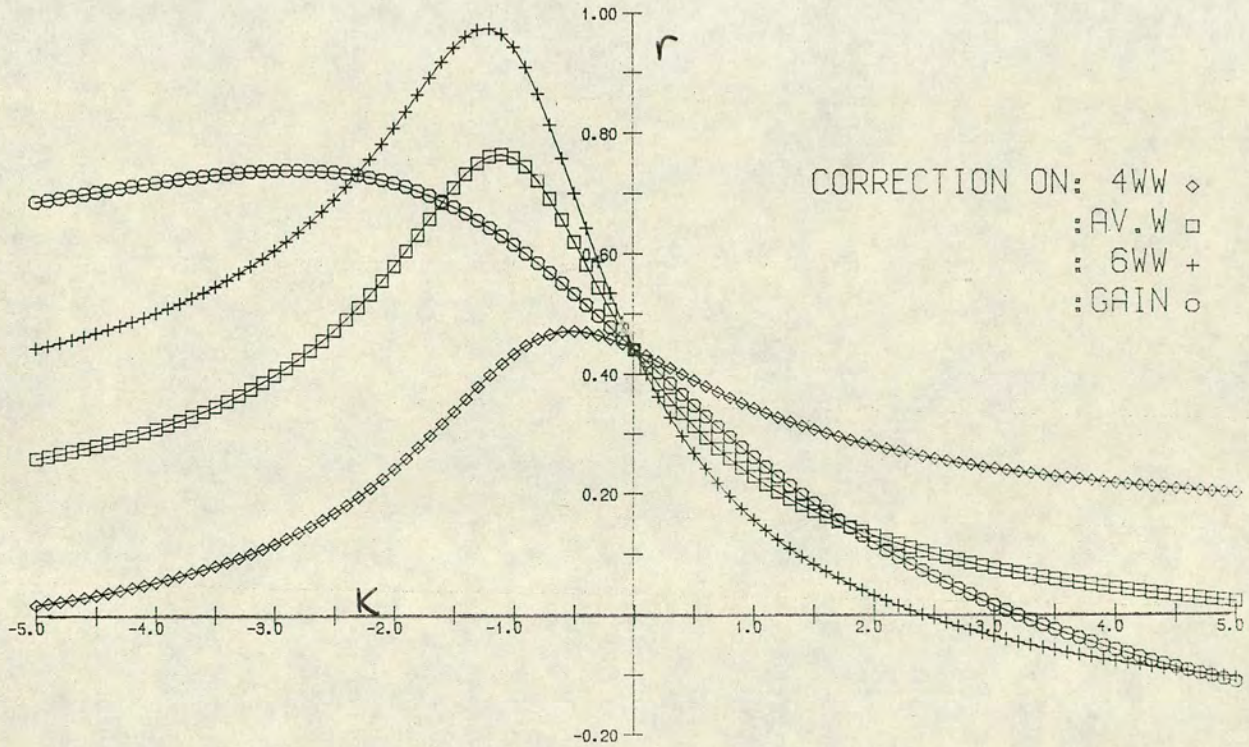


Fig 5.10 CORRELATION OF CAT/LM AND CORRECTED FI vs K



body weight is very highly correlated with CAT/BW and CAT/LM, respectively. The standard error of the k values maximising the correlations between the indices and CAT/LM were bootstrapped to determine the variability of these points. The results are as follows:

correction on 4WW, correlation = .47, k = - .50 s.e. = .18
AV.W, correlation = .77, k = -1.11 s.e. = .07
6WW, correlation = .97, k = -1.12 s.e. = .02

Finally, in fig. 5.11 the individual values of energy used to deposit fat are plotted against individual EFORGN. EFORGN is of course comprised of the energy required to deposit fat plus the energy required to deposit lean. The line labelled L is the linear regression of energy used to deposit fat on EFORGN, and formal analyses also reveal a small but significant quadratic regression effect. The F line is the average of the equivalent regressions for the FH and FL lines in section 4, corrected for the fact that the measurement period in this study was 14, as opposed to 18, days.

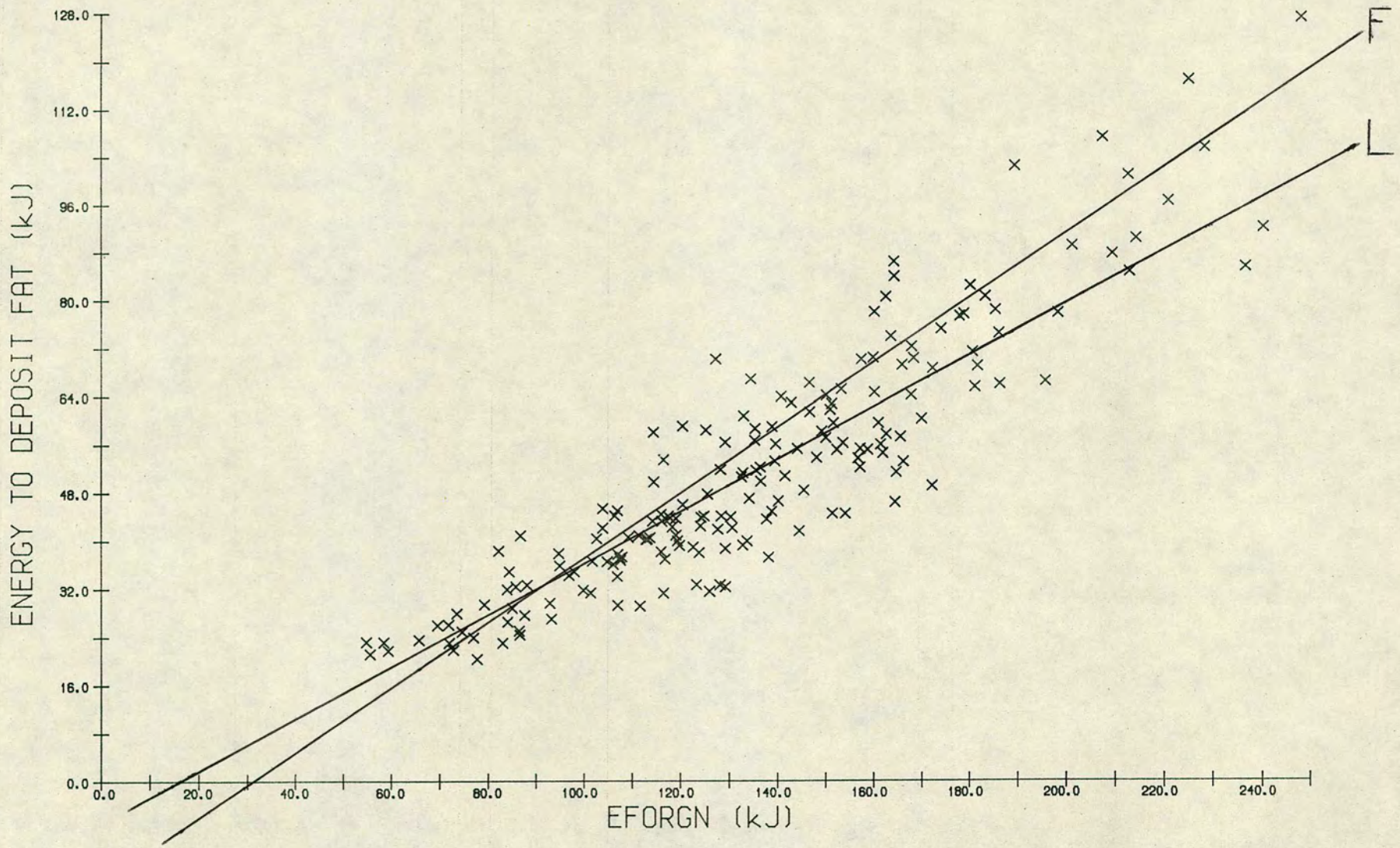
5.4 DISCUSSION

5.4.1 Food Intake and Carcass Composition

The hypothesis that the restriction on 4WW caused the "unexpected" carcass composition changes in the A lines appears to be correct, as both FAT% and DM% show a rapid decrease in their correlations with the selection index, as the value of k becomes more negative. The results for DM% perhaps agree more closely with the A line results than the FAT% results do, however as was mentioned above, DM% may be a more reliable indicator of carcass fatness than FAT% in this study.

A tentative explanation for this restriction effect was proposed in section 2, and from the results of the subsequent investigations it would still appear to be valid. It is as follows: maintenance requirements have been shown to comprise the greater part of a growing mouse's intake, and they also appear to vary in proportion to lean mass (or lean mass$^{.75}$) rather then body weight (sections 2 and 3). Therefore, the mice with the greater intake and hence maintenance requirements, at the same 4WW, will tend to be the leaner mice. These

Fig. 5.11


ENERGY TO DEPOSIT FAT VS EFORGN
ENERGY TO DEPOSIT FAT (kJ)
128.0
112.0
96.0
80.0
64.0
48.0
32.0
16.0
0.0
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0
160.0
180.0
200.0
220.0
240.0
EFORGN (kJ)
F
L

will have been the mice selected by the A line criterion.

The results obtained in this study also provide an explanation for the replicate and generation differences in carcass composition in the A lines. The results from the 17 week carcass composition analyses show that whilst in replicates 1 and 3 the AH lines were leaner than the AL lines, in replicate 2 the reverse is true. In addition, the composition trends appear to have changed slightly over the generations of selection. In general, the H-L and C-L differences do not appear to have increased with generation of selection (section 2) as is generally expected, and they may actually have decreased after perhaps generation 10. This is demonstrated by the fact that whilst the composition determinations at generations 5 (S. Copeland, unpublished) and 7 (Sharp et al, 1984) showed the AL mice to be fatter the AC mice, by generation 14 this was no longer true (section 2). Moreover, the gonadal fat pad determinations on generation 20 mice (section 3.2) found no H-L differences.

With reference to these two points, it can be observed from figs. 5.1 and 5.2 that the correlations between the index and carcass fatness are extremely sensitive to the values of k (or b) in the index. In other words, a very small change in the k value, or the regression coefficient b, has a large effect on the correlation obtained. Thus even small initial sampling differences between replicates would cause large differences in the expected carcass composition changes. In addition, the A line criterion has remained constant throughout the selection experiment, whereas with selection on FI and 4WW the b value (the regression of FI on 4WW) would have been expected to change -both within and between lines. Therefore, by the later generations the criterion probably differed in its true effects from that initially used, and thus slight changes in the patterns of the carcass composition responses may well have been expected to occur as the generations of selection proceded. To answer this conjecture a study of the within line relationships between FI and 4WW, for each generation of the A lines, could perhaps be undertaken. In contrast to the A line criterion an index comprising, for example, FI plus 4WW would have given more predictable responses in carcass composition, between both replicates and generations.

Restricting AV.W or 6WW, instead of 4WW, when selecting for FI would

have lead to a progressively increased fat deposition. Presumably this results from a trade off between (i) increasing lean percentage or mass to increase maintenance requirements, and (ii) increasing fat deposition as a means of increasing intake above maintenance without greatly increasing body size. This latter effect would become more important as the age at which weight is restricted increases.

5.4.2 Further Results

5.4.2.1 Simple correlations

From sections 2 and 3 it was concluded that (i) maintenance and intake in excess of maintenance vary independently of each other, (ii) maintenance itself is proportional to lean mass rather than bodyweight, and (iii) the intake ratio (CATRAT) is far more important than carcass composition in defining efficiency for mice. Given (i), the intake ratio should be independent of intake, and therefore intake and efficiency should be uncorrelated in a given population (as was implied by the A line criterion). Verifications of most of these conclusions can be seen in the correlations in table 5.1.

Firstly, as predicted by (i), the correlation between CATAB and EFORGN (r=-.10) is not significantly different from zero, and in agreement with (iii), EFF and CATRAT are very strongly correlated (r=.90). FI is highly correlated with CATAB (r=.85) and EFORGN (r=.43), however as predicted it is uncorrelated with CATRAT (r=.04) and EFF(r=.00). The conclusions about the relationships between intake, maintenance, intake in excess of maintenance and efficiency therefore appear to be true at the phenotypic level, within a population.

The relationships concerning carcass composition and maintenance (or catabolism) are not so pronounced, due to the small differences and high correlation (r=.98) between lean mass and body weight. They can be seen, however. Firstly, despite the very high correlation (r=.98) between CAT/BW and CAT/LM, the correlations of FAT% and DM% with (i) CAT/BW and (ii) CAT/LM differ from each other. Although by definition the correlations of FAT% and DM% with CAT/LM must be more positive than those with CAT/BW, the former correlations do not differ from

zero whereas the correlation of FAT% with CAT/BW is negative -as expected. Secondly, whilst DM% is positively correlated with AV.W, DM% and FAT% are not significantly correlated with either CATAB or AV.LM - in agreement with the F line results. Thirdly, the partial correlations of CATAB with AV.LM, given a constant AV.W is positive (r=.21), whereas the partial correlation of CATAB with AV.W, given a constant AV.LM, is zero (r=-.Ø5). The same trends can be shown for the partial correlations of CATAB and FAT%, given constant AV.W's and AV.LM's, respectively. It does appear, therefore, that maintenance requirements are more closely related to lean mass than to body weight, although lean mass and bodyweight are of course similar and highly correlated.

5.4.2.2 Index correlations

Figs. 5.1 to 5.1Ø will now be discussed. The main features of figs. 5.1 and 5.2 have been discussd, however there are still two points of interest. Firstly, an index of FI corrected for GAIN would presumably result in a greater increase in fatness than would FI per se, in contrast to the indices restricting body weights. This is simply because depositing energy as fat is a means of increasing intake without greatly increasing GAIN. Secondly, the older the mouse the greater the correlation between fatness and body size. This is a demonstration of the "Clarke effect" described in section 1.3.2.2.

The correlations of FI with the indices are shown in fig 5.3. It can be seen that the correlation of the A line criterion with FI is approximately .7Ø, and thus about 3Ø% of the potential increase in FI may have been lost by restricting 4WW. Indices using AV.W and 6WW have similar effects, although the index using gain is less sensitive to changes in k. From fig. 5.4 it is apparent that GAIN would be increased by restricting 4WW, in other words the A line criterion would select (and has selected) mice growing quickly from 4 to 6 weeks. If AV.W were to be restricted (i.e. k=-1) there would be little effect on gain, as compared to selection on FI per se, and restricting 6WW would reduce GAIN. EFORGN (fig. 5.8) shows similar trends to GAIN, as would be expected, with the discrepancies being accounted for by the type of tissue being deposited (fat or lean) -and, of course,

errors in the assumptions used to calculate EFORGN.

The A line criterion would be expected to increase efficiency (fig. 5.5) from 4 to 6 weeks of age (as was observed by Sharp et al,1984) whereas, predictably, an index restricting 6WW would be expected to reduce efficiency -due to a decreased GAIN. Selection indices incorporating AV.W would not affect EFFIC at all, as selection would presumably put equal pressure on CATAB and EFORGN (as both are closely related to AV.W). The index incorporating GAIN shows predictable trends. The correlations for CATRAT (fig. 5.6) show almost exactly the same patterns as those of EFF, and this again underlines the equivalence of these two traits. The discrepancies between the two graphs are again due to the differences in the costs of fat and lean deposition, and errors in the assumptions made.

In fig. 5.7 the trends for CATAB can be seen, and two features are of interest. Firstly, FI corrected for GAIN is almost perfectly correlated with CATAB -as this is almost the definition of catabolism. Secondly, restricting 6WW would have had a neglible effect on CATAB, compared to FI per se, whereas restricting 4WW has a major effect. These results are put in perspective, however, by the CAT/BW and CAT/LM results.

Figs. 5.9 and 5.1Ø show the correlations of the indices with CAT/BW and CAT/LM, respectively, and the correlations for CAT/LM are nearly always slightly greater than those for CAT/BW. The outstanding feature of the graphs are the very high correlations of FI corrected for AV.W and 6WW with CAT/BW and CAT/LM, respectively. The standard errors of the k values maximising these correlations (see results) are very small, indicating that these points are quite precisely defined. These indices would therefore be suitable criteria for future selection experiments designed to study maintenance requirements. It can be seen, however, that the A line criterion and FI per se have similar correlations with these catabolism traits, and thus possibly nothing was gained in terms of the response in maintenance requirements in the A lines, by restricting 4WW. The index using GAIN is somewhat insensitive to changes in the k value, as would be expected from the lack of correlation between maintenance and intake in excess of maintenance.

Several points can be drawn from these investigations. Firstly,

changing the value of k from Ø to -1 nearly always has dramatic effects on the correlated responses expected, and thus the A line criterion is quite different from FI per se in terms of the expected correlated responses to selection. Secondly, if it is desired to alter FI in relation to body weight, the choice of the reference weight, e.g. 4WW, 6WW etc, has dramatic effects in terms of the expected correlated responses. Indices correcting FI for GAIN would appear to be less sensitive to changes in k. Thirdly, and importantly, it appears that an explanation for most of these "static" correlations obtained can be invoked from the metabolic growth model used in these studies. This adds further confidence to the use of this model and the description of growth in simple input and output terms. Finally, from these results it is possible to suggest selection criteria for further experiments, for the areas in which selection experiments might be appropriate. The obvious example is FI corrected for 6WW (k = -1.12) to select for increased maintenance requirements, as this probably is a suitable area.

The first and second points highlight the care that must be taken when generalising the results of selection for a criterion such as the A line index. Criteria such as these which combine both input and output components of growth appear to have quite different implications from selection for FI per se.

5.4.2.3 Energy partition in excess of maintenance

Fig. 5.11 represents the attempt made to verify the conclusions drawn in section 4 concerning the partition of energy in excess of maintenance between lean and fat deposition. These studies differed in two important respects. Firstly, the experimental techniques for the two experiments were quite different. In section 4, the mice had restricted intakes and the mean value of energy to deposit fat was close to zero, whereas in this study all mice were fed ad libitum and the estimated fat gains were much larger. Secondly, the assumptions used to calculate fat and lean gain in the two experiments somewhat different. These differences will obviously affect the absolute values of EFORGN and energy to deposit fat, so it is the overall trends that are of more interest. The averaged regression line from the section 4

study is plotted so that these trends can be compared.

It can be seen that similar, but not identical, trends have emerged from these two investigations, with the section 4 regression line passing through the observed points in this study. Although the two regression lines do differ significantly from each other, the values of EFORGN at which fat deposition commences (i.e. the X axis intercepts, 16.25 for the L line, 40x14/18 = 31.11 for the F line) do not differ significantly from each other. Much higher levels of intake can be seen in this experiment than were studied in section 4, and it appears that the proportion of energy deposited as fat may increase slightly as energy intake becomes very large. This could not be shown in section 4, however it is shown in this experiment by the small but significant quadratic regression effect, and it is in agreement with generalised models of pig growth (C.T.Whittemore, pers. comm.) A final point of interest is that although energy to deposit fat and EFORGN appear to be quite closely related (r = .88), the results from figs. 5.7 and 5.8 do imply exploitable variation between individuals. For example, for FI corrected for 4WW a large increase in EFORGN is predicted, whereas with the index of FI corrected for 6WW a decrease in EFORGN may be expected -however both indices have similar predicted carcass composition changes. In summary, the most important finding from this section is the fact that the results appear to be compatable with those concerning energy partition in section 4. The fact that the results obtained are quite dependent upon the assumptions made, unfortunately makes it difficult to draw stronger or more definite conclusions.

5.4.3 Summary

Satisfactory answers appear to have been obtained for both sections of this study.

Firstly, the hypothesis was made that the unexpected carcass composition changes in the A lines were a result of the corrections applied to 4WW, and this hypothesis was found to be correct. Although it has not been possible to demonstrate that the phenotypic correlation between carcass fatness and the A line criterion is negative, this correlation is nevertheless significantly less than the

correlation between carcass fatness and FI per se. Furthermore, this correlation between carcass fatness and FI corrected for 4WW is extremely sensitive to changes in the degree of correction employed, and this could explain the inconsistent carcass composition results in the A lines, both between replicates and between generations.

More generally it has also been possible to demonstrate the effects of placing a restriction on an output component of growth, i.e. body weight per se, when selecting for the input component, FI. It appears that indices of FI per se and FI in relation to body weight have quite different implications from each other in terms of their correlated responses.

Secondly an attempt was made to verify at the phenotypic level some of the relationships between the components of growth implied by the results from sections 2, 3 and 4. In general this has been successful, and there are no major discrepancies between these results and the findings from those studies. The most important results are as follows:

(i) The relationships between intake, catabolism, intake in excess of catabolism and efficiency, concluded from section 2, were found to be true in this population.

(ii) It was possible to show that catabolism was more closely related to lean mass than to body weight, however the improvement in the relationship was only small -presumably because lean mass comprises approximately of 91% of body weight, anyway.

(iii) The patterns of energy partition between lean and fat deposition were found to be in general agreement with those observed in section 4. These patterns were studied at a very crude level, however, being very dependent on the assumptions used in the calculation of fat and lean deposition, so no further conclusions could be drawn.

(iv) From the general investigations into the correlations between various traits of interest and different selection criteria, it was found that the simple metabolic, or components of growth, model was adequate to explain most of the results. This model can therefore be viewed with confidence as an adequate descriptor of the growth of a mouse.

Section VI GENERAL DISCUSSION

6.1 BACKGROUND

The primary aim of this thesis has been to study some of the genetic aspects of growth in mice, and from the results obtained to derive general relationships or patterns which could be extrapolated to other species. So far the growth of mice has been studied, but as yet little attempt has been made to extrapolate the results to other species. In this section, therefore, the results which have been obtained will be summarised and their relevance to other species will be discussed.

From the general consideration of growth in section 1 it was decided to describe growth within a metabolic framework, and therefore to design and analyse all the experimental investigations within this framework. The model used to describe growth, or the usage of energy for growth, is shown in fig. 1.3 and it describes growth in terms of input and output components. The input component is of course food intake, and the major output components are maintenance requirements and fat and lean gain.

The mice used as the experimental units for these investigations, the A, P and F lines, are excellent material for investigations of this type as they represent lines of mice selected for, and differing widely in, these input and output components of growth. The A line results do need to be treated and interpreted with some caution, however, as although they have been selected primarily for intake, their selection criteria is confounded with body weight -an output component. In section 5 it was demonstrated how this confounding can have large effects on the expected correlated changes in the components of growth. The P and F lines have been selected for absolute and relative output components of growth, and thus they are free of these confounding effects.

6.2 EXPERIMENTAL FINDINGS

Firstly consider the effects of selecting for the input components of growth, i.e. the A lines. Notwithstanding the comments made in section 6.1 about the A line criterion, in section 2 it was shown that changing intake appears to change estimated maintenance requirements and intake in excess of maintenance, proportionately. These results

were verified by the fasting heat production differences between the AH and AL lines shown in sections 3.1 and 3.3. The A lines therefore demonstrate additive genetic variation for maintenance requirements - a result which has been implied (e.g. from the results of selection for efficiency), but rarely demonstrated, in mice.

The A lines appear to have between line variation in their maintenance requirements over and above that accounted for by lean mass changes (the F and P line result), however, so in sections 3.2. and 3.3 possible causes of these differences were studied. It was hypothesized that the changes in their maintenance requirements may be associated with either changes in the active component of brown adipose tissue or temperature adaptation effects. Neither factor was found to be important, however, so the reasons for the observed fasting heat production and maintenance changes still have to be resolved.

Finally, the possible effects of a restriction on body weight whilst selecting for food intake were studied in section 5. The importance of the results obtained in this section was to explain some of the anomalies and inconsistencies in the A line results, and to demonstrate the effects of confounding intake with body weight when selecting for intake, rather than to make new findings about the components of growth. It was successfully demonstrated that the A line criterion could conceivably lead to decreased, rather than increased, carcass fatness with upward selection, but it was also shown how the expected change could be inconsistent between lines.

Secondly consider the P lines. Selection for estimated lean mass has resulted in large changes in lean mass, but small and often insignificant changes in carcass composition and in food intake, maintenance requirements and fasting heat production in relation to metabolic body weight. Small changes in relative food intake and maintenance requirements obviously have occurred, however, as the intake ratio (intake/maintenance) has been changed during the fast growing periods (to allow the changes in body size), but it is not clear which component is the greater contributer. Changing the lean mass output component of growth therefore appears to do precisely as intended, i.e. it changes lean mass, but it leaves the other components largely unchanged.

Lastly consider the effects of selecting for estimated fat percentage, i.e. the F lines. It was found in section 2 that although this selection had resulted in large changes in fat weight and percentage, actual lean mass remained unchanged. Furthermore, it was found that total maintenance requirements were unchanged, and hence that maintenance requirements in relation to lean mass were also unchanged. Verification of this finding was obtained in section 3.1. In other words, changing fat percentage has merely changed intake in excess of maintenance.

The investigation in section 4 was undertaken to study how the patterns of energy partition change as the absolute level of intake in excess of maintenance, and more importantly, the proportion of this energy deposited as fat, are genetically changed. It was found that the fatness changes were created by a complex interaction of food intake and energy partitioning changes. Although the FH and FL lines do partition their energy differently between lean and fat deposition, above the level at which fat deposition commences (which was equivalent for the two sets of lines), they nevertheless continue to have ad libitum intakes which allow the same total quantity of lean tissue to be deposited.

The implications of these A, P and F line results in terms of the relationships between the components of growth have been discussed throughout the thesis, and they will now be briefly summarised.

Firstly, there appears to be genetic variation for all of the components of growth. This is the fundamental observation around which the results and conclusions of this thesis are drawn.

Secondly, many of the components appear to be able to vary independently of each other, in other words they may be uncorrelated, or only weakly correlated. This has been demonstrated in many of the studies. For example, the F lines show that intake in excess of maintenance can be changed without changing maintenance requirements, whereas in the A lines both components are changed proportionately. The P and F lines also demonstrate that it is possible to change either lean mass or carcass composition and yet leave the other component unchanged.

Thirdly, maintenance requirements have been shown to be more closely related to lean mass than than to body weight. Variation still exists

in maintenance over and above that explained by lean mass (for example in the A lines), however, but possible causes of this variation have yet to be found.

Fourthly, changes in the usage of energy in excess of maintenance which lead to carcass composition changes are a complex interaction of food intake and energy partition effects, with both factors being important. Increasing fatness will increase intake as well as causing proportionately more energy to be partitioned towards fat deposition.

Conclusions 2, 3 and 4 are obviously closely interrelated. For example, if lean mass is to remain unchanged with increasing fat content, then intake in excess of maintenance must increase regardless of whether or not partitioning changes occur. In addition, if total maintenance requirements and total lean mass are highly correlated, and if fat content is uncorrelated with one of these components, then fat content should also be uncorrelated with the other component.

Fifthly and finally, it has been shown for mice that efficiency is closely related to the intake ratio, and that the type of tissue being deposited is a far less important determinant of efficiency. To demonstrate this, the A lines show little change in either the intake ratio or efficiency, whereas the P and F lines have corresponding changes in both. For the F lines, however, it is the fatter FH lines which have the greater intake ratio and are the more efficient - despite the fact that fat is energetically more expensive to deposit than lean.

These results will now be discussed in relation to previously reported experiments from mice and other domestic species.

6.3 COMPARISONS WITH OTHER STUDIES

The results obtained in these studies appear to be totally compatable with those of the experiments described in the review of literature, for mice, and the relationships described above can account for the previously reported correlated responses. For example, consider selection for body weight per se. The results from these studies would predict a general increase in intake in excess of maintenance, fatness and efficiency, but little change - or perhaps slight decreases - in maintenance requirements. These appear to be

precisely the changes that have occurred in most of the reported studies. The experimental results also back the tentative suggestions made in the review of literature to account for the correlated responses to selection for efficiency. In most studies the expectation prior to selection had been that leaner mice would be obtained, however the reverse usually appears to have happened, with actual increases in fatness accompanying the increases in efficiency. The explanation for these results is that the mice probably became more efficient by increasing the proportion of their energy in excess of maintenance deposited as fat, and thereby reducing their relative maintenance requirements. This in turn would increase their intake in excess of maintenance, their intake ratio and hence their efficiency, at any given level of intake.

Of greater importance than the comparisons with other mouse experiments, however, is how the obtained results compare with those of other species. In the review of literature several differences between species in the correlated responses were apparent when selection was for individual components of growth. For example, compared to mice increasing intake in poultry increased maintenance requirements and fatness but it reduced efficiency; increasing fatness reduced efficiency rather than increase it, and increasing efficiency reduced maintenance requirements but it also reduced fatness. In pigs it appears that increased efficiency is associated with a reduced fatness, a reduced intake, but an increase in maintenance requirements. Whilst these differences between mice, poultry and pigs seem to be large, it may be possible to use the results outlined above to account for them.

When the relationships between the components of growth for mice are compared to those which may be relevent to, for example, pigs, it is found that all relationships could conceivably be the same except for the absolute level of intake in relation to maintenance, i.e. the intake ratio. Pigs appear to have a much larger intake ratio than do mice during the fast period of growth (e.g. 3.Ø vs 1.3), and hence they are much more efficient. This difference in intake ratios will affect the expected relationships between intake, carcass composition and efficiency in the following way:

Mice have a small intake ratio and they become more efficient by

depositing more and more fat simply because after 4 weeks of age their efficiency (at any given level of intake) generally appears to be less than the efficiency of fat deposition (1g fat/53.1kJ, or .2 g/g given the experimental diet). Therefore, ingesting an extra unit of energy and depositing it as fat will increase their efficiency. Pigs, on the other hand, with an efficiency greater than this value, would become less efficient by depositing an additional unit of energy intake as fat.

A small scale investigation to test the validity of extrapolating these results obtained for mice to pigs, by changing only the intake ratio, was undertaken using an interactive computer model of the growth of an animal from 4 to 6 weeks of age. Firstly, the growth of a mouse was modelled using the values for maintenance and the intake ratio obtained in section 2 (average ad libitum intake ratio = 1.27). Maintenance was assumed to be a fixed cost per unit of either $BW^{.75}$ or $LM^{.75}$, and was calculated daily for the current BW or LM. The absolute value of the intake ratio followed the trends from 4 to 6 weeks of age observed in section 2, and the results from section 4 were used to model the partition of energy between lean and fat deposition. The assumption was made that lean deposition ceases at normal ad libitum intake, and that only fat deposition occurrs with increments of energy intake above this level.

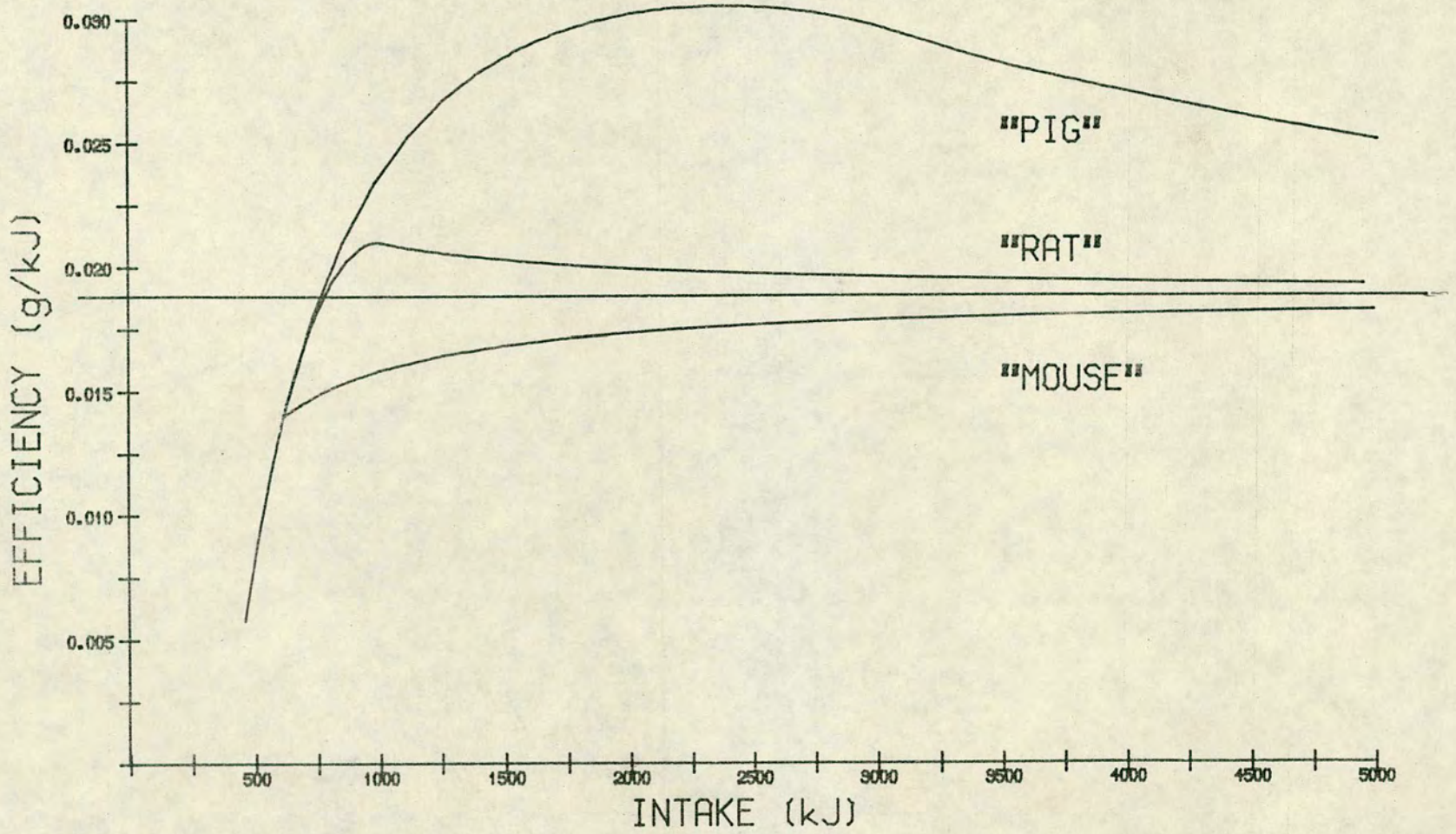
This model was then ammended, altering the assumptions about the intake ratio, to create an animal which "ate like a pig" (average ad libitum intake ratio approximately 3.4) and an intermediate animal (a "rat", average ad libitum intake ratio approximately 1.8). For the "pig" model it was assumed that as intake approached ad libitum mainly fat was deposited, and above ad libitum intake only fat was deposited. The "rat" model used the same assumptions as the mouse model.

Fig. 6.1 shows the results of this study, for the model assuming maintenance to be proportional to lean mass, with efficiency (g/kJ) being plotted against intake (kJ). (N.B. for the mouse model the intake, growth and efficiency results were in agreement with those of sections 2 and 5). When maintenance was assumed to be related to BW rather than LM similar overall trends were observed.

Consider, firstly, the line depicting the efficiency of a normal mouse. As expected, efficiency increases as intake and fat deposition

Fig 6.1

EFFICIENCY vs INTAKE
For the "PIG", "RAT" and "MOUSE" MODELS



increase (lean deposition ceases at ad libitum intake i.e. 612.7kJ), but it never maximises. As intake becomes very large, however, efficiency approaches the value of .0188, which is of course 1/53.4 - the efficiency of depositing fat. Consider now the mouse which "eats like a pig". There are three points of interest for this genetically engineered creature. Firstly it has an efficiency much greater than the .0188 value, secondly its efficiency is maximised somewhat below ad libitum intake, and thirdly it has a negative relationship between efficiency and intake at and around ad libitum intake values (ad libitum intake is approximately 3100 kJ). These are the same efficiency patterns as those demonstrated for pigs by Davies and Lucas (1972a). Again the limiting efficiency is .0188 as the rate of fat deposition becomes very large. Finally, the mouse which "eats like a rat" shows intermediate trends (ad libitum intake approximately 1000 kJ), with efficiency once again approaching .0188 as intake becomes very very large.

These findings are in agreement with the results obtained for both mice and pigs, and they therefore demonstrate the effects that the magnitude of the intake ratio has on intake, carcass composition and efficiency. For animals with a low inherent rate of lean gain (i.e. mice) the intake ratio is more closely related to efficiency than is the type of tissue being deposited, whereas for animals with a high inherent rate of lean gain the type of tissue being deposited may be more important than the intake ratio.

The results quoted above for poultry are compatable with those for pigs if, for poultry, there is more variation in maintenance requirements over and above that explained by lean mass. This would explain the inconsistency that for pigs lean mass and maintenance requirements appear to change in the same direction with selection, whereas for poultry increases in lean mass appear to be accompanied by decreases in maintenance requirements, with selection for increased efficiency.

Finally, the results and patterns discussed in this section are quite general, and more emphasis has been placed on the relative level of intake than such factors as age or degree of maturity. These two factors are intrinsic to the model, however, as the intake ratio and the partition of energy between lean and fat are a function of both

age and maturity. Therefore the effects of, for example, the age at selection on the expected correlated responses, can easily be studied in a model using the obtained results. In addition, the investigations made in this thesis show the relationships between the components of growth from weaning until maturity, so the complete growth of a mouse from weaning until maturity can be modelled using the observed results.

6.4 GENERAL IMPLICATIONS

Several genetic relationships between the components of growth have been discovered or verified for mice, and an important finding is that it does appear to be possible to extrapolate these findings to other species. For example, merely by modelling the growth of a mouse, but altering the assumptions about its intake ratio and hence its inherent rate of lean growth, a mouse with the intake and efficiency patterns of a pig was obtained. It therefore appears to be valid to extrapolate findings between species -provided care is taken. The use of laboratory animals to model the growth of domestic animals is thus justified.

The observation of genetic variation for all the components of growth (including maintenance), and the fact that they generally appear to be able to vary independently of each other, obviously has implications for animal breeding. The major complicating factor is, however, the fact that lean mass and maintenance requirements are strongly correlated, as in practical situations it may be desired to increase lean mass and percentage yet reduce maintenance requirements. If it is desirable to select for increased lean mass or decreased fatness, then the overall gains from selection will be a tradeoff between the (desirable) increase in lean mass or percentage and the (undesirable) increase in the costs of maintaining this lean mass. Although the overall effects of this tradeoff will vary between species, being dependent to a large extent on the intake ratio of each species, they will still offset much of the predicted gain from selection.

The results that have been obtained and their implications also highlight the fact that care should also be taken when formulating

selection indices to "improve" animals. For example, with pigs a negative correlation between intake and fatness may be true of unselected populations, however after selection for increased efficiency or decreased intake or partition of energy towards lean, the rate of fat gain may be reduced to such an extent that the correlation becomes positive. Once this happens the old index would no longer be appropriate and a new index would have to be formulated.

In conclusion, therefore, whilst selection strategies (and hence selection indices) for the improvement of domestic animals can and perhaps should be derived, the relationships between the components of growth must always be taken into consideration when these strategies or indices are being used. As each of these components of growth are gradually changed by selection, the biological relationships and statistical correlations between the components will change and it will become necessary to formulate new selection objectives and strategies, and hence new selection indices. It is from experiments on laboratory animals, such as those that have been described in this thesis, that the important relationships between the components of growth will be discovered. It is also from such experiments that it will be possible to deduce the effects that changing the individual components will have on the overall relationships.

REFERENCES

Abplanalp,H; F.X.Ogasawara and V.S.Asmundson(1963). Influence of selection for body weight at different ages on growth of turkeys. British Poultry Science,4:71-82

Agricultural Research Council(1981). The Nutrient Requirements of Farm Livestock. No.2. Ruminants. Agricultural Research Council,London.

Agricultural Research Council(1982). The Nutrient Requirements of Farm Livestock. No.3. Pigs. Agricultural Research Council,London

Ahmed,Abdel Aziz M.T.(1982). Effects of selection on the energetic efficiency of growth. Ph.D. Thesis. University of Bristol.

Allen,P. and J.C.McCarthy(1980). The effects of selection for high and low body weight on the proportion and distribution of fat in mice. Animal Production,31:1-11

Atkinson,T;V.R.Fowler;G.A.Garten and A.K.Lough(1972). A rapid method for the accurate determination of lipid in animal tissues. Analyst,97:562-567

Baker,R.L. and A.B.Chapman(1975). Correlated responses to selection for postweaning gain in the rat. Genetics,80:191-203

Bateman,N. and J.Slee(1979). Growth,food intake and cold exposure in mice. Animal Production,28:157-170

Biondini,P.E.;T.M.Sutherland and L.H.Haverland(1968). Body composition of mice selected for rapid growth rate. Journal of Animal Science,27:5-12

Blaxter,K.L.(1962). The Energy Metabolism of Ruminants. Hutchinson. London.

Blaxter,K.L.(1979). Use of Energy for Maintenance and Growth. Proceedings of 8th Symposium on Energy Metabolism, E.A.A.P. pp183-187. Butterworth, London.

Boshowers,F.M.G. and Elly Nicaise(1981). Measurement of the respiratory metabolism of the fowl. British Poultry Science,22:52-69

Brown,M.A. and R.R.Frahm(1975). Feed efficiency in mice selected for preweaning and postweaning growth. Journal of Animal Science,41:1002-1007.

Chaffee,R.J. and J.C.Roberts(1971). Temperature acclimation in birds and mammals. Annual Review of Physiology,33:157-197

Conolty,N.L. and L.J.Koong(1976). Utilization of energy for maintenance and for

fat and lean gains by mice selected for rapid postweaning growth rate. Journal of Nutrition,106:1202-1208

Clarke,J.N.(1969). Studies on the genetic control of growth in mice. Ph.D. Thesis. University of Edinburgh.

Crooke,W.M. and W.E.Simpson(1971). Determination of ammonium in Kjeldahl digests of crops by an automated procedure. Journal of the Science of Food and Agriculture,22:9-10

Davies,J.L. and I.A.M.Lucas(1972). Responses to variation in dietary energy intakes by growing pigs. 2. The effects on feed conversion efficiency of changes in level of intake above maintenance. Animal Production,15:117-125

Davies,J.L. and I.A.M.Lucas(1972). Responses to variation in dietary energy intakes by growing pigs. 3. Effect of level of intake of diets of differing protein and fat contents on the performance of growing pigs. Animal Production,15:127-137

Diaconis,P. and B.Efron(1983). Computer-intensive methods in statistics. Scientific American,248:96-108

Dickerson,G.E.(1978). Animal size and efficiency:basic concepts. Animal Production,27:363-379

Efron,B.(1982). The Jackknife, the Bootstrap, and Other Resampling Plans. Society for Industrial and Applied Mathematics, Philadelphia, Pennsylvania. J.W.Arrowsmith Ltd.

Eisen,E.J.(1976). Results of growth curve analyses in mice and rats. Journal of Animal Science,42:1008-10023

Eisen,E.J.(1977). Restricted selection index:an approach to selecting for feed efficiency. Journal of Animal Science,44:958-972

Eisen,E.J. and T.Bandy(1977). Correlated responses in growth and body composition of replicated single-trait and index selected lines of mice. Theoretical and Applied Genetics,49:133-144

Eisen,E.J;B.J.Lang and J.E.Legates(1969). Comparisons of growth functions within and between lines of mice selected for large and small body weight. Theoretical and Applied Genetics,39:251-260

Ellis,M;W.C.Smith;R.Henderson;C.T.Whittemore and R.Laird(1983). Comparative performance and body composition of control and selection line large white pigs. 2.Feeding to appetite for a fixed time. Animal Production,36:407-413

Ellis,M;W.C.Smith;R.Henderson;C.T.Whittemore;R.Laird and P.Phillips(1983).

Comparative performance and body composition of control and selection line large white pigs. 3.Three low feeding scales for a fixed time. Animal Production,37:253-258

Falconer,D.S.(1953). Selection for large and small size in mice. Journal of Genetics,51:470-501

Falconer,D.S.(1973). Replicated selection for body weight in mice. Genetical Research,22:291-321

Falconer,D.S.(1981). Introduction to Quantitative Genetics. Second Edition. Longman. London and New York.

Farrell,D.J;S.I.Atmamihardja and R.A.E.Pym(1982). Calorimetric measurements of the energy and nitrogen metabolism of Japanese Quail. British Poultry Science,23:375-382

Fowler,R.E.(1962). The efficiency of food utilisation, digestibility of foodstuffs, and energy expenditure of mice selected for large or small body size. Genetical Research,3:51-68

Fowler,V.R;M.Bichard and A.Pease(1976). Objectives in pig breeding. Animal Production,23:365-387

Frahm,R.R. and M.A.Brown(1975). Selection for increased preweaning and postweaning weight gain in mice. Journal of Animal Science,41:33-42

Frisch,J.E. and J.E.Vercoe(1980). Changes in fasting metabolism in cattle as a consequence of selection for growth rate. in Energy Metabolism, L.E.Mount ed. Butterworths, London. pp431-434

Gall,G.A.E. and W.H.Kyle(1968). Growth of the laboratory mouse. Theoretical and Applied Genetics,38:304-308

Gunsett,F.C;D.H.Baik;J.J.Rutledge and E.R.Hauser(1981). Selection for feed conversion on efficiency and growth in mice. Journal of Animal Science,52:1280-1285

Hayes,J.F. and J.C.McCarthy(1976). The effects of selection at different ages for high and low body weight on the pattern of fat deposition in mice. Genetical Research,27:389-403

Henderson,Ruth.C;C.T.Whittemore;M.Ellis;W.C.Smith and P.Phillips(1983). Comparative performance and body composition of control and selection line large white pigs. 1.On a generous fixed feeding scale for a fixed time. Animal Production,36:399-405

Hetzel,D.J.S. and F.W.Nicholas(1978). Growth and body composition of mice selected for growth rate under ad libitum or restricted feeding. Proceedings of the Australian Society of Animal Production,12:194

Hetzel,D.J.S. and F.W.Nicholas(1982). Direct and correlated responses to selection for post-weaning weight gain on ad libitum or restricted feeding in mice. Theoretical and Applied Genetics,63:145-150

Hull,P(1960). Genetic relations between carcass fat and body weight in mice. Journal of Agricultural Science,55:317-321

Kielanowski,J.(1976). Energy cost of protein deposition. in Protein Metabolism and Nutrition,D.J.A.Cole ed. E.A.A.P. Publ.No.16. Butterworths,London.pp207-216

Kirkwood,J.K. and A.J.F.Webster(1984). Energy-budget strategies for growth in mammals and birds. Animal Production,38:147-155

Kownacki,M. and J.Keller(1978). The basal metabolic rate in selected and unselected mice. Genetica Polonica,19:339-343

Kownacki,M;J.Keller and E.Gebler(1975). Selection of mice for high weight gains -its effect on the basal metabolic rate. Genetica Polonica,16:359-363

Lacy,R.L. and Carol B. Lynch(1979). Quantitave genetic analysis of temperature regulation in mus musculus. I. Partitioning of variance. Genetics,91:743-753

Lang,B.J. and J.E.Legates(1969). Rate, composition and efficiency of growth in mice selected for large and small body weight. Theoretical and Applied Genetics,39:306-314

LaSalle,T.J;J.M.White and W.E.Vinson(1974). Direct and correlated responses to selection for increased postweaning gain in mice. Theoretical and Applied Genetics,44:272-277

Leclerq,B;J.C.Blum and J.P.Boyer(1980). Selecting broilers for low and high abdominal fat: initial observations. British Poultry Science,21:107-113

Lindberg,O.(1970). Brown Adipose Tissue. Elselvier, New York.

Lynch,Carol B. and R.C.Roberts(1984). Aspects of temperature regulation in mice selected for large and small size. Genetical Research,43:299-306

Lynch,Carol B. and D.S.Sulzbach(1984). Quantitave genetic analysis of temperature regulation in mus musculus II. Diallel analysis of individual traits. Evolution,38:527-540

Meltzer,A;G.Goodman and J.Fistol(1982). Thermoneutral zone and resting metabolic rate of growing white leghorn-type chickens. British Poultry Science,23:383-391

Miller,B.G;J.K.Kirkwood;K.Howard;A.Tuddenham and A.J.F.Webster(1981). A self-compensatory, closed circuit respiration calorimeter for small

mammals and birds. Laboratory Animals,15:313-317

McCarthy,J.C.(1982). The laboratory mouse as a model for animal breeding: a review of selection for increased body weight and litter size. 2nd World Congress on Genetics Applied to Livestock Production,5:66-83

McCarthy,J.C.(1984). Studies of the effects of selection for growth and efficiency in mice. Proceedings of the 26th British Poultry Breeders Roundtable Conference,7-28.

McCarthy,J.C. and H.Bakker(1979). The effect of selection for different combinations of weights at two ages on the growth curve of mice. Theoretical and Applied Genetics,55:57-64

McCarthy,J.C. and D.J.Doolittle(1977). Effects of selection for independent changes in two highly correlated body weight traits of mice. Genetical Research,29:133-145

McLellan,C.R. and R.R.Frahm(1973). Direct and correlated responses to two-way selection for hindleg muscle system weight in mice. Journal of Animal Science,36:442-451

McPhee,C.P. and A.R.Neill(1976). Changes in body composition of mice selected for high and low eight week weight. Theoretical and Applied Genetics,47:21-26

McPhee,C.P;P.C.Trappett;A.R.Neill and F.Duncalfe(1980). Changes in growth, appetite, food conversion efficiency and body composition in mice selected for high post-weaning weight gain on restricted feeding. Theoretical and Applied Genetics,57:49-56

Notter,D.R;G.E.Dickerson and J.A.DeShazer(1976). Selection for rate of lean gain in the rat. Genetics,84:125-144

Parks,J.R.(1970). Growth curves and the physiology of growth. American Journal of Physiology,219:833-843

Pennycuik,P.R.(1967). A comparison of the effects of a variety of factors on the metabolic rate of the mouse. Australian Journal of Experimental Biological and Medical Science,45:331-346

Proudman,J.A;W.J.Mellen and D.L.Anderson(1970). Utilization of feed in fast and slow growing lines of chickens. Poultry Science,49:961-972

Pullar,J.D. and A.J.F.Webster(1974). Heat loss and energy retention during growth in congenitally obese and lean rats. British Journal of Nutrition,31:377-392

Pullar,J.D. and A.J.F.Webster(1977). The energy cost of fat and protein deposition in the rat. British Journal of Nutrition,37:355-363

Pym,R.A.E.(1985). Direct and correlated responses to selection for improved food efficiency. in Poultry Genetics and Breeding. W.G.Hill, J.M.Manson and D.Hewitt eds. British Poultry Science Ltd. Longman. pp97-112

Pym,R.A.E. and D.J.Farrell(1977). A comparison of the energy and nitrogen metabolism of broilers selected for increased growth rate,food consumption and conversion of food to gain. British Poultry Science,18:411-426

Pym,R.A.E. and P.J.Nicholls(1979). Selection for food conversion in broilers: Direct and correlated responses to selection for body weight gain, food consumption and food conversion ratio. British Poultry Science,20:73-86

Pym,R.A.E. and A.J.Solvyns(1979). Selection for food conversion in broilers: Body composition of birds selected for increased body weight gain, food consumption and food conversion ratio. British Poultry Science,20:87-97

Ricard,F.H.(1975). Essai de selection sur la forme de la courbe de croissance chez le poulet. Annales de Genetique et de Selection Animale,7:427-443

Richards,F.J.(1959). A flexible growth function for empirical use. Journal of Experimantal Botany,10:290-300

Roberts,R.C.(1961). The lifetime growth and reproduction of selected strains of mice. Heredity,16:369-381

Roberts,R.C.(1981). The growth of mice selected for large and small size in relation to food intake and the efficiency of conversion. Genetical Research,38:9-24

Rothwell,N.J;M.E.Saville and M.J.Stock(1982). Effects of feeding a "cafetaria" diet on energy balance and diet-induced thermogenesis in four strains of rat. Journal of Nutrition,112:1515-1524

Rothwell,N.J. and M.J.Stock(1979). A role for brown adipose tissue in diet-induced thermogenesis. Nature,281:31-34

Saxton,A.M. and E.J.Eisen(1984). Genetic analysis of brown adipose tissue, obesity and growth in mice. Genetics,104:705-718

Sharp,G.L;W.G.Hill and A.Robertson(1984). Effects of selection on growth, body composition, and food intake in mice. 1. Responses in selected traits. Genetical Research,43:75-92

Simon,J. and B.Leclerq(1982). Longitudinal study of adiposity in chickens selected for high or low abdominal fat content: further evidence of a glucose-insulin imbalance in the fat line. Journal of Nutrition,112:1961-1973

Stanier,M.W. and L.E.Mount(1972). Growth rate,food intake, and body composition before and after weaning in strains of mice selected for mature body weight. British Journal of Nutrition,28:307-325

Stephenson,S.K. and R.C.Malik(1984). Energy partitioning and growth in mice selected for high and low body weight. Genetical Research,43:323-337

Sulzbach,D.S. and Carol B. Lynch(1984). Quantitative genetic analysis of temperature regulation in mus musculus. III. Diallel analysis of correlations between traits. Evolution,38:541-552

Sundstol,F;N.Stangen and O.Vangen(1979). Energy metabolism in lines of pigs selected for thickness of backfat and rate of gain. Acta Agriculturae Scandinavica,29:337-345

Sutherland,T.M.(1965). The correlation between feed efficiency and rate of gain, a ratio and its denominator. Biometrics,21:739-749

Sutherland,T.M;P.E.Biondini;L.H.Haverland;D.Pettus and W.B.Owen(1970). Selection for rate of gain, appetite and efficiency of feed utilization in mice. Journal of Animal Science,31:1049-1057

Sutherland,T.M;P.E.Biondini and G.M.Ward(1974). Selection for growth rate, feed efficiency and body composition in mice. Genetics,78:525-540

Taylor,StC.S.(1965). A relation between mature weight and time taken to mature in mammals. Animal Production,7:203-220

Thonney,M.L;R.W.Touchberry;R.D.Goodrich and J.C.Meiske(1976). Intraspecies relationship between fasting heat production and body weight:a reevaluation of W.75. Journal of Animal Science,43:692-704

Timon,V.M. and E.J.Eisen(1969). Comparison of growth curves of mice selected and unselected for postweaning gain. Theoretical and Applied Genetics,39:345-351

Timon,V.M. and E.J.Eisen(1970). Comparisons of ad libitum and restricted feeding of mice selected and unselected for postweaning gain. I.Growth, feed consumption and feed efficiency. Genetics,64:41-57

Timon,V.M;E.J.Eison and J.M.Leatherwood(1970). Comparisons of ad libitum and restricted feeding of mice selected and unselected for postweaning gain. II.Carcass composition and energetic efficiency. Genetics,65:145-155

Titus,H.W;M.A.Jull and W.A.Hendricks(1934). Growth of chickens as a function of feed consumption. Journal of Agricultural Research,48:817-835

Wang,C-T;G.E.Dickerson;S.E.Hadden and J.A.DeShazer(1980). Feed utilization of rats selected for efficiency of lean gain. Zeitschrift fur Tierzuchtung und Zuchtungsbiologie,97:217-240

Webster,A.J.F.(1977). Selection for leanness and the energetic efficiency of growth in meat animals. Proceedings of the Nutrition Society,36:53-59

Webster,A.J.F.(1980). The energetic efficiency of growth. Livestock Production Science,7:243-252

Webster,A.J.F.(1981). The energetic efficiency of metabolism. Proceedings of the Nutrition Society,40:121-127

Webster,A.J.F.(1983). Energetics of maintenance and growth. in Mammalian Thermogenesis. L.Giradier and M.J.Stock ed. Chapman and Hall, London

Whittemore,C.T. and R.H.Fawcett(1974). Model responses of the growing pig to the dietary intake of energy and protein. Animal Production,19:221-231

Whittemore,C.T. and R.H.Fawcett(1976). Theoretical aspects of a flexible model to simmulate protein and lipid growth in pigs. Animal Production,22:87-96

Williams,J.C.(1984). Influence of selection for body weight and testis weight on the growth of mice. Ph.D. Thesis. University of Edinburgh

Wilson,S.P.(1969). Genetic aspects of feed efficiency in broilers. Poultry Science,48:487-495

Wilson,S.P.(1973). Selection for a ratio of body weight gains in mice. Journal of Animal Science,37:1098-1103

Yuksel,E;W.G.Hill and R.C.Roberts(1981). Selection for efficiency of feed utilisation in growing mice. Theoretical and Applied Genetics,59:129-137