

THE INFLUENCE OF THE MATERNAL
ENVIRONMENT ON GROWTH IN MICE

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INTRODUCTION

During the last fifty years many reports and essays have appeared concerning the inheritance of body size in mammals and several reviews of these studies are now available (Venge 1950, Gruneberg 1952). An examination of these and of the detailed reports on which they are based shows that the inheritance of body size has been examined in three main ways, i.e.

1. The inbreeding and crossbreeding of animals of diverse size.
2. The continued selection of animals of large and small size from a common parent stock.
3. The analysis of pedigree records to establish the genetic and environmental components of the phenotypic variation.

Each of these techniques has produced data leading to the conclusion that body size is determined by many genes in the manner common to many quantitative characters. Following upon this general conclusion many studies of body size have been concerned with the development of a reasonable theory of quantitative inheritance, and to this end they have been directed towards a comparison of observed experimental results with expectation as determined by theory. By such means the first concept of a simple additive nature of gene effects was extended to one in which dominance relations were added, thus allowing of an/

an explanation of heterosis and inbreeding depression in terms of homozygous and heterozygous allelic pairs.

But in spite of these advances many complications remain in the interpretation of experimental data on body size. Some of these such as scale effects and changes in the degree of dominance with selection have been discussed in detail (Mather 1949, Fisher 1930). The importance of many others is still obscure. For example, in the treatment of the data recorded in many experiments a number of simplifying assumptions have had to be made. Those commonly encountered include the absence of cytoplasmic and perhaps maternal effects, lack of interactions between the genotype and environment, the stability of environmental conditions for successive generations and the lack of a correlation between the genotype and environment.

Of the factors known to be associated with mammalian growth maternal effects appear to be of widespread importance. They have been reported to influence the growth of horses (Walton and Hammond 1938), cattle (King and Donald 1955, Brumby and Hancock 1956), sheep (Hunter 1956), rabbits (Venge 1953) and mice (Bateman 1954). Although of widespread occurrence the manner in which the maternal effect influences growth is far from clear. The possible mechanisms that have been suggested to explain the effect include cytoplasmic inheritance, nutrition and endocrine factors (for review, see Hunter 1956).

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The importance of maternal effects to mammalian growth emphasizes a particular problem encountered when selection experiments are undertaken, namely that when a selection response is observed there is a probable consequent change in the maternal environment provided for the next generation. It may be argued that by selecting within the litters of multiparous animals it is possible to avoid directly selecting for maternal environment but the problem of a possible genetic correlation between the character selected and the subsequent maternal performance then arises. Similarly it is apparent that an examination of inbreeding depression, and heterosis in mammals is greatly complicated by differences in the maternal environment provided for different crosses.

The experiments discussed here were intended to clarify the importance of the maternal environment to the growth of a large and small strain of mice selected by Falconer from a common base population, and further, to endeavour to clarify the nature of the maternal influence operating.

The experimental programme planned was made possible by the recent successful development of techniques of egg transplantation in mice (for review, see McLaren and Michie 1956). The use of this technique enabled the prenatal and postnatal maternal environment to be varied at will.

In brief, an attempt was made to answer the questions:

1. Are maternal effects of importance in explaining the asymmetrical selection response in body weight recorded by Falconer (1955)?

2. In what manner are these maternal effects related to body size?
3. What is the possible nature of the maternal mechanism involved?

MATERIALS AND METHODS

a. Stocks used

The large and small strains of mice used in this work originated from the same base population formed by crossing four highly inbred strains (CBA, RIII, A and C57BC). Selection for body weight at six weeks of age was made within litters for some 40 generations in the up direction and thirty generations in the down direction. At generation 31 in the up line and generation 20 in the down line reverse selection lines were started. In the small line this resulted in an immediate response and was accompanied by an increase in fecundity and a decline in the variability of body weight (Falconer 1955). In the large line the response to reverse selection was slower (Falconer, unpublished). The parental lines chosen were the large strain animals from generations 37 and 38, and the reverse small strain animals from generations 30 and 31. The reverse selected small line animals were chosen rather than the small line animals because of their greater fecundity and lower variability.

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The unselected stock originated from a cross of several heterogeneous stocks. This cross had been maintained for eighteen generations with minimum inbreeding and without conscious selection for any character. The three stocks will be referred to as the L, S, and U strains respectively. All litters were weaned at 21 days after birth.

b. Egg transfers

Immature female mice aged 22-25 days were used as donors. Ovulation was induced by combined treatment with follicle stimulating and luteinising hormone. Three I. U. of F.S.H. (Serum Gonadotrophin B.P. Organon) were used as the priming dose followed by 3 I. U. of L.H. (Chorionic Gonadotrophin B.P. Organon) 48 hours later. Ovulation is believed to occur some 12 hours later (Runner and Palm 1953). The occurrence of mating was detected by the presence of a vaginal plug on the following morning. Three days later the plugged donors were killed, the uterine horns dissected out and washed through with a small volume of Ringer phosphate saline (Pannett and Compton 1924). The eggs, usually in the early blastocyst stage, were collected in a watchglass and identified under a binocular.

Recipient mice of the large and small strain were primed with F.S.H. and L.H. in exactly the same manner as the donor mice, then mated to a vasectomised male. Recipients of the unselected/

unselected strain, owing to the much larger number of females available, were mated to vasectomised males and those found with plugs on any given day used as recipients. All egg transfers were made into recipients $2\frac{1}{2}$ days after mating, for McLaren and Michie (1956) reported a better conception rate using $2\frac{1}{2}$ day recipients rather than fully synchronised donors and recipients.

Recipient animals were anaesthetised with ether and a dorsal skin incision made over the region of the right ovary. The abdominal wall was then opened and the ovarian fat pad, ovary and Fallopian tubes exteriorised. Slightly below the tubo-uterine junction an incision was made in the uterus with a needle and through this the end of a fine pipette carrying the eggs was inserted. In this manner approximately 10-15 eggs were inserted into the right uterine horn of each recipient. The ovary and fat pad were then returned to the abdominal cavity and the skin incision closed with a cotton suture.

c. Analysis of growth data

The variation in weight of individual animals at a given age was influenced by a number of components of which genotype, maternal effect and litter size were the most important. Of these three major sources of variation, litter size was of little interest and added an unnecessary complication to the interpretation of results. From an experimental viewpoint it was impossible to completely standardize the size of litters, but by statistical manipulation/

manipulation the same end was achieved. Each mean weight and variance was adjusted to that equivalent to a litter size of 5 animals.

Details of the analysis are as follows:

Analyses of the variances, and of the covariance of the mean weight of litters and litter size, were made on birth weights, then weekly weights to six weeks of age, thereafter at 8, 10 and 12 weeks of age. Separate analyses were performed for male and female mice after three weeks of age. In each analysis the error variance and group mean was adjusted to a mean litter size of 5. Then the mean of the separate male and female mice was estimated and the male and female error variance combined. From this combined error variance for each separate experimental group of mice a pooled error variance and an average standard error for the group means was computed. From this the approximate difference required for significance between any two groups was estimated.

Approximately 10 litters were produced in each experimental group for it was argued that with an average litter size of 5 and a coefficient of variation of the body weights of the order of 15%, group sizes of this magnitude would provide sufficient material to detect, with a probability of 75%, differences of the order of 10% or more in mean body weight (Snedecor 1956).

d. Experiments performed and notation used

As/

As already pointed out, the letters L, S and U were used to denote the large, small and unselected strains respectively. To describe each experimental group a minimum of three letters was used, e.g. S / L / U.

The first letter indicates the strain of the embryo implanted in the female, the second letter indicates the strain of the female in which the embryos were reared to parturition, and the third letter indicates the strain of the female which suckled the embryos after birth. When a transplantation or fostering took place the appropriate letter is underlined. For example, the above three letters indicate that small strain eggs were implanted in large strain females and the resulting young were fostered onto U strain females which reared them. Where crosses were made the female member is noted first.

Fifteen groups in all were compared in the course of 6 separate experiments. For convenience to the reader each experiment is tabulated below with a symbolised representation of the groups compared.

1. The influence of transplantation of fertilized eggs upon the subsequent growth of the resulting mice.

U / U / U and U / U / U

2. The influence of fostering within strains upon the weaning weight,

a. S / S / S and S / S / S

b. L / L / L and L / L / L

3./

3. The relative importance of maternal effects in the large and small strains,

- a. $\underline{U} / L / L$ and $\underline{U} / S / S$
 b. $\underline{S} / L / L$ and $S / S / S$
 c. $\underline{L} / S / S$ and $L / L / L$

4. The relationship of the maternal performance to body size,

- a. $U / U / U$, $\underline{U} / L / L$ $\underline{U} / S / S$
 b. $\underline{L} / U / U$ and $L / L / L$
 c. $\underline{S} / U / U$ and $S / S / S$

5. The partitioning of the prenatal and postnatal maternal environment,

- a. $S \times L / S / S$, $S \times L / S / \underline{U}$ and $\underline{S \times L} / U / \underline{U}$
 b. $S / S / \underline{L}$ and $S / S / S$
 c. $L / L / \underline{S}$ and $L / L / L$
 d. $S / S / \underline{U}$ and $S / S / S$
 e. $U / U / \underline{S}$ and $U / U / U$

6. The role of cytoplasmic inheritance and sex linkage in the determination of body size,

- a. $\underline{S \times L} / U / U$ and $\underline{L \times S} / U / U$
 b. $S \times L / S / S$ and $L \times S / L / L$

RESULTS/

RESULTSa. The influence of transplantation of fertilized eggs upon the subsequent growth of the resulting mice

The work of Gates (1956) established that fertilized eggs obtained from immature mice as a result of treatment with gonadotrophins were viable and capable of normal development. It was also observed that the transplantation of $3\frac{1}{2}$ day mouse eggs did not appreciably effect their embryonic weight at 18 days. This study did not, however, include the postnatal growth phase of the young resulting from transferred eggs, nor was anything known of the impact of the transplantation procedure upon the postnatal maternal performance of the host female. For these reasons it was considered desirable to compare the postnatal growth of embryos resulting from egg transplants with that of normal native embryos. Fertilized eggs from immature U strain mice were transplanted to mature $2\frac{1}{2}$ day pseudo-pregnant females of the same strain and the consecutive weights of the resulting embryos compared with those of embryos of the U strain conceived and born in the normal manner. The relevant growth data for this comparison are presented in lines 1 and 2 of

Table I.

No difference between the two groups was apparent at any stage of growth. Although this comparison was made in the U strain/

strain only, the conclusion that transplantation per se is without effect on the subsequent growth potential of the embryo, has been extended to the large and small strains as well.

TABLE I

Summary of Body Weights (in Gms.) Adjusted to a Mean Litter Size of 5 Young

Group	Mean Litter Size	No. of individuals	Week									
			0	1	2	3	4	5	6	8	10	12
1. U / U / U	5.9	59	1.69	4.81	7.47	10.63	16.99	22.29	24.62	27.32	28.92	30.22
2. U / U / U	5.75	46	1.79	4.88	7.53	10.53	16.02	21.46	24.09	27.11	28.79	29.96
3. U / S / S	3.77	49	1.43	3.78	6.16	8.86	14.96	19.36	21.73	24.20	25.34	26.67
4. U / L / L	4.0	40	1.70	4.75	7.63	10.73	17.14	21.37	24.28	27.32	28.89	29.84
5. S / S / S	4.5	44	1.16	3.23	5.41	6.70	9.28	12.00	13.45	15.04	15.81	16.64
6. S / L / L	4.1	37	1.31	3.65	5.81	7.42	10.05	12.02	13.84	15.85	17.36	18.50
7. L / L / L	6.1	55	1.58	4.76	7.34	9.73	16.67	24.24	27.61	29.98	32.02	33.27
8. L / S / S	3.0	32	1.36	3.46	5.48	7.55	14.37	22.00	24.89	28.75	30.82	32.86
9. L / U / U	5.8	58	1.43	4.32	6.88	8.76	13.03	15.55	16.72	18.94	20.57	21.88
10. L / U / U	3.3	31	1.74	5.28	8.33	11.42	20.69	26.14	28.70	31.68	33.76	35.05
11. S x L / U / U	4.3	43	1.66	4.65	7.71	9.92	16.29	21.36	23.57	26.24	27.71	29.37
12. L x S / U / U	5.25	63	1.58	4.29	6.99	9.70	14.97	19.57	21.79	24.00	25.68	27.09
13. S x L / S / S	4.80	48	1.24	3.65	6.22	9.07	14.80	19.45	21.41	23.62	25.33	26.52
14. L x S / L / L	6.67	60	1.50	3.76	6.54	8.96	13.42	18.74	20.94	23.83	25.71	26.91
15. S x L / S / U	5.20	52	1.29	4.66	8.10	10.58	16.27	20.94	22.52	24.62	26.62	28.18
Pooled Regression of Weight on Litter Size Within Groups						♀ only	-0.6975	-0.5469	-0.5226	-0.5075	-0.5062	-0.4957
			-0.0388	-0.1543	-0.4012	-0.6066						
						♂ only	-0.6203	-0.6516	-0.6592	-0.6259	-0.6341	-0.6934
Pooled Error Mean Square Within Groups Corrected for Litter Size			0.0283	0.2979	1.3564	2.6687	7.0338	8.3643	6.6364	7.1386	8.2224	8.8000
Approximate Difference Required for Significance Between any Two Groups (P.0.05)			0.13	0.41	0.87	1.23	1.97	2.15	1.85	1.99	2.13	2.21

b. The influence of fostering within strain upon the weaning weight

At birth many litters were cross-fostered to females of another strain, the rationale for which rested on the hypothesis that cross-fostering is without detrimental effects to subsequent growth rates. The evidence available concerning this question appeared to be confined to two reports. In 1950 Butler and Metrakos produced data suggesting that fostering had a detrimental effect on pre-weaning growth, though the data available was limited. Conversely, Bateman (1954) reported that fostering per se had no influence upon the 12 day weight of suckling mice.

In view of the discrepancy between the conclusions of these two reports it was considered advisable to investigate the problem in the stocks used in this work.

Tables 2 and 3 list the weaning weights of control and fostered litters, these being subdivided into litter sizes. As no systematic difference existed between the means or variance of litters of the same size within the two groups, it was concluded that the influence of fostering per se is not an appreciable source of variation when considering the weight increments of the large and small strains of mice.

Two other conclusions may be drawn from these tables:

1./

1. Litter size does not appear to influence the amount of variation within the litters.
2. The within litter variation and coefficient of variation is greater in the large strain than in the small. Expressed as a percentage of the total variation, however, the within litter variation of the large strain accounts for only 10% of the total variation whereas the within litter variation of the small strain accounts for 27% of the total variation.

TABLE 2A comparison of weaning weights of normal and fostered litters of the small strain

<u>Litter Size</u>	<u>Total No. Animals</u>	<u>Control</u>		<u>Fostered</u>		<u>Variance Within Litters</u>
		<u>Mean (Gm.)</u>	<u>Variance Within Litters</u>	<u>Total No. Animals</u>	<u>Mean (Gm.)</u>	
2	20	8.72	0.248	10	8.91	0.578
3	30	8.34	0.562	21	8.74	0.160
4	40	7.93	0.120	28	7.83	0.228
5	50	7.76	0.298	45	7.46	0.263
6	60	7.70	0.283	42	7.22	0.438
7	70	7.06	0.392	56	6.86	0.537

Components of variance:

Between size	0.425	0.436
Between litters	0.429	0.437
Within litters	0.320	0.393

TABLE 3

A comparison of weaning weights of normal and fostered litters of the large strain

<u>Litter Size</u>	<u>Total No. Animals</u>	<u>Control</u>		<u>Fostered</u>		
		<u>Mean (Gm.)</u>	<u>Variance Within Litters</u>	<u>Total No. Animals</u>	<u>Mean (Gm.)</u>	<u>Variance Within Litters</u>
3	30	11.5	1.134	6	13.32	0.263
4	40	12.0	0.724	20	12.37	1.296
5	50	10.91	0.571	30	10.82	1.537
6	60	10.28	0.680	24	10.26	1.192
7	70	9.95	1.160	28	11.17	0.699
8	80	8.88	0.628	32	9.01	0.569

Components of variance:

Between size	0.864	0.979
Between litters	3.222	2.048
Within litters	4.728	5.552

c. The relative importance of maternal effects in the large and small strains

As already pointed out in the introduction, Falconer selected these large and small strains of mice using a within litter selection technique, the criteria of selection being the deviation of each individual from the mean value of the family to which it belonged. Assuming random drift to be small, it follows that any difference in the maternal environment provided by the two selected lines must be a consequence of a correlation between body size and maternal environment.

An appraisal of the difference in the maternal environment of the two lines was made in two ways. In the first experiment fertilized eggs of the U strain were implanted in both large and small strain females, and the resulting embryos compared in growth rate. In the second experiment fertilized eggs of the small strain were implanted in large strain mothers, while fertilized eggs of the large strain were implanted in small strain mothers. The subsequent growth of the embryos was compared with that of normally born large and small strain mice. The results of the first experiment are presented in lines 3 and 4 of Table I, and in figure 1. The results of the second experiment are presented in lines 5 and 6, and 7 and 8 of Table I, and figures 2 and 3.

The/

The results of the first experiment (figure 1) indicate beyond all doubt that a substantial difference existed between the maternal environment provided by the two strains. A large difference in weight was already apparent at birth, a difference which steadily increased up to eight weeks of age at which stage it appeared relatively stable.

The results of the second experiment (figures 2 and 3) substantiate those of the first and indicate that at least part of the difference in body weight observed between the large and small strain lines was due to a difference in the maternal environment provided by the two strains. Because of the nature of the selection programme used in developing these stocks it would appear that this difference in maternal environment originated because of a change in the body weight of the selected parental stocks.

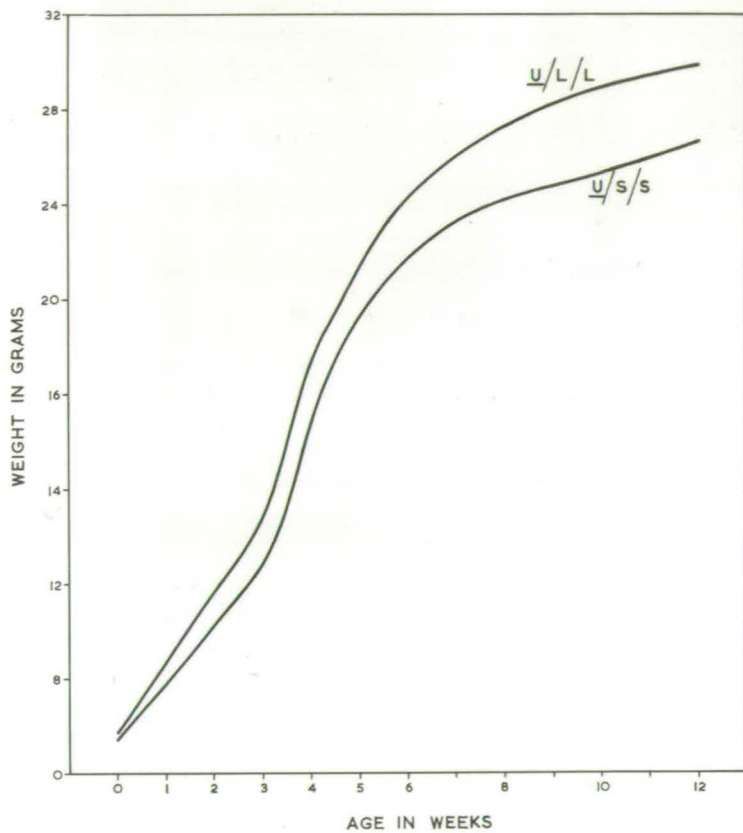


Fig. 1. A comparison of the growth of U strain young when reared in and suckled by large and small strain females.

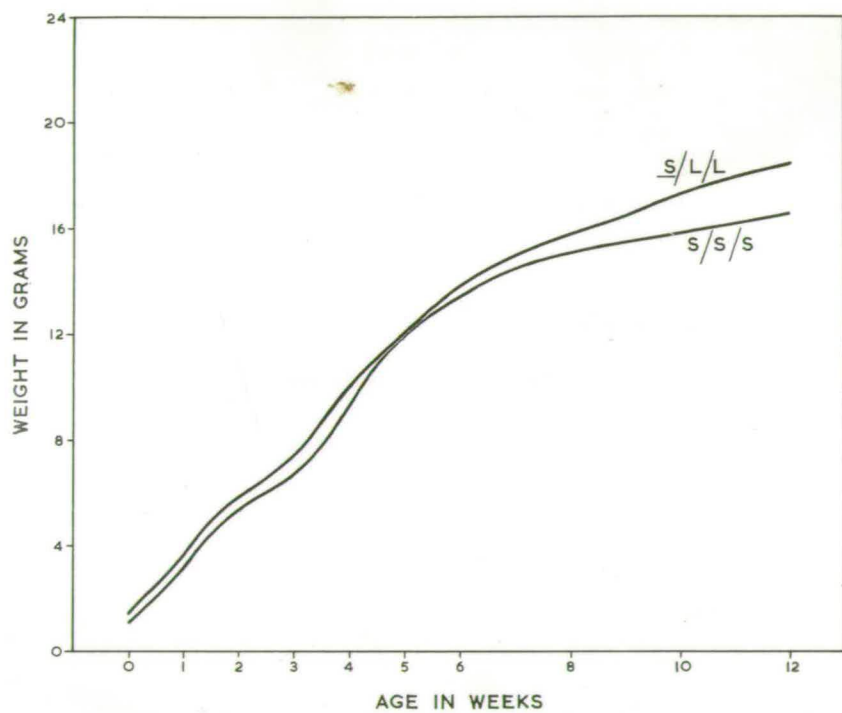


Fig. 2. A comparison of the growth of S strain young reared in and suckled by large and small strain females.

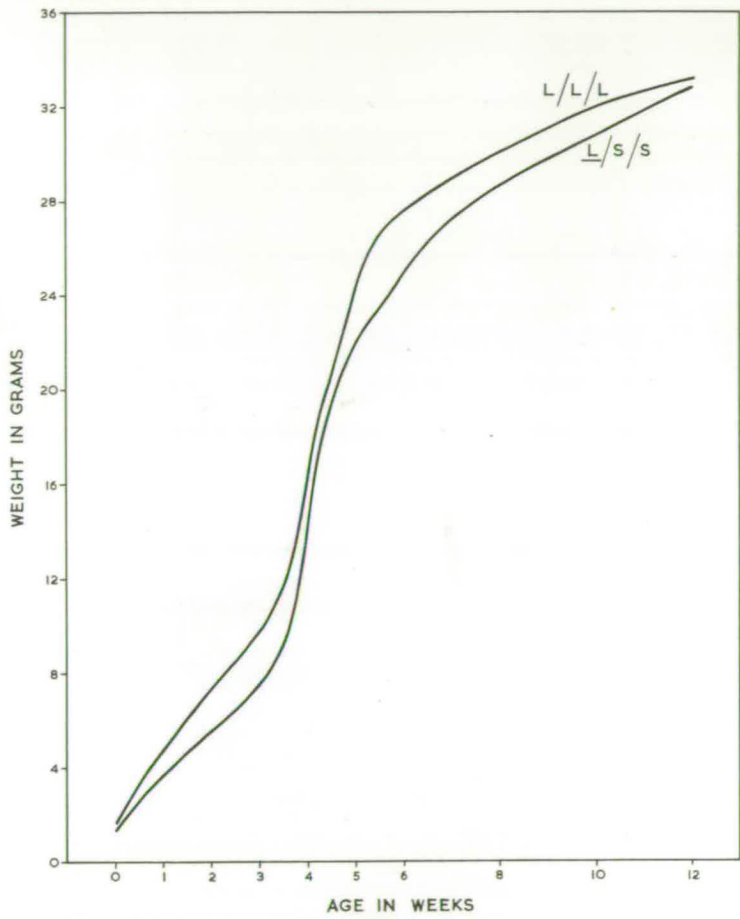


Fig. 3. A comparison of the growth of the L strain young reared in and suckled by large and small strain females.

d. The relationship of the maternal influence to body size

The experiments described in the previous section indicated that a substantial difference existed in the maternal environment provided for the two strains. This difference was attributed to the change of body size produced by selection. The question remains whether or not this difference in the maternal environment is simply related to body size, for it might be supposed that while the small strain animals provide a poorer environment than the large strain, the large strain animals might provide no better environment than that provided by mice unselected for size.

Some evidence for an asymmetrical maternal effect was provided by a comparison of large and small strain females as host mothers of U strain young (Table I, lines 2, 3 and 4, and figure 4). Reared in large strain host mothers these U strain young grew at the same rate as those reared in their own U strain mothers, but reared in small strain host mothers they grew much more slowly. In other words, large strain females used as host mothers were equal in maternal performance to the U strain females; but small strain females used as host mothers recorded a much poorer performance.

Further evidence was obtained by implanting both large and small strain eggs in U strain females and comparing the growth of the resultant embryos with the control stocks of the large and/

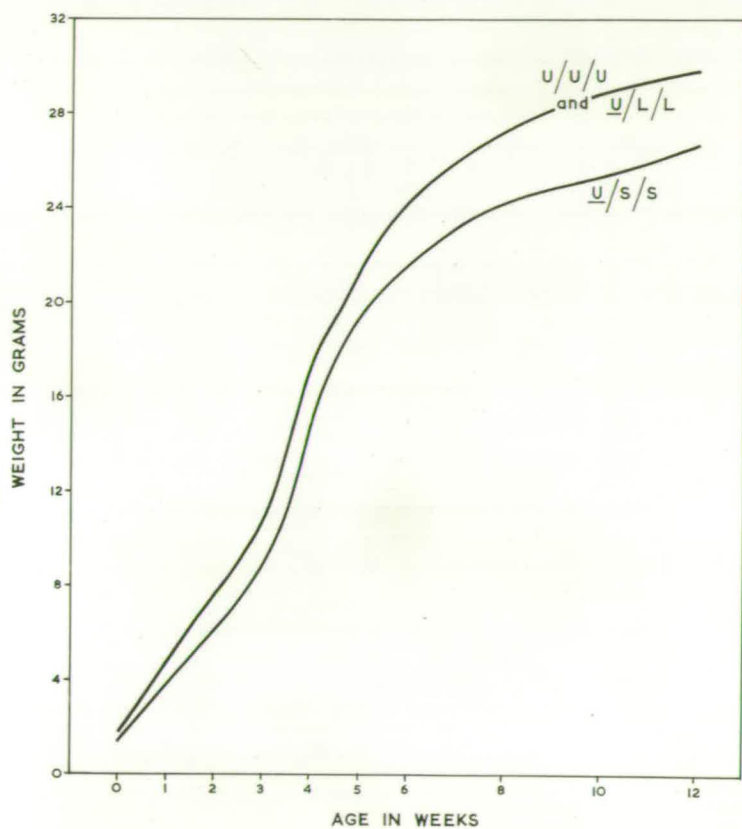


Fig. 4. A comparison of the growth of U strain young reared in and suckled by U strain, L strain and S strain females.

and small strains. The results of this comparison are presented in Table I (lines 5 and 9, and 7 and 10) and figure 5.

Rather surprisingly perhaps both the large strain and the small strain animals were found to be greatly increased in size when implanted in U strain females, even though the U strain females were smaller in size than the large strain females. It follows that the maternal environment provided by the large strain females must be inferior to that provided by the U strain females when rearing large and small strain embryos. On the other hand, it was shown that the maternal performance of large strain females was equivalent to that of the U strain females when both were rearing U strain embryos. In other words, an interaction exists between the genotype of the embryo and the maternal environment provided. Two other conclusions emerge from these results:

1. The difference in maternal environment produced by changes in body weight is asymmetrical.
2. Though body size and maternal effect are related the fact that the maternal environment provided by the U strain stock is superior to that provided by the large strain stock indicates that there are factors associated with a good maternal environment that are unrelated to body size.

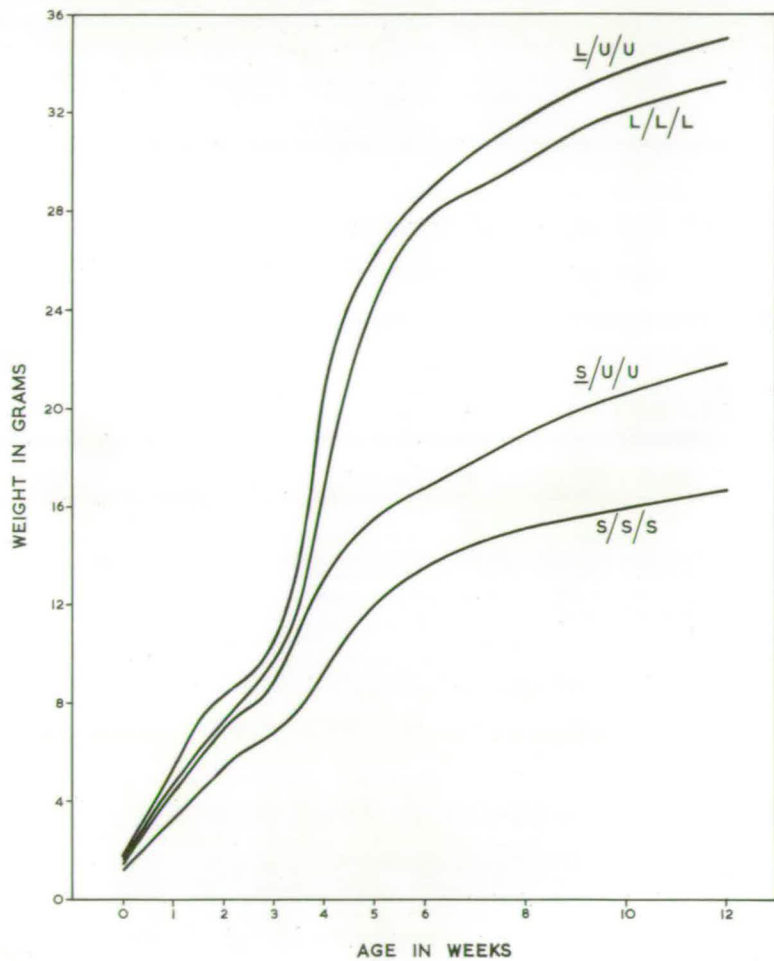


Fig. 5. A comparison of the growth of large and small strain young reared in and suckled by U strain females.

e. The partitioning of the prenatal and postnatal maternal environment

The maternal environment provided by the female may be split into two major phases, i.e. the prenatal (the period from ovulation to parturition) and the postnatal (the period from parturition to weaning). A separation of the total maternal environment into these two phases is of considerable practical interest for though the prenatal phase is relatively difficult to influence save by severe changes in nutrition (Wallace 1948) the postnatal period readily lends itself to environmental modification.

A partitioning of the maternal environment into the two phases was achieved in two separate experiments.

In the first experiment F_1 hybrids of small strain female, large strain male crosses were normally reared and compared to the same crosses fostered to U strain females. They were also compared to the same crosses implanted in and reared by U strain females. Three separate environments were thereby achieved, i.e. the normal, an alien postnatal, and an alien pre- and postnatal combined. Results for the growth of the three groups are tabulated in Table I (lines 11, 13, 15) and in figure 6.

As expected a difference in birth weight between the normal cross and those reared in U strain females was apparent. In the/

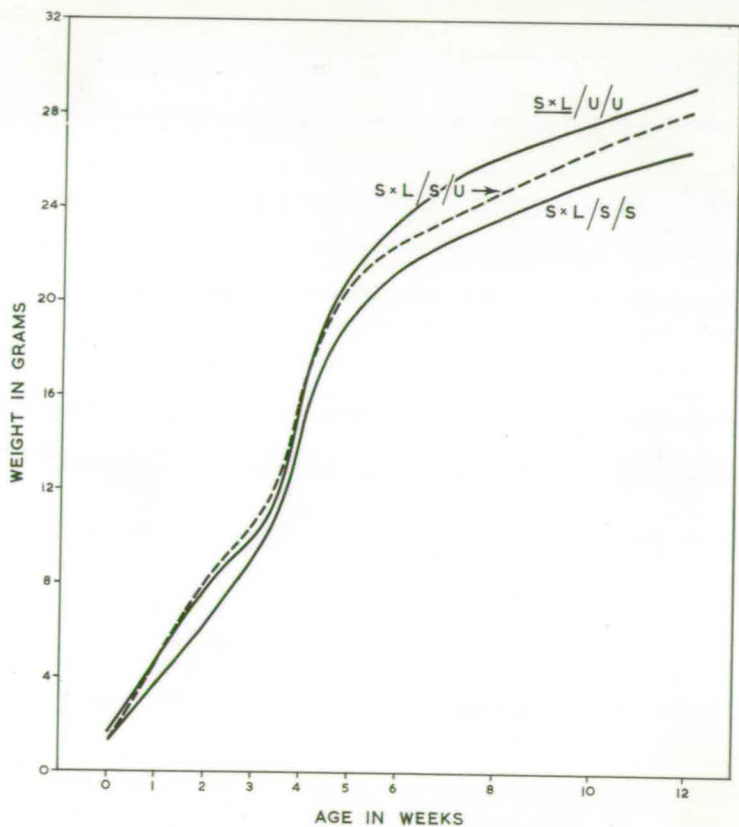


Fig. 6. A comparison of the growth of mice of one genotype reared under conditions where the prenatal and postnatal environment were varied independently.

the groups born of small strain females but reared by the U strain females this difference was quickly eliminated and did not again appear until the animals were 6 weeks of age. At this stage the weight of the animals implanted in U strain females surpassed that of those merely reared by U strain females. Throughout, the young mice born and reared by small strain females grew at a slower rate. From six to twelve weeks of age the relative difference between the three groups did not change appreciably, the position of the three groups suggesting that for this particular situation the postnatal environment accounted for about one-half of the total measureable maternal difference.

In the second series of experiments small and large strain embryos were mutually cross-fostered, as were small strain and U strain embryos, and weaning weights recorded. Limitations in the cage space available did not allow these animals to be retained beyond 3 weeks of age. The relevant weaning weights are tabulated in Tables 4a, 4b and 4c.

The performance of small strain young reared by large strain females proved no better than that of small strain young reared by small strain females, whereas small strain young reared by U strain females were appreciably heavier at weaning. This observation suggested that the large strain females do not/

TABLE 4a

The influence of cross fostering of small, large and unselected strains on body weight at 21 days

Small Strain

<u>Litter size</u>	<u>Control</u>		<u>Suckled by L strain</u>		<u>Suckled by U strain</u>	
	<u>No. of indiv.</u>	<u>Weight (gms.)</u>	<u>No. of indiv.</u>	<u>Weight (gms.)</u>	<u>No. of indiv.</u>	<u>Weight (gms.)</u>
2	20	8.72				
3	30	8.30	9	8.00	3	9.03
4	40	7.93	20	7.56	8	9.19
5	50	7.76	10	7.54	10	8.52
6	60	7.70	42	7.91	36	8.66
7	70	7.06	35	6.94	21	8.74

Components of variance:

Between litters	0.43	0.20	0.27
Within litters	0.35	0.31	0.67

TABLE 4b

The influence of cross fostering of small, large and unselected strains on body weight at 21 days

Large Strain

<u>Litter size</u>	<u>Control</u>		<u>Suckled by S strain</u>	
	<u>No. of indiv.</u>	<u>Weight (gms.)</u>	<u>No. of indiv.</u>	<u>Weight (gms.)</u>
3	30	11.5	3	11.07
4	40	12.0	24	9.89
5	50	10.91	35	9.36
6	60	10.28	54	9.49
7	70	9.95	28	7.73
8	80	8.88	16	6.56
<u>Components of variance:</u>				
		0.32		0.84
		0.86		0.60

The influence of cross fostering of small, large and unselected strains on body weight at 21 days

<u>Litter size</u>	<u>U Strain</u>		<u>Suckled by S strain</u>	
	<u>No. of indiv.</u>	<u>Weight (gms.)</u>	<u>No. of indiv.</u>	<u>Weight (gms.)</u>
4	12	11.51	12	11.00
6	30	10.40	24	10.09
7	21	9.00	21	9.30
Components of variance:				
Between Litters		4.33		1.06
Within Litters		0.68		0.43

not markedly differ from the small strain in lactational capacity, from which it follows that the difference in maternal performance observed between the large and small strain must largely originate in the prenatal environment.

In contrast large strain young reared by small strain females were somewhat smaller at weaning than the large strain controls, an observation that suggested the small strain were actually inferior to the large strain in lactational capacity. Yet, this conclusion is difficult to justify when the performance of U strain young reared by small strain females is considered for the weaning weights of these U strain young were apparently normal.

From this apparent anomaly in the results obtained it appears that an interaction exists between the lactational performance of the female and the type of young being reared. But whatever the nature of such an interaction it may be concluded that the inferiority of the maternal performance of the small strain, for large strain embryos, appears to be determined in part by postnatal factors whereas the superiority of the large strain maternal performance for small strain animals appears to be almost solely determined by prenatal factors.

In general then it may be said that in each of the situations examined the prenatal maternal influence was of marked importance, whilst the postnatal contribution to the maternal/

maternal performance varied according to the genotype of both the female and the young being suckled. This general conclusion is in agreement with that of Bateman (1954) who analysed the causes of variation in the 12 day weight of mice. He found that the prenatal influence was greater than the postnatal influence while the combined total maternal influence (in litters of eight) amounted to 73% of the total variation present.

f. The role of the cytoplasm and sex linkage in the determination of body size

It is a fairly common observation that reciprocal crosses between animals of different sizes lead to F_1 progeny that differ in size, the hybrid tending to resemble the size of the female rather than the male. There are three possible causes for the reciprocal difference: maternal effects, sex linkage and cytoplasmic inheritance.

The analysis of the role of sex linkage does not normally provide a particularly difficult problem. The first step of such an analysis involves a comparison of the reciprocals in the heterogametic sex; if these do not differ significantly then a sex-linked difference is unlikely. The distinction between the maternal effect and the cytoplasmic influence is more difficult to make, especially in mammals. The situation is further complicated by possible differences in the cytoplasmic specificity, three types of which have been distinguished, i.e. specificity through ancestral continuity, through genetic conditioning in the egg stage, and through experimental change, i.e. dauermodification (Goldschmidt 1955). Of these only the first may be considered as cytoplasmic heredity.

A distinction between the contribution of the collective cytoplasmic influence and the maternal environment may be made by standardizing the maternal environment for each of the reciprocal crosses. This possible approach to the problem was used/

used here.

Reciprocal crosses were made between the large and small strains and the resulting fertilized eggs transplanted to U strain females. The weights of the resulting young are presented in Table I (lines 11, 12) and in figure 7.

At birth a significant difference in weight was apparent, in favour of the young resulting from the small females and large males. This difference persisted throughout the 12 weeks body weights were recorded, resulting in a difference of weight of the order of 8% at 12 weeks of age.

Table 5 presents data for the body weights of the hybrid male and female mice computed separately and shows that the difference observed between the two reciprocal hybrids existed in the female mice as well as the males. Thus sex linkage does not appear to be the cause in the difference observed. Rather it appears that the cytoplasm of the small strain animals enhances body size to a greater degree than does the cytoplasm of the large strain.

As a consequence of this result reciprocal crosses were made between the large and small strains and allowed to develop and suckle normally. Growth data for these are tabulated in Table I (lines 13, 14). A difference in birth weight reflecting differences in prenatal environment was apparent but on weaning at 21 days this difference was negligible. Thereafter no apparent difference existed between the two crosses. The previous experiments/

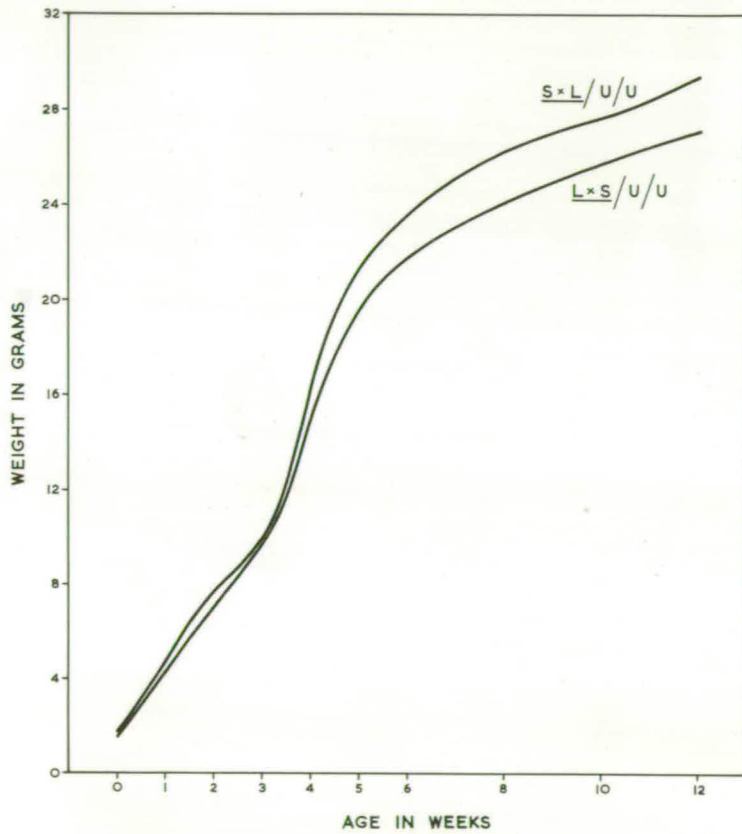


Fig. 7. A comparison of the growth of reciprocal crosses of the large and small strain when the maternal environment was constant.

experiments recorded here established that the difference in maternal environment in the two strains would lead to the expectation that the large female, small male cross would actually be larger than its reciprocal, but this was not the case. This apparent anomaly may be explained in terms of the counter-balancing of the poorer maternal environment of the small strain by a greater cytoplasmic contribution of the small strain to growth.

TABLE 2

Body weights of reciprocal F₁ hybrids of the large and small strains (grams)

<u>Cross</u>		<u>Age in weeks</u>					
		4	5	6	8	10	12
<u>S x L</u> / U / U	♀	15.0	19.89	21.51	23.86	24.73	25.78
<u>L x S</u> / U / U	♀	14.31	17.89	19.70	21.55	22.76	23.89
<u>S x L</u> / U / U	♂	17.57	22.82	25.62	28.62	30.68	32.96
<u>L x S</u> / U / U	♂	15.97	21.25	23.87	26.45	28.60	30.29

DISCUSSIONa. Analysis of data

In the treatment of the data presented several simplifying assumptions were made without prior discussion of their validity. Some comment on these points is called for.

In the first place the relationship of body weight and litter size was treated as a linear one. Though this condition is not strictly true the actual departure from linearity over the range of mean litter sizes considered, as indicated in Tables 2 and 3, is so small as to make this criticism of minor significance.

In the second place litter size has been taken as the number of living young the female reared beyond 24 hours, but because appreciable mortality occurred at the time of parturition this measure of litter size actually underestimates the true litter size. This approximation was made for two reasons:

1. The weight of young at any weighing prior to weaning was largely dependent upon the number of young being reared by the female at that period of time, rather than on the number of young born in the litter.
2. As there was no reason to believe marked differences occurred in the percentage loss of young within litters in the various groups it was considered unlikely that any serious bias would be introduced by using the 24 hour post partum number of young.

The/

The procedure of using the size of litter at 24 hours thus appeared a reasonable compromise between the two conflicting alternatives of number born and number reared.

The third query that may be raised concerns the validity of pooling regressions and variances within groups when there was prior evidence, illustrated in Table 2, suggesting that the variances of the large and small strains were different. The alternative to pooling the within group estimates was to use each separately in adjusting the group mean and its variance for a standard litter size of 5 young. As each group comprised approximately 10 litters, a considerable amount of sampling variation entered into individual within group estimates. Thus it was argued that the pooling of the data would be less likely to bias the adjusted means and variances than by using individual group estimates. In fact the corrections applied to final body weights in each group were very small (about 0.5 gram), while the comparisons of interest between the various groups were usually sufficiently clear cut to give a definite answer to the problem posed.

b. Reproductive physiology

An examination of various aspects of the reproductive physiology of the strains of mice used which became apparent in the course of this work is not strictly relevant to the object of this study. Nevertheless, several points appear worthy of mention; in particular, the recovery of fertilized ova, and the success achieved in causing these to implant.

The number of ova recovered from immature females following superovulation showed a marked difference between strains, small strain and U strain females providing many more eggs per female than large strain females. There was also an appreciable difference in the uniformity of development of these eggs at the time of recovery. Eggs from the small and U strains were usually in the blastocyst stage, whereas many large strain eggs were in the late morulla stage and many others appeared to be fragmenting. Coupled with this problem of a lower available number of viable eggs from fertile matings of the large strain, males of this strain showed marked variability in their mating performance, many exhibiting little desire to mate with immature superovulated females. No trouble in this respect was experienced with U or small strain males.

The percentage of successful pregnancies resulting from egg transplantation was high when using U strain recipients; about 80% of operations resulting in pregnancy. On the other hand small strain females proved refractory in this regard, for only/

only 20% of transfer operations resulted in successful implantations. With both of these strains pregnancy was normally accompanied by successful parturition and lactation performance. This was not the case with the large strain recipients. Though the percentage of transplants resulting in pregnancy appeared satisfactory, i.e. about 60% of operations, the incidence of death at parturition was very high. Many young appeared to be suffocated during the birth process and many others, both dead and alive, were eaten by the recipient female. Even amongst large strain females successfully littering, a number of litters up to a week of age were suddenly killed and eaten by the female for no obvious reason. This problem occurred to a lesser degree in the large strain parental stocks and entailed keeping a much larger parental stock than was envisaged in the original design of the experiment.

c. The variation in maternal performance

From the results of the egg transplants between the various strains four main conclusions emerge:

1. There is a difference in the maternal environment provided by the large and small strain which has resulted from changes in body size.
2. This difference in maternal environment between the two strains comes about mainly by a reduction in the maternal performance of the small strain.
3. The genetic make-up of the embryo influences the maternal performance rating of the female, i.e. the embryo creates a specific demand both prenatally and postnatally.
4. A major portion of the maternal influence of the female on the postnatal growth of her young occurs during the prenatal period.

The problem remains of examining the possible mechanisms underlying these observations.

Perhaps the most surprising feature of the result of these experiments is the asymmetry of the change in maternal performance resulting from selection for body size and it is of considerable interest to enquire how it is that an increase in body size fails to increase maternal performance to the same degree as an equivalent decline in size decreases it.

Falconer/

Falconer (1955) sought to explain an asymmetry of the postnatal maternal performance in the following way. He suggested that there were two components in maternal performance, one related to anatomical development (i.e. size of mammary glands), the other to physiological efficiency. The anatomical component would be expected to be directly related to body size, whereas the physiological component would not. Rather, as this physiological component is in turn a component of natural fitness, it would show overdominance as postulated by Lerner (1954). An increase in homozygosis brought about by changes in gene frequency as a result of selection would then produce a decline of the physiological component in both lines. The result in the large line of the simultaneous changes in the anatomical and physiological components would be a counterbalancing of increased size and decreased lactational efficiency. In the small line there would be a decline in both size and lactational efficiency resulting in the large net decline of maternal performance observed.

As it stands this attractive explanation cannot be reconciled with the present situation for it was shown that the prenatal maternal effect was at least of equal importance to the postnatal. However, it seems possible that an analogous situation applies during the prenatal embryonic period. It may be argued that the anatomical component is represented by the size of the foetal/

foetal placenta, and the physiological component is represented by the efficiency of the placenta as an organ of interchange. If this were the case a close parallel of Falconer's explanation would be expected.

This hypothesis rests largely on two basic premises,

(a) that embryo size and placenta size are related, (b) that a variation occurs in the functional efficiency of the combined maternal and foetal placenta as an organ of interchange.

Hammond (1935) investigated the relationship between the weight of the foetal placenta and the individual foetus in rabbits. He found that there was no relationship between the two in the early stages of pregnancy, but at later stages the weight of the foetus and the weight of its placenta became closely correlated. A similar observation was made by Ibsen (1928) in guinea pigs, while McKenzie and Bogart (1934) found that the number of cotyledons on the foetal placenta of the pregnant ewe was closely correlated with the weight and thrift of the new born lamb. It is in the latter stage of pregnancy that the foetus makes the greatest growth and it is only then that differences in weight due to varying litter size become apparent (Hammond 1935, Winters and Feuffel 1936). Of particular interest is the observation that prior to the establishment of differences in foetal weight, it is the weight of the foetal placenta that is first increased by a decrease in the number of ova fertilized (Hammond 1935). From this observation it is reasonable to imply that/

that because changes of placental weight precede changes of foetal weight, the weight of the placenta is a causal factor in determining foetal weight. The ratio of the weight of the foetal to the maternal placenta varies with the stage of pregnancy, for the growth of the maternal placenta declines in the latter stage of pregnancy whereas the foetal placenta continues to grow rapidly (Hammond 1935). The weight of the maternal placenta bears no relation to the weight of the young born (Hammond 1935). Thus it appears that the foetal rather than the maternal placenta is the main controlling factor in the nutrition of the embryo, and further, that it is the size to which the foetal placenta grows in the early stages of pregnancy that determines the availability of the nutrient supply to the foetus during the latter stages of pregnancy.

The second premise that requires examination is that concerning variation in the interchange efficiency of placenta of different strains of mice. Unfortunately there is little direct evidence on this point.

The transmission of material across the placental barrier is known to depend on (a) the substance transmitted, (b) the placental structure of the animal, (c) the stage of pregnancy. A marked variation between species in the morphogenesis of the foetal membrane was established by Mossman (1937) who was able to/

to classify placentae according to (a) the number and type of tissue between the maternal and foetal blood circulations and (b) the diminution in layers as pregnancy advances. A difference in permeability between these placental types has been demonstrated.

Flexner and Gellhorn (1942) labelled sodium chloride with Na^{24} and used this as a marker to measure the relationship of the permeability of the placental barrier to the type of structure of the placenta. They succeeded in demonstrating a marked variation between species in the amount of Na^{24} transferred per gram of placental tissue.

These reports refer to differences between species rather than to differences between strains within species but it is not unreasonable to envisage various strains of mice establishing minor changes in placental structure affecting permeability or or alternatively of changing the permeability of the cell barriers already established. It seems reasonable therefore to accept with reservation this second premise, in which case it follows that the above explanation of the nature of the prenatal maternal effect offers a reasonable working hypothesis upon which further experimental work might be based.

Granted that the suggested explanation of the nature of the asymmetrical maternal response is reasonable, it then becomes easier to visualize a possible mechanism underlying the interaction that was demonstrated between the strain of the embryo and the strain/

strain of the host female. The nutrient supply the embryo draws upon is provided solely by the female, whereas the capacity of the embryo to use these nutrients depends upon the type and size of placenta it is able to form during the early stage of pregnancy. If, as seems likely, the functional efficiency of the placenta differs between strains of mice then it is reasonable to expect embryos of one strain to make greater use of the nutrients available than embryos of a different strain.

As discussed above, Falconer has provided an attractive hypothesis concerning the mechanism of the asymmetrical response in the postnatal maternal period, and it only remains to comment upon the interaction observed in the postnatal maternal performance. To do this satisfactorily a brief outline of the physiology of pituitary function is first required.

Much recent experimental work in endocrinology has sought to clarify the relationship of the nervous system to the endocrine system. The problem of finding the mechanism of integration of the two control systems has largely centred about the anterior pituitary gland for this is an endocrine organ of paramount importance subject in part to neural control but without the direct involvement of nerve fibres. The key to the explanation of this anomaly appears to lie in the posterior lobe of the pituitary gland (Benson and Cowie 1957). This is an endocrine gland composed mainly of neural tissue and serves as a storage organ/

organ for the products of synthesis of the paraventricular and supraoptic nuclei contained in the hypothalamus. Neural stimulation of certain types initiates the release of the active peptides of the posterior pituitary which in turn pass through the anterior lobe en route to the systemic circulation. It is the passage of these active peptides through the anterior pituitary that is now believed to trigger the release of the endocrine factors characteristic of the anterior lobe.

An understanding of this mechanism provides a basis for understanding the nature of the interaction observed between the strain of embryo and strain of suckling female. The demand of the embryo is reflected in the transmission of neural stimuli from the nipples of the female to the posterior pituitary. The end result of such a stimulus is twofold: (a) posterior lobe factors are released which initiate contraction of the myoepithelial cells about the alveoli of the mammary tissue and result in milk ejection; (b) the passage of these posterior lobe factors through the anterior pituitary initiates the release of anterior lobe factors such as prolactin and growth hormone which are known to influence the activity of the mammary tissue in synthesising milk. In such a manner the greater demand of one type of young mouse compared to that of another may be translated into differences in the functional activity of the mammary tissue of the females suckling them.

d. The cytoplasmic influence

The difference that was established between the reciprocal crosses reared in the same environment provides, apparently, the first clear case in which cytoplasmic factors have been shown to influence growth. How unique this observation is likely to be is yet unknown but the demonstration of an appreciable cytoplasmic influence on growth is of considerable interest. The nature of the influence operating is obscure, but it seems unlikely that actual cytoplasmic inheritance is involved. The most favourable cytoplasm for growth was provided by the small strain. If the inheritance of particular cytoplasmic agents increasing body size were involved it would be difficult to comprehend their accumulation in greater quantity in the small strain than in the large. Rather, it appears more likely that this cytoplasmic difference originates as a modification of the cytoplasm determined by the nuclear genetic structure of the small strain, and is dependent upon the continued genetic identity of the small strain.

e. Maternal effects and changes in gene frequency

The final problem to be considered is the relevance of the maternal influence to the interpretation of genetical studies in mammals for just as cytoplasmic influences may be confounded with maternal effects, so too may changes in gene frequency accompanying selection and inbreeding.

For convenience in discussion the problem may be thought of in two stages:

1. the correlation of the maternal effects with the character selected;
2. the importance of this changed maternal performance to the offspring of the selected animal.

As already pointed out the selection carried out by Falconer for body size in the large and small strains was made within litters and equal numbers of individuals were selected from all available families. By the use of such a technique the environmental component of variation common only to members of one litter, i.e. maternal effect, was subject to no direct selection at all, since it is a character of the mother and no selection between families was practiced. Thus, neglecting random drift, the change in maternal performance was a correlated response resulting from the genetic correlation between body weight and mothering ability.

An expectation of a general asymmetrical correlation between body/

body size and maternal effect such as demonstrated in this experiment would be a departure from symmetry in the response to selection for body size, selection for large size being less effective than selection for small size. Such an asymmetry was observed by Falconer (1955), the realized heritabilities being $17.5 \pm 1.6\%$ in the large line and $51.8 \pm 2.3\%$ in the small line.

Though rather elaborate genetical interpretations may be evoked to explain such an asymmetrical selection response, a realization of the nature and magnitude of the role of the maternal environment and of the manner in which it varies as selection proceeds appears to obviate the need for such complicated hypotheses.

In a like manner the correlation of maternal effect and body size raises a further interesting point in relation to selection limits. Thus it is believed that genetic variation still exists in both the large and small selected lines yet response to selection has virtually ceased. In such a situation one might envisage the upper selection limit being imposed, not by the exhaustion of genetic variation for body size per se, but rather by a limitation of size imposed by the maternal performance of the female.

However, this point is speculative and requires further investigation.

A somewhat similar problem of interpretation was reported

by/

by Falconer (1955), who noted a rather more indirect correlation between body weight and litter size. He observed that females resulting from large litters were smaller at mating than females from small litters. As a correlation between the number of eggs shed and body size was also apparent the preliminary stages of selection for increased litter size actually resulted in smaller litters than those resulting from matings in the line selected for small litter size. Thus in this case the correlated change in maternal performance completely masked the genetic changes resulting from selection.

A further aspect of the problem of the correlated maternal response is the relationship between maternal effect and fitness. Bateman (see Falconer 1955) selected for lactational performance in mice but realized slow progress in both the upward and downward direction, yet reverse selection in each line made at each successive generation yielded a marked response. Preliminary results of relaxing selection at each generation showed a regression of the means of both high and low lines to an intermediate level. Results such as these would be expected if an intermediate genotype were optimal for fitness and natural selection was opposing change in either direction. Why it should be that increased lactational performance apparently lowers fitness remains obscure. The important point so far as this discussion is concerned is that a correlated change in maternal performance accompanying selection for a character may result in a change of fitness of the selected population quite apart from that/

that due to a direct correlation of the character selected with fitness.

In the same manner and for similar reasons to those outlined above the interpretation of inbreeding and crossbreeding experiments has been complicated. The increasing homozygosity of the embryo which accompanies inbreeding is inevitably accompanied by increasing homozygosity of the female bearing the embryo. This increased homozygosity of the female undoubtedly influences her maternal performance which in turn affects the development of the embryo to a variable degree. Thus the separation of the influence of inbreeding upon the embryo compared to the female is confounded.

In this situation as with those previously commented upon the technique of egg transplantation appears to offer the most suitable practical technique for disentangling the effects of otherwise confounded variables and of studying their interaction with each other.

SUMMARY

1. The technique of ova transplantation was used in an investigation of the importance and nature of the maternal influence upon the growth of a large and small strain of mice. The strains of mice used had been established by Falconer using within litter selection for approximately 35 generations.
2. Preliminary experiments established that neither transplantation nor fostering of young within strains influenced the growth potential of the embryos.
3. A marked difference was demonstrated in the maternal environment provided by the large and small strain females to embryos of a non-related unselected strain.
4. Compared to an unselected outbred strain both the large and small strain females proved inferior in maternal performance, but the main difference between the maternal performance of the large and small strains came about by a reduction in the maternal performance of the small strain.
5. An interaction between the prenatal maternal environment of the female and the genotype of the embryo implanted was apparent.
6. The partitioning of the total maternal environment into prenatal and postnatal phases, demonstrated the marked importance of the prenatal phase to growth. The postnatal contribution varied according to the genotype of both the female/



female and the young being suckled. An interaction between the lactational performance of the female and the genotype of the young was apparent.

7. Sex linked genes were not responsible for any marked effect on body size, but evidence was found showing that the cytoplasmic influence on growth was greater in the small strain than in the large strain.
8. A possible explanation of the asymmetry and interaction of maternal effects is provided, and the results are discussed in relation to the interpretation of selection and inbreeding experiments in mammals.

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