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THE INFLUENCE OF THE MATERNAL ENVIRONMENT ON GROWTH IN MICE

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1. 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 ; Grüneberg, 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 other 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 simple additive gene effects was extended to one in which dominance relations were included thus allowing of 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.

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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).

The importance of maternal effects on mammalian growth emphasises 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 matings.

The experiments discussed here were intended to investigate 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 reviews, see McLaren and Michie, 1956). The use of this technique enabled the prenatal 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 mechanism involved ?

2. MATERIALS AND METHODS

(i) *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 C57BL). Selection for body weight at six weeks of age was made within litters for some forty generations in the upward direction and thirty generations in the downward direction. At generation 31 in the up line and generation 20 in the down

lines 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 fecundity and lower variability.

The unselected stock originated from a cross of several heterogeneous strains. 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 large (L), small (S) and unselected (U), strains. In all three strains litters were weaned at 21 days after birth.

(ii) *Egg transfer*

Immature female mice aged 22-25 days were used as donors. Ovulation was induced by 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 thereafter (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 mated 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 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 2½ days after they were mated, for McLaren and Michie (1956) reported a better conception rate using 2½ day recipients rather than fully synchronised donors and recipients.

Recipient animals were anæsthetised 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 tube-uteral 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.

(iii) *Analysis of growth data*

The 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 standardise completely the size of litters, but by statistical manipulation the same end was aimed at. 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 variances, and of the covariance of the mean weight of litters and litter size, were made on birth weights, then on weekly weights to 6 weeks of age, thereafter at 8, 10 and 12 weeks of age. Separate analyses were performed for male and female mice after 3 weeks of age. In each analysis the error variance and group mean were 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 estimated that with an average litter size of 5 and a coefficient of variation of the body weights of the order of 15 per cent., group sizes of this magnitude would provide sufficient material to detect ($P<0{\cdot}05$), with a probability of 75 per cent. differences of the order of 10 per cent. or more in mean body weight (Snedecor, 1956).

(iv) *Experiments performed and notation used*

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 embryo implanted in the female, the second the strain of female in which the embryos were implanted and the third the strain of female which suckled the young after birth. When a transplantation or fostering took place the appropriate letter is italicised. 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 six 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 fertilised 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. The relative importance of maternal effects in the large and small strains,

   (*a*) *U*/L/L and *U*/S/S
   (*b*) *S*/L/L and S/S/S
   (*c*) *L*/S/S and L/L/L

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

   (*a*) U/U/U, *U*/L/L, *U*/S/S
   (*b*) *L*/U/U and L/L/L
   (*c*) *S*/U/U and S/S/S

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

   (*a*) S×L/S/S, S×L/S/U and *S*×*L*/U/U
   (*b*) S/S/*L* and S/S/S
   (*c*) L/L/*S* and L/S/L
   (*d*) S/S/*U* and S/S/S
   (*e*) U/U/*S* and U/U/U

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

   (*a*) *S*×*L*/U/U and *L*×*S*/U/U
   (*b*) S×L/S/S and L×S/L/L

3. RESULTS

(i) *The influence of transplantation of fertilised eggs upon the subsequent growth of the resulting mice*

The work of Gates (1956) established that fertilised 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 affect 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. Fertilised 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 1.

No difference between the two groups was apparent at any stage of growth. Although this comparison was made in the U strain only, it appears reasonable to extend the conclusion that transplantation *per se* is without effect on the subsequent growth potential of the embryo to the large and small strains as well.

(ii) *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 were 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.

Table 2 lists 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 this table :

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 per cent. of the total variation whereas the within litter variation of the small strain accounts for 27 per cent. of the total variation.

TABLE 1

Summary of body weights (in gm.) 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. *S*/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*×*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*×*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×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×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×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			−0·039	−0·154	−0·401	♀ only −0·607 ♂ only	−0·698 −0·620	−0·547 −0·652	−0·523 −0·659	−0·508 −0·626	−0·506 −0·634	−0·496 −0·693
Pooled error mean square within groups corrected for litter size			0·028	0·298	1·356	2·669	7·034	8·364	6·636	7·139	8·222	8·800
Approx. 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

(iii) *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 within litter selection, 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 fertilised eggs

TABLE 2

A comparison of weaning weights of normal and fostered litters of the small and large strains

Litter size	Small strain						Large strain					
	Control			Fostered			Control			Fostered		
	N	W	V	N	W	V	N	W	V	N	W	V
2	20	8·72	0·248	10	8·91	0·578	...	...	...	...	...	...
3	30	8·34	0·562	21	8·74	0·160	30	11·5	1·134	6	13·32	0·263
4	40	7·93	0·120	28	7·83	0·228	40	12·0	0·724	20	12·37	1·296
5	50	7·76	0·298	45	7·46	0·263	50	10·91	0·571	30	10·82	1·537
6	60	7·70	0·283	42	7·22	0·438	60	10·28	0·680	24	10·26	1·192
7	70	7·06	0·392	56	6·86	0·537	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			0·43			0·44			4·73			5·55
Between litters			0·43			0·44			3·22			2·05
Within litters			0·35			0·40			0·86			0·98

N = Number of individuals. W = Mean weight in gm. V = Variance within litters.

of the U strain were implanted in both large and small strain females, and the resulting young compared in growth rate. In the second experiment fertilised eggs of the small strain were implanted in large strain mothers, while fertilised 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 1 and in the graphs marked *U*/L/L and *U*/S/S of fig. 1 (*a*). The results of the second experiment are presented in lines 5 and 6, and 7 and 8 of table 1, and in the remaining graphs of fig. 1 (*a*).

The results of the first experiment 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 8 weeks of age at which stage it appeared relatively stable.

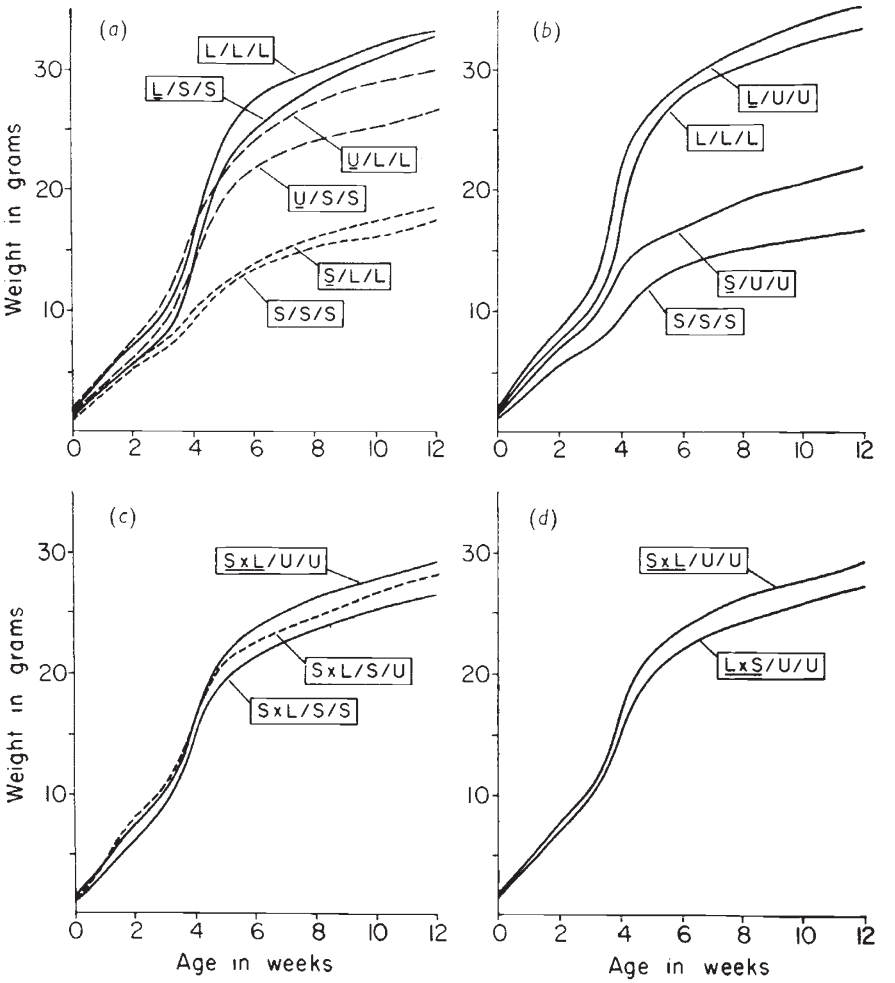

FIG. 1.—(*a*) Joint effects of prenatal and postnatal environment. Growth curves of young of the three strains when reared in and suckled by L-strain or by S-strain females. (*b*) Joint effects of prenatal and postnatal environment. Growth curves of L-strain and S-strain young when reared in and suckled by their own mothers or by U-strain females. (*c*) Separate effects of prenatal and postnatal environment. Growth curves of hybrid young (S ♀ × L ♂) when reared in and suckled by S-strain females (lower curve); when reared in S-strain females but suckled by U-strain females (middle curve); and when reared in and suckled by U-strain females (upper curve). (*d*) Difference in growth between reciprocal crosses of Large and Small strains when reared in and suckled by U-strain females.

The results of the second experiment 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.

(iv) *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 larger 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 mothres of U strain young (table 1, lines 2, 3 and 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. The comparison may be seen in the graphs marked *U*/L/L and *U*/S/S in fig. 1 (*a*). The graph of U/U/U was indistinguishable from *U*/L/L, and is not shown separately.

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 small strains. The results of this comparison are presented in table 1 (lines 5 and 9, and 7 and 10) and fig. 1 (*b*).

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 conditions 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.

(v) *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 given in table 1 (lines 11, 13, 15) and in fig. 1 (*c*).

As expected a difference in birth weight between the normal cross and those reared in U strain females was apparent. In 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 6 to 12 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 measurable 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 table 3.

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, although small strain young reared by U strain does were appreciably heavier at weaning. This observation suggested

that the large strain females do 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 appears untenable 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.

TABLE 3

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

Litter size	Small strain						Large strain				U strain			
	Control		Suckled by L strain		Suckled by U strain		Control		Suckled by S strain		Control		Suckled by S strain	
	N	W	N	W	N	W	N	W	N	W	N	W	N	W
3	30	8·30	9	8·00	3	9·03	30	11·5	3	11·07	...	...	...	...
4	40	7·93	20	7·56	8	9·19	40	12·0	24	9·89	12	11·51	12	11·00
5	50	7·76	10	7·54	10	8·52	50	10·91	35	9·36	...	...		...
6	60	7·70	42	7·91	36	8·66	60	10·28	54	9·49	30	10·40	24	10·09
7	70	7·06	35	6·94	21	8·74	70	9·95	28	7·73	21	9·00	21	9·30
8	...	...	...	...		...	80	8·88	16	6·56	...	...	...	...
Components of variance :														
Between litters .	0·43		0·20		0·77		3·22		0·84		4·33		1·06	
Within litters .	0·35		0·31		0·67		0·86		0·60		0·68		0·43	

N = Number of individuals. W = Mean weight in gm.

From this apparent anomaly 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 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 per cent. of the total variation present.

(vi) *The role of the cytoplasm and sex linkage in the determination of body size*

It is a fairly common observation that reciprocal crosses between mammals 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. The situation is 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 standardising the maternal environment for each of the reciprocal crosses. This approach to the problem was used here.

Reciprocal crosses were made between the large and small strains and the resulting fertilised eggs transplanted to U strain females. The weights of the resulting young are presented in table 1 (lines 11, 12) and in fig. 1 (*d*).

At birth an appreciable 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 that body weights were recorded, resulting in a difference of weight of the order of 8 per cent. at 12 weeks of age.

Table 4 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 of the difference observed. Rather it appears that the cytoplasm of the small strain animals enhance 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 1 (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 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

TABLE 4

Body weights of reciprocal F_1 hybrids of the large and small strains (gm.)

Cross	Age in weeks					
	4	5	6	8	10	12
$S \times L/U/U$ ♀	15·0	19·89	21·51	23·86	24·73	25·78
$L \times S/U/U$ ♀	14·31	17·89	19·70	21·55	22·76	23·89
$S \times L/U/U$ ♂	17·57	22·82	25·62	28·62	30·68	32·96
$L \times S/U/U$ ♂	15·97	21·25	23·87	26·45	28·60	30·29

anomaly might 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.

4. DISCUSSION

(i) *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 linear. Though this condition is not strictly true, the actual departure from linearity over the range of mean litter sizes considered, as indicated in table 2, 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 postnatal 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 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 five young. As each group comprised approximately ten 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 gm.), while the differences of interest between the various groups were usually sufficiently clear cut to give a definite answer to the problem posed.

(ii) *Reproductive physiology*

An examination of various aspects of the reproductive physiology of the strains of mice used 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 fertilised 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 morula 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 per cent. of operations resulting in pregnancy. On the other hand small strain females proved refractory in this regard, for only 20 per cent. 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 per cent. 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.

(iii) *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 deterioration 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 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 (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 fœtal 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 fœtal placenta as an organ of interchange.

The literature available covering these phases of the physiology of the placenta has been reviewed in detail by Huggett and Hammond (1952). Suffice to state here that reasonable evidence establishing the validity of both points may be found therein, 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.

(iv) *The cytoplasmic influence*

Though the difference that was established between the reciprocal crosses reared in the same environment provides apparent evidence that cytoplasmic factors are influencing growth, three further possible explanations may be invoked. The first lies in the fact that the eggs spent $3\frac{1}{2}$ days post-ovulation in their own dam prior to transplantation. Thus it may be argued that the difference observed in the reciprocal crosses is merely a consequence of this early maternal environment. Some evidence supporting this explanation is provided by the observation that the eggs of the small strain appeared slightly further developed at $3\frac{1}{2}$ days' post-ovulation than did those of the large strain. On the other hand it was shown that the post-implantation environment of the small strain was poorer than that of the large strain and it seems unlikely that the reverse would be true of the pre-implantation environment.

A further possible explanation lies in terms of differential mortality of eggs actually implanted but in view of the success achieved in causing eggs to implant in the U strain females this too seems unlikely. A third possibility is that it is merely a chance result. Obviously, further work on this point is required.

5. 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 material 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 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 suggesting that the cytoplasmic influence on growth was greater in the small strain than in the large strain.

8. The results are discussed in relation to the interpretation of selection experiments.

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