The size and endocrine activity of the pituitary in mice selected for large or small body-size

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The large alterations in body-weight arising after several generations of selection for large or small body-size clearly involve basic physiological processes. In previous papers we have described various physiological differences between mice of large and small body-size, using two strains (*N* and *C*) selected by Falconer (1953, 1960). Mice of the large line of strain *N* (*NL*) grow considerably faster and contain a higher proportion of body fat than mice of the small line (*NS*) (Fowler, 1958). *NL* mice consume more food and absorb a greater proportion of nitrogen from it, but differences in food utilization appear to be largely a consequence of the different growth rates of the two lines (Fowler, 1962).

Physiological changes of a more fundamental nature are clearly involved in determining adult body-weight, and the basic endocrine pattern of large and small mice is an obvious choice for study. Earlier work on the reproductive physiology of strains *N* and *C* indicated that differences existed between mice of the large and small lines of both strains in the amounts of pituitary gonadotrophins secreted (Fowler & Edwards, 1960). Sterility in line *NS*, which appeared to be due to a general deficiency in the secretion of the gonadotrophic and pregnancy hormones, became acute during the present experiment, and severely limited the number of *NS* mice available for study. *NL* mice have a higher energy expendtiure than *NS* mice, though the expenditure is similar in the two lines and in the control line (*NC*) when animals of the same weight are compared (Fowler, 1962). In the present experiments, adult mice of strain *N* were used in studies of pituitary weights, gonadotrophic activity of the anterior pituitary, thyroid activity, and alterations in growth rates following the injection of growth hormone. Mice related to line *CL* were also available and were included in some of the studies.

MATERIAL

Mice of strain *N* and of a line related to strain *C* were obtained from Dr D. S. Falconer, Institute of Animal Genetics, Edinburgh. The construction of the foundation population, and the methods and response to selection have been described by Falconer (1953, 1960). At the beginning of the study, lines *NL* and *NS* had been selected for 50 and 41 generations respectively. Two further generations were reared during the present work, no attempt being made to select the mating pairs. Mice related to strain *C* (referred to here as *CL*) came from a cross between

CRL and a strain carrying the gene *pituitary dwarf* (Falconer & Isaacson, 1959). Despite this cross and a relaxation of selection, these mice still grew very rapidly. Some of them weighed 40 g. or more at 3 months of age, while their litter mates which were homozygous for *pituitary dwarf* were less than 10 g. Both types of mice were used.

METHODS AND RESULTS

Pituitary weights

The pituitaries of *NL* mice weighed between 0·6 and 2·7 mg., those of *NS* mice between 0·4 and 1·2 mg. Pituitary weights were highly correlated with body-weight and with age in mice of both lines, the correlations being lowest in *NS* males (Fig. 1; Table 1). The greater variability in *NS* pituitaries might have been due to difficulty
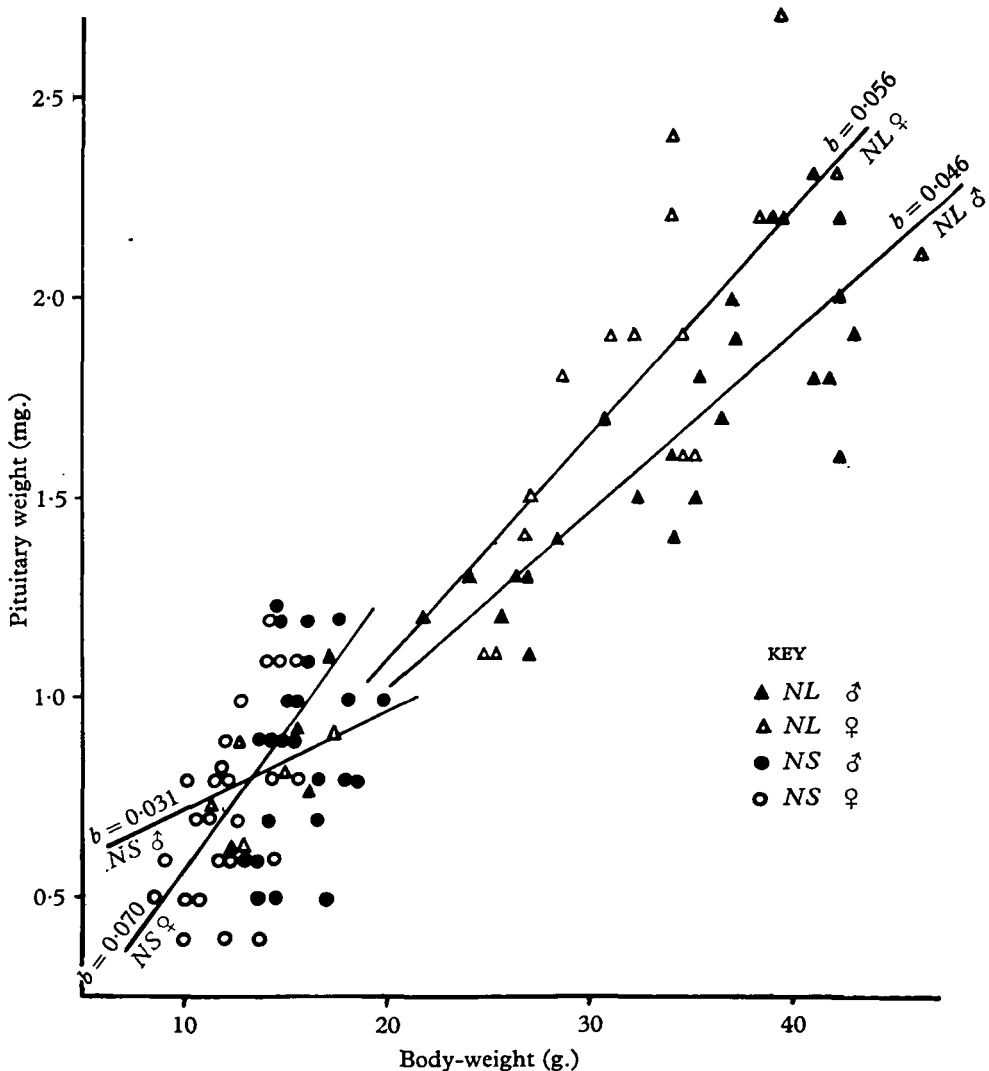

Fig. 1. Relationship between body-weight and pituitary weight in mice of strain *N*.

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Table 1. *Correlation between pituitary weight and body-weight or age in* NS, NL, *and* CL *mice*

	Correlation coefficient between pituitary wt. (mg.) and body-weight (g.)		Correlation coefficient between pituitary wt. (mg.) and age (months)	
	Males	Females	Males	Females
NS	0·245	0·579†	0·096	0·525*
NL	0·867†	0·906†	0·728†	0·626†
CL	0·480*	0·660†	0·270	0·497†

*P < 0·01.   † P < 0·001.

in the clean removal of small pituitaries. Pituitary weights of *CL* mice were between 1·5 and 4·4 mg., those of female mice being heavier (Fig. 2), and were also highly correlated with body-weight and age (Table 1). Six *dwarf* pituitaries were weighed, and are shown in the inset of Fig. 2. There was evidently proportionally less

Table 2. *Analysis of the regression of pituitary weight (mg.) on body-weight (g.) in* NS, NL *and* CL *mice*

I. Regression coefficients (*b*) and number of mice (*N*):

		Males		Females	
Strain	N	b	N	b
NS	23	0·031	25	0·070†
NL	29	0·046‡	21	0·056‡
CL	42	0·032†	48	0·068‡

Mean slope   0·044 ± 0·005   0·059 ± 0·005
Difference between mean slopes: $t_{176} = 2.48$*

II. Analysis of differences between strains:

	Males			Females		
Source of variance	d.f.	m.s.	F	d.f.	m.s.	F
A. Comparison of NS, NL, *and* CL						
Differences in slope	2	0·081	1·05	2	0·079	0·61
Error	88	0·077		88	0·129	
Between adjusted means	2	1·209	15·70‡	2	14·925	116·60‡
Error	90	0·077		90	0·128	
B. Comparison of NS *and* NL						
Differences in slope	1	0·010	0·26	1	0·021	0·39
Error	48	0·039		42	0·054	
Between adjusted means	1	0·015	0·38	1	0·014	0·26
Error	49	0·039		43	0·053	

*P < 0·05.   † P < 0·01.   ‡ P < 0·001.

pituitary tissue in dwarfs, their pituitary weights being well below the regression lines for their normal sibs. Data from these six *dwarfs* are not used in any of the following calculations.

The regression coefficients of pituitary weight on body weight in *NL*, *NS*, and *CL* mice are given in Table 2. Each of the values for females was greater than for
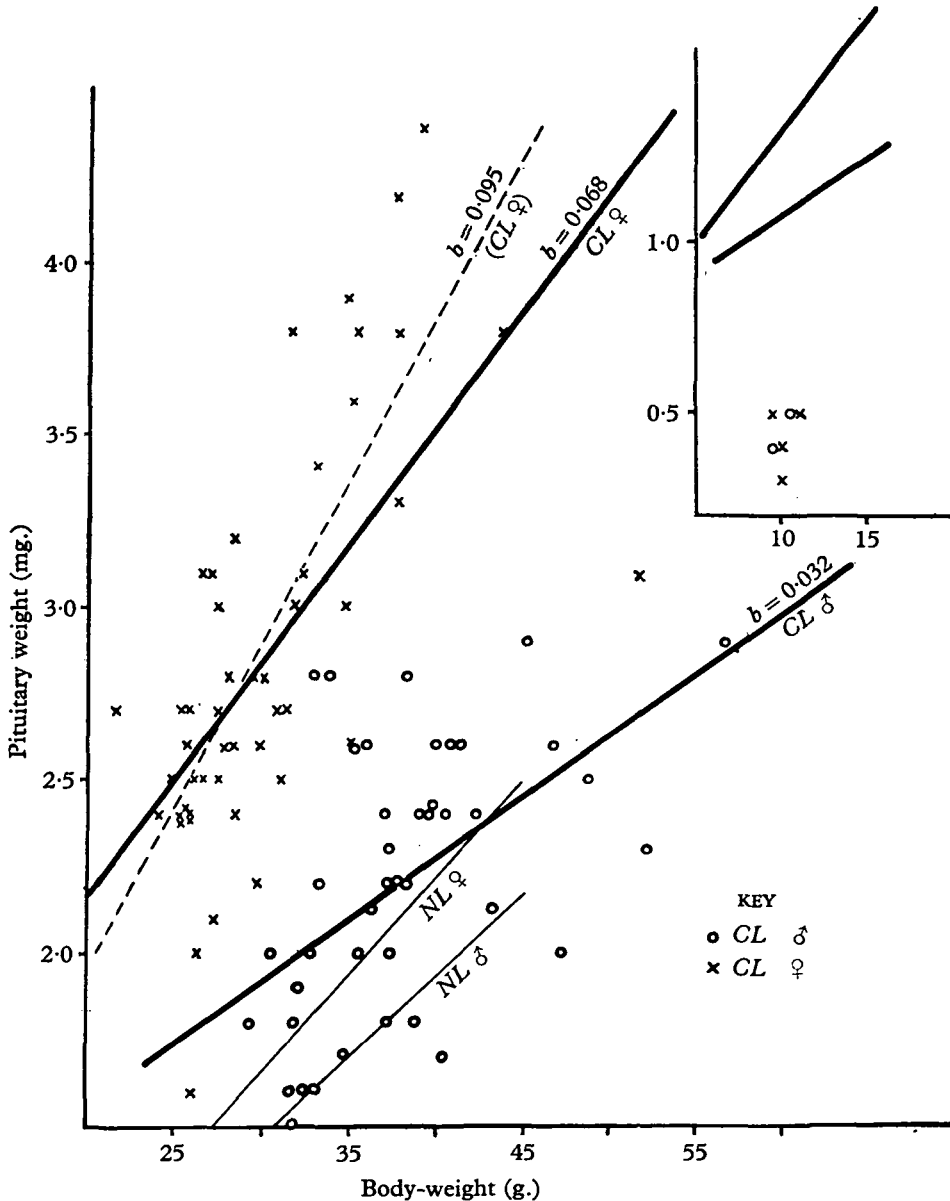

FIG. 2. Relationship between body-weight and pituitary weight in *CL* mice. The inset shows the relationship in *dwarf* mice. The dotted line shows the regression for *CL* females when the pituitary weighing 3·1 mg. taken from a female weighing 51·8 g. is excluded. The regression lines for males and females of line *NL* are shown for comparison.

the corresponding males. When analysed within sexes, the differences between strains were not significant, hence the mean slopes for each sex were calculated. In this calculation, the separate slopes for the three strains were weighted by the reciprocal of their variances. The mean slopes for males and females were found to differ significantly (Table 2). Sex differences in pituitary size have been reported previously (e.g. Evans & Simpson, 1929).

Significant differences existed between the strains in the position of the regression lines. Differences between the adjusted means were highly significant when data from *NS*, *NL* and *CL* mice were tested, whereas data from *NS* and *NL* mice alone showed no difference between the adjusted means (Table 2). The pituitaries of *CL* mice were thus different from those of *NL* and *NS*, being much heavier per unit of body-weight (Fig. 2).

Gonadotrophin content of the anterior pituitary

The total gonadotrophin content of the anterior pituitary was assayed using techniques developed for the assay of gonadotrophin preparations (Claringbold & Lamond, 1957). In preliminary experiments, pituitaries from female outbred mice
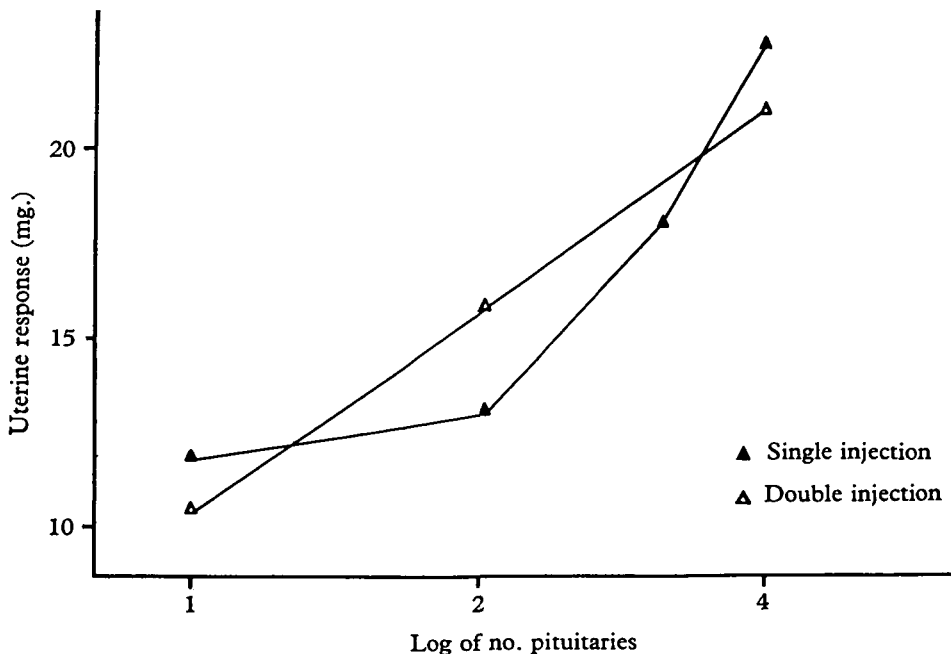

Fig. 3. Response of the uterus of immature mice to single or double injections of pituitary tissue from outbred mice.

were injected subcutaneously into female mice aged 3 weeks and weighing 8 to 10 g. Forty-eight hours later, the uteri of the recipients were removed, dried by blotting with filter paper, and weighed. Neither freezing and thawing nor homogenization of the pituitaries gave a more consistent response than whole untreated pituitary,

hence subsequent injections were made with fresh pituitaries. Claringbold and Lamond showed that the uterine response was less variable if the injection of gonadotrophin was divided; the uterine response to single or to double injections of pituitary given at an interval of 20 hours are plotted in Fig. 3. With double injections the slope of the regression line of uterine weight on log dose was slightly steeper (15·74), and the index of precision (λ) (Bliss, 1952) considerably smaller (0·258) than with a single injection (13·50 and 0·516 respectively). Double injections were therefore adopted for assaying the pituitaries of mice of strain N. The injection of pituitary tissue could stimulate extra growth in immature mice, and thus alter the uterine sensitivity to gonadotrophins. There was little or no evidence of this, however. The correlations between the body-weights of the recipients at autopsy and their uterine weights were not significant, and there was no evidence of an increase in the variance of uterine weight with increasing dose.

Table 3. *Analysis of the response of the uterus of immature mice to the injection of* NL *or* NS *pituitary tissue*

I. Regression of uterine weight (mg.) on log weight
of pituitary injected (g.)

	Males	Females
NS	23·90 ± 6·72	13·66 ± 6·42
NL	23·53 ± 3·01	19·54 ± 3·96

II. Potency estimate (in I.U./mg) of *NS* and *NL* pituitaries
in terms of PMS

	Males	Females
NS	0·130	0·076
NL	0·150	0·064

Based on data obtained in the preliminary experiments, recipient females were injected with one or two *NL*, or with two or four *NS* pituitaries in two equal injections. The pituitary donors were aged between 6 weeks and 15 months, and a total of 59 recipients were injected. Uterine responses to injections of *NS* or *NL* pituitary were variable and are plotted against the amount of tissue injected in Fig. 4. The age of the donor was not a major factor influencing the response. A significant regression of uterine response on log weight of pituitary occurred in all groups except *NS* females (Table 3). The slopes of the regression lines for males appeared to be greater than those for females, although the differences were not statistically significant ($F^1_{57} = 0·89$). Differences in the elevation of the lines for males and females were significant ($F^1_{58} = 8·26, P < 0·01$). A sex difference in the gonadotrophic potency of the pituitary also occurs in rats (Evans & Simpson, 1929) and in man (Witschi & Riley, 1940).

The sex difference in the gonadotrophin content of the pituitary made it necessary to compare *NL* and *NS* pituitaries within sexes. No differences could be found between the large and small mice in the slopes or mean elevations of the regression lines; the combined slopes for males and for females are given in Fig. 4. The potency
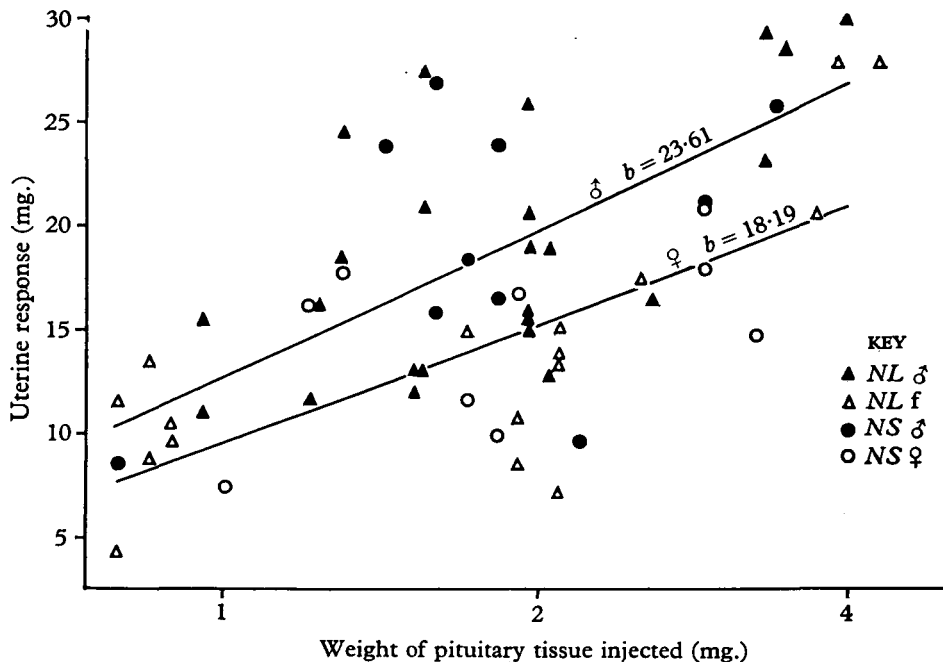
Fig. 4. Response of the uterus of immature mice to injections of pituitaries from strain N mice.
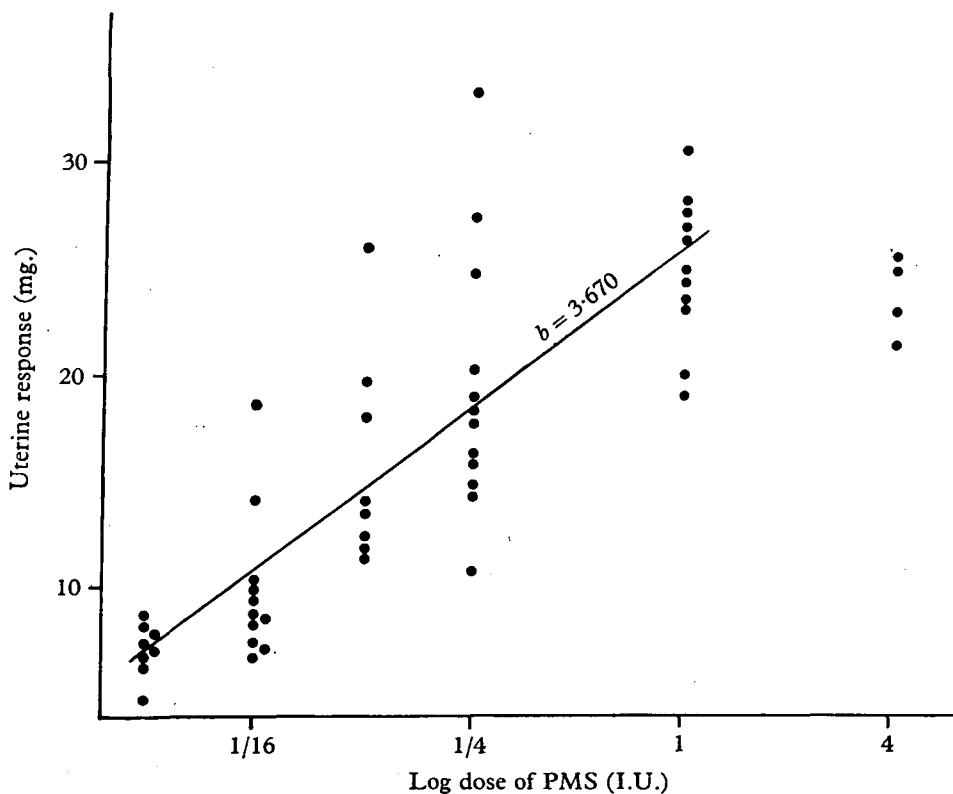

Fig. 5. Response of the uterus of immature mice to a single injection of PMS.

of *NL* and *NS* pituitaries was expressed in terms of the response to an intraperitoneal injection of pregnant mares' serum (PMS) (Fig. 5), using the standard statistical techniques for parallel line assays (Finney, 1952). Results obtained with 4 I.U. PMS were excluded, since the maximum response had evidently been reached with 1 I.U. The slope of the log dose response line for PMS was not significantly different from those obtained from injections of pituitary except for *NL* males ($P < 0.05$), hence the potency estimate for these males is not strictly valid. The calculated potencies are given in Table 3.

Estimation of thyroid activity

Sections of the thyroids of *NL* and *NS* mice showed them to be histologically normal.

[131]I was used to estimate thyroid activity. The mice were fed with Purina Rat Chow, a diet low in iodine, for two weeks, and were then injected with 2 μc. [131]I (Radiochemical Centre, Amersham). The uptake of ^{131}I into the thyroid was measured 12 to 15 hours later, further measurements being made twice daily for 3 days, and once daily thereafter. The Ekco scintillation counter, Type 559D, was modified by insertion of a steel collimator to give a distance of 7·7 cm. between the crystal face and the tissues of the animal. The aperture of the collimator was 0·96 cm. and its half angle was 15°. At each measurement, three counts were taken on the neck, and three on the abdomen. Radioactivity in the thyroid was then estimated in the conventional manner by subtracting natural background plus one-half of the count of the abdomen from the count of the neck (Wolff, 1951). Corrections were made for decay of tracer.

Thyroid activity of strain *N* mice was estimated in two ways. The *maximum uptake* of ^{131}I was calculated for each mouse, and was found to occur on the first or second day after injection. In the second method, the *thyroid secretion rate* (TSR) was found according to the method described by Amin, Chai & Reineke (1957). In this method increasing amounts of 1-thyroxine are injected daily into each mouse until the loss of radioactivity from the thyroid is reduced to 5 % or less during a two-day interval.

The maximum uptake of ^{131}I varied considerably between mice (Fig. 6). A significant correlation could not be established between uptake of ^{131}I and sex, age or body weight, hence the mean uptake was calculated for each line. The means were 51·5 and 26·8 c./sec. in *NL* and *NS* mice respectively. The uptake for each mouse was transformed into logs, and the means (1·684 and 1·395) differed significantly ($t_{53} = 6.99$; $P < 0.001$). Expressed per gram of body-weight, the uptake was slightly higher in *NS* mice (Fig. 7). If fat is excluded from the body-weights, however, uptakes per gram were almost identical in *NL* and *NS* mice, for Fowler (1958) found that the fat-free carcasses of *NL* mice weighed approximately double those of *NS* mice at all ages after 21 days. Uptake per unit weight declined with age (Fig. 7) which is to be expected since total uptake was fairly constant over all age groups.
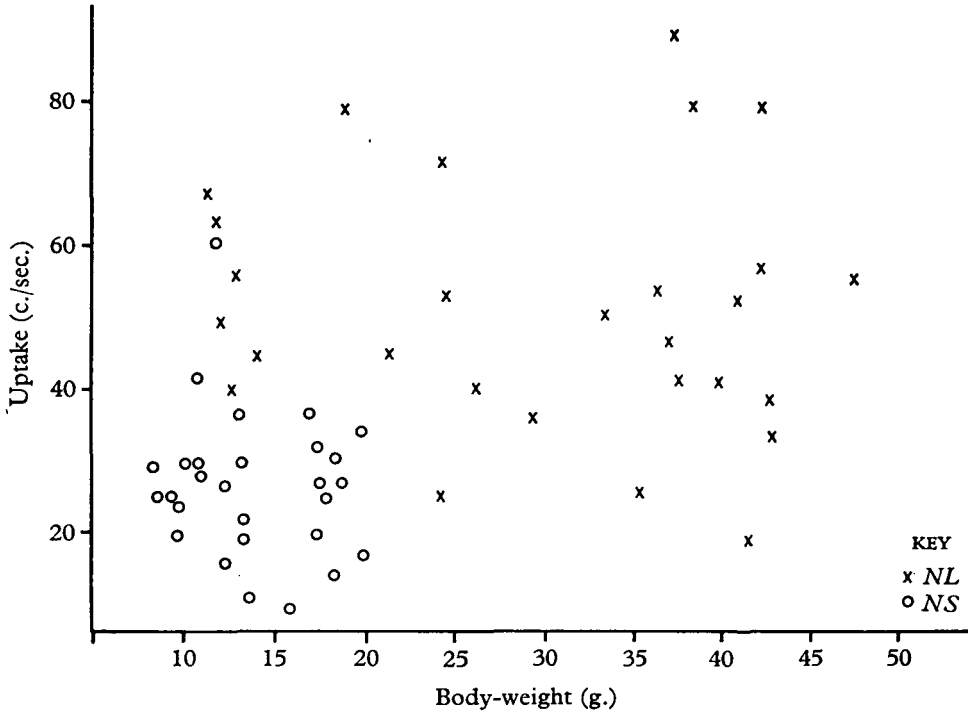
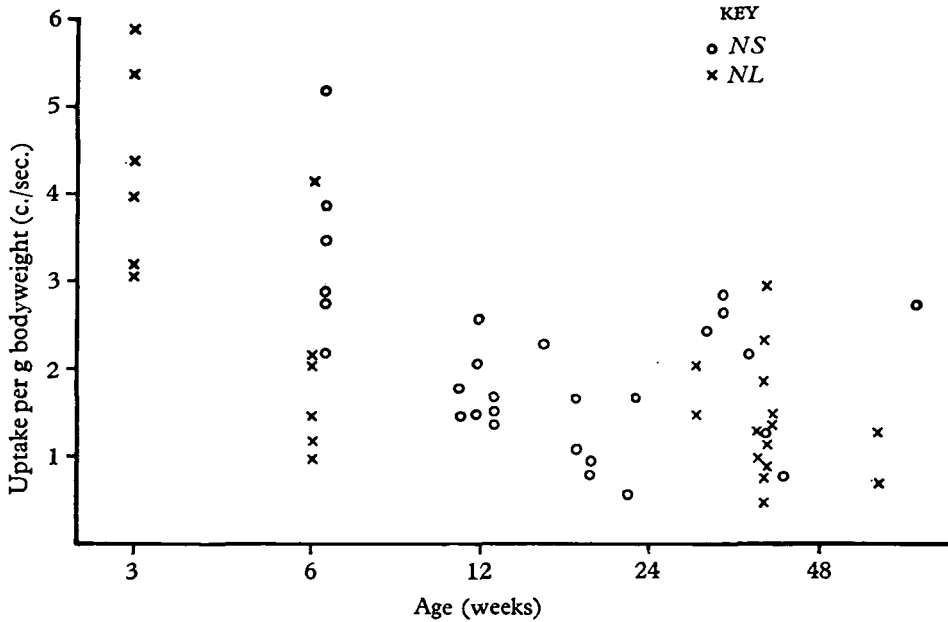Fig. 6. Maximum uptake of ^{131}I into the thyroids in relation to body-weight in mice of strain N.

Fig. 7. Maximum uptake of ^{131}I per unit body-weight in relation to age in mice of strain N.

Ten *CL* mice and 5 *dwarfs* were also injected with [131]I. Uptake by *CL* mice was higher than that of *NL* mice, being 114.3 ± 11.1 c./sec. in four males (mean weight 43.0 g.) and 56.2 ± 6.1 c./sec. in six females (mean weight 32.7 g.). In contrast, there was practically no uptake at all into *dwarf* thyroids, the count on the neck region being the same as that on the abdomen. A similar finding was reported by Wykes, Christian & Andrews (1958) and Wegelius (1959). *Dwarf* mice clearly had a less active thyroid than *NS* mice.

The TSR was difficult to measure in *NS* mice because of the low uptake of ^{131}I, and this probably led to overestimates. The TSR varied greatly between mice,

Table 4. *Mean TSR of* NL *and* NS *mice of various ages*

NL mice

No.	Age (days)	Mean body-weight (g.)	Mean TSR	Mean TSR/100 g. body-weight
6	21	12.4 ± 0.4	3.1 ± 0.4	26.2 ± 2.1
4	43	23.4 ± 1.7	3.9 ± 0.8	16.9 ± 1.2
8	204–274	39.5 ± 1.5	3.8 ± 0.3	9.5 ± 0.6

NS mice

No.	Age (days)	Mean body-weight (g.)	Mean TSR	Mean TSR/100 g. body-weight
6	45	10.4 ± 0.6	3.1 ± 0.3	38.6 ± 5.0
6	78–108	14.2 ± 1.3	3.6 ± 0.3	24.3 ± 3.1
4	215–268	11.5 ± 0.7	3.0 ± 0.3	29.3 ± 3.7

although the mean TSR was similar in both lines and independent of body-weight (Table 4). Mean TSR per 100 g. body-weight appeared to be higher in *NS* mice at all ages, but was comparable in both strains for animals of similar body-weight.

Injection of pituitary tissue or growth hormone into strain N mice

The response to growth hormone or to injections of pituitary tissue taken from outbred mice was studied in *NS* mice aged 24 to 27 days, and in *NL* mice of similar weight. Whole pituitaries, or extracts obtained by grinding with silver sand at pH 8 were injected subcutaneously into the recipients. Various amounts of growth hormone were injected into other mice, the hormone being similar to the International Standard or obtained from Armour Laboratories.

Extracts of pituitary glands produced no increase in the growth rate of *NS* mice. The injection of one living pituitary every 3 days also failed to stimulate additional growth in three *NS* mice. Five *NL* and seven *NS* mice were injected daily with one

or three fresh pituitaries. Siblings from the same litter were not injected or were injected with saline to serve as controls. Each of the treated *NS* mice grew more rapidly than control litter mates, the mean increase in body-weight being 3·8 g. in treated and 2·6 g. in control mice over a period of 2 weeks (Table 5). *NL* mice did not respond to the injection of similar amounts of pituitary tissue.

Table 5. *Mean increase in weight of* NL *and* NS *mice after the daily injection of pituitary tissue*

Line		No. of mice	Initial weight (g.)	Gain in weight (g.)	
				Week 1	Week 2
NL	Treated	5	7·2	5·0	5·4
	Controls	7	7·4	5·1	5·3
NS	Treated	7	6·0	2·0	1·8
	Controls	9	6·5	1·2	1·4

Most of the following data using growth hormone were obtained by Dr R. E. Fowler, and I am indebted to her for permission to quote it. Bovine somatotrophin was injected subcutaneously into *NL* and *NS* mice. Amounts of less than 1 mg.
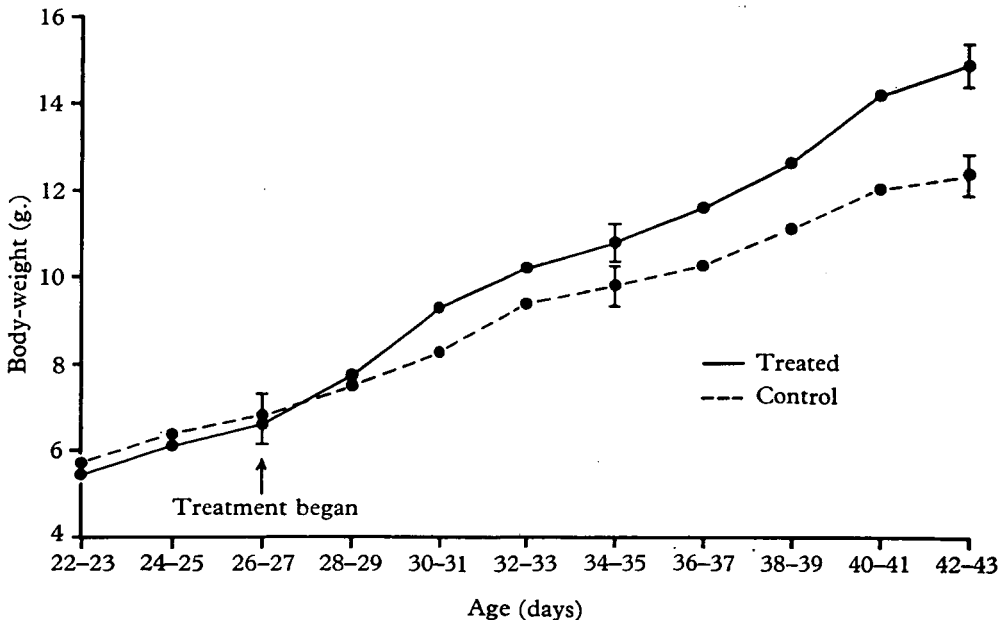

Fig. 8. Mean growth of *NS* mice treated with growth hormone and of their untreated sibs. One standard error is plotted above and below the mean.

per day did not promote extra growth. Eleven *NS* mice were given 1 mg. per day for 16 days from 26 days of age, 17 siblings being used as controls. Each treated mouse grew more rapidly than its controls. The mean increase in weight was from 6·3 to 14·9 in treated mice (an increase of 8·6 g.), and from 6·8 to 12·4 in controls (i.e. 5·6 g.) (Fig. 8). The carcasses of two treated and three control mice were

analysed for fat, protein and water (Fowler, 1958). The increased weight of the treated mice was largely due to protein and associated water (Table 6). Two *NL* mice were given 3 mg. somatotrophin from 27 to 40 days of age. They also increased in weight (mean 15·1 g.) more rapidly than three controls (11·0 g.).

Table 6. *Increase in weight and carcass composition of two* NS *mice given 1 mg. growth hormone daily from 26 to 42 days of age. Data obtained by Fowler* (*unpublished*)

Animal	Body-weight (g.)				% increase 26–42 days	Body components (g.) at 42 days of age		
	21 days	26 days	35 days	42 days		Fat	Protein	Water
Treated	4·7	7·2	11·3	15·0	155	1·17	2·45	8·30
,,	6·1	7·1	13·4	16·8	137	1·10	2·76	9·49
Control	5·0	6·4	9·2	11·2	75	1·02	1·80	6·11
,,	5·7	7·2	11·2	13·8	92	1·54	2·28	7·83
,,	5·8	7·0	11·0	13·4	92	0·91	2·15	7·70

DISCUSSION

At first glance, the primary physiological change in adult mice selected for large or small body-size appears to have been in the size of the pituitary gland. The pituitaries of mice of the large line were larger than those of the small line at all ages. But the regression of pituitary weight on body-size was common to the two lines. The total amount of pituitary per unit of body-weight was thus identical in both *NL* and *NS* mice, and it was necessary to determine the endocrine activity per unit weight of pituitary tissue ('unit potency').

The unit potency of pituitary gonadotrophin was equivalent in large and small mice. This was found by direct measurement, using the uterine weight assay. The *secretion* rate of gonadotrophins per unit of pituitary weight was also probably similar in *NL* and *NS* mice, as judged by the regressions of egg number on body-weight (Fowler & Edwards, 1960). The unit potency of thyrotrophin was presumably equivalent in the large and small mice, for thyroid activity and energy expenditure (Fowler, 1962) were almost identical in *NL* and *NS* mice when expressed per gram of the fat-free carcass. It is difficult to draw definite conclusions about growth hormone from the available experimental data. *NS* mice responded more strongly than *NL* mice to identical amounts of pituitary tissue or growth hormone, which presumably indicates that relatively less endogenous hormone was secreted by the small mice. The amount of hormone needed to induce a significant increase in growth (1 mg. per day, or more) might have been to the species specificity of this hormone (e.g. Knobil & Greep, 1959; Li, Papkoff & Jordan, 1959). In rats, both pituitary size and the unit potency of growth hormone in the pituitary are similar over wide differences in age or body-weight (Solomon & Greep, 1958; Bowman, 1961); the common regression line of pituitary weight on body-weight in *NL* and *NS* mice indicates that mice are similar to rats in this respect. Thus it

appeared that the endocrine activity of unit amounts of pituitary tissue was similar in both lines, and that the overall change in pituitary size was the primary response to selection.

But the change in pituitary size might conceivably be determined by the change in body-size, and not *vice versa*. Evidence exists both for and against this proposition. Results obtained from *pituitary dwarf* mice, and from the injection of growth hormone into rats, suggest that the pituitary determines body-size. The stunted growth of *pituitary dwarf* mice is due to a lack of growth hormone caused by the absence of normal acidophils in the anterior pituitary (Smith & MacDowell, 1930, 1931; Francis, 1944; Elftman & Wegelius, 1959); the reciprocal exchange of pituitaries between normal and *dwarf* mice produces stunted growth in the former and near-normal growth in the latter (Carsner & Rennels, 1960). Rats injected with growth hormone increase in body-weight proportionally to the amount of hormone injected, and this elevated weight is maintained for as long as that amount of hormone is given. Increasing the amount of hormone results in growth to a new plateau whereas cessation of the injections results in a rapid decline in body-weight to the original size (Emerson & Emerson, 1960). On the other hand, pituitary size is known to be influenced by physiological changes and other factors, e.g. pregnancy (Ladman & Runner, 1953; Grosvenor & Turner, 1960), and injections of oestrogen (Greep & Jones, 1950). Gross damage to the hypothalamus results in decreases in both body and pituitary weights to two-thirds of their original size (Reichlin, 1960). Moreover, the high correlation found between pituitary size and body-weight is not unexpected, for the weights of many organs bear a constant relationship to body-weight (e.g. Robinson & Wilber, 1961; Widdowson & McCance, 1960). It is of interest that a similar relationship may hold in another physiological system, for maternal pituitary weight is positively correlated with the number of implanted embryos, possibly via the secretion of gonadotrophins (Ladman & Runner, 1959).

Selection for body-size in strain N has thus produced alterations in both adult pituitary weight and adult body-weight, though it is not clear which of the two is the primary change. Differences in pituitary size would *a priori* be considered to be closer to the primary genetic change. Although there was no evidence of differences in unit potency of pituitary tissue in NL and NS mice, such differences have been reported in pigs. Pigs selected for rapid gain in weight had a higher unit potency of pituitary growth hormone at all ages than those selected for slow gain, the pituitary weight/body-weight ratios being the same in both lines (Baird, Nalbandov & Norton, 1952). Since this ratio was similar in both lines, it follows that pigs showing rapid gain also possessed a large pituitary. It is not clear from data given by Baird *et al.* whether the two lines of pigs were derived from the same foundation population, or whether genetic differences existed between the two lines before selection began. The present work has shown that considerable differences in pituitary size exist between animals of the same body-weight derived from different foundation populations, i.e. NL vs. CL. The higher pituitary weight/body-weight ratio in CL mice could be interpreted as evidence of a lower unit potency of their pituitary. But this interpretation would be misleading, since the higher pituitary weight in

CL mice is associated with a superior physiological background in that they grow more rapidly, have a greater proportion of carcass protein and water (Fowler, 1958), shed more eggs per unit body-weight (Fowler & Edwards, 1960) and evidently have a greater thyroid activity.

Many previous studies have shown that differences occur in the thyroid activity of inbred and hybrid mice, although no correlation was found between thyroid activity and body-weight in various strains (Wykes *et al.*, 1958; Silverstein, Sokoloff, Mikelsen & Jay, 1960). Estimates of TSR in the present work are higher than those of Amin *et al.* (1957) and much higher than those of Wada, Berswordt-Wallrabe & Turner (1959). The cause of these discrepancies is not clear, but could be due to differences in strain, diet, laboratory conditions, etc. Mendelian recessives also influence pituitary function in mice and other species. *Dwarf* mice, men and cattle secrete less growth hormone than normals (e.g. Raben, 1959; Marlowe, 1960; Foley, Heidenreich & Lasley, 1960). Homozygous *obese* mice produce smaller amounts of gonadotrophins than normal siblings (Lane, 1959), seldom come into oestrus, and fail to maintain their pregnancies in the absence of exogenously administered hormones (e.g. Smithberg & Runner, 1957). Large mice of strain *N* and *C* are quite different, the large body-size interfering with neither oestrus nor pregnancy (Fowler & Edwards, 1960).

SUMMARY

Mice of two strains, *N* and *C*, were used in studies on body-size, pituitary size, and endocrine potency of the pituitary. Strain N had been selected for large (*NL*) and small (*NS*) body-size; strain *C* had also been selected for large body-size (*CL*) but had been crossed to an outbred strain segregating pituitary dwarfism.

Pituitary weights and body-weights were highly correlated, the regression lines being common in *NL* and *NS* mice. Female pituitaries were considerably heavier than male pituitaries in *CL* mice. In relation to body-weight, *CL* pituitaries were consistently heavier than those of *NL* or *NS* mice.

No differences were detected in the unit potency of gonadotrophins in the pituitaries of *NL* and *NS* mice as estimated by the uterine response of immature outbred mice to subcutaneous injections of pituitary tissue. The uptake of ^{131}I into the thyroid was comparable in *NL* and *NS* mice per unit of body-weight, and the thyroid secretion rate was also similar using animals of the same body-weight. Immature mice of both lines responded by increased growth to injections of growth hormone or fresh mouse pituitary, though the response was greater in *NS* than in *NL* mice.

The primary response to selection has probably been in the size of the pituitary rather than in its unit potency. The interrelationships between body-size, body components, organ size and endocrine levels are discussed.

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