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# Cell numbers and cell sizes in organs of mice selected for large and small body size

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SUMMARY

Cell numbers in four organs of large, control and small mice were estimated by nuclear counts. Average cell mass was estimated from the cell number and the organ weight. The mice were from the selected Q-strain with six replicate lines in each size-group. The organs were lung, liver, spleen and kidney. At 6 weeks of age the large mice had more cells and larger cells than the controls in all organs; the small mice had fewer and smaller cells than the controls. The regression of log cell-number on log-organ weight provides a measure of how much, proportionately, cell number contributes to the differences in organ weight. In the lung and spleen, cell number contributed about 70% of the strain differences in organ weight, cell mass contributing about 30%; in the liver and kidney the relative contributions were about equal, at 50%.

Cell counts at different ages from 3 to 15 weeks showed that cell number and cell mass contributed to the increases of organ weights during growth in roughly the same proportions as stated above. From this it is concluded that the main effect of selection for body weight has been to speed up or slow down the normal processes of cellular growth.

## 1. INTRODUCTION

When body size is changed by selection, the response might be partitioned into changes in the number of cells and in average cell size. These changes could then be described formally in terms of the genetic correlations of cell number and cell mass with body weight. An alternative viewpoint is to consider a genetic change in body weight as an adjustment of the regulation of growth, and to ask whether this regulation operates by changing cell number or by changing cell size, or by both.

The relation of body size to cell number and cell size in *Drosophila* has been very thoroughly studied by Robertson (1959*a*, *b*). Genetic variation of both number and size was found, with the interesting difference that the genetic variation of cell number was mainly additive, but that of cell size was non-additive. Information about mammals is much less complete, though genetic variation in both cell number and cell size have been reported. Robinson & Bradford (1969) found that the larger sizes of seven organs in a strain of mice selected for increased post-weaning growth were due to increased cell number. Hanrahan, Hooper & McCarthy (1973)

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studied muscles in strains of mice selected up and down for growth rate, and found the changes in muscle size were mainly due to fibre number though there were some changes also in fibre diameter. Krzanowska (1967) compared $F_1$ hybrid mice with the parental inbreds and found that the heterosis in the growth of the embryonic liver was due to cell number. Musialek (1974) similarly compared $F_1$ and inbred mice and found postnatal heterosis due mainly to cell number, with smaller effects due to cell size. Priestley & Robertson (1973), studying the same strains as are described in this paper, though by different methods, found the main differences to be in cell number, but there were smaller differences in cell size. The general conclusion from the previous work on mice is that genetic differences of size are mainly, but not exclusively, due to cell number.

There have been many studies of environmental effects on cellular properties. The general conclusion from the work on nutrition in rats is that the level of nutrition early in postnatal life affects cell number and not cell size, but later in life it affects cell size and not cell number (Winick & Noble, 1966, 1967; Winick, Fish & Rosso, 1968). Musialek (1974) compared the effects of nutritional level with those of heterosis and found both affected cell number and cell size in the same ways.

Differences of size between mammalian species are mainly differences of cell number (Berrill, 1955), so one might expect artificial selection to affect mainly cell number. Cell size does, however, differ between species; the cells of mice and elephants differ by a factor of 2 in linear dimensions, and so by a factor of 8 in volume (Berrill, 1955). Furthermore, cell size increases during the growth of the individual, a 4-fold increase occurring in the rat kidneys after birth (Winick & Noble, 1965).

From the evidence of previous work it seems, therefore, that one should expect there to be genetic variation of both cell number and cell size on which artificial selection for body weight might act. This paper examines the differences of cell number and cell size between strains of mice previously selected up and down for body weight. The weight of the large mice was about twice that of the small at 6 weeks of age. An important aspect of the material was that the selection was replicated. There were six lines selected independently for large size, six selected for small size and six unselected controls. Any change that is found regularly in all the replicates can with more confidence be ascribed to the genetic differences in growth rate, whereas irregular changes, differing from line to line, are more likely to be the consequences of random drift, perhaps unrelated to the character selected for.

## 2. MATERIALS AND METHODS

### (i) *Sources of mice*

Two sets of data were obtained. The first, or main, experiment was a 'cross-sectional' study in which the material was obtained from mice all aged 6 weeks. The second, or subsidiary, experiment was a 'longitudinal' study in which material was obtained from a smaller number of mice at six different ages. All the mice

came from the replicated Q-lines selected for body weight at 6 weeks of age (Falconer, 1973). There were six Large (L) lines, six Small (S) lines and six unselected Control (C) lines. The six replicates within each size-group (i.e. L, C, or S) were labelled A–F, so that, for example, LA and SA are the large and small lines of the A-replicate. The three lines (L, C and S) of each replicate shared some common ancestry in the base population, but no resulting correlations in any feature were found. The 18 lines are therefore best regarded as 6 random replicates in each of the three size-groups.

The mice for the main experiment came from the 14th and 15th generations, when the lines had made about 85 % of the total response achieved by generation 21. After generation 21 all the lines were maintained without selection, and the mice for the subsidiary experiment came from generation 31. For the main experiment each of the 18 lines provided 8 males and 8 females taken equally from two litters in each of the two generations. There were thus in all 144 mice of each sex. The subsidiary experiment was restricted to males of only two replicates, B and E, in each size-group, making 6 lines in all. Material was obtained from mice at six ages, namely 3, 5, 6, 7, 9 and 15 weeks. Each line provided 4 mice, all males, at each age, taken from two litters. Some litters contributed to several ages, others to only one. The total number of mice was 144.

### (ii) *Cell-counting*

The procedure for preparation of the tissues and counting of the cells was the same in both experiments. After the mice were killed, they were bled thoroughly. Four organs – lung, liver, spleen and kidneys – were weighed, and homogenized at constant speed for exactly 1 min in 25 ml (50 ml for liver) of 0·01 N-HCl. The method was basically that described by Zumoff & Pachter (1964) for releasing nuclei for counting. The homogenate was examined microscopically for residual clumping of cells. In a few cases, further homogenization was carried out, but the practice was avoided in marginal cases to reduce the risk of breaking nuclei. The homogenate was stored at 4 °C to await counting; the storage period did not affect mean nuclear counts. Care was taken that the size-groups did not differ much in their mean duration of storage, or in the range of dates over which their cells were counted. Five samples were taken from each aliquot, and each sample was counted on a haemocytometer slide. The nuclei were counted in five areas spaced systematically on each slide. The total volume in which nuclei were counted for each organ was $1 \times 10^{-4}$ ml. Multiplying the total count by $25 \times 10^4$ (or by $50 \times 10^4$ for liver) gave the estimate of the cell-number in the organ. An estimate of cell size was obtained by dividing the organ weight by the number of cells in that organ. All extracellular components will of course affect the estimate, though there is no particular reason for this error to affect the size-groups differentially. The measure of cell size is thus the weight of organ associated with each nucleus, for which we use the term 'cell mass', expressed in nanograms ($g \times 10^{-9}$).

### (iii) *Error variance*

The design of the experiment provided estimates of the error variance of the counts of nuclei, which could be partitioned into components between squares within slides, and between slides. It did not, however, allow the whole of the error variance between individual mice to be estimated, because each organ was homogenized as a whole. After the data had been collected it appeared that there were some differences between mice that were far too great to be real. These differences must have arisen, in part, from 'error' in the preparation of the suspension of nuclei for counting, but we have no means of estimating this error variance, and so we cannot assess the significance of differences between individual mice. The between-mice component is used as the error for assessing differences between lines.

For each organ we have three parameters whose interrelations are to be studied: organ weight, cell number and cell mass. It is important to note, however, that we have only two independent variables: organ weight and cell number, cell mass being derived directly from these two. There is no reason to suppose that the error deviations in organ weight and cell number will be correlated, but the error deviations in cell number and cell mass are correlated negatively. For this reason we can get no information about any real correlation that there may be between cell number and cell mass.

Table 1

(*a*) Six-week body weight (g) of the mice used in the main study, 16 mice per line, sexes averaged

| | A | B | C | D | E | F | Mean |
|---|---|---|---|---|---|---|---|
| L | 32·86 | 32·76 | 32·06 | 31·57 | 30·54 | 34·26 | 32·34 |
| C | 25·11 | 28·42 | 22·39 | 24·80 | 23·36 | 22·78 | 24·48 |
| S | 16·12 | 17·56 | 17·22 | 15·99 | 17·00 | 15·14 | 16·51 |

(*b*) Mean weights (g) of the organs of the mice used, pooled over replicates

| | Lung | Liver | Spleen | Kidney |
|---|---|---|---|---|
| L | 0·221 | 2·438 | 0·136 | 0·549 |
| C | 0·167 | 1·683 | 0·103 | 0·405 |
| S | 0·122 | 1·038 | 0·058 | 0·252 |

## 3. RESULTS

The results of the main experiment will be presented first; those of the longitudinal study are presented in section (v) below.

### (i) *Body weight and organ weight*

The mean body weights of all the lines at 6 weeks are given in Table 1(*a*). Except for a rather high value in the CB line, the samples are representative of the lines from which they were drawn, as described by Falconer (1973). Table 1(*b*) gives the mean organ weights in the three size-groups.

In order to see how the organ weights were related to body weight, the mean log organ-weight of each line was plotted against the mean log body-weight,

plotting each sex separately but on the same graph. These plots, shown in Fig. 1, are all clearly linear, justifying the calculation of linear regressions. In no organ was the slope of the regression line significantly different between the sexes. Common regressions pooled within sexes were therefore calculated and these are

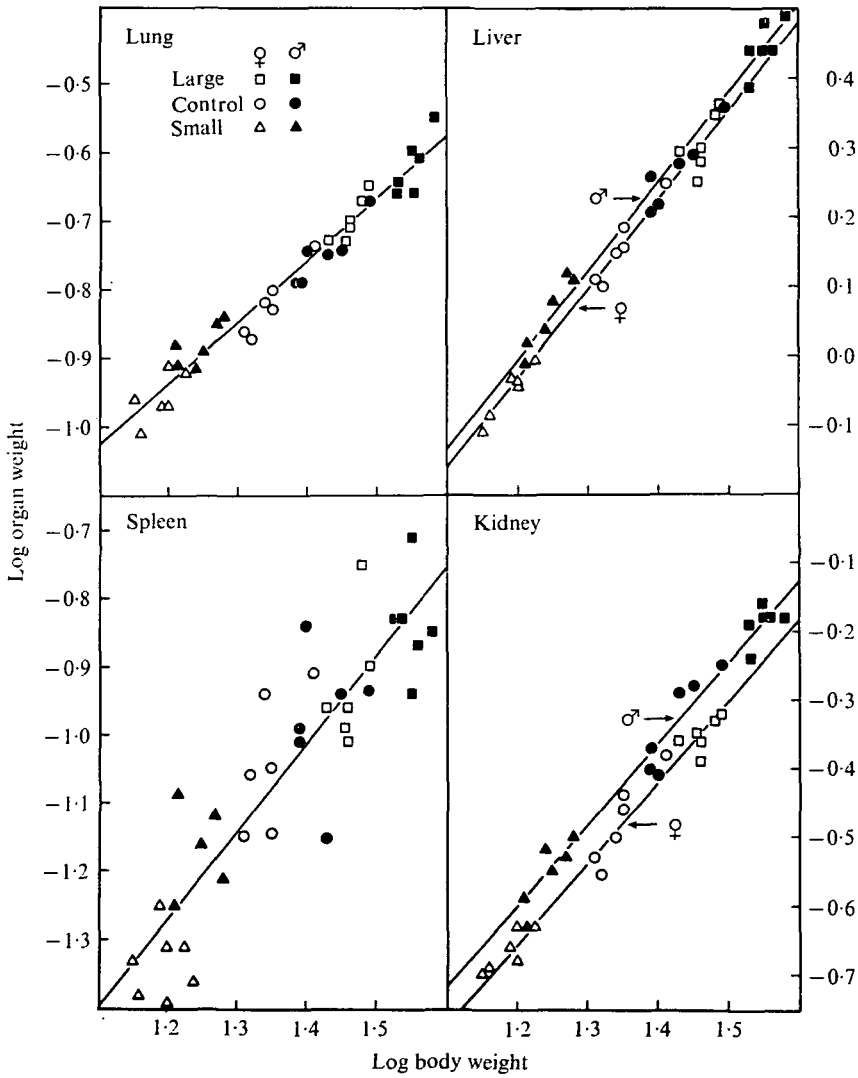


Fig. 1. Relation of organ weight to body weight, with regression of $\log_{10}$ organ-weight on $\log_{10}$ body-weight, in mice aged 6 weeks. The scales for liver and kidney are at the right.

shown in the figure. The elevations of the regression lines of the two sexes did not differ significantly in lung and spleen, and single lines are drawn for these organs. In the liver and kidney, however, the sexes differed significantly in elevation ($P < 0{\cdot}001$ in both cases), males having relatively larger organs than females. Males had livers 6·1 % heavier than females and kidneys 13·3 % heavier.

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For each organ the regression equation $\log y = \log a + b(\log x)$ was calculated, $y$ being organ weight and $x$ body weight. This gave the allometric relation $y = ax^b$. The values of $a$ and $b$ are given in Table 2. All the estimates of the common $b$ are significantly different from 1, being less than 1 for lungs and greater than 1 for the other organs. In other words, large mice have relatively smaller lungs, and relatively larger livers, spleens and kidneys.

Table 2. *Relation between organ weight* ($y$) *in grams and body weight* ($x$), *in grams, from* $y = ax^b$, *in mice aged 6 weeks*

($a$ and $b$ were estimated from regressions of $\log y$ on $\log x$. The values of $b$ are the common regression coefficients within sexes. The values of $a$ are derived from the common $b$ and the separate means of each sex.)

| | $a_♀$ | $a_♂$ | $b \pm$ S.E. |
|---|---|---|---|
| Lung | 0·0098 | 0·0100 | 0·888 ± 0·033 |
| Liver | 0·0273 | 0·0290 | 1·277 ± 0·029 |
| Spleen | 0·0016 | 0·0016 | 1·274 ± 0·120 |
| Kidney | 0·0087 | 0·0098 | 1·173 ± 0·032 |

(ii) *Cell size and cell mass*

The changes in cell size and cell mass that have resulted from selection for body weight are first illustrated diagrammatically in Fig. 2. The three line-means (L, C, S) in each replication (A–F) are connected as if they were one-step correlated responses in six separate selection experiments, with the Control line as the starting point. The changes of cell number are very consistent, the order of the lines being L > C > S in all four organs and all replicates, except for the liver in two replicates. The changes in cell mass are also consistent in showing that downward selection has decreased cell mass, i.e. C > S, to which there are two exceptions; upward selection was less consistent, with five exceptions to the order L > C. The presentation in Fig. 2 thus leaves no doubt that both cell number and cell mass have been changed in all the organs. As noted earlier, however, the significance of the changes should be assessed by treating replicates as random lines within size-groups. This was done as follows.

The means of the size-groups were calculated from the six line-means in each, and the differences tested by $t$-tests. The results are given in Table 3. These again leave no doubt that the selection for body weight has changed both cell number and cell mass in all of the four organs. The proportionate changes are given in Table 4, with the changes in organ weight for comparison. The proportionate changes are rather more in cell number than in cell mass, particularly in lung and spleen. We shall return in the next section to the question of how much of the changes of organ weight are attributable to cell number and how much to cell mass.

Hierarchical analyses of variance were also carried out to see whether there were significant differences between lines within size-groups. The analyses of variance are not given in full, but only a summary of the components, in Table 5. The components of cell number and cell mass between replicate lines within size-groups

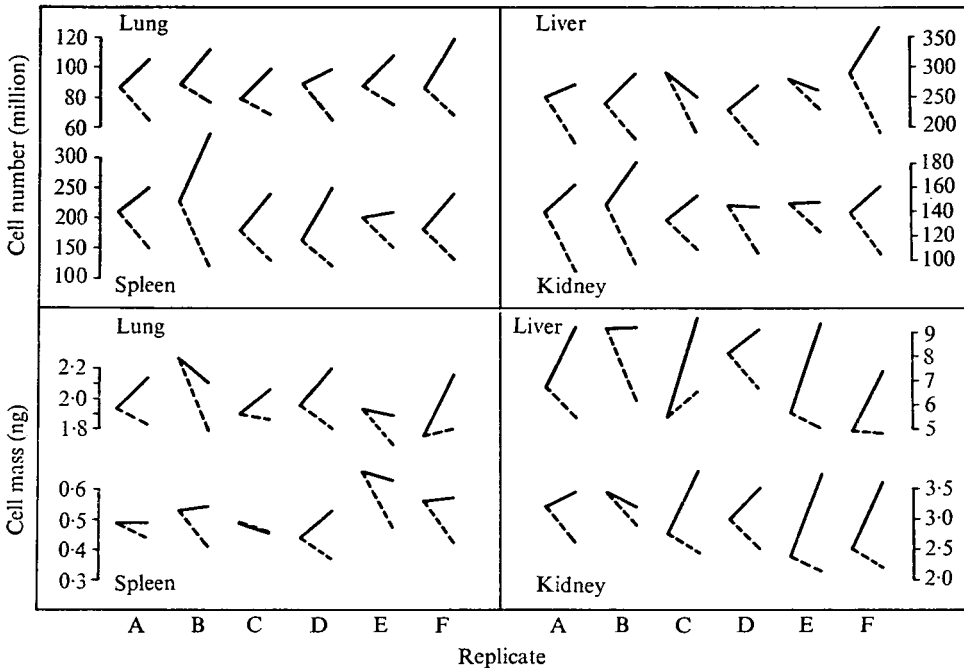


Fig. 2. Changes in cell number and cell mass in mice aged 6 weeks brought about by selection for body weight. The six replicates are depicted as separate 'one-step' selection responses, the control levels being taken as the starting points. Solid lines represent the responses to selection for large body size, broken lines selection for small body size. The sexes are averaged. The scales for liver and kidney are at the right.

Table 3. *Mean cell number and cell mass, with standard errors, in the three size-groups, Large (L), Control (C) and Small (S)*

(Each mean is based on six line-means, sexes averaged. The stars give the significance of the differences between size-groups.)

| | Lung | Liver | Spleen | Kidney |
|---|---|---|---|---|
| | | Number (millions) | | |
| L | 107·1 ± 3.2 | 286·6 ± 18·1 | 257·5 ± 19·1 | 158·6 ± 5·5 |
| L–C | ** | | * | * |
| C | 86·1 ± 1·5 | 264·8 ± 10·5 | 193·9 ± 10·6 | 142·0 ± 2·1 |
| C–S | *** | *** | *** | *** |
| S | 69·1 ± 2·3 | 191·5 ± 8·3 | 132·9 ± 5·7 | 104·2 ± 4·6 |
| L–S | *** | *** | *** | *** |
| | | Mass (ng) | | |
| L | 2·094 ± 0·044 | 9·018 ± 0·336 | 0·537 ± 0·024 | 3·548 ± 0·086 |
| L–C | | * | | ** |
| C | 1·956 ± 0·069 | 6·681 ± 0·678 | 0·530 ± 0·031 | 2·895 ± 0·162 |
| C–S | * | | * | |
| S | 1·792 ± 0·026 | 5·739 ± 0·286 | 0·432 ± 0·015 | 2·464 ± 0·112 |
| L–S | *** | *** | ** | *** |

* $P < 0·05$, ** $P < 0·01$ *** $P < 0·001$

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were all significant at the 5% or higher level in both sexes in all organs, with the exception only of cell number in female lungs.

The component between size-groups was greater than the component between replicates within size-groups in all cases, but the difference was much greater for cell number than for cell mass. These comparisons are given at the foot of Table 5.

Table 4. *Proportionate changes in organ weight, cell number and cell mass, based on the size-group means in Table 3*

(Each entry is the percentage difference from Control.)

| | | Lung | Liver | Spleen | Kidney | Mean |
|---|---|---|---|---|---|---|
| Organ weight | L–C | 33 | 46 | 34 | 38 | 38 |
| | S–C | −26 | −38 | −44 | −38 | −36 |
| Cell number | L–C | 24 | 8 | 33 | 12 | 19 |
| | S–C | −20 | −28 | −32 | −27 | −27 |
| Cell mass | L–C | 7 | 35 | 1 | 23 | 16 |
| | S–C | −8 | −14 | −18 | −15 | −14 |

Table 5. *Components of variance of cell number and cell mass in mice aged 6 weeks, sexes averaged*

(The total variance given is the sum of the components in actual units. The components are given in percentages of the total. The components between replicates are within size-groups, and those of individuals are within replicates. For explanation of the ratio of components, see text.)

| | Lung | Liver | Spleen | Kidney | Mean |
|---|---|---|---|---|---|
| Cell number | | | | | |
| Total (millions)² | 534 | 6426 | 6420 | 1394 | — |
| Size-groups (%) | 67 | 37 | 59 | 55 | 54 |
| Replicates (%) | 5 | 10 | 14 | 5 | 9 |
| Individuals (%) | 28 | 53 | 27 | 40 | 37 |
| Cell mass | | | | | |
| Total (ng)² | 0·1093 | 7·735 | 0·0157 | 0·7364 | — |
| Size-groups (%) | 20 | 34 | 18 | 38 | 27 |
| Replicates (%) | 10 | 11 | 16 | 10 | 12 |
| Individuals (%) | 70 | 55 | 66 | 52 | 61 |
| Ratio of components | | | | | |
| Size-groups/replicates | | | | | |
| Cell number | 13·4 | 3·7 | 4·2 | 11·0 | 6·0 |
| Cell mass | 2·0 | 3·1 | 1·1 | 3·8 | 2·25 |

In the lung, for example, the ratio of the component between size-groups to the component between replicates is 13·4 for cell number but only 2·0 for cell mass. The differences are in the same direction in the other organs, though quite small in the liver. The differences between size-groups were the result of selection while the differences between replicates were mainly the result of random drift. It seems, therefore, that the genetic changes brought about by selection have affected cell number relatively more than have the genetic changes resulting from random drift.

(iii) *Relative importance of cell number and cell mass*

The proportionate changes described in the previous section suggest that the differences of organ weights have been brought about on the whole more by changes in cell number than by changes in cell mass. The relative contribution that each has made to the differences of organ weight can be quantified from the regression of log cell-number on log organ-weight, as the following considerations will show.

If the logarithms of cell number, cell mass, and organ weight are denoted by $n$, $m$, and $w$ respectively, then

$$n+m = w,$$

$$\text{cov}_{nw} = \text{cov}_{(w-m)w} = \text{var}_w - \text{cov}_{mw}$$

Dividing both sides by $\text{var}_w$ gives

$$b_{nw} = 1-b_{mw},$$

$$b_{nw}+b_{mw} = 1,$$

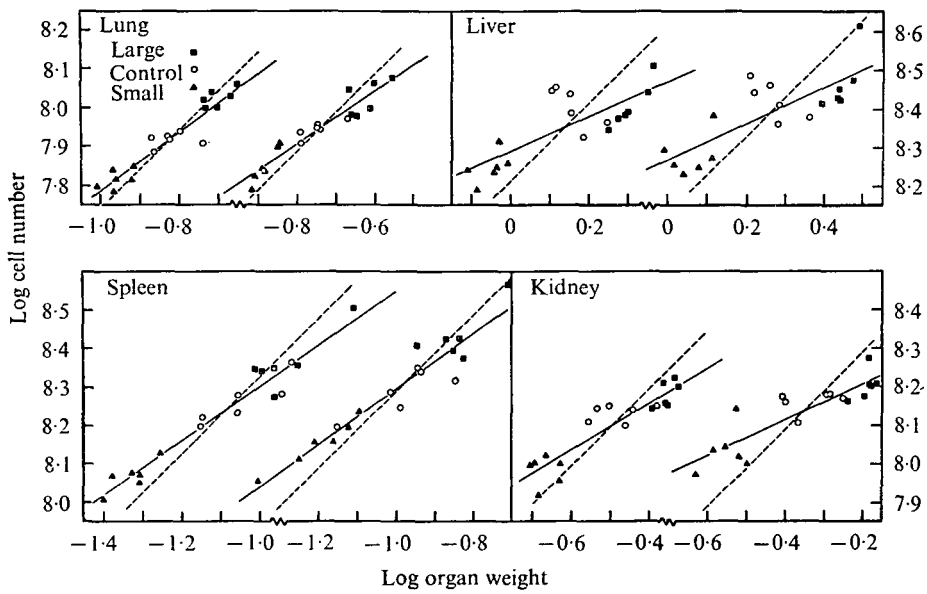


Fig. 3. Regressions of $\log_{10}$ cell-number on $\log_{10}$ organ-weight in mice aged 6 weeks. The continuous straight lines are the calculated regressions. The broken lines have a slope of 1, as expected if the differences of cell number explained all the differences of organ weight. Females are on the left, males on the right, in each organ.

where $b_{nw}$ and $b_{mw}$ are the regression coefficients of log cell-number and of log cell-mass respectively on log organ-weight. As noted earlier, there was error of unknown amount in estimating cell number. The error in estimating organ weight, however, was negligible, so the estimation of $b_{nw}$ is valid.

The regression $n$ on $w$ therefore provides a measure of the relative contribution of cell number to the differences of organ weight, ranging from $b_{nw} = 0$, when the

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whole difference is due to cell mass, to $b_{nw} = 1$, when the whole difference is due to cell number.

Plots of log cell-number on log organ-weight are shown in Fig. 3. Linear regressions were calculated from the line-means separately for each sex, and are shown by solid lines in the figure. The broken lines have slopes of 1, showing where the regressions would lie if cell number were wholly responsible for the differences of organ weight. The corresponding numerical values are given in Table 6A. The intercept, log $a$, is the predicted log cell-number of an organ weighing 1 g, and it provides a measure of the elevation of the regression line. The regression coefficients, $b$, are all less than 1. All four regressions in each sex are significantly different from both 1 and zero with $P < 0{\cdot}001$ in every case. The regressions in the two sexes are not significantly different in any organ and are combined in the common regression given in Table 6A. These show that changes in cell number

Table 6. *Relations of cell number ($N$) to organ weight ($W$) in grams by the regression $\log N = \log a + b \log W$, calculated from line-means, with standard errors*

(A: All mice aged 6 weeks. B: Mice aged 3–15 weeks (3–6 weeks for liver). The values of $a$ for the two sexes at 6 weeks are calculated from the common regression.)

| | Lung | Liver | Spleen | Kidney |
|---|---|---|---|---|
| | (A) Age 6 weeks | | | |
| Females | | | | |
| Log $a$ | 8·544 ± 0·049 | 8·291 ± 0·019 | 9·004 ± 0·046 | 8·400 ± 0·035 |
| Log $b$ | 0·757 ± 0·059 | 0·453 ± 0·090 | 0·704 ± 0·041 | 0·603 ± 0·068 |
| Males | | | | |
| Log $a$ | 8·452 ± 0·041 | 8·270 ± 0·028 | 9·018 ± 0·057 | 8·298 ± 0·025 |
| Log $b$ | 0·682 ± 0·054 | 0·470 ± 0·091 | 0·723 ± 0·056 | 0·460 ± 0·064 |
| Common $b$ | 0·719 ± 0·040 | 0·462 ± 0·063 | 0·712 ± 0·034 | 0·521 ± 0·048 |
| ♀ log $a$ | 8·512 | 8·289 | 9·014 | 8·359 |
| ♂ log $a$ | 8·480 | 8·272 | 9·007 | 8·320 |
| $N$ (♀)/$N$ (♂) = $M$ (♂)/$M$ (♀) | 1·076 | 1·040 | 1·016 | 1·094 |
| | (B) Age 3–15 weeks* | | | |
| Males | | | | |
| Log $a$ | 8·490 ± 0·052 | 8·204 ± 0·025 | 9·157 ± 0·058 | 8·405 ± 0·019 |
| Log $b$ | 0·603 ± 0·059 | 0·269 ± 0·090 | 0·868 ± 0·053 | 0·552 ± 0·037 |

* 3–6 weeks for the liver.

account for about 70% of the differences of organ weight in lung and spleen, and for about 50% in liver and kidney. (Lung and spleen are not significantly different from each other, and nor are liver and kidney; but both lung and spleen are significantly different from both liver and kidney, with $P < 0{\cdot}01$, or $P < 0{\cdot}001$.) Complementarily, the relative contribution of cell mass to the differences of organ weight between lines, measured as $1 - b_{nw}$, was about 30% for lung and spleen and about 50% for liver and kidney. These estimates confirm and quantify the impression given by the simple treatment in the previous section.

### (iv) *Comparison of sexes*

Males have larger organs than females. How much of this difference is due to cell number and how much to cell mass? As noted earlier, the regressions of log cell-number on log organ-weight in males and in females did not differ in slope. The common regression was calculated and the two regression lines were tested for differences in elevation to make the comparison of log cell-number at the same organ weight. The elevations were not significantly different in liver or in spleen, but they were in lung ($P < 0{\cdot}01$) and in kidney ($P < 0{\cdot}05$). In these organs males had fewer and larger cells than females. As a measure of elevation the intercepts were calculated from the common regression. The antilog of the difference between the intercepts gives the cell number in one sex relative to that in the other when adjusted to the same organ weight. The cell number in females relative to males is the same as the cell mass in males relative to females. These relative values are given in Table 6A. Expressed in terms of cell mass, males had larger cells than females in all organs; in the lung they were 7·6 % larger and in the kidney 9·4 % larger. The differences of 4·0 % in the liver and 1·6 % in the spleen were not significant, as noted earlier.

To estimate the relative contribution of cell number and cell mass to the sex-difference in organ weight, we need the regression based on the sex-means, i.e. the between-sex regression. This was 0·30 in lungs and 0·25 in kidneys, so the difference between the sexes in the weights of these organs was 70 and 75 % due to cell mass, in contrast to 22 and 48 % for the differences between the lines.

### (v) *Changes during growth*

The main experiment has shown that the Large, Control and Small mice differed in cell mass in the four organs, when compared at the fixed age of 6 weeks. Data for the longitudinal study, to be described now, were collected with the object of finding out if the cellular changes during growth resembled those brought about by selection. Cell mass is known to increase during growth in several organs and tissues of rats (Enesco & Lablond, 1962; Winick & Noble, 1965). If the same is true of the organs studied in our mice, selection could have produced the observed differences of cell mass by speeding up or slowing down this normal increase of cell mass during growth.

Fig. 4 shows the changes of cell number and cell mass during growth from 3 to 15 weeks. The two replicates in each size-group have been averaged since the mean of each line at each age was based on only four animals. Many irregularities remain in the graphs, but three features seem clear, if some exceptions are disregarded. (1) The size-groups differ in cell number in the expected direction at all ages. (2) Cell number increases from 3 to 6 or 7 weeks and then remains constant, or declines. It is hard to understand the decline of cell number in the lung; in the spleen it was accompanied by a reduction in organ weight; in the liver, where it is most marked, it could be due to the formation of polyploid cells. (3) Cell mass increases fairly regularly in all organs throughout the period from 3 to 15 weeks.

Except for the reduction of cell numbers, these changes during growth resemble in general outline those found by Enesco & Lablond (1962) and by Winick & Noble (1963) in rats, organ growth being mainly by cell number initially and by cell mass later. The increase of cell mass during the growth of the organs shows that the differences found at 6 weeks could be simply the developmental consequences of

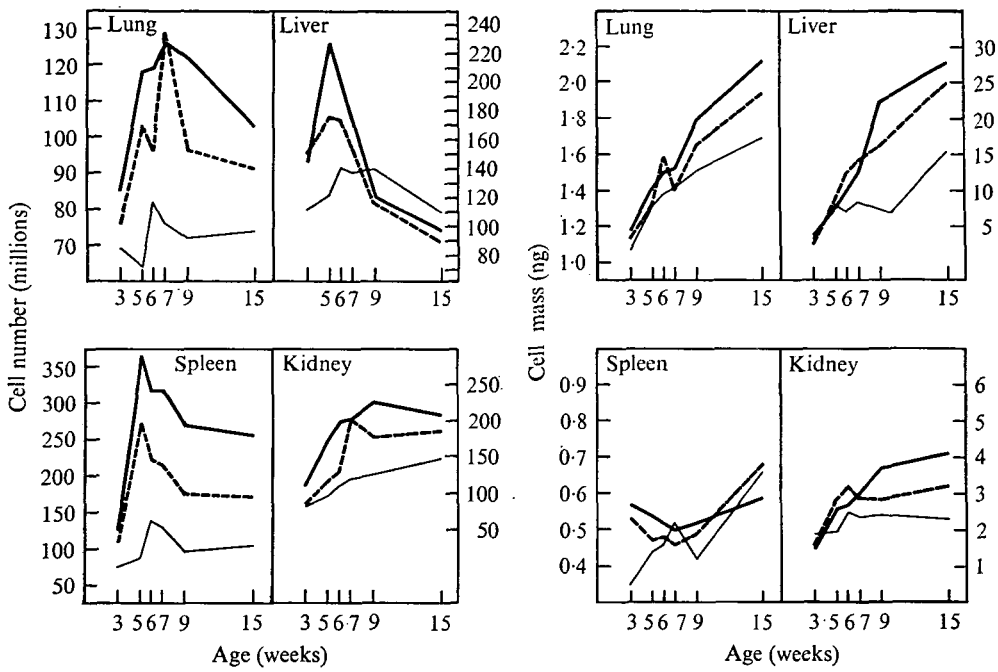


Fig. 4. Changes of cell number and cell mass in males during growth from 3 to 15 weeks. Means of two replicates each of Large (thick lines), Control (broken lines) and Small (thin lines).

the changes of organ weight brought about by selection. To test this possibility we analysed the data by regression in the manner described for the main experiment.

Fig. 5 shows the plots of log cell-number against log organ-weight, the points being line-means at each age. The essential difference between these graphs and those in Fig. 3 is that in Fig. 3 the differences of organ weight are due to the selection-history of the lines, whereas here (Fig. 5) they are due also to age-differences. The regression lines fitted to the points are shown on the graphs and the regression coefficients are given in Table 6B. The graph of the liver is confusing because of the marked reduction of cell numbers after about 6 weeks despite continued increase of organ weight. Because of the obvious non-linearity at the higher ages, the calculation of the regression in the liver was based on the points for 3, 5 and 6 weeks only.

With the possible exception of the liver, two main features of the results are clear. First, the points for the three size-groups and all ages fall reasonably well on

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the same lines, showing that, in the main, organs of the same weight have the same cell mass, irrespective of age or of size-group. In the main, therefore, selection has not changed cell mass except as a concomitant to the change of organ weight during growth. Secondly, the slopes of the regressions do not differ much from those obtained from mice all aged 6 weeks given in Table 6A. The difference between the two regressions in males is not significant in any organ. The similarity of the two regressions shows that cell number and cell mass make roughly the same relative contribution to the increase of organ weight during growth as they do to the differences produced by selection.

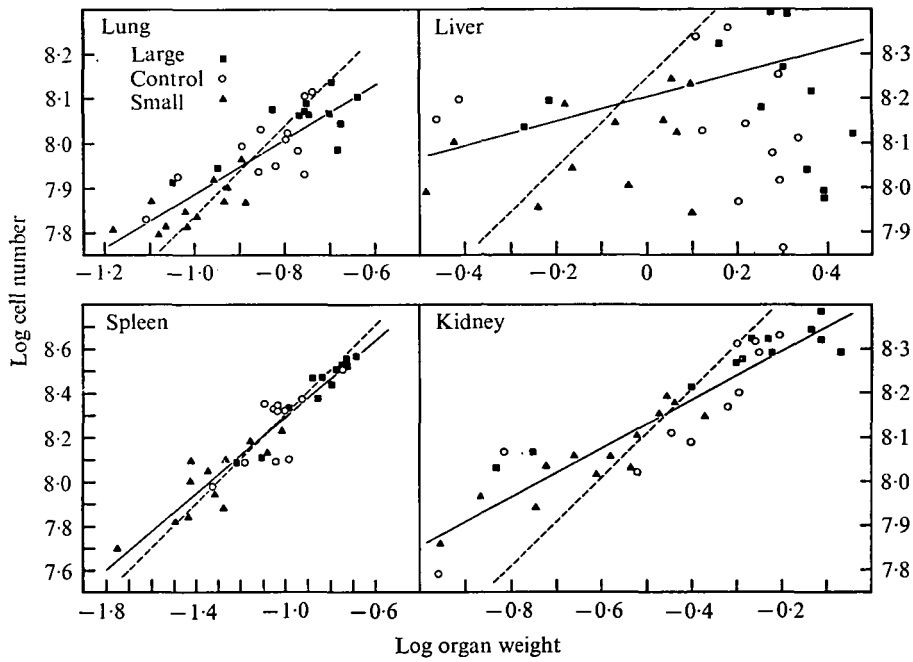


Fig. 5. Regressions of $\log_{10}$ cell-number on $\log_{10}$ organ-weight in male mice aged 3–15 weeks. The continuous straight lines are the calculated regressions (in the case of the liver, based on 3, 5 and 6 weeks only). The broken lines have a slope of 1, as expected if organs grew only by increase of cell number. Both scales in the graphs of spleen are half those of the other organs.

The conclusion to be drawn from the study of mice at different ages is that cell size increases during growth, and the difference in cell size between the selected lines is what would be expected from the different amounts of growth that they have made.

## 4. DISCUSSION

In the context of selection responses, the question asked was: did the response of body weight take place by changes of cell number or of cell size, and the answer was by both, in the four organs studied. But these two changes were themselves the consequence of a single effect of selection, the change in the rate of growth.

During the growth of any mouse the cells increase both in number and in size, the increase in cell size differing in amount between the organs. The effect of upward selection has been to make the mice grow faster so that at 6 weeks of age their cells are both more numerous and larger than those of the unselected controls. Downward selection had the opposite effect, resulting in mice at 6 weeks having fewer and smaller cells. When compared at the same body weight, and consequently at different ages, the Large, Control and Small strains had cells of roughly equal number and size in all the organs studied. The effect of selection might be summed up as a change in the relation of developmental age to chronological age.

The effects of selection for body size on the numbers and sizes of the cells of the lung, liver, spleen and kidney, described here, are the same as the effects on the numbers and diameters of muscle fibres reported by Byrne, Hooper & McCarthy (1973). The strains selected for increased and decreased growth rate, with which these authors worked, were derived from the same base population as the Q-stocks with which we worked. They measured the fibre number and diameter in seven muscles and found the large mice had consistently more and larger fibres than the controls, while the small mice had consistently fewer and smaller fibres. The muscle fibres of mice stop increasing in numbers soon after birth, and the subsequent increase of muscle size takes place by increase of the diameter of the fibres. Thus the developmental process in muscle fibres and in cells is similar in that both increase first in numbers and later in size. When the mice studied by Byrne, Hooper and McCarthy were compared at the same body weight the results were somewhat different from ours. At the same body weight, when the large mice were younger than the small, the large mice had more fibres than the small but with smaller diameters (Hooper & McCarthy, 1976). These results can be interpreted in the same way as ours: the large mice have gone through their developmental process faster than the small, but in this case fibre diameter increases with age independently of body weight. Consequently the younger large-strain mice have smaller fibres than the older small-strain mice.

A similar picture of the effect of selection on fatness was described by Clarke (1969). He studied the fat content of the same Q-strains after 14 generations of selection. The large mice had relatively more fat than the small at a fixed age, but when compared at the same weight there was little difference.

These three studies on the Q-strain mice show that selection for body weight has produced correlated responses in the numbers and sizes of cells in four organs, in the numbers and diameters of muscle fibres, and in the relative amount of fat. All these correlated responses have resulted from a single effect of selection in altering the timing of the normal developmental processes of growth.

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