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Protein and nucleic acid metabolism in organs from mice selected for larger and smaller body size

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SUMMARY

Studies of the growth and composition of Q-strain mice selected over 20 generations for high and low body weight at 6 weeks of age, and their unselected controls, were made on livers and kidneys of males from the five selection replicates A, B, C, D and F. Differences in growth rate between Large and Small QD mice were confirmed from 2 to 9 weeks of age, but were greatest in the third, fourth, sixth and seventh weeks. Total amounts of dry matter, protein, free amino acids, bulk RNA and ribosomes were increased or decreased from control values in proportion to organ weight. A less-perfect relationship between DNA content and organ weight suggested that some small changes in average cell mass had accompanied the main change in cell number in organs from the selected lines. Absorbance profiles of polyribosomes from both organs were identical in selected and control mice: selection had not operated on the proportion of single (currently inactive) ribosomes. Attempts to relate the observed differences in growth rate in QD mice to differences in the rate of protein synthesis produced an unexpected result: incorporation of radioactively labelled amino acids was consistently higher in the organs of the Small mice. Measurements of rates of protein turnover, and calculated rates of protein degradation, suggested that protein might also be degraded more rapidly in the small mice.

1. INTRODUCTION

In the search to identify the factors responsible for the quantitative control of mammalian growth, a considerable body of data has now accumulated describing many aspects of protein and nucleic acid metabolism during the normal development of cells and organs, in situations of hormonal or dietary imbalance, and in the changes following a stimulus to regenerative or chemically induced growth. Although the considerable potential for genetic manipulation of growth rate has long been evident in domestic and laboratory animals, the cellular and intracellular mechanisms concerned have seldom been studied. In this paper we describe some consequences of increasing or decreasing growth rate in mice by long-term selection, in terms of the protein and nucleic acid components of two organs, liver and kidney. We were particularly interested in the possibility that selection had altered the abundance of ribosomes or their engagement in protein synthesis.

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In addition we have attempted to compare gross rates of protein synthesis and protein degradation in the selected and control mice.

2. MATERIALS AND METHODS

(i) *Mice*

The origin of the Q selected stocks is described in detail by Falconer (1974). Briefly, two-way selection for high and low body weight at 6 weeks of age in six replicate samples (A–F) from the random-bred Q-strain produced in each replicate a selected large (L), selected small (S) and an unselected control (C) line. Each line was maintained by eight single pair matings with minimal inbreeding. During these experiments, in the twenty-first and subsequent generations, selection was relaxed and there was no further divergence from the controls.

The main tissue components of livers and kidneys were examined briefly in all replicates except E, with more detailed studies on B and D. Protein synthesis and protein turnover were estimated in D only. Only male mice were used throughout this study. They were housed at 21 ± 2 °C, in groups of three to six, and fed a standard cube diet with free access to water. Mice were killed between 9.0 and 11.0 a.m., except those used for preparation of liver ribosomes, which were killed at 4.0 p.m. In each type of experiment, L, C and S mice of the same age were examined simultaneously to minimize the effect of any day-to-day variation in technique or environment.

(ii) *Measurement of growth*

Body-weight and growth-rate data were collected in a single group of QD male mice comprising 69 QLD, 47 QCD and 33 QSD, in generation 35. The mice were weighed individually twice weekly and averaged growth curves for each line were built up from individual data. Growth increments in successive 3·5-day periods on the averaged curves were used to compute relative growth rates, defined as gain per 3·5 days/weight at the beginning of 3·5 days. Organ weight curves were compiled from data obtained in routine experiments with additional material from ages not covered.

(iii) *Biochemical methods*

Techniques used to estimate tissue constituents were essentially those described in Priestley & Malt (1968). Water content was determined when weighed organs were dried to constant weight under vacuum. Total protein, RNA and DNA were assayed using a single homogenate from each organ. Tissue was thoroughly homogenized in either a Dounce or a Tenbroeck glass homogenizer containing homogenization buffer (0·25 M sucrose, 0·1 M Tris, 0·025 M-KCl, 0·0015 M-$MgCl_2$, pH 7·6) at 4 °C. Duplicate aliquots were withdrawn for protein and RNA determination; components soluble in 0·2 N perchloric acid (PCA) at 4 °C were rejected and the precipitate hydrolysed in 0·3 N-KOH at 37 °C for 60 min. Protein was measured in the hydrolysate with Biuret reagent after extraction of lipids with

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ether and centrifugation for 10 min at 12000 *g*. RNA in the hydrolysate was estimated from optical density (O.D.) at 260 nm (32 μg/ml RNA equivalent to 1·00 O.D. units: Munro & Fleck, 1966) after precipitation of DNA and protein with cold PCA and centrifugation of the supernatant at 12 000 *g*.

In DNA estimation, duplicate 0·5 ml samples of the original homogenates were mixed with 5 ml cold 0·5 N-PCA and centrifuged at 6000 *g* for 10 min. The precipitates were extracted for 15 min with 0·5 N-PCA at 70 °C. DNA in the extract was measured by the diphenylamine method of Burton (1956), using a calf thymus standard (Sigma Chemical Co., London, DNA Grade I) corrected for water and sodium content. The efficiency of the hot PCA extraction was estimated using four mice each injected 24 and 48 hr previously with a total of 0·25 mCi [^{3}H]-6-thymidine (Radiochemical Centre, Amersham). The extraction was found to remove an average of 70 % of the label in precipitates from livers and 74 % of that in precipitates from kidneys. DNA values were corrected to 100 % efficiency of extraction on the basis of these figures.

In later experiments (including those dealing with protein synthesis and turnover), where protein alone was measured and loss of RNA was unimportant, a more rigorous lipid extraction was performed. The initial precipitation was in 10 % trichloracetic acid (TCA) instead of PCA. Precipitates were extracted with ethanol containing 2 % sodium acetate, then with ethanol–ether (3:1), and finally with ether *before* hydrolysis in KOH, as suggested by Lowry *et al.* (1951). A standard curve prepared with duplicate dilutions of bovine serum albumin (fraction V) was used in all protein determinations.

Free amino acid concentrations were measured in postmitochondrial supernatants (20000 *g* for 5 min, Sorvall RC 2 centrifuge) by the ninhydrin method of Moore & Stein (1948), as described in Priestley & Malt (1968), using a sample:reagent ratio of 1:10 by volume, and 50 % aqueous ethanol as diluent. The standard curve was prepared with leucine and results were expressed relative to that amino acid.

(iv) *Preparation of polyribosomes*

Weighed pairs of kidneys or individual livers were disrupted in homogenization buffer at 4 °C in Dounce homogenizers with five strokes of the A pestle and three strokes of the B pestle. If the final yield was to be recorded, the volume of homogenate was adjusted before a postmitochondrial supernatant was prepared by centrifugation at 20000 *g* for 5 min. With kidneys a 0·8 ml aliquot of supernatant was layered directly over a 16·6 ml gradient of 15–35 % sucrose (w/w) in RSB (reticulocyte standard buffer: 0·01 M Tris, 0·01 M-KCl, 0·0015 M-$MgCl_2$, pH 7·6) for centrifugation at 27000 rev/min for 170 min (Beckman SW 27.1 rotor, 4°). Mice to be used for preparation of liver ribosomes were fasted for 6 h to reduce glycogen, and either 0·5 % yeast RNA (British Drug Houses) (Haschemeyer & Gross, 1967) or 10 % high-speed supernatant (55000 *g* for 5 h) from mouse liver (Blobel & Potter, 1967; Priestley & Malt, 1969) was included in the homogenization buffer to combat RNase activity. Postmitochondrial supernatants from livers were

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made 1 % with sodium deoxycholate to release ribosomes from membranes, then diluted to a standard volume with homogenization buffer. From the diluted supernatant 12 ml was layered over a discontinuous gradient of 8 ml 0·5 M sucrose and 5 ml 2·0 M sucrose in RSB, and the gradients were centrifuged at 25 000 rev/min for 16 h (Beckman Type 30 rotor, 4°). The clear pellet of mixed free and membrane-bound ribosomes was dispersed by stirring briefly in homogenization buffer and layered over a 15–35 % sucrose gradient for final centrifugation as described for kidney. After centrifugation O.D. at 260 nm in effluent from the bottom of the gradients was monitored continuously in a Beckman DB-GT spectrophotometer with a 10 in. recorder. Yields of single ribosomes and polyribosomes were estimated by measuring appropriate areas under the O.D. profiles using a compensating planimeter. A correction for loss of the fastest-sedimenting polyribosomes was applied by measuring the area under the extrapolated leading edge of the O.D. trace beyond the point corresponding to the bottom of the gradient, and adding this area to that of the polyribosomes (Blobel & Potter, 1967). Further corrections to yield were made for any small variations in flow rate.

(v) *Protein synthesis*

Rates of protein synthesis in QLD, QCD and QSD male mice were compared by three different methods. First, incorporation of radioactive amino acids by free ribosomes was measured in whole kidneys *in vitro* (Priestley & Malt, 1968). Weighed pairs of kidneys were decapsulated and gently agitated at 37 °C, for 15 min in Hanks solution (pH 7·4) containing 10 μCi [^{14}C]protein hydrolysate (Radiochemical Centre, England; 45 mCi per milliatom carbon). Incorporation was halted by adding a large excess of ice-cold Hanks solution, and after further rinsing the kidneys were homogenized for preparation and display of ribosomes on sucrose gradients, as described earlier. Aliquots of each postmitochondrial supernatant were retained for estimation later of free amino acid concentration and radioactivity. Equal fractions of approximately 1 ml were collected from each gradient, and to each was added 1 ml N-NaOH plus two drops of 2 % bovine serum albumen as carrier protein, followed by 2 ml cold 20 % TCA. After standing at 4 °C for at least 60 min precipitates were collected on Oxoid membrane filters, dried and counted in a Nuclear-Chicago gas-flow counter. Results were expressed in two ways:

(A) As the ratio cpm (supernatant)/cpm (ribosomes) representing the ratio of counts in completed peptides released from the ribosomes to the counts in nascent polypeptides still attached to ribosomes.

(B) As the ratio total cpm/cm^2 polyribosome O.D. on the recorder chart. Adjustment of these (B) results for differential dosage in the three sizes of kidneys, and for minor differences in dilution of the label in the free amino acid pools of individual organs, was calculated from the specific activities of the free amino acid pool in each preparation. The final results therefore represent incorporation rate at a standard specific activity of the precursor pool. Concentrations of free amino acids were measured in acid-soluble fractions prepared in duplicate from

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each postmitochondrial supernatant; aliquots of the same solutions were applied to a filter membrane for counting in the gas-flow counter.

Secondly, incorporation of labelled amino acids into total kidney and liver protein was measured 1 h after intraperitoneal injection of 10 μCi [^{14}C]protein hydrolysate in 0·1 ml of buffered saline. Total organ protein was estimated in duplicate as described earlier. TCA-precipitates of duplicate aliquots from the solutions of protein in KOH were collected on membrane filters for counting in the gas-flow counter. The specific activities of the free amino acid pools were measured and used to compensate for differential dosage and dilution of the isotope in the precursor pool of each individual organ. Results were expressed as cpm/mg protein.

A third set of comparisons was derived from the experiments on protein turnover described below. Zero time values for specific activity were read from linear regressions of log specific activity on time. In this case it was impossible to measure specific activities of the precursor pools, which decline within minutes of injection, but a crude correction for the effects of differential dosage was devised from the relationship between organ weight and specific activity of the amino acid pool following injection of labelled amino acid recorded in the previous experiment. It was found that in QLD and QSD the specific activity of the amino acid pools differed from QCD by about half the difference in mean organ weight. Where QLD organs were 40 % heavier, for instance, the specific activities of the pools were taken to have been 20 % less, or 80 % of QCD.

(vi) *Protein turnover and degradation*

Protein turnover was estimated as the half-life ($T_{\frac{1}{2}}$) in days of the decline in specific activity of kidney and liver protein (cpm/mg protein) after intraperitoneal injection of [^{14}C]guanido-L-arginine (Radiochemical Centre, England; 25–50 mCi/mmol) (Swick, 1958; Schimke *et al.* 1968). Groups of 16 QLD, 16 QCD and 16 QSD male mice were compared at two different ages. Those aged 28 days each received a dose of 5 μCi and those aged 56 days received 10 μCi per mouse. Four mice from each line were killed 1, 2, 3 and 6 days later, with precautions to ensure that each group of four were not litter-mates. Livers and kidneys were removed immediately, chilled, weighed and homogenized for duplicate determination of total protein and acid-precipitable radioactivity in protein, as described for protein synthesis experiments. Some organs were stored at -25 °C after weighing for processing later. Half-lives were taken from semilogarithmic plots of specific activity against time, based on computation of linear regression coefficients. This half-life expresses the overall rate of protein replacement and masks variation between proteins.

It is influenced both by rate of protein degradation and by accumulation of new (unlabelled) protein. To estimate protein degradation it is necessary to correct for any dilution of the labelled protein due to increase in protein content during the 6-day period of assessment. Our corrections were based on the increases in body weight in QD male mice of the same ages seen in Fig. 1. Changes in organ

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weight were assumed to be directly proportional to change in body weight, and the total concentration of protein in each organ was assumed to remain constant over the 6-day period (Fig. 4 shows that this is substantially accurate). From the relative growth increments (increase in weight over 6 days/weight at beginning of 6 days) we calculated the decline in specific activity solely due to growth, and subtraction of this rate of decline from the observed rate left a decline due to protein degradation. The correction was large in mice aged 28 days and relatively minor in those aged 56 days. The rate of degradation was also expressed as a half-life $DT_{\frac{1}{2}}$.

(vii) *Statistical analyses*

The data in Tables 2, 4 and 5 featuring the five replicates (A, B, C, D, F) and the three size-groups (Large, Control, Small) were analysed as follows. A cross-classified analysis of variance was carried out in which the two main effects, replicates and size-groups, were treated as fixed factors, the mean squares being tested against the residual mean square. Significant differences between replicates are probably not meaningful because the experiments on the different replicates were done at different times. The object of the analyses, in addition to testing the significance of differences between size-groups, was to set 95 % confidence limits on the difference between the Large and Small groups. This was done as follows. The standard error (σd) of the difference was estimated as $(2\sigma^2/n)$, where σ^2 is the error variance and n is the number of observations in each size-group. The error variance σ^2 was the residual mean square where there was no significant interaction between replicates and size-groups, or the interaction mean square in the one case where the interaction was significant. The 95 % confidence limits were estimated as $d+2\sigma_d$, where d is the observed difference (Large − Small). The confidence limits are given in the form of L/S %; that is, the limits of the mean of Large as a percentage of the mean of Small. Thus, for example, limits of 106 % and 97 % indicate (with 95 % confidence) that the mean value of Large mice is not more than 6 % greater, or more than 3 % less, than the mean value of Small mice.

3. RESULTS

(i) *Growth rate*

Figs. 1 and 2 illustrate the effects of selection on the size and growth rate of male QD mice. The data were obtained with generation 35, but curves from generation 17 show similar growth rates. These QLD mice were about 59 % heavier than their unselected controls when 6 weeks old, while QSD were about 18 % lighter. Slight differences in the amount of body fat contribute to this effect (Clark, 1969), but almost the same degree of difference from controls is maintained when fat-free organ weights are compared (Fig. 3). The curves showing rates of relative growth (in this case gain in body weight per half week/body weight at the beginning of each half-week) in Fig. 2, and in particular the ratio of relative growth rate in QLD/relative growth rate in QSD, explain how the twofold difference in mature body weight between QLD and QSD was achieved. Some difference was

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already present at birth, when weights were 1·65 g for QLD, 1·55 g for QCD and 1·37 g for QSD. Similar relative growth rates in the first 2 weeks after birth were followed by a more than twofold difference in the third and fourth weeks. In the period of most rapid growth (in absolute terms, gain/day) from 3 to 6 weeks, the QLD mice averaged only 137 % of the relative growth rate shown by QSD, but in the seventh week there was again a twofold difference. Thus in QLD the phase of most rapid growth appears to begin slightly earlier and to persist slightly later. Organ weights were virtually static after 60 days and subsequent increases in body weight may have been limited to addition of fat.

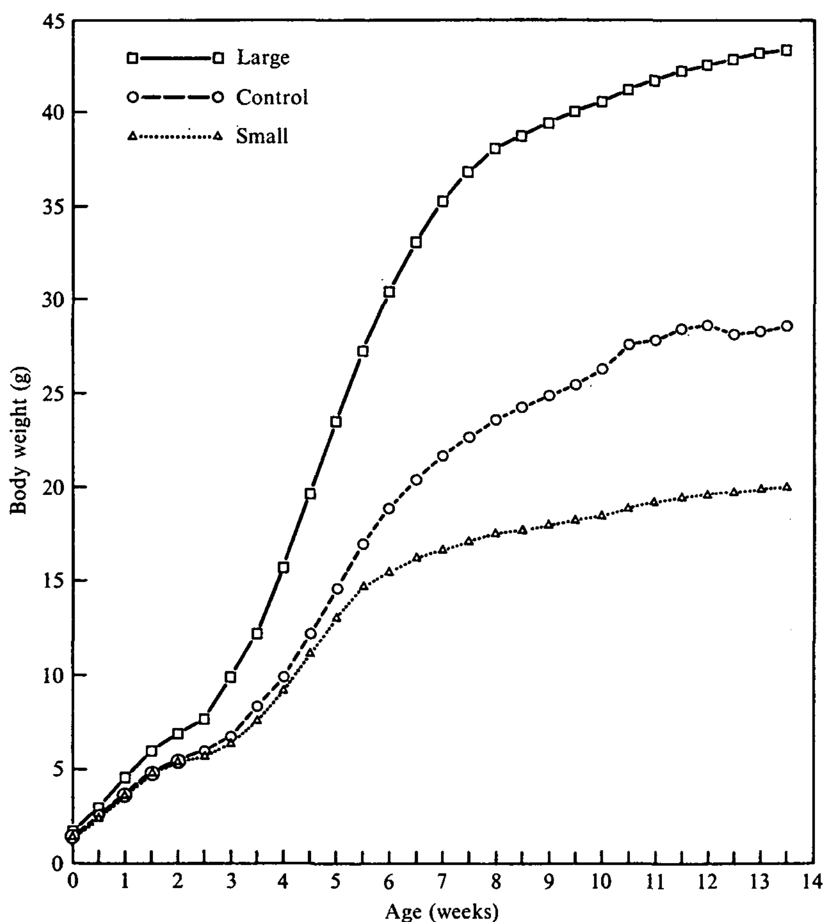

Fig. 1. Growth of QD male mice.

(ii) *Water*

Table 1 shows that the water content of the tissues – another factor which might complicate comparisons of growth rate – remained unchanged in livers and kidneys of adult selected mice. Water content in spleens and hearts was also examined: pooled mean values (all six replicates) were all 77–78 % of the wet

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weight for spleen and 76–77 % for heart in selected and control lines. The differences in wet organ weights therefore represent differences in true growth.

(iii) *Protein and free amino acids*

Total protein concentration in livers and kidneys of mature mice of five replicates are given in Table 2. There were no notable differences between selected and control mice, either in these values or in comparisons made at several younger

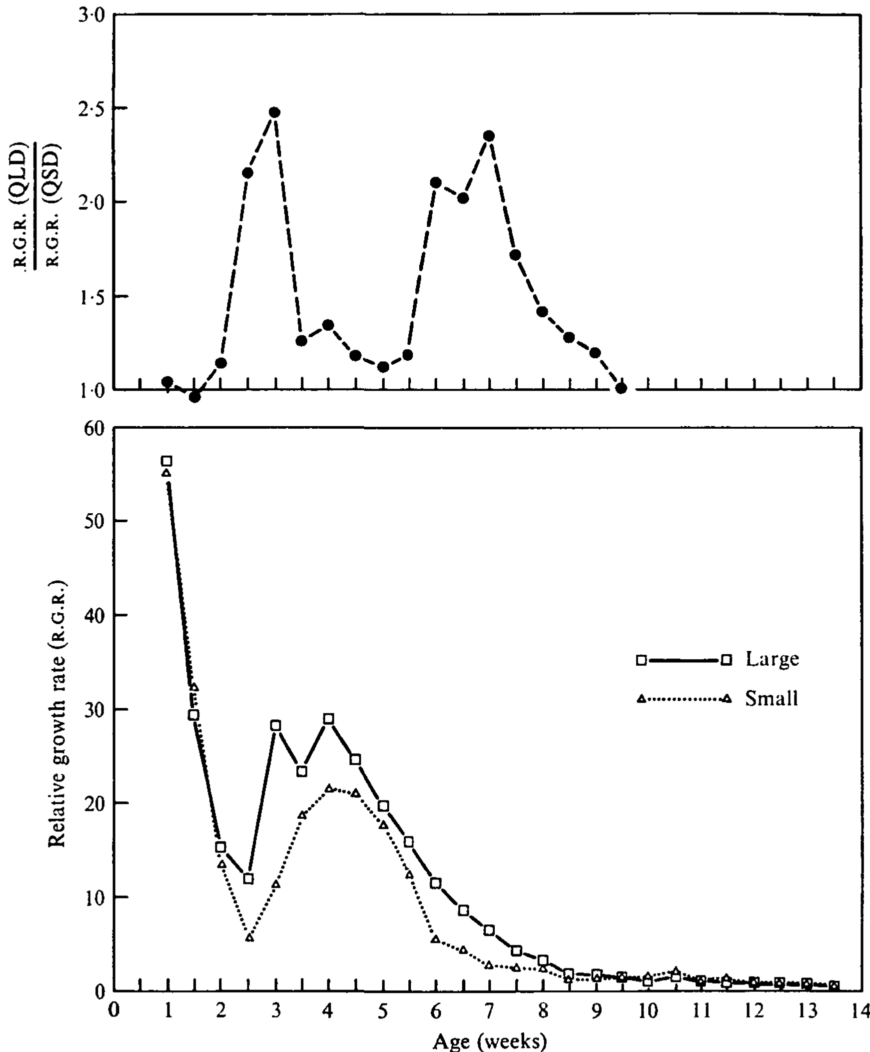

Fig. 2. Growth-rate of QD male mice. Relative growth per 3·5 days is shown for L and S and also (above) the ratio relative growth in L: relative growth in S.

ages in QB and QD (Fig. 4). The actual concentrations encountered, mostly 15–16 % of the wet weight in kidney (the kidneys in replicates A, C and F gave exceptionally high values) and around 20 % for liver, and the pattern of slight

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increases in concentration with increasing age agree with previous work (Priestley & Malt, 1968; Doljanski, 1960).

Table 3 shows the concentration of free amino acids in livers and kidneys. These data, and many additional sets which are not shown, indicate little or no difference between selected and control material, with some differences between mice of different ages.

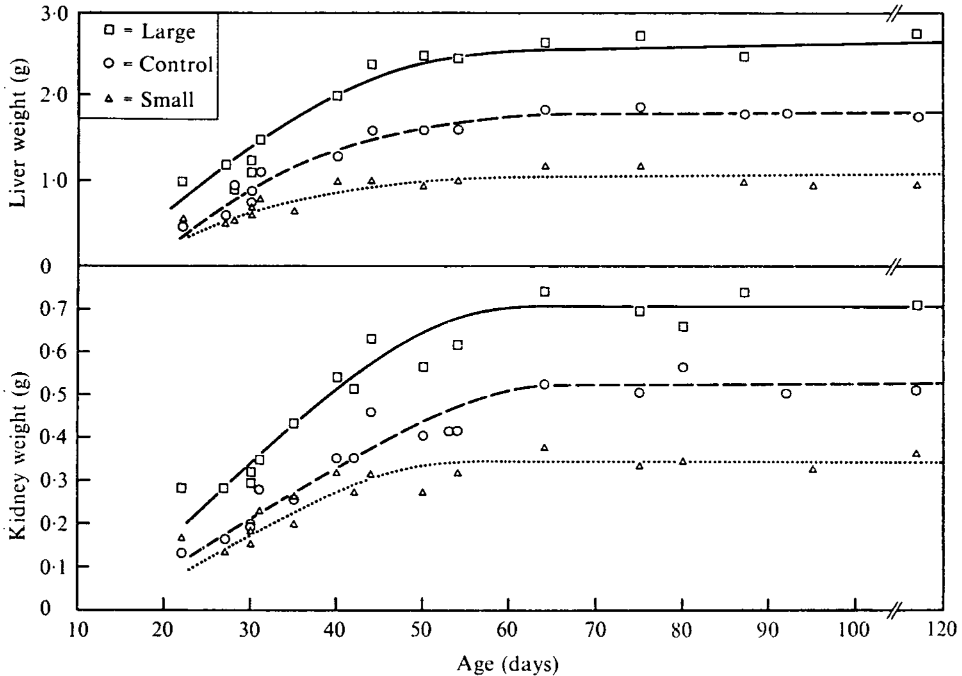

Fig. 3. Organ weight in QD male mice. Each point is a mean from between 5 and 16 mice.

Table 1. *Water content of liver and kidneys as percentage of wet weight*

(Each value shown is a mean from separate determinations on five or six organs. Mice were between 70 and 100 days old.)

	Kidney			Liver		
Replicate	L	C	S	L	C	S
A	74·4	75·0	73·5	66·9	67·9	68·0
B	75·2	73·8	73·3	68·7	67·2	65·7
C	73·5	72·8	72·5	69·3	66·7	66·8
D	74·4	74·4	72·7	66·6	66·2	67·6
E	74·4	73·4	71·9	67·3	66·0	64·8
F	73·9	73·5	73·7	64·5	64·7	69·4
Pooled mean	74·6	73·7	72·9	67·2	66·5	67·1

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Table 2. *Total protein concentration as percentage of wet weight* (± s.e.) *in organs of mice more than 60 days old*

(n is the number of separate determinations contributing to the mean for each line (L, C or S). Determinations were duplicated, with an average difference of 6 % between subsamples from the same homogenate.)

Replicate	n	L	C	S
		Kidney		
A	4	18·0 ± 0·4	17·8 ± 0·3	18·7 ± 0·4
B	14	15·8 ± 0·2	15·1 ± 0·3	15·1 ± 0·3
C	4	18·0 ± 1·6	18·1 ± 0·5	18·5 ± 1·8
D	16	15·9 ± 0·2	15·7 ± 0·3	15·5 ± 0·4
F	4	19·1 ± 1·5	19·5 ± 1·6	19·7 ± 2·1
Weighted means (s.e. ± 0·26)	42	16·58	16·29	16·35
		Liver		
A	4	19·3 ± 0·1	18·4 ± 1·0	21·0 ± 0·6
B	4	20·0 ± 0·8	20·1 ± 0·2	20·4 ± 0·4
C	4	20·3 ± 0·4	20·2 ± 0·5	21·3 ± 0·3
D	4	19·1 ± 0·3	20·1 ± 0·1	20·9 ± 0·7
F	4	19·4 ± 0·3	19·7 ± 0·1	20·3 ± 0·5
Weighted means (s.e. ± 0·23)	20	19·57	19·71	20·88

Differences between size-groups were significant ($P < 0·1$ %) in liver. 95 % confidence limits of L/S: kidney 106–97 %; liver 97–91 %.

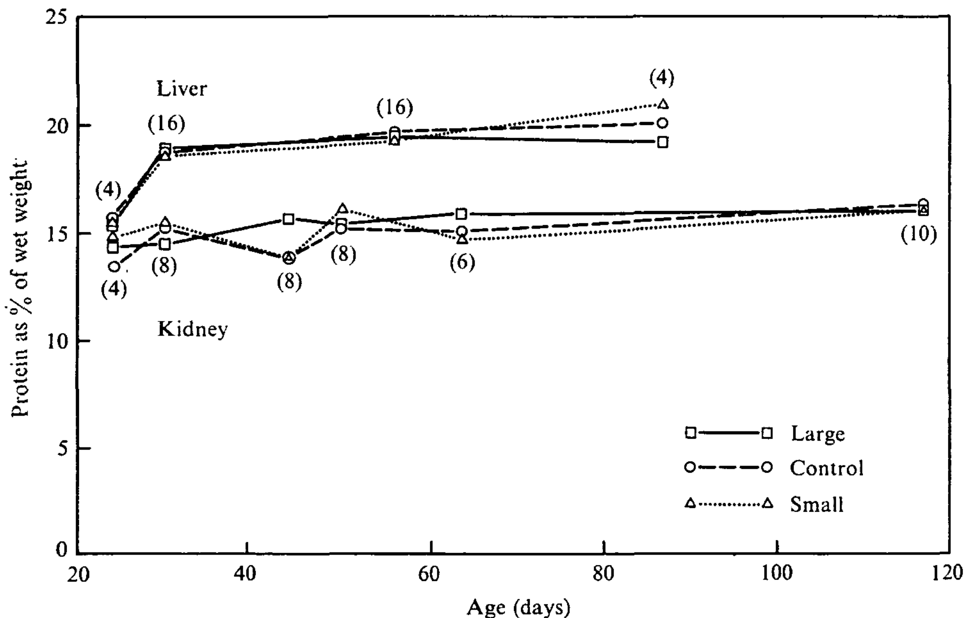

Fig. 4. Protein as percentage of wet weight in QD liver and kidney. Each point is a mean from the number of separate duplicated determinations indicated, with an average difference between duplicates in the same determination of 6 %.

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(iv) *Nucleic acids*

Concentrations of bulk RNA (that is, RNA of all types) in organs from adult mice are given in Table 4. Again there were no consistent differences in the selected mice. Fig. 5 shows RNA concentrations in QD mice at several ages; QLD kidneys exceeded QCD and QSD by about 2 %, but this effect was absent in liver and not

Table 3. *Free amino acid concentration* (μM/*g wet weight* ± S.E.) *in QD mice*

(All determinations were duplicated, with average difference between subsamples 2%.)

Organ	Age (days)	n	L	C	S
Kidney	75	6	57·05 ± 1·38	59·84 ± 1·82	58·93 ± 5·32
Liver	75	6	49·73 ± 3·40	51·41 ± 0·88	50·42 ± 1·38
Kidney	40	4	40·36 ± 0·97	37·67 ± 1·03	37·81 ± 0·62
Liver	40	4	33·47 ± 0·69	36·23 ± 1·42	37·13 ± 0·46

Table 4. *Total RNA concentrations* (*mg/g* ± S.E.) *in mice over 60 days old*

(All determinations were duplicated, with average difference between subsamples 3%.)

Replicate	n	L	C	S
		Kidney		
A	4	5·235 ± 0·16	5·188 ± 0·14	5·320 ± 0·09
B	14	5·188 ± 0·08	4·992 ± 0·08	5·186 ± 0·11
C	4	4·543 ± 0·09	4·902 ± 0·24	4·743 ± 0·10
D	16	5·288 ± 0·08	5·136 ± 0·07	5·080 ± 0·10
F	4	5·017 ± 0·14	5·276 ± 0·21	5·070 ± 0·20
Weighted means (S.E. 0·049)	42	5·130	5·084	5·106
		Liver		
A	4	10·386 ± 0·33	10·285 ± 0·41	10·436 ± 0·28
B	14	9·024 ± 0·19	9·956 ± 0·26	8·227 ± 0·16
C	4	8·964 ± 0·53	8·668 ± 0·23	9·682 ± 0·26
D	16	8·743 ± 0·23	8·716 ± 0·21	9·025 ± 0·25
F	4	8·925 ± 0·37	7·936 ± 0·14	8·064 ± 0·10
Weighted means (S.E. ± 0·123)	42	9·030	9·198	8·843

Differences between size-groups were not significant. 95 % confidence limits of L/S: kidney 103–98 %; liver 111–94 %.

seen in estimations on QB organs at several ages (results not shown). The actual RNA concentrations, and their tendency to decline slowly after puberty agree with previous findings (Baserga, Petersen & Estensen, 1966; Priestley & Malt, 1968). Concentrations of DNA in older mice are listed in Table 5 and compared with values from younger mice in Fig. 6. Here the data show a general tendency, more apparent in liver than kidney, and with QLA mice an exception, for DNA concentration to be highest in small mice and lowest in Large mice. When the replicates in Table 5 are pooled and compared with the controls the mean differences are − 1·4 % for Large kidneys and + 4·5 % for Small kidneys, with − 0·3 % for Large livers and + 13·0 % for Small livers. If selection has not affected cell ploidy and the DNA complement of the diploid nucleus has remained constant

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(Vendrely, 1955), comparisons of DNA per unit weight give valid comparisons of average cell mass. Our data then show that cell mass has been altered by the selection, though only slightly compared to the change in cell number, and more in the Small lines than in the Large. An increase in DNA concentration of, say, x % indicates a decrease in cell mass also of x %. Thus in Table 5 the average cell

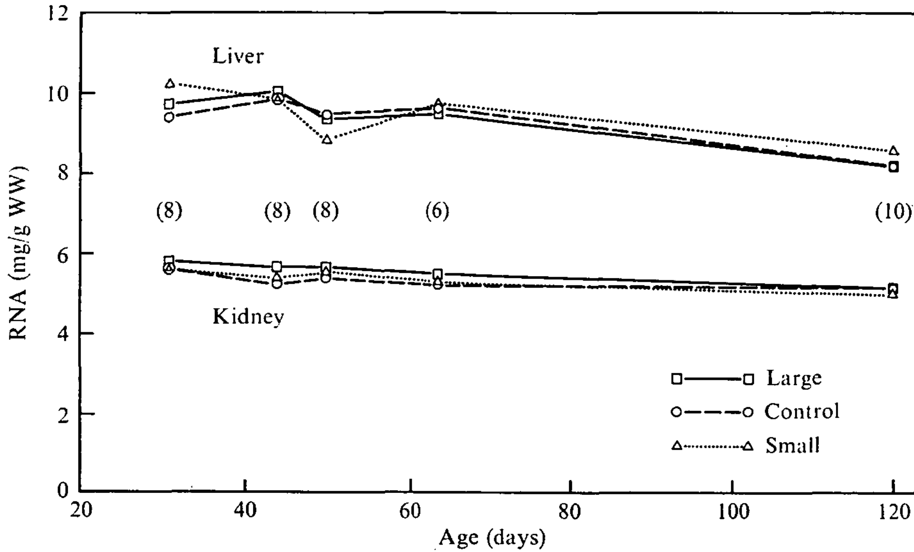

Fig. 5. Bulk RNA concentration in QD liver and kidney. Each point is a mean from the number of separate duplicated determinations indicated, with an average difference between duplicates in the same determination of 3 %.

Table 5. *DNA concentrations (mg/g wet weight* ± S.E.*) in mice aged more than 60 days*

(All determinations were duplicated with average difference between subsamples 6 %.)

Replicate	n	L	C	S
		Kidney		
A	4	5·004 ± 0·22	4·205 ± 0·08	4·799 ± 0·08
B	14	4·308 ± 0·19	4·290 ± 0·20	4·719 ± 0·13
C	4	3·724 ± 0·23	4·446 ± 0·10	4·332 ± 0·16
D	14	4·547 ± 0·15	4·508 ± 0·19	4·582 ± 0·14
F	4	3·996 ± 0·25	4·696 ± 0·18	4·736 ± 0·14
Weighted means (S.E. ± 0·094)	40	4·377	4·435	4·642
		Liver		
A	4	2·947 ± 0·18	2·379 ± 0·12	2·456 ± 0·12
B	14	2·136 ± 0·15	2·241 ± 0·15	2·407 ± 0·06
C	4	1·790 ± 0·20	2·162 ± 0·14	2·454 ± 0·12
D	14	2·077 ± 0·14	2·135 ± 0·12	2·605 ± 0·13
F	4	2·337 ± 0·27	2·296 ± 0·07	2·721 ± 0·17
Weighted means (S.E. ± 0·071)	40	2·181	2·215	2·517

Differences between size-groups almost significant ($P \sim 5$ %) in kidney; significant ($P < 1$ %) in liver. 95 % confidence limits of L/S: kidney 100–89 %; liver 95–79 %.

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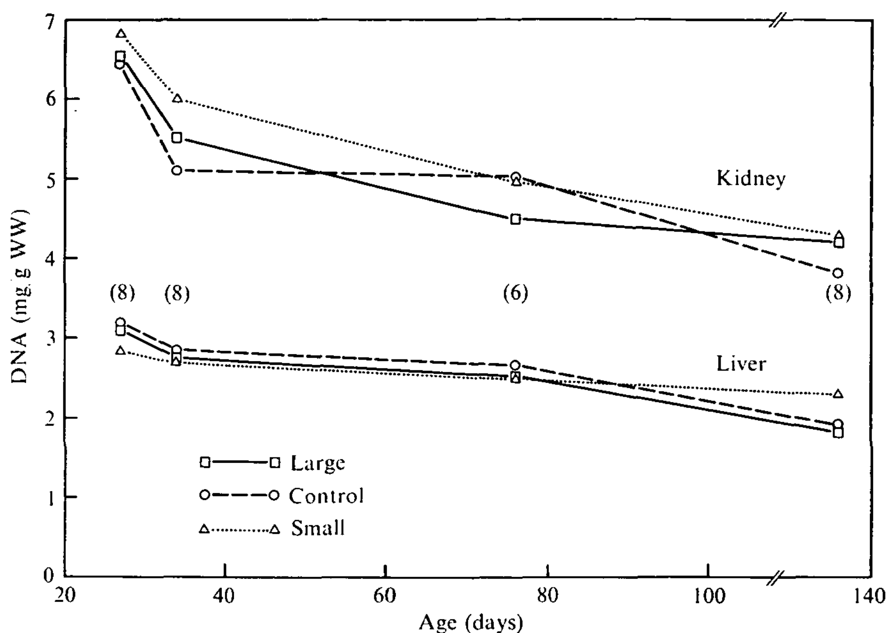

Fig. 6. Concentration of DNA in QB liver and kidney. Each point is a mean from the number of separate duplicated determinations indicated, with an average difference between duplicates in the same determination of 6 %.

Table 6. *Ribosome content*

(Mean values from *n* separate determinations are shown. Each represents recovery of ribosomes, recorded in O.D., in a sucrose gradient, after correction for organ weight, i.e. as cm^2 O.D.^{260}/g wet weight ± S.E. Recoveries from liver and kidney should not be compared; results refer to free ribosomes only in kidney, total (free plus membrane-bound) ribosomes in liver, and loss of liver ribosomes during preparation is proportionately greater.)

Replicate	Age	*n*	L	C	S
		Kidney			
B	35	10	216 ± 10	202 ± 10	152 ± 14
D	40	13	274 ± 19	249 ± 17	207 ± 15
B + D	70–95	11	223 ± 20	277 ± 14	181 ± 13
Weighted means		33	239	244	182*
		Liver			
D	30	10	99 ± 14	95 ± 11	100 ± 9
D	90	8	84 ± 15	111 ± 7	83 ± 9
Weighted means		18	92	102	92

* For reasons given in the text these differences are not regarded as significant.

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mass has been reduced by 4·5% in Small kidneys, and by 13% in Small livers. This point will be considered further in the discussion.

(v) *Ribosomes and polyribosomes*

The ribosome contents of livers and kidneys, as judged by recoveries in sucrose gradient analyses, are compared in Table 6. Mean recoveries per unit weight of Large kidneys exceeded those from controls while recoveries from Small kidneys

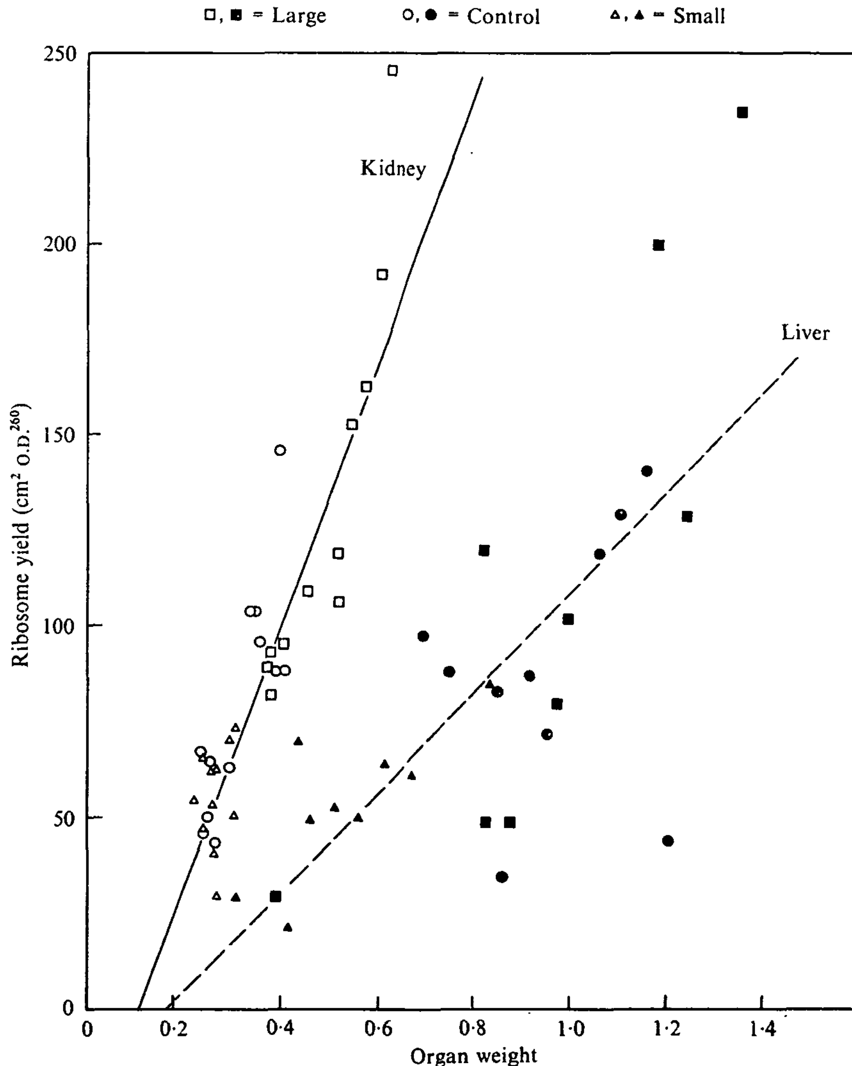

Fig. 7. Ribosome content in QD liver and kidney.

were less than controls. Recoveries of ribosomes from livers were all substantially the same. Although consistent, the significance of the differences recorded for kidney is very doubtful. First, this type of estimation is essentially semi-quantita-

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tive and there were marked differences between successive experiments in the yields of ribosomes obtained. Secondly, when recoveries were plotted against kidney weight, as in Fig. 7, all three sets of data (L, C and S) lay on or close to the same regression line. Finally, since ribosomal RNA accounts for about 80% of total RNA, any real difference in ribosome concentration would be revealed in the values for total RNA (Table 4). In fact, QLB and QSB gave identical value for total RNA while QLD and QSD differed by only 3–4%, showing that the 24% difference in mean ribosome recovery should be disregarded.

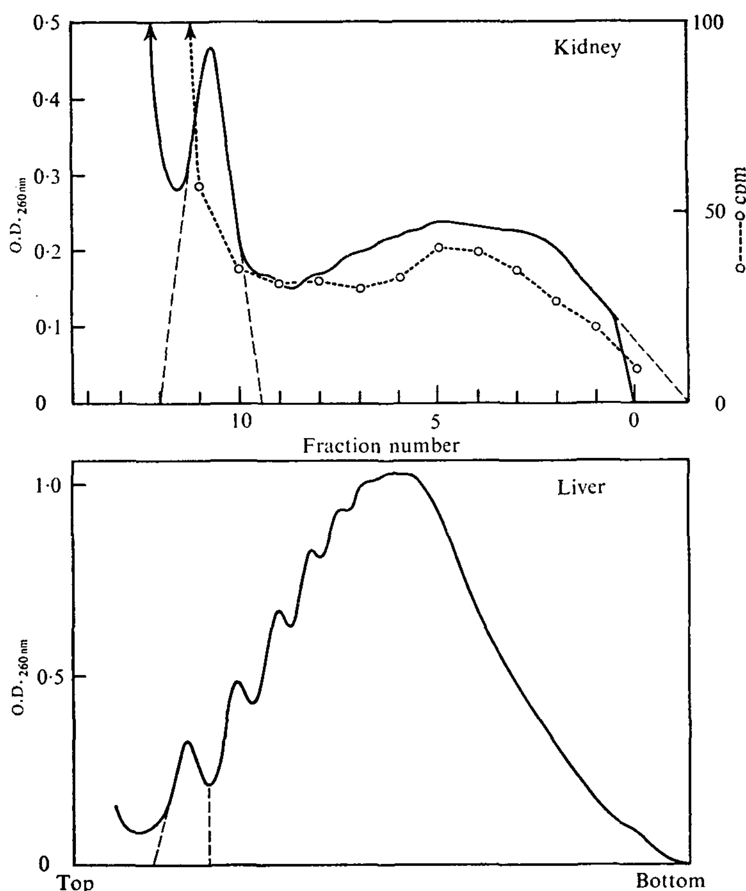

Fig. 8. Polyribosomes from QCD kidney and liver. The area between the dashed lines on the left of each Figure was taken to represent single ribosomes, and the total area of OD to the right of this was interpreted as polyribosomes. The triangular area beneath the extrapolated portion of the OD trace on the right of the kidney diagram was included with the polyribosomes. Radioactivity in the kidney diagram is acid-precipitable radioactivity incorporated during a 15 min incubation of decapsulated kidneys with ^{14}C-labelled amino acids.

Fig. 8 shows representative absorbance profiles of kidney and liver polyribosomes. That from kidney closely resembles profiles of free polyribosomes obtained from kidneys of Charles River mice (Priestley & Malt, 1968, 1969), while the liver

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polyribosomes illustrated resemble profiles obtained from rat liver by other workers (e.g. Blobel & Potter, 1967) except that the exaggerated dimer peak characteristic of rat liver polyribosomes is absent. Apart from differences in total OD, the overall appearance of the profiles did not vary and the same proportions of ribosomes sedimented as polyribosomes (Table 7) in selected and control mice.

Table 7. *Polyribosomes, as percentage of total ribosomes* (±S.E.)

Replicate	Age (days)	*n*	L	C	S
B Kidney	35	10	74±1	76±1	76±1
B+D Kidney	70–95	11	80±1	80±1	81±1
D Liver	30	9	92±1	91±1	92±2
D Liver	90	8	94±1	94±1	92±1

Table 8. *Amino acid incorporation in kidneys and livers of QD mice*

Expt	Age (days)	Organ	*n*	QLD±S.E.	QCD±S.E.	QSD±S.E.
1	35–40	K	14	(A) 5·44±0·33	5·00±0·25	6·34±0·36
				(B) 29·63±1·82	43·50±5·67	41·92±3·16**
2	30	K	10	906±62	1119±80	1143±85*
		L	10	1486±125	1951±77	2193±152**
3	28	K	(16)	150	210	257
		L	(16)	31	58	43
4	56	K	(16)	275	261	347
		L	(16)	42	44	54

Incorporation was *in vitro* in (1); values shown are (A) ratios of cpm in supernatant (peptides released from ribosomes) to cpm in ribosomes (nascent peptides), and (B) total cpm/cm^2 polyribosome O.D.^{260}. Each value is a mean from the number of separate determinations indicated (*n*). Other data (2–4) are specific activities (cpm/mg organ protein) after intraperitoneal injection of [^{14}C]protein hydrolysate (2) or [^{14}C]guanido-L-arginine (3, 4). Results in (3) and (4) are zero time values from the linear regression of log specific activity on time, each regression based on 16 organs (see Fig. 9). Mice in (3) received 5 μCi, those in (4) 10 μCi and the apparent effect of age (shown in days) should be ignored.

Except for 1A, where both parts of the ratio are affected proportionately, all results have been adjusted for differences between L, C and S in dosage and dilution of the label in the amino acid pool of the organ, as described in the text.

* Significantly greater than QLD value ($P < 0·05$).

** Significantly greater than QLD value ($P < 0·01$).

Monomeric ribosomes (that is, those not currently active in protein synthesis) represented 20–25 % of the total in kidneys, as in ICR mice (Priestley & Malt, 1968, 1969), and about 8 % in livers. Absorbance peaks interpreted as single ribosomes in liver gave zero OD at 320 nm, confirming that there was no confusion with ferritin (Wilson & Hoagland, 1965), which may exaggerate or obscure the single ribosome peak in crude preparations of liver ribosomes.

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(vi) *Protein synthesis*

Rates of amino acid incorporation in QLD, QCD and QSD mice are compared in Table 8. Experiment 1 was an attempt to compare the efficiency of the ribosomes in incorporating amino acids into peptides *in vitro*, and results are essentially translation per unit of polyribosomes in 15 min. Although the two methods of assessment (A and B) give slightly different results relative to the controls, in both cases QSD exceeds QLD. This pattern of Small exceeding Large was repeated in the other experiments, where incorporation is related to total organ protein (cpm/mg), again with considerable variation in the results relative to the controls. The average QSD/QLD ratio for the five sets of data is 1·36 in kidney and 1·38 in liver.

Table 9. *Rates of protein turnover and degradation in QD mice*

Age (days)	Organ	Turnover in days ($T_{\frac{1}{2}} \pm$ S.E.)			Degradation in days ($DT_{\frac{1}{2}}$)		
		QLD	QCD	QSD	QLD	QCD	QSD
28	K	6·6 ± 4·2 (11 %)	4·4 ± 1·9 (16 %)	4·5 ± 1·9 (15 %)	18 (4 %)	10·5 (7 %)	10·4 (7 %)
	L	5·1 ± 1·9 (14 %)	3·1 ± 0·6 (22 %)	4·2 ± 1·2 (17 %)	13·5 (5 %)	6·7 (10 %)	9·5 (7 %)
56	K	3·7 ± 0·5 (19 %)	4·2 ± 0·4 (17 %)	4·7 ± 0·5 (15 %)	4·0 (17 %)	4·7 (15 %)	4·9 (14 %)
	L	5·0 ± 1·0 (14 %)	3·6 ± 0·5 (19 %)	3·8 ± 0·3 (18 %)	5·4 (13 %)	4·1 (17 %)	3·9 (18 %)

Rate of protein turnover (half-life $T_{\frac{1}{2}}$) was calculated as the exponential decline in specific activity (cpm/mg protein) after injection of [^{14}C]guanido-arginine (Fig. 9). Turnover is also shown (in parentheses) as the percentage daily decline in the residual specific activity; this percentage was read from semilogarithmic plots of specific activity against time, with the extrapolated day 0 specific activity as 100 %. Turnover includes both degradation of protein and dilution of the labelled protein due to growth during the 6-day experiment. The dilution was calculated by assuming that net increase in organ protein (growth) was proportional to increase in body weight of QD mice (Fig. 1) in 6 days from the ages shown. For instance, a 50 % increase in protein content would decrease specific activity by 33 %. This calculated decline due to dilution was then subtracted from the decline observed in 6 days to leave the decline solely due to degradation, which was then re-expressed as a half-life ($DT_{\frac{1}{2}}$) and the corresponding daily increment.

(vii) *Protein turnover and degradation*

The turnover rates of organ protein after labelling with ^{14}C-guanido-arginine are shown in Table 9. The half-lives ranged from 3·0 to 6·6 days with an average of 4·4 days, which agrees with previous estimates for liver and kidney in rats and mice (Tomashefsky & Tannenbaum, 1970; Schimke *et al.* 1968; Scornick, 1972). In the kidneys of 56-day-old mice half-lives increased slightly from Large to Control to Small, but the three other sets of data agree in showing the longest half-lives in QLD. Although these differences in half-life of QLD and QCD protein seem impressive, variation between individual mice within each line was con-

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siderable, and statistical comparison of the slopes of the regression lines from which $T_{\frac{1}{2}}$ values were taken failed to confirm a significant difference.

Table 9 also shows the calculated rates of protein degradation ($DT_{\frac{1}{2}}$). In the older age-group correction of turnover rates for some slight dilution of the labelled protein during growth preserved the same patterns of difference between selected

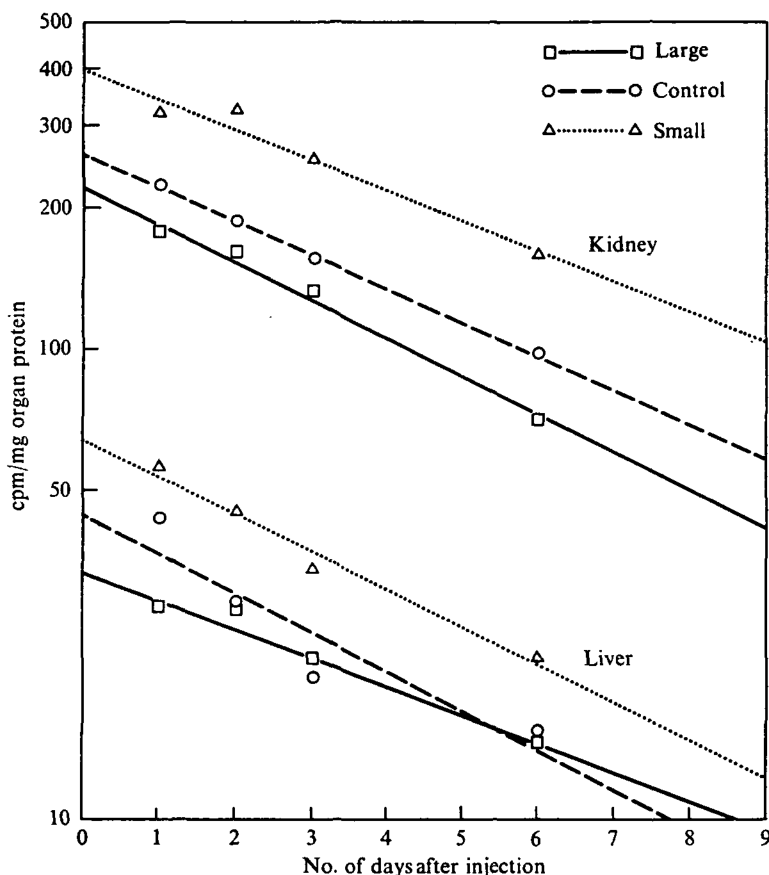

Fig. 9. Protein turnover in QD kidney and liver, shown as decline in specific activity of organ protein after injection of [^{14}C]guanido-arginine in mice 56 days old. Each point is a mean representing four separate determinations. Regression lines were computed using all 16 values. Zero time values were used as estimates of rates of protein synthesis after correction for differential dosage.

and control lines, with only small increase in half-life. In the younger mice, however, as much as 50 % of the observed decline in specific activity was attributed to dilution by growth, and elimination of this contribution substantially increased the half-lives from the $T_{\frac{1}{2}}$ values. Degradation rates ($DT_{\frac{1}{2}}$) for this group were two to three times those for the older group, indicating a much slower rate of degradation in the younger mice.

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4. DISCUSSION

The simplest explanation of the effects of selection on growth-rate in Q mice would be that a single rate-controlling factor had been increased in Large and decreased in Small. For instance, any significant change in the concentration of ribosomes in the tissues, other things being equal, would affect growth rate. In fact, none of the basic factors examined here – concentration of ribosomes and total RNA, proportion of ribosomes aggregated with mRNA as polyribosomes, and the supply of free amino acids for protein synthesis – were significantly changed in selected mice. Absolute amounts of all components except DNA appear to have increased or decreased in proportion to weight.

Slight deviations from a strict relationship between DNA content and weight in Large, Control and Small mice suggest that some changes in cell size, in addition to those in cell number, had contributed to the changed organ weights of selected mice. We will return to this later.

The probability that selection would focus on any one factor was not high. Growth rate, as reflected in body size in mammals, is thought to be controlled by several, perhaps many, different genes (Falconer, 1960, Roberts, 1966), and where changes in organ growth-rate can be explained in intracellular terms several factors seem to work together. In kidneys regenerating after contralateral nephrectomy, for instance, increased protein synthesis follows increased RNA synthesis and attainment of higher RNA concentrations (Bucher & Malt, 1971), and there is also decreased protein catabolism (Tomashefsky & Tannenbaum, 1969); virtually the same processes operate in regenerating liver (Bucher & Malt, 1971, Scornick, 1972). The anabolic action of growth hormone appears to involve a very rapid increase in both amino acid transport and in the rate at which ribosomes assemble amino acids into polypeptide, with a later increase in RNA synthesis (Korner, 1968). If differences in the size of organs from Q mice are of this multifactorial nature then the change in each individual factor might be only a few per cent and might be undetected in our estimations, or at best inconspicuous in our results. Inevitably, many differences of this small order were recorded, but since few were consistently present even in one organ of one replicate, they may represent errors in technique or sampling.

Some attempt must be made to assess the possibility that real differences between selected and control Q mice exist, but went undetected because we failed to examine the right parameters, or the critical stage of growth, or chose unrepresentative organs for study. First, although the parameters used in our experiments are those which, on current evidence, seem most implicated in quantitative control of growth, important contributions may, of course, be made by other factors or components. For example, altered availability of a factor promoting translation of messenger RNA would not appear in our results, although the consequence would presumably register in the comparisons of amino acid incorporation. Alterations in some other components would probably have shown indirectly: shortage of initiation or termination factors, for instance, or of mRNA

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generally, might change polyribosome profiles. We have attempted to counter the second possibility – that differences introduced by selection are transient, present for only a short phase of growth – by examining mice at several different ages, where this was compatible with maintaining a reasonable sample size. Most mice used were between 4 and 9 weeks old, when differences in rates of relative growth are well established. Thirdly, as to the degree to which liver and kidney reflect the general pattern of growth, the effects of selection on the growth of these organs appears to match the effect on body weight when different replicates or different ages are compared (Figs. 1, 3; also Gauld, unpublished). In histological or biochemical terms no organ is more representative of the body mass than any other, except in the sense that muscle or skin account for the largest proportion. Liver and kidney were chosen for convenience of removal and homogenization, and because much data was already available from each for comparison with our own results.

A fourth possibility is that because of the variation encountered within each line and the small size of the differences anticipated between lines, our samples of mice were too small to establish statistically valid differences. This possibility is largely discounted by the analysis of variance, which shows that quite small differences would have been established had they been consistently present. The two-way selection in several replicates clearly limited number of mice per group. However, except in our surveys of replicates A, C and F, the number of mice used to compare each component was still extremely high by most standards, particularly when data gathered at several ages could be pooled. For instance, the total sample *for each* of the three QD lines was 40 mice for RNA estimation and 38 for DNA estimation. Since this was in each of the two organs, and the B replicate was studied in similar detail, our RNA estimations alone totalled over 300 (each in duplicate) and further increase in numbers was scarcely practicable.

Two suggested targets for selection can be considered briefly in the light of these results. The first is increased synthesis (or release?) of pituitary hormones, particularly growth hormone (Baird, Nalbandov & Norton, 1952; Nalbandov, 1963) which might be selected for in the Large mice and selected against in the Small mice. If this were the case, the Small mice might resemble, in some features, hypopituitary dwarf mice, or hypophysectomized rats. We cannot see any common features. The softer hair, rounder body and sharper face of the dwarf (*dw/dw*) mouse is not seen in QS mice. Proportions of single ribosomes are raised, and total RNA concentrations are lower both in dwarf mice (Priestley & Robertson, 1972; Chen *et al.* 1972) and in hypophysectomized rats (Korner, 1968) but are normal in QS mice. Many hepatocytes are binucleate and many nuclei appear to be polyploid in liver from QS mice, in contrast to the dwarf mouse where the cells remain mononucleate and diploid (Leuchtenberger, Helweg-Larsen & Murmanis, 1954). A second possibility is that selection has been for or against appetite. Differences in appetite are known in Q (Roberts, unpublished data) and other mice selected for body size (Fowler, 1958). It is difficult to predict the intracellular effects, if any, of changes in appetite, except perhaps alteration in the size of free amino

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acid pools or in blood glucose, neither of which show any change in Q selected mice (Table 3, and Gauld, unpublished results). Appetite might also affect cell size, but this too was rather stable.

The changes in average cell mass calculated from all the DNA data obtained from replicates B and D are set out in Table 10. The calculations assume constant cell ploidy (that is ploidy × number of nuclei per cell), constant DNA content of diploid nuclei, and a constant proportion of extracellular mass. The weakest of these assumptions, in our view, concerns cell ploidy in hepatocytes (roughly 50 % of liver cells). No detailed study of ploidy in Q mouse liver has been made. Our own brief examination of cell suspensions merely confirmed that in Large, Control

Table 10. *Changes in average cell mass estimated from DNA concentrations, expressed as a percentage of controls*

(Replicate B: 30 L, 30 C and 30 S mice at ages 27–135 days. Replicate D: 38 L, 38 C and 38 S mice at ages 32–120 days. Mean changes in organ weight in the same mice are given for comparison.)

		Large		Small	
Replicate		Liver	Kidney	Liver	Kidney
QB	Cell mass	+ 5	− 2	0	−11
	Weight	+46	+31	−32	−30
QD	Cell mass	+ 4	− 2	−16	− 2
	Weight	+47	+36	−40	−28

and Small many hepatocytes were binucleate, with nuclei of various sizes. If mean cell ploidy increases with liver weight, as in Swiss mice (Epstein, 1967), not just within, but across the Q lines, then cell ploidy will increase from QS to QC to QL and the values in Table 10 must underestimate the degree of change in the mass of the average liver cell. With this important reservation then, the DNA data indicate a small contribution from changed cell mass towards the change in organ mass. There is considerable variation between lines, but cell mass appears to have shifted more in QS than QL, and more in liver than in kidney. This general pattern agrees substantially with comparisons of cell size in Q mice using a nuclear count method (Gauld, Falconer & Roberts, unpublished) when results for B and D replicates are compared. If anything, the DNA values indicate slightly less change in cell mass. The general conclusion that selection for size operates more on cell replication than on cell size finds broad agreement in a range of previous studies with selected mice (Robinson & Bradford, 1968), chicks (Lepore, Siegel & Siegel, 1965) and Drosophila (Robertson, 1959).

The result of the protein synthesis measurements seems very clear: at 28, 40 and 56 days old QSD mice synthesized protein at a much faster rate than QLD. However, since growth curves plainly show QLD growing faster at these ages, this result is somewhat difficult to accept. A possible source of error in the results applies to Expt 1 B. It arises from the main difficulty in measuring protein synthesis in animals of different sizes, which is that of standardizing dosage. Because we

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were unable to grade minute volumes of innoculate closely to body weight, particularly when many mice were to be injected rapidly to maintain the same duration of labelling, selected and control mice were given identical doses per mouse, corresponding to different doses per body or organ weight. Our remedy was to measure the concentration of isotope in the free amino acid pool of the organ so that, for instance, greater dilution in a Large (QLD) kidney could be compensated by proportionate adjustment of the final result. In practice, differences between selected and control mice in dilution of the radioactive amino acids in the precursor pools were small, and always less than the differences in organ weight. However, it is only possible to make one, terminal, measurement of the free amino acid pool, and in the *in vitro* conditions of the first experiment attainment of the recorded concentration may have been quicker in Small than in Large kidneys due to more rapid penetration of the smaller tissue mass. Experiment 1A, where counts were related to counts, is independent of dosage, and this effect would not affect the *in vivo* experiments, since the blood supply to each organ would ensure efficient distribution of the labelled amino acids.

A higher rate of protein synthesis is QSD mice might be compatible with their slower growth if it was outweighed by even more rapid protein degradation in QSD. The results do hint at slower turnover in QLD: both organs at 28 days and livers at 56 days show QLD with slower turnover and degradation rates than QCD and QSD. Unfortunately, as elsewhere in this study, variation between individual mice within each group was considerable and no statistically valid difference was established for the Large line. In view of the very different rates of protein synthesis recorded in Table 8 we cannot therefore explain the faster growth of the Large line in a way compatible with all the data. If, however, the differences in protein synthesis could be traced to some artifact of technique, so that the rates were, in fact, the same, or nearly the same, in all lines, then our failure to detect differences in cellular composition related to protein synthesis would be understandable. Furthermore even large differences in organ growth-rate could then be explained by quite small differences in rate of protein degradation, particularly if, as the rapid rates of protein turnover suggest, rates of synthesis and degradation are fairly evenly balanced. At present the results in Table 8 prevent us from proposing such an explanation with any confidence.

One clear conclusion from the degradation estimations ($DT_{\frac{1}{2}}$ values) is that degradation proceeded much faster in the older mice. While the assumption used in calculating $DT_{\frac{1}{2}}$ is slightly questionable, in that organ weight does not necessarily follow body weight precisely, particularly in older mice accumulating fat, the error introduced is clearly small relative to the extremely large difference in degradation rate between the two age-groups. If this is truly an age difference, it suggests that normal termination of growth at maturity may be controlled by an increasing rate of protein degradation, until at the age when the rates of protein synthesis and degradation are equal growth ceases. This aspect of the results will be discussed and extended in a separate publication.

The Q selected mice appear to provide an ideal opportunity to identify any

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changes in tissue components resulting from selection for body size. The availability of several replicates each with two-way divergence from controls minimizes the risk of confusion from differences in variables unrelated to growth – a danger always present in systems with single selected and control lines. However, with the likelihood of an interaction between several different factors, each influencing growth to a small extent, our essentially negative findings cannot be completely conclusive. To be more certain that the cellular and intracellular composition of animals selected for larger or smaller body size has remained the same as in the original parent population, animals showing a much greater size range, say several hundred per cent, need to be available.

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