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Environmental Maternal Influences on Body Composition in Mice Selected for Body Weight *

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Summary. The effect of the postnatal maternal environment, simulated by rearing mice in litters of three, six or nine, on body weight and body composition was investigated in three lines of mice differing widely in growth rate. The lines were selected for high (H_6) and low (L_6) 6-week body weight while the control line was maintained by random selection. Body weight and weights and percentages of ether extract, water, ash and protein at 21, 42, 63 and 84 days were recorded. With few exceptions, there were positive correlated responses to selection in body weight and in weights of body components. At 21 and 42 days the correlated responses were larger in L_6 mice than in H_6 mice. Body weight and weights of body components were larger for mice reared in litters of three than for those reared in litters of nine. Also, mice reared in litters of six were intermediate in body weight and weights of some of the body components between those reared in litters of three and nine. Differences in body weight and weights of body components due to postnatal maternal environment were small by comparison with differences due to genetic line. There were significant line by maternal environment interactions in body weight at 21 days and in ether extract weight at 21 and 63 days. Line and maternal environment differences in percentages of body components did not follow any consistent trend. The results for percentages of body components were further complicated by line × maternal environment interactions. In general, both line and postnatal maternal environmental differences in percentages of body components diminished with age.

Key words: Maternal effects – Body composition – Selection – Growth – Mice

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Introduction

Postnatal environmental maternal effects are often implicated as a possible causal component of observed variation in growth. For example, many authors list environmental maternal effects as one of the causal components of the observed postnatal maternal variance in body weight in crossfostering studies. Most studies yield little information on the nature of maternal environmental influences since known differences in the maternal environment are not part of the experimental design. Nevertheless, there have been reports that indirectly have yielded useful information on postnatal environmental maternal effects and suggested that more detailed studies of this source of variation would be informative (Falconer 1963; Eisen 1970).

There are reports indicating the importance of postnatal environmental maternal influences on body composition mediated through controlling the energy available to pups during the nursing period by manipulation of postnatal litter size. Widdowson and McCance (1960) studied the growth patterns of several tissues and organs in rats reared in litters of three or of 15 to 20. At weaning and at later ages rats reared in small litters exceeded those reared in large litters in body length and in weights of several organs. At weaning and all later ages fat percentage was much higher in rats reared in the small litter size. Eisen and Leatherwood (1978) reported that percentage fat of mice reared in litters of 14 was consistently less than in litters of eight from six to 30 weeks of age.

There is evidence that postnatal environmental maternal influences on cell number and cell size in various fat depots in rats and mice are substantial. Knittle and Hirsch (1968) reared rats in litter sizes of four and 22. At 5, 10, 15 and 20 weeks of age, rats reared in the smaller litter size had significantly larger epididymal fat pads caused by both more and larger cells in the pad. Eisen and Leatherwood (1978) reported that both a polygenic obese and control strain of mice had fewer and smaller epididymal fat cells when reared in large litters. In addition, environ-

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mental maternal influences may depend on the particular strain. Johnson et al. (1973) found that rearing rats in small or large litters affected cell size and number in various fat depots of control rats but not in obese rats. However, Eisen and Leatherwood (1978) reported no important interaction between genetic strain and number reared.

These results suggest the need for determining the relative importance of postnatal environmental maternal effects, genetic effects due to selection for growth and their interaction on growth and body composition at different ages. The present study was undertaken to investigate the effects of rearing high and low body weight selected and control lines of mice in different litter sizes on body weight and body composition at 21, 42, 63 and 84 days of age.

Material and Methods

Genetic Stocks

Parents of mice used in the present study were from generations 75 and 76 of an experiment in which mice were selected for high (H_6) and low (L_6) 6-week body weight. Parents of control mice (C_2) were from generations 44 and 45 of a population that had been maintained contemporaneously with the selected lines by random mating. The control line, which consisted of 30 females and 15 males, was maintained by randomly choosing a female from each litter and a male from two litters having the same sire. The selected lines, each consisting of 20 females and 10 males, were developed by selecting the largest (H_6) or smallest (L_6) female in each of 20 litters and the largest or smallest male from two litters by the same sire. After a number of generations the control line was reconstituted from the original stocks. A more detailed description of the origin and selection history of the present lines has been given by Legates (1969).

Experimental Design

After littering, dams were assigned to rear three, six or nine offspring from their own line. To avoid associations between the dam's body weight and the litter size she reared, the method of assignment was to select at random within a line three dams that had littered within a twelve-hour period and to form a pool of the progeny from these three dams. Then one of the dams was randomly selected to rear nine young also randomly selected from the pool, a second dam to rear six and the third one to rear three. In order that approximately equal numbers of offspring were allotted to each number-reared treatment, two of the dams were randomly assigned to rear three young and the third one to rear six in some groups. Males and females were assigned to each of the three dams so that postnatal litters contained approximately equal numbers of males and females. Mice were identified by toe-clipping at twelve days of age and weaned at 21 days. Mice of like sex, postnatal litter size and line were randomly assigned to cages in groups of four. Dams were fed Purina Mouse Chow ad libitum during lactation; after weaning the progeny were fed Purina Laboratory Chow ad libitum. All mice were individually weighed at 21, 42, 63 and 84 days of age.

Sampling of Mice for Chemical Analysis

Ten males and ten females were randomly selected from each line and postnatal litter size for body chemical composition analysis at 21, 42, 63 and 84 days of age. The number of mice sampled are shown in Table 1. At each age, the sampled mice were killed by overexposure to ether. Using the methods outlined by Eisen and Leatherwood (1976), empty body weight and weights of ether extract, water, ash and protein were obtained on individual mice. Percentage of each body component was obtained by dividing each component by empty body weight.

Statistical Methods

At each age, it was assumed that an observation, $Y_{ijk\ell}$, could be described by the following model:

$$Y_{ijkm} = \mu + \ell_i + s_j + n_k + (\ell s)_{ij} + (\ell n)_{ik} + (sn)_{jk} + (\ell sn)_{ijk} + e_{ijk\ell}$$

where μ = overall mean, ℓ_i = fixed effect of the i^{th} line (i = 1, 2, 3), s_j = fixed effect of the j^{th} sex (j = 1, 2), n_k = fixed effect of the k^{th} number-reared treatment (k = 1, 2, 3), $(\ell s)_{ij}$, $(\ell n)_{ik}$, $(sn)_{jk}$ and $(\ell sn)_{ijk}$ are two-way and three-way interactions, respectively, and e_{ijkm} = random error assumed NID (0, σ^2). The data were unequally distributed in the subclasses and statistical methods appropriate for non-orthogonal data were used (Harvey 1960).

Certain linear contrasts (Table 2) were decided upon, a priori, because they have specific biological interpretations. The first two contrasts among the lines measure asymmetry of response to selection and the divergence between the selected lines, respectively,

Table 1. Numbers of mice sampled from the treatment subclasses for body composition analysis at 21, 42, 63 and 84 days of age

Age at slaughter (days)	Line																	
	C_2 — number reared						H_6 — number reared						L_6 — number reared					
	3		6		9		3		6		9		3		6		9	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
21	10	10	10	10	12	9	10	10	10	10	10	10	10	10	10	10	10	10
42	10	9	11	10	11	10	11	10	9	10	10	10	10	10	9	9	11	12
63	11	9	10	10	10	10	10	7	6	10	11	11	9	10	7	6	10	10
84	10	10	10	11	10	10	10	9	7	8	10	10	10	10	10	4	10	10

Table 2. Linear contrasts among the treatment means in the analysis of environmental maternal effects at each age

Linear contrast	Line X sex X number-reared combination																	
	CM3	CM6	CM9	CF3	CF6	CF9	HM3	HM6	HM9	HF3	HF6	HF9	LM3	LM6	LM9	LF3	LF6	LF9
Line contrasts																		
$2C_2-H_6-L_6$	2	2	2	2	2	2	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
H_6-L_6	0	0	0	0	0	0	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1
C_2-H_6	1	1	1	1	1	1	-1	-1	-1	-1	-1	-1	0	0	0	0	0	0
C_2-L_6	1	1	1	1	1	1	0	0	0	0	0	0	-1	-1	-1	-1	-1	-1
Sex contrast																		
M–F	1	1	1	-1	-1	-1	1	1	1	-1	-1	-1	1	1	1	-1	-1	-1
Number reared contrasts																		
$2N_6-N_3-N_9$ (Quadratic)	-1	2	-1	-1	2	-1	-1	2	-1	-1	2	-1	-1	2	-1	-1	2	-1
N_3-N_9 (Linear)	1	0	-1	1	0	-1	1	0	-1	1	0	-1	1	0	-1	1	0	-1
N_6-N_3	-1	1	0	-1	1	0	-1	1	0	-1	1	0	-1	1	0	-1	1	0
N_6-N_9	0	1	-1	0	1	-1	0	1	-1	0	1	-1	0	1	-1	0	1	-1

while the second two measure correlated responses to selection in the H_6 and L_6 lines, respectively. The first two number-reared contrasts measure quadratic and linear effects of number-reared, respectively. The interaction contrasts are obtained by multiplying the coefficients of one main effect contrast by a contrast involving a second main effect. Interaction contrasts are given in the results section when considered biologically significant. All linear contrasts are expressed as a percentage of the control line.

Results

Body Weight

Least squares means for body weight at 21, 42, 63 and 84 days are plotted by line × sex × number-reared subclass in Figure 1. Linear contrasts among treatment means are

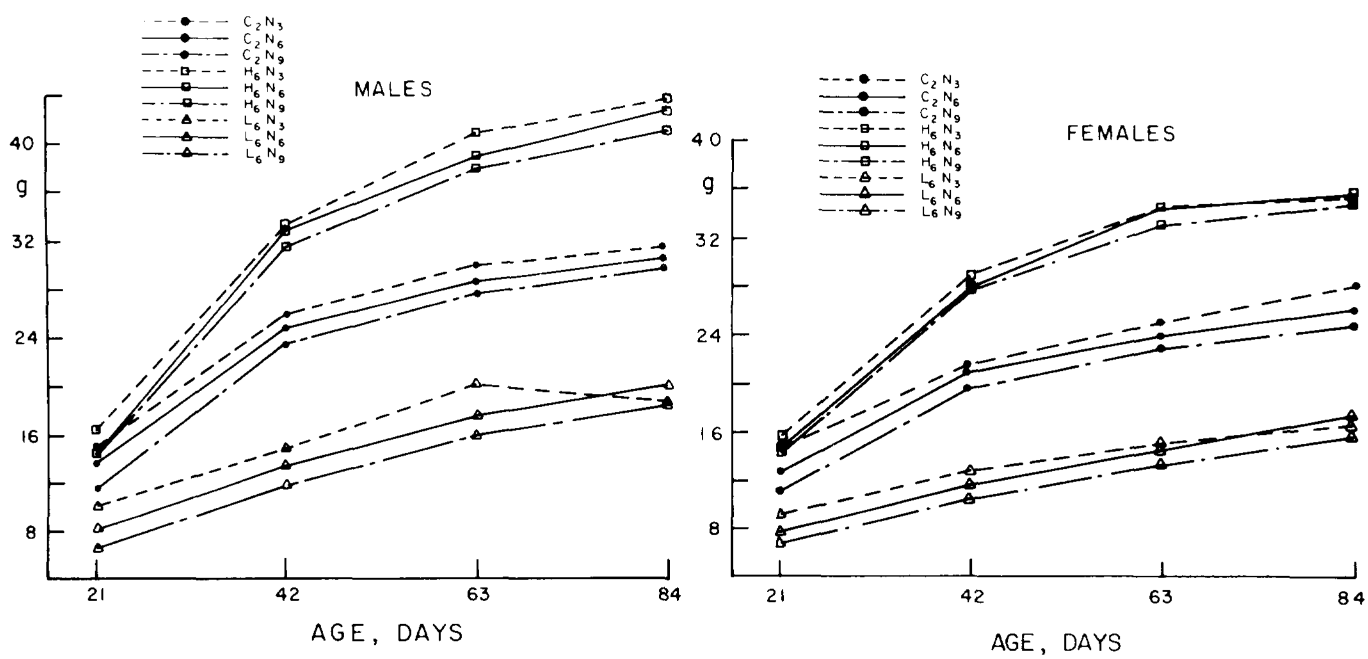

Fig. 1. Least squares means, plotted by line X sex X number-reared subclasses, for body weights (g) at 21, 42, 63 and 84 days

Table 3. Linear contrast and their standard errors (as a percentage of the control mean for each age) among treatment means for body weight at 21, 42, 63 and 84 days of age

Linear contrast	Body weight (g) at day			
	21	42	63	84
Line contrasts				
$2C_2 - H_6 - L_6$ (1)	3.27 ± 0.34[b]	2.77 ± 0.50[b]	0.09 ± 0.77	0.42 ± 0.75
$H_6 - L_6$ (2)	6.75 ± 0.21[b]	18.07 ± 0.32[b]	20.39 ± 0.50[b]	20.98 ± 0.51[b]
$C_2 - H_6$	−1.74 ± 0.20[b]	− 7.65 ± 0.30[b]	−10.15 ± 0.46[b]	−10.28 ± 0.45[b]
$C_2 - L_6$	5.01 ± 0.19[b]	10.42 ± 0.29[b]	10.24 ± 0.46[b]	10.70 ± 0.45[b]
Male-female (3)	0.35 ± 0.16[a]	3.41 ± 0.25[a]	4.57 ± 0.39[b]	4.77 ± 0.39[b]
Number-reared contrasts				
$2N_6 - N_3 - N_9$ (4)	−0.51 ± 0.36	0.30 ± 0.55	0.05 ± 0.89	0.72 ± 0.90
$N_3 - N_9$ (5)	2.86 ± 0.19[b]	2.05 ± 0.29[b]	2.42 ± 0.44[b]	1.49 ± 0.42[b]
$N_6 - N_3$	−1.68 ± 0.21[b]	− 0.87 ± 0.31[b]	− 1.18 ± 0.51[b]	− 0.38 ± 0.52
$N_6 - N_9$	1.17 ± 0.19[b]	1.18 ± 0.30[b]	1.23 ± 0.48[b]	1.10 ± 0.48[a]
Line × sex contrasts				
(1) × (3)		1.73 ± 1.0		− 1.00 ± 1.50
(2) × (3)		2.50 ± 0.63[b]		4.91 ± 1.02[b]
Line × number-reared contrasts				
(1) × (4)	1.69 ± 1.44			
(1) × (5)	2.46 ± 0.81[b]			
(2) × (4)	−0.55 ± 0.91			
(2) × (5)	−1.11 ± 0.48[a]			

[a] $P < 0.05$
[b] $P < 0.01$

shown in Table 3. The divergence of the selected lines was highly significant at all ages, but asymmetry was significant only at 21 and 42 days. The correlated responses in H_6 and L_6 were significant at all ages. Males were consistently larger than females. N_3 mice (reared in litters of size three) were significantly heavier and N_9 mice (reared in litters of size nine) significantly lighter than N_6 mice (reared in litters of size six). Body weight increased linearly with smaller postnatal litter size. There was a significant line by number-reared interaction at 21 days of age, but the interaction was due to line differences changing in magnitude from one number-reared class to another, without any change in the ranking of the lines. The significant (1) × (5) contrast was attributed to the difference between C_2 mice and the average of selected mice being greater for mice reared in litters of size three than it was for mice reared in litters of size nine ($2C_2 - H_6 - L_6 = 4.2$ g and 1.76 g for mice reared in litters of size three and nine, respectively). The significant (2) × (5) contrast was caused by the difference between H_6 and L_6 being smaller for mice reared in litters of size three than it was for mice reared in litters of size nine ($H_6 - L_6 = 6.29$ g and 7.39 g for mice reared in litters of size three and nine, respectively).

Weights of Body Components

Since estimation of various two- and three-fractor interactions was permitted by the design of the present experiment (Materials and Methods), these interactions were estimated in the present data. Many of these interactions were statistically significant, but were small and considered to be of little, if any, biological interest. Consideration of these interactions in the results would divert attention from the more important features of the results. Therefore, several statistically significant interactions are given little emphasis in describing the results except in cases where interpretation of main effects are influenced by the presence of the interactions.

Least squares means for ether extract, water, ash and protein weights are plotted by line × sex × number-reared subclass in Figures 2 to 5, respectively. Linear contrasts among the subclass means are shown in Tables 4 to 7. The H_6 and C_2 lines had significantly greater ether extract weights than the L_6 line at all ages. The C_2 line had a significantly greater ether extract weight than H_6 at 21 days, no significant difference at 42 days and significantly less at 63 and 84 days. The asymmetric response in ether extract weight at 21 and 42 days was due to a greater

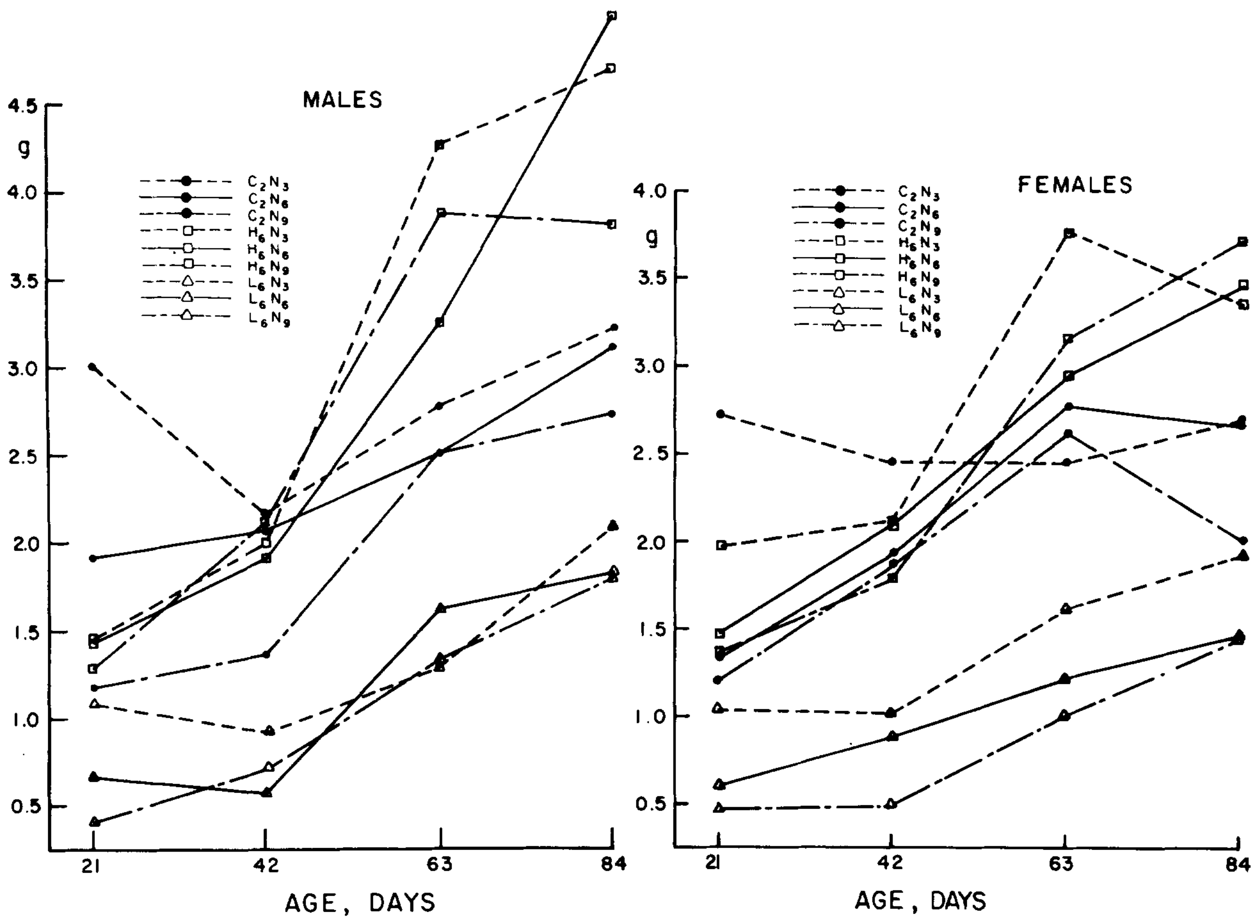

Fig. 2. Least squares means, plotted by line X sex X number-reared subclasses, for ether extract weights (g) at 21, 42, 63 and 84 days

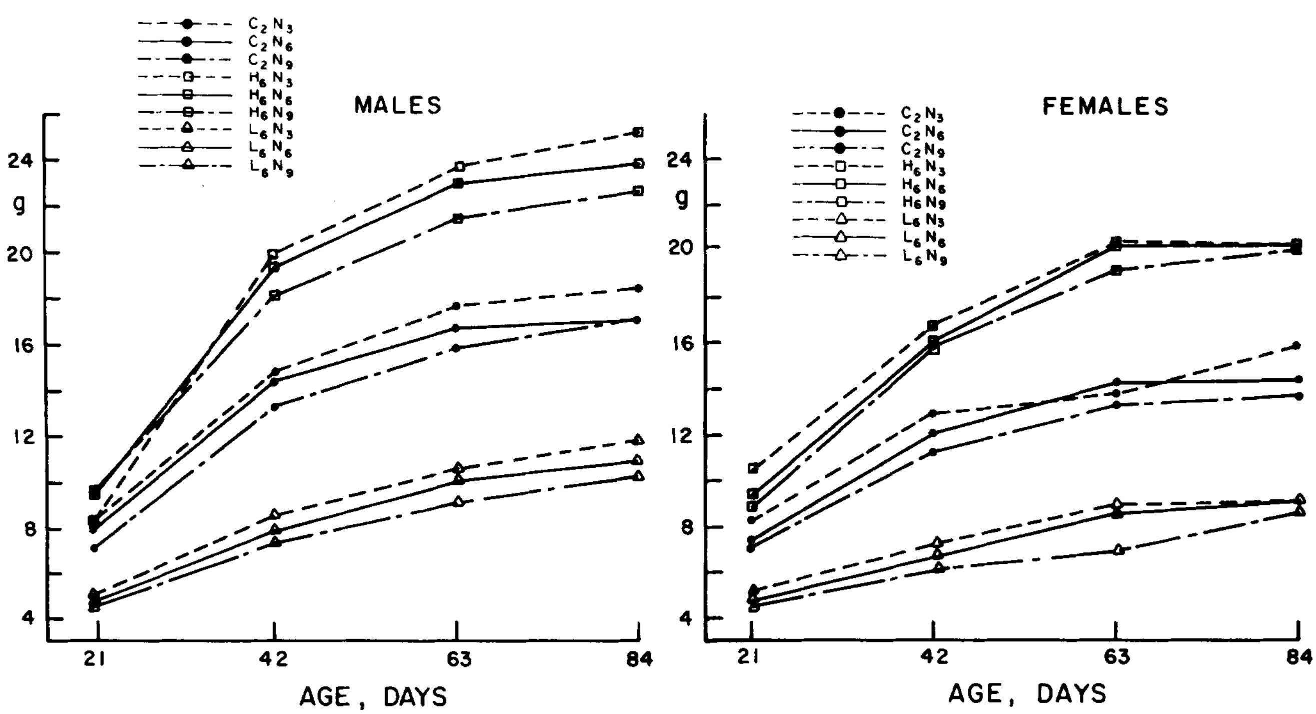

Fig. 3. Least squares means, plotted by line X sex X number-reared subclasses, for water weights (g) at 21, 42, 63 and 84 days

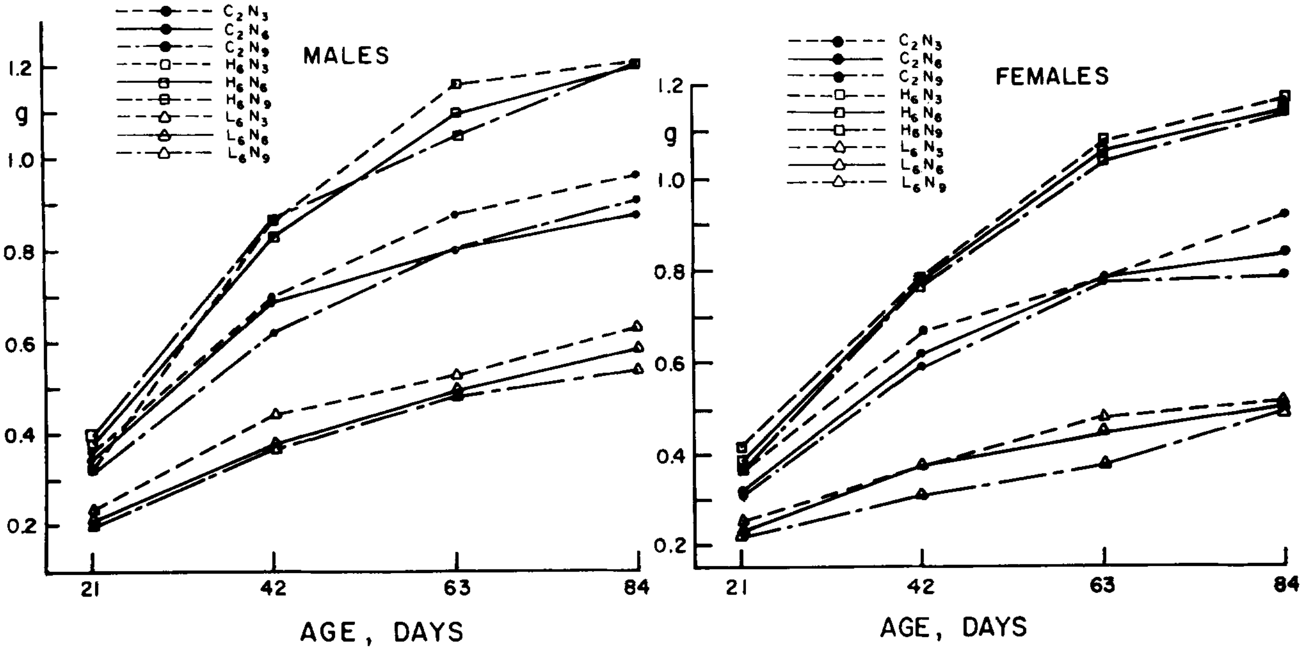

Fig. 4. Least squares means, plotted by line X sex X number-reared subclasses, for ash weights (g) at 21, 42, 63 and 84 days

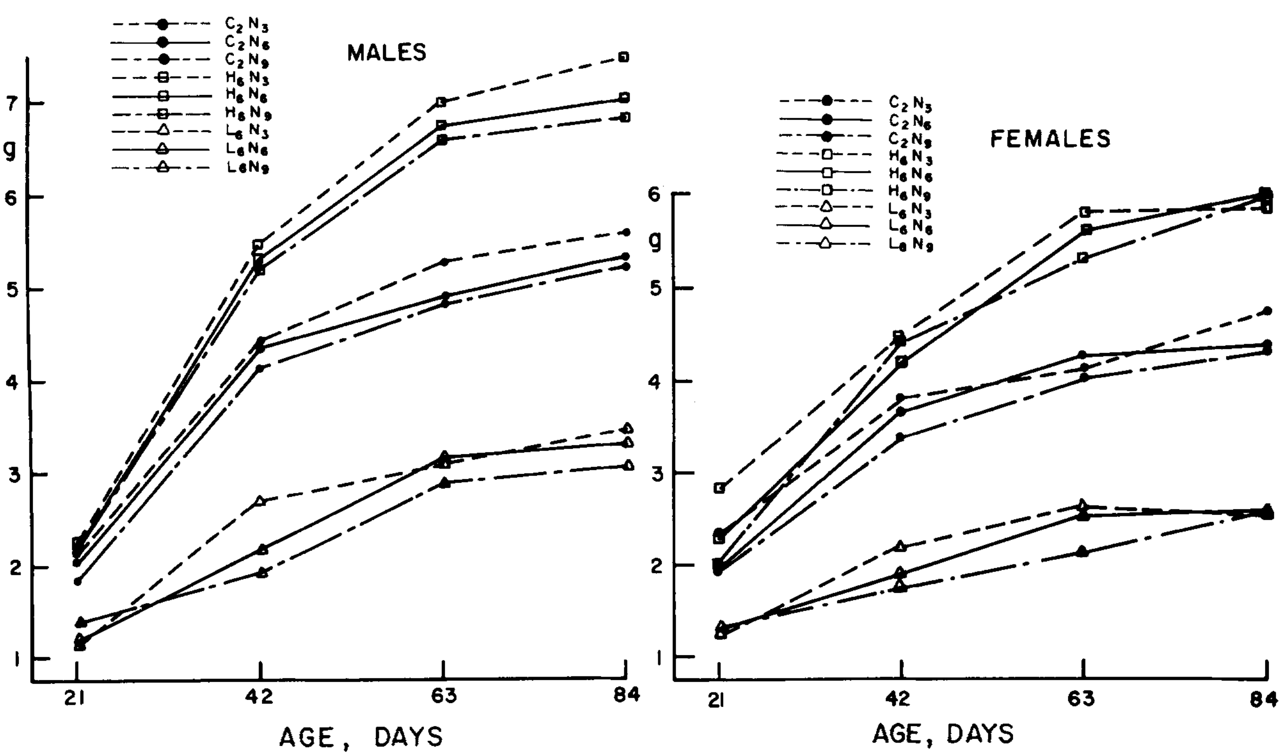

Fig. 5. Least squares means, plotted by line X sex X number-reared subclasses, for protein weights (g) at 21, 42, 63 and 84 days

response in L_6 than in H_6; this difference was absent at later ages. Mice showed a linear effect of postnatal litter size on ether extract weight at all ages except 21 days when a quadratic effect was evident. In general, individuals reared in larger litters had a reduced ether extract weight, although some exceptions were observed. At 21 days, the significant line by number-reared and line X sex interactions were not caused by changes in ranking of subclass means. The line X sex X number-reared interaction at 42 days was large and could be attributed to the

Table 4. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for body components at 21 days

Linear contrast		Ether extract	Water	Ash	Protein
$2C_2-H_6-L_6$	(1)	82.52 ± 8.10[b]	16.55 ± 5.05[b]	21.92 ± 5.64[b]	25.85 ± 5.85[b]
H_6-L_6	(2)	41.95 ± 4.66[b]	58.39 ± 2.92[b]	44.89 ± 3.27[b]	51.01 ± 3.39[b]
C_2-H_6		20.29 ± 4.66[b]	−20.92 ± 2.92[b]	−11.50 ± 3.26[b]	−12.58 ± 3.39[b]
C_2-L_6		62.24 ± 4.66[b]	37.47 ± 2.92[b]	33.41 ± 3.27[b]	38.43 ± 3.39[b]
Male-female	(3)	1.16 ± 3.81	− 1.76 ± 2.37	2.94 ± 2.64	
$2N_6-N_3-N_9$	(4)	−19.60 ± 8.10[b]	− 0.44 ± 5.06	1.37 ± 5.65	− 3.44 ± 5.85
N_3-N_9	(5)	47.67 ± 4.66[b]	8.48 ± 2.92[b]	7.06 ± 3.27[a]	9.19 ± 3.39[b]
N_6-N_3		−33.63 ± 4.66[b]	− 4.46 ± 2.92	− 2.84 ± 3.27	− 6.29 ± 3.39
N_6-N_9		14.04 ± 4.66[b]	4.02 ± 2.92	4.22 ± 3.25	2.85 ± 3.39
(1) × (3)		41.05 ± 16.15[b]			
(2) × (3)		−11.12 ± 9.37			
(1) × (4)		−66.20 ± 34.32[a]			
(2) × (4)		124.20 ± 19.75[b]			
(1) × (5)		7.36 ± 19.86			
(2) × (5)		−10.91 ± 11.44			

[a] $P < 0.05$
[b] $P < 0.01$

Table 5. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for body components at 42 days

Linear contrast		Ether extract	Water	Ash	Protein
$2C_2-H_6-L_6$	(1)	59.21 ± 7.64[b]	10.52 ± 3.63[b]	16.87 ± 3.78[b]	25.11 ± 4.00[b]
H_6-L_6	(2)	62.65 ± 4.45[b]	79.02 ± 2.10[b]	68.42 ± 2.19[b]	70.33 ± 2.33[b]
C_2-H_6		− 1.72 ± 4.45	−34.25 ± 2.10[b]	−25.85 ± 2.19[b]	−22.61 ± 2.30[b]
C_2-L_6		60.93 ± 4.40[b]	44.76 ± 2.09[b]	42.57 ± 2.18[b]	47.72 ± 2.30[b]
Male-female	(3)		15.59 ± 1.72[b]	8.67 ± 1.79[b]	16.69 ± 1.90[b]
$2N_6-N_3-N_9$	(4)	0.25 ± 7.74	0.95 ± 3.67	− 0.31 ± 3.82	− 1.44 ± 4.05
N_3-N_9	(5)	19.59 ± 4.40[b]	10.80 ± 2.07[b]	7.59 ± 2.15[b]	8.27 ± 2.30[b]
N_6-N_3		− 9.67 ± 4.50[a]	− 4.92 ± 2.13[a]	− 3.87 ± 2.22	− 4.86 ± 2.35[a]
N_6-N_9		9.92 ± 4.40[a]	5.88 ± 2.10[b]	3.56 ± 2.18	3.41 ± 2.30
(1) × (3)			1.48 ± 7.26		2.00 ± 7.99
(2) × (3)			12.46 ± 4.21[b]		15.88 ± 4.65[b]
(1) × (3) × (4)		176.16 ± 65.08[b]			
(1) × (3) × (5)		61.23 ± 37.45			
(2) × (3) × (4)		7.64 ± 38.46			
(2) × (3) × (5)		6.88 ± 21.26			

[a] $P < 0.05$
[b] $P < 0.01$

difference between C_2 mice and the average of selected mice being greater for N_6 mice (3.28 g) than for the average of N_3 and N_9 mice (1.32 g) in the case of males, whereas in the case of females the reverse was true (1.70 g vs 3.21 g). The line × number-reared interaction at 63 days was attributed to the difference between H_6 and L_6 mice being less for N_6 mice (1.16 g) than for the average of N_3 and N_9 mice (2.72 g).

A more consistent pattern was evident for water weight. At all ages C_2 mice contained significantly less water than H_6 mice and more than L_6 mice. After 21 days, N_3 mice contained more water than N_6 mice, and

Table 6. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for body components at 63 days

Linear contrast		Ether extract	Water	Ash	Protein
$2C_2-H_6-L_6$	(1)	11.22 ± 8.76	0.82 ± 3.00	7.47 ± 3.73[a]	4.26 ± 3.53
H_6-L_6	(2)	84.49 ± 5.34[b]	80.61 ± 1.83[b]	76.19 ± 2.24[b]	75.32 ± 2.15[b]
C_2-H_6		−36.65 ± 5.11[b]	−39.90 ± 1.83[b]	−34.36 ± 2.12[b]	−35.52 ± 2.04[b]
C_2-L_6		47.83 ± 5.15[b]	40.70 ± 1.77[b]	41.70 ± 2.12[b]	39.80 ± 2.11[b]
Male-female	(3)		16.02 ± 1.46[b]	6.47 ± 1.74[b]	19.33 ± 1.71[b]
$2N_6-N_3-N_9$	(4)	−12.56 ± 9.34	4.16 ± 3.20	− 1.62 ± 3.98	2.44 ± 3.75
N_3-N_9	(5)	10.68 ± 4.99[a]	9.90 ± 1.71[b]	8.09 ± 2.12[b]	8.07 ± 2.00[b]
N_6-N_3		−11.60 ± 5.38[a]	− 2.87 ± 1.84	− 4.85 ± 2.24[a]	− 2.83 ± 2.15
N_6-N_9		− 0.92 ± 5.23	7.03 ± 1.79[b]	3.24 ± 2.24	5.27 ± 2.11[a]
(1) × (3)					− 2.39 ± 7.04
(2) × (3)					12.22 ± 4.27[b]
(1) × (4)		52.60 ± 37.88			
(1) × (5)		−27.55 ± 21.02			
(2) × (4)		−59.90 ± 23.82[a]			
(2) × (5)		8.30 ± 12.29			

[a] $P < 0.05$
[b] $P < 0.01$

Table 7. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for body components at 84 days

Linear contrast		Ether extract	Water	Ash	Protein
$2C_2-H_6-L_6$	(1)	−10.85 ± 9.47	1.11 ± 2.88	4.85 ± 3.73	8.23 ± 3.58[a]
H_6-L_6	(2)	81.43 ± 5.79[b]	74.85 ± 1.77[b]	78.68 ± 2.24[b]	78.70 ± 2.19[b]
C_2-H_6		−46.14 ± 5.46[b]	−36.87 ± 1.65[b]	−36.85 ± 2.12[b]	−35.24 ± 2.06[b]
C_2-L_6		35.29 ± 5.61[b]	37.98 ± 1.72[b]	41.83 ± 2.24[b]	43.46 ± 2.13[b]
Male-female	(3)	22.29 ± 4.59[b]	17.81 ± 1.40[b]	8.59 ± 1.74[b]	20.25 ± 3.86[b]
$2N_6-N_3-N_9$	(4)	10.05 ± 10.23	− 2.07 ± 3.12	− 3.48 ± 3.98	− 1.05 ± 3.86
N_3-N_9		15.15 ± 5.35[b]	8.87 ± 1.62[b]	6.85 ± 2.12[b]	10.90 ± 2.02[b]
N_6-N_3		− 2.55 ± 5.79	− 5.47 ± 1.76[b]	− 5.10 ± 2.24[b]	− 3.33 ± 2.19
N_6-N_9		12.60 ± 5.75[a]	3.40 ± 1.76	1.61 ± 2.24	2.28 ± 2.17
(1) × (3)		− 5.13 ± 18.89	− 2.53 ± 5.76		− 1.82 ± 7.15
(2) × (3)		24.90 ± 11.61[a]	10.22 ± 3.54[b]		10.90 ± 2.17[b]
(1) × (3) × (4)		−93.94 ± 82.07			
(1) × (3) × (5)		−55.60 ± 45.26			
(2) × (3) × (4)		55.02 ± 54.61			
(2) × (3) × (5)		51.52 ± 26.25[a]			

[a] $P < 0.05$
[b] $P < 0.01$

N_6 mice more than N_9 mice, though not always significantly so. No quadratic effect of number-reared was found for water weight. At all ages H_6 mice had significantly more ash and L_6 significantly less than C_2 mice. N_3 mice had significantly more ash than N_9 mice at 21 and 42 days, and significantly more than N_9 and N_6 mice at 63 and 84 days. The decline in ash weight with increased postnatal litter size was linear at all ages.

The H_6 mice contained significantly more and L_6 mice significantly less protein than C_2 mice at all ages. The response was asymmetric at all ages except 63 days. N_3 mice had significantly more protein than N_9 mice at all ages. N_3 mice had significantly more protein than N_6 mice at 42 days, and at 63 days N_6 mice had significantly more protein than N_9 mice. Protein weight declined linearly with increased postnatal litter size.

Line by number-reared and line by number-reared by sex interaction contrasts were not significant for water, ash and protein weights at any of the ages studied.

Body Components Expressed as Percentages

Least squares estimates of the means for ether extract, water, ash and protein percentages at 21, 42, 63 and 84 days are plotted by line × sex × number-reared subclasses in Figures 6-9. Linear contrasts are shown in Tables 8-11. Ether extract percentage showed a significant negative correalated response in L_6 at 21, 42 and 63 days with no difference at 84 days. The H_6 line had lower fat percentages than C_2 at 21 and 42 days but no differences were found at later ages. N_3 mice were significantly higher in ether extract percentage than N_6 mice at 21 and 42 days, and N_6 mice were significantly higher than N_9 mice at 21 days. Postnatal litter size differences were negligible at later ages. The line by number reared interaction at 21 days did not involve a change in ranking of the number-reared classes from one line to the next. At 42 and 63 days, interaction effects for ether extract percentage were large in comparison with main effects. The line × sex × number-reared interaction contrast (1) × (3) × (4) of Table 9 at 42 days was due to the male difference between C_2 mice and the average of selected mice being greater for N_6 mice (13.10%) than for the average of N_3 and N_9 mice (3.87%), whereas in females the oppostite was found (4.51% for N_6 and 13.64% for the average of N_3 and N_9). The linear contrast (1) × (3) × (5) of Table 9 was due to the male difference between C_2 mice and the average of selected mice being greater for N_3 mice (4.77%) than for N_9 mice (–0.89%), while in females the differences were 5.78% and 7.86% for N_3 and N_9, respectively. The line × sex × number-reared interaction at 63 days could be attributed to the difference between C_2 mice and the average of selected mice being greater for N_3 mice (3.77%) than it was for N_9 mice (2.46%) for males, whereas for females the difference between C_2 mice and the average of selected mice was negative for N_3 mice (–4.21%) and positive for N_9 mice (1.76%) (contrast (1) × (3) × (5) of Table 10).

Line differences in water percentage were opposite in

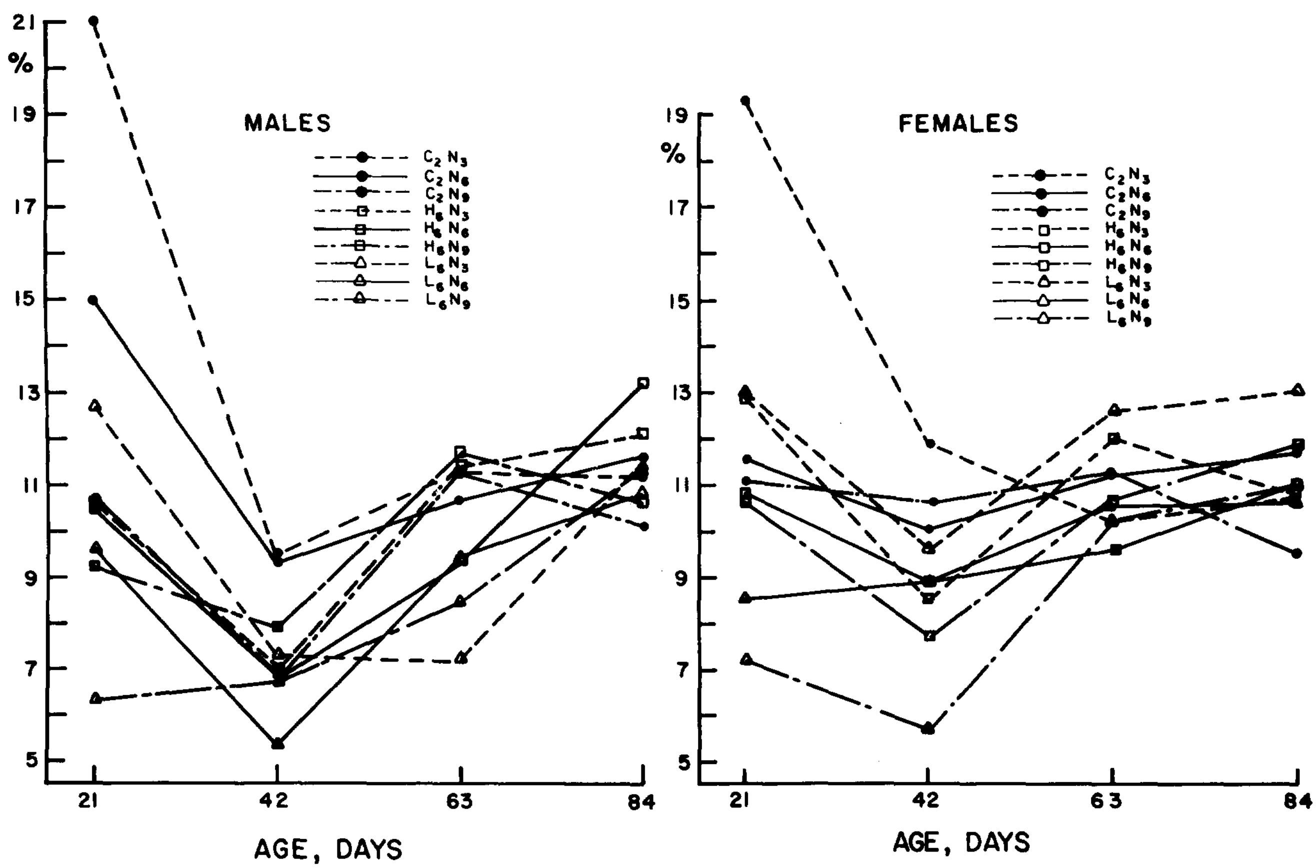

Fig. 6. Least squares means, plotted by line × sex × number-reared subclasses, for percentages (%) of ether extract at 21, 42, 63 and 84 days

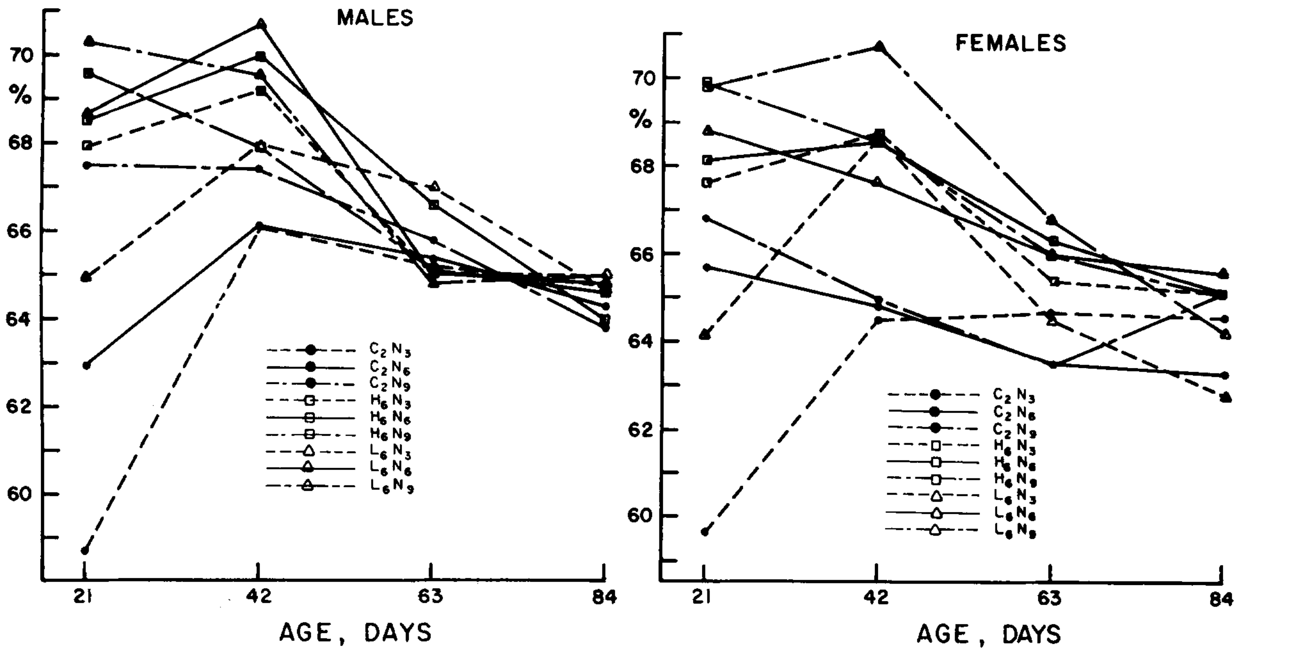

Fig. 7. Least squares means, plotted by line X sex X number-reared subclasses, for percentages (%) of water at 21, 42, 63 and 84 days

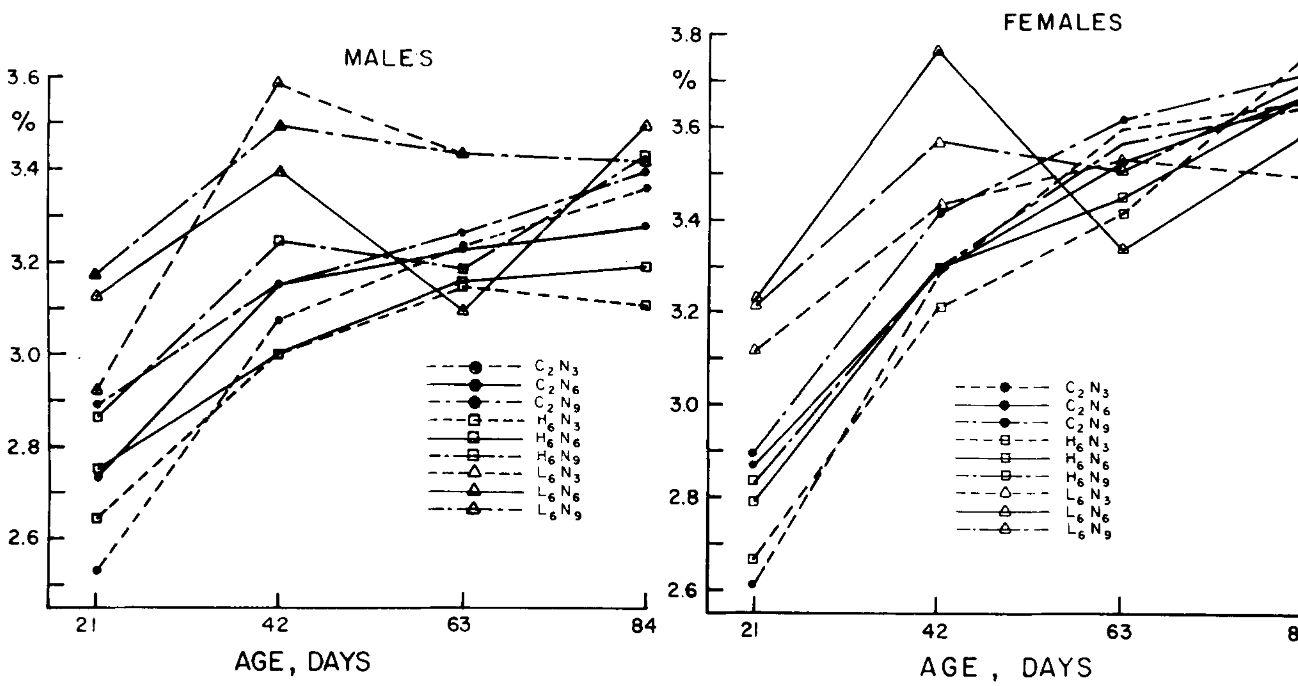

Fig. 8. Least squares means, plotted by line X sex X number-reared subclasses for percentages (%) of ash at 21, 42, 63 and 84 days

sign of ether extract percentage but were smaller as a percentage of the control line mean. Number-reared effects for water percentage were significant at 21-days only. The N_6 mice were significantly higher in water percentage than N_3 mice, and N_6 mice were lower than N_9 mice. The line × number-reared interactions at 21 and 42 days were large and involved lines ranking differently for mice from the different litter sizes. The line × number-reared interaction at 21 days could be attributed to (a) the difference in water percent between C_2 mice and the average of selected mice, being −13.87 g for N_3 mice and −5.37 g for N_9 mice (contrast (1) × (5) of Table 8) and (b) the difference between H_6 and L_6 mice, being positive for N_3 mice (3.29 g) and negative for N_9 mice (−0.30 g)

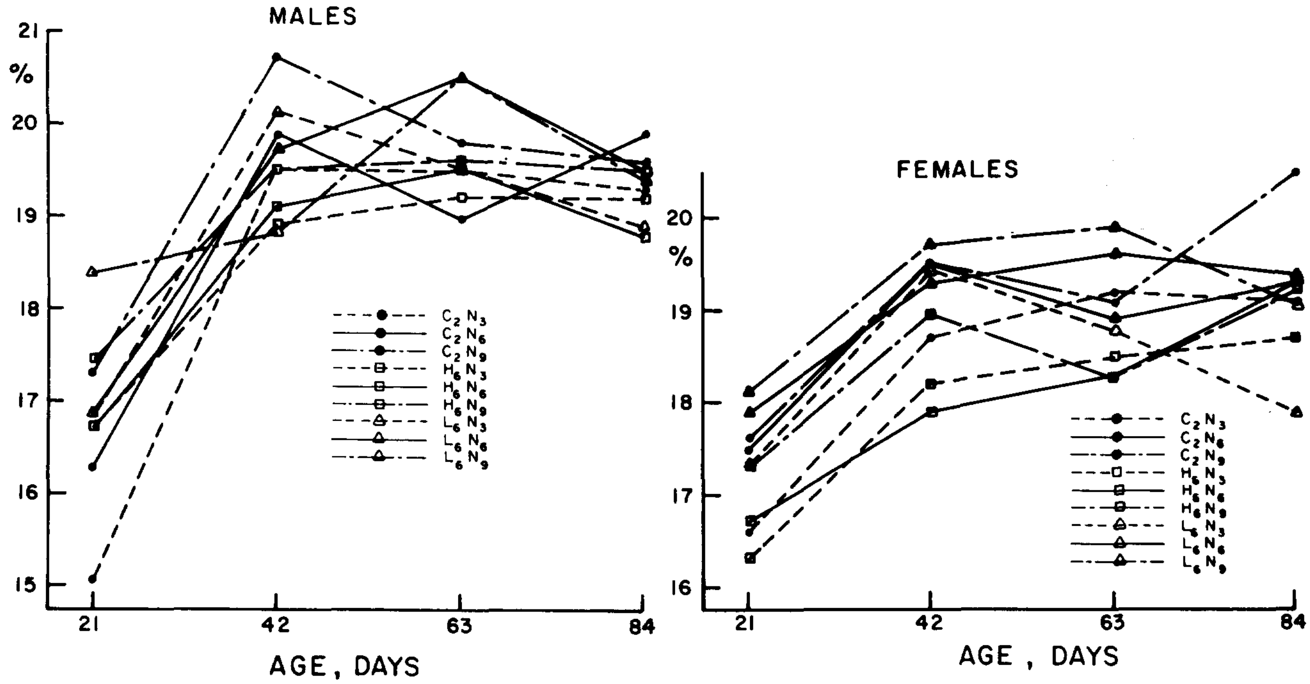

Fig. 9. Least squares means, plotted by line X sex X number-reared subclasses, for percentatges (%) of protein at 21, 42, 63 and 84 days

Table 8. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for percentages (%) of body components at 21 days

Linear contrasts		Ether extract %	Water %	Ash %	Protein %
$2C_2-H_6-L_6$	(1)	62.43 ± 5.86[b]	−14.53 ± 1.47[b]	−13.39 ± 3.16[b]	− 5.97 ± 3.19
H_6-L_6	(2)	8.33 ± 3.40[a]	1.31 ± 0.85	−13.54 ± 1.84[b]	− 4.40 ± 1.85[b]
C_2-H_6		27.04 ± 3.39[b]	− 7.92 ± 0.85[b]	0.08 ± 1.95	− 0.79 ± 1.84
C_2-L_6		35.38 ± 3.39[b]	− 6.61 ± 0.85[b]	−13.47 ± 1.95[b]	− 5.18 ± 1.84[b]
Male-female	(3)	0.45 ± 2.77			
$2N_6-N_3-N_9$	(4)	−14.02 ± 5.89[a]	2.32 ± 1.48	− 3.84 ± 3.17	− 1.09 ± 3.20
N_3-N_9	(5)	38.87 ± 3.39[b]	− 8.12 ± 0.85[b]	− 8.34 ± 1.83[b]	− 7.25 ± 1.84[b]
N_6-N_3		−26.45 ± 3.40[b]	5.22 ± 0.85[b]	6.09 ± 1.84[b]	3.08 ± 1.85
N_6-N_9		12.42 ± 3.39[b]	− 2.90 ± 0.85[b]	− 2.26 ± 1.83	− 4.17 ± 1.84[a]
(1) X (3)		30.56 ± 11.73[b]			
(2) X (3)		− 8.40 ± 6.80			
(1) X (4)		−48.73 ± 24.94	3.63 ± 6.26		
(1) X (5)		72.25 ± 14.35[b]	−13.39 ± 3.61[b]		
(2) X (4)		7.67 ± 14.42	− 6.23 ± 3.62		
(2) X (5)		−28.52 ± 8.32[b]	5.66 ± 2.09[b]		

[a] $P < 0.05$
[b] $P < 0.01$

(contrast (1) × (5) of Table 8). At 42 days the line × number-reared interaction could be attributed to the difference between H_6 and L_6 mice being positive for N_3 mice (0.60 g) and negative for N_9 mice (−1.96 g) (contrast (2) × (5) of Table 9). At 63 days, the line × sex × number-reared interaction was very large when compared with main effects and could be attributed to the difference between C_2 mice and the average of selected mice, being negative for N_3 mice (−1.53 g) and positive for N_9 mice (1.51 g) in the case of males, whereas for females the corresponding values were −0.45 g and −5.59 g (contrast (1) × (3) × (5) of Table 9).

The C_2 mice were significantly lower in ash percent than L_6 mice at 21 and 42 days but did not differ from H_6 mice. At 21 days, N_3 mice had a significantly lower ash percentage than N_9 and N_6 mice. At 63 days, N_6 mice had a significantly lower ash percentage than N_9 mice. Females were higher in ash percent than males at all ages.

H_6 mice were significantly lower in protein percentage than C_2 mice at 42 and 84 days. L_6 mice were significantly higher than C_2 at 21 and 63 days, but lower at 84 days. H_6 mice were significantly lower than L_6 mice at all ages except 84 days. At 21 days, N_9 mice were significantly higher in protein percent than N_6 mice, and N_6 mice were significantly higher than N_3 mice. At 84 days, N_9 mice were significantly higher than N_3 mice.

Discussion

The positive correlated responses in body weight at all ages due to selection for high and low body weight are typical of most selection studies with mice; for reviews see Roberts (1965) and Eisen (1974). The asymmetrical response in body weight at 21 and 42 days of age is also a common finding in selection studies and was observed in earlier generations of the present selection lines (Legates 1969). The higher and lower body weights of mice reared in litters of size three and nine, respectively, as compared with mice reared in litters of size six is in complete agreement with the studies in rats of Widdowson and McCance (1960, 1963) and Widdowson and Kennedy (1962). However, these other studies were limited to rats from a single genetic line. The present study suggests that the magnitude of the effect on body weight of rearing mice in a given litter size may depend on the genetic line in question, albeit only to a small extent. However, differences in body weight due to number-reared treatments, though they persisted with age, were small in comparison with differences due to genetic line. Similar findings were reported in comparing a polygenic obese and control strain of mice (Eisen and Leatherwood 1978).

The positive correlated responses observed for weights of body components at all ages, except for ether extract of H_6 mice at 21 days, reflect to a large extent the pattern of body weight differences among the lines. Similar findings have been reported in mice by Fowler (1958), Timon et al. (1970), Biondini et al. (1968) and Hayes and McCarthy (1976), in rats by Baker and Chapman (1975) and in *Tribolium castaneum* by Medrano and Gall (1976). Water and protein were the components that contributed most to the differences in body weight among the lines,

Table 9. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for percentages (%) of body components at 42 days

Linear contrast		Ether extract %	Water %	Ash %	Protein %
$2C_2-H_6-L_6$	(1)	45.18 ± 5.98[b]	−10.39 ± 1.13[b]	− 8.00 ± 2.98[b]	4.93 ± 2.25[a]
H_6-L_6	(2)	5.53 ± 3.44	− 0.59 ± 0.65	−11.32 ± 1.71[b]	− 3.92 ± 1.31[b]
C_2-H_6		19.82 ± 3.45[b]	− 4.90 ± 0.65[b]	1.67 ± 1.71	4.42 ± 1.30[b]
C_2-L_6		25.35 ± 3.45[b]	− 5.49 ± 0.65[b]	− 9.64 ± 1.71[b]	0.50 ± 1.30
Male-female	(3)	−17.24 ± 2.82[b]	1.29 ± 0.53[a]	− 5.17 ± 1.40[b]	2.92 ± 1.30[a]
$2N_6-N_3-N_9$	(4)	− 0.73 ± 6.04			
N_3-N_9	(5)	14.01 ± 3.41[b]			
N_6-N_3		− 7.37 ± 3.50[a]			
N_6-N_9		6.64 ± 3.45			
(1) × (4)		0.29 ± 25.38	− 3.52 ± 4.79		
(1) × (5)		18.53 ± 14.61	− 1.66 ± 2.76		
(2) × (4)		6.10 ± 15.00	2.61 ± 2.83		
(2) × (5)		−24.12 ± 8.30[b]	3.91 ± 1.56[a]		
(3) × (4)			5.03 ± 2.29[a]		
(3) × (5)			0.38 ± 1.29		
(1) × (3) × (4)		189.28 ± 50.76[b]			
(1) × (3) × (5)		79.79 ± 29.22[b]			
(2) × (3) × (4)		35.18 ± 29.98			
(2) × (3) × (5)		14.99 ± 16.59			

[a] $P < 0.05$
[b] $P < 0.01$

though when expressed as a percentage of C_2 line means for each component all components contributed more equally.

Again, the differences in weights of body components at all ages among mice reared in the three litter sizes reflect body weight differences to a large extent, i.e., mice reared in small litter sizes had heavier weights of all body components. Widdowson and McCance (1960) observed higher fat weights for rats reared in small litter sizes. In the present study, water was the component that contributed most to the body weight differences among the mice reared in the different litter sizes, with ether extract being the next most important component. When expressed as a percentage of the control line mean for each trait, ether extract was the component that was changed most by rearing mice in the large and small litter sizes.

In the case of ether extract weight, the interactions involving lines and number-reared treatments were a notable feature of the results, particularly after 21 days. Differences in ether extract due to rearing in different litter sizes depended on the particular line. Despite the complications of interactions, particularly for ether extract, it is clear that genetic lines were more important than number-reared treatments in causing differences in weights of body components. Differences in weights of body components due to rearing in different litter sizes did not disappear with age, however. Eisen and Leatherwood (1978) reported that genetic line and postnatal litter size effects were of equal magnitude at four weeks but by six weeks differerences between genetic lines were larger than between litter size reared for fat, lean, and ash weights. The effect of the postnatal maternal environment persisted until 30 weeks of age. In contrast to the present study, no line by postnatal litter size interactions were obtained for fat weight.

Comparisons among the lines for percentages of body components depended to a great extent on the age at which the comparisons were made, regardless of the number-reared. This is typical of most studies with mice in which percentages of body components have been recorded at more than one age, for example, Fowler (1958), Biondini et al. (1969), Lang and Legates (1969), Hayes and McCarthy (1976) and Eisen et al. (1977), and emphasizes the need to assess body composition at many ages. For ether extract and water percentage, differences among mice reared in the different litter sizes depended to a large extent on the particular line considered in the present data. At 21 days, however, ether extract percentage was highest in mice reared in litters of size three, and intermediate in mice reared in litters of size six in all lines.

Table 10. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for percentages (%) of body components at 63 days

Linear contrast		Ether extract %	Water %	Ash %	Protein %
$2C_2-H_6-L_6$	(1)	13.25 ± 65.07	− 3.15 ± 1.19[b]		−1.16 ± 2.09
H_6-L_6	(2)	9.50 ± 4.16[a]	0.06 ± 0.73		−4.63 ± 1.27[b]
C_2-H_6		1.87 ± 3.98	− 1.61 ± 0.70[b]		1.73 ± 1.22
C_2-L_6		11.37 ± 4.02[b]	− 1.54 ± 0.70[a]		−2.89 ± 1.23[a]
Male-female	(3)	− 7.38 ± 3.31[a]		−7.83 ± 1.20[b]	3.77 ± 1.01[b]
$2N_3-N_6-N_9$	(4)			−6.69 ± 2.64[a]	
N_3-N_9	(5)			−1.11 ± 1.41	
N_6-N_3				−2.79 ± 1.52	
N_6-N_9				−3.90 ± 1.47[b]	
(1) × (3)		27.94 ± 13.69[a]	5.43 ± 2.39[a]		
(2) × (3)		25.50 ± 8.32[b]	− 0.26 ± 1.45		
(1) × (4)		28.11 ± 29.61			
(1) × (5)		−21.11 ± 16.42			
(2) × (4)		−43.60 ± 18.61[b]			
(2) × (5)		5.09 ± 9.60			
(1) × (3) × (4)		−69.37 ± 59.21	4.53 ± 10.34		
(1) × (3) × (5)		66.09 ± 32.81[a]	−12.57 ± 5.73[a]		
(2) × (3) × (4)		−53.54 ± 37.22	6.79 ± 6.50		
(2) × (3) × (5)		19.91 ± 19.20	− 5.83 ± 3.35		

[a] $P < 0.05$
[b] $P < 0.01$

Table 11. Linear contrasts and their standard errors (as a percentage of the control mean for each trait) among treatment means for percentages (%) of body components at 84 days

Linear contrast		Ash %	Protein %
$2C_2-H_6-L_6$	(1)		6.04 ± 2.23^b
H_6-L_6	(2)		0.89 ± 1.38
C_2-H_6			2.57 ± 1.29^a
C_2-L_6			3.47 ± 1.33^b
Male-female	(3)	-9.17 ± 1.14^b	
$2N_6-N_3-N_9$	(4)		1.04 ± 2.41
N_3-N_9	(5)		-3.67 ± 1.26^b
N_6-N_3			2.36 ± 1.37
N_6-N_9			-1.31 ± 1.36
(1) × (3)		-1.68 ± 4.61	
(2) × (3)		-8.60 ± 2.84^b	
(1) × (3) × (4)		-43.89 ± 20.06^a	
(1) × (3) × (5)		-2.42 ± 11.07	
(2) × (3) × (4)		-18.50 ± 12.89	
(2) × (3) × (5)		-6.89 ± 6.40	

[a] $P < 0.05$
[b] $P < 0.01$

This result agrees with that of Widdowson and McCance (1960) for rats at weaning. A notable feature of the present data after weaning was the inconsistency among lines in the ranking of the number-reared classes for ether extract percentage. Widdowson and McCance (1960) found the same ranking at all ages for the number-reared subclasses, but only a single genetic line was involved. Studies of cell number and cell size in fat depots of mice reared in small and large litter sizes have usually indicated increased cell size and number, or both, in mice reared in the smaller litter sizes (Knittle and Hirsch 1968). Some studies, however, have indicated much variation from one depot to another in cellularity characteristics, and the results of one study indicated that the effects of high and low preweaning nutritional levels were different in obese mutant rats than in normal rats (Johnson et al. 1973). In contrast, both polygenic obese and non-obese mice had more and larger fat cells when reared in smaller size litters (Eisen and Leatherwood 1978).

The relative differences in percentage ether extract among the H_6, C_2 and L_6 lines were generally small and diminished to a negligible amount at later ages, as did the differences in percentage ether extract among the three postnatal litter size groups. The lack of any sizeable correlated response in percentage body fat in the H_6 line agrees with the data of Lang and Legates (1969) and Eisen et al. (1977). The observed interaction of line and postnatal litter size for ether extract may be the result of the small influence of selection on this trait which may make it more susceptible to postnatal environmental maternal influences. Eisen and Leatherwood (1978), comparing an obese and non-obese line, found that mice reared in litters of eight had consistently higher fat percentage than in litters of 14 from 6 to 30 weeks of age. The magnitude of the difference was larger for the obese line and no interaction was detected. Another marked contrast to the present study was the delayed manifestation of the genetic line and postnatal environmental maternal effect on fat percentage, being negligible at four weeks and increasing subsequently. Postnatal environmental maternal effects contributed little to differences in lean and ash percentages.

General conclusions based on the present study and that of Eisen and Leatherwood (1978) are as follows. The weight of protein, fat, ash and water are permanently affected by the postnatal maternal environment, regardless of whether selection has been for large or small body size and regardless of whether there has or has not been a correlated response in percentage body fat. However, the magnitude of line differences tend to be larger when mice are reared in small litters. On a percentage basis, the results are somewhat more variable and depend to a great extent on the variation in percentage body fat among lines. If the differences among lines in percentage body fat are small, then it is difficult to predict precisely what will happen other than to say that postnatal environmental maternal effects are likely to be large at weaning and diminish markedly with maturity. In contrast, lines selected for body weight and showing a large correlated response in percentage fat continue to exhibit clear differences between postnatal litter size groups in percentage body fat to at least 30 weeks of age. The effects of postnatal litter size on the percentages of protein and ash are relatively small and likely are influenced primarily by variation in percentage fat.

Literature

Baker, R.L.; Chapman, A.B.: Correlated responses to selection for postweaning gain in the rat. Genetics **80**, 191-203 (1975)

Biondini, P.E.; Sutherland, T.M.; Haverland, L.H.: Body composition of mice selected for rapid growth rate. J. Anim. Sci. **27**, 5-12 (1968)

Eisen, E.J.: Maternal effects on litter size in mice. Can. J. Genet. Cytol. **12**, 209-216 (1970)

Eisen, E.J.: The laboratory mouse as a mammalian model for the genetics of growth. Proceedings First World Congress on Genetics Applied to Livestock Production. 1, 467-492 (1974)

Eisen, E.J.; Leatherwood, J.M.: Effects of early pregnancy on growth, body composition and efficiency in mice. J. Anim. Sci. **42**, 52-62 (1976)

Eisen, E.J.; Leatherwood, J.M.: Adipose cellularity and body composition in polygenic obese mice as influenced by preweaning nutrition. J. Nutr. **108**, 1652-1662 (1978)

Eisen, E.J.; Bakker, H; Nagai, J.: Body composition and energetic

efficiency in two lines of mice selected for rapid growth rate and their F_1 crosses. Theor. Appl. Genet. **49**, 21-34 (1977)
Falconer, D.S.: Maternal effects and selection response. In: Genetics Today (ed. Geerts, S.J.). Proc. of XI. International Congress of Genetics. New York: Pergamon Press 1963
Fowler, R.E.: The growth and carcass composition of strains of mice selected for large and small body size. J. Agric. Sci. **51**, 137-148 (1958)
Harvey, W.R.: Least squares analysis of data. United States Department of Agriculture Publication ARS-20-80 (1960)
Hayes, J.F.; McCarthy, J.C.: The effects of selection at different ages for high and low body weight on the pattern of fat deposition in mice. Genet. Res. **27**, 389-403 (1976)
Johnson, P.R.; Stern, J.S.; Greenwood, M.R.C.; Zucker, L.M.; Hirsch, J.: Effect of early nutrition on adipose cellularity and pancreatic insulin release in the Zucker rat. J. Nutr. **103**, 738-743 (1973)
Knittle, J.L.; Hirsch, J.: Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism. J. Clin. Invest. **47**, 2091-2098 (1968)
Lang, B.J.; Legates, J.E.: Rate, composition and efficiency of growth in body weight. Theor. Appl. Genet. **39**, 306-314 (1969)
Legates, J.E.: Direct and correlated responses to selection in mice. In: Genetics Lectures (ed. Bogart, R.), vol. 1, pp. 149-165. Corvallis, Oregon: Oregon State University Press 1969
Medrano, J.F.; Gall, G.A.E.: Growth rate body composition, cellular growth and enzyme activities in lines of *Tribolium castaneum* selected for 21-day pupa weight. Genetics **83**, 379-391 (1976)
Roberts, R.C.: Some contributions of the laboratory mouse to animal breeding research. Anim. Breed. Abstr. **33**, 339-353 (1965)
Timon, V.M.; Eisen, E.J.; Leatherwood, J.M.: Comparisons of ad libitum and restricted feeding of mice selected and unselected for postweaning gain. II. Carcass composition and energetic efficiency. Genetics **65**, 145-155 (1970)
Widdowson, E.M.; Kennedy, G.C.: Rate of growth, mature weight and lifespan. Proc. Roy. Soc. (Lond.) **B156**, 96-108 (1962)
Widdowson, E.M.; McCance, R.A.: Some effects of accelerating growth. 1. General somatic development. Proc. Roy. Soc. (Lond.) **B152**, 188-206 (1960)
Widdowson, E.M.; McCance, R.A.: The effect of finite periods of undernutrition at different ages on the composition and subsequent development of the rat. Proc. Roy. Soc. (Lond.) **B158**, 329-342 (1963)

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