ORGAN WEIGHTS AND BODY COMPOSITION OF PARTURIENT AND LACTATING MICE, AND THEIR YOUNG, AT 21° C AND –3° C

S. A. BARNETT AND E. M. WIDDOWSON

*Zoology Department, Glasgow University, and
Dunn Nutritional Laboratory, Cambridge*

(Received 13th August 1970)

Summary. Four classes of A2G/Tb mice were studied: (i) controls kept in an environmental temperature of 21° C; (ii) mice transferred at mating from 21 to –3° C; (iii) mice of the first generation reared at –3° C; (iv) mice of the twenty-fifth to the twenty-ninth generations of a stock kept permanently at –3° C. Of each class, some were killed, with their litters, at either first or second parturition, and some on the 10th day of either the first or second lactation. Litters kept for 10 days were reduced to four young at birth.

The females at their first parturition were compared with virgins of a previous study. Pregnancy was accompanied by increase in the relative weight of heart, liver and stomach, and in the length of the small intestine; but the small intestine lost weight, and there was no change in kidney weight. The percentage of collagen and calcium in the body declined, but the absolute amount of nitrogen in the body increased.

During lactation, the liver and stomach, but not the heart, increased in relative weight still further; and the weight of the small intestine also rose. The percentage of fat and calcium in the body declined.

The relative weights of the heart, kidneys and small intestine were higher in the cold environment than at 21° C, but liver weight was not affected. The proportion of fat, nitrogen and collagen in the body was lower in the cold.

The young had a much higher proportion of fat in their bodies at 10 days after birth than the neonates. They also contained more collagen and calcium and less water per unit body weight. The main effect of cold on the young was a higher fat content at birth.

INTRODUCTION

Pregnancy and lactation are especially exacting tests of cold-adaptation since, of all normal conditions, they require the highest rates of metabolic turnover. To maintain these rates, the functional capacity of certain organs must be increased. Accordingly, we have studied the effects of three kinds of exposure to a cold environment on the organ weights and body composition of parturient

and lactating female mice and their young. Our findings illustrate the responses of a number of tissues during physiological adaptation to a double strain on bodily resources.

METHODS

The mice and their environment

The mice were of the highly inbred strain, A2G/Tb, originally acquired as A2G from Glaxo Laboratories. Controls were kept in a temperature-regulated room at about 21° C, and were of the thirty-seventh to the forty-fourth generations bred in Glasgow. The room used for experimental groups was kept at -3° C, with a differential of less than 1° C above and below this temperature. Every 6 hr, the temperature rose briefly to just below 0° C during automatic defrosting. There were also brief rises each morning when work was going on. Once a week, when cages were cleaned, there was a more prolonged rise, sometimes above 0° C. In both rooms, there was a 12-hr light-dark cycle. There was no control of humidity.

There were three classes of experimental mice. (i) The transferred pairs were the young of controls; they were reared at 21° C, and transferred on mating to -3° C. (ii) The new stock were the offspring of transferred mice, that is, they were of the first generation reared in the cold. (iii) The old stock were from a colony which had been bred continuously at -3° C for twenty-five to twenty-nine generations. All pairs were litter mates; the litters were separated into groups of like sex at the age of 5 weeks; pairs were mated at 10 weeks and thereafter remained together throughout the experiment. The controls, reared at 21° C, constituted a fourth class. Each class was divided into four subclasses: females killed (i) at the birth of, or (ii) 10 days after the birth of their first litters, or (iii) at the birth of, or (iv) 10 days after the birth of their second litters. 'At birth' signifies litters found newly born on inspection of mated pairs at about 10.00 hours. 'Ten days' signifies 10 days after discovery of a new litter. Litters killed at birth were weighed and used as a whole for analysis. Litters to be kept to 10 days were reduced to four young at birth, so that conditions in the nest should be as uniform as possible. A pair whose second litter was to be used was allowed to rear the first litter to the age of 3 weeks. This sometimes required the female to provide milk for one litter during the gestation of a second.

The mice were kept in standard metal cages of the 'shoe box' type, 35 × 10 × 10 cm high, with wire lids. The floor of each cage was covered with sawdust, and there was also cotton wool bedding. Conditions in such cages, in both environmental temperatures, have been described by Barnett (1956). There was never any sign of cold injury.

All mice had Diet 41 (Bruce & Parkes, 1949) supplied in excess. At 21° C, they had water at all times but, at -3° C, they had ice blocks which they licked; mice given water in this way have the same breeding performance as those which receive it in open jars in which it is allowed to freeze (Stoddart, 1966).

Techniques

The mice were killed with ether and weighed to the nearest 0.1 g. Each

female was skinned and the skin, with hair, was weighed after removal of adherent fat and muscle. The liver, kidneys, spleen and heart were removed and weighed. The gastrointestinal tract from below the diaphragm was removed. The stomach was emptied and weighed. The small intestine was removed, laid out on a large sheet of paper and measured with a millimetre rule; it was then emptied and weighed. The large intestine, including the caecum, was also emptied and weighed. The uterus was removed and weighed.

The organs were returned to the rest of the skinned body, and the whole was dried at 95° C to a constant weight. To remove the fat, the dried body was covered with light petroleum (b.p. 40 to 60° C) and left to stand overnight at room temperature. The petroleum was decanted, and the process repeated until the extract was colourless. Fat was taken as the difference between dry weight and fat-free dry weight. The dry fat-free residue was ground in a Culatti hammer mill and the powder sampled and analysed for total nitrogen, collagen, calcium and phosphorus (Dickerson, 1962). The skin was weighed and dried to a constant weight. Subsequent treatment of the skin was the same as that for the carcass.

The young were killed with ether. Their stomachs were emptied, and the whole litter was dried at 95° C to constant weight. Fat was removed as described above, and the residue analysed for nitrogen, collagen and calcium.

Statistical analysis

Statistical analyses were carried out by a KDF9 computer. Means and standard errors of organ weights were calculated, both for absolute values and also for the values relative to 100 g body weight. Relative organ weights are based on 'empty' body weight, that is, total weight minus weight of gut contents. Student's *t* test (two-tailed) was used for comparisons between means. Wherever a statement is made about the significance of differences between means of organ weights, it is based on relative weights. To check that these are valid, and not merely a result of differences in body weight between classes, regressions of organ weights on body weight were calculated within classes; hardly any of these was statistically significant and so covariance analysis was not required (Angervall & Carlström, 1963).

RESULTS

General

An overview of our findings is given in Tables 1, 19 and 20. For some organs and substances, there are comparable figures from previous work on virgin females aged 16 weeks (Barnett & Widdowson, 1965); Table 1 summarizes the differences between the virgins and the females of the present work at the end of their first pregnancy when they were about the same age as the virgins. Since no virgins were studied immediately after transfer to the cold environment, only three classes can be compared. The original findings from the previous study were no longer available, and so no statistical analysis of the differences is possible; we therefore give only percentage differences.

All tables, except Tables 1, 19 and 20, are arranged on the 'diallele' system.

TABLE 1
COMPARISON OF PRIMIPAROUS MICE WITH VIRGIN FEMALES

	21° C	-3° C	
		Generation 1 (new stock)	Generations 25 to 29 (old stock)
Body weight	+11	+5	+13
Heart weight	+121	+114	+65
Liver weight	+90	+49	+59
Kidney weight	-1	+1	-2
Stomach weight	+25	+6	+19
Small intestine: weight	-17	-30	-14
length	+20	+16	+15
Spleen weight	+8	+7	+55
Body fat	-4	+16	0
Water (carcass)	+4	+2	+2
(skin)	0	-7	+3
Nitrogen (carcass)	+2	0	+2
(skin)	+10	+23	+6
Collagen (carcass)	-22	-13	-24
(skin)	-31	-40	-45
Calcium (carcass)	-35	-35	-38

The figures represent percentage change in relative weights, resulting from pregnancy.

Reading across gives the effect of reproductive state; reading down shows the effects of different kinds of cold exposure.

Numbers of mice and mean body weights are given in Table 2. The mice at 21° C were consistently heavier than those of any of the classes at -3° C, but the differences between them and the mice of long ancestry in the cold were small. The mice of the first generation reared in the cold were generally the lightest. This conforms with earlier observations (reviewed by Barnett, 1965).

TABLE 2
NUMBERS OF FEMALE MICE AND THEIR BODY WEIGHTS

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	No. of mice B.wt (g)	13 27.2 ± 1.1	14 27.8 ± 1.1	10 28.4 ± 0.2	11 30.6 ± 1.0
21 → -3° C	No. of mice B.wt (g)	13 24.6 ± 0.5	16 27.2 ± 0.5	10 26.9 ± 1.1	10 23.7 ± 1.7
-3° C, Generation 1	No. of mice B.wt (g)	9 22.9 ± 0.5	6 23.9 ± 2.0	11 25.5 ± 0.9	12 24.2 ± 1.9
-3° C, Generations 25 to 29	No. of mice B.wt (g)	10 26.2 ± 0.8	9 26.6 ± 0.8	10 27.9 ± 0.7	9 29.3 ± 0.9

Values expressed as means ± S.E.

TABLE 3
HEART WEIGHTS OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg g/100 g b.wt	278 ± 27 1.07 ± 0.03	276 ± 23 0.99 ± 0.03	313 ± 37 1.07 ± 0.04	293 ± 15 0.97 ± 0.04
21 → -3° C	mg g/100 g b.wt	322 ± 29 1.28 ± 0.06	329 ± 23 1.23 ± 0.04	342 ± 39 1.22 ± 0.05	352 ± 45 1.50 ± 0.07
-3° C, Generation 1	mg g/100 g b.wt	311 ± 40 1.34 ± 0.04	317 ± 65 1.38 ± 0.14	322 ± 35 1.30 ± 0.05	332 ± 31 1.38 ± 0.07
-3° C, Generations 25 to 29	mg g/100 g b.wt	302 ± 35 1.15 ± 0.05	333 ± 45 1.26 ± 0.09	356 ± 42 1.24 ± 0.04	382 ± 57 1.32 ± 0.12

Values expressed as means ± S.E. Numbers of mice as in Table 2.

Weights of organs

The relative heart weight of primiparous females was about twice that of corresponding virgins. There was little evidence of further enlargement during lactation or during the second reproductive cycle (Table 3). The hearts of mice of all groups in the cold were larger, absolutely and relatively, than those of the corresponding control groups.

The relative weight of the liver of primiparous mice at 21° C averaged nearly twice that of the control virgins; this accords with the observations of Peters, Krijnen & Boyd (1967) on rats. At -3° C the difference was less but still marked. There was evidence of a further rise during first lactation; this too confirms previous work on rats (Kennedy, Pearce & Parrott, 1958; Peters & Krijnen, 1966). But during second pregnancy and lactation relative liver weight underwent no further increase, and even tended to decline, especially at 21° C (Table 4). The livers of the mice at -3° C were not consistently larger than those of the corresponding controls. Hence the enlargement resulting from pregnancy was not enhanced in the cold environment.

TABLE 4
LIVER WEIGHTS OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	g g/100 g b.wt	2.37 ± 0.24 8.49 ± 0.21	2.71 ± 0.23 9.63 ± 1.07	2.74 ± 0.33 9.54 ± 0.39	2.68 ± 0.16 8.17 ± 0.25
21 → -3° C	g g/100 g b.wt	1.93 ± 0.19 7.64 ± 0.64	2.51 ± 0.18 9.60 ± 0.51	2.38 ± 0.29 8.46 ± 0.13	1.99 ± 0.27 8.55 ± 0.11
-3° C, Generation 1	g g/100 g b.wt	2.01 ± 0.27 8.29 ± 0.43	2.39 ± 0.53 10.05 ± 0.51	2.27 ± 0.24 9.21 ± 0.45	2.07 ± 0.26 8.80 ± 0.68
-3° C, Generations 25 to 29	g g/100 g b.wt	2.41 ± 0.28 8.96 ± 0.35	2.54 ± 0.33 9.65 ± 0.32	2.66 ± 0.32 9.66 ± 0.18	2.63 ± 0.34 8.83 ± 0.20

Values expressed as means ± S.E. Numbers of mice as in Table 2.

TABLE 5
KIDNEY WEIGHTS OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg mg/100 g b.wt	412 ± 40 1.47 ± 0.04	465 ± 39 1.67 ± 0.06	428 ± 71 1.67 ± 0.09	477 ± 27 1.44 ± 0.03
21 → -3° C	mg mg/100 g b.wt	439 ± 54 2.00 ± 0.07	558 ± 38 2.09 ± 0.06	557 ± 63 2.02 ± 0.11	573 ± 84 2.55 ± 0.23
-3° C, Generation 1	mg mg/100 g b.wt	484 ± 64 2.02 ± 0.06	551 ± 115 2.35 ± 0.14	547 ± 62 2.37 ± 0.04	558 ± 56 2.43 ± 0.09
-3° C, Generations 25 to 29	mg mg/100 g b.wt	443 ± 51 1.69 ± 0.04	495 ± 66 1.89 ± 0.07	473 ± 79 1.75 ± 0.08	525 ± 71 1.77 ± 0.08

Values expressed as means ± S.E. Numbers of mice as in Table 2.

The relative weights of the kidneys of the primiparous mice were, by contrast, no greater than those of the corresponding virgins, except those of the first generation at -3° C; but they showed a consistent tendency to increase during first lactation (Table 5). Kidneys were heavier at -3° C than at 21° C. The effect of the cold environment on the kidneys was most marked among the transferred mice and those of the first generation at -3° C. The mice of long ancestry at -3° C had consistently lower relative kidney weights than those of the other two groups in the cold: the differences of the four classes of Generations 25 to 29 from Generation 1 are all statistically significant ($P < 0.001$, 0.02, 0.001, 0.001).

The stomachs of primiparous mice were heavier than those of virgins. The figures in Table 6 suggest that lactation generally led to a further increase, but none of the differences reaches even the 1% level. Cold made little difference to the relative stomach weights of the parturient and lactating mice, though it caused higher stomach weights in virgins (Barnett & Widdowson 1965). The small intestine presented a contrasting and complex picture. At the

TABLE 6
STOMACH WEIGHTS OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg g/100 g b.wt	199 ± 22 0.73 ± 0.08	226 ± 19 0.80 ± 0.04	255 ± 32 0.85 ± 0.03	293 ± 14 1.02 ± 0.10
21 → -3° C	mg g/100 g b.wt	191 ± 17 0.73 ± 0.03	254 ± 29 1.06 ± 0.21	239 ± 30 0.82 ± 0.05	242 ± 32 1.07 ± 0.07
-3° C, Generation 1	mg g/100 g b.wt	207 ± 32 0.80 ± 0.03	230 ± 49 1.01 ± 0.13	202 ± 22 0.81 ± 0.03	248 ± 30 0.98 ± 0.10
-3° C, Generations 25 to 29	mg g/100 g b.wt	224 ± 27 0.86 ± 0.05	240 ± 32 0.93 ± 0.04	246 ± 30 0.83 ± 0.06	241 ± 38 0.74 ± 0.08

Values expressed as means ± S.E. Numbers of mice as in Table 2.

TABLE 7
WEIGHTS AND LENGTHS OF SMALL INTESTINE OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg g/100 g b.wt mm	643 ± 75 2.54 ± 0.16 559 ± 51	1002 ± 87 3.65 ± 0.22 561 ± 45	736 ± 115 2.86 ± 0.37 685 ± 77	760 ± 59 2.26 ± 0.16 661 ± 15
21 → -3° C	mg g/100 g b.wt mm	843 ± 79 3.34 ± 0.21 592 ± 50	1193 ± 95 4.63 ± 0.37 601 ± 41	604 ± 81 2.11 ± 0.17 668 ± 75	767 ± 113 3.59 ± 0.25 614 ± 79
-3° C, Generation 1	mg g/100 g b.wt mm	665 ± 105 3.12 ± 0.36 600 ± 75	883 ± 208 3.73 ± 0.35 602 ± 123	701 ± 90 2.85 ± 0.13 648 ± 68	884 ± 110 3.90 ± 0.33 653 ± 60
-3° C, Generations 25 to 29	mg g/100 g b.wt mm	900 ± 113 3.74 ± 0.11 615 ± 69	989 ± 172 3.91 ± 0.45 630 ± 80	760 ± 106 2.87 ± 0.28 659 ± 79	848 ± 141 3.00 ± 0.29 700 ± 88

Values expressed as means ± S.E. Numbers of mice as in Table 2.

end of first pregnancy absolute and relative weights had declined from those of virgins of all classes, but length had increased; the small intestine again declined in weight during second pregnancy, but it continued to lengthen (Table 7): hence weight per unit length was consistently low at parturition; this must have been due to thinning of the gut wall. The intestine weight (but not length) of most classes increased during lactation, as was to be expected from previous work (Fell & Campbell, 1964). Hence the weight (absolute or relative) of the small intestine fluctuated with reproductive state, but absolute length tended to rise throughout. Whether the change in length was related to age rather than parity cannot be determined on this evidence. The effect of cold on the small intestine was usually a higher relative weight and, during first pregnancy and lactation, greater length also.

TABLE 8
WEIGHTS OF LARGE INTESTINE OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg g/100 g b.wt	442 ± 45 1.68 ± 0.15	609 ± 50 2.14 ± 0.07	505 ± 65 1.74 ± 0.13	562 ± 26 1.92 ± 0.12
21 → -3° C	mg g/100 g b.wt	473 ± 50 1.73 ± 0.09	591 ± 43 2.20 ± 0.06	387 ± 48 1.33 ± 0.06	408 ± 59 1.78 ± 0.09
-3° C, Generation 1	mg g/100 g b.wt	431 ± 66 1.99 ± 0.23	414 ± 85 1.79 ± 0.16	333 ± 39 1.23 ± 0.09	389 ± 37 1.62 ± 0.07
-3° C, Generations 25 to 29	mg g/100 g b.wt	387 ± 46 1.51 ± 0.11	488 ± 77 1.94 ± 0.19	383 ± 46 1.33 ± 0.09	435 ± 58 1.49 ± 0.10

Values expressed as means ± S.E. Numbers of mice as in Table 2.

TABLE 9
SPLEEN WEIGHTS OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg g/100 g b.wt	157 ± 17 0.53 ± 0.05	222 ± 20 0.79 ± 0.06	152 ± 21 0.55 ± 0.07	208 ± 23 0.66 ± 0.06
21 → -3° C	mg g/100 g b.wt	167 ± 17 0.66 ± 0.07	183 ± 15 0.72 ± 0.04	165 ± 22 0.62 ± 0.09	117 ± 22 0.46 ± 0.04
-3° C, Generation 1	mg g/100 g b.wt	144 ± 21 0.61 ± 0.07	165 ± 39 0.71 ± 0.08	172 ± 24 0.82 ± 0.03	128 ± 19 0.59 ± 0.07
-3° C, Generations 25 to 29	mg g/100 g b.wt	139 ± 16 0.53 ± 0.04	181 ± 25 0.68 ± 0.04	153 ± 22 0.53 ± 0.05	169 ± 25 0.59 ± 0.07

Values expressed as means ± S.E. Numbers of mice as in Table 2.

There are no figures for the large intestine of virgin mice. The relative weight of the large intestine usually rose during lactation (Table 8). The mice in the cold tended to have lighter large intestines than those of the controls, but the differences in relative weights were small and inconsistent.

The spleen of primiparous mice of long ancestry at -3° C was much heavier than that of virgins. Peters *et al.* (1967) report a similar effect of pregnancy on the spleen of rats. At 21° C absolute and relative spleen weight increased during both lactations, but there was no consistent effect at -3° C (Table 9). Nor was there any consistent effect of cold.

The relative weight of the uterus declined during lactation, except that of the controls with their second litter. It rose again during second pregnancy, but the lowest mean relative uterine weights recorded were those of all groups at -3° C during second lactation (Table 10). Uterine weight tended to be lower at -3° C than at 21° C.

The figures of skin weights in Table 11 represent the weight of the fresh skin with its hair, but without fat. The weights, absolute or relative, were all

TABLE 10
UTERUS WEIGHTS OF MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	mg g/100 g b.wt	664 ± 66 2.55 ± 0.17	553 ± 69 2.03 ± 0.34	576 ± 79 2.05 ± 0.16	665 ± 197 2.24 ± 0.25
21 → -3° C	mg g/100 g b.wt	569 ± 73 1.87 ± 0.30	413 ± 56 1.52 ± 0.28	572 ± 85 2.23 ± 0.21	282 ± 77 1.18 ± 0.33
-3° C, Generation 1	mg g/100 g b.wt	422 ± 72 2.02 ± 0.25	356 ± 84 1.60 ± 0.31	524 ± 70 2.15 ± 0.23	240 ± 74 1.15 ± 0.42
-3° C, Generations 25 to 29	mg g/100 g b.wt	535 ± 78 1.88 ± 0.22	426 ± 69 1.68 ± 0.21	662 ± 97 2.03 ± 0.23	357 ± 76 1.12 ± 0.20

Values expressed as means ± S.E. Numbers of mice as in Table 2.

TABLE 11
WEIGHTS OF FAT-FREE SKINS OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C	g/100 g b.wt	3.2 ± 0.31 11.4 ± 0.2	3.3 ± 0.28 11.3 ± 0.4	3.3 ± 0.38 11.8 ± 0.2	3.8 ± 0.41 13.4 ± 0.3
21 → -3° C	g/100 g b.wt	3.2 ± 0.32 11.9 ± 0.9	3.0 ± 0.22 11.1 ± 0.4	3.5 ± 0.41 12.4 ± 0.3	3.1 ± 0.45 13.0 ± 0.3
-3° C, Generation 1	g/100 g b.wt	3.0 ± 0.39 12.2 ± 0.4	3.1 ± 0.63 14.2 ± 2.0	3.3 ± 0.36 13.5 ± 0.7	3.0 ± 0.45 12.6 ± 0.5
-3° C, Generations 25 to 29	g/100 g b.wt	3.1 ± 0.36 11.6 ± 0.4	3.1 ± 0.41 12.4 ± 0.7	3.4 ± 0.38 11.9 ± 0.3	3.7 ± 0.47 12.2 ± 0.3

Values expressed as means ± S.E. Numbers of mice as in Table 2.

TABLE 12
FAT CONTENT OF FEMALE MICE

		After litter 1		After litter 2	
		0 days	10 days	0 days	10 days
21° C		13.6 ± 0.9	9.5 ± 0.1	11.0 ± 0.6	12.1 ± 1.2
21 → -3° C		9.3 ± 0.5	9.2 ± 0.8	11.4 ± 1.4	5.8 ± 1.0
-3° C, Generation 1		9.9 ± 0.5	7.6 ± 0.1	7.2 ± 0.1	7.7 ± 1.3
-3° C, Generations 25 to 29		10.9 ± 0.3	10.8 ± 0.6	12.3 ± 0.7	10.3 ± 0.9

Numbers of mice in each class as in Table 2.
Fat content expressed as mean % b.wt ± S.E.

TABLE 13
WATER CONTENT OF FAT-FREE CARCASS AND SKIN OF FEMALE MICE

		Litter 1		Litter 2	
		0 days	10 days	0 days	10 days
21° C	Carcass	74.8 ± 0.12	75.6 ± 0.16	75.9 ± 0.25	76.6 ± 0.41
	Skin	65.8 ± 0.66	66.0 ± 0.60	66.0 ± 0.65	67.6 ± 0.91
21 → -3° C	Carcass	74.6 ± 0.21	75.2 ± 0.36	75.2 ± 0.45	74.6 ± 0.55
	Skin	63.6 ± 0.40	63.3 ± 0.46	65.0 ± 0.91	62.8 ± 1.90
-3° C, Generation 1	Carcass	74.4 ± 0.29	74.9 ± 0.31	75.0 ± 0.38	75.2 ± 0.23
	Skin	63.4 ± 0.66	64.2 ± 0.91	63.6 ± 0.72	62.1 ± 0.63
-3° C, Generations 25 to 29	Carcass	74.5 ± 0.25	75.5 ± 0.35	75.2 ± 0.39	74.2 ± 0.79
	Skin	65.5 ± 0.50	64.9 ± 0.70	66.5 ± 1.23	66.3 ± 0.72

Numbers of mice in each class as in Table 2.
Values expressed as mean % ± S.E. of water in fat-free carcass and skin.

TABLE 14
NITROGEN, COLLAGEN AND CALCIUM CONTENT OF THE FAT-FREE CARCASS AND SKIN OF FEMALE MICE AFTER FIRST LITTERS

		Nitrogen		Collagen		Calcium	
		0 days	10 days	0 days	10 days	0 days	10 days
21° C	Carcass	3.08 ± 0.12	2.93 ± 0.07	2.17 ± 0.05	1.92 ± 0.04	1.24 ± 0.05	1.07 ± 0.03
	Skin	4.85 ± 0.16	4.88 ± 0.09	6.13 ± 0.15	5.89 ± 0.24	—	—
21 → -3° C	Carcass	2.94 ± 0.11	2.80 ± 0.08	1.99 ± 0.05	1.82 ± 0.05	1.23 ± 0.05	1.08 ± 0.04
	Skin	5.21 ± 0.10	5.30 ± 0.08	5.47 ± 0.15	6.14 ± 0.15	—	—
3° C, Generation 1	Carcass	2.66 ± 0.09	2.95 ± 0.17	2.12 ± 0.04	1.88 ± 0.07	1.27 ± 0.04	1.19 ± 0.10
	Skin	5.16 ± 0.13	5.14 ± 0.17	5.10 ± 0.13	5.33 ± 0.16	—	—
-3° C, Generations 25 to 29	Carcass	2.91 ± 0.15	2.52 ± 0.12	1.95 ± 0.05	1.97 ± 0.05	1.17 ± 0.03	1.16 ± 0.04
	Skin	4.91 ± 0.07	4.97 ± 0.15	5.40 ± 0.24	5.54 ± 0.20	—	—

Numbers of mice in each class as in Table 2, for first litters.
Values expressed as mean % ± S.E. of fresh weight of fat-free carcass and skin.

higher after first pregnancy than those given by Barnett & Widdowson (1965) for skin plus hair of virgins. The relative weight of skin plus hair of parturient and lactating mice was not consistently altered either by reproductive state or by the cold environment.

Chemical composition

The percentage of fat in the primiparous mice was similar to that in virgin females. The figure tended to decline during first lactation but, except in the first generation at -3°C , was partly or wholly restored by the time of second parturition (Table 12). During second lactation the proportion of fat in the transferred mice again declined but it did not do so in the mice of the other groups.

TABLE 15
TOTAL NITROGEN, COLLAGEN AND CALCIUM IN THE BODIES OF FEMALE MICE

		<i>Virgin</i> *	<i>Parturient</i> †	<i>Lactating</i> †
21° C	Nitrogen	0.53	0.84	0.83
	Collagen	0.59	0.70	0.62
	Calcium	0.28	0.28	0.25
21 → -3°C	Nitrogen	—	0.73	0.74
	Collagen	—	0.56	0.56
	Calcium	—	0.25	0.23
-3°C , Generation 1	Nitrogen	0.43	0.65	0.77
	Collagen	0.47	0.54	0.56
	Calcium	0.26	0.24	0.24
-3°C , Generations 25 to 29	Nitrogen	0.49	0.75	0.65
	Collagen	0.53	0.57	0.56
	Calcium	0.27	0.24	0.23

Values expressed in g.

* From Barnett & Widdowson (1965). † First litter.

A fall in the proportion of fat in mice lactating at 21°C has also been observed by Pennycuik (1966); even when her mice were kept at 34°C there was some decline. There was a tendency for the mice at -3°C to have a lower proportion of fat than those at 21°C , but each class in the cold evidently had its own distinctive pattern of fat metabolism; in particular, the mice of long ancestry in the cold had consistently more fat per unit body weight than the other two classes at -3°C .

There was a slightly higher percentage of water, and hence a lower percentage of solids, in the fat-free carcass of all groups of primiparous mice than in virgins of the same class. The lower figure for solids corresponds to the lower proportion of collagen and calcium also found in the primiparous mice; this must reflect a lower relative weight of bone. There was no consistent effect of lactation or of second pregnancy on water content (Table 13). Nitrogen, expressed as a percentage of carcass weight, was unaffected by lactation, but carcass collagen and calcium tended to decline (Table 14).

Further information on changes in the content of solids may be obtained from the absolute amounts present in the body. Table 15 compares the figures for virgins with those of pregnant and lactating mice at both temperatures: there was a substantial increase, even at -3°C , in the amount of nitrogen in the body during pregnancy; this was in addition to the nitrogen laid down in the foetuses. This confirms the observations of Spray (1950). By contrast, the figures for collagen show little change; and those for calcium show none. Hence it is the gain in body protein, not loss of calcium, which accounts for the lower concentration of calcium in the parturient female.

The controls had nearly always a higher concentration of nitrogen and collagen in their bodies, and of collagen in their skins, than the groups at -3°C , but almost the same proportion of calcium.

TABLE 16
LITTER SIZES AND WEIGHTS

		Litter 1		Litter 2	
		0 days	10 days*	0 days	10 days*
21° C	Young/litter	6.0 ± 0.48 (12)	4.0 (11)	5.5 ± 1.11 (10)	4.0 (8)
	Wt (g)	8.5 ± 0.53	28.4 ± 1.06	8.4 ± 1.69	26.2 ± 1.57
21 → -3° C	Young/litter	5.5 ± 0.49 (13)	4.0 (11)	6.0 ± 0.77 (10)	4.0 (5)
	Wt (g)	8.3 ± 0.66	26.8 ± 1.26	9.8 ± 1.28	29.3 ± 2.32
-3° C, Generation 1	Young/litter	5.2 ± 0.81 (9)	4.0 (3)	5.4 ± 0.51 (11)	4.0 (9)
	Wt (g)	7.5 ± 1.12	26.6 ± 1.84	7.9 ± 0.70	23.1 ± 1.37
-3° C, Generations 25 to 29	Young/litter	5.2 ± 0.71 (10)	4.0 (7)	6.2 ± 0.75 (10)	4.0 (7)
	Wt (g)	8.1 ± 0.94	26.6 ± 1.04	9.2 ± 0.96	28.4 ± 2.09

Values expressed as means ± S.E. Number of litters in parentheses.

* Wholly surviving litters only.

Effects on young

The observations on the newborn and 10-day young are summarized in Tables 16 to 18. The increase in weight from birth to 10 days was accompanied by an increase in the proportion of body fat from 2.5%, or less, to about 15%. There were also increases in the concentration of nitrogen, collagen and calcium (Tables 17 and 18), and a corresponding decrease in water. These findings conform with those of Bailey, Kitts & Wood (1960) on mice, and with those of Spray & Widdowson (1950) and Widdowson & Dickerson (1964) on a number of other species.

The cold environment had little effect on birth weight, the number of young born per litter, or on the weight of the four mice of each litter kept to 10 days (Table 16). The principal influence of cold on the composition of the young was a higher fat content at birth (Table 17). This unexpected finding applies to all three classes in the cold, and to both first and second litters.

TABLE 17
WATER AND FAT CONTENT OF YOUNG MICE

		Litter 1		Litter 2	
		0 days	10 days	0 days	10 days
21° C	Water*	85.3 ± 0.17 (12)	80.0 ± 2.12 (14)	84.5 ± 0.38 (10)	78.6 ± 0.62 (10)
	Fat†	0.9 ± 0.27	17.5 ± 1.78	1.3 ± 0.34	13.5 ± 1.50
21 → -3° C	Water*	84.2 ± 0.27 (13)	79.3 ± 0.20 (16)	85.5 ± 0.39 (10)	80.2 ± 0.76 (9)
	Fat†	2.1 ± 0.39	17.7 ± 0.75	2.1 ± 0.50	19.0 ± 1.36
-3° C, Generation 1	Water*	84.0 ± 0.35 (9)	79.6 ± 0.09 (6)	84.6 ± 0.30 (11)	79.8 ± 0.23 (12)
	Fat†	2.5 ± 0.66	17.7 ± 0.91	1.3 ± 0.52	14.9 ± 0.74
-3° C, Generations 25 to 29	Water*	84.3 ± 0.21 (10)	79.3 ± 0.24 (9)	85.1 ± 0.17 (10)	79.5 ± 0.44 (9)
	Fat†	2.0 ± 0.50	18.2 ± 0.81	1.6 ± 0.25	17.8 ± 1.31

Number of litters in parentheses.

* Expressed as mean % ± S.E. of the fat-free body

† Expressed as mean % ± S.E. of b.wt.

DISCUSSION

Effects of pregnancy

The greatest effects on organ weights and body composition were those associated with pregnancy. This is shown by comparing the virgins of the previous study (Barnett & Widdowson, 1965) with the females at their first parturition (Tables 1 and 15). The relative weight of the heart doubled during pregnancy, in both environments. The relative weight of the liver, too, nearly doubled at 21° C; at -3° C, the increase was about 50%. Evidently, the increase in relative liver weight due to the cold environment and that due to pregnancy were not simply additive. By comparison, the increase in relative stomach weight was small. These processes of adaptive growth were accompanied by an increase of about 50% in the amount of nitrogen in the body.

There were two anomalies. First, despite the increase in food intake (Barnett & Little, 1965), the weight of the kidneys was unaffected by pregnancy. Second, the small intestine of parturient mice was lighter than that of the virgins, though it evidently lengthened during pregnancy. Both these observations suggest the need for further histological and functional studies of these organs under various conditions of demand upon them.

Effects of lactation

Compared with those of pregnancy, the changes which accompanied lactation (Table 19) were small. The heart, whose weight increased so greatly in pregnancy, was evidently able to cope with the additional demands of lactation without further enlargement. On the other hand, the liver enlarged further during first lactation, and the weight of the small intestine, but not its length, also increased. These changes correspond to the steep rise in food consumption

TABLE 18
NITROGEN, COLLAGEN AND CALCIUM CONTENT OF THE FAT-FREE BODIES OF YOUNG MICE (FIRST LITTERS)

	Nitrogen		Collagen		Calcium	
	0 days	10 days	0 days	10 days	0 days	10 days
21° C	1.73 ± 0.06 (12)	2.40 ± 0.10 (14)	0.64 ± 0.03 (12)	1.16 ± 0.07 (14)	0.31 ± 0.02 (12)	0.51 ± 0.03 (14)
21 → -3° C	1.91 ± 0.08 (13)	2.42 ± 0.05 (16)	0.69 ± 0.03 (13)	1.21 ± 0.08 (16)	0.30 ± 0.02 (13)	0.50 ± 0.03 (16)
-3° C, Generation 1	1.78 ± 0.05 (9)	2.39 ± 0.03 (6)	0.75 ± 0.05 (9)	1.21 ± 0.02 (6)	0.30 ± 0.03 (9)	0.59 ± 0.03 (6)
-3° C, Generations 25 to 29	1.75 ± 0.13 (10)	2.45 ± 0.10 (9)	0.71 ± 0.03 (10)	1.29 ± 0.05 (9)	0.28 ± 0.01 (10)	0.52 ± 0.06 (9)

Values expressed as mean % ± S.E. of fresh weight of fat-free bodies.
Numbers of litters in parentheses.

TABLE 19
SIGNIFICANCE OF DIFFERENCES DUE TO REPRODUCTIVE STATE

	Lactating females and their young compared with parturient; first litters	Females at second parturition and their young compared with first	Females at second lactation and their young compared with first
Heart weight	- *** 0 0 * 0 0	0 + * 0 0 0 0	0 + ** 0 0 - *
Liver weight	+ ** 0 + * + * 0	+ * + * 0 + *** 0	- *** - 0 0 0
Kidney weight	+ *** + ** 0 0 0	0 - *** 0 + *** 0	- *** + * 0 0
Small intestine weight	+ *** + *** 0 + 0	0 - ** - ** 0 0	0 - *** 0 0
Large intestine weight	0 + * 0 0 0 0	0 - * 0 0 0 0	0 - 0 0 - *
Uterus weight	0 0 0 0 0 0	0 0 0 0 0 0	+ *** + *** 0 0
Skin weight	- ** 0 0 - * 0 0	- * 0 0 - ** 0 0	+ * 0 0 0
Fat	+ *** 0 + * + * 0	+ *** 0 0 + 0	+ * 0 0 0
Water: carcass	- *** - * - ** 0		
Collagen: carcass	- * - * 0 0		
Calcium: carcass	- *** - *** - *** - ***	0 + ** + * 0	0 0 0 - *
Young: water	+ *** + *** + *** + ***		
fat	+ *** + *** + *** + ***	0 0 0 0	0 0 0
nitrogen	+ *** + *** + *** + ***		
collagen	+ *** + *** + *** + ***		
calcium	+ *** + *** + *** + ***		

Of each group of four symbols, the first refers to controls, the second to mice transferred to the cold, the third to mice of the first generation reared in the cold and the fourth to mice of long ancestry at -3°C . All weights of organs compared are relative weights. 0 = no statistically significant difference; + = increase ($P < 0.1$); - = decrease ($P < 0.1$); ... = no information. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 20
SIGNIFICANCE OF DIFFERENCES OF MICE IN COLD ENVIRONMENT FROM CONTROLS

	21 → -3° C		-3° C, Generation 1		-3° C, Generations 25 to 29				
Body weight	-*	0	-**	-*	0	0	0	0	0
Heart weight	+**	+***	+***	+**	0	+**	0	+**	0
Kidney weight	+***	+***	+***	+***	+***	+***	0	0	+***
Small intestine weight	+**	+	0	0	+	+	0	0	+
length	+	0	0	0	0	0	0	0	0
Uterus weight	-	0	0	0	0	0	0	0	0
Fat	-***	0	-***	-*	-*	+*	0	0	0
Nitrogen: carcass	0	-*	...	...	0	-***	...	...	...
Collagen: carcass	-*	0	0	...	0	-**	0	...	...
skin	-***	0	...	...	-*	0	...	...	...
Young: water	0	0	0	0	-	0	0	0	0
fat	+	0	+	0	+	0	0	0	+

Of each group of four symbols, the first refers to mice at first parturition, the second to first lactation, the third to second parturition and the fourth to second lactation. All weights of organs compared are relative weights.

0 = no statistically significant difference; + = increase ($P < 0.1$); - = decrease ($P < 0.1$); ... = no information.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

which occurs during the early days of lactation (Barnett & Little, 1965). The changes during lactation may in fact be supposed to be responses to metabolic demand, especially since activity also probably rises during lactation: the time of minimum activity is around parturition (McEwan, unpublished data; see Barnett & Burn, 1970). But, since metabolic turnover is highest during lactation, the largest changes might be expected then, and not during pregnancy. Probably the tissue hyperplasia and hypertrophy that take place during pregnancy reflect, in part, not metabolic demand but endocrine effects (reviewed by Dewar, 1969) and are, in effect, anticipating the needs of lactation.

Effects of cold

Differences attributable to the cold environment (Table 20) were also less marked than those of pregnancy, but generally agreed with those observed in unmated laboratory mammals (reviewed by Barnett & Mount, 1967). Body weight was consistently lower in the cold than at 21° C, and lowest among the mice of the first generation in the cold. This accords with previous observations (reviewed by Barnett, 1965) on the poor performance of mice reared by parents which had been transferred as adults to the cold environment. The relative heart weight was also consistently higher at -3° C; hence the effects of pregnancy and of cold on this organ were additive, and illustrate the extent to which it responds by enlargement to the demands put on it. Most studies of adaptive growth of heart muscle have used special experimental procedures or have involved forced, strenuous exercise (Grande & Taylor, 1965); Jansky & Hart (1968) have, however, recorded the increased cardiac output of rats exposed to cold. Like theirs, our observations illustrate the lability of the heart in its response to more natural demands.

The kidneys, too, were consistently larger at -3° C than at 21° C, in conformity with many other observations (Barnett & Mount, 1967), though the kidneys of the mice of long ancestry in the cold were less affected than those of the other two groups. Unlike the heart, the kidneys did not enlarge during pregnancy but did so during lactation; hence, on this evidence, the kidneys respond to metabolic demand by enlargement, but not to the hormonal changes of pregnancy. Nevertheless, kidney function may have altered during pregnancy, since the mouse kidney can cope with added metabolic turnover without enlarging; in the previous study, the male mice did not have larger kidneys at -3° C than at 21° C (Barnett & Widdowson, 1965).

The small intestine also presented some anomalies. It was both heavier and longer in the cold, at least during first pregnancy and lactation; but in pregnancy it only lengthened, while lactation led to a consistent increase only in weight. Fell & Campbell (1964) have reviewed work on the alimentary tract during lactation, especially that of mice, rats and ewes. The stomach, small intestine and caecum all become heavier, and decline after the young are weaned. Evidently, the gross effects of cold exposure and lactation are, in this respect, similar. On the evidence of Fell & Campbell (1964), the hypertrophy of the intestine in both conditions is a local response to the presence of increased amounts of some constituents of food. The lengthened, thin-walled intestine of

the period around parturition is subject to intussusception; and this is probably responsible for some deaths of mated females in our colony (K. M. H. Munro, unpublished data).

The most notable negative finding was the failure of the weight of the liver to reflect the increased metabolism in the cold, although virgin females at -3°C have relative liver weights about 25 % higher than those of controls (Barnett & Widdowson, 1965). Evidently, the large increase during pregnancy enables the liver to cope, without further growth, with the extra demand imposed by a cold environment, as well as that arising from pregnancy.

ACKNOWLEDGMENTS

The work was aided by generous grants to S.A.B. by the Medical Research Council and the Royal Society. Indispensable statistical advice was given by Mr R. A. Elton. We are also very grateful to Miss J. M. Kendall, Mrs A. C. Neil, Mr R. C. Stoddart and Miss A. L. Walmsley for technical help.

REFERENCES

- ANGERVALL, L. & CARLSTRÖM, E. (1963) Theoretical criteria for the use of relative organ weights and similar ratios in biology. *J. theor. Biol.* **4**, 254.
- BAILEY, C. B., KITTS, W. D. & WOOD, A. J. (1960) Changes in the gross chemical composition of the mouse during growth in relation to the assessment of physiological age. *Can. J. Anim. Sci.* **40**, 143.
- BARNETT, S. A. (1956) Endothermy and ectothermy in mice at -3°C . *J. exp. Biol.* **33**, 124.
- BARNETT, S. A. (1965) Adaptation of mice to cold. *Biol. Rev.* **40**, 5.
- BARNETT, S. A. & BURN, J. (1970) *Maternal and infant behavior*. In: *Reproduction and Breeding Techniques for Laboratory Animals*, p. 177. Ed. E. F. E. Hafez. Lea & Febiger, Philadelphia.
- BARNETT, S. A. & LITTLE, M. J. (1965) Maternal performance in mice at -3°C ; food consumption and fertility. *Proc. R. Soc. B*, **162**, 492.
- BARNETT, S. A. & MOUNT, L. E. (1967) *Resistance to cold in mammals*. In: *Thermobiology*, p. 411. Ed. A. S. Rose. Academic Press, London.
- BARNETT, S. A. & WIDDOWSON, E. M. (1965) Organ-weights and body-composition in mice bred for many generations at -3°C . *Proc. R. Soc. B*, **162**, 502.
- BRUCE, H. M. & PARKES, A. S. (1949) Feeding and breeding of laboratory animals. 9. A complete cubed diet for mice and rats. *J. Hyg., Camb.* **47**, 202.
- DEWAR, A. D. (1969) The mechanism and nature of the extra-uterine weight gain of pregnancy in the mouse. *J. Reprod. Fert. Suppl.* **9**, 17.
- DICKERSON, J. W. T. (1962) The effect of development on the composition of a long bone of the pig, rat and fowl. *Biochem. J.* **82**, 47.
- FELL, B. F. & CAMPBELL, R. M. (1964) *Gastrointestinal hypertrophy in lactation and its relation to diet*. In: *The Role of the Gastrointestinal Tract in Protein Metabolism*, p. 199. Ed. H. N. Munro. Blackwell Scientific Publications, Oxford.
- GRANDE, F. & TAYLOR, H. L. (1965) Adaptive changes in the heart, vessels, and patterns of control under chronically high loads. *Handb. Physiol.* **3**, 2615. Ed. W. S. Hamilton. American Physiological Society.
- JANSKY, L. & HART, J. S. (1968) Cardiac output and organ blood flow in warm- and cold-acclimated rats exposed to cold. *Can. J. Physiol. Pharmac.* **46**, 653.
- KENNEDY, G. C., PEARCE, W. M. & PARROTT, D. M. V. (1958) Liver growth in the lactating rat. *J. Endocr.* **17**, 158.
- PENNYCUK, P. R. (1966) Differences between mice gestating and lactating at 21°C and at $34^{\circ}\text{--}36^{\circ}$ with particular reference to the mammary gland. *Aust. J. exp. Biol. med. Sci.* **44**, 419.
- PETERS, J. M. & KRIJNEN, C. J. (1966) Organ weights and water contents of lactating rats. *Growth*, **30**, 295.
- PETERS, J. M., KRIJNEN, C. J. & BOYD, E. M. (1967) Role of concepta in the production of hypertrophy and hydration of maternal body organs in the third week of pregnancy in albino rats. *J. Reprod. Fert.* **14**, 235.
- SPRAY, C. M. (1950) A study of some aspects of reproduction by means of chemical analysis. *Br. J. Nutr.* **4**, 354.

- SPRAY, C. M. & WIDDOWSON, E. M. (1950) The effect of growth and development on the composition of mammals. *Br. J. Nutr.* **4**, 332.
- STODDART, R. C. (1966) Ice blocks as water supply for mice at -3°C . *J. Inst. Anim. Techns*, **17**, 158.
- WIDDOWSON, E. M. & DICKERSON, J. W. T. (1964) *Chemical composition of the body*. In: Mineral Metabolism, Vol. 2A, p. 1. Eds. C. L. Comar and F. Bronner. Academic Press, New York.