Changes in adipose tissue of the rat due to early undernutrition followed by rehabilitation

1. Body composition and adipose tissue cellularity

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1. Male Black and White Hooded rats were allocated at birth to foster mothers in litters of three, nine or sixteen. At weaning animals from each of these litter sizes were *ad lib.*-fed on a stock diet forming three *ad lib.*-fed control groups. At weaning further animals from litters of nine and sixteen were fed on the stock diet in restricted amounts until 12 weeks of age. These undernourished animals were then rehabilitated by being allowed *ad lib.* access to the stock diet.

2. Five animals from each group were killed at various stages of the experiment, their bodies analysed for fat and nitrogen, and the size and number of cells determined in four specific fat depots.

3. The previously undernourished rats failed to make a complete recovery and were significantly smaller than *ad lib.*-fed animals from the same litter size at 32 weeks of age when the experiment was terminated.

4. The previously undernourished rats from litters of nine deposited a significantly greater proportion of fat in their bodies during rehabilitation than *ad lib.*-fed animals from litters of nine over the same gain in body-weight. The previously undernourished rats from litters of sixteen deposited the same proportion of fat in their bodies during rehabilitation as *ad lib* -fed animals from litters of three, nine and sixteen over the same gain in body-weight.

5. There were no significant differences in apparent or total fat cell numbers between *ad lib.*-fed animals and undernourished–rehabilitated animals at any of the four sites studied at 32 weeks of age.

It has recently been demonstrated by Harris & Widdowson (1978) that undernutrition of Black and White Hooded rats from 3 to 12 weeks of age followed by rehabilitation caused a rapid deposition of fat in the bodies of both males and females. However, these animals had not stopped growing at the termination of the experiment, so it could not be stated whether or not this effect lasted until adult weight was attained. Work of Meyer & Clawson (1964), who also used the rat, suggested that the increase in the proportion of fat deposited during rehabilitation from undernutrition was proportional to the severity of the undernutrition and the length of time it lasted. Thus, it might be anticipated that a greater deposition of excess fat would occur during rehabilitation if Black and White Hooded rats were undernourished from birth to 12 weeks of age instead of from 3 weeks to 12 weeks of age.

At the cellular level a possible effect of undernutrition is to decrease the rate of fat cell replication while simultaneously lengthening the replication period (Kirtland & Gurr, 1978). This would allow complete cell replication to occur with no final change in cell number compared with that present in normally reared animals. This concept is supported by the work of Haugeback *et al.* (1974) and Harris & Widdowson (1978), where early undernutrition was carried out during the phase of cell replication but no final effect on cell numbers was demonstrated. However, when Dobbing (1968) postulated the existence of a 'vulnerable period' in the development of organs in an animal, he stipulated that to cause

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a permanent change in cell number a 'substantial proportion of the period of multiplication is interfered with'. It seemed possible that the earlier work on undernutrition of the Black and White Hooded rat (Harris & Widdowson, 1978) had no permanent effect on fat cell number, not because of a lengthening of the replication period, but because the majority of the period of fat cell replication had taken place before undernutrition had begun.

In an attempt to include the 'vulnerable period' of the development of adipose tissue it was decided to undernourish rats from birth to 12 weeks of age. Three methods have been described to undernourish rats from birth to weaning; restricting the mother's food intake with a resultant decrease in milk flow (Stephan *et al.* 1971); removing the young from the mother for certain periods each day (Oscai *et al.* 1972); and increasing the number of young to be suckled so that less milk is received by each of the young (Widdowson & McCance, 1960). This latter technique allows a more quantitative variation in the extent of nutrition of each pup. Taking this into consideration, together with the fact that this technique has been widely used in similar work (Winick & Noble, 1966; Knittle & Hirsch, 1968), it was decided that it was the most suitable of the three for the present experiment. It was also decided to include not only two extremes of litter size (three and sixteen), but litters of nine as well. This was intended to clarify interpretations of the results; whether they arose from a comparison of well-nourished with average-nourished, of undernourished with average-nourished, or the less physiologically realistic comparison of well-nourished with undernourished.

MATERIALS AND METHODS

Experimental design

Animals born on the same day were removed from their mothers, sexed, and males randomly allocated to their own or foster mothers in groups of three (ten mothers), nine (five mothers) or sixteen (five mothers). Female young were given to spare mothers and discarded from the experiment. At weaning twenty males from litter sizes three and nine, and five males from litter size sixteen were allowed *ad lib.* access to the stock diet and formed the groups L3/C (animals from litters of three, i.e. well-nourished before weaning and *ad lib.*-fed after weaning), L9/C (animals from litters of nine, i.e. average-nourished before weaning and *ad lib.*-fed after weaning). A further five males from litters of nine were placed on a restricted intake of the stock diet as described by Harris & Widdowson (1978). These animals were severely undernourished from 3 to 12 weeks of age and then rehabilitated by being allowed *ad lib.* access to the stock diet. These animals formed the group L9/U (animals from litters of nine), i.e. average-nourished before weaning and undernourished from 3 to 12 weeks). At weaning twenty animals were taken from litters of sixteen and placed on a restricted diet identical to that used for the L9/U group. At 12 weeks these animals were rehabilitated by being allowed *ad lib.* access to the stock diet. These animals formed the L16/U group (animals from litters of sixteen, i.e. undernourished before weaning and further undernourished from 3 to 12 weeks).

The L9/U group was essentially the same as the treatment group in Harris & Widdowson (1978), and so it was anticipated that the animals in it would exhibit growth and body composition changes similar to those found by Harris & Widdowson (1978). It was decided to test these animals for the effects of treatment only at the end of the experiment. Animals reared in litters of sixteen and then well-nourished throughout life (group L16/C) were also sampled only at the end of the experiment. All other groups were sampled at 3 weeks (the end of litter manipulation), 12 weeks (the end of undernutrition), 14 weeks (early in rehabilitation), 21 weeks (as growth slowed) and 32 weeks (when growth was complete for all groups).

Killing and dissection of the animals

The animals were killed by an intraperitoneal injection of Sagatal (sodium pentabarbitone; Abbott Laboratories Ltd, Queensborough, Kent). The subcutaneous fat which extended along the front of the thigh and across the abdomen was removed from both sides of the animal (abdominal sample). The pad of fat across the scapular region was also removed (scapular sample). The abdomen was opened and the epididymal fat pads removed (epididymal sample). The remainder of the fat in the abdomen, including that around the kidneys, but excluding that in the mesentery, was also removed (perirenal sample). All visible fat at each of these sites was dissected out, divided into three parts and each part weighed. The sum of the three weights gave the total weight of adipose tissue at that site. One part was immersed in formol saline (9 g sodium chloride/l) to be used to measure the size of observable fat cells, and a further sample in diethyl ether to be used to determine triglyceride; the third sample was frozen at $-20°$ to be used to determine the DNA in the tissue. The gastro-intestinal tract was removed from the carcass, emptied, and returned to the carcass, and the carcass was stored at $-20°$ until analysed.

Analytical methods

The frozen carcasses were forced twice through a power-driven mincer which resulted in a uniform mince of tissue, bone and fur. Weighed samples were oven dried at 100° and used to determine body crude protein (nitrogen $\times$ 6·25) and total body lipid.

Protein. N was determined by macro-Kjeldahl digestion, using mercuric oxide as a catalyst, followed by an automated colorimetric determination of N by the method of Munro & Fleck (1969).

Total lipid. Total lipid was determined by the method of Southgate (1971).

Apparent cell number. Measurement of apparent fat cell size, determination of triglyceride and the estimation of apparent fat cell number were as described by Kirtland *et al.* (1976).

Total cell number. DNA was extracted from the adipose tissue samples by the method of Zamenhof *et al.* (1964) with some modifications to allow initial removal of the lipid as suggested by Munro & Fleck (1969). DNA was estimated by the colorimetric assay of Burton (1956). Total cell number was calculated from total DNA by assuming that 1000 cells contained 6·2 ng DNA.

Statistical analyses. Analyses of variance between treatment groups at each age were made using appropriate contrasts to derive any significant effects of treatments ($P < 0·05$) with respect to all measured variables. Five animals from each group were killed at the various stages of the experiment.

RESULTS

Gross changes

Fig. 1 shows curves of mean body-weights throughout the experiment of the five groups of animals that were alive until the end of the experiment. Animals reared in litters of three were significantly heavier throughout the experiment than those reared in litters of nine, which were significantly heavier than animals reared in litters of sixteen. When animals from litters of nine or sixteen were undernourished from 3 to 12 weeks and rehabilitated (groups L9/U and L16/U) they grew rapidly, but did not attain the mean body-weight of *ad lib.*-fed animals of the same litter size.

Fig. 2 illustrates the mean weekly food intakes of the *ad. lib*-fed and rehabilitating animals up to 21 weeks of age. Animals in groups L9/U and L16/U achieved the same intake as control-fed animals of the same age and litter size after 5–6 weeks of rehabilitation, i.e.

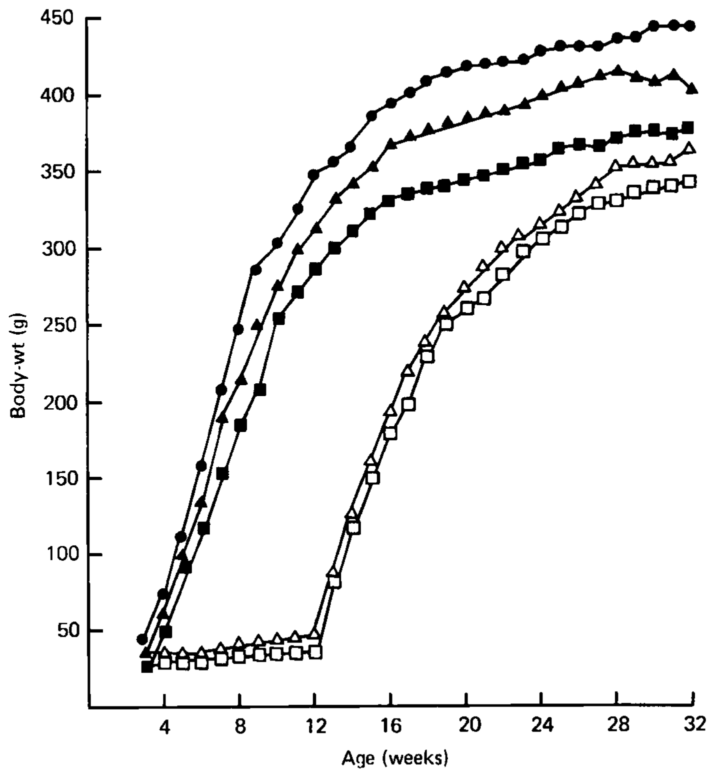

Fig. 1. Growth curves of *ad lib.*-fed and undernourished–rehabilitated rats (for details of feeding regimen, see p. 16). ●, Animals from litters of three, *ad lib.*-fed from weaning; ▲, animals from litters of nine, *ad lib.*-fed from weaning; ■, animals from litters of sixteen, *ad lib.*-fed from weaning; △, animals from litters of nine, undernourished from 3 to 12 weeks; □, animals from litters of sixteen, undernourished from 3 to 12 weeks.

slightly faster than the period of time taken for control-fed animals of the same body-weight to achieve the same levels of food intake.

Table 1 shows the average weights of protein present in the bodies of animals of each group sampled. By the end of the period of undernutrition the animals in group L16/U had a larger weight of protein than the animals in groups L3/C, L9/C and L16/C had at weaning, showing that some protein had been deposited during the 12 weeks of undernutrition. At 32 weeks the *ad lib.*-fed animals which had been reared in litters of three had significantly more body protein than those reared in litters of nine, which had more than those reared in litters of sixteen. Animals which had been undernourished and rehabilitated failed to attain the weight of body protein found in *ad lib.*-fed animals from the same litter size.

Table 2 shows the weights of crude fat in the bodies of the animals of each group sampled. *Ad lib.*-fed animals reared in litters of three had significantly more fat than *ad lib.*-fed animals reared in litters of nine throughout the experiment, and both these groups had significantly more fat than *ad lib.*-fed animals reared in litters of sixteen at the end of the experiment. At the end of the period of undernutrition the animals of group L16/U had significantly less fat than the *ad lib.*-fed animals at both 3 and 12 weeks of age. On rehabilitation all animals in group L16/U deposited fat rapidly, although they failed to attain the

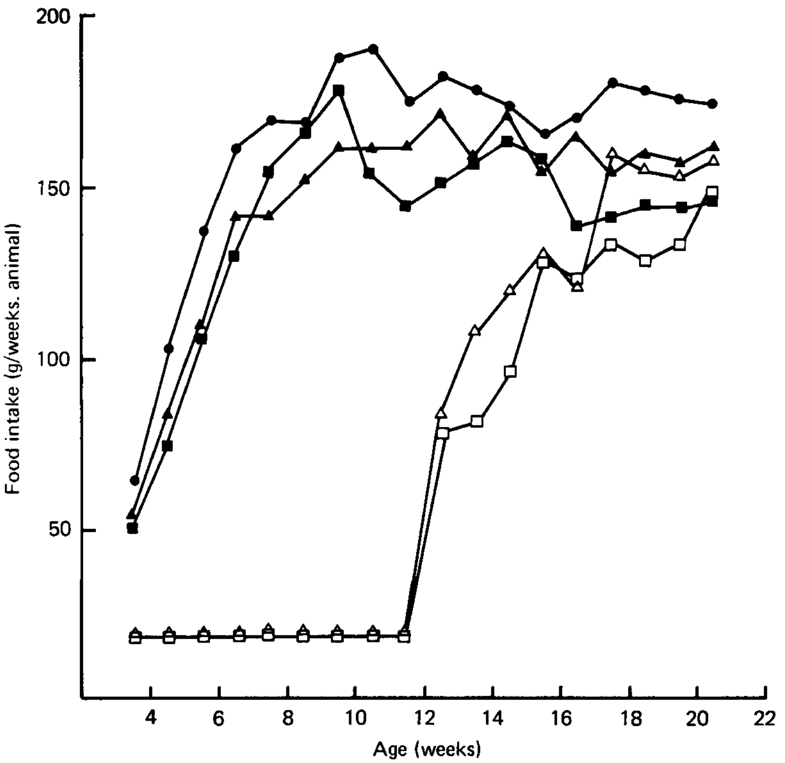

Fig. 2. Weekly food intakes (g/animal) of *ad lib.*-fed and undernourished–rehabilitated rats (for details of feeding regimen, see p. 16). ●, Animals from litters of three, *ad lib.*-fed from weaning; ▲, animals from litters of nine, *ad lib.*-fed from weaning; ■, animals from litters of sixteen, *ad lib.*-fed from weaning; △, animals from litters of nine, undernourished from 3 to 12 weeks; □, animals from litters of sixteen, undernourished from 3 to 12 weeks.

Table 1. *Weights (g) of crude protein (nitrogen* ×6·25) *in carcasses of groups of Black and White Hooded rats from litters of three, nine or sixteen fed on the control diet (groups* L3/*C*, L9/*C and* L16/*C respectively) or litters of nine and sixteen undernourished* (L9/*U and* L16/U *respectively*)*

(Mean values with their standard errors for five animals)

Group ...	L3/C		L9/C		L16/C		L9/U		L16/U	
Age (weeks)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3	6·5	0·1	5·3	0·2	4·6	0·2	—	—	—	—
12	58·7	4·3	51·3	3·7	—	—	—	—	8·6	0·4
14	64·2	4·7	56·7	4·2	—	—	—	—	21·2	1·4
21	71·8	4·9	64·9	4·7	—	—	—	—	43·4	3·3
32	82·1	5·6	76·8	5·5	65·4	5·8	62·3	5·6	56·4	5·1

* For details of feeding regimens, see p. 16.

weights of body fat found in any of the *ad lib.*-fed groups by the end of the experiment. At the end of the experiment the animals of the L9/U group had deposited fat to a weight which was not significantly different from that found in *ad lib.*-fed animals of the same age and litter size, although the latter were significantly heavier.

Table 2. *Weights of fat (g) in carcasses of groups of Black and White Hooded rats from litters of three, nine or sixteen fed on the control diet (groups L3/C, L9/C and L16/C respectively) or litters of nine and sixteen undernourished (L9/U and L16/U respectively)**

(Mean values with their standard errors for five animals)

Group ...	L3/C		L9/C		L16/C		L9/U		L16/U	
Age (weeks)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3	4·9	0·5	2·1	0·2	1·1	0·1	—	—	—	—
12	21·2	2·3	19·7	4·1	—	—	—	—	0·7	0·2
14	27·0	2·8	23·2	4·7	—	—	—	—	4·3	1·1
21	48·4	5·0	31·4	7·1	—	—	—	—	16·1	4·2
32	55·0	5·7	49·5	9·6	36·0	3·3	48·8	5·8	27·1	7·0

* For details of feeding regimens, see p. 16.

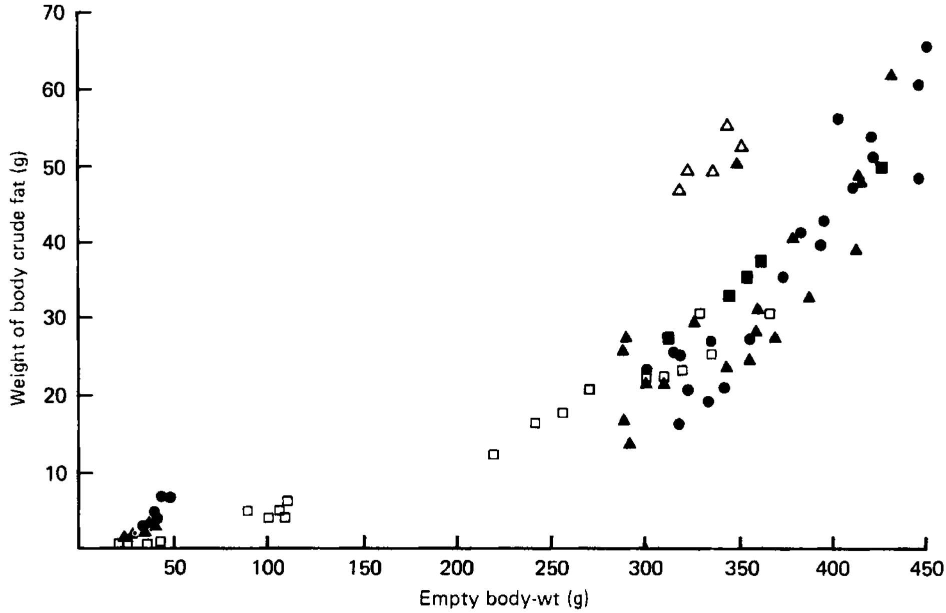

Fig. 3. Weight of body crude fat (g) compared with empty-body-weight (g) in *ad lib.*-fed and undernourished–rehabilitated rats (for details of feeding regimen, see p. 16). ●, Animals from litters of three, *ad lib.*-fed from weaning; ▲, animals from litters of nine, *ad lib.*-fed from weaning; ■, animals from litters of sixteen, *ad lib.*-fed from weaning; △, animals from litters of nine, undernourished from 3 to 12 weeks; □, animals from litters of sixteen, undernourished from 3 to 12 weeks.

Fig. 3 shows weights of total body fat *v.* empty-body-weights and demonstrates that the variation in total body fat found between the *ad lib.*-fed animals and animals of group L16/U, when comparisons are made at the same ages, disappears when comparisons are made at the same body-weight, i.e. undernutrition from birth to 12 weeks affected body size but not the proportion of fat in the body at any given weight. However, on the same plot of total body fat *v.* empty-body-weight (Fig. 3) it is clear that the animals of group L9/U had significantly more fat at equal body-weights than any of the *ad lib.*-fed animals, including those animals reared in the same-size litters. This suggests that undernutrition from 3 to 12 weeks not only affected body size but, in contrast to undernutrition from birth

Table 3. *Weights (mg) of adipose tissue at every site sampled in groups of Black and White Hooded rats from litters of three, nine or sixteen fed on the control diet (groups L3/C, L9/C and L16/C respectively) or litters of nine and sixteen undernourished (L9/U and L16/U respectively)**

(Mean values with their standard errors for five animals)

Group ... Age (weeks)	Site	L3/C Mean	SE	L9/C Mean	SE	L16/C Mean	SE	L9/U Mean	SE	L16/U Mean	SE
3	Abdominal	790	150	290	50	90	23	—	—	—	—
	Scapular	254	64	52	12	9	1	—	—	—	—
	Perirenal	104	32	30	2	—	—	—	—	—	—
	Epididymal	68	16	43	13	—	—	—	—	—	—
12	Abdominal	5690	410	5480	570	—	—	—	—	120	20
	Scapular	856	102	786	113	—	—	—	—	23	5
	Perirenal	4341	290	3250	567	—	—	—	—	—	—
	Epididymal	3965	221	3117	191	—	—	—	—	—	—
14	Abdominal	6930	670	5670	740	—	—	—	—	1540	170
	Scapular	1043	159	829	122	—	—	—	—	296	36
	Perirenal	5635	917	4184	592	—	—	—	—	260	40
	Epididymal	4581	482	4025	335	—	—	—	—	470	125
21	Abdominal	11330	530	8430	520	—	—	—	—	4050	590
	Scapular	1884	91	1582	149	—	—	—	—	619	121
	Perirenal	9318	503	5699	300	—	—	—	—	2449	480
	Epididymal	7655	181	5133	289	—	—	—	—	2254	235
32	Abdominal	10610	1060	10480	970	7340	840	7970	400	6250	350
	Scapular	2673	192	2366	353	1739	244	1789	167	1504	148
	Perirenal	9157	1167	7196	492	5551	799	6139	332	4390	284
	Epididymal	7286	584	6455	356	4850	613	5088	444	4238	275

* For details of feeding regimens, see p. 16.

to 12 weeks, also affected the proportion of fat found in the body at equal body-weights. This has been demonstrated by Harris & Widdowson (1978) at earlier stages of rehabilitation, and the present experiment suggests that this effect lasts until the animals are fully grown.

Cellular changes

Table 3 shows weights of adipose tissue at each site sampled in all treatment groups. There was a rapid increase in the weight of tissue at all sites in the *ad lib.*-fed animals (groups L3/C and L9/C) between 3 and 12 weeks of age. Between 12 and 32 weeks of age there was a gradual increase in weight at every site, with most of the increase occurring by 21 weeks of age. Undernutrition from birth to 12 weeks of age caused a smaller weight of adipose tissue to be present than in *ad lib.*-fed animals of the same age. At the deep body sites there was insufficient material for sampling throughout undernutrition. On rehabilitation of the undernourished animals adipose tissue was deposited rapidly. However, at no site did the weight of adipose tissue attain that found in the *ad lib.*-fed animals of the same age.

Table 4 shows changes in mean volumes of apparent fat cells (mature fat cells with diameters greater than 10 μm) in the *ad lib.*-fed normally reared animals and in the undernourished and rehabilitated animals. *Ad lib.*-fed animals showed a gradual increase in apparent cell volume at all sites up to 21 weeks. Undernutrition from birth to 3 weeks and from birth to 12 weeks caused, at the sites where there was sufficient tissue available for sampling, apparent cells to be smaller than in *ad lib.*-fed animals of the same age. On

Table 4. *Volumes ($\mu m^3 \times 10^3$)) of apparent cells at every site sampled in groups of Black and White Hooded rats from litters of three, nine or sixteen fed on the control diet (groups L3/C, L9/C and L16/C respectively) or litters of nine and sixteen undernourished (L9/U and L16/U respectively)**

(Mean values with their standard errors for five animals)

Group ... Age (weeks)	Site	L3/C Mean	SE	L9/C Mean	SE	L16/C Mean	SE	L9/U Mean	SE	L16/U Mean	SE
3	Abdominal	145	25	75	19	29	6	—	—	—	—
	Scapular	172	28	48	16	10	3	—	—	—	—
	Perirenal	214	40	75	8	—	—	—	—	—	—
	Epididymal	74	15	34	3	—	—	—	—	—	—
12	Abdominal	282	54	334	47	—	—	—	—	14	6
	Scapular	303	128	160	34	—	—	—	—	6	2
	Perirenal	548	139	736	66	—	—	—	—	—	—
	Epididymal	472	51	367	46	—	—	—	—	—	—
14	Abdominal	351	60	402	98	—	—	—	—	171	30
	Scapular	194	39	210	33	—	—	—	—	154	27
	Perirenal	784	122	723	54	—	—	—	—	208	35
	Epididymal	638	90	458	42	—	—	—	—	149	20
21	Abdominal	612	32	367	58	—	—	—	—	246	57
	Scapular	313	77	280	31	—	—	—	—	159	15
	Perirenal	1169	114	837	95	—	—	—	—	467	94
	Epididymal	814	66	620	67	—	—	—	—	294	33
32	Abdominal	517	104	548	75	363	78	448	36	397	34
	Scapular	512	65	417	44	305	30	348	39	237	52
	Perirenal	1202	92	1053	84	893	136	1027	126	958	90
	Epididymal	809	97	936	116	540	51	676	34	549	72

* For details of feeding regimens, see p. 16.

rehabilitation the apparent fat cells in the previously undernourished animals increased rapidly in volume. In spite of this, by the end of the experiment the cells of the previously undernourished animals were significantly smaller than those in *ad lib.*-fed animals reared in litters of three at all sites. There was a trend for the cells of the previously undernourished animals (group L16/U) to be smaller than those of the *ad lib.*-fed animals reared in litters of nine, but to be the same size as those in animals reared in the same-size litters and *ad lib.*-fed (group L16/C).

Table 5 gives values for numbers of apparent fat cells found at each site in each group of animals. The two groups of *ad lib.*-fed animals (L3/C and L9/C) showed a rapid increase in apparent cell numbers between 3 and 12 weeks of age at every site. This increase was followed by a slow increase or a stabilization in cell numbers after 12 weeks of age. Undernutrition from birth to 3 weeks of age (group L16/C) had no effect on apparent cell number compared with animals in groups L3/C and L9/C, whereas undernutrition continued until 12 weeks of age resulted in fewer apparent cells compared with those in *ad lib.*-fed animals of the same age. During rehabilitation from undernutrition apparent cell numbers increased rapidly and appeared to be still increasing at the end of the investigation. There was a trend for apparent cell numbers to be lower in the previously undernourished animals than in *ad lib.*-fed animals of the same age. However, by 32 weeks of age this difference was not significant at any site. There was no significant difference in apparent cell number between *ad lib.*-fed animals and undernourished then rehabilitated animals at the epididymal site, which is the site where differences have been demonstrated by other investigators (Knittle & Hirsch, 1968).

Table 5. *Numbers* ($\times 10^5$) *of apparent cells at every site sampled in groups of Black and White Hooded rats from litters of three, nine or sixteen fed on the control diet (groups L3/C, L9/C and L16/C respectively) or litters of nine and sixteen undernourished (L9/U and L16/U respectively)**

(Mean values with their standard errors for five animals)

Group .. Age (weeks)	Site	L3/C Mean	L3/C SE	L9/C Mean	L9/C SE	L16/C Mean	L16/C SE	L9/U Mean	L9/U SE	L16/U Mean	L16/U SE
3	Abdominal	39	4	30	6	25	9	—	—	—	—
	Scapular	10	2	8	1	13	9	—	—	—	—
	Perirenal	4	1	2	2	—	—	—	—	—	—
	Epididymal	6	1	8	2	—	—	—	—	—	—
12	Abdominal	113	24	116	12	—	—	—	—	31	11
	Scapular	24	6	33	4	—	—	—	—	11	7
	Perirenal	79	4	41	4	—	—	—	—	—	—
	Epididymal	78	3	85	7	—	—	—	—	—	—
14	Abdominal	151	18	109	13	—	—	—	—	68	10
	Scapular	42	9	26	6	—	—	—	—	14	2
	Perirenal	69	5	55	5	—	—	—	—	10	2
	Epididymal	71	5	84	8	—	—	—	—	29	7
21	Abdominal	154	5	197	22	—	—	—	—	124	11
	Scapular	51	10	36	3	—	—	—	—	25	6
	Perirenal	80	5	69	5	—	—	—	—	38	3
	Epididymal	95	8	82	8	—	—	—	—	74	6
32	Abdominal	185	28	190	42	178	33	146	4	129	14
	Scapular	39	2	42	2	41	4	42	5	65	18
	Perirenal	73	6	75	5	64	9	60	6	41	1
	Epididymal	87	6	83	3	68	5	71	4	78	11

* For details of feeding regimens, see p. 16.

Table 6 shows changes in total cell numbers, derived from DNA levels, for all sites in each group of animals. There was an initial increase in total cell numbers at all sites between 3 and 12 weeks of age in the *ad lib.*-fed animals, although there was no further increase after 12 weeks of age. Undernutrition from birth to 3 weeks (group L16/C) caused fewer cells to be present at the abdominal site than in *ad lib.*-fed animals (group L3/C) of the same age. At the end of 12 weeks of undernutrition all tissue available was used to determine apparent cell numbers and so results for total cell number are only available after 2 weeks of rehabilitation. At this time there were fewer cells at all sites in group L16/U than in *ad lib.*-fed animals of the same age. By the end of rehabilitation there were no significant differences in total cell number between *ad lib.*-fed animals and undernourished–rehabilitated animals at any site.

Although apparent cell numbers and total cell numbers follow similar trends with age and undergo similar changes due to undernutrition, they are of very different orders of size. In the two subcutaneous tissues apparent cells comprise only 5% of the total number of cells, and in the deep body tissues apparent cells comprise only 10% of the total number of cells.

DISCUSSION

From the analyses of gross body composition two main effects are clear; first, rehabilitation after undernutrition from 3 to 12 weeks caused a rapid deposition of fat in the body so that at equal body-weight the rehabilitated animals had more fat than the *ad lib.*-fed animals,

Table 6. *Total numbers* ($\times 10^5$) *of cells at every site sampled in groups of Black and White Hooded rats from litters of three, nine or sixteen fed on the control diet (groups L3/C, L9/C and L16/C respectively) or litters of nine and sixteen undernourished (L9/U and L16/U respectively)**

(Mean values with their standard errors for five animals)

Group ...		L3/C		L9/C		L16/C		L9/U		L16/U	
Age (weeks)	Site	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3	Abdominal	890	140	390	90	340	30	—	—	—	—
	Scapular	240	70	160	50	—	—	—	—	—	—
	Perirenal	90	30	80	30	—	—	—	—	—	—
	Epididymal	90	30	80	40	—	—	—	—	—	—
12	Abdominal	3570	920	5740	770	—	—	—	—	—	—
	Scapular	840	50	1120	370	—	—	—	—	—	—
	Perirenal	770	80	650	130	—	—	—	—	—	—
	Epididymal	600	110	950	240	—	—	—	—	—	—
14	Abdominal	4330	860	3250	650	—	—	—	—	1610	90
	Scapular	1060	190	720	150	—	—	—	—	530	80
	Perirenal	580	20	630	90	—	—	—	—	370	40
	Epididymal	730	50	790	100	—	—	—	—	520	200
21	Abdominal	3420	940	4260	290	—	—	—	—	2440	550
	Scapular	1280	120	810	60	—	—	—	—	570	150
	Perirenal	900	140	650	130	—	—	—	—	500	90
	Epididymal	750	60	940	130	—	—	—	—	640	90
32	Abdominal	3140	460	3140	350	2910	510	3010	360	2900	320
	Scapular	1040	130	760	170	950	240	1010	130	820	190
	Perirenal	750	50	510	110	500	130	570	40	520	160
	Epididymal	660	100	470	110	440	90	490	60	470	60

* For details of feeding regimens, see p. 16.

and secondly, rehabilitation after undernutrition from birth to 12 weeks caused no change in the proportion of fat deposited compared with that in *ad lib.*-fed animals of equal body-weight. In both instances the weight of body protein remained appropriate for the body-weight of the animal.

The changes in mean body-weight found for each group in the present experiment confirmed that it is the early period of undernutrition, before weaning, which has the greatest effect on body size; later nutritional manipulation was followed by a compensatory response which tended towards the weights attained by animals with the same pre-weaning history. That is, animals undernourished from 3 to 12 weeks from litters of nine tended to approach the body-weights of *ad lib.*-fed animals from litters of nine, while undernourished animals from litters of sixteen approached the body-weights of *ad lib.*-fed animals from litters of sixteen. Thus, it would seem that ultimate body size is determined in the period from birth to 3 weeks of age, and therefore appetite for the rest of the animal's life must be determined from the speed at which it is growing during this period. Any further manipulation must be extreme to alter this present pattern.

The age at which undernutrition is imposed has been reported to influence the food intake during the recovery period (Allden, 1968), and this is borne out by the results of the present work, in which later commencement of undernutrition led to a higher food intake during rehabilitation. The initial food intake during rehabilitation was related to the time of onset of undernutrition; that is, the first influence.

It has been suggested (McCance, 1976) that the strong influence which nutrition from birth to 3 weeks exerts on subsequent food intake and growth in the rat is a result of the hypothalamic control centres of appetite being 'fixed' relative to appetite during this period. If this is so, then when animals are undernourished during this period the appetite would be set at a level appropriate to their small size and slower rate of growth. Similarly, good nutrition during this period would allow appetite to be set at a higher level, appropriate to a greater body size and speed of growth. If such animals were undernourished after this critical period (as was group L9/U), and then rehabilitated, they would possibly eat more food than could be utilized for the proportional development of the body. If this happened the extra energy would be deposited in the body as fat, and this is a possible explanation for the results obtained in the present work.

Whatever the reasons for the differences in fat deposition shown in this experiment, the over-all results agree with the allometric theories of growth proposed by Elsley *et al.* (1964) and Tulloh (1964); the major change in body proportions which results from early nutritional manipulation is observed in the adipose tissue. In the rat and the sheep (Meyer & Clawson, 1964) this is only a minor change. However, there appear to be species differences, as the pig has been shown (Lister & McCance, 1967) to become extremely fat during rehabilitation from early, severe undernutrition.

The results of the study of changes in size and number of fat cells showed that undernutrition from birth to 12 weeks of age, while causing a reduction in the size of the fat cells at most sites compared with normal animals, did not cause any consistent alteration in apparent fat cell numbers. This result was unexpected in view of the clear differences in apparent fat cell numbers found at the epididymal site of animals undernourished from birth to 3 weeks by Knittle & Hirsch (1968). The present results are also unexpected in view of the results of Greenwood & Hirsch (1974), which showed a high rate of fat cell replication over the period from birth to 3 weeks.

There are three possible interpretations of the results on fat cells in this present experiment; firstly that final fat cell numbers were already present at birth in the Black and White Hooded rat; secondly that slow replication of fat cells occurred throughout the period of undernutrition (i.e. there was a lengthening of the sensitive period due to undernutrition); thirdly that replication ceased during undernutrition but recommenced with rehabilitation for a period sufficiently long to allow complete catch-up to normal cell numbers

The first possibility, that sufficient cells were already present by birth, seems unlikely. In the Black and White hooded rat it is known that vital organs such as the liver, kidney and brain are still replicating between birth and weaning (Widdowson, personal communication) and it seems unlikely, teleologically, that a greater priority would be given to adipose tissue with its apparent lack of vital function. It also seems unlikely in view of the fact that so little DNA was found at the four sites sampled at weaning (in some instances there was too little tissue for analysis). This suggests that some fat cell replication occurs after birth, especially at the deep body sites. Similarly, the second possibility also seems unlikely when it is considered that at the end of undernutrition tissue was only able to be collected from the abdominal and scapular sites (the deep body sites yielded nothing that could be validly claimed as adipose tissue). It would appear, therefore, that during rehabilitation either replication, or recruitment from other tissue, or both, must occur at least at the deep body sites, and that over all there is insufficient adipose tissue at any site to support a postulate of slow replication throughout undernutrition. Thus the final possibility, that replication ceases throughout undernutrition but starts again with rehabilitation, appears to be the most likely. This suggests that either there is no sensitive period as postulated by Brook (1972), or that the sensitive period is dependent upon body-weight, not age.

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