Deposition of fat in the body of the rat during rehabilitation after early undernutrition

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(*Received* 18 *March* 1977 – *Accepted* 17 *May* 1977)

1. Male and female rats aged 3 weeks were divided into two groups. One group of each sex was allowed unlimited access to the stock diet, the other group was given the stock diet in restricted amounts for 10 weeks so that the males gained only 19 g and the females 21 g in comparison with 176 g and 116 g for the well-nourished males and females respectively. The undernourished animals were then rehabilitated by being allowed the stock diet *ad lib.*

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

3. The undernourished rats failed to make a complete recovery and were significantly smaller than the controls of the same sex at 172 d of age when the experiment terminated.

4. The previously undernourished rats deposited significantly more fat in their bodies during rehabilitation than the control animals in the same number of days and over the same gain in body-weight.

5. There were no significant differences in the number of cells containing fat at the abdominal fat site between the undernourished and rehabilitated animals and the controls at any stage, nor were there any significant differences in apparent fat cell numbers between the control and rehabilitated animals at any of the other sites studied when the experiment ended at 172 d.

It is often suggested that overfeeding in early life predisposes to adult obesity. Brook (1972) postulated that the last 3 months of foetal life and the first year after birth is the sensitive period for the formation of adipose cells in man, and that growth of the adipose organ during this time is due to an increase in the number of cells. After the end of the first year an increase in size rather than number of fat cells predominates. Thus the effects of overnutrition after the sensitive first year are more easily reversed than if the overnutrition occurs earlier. This was an attractive hypothesis, but there are difficulties. Dauncey & Gairdner (1975) showed that the increase in size of the fat cells observed to take place during the first year after birth was fully adequate to accommodate all the fat laid down in the body, and Widdowson & Shaw (1973) pointed out that it is not only overnutrition early in life that leads to rapid deposition of fat, but that undernutrition followed by rehabilitation does the same thing. This observation was based on studies with pigs (Widdowson, 1974), and it had been made earlier on this species by McMeekan (1940) and Lister & McCance (1967), and on rats by Meyer & Clawson (1964). Ashworth (1969), in a study of the growth rates of children recovering from protein-energy malnutrition, also observed that there was a specific increase in fat in the body when the expected 'weight-for-height' had been reached, so that after recovery, previously malnourished children were fatter than well-nourished children of the same age.

The present investigation was undertaken to answer two questions: (1) what is the composition of the weight gain in terms of adipose and lean tissue of rehabilitating male and female rats severely undernourished for 10 weeks after weaning; (2) if there is a particularly rapid deposition of fat, is this associated with an increase in number or size of fat cells or both?

MATERIALS AND METHODS

Experimental design

Forty male and forty female Black-and-White Hooded rats aged 21 d were used. Five animals of each sex were killed at the beginning of the study, fifteen of each sex were given the stock diet (Oxoid Breeding Diet, Oxoid Ltd, Southwark Bridge Road, London) *ad lib.* throughout the experimental period and the remaining twenty of each sex were undernourished by being given only 2·5 g stock diet/d. This enabled them to gain weight very slowly over the 10 weeks of treatment, but the gain was much less (19 and 21 g for males and females respectively) than the corresponding values for the control animals (176 and 116 g respectively). After the 10-week period of undernutrition the animals were allowed *ad lib.* access to the stock diet.

The control and rehabilitating rats were caged individually. Severely undernourished animals are very susceptible to cold, and the rats that were being undernourished were kept four to a cage so that they could huddle and keep warm.

Killing and dissection of the animals

Five males and five females were killed at the beginning of the study (21 d of age), and five of each sex and group at the end of the period of undernutrition (92 d of age) and after 20 and 80 d of rehabilitation of the undernourished animals (112 and 172 d). The animals were killed by an intraperitoneal injection of Nembutal (sodium pentabarbitone; Abbott Laboratories Ltd, Queenborough, Kent). After each animal was killed it was weighed. The subcutaneous fat which extended along the front of the thigh and across the abdomen (inguinal abdominal sample) was removed from both sides of the animal. The pad of fat across the scapular region was also removed. The abdomen was opened and in the female rats the parametrial fat attached to the uterus was dissected out, while in the male the epididymal fat pads were removed. The remainder of the fat in the abdomen (perirenal fat), including that round the kidneys, but excluding that in the mesentery, was also removed. All samples were divided into two and each part weighed. One part was immersed in formal saline (9 g sodium chloride/l formalin) for measurement of size of cells, and the other part in diethyl ether for determination of triglyceride. The gastrointestinal tract was removed and weighed; it was then emptied and reweighed, and returned to the carcass. The 'empty' carcass was weighed and stored at $-20°$ for analysis.

Analysis of carcasses

Water. Total water was determined by vacuum-drying the carcass at 20° for 3 d to constant weight and calculating the loss of weight.

Protein. The dried carcasses were forced twice through a power-driven mincer. This resulted in a fine powder with fur distributed throughout. All samples were then re-dried. Nitrogen was determined by macro-Kjeldahl digestion 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).

Analysis of adipose tissue

Size of fat cells. Samples previously fixed in formal saline were frozen, sectioned, floated on water and the fat cells measured immediately. All sections were cut thicker than the largest cell diameter (117 μm). Measurements were made at $\times 100$ magnification against a calibrated eyepiece, after focusing to the site of maximum diameter. To allow for distortion, measurements of cell diameter were taken in two horizontal directions at 90° to

each other. Ten cells were measured in each direction for each section and four sections were cut from each sample; thus eighty measurements were made on each sample. All measurements were made within 7 d of removing the samples from the animals.

Determination of triglyceride. Samples of fat which had been stored in diethyl ether, and weighing between 30 and 2000 mg according to the amount available, were homogenized and filtered through glass-wool. The diethyl ether was removed by evaporation, and the remaining triglyceride weighed.

Statistical analyses

Analyses of variance between control and rehabilitated groups at each age were made using orthogonal contrasts to derive effects of sex, treatment and treatment–sex interactions with respect to all measured variables (Ridgman, 1975).

Polynomial analyses of plots of empty weights, total lipid (%) and weights of protein were carried out, using manual methods to derive the necessary irregularly-spaced orthogonal polynomial coefficients (Ridgman, 1975).

A computer program based on analysis of variance using orthogonal contrasts to derive effects of sex, treatment and treatment–sex interactions was used to analyse a series of factors derived from cell measurements. These factors were derived as follows: mean cell diameter was the average of eighty measurements; mean cell volume was obtained by calculating the volume of each cell $\left(=\frac{\pi d^3}{6}\right)$ and averaging over the eighty cells; weight of triglyceride (/cell) = mean cell volume × density of triglyceride = mean cell volume × 0·915 (density of triolein); cell number $= \frac{\text{weight of triglyceride in sample} \times \text{weight of whole tissue}}{\text{weight of tissue sample} \times \text{weight of triglyceride per cell}}$.

RESULTS

Body-weights and composition of the whole body

Fig. 1 shows the mean body-weights throughout the experiment of the four groups of animals that were alive until the end of the experiment. After 10 weeks of undernutrition both males and females grew rapidly in response to unlimited food, but did not reach the same weight as the well-nourished controls by the end of the experiment at 172 d. Fig. 1 suggests that the rehabilitating female rats caught up their well-nourished controls better than the corresponding males. This is true in terms of absolute body-weight, but when the difference between the weights of the control and rehabilitated animals at 172 d is expressed as a percentage of the weight of the controls the sex difference is not statistically significant.

Fig. 2 shows the polynomials calculated from the 'empty' carcass weights of all groups killed at each stage of the experiment. The ages at which the control animals weighed the same as the rehabilitated animals of 92, 112 and 172 d of age were determined from these polynomials. These ages were used as the starting points to derive the gain in body components of the controls (*a*) while they gained the same amount of weight and (*b*) in the same number of days as the treatment rehabilitated animals from 92 to 112 d and from 92 to 172 d. Table 1 shows the results for the 92–112 d period in the rehabilitating animals and corresponding controls, and Table 2 shows similar information for the 92–172 d period in the rehabilitating animals and corresponding controls. The values in Tables 1 and 2 for the amounts of protein and the amounts of total lipid were derived from the polynomials shown in Figs. 3 and 4. Tables 1 and 2 show that the same gain in weight was achieved by the rehabilitating animals in a shorter period than the controls. Less protein was deposited by the rehabilitating animals during the first 20 d of rehabilitation than by

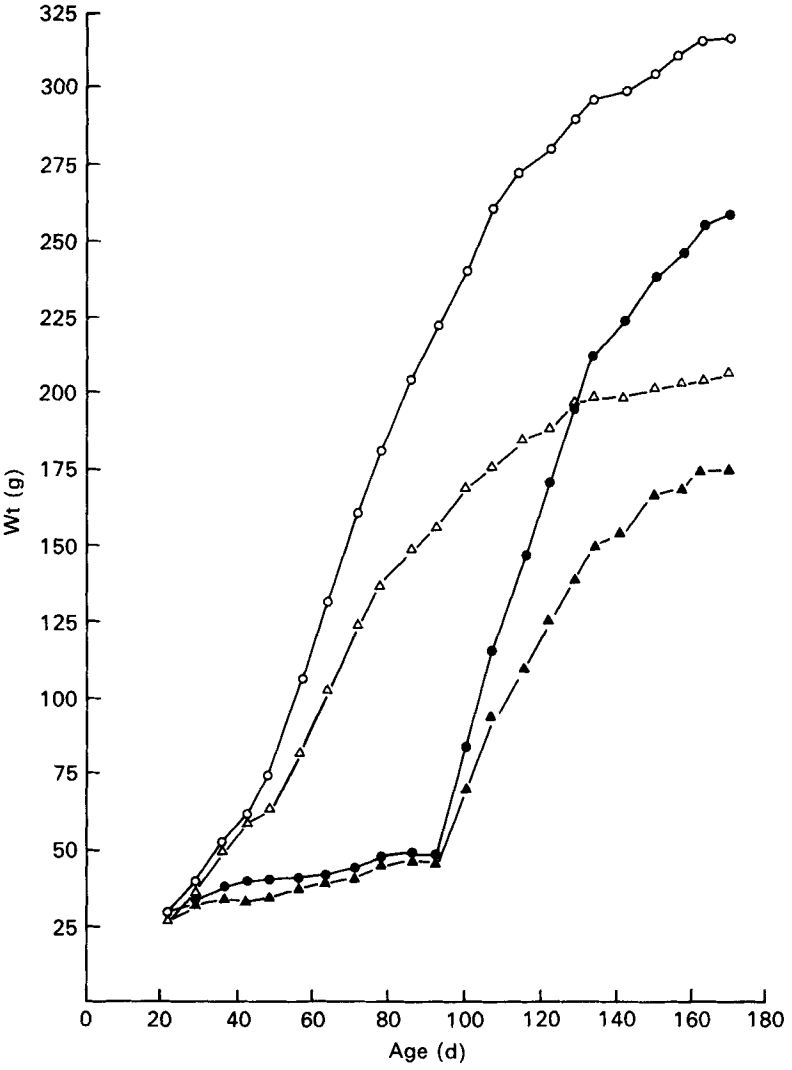

Fig. 1. Growth curves of well-nourished and undernourished-rehabilitated rats (for details of feeding regimen, see p. 202). (○), Control males; (△), control females; (●), undernourished-rehabilitated males; (▲), undernourished-rehabilitated females.

control animals gaining the same amount of weight, but the rehabilitating animals deposited as much, or more, protein than the control animals over the same period of time. Over the entire period of rehabilitation there was no difference in the amounts of protein deposited by control and rehabilitating animals (Table 2). However, significantly more total lipid was deposited in the rehabilitating animals than the control animals whether results are compared over the same increase in body-weight or the same period of time. This resulted in animals of the same body-weight having different body composition (Table 3).

There were no significant differences in the concentration of protein in the body within each sex, but there were significant differences in the concentration of total lipid. The rehabilitating animals had a significantly greater concentration of total lipid than the control animals of the same body-weight, and also greater than the heavier control

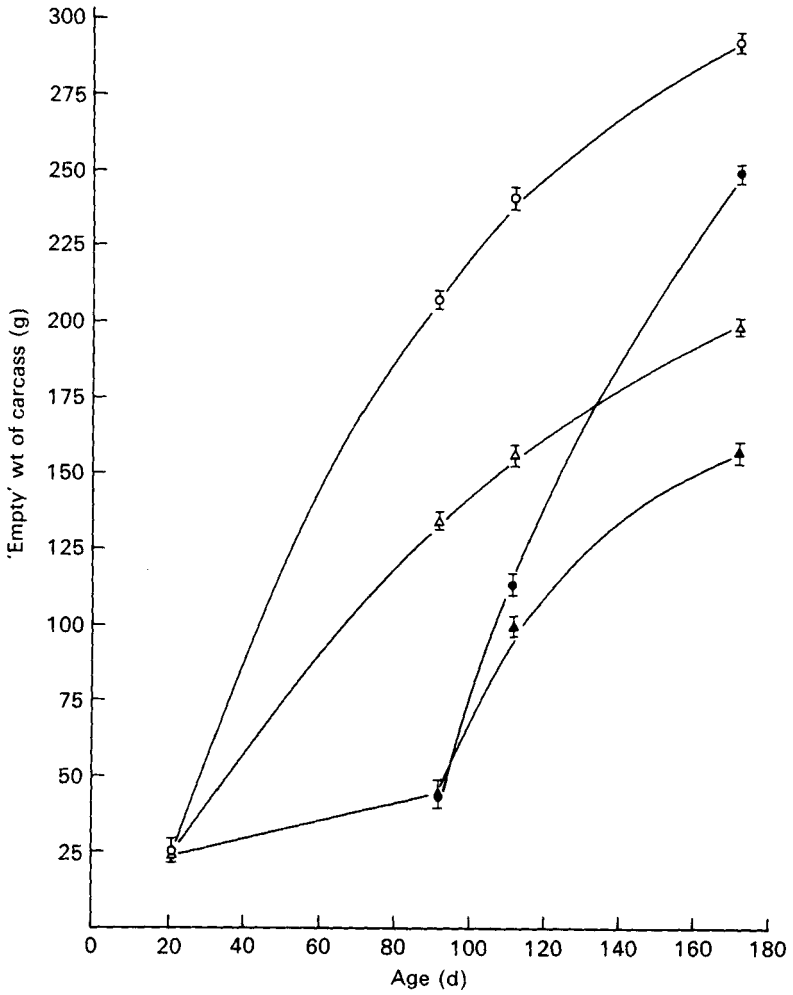

Fig. 2. Polynomials calculated for 'empty' weights (g) of carcasses of well-nourished and undernourished-rehabilitated rats (for details of dietary regimen, see p. 202). Points are mean values with their standard errors represented by vertical bars. (○) Control males; (△) control females; (●), undernourished-rehabilitated males; (▲), undernourished-rehabilitated females.

animals of the same age. Fig. 5 shows the polynomial calculated for the weight of total lipid in 100 g dry carcass and illustrates the rapid deposition of lipid. Fig. 5 suggests that the concentration of total lipid in the bodies of the males reached a maximum and had begun to decrease by the end of the experiment.

Size and number of fat cells

The only site from which adipose tissue could be dissected at every stage of the investigation was the abdominal. Results from this site for average volumes and number of cells for each group of animals are shown in Table 4. There was a large increase in cell volume in the controls between 21 and 92 d in both the males and females. Changes after this age were not significant in either sex because of the wide variation in cell volumes between animals. During the period of undernutrition the fat cells decreased in average volume, but there was a much greater variation in size of cells in the undernourished animals than in those of any

Table 1. *Composition of the weight gain in rats during the first 20 d of rehabilitation after a period of undernutrition, and in control rats of the same starting weight for the same weight gain (groups B and E respectively) and in the same period of time (groups C and F respectively)*

	Male			Female		
Group ...	A Re-habilitated	B Control	C Control	D Re-habilitated	E Control	F Control
Age range (d)	92–112	27–52	27–47	92–112	31–67	31–51
Period of feeding (d)	20	25	20	20	36	20
Wt gain (g)	70	70	55	55	55	32
Crude protein (nitrogen × 6·25) gain (g)	10·9	18·2	14·0	11·5	12·7	7·0
Total lipid gain (g)	9·6	3·2	2·2	6·8	2·7	1·0

Table 2. *Composition of the weight gain in rats during the entire period of rehabilitation after a period of undernutrition, and in control rats of the same starting weight for the same weight gain (groups B and E respectively) and over the same period of time (groups C and F respectively)*

	Male			Female		
Group ...	A Re-habilitated	B Control	C Control	D Re-habilitated	E Control	F Control
Age range (d)	92–172	27–117	27–107	92–172	31–119	31–111
Period of feeding (d)	80	90	80	80	88	80
Wt gain (g)	204	204	189	119	119	112
Crude protein (nitrogen × 6·25) gain (g)	52·6	53·5	48·2	30·7	27·5	26·2
Total lipid gain (g)	23·0	21·3	18·9	15·9	13·0	12·1

other group. As soon as rehabilitation was started the cells began to fill with fat. After 20 d rehabilitation the mean volume of the cells in the rehabilitating animals was not significantly different from that of the controls of the same age, and there was no further significant increase in cell volume at the abdominal site in the rehabilitating animals. The apparent increase in cell numbers in the abdominal site between 21 and 92 d was significant in the control animals ($P < 0.05$), but not in those that were undernourished, because of the wide individual variation. There was no significant difference between the numbers of identifiable cells in the control and rehabilitating animals at 92 d or during the rehabilitation period. However, there was an apparent over-all increase in cell numbers at the abdominal site between 21 and 172 d in both control and rehabilitating animals ($P < 0.05$ for both groups).

Table 5 shows the mean volumes and numbers of cells in the adipose tissue from the scapular, perirenal, epididymal and parametrial sites in all groups of animals at 172 d. There were no significant differences in either volume or number of cells between the rehabilitated and control animals in any of the sites. There were, however, large and consistent differences between the volume of the cells in the subcutaneous and deep body sites of both groups of animals, scapular fat having smaller cells than fat from the perirenal, epididymal or parametrial regions. As the amount of tissue sampled in all sites represented only 10% of the total body fat the results did not contradict that for gross body analysis, which showed that the rehabilitating animals had deposited more fat than the control animals by 172 d. In Tables 4 and 5 it can be seen that there is a consistent difference between cell volumes

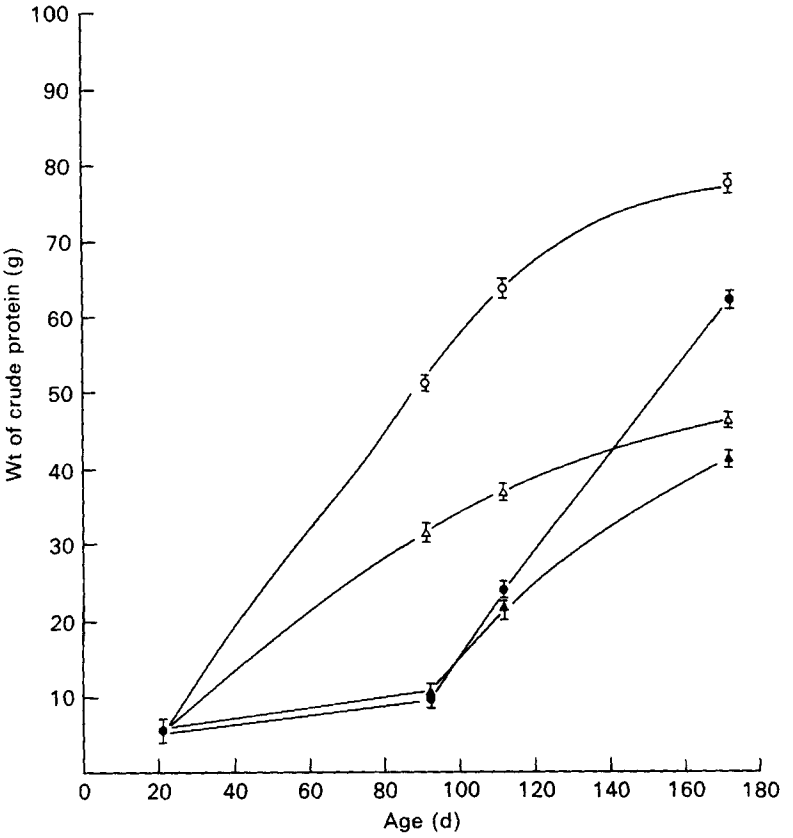

Fig. 3. Polynomials calculated for weight (g) of crude protein (nitrogen × 6·25) in the carcasses of well-nourished and undernourished-rehabilitated rats (for details of dietary regimen, see p. 202) Points are means with their standard errors represented by vertical bars. (○), control males; (△), control females; (●), undernourished-rehabilitated males; (▲), undernourished-rehabilitated females.

in the male and female control and fully rehabilitated animals; males had larger cells than females in all three sites examined.

DISCUSSION

One of the most striking features of the realimentation of animals from the undernourished state is the greater proportion of fat in the body compared with that in animals maintained on a high plane of nutrition throughout life (McMeekan, 1940; Meyer & Clawson, 1964; Lister & McCance, 1967; Widdowson, 1974). In the present experiment the realimented animals were significantly smaller than the controls of the same sex and age but they had the same amounts of total lipid. They therefore had a higher proportion of body fat than the controls that had never been undernourished. The relationships calculated for the deposition of fat cannot be extrapolated validly, so it cannot be stated that there would be more fat over a longer period. Allden (1970), suggested that, while the amount of fat increases more than any other tissue in response to high nutrient intakes, this effect was transient. The male rats in the present study appear to confirm Allden's (1970) suggestion; the proportion of fat in their bodies showed an initial surge on refeeding followed by a

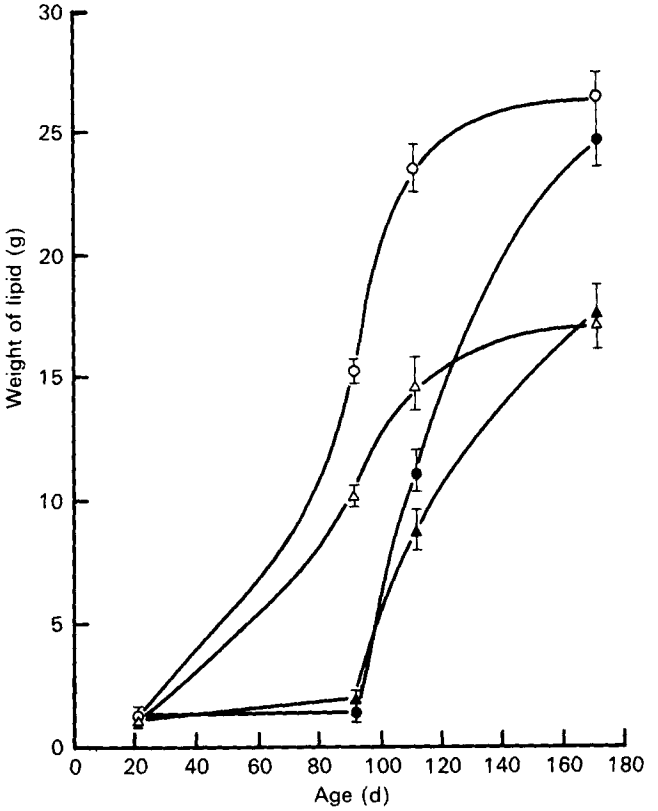

Fig. 4. Polynomials calculated for weight (g) of total lipid in the carcasses of well-nourished and undernourished-rehabilitated rats (for details of dietary regimen, see p. 202). Points are mean values with their standard errors represented by vertical bars. (○), control males; (△), control females; (●), undernourished-rehabilitated males; (▲), undernourished-rehabilitated females.

Table 3. *Body composition (g/kg wet weight) at the end of rehabilitation in the rats previously undernourished, and in the control rats of the same body-weight (groups B and E respectively) and the same age (groups C and F)*

Group ...	Male			Female		
	A Re-habilitated	B Control	C Control	D Re-habilitated	E Control	F Control
Age (d)	172	117	172	172	119	172
Wt (g)	249	249	292	163	163	197
Crude protein (nitrogen × 6·25) (g/kg)	251	252	264	252	232	235
Total lipid (g/kg)	106	88	86	99	84	82

decrease. The females did not show this decrease, and whether this is a permanent characteristic, or whether the decrease is merely delayed cannot be determined from the present results. Female pigs severely undernourished for 1, 2 and 3 years and then rehabilitated became progressively fatter the longer they had been undernourished, and in this species the fatness persisted into adult life (Widdowson, 1974).

At the end of the experiment the proportion of protein in the body tissue in the control

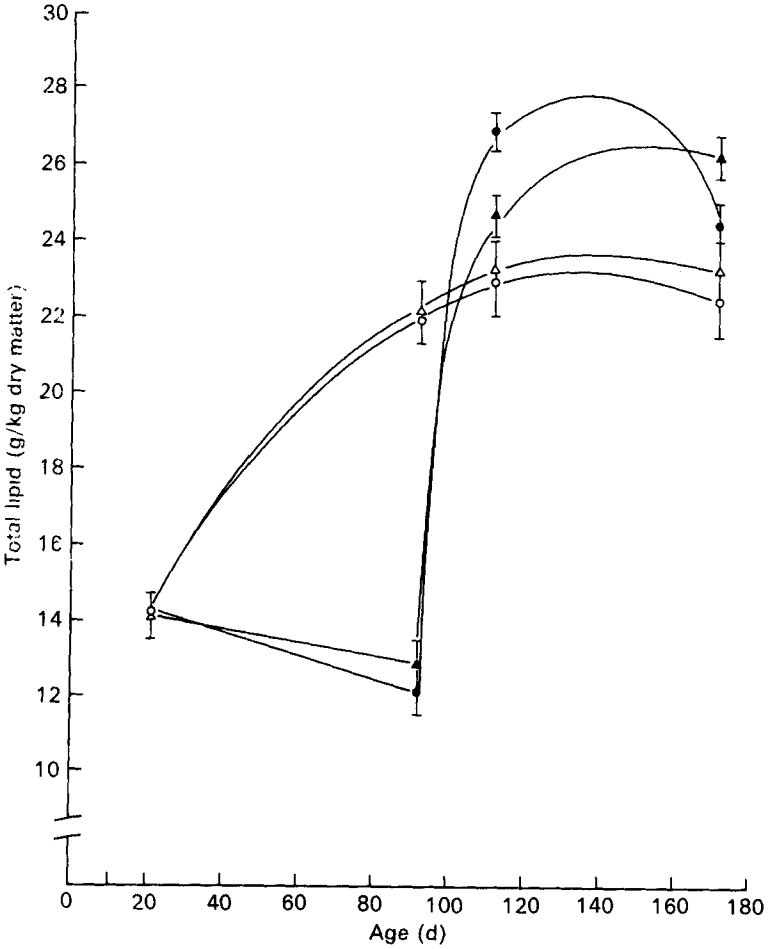

Fig. 5. Polynomials calculated for weight (g/kg dry matter) of total lipid in the carcasses of well-nourished and undernourished-rehabilitated rats (for details of dietary regimen, see p. 202). Points are mean values with their standard errors represented by vertical bars. (○), control males; (△), control females; (●), undernourished-rehabilitated males; (▲), undernourished-rehabilitated females.

and rehabilitated animals was not significantly different. In contrast to this, the proportion of lipid deposited in the rehabilitating animals was greater than that deposited in the control animals. This agrees with the concepts of growth postulated by Elsley, McDonald & Fowler (1964), who suggested that restricted nutrition, followed by complete rehabilitation, leads to a permanent change only in proportions of fat tissue, and not in that of muscle or bone. There is normally a maximum to the rate of protein deposition, and any excess of nutrients must be converted to fat. It seems that animals rehabilitating after severe under-nutrition eat more than will satisfy the demands for maintenance and the growth of lean tissue together with the appropriate amount of fat, so that excess fat is deposited in the body.

The different patterns of protein deposition observed in the rehabilitating rats are primarily due to the influence of the sex hormones. The rats were undernourished during the period when they normally reach puberty. Some sexual development went on (Widdowson, Mavor & McCance, 1964), but they did not reach sexual maturity until they were rehabilitated. The rapid deposition of protein in the realimenting males appears to be the

Table 4. *Average volumes ($\mu m^3 \times 10^3$) and numbers of cells ($\times 10^5$) at the abdominal site at each time of sampling in rats rehabilitated after a period of undernutrition and in control rats well nourished throughout the experimental period*

(Mean values with their standard errors)

		Male				Female			
Group ...		Rehabilitated		Control		Rehabilitated		Control	
Age (d)		Mean	SE	Mean	SE	Mean	SE	Mean	SE
21	Volume			53	9			46	5
	No.			25·2	4·1			18·5	1·9
92	Volume	14	7	152	16	33	13	121	7
	No.	94·1	24·2	83·9	13·1	61·4	13·1	47·9	7
112	Volume	169	22	347	106	180	37	141	32
	No.	67·9	3·3	60·9	13·3	52·5	16·8	78·1	13·5
172	Volume	250	50	254	34	168	30	110	17
	No.	136	13·5	128	16·3	91·8	17·1	134	12·6

Table 5. *Average volumes ($\mu m^3 \times 10^3$) and numbers of cells ($\times 10^5$), sampled at the scapular, perirenal, epididymal and parametrial sites at 172 d of age in rats rehabilitated after a period of undernutrition and in control rats well-nourished throughout the experimental period*

(Mean values with their standard errors)

		Male				Female			
Group ...		Rehabilitated		Control		Rehabilitated		Control	
Site of sampling		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Scapular	Volume	178	18	151	14	154	10	128	11
	No.	23·2	3·9	25·0	4·4	21·5	2·6	24·6	3·2
Perirenal	Volume	500	74	547	117	413	50	356	53
	No.	44·1	5·0	39·4	5·9	38·0	4·7	36·7	3·9
Epididymal	Volume	422	32	492	56		—		—
	No.	54·5	4·6	60·9	6·7		—		—
Parametrial	Volume		—		—	400	46	393	125
	No.		—		—	50·7	4·4	58·4	6·6

result of the androgens causing a non-specific increase in body proteins with growth. The initial rapid increase due to oestrogens in the female is transient and female protein deposition settles down to a slower pattern under the influence of growth hormone, insulin and possibly glucagon. Whether these rates of deposition can be altered by dietary or hormonal manipulation is presently being studied.

In discussing the results on the fat cells, it must be emphasized that it is by no means certain that the number of cells seen on a microscope slide represents the true number of fat cells in the sample. There may be other cells which do not contain enough fat to be identified. There is evidence that the number of cells containing fat remains constant throughout adult life in man and the rat (Hirsch & Han, 1969; Hirsch & Knittle, 1970), and any changes in the size of the adipose organ during adult life are due to changes in the volume of the cells. The results of the present experiment show that the number of fat cells at the abdominal site was not significantly different in undernourished, rehabilitated or well-nourished control animals at any stage of the experiment. Moreover, the number of

cells in the other sites sampled was the same in rehabilitated and control animals at 172 d when the experiment terminated. There are two possible explanations for these results. One is that cell replication continued until 172 d in all animals; but this seems unlikely in view of the results of Greenwood & Hirsch (1974), who used a tritiated-thymidine technique to show that the major phase of cell replication was complete by 40 d. If this timing of the replication period is correct, the severe undernutrition in the present experiment did not permanently affect the number of fat cells because the majority of cells had already been formed during the suckling period before undernutrition began, and it is this alternative explanation which appears to be most likely.

If the critical period of multiplication of fat cells in the human infant ends considerably before 1 year of age, the age suggested by Brook (1972), it is probable that most children who become marasmic have already developed the full number of fat cells before they became undernourished. An earlier termination of the critical period is not unreasonable, and it has even been suggested (Widdowson, 1977) that all the fat cells are formed by the time of birth. If this is so, then undernutrition will merely result in already existing fat cells being emptied, as was the situation in the undernourished rats. On refeeding there is the potential to lay down fat by expansion of these existing cells to an over-all level which is as great as or greater than in the normal child. There is no contradiction therefore between the rapid deposition of fat in infants recovering from undernutrition as found by Ashworth (1969) and the concept of a critical period postulated by Brook (1972). The critical period probably ends earlier than Brook suggested, and it seems likely that malnutrition of the human infant generally occurs after termination of proliferation of the fat cells.

The authors wish to thank Dr J. Kirtland for the help and facilities she made available, and Mr W. J. Ridgman for advice over the statistical analysis of the results. P.M.H. was supported by a Sir Walter Mulholland Fellowship and E.M.W. received a grant from the Medical Research Council.

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Printed in Great Britain