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The development of the weanling rat during nutritionally-induced growth retardation and during early rehabilitation

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1. Weanling (24-d-old) male rats were maintained at their body-weight for 1 month by restricting the intake of their normal diet. The animals were then rehabilitated for 0, 3, 7, 10 or 16 d. Control animals were given an unrestricted diet and some killed at the same body-weight as the experimental animals and others at the same age.

2. The forebrain, cerebellum, brain stem, heart, lungs, liver, spleen, kidneys, testes, and three sections of the alimentary tract were weighed, and DNA, RNA and protein contents were determined in the brain parts and liver.

3. During rehabilitation the weight of the body, corrected for the weight of the gut contents, showed a rapid gain between 7 and 10 d, which was synchronous with a rapid gain in weight of the testes.

4. The weight of the majority of organs relative to body-weight was maintained during both undernutrition and rehabilitation, the most marked exceptions being the stomach, which increased in relative weight during undernutrition, and maintained a high relative weight during rehabilitation, and the spleen, which lost weight during undernutrition, and on rehabilitation gained weight very rapidly to achieve a high relative weight.

5. The weight of the forebrain fell during undernutrition, due to a loss of water, and the weight of the brain stem rose. In the forebrain, DNA and the protein: DNA ratio were unchanged throughout, whereas a marked loss of RNA occurred during undernutrition, which was restored during rehabilitation.

6. The weight of the liver remained unchanged during undernutrition, despite increases in the amounts of DNA and protein. The amount of liver RNA decreased during undernutrition, but on rehabilitation showed an immediate and rapid increase. The variables measured in the liver were normal relative to body-weight, within 10 d of rehabilitation.

7. It is suggested that the growth occurring on rehabilitation is a balanced response to a single stimulus, partly mediated at the cellular level by RNA.

Undernutrition retards the rate of growth of young animals and children, and the permanency of the effect depends upon the timing, duration and severity of the insult (Widdowson & McCance, 1963). When nutritionally-retarded animals are rehabilitated, they initially gain weight faster than normal (Osborne & Mendel, 1915, 1916). The causes of this rapid weight gain are unknown, although it may be partly due to an increase in gut contents, as a result of gorging (McMeekan, 1940).

Rats that had been held at constant body-weight for 1 month by feeding a protein-deficient diet showed a rapid weight gain on rehabilitation (Dickerson, Hughes & McAnulty, 1972). Their body-weights did not, however, return to normal by 140 d of age, although the bone maturity score and liver weight did do so. Similar restriction of growth potential results from feeding restricted amounts of a normal diet after weaning (Løvtrup & Swanson, 1958; Winick & Noble, 1966). In these, and many other

nutritional studies, the rehabilitated animals were compared with controls of the same age, and little attention was paid to the relationship of the weights of the organs to the body-weight, although there is some evidence that the relative weights of the organs tend to remain normal (Winters, Smith & Mendel, 1927).

The ability of an organ to return to normal weight for age on rehabilitation is dependent on the behaviour of the cell populations that constitute the organs and tissues of the body. If malnutrition occurs during periods of cell proliferation, the possibility of complete recovery is said to be unlikely, but if it occurs during periods of cell enlargement, the chances of recovery are much greater (Winick & Noble, 1966).

The present paper reports part of a study of changes in different organs and tissues during the first 16 d of rehabilitation of rats whose body-weight had been held constant for 4 weeks from weaning. Control animals of the same body-weight and of the same age have been used throughout.

MATERIALS AND METHODS

Male black hooded rats were raised in litters of eight pups, weaned at 21 d of age, and at 24 d of age allocated randomly to one of three groups. The first group, consisting of fifty animals ('experimental'), were given amounts of their normal diet, so restricted that there was no significant change in body-weight for 28 d. Ten animals were killed at the end of this period of undernutrition, and the remainder allowed *ad lib.* access to the stock diet. Ten rats were then killed after 3, 7, 10 and 16 d of rehabilitation. The experimental animals were allowed free access to water. Control animals were allowed free access to food and water, and ten animals were killed at the same age as the undernourished ones ('age controls') and ten at the same body-weight ('weight controls'). A total of 150 rats were used.

The animals were killed with chloroform, and the brain (dissected into forebrain, cerebellum and brain stem (Dickerson & McAnulty, 1972)), liver, heart, lungs, spleen, stomach, small intestine, large intestine, kidneys and testes were removed. The brain parts and liver were weighed immediately, cooled rapidly on solid CO_2, and stored at $-15°$ until analysed. The heart, lungs, spleen, kidneys and testes were weighed and discarded. The sections of the alimentary tract were weighed, the contents were washed out, and the sections were blotted dry and reweighed. The length of the small intestine was measured before emptying.

DNA and RNA were extracted from the brain parts and liver by the method of Munro & Fleck (1966). DNA was determined by the diphenylamine method (Burton, 1956), as modified by Giles & Myers (1965), and RNA by the ultraviolet absorption method (Munro & Fleck, 1966). Protein was determined in 0·1 M-sodium hydroxide extracts by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

The body-weights of the undernourished animals were maintained practically constant during the 4 weeks of food restriction, whereas those of the control animals

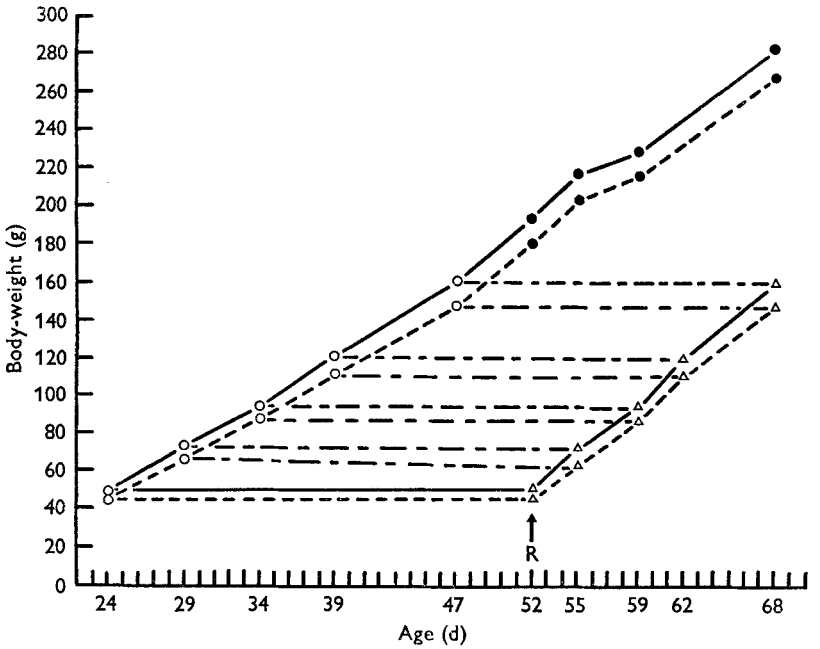

Fig. 1. Effect of undernourishing weanling male rats for 28 d and of subsequent rehabilitation, on body-weight. Both the body-weight (——) and the body-weight minus the weight of the gut contents (– – – –) are shown; the relationship between the experimental animals and the weight controls is also indicated (— – —); (△), experimental; (○), 'weight controls'; (●), 'age controls'; R, rehabilitation.

rose by 148 g (Fig. 1). On rehabilitation, the body-weights of the experimental animals rose by 110 g in 16 d, and those of the controls by 87 g in the same period. The mean rate of increase (± SEM) was 7·0 ± 0·2 g/d in the experimental animals, compared with 5·4 ± 0·3 g/d in normal animals of the same age ('age controls'), and 5·1 ± 0·2 g/d in normal animals of the same weight ('weight controls').

The experimental animals rehabilitated for 3 d had a greater amount of material in their alimentary tract than their 'weight controls' ($P < 0·001$). Thus, the body-weight minus gut contents was below that of the 'weight controls'. Subsequently the weight of the gut contents of the experimental animals did not differ significantly from that of the 'weight controls'. Corrected values for the rate of increase of body-weight were 6·2 ± 0·2 g/d for the experimental animals, 5·4 ± 0·2 g/d for the 'age controls' and 4·4 ± 0·2 g/d for the 'weight controls'. The differences between the rates for the experimental animals and both sets of controls were significant ($P < 0·02$ and $P < 0·001$, respectively).

The rate of increase in body-weight, uncorrected for gut contents, showed two peaks, between 52 and 55 d of age and between 59 and 62 d of age, respectively (Fig. 2), whereas there was only one peak in the rate of increase of the corrected body-weight, between 59 and 62 d of age.

Of the organs weighed, the weight of the brain was the least affected. The growth of the three brain parts of the undernourished rats was retarded to a similar degree

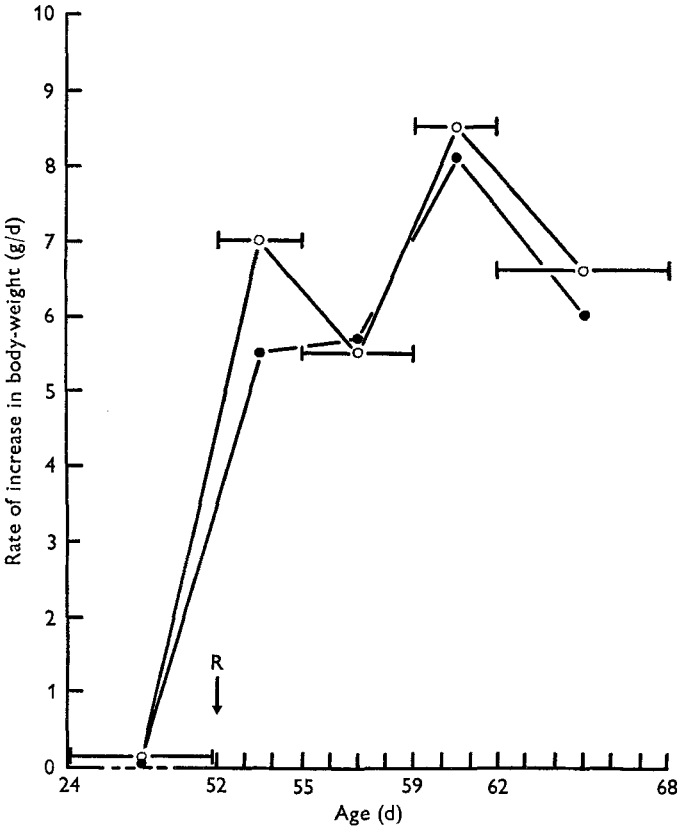

Fig. 2. Rate of increase in body-weight, and in body-weight minus the weight of the gut contents, during rehabilitation of male weanling rats following 28 d undernutrition. Each point represents the mean of weights from ten animals. The horizontal bars indicate the periods over which the rates of increase are calculated; (○), body-weight; (●), body-weight minus weight of gut contents; R, rehabilitation.

(82–84%), when compared with that of the 'age controls'. However, after 16 d of rehabilitation, the weight of the brain stem had increased to that of the 'age controls', whereas the forebrain ($P < 0{\cdot}01$) and cerebellum ($P < 0{\cdot}001$) weighed significantly less than normal. When compared with the brains of the 'weight controls' (Table 1) the weight of the whole brain was slightly less than at the start of undernutrition, and this was due to a very significant decrease in the weight of the forebrain, caused by a significant fall in the percentage of water ($P < 0{\cdot}001$). In contrast, the weight of the cerebellum was the same as in the 'weight controls', whilst the brain stem was slightly heavier. The weight of the forebrain had increased to that of the 'weight controls' by the 16th day of rehabilitation, and the brain stem by the 7th day.

Neither undernutrition nor rehabilitation affected the total amount of DNA or the protein:DNA ratio in the brain parts. The amount of protein in each brain part was not significantly different from that of the 'weight controls', but was significantly less than that of the 'age controls'. On rehabilitation, the amount of protein in the brain stem had increased to the value for the 'age controls' within 7 d, whereas the amounts

Table 1. *Effect on the brain of undernourishing weanling male rats for 28 d and of subsequent rehabilitation for 3, 7, 10 or 16 d, compared with 'weight controls' and 'age controls' (animals killed at the same weight or age as undernourished rats)*

(Mean values and standard deviations for ten animals)

			Controls			Controls			Controls			Controls			Controls	
		0 d	'Weight'	'Age'	3 d	'Weight'	'Age'	7 d	'Weight'	'Age'	10 d	'Weight'	'Age'	16 d	'Weight'	'Age'
Age (d)	...	52	24	52	55	29	55	59	34	59	62	39	62	68	47	68
Forebrain:																
Wt (g)	Mean	0·85	0·92***	1·01***	0·89	0·93**	1·04***	0·90	0·95**	1·04***	0·92	0·96*	1·02***	1·00	0·98	1·07**
	SD	0·03	0·04	0·05	0·03	0·02	0·04	0·04	0·03	0·04	0·04	0·04	0·05	0·06	0·03	0·04
RNA (mg)	Mean	1·04	1·99**	1·47*	1·30	1·66	1·48	1·35	1·81***	1·52	1·63	1·73	1·44	1·89	1·56	1·55
	SD	0·37	0·73	0·32	0·45	0·25	0·51	0·19	0·17	0·22	0·47	0·49	0·56	0·41	0·30	0·34
Cerebellum:																
Wt (g)	Mean	0·18	0·17	0·22***	0·19	0·18	0·23***	0·19	0·20	0·23***	0·20	0·19	0·23***	0·21	0·20	0·24***
	SD	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·01	0·02	0·02	0·01
RNA (mg)	Mean	0·74	0·86	1·13***	0·85	0·91	1·20*	0·95	1·01	1·15	1·06	1·08	1·20	1·15	1·18	1·23
	SD	0·20	0·15	0·10	0·21	0·16	0·26	0·41	0·11	0·16	0·24	0·24	0·19	0·16	0·26	0·24
Brain stem:																
Wt (g)	Mean	0·24	0·22*	0·29***	0·25	0·24*	0·30***	0·26	0·25	0·29**	0·27	0·27	0·31**	0·29	0·28	0·31
	SD	0·01	0·02	0·01	0·01	0·01	0·01	0·01	0·02	0·02	0·02	0·02	0·02	0·03	0·02	0·03
RNA (mg)	Mean	0·60	0·70	0·76*	0·64	0·72	0·90**	0·70	0·92*	0·80	0·78	0·88	0·78	0·80	0·82	0·92
	SD	0·16	0·16	0·22	0·12	0·10	0·14	0·18	0·10	0·34	0·26	0·16	0·24	0·22	0·18	0·10
Whole brain:																
Wt (g)	Mean	1·27	1·32*	1·52***	1·33	1·34	1·56***	1·35	1·40*	1·56**	1·39	1·42	1·56***	1·51	1·47	1·62**
	SD	0·04	0·04	0·07	0·04	0·03	0·05	0·05	0·03	0·06	0·06	0·05	0·06	0·09	0·04	0·06
RNA (mg)	Mean	2·43	3·31**	3·35***	2·74	3·33**	3·52*	3·01	3·68**	3·49	3·59	3·79	3·37	3·90	3·64	3·75
	SD	0·32	0·75	0·30	0·53	0·32	0·54	0·45	0·22	0·46	0·71	0·65	0·71	0·40	0·59	0·32

Difference between mean values for experimental and control groups was statistically significant: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

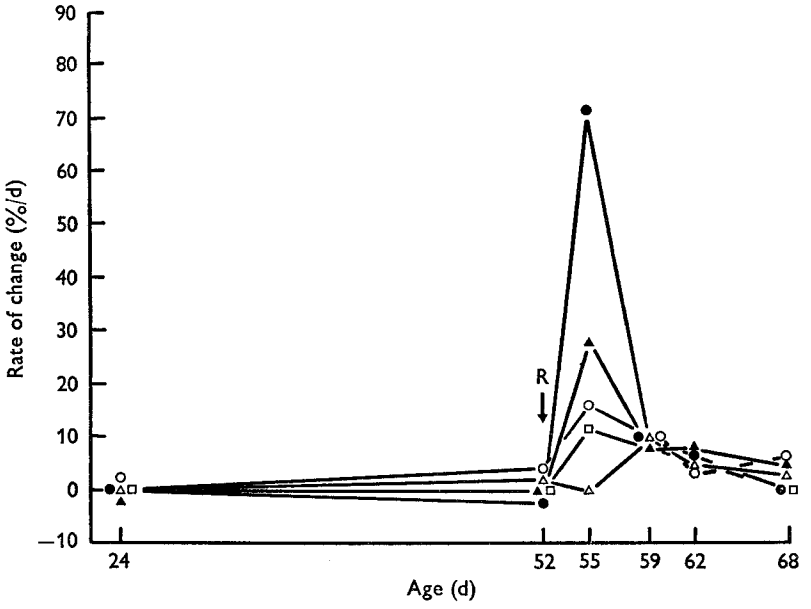

Fig. 3. Rates of change in the variables measured in the liver during the undernutrition and rehabilitation of weanling male rats. Values are the mean daily percentage increases over the period immediately preceding each point; (▲), weight; (△), total DNA; (●), total RNA; (○), total protein; (□), protein:DNA ratio; R, rehabilitation.

in the forebrain and cerebellum had not returned to these values by 16 d. The absolute amount of RNA in the forebrains of the undernourished animals was less than that in the 'weight controls' (Table 1), whereas there was no significant difference in that in the cerebellum and brain stem. On rehabilitation, the amount of RNA in the forebrain increased rapidly, and was similar to that of the 'weight controls' by the 10th day. The forebrain, cerebellum and brain stem of the undernourished animals contained significantly less RNA than those of the 'age controls'. The amount of RNA in the cerebellum and brain stem increased to that of the 'age controls' within 7 d of rehabilitation and that in the forebrain reached the 'age control' values within 10 d.

The values for all the variables measured in the livers of the undernourished animals were considerably lower than those of the 'age controls'. Of the organs discussed in this paper, only the weight of the spleen was retarded more than that of the liver at the end of the 28 d of undernutrition. On rehabilitation, the values for all the variables in the liver rose (Fig. 3), but the amount of RNA showed the greatest rise. The protein: DNA ratio returned to that of the 'age controls' within 10 d, whereas the liver weight, DNA, RNA and total protein were only about 60% of the values for the 'age controls' after 16 d rehabilitation.

The weight of the liver in the undernourished animals was similar to that of the 'weight controls' (Table 2), whilst the amounts of DNA and protein were significantly greater, and the amount of RNA was only about 50% of that of the 'weight controls'. Undernutrition did not alter the protein: DNA ratio. Most of the values for these measurements reached those of the 'weight controls' within 3 d of rehabilitation, but

Table 2. *Effect on the liver of undernourishing weanling male rats for* 28 *d and of subsequent rehabilitation for* 3, 7, 10 *or* 16 *d, compared with 'weight controls' and 'age controls' (animals killed at the same weight or age as the undernourished rats)*

(Mean values and standard deviations for ten animals)

			Controls			Controls			Controls			Controls			Controls	
		0 d	'Weight'	'Age'	3 d	'Weight'	'Age'	7 d	'Weight'	'Age'	10 d	'Weight'	'Age'	16 d	'Weight'	'Age'
Age (d)	...	52	24	52	55	29	55	59	34	59	62	39	62	68	47	68
Wt (g)	Mean	2·58	2·56	12·58***	4·72	4·19	13·88***	6·15	5·46*	13·99***	7·60	7·10	12·91***	9·88	9·80	16·68***
	SD	0·32	0·29	1·49	0·64	0·64	2·62	0·57	0·62	2·83	0·87	0·54	2·52	1·40	1·09	1·87
DNA (mg)																
	Mean	6·04	5·29*	15·05***	6·64	6·99	16·55***	9·37	9·06	17·06***	11·13	10·13	17·45***	13·39	12·95	22·76***
	SD	0·74	0·65	1·90	0·81	0·64	2·86	1·80	1·29	3·69	1·92	1·80	3·18	0·95	1·96	4·01
RNA (mg)																
	Mean	7·78	15·51***	62·95***	24·44	26·80	66·27***	33·82	31·44	68·10***	40·95	39·31	72·62***	47·38	49·07	86·83***
	SD	2·02	2·68	14·05	5·45	6·10	10·55	6·57	6·63	9·51	7·67	7·35	18·00	9·66	7·83	17·55
Protein (g)																
	Mean	0·53	0·42***	2·07***	0·79	0·68	2·36***	1·09	0·84***	2·58***	1·29	1·30	2·30***	1·69	1·63	3·03***
	SD	0·07	0·06	0·29	0·14	0·11	0·39	0·14	0·13	0·56	0·21	0·12	0·56	0·25	0·30	0·44
Protein: DNA																
	Mean	89	80	138***	120	99*	146**	119	93**	154**	117	121	132*	126	127	135
	SD	12	14	18	19	20	19	20	15	28	17	11	19	17	22	19

Difference between mean values for experimental and control groups was statistically significant: * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Table 3. *Effect on the weight (g) of various organs of undernourishing weanling male rats for 28 d and of subsequent rehabilitation for 3, 7, 10 or 16 d, compared with 'weight controls' and 'age controls' (animals killed at the same weight or age as undernourished rats)*

(Mean values and standard deviations for ten animals)

		Controls			Controls			Controls			Controls			Controls	
	0 d	'Weight'	'Age'	3 d	'Weight'	'Age'	7 d	'Weight'	'Age'	10 d	'Weight'	'Age'	16 d	'Weight'	'Age'
Heart:															
Mean	0·20	0·20	0·64***	0·26	0·28*	0·66***	0·38	0·36	0·68***	0·43	0·45	0·71***	0·55	0·55	0·82***
SD	0·02	0·02	0·08	0·02	0·02	0·05	0·02	0·03	0·06	0·04	0·04	0·08	0·06	0·04	0·08
Lungs:															
Mean	0·37	0·36	0·87***	0·44	0·49*	0·92***	0·62	0·59	1·06***	0·71	0·71	0·95***	0·83	0·83	1·19***
SD	0·03	0·05	0·15	0·05	0·03	0·05	0·05	0·06	0·16	0·07	0·11	0·12	0·07	0·07	0·18
Spleen:															
Mean	0·12	0·27***	0·63***	0·24	0·33***	0·60***	0·48	0·47	0·63*	0·55	0·59	0·69*	0·75	0·56**	0·63
SD	0·03	0·03	0·09	0·05	0·05	0·05	0·08	0·22	0·29	0·09	0·25	0·22	0·16	0·07	0·11
Kidneys:															
Mean	0·67	0·63	1·99***	0·90	0·94	2·14***	1·12	1·15	2·17***	1·32	1·37	2·20***	1·65	1·64	2·62***
SD	0·05	0·05	0·12	0·07	0·06	0·21	0·10	0·08	0·31	0·13	0·11	0·30	0·12	0·16	0·22
Testes:															
Mean	0·52	0·33*	2·21***	0·75	0·57***	2·25***	0·93	0·88	2·32***	1·38	1·32	2·34***	1·57	1·66	2·57***
SD	0·30	0·03	0·18	0·09	0·05	0·23	0·22	0·13	0·21	0·24	0·12	0·30	0·24	0·22	0·31
Stomach (empty):															
Mean	0·61	0·37***	1·02***	0·72	0·53***	1·04***	0·87	0·60***	1·06***	0·92	0·77***	1·07**	1·09	0·88*	1·25**
SD	0·08	0·04	0·07	0·07	0·04	0·08	0·07	0·04	0·09	0·07	0·08	0·15	0·13	0·08	0·12
Small intestine (empty):															
Mean	2·32	2·04	6·71***	3·59	3·45	6·96***	4·55	4·13**	7·10***	4·99	4·94	6·47***	6·76	5·56***	7·45*
SD	0·42	0·35	0·68	0·31	0·38	0·85	0·21	0·30	0·64	0·48	0·63	0·81	0·59	0·53	0·73
Large intestine (empty):															
Mean	0·33	0·25***	0·87***	0·51	0·43*	0·87***	0·66	0·57*	0·97***	0·69	0·64	0·94***	0·80	0·77	1·10***
SD	0·06	0·02	0·13	0·08	0·07	0·13	0·07	0·11	0·10	0·08	0·12	0·13	0·08	0·05	0·17

Difference between mean values for experimental and control groups statistically significant: * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

between 3 and 7 d the amount of protein rose above that of the 'weight controls', resulting in increased values for organ weight and for the protein:DNA ratio.

Of the remaining organs, the weights of the various sections of the alimentary tract, especially the stomach, were the least retarded by undernutrition, compared with the 'age controls'. The spleen was the most retarded, being only 19% of the normal weight. The weights of the heart, lungs, kidneys and small intestine in the undernourished animals were similar to those of the 'weight controls' (Table 3), whilst the weight of the spleen was much less, and that of the stomach, large intestine and testes was greater.

On rehabilitation, the weight of the spleen increased rapidly, and after 16 d was heavier than that of the 'weight controls' (Table 3). The stomach, small intestine and large intestine were significantly lighter than those of the 'age controls' after 16d rehabilitation. The weight of the stomach was, however, greater than that of the 'weight controls' throughout the 16 d of rehabilitation, whereas that of the small intestine was greater at 7 and 16 d, and that of the large intestine was greater initially, but was normal from the 10th day (Table 3). The weight of the testes reached the 'weight control' value within 7 d, whereas those of the heart, lungs and kidneys were comparable with those of the 'weight controls' throughout the period studied. The weights of the testes, heart, lungs and kidneys were approximately 65% of the values for the 'age controls' after 16 d rehabilitation.

The length of the small intestine increased slightly but significantly ($P < 0{\cdot}01$) during undernutrition, and during rehabilitation was similar to that of the 'weight controls'. After 16 d rehabilitation it was only 8% shorter than that of the 'age controls'.

DISCUSSION

The body-weights of weanling male rats that had been kept constant for 4 weeks by giving them reduced amounts of their normal diet showed an immediate rapid increase in weight when given free access to food, and part of this increase was due to gut fill. However, between 7 and 10 d of rehabilitation, a second peak in the rate of weight gain occurred, which was independent of the gut contents. Few of the organs studied were growing rapidly during this period (Table 3), and it seems likely that the rapid weight gain was due to muscle growth, for the gastrocnemius and quadriceps muscles have been shown to increase rapidly in weight between 7 and 10 d of rehabilitation, accompanied by an increased rate of DNA synthesis (Dickerson & McAnulty, unpublished results). In male rats there is normally a greater replication of muscle DNA than in either castrated males or in females (Buchanan & Pritchard, 1970). The effect in the male is similar to that caused by exogenous androgens (Cheek, Brasel & Graystone, 1968). The rapid increase in growth of skeletal muscle and in DNA synthesis (Dickerson & McAnulty, unpublished results) coincided with that in the testes in the present study, and a similar relationship has also been found between femur length and testes weight (Dickerson & Widdowson, 1960; Widdowson & McCance, 1960).

During undernutrition, the weight of many of the organs investigated maintained a

normal relationship to the body-weight. This has been reported previously in rats fed low-protein diets (Winters *et al.* 1927; Dickerson *et al.* 1972), but during protein restriction the heart and liver increased in weight. The muscles of the rat apparently maintain their correct weight relative to body-weight during both protein and energy restriction (Mendes & Waterlow, 1958; Dickerson *et al.* 1972; Dickerson & McAnulty, unpublished results). On rehabilitation, the organs that had deviated from the normal weight relative to body-weight during undernutrition tended to return to the normal relationship. After 16 d rehabilitation, the spleen, stomach and small intestine were the only organs with significantly abnormal weights relative to body-weight, and all three were heavier than normal. The thymus (McAnulty & Dickerson, 1973), anterior tibialis muscle and quadriceps muscle had normal relative weights after 16 d rehabilitation, whereas at that time the gastrocnemius was light in relation to body-weight (Dickerson & McAnulty, unpublished results).

The testes increased in weight during undernutrition, confirming earlier findings (Winters *et al.* 1927; Clarke & Smith, 1938; Widdowson & McCance, 1963; Widdowson, Mavor & McCance, 1964), but on rehabilitation, the weight relative to body-weight returned to normal within 7 d, due to the testes increasing in weight relatively more slowly than the body.

An increase in weight of the stomach during undernutrition has been reported before (Widdowson & McCance, 1963). The large intestine also increased in weight, whereas the weight of the small intestine did not change significantly. The marked increase in weight of the stomach is probably due to the fact that undernourished rats eat all their daily allowance of food at one time. During rehabilitation, the stomach remained heavier than that of the 'weight controls', and the small intestine also became heavier. This may be a response to a more rapid rate of food consumption during rehabilitation. The weight of the large intestine, however, returned to that of the 'weight controls'.

A marked decrease in weight of the spleen during undernutrition occurs in the rat (Mulinos & Pomerantz, 1940; Widdowson & McCance, 1960, 1963) and man (Trowell, Davies & Dean, 1954; Mugerwa, 1971), and is due principally to atrophy of the lymphoid tissue, although the Malpighian corpuscles are also reduced in size (Mulinos & Pomerantz, 1940; Stekel & Smith, 1970; Mugerwa, 1971). The thymus also decreases in weight (McAnulty & Dickerson, 1973). On rehabilitation the spleen rapidly increased in weight, and after 16 d the weight was greater than in the 'weight controls'. A similar rapid increase in weight also occurs in the thymus (McAnulty & Dickerson, 1973), but this organ returns to a normal weight relative to body-weight on rehabilitation.

It was found that the spleen showed a considerable variation in weight within groups, and this has been commented on previously (Widdowson & McCance, 1960). It is therefore necessary to interpret results obtained on the spleen with caution, as in some instances twice the standard deviation of the mean weight of the spleen almost equalled the mean itself (Table 3). The reason for the large standard deviations is unknown, but it was usually only one or two individual spleens in each group that caused the high value. The spleen is affected by stress and infections, and it may have

been undetected variables from these sources, operating on individual animals, that caused the idiosyncratic weights.

The small loss in brain weight that occurred during undernutrition was due to a significant fall in the percentage of water in the forebrain. There was no change in the total amount of DNA (a measure of cell number) or in the protein:DNA ratio (a measure of cell size). The weight of the forebrain has previously been found to fall (Dickerson & Walmsley, 1967; Graystone & Cheek, 1969; Dickerson *et al.* 1972), though this was not commented upon. The increase in weight of the brain stem is similar to that which occurs in the spinal cord (Dickerson & Walmsley, 1967; Dickerson, Dobbing & McCance, 1967). After rehabilitation, the brain stem was the only part of the brain whose weight returned to that of the 'age controls'.

The relative weight of the liver remained normal during both undernutrition and rehabilitation, except at 7 d of rehabilitation, when an increase in the amount of protein caused the liver weight to exceed that of the 'weight controls'. Despite the constancy of liver weight during undernutrition, the amounts of DNA and protein increased, and thus other liver constituents, including glycogen (Deane, 1944; Cardell, 1971) must have been decreasing.

The amount of liver RNA fell during undernutrition, increased very rapidly during the first 3 d of rehabilitation, and thereafter increased at a slower rate. A similar initial rapid rate of RNA synthesis during rehabilitation occurred in the thymus and muscles of these rats (McAnulty & Dickerson, 1973; Dickerson & McAnulty, unpublished results). This initial rapid increase in RNA synthesis was proportional to the rate of growth of the organ, suggesting that RNA plays an important role in the growth of rehabilitating organs. It is interesting to note, in this respect, that increased RNA synthesis is found in many rapidly growing systems, such as regenerating liver (Dykstra & Herbst, 1965) and developing embryos (Caldarera, Barbiroli & Moruzzi, 1965).

In conclusion, the nutritional conditions imposed in this study resulted in an adaptive pattern of growth, which tended to maintain the majority of organs at a correct weight relative to body-weight. Deviations from this general pattern appeared to be specific adaptations to the nutritional conditions, with the exception of the deviations of the spleen, and thymus (McAnulty & Dickerson, 1973), which do not appear to adapt in the same way. The relationship to body-weight was maintained during rehabilitation, despite the fact that the body-weight was increasing much more rapidly than normal. It would therefore appear that the rapid growth of the organs following undernutrition is a balanced response, under the control of a single stimulus. The immediate response of RNA to rehabilitation suggests that RNA may be involved in the mediation of this stimulus in the individual organs.

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REFERENCES

Buchanan, T. A. S. & Pritchard, J. J. (1970). *J. Anat.* **107**, 185.
Burton, K. (1956). *Biochem. J.* **62**, 315.
Caldarera, C. M., Barbiroli, B. & Moruzzi, G. (1965). *Biochem. J.* **97**, 84.
Cardell, R. R. Jr (1971). *Am. J. Anat.* **131**, 21.
Cheek, D. B., Brasel, J. A. & Graystone, J. E. (1968). In *Human Growth* p. 306 [D. B. Cheek, editor]. Philadelphia: Lea and Febiger.
Clarke, M. F. & Smith, A. H. (1938). *J. Nutr.* **15**, 245.
Deane, H. W. (1944). *Anat. Rec.* **88**, 39.
Dickerson, J. W. T., Dobbing, J. & McCance, R. A. (1967). *Proc. R. Soc.* B **166**, 396.
Dickerson, J. W. T., Hughes, P. C. R. & McAnulty, P. A. (1972). *Br. J. Nutr.* **27**, 527.
Dickerson, J. W. T. & McAnulty, P. A. (1972). *Resuscitation* **1**, 61.
Dickerson, J. W. T. & Walmsley, A. L. (1967). *Brain* **90**, 897.
Dickerson, J. W. T. & Widdowson, E. M. (1960). *Proc. R. Soc.* B **152**, 207.
Dykstra, W. G. Jr & Herbst, E. J. (1965). *Science, N.Y.* **149**, 428.
Giles, K. W. & Myers, A. (1965). *Nature, Lond.* **206**, 93.
Graystone, J. E. & Cheek, D. B. (1969). *Pediat. Res.* **3**, 66.
Løvtrup, S. & Swanson, V. L. (1958). *Acta physiol. scand.* **43**, 51.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
McAnulty, P. A. & Dickerson, J. W. T. (1973). *Pediat. Res.* **7**, 778.
McMeekan, C. P. (1940). *J. agric. Sci., Camb.* **30**, 276.
Mendes, C. B. & Waterlow, J. C. (1958). *Br. J. Nutr.* **12**, 74.
Mugerwa, J. W. (1971). *J. Path.* **105**, 105.
Mulinos, M. G. & Pomerantz, L. (1940). *J. Nutr.* **19**, 493.
Munro, H. N. & Fleck, A. (1966). *Meth. biochem. Analysis* **14**, 113.
Osborne, T. B. & Mendel, L. B. (1915). *J. biol. Chem.* **23**, 439.
Osborne, T. B. & Mendel, L. B. (1916). *Am. J. Physiol.* **40**, 16.
Stekel, A. & Smith, N. J. (1970). *Am. J. clin. Nutr.* **23**, 896.
Trowell, H. C., Davies, J. N. P. & Dean, R. F. A. (1954). *Kwashiorkor*. London: Arnold.
Widdowson, E. M. & McCance, R. A. (1960). *Proc. R. Soc.* B **152**, 188.
Widdowson, E. M. & McCance, R. A. (1963). *Proc. R. Soc.* B **158**, 329.
Widdowson, E. M., Mavor, W. O. & McCance, R. A. (1964). *J. Endocr.* **29**, 119.
Winick, M. & Noble, A. (1966). *J. Nutr.* **89**, 300.
Winters, J. C., Smith, A. H. & Mendel, L. B. (1927). *Am. J. Physiol.* **80**, 576.

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