Polyamines and their biosynthetic decarboxylases in various tissues of the young rat during undernutrition

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1. Male weanling rats were maintained at a constant body-weight by feeding them reduced amounts of the normal diet for various periods up to 4 weeks. Control male rats were allowed free access to the normal diet and some were killed at the beginning of the experiment and others at the same ages as the experimental rats.

2. After killing by cervical dislocation the rats had their liver, quadriceps muscles and spleen removed. The tissues were weighed and the activities of the enzymes ornithine decarboxylase (ODC; *EC* 4.1.1.17) and *S*-adenosylmethionine decarboxylase (SAMD; *EC* 4.1.1.50) assayed in each tissue. In the liver the content of the polyamines (spermidine and spermine) and putrescine was also measured.

3. The liver and quadriceps muscles showed an over-all maintenance of weight during undernutrition, but the spleen lost weight during the first 7 d of undernutrition and then remained constant. The weight of the liver increased by approximately 50 % following the daily maintenance feed, but returned to its pre-feeding value by 24 h after feeding.

4. During the first 7 d of undernutrition ODC activity decreased in all three tissues, and remained fairly constant thereafter. In the liver there were marked increases in the activity of ODC during the first 4 h after the daily feed, but the activity then decreased to prefeeding values. SAMD activity tended to remain normal in the liver, decreased initially and then returned to normal in the quadriceps muscles, and remained normal initially and then decreased in the spleen. Hepatic SAMD activity showed no consistent response to the daily feed, but quadriceps SAMD activity increased significantly between 1 and 8 h after feeding.

5. Hepatic putrescine content remained constant during undernutrition whilst spermine increased slightly and was then maintained above normal for liver size. Hepatic spermidine content decreased initially and then remained constant. Putrescine increased slightly in response to the daily feed and spermidine increased considerably. Spermine content was unaffected by the daily feed.

6. It is suggested that the response of polyamine synthesis in the various tissues is primarily dependent upon the way in which nutrients are made available to the tissues. The maintenance of spermine content in the liver at the expense of spermidine may be related to differential changes in the nucleic acids.

The polyamines, spermidine and spermine, have been found to exhibit effects at most stages of nucleic acid and protein synthesis. They bind to and stabilize both DNA and RNA (Agrell & Heby, 1968, 1971), stimulate magnesium-dependent DNA and RNA polymerases (Raina & Jänne, 1970; Chiu & Sung, 1972), affect the methylation and aminoacylation of transfer RNA (Leboy, 1970; Aoyama & Chaimovich, 1973), stimulate the uptake of amino acids into the cell (Gibson & Harris, 1974), stabilize ribosomes and polyribosomes (Siekevitz & Palade, 1962), and facilitate the binding of ribosomes to the endoplasmic reticulum (Khawaja, 1971).

The polyamines are synthesized from ornithine and *S*-adenosylmethionine. Ornithine is decarboxylated by ornithine decarboxylase (ODC; *EC* 4.1.1.17) to form putrescine (Jänne & Raina, 1968; Russell & Snyder, 1968; Pegg & Williams-Ashman, 1968*a*), and this forms the diaminobutane backbone of the polyamines. *S*-Adenosylmethionine is decarboxylated by *S*-adenosylmethionine decarboxylase (SAMD; *EC* 4.1.1.50) to form *S*-adenosyl-(5′)-3-methylthiopropylamine (Pegg & Williams-Ashman, 1968*b*). A propylamine

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group from this compound is joined either to putrescine by spermidine synthase to form spermidine (Jänne, Schenone & Williams-Ashman, 1971), or to spermidine by spermine synthase to form spermine (Raina & Hannonen, 1971).

During undernutrition of weanling rats, increase of tissue DNA and protein contents is inhibited, whilst RNA content decreases (McAnulty & Dickerson, 1973, 1974; Dickerson & McAnulty, 1975). As the polyamines produce so many effects on nucleic acid and protein synthesis it is of interest to know how they react to a period of undernutrition. Short periods of starvation cause reductions in hepatic spermidine and putrescine concentrations (Siimes, 1967; Domschke & Söling, 1973), whilst SAMD activity is reduced and ODC activity completely lost (Jänne & Raina, 1969; Schrock, Oakman & Bucher, 1970; Domschke & Söling, 1973). Preliminary observations have also shown that hepatic ODC activity is lost during undernutrition, but can rapidly reappear in response to a single feed or rehabilitation (McAnulty & Williams, 1975*a*, *b*).

The purpose of the present study was to examine, in detail, changes in polyamine synthesis in various tissues during undernutrition. The method of undernutrition and the tissues selected for investigation were chosen to correspond with previous studies on nucleic acid and protein accumulation (McAnulty & Dickerson, 1973, 1974; Dickerson & McAnulty, 1975), to make direct comparisons possible.

MATERIALS AND METHODS

Black-hooded rats were reared in litters of eight pups for the first 3 weeks of postnatal life. At the end of this time the mother was removed and the pups left for 3 d to become accustomed to the mother's absence and to a completely solid diet. Male pups falling within a weight-range of 45–55 g on the 24th day of age were separated from the litters and caged individually. A total of 120 of these rats were then fed amounts of the normal diet which allowed only maintenance of the initial body-weight. The daily ration of food was given at 10.00 hours. On the 7th, 14th, 21st and 28th days of the restricted diet thirty of the rats were killed. On each of these days the thirty rats were divided into five groups of six. One group was killed before the daily feed, and the others 1, 4, 8 and 24 h after the daily feed. A group of six rats was killed at 24 d of age for initial controls, and four groups of six rats were allowed to feed normally and killed at 31, 38, 45 and 52 d of age to act as age controls. All control rats were killed at 10.00 hours, and all animals were killed by cervical dislocation.

Immediately after killing, the liver, spleen and both quadriceps muscles were removed, weighed, and used immediately for analysis. The livers and spleens were processed without any initial treatment, but the muscles were minced with scissors.

A membrane-free supernatant was prepared from the tissues by a modification of the method of Russell & Snyder (1968). The tissues were homogenized in a cooled Thomas glass homogenizer with a teflon pestle on a stainless steel handle in 5 vol. (w/v) of ice-cold 50 mM-sodium/potassium phosphate buffer (pH 7·0), containing 5 mM-pyridoxal-5′-phosphate, 5 mM-dithiothreitol and 2 mM-EDTA. The homogenizer was surrounded by ice throughout. After homogenization of liver samples, 3 ml of the homogenate was removed for extraction of the polyamines (see p. 75). The homogenates were then centrifuged for 20 min at 20000 *g* at 4°, and the resulting supernatants were used for enzyme analyses and soluble protein determinations.

The activity of ODC was measured by the $^{14}CO_2$ release method of Russell & Snyder (1968), but with 5 mM-dithiothreitol and 2 mM-EDTA added to the incubation medium. The incubation vessels were a modification of those described by Jones, Hampton & Preslock (1972), and were plastic liquid scintillation counting vials (Packard Instrument International S.A., Breda, Holland) with a 1·5 cm 3MM grade filter-paper circle (Reeve

Angle Scientific Ltd, London) affixed to the raised plastic ridge in the vial cap with three spots of Copydex. The filter paper discs were soaked with a 1·0 M solution of Hyamine hydroxide in methanol to absorb the released CO_2. The enzyme substrate was DL-ornithine-1-[carboxy-^{14}C]monohydrochloride (Radiochemical Centre Ltd, Amersham, Bucks.), diluted with L-ornithine monohydrochloride to give a final specific activity of 0·49 mCi/mmol.

The activity of SAMD was also measured by a $^{14}CO_2$ release method (Jänne & Williams-Ashman, 1971*a*). The incubation vessels were 15 ml conical glass centrifuge tubes with a silicone rubber vaccine cap (Jencons (Scientific) Ltd, Hemel Hempstead, Herts.). Pushed through the centre of the vaccine cap was a 50 mm disposable hypodermic needle filled with Copydex to make it gas-tight. Impaled on the needle was a filter paper circle similar to those used in the ODC assay, again soaked with 1·0 M-Hyamine hydroxide in methanol. The enzyme substrate was *S*-adenosyl-L-[carboxy-^{14}C]methionine (Radiochemical Centre Ltd, Amersham, Bucks.) diluted with *S*-adenosyl-L-methionine chloride to give a final specific activity of 6·19 or 6·3 mCi/mmol, depending on the isotope batch.

In both enzyme assays the reaction was stopped by injecting 1 ml of 2·4 M-citric acid into the incubation mixture. After a further 30 min incubation the filter paper discs were placed in liquid scintillation counting vials with 10 ml of scintillation fluid (toluene containing 36·2 mM-2,5-diphenyloxazole and 0·27 mM-1,4-di(2-(5-phenyloxazolyl)) benzene). Radioactivity was measured in a Philips Automatic Liquid Scintillation Analyser, which automatically corrected for quenching with an external standard. The activity of both enzymes was expressed as pmol $^{14}CO_2$ released during 30 min of incubation by 1 mg of soluble protein. Soluble protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

The polyamines and putrescine were extracted from the liver homogenates with alkaline *n*-butanol by the method of Raina (1963), as modified by Jänne, Raina & Siimes (1964). The amines were separated by paper electrophoresis and quantified colorimetrically after staining with ninhydrin (Raina, 1963; Raina & Cohen, 1966). Spermidine and spermine for standards were obtained from the Aldrich Chemical Co., Milwaukee, Wisconsin, USA, and putrescine from Koch-Light Laboratories, Colnbrook, Bucks.

Differences between the various periods of undernutrition and between different lengths of time after the daily feed were examined using a two-way analysis of variance for each of the parameters measured.

RESULTS

During the period of undernutrition imposed in this study there were no significant over-all changes in the body-weight of the experimental rats. The body-weight was kept at between 47 and 53 g throughout. The body-weight increased in response to the daily feed, but returned to its prefeeding range before the following day's feed.

The results of the analyses of variance are shown in Table 1. The pooled SEM for each parameter (derived from the analysis of variance) is also shown, and the pooled SEM's for periods of undernutrition and times after feeding are also shown in the figures and tables relating to each parameter.

The weights of the liver and quadriceps muscles showed no over-all change throughout the period of undernutrition (Fig. 1). The spleen, however, decreased to 39 % of its initial weight during the first 7 d of undernutrition, and then remained constant up to 28 d (Fig. 1). Despite over-all constancy of weight, the liver showed marked fluctuations in weight following the daily feed (Fig. 2, Table 1). The weight began to increase within the first hour after feeding, and continued to increase up to 8 h after feeding. This increase in weight was in the region of 50 % of the initial weight. By 24 h after feeding the liver had returned to its prefeeding weight. There was a tendency for the weight reached at 8 h to become greater as

Table 1. *Analysis of variance of differences between periods of undernutrition and between lengths of time after the daily feed for each of the parameters measured in this study*†

(Values represent the variance ratio *F*)

	SEM‡	Between periods of undernutrition (P) (df = 3 and 100)	Between times after feeding (T) (df = 4 and 100)	Interaction (P × T) (df = 12 and 100)
Liver weight	0·098	18·33***	68·67***	2·17*
Spleen weight	0·013	1·30	4·60**	3·40***
Quadriceps weight	0·026	18·44***	15·33***	5·78***
Liver ODC§	37·1	25·40***	258·28***	21·20***
Spleen ODC	6·2	4·04*	10·35***	3·48***
Quadriceps ODC	0·9	4·75**	3·75**	2·50**
Liver SAMD§	10·5	28·00***	37·00***	20·50***
Spleen SAMD	13·7	23·80***	13·20***	17·00***
Quadriceps SAMD	11·7	25·67***	29·00***	5·33***
Liver putrescine	0·024	10·00***	26·80***	8·13***
Liver spermidine	0·084	6·00**	69·50***	7·00***
Liver spermine	0·092	11·25***	3·88**	2·88**

Statistical significance: * $P < 0{\cdot}025$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

† For details see p. 75.

‡ Pooled SEM for each parameter derived from the analysis of variance.

§ ODC, ornithine decarboxylase (*EC* 4.1.1.17); SAMD, *S*-adenosylmethionine decarboxylase (*EC* 4.1.1.50).

undernutrition progressed. Thus the weight reached after 28 d was about 25 % higher than the weight reached after only 7 d (the difference being more than three times the pooled standard deviation). The significant interaction (Table 1) for liver weight indicates that the increase in weight attained with longer periods of undernutrition did not occur at all time points, and this is evident in Fig. 2. There were no marked fluctuations in the weights of the quadriceps muscles and spleen in response to the daily feed.

In all three tissues examined there were marked reductions in the activity of ODC in response to undernutrition (Fig. 3). The reductions occurred during the first 7 d of undernutrition, and thereafter there were only slight over-all changes in the activity of the enzyme. Towards the end of the undernutrition period the activity of ODC returned to the normal range, due to a reduction in enzyme activity in all three normal tissues beginning between 31 and 38 d of age. Like the weight of the liver, the activity of hepatic ODC showed marked fluctuations in response to the daily feed (Fig. 4, Table 1). ODC activity increased, on average, 85-fold during the first hour following feeding, and this took the activity to above both initial control and age control values. In all the animals except those undernourished for 14 d, the activity continued to increase and reached a maximum 4 h after feeding. The rats undernourished for 14 d showed no change in ODC activity between 1 and 4 h after feeding. In the same way that the peak liver weight that was reached after feeding tended to become greater as undernutrition progressed, the peak in hepatic ODC activity also tended to become greater (the activities at 4 h in the 7 and 28 d groups are separated by more than three times the pooled standard deviation). From 8 h onwards the hepatic ODC activity decreased and returned to the prefeeding values by 24 h after feeding. The significant interaction for liver ODC activity (Table 1) is due to the 14 d group not following the same pattern as the other three groups, and the reversal of the magnitude of enzyme activities

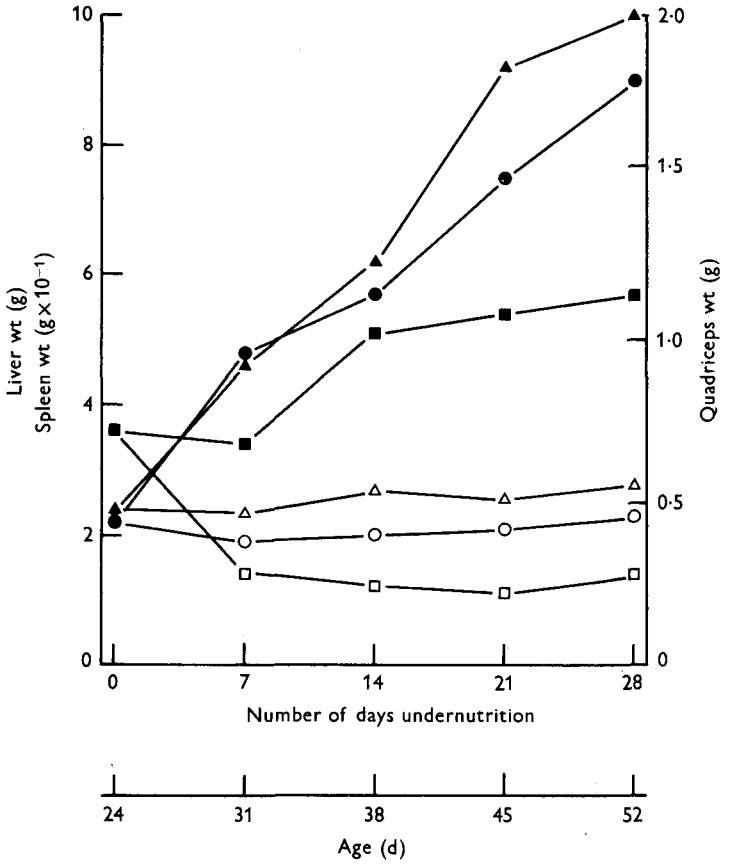

Fig. 1. The weights of the liver, spleen and paired quadriceps muscles of undernourished male weanling rats after various periods of undernutrition, and of control rats of the same age. The undernourished rats were not given their daily maintenance food quota on the day they were killed. Values are means for six animals; (●), control liver; (○), experimental liver; (▲), control quadriceps muscles; (△), experimental quadriceps muscles; (■), control spleen; (□), experimental spleen.

between 4 and 8 h. The activity of quadriceps muscle and spleen ODC showed no marked fluctuations in response to the daily feed.

The activity of SAMD during undernutrition was different in each of the tissues examined (Fig. 5). In the liver the activity of SAMD tended to remain within the normal range. In the quadriceps muscles the activity of SAMD decreased during early undernutrition, but then increased again and was within the normal range between 21 and 28 d of undernutrition. SAMD activity in the spleen remained within the normal range during the first 14 d of undernutrition, but then fell sharply and remained at a low activity for the rest of the period of undernutrition.

In the livers and spleens of the undernourished rats there were no marked fluctuations in the activity of SAMD after the daily feed, and no consistent patterns of response could be detected (Tables 1, 2). In the quadriceps muscles a pattern of response tended to occur (Fig. 6, Table 1). During the first hour after feeding the activity remained constant or fell, but then increased steadily over the next 7 h. At 8 h after feeding the activity of SAMD was within the normal range for age, however long the period of undernutrition. Thereafter the

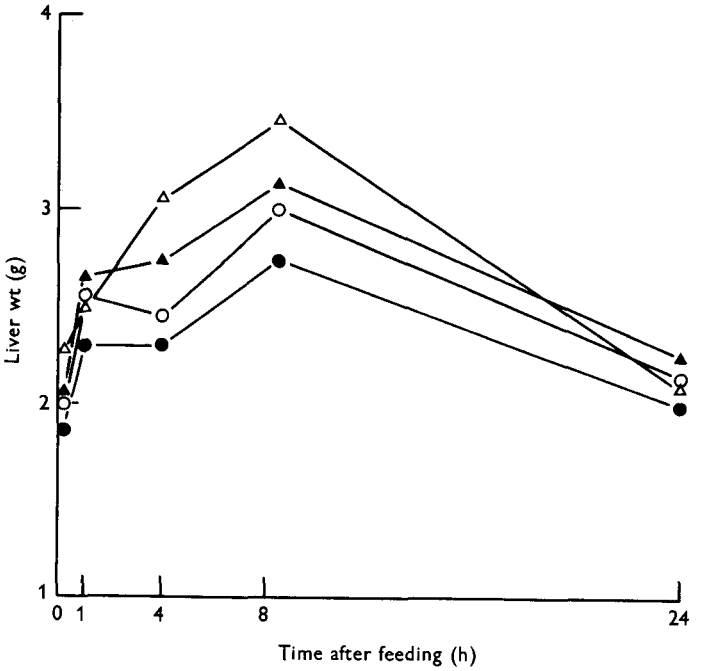

Fig. 2. The weight of the liver of undernourished male weanling rats at various times after the daily feed, and after various periods of undernutrition. Values are means for six animals; (●), animals undernourished for 7d; (○), 14d; (▲), 21d; (△), 28d. The pooled SEM for periods of undernutrition was 0·044, for times after feeding 0·049, and for the interaction 0·098.

enzyme activity tended to decrease. The slightly differing pattern of the response in the animals undernourished for 28 d resulted in a significant interaction (Table 1).

The hepatic content of the polyamines and putrescine showed a different response for each amine during undernutrition (Fig. 7). Putrescine content remained fairly constant throughout undernutrition, and thus fell below the age controls in which putrescine increased slowly. The content of spermidine decreased by about 50% during the first 7 d of undernutrition, and then remained fairly constant. It was below both age control and initial control levels throughout undernutrition. The spermine content of the liver continued to increase during the first 7 d of undernutrition, but then decreased between 7 and 14 d. Thereafter spermine remained fairly constant, and was maintained at a higher content than in the initial controls.

There were fluctuations in the hepatic putrescine content following the daily feed (Tables 1, 3), but no consistent pattern was evident. There was a tendency for putrescine to rise to a peak 8 h after feeding, but in the rats undernourished for 7 and 14 d there was an equally high content at 1 h. The spermidine content of the liver increased during the first 8 h after feeding (Fig. 8, Table 1), the average increases being 57 % of the prefeeding content. Between 8 and 24 h the spermidine content returned to values similar to those before feeding. The fluctuations in hepatic spermine content following the daily feed were only slight (Tables 1, 4). Because of this, the concentration of spermine in the liver decreased as liver weight increased, so that at 8 h spermine concentration was within the normal range of the initial controls. As the weight of the liver decreased between 8 and 24 h, the spermine concentration returned to its prefeeding value.

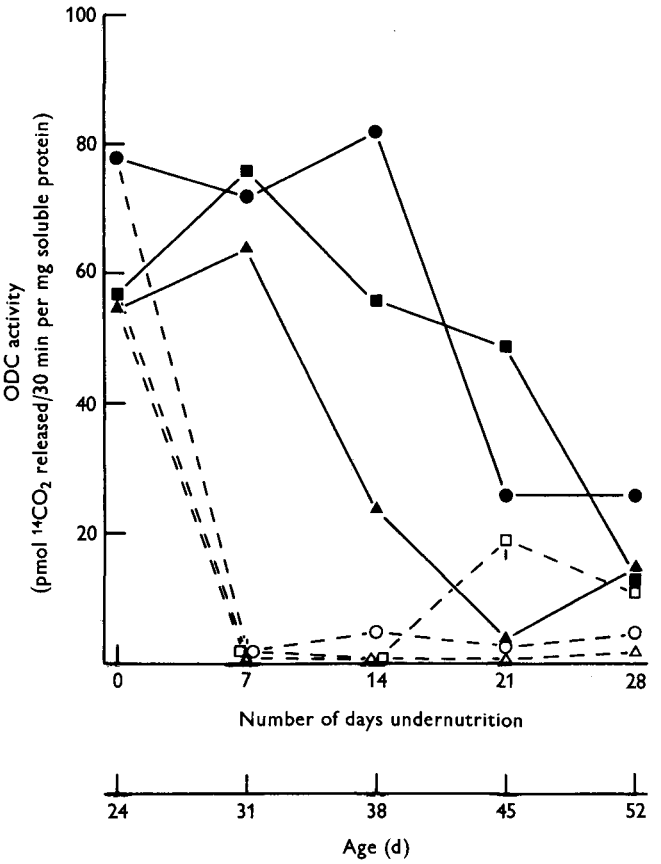

Fig. 3. The activity of ornithine decarboxylase (ODC, *EC* 4.1.1.17) in the liver, spleen and paired quadriceps muscles of undernourished male weanling rats after various periods of undernutrition, and of control rats of the same age. The undernourished rats were not given their daily maintenance food quota on the day they were killed. Values are means for six animals; (●), control liver; (○), experimental liver; (▲), control quadriceps muscles; (△), experimental quadriceps muscles; (■), control spleen; (□), experimental spleen.

DISCUSSION

During the period of undernutrition imposed in this study there was no over-all change in the body-weight of male weanling rats for 4 weeks. As has been shown previously (McAnulty & Dickerson, 1974; Dickerson & McAnulty, 1975), there were no over-all changes in the weights of the liver and quadriceps muscles during undernutrition, but the spleen lost about 60 % of its initial weight.

Despite the over-all maintenance of liver weight there were marked fluctuations in both weight and polyamine synthesis of the liver in response to the daily feed. The weight of the liver increased by approximately 50 % during the first 8 h after feeding, and the increase tended to become greater as undernutrition progressed. A similar rapid increase in liver weight has been reported in meal-fed rats (Leveille & Chakrabarty, 1967), and it has been shown that some of the increase is due to a reduction in protein breakdown (Garlick, Millward & James, 1973). Also in meal-fed rats there is an uptake of glucose from the meal into the liver, and this is incorporated into fatty acids (Leveille, 1967) or stored in the hepatocyte as glycogen (Deane, 1944). Thus the marked increase in weight of the liver of

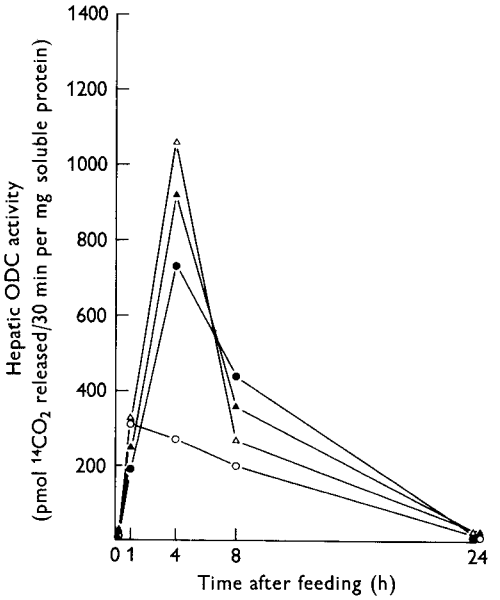

Fig. 4. The activity of ornithine decarboxylase (ODC, *EC* 4.1.1.17) in the liver of undernourished male weanling rats at various times after the daily feed, and after various periods of undernutrition. Values are means for six animals; (●), animals undernourished for 7d; (○), 14d; (▲), 21d; (△), 28 d. The pooled SEM for periods of undernutrition was 16·6, for times after feeding 18·6, and for the interaction 37·1.

the undernourished rats was probably due to uptake and storage of nutrients from the meal, combined with alterations in the rate of protein catabolism. Following metabolism the products of the nutrients would pass into the general circulation, and the weight of the liver return to its prefeeding value.

A reduction in the rate of protein catabolism would partly explain the marked increases that occur in hepatic ODC after feeding, due to a general increase in the amount of protein. However, in meal-fed rats the increase in liver protein is of the order of 20 % in 8 h (Garlick *et al.* 1973), but the increase in ODC activity in this study was of the order of 30000 %. It therefore seems likely that specific induction of the enzyme was occurring.

The stimulus causing the increase in hepatic ODC activity after feeding is unknown. In meal-fed rats a similar increase in hepatic ODC activity occurs 4 h after the daily feed (Hayashi, Aramaki & Noguchi, 1972; Yager, Lichtenstein, Bonney, Hopkins, Walker, Dorn & Potter, 1974). If the increase in activity is hormonally-mediated it would require, at the time of feeding, a marked secretion of one of the hormones that affect hepatic ODC (principally growth hormone and corticosteroids), because an activity peak of the magnitude obtained in this study requires a substantial hormonal stimulus 4 h previously (Morris & Fillingame, 1974). Whilst the possibility that such an hormonal stimulus does occur cannot be dismissed, it seems unlikely. It is more probable that ODC activity is stimulated by the flow of nutrients via the hepatic portal system. In starved rats hepatic ODC can be stimulated by the intubation of casein hydrolysate into the stomach (Fausto, 1969), and a comparable result is obtained if only histidine, lysine and arginine are intubated (Fausto, 1971). Arginine intubated individually also causes a marked increase in ODC activity, but ornithine has only a small effect (Fausto, 1971). It may be, therefore, that the supply of arginine in the diet is important in changes in the activity of ODC. It is unlikely that the changes in ODC activity are due to alterations in the pyridoxine content of the diet (ODC activity being

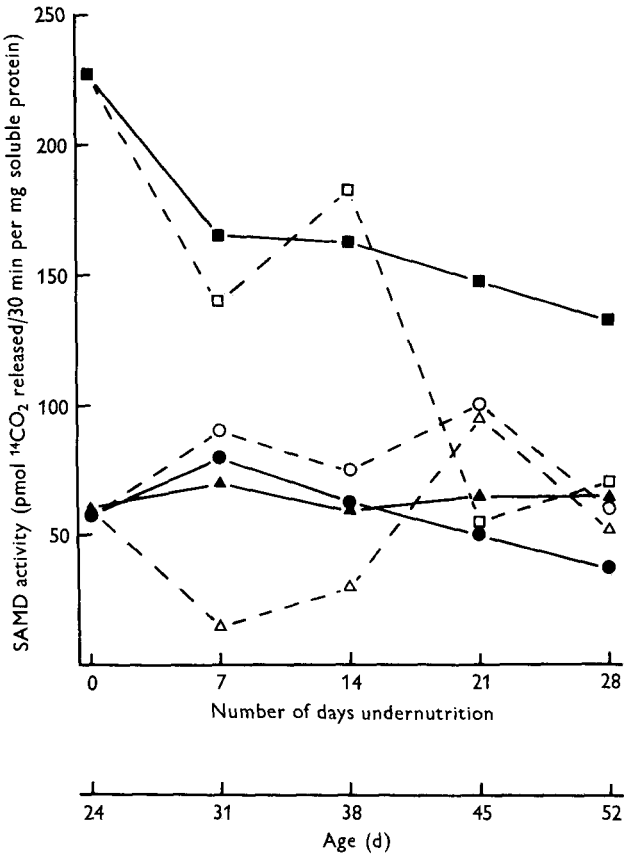

Fig. 5. The activity of *S*-adenosylmethionine decarboxylase (SAMD, *EC* 4.1.1.50) in the liver, spleen and paired quadriceps muscles of undernourished male weanling rats after various periods of undernutrition, and of control rats of the same age. The undernourished rats were not given their daily maintenance food quota on the day they were killed. Values are means for six animals (●), control liver; (○), experimental liver; (▲), control quadriceps muscles; (△), experimental quadriceps muscles; (■), control spleen; (□), experimental spleen.

Table 2. *The activity of S-adenosylmethionine decarboxylase (pmol $^{14}CO_2$ released/30 min per mg soluble protein) in the liver and spleen of undernourished male weanling rats at various times after the daily maintenance feed, and after various periods of undernutrition.*

(Mean values for six animals)

Time after feeding (h)	Liver Period of undernutrition (d)				Spleen Period of undernutrition (d)			
	7	14	21	28	7	14	21	28
0	91·2	73·8	101·1	60·2	141·8	183·2	54·9	68·7
1	115·2	108·9	156·2	99·9	71·6	99·2	70·6	76·4
4	56·2	64·5	73·4	135·0	57·8	98·6	141·4	125·8
8	48·5	81·5	120·2	68·0	130·4	116·9	132·2	84·0
24	102·9	67·8	95·6	55·9	150·7	167·3	95·9	76·4

SEM for periods of undernutrition = 4·7.
SEM for times after feeding = 5·3.
Pooled SEM for interaction = 10·5.

SEM for periods of undernutrition = 6·1.
SEM for times after feeding = 6·9.
Pooled SEM for interaction = 13·7.

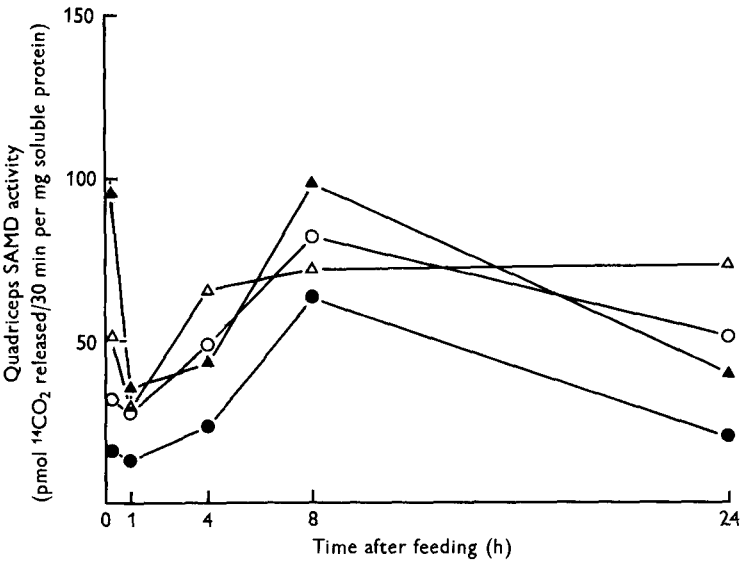

Fig. 6. The activity of *S*-adenosylmethionine decarboxylase (SAMD, *EC* 4.1.1.50) in the paired quadriceps muscles of undernourished male weanling rats at various times after the daily feed, and after various periods of undernutrition. Values are means for six animals; (●), animals undernourished for 7d; (○), 14d; (▲), 21d; (△), 28d. The pooled SEM for periods of undernutrition was 5·2, for times after feeding 5·9, and for the interaction 11·7.

dependent on pyridoxal-5'-phosphate (Jänne & Williams-Ashman, 1971*b*)), because after 5 weeks on a pyridoxine-deficient diet the activity of hepatic ODC of rats is slightly above normal (Sturman & Kremzner, 1974).

Unlike ODC, the activity of hepatic SAMD was maintained at normal levels during undernutrition. This is different to starvation during which SAMD activity becomes low or undetectable (Domschke & Söling, 1973). This difference is probably due to the fact that putrescine is an essential co-factor for SAMD (Pegg & Williams-Ashman, 1969) and remains normal for the size of the liver during undernutrition, but becomes undetectable during starvation (Domschke & Söling, 1973). The putrescine content of the liver would be maintained during undernutrition by the daily bursts in ODC activity. Despite the bursts in ODC activity, the putrescine content of the liver showed only slight elevations after the daily feed. This suggests that putrescine is metabolized almost as soon as it is synthesized, and this partly explains the marked increase in the spermidine content of the liver during the first 8 h after feeding. This increase cannot be entirely due to putrescine formation because before feeding spermidine content is below normal despite a normal putrescine content and normal SAMD activity. During starvation there is a marked reduction of hepatic *S*-adenosylmethionine (Schlenk, 1965), and thus the provision of this compound from methionine is probably also affected during undernutrition. The spermidine formed during the first 8 h after feeding is in turn converted to spermine, as evidenced by the decrease in hepatic spermidine between 8 and 24 h after the daily feed and the maintenance of spermine content above normal for liver size throughout undernutrition. During the first 7 d of undernutrition the content of spermine increased at a nearly normal rate, but this was halted as the rate of spermidine formation fell. This ability of the liver to continue converting spermidine into spermine has also been reported during starvation (Siimes, 1967), and suggests that the enzyme spermine synthase is unaffected by dietary alterations.

The maintenance of spermine at the expense of spermidine in the liver during under-

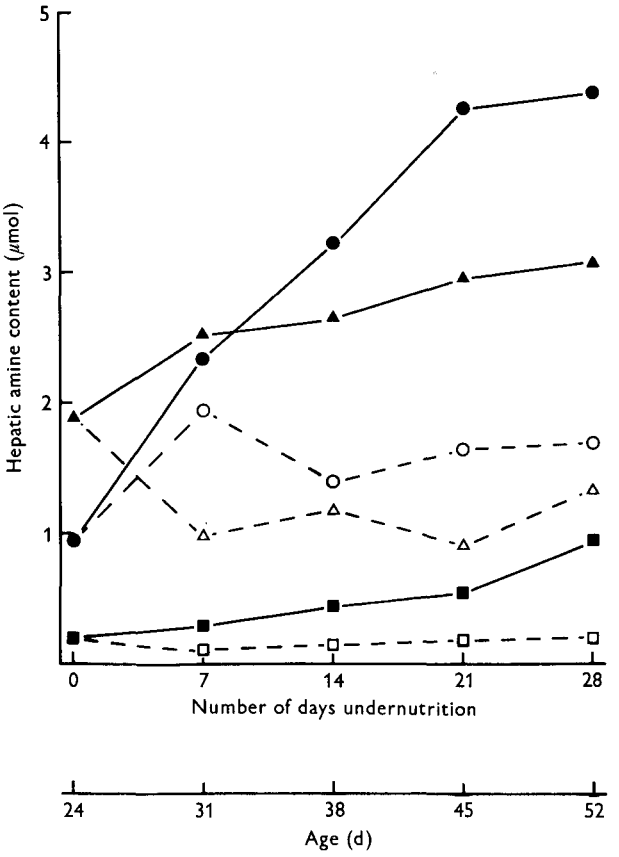

Fig. 7. The content of polyamines and putrescine in the liver of undernourished male weanling rats after various periods of undernutrition, and of control rats of the same age. The undernourished rats were not given their daily maintenance food quota on the day they were killed. Values are means for six animals; (●), control spermine; (○), experimental spermine; (▲), control spermidine; (△), experimental spermidine; (■), control putrescine; (□), experimental putrescine.

Table 3. *The hepatic content of putrescine (μmol) in undernourished male weanling rats at various times after the daily maintenance feed, and after various periods of undernutrition*

(Mean values for six animals)

Time after feeding (h)	Period of undernutrition (d)			
	7	14	21	28
0	0·128	0·147	0·176	0·233
1	0·285	0·262	0·157	0·232
4	0·167	0·145	0·241	0·178
8	0·274	0·215	0·272	0·289
24	0·132	0·146	0·172	0·248

SEM for periods of undernutrition 0·011.
SEM for times after feeding 0·012.
Pooled SEM for interaction 0·024.

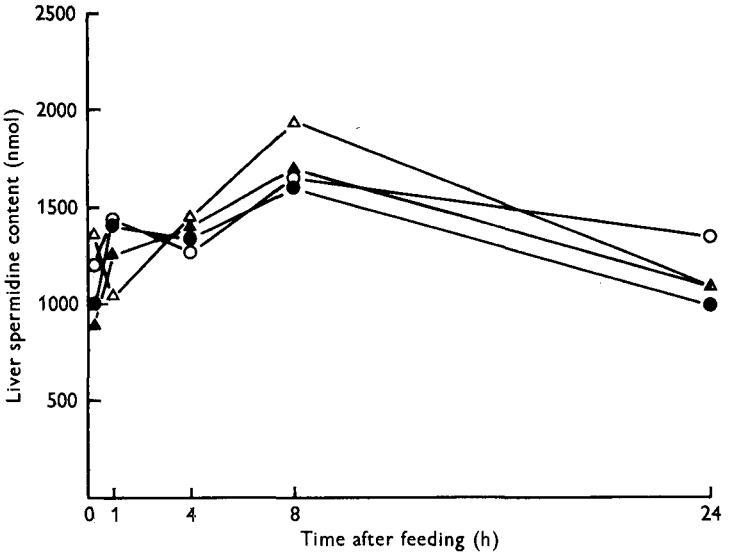

Fig. 8. The content of spermidine in the liver of undernourished male weanling rats at various times after the daily feed, and after various periods of undernutrition. Values are means for six animals; (●), animals undernourished for 7d; (○), 14d; (▲), 21d; (△), 28d. The pooled SEM for periods of undernutrition was 0·038, for times after feeding 0·042, and for the interaction 0·084.

Table 4. *The hepatic content of spermine (μmol) in undernourished male weanling rats at various times after the daily maintenance feed, and after various periods of undernutrition.*

(Mean values for six animals)

Time after feeding (h)	Period of undernutrition (d)			
	7	14	21	28
0	1·945	1·103	1·667	1·719
1	1·410	1·471	1·343	1·480
4	1·396	1·272	1·732	1·837
8	1·725	1·426	1·631	1·662
24	1·855	1·383	1·219	1·236

SEM for periods of undernutrition = 0·041.
SEM for times after feeding = 0·046.
Pooled SEM for interaction = 0·092.

nutrition is of especial interest because it has often been noted that changes in spermine tend to parallel those in DNA whilst changes in spermidine parallel those in RNA (Raina & Jänne, 1970). In the livers of rats undernourished in the same way as in this study, DNA remains constant throughout whilst RNA decreases (McAnulty & Dickerson, 1974). Whether this relationship between the polyamines and nucleic acids is of any significance remains to be elucidated.

The weight of the quadriceps muscles was maintained constant throughout undernutrition, and, unlike the liver, there were no fluctuations in muscle weight following the daily feed. This is probably due to the fact that the muscles receive a more uniform supply of nutrients than the liver during this type of undernutrition. The activity of ODC in the quadriceps muscles became low or undetectable during undernutrition, and showed no response to the

daily feed. This low ODC activity reflects the decrease in muscular protein synthesis that occurs during nutritional deficiencies (Waterlow & Stephen, 1968), and also the increase in pyridoxal-enzyme-specific protease activity (Katunuma, Kominami, Kobayashi, Hamaguchi, Banno, Chichibu, Katsunuma & Shiotani, 1975). The activity of SAMD, however, is not lost, and after an initial reduction in activity is maintained within the normal range for muscle size during the later stages of undernutrition. In addition, the activity of SAMD shows an increase between 1 and 8 h after the daily feed.

There are several possible reasons for this differing response of quadriceps SAMD to that of quadriceps ODC. It may be that SAMD activity is not present in the muscle in vivo, because putrescine content of the muscle is likely to be reduced as a result of the low ODC activity, and SAMD is putrescine-dependent (Pegg & Williams-Ashman, 1969). Therefore when the enzyme is assayed with exogenous putrescine, enzyme molecules that may have been inactive in vivo would be activated. However, this does not explain why SAMD activity increases after the daily feed. It is unlikely that the enzyme responds to a specific hormonal stimulus because ODC and SAMD invariably respond to the same hormones, and ODC exhibits no response. It may be that SAMD responds to a specific nutrient, possibly methionine via *S*-adenosylmethionine, which becomes available to muscle after the daily feed. The increase in SAMD activity despite increased muscle protein catabolism (Waterlow & Stephen, 1968) may be due to the stronger binding of pyridoxal-5′-phosphate to the SAMD molecule than to the ODC molecule (Feldman, Levy & Russell, 1972). It has been claimed that proteases attack pyridoxal-dependent enzymes at the lysine residue at which pyridoxal-5′-phosphate also binds (Katunuma *et al.* 1975), and if so, the pyridoxal-5′-phosphate would protect the enzyme against degradation.

The weight of the spleen decreased during the first 7 d of undernutrition, and then remained constant with no daily fluctuations in response to feeding. A similar decrease in weight occurs during protein restriction (Dickerson, McAnulty & Pope, unpublished results). This decrease in weight is due principally to a loss of lymphatic tissue (Mulinos & Pomerantz, 1940). The activity of ODC decreased to negligible levels during the first 7 d of undernutrition, and then showed little over-all or daily variation. The activity of SAMD did not decrease immediately, but remained at normal levels until the 14th day of undernutrition and then decreased. There is some evidence that in the thymus the activity of ODC may be confined to the lymphocytes (Atkins & Beaven, 1975). If polyamine-synthesizing enzyme activities are also higher in the lymphatic tissue of the spleen than in other splenic constituents then the loss of lymphocytes during undernutrition would partly explain the decrease of the enzyme activities. SAMD would remain elevated for longer than ODC because of its longer half-life and greater affinity for pyridoxal-5′-phosphate, and would only decrease markedly when lymphocyte loss became acute.

This study has shown that polyamine synthesis is reduced in various tissues of the young male rat as a result of undernutrition. The way in which polyamine synthesis is affected in each tissue depends upon the nutrient supply to the tissue and the response of the cells of the tissue to undernutrition. In the liver the content of spermine is maintained at the expense of spermidine, and this may be related to changes that occur in the nucleic acids.

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