

## Polyamines and their Biosynthetic Decarboxylases in Various Tissues of the Young Rat during Recovery from Undernutrition

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1. Weanling male and female rats were undernourished for 4 weeks and then rehabilitated by allowing *ad libitum* feeding. 2. During rehabilitation polyamine-biosynthetic enzymes were examined in the liver, spleen and quadriceps and gastrocnemius muscles. 3. During the first few hours of rehabilitation there was a marked increase in liver weight, accompanied by a very marked increase in ornithine decarboxylase activity. Increases in the activity of this enzyme in other tissues did not occur until between 2 and 7 days of rehabilitation, at which time there were further increases in enzyme activity in the liver. 4. *S*-Adenosylmethionine decarboxylase activity also showed marked fluctuations in activity in all the tissues examined. 5. Hepatic putrescine and spermidine concentrations also varied during rehabilitation, but spermine concentration remained relatively constant. Both spermine and spermidine were at normal concentrations in the liver from the 10th day of rehabilitation onwards. 6. In all of the tissues examined there were marked sex differences in the parameters studied, particularly in splenic and muscular ornithine decarboxylase activity. 7. In the tissues of the male rats, changes in polyamine synthesis paralleled changes in nucleic acid and protein synthesis.

Enhanced rates of polyamine synthesis are a common feature of rapidly growing tissues (Russell, 1973*a*), and this probably relates to the effects that the polyamines have on nucleic acid and protein synthesis. The polyamines have been shown to exert effects on transcription (Singh & Sung, 1972; Moruzzi *et al.*, 1974; Caldarera *et al.*, 1975), translation (Moruzzi *et al.*, 1968; Giorgi, 1970; Konecki *et al.*, 1975) and the methylation and aminoacylation of tRNA (Leboy, 1970; Aoyama & Chaimovich, 1973). In addition the polyamines bind to and stabilize the cell organelles involved in these processes: the nucleus (Busch *et al.*, 1967; MacGregor & Mahler, 1967), nucleolus (Gfeller *et al.*, 1972), ribosomes, polyribosomes and microsomal fraction (Siekevitz & Palade, 1962; Khawaja, 1971).

During rapid tissue growth the accumulation of polyamines is accompanied by marked changes in some of the enzymes that synthesize the polyamines. In particular, ornithine decarboxylase (EC 4.1.1.17), which forms putrescine from ornithine (Jänne & Raina, 1968), exhibits very marked increases in activity. *S*-Adenosylmethionine decarboxylase (EC 4.1.1.50), which forms *S*-adenosyl-(5′)-3-methylthio-

propylamine from which the propylamine groups of the polyamines are derived (Pegg & Williams-Ashman, 1968), also shows changes in activity, but these are less marked than those of ornithine decarboxylase. As yet there is little information on the other polyamine-synthesizing enzymes, spermine synthase and spermidine synthase, although their activities do alter in the regenerating liver after partial hepatectomy (Hannonen *et al.*, 1972).

A phase of rapid tissue growth occurs in the rat after a period of growth restriction caused by undernutrition (McAnulty & Dickerson, 1974), and this rapid growth is associated with rapid accumulation of nucleic acids and protein, especially RNA (McAnulty & Dickerson, 1973, 1974; Dickerson & McAnulty, 1975). This suggested that the polyamines might also be involved. It was shown that the activities of polyamine-synthesizing enzymes were decreased in various tissues during undernutrition, although there was a burst of increased enzyme activity in the liver after the daily maintenance feed (McAnulty & Williams, 1976). Preliminary studies have shown that the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase do show marked fluctuations in liver and skeletal muscle during recovery from undernutrition (McAnulty & Williams, 1975*a,b*). The purpose of the study reported in the present paper was to examine these changes more extensively in the tissues of rehabilitating rats, and to

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relate changes in the hepatic enzymes to changes in hepatic polyamine content.

## Materials and Methods

### Animals

Black-hooded rats from original Medical Research Council stock were used throughout. All rats were reared normally in litters of eight pups until 21 days old. The mother was then removed and the litters left for a further 3 days to allow adjustment to a solid diet and the mother's absence.

### Experimental procedure

At 24 days of age the rats were caged individually and allowed either free access to the normal diet (control group) or fed a restricted diet (experimental group). The experimental rats were allowed only enough of the normal diet (Oxoid Breeding Diet) to maintain their body weight constant for 28 days. The food was given in a single feed at 10:00h each day. After 28 days the rats were rehabilitated by allowing them free access to the normal diet. A total of 156 rats were subjected to this regimen, 78 of each sex. Six animals of each sex were killed after 0, 1, 4 and 8 h of rehabilitation, and after 1, 2, 3, 4, 5, 10, 15, 20 and 25 days of rehabilitation. Six control rats of each sex were killed on each of the days that corresponded to 0, 5, 10, 15, 20 and 25 days of rehabilitation in the experimental group (i.e. at 52, 57, 62, 67, 72 and 77 days of age).

The rats were killed at 10:00h (except those killed during the first day of rehabilitation) by cervical dislocation. The liver, spleen, both quadriceps muscles and both gastrocnemius muscles were rapidly removed and weighed.

### Preparation of tissue extracts

The excised tissues were processed immediately. Liver and spleen required no initial treatment, but muscle was minced with scissors. The tissues were homogenized in 5 vol. of cold 50mM-sodium/potassium phosphate buffer (pH7.0), containing 5mM-pyridoxal 5'-phosphate, 5mM-dithiothreitol (both obtained from Koch-Light Laboratories, Colnbrook, Bucks., U.K.) and 2mM-EDTA, in a cooled Thomas homogenizer. After homogenizing the liver samples, 3 ml of homogenate was removed for polyamine extraction (see below). The homogenates were centrifuged for 20 min at 4°C and 20000g ( $r_{av}$ , 10 cm). The resulting supernatants were used for ornithine decarboxylase and *S*-adenosylmethionine decarboxylase assays, and for soluble protein determinations.

Polyamines and putrescine were extracted from the samples of liver homogenate with alkaline butan-1-ol

by the method of Raina (1963), as modified by Jänne *et al.* (1964).

### Ornithine decarboxylase assay

The method used was a modification of that described by Russell & Snyder (1968), an adapted version of the incubation vessels described by Jones *et al.* (1972) being used. The vessels were plastic liquid-scintillation vials (Packard Instrument International S.A., Breda, Holland) with a hole bored near the top covered with Sellotape. Into these were placed 1.6 ml of the same buffer as was used for tissue homogenization, and 400  $\mu$ l of the enzyme supernatant preparation. After preincubation for 10 min at 37°C in a shaking water bath, 0.5  $\mu$ Ci (100  $\mu$ l) of DL-[carboxy-<sup>14</sup>C]ornithine monohydrochloride (The Radiochemical Centre Ltd., Amersham, Bucks., U.K.), diluted with unlabelled L-ornithine monohydrochloride (Koch-Light Laboratories) to give a specific radioactivity of 0.49 mCi/mmol, was added to each vial to give a final concn. of 0.5 mM-ornithine. The vial was immediately sealed with its plastic cap. Fixed in the cap with three spots of Copydex was a 1.5 cm 3MM-grade filter-paper disc (Reeve Angel Scientific Ltd., London E.C.4, U.K.), on to which three drops of a 1.0 M solution of Hyamine hydroxide in methanol (Koch-Light Laboratories) had been placed. The vials were incubated at 37°C for 30 min in the shaking water bath. The reaction was stopped by injecting 1 ml of 50% (w/v) citric acid through the Sellotape-covered hole in the vial, and the hole resealed with a further piece of Sellotape. Incubation was continued, with shaking, for a further 30 min. The filter-paper discs were removed from the vial caps and placed in another plastic scintillation vial containing 10 ml of toluene with 0.8% (w/v) 2,5-diphenyl-oxazole and 0.01% (w/v) 1,4-bis-(5-phenyloxazol-2-yl)benzene (both from Hopkin and Williams, Chadwell Heath, Essex, U.K.). Radioactivity was measured in a Philips automatic liquid-scintillation analyser, which automatically corrected for quenching by the use of an external standard. Ornithine decarboxylase activity was expressed as pmol of <sup>14</sup>CO<sub>2</sub> produced in 30 min by 1 mg of soluble protein.

### *S*-Adenosylmethionine decarboxylase assay

The activity of this enzyme was also measured by a <sup>14</sup>CO<sub>2</sub>-release method, that of Jänne & Williams-Ashman (1971a). For this assay the incubation vessels were 15 ml conical glass centrifuge tubes. The incubation medium was 40  $\mu$ l of 100mM-sodium/potassium phosphate buffer (pH7.4) containing 3mM-EDTA, 5mM-dithiothreitol and 2mM-putrescine. Samples (100  $\mu$ l) of the enzyme supernatant preparation were added to the incubation medium, and the tubes preincubated for 10 min at 37°C in the

shaking water bath. *S*-Adenosyl-L-[carboxy- $^{14}\text{C}$ ]-methionine (The Radiochemical Centre) ( $0.5\ \mu\text{Ci}$ ;  $10\ \mu\text{l}$ ) diluted with *S*-adenosyl-L-methionine chloride (Koch-Light Laboratories) to give a specific radioactivity of 6.19 or 6.36 mCi/mmol (depending on the isotope batch), was added to each tube to give a final concn. of 0.2 mM-*S*-adenosylmethionine. The tubes were sealed immediately with a silicone rubber vaccine cap [Jencons (Scientific) Ltd., Hemel Hempstead, Herts., U.K.], through the centre of which was a 50 mm disposable hypodermic needle (Gillette Surgical, Isleworth, Middx., U.K.) filled with Copydex to make it gas-tight. Impaled on the needle was a filter-paper disc similar to those used for the assay of ornithine decarboxylase activity, and again treated with 1.0 M-Hyammine hydroxide in methanol. The incubation conditions, method of stopping the reaction and measurement of  $^{14}\text{CO}_2$  released were the same as for ornithine decarboxylase. The citric acid was injected through the vaccine cap which resealed itself.

#### *Soluble protein determination*

Protein was measured in the enzyme supernatant preparations by the method of Lowry *et al.* (1951). Standards were prepared from bovine serum albumin (BDH Chemicals, Poole, Dorset, U.K.).

#### *Polyamine and putrescine determinations*

The polyamines and putrescine were separated and quantified by the method of Raina (1963) as modified by Raina & Cohen (1966). The amines were separated by electrophoresis on Whatman no. 1 filter-paper strips ( $25\text{ cm} \times 3\text{ cm}$ ), by using, as electrolyte, 0.1 M-citric acid/trisodium citrate buffer (pH 4.3). Portions of volume  $20\ \mu\text{l}$  of the amine extracts were used, and electrophoresis was conducted at a constant voltage of 8 V/cm for 2.5 h at ambient temperature ( $22^\circ\text{C}$ ). The electrophoretic mobility ( $m$ ) of spermine was  $1.25 \times 10^{-4}\text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ , that of spermidine was  $1.39 \times 10^{-4}\text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$  and that of putrescine was  $1.74 \times 10^{-4}\text{ cm}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ . The filter-paper strips were dried at  $70^\circ\text{C}$ , dipped in a solvent system of acetone/water/acetic acid (20:2:1, by vol.) containing 0.87% (w/v) ninhydrin and 0.09% (w/v) cadmium acetate, and developed at  $70^\circ\text{C}$  for 1.5 h. The red bands that developed were cut from the filter-paper strip, eluted in the dark for 1 h with a solvent system of acetic acid/ethanol/water (5:4:1, by vol.) containing 5% (w/v) cadmium acetate, and the  $A_{505}$  of the colour was measured. Spermidine and spermine for standards were obtained from the Aldrich Chemical Co., Milwaukee, WI, U.S.A., and putrescine was from Koch-Light Laboratories.

#### *Statistical treatment of results*

Means ( $\pm$ S.E.M.) were calculated, and the statistical significance of differences between means was tested by use of Student's *t* test.

## Results

#### *Body weight*

During the period of 28 days undernutrition there was no significant overall change in the body weight of the experimental animals. There were increases in body weight after the daily feed, but the weight returned to its pre-feeding value long before the next feed. At the end of the period of undernutrition the body weight of the males was 28% of that of the age controls, and that of the females was 38%. On rehabilitation the body weight increased rapidly in both sexes, and at a rate that was higher than that of the age controls and also normal animals growing over the same body-weight range. The rate of increase in weight was similar in both sexes for the first 13 days of rehabilitation, but thereafter the females gained weight more slowly than the males. After 25 days rehabilitation the deficit in weight of the males, compared with age controls, was 30%, and that of the females 13%. The females made a relatively better recovery than the males because of the lower weight of the control females.

#### *Organ weight*

The weight of the liver of both sexes was considerably below that of the age controls at the end of undernutrition (25 and 32% of control liver weights for males and females respectively). In both sexes the weight of the liver increased by about 50% during the first 8 h of rehabilitation, but then showed no further significant change between 8 h and 2 days. Liver weight increased steadily from 2 days onwards, and returned to normal in the females by the 20th day of rehabilitation. In the males liver weight had reached 82% of that of the controls ( $P < 0.02$ ) by the 25th day of rehabilitation.

At the end of the period of undernutrition, the weight of the spleen was 38 and 33% of that of age controls, for males and females respectively. During the first day of rehabilitation there was little variation in the weight of the spleen, but thereafter spleen weight began to increase in both sexes. The rate of increase became faster between 2 and 5 days in the males and between 3 and 5 days in the females. On the 5th day of rehabilitation there was no significant difference between control and experimental spleen weights, although in the males this was more likely due to large variations in control spleen weight than to a return to normal. The mean spleen weights of the male experimental and control rats were not similar until the 15th day of rehabilitation.

The weights of both muscles examined in the male rats at the end of undernutrition were 27% of those of the controls, whereas those of the female rats were 30% of controls for the quadriceps muscles and 33% for the gastrocnemius muscles. Neither muscle showed any change in weight during the first 2 days of rehabilitation, and in the female gastrocnemius no change during the first 3 days. Thereafter the muscles increased in weight, and from 20 days onwards the weights of both muscles were greater in the males than in females. Neither muscle returned to a normal weight for age in either sex during the 25 days of rehabilitation studied. The muscles of the males reached a weight that was about 70% of that of the controls, and the muscles of the females reached about 78%.

#### Ornithine decarboxylase activity

In all four tissues examined the optimum pH for measuring ornithine decarboxylase activity was found to be 7.0, the presence of pyridoxal 5'-phosphate was essential, 2mM-EDTA gave an average increase in the activity measured of 27%, and 5mM-

dithiothreitol gave an approximately 5-fold increase in the activity measured. In the experimental rats, in which ornithine decarboxylase had been stimulated by feeding, Michaelis-Menten kinetics were obeyed, and a  $K_m$  of about 0.125 was obtained for each tissue examined.

The response of hepatic ornithine decarboxylase activity during rehabilitation has been described elsewhere (McAnulty & Williams, 1975a), and is only described briefly here. Immediately before rehabilitation the activity of hepatic ornithine decarboxylase of male rats was not significantly different from that of controls, but that of the females was below normal. On rehabilitation the enzyme activity increased rapidly in the livers of both sexes, but much faster in the females than in the males. In both, the activity reached a peak at 4h, with the peak in the female livers being about twice that in the male livers. Between 8h and 2 days the enzyme activity remained fairly constant in the male livers, decreased between 2 and 3 days, but then increased again and remained constant between 4 and 10 days. The activity then decreased again, and returned to control values at 20 days. In the females the activity decreased between 1

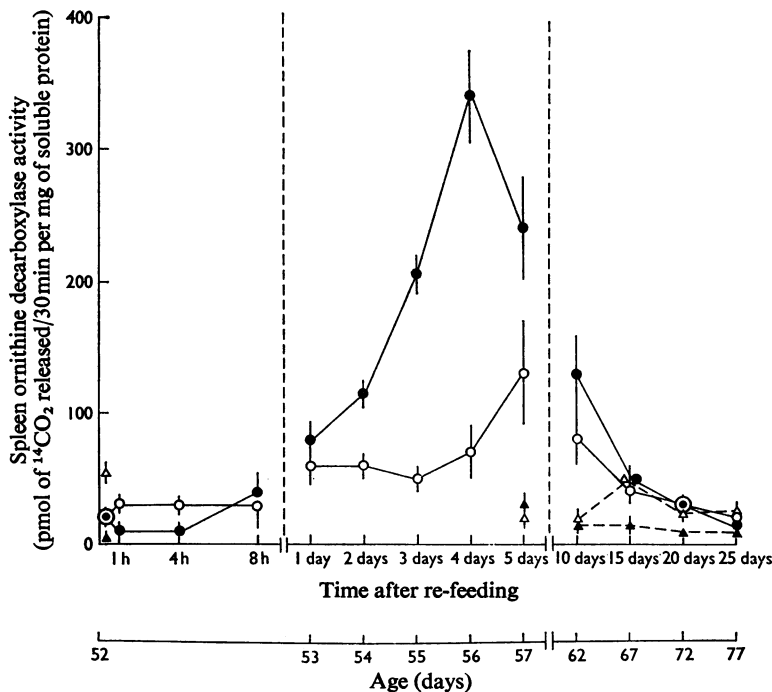


Fig. 1. Splenic ornithine decarboxylase activity during rehabilitation. Ornithine decarboxylase activity was measured in the spleens of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the s.e.m.

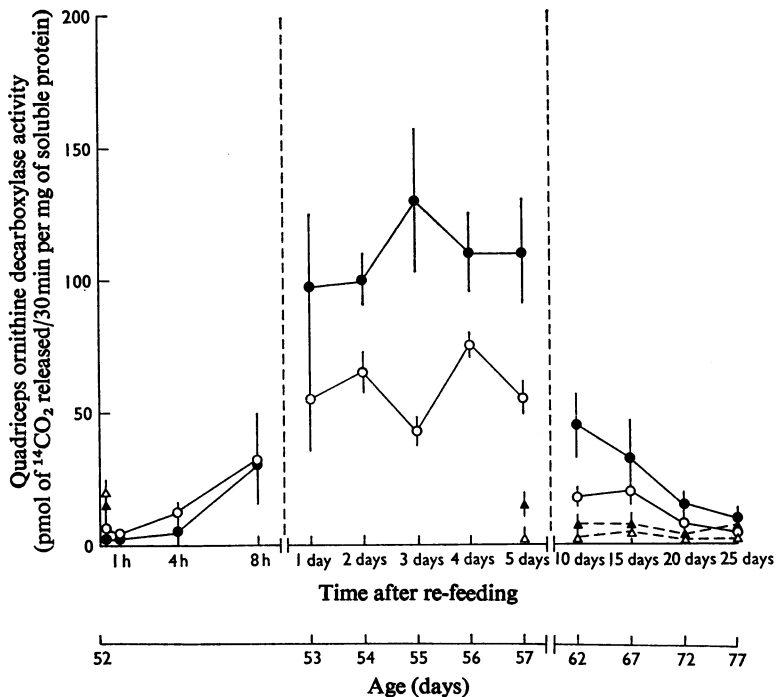


Fig. 2. *Quadriceps-muscle ornithine decarboxylase activity during rehabilitation*

Ornithine decarboxylase activity was measured in the paired quadriceps muscles of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the S.E.M.

and 2 days, remained constant up to 5 days, and then, after a slight increase, returned to control values at 15 days.

Before rehabilitation the activity of ornithine decarboxylase was normal for age in the spleens of the males, but slightly below normal in the spleens of the females ( $P < 0.002$ ) (Fig. 1). In both sexes there was a slight increase in enzyme activity during the first day of rehabilitation. Thereafter the enzyme activity in the spleens of the males increased rapidly to a peak at 4 days, and then returned to control values by the 20th day of rehabilitation. In the spleens of the females, there was no marked increase in enzyme activity until after 3 days. The peak activity reached by the females at 5 days was only 38% of that of the males, and the activity then decreased to control values by the 15th day of rehabilitation.

In both muscles examined (Figs. 2 and 3), the activity of ornithine decarboxylase was normal for age in the males before rehabilitation, but slightly below normal in the females ( $P < 0.02$ ). In both muscles of both sexes the enzyme activity began to increase during the first 8 h of rehabilitation. During sub-

sequent rehabilitation muscular ornithine decarboxylase responded in a similar way to the spleen enzyme, the enzyme activity reaching maxima in the male muscles which were approximately twice those in the female muscles. In the females the peak in enzyme activity was reached at the same time in both muscles, but in the males the peak occurred at 3 days in the quadriceps and at 5 days in the gastrocnemius. In the gastrocnemius of the males the peak activity was higher than that in the quadriceps, but in the female muscles the peak activities were similar. The enzyme activity returned to normal between 15 and 25 days of rehabilitation.

#### *S-Adenosylmethionine decarboxylase activity*

For the conditions used in this study, the optimum pH for measuring *S*-adenosylmethionine decarboxylase activity was 7.4 for all four tissues. The presence of putrescine was essential in the incubation medium ( $K_m = 0.1$  mM), but pyridoxal 5'-phosphate was inessential. The activity of *S*-adenosylmethionine decarboxylase was found to obey Michaelis-Menten

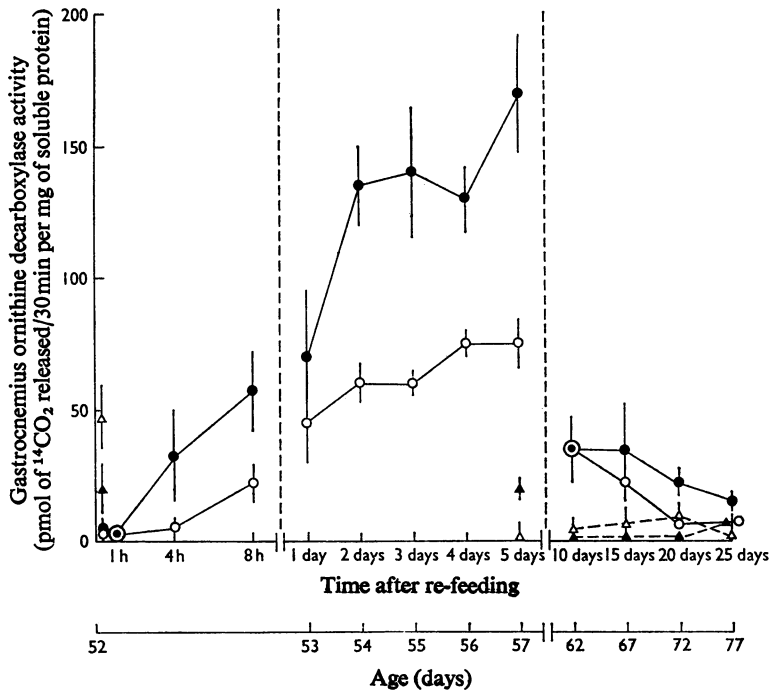


Fig. 3. *Gastrocnemius-muscle ornithine decarboxylase activity during rehabilitation*

Ornithine decarboxylase activity was measured in the paired gastrocnemius muscles of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the s.e.m.

kinetics in both normal and rehabilitating tissues, and a  $K_m$  of 0.067 mM was obtained for all four tissues.

The activity of hepatic *S*-adenosylmethionine decarboxylase during rehabilitation, like hepatic ornithine decarboxylase, has been described elsewhere (McAnulty & Williams, 1975a), and is only summarized here for completeness. At the end of the period of undernutrition the activity of hepatic *S*-adenosylmethionine decarboxylase was normal for age in both sexes. The activity of the enzyme did not change during the first 8 h of rehabilitation in the males, but in the females the activity increased and then fell sharply to below control values. In both sexes there was an increase in enzyme activity between 8 and 24 h, and a peak was reached at 24 h, which was higher in the livers of the females than of the males. The enzyme activity in the livers of the males then decreased to control values on the third day of rehabilitation, and thereafter followed a similar pattern to that of ornithine decarboxylase. In the female livers the activity decreased between 1 and 2 days, and then remained constant at control values until 5 days after re-feeding. The activity then

increased to a peak at 15 days, and returned to control values by 25 days. Throughout the period investigated the activity of the enzyme was higher in the livers of the female controls than in the male controls.

Before rehabilitation the activity of *S*-adenosylmethionine decarboxylase in the spleens of the males was below that of controls ( $P < 0.05$ ), but in the females the difference was not significant (Fig. 4). The activity of the splenic enzyme was similar in the males and females during rehabilitation, except that at day 1 the activity was higher in females than in males, and at 4 days the activity was higher in males. The enzyme reached a peak of activity at 4 days in the males and at 10 days in the females. Thereafter the activity decreased, and there was no significant difference between any of the groups 20 days after re-feeding.

At the end of the period of undernutrition the activity of *S*-adenosylmethionine decarboxylase was not significantly different from that of controls in both of the muscles examined in the male rats (Figs. 5 and 6). The enzyme activity was below normal, however, in both muscles of the female rats ( $P < 0.01$ ).

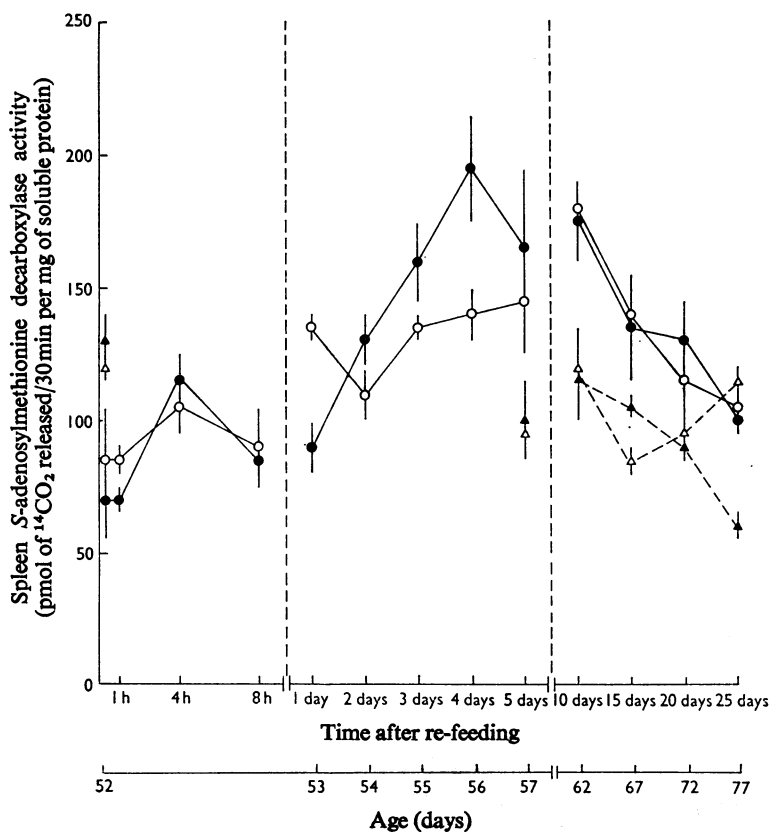


Fig. 4. Splenic *S*-adenosylmethionine decarboxylase activity during rehabilitation

*S*-Adenosylmethionine decarboxylase activity was measured in the spleens of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the S.E.M.

Except for the female quadriceps, the pattern of enzyme activity during rehabilitation was similar in both muscles of both sexes. The activity increased to a peak during the first day of rehabilitation, and then fell to below control values for the period between 3 and 5 days. The activity returned to normal by the 15th day of rehabilitation and remained normal thereafter. In the quadriceps of the females there were only slight variations in enzyme activity between 4h and 25 days after re-feeding. During this time the activity was the same as, or slightly below, that of the controls.

#### Hepatic putrescine, spermidine and spermine

The concentration of putrescine was normal in the livers of both sexes at the end of undernutrition (Fig. 7), although the concentration in female liver was much lower than in the male. After an initial decrease,

the concentration of putrescine increased between 1 and 4h in the livers of both sexes. A normal putrescine concentration was maintained between 4h and 5 days in the livers of the males, but in the females the concentration increased to a peak at 4 days, which was considerably above normal. Between 5 and 10 days, putrescine concentration increased in the livers of both sexes, and then steadily declined. The concentration in the female livers was not significantly different from normal on the 25th day of rehabilitation, but the concentration in the male livers fell below normal, owing to a marked increase in the concentration of putrescine in the control livers.

At the end of undernutrition the concentration of spermidine in the livers of the males was higher than normal ( $P < 0.01$ ), but that of the females was not significantly different from controls (Fig. 8). During the first day of rehabilitation there were slight increases in spermidine concentration in the livers of

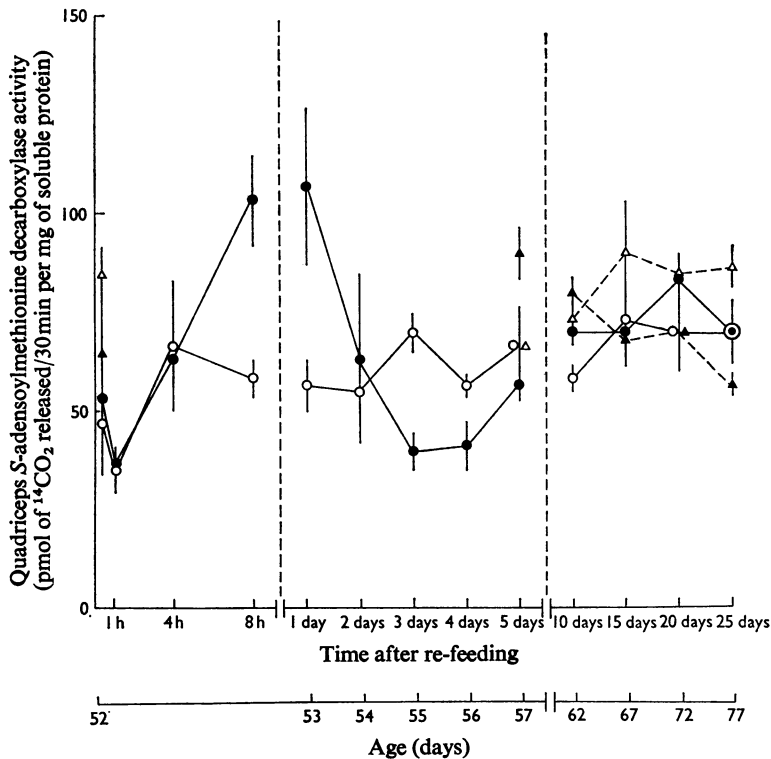


Fig. 5. *Quadriceps-muscle S-adenosylmethionine decarboxylase activity during rehabilitation*

*S-Adenosylmethionine decarboxylase activity was measured in the paired quadriceps muscles of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the S.E.M.*

both sexes. Up to the 5th day of rehabilitation there were no further significant changes in the spermidine concentration of the female liver, but in the male liver the concentration increased between 2 and 4 days and then decreased between 4 and 5 days. During this time the concentration of spermidine in the liver of both sexes was above normal. Between 10 and 25 days the concentration of spermidine decreased slowly in both sexes and returned to normal values.

Spermine concentration in the livers of the females was normal before rehabilitation (Fig. 9), but that of the males was above normal ( $P < 0.01$ ). On re-feeding, spermine concentration initially decreased and then remained relatively constant during the first day. Slight increases occurred during the next 4 days, and from the 10th day of rehabilitation onwards there were only slight deviations from normal liver spermine concentrations.

## Discussion

During the period of rapid tissue growth that attends the period of rehabilitation after undernutrition, there was an increase in the activities of polyamine-biosynthesizing enzymes. This is in agreement with observations on other rapidly growing tissues, such as regenerating liver (Jänne, 1967), foetal tissues (Russell & McVicker, 1972; Williams & McAnulty, 1976) and neoplastic tissue (Russell, 1973*b*). The characteristics of the enzymes synthesizing the polyamines were similar to those reported by other workers (Jänne & Williams-Ashman, 1971*a,b*), and thus it is likely that polyamine synthesis during nutritional rehabilitation is by the same mechanisms as in other tissues.

The weight of the liver increased markedly during the first few hours of rehabilitation, and this was



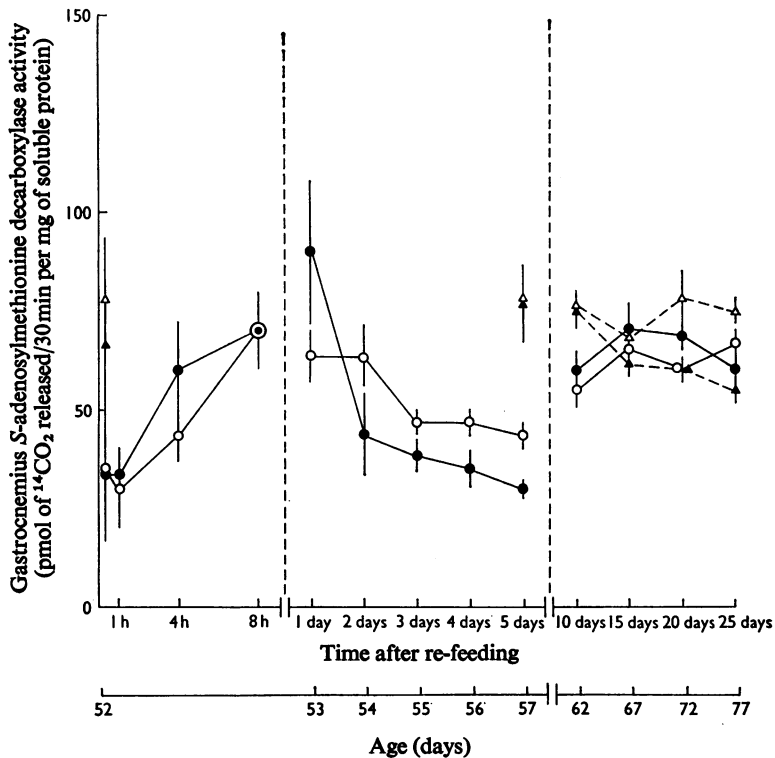


Fig. 6. *Gastrocnemius-muscle S-adenosylmethionine decarboxylase activity during rehabilitation*  
*S-Adenosylmethionine decarboxylase activity was measured in the paired gastrocnemius muscles of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the S.E.M.*

accompanied by rapid increases in the activity of ornithine decarboxylase and the concentration of putrescine. Similar events occur in the liver after the daily maintenance feed during the period of undernutrition (McAnulty & Williams, 1976), and also occur, after feeding, in the livers of rats trained to eat their food in a single meal each day (Hayashi *et al.*, 1972; Yager *et al.*, 1974; Yanagi *et al.*, 1976). The rapid increase in weight of the liver after a feed has been noted by other workers (Leveille & Chakrabarty, 1967; Garlick *et al.*, 1973; Jasper & Brasel, 1974). The increase is not due to DNA (Jasper & Brasel, 1974) or to water content (J. P. G. Williams, unpublished work), and it has been suggested that the increase may be due to a decrease in the rate of protein catabolism (Garlick *et al.*, 1973). We have also suggested that some of the increase is due to storage of nutrients from the meal (McAnulty & Williams, 1976); for example, glucose may be stored as fatty acids (Leveille, 1967) or glycogen (Deane, 1944). After the initial increase in liver weight during

rehabilitation, there was no further change in weight until 2 days after re-feeding. In the undernourished rats the weight decreased after the increase in weight that resulted from the daily feed (McAnulty & Williams, 1976), and this difference between the two situations further suggests that the liver's storage function partly accounts for the marked changes in weight.

The increase in activity of hepatic ornithine decarboxylase immediately after re-feeding is not as great as the increase that follows the daily feed during undernutrition (McAnulty & Williams, 1976). This can be only said for the livers of the males, for in the previous study females were not examined. In the males the increase in enzyme activity after rehabilitation is only about half that in the undernourished rats. The reason for this is unclear, especially as the undernourished rats receive only a small amount of food, whereas the rehabilitating rats have unlimited access to food. One possible reason for the difference is that the rehabilitating rats gorge their food until it

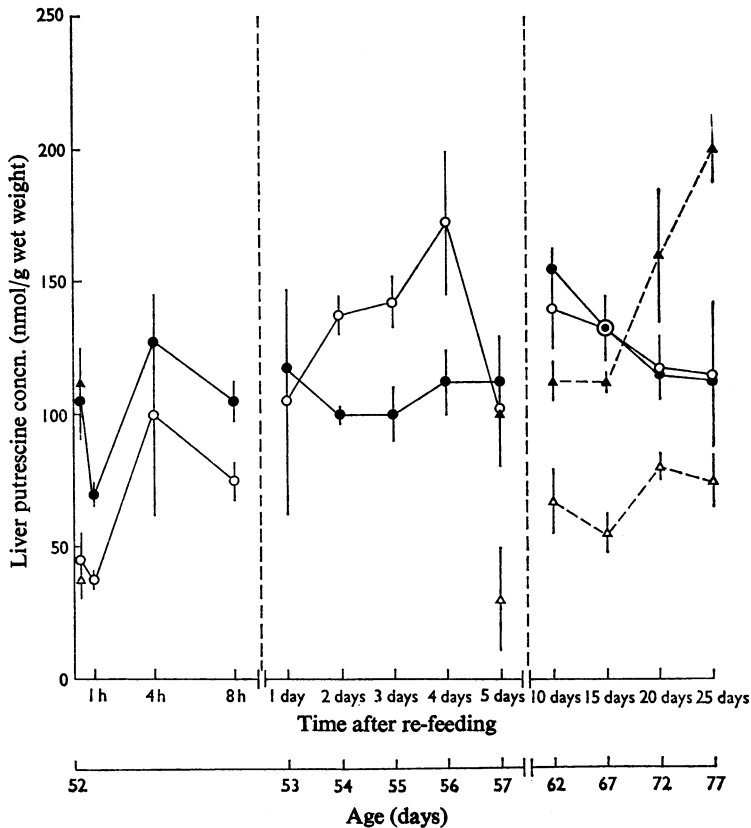


Fig. 7. Hepatic putrescine concentration during rehabilitation

Putrescine concentration was measured in the livers of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the S.E.M.

becomes physically impossible to consume any more. This may result in physiological stress, which could affect the activity of the enzyme.

The reason for the marked increase in the activity of hepatic ornithine decarboxylase in the first few hours of rehabilitation is probably due to the flow of nutrients via the hepatic portal system. It is unlikely that the increase in activity is hormonally mediated, because spleen and muscle do not show a similar response, and it is doubtful whether a large enough hormonal stimulus could occur so soon after the beginning of rehabilitation. Fausto (1969) has shown that hepatic ornithine decarboxylase can be stimulated by the intubation of an amino acid mixture into the stomachs of starved rats, and arginine, but not ornithine, can also cause a marked stimulation if intubated individually (Fausto, 1971). Therefore arginine is possibly one of the dietary constituents responsible for the increase in enzyme activity.

The later changes in polyamine synthesis in all tissues examined are more likely to be hormonally mediated, although nutrient flow probably still has some effect. Hepatic polyamine synthesis is primarily controlled by growth hormone and corticosteroids (Jänne & Raina, 1969; Russell *et al.*, 1970; Richman *et al.*, 1971), and during recovery from undernutrition serum growth-hormone concentrations are elevated in rats (Sinha *et al.*, 1973). Corticosteroid concentrations are elevated during undernutrition, and return to normal during rehabilitation (Adlard & Smart, 1972). Although it is possible that hepatic polyamine synthesis is affected by these hormones during rehabilitation, the situation with the spleen and skeletal muscle is less clear. Neither spleen nor muscle ornithine decarboxylase responds to growth hormone (Sogani *et al.*, 1972), and the effect of corticosteroids on polyamine synthesis in these tissues has not been examined. Prolactin can stimulate spleen

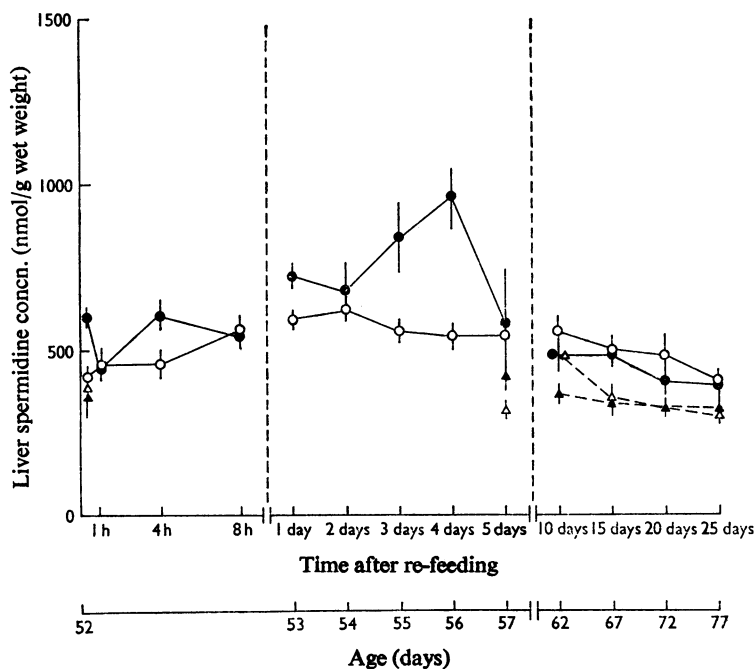


Fig. 8. *Hepatic spermidine concentration during rehabilitation*

Spermidine concentration was measured in the livers of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the S.E.M.

ornithine decarboxylase in male rats, but has no effect on skeletal muscle (Richards, 1975), and its response to nutritional rehabilitation is unknown.

In all three tissues examined there were marked sex differences in the activities of the polyamine-synthesizing enzymes during rehabilitation, particularly in spleen and skeletal muscle. Sex hormones do not affect hepatic polyamine synthesis (Kaye *et al.*, 1971; Grahn *et al.*, 1973), and their effect on spleen and muscle is not known. It may be that the sex hormones affect the production of hormones that do have an effect on polyamine synthesis, or else, and perhaps more likely, the sex hormones may affect these tissues during early life so that their response to other hormones is different later in development. Prolactin stimulates ornithine decarboxylase in the spleens of male rats but not of females (Richards, 1975), and thus there is a possibility that it is involved in the sex difference during rehabilitation.

The question remains whether these changes in polyamine biosynthesis during nutritional rehabilitation are related to changes in nucleic acids and protein. In liver and muscle of male rats there is a marked rate of accumulation of RNA during the

first 3 days of rehabilitation (McAnulty & Dickerson, 1974; Dickerson & McAnulty, 1975). Protein also accumulates during these first 3 days in the liver and quadriceps muscle, but not in the gastrocnemius muscle. In the male liver there are increases in ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activities, and putrescine and spermidine concentrations, at this time, and in muscle there are also increases in *S*-adenosylmethionine decarboxylase activity. DNA does not begin to accumulate until 3 days after the beginning of rehabilitation in the liver of male rats, and not until after 7 days in the muscles (McAnulty & Dickerson, 1974; Dickerson & McAnulty, 1975). This corresponds to the second burst of increased activities of the polyamine-synthesizing enzymes in the liver, and is just after the peak in ornithine decarboxylase activity and at the same time as the increase in *S*-adenosylmethionine decarboxylase activity in the muscles. Female rats cannot be compared as changes in tissue nucleic acids and protein have not been examined.

Whether the coincidence of events in macromolecular and polyamine synthesis during nutritional rehabilitation is of significance is unknown. The large

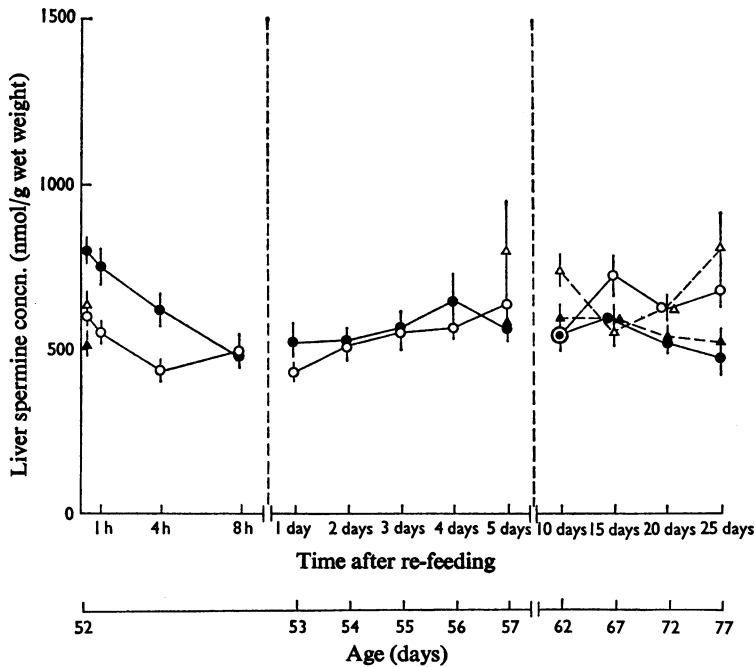


Fig. 9. Hepatic spermine concentration during rehabilitation

Spermine concentration was measured in the livers of male (●) and female (○) rats during recovery from 28 days of undernutrition, and compared with male (▲) and female (△) controls of the same age. Each point is the mean of six animals and the vertical bars indicate the s.e.m.

body of evidence implicating the polyamines in macromolecular synthesis suggests that this relationship is worth investigating.

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