Polyamine Metabolism in Liver of Young Rats

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Rat liver undergoes a phase of rapid growth during weaning. We followed the changes in polyamine metabolism occurring during this period of natural growth, and compared them with changes in DNA and RNA accumulation. There was a 2.5-fold increase in the number of cells per liver between suckling (18–19 days old) and weanling (30–32 days old) rats. Ornithine decarboxylase activity increased from the low value in 18-day-old rat pups and remained significantly higher (approx. 5–10-fold) than that in adult rats from day 21 to day 34. Putrescine-dependent *S*-adenosylmethionine decarboxylase activity was slightly but significantly increased during most of this period. Spermidine and RNA concentrations fluctuated in concert, whereas spermine content per cell doubled during the period from day 23 to day 30.

Ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) is the initial enzyme in the synthesis of the polyamines spermidine and spermine. These polycations are thought to be involved in the regulation of nucleic acid metabolism (for review see Jänne *et al.*, 1976). All known growth-promoting hormones increase the synthesis of spermidine in parallel with that of RNA and protein. In liver, stimulated to proliferate after extirpation of two-thirds of the organ (Jänne & Raina, 1968; Russell & Snyder, 1968) or after injection of somatotropin (Jänne & Raina, 1969), a rapid rise in the activity of ornithine decarboxylase occurs, followed by accumulation of polyamines and RNA. Although the initial marked increase in ornithine decarboxylase activity is maintained for only a short time, activity is generally maintained well above the baseline until the growth stimulus is removed. These growth stimuli are, however, imposed on slowly growing tissue. Little work has been reported on polyamine-synthesizing enzymes or on polyamine concentrations during the normal phase of rapid growth in young animals.

It is known that the liver of young rats undergoes a phase of rapid growth as the animals wean themselves from their mothers' milk, between the ages of 2.5 and 4 weeks (Oliver *et al.*, 1962). The stimulus for growth comes naturally and thus provides a model for the study of polyamine metabolism during a physiological growth period. We have used this model to study the activity of ornithine decarboxylase and putrescine-dependent *S*-adenosylmethionine decarboxylase (EC 4.1.1.50), the first two enzymes of

polyamine synthesis. We have also monitored polyamine and nucleic acid concentrations during this period of rapid growth.

Materials and Methods

Animals

Sprague–Dawley rats, bred in the Animal Unit, Health Sciences Complex, Memorial University of Newfoundland, were used for all experiments. Both male and female rat pups were used for studies up to 30 days of age; thereafter only male animals were chosen. All rats had free access to Purina rat chow (Ralston Purina of Canada, Don Mills, Ont., Canada) and water. Animals were maintained in the same room at constant temperature (25°C) and constant lighting conditions (12h light/12h dark). Suckling animals began to show an interest in the chow at 18–19 days of age and appeared to be completely weaned by 28 days, although they remained in the same cage with their mothers throughout the study. The six litters included in this study contained from 10 to 13 pups per mother. Male rats, 8–12 weeks of age, were obtained from the same colony.

Chemicals

Omnifluor, L-[1-¹⁴C]ornithine (56.6mCi/mmol) and *S*-adenosyl-L-[carboxy-¹⁴C]methionine (55mCi/mmol) were from New England Nuclear, Dorval, Canada. Diphenylamine and acetic acid (both AnalaR grade) were from BDH Chemicals, Poole, Dorset, U.K., and orcinol monohydrate was from Aldrich Chemical Co., Milwaukee, WI, U.S.A.

All other chemicals were of the highest analytical grade available and were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Centre wells and rubber stoppers used in $^{14}\text{CO}_2$ trapping were from Kontes Glass Co., Vineland, NJ, U.S.A.

Tissue preparation

Rats were killed by cervical dislocation between 10:00 and 12:00h. Livers were rapidly removed, blotted and weighed. They were immediately placed in 10 vol. of ice-cold buffer A (0.25M-sucrose/2mM-EDTA/2mM-Hepes [4-(2-hydroxymethyl)-1-piperazine-ethanesulphonic acid], pH7.4) per g of tissue, chopped and homogenized by five passes of a hand-held Potter-Elvehjem homogenizer (clearance 0.15mm). Dithiothreitol (5mM) was present in buffer A if enzyme assays were not to be performed the same day that rats were killed (see the next section).

A portion of homogenate was centrifuged at 105000g for 70min to remove organelles. The supernatant of this fraction is referred to as cytosol.

Assay of enzymes

Ornithine decarboxylase activity in cytosol was measured by following the release of $^{14}\text{CO}_2$ from L-[1- ^{14}C]ornithine, as described previously (Murphy & Brosnan, 1976). L-Ornithine was present in the incubation medium at a concentration of 0.2mM and a specific radioactivity of 1 $\mu\text{Ci}/\mu\text{mol}$.

Putrescine-dependent S-adenosylmethionine decarboxylase in cytosol was assayed by following the release of $^{14}\text{CO}_2$ from S-adenosyl-L-[carboxyl- ^{14}C]methionine (Symonds & Brosnan, 1977). S-Adenosyl-L-methionine was present in the incubation medium at a concentration of 0.2mM and a specific radioactivity of 0.5 $\mu\text{Ci}/\mu\text{mol}$. Putrescine concentration was saturating (50 μM).

For all enzyme assays, activity was linear with protein concentration and time under the conditions used. If enzyme assays were performed as soon as the cytosol fraction was prepared (approx. 2–4h after death), the addition of 5mM-dithiothreitol to the homogenizing medium, as suggested by Williams-Ashman and co-workers (Jänne & Williams-Ashman, 1970; Coppoc *et al.*, 1971) had no significant effect on activity of either enzyme in these young rats. If, however, assays were to be performed the next day, the addition of dithiothreitol was essential to maintain enzyme activity, especially in adult rats.

Analysis of nucleic acids, protein and polyamines

Liver homogenate was used for these determinations. DNA was extracted into hot HClO_4 , as described by Schneider (1945), and the concentration was determined with diphenylamine reagent (Burton, 1956) with calf thymus DNA as standard. RNA was

precipitated from homogenates with 0.5M- HClO_4 and hydrolysed in 0.3M-KOH, as described by Seiler & Schmidt-Glenewinkel (1975). The orcinol method (Ashwell, 1957) was used to determine RNA, with *Torula* yeast RNA (type VI; Sigma Chemical Co., St. Louis, MO, U.S.A.) as standard. Protein (in homogenate and cytosol) was determined as described by Lowry *et al.* (1951) after solubilization of membranous material with deoxycholate (Jacobs *et al.*, 1956). Bovine serum albumin was used as standard. It was essential to include dithiothreitol in the standards and blanks when it was included in the homogenizing medium.

For analysis of polyamine concentrations, a portion of homogenate was deproteinized by addition of solid sulphosalicylic acid (40mg/ml of homogenate), protein was removed by centrifugation (20000g for 10min at 4°C), and the clear supernatant was applied to a Beckman amino acid analyser, as described previously (Hall *et al.*, 1978).

Statistical analysis

Differences are described as significant when $P < 0.05$, calculated by Student's *t* test.

Results

Growth of liver in neonatal rats

The liver of the neonatal Sprague-Dawley rat grows relatively slowly for the first 2.5 weeks after birth. During the next 2 weeks the liver undergoes a phase of rapid growth, during which it increases in weight by approx. 350%. These changes are recorded in Table 1. The rate of growth of the liver exceeds that of the animal, and thus the hepatosomatic index (liver weight as a percentage of body weight) increases. The rate of increase (daily percentage increase) in weight of liver over this period is illustrated in Fig. 1(a). The rate of growth then slows to that seen in the adult Sprague-Dawley rat.

Concentration of nucleic acids, protein and polyamines in liver during postnatal development

The concentration of DNA per g decreased from day 18 to day 22 (Fig. 1b), but the total content of

Table 1. *Growth of liver during weaning*
Values are the means $\pm$ s.d. for four rats. Hepatosomatic index is (liver weight/body weight) $\times$ 100.

Parameter	Age of rat (days)	
	18–19	31–32
Rat weight (g)	38 $\pm$ 6	100 $\pm$ 5
Total liver weight (g)	1.19 $\pm$ 0.07	4.35 $\pm$ 0.16
Hepatosomatic index	3.2 $\pm$ 0.3	4.3 $\pm$ 0.2
DNA (mg/total liver)	3.74 $\pm$ 0.53	9.23 $\pm$ 0.32

Fig. 1. *Hepatic growth rate, nucleic acid, protein and polyamine contents and activities of polyamine-synthesizing decarboxylases in rat liver during and after weaning*

Analytical procedures are described in the Materials and Methods section. Each point in parts (b)–(h) is the mean of duplicate assays from four to ten different rats; vertical lines represent the S.E.M. The hatched horizontal bar in parts (d) and (h) represents the mean $\pm$ S.D. for the basal decarboxylase activity from 15 adult rats (8–12 weeks of age). (a) Rate of increase of liver weight, expressed as a percentage of previous day's liver weight; (b) DNA content per g of liver; (c) spermine; (d) ornithine decarboxylase activity in liver cytosol; (e) RNA; (f) total protein; (g) spermidine; (h) putrescine-dependent S-adenosylmethionine decarboxylase activity in liver cytosol.

DNA per liver increased by about 2.5-fold (Table 1). The concentration of RNA, protein and spermidine relative to DNA increased significantly from day 21 to day 25 (Figs. 1e, 1f and 1g respectively). Spermine concentration relative to DNA more than doubled during weaning (Fig. 1c). This increase was also significant if expressed per g of liver. There was a negative correlation between spermine concentration and growth rate after day 23, as shown in Fig. 2.

Ornithine decarboxylase activity

The changes in activity of ornithine decarboxylase in liver cytosol during the phase of rapid growth are illustrated in Fig. 1(d). In suckling rats, ornithine decarboxylase activity was within the low range characteristic of adult rats. By day 21, mean activity was significantly increased over that in suckling or adult rats (approx. 5-fold). Within individual litters the activity appeared to undergo cyclic fluctuations between the ages of 20 and 36 days. Since the periodicity of these cycles was different from one litter to the next, the range of activities on individual days was large but all remained consistently above adult values until day 30 ($P < 0.05$ when compared with adult rats). As long as the animals were not stressed, we did not see sharp rapid changes in enzyme activity, but rather a prolonged increase of activity throughout the growth phase.

Putrescine-dependent *S*-adenosylmethionine decarboxylase activity

There was a slight but significant increase in the activity of putrescine-dependent *S*-adenosyl-

Fig. 2. Correlation between hepatic spermine concentration and growth rate of liver in rats from 23 to 34 days of age. Data from Figs. 1(a) and 1(c) have been replotted and have been subjected to correlation analysis. Correlation coefficient was -0.96 . The regression line was calculated by least-squares analysis.

Fig. 3. Putrescine concentration and ratio of activity of ornithine decarboxylase to *S*-adenosylmethionine decarboxylase in rat liver during and after weaning.

Putrescine (○) was measured on a Beckman model 121 amino acid analyser (Hall *et al.*, 1978). Each point is the mean $\pm$ S.E.M. of putrescine concentrations from three to seven different rats. * $P < 0.05$ compared with 18–19-day-old rats. The ratio of ornithine decarboxylase to *S*-adenosylmethionine decarboxylase (●) was calculated from the data in Figs. 1(d) and 1(h).

methionine decarboxylase throughout most of the period of rapid growth of liver as shown in Fig. 1(h). The activity of this enzyme is generally well in excess of that measured for ornithine decarboxylase when both enzymes are measured under optimal conditions *in vitro*. If the assays *in vitro* reflect the activities *in vivo*, there would be excess capacity of the former enzyme and a stimulation of the pathway would not require an increase in its concentration, since it would not be rate-limiting for spermidine synthesis, until the activity of ornithine decarboxylase was markedly increased. This would appear to be the case, since significant accumulation of putrescine *in vivo* is only observed when the activity of ornithine decarboxylase exceeds that of putrescine-dependent *S*-adenosylmethionine decarboxylase (see Fig. 3).

Discussion

The livers of neonatal rats contain haematopoietic cells, which are lost soon after birth (Oliver *et al.*, 1962). During the first 2–3 weeks of life the rate of growth of the liver is relatively slow, and the hepatosomatic index is low. At approx. 18 days of age, the rate of growth of liver begins to exceed that of the animal as a whole. This phase of rapid growth in liver continues until approximately day 30–32, at which time the growth rate of liver becomes similar to that of the whole animal. During this 10–14-day period of rapid growth, the liver weight increased approx. 3.5-fold. The total DNA per liver increased approx. 2.5-fold (Table 1). Since the DNA

content per cell is constant (Weber *et al.*, 1971), this would imply a 2.5-fold increase in cell number in the liver during this phase of rapid growth. At the same time, there was a decrease in the DNA content, and thus in the number of cells, when expressed per g of liver. Our results are in agreement with the histological observations of Weber *et al.* (1971) in rats of similar age.

The rate of growth of liver observed during this period of postnatal development approximates to that observed by Haug *et al.* (1973) in liver during regeneration after partial hepatectomy, a classic model of liver growth. Thus this period of hepatic development presents a model for rapid growth of liver without the attendant stresses of either surgery, as in liver regeneration, or of injection, as in hormone treatment.

All known stimuli for growth of any tissue studied to date give rise to increased ornithine decarboxylase activity (Jänne *et al.*, 1976). If this increase is in fact related to growth, then it should also be observed in liver during this period of rapid growth at weaning. Studies have been reported (Jänne & Hölttä, 1973; Raina *et al.*, 1976) which related the hepatic ornithine decarboxylase activity to the age of the rat, but these did not include animals between 2 and 4 weeks of age. We have now shown that during a naturally occurring period of rapid growth there is a significant increase in activity of ornithine decarboxylase and, to a lesser extent, of putrescine-dependent S-adenosylmethionine decarboxylase.

Jänne & Hölttä (1973) have reported increased activity of ornithine decarboxylase in thymus and small intestine of rats at 1 month of age, when compared with rats 2 weeks old or adult rats. In their study, the activity of this enzyme was low in liver of 1-month-old rats. It is possible that the growth response, and thus polyamine synthesis, occurs earlier in liver than in other organs, or that the response is more prolonged in tissues such as thymus and small intestine than it is in liver.

Many conditions that result in increased accumulation of RNA in liver cells are also associated with increased spermidine concentration (Jänne *et al.*, 1976). In the present example of rapid growth, RNA concentration increased by about 35%. After an initial fall, spermidine concentration per cell increased in concert with RNA ($r = +0.63$).

Our data show a strong negative correlation between growth rate of liver from 23 to 34 days of age and hepatic spermine content. Hence there is an inverse relationship between the rate of liver growth and the spermine concentration in a situation where the growth rate is naturally decreasing. These data lend support to the suggestion of Jänne *et al.* (1976) that spermine concentration could be negatively related to the rate of growth.

We do not know what signal initiates the growth

response in these young rats, and thus, what signal, presumably, is responsible for the increased putrescine synthesis in this tissue. Marked changes occur in both metabolic and endocrine status of the rat during this period. Suckling rats ingest a diet high in fat (69% of energy) and low in carbohydrate (8%) (Dymsza *et al.*, 1964). At approximately day 18 or 19 they begin to nibble at the pelleted chow that their mother has been eating and over the next 10 days their intake shifts completely from mother's milk to chow. Thus they gradually give up their high-fat low-carbohydrate diet for one high in carbohydrate (69%) and low in fat (9%). Total protein content of both diets remains similar (23 and 22%, respectively), but the concentration of individual amino acids in the proteins could be different. At the same time numerous metabolic changes are occurring, since the pup must now begin to synthesize more lipid for itself, and must utilize and store more carbohydrate. Insulin becomes progressively more important and the plasma concentrations of insulin are reported to increase at this time (Blázquez *et al.*, 1970). Somatotropin and thyroid hormone (thyroxine and triiodothyronine) concentrations in rat plasma also increase at the time of weaning (Blázquez *et al.*, 1974; Dussault & Labrie, 1975). It is possible that one or more of these hormones is responsible for triggering the rapid growth seen in our rats. All three hormones have been shown to increase ornithine decarboxylase activity in rat liver (Jänne & Raina, 1969; Panko & Kenney, 1971).

In conclusion, the phase of rapid growth of liver in weanling rats serves as a model of normal physiological growth, unaccompanied by the stresses of surgery or injection. We have observed an increased activity of hepatic ornithine decarboxylase during this phase of rapid liver growth. Pronounced increases in spermidine concentration are observed, and these correlate well with increases in total RNA. Spermine remains at a low concentration during the period of increasing hepatic growth rate, but increases as the growth rate decreases. The results are in accord with the ideas that increased spermidine concentrations are associated with rapid growth of tissues and that spermine concentrations are negatively related to the rate of growth.

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