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Ornithine Decarboxylase Activity and the Onset of Deoxyribonucleic Acid Synthesis in Regenerating Liver

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Compared with normally fed animals, rats fed on a low-protein diet for 3 days exhibit a considerable delay in DNA synthesis after partial hepatectomy. In the regenerating livers of these animals (*a*) the timing of the first peak of ornithine decarboxylase activity is not altered and (*b*) the second peak of enzyme activity is delayed by a few hours, but polyamine concentrations are similar to those of normally fed rats. The results suggest that regardless of the possible effect of polyamines on DNA synthesis, the time course of ornithine decarboxylase activity appears to be independent of the onset of DNA replication in regenerating livers.

After partial hepatectomy in rats a striking change in ornithine decarboxylase activity occurs during the pre-replicative phase of the regenerative process (Russell & Snyder, 1968; Fausto, 1969; Hölttä & Jänne, 1972). Although the time course of putrescine synthesis in the early stages of liver regeneration coincides with changes in the incorporation of orotic acid into ribosomal RNA (Fausto, 1969), it has been suggested that the biphasic rise in ornithine decarboxylase activity may be related to DNA synthesis (Gaza *et al.*, 1973). Thus it is of interest to determine if the activation of the polyamine-synthetic pathway is coupled with the onset of DNA replication in experimental situations where the timing of DNA synthesis in regenerating livers has been altered. We measured ornithine decarboxylase activity and polyamine concentrations in regenerating livers of protein-deprived and hypophysectomized rats. In rats maintained on a protein-free diet for 3 days or in hypophysectomized animals the start of DNA synthesis after partial hepatectomy is delayed by many hours.

Materials and Methods

Animals and diets

Male Holtzman rats (100–140g) were from Charles River Laboratories (Wilmington, MA, U.S.A.) and kept under a controlled lighting schedule with light from 06:00 to 18:00h. The normal diet contained 24% protein (w/w). For protein-deprivation studies animals were kept for 3 days on a protein-free diet from Nutritional Biochemicals (Cleveland, OH, U.S.A.). The diet contained 70% corn starch, 15% cellulose, 10% vegetable oil, 4% U.S.P. XIV salt mixture, 1% cod liver oil and vitamin supplementation. This diet was offered *ad libitum* after overnight deprivation of food, and 5% (w/v) sucrose was supplied in the drinking water. Animals deprived of dietary protein lost up to 20% of their body weight over the 3-day feeding period. The size of the rats to be used in the normally fed and protein-deprived groups were selected to provide animals of comparable weight at the end of the feeding period. Hypophysectomized rats (Charles River Laboratories) were kept in the laboratory for 10–14 days before the experiments and received NaCl in the drinking water. Partial hepatectomies were performed by the procedure of Higgins & Anderson (1931) by using a mixture of diethyl ether and O_2 for anaesthesia (Bucher & Swaffield, 1966; Fausto & Butcher, 1976). All animals were deprived of food for 16h before being killed.

Ornithine decarboxylase assay

The enzyme was assayed *in vitro* (Fausto, 1969, 1971) by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine (43.04mCi/mmol; New England Nuclear, Boston, MA, U.S.A.). Excised livers were homogenized in 0.25M-sucrose containing 0.5mM-EDTA, 10mM-mercaptoethanol and 10mM-Tris/HCl buffer, pH7.4. The homogenates were centrifuged for 48min at 105000*g* in an International B-60 ultracentrifuge and samples of the cytosol were assayed for enzyme activity. The incubation mixture (1ml total) consisted of 0.4ml of cytosol, 0.2μmol of pyridoxal phosphate, 5mM-dithiothreitol, 10mM-Tris/HCl buffer, pH8.0, and 0.8μCi of [1-^{14}C]ornithine and unlabelled L-ornithine to 2mM. After preincubation for 10min at 37°C, labelled ornithine was added and the tubes were capped and incubated for 30min. The $^{14}CO_2$ evolved during the reaction was trapped in ethanolamine/ethylene glycol (1:2, v/v). For radioactivity determination a sample of the

trapping solution was pipetted directly into a vial containing scintillation fluid as previously described (Fausto, 1969, 1971).

Polyamine determination

The polyamines in deproteinized liver extracts were separated on Dowex 50 W columns (1 cm × 4 cm) essentially by the procedure described by Inoue & Mizutani (1973). A sample of the liver (0.1–1.0 g) was homogenized in 5 ml of cold 0.6 M-perchloric acid. After centrifugation the pellets were resuspended in 0.2 M-perchloric acid and the total acid-soluble fraction was collected. Portions of the supernatants were applied to the Dowex columns. After washing the columns with phosphate buffer and 1 M-HCl the polyamines were eluted with 6M-HCl. Samples (5–30 μl) of the concentrated 6 M-HCl eluates were applied to paper strips and electrophoresis was carried out for 2 h at 250 V (Jänne, 1967). The bands corresponding to polyamines were extracted with 5 ml of a mixture containing 0.2 g of cadmium acetate, 40 ml of ethanol, 50 ml of acetic acid and 10 ml of water. The A_{505} was determined and compared with that of standards. The recovery of the polyamines was approx. 90%

Incorporation of [^3H]thymidine into DNA

All animals were killed between 08:00 and 11:00 h. Each rat received 5 μCi of [*methyl*-^{3}H]thymidine (6.7 Ci/mmol, New England Nuclear) via the tail vein and was killed 1 h after the injection. After separation of nuclei from the liver homogenates (Uchiyama *et al.*, 1966) DNA was extracted by the procedure of Munro & Fleck (1966). Samples of the extracted DNA were added to Aquasol (New England Nuclear) for the determination of radioactivity. To compare the uptake of thymidine in normally fed and protein-deprived animals, samples of the homogenates were treated with cold 0.6 M-perchloric acid and the pellets washed with 0.2 M-perchloric acid. The amount of radioactivity detected in the total acid-soluble fractions of normally fed and protein-deprived rats was approximately the same.

DNA and protein determinations

DNA was determined by the diphenylamine procedure by using the modifications described by Burton (1956). Calf thymus DNA was used as a standard. Protein was determined by the procedure

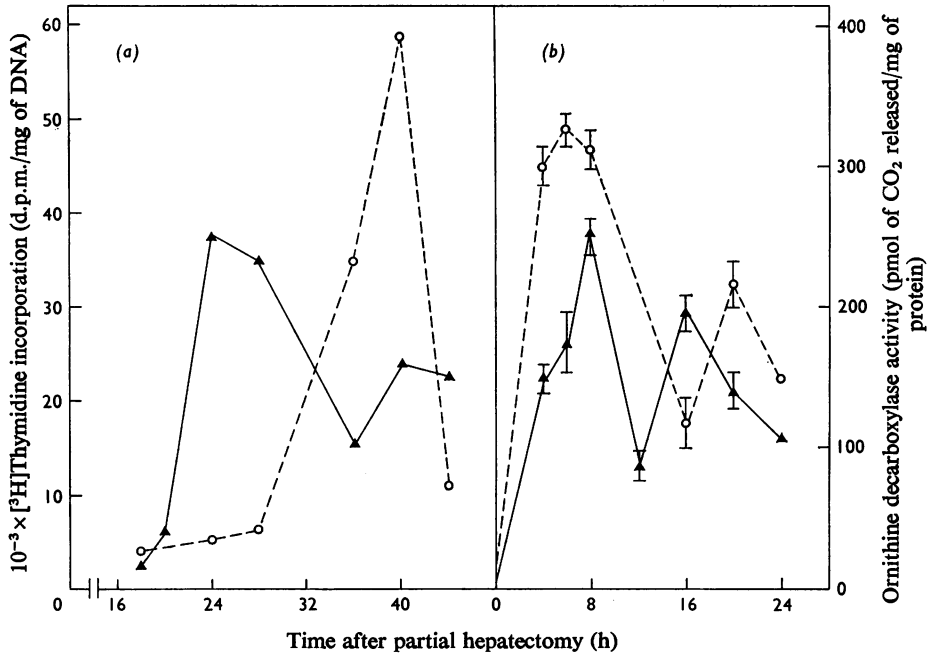

Fig. 1. *Ornithine decarboxylase activity and DNA synthesis in regenerating livers of protein-deprived and normally fed rats*
Rats were kept on normal (▲) or protein-free (○) diets before partial hepatectomy. (*a*) Animals were injected with 5 μCi of [^{3}H]thymidine via the tail vein and killed 1 h later as indicated on the abscissa. (*b*) Liver ornithine decarboxylase was assayed at the times indicated on the abscissa in two sets of identical experiments. In each set, ornithine decarboxylase activity was measured in duplicate samples from the pooled cytosols of three livers. The vertical bars indicate the range of variation.

of Lowry *et al.*, (1951) with crystallized bovine albumin as a standard.

Results

Rats kept on standard diets before partial hepatectomy show a major peak of DNA synthesis 24h after the operation. In contrast, if animals are maintained for 3 days on a protein-free diet before partial hepatectomy, the incorporation of [^{3}H]thymidine into DNA is not enhanced until 26–28h after the operation and reaches a maximum between 36 and 40h (Fig. 1). After partial hepatectomy the protein-deprived rats show a rapid increase in liver RNA, but a lag in the restoration of protein, which only reaches normal values at 36h after the operation, when DNA synthesis is at a maximum (J. A. McGowan & N. Fausto, unpublished work). Alterations in DNA synthesis in the regenerating liver of protein-deprived rats have been previously reported (Hilton & Sartorelli, 1970; Stirling *et al.*, 1975). Ornithine decarboxylase activity was determined at various times after partial hepatectomy in animals kept on normal or protein-free diets (Fig. 1). In normally fed rats, ornithine decarboxylase activity showed a biphasic increase in activity during liver regeneration [confirming the observations of Hölttä & Jänne (1972)], with maxima at 8 and 16h after the operation. Protein-deprived rats displayed an initial peak of activity at 4–6h after partial hepatectomy and a second increase at 20h. Thus, although the timing and the magnitude of the first increase in ornithine decarboxylase activity is not altered in protein-deprived rats, the second rise in enzyme activity after partial hepatectomy is delayed by approx. 4h in these animals. Since DNA synthesis is delayed by 16h in the protein-deprived rats, it is questionable if the moderate retardation in the second rise in enzyme activity would correlate with the long delay in DNA replication. If this delay is of importance for the timing of DNA synthesis, it would be expected that the hepatic concentrations of putrescine, spermidine and spermine measured 24h after partial hepatectomy might be below normal in protein-deprived rats. However, as shown in Table 1 the polyamine concentrations in 24h-regenerating livers of normally fed and protein-deprived rats are similar.

Hypophysectomized rats kept on normal diets also exhibit a delay in DNA synthesis after partial hepatectomy. In hypophysectomized partially hepatectomized rats the major peak in DNA synthesis occurs 36h after the operation (Rabes & Brandle, 1969) and ornithine decarboxylase activity is low in 4h-regenerating liver (Russell & Snyder, 1969). Because of similarities between this experimental model and the protein-deprivation experiments, a more detailed study of the time course of ornithine decarboxylase activity during liver regeneration was undertaken in hypophysectomized rats. The results shown in Table 2 confirm the observation of Russell & Snyder (1969) that ornithine decarboxylase activity is lower than normal in 4h-regenerating liver of hypophysectomized rats. However, as early as 12h after partial hepatectomy ornithine decarboxylase activity in the liver of hypophysectomized rats is

Table 1. *Polyamine concentrations in the 24h-regenerating liver of protein-deprived and normally fed rats*

Polyamines were measured as described in the Materials and Methods section with six rats per group. Mean values are presented±S.E.M. All determinations were done in duplicate samples.

Treatment	Polyamine concentration (μmol/g)		
	Putrescine	Spermidine	Spermine
Non-regenerating, normally fed	<0.10	1.11±0.09	0.96±0.05
24h-regenerating, normally fed	0.29±0.04	1.78±0.07	0.67±0.03
24h-regenerating, protein-deprived	0.27±0.03	1.71±0.14	0.65±0.02

Table 2. *Ornithine decarboxylase activity in regenerating livers of normal and hypophysectomized rats*

Ornithine decarboxylase activity was determined as described in the Materials and Methods section with four rats per group. Mean values are presented±S.E.M. All determinations were done in duplicate samples. Statistical differences were assessed by Student's *t* test.

Treatment	Ornithine decarboxylase activity (pmol of CO_2/mg of protein)			
Time after partial hepatectomy (h) ...	4	8	12	16
None	165±6	151±27	140±2	165±22
Hypophysectomy	47±9*	61±20†	129±16	144±17

* Different from corresponding values of non-hypophysectomized rats at $P<0.01$.
† Different from corresponding values of non-hypophysectomized rats at $P<0.05$.

similar to that present in the regenerating liver of a normal rat.

Discussion

In studies of ornithine decarboxylase activity and DNA synthesis in the liver of rats subjected to a variety of stimuli, Schrock *et al.* (1970) concluded that putrescine synthesis did not correlate well with DNA replication, but could be involved in some step of RNA metabolism. We have also shown previously that doses of X-irradiation, which severely inhibit DNA synthesis after partial hepatectomy, do not prevent the initial rise of ornithine decarboxylase activity in regenerating livers (Fausto, 1969). Moreover, various agents such as amino acids and hormones cause a very large stimulation in the activity of this enzyme without an appreciable change in the mitotic index of the liver of the injected rats (Fausto, 1971). Gaza *et al.* (1973) suggested, however, that the second rise in ornithine decarboxylase activity may have some relationship with further DNA synthesis. More recently, Pösö & Jänne (1976) have shown that inhibition of polyamine synthesis by diaminopropane is accompanied by a large decrease in DNA synthesis in regenerating rat liver. In the present work we show that in two situations where the timing of DNA synthesis is altered after partial hepatectomy the changes in ornithine decarboxylase activity do not parallel DNA replication. In protein-deprived rats the peak of DNA synthesis occurs approx. 16h later than usual. The first rise in ornithine decarboxylase activity is not changed in the liver of these animals, but the second rise is delayed by about 4h. This causes no changes in polyamine concentrations 24h after partial hepatectomy, a time when the normally fed animals show maximal incorporation of [^{3}H]thymidine into DNA, but protein-deprived rats show negligible DNA replication. In hypophysectomized partially hepatectomized rats, DNA synthesis is also delayed by 12–16h during liver regeneration (Rabes & Brandle, 1969). In these animals, we find, the first rise in enzyme activity is inhibited, but by 12h ornithine decarboxylase activity is similar to that of non-hypophysectomized rats. An apparent relationship between polyamine concentrations and DNA synthesis has been described in a variety of experimental systems in which polyamine synthesis was blocked by inhibitors (Fillingame *et al.*, 1975; Boynton *et al.*, 1976; Mamont *et al.*, 1976; Krokan & Eriksen, 1977; Sunkara *et al.*, 1977). In most of these experiments, the effects of the inhibitor methylglyoxal bis(guanylhydrazone) were reversed by spermidine addition, but in some situations the action of the inhibitor was blocked by putrescine, spermine or cadaverine, a diamine not normally found in mammalian cells (Krokan & Eriksen, 1977). Other investigations have failed to detect a correlation between polyamine concentrations and DNA replication (Clark & Duffy, 1976), and Goldstein *et al.* (1976) concluded that polyamine synthesis is possibly regulated separately from DNA and ribosomal RNA synthesis. It is likely that basal concentrations of polyamines are essential for many cellular functions, including DNA synthesis, and that cellular depletion of polyamines leads to growth arrest. However, there is no direct proof at this time that the increased polyamine synthesis universally found in cells stimulated to grow is the trigger for the initiation of DNA replication. The present results indicate that the time course of ornithine decarboxylase activity is not directly co-ordinated with the onset of DNA synthesis in regenerating rat liver.

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References

Boynton, A. L., Whitfield, J. F. & Isaacs, R. J. (1976) *J. Cell. Physiol.* **89**, 481–488
Bucher, N. L. R. & Swaffield, M. (1966) *Biochim. Biophys. Acta* **129**, 445–459
Burton, K. (1956) *Biochem. J.* **62**, 315–323
Clark, J. L. & Duffy, P. (1976) *Arch. Biochem. Biophys.* **172**, 551–557
Fausto, N. (1969) *Biochim. Biophys. Acta* **190**, 193–201
Fausto, N. (1971) *Biochim. Biophys. Acta* **238**, 116–128
Fausto, N. & Butcher, F. R. (1976) *Biochim. Biophys. Acta* **428**, 702–706
Fillingame, R. H., Jorstad, C. M. & Morris, D. R. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4042–4045
Gaza, D. J., Short, J. & Leiberman, I. (1973) *FEBS Lett.* **32**, 251–253
Goldstein, D. A., Heby, O. & Marton, L. J. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4022–4026
Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202
Hilton, J. & Sartorelli, A. (1970) *Adv. Enzyme Regul.* **8**, 153–166
Hölttä, E. & Jänne, J. (1972) *FEBS Lett.* **23**, 117–121
Inoue, H. & Mizutani, A. (1973) *Anal. Biochem.* **56**, 408–416
Jänne, J. (1967) *Acta Physiol. Scand. Suppl.* **300**
Krokan, H. & Eriksen, A. (1977) *Eur. J. Biochem.* **72**, 501–508
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Mamont, P. S., Böhlen, P., McCann, P. P., Bey, P., Schuber, F. & Tardif, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1626–1630
Munro, H. N. & Fleck, A. (1966) *Methods Biochem. Anal.* **14**, 113–176
Pösö, H. & Jänne, J. (1976) *Biochem. J.* **158**, 485–488
Rabes, H. M. & Brandle, H. (1969) *Cancer Res.* **29**, 817–822

Russell, D. H. & Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **60**, 1420–1427
Russell, D. H. & Snyder, S. H. (1969) *Endocrinology* **84**, 223–228
Schrock, T. R., Oakman, N. J. & Bucher, N. L. R. (1970) *Biochim. Biophys. Acta* **204**, 564–577
Stirling, G. A. Bourne, L. D. & March, T. (1975) *Br. J. Exp. Pathol.* **56**, 502–509
Sunkara, P. S., Rao, P. N. & Nishioka, K. (1977) *Biochem. Biophys. Res. Commun.* **74**, 1125–1133
Uchiyama, T., Fausto, N. & VanLancker, J. L. (1966) *J. Biol. Chem.* **241**, 991–996