Amine Synthesis in Regenerating Rat Liver: Extremely Rapid Turnover of Ornithine Decarboxylase

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(Received December 10, 1968)

SUMMARY

Following partial hepatectomy, there is a dramatic enhancement in the residual liver in the activity of ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17), an enzyme involved in polyamine synthesis. This increase in enzyme activity is completely prevented by treatment of the animals with puromycin at the time of hepatectomy, or by treatment with cycloheximide at the time of hepatectomy or up to 1 hr after hepatectomy. Actinomycin D administration at the time of operation prevents the subsequent rise in ornithine decarboxylase activity, but is only partially effective when given 30 min post-hepatectomy and ineffective 1 hr after operation. When cycloheximide is administered to unoperated rats or to animals 4 or 24 hr after hepatectomy, ornithine decarboxylase activity declines rapidly, with a half-life of about 11 min. After puromycin treatment, the decline of ornithine decarboxylase activity also shows a half-life of about 11 min. These findings suggest that the turnover of hepatic ornithine decarboxylase is extremely rapid.

INTRODUCTION

The polyamines spermidine and spermine, and their precursor putrescine, appear to play important roles in rapid tissue growth. Their concentrations increase markedly in regenerating rat liver (1, 2) and during certain growth phases of the chick embryo (3, 4), and are influenced by pituitary secretion of growth hormone (5). Mammalian tissue levels of polyamines are highest in organs with high rates of RNA synthesis, such as bone marrow, prostate, and pancreas (6). These compounds stabilize ribosomes and nucleic acids (7) and, in microorganisms, can directly affect rates of RNA synthesis (8).

Polyamine synthesis in bacteria (7, 9) and mammals (10, 11) involves the decarboxylation of ornithine to putrescine, which is then converted to spermidine by condensation with a propylamine moiety derived from the decarboxylation of *S*-adenosylmethionine. The enzymatic steps in the conversion of spermidine to spermine have not been characterized (7).

We have estimated the activity of ornithine decarboxylase (L-ornithine carboxylyase, EC 4.1.1.17) in a variety of rapidly growing tissues (12–14). In regenerating rat liver there is a very rapid and dramatic enhancement of ornithine decarboxylase activity. As early as 1 hr after partial hepatectomy, ornithine decarboxylase activity is tripled, is 10 times the basal level by 4 hr, and attains a peak activity 25 times

This research was supported by United States Public Health Service Grants 1-R01-NB-07275 and 1-PO1-GM-16492, by a grant from the American Cancer Society, and by Research Career Development Award 1-K3-MH-33128 to Solomon H. Snyder from the National Institute of Mental Health.

[1] Postdoctoral Fellow of the National Institutes of Health (GM-1183).

greater than control values at 16 hr (12, 13). In chick embryo, during the period of maximal limb bud formation, we found very high ornithine decarboxylase activity, which gradually declined to negligible levels by the time of hatching (12, 13). Certain rapidly growing tumors also showed ornithine decarboxylase activity far greater than that of nonmalignant tissues (12, 13). Also, administration of growth hormone markedly stimulates ornithine decarboxylase activity in the livers of normal rats (14).[2] Hypophysectomy abolishes the early enhancement of ornithine decarboxylase activity in rat liver after partial hepatectomy, an effect that is reversed by growth hormone (14).

After partial hepatectomy or growth hormone treatment, the increase in ornithine decarboxylase activity was extremely rapid. We wondered if this reflected protein synthesis *de novo* or activation of existing enzyme molecules, and accordingly have examined the effect of inhibitors of protein and nucleic acid synthesis on the response of ornithine decarboxylase activity to partial hepatectomy.

The association of striking changes in ornithine decarboxylase activity with alterations in tissue spermidine and putrescine concentrations suggests that ornithine decarboxylase activity may regulate the rate of synthesis of polyamines. In order to shed light on the dynamic regulation of polyamine synthesis, we studied the rate of turnover of ornithine decarboxylase, using inhibitors of protein synthesis.

MATERIALS AND METHODS

DL-Ornithine-1-^{14}C monohydrochloride (2.3 mC/mmole) was obtained from the New England Nuclear Corporation. Actinomycin D was obtained from Merck Sharp and Dohme Research Laboratory, and cycloheximide and puromycin hydrochloride, from Nutritional Biochemicals Corporation. NSD-1055 (4-bromo-3-hydroxybenzyloxamine dihydrogen phosphate) was generously donated by Dr. David J. Drain of Smith and Nephew Research, Ltd.

Sprague-Dawley male rats (150–200 g) were lightly anesthetized with ether. Partial hepatectomy was performed by the method of Higgins and Anderson (15). Sham-operated animals were anesthetized and laparotomized. Rats were killed by cervical fracture and decapitation, and the liver remnants (caudate and right lateral lobes) were removed, chilled immediately on ice, and homogenized in 5 volumes of 0.05 M sodium potassium phosphate buffer at pH 7.2. The homogenate was centrifuged at 20,000 $\times$ *g* for 20 min, and the supernatant fluid was used for enzyme assay.

Ornithine decarboxylase activity was determined by measuring the liberation of $^{14}CO_2$ from carboxyl-labeled substrate as previously described (13).

RESULTS

Effect of cycloheximide and puromycin on the increase in ornithine decarboxylase activity after partial hepatectomy. Groups of rats received cycloheximide injections 20 min prior to hepatectomy, at the time of hepatectomy, or 30 or 60 min following hepatectomy. They were killed 4 hr after hepatectomy, and their livers were assayed for ornithine decarboxylase activity. Enzyme activity 4 hr after sham operation was 7.30 ± 0.86 mμC of $^{14}CO_2$/30 min/g. Ornithine decarboxylase activity in partially hepatectomized rats was about 10 times greater than that of the sham-operated animals. Regardless of the time of its administration, cycloheximide completely prevented the increase in ornithine decarboxylase activity after partial hepatectomy (Fig. 1). To ascertain whether cycloheximide could affect the enzyme itself, cycloheximide (10^{-4} M) was incubated with enzyme preparations in the assay system for 10 min before addition of substrate. Under these conditions, no change in ornithine decarboxylase activity was detected.

These results suggested that the increased ornithine decarboxylase activity in regenerating rat liver represented synthesis of this enzyme *de novo*. In order to substantiate

[2] J. Janne and A. Raina, personal communication.

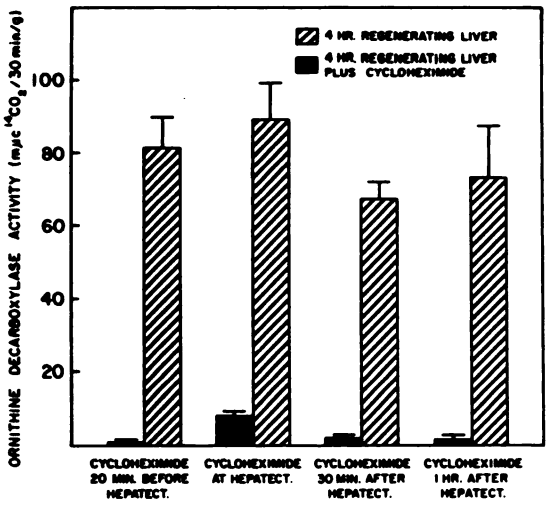

FIG. 1. *Effect of cycloheximide on the increase in ornithine decarboxylase activity after partial hepatectomy*

Rats received cycloheximide (50 mg/kg intraperitoneally) or 0.9% NaCl at various times and were killed 4 hr after partial hepatectomy. Each column represents the mean and standard error for 5–10 animals.

the data obtained with cycloheximide, using another drug, an experiment employing puromycin was designed. A group of rats received puromycin at the time of hepatectomy, and another group received injections of 0.9% NaCl. Both groups were killed 2 hr after operation, along with sham-operated animals that had received NaCl injections, and their livers were assayed for ornithine decarboxylase activity. Partial hepatectomy produced a 4.6-fold increase in ornithine decarboxylase activity in the NaCl-treated rats as compared with sham-operated animals. Puromycin completely abolished this increase (Fig. 2).

Effect of actinomycin D on the increase of ornithine decarboxylase activity after partial hepatectomy. Groups of rats received actinomycin D at the time of hepatectomy or 30 or 60 min following hepatectomy and were killed 4 hr after operation, and their livers were assayed for ornithine decarboxylase activity. Actinomycin D administered at the time of hepatectomy completely abolished the increase in ornithine decarboxylase activity, as had occurred with cycloheximide and puromycin (Fig. 3). However, in contrast to the results with cycloheximide, when actinomycin D was given 30 min after operation it only partially prevented the increase in ornithine decarboxylase activity. When actinomycin D was administered 1 hr after hepatectomy, ornithine decarboxylase activity at 4 hr did not differ from that of the NaCl-treated control animals.

Determination of the half-life of ornithine decarboxylase using cycloheximide and puromycin. In order to ascertain the rate of turnover of ornithine decarboxylase in rat liver, cycloheximide or puromycin was administered and the rate of decline of ornithine decarboxylase activity was examined. In an initial experiment, groups of rats were subjected to partial hepatectomy and received injections of cycloheximide 30, 60, 90, and 120 min prior to being killed 24 hr after operation, along with NaCl-treated controls (Table 1). In as little as 30 min after cycloheximide treatment, ornithine decarboxylase activity was

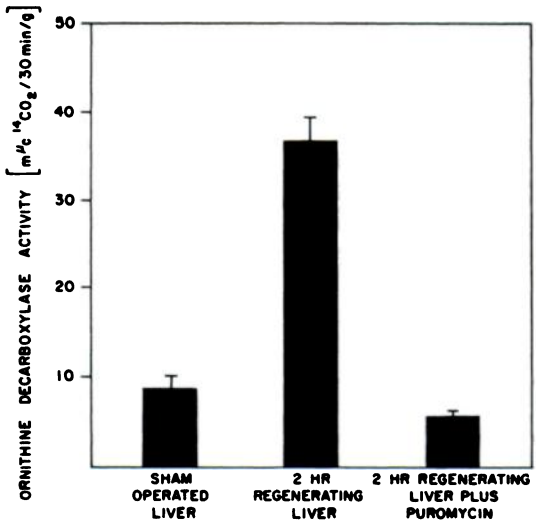

FIG. 2. *Effect of puromycin on the increase in ornithine decarboxylase activity after partial hepatectomy*

Ornithine decarboxylase activity was measured in sham-operated rat liver, 2-hr regenerating rat liver, and 2-hr regenerating rat liver with a 100 mg/kg dose of puromycin given intraperitoneally at the time of the operation. Each column represents the mean and standard error for 5–10 animals.

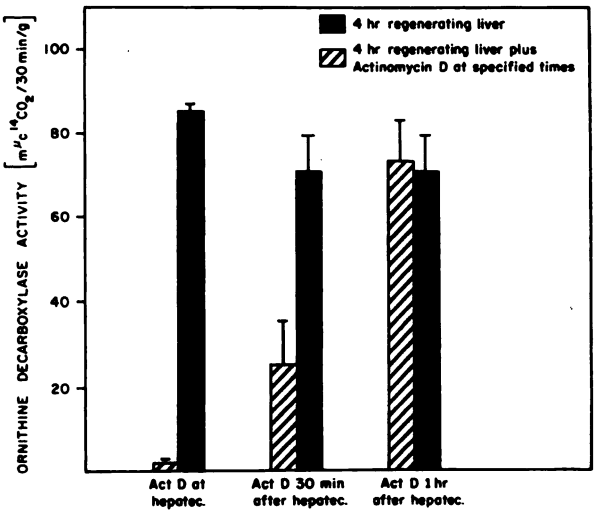

FIG. 3. *Effect of actinomycin D on ornithine decarboxylase activity of 4-hr regenerating rat liver*
A 2 mg/kg dose of actinomycin D was administered intraperitoneally at the times indicated. Each column represents the mean and standard error for 5–10 animals.

reduced to basal levels. At 90 and 120 min after cycloheximide, enzyme activity was still lower than the levels at 30 min, although only at 90 min after treatment was it significantly lower ($p < 0.02$) than at 30 min. In order to study the effect of cycloheximide over shorter time intervals, groups of rats received cycloheximide 10, 20, and 30 min before being killed 24 hr after operation, along with controls given 0.9% NaCl. There was a very rapid decline of ornithine decarboxylase activity after cycloheximide treatment, with a half-life of 11.5 min (Fig. 4).

TABLE 1
Effects of cycloheximide on ornithine decarboxylase in 24-hr regenerating rat liver
Rats treated with cycloheximide received 50 mg/kg doses intraperitoneally. There were 5–10 rats in each group.

Treatment of animals	$^{14}CO_2$ production (mean ± SE)
	mμC/30 min/g
Controls (0.9% NaCl)	40.6 ± 8.3[a]
Cycloheximide ½ hr prior to decapitation	5.6 ± 1.9[b]
Cycloheximide 1 hr prior to decapitation	3.5 ± 0.85
Cycloheximide 1½ hr prior to decapitation	0.92 ± 0.80[b]
Cycloheximide 2 hr prior to decapitation	1.20 ± 0.90

[a] Controls differed from all other groups ($p < 0.001$).
[b] These values differed significantly ($p < 0.02$).

The extremely rapid turnover rate obtained in the 24-hr regenerating liver raised the question whether the turnover rate of ornithine decarboxylase varied at different times after hepatectomy or in the basal condition. Accordingly, groups of rats were partially hepatectomized and treated with cycloheximide 10, 20, and 30 min before being killed 4 hr after operation (Fig. 4). Just as had been observed with the 24-hr regenerating liver, in the 4-hr regenerating liver there was a very rapid decline of enzyme activity, with a half-life of 11.5 min.

In another experiment, unoperated rats received cycloheximide and were killed 5, 10, and 15 min later, and their livers were assayed for ornithine decarboxylase activity. Enzyme activity declined exponentially, with a half-life of 10 min, similar to the half-life observed in both the 4-hr and 24-hr regenerating livers.

In an experiment designed to detect the

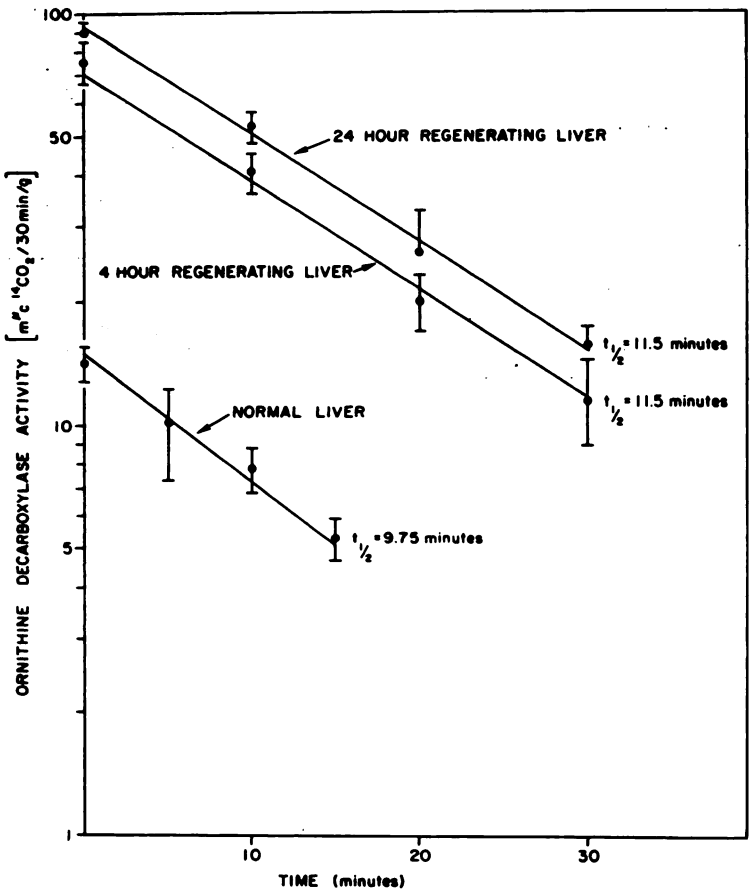

FIG. 4. *Time course of the decline of ornithine decarboxylase activity in 4-hr regenerating rat liver, 24-hr regenerating rat liver, and normal rat liver after administration of 50 mg/kg of cycloheximide intraperitoneally*

The lines were plotted by the least squares method. Each point represents the mean ± standard error for five animals.

possible presence of an inhibitor of ornithine decarboxylase in livers of cycloheximide-treated rats, enzyme preparations from 4-hr regenerating rat livers after injection of 0.9% NaCl and 4-hr regenerating rat livers 30 min after treatment with cycloheximide (50 mg/kg) were mixed together and assayed for ornithine decarboxylase activity. Enzyme activity was additive in all cases, indicating the absence of an inhibitor in livers of the cycloheximide-treated rats.

The substrate concentration used in most experiments (50 μM as L-ornithine) was nonsaturating. It is conceivable that after cycloheximide treatment, levels of free ornithine in the liver might change. Accordingly, in some experiments saturating amounts of L-ornithine (2 mM) were used as substrate, and the same rapid decline in ornithine decarboxylase activity, with a half-life of 11 min, was observed. This indicates that changes in free hepatic L-ornithine cannot account for the cycloheximide-produced decrease in ornithine decarboxylase activity.

To determine whether the short half-life for ornithine decarboxylase obtained with cycloheximide was related primarily to inhibition of protein synthesis by the drug rather than to some other effect of cycloheximide, we designed an experiment using

puromycin, a different inhibitor of protein synthesis. Groups of rats were partially hepatectomized and received puromycin 10, 20, and 30 min before being killed 4 hr after operation (Fig. 5). Just as had been found in the experiments with cycloheximide, ornithine decarboxylase activity fell quickly after puromycin treatment, with a half-life of 11.5 min.

Effect of actinomycin D on ornithine decarboxylase activity of regenerating rat liver. Actinomycin D inhibits DNA-dependent synthesis of RNA (16). Tschudy *et al.* (17) administered actinomycin D to rats in order to measure the half-life of the messenger RNA for hepatic δ-aminolevulinic acid synthetase. We examined whether such an approach might be applicable to ornithine decarboxylase in the following experiment. Groups of rats were partially hepatectomized and treated with actinomycin D at eight time intervals between ½ hr and 4 hr before being killed 24 hr after operation. Ornithine decarboxylase activity was not altered at any time by actinomycin D treatment (Table 2). In another experiment, rats were partially hepatectomized and received actinomycin

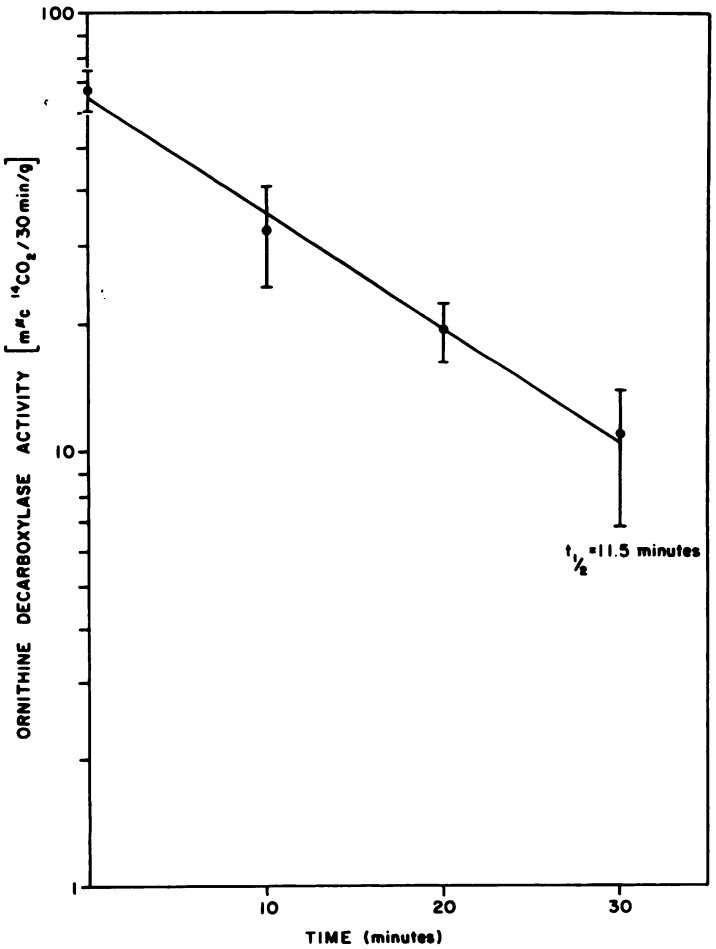

FIG. 5. *Time course of the decline of ornithine decarboxylase activity in 4-hr regenerating rat liver after the administration of 100 mg/kg of puromycin intraperitoneally*

The line was plotted by the least squares method. Each point represents the mean ± standard error for five animals.

TABLE 2
Effects of actinomycin D on ornithine decarboxylase activity of regenerating rat liver

Rats treated with actinomycin D received 2 mg/kg doses intraperitoneally. There were 5–10 rats in each group.

Experimental animals 24 hr after partial hepatectomy	$^{14}CO_2$ production (mean ± SE)
	mμC/30 min/g
Controls (0.9% NaCl)	48.4 ± 5.8
Actinomycin D ½ hr before decapitation	50.8 ± 7.4
Actinomycin D 1 hr before decapitation	64.2 ± 11.7
Actinomycin D 1½ hr before decapitation	46.2 ± 7.2
Actinomycin D 2 hr before decapitation	43.9 ± 6.7
Actinomycin D 2½ hr before decapitation	46.9 ± 5.7
Actinomycin D 3 hr before decapitation	51.8 ± 18.7
Actinomycin D 3½ hr before decapitation	41.6 ± 3.7
Actinomycin D 4 hr before decapitation	51.8 ± 14.6

D or 0.9% NaCl 6 hr before being killed 7 hr after operation. Enzyme activity in the control hepatectomized rats was 118.0 ± 17.3 mμC of $^{14}CO_2$/30 min/g, but was reduced in the actinomycin D-treated rats to 27.3 ± 17.3 mμC of $^{14}CO_2$/30 min/g of tissue.

DISCUSSION

In the present study we have examined whether the very rapid increase in ornithine decarboxylase activity after partial hepatectomy involves new protein synthesis. Since puromycin and cycloheximide, drugs that inhibit protein synthesis by different mechanisms, both completely prevented the increase in ornithine decarboxylase activity after partial hepatectomy, it would appear that this increase in enzyme activity reflects synthesis *de novo* of ornithine decarboxylase. Our finding that the apparent degradation rate of ornithine decarboxylase is the same in the basal and in the induced state also suggests that the increase in ornithine decarboxylase activity after partial hepatectomy represents synthesis of new enzyme, rather than a slowing of its degradation.

In experiments measuring the decline of ornithine decarboxylase activity after puromycin or cycloheximide treatment, the half-life of decline was extremely short, about 10–11 min, whether studied in the basal or the induced state. This appears to be the shortest half-life reported for a mammalian enzyme (Table 3). In the literature we reviewed, δ-aminolevulinic acid synthetase had the fastest recorded turnover rate, with a half-life of 67–72 min, which is more than 6 times greater than the half-life of hepatic ornithine decarboxylase. Studies of enzyme turnover rate based solely on the measurement of enzyme activity must be interpreted with caution, since drugs such as cycloheximide may block enzyme degradation as well as enzyme synthesis, and may produce effects unrelated to protein synthesis (29). However, for at least two hepatic enzymes, tyrosine transaminase (19, 22, 30) and tryptophan pyrrolase (23, 31), turnover rates calculated from the fall in enzyme activity after treatment with inhibitors of protein synthesis appear to be valid measures, since they are the same as when

TABLE 3
Half-lives of some mammalian enzymes

Enzyme	$t_{1/2}$
Ornithine decarboxylase	11 min
δ-Aminolevulinic acid synthetase (18)	67–72 min
Tyrosine transaminase (19)	1.5 hr
Histidine decarboxylase (20)	1.8 hr
Ribonucleotide reductase (21) (conversion of CMP to dCMP)	2.0 hr
Tryptophan pyrrolase (22, 23)	2.5 hr
Threonine dehydratase (24)	3.0 hr
Serine dehydratase (25)	5.2 hr
Catalase (26)	1–1.5 days
Glutamic-pyruvic transaminase (27)	3.5 days
Arginase (28)	4–5 days

determined by changes in amount of isotopically labeled enzyme.

When an enzyme is "induced" by a stimulus which is present continuously until a new steady-state enzyme level is established and the "inducing" stimulus ceases quickly, the half-times for the degradation of the enzyme in the presence and absence of the inducing stimulus can be estimated from the half-times for the shift to and from the new steady state. In regenerating liver the stimulus for the enhancement of ornithine decarboxylase activity is unknown, as is the duration of its presence. Accordingly, rates of changes in ornithine decarboxylase activity after partial hepatectomy could not be used to confirm the half-lives estimated from experiments with cycloheximide and puromycin.

The synthesis of most mammalian enzymes is a linear function of time, whereas enzyme degradation is an exponential function of time. Therefore, rates of change of enzyme levels from one steady state to another are determined solely by the degradation rate of the enzyme (27, 29, 32). The very high degradative rate of ornithine decarboxylase suggests that its activity changes rapidly in response to stimuli to altered synthesis. Since ornithine decarboxylase activity may be rate-limiting in the biosynthesis of the polyamines (13), our results suggest that polyamine synthesis in mammalian liver is a finely modulated process. Inasmuch as the physiological function of the polyamines is not established, it is difficult to ascertain the importance of such a sensitive regulation of their synthesis.

Knowing the half-life for the turnover rate of ornithine decarboxylase, it should be possible to calculate the synthesis rate of this enzyme after partial hepatectomy. Assuming that enzyme synthesis conforms to zero-order kinetics and that degradation is first-order with decay constant k, the simplest expression for enzyme levels would be

$$\frac{dP}{dt} = S - kP \qquad (1)$$

In this equation S is synthesis rate and P is enzyme level. Up to 24 hr after partial hepatectomy, the mass of the liver does not change significantly, so that mass effects can be ignored in the above equation.

Since the half-life of ornithine decarboxylase does not change after hepatectomy, k remains constant. If during induction S changes in a single-step fashion to a new level, S', then

$$S' = \frac{k(P' - Pe^{-kt})}{1 - e^{-kt}} \qquad (2)$$

In Eq. 2, P' is the enzyme level at any given time and P is the basal enzyme level. Since the half-life of ornithine decarboxylase is very short, the exponential factors will be negligible at values of kt greater than about 4. If the enzyme level is at a steady state, ornithine decarboxylase synthesis rate could be calculated as follows:

$$S' = kP \qquad (3)$$

Equation 2 could be applied to calculating the synthesis rate of ornithine decarboxylase only if hepatectomy produced a single-step rather than a graduated increase in enzyme synthesis, so that after hepatectomy at various times the enzyme synthesis rate should be constant even though enzyme activity varies. In preliminary attempts to calculate S' using ornithine decarboxylase activity at different intervals after hepatectomy (13), we obtained varying values of S'. This suggests that the rate of ornithine decarboxylase synthesis does not change in a single-step fashion after hepatectomy.

If enzyme activity is constant, the synthesis rate can be calculated using Eq. 3. After partial hepatectomy, however, enzyme activity fluctuates continuously (13), so that the rate of ornithine decarboxylase synthesis cannot be calculated by this method from changes in its activity after hepatectomy.

Berlin and Schimke (33) have calculated changes in synthesis rates of several inducible hepatic enzymes by examining the initial rate of increase of enzyme activity after administration of the inducing hormone. For such calculations to be applicable, however, the time interval during

which increase in enzyme activity is followed must be much shorter than the half-life of the enzyme, so that $1 - e^{-kt} \simeq kt$. The extremely short half-life of ornithine decarboxylase would preclude such determinations for this enzyme. Interestingly, for some enzymes examined by Berlin and Schimke (33), the half-lives also appear to be too brief to permit an accurate calculation of enzyme synthesis by this method.

Actinomycin D, when administered at the time of hepatectomy, completely prevented the enhancement of ornithine decarboxylase activity when measured 4 hr after operation. When given 30 min after hepatectomy, however, it only partially inhibited the increase in enzyme activity, and when given at 1 hr it was without effect. Actinomycin D inhibits DNA-dependent RNA synthesis (16) and has been used by numerous investigators to study the role of RNA synthesis in the regulation of a variety of mammalian enzymes. If we assume that the effect of actinomycin D on ornithine decarboxylase activity was mediated by its action on RNA synthesis, then it would appear that virtually all of the messenger RNA for the levels of ornithine decarboxylase measurable at 4 hr was synthesized in the first hour after hepatectomy. This finding is interesting in light of the observation of Church and McCarthy (34) that during the first hour after hepatectomy in the mouse, a new species of very short-lived RNA is synthesized.

When actinomycin D was given to rats 24 hr after hepatectomy, there was no decline in ornithine decarboxylase activity for up to 4 hr. Tschudy *et al.* (17) treated rats with actinomycin D and measured the activity of δ-aminolevulinic acid synthetase at various time intervals. After a lag phase of 3–4 hr, enzyme activity declined rapidly, and a half-life for the messenger RNA of δ-aminolevulinic acid synthetase of 40–70 min was calculated. Using the assumptions of Tschudy *et al.* (17), it would seem that the half-life for ornithine decarboxylase messenger RNA is longer than that of δ-aminolevulinic acid synthetase messenger RNA. Our experiments were not carried out for long enough periods to determine definitively whether or not the messenger RNA half-life for ornithine decarboxylase is shorter than that of the bulk of cytoplasmic rat liver messenger RNA, which is stable for at least 40 hr (35). In one experiment, we found a considerable decline of ornithine decarboxylase activity 6 hr after administering actinomycin D, which suggests that messenger RNA for ornithine decarboxylase may be relatively short-lived.

ACKNOWLEDGMENTS

The authors would like to thank Michael Kuhar and Dr. M. Walser for their assistance in describing kinetic considerations of turnover rates.

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