METABOLIC ALTERATIONS OF LIVER REGENERATION. III.*

L-TRYPTOPHAN MEDIATED INCREASE OF THYMIDINE PHOSPHORYLATION IN REGENERATING RAT LIVER

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The activity of thymidine kinase in regenerating rat liver is maximal at 24—30 h following partial hepatectomy. The dietary administration of L-tryptophan during this stage of regeneration prevents the decrease of thymidine and thymidylate kinase activities which occurs at later phases of liver regeneration. Simultaneously the incorporation of thymidine into liver DNA is increased. The stabilizing effect of tryptophan on the induced liver enzymes is discussed.

Among amino acids L-tryptophan has a special role on account of its effect on the synthesis of liver proteins and nucleic acids[1-3]. The administration of tryptophan *in vivo* results in the stimulation of liver protein synthesis associated with the simultaneous changes of liver polyribosome distribution[4-6]. It seems likely that the effect of L-tryptophan on polyribosome profile and on liver proteosynthesis is achieved by a cytoplasmic mechanism[2,4].

This contention is further strengthened by the investigations concerning the effect of dietary tryptophan on the level of different liver enzymes. Following tryptophan the enhancement of the activity of tryptophan pyrrolase[7-9], tyrosine α-ketoglutarate transaminase[10-12], serine dehydratase[13], ornithine δ-transaminase[13] and phosphoenolpyruvate carboxykinase[14] has been observed. Employing the method of a specific immunoprecipitation it has been shown[12,15] that the increase of enzyme activity is not always accompanied by the increase of the rate of enzyme synthesis. An analogous stabilizing effect of tryptophan is probably operating in case of DNA-dependent RNA polymerase observed in liver nuclei following the administration of tryptophan *in vivo*[16]. This report deals with the effect of tryptophan on thymidine phosphorylation in regenerating rat liver.

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EXPERIMENTAL

Chemicals. Adenosine 5'-triphosphate, disodium trihydrate, thymidine 5'-phosphate and thymidine 5'-triphosphate were delivered by Calbiochem (Luzern). L-Tryptophan, L-histidine and L-lysine were purchased from Loba-Chemie (Vienna). Thymidine-[2-^{14}C] (25 μCi/μmol) was obtained from the Institute for Research, Production and Uses of Radioisotopes, Prague.

Animals. For the experiments female rats (170–175 g) kept under standard conditions were used. 70 per cent hepatectomy[17] was performed under ether narcosis in groups including 4–6 animals. L-Tryptophan was dissolved in 1M-NaOH, neutralized with 1N-HCl (pH 8·5–9·0) and intubated in a maximal volume of 5 ml under ether narcosis.

Liver cell-free extract. The animals were killed by decapitation and bled at different phases following partial hepatectomy. The excised livers were homogenized in the cold with 3 volumes of 0·025M Tris-HCl buffer (pH 7·5) with 0·005M Mg^{2+}-ions and 0·025M-KCl. The homogenate was centrifuged (10000 *g*, 20 min, 3°C) and the defatted supernatant fraction was immediately used for the phosphorylation reactions.

Thymidine phosphorylation was carried out[18] for 10 min at 37°C in 0·5 ml of reaction mixture in $5 \cdot 10^{-2}$M Tris-HCl buffer (pH 8·0). $5 \cdot 10^{-5}$M Thymidine-[2-^{14}C] and $3 \cdot 10^{-3}$M adenosine 5'-triphosphate with equimolar Mg^{2+}-ions were incubated with 0·1 ml of a cell-free fraction from liver. The newly formed 5'-phosphates of thymidine were separated chromatographically with standards on a Whatman paper No 1 in a solvent system composed of isobutyric acid–water–ammonium hydroxide (66 : 33 : 1·5). The radioactivity was determined in a liquid scintillation spectrometer (Packard).

Incorporation of thymidine. Thymidine-[2-^{14}C] was administered *i.p.* at the dose level of 3 μCi/0·5 μmol per animal to groups of 4 female rats 24 h following partial hepatectomy. 2 h later the animals were killed by decapitation, bled and the livers homogenized with 5 volumes of ice-cold 0·2M-$HClO_4$. The acid-soluble pool was extracted by repeated washing in 0·2M-$HClO_4$ and the sediments were subjected to alkaline hydrolysis with 1M-KOH (16 h, 20°C). Neutralization was carried out with 70% $HClO_4$, and after centrifugation the sediments were dried and hydrolysed 1 h at 100°C with 3 volumes of 70% $HClO_4$. The hydrolysates were neutralized with 3M-KOH, centrifuged and subjected to chromatography on Whatman paper No 3 in a mixture composed of 1-butanol–acetic acid–water (10 : 1 : 3). Strips of paper were eluted with water, and eluates rechromatographed in a solvent system composed of isopropylalcohol–ammonium hydroxide–water (7 : 1 : 2).. Spectroscopically pure thymine was eluted and assayed with a Unicam SP 700 spectrophotometer and radioactivity was determined by a liquid scintillation counter (Packard). The incorporation of thymidine-[2-^{14}C] is expressed as specific radioactivity of isolated thymine in c.p.m./μmol.

RESULTS AND DISCUSSION

Liver regeneration is mediated by short-lived RNA molecules synthesized soon after hepatectomy; their formation is discontinued at various stages and ceases at various times during regeneration[19,20]. The first stages of regeneration are characterized by the increased synthesis of ribonucleic acids (*e.g.*[21–23]) followed by the enhanced DNA synthesis (*e.g.*[24–26]). Table I indicates that the administration of L-tryptophan 24 h after partial hepatectomy prevents the decrease[27,28] of thymidine kinase and thymidylate kinase activities which occurs at later phases of regeneration[29–31]. No similar effect has been found with L-histidine or L-lysine administered at a similar

dose level. The application of tryptophan to sham-operated animals under these conditions has no effect on thymidine phosphorylation although the stimulatory action of dietary proteins on liver thymidine kinase has been observed in animals kept on 0% protein diet[32].

The action of tryptophan on the phosphorylation of thymidine during different

TABLE I

Phosphorylation of Thymidine in an Extract from Regenerating Rat Liver after Application of Different Amino Acids *in vivo*

Groups of 5 female rats (170 g) were intubated 24 h following partial hepatectomy with a solution of amino acids (5 ml, 1 g per kg body weight) and 24 h later they were killed. Phosphorylation of thymidine-[2-^{14}C] (5 . 10^{-5}M) was carried out for 10 min at 37°C in 0·5 ml reaction mixture containing 0·05M Tris-HCl (pH 8·0), 3 . 10^{-3}M adenosine 5′-triphosphate with equimolar Mg^{2+}-ions and 0·1 ml of a supernatant fraction from rat liver.

Regeneration h	Application 1 g/kg	TTP %	TDP %	TMP %	Total phosphorylation %
Sham-operated	0	0·36	0·75	5·35	100
Sham-operated	L-Try	0·42	0·83	5·27	101
24	0	5·07	2·72	34·18	651
48	0	2·35	1·32	12·76	254
48	L-Try[a]	5·17	4·25	29·36	601
48	L-Lys[a]	3·08	1·71	13·90	289
48	L-His[a]	3·14	1·83	14·59	302

[a] Amino acids were administered *per os* 24 h following partial hepatectomy.

TABLE II

Incorporation of Thymidine into DNA of Regenerating Rat Liver Following the Administration of L-Tryptophan

L-Tryptophan was intubated at the dose level of 1 g per kg body weight in a maximal volume of 5 ml 24 h following partial hepatectomy to groups of 4 female rats. Thymidine-[2-^{14}C] was injected intraperitoneally 24 h later at a dose level of 3 μCi/0·2 μmol and the animals were killed 2 h thereafter. Control = 100%.

Experiment No	Thymidine incorporation, c.p.m./μmol		Increase %
	control	tryptophan-treated	
1	26 040 ± 3 530	36 090 ± 6 940	138
2	24 213 ± 2 060	29 870 ± 5 460	123

stages of liver regeneration is given in Fig. 1. A single dose of tryptophan 2 h following hepatectomy results in a slight increase of enzyme activity at 24 h; at later periods of regeneration thymidine phosphorylation decreases in a similar manner as in the controls. The administration of tryptophan during the stage of a maximal thymidine kinase activity prevents almost totally the decrease of thymidine phosphorylation during the next 24 h period.

The relation of thymidine phosphorylation in 48 h regenerating liver to the dose level of tryptophan administered 24 h following partial hepatectomy is given in Fig. 2. Under these conditions thymidine kinase is stimulated 2–3 times, and the formation of corresponding 5'-triphosphate and 5'-diphosphate is increased nearly 3 times. At suitable time interval between hepatectomy and application of tryptophan even higher levels of phosphorylation may be achieved. However, repeated tryptophan administration at different stages of regeneration does not prevent the final decrease of thymidine phosphorylation to the levels which occur in control non-regenerating livers (Fig. 3).

In accordance with the enhanced activity of thymidine kinase and thymidylate kinase the administration of tryptophan leads to the higher incorporation of thymidine-[2-^{14}C] into liver deoxyribonucleic acids (Table II). This effect is disappearing at later stages of regeneration; at 72 h of regeneration the incorporation of thymidine into control and tryptophan-treated livers is the same.

The effect of tryptophan depends apparently on the primary induction, *i.e.*, on the partial hepatectomy. Figs 1 and 3 suggest that the most considerable effect of tryptophan is observed at the stage of maximal enzymatic activity and probably also during

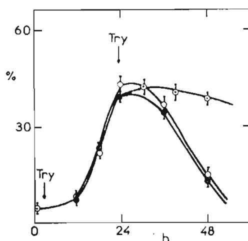

FIG. 1

Effect of L-Tryptophan Administered at Different Time Intervals Following Partial Hepatectomy on Thymidine Phosphorylation

L-Tryptophan (1 g per kg body weight) was intubated 2 (○) or 24 (⊙) h following partial hepatectomy as indicated to groups of 6–9 rat females (170 g). Controls without tryptophan (●). %, Total phosphorylation of thymidine-[2-^{14}C] during 10 min incubation; h, time intervals following partial hepatectomy.

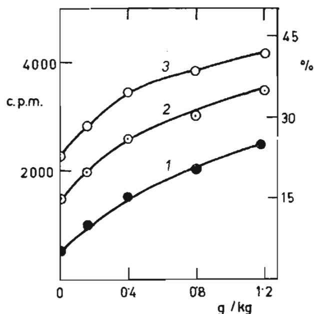

FIG. 2

Increase of Thymidine Phosphorylation in 48 h Regenerating Rat Liver in Relation to the Dose Level of L-Tryptophan

L-Tryptophan was intubated to groups of 3—6 rat females (175 g) 24 h following partial hepatectomy. The animals were killed 24 h later and phosphorylation of thymidine was determined. *1* Radioactivity of thymidine 5'-triphosphate and 5'-diphosphate (c.p.m.); *2* thymidine 5'-monophosphate (c.p.m.); *3* total phosphorylation (%). Dose level of L-tryptophan is given in g per kg.

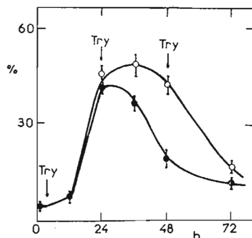

FIG. 3

Time Course of Thymidine Phosphorylation during Liver Regeneration Following Repeated Application of L-Tryptophan

L-Tryptophan (○; 1 g per kg body weight) was intubated to groups of 4—6 rat females 2, 24 and 48 h following partial hepatectomy as indicated. Controls without tryptophan (●). The animals were killed at different time intervals after hepatectomy (h) and the total phosphorylation of thymidine was determined during 10 min incubation (%).

the period of a maximal synthesis of the corresponding messenger RNA. It remains to be elucidated whether the described enhancement of thymidine and thymidylate kinase is caused by tryptophan alone or its metabolites (*e.g.*[33]). The increase of thymidine phosphorylation reminds the induction of amino acid metabolizing enzymes in normal liver. It seems that the mechanism operating in case of regenerating liver consists also in the tryptophan-mediated *stabilization* either of the synthesized enzyme or of the messenger RNA rather than in the stimulation of their synthesis. The presence of tryptophan may be a more general requisite for the regulation of the turnover of some proteins prolonging the half life of their biologic activity.

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