circulating blood. Urinary excretion accounts for only approximately 20% of injected heparin and therefore does not account for the rapid elimination of anticoagulant from the blood. Liver and spleen appear to retain isotope activity after anticoagulant and isotope activity have been eliminated from the plasma.

Mast cells are believed to be a site of synthesis or storage of heparin in the intact animal. Spolter and Marx(8) have shown that isolated mouse mastocytoma is capable of synthesizing heparin from inorganic sulfate. These workers have also shown that phosphoadenosine-phosphosulfate serves as the sulfate donor for synthesis of heparin by the mouse mastocytoma. The intense metachromatic activity of mast cell granules is believed to be due to presence of mucopolysaccharides having heparin-like activity. The current experiments indicating that injected heparin $S^{35}$ appears in the granules of peritoneal mast cells of the rat suggest that the mast cells may function as a storage depot for administered heparin.

*Summary.* Heparin $S^{35}$ was extracted from the liver of dogs administered inorganic $Na_2S^{35}O_4$. Distribution of this heparin was determined in rats. The mast cells were found to take up injected heparin $S^{35}$, and radioactivity persisted in liver and spleen after virtual disappearance from plasma. Anticoagulant activity was found to parallel isotope activity of the plasma of dogs injected with heparin $S^{35}$. Urinary excretion in dogs accounted for only about 20% of the injected isotope at a time when plasma anticoagulant activity had returned to normal.

Technical assistance of Miss C. S. Spaeth is gratefully acknowledged.

1. Loomis, T. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101. 447.

2. Charles, A. F., Scott, D. A., *J. Biol. Chem.*, 1933, v102, 431.

3. Eiber, H. B., Danishefsky, I., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 801.

4. ———, *J. Biol. Chem.*, 1957, v226, 721.

5. Tocantins, L. M., The Coagulation of Blood, Grune and Stratton, 1955, 27.

6. Walkenstein, S. S., Knebel, C. M., *Anal. Chem.*, 1957, v29, 1516.

7. Glick, D., Bonting S. L., den Boer, D., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 357.

8. Spolter, L., Marx, W., *Biochem. Biophys., Acta*, 1959, v32, 291.

Received December 1, 1960. P.S.E.B.M., 1961, v106.

## Effect of Thyroxine on Enzymes of the Urea Cycle in Regenerating Rat Liver.* (26380)

M. NAZARIO† AND PHILIP P. COHEN

*Department of Physiological Chemistry, University of Wisconsin, Madison*

Studies in this laboratory have shown(1,2) that treatment of *R. catesbeiana* tadpoles with low concentrations of L-thyroxine ($2.6 \times 10^{-8}$M) resulted in a marked increase in the specific activity of liver carbamyl phosphate synthetase. This increase has been shown to be due to *de novo* synthesis of the enzyme(3). An increase in specific activity has also been observed for 2 other urea cycle enzymes, namely ornithine transcarbamylase and arginase, during thyroxine-induced metamorphosis (unpublished studies).

In view of these findings, it seemed desirable to determine whether the thyroid hormone played a similar role in mammalian tissues. For this purpose, the effect of thyroidectomy on the activity of carbamyl phosphate synthetase, ornithine transcarbamylase and arginase in regenerating rat liver was investigated.

* This study was supported in part by grants from the Nat. Cancer Inst., N.I.H., U. S. Public Health Service and Wisconsin Alumni Research Foundation.

† Fellow of World Health Organization, on leave of absence from Inst. Nacional de Microbiol., Buenos Aires, Arg.

TABLE I. Liver Weight during Regeneration in Athyroid Rats.

| Animal No. | Preoperative body wt (g) | Liver removed (g) | Body wt before sacrifice (g) | | Wt of liver after regeneration (g) | | | g liver/100 g body wt |
|---|---|---|---|---|---|---|---|---|
| | | | Athyroid | Athyroid plus thyroxin | 48 hr | 72 hr | 144 hr | |
| 14 | 173 | 3.35 | 163 | | 2.75 | | | 1.69 |
| 15 | 193 | 3.50 | 190 | | 2.55 | | | 1.34 |
| 22 | 160 | 3.45 | 160 | | 2.60 | | | 1.62 |
| 12 | 168 | 2.55 | | 153 | 3.30 | | | 2.16 |
| 20 | 175 | 3.00 | | 160 | 3.40 | | | 2.13 |
| 21 | 152 | 3.15 | | 142 | 2.70 | | | 1.90 |
| 1 | 160 | 2.55 | 160 | | | 2.30 | | 1.44 |
| 2 | 155 | 3.10 | 152 | | | 2.25 | | 1.48 |
| 3 | 175 | 3.70 | 172 | | | 3.10 | | 1.80 |
| 5 | 150 | 2.70 | | 126 | | 4.10 | | 3.25 |
| 6 | 165 | 2.95 | | 149 | | 4.75 | | 3.19 |
| 10 | 175 | 3.55 | 178 | | | | 4.90 | 2.75 |
| 19 | 175 | 3.80 | 185 | | | | 4.40 | 2.38 |
| 8 | 165 | 3.55 | | 125 | | | 3.35 | 2.68 |
| 9 | 152 | 2.85 | | 128 | | | 8.30* | 6.48 |
| 17 | 240† | 5.20 | | 230 | | | 8.10 | 3.52 |

* Histochemical examination showed fat infiltration.
† Body wt was 160 g when experiment was started.

*Methods.* Male albino rats,‡ weighing 145-180 g were used. One week after surgical thyroidectomy, each rat received an intraperitoneal injection of 500 μcuries carrier-free $I^{131}$. Following this, the animals were kept in metabolic cages and were fed a low iodine diet§ enriched in protein by the addition of 10% crude casein. A second dose of 250 μcuries $I^{131}$ was administered 2 weeks after the first injection. Partial hepatectomy by the usual technic(4), with removal of 65 to 75% of the liver, was performed under light ether anesthesia 6 to 8 weeks after administration of the last dose of radioactive iodine.

Some of these animals (designated "athyroid plus thyroxine") received subcutaneous injections of 0.5 mg L-thyroxine during the period that elapsed between partial hepatectomy and sacrifice. Rats 4-9, and 12 were given an additional injection of L-thyroxine 12 hours before partial hepatectomy. The liver samples were carefully blotted with filter paper, weighed, and the enzymes assayed in duplicate.

Assay procedures and units of enzyme activity are those previously described(5). Protein determinations were carried out according to Lowry *et al.*(6) and liver glycogen was estimated by the anthrone method(7).

*Results and discussion.* The rate of liver regeneration is shown in Table I and Fig. 1. It is apparent from the table that the athyroid animals show a slower rate of liver regeneration and that injection of thyroxine improves the proliferative capacity to levels that compare with those of normal rats kept on the same diet (Fig. 1). Following partial hepatectomy average daily food intake dropped to about one-half and one-third that of the intact animals, respectively, for the athyroid and "athyroid plus thyroxine" rats. No significant difference could be found for total liver protein content on a wet weight basis among all 3 groups of rats. Glycogen estimations were used as an additional indication of degree of response to thyroxine administration(8). The glycogen content was below level of detection in all thyroxine-injected animals.

The influence of thyroidectomy on liver regeneration in the rat has been studied by Christensen and Jacobsen(9) and by Drabkin(10) who concluded that thyroidectomy did not impair liver regeneration. However, these investigations dealt with liver weight

‡ Supplied by Holtzman Co., Madison, Wisc.

§ The low iodine diet used and the casein used for enrichment were purchased from Nutritional Bio-Chemicals Corp., Cleveland, Ohio.

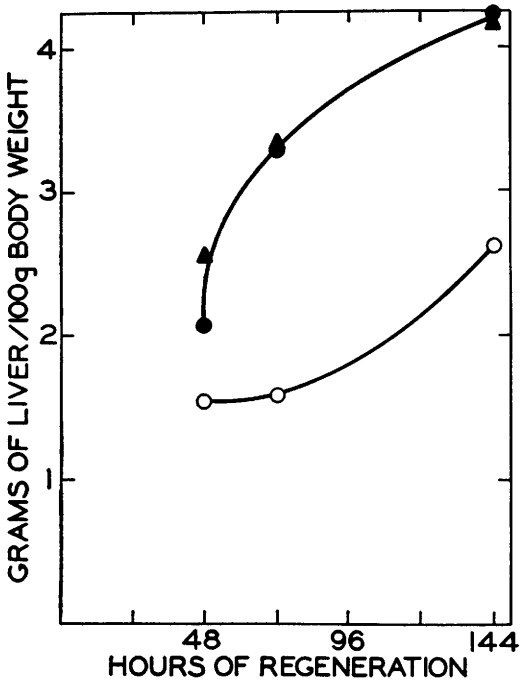


FIG. 1. Rate of liver regeneration in normal (▲—▲), athyroid (○—○) and athyroid plus thyroxine (●—●) rats.

estimations in the time period starting 2 weeks after partial hepatectomy and thus did not deal with the early stages of the regeneration process. Further, only surgical thyroidectomy was employed in these studies.

As to the activity of the enzymes measured,‖ no significant differences between normal, athyroid and "athyroid plus thyroxine," could be found in the livers of these 3 groups of rats at time of hepatectomy (excised liver), or during regeneration with the possible exception of ornithine transcarbamylase (Table II).

Values for activity of ornithine transcarbamylase in excised liver did not show significant differences among the 3 groups of rats (Table II) However, athyroid animals failed to reproduce the consistent fall in specific activity that is known to occur during regeneration in the livers of normal rats(11). Furthermore, injection of thyroxine to these animals resulted in a steady decrease in specific activity of ornithine transcarbamylase. The hypothesis has been previously proposed (11) that the decrease in specific activity of ornithine transcarbamylase that takes place during liver regeneration, together with a simultaneous increase in specific activity of carbamyl phosphate-aspartate transcarbamylase(12) might represent a regulatory mechanism by which more carbamyl phosphate is made available for pyrimidine biosynthesis.

The present results, considered in relation to the regeneration process, seem to lend further support to this proposal.

An additional point deserves some comment. While in *R. catesbeiana* tadpoles, thyroxine apparently "unlocks" a mechanism leading to an increased rate of synthesis of carbamyl phosphate synthetase, ornithine transcarbamylase and arginase, the thyroid hormone is not required for production of these urea cycle enzymes by newly formed rat liver cells. The different response of the amphibian and mammalian species is better appreciated when it is realized that thyroxine injection to athyroid, partially hepatectomized rats, resulted in a non-specific stimulation of liver protein synthesis.

*Summary.* The activities of carbamyl phosphate synthetase, ornithine transcarbamylase and arginase have been found to be unchanged in regenerating liver of thyroidectomized rats. Complete thyroidectomy results in an impaired rate of liver regeneration. Daily injection of thyroxine during the

TABLE II. Ornithine Transcarbamylase Activity in Liver of Athyroid Rats.*

| Time after hepatectomy (hr) | Athyroid (specific activ.) | Athyroid plus thyroxin (specific activ.) |
|---|---|---|
| 0 | 323 ± 62 (13) | 331 ± 58 (8) |
| 48 | 301 ± 7 (3) | 244 ± 55 (3) |
| 72 | 338 ± 43 (3) | 232 ± 25 (2) |
| 144 | 258 ± 30 (2) | 186 ± 17 (3) |

* Values represent avg specific activity ± stand. dev. Figures in parentheses indicate No. of animals.

‖ Other enzymes measured in some of these animals include glutamic dehydrogenase and carbamyl phosphate-aspartate transcarbamylase. Values obtained for specific activity of these enzymes were within the expected range. We are grateful to Dr. N. de Groot for glutamic dehydrogenase assays, and to Dr. S. Kim for aspartate transcarbamylase assays.

regeneration period restores the capacity of the liver to regenerate.

1. Brown, G. W., Jr., Brown, W. R., Cohen, P. P., *J. Biol. Chem.*, 1959, v234, 1775.
2. Paik, W. K., Cohen, P. P., *J. Gen. Physiol.*, 1960, v43, 683.
3. Metzenberg, R. L., Marshall, M., Paik, W. K., Cohen, P. P., *J. Biol. Chem.*, 1960, v236, 162.
4. Higgins, G. M., Anderson, R. M., *Arch. Pathol.*, 1931, v12, 186.
5. Brown, G. W., Jr., Cohen, P. P., *J. Biol. Chem.*, 1959, v234, 1769.
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *ibid.*, 1951, v193, 265.
7. Hassid, W. Z., Abraham, S., in Colowick, S. P., Kaplan, N. O. (editors) Methods in Enzymology, Vol. III, 351, 1957, Academic Press, New York.
8. Karp, A., Stetten, D., Jr., *J. Biol. Chem.*, 1949, v179, 819.
9. Christensen, B. G., Jacobsen, E., *Acta Med. Scand.*, 1949, v136, Suppl. 234, 103.
10. Drabkin, D. L., *J. Biol. Chem.*, 1950, v182, 335.
11. Kim, S., 1960, Ph.D. Thesis, Univ. of Wisconsin.
12. Calva, E., Cohen, P. P., *Cancer Res.*, 1959, v19, 679.

Received January 3, 1961. P.S.E.B.M., 1961, v106.

## Modification of Lethality of Endotoxin in Mice by Zymosan. (26381)

HENRY H. FREEDMAN AND BARNET M. SULTZER
(Introduced by A. S. Gordon)
*Princeton Labs., Inc., Princeton, N. J.*

Benacerraf *et al.*(1) have shown that pre-treatment of mice with the yeast extract, zymosan, increases susceptibility to lethality of *intravenously* administered endotoxin, despite the concomitant stimulation of phagocytic activity of the reticuloendothelial system commonly associated with refractoriness to endotoxic effects. We decided to determine whether plasma of such RES-stimulated but endotoxin-susceptible mice would protect normal recipients against lethality of endotoxin, as does plasma of RES-stimulated endotoxin-tolerant donors(2). Mice were treated with an available zymosan preparation, plasma taken from some, the others being challenged *intraperitoneally* with endotoxin. Surprisingly, these zymosan-treated donors showed increased resistance to the *i.p.* endotoxin challenge.

The present report confirms the finding of Benacerraf *et al.*(1), for the experimental model they used, and describes the modification of endotoxin susceptibility by zymosan pre-treatment, ranging from increased resistance to increased susceptibility, depending upon the zymosan employed, method of preparation for injection, and route of administration of the endotoxin challenge. Stimulation of phagocytic activity was observed uniformly and the protection seen was not attributable to contamination by bacterial endotoxin or to stimulation of antibody formation to the challenging endotoxin.

*Materials and methods.* For studies on modification of endotoxin lethality and phagocytic activity, mice of both sexes, weighing 16-20 g, were used. Experiments to determine possible endotoxin contamination of zymosan were performed on male rabbits weighing 2 to 2.5 kg.

The results obtained with 2 zymosan preparations, designated "A" and "B",* are reported herein. The suspension of zymosan was prepared either exactly as described by Benacerraf *et al.*(1) or by substituting vigorous boiling directly on a hot plate for the lesser degree of heating in a boiling water-bath. Precautions to avoid endotoxin contamination were observed: all vials, beads, syringes, and needles were heated in an oven at 175°-180°C for 2-3 hours before use. Saline for dilutions was demonstrated to be pyrogen-free by test in rabbits. Pre-treatment of mice was standardized at 1 mg in 0.2 ml,

* Zymosan "A" was obtained from Mann Res. Labs., lot #4393, and "B" from Fleischmann Labs., lot #9B551.