thromboplastic, hemolytic or hemagglutinating, thus contraindicating their clinical use in crude form. Fractionation of whole venoms has been reported(19,20) and it seems conceivable that a pure fibrinolytic fraction, devoid of toxic activities, might be separated and prove of therapeutic value.

Summary. Of the 16 snake venoms studied, 11 actively lysed human blood clots. However, only one of these, *C. basiliscus,* was devoid of thrombic, hemolytic, and hemagglutinating properties. This venom was fibrinogenolytic as well as fibrinolytic. The possible therapeutic use of certain venoms as dissolving agents for intravascular clots presents a theoretical advantage over most other fibrinolytic agents in that their fibrinolytic activity is not readily inhibited by human serum. Fractionation of a pure fibrinolytic principle may be possible.

Acknowledgement is gratefully made of the valuable bibliographic assistance of Miss Iris Kiem, Department of Epidemiology, University of Pittsburgh Graduate School of Public Health.

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Received July 23, 1956. P.S.E.B.M., 1956, v93.

Effect of Thyroxine on Liver Tyrosine-glutamic Acid Transaminase. (22648)

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In the course of studying the effects of different hormonal states and hormone administration upon activity of several rat liver enzymes it was observed that dietary treatment with 1% thyroid for 8 days or more resulted in marked inhibition of liver tyrosine oxidation(1). Furthermore, the degree of inhibition was equally significant in normal animals and in animals under conditions of varying hormonal state, whereas certain of the other enzymes were affected by thyroid only under restricted conditions. This suggested a direct effect of the hormone* upon the tyrosine oxidase system. Since low dietary protein decreased activity of homogenate preparations (1) and inanition has been reported to enhance tyrosine oxidation in soluble preparations(2) this effect was further investigated

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* Reference to thyroxine hormone means thyroxine itself or whatever active form to which it may be converted.

using *in vitro* technics.

Methods and materials. Mature Long-Evans strain male rats weighing between 200 and 400 g were used as a source of liver tissue. The enzyme was prepared by homogenizing fresh, chilled liver with 2 volumes of cold 0.14 M KCl and centrifuging at 30,000 rpm for 45 minutes in a refrigerated Spinco centrifuge with a No. 40 rotor. The clear supernatant was used as the enzyme preparation. Enzyme activity was measured by use of an automatic recording Beckman spectrophotometer with a thermostat-controlled heating chamber at 37°C. Tracings from the recorder measured changes in optical density at 320 mμ (U. V. Light) which measured appearance of the enol form of p-hydroxyphenyl pyruvate (HPP) and tautomerization of the keto form to the enol form of HPP by tautomerase(3). Keto HPP shows end absorption in U.V. light while the enol form absorbs maximally at 300 mμ. The transaminase-tautomerase system included: 6 μM tyrosine in phosphate buffer(3), 0.1 ml of 0.1 M α-keto glutarate at pH 7.0, 1 ml of 0.2 M phosphate buffer at pH 7.0, 0.3 ml of soluble enzyme preparation (pH 7.0) and water to make a total volume of 3.6 ml. The final pH of the system was 7.0. The system used to measure tautomerase activity included: 6 μM keto HPP, pH 6.8, 1.0 ml 0.2 M phosphate buffer at pH 7.0, 0.3 ml enzyme and water to make 3.6 ml. L-thyroxine was dissolved in 0.02 N NaOH (pH 8)(4) and was added to the system in 0.1 ml and pre-incubated with the enzyme for 2 minutes at 25°C before addition of substrate, however, this procedure was not necessary to obtain the hormone action. The reaction was carried out in 1 cm quartz cells and the complete system was set to zero optical density immediately after the addition of the substrate. Either tautomerase or transaminase-tautomerase activity has been expressed as the change in optical density at 320 mμ in 10 min. (Δ O.D. 320 mμ /10 min.). One ml of the enzyme contained approximately 1.8 mg Kjeldahl nitrogen.

Results. When the fresh enzyme preparation was used to measure enol HPP accumulation with tyrosine as substrate, equilibrium enol HPP formation was reached in about 10 min. Further oxidation of HPP by oxidative enzymes could be seen in 20 minutes when enol HPP had nearly disappeared. In the presence of 10^{-4} M thyroxine the overall reaction rate was reduced by about 60%. Since Knox and Knox(6) have demonstrated that the transaminase reaction limited the overall rate of the system converting tyrosine to fumarate and acetoacetate and from kinetic studies of this system measuring QO_2(1) it appeared that the site of inhibition by thyroxine must be early in the tyrosine metabolizing sequence. After 48 hours of aging the enzyme at 5°C, further oxidation of enol HPP could no longer be detected during 60 minutes of reaction with tyrosine and α-ketoglutarate in the system as substrates. This meant that the residual activities were those of transaminase (E_1) and tautomerase (E_2) whose activity had not decreased significantly during this time:

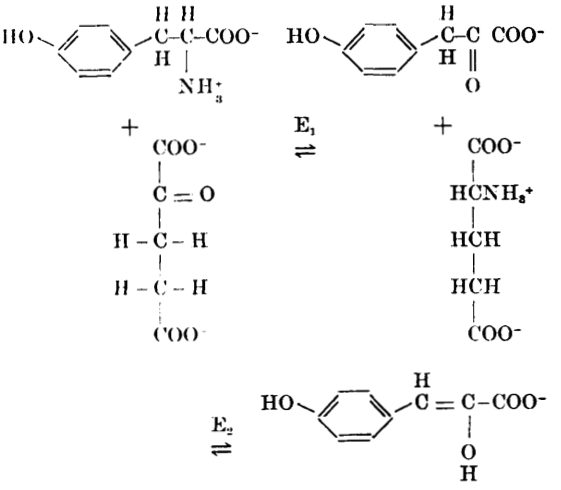

Table I shows the effect of increasing concentrations of thyroxine on coupled transaminase-

TABLE I. Effect of Increasing Concentrations of Thyroxine upon Tyrosine Glutamic Acid Transaminase-Tautomerase Activity.

Enzyme* concentration (mg N)	L-Thyroxine concentration (M)	Transaminase-tautomerase activity (Δ O.D. 320 mμ/10 min.)
.54	—	.274
"	5.10^{-5}	.121
"	1.10^{-4}	.075
"	2.10^{-4}	.051

* 3 day aged enzyme.

TABLE II. Effect of Thyroxine upon Activities of Coupled Transaminase-Tautomerase and Tautomerase.

Thyroxine conc. (M)	Substrate (6 μM)	Enzyme activity* (Δ O.D. 320 mμ/10 min.)	% inhibition
None	L-tyrosine	.220	—
10^{-4}	"	.082	63
None	keto HPP	.330	—
10^{-4}	"	.300	9

* 3 day aged enzyme.

tautomerase activity. Since the results showed significant effects upon this system it was essential to determine which enzyme was being inhibited by the hormone.

Table II shows the effect of added thyroxine to the coupled enzyme system and also upon the tautomerase reaction which could be isolated from E_1 by omitting α-ketoglutarate from the system and supplying keto HPP as substrate. The formation of enol HPP was then measured in the aged preparation which was free of detectable oxidative steps during the experimental period. While only a small inhibition of E_2 activity was noted in the presence of thyroxine a large inhibition occurred in the E_1E_2 reaction which indicated that the site of thyroxine action occurred at the transaminase level. In addition, this inhibition appeared to be non-competitive in nature as determined by the method of Ackermann and Potter(5).

Discussion. The possibility that thyroxine may act as an inhibitor of other transaminating enzymes is being investigated. The effective levels of thyroxine indicate that this reaction may be of physiological importance. *In vivo* data(1) on tyrosine oxidation add credence to this point. The significance of such a mechanism in regard to protein synthesis is obvious. Previous work with diiodotyrosine(1) indicates that this substance inhibits at the substrate level (competitive inhibition) while thyroxine is more effective at lower concentrations and appears to be binding the enzyme (non-competitive inhibition) perhaps analogous to thyroglobulin formation. Binding of thyroxine has been observed to occur in serum by a "thyroxine binding protein"(7) presumably of the G_1 globulin fraction. A report of *in vivo* studies and mechanism of inhibition will appear later.

Summary. Thyroxine in concentrations as low as 5.10^{-5}M has been shown to markedly inhibit transaminase-tautomerase activity of the liver tyrosine oxidase system. The transaminase enzyme appears to be the most directly involved in the inhibition as determined by difference studies.

The author is indebted to Dr. J. B. Neilands for the use of his facilities and for stimulating discussions in the course of the work.

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Received July 23, 1956. P.S.E.B.M., 1956, v93.