and 1.10) or litter size (7.67 ± 0.19 and 7.69 ± 0.29) between the second and third litters. Furthermore, the sex ratio of both the second and third litters is not significantly different from that of the first litter (1.08).

The material is too small for any detailed analysis and cannot be compared at the present time with the results of Hertwig (*6*), who used larger dosages. No attempt, therefore, will be made at this time to interpret the above results; however, several theories have been proposed, and experiments are now being conducted in order to test these theories.

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The Inhibition of Blood Clotting *in Vitro* and *in Vivo* by Tyrosinase

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Mushroom tyrosinase has been found to inactivate several biologically active proteins such as invertase, pepsin, trypsin, chymotrypsin, and insulin (*1–3*) and more recently has been shown to inactivate thrombin and to so modify fibrinogen that with thrombin it clots very slowly to form a clot which is decreased in amount and amorphous instead of fibrous in structure (*4*). In view of the marked effect of tyrosinase on thrombin and fibrinogen, it became a problem of considerable interest to study the action of tyrosinase on the clotting system of whole blood and plasma. Tyrosinase was also injected into the blood stream of mice in order to investigate *in vivo* effects of tyrosinase on the blood clotting mechanism of animals.

Nine ml human[2] blood was obtained by venipuncture and mixed with 1 ml 0.1 *M* sodium oxalate. To 2 ml blood was added 0.2 ml tyrosinase (Treemond, 10,000 Miller-Dawson u/ml or 1500 u/mg protein), and the system was incubated in a water bath at 37°. A control was run simultaneously which contained tyrosinase inactivated by boiling. At successive intervals aliquots were removed for the measurement of clotting time and added to an equal volume of 0.02 *M* $CaCl_2$ in a small test tube at room temperature. The tube was tilted at intervals, and the clotting time was taken as the time required for the blood to cease to flow when the tube was tilted (*5*). The inactivation by tyrosinase of one or more components of the clotting system is shown in Fig. 1. The exponential character of the curve for inactivation is very similar to that described for the action of tyrosinase on thrombin or fibrinogen (*4*).

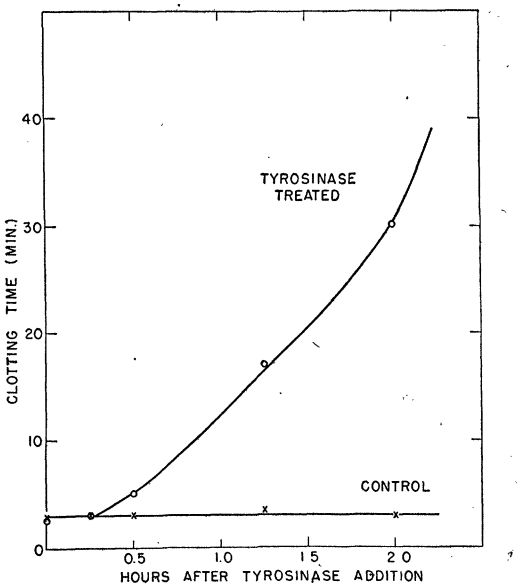

FIG. 1. Action of 0.2 ml tyrosinase on the clotting mechanism of 2.0 ml human blood at 37°. The control contains inactivated tyrosinase.

The inactivation of the clotting system of whole human blood is independent of the presence of the blood cells, since results similar to those of Fig. 1 were obtained with plasma prepared by centrifuging the above blood sample or with reconstituted lyophilized human plasma (Sharpe & Dohme).

In order to study the effects of tyrosinase on the clotting mechanism *in vivo* the enzyme was injected intracardially into laboratory white mice (either Harvard or Swiss strain) weighing about 25 g. For injection purposes the tyrosinase was dissolved in 0.9% NaCl. As in the *in vitro* studies, inactivated tyrosinase was used in the control experiments. Blood samples from the tip of the tail were drawn into fine capillary glass tubes at successive intervals for measurement of clotting time. Two different methods for measuring the rate of clotting were used. In the first, clotting time was taken as the time required for the column of blood to cease to flow in the capillary when gently moved to and fro by variations in air pressure. The second method was the standard capillary tube technique for measuring the rate of clotting (*6*) and involved breaking off pieces from the tube at successive intervals and measuring the time required before a fibrin thread forms when the broken pieces are slowly separated. Neither method was ideal, and somewhat variable results were obtained, especially with samples of blood that required a long time to clot. This difficulty was related to the fact that tyrosinase-treated

[1] Aided by a grant from Ethicon Suture Laboratories.

[2] I.W.S., Type B, Rh positive.

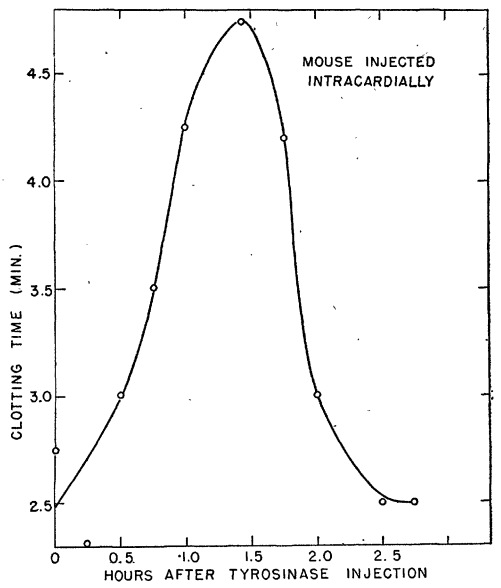

FIG. 2. Effect of 0.2 ml tyrosinase, injected intracardially in a 25-g mouse, on the clotting system of the blood. Blood samples were removed at successive intervals from the tip of the tail for measurement of clotting time.

blood not only clotted slowly but never produced as firm and rigid a clot as did normal blood.

Typical changes in clotting time resulting from the injection of 0.2 ml tyrosinase into a 25-g mouse are shown in Fig. 2. Although the clotting time is about doubled within 1.5 hr after the injection, the clotting mechanism returns to normal in 3 hr. In a series of experiments the clotting time showed a two- to four-fold increase within 1.0–1.5 hr after the tyrosinase was injected. The restoration of a normal clotting system in the treated mouse was often delayed for 8 or even 24 hr in some experiments. Animals which showed the greatest effect of tyrosinase on the blood often appeared sick and occasionally died in 24–48 hr. In control animals which were injected with boiled tyrosinase the clotting system was unaffected during the 8 hr of the experiment.

The problem of immunization of mice to tyrosinase was investigated by using a single animal for a series of injections over a period of 1–2 months. Results with several animals clearly show that injections of tyrosinase cause the production of antibodies which inhibit the enzyme *in vivo* (*7*). For example, in one mouse the clotting time increased to 11 min when tyrosinase was injected the first time; 15 days later when it was injected again the increase was up to 5 min, and 8 days later a third injection did not noticeably change the clotting time from the normal value of 2.6 min.

These studies show that tyrosinase rapidly inactivates the clotting system of plasma, whole blood, and of circulating blood of the mouse. In the living animal the clotting system returns to normal after several hours. Experiments are in progress to determine which components of the clotting system of blood *in vivo* and *in vitro* are inactivated by tyrosinase and to determine whether the eventual restoration *in vivo* of the system to normal represents a reversal of the inactivation or a replenishment by the blood-synthesizing organs of the inactivated components.

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The Nonsurgical Treatment of Cataract[1]

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Because of the high incidence of cataracts and the hazards associated with their surgical removal (disregarding psychological and economic factors), a nonsurgical treatment of cataracts has been sought by ophthalmologists for many years. At one time it was thought that the administration of bovine lenticular extracts might be of therapeutic value (*1*), but this was not established (*2*). Subsequently, and because of the differences that were noted between lenticular extracts from fish and the proteins of mammalian lenses (*3–5*), the possibility of fish lens proteins being of therapeutic value was considered.

In November 1950, experimental work was started to study the ocular changes in rats subjected to high dietary stresses. The results, as yet unpublished, cover more than a year's work on the lens changes in rats under controlled conditions. Groups were large enough (24 or more) to compensate for individual biological differences. Selected groups of individually caged rats were placed under a physiological stress such that changes might, and did, occur in the eyes. These eye changes were ophthalmoscopically observed and critically described each week. Concurrently, blood and urine chemistry, employing microtechniques supplemented by paper chromatography, was carried on. Initial experiments were performed on rats 6 weeks of age; but before the work was completed, not only were all age groups studied, but stresses applied to females at time of breeding were finally evaluated, in order to differentiate between the effect on the offspring during fetal life and during lactation, by examining the eyes of baby rats at weaning. These experiments, which involved many hundreds of rats whose eyes were examined ophthalmoscopically weekly, ultimately made it possible to induce cataracts

[1] The patients were treated at the office of Dr. Ginsberg, 705 New Jersey Ave., Brooklyn, N. Y.