Technical Papers

Sex Ratio of Offspring from Irradiated Male Mice[1]

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A group of interrelated phenomena (visible mutations, sperm lethality, semi- and total sterility, reduction of litter size, etc.) resulting from irradiation of male or female mice has been reported by Snell (*1–4*), Hertwig (*5–7*), Russell (*8*), and by many others in this field. In the present paper some preliminary data on the alteration of the sex ratio of offspring produced by irradiated male mice are presented.

were born within 40 days after irradiation of the male constitute the "control" and "experimental" groups, respectively. Although the first litters are usually below par both as to number and weight, the second and third litters are quite comparable (*9*).

Twenty pairs of mice produced three successive litters, the third one within 40 days of irradiation of the male. The mean litter size (± SE) and sex ratio of the 60 litters are given in Table 1. It will be noted that the mean litter size of the third litters (5.95 ± 0.31) is significantly lower ($P = < .001$) than that of the second litters (7.65 ± 0.22). Such a reduction in litter size has been reported previously (*1*) and appears to be proportional to the dosage. However, it is here further noted that the mean reduction of litter size (1.70) is largely due to the mean reduction of females (1.45).

TABLE 1

MEAN LITTER SIZE (± SE) AND SEX RATIO OF THE FIRST THREE LITTERS OF 20 PAIRS OF A/HEJAX MICE, AND A STATISTICAL COMPARISON OF SECOND AND THIRD LITTERS

Litter order	No.	Females	Males	Total	Sex ratio
First	20	3.05 ± 0.27	3.35 ± 0.26	6.40 ± 0.34	1.10
Second	20	3.80 ± 0.20	3.85 ± 0.21	7.65 ± 0.22	1.01
Third	20	2.35 ± 0.24	3.60 ± 0.27	5.95 ± 0.31	1.53
3rd/2nd%		61.8%	93.5%	77.8%	151.5%
Student's t		4.64	0.73	4.47	
P (38 d.f.)		< .001	.5	< .001	

TABLE 2

MEAN LITTER SIZE (± SE) AND SEX RATIO OF THE FIRST THREE LITTERS OF 39 PAIRS OF A/HEJAX MICE, AND A STATISTICAL COMPARISON OF SECOND AND THIRD LITTERS

Litter order	No.	Females	Males	Total	Sex ratio
First	39	3.18 ± 0.23	3.44 ± 0.18	6.62 ± 0.24	1.08
Second	39	3.64 ± 0.21	4.03 ± 0.22	7.67 ± 0.19	1.11
Third	39	3.67 ± 0.24	4.02 ± 0.23	7.69 ± 0.29	1.10
3rd/2nd%		100.8%	99.8%	100.3%	99.1%
Student's t		.09	.01	.02	
P (76 d.f.)		.9	.9	.9	

Littermates of the highly inbred A/HeJax strain of mice were paired and mated when 60 days old. The first and second litters were weighed, sexed, and discarded. One or two days before the birth of the second litter, the male was separated from the female. Within 24 hr of the birth of the second litter the genitalia of the male parent were irradiated with an x-ray tube rendering a dose of 150 or 300 r. Immediately after irradiation the paired mice were reunited. Although some differences were found between the two dosages, in this brief report only the pooled results will be presented. The second litters and those litters that

This alteration of the sex ratio between the control and experimental litters appears to be a real one, for whereas the difference between the mean number of males in the control (3.85 ± 0.21) and experimental litters (3.60 ± 0.27) is not significant ($P = .5$), that between the mean number of females in the same two groups (3.80 ± 0.20 and 2.35 ± 0.24) is highly significant ($P = < .001$).

As litter size and sex ratio are extremely difficult characters to analyze, the present data were compared with a second and more independent "control" group. Table 2 gives the mean litter size (± SE) and sex ratio for the first three litters of 39 A/HeJax mice living in the laboratory during the course of this experiment. The main point to be noted here is that there is no significant change ($P = .9$) in the sex ratio (1.11

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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*).

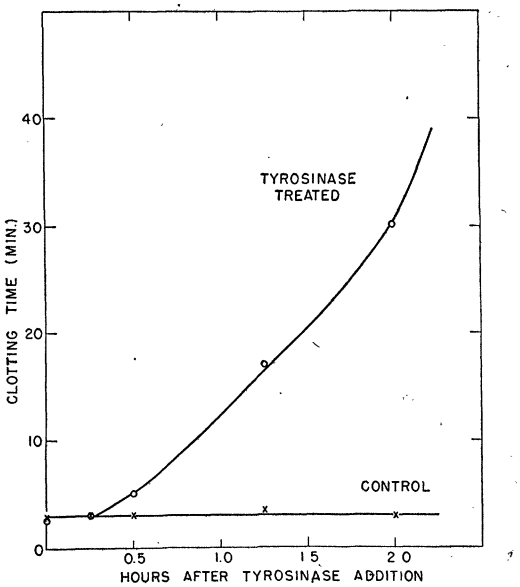

FIG. 1. Action of 0.2 ml tyrosinase on the clotting mechanism of 2.0 ml human blood at 37°. The control contains inactivated tyrosinase.

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

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