16893

Histamine in Rat Plasma; Correlation with Blood Pressure Changes Following X-irradiation.

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Although considerable has been written to indicate that there is a close correlation between the conditions of shock and the liberation of a histamine like substance from damaged tissue[1,2,3] it is only recently that the publications of Prosser, Painter and Moore[4] and others[5,6] suggest that a similar histamine shock syndrome can consistently be produced with mid-lethal dosages of X-rays. Their observations indicate that with such concentration of X-rays the toxic products of tissue breakdown reach their highest concentration at about 2 hours after irradiation.

In view of the fact that no definite statement is available as to whether the toxic product liberated is actually histamine or not, it will be the object of this investigation to demonstrate that the toxic product in the blood stream following irradiation is histamine, and that the level of assayable histamine in blood is highest about 2 hours after irradiation and again about 5 days later, at which times the blood pressure of the animals is at its lowest point.

Experimental. In the experiments to be reported, adult rats were used irrespective of sex. After irradiation, the rats were used either for the collection of blood samples (enough for 2 cc of plasma) to be assayed for histamine at various time intervals, or for blood pressure determinations over the corresponding time interval. In making these determinations a total of 21 rats were used for making histamine assays at various time intervals, varying from .5 to 24 hours after irradiation; 5 other rats were used to follow variations in blood pressure, determined at 15 minute intervals for 4 to 5 hours after the radiation was given. After once being irradiated, never was a rat used for a second experiment.

The irradiation techniques used were developed by Quastler and Kirschner.[3] The rats were placed in a cell cut out of presswood, large enough to accommodate 3 rats. The factors for the raying were: 200 KVp without a filter. The distance from the target electrode to the top of the rats' cell was 50 cm. One-half of the dose was administered from the top and one-half from the bottom. By raying in this fashion a fairly homogeneous distribution of dose is attained. The dose rate was 100 r per 2 minutes and 24 seconds, and the actual mid-lethal dose given each rat was 600 r.

When blood pressure measurements were taken on the intact rat to record the severity of the effects of irradiation, a slight modification of the method described by Byrom and Wilson[7] was used. It consisted essentially in using a plethysmograph especially designed to fit the rat's tail.

The method used for preparing extracts for histamine assay was that developed by McIntire, Roth and Shaw.[8] This method appears to be superior to others available[9,10,11]

1 Moon, V. H., Oxford University Press, 1938.

2 Wiggers, C. J., *Physiol. Rev.*, 1942, **22**, 74.

3 Kirschner, L. B., Univ. of Ill., 1947, (Master's Thesis).

4 Prosser, C. L., Painter, E. E., and Moore, M. C., to be published in Atomic Energy Commission Technical Series.

5 Painter, E. E., and Moore, M. C., *Fed. Proc.*, 1948, **7**, 90.

6 Prosser, C. L., Painter, E. E., Lisco, H., Brues, A. M., Jacobson, L. O., and Swift, M. N., *Radiology*, 1947, **49**, 299.

7 Byrom, F. B., and Wilson, C., *J. Physiol.*, 1938, **93**, 301.

8 McIntire, F. C., Roth, L. W., and Shaw, J. L., *J. Biol. Chem.*, 1947, **170**, 537.

9 Code, C. F., *J. Physiol.*, 1937, **89**, 257.

10 Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, **85**, 1.

11 Schild, H. O., *J. Physiol.*, 1942, **101**, 115.

in that very small amounts of histamine can be detected and also that only histamine is said to be extracted from the blood sample being studied and not other impurities. The essential features of the method are that the histamine can be removed from the aqueous sample almost in its entirety with N-butanol, and that then the histamine can be eluted from the butanol by means of the cation exchange medium, cotton acid succinate. Following this the histamine is washed from the

TABLE I.
Amount of Histamine Present in 2 cc Samples of Blood Plasma After Irradiation (600r).

Rat No.	Sampling time after x-ray (hr)	Histamine present (γ)
A4	.5	.0
A13	.5	.0
B1	1.5	1.0
A2	2.	1.0
A3	2.	2.0
A5	2.	2.0
A15	2.	1.2
A20	2.	2.0
A22	2.	1.4
B2	2.	1.2
B3	2.	1.2
C1	2.	1.6
C2	2.	1.6
B4	2.5	.4
A21	3.	.0
A23	3.	.0
A24	4.	.0
C3	10.	.0
C4	16.	.0
A25	18.	.0
A26	24.	.0

cotton acid succinate with a small volume of dilute hydrochloric acid, and the resulting solution is neutralized with weak sodium hydroxide to give an isotonic solution suitable for bioassay. The actual assay for histamine is carried out by recording blood pressure changes in the anesthetized cat (Nembutal).

Results. In reporting the results obtained, particular emphasis will be placed on the correlation between the appearance of assayable histamine in the blood plasma and the maximum fall in blood pressure occurring approximately 2 hours after the mid-lethal dose of radiation was given (600 r). The data obtained for the actual amount of histamine present in terms of gamma per 2 cc of blood plasma is presented in Table I. That the assayable compound in these experiments is histamine is supported by the fact that the method used for isolating the compound is specific for histamine. It was also found that if a known amount of assayable histamine in gamma concentration which was previously extracted from a blood sample of an irradiated rat, is added to a similar amount of histamine diphosphate, the fall in blood pressure in the test cat was consistently twice that which

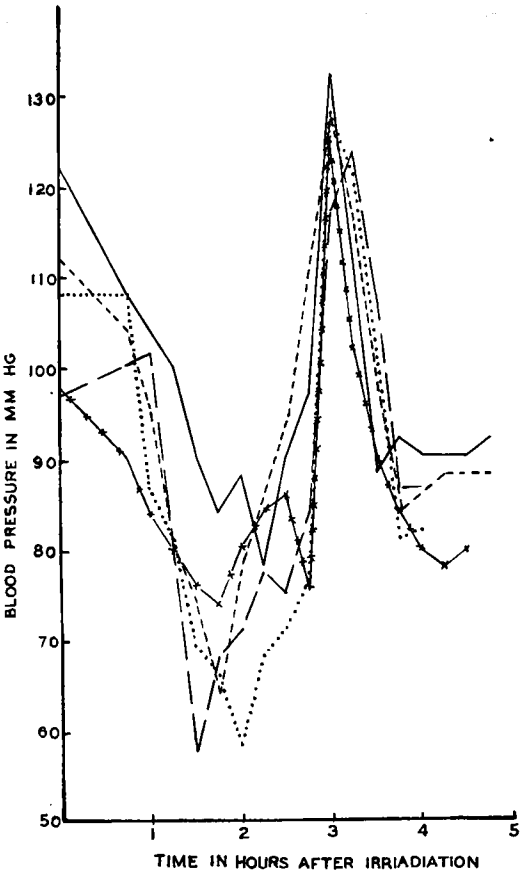

FIG. 1.
Effects of irradiation on blood pressure in the rat.

occurs when the histamine diphosphate is injected alone. It is believed that if the extractive is not actually histamine it should not produce so clear cut an additive effect as these experiments indicated.

It was interesting to note that along with the appearance of histamine there occurred a marked fall in blood pressure which showed an excellent correlation between the highest

level of histamine concentration in the blood and the lowest point of blood pressure fall (Fig. 1). As the graph indicates, following this fall in blood pressure there occurs a marked compensation within an hour that exceeds the normal pressure at the beginning of the experiment. Although there is no satisfactory explanation for this change in blood pressure after the marked fall, it should be mentioned that when this rise does occur there is no measurable histamine present in the blood stream.

In a limited number of experiments in which the rats were followed for a longer time after irradiation than those just described, it was interesting to find that following the first 4 or 5 hours period after mid-lethal dosage of X-rays, even though the histamine in the blood was not measurable, the animals' blood pressure remained relatively low compared with normal, and continued to fall slowly to levels of 60 to 70 mm of Hg by the 4th to 6th day after irradiation. There also occurred in all the animals studied a marked loss in appetite and weight during this same period.

From this critical point, however, the rats either became progressively more depressed and died at the end of about 10 days, or showed a marked recovery in appetite, weight, blood pressure, and general appearance and survived the 600 units of X-irradiation. It was also interesting to note that if histamine assays were run on rats in these experiments there occurred a gradual increase in assayable histamine starting the 2nd day after irradiation, until on the 5th day, the rats showed nearly 1.0 γ of histamine (by assay) per 2 cc of blood plasma extracted. If the rats survived this critical 4 to 6 day period and showed signs of recovery, there was no evidence of histamine present in the blood by the 9th or 10th day after irradiation.

From these experiments it would appear that the low blood pressure correlates with high levels of histamine in the blood plasma which is very suggestive of the theory that the cause of death after irradiation is of the nature of circulatory failure or a condition simulating shock, an observation that was earlier suggested by Prosser, Painter and Moore.[4] Although no explanation can be advanced concerning the mechanism responsible for the appearance of histamine after irradiation, it is believed that the cyclic appearance of histamine at a 2 hour and a 5 day period after irradiation offers suggestions for further investigation of the problem in question.

Summary. 1. Rats, after X-irradiation with mid-lethal dosage (600 r) show assayable histamine in concentrations of 1 to 2 γ per 2 cc of blood plasma at 2 hour and 5 day intervals following the irradiation.

2. Rats irradiated similarly to those used in the histamine assay experiments show a fall in blood pressure that correlates with the appearance of histamine in blood plasma and strongly suggests that histamine is responsible for the lowered blood pressure.

3. Rats, from 4 to 6 days after a mid-lethal dosage of irradiation show a loss in appetite and weight, and a critical fall in blood pressure from which only 50% will recover. The rest die within 9 or 10 days after the irradiation was given.

16894

Chorio-meningo-encephalitis Following Inoculation of Newcastle Disease Virus in Rhesus Monkeys.*

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Newcastle Disease Virus (NDV), commonly the cause of a disease of fowl, patently, may, because of intimate association with man, cause human disease. Recognized syndromes in man caused by NDV include conjuncti-

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