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THE EFFECT OF HEPARIN UPON THE LD-50% OF THE X-IRRADIATED RAT

BY

HENRY I. KOHN*

and

PAUL ROBINETT **

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ABSTRACT

The LD-50% of the dog is about 300-350 r (200 KV X-rays), for the rat about 550-600 r. The dog experiences an heparinemia, the rat does not. Could the difference in sensitivity be due to the failure of the rat to release heparin?

By the judicious administration of heparin we have reduced the LD-50% of the rat to that of the dog. While heparinization increases hemorrhage, it does not change its distribution. Arguments are advanced which indicate that heparin is not the primary cause of the difference in sensitivity between the two species.

THE EFFECT OF HEPARIN UPON THE LD-50% OF THE X-IRRADIATED RAT

Henry I. Kohn and Paul Robinett

Among the mammals, the great differences in sensitivity to ionizing radiations have not been explained, no doubt because we know so little about the pathologic physiology of the irradiation injury syndromes. The recent work of Allen et al (1), however, offers a new possibility in this connection. They reported that an heparinemia occurs in the dog during the second week following heavy irradiation (LD-100%, 200 KV, 450 r), that it is coincident with a remarkable rise in the clotting time as well as other hemorrhagic manifestations, and that it can be controlled by the administration of toluidine blue. Now under comparable conditions, the LD-50% for the rat is about twice that for the dog (Prosser, 4). Might not this difference in sensitivity be due to the failure of the injured rat to release heparin?

Several points should be considered in the design and interpretation of experiments to test this hypothesis (Allen and cs., 1) employed an exposure of 450 r, which consistently kills all of the animals in about 10 - 14 days, and which for them is 150 r more than the LD-50%. No report is given as to the extent of heparinemia at the LD-50%. Prosser (4), in his review of the Argonne Laboratory data, states that the clotting time may be doubled at 200 r and above; presumably the changes are not great at or under the LD-50%. As matters stand at present, then, markedly prolonged clotting times occur in dogs subjected to more than the LD-50%, after the lapse of one week or more; the r-dosage for this effect, however, is only three-quarters of that equivalent to the LD-50% in the rat.

As to the site of hemorrhage in the dog, Allen and cs (1), state that at 450 r the mouth, soles of the feet and subcutaneous tissues of the sides -

sites most likely subject to trauma - bleed first. Later there may be bleeding from the rectum, vagina and urinary tract. Autopsy reveals hemorrhage along the G. I. tract, especially in the colon and the proximal 4/5 of the stomach; the heart muscle, especially along the coronaries, is frequently involved, as are the parietal pleura, diaphragm, and lungs. Field and Rekers (3) found at the LD-50% (350 r) "generalized purpura and petechiae formation, intra-pulmonary, gastric and intestional hemorrhage". Curiously, they state that less hemorrhage was seen at doses higher than at the LD-50%. This distribution of hemorrhage is somewhat different from that seen in the rat (see below).

METHODS

The animals were caged either individually or in pairs, and were fed Purina dog checkers and water ad libitum. The various groups in a given experiment were arranged to have the same average weight.

For irradiation, they were exposed in a small box, divided into individual stalls, with a thin lucite top. The exposure box was placed on a scatter block of masonite (10" x 10" x 4"). The source of irradiation was a G. E. Maximar outfit, run at 250 K.V., 15 ma., with intrinsic filtration of 0.4 mm. of Cu. and delivering approximately 40 r per minute at the exposure box. The exposures given in the tables are based on the readings of a Victoreen thimble chamber placed on the floor of the exposure box and therefore include the back-scatter. At the same level in air the reading would be only 70 per cent as great.

Clotting times were determined by a moist chamber method. The tail was warmed in water of 46° C. for about 15 seconds, wiped dry, and the tip cut off. After discarding the first drop or two, a drop was placed on a clean glass slide, which was then placed in a small moist chamber. The presence of coagulation was determined from time to time by briefly probing the drop with a

needle. Five to 10 minutes was the normal clotting time by this method. Heparinized animals can have blood which does not clot in 10 hours, or more.

The heparin used was the sodium salt (Upjohn), 1,000 units per mgm.

EXPERIMENTS

Experiments were done with hooded (Tumblebrook Farm) and albino (Sprague-Dawley) rats. The general plan of all the experiments was the same. Two series were employed, one usually injected with saline i.p., the other with heparin. In each series, groups of about 10 animals were exposed to varying amounts of X-rays. The injections were begun on about the 6th day after irradiation and were continued 5 to 10 days.

Clotting Times. We have found no change in the clotting times following irradiation. For example, 5 hooded and 5 albino rats were exposed to 1,000 r and 1,250 r. Clotting was evident within 5 - 10 minutes, as in the normal animal. All of these animals died within 5 days of exposure, which was 125 - 150 per cent of the LD-50%. Such animals, of course, are dehydrated, and the blood does not flow freely from the tail. This complication renders the determinations less accurate. We know from our experience with heparin, however, that the clotting time can be prolonged for hours, and we therefore feel that the present result indicates that the change in clotting time in the rat must be small, if it exists, compared to what has been reported in the dog.

Hooded Tumblebrook Strain. Preliminary trials indicated that in a 150 gram rat, 1 mgm. of heparin i.p. prolonged the clotting time for about 3 hours, 2 mgm. for about 7, and 5 mgm. for about 24. The first two experiments failed because the heparin dosage was sufficiently large to kill a number of the unirradiated controls. It was concluded that 3 mgm. of heparin b.i.d. could not be tolerated by the female hooded rat. A third experiment, summarized in Table I, involved a dosage of 2 mgm. of heparin once a day and suggested that heparin can reduce the LD-50%, though in this case the reduction was only about 50 r. The injection per se of saline into the controls did not change the LD-50%, when compared to previously obtained data.

The animals which died in the first two experiments (involving more than 100 animals) were autopsied. The gross findings were affected by heparin in only one respect, namely, the production of hemorrhage in the abdominal wall, and usually in the peritoneal cavity, at the sites of injection.

Albino Sprague-Dawley Strain. The results with the hooded animals suggested that lower dosages of heparin should be employed, and it was also thought advisable to try another strain. Clotting times showed that in the Sprague-Dawley male, of 275 - 325 grams, 2 or 3 mgm. of heparin i.p. will render the blood practically incoagulable for several hours. Four hours after injection the clotting time will be 30 - 45 minutes, and this will fall to the normal range of 5 - 10 minutes at 6 hours. The injection of 2 mgm. in the morning and 3 mgm. in the afternoon allows complete recovery between injections, and additional tests showed no cumulative effect. This dosage of 15 mgm. per kilo per day, which in the rat prolongs the clotting time for 8 - 10 hours, would probably be twice as effective in man. Little is known about the metabolism of heparin; Astrup (2) was able to recover only about 10 per cent from the urine of patients receiving the drug intravenously.

The results of the first experiment are given in Table II, where it is

Table I

Effect of Heparin Upon the X-ray Tolerance of the Hooded Rat

The animals were exposed on Day 0, and were injected on Days 7 through 14 with 2 mgm. of heparin i.p., or with saline. Weight: males, 200-250 grams; females, 175-200 grams.

Exposure* r	Treatment	Sex	Survivors on day				
			0	6	14	18	Percent
0	H	F	9	9	9	9	100
450	S	M	4	4	4	4	100
	H	M	8	8	7	7	88
550	S	M	7	7	7	6	86
	H	M	7	6	6	5	72
650	S	M	8	8	5	5	63
	H	M	8	8	3	3	38

* Includes backscatter. Air dose equals 70 per cent.

Table II

Heparin and the X-ray Tolerence of the Sprague-Dawley Rat

The mean weight of each group (males) varied from 319 to 324 grams, with standard deviations between 26 and 40. The animals were exposed on Day 0 and were injected on Days 6 through 10. Of each heparin group, 5 received 3 mgm. b.i.d., the others 5 mgm. b.i.d. The difference in dosage made no difference and both are recorded together. The control series was not injected.

Exposure* r	Series	Survivors on day				
		0	6	11	14	28
0	Heparin	10	10	10	10	10
700		10	10	1	0	0
800		9	9	0	0	0
900		10	8	0	0	0
700	Control	10	10	9	7	7
800		10	10	3	3	3
900		10	8	1	1	1

* Includes backscatter. Air dose equals 70 per cent.

obvious that the LD-50% has been reduced by heparin from 750 r to well under 700 r, and perhaps very much lower. The data of the second experiment in Table III shows that heparin reduced the LD-50% from 800 r to 500 r.

The autopsy findings are summarized in Table IV. The animals listed were those dying during the first 11 days of the experiment, i. e., during the period of heparin administration. Five autopsies were lost in the first experiment, none in the second. The positive findings of the table have the following significance: mouth, minimal or moderate signs of hemorrhage at the gingival margins; nose, from minimal signs to occasional blood caked whiskers; eyes, crusting of pigment along the lids; anus, signs of black stools; G.I. tract, plainly evident signs of gross hemorrhage anywhere from stomach to colon; skin, 50 or more petechiae in the under surface of the skin of the abdomen and back (extensive hemorrhage was never seen independent of the sites of injection).

Hemorrhage at the site of injection of heparin (but not of other injections), both subcutaneous and intramuscular, was present to a greater or lesser degree in 95 per cent of the cases, and is not listed in the table. Neither is hemorrhage within the abdominal cavity listed: it was much less frequent.

Retroperitoneal hemorrhage was noted in 45 per cent of the cases of the second experiment, heparin series, but never in the controls. It generally began in the region of the bifurcation of the aorta and spread upwards, especially on the left, sometimes as far as the kidney. Half of these animals also showed some hemorrhage in the seminal vesicle or in the neighborhood of the vas deferens.

Two cases of the second experiment, heparin series, both of which died after the first day of injection, were not included in the tabulation because it was probable that they bled to death as a result of a traumatic injection (one had received no irradiation).

Eight brains were examined in the heparin series each showing several petechial hemorrhages. The meninges showed some injection

Table III

Heparin and the X-ray Tolerance of the Sprague-Dawley Rat

The mean weight of each group (males) varied from 275 - 277 grams, with standard deviations between 14 and 33. The animals were exposed on Day 0, and the heparin series received 2 mgm. each morning and 3 mgm. each afternoon on Days 6 through 10.

Exposure* r	Series	Survivors on day				
		0	6	11	14	22
0	Heparin	8	8	7	7	7
350		8	8	8	8	8
450		10	10	6	6	6
550		10	10	2	2	2
650		10	10	0	0	0
700	Control	10	10	9	8	8
800		10	10	6	5	4

* Includes backscatter. Air dose equals 70 per cent.

Table IV

Positive Findings at Autopsy in Sprague-Dawley Rats

Experiment	Series	No.	Percentage					
			Mouth	Nose	Eyes	Anus	G.I.Tract	Skin
1	Control	13	77	62	54	31	100	31
	Heparin	29	21	41	41	35	90	10
2	Control	5	40	60	80	20	100	20
	Heparin	21*	13	27	13	95	100	5

* Two are not included, but are discussed separately in the text.

but hemorrhage was not striking. The voluntary musculature, the adrenals, and the bladder (invariably filled with urine) were consistently negative. The liver was striking only because of its pallor. The lungs were not remarkable when the positive and general controls (of past experience) were considered.

The principal positive findings are listed in Table IV, where it may be seen that the G. I. tract is by far the most prominent. Grossly, hemorrhage into the stomach, which appears distended and blue, is in some ways the most characteristic sign. The petechiae and small hemorrhages are confined to the glandular portion, and often appear disproportionately small compared to the amount of blood present. Occasionally the wall of the cecum shows hemorrhage. The contents of the intestines are variously colored from black to red. The regional nodes are red or black, and are swollen.

All in all, there is no obvious difference in the distribution of hemorrhage between the heparin and control animals, except retroperitoneally on the dorsal wall. In general, the heparin animals tend to die a little sooner, and consequently the frequency of hemorrhage outside of the G. I. tract is somewhat less.

DISCUSSION

By the judicious use of heparin, the LD-50% of the rat has been reduced by about 1/3, and it is possible that further trials could increase this figure. Measured in air, this brings the LD-50% of our albino rats from about 550 r to about 350 r; the latter figure is the LD-50% for the dog.

We do not think then, that heparin is the decisive factor which distinguishes the reaction of the dog from that of the rat. In the first place, the distribution of hemorrhage in the heparinized rat is essentially unchanged. What has occurred is more of the original type of hemorrhage, plus that in the body wall at the sites of injection. The net effect is to place an added stress upon a

critically injured organism. The distribution of hemorrhage in the rat, therefore, is a direct reflection of the local injury; latent injuries requiring heparin for their development are not grossly important.

In the second place, the administration of toluidine blue to the irradiated dog, according to Allen et al (1), dramatically diminishes hemorrhage, but does not change the fact that 450 r is an LD-100% exposure. Although the final interpretation of this experiment must await further details, it appears now that the dog protected against circulating heparin continues to be more sensitive than the rat.

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