Mechanism of Acute Hypotension Following Total Body Irradiation.* (18930)

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Large doses of total body X-ray have been known to result in a shock-like state followed by death. That this true shock-like state is preceded by an acute, moderately severe and rather persistent hypotension is not generally appreciated. The purpose of this paper is to present data on the blood pressures, blood volumes and hematocrits of rats following large doses of total body irradiation; and to determine by the presentation of further data on adrenalectomized and spinal, irradiated and nonirradiated rats the resulting mechanism of acute hypotension.

Methods. All rats used in this study were normal young adult male or female stock white rats. Their weights varied from 339 g to 120 g with the average approximately 200 g. The rats were kept in wire cages and fed the standard diet employed in this institution.

All of the irradiation was given with the same machine at 200 Kv with an inherent filtration of 3 mm Al. In general the rats were irradiated 4 at a time in a small pasteboard box. In all cases following the irradiation, the rats were denied food and water. This was the only time during the course of the experiments that food and water was not readily available to all the animals.

The blood volumes were determined by the method using P^{32}† labeled red cell technic (to be reported). The average adult white rat's blood volume was found to be 6% of body weight.

The hematocrits were determined on whole fresh heparinized blood obtained with the blood volume sample by direct cardiac puncture. Wintrobe hematocrit tubes were used.

The blood pressures were determined in all cases by direct aortic puncture with a 25-gauge needle connected to a saline mercury manometer.

In the bilaterally adrenalectomized animals reported, the adrenalectomies were done singly, and transperitoneally, through lateral abdominal incisions. The first adrenal was removed in each case 5 days before the second in order to insure a nearly normal animal at the time of the second procedure, and to obviate as far as possible the influence of trauma.

The spinal animals were transected at the level of C6 in all cases. The transections were performed with a small pair of dissecting scissors and complete transection was invariably followed by complete paralysis from the low neck down.

Complete autopsies were performed upon all the animals, and H and E sections of the Zenker-fixed material from each organ studied microscopically. The brains and spinal cords were fixed in Zenker's Formol and stained with Phloxine Methylene Blue.

Results. From the data presented in Table II, it can readily be seen that normal rats develop hypotension within 6 hours following 1,500 roentgens of total body irradiation. This hypotension persists for at least 30 hours and is not accompanied by any significant change in either the blood volume or the hematocrit. A total of 23 rats were used in this study.

From Tables IIIa and IIIb it is seen that 2,000 to 2,500 roentgens of total body radiation respectively, result in a hypotension which

TABLE I. Normal Control Rats.

BP mm Hg	Hematocrit	Blood vol (cc)	% Body wt
90	54	12.3	5.6
92	46	15.5	7.4
92	56	13.3	7
95	50	15.5	7
94	52	15.5	7.7
100	50	18.2	7.6
102	54	15.7	6.9

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† The P^{32} was supplied by the Oak Ridge National Laboratories, Oak Ridge, Tenn.

TABLE II. 1500r Total Body Radiation.

Time interval, hr	BP mm Hg	Hematocrit	Blood vol (cc)	% body wt
6	68	46	14.7	6.95
6	78	47	10.75	4.4
6	72	52	11.85	6
6	77	42	16.65	7.85
6	68	50	16.3	6.95
6	78	48	12.3	6.2
6	78	46	16.3	7.4
6	67	50	17.5	7.45
17	68	38	12	5.85
17	72	44	9.95	4.75
17	64	43	12.2	5.9
17	65	44	9.65	5.35
17	68	41	11.8	5.7
17	68	45	13.25	5.9
17	—	42	14.5	6.15
30	52	45	9.7	4.9
30	58	43	11.15	5.8
30	75	42	12.35	5.8
30	60	43	12.35	5.45
30	48	43	11.35	6.15
30	75	43	11.35	5.7
30	50	40	4.4	2.15
30	58	43	12.25	5.75

TABLE IIIa. 2000r Total Body Radiation—6 Hr Interval.

BP mm Hg	Hematocrit	Blood vol (cc)	% Body wt
—	50	13.5	5.75
73	52	12.75	6
74	49	12.15	5.85
70	50	13.5	6.25
78	50	14.75	6.45
71	49	13.9	6
74	50	12.35	5.85
69	48	13	5.95
70	48	14.35	6.35
71	49	14.65	6.7
70	46	15.35	6.4
70	47	15.2	6.9
68	47	14.65	6.25

TABLE IIIb. 2500r Total Body Radiation—6 Hr Interval.

70	50	10.75	5
75	46	10.9	5.2
68	48	12.35	5.7
84	49	12.3	5.4
72	49	10.9	5.35
73	50	11.3	5.45

is of a similar order of magnitude at the end of 6 hours as that produced by 1,500 roentgens. Nineteen rats were used in this series.

Autopsies upon these rats failed to demonstrate any significant gross changes. The microscopic changes in all rats at the end of 6 hours were similar regardless of the dose group from which they came. These consisted of early destruction, fragmentation and pyknosis of the lymphoid tissue wherever it was found. The mucosal cells in the bases of the crypts of the small intestine showed evidence of early degeneration in the form of nuclear fragmentation, pyknosis and karyorrhexis. The sex cells in the ovaries and testes showed evidence of early disintegration similar to that seen in the lymphoid cells. The H and E bone marrow sections showed evidence of moderate cellular destruction. The lymphocytes show changes similar to those described in the gastrointestinal tract, and the early white cell forms showed some evidence of nuclear pyknosis, the younger cells in general being the most severely damaged. No attempt was made to quantitate this damage. These changes have been described in detail by Warren(2) and more recently by Bloom(3) and the changes mentioned here differ in no way from those described. Other organs and tissues including the brains and spinal cords did not show any abnormalities.

At the end of 17 hours the rats showed a minimal serosal surface hyperemia, but otherwise appeared grossly normal. Microscopically the changes found were identical with those described above, but somewhat more advanced. The germinal centers of the lymphoid tissue were filled with debris and the lymphoid tissue fragments were beginning to disappear. The nervous systems of these animals were not examined.

By the end of 30 hours there was marked destruction and removal of the lymphoid tissue. Some of the mucosal cells of the small bowel showed fairly pronounced fragmentation. There was no sloughing of the mucosa. For the first time, evidence of adrenal cortical change became evident. This consisted of a striking vacuolization of the outer rim of cells of the zona fasciculata. Their cytoplasm was clear and the cells appeared swollen with their nuclei pushed to one side. The H and E bone marrow sections showed moderate evidence of

1. Montgomery, P. O'B. Data to be published.

2. Warren, S., *Arch. Path.*, 1942, v34, 443; 1943, v35, 121.

3. Bloom, W., *Histopathology of Irradiation*, 1948, v22, I, McGraw-Hill Book Co., Inc., New York.

TABLE IV. Spinal Rats 30 Hr After Cord Transections.

BP mm Hg	Hemato-crit	Blood vol (cc)	% Body wt
85	46	11.6	4.8
80	48	15.9	5
50	50	14.95	5.95
70	51	18.8	6.95
72	48	18.25	6.9
78	52	13.25	5.35
78	47	12.4	4.75
70	39	22.95	7.85
80	56	15.15	5.2
70	37	16.3	6
Spinal Rats 72 Hr After Cord Transections			
40	—	6.4	5.35
68	55	8.95	4.5
70	46	14.7	5.9
80	38	10	6.65
65	41	11.35	5.75
45	51	8.45	4.2
65	51	11.75	5.1
55	52	12.9	6.9

TABLE V. Spinal Rats 1500r Total Body Radiation. BP, BV and hematocrit done 30 hr after cord transections; 6 hr after X-ray.

BP mm Hg	Hemato-crit	Blood vol (cc)	% Body wt
78	57	17.2	5.25
80	52	16.45	7
82	52	18	6.1
70	50	14	5.6
70	51	10.75	6.15
60	45	17.45	5.15
80	53	16.8	5.65
72	47	17.2	6

cell destruction, but no attempt was made to study the cell type or degree of maturation at which the effect was most pronounced. The ovaries showed destruction of ripening eggs and follicular cells, while the testes showed partial and occasionally complete destruction of spermatocytes to the level of the primary spermatocyte. In no case was destruction of ova or spermatocytes complete. Other organs and tissues were normal. The nervous systems of these animals were not examined.

In Table IV are recorded the blood pressures, blood volumes, and hematocrits of the non-irradiated spinal rats. It is at once apparent that at the end of each time interval the results are strikingly similar to those found in the normal total body irradiated rats. Autopsies upon these rats revealed marked distention of the bladder and colon, and the viscera were cool to the touch. No microscopic sections were made.

In Table V are the values for the blood pressures, hematocrits, and blood volumes of the spinal rats subjected to 1,500r total body irradiation. The irradiation was given 30 hours after the transections were performed and the measurements made 6 hours after the irradiation. It is evident that these animals do not differ from their controls in regard to blood pressure, blood volume and hematocrit. Thus, there is no immediate effect upon these values in the irradiated spinal rat, despite the fact that autopsies revealed tissue destruction identical in amount and disposition with that seen in the normal rat given 1,500r total body irradiation.

In Table VI are data showing the effects of bilateral adrenalectomy upon the blood pressure, blood volume and hematocrit. At the end of 6 hours the animals are still hypotensive —a response which the unirradiated rat shows to a similar operative procedure whether or not the adrenals are actually removed—but at the end of 30 hours the blood pressure has returned to relatively normal levels. Autopsies upon these animals revealed no pathological changes.

In Table VII are similar data upon irradiated bilaterally adrenalectomized rats. The 1,500r of total body irradiation was given 30 hours after the bilateral adrenalectomy and the determinations performed 6 hours after the irradiation. The data indicate that these rats behave in a fashion identical to normal

TABLE VI. Bilateral Adrenalectomized Rats. 6 hr interval

BP mm Hg	Hemato-crit	Blood vol (cc)	% Body wt
62	61	17	6.35
74	65	12.9	4.8
85	62	16.45	6
80	56	16.2	5.5
90	56	20	6.5
92	57	23.2	8.3
30 hr interval			
87	51	13.7	6
85	47	14.35	5.85
100	52	13.75	5.7
85	51	14.1	6.2
90	52	18.3	6.85
98	51	16.2	6.1

TABLE VII. Bilateral Adrenalectomized Rats 6 Hr After 1500r TBR and 30 Hr After Adrenalectomy.

BP mm Hg	Hemato-crit	Blood vol (cc)	% Body wt
70	54	12.2	4.95
80	50	11.85	4.95
80	51	11.5	4.3
—	54	10.9	3.6
80	52	13.55	5.75
75	53	13.1	4.4
75	56	12.2	4.6
65	52	13.7	4.45
80	56	14.35	4.8
90	51	11.2	4.4
72	51	13.1	4.95

rats given 1,500r total body irradiation. The tissue changes at autopsy of these animals were similar grossly and microscopically to the intact rats 6 hours after 1,500r total body irradiation.

The rats represented by the data in Table I were run as a control group against all the rats receiving irradiation, in order to eliminate any question of blood volume, blood pressure or hematocrit change as a result of depriving the animals of water, and crowding them together in a small box during the time they were irradiated. The animals were put into the radiation box six hours before the determinations were carried out, and left for the same length of time taken by the actual radiation process; they were also deprived of food and water during the six hours preceding the determinations. As shown by the results in Table I no appreciable differences were produced.

Discussion. Normal adult rats develop hypotension within 6 hours following large doses of total body X-ray. This hypotension persists for at least 30 hours. That this hypotension is not a manifestation of clinical shock, as the syndrome is ordinarily defined, is reflected in the failure of either the blood volume or the hematocrit to change during the observed period of hypotension(4).

If this hypotension is not a manifestation of clinical shock, of what then is it a manifestation?

It has been considered by Moon(5) to be due to the toxic effects of the absorption of degenerated intestinal mucous membrane and intestinal contents. That the late true shock picture of irradiation sickness may be due in part to this we do not wish to take exception, but we do not agree that it plays any role in the early acute hypotension following large doses of total body X-ray. Our evidence for this rests on the failure to demonstrate histologically any more than very early evidences of degeneration of the mucosal cells of the gastro-intestinal tract, and the fact that the hypotension continues almost at the same level for the first 30 hours, while histological evidence of cellular destruction both in the gastro-intestinal tract and the lymphoid tissue steadily increases. Furthermore, increasing the dose from 1,500r to 2,500r does not change the response of the blood pressure, and were tissue destruction responsible one would certainly expect more tissue destruction and hence more hypotension with 2,500r than with 1,500r.

The adrenal cortex exerts a profound effect upon the normal blood pressure regulating mechanisms, and removal of the adrenal cortices has long been known to produce severe and constant hypotension. We do not believe, however, that the adrenal cortex plays any significant role in the production of the acute hypotension seen following total body irradiation. The non-irradiated bilaterally adrenalectomized rat goes through a brief period of hypotension due to the operative trauma, which is over within 24 hours. At the end of this time his blood pressure, blood volume, and hematocrit are normal. If he is given a large dose of total body irradiation at this time, he responds as does the non-adrenalectomized irradiated rat. Furthermore, we have failed to demonstrate any histological abnormality in the irradiated animals' adrenals at the end of either 6 or 17 hours following irradiation. The earliest histological change is noticeable only at the end of 30 hours. Thus it is difficult to see how either adrenal failure or abnormal secretion could account for the observed data.

A direct effect of the radiation upon the

4. Moon, V. H., *Ann. Int. Med.*, 1939, v13, 451.

5. Moon, V. H., Kornblum, K., Morgan, D. R., *J. A. M. A.*, 1941, v116, 489.

cardio-vascular system might account for the hypotension. Indeed this point of view can be defended even in the face of an obvious lack of histological evidence of such damage. An inspection of the data with regard to the spinal rats, however, excludes a possible direct effect upon the cardiovascular system. The 30-hour spinal rat's blood pressure, blood volume and hematocrit are not changed by irradiation. Should there be a direct effect one would expect the irradiated spinal animal to show a more profound hypotension than his non-irradiated control. Thus it is also difficult to conceive of histamine-like substances being the responsible agents, as has been claimed by Painter(6); for again one would expect a further lowering of the blood pressure in the spinal rat following radiation.

It is the remarkable failure of the irradiated spinal rat to differ from his non-irradiated spinal control—in so far as blood pressure, blood volume, and hematocrit are concerned—that lead us to the conclusion that depression of the nervous system is the cause of the immediate hypotension produced by large doses of total body X-ray which are not accompanied by changes in the blood volume or the hematocrit. Additional support for this hypothesis is found in the observations of Prosser *et al.*(7), that the immediate hypotension in rabbits after total body irradiation may be largely guarded against by vagotomy or atropinization; and that it can be antagonized by adrenalin. We have been unable to observe any histological changes in the central nervous system of the irradiated rats by conventional technics. We do not, however, feel that this is any assurance that there has been no change in function. That the autonomic nervous system can be damaged by external radiation has been demonstrated by Suzuki(8). However, as shown by Griffith and Pendergrass(9) the dose must be high, *i.e.* in excess of 800r.

Summary and conclusions. 1. The effects of large doses of total body radiation upon the blood pressure, blood volume, and hematocrit of normal adrenalectomized and spinal adult rats have been studied. 2. From the data presented it appears that the cause of the immediate hypotension following such irradiation is depression of the nervous system.

We wish to acknowledge the technical assistance of Miss Blair Burwell.

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A Simple Assay of Mammalian Pineal Extracts. (18931)

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Amphibian larvae react to feeding with, or injection of, pineal substance with a transient contraction of the skin melanophores. This reaction has been described by McCord and Allan(1) and subsequently by Hogben(2), Engel(3), and others, as occurring in tadpoles only until metamorphosis. At that period the response of the melanophores to pineal extract ceased in the species observed by these authors.

In the following report experiments are described, in which extracts of mammalian

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2. Hogben, L. T., Pigmentary Effector System III, *Amphibia,* Oliver & Boyd, Edinburgh, 1924.

3. Engel, P., *Endocrinology,* 1939, v25, 144.