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Hypothromboplastinemia Following Total Body Irradiation.

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(Introduced by R. W. Heinle.)
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Allen and Jacobson[1] published a report concerning the hemorrhagic syndrome associated with total body exposure to ionizing irradiation. They were able to demonstrate a circulating anticoagulant which was indistinguishable from heparin. A more detailed study of the same and similar experiments was published by Allen, Sanderson, Milham, Kirschon, and Jacobson.[2] These studies are of considerable importance with respect to the effect of irradiation upon the human being and also because of their relation to idiopathic thrombocytopenic purpura and thrombocytopenia secondary to aplasia or destruction of the bone marrow. Interest has been stimulated among radiologists, investigators working in the field of atomic irradiation, hematologists, and various others concerned with the coagulation of blood.

The syndrome that Allen and his associates produced in the dog with a total body exposure of 450 r (LD-100%) consisted principally of extensive hemorrhage throughout all organs of the body, neutropenia, thrombocytopenia, anemia, prolongation of the clotting time of whole blood, and prolongation of the bleeding and clot retraction times. Prothrombin times were within normal limits up to 24 hours prior to death. Blood fibrinogen, calcium, phosphorus, and magnesium levels were normal. The animals succumbed on an average of 11 days following irradiation. This syndrome resembles that of idiopathic thrombocytopenia with the exception of the neutropenia and prolonged clotting time. The decreased coagulability of the blood was thought to be due to a circulating heparin-like anticoagulant. This was demonstrated by prolonging the clotting time of normal plasma by the addition of plasma from an irradiated animal and by *in vivo* and *in vitro* titrations of the irradiated blood with toluidine blue and protamine sulphate.

Kohn and Robinett[3] reported no change in the clotting time of rats subjected to 1,000-1,250 r, which was 125-150% of the LD-50%. By administering heparin to the rats, the LD-50% could be reduced about one-third. Allen and his associates routinely used the LD-100%.

Our interest in this problem arose in an attempt to determine whether the reputed decreased coagulability of the blood following irradiation of the dog was due to circulating heparin or an increase of the heparin cofactor. Our experiments have demonstrated no evidence of heparinemia or increase in the heparin cofactor. A definite change in the amount of thromboplastic protein that could be extracted from plasma subjected to high-speed centrifugation was found.

Method. Studies were carried out prior to irradiation and for the most part, every other day after irradiation. Determinations consisted of the hemoglobin, erythrocyte count, leucocyte count, platelet count (blood smears, 2.5% sodium citrate), clotting time,[4] clot retraction, heparin cofactor,[5] protamine titration where indicated, and thromboplastin assay.

Healthy mongrel dogs were subjected to total body irradiation with 450 r (LD-100%) from a 220 K.V.P. machine at 15 milliamperes through a 0.5 mm copper filter and a 1.0 mm aluminum filter. H.V.L. was 1.2 mm

[1] Allen, J. G., and Jacobson, L. O., *Science*, 1947, **105**, 388.

[2] Allen, J. G., Sanderson, M., Milham, M., Kirschon, A., and Jacobson, L. O., *J. Exp. Med.*, 1948, **87**, 71.

[3] Kohn, H. I., and Robinett, P., *Oak Ridge National Laboratory*, 1948, unclassified.

[4] Lee, R. I., and White, P. D., *Am. J. Med. Sci.*, 1913, **145**, 495.

[5] Holden, W. D., Cole, J. W., and Davis, J. H., Jr., *Surg., Gyn., and Obs.*, 1949, in press.

TABLE I.
Hematologic and Coagulation Changes in Exp. No. 5.

Date	11/22	11/24	11/26	11/29	12/1	12/3	12/6	12/8
Hb. g/100 ml	16.0		15.5	15.0	13.5	13.0	11.0	9.0
RBC/c.mm. × 10^6	6.56		6.23	6.03	6.12	5.59	4.02	3.62
WBC/c.mm.	15,275		10,700	2,000	600	760	175	50
Platelets/c.mm. × 10^3	308		236	223	110	16	4	
Clotting time, min.	7		5	5	9	9	9	9
Heparin cofactor. % thrombin destroyed	92.2	Irradiated 450 r.	95	93.5	92	92.3	94.9	85
Clotting time supernatant plasma (sec.)	138		190	283	403	348	510	600
Clotting time (sec.) thromboplastin + supernatant plasma	24		21	22	29	22		
Clotting time (sec.) suspended sediment and supernatant plasma	84		89	87	120	120	132	
Clot retraction	Normal							None

of copper. Irradiation was delivered at a rate of 6-7 r per minute.

Chargaff and West[6] demonstrated that a sediment exhibiting thromboplastic activity could be removed from oxalated plasma subjected to high-speed centrifugation. The clotting time of the supernatant plasma was an index of the amount of thromboplastic protein remaining after centrifugation. The clotting time of the supernatant plasma after portions of the sediment were added to it also indicated the amount of thromboplastic activity in the original plasma. In this way, minute changes in the thromboplastic protein, measured in gammas, could be demonstrated.

The following procedure was used to assay the thromboplastic protein of the plasma. Twenty ml of blood were removed from the jugular vein with as little tissue trauma as possible and quickly mixed with 2.0 ml of a 0.1 M sodium oxalate solution. The blood was centrifuged at 4,000 r.p.m. (2,400 g) for 20 minutes at a temperature of 4°C in a refrigerated International centrifuge. The supernatant plasma was carefully pipetted off, and this was centrifuged for 150 minutes at 20,000 r.p.m. (33,000 g) at the same temperature. The plasma was decanted. The sediment remaining in the base of the centrifuge tube was then suspended in normal saline. The amount of saline used was one-tenth the volume of supernatant plasma. To 0.1 ml of the supernatant plasma was added 0.1 ml of normal saline, and the mixture was placed in a water bath at 37°C for one minute. One-tenth milliliter of 0.022 M calcium chloride solution was added and a stopwatch started. The clotting time was determined in seconds. When short clotting times were obtained, solid clot formation occurred and this was used as the end point. When the clotting time was prolonged, the formation of a solid clot was preceded by fibrin strands. End points were indicated by the first appearance of the latter. One-tenth milliliter samples of the supernatant plasma were then added to saline and various concentrations of a suspension of thromboplastin* and the clotting times determined after incubation for one minute at 37°C and recalcification. The suspension of thromboplastin was prepared by mixing 50 mg of the thromboplastin with 12 ml of normal saline and heating it for 10 minutes at 50°C. Following the above procedure, 0.1 ml of the suspended sediment was added to 0.1 ml of the supernatant plasma. The mixture was incubated for one minute at 37°C and then quickly recalcified by adding 0.1 ml of the 0.022 M calcium chloride solution.

Results. Five dogs were irradiated. One lived 5 and another 7 days, and neither showed any changes in the clotting mech-

[6] Chargaff, E., and West, R., *J. Biol. Chem.*, 1946, **166**, 189.

* The Maltine Company.

TABLE II.
Average of Blood Determinations on the Day Before Irradiation and the Day of Death.

	Before irradiation	Day of death
Hb. g/100 ml	13.4	10.1
RBC/c.mm. × 10^6	6.5	3.8
WBC/c.mm.	12,300	175
Platelets/c.mm.	236,000	6,630
Clotting time (min.)	8	8
Clot retraction	Normal	0
Heparin cofactor. % thrombin destroyed	89	83
Clotting time (sec.) supernatant plasma	147	440
Prothrombin time (sec.)	14.8	17.4
Clotting time (sec.) supernatant plasma + sediment	70	107

anism or hematologic picture that could be subjected to a critical analysis. The other 3 dogs survived 9, 10, and 11 days respectively and displayed the same clinical changes and similar alterations in the studies of their blood. At autopsy the following findings were observed. In general, multiple focal pulmonary and epicardial hemorrhages were present. Submucosal hemorrhages were scattered throughout the gastrointestinal tract, and were routinely present in the urinary bladder. Small areas of bronchopneumonia were present in one dog, while in another, pneumonic consolidation was widespread. A hemothorax (50 ml) was present in one dog and a bilateral pleural effusion in another.

Table I shows the studies performed during the course of one experiment, while Table II shows the average determinations of 3 experiments before irradiation and on the day of death. The significant findings so far as the hemorrhagic tendency is concerned are the reduction in thrombocytes, the increase in the clotting time of the supernatant plasma, and the increase in the clotting time of the supernatant plasma after the addition of the suspended sediment (Fig. 1). These latter 2 determinations represent thromboplastic protein in the blood stream and indicate a definite diminution following irradiation. The gradual reduction of thromboplastic activity in the plasma parallels the reduction in thrombocytes, but it cannot be stated from these observations that the thromboplastic protein was derived from the thrombocytes. The following determinations demonstrate that the prolongation of the clotting time was not due to the presence of an anticoagulant or the depletion of either prothrombin or fibrinogen.

Protamine sulphate† in amounts from 0.02 mg to 0.06 mg in 0.1 ml of physiological saline was added to 0.1 ml of the supernatant plasmas after their clotting times had become prolonged. After adding 0.1 ml of 0.022 M calcium chloride solution, there was in no instance a shortening of the control clotting time. In other experiments[7] plasma obtained from heparinized blood showed a return of the prolonged clotting time to normal or near normal values after the addition of protamine sulphate. The above titrations suggest that no anticoagulant subject to neutralization by protamine was effecting the deceleration of clot formation in the supernatant plasma.

When 0.1 ml of commercial thromboplastin and 0.1 ml of 0.022 M calcium chloride solution were added to the supernatant plasma, a satisfactory estimate of the available prothrombin was determined. Clotting times varied from an average of 14.7 seconds before irradiation to 17.4 seconds on the day of death. Although this represents a slight reduction in the amount of prothrombin in the supernatant plasma, it hardly accounts for either the marked prolongation of the clotting time of the recalcified supernatant plasma or the hemorrhagic manifestations exhibited by the animals.

The fact that a tenacious solid clot could always be obtained from the recalcification of the supernatant plasma indicated that an adequate quantity of fibrinogen was present. This was further demonstrated by adding 10

† The Upjohn Company.

[7] Portmann, A. F., and Holden, W. D., to be published.

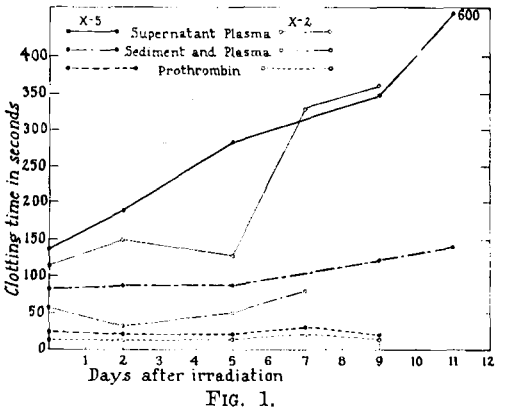

FIG. 1.

Graphic representation of the changes in supernatant plasma, sediment and plasma, and prothrombin in two experiments.

units of thrombin‡ to 0.1 ml of the supernatant plasma and 0.1 ml of 0.9% sodium chloride solution and obtaining satisfactory clots in less than 5 seconds.

Clotting times performed on whole blood throughout all experiments showed no significant changes. Determinations of the heparin cofactor likewise showed no change of importance. The failure to demonstrate any prolongation of the clotting time of the blood is at variance with the observations of Allen and his associates.[1,2] We have no satisfactory explanation of this. The determination of the clotting time of whole blood by any of the various test tube methods is notoriously difficult to perform and interpret. For this reason special precautions were taken. The same physician drew all the blood and performed all the clotting times under the same circumstances. We do not wish to deny that heparinemia may exist after irradiation of this character, but up to the present we have been unable to satisfy ourselves that it exists, whereas we have been able to demonstrate a hypothromboplastinemia and thrombocytopenia which explain the clinical manifestations exhibited by these animals.

Conclusions. 1. A hemorrhagic syndrome in dogs was produced by total body irradiation (450 r). Manifestations consisted of multiple areas of hemorrhage in the pulmonary, gastrointestinal, and urinary systems, anemia, leucopenia, thrombocytopenia, prolongation of clot retraction, and hypothromboplastinemia.

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‡ Parke, Davis and Company.

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Fluorescence of Intravenously Administered Rose Bengal Appears Only in Hepatic Polygonal Cells.

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Although certain intravenously administered dyes have been employed in the study of hepatic function over the past 3 decades, the exact mechanisms by which they are removed from the blood are poorly understood. Earlier work indicated that the entire reticuloendothelial system participated in the removal of these substances. This concept is still widely prevalent, although more recently indirect evidence suggests that the normal liver alone is responsible for the uptake of such dyes as the disulfonate of phenoltetrabromphthalein (Bromsulfalein).[1] Now that hepatic blood flow in man is being estimated by a technique based on the extraction of Bromsulfalein by the liver,[2] it is essential to determine whether the removal of this dye, or of others similarly handled, measures the

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1 Ingelfinger, F. J., *Bull. New Eng. Med. Cen.*, 1947, **9**, 25.