Plasma Antihemophilic Activity Following Total Body Irradiation.*† (19198)

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There is no general agreement at present regarding the nature of the coagulation defect which develops following total body irradiation. No deficiency of fibrinogen exists(1); instead, fibrinogen levels are often elevated. Likewise there is no deficiency of Ac-globulin (2) or of prothrombin(1,2). The severe thrombocytopenia appears to be of major importance in the delayed clotting, although many workers have denied that the low platelet level is the sole basis of the coagulation defect. It has been suggested that either an excess of a heparin-like circulating anticoagulant(1) or a deficiency of plasma thromboplastin(3) may contribute to the clotting abnormality. Recently it has been shown in irradiated dogs that thrombin is formed slowly during clotting(2). One component required for prompt evolution of thrombin in clotting blood is the antihemophilic factor (AHF). With the recent development of an assay procedure for AHF(4), it became possible to determine if alterations in this clotting factor occur after whole body irradiation.

Procedures. Ten healthy mongrel dogs were given single doses of total body X-irradiation (600 r, 2000 kv) by a procedure described previously(5). Platelet and leucocyte counts and hematocrit values were determined at frequent intervals on all dogs. For platelet counts the method of Brecher and Cronkite (6) was used on five of the dogs, and a modification of Nygaard's method(7) on the other animals. Two measures of the clotting defect

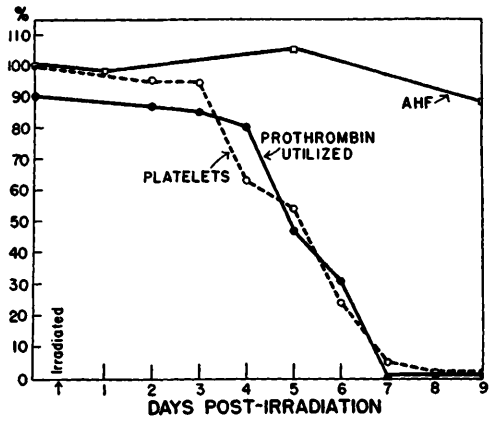

FIG. 1. Thrombocytopenia, impaired prothrombin utilization and stable plasma AHF following irradiation of 19.5 kg ♂ dog. Only the 1 hr prothrombin data are plotted. Control platelet count 614,000/mm³, Nygaard method.

were used: (a) clotting times of venous blood, using either a modified Lee-White procedure (two tubes, 10 x 75 mm, 28°C) or the procedure of Jackson *et al.*(2) (three tubes, 12 x 75 mm, 37°C) and (b) the rate of loss of prothrombin from clotting blood, as determined by the prothrombin utilization test(8). Prothrombin determinations were made by a modified two-stage procedure(4). AHF assays were performed by a method recently described(4).

Results and discussion. All the dogs developed a severe irradiation reaction and died within 7-15 days. In all animals at the height of the reaction, severe anemia, leucopenia and thrombocytopenia (0-12,000 per mm³) were present. Impaired clotting was observed. The clotting time by the Lee-White procedure ranged from 12-40 min (normal controls, 6-8 min) and by the Jackson procedure ranged from 70-300 min (normal controls, 10-20 min). In clotting blood, prothrombin utilization was impaired; less than 10% was consumed in one hour. The response of one animal, typical of this group of dogs, is shown in

* This investigation was supported by a research grant as part of the National Blood Program, National Institutes of Health, U. S. Public Health Service.

† The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of the Navy or of the Naval Service at large.

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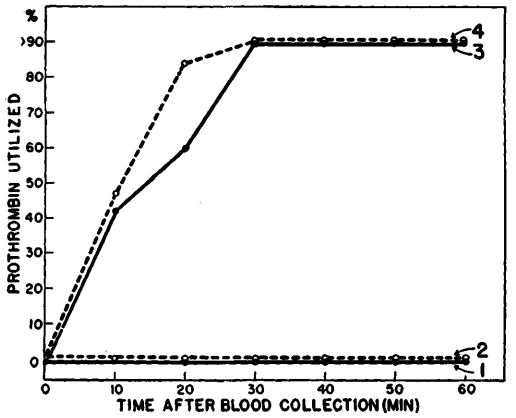

FIG. 2. Transfusions of hemophilic dog with plasmas from irradiated and normal dogs. Recipient, ♀, 21.6 kg; dose 2 ml/kg. See text for explanation of curves.

Fig. 1. Beginning on the fourth day after irradiation, a progressively increasing thrombocytopenia developed. Concomitantly, there was impaired utilization of prothrombin in clotting blood. Plasma AHF levels, however, remained within normal limits. This dog died on the 9th day following massive hemorrhage into the intestine. Autopsy studies revealed in addition widespread destruction of lymphoid tissue similar to that observed by Tullis(9) in swine used in the Bikini atomic bomb tests.

Plasma AHF assays were done on 9 of the 10 dogs at the time the clotting defect was most pronounced (7-13 days after irradiation). The mean value obtained was 97.5% of normal plasma; 95% confidence limits were 81.6-113.4. This value for AHF is comparable to that observed in a series of normal dogs(4).

Further tests of antihemophilic activity of the irradiated dogs were made by transfusing their plasmas into hemophilic dogs and comparing the changes in the hemophilic clotting defect with those produced by normal plasma transfusions. The results of one of 3 experiments of this type are shown in Fig. 2. In this experiment whole blood obtained from a dog 12 days after total body irradiation showed no detectable utilization of prothrombin in one hour (Curve 1, Fig. 2). Citrated plasma, prepared from this dog at the same time by the silicone technic(8), was transfused promptly into a hemophilic dog. Like the irradiated dog, the hemophilic recipient prior to transfusion showed no prothrombin utilization (Curve 2, Fig. 2). Immediately after transfusion, however, the prothrombin in the hemophilic blood disappeared rapidly (Curve 3, Fig. 2). This corrective effect was similar to that produced by a transfusion of citrated normal plasma (Curve 4, Fig. 2), given to the same hemophilic animal after the beneficial effects of the original transfusion had disappeared.

Previous work has indicated that formed elements, particularly platelets, are necessary for AHF to manifest itself(10). In the above experiments, plasma AHF was active in the presence of the full complement of platelets furnished by the hemophilic blood. In the following experiment, a study was made of the clotting rate in the mixtures of irradiated dog's blood with either hemophilic platelet-poor plasma or whole blood. With platelet-poor hemophilic plasma (Series 1, Table I), it will be observed that rapid conversion occurred when platelets were abundant in the irradiated dog's blood. When thrombocytopenia developed, however, prothrombin utilization was retarded. On the other hand, the addition of the irradiated dog's blood to whole hemophilic blood resulted in prompt utilization of prothrombin throughout the post-irradiation period (Series 2, Table I). A similar mutually corrective effect of blood from patients with idiopathic thrombocytopenia and with hemophilia has been observed previously(11). It would appear that the blood from irradiated dogs is potentially capable of clotting at a normal rate, provided that an adequate number of platelets is available.

In view of evidence that certain globulins are produced by lymphoid tissue(12), it is of interest that AHF remained at normal levels in these experiments despite severe damage to the lymphoid system. Previous data indicated that AHF, unlike some other proteins of the clotting reaction, is not reduced in severe liver damage(4). Apparently neither an intact lymphoid system nor an intact liver is needed for the maintenance of the antihemophilic activity of plasma.

Summary. 1. Plasma antihemophilic ac-

TABLE I. Effect of Dog Blood Collected Daily Following Irradiation on Platelet-Poor Hemophilic Plasma and Whole Hemophilic Blood.

Days after irradiation	Test series 1: .5 ml irradiated dog whole blood* .7 ml platelet-poor hemophilic plasma†		Test series 2: .5 ml irradiated dog whole blood* 1.2 ml hemophilic whole blood*‡	
	% prothrombin utilized in 1 hr	No. platelets per mm³ mixture	% prothrombin utilized in 1 hr	No. platelets per mm³ mixture
2	90	243000	90	432000
3	90	240000	82	404000
4	85	161000	90	356000
5	81	138000	90	308000
6	79	61300	90	302000
7	17	12750	90	204000
8	28	2300	90	198000
9	18	1275	90	218000

* Whole blood freshly drawn, no anticoagulant, immediate mixing. Irradiated dog same as in Fig. 1.

† Prepared by centrifugation at 5°C at about 14000 g for 2 hr, silicone technic, no anticoagulant.

‡ Equivalent to .7 ml plasma.

tivity of dogs subjected to total body irradiation remained at normal levels. 2. Whole hemophilic blood with a full complement of platelets accelerated the clotting of irradiated dog's blood, while platelet-poor hemophilic plasma did not.

1. Allen, J. G., Sanderson, M., Milham, M., Kirschon, A., and Jacobson, L. O., *J. Exp. Med.*, 1948, v87, 71.

2. Jackson, D. P., Cronkite, E. P., Jacobs, G. J., and Behrens, C. F., to be published.

3. Holden, W. D., Cole, J. W., Portmann, A. F., Storaasli, J. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 553.

4. Graham, J. B., Collins, D. L., Jr., Godwin, I. D., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 294.

5. Chapman, W. H., Cronkite, E. P., Chambers, F. W., and Morgan, J. E., *NMRI Project* No. NM 006 012.05.03, 1950.

6. Brecher, G., and Cronkite, E. P., *J. App. Physiol.*, 1950, v3, 365.

7. Nygaard, K. K., *Proc. Staff Meet., Mayo Clin.*, 1933, v8, 365.

8. Buckwalter, J. A., Blythe, W. B., and Brinkhous, K. M., *Am. J. Physiol.*, 1949, v159, 316.

9. Tullis, J. L., *Am. J. Path.*, 1949, v25, 829.

10. Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 117.

11. Stefanini, M., and Crosby, W. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 301.

12. Harris, T. N., and Harris, S. J., *J. Exp. Med.*, 1949, v90, 169.

Received October 31, 1951. P.S.E.B.M., 1951, v78.

Studies on Purine and Pyrimidine Requirements of a Strain of *Streptococcus faecalis*. (19199)

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In an earlier paper(1) it was reported that a strain of *Streptococcus faecalis*, subsequently designated *S. faecalis* AB, required uridine or other pyrimidine nucleosides and nucleotides for optimum early growth. In that study, the authors used a basal medium containing free purine and pyrimidine bases to obtain maximum growth response to uridine. Snell *et al.*(2-4) reported that purine and pyrimidine bases had no stimulatory effect on the growth of *S. faecalis*, while Barton-Wright (5) and Luckey *et al.*(6), without discussion