J. Exp. Med., 1954, v99, 89.
8. Meyer, A. S., *J. Biol. Chem.*, 1952, v203, 469.
9. Zaffaroni, A., *Recent Progress Hormone Research*, 1953, v8, 51.
10. Chen, C., Wheeler, J., and Tewell, H. E., Jr., *J. Lab. and Clin. Med.*, 1953, v42, 749.
11. Simpson, S. A., *et al., Experientia*, 1953, v9, 333.
12. Simpson, S. A., Tait, J. F., and Bush, I. E., *Lancet*, 1952, v263, 226.
13. Kass, E. H., and Finland, M., *Ann. Rev. Microbiol.*, 1953, v7, 361.
14. Hechter, O., *Ciba Foundation Coloquia on Endocrinology*, 1953, v7, 161, Churchill, London.
15. ———, Trans. 3rd Conf. Adrenal Cortex. Macy Foundation, Ed. E. Ralli, 1952, p. 115.
16. Stone, D., and Hechter, O., *Abstr. XIX Int. Physiol. Congress* (Montreal), 1953, p. 805.

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Changes in Clot Retraction Time and Platelet Adhesiveness Following Whole Body Irradiation.* (20960)

J. PHILIP SAVITSKY AND SOL SHERRY.

From the May Institute for Medical Research of the Jewish Hospital Association, Cincinnati, O.

The exact nature of the clotting defect in the hemorrhagic state following whole body radiation is unclear. It is apparent that the concentrations of fibrinogen(1), calcium, prothrombin(1), ac-globulin(2), antihemophilic globulin(3) and the plasma precursor of serum prothrombin conversion accelerator(4) are normal in the irradiated animal. The most significant concomitant of the prolonged clotting time and the hemorrhagic syndrome is the marked thrombocytopenia. Emphasizing the significance of the thrombocytopenia have been the studies demonstrating temporary reversal of the clotting defect following transfusion into these animals of normal platelets (5,6). It is not known whether there are qualitative changes in platelets of irradiated animals or whether these changes may precede onset of thrombocytopenia. Cohn has shown in his charts that there is a delay in initiation of clot retraction by the third post radiation day prior to onset of thrombocytopenia but no reference is made to this observation in the text(7). There is a very close relation between the rate of initiation of clot retraction as measured by clot retraction time and level of platelet adhesiveness(8,9).

In this study evidence is presented of changes in platelet adhesiveness and clot retraction time preceding onset of thrombocytopenia in the irradiated dog. There is also present in the irradiated animal a circulating plasma factor which inhibits platelet adhesiveness and prolongs the initiation of clot retraction.

Methods. Citrated venous blood was placed in a lusteroid tube and immediately after drawing blood a sample was drawn in a red-cell diluting pipette. The diluting fluid was 3.8% sodium citrate and 0.2% formalin. The pipette was placed in shaker for 10 minutes and the fluid then examined directly on a counting chamber. Platelet adhesive index was determined by the glass-wool method of Moolten and Vroman(10). The normal index in the dog varied from 1.15 to 1.35 and is reproducible with less than 10% error. Clot retraction time was determined by the method of recalcified venous blood in castor oil(8). This was modified to account for the more rapid clotting of recalcified dog's blood. 4.5 ml of venous blood was drawn into a syringe containing 0.5 ml of 3.8% sodium citrate. One ml of this blood was placed in each of 2 glass tubes in a water bath at 18°C and .06 ml of a 5% $CaCl_2 \cdot 2H_2O$ solution was added to each tube. Immediately following addition of calcium, blood was drawn up in a siliconized capillary pipette and deposited as a single drop in castor oil. The tubes of castor oil were kept at 18°C and the test done in quadruplicate. The clot retraction time was

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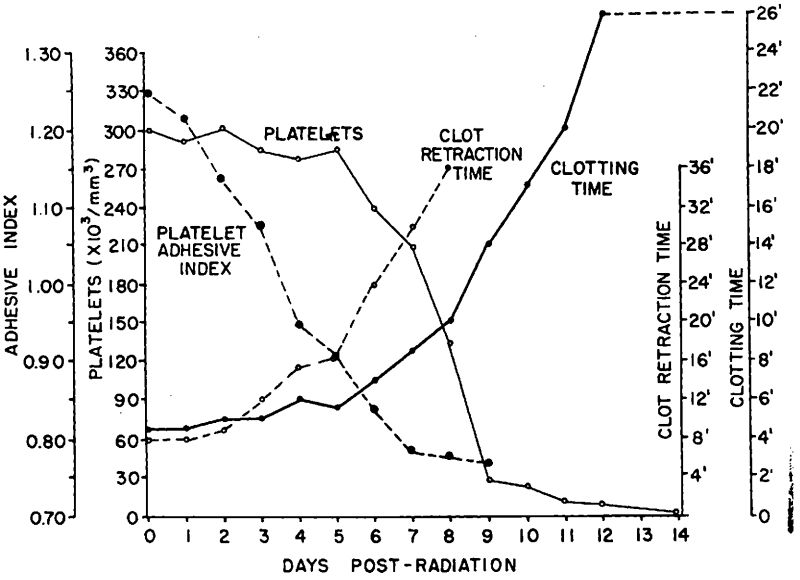

FIG. 1. Average daily values of platelet adhesive index, platelet count, clot retraction time and clotting time of 8 dogs following 400 r whole body radiation.

the time from clotting of recalcified blood to exudation of serum from the drop in castor oil. The normal range, as performed on 15 dogs, was 6-10 minutes, mean value 7.5 minutes. Clotting time was done in a glass clotting tube at room temperature. Blood was drawn using 2-syringe technic. Normal clotting time in dogs varied from 3 to 6 minutes. Dogs weighing 20 to 30 lb received 400 r, 250 kv. peak, 15 ma, dose rate 25 r/min. Target skin distance, 100 cm. Dosage of 400 roentgens was LD_{90}.

Results. Eight healthy dogs were given 400 r of whole body radiation and studied daily until time of death. Daily determinations were made of platelet count, platelet adhesive index, clot retraction time and clotting time. Results are shown in Fig. I. There was a significant decrease in platelet adhesiveness by the third and fourth post radiation days and a concomitant prolongation of clot retraction time. These changes occurred prior to any demonstrable changes in platelet count or clotting time. When the platelet count dropped below 100000/mm^3, clot retraction did not occur and it was difficult to determine platelet adhesive index.

It was observed accidentally that *in vitro* addition of excess calcium to blood from an irradiated dog returned a prolonged clot retraction time to normal. The effect on clot retraction time, of varying the concentration of calcium was then determined. Different

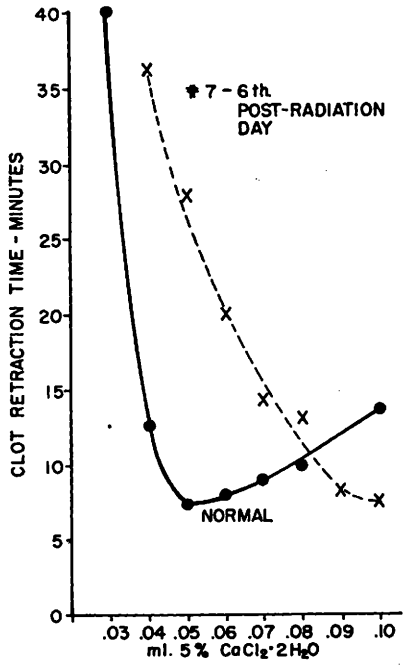

FIG. 2. Effect of varying concentrations of calcium on clot retraction time of normal and irradiated dogs blood.

TABLE I. Inhibition of Clot Retraction and Platelet Adhesiveness by Plasma from an Irradiated Dog.

	Clot retraction time, min.	Platelet adhesive index
1 cc N*	7	1.23
Idem + .1 cc citrate-saline	6	—
" .2 *idem*	8	1.26
" .1 normal plasma	6	—
" .2 *idem*	7	1.22
" .1 plasma (irrad.)	8	—
" .2 *idem*	18	.87

* N = Normal blood.

amounts of calcium chloride solutions were added *in vitro* to normal dog's citrated blood and to citrated blood from irradiated animals. Blood from irradiated dogs was drawn on 5th to 7th days post radiation when they had a markedly prolonged clot retraction time but with at least 50% of the platelets still present. The results of a typical experiment are shown in Fig. 2. The curve for normal blood is similar to that observed for coagulation time with variation in calcium concentration. The shift of the clot retraction time curve in the irradiated animals to a higher concentration of calcium suggests a defect in the use of calcium in initiation of clot retraction. The calcium concentration in blood when 0.1 ml of a 5% $CaCl_2$ solution is added, is far in excess of physiological concentration. These results suggested that not only was there a defect in the platelet prior to onset of thrombocytopenia but that perhaps there might be a substance present in plasma of these irradiated animals which damaged the platelets and inhibited initiation of clot retraction. This was tested by adding plasma from a thrombocytopenic dog (12th post radiation day) to normal blood *in vitro*. To one ml of citrated blood from a normal dog, 0.2 ml of thrombocytopenic plasma and 0.7 cc of 5% $CaCl_2$ solution were added. This was found to prolong clot retraction time of normal blood whereas addition of normal plasma to normal blood had no effect. A typical experiment is shown in Table I. Further studies showed that this prolongation of clot retraction time of normal blood could be prevented by addition of excess calcium with a similar shift of the curve to higher concentrations of calcium as shown in Fig. 2. Also plasma from irradiated dogs markedly decreased platelet adhesiveness of normal blood in the same proportions of added plasma to normal blood (Table I).

The properties of this clot retraction inhibitor present in plasma of irradiated dogs were investigated. It was found to be nondialyzable, water soluble, stable at 4°C for several weeks, stable at 56°C for at least 30 minutes and at 60°C for at least 20 minutes. This substance was destroyed in 5 minutes at 65°C. It was stable over a pH range of 5-9 and was not precipitated with globulins at pH 5.2 (diluted with distilled water). It was not adsorbed by $BaSO_4$, $Ca_3(PO_4)_2$ or $Al(OH)_3$. All attempts at extraction with lipid solvents were unsuccessful.

The inhibitor of the initiation of clot retraction and of platelet adhesiveness was not demonstrable in the plasma of the irradiated dog until 8th post radiation day. It was most easily shown when the animal became thrombocytopenic at about 10th to 12th post radiation days, although it was never possible to add less than 0.2 cc of plasma to one ml normal blood and produce inhibition of clot retraction time.

Discussion. Our data indicate that qualitative changes in platelets occur prior to development of thrombocytopenia associated with radiation hemorrhage. Concomitant with the decrease in platelet adhesiveness is a prolongation of clot retraction time, which is a measure of the rate of initiation of clot retraction. Platelets start to disappear from circulation by 5th to 6th post radiation days at a time when platelet adhesive index is extremely low and clot retraction time markedly prolonged. It may be that changes in the surface properties of the platelets play some role in subsequent rapidly developing thrombocytopenia.

There is present in the plasma of the irradiated dog, a substance which lowers the platelet adhesiveness and inhibits clot retraction time of normal blood. The demonstration of a circulating factor present 8 to 15 days following radiation which can induce changes in normal blood similar to those occurring in radiated animal raises again the

question of the "indirect" effect of radiation. The extensive investigations of Lawrence and his coworkers(11) of lack of effect of cross transfusions from radiated animals into normal have provided strong negative evidence. These studies have been done *in vivo,* whereas the evidence presented here for a plasma clot retraction time inhibitor has been entirely *in vitro.* The inability to demonstrate this factor before the 8th post radiation day is significant because the studies of Lawrence *et al.* extended only through the 5th post radiation day.

Conclusions. 1. There is an inhibition of platelet adhesiveness and a prolongation of clot retraction time following whole body radiation of the dog. These changes are demonstrable 3 days after radiation, prior to thrombocytopenia or prolongation of clotting time which is associated with hemorrhage. 2. Inhibition of clot retraction time can be reversed *in vitro* by addition of excesses of calcium. This suggests a defect in the radiated animal in the use of calcium in initiation of clot retraction. 3. There is a substance in the plasma of the radiated dog which acts on platelets to decrease platelet adhesiveness and inhibit clot retraction time. This factor is not demonstrable before 8th post radiation day and is most easily shown when the animal is thrombocytopenic.

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1. Jackson, D. P., Cronkite, E. P., Levoy, G. V., and Halpern, B., *J. Lab. and Clin. Med.,* 1952, v39, 449.

2. Jackson, D. P., Cronkite, E. P., Jacobs, G. J., and Behrens, C. F., *Am. J. Physiol.,* 1952, v169, 208.

3. Penick, G. D., Cronkite, E. P., Godwin, I. D., and Brinkhous, K. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 732.

4. Jacobs, G. J., and Cronkite, E. P., (Unpublished). Quoted by Cronkite, E. P., and Brecher, G., *Defects in Hemostasis Produced by Whole Body Irradiation. Blood Clotting and Allied Problems,* Trans. 5th Josiah Macy Conference. Josiah Macy Foundation, New York, 1952.

5. Brecher, G., and Cronkite, E. P., *Proc. New York Path. Soc.,* 1950-1951, v116.

6. Woods, M. C., Gamble, F. N., Furth, J., and Bigelow, R. R., *Blood,* 1953, v8, 545.

7. Cohn, S. H., *ibid.,* 1952, v7, 225.

8. Savitsky, J. P., and Werman, R., *Am. J. Clin. Path.,* 1952, v22, 1175.

9. Savitsky, J. P., *Blood,* 1953, v8, 1091.

10. Moolten, S. E., and Vroman, L., *Am. J. Clin. Path.,* 1949, v19, 701.

11. Lawrence, J. S., Valentine, W. N., and Dowdy, A. H., *Blood,* 1948, v3, 593.

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Production of Renal Hypertension by Injection of Finely Divided Silica into Renal Artery.* (20961)

CAMPBELL MOSES.

From the Addison H. Gibson Laboratory, University of Pittsburgh School of Medicine.

The frequent occurrence of sustained hypertension in the course of chronic renal disease and the need for a procedure to produce experimental hypertension by an intrarenal lesion occasioned this study. The development of marked fibrosis as a reaction to silica in the liver(1-3) indicated that a similar technic applied to the kidney might produce sustained hypertension.

Methods. Mongrel dogs prepared with an exteriorized carotid artery to permit the ready determination of arterial systolic pressure in the unanesthetized state were kept in the laboratory under uniform conditions. The systolic blood pressure was determined by palpation of the exteriorized carotid distal to an occluding pneumatic cuff at twice weekly intervals for 10 months. After preliminary trials in 24 dogs had established suitable technic and dosage, 5 dogs anesthetized with

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