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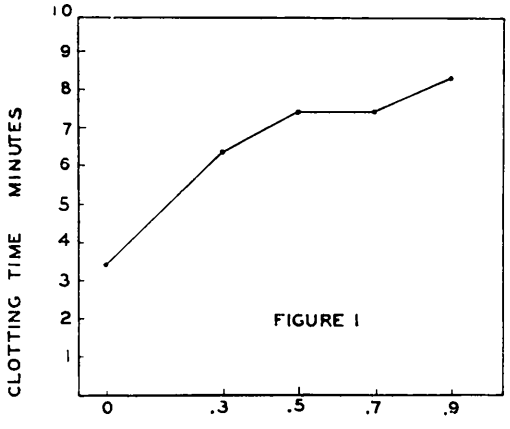
Platelet Fragility in Man.

LEWIS R. DAY. (Introduced by Henry N. Harkins.)
From the School of Medicine, Johns Hopkins University.

For many years it has been known that a certain strain of swine demonstrates an abnormality of blood coagulation similar to that found in human hemophiliacs. The genetics of this abnormality and its mechanism have been extensively studied.[1-3]

Working with this strain of swine, Muhrer, Bogart and Hogan[4] have developed a method of testing platelet fragility. It is the purpose of the present study to determine (1) whether the method is applicable to the determination of platelet fragility in man, and (2) whether human platelets react similarly to those of swine.

The method resembles that used for testing erythrocyte fragility. Platelet-rich plasma is obtained by moderate centrifugation of whole blood, drawn with special precautions to minimize trauma to the formed elements. The blood is drawn through a petrolatum-lined No. 20 McCrae needle directly into cold parafinned centrifuge tubes containing sodium citrate. This plasma is added to various hypotonic saline solutions, recalcified, and the time of coagulation measured. It is assumed that the mechanism of coagulation is the classical 2-stage process postulated by most workers in the field, and that coagulation time is quantitatively related to the speed of thrombin formation, which is in turn directly dependent upon the amount of thromboplastin released by platelets. One assumes further that if platelet-rich plasma exposed to hypotonic solutions clots faster than normal plasma, this is due to a more complete lysis of platelets. Conversely, if 2 different specimens, after similar exposure to saline solutions of the same tonicity, have different clotting times, there is presumably a difference in platelet fragility.



Effect of saline solutions on clotting time of normal platelet-rich plasma; reaction taking place in 0.9% saline.

Critique of Method. (1) To determine whether the effect of the hypotonic solutions was on some phase of the clotting mechanism other than the platelets, the specimens, after exposure to the various saline solutions, were all brought up to 0.9% NaCl, prior to recalcification. Under such circumstances, clotting occurred in a normal saline solution. As is shown in Fig. 1, the specimens exposed to hypotonic solutions clotted faster, even though clotting took place in a normal saline solution; and the more hypotonic the solution, the faster was the coagulation on eventual recalcification. The longer the time of exposure to the hypotonic solution, prior to recalcification, the more coagulation was accelerated. (Fig. 2). This suggests that, as in the case of erythrocytes, platelets do not disintegrate all at once, but are slowly lysed over a period of about 15 minutes.

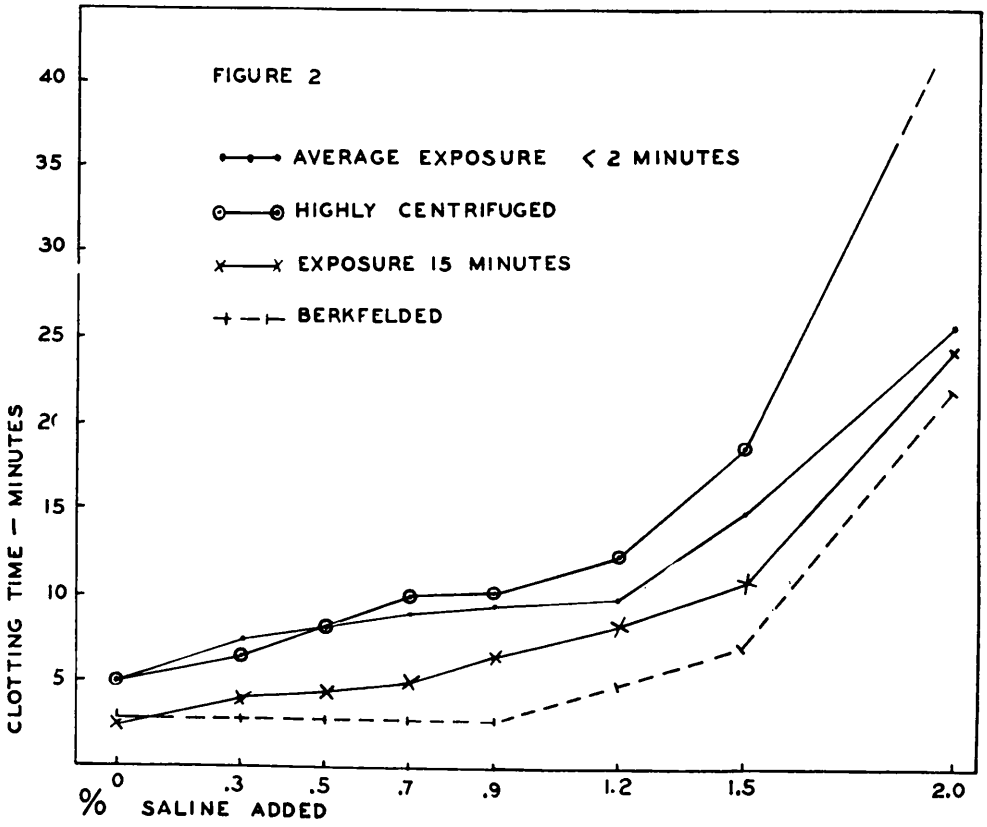
Similar results were obtained with highly centrifuged specimens (Fig. 2). Although smears of these plasmas showed no cells, the

1 Hogan, A. G., Muhrer, M. E., and Bogart, R., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 217.

2 Bogart, R., and Muhrer, M. E., *J. Hered.*, 1942, **33**, 59.

3 Muhrer, M. E., Hogan, A. G., and Bogart, R., *J. Physiol.*, 1943, **136**, 355.

4 Muhrer, M. E., Bogart, R., and Hogan, A. G., *Am. J. Physiol.*, 1944, **141**, 449.



Effect of saline solutions on clotting time of normal platelet-rich, platelet-poor, and platelet-free plasma and the effect of varying the exposure time; reactions taking place in solutions of varying tonicity.

results were essentially the same. However, it must be assumed that they contained platelets, since when specimens were passed through a Berkefeld N filter, exposure to hypotonic solution no longer affected clotting time significantly (Fig. 2).

These data suggest that the method as described by Muhrer does test the resistance of the platelets to hypotonic saline solution.

Platelet Fragility in Man. (2) The results with several normal human specimens are shown in Fig. 3. These were taken from normal healthy medical students and from white male patients, all of whom had apparently normal clotting mechanisms.

Specimens were also drawn from a known hemophiliac who is 40 years old and has been treated at the Johns Hopkins Hospital since the age of 5. These results are shown in Fig. 3. The curves are similar to those obtained in hemophilic swine, the difference being that in the latter the clotting time was reduced to normal levels by exposure to distilled water, while in man the clotting time was shortened, but not to normal levels.

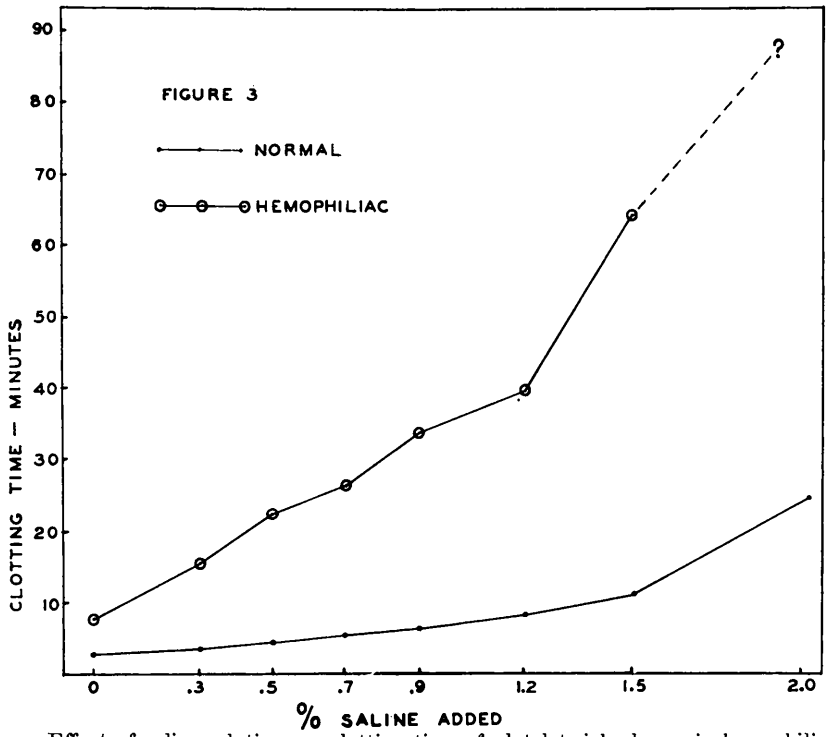
Discussion. Extensive studies of the clotting mechanism in normal humans and hemophiliacs to determine the fundamental abnormality have evolved several theories.[5-8] The present study on the resistance of normal and hemophilic platelets is offered as additional but not conclusive evidence that the difference between hemophilic and normal blood clotting lies in the platelets and not the plasma. Further study is necessary,

[5] Minot, G. R., and Lee, Roger I., *Arch. Int. Med.*, 1916, **18**, 474.

[6] Howell, W. H., and Cekada, E. B., *Am. J. Physiol.*, 1926, **78**, 500.

[7] Patek, A. J., and Stetson, R. P., *J. Clin. Invest.*, 1936, **15**, 531.

[8] Kark, R., *Clinics*, 1943, **2**, 15.



Effect of saline solutions on clotting time of platelet-rich plasma in hemophilia as compared with normal; reactions taking place in solutions of varying tonicity.

however, for it is entirely possible that the increased lysis of platelets by supplying an excess of thromboplastin serves to counteract some other deficiency or excess in hemophilia.

As suggested by Muhrer, one of the chief drawbacks in the testing of substances active in reducing clotting time in hemophilia is the lack of suitable experimental animals. These findings with human blood are sufficiently close to those obtained with the blood of hemophilic swine to suggest the possibility that study of the latter may throw light on the human disease.

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Present address: Presbyterian Hospital, Chicago.

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Effect of 2-Methyl-1,4-Naphthoquinone on Glycolysis of *Schistosoma mansoni*.*

ERNEST BUEDING, LAWRENCE PETERS, AND JEAN F. WAITE.

From the Department of Pharmacology, Western Reserve University School of Medicine, Cleveland.

Earlier observations indicated that oxidative metabolism is not essential for the survival of *Schistosoma mansoni*.[1] Since schistosomes have a high rate of both anaerobic

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[1] Peters, L., Welch, A. D., and Bueding, E., unpublished data.