animals made hypertensive by partial renal artery occlusion has previously been reported (12). The present investigation further suggests that the kidney is involved in the etiology of experimental renal hypertension at a metabolic level. The exact mechanism whereby the kidney produces its action cannot be determined from this study.

The endogenous dehydrogenase enzyme activity of the heart was raised in the presence of hypertensive blood pressure levels. This appears to have some relationship to the degree of blood pressure attained. Conversely reserpine lowered this activity significantly. Reserpine has been shown to produce a reduction in amplitude and frequency of contractions in the isolated cat and rabbit hearts (13). Thus the dehydrogenase enzyme activity may be a reflection of the work being done by the heart. This would account for the increased enzyme activity of the hypertensive heart. Whether reserpine produces this reduced enzyme activity in the heart directly or indirectly necessitates further investigation. Reduced enzyme activity resulting in reduced cardiac amplitude and frequency of contractions could possibly contribute to the hypotensive action of this substance.

Summary. 1) The endogenous dehydrogenase enzyme activity, as measured by the tetrazolium technic, was determined in kidney, adrenal, thyroid, pituitary and heart tissue from untreated and reserpine treated hypertensive animals. Experimental renal hypertension was produced in rats by 3 different methods. 2) A reduction in endogenous dehydrogenase enzyme activity of kidney tissue was observed in hypertensive animals while the activity of cardiac muscle was increased. Treatment with reserpine resulted in a depression of the endogenous dehydrogenase enzyme activity in cardiac tissue of both hypertensive and normal animals.

1. Braun-Menendez, E., *Stanford Med. Bull.*, 1952, v10, 65.
2. Wakerlin, G. E., *Physiol. Revs.*, 1955, v35, 555.
3. Goldblatt, H., *ibid.*, 1947, v27, 120.
4. Gross, F., Neolpp, B., Sulser, F., Doebelin, R., and Kundig, H., *Klin. Wchnschr.*, 1955, v33, 372.
5. Grollman, A., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 102.
6. Hartroft, W. S., and Best, C. H., *Brit. Med. J.*, 1949, v1, 423.
7. Page, I. H., *Science*, 1939, v89, 273.
8. Brecht, K., and Boucke, H., *Arch. Exp. Path. and Pharmakol.*, 1953, v217, 399.
9. Kun, E., and Abood, L., *Science*, 1949, v109, 144.
10. Black, M. M., Zweifach, B. W., and Speer, F. D., *Am. J. Clin. Path.*, 1952, v23, 332.
11. Kivey-Rosenberg, E., Cascarano, J., and Zweifach, B. W., *Trans. N. Y. Acad. Sc.*, 1952, v17, 402.
12. Raska, S. B., *J. Exp. Med.*, 1945, v82, 227.
13. Tripod, J., and Meier, R., *Arch. Internat. Pharmacodyn. Therap.*, 1954, v97, 251.

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Clotting of Hemophilic Blood with Purified Platelet Cofactor I, Platelet Factor 3 and Threone.* (23299)

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When blood clots a large number of substances are involved in many chemical interactions. These are as numerous and complex as intracellular processes, and one way to satisfy our curiosity about them is to study each chemical reaction separately. Before that can be done the substances involved must first be obtained in purified form. With that accomplished there is an additional opportunity in finding out what happens when such substances are added to blood as found in the

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veins of the average person or of one with a bleeding or thrombosing tendency. Two substances of particular interest in the clotting of blood are platelet factor 3 and platelet cofactor I. Both have recently been obtained in purified form(1,2). One is a lipoprotein of platelets and the other a plasma protein. The two together in solution, but still as unaltered independent entities, are by definition called threone(3). The activity is concerned with conversion of prothrombin to thrombin and can be represented by equation:

$$\text{Prothrombin} \xrightarrow[\text{Ac-globulin}]{\text{Ca}^{++}\ \text{Threone}} \text{Thrombin}$$

Threone activity is not found in serum even if platelet factor 3 is supplied. This is explained on the basis that platelet cofactor I combines with an inhibitor during the clotting of blood. Ether extraction presumably removes this inhibitor to set the platelet cofactor I free; for, ether extracted serum *does* have platelet cofactor I activity(4). The low threone activity of hemophilia A can also be increased by extracting the plasma with ether. This is consistent with the view that an inhibitor retards the function of platelet cofactor I in clotting of hemophilic blood. From this viewpoint we wondered what would be observed upon adding purified platelet cofactor I or purified platelet factor 3 to the whole blood of hemophiliacs.

Methods. Platelet factor 3: This was purified from bovine plasma and assayed as described by Alkjaersig, Abe and Seegers(1). *Platelet cofactor I*: This was purified from bovine plasma as described by Seegers, Landaburu and Fenichel(2). The preparation had a high activity and with the ultracentrifuge, products of this kind develop virtually a single boundary pattern. *Hemophilic blood and plasma*: Siliconed syringes and glassware were used throughout. Blood was drawn with 2 syringe technic. The plasma was obtained by centrifugation of blood mixed with 19% sodium citrate in the proportion of 49 to 1 respectively. The hemophiliacs were carefully studied in extensive laboratory tests and clinical observations and typed and graded in accordance with previously described criteria.

TABLE I. Clotting Time of Blood in Seconds at 37°C, and in Siliconed Tubes.

Patient	Whole blood	Whole blood plus Saline*	Whole blood plus Co-factor I†	Whole blood plus Platelet factor 3‡
Hemophilia A				
Grade 3	10,000	8,500	1,475	<410
" 1	7,150	5,050	1,575	195
" 2 to 3	20,000	20,000	620	185
" 2	12,600	9,600	1,075	270
Control	1,200	1,080	150	175

* .1 ml 0.9% NaCl plus 1 ml blood.
† Five mg platelet cofactor I dissolved in 1 ml 0.9% NaCl. Take 0.1 ml plus 1 ml blood.
‡ Purified platelet factor 3 in conc. of 720 units per ml. Take 0.1 ml plus 1 ml blood.

Grade 1 is the least severe disease, 2 and 3 more severe and 4 the most severe. *Clotting tests*: These tests are outlined in footnotes to the 2 tables.

Results. Whole blood clotting time is somewhat longer than blood diluted simply with saline (Table I). The clotting time was however, much shorter when the saline contained purified platelet cofactor I or purified platelet factor 3. Each of these factors added separately thus accelerates the clotting of normal blood. With all of the blood samples obtained from the hemophiliacs the whole blood clotting time was between 2 and 5 hours even on slight dilution with saline. With the addition of platelet cofactor I clotting time was approximately equal to that of normal whole blood, but not at all equal to that of normal blood to which platelet cofactor I had also been added. Purified platelet factor 3, in the selected concentration, was far more effective than platelet cofactor I. In fact, in certain instances, the clotting times were almost as low as with normal blood.

In the recalcified clotting time experiments (Table II) practically all of the results could have been predicted from the work with whole blood. The platelet factor 3 was most effective and purified platelet cofactor I reduced clotting time to normal; that is, to what the normal is without added cofactor I. One of the clotting times with cofactor I was quite long. A mixture of platelet cofactor I and platelet factor 3 was made so that the concentration of each would be exactly half of that used in other experiments. Thus the

TABLE II. Recalcified Plasma Clotting Times, in Seconds at 37°C, and in Siliconed Tubes.

Patient	Plasma only	Plasma* plus Platelet factor 3†	Plasma* plus Platelet cofactor I‡	Plasma* plus Threone §
Hemophilia A				
Grade 3	>20 hr	98	1,500	141
" 1	"	98	1,530	144
" 2 to 3	"	97	1,090	128
" 2	"	115	6,700	205
Control	1,120 sec.	82	125	47

* .1 ml of .1 M $CaCl_2$ plus .5 ml plasma.

† In .1 M $CaCl_2$ platelet factor 3 in conc. of 360 units/ml. Take .1 ml of this platelet factor 3 solution plus .5 ml plasma.

‡ .5 mg platelet cofactor I in .1 ml of .1 M $CaCl_2$ plus .5 ml plasma.

§ In .1 M $CaCl_2$ 360 units platelet factor 3/ml plus equal vol of 5 mg platelet cofactor I dissolved in .1 M $CaCl_2$. Take .1 ml of this mixture plus .5 ml plasma.

concentration of each was reduced by 50%, but both were present simultaneously. Hence we had threone. The clotting time found was below that with platelet cofactor I alone, but not as low as with platelet factor 3. A comparatively low clotting time was observed with the normal plasma.

Discussion. Recalcified clotting times after addition of our purified platelet cofactor I, platelet factor 3 or with threone have not been measured previously. There are variations in the figures obtained, that arouse curiosity and it would be most interesting to extend a study of this kind to include numerous hemophiliacs and individuals with thrombosing tendencies. Quite possibly such simple empirical tests would uncover helpful correlations not detected by other tests. We can readily see that hemophilic blood and plasma are antagonistic to cofactor I, platelet factor 3 and threone, in accord with the fact that in hemophilia A an inhibitor of the first phase of clotting is present in excess(5).

Perhaps occurrences in test tubes cannot serve as a basis for accurate predictions of therapeutic effectiveness *in vivo*. There are many substances (tissue thromboplastin, urine concentrates, fresh serum, thrombin) which when added to hemophilic blood will reduce its clotting time. These materials act probably by bypassing the fundamental defect, and the substances here described may well belong in this group. However, large quantities of platelet cofactor I given intravenously might be of help for short periods of time in hemophilia. Favorable results would be more likely with platelet factor 3 or with threone. In the purity and concentration used by us these are powerful coagulants.

Summary. Purified platelet cofactor I, purified platelet factor 3 or the two together reduce the clotting time of hemophilic blood or recalcified plasma; but, not to the degree observed with normal blood or plasma. In the concentrations used platelet factor 3 was more effective than platelet cofactor I.

1. Alkjaersig, N., Abe, T., and Seegers, W. H., *Am. J. Physiol.*, 1955, v181, 304.

2. Seegers, W. H., Landaburu, R. H., and Fenichel, R. L., *ibid.*, in press.

3. Murray, M., Johnson, S. A., and Seegers, W. H., *Am. J. Physiol.*, 1954, v178, 10.

4. Johnson, S. A., and Seegers, W. H., *J. Appl. Physiol.*, 1954, v6, 429.

5. Tocantins, L. M., *Blood*, 1954, v9, 281.

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