large numbers of compounds across the tubular epithelium. Most recently Margoshes and Vallee(9) have reported the isolation of a cadmium-containing protein from the kidney cortex of the horse, thus furnishing more evidence that the presence of cadmium in the renal cortex reflects a biological need. It may be postulated that a possible function of cadmium in the kidney is concerned with water reabsorption, since mercury, a closely related element in physico-chemical properties, is known to inhibit water reabsorption in nontoxic doses.

Summary. Using radioisotopes another element, cadmium, has been found to selectively accumulate in one tissue, the kidney cortex of the rat, in concentrations sufficient to warrant its use as a research tool. The cortex of the young rat, which contains only one-third the number of nephron units found in the adult, is unable to concentrate Cd^{115} at the adult level. The studies reported indicate a possible physiological role of cadmium in renal function.

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Received October 7, 1957. P.S.E.B.M., 1957, v96.

Differentiation of Antiheparin and Thromboplastin-Generating Factors in Human Platelets.* (23620)

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Several authors(1,2,3,4) have suggested that platelets contain a factor which antagonized the effect of heparin in delaying blood coagulation. It has also been suggested that this factor is not the same as the thromboplastin-generating factor (Factor 3, of Seegers) on the basis of differential ultracentrifugation.

Materials. Platelet factors. Lyophilized human platelet material (LPM), prepared by the method of Klein *et al.*(5) was extracted with alcohol-ether (3:1 v/v) and the thromboplastin-generating material precipitated by adding three volumes of acetone in the cold. The precipitate was suspended in a small volume of water and lyophilized. This lyophilized platelet material (platelet lipid) was suspended in imidazole buffer pH 7.4 to a concentration of 1.0 mg/ml. This is the platelet lipid extract containing thromboplastin-generating activity(6). The residue of platelets after lipid extraction (lipid extracted platelet material) was freed of organic solvents and resuspended in imidazole buffer to a concentration of 3 mg/ml unless otherwise stated. *Brain lipid extract.* This was the alcohol-ether soluble acetone precipitated cephalin fraction of beef brain(7). Commercially available heparin sodium,‡ bovine fibrinogen (Fraction I§), and Thrombin Topical‖ were used.

Methods. The different materials were

* This investigation was supported in part by the Atomic Energy Contract AT(30-1), and The Children's Cancer Research Fn.

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‖ Parke Davis and Co., Detroit, Mich.

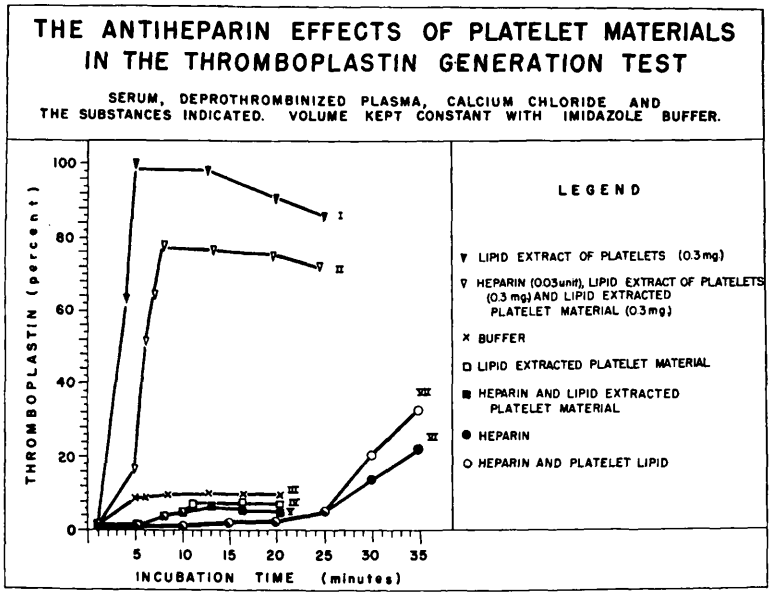

FIG. 1. Antiheparin effects of platelet materials in the thromboplastin generation test. .3 cc serum (dil. 1:10). .3 cc deprothrombinized plasma (dil. 1:5). .3 cc $CaCl_2$. .1 cc respectively of: (a) lipid extract of platelets (.3 mg/cc) of imidazole buffer; (b) heparin (.03 unit/cc); (c) LPM (.3 mg/cc of imidazole buffer). Volume kept constant at 1.2 cc with imidazole buffer.

tested in several systems to determine their effects on activity of heparin at different stages in the coagulation process. These included a simplification(8) of the thromboplastin-generation test(9), thrombin clotting times with plasma and fibrinogen(10), and recalcification times(11). To show the general property of platelets in removing anticoagulant activity from a solution of heparin, 6 mg of LPM were added to 1 cc of imidazole buffer containing 0.1 unit of heparin. The mixture was allowed to stand at room temperature for 5 minutes, then centrifuged at 30,000 g for one hour. The supernatant was used in the thromboplastin-generation test in comparison with the sediment, with the same solution of heparin, and with a suspension of platelets in buffer treated similarly. Lipid-extracted LPM was also tested in the same way. The thromboplastin-generation test was used to investigate the antiheparin property of LPM as compared to fresh platelets in more detail, as follows: first, 0.1 ml of 0.3 unit/ml of heparin and of heparin incubated with LPM, were added to the incubation mixture at the beginning of the experiment to examine its effect on thromboplastin production. Various amounts of heparin were used in the thrombin and recalcification times according to the sensitivity to heparin of the plasma employed.

Results. In the simple test of the property of platelets to remove heparin activity from a solution, it was found that 6 mg of LPM or lipid-extracted platelets completely neutralized the effects of 0.1 unit of heparin, similar to equivalent amounts of fresh platelets. The results of the more detailed studies are shown in Fig. 1 and 2.

(1) *Thromboplastin-generation test.* Fig. 1 shows the results of a representative system of tests expressed as percentage of thromboplastin formed at different periods of incubation. Comparison of the curves shows the following: thromboplastin was rapidly generated when the lipid extracted from platelets was used (Curve I). Only a small amount of thromboplastin was very slowly formed when heparin (0.03 unit) was added to this system (Curve VII). Formation of thromboplastin was restored to nearly normal by the addition of lipid-extracted platelet material to the system (Curve II). This lipid-extracted material did not, itself, have any thromboplastin-gen-

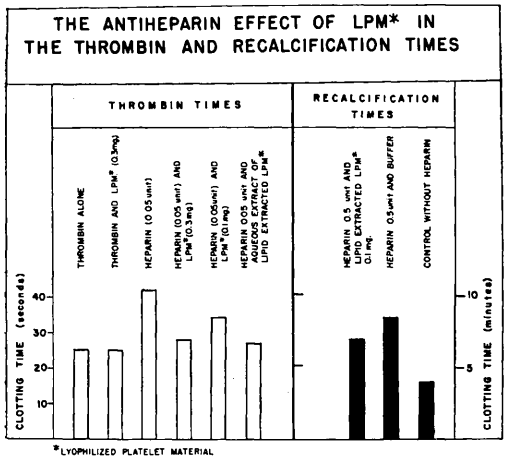

FIG. 2.

erating potency, the curve obtained when it, alone, was added to the system (Curve IV) being similar to that obtained when buffer was used (Curve III). However, when used alone, the lipid-extracted platelet material was partially effective in neutralizing heparin in the earlier stages of incubation (Curve V compared with Curves VI and III). Similar results were obtained when brain lipid extract was used in place of the platelet lipid in these experiments. A combination of brain lipid extract and lipid-extracted platelets reversed the effects of heparin. Whole platelets have the same antiheparin effect as the combination of brain lipid and lipid-extracted platelet material. Increasing the concentration of brain lipid extract did not, by itself, reverse the action of the heparin.

(2) *Thrombin clotting times.* Results in these tests are shown in Fig. 2, and demonstrate phenomena essentially similar to those observed in the thromboplastin-generation test. It will be seen that platelet materials do not have any accelerating effect on the thrombin time alone, but that in suitable concentrations they are able to antagonize the effect of heparin on it. Similar results were obtained in studying the reversibility of the effects of heparin on the clotting time of recalcified plasma. The corresponding data in Fig. 2 represent the averages from over 200 individual determinations on the plasma samples obtained from 58 normal donors.

Summary. Lyophilized human platelet material can be separated into thromboplastin-generating and heparin-antagonizing fractions. The heparin-antagonizing fraction does not, in itself, possess any thromboplastic activity but it reverses the effects of heparin on thromboplastin formation and thrombin activity. The material responsible is present in an aqueous extract of lipid-extracted platelets.

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Received May 31, 1957. P.S.E.B.M., 1957, v96.