a biphasic response with DCA, which elicited a net loss of sodium in adrenalectomized rats. The data of Forsyth(10) suggest the strong influence of this phenomenon with DCA, by showing increased excretion with small doses, and retention with larger doses in adrenalectomized mice.

Summary. 1. The influence of time and dosage upon sodium excretion by rats was investigated after cortisone and hydrocortisone administration. 2. Results of time-response studies show that large doses cause initially weak and transient retention of sodium and subsequently strong excretion of the ion. Smaller doses reduce progressively duration of retention phase and hasten onset of secondary diuresis. 3. Two factors appear to influence the response of sodium excretion. During a fixed time-interval of urine collection, increasing doses cause excretion, no change and retention of sodium. Similar changes in excretion of the ion are seen with a given dose when collection interval is progressively shortened.

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Comparison of Tissue and Plasma Thromboplastic Activities.* (23052)

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Since introduction of the thromboplastin generation test by Biggs and Douglas(1) a great many studies have been carried out dealing with defective formation of "plasma thromboplastin" in various coagulation abnormalities. This plasma thromboplastin is defined as activity developed in a dilute mixture of adsorbed plasma ($Al(OH)_3$ or $BaSO_4$), serum, platelets and calcium. When samples of such a mixture, together with additional calcium, are added to normal plasma "thromboplastic" activity develops during 5-minute incubation as clotting times decrease from over a minute to approximately 10 seconds. Relatively little attention has been paid to the activity of fully formed normal plasma thromboplastin in the clotting mechanism. A distinct difference between plasma and tissue thromboplastin was demonstrated by Gollub, Ulin and Black who showed that the bacterial enzyme thromboplastinase would inactivate tissue thromboplastins, but not plasma thromboplastin, although it did have some inhibitory effects in early stages of plasma thromboplastin generation.

The simple experiments presented in this study show certain striking differences between tissue and plasma thromboplastin.

Materials and methods. Tissue thromboplastin: Human Brain: 300 mg acetone-dried human brain powder suspended in 5

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ml 0.85% sodium chloride, and incubated 10 min. at 37°C. The material was allowed to sediment for 30 min. and the supernatant employed. Rabbit Tissue: Simplastin (kindly supplied by Warner-Chilcott), used as directed, without additional calcium. *Plasma thromboplastin*: 1 ml normal platelet suspension plus 1 ml normal $BaSO_4$ plasma (dil. 1-5) plus 1 ml normal oxalated serum (dil. 1-10) plus 1 ml 0.025 M $CaCl_2$. All tests were carried out in 15 min. after the mixture had been allowed to activate for 8 min. The same clotting time (10 sec.) on normal citrated plasma was obtained at beginning and end of experiment. *Test plasmas*: Unless otherwise stated all normal and patients' plasmas were citrated and had been stored frozen 2 weeks to 6 months. The PTA deficient plasma was obtained from patient previously described (A. A.(2)) who was seen through the courtesy of Dr. Robert L. Rosenthal. The Hageman factor deficient plasma was obtained from a patient, also previously described (M.R.(3)), and studied through the courtesy of Dr. Oscar Ratnoff. The proaccelerin deficient plasma was from a girl previously described (D.H.(4)). The proconvertin deficient plasma, lacking "Stuart factor," was a lyophilized preparation supplied through the kindness of Dr. John Graham. This patient has been described by one of us(5) and by Dr. Graham(6). The plasma designated Dicumarol was from a 63-year-old man on long-term, poorly controlled therapy who showed hemorrhagic symptoms. The patient with vit. K deficiency was a 4-month-old infant with hemorrhagic symptoms one month and signs of intracranial hemorrhage 2 days. A prompt response followed vit. K therapy. The patient used as example of liver disease suffered from severe post-hepatitic cirrhosis confirmed at autopsy. The heparinized plasma was obtained from normal subject one hour after 50 mg heparin was administered i.v. Recalcification times were carried out in duplicate by adding 0.1 ml thromboplastin or saline, simultaneously with 0.1 ml 0.02 M $CaCl_2$ to 0.1 ml plasma at 37°C. Results of other test procedures are from the initial patient workup by our standard methods(7,8).

TABLE I. Recalcification Times.

Type of plasma	Recalcification times (sec.) in presence of Saline	Rabbit tissue	Human brain	Plasma thromboplastin
Normal	145	12.2	15.0	10.0
" platelet-poor	390	12.0	14.2	10.0
AHF Deficient	1,350	12.2	14.2	9.8
PTC "	1,620	12.0	17.5	10.1
PTA "	570	12.0	15.1	10.4
Hageman " factor	960	13.0	16.2	9.8
Proaccelerin "	820	32.2	70.0	9.3
Proconvertin " (Stuart)	745	53.6	117.0	9.2
Mixed deficiencies				
Dicumarol	695	44.5	98.5	39.5
Vit. K deficient	3,600	33.0	69.0	32.0
Liver disease	695	30.8	60.2	16.8
Normal— heparinized	>1,000	14.8	22.2	40.0

Results. The Table shows recalcification times of various plasmas in the presence of saline, rabbit tissue, human brain and plasma thromboplastins. All saline times are significantly longer than that of normal plasma indicating that platelets, AHF (anti-hemophilic factor), PTC (plasma thromboplastin component), PTA (plasma thromboplastin antecedent), Hageman Factor, proaccelerin and proconvertin (Stuart) are all factors essential to normal coagulation. This was borne out by initial examinations on these patients, all of whom showed abnormal thromboplastin generation curves as well as other coagulation abnormalities. The patients suffering from mixed deficiencies: dicumarol intoxication (prothrombin 2%, proconvertin 1%, PTC 20%), vit. K deficiency (prothrombin 5%, proconvertin 5%, PTC 15%) and hepatic cirrhosis (prothrombin 26%, proconvertin 3%, proaccelerin 39%, and PTC 10%), also showed abnormal thromboplastin generation curves on the initial examinations and prolonged recalcification times.

Human brain and rabbit tissue thromboplastin times were normal in platelet, AHF, PTC, PTA and Hageman factor deficiencies, but markedly prolonged in proaccelerin, proconvertin and the mixed deficiencies, and in

heparinized plasma. The plasma thromboplastin times were normal in all except the mixed deficiencies in which prothrombin itself was low, and in heparinized plasma.

In another experiment plasma thromboplastin was prepared as usual and then centrifuged at 20,000 g for 30 min. When the sediment was resuspended in fresh saline and calcium, it had almost all the activity of the original mixture, when tested against either normal or deficient plasmas. If the sedimented material was suspended in saline, without calcium, no clot was formed when it was added to plasma but a clot did form when additional calcium was simultaneously added.

Discussion. From the foregoing experiments it appears that "plasma thromboplastin" is a complex, sedimentable at high speed centrifugation, separable from calcium ion, inhibitable by heparin, which can substitute for the activities of platelets, AHF, PTC, PTA, Hageman factor, proaccelerin and proconvertin (Stuart), and which, in the presence of calcium, appears to convert prothrombin to thrombin directly. This latter premise is based upon the observation that only the plasmas low in prothrombin gave prolonged plasma thromboplastin times. Recent experiments suggest the possibility that the SPCA deficient patient of Alexander(9) and the hypo-proconvertinemic patient described herein and previously(5), deficient in the so-called "Stuart Factor" (Hougie and Graham(6)), may suffer from different deficiencies although both show prolonged prothrombin times and abnormal proconvertin levels as assayed by the Owren method. Alexander's patient is said to have a normal thromboplastin generation, and therefore it is possible that this factor is not necessary for plasma thromboplastin generation, but acts at a later stage. If this is the case it would seem likely that this patient's plasma might have a prolonged plasma thromboplastin time.

Brain thromboplastin and plasma thromboplastin are similar in their abilities to shorten recalcification times in platelet, AHF, PTC, PTA and Hageman factor deficiencies. Both give long recalcification times in the presence of prothrombin deficiencies. Both appear to be inhibited by heparin, although these experiments do not clearly differentiate this effect from an anti-thrombic one. Brain and plasma thromboplastin differ strikingly when tested against proaccelerin and proconvertin deficient plasmas, for only plasma thromboplastin is able to clot these plasmas in normal times. That this effect is not simply due to carrying over of these factors in plasma-thromboplastin mixture is shown by the observation that after high speed centrifugation and resuspension of the "plasma thromboplastin" it is still active in these deficiencies.

Summary. 1. A lengthening of saline-recalcification time beyond normal occurred in plasma deficient in any one of the following: platelets, AHF, PTC, PTA, Hageman factor, proaccelerin, or proconvertin. It also occurred in certain acquired multiple factor deficiencies, and in heparinized plasma. 2. Brain thromboplastin recalcification times were normal in platelet, AHF, PTC, PTA and Hageman factor deficiencies, but prolonged in proconvertin or proaccelerin deficiency or heparinized plasma. 3. Plasma thromboplastin recalcification times were normal in these same deficiencies and, in addition, in proconvertin or proaccelerin deficiency. This latter activity seemed due to incorporation of (pro) convertin and (pro) accelerin into the plasma thromboplastin complex, rather than to any free proconvertin and proaccelerin in solution. 4. Formed plasma thromboplastin is inactive in absence of optimal calcium concentration.

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Cardiac Retroperfusion with Induced Asystole for Open Surgery upon the Aortic Valve or Coronary Arteries.* (23053)

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The concomitant use of a pump-oxygenator (1,2) for circulatory support during total by-pass of the heart together with retrograde perfusion via coronary sinus permits open surgery on the ascending aorta and aortic valves for periods of 15 to 30 minutes. This technic has been tested experimentally and successfully applied subsequently in man(3,4). Retrograde perfusion via coronary sinus of arterial blood supports myocardial activity and prevents coronary air embolism during interval that the ascending aorta remains open. As a rule almost no coronary venous drainage occurs from the right ventricle into the coronary sinus in the dog. Therefore, virtually no retroperfusion of this myocardial area takes place. Despite this fact the canine right ventricle appears to tolerate 15 to 20 minutes of by-pass assisted by retroperfusion, because cardiac workload is almost completely eliminated, and the left ventricular muscle remains oxygenated. In man the anatomical pattern of coronary veins usually permits more adequate retroperfusion of the right heart through the coronary sinus. Previously we reported on retroperfusion technic (4). There were 12 long-term survivals among 16 dogs. submitted to this technic, for intervals of 15 to 20 minutes. Only one of 4 deaths was related to cardiac complication. Four additional animals were submitted to 30 minutes of retroperfusion. Three of them died apparently from irreversible fibrillation appearing at termination of the retroperfusion and presumably induced by intolerable state of right ventricular hypoxia. Blanco *et al.* (5) reported survivals in dogs after retroperfusion of coronary veins up to 7¼ minutes.

This duration of retroperfusion, safe for dogs and probably for man, must certainly be extended to 30 minutes or more if we are to realize its full usefulness. Therefore, we have tested the potential advantages of combining retroperfusion of the coronary sinus with asystole induced with potassium citrate, hoping thereby to reduce further myocardial oxygen consumption. Also, the advantages of working in a completely still operative field would be realized. And finally, both experimentally and clinically it has been found dangerous to attempt to re-start a deliberately arrested heart by forward coronary perfusion unless vents into the left ventricle and left atrium have been provided to keep those chambers decompressed. In patients without septal defects it may not be technically feasible to establish these vents; and therefore, a method for restoring the beat (by means of retroperfusion) with the aorta still open becomes valuable. The results of these studies comprise the subject matter of this paper.

Method of study. Forty dogs had their heart and lungs by-passed utilizing a pump oxygenator(1,2). They were subdivided into 4 groups. *Group I* consists of 10 dogs sub-

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