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A Method for Assay of Stuart Factor.* (23570)

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Stuart clotting defect was first described by Hougie, Barrow, and Graham in 1957(1). The name was applied after the surname of the patient in whom the deficiency was first identified, and who had previously been reported by Lewis, Fresh, and Ferguson(2) as a case of proconvertin deficiency. Although the patient showed the laboratory features of proconvertin deficiency, there was found to be cross correction of this patient with the original proconvertin deficient patient of Alexander, Goldstein, Landwehr, and Cook(3), indicating that the two patients were deficient in separate clotting entities. It was demonstrated, moreover, that this new factor was necessary not only for the action of blood thromboplastin but also for the action of tissue thromboplastin. It was present in serum, adsorbable by barium sulfate and Seitz filters, and relatively stable to heat and changes in pH. During early dicumarol therapy the concentration is relatively high, whereas later it is depressed. While in proconvertin deficiency the Russell viper venom time is normal(4), in Stuart deficiency the Russell viper venom time is prolonged.

The latter finding suggested that adsorbed beef plasma as prepared for the proconvertin test could be used as a substrate for the assay of Stuart factor if Russell viper venom was used as the thromboplastin. Consequently, the following experiments were performed using the proconvertin test as described by Owren and Aas(5) and modified by Adamis, Sise and Kimball(6).

Methods. The assay was performed in the same fashion as for proconvertin with the exception that Russell viper venom fortified with a lipoid extract of human brain (crude cephalin) was used as the thromboplastin. A chloroform extract of acetone dried human brain was prepared by the method of Bell and Alton(7). A dilution of one part of this crude cephalin preparation was made with 30 parts of veronal buffer pH 7.4, ionic strength 0.154.

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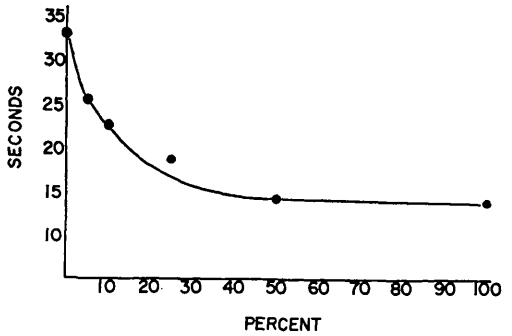

FIG. 1. Dilution curve showing clotting times in the assay system for various dilutions of pooled normal plasma in buffered potassium oxalate diluting solution. One hundred percent is a 1 to 10 dilution.

This diluted crude cephalin was used to dissolve the dried Russell viper venom (Stypven) in a strength of 1:40,000. The thromboplastin material thus prepared was frozen and stored at −20°C in aliquots sufficient for one day's use. The preparation is stable in the frozen state but deteriorates at room temperature sufficiently that refreezing for later use is not possible. A new dilution curve is necessary for each batch of viper venom cephalin mixture as well as reagent. Before recalcification of the mixture of reagent, 1:10 plasma dilution, and viper venom-cephalin mixture, it is allowed to incubate for 5 minutes. Conversion from clotting time of this mixture to percent normal is made on the basis of the standard dilution curve procedure.

Results. A sample dilution curve of a pooled sample of 5 normal plasmas is shown in Fig. 1. Although the clotting times do not show an ideally wide spread from the zero to 100% value, the reproducibility of the results have indicated that the test is accurate enough for ordinary purposes. Undoubtedly, Stuart factor is not completely adsorbed from the beef plasma reagent.

TABLE I. Clotting Times of the Assay System and Calculated Value of Stuart Factor in Various Plasmas and Sera.

Material	Clotting time, sec.	% normal
Normal plasma	14.0–16.5	100
Saline	30.3–42.5	0
Congenital proconvertin deficiency plasma	15.3	100
Congenital Stuart deficiency plasma	36	0
Congenital PTC deficiency plasma	16.2	80
Normal serum	15.1	100
Congenital Stuart deficiency serum	31.8	0
Congenital PTC deficiency serum	18.2	33

The clotting times and calculated value of Stuart factor for various plasmas are shown in Table I. We are indebted to Dr. John B. Graham for sending lyophilized samples of plasma and serum from the patient Stuart.

In Fig. 2 there is shown the response of Stuart factor and proconvertin to a dose of Warfarin, 300 mg intravenously, in 5 patients. The decrease in Stuart factor occurs gradually compared to proconvertin. This agrees with the findings of Hougie *et al.*(1).

The results indicate that the test is sensitive to Stuart factor, and insensitive to proconvertin and prothrombin. Owren and Aas(5) have previously shown the system to be insensitive to ac-globulin. It may be tentatively concluded that the test, therefore, is a measure of Stuart factor.

Discussion, Since in Stuart factor deficiency, the clotting time is prolonged with either

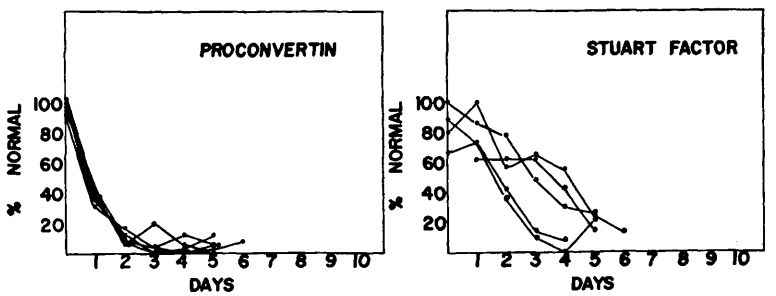

FIG. 2. Changes in proconvertin and Stuart factor after 300 mg of Warfarin intrav. in 5 patients.

tissue thromboplastin or viper venom, it is apparent that this factor is necessary for the action of both of these thromboplastic materials. It has been suggested that viper venom contains proconvertin or acts in place of proconvertin(4). It is also possible that there are two distinct systems for activation of the prothrombin conversion enzyme, one through the tissue thromboplastin route where proconvertin is necessary, and the other through the plasma thromboplastin route where proconvertin is not necessary. In this sense, proconvertin may be thought of as a true "cothromboplastin" but only for the tissue thromboplastin. Viper venom in this scheme appears to act in the same manner as plasma thromboplastin (platelets, antihemophilic globulin, PTC, PTA, and possibly other factors) for it does not require the presence of proconvertin for its normal action(8).

It seems probable from the findings that Hjort, Rapaport, and Owren's(9) method of assaying prothrombin by viper venom is also sensitive to Stuart factor.

Summary. The modification of Owren and Aas' test for proconvertin by substitution of viper venom-cephalin mixture for tissue thromboplastin is described as a method of assay for Stuart factor.

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Effect of Adrenocortical Hormones on Serum Proteins of Adrenalectomized Mice Infected with *M. tuberculosis*.* (23571)

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The electrophoretic distribution of serum proteins following experimental infections with *M. tuberculosis* has been studied in rabbits(1), guinea pigs(2-4), and rats(3). Also, the effects of adrenocortical hormone administration on serum protein patterns have been reported on rabbits(5,6), guinea pigs(7), rats(8), and dogs(6). It has been difficult to evaluate these results due to species variation, variation in severity and length of the infection, and differences in amounts and types of hormones administered.

This study was designed to investigate the effects of adrenocortical hormones on serum proteins of infected, adrenalectomized mice where there is no buffering effect on the intact adrenal gland, and to compare the effects of similar amounts of a glucocorticoid and a mineralocorticoid in this animal.

Materials and methods. Bilateral adrenalectomies were performed on 260 CFl albino mice. One hundred thirty of these mice and

* Hydrocortisone, desoxycorticosterone, and 2-methyl-9-fluorohydrocortisone were generously donated by Dr. Robert O. Stafford, Dept. of Endocrinology, Upjohn Co., Kalamazoo, Mich. Total protein determinations were performed by Jack A. Moorman and Jeanne LaCerte.

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