TABLE I. Reactions of Canine Erythrocytes with 2 Types of Coombs Sera on Representative Days following a Single Intravenous Dose of Acetylphenylhydrazine.

Dog #	1			2			3			4		
Day after phenyl-hydrazine	Het, %	Serum A*	Serum B†	Het, %	Serum A	Serum B	Het, %	Serum A	Serum B	Het, %	Serum A	Serum B
0	49	—		45	—	—	56	—	—	51	—	—
1	41	—	—	34	—	—	51	—	—	44	—	—
4	31	—	—	26	—	—	40	—	—	36	—	—
7	27	—	—	28	—	—	27	—	—	28	—	—
9	30	±	—	31	±	—	34	1+	—	30	—	—
11–14	39	—	—	37	—	—	42	—	—	38	—	—
15–18	42	—	—	37	—	—	43	—	—	41	—	—
19–24	49	—	—	47	—	—	51	—	—	46	—	—

Dog #	5			6			7			8	
Day after phenyl-hydrazine	Het, %	Serum A*	Serum B†	Het, %	Serum A	Serum B	Het, %	Serum A	Serum B	Het, %	Serum B
0	46	—	—	41	—	—	53	—	—	44	—
1	37	—	—	36	—	—	42	—	—	23	—
4	33	—	—	23	—	—	31	—	—	29	—
7	31	—	—	30	—	—	27	—	—	26	—
9	33	—	—	30	—	—	27	—	—	25	—
11–14	36	—	—	33	—	—	35	—	—	34	—
15–18	38	—	—	35	—	—	39	—	—	34	—
19–24	46	—	—	46	—	—	44	—	—	42	—

* Serum A: Antiserum against whole dog serum.
† Serum B: " " dog globulin (see text).

contradiction to the results of others.

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Effect of Russell's Viper Venom (Stypven) on Stuart Clotting Defect. (22823)

CECIL HOUGIE (Introduced by Cornelia Hoch-Ligeti)
Department of Clinical Pathology, University of Virginia, Charlottesville.

In a study of patient (R.S.) with congenital hemorrhagic disorder of moderate severity, Lewis, *et al.*(1) showed he had a prolonged one-stage prothrombin time which was corrected by normal plasma or serum but not by barium sulphate-adsorbed plasma or serum. The 2-stage prothrombin was normal and prothrombin consumption abnormal. These authors believed that the factor deficient in this patient was identical to that previously described by Alexander, *et al.*(2) Koller, *et al.*(3) and Owren(4) and now referred to as SPCA, factor VII or proconvertin (the 3 terms being used synonymously). It has now been established that factor VII (SPCA or proconvertin) is not essential for generation of blood thromboplastin(5,6). In a re-study of patient (R.S.) it has been found that the patient's serum is deficient in a factor essential for blood thromboplastin formation(7). Thus if various concentrations of normal serum in the patient's serum are prepared and incubated with normal $Al(OH)_3$-adsorbed plasma and platelets in the thromboplastin generation test, maximum yield of thromboplastin generated

is proportional to amount of normal serum in the mixture. The factor deficient in this patient therefore differs from factor VII. However the two factors appear to have many properties in common, both being relatively heat stable, present in serum as well as plasma and necessary for a normal one-stage prothrombin time(7).

Since Russell's viper venom (Stypven) completely corrects the prolonged one-stage prothrombin time of plasma deficient in factor VII(8,9) it appeared reasonable to determine whether Stypven would also correct the clotting defect of the patient (R.S.)

Materials and methods. Whole blood was collected in glass tubes, and allowed to clot and stand at 28°C for 24 hours before separating the serum. Plasma was obtained by adding 9 parts whole blood to 1 part 3.8% trisodium citrate and centrifuging at 3,000 rpm. Russell's viper venom (Stypven), obtained from Burroughs Wellcome and Co., was diluted with distilled water. Platelet suspensions were prepared from a normal subject by the technic used in the thromboplastin generation test(10). A 0.1% suspension of crude cephalin(11) in distilled water was used. Modified one-stage prothrombin times were determined by mixing 0.1 ml 0.1% cephalin suspension, 0.1 ml platelet suspension or fresh thromboplastin solution (Difco), 0.1 ml Stypven solution, 0.1 ml plasma and 0.1 ml 0.025M $CaCl_2$ in the order named.

Results. Effect of varying concentrations of Stypven on modified one-stage prothrombin time of patient's plasma. Various concentrations of Stypven were prepared ranging from 1 in 1,000 to 1 in 100,000 and the modified one-stage prothrombin time of normal or patient's (R.S.) plasma determined using Difco brain thromboplastin and each dilution of Stypven (Table I). It is to be noted that varying the concentration of Stypven did not result in any significant correction of the patient's prothrombin time, which remained between 2½ and 3 times that of normal control for the particular dilution of Stypven under test. Similar results were obtained if either a platelet suspension or a 0.1% suspension of crude cephalin was substituted for the brain thromboplastin used in the above experiment.

TABLE I. Effect of Concentration of Stypven on Modified One-Stage Prothrombin Time Using Difco Rabbit Brain Thromboplastin.

.1 ml plasma, .1 ml Difco brain thromboplastin, .1 ml of Stypven in conc. listed below, .1 ml 0.025M $CaCl_2$,	Clotting times, sec. Normal plasma	Patient's plasma
1 in 1,000	8.9	23.5
2,500	5.5	15
5,000	5	14.6
10,000	6.6	18.8
20,000	8.5	22
50,000	11	27
100,000	11.8	35
Saline control	13.8	42

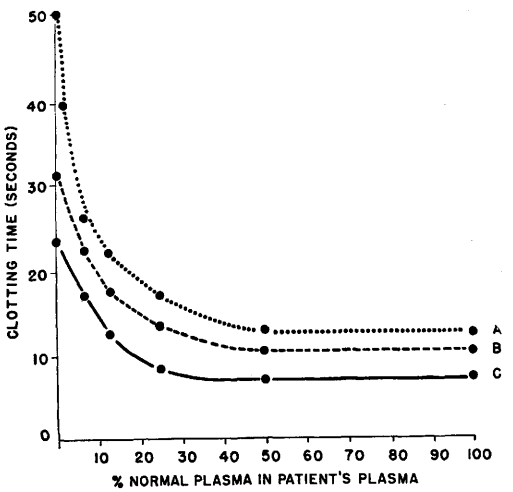

FIG. 1. Dilution curves obtained by determining one-stage "prothrombin" times of various dilutions of normal plasma in patient's (R.S.) plasma using, as sources of thromboplastin, a mixture of brain thromboplastin and (A) saline, (B) "Stypven" diluted 1 in 60,000 and (C) "Stypven" diluted 1 in 10,000.

Effect of diluting patient's plasma with normal plasma. Serial dilutions of patient's plasma in normal plasma were prepared and the modified one-stage prothrombin times of each dilution determined using Difco brain thromboplastin with either Stypven or saline (Fig. 1). Two concentrations of Stypven were used, 1 in 10,000 which was found to be the optimal dilution for the batch of Stypven used in this experiment and a 1 in 60,000 dilution. The result of such an experiment is shown in Fig. 1, where it can be seen that the

3 curves are roughly parallel: the one-stage prothrombin time and modified one-stage prothrombin, increasing in the same proportion when the concentration of normal plasma in the patient's plasma is diminished.

Discussion. It has already been shown that the patient (R.S.) is not deficient in either PTC or prothrombin and that mixtures of plasma from a case of congenital deficiency of SPCA (factor VII) and the patient's plasma are mutually corrective(7). The factor deficient in the patient differs from factor X since this factor affects the yield but not the rate of blood thromboplastin formation. The factor, deficient in the patient, also differs from AHF and factor V which are not present in normal serum and from the Hageman factor(12), PTA(13) and the fourth thromboplastin factor(14) since deficiencies of these factors do not affect the one-stage prothrombin time.

Telper, *et al.*(15) have recently described a patient with a congenital hemorrhagic diathesis due to deficiency of a factor which resembles factor VII but is necessary for blood thromboplastin formation. This factor, which is referred to as the Prower factor, resembles that deficient in our patient. The clotting defect is, however, completely corrected by Stypven while the present results show clearly that the clotting defect in the patient (R.S.) is not corrected by Stypven. This difference cannot be explained on a technical basis as one of the technics used in the present work was the same as that employed by Telper, *et al.*, and similar results were obtained using modifications of this technic. Moreover the difference is not merely a quantitative one for the patient of Telper, *et al.* had a one-stage prothrombin time which was more than twice the normal control indicating that the clotting deficiency was a moderately severe one. The two clotting defects must therefore be distinct and the factor deficient in the patient (R.S.) is referred to as the Stuart factor after the patient's surname.

The possibility that the patient (R.S.) is deficient in two factors has to be considered. A multiple congenital defect would imply that the patient was deficient in the Stuart factor and one or another *new* clotting factor including possibly the Prower factor since a deficiency of all the other known coagulation factors including Christmas factor (PTC) has been excluded. Since 2 other unrelated patients suffering from congenital hemorrhagic diatheses have been studied and found to have identical clotting defects(1,7), the multiple deficiency hypothesis is highly unlikely. The probabilities of the same double homozygosity or homozygosity at one locus, and heterozygosity at the other occurring independently 3 times in a small population are exceedingly small. The possibility of a multiple congenital deficiency can therefore be discarded with a reasonable degree of safety.

The finding that Stypven does not correct the Stuart defect however, does more than establish its separate identity. It is of practical importance since a simple prothrombin assay method has been described in which it is assumed that the one-stage "prothrombin time" is a true measure of prothrombin when Stypven is added to the brain thromboplastin (16). Such an assay procedure however is a function of both prothrombin and Stuart factor. It has been pointed out elsewhere(7) that the usual assay procedures for factor VII (proconvertin) as described by Owren(17) and Koller *et al.*(3) measure both Stuart factor and factor VII. These asbestos filtered plasmas retain considerable prothrombin but are deficient not only in factor VII (proconvertin) as these workers have assumed but Stuart factor and probably Prower factor. However, the simple addition of Stypven to such substrate plasmas should allow these assay technics to measure specifically the Stuart factor.*

Summary. It is shown that Russell's viper venom (Stypven) does not correct the prolonged one-stage prothrombin time of patient with a congenital hemorrhagic diathesis previously thought to be deficient in factor VII. The factor deficient in this patient which is referred to as the Stuart factor, is readily distinguishable from both the Prower factor and factor VII since the prolonged prothrombin times of plasma deficient in either of these

* To be published.

two factors are completely corrected by Stypven. The finding that Stypven does not correct the clotting defect of Stuart factor deficient plasma implies that certain modified one-stage "specific" assay methods for prothrombin using Stypven as a source of factor VII are not, in fact, specific, and measure both prothrombin and Stuart factor.

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Oxygen Uptake and Lactate Formation of HeLa Cells.*† (22824)

H. J. PHILLIPS AND H. L. MCCARTHY‡ (Introduced by C. M. Wilhelmj)
Department of Physiology and Pharmacology, Creighton University School of Medicine, Omaha, Neb.

HeLa cells were obtained from an epidermoid carcinoma of the cervix and started as a tissue culture in 1951(5). After 4 years *in vitro*, smears stained by Papanicolou's technic (4) indicated that HeLa had malignant features. It has been grown by Toolan in cortisone-treated rats and in human subjects in terminal stages of another neoplastic process (5). The cells are uniform in appearance as compared to mixed cell types found in tissue. They can be easily separated without apparent injury. Cell number or weight can be determined quite accurately since there is little interstitial material. For these reasons HeLa makes an ideal cell suspension on which to study metabolism. Our primary objective was to compare metabolism of human HeLa cells to that reported characteristic of animal tumors(2). Originally Gey started HeLa in medium which contained human serum. Other laboratories§ adapted a line of HeLa cells to grow in medium containing horse serum. Our second objective was to compare metabolism of cells cultured in human serum‖ with that of cells cultured in horse serum.

Procedure. At start of this study 2 lines of HeLa were carried in medium containing 40% Earle solution, 20% chick embryo extract (50:50) and 40% horse or human serum. It was desired to carry one line of cells

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‡ Part-time Medical Student Research Fellow, U.S.P.H.S.

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