response could usually be detected since epidermal destruction was usually more advanced in the homograft from the red cell or first-set skin graft donor. The somewhat reduced survival time of unrelated homografts on immunized hosts may have been the result of a relatively small number of antigenic factors shared in common between the homografts(8). Autografts remained viable and apparently were not influenced by adjacent degenerating homografts. This observation parallels that of Eichwald and his co-workers(9).

Summary. Skin homografts were made between individuals of a single line of random-bred and between 3 strains of inbred rats. Graft survival was determined from histological sections of graft epidermis, and presence or absence of circulation. Survival time of skin homografts was reduced in individuals that had received a prior inoculation of packed erythrocytes.

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Isolation of Coagulation Factors by Continuous Flow Electrophoresis.* (25082)

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Continuous flow paper electrophoresis, recently(1) applied to study of blood coagulation proteins, achieved partial separation and recovery of these factors. The present studies used this technic, confirmed and extended the findings of Lewis, *et al.*(1), and provided new data on the Stuart, proconvertin, and Hageman clotting factors.

Materials and methods. The principles of continuous flow paper electrophoresis are described by Block, Durrum, and Zweig(2). The Beckman/Spinco model CP was used with barbital buffer pH 8.6, ionic strength 0.02, and 14°C. 15-20 ml samples, previously dialyzed overnight against the buffer at 4°C were fractionated by constant current of 40 milliamps which developed 500 volts. 32 fractions were collected during the usual run of 16-20 hours. At the end of this time the curtain was removed, dried 30 minutes at 110°C, and dyed with bromphenol blue. Protein in each sample was determined by a biuret method. Fibrinogen, prothrombin, proconvertin, PTC, AcG, and AHF were measured by standard assays(3). The last 2 factors, found labile under the conditions in early experiments, were excluded from the later routine. Hageman factor was estimated using Hageman deficient substrate in partial thromboplastin time test(4). Stuart factor was assayed by specific assay(5) which used a substrate deficient only in Stuart factor. *Refined preparations.* In many instances, fraction collecting tubes could be selected which contained only Stuart, proconvertin, or Hageman factor. These selected fractions were concentrated by overnight dialysis against 10% gelatin, which removed 25-40% of water and buffer salt. The dialysate was lyophilized and stored at −20°C until reconstituted with distilled water. To refine Hageman factor, the pH was adjusted to 5.2 with dilute acetic or hy-

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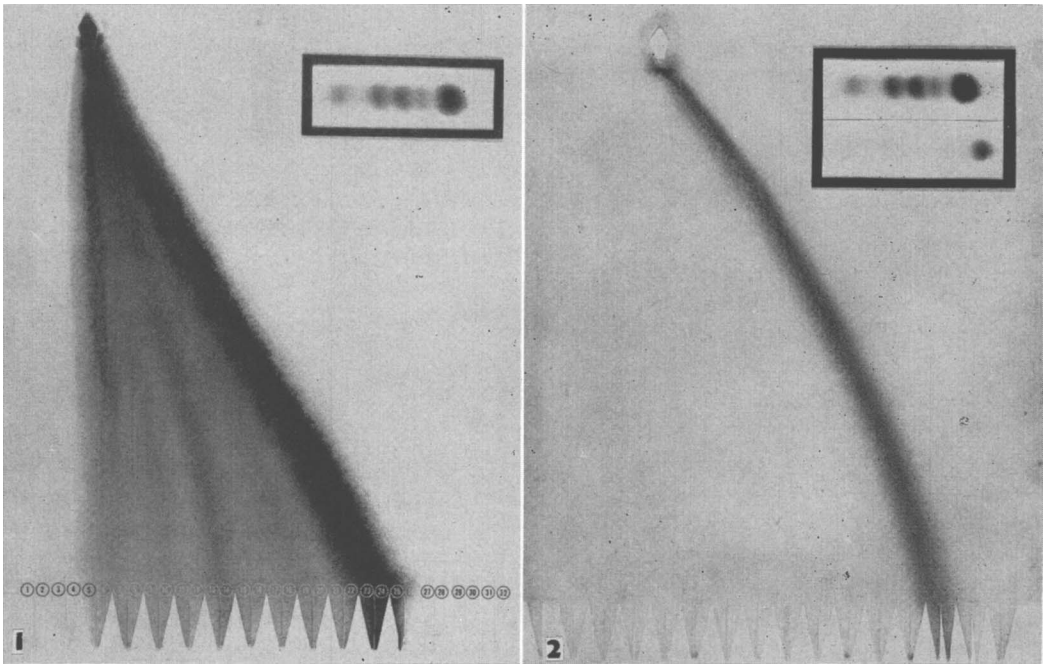

FIG. 1. Normal plasma continuous flow electrophoresis pattern with paper strip pattern (inset) for comparison. Bands, left to right, represent gamma, beta, alpha 2, alpha 1 globulins, and albumin.

FIG. 2. Continuous flow pattern of refined Stuart factor preparation. Inset shows paper strip pattern of preparation compared to normal plasma.

drochloric acid and the precipitate, recovered in barbital-saline buffer, lyophilized. For Stuart or proconvertin, the appropriate gelatin dialysate was adjusted to pH 7.0 and adsorbed with $BaSO_4$. The sediment was eluted with 0.2 M trisodium citrate, dialyzed against 0.3% NaCl until free from citrate, and then lyophilized. It is recognized that these products are not physicochemically pure. They are, however, concentrated and freed from much inactive protein and inhibitory actions of excess buffer salt. These refined preparations serve as excellent materials in studies of basic clotting factor reactions(6).

Results. Fractionation. A typical normal plasma pattern is shown in Fig. 1. For comparison, a routine paper strip pattern of the same plasma is shown in the inset. The bands correspond to usual protein zones, with albumin the darkest band on the right. The numbered tabs are shown at bottom of continuous flow pattern. In this run, tubes 5-26 contained protein. Clotting factor assays showed results similar to those of Lewis *et al.*(1), namely: incomplete resolution, with fibrinogen and Hageman in beta and gamma globulin areas; prothrombin, proconvertin, and PTC in the alpha 2, alpha 1, and late albumin areas; and AcG in the albumin area. Our specific assay showed Stuart factor in the albumin and pre-albumin areas, thus different from proconvertin. Stuart factor alone was found in tubes 25-26; these were pooled and refined further, as described above. The product was reconstituted with distilled water in 1/10 the original buffer volume and rerun on the curtain. Fig. 2 shows the resultant pattern. Stuart activity was found only at points stained by the dye. The inset in Fig. 2 shows the paper strip pattern of this preparation, compared with normal plasma. A single zone, corresponding to the curtain band, is seen, which overlaps the albumin and pre-albumin areas of the normal plasma. Thus, Stuart factor is found in the albumin and pre-albumin areas of normal plasma.

Testing of refined fractions. Table I shows assays for prothrombin (ProT), proaccelerin (AcG), proconvertin (ProC*), Stuart, and Hageman (Hag.), performed on refined preparations. The results are compared with the substrate control (0%) and normal plasma (100%). All refined preparations clearly lack prothrombin and proaccelerin. Each refined preparation contains the factor in question, essentially free from other clotting factors. In the Hageman assay, the refined Hageman preparation had activity similar to normal plasma, whereas the other products were Hageman free. The minor shortening of clotting-times in some of the other tests with the Hageman preparation is a known characteristic of excess Hageman factor(4). In the specific Stuart assay, the results with the refined preparations are negative except for Stuart, which is comparable to normal plasma. The proconvertin assay (ProC*) is the non-specific Owren method, which shows minor correction with either proconvertin or Stuart alone. In fact, the sum of the independent substrate corrections is less than 15% normal plasma concentration. However, a combination of the *2* refined preparations corrects to better than 75%. These findings have been confirmed by correction of plasmas specifically deficient in proconvertin (Proc.–) or Stuart factor (Stuart–). The results are seen in Table II. In these experiments, the assays reflect proconvertin deficiency in *3, 6, 8*; Stuart deficiency in *4, 7, 9*; a deficiency of both in *1*; and a deficiency of neither in *2, 5, 10*. Each preparation corrects the existent deficiency in that factor, *5*; *10*. A mixture of both preparations gives a normal assay, as shown previously in Table I. However, if either factor is lacking,

TABLE I. Assays of Clotting Factor Preparations. Clotting-times, sec. at 37°C.

Preparation	Assay ProT	AcG	Hag.	Stuart	ProC*
Substrate control	113.4	70.5	>600	40.4	115.0
Normal plasma	18.5	16.6	122.0	15.7	16.9
Hageman	94.5	81.5	110.4	38.0	82.0
Proconvertin	114.0	82.6	>600	38.7	45.0
Stuart	114.2	81.4	"	15.6	63.6
ProC + Stuart	115.0	"	"	15.7	19.7

* Non-specific Owren type assay.

TABLE II. Assays on Mixtures of Factor Deficient Plasmas and Refined Preparations. Clotting-times, sec. at 37°C.

Preparation	Assay ProC*	Stuart
1. Substrate control	105.6	46.6
2. Normal plasma	17.4	16.4
3. Proc.– "	58.4	20.0
4. Proc. prep.	45.5	42.7
5. *3* + *4*	17.0	17.8
6. *3* + *8*	52.0	15.5
7. Stuart– plasma	59.7	45.5
8. Stuart prep.	71.4	15.6
9. *7* + *4*	42.8	43.2
10. *7* + *8*	18.0	16.2

* Non-specific Owren type assay.

an abnormal proconvertin assay (ProC.*) results. Further tests showed the proconvertin preparation to be active in our *specific* Factor VII test(5), whereas the Stuart preparation was inactive. This is additional proof that (a) the Owren technic is dependent upon both factors, and (b) complete separation of proconvertin and Stuart has been accomplished.

Discussion. The purpose of our study was to obtain individual clotting factors essentially free from others. The above tests show achievement of this goal in the case of proconvertin, Stuart, and Hageman factor. No serious consideration of concentration was raised in this part of the work. However, potencies approximating those in normal plasma were obtained for each of the refined preparations despite considerable dilution accompanying the technic.

The previous findings of Lewis and colleagues(1) regarding electrophoretic mobilities of many clotting factors have been confirmed. New information concerns the rapid migration of the Stuart factor even in advance of albumin. Others(7) have noted this in zone electrophoresis on starch gel. The present methods are only the beginnings of an approach to the production of high potency preparations suitable for physico-chemical study. In advance of this biochemical goal, they do find immediate application to studies on thrombin and thromboplastin formation *in vitro*(6).

Summary. Continuous flow paper electrophoresis has been applied to isolation of blood clotting factors. Stuart factor has been separated from proconvertin and shown to migrate

in the albumin and pre-albumin area. Data presented show factor specificity and freedom from other clotting factors in the case of proconvertin, Stuart, and Hageman preparations.

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Effect of Antispasmodic and Ganglionic Blocking Agents on Mortality Following Electroshock Convulsions in Mice.* (25083)

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Sporadic reports have appeared which indicate that certain agents produce a high incidence of post seizure mortality following electroshock convulsions in mice. Jenney(1) has shown that reserpine produces such an effect, while Berger(2) demonstrated a similar increase in post seizure mortality with benactyzine. Toman *et al.*(3) stated that an increase in post seizure mortality occurs with a variety of apparently unrelated agents and is nonspecific. Vernier and Meckelnburg (personal communication) observed that atropine, scopolamine, and other antispasmodics also increased post seizure mortality, and we found that ganglionic blocking agents were capable of eliciting the same response. Since no studies relating to the mechanism and site of action by which such drugs act have been reported, it was of interest to study the phenomena in more detail. The present studies were undertaken to determine if post seizure lethal effect of antispasmodic and ganglionic blocking agents was related to their peripheral anticholinergic activity and to determine if such agents altered the electroconvulsive threshold.

Method. Female mice of CF#1 strain were chosen to study ability of antispasmodic and ganglionic blocking drugs to increase mortality following supramaximal electroshock convulsions. The electroshock method was a modification of that described by Swinyard *et al.*(4). A supramaximal alternating current stimulus of 120 volts was passed for 0.3 second through saline-wick corneal electrodes from a Medcraft apparatus, type B_2, series 210. Care was taken to keep the wick electrodes saturated with physiological saline at all times. Five to 7 graded doses of the agents were administered intraperitoneally, in groups of 10 mice each, to establish response relating dose and mortality following maximal seizure. Time allowed for absorption of drugs before electroconvulsive shock was determined from average time required for maximal pupil dilatation (see below). The amount of drug resulting in mortality for 50% of mice tested was expressed as post seizure lethal dose$_{50}$ (P.S. LD_{50}) and was derived according to Litchfield-Wilcoxon procedure(5). To determine if antispasmodic and ganglionic blocking drugs altered the electroconvulsive threshold, mice were pretreated intraperitoneally with either 3.84 mg/kg atropine sulfate or 3.2 mg/kg mecamylamine. Control animals received saline, and 30 minutes after treatment groups

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