Synergism of Thromboplastic Extracts: Synergistic Activity of Rabbit Lung and Brain Thromboplastic Extracts

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In the course of attempts to standardize thromboplastic materials, an apparent synergism of some of these materials was encountered. The purpose of this paper is to present evidence of such an effect in the thromboplastic activities of rabbit lung and rabbit brain mixtures.

Acetone-dried rabbit brain and acetone- or air-dried rabbit lung were extracted with 0.85% NaCl and tested on frozen plasma from normal human subjects, utilizing our modified one-stage technique of Quick. Results of volumetric mixtures of the extracts when tested in this way are shown in Table 1 and graphically in Fig. 1, curves A, B, and C.

The data demonstrate that there is increased thromboplastic activity of mixtures of brain and lung extracts above that which would be anticipated by the process of mixing. This is particularly well demonstrated by curve B, where an extremely "slow" lung preparation made by acetone drying was mixed with a "rapid" brain

TABLE 1

Brain-lung ratio	PLASMA A*		PLASMA B†		PLASMA C*	
	Observed clotting time (C.T.) in sec	Calc. add. C.T. in sec	Observed C.T. in sec	Calc. add. C.T. in sec	Observed C.T. in sec	Calc. add. C.T. in sec
100B 0L	14.8		14.7		21.0	
90B 10L	—	—	14.5	17.3	17.0	20.4
80B 20L	13.2	14.8	13.8	19.8	16.7	19.8
70B 30L	12.9	14.8	13.1	22.3	15.6	19.2
60B 40L	12.8	14.8	11.9	24.8	14.0	18.6
50B 50L	12.3	14.9	12.6	27.3	15.0	18.0
40B 60L	12.2	14.9	12.7	29.8	13.7	17.4
30B 70L	13.4	14.9	13.0	32.3	14.2	16.8
20B 80L	14.1	14.9	13.8	34.8	13.7	16.2
10B 90L	14.0	14.9	15.5	37.3	13.5	15.7
0B 100L	14.9		40.4		15.1	

* Clotting time obtained with air-dried lung thromboplastin.

† Clotting time obtained with acetone-dried lung thromboplastin.

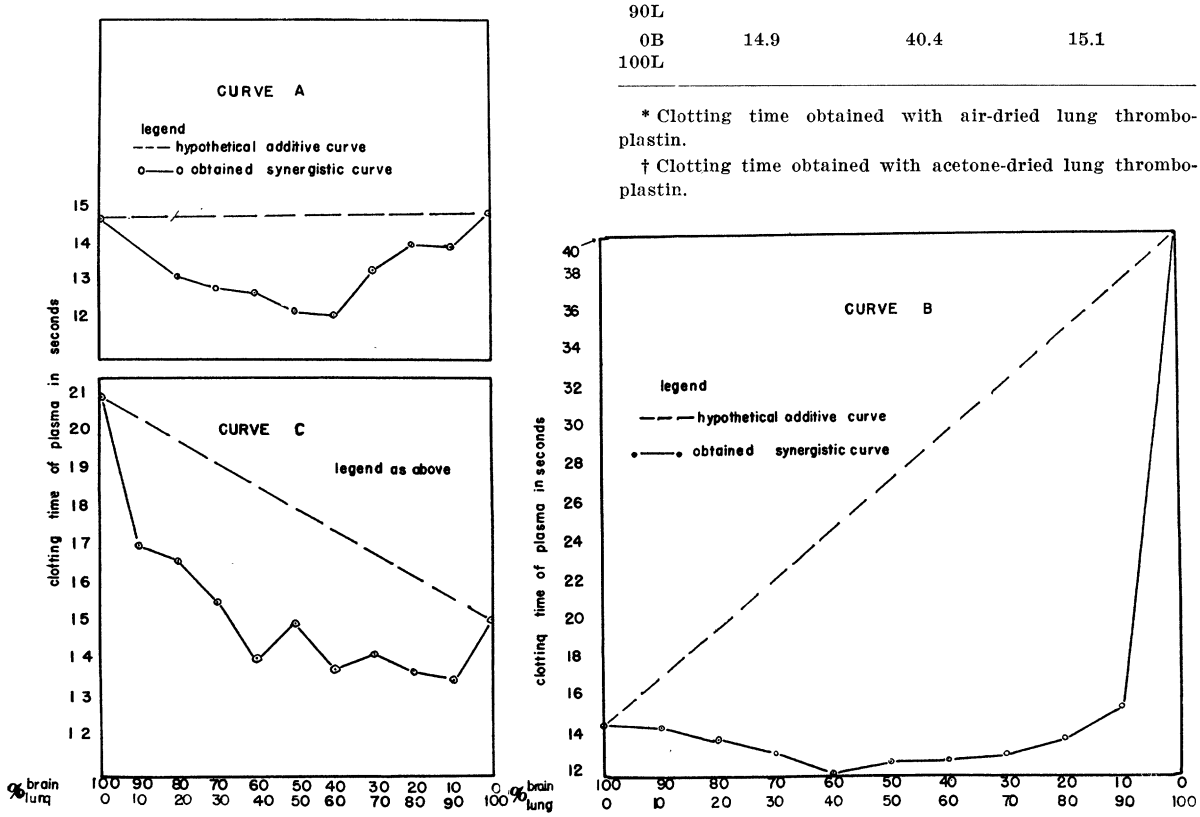

FIG. 1.

preparation. A mixture of one part of brain extract with nine parts of lung extract exhibited sufficient synergistic action to shorten the clotting time from 40 to 15 sec. The same effect is apparent in curves A and C, using more rapid initial preparations. This effect may be interpreted as due to a reshuffling of components in a thromboplastin complex, or a rebalancing of functionally different thromboplastins. The effect of various clinical conditions on the prothrombin time determined with these thromboplastins is being investigated for possible application to routine prothrombin time determinations.

The practical economic value of this effect is the possibility of increasing the yield of material from one animal several fold, and also of making available a more active preparation of thromboplastin.

These considerations apparently apply also to mixtures of brain thromboplastin. Preliminary experience in this laboratory indicates that mixtures of "deteriorated slow" brain thromboplastin with fast thromboplastin in about equal proportions give prothrombin times equal to those obtained with 100% fast brain thromboplastin. If this is borne out, much thromboplastin, now discarded, may be salvaged.

Chromatographic Analysis of a Mixture of Proteins from Egg White

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Successful separations and analyses of a wide variety of compounds have been achieved with the use of ion-exchange resins (*4*). As a result of the utilization of refractometric optical methods of observation (*2*), the chromatographic separation of protein mixtures has been predicted (*10, 15*). In fact, Tiselius has reported distinct fractionation of protein mixtures on paper by the somewhat different technique of salting-out adsorption (*14*).

This preliminary paper will outline briefly the experimental conditions used for a chromatographic analysis of a protein mixture, and its correlation with the results of an electrophoretic analysis of the same mixture, using the standard Tiselius apparatus (*12*).

The protein system studied, egg white albumin fraction (*5*), was prepared from hen's egg white by the addition of an equal volume of saturated ammonium sulfate and the subsequent removal of the precipitate formed. Just prior to use, the supernatant, which had been stored in the refrigerator, was dialyzed extensively against running tap water and two changes of 20 volumes of cold distilled water. The pH of the final preparation was 6.8.

In preparation for use, the resin, Dowex 50,[1] a cation exchanger in the 200–500 mesh size, was given successive overnight treatments with 4% ammonium hydroxide, 4% sulfuric acid, and 4% ammonium hydroxide, with extensive washing with distilled water between treatments, and a final washing with freshly boiled distilled water. The resin column was formed in one arm of a U-tube which contained a fused-in fine sintered glass disk to support the adsorbent, and was connected at the top to one arm of a standard tall center section Tiselius electrophoresis cell (*7*) through a 1-mm-diam hole in a specially made bottom section. The adsorbent column was 19.5 cm long and 6.9 mm in diam and was covered by a glass wool plug just below the sliding plate interface, connecting bottom and center sections of the modified electrophoresis cell. Just prior to the analysis, the U-tube was washed and filled with protein mixture below the sintered glass disk. During operation, protein was introduced to the bottom of the column through the U-tube from a liquid reservoir above the other vertical arm. The whole assembly, except

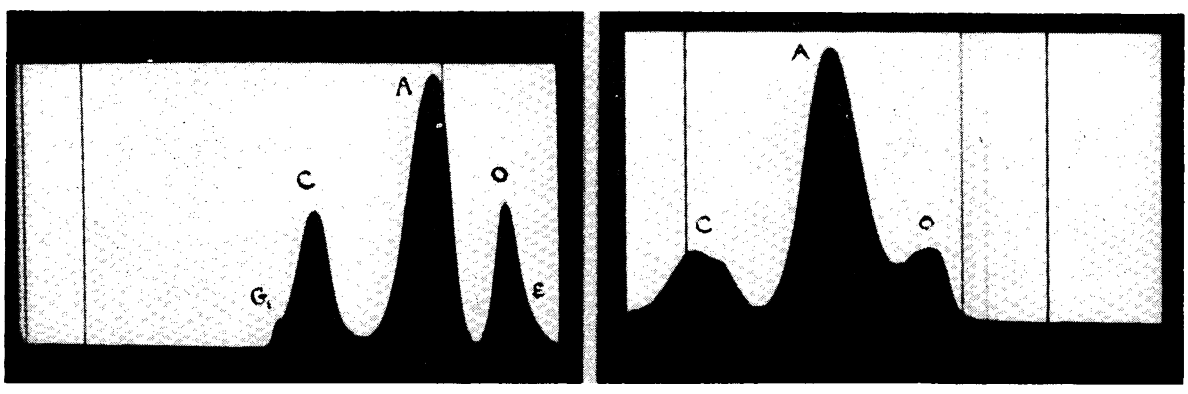

FIG. 1. *Left,* electrophoretic pattern of descending boundaries of egg white albumin fraction in 0.1 M sodium acetate —0.05 M acetic acid buffer at pH 3.90, after 8,520 sec electrolysis at 6.17 v/cm. *Right,* schlieren scanning pattern of effluent from chromatographic column during frontal analysis, flow rate 0.39 ml/hr.

G_1: globulin, C: conalbumin, A: ovalbumin, O: ovomucoid, ε: false buffer boundary. Arrows below patterns represent direction of electrophoretic migration or hydrodynamic flow.

[1] Dow Chemical Company, Midland, Mich.