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

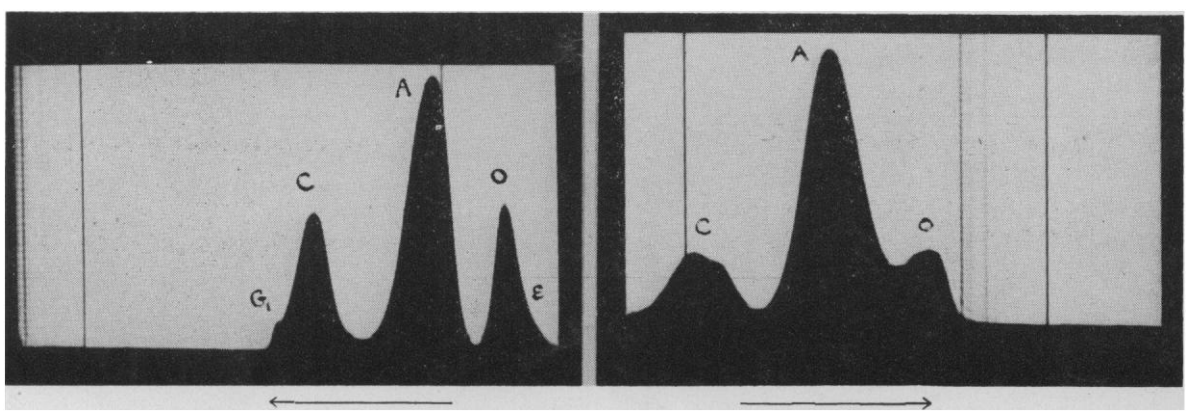

FIG. 1. *Left,* electrophoretic pattern of descending boundaries of egg white albumin fraction in 0.1 M sodium acetate —0.05 M acetic acid buffer at pH 3.90, after 8,520 sec electrolysis at 6.17 v/cm. *Right,* schlieren scanning pattern of effluent from chromatographic column during frontal analysis,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

for the protein reservoir, was immersed in an electrophoresis bath thermostatically controlled at 4° C. Refractometric optical measurements were made on the effluent solution in the Tiselius cell above the column, using the schlieren scanning technique of Longsworth (*6*). This arrangement permits the application of the frontal analysis method of Tiselius and Claesson (*1, 2, 13*).

The electrophoretic analysis of the original albumin fraction is shown in Fig. 1, *Left*. The separation into three major components parallels the results of Longsworth, Cannan, and MacInnes (*9*).

In Fig. 1, *Right* is shown the schlieren diagram of the material emerging from the column during frontal analysis. The separation into three major components is again apparent. By means of a capillary, a sample was drawn from between the second and third peaks. Resolution of this sample in the microelectrophoresis cell (*12*) established the identity of proteins giving rise to the boundaries observed in frontal analysis.

TABLE 1

ANALYSES OF EGG WHITE ALBUMIN FRACTION

Method and sample	Ovomucoid %	Albumin %	Conalbumin %	Total protein concn.*
Frontal analysis (original)	13.2	68.5	18.3	1.95%
Electrophoresis (original)	19.9	56.2	22.8	2.05%†
Electrophoresis (column effluent)	19.7	57.5	21.4	.....

* After water dialysis, assuming specific refractive index increment of 0.00185.

† From freely diffusing boundary.

To investigate the possibility of protein denaturation by the resin, electrophoretic and ultracentrifugal analyses were also performed on material collected from the column after no further chromatographic resolution was indicated by the optical system. The schlieren diagrams obtained from electrophoretic analysis of the effluent reproduced in every detail the patterns obtained from the original egg white albumin fraction, indicating that adsorption onto and desorption from the ion-exchange resin had not altered the charge distribution of the several protein components. However, the ultracentrifuge experiments appeared to indicate that there had been small changes in the size distribution, and this remains to be investigated in more detail.

Comparison of the chromatographic analysis with the electrophoretic analyses of the original albumin fraction and of the effluent from the column is made in terms of percentage composition in Table 1. In addition, the total protein concentration of the sample shown in Table 1 was determined separately from the schlieren scanning diagram of a freely diffusing boundary which had been formed against water and compensated into view in the electrophoresis cell.

Although there is qualitative agreement between the electrophoretic and chromatographic results, it should be noted that both electrophoretic analysis and chromatographic frontal analysis are subject to quantitative errors (*1, 3, 8, 11*), which have not been evaluated for the protein system used here. An investigation of the possible magnitude of the errors involved in the frontal analysis of protein mixtures with this ion-exchange resin is being undertaken by the analysis of synthetic protein mixtures. The feasibility of extending the present technique to larger scale separation of proteins is also under study.

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