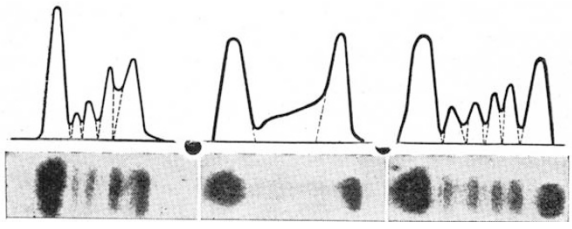
Effect of Ultrasound on the Protein of Human Serum Denatured with Tannin

Tannin—the natural tannin glycoside ester which occurs in many plants—is a weakly acidic compound. An aqueous solution of tannin gives an insoluble precipitate with solutions of salts of heavy metals, and with solutions of organic substances, such as alkaloids, gelatine and protein. Numerous investigators have studied these complexes, using various means to break them up. We have used ultrasonic vibration, with which we previously broke up the tannin-alkaloid complex in an alcoholic extract quinine bark.[1] Other workers[3] have studied the effect of ultrasound on the structure of organic substances, particularly molecular size. The present work was concerned with the complex of tannin with human serum protein.

To 0·3 ml. blood serum was added 0·3 ml. 1 per cent aqueous solution of tannin. The complex was then subjected to ultrasound for 30 minutes. A piezoelectric generator with frequency 780 kc./s. and power 30 W./cm.[2] was used. A stream of water kept the temperature in the range 12·5–17°C.

The serum was then investigated by electrophoresis on Whatman No. 1 filter paper, with veronal buffer *p*H 8·6 and ionic strength 0·10. 8 μl. serum were placed on the strips and electrophoresis was carried out for 16 hours at 8 V./cm. (0·19 m.amp./cm.-wide strip).

Electrophoresis patterns of normal serum, the complex with tannin, and the complex after the action of ultrasound are shown in Fig. 1.



(*a*) (*b*) (*c*)

Fig. 1. Electrophoresis of human serum protein. (*a*), Normal; (*b*), after treatment with tannin; (*c*), as (*b*) after 30 min. treatment with ultrasound.

Our results suggest that tannin forms a permanent insoluble complex with the serum globulins rather than with albumen. Globulins bound with tannin do not migrate in the electric field and remain on the starting line, which indicates that they are denatured by tannin. The complex appears to be broken up by ultrasound, since after this treatment we found four globulin fractions migrating at the speeds of normal α_1-, α_2-, β-, and γ-globulins. Treatment for 30 minutes did not break up all the complex, since some protein remained on the starting line. After the break up of the complex under the influence of ultrasound the size of α_1- and α_2-globulin fractions increased slightly while there was a slight decrease in the β- and γ-globulin fractions (Fig. 1). This suggests that some change in structure takes place, but it does not appear to be fundamental. The albumen fraction also appears to be slightly affected.

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A New Two-Dimensional Paper Chromatographic Method applied to the Separation of Amino-acids

One of the advantages of the ion-exchange cellulose papers as chromatographic media is their ability to exhibit both ion-exchange and cellulosic properties. Thus, it has been shown that ion-exchange separations can be combined with those of a partition type as a two-dimensional chromatogram to form a rapid and efficient means of resolving mixtures of inorganic ions[1].

This method has been applied to the separation of synthetic mixtures of amino-acids. The success of the method depends largely on the ability to suppress the ion-exchange characteristics of the cellulosic materials, in such a way that a purely partition separation of amino-acids can be made to take place in one direction. This has been achieved by using developing conditions under which either the functional groups of the exchange material are no longer ionized or, under which the amino-acids carry the same charge as the exchanger.

A two-dimensional separation on cellulose phosphate paper, which is a medium strength cation exchange material, is illustrated in Fig. 1. The amino-acid solution was applied to the dry paper as a single large spot and development was carried out according to conventional techniques by a descending method. In the partition solvent, *m*-cresol/ammonia, the amino-acids were anionic and separated as on unmodified cellulose. The separated spots on this and all other chromatograms were detected by spraying with an 0·2 per cent solution of ninhydrin in chloro-

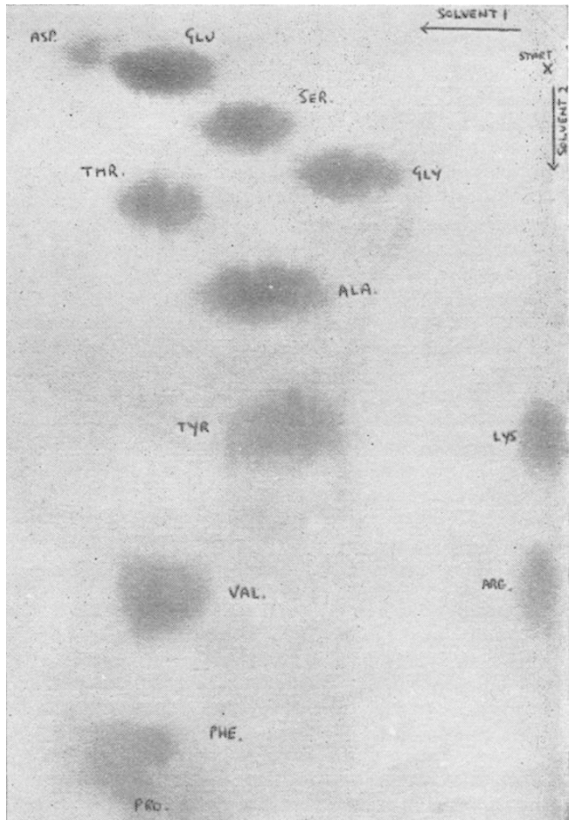

Fig. 1. A two dimensional separation of amino-acids on cellulose phosphate paper in the hydrogen form. Solvent 1:0·02*M* sodium buffer at *p*H 4·7. Solvent 2:*m*-cresol/1 per cent ammonia.