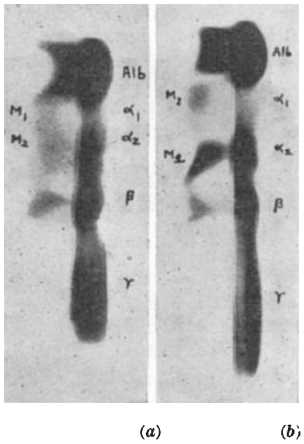
Two-Dimensional Agar Electrophoresis of Serum Mucoproteins

The agar electrophoresis technique developed in this laboratory[1,2] has been adapted to two-stage and two-dimensional procedure for demonstrating the micro-heterogeneity of the main protein components of human serum[3]. Recently, Markham[4] reported a method of two-dimensional paper electrophoresis for the separation of mucoproteins. The method is elaborate, and the separations achieved are not satisfactory, diffused zones being obtained as a result of trailing due to adsorption of the proteins on paper at the acid *p*H. The present communication gives a brief description of a simple method of two-dimensional agar electrophoresis, by which the mucoproteins can be separated into well-defined zones.

The method consists in the separation of the main protein components of serum at *p*H 8·6 (veronal–acetate buffer, 0·05 ionic strength) on agar gel (1 per cent, 7–8 c.c.) layered into the space (18 cm. × 2·5 cm.) provided by a 'Perspex' frame kept on a square plate glass (18·5 cm. × 18·5 cm.). The serum sample (15 µl.) is applied to a small Whatman No. 1 paper disk (6 mm. diam.) and placed at the centre on the surface of the agar gel. Another paper disk of the same size is soaked in 0·2 per cent amidoschwarz dye and superimposed on the serum paper disk. The dye serves as marker for albumin. After electrophoresis (200 V.; 7–8 m.amp., 4 hr., 22–24° C.) the paper disks and the excess of the gel layer on both sides of the protein zone are removed. This can be easily achieved by noting the position of the albumin spot which is stained by the dye. The gel strip (8 mm. wide) containing the main protein components is retained on the plate glass. Another lot of agar gel (1 per cent, 20 c.c.) containing acetic acid–acetate buffer of *p*H 4·5 and ionic strength 0·05 is layered into the space (14·5 cm. × 14·5 cm.) provided by a 'Perspex' frame so as to immerse completely the agar gel strip containing the main protein fractions. The electrophoresis is carried out at right angles to the first direction for 6–8 hr. at 100–120 V.; 8–9 m.amp. After electrophoresis the agar gel layer is dried in a current of air at room temperature and subjected to the staining procedure using amidoschwarz dye[1,2]. Typical patterns of normal and cancer serum are shown in Fig. 1*a* and *b*. The patterns show the separation of four protein components having the mobilities of albumin, α_1-, α_2- and β-globulins at *p*H 8·6 and migrating towards the anode after the second electrophoresis at *p*H 4·5. In the case of cancer serum the intensity of the spots relating to M_1 (having the mobility of α_1-globulin at *p*H 8·6) and M_2 (mobility of α_2 at *p*H 8·6) mucoproteins is more than the corresponding protein fractions of normal serum.



(*a*) (*b*)

Fig. 1. Two-dimensional agar electrophoresis patterns of human serum. (*a*) Normal serum; (*b*) cancer (check) serum

The mucoproteins can be stained with fuchsin sulphite after periodate oxidation. The marker is not added when the agar film is stained for carbohydrates. The mucoprotein M_1 contains a large percentage of carbohydrate, as indicated by the intensity of the colour obtained after staining with fuchsin. When the agar plate is subjected to the successive staining procedure with fuchsin sulphite followed by amidoschwarz, the mucoprotein M_1 is stained more densely for carbohydrate than for protein as indicated by the predominance of pink colour of the spot, thereby indicating the large percentage of carbohydrate in this component. The other component (M_2) having the mobility of α_2-globulin at *p*H 8·6 also gives a test for carbohydrate; but the concentration of the carbohydrate is lower than in the M_1 component, while the protein concentration is high. The components having the mobilities of β-globulin and albumin at *p*H 8·6 also give a reaction for carbohydrate. Thus four protein components of anodic mobility at *p*H 4·5 and having the mobilities of albumin, α_1-, α_2- and β-globulins at *p*H 8·6 are present in human serum. Further work on the development of a quantitative method for the analysis of these proteins by eluting the colour of the spots after electrophoresis on 'Cellophane' or polyester films[5,6] is in progress. The technique, being simple and elegant, can be used for routine examination of the changes in mucoproteins in pathological samples of blood serum. Further investigations on the isolation and characterization of the four serum protein components having anodic mobility at *p*H 4·5 and their occurrence in different animals are in progress. It is hoped that the use of this technique will facilitate the investigations of mucoproteins in serum and urine.

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Relationship of Complement to Blood Coagulation

The factors that constitute the complement system and those essential for blood coagulation are proteins which have very similar physical properties. These similarities have attracted the attention of numerous workers who have attempted to define the interrelationship of these factors. It was originally thought that prothrombin and the C^11 component of complement were identical[1], but this has been since shown to be erroneous[2,3].

Conflicting evidence exists, however, to show that when serum or plasma is treated by heat, ammonia, zymin heparin or decalcifying salts both the activity of complement and fibrin formation may be impaired[4].

These observed facts suggested that one of the other factors essential for normal blood coagulation