official or reflecting the views of the U.S. Navy Department or the Naval Service at large.

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Two-Dimensional Electrophoresis of Serum Proteins

A METHOD of zone electrophoresis in starch gels has recently been described by one of us[1]. The high degree of resolution obtained with this method when applied to serum appears to be due to the use of a supporting medium the pore size of which approaches the molecular dimensions of some of the proteins involved, so that resolution by molecular sizes is superimposed on resolution by free solution mobilities. Consequently the starch-gel electrophoretic separations result from a qualitatively different process from that involved in free-solution electrophoresis. A combination of the two electrophoretic processes at right-angles should therefore give a much greater degree of resolution than is possible with either separately. Such a two-dimensional electrophoretic system has been tested by us, using electrophoresis on filter paper as a close approximation to that occurring in free solution. The present communication illustrates the type of result which can be obtained; a detailed description of the method will be published later.

The first electrophoresis is carried out on a 5-mm. wide strip of Schleicher and Schüll 589 *YD* filter paper using a buffer of *p*H 8·55 (Poulik, M. D., unpublished work) and of ionic strength approximately 0·1, prepared from Michaelis stock solution[2] by the addition of hydrochloric acid. The strip with the serum proteins separated according to their free-solution mobilities is then inserted into a starch gel 12 cm. in width, and a second electrophoresis is carried out at right-angles to the first.

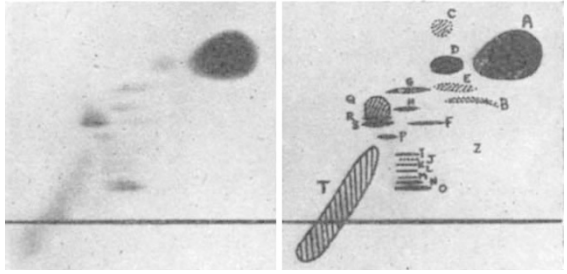

Fig. 1. Two-dimensional electrophoresis of normal human serum of group IIB (left) and key (right). *A*, albumin; *B*, a component unresolved from albumin and α_1-globulin on filter paper; *C*, *D* and *E*, α_1-globulin; *F*, a component unresolved from α_1- and α_2-globulins on filter paper; *G* to *O*, α_2-globulin; *P*, a component unresolved from α_2- and β-globulins on filter paper; *Q*, *R* and *S*, β-globulin; *T*, γ-globulin. *Z* is a shadow introduced in the photographing and should be disregarded. The black line across the photograph indicates the position of insertion of the filter-paper strip

The gel is made and stained in the way described in the previous publication[1] and the starch-gel electrophoretic conditions are the same as described there for serum protein separations.

A photograph and explanatory diagram of the results obtained with 0·01 ml. of normal human serum of group IIB [1,3] are shown in Fig. 1. More than fifteen resolved components can be seen even at the present stage of development of the method. The full significance of these results will be discussed in the later paper. However, we should like to point out that, in addition to an improvement in the resolutions which can be obtained, information on the distribution of molecular sizes in the various electrophoretic fractions results from this new method. For example, the α_2-globulin fraction obtained in the first filter-paper electrophoresis is resolved into more than six components by the second starch-gel electrophoresis. This indicates that there are more than six molecular size-groups present in the α_2-globulin of serum from the individual here concerned.

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Fractionation of Deoxyribonucleic Acid by Physical Procedures

THE use of an ultra-violet light absorption system for determining the sedimentation characteristics of deoxyribonucleic acid at low concentrations has already been described[1]. Further work has shown that the sedimenting boundary is stable and that the measurements can be made at concentrations of deoxyribonucleic acid as low as 10^{-5} gm./ml. It has been found for five preparations from calf thymus which have been studied[2] that the distributions of sedimentation constants at infinite dilution (for solutions in 0·2 *M* sodium chloride) covered a range from $S_0 = 10$ to at least $S_0 = 40$, and that the individual distributions differed significantly, the average sedimentation constant varying from $S_0 = 23$ to $S_0 = 29$.

To confirm that this observed distribution was due to a true heterogeneity, a solution of deoxyribonucleic acid (0·007 per cent) in 0·2 *M* sodium chloride was centrifuged in a bucket rotor for a sufficiently long time to remove all the material with $S > 20$. The supernatant was allowed to stand for 24 hr. at 4° C. and the distribution of the sedimentation coefficients was then determined. It was found that the average sedimentation coefficient was reduced from $S = 19$ to $S = 14$, and only 4 per cent of the deoxyribonucleic acid had a sedimentation coefficient greater than $S = 20$ (Fig. 1*a*). This shows that the observed distribution of sedimentation coefficients is real and also that there is no tendency for the particles of low sedimentation coefficients to aggregate on standing.

In order to determine to what extent variations in the distribution can arise during the preparative procedure, curves for the distribution of the sedimentation coefficients for the deoxyribonucleic acid in the nucleoprotein and at various stages during the removal of protein have been determined by carrying out experiments in 2·5 *M* sodium chloride, a salt concentration at which the dissociation into deoxy-