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'Crossing' Paper Electrophoresis for the Detection of Immune Reactions

Grassmann and Hübner[1] have pointed out the possibility of applying their continuous paper electrophoresis to the detection of loosely bound molecular compounds. The example they gave was a pair of dyes, orange II and methylene blue. These substances were introduced continuously on to a hanging paper at two separate points. They ran down vertically due to gravity and at the same time migrated horizontally towards each other under the influence of the applied electrical field. It was anticipated that the procedure would be applicable not only to the molecular compounds of dyestuffs but also to antigen–antibody complex formation. However, simpler methods have been devised for the antigen–antibody reaction[2–4].

We have succeeded in simplifying and improving the procedure of Grassmann and Hübner merely by making use of ordinary paper electrophoresis. The substances to be tested are applied not as spots but as streaks drawn obliquely to each other (crossed or not crossed). The angle between the streaks remains the same during electrophoresis, but the two lines cross over, since their mobilities differ. (The procedure may thus be called the 'crossing' paper electrophoresis.) The moving force in the direction at right angles to the electric field is not gravity but the difference in the mobilities and the geometry of the lines of the substances. The final result, however, is the same as is obtained by continuous paper electrophoresis.

By this method, the antigen–antibody reaction has been demonstrated on filter paper. As can be seen from Fig. 1, rabbit antiserum (anti-bovine serum) ran ahead and the antigen (bovine serum) ran after. Gamma-globulin of the antiserum was overtaken by the following antigen proteins and crossed over. Thus several lines of precipitates formed gradually alongside the γ-globulin, down to the crossing points of the antigens with it. If the electrophoresis is carried out two-dimensionally, antibody containing γ-globulin was crossed over on a different stretch with each antigenic protein. The ultimate results are almost the same as those obtained by the immunoelectrophoresis of Grabar. The advantages of 'crossing' paper electrophoresis over immunoelectrophoresis are first its simplicity, and second the reduction of material and time needed.

A detailed report and other applications will appear elsewhere.

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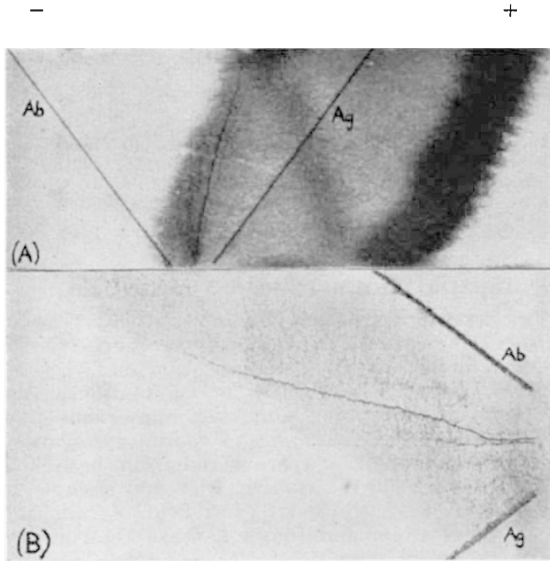

Fig. 1. Detection of antigen–antibody reaction by 'crossing' paper electrophoresis. 0·0025 ml./cm. of bovine serum was applied on the line *Ag*, and 0·005 ml./cm. of rabbit antiserum on the line *Ab*. Electrophoresis was carried out for 8 hr., 3 m.amp., 100 V., with veronal buffer, *p*H 8·6, and ionic strength 0·05. Stained with bromophenol blue. (*A*) Filter paper, not rinsed; (*B*) rinsed in water before staining. Enlarged at the gamma-globulin zone of the antiserum

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Mechanism of Oxidative Phosphorylation

THE phenothiazine tranquillizer chlorpromazine has the following actions: (1) It inhibits electron transport between reduced diphosphopyridine nucleotide and cytochrome *c* in appropriate systems; the efficiency of the coupled phosphorylation is not affected. (2) Chlorpromazine is not effective in mitochondria treated with water or in non-phosphorylating reduced diphosphopyridine nucleotide – cytochrome *c* reductase systems. (3) Chlorpromazine prevents the stimulation of respiration in systems deficient in adenosine diphosphate by 2 : 4 dinitrophenol (Table 1). (4) It causes some stimulation of respiration in mitochondrial systems deficient in inorganic phosphate, but not a maximal stimulation (Table 1), without causing any detectable increase in the level of inorganic phosphate. (5) It inhibits powerfully the exchange reaction phosphorus-32 – adenosine triphosphate[1], under conditions in which electron transport cannot be affected (Table 2). (6) It has no effect on succinate – cytochrome *c* reductase in any system.

These findings are interpreted to mean that chlorpromazine inhibits electron transport between reduced diphosphopyridine nucleotide and cytochrome *c* by inhibiting an intermediate in oxidative