Reports

New Approach to Continuous Electrophoresis

Electrophoresis has long been a powerful tool in the laboratory for separating proteins, amino acids, colloids, and other complex mixtures of electrically charged particles. Though various types of apparatus are in use, the basic principle of operation is the same; individual components separate by virtue of differences in direction and rate of migration in electrical fields of up to several hundred volts. The recent trend toward continuous electrophoresis, as exemplified by the work of Durrum (*1*), Brattsten (*2*), and Bier (*3*), suggests that what was mainly an analytical method some 20 years ago may emerge as a preparative technique in its own right. Indeed, our whole concern was with the development of large-scale apparatus such as might be used to fractionate mixtures at the rate of liters per hour (*4*).

A central problem in electrophoresis is the remixing of partially separated fractions or layers by local density differences or thermal convection of the liquid as a whole. To cope with this problem, electrophoresis is often performed in anticonvection media such as filter paper, starch, or glass powder. Unfortunately, all such solids reduce capacity by their presence in the fractionation space, and they introduce such further complications as endosmosis, evaporation, adsorption, and "fanning" or "packing diffusion." In the hands of Brattsten and co-workers (*5*) these side effects have been minimized, but truly large-scale operation by their technique seems remote.

Stray convection currents can be especially detrimental in the Kirkwood-Cann method of electroconvection (*6*). Since the separation depends on convection transport by gravity in a liquid free of solid media, thermal effects must be minimized by close temperature control and operation at a density near the maximum density of water. In large apparatus the heat evolved by passage of electricity cannot be readily dissipated, and the problem is therefore intensified. A further drawback of electroconvection is that only one component at a time can be separated, and this criticism also applies to the continuous procedure of Bier (*3*).

We started from the premise that forced laminar flow was the most suitable way to suppress unwanted eddies. Furthermore, if the liquid flows upward, any slight decrease in its density by electrical heating as it passes through the potential field will tend to aid rather than oppose the motion. Thickening agents like Methocel or dextran permit mobilities practically equal to those in pure water and yet they act to suppress eddies.

Considerable preliminary work led to the design of an apparatus made of Lucite, incorporating some of these ideas (*7*). The principle of operation is shown in Fig. 1. Separation takes place in a 4-foot vertical column of rectangular cross-section (about 3 by 2 in.) filled with a viscous fluid in upward laminar motion (*S*). The mixture to be separated is injected at the bottom of the column through a centrally located duct with a slit opening (40 by 2 mm). This duct was designed to distribute the mixture over its entire area at the slit opening. Knife-edges ensure that there is minimum disturbance to the general pattern of viscous flow at the point of entry (*I*).

At the top of the column is a series of seven similar ducts (45 by 3 mm) centrally located and separated from one another by single knife-edges (*O*). These are oriented in the same direction as the feed duct and serve as flow splitters. The potential field is applied transverse to the direction of flow at electrodes located in special compartments (*E*) which are separated from the main column by rigid porous walls of a thermoplastic resin (*PW*). The potential field extends to a height of 40 in. Provisions are made for balancing pressures between electrode compartments and the main column.

When no potential is applied, the mixture proceeds up the center of the column as a single ribbon and leaves through the middle duct at the top of the column. However, when a potential is applied, the single band splits up into several individual bands corresponding to the number of separable components. Under favorable conditions these can be withdrawn through separate ducts at the top.

One set of typical results, shown at the right in Fig. 1, was obtained with a mixture of basic fuchsin, bromthymol blue, and bromphenol blue at a flow rate of about 5 ml/min. Photographs were taken at the top of the column, just beyond the potential field. The mixture split up into three distinct bands (from left to right, basic fuchsin, bromthymol blue, and bromphenol blue) which appeared red, yellow, and blue, respectively, on color photographs. Unfortu-

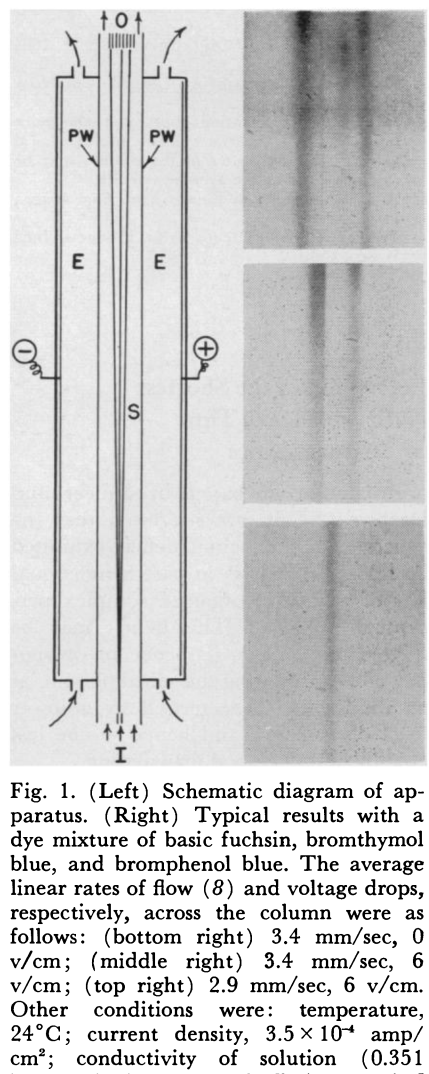

Fig. 1. (Left) Schematic diagram of apparatus. (Right) Typical results with a dye mixture of basic fuchsin, bromthymol blue, and bromphenol blue. The average linear rates of flow (*8*) and voltage drops, respectively, across the column were as follows: (bottom right) 3.4 mm/sec, 0 v/cm; (middle right) 3.4 mm/sec, 6 v/cm; (top right) 2.9 mm/sec, 6 v/cm. Other conditions were: temperature, 24°C; current density, 3.5×10^{-4} amp/cm^2; conductivity of solution (0.351 Methocel plus acetate buffer), 5.5×10^{-5} ohm^{-1} cm^{-1}; viscosity, 10 cp; *p*H, 5.3.

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nately, the yellow color was almost entirely lost in the black-and-white reproduction. At the lowest rate of flow, distances between the centers of color bands were 7 mm, red to yellow, and 3 mm, yellow to blue, with colorless spaces in between. Thus it was possible to perform a complete separation with the knife-edged flow splitter at the top of the column.

It should be emphasized that these are preliminary results; no protein mixtures have been tested as yet. The technique seems sufficiently novel, however, to merit early reporting (*9*).

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Perception of the Shortest Noticeable Dark Time by Schizophrenics

In an earlier paper by Saucer and Deabler (*1*), it was suggested that the generalized intellectual deficit exhibited by schizophrenics is in part a functional loss of ability to organize complex perceptual processes. This deficit may be related to Koffka's (*2*) concept of vigilance by considering the schizophrenic as an individual characterized by a lower level of vigilance and hence as one less able to produce good articulation.

Concerning vigilance, Koffka has said: "When the organism is active, at a high state of vigilance to use Sir Henry Head's term, it will produce good articulation; when it is passive, in a state of low vigilance, it will produce uniformity" (*2*).

Articulation may be defined as (i) a sharp separation between figure and ground or (ii) the isolation and preservation of *Gestalten* in an ambiguous situation. Our concern here is largely with the first definition, since critical flicker fusion (CFF) is studied in terms of a rigidly organized figure-ground relationship, although it must be stated that the light phase may be either figure or ground.

Critical flicker fusion clearly provides the necessary poles of articulation as flicker and uniformity as fusion. Therefore, CFF techniques may be expected to show a lowered ability to produce good articulation in schizophrenia.

Such a relationship has not yet been clearly demonstrated. This may be in part due to the choice of stimulus parameters. Conventional CFF studies have for the most part been carried out by the use of the General Radio Strobotac which has a light-dark ratio of approximately 1:5000 at fusion or by the use of the Tachisticope with a 1:1 light-dark ratio. Tanner (*3*) reported a study in which the light time was held constant and fusion was obtained by shortening the dark interval between flashes. He found that, under these conditions, correlations as high as .513 at the 5 percent level of significance between the shortest noticeable dark time and American College Entrance test scores could be demonstrated. It is his suggestion that neither a short light-dark ratio nor a 1:1 light-dark ratio presents optimal stimulus conditions for the study of relationships between CFF and psychological variables.

It is hypothesized that if Tanner's technique is applied to the question of articulation by schizophrenics, then the expected significant relationship between schizophrenia and CFF may be shown to exist. This paper is the report of an experiment in which schizophrenic and control subjects are compared with respect to the shortest noticeable dark time, it being expected that the schizophrenics will have a significantly longer shortest noticeable dark time at fusion.

Stimulation was provided by a fluorescent neon bulb (Westinghouse Nitelite) arranged to shine through a ¾-in. aperture. A ⅛-in. opal Lucite filter was interposed between the light and the aperture to provide uniform and diffuse illumination. The length of the light flash was fixed at 50 msec (a compromise between the 38- and 84-msec periods used by Tanner), and the intensity adjusted until a pilot group of normal observers reported fusion at SND times approximately the same as the 6.44-msec time reported by Tanner.

The light was gated by an asymmetrical multivibrator (*4*). On and off times could be varied independently over a range of 0.4 to 400 msec. The light fraction and the dark fraction were measured by means of a Tektronix 360 cathode-ray synchroscope and 162 pulse generator.

Control subjects were seven psychologically naive hospital staff members, six nonpsychotic neuropsychiatric patients, and six nonneuropsychiatric patients from the medical service of the Perry Point (Md.) VA Hospital. The experimental subjects were 20 acute and chronic schizophrenic patients from the neuropsychiatric wards of the hospital.

Subjects were brought into the laboratory and given a brief period of dark adaptation. For half the subjects, this period was 1 minute, for the other half, 3 minutes. After the dark-adaptation procedure was finished, it was demonstrated that the operator could vary the light from a clearly flickering state to a state of fusion.

The subjects were again shown the flickering phase and asked to report "when the light stops flickering." The shortest noticeable dark time was recorded, and the dark fraction was decreased well beyond this point. The subject was then asked to report "when the light starts to flicker." No further instructions were given. An ascending-descending series of six measures was taken.

There were no significant differences due to dark adaptation. The mean shortest noticeable dark time for the schizophrenic group was 11.58 msec with a standard deviation of 5.87 msec. The mean shortest noticeable dark time was 7.47 msec for the control group with a standard deviation of 0.833 msec. Although it is evident that there are gross differences in variance between the two groups (F ratio = 37.71 for 19 degrees of freedom), a t of 3.12, significant at the .002 level for a one-tailed test was computed.

Since the variances were obviously noncommensurate, nonparametric statistics were applied. For the rank order differences a Mann-Whitney CR of 3.72, significant at the .0001 level of confidence, was obtained. If the 19 high scores were taken as "normal" and the 20 low scores were taken as "schizophrenic," then 34/39 of the group could be correctly identified.

We feel that the null hypothesis of no true difference in shortest noticeable dark time between the control and schizophrenic group can be rejected at an adequate level of confidence.

Under these conditions of stimulus presentation, the schizophrenic is evidently less able to maintain good articulation. It is believed that the term *lowered vigilance* may be applicable to the schizophrenic. As a clinical concept, vigilance is useful in that it may serve to unify demonstrated relationships between CFF and a variety of psychological factors.

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