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Electrophoretic Interaction Studies by the Stable-Flow Free-Boundary Method

Abstract. A new method is described for rapidly mixing and unmixing (separating) components in free solution, enabling studies to be carried out on interactions of the components during their time of contact (presently as short as a few seconds). This method combines a multilayer, stable-flow fluid system with one (or more) transversely acting force fields, commonly an electric field, and is applicable to small molecules, large molecules, and cells.

The stabilized flow system recently reported (*1*) permits continuous electrophoretic separations and concentrations of large sample volumes in free solution without supporting medium. This achievement also permits other preparative and analytical investigations (*2*), including interaction studies, a type of which is the present subject. Several other interesting approaches to continuous solution separations have appeared recently. By isoelectric point immobilization and countercurrent flow, Bier separates the slowest or fastest component of a mixture (*3*). By hyperkinetic sample flow and high-viscosity polymer stabilization, Dobry and Finn fractionate dyes (*4*). [See also Bier's review (*5*)]. In none of these other methods, however, nor apparently in Philpot's early work (*6*), has true flow stability been realized in the sense described below.

A large number of independent, contiguous liquid strata can now be maintained in steady-state laminar flow through a separation (or analytical) apparatus and out into individual containers, with or without simultaneous transverse migration of components under the influence of applied forces. Flow rates from 0 to many milliliters per minute are practical. Flow stability is primarily due to the self-balancing nature of the system, the separate collection containers forming a single hydrodynamic unit with the flow cell. Significant inequities in flow rate (that is, in levels of identical bottles sharing a common horizontal) are thus precluded over a wide variety of operating conditions, that is, the laminar flows are stabilized by "hydrodynamic feedback."

The current apparatus is of the symmetrical 12 inlet–12 outlet form (Fig. 1*A*). Flows are horizontal through the main migration chamber from right to left. Single or multiple samples and background fluids can be injected through a variety of inlet combinations; thus great flexibility of experimental design is obtained. For electrophoresis, a voltage is applied between the top and bottom electrodes (commonly platinum foil or mesh). Force fields other than electric also show promise, but will not be further discussed here. The electrode compartments are hydrodynamically (not electrically) isolated from the main chamber by membranes. Thus, flows through them can be independently varied without disturbing main chamber flows, for example, to prevent diffusion into the main chamber or to set up steady-state *p*H gradients. Pumping is usually by a motor-driven syringe rack, though a much simpler gravity feed system also appears feasible.

Fig. 1*B*, a photograph of a steady-state pattern without electric field (30-cm apparatus), attests to the excellent stability of the different flowing layers. (Spectral analyses on collected fractions also verify this stability.) Similar pictures have been taken with 12 alternating color streams. Cresyl violet enters via inlet No. 5; bromphenol blue, via inlet No. 7. Small density gradients assist in eliminating turbulence that might be caused by uneven pumping, shock, and so forth. Flows pictured are 1.2 ml/min per outlet, 14.4 ml/min over-all. (Sucrose concentrations in inlet streams are: Nos. 1 to 4, none; No. 5, 0.4 percent; No. 6, 0.6 percent; No. 7, 0.8 percent; No. 8, 1.0 percent; Nos. 9 to 12, 2 percent.)

If an electric field is applied to a two-sample system as in Fig. 1*B*, various migration principles can apply. Some discussion of these has already been given (*2*), and it is beyond the scope of the present report to consider them in detail. Suffice it to say that concentrations, *p*H values, densities, flow rates, and field strength can generally be chosen to cause the migration paths of the two components to cross. The time of contact will depend upon the flow rates and electrophoretic migration velocities, both of which can be varied. If during this time, reaction occurs which gives rise to a new component with different properties, it may be separated from the original components at the outlets. If desired, the migration paths after the crossover can be altered by conductivity discontinuities in solution. In the following example, inlet solutions 1 to 4 and 9 to 12 are of higher conductivity than 5 to 8, essentially eliminating further vertical migration of samples above the 4 to 5 and below the 8 to 9 free-boundary positions (*2*, *7*).

Figure 1*C* shows this situation for the dye system of Fig. 1*B*: 0.004 percent cresyl violet enters via inlet No. 5, and 0.001 percent bromphenol blue enters via No. 7 (sucrose concentrations as above). The solution in the

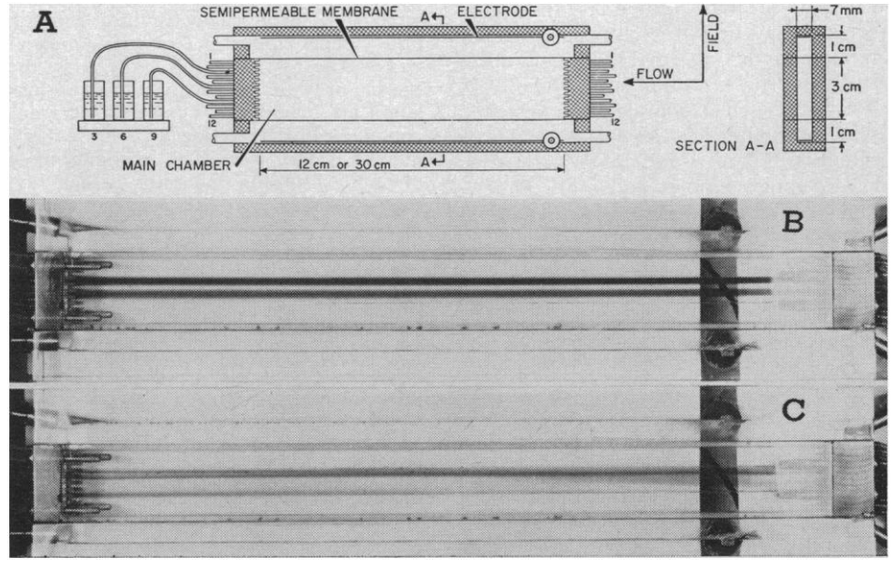

Fig. 1. (*A*) Free-flow apparatus. (*B*) Steady-state flow pattern without electric field. Dye samples admitted through inlets 5 and 7. (*C*) "Crossover" dye experiment with reaction product (middle component) separated at outlet.

top electrode compartment and inlets Nos. 1 to 4 is standard *p*H 4 phthalate buffer diluted 50 to 1 to ionic strength approximately 0.001. In the bottom electrode compartment and inlets Nos. 9 to 12 the solution is standard *p*H 7 phosphate buffer diluted 50 to 1 to ionic strength approximately 0.002; the potential is 11 volts over-all (top positive), the current 1.2 ma; the flow is 1 ml/min per inlet, or 12 ml/min overall. The basic cresyl violet moves down, the unreacted portion appearing as the bottom "ribbon" at the outlet (left). The acidic bromphenol blue migrates upward, its unreacted portion appearing as the uppermost ribbon. During their "crossover" time they react, forming the middle ribbon shown at the outlet. Depending upon initial sample concentrations, the reaction product may be soluble and subject to isoelectric-point stabilization, or it may be (partly) a precipitate with its vertical position stabilized by a suitable density gradient (*2, 7*). With present apparatus and parameters the contact time during "crossover" can be varied from a few seconds to arbitrarily long times. Thus reactions can be studied over discrete time segments, for instance, during the first few seconds, after which reaction ceases upon unmixing of unreacted or dissociated components. The various layers can be analyzed during flow (for example, optically) or after collection, leading to information on the basic interaction itself.

Where the "reaction product" is a weak complex or association product, the stable-flow free-boundary method may offer unique advantages for its study. Migration in free solution in a relatively low electric field is probably one of the least disruptive procedures one can apply to species under study. Physical properties such as absorption spectra may be but slightly modified by weak complexing, making quantitative study in the mixture very difficult. If, however, such a complex can be completely and rapidly separated while its integrity is preserved, investigation becomes much simpler and more direct.

This dye experiment is presented as a model for macromolecular and cellular interaction studies (for example, enzyme-substrate systems) rather than as a complete study in itself. The feasibility of protein and cellular migration studies by the stable-flow, free-boundary method has already been established (*2*), and additional work with both is now under way. Interaction studies of this general type can to some extent be carried out on supporting media such as paper but the times required are generally much longer and the results not necessarily representative of those in free solution.

In conclusion, it should be emphasized that this work is in its early stages and the theoretical and experimental limits for the method are not yet clearly defined. This multivariable system shares many of the complexities discussed by Dobry and Finn (*4*), and Svensson (*8*). Higher sample concentrations will certainly be desirable for some applications; density-gradient column analyses by Svensson and coworkers are helpful in estimating these possibilities (*8*). Even at this stage, however, it appears to offer a new method for study of interactions in free solutions, including weak interactions, by rapid mixing and unmixing accompanied by low-stress separations of reactants and products (*9*).

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Chromosomal Polymorphism in the Tumorous-Head Strain of Drosophila melanogaster

Abstract. The Payne inversion is scattered throughout many natural and laboratory populations of *Drosophila melanogaster*. The results of this study show that the majority of flies in the tumorous-head strain are heterozygous for a chromosome containing this inversion and the recessive mutant, scarlet eyes. This chromosome is maintained in the laboratory stock at a high frequency through a heterotic mechanism.

Flies with the tumorous-head trait in *Drosophila melanogaster* are largely characterized by abnormal growths in various regions of the head. These growths, which often appear to be homoeotic in nature, vary in size from small protuberances, affecting a small area of the eye or antenna, to massive amorphous growths occupying most of the head region. Some flies with the trait lack these growths and show an eyeless phenotype. The trait first appeared in 1945 at the University of Texas within a strain of flies that originally came from a sample of a wild population collected at Acahuizotla, Mexico, in 1941. The genetics of the abnormality was first described in 1949 by Gardner and Woolf (*1*). Since that time the trait has been the subject of many articles by Gardner and co-workers. A recent article giving references was published in 1959 by Gardner (*2*).

The genetic mechanism involved consists of a third chromosome, semidominant gene (*tu-3*) in the right arm at about position 58, and a sex-linked recessive gene (*tu-1*) near or in the heterochromatic region, which is responsible for a maternal effect (*2*). The tumorous-head strain, which is homozygous for *tu-1* and *tu-3*, is symbolized by *tu-h*.

Experiments have shown that the recessive gene for scarlet eyes (*st*), located at position 44 in the left arm of the third chromosome, is found in the heterozygous condition in the majority of the flies of the strain. From 300 single-pair matings between *tu-h* and *st* flies, offspring were obtained in 251 of the cultures. A total of 37 of the cultures showed all nonscarlet offspring, while 214, or 85 percent, showed segregation for scarlet and nonscarlet. A sample of 2716 flies from these latter cultures yielded 1389 nonscarlet and 1327 scarlet, which approximates a 1:1 ratio ($0.20 < P < 0.30$).

The results of these crosses indicate that there are two different third chromosomes in the flies of the *tu-h* strain. The chromosome not containing scarlet was tentatively called IIIA, while the one containing scarlet was symbolized by IIIB. The above results also suggest that IIIB is homozygous lethal since the scarlet phenotype does not occur in any of the flies in the *tu-h* strain. This was demonstrated experimentally from matings between *tu-h* flies and those with the following third chromosome markers: *ru h* D^3 *st ri InRC e l3e/Me' Ins ri* Sb^1. From the progeny, flies were selected that were nonscarlet, dichaete (IIIA/*ru h* D^3 *st ri InRC e l3e*) and scarlet, dichaete (IIIB/*ru h* D^3 *st ri InRC e l3e*). Each type was then inbred in an attempt to obtain nondichaete offspring that were IIIA/IIIA and IIIB/IIIB. The IIIA/IIIA types were found in the expected frequency but the offspring from the other cross were all scarlet, dichaete, like the parents, indicating the lethality of the IIIB chromosome when homozygous.

Evidence for the presence of an inversion in the left arm of the IIIB chromosome came from crossing females that were IIIA/*ru h th st* p^p *cu sr* e^s *ca* and IIIB/*ru h th st* p^p *cu st* e^s *ca* with *ru h th st* p^p *cu sr* e^s *ca* males. Crossing-over occurred along the entire length of the IIIA chromosome but was suppressed in the left arm of the IIIB chromosome. The salivary gland chro-