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-

mosomes of IIIB/*ru h D*^{3} *st ri InRC e l3e* heterozygotes were then examined. A large paracentric inversion was observed in the left arm of the IIIB chromosome with distal and proximal breaks corresponding to those for the Payne inversion (*3*), symbolized by *In(3L)P*.

Genetic evidence for the presence of the Payne inversion in the *tu-h* strain was obtained by crossing IIIB/*ru h D*^{3} *st ri InRC e l3e* flies with those with the markers *R Ly/In(3L)P*, and picking out in the offspring flies that were IIIB/*In(3L)P*. These flies were viable, showing that different lethals are associated with the two chromosomes. Inbreeding these types gave rise to about 30 percent recombinant scarlet offspring, resulting from the freeing of *st* by crossing-over from the lethal or lethals associated with the left arm of the IIIB chromosome. The occurrence of scarlet offspring demonstrates that pairing of the chromosomes in meiosis is normal and, therefore, that the inversion in the IIIB chromosome is *In(3L)P*.

The Payne inversion is widespread in many natural populations and laboratory stocks of *Drosophila melanogaster* (*3*). The interesting problem is what advantage the chromosome containing this inversion bestows upon its bearers in the *tu-h* strain, for it represents a remarkable case of natural selection for the heterozygote in a laboratory population. All *tu-h* strains maintained at the University of Utah contain the IIIB chromosome, and from the results of outcrosses the scarlet gene is known to have been heterozygous in all these strains since at least 1951. The techniques of maintaining the stocks have varied over the years. In some generations flies showing the tumorous-head trait have been selected as parental types; in others, mass mating procedures have been employed. Under either method, and even when the number of parental flies utilized has been small, the IIIB chromosome has been kept at a high frequency. The suppression of crossing-over in the left arm of chromosome III by the Payne inversion has likely led to the establishment of a heterotic mechanism.

Since the *tu-1* gene has been found in the homozygous condition in several different natural and laboratory populations (*4*), it seems likely that the original flies collected in Mexico possessed *tu-1* and were also undergoing segregation for the Payne inversion. The mutations to *tu-3* and *st* occurred later in the laboratory stock, although *st* might have been present on the same chromosome as the inversion in the original flies.

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Appearance of Genetic Transforming Activity in Pneumococcal Cultures

Abstract. Growing populations of *Pneumococcus* were found to release into the culture medium deoxyribonucleate-containing material with genetic transforming activity. Active material was maximally produced at the time when the culture was most responsive to added deoxyribonucleate. Since mixed cultures thus give rise to recombinants, it may be that transformation provides a natural mechanism of genetic recombination for *Pneumococcus*.

In 1944, Avery, MacLeod, and McCarty (*1*) established the genetic transforming principle of *Pneumococcus* to be deoxyribonucleic acid. Since that time most transformations, in pneumococci and in other transformable bacteria, have been carried out with purified soluble deoxyribonucleate, with the principal exception of the lysate transformation system described by Hotchkiss (*2*). In this system, penicillin, or later streptomycin, was used to kill sensitive organisms carrying a suitable genetic marker. If appropriate recipient cells resistant to the drug were mixed with the sensitive population, a fraction of the resistant recipients were transformed by genetic material from the dying population. This method was used mainly as a tool for simplified transformation tests until we recently began investigations on genetic and physiologic aspects of transformation by lysed cells.

The bacteria used in this investigation were pneumococcal strain R_6 [a single-colony derivative of R36A (*1*)] and derived variants genetically resistant to streptomycin, sulfonamide, micrococcin, and amethopterin, singly or in combination. These variants were obtained by transformation of the R_6 wild type with the appropriate deoxyribonucleate extracted from cultures of spontaneously arising resistant mutant strains.

Deoxyribonucleate was prepared by the method described by Hotchkiss (*3*), based on the method of McCarty and Avery (*4*). The bacteria were grown in a casein hydrolyzate medium enriched with vitamins, minerals, glucose, bovine serum albumin, and neopeptone. Cultures to be used as recipients were frozen at −20°C in 10-percent glycerol when they had reached the transformable state (*5*) and maintained at this temperature. The frozen cultures retained their transformability during 2 to 3 months of storage.

Bacteria to be used as donors were freshly grown (starting with an inoculum from a culture thawed at 0°C) on the day of use. Total population size and number of transformants were estimated by colony counts in broth containing specific antibody in the presence of which each viable unit grows as a visible aggregate (*6*). Transformations were initiated by exposing the freshly thawed diluted recipient cells to donor material for 30 minutes at 30°C (*5*). At this time pancreatic deoxyribonuclease was added to terminate the reaction by destroying any deoxyribonucleate not taken up by the cells. The cultures were then incubated at 37° for 30 to 90 minutes to permit expression of the newly acquired traits, after which aliquots were diluted into the appropriate scoring medium. Colonies were counted after 16 to 20 hours' incubation at 37°C. When cells were to be separated from culture fluid, Millipore filters of porosity 0.45 ± 0.02 μ (Millipore Filter Corp.) were used.

During the course of experiments on the mechanism of streptomycin-induced lysate transformation it was found that cell-free filtrates of control cultures not treated with drug, and therefore presumably normal, were often able to effect transformation of recipient cultures. When this phenomenon was investigated further it was found that all of the drug-resistance properties mentioned earlier could be regularly induced in recipients by using filtrates of appropriate living cultures, and that the activity in the filtrates was probably due to some deoxyribonucleate-containing material, since it, like the activity released by drugs, could be destroyed by pancreatic deoxyribonuclease. The deoxyribonucleate in such material was of sufficient intactness to transfer linked determinants of sulfonamide resistance (*6*), the frequency of double transformants being as much as 1000 times that expected on the basis of chance.

It was also found that the amount of active transforming material present in any culture filtrate was related to the stage of growth, the maximum activity appearing at about the middle of the logarithmic phase (about 2×10^7 colony-forming units per milliliter). Because transforming activity and the stage of growth of the culture were clearly related, and because transformability by exogenous deoxyribonucleate also varies with stage of growth of the culture, it was of interest to study the relation between the abilities to serve as