Alkaline phosphatase in *Drosophila* seems to be a family of enzymes whose members (except for band 1) are organ or even tissue specific. Indeed, electrophoretic variants for larval hypodermis and adult hindgut phosphatase are controlled by loci on different chromosomes.

The increase of larval hypodermal activity prior to laying down of new cuticle, most dramatic in third instar larvae before the secretion of pupal cuticle, suggests a role for this enzyme in cuticle formation and the possibility of its regulation by the ring gland.

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15 October 1965

Transverse Gradient Electrophoresis: Protein Homogeneity Test and Subfractionation Technique

Abstract. *Electrophoresis of protein (including enzyme) was conducted in a gel medium across which, transverse to the direction of protein migration, a continuous* p*H gradient extends. Any splitting of a resultant trace for the change of protein mobility with* p*H suggests both protein heterogeneity and the* p*H conditions under which further purification and subfractionation may best be pursued.*

Electrophoresis, especially in gel media, is perhaps the most valuable single method for subfractionating proteins or testing protein homogeneity. Its usefulness is considerably enhanced when the protein is studied electrophoretically under different *p*H or other conditions, a time-consuming process. However, with one continuous *p*H gradient across the gel, transverse to the direction of protein migration, there is an infinite series of changes in *p*H, and maximum fractionation power and information may be attained. The proposed method, named *transverse gradient electrophoresis*, is different from the isoelectric spectra method of Kolin (*1*) who used a *p*H gradient in the same direction as protein migration.

The typical procedure incorporates the original starch-gel techniques of Smithies (*2*). The combination gel mold and electrophoresis cell made of acrylic plastic, 12.3 cm wide by 25 cm long by 0.65 cm deep (*3*), is bisected lengthwise into equal compartments separable by a removable close-fitting divider strip. A suspension of solubilized starch (Connaught Medical Laboratories, Toronto), 30 g/200 ml, is prepared in each of two solutions: (i) 0.010*N* sodium acetate (*p*H 8.6, specific conductance 6.8×10^{-4} mho/cm); and (ii) 0.010*N* sodium acetate, 0.125*N* acetic acid (*p*H 3.7, specific conductance 7.7×10^{-4} mho/cm). Each suspension, with continuous swirling, is heated just to the boiling point and maintained at this temperature until it forms a homogeneous, viscous, translucent fluid; then, in rapid succession, it is evacuated to remove air bubbles and poured to overflowing into its respective compartment in the warmed gel mold. The divider strip is removed, and the two solutions, while still fluid, are knitted together carefully along their common interface by a gentle zigzagging motion of a glass rod. A glass plate is pressed on top and weighted; the assembly is set aside for 12 to 18 hours to permit gelling and diffusion to a uniform *p*H gradient. The *p*H distribution in the gel and gradient uniformity can be observed with the naked eye if an appropriate indicator is also included in the gel solutions.

A transverse slit (7.5 by 0.55 cm) is cut vertically into the reexposed gel surface. A fitting filter-paper strip, moistened with the sample protein solution (such as blood serum) is inserted into the slit. The glass cover plate is replaced, and the vertical gel electrophoresis apparatus (*3*) is assembled, with a mixture 1 : 1 of solutions (i) and (ii) in the electrode chambers. After electrophoresis has proceeded for 16 hours at 6 volt/cm, the gel is removed and indexing teeth are cut along its edges to assist in later comparisons. A special corrugated blade, 5.5 teeth per centimeter, mounted on a 30-cm-long holder is convenient. Three similar slices, taken from the gel (with a piano-wire gel slicer, *3*), are made visible by histochemical methods (*4*) for protein (naphthalene black dye), aminopeptidase (alanyl β-naphthylamide substrate and diazotized *o*-aminoazotoluene coupling agent), and esterase (β-naphthyl acetate substrate and diazo blue B coupling agent).

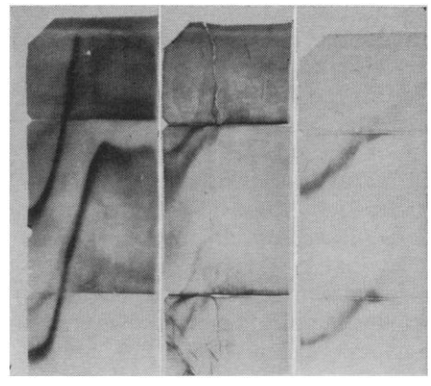

Fig. 1. Three slices from a transverse-gradient starch-gel electrophoresis of human serum (acetic acid–sodium acetate buffer with final gel gradient (left to right) from *p*H 5.4 to 4.0). Patterns (left to right) are for protein, aminopeptidase, and esterase. For convenient comparison, duplicate samples were run in tandem on the same gel (not recommended for maximum reproducibility).

The typical pattern (Figs. 1 and 2) is one of many traces and branchings, with localized narrowing and intensification along some segments or broadening and fading out along others, with many crossovers. The final gel gradient extends from *p*H 4.0 to 5.4. Principal characteristic features are reproducible for the duplicate samples of Fig. 1. The patterns provide the following important observations and implications:

1) While the patterns detect heterogeneity in many traces as expected, they simultaneously indicate the optimal *p*H regions where particular separations best occur, and valuable information to guide more intensive subfractionation work by conventional methods is provided.

2) For practical value, if a *p*H region is to be optimum, not only must a separation occur, but the separating minor constituent must maintain a vis-

ible trace. The patterns show that these conditions are sometimes met over only a narrow range (less than a *p*H unit), evidence that sole reliance upon tests at arbitrary *p*H intervals could result in the exclusion of optimum or critical conditions for separation.

3) A trace of the change of mobility with *p*H is a distinguishing characteristic of a protein, the trace being a type of two-dimensional analysis or "fingerprint," analogous to an absorption spectrum and with similar value for identification or molecular structural information. In Fig. 2 is emphasized, for example, the sharp contrast that the long sweeping (impure) albumin trace makes with other protein traces and with the gently curving esterase trace. Each configuration reflects such fundamental molecular conditions as amino acid composition of the protein, the attachment of other ionizable groups to the protein, and molecular shape and size—structural factors that determine relations between *p*H and mobility in gel media. Each pattern can give considerable enlightening information on many proteins simultaneously.

4) The patterns can provide information on intermolecular associations. Even for a complex protein mixture like serum, Fig. 2 shows that most of the enzyme traces move quite independently of one another and bulk proteins (freely crossing over). On the other hand, at least two aminopeptidase traces do follow closely along corresponding protein traces, suggesting association, and one esterase trace clings to the albumin trace over almost its entire cathodic-anodic sweep.

5) The patterns can provide information on optimal *p*H conditions for isolating a given enzyme (or other protein) with minimum contamination by other enzymatic activity and with maximum specific activity. Thus the major esterase activity is well separated from both aminopeptidase and bulk protein in a *p*H region somewhat left of center in the patterns illustrated.

Transverse gradient electrophoresis offers a fundamental advantage in that a single unidirectional run can submit a mixture to an entire series of different conditions of fractionation. In contrast, the isoelectric spectra method provides only one condition, isoelectric point. Conventional two-dimensional methods provide two conditions, a different *p*H for each of the two dimensions (which actually involves two successive right-angle runs).

While the method as described has manifest effectiveness for qualitative separations, the technique has additional potential for quantitative measurements of *p*H-mobility relations. Other types of gradients containing agents that differentially affect electrophoretic mobility might be even more useful. To our knowledge, this technique has not been described or performed previously. Bitancourt and Nogueira (*5*) have simultaneously made chromatographic separations at four discrete *p*H conditions on a paper sheet, but they did not provide a continuous *p*H gradient.

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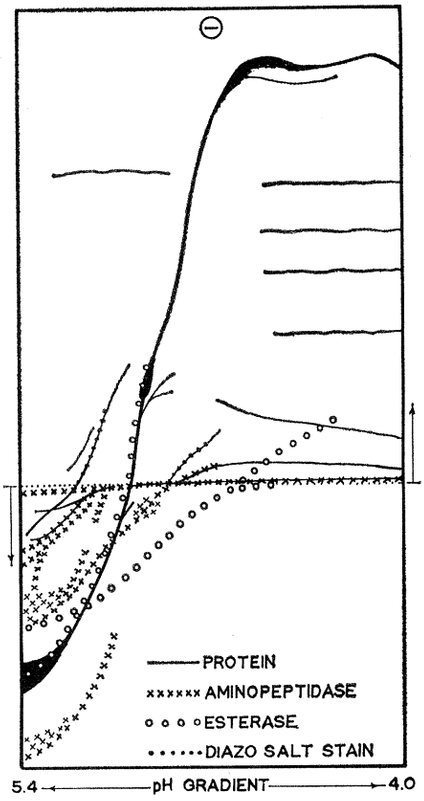

Fig. 2. Composite drawing for transverse-gradient starch-gel electrophoresis of human serum, bringing out *p*H-mobility relations among protein and enzyme traces (*6*); taken from Fig. 1 and other patterns. (The horizontal solid-line segments coarsely locate several rather broad and diffuse protein traces.)

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7. Aided by grants T-77 and T-252 from the American Cancer Society, PHS grant No. CA-06730, and gifts from Mollie E. Swinney. For assistance, I thank Charlotte Vivonia and Jean M. Hilburn (for work done at Los Angeles Veterans Administration Center) and E. R. May.

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Tabashir: An Opal of Plant Origin

Abstract. *A specimen of tabashir, a variety of opal found in bamboo, contained more water but smaller amounts of alkalis and alkaline earths than most opals. It consisted of particles of about 100 angstroms in diameter, linked together in clumps, which appeared in fractured surfaces as irregularities. The tabashir was amorphous, but its microstructure differed from that of silica gel and amorphous opal of inorganic origin.*

Many plants, such as grasses, *Equisetum*, bamboo, and certain tropical trees, absorb large quantities of dissolved silica, and as a result, solid silica is deposited in the cell walls of the plants (*1*). This silica, which can be isolated from the plant tissues as particles of the same dimensions as cells, has been identified as opal by optical and x-ray techniques (*2*). The particles are known as opal phytoliths, and, although long neglected in mineralogical studies of soils, they have been frequently reported in recent years (*3*).

In contrast to the small phytoliths, relatively large masses of silica have been found in the hollow stems of bamboo; this material is called tabashir (also tabasheer and tabaschir). Although recognized as a curiosity since antiquity, tabashir has not previously been examined by modern techniques, and little is known about it except that it is a porous, hydrous silica with a reported index of refraction of 1.11 to 1.18 (*4, 5*). We have examined tabashir and compared its structure with that of opal phytoliths, silica gel, and opal of inorganic origin.

A single piece of tabashir weighing