tinuous function of cell position in the cell cycle. Therefore, it seemed reasonable that experiments with synchronized cells could help explain the effects of serum on randomly dividing populations. Cells were synchronized according to the method of Terasima and Tolmach (*4*), except that 3 hours prior to harvest, the cells were placed in a refrigerator at 5°C for 1 hour (*5*). The cells were harvested in media of different FBS concentrations, planted either in T-15 flasks for growth experiments or in plastic petri dishes for studies on C^{14}-thymidine uptake and for exposure to 500-r irradiation at various times after harvest (Table 1). The average intermitotic period (2nd column) is within about 10 percent of the doubling time obtained from randomly dividing cells. A more important result is that the duration of the DNA synthetic phase (4th column), its position in the cell cycle with respect to the onset of the following mitosis (5th column), and the peak rate of DNA synthesis (6th column) are essentially independent of FBS concentration. The data on survival after x-irradiation show that the cells were sensitive at the time of harvest and then entered an insensitive phase. The duration of this insensitive phase increased as FBS concentration decreased. The response of all the cells during the last 15 hours of their cycle was almost identical and, therefore, also independent of FBS concentration. Sinclair and Morton (*5*) have reported on the survival of synchronized Chinese hamster cells whose generation time was about 12 hours. The response of their cells was quite similar to ours, if only the last 12 hours (Fig. 3) are used for comparison. Figure 3 is drawn to accentuate this common behavior during the later part of the cell cycle.

The concurrent measurement of C^{14}-thymidine uptake (0.008 μc/ml, administered to cells for 1 hour) showed that a sharp rise in the rate of DNA synthesis preceded the rise in survival by about 1 hour. While some variation in G_2 and M cannot be ruled out by these data, the major contribution to the serum-induced variation in doubling times is the change in the duration of G_1. Such a dependence has been suggested (*7*) on the basis of autoradiographic studies on mouse epithelial cells and has been confirmed in varying degrees and in various tissues by others (*8*).

The growth cycle data and the radiation response of the synchronously growing hamster cells can be used to calculate the response of the exponentially growing cultures (*9*). At FBS concentrations of 5, 10, and 15 percent, the experimentally obtained D_{10}'s were 368 ± 10, 346 ± 8, and 322 ± 8 r, as compared to the calculated (*9*) values of 362, 342, and 331 r. The excellent agreement between the experimental and calculated values of D_{10}'s leads to the conclusion that the serum-induced change in D_{10}'s is a result of redistribution of cells around the cell cycle as the duration of the G_1 phase of the cycle changes with FBS concentration.

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Patterns of Alkaline Phosphatase in Developing Drosophila

Abstract. *Electrophoretic study of alkaline phosphatase in developing* Drosophila *shows that different stages are characterized by the appearance and disappearance of organ-specific enzyme bands. A new electrophoretic variant, from adult hindgut, is controlled by the second chromosome locus* Aph-2.

Alkaline phosphatase has long been the subject of ontogenetic and cytochemical studies. The discovery of gene-controlled electrophoretic variants of this enzyme in *Drosophila melanogaster* larvae (*1*) raises the possibility of using this system for the study of the genetic control of enzyme regulation in a diploid organism. We have studied phosphatase electrophoretic patterns during development from egg to adult and have discovered new electrophoretic variants.

Flies of the Woodbury (New Jersey) strain were grown in half-pint milk bottles at 25°C on standard medium of corn meal, dextrose, and agar to which live yeast was added. To collect eggs of uniform age, about 50 to 75 females were allowed to lay eggs on petri plates containing 2 percent agar smeared with a honey-yeast mixture. The first clutch of eggs, collected for 2 hours, was discarded.

Homogenates of many eggs (50 to 100) and first instar larvae (10 to 15) were required to produce adequate intensity of staining, but enzyme bands of subsequent stages were produced from homogenates of single whole flies or organs. All samples were ground in microscale homogenizers in 0.03 ml of distilled water, and the crude homogenates were loaded into Buchler vertical starch-gel molds. Tris-HCl, 0.05*M*, at *p*H 8.6, was used as buffer in both vessels and gels. Electrophoresis was carried out at 7 to 9 volt/cm for 2 to 4 hours at 4°C. Sliced gels were stained (*2*) for 18 hours at room temperature (23°C). Frozen sections for histochemical staining, 7 to 8 μ thick, were cut from unfixed and Formalin-fixed whole larvae (*3*) and incubated for 1 hour in the same staining solution. Figure 1 demonstrates characteristic electrophoretic patterns in different developmental stages. The main enzyme bands have been numbered in order of appearance during development. Identity of bands in different stages is based on identity of electrophoretic mobility and other criteria.

Band 1 is a faint band present in all stages of development. Bands 2, 3, and 4 appear in that order, are all strongly developed in mature eggs (18 to 20 hours after fertilization), and persist through all three larval instars.

Evidence points to larval hypodermis

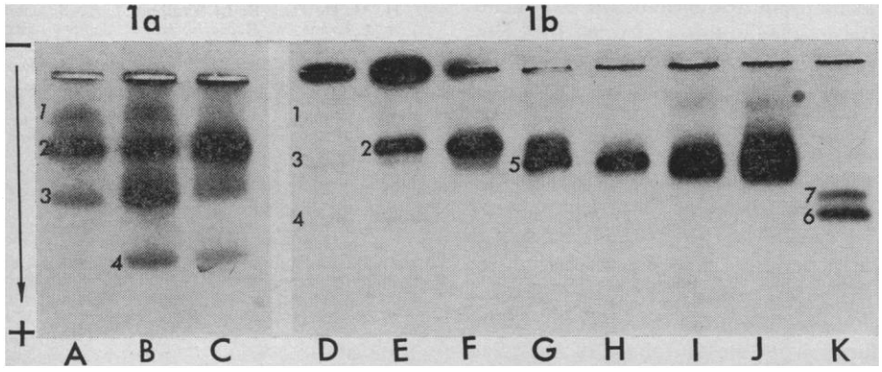

Fig. 1. The gel in 1*a* was run 1 hour longer than the gel in 1*b*. (*a*) *A*, young eggs; *B*, mature eggs; *C*, a late third instar larva for comparison. (*b*) *D*, Second instar; *E*, late third instar; *F*, prepupa; *G*, late prepupa; *H*, pupa; *I*, late pupa; *J*, young adult female; *K*, mature adult female. Bands are numbered 1 to 7 in order of first appearance in development.

as the source of band 2. In Yao's histochemical study of developing *Drosophila* (*4*), alkaline phosphatase was first seen in eggs at 9 to 10 hours after fertilization and was at that time localized to hypodermis. Hypodermal staining diminished in first instar larvae, remained low during second and young third instars, but increased markedly in mature third instars (72 hours after hatching). Yao stained alcohol-fixed material with Danielli's stain for alkaline phosphatase, but we have confirmed his results in third instars with unfixed and Formalin-fixed frozen sections stained with the method of Lawrence *et al.* (*2*). Similarly, we find that in electrophoretic runs heavy staining of band 2 appears at about 10 hours, diminishes in relative intensity in multiple homogenates of first instars (24 hours after hatching), remains barely visible in single second and young third instars (48 hours after hatching), and increases markedly in older third instars (72 hours after hatching).

In addition to the parallel timing of histochemical sections and zymograms, more direct evidence is obtained from dissection. If the internal organs of second or third instar larvae are dissected free there is left a shell containing cuticle, hypodermis, and muscle. Only this shell, homogenized and subjected to electrophoresis, produces band 2. This band is very light-staining in shells from second and young third instars and dark-staining in shells from mature third instars. The electrophoretic variants reported by Beckman and Johnson (*1*) are of this enzyme band.

Simple dissection reveals that band 3 comes from midgut. In favorable runs, band 4 can be split into two bands, 4*a* from hindgut, and 4*b* from midgut. These bands disappear in the prepupal stage earlier than band 2 (about 96 hours after hatching).

Band 5 appears in young pupae or late prepupae while band 2 is fading, and lasts until about 18 hours after emergence of young adults. At first narrow, it widens considerably in late pupae. This band is derived from the yellow body—a pellet formed from sloughed larval gut cells—which comes to lie inside the newly developed imaginal gut and is passed as meconium in the first day after emergence (*5*). We assume, therefore, that yellow-body phosphatase is larval gut enzyme, probably modified during histolysis. However, an attempt to modify the larvel-gut enzyme by incubating mixtures of crude homogenates of third instar larvae and pupae for periods up to 24 hours at 25°C did not result in loss of larval gut band activity or in difference of mobility.

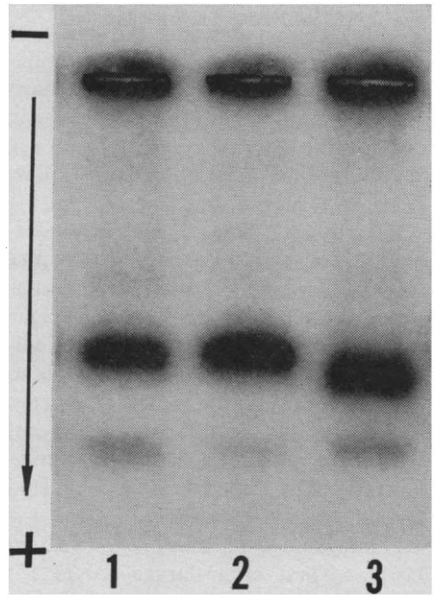

Fig. 2. Electrophoretic variants of adult hindgut phosphatase. No. 1 is an Aph^B/Aph^B female; No. 3 is an Aph^A/Aph^A male; No. 2 is an F_1 Aph^A/Aph^B female.

Band 6 derives from adult midgut, and band 7 from adult hindgut. The midgut band appears first, followed by the hindgut band which appears at about 20 hours, and both bands persist at high intensity throughout adulthood. Although flies in culture feed on live yeast, yeast alkaline phosphatase is not involved in the formation of either band. Flies isolated as pupae and starved for 48 hours after emergence are indistinguishable electrophoretically from fed controls. In addition, yeast phosphatase migrates as a band distinct from bands 1 to 7.

Two inherited electrophoretic variants of band 7 have been identified. When flies homozygous for the fast band (farther toward the anode) are mated with flies homozygous for the slow band, the resulting heterozygotes produce a band which migrates at the same rate as that of the slow parent (Fig. 2). In the F_2 generation, segregation occurs in a 3 to 1 ratio (41 "slow" flies to 122 "fast" flies). The locus, designated *Aph-2*, with variants $Aph\text{-}2^A$ (fast) and $Aph\text{-}2^B$ (slow), is on chromosome II (*6*). The locus for larval phosphatase, *Aph*, is on chromosome III (*1*).

Bands 2 to 7 all have adenosine triphosphatase, adenosine monophosphatase, and glucose-6-phosphatase activity, as stained by the Wachstein and Meisel methods (*7*). The qualitative and crudely quantitative analysis possible with our method shows no differences of substrate specificities between these bands. Band 1 is too weak for testing with our technique.

A comparison of larvae, pupae, and adults of *D. melanogaster* with other species of *Drosophila* reveals that phosphatase bands of *D. simulans*, a sibling species, are electrophoretically almost identical in pattern, mobility, and intensity, while species belonging to other subgenera, such as *D. virilis* and *D. funebris*, are somewhat different from *D. melanogaster* but resemble each other rather closely.

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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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).

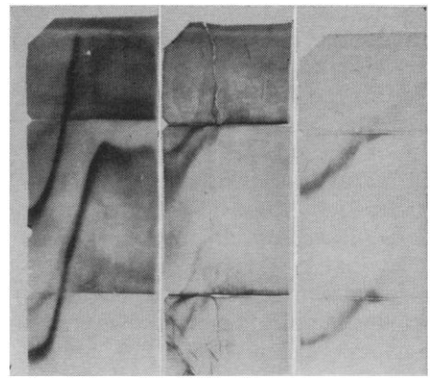

Fig. 1. Three slices from a transverse-gradient starch-gel electrophoresis of human serum (acetic acid–sodium acetate buffer with final gel gradient (left to right) from *p*H 5.4 to 4.0). Patterns (left to right) are for protein, aminopeptidase, and esterase. For convenient comparison, duplicate samples 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-