tration rose to more than 2.5 mM. This may well have affected the relative rates of ammonia and urea excretion. Enzyme activities reported are thus consistent with the predominantly ammonotelic metabolism of lungfish in an aquatic environment.

In lungfish, which are capable of alternating between periods of ammonotelism and ureotelism, it is quite probable that levels of activity of the enzymes ornithine carbamoyltransferase and arginase would not undergo such striking changes as would the rate-limiting enzymes, carbamoyl phosphate synthetase and argininosuccinate synthetase. Thus a comparison of the levels of activity of ornithine carbamoyltransferase in lungfish with that in elasmobranchs may not be appropriate in the context of ascribing an ammonotelic or ureotelic status. Validation of this position will have to await the outcome of experiments recently initiated to determine levels of activity of the enzymes of the ornithine-urea cycle in aestivating lungfish when the animal is in the ureotelic phase.

In previous experiments, Janssens (*9*) demonstrated synthesis of urea from ammonia and bicarbonate by liver slices of *Protopterus* at a rate of approximately 2 μmole per gram of tissue per hour. This is consistent with the activity of the rate-limiting enzyme, argininosuccinate synthetase, which is reported here, and it is therefore not surprising that addition of ornithine to the incubation medium did not produce an increase in the rate of synthesis of urea (*9*).

Goldstein and Forster (*16*) recently suggested that, in lungfish, urea might be formed from uric acid and not by way of the ornithine-urea cycle. While this remains a possibility, our demonstration of the presence of all enzymes of the ornithine-urea cycle in *Protopterus* establishes this pathway for urea biosynthesis. Further investigation will be necessary to determine whether it is the major pathway.

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$$ATP + NH_3 + CO_2 \rightleftharpoons ADP + \text{carbamoyl phosphate} \quad (1)$$

This reaction is not dependent on acetylglutamate. Carbamoyl phosphate synthetase catalyzes the sequential reaction:

$$ATP + CO_2 \xrightarrow{\text{acetyl-glutamate}} ADP + P_i + \text{"active } CO_2\text{"} \quad (2)$$

$$ATP + \text{"active } CO_2 + NH_3 \xrightarrow[\text{acetyl-glutamate}]{} ADP + \text{carbamoyl phosphate} \quad (3)$$

If reactions 2 and 3 are summed, then

$$2ATP + CO_2 + NH_3 \xrightarrow{\text{acetyl-glutamate}} 2ADP + P_i + \text{carbamoyl phosphate} \quad (4)$$

where reaction 2 is irreversible and reactions 2 and 3 are dependent on the presence of acetylglutamate [see review by P. P. Cohen in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrbäck, Eds. (Academic Press, ed. 2, New York, 1962), vol. 6, p. 477].
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Sedimentation of an Initially Skewed Boundary

Abstract. *An asymmetric initial boundary is often formed when a synthetic boundary cell is used in the analytical ultracentrifuge. Model calculations show that, when such a boundary diffuses, the peak of the corresponding gradient pattern moves. The movement of the peak resulting from diffusion, when superimposed on the sedimentation of the boundary, may produce errors in the measurement of the sedimentation coefficient.*

The sedimentation velocity of a solute in the ultracentrifuge is correctly evaluated by measuring the rate of movement of the square root of the second moment ($\bar{r}$) of the gradient of solute concentration. The identification of the weight-average sedimentation coefficient (S) in the plateau region with $1/\omega^2 \cdot d \ln \bar{r}/dt$, where ω is the angular velocity and t is time, is correct for any boundary shape (*1*). If the gradient curve is symmetrical, then, unless the boundary is unusually broad, the position of the maximum gradient (r_{max}) lies within a few microns of $\bar{r}$ (*2*). Conditions giving rise to symmetrical boundaries are quite often met; it is, therefore, a common practice to derive sedimentation coefficients from measurements of r_{max} rather than $\bar{r}$, since the peak position is more easily located than is the square root of the second moment.

In order to produce a symmetrical gradient curve, a solute must be homogeneous with respect to sedimentation coefficient. Its diffusion coefficient (D) must be independent of concentration (*3*), and its sedimentation coefficient must either be independent of concentration or else be a linear function of concentration (*4*). In addition, the shape of the boundary must not be perturbed by restricted diffusion against the top of the solution column (*5*). This last condition generally requires the use of a synthetic boundary cell, which is designed to form, at the beginning of the experiment, a sharp boundary between solvent and solution some distance from the meniscus.

Synthetic boundary cells do not always function properly. There may be some mixing when the solvent compartment empties; moreover, convection may occur during acceleration of the centrifuge rotor after the boundary has been formed. Whatever the cause, it is not unusual to find that the boundary is somewhat skewed by the time the rotor reaches the speed at which sedimentation is to be observed. I now report that, in such a case, the use of successive peak positions to calculate the sedimentation coefficient may lead to serious errors.

The behavior of asymmetric boundaries has been studied theoretically by a simulation of the diffusion of such boundaries with a simple numerical model and a digital computer. The model, resembling one suggested by Vink (*6*), has been somewhat modified to take account of the sector shape of

the usual ultracentrifuge cell. The simulation method has been shown to describe very accurately the diffusion of an initially sharp boundary (*7*). Attention has been focused on initial boundaries giving single gradient peaks without shoulders and on solutes whose diffusion coefficients are independent of concentration; within these limits, the diffusion of a variety of boundaries of different widths and different degrees of skewing has been simulated. The results allow a few general observations on the probable effect of a skewed initial boundary on the measurement of sedimentation coefficients.

A series of concentration-gradient distributions derived from the simulated diffusion of a skewed initial boundary is presented in the figure. The general course of this calculation is typical of all of the skewed boundaries whose behavior has been examined by the use of the model. As the initially asymmetric boundary diffuses, the corresponding gradient curve tends to become progressively more symmetrical. As the gradient curve proceeds toward symmetry, its maximum moves. The direction in which r_{max} moves depends on the direction in which the original boundary is skewed. In the case shown in Fig. 1, the boundary is skewed to the left, and the peak moves toward the right. The peak asymptotically approaches the position it would have occupied if the original boundary had been symmetrical. The rate at which the migration of the maximum gradient proceeds is directly proportional to the diffusion coefficient. In many realistic cases, the shift of r_{max} to its limiting position is essentially complete within the time required for an ordinary sedimentation velocity experiment.

If a skewed gradient is formed by a synthetic boundary cell in the ultracentrifuge, the migration of r_{max} resulting from diffusion will be superimposed on the movement of the boundary as a whole in the centrifugal field. The upper limit on the distance that the maximum gradient can move as a consequence of diffusion is set by the difference (Δr) between the initial location of the peak and r_{max} of the corresponding symmetrical boundary. The latter position coincides with, or is very slightly less than, the square root of the second moment of the gradient curve. The maximum relative error introduced, where the initial boundary is skewed, by the use of peak positions to measure the sedimentation coefficients, should depend on the ratio of Δr to the distance the entire boundary moves as a result of sedimentation. The error introduced by the diffusion shift of r_{max} will be most important for solutes which sediment slowly and diffuse rapidly. These are precisely the solutes with which the synthetic boundary cell is most often used.

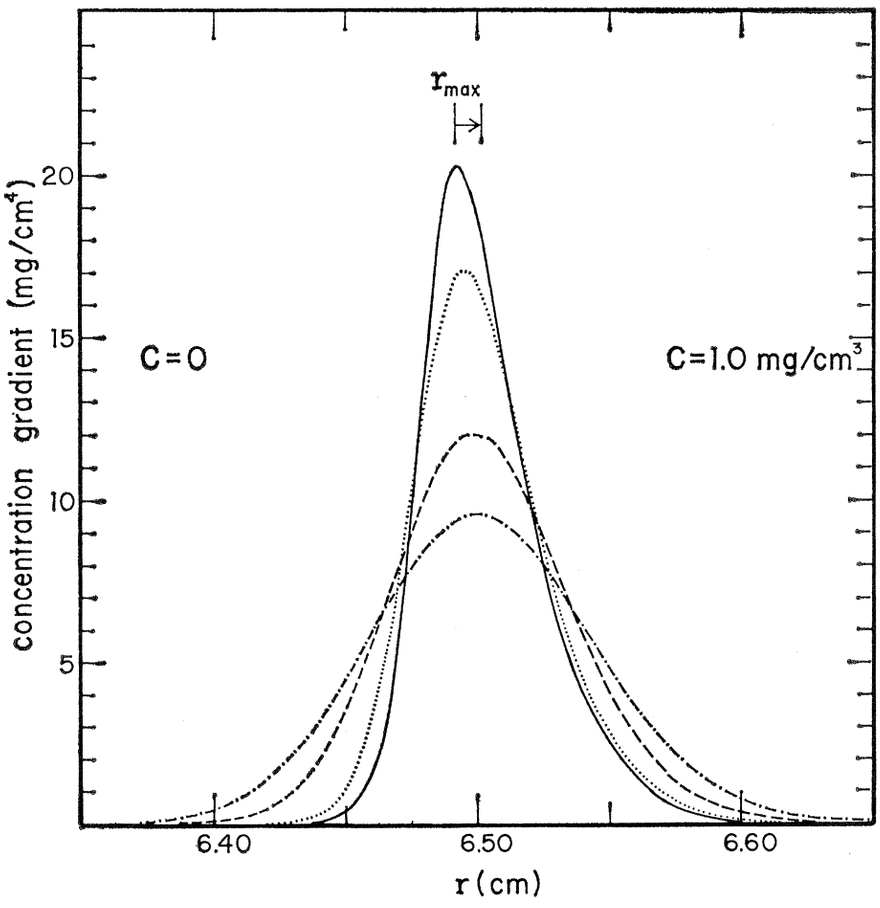

Fig. 1. Concentration gradient distribution during the simulated diffusion of an initially skewed boundary. $D = 10.0 \times 10^{-7}$ cm^2/sec. Gradient patterns shown at zero time (——); after 1 minute (. . . .), 5 minutes (- - - -), and 10 minutes (·····). The bracket labeled r_{max} shows the movement of the maximum ordinate (about 80 μ) between zero time and 10 minutes. Subsequent patterns would show continued slow movement of the peak toward the square root of the second moment, which lies about 20 μ to the right of the 10-minute position of the peak.

A gradient curve with a given Δr may be either relatively narrow and sharply asymmetric, or relatively wide and more moderately skewed. Examination of the diffusion of such boundaries by model calculations showed that the peak moves more rapidly in the former case than in the latter. If D has a value typical for a protein of moderate size, then, in a narrow and very asymmetric boundary, the peak movement may be essentially complete in a few minutes; in a wider boundary with the same Δr, the migration of r_{max} may be spread in time over a period comparable with that required to complete a velocity sedimentation experiment.

The effect of the diffusion shift of r_{max} on the results of an ultracentrifuge experiment should differ somewhat in the two cases. With a symmetrical boundary (ignoring the fact that r_{max} and $\bar{r}$ do not quite coincide, and neglecting also the effect of radial dilution on S in concentration-dependent systems), a curve of r_{max} plotted against time is linear, and the slope is $\omega^2 S$. If the initial boundary is narrow and highly asymmetric, the movement of the maximum gradient due to diffusion will be concentrated at the beginning of the run. As a result, the first few points taken will deviate from the linear curve of ln r_{max} plotted against time defined by the later points. Observations of this kind are not unusual; they are especially puzzling since the "bad" points are derived from measurements taken early in the run, when the gradient curve is sharp and its maximum is easy to locate precisely.

If the initial boundary is wide and

only moderately asymmetric, the effect of diffusion on r_{max} may be easier to overlook. The shift will continue at a decreasing rate throughout the run. As a result, the experimental curve of ln r_{max} plotted against time will be nonlinear. The curvature may not be obvious, however, particularly if there is any uncertainty in the location of r_{max}, as there may be with the relatively wide boundaries observed late in the run. If the best straight line is fitted to the points, an erroneous sedimentation coefficient will be obtained. Behavior of this kind is probably less common than that associated with narrow, highly asymmetric boundaries. Wide and moderately skewed initial boundaries seem to be most often encountered when the solute concentration is low and the boundary is not well stabilized against convection during the acceleration of the centrifuge rotor.

Fujita and MacCosham (*5*) have described the behavior of a sedimenting boundary in a standard ultracentrifuge cell, in which the gradient curve is distorted by restricted diffusion at the meniscus. A maximum in the gradient curve does not appear until the experiment has been under way for some time, and the boundary separating from the meniscus is skewed. As the boundary moves away from the distorting influence of the meniscus, the peak proceeds to overtake the position it would have occupied if the boundary had not been subjected to restricted diffusion. This interesting effect probably differs from the diffusion shift that has been described here only in the cause of the original skewing of the gradient curve. In both cases, the anomalous rate at which r_{max} moves down the cell is due to the tendency of diffusion to restore the symmetry of the gradient curve.

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Drosophila melanogaster: Inheritance of a Deficiency of Alkaline Phosphatase in Larvae

Abstract. *A deficiency of the normally prominent alkaline phosphatase zone (by starch-gel electrophoresis) has been discovered in a newly investigated laboratory strain of* Drosophila melonogaster. *Mating experiments indicate that genetic control is by an allele of a previously described electrophoretic variation. Heterozygotes resulting from crosses of the deficient type and the fast electrophoretic variant show only the fast phenotype. In deficient × slow heterozygotes, however, there is a new band that does not correspond in electrophoretic mobility with any of the bands of other heterozygous or homozygous types. It is suggested that the allele responsible for the deficiency leads to the manufacture of an inactive subunit that is able to hybridize with the subunits of the slow electrophoretic form.*

During the later hours of larval development in *Drosophila melanogaster*, a densely staining alkaline phosphatase (APH) zone is detectable when single individuals are electrophoresed in starch gels. Previously described (*1*) are the genetic control of an electrophoretic variation in that zone and the appearance of a hybrid enzyme in heterozygotes; the observations are consistent with the hypothesis that the active enzyme is a dimer consisting of two identical subunits in homozygotes, and that combination of unlike subunits in heterozygotes produces an enzyme molecule of intermediate mobility.

Investigation of the various isozyme patterns of an inbred *car* strain of *Drosophila* revealed a complete lack of demonstrable APH activity in the area of the normally dense APH component. After the deficiency was confirmed in several consecutive generations, adult flies from the stock were mated with homozygous fast (Aph^F/Aph^F) and slow (Aph^S/Aph^S) strains, and the F_1 larval offspring were examined. The deficient × fast hybrid produces only a fast band, the intensity of which is no more than slightly less than that of Aph^F/Aph^F type (Fig. 1). Examination of deficient × slow hybrid larvae reveals two bands: one corresponding to the expected slow band; the other migrating slightly faster than the hybrid band of Aph^F/Aph^S (Figs. 1, 2).

Except for the new band in the deficient × slow heterozygotes, the phenotypes are compatible with production of the deficient phenotype by a "silent" Aph^O allele. This finding is supported by the segregation ratios resulting from backcrosses, outcrosses, and $F_1 \times F_1$ matings of deficiency heterozygotes. The mating experiments are summarized in Table 1. Preliminary support for allelism has been obtained by comparing map distances between *Aph* and the *Est 6* locus, using the deficiency as well as the electrophoretic variation.

The presence of the "extra" band in Aph^S/Aph^O heterozygotes possibly results from combination of an S subunit and the product of the "silent" allele, in which case Aph^O produces protein that cannot dimerize, or does dimerize but is inactive for some reason under the test conditions employed. No indication of a double band has yet been found in Aph^F/Aph^O larvae; all attempts show only the single band, in starch gels with continuous and discontinuous buffer systems at *p*H 9.5, 8.5, 7.5, 7.0, 6.5, 6.0, and 3.0; in poly-

Table 1. Numbers of offspring from matings of various parental phenotypes, demonstrating segregation of Aph^O. Phenotypes designated F^F are from homozygous Aph^F/Aph^F flies. The designation F^O is used for F_1 hybrids from a cross between homozygous fast and deficient strains. APH, alkaline phosphatase.

Parental APH	Phenotypes of Offspring					Offspring (No.)
	F	O	SO	FS	S	
$F^F \times O$	31					31
$S \times O$			29			29
$F^O \times O$	74	52				126
$F^O \times F^F$	84					84
$SO \times O$		97	69			166
$SO \times S$			83		87	170
$SO \times F^F$	24			21		45
$F^O \times S$			70	67		137
$F^O \times F^O$	84	29				113
$SO \times SO$		27	60		20	107