The developmental study has shown that 1st instar larvae give a negative Acph reaction, while 2nd instar larvae rarely give a positive reaction. This situation is identical in all 3 phenotypes. On the other hand, positive reaction (for all 3 phenotypes) was observed in all other stages studied. However, variations in the staining intensity of the various bands were found, and these variations appeared to be stage-specific. Thus, the A phenotype showed that band 1 of 3rd instar larvae (early and late) was more intense than bands 2 and 3 which stained identically. The situation in prepupae and pupae was the same but the difference between band 1, and bands 2 and 3 was less evident. In virgin individuals (3 h after emergence) bands 2 and 3 appeared more intense and band 1 continued staining as intensely as in pupae. Thereafter, the intensities of the 3 bands could be classified as $3 > 2 > 1$ with the intensity of band 1 declining as compared to earlier stages, and the intensity of band 3 increasing; band 2 remained about the same. The B phenotype showed that band 3 of 3rd instar larvae (early and late) immediately appeared more intense and remained more intense (gradually increasing) as the individuals became older. On the other hand, band 4 increased in intensity in 3rd instar larvae, prepupae and pupae, but remained constant thereafter. Finally, band 5 appeared at first in late 3rd instar larvae, and increased through the pupal and adult stage. Overall, the order of intensity was $3 > 4 > 5$.

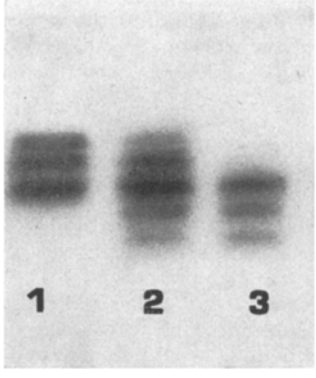

Fig. 2. Photograph of a zymogram of the 3 different acid phosphatase phenotypes of *D. auraria* (1 = phenotype B; 2 = phenotype AB; 3 = phenotype A). The zymogram was obtained from adult individuals.

Phenotype AB is produced from the cross of phenotypes A and B (Figures 1 and 2). The intensities of the 5 bands produced followed the relationship $3 > 4 > 2 > 5 \geq 1$ in adult individuals.

In order to determine the mode of inheritance of the genes controlling the Acph phenotypes, all possible crosses were performed and the results showed that the 3 phenotypes appear to be controlled by simple Mendelian inheritance. However, the question that needs to be answered is: what controls the appearance of the various Acph bands in each phenotype?

Various hypotheses could be proposed in attempting to explain the data presented in this report. The results of the crosses indicate that the acid phosphatases of *D. auraria* are controlled by 2 alleles of a single autosomal gene. This, however, is not sufficient to explain the existence of the 3 and the 5 bands that appear in the 2 homozygotes and the heterozygote respectively. This can be explained only if we were to assume that the enzymes are polymers consisting of 2 or 4 subunits and that they are controlled by 2 pairs of closely linked autosomal genes, Acph-A and Acph-B. Of these, gene Acph-A is represented by alleles Acph-A^1 and Acph-A^2 controlling enzymes with the mobility of bands 1 and 3 respectively, and gene Acph-B is represented by alleles Acph-B^1, and Acph-B^2 controlling enzymes with the mobility of the bands 3 and 5 respectively. The hypothesis requires that the one gene (Acph-A or Acph-B) is the product of a duplication of the other (as has already been described for amylases of *Drosophila*[7] and aspartate aminotransferase in fishes[8]) and that mutations created the alleles Acph-A^1 and Acph-B^2. Thus, genes Acph-A^2 and Acph-B^1 have the same origin and structure, and this explains the similar behavior of band 3 that they control in all phenotypes and in the various developmental stages.

According to this hypothesis, phenotypes A, B and AB are derived from genotypes $\frac{\text{Acph-A}^1\ \text{Acph-B}^1}{\text{Acph-A}^1\ \text{Acph-B}^1}$, $\frac{\text{Acph-A}^2\ \text{Acph-B}^2}{\text{Acph-A}^2\ \text{Acph-B}^2}$, and $\frac{\text{Acph-A}^1\ \text{Acph-B}^1}{\text{Acph-A}^2\ \text{Acph-B}^2}$ respectively.

The different activity of the Acph bands in the various developmental stages could be explained by the existence of regulatory mechanisms[7] determining the amount of enzyme to be produced by the action of each gene.

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Unstirred Layer Thickness in Perfused Rat Jejunum in vivo

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Summary. The effective unstirred layer thickness in the rat jejunum perfused in vivo amounts to at least 530 μm. This value has been estimated from the absorption increase of L-phenylalanine due to better mixing of the luminal solution by air bubbles.

The unstirred layer adjacent to the mucosal surface of the intestine may be the source of biased results: reduced permeability coefficients[1-3], raised K_m-values[4-7], shift of the pH-absorption curves to the right or the left[8-11]. When the effective thickness of the unstirred layer is known, corrections can be applied[1,2,5]. The unstirred layer thickness in the intestine can be determined in vitro by measuring the change of the potential difference after raising or lowering the osmolarity in the bulk phase[1,3,6,12,13]. In vitro the unstirred layer can be reduced by vigorous stirring[13,14] or shaking[7]. In vivo, these methods cannot be applied. By adapting the in

vitro method of FISHER and GARDNER[15], a better mixing of the luminal solution and a reduction of the unstirred layer can also be achieved in vivo: a buffered solution and air are perfused simultaneously through the intestinal lumen, so that alternately segments of fluid and air pass the lumen ('segmented flow'). From the change of the absorption rate using segmented and non-segmented flow, the reduction of the unstirred layer thickness can be estimated. This result represents the minimal value in perfusion experiments in vivo.

Jejunal loops (length about 5 cm, distance from the duodenojejunal flexure 16 to 30 cm) of male rats (330–350 g) anaesthetized with urethane were perfused (single pass) with a buffered solution (1/15 M KH_2PO_4–Na_2HPO_4, pH 6.8, 1.5 mg/ml urethane, adjusted to 320 mosm/kg with NaCl) containing ^{14}C-labelled L-phenylalanine (100 μM) and 0.1 mg/ml phenol red as volume marker. After injection of heparin into the jugular vein, the descending jejunal vein was punctured and the outflowing blood collected and weighed. The blood loss was compensated by jugular infusion of fresh heparinized rat blood. The absorption rate (nmole/min · g), more precisely the appearance rate of L-phenylalanine in the intestinal venous blood, was calculated from the weight (g) and the ^{14}C-activity (μCi/ml) of the collected jejunal blood using its specific weight (g/ml), the specific activity (μCi/μmole), the duration of the sampling period (min), and the wet tissue weight of the loop (g) obtained at the end of the experiment; details see elsewhere[16,17]. In a preperiod of 30 min, the intestinal lumen was perfused (0.5 ml/min) with a buffered solution without L-phenylalanine to suppress the transient initial absorption peak (unpublished). In 3 consecutive experimental periods (15 min each), the L-phenylalanine-containing solution was perfused in the following manner: 0.5 ml/min (treatment A), 0.5 ml/min + 0.5 ml/min air (treatment B), 1 ml/min (treatment C). The order of the 3 treatments was changed according to the 6 possible permutations of 3 elements. The order of the permutations was randomized (1 permutation per 1 rat). By this cross-over design, the 3 treatments could be compared in the same loop and the variability between rats and periods could be eliminated by an appropriate analysis of variance. In treatment B, the air and the fluid were mixed by means of a Y-shaped tube tied into the proximal end of the loop, otherwise the air inflow was interrupted. The length of the air and fluid segments amounted to 0.5 to 1 cm. The blood of the first 5 min in each experimental period was discarded. The concentration of the absorbed L-phenylalanine in the intestinal venous blood did not differ significantly in the second and third 5 min, so that an average value was calculated.

The following appearance rates of L-phenylalanine in the intestinal venous blood were measured: A) 7.4 ± 0.5; B) 12.9 ± 0.5; C) 8.5 ± 0.5 nmole/min · g (N = 6, mean ± SE based on rest variance). The increase of the perfusion rate from 0.5 to 1 ml/min (A–C) causes only a small – insignificant – increase of the absorption rate. The segmented flow increases the absorption rate significantly ($p < 0.001$) by a factor of 1.74 (B/A) or 1.52 (B/C). The reduction of the unstirred layer thickness corresponding to the increase of the absorption rate can be calculated by means of the following equation:

$$\Delta\delta = C_b \cdot D \cdot \frac{A_{UL}}{W} \cdot \left[\frac{1}{\Phi_A} - \frac{1}{\Phi_B}\right],$$

$\Delta\delta$ = change of unstirred layer thickness (μm); C_b = luminal concentration (bulk phase), here 100 μM; D = diffusion constant, here for phenylalanine at 38 °C = $0.9654 \cdot 10^{-5}$ cm²/sec (extrapolated from the 25 °C-value[18,19]); ϕ = absorption rate (nmole/min · g); A_{UL}/W = unstirred layer area related to wet tissue weight (cm²/g). The unstirred layer area was determined in situ: The loop was approximated by a cylinder. Its length was measured by means of a thread placed on the loop along its curved axis. The outer circumference was measured by a thread leading around the loop through a small hole in the mesentery closely attached to the surface. The inner circumference was obtained by subtraction of $2 \cdot \pi \cdot 0.07$ = 0.45 cm (0.07 cm = average thickness of jejunal wall). Inner circumference times length results in the area of the cylinder touching the tips of the villi. This area is considered the average area of the unstirred layer in situ and is related to the wet tissue weight of the loop: 16.0 ± 0.3 cm²/g. The reduction of the unstirred layer thickness due to the segmented flow amounts to 530 ± 90 μm. This value represents the lower limit of the effective thickness of the unstirred layer in the perfused jejunum of the rat, since the mixing in the lumen by the air bubbles is presumably not complete and does not reach into the intervillous spaces.

The unstirred layer thickness of about $^1/_2$ mm determined in vivo exceeds the thickness measured in vitro[3,6]: 150 to 200 μm. Thus, the higher K_m-values obtained in vivo[20–23] for carrier mediated transport processes in the intestine may be explained at least partly by the larger effective thickness of the unstirred layer.

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