changes in membrane structure in uranyl-treated roots provide such markers. Because uranyl is toxic, our results do not establish pinocytosis as a normal mechanism of intake of material by plants. However, the best evidence of pinocytosis was with exposures to 1 m*M* uranyl for 1 hour (Fig. 1, F and G) or to 0.1 m*M* for 20 hours (Fig. 1E). Roots were not killed by these treatments; they grew at 30 to 50 percent of the normal rate when transferred to water.

In animals, salts and proteins induce pinocytosis (*8*), and this may account for active uptake of soluble macromolecular substances (*9*). In plants, a role for pinocytosis in active ion uptake has been postulated (*10*), and an increase in numbers of pinocytotic vesicles in roots exposed to salts has been reported (*11*). Chemically characterized plant-root secretions include acidic polysaccharides and protein-polysaccharide complexes of high viscosity (*12*). Such anionic polymers would not only bind UO_2^{2+} and other cations but also could account for cell wall swelling and invaginations of the plasmalemma. Released pinocytotically they could transport bound uranyl to vacuoles. Freed of uranyl, the carriers could be reused in a cyclic system of uptake. The driving force for such a process could be the creation of complex macromolecules (*13*). In view of evidence that uranyl and calcium ions bind to similar sites (*5*), our results may represent an exaggerated and abnormal picture of a transport system which functions less conspicuously in normal plants.

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References and Notes

1. Intact germinated oat (*Avena sativa* L.) seedlings with roots 5 to 7 mm long were placed for various periods of time in solutions of uranyl acetate. After removal and desorption in three changes of distilled water, the apical 3 mm of the root was cut off and immediately fixed at 23° to 25°C in 6 percent glutaraldehyde buffered with 0.03*M* potassium phosphate (*p*H 7.4) for 1 hour, then washed twice in buffer and fixed in 2 percent osmium tetroxide in the same buffer for 2 hours. After dehydration in a graded ethanol series, the root tips were embedded in Epon [J. H. Luft, *J. Biophys. Biochem. Cytol.* **9**, 409 (1961)]. Thin sections were mounted on noncoated copper grids and stained for 5 minutes in 2 percent lead citrate [E. S. Reynolds, *J. Cell Biol.* **18**, 208 (1963)]. The magnification standard was a 28,000-line diffraction-grating replica. At least two and in most cases three or more roots of each of two oat cultivars, Compact (CI 8280) and a Victorgrain mutant (CI 7418), were examined for each treatment, and the illustrations are typical of results with these two varieties. Preliminary work with bean, barley, maize, and wheat roots has yielded results similar to those with oats, but are not extensive enough to justify conclusions about possible differences among species. The procedure for germinating oat seedlings has been published [H. H. Luke and H. Wheeler, *Phytopathology* **45**, 453 (1955)].
2. The ultrastructure of the cap region of untreated oat roots has been described (*14*) and is very similar to that of maize and wheat (*15*). The outer three to five layers of cells of the oat rootcap exhibit high secretory activity and, in this area, dictyosomes are hypersecretory, secretory vesicles are common in the cytoplasm, and intercellular spaces are filled with slime. Occasionally, accumulations of secretory products are found between the cell wall and plasmalemma in secretory cells of untreated oat roots. When treated with uranyl acetate, outer rootcap secretory cells undergo changes quite different from those in cells in the cap interior which normally do not exhibit secretory activity. Because of this, the effects of UO_2^{2+} on cells in these two regions will be discussed in separate sections. The rootcap was chosen for this study partly because its ultrastructural features were well known and partly because it represents a mass of cells isolated from the vascular system of the plant. We emphasize that the course of events found in the rootcap may not apply to other regions of the root. In preliminary work, crystals have been found localized in vacuoles in the root hair region of roots exposed to uranyl for as little as 10 minutes. The fragility of root hairs and their tendency to rupture when placed in hypotonic solutions preclude any conclusion as to how such localization occurred.
3. We will use pinocytosis as a general term for the intake of water or solids by invagination of the plasmalemma as defined by F. A. L. Clowes and B. E. Juniper [*Plant Cells* (Blackwell, Oxford, 1968), p. 490].
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Developmental Variation in the Isoenzymes of Human Liver and Gastric Alcohol Dehydrogenase

Abstract. *The isoenzyme patterns of alcohol dehydrogenase from human liver and stomach are different in the fetal, newborn, childhood, and adult periods of life. This provides additional evidence that the fetal and adult alcohol dehydrogenases are qualitatively different. Gastric and hepatic isoenzyme patterns also differed from each other.*

Our understanding of the mechanism of differentiation may be advanced by studies which use specific developmental markers such as proteins that are present in the wild type of a species during a restricted period of development. Developmental markers already known in man include embryonic and fetal hemoglobins (*1*), the lactate dehydrogenases (*2*), the esterases (*3*), and creatine phosphokinases (*4*).

The activity of liver alcohol dehydrogenase (LADH) increases with fetal development in rats, guinea pigs, and man (*5*, *6*). Values of the Michaelis constant (K_m) and optimum values of *p*H differ between the human fetal and adult forms of this enzyme (*6*), and developmental variation in the isoenzymes of human LADH has also been reported (*7*). This study shows that the developmental variation of human LADH and human alcohol dehydrogenase from gastric mucosa (GADH) continues up to puberty.

Tissues were obtained 12 to 36 hours after death. Specimens from spontaneously aborted fetuses were obtained 12 to 24 hours after passage. An occasional fetal specimen was obtained by hysterotomy. All fetuses used had no visible external or internal malformations and tissues were analyzed immediately or stored at −20° to −25°C.

Tissues were homogenized at 0° to 4°C. The homogenate was diluted 1 : 5 with buffer prior to enzyme assay (*8*) and was applied directly to agarose gel for electrophoresis. Agar and agarose gel electrophoresis was performed according to a modification of the method described by Ursprung (*9*), and protein concentration was measured by a micromodification of the Lowry method (*10*).

Table. 1. Number and proportion of adult, newborn, and fetal samples in different ranges of LADH activity. The mean and range of LADH activity in milliunits per milligram of protein in adults was 79.8 (3 to 319), in newborns was 45.5 (2 to 164), and in fetuses was 15 (1 to 24).

Range of LADH activity in milliunits per milligram of protein	Analysis					
	Adult		Newborn		Fetal	
	No.	%	No.	%	No.	%
0–14	15	30	7	31.8	12	80
15–79	16	32	11	50.0	3	20
80+	19	38	4	18.2	0	00

$\chi^2 = 17.378$, $.001 < P < .01$.

The electrophoresis bands of alcohol dehydrogenase were identified by comparison with partially purified enzyme and by comparison of staining intensity with and without ethanol. Agar or agarose gel was used to reduce the likelihood that differences in banding patterns might be due to the separation of different enzyme-coenzyme complexes through the sieving properties of starch gel (*11*).

Fifty percent (weight/volume) homogenates from adult human brain, muscle (skeletal and cardiac), skin, spleen, leukocytes, fibroblasts, stomach, and liver were tested, but only homogenates of gastric tissue and liver showed readily defined patterns after the electrophoresis.

The agar or agarose gel pattern of human fetal LADH consisted of two anodal zones and a single, slow moving cathodal zone (Fig. 1, channel 1). Fifty fetuses (21 to 260 mm crown-to-rump; estimated gestational age, 45 to 184 days) showed this pattern (LADH 1) at *p*H 6.0 to *p*H 9.0. Later in gestation, a second, less intense, but more cathodal band appeared (LADH 2) (Fig. 1, channel 2). This band was also found in the patterns of most premature infants (870 to 2000 g birth weight). A third, still more cathodal band (LADH 3), varying in intensity, appeared at full term and was also detected in older infants (Fig. 1, channel 3). One of the 50 fetal specimens showed, on repeated study, a single cathodal band with slightly lower mobility than the normal fetal band, and this slower band might represent a structural variant of the "fetal" LADH.

Additional evidence that variation in banding pattern was related to age came from studies of liver homogenates obtained at autopsy from one Caucasian family consisting of the two parents and their six children (*12*). One child (aged 3 years) had a pattern similar to that seen in newborn infants, showing LADH bands 1, 2, and 3 (Fig. 1, channel 3). Three of the children (aged 5, 7, and 8½ years) had patterns showing LADH 1, 2, and 3 as intense bands and in addition a less intense band (LADH 4), which had the greatest mobility toward the cathode (Fig. 1, channel 4). The two other children (aged 11 and 14 years) had patterns like those of their parents, with weak LADH bands 1 and 4 and a strong LADH 2 band (Fig. 1, channel 5). Specimens from two children (aged 4 years) unrelated to the above-mentioned family had patterns like that of the 3-year-old child (Fig. 1, channel 3).

Two common LADH isozyme patterns were found in homogenates from different adult livers. Both types showed two weak anodal zones of LADH activity. The most frequent pattern showed three cathodal zones (Fig. 1, channel 5) corresponding in mobility to LADH 1, 2, and 4. In this pattern the LADH 2 band always showed the most intensity, and prolonged electrophoretic runs suggested that the LADH 1 in some adults moved slightly faster than LADH 1 in fetal specimens. The other frequent adult LADH pattern had only two bands, LADH 2 and 4 (Fig. 1, channel 6). In this pattern LADH 4 was always most intense, and an LADH 1 band was not present.

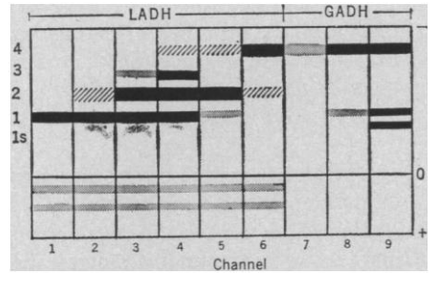

Fig. 1. The electrophoretic banding pattern of LADH and GADH in homogenates of human livers and stomachs at different ages on agarose gel at *p*H 8.7. As much blood as possible (blood does not contain alcohol dehydrogenase activity) was removed, and the tissue was weighed. A 20 percent (weight to volume) proportion was used. Tissues were homogenized in 0.25*M* KCl, *p*H 7.4, containing $10^{-3}M$ β-mercaptoethanol and $10^{-4}M$ diisopropyl fluorophosphate. One milligram of NAD was added to each 100 ml of agarose gel. Channel 1, fetus; channel 2, premature infants; channel 3, full-term, newborn infants up to 5-year-old children; channel 4, 5- to 9-year-old children channel 5, 11-year-old children to adult (most common type); channel 6, adult (less common type); channel 7, fetus; channel 8, newborn infants; channel 9, adults.

Homogenates of adult liver specimens obtained by needle biopsy or open surgical biopsy showed the same electrophoretic patterns as autopsy specimens from adults. Any given specimen showed the same banding pattern over a 1-year period; and no interconversion of banding pattern was produced by storage, addition or removal of nicotinamide adenine dinucleotide (NAD) (*13*), heat, or proteolytic enzymes. Mixing homogenates of adult and fetal livers produced the sum of the individual banding patterns.

Developmental variation was also noted in the GADH isoenzymes (Fig. 1, channels 7, 8, 9). Homogenates of gastric specimens from embryos (100 to 195 mm crown-to-rump) had a single, low intensity, rapid-moving cathodal band with a mobility like that of the LADH 4 band in adult liver homogenates (Fig. 1, channel 7); and therefore this band has been designated GADH 4. In the newborn, a second cathodal GADH band, which moved like LADH 1, appeared and the GADH 4 band increased in intensity (Fig. 1, channel 8). The fetal, newborn, and adult gastric isoenzyme patterns were all different from the hepatic pattern at the corresponding ages. The adult pattern of GADH consisted of one very rapid and two much slower cathodal bands, the slowest of which was slower than LADH 1 and has been called GADH 1s.

No qualitative change in GADH banding pattern was noted after storage for up to 7 months at −20°C. The intensity of GADH bands was strikingly reduced beyond 18 hours postmortem, but again no qualitative change in banding pattern was noted.

Our study, in contrast to an earlier report (*6*), found no linear correlation between LADH enzyme activity and the crown-to-rump length of the fetuses, weight of the newborns, or age of the adults. This difference may be related to the fact that fetal tissue was obtained by therapeutic abortion in the Scandanavian study, while spontaneous abortuses comprised the majority of our samples.

The differences in the distribution of LADH enzyme activity in adult, newborn, and fetal samples are statistically significant ($\chi^2 = 17.378$, $.001 < P < .01$) (Table 1). The significance is due to differences in the distribution of adult and newborn samples versus the fetal samples ($\chi^2 = 10.827$, $P < .001$). There is no significant difference in the distribution of enzyme levels between adult and newborn samples.

In another investigation, gradient elution from a carboxymethyl cellulose column demonstrated three peaks with LADH activity in some individuals and two LADH activity peaks in others (*8*). This variation was similar to the variation in number of cathodal adult isoenzymes found in our study.

Our results indicate at least three transitional periods in the ontogeny of LADH in man; the first occurs during late fetal life, the second at from 4 to 6 years of age, and the third between 11 and 14 years of age. Although the developmental variation in creatine phosphokinase (*4*), esterase (*3*), lactate dehydrogenase (*2*), and hemoglobins (*1*) seems to be almost complete or complete by the newborn period, this study indicates that developmental change probably continues for a longer period in human LADH than in other developmental markers thus far described in man. Since the primary metabolic function of LADH in man is unknown, no reason can be given for this variation.

Because LADH from human fetuses has not yet been purified, any differences in primary structure between the fetal and adult enzyme are still unknown. However, the finding that, compared with the adult enzyme, fetal LADH has a different K_m and optimum *p*H value as well as a characteristic electrophoretic pattern (*6*) suggests that fetal and adult LADH are structurally different proteins.

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Krabbe's Globoid Cell Leukodystrophy: Deficiency of Galactocerebrosidase in Serum, Leukocytes, and Fibroblasts

Abstract. *The activity of galactocerebroside β-galactosidase was extremely low in serum, leukocytes, and cultured fibroblasts of patients with Krabbe's disease. Antemortem diagnosis is possible without organ biopsies. The parents of patients showed enzyme activities generally lower than that of normal controls. This finding provides supportive evidence that the deficient activity of galactocerebroside β-galactosidase is the genetically determined enzymatic defect underlying the disease. Demonstration of this deficiency requires the use of the specific substrate, galactocerebroside. Assays carried out with synthetic, unnatural substrates, such as 4-methylumbelliferyl β-galactoside, do not distinguish patients or heterozygous carriers from normal individuals.*

Krabbe's globoid cell leukodystrophy is a genetically determined and rapidly fatal neurological disorder of infants, characterized by almost total loss of myelin, severe gliosis, and massive infiltration of the unique multinucleated globoid cells in white matter. Deficient activity of galactocerebroside β-galactosidase has been demonstrated in this disease (*1*). We have extended our study to readily available materials such as peripheral blood and cultured fibroblasts in an attempt to establish a means of antemortem diagnosis of the disease. We also assayed samples from parents of patients with Krabbe's disease, because a partial deficiency of the enzyme would be expected in heterozygous carriers, if the lack of galactocerebroside β-galactosidase activity is indeed the genetically determined defect.

Serum was separated from blood clot by centrifugation, and the enzyme assays were performed immediately, because both galactocerebroside β-galactosidase and 4-methylumbelliferyl β-galactosidase were unstable in serum and rapidly lost activities even if serum samples were stored frozen or refrigerated (Fig. 1). A similar instability of enzyme in serum was reported for another specific galactosidase, galactosylgalactosylglucosylceramide:galactosyl hydrolase (*2*). Leukocyte suspensions were prepared essentially by the method of Snyder and Brady (*3*), with minor modifications (*4*). Fibroblasts were cultured from skin (*5*) and collected by trypsinization. Both leukocytes and fibroblasts were frozen and thawed several times before enzyme assays, and fibroblasts were disrupted with high-frequency sound. β-Galactosidases in leukocytes were stable when kept frozen, with only a slight loss of galactocerebroside β-galactosidase activity over an 8-week period (Fig. 1). The protein concentrations of leukocyte and fibroblast preparations were determined

Table 1. Activity of β-galactosidases in serum.

Subject	Galactocerebroside β-galactosidase (nmole/hr/100 ml)	4-Methylumbelliferyl β-galactosidase (nmole/hr/ml)
Patient	0	13.8
Father	6.5	4.8
Mother	3.2	5.6
Brother 1	5.8	4.8
Brother 2	27.7	6.0
Child control	15.5	6.0
Adult control 1	19.2	8.6
Adult control 2	23.6	5.0
Adult control 3	15.1	7.8