perature of 20° C. No food was provided.

Previous studies of populations of this species indicate that freshly collected animals show an activity rhythm characterized by two (usually unequal) bursts of activity per day, synchronized with the tides on the shore at the point of collection. This rhythm can be entrained in the laboratory by mechanical stimuli (*4*). Several features of the record in Fig. 2 are of biological interest: (i) a tendency for more intense activity to recur at intervals of about 14 to 16 days; (ii) a free-running period of the rhythm which shortened during the recording; and (iii) an apparent drifting together of two components of the rhythm about midway through the record. A simultaneous recording from another, adjacent animal which survived about 60 days indicates that neither the days nor the times of most intense activity coincided for the two animals (most intense activity for the other animal: 10 to 12 p.m., 4 April to 9 April; and midnight to 4 a.m., 8 and 9 May). Such results demonstrate that these features of the recordings are not due to influences of the environment on either the animals or the activity sensors.

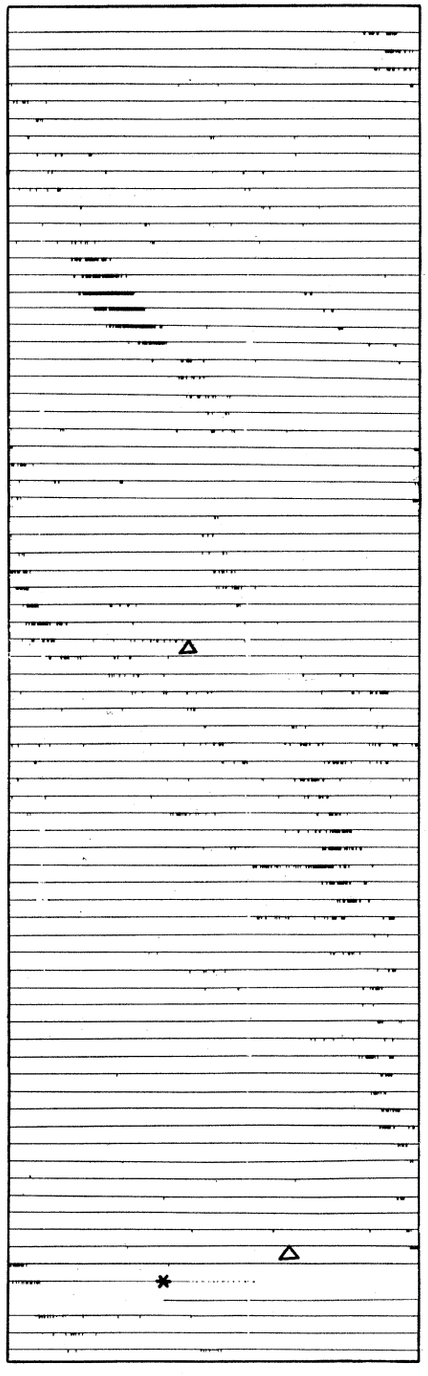

Fig. 2. Record (77 days) of the swimming activity of an isolated adult isopod, *Excirolana chiltoni,* under constant conditions. Successive 24-hour strips of record (midnight to midnight) mounted beneath each other. Triangles indicate times at which distilled water (about 30 ml) was added to replace evaporation; asterisk indicates pen failure, corrected about 24 hours later.

A subsequent recording from another isopod indicates that repetition of sessions of intense activity at intervals of about 2 weeks is not a unique feature of the behavior of the animal whose activity is shown in Fig. 2. During a recording from 9 June to 5 July 1966, this third animal showed very intense activity between 4 and 8 a.m. 11 to 13 June, and between 5 and 8 a.m. 25 to 28 June.

The device described here could obviously be used in its present form for long-term, inexpensive monitoring of the activity of larger aquatic organisms than are considered here (for example, fish of any size in a larger aquarium). Modification of the apparatus for use with much smaller aquatic animals also appears to be possible by using thermistors of much smaller thermal inertia, or by inserting an additional transistor in the transducer circuitry. Such modification has not yet, however, been attempted; environmental factors, including temperature, vibration and evaporation, as well as instrumental noise and drift will ultimately place a lower size limit on the organisms for which the sensing principle is useful (*5*).

A. A. HEUSNER*
Center for Health Sciences, University of California, Los Angeles
J. T. ENRIGHT†
Department of Zoology, University of California, Los Angeles

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* Present address: Institut de Physiologie, Faculté de Médecine, Strasbourg, France.
† Present address: Department of Oceanography, Scripps Institution of Oceanography, University of California, La Jolla.

18 July 1966

Electrophoretic Heterogeneity of Mammalian Galactose Dehydrogenase

Abstract. *Electrophoretically distinct forms of galactose dehydrogenase were demonstrated in various tissues of the rat. Phylogenetic comparisons revealed considerable variation with species in mammalian liver zymograms; virtually no activity was demonstrable in fish, pigeon, and frog liver. Ontogenetic studies of the rat revealed sequential appearance of liver isoenzymes.*

D-Galactose can be converted to D-xylulose by rat liver enzymes (*1*); the first enzyme involved, galactose dehydrogenase, has been purified 100-fold and its properties have been studied (*2*); it is a nicotinamide adenine dinucleotide (NAD)–requiring enzyme found in the soluble cellular fraction of the liver of several mammalian species. We now describe electrophoretically distinct forms (isoenzymes) of galactose dehydrogenase in rat tissues, compare the electrophoretic patterns of liver extracts from different species, and describe changes that occur in the developing rat liver.

Vertical starch-gel electrophoresis was performed with 0.0053*M* phosphate buffer, *p*H 6.7, at 5 volt/cm for about 15 hours at 4°C. A thin layer of gel was then sliced and incubated at room temperature for about 45 minutes in a mixture, modified from that used for staining lactic dehydrogenase (LDH) isoenzymes (*3*), that contained phenazine methosulfate (0.02 mg/ml), NAD (1 m*M*), nitroblue tetrazolium (0.5 mg/ml), sodium cyanide (1 m*M*), tris buffer, *p*H 8.4 (50 m*M*), and galactose (50 m*M*).

Tissues of Sprague-Dawley rats were homogenized in 0.01*M* phosphate buffer, *p*H 7.0, and centrifuged at 32,000*g* for 90 minutes. The dialysed supernatant was separated by starch-gel electrophoresis. Liver microsomal prep-

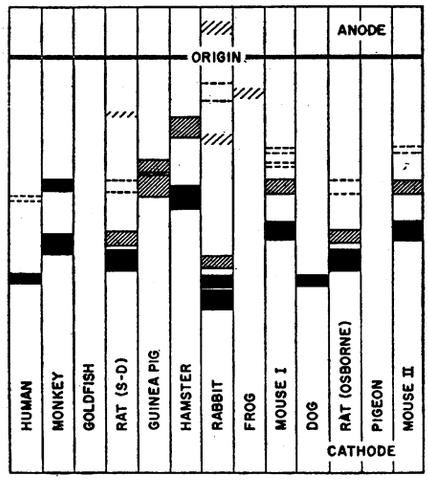

Fig. 1. Diagram of galactose dehydrogenase isoenzymes from liver preparations from 11 vertebrate species, separated by starch-gel electrophoresis; relative intensity of staining of the bands is not related to galactose concentration.

arations were obtained by ultrasonic treatment of the 8000 to 100,000g pellet. Enzyme activity was assayed spectrophotometrically by following the rate of reduction of NAD at 340 mμ (*2*).

The galactose dehydrogenase isoenzymes are basic proteins, as indicated by their migration toward the cathode at *p*H 5.3 to 8.1; five were found in rat liver preparations. The most rapidly migrating bands stained darkest, indicating greater enzyme activity. The slowest band (band 5) stained very lightly and was lost during purification procedures. Kidney, testis, gut, and mammary gland preparations had only band 5. The specific activity of these tissues was less than 10 percent of that obtained with liver extracts; brain, muscle, spleen, lens, red cells, white cells, and plasma had no measurable activity. Liver microsomal preparations produce only one band, which was identical with band 5. Eighty-five percent of the galactose dehydrogenase activity of liver cells was soluble (*2*), in contrast with mammalian glucose dehydrogenase, which is found exclusively in microsomal fractions of liver (*2, 4*). Rat hemoglobin migrated near band 5, and the most cathodal LDH band (LDH-5) migrated slightly slower.

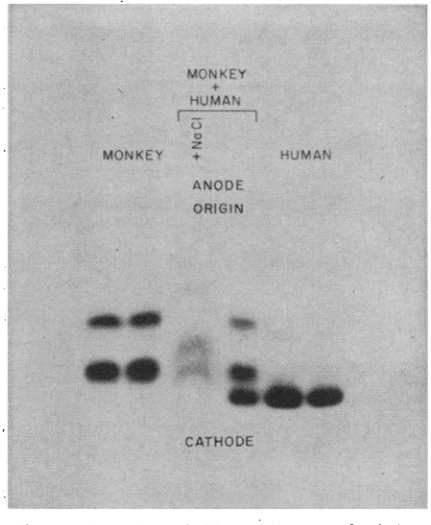

Fig. 2. Starch gel illustrating typical isoenzyme patterns obtained by mixing liver extracts of different species. Equal parts (50 μl) of the extracts were mixed, and 50 μl of 0.1M phosphate buffer (*p*H 6.9) or of 3M NaCl was added; the mixtures were frozen for 4 hours at −20°C.

Human liver preparations show one major fast-moving galactose dehydrogenase band and one very weakly staining band similar in mobility to rat-liver galactose dehydrogenase band 4. (Fig. 1). Human red and white blood cells, obtained by fibrinogen sedimentation (*5*) and ultrasonically disrupted, lack activity and show no bands on electrophoresis.

Comparative study of the electrophoretic mobilities of liver preparations from 11 vertebrate species (Fig. 1) indicated marked heterogeneity of the enzyme, as has been described for red-cell LDH (*6*); at least 16 electrophoretically distinguishable isoenzymes are visualized, and no strain differences were detected in two rat and three mouse strains. Three adult human liver extracts showed identical patterns. The major bands of human and dog liver were electrophoretically indistinguishable. Two of the nonmammalian species (goldfish and pigeon) showed no detectable activity on starch gels, and frog demonstrated only one very weak band. All mammalian livers assayed had substantial galactose dehydrogenase activity; activity is undetectable in frog or goldfish liver extracts and weak in pigeon liver. It is not clear from these studies whether the phylogenetic differences are somehow related to the exposure of mammals to dietary galactose.

The electrophoretic species differences probably did not reflect differences in the environmental conditions of the homogenates, since mixture of homogenates from different species resulted in patterns that are strictly additive (Fig. 2). After freezing of the enzyme mixtures in 1M NaCl, a technique used to obtain subunit recombinations of LDH (*7*), the number of bands was the same but the mobilities were slower and the bands were distorted, suggesting that protein denaturation had occurred. Progressive dilution of liver extracts resulted in lighter-staining bands without change in mobility, indicating that differences in protein concentration do not account for the differences in electrophoretic mobility. Elution and electrophoresis of the faster-moving galactose dehydrogenase band of monkey liver resulted in a single weak band of similar mobility. Recovery of enzymatic activity by elution was very low, and reruns of the eluted minor bands lacked enough activity for detection.

The zymogram patterns of rat liver extracts obtained during various stages of development demonstrated sequential changes (Fig. 3). Extracts of liver obtained from 18- and 19-day fetuses showed only a very weak galactose dehydrogenase band 4, and the 20-day fetal liver demonstrated in addition a light-staining band 1; band 1 increased sharply in intensity in the liver of newborn rats. Band 2 first appeared in the 5-day animal. The zymograms from the 15-day and adult rats were identical except for the presence of band 5 in the adult. These changes are similar to those observed for LDH of chick tissues, but in the latter case all isoenzymes are present at all stages of development, and alterations occur in the distribution and intensity of activ-

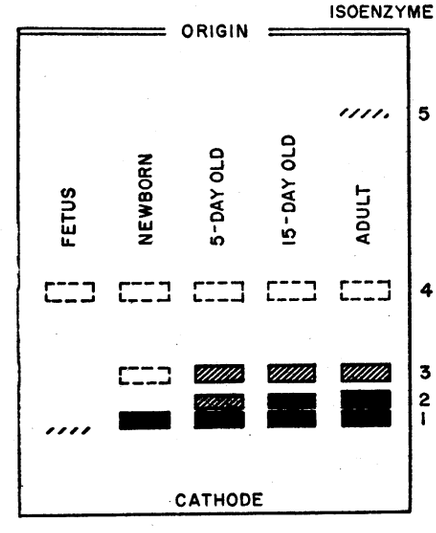

Fig. 3. Diagram of the galactose dehydrogenase isoenzymes from rat liver, obtained during various stages of development and separated by starch-gel electrophoresis. The fetus was 20 days old; the adult pattern came from livers from animals older than 30 days.

ity rather than in the appearance of new bands (*8*). Galactose dehydrogenase specific activity is barely detectable before birth, whereupon a sudden increase results in a maximum in animals 5 days old, which falls slowly to the adult level in animals aged 30 days (*2*). The zymogram patterns suggest that the fall in specific activity after 5 days of age does not result from selective depressions of isoenzymes.

Differences among the various isoenzymes, other than electrophoretic mobility, remain to be determined. No differences in Michaelis-Menten kinetic constants, *p*H optimum, or heat-inactivation behavior were found between liver galactose dehydrogenase isolated from newborn and adult rats (*2*); it is not known whether the enzyme contains a subunit type of structure such as that in LDH. Further purification of the enzyme, and isolation of the individual isoenzymes in high yield, will probably be necessary to resolve these questions. Search for variants of human isoenzymes will be hampered by the limited availability of tissue for study: only liver contains appreciable activity.

PEDRO CUATRECASAS
STANTON SEGAL
Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland

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31 August 1966

Polymorphism of Heavy-Chain Genes in Immunoglobulins of Wild Mice

Abstract. *The serums of 123 wild mice from six different geographic locations in the United States contain five of the six known heavy-chain antigenic determinants that have been identified in immunoglobulin of inbred laboratory strains of mice. On the basis of the distribution of determinants in inbred strains, 44 of the mice were judged to be heterozygotes of various combinations and two had combinations of determinants that were unusual and could only have occurred in laboratory inbred mice by recombination.*

We have examined the serums of wild mice for antigenic determinants present in immunoglobulins of inbred laboratory strains. One of the determinants was not found, while other determinants were present in various combinations suggestive of heterozygosity and, in two instances, of recombination in mice.

The wild mice were trapped in different geographic areas of the United States. Antigenic determinants were identified with precipitating isoantiserums; that is, antiserums prepared by immunization of inbred mice with the immunoglobulins from genetically different inbred mice (*1–7*). Some of the isoantiserums provided a means of identification of antigenic determinants on specific heavy-chain immunoglobulin molecules (*4, 6*), while others were used to identify determinants on immunoglobulins not yet assigned to a specific class (*2, 5*). These isoantiserums have made it possible to study the genetic control of heavy-chain antigenic determinants. In the mouse the immunoglobulin genes A, G, and H, controlling the three heavy chains of γA, γG, and γH immunoglobulins, respectively, are closely linked (*3, 4, 8*). Each of four isoantiserums provided identification for specific determinants on proteins that were shown by immunoelectrophoresis to be immunoglobulins (probably γG) (*2*). These determinants, called 2, 3, 4, and 5, were found separately among different groups of homozygous inbred strains designated Asa2, Asa3, Asa4, and Asa5 (Table 1) (*2*; see *9*). Homozygous mice carry one of these determinants, heterozygotes carry two, but no inbred mouse has been found which carries three. In 14 of the 38 inbred strains studied, the 2, 3, 4, and 5 determinants were not found, and this group was designated Asal (al) (*2*; see also *9*). Isoantiserums reacting with a determinant only present in the a1 group are difficult to prepare since most immunoglobulin determinants of the a1 strain are widely distributed among the inbred strains carrying the immunoglobulin determinants 2, 3, 4, and 5. However, we were able to identify antigenic determinants on immunoglobulins in the a1 group with two specific isoantiserums. With one, we identified a determinant G6 on the heavy chain of the γG immunoglobulin, while with the other isoantiserum we identified a determinant H9 on the heavy chain of the γH immunoglobulin (*4, 6*). The specificity of these two isoantiserums could be determined because immunoglobulins carrying specific determinants can be isolated in pure form from BALB/c mice with transplantable plasma cell tumors (*10*). The BALB/c belongs to the a1 group of mice (*2*).

The determinants identified in 38 homozygous inbred strains by use of the six isoantiserums described (Table 1) are G6, H9 for group a1; 2, H9 for a2; 3, H9 for a3; 4, G6 for a4; and 5, H9 for a5.

Most inbred strains now in use are derived from assorted European and Asian stocks (*11*), and the distribution of immunoglobulin genes in domesticated stock and "wild type" *Mus musculus* should be quite different. We therefore determined the immunoglobulin phenotypes and genotypes of wild *Mus musculus*. Serums from wild *Mus musculus* and *Peromyscus* were collected in 1961–62 (*12*). None of the *Peromyscus* serums tested with a variety of specific determinant antiserums (Table 1) showed a precipitin reaction. The serums were diluted 1:5 and 1:10 when collected, and were then frozen until tested.

Serums collected from 123 wild *Mus Musculus* were each tested in Ouchterlony plates (Table 1). The distribution of determinants among the wild mice and their genotypes based on the determinants present was investigated (Table 2). We see that none of the 123 wild mice showed the 2 determinant found in the a2 group of inbred strains. Among the inbred strains, the incidence of the determinant 2 is fairly high, and of 38 inbred strains nine showed this determinant (*2*). Isoantiserums to determinant 2 are the easiest to prepare, and immunoglobulins of a2 mice appear to be equally highly anti-