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Developmental Changes of Mammalian Lactic Dehydrogenases

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Evidence is presented supporting the view that the five electrophoretic forms of lactic dehydrogenase found in animals represent two distinct types of enzymes (M and H) with three intermediate hybrids. Data indicating that the hybrids are formed at random are given. Evidence is also given showing that, when the electrophoretic patterns do not follow a binomial distribution, more than one cell type is involved. In the rat the embryonic form of lactic dehydrogenase is the M form; during development and maturation in rat heart, there is a shift from M type of lactic dehydrogenase units to H type. Data have also been obtained indicating that the rabbit and bovine embryonic forms are the M type of lactic dehydrogenase. In contrast, the human embryonic type is the H form. Great variations in the composition of the two types of lactic dehydrogenase in the livers of adult mammals as well as in other animals have been found. The significance of the two distinct lactic dehydrogenases and the possible factors that control the changes in development are discussed.

In recent reports, we presented evidence indicating that two types of lactic dehydrogenase (LDH) exist in most animals (Cahn *et al.*, 1962b; Kaplan and Ciotti, 1961a). These two types appear to be controlled by separate genes and are different in their amino acid compositions, catalytic characteristics, physical properties, and immunologic reactions. We have designated one type as M, since it occurs largely in skeletal muscle, and the second, which is usually present in cardiac muscle, as H. Both types appear to exist as tetramers consisting of identical polypeptide units. We have designated these aggregate forms as M_4 and H_4. In addition to the two homologous forms, hybrids between the two types also containing four units have been identified as intermediate electrophoretic entities. We have designated these hybrids M_3H_1, M_2H_2, and M_1H_3. The form M_3H_1 contains three M subunits and one H unit, M_2H_2 two units of each type, and M_1H_3 one M peptide chain and three of the H type. These hybrids have intermediate immunologic, catalytic, and physical properties.[1]

We have previously reported that the lactic dehydrogenase of the heart of the newborn rat was different from that of the adult. This difference was deter-

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mined by a comparison of the rates of reaction with analogs of NAD[2] (Kaplan and Ciotti, 1961b). These variations led us to make a more thorough study of the changes in lactic dehydrogenase in the prenatal and postnatal animal. It is the purpose of this paper to present detailed data on the changes that occur in rat heart and also to discuss the changes in molecular forms of the lactic dehydrogenase which take place in other tissues of the rat—as well as in other animals.

Materials and Methods

Preparation of Tissue Extracts.—Freshly dissected or frozen tissues were diced, added to cold 0.25 M sucrose in a ratio of 200 mg tissue per ml of sucrose, and ground in an all-glass Potter-Elvejhem motor-driven homogenizer. Centrifugation of these extracts for 1 hour at a maximum centrifugal force of 144,000 $\times g$ yielded a supernatant fluid which we have used for both the catalytic and electrophoretic assays.

Separation on Starch Grain.—The crude extract, 1.5 ml, was separated electrophoretically on a starch medium in barbital buffer, *p*H 8.6, with an ionic strength of 0.05. The electrophoretic method used was that described by Kunkel (1954) except for the following changes: Blocks were prepared in lucite molds (53 × 7 × 0.5 cm) which had a cellophane sheet pressed to the inner surfaces and extended over the edges. Excess buffer was removed by pressing blotting paper to the top of the block. After application of the sample, wicks made of eight thicknesses of Whatman No. 1 filter paper cut to fit the width of the block were dampened with buffer and pressed to the top of the block at both ends. The cellophane sides were folded over the top of the block and cut at the outer edges of the wicks, thus completely encasing block and wicks. Separation was carried out at 4° with a voltage of 410 volts for 20 hours.

At the conclusion of the separation experiment, the bands of lactic dehydrogenase were localized in the following manner: The cellophane was folded back, the wicks were removed, and the block was blotted to withdraw excess moisture. A spatula was used to cut a narrow slit, about 5 mm from one side, along the length of the block. The slit penetrated no deeper than just below the surface of the block. A solution consisting of 0.0055 M acetylpyridine adenine dinucleotide, 0.08 M lithium lactate, and 0.1 M Tris-HCl, *p*H 9, was applied with a syringe along the slit. The block was rewrapped with the cellophane and allowed to develop at 4° for 1 hour. When viewed under long-wave ultraviolet light, the bands of lactic dehydrogenase were indicated by the fluorescent spots of reduced acetylpyridine adenine dinucleotide along the slit. The block was sliced on both sides of the fluorescent spots and the segments medial to the fluorescent spots were taken. Each segment was packed in a medium grain-sintered glass filter, and the lactic dehydrogenase was eluted by the addition of aliquots of 0.1% bovine serum albumin in 0.05 M potassium phosphate buffer, *p*H 7.5, above the starch and then application of gentle suction. Each segment was washed three times with 2-ml aliquots of the buffer. This procedure resulted in total displacement of the lactic dehydrogenase from the starch.

Starch Gel Electrophoresis.—Starch gel electrophoresis was carried out by a modification of the method of Smithies (1955). A 14% gel of the dimensions 19.5 × 6.9 × 0.6 cm was used for all experiments. The gel was made up in the following *p*H 7.0 buffer: 7.0 ml of 0.2 M citric acid plus 43.0 ml of 0.2 M Na_2HPO_4 plus 950 ml of H_2O. Approximately 0.02 – 0.04 ml of enzyme solution was applied on 2 – 4 thicknesses of Whatman No. 1 filter paper which was then inserted at the origin. Wicks made of 8 thicknesses of Whatman No. 1 filter paper were used for electrical connections to the gel. Liquified petroleum jelly was poured on the exposed surface of the cooled gel and allowed to solidify, thus protecting the gel from loss of water by evaporation. The electrode buffer of *p*H 7.0 was made by adding to 180 ml of H_2O 20 ml of 0.2 M citric acid and 160 ml of 0.2 M Na_2HPO_4. Agar-saturated KCl bridges were used to connect the electrode chambers to the chambers in which the wicks were immersed. Separation was carried out for 18–20 hours at 4° with a voltage gradient of 7.5–12.5 volts/cm across the gel and a constant current of 24–27 milliamps. After the separation experiment the gel was cut into 3 slices. Lactic dehydrogenase was localized in the gel by incubating the center slice, 2–3 mm thick, in the following solution for 15–120 minutes at 37° in the dark: Tris buffer, *p*H 8.0, 0.1 M, 26.5 ml; lithium lactate, 2.0 M, 1.5 ml; phenazine methosulfate, 5 mg/ml, 0.12 ml; nitro blue tetrazolium, 10 mg/ml, 1.0 ml; NAD, 30 mg/ml, 0.6 ml. This staining procedure is a modification of the method of Dewey and Conklin (1960).

Enzyme Assay Method.—All kinetic measurements were made on a Zeiss spectrophotometer, model PMQ II, with quartz cuvets with a light path of 1 cm and at a temperature of 26°. Levels of lactic dehydrogenase activity in the extracts and in the eluates from the starch grain experiments were determined by measuring the oxidation of NADH. The reaction mixtures consisted of 0.1 M potassium phosphate buffer, *p*H 7.5, 1.5×10^{-4} M NADH, and 3×10^{-4} M pyruvate in final concentrations. The reaction usually was started by the addition of a solution containing the lactic dehydrogenase.

For a characterization of the lactic dehydrogenase composition, we have used a comparison of the rate of reaction obtained with NHXADH and a low concentration with that of NADH with a high level of pyruvate. The low pyruvate level was at a concentration of 1×10^{-4} M, and the high amount was 3.3×10^{-3} M. The amount of reduced coenzyme added in all cases was the same as that used in the standard assay. Measurements were usually compared between 30 and 90 seconds after the initiation of the reaction. Readings were taken at 340 mμ. The ratio, $NHXDH_L/NADH_H$, signifies the rate with the reduced hypoxanthine analog at the lower pyruvate concentration divided by the rate with the reduced natural coenzyme at the higher keto acid level.

Results

Lactic Dehydrogenases of the Rat.—In the rat five electrophoretic bands of lactic dehydrogenase can be

[1] In a recent report Millar (1962) has reported that the beef H lactic dehydrogenase consists of two subunits and that the molecular weight of the enzyme is 72,000. This conclusion is based partly on the observation that there was a concentration dependence on the sedimentation constant. Mr. A. Pesce of our laboratory has not been able to confirm Millar's finding on the concentration dependence and has consistently obtained a molecular weight of approximately 140,000. This value seems to be concentration independent. Appella and Markert (1961), in two sentences at the conclusion of their article, suggested the possibility that the five forms of lactic dehydrogenase in animals are due to the presence of two polypeptides with a slightly different charged group.

[2] In this paper, NAD is the abbreviation of nicotinamide adenine dinucleotide and NHXD the abbreviation of its hypoxanthine analog.

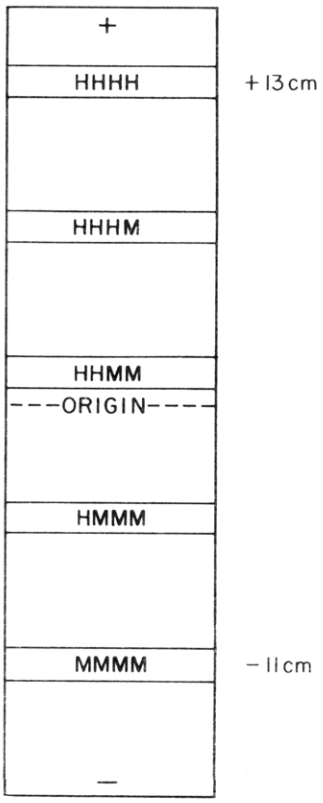

FIG. 1.—Migration of various lactic dehydrogenase forms of the rat on starch grain.

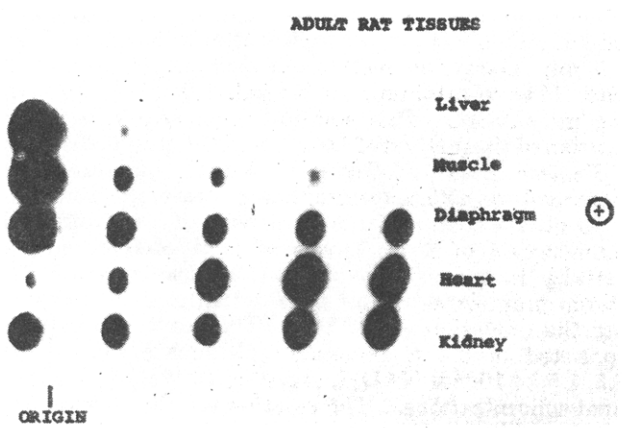

FIG. 2.—Electrophoretic pattern of different rat tissues on starch gel. See Methods section for description of procedure.

distinguished. Their relative mobilities on starch grain are illustrated in Figure 1.

The $NHXDH_L/NADH_H$ ratios for the pure M (M_4) and H (H_4) types are extremely different. This is illustrated in Table I, which gives data on various electrophoretic forms obtained after elution from the starch grain. The hybrids have intermediate ratios and, as indicated in the table, the experimental values are close to the ratios which are predicted on the basis that the individual subunits are catalytically independent. Since this independence appears to exist, it is possible to compute the relative amount of H and M subunits in a given extract. For example, if a ratio of 1.98 is obtained, then there would be 50% of the H form and 50% of the M form. In general, the calculation from the ratio has been in good agreement with that computed by measuring the actual levels of the various forms after starch grain electrophoresis. In our calculations we have assumed a linear relationship between the ratios obtained and the relative percentage of the two lactic dehydrogenase forms. Four of the electrophoretic bands can be observed in the adult rat heart on starch grain. Figure 2 gives the pattern of lactic dehydrogenase types, after electrophoresis on starch gel, from crude extracts of adult rat heart, kidney, liver, diaphragm, and gastrocnemius muscle. In Table II are presented the $NHXDH_L/NADH_H$ ratios of the same extracts that were used for the studies in Figure 2. The relative percentage of H subunits is calculated on the basis of these ratios and is calculated from the data shown in Table I. There is a good correlation between the electrophoretic distribution and the ratios. Hence, adult rat liver contains almost completely M-type lactic dehydrogenase; there are somewhat more H units in the gastrocnemius muscle, whereas the diaphragm has a still higher percentage of H subunits. Heart and kidney of the adult rat contain a large excess of H subunits. As we have pointed out previously, the occurrence of hybrids depends on the two genes operating in one cell, and their formation is random; hence, the relative levels should follow a binomial distribution. Examination of the gel pattern for the heart extracts[3] indicates a typical binomial distribution. However, the randomness is not present in the kidney extracts, since, as can be observed, there is too much of the M_4 form for the distribution to be binomial. We therefore thought that the difference in patterns between the kidney and heart extracts was due to the fact that we were dealing with a number of different cell types in the kidney. This view appears to be correct, since, as also indicated in Table II, the papilla, medulla, and cortex of the kidney all appear to have different relative amounts of the two lactic dehydrogenase types. The papilla contains

TABLE I

FOUND AND CALCULATED VALUES FOR $NHXDH_L/NADH_H$ RATIOS FROM ELECTROPHORETIC BANDS OF RAT TISSUES[a]

	Found	Predicted
H_4	3.15	—
M_1H_3	2.42	2.56
M_2H_2	1.85	1.98
M_3H_1	1.27	1.37
M_4	0.80	—

[a] The ratios reported here are slightly different from those reported in an earlier study. These discrepancies appear to be due to the presence of varying amounts of inhibitors in different reduced coenzyme preparations (Fawcett *et al.*, 1961).

TABLE II

COMPOSITION OF LACTIC DEHYDROGENASES OF DIFFERENT RAT TISSUES

	$\frac{NHXDH_L}{NADH_H}$	Calculated % H Type
Heart	2.63	78
Liver	0.84	2
Leg muscle	1.05	11
Diaphragm	1.31	28
Kidney	2.76	84
Renal zones		
Papilla	1.00	9
Cortex	3.12	98
Medulla	1.82	44

[3] The same comparative phenomenon can also be observed with kidney and heart extracts on starch grain.

TABLE III
DEVELOPMENT OF LACTIC DEHYDROGENASE IN THE RAT HEART

	% Total Units Recovered from Starch Grain Block[a]				
	M_4[b]	M_3H_1	M_2H_2	M_1H_3	H_4
Fetus					
9 day	61	39	0	0	0
13 day	36	27	23	14	0
19 day	<3	30	37	27	6
Postnatal					
24 hours	<1	31	34	22	13
2 weeks	<0.1	17	17	44	22
3 weeks	<0.1	9	16	41	34
5 weeks	<0.1	5	12	49	33
3 months	<0.1	7	9	31	53
6 months	<0.1	3	6	32	59
12 months	<0.1	2	5	36	57

[a] See text for method of recovery. [b] The M_4 form has been found to be the most labile of the various bands; this may be responsible for the lack of strict adherence to the random distribution.

almost completely M subunits, and, although it represents only a small amount (less than 10%) of the total kidney lactic dehydrogenase, it accounts for the nonrandom pattern observed with the crude kidney extracts. There is a relatively high percentage of lactic dehydrogenase hybrids in the medulla, whereas the cortex has a large excess of H subunits. We feel, on the basis of the results such as those obtained with the kidney as well as other data, that a nonrandom electrophoretic lactic dehydrogenase pattern of a tissue or a body fluid indicates the contributions of more than one cell type.

Changes of Rat Heart Lactic Dehydrogenase During Development.—Table III illustrates the changes in composition of the rat heart lactic dehydrogenase from early embryo to the fully matured adult after separation on starch grain. The earliest embryonic heart that we could obtain had a large percentage of the pure M type (M_4) as well as some of the hybrid containing 3 M and 1 H subunits. As development continues, there is a gradual shift from production of M subunits to production of the H type. It is important to emphasize that the H gene is first expressed by the formation of the hybrids and then finally by the appearance of the pure H type (H_4). Another interesting point is the considerable change in the composition of the two forms even after birth. The data indicate that the composition of the various forms appears to follow a binomial distribution and the formation of the hybrids appears to be a random phenomenon.

Since the subunits appear to act as independent entities in the hybrid form, it is possible to calculate the amount of M and H subunits in a given tissue by catalytic measurements. This is illustrated in Table IV, where the changes with development and aging of the rat heart can be followed by such measurements. We have calculated from these ratios the relative amounts of H subunits. It is of interest that the electrophoretic pattern can be predicted by such catalytic determinations. Furthermore, this ratio or comparative rates of reaction with other NAD analogs has provided simple methods to compute the relative composition of M and H subunits in a given tissue.

The fetal lactic dehydrogenase in the rat appears to be M type. This is indicated by the fact that, when a 7-day whole rat embryo extract was analyzed, evidence for the presence of nearly all M_4 was obtained. The rat kidney, which, like the heart in the adult, is primarily H type, has a large percentage of M subunits in the late fetal stages.

Development in the Rabbit.—The heart of the adult rabbit, in contrast to that of the mature rat, contains nearly all pure H type (H_4) (see Table V). Only traces of the hybrids of the pure M type can be observed on electrophoresis. It is of interest that the heart of the 15-day-old rabbit fetus has about 48% H-type subunits. The gestation period of the rabbit is 30 days. Our earliest data for the rat heart are also at the half-gestation period (see Table IV), and at this time the rat heart contains only 10% H subunits. The newborn rat heart contains only about 30% H type as compared to 81% found in the newborn rabbit heart.

The adult rat liver contains almost all M type, and hence during development very little change can be observed in the lactic dehydrogenase composition of this organ. However, the adult rabbit liver has a relatively large per cent of H subunits, and there is a shift from M to H type during development (see Table V). The data for the rabbit indicate that this mammal, like the rat, has the M type as the embryonic form, and that there is a shift in some cells of the M to H type during development. This shift occurs in kidney and brain, which contain relatively large amounts of H type in both the mature rabbit and rat.

Lactic Dehydrogenase Types in Bovine Tissues.—Although we have not been able to obtain early fetal calf specimens, we have carried out analyses on the

TABLE IV
CHANGE IN RAT HEART LACTIC DEHYDROGENASE DURING DEVELOPMENT AND MATURATION

	$\frac{NHXDH_L}{NADH_H}$	Calculated % H Subunits
Prenatal		
14 days	1.02	9
19 days	1.37	25
Postnatal		
24 hours	1.55	33
3 weeks	2.06	53
3 months	2.30	64
1 year	2.54	74

TABLE V
PER CENT H SUBUNITS IN HEART AND LIVER OF RABBITS OF DIFFERENT AGES

Tissue	15-Day-Old Fetus	Newborn	Adult
Heart	48.2	81.0	>99.0
Liver	12.5	39.5	63

late fetus (Table VI). The data in the table are very suggestive that the embryonic form of the beef is M and that there is a shift to H units during development and maturation. It is of interest that the adult beef liver lactic dehydrogenase consists largely of H subunits; this will be discussed below in greater detail.

TABLE VI
PER CENT H SUBUNITS IN BEEF TISSUES

	Heart	Liver	Kidney
Fetal[a]	76.3	67	82.6
Adult	85.9	97.4	96.5

[a] This fetal material was obtained from a late embryo.

Nature of the Human Embryonic Lactic Dehydrogenase.—Although the embryonic forms of the rabbit, beef, and rat are the M type, the human embryo appears to begin with the H form. This is illustrated in Table VII, in which the earliest embryo observed was almost completely H subunits in all of its tissues. This is true for tissues such as leg muscle and liver, which are largely M-type tissues in the adult human. Hence development and maturation in the human, in contrast to other mammals, are associated with the appearance of the M form of lactic dehydrogenase. The first indication of the M type is the appearance of the hybrid M_3H_1. Although the composition of the heart lactic dehydrogenase of both human and the rat with respect to the two types of lactic dehydrogenase is roughly the same, the manner in which this final composition is obtained is quite different.

TABLE VII
PER CENT H SUBUNITS IN HUMAN LIVER AND SKELETAL MUSCLE[a]

		Liver	Muscle
Fetus	6 weeks[b]	>99	>99
Fetus	3 months	94	85
Fetus	7 months	73.6	60
Adult no. 1		14.1	22
Adult no. 2		4.3	12
Adult no. 3		2.7	26

[a] Leg muscle. [b] The time periods for the fetuses are approximate.

Type of Lactic Dehydrogenase in Livers of Different Animals.—In surveying a variety of animals, we became aware of the differences in the relative levels of the two lactic dehydrogenase types in livers of different species. As a comparative measure, we have tabulated the relative amounts of H subunits present in the adult liver (Table VIII). The human liver contains mainly the M polypeptide, as indicated by the small amount of H subunits. The three closely related rodents—rat, mouse, and hamster—also have nearly all M-type units in the liver. On the other hand, the guinea pig has a higher percentage of the H form than the other rodents studied. The rabbit has about two thirds of its lactic dehydrogenase in the liver as the H form, whereas the pig has about 78% of the total as H polypeptides. It is of interest that the four ruminants—sheep, goat, beef and deer—have almost completely the H form in their livers. In contrast, the horse has only a trace of the H-type catalyst. The cat and dog have principally the M subunits.

Variation in the livers of different birds has also been found (Table IX).

TABLE VIII
PER CENT H SUBUNITS IN "ADULT" LIVER OF DIFFERENT MAMMALS[a]

Man	5	Dog	10
Rat	<5	Cat	8
Mouse	<5	Pig	78
Hamster	<5	Sheep	90
Bat	<5	Beef	91
Guinea pig	22	Deer	95
Rabbit	60	Goat	94
		Horse	<5

[a] The values were calculated from the $NHXDH_L/NADH_H$ ratios, after comparison with the ratios obtained from M_4 and H_4 types of each species.

TABLE IX
PER CENT H SUBUNITS IN LIVERS OF DIFFERENT ADULT AVIAN SPECIES

Chicken	68	Guinea hen	72
Pigeon (domestic)	72	Auk	70
Domestic goose	21	Woodcock	50
Domestic duck	24	Petrel	>95
Pintail duck	23	Ruffed grouse	25
Wild mallard duck	26	Sparrow	19
Black duck	22	Starling	49
Murre	90	Blue jay	55
Blue heron	92	Virginia rail	31
Pheasant	25		

DISCUSSION

The data in this paper support the previously advanced view that most animals possess two distinct lactic dehydrogenases, each one composed of four subunits, and that these subunits (M and H) are probably elaborated by two different genes (Cahn *et al.*, 1962b; Kaplan and Ciotti, 1961a). Furthermore, hybrids of these two types appear and progress characteristically during development in the embryo as well as during maturation after birth. All of our results indicate that the hybrids are formed at random and their concentrations follow a binomial distribution. We again emphasize that the occurrence of the hybrids appears to be due to the fact that both genes are operating in one cell during the change of lactic dehydrogenase from the embryonic form to the adult form.

As reported earlier (Cahn *et al.*, 1962a,b), the embryonic form in the chicken is the H type. In contrast, the three mammals that we have studied—rat, rabbit, and beef—all appear to have the M form as the embryonic type. Dr. C. A. Markert has informed us that the mouse and pig also appear to have the M unit as the embryonic form. Most unexpected was the finding that in the human the embryonic form is the H type of lactic dehydrogenase. Whether the human is unique among the mammals remains to be elucidated, since only a limited number of mammals have been studied. We hope to study the embryos of other primates, as well as of other mammals. It would also be of considerable interest to know whether the H form is the embryonic type of other birds besides the chicken.

Our studies certainly show the great variability with which different species select the type of lactic dehydrogenase in the liver. The early human embryonic liver is entirely H type, but the adult liver contains only a very small percentage of this type. In the rat, on the other hand, the embryonic form in the liver, even in the earliest embryo studied, is the M subunit, and there is relatively no change in the composition of the lactic dehydrogenase of the adult rat liver. The rabbit and beef, however, have the same embryonic

form as the rat, but show individually different amounts of H type in the adult liver.

The shifts in types of lactic dehydrogenase appear to be similar to those that take place with hemoglobin. These shifts, furthermore, appear to be species specific. It appears to us that in different species the characteristics of repression and de-repression of the two genes controlling the synthesis of the two types of lactic dehydrogenase are different and that these characteristics may be of considerable importance for the development as well as the survival of the species. Our results further indicate that all of the cells of one animal have the genetic capacity to produce both types of lactic dehydrogenase, but their expression is controlled by unknown factors which are associated with the underlying mechanisms involved in differentiation and development.

A somewhat surprising result of this study was the finding that the type of lactic dehydrogenase present in the livers of adults of different species showed such great diversification. However, it appears to us that there is some significance to this variation and that the presence of the particular composition of lactic dehydrogenase is not a random occurrence but is of functional importance in liver metabolism and has developed from natural selection. For example, the four ruminants—deer, beef, sheep, and goat—all have largely H-type lactic dehydrogenase units, whereas the three closely related rodents—rat, mouse, and hamster—all possess almost all M type units. This would suggest that closely related species may have similar patterns of lactic dehydrogenase distribution in their tissues.

Previously we have advanced the view that the two types of lactic dehydrogenase have different physiological roles (Kaplan, 1961a, b). Because of their differences with respect to inhibition by excess pyruvate, we have ascribed a function to the M type enzyme in tissues where glycolysis is of importance, such as most mammalian skeletal muscle, and the H type in tissues which have a relatively constant rate of metabolism and which are more aerobic, such as heart. The M-type enzymes of vertebrates show fewer properties of substrate inhibition than the corresponding H form. In our laboratory, we have been able recently to gain further support for the functional significance of the two forms. We have found that birds that are poor fliers have the M type of enzyme in their breast muscles, whereas in those birds whose flight is extensive and sustained a large percentage of the H type of lactic dehydrogenase is present (Wilson *et al.*, 1962). An almost perfect correlation has been obtained between the strength of sustained flying and the amount of H type of lactic dehydrogenase present in the pectoral muscle.

It is difficult to explain the differences in distribution of the two forms of lactic dehydrogenase in the livers of different animals. There are some basic differences in metabolism between ruminants and a number of other mammals that have been studied. The ruminants are known to absorb fatty acids from the intestine. These fatty acids are converted to glucose in the liver, and it is this conversion which is largely responsible for the blood sugar of the cow. It is possible that oxidative metabolism is greater in the livers of ruminants than of other mammals (*i.e.*, human, rat, and mouse) and that pyruvate is an intermediate in the formation of glucose from the fatty acids. In order to prevent the pyruvate from being converted to lactate, it may be essential to have the H form of lactic dehydrogenase, which is strongly inhibited by an excess of pyruvate. Although fatty acid conversion to glucose may be the reason for the presence of the H type of lactic dehydrogenase in the livers of ruminants, we are at a loss to give a resonable explanation for the presence of the M type in the livers of other mammals. We believe, however, that it may be valuable to compare the metabolic patterns of the liver in different species in somewhat more detail in light of the findings with the lactic dehydrogenase distribution.

The greatest emphasis in comparative biochemical studies has been on the similarities between different organisms. It is important to stress the biochemical unity of living systems but, as our studies indicate, the differences in biochemical properties may be of prime consideration in understanding speciation. This work also points to the dangers of using indiscriminately different species in biochemical studies. Studies on the same reaction or mechanism in the rat or beef may not necessarily give the same answer. Furthermore, our work indicates the importance in enzymatic investigations of controlling the age of the animals. We feel that the two forms of lactic dehydrogenase are very useful indicators in following the course of molecular changes in development and maturation.

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