UNPUBLISHED PRELIMINARY DATA

STRUCTURAL AND FUNCTIONAL PROPERTIES OF THE H AND M SUBUNITS OF LACTIC DEHYDROGENASES*

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Introduction

Five electrophoretically distinct forms of lactic dehydrogenase (LDH) have been observed in most animals so far examined.[1-6] These multiple molecular forms arise from the combination of two distinct types of protein subunits into the tetrameric forms of active LDH. One type is designated as "H" type and the other as "M" type. The nomenclature of the subunits is derived from the fact that the LDH form composed of four identical "M" subunits (M_4) is found in skeletal muscle of adult organisms, whereas the H_4 form is characteristic of adult heart type tissues.[1] The intermediate electrophoretic forms of LDH thus are molecular hybrids of muscle type and heart type LDH, and exhibit structural and functional properties that are a combination of the two extreme forms.[7,8]

In order to establish the structural relationships between H and M subunits we have investigated H_4, M_4, and hybrid LDH's from 20 species. Amino acid analyses, fingerprint patterns, immunological cross reactions, temperature stabilities, and molecular weights have been employed to compare the structures of the subunits. Comparative active site determinations on heart and muscle type LDH's as well as <u>in vitro</u> hybridization of subunits have given some indications of the structural features required for the catalytic integrity of the enzyme.

The functional distinction between the two types of subunits has been established by determination of optimal substrate concencentrations, comparative K_m values, analogue ratios, and turnover numbers. This functional distinction appears to be of considerable importance in the metabolism of the tissue involved. The synthesis of the two types of subunits is under the control of separate genes.[1,4,9]

Independent regulation of these genes can give rise to varying proportions of H and M subunits, thereby controlling the relative distribution of the five LDH forms within a given tissue. The distinctive catalytic properties of the two types of subunits are adapted to the metabolic environment of the tissues in which each subunit predominates.[10]

Structural Distinctions between H and M Subunits

The multiple molecular forms of LDH can be identified on the basis of their different electrophoretic mobilities, and the two types of subunits may be expected to exhibit other distinctive structural features. Structural dissimilarities between H and M subunits have been investigated by amino acid analysis, peptide mapping, immunological cross reaction, and temperature stability.

Amino Acid Compositions of H and M Subunits.--The relationship between the primary sequences of heart and muscle types of LDH's from several species was investigated by comparative amino acid analysis[7,11,12] and the results are shown in Table 1. The H_4 and M_4 enzymes within a given species are quite distinct in their amino acid compositions. In contrast, the H_4 enzymes from either the birds or the mammals show marked similarities to other avain or mammalian H_4 LDH's. Likewise, the M_4 enzymes from closely related species show similarities linking them more closely to one another than to their corresponding H_4 types. This distinction between the heart type and the muscle type of LDH is particularly striking among bird enzymes in their values for histidine, threonine, glutamic acid, isoleucine, leucine, and tyrosine.

Table 1

Comparative Fingerprint Patterns of H and of M Subunits.--Additional evidence for the structural distinction between heart

and muscle type LDH is obtained from peptide maps of tryptic digests. For two proteins with very closely related primary sequences, the number of ninhydrin-positive spots on a peptide map should remain approximately the same whether the proteins are fingerprinted separately or as an equimolar mixture. For proteins that are more divergent in their primary sequences, the number of peptides common to both should be fewer. Thus, a tryptic fingerprint of a 1:1 mixture of the more diverse proteins should give a marked increase in ninhydrin-positive spots. In Table 2 is given a peptide map comparison of heart and muscle type LDH's. The LDH from chicken heart appears to be more closely related to the turkey heart enzyme than to LDH from chicken muscle. While the beef heart and chicken heart enzymes have undergone considerable evolutionary change with respect to one another, the two heart enzymes still appear to have retained in common a larger percentage of their primary sequences than have the H_4 and M_4 LDH's isolated from the same species.

Table 2

Immunological Relationship between H and M Subunits.--The most sensitive measure so far employed for structural comparisons among LDH's is the micro complement (C') fixation technique of Wasserman and Levine[13] as applied to LDH's in our laboratory.[3,14] The amount of excess anti-chicken-LDH required to achieve a unit cross reaction with other LDH's acting as antigens becomes a measure of the structural relationship among the antigens. Table 3 clearly demonstrates that the LDH's may be grouped into two distinct series of structurally related proteins, those which are heart type LDH's, and those which are muscle type enzymes. Moreover, the degree of structural dissimilarity between the H and the M subunits from a single species, the chicken, is at least as extensive as the dissimilarity between the H subunits from chicken and beef. This point follows from the

Table 3

fact that M subunits from chicken will not cross react with antibodies to chicken H subunits, whereas H subunits from beef will so cross react.

Differential Temperature Stabilities of H and of M Subunits.--A marked distinction in temperature stability is apparent between heart and muscle types of LDH. Figure 1 shows that the H_4 enzymes from chicken or beef retain enzymatic activity at elevated temperatures under conditions that rapidly inactivate the M_4 forms. The subunits retain this structural distinction in the hybrid forms of LDH. This difference in temperature stability between H and M subunits suggests structural differences that permit the H type of LDH to retain conformation and catalytic integrity, whereas the M type is not so structurally stabilized.

Fig.1

Structural Similarities between H and M Subunits

While considerable distinction between H and M subunits of LDH is unmistakable, similarities are also presupposed on the basis that both types of subunits operate as LDH's. The similarities between heart type LDH and muscle type LDH extend to molecular weight and shape, active-site sulfhydryl peptides, and subunit association.

Molecular Weight and Shape of LDH's.--All the LDH's so far examined in our laboratory, except the beef H_4 enzyme, exhibit essentially the same molecular weights regardless of whether the enzyme tested is a heart type, muscle type, or hybrid LDH. The values obtained for a series of LDH's are given in Table 4. With the exception of the H_4 enzyme from beef, all the molecular weights seem, within the range of error, to be around 147×10^3. This molecular size extends even to the L-(+)-lactate dehydrogenase isolated from *Lactobacillus arabinosus*. The values in Table 5 were all determined

Table 4

Table 5

under identical conditions by means of the Ehrenberg method of approach to sedimentation equilibrium.[11,15] Independent determinations of molecular weight by sedimentation and diffusion constants gave absolute values about 10% lower than the molecular weights listed in Table 5. While the absolute values of the molecular weights for these LDH's is still in question, the essential constancy of the molecular weight from species to species, for both H and M subunits, is well established.

Sedimentation constants for a number of LDH's have also been determined.[12] These constants appear to be the same for all the LDH's examined. This fact suggests that these enzymes have the same molecular shape (Table 5).

Active-site Sulfhydryl Peptide from H_4 and M_4 LDH's.--The operation of a sulfhydryl group at the active-site of LDH has been established by several workers.[16-23] The observation that the M_4 enzyme from bullfrog leg muscle has only one or two sulfhydryl groups per subunit has permitted the identification of the region of the primary sequence that contains the active site thiol. A dodecapeptide containing the essential thiol group labeled with iodoacetate-1-C^{14} has been isolated from a tryptic digest of the frog muscle enzyme. A sulfhydryl peptide of virtually identical sequence has also been isolated from tryptic digests of four other LDH's. These four LDH's included M_4 enzymes from chicken and dogfish and H_4 enzymes from chicken and beef. In Table 6 are shown the amino acid compositions of the sulfhydryl peptides common to these four LDH's and present at the active-site of frog M_4 LDH. After partial acid hydrolysis, identical peptide mixtures were obtained for each of the five peptide. This fact demonstrates that the sequences of these peptides are also identical.[22,23] If this sole common sulfhydryl peptide represents

Table 6

an essential thiol peptide in all these LDH's, then its presence in both types of LDH is a critical point of similarity shared by both H and M subunits.

Table 7 Table 7 shows the sequence of this important thiol peptide as isolated from chicken heart LDH.[23] The comparison of this peptide to active site thiol peptides previously identified in triosephosphate dehydrogenase (TPD)[24,25] and in yeast alcohol dehydrogenase (Y-ADH) and horse liver alcohol dehydrogenase (HL-ADH)[26,27] is also given in Table 7. If the relationship among these active site thiol peptides can be further substantiated, the common sulfhydryl peptide from the LDH's may become a feature relating the H and M subunits not only to one another but also to some other dehydrogenases.

In vitro Hybridization of H and M Subunits.--The ability of H and M subunits from a single species to associate into the tetrameric form of the active enzyme is implicit in the concept of hybrid LDH's. The association of the two types of subunits from the same species into the intermediate forms of LDH has been achieved *in vitro* by freezing and thawing under appropriate conditions.[28-29] This ability of H and M subunits to associate into the tetrameric forms of LDH

Fig.2 extends also to subunits from quite distinct species. Figure 2 reproduces the starch gel electrophoresis patterns demonstrating interspecies hybridization between dogfish M_4 and chicken H_4 LDH's, between dogfish M_4 and beef H_4 LDH's, and between sturgeon M_4 and beef H_4 LDH's. Interspecies hybridization between H and M subunits has also been

Table 8 achieved for many other species. Table 8 lists the attempts at interspecies hybridization that have succeeded under the conditions of multiple freezing and thawing of equimolar mixtures of LDH's in the presence of sodium chloride and sodium phosphate buffer at pH 7.0. Where a binomial (random) distribution of subunits has been obtained,

this fact is indicated. Species that failed to hybridize with one another under these conditions are also listed.

The M subunits from bullfrog LDH did not hybridize with H subunits from the same species. A similar observation was made with amphiuma LDH's. This unusual occurrence confirms the finding that neither frog nor amphiuma LDH's show the existence of hybrid forms in vivo. Only the H_4 and M_4 forms have been observed. Although frog and amphiuma LDH's failed to form hybrids between their own corresponding H and M subunits, the M type from both species can form hybrids with LDH subunits (either M or H) from quite distinct species.[30]

Interspecies hybridization of H and M subunits demonstrates that in spite of the considerable structural difference between the two types of subunits, the features required for subunit association into active tetramers are retained. This fact is especially striking when the subunits being hybridized are derived from species that are taxonomically quite remote.

The failure to obtain hybridization in some cases may reflect a difference in rate of hybridization under the conditions employed. Even in cases where hybridization succeeded, differences in rate were apparent in the varying number of freezings and thawings required to achieve binomial distribution of subunits. Whatever the reason for failure to achieve hybridization in some cases, the mechanism of subunit association is clearly not simple and involves some distinct species specificity. This specificity is further demonstrated by the failure of beef H_4 LDH to hybridize with chicken muscle TPD or with bovine serum albumin. Moreover, in vitro hybridization with crude extracts of LDH shows no interference with hybridization by other proteins present in the crude extract.[31]

Functional Significance of H and M Subunits

The very presence of two types of LDH within a single organism suggests a specific functional role for each and a catalytic distinction between them. Distinct catalytic differences can be observed for H subunits and M subunits in their optimal substrate concentrations, K_m values, reactivities toward coenzyme analogues, and turnover numbers. Based on these parameters, M types of LDH appear to be adapted to operation in a relativity anaerobic environment where a high concentration of pyruvate may accumulate quickly.[1,11,32,33] The concept of such a functional role is supported by the differential repression of synthesis of M subunits under conditions of increasing oxygen tension.[34] This suggested functional role as well as this differential repression of synthesis may be reflected in the fact that the proportion of M subunits is higher in those tissues that function by means of an anaerobic metabolism.[1,3,4,35]

Optimal Substrate Concentrations and K_m Values for H and M Subunits.--Table 9 summarizes the effects of pyruvate and lactate on the operation of various heart and muscle type LDH's. The optimal pyruvate concentration is about five times higher for M_4 enzymes than for the corresponding H_4 forms. Similarly, the M_4 forms can function in an environment considerably higher in lactate concentration than can the H_4 forms. The relative K_m values for both lactate and pyruvate indicate that the M_4 forms bind the substrates less tightly than do the H_4 LDH's.[11,12]

Table 9

Coenzyme Analogue Ratios and Turnover Numbers of H and M Subunits.--By means of the preferential inhibition of H types of LDH with high concentrations of pyruvate it is possible to construct a ratio of rates of reaction that distinguishes the H_4 enzyme from the

M_4 enzyme.[1,32,33,36] This ratio with the hypoxanthine analogue of reduced nicotinamide adenine dinucleotide at a low pyruvate concentration ($NHXDH_L$) versus the reduced natural coenzyme at a pyruvate concentration sufficient to inhibit the H_4 enzyme ($NADH_H$) is shown in Table 10. Use of the hypoxanthine analogue at a low pyruvate concentration increases the sensitivity of this ratio but is not critical. These analogue ratios are useful for distinguishing between the catalytic operation of the H and M types of LDH. The ratios are important parameters for the determination of the proportions of H and M subunits present in crude extracts of LDH.

Table 10

Table 10 also shows the division between H_4 and M_4 LDH's based on their turnover numbers. For the H_4 enzymes these values remain around 50,000, whereas for the M_4 forms they reach twice that value. For some fish M_4 LDH's the turnover numbers reach values close to 150,000.[11,12]

Differential Effect of Oxygen Tension on the Synthesis of H and M Subunits in Tissue Cultures.--The rates of synthesis of H and M subunits of LDH have been investigated with tissue cultures of heart cells from monkeys, heart and muscle cells from chicken embryos, and kidney cells from new-born mice.[34] Variation in oxygen tension affected the rate of synthesis of M subunits without a corresponding effect on the rate of synthesis of H subunits. The results of this tissue culture study are given in Table 11. Decreasing oxygen tension from one atmosphere to 2.5% of one atmosphere caused an increase in total LDH synthesis. The proportion of H and M subunits present in the LDH was determined by means of the ratio of rates of reaction at "low" to "high" pyruvate concentration, as defined in Table 10. The amount of H subunits present remained approximately unchanged, and there was a preferential synthesis of M subunits. Decreasing

Table 11

oxygen tension caused the percentage of M subunits present in the total LDH to rise from 54 to 69%.

The stimulating effect of low oxygen concentration persisted for 48 hours after a brief exposure to the low oxygen tension. This suggests that the effect of oxygen tension is mediated by some factor with an effective lifetime of 48 hours.

Since chemical agents causing a shift to anaerobic metabolism do not mimic the effect of low oxygen tension, that effect is likely to be independent of immediate relationship to any carbohydrate metabolites.

Thus, decreasing oxygen tension preferentially elicits the synthesis of the LDH type which is better suited to function under more anaerobic conditions. The precise significance of this observation is still obscure, but the correlation between function and rate of synthesis of multiple molecular forms of an enzyme is of considerable interest.

Correlation Between Metabolic Environment and Proportion of H and M Subunits.--The ability of M subunits to operate under conditions of pyruvate concentration in which the corresponding H type is inhibited has been pointed out. This selective substrate inhibition can be placed on a quantitative basis by use of the analogue ratio defined in Table 10. The analogue ratios established for crystalline enzymes can then be employed to determine the relative proportion of H and M subunits in crude extracts. The proportion of H and M subunits determined by such an approach agrees with the results obtained by starch gel electrophoresis and micro C' fixation.[1,3,4,7,35]

With the analogue ratio as a measure of the relative amounts of H and M subunits in various tissues, it is possible to establish

a correlation between the percentage of H subunits in a given tissue and the type of metabolism of the tissue.[4] Table 12 lists such a correlation for various rat tissues. Tissues that operate in a relatively oxygen-rich environment (heart, kidney) have a high percentage of H subunits. The leg muscles have a high proportion of M subunits in keeping with the relatively anoxic conditions under which they operate. Diaphragm has an intermediate value. The results obtained for liver vary widely depending on the species examined.

Table 12

The relationship between oxygen tension and LDH type within a single organ, the kidney, is also shown in Table 12. The interior layers of the kidney depend more on glycolysis than does the cortex.[36] Similarly, oxygen tension is known to decrease toward the center of the kidney.[37] These facts are consistent with the high percentage of M type LDH observed in the interior layers of the rat kidney.

This correlation between the function of various tissues and the type of LDH that predominates in the tissue has been further refined with individual muscles from rabbit, chicken, and humans.[8] It has been observed that postural muscles or those involved in rhythmic contractions have more H subunits than those muscles that are subject to sudden bursts of activity. This observation is consistent with the remarkable correlation obtained between the amount of H subunits in the breast muscle of various birds and the flight habits of those birds.[35] Birds capable of sustained flight, such as the humming bird or storm petrel, had almost solely H type subunits in their breast muscles. The breast muscles of flightless birds, such as the chicken, or of poor fliers, like the pheasant, were found to be devoid of H subunits.

In human cancers of the thyroid and colon, the adjacent, uninvolved tissue is substantially lower in the proportion of M subunits

Table 13

than is the tumor,[8,38] as shown in Table 13. In tumors of both thyroid and colon, the proportion of M subunits increased from 32% and 66%, respectively, to about 85%. Thus, the high rate of glycolysis that takes place in malignant tissue is accompanied by a striking increase in the type of LDH that is better suited to function in glycolysis.

Summary

On the basis of structural characteristics, the lactic dehydrogenases (LDH's) may be divided into two basic types, the H type and the M type. The structural distinctions between these two basic types extend to their amino acid compositions, to their primary sequences as measured by comparative fingerprint patterns and immunological cross reactions, and to their temperature stabilities.

In spite of the marked differences between the two types of LDH subunits, important structural similarities are retained. The H and M subunits are similar in molecular weight and shape. They possess the identical active site sulfhydryl peptide, and they can associate with LDH subunits from a wide variety of species into the hybrid forms of active LDH.

The H and M subunits of LDH show distinctive catalytic properties. The M types are adapted to function at higher substrate concentrations than are the H types. The synthesis of M subunits is stimulated by low oxygen tension, whereas the synthesis of H subunits is not affected in this manner. The M type of LDH predominates in tissues that operate by a relatively anaerobic metabolism.

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Footnotes

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Table 1

Amino Acid Composition of Heart Type Lactic Dehydrogenase and Muscle Type Lactic Dehydrogenase from Several Species of Birds and Mammals[12]

	Chicken		Turkey		Pheasant		Rabbit		Beef	
	H_4	M_4	H_4	M_4	H_4	M_4	H_4	M_4	H_4	M_4
Lys	99	112	87	97	97	97	97	104	96	103
His[a]	**30**	**63**	**25**	**73**	**27**	**61**	**27**	**41**	**26**	**33**
Arg	35	35	34	35	38	45	**33**	**37**	**34**	**42**
Asp	129	125	132	122	131	110	140	121	132	127
Thr	**75**	**51**	**66**	**47**	**64**	**51**	48	46	56	48
Ser	107	110	100	103	79	92	87	84	97	87
Glu	**122**	**102**	**124**	**98**	**120**	**100**	120	121	129	121
Pro	38	44	43	57	44	51	49	41	46	51
Gly	96	104	99	104	109	97	92	109	98	100
Ala	88	81	94	75	107	93	86	86	80	78
Val	125	121	142	129	128	118	135	131	138	115
Met	25	31	28	28	25	30	32	34	36	32
Ileu	**66**	**85**	**69**	**90**	**67**	**89**	84	82	85	91
Leu	**149**	**121**	**139**	**124**	**132**	**114**	138	142	143	136
Tyr	**31**	**19**	**26**	**20**	**31**	**21**	28	26	29	29
Phe	19	27	21	24	30	29	24	26	21	29
Try	22	24	--	22	--	24	--	--	22	24
Cys	26	26	--	--	--	--	--	16	17	26

[a] Values listed in boldface show the distinction between the H_4 and M_4 forms and the similarity among the H_4 forms and among the M_4 forms.

Table 2

Fingerprint Patterns of Heart and Muscle Type Lactic Dehydrogenases

The fingerprinting procedure is described in Fondy *et al.*[7]

LDH type	Number of ninhydrin-positive spots
Chicken H_4	34-38
Chicken M_4	35-38
Turkey H_4	33-35
Beef H_4	33-35
Chicken H_4 + chicken M_4	50-53
Chicken H_4 + turkey H_4	35-40
Chicken H_4 + beef H_4	45-47

Table 3

Cross Reaction of Various Lactic Dehydrogenases with Antibodies to Chicken Heart and Chicken Muscle Lactic Dehydrogenase[3]

LDH type	Antibody concentration required for 50% C'fixation	
	Anti-chicken heart	Anti-chicken muscle
Chicken H_4	1.0	NCR[a]
Turkey H_4	1.5	
Pigeon H_4	2.2	
Frog H_4	13	
Beef H_4	80	
Dogfish H_4	NCR	
Chicken M_4	NCR	1.0
Turkey M_4		1.2
Pigeon M_4		2.2
Frog M_4		40
Beef M_4		NCR
Dogfish M_4		NCR

[a] NCR = No cross reaction.

Table 4

Molecular Weights of H_4, M_4, and Hybrid Lactic Dehydrogenases and of Lactic Dehydrogenase from *Lactobacillus arabinosus*[12]

Species	M.W.[a] x 10^3 H_4	M_4
Beef	131	153
Human	146	---
Chicken	151	140
Bullfrog	---	154
Dogfish	---	141

Lactobacillus arabinosus[a]	153
Chicken H_2M_2	154
Chicken HM_3	146

[a] All values ± 7.

Table 5

Sedimentation Constants for H_4 and M_4 Lactic Dehydrogenases and for Lactic Dehydrogenase from *Lactobacillus arabinosus*[12]

Species	$S_{20,w}$	
	H_4	M_4
Beef	7.45	7.32
Human	7.46	----
Chicken	7.31	7.33
Turkey	7.49	7.52
Bullfrog		7.56
Dogfish		7.54
Lobster		7.40
Lactobacillus arabinosus	7.50	

Table 6

Amino Acid Compositions of Thiol Peptides Common to Five Lactic Dehydrogenases[22,23]

Chicken H_4 LDH :	^{14}CMCys,	Arg,	Asp(2),	Gly(2),	Ala,	Ileu,	Leu,	Ser, Thr,	Val
Chicken M_4 LDH :	^{14}CMCys,	Arg,	Asp(2),	Gly(2),	Ala,	Ileu,	Leu,	Ser(2) ,	Val
Beef H_4 LDH :	^{14}CMCys,	Arg,	Asp(2),	Gly(2),	Ala,	Ileu,	Leu,	Ser(2) ,	Val
Frog M_4 LDH :	^{14}CMCys,	Arg,	Asp(2),	Gly(2),	Ala,	Ileu,	Leu,	Ser(2) ,	(Val),(Glu)
Dogfish M_4 LDH :	^{14}CMCys,	Arg,	Asp(2),	Gly(2),	Ala,	Ileu,	Leu,	Ser(2) ,	(Glu)

Table 7

Amino Acid Sequences Around Essential Thiol Groups in Four Pyridine Nucleotide Dehydrogenases

LDH[a]	:	- Val -	Ileu-	Ser -	Gly -	(Gly, CMCys)-	Asn -	Leu -	Asp[b]-	Thr -	Ala -	Arg -
HL-ADH[c]	:	- Val -	Ala -	Thr -	Gly -	Ileu -	CMCys -	Arg -	Ser -	Asp -	Asp -	His - Val -
Y-ADH[d]	:		- Tyr -	Ser -	Gly -	Val -	CMCys -	His -	Thr -	Asp -	Leu -	His - Ala -
TPD[e]	:	- Val -	Ser -	Asn -	Ala -	Ser -	CMCys -	Thr -	Thr -	Asn -	Cys -	Leu - Ala -

[a] Chicken H_4 LDH.[22,23]

[b] Presence or absence of amide not yet established.

[c] See Refs. 26 and 27.

[d] See Ref. 26.

[e] Rabbit and pig muscle and yeast TPD.[24,25]

Table 8

In vitro Hybridization of Lactic Dehydrogenase Subunits[29,30]

	Hybridization	Binomial Pattern
Chicken M_4 + chicken H_4	+	+
Chicken M_4 + rabbit H_4	+	+
Chicken H_4 + beef H_4	+	+
Dogfish M_4 + chicken H_4	+	+
Dogfish M_4 + beef H_4	+	+
Dogfish M_4 + lobster M_4	+	-
Halibut M_4 + rabbit H_4	+	+
Sturgeon M_4 + beef H_4	+	+
Bullfrog M_4 + beef H_4	+	+
Bullfrog H_4 + chicken H_4	+	-
Bullfrog M_4 + bullfrog H_4	-	
Amphiuma M_4 + amphiuma H_4	-	
Amphiuma M_4 + beef H_4	+	+

Table 9

Optimal Substrate Concentrations and K_m Values for Various Heart and Muscle Type Lactic Dehydrogenases[11,12]

	Optimal Pyruvate ($M \times 10^4$)		Optimal Lactate ($M \times 10^4$)	
Species	H_4	M_4	H_4	M_4
Beef	6	30	400	2000
Chicken	4	30	300	2500
Turkey	-	20	-	-
Frog	-	20	-	-
Dogfish	-	30	-	-
Rabbit	7	-	-	-

	K_m[a] Pyruvate ($M \times 10^4$)		K_m[a] Lactate ($M \times 10^4$)	
Species	H_4	M_4	H_4	M_4
Beef	1.4	10.0	90	250
Chicken	0.9	3.2	70	400
Turkey	-	2.2	-	370
Frog	-	1.4	-	830
Dogfish	-	3.3	-	1100
Rabbit	1.1	-	140	-

[a] Determined by reciprocal plots.

Table 10

Analogue Ratios and Turnover Numbers of Various Heart and Muscle Type Lactic Dehydrogenases[11,12]

	$(NHXDH_L/NADH_H)$[a]		Turnover numbers[b]	
Species	H_4	M_4	H_4	M_4
Beef	2.78	0.63	49,400	80,200
Chicken	3.02	0.40	45,500	93,400
Frog	-	-	-	86,000
Dogfish	-	-	-	109,000
Rabbit	-	-	41,500	-

[a] Ratio of rates of reaction of reduced hypoxanthine analog of nicotinamide adenine dinucleotide (NHXDH) at a pyruvate concentration of 3×10^{-4} M and of NADH at a pyruvate concentration of 1×10^{-2} M.

[b] Represents moles NADH oxidized per mole of enzyme per minute at 25° at pH 7.5 with pyruvate at V_{max}.

Table 11

Effect of Oxygen Tension on Synthesis of H and M Subunits of Lactic Dehydrogenase[34]

Oxygen Tension (% Atmospheric Pressure)	Total LDH Units/Plate	Total M Subunits	Total H Subunits	% M Type
95	0.99	0.54	0.45	54
20	1.07	0.59	0.48	55
10	1.26	0.80	0.46	63
5	1.64	1.05	0.59	64
2.5	1.77	1.21	0.56	69
0	1.06	0.73	0.33	69

Table 12

Percentage of H Subunits in Various Rat Tissues[4]

Tissue	$NHXDH_L/NADH_H$[a]	Calculated % H Type
Heart	2.63	78
Kidney	2.76	84
Diaphragm	1.31	28
Leg Muscles	1.05	11
Liver	0.84	2
Renal Zones		
Cortex	3.12	98
Medulla	1.82	44
Papilla	1.00	9

[a] Defined in Table 10.

Table 13

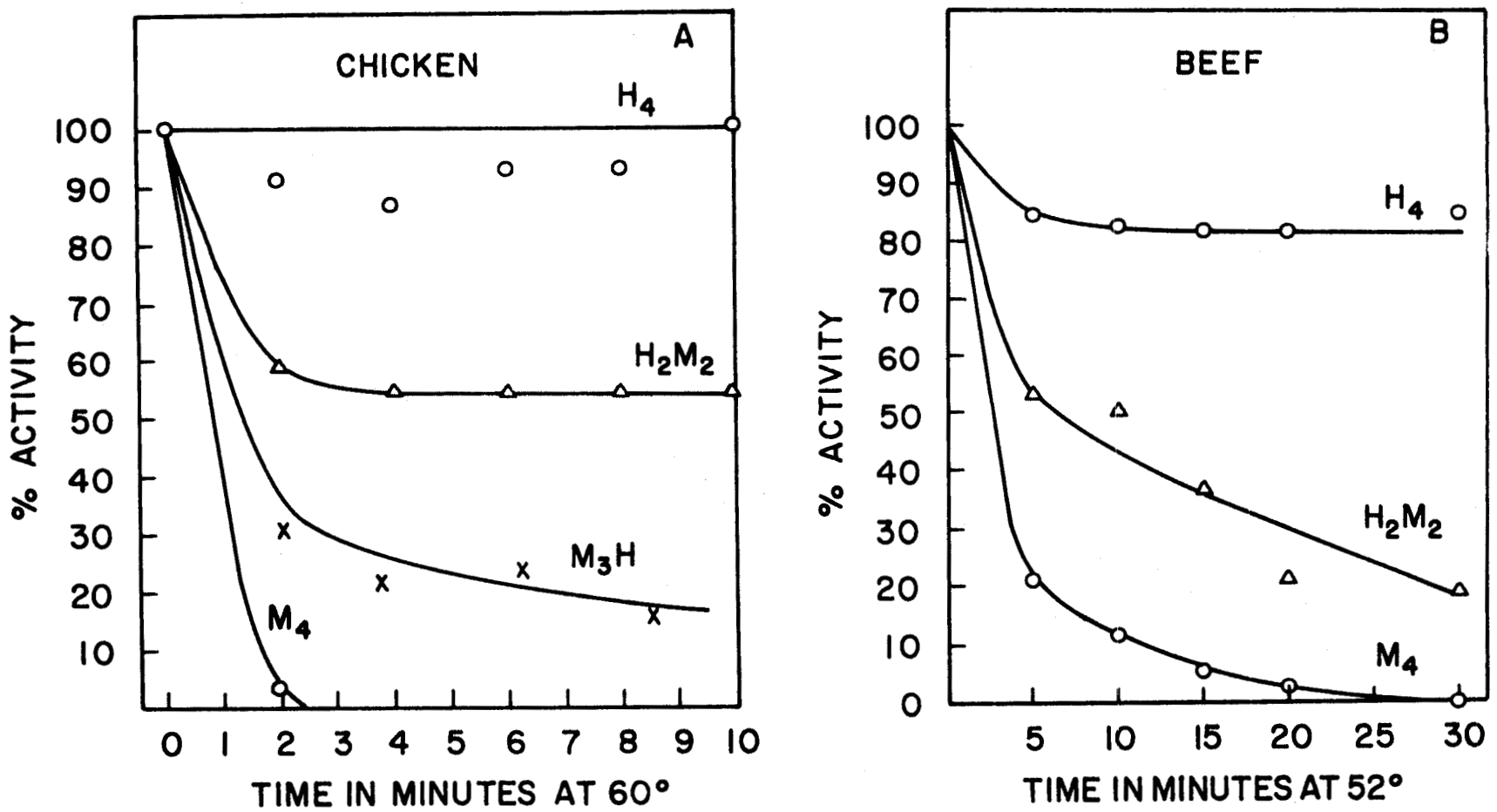
Lactic Dehydrogenase in Human Tumors[38,8]

Tissue	% of M subunits	LDH (enzyme units/g) M	H
	Thyroid		
Uninvolved	32	47	100
Tumor	82	492	108
	Colon (1)		
Uninvolved	66	88	46
Tumor	85	260	46
	Colon (2)		
Uninvolved	51	88	82
Tumor	93	847	65

Figure Legends

Fig. 1. Heat stabilities of crystalline LDH's from chicken and beef removed from starch grain. The activities of aliquots at various intervals were measured with 3×10^{-4} M pyruvate and 1×10^{-4} M reduced nicotinamide adenine dinucleotide (NADH).

Fig. 2. <u>In vitro</u> hybridization of H and M subunits of LDH's from diverse species. Hybridization was achieved by "quick" freeze and "slow" thaw of equimolar mixtures of the H_4 and M_4 enzymes in 0.25 M sodium chloride and sodium phosphate buffer, pH 7.0, as described by Chilson, Costello, and Kaplan.[29,31]


A
CHICKEN
H_4
H_2M_2
M_3H
M_4
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9 10
TIME IN MINUTES AT 60°
B
BEEF
H_4
H_2M_2
M_4
% ACTIVITY
100
90
80
70
60
50
40
30
20
10
0
5 10 15 20 25 30
TIME IN MINUTES AT 52°

IN VITRO HYBRIDIZATION OF
HEART- AND MUSCLE-TYPE LACTIC DEHYDROGENASES