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THE RATES OF DISAPPEARANCE OF L-LACTATE DEHYDROGENASE ISOENZYMES FROM PLASMA

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SUMMARY

1. The M_4 and H_4 isoenzymes of lactate dehydrogenase (L-lactate:NAD^+ oxidoreductase, EC 1.1.1.27) purified from sheep skeletal muscle and sheep heart, respectively, were injected intravenously into lambs, and their rates of disappearance from plasma were determined.

2. Biphasic exponential curves were fitted to the rates of disappearance of both isoenzymes. The disappearance of isoenzyme M_4 activity occurred in two phases with half-lives of 2.0 and 8.0 h. The corresponding values for isoenzyme H_4 were 2.4 and 48.0 h.

3. In lambs with nutritional muscular dystrophy there were elevated levels of plasma isoenzymes due to their release from dystrophic muscles. Therapeutic treatment with vitamin E caused an exponential decline of plasma isoenzymes to normal levels. The half-lives for isoenzymes H_4, H_3M_1, H_2M_2, H_1M_3 and M_4 were 48, 31, 20, 13 and 7 h, respectively. This decline in plasma activity is due to the arrest of abnormal leakage from damaged tissues.

4. Two hypothetical mathematical model systems consistent with the results were devised. These were used to estimate the rate constants for the distribution of the isoenzymes in the body fluids and for their elimination or inactivation. Normal and increased rates of isoenzyme release from tissue were estimated. In order to reach the same level in plasma, isoenzyme M_4 must be released from tissues at a rate 7.5 or 15 times greater than isoenzyme H_4, depending on the model system adopted.

5. The more rapid disappearance rate of isoenzyme M_4 accounts for its relatively low activity in plasma from both normal and dystrophic animals.

INTRODUCTION

The five isoenzymes of lactate dehydrogenase (L-lactate: NAD^+ oxidoreductase, EC 1.1.1.27) can be separated by electrophoresis. These isoenzymes are tetramers

Abbreviation: LDH, lactate dehydrogenase.

composed of different proportions of two monomers[1]. The monomers have been called H and M and the tetramers H_4, H_3M_1, H_2M_2, H_1M_3 and M_4 (ref. 2). The H-type isoenzymes predominate in cardiac muscle and the M-type isoenzymes in skeletal muscle.

Plasma lactate dehydrogenase activity is increased in muscular dystrophy presumably because of leakage of the tissue isoenzymes into the blood stream. However, in man[3] and lambs[4,5] with muscular dystrophy the plasma often contains more of the H-type than of the M-type LDH isoenzymes. The following experimental observations suggest some possible reasons for this difference between muscle and plasma LDH isoenzyme patterns. (1) The M-type isoenzymes are more labile during storage of frozen tissue homogenates[6]. (2) The proportion of M-type isoenzymes is reduced in dystrophic muscle from humans[7,8] and chickens[9], although it is normal in dystrophic mice[8] and lambs[4,5]. (3) There appears to be some release of H-type LDH from heart and liver in dystrophic lambs[4,5]. However, the plasma level of M-type LDH seems low even when precautions are taken to prevent loss of activity *in vitro* and allowance is made both for any changes in the LDH pattern of affected muscle and for release of LDH isoenzymes from other tissues. There remain two other possible explanations which have been proposed for the plasma LDH pattern[7]: (1) selective release of isoenzymes from the damaged muscle and (2) selective clearance of isoenzymes from the blood stream. There is no evidence available on the rates of release of different isoenzymes from dystrophic muscle, but it has recently been reported that injected heterologous isoenzymes H_4 and M_4 disappear at different rates from the plasma of mice[10]. The present study of which preliminary reports have been made[11,12] provides the first experimental evidence that homologous LDH isoenzymes disappear from the blood stream at different rates.

METHODS

LDH assay

Total LDH activity was determined by the method of HENRY *et al.*[13] using a recording spectrophotometer with a cuvette housing maintained at 25°. The enzyme units are the μmoles of $NADH_2$ oxidized per min at this temperature.

LDH electrophoresis and staining

Electrophoresis of 2-μl samples of plasma was performed in agar gel using 0.07 M barbitone buffer (pH 8.6) with a potential difference of 25 V/cm for 35 min (ref. 14). The isoenzyme bands were stained by incubating the gels at 37° for 30 min in a substrate–tetrazolium mixture[5]. The blue isoenzyme bands in the dried gels were scanned in an automatic recording and integrating densitometer (Chromoscan: Joyce, Loebl and Co. Ltd., Newcastle-on-Tyne, England). The densitometer readings were converted to isoenzyme activities (units/l) by means of a calibration curve (Fig. 1).

Purification of M_4 isoenzyme from sheep skeletal muscle

(1) LDH was extracted from minced muscle into 0.1 M phosphate buffer in a high-speed blendor and particulate material removed by centrifuging.

(2) LDH was precipitated from the extract by $(NH_4)_2SO_4$ between 35 and 65% saturation at 4°.

(3) The LDH was dissolved and dialyzed against 5 mM Tris–1.5 mM EDTA

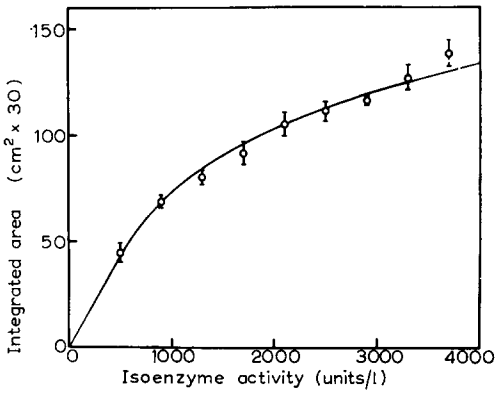


Fig. 1. Calibration curve prepared by plotting densitometer readings of stained M_4 and H_4 isoenzyme bands of plasma of lambs in the injection experiment against LDH activity determined spectrophotometrically (less the endogenous activity of the other isoenzymes in plasma). Above 50 units/l an exponential curve ($\log x = (y + 233)/102$) has been fitted by the least-squares method to the results which are shown as means and their standard errors ($\dot{\phi}$).

buffer (pH 8.4). It was then applied to a DEAE-cellulose column and eluted with the same buffer. LDH isoenzyme M_4 passed through the column while the other isoenzymes and most of the other protein were adsorbed.

Purification of H_4 isoenzyme from sheep heart

The same procedure was followed as for M_4 LDH except that the DEAE-cellulose column was washed with buffer containing an increasing NaCl gradient. Isoenzyme H_4 was the last to be eluted, appearing in the effluent when the salt concentration reached 0.15 M.

Injection of purified isoenzymes into lambs

A series of experiments was performed in which the purified LDH isoenzymes were injected intravenously into Clun Forest lambs of various ages. Blood samples were collected from the jugular vein at intervals before and after injection. Plasma from the heparinized blood samples was assayed for total LDH activity and the individual isoenzymes were estimated by electrophoresis, staining and scanning.

Experimental production of acute muscular dystrophy in lambs[5]

Four lambs (Nos. 84, 85, 188 and 189) were fed a liquid diet of 20% skim milk and 5% cod-liver oil (w/v). Lamb 85, which was given 100 mg α-tocopheryl acetate injections on days 0, 7 and 14, remained clinically normal and no abnormal changes were observed in plasma LDH. The other three animals developed acute muscular dystrophy within 1 or 2 weeks of commencing feeding the vitamin E-deficient diet. At the same time plasma LDH isoenzyme activities rose to abnormally high levels.

Mathematical

After intravenous administration of isolated isoenzymes, the concentration of LDH in plasma can be described by double exponential functions

$$C_t = C_0 (Ae^{-\alpha_1 t} + Be^{-\alpha_2 t})$$

TABLE I

SINGLE INTRAVENOUS INJECTIONS OF H_4 AND M_4 LDH ISOENZYMES INTO LAMBS

Plasma isoenzyme activity at time t, $C_t = C_0 (Ae^{-a_1 t} + Be^{-a_2 t})$, see text.

Isoenzyme	*Lamb No.*	*Body weight (kg)*	*Isoenzyme injected (R)*		C_0 *(units/l)*	A	B	α_1 (h^{-1})	α_2 (h^{-1})	V_1 *(ml/kg)*
			Activity (units)	*Protein (mg)*						
H_4	190	34.5	4040	47	1913	0.244	0.756	0.9325	0.0177	61
	191	27.4	3380	43	3468	0.447	0.553	0.2256	0.0095	36
	1	8.2	1210	14	2264	0.637	0.363	0.1177	0.0096	65
	2	7.0	1010	12	1769	0.730	0.270	0.0498	0.0063	82
	22	11.2	2070	34	3462	0.372	0.628	0.2297	0.0176	53
	116	8.5	1960	27	3819	0.471	0.529	0.2223	0.0103	74
Mean						0.483	0.517	0.2963	0.0118	61.8
S.E.						0.065	—	0.1193	0.0018	6.0
Pooled data						0.379	0.621	0.2868	0.0144	—
M_4	190*	27.5	4940	38	3582	0.500	0.500	0.4717	0.1059	38
	191	20.5	2475	19	2947	0.267	0.733	2.5920	0.1399	41
	1	10.5	2720	45	5876	0.223	0.777	8.4360	0.2001	50
	1	12.0	1360	22	2114	0.695	0.305	0.3038	0.0767	54
	2	10.5	2880	19	5025	0.201	0.799	0.4316	0.0942	60
	2*	11.2	1360	22	2414	0.563	0.437	0.3917	0.0779	63
Mean						0.408	0.592	2.1044	0.1158	51.0
S.E.						0.076	—	1.2017	0.0174	3.7
Pooled data						0.553	0.447	0.3427	0.0867	—

* In these cases the first approximations in curve fitting obtained by curve stripping could not be improved by the iterative least-squares method (see text).

where C_t is the plasma concentration of isoenzyme at any time t after injection, and C_0 is the uniform concentration of isoenzyme in plasma immediately after injection.

Such curves were fitted to experimental data by the following methods, using the I.C.T. Orion computer (Rothamsted Experimental Station, Harpenden, Herts., England). First approximations were made by a curve-stripping method using logarithms of plasma enzyme concentration (*e.g.* RIGGS[15]). These approximations were improved using an iterative least-squares method based on that of DEMING[16]. In two cases this method did not converge satisfactorily (see Table I).

This behaviour of administered isoenzymes is consistent with the hypothesis that the enzyme is distributed in at lease two miscible compartments. The simplest model of such distribution is shown in Fig. 2. The isoenzymes normally enter an extravascular compartment, volume V_2 and isoenzyme concentration C_2, and the rate of entry of isoenzyme is S. The extravascular compartment can exchange isoenzyme with an intravascular compartment, volume V_1 and isoenzyme concentration C_1. In the absence of direct experimental evidence, it must be assumed that the isoenzyme can disappear irreversibly from both compartments. The movement of isoenzyme into and out of the compartments can be described by

$$V_1 \frac{dC_1}{dt} = k_{21} V_2 C_2 - (k_{10} + k_{12}) V_1 C_1 \quad (1)$$

$$V_2 \frac{dC_2}{dt} = S + k_{12} V_1 C_1 - (k_{20} + k_{21}) V_2 C_2 \quad (2)$$

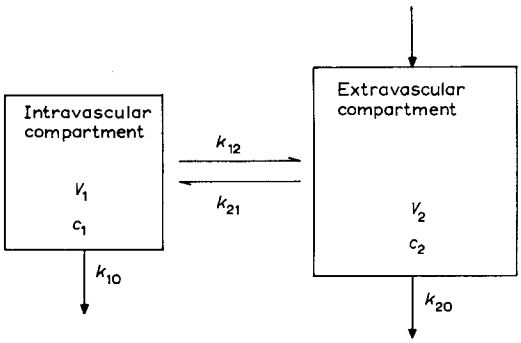


Fig. 2. Hypothetical mathematical model of the distribution of enzymes in body fluids.

These expressions can be rearranged to a second-order linear differential equation in C_1

$$\frac{d^2C_1}{dt^2} + (k_{10} + k_{12} + k_{20} + k_{21})\frac{dC_1}{dt} + (k_{10}k_{20} + k_{10}k_{21} + k_{12}k_{20})C_1 = \frac{Sk_{21}}{V_1} \quad (3)$$

The complementary function is a double exponential,

$$C_1 = X_1e^{-\alpha_1 t} + X_2e^{-\alpha_2 t}$$

where X_1 and X_2 are arbitrary constants which can be solved from the initial conditions, and α_1 and α_2 are given by

$$\alpha = 0.5\ \{(k_{10} + k_{12} + k_{20} + k_{21}) \pm \sqrt{(k_{10} + k_{12} + k_{20} + k_{21})^2 - 4(k_{10}k_{20} + k_{10}k_{21} + k_{12}k_{20})}\} \quad (4)$$

the sign before the square root being *plus* for α_1 and *minus* for α_2. Three kinetic states of the model can be considered, and the relevant solutions of the general equation (Eqn. 3) can be obtained.

Injection of isoenzyme into plasma: In this case it is assumed that endogenous isoenzyme is negligible compared with the administered isoenzyme, so the initial conditions are that $S = 0$, $C_2 = 0$ and $C_1 = R/V_1$ where R is the amount of isoenzyme injected into V_1. The solution of Eqn. 3 is then

$$C_1 = \frac{R}{V_1}\left\{\left(\frac{k_{10} + k_{12} - \alpha_2}{\alpha_1 - \alpha_2}\right)e^{-\alpha_1 t} - \left(\frac{k_{10} + k_{12} - \alpha_1}{\alpha_1 - \alpha_2}\right)e^{-\alpha_2 t}\right\} \quad (5)$$

Discontinued high entry rate: This case may be exemplified by treatment of a disorder which results in a high entry rate of isoenzyme into V_2. It is assumed that the rate of decrease of S is rapid, relative to the disappearance of isoenzyme which has accumulated in V_1 and V_2. The initial conditions in this case are that $C_1 = Sk_{21}/V_1\alpha_1\alpha_2$ and that S becomes zero. The solution of Eqn. 3 is then

$$C_1 = \frac{Sk_{21}}{V_1\alpha_1\alpha_2}\left(-\frac{\alpha_2}{(\alpha_1 - \alpha_2)}e^{-\alpha_1 t} + \frac{\alpha_1}{(\alpha_1 - \alpha_2)}e^{-\alpha_2 t}\right) \quad (6)$$

Steady entry of isoenzyme into V_2: This case may be exemplified by the normal entry of isoenzyme into the system, producing a normal plasma concentration of the isoenzyme. Alternatively, the entry rate may be increased by induced tissue breakdown, producing elevated plasma concentrations of isoenzyme. In both cases it is

assumed that the rate of entry of isoenzyme is fairly constant, when entry rate and plasma concentration of isoenzyme are related by

$$C_1 = \frac{Sk_{21}}{V_1(k_{10}k_{20} + k_{10}k_{21} + k_{12}k_{20})} \tag{7}$$

or

$$C_1 = \frac{Sk_{21}}{V_1\alpha_1\alpha_2} \tag{7a}$$

A hydrodynamic model similar to that of ZILVERSMIT AND SHORE[17] gave results which agreed with those predicted by the application of these analytical solutions. (The present practical application of these analytical solutions will be described in their relevant contexts.)

RESULTS

Inactivation of LDH isoenzymes in vitro

When the purified isoenzymes were incubated with either 0.1 M phosphate buffer (pH 7.4) or dialyzed sheep plasma at 39° for 2 days, inactivation was negligible. Incubation with undialyzed plasma had only a slight effect on the activity of the H_4 isoenzyme but caused complete inactivation of the M_4 isoenzyme in less than 24 h. However, it was found that the pH of plasma increased to 7.8 or 7.9 during incubation. The pH of plasma could be controlled at 7.4 by collecting blood, separating the plasma and incubating under liquid paraffin, or more simply by adding 0.1 M phosphate buffer to it. When this was done the rate of inactivation of M_4 LDH was considerably slower ($t_{\frac{1}{2}} = 45$ h).

Single-injection experiments

Following the intravenous injection or either M_4 of H_4 LDH into lambs, the only change in plasma isoenzyme pattern was an increase in the activity of the injected isoenzyme. The disappearance of the injected isoenzyme from plasma was therefore studied by following the decline in total LDH back to its normal level in plasma. Double exponential curves were fitted to the data from each experiment (Table I,

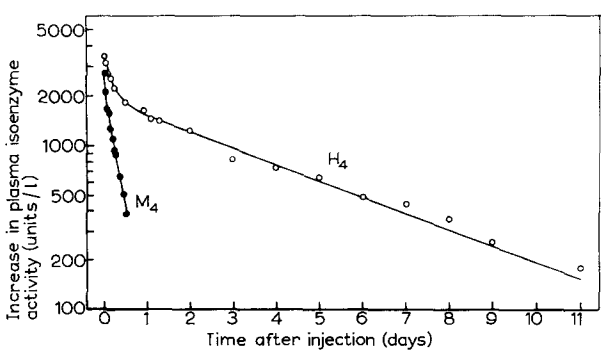


Fig. 3. Semi-log plots of the results of two experiments in which purified sheep LDH isoenzymes were injected intravenously into lamb 191. M_4 LDH (●) was injected in the earlier experiment and and H_4 LDH (○) in the later one. The increase of plasma LDH activity above the normal level is plotted against time.

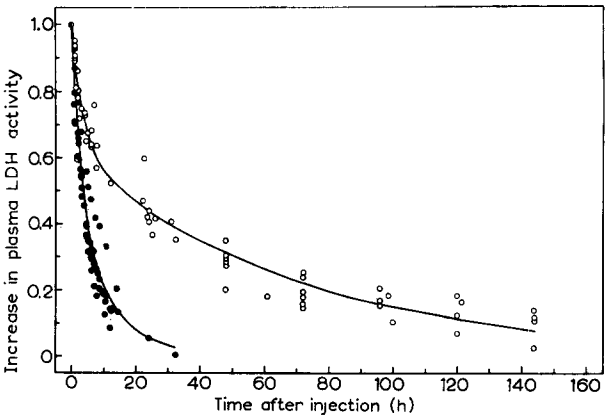


Fig. 4. Intravenous injection of purified sheep LDH isoenzymes into lambs. Isoenzyme H_4 (○) and isoenzyme M_4 (●) activities are plotted as fractions of the increased plasma activity shortly after injection. Double exponential curves have been fitted to data pooled from the experiments listed in Table I.

Fig. 3) and to the pooled data (Table I, Fig. 4). The values obtained for R, V_1, α_1 and α_2 were used to solve Eqn. 5 for k_{12}, k_{21}, and k_{20}. There can be no unique solution of Eqn. 5 in the absence of independent evidence concerning the rates k_{10} and k_{20}. As a first approximation for both isoenzymes, it will be assumed that k_{10} is zero (Table II,a). In the case of M_4, another simple approach is to assume that the difference from H_4 results from a difference in k_{10} only (Table II,b).

Treatment of muscular dystrophy (discontinued high entry rate)

Three lambs with experimental nutritional muscular dystrophy all had elevated plasma LDH isoenzyme levels, presumably due to release of LDH from damaged

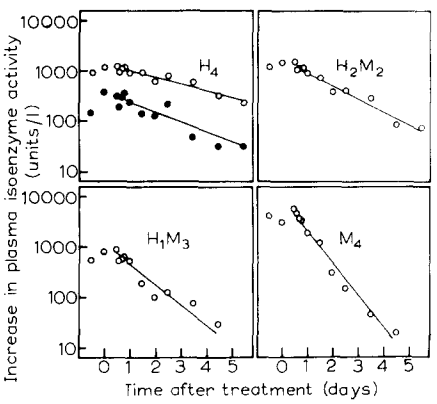
TABLE II

CALCULATION OF RATE CONSTANTS FOR H_4 AND M_4 LDH ISOENZYMES

	k_{10} (h^{-1})	k_{12} (h^{-1}) $[A(\alpha_1-\alpha_2)+\alpha_2]$	k_{20} (h^{-1}) $\left(\frac{\alpha_1\alpha_2}{k_{12}}\right)$	k_{21} (h^{-1}) $[\alpha_1+\alpha_2-(k_{12}+k_{20})]$	S/C_1* (ml/h/kg) $\left(\frac{V_1\alpha_1\alpha_2}{k_{21}}\right)$
(a) Assume $k_{10}=0$ for both H_4 and M_4					
H_4	0	0.118	0.035	0.148	1.57
M_4	0	0.228	0.130	0.071	23.6
(b) Assume $k_{10}=0$ for H_4, k_{12}, k_{21} and k_{20} for M_4 are same as for H_4					
H_4	0	0.118	0.035	0.148	1.57
M_4	0.128				11.32

* C_1 and therefore S vary between experiments. Their ratio S/C_1 can be regarded as a metabolic clearance rate (ml plasma/h per kg body weight). Turnover of LDH is equivalent to complete clearance of (S/C_1) ml/h per kg.

muscle. Treatment of these animals with α-tocopheryl acetate by intramuscular injection caused the exponential decline of plasma LDH isoenzyme activities, *e.g.* lamb 188, Fig. 5. The isoenzyme activities were determined by scanning stained isoenzymes after electrophoresis of plasma (see METHODS). The half-life for the decline in each isoenzyme was determined by fitting a curve to logarithms of activities by the method of least squares. The mean half-lives $\pm$S.E. for isoenzymes H_4, H_3M_1, H_2M_2, H_1M_3 and M_4 were 48.6$\pm$5.7, 30.8$\pm$3.1, 20.0$\pm$4.1, 12.6$\pm$2.7 and 7.4$\pm$1.8 h, respectively. The pooled data from all three animals for isoenzymes H_4 and M_4 are plotted in Fig. 6. The curves on this figure were obtained by assuming a 12-h time lag between the injection of vitamin E and its effect, and then evaluating Eqn. 6 using the exponential coefficients derived from the single-injection experiments. A control experiment showed that plasma α-tocopheryl acetate is approaching its maximum 12 h after intramuscular injection.



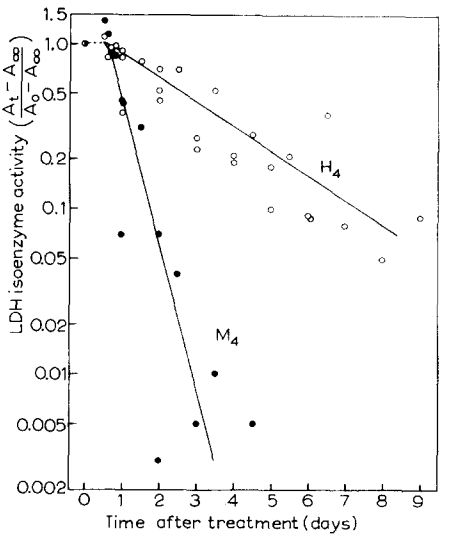


Fig. 5. Semi-log plot of the decrease in plasma LDH isoenzyme activities in dystrophic lamb 188 after treatment on day 0 with 200 mg α-tocopheryl acetate intramuscularly. The curves were fitted by the method of least squares.

Fig. 6. Semi-log plot of the decrease in plasma H_4 isoenzyme (○) and M_4 isoenzyme (●) in three dystrophic lambs treated with α-tocopheryl acetate (200 mg, intramuscular). The activities are fractions of the increase of activity above normal at the time of treatment, day 0. A_∞, endogenous activity; A_0, activity on day 0 and A_t = activity at time t after treatment. The curves were calculated from rate constants derived from the isoenzyme-injection experiments.

Rates of release of endogenous isoenzymes

The normal plasma isoenzyme activities are presumably due to leakage from the tissues mainly into V_2, the volume of the interstitial fluid. The rate of leakage (S), when fairly constant, can be derived from Eqn. 7a (Table II). The rate of leakage responsible for constantly elevated plasma activities can be calculated in the same manner. When the original tissue activity is known the rate of tissue destruction can be estimated. Typical H_4 and M_4 activities in lamb skeletal muscle are 6.24 and 81.8 units per g wet wt., respectively. Thus normal plasma M_4 LDH activity (C_1 = 70

units/l) could be maintained by the breakdown of a mere 0.48 g of skeletal muscle per day per kg body weight, if $k_{10} = 0$ (Table II,a) or of 0.23 g if $k_{10} = 0.128$ (Table II,b). These rates are approximately equivalent to a turnover of 0.1 or 0.05% of skeletal muscle per day, assuming muscle to be 50% of the body weight. In muscular dystrophy the degeneration of 1 g of skeletal muscle per h per kg body weight would maintain plasma H_4 and M_4 activities at several thousand units per l. Activities of this order of magnitude are in fact observed experimentally.

DISCUSSION

The previous reports of disappearance rates of various injected enzymes (transaminases, dehydrogenases, *etc.*) have been reviewed by HESS[18]. The disappearance rates of enzymes generally resemble those of other proteins in following a bi-phasic exponential course. The first exponential phase probably represents the distribution of the enzyme between an intravascular and an extravascular compartment. The second phase is mainly due to the actual removal or inactivation of the enzyme and is a measure of its turnover rate. The enzymes have faster turnover rates than the plasma proteins. The half-lives of the two phases of disappearance of LDH activity from plasma in lambs are 2.0 and 8.0 h for isoenzyme M_4, and 2.4 and 48.0 h for isoenzyme H_4. The more rapid disappearance of the M_4 LDH is not exceptional since FLEISHER AND WAKIM[19] found that the disappearance rate of one isoenzyme of L-aspartate:2-oxoglutarate amino-transferase (EC 2.6.1.1) was much faster than that of the other. Unfortunately there are no reports in the literature with which to compare the half-lives calculated for sheep H_4 LDH. However, half-lives of 1.4 and 2.5 h obtained by AMELUNG[20] for LDH disappearance in rabbits can be compared to the present M_4 results since he injected rabbit muscle LDH which contains mainly isoenzyme M_4 (ref. 21). The disappearance of injected LDH isoenzymes has not been studied in man. However, a half-life of 14.4 h has been calculated for the decline in plasma LDH after toxic liver damage in man, whereas during recovery from myocardial infarction the half-life is 36 h (ref. 18). This 2.5-fold difference is probably due to different disappearance rates of isoenzymes since in man liver damage releases mainly M_4 LDH (ref. 22), while cardiac damage releases mainly the more slowly disappearing H_4 isoenzyme[23]. The very rapid disappearance of M_4 LDH from human plasma is well illustrated in a case described by COHEN *et al.*[24].

In the present work the mean volume (56.4 ml per kg body wt.) estimated for V_1, the first compartment of the mathematical model, is consistent with the hypothesis that the injected isoenzymes mix rapidly in the plasma volume. The size of the extravascular compartment cannot be derived from the equations without knowing the concentration of isoenzymes therein. There are technical difficulties in obtaining representative samples of extravascular fluids. The rate constants k_{12} and k_{21} represent the filtration of isoenzymes from plasma into interstitial fluids and return *via* lymph, respectively. The rate constants k_{10} and k_{20} represent the elimination or inactivation of isoenzyme from the two compartments. From the work of AMELUNG *et al.*[25] it appears that excretion of LDH in bile and urine is of minor importance. The preliminary incubation experiments *in vitro* revealed that inactivation of sheep LDH isoenzymes by plasma at pH 7.4 was slow compared to the rates of disappearance observed *in vivo*. Even with the more labile M_4 LDH only one sixth of the rate of

disappearance of activity could be attributed to inactivation. Evidence is accumulating which suggests that the reticulo-endothelial system removes enzymes from the circulation. Reticulo-endothelial system blocking agents, *e.g.* thorotrast, zymosan and cholesterol oleate, at dose levels which reduce carbon clearance rates greatly depress LDH clearance[26,27]. Furthermore, infection of mice with Riley's virus depresses carbon clearance and the disappearance rate of both rabbit muscle LDH (ref. 28) and M_4 isoenzyme isolated from rabbit muscle[10]. The absence of any significant effect on pig heart H_4 LDH disappearance in infected mice indicates that different mechanisms control the disappearance of these two isoenzymes. This conclusion is supported by the observation that when an isoenzyme is injected into lambs the endogenous levels of the other four are not depressed. In a control experiment of which no details are given in this paper, H_4 and M_4 disappeared at their normal rates when they were both injected simultaneously.

It can be postulated that the differences between M_4 and H_4 LDH disappearance are due to the more rapid distribution of the M_4 isoenzyme between plasma and interstitial fluid and its more rapid removal from interstitial fluid by the reticulo-endothelial system. Having assumed that k_{10} is zero for both isoenzymes, rate constants can be calculated (Table IIa). Alternatively it can be assumed that the two isoenzymes are both distributed between body fluids and catabolised from interstitial fluid at the same rates and differ in that only M_4 LDH is irreversibly removed from plasma by the reticulo-endothelial system. In these circumstances the rate constants for both isoenzymes are identical except that $k_{10} = 0$ for H_4 LDH, and k_{10} for M_4 LDH is then 0.128 (Table II,b). This second hypothesis is favoured since it seems more reasonable to postulate that two isoenzymes of the same molecular weight are distributed between plasma and tissue fluid at the same rate. Moreover, the effect of particulate reticulo-endothelial system blocking agents on M_4 LDH (ref. 27) can then be attributed to a blocking of the irreversible removal of LDH from plasma. There is evidence that Riley virus infection in mice depresses the rate of M_4 LDH disappearance rate to the same value as for H_4 LDH (ref. 10). Thus this virus could also be blocking the irreversible removal from plasma. With either hypothetical model the calculated rates of release (S) of isoenzymes from tissues of normal and dystrophic animals appear to be reasonable when expressed as the amount of tissue undergoing destruction. However, it must be borne in mind that enzymes can leak from apparently normal cells[29] and that damaged cells may eventually regenerate. These results indicate that M_4 isoenzyme must leak from tissues at a rate either 7.5 or 15 times greater than that of H_4 isoenzyme in order to maintain the same level in plasma. These calculations are consistent with the observation that M_4 LDH activity in normal plasma is lower than that of H_4 LDH, despite the fact that it predominates in the tissues of the body as a whole. They are also consistent with the relatively low plasma M_4 LDH levels often found in muscular dystrophy[5,7–9]. The actual rates of decline of plasma LDH isoenzymes in lambs with nutritional muscular dystrophy treated with vitamin E are in good agreement with those predicted from the known behaviour of administered homologous isoenzymes, allowing a 12-h time lag for the intramuscular injection to take effect (Fig. 5). The progressively faster exponential disappearance rates of the five isoenzymes from H_4, H_3M_1, H_2M_2, H_1M_3 to M_4 in this experiment are reminiscent of the progressive differences between isoenzymes in pyruvate concentration optima,

charge density, antibody inhibition, pH inactivation and temperature stability[30,31] and relate to the tetrameric molecular structure of LDH (ref. 2).

The results of these experiments with vitamin E confirm the claim that plasma enzyme levels can be used to assess the therapeutic value of drugs in muscular dystrophy[32]. Although slowly disappearing enzymes and isoenzymes will accumulate to give higher plasma levels in muscular dystrophy, the rapidly disappearing ones will be more obviously sensitive to therapeutic agents, especially those with only a transient effect.

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