DIAGNOSTIC DISSECTION BY ISOENZYMES

F. WRÓBLEWSKI

Section of Medical Enzymology, Sloan-Kettering Institute and Department of Medicine, Memorial-Sloan-Kettering Cancer Center, New York, U.S.A.

INTRODUCTION

Alterations to plasma and serum enzymes represent subtle protein modifications associated with disease states. It appears that these enzyme activities are associated with two or more closely-related multimolecular forms which are, nevertheless, chemically, physically and immunologically distinct[1-10]. These components, isoenzymes, can be measured individually. It is the purpose of this presentation to report on the isoenzyme composition of lactic dehydrogenase (LDH) in the tissues and plasma of normal individuals and of patients with various disease states.

METHODS AND MATERIALS

Lactic dehydrogenase activity was determined by the method previously described[11]. Zone electrophoresis was used to fractionate the LDH activities by a modification[12] of Smithers' method as described previously[13]. The patients studied were observed at the Memorial and James Ewing Hospitals in New York City, and diagnoses were confirmed by the usual clinical criteria and/or *post mortem* examination.

RESULTS

Heparinized plasma obtained from normal adults had a total LDH activity of 360 $\pm$ 60 units/ml, within a range of activity from 240 to 480 units/ml at 30°. The plasma of normal adults contained all five plasma isoenzymes (LDH_1, LDH_2, LDH_3, LDH_4, and LDH_5).

Table 1. Relative electrophoretic mobilities of human LDH isoenzymes (mobility of purified bovine haemoglobin = 1·00), determined by the method of Smithers[12,13]

Isoenzyme	*Electrophoretic migration rate*
LDH_1	−0·21 $\pm$ 0·02
LDH_2	0·39 $\pm$ 0·03
LDH_3	0·75 $\pm$ 0·02
LDH_4	1·14 $\pm$ 0·05
LDH_5	1·53 $\pm$ 0·05

Table 2. The isoenzyme composition of normal human plasma LDH

Isoenzyme	LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
Percentage of total LDH*	0–5	0–15	0–37	23–50	6–36

* Range includes 2 standard deviations from mean value.

Table 1 lists the rates of electrophoretic migration of the human LDH isoenzymes relative to that of purified bovine haemoglobin. *Table 2* gives the relative amounts of the isoenzymes present in the plasma of normal adults, expressed as approximate percentages of the total LDH, as determined by electrophoresis. In all cases, plasma LDH_4 was present in greater amounts than was plasma LDH_5.

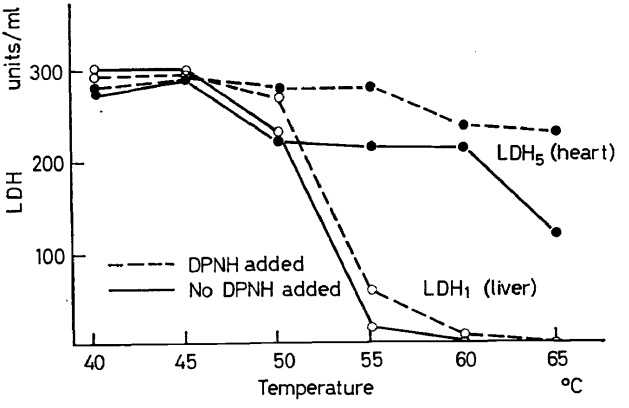


Figure 1. Thermal stabilities of human LDH_1 and LDH_5 in serum

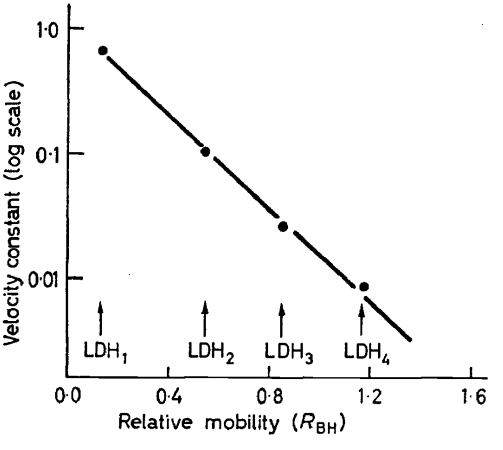


Figure 2. The velocity constants of inactivation at 53° of LDH_1, LDH_2, LDH_3 and LDH_4, as a function of the relative electrophoretic mobilities of these isoenzymes

The human isoenzymes can be differentiated by physical, chemical and immunological characteristics. *Figure 1* contrasts the thermal stabilities of LDH_1 and LDH_5, while *Figure 2* contrasts the velocity constants of inactivation of four of the human isoenzymes at 53°. *Figure 3* depicts the apparent energies of activation for the five isoenzymes relative to their mobilities on starch-gel. The percentage inhibition obtained immunologically using purified anti-LDH_1 and anti-LDH_5 against all five human isoenzymes is depicted in *Figure 4*.

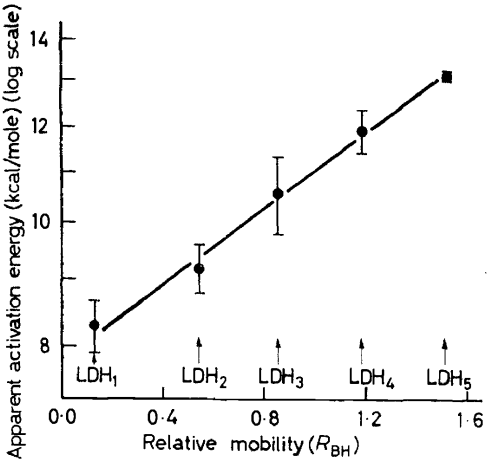


Figure 3. The apparent energies of activation (for the reaction: lactate⟶pyruvate) using LDH_1, LDH_2, LDH_3, LDH_4 and LDH_5, as a function of the relative electrophoretic mobilities of these isoenzymes

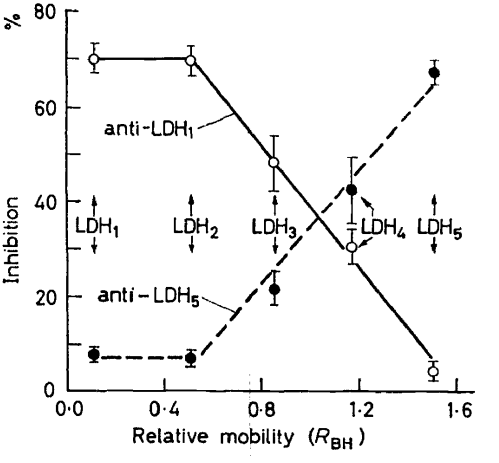


Figure 4. The degrees of inhibition of LDH_1, LDH_2, LDH_3, LDH_4 and LDH_5 by anti-LDH_1 and anti-LDH_5 respectively, as a function of the relative electrophoretic mobilities of these isoenzymes

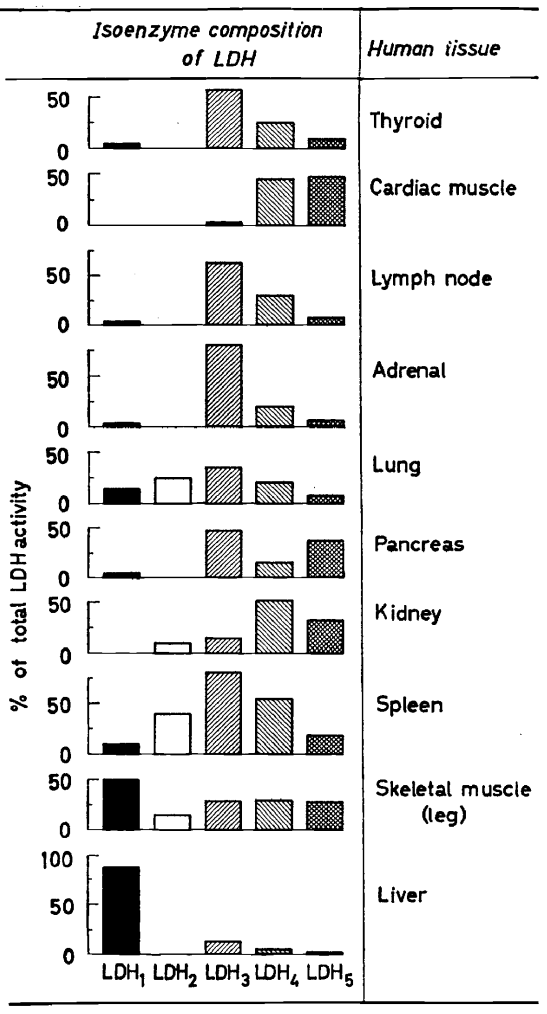
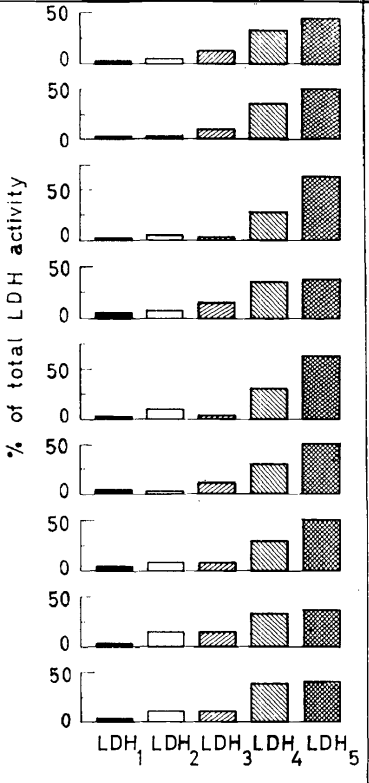


Figure 5. The distribution patterns of LDH isoenzymes in various normal human tissues

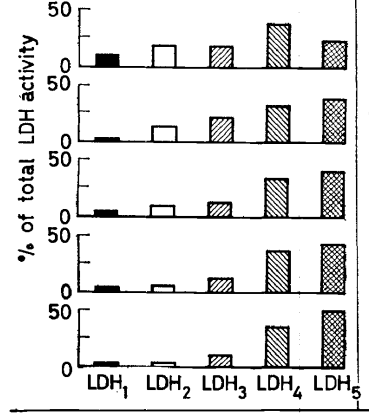
Examination of the LDH isoenzymes of normal human tissues indicates that different tissues contain different proportions of the five isoenzymes and that these occur in distinct patterns. *Figure 5* shows the LDH compositions and the distribution patterns of the isoenzymes in various normal human tissues.

Plasma obtained from patients with myocardial infarction showed an increased content of plasma LDH_4 and LDH_5 with the preponderant increase in the latter. *Figure 6* lists the total plasma LDH and glutamic–oxaloacetic transaminase (GOT) and the relative distribution of the five LDH isoenzymes observed in patients with myocardial infarction. *Figures 7, 8* and *9* depict the serial observation in patients who incurred transmural myocardial infarction. The plasma isoenzyme changes appear earlier in the course of myocardial infarction, and last longer following coronary occlusion, than the alteration in total plasma enzyme activity.



Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)	Diagnosis
	1300	94	Anterior myocardial infarction
	930	110	Anterior myocardial infarction
	2000	50	Anteroseptal myocardial infarction
	340	20	Posterior myocardial infarction
	780	14	Anterolateral myocardial infarction
	640	22	Posterior myocardial infarction
	760	34	Subendocardial myocardial infarction (shock)
	1000	77	Staphylococcal cardiac abscess
	240	20	Benign pericarditis

Figure 6. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in 9 patients with myocardial infarction



Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)	Days following onset
	440	25	24
	430	22	19
	440	25	13
	740	30	5
	930	74	2

Figure 7. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (G.B., 50 yrs. ♂) with posterior myocardial infarction

Angina pectoris causes no significant change in plasma isoenzyme composition (*Figure 10*), though subendocardial infarction is associated with an increase in plasma LDH_5 (*Figure 11*). The changes in myocardial infarction are to be contrasted with those seen in pulmonary infarction (*Figure 12*).

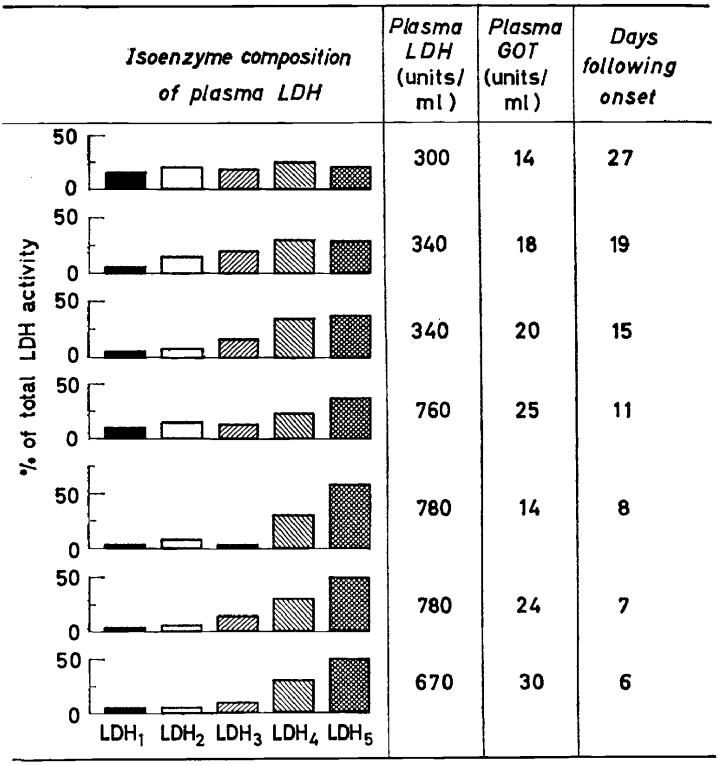


Figure 8. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (J.W., 49 yrs. ♂) with posterior myocardial infarction

Figures 13–21 depict the isoenzyme patterns observed when the plasma of patients with various diseases are examined. It appears that the isoenzyme composition of the diseased tissue or organ is contributed to the plasma, and is in part added to the isoenzyme components normally found in the plasma.

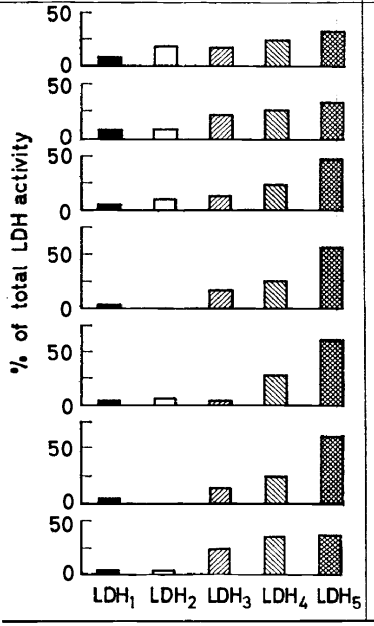
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)	Days following onset
	360	18	20
	330	25	15
	350	20	13
	1,100	30	5
	2,000	50	3
	740	44	1d–5h
	480	26	1h

Figure 9. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (F.R., 49 yrs. ♂) with anteroseptal myocardial infarction

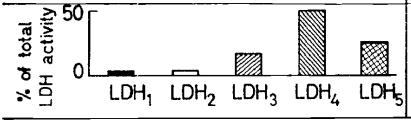
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)
	500	38

Figure 10. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (H.F., 72 yrs. ♂) with angina pectoris and congestive failure

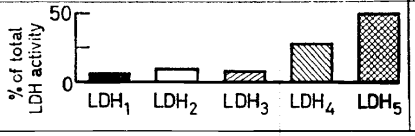
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)
	760	25

Figure 11. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (I.G., 70 yrs. ♂) with subendocardial infarction (secondary to shock)

Isoenzyme composition plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)
	400	48

Figure 12. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (I.McA., 55 yrs. ♂) with pulmonary infarction

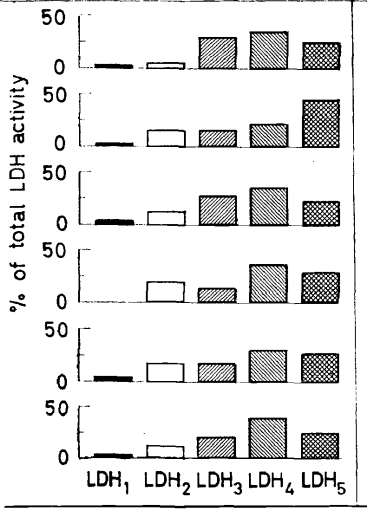
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Diagnosis (Patient)
	3,000	Infectious hepatitis (K.V.)
	490	Serum hepatitis and leukaemia (J.O.)
	600	Serum hepatitis (C.C.)
	500	Choledochal cyst with cirrhosis (J.P.)
	340	Cirrhosis (W.L.)
	1,900	Metastatic adenocarcinoma to liver (L.B.)
	2,600	Metastatic adenocarcinoma to liver (C.C.)
	360	Metastatic adenocarcinoma to liver (L.G.)

Figure 13. The isoenzyme compositions and total concentrations of plasma LDH in 8 patients with various diseases of the liver

Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Date	Plasma GOT (units/ml)	Plasma GPT (units/ml)
	2,600	7.4.60	720	1,900
	3,000	5.4.60	980	2,600
	1,100	4.4.60	600	1,800

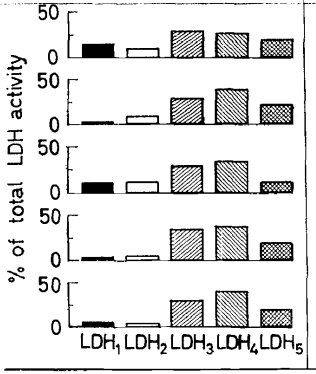
Figure 14. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH, GOT and GPT* in a patient (K.V., 56 yrs. ♀) with infectious hepatitis

* Glutamic-pyruvic transaminase.



Plasma LDH (units/ml)	Diagnosis (Patient)
220	Stone obstructing common duct (J.H.)
420	Reticulum sarcoma obstructing common duct (R.R.)
780	Hodgkin's disease obstructing common duct (R.W.)
380	Primary adenocarcinoma of common duct (N.B.)
540	Medullary duct carcinoma obstructing common duct (B.J.)
1,800	Adenocarcinoma of gall-bladder obstructing common duct (V.S.)

Figure 15. The isoenzyme compositions and total concentrations of plasma LDH in 6 patients with various diseases involving obstruction of bile ducts



Plasma LDH (units/ml)	Diagnosis (Patient)
560	Subacute pancreatitis with fistula (R.U.)
410	Subacute pancreatitis (T.K.)
750	Acute pancreatitis (C.B.)
900	Chronic pancreatitis and adenocarcinoma of pancreas (C.C.)
410	Acute pancreatitis (G.K.)

Figure 16. The isoenzyme compositions and total concentrations of plasma LDH in 5 patients with various diseases of the pancreas

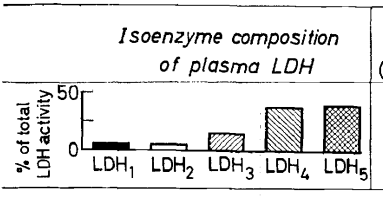


Figure 17. The isoenzyme composition and total concentration of plasma LDH in a patient (N.K., 18 yrs. ♀) with acute glomerulonephritis

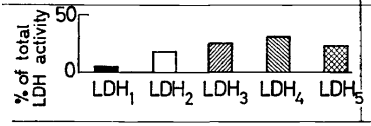
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Plasma GOT (units/ml)
	370	10

Figure 18. The isoenzyme composition of plasma LDH and the concentrations of total plasma LDH and GOT in a patient (C.M., 74 yrs. ♂) with follicular carcinoma of the thyroid

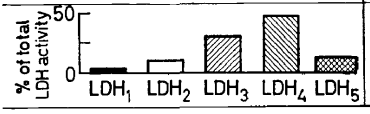
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)
	1,200

Figure 19. The isoenzyme composition and total concentration of plasma LDH in a patient (E.S., 35 yrs. ♀) with adenocarcinoma of the adrenal cortex

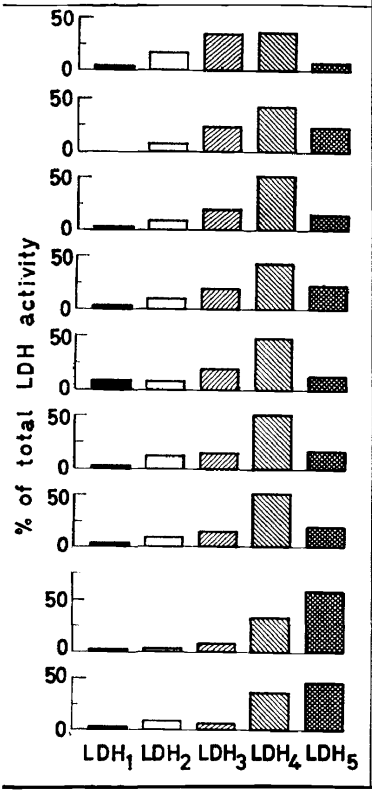
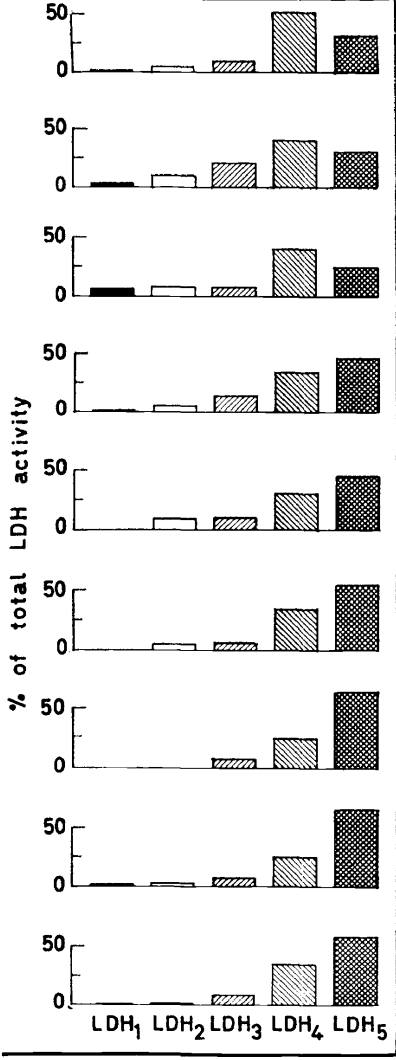
Isoenzyme composition of plasma LDH	Plasma LDH (units/ml)	Diagnosis (Patient)
	830	Agnogenic myeloid metaplasia (H.M.)
	900	Chronic myelogenous leukaemia pancytopenia due to myleran and 6-M.P. (S.T.)
	1,000	Chronic myelogenous leukaemia (J.P.)
	960	Acute leukaemia (G.G.)
	700	Subacute leukaemia (R.F.)
	270	Acute leukaemia (H.U.)
	1,200	Sickle cell anaemia (A.L.)
	1,200	Pernicious anaemia (L.F.)
	1,900	Macrocytic anaemia associated with total gastrectomy (F.W.)

Figure 20. The isoenzyme compositions and total concentrations of plasma LDH in 9 patients with various blood diseases



Isoenzyme composition of plasma LDH	*Plasma LDH (units/ml)*	*Date*	*Reticulocytes (%)*
	300	18.1.61	10·4
	600	3.1.61	1·2
	450	29.12.60	2·5
	1,100	27.12.60	4·0
	1,100	22.12.60	11·5
	1,000	19.12.60	25·5
	1,300	9.12.60	3·4
	1,100	7.12.60	3·0
	1,200	5.12.60	6·4

Figure 21. The isoenzyme composition and total concentration of plasma LDH and the reticulocyte level in a patient (L.F., 31 yrs. ♀) with pernicious anaemia

CONCLUSIONS

Human tissues each contain one or more of these LDH isoenzymes, and each tissue has an individual and characteristic isoenzyme pattern.

The plasma isoenzyme pattern seen during the course of myocardial infarction appears to be a more sensitive, specific and lasting parameter of myocardial necrosis than the measurement of total serum or plasma enzyme activity.

Diseases of various other organs cause characteristic alterations of plasma isoenzyme composition, reflecting in part the isoenzyme composition of the diseased tissue. Further studies are essential to define the usefulness of plasma and tissue isoenzyme estimation in clinical situations.

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