STUDIES ON LACTIC DEHYDROGENASE IN HEALTH AND IN DISEASES

By

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The enzyme, lactic dehydrogenase (LDH), is present in most animal and human tissues as well as in body fluids such as urine, serum, serous effusion and cerebrospinal fluid. By different mechanisms, LDH activity varies in body fluids in response to pathological process in tissues bathed by these fluids. The elevation of serum LDH activity has been observed in association with a variety of diseases, such as myocardial infarction, homologous serum hepatitis, carcinoma of various sites, metastatic carcinoma of the liver, diabetic acidosis, meningitis, myelogenous leukemia and various anemias (7,5, 9, 8 and 4). Necrosis of tissue results in the release of intracellular LDH which finds access into the extracellular fluid compartment resulting in an increase in serum LDH. The alterations in the serum LDH activity associated with tissue necrosis are influenced by many factors. A study was undertaken to find out the levels of serum LDH activity in patients with diseases of the liver, in whom other liver function tests were simultaneously carried out. Normal subjects were also studied for their serum LDH activity as a control group for comparison. The results of this study have been reported herewith.

MATERIALS AND METHOD

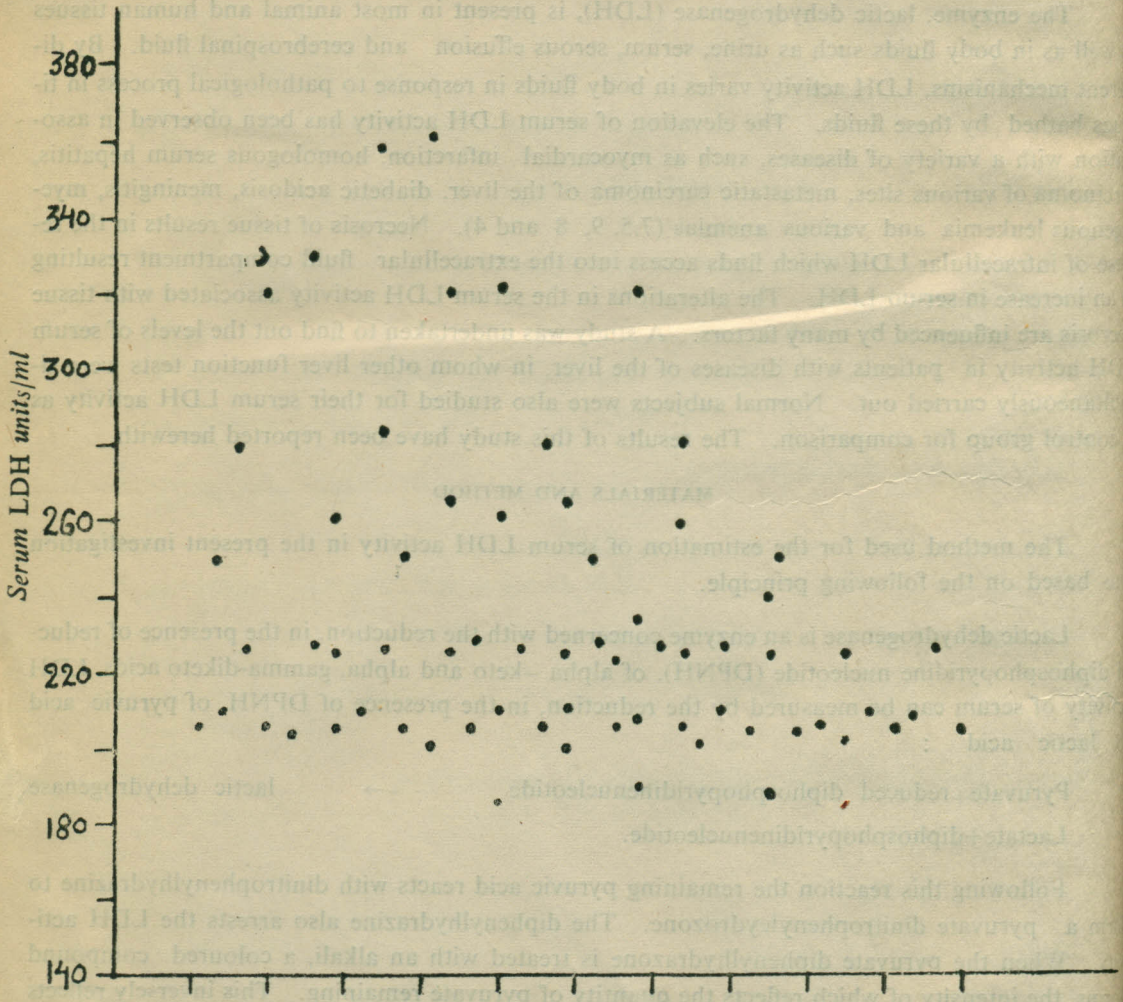
The method used for the estimation of serum LDH activity in the present investigation was based on the following principle.

Lactic dehydrogenase is an enzyme concerned with the reduction, in the presence of reduced diphosphopyridine nucleotide (DPNH), of alpha –keto and alpha, gamma-diketo acids. LDH activity of serum can be measured by the reduction, in the presence of DPNH, of pyruvic acid to lactic acid :

Pyruvate+reduced diphosphopyridinenucleotide $\xrightarrow{\text{lactic dehydrogenase}}$ Lactate+diphosphopyridinenucleotide.

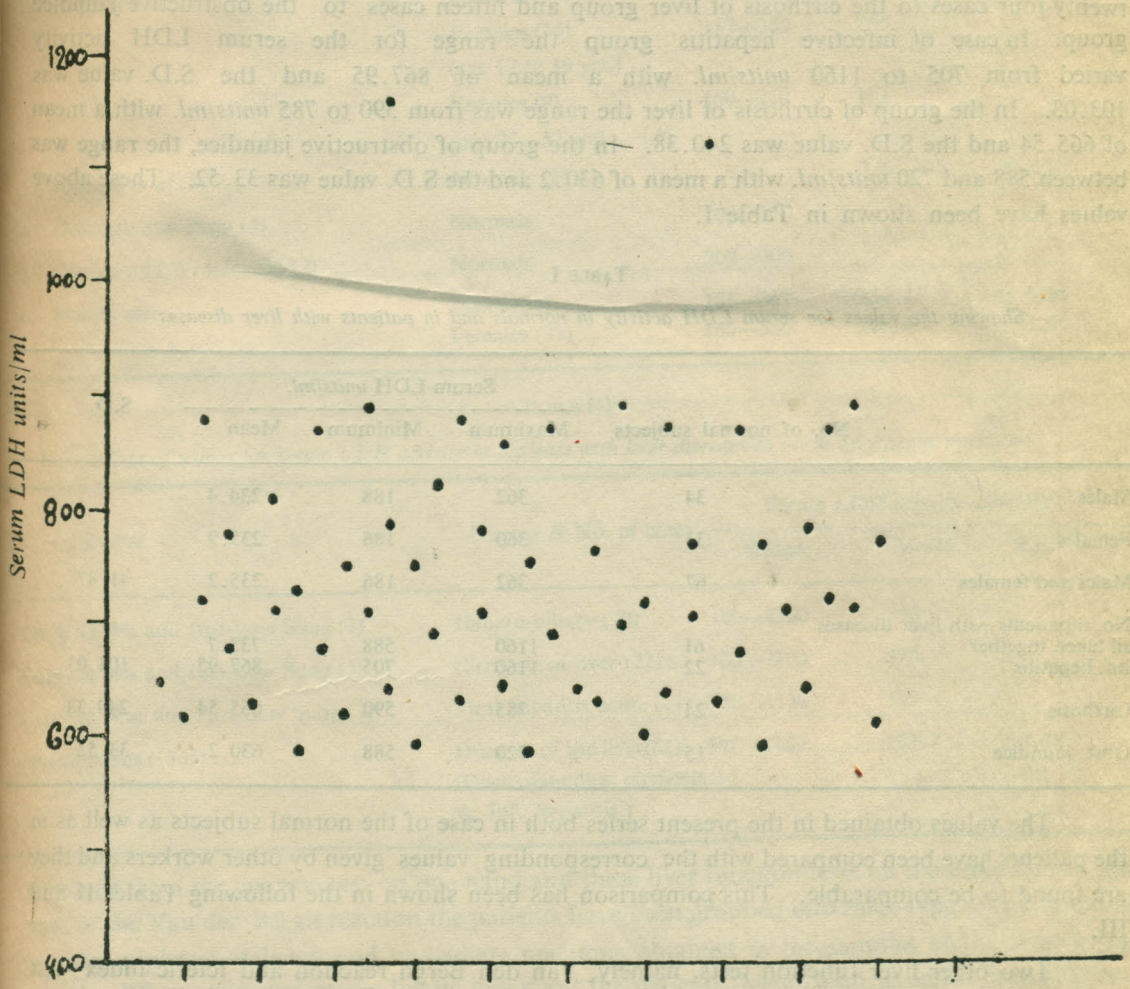
Following this reaction the remaining pyruvic acid reacts with dinitrophenylhydrazine to form a pyruvate dinitrophenyleydrozone. The diphenylhydrazine also arrests the LDH activity. When the pyruvate diphenylhydrazone is treated with an alkali, a coloured compound forms, the intensity of which reflects the quantity of pyruvate remaining. This inversely reflects the level of LDH activity. The intensity of the colour of the pyruvate dinitrophenylhydrazone formed was measured in terms of per cent transmittance at 550 $m\mu$ in a spectrophotometer. The

per cent transmittance was converted to units of lactic dehydrogenase activity through the stan dard curve obtained by setting up solutions containing decreasing amounts of pyruvic acid re flecting increasing LDH activities and treating them for the colour development according to th above procedure (2 , 10). Sixty-seven normal healthy adult subjects ranging in their age fro 28 to 57 years were investigated for their serum LDH activity. This group included thirty-fou male subjects and thirty-three female ones. Each of the estimation was done in duplicate and th average was taken thereof. Similarly sixty-one cases of diseases of liver whose diagnosis ha been given separately (Table VII) with their age ranging between 37 and 53 years, were investi gated for their serum LDH activity. These patients were studied for their serum enzyme activit



Graph 1 *showing the scatter points for the values of serum LDH in patient with hepatic diseases.*

levels at the time of onset of the disease before any kind of treatment was given and during their stay in the wards of the Hospital, where they were admitted for their complaints and for the purpose of further laboratory investigations. These patients belonged to the S.S.G. Hospital, Baroda and the Infectious diseases Hospital. Other liver function tests, namely, Van den Bergh test and Icteric index test were also performed simultaneously in the samples of sera obtained from the same patients (6). A comparison has been made between the results obtained of the serum LDH activity on one hand and those of the above two liver function tests performed on the other hand. These detailed results are shown in Tables VI, VII VIII and IX and are represented in Graphs I and II.



Graph 2 showing the scattered points for the values & serum LDH *in normal healthy subjects.*

RESULTS AND DISCUSSION

The values obtained for the serum LDH activity in normal male and female subjects ra from 186 to 362 *units/ml.* with a mean of 235.2 *units/ml.* The S.D. value was 41.87. In ca the normal male subjects the range was found to be from 188 to 362 with a mean value of 2 and in case of the females, the range was from 186 to 360 *units/ml.* with a mean of 235.9 *unit* There was no significant difference between the values for males and females. In case of the tients, all taken together, the range was found to be from 588 to 1160 *units/ml.* with a mea 733.7 *units/ml.* There were twenty-two cases which belonged to the infective hepatitis gr twenty-four cases to the cirrhosis of liver group and fifteen cases to the obstructive jaun group. In case of infective hepatitis group the range for the serum LDH act varied from 705 to 1160 *units/ml.* with a mean of 867.95 and the S.D. value 103.03. In the group of cirrhosis of liver the range was from 590 to 785 *units/ml.* with a n of 665.54 and the S.D. value was 240.38. In the group of obstructive jaundice, the range between 588 and 720 *units/ml.* with a mean of 630.2 and the S.D. value was 33.52. These a values have been shown in Table I.

TABLE I

Showing the values for serum LDH activity in normals and in patients with liver diseases

	No. of normal subjects	Serum LDH *units/ml.*			S.D.
		Maximum	Minimum	Mean	
Males	34	362	188	234.4	
Females	33	360	186	235.9	
Males and females	67	362	186	235.2	41.87
No. ofpatients with liver diseases					
all taken together	61	1160	588	733.7	
Inf. hepatitis	22	1160	705	867.95	103.0
Cirrhosis	24	785	590	665.54	240.3
Obst. Jaundice	15	720	588	630.2	33.52

The values obtained in the present series both in case of the normal subjects as well a the patients have been compared with the corresponding values given by other workers and are found to be comparable. This comparison has been shown in the following Tables II III.

Two other liver function tests, namely, Van den Bergh reaction and Icteric index were also simultaneously performed in the samples of sera, obtained from these patients, w they were investigated for their serum LDH activity. A comparison was made between the re

TABLE II

Showing comparison of values for serum LDH activity in normal healthy subjects as given by different workers

	Author	Sex and No. of subjects Studied	Serum LDH activity *units/ml* Range	Mean	S.E.
1	Chi. Ping, Wu and Juei-Low Sung (3)	Males (18)	107—267	199	—
		Females (18)	100—330	172	
2	Rapp and Bell (8)	Males (30)	150—260	189	..
		Females (35) (age 17 to 19 yrs.)	187—350	165	..
		Females (9) (age 24—52 yrs)	160—210	181	..
3	Albaum *et al* (1)	Normals (26)	..	189	± 13.3
4	Goldfarb and Papp (4)	Normals	100—350	..	..
5	Cabaud and Wroblewski (2)	Normals	200—500	..	..
6.	Present series	Males (34)	188—362	234.4	± 6.84
		Females (33)	186—360	235.9	± 7.54

TABLE III

Comparison of values for serum LDH activity in patients with liver diseases as given by diffeent workers

Author	Disease & No. of cases	Serum LDH activity *units/ml.* Range	Mean	S.E.
Chi-Ping Wu and Juei-Low Sung (3)	Hepato-biliary (70)	100—2100	356	...
Chi-Ping Wu and Juei-Low Sung (3)	Cirrhosis of liver (27)	100—2100	374	...
Chi-Ping Wue and Juei-Low Sung (3)	Viral hepatitis acute (13)	250—1130	507	...
Present series (1965)	Diseases of the liver(61) (Obst. jaundice, cirrhosis & Inf. hepatitis.)	588—1160	733.7	±8.75

obtained from the enzyme test on one hand and these liver function tests on the other hand. In case of the Van den Bergh reaction the patients have been grouped into three types of the reaction, namely, biphasic, delayed and immediate reactions obtained in the samples of the sera, when tested. Whereas in case of the Icteric index test the patients have been grouped into four arbitrary groups according to the values of the Icteric index units obtained in the samples of sera,

when tested. In Tables IV and V are shown the values of the serum LDH activity obtai these patients grouped as described above.

TABLE IV

Showing values of serum LDH activity with their range, mean and S.D. values in patients with liver diseases gro according to the response obtained with Van den Bergh test

		Group I	*Group II*	*Group I*
No. of Patients		22	24	15
Serum LDH *units/ml.*	Maximum	1160	785	720
	Minimum	705	590	588
	Mean	865.23	682.21	630.
	S.D.	111.27	176.49	33.5

Group. I—biphasic ; Group. II—delayed; Group. III—Immediate.

TABLE V

Showing values of serum LDH activity with their range, mean and S.D. values in patients with liver diseases grou according to the response obtained with Icteric index test

		Group I	*Group II*	*Group III*	*Group*
No. of patients		16	24	12	9
Serum LDH *units/ml.*	Maximum	720	870	960	1160
	Minimum	588	620	610	782
	Mean	641.69	714.54	822.33	904.1
	S.D.	60.19	74.58	86.95	132.9

Group. I Icteric index 14-27 units Group. III ,, ,, 41-54 units
Group. II ,, ,, 28-40 ,, Group. IV ,, ,, 55 units and abov

Statistical analysis of the data : The data has been statistically analysed to find out whet the differences observed were of any significant order. In Table X are shown the results of t analysis. It can be seen that the differences are statistically significant both according to t test and Behren's test. The variance ratio has also been shown in the table.

Wroblewski and La Due (10) noted elevations in lactic dehydrogenase activity in serum patients of myocardial infarction, leukemia and hepatitis. Zimmerman and Weinstein (

TABLE VI

Showing values for serum lactic dehydrogenase (units/ml.) activity in normal healthy subjects

S. No.	Sex	LDH *units/ml.*	S.No.	Sex	LDH *units/ml.*	S. No.	Sex.	LDH *units/ml.*
1	M	360	24	M	205	46	F	205
2	F	360	25	F	205	47	M	205
3	M	280	26	M	205	48	F	321
4	F	280	27	M	204	49	M	250
5	F	210	28	F	205	50	F	205
6	M	225	29	F	204	51	F	205
7	F	223	30	M	320	52	F	262
8	M	200	31	F	320	53	M	240
9	F	205	32	M	250	54	M	228
10	M	210	33	M	210	55	M	227
11	M	205	34	F	225	56	F	226
12	F	201	35	F	226	57	M	260
13	M	226	36	M	210	58	F	260
14	F	225	37	M	190	59	M	250
15	F	227	38	F	226	60	F	330
16	M	226	39	M	225	61	M	321
17	M	206	40	F	201	62	F	265
18	F	225	41	M	188	63	M	261
19	F	205	42	F	210	64	F	280
20	M	205	43	M	226	65	M	283
21	M	210	44	F	224	66	F	208
22	F	188	45	M	250	67	M	233
23	F	205						

TABLE VII

Showing values for serum lacitic dehydrogenase (units/ml.) activity in patients with hepatic diseases.

S. No.	LDH *units/ml.*	Clinical diagnosis	S. No.	LDH *units/ml.*	Clinical diagno
1	620	Obst. Jaundice	32	705	Inf. hepatitis
2	588	,, ,,	33	632	Cirrhosis
3	750	Cirrhosis	34	646	Obst. Janudice
4	870	Inf. hepatitis	35	677	Cirrhosis
5	730	Cirrhosis	36	605	Obst. Jaundice
6	770	Inf. hepatitis	37	590	,, ,,
7	780	,, ,,	38	648	Cirrhosis
8	820	,, ,,	39	622	Obst. Jaundice
9	860	,, ,,	40	647	Cirrhosis
10	700	Cirrhosis	41	676	Obst. Jaundice
11	782	Inf. hepatitis	42	885	Cirrhosis
12	785	,, ,,	43	720	,,
13	720	Cirrhosis	44	630	Obst. Jaundice
14	675	,,	45	690	Cirrhosis
15	765	Inf. hepatitis	46	872	Inf. hepatitis
16	810	,, ,,	47	708	Cirrhosis
17	750	Cirrhosis	48	712	,,
18	890	Inf. hepatitis	49	642	Obst. Jaundice
19	870	,, ,,	50	872	Inf. hepatitis
20	750	Cirrhosis	51	708	Cirrhosis
21	770	,,	52	712	,,
22	610	Obst. Jaundiee	53	642	Obst. Jaundice
23	770	Cirrhosis	54	709	Cirrhosis
24	870	Inf.hepatitis	55	885	Inf. hepatitis
25	890	,, ,,	56	592	Obst. Jaundice
26	630	Obst. Jaundice	57		Inf. hepatitis
27	720	,, ,,	58	882	,, ,,
28	630	Cirrhosis	59	960	,, ,,
29	590	,,	60	1160	,, ,,
30	640	Obst. Jaundice	61	1120	,, ,,
31	69u	Cirrhosis			

TABLE VIII

Relationship of serum lactic dehydrogenase (units/ml.) activity with liver function test-Van den Bergh reaction.

Group I		*Group II*		*Group III*	
S. No.	LDH *units/ml.*	Sr. No.	LDH *units/ml.*	Sr. No.	LDH *units/ml.*
1	870	1	750	1	620
2	770	2	730	2	588
3	780	3	700	3	610
4	820	4	720	4	630
5	860	5	675	5	720
6	782	6	750	6	640
7	785	7	750	7	646
8	765	8	770	8	605
9	810	9	770	9	590
10	890	10	630	10	622
11	870	11	590	11	676
12	870	12	690	12	630
13	890	13	632	13	642
14	705	14	677	14	642
15	872	15	648	15	592
16	872	16	647		
17	885	17	785		
18	877	18	720		
19	862	19	690		
20	960	20	708		
21	1160	21	712		
22	1120	22	708		
		23	712		
		24	709		

Group I :—Biphasic: Group II:—Delayed; Group III:—Immediate.

TABLE IX

Relationship of serum lactic dehydrogenase (units/ml.) activity with liver function test-Icteric Index.

Group I		*Group II*		*Group III*		*Group IV*	
S. No.	LDH *units/ml.*	S. No.	LDH *units/ml.*	S. No.	LDH *units/ml.*	Sr. No.	LDH *units/ml.*
1	588	1	620	1	770	1	860
2	700	2	750	2	870	2	782
3	720	3	870	3	820	3	785
4	675	4	730	4	765	4	890
5	630	5	810	5	770	5	870
6	590	6	750	6	610	6	785
7	690	7	870	7	890	7	885
8	705	8	750	8	872	8	1160
9	646	9	770	9	872	9	1120
10	605	10	630	10	887		
11	590	11	720	11	882		
12	622	12	640	12	960		
13	630	13	632				
14	642	14	677				
15	642	15	648				
16	592	16	647				
		17	676				
		18	720				
		19	690				
		20	708				
		21	712				
		22	708				
		23	712				
		24	709				

Group I Icteric Index 14 —27 Group III „ „: 41 —54
Group II „: 28 —40 Group IV „ „: 55 and above

TABLE X

Table showing the statistical analysis of the data for the values of serum LDH activity (units/ml.) in normals and in patients.

	Sample size	*A·M.*	*S.D.*	*(S.E.)2*	*t-test*	*Behren's test*	*Variance Ratio*
Normals	67	235.16	41.87	26.17			
Patients :—							
Obst. Jaundice	15	630.2	33.52	74.92	Significant	Significant	Not significant
Cirrhosis	24	665.54	240.38	240.6	,,	,,	Significant 0.1%
Inf. hepatitis	22	867.95	103.03	482.49	,,	,,	,, 0.1%
Van den Bergh reaction :—							
Group I	22	865.23	111.27	562.73	,,	,,	,, 0.1%
Group II	24	682.21	176.49	129.79	,,	,,	,, 0.1%
Group III	15	630.2	33.52	74.92	,,	,,	Not significant.
Icteric index test :—							
Group I	16	641.69	60.19	226.44	,,	,,	Significant 5%
Group II	24	714.54	74.58	231.75	,,	,,	,, 0.1%
Group III	12	822.33	86.95	630.05	,,	,,	,, 0.1%
Group IV	9	904.11	132.94	1963.77	,,	,,	Significant

A.M. :—Arithmetic mean. S.D. :—Standard deviation. S.E. :—Standard error.
Difference between the two means (for normals and patients) is highly significant.

observed elevated levels of LDH in serum of patients with carcinoma, acute myocardial infarction, sickle cell anemia, megaloblastic anemia and acute infectious mononucleosis. Hsieh and Bluementhal (7) reported an elevation of serum LDH activity in neoplastic diseases like stem cell and granulocytic leukemia, lymphoma and those involving hepatic metastasis as also in non-neoplastic diseases like myocardial infarction, infectious mononucleosis thrombocytopenia, hepatitis and obstructive jaundice whether intra or extra-hepatic.

Although different disease states produce quantitatively similar serum LDH alterations, it appears that different mechanisms contribute to the serum enzyme increments. In the course of homologous serum hepatitis huge increments in serum glutamic oxaloacetic transaminase are seen with only minimal serum LDH alterations. These serum enzyme changes occur, although the liver is richer in LDH than human heart tissue whereas GOT is present more abundantly in human heart tissue than in hepatic tissue. In situations where tissue necrosis occurs, intracellular LDH is released into the circulation. In inflammatory states involving organs rich

in LDH, little serum LDH alterations may occur unless cellular necrosis accompanies the inf matory process. When both inflammatory and necrotic factors are present, serum LDH vity may be elevated, but not necessarily in proportion to the tissue enzyme concentration. these conditions the factors other than the level of tissue enzyme activity are influencing th rum enzyme alterations. It may perhaps appear that inflammatory processes may selecti alter intracellular enzyme permeability, as well as the mechanisms for serum enzyme degrada In an experiment for myocardial infarction, muscle necrosis was induced experimentally in s groups of rats (5). The authors were able to prove in such animal experiments that, follo acute lesion, extensive enzyme activity changes occur in the liver, an organ of great import in enzyme metabolism, at the same time when the change is noted in the serum enzyme acti The enzyme released from the necrotic tissues in the myocardium has a ready access to the flo blood stream in the cardiac chambers thus resulting in an elevated serum LDH,which will n so in other cases where the released enzyme does not have a ready access to a blood supply, in w case the enzyme is destroyed or inactivated before reaching the circulatory system.

In the present series it can be seen from the Tables and the Graphs that the serum L activity has been found to be increased in cases of the patients with liver diseases studied, p cularly so in cases of infective hepatitis, as compared to the normals. In infective hepatitit increase is of the highest order; whereas in cases with cirrhosis and obstructive jaundice, elevation is of a relatively lower order. In the obstructive jaundice, the rise was the minim of the rise observed in these three conditions. The data has been statistically analysed and differences have been found to be statistically significant.

The variations of enzyme activity in serum following the damage to the tissues e.g. *etc.* are not to be interpreted solely as consequences of enzyme leakage from the necrotic ti cells. These changes should rather be regarded as a more general phenomenon. A com reaction of the organism begins to occur after the damage of the above type as it does in m other acute diseases. It is this complex reaction that gives rise to a number of symptoms causes the changes in enzyme activities. Thus there will be several factors influencing the zyme level both in the cell and in the serum. The degradation and formation of the enz in the cell membranes may be responsible for the level of the enzyme in the cells of the or especially in the liver, and the permeability of the cell membranes may be responsible for level of the enzyme in the cell. Certain enzymes are found to exist in two forms, an active f and its inactive or precursor form; and this behaviour has got to be taken into account when c sidering the factors influencing the serum enzyme level. Further, the permeability of cell me ranes, the elimination of the enzyme in urine and in bile and the degradation or the inhibitio the enzyme in the serum will also be the influencing factors in addition as well (Fig. 1).

(Fig. 1)

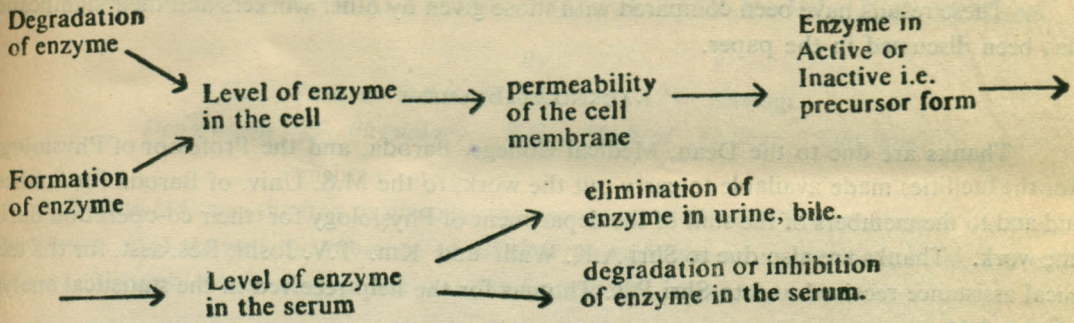


Fig 1:– illustrating in tho form of a diagramms the various factors influencing the level of enzyme in the cell and the serum.

The above factors will help explain the non-uniform behaviour of the enzymes *e.g.* lactic dehydrogenase, transaminases, etc. in respect of their levels in serum in cases of tissue damage, despite of the liberation of considerable amounts of intracellular enzymes into circulation. In another studies where the levels of SGOT and SGPT have been studied both in health and in diseases of liver, a similar finding was observed (unpublished data).

SUMMARY

A study was undertaken to find out the activity of lactic dehydrogenase (LDH) in serum of sixty-seven normal healthy subjects and to compare this activity with that similarly estimated in sixty-one patients with hepatic diseases. In the latter group, other liver function tests, namely, Van den Bergh reaction and Icteric index test were also performed simultaneously in the same samples of their sera and an attempt was made to compare the results of these two tests with those of the enzyme estimation.

The age of the normal subjects ranged between 28 and 57 years and they comprised of thirty-four males and thirty-three females. The serum LDH activity in this group was found to range between 186 and 362 *units/ml.* with a mean of 235.2 and the standard deviation of 41.87. No significant difference was found between the average values of LDH activity in the males and the females.

In patients whose age ranged between thirty-seven and fifty-three years, the corresponding range was from 588 to 1160 *units/ml.* with an average of 733.7. The activity of serum LDH has been found to be increased in patients particularly in patients with infective hepatitis, where high serum LDH activity has been observed as compared to the normals. The difference between the two means was statistically significant. The comparison has been made between the values obtained in different groups classified according to the response given by both the Van den Bergh test and the Icteric index reaction. And here also the differences found for the values of serum

LDH activity in these groups were statistically significant.

These results have been compared with those given by other workers and their significan has been discussed in the paper.

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