Serum Lactic Dehydrogenase Isoenzymes in Leprosy[1, 2]

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Serologic studies in leprosy have been primarily directed toward characterization and identification of various antibodies present in the sera of leprosy patients ([9, 10, 11]). In recent years efforts have been made to determine the possibility of establishing a serodiagnostic test for the disease, without much success although these studies have demonstrated a specific type of distribution of some of the antibodies in relation to the status of the disease ([9, 12]). It was, therfore, felt that estimation of the lactic dehydrogenase in the sera of leprosy patients and their distribution, qualitative or quantitative, might afford a system through which an early diagnosis could be made. If successful, such a test would then act as an auxiliary aid to the diagnostic technics currently in vogue.

Sera from leprosy patients in different stages of the disease and from contacts of leprosy patients were therefore obtained from India[4] and were subjected to isoenzyme estimation, to determine deviation, if any, in the pattern of the various lactic dehydrogenases (LDH) as compared to normal serum distribution.

MATERIALS AND METHODS

Test sera from:[5]

Lepromatous cases	7
Dimorphous cases	4
Tuberculoid Cases	7
Total	18

Control sera from:[5]

Contacts of leprosy cases	3
Normal individuals	2
Total	5

Enzyme assays. Lactic dehydrogenase was determined by a modification of the Kornberg technic ([6]). The reaction mixtures consisted of the following substrates per milliliter:

Potassium pyruvate	10.0 micromoles
Reduced diphosphopyridine nucleotide (DPNH)	0.1 micromoles
Potassium phosphate buffer (pH 7.4)	50.0 micromoles
Test serum	0.1 ml.

The rate of disappearance of DPNH was determined at 340 millimicrons in a Gilford Model 2000 Automatic Recording Photometer.

Electrophoresis. A commercial vertical gel electrophoresis apparatus (E. C. Corporation, Philadelphia) was used with a polyacrylamide gel support. The buffer (pH 9.2) used consisted of the following:

tris(hydroxymethyl)-aminonethane (TRIS)	20.0 gm.
Disodium EDTA	2.0 gm.
Boric acid	0.76 gm.
Distilled water	4 liters

Twenty microliter samples of the test sera were applied to the gel and the electrophoresis was run for two hours at 70 ma. After completion of the run, the gels were

1 Received for publication 24 July 1970.
2 This investigation was supported by the United States-Japan Cooperative Medical Science Program administered by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, Department of Health, Education, and Welfare (Grant AI-08647 to one of us (RGN), Bethesda, Maryland 20014.
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4 The sera were obtained under frozen condition by air mail transport.
5 The status of the patients was based on clinical and bacteriologic examinations done at intervals prior to obtaining the sera. Lepromin test was done on some, employing the Dharmendra antigen prepared at the Acworth Leprosy Hospital.

stained for LDH enzymes by the Goldberg method ([3]).

Hemoglobin determination. Hemoglobin was determined by the method of Evelyn and Malloy ([4]).

RESULTS

Table I gives the quantitative assay for lactic dehydrogenase. The values ranged from 40 to 186 units (millimicromoles per minute) per ml. of serum. It will be noted that there is no significant correlation between the type of leprosy and the concentration of the enzyme. The sera had been maintained in frozen state for a considerable period before they were used for analysis.

Since the serum LDH assays can be seriously affected by alterations caused by lysed erythrocytes, it was felt necessary to determine the extent of hemolysis in the sera samples used. Table I also shows the hemoglobin assay. It will be seen that the level of hemoglobin is low, indicating insignificant hemolysis.

Figure 1 is a representation of the electrophorogram of some of the sera tested for LDH activity. All of the sera tested exhibited isoenzyme patterns of the alpha type, as described by Cohen *et al.* ([2]).

DISCUSSION

Selection of the lactic dehydrogenase isoenzyme pattern as a possible serodiagnostic tool was based on the fact that this particular enzyme system has been well characterized ([2, 5, 7, 8]) and exhibits not only tissue specificity but also at times correlates progressive stages of some infections.

The level of LDH in unhemolyzed serum is normally very low and the isoenzyme pattern is almost identical to that of erythrocytes. Appearance of readily detectable cellular LDH isoenzymes in the serum is therefore indicative of tissue damage.

Brody ([1]) has shown that even nerve damage can cause a change in LDH distribution. This change presents a pattern characteristic of the newborn organism.

In leprosy, where there is a very definite involvement of internal organs as well as nerves, it was considered possible that a very significant deviation in both the total LDH and the specific isoenzymes would be easily demonstrable. However, the results did not indicate significant deviation from normal isoenzyme patterns. This may indicate that the tissue damage occurring in leprosy in all probability does not lead to the release of isoenzymes of the beta-gamma pattern, which have been shown by Cohen *et al.* ([2]) to be associated with damage to liver, kidney, skeletal muscle and other tissues. The available test materials, however, were too small to permit any definite conclusion in this regard. The isoenzyme seen in the test sera was of the alpha pattern and the concentration of this

TABLE 1. *Assay of lactic dehydrogenase and hemoglobin in serum.*

Test sera from	No. of sera tested	Lactic dehydrogenase (n moles/min./ml.) Range	Hemoglobin (mg./100 ml.) Range
Lepromatous patients	7	40–186	0.0–0.7
Dimorphous patients	4	81–117	0.2–0.6
Tuberculoid patients	7	65–158	0.0–1.6
Control sera from			
Normal healthy individuals	2	202[a]–339[b]	Not done
Contacts of leprosy patients	3	47– 97	Not done

[a] Freshly obtained serum (obtained from Hubbard Hospital).
[b] Stored frozen for one year (obtained from India).

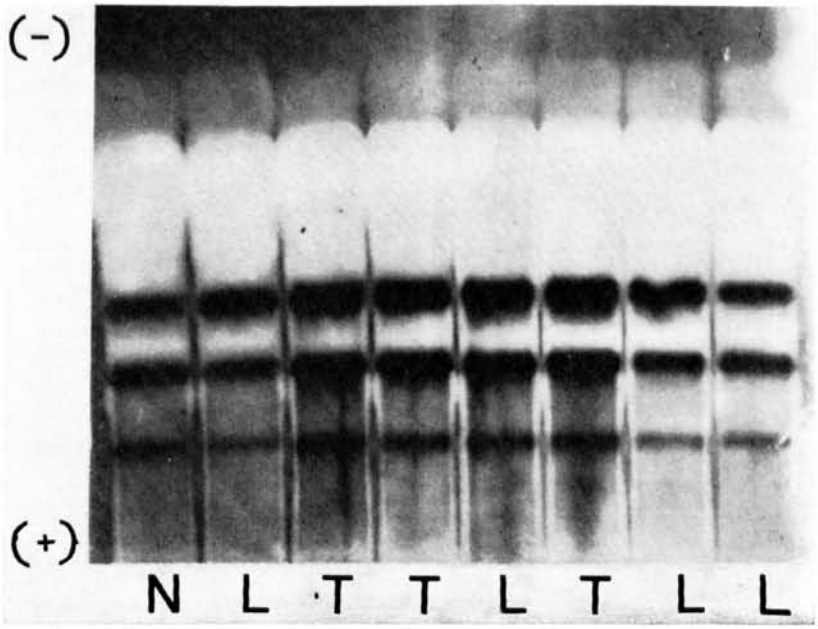

FIG. 1. Electrophoretic patterns, on acrylamide gel, of lactic dehydrogenase acitivity in sera of leprosy patients. **N** = normal serum; **L** = serum from lepromatous patient; **T** = serum from tuberculoid patient.

enzyme was similar to that seen in sera from contacts of leprosy patients.

As can be seen from the data presented in Table I, quantitative assay yielded no correlation between LDH concentration and type of leprosy although there appears to be a depletion of the enzyme as compared to the normal sera. One of the reasons could be the effect of prolonged cold storage.[6] Cohen *et al.* ([2]) have shown that the unit values for healthy controls ranged from 200 to 400 units per ml. when fresh sera are employed. These values, however, appear to diminish approximately by 40 per cent on storage at —10°C for a period of three weeks or more.

The other possibility, although unlikely, may be the preferential degradation of the beta-gamma isoenzymes during storage. The study of Wroblewski and Gregory ([13]) indicated greater lability of LDH_1 than LDH_5 at 55°C, although the two isoenzymes exhibited equal stability at 45°C. Use of fresh unstored sera from leprosy patients and from normal healthy individuals could help to resolve this point. In the present studies only one of the normal sera used for LDH determination was a freshly obtained sample. The loss of enzyme activity in the sera from the patients and contacts appears to be between 60 to 90 per cent as compared to the activity in the normal serum.

The extent of nerve involvement and muscle damage in tuberculoid leprosy and the involvement of liver and other internal organs in advanced lepromatous leprosy, which at times is rather severe, would suggest that isoenzymes might be liberated to a high level in the sera of such patients. This might be especially so in lepromatous leprosy, and these enzymes might be distributed in a manner different from that in normal serum. Studies of the LDH patterns in other diseases have indicated changes which could be correlated with the clinical status of the disease.

Information regarding the patients studied, did not include data other than their clinical status, age and sex. It is therefore not possible to state at this time whether the patients had involvement of liver or other internal organs, although such damage may be assumed to exist as a normal sequel to progressive infection.

It appears from these preliminary studies

[6] For approximately one year prior to testing.

that tissue damage in leprosy is not of the type which causes release of LDH into the circulatory system. It is not unlikely that the tissue damage leading to associated changes in the LDH and isoenzymes patterns in other chronic infections is different from that observed in leprosy. Detection of the alpha isoenzyme in almost all the cases studied, in contrast to the detection of the beta-gamma type shown by Cohen *et al.* ([2]) in other infections, lends credence to this hypothesis. The possibility of preferential degradation notwithstanding, if the beta-gamma pattern had been present, there would have been some indication of it at least in a few, if not all, of the test sera.

SUMMARY

Lactic dehydrogenase isoenzymes (LDH) were determined in sera from patients with different types of leprosy.

No significant variations were observed in the pattern of LDHs within the different types of the disease.

Quantitative changes noticed in the total enzyme concentration have been attributed to a possible depletion of the enzyme due to prolonged storage rather than due to the chronic infection itself.

It is suggested that tissue damage associated with leprosy is of a different nature from that seen in other chronic infections, in relation to changes in the LDH and isoenzyme patterns observed in these diseases.

RESUMEN

Se determinaron los niveles de isoenzimas de deshidrogenasa láctica (DHL) en sueros de pacientes con diferentes tipos de lepra.

No se observaron variaciones significativas en el patrón de DHLs entre los diferentes tipos de la enfermedad.

Los cambios cuantitativos encontrados en la concentración total de enzimas se han atribuido a una posible depleción de la enzima debido a un almacenamiento prolongado, más que a la infección crónica misma.

Se sugiere que el daño tisular asociado a la lepra es de una naturaleza diferente al que se ve en otras infecciones crónicas, en relación con las variaciones de los patrones de DHL e isoenzima observadas en estas enfermedades.

RÉSUMÉ

On a procédé à la détermination des isoenzymes de la déshydrogenase lactique (LDH) dans des échantillons de sérum provenant de malades atteints de différents types de lèpre.

Aucune variation significative n'a été observée dans le profil des LDH en rapport avec les différents types de la maladie.

Les modifications quantitatives qui ont été relevées dans la concentration totale de l'enzyme, ont été attribuées à une perte possible de l'enzyme par suite d'un entreposage prolongé, plutôt qu'à l'infection chronique elle-même.

On suggère que les lésions tissulaires associées à la lèpre sont d'une nature différente de celles qui sont observées dans d'autres infections chroniques; elles seraient en relation avec des modifications dans les profils de la LDH et de l'isoenzyme qui sont observées dans ces affections.

Acknowledgments. The authors would like to thank the Superintendent and staff of the Acworth Leprosy Home, Bombay, India, for supplying the sera (patients and contacts) used in the study. Participation of R. P. Warick is sincerely appreciated.

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