Lactate Dehydrogenase Zymograms of Skin Biopsies in Patients with Leprosy. A Preliminary Report[1]

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Isoenzymes are enzymes with functional unity but electrophoretic diversity. The isoenzymes of lactate dehydrogenase (LDH) have been extensively studied in human sera. Alterations of LDH patterns are established as pathogenetic markers in the diagnosis of necrotic parenchymal diseases ([3]). We have studied isoenzymes of microbes and have found characteristic definable isoenzymes of LDH in *Staphylococcus* ([16]) and *Pseudomonas*. Lepromatous leprosy is an unusual host-parasitic interaction in which the causative organism, *M. leprae*, accounts for a major part of the weight of the dermis. It appeared worthwhile to investigate isoenzymes of LDH in tissues of patients with different varieties of leprosy with two intentions. First, we wished to define LDH isoenzyme patterns (zymograms) of the skin in different forms of leprosy. A careful scrutiny of the literature has revealed only four reports on LDH in leprosy ([9, 13, 14, 20]). Second, we wished to explore the possibility that *M. leprae* has distinctive isoenzymes which might be markers for viable organisms in the tissues. Currently, the effect of therapy is judged by, among other things, the morphological index ([6]), but even this has not been universally accepted as a reliable marker of the viability of the organisms ([5]).

MATERIALS AND METHODS

Cases. Seventy-eight cases of leprosy attending the Sir J. J. Group of Hospitals, Bombay, were studied. In each case a detailed history to include the duration and type of treatment and a careful clinical examination were carried out. Smears were obtained, and the bacterial (BI) and morphological (MI) indexes determined by Ziehl-Neelsen staining ([6, 11]) and by fluorescence microscopy ([8]). Lepromin testing with armadillo lepromin was done. A biopsy of a lesion in the skin was taken for a histological study and for the determination of the LDH zymogram. Each case was classified according to the scheme of Ridley and Jopling ([12]). Cases of lepromatous leprosy (LL) were further divided on the basis of MI, therapy, and clinical features into the subvarieties "active" and "regressing."

Normal tissues for LDH zymograms. Twenty-five skin samples were obtained. Fifteen were from cadavers and ten from normal patients undergoing plastic surgical procedures.

Tissue extraction for LDH zymograms. A piece of dermis together with the epidermis weighing 100 mg was carefully trimmed from the biopsy and washed thoroughly with sterile saline to remove blood components. The tissue was then homogenized in 2 ml of 0.1 M tris glycine buffer, pH 8.4, in a manual Teflon-tipped tissue homogenizer of 20 ml capacity. Homogenization was carried out by the same operator and timed for 10 min. The fluid and tissue debris were centrifuged at 12,000 × *g* for 20 min at 4°C and the clear supernatant immediately studied for the LDH isoenzymes.

LDH isoenzyme separations. This was by polyacrylamide disc gel electrophoresis in a miniaturized apparatus ([4, 15]). The gel contained 7 percent monomer in the same tris glycine buffer used for extraction. Separations were in gels cast in glass tubes of 2 mm internal diameter. The inoculum of the tissue extract was 10 μl, the current 1 mA/gel, and the movement was monitored by the movement of albumin in a parallel human serum sample stained with bromophenol blue. The gels were immediately re-

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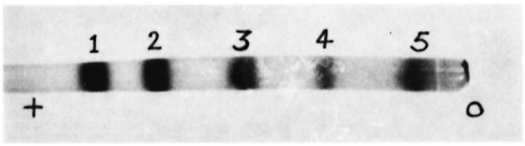

FIG. 1. Lactate dehydrogenase zymogram of normal tissue on polyacrylamide disc gel electrophoresis. The gel was 2 mm in diameter. O = origin. Five distinct bands are seen. These are numbered and correspond to the bands observed in normal human serum.

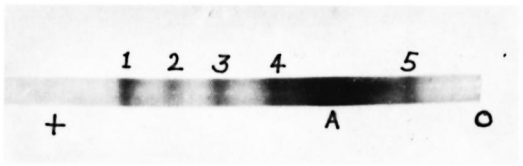

FIG. 2. Lactate dehydrogenase zymogram of tissue from a case of active lepromatous leprosy. O = origin. The usual five bands are seen and numbered as in Fig. 1. An additional, anomalous band (A) of very great intensity is seen between band 4 and 5. This probably represents an LDH of *M. leprae*.

moved and stained for LDH isoenzymes ([7]). Briefly, the method was as follows: Each gel was incubated at 37°C in darkness in a solution of lithium lactate (substrate), nicotinamide adenine dinucleotide (NAD) (coenzyme), phenazine methosulfate (PMS), and nitroblue tetrazolium chloride (NBT). LDH releases H^+ ions from the substrate, which are transferred by PMS and reduced NBT to a purple colored triphenyl formazan. The zymogram was scrutinized visually and eF values for each calculated by the following formula:

$$\text{eF of LDH isoenzyme} = \frac{\text{Distance travelled by LDH band}}{\text{Distance travelled by albumin marker}}$$

RESULTS

Normal tissue LDH zymogram. Extracts of normal skin invariably showed five LDH isoenzymes with eF values of 0.66, 0.5, 0.37, 0.22, and 0.08 (all ±0.04). These have been conveniently labeled in the European system as 1, 2, 3, 4, 5, respectively ([3]) (Fig. 1). These corresponded with the LDH zymogram described for normal human serum, but the densities of the bands were different and variable.

Zymograms of skin from patients with leprosy. All tissues of cases showed the five bands seen in normal tissues. In addition, 17 cases showed extra LDH isoenzymes (anomalous bands) (Fig. 2). In five cases, two anomalous bands were seen. The eF values of these anomalous bands were 0.125 (±0.015) and 0.525 (±0.015). The Table summarizes these results.

Of the total of 78 cases studied, 27 were positive for acid-fast bacilli (AFB). Anomalous LDH isoenzymes were exclusively seen in cases positive for AFB and occurred in 17 of them. All these 17 cases had a BI of 2 or more and a MI of 5 percent or more. Thus the anomalous LDH isoenzymes correlated with large numbers of viable microorganisms.

Details of the ten cases showing AFB but no anomalous bands were also quite interesting. Two cases had BT leprosy and had a low bacterial load (BI 1). Four cases had LL disease with BIs of 5, 5, 3, and 2 but were on prolonged treatment with no viable organisms as judged by the MI. These six cases therefore had few viable organisms. Only four cases in this group had evidence of numerous viable organisms. One case had LL with ENL (BI 6 and MI 10%), and three cases had LL with BI 6, MI 10%; BI 5, MI 8%; and BI 5, MI 15%, respectively.

DISCUSSION

We have been able to obtain only four reports from the literature regarding anomalous (additional) isoenzymes of LDH other than the five classical varieties seen in human serum samples. Lubrano ([10]) described additional bands which he designated as T bands between LDH 4 and 5 in 42 of 76 cases of liver diseases. An additional band between LDH 4 and 5 was described in some sera containing the hepatitis B antigen ([1]), and this was later correlated with the hepatitis B "e" antigen ([17]). Lastly, anomalous bands were described in sera of patients with malignant tumors, as reported by Wilkinson ([19]).

Four reports are available dealing with leprosy and LDH. Zuravieva ([20]) studied tissues of 69 patients by histochemical methods and observed a loss of LDH staining activity. This was ascribed to depres-

THE TABLE. *Type of leprosy and additional (anomalous) LDH isoenzymes in skin extracts.*[a]

Variety of leprosy (Ridley-Jopling) ([12])	No. of cases	Additional LDH isoenzymes		Total cases with anomalous isoenzymes
		eF 0.125	eF 0.525	
LL Total	45	11	5	12
a. active	17	9	4	10
b. active, ENL	3	2	1	2
c. regressing	25	0	0	0
BL	5	5	1	5
BT	8	0	0	0
TT	19	0	0	0
Indeterminate	1	0	0	0
Total	78	16	6	17[b]

[a] Every tissue showed the five LDH isoenzymes seen in extracts of normal skin.

[b] Five cases showed both anomalous bands.

sion of anaerobic glycolysis in the leprosy granuloma. Isoenzymes of the tissues were not studied. Levitch ([9]) studied sera from cases of leprosy and did not observe any significant changes in the serum LDH zymograms. The only report of zymograms in tissue homogenates is by Saito ([13]). In 20 tissues, this observer demonstrated a rise of LDH 3 and 4 in AFB-containing tissues, of LDH 3 in LL with ENL, and of LDH 5 in tuberculoid leprosy. Anomalous bands were not observed. Lastly, the same observer studied serum LDH isoenzymes in leprosy and found similar changes as in the tissue homogenates ([14]). The changes were ascribed to tissue damage and lowered clearance of isoenzymes by a deranged reticuloendothelial apparatus.

The origin of LDH isoenzymes in skin is obscure. Trace contamination from blood cannot be excluded. Two reports in the literature describe LDH isoenzymes of the skin ([2, 18]). One suggests that LDH 4 and 5 originate mainly from cells of the epidermis and the others from the dermis. Undoubtedly, isoenzymes differ from tissue to tissue. The liver and voluntary muscle are the principal sources of LDH 5 and 4 and the heart of LDH 1–2. So far, a possible origin from microbes has not been thought of.

That microbes might contribute to distinctive isoenzymes became vividly apparent to us from our studies on sonicated *Staphylococcus aureus* ([16]). This organism had up to three LDH isoenzymes, and there was even a correlation between antibiotic sensitivity and LDH isoenzymes. In view of the well known heavy bacterial load in lepromatous leprosy, it seemed worthwhile to investigate leprosy tissues. Perhaps the miniaturized technique for polyacrylamide disc gel electrophoresis (PADGE) that we used with its increased sensitivity enabled us to detect two additional LDH isoenzymes in tissue extracts of dermal leprosy lesions. Further, the bands occurred exclusively in lesions loaded with viable organisms. There were, however, four cases which were exceptions. It seems reasonable to infer that the anomalous bands were derived from *Mycobacterium leprae*. Unfortunately, the literature does not contain reports of LDH isoenzyme patterns of mycobacteria that enable any valid comparisons or inferences to be drawn.

The presence of anomalous LDH isoenzymes in tissues correlated well with the viability of the microorganisms as determined by the MI. If these observations are reproducible in other laboratories, then there would seem to be two practical uses. First, in an individual case, LDH isoenzymes in sequential biopsies might be useful to monitor the effect of treatment. Second, it might easily help to define cases with drug-resistant organisms. We are undertaking studies on these lines. Certainly, the technology is less complicated and time consuming than the growth of *M. leprae* in the mouse foot pad.

SUMMARY

LDH isoenzymes were studied in tissue extracts of 78 cases of leprosy. All 25 control tissues showed five LDH isoenzymes corresponding to those of human sera. All tissues from the leprosy cases showed five similar bands. Seventeen cases showed additional LDH isoenzymes (anomalous bands). In 12 cases there was a single extra band with an eF value of either 0.125 ± 0.015 or 0.525 ± 0.015, and five cases showed both these bands. Additional bands were observed only in cases positive for acid-fast microorganisms (17 of 27 cases), and their presence correlated well with bacterial load (as judged by the BI) and viable

organisms (as judged by the MI). Four cases with a high BI and MI did not show anomalous bands, however. A plausible explanation for these bands is that they originate from viable *M. leprae*.

RESUMEN

Se estudiaron las isoenzimas de la deshidrogenasa láctica (LDH) en extractos tisulares de 78 casos de lepra. Los 25 extractos tisulares usados como controles mostraron cinco isoenzimas de la LDH correspondientes a las encontradas en el suero humano. Todos los tejidos de los casos de lepra mostraron five bandas similares. Diecisiete casos mostraron isoenzimas adicionales de la LDH (bandas anormales). En 12 casos hubo una sola banda extra con un valor eF de 0.125 ± 0.015 o de 0.525 ± 0.015 y cinco casos tuvieron ambas bandas. Otras bandas adicionales sólo se observaron en casos positivos para microorganismos ácido resistentes (17 de 27 casos) y su presencia correlacionó bien con la carga bacteriana (estimada por el índice bacteriológico, BI) y con la presencia de organismos viables (según el índice morfológico, MI). Sin embargo, cuatro casos con BI y MI elevados no mostraron bandas anormales. Una posible explicación sobre la presencia de estas bandas es que se originen de *M. leprae* viables.

RÉSUMÉ

On a étudié les isoenzymes LDH dans des extraits tissulaires obtenus chez 78 cas de lèpre. Les tissus prélevés chez 25 témoins ont présenté dans tous les cas des taux d'isoenzymes LDH cinq correspondant à ceux observés dans le serum humain. Tous les tissus recueillis dans les cas de lèpre montraient cinq bandes semblables. Chez 17 cas, on a observé des isoenzymes LDH supplémentaires, qui se traduisaient par des bandes anormales. Dans 12 cas, on a observé une bande supplémentaire unique et des valeurs eF qui se situaient soit à 0.125 ± 0.015 ou à 0.525 ± 0.015. Cinq cas présentaient l'une et l'autre de ces deux bandes. Des bandes supplémentaires ont été observées uniquement dans des cas où l'on pouvait mettre en évidence des micro-organismes acido-résistants, soit 17 des 27 cas présentant des isoenzymes LDH supplémentaires. L'apparition de ces bandes présentait une corrélation élevée avec la charge bactérienne, telle qu'on l'estimer par l'Index Bactériologique (IB), de même qu'avec la présence de micro-organismes viables, estimés d'après l'Index Morphologique (IM). Toutefois quatre cas présentant un Index Bactériologique élevé, de même qu'un Index Morphologique élevé, ne présentaient pas de bandes anormales. L'explication plausible pour ces bandes est qu'elles trouvent leur origine des *M. leprae* viables.

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REFERENCES

1. Baxi, A. J., Bapat, J. P., Damle, S. R., Talvadekar, R. V., Rajpal, R. M. and Dave, J. K. New laboratory method to detect hepatitis B (Australia) antigen based on anomalous lactate dehydrogenase isoenzyme. Vox Sang. **31** (1976) 70–74.
2. Carr, A. and Skilien, A. W. Lactate dehydrogenase isoenzymes in skin. Br. J. Dermatol. **75** (1968) 331–336.
3. Coodley, E. L. Isoenzymes in diagnosis. In: *Diagnostic Enzymology*. Coodley, E. L., ed. Philadelphia: Lea & Febiger, 1970, pp. 223–255.
4. Davis, B. J. Disc electrophoresis. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121** (1964) 404–427.
5. Desikan, K. V. Correlation of morphology with viability of *Mycobacterium leprae*. Lepr. India **48** (1976) 391–397.
6. Dharmendra. Recent advances in microbiology in leprosy. Lepr. India **49** (1977) 10–35.
7. Dietz, A. A., Lubrano, T. and Rubinstein, H. M. Disc electrophoresis of lactate dehydrogenase isoenzymes. Clin. Chim. Acta **27** (1970) 225–228.
8. Jariwala, H. J. and Kelkar, S. S. Fluorescence microscopy for detection of *M. leprae* in tissue sections. Int. J. Lepr. **47** (1979) 33–36.
9. Levitch, M. E. and Navalkar, R. G. Serum lactic acid dehydrogenase in leprosy. Int. J. Lepr. **38** (1970) 368–372.
10. Lubrano, T., Dietz, A. A. and Rubinstein, H. M. Extra lactate dehydrogenase isoenzyme band in serum of patients with severe liver disease. Clin. Chem. **17** (1971) 882–887.
11. Ridley, D. S. Bacterial indices. In: *Leprosy in Theory and Practice*. 2nd ed. Cochrane, R. G. and Davey, T. F., eds. Bristol: John Wright & Sons, Ltd., 1964, p. 620.
12. Ridley, D. S. and Jopling. W. H. Classification of leprosy according to immunity. A five-group system. Int. J. Lepr. **34** (1966) 255–273.
13. Saito, N. Lactic dehydrogenase in leprosy patients. Kinetics of damageable tissue in leprosy patients. Int. J. Lepr. **40** (1968) 251–259.
14. Saito, N. Lactate dehydrogenase isoenzymes in leprosy patients. Lepr. India **44** (1972) 82–89.
15. Saoji, A. M. and Kelkar, S. S. Miniaturization of electrophoretic separation in polyacrylamide gel electrophoresis. Indian J. Pathol. and Microbiol. **22** (1979) 291–294.
16. Saoji, A. M., Mondkar, A. D. and Kelkar, S. S. Lactate dehydrogenase isoenzyme pattern and drug resistance in *Staphylococcus*. Indian J. Med. Res. **69** (1979) 32–36.
17. Vyas, G. N., Peterson, D. L., Townsend, R. M., Damle, S. R. and Magnius, L. O. Hepatitis B "e" antigen: An apparent association with lactate dehydrogenase isoenzyme-5. Science **198** (1977) 1068–1070.
18. Weber, G. and Pfleiderer, G. Isoenzymes in

the human epidermis. Ann. N.Y. Acad. Sci. **94** (1961) 933–936.

19. WILKINSON, J. H. Chemistry of enzymes of diagnostic tests. In: *The Principles and Practice of Diagnostic Enzymology*. Wilkinson, J. H., ed. London: Edward Arnold, 1976, p. 50.
20. ZURAVIEVA, G. F. Histochemical investigation of the activity of oxido reductase in the skin lesions of lepromatous leprosy. Bull. WHO **46** (1972) 813–819.