

# Lactic Dehydrogenase Isozymes in Leprosy Patients

## II. Kinetics of Damageable Tissues in Leprosy Patients<sup>1</sup>

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Lactic dehydrogenase isozymes (l-lactate, NAD oxidoreductase), which are key enzymes in glycolysis, have been the object of extensive investigation. Five LDH isozymes have been found. The five isozymes represent the five possible tetrameric combinations of the two polypeptides. Thus, the five tetramers range from LDH-1(H<sub>4</sub>) to LDH-5(M<sub>4</sub>), the three hybrid enzymes being H<sub>3</sub>M<sub>1</sub>, H<sub>2</sub>M<sub>2</sub>, and H<sub>1</sub>M<sub>3</sub>. Each isozyme is composed of four subunits which may be one or both of the two polypeptides called subunit H and subunit M. The synthesis of the polypeptides is controlled by different genes. Each subunit confers on the tetramer distinct catalytic, physical, and immunological properties according to its relative position in the individual isozymes. These findings have led to a better understanding of the physiological significance of isozymes (1, 4, 6, 9, 17, 18, 23).

For a number of years research on LDH in cancer has concentrated on the changes which can be demonstrated in body fluids in progressive malignancies. The results indicate that the isozyme activity in malignant tissues shifts towards the cathodic isozymes LDH-5 and LDH-4 and is thought partly to explain the high capacity for anaerobic glycolysis in malignancies (12, 24).

Only a few studies have been reported (15) on LDH isozymes in leprosy, a chronic granulomatous disease.

We reported previously that the amount of lactic acid in the blood of leprosy patients, especially lepromatous cases, increased to more than twice that of normal subjects, and that serum LDH isozymes showed a tendency to an increase in M-containing isozymes in contrast to normal serum (22).

In the present study the LDH isozyme patterns of tissues injured in leprosy patients—dermis, peripheral nerves, testis (parenchyma), lymph node and lymphocytes obtained from lymph nodes—are reported.

### MATERIALS AND METHODS

The investigation was carried out on 20 leprosy patients, of whom 12 were men and 8 were women. Eighteen were lepromatous and two tuberculoid. Eight healthy adults were employed as control subjects. The subjects ranged from 32-78 years in age.

**Specimens.** One to two grams of dermis, peripheral nerve, testis (parenchyma), and lymph nodes were obtained at necropsy within 16 hours after death. These tissues (except autopsies on leprosy patients having various cancers or liver injury) were obtained from the National Leprosaria Tama Zensho-en and Amami Wako-en. Control tissues were obtained from autopsy cases at the Tokyo Medical Examiner's Office. The tissues were washed thoroughly in cold veronal buffer to lyse any remaining erythrocytes. Then they were ground in a glass homogenizer with 2-5 ml of veronal buffer (diluted to 200-300 unit/min/ml activity) pH 8.4, ionic strength 0.1, and centrifuged at 10,000 rpm for 30 minutes. The extracts were analyzed for isozyme distribution and the precipitates were used for bacteriologic examination. Additionally, lymphocytes were separated from one half of each lymph node by the glass slide method (11).

**Isozyme separation** (25). One hundredth milliliter of the supernatant was separated electrophoretically on an agar gel supporting medium in veronal buffer at pH 8.4. After electrophoresis at 4°C for one hour at 100-120 volts and 50 mA, the location of LDH activity on the agar gel was visualized by incubating slices of the gel in the following medium:

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0.7%	tris buffer pH 7.4	56 ml
0.4 g/dl	potassium cyanide	5 ml
2M	sodium lactate	4 ml
20 mg/dl	phenazin methosulphate	5 ml
NAD (sigma)		40 mg
nitroblue tetrazolium		30 mg

Incubation was carried out in the dark at 37°C for one hour. The most favorable development of zymograms generally occurred within an hour.

Zymograms are preserved indefinitely in a solution composed of ethanol 70 ml, acetic acid 5 ml, and distilled water 25 ml. The band is quantitated in a densitometer.

**Mean ratio** (<sup>12</sup>). The relative isozyme activity was expressed by the ratio LDH-4/LDH-2 ( $M_3H_1/M_1H_3$ , tetramers of subunits M and subunits H). The choice of this ratio instead of the total M/H ratio was necessary because of hemoglobin contamination of the samples. The blood content of tissue, however, was very low and fairly constant. The isozyme activity of lymphocytes was presented by the ratio LDH-3/LDH-2 as the mean ratio, because LDH-4 was very low.

**LDH activity** (<sup>8, 25</sup>). One-tenth milliliter of the supernatant was transferred to a cuvette having a 1 cm path length to which was added 0.1 ml of 0.25 M-lithium pyruvate, 2.5 ml of phosphate buffer and 0.4 ml distilled water. The decrease in absorption at 340 m $\mu$  was measured in a spectrophotometer.

## RESULTS

Tables 1 and 2 present the distribution of LDH isozymes and their mean ratios in extracts of dermis, peripheral nerve, testis, lymph node and lymphocytes in leprosy patients and normal adults.

Most LDH isoenzyme activities in *M. leprae*-positive dermis of lepromatous type appeared in the LDH-4 and LDH-3 portions; whereas in bacilli-negative dermis of lepromatous type, tuberculoid cases and normal adults, the highest LDH activity was in the LDH-5 portion. Isozyme patterns of lepromatous nodules showed higher LDH-3 activity than that found in lepromatous dermis. Likewise, isozyme patterns in lepromatous peripheral nerves showed

TABLE 1. Distribution of LDH isozymes observed in leprosy patients.

Specimen	Type <sup>a</sup>	Lepra bacilli	No. of cases	LDH-1 (H4) %	LDH-2 (M1H3) %	LDH-3 (M2H2) %	LDH-4 (M3H1) %	LDH-5 (M4) %	LDH-4/LDH-2 mean ratio
Dermis	L	(+)	6	2.4±0.66	14.5±0.66	25.0±1.2	24.0±0.62	34.4±1.29	1.65
Dermis	L	(-)	12	1.0±0.35	7.3±0.71	16.3±0.79	25.7±0.94	49.9±1.21	3.50
Dermis	T	(-)	2	1.0±0.50	6.8±0.38	18.8±0.60	24.9±0.42	48.5±0.89	3.60
Leproma	L	(+)	10	1.1±0.37	17.0±0.81	29.8±1.50	25.0±1.35	27.3±2.18	1.40
Peripheral nerve	L	(+)	8	5.2±0.93	18.7±0.85	33.1±1.11	28.2±1.0	14.8±0.58	1.5
Peripheral nerve	L	(-)	2	4.6±0.42	18.5±0.71	38.4±0.07	25.8±0.64	12.8±0.04	1.4
Peripheral nerve	T	(-)	2	8.8±0.58	21.6±0.45	32.0±0.10	24.0±0.70	13.3±0.06	1.1
Testis	L	(+)	3	2.6±0.28	16.6±0.62	37.5±2.0	27.7±0.5	15.2±1.27	1.7
Lymph node	L	(+)	5	9.3±4.06	20.5±3.5	28.1±2.82	26.0±5.1	16.0±4.39	1.3
Lymph node	L	(-)	1	9.0	23.5	34.5	21.3	11.7	0.91
Lymphocyte	L	(+)	5	3.4±1.02	20.9±0.77	42.6±1.09	19.1±1.49	11.7±1.44	2.04 <sup>b</sup>

<sup>a</sup> L = lepromatous; T = tuberculoid.

<sup>b</sup> LDH-3/LDH-2 : mean ratio.

TABLE 2. Distribution of LDH isozymes observed in normal subjects.

Speci- men	No. of cases	LDH-1 (H4) %	LDH-2 (H3M1) %	LDH-3 (H2M2) %	LDH-4 (H1M3) %	LDH-5 (M4) %	LDH-4/LDH-2 mean ratio
Dermis	8	0	7.3±0.23	15.3±0.59	25.6±2.12	51.7±0.59	3.55
Peripheral nerve	5	11.0±0.45	22.5±0.91	31.1±0.48	21.0±0.55	11.3±0.48	0.93
Testis	4	5.7±1.10	33.9±1.25	39.4±2.39	20.1±0.82	1.6±0.46	0.60
Lymph node	2	8.9±0.46	25.5±1.32	33.4±1.86	22.2±2.08	9.3±1.14	0.80
Lympho- cyte	2	6.2±0.50	19.8±0.92	67.0±0.90	8.3±0.35	0	3.3 <sup>a</sup>

<sup>a</sup> LDH-3/LDH-2 : mean ratio.

higher LDH-3, LDH-4, and LDH-5 activities than the nerves of tuberculoid patients and normal adults. Except for LDH-5 activity, no marked difference was observed between isozyme patterns in lepromatous lymph nodes and those of control subjects. In a mature normal testis, LDH-5 activity was low, and sub-band "X" (<sup>2, 16, 20</sup>) was seen in the area of LDH-3 and LDH-4; however, band "X" was not observed in a lepra bacilli-positive testis. The number of samples was not enough to decide whether this absence of band "X" was caused by testicular damage by lepra bacilli or by other causes.

From these results, it appears that there is a significant difference between the distribution of LDH isozymes in lepra bacilli-positive tissues and those in lepra bacilli-negative tissues including control tissues. Tables 1 and 2 give the mean ratios (LDH-4/LDH-2) of isozymes. It appears that

the mean ratios in all lepra bacilli-positive tissues were 1.3-1.7, while bacilli-negative dermis showed ratios of 3.5-3.6. Bacilli-negative peripheral nerves, testis and lymph node showed ratios of 1.4-0.6. On statistical analysis of the data, the differences of mean ratios between all bacilli-positive tissues and control tissues were found to be significant ( $p < 0.001$ ).

Lymphocytes separated from bacilli-positive lymph nodes showed a tendency to increased M-containing isozymes. The mean ratio for these lymphocytes was 2.04, but that for normal lymphocytes was 3.30.

The basic data for lepromas are presented in Table 3.

The zymograms of the tissues in each case are given in Figures 1, 2 and 3. Normal tissues are shown in Figure 1 as controls. The zymograms in a lepromatous case with recent *erythema nodosum leprosum* obtained ten hours after his death are

TABLE 3. Distribution of LDH isozymes observed in lepromas.

Patient no.	LDH-1 (H4) %	LDH-2 (M1H3) %	LDH-3 (M2H2) %	LDH-4 (M3H1) %	LDH-5 (M4) %	LDH-4/LDH-2 mean ratio
1	0.4	13.7	28.2	28.2	29.4	2.12
2	0	14.3	29.0	27.5	29.2	1.90
3	2.0	16.7	24.3	26.9	31.0	1.60
4	0	17.0	37.4	34.0	12.0	2.0
5	2.5	15.6	29.4	21.8	31.2	1.4
6	2.3	18.4	28.4	22.0	28.8	1.20
7	0	18.0	38.3	21.2	22.7	1.18
8	2.5	15.8	26.6	24.4	30.7	1.54
9	0	12.4	26.4	26.3	34.9	2.11
10	0.8	17.6	30.4	27.8	23.4	1.58

given in Figure 2. Similar isozyme patterns are seen in each tissue from this patient. Electrophoretic patterns of LDH isozymes in lymphocytes from lepra bacilli-positive lymph node and in control lymphocytes are given in Figure 3. In lymphocytes of lepromatous cases, there was a pattern similar to that in lepra bacilli-positive tissues.

## DISCUSSION

It has been recently reported <sup>(20)</sup> that LDH is synthesized both in the cell nucleus and in the cytoplasm. There is some evidence for the isozymes playing a physiological part in the regulatory process of the metabolism of the cell. The LDH isozymes that show different kinetic properties may regulate the ratio of NADH<sub>2</sub> to NAD in the transformation of pyruvate into lactate which is important for controlling many biochemical reaction rates in the cell. Furthermore, the observation <sup>(7)</sup> that LDH-5 showed allosteric regulatory ability offers a method for regulating their relative contribution to the total LDH activity.

According to Kaplan <sup>(10)</sup>, whose hypothesis is based on the fact that LDH-1 is inhibited by pyruvate concentrations to which LDH-5 is resistant, LDH-1 prevails in tissues with aerobic metabolism, while LDH-5 prevails in tissues with anaerobic metabolism. In this connection, an increased amount of LDH in the cytoplasm compared with the nucleus seems to indicate that the soluble fraction of the cytoplasm plays a principal part in the anaerobic method of energy supply in the cell.

Oshima <sup>(21)</sup> recently demonstrated that isozyme patterns are different in the various tissues of an individual, as observed in the process of cytodifferentiation and development of the individual. He reported that LDH isozymes are not affected by variation in physiologic conditions such as the sex cycle, change of temperature, administration of automatic blocking agent or adreno-cortical hormone, unless there is subsequent cytodifferentiation or development.

As shown in Table 1, injured tissues such as dermis containing lepra bacilli, peripheral nerve, testis and lymph node have a similar distribution pattern of LDH iso-

zymes, and the mean ratios of LDH isozymes in these tissues were 1.3-1.7, irrespective of the tissues from which they were derived. In particular, lymphocytes from lepra bacilli-positive lymph nodes showed a tendency to have elevated M-containing isozymes and its mean ratio was lower than the normal ratio.

Bloom *et al* <sup>(3)</sup> found that in cell culture the initial pathway was aerobic, while anaerobic glycolysis occurred after 46-76 hours together with the shift of the LDH isozyme patterns. The predominant cell in short-time leukocyte cultures has been observed to be the small lymphocyte.

On the other hand, Larson <sup>(13)</sup> described LDH isozyme patterns determined in extracts of lymphocytes which had been cultured with and without phytohemagglutinin (PHA). During the PHA-induced transformation of the lymphocytes, the proportion of subunits M in their LDH increased; in cultures without PHA this change was not observed. Continuous aeration of the culture flasks with 9% oxygen resulted in an increase of M-containing isozymes in the PHA-stimulated cell, whereas with 50% oxygen there tended to be an increase in the H-containing isozymes. As shown in Figure 3, the proportion of subunits M in their LDH of the lymphocytes from positive bacilli lymph node increased. It is thought that the increase occurs during the changes of the lymphocyte response to lepra bacilli in the lymph node. In leucocyte culture, we have found that the lepromatous patients have impaired lymphocyte transformation to non-specific nitrogens (PHA). It is thought that there is some relationship to the immunological impairment of the circulating lymphocytes in the lepromatous patients and the increase of M-containing isozymes of the lymphocytes from positive bacilli lymph node.

These facts indicate that the LDH isozyme pattern is closely related to cytodifferentiation in the cell itself, and such an alteration in response to metabolic requirements is attributed to some external cause. This might be the invasion by lepra bacilli or some internal factor relating to the immunologic response of the host.

(Fig. 1)

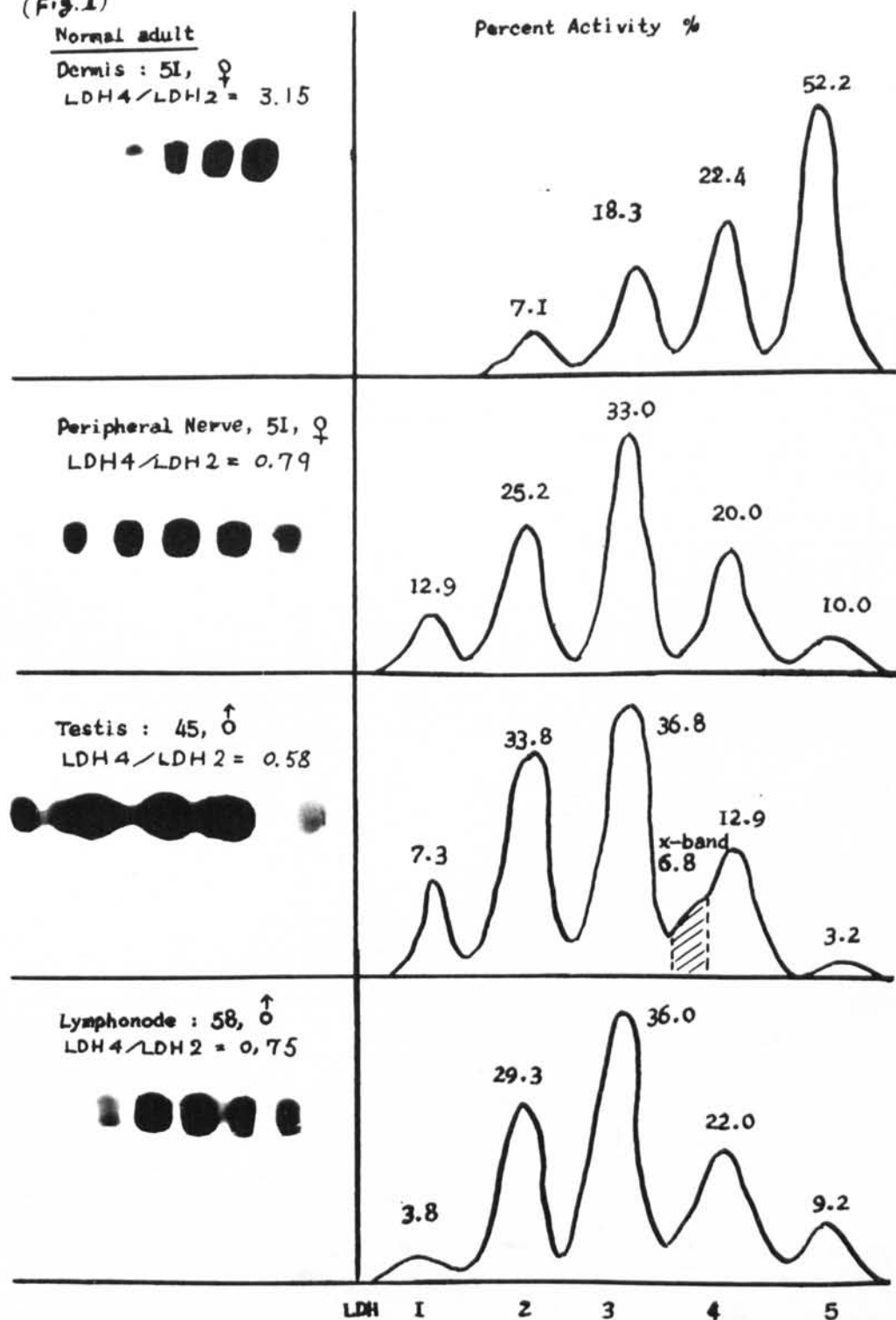


FIG. 1. Electrophoretic patterns on agar gel of LDH isozymes in various tissues of normal adults.

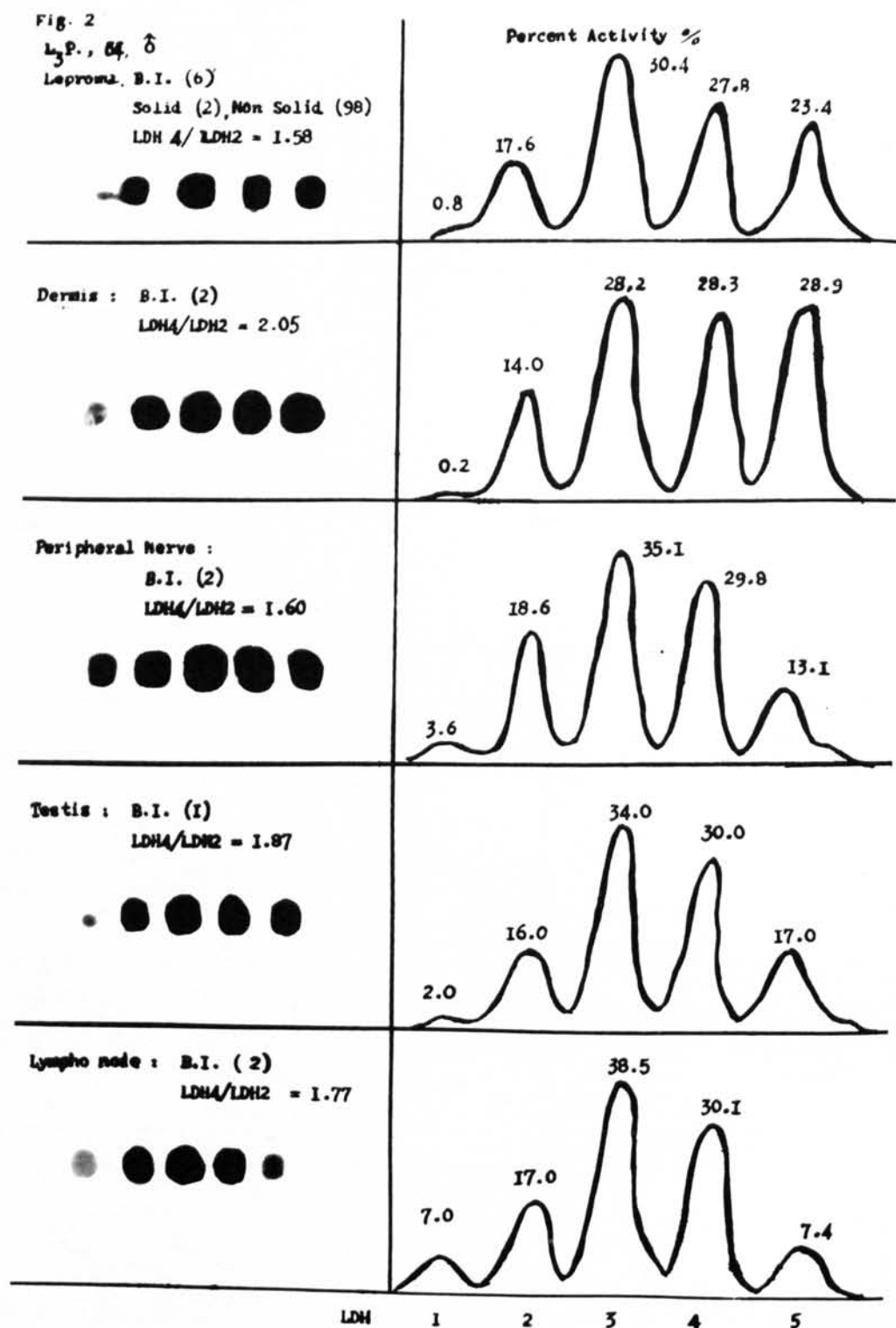


FIG. 2. Electrophoretic patterns on agar gel of LDH isozymes in various tissues of lepromatous casea.



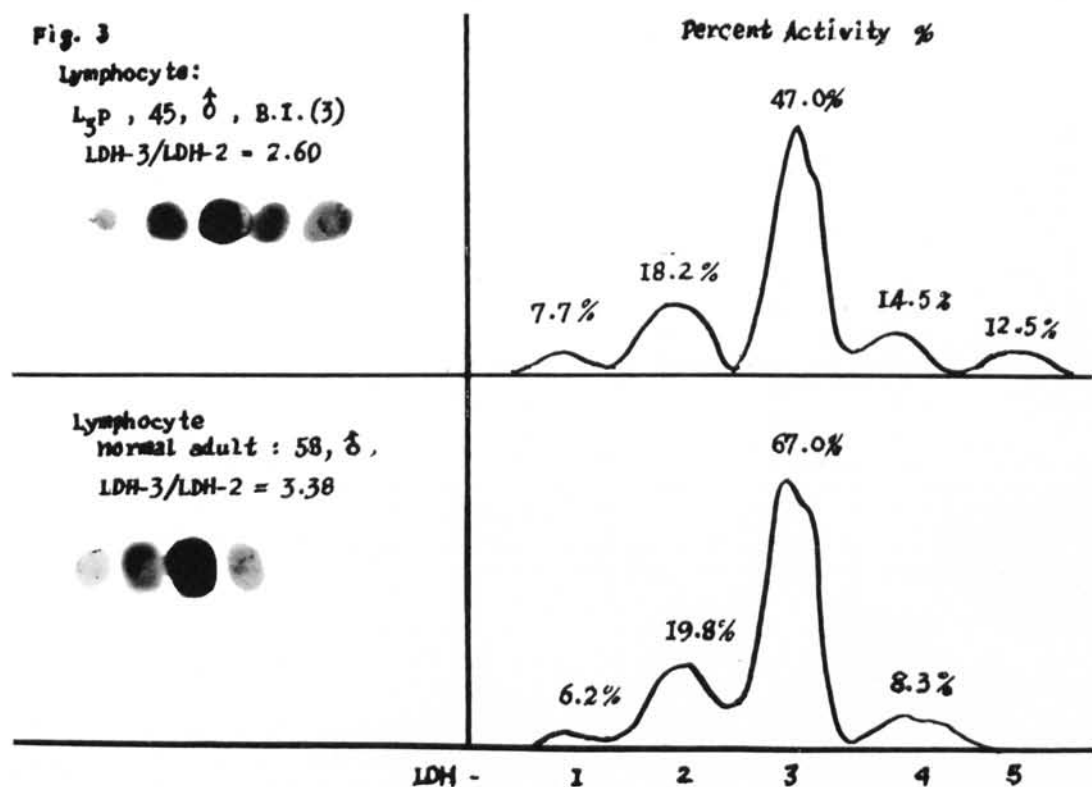


FIG. 3. Electrophoretic patterns on agar gel of LDH isozymes in lymphocytes of a lepromatous case and a normal adult.

It is thought that the LDH isozyme pattern changes occurred first, before changes in the activity of lactate, glucose-6-phosphate or 6-phosphogluconate dehydrogenase (<sup>14</sup>). LDH isozyme patterns of the skin and serum might be useful in determining prognosis in leprosy. In progressive cases the increase of M-containing isozymes in the serum LDH results from the release of injured tissue materials, and there are correlations between the serum isozyme patterns and status of leprosy in patients (<sup>21</sup>).

If changes in enzymatic activity and the distribution of LDH isozymes are cellular metabolic changes, their study may provide clues to cellular development and specialization in leprosy. Thus LDH isozymes could prove to be a sensitive index of physiological activities of cells grown in tissue culture of lepra bacilli, and they may provide a useful method of showing cellular alterations *in vitro* as well as *in vivo*.

## SUMMARY

The LDH isozyme patterns of injured tissues in leprosy patients such as dermis, peripheral nerves, testis and lymph nodes, are reported.

1. Lepromas of dermis in which lepra bacilli were present, peripheral nerves, testis and lymph nodes of lepromatous patients showed a similar distribution pattern of LDH isozymes.

2. The mean ratios for lepromatous tissues containing lepra bacilli were 1.3-1.7, but for the bacillary negative tissues were 0.60-1.40. On the other hand, the dermis of normal subjects and of tuberculoid patients showed ratios of 3.55-3.60.

3. Band "X" was seen in a normal testis, while this band was not detectable in a lepromatous testis. Its appearance is unexplained.

4. Lymphocytes separated from the lepra bacilli-positive lymph node showed a

tendency to an increase in M-containing isozymes.

5. Such changes of LDH patterns observed in lepromatous tissues have been attributed to the damage caused by lepra bacilli.

6. If these alterations in isoenzyme distribution patterns reflect cellular metabolism changes, their study may provide clues to cellular development and specialization and may be useful in tissue culture of lepra bacilli and in prognosis.

## RESUMEN

Se describen los patrones de isozimas LDH de tejidos lesionados en pacientes de lepra, tales como dermis, nervios periféricos, testículos y ganglios linfáticos.

1. Los lepromas del dermis en los cuales se encontraban presentes bacilos de lepra, los nervios periféricos, los testículos y los ganglios linfáticos de los pacientes lepromatosos mostraron un patrón similar de distribución de isozimas LDH.

2. Las relaciones promedio para tejidos lepromatosos que contenían bacilos de lepra fueron de 1,3—1,7; pero para los tejidos bacilarmente negativos fueron de 0,60—1,40. Por otra parte, los dermis de sujetos normales y de pacientes tuberculoides mostraron relaciones de 3,55—3,60.

3. La banda "X" fué observada en un testículo normal, mientras que esta banda no fué detectable en un testículo lepromatoso. Su aparición no se puede explicar.

4. Los linfocitos separados de los ganglios linfáticos que contienen bacilos de lepra mostraron una tendencia hacia un aumento de isozimas que contienen M.

5. Estos cambios en los patrones de LDH observados en los tejidos lepromatosos han sido atribuidos al daño producido por los bacilos de la lepra.

6. Si estas alteraciones en los patrones de distribución de isoenzima reflejan cambios en el metabolismo celular, su estudio puede proporcionar claves para el desarrollo y la especialización celular y puede ser útil en el cultivo del bacilo de la lepra en tejidos y en el pronóstico de la enfermedad.

## RÉSUMÉ

On rapporte dans cet article les profils de l'isozyme LDH dans les tissus affectés par la lèpre, chez des malades atteints de cette maladie, tels que le derme, les nerfs périphériques, les testicules et les ganglions lymphatiques.

1. Les lépromes du derme dans lesquels on peut mettre en évidence des bacilles de la lèpre, les nerfs périphériques, les testicules et les ganglions lymphatiques des malades lépromateux présentent des profils de distribution similaires pour les isozymes LDH.

2. Le rapport moyen observé dans les tissus lépromateux contenant des bacilles de la lèpre a été de 1,3 à 1,7; pour les tissus négatifs pour les bacilles, ce rapport était de 0,60 à 1,40. Par ailleurs, le derme d'individus normaux, de même que le derme de malades tuberculoides, présentaient des rapports de 3,55 à 3,60.

3. La bande "X" a été observée dans un testicule normal, alors que cette bande n'a pas été décelée dans un testicule lépromateux. Son apparition n'est pas expliquée.

4. Des lymphocytes recueillis à partir de ganglions lymphatiques positifs pour le bacille de la lèpre, on montra une tendance à présenter un taux augmenté d'isozymes contenant le facteur M.

5. Les modifications des profils LDH observées dans les tissus lépromateux ont été attribuées aux lésions causées par le bacille de la lèpre.

6. Si ces altérations dans les profils de distribution de l'isoenzyme reflètent des modifications métaboliques cellulaires, leur étude pourrait fournir une explication au développement et à la spécialisation cellulaire et pourrait se révéler utile pour les essais de culture sur tissus du bacille de la lèpre, ainsi que pour le pronostic.

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