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Effects of DDS on Lysosomal Enzymes from Leprosy Tissues[1]

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The term "lysosome" was first proposed by De Duve and associates ([3]) when a special group of cytoplasmic particles was found in rat liver containing within them as many as five distinct acid hydrolases with widely differing specificities.

Weinstock *et al.* ([19]) showed that the muscle wasting that occurs in muscular dystrophy is primarily a hydrolytic and catabolic process in which the activity of cathepsin (one of the lysosomal enzymes) was observed to be increased. Zalkin *et al.* ([21]) and Tappel *et al.* ([17]) have reported greatly elevated activities of lysosomal enzymes in muscular dystrophy of laboratory animals.

Brieger and Allen ([1]) showed increased acid phosphatase activity in leprosy tissues, where there is active bacterial lysis. Prabhakaran and Bapat ([12]) reported that inoculation *in vivo* and *in vitro* of ICRC bacilli, which had been originally isolated from human leprosy, caused an increase in acid phosphatase of mouse macrophages. Wade ([18]) has considered the possibility of the presence of lysosomes in various types of leprosy. Palekar and Magar ([10]) have reported increased activities of four lysosomal enzymes, viz., acid phosphatase, cathepsin, ribonuclease, and aryl sulfatase, in leprosy tissues.

Sulfone therapy of leprosy, first introduced more than 20 years ago by Faget and associates ([5]), is one of the triumphs of modern medicine. Treatment in large doses leads to anemia, but according to Dharmendra *et al.* ([4]) in doses of 100 mgm. a day oral DDS does not produce any marked hematologic changes, even on prolonged administration. Smith ([16]) presented evidence indicating that the effective action of sulfone compounds in treatment is due to their breakdown into DDS in the body.

The present paper presents a report of the specific activities of a number of lysosomal enzymes, viz., acid phosphatase, cathepsin, ribonuclease, and aryl sulfatase, derived from normal tissues as well as tissues of leprosy patients who had not undergone DDS treatment. This paper also reports the results of a study of the effect of DDS administration on lysosomal enzymes, in which specific activities were determined for the same enzymes in the tissues of leprosy patients under DDS treatment and the tissues of leprosy patients who had undergone DDS treatment for 6-10 years and been found bacteriologically negative for *M. leprae*.

MATERIALS AND METHODS

Tissues examined. These were obtained from the following groups:

1. Normal persons.
2. Leprosy patients who had not undergone DDS treatment.
3. Leprosy patients who were under DDS treatment.
4. Leprosy patients who had been under regular DDS treatment for 6-10 years and found to be bacteriologically negative for *M. leprae*.

Tissue specimens weighing 250-300 mgm. were obtained by biopsy from the arms of normal persons and from the lesions on the arms of the patients with lepromatous, tuberculoid and maculoanesthetic types of leprosy.

Six persons from group 1 and 18 each from groups 2, 3 and 4 (6 each of the

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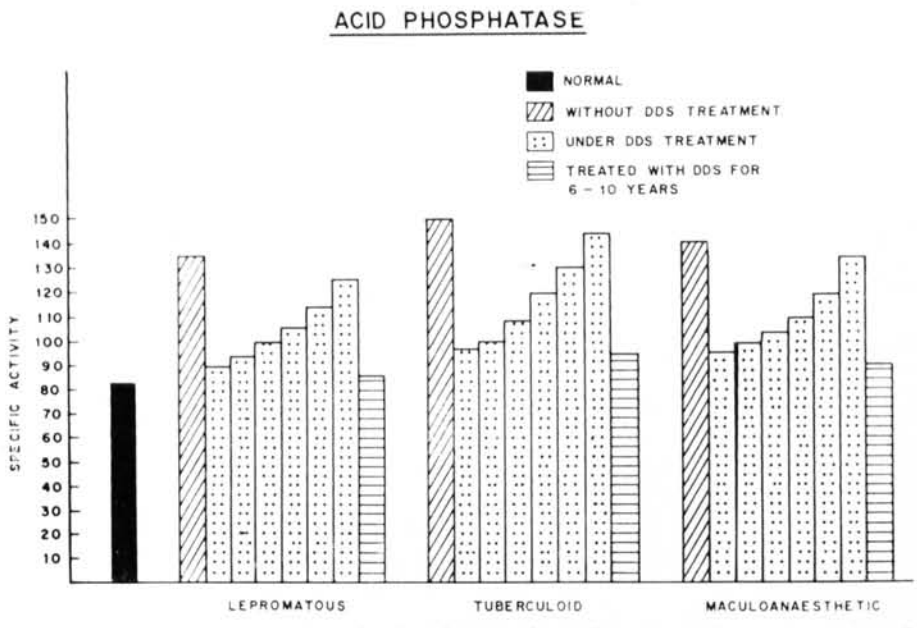

FIG. 1. Specific activity of acid phosphatase from lysosomal fraction (L). Values of normal, without DDS treatment, and treated with DDS for 6-10 years are mean of six observations ± S.D. (Standard deviation).

lepromatous, tuberculoid and maculoanesthetic types of leprosy patients) were studied.

Treatment schedule of patients. The schedule was as follows: Oral therapy with DDS in a dosage of 25 mgm. daily for 6 days per week for 3 months, increased by 25 mgm. (daily for 6 days per week) every 3 months until the maximum of 100 mgm. daily for 6 days per week was reached, which was continued.

Collection and preparation of tissues. Tissues were collected in 0.25 M sucrose containing 0.001 M tetrasodium salt of ethylene-diamine-tetra acetic acid. The suspension was adjusted to pH 7.0, and frozen immediately at -60°C with dry ice, acetone mixture being used to minimize changes in the lysosomes. The tissues were homogenized with a Potter and Elvehjem glass homogenizer ([11]). The homogenate (H) was filtered and fractionated further according to the method of Sawant *et al.* ([15]) in order to obtain light mitochondrial fractions (F I, F II, F III) and the lysosomal fraction (L). All the steps from collection to incubation for enzyme assay were carried out at 0-4°C.

Enzyme assays. Specific activities of all the enzymes were determined as follows from all the fractions after treatment with the detergent Triton x-100:

1. Acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2)[3] activity was assayed by the method of Gianetto and De Duve ([6]). Released inorganic phosphorus was measured by the method of Lowry *et al.* ([7]).

2. Cathepsin (EC 3.4.4.9) activity was assayed by the method of Gianetto and De Duve ([6]). Released tyrosine was measured by Miller's method ([9]).

3. Ribonuclease (polyribonucleotide-2-oligonucleotide transferase, (cyclizing) EC 2.7.7.16) activity was measured by the method of De Duve *et al.* ([3]).

4. Aryl sufatase (aryl sulfate sulfohydrolase, EC 3.1.6.1) was assayed by the method of Roy ([14]).

RESULTS

It was found that specific activities of all the four lysosomal enzymes increased grad-

[3] Enzyme Classification. According to International Union of Biochemistry; Report of the Commission on Enzymes, Oxford, Pergamon Press, 1961.

TABLE 1. *Acid phosphatase: specific activity in terms of mμ moles of Pi released/min./mgm. N.*

Group	Type	H	F I	F II	F III	L
Normal[a]	—	14.95 ± 0.45	21.25 ± 0.96	32.85 ± 1.62	49.64 ± 2.06	82.01 ± 3.93
Without treatment	L[a]	19.10 ± 0.63	26.61 ± 0.34	42.34 ± 0.98	57.45 ± 1.21	134.33 ± 6.89
	TM[a]	23.05 ± 0.76	30.47 ± 1.25	48.84 ± 2.02	66.81 ± 2.96	149.23 ± 10.54
	MA[a]	21.40 ± 0.91	28.91 ± 0.38	46.04 ± 6.15	62.45 ± 3.26	140.17 ± 10.05
Treated bact. neg.	L[a]	15.70 ± 1.09	23.55 ± 1.21	35.56 ± 1.28	53.17 ± 2.05	86.85 ± 0.66
	TM[a]	16.48 ± 2.22	24.10 ± 1.86	36.58 ± 1.37	56.26 ± 1.59	92.42 ± 0.65
	MA[a]	16.30 ± 0.92	24.03 ± 0.92	36.32 ± 0.88	55.04 ± 1.12	89.71 ± 0.44
Under treatment	L1	15.79	21.99	34.62	52.32	89.44
	2	16.54	22.03	36.21	53.13	92.37
	3	16.84	23.92	36.57	53.18	98.94
	4	17.62	23.94	38.43	54.85	104.43
	5	18.14	24.83	40.12	55.38	113.93
	6	18.52	25.60	41.30	56.29	123.71
	TM1	17.18	23.43	36.65	54.35	96.99
	2	17.54	24.65	38.33	56.16	99.94
	3	18.43	25.28	39.39	57.98	106.23
	4	19.73	26.68	42.47	59.99	117.18
	5	20.88	28.31	44.01	62.27	128.97
	6	21.73	29.03	46.26	64.28	141.12
	MA1	15.92	22.25	35.83	53.33	96.45
	2	17.09	22.72	37.80	54.46	98.20
	3	17.32	23.43	39.54	55.70	103.18
	4	17.40	24.77	41.57	57.20	109.03
	5	18.91	26.09	43.07	59.16	118.70
	6	20.20	28.01	44.34	61.12	132.30

[a] Results are mean of six observations ± S.D.

L = Lepromatous
TM = Tuberculoid
MA = Maculoanesthetic

S.D. = Standard deviation
Pi = Inorganic phosphorus

TABLE 2. *Cathepsin: specific activity in terms of mμ moles of tyrosine liberated/min./mgm./N.*

Group	Type	H	F I	F II	F III	L
Normal[a]	—	4.13 ± 0.35	7.28 ± 0.60	8.76 ± 0.47	12.02 ± 0.87	57.84 ± 3.86
Without treatment	L[a]	5.95 ± 0.23	9.81 ± 0.31	15.52 ± 0.59	20.65 ± 0.32	90.90 ± 1.68
	TM[a]	6.63 ± 0.69	11.44 ± 0.52	17.59 ± 0.72	23.55 ± 0.31	111.48 ± 4.59
	MA[a]	6.47 ± 0.19	10.90 ± 0.20	16.84 ± 0.74	23.12 ± 0.51	97.93 ± 3.56
Treated bact. neg.	L[a]	4.78 ± 0.47	7.88 ± 0.40	10.64 ± 0.59	14.71 ± 0.62	66.63 ± 1.03
	TM[a]	6.08 ± 0.58	8.82 ± 0.27	13.37 ± 0.99	16.08 ± 0.91	73.11 ± 0.74
	MA[a]	5.62 ± 0.34	8.54 ± 0.27	11.68 ± 1.16	15.62 ± 0.43	70.98 ± 0.65
Under treatment	L1	4.82	7.72	9.92	15.04	68.51
	2	4.95	8.09	10.58	15.77	69.38
	3	5.20	8.32	11.72	17.24	73.25
	4	5.40	8.64	12.50	17.73	74.76
	5	5.43	8.96	13.36	18.71	78.12
	6	5.60	9.29	14.16	19.59	84.02
	TM1	4.88	8.12	10.67	15.22	76.01
	2	5.15	8.63	11.25	16.24	80.76
	3	5.41	8.84	11.91	17.19	89.38
	4	5.68	9.23	12.72	17.71	90.40
	5	6.01	9.79	14.26	19.03	97.28
	6	6.23	10.74	15.49	20.71	104.33
	MA1	4.87	7.94	10.18	14.85	69.01
	2	5.11	8.42	10.95	16.08	73.50
	3	5.24	8.56	11.82	16.78	77.65
	4	5.72	9.51	13.21	18.26	79.59
	5	5.84	9.85	13.99	18.76	84.20
	6	6.17	10.47	14.86	19.88	90.72

[a] See footnote Table 1.

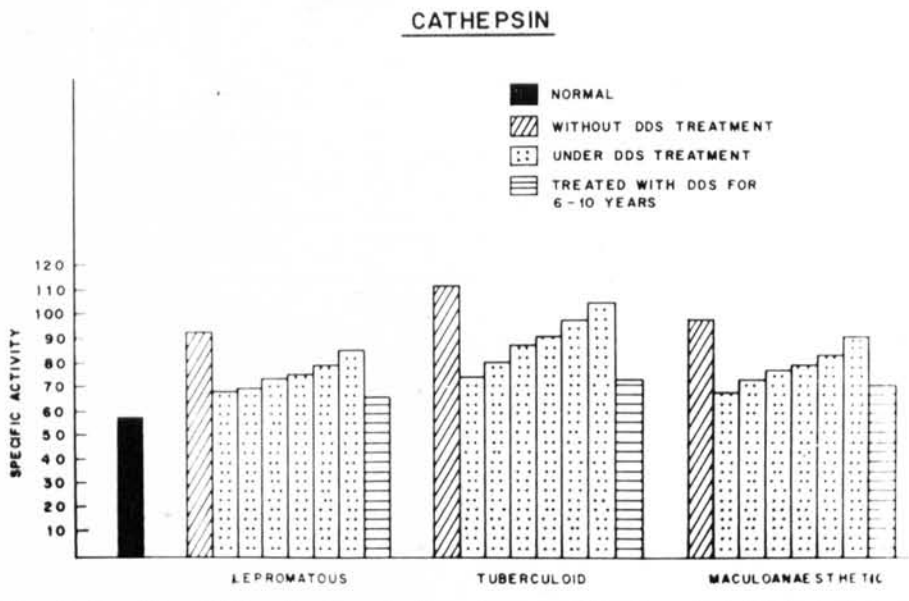

FIG. 2. Specific activity of cathepsin from lysosomal fraction (L). Values of normal, without DDS treatment, and treated with DDS for 6-10 years are mean of six observations ± S.D.

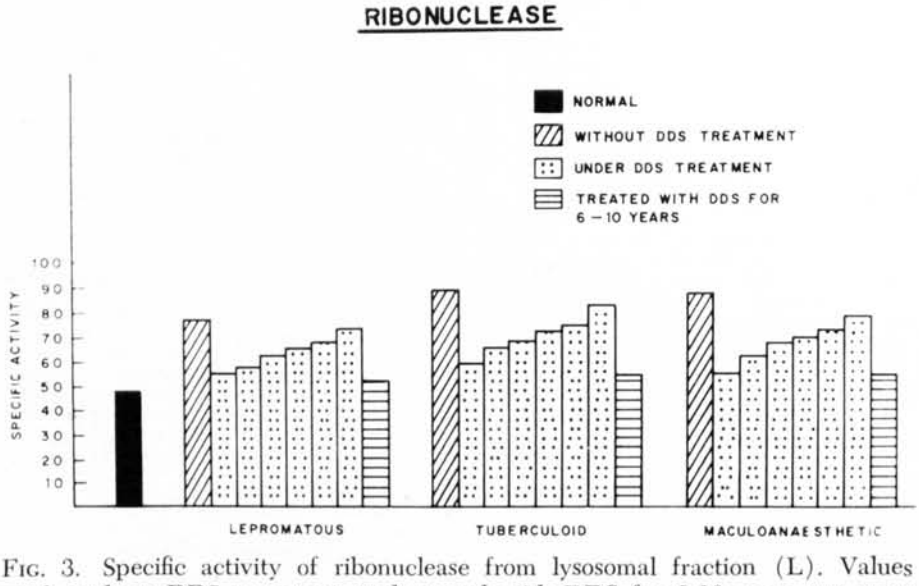

FIG. 3. Specific activity of ribonuclease from lysosomal fraction (L). Values of normal, without DDS treatment, and treated with DDS for 6-10 years are mean of six observations ± S.D.

ually as the fractionation proceeded from the homogenate (H) to the lysosomal fraction (L), which showed the highest activity. In the case of aryl sulfatase the homogenate and light mitochondrial fractions showed very low activity as compared with the lysosomal fraction.

The lysosomal fraction from the tissues of leprosy patients of all types who had not undergone DDS treatment showed significantly high ($P = 0.01$) activity for all the enzymes when compared with the same fraction from normal tissues. (Tables 1 to 4).

The lysosomal fraction from the tissues of leprosy patients of all types who had undergone DDS treatment for 6-10 years, and who had been found bacteriologically negative for *M. leprae*, showed significantly low ($P = 0.01$) activity as compared with the corresponding fraction from patients who had not undergone DDS treatment, indicating thereby a tendency to move toward normal values (Figs. 1 to 4).

Specific activites of the lysosomal enzymes from the lysosomal fraction of tissues of leprosy patients who were under DDS treatment at the time were lower than those of patients who had not received treatment, and higher than those of patients who had undergone prolonged (6-10 years) treatment and been found bacteriologically negative for *M. leprae* (Fig. 4)

These results indicate that the specific activities of lysosomal enzymes from the tissues of leprosy patients of all types decreased significantly after DDS treatment, showing a tendency to attain normal values.

DISCUSSION

In the course of his description of lysosomes as biochemical and morphologic entities, De Duve ([2]) suggested that the enzymes ordinarily sequestered within these organelles might be released in various physiologic and pathologic states. It was considered possible by Weissmann ([20]) that acid hydrolases freed into the cell sap or surrounding tissues might indeed play a role in local tissue degeneration and in the catabolism of host cell constituents as well as of the invasive bacteria.

Malfatti and Jonquieres ([8]), in their study of modifications of *M. leprae* during chemotherapy, reported swelling of cytoplasm, a granular state, and the disappear-

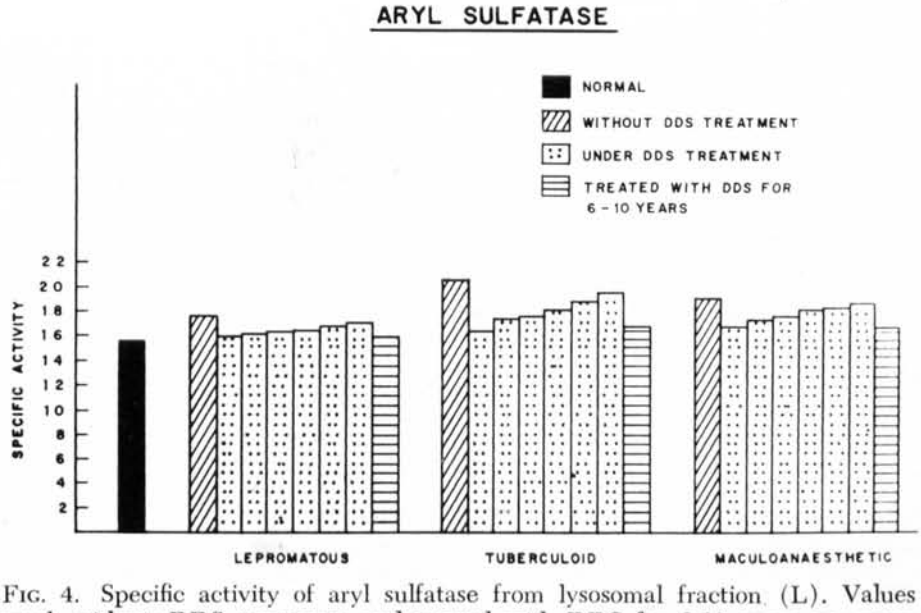

FIG. 4. Specific activity of aryl sulfatase from lysosomal fraction (L). Values of normal, without DDS treatment, and treated with DDS for 6-10 years are mean of six observations ± S.D.

TABLE 3. *Ribonuclease: specific activity in terms of mμ moles of substrate hydrolyzed/min./mgm./N.*

Group	Type	H	F I	F II	F III	L
Normal[a]	—	3.06 ± 0.32	4.31 ± 0.35	5.51 ± 0.47	10.30 ± 0.51	48.03 ± 4.11
Without treatment	L[a]	3.83 ± 0.18	5.57 ± 0.25	8.08 ± 0.35	19.47 ± 0.34	76.81 ± 0.42
	TM[a]	4.57 ± 0.35	6.24 ± 0.22	9.70 ± 0.49	22.80 ± 0.49	87.39 ± 4.49
	MA[a]	4.36 ± 0.28	6.00 ± 0.12	9.01 ± 0.08	21.76 + 0.60	87.52 ± 3.47
Treated bact. neg.	L[a]	3.41 ± 0.37	4.76 ± 0.17	6.09 ± 0.40	11.30 ± 0.73	52.53 ± 0.78
	TM[a]	3.94 ± 0.28	5.29 ± 0.54	7.03 ± 0.24	14.12 ± 0.65	55.99 ± 0.53
	MA[a]	3.78 ± 0.11	4.97 ± 0.24	6.90 ± 0.20	13.62 ± 0.65	55.30 ± 0.36
Under treatment	L1	3.28	3.89	6.18	11.25	55.14
	2	3.36	3.98	6.22	11.94	59.61
	3	3.39	4.02	6.53	13.36	63.85
	4	3.41	4.09	6.78	15.50	66.20
	5	3.47	4.10	6.95	17.26	68.60
	6	3.65	4.21	7.24	18.25	72.14
	TM1	3.65	4.39	7.77	14.34	58.04
	2	3.75	4.56	8.13	15.47	65.58
	3	3.87	4.57	8.47	15.50	68.56
	4	3.94	4.63	8.61	16.13	72.47
	5	4.04	4.72	8.94	17.74	74.91
	6	4.17	4.93	9.02	19.99	82.54
	MA1	3.44	4.18	7.04	12.54	56.92
	2	3.61	4.81	7.10	13.34	62.25
	3	3.68	4.36	7.14	14.10	69.36
	4	3.85	4.40	7.62	15.18	70.37
	5	3.90	4.46	7.80	18.13	73.96
	6	4.01	4.60	7.84	19.21	79.69

[a] See footnote Table 1.

TABLE 4. *Aryl sulfatase: specific activity in terms of mμ moles of substrate hydrolyzed/min./mgm./N.*

Group	Type	H	F I	F II	F III	L
Normal[a]	—	0.659 ± 0.063	0.724 ± 0.086	0.986 ± 0.043	1.481 ± 0.048	15.829 ± 0.690
Without treatment	L[a]	0.663 ± 0.021	0.948 ± 0.034	1.345 ± 0.084	1.937 ± 0.034	17.776 ± 0.290
	TM[a]	0.732 ± 0.050	1.040 ± 0.049	1.681 ± 0.078	2.070 ± 0.053	20.439 ± 1.380
	MA[a]	0.753 ± 0.031	1.056 ± 0.058	1.604 ± 0.069	2.021 ± 0.057	19.378 ± 0.668
Treated bact. neg.	L[a]	0.660 ± 0.011	0.738 ± 0.049	1.130 ± 0.154	1.563 ± 0.666	16.330 ± 0.481
	TM[a]	0.691 ± 0.021	0.849 ± 0.052	1.341 ± 0.106	1.698 ± 0.078	17.069 ± 1.387
	MA[a]	0.678 ± 0.010	0.805 ± 0.039	1.307 ± 0.081	1.649 ± 0.088	16.903 ± 0.338
Under treatment	L1	0.659	0.743	1.115	1.543	16.351
	2	0.664	0.747	1.136	1.587	16.424
	3	0.669	0.752	1.154	1.631	16.509
	4	0.673	0.761	1.178	1.677	16.789
	5	0.678	0.774	1.214	1.724	16.907
	6	0.684	0.785	1.251	1.759	17.292
	TM1	0.675	0.771	1.389	1.592	16.567
	2	0.682	0.779	1.422	1.654	17.637
	3	0.684	0.786	1.447	1.733	17.911
	4	0.691	0.794	1.478	1.796	18.364
	5	0.693	0.801	1.503	1.822	18.981
	6	0.704	0.809	1.532	1.851	19.762
	MA1	0.687	0.778	1.354	1.578	16.944
	2	0.689	0.785	1.369	1.676	17.453
	3	0.692	0.792	1.381	1.684	17.929
	4	0.695	0.798	1.397	1.729	18.360
	5	0.702	0.801	1.502	1.785	18.423
	6	0.716	0.814	1.578	1.834	18.715

[a] See footnote Table 1.

ance of the peripheral halo, which is apparently dependent upon the normal bacillary metabolism, facts indicating disturbances in normal bacillary metabolism during chemotherapy.

Prabhakaran and Bapat ([12]) provided a clue to a probable mode of action of DDS in the treatment of leprosy in suggesting that the drug induces bacterial lysis indirectly by effecting release of lysosomal enzymes in the Virchow cells where *M. leprae* proliferates.

Rath de Souza and de Souza Lima ([13]), in a study of the mechanism of sulfone derivatives in lepromatous leprosy, stated that, although the sulfones exercise a definite beneficial effect, they do not go further than to start, accelerate and intensify some mechanism that seems to be present already in the Virchow cell-leprosy bacillus complex.

Our results have been gratifying, suggesting that the biochemical evidence could be integrated in understanding of the general physiology of the cell as an effective device allowing the cell to utilize organic matter incorporated after the DDS treatment. The DDS may be acting principally on the lysosomal component, increasing its hydrolase activity, altering cell metabolism in some way, and rendering the cell cytoplasm unsuitable for the multiplication and survival of the bacillus.

SUMMARY

The specific activities of four lysosomal enzymes, viz., acid phosphatase, cathepsin, ribonuclease and aryl sulfatase, were found to increase with fractionation from an original tissue homogenate to the lysosomal fraction.

In order to determine the effect of DDS administration on lysosomal enzymes, a study was made of the specific activities of these enzymes in tissues from leprosy patients who were under DDS treatment at the time, and leprosy patients who had undergone DDS treatment for 6-10 years and been found bacteriologically negative for *M. leprae*.

The results indicated that the specific activities of lysosomal enzymes from tissues of leprosy patients of all types decreased significantly after DDS treatment, showing a tendency to attain normal values.

RESUMEN

Las actividades específicas de cuatro enzymas lysosomales, ej., ácido fosfatasa, cathepsin, ribonuclease y aryl sulfatase, se encontraron que aumentaban con el fraccionamiento de un tejido original homogenizado a la fracción lysosomática.

Para determinar el efecto de la administración de DDS en enzymas lysosomales, se hizo un estudio de las actividades específicas de estas enzymas en tejidos de pacientes de lepra que habían tenido tratamiento con DDS en ese momento, y pacientes de lepra que habían recibido tratamiento con DDS por 6-10 años y que había sido encontrados bacteriologicamente negativos para *M. leprae*.

Los resultados indican que las actividades específicas de las enzymas lysosomales de los tejidos de pacientes de lepra de todos los tipos, disminuyó significativamente después del tratamiento con DDS, mostrando una tendencia a acercarse a valores normales.

RÉSUMÉ

Après fractionnement d'un homogénéisat de tissu originel, permettant d' obtenir la fraction lysosomique, on a observé une augmentation dans les activités spécifiques de quatre enzymes des lysosomes: la phosphatase acide, la cathepsine, la ribonuclease et l'aryl sulfatase.

En vue de déterminer l'effet de l'administration de DDS sur les enzymes des lysosomes, on a mené une étude des activités spécifiques de ces enzymes dans des tissus obtenus chez des malades de la lèpre qui, à ce moment, étaient soumis au traitement par la DDS, ainsi que chez des malades qui avaient reçu un traitement par la DDS pendant 6 à 10 ans et étaient devenus bactériologiquement négatifs pour *M. leprae*.

Les résultats indiquent que les activités spécifiques des enzymes des lysosomes obtenus à partir de tissue de malades de la lèpre de n'importe quel type, diminuaient significativement après le traitement par la DDS et montraient une tendance à se stabiliser à des valeurs normales.

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