Acid Phosphatase Activity of Liver Homogenates and its Relation to Drug Activity in Rats Infected with *Mycobacterium lepraemurium*[1, 2]

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Chatterjee ([8]) and Rees and Waters ([21]) suggested the value of examination of acid phosphatase activity in experimental and human leprosy. Hence certain well known antimycobacterial agents like isonicotinic acid hydrazide (isoniazid, INH), 2-ethylisothionicotinamide (Ethionamide, Trescatyl, 1314TH, Thioamid), 4, 4'-diaminodiphenyl sulfone (DDS), 1-(p-N-N' - dimethylaminophenyl) - 3 - (p-n-butoxyphenylthiourea (Su. 1906) and p-acetylaminobenzaldehyde thiosemicarbazone (Conteben, TBI/698) were reassayed in experimental murine leprosy of rats with a view to determining if there is any relation between organ weights, necropsy findings, histopathologic findings and acid phosphatase activity.

MATERIALS AND METHODS

Forty-eight female albino rats (C.D.R.I.-Duckery strain), weighing about 50 gm. each, were divided into six equal groups. The spleen and liver of a rat infected 200 days previously with *Mycobacterium lepraemurium* were weighed and aseptically cut into small pieces, ground with an equal quantity of sterile sand by means of mortar and pestle, and finally suspended in Hanks' balanced salt solution (BSS) on the basis of 5 ml. BSS/gm. of tissue. The suspension was centrifuged at 700 x g for 5 minutes and then the bacilli in the supernatant were counted by the method described by Hanks ([15]). The suspension contained 5 x 10^8 bacilli per ml. Four-tenths ml. of suspension was inoculated intraperitoneally into each rat. The first group was left as untreated controls. The second group was fed orally with INH dissolved in distilled water at a dosage level of 10 mgm./kgm. The other groups were each fed *per os* respectively with 1314TH, DDS, Su. 1906 and TBI/698 at dosage levels of 100 mgm./kgm. The insoluble drugs were made into a thick paste with a few drops of 10 per cent Tween 80 in a mortar and pestle and then diluted with distilled water. Two-tenths ml. of the suspension of each drug was fed orally by means of a blunt, bent needle (18 gauge) and a tuberculin syringe for 6 days a week. The rats were weighed weekly and the experiment terminated on the 159th day of infection after 133 days of therapy by which time 50 per cent of the control rats had died of advanced murine leprosy infection. The animals were sacrificed and at autopsy the weights of the omentum together with pelvic fat, spleen, liver and lung were recorded and a score of 0 to 4 was given to these organs by the method of Gupta and Mathur ([13]). The tissues were fixed in 10 per cent formol saline and 5 μ thick sections were cut and stained with hematoxylin-eosin. Those animals which died before the termination of the experiment were examined but left out of consideration.

Acid phosphatase activity. This activity was determined in the liver of six rats from each of the six groups and was compared with that of the livers of 10 normal female rats of the same weight. A part of the liver was washed in chilled distilled water, dried between filter papers and weighed. Ten per cent (wet weight/volume) homogenates were made in 0.25 M sucrose in a Potter Elvehjem homogenizer by 1 minute

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TABLE 1. *Acid phosphatase activity of liver homogenates of untreated and treated rats.*

Group	No. rats	Specific activity (mean ± S.E.) (Units per mgm. protein)	Per cent of normal
Normal	10	0.78 ± 0.04	100
Control	4	1.11 ± 0.05	142[a]
INH	6	0.69 ± 0.05	89
1314TH	6	0.83 ± 0.04	106
DDS	6	1.04 ± 0.06	133[a]
SU-1906	6	1.15 ± 0.05	147[a]
TB1/698	6	1.08 ± 0.03	139[a]

[a] Significantly different from normal at 5% level.

homogenization. The supernatant obtained after centrifugation of the homogenate at 700 x g for 5 minutes, to remove nuclei and cell debris, was used as the enzyme source. All these operations were performed between 0-4°C. The protein content of the homogenate was estimated by the method of Lowry and his co-workers ([18]).

The method of Appelmans and DeDuve ([1, 2]) using sodium-β-glycerophosphate as the substrate was used for the assay of acid phosphatase. The incubation mixture consisted of the following agents: 1.0 ml. of 0.5 M acetate buffer at pH 5.0; 0.5 ml. of 0.1 M sodium-β-glycerophosphate and 0.1 ml. of the 10 per cent liver homogenates. The volume of the mixture was adjusted to 2.0 ml. with distilled water. After allowing the reaction to proceed for 30 minutes at 38°C in a water bath it was stopped by adding 1.0 ml. of 10 per cent (vol./vol.) of trichloracetic acid. After centrifugation at 1,400 x g for 5 minutes, 1.0 ml. of the clear supernatant was taken for the determination of liberated inorganic phosphate by the method of Sumner ([24]). Appropriate substrate, buffer and tissue blanks were subtracted from the total phosphorus values obtained. The liberation of 1 μg. of phosphorous per minute per milligram protein was taken as one unit of enzyme. The result is expressed as percentages of activity of the treated or untreated group against the activity of the normal group (Table 1).

RESULTS

Mortality. Four control rats died of moderate to massive leprous lesions in the organs of predilection on the 147th, 154th, 155th and 159th days of infection. Similarly two rats from each of the Su. 1906 and TBI/698 groups and one rat from the DDS group died between the 133rd to 155th days of infection with moderately advanced leprous disease. One rat of the

TABLE 2. *The organ weights in treated and untreated rats.*

Group	No. rats	Median organ weights in gm. with standard error			
		Lung	Liver	Spleen	Omentum and pelvic fat
Control	4	2.0 ± 0.32 (1.5–3.0)[a]	13.3 ± 2.8 (6.5–19.4)	2.1 ± 0.63 (0.7–3.4)	12.8 ± 0.43 (10.0–15.9)
INH	8	1.3 ± 0.07[b] (1.0–1.6)	3.7 ± 0.19[b] (3.0–4.4)	0.4 ± 0.03[b] (0.2–0.6)	2.8 ± 0.46[b] (0.2–4.2)
1314TH	7	1.4 ± 0.24 (1.0–1.7)	4.8 ± 0.57[b] (4.2–7.6)	0.5 ± 0.09[b] (0.4–1.0)	4.3 ± 0.84[b] (1.5–6.8)
DDS	7	1.1 ± 0.08[b] (0.9–1.2)	6.2 ± 0.81[b] (4.5–10.8)	1.05 ± 0.09[b] (0.8–1.3)	4.8 ± 1.17[b] (1.8–10.3)
Su-1906	6	2.1 ± 0.39 (1.2–3.1)	7.1 ± 1.2 (6.1–10.1)	1.1 ± 0.19 (1.0–1.3)	4.3 ± 0.9[b] (2.5–7.7)
TBI/698	6	1.4 ± 0.18 (1.2–1.8)	7.9 ± 1.17 (4.7–13.0)	1.1 ± 0.27 (0.6–1.6)	6.1 ± 2.35[b] (0–15.0)

[a] Figures in parenthesis denote range.
[b] Significantly different from control at 5% level.

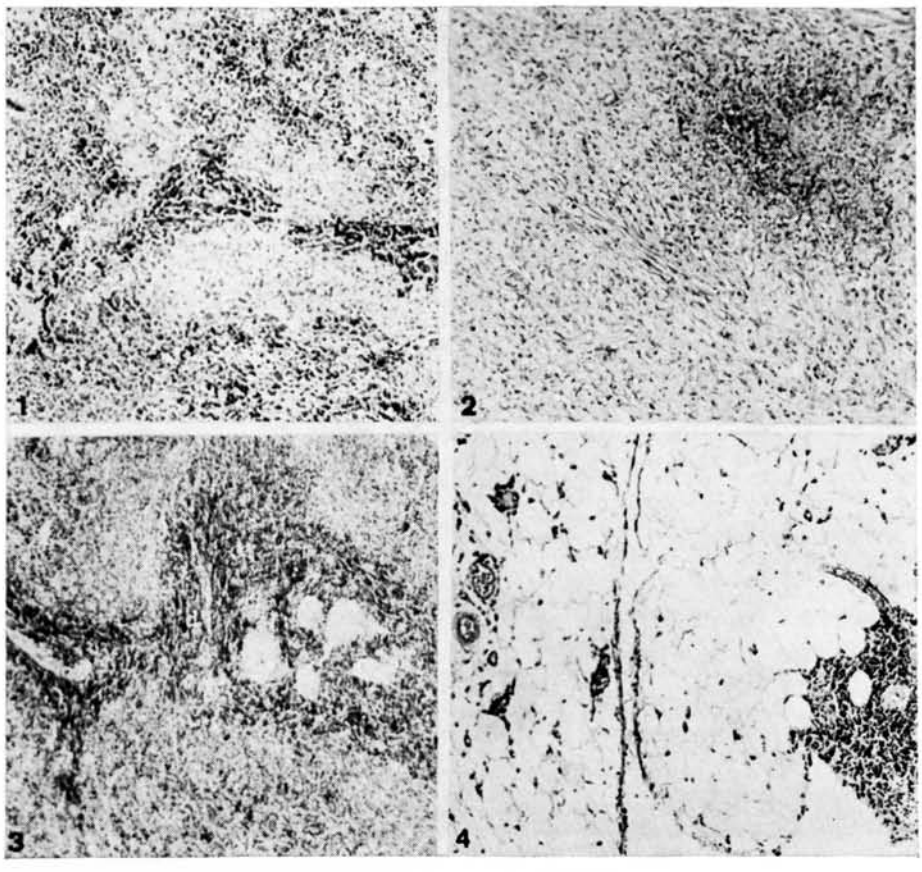

FIG. 1. Photomicrograph of the spleen of control rat showing complete loss of normal follicular pattern and confluent nodules of lepra-epithelioid cells. (Hematoxylin & eosin stain, magnification X80)

FIG. 2. Photomicrograph of the spleen of INH treated rat showing one or two minute collections of lepra epithelioid cells. (Hematoxylin & eosin stain, magnification X80)

FIG. 3. Photomicrograph of the omentum of control rat showing confluent lepra nodules with pyknotic degeneration. (Hematoxylin & eosin stain, magnification X80)

FIG. 4. Photomicrograph of the omentum of 1314TH treated rat showing one or two minute areas of lepra-epithelioid cells. (Hematoxylin & eosin stain, magnification X80)

1314TH group died on the 12th day of infection of causes other than murine leprosy.

Organ weights. Table 2 shows that all treated groups had omental and pelvic fat weights about 50 to 80 per cent less than that of the controls. Similarly there was about a 40 to 80 per cent decrease in the spleen and liver weights of the drug treated groups as compared to those of the untreated controls. The INH, 1314TH and DDS treated groups showed significantly lower liver and spleen weights when compared with the untreated controls ($P = < 0.05$). As judged by organ weights, the response of INH, 1314TH and DDS was superior to Su. 1906 and TBI/698.

Necropsy scores. Table 3 shows that the necropsy scores of the INH, 1314TH, DDS and Su. 1906 treated groups were significantly different from that of the control group ($P = < 0.05$). The Su. 1906 and TBI/698 treated groups showed liver scores insignificantly different from that of the control group.

Histopathologic scores. Table 3 shows that histopathologically only the INH and 1314TH groups had significantly lower

TABLE 3. *The necropsy and histopathologic scores*[a] *in treated and untreated rats.*

Group	Necropsy scores ± S.E.					Histopathologic scores ± S.E.				
	Lung	Liver	Spleen	Omentum & pelvic fat	Total	Lung	Liver	Spleen	Omentum & pelvic fat	Total
Control	0.5 ± 0.28 (0–1)[a]	2.7 ± 0.79 (1–4)	2.7 ± 0.87 (0–4)	3.2 ± 0.57 (2–4)	9.2 ± 1.96 (4–13)	1.7 ± 0.66 (1–2)	1.7 ± 0.66 (1–2)	2.2 ± 0.36 (2–3)	2.2 ± 0.36 (2–3)	8.0 ± 0.70 (6–9)
INH	0.1 ± 0.12 (0–1)	0	0	0	0.1 ± 0.12[b] (0–1)	0	0	1.5 ± 0.28 (1–2)	0.25 ± 0.24 (0–1)	1.75 ± 0.24[b] (1–2)
1314TH	0	0.28 ± 0.18 (0–1)	0.14 ± 0.13 (0–1)	0.28 ± 0.18 (0–1)	0.7 ± 0.28[b] (0–2)	0	0	0.25 ± 0.24 (0–1)	0.25 ± 0.24 (0–1)	0.50 ± 0.49[b] (0–2)
DDS	0	0.14 ± 0.13 (0–1)	0.14 ± 0.13 (0–1)	0.85 ± 0.39 (0–3)	1.1 ± 0.55[b] (0–4)	0.25 ± 0.24 (0–1)	1.7 ± 0.24 (1–2)	2.0 ± 0	1.5 ± 0.89 (0–3)	5.5 ± 0.94 (3–7)
Su-1906	0	2.0 ± 0.35 (1–3)	0.80 ± 0.19 (0–1)	1.10 ± 0.5 (0–3)	4.0 ± 0.78[b] (2–5)	0.25 ± 0.24 (0–1)	2.2 ± 0.36 (2–3)	2.0 ± 0	1.5 ± 0.64 (0–3)	6.0 ± 0.90 (4–8)
TBI/698	0	2.0 ± 0.62 (0–3)	0.50 ± 0.42 (0–1)	2.0 ± 0.67 (0–4)	4.5 ± 1.22 (0–8)	2.0 ± 0.40 (1–3)	3.0 ± 0	2.0 ± 0	2.2 ± 0.79 (0–3)	9.2 ± 1.2 (6–11)

[a] Figures in parenthesis denote range.

[b] Significantly different from control at 5% level.

Necropsy score
0 = no lesion
1 = slight lesion (miliary without caseation)
2 = moderate " (discrete, nodular)
3 & 4 = severe " (confluent, nodular with moderate to severe necrosis)

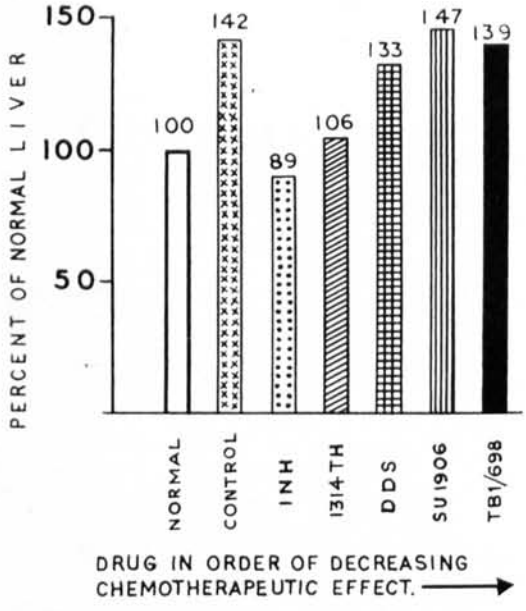

FIG. 5. Acid phosphatase activity of the liver homogenates of treated and untreated rats.

scores when compared with that of controls ($P = < 0.05$). The spleens of control rats showed complete disorganization of normal follicular structure with replacement by confluent nodules of lepra-epithelioid cells (Fig. 1). The spleens of the INH and 1314TH treated groups showed minute foci of lepra-epithelioid cells (Fig. 2). The omentum of control rats showed gross disorganization of structure with confluent lepra-epithelioid nodules having pyknotic degenerated centers surrounded by lepra-epithelioid and spindle cell histiocytes (Fig. 3). The omentums of INH and 1314TH treated groups showed minute epithelioid cell collections (Fig. 4).

Acid phosphatase activity. There was a good correlation between acid phosphatase activity and liver histopathologic scores. The acid phosphatase activities of liver homogenates of the INH and 1314TH treated groups were within normal limits. The rest of the treated groups showed an elevated acid phosphatase activity compared with that of the untreated control (Fig. 5).

DISCUSSION

Acid phosphatase provides a direct measure of lysosomal activity. Its presence has been demonstrated in lepra cells histochemically [4]. The demonstration of acid phosphatase activity in tuberculoid leprosy and not in lepromatous leprosy is possible due to the greater ability of the hosts cells in tuberculoid leprosy to destroy the lepra bacilli [8]. Although murine leprosy resembles lepromatous leprosy more than tuberculoid leprosy there was a significant increase in acid phosphatase activity in the rat liver homogenates in advanced murine leprosy five months after infection. This increase could possibly be due to the release of "bound" acid phosphatase as a result of liver damage by *M. lepraemurium* infection [12]. Histopathologic examination of the liver tissue strengthens this assumption as the increase in acid phosphatase activity was directly proportional to the extent of liver damage.

An increase in lactic acid dehydrogenase activity [26] and a decrease in succinic dehydrogenase [19] and histidase activities [19] in *M. lepraemurium* infections have been previously reported. At present with the fragmentary data in hand, it is difficult to establish the biochemical basis of murine leprosy. Future studies with purified enzyme preparations from normal and infected rats could provide suitable explanations for changes in the activities of enzymes induced during infection with *M. lepraemurium*.

The beneficial activity of 1314TH in human tuberculosis has been adequately established [20, 25]. The effectiveness of 1314th has not been established in mice infected intraperitoneally with *M. lepraemurium* [5, 6, 7] or via the foot pad with *M. leprae* [10, 22, 23] although 3264TH (isothinonicotinamide) and 1314TH are effective in human leprosy [9, 17]. In the present experiments 1314TH showed an activity comparable to INH at a dose 10 times higher than INH. TBI/698 and Su.1906 have been shown to be ineffective in *M. lepraemurium* infections by Chang [7]. In foot pad infections with *M. leprae* contradictory results were obtained [10, 22, 23]. DDS proved subeffective in our experiment. This is understandable because in the experiment of Hadler and Ziti [14] at least 240 days treatment with DDS was necessary to show histologic regression of

the disease. Many workers have reported low activity of DDS in *M. lepraemurium* infections ([3, 5, 11, 12, 16]).

SUMMARY

There was an elevation of hepatic acid phosphatase activity in rats infected with *M. lepraemurium* 159 days after infection. This elevation of acid phosphatase activity was similar in an untreated control group and in ineffectively treated groups. In INH and 1314TH treated animals the acid phosphatase activity was within normal limits. It was possible to correlate increased acid phosphatase activity with increased liver damage as shown histologically.

1314TH and INH proved equally effective in *M. lepraemurium* infection of rats.

RESUMEN

Se encontró un aumento de la actividad de fostatasa ácida en ratones infectadas con *M. lepraemurium* 159 días después de la infección. Este aumento de la actividad de fosfatasa ácida fué similar en un grupo control no tratado y en grupos en los cuales el tratamiento no fué efectivo. En los animales tratados con INH y 1314TH la actividad de fosfatasa ácida estaba dentro de límites normales. Es posible relacionar el aumento de la actividad de fosfatasa ácida con aumento del daño hepático, como se demuestra histológicamente.

El 1314TH y el INH demostraron ser igualmente efectivos en la infección por *M. lepraemurium* en ratones.

RÉSUMÉ

Chez des rats infectés par *M. lepraemurium*, on a constaté une élévation de l'activité de la phosphatase acide hépatique, 159 jours après l'infection. Cette élévation de l'activité en phosphatase acide était semblable à celle notée dans un groupe témoin non traité et dans les groupes traités de manière inefficace. Chez des animaux traités par l'INH, et par le 1314TH, l'activité en phosphatase acide était dans les limites normales. On a pu établir une corrélation entre l'augmentation de l'activité en phosphatase acide avec un dommage hépatique accru tel qu'il apparaît histologiquement.

L'INH et le 1314 TH se sont révélés dotés d'une efficacité équivalente dans l'infection des rats par *M. lepraemurium*.

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