INTERNATIONAL JOURNAL OF LEPROSY

Volume 53, Number 2
Printed in the U.S.A.

Biochemical Alterations in the Serum of Armadillos (*Dasypus novemcinctus*) Infected with *Mycobacterium leprae*. A Preliminary Report[1]

Oscar Rojas-Espinosa, F. Quesada-Pascual, Antonina Oltra, Patricia Arce, S. Estrada-Parra, and Thomas M. Buchanan[2]

Hanseniasis in its lepromatous form is a systemic disease in which, besides the skin and peripheral nerves, lesions affect the testes, eye structures, lymphoid organs, and liver. In the liver, the presence of lepromas within the periportal areas, with bacillary infiltration in Kupffer cells and endothelial cells without involvement of the hepatocyte, has been described ([18]). These histopathological findings have also been observed in armadillos with experimental leprosy ([7]) and in armadillos bearing the natural leprosy-like disease ([17]).

Several groups have studied liver function in Hansen's disease patients. In general terms, severe hepatic dysfunction has not been found in the quiescent forms of the disease, while significant alterations have been reported in reactional leprosy. Here, an increase in the serum concentrations of glutamate-oxalacetate (GOT) and glutamate-pyruvate (GPT) transaminases has been reported ([10]). Measurement of serum lactate dehydrogenase (LDH) in leprosy has given variable results; the most consistent alterations involve isozymes IV and V ([16]) which have been found increased ([14]), decreased ([16]), or normal ([8]). Additional abnormal isozymes have only been detected in cases with a high bacterial index (BI), and this has led to the suggestion that they originate from viable *Mycobacterium leprae* ([15]). LDH activity or LDH isozymes in leprosy have not shown correlation with any clinical form, whether lepromatous, tuberculoid, dimorphous, or undetermined, nor with their clinical status (quiescent, active, or reactional). Similar variable results have been observed with the serum levels of alkaline phosphatase (AlkP) in leprosy. Recent communications on this enzyme indicate a rise in its activity during reactional episodes, regardless of the clinical classification of the disease ([5]). Based on this, it has been suggested that the increase in the serum level of AlkP was produced by the systemic damage generated during the reactional episode more than by the disease itself ([11]).

In all, these discrepancies in humans are probably due to the heterogeneity of the patients in regard to age, exact form of leprosy and degree of involvement, type, time, and dosage of treatment, and to the presence of other clinical or subclinical, nonleprosy-associated, infectious or noninfectious, pathologic conditions. Contrary to this situation, in a recent study on the levels of GPT, GOT, LDH, and AlkP in mice infected with *M. lepraemurium*, we found during the course of the infection ([13]) an early increase (around 1 month post-inoculation) in the levels of serum GPT and GOT, and a more delayed (around 2.5 months post-infection) but more marked increase in the levels of LDH. From this, it was suggested that the mycobacterial infection of mice could be monitored by measuring the levels of these enzymes, watching for increases in their activities as an early indication of infection. This can be a useful tool for workers interested in murine leprosy, but it may also be useful for workers in human leprosy who use experimental models such as the armadillo. In this report, we present our results on the measurement of GOT, GPT, LDH, and AlkP levels in the sera of ar-

[1] Received for publication on 24 May 1984; accepted for publication in revised form on 27 November 1984.

[2] O. Rojas-Espinosa, D.Sc.; F. Quesada, Ph.D.; A. Oltra, Q.B.P.; S. Estrada-Parra, Ph.D., Departamento de Inmunología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, 11340 México, D.F. P. Arce, I.B.Q., Laboratorio de Bioquímica, Instituto Nacional de Perinatología, 11000 México, D. F., México. T. M. Buchanan, M.D., Immunology Research Laboratory, Pacific Medical Center, Seattle, Washington 98144, U.S.A.

madillos inoculated with *M. leprae* at various periods post-inoculation.

MATERIALS AND METHODS

Animals. Wild, nine-banded armadillos (*Dasypus novemcinctus,* Linn.) were captured in the state of Nuevo Leon in the northern part of Mexico and sent to Mexico City. Here the animals were housed in ad hoc facilities for periods that ranged from 3–6 months, until they adapted to the new environmental and diet conditions. Armadillos were then selected and examined for natural mycobacterioses or other unwanted diseases. Physical examinations and searches for bacilli in nasal exudates, cutaneous lymph, and ear imprints were routinely performed. So far, no natural mycobacterioses have been detected.

Inoculation with *M. leprae.* Inoculation was done with *M. leprae* isolated according to Draper's method ([3]) from lepromas taken from untreated lepromatous patients (inoculum H) or with *M. leprae* isolated from the spleen of one armadillo previously infected with human bacilli (inoculum A). This latter material was obtained from the National Hansen's Disease Center, Carville, Louisiana, U.S.A. Each animal was inoculated with 10^8 *M. leprae* by the intravenous route, leaving part of the inoculum deposited intradermally at the site of the injection. This allowed us to predict the systemic infection; in general, when a leproma appeared and grew at the site of inoculation, the systemic infection occurred sooner or later.

For this study, 15 armadillos were inoculated with *M. leprae* H or A. Inoculation, however, was done at different times, depending on the availability of armadillos and the source of bacilli. Evidence of infection was looked for by periodic physical examinations and by searching for acid-fast bacilli (AFB) in smears prepared from nasal exudate, cutaneous lymph, and ear imprints. Blood samples were simultaneously taken for serum enzyme determinations.

Enzyme determinations. Enzyme determinations in serum were based on the methods described by Wroblewski and LaDue ([19]) for LDH; Karmen ([6]) for GOT; Reitman and Frankel ([12]) for GOT and GPT; and Bessey, *et al.* ([2]) for alkaline phosphatase (AlkP). Assays were carried out in the Abbott automated clinical chemistry analyzer, ABA 100, using commercial Beckman kits. For GPT, GOT, and AlkP, direct determinations were done in undiluted 5 μl samples, while for LDH the kinetic method in 5 μl of 1:5 or 1:10 diluted serum was required. Calibration of the analyzer was performed with reagents intended for the analysis of human serum samples. LDH isozymes were quantified according to Mager, *et al.* ([9]).

RESULTS

The results of a first experiment with 15 armadillos having variable periods of inoculation with *M. leprae* H or A are shown in Table 1. At the time this experiment was performed we had no data on the normal levels of GOT, GPT, LDH, or AlkP in armadillos, so that the results on the enzyme levels in the *M. leprae*-inoculated animals were compared with the ones obtained from a single, healthy, noninoculated armadillo (A10). When this was done, we observed that two animals (A03 and A04), both having been injected with human material 10 months before, showed an impressive increase in their levels of LDH (18,500 U/l and 11,966 U/l, respectively, vs 6266 U/l in the noninoculated A10 animal). These same animals plus armadillo A05 showed elevated levels of GOT and GPT. Armadillo A05, however, did not show any increase in serum LDH level. All of the other animals gave results similar to those found in armadillo A10 for all of the enzyme activities tested. A few months later, armadillos A03 and A04 were sacrificed because of obvious signs of leprous infection (Fig. 1).

In a second experiment performed 10 months later, sera from the six surviving armadillos (A01, A05, A07, A09, A12, and A14) and from six other noninoculated armadillos (A16–A22) were tested for enzymatic activity. This time AlkP was not studied since their levels were not modified by the leprosy infection (Table 1). The results are shown in Table 2. It can be observed that within the inoculated group, only armadillo A05 showed clear alterations in levels of GOT and GPT but not in total LDH. However, as shown in Figure 2 and

TABLE 1. *Serum levels of lactate dehydrogenase (LDH), glutamate-pyruvate transaminase (GPT), glutamate-oxalacetate transaminase (GOT), and alkaline phosphatase (AlkP) in armadillos (*D. novemcinctus*) inoculated with* M. leprae.[a]

Armadillo no.	Infection time (mo)	Source of bacilli	LDH U/l	GPT U/l	GOT U/l	AlkP U/l	AFB[b]
A10	0	None	6,266	12	98	1.3	−
A01	34	H[c]	5,060	29	90	1.7	−
A02	11	H	4,460	27	101	1.4	−
A03	10	H	18,500	105	440	1.8	+
A04	10	H	11,966	53	261	3.1	−
A05	10	H	5,100	109	276	3.5	−
A07	8	H	4,400	11	65	1.1	−
A08	8	H	4,300	13	101	0.6	−
A09	8	H	4,066	5	60	1.0	−
A06	2	A[d]	4,666	19	105	1.1	−
A11	2	A	5,130	17	143	0.6	−
A12	2	A	4,530	12	69	0.8	−
A13	2	A	6,830	15	121	3.8	−
A14	2	A	5,120	19	132	1.2	−
A15	2	A	11,866	29	139	2.5	−

[a] Each animal received about 10^8 bacilli intravenously.
[b] Presence of acid-fast bacilli in either nasal exudate, cutaneous lymph, or ear imprints.
[c] H = human, untreated lepromatous patients.
[d] A = armadillo-derived *M. leprae*.

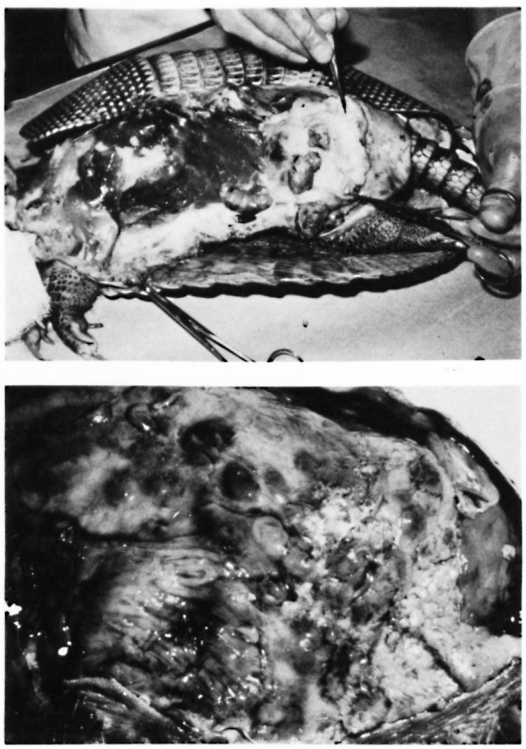

FIG. 1. Massive systemic nodular leprosy infection in nine-banded armadillos A03 and A04 (*Dasypus novemcinctus*) after one year of inoculation with about 10^8 *M. leprae* of human origin.

Table 3, when LDH isozymes were looked for, an increase of isozyme V was observed. This increase was almost three times the normal value (20.6% in A05 vs 7.6 ± 2% in uninfected armadillos). In the absence of an obvious increase in the total LDH activ-

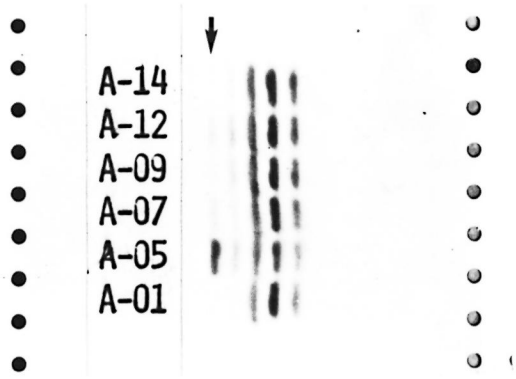

FIG. 2. LDH isozymes in sera from armadillos after varying periods of inoculation with *M. leprae* (see text). Isozyme V (arrow) appears increased in armadillo A05, an animal inoculated with leprosy bacilli 20 months before (LDH isozymes determined by the method of Mager, *et al.* [9]).

TABLE 2. *Serum levels of lactate dehydrogenase (LDH), glutamate-pyruvate transaminase (GPT), and glutamate-oxalacetate transaminase (GOT) in noninoculated or* M. leprae-*inoculated armadillos (*D. novemcinctus*).*[a]

	LDH U/l	GPT U/l	GOT U/l	AFB[b]
		Noninoculated armadillos		
A16	4160	0	104	
A17	4013	10	126	
A19	4273	22	131	
A20	4260	26	203	
A21	4300	5	144	
A22	3593	7	88	
Mean ± S.D.	4099 ± 269	12 ± 10	132 ± 40	
		Inoculated armadillos		
A01 (43, H)[c]	3126	1	27	−
A05 (20, H)	3546	142	425	−
A07 (18, H)	3053	2	57	−
A09 (18, H)	3666	4	40	±
A12 (12, A)	3986	8	87	−
A14 (12, A)	3946	1	38	−

[a] Each inoculated animal received about 10^8 bacilli from human (H) or armadillo (A) sources.
[b] Presence of acid-fast bacilli in either nasal exudate, cutaneous lymph, or ear imprints.
[c] Months of infection and source of bacilli (H or A).

ity in armadillo A05, the rise in the level of isozyme V seemed to be at the expense of isozymes I and III which showed a measurable decrease relative to the normal average values found in noninoculated armadillos A16–A22 (Table 3). Also, the greatest variations within the armadillos were observed in isozymes IV and V, regardless of whether or not the animals were inoculated.

TABLE 3. *Isozymes of lactate dehydrogenase (LDH) in noninoculated or* M. leprae-*inoculated*[a] *armadillos.*

	LDH isozymes				
	I	II	III	IV	V
			Noninoculated armadillos		
A16	26.0[b]	25.6	24.5	15.4	8.4
A17	27.6	24.9	23.9	14.8	8.7
A19	27.6	27.2	26.0	11.8	7.3
A20	27.9	25.9	25.5	12.7	7.9
A21	30.9	28.0	23.6	6.5	10.9
A22	32.3	32.8	24.5	7.8	2.6
Mean ± S.D.	28 ± 2	26 ± 1	27 ± 1	11.5 ± 3.6	7.6 ± 2
			Inoculated armadillos		
A01 (43, H)[c]	26.6	34.2	29.1	7.5	2.5
A05 (20, H)	20.6	24.5	23.7	10.5	20.6
A07 (18, H)	28.6	29.9	26.7	6.9	7.8
A09 (18, H)	28.0	30.0	28.4	9.6	4.1
A12 (12, A)	26.7	29.3	26.3	10.5	7.0
A14 (12, A)	30.2	32.7	27.2	6.9	2.9
Humans[d]	30 ± 2	34 ± 4	27 ± 5	4.5 ± 2	3.6 ± 2

[a] Each inoculated animal received about 10^8 human (H) or armadillo (A) derived *M. leprae.*
[b] Figures are the percent fractions of total LDH activity.
[c] Months of infection and bacilli source (H or A).
[d] Isozyme values found in human beings.

Compared to humans, armadillos do not seem to differ in their relative proportion of isozymes I and III, but they seem to have higher levels of isozyme IV and perhaps of isozyme V and somewhat lower levels of isozyme II. Whether these differences are real or not (because of the small number of armadillos studied) is a matter for further study.

Armadillo A09 did not show alterations in its enzyme levels 18 months after inoculation, and yet at that time it presented a few bacilli in the smears prepared from nasal exudate and from cutaneous lymph. When this animal was sacrificed (26 months post-inoculation, 8 months after the enzyme analysis) because of marked signs of prostration, a rather moderate leprosy infection, almost limited to the presence of a few, small-sized, subcutaneous lepromas, was detected. No obvious morphological or size alterations in the liver or spleen were observed, and only scarce AFB were found in imprints made from these organs.

It can also be observed (Table 1) that armadillo A15 showed an increase in its LDH level comparable to the increase in the LDH levels found in armadillos A03 and A04. Armadillo A15, however, did not show any alteration in its GOT or GPT levels. Six to eight days after the blood sample was taken, this animal showed a severe, extensive pyogenic infection on the left leg which killed the animal three days later. It is quite possible that the observed increase in the LDH value reflected the tissue destruction caused by the ongoing acute pyogenic infection rather than the effect of the very incipient (three-month old) leprous infection.

Except for these two cases (armadillos A09 and A15), there was a good correlation between infection (presence of bacilli in any sample taken) and an increase in the serum GOT, GPT, and/or LDH levels.

DISCUSSION

The present results indicate that armadillos that develop leprosy after inoculation with *M. leprae* showed increases in their levels of serum GOT, GPT, and LDH.

In some cases total LDH activity clearly increased; in others (armadillo A05), no such increase in total LDH activity was observed, although isozyme V increased. It is likely that the increase in total LDH activity observed in these animals was the result of an increase in the level of isozyme V. In humans, this isozyme has been reported to largely predominate in the liver (30–85% of total activity) and skeletal muscle ([4]). The liver in armadillos infected with *M. leprae* is one of the most affected organs.

Liver damage may also explain the more moderate but, at the same time, consistent alterations in the serum levels of GOT and GPT. Human GOT exists in large amounts in myocardium and skeletal muscle and in lesser amounts in the liver so that, apart from being a marker for myocardial infarction, serum GOT is useful in the early detection of liver disease ([4]). On the other hand, since liver tissue in humans contains about three times the amount of GPT as does heart, muscle, or kidney tissue, a rise of GPT is more likely to be due to liver disease. For GPT in humans, normal values in serum are 1–29 U/l at 30°C, rising to 1000–4000 U/l in patients with viral hepatitis. A GOT/GPT ratio of 0.64, on the average, is characteristic of acute hepatitis ([4]), while for all other healthy or disease states the ratio is >1.0. If this holds true in the armadillo, our results indicate that the animals that developed systemic leprosy had a chronic rather than an acute liver disease. This is what might be expected from leprosy. In these animals, both transaminases increased but the ratio GOT/GPT was always higher than 3.0.

In summary, those animals that became heavily infected with leprosy showed marked liver and spleen alterations and obvious increases in their serum LDH levels with moderate but consistent elevations in their GOT and GPT levels. It is likely that a mild infection will provoke moderate visceral enlargement and less pronounced enzyme alterations, while the very incipient, subclinical, or aborted infection will cause no measurable alterations.

It is clear that a more detailed follow-up of the modifications in the levels of GPT, GOT, and LDH at more frequent intervals, and a more strict correlation with clinical, pathological, and bacteriological data during the course of the infection, in a larger group of animals are needed before attempting to propose detection of enzymatic changes as a biochemical marker (not nec-

essarily specific) of the mycobacterial infection.

SUMMARY

Armadillos (*Dasypus novemcinctus*) were inoculated with *Mycobacterium leprae* isolated from lepromas taken from untreated lepromatous patients or from the spleen of an armadillo previously infected with human *M. leprae.* The effect of the infection on the serum levels of lactic dehydrogenase (LDH), alkaline phosphatase (AlkP), glutamate-oxalacetate (GOT) and glutamate-pyruvate (GPT) transaminases was investigated. In general, there was a good correlation between positive evidences of infection and alterations in the levels of LDH, GOT, and GPT. Although elevations in LDH levels were more striking, elevations in GOT and GPT levels were more consistent with the disease. When an absolute increase in the total LDH activity was not observed in a *M. leprae*-infected animal, an increase in the level of LDH isozyme V was still clearly evident. Serum levels of alkaline phosphatase were not affected by the disease. The ratio GOT/GPT (>1.0) in the infected animals reflected and supported the chronic nature of the disease and the liver involvement. The enzymatic alterations are not, however, specific for leprosy.

RESUMEN

Se inocularon armadillos (*Dasypus novemcintus*) con bacilos de la lepra (*Mycobacterium leprae*) aislados de lepromas tomados de pacientes con lepra lepromatosa no tratada o del bazo de un armadillo previamente infectado con *M. leprae.* Se investigó el efecto de la infección leprosa sobre los niveles séricos de las enzimas deshidrogenasa láctica (LDH), fosfatasa alcalina (AlkP), y las transaminasas glutámico-oxalacética (TGO) y glutámico-pirúvica (TGP). Aunque las alteraciones en los niveles de LDH fueron más marcadas, las elevaciones en los niveles de TGO y TGP fueron más consistentes con la existencia de enfermedad. En un animal infectado con *M. leprae,* aún cuando no se observó un incremento en la actividad total de LDH, ocurrió una clara elevación en la concentración de la isoenzima LDH-V. Los niveles de la fosfatasa alcalina no se modificaron por la enfermedad. En los animales infectados, la relación TGO/TGP (>1.0) reflejó la naturaleza crónica de la enfermedad y la afección hepática. Las alteraciones enzimáticas son, sin embargo, no específicas de la lepra.

RÉSUMÉ

Des tatous (*Dasypus novemcinctus*) ont été inoculés par des bacilles *Mycobacterium leprae* récoltés soit à partir de lépromes de malades lépromateux non traités, soit dans la rate d'un tatou déjà infecté par *M. leprae* humain. On a recherché les effets de l'infection sur les taux sériques de la déhydrogénase lactique (LDH), de la phosphatase alcaline (AlkP), de la glutamate-oxalacetate transaminase (GOT), et la glutamate-pyruvate transaminase (GPT). De manière générale, on a observé une bonne corrélation entre les signes d'infection et les modifications dans les taux de LDH, GOT, et GPT. Quoique l'élévation des taux de la LDH était la plus impressionante, les élévations des taux des GOT et GPT étaient mieux corrélées avec la maladie. Lorsque l'animal infecté par *M. leprae* ne présentait pas d'augmentation absolue de l'activité totale en LDH, il était cependant encore possible de mettre clairement en évidence une augmentation des taux de l'isozyme LDH V. Les taux de la phosphatase alcaline dans le sérum n'étaient pas affectés par la maladie. Le rapport GOT/GPT (>1,0) chez les animaux infectés reflètait et confirmait la nature chronique de la maladie et l'atteinte hépatique. Les altérations enzymatiques mises en évidence ne sont cependant pas spécifiques de la lèpre.

Acknowledgments. This investigation received partial financial support from the Dirección de Graduados e Investigación del Instituto Politécnico Nacional, México (Project 81009, Fragocitosis en lepra humana y murina, alteraciones serológicas en modelos experimentales de la lepra); from the Consejo Nacional de Ciencia y Tecnología, México, D.F. (Project: Inmunologia de la lepra y de los ácidos nucleicos, PCSAB-NA-005363); and from the Department of Health and Human Services, United States Public Health Service (Project: Serodiagnosis of subclinical leprosy, ICIDR Program 5R21 A1-16290). The authors are indebted to Irais Méndez, Q.B.P., for her help in part of this work.

O. Rojas-Espinosa, F. Quesada-Pascual, and S. Estrada-Parra are research fellows from the COFAA, I.P.N.

REFERENCES

1. Balakrishnan, S. Biochemical aspects of reactional states in leprosy. Lepr. India **48** (1976) 402–412.
2. Bessey, O. A., Lowry, O. H. and Brock, M. J. Method for rapid determination of alkaline phosphatase with 5 cubic millimeters of serum. J. Biol. Chem. **164** (1946) 321–329.
3. Draper, P. Purification of *M. leprae,* protocol 1/79., Scientific working group meeting. Geneva, February 7–8 (1979).
4. Guilbault, G. G. *Handbook of Enzymatic Methods of Analysis. (Clinical and Biochemical*

Analysis, Vol. 4). New York: Marcel Dekker, Inc., 1976, pp. 118–121, 121–123, and 131–136.

5. KAPOOR, K. K. and GUPTA, S. C. Serum cholesterol and alkaline phosphatase in different types of leprosy. Lepr. India **46** (1974) 152–156.
6. KARMEN, A. A note on the spectrophotometric assay of glutamic-oxalacetic transaminase in human blood serum. J. Clin. Invest. **34** (1955) 131–133.
7. KIRCHHEIMER, W. F., STORRS, E. E. and BINFORD, C. H. Attempts to establish the armadillo (*Dasypus novemcinctus,* Linn.) as a model for the study of leprosy. II. Histopathologic and bacteriologic post-mortem findings in lepromatoid leprosy in the armadillo. Int. J. Lepr. **40** (1972) 229–242.
8. LEVITCH, M. E. and NAVALKAR, R. G. Serum lactic dehydrogenase isoenzymes in leprosy. Int. J. Lepr. **38** (1970) 368–372.
9. MAGER, M., BLATT, W. S. and ABELMAN, W. H. The use of cellulose acetate for the electrophoretic separation and quantitation of serum lactic dehydrogenase isozymes in normal and pathologic states. Clin. Chim. Acta **14** (1966) 689–697.
10. PACINI, A. and FLIESS, E. L. Estudios enzimáticos en pacientes hansenianos. 1. Actividad de las transaminasas glutámico pirúvica y glutámico oxalacética. Hansenol. Int. **3** (1978) 151–159.
11. PACINI, A. and FLIESS, E. L. Estudios enzimáticos en pacientes hansenianos: Actividad sérica de la fosfatasa alcalina. Leprología **21** (1979) 46–50.
12. REITMAN, S. and FRANKEL, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. **28** (1957) 56–63.
13. ROJAS-ESPINOSA, O., OLTRA, A. and MENDEZ, I. Serum enzymatic changes following infection of mice with *Mycobacterium lepraemurium.* Int. J. Lepr. **53** (1985) 258–261.
14. SAITO, N. Lactic dehydrogenase isozymes in leprosy patients. I. Serum lactic dehydrogenase. Lepr. India **44** (1972) 82–89.
15. SAOJI, A. M., JARIWALA, H. J. and KELKAR, S. S. Lactate dehydrogenase zymograms of skin biopsies in patients with leprosy. A preliminary report. Int. J. Lepr. **48** (1980) 426–430.
16. SAOJI, A. M. and KELKAR, S. S. Qualitative studies of serum lactate dehydrogenase isozyme patterns in leprosy. Lepr. Rev. **52** (1981) 309–314.
17. SMITH, J. H., FILE, S. K., NAGY, B. A., FOLSE, D. S., BUCKNER, J. A., WEBB, L. J. and BEVERDING, A. Leprosy-like disease of wild armadillos in French Acadiana, Louisiana. J. Reticuloendothel. Soc. **24** (1978) 705–719.
18. VERGHESE, A., JOB, C. K. and PATH, M. C. Correlation of the liver function with pathology of the liver in leprosy. Int. J. Lepr. **33** (1965) 342–348.
19. WROBLEWSKI, F. and LADUE, J. S. Lactic dehydrogenase activity in blood. Proc. Soc. Exp. Biol. Med. **90** (1955) 210–213.