INTERNATIONAL JOURNAL OF LEPROSY

Volume 43, Number 1
Printed in the U.S.A.

INTERNATIONAL JOURNAL OF LEPROSY

And Other Mycobacterial Diseases

VOLUME 43, NUMBER 1 JANUARY-MARCH 1975

Acid Mucopolysaccharide Metabolism in Leprosy 3. Hyaluronic Acid Mycobacterial Growth Enhancement, and Growth Suppression by Saccharic Acid and Vitamin C as Inhibitors of ß-Glucuronidase[1, 2]

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Histochemical studies of leprosy skin biopsies ([8, 16]) demonstrated hyaluronic acid (HA) accumulation in lepromas, presumably due to lepra cell deficiency in β-glucuronidase. *Mycobacterium leprae,* it was found, seemed to be able to utilize HA as a nutrient. In tuberculoid lesions, however, deposition of HA does not last long because epithelioid and Langhans type giant cells have the necessary degrading enzymes. The present study was, therefore, designed to provide experimental information on the enhancing effect of HA on *in vivo* growth of *M. leprae* as well as the possible effect of β-glucuronidase inhibitors on the development of human and murine leprosy. These first sought histochemical evidence of the presence of HA also in murine lepromas and the presence of β-glucuronidase in either *M. lepraemurium* or in epithelioid cells in the tuberculoid granuloma produced in guinea pigs in response to *M. lepraemurium* infection. Secondly, evidence for the growth enhancing effect of HA and similar acid mucopolysaccharides (AMPS) on the *in vivo* growth of *M. lepraemurium* was elicited. Thirdly, growth enhancement of HA for *M. leprae* inoculated in mice was determined in pilot studies and, finally, the effects of saccharic acid, saccharolactone and ascorbic acid as β-glucuronidase inhibitors was examined.

MATERIALS AND METHODS

AMPS and β-glucuronidase in murine leprosy. Spleens, livers and subcutaneous lepromas of C3H and Swiss mice infected for six months with *M. lepraemurium* by the intraperitoneal route were examined by light- and electron microscopic histochemistry for AMPS and β-glucuronidase. The methods utilized were those described previously ([8, 16]). Experimental granulomas were produced in four female guinea pigs by the inoculation of 2×10^7 of *M. lepraemurium* into each of four subcutaneous locales about umbilici. These animals were sacrificed one month later. The granulomas were fixed for 24 hours in formol chloral hydrate ([18]). After washing the tissue with cacodylate buffer containing 7% sucrose,

[1] Received for publication 20 September 1974.

[2] Supported by U.S.P.H.S. Research Grant AI-10034 and by American Leprosy Missions, Inc.

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cryostat sections were stained for β-glucuronidase for light microscopy examination ([18]). The residual portions of the tissue were processed for H & E, acid-fast and Mowry's stains.

In vivo bacillary growth enhancement by HA. Male Swiss mice from six to seven weeks of age were divided into four groups consisting of five animals each. These groups were utilized separately to test the growth enhancing effects of hyaluronic acid from umbilical cord (No. H-1876 sodium salt, grade III-5), heparin (No. H-3125 from hog intestinal mucosa, grade I), and chondroitin sulfate (No. C-3254 whale and shark cartilage sodium salt, mixed isomers grade III),[4] respectively for subcutaneously inoculated *M. lepraemurium.*

The suspensions of *M. lepraemurium* and AMPS were thoroughly mixed and inoculated subcutaneously into each group of mice in the lower abdomen according to the method of Nishimura ([10]). Each mouse received 10^7 *M. lepraemurium* and 5 mg of AMPS. The fourth group of mice received 10^7 *M. lepraemurium* only and served as a control. The animals were fed Purina chow and water *ad libitum* and sacrificed three months later. On sacrifice, the lepromas were weighed separately and cut in half. Half of each leproma was utilized for determination of the bacillary count and its Morphologic Index as utilized in our laboratory ([19]). The other halves of the lepromas were processed histopathologically for staining with hematoxylin-eosin and acid-fuschin. The other organs were also systematically examined histopathologically for possible significant growth of *M. lepraemurium.*

In vivo growth enhancement of M. leprae in mice. Five female C3H mice were subcutaneously inoculated, in a pilot study, with *M. leprae* suspensions containing HA into lower abdomen and left foot pads. The inocula contained 10^7 *M. leprae* plus 2 mg of HA and 2×10^6 *M. leprae* plus 0.1 mg of HA respectively. The animals were kept under the conditions noted above except for the additional intraperitoneal injection of HA solution containing 3 mg of HA in 0.3 ml of distilled water into each mouse on days 6, 19, 35, 50, 85, and 112 of the experiment and additionally subcutaneously at the abdominal site of inoculation on day 64. One mouse received the same amount of *M. leprae* in the same places of the body as the above but without the addition of HA at any time and served as a control. A single control is, of course, minimal, save for the fact that *M. leprae* inoculations have long been tried in mouse foot pads and elsewhere. The original *M. leprae* suspension was made from skin biopsy specimens of three early lepromatous cases from Hale Mohalu Hospital in Honolulu. The skin was trimmed leaving lepromas which were homogenized together in a 1:5 dilution of Hank's biological saline solution and partially purified according to the method utilized above ([10]). The Morphologic Index of the bacillary suspension was 12%. A mouse was sacrificed on the 34th day and the rest were sacrificed on the 120th day. The autopsied mice were histopathologically examined.

Established murine leprosy treated by enzyme inhibitor. Seventeen albino Swiss mice (11 female, 6 male), having established *M. lepraemurium* infections of ten months' duration following intraperitoneal inoculation of 3×10^6 bacilli, were available from *M. lepraemurium* maintenance stock animals. Of these, five (3 female, 2 male) were retained as controls and twelve (8 female, 4 male) were given 20 mg saccharic acid intraperitoneally, six times a week for two months. At the end of this period the ten surviving animals (7 experimental, 3 control) were autopsied, as had been the others at the times of their deaths.

Treatment of M. lepraemurium inoculum with enzyme inhibitors. Seventy-two, five to six week old female Swiss albino mice were inoculated with *M. lepraemurium* and treated daily thereafter for either two or three months with saccharic acid, saccharolactone or ascorbic acid according to the scheme presented in Table 1.

Preparation of inhibitors. The β-glucuronidase inhibitors utilized were the competitive inhibitors D-saccharic acid (glucaric acid) as the calcium salt and saccharo-1-4-lactone monohydrate, and the noncompetitive inhibitor L-ascorbic acid (vitamin C).

Saccharic acid was first dissolved in 1 N HCl. The solution was then raised to pH 5.2 with the addition of 1 N NaOH followed by dilution to the desired concentration by phosphate buffered saline to pH 7.4. The other compounds were dissolved directly in the phosphate buffered saline. The solutions

[4] Chemicals from Sigma Chemical Company.

TABLE 1. *Effect of β-glucuronidase inhibitors on murine leprosy.*

Item	Foot pad			Inguinal subcutaneous			Abdominal wall	
Inhibitor [a] & dose	SA 1 mg	AA 1 mg	Control 0	SA 20 mg	AA 20 mg	Control 0	SL 2 mg	Control 0
Inoculum	10^7	10^7	10^7	10^9	10^9	10^9	10^7	10^7
No. animals	10	10	10	10	10	10	8	4
Surviving mice	10	10	10	9	10	10	8	4

[a] SA = saccharic acid; AA = ascorbic acid; SL = saccharolactone. Dose = daily.

TABLE 2. *Vitamin C treated lepromatous leprosy.*

Case number	1	2	3	4	5
Leprosy duration (years)	2	2	12	5	2
DDS/week (dosages: mg × frequency per week)	none	12.5–×25 × 2	25 × 2	25 × 3	25 × 2
Lepromin reactions (mm)					
Fernandez	4	8	3	7	2
Mitsuda	0	0	0	3	0
Histopathologic regression	+	+	+	+	+
Site of biopsy	right shoulder	left arm	left deltoid	right forearm	right thigh
Interval 1st to 3rd biopsy (months)	4.5	8	24	24	7
BI decrease	4 → 3.3	4 → 0.75	3.8 → 3.5	4.5 → 2.6	5.1 → 3.5
MI decrease	58 → 9	13.5 → 0	54 → 2.2	1.8 → 0	45 → 2
Time: BI & MI decrease (months)	2.5	5.5	13	2	3.5

were adjusted so that the desired inhibitor dose (Table 1) was contained in one milliliter. All inhibitors were delivered intraperitoneally six times a week for the duration of the experiment.

Enzyme inhibition in human leprosy. In the course of providing routine processing of sequential biopsies from leprosy patients in Zaire, we encountered, without prior alerting, one lepromatous patient who had been given 1.5 gm vitamin C per day for 4.5 months without receiving any specific antileprosy therapy. His histopathologic lesion regression was so striking and unexpected that review of other biopsy material was prompted and an additional four instances were found who had received the same vitamin supplement concomitant with DDS therapy for periods varying from 7 to 24 months. The accompanying biopsy notes were expanded by inquiry[5] and the data is summarized in Table 2.

RESULTS

AMPS and β-glucuronidase in murine leprosy. Murine lepra cells contained much

[5] Dr. Wayne M. Meyers generously obtained for us the additional data and obtained for our review earlier biopsies on some of these cases which had been sent to Koninklijk Instituut voor de Tropen, Amsterdam and the Armed Forces Institute of Pathology, Washington, D.C. for evaluation.

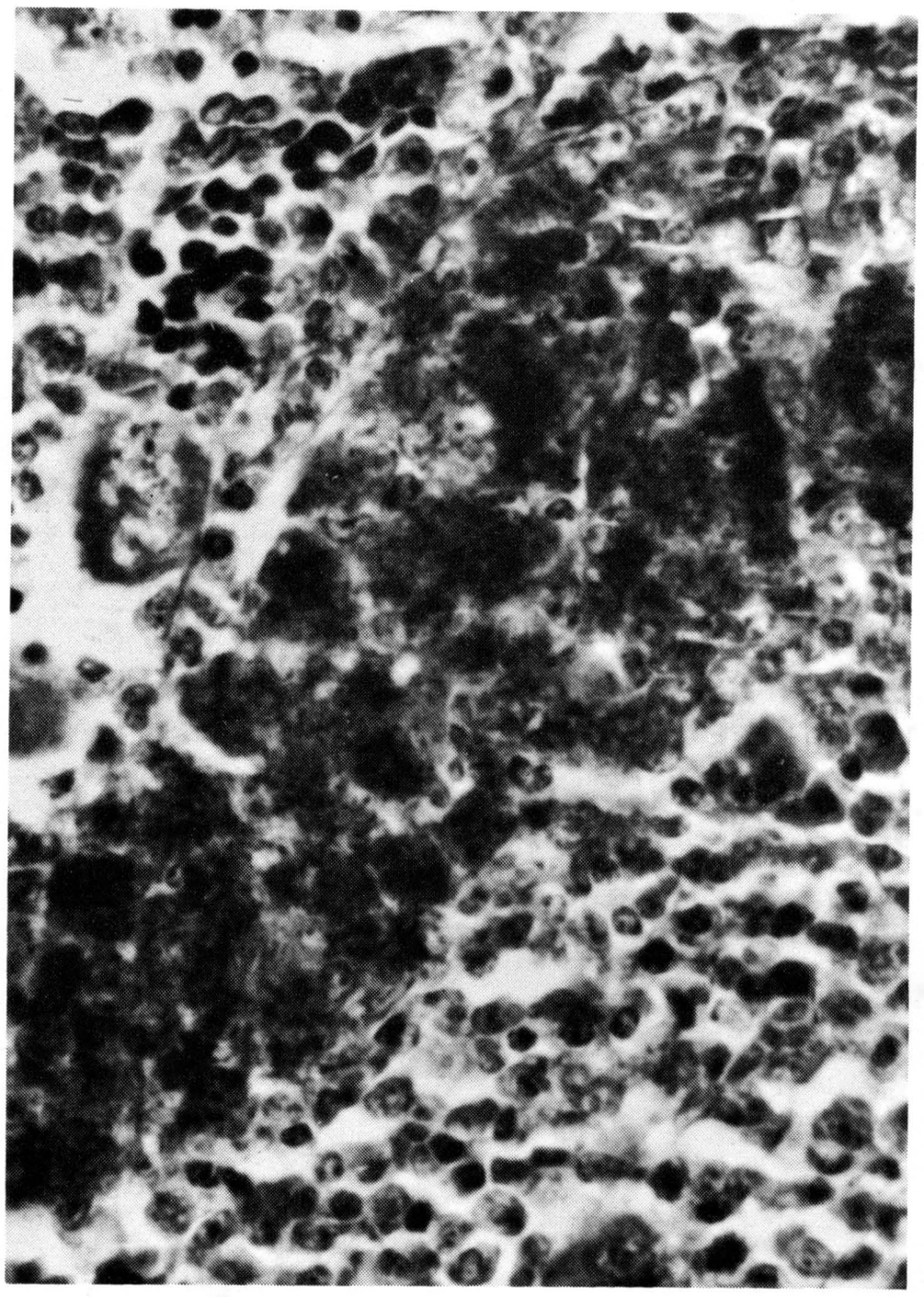

FIG. 1. Mouse spleen leproma containing abundant AMPS. Mowry's stain counterstained with PAS. ×400.

AMPS (Fig. 1). This was regarded as HA since it was characterized by positive Mowry's stain, heavier staining with alcian blue at pH 2.5 than at lower pH, and because of its hyaluronidase digestibility ([8]). Electron microscopy also revealed iron deposition by Rinehart Abul-Haj staining, as shown in Figure 2. The deposition was diffuse in the cytoplasm of the murine lepra cells and thus differed from its distribution in human lepra cells as noted previously ([3, 8, 15]). The bacilli grew diffusely in the lepra cells without forming large vacuoles stuffed with HA ([8]). *M. lepraemurium* was associated with β-glucuronidase (Fig. 3) since spotty, electron-dense deposits were found closely surrounding the bacilli following Smith and Fishman's ([18]) staining for this enzyme. Stamp impressions of the spleen stained by the same method for light microscopy revealed positive bacillary staining for β-glucuronidase (Fig. 4). The lepra cell cytoplasm did not seem to have this enzyme activity. Epithelioid cells produced in guinea pigs, however, showed strong activity of β-glucuronidase in the cytoplasm, as shown in Figure 5.

In vivo bacillary growth enhancement by HA. As noted in Table 3, hyaluronic acid and heparin seemed to increase the Morphologic Index of *M. lepraemurium* markedly. The Bacillary Index did not show homogenous results but it still showed increased numbers of bacilli. Chondroitin sulfate did not show marked growth enhancement for the bacilli as did the above two AMPS.

Histopathologic observation showed marked extracellular growth of *M. lepraemurium* with either HA or heparin, as shown in Figure 6, besides the usual growth of this organism in the lepra cells. This was not seen in either the control animals or those given chondroitin sulfate.

In vivo growth enhancement of M. leprae in mice. As shown in Figure 7, tiny subcutaneous nodules developed at about the 30th experimental day in mice inoculated with the mixtures of *M. leprae* and HA. These nodules remained the same in size throughout the experiment in spite of the additional injections of HA. The foot pads did not show nodules or swelling in the course of study. The control did not show any nodules throughout. Histopathologically, a mouse sacrificed on the 34th experimental day showed a type of granulomatous inflammation with numerous acid-fast bacilli, a fairly large proportion of which were granulated. On day 120, however, an astonishing growth of the bacilli was observed (Fig. 7). Among these acid-fast bacilli there were many solid forms and bacilli were often seen extracellularly. The foot pads also showed fairly strik-

TABLE 3. *AMPS enhancement of* M. lepraemurium *growth in three months.*

	Bacillary Index	Morphologic Index
Hyaluronic acid	$(4.14 \pm 4.00) \times 10^9$	$76.0 \pm 4.9\%$
Heparin	$(2.07 \pm 1.08) \times 10^9$	$70 \pm 0\%$
Chondroitin sulfate	$(2.77 \pm 1.17) \times 10^8$	$61.7 \pm 3.7\%$
Control	$(3.32 \pm 1.92) \times 10^8$	$44 \pm 7.3\%$

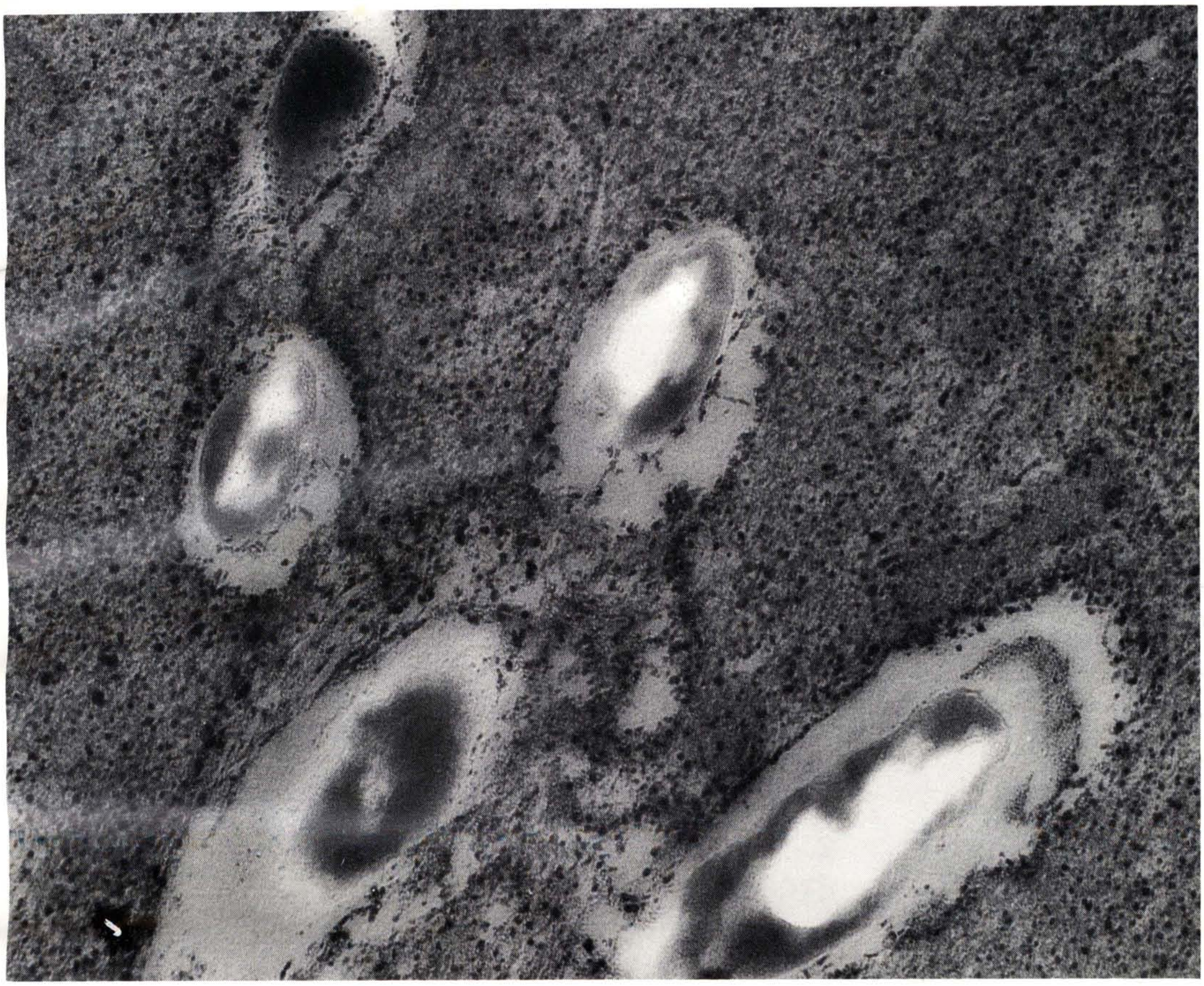

FIG. 2. Electron micrograph, mouse *M. lepraemurium* infected spleen. Reinhart Abul-Haj modification of Mowry's iron solution. AMPS represented by diffuse, electron dense, iron particle deposition. ×9,500.

ing growth but bacilli were seen only in the histiocytes. Control did not show the bacilli in the injected area of abdominal skin and only mild and localized growth in the foot pad. No spread of the organisms to the distant organs was observed.

Saccharic acid treatment of established murine leprosy. The animals were, in many instances, virtually moribund at the beginning of this pilot experiment. There being too few available for good matching as to control versus treated and the course of murine leprosy being well known, those that were most severely ill were placed in the group for treatment. Within three to four days the treated animals recovered remarkably and were described by the attendants as "dancing about." In any case, they again became very active and their lepromas regressed markedly in size. Many of them, males especially, had ulcerated lepromas which now healed from the margins. Though not every one attained complete re-epithlialization, virtually all showed marked scarring.

After two months' treatment the surviving animals were sacrificed and autopsied, the others having died due to tumors, bronchopneumonia, etc. Their livers and spleens were significantly smaller (Fig. 8) and weighed less (Table 4) than those of the controls. Histopathologically, the treated lesions were regressed, with lepra cells being smaller where present, and with a significant tendency toward morphologic change to granuloma rather than leproma structure (Fig. 9). These granulomas were made up of slightly vacuolated epithelioid-like histiocytes containing few bacilli and surrounded by a few lymphocytes. Where bacilli were present, both intracellularly and extracellularly, there was a striking shift in bacillary

TABLE 4. *Ratio of liver and spleen to total body weight.*

Treatment [a]	Ratio organ/body weights [b]	
	Liver	Spleen
Saccharic acid (4)	6.96 ± 0.57%	1.10 ± 0.26%
Control (2)	8.31 ± 0.85%	2.34 ± 0.08%

[a] Numbers in parenthesis refer to number of animals.
[b] Ratio with standard deviation.

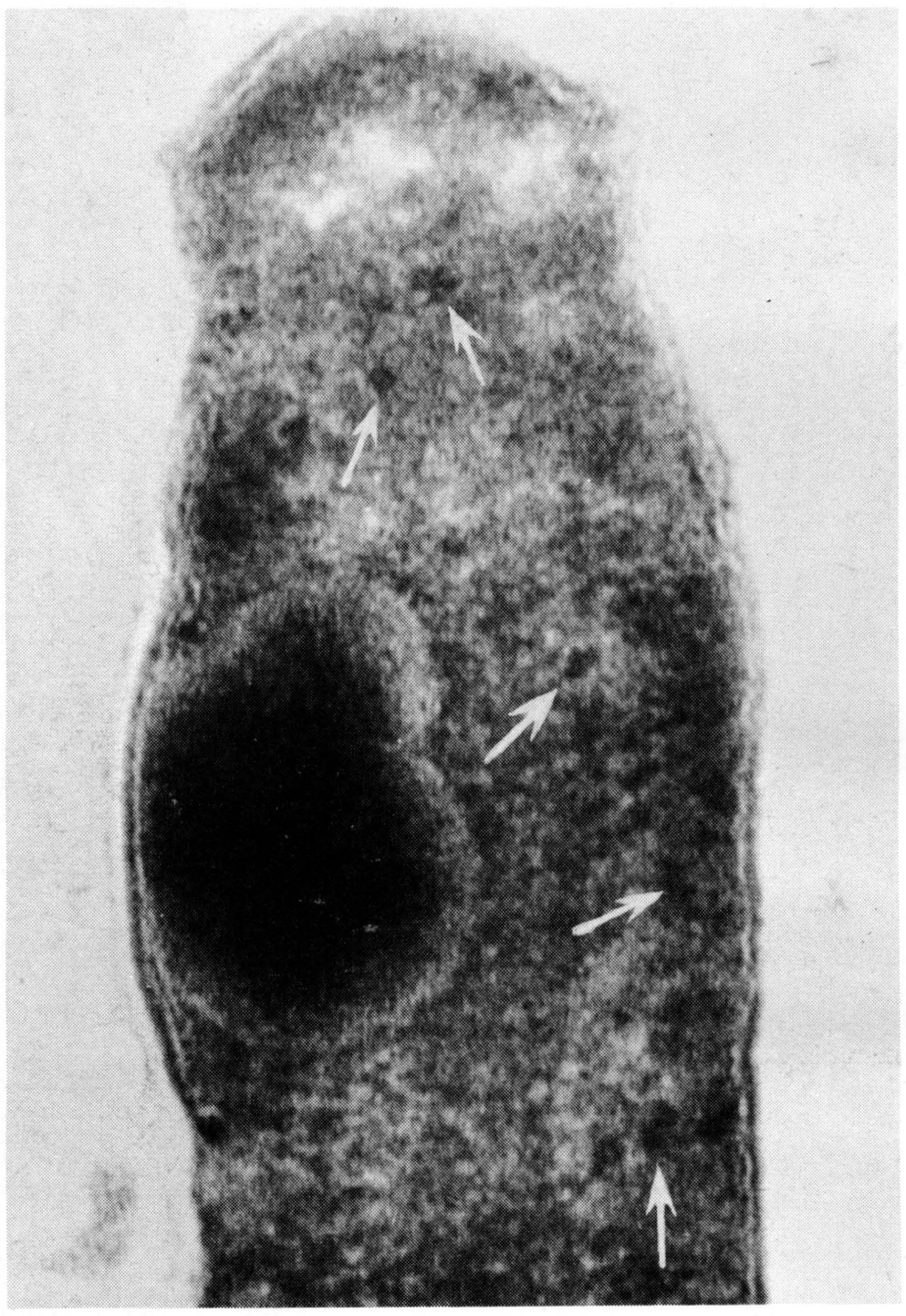

FIG. 3. Electron micrograph of sectional *M. lepraemurium* showing β-glucuronidase activity (arrows) by the Smith-Fishman technic. ×28,000.

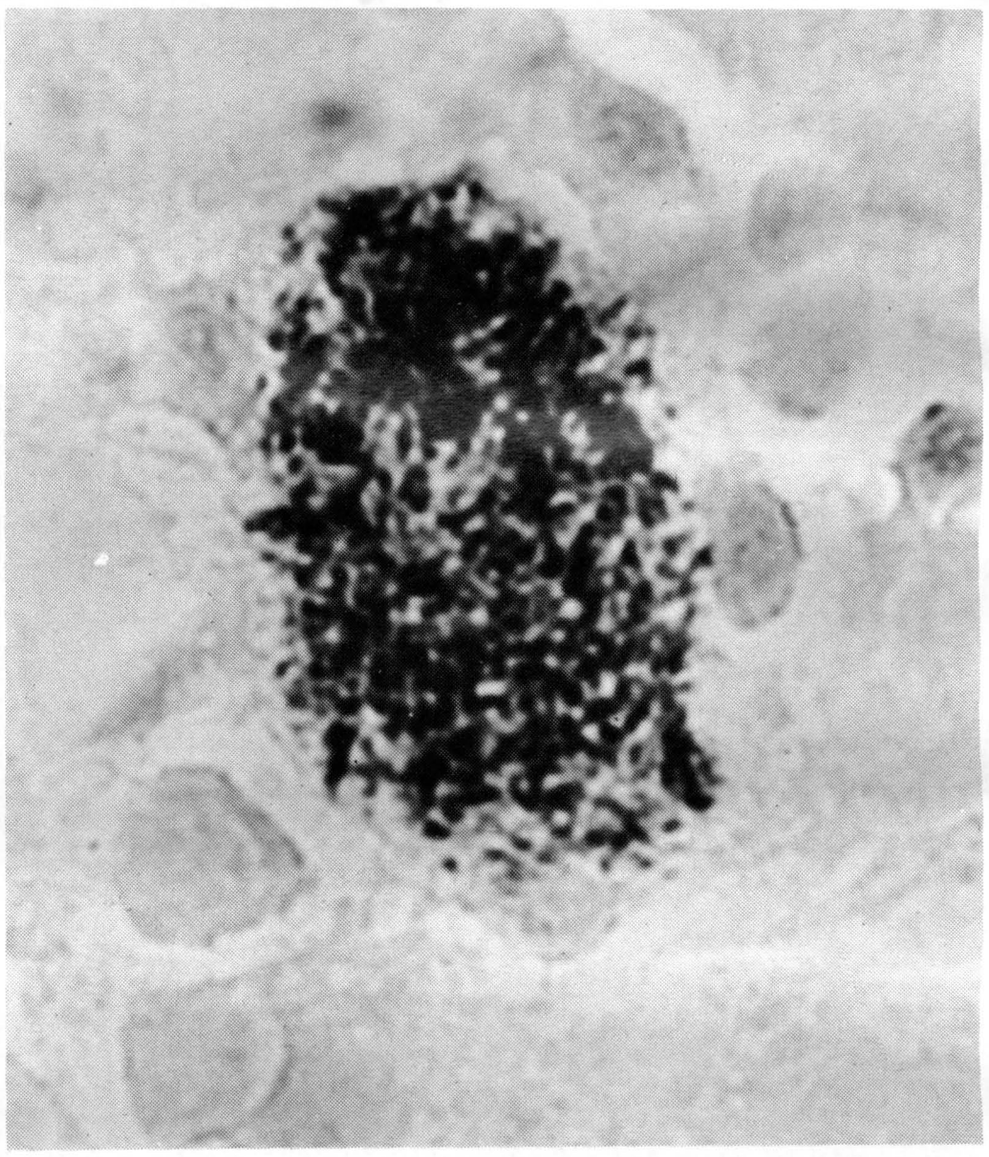

FIG. 4. *M. lepraemurium* β-glucuronidase activity from mouse spleen smear treated with lysozyme. Activity is present outside the bacilli and more intensely within them. ×1,000.

morphology in that a high proportion showed "tadpole" shapes similar to those of Figure 11, easily seen in paraffin sections. When lying in extracellular spaces, they were so numerous as to give the impression of a swarm of tadpoles in a small pond. The essential morphologic change, on acid-fast stain, consisted of dark round swelling at one end of the bacillus whereas the rest of the organism stained relatively faintly.

Treatment of early murine leprosy with enzyme inhibitors. The results were similar to, but less striking than those noted in relation to treatment of well-established infections. The animals inoculated in the foot pads seemed to respond better than those inoculated into the abdominal wall or inguinal area; and those treated with saccharic acid had better results than those treated with ascorbic acid, though both groups did better than the control animals (Fig. 10, Table 5). Though the bacilli multiplied in both the experimental groups, there were more fragmented forms in these groups and the bacillary "tadpole" appearance (Fig. 11) was again in evidence. The treated groups showed more localized histiocytic infiltrates and larger abscess-like areas containing considerable polymorphonuclear leucocytes but without specific necrosis. Two of the eight animals treated with saccharolactone and inoculated in the abdominal wall did not have any lesions at autopsy whereas the

TABLE 5. *Enzyme inhibitor treated* M. lepraemurium *infected foot pads.*

Inhibitor	Foot pad weight (gm)[a]
Saccharic acid	0.21 ± 0.05
Ascorbic acid	0.24 ± 0.02
None (control)	0.26 ± 0.07

[a] Mean ± standard deviation.

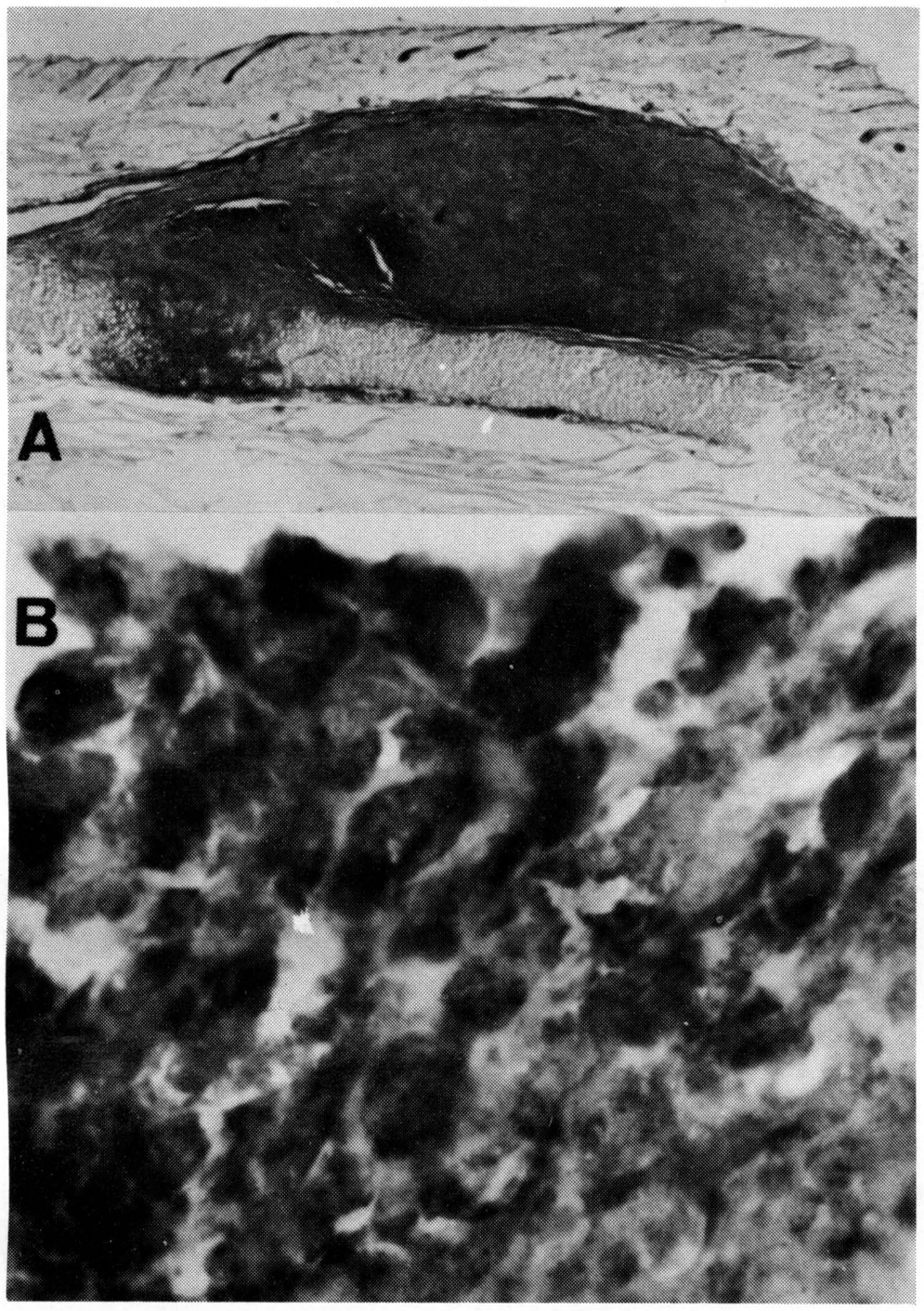

FIG. 5. Guinea pig *M. lepraemurium* induced granuloma showing β-glucuronidase activity localized in (A) the granuloma (×2.5), and (B) granuloma macrophages (×400).

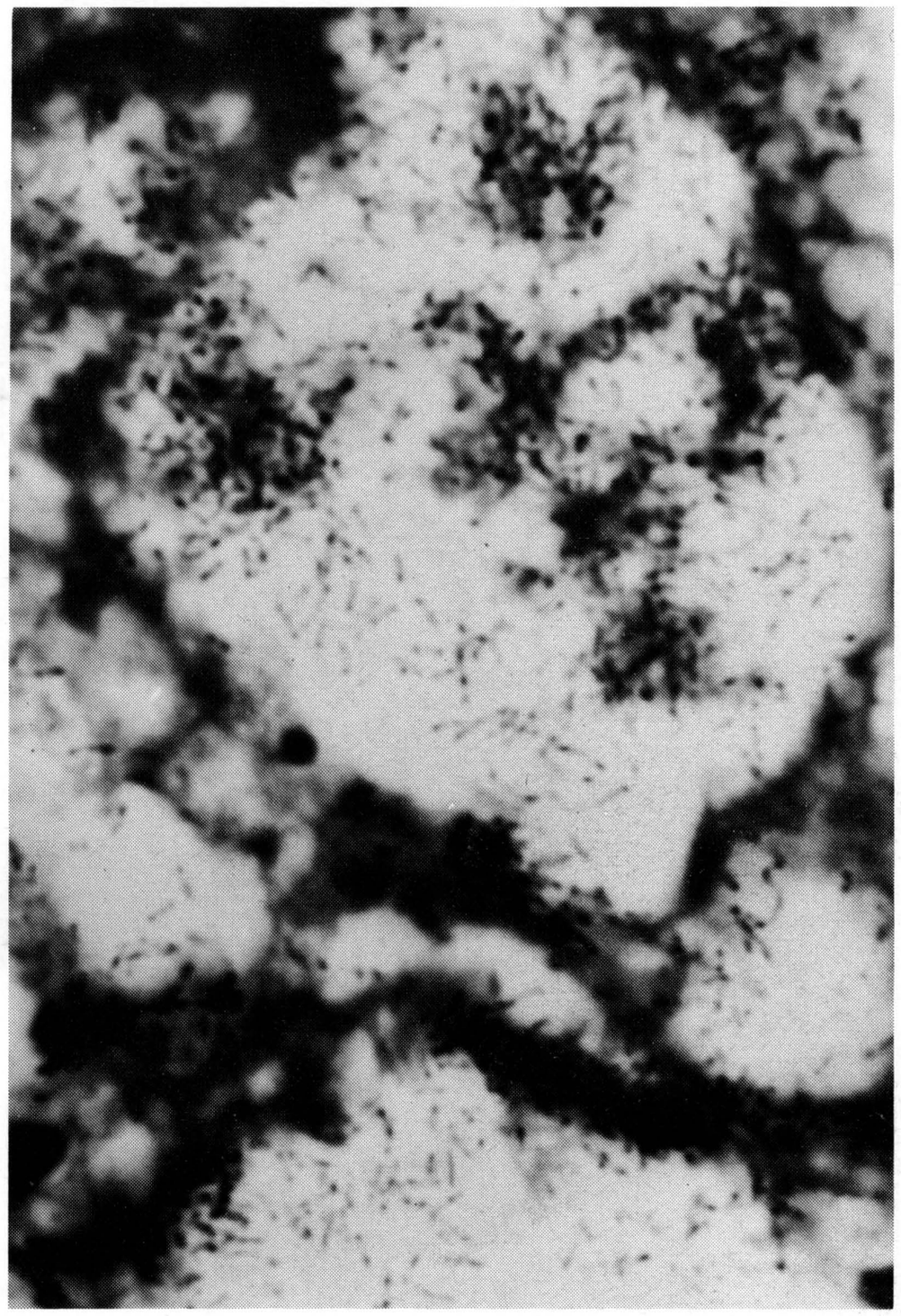

FIG. 6. Pronounced extracellular, as well as intracellular, proliferation of *M. lepraemurium* subcutaneously in lesion fed HA. Acid-fast stain. ×400.

others again showed a tendency to the granulomatous morphology described in association with the treated, established infections. This tendency toward granuloma formation was not evident in the saccharic acid and ascorbic acid treated animals inoculated in the inguinal area.

Enzyme inhibition in human leprosy. All five cases showed lesion regression histopathologically which was associated with striking decreases in the Morphologic Indices as recorded in Table 2, as well as decreases in Bacterial Index determinations. The initial and final biopsies at 4.5 months on patient No. 1 are presented in Figure 12, and Figure 13 compares the bacillary appearances in these biopsies. Such changes are not unknown in the treatment of lepromatous leprosy with DDS. However, when the same changes occurred in a patient not receiving specific antileprosy therapy but only high dosage vitamin C, the finding can be said to be unusual and somewhat remarkable. This case also presented a striking number of "tadpole" shaped bacilli as compared to the others where degenerative changes were more conventional.

DISCUSSION

Prior studies ([8, 16]) having demonstrated

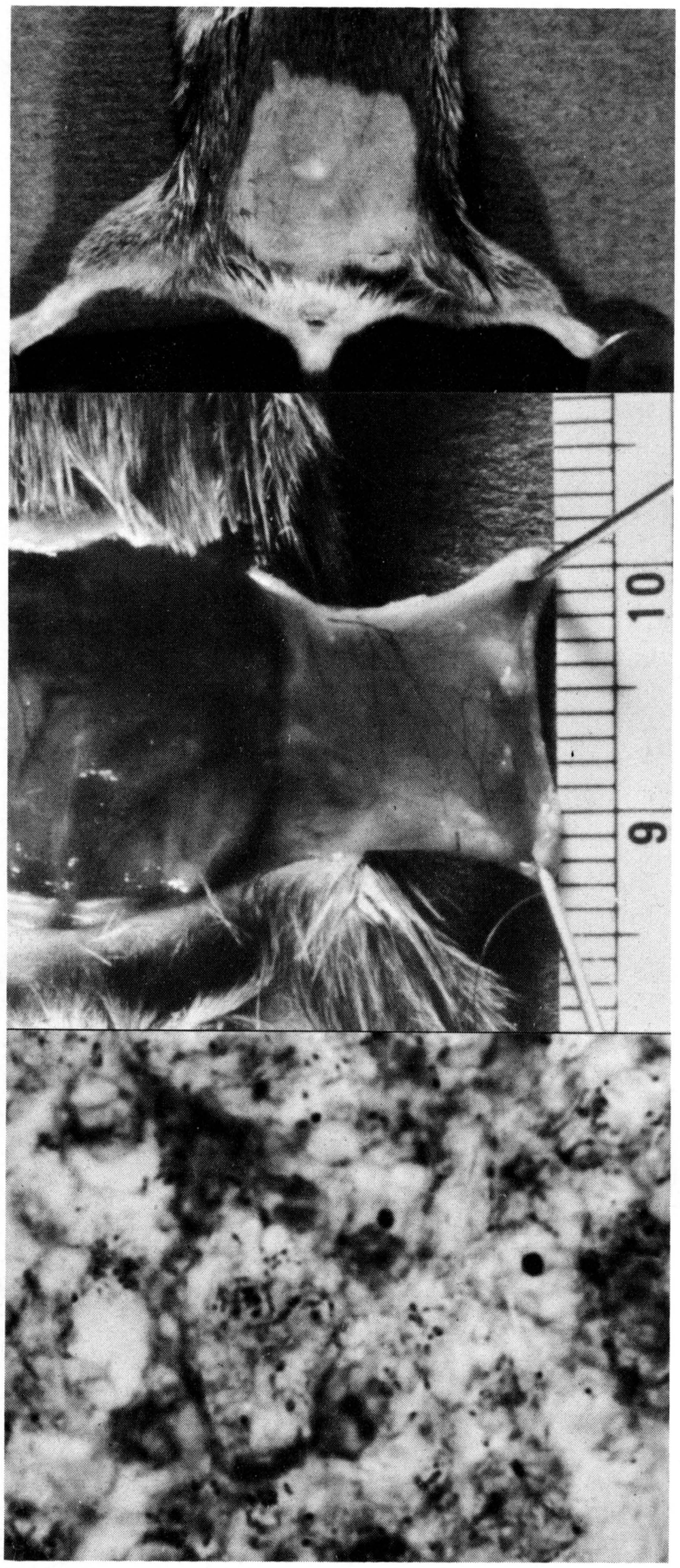

FIG. 7. Subcutaneous, abdominal wall, *M. leprae* leproma (A & B) 35 days following inoculation of bacilli together with HA. Extracellular and intracellular bacilli (C), with many granulated forms in this nodule. Acid-fast stain. ×1,000.

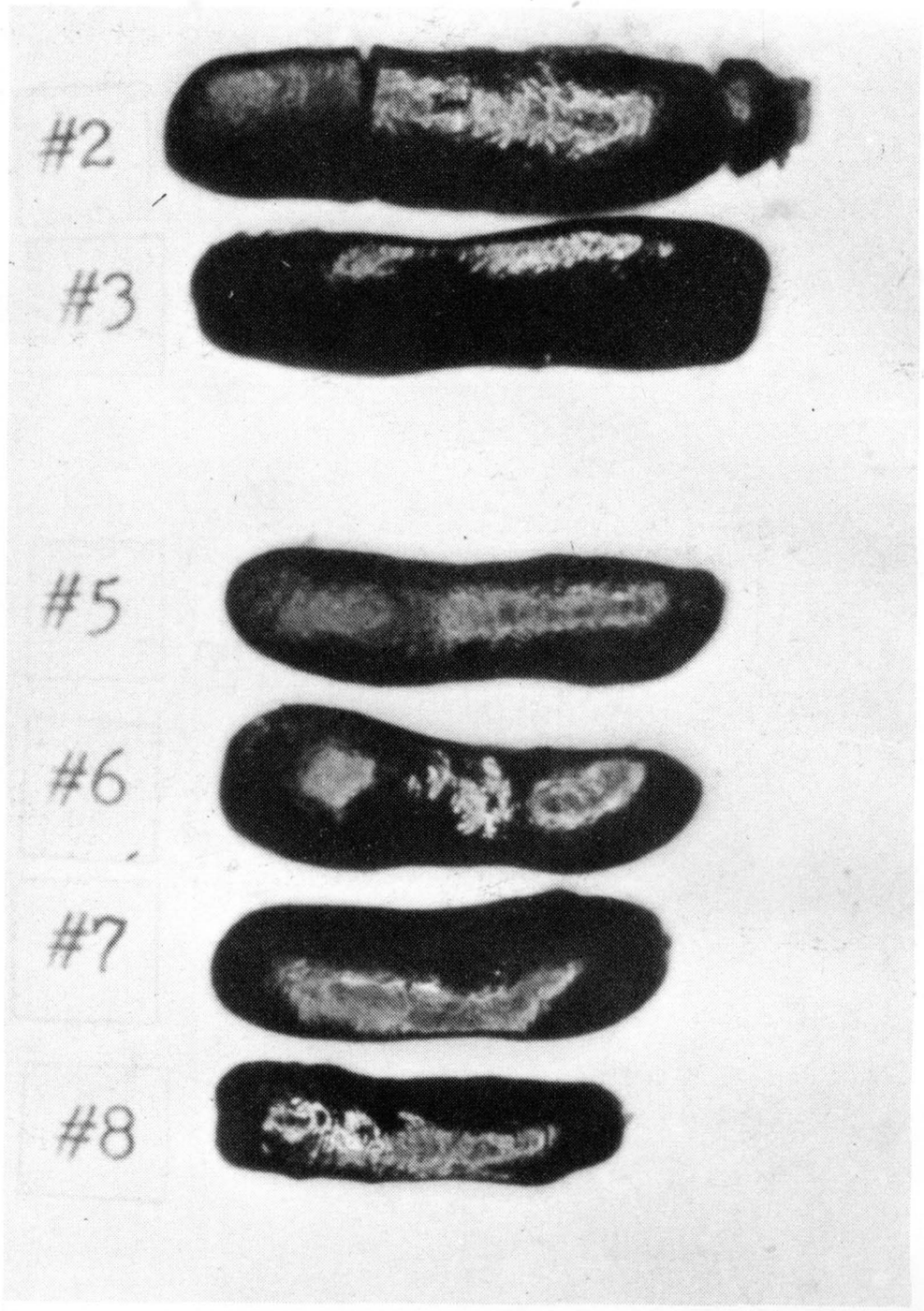

FIG. 8. Mouse spleens at 12 months *M. lepraemurium* infection without treatment (2 & 3) and with saccharic acid treatment (5-8) for final two months.

hyaluronic acid degrading ability on the part of leprosy epithelioid and Langhans type giant cells in contrast to a lack of similar ability on the part of lepromatous foam cells, the possibility arose that hyaluronic acid may serve as a growth promoting substrate for *M. leprae* and *M. lepraemurium.* The hypothesis was reinforced by the demonstration that both of these mycobacteria showed β-glucuronidase activity. Additional reinforcement as well as a possible explanatory factor for the areas of lesion predilection in leprosy was found in the known concentration of hyaluronic acid in tissues such as nerves and the stroma of testis as demonstrated by a number of studies, in particular by Hollander and Sommers [5], Skinsnes and Matsuo [8, 16], Abood and Abul-Haj [1], Adams [2], and Skinsnes and Yamashiro [17]. The present pilot studies are presented as preliminary investigations to a broader series of studies now in progress since the slow generation times of both these mycobacteria often require experimental study over a period of several months.

The first pilot study, utilizing *M. lepraemurium,* demonstrated that the distribution of HA concentration is similar in murine leprosy to the storage of lipid in this infection as previously noted [9, 13, 14]. This presence of HA in murine lepra cells and the presence of β-glucuronidase activity on the part of *M. lepraemurium* suggests a similar-

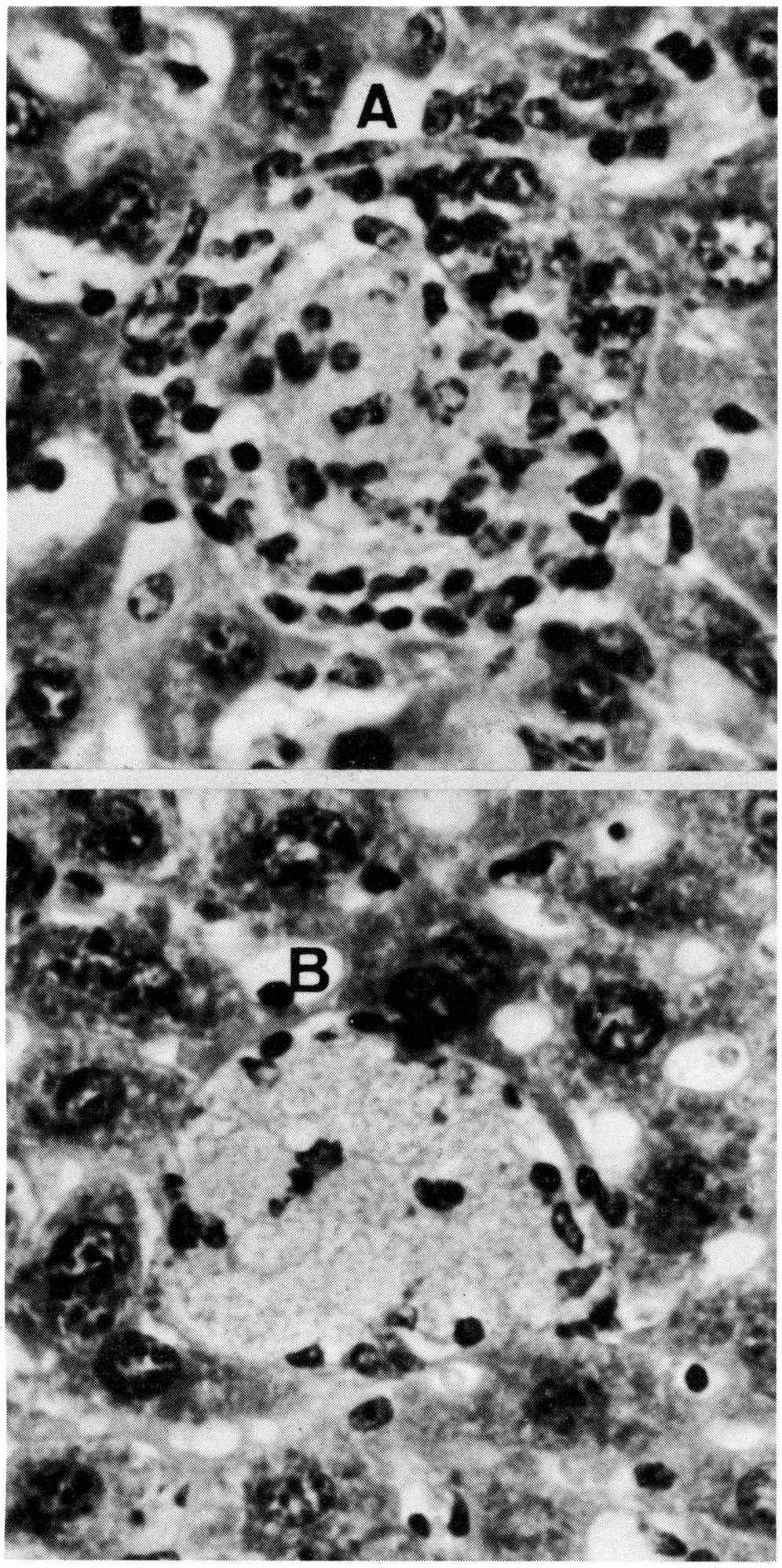

FIG. 9. "Granuloma" development in mouse liver following two months saccharic acid treatment (A) and usual leproma (B) in untreated *M. lepraemurium* infection; both 12 month old infections. H & E. ×400.

ity to the metabolism of *M. leprae* and that the murine infection can be utilized to some degree at least as a model for the study of lepromatous leprosy in this respect. Hadler ([4]) noted an inability of *M. lepraemurium* to grow in guinea pigs and this may be due to the ability of the responding guinea pig epithelioid cells to metabolize HA and thus deprive the bacillus of a needed energy source. Similarly, differences in mouse strain susceptibility may be related to the finding of Smith and Fishman ([18]) that the tissues (liver, spleen and prepuce) of C-57 black mice showed a higher β-glucuronidase value than do those of C3H mice and, as reported by Kawaguchi ([6]), the former tend to develop a more benign murine leprosy infection than do the latter.

As indicated in Table 3, all the acid mucopolysaccharides employed (hyaluronic acid, chondroitin sulfate and heparin) seemed to stimulate the growth of *M. lepraemurium* but HA and heparin appear to be more effective than chondroitin sulfate, and further studies are concentrating on the use of HA. The relatively massive extracellular appearance of bacilli as related to the presence of either HA or heparin suggests the strong possibility that these substances act as nutrients for this organism. Nakamura ([9]) reported a growth enhancement effect on *M. lepraemurium* in mice by the inoculation of Ehrlich ascites carcinoma. This tumor has been found to have a high concentration of HA ([7]) and it is possible that this was responsible for the effect noted. Further studies in progress suggest that one effect of AMPS may be to shorten the lag phase of *in vivo* inoculum growth.

Prior studies ([8, 16]) suggested that HA might have a prime role in the metabolism of *M. leprae*. These pilot study results indicate that HA does have a growth promoting effect on *M. leprae* inoculated subcutaneously into mouse abdominal walls. Studies in progress suggest that delivery of HA to the site of infection is considerably more effective than general delivery as by intraperitoneal injection. Since these pilot studies were essentially conducted "in the dark" with respect to possibly effective doses, route of delivery and frequency of delivery, the mixing of HA with the inoculum and its subsequent delivery subcutaneously to the site of infection on day 64 rather than by intraperitoneal injection may have been fortuitous in providing a guideline. Experiments in progress involving culture media growth of *M. leprae* and *M. lepraemurium* on differing but possibly suitable media fortified with HA are tentatively supportive of these findings.

Clearly, a crucial question in these and the companion studies in progress relates to the necessity of identifying the microorganisms described as being the organisms they are stated and believed to be; especially so with respect to *M. leprae*. This laboratory is critically aware of past errors in this respect in the history of leprosy research.

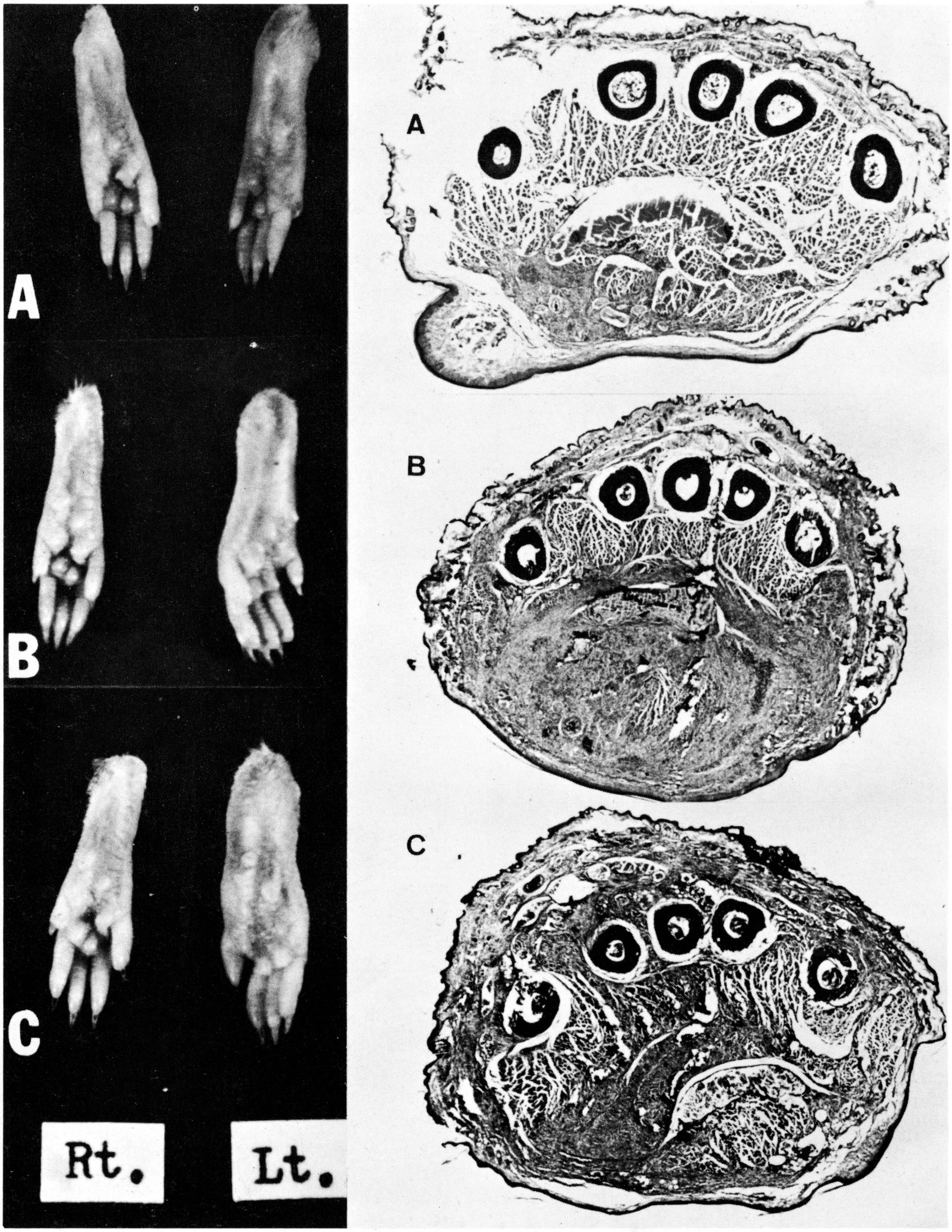

FIG. 10. *M. lepraemurium* infected mouse foot pads treated with (A) saccharic acid, (B) ascorbic acid, and (C) control. Control infection extends both dorsally and ventrally whereas ascorbic acid treated is limited to the ventral pad while the saccharic acid treated lesion is minimal and in ventral pad only. Saffron trichrome stain.

A variety of local factors have mitigated against the present use of the mouse foot pad inoculation of bacilli harvested from the experiments. Careful isolation of inoculum from source material and great care in maintaining the identity of the isolates give some

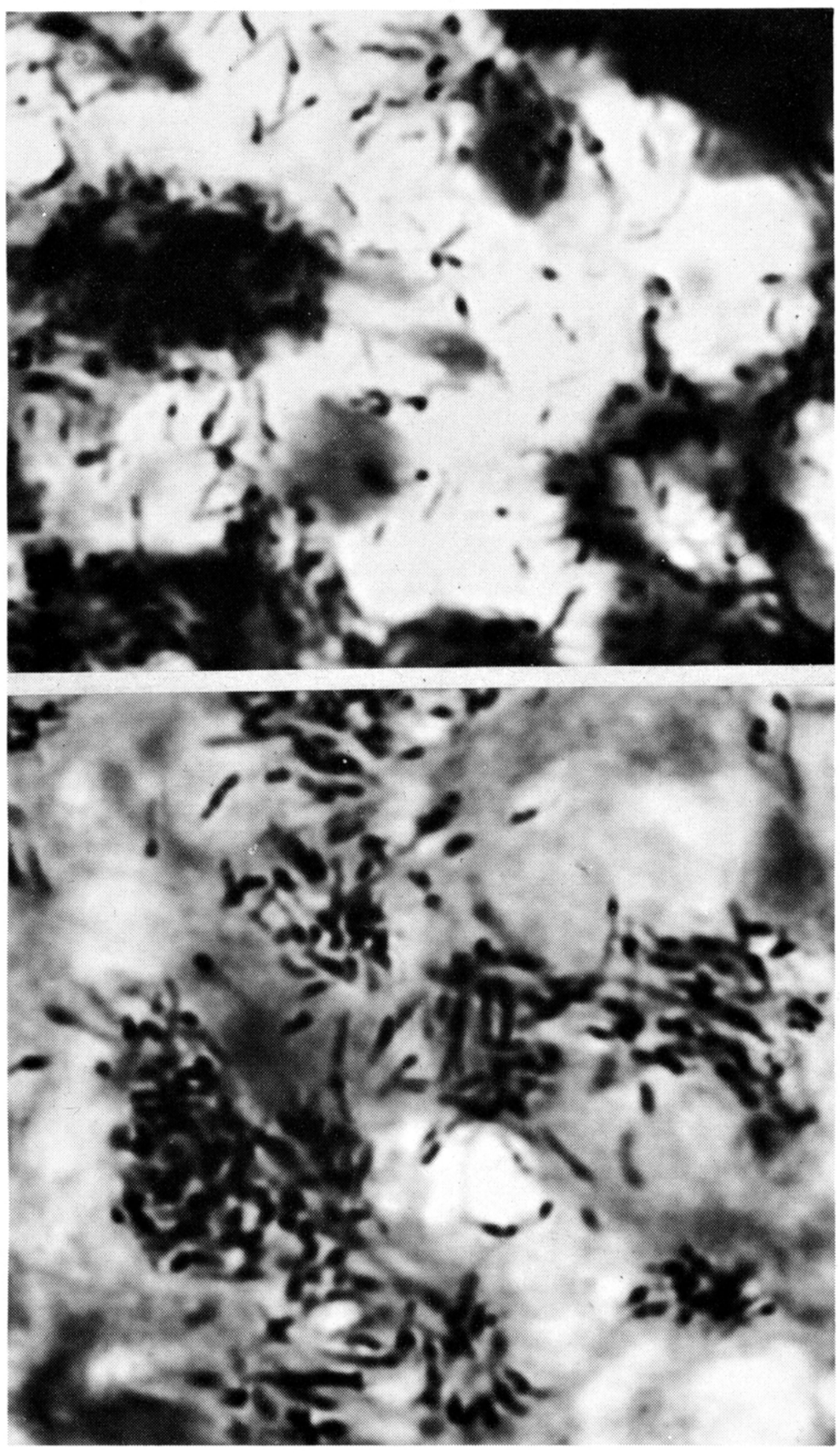

FIG. 11. "Tadpole" shaped *M. lepraemurium*, extracellular and intracellular, following saccharic acid treatment. Acid-fast stain. ×540 & ×1,000.

confidence, as does the lack of growth on standard *in vivo* culture media. Limitation of the amount of organism provided by the experimental growth and their slow growth lend yet more confidence while at the same time presenting difficulties with attempts to obtain adequate material to be utilized, for example, in adequate foot pad inoculation. Accordingly, another approach is being ap-applied in that the D-dopaoxidase test described by Prabhakaran ([11]) has been adapted for small quantity bacillary examination by both light and electron microscopy. This is currently being utilized as a basic guide.

Clear determination of mycobacterial content of β-glucuronidase was made feasible by the use of chloroform and/or lysozyme. The readiness with which this is accomplished suggests that the bacillary capsules and cell walls have a considerable poly-

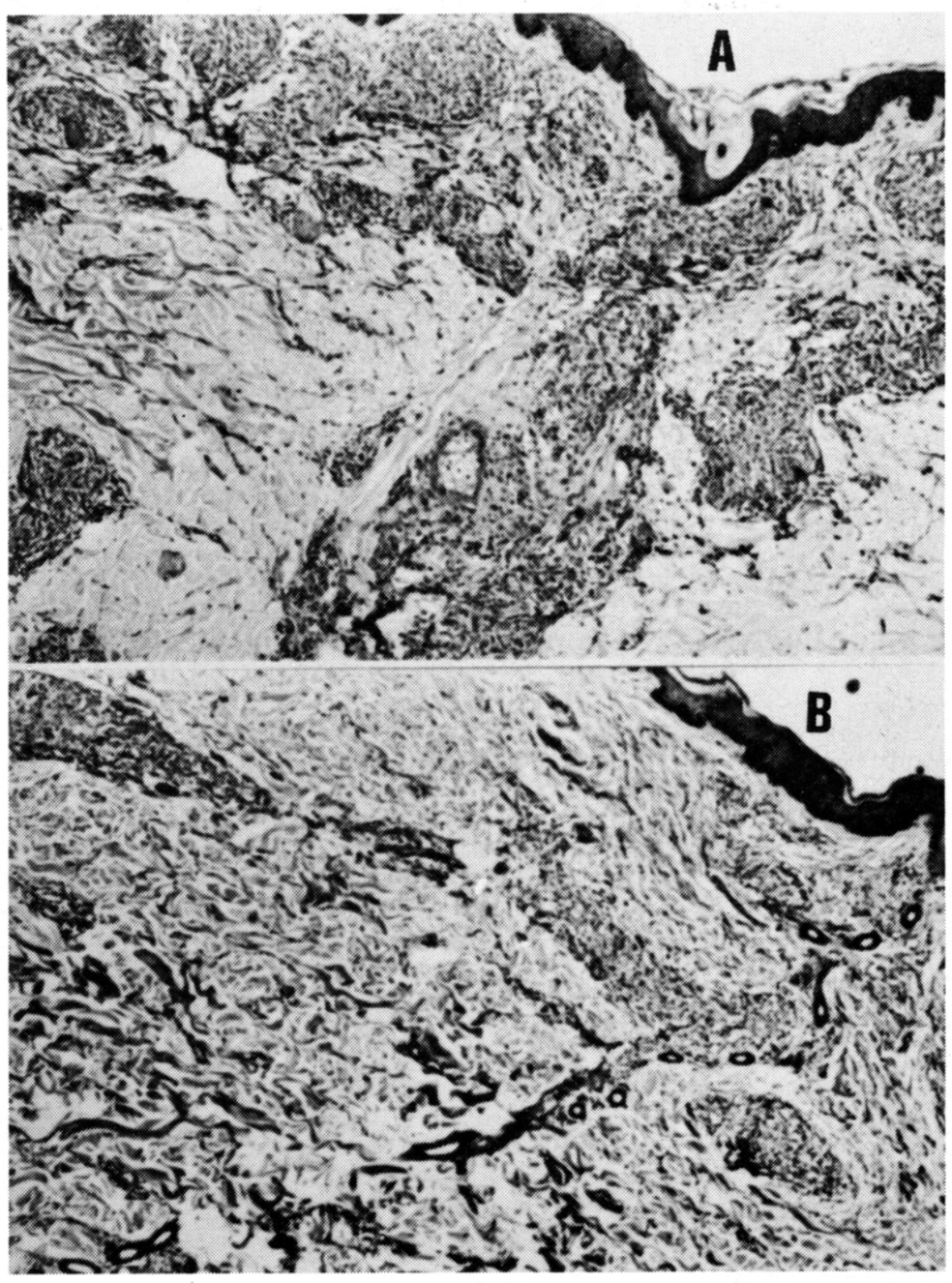

FIG. 12. Vitamin C treated lepromatous leprosy. (A) Initial biopsy, (B) biopsy after 4.5 months vitamin C treatment only. Acid-fast stain. ×25.

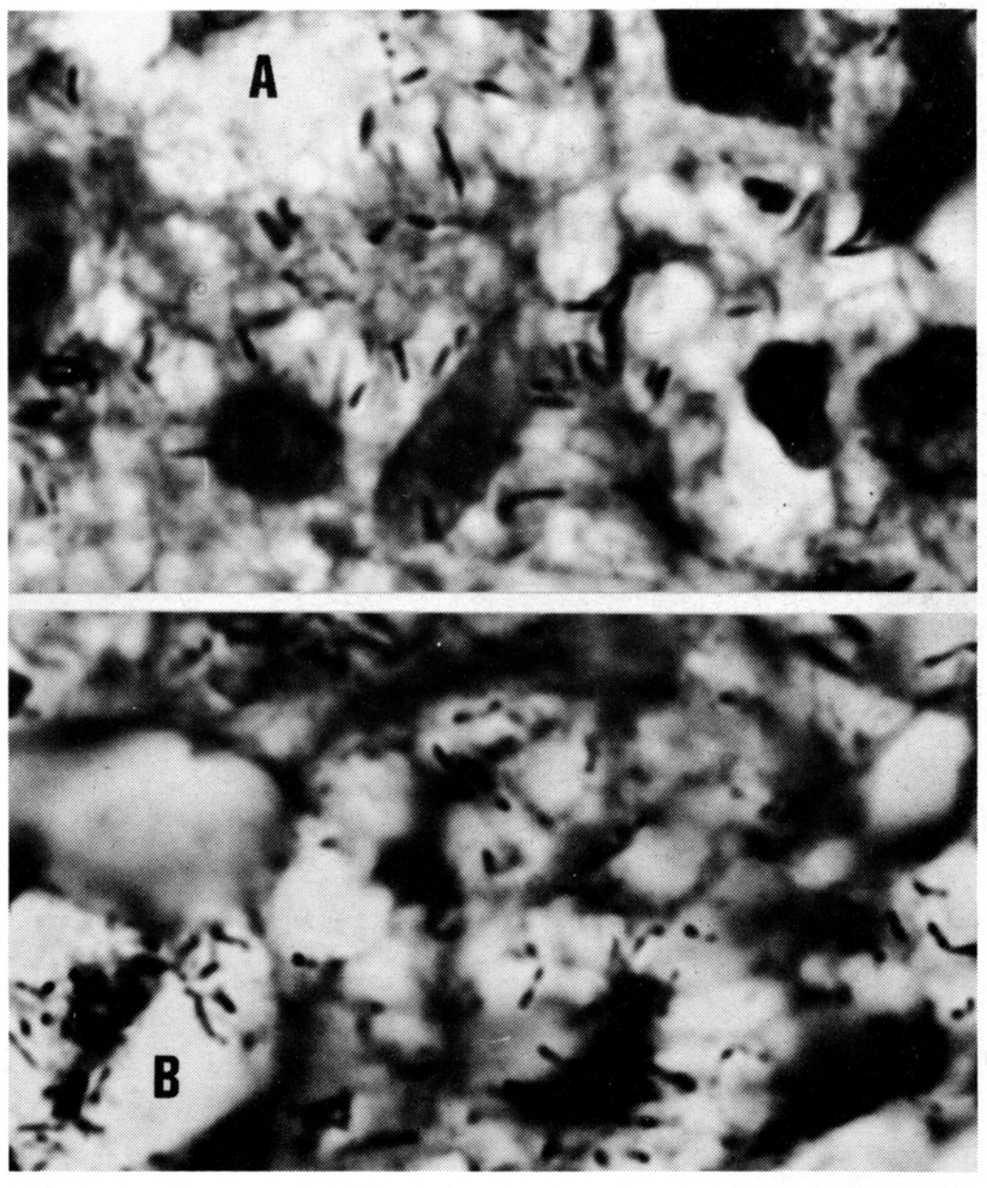

FIG. 13. Vitamin C treated lepromatous leprosy. (A) Bacilli in initial biopsy and (B) in 4.5 months biopsy. Acid-fast stain. ×1,000.

saccharide component. Inability of lepra cells to metabolize these polysaccharides, or their incomplete breakdown, may possibly result in their complexing with lipids to yield relatively insoluble complexes responsible for lipid storage in these cells.

The cases of lepromatous leprosy who received vitamin C as a treatment supplement are clearly too few to provide more than suggestive findings. However, the results, particularly in case No. 1 who received vitamin C only, are consonant with the experimental experiences reported with β-glucuronidase inhibitors. The changes in bacillary morphology, described as "tadpole shaped" bear a resemblance to some of the varieties which have been described and discussed as "club forms" ([12]) and which have been postulated by some to be degenerate, dead forms and by others to be concerned with bacillary regeneration. In the present variety and occurrence they probably represent morphologic abnormality due to enzyme inhibitor regression and interference with their metabolism. Their metabolic status is being investigated histochemically.

These preliminary reports are presented without delaying for the additional year or two of work which would help resolve some of the tentative conclusions because the findings are consonant with additional studies in process, because they are consonant with our findings regarding the pathology of leprosy, and particularly because they may be helpful to the current extensive efforts in many laboratories to devise practical means of *in vitro* cultivation of *M. leprae.*

SUMMARY

A series of pilot studies are presented utilizing mouse and human infections with *M. leprae* and mouse infections with *M. lepraemurium* relating to the previously reported finding that hyaluronic acid seems to be a major nutrient substrate for these bacilli. The "feeding" of hyaluronic acid to the bacilli enhanced the growth of *M. leprae* in mouse abdominal walls and increased the Morphologic Index of *M. lepraemurium* infection. Saccharic acid, an inhibitor of β-glucuronidase previously reported as present in these leprosy bacilli, caused marked regression of advanced *M. lepraemurium* infection, inhibited early infections and was accompanied by marked morphologic changes in the bacilli. Ascorbic acid (vitamin C), also an inhibitor of β-glucuronidase, given at a level of 1.5 gm/day for 4.5 months to one lepromatous patient without other treatment and for up to 24 months to four other lepromatous patients receiving DDS, was accompanied by lesion regression and changes in bacillary morphology similar to those seen in the inhibitor treated mice.

If these observations are confirmed the possible use of β-glucuronidase inhibitors as a useful adjunct to other leprosy therapy is raised as is also the likelihood of developing new therapies.

RESUMEN

Se presentan una serie de estudios pilotos utilizando infecciones murinas y humanas con *M. leprae* e infecciones murinas con *M. lepraemurium.* Estos estudios están relacionados con trabajos prévios donde se halló que el ácido hialurónico podría ser el mayor substrato nutritivo para estos bacilos. Suplementación de ácido hialurónico a los bacilos aumentó el crecimento de *M. leprae* en las paredes abdominales de lauchas y aumentó el Indice Macrofágico en la infección con *M. lepraemurium.* El ácido sacárico, un inhibidor de la β-glucoronidasa hallada previamente en estos bacilos leprosos, causó una marcada regresión de la infección avanzada por *M. lepraemurium,* inhibió infecciones precoces y fue acompañado de marcados cambios morfológicos en los bacilos. El ácido ascórbico (vitamina C), tambien inhibidor de la β-glucoronidasa, administrado a un nivel de 1.5 gramo/dia durante 4.5 meses a un paciente lepromatoso sin ningún otro tratamiento, y durante 24 meses a otros pacientes lepromatosos que recibieron además DDS, se acompañó de regresión lesiva y de cambios en la morfología bacilar similares a los observados en las lauchas tratadas con el inhibidor.

Si estas observaciones fueran confirmadas podrían señalar a los inhibidores de la β-glucoronidasa como útiles complementos de otros tratamientos leprosos como asi también la posibilidad de desarrollar nuevos tratamientos.

RÉSUMÉ

Selon des études déjà rapportées l'acide hyaluronique semble servir comme un aliment pour *M. leprae* et *M. lepraemurium.* À l'égard de cette découverte on présente ici les résultats de plusiers études préliminaires sur l'infection à *M. leprae* chez la souris et l'homme, et à *M. lepraemurium* chez la souris. La croissance de *M. leprae* aux parois abdominales des souris a été augmentée dans la présence de l'acide hyaluronique supplémentaire. Chez la souris traitée d'une façon semblable et infectée avec *M. lepraemurium* l'index morphologique des bacilles a été agraıdi.

L'acide saccharique, un inhibiteur de β-glucuronidase, rapporté préalablement aux bacilles de leprae, a provoqué une inhibition précoce des infections et des changements remarquable de la morphologie des bacilles. L'acide ascorbique (vitamine C), aussi un inhibiteur de β-glucuronidase, à raison de 1,5 gm par jour pendant 4,5 mois à un patient lépromateux sans autre traitement, et pendant 24 mois à quatre patients lépromateux recevants DDS, a provoqué des régressions des lésions et changements morphologiques des bacilles. Ces modifications ont été similaires aux changements chez la souris traitée par l'inhibiteur.

Ces observations lorsqu'elles sont confirmées suggèrent l'emploi des inhibiteurs de β-glucuronidase comme traitement accessoire aux thérapies courantes. De plus, la possibilité du développement de nouvelles thérapies se présente.

Acknowledgments. The highly competent histopathological technical assistance of Kazuko Matsuo and the talented illustrative and photographic assistance of Johnny Shek are fully recognized.

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