PATHOGENESIS OF PARATUBERCULOSIS (JOHNE'S DISEASE) IN THE GOLDEN SYRIAN HAMSTER

by

Arlis Dormon Boothe

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
MASTER OF SCIENCE

Major Subject: Veterinary Pathology

Iowa State University
Ames, Iowa
1967

TABLE OF CONTENTS

INTRODUCTION

Paratuberculosis is a specific infectious disease of ruminants caused by an acid-fast organism, Mycobacterium paratuberculosis, and was first recognized as a specific disease identity in 1906 (7). It has also been suspected of causing naturally occurring disease in horses (95), swine (30,31), and monkeys (82). Often, it is a wasting illness with a prolonged course, during which intractable diarrhea results in dehydration, emaciation, and eventually death. The disease is an important economic problem in cattle but is of lesser importance in other species.

Experimental work on M. paratuberculosis has been greatly hampered because attempts to infect laboratory animals have not been consistently successful. The results of previous investigations of its pathogenicity for laboratory animals may be arranged roughly into 3 groups: a large number of apparent failures was noted; a group in which lesions quite unlike those found in cattle was produced; and few experiments in which an intestinal infection resembling the natural disease were obtained.

For economic reasons, more efficient utilization of isolation facilities, larger numbers of animals per experiment, and shorter-termed experiments, it was concluded that a laboratory animal was needed for the study of paratuberculosis. The pathogenicity of M. paratuberculosis has not been thoroughly investigated in laboratory animals. In evaluation of a disease process, it is necessary to know the extent to which the etiologic agent is disseminated in the body and how it becomes localized in particular organs after exposure. This study was undertaken

to gain more information about the pathogenesis of paratuberculosis in a laboratory animal that has an alimentary system with features similar to those of the ruminant.

The golden hamster, (Mesocricetus auratus, formerly Cricetus auratus Waterhouse, 1839), is a rodent belonging to the group of white-footed mice and possesses a forestomach. In its forestomach, which is the simplest among the polygastric animals, microorganisms are present that are similar to the microorganisms of the rumen (62). Further, it has been shown that the addition of urea to the ration is nutritive for the hamster (65). Anatomically and physiologically, the hamster resembles the ruminant closer than any other available laboratory animal and lends further justification for its use in this study.

Previous workers (36,37) have noted evidence of multiplication of M. paratuberculosis in the hamster. Their studies were based on cultural examinations with very limited histopathology. Since it requires about 3 months to grow cultures of M. paratuberculosis, evaluations of assay animals by histopathologic means may be faster. Therefore, an extensive histopathologic evaluation of the pathogenesis of paratuberculosis in the golden hamster was undertaken.

REVIEW OF LITERATURE

History of Domestication of the Golden Hamster

In 1930, the Department of Parasitology of the Hebrew University of Jerusalem was engaged in the investigations on Mediterranean kala azar. The only suitable experimental animal then known was the Chinese hamster which had the disadvantages of being imported from the Far East, not breeding in captivity, and being very susceptible to Pasteurella.

In 1930, the late Professor Aharoni of the Department of Zoology of the Hebrew University went on a zoological expedition to Syria and brought back Cricetulus phoeus for kala azar studies. In addition to these animals (which were also unsatisfactory because they did not breed in captivity), Professor Aharoni brought back a litter of 8 golden hamsters collected near Aleppo which he reared and presented to the Department of Parasitology.

Four of the hamsters escaped and 1 female was killed by a male, thus leaving only 1 male and 2 females. From these, several litters were reared, establishing the ease of breeding this species in captivity.

The hamster was first used on kala azar research in November 1930, and it was later found valuable for work on tuberculosis and brucellosis. From Jerusalem the hamsters were sent to France, England, India, and Egypt. From India, I. J. Kligler sent a group to America. To the best of recorded knowledge, all the golden hamsters now being used as

laboratory animals in Europe and America originated from the littermates first raised in captivity at the Hebrew University of Jerusalem in 1930 (2).

Historical Work on Paratuberculosis

Paratuberculosis (Johne's disease) is a naturally occurring chronic infectious enteric disease of ruminants caused by an acid-fast organism, M. paratuberculosis. Jöhne and Frothingham (50) in 1895 were the first to demonstrate the causative organisms in the thickened mucosa of the intestine of cattle suffering from chronic diarrhea. They believed it to be an unusual type of avian tubercle bacillus. Koch was also in complete agreement with them.

In 1906, Bang (7) showed that the organism could be transmitted to cattle, would produce disease after a long incubation period, and was a separate entity from tuberculosis.

Paratuberculosis in the United States was first described in 1908 by Pearson (81) in Pennsylvania and again later the same year by Beebe (8) of Minnesota.

The causative organism was never cultured on artificial media until 1912 when Twort and Ingram (106) reported their success with media containing dried and powdered growth factors of certain acid-fast bacilli--timothy grass bacillus, smegma bacillus of Moeller, nasenschleim bacillus of Karlinski, and a human type of tubercle bacillus. Media containing fractions of tubercle bacilli isolated from cats, avian type, and bovine strains produced no growth of paratuberculosis bacillus.

Morphological and Physiological Characteristics of M. paratuberculosis

Mycobacterium paratuberculosis is a short, thick, non-sporulating, Gram-positive rod, one-half micron in diameter by 1 to 2 μ in length and comparable in size to the avian tubercle bacillus (66). When stained with an appropriate dye, such as carbol fuchsin, they are not decolorized by the action of acids diluted in alcohol. Most of the bacilli are stained evenly but a beaded form may occasionally be observed. In tissue sections, the organisms are usually found in variable size clumps within "fixed" phagocytic cells. However, numerous bacteria may be dispersed singly within the macrophages. The bacillus is aerobic, grows best at 39° C., and is difficult to cultivate on artificial media.

Previous Experimental Paratuberculosis in the Golden Hamster

Daubney (51) in 1938, was the first to suggest that the golden Syrian hamster might be used as an experimental animal in Johne's disease research since it had been found of value in the study of leprosy by Adler. In the same year, Dunkin (51) used hamsters in the study of Johne's disease and stated that he had not succeeded in infecting them.

Francis (33,34) in 1943, inoculated 10 hamsters intraperitoneally and 4 orally with M. paratuberculosis as follows:

1. Five 23-day-old hamsters with an average weight of 42 g. received intraperitoneally an initial 2.5 mg. dose and another 2.5 mg. dose 7 days later. Postmortem examinations were performed at 9, 10, 22, 31, and 32 weeks post-inoculation (PI).
2. Four 12-day-old hamsters with an average weight of 11 g. and one 3-month-old 95 g. hamster received an initial 0.1 cc. and 0.2 cc. intraperitoneal dose respectively of a thick emulsion made from the spleen of a hamster from group 1 killed at 9 weeks. The same dosage was repeated in 7 days using the spleen of a hamster from group 1 killed at 10 weeks. Two of the 12-day-old hamsters died soon after inoculation and no postmortem examination was made. Two of the 12-day-old hamsters survived and were examined at 12 and 25 weeks. The 3-month-old hamster was examined at 26 weeks PI.
3. Four 7-day-old hamsters with an average weight of 10.2 g. were inoculated *via* stomach tube with a 0.25 cc. suspension of the intestinal mucosa of a hamster from group 1 killed at 22 weeks PI. Postmortem examinations were made on 1 hamster each at 5 and 17 weeks PI. No report was made of 2 hamsters.

Results of the hamsters inoculated by Francis are summarized in Table 1. Francis concluded that the results in hamsters were less consistent than in mice, although the dosage was 3 times lower in

Table 1. Distribution of M. paratuberculosis after intraperitoneal (groups 1 and 2) and oral (group 3) inoculation as reported by Francis (34)

Group number	Time after inoculation (weeks)	Smear of tissue (number of bacilli)				Culture of tissue		
		Mesenteric lymph node	Liver	Spleen	Intestine	Liver	Spleen	Intestine
1	9	0	Few	Several	0	0	0	0
	10	0	None	Few	0	0	0	0
	22	0	None	None	Several	+	+	0
	31	0	None	None	None	-	-	0
	32	Many	None	None	Few	0	0	0
2	12	0	None	None	None	+	+	0
	25	0	Few	0	Several	0	0	0
	26	None	None	None	None	0	0	0
3	5	None	None	None	None	-	-	0
	17	None	Few	None	None	0	0	0

0 = Not reported
+ = Growth
- = No growth

the hamsters than that given to the mice. The bacilli remained viable after animal passage. The cellular reactions in infected mice and hamsters were similar to those in naturally occurring disease.

Hirch (47) in 1956, inoculated the following groups with cultures of M. paratuberculosis grown in Glover's modification of Dubos and Davis liquid medium:

1. Eleven adult hamsters were given 9 daily oral doses of 0.5 ml. each of the undiluted culture amounting to 1.5 mg. of moist organisms. Postmortem examinations were made at intervals from 4 to 40 weeks PI.
2. Four adult hamsters were given 6 daily oral doses of 0.5 ml. each of the undiluted culture amounting to 0.6 mg. of moist organisms. Postmortem examinations were made at 6, 10, 20, and 40 weeks PI.
3. Fourteen adult hamsters, divided into 4 groups, were inoculated once intraperitoneally with variable quantities of a culture recently isolated: (1) from a goat, (2) after first passage through a hamster, (3) after second passage through a hamster, or (4) after second passage through a rabbit.
4. Twelve 2- or 3-day-old hamsters, divided into 3 equal groups, were inoculated intraperitoneally once with 0.1 to 0.2 ml. of culture: (1) recently isolated from a goat, (2) after second passage through a hamster, and (3) several subsequent subcultures after passage through a rabbit.

Hirch reported that the liver from the hamsters which received the oral inoculations (groups 1 and 2) was most consistently positive as organisms were isolated from 10 of the 14 examined. Positive cultures were also obtained from 7 of 13 lungs examined. Viable organisms gradually disappeared from the lungs and none were isolated after 40 weeks PI. Only 1 hamster, killed after 40 weeks PI, yielded a positive culture from the kidney. Typical clumps of acid-fast bacilli were found in smears of the intestinal mucosa only after 40 weeks PI.

In the hamsters that received 0.6 mg. of organisms, the cultures of the organs were all negative at 6, 10, and 20 weeks PI, but a small number of colonies grew from the liver, kidney, and intestine of the hamster examined at 40 weeks PI.

The results of culturing and intestinal films from groups 3 and 4 are summarized in Table 2. In group 3, there was an increase in the number of colonies with longer duration after inoculation. A difference was noted between the source of the inoculum and the number of colonies obtained. In group 4, a small number of typical acid-fast bacilli were seen in films made from mucosal scrapings of the small intestine of nearly all of the animals. No real increase was observed in colony counts of cultures of livers and intestines until 26 to 35 weeks after inoculation. The organisms from the second hamster passage and from the goat appeared to become established in the hamster. The organisms, from the rabbit, that had been subsequently subcultured many times, did not multiply in the hamster.

Table 2. Distribution of M. paratuberculosis after intraperitoneal inoculations of hamsters by Hirch (47)

Group number	Dose (mg.)	Time after inoculation (weeks)	Results: Culture (number of colonies) Liver	Results: Culture (number of colonies) Intestine	Results: Intestinal films Number positive/total
3		12.5	0,0,0,0,100	18,97,29,49,0	0/5
From	0.3	25	Many	Many	4/6
goat		40	Many	Many	4/6
3		11.5	99,0,0	0,27	1/5
Second passage hamster	0.8	40	3,1,3	91,35,77,57	1/6
3		12	0,43,12,7,84	0,0,7,13,10	3/5
Second passage rabbit	0.5	18	50,14,21	Many	0/8
3		3	1,0,0	0,0,0	0/6
From	0.3	13	84,87,106	32,34	1/6
goat		27	Many	9,100	6/7
		35	Many	Many	1/6
3		12	5,0,0	Not done	0/6
First		19.5	Many	Many	1/6
passage hamster	0.2	35	0,0,0	4,3,0	1/6
4	0.08	6	0,0	3,0,1	2/6
From		15	10,0,0	0,0,0	1/6
goat		26	1,12,0	75,114,100	2/5
		35	19,2,35,20	5,0,25,26	2/6
4		7	0,0,2	0,0,2	1/6
Second		16	0,0,0	0,0,0	0/6
passage	0.04	26	50,12,0	Many	2/6
hamster		35	2,0,0	Many	2/6
4		6	0,4,0	0,0,0	1/6
From	0.08	15	0,0,0	0,0	3/6
rabbit		24.5	0,0,0	10,0,0	1/6
		35	0,0,0	1,0,0,0,0	0/6

Although typical acid-fast organisms were demonstrated in the small intestine and liver, sometimes in large numbers, the animals appeared quite healthy, and apart from a slight thickening of the small intestine and enlargement of the mesenteric lymph nodes, no obvious changes were seen at the postmortem examination. No clinical disease was produced in animals maintained for 40 weeks after inoculation. Hirch concluded that there was fairly definite evidence of the multiplication of the bacteria somewhere in the organs of the hamster with a slow subsequent establishment in the small intestine. There was apparently a variation in the "infectivity" of different strains.

Harding (45) in 1959, reported histopathologic studies on the hamsters used in the experiments by Hirch (47). The frequency of lesions in organs is tabulated in Table 3, but the distribution of acid-fast bacilli without the presence of lesions was not recorded.

Harding found that lesions in the liver parenchyma were smaller and less numerous than those in the interlobular connective tissue and portal tracts. However, the lesions of the portal triads were much more extensive and often the only changes detected. Bile duct proliferation was particularly marked. The splenic lesions were a diffuse hyperplasia of the epithelioid cells involving only the red pulp in 2 cases, whereas in the other 4 cases, the lesions consisted of clearly defined foci of epithelioid cells with tendency to form symplasmata and giant cells in both lymphoid follicles and red pulp. Five of the 9 hamsters inoculated orally showed lung lesions, thus raising the question of inhalation. These lung lesions demonstrated

Table 3. Frequency of lesions in hamsters produced by *M. paratuberculosis* as reported by Harding (45)

Organs	Number of organs with lesions	Total number of organs examined
Liver	60	80
Spleen	6	62
Lungs	10	74
Kidneys	3	75
Intestines	9	65
Mesenteric lymph nodes	2	2
Peritoneum covering organs	24	NR

NR = Not reported

definite regression after 3 months. One of the 3 cases in the kidney involved only the visceral peritoneum. Three cases of the intestinal lesions were confined to the lymphoid follicles, whereas all the others had extended through the muscularis mucosa and involved the overlying villi. No mucosal lesions were observed except immediately over an infected lymphoid follicle. The lesions in the mesenteric lymph nodes were in the cortex and tended to form symplasma.

Harding concluded that the organisms were fairly numerous during the period following infection, subsequently decreasing in numbers and surviving for an interval in the lymphoid tissue of the small intestine, but again becoming numerous after a latent period of many months. Of the species studied, mouse, rabbit, and hamster, Harding considered the hamster to have an organ susceptibility most like that of the ruminant.

Gilmour and Brotherston (36) in 1961, orally inoculated 3 groups of hamsters of about 20 g. in weight with 1 dose respectfully of 10^9, 10^8, and 10^7 viable units of M. paratuberculosis from a sixth subculture from a clinical case of Johne's disease in a sheep. Five or 6 hamsters from each group were killed at 1 and 2 months after inoculation. Viable counts were made of the liver, spleen, and of the intestinal walls of each animal. The minimum detectable infections were $\log_{10}$ 2.39 viable units in the livers and spleens and 3.49 viable units in the intestines. The mesenteric lymph nodes and livers were examined histologically.

At 1 month, intestinal infections were reported in 1 of 6 hamsters given 10^7 viable units, in 3 of 6 given 10^8 viable units, and in 5 of 6 hamsters given 10^9 viable units. After 2 months, intestinal infections were present in 1 of 6, 6 of 6, and 5 of 5 hamsters at the 10^7, 10^8, and 10^9 dose levels respectively. When detectable infections were present in the small intestine, the mean group counts at 2 months were at least 1 $\log_{10}$ units greater than those at the 1-month examination which, according to Gilmour and Brotherston, indicated that progressive infection had been established. Infections were not progressive in the large intestines.

Organisms were recovered from all hamsters examined at 3 months that had received 10^8 and 10^9 viable units. However, the viability counts at 2 months were not considered significantly greater when compared with those obtained at 1 month.

At 1 month following inoculation, lesions were present in the mesenteric lymph nodes of 1 hamster in each of 2 groups given 10^8 and 10^9 viable units. After 2 months, lesions were reported in 5 of 6 given 10^8 viable units and in 5 of 5 given 10^9 viable units. No microscopic lesions were detected in the liver of any hamster.

In 1963, Gilmour et al. (37) reported the results obtained from 33 hamsters weighing about 50 g. each that were inoculated orally at 8 weeks of age with 6×10^7 viable units of M. paratuberculosis of the bovine type that required the M. phlei growth factor and grown on William Smith solid medium. Viable unit counts were carried out on 0.1 ml. of a 1:200 dilution of weight-volume suspensions of spleens,

kidneys, lungs, livers, mesenteric lymph nodes, and both small and large intestines of animals killed 1, 6, and 24 hours, 1 week, and 1, 4, 8, 9, and 11 months after dosing. The cultural examination results are summarized in Table 4. They obtain no clutures of M. paratuberculosis from organs of inoculated hamsters after 6 hours and before 1 month PI. Histologic examinations were reported on sections from the livers and small intestines of the hamsters killed from 24 hours and later. The lesions in the livers were located close to the portal tracts and consisted of a central mass of epithelioid cells and peripheral zone of lymphocytes. Lesions were found only in 1 section of the intestinal tract that included a Peyer's patch. The lesions were discrete, round foci of epithelioid cells with abundant pale, eosinophilic cytoplasm. Smaller groups of epithelioid cells were present in the mucosa overlying the lymphoid tissue which was somewhat thickened. Numerous lesions were present in a small piece of attached lymph node. However, it was noted that no acid-fast bacilli were observed in any of the lesions in sections stained by the Ziehl-Neelsen technique.

Gilmour et al. (37) reported another experiment where 24 hamsters weighing about 65 g. were dosed orally once weekly for 4 weeks and received a total dose of 4×10^5 viable units of M. paratuberculosis. Cultural counts were made of the livers, spleens, mesenteric lymph nodes, and small and large intestines at 1 week and 1, 4, 6, and 8 months. The cultural examination results are summarized in Table 5. Histologic examinations were made of all the livers and small intestines. No

Table 4. Distribution of M. paratuberculosis after oral dosing as reported by Gilmour et al. (37)

Time after dosing	Hamster number	Spleen	Kidney	Lung	Liver	Mesenteric lymph node	Intestine	
							Small	Large
1 hour	1	---	---	C	---	C	3.8	4.1
	2	2.4	---	C	3.4	C	---	3.8
	3	---	---	C	---	C	---	4.2
6 hours	4	2.7	---	C	3.3	C	---	---
	5	---	---	C	---	---	---	4.3
	6	---	---	C	---	---	---	3.9
24 hours	7,8,9	---	---	---	---	---	---	---
1 week	10,11, 12,13	---	---	---	---	---	---	---
1 month	14,15, 16,18	---	---	---	---	---	---	---
	17	3.8	---	3.9	3.8	C	3.5	---
	19,30	---	---	---	---	---	---	---
4 months	22	4.5	---	C	4.1	C	5.9	---
	20	2.4	---	C	---	---	---	4.4
	21	---	---	C	---	4.5	---	4.8
	25	---	---	---	---	3.1	---	---
8 months	23	5.0	2.9	---	3.6*	5.9	4.9	---
	33	5.3	---	---	3.8	6.8+	4.8	3.7
9 months	34	5.7	---	3.4	4.3*	6.8+	4.3	5.0
11 months	28	0	0	0	0	0	3.5	4.1
	24	2.9	---	---	---	6.8	4.6	4.8
	26	2.7	---	---	---	6.8	3.9	3.5
	27	5.5	---	---	3.5	5.4	3.5	3.8
	29	5.3	2.4	---	3.5*	6.4*	3.8*	4.8
	35	5.4	2.4	3.0	5.4*	6.7	4.1	5.1

Counts expressed as $\log_{10}$ viable units per organ
C = Contaminated
0 = Not examined
* = Organ contained lesions
+ = Greater than
--- = Less than minimum detectable levels = $\log_{10}$ 2.4 viable units for the lymph nodes, spleens, livers, kidneys, and lungs, and $\log_{10}$ 3.5 for the intestines

Table 5. Distribution of M. paratuberculosis after 4 weekly doses as reported by Gilmour et al. (37)

Time after last dose	Hamster number	Liver	Spleen	Mesenteric lymph node	Intestine	
					Small	Large
1 week	1 - 6	---	---	---	---	---
1 month	7	---	---	2.4	4.9	---
	8 - 12	---	---	---	---	---
4 months	14, 16	---	---	---	---	---
	15	C	---	5.9	---	3.5
	17	---	---	3.4	---	---
	18	---	---	---	---	3.5
	19	2.4	3.5	C	3.5	---
6 months	22	---	---	0	---	---
8 months	23 - 25	---	---	---	---	---
	26	---	5.4	+6.8	6.1	3.8
	27	2.4	2.4	5.9	5.9	4.9

Counts expressed as $\log_{10}$ viable units per organ
C = Contaminated
\+ = Greater than
0 = Not examined
--- = Less than minimum detectable levels = $\log_{10}$ 2.4 viable units for the lymph nodes, livers, spleens, and $\log_{10}$ 3.5 for the intestines

specific lesions were detected in any of the histologic sections.

Avian tuberculin sensitivity tests were made on all hamsters in the above 2 experiments. There was no correlation between the development of tuberculin reaction and the viable unit counts. However, there was some indication of an association between sensitivity and finding of specific lesions. Lesions were found in 4 of 5 hamsters that reacted to the test immediately prior to necropsy. No lesions were found in 4 hamsters that did not react.

In contradistinction to findings in sheep and cattle, Brotherston and Gilmour (19) reported that in hamsters, infections of the livers and spleens were present and were frequent. They also attempted vaccination evaluation by utilization of the hamster as a test animal. They vaccinated 7 recently weaned hamsters with 0.05 ml. of Weybridge adjuvant vaccine containing approximately 0.17 mg. of heat-killed organisms. One month later, the vaccinated and 7 unvaccinated hamsters were challenged once weekly for 10 weeks with 10^6 viable units for a total of 10^7 viable units of M. paratuberculosis. The results are summarized in Table 6.

Brotherston and Gilmour considered the reduction in the level of infection in the small intestines of the vaccinated hamsters as significant. Also, the results were similar to that in sheep as invasion of the mesenteric lymph nodes was not prevented.

Table 6. Viable unit counts in vaccinated and unvaccinated hamsters 6 weeks after last challenged as reported by Brotherston and Gilmour (19)

	Hamster number	Liver	Spleen	Mesenteric lymph node	Intestine	
					Large	Small
Vaccinated	1	---	---	---		---
	2	---	3.2	4.2		---
	3	---	---	5.4		3.9
	4	---	---	6.0		---
	5	---	---	---		---
	6	---	---	---		---
	7	---	---	5.5		---
Controls	8	---	---	---		---
	9	---	2.4	5.7		6.1
	10	---	---	3.3		6.4
	11	---	2.7	4.3		6.6
	12	---	---	5.5		---
	13	---	2.7	4.7		4.3
	14	---	2.9	4.0		6.8

Counts expressed as $\log_{10}$ viable units.
--- = Less than minimum detectable levels = $\log_{10}$ 2.4 in mesenteric lymph node, liver, and spleen, and $\log_{10}$ 3.4 in intestines

Paratuberculosis in Other Laboratory Animals

Rabbits

Markus (64) in 1904, Bang (7) in 1906, Meyer (68) in 1913, and Datta (51) in 1938 reported that they were unable to establish infections in rabbits. Twort and Ingram (105) in 1913 inoculated a group of rabbits and obtained 1 case resembling paratuberculosis in cattle. This was 1 of a few reported cases in laboratory animals that resembled the disease of cattle. Twort (101, 102, 103) in 1912, 1913, and 1914 found lesions consisting chiefly of lymphoid cells in the intestine, liver, spleen, and thoracic and mesenteric lymph nodes in 5 of 23 rabbits inoculated with M. paratuberculosis. Intra- and extracellular organisms were found in all affected organs and were cultured from the intestine and the mesenteric lymph nodes. Similar results have been obtained by Andersen (5) in 1921, Boquet (13) in 1929, Mohler (71) in 1939, Sahai (88) in 1943, Francis (33, 34) in 1943, Alikaeva (3) in 1940, Rankin (84) in 1958, Hirch (47) in 1956, and Harding (45) in 1959. Taylor (99) in 1951 failed to demonstrate lesions but was able to culture organisms from 2 of 5 rabbits that had been inoculated with M. paratuberculosis. Twort and Craig (104) in 1913 demonstrated identical lesions in rabbits that had been inoculated intraperitoneally with either dead M. paratuberculosis or M. tuberculosis.

Guinea pigs

Markus (64) in 1904, Twort and Ingram (105) in 1913, Meyer (68) in 1913, Twort (102, 104) in 1914, Andersen (5) in 1922, Datta (51) in 1938,

Popp (83) in 1940, and Vallée et al. (107) in 1941 were not successful in establishing infections in guinea pigs that had been inoculated with either M. paratuberculosis or suspensions of infected tissue containing M. paratuberculosis.

Jöhne and Frothingham (50) in 1885 inoculated guinea pigs with intestinal scrapings from an affected cow. Although these guinea pigs did not die, they did demonstrate atypical lesions of tuberculosis and thus led both Johne and Frothingham, along with Koch, to believe the condition to be produced by an unusual type of avian tubercle bacillus. Bang (7) in 1906 also demonstrated acid-fast organisms in lesions in guinea pigs inoculated with suspensions of tissue containing the acid-fast bacilli described by Johne and Frothingham.

Boquet (13, 14, 15, 16, 17) in 1929 and 1930 inoculated guinea pigs orally, intratracheally, subcutaneously, intraperitoneally, and intravenously with variable quantities of culture of M. paratuberculosis. He recovered the organisms by culture from the blood, bile, spleen, liver, lung, and various lymph nodes. Hagan and Mansfield (43) in 1930, and Hagan and Levine (42) in 1932 observed epithelioid-cell proliferation in the lymphatic tissue and giant cell formation when they inoculated guinea pigs with living or killed cultures of M. paratuberculosis and different strains of non-pathogenic mycobacteria. In 1939, Mohler (71) observed what probably was the first recorded instance of a fetal transmission of M. paratuberculosis. This report was based on microscopic observation of liver smears from the offspring of a female guinea pig that had been inoculated intraperitoneally with a culture of

M. paratuberculosis 2 months prior to mating. Lesions have been noted in experimentally inoculated guinea pigs by Taylor (98, 99) in 1940 and 1951, Sahai (88, 89) in 1940 and 1941, Vallée et al. (107) in 1941, Francis (33, 34) in 1943, Konst and Watson (56) in 1943, Glover (38, 39) in 1941, Levi (58) in 1941, and Verlinde and Bekker (109) in 1945.

Mice

Twort and Ingram (105) in 1913 and Andersen (5) in 1921 have reported failures in attempting to infect mice with M. paratuberculosis.

Twort (102, 103, 104) in 1913 and 1914 demonstrated lesions and acid-fast organisms in intestinal lesions consisting of lymphoid cells, liver, spleen, and lymph nodes. Mycobacterium paratuberculosis was cultured from the intestine and lymph nodes. Similar results were also obtained in mice by Boquet (12) in 1925 who considered them to be less sensitive than rats, Francis (33, 34) in 1943, Popp (83) in 1940, Lominski et al. (60) in 1956, Harding (45) in 1959, Brotherston and Gilmour (36) in 1961, and Chandler (21, 23, 24, 25) in 1961.

Rats

Twort and Ingram (105) in 1913, Meyer (68) in 1913, and Andersen (5) in 1921 were not successful in establishing infections in rats that had been inoculated with either cultures of M. paratuberculosis or suspensions of infected tissue containing M. paratuberculosis. In 1914, Twort (102) reported that a few bacilli were found in the ileum in 2 and in the duodenum of 1 of 12 rats that had been inoculated intraperitoneally with cultures of M. paratuberculosis. Boquet (12) reported nodules on

the peritoneum and omentum that contained many acid-fast bacilli, hypertrophy of the lymph nodes, and organisms in the liver, spleen, and lymph nodes of rats that had been inoculated intraperitoneally with cultures of M. paratuberculosis. Chandler (22) in 1961 reported low grade infections in rats but stated that mice were of greater promise as experimental animals.

Voles

Levi (59) in 1950 reported producing granulomatous foci consisting of epithelioid cells and lymphocytes, in the intestine, mesenteric lymph nodes, liver, spleen, and less frequently, in the lungs of voles 6 weeks to 15 months following intraperitoneal inoculation with 2 to as little as 0.0001 mg. of M. paratuberculosis. In voles, Levi described Johne's disease as a chronic disease characterized by an absence of clinical symptoms.

Fowls

Markus (64) in 1904, Bang (7) in 1906, Twort and Ingram (105) in 1913, Meyer (68) in 1913, Andersen (5) in 1921, and Heelsbergen (46) in 1931 were not successful in establishing infection in chickens or pigeons that had been inoculated with M. paratuberculosis. Mohler (71) in 1939 also was unable to establish infection in chickens but did produce lesions containing acid-fast bacilli in pigeons that had been inoculated intraperitoneally with oil emulsions of both cultures and affected tissues from guinea pigs. In 1940, Sahai (88) described pinpoint nodules in the mesentery and liver of fowls that received

intraperitoneally 4 mg. of culture of M. paratuberculosis. Localized necrotic lesions in the breast muscle were observed in 1943 by Meyn and Weiske (69) in hens after intramuscular inoculation of M. paratuberculosis suspended in a mixture of Kieselguhr, trypan blue, potassium iodide, and saline.

Chick embryos

Stavitsky and Beck (97) in 1946 inoculated chick embryos with several typical strains and 1 atypical strain (grew without the phlei factor) of M. paratuberculosis. Both types caused proliferative lesions of the chorioallantoic membrane and fatty degeneration of the liver. The atypical strain multiplied on the chorioallantoic membrane but not within the embryo. The typical strains did not multiply anywhere on the chorioallantoic membrane or in the embryo.

Cats

In 1944, Johnson and Pratt (52) reported feeding pure cultures and infected bovine intestinal tissue containing M. paratuberculosis to 14 cats. Six cats developed sensitivity to johnin. Two of these developed diarrhea and were examined 4 weeks after inoculation. Gross lesions were suggestive of Johne's disease but the microscopic study was less convincing as only a few epithelioid cells containing acid-fast bacilli were found. Johne's disease was not observed in the other cats.

Monkeys

Five natural cases of paratuberculosis have been reported in the

rhesus monkey by Pitcock and Gisler (82) in 1961. All 5 instances were in animals 3 to 10 years of age. Four monkeys demonstrated a short interval of diarrhea prior to death. Multiple tuberculin tests were negative. All 5 were histologically verified as paratuberculosis but no attempt was made to culture the organisms. Masses of acid-fast bacilli were demonstrated within macrophages in the lamina propria of the jejunum, ileum, cecum, and colon.

METHODS OF PROCEDURE

Source and Care of Animals

Sixty healthy female Syrian golden hamsters with an average weight of 50 g. were acquired from a commercial source. The hamsters were randomly divided into 4 groups of 15 each. They were maintained in an animal room for a period of 10 days prior to inoculation to permit them to adapt to their new environment and recover from any possible stress of shipping. After inoculation, each hamster was placed in a separate cage to prevent cannibalism.

Four hamsters, 3 females and 1 male, were obtained later for electron microscopic studies.

The cage dimensions were 7 in. wide x 10 in. deep x 7 in. high. The front and bottom of the cages were wire cloth, 3-mesh 16 gauge and galvanized after weaving. The wire cloth was lock-seamed into 24-gauge galvanized sheet metal back and sides. The sides of the cage were double folded for added stiffness. The cages were so constructed as to offer no obstruction which would prevent excreta dropping into the pans resting on the shelf beneath. The cages[1] were arranged on glides in a stand 57 in. long, 24-1/4 in. wide, and 66 in. tall. The stand was mounted on 5 in. heavy duty hard rubber swivel casters and had a capacity of 60 cages (Figure 1).

---

[1]George H. Wahmann Manufacturing Company, Baltimore 2, Maryland.

The temperature and relative humidity were kept constantly at 70° F. and 45% respectively. Strict sanitation was maintained throughout the experiment to prevent the spread of organisms between cages.

The hamsters were given water and Purina Laboratory Chow[1] cubes free choice throughout the experiment. The approximate analysis of Purina Laboratory Chow is as follows:

Protein, %	23.40
Argine, %	1.15
Glycine, %	.77
Lysine, %	1.27
Methionine, %	.36
Tryptophan, %	.22
Cystine, %	.25
Histidine, %	.44
Leucine, %	1.40
Isoleucine, %	1.03
Phenylalanine, %	.82
Threonine, %	.78
Valine, %	1.02
Fat, %	3.78
Fiber, %	4.86
Ash, %	7.72
Nitrogen-Free Extract, %	50.58
Calcium, %	1.42
Phosphorus, %	.96
Potassium, %	.72
Magnesium, %	.23
Sodium, %	.46

[1]Ralston Purina Company, St. Louis, Missouri.

Chlorine, %	.52
Iron, ppm.	275.00
Zinc, ppm.	60.64
Manganese, ppm.	49.45
Copper, ppm.	18.20
Iodine, ppm.	1.18
Carotene, ppm.	5.28
Vitamin A, I.U./g.	12.00
Vitamin D, I.U./g.	5.31
Alpha-tocopherol, I.U./lb.	38.35
Thiamin, ppm.	16.55
Riboflavin, ppm.	8.30
Niacin, ppm.	98.34
Pantothenic Acid, ppm.	21.59
Choline, ppm.	1665.00
Folic Acid, ppm.	6.46
Pyridoxine, ppm.	5.00
Biotin, ppm.	.06
Vitamin B-12, mcg./lb.	15.89

Source of Inoculum

The M. paratuberculosis inoculum used in this study was obtained by tryptic digestion of the intestinal mucosa from an experimentally infected steer that developed clinical paratuberculosis. The steer had been infected intravenously with organisms that had never been cultured

in vitro. The organisms used to inoculate the steer had been obtained by the tryptic digestion of the intestinal mucosa from a naturally infected cow as described below.

The steer was electrocuted. The intestine was removed and the mucosa was washed with water before being removed by scraping. Approximately 4 liters of mucosa were obtained. Two liters of this were digested by mixing aliquots of mucosa with equal volumes of a 2.5% aqueous trypsin solution and chopping the host cells (57). The temperature of the digesting mixture was raised to 38° C. and it was stirred constantly. The pH was maintained between 7.5 to 8.0 during digestion by adding sodium hydroxide. Digestion required up to 30 min. After digestion, the suspension was filtered through cheesecloth and centrifuged for 30 min. at 3,000 r.p.m. in a centrifuge maintained at 10° C. The sediment was resuspended in water and again centrifuged. A sample of the final sediment was stained by the Ziehl-Neelsen method and apparently contained only *M. paratuberculosis*.

The total sediment was placed between stacked filter paper and blotted under a 1 kg. weight for 2 hr. A sample of the blotted material was weighed and dried in an oven for 3 hr. at 100° C. and again weighed. This was repeated until constant weight was obtained. The ratio of blotted to oven-dried weight was used to calculate the amount of blotted material to be resuspended for the inoculum. The inoculum was suspended in 1% sodium hydroxide for 30 min. and neutralized with 0.25 N hydrochloric acid. The neutralized mixture was centrifuged and resulting sediment was resuspended in physiological saline until a

concentration was reached in which 1 ml. contained 1 mg. of dried M. paratuberculosis. The aliquot was divided into 2 parts, and 1 part was heat-killed by placing in an autoclave with flowing steam (100° C.) for 2 hr. This material was refrigerated overnight and the hamsters were inoculated the following day.

Inoculation of Hamsters for Study by Light Microscopy

The 60 female Syrian golden hamsters were randomly divided into 4 groups and inoculated as follows with the suspension of M. paratuberculosis:

1. One mg. of living organisms was administered intragastrically via celiotomy incision to 15 hamsters (Table 7).
2. One mg. of heat-killed organisms was administered intragastrically via celiotomy incision to 15 hamsters (Table 8).
3. One mg. of living organisms was administered intraperitoneally by injection to 15 hamsters (Table 9).
4. One mg. of heat-killed organisms was administered intraperitoneally by injection to 15 hamsters (Table 10).

The hamsters inoculated by the intragastric route were anesthetized with ether and secured to an Animal Surgical Apparatus.[1] A midline incision was made into the peritoneal cavity. The stomach was lifted from beneath the edge of the liver with a spay hook and then the suspension of organisms was injected by a 25-gauge needle through

[1]Brookline Surgical Specialties, Brookline 46, Massachusetts.

the thick muscular wall of the greater curvature of the stomach as illustrated in Figure 2.

The intraperitoneal injection was made through the flank and particular attention was taken to avoid injection into either the urinary bladder or the intestinal tract

Necropsy Procedure of Hamsters for Study by Light Microscopy

One hamster from each group was killed by overdosing with ether and thorough necropsies were performed at 2, 4, 8, 14, 28, 56, 98, 154, 210, 266, and 329 days following inoculation. Any hamster from the above respective groups exhibiting illness was selected for examination at the next time interval. Otherwise, a hamster was selected at random from each group.

All antemortem signs and gross postmortem lesions were described for each hamster. The following organs or tissue specimens were selected for histologic examination using the terminology of Michel (70), Brosseau (18), and Trautmann and Fiebiger (100).

Lymphatic system

Both prefemoral (subiliac) lymph nodes

Both axillary lymph nodes

Both mandibular (submental) lymph nodes

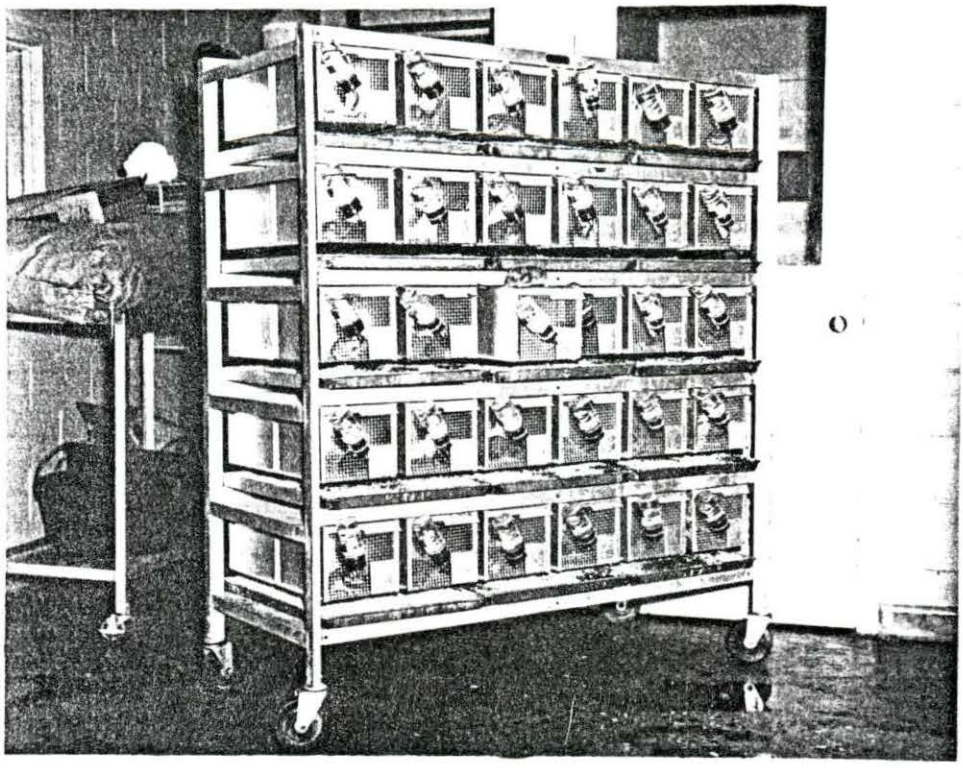
Both parotid lymph nodes

Anterior mediastinal lymph nodes

Anterior mesenteric lymph node

Figure 1. A 60-mouse cage unit with wire mesh fronts and bottoms used for keeping hamsters during this experiment.

Figure 2. Surgical approach used when inoculating hamsters intragastrically with M. paratuberculosis.



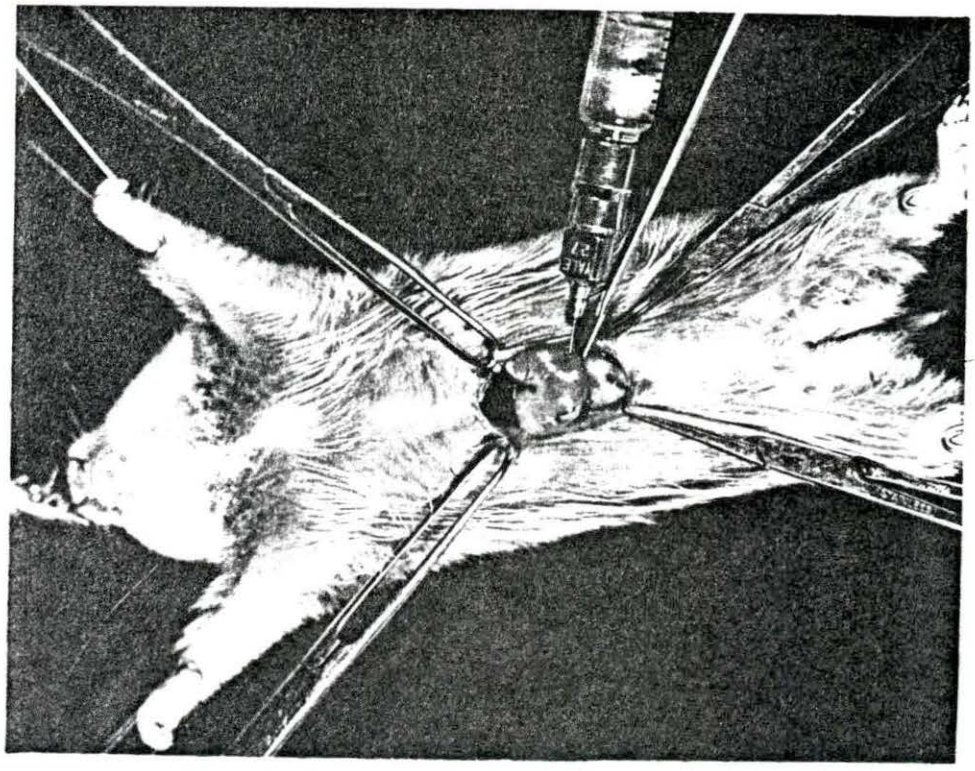


Table 7. Hamsters inoculated intragastrically with 1 mg. of living M. paratuberculosis

Hamster number	Date inoculated	Date examined	Duration following inoculation (days)
88	4-23-63	4-25-63	2
89	"	4-27-63	4
90	"	4-30-63	8
91	"	5-7-63	14
92	"	5-22-63	28
93	"	6-16-63	56
94	"	6-18-63	56
95	"	7-22-63	98
96	"	9-23-63	154
98	"	10-4-63	154
99	"	11-20-63	210
100	"	1-22-64	266
101	"	3-23-63	329
102	"	3-23-63	329
103	"	3-23-63	329

Table 8. Hamsters inoculated intragastrically with 1 mg. of heat killed M. paratuberculosis

Hamster number[a]	Date inoculated	Date examined	Duration following inoculation (days)
120	4-24-63	4-26-63	2
121	"	4-28-63	4
122	"	5-1-63	8
123	"	5-8-63	14
124	"	5-22-63	28
125	"	6-18-63	56
126	"	7-29-63	98
127	"	9-24-63	154
130	"	11-21-63	210
131	"	1-22-64	266
132	"	3-24-64	329
133	"	3-24-64	329
134	"	3-24-64	329
135	"	3-24-64	329

[a]One hamster died of ileitis early in the experiment and was not included in the results.

Table 9. Hamsters inoculated intraperitoneally with 1 mg. of living M. paratuberculosis

Hamster number[a]	Date inoculated	Date examined	Duration following inoculation (days)
104	4-23-63	4-25-63	2
105	"	4-27-63	4
106	"	4-30-63	8
107	"	5-7-63	14
108	"	5-22-63	28
109	"	6-18-63	56
110	"	7-29-63	98
111	"	9-23-63	154
112	"	11-20-63	210
113	"	1-10-64	266
114	"	3-23-64	329

[a]Three hamsters died of ileitis early in the experiment and were not included in the results. One hamster was killed accidentally and not included in results.

Table 10. Hamsters inoculated intraperitoneally with 1 mg. of heat killed M. paratuberculosis

Hamster number[a]	Date inoculated	Date examined	Duration following inoculation (days)
136	4-24-63	4-26-63	2
137	"	4-28-63	4
138	"	5-1-63	8
139	"	5-8-63	14
140	"	5-20-63	28
141	"	6-20-63	56
142	"	7-30-63	98
143	"	9-24-63	154
144	"	11-21-63	210
145	"	1-24-64	266
146	"	3-24-64	329
147	"	3-24-64	329
148	"	3-24-64	329

[a]Two hamsters died of ileitis early in the experiment and were not included in the results.

Ileocecal or mesenteric lymph nodes other than the anterior mesenteric lymph node

Both deep inguinal (hypogastric sacral) lymph nodes

Both medial iliac lymph nodes

Spleen

Thymus

Base of the tongue (tonsil) including the hyoid bone

Alimentary system

Both buccal pouches

Both mandibular salivary glands

Both parotid salivary glands

Forestomach

Stomach

Three segments of the duodenum

Peyer's patches including its respective segment of the jejunum or ileum

Cecum

Segments of the spiral colon

Segments of the terminal colon

Three transverse samples of the liver, one of which contained the gall bladder

Pancreas

Omentum

Cardiovascular system

Bone marrow of the sternebrae and lumbar vertebrae

Nervous system

The brain was divided on a midsaggital plane and a saggital sample was taken from each half including the cerebral cortex, cerebellar cortex, thalamus, and medulla oblongata

Lumbar spinal cord

Respiratory system

Both lungs including the trachea and the arch of the aorta

Urogenital system

Both kidneys

Urinary bladder

Uterus with the attached ovaries

Skeletal system

Lumbar vertebra

Hyoid bone

Sternum

Special sense organs

Both eyes

Both exorbital lacrimal glands

Both infraorbital lacrimal glands

Processing of Tissue for Study by Light Microscopy

All tissue specimens, except the kidneys and heart which were divided on the median plane, were submerged as whole organs in 10% formalin for a minimum of 24 hr. at 70° F. Following fixation, specimens containing bone were demineralized by placing in 30% formic

acid for 3 to 6 days, then washed overnight in running tap water. All fixed tissues were dehydrated in graduated concentrations of ethyl alcohol, cleared in Clearing Agent[1] and infiltrated with Paraplast[2] in the routine manner in an Autotechnicon. The tissues were embedded in Paraplast and 2 sections, 8 μ thick, were cut from each block and mounted on glass slides for staining. One tissue section from each block, stained by a modified warm Ziehl-Neelsen method, was thoroughly examined for the distribution of M. paratuberculosis. When any significant tissue alterations were found, the other tissue section was stained with hematoxylin and eosin or a comparable stain for the study of the cellular response to M. paratuberculosis.

Inoculation of Hamsters for Electron Microscopic Studies

Two groups of hamsters were used for tissue studies by electron microscopy and were inoculated as follows:

1. Three 40 to 60 g. female golden Syrian hamsters were inoculated intraperitoneally with 30 mg. of M. paratuberculosis.
2. One 40 to 60 g. male golden Syrian hamster was inoculated intraperitoneally with 30 mg. of M. paratuberculosis. The same dosage was repeated via the same route 9 weeks later for a total dose of 60 mg.

---

[1]The Technicon Company, Chauncey, New York.

[2]Aloe Scientific, St. Louis, Missouri.

The organisms of M. paratuberculosis were obtained at the same time and from the same source as that used to inoculate hamsters for light microscopic studies. The only difference was that the organisms used in the electron microscopic studies had been kept at -60° C. for 27 months for the first group and 39 to 41 months for the second group.

Necropsy Procedures of Hamsters for Tissue Studies by Electron Microscopy

The hamster was anesthetized with ether and then secured to an Animal Surgical Apparatus. The anterior mediastinal or anterior mesenteric lymph nodes were surgically removed via a laporatomy procedure, after which the hamster was euthanatized by overdosing with ether.

One hamster each was examined at intervals following inoculation as illustrated in Table 11.

Fixation and Embedment of Tissues for Study by Electron Microscopy

About 30 mg. of the frozen M. paratuberculosis were thawed and fixed in cold 1% osmium tetroxide with Palade's Veronal-acetate buffer. The cells were then rinsed in the buffer and pelleted in a centrifuge tube of warm agar according to the method of Cheville.[1] The bacterial cells,

---

[1]Cheville, N. F., Ames, Iowa. Data on the embedment of tissue culture cells for study by electron microscopy. Personal communication. 1966.

Table 11. Hamsters inoculated intraperitoneally with living *M. paratuberculosis* and used for tissue examination by electron microscopy

Group number	Date inoculated: First inoculation with 30 mg.	Date inoculated: Second inoculation with 30 mg.	Date examined	Duration following inoculation: First inoculation	Duration following inoculation: Second inoculation
1	7-28-65	ND	9-10-65	42 days	NA
1	7-28-65	NA	10-28-65	90 days	NA
1	7-28-65	ND	1-20-65	168 days	NA
2	7-21-66	9-29-66	2-1-67	175 days	98 days

ND = Not done
NA = Not applicable

embedded in agar, were cut into 1 mm.^{3} blocks, and treated as tissues that had been post-fixed in osmium tetroxide.

Immediately upon surgical removal, the lymph nodes were cut approximately into 1 mm.^{3} blocks while immersed in cold 4% glutaraldehyde in a 0.2 M sodium cacodylate buffer. This was followed, with the exception of those tissues used for acid phosphatase studies, by post-fixation for 1 hr. in cold 1% osmium tetroxide buffered in 0.2 M sodium cacodylate.

Tissues from the hamster of group 2 that were examined at 175 days after the first or 98 days following the second inoculation were utilized for acid phosphatase studies. After the fixation in glutaraldehyde, the 1 mm.^{3} blocks were sliced as thin as possible with a razor blade and incubated 30 min. in Gomori's medium (41). The tissues were given a 2% acetic acid wash and a buffer wash (0.2 M sucrose in 0.1 M sodium cacodylate) followed by post-fixation for 1 hr. in cold 1% osmium tetroxide buffered in 0.2 M sodium cacodylate.

Following dehydration, all tissue blocks were embedded in Epon 812 according to the method of Luft (61). Thin sections were cut in the LKB 4802A ultramicrotome[1] with a diamond knife[2] and picked up on uncoated 200-mesh copper grids.[3] The sections were stained with a modified

---

[1]LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, Maryland.

[2]E. I. duPont de Nemours and Company, Instrument Products Division, Wilmington, Delaware.

[3]Polysciences, Inc., Rydal, Pennsylvania.

Reynolds' lead citrate stain according to the method of Venable and Coggeshall (108). Sections were examined with a Philips EM-200 electron microscope[1] at 60 kv.

---

[1]Philips Electronic Instruments, 750 South Fulton Avenue, Mount Vernon, New York.

FINDINGS

Classification of Lesions

For simplicity, lesions were classified as 1-plus (+), 2-plus (++), 3-plus (+++), and regressing (°). See Tables 12, 14, 16, 18, and 20.

One-plus lesions (Figure 3) were characterized by the presence of isolated reticuloendothelial cells containing acid-fast bacilli.

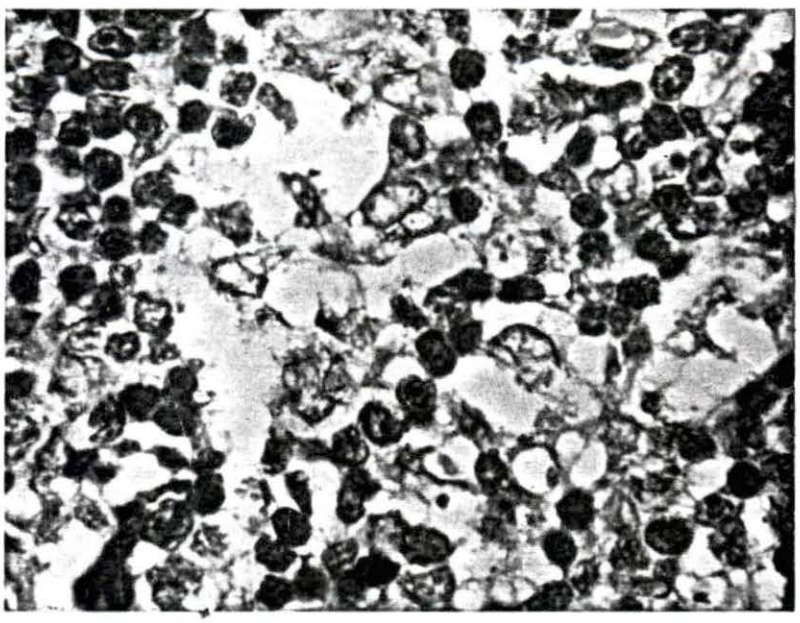
Two-plus lesions (Figure 4) were characterized by the presence of acid-fast bacilli within the cytoplasm of reticuloendothelial cells that were adjacent to each other and formed small foci of reticuloendothelial tissue hyperplasia that occupied less than 3% of the lymphoid tissue contained in the sections that were examined.

Three-plus lesions (Figures 5 and 6) were characterized by the presence of acid-fast bacilli within the cytoplasm of reticuloendothelial cells that were aggregated into foci of reticuloendothelial tissue hyperplasia that occupied more than 3% of the lymphoid tissue contained in the sections that were examined. In addition, Schaumann's bodies and/or foreign body giant cells were often present.

Old regressing lesions were characterized by indistinct foci of reticuloendothelial cells that demonstrated no acid-fast bacilli. Usually there was an infiltration of lymphocytes and plasma cells into the area of hyperplasia. Degenerating Schaumann's bodies or foreign body giant cells were often present. Most of the reticuloendothelial cells in these lesions contained large amounts of hemosiderin.

Figurs 3. One-plus (+) lesion. Acid-fast bacilli within isolated reticuloendothelial cells in the anterior mesenteric lymph node at 98 days after intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x1250.

Figure 4. Two-plus (++) lesion. Acid-fast bacilli within reticuloendothelial cells in a small indistinct focus of reticuloendothelial tissue hyperplasia in the anterior mesenteric lymph node at 98 days following intragastric inoculation with living M. paratuberculosis. Compare with Figure 3. Ziehl-Neelsen method. x1250.



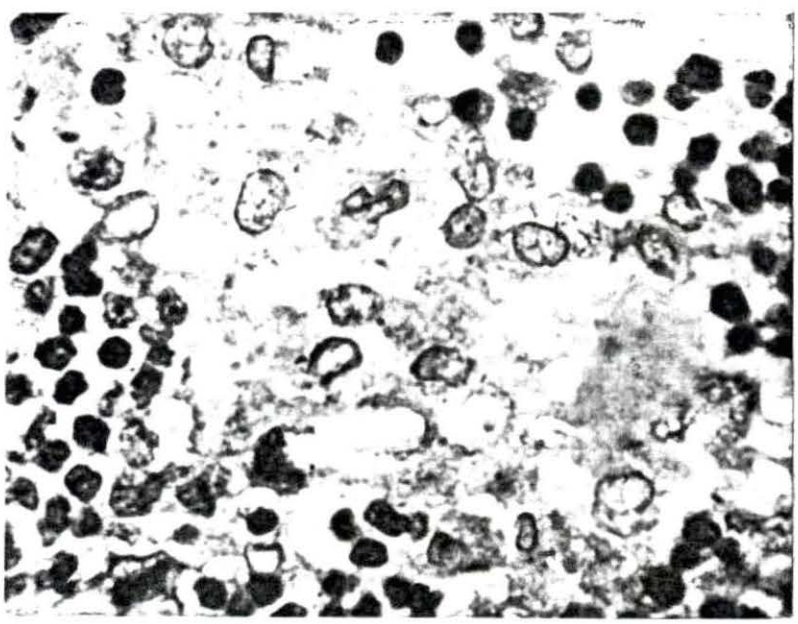
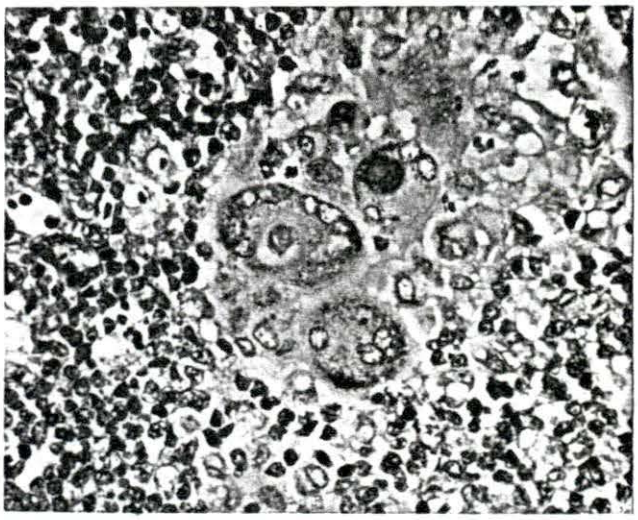
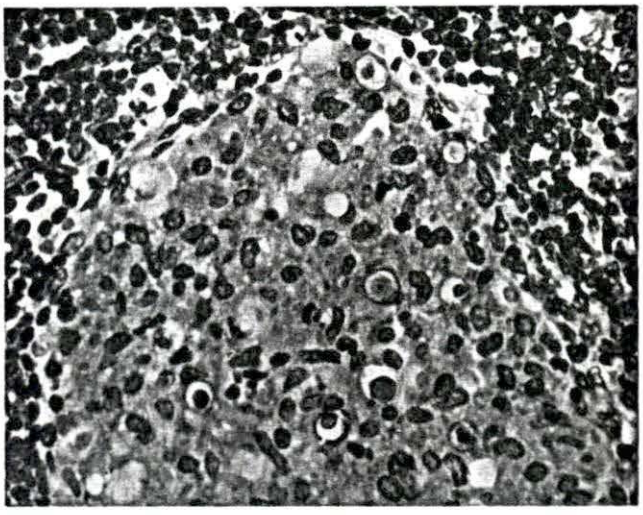


Figure 5. Three-plus (+++) lesion. Intracytoplasmic acid-fast bacilli and multinucleated foreign body giant cells in a focus of reticuloendothelial tissue hyperplasia in the anterior mesenteric lymph node at 98 days following intragastric inoculation with living M. paratuberculosis. Compare with Figures 3 and 4. x500.

Figure 6. Three-plus lesion in an axillary lymph node at 154 days following intragastric inoculation with living M. paratuberculosis. Note the small concentric laminated bodies (Schaumann's bodies) in the area of reticuloendothelial tissue hyperplasia and the distinct boundary between the normal lymphoid tissue and the area of hyperplasia. Harris' hematoxylin and eosin stain. x500.





Figures 57 and 58 illustrate regressing lesions in the anterior mesenteric lymph node from a hamster examined 329 days following inoculation with heat-killed M. paratuberculosis.

Living Organisms Administered Intragastrically

Clinical findings

The site of the celiotomy incision had healed. The hair coat was normal and there was no discharge from the eyes or any of the body orifices until 28 days PI. At approximately 28 days, a diarrhea, presumedly due to ileitis, that persisted 5 to 10 days, was observed in several hamsters. Three of these animals had a reoccurrence of the diarrhea at a later date (56, 56, and 98 days). No further diarrhea was observed in the group throughout the remaining portion of the experiment. No clinical signs of paratuberculosis were observed in any of the hamsters included in this group. The diarrhea that was observed was attributed to ileitis.

Necropsy findings

Lymphatic system Macroscopically, no enlargement of the lymph nodes was observed until 56 days. At 56 days, the regional lymph nodes (prefemoral, axillary, mandibular, and parotid) were slightly enlarged and the visceral lymph nodes (anterior mediastinal, anterior mesenteric, ileocecal, deep inguinal, and medial iliac) were moderately to markedly enlarged (1 to 2 times larger than normal). This degree of enlargement was consistently about the same at 98 and 154 days as that observed at

56 days. At 210, 266, and 329 days following inoculation, both the regional and mesenteric lymph nodes were 2 to 4 times larger than normal.

The thymus was normal in size and shape throughout the entire experiment. Involution was first noted at 154 days or when the hamster was about 6 months old.

No gross alterations were observed in the spleen or in the tissues adjacent to or in the root of the tongue.

Microscopically (Table 12), acid-fast bacilli were first observed in the anterior mesenteric and ileocecal lymph nodes (Figure 7) at 14 days and in the iliocecal lymph nodes (Figure 8) at 28 days following inoculation. At 56 days, organisms were present in the anterior mediastinal and mandibular lymph nodes, and acid-fast bacilli as well as reticuloendothelial tissue hyperplasia were present in the ileocecal and axillary lymph nodes (Figure 9). At 98 days, acid-fast bacilli were observed in all lymph nodes except the deep inguinal lymph nodes. Reticuloendothelial tissue hyperplasia was present in all lymph nodes examined except the deep inguinal and prefemoral lymph nodes (Figures 10 and 11). Acid-fast bacilli and extensive reticuloendothelial hyperplasia were observed in all of the regional and visceral lymph nodes examined at 154 (Figures 12, 13, 14, 15, 16, 17, and 18), 210 (Figures 19 and 20), 266 (Figures 21 and 22), and 329 days (Figure 33) following inoculation. The reticuloendothelial tissue hyperplasia involved as much as 30% of some of the sections examined (Figures 12, 13, 16, 19, 20, 21, 22, and 23). Schaumann's bodies were first observed as

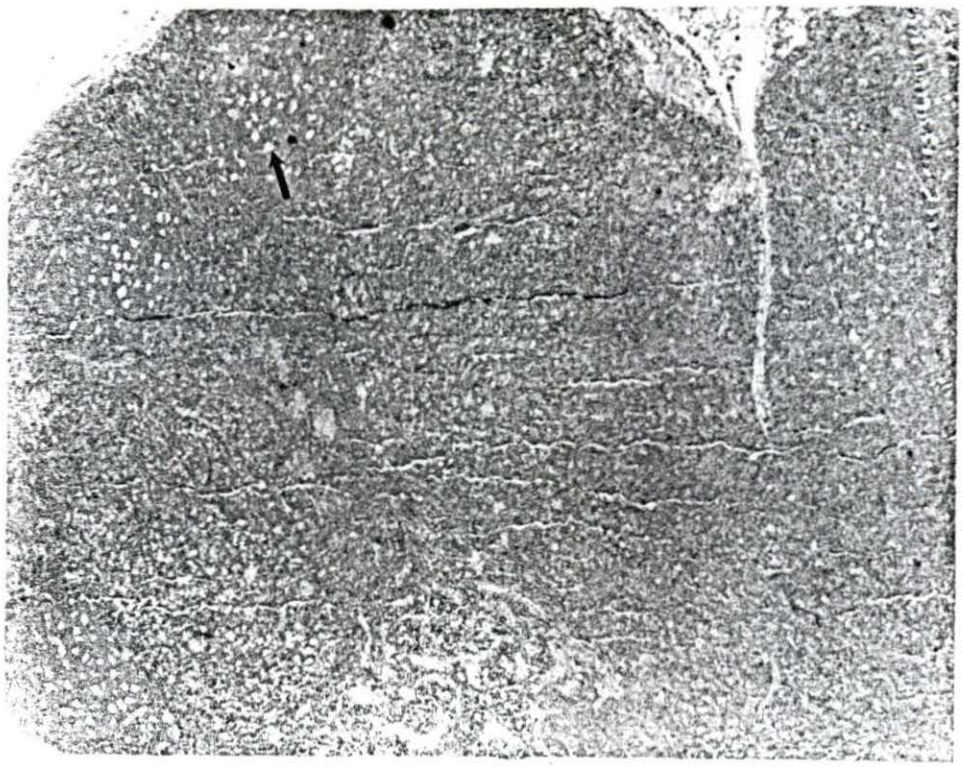
Table 12. Distribution of organisms and lesions in hamsters inoculated intragastrically with 1 mg. of living M. paratuberculosis

Organ	Time (days)										
	2	4	8	14	28	56	98	154	210	266	329
Ant. mediastinal lymph nodes	-	-	-	-	-	+	++	+++	+++	+++	+++
Ant. mesenteric lymph node	-	-	-	+	-	-	+++	+++	+++	+++	+++
Mesenteric lymph nodes, other	-	-	-	+	+	++	++	+++	+++	+++	+++
Deep inguinal lymph nodes	-	-	-	-	-	-	-	+++	+++	+++	+++
Medial iliac lymph nodes	-	-	-	-	-	-	++	+++	+++	+++	+++
Prefemoral lymph nodes	-	-	-	-	-	-	+	+++	+++	+++	+++
Axillary lymph nodes	-	-	-	-	-	++	++	+++	+++	+++	+++
Mandibular lymph nodes	-	-	-	-	-	+	++	+++	+++	+++	+++
Parotid lymph nodes	-	-	-	-	-	-	++	+++	+++	+++	+++
Spleen	-	-	-	-	-	+	++	+++	+++	+++	+++
Thymus	-	-	-	-	-	-	++	+++	+++	+++	++
Base of tongue	-	-	-	-	-	-	-	-	-	-	-
Buccal pouches	-	-	-	-	-	-	-	-	-	-	-
Mandibular salivary glands	-	-	-	-	-	-	-	-	-	-	-
Parotid salivary glands	-	-	-	-	-	-	-	-	-	-	-
Fore stomach	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-
Duodenum (lymph nodules)	+	-	-	-	-	-	-	-	++	-	-
Peyer's patches	-	-	-	+	-	+	++	+++	+++	+++	+++
Cecum (lymph nodules)	-	-	-	-	-	-	+	++	-	+++	+++
Spiral colon (lymph nodules)	-	-	-	-	-	-	-	++	++	-	++
Terminal colon (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Liver	-	-	-	++	++	-	-	+++	++	++	+++
Pancreas	-	-	-	-	-	-	-	-	-	-	-
Omentum	-	-	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-	-
Sternal bone marrow	-	-	-	-	-	-	-	++	-	-	-
Vertebral bone marrow (lumbar)	-	-	-	-	-	-	-	++	-	-	-
Brain	-	-	-	-	-	-	-	-	-	-	-
Spinal cord (lumbar)	-	-	-	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	+	-	-	++
Kidneys	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	-	-	-	-
Eye	-	-	-	-	-	-	-	-	-	-	-
Infraorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-
Exorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-

- = No organisms or tissue reaction
+ = Organisms within individually located macrophages
++ = Tissue reactions occupying less than 3% of the normal tissue
+++ = Tissue reactions occupying more than 3% of the normal tissue

Figure 7. Ileocecal lymph node at 14 days following intragastric inoculation with living M. paratuberculosis. Note the absence of germinal centers and the presence of prominent reticular cells (arrow). Ziehl-Neelsen method. x60.

Figure 8. Ileocecal lymph node at 28 days following intragastric inoculation with living M. paratuberculosis. Note the lack of demonstrable reticuloendothelial tissue hyperplasia and the similarity of the tissue when compared with Figure 7. Ziehl-Neelsen method. x60.



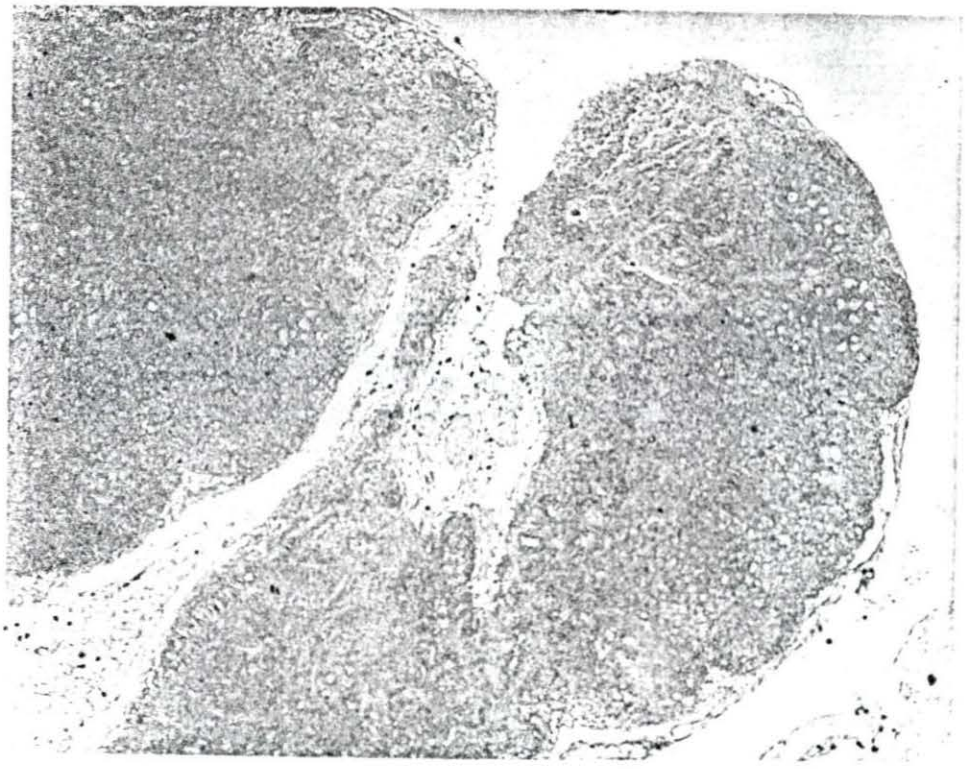
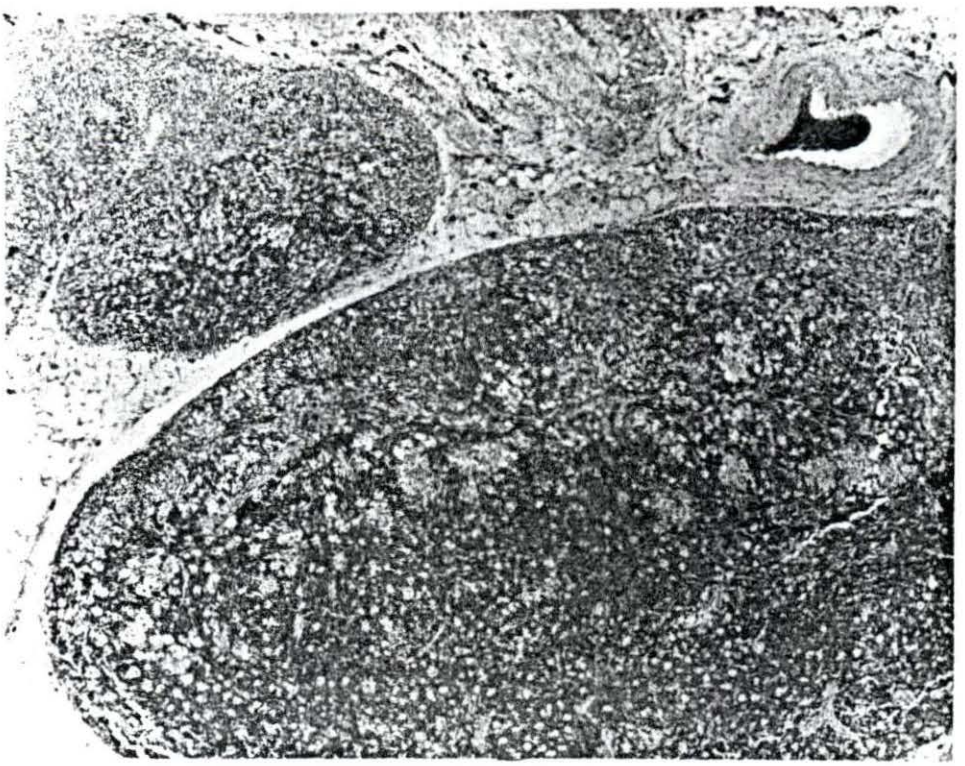


Figure 9. Ileocecal lymph node at 56 days following intragastric inoculation with living M. paratuberculosis. Two-plus lesions of reticuloendothelial tissue hyperplasia (arrows). Note that the small areas of hyperplasia appear around the prominent reticular cells. Ziehl-Neelsen method. x60.

Figure 10. Anterior mesenteric lymph node at 98 days after intragastric inoculation with living M. paratuberculosis. Note the increase in the number and the distinctiveness of the foci of reticuloendothelial tissue hyperplasia. Ziehl-Neelsen method. x60.



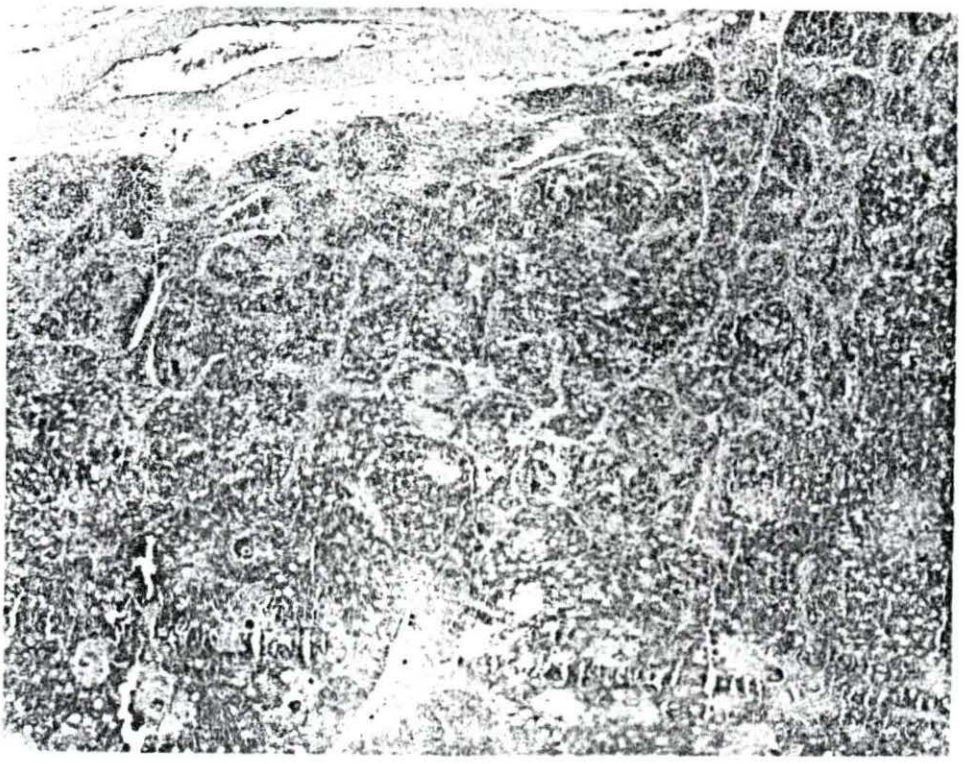
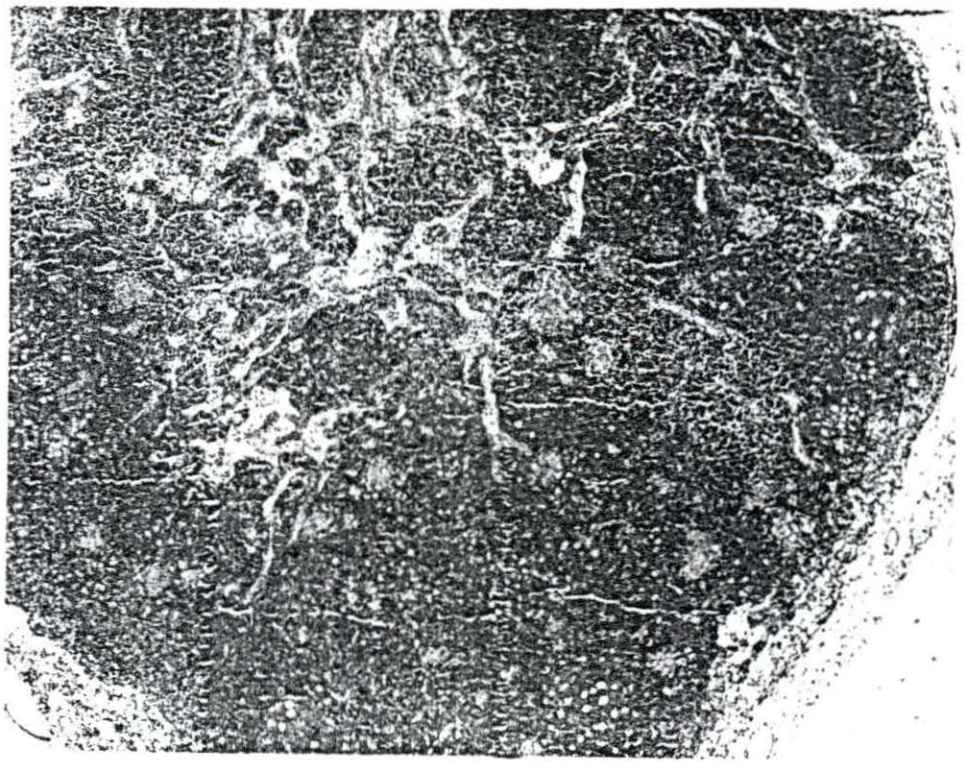


Figure 11. Ileocecal lymph node at 98 days following intragastric inoculation with living M. paratuberculosis. Two-plus lesions are present. Ziehl-Neelsen method. x60.

Figure 12. Anterior mesenteric lymph node at 154 days following intragastric inoculation with living M. paratuberculosis. Two portions of the pancreas and the anterior mesenteric artery are included in this section. Note the Schaumann's bodies in the areas of reticuloendothelial tissue hyperplasia. Ziehl-Neelsen method. x60.



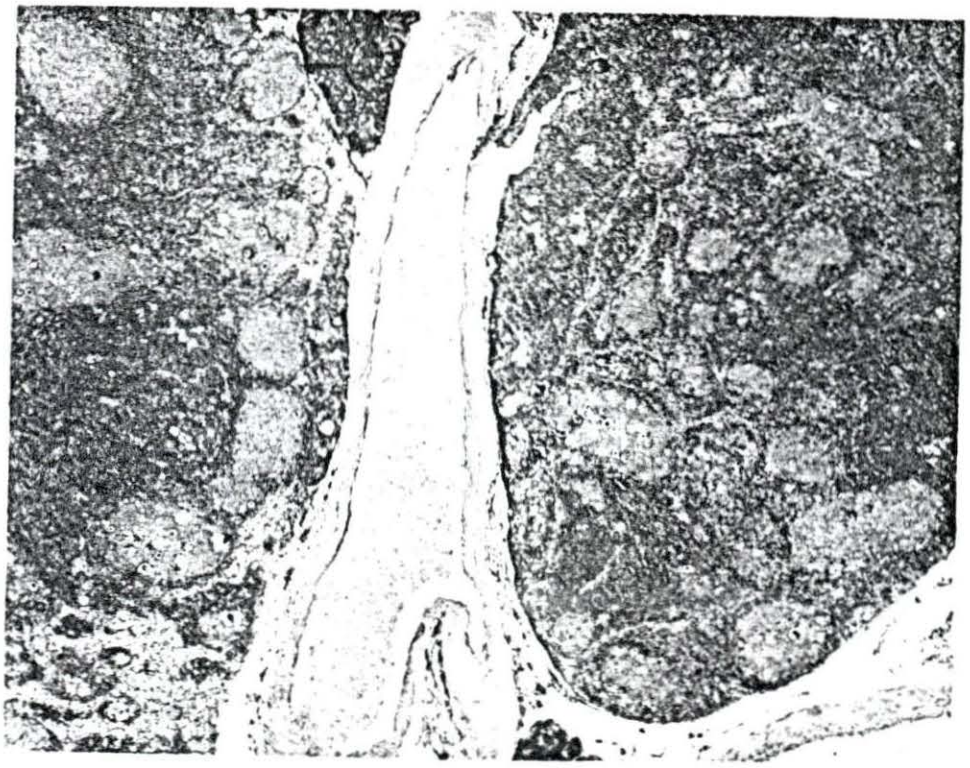
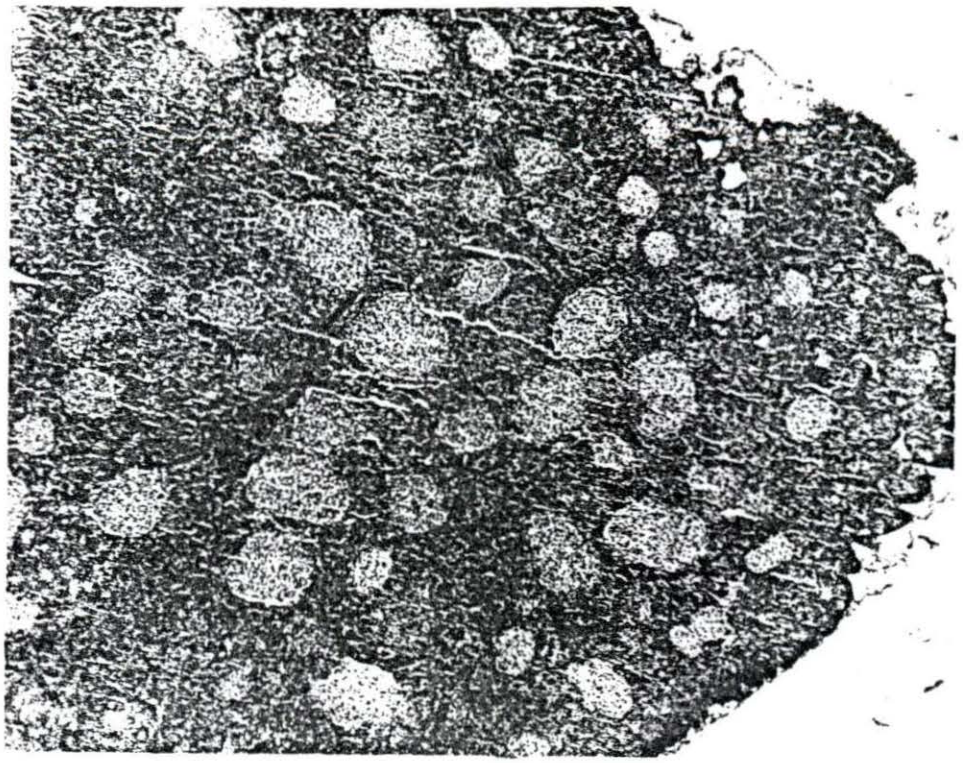


Figure 13. Extensive reticuloendothelial tissue hyperplasia in the mandibular lymph node at 154 days after intragastric inoculation with living M. paratuberculosis. Harris' hematoxylin and eosin stain. x60.

Figure 14. Higher magnification of 1 of the foci of reticuloendothelial tissue hyperplasia of Figure 13. Note the density of the reticuloendothelial cell cytoplasm, the small Schaumann's bodies surrounded by an artifactitious halo, the remarkable absence of lymphocytes in the area of hyperplasia, and the distinct border between the hyperplastic tissue and the normal lymphoid tissue. Harris' hematoxylin and eosin stain. x600.



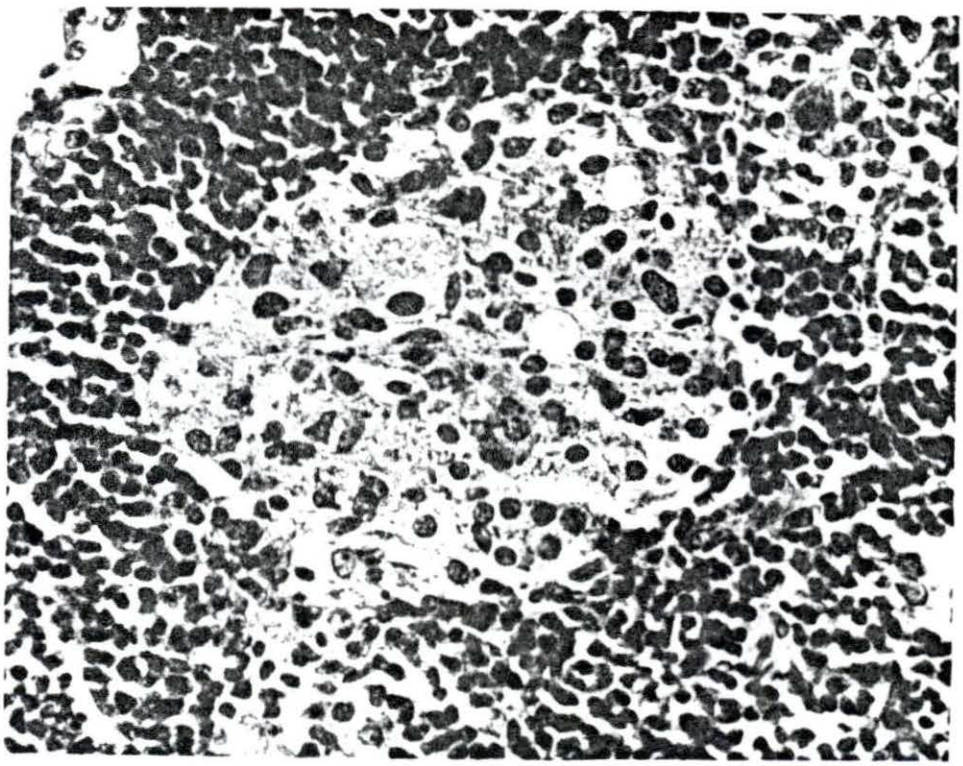
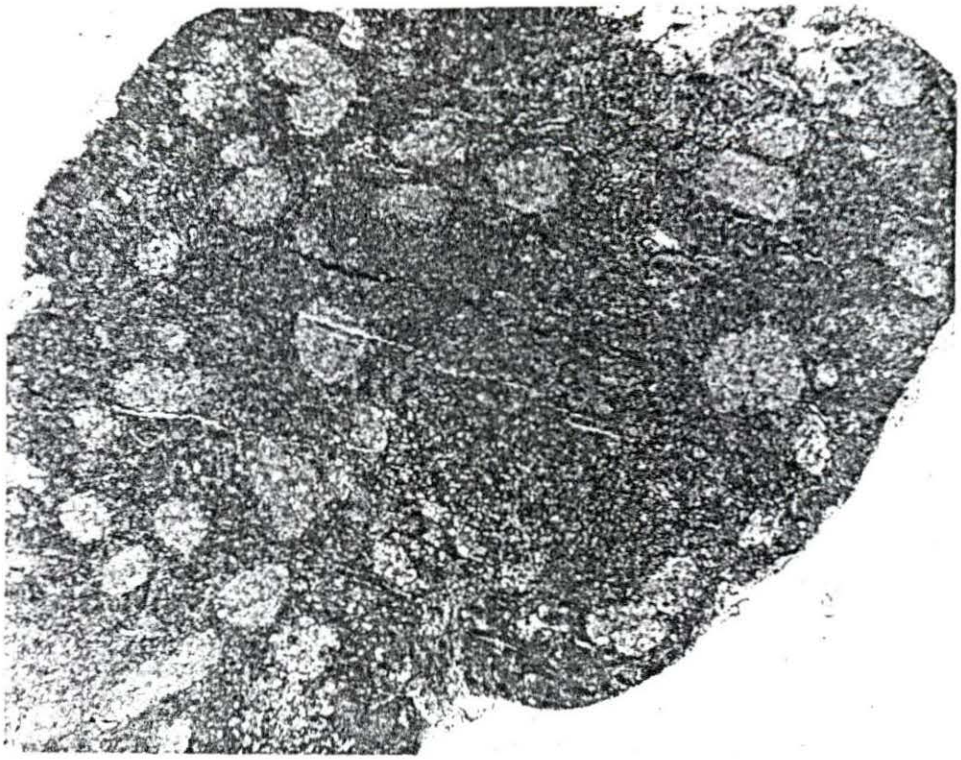


Figure 15. Section illustrating the extensive reticuloendothelial tissue hyperplasia in a prefemoral lymph node at 154 days following intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.

Figure 16. Multifocal reticuloendothelial tissue hyperplasia in an axillary lymph node at 154 days following intragastric inoculation with living M. paratuberculosis. See Figure 6 for a higher magnification of 1 of the foci of hyperplasia from this same lymph node. Ziehl-Neelsen method. x60.



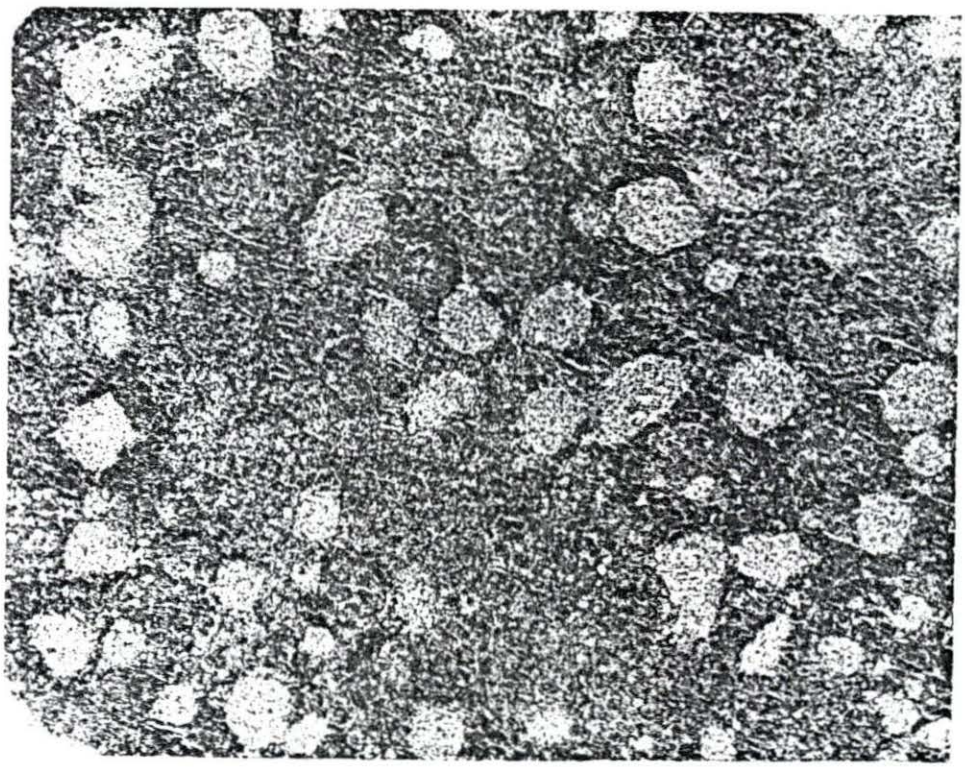
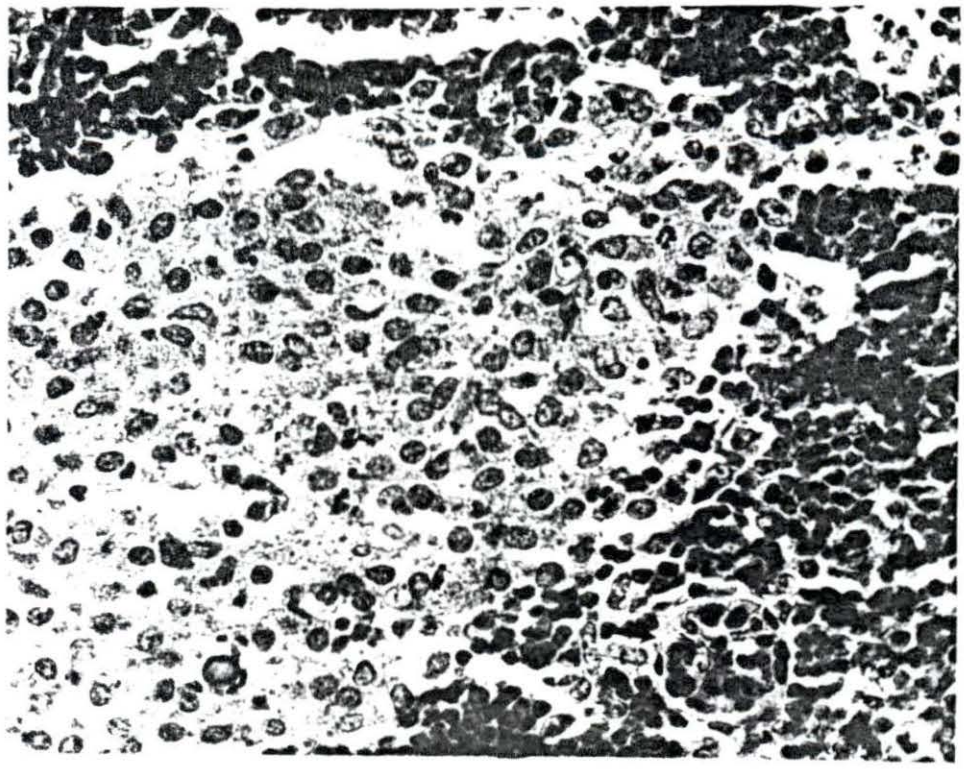


Figure 17. Reticuloendothelial tissue hyperplasia in the medial iliac lymph node at 154 days following intragastric inoculation with living M. paratuberculosis. Note the lack of lymphocytes in the area of reticuloendothelial tissue hyperplasia and the post-capillary venule (arrow) in the adjacent normal lymphoid tissue. Harris' hematoxylin and eosin stain. x600.

Figure 18. Higher magnification of the post-capillary venule in Figure 17. Note the distinct border between the normal lymphoid tissue and the reticuloendothelial tissue hyperplasia. x1050.



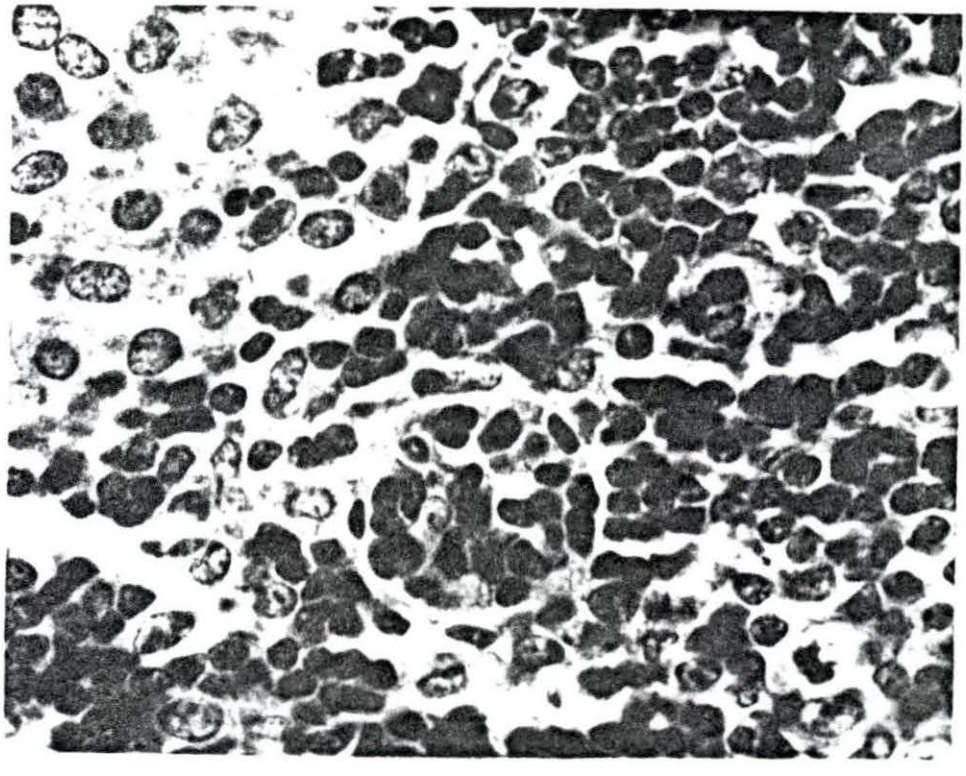
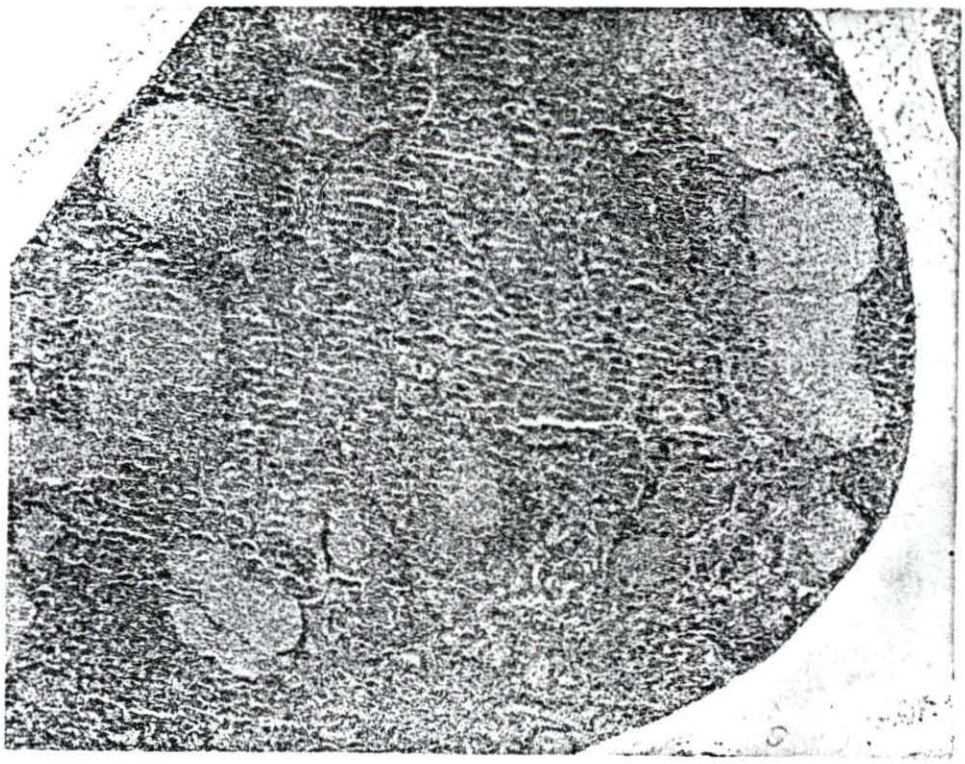


Figure 19. Extensive reticuloendothelial tissue hyperplasia of the anterior mesenteric lymph node at 210 days following intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.

Figure 20. Multifocal reticuloendothelial tissue hyperplasia in the ileocecal lymph node at 210 days following intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.



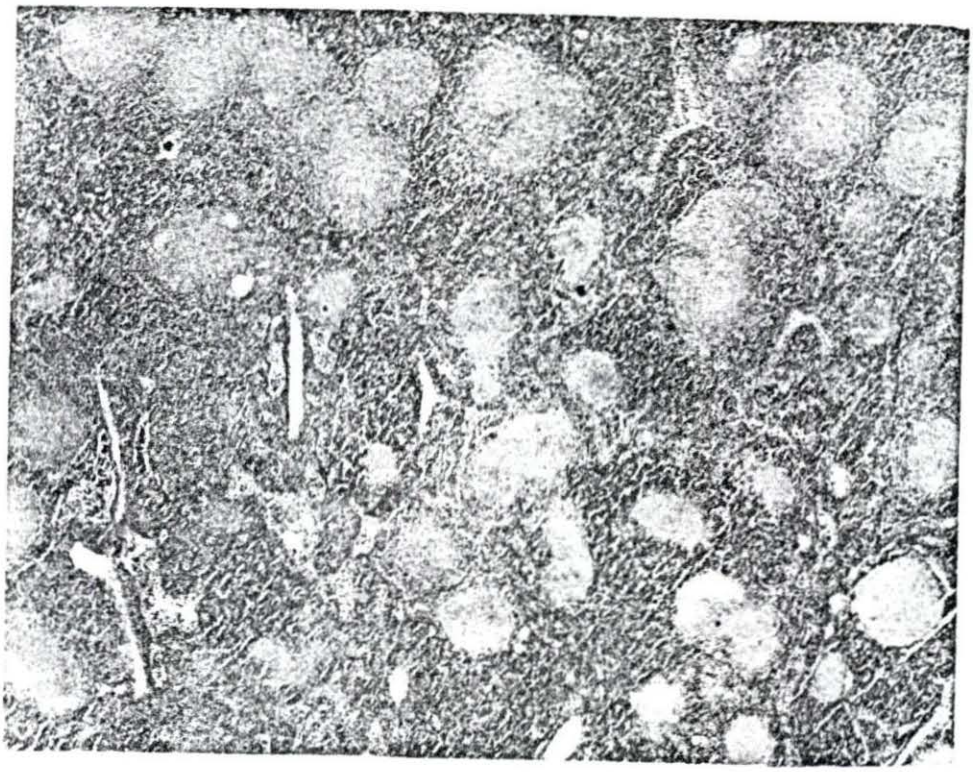
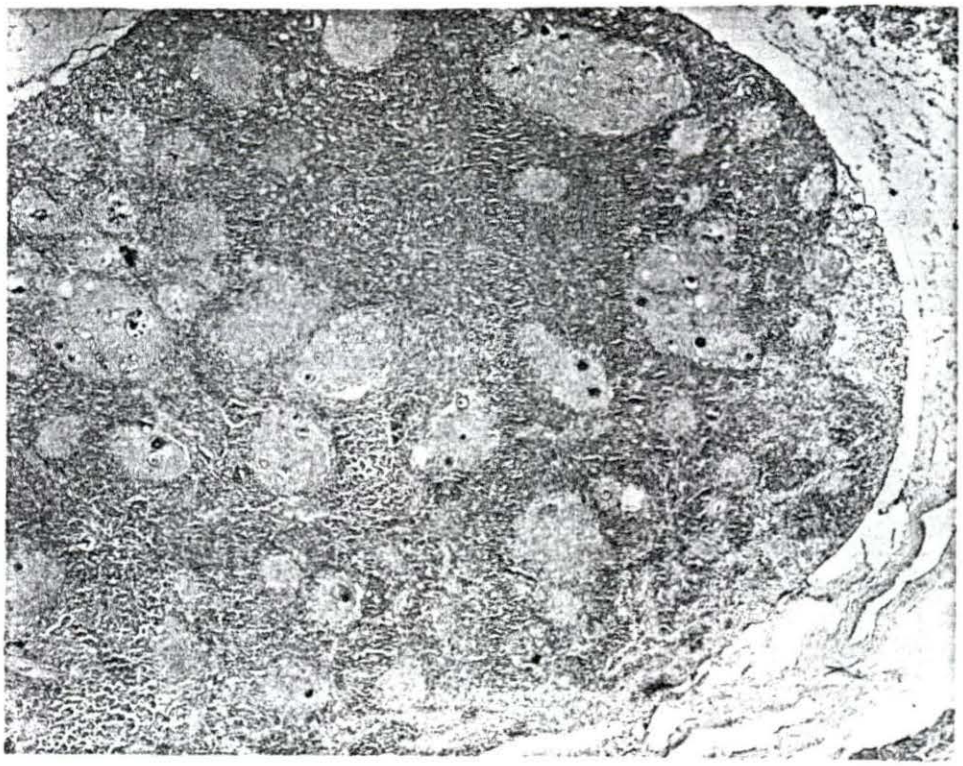


Figure 21. Anterior mesenteric lymph node at 266 days after intragastric inoculation with living M. paratuberculosis. Note the large foci of reticuloendothelial tissue hyperplasia containing large Schaumann's bodies. Ziehl-Neelsen method. x60.

Figure 22. Reticuloendothelial tissue hyperplasia of the ileocecal lymph node at 266 days following intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.



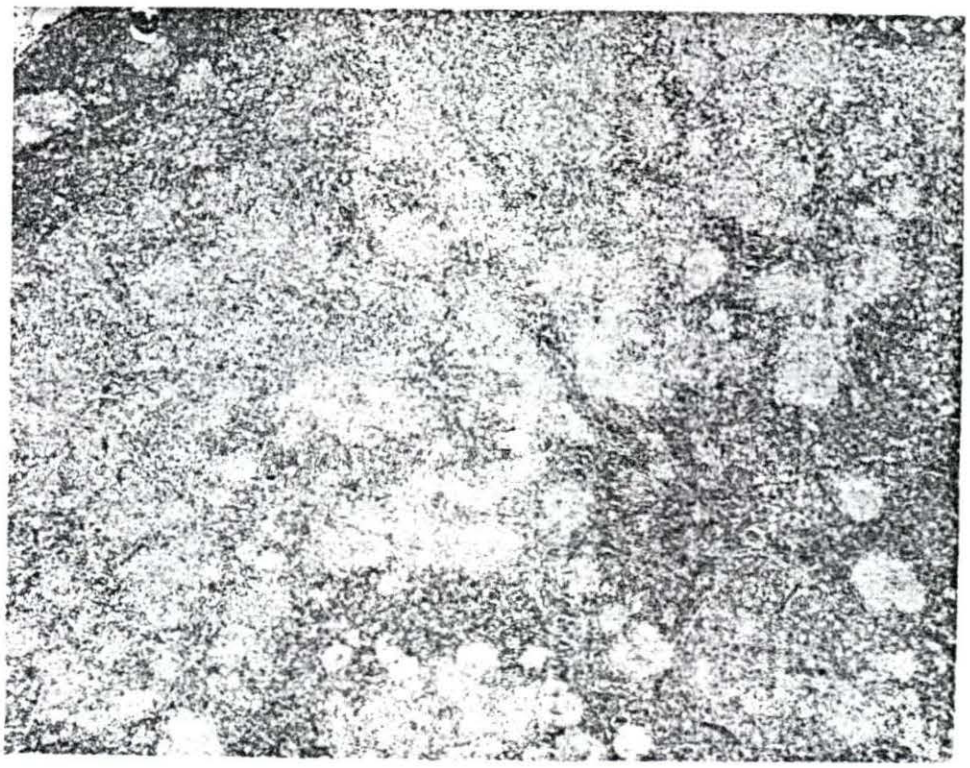
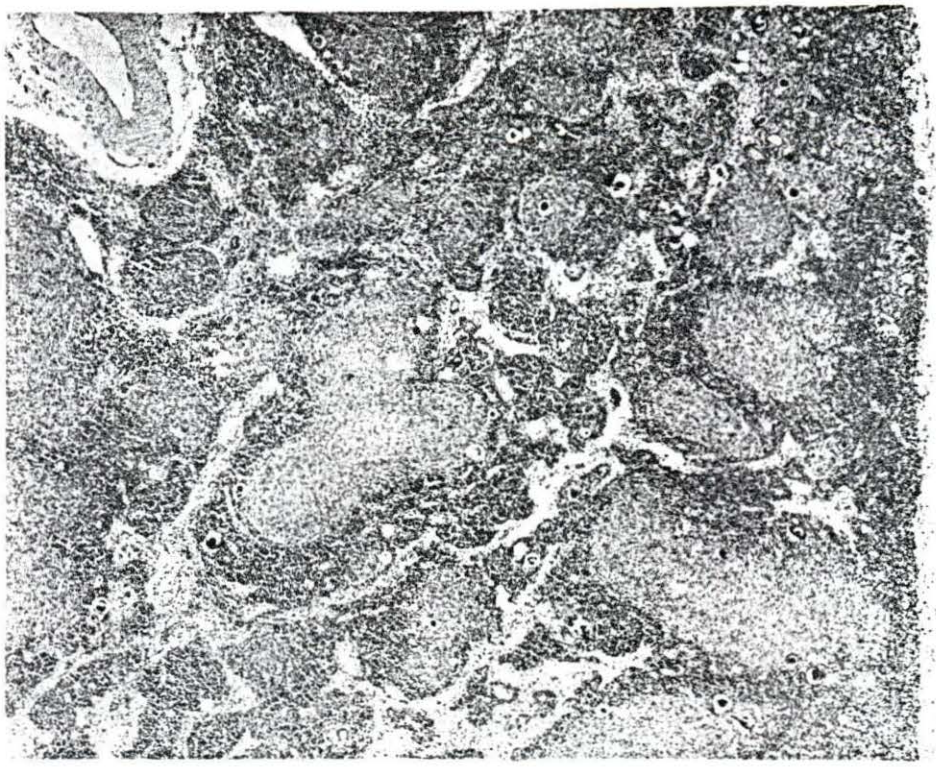


Figure 23. Extensive reticuloendothelial tissue hyperplasia occupying approximately 50% of the normal lymphoid tissue in the anterior mesenteric lymph node at 329 days after intragastric inoculation with living M. paratuberculosis. Note that the Schaumann's bodies are not always confined to the areas of hyperplasia. Ziehl-Neelsen method. x60.

Figure 24. Mandibular lymph node at 154 days after intragastric inoculation with living M. paratuberculosis. Post-capillary venules (arrows). Ziehl-Neelsen method. x600.



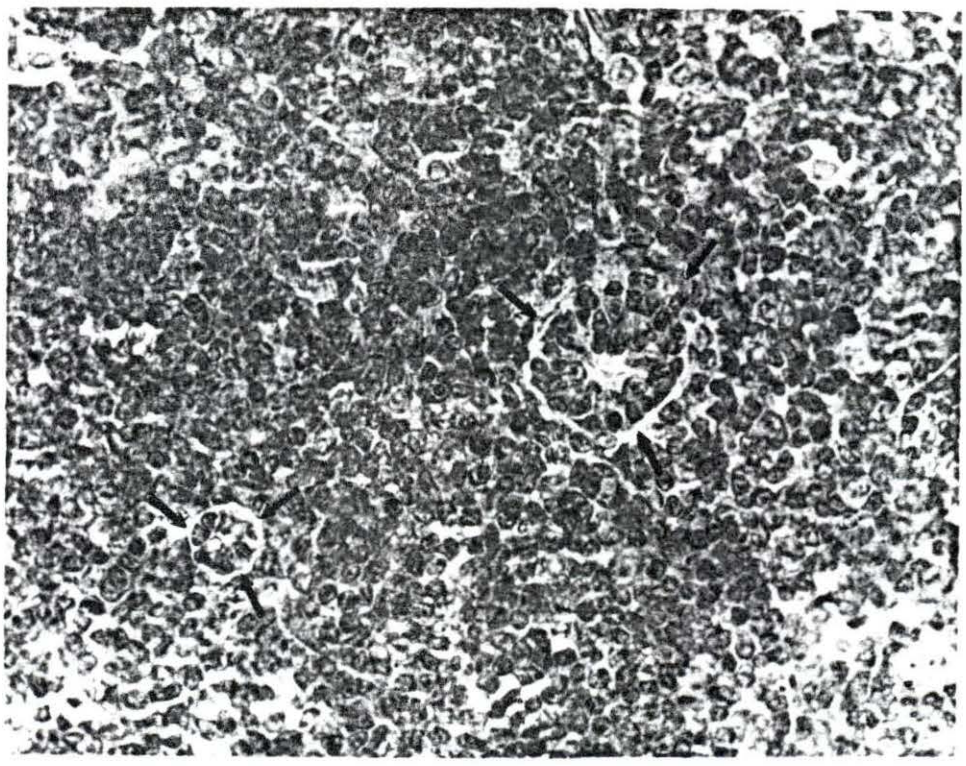
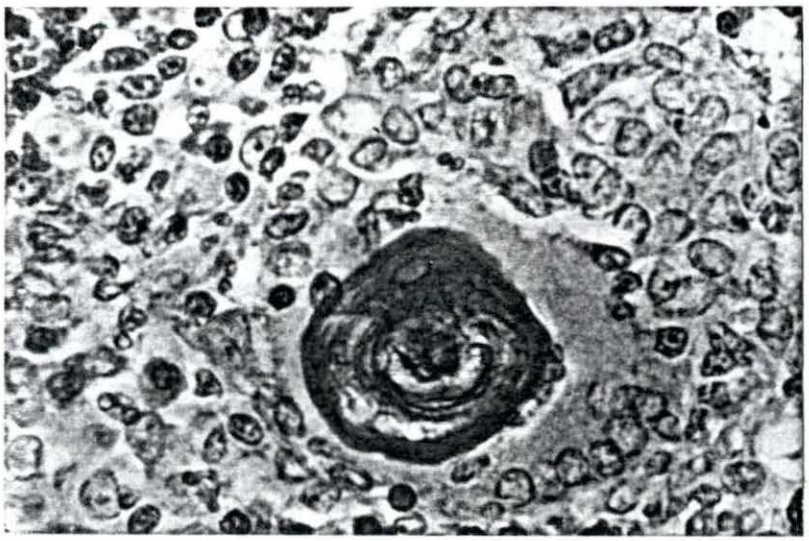
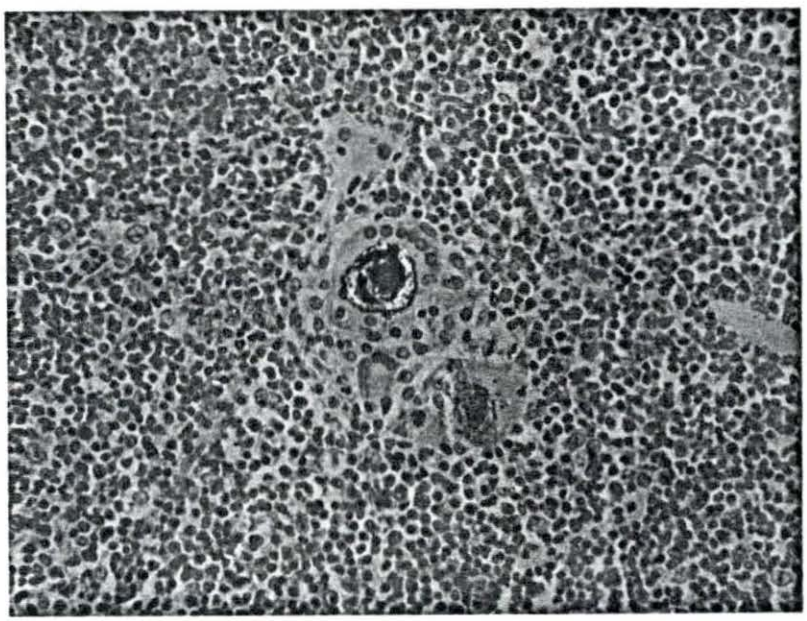


Figure 25. Large Schaumann's body in an ileocecal lymph node at 329 days after intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. xl,250.

Figure 26. Schaumann's body stained for calcium. Note that the Schaumann's body is partially surrounded by a multinucleated foreign body giant cell of the Langhan type. Von Kossa method. x600.





small, round, pale eosinophilic staining bodies (Figure 6) in the ileocecal lymph node at 98 days following inoculation. Observations at later intervals following inoculation revealed larger, more basophilic, and concentrically laminated bodies that were sometimes located within large multinucleated giant cells of the Langhan type (Figures 21, 23, 25, and 26).

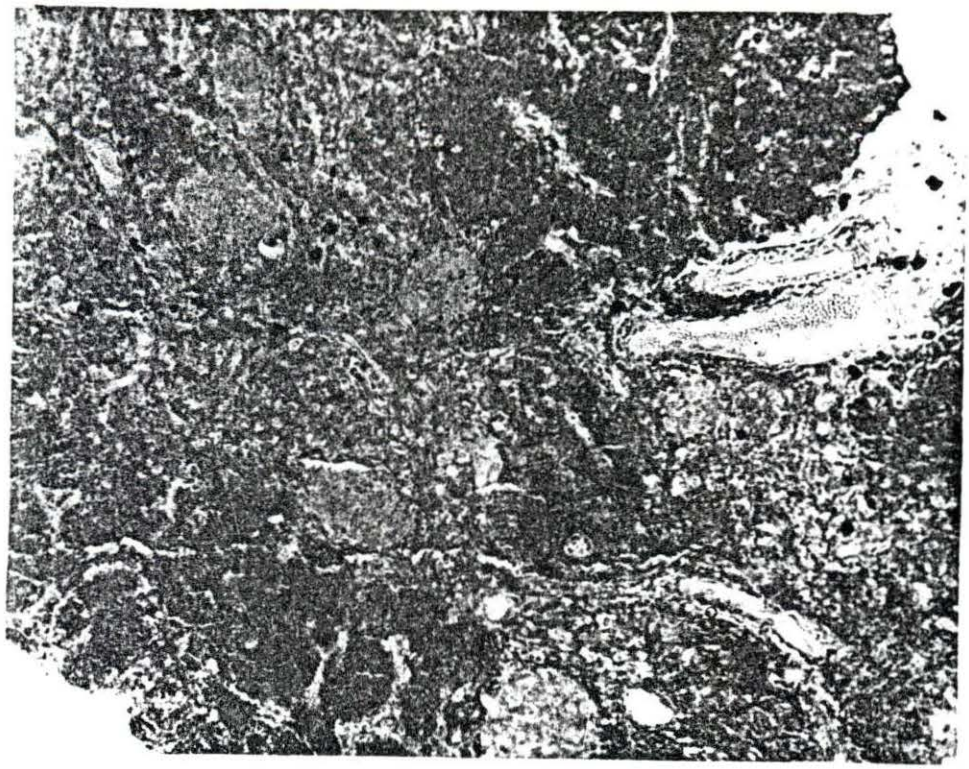
Post-capillary venules were noted in lymph nodes from several hamsters of this group. They were most prevalent in the hamster examined at 154 days PI (Figures 17, 18, and 24). They were more prominent in regional lymph nodes than in visceral lymph nodes.

Acid-fast bacilli and reticuloendothelial tissue hyperplasia were observed in thymus from 98 days through 329 days. Lesions were never as extensive as those observed in the lymph nodes and were usually confined to the medulla of the thymus (Figures 27 and 28).

A moderate increase in neutrophils was noted in the white pulp of the spleen at 28 days. Reticuloendothelial tissue hyperplasia was observed in the spleen beginning at 98 days and persisted through 329 days. Lesions were always located in white pulp and consisted of small multifocal areas of reticuloendothelial tissue hyperplasia at 98 days. Many large round circumscribed foci of reticuloendothelial tissue hyperplasia were present at 154 days (Figure 29). This advanced state of tissue alteration was also present at 210, 266, and 329 days. At 266 and 329 days, variable amounts of hemosiderin were contained within reticuloendothelial cells in areas of hyperplasia. Schaumann's

Figure 27. Foci of reticuloendothelial tissue hyperplasia in the thymus at 154 days after intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.

Figure 28. Small distinct foci of reticuloendothelial tissue hyperplasia in the medulla of the thymus at 154 days after intragastric inoculation with living M. paratuberculosis. Harris' hematoxylin and eosin stain. x150.



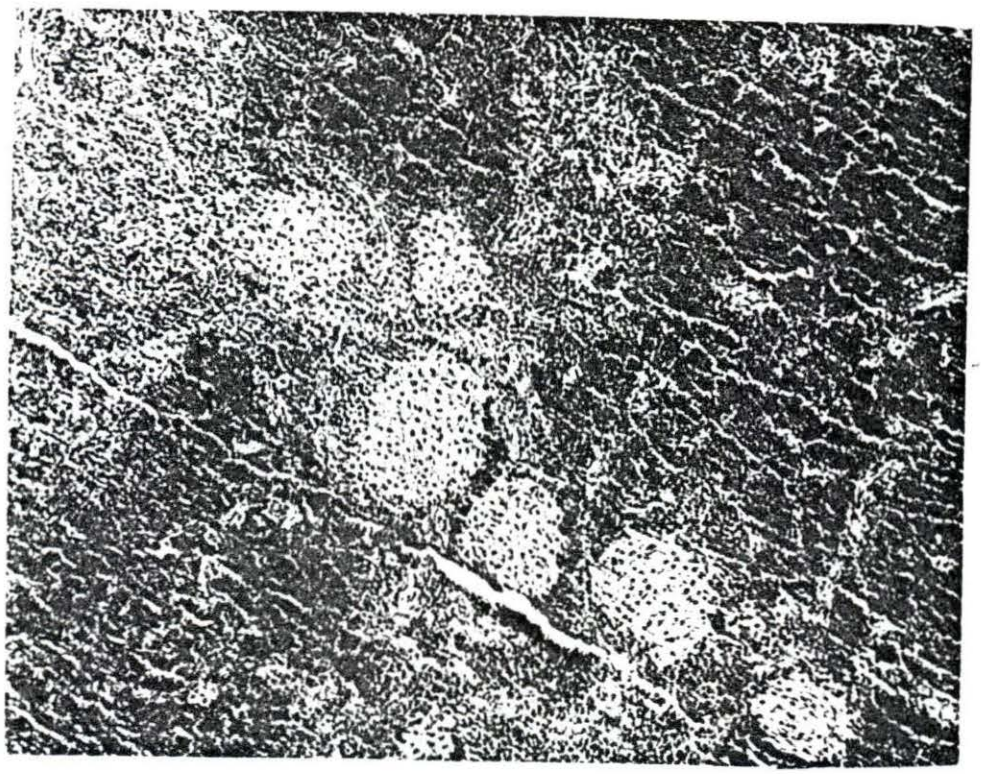
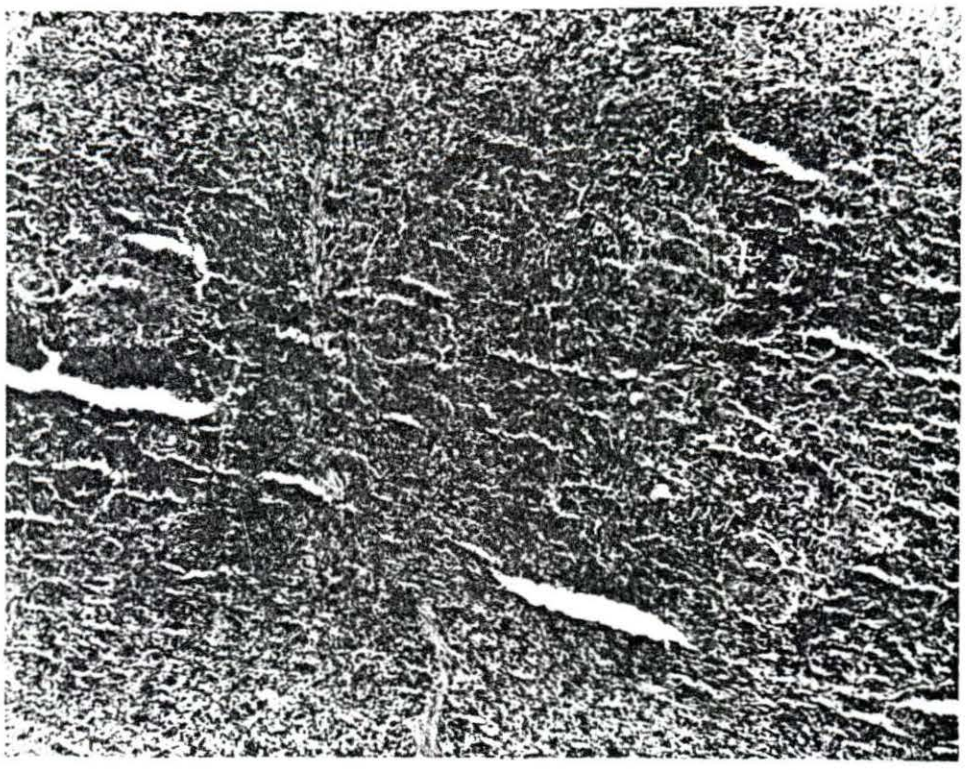
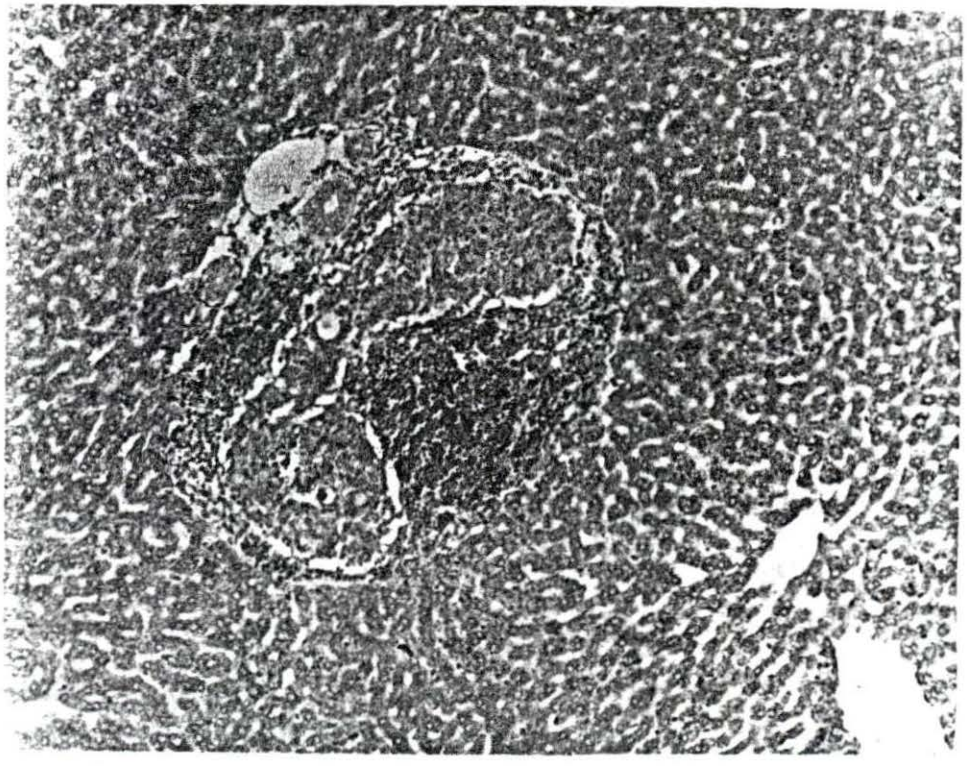


Figure 29. Foci of reticuloendothelial tissue hyperplasia in the white pulp of the spleen at 154 days after intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x150.

Figure 30. Focus of reticuloendothelial tissue hyperplasia in a periportal focus of lymphocytic infiltration in the liver at 154 days following intragastric inoculation with living M. paratuberculosis. Note the presence of the Schaumann's bodies. Ziehl-Neelsen method. x150.





bodies were usually, but not entirely, confined to the areas of reticuloendothelial tissue hyperplasia at and following 154 days.

No acid-fast bacilli, lesions, or lymphoid tissue were found in tissues adjacent to or in the root of the tongue from hamsters included in this group.

Areas of hyperplasia in lymphoid tissue consisted almost entirely of reticuloendothelial cells (Figures 6, 14, 17, and 32) that contained from few to several small acid-fast bacilli in their cytoplasm (Figures 4 and 5). The border of these foci abruptly joined the normal lymphoid tissue and there was a very limited zone that contained both hyperplastic reticuloendothelial cells and lymphocytes (Figures 6, 14, 17, 18, and 32). The reticuloendothelial tissue hyperplasia was usually very extensive for the number of acid-fast bacilli present and occupied up to 25% of the white pulp of the spleen at 154 days or 25 to 30% of the lymph nodes at 154, 210, 266, and 329 days. The ratio of reticuloendothelial tissue hyperplasia to the number of acid-fast bacilli increased with the duration following inoculation. At 266 and 329 days, the cytoplasm of the hyperplastic reticuloendothelial cells stained more deeply eosinophilic and the borders of the foci were less circumscribed, the peripheral zone containing a few more lymphocytes and plasma cells than the same type of lesion at 154 and 210 days.

Alimentary system No macroscopic tissue alterations were observed in the buccal pouches, mandibular and parotid salivary glands, forestomach, stomach and duodenum.

The Peyer's patches were observed as whitish, round, raised subserosal areas located anywhere between the duodenum and the ileocecal junction. The largest of the Peyer's patches was located near the anterior end of the jejunum. There was a gradual decrease in size with the smaller, also the more numerous, being in the ileum. The number, location, and size of the Peyer's patches were fairly constant in all hamsters examined.

The jejunum was grossly normal throughout the experiment. However, the terminal ileum was observed to be rigid and twice its normal diameter at 14 days and 3 to 4 times at 28 days. Several whitish subserosal foci, about 1 mm. in diameter, were observed. Adhesions between the ileal serosa and the adjacent parietal and visceral peritoneum were present. The diameter of the lumen was 2 times larger than normal and the ileal wall was markedly thickened. At 56 days, there was 1 case with enlargement of the ileum (about 3 times that of normal size) and another case where the ileum was smaller in diameter and whitish in color. After 98 and 154 days, the diameter of the ileum was smaller than normal, whitish, and the ileal wall was firmer and thicker than normal. The lumen was virtually non-patent. The ileum was grossly normal at 210, 266, and 329 days following inoculation.

Grossly, the cecum, spiral and terminal colon were normal throughout the 329 days of the experiment.

The liver was normal in all hamsters of this group except 1 of the 3 examined at 47 weeks. In this case, the liver contained several

raised areas about 1 cm. in diameter. Incision of the areas revealed cysts filled with a reddish fluid.

The pancreases were normal. A few adhesions of the omentum to the incision site of the celiotomy were noted.

Microscopically, no acid-fast bacilli or lesions were observed in the buccal pouches, mandibular and parotid salivary glands, forestomach and stomach. Two acid-fast bacilli were present within a macrophage in the lamina propria of a villus in the duodenum at 2 days. No other acid-fast bacilli were observed in the duodenum of hamsters from this group. However, 3 small foci of reticuloendothelial tissue hyperplasia were observed in lymph nodules of the duodenal submucosa near the pylorus in the hamster examined at 210 days.

A mild indistinct reticuloendothelial tissue hyperplasia, in the absence of acid-fast bacilli, was first observed in 6 of 8 Peyer's patches from the jejunum and ileum examined at 14 days. Occasional acid-fast bacilli were first observed within phagocytes in the submucosa of the jejunum at 56 days and were not associated with either Peyer's patches or reticuloendothelial hyperplasia.

A few acid-fast bacilli were observed within the cytoplasm of reticuloendothelial cells located in small indistinct foci of hyperplasia in all of the Peyer's patches examined at 98 days. Several acid-fast bacilli were in the cytoplasm of hyperplastic cells located in medium to large multifocal areas of reticuloendothelial tissue hyperplasia and occupied up to 25 to 30% of the lymphoid tissue in the sections of the Peyer's patches examined at 154 (Figures 31 and 32),

210, 266, and 329 days. The distinctiveness of the foci of reticuloendothelial tissue hyperplasia was reduced due to infiltration with lymphocytes and plasma cells at 329 days. Schaumann's bodies, first observed at 210 days, were larger at 266 days and remained about the same size at 329 days.

Table 13 illustrates the number and distribution of aggregates of lymph nodules or Peyer's patches in the submucosa of the jejunum and ileum and solitary lymph nodules of the duodenum, cecum, and spiral and terminal colon for each hamster inoculated intragastrically with living M. paratuberculosis.

Acid-fast bacilli were observed in lymph nodules of the cecum at 98 days and both acid-fast bacilli and reticuloendothelial tissue hyperplasia were present in the lymph nodules at 154, 266, and 329 days.

Acid-fast bacilli and foci of reticuloendothelial tissue hyperplasia were observed in the lymph nodules of the spiral colon at 154, 210, and 329 days PI. No lesions or acid-fast bacilli were observed in the tissues adjacent to the lymph nodules located in the spiral colon.

No lesions or acid-fast bacilli were observed in any of the lymph nodules or adjacent tissues of the terminal colon from hamsters inoculated intragastrically with living M. paratuberculosis.

No lesions or acid-fast bacilli were found in the liver at 2 and 4 days. After 8 days, several small periportal foci of lymphocytes were observed. At 14 and 28 days, there were small foci of reticuloendothelial tissue hyperplasia within the periportal foci of lymphocytes. Very few acid-fast bacilli were observed within the cytoplasm of

Table 13. The frequency of encountering lymphoid tissue in the intestinal tract of hamsters inoculated intragastrically with living M. paratuberculosis

Duration following inoculation[a] (days)	Organ examined				
	Duodenum[b]	Peyer's patches[c]	Cecum[b]	Spiral colon[b]	Terminal colon[b]
2	0/0	5/6	0/1	0/6	0/4
4	0/6	8/9	4/1	0/6	0/4
8	0/6	9/9	2/1	0/4	0/3
14	0/5	8/8	2/1	0/4	1/3
28	0/4	6/9	0/1	1/6	0/2
56	0/4	6/8	0/0	0/6	0/3
56	0/5	3/6	0/1	0/6	0/2
98	0/6	5/9	3/1	0/6	1/5
154	0/0	7/8	1/1	2/6	1/3
154	0/4	3/6	0/1	1/6	0/2
210	3/5	9/9	1/1	1/6	1/3
266	0/5	7/8	6/2	1/6	0/4
329	0/5	8/12	4/1	0/6	0/3
329	0/5	9/9	4/2	1/6	0/4
329	0/5	10/10	5/1	3/8	0/3

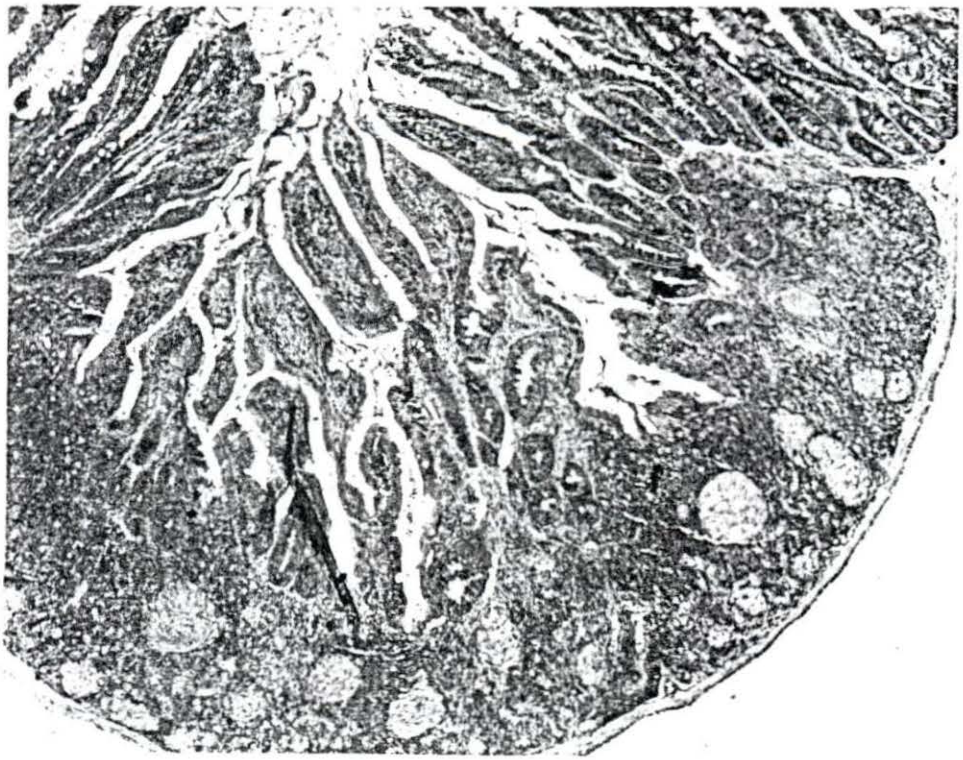
[a]One hamster was examined at each time interval.

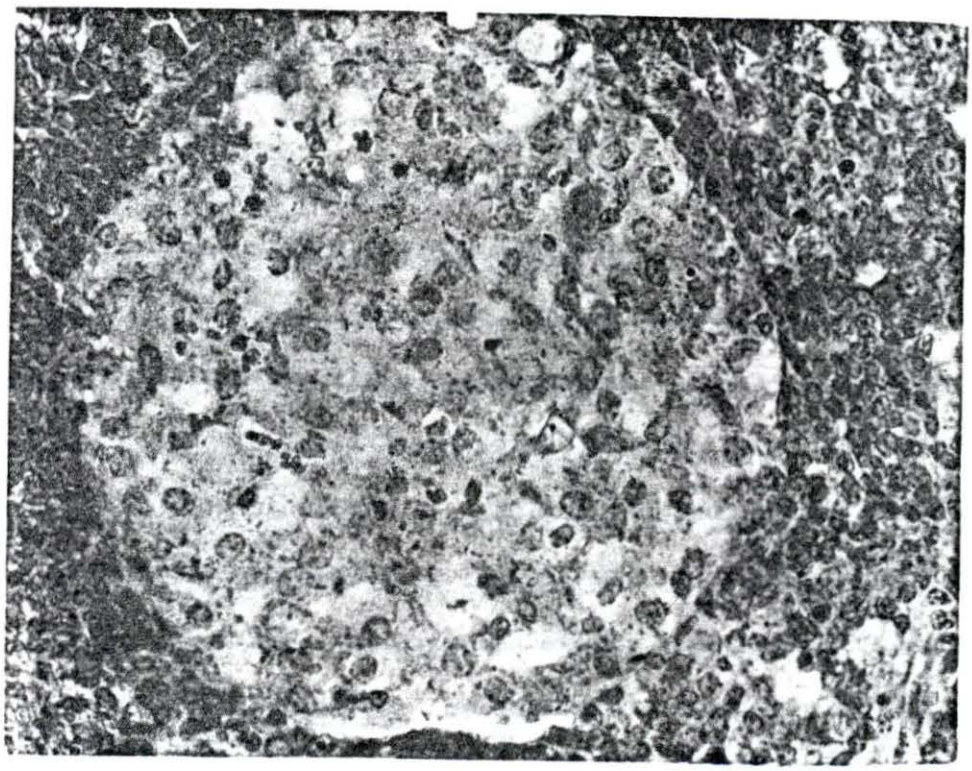
[b]Number of lymph follicles demonstrated/number of cross-sections examined from specified segment of intestinal tract.

[c]Number of Peyer's patches histologically demonstrated/number of Peyer's patches that could be observed grossly and were embedded for sectioning.

Figure 31. Peyer's patch containing foci of reticuloendothelial tissue hyperplasia in the jejunum of a hamster at 154 days following intragastric inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.

Figure 32. Higher magnification of a focus of reticuloendothelial tissue hyperplasia in the Peyer's patch in Figure 31. x600.





reticuloendothelial cells at 14 days. The few lymphocytes in the periportal tissues at 56 and 98 days were considered normal. Several areas of periportal lymphocytic infiltration that contained from 1 to 2 small or medium size foci of reticuloendothelial hyperplasia were present at 154 (Figure 30), 210, 266, and 329 days. Few intracytoplasmic located acid-fast bacilli and few multinucleated foreign body giant cells were present in several of the hyperplastic foci. Schaumann's bodies were also present at 210, 366, and 329 days. There was marked bile duct proliferation, portal fibrosis, mild generalized centro-lobular fibrosis, and sinusoidal dilatation in the liver that contained hepatic cysts from the hamster examined at 329 days.

No microscopic lesions or acid-fast bacilli were observed in the pancreas or the omentum from any of the hamsters examined in this group.

Cardiovascular and hematopoietic system No gross lesions were observed in the heart. Bone marrow of lumbar vertebrae and sternebrae was grossly normal.

Microscopically, no lesions or acid-fast bacilli were found in the heart. No lesions or acid-fast bacilli were present in the bone marrow of the lumbar vertebrae and sternebrae except at 154 and 329 days. Several acid-fast bacilli were present in 1 of the 2 hamsters examined at 154 days. The acid-fast bacilli occurred singly in the cytoplasm of reticuloendothelial cells in areas of mild hyperplasia of the bone marrow of both the lumbar vertebrae and the sternebrae. There was a mild increase in the neutrophils in the bone marrow. The bone marrow of the lumbar vertebrae and sternebrae of 1 of the 3

hamsters, examined at 329 days, contained several acid-fast bacilli in the cytoplasm of reticuloendothelial cells in areas of mild hyperplasia.

Nervous system The brain and lumbar spinal cord were grossly normal in all of the hamsters of this group.

Microscopically, no acid-fast bacilli or lesions were observed in the cerebrum, cerebellum, lumbar spinal cord, or the meninges from any of the hamsters in this group. However, at 154 days, corpora amylacea were present in the white matter of the occipital lobe of the cerebrum and in the granular layer of the cerebellum.

Respiratory system Grossly, lungs of most hamsters were very moist and mottled colored varying from pale pink to areas of purplish red. No excess fluid or adhesions were observed in the pleural cavity.

Microscopically, a moderate increase was noted in the neutrophils in the capillaries and venules of the lungs examined at 28 days. Lungs of 1 hamster examined at 154 days contained considerable edema and blood in alveoli and a marked hyperplasia of epithelial lining of the bronchioles. One acid-fast bacillus was observed in cytoplasm of a reticuloendothelial cell in the septal wall of the lung from 1 hamster examined at 154 days. One hamster examined at 329 days contained small foci of reticuloendothelial tissue hyperplasia with moderate amounts of hemosiderin and an occasional Schaumann's body. No acid-fast bacilli were found in any of these hyperplastic foci. Alternating areas of alveolar atelectasis and emphysema were present in the lungs of all

3 hamsters examined at 329 days.

Urogenital system With the exception of 1 of the 3 examined at 329 days, the kidneys were grossly normal in all hamsters examined in this group. The kidneys of the hamster with hepatic cysts were a pale yellow and slightly enlarged.

The urinary bladder from hamsters in this group was grossly normal and contained 1 to 3 cc. of yellowish viscous fluid.

Macroscopically, the uterus was grossly normal in all hamsters inoculated intragastrically with living organisms.

Microscopic examination revealed no acid-fast bacilli or lesions of paratuberculosis in any of the kidneys. With the exception of the hamster with hepatic cysts, the kidneys were histologically normal. The kidneys from this hamster, examined at 329 days, contained many hyaline casts in the collecting tubules and several plasma cells in the interstitial tissue of the renal pelvis.

No lesions or acid-fast bacilli were observed in the urinary bladder from any of the hamsters in this group.

No acid-fast bacilli, lymphoid tissue, or tissue alterations resembling paratuberculosis were observed in the uterus, ovary or oviduct of any of the hamsters examined in this group. The hamster examined at 329 days and found to have hepatic cysts also contained many neutrophils in the uterine horns. Moderate cystic degeneration of the uterine glands was present in the uterus of another of the hamsters examined at 329 days.

Skeletal system No gross or microscopic lesions were present in the osseous tissue of the lumbar vertebrae, hyoid bone, or sternebrae of any of the hamsters inoculated intragastrically with living M. paratuberculosis.

Special sense organs No macroscopic lesions were observed in eyes, infraorbital or exorbital lacrimal glands from hamsters of this group.

Histologically, no acid-fast bacilli or lesions of paratuberculosis were present. Several corpora amylacea were present in the ducts of the infraorbital lacrimal glands of 1 of the 3 hamsters examined at 329 days.

Heat-Killed Organisms Administered Intragastrically

Clinical findings

The celiotomy incision had healed without evidence of any infection. The hair coat was normal and there was no discharge from the eyes or any of the body orifices, except for hamster examined at 8 days. This hamster had diarrhea and the eyelids were matted together with a conjunctival discharge. The skin was dry and nonelastic. No clinical signs of paratuberculosis were observed in any hamsters included in this group.

Necropsy findings

Lymphatic system Macroscopically, the lymph nodes from all the hamsters that were examined at 2, 4, 14, 98, 154, 210, 266, and 329 days PI were normal. The left parotid, left mandibular, and left axillary lymph nodes were hyperemic, and the anterior mediastinal, ileocecal,

and medial iliac lymph nodes were enlarged about 3 times normal size in the hamster examined at 8 days. Other lymph nodes in this hamster were normal. After 28 and 56 days PI, the regional lymph nodes were normal but the visceral lymph nodes were slightly enlarged.

The thymus was normal in all animals in this group.

No gross alterations were observed in the spleen or in the tissues adjacent to or in the root of the tongue.

Microscopically (Table 14), no acid-fast bacilli or reticulo-endothelial tissue hyperplasia were observed in the lymphoid tissue of the hamsters that had been inoculated intragastrically with heat-killed M. paratuberculosis (Figures 33, 34, 35, 36, 37, and 38).

Post-capillary venules were present in the majority of the lymph nodes from all hamsters included in this group. These venules were more prominent in the regional than in the visceral lymph nodes and were most accentuated from 14 to 154 days.

Several megalokaryocytes were prominent in the lymph nodes of the hamster examined at 2 days and persisted in moderate numbers up to 98 days. There was a gradual decrease in the number of these cells after 98 days. These megalokaryocytes were more prominent in the visceral lymph nodes, especially the ileocecal lymph nodes.

There were accumulations of erythrocytes in the sinusoids of the left parotid, left mandibular, and left axillary lymph nodes from the hamster examined at 8 days following inoculation.

Although no suppurative foci were observed, there was an increased accumulation of neutrophils in the sinusoids of the anterior mesenteric,

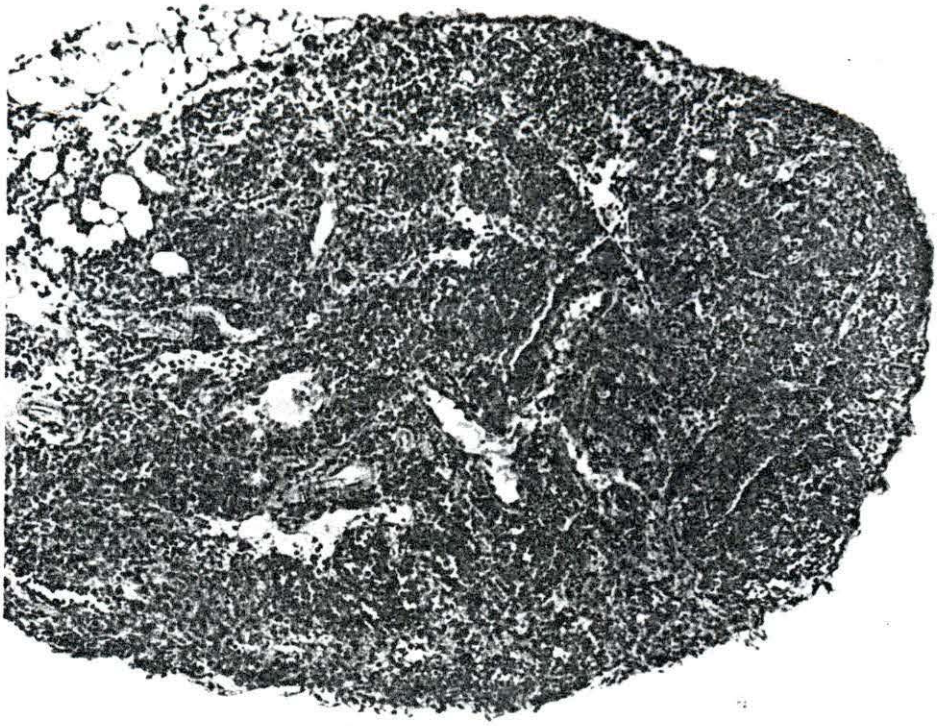
Table 14. Distribution of organisms and lesions in hamsters inoculated intragastrically with 1 mg. of heat killed M. paratuberculosis

Organ	Time (days)										
	2	4	8	14	28	56	98	154	210	266	329
Ant. mediastinal lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Ant. mesenteric lymph node	-	-	-	-	-	-	-	-	-	-	-
Mesenteric lymph nodes, other	-	-	-	-	-	-	-	-	-	-	-
Deep inguinal lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Medial iliac lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Prefemoral lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Axillary lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Mandibular lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Parotid lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	-	-	-	-	-
Base of tongue	-	-	-	-	-	-	-	-	-	-	-
Buccal pouches	-	-	-	-	-	-	-	-	-	-	-
Mandibular salivary glands	-	-	-	-	-	-	-	-	-	-	-
Parotid salivary glands	-	-	-	-	-	-	-	-	-	-	-
Fore stomach	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-
Duodenum (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Peyer's patches	-	-	-	-	-	-	-	-	-	-	-
Cecum (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Spiral colon (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Terminal colon (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-	-	-	-
Pancreas	-	-	-	-	-	-	-	-	-	-	-
Omentum	-	-	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-	-
Sternal bone marrow	-	-	-	-	-	-	-	-	-	-	-
Vertebral bone marrow (lumbar)	-	-	-	-	-	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-	-	-	-	-
Spinal cord (lumbar)	-	-	-	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	-	-	-	-
Kidneys	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	-	-	-	-
Eye	-	-	-	-	-	-	-	-	-	-	-
Infraorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-
Exorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-

- = No organisms or tissue reaction present

Figure 33. Anterior mesenteric lymph node at 98 days following intragastric inoculation with heat-killed M. paratuberculosis. Compare with Figure 10. Ziehl-Neelsen method. x150.

Figure 34. Ileocecal lymph node at 98 days following intragastric inoculation with heat-killed M. paratuberculosis. Note the absence of germinal centers and the presence of prominent reticular cells (arrow). Compare with Figure 11. Ziehl-Neelsen method. x150.



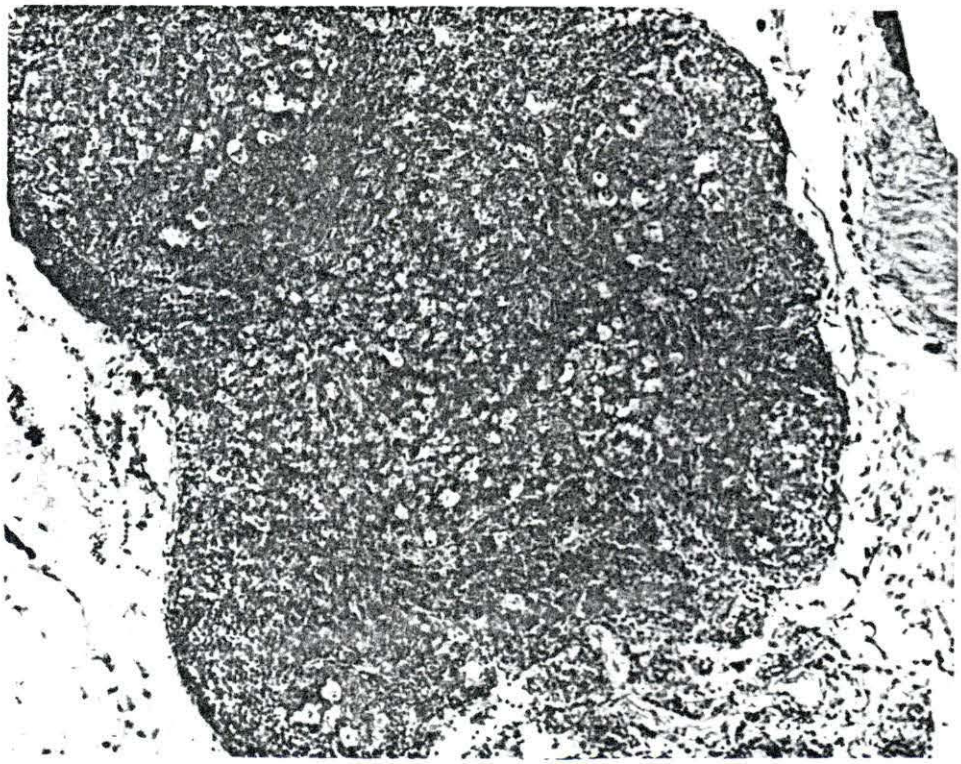
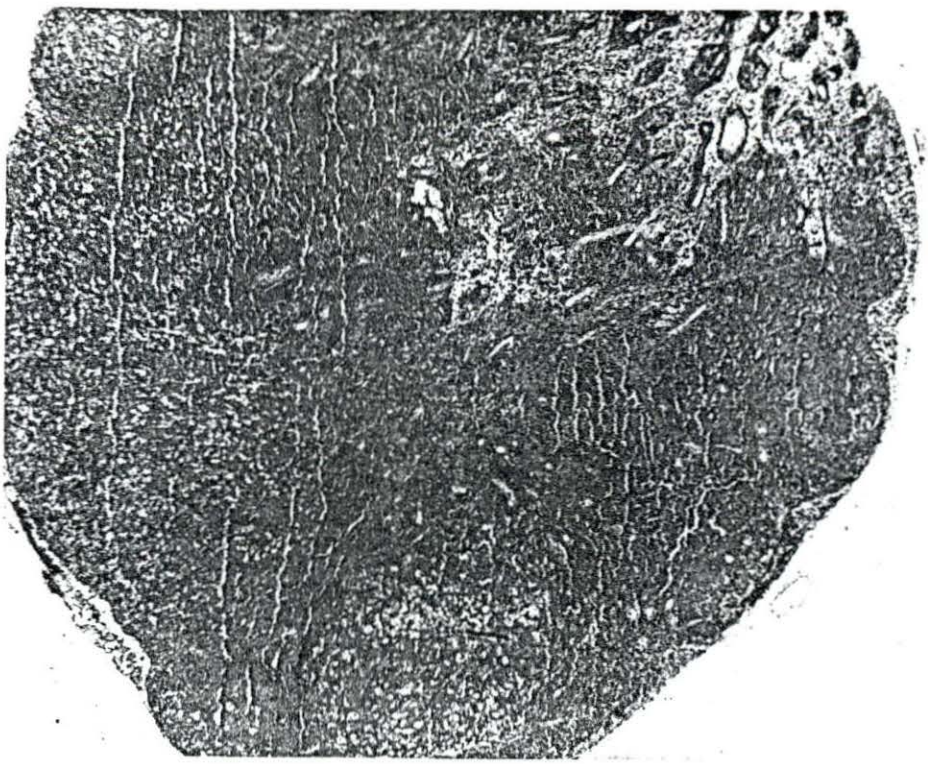


Figure 35. Ileocecal lymph node at 154 days following intragastric inoculation with heat-killed M. paratuberculosis. Compare with Figure 12. Ziehl-Neelsen method. x60.

Figure 36. Ileocecal lymph node at 210 days following intragastric inoculation with heat-killed M. paratuberculosis. Compare with Figures 19 and 20. Ziehl-Neelsen method. x60.



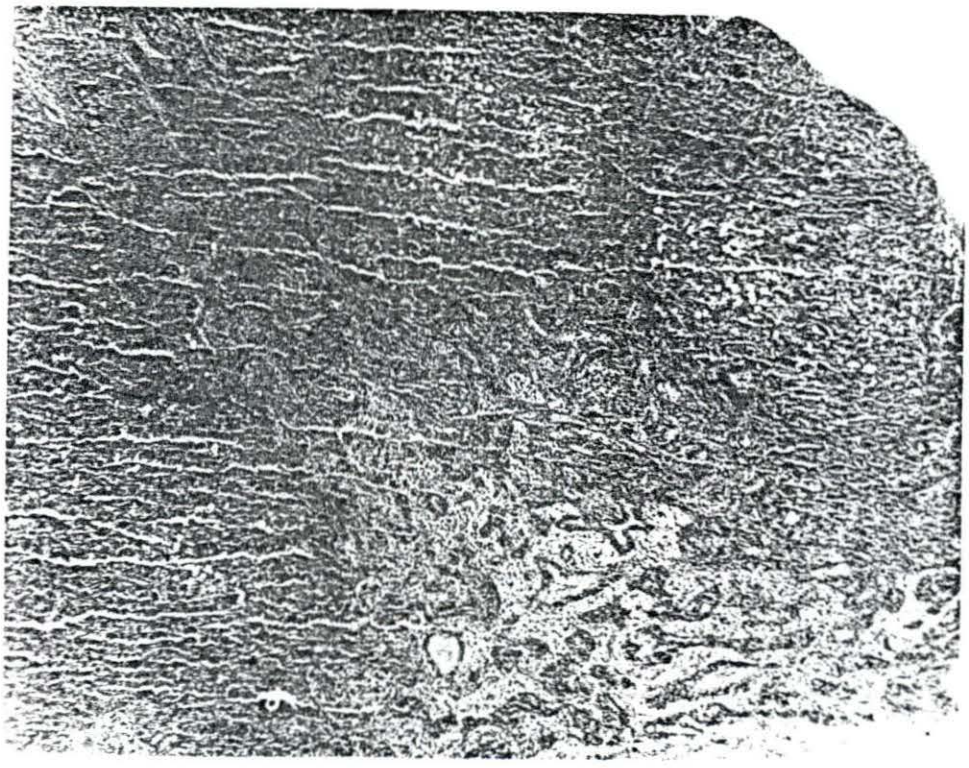
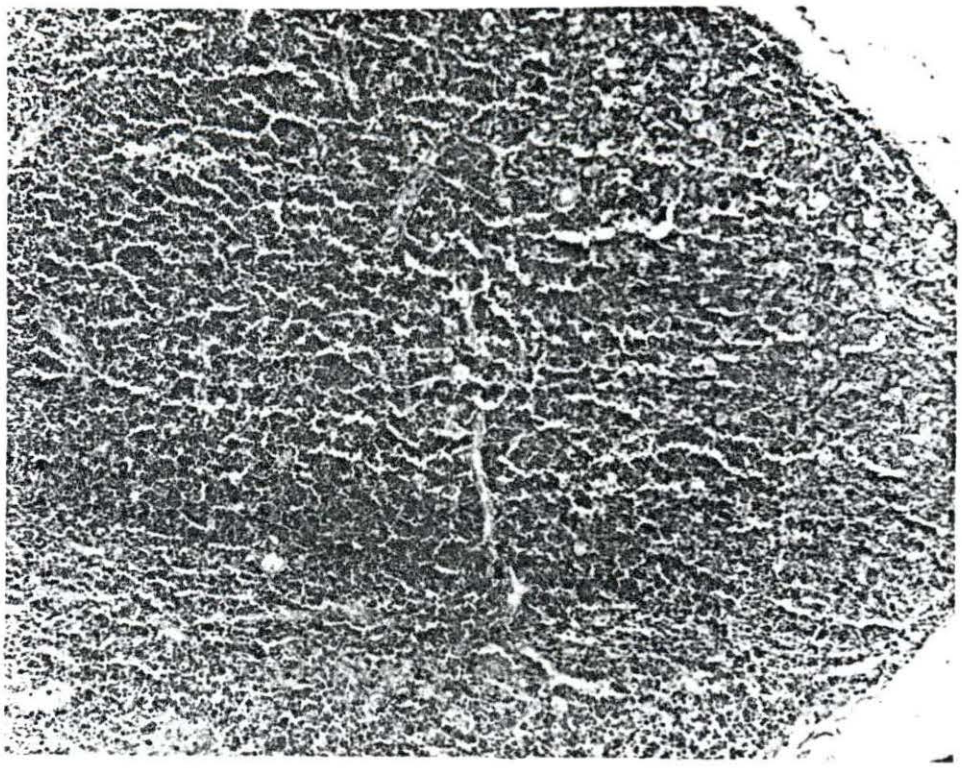
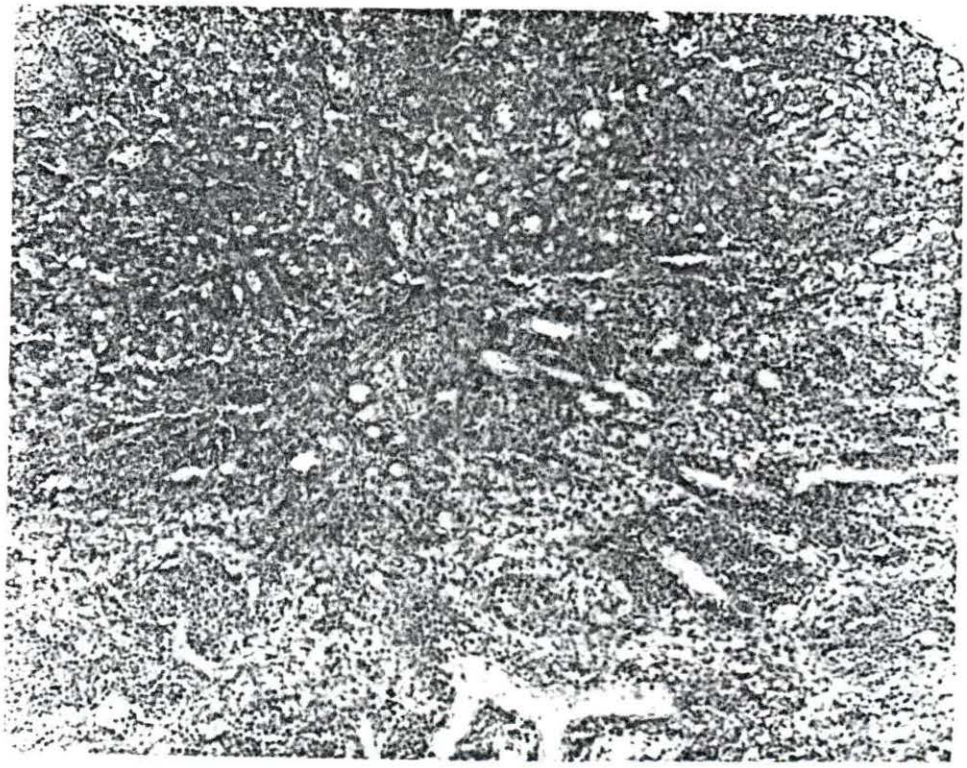


Figure 37. Anterior mesenteric lymph node at 266 days following intragastric inoculation with heat-killed M. paratuberculosis. Note the absence of reticuloendothelial tissue hyperplasia when compared with Figures 21 and 22. Ziehl-Neelsen method. x150.

Figure 38. Anterior mesenteric lymph node at 329 days following intragastric inoculation with heat-killed M. paratuberculosis. Compare with Figure 23. Ziehl-Neelsen method. x150.





iliocecal, and medial iliac lymph nodes from the hamster examined at 8 days and in the anterior mediastinal and iliocecal lymph nodes from the hamster examined 14 days following inoculation.

No acid-fast bacilli or lesions of paratuberculosis were present in the thymus from any of the hamsters included in this group.

The spleen and the tissues adjacent to or in the root of the tongue were histologically normal for all hamsters included in this group.

Alimentary system No macroscopic tissue alterations were observed in the buccal pouches, mandibular, and parotid salivary glands, forestomach, and stomach.

The small intestine was grossly normal except in a few cases where there was enlargement of the ileum. The ileum was enlarged 3 to 4 times its normal size at 8 days, about 2 times at 14 days, and only slightly at 28 days. This enlargement extended to the distal jejunum in the hamster examined at 8 days. The affected ileums were rigid and enlarged due to thickening of its walls and an increase in the diameter of the lumen. There were several whitish subserosal foci, about 1 mm. in diameter, and varying degrees of adhesions of the ileal serosa to adjacent parietal and visceral peritoneum. The ileum was white and small in diameter in the hamsters examined at 56 and 266 days.

Grossly, the cecum, spiral and terminal colon, liver, pancreas, and omentum were normal in all hamsters of this group.

Histologically (Table 14), no acid-fast bacilli or lesions of paratuberculosis were observed in the buccal pouches, mandibular and

parotid salivary glands, forestomach, stomach, duodenum, Peyer's patches, cecum, spiral and terminal colon, liver, pancreas, and omentum.

Table 15 illustrates the number and distribution of aggregates of lymph nodules as Peyer's patches in the submucosa of the jejunum and ileum and the solitary lymph nodules of the duodenum, cecum, and spiral and terminal colon for each of the hamsters included in this group.

Multifocal areas of chronic suppurative inflamation were present in the submucosa and subserosa of the ileum of the hamsters examined at 8, 14, and 28 days. Fissures, filled with purulent exudate, extended from the intestinal crypts through the muscularis to the subserosa. False diverticuli of the lamina epithelialis were often extended through the tunica muscularis by the fissures to the subserosa.

Occasional small foci of periportal accumulation of lymphocytes were present in the liver at 14 days. At 28 days, there were several small foci of lymphocytes scattered throughout the liver and not necessarily confined to the periportal region. The liver from hamster examined at 56 days had a marked bile duct proliferation and periportal lymphocytic infiltration.

No lesions were observed in the pancreas or omentum from any of the hamsters in this group.

Cardiovascular and hemopoietic system No gross or microscopic lesions were present in the heart or bone marrow of the lumbar vertebrae

Table 15. The frequency of encountering lymphoid tissue in the intestinal tract of hamsters inoculated intragastrically with heat-killed M. paratuberculosis

Duration following inoculation[a] (days)	Organ examined				
	Duodenum[b]	Peyer's patches[c]	Cecum[b]	Spiral colon[b]	Terminal colon[b]
2	0/0	5/8	1/1	1/8	1/3
4	0/5	8/8	2/1	2/6	0/3
8	0/5	8/8	1/1	2/5	0/3
14	0/5	5/8	1/1	1/6	0/0
28	0/4	6/8	1/1	1/6	1/3
56	0/6	5/9	1/1	0/6	1/5
98	0/5	6/9	1/1	2/8	1/4
154	0/5	10/10	1/1	1/6	0/4
210	0/5	8/9	4/1	1/6	1/5
266	0/6	9/10	3/2	2/8	1/3
329	0/5	7/10	5/2	1/8	1/4
329	0/5	10/10	2/1	1/6	2/4
329	0/5	9/9	1/1	1/8	0/4
329	0/5	6/9	0/1	1/6	0/4

[a]One hamster was examined at each time interval.

[b]Number of lymph follicles demonstrated/number of cross-sections examined from specified segment of intestinal tract.

[c]Number of Peyer's patches histologically demonstrated/number of Peyer's patches that could be observed grossly and were embedded for sectioning.

and sternebrae of any of the hamsters inoculated intragastrically with heat-killed M. paratuberculosis.

Nervous system No gross or microscopic lesions were observed in the spinal cord, cerebrum, cerebellum, or meninges of the hamsters inoculated intragastrically with heat-killed M. paratuberculosis.

Respiratory system The lungs were grossly normal, moist on the surface, and varied from pale pink to pale red. They were all histologically normal with the exception at 2 days when a moderate hyperplasia of the respiratory epithelium was observed and at 56 days when a moderate edema and a mild emphysema were present.

Urogenital system The right kidney of the hamster examined at 8 days was normal in color but about 25% of its normal size. Histologically, the hypoplasia involved the cortex more than the medulla and there were fewer glomeruli than normal (probably not caused by M. paratuberculosis). All of the other kidneys examined from hamsters inoculated intragastrically with heat-killed organisms were normal.

The urinary bladder of all hamsters in this group was normal. Each bladder contained from 1 to 3 cc. of yellowish viscous fluid.

No lesions or acid-fast bacilli were observed in the uterus from any of the hamsters in this group.

Skeletal system No lesions or acid-fast bacilli were observed in the osseous tissue of the lumbar vertebrae, hyoid bones, or sternebrae from any of the hamsters inoculated intragastrically with heat-killed M. paratuberculosis.

Special sense organs The eyes were all normal except in the hamster examined at 8 days. In this case, the eyelids were matted with conjunctival discharge and the cornea of 1 eye was cloudy. Histologically, several neutrophils and lymphocytes were present in the tunics of this eye and in places this chronic inflammation extended into the muscles of the orbit.

The infraorbital lacrimal glands were grossly normal. Histologically, several corpora amylacea were present in the ducts of the infraorbital glands of 2 of the hamsters examined at 329 days. No other lesions or acid-fast bacilli were observed in any of these glands from hamsters of this group.

No lesions or acid-fast bacilli were observed in the exorbital lacrimal glands from hamsters inoculated intragastrically with heat-killed M. paratuberculosis.

Living Organisms Administered Intraperitoneally

Clinical findings

The hamsters examined at 2, 4, 98, 154, and 329 days were normal. Each had a healthy appearing skin and hair coat. No discharge was present from the eyes or any of the body orifices. The hamsters examined at 98, 154, and 329 days weighed less than normal hamsters would at this age. Eversion of the rectum was present in the hamster examined at 8 days. The hamster examined at 14 days was lethargic and had a diarrhea. No diarrhea was present in the hamsters examined at 28, 56, and 266 days, but they were small for their age and had a history of a previous diarrhea at 7, 21, and 14 days respectively prior to

postmortem examination. The hamster examined at 210 days had died without any apparent cause. Although no clinical signs of paratuberculosis were observed, this group of hamsters did have the highest mortality of any of the 4 groups in this study.

Necropsy findings

Lymphatic system Grossly, all of the lymph nodes were normal in the hamsters examined at 2, 4, 14, 28, and 56 days PI. The medial iliac lymph nodes were enlarged about 3 times their normal size at 8 days, while all the other lymph nodes were within normal limits. The prefemoral lymph nodes were slightly enlarged at 98 days and all of the visceral lymph nodes were about 2 times larger than normal. The anterior mediastinal, anterior mesenteric, and ileocecal lymph nodes were slightly enlarged while the deep inguinal and medial iliac lymph nodes were moderately enlarged at 154 days. At 210 days, the parotid, mandibular, anterior mesenteric, ileocecal, deep inguinal, and medial iliac lymph nodes were slightly enlarged, but the mediastinal lymph nodes were enlarged about 2 times. All of the regional lymph nodes were twice their normal size at 266 days while the mediastinal lymph nodes were enlarged slightly and the anterior mesenteric, deep inguinal, and medial iliac lymph nodes were enlarged about 3 times. However, the ileocecal lymph nodes were 4 to 5 times normal size. The parotid lymph nodes were about 2 times normal size at 329 days; all the other lymph nodes were within normal limits.

The thymus was grossly normal in all of the hamsters included in this group.

No gross tissue alterations were noted in the spleen or the tissues adjacent to or in the root of the tongue from the hamsters inoculated intraperitoneally with living M. paratuberculosis.

Histologically (Table 16), acid-fast bacilli were observed within reticuloendothelial cells in all 3 of the anterior mediastinal lymph nodes and 2 acid-fast bacilli were found within the cytoplasm of a reticuloendothelial cell in a medullary sinus of 1 of the 2 mandibular lymph nodes from the hamster examined 2 days PI. Acid-fast bacilli were also found within macrophages in the loose areolar connective tissue on the surface of the capsule of the ileocecal and anterior mesenteric lymph nodes. A few acid-fast bacilli were found within the reticuloendothelial cells in the subcapsular sinus of the ileocecal lymph node from the hamster examined at 4 days. Several small and medium size clumps of acid-fast bacilli were present in hypertrophied reticuloendothelial cells in the sinusoids of the ileocecal lymph nodes and in 2 of 4 of the anterior mediastinal lymph nodes from the hamster that was examined 8 days PI.

In the anterior mediastinal lymph nodes examined at 14 days PI, many acid-fast bacilli were observed occurring singly and in clumps in the cytoplasm of reticuloendothelial cells. Many small and medium size foci of reticuloendothelial tissue hyperplasia occupied between 35 to 70% of the lymphoid tissue (Figure 39). There was an increased accumulation of neutrophils in the sinusoids of these lymph nodes. Acid-fast bacilli were more numerous per unit area in the reticuloendothelial tissue hyperplasia of these lymph nodes when compared with

Table 16. Distribution of organisms and lesions in hamsters inoculated intraperitoneally with 1 mg. of living M. paratuberculosis

Organ	Time (days)										
	2	4	8	14	28	56	98	154	210	266	329
Ant. mediastinal lymph nodes	+	-	+	+++	-	+++	+++	+++	+++	++	+++
Ant. mesenteric lymph node	-	-	-	+	-	+++	+++	+++	+++	+++	+++
Mesénteric lymph nodes, other	-	+	+	++	-	+++	+++	+++	+++	+++	+++
Deep inguinal lymph nodes	-	-	-	+	-	+++	+++	+++	+++	+++	+++
Medial iliac lymph nodes	-	-	-	+	-	+++	+++	+++	+++	+++	+++
Prefemoral lymph nodes	-	-	-	-	-	+++	+++	+++	+++	+++	+++
Axillary lymph nodes	-	-	-	-	-	++	+++	+++	+++	+++	+++
Mandibular lymph nodes	+	-	-	-	-	+++	+++	+++	+++	+++	+++
Parotid lymph nodes	-	-	-	-	-	+++	+++	+++	+++	+++	+++
Spleen	-	-	-	+	-	+++	+++	+++	+++	+++	+++
Thymus	-	-	-	-	-	++	+++	+++	++	++	+++
Base of tongue	-	-	-	-	-	-	-	-	-	-	-
Buccal pouches	-	-	-	-	-	-	-	-	-	-	-
Mandibular salivary gland	-	-	-	-	-	-	-	-	-	-	-
Parotid salivary gland	-	-	-	-	-	-	-	-	-	-	-
Fore stomach	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-
Duodenum (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Peyer's patches	-	-	-	-	-	+++	+++	+++	+++	++	++
Cecum (lymph nodules)	-	-	+	-	-	++	++	-	++	++	-
Spiral colon (lymph nodules)	-	-	-	-	-	-	-	-	-	++	-
Terminal colon (lymph nodules)	-	-	-	-	-	-	-	++	-	-	-
Liver	+	-	+	++	-	++	+++	+++	+++	++	++
Pancreas	-	-	-	-	-	-	-	-	-	-	-
Omentum	++	+	++	+++	-	+++	+++	+++	+++	-	-
Heart	-	-	-	-	-	-	-	-	-	-	-
Sternal bone marrow	-	-	-	-	-	+++	+++	-	+++	-	-
Vertebral bone marrow (lumbar)	-	-	-	-	-	++	+++	-	++	++	+++
Brain	-	-	-	-	-	-	-	-	-	-	-
Spinal cord (lumbar)	-	-	-	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	-	-	-	-
Kidneys	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	-	-	-	-
Eye	-	-	-	-	-	-	-	-	-	-	-
Infraorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-
Exorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-

- = No organisms or tissue reaction
+ = Organisms within individually located macrophages
++ = Tissue reactions occupying less than 3% of the normal tissue
+++ = Tissue reactions occupying more than 3% of the normal tissue

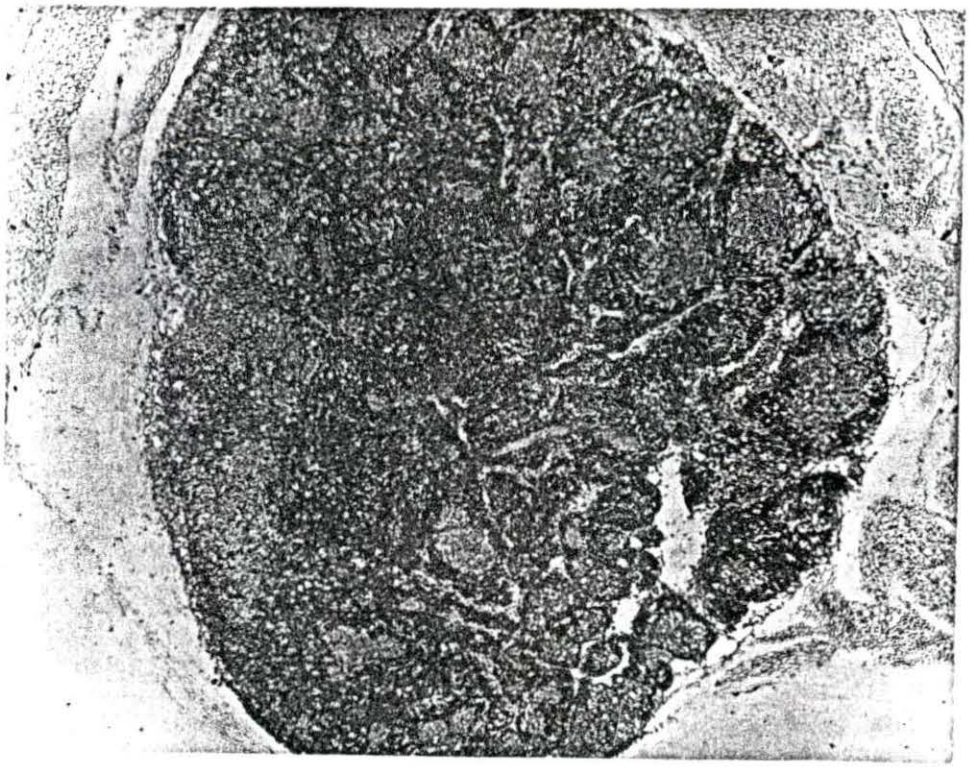
lesions in the hamsters exposed intragastrically with living organisms. Smaller lesions, containing acid-fast bacilli, were also found in the ileocecal lymph nodes. Few small clumps of acid-fast bacilli were present within the cytoplasm of isolated reticuloendothelial cells located on the walls of the sinusoids in the medullary portion of the anterior mesenteric, deep inguinal and medial iliac lymph nodes, and in the red pulp of the spleen.

No lesions or acid-fast bacilli were present in the lymphoid tissue of the hamster examined 28 days following inoculation. All of the lymph nodes from the hamsters examined at 56, 98 (Figures 40, 41, and 42), 154 (Figures 45 and 46), 210 (Figure 47), 266 (Figures 48 and 49), and 329 days (Figure 51) PI contained acid-fast bacilli that occurred singly or in small clumps within reticuloendothelial cells.

The reticuloendothelial tissue hyperplasia of the axillary lymph node from the hamster examined at 56 days and of the anterior mediastinal lymph nodes from the hamster examined at 266 days occupied less than 3% of the lymphoid tissue examined. The reticuloendothelial tissue hyperplasia of all of the other lymph nodes examined at 56 and 266 days consisted of areas that occupied more than 3% of the lymphoid tissue in the sections. Reticuloendothelial tissue hyperplasia was extensive in the visceral lymph nodes and occupied as much as 50% of the lymphoid tissue in the sections of the anterior mediastinal lymph nodes at 56, 98 (Figures 43 and 44), and 329 days and of the anterior mesenteric, and ileocecal lymph nodes at 329 days (Figure 51). The reticuloendothelial tissue hyperplasia of the regional lymph nodes occupied 20 to 30% of the

Figure 39. Anterior mediastinal lymph node at 14 days after intraperitoneal inoculation with living M. paratuberculosis. Compare with Figure 7. Ziehl-Neelsen method. x60.

Figure 40. Distinct foci of reticuloendothelial tissue hyperplasia in the parotid lymph node at 98 days following intraperitoneal inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.



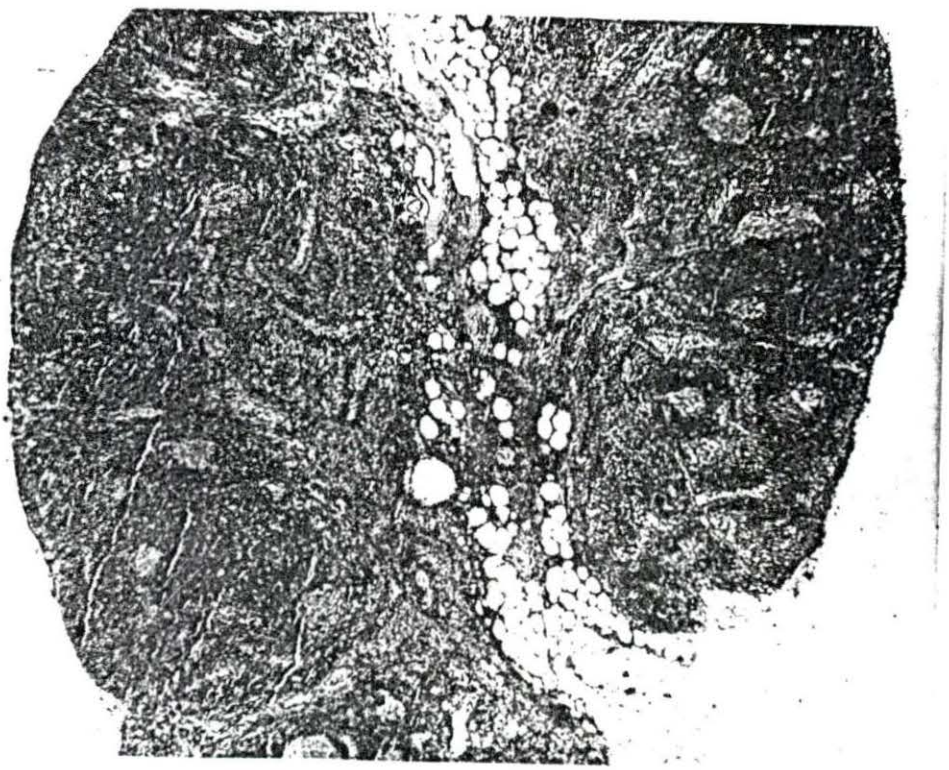
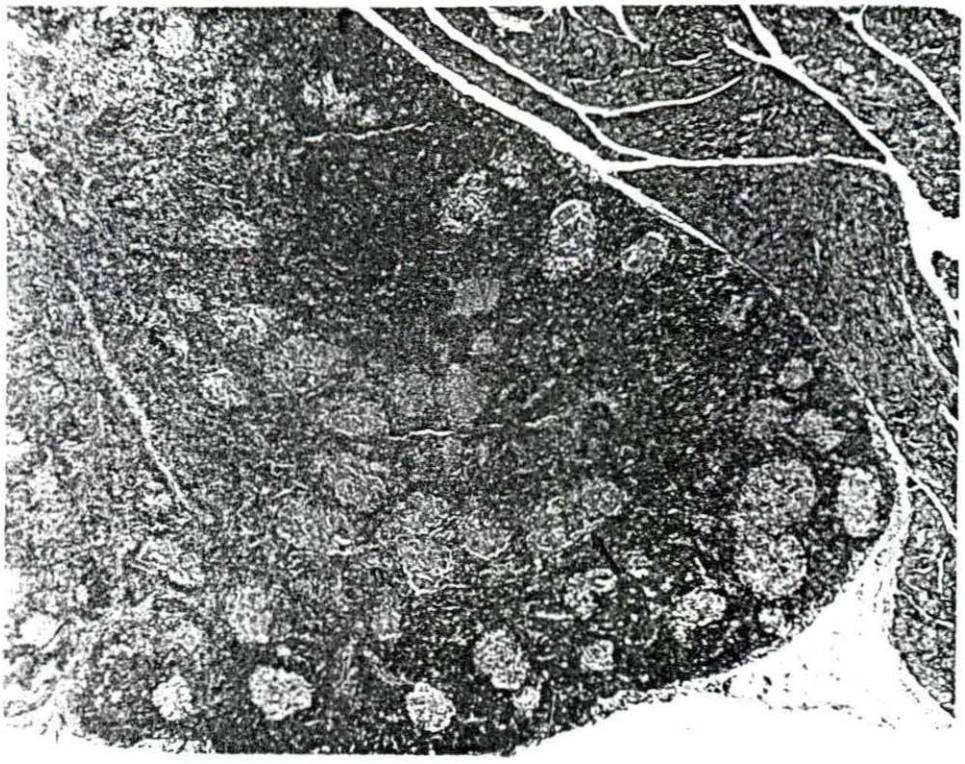


Figure 41. Multifocal reticuloendothelial tissue hyperplasia in the anterior mesenteric lymph node 98 days following intraperitoneal inoculation with living M. paratuberculosis. An adjacent portion of the pancreas is included in this section. Compare with Figures 10 and 33. Ziehl-Neelsen method. x60.

Figure 42. Higher magnification of the large foci of reticuloendothelial tissue hyperplasia indicated by arrow in Figure 41. Note the presence of many small Schaumann's bodies confined to the area of hyperplasia. Ziehl-Neelsen method. x375.



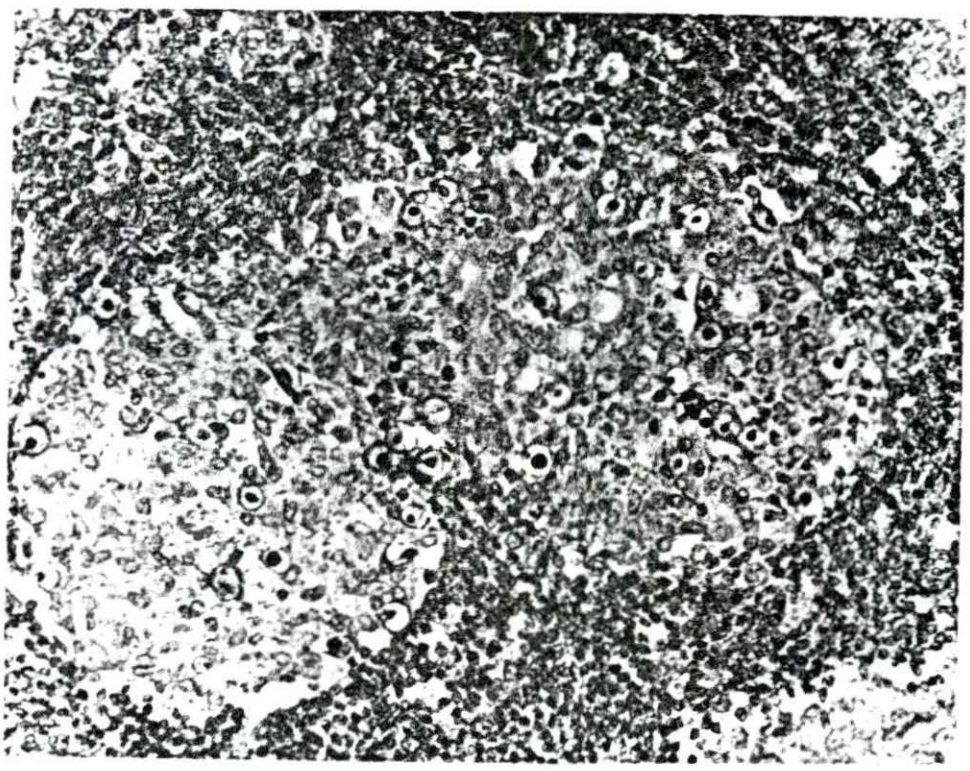
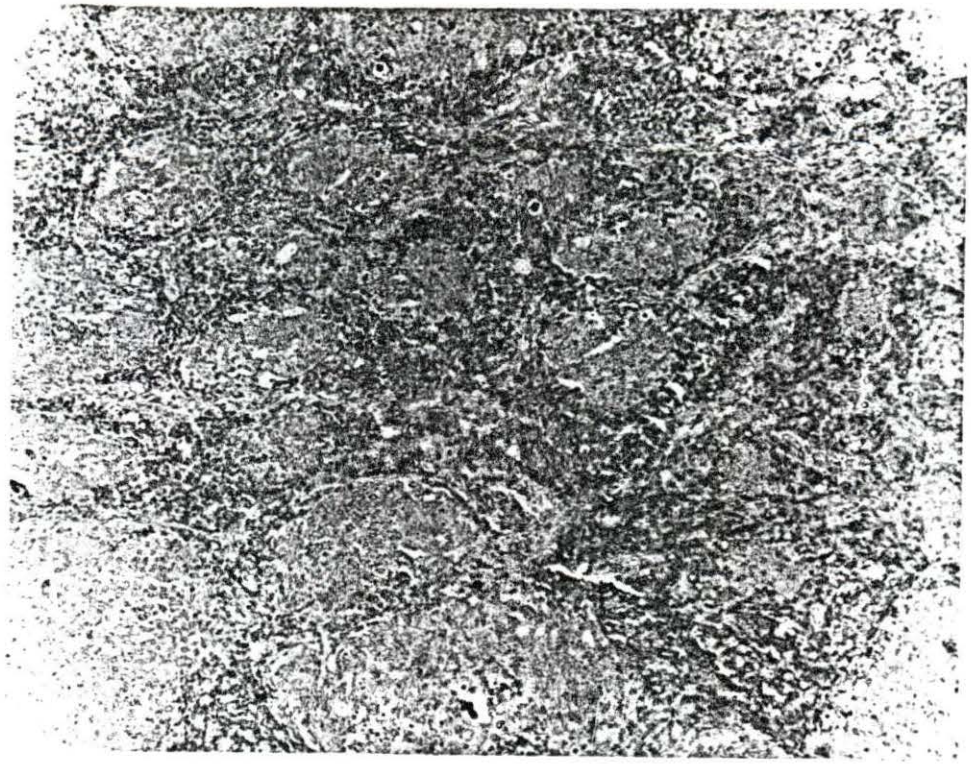


Figure 43. Extensive reticuloendothelial tissue hyperplasia in the anterior mediastinal lymph node 98 days following intraperitoneal inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x150.

Figure 44. Higher magnification of an area from the same lymph node in Figure 43. Note the many small Schaumann's bodies whose size is about the same as that of a lymphocyte nucleus. Ziehl-Neelsen method. x600.



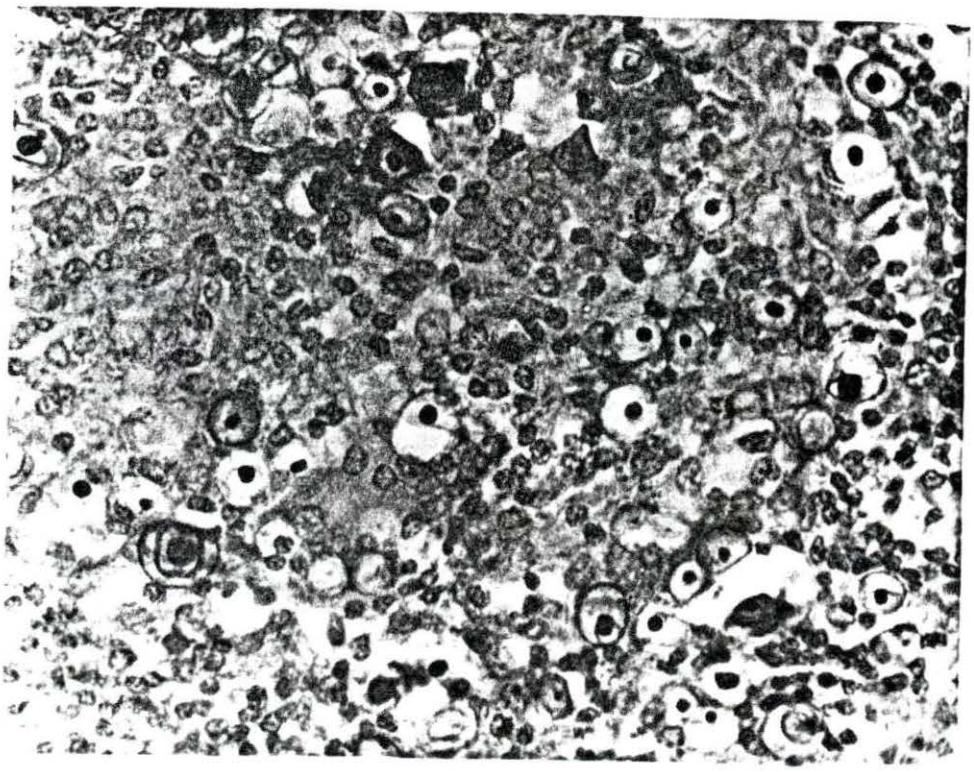
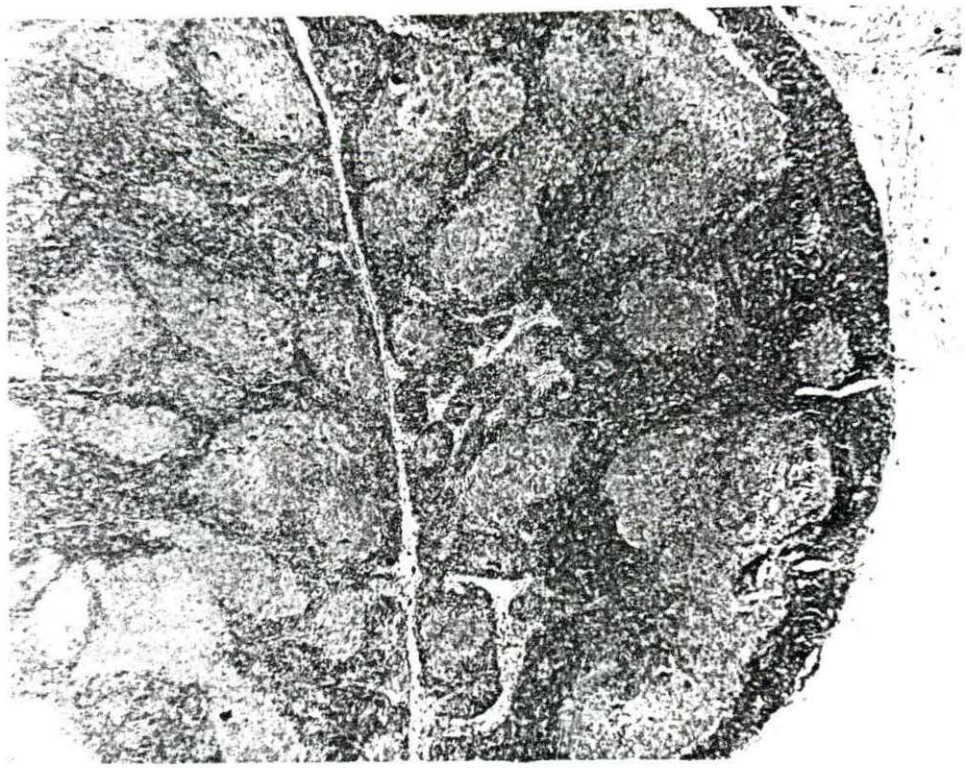


Figure 45. Anterior mesenteric lymph node at 154 days after intraperitoneal inoculation with living M. paratuberculosis. Note the extensive reticuloendothelial hyperplasia and compare with Figures 12 and 35. Ziehl-Neelsen method. x60.

Figure 46. Extensive hyperplasia of the mandibular lymph node 154 days following intraperitoneal inoculation with living M. paratuberculosis. Compare the lesions with those in Figures 13, 15, and 16. Ziehl-Neelsen method. x60.



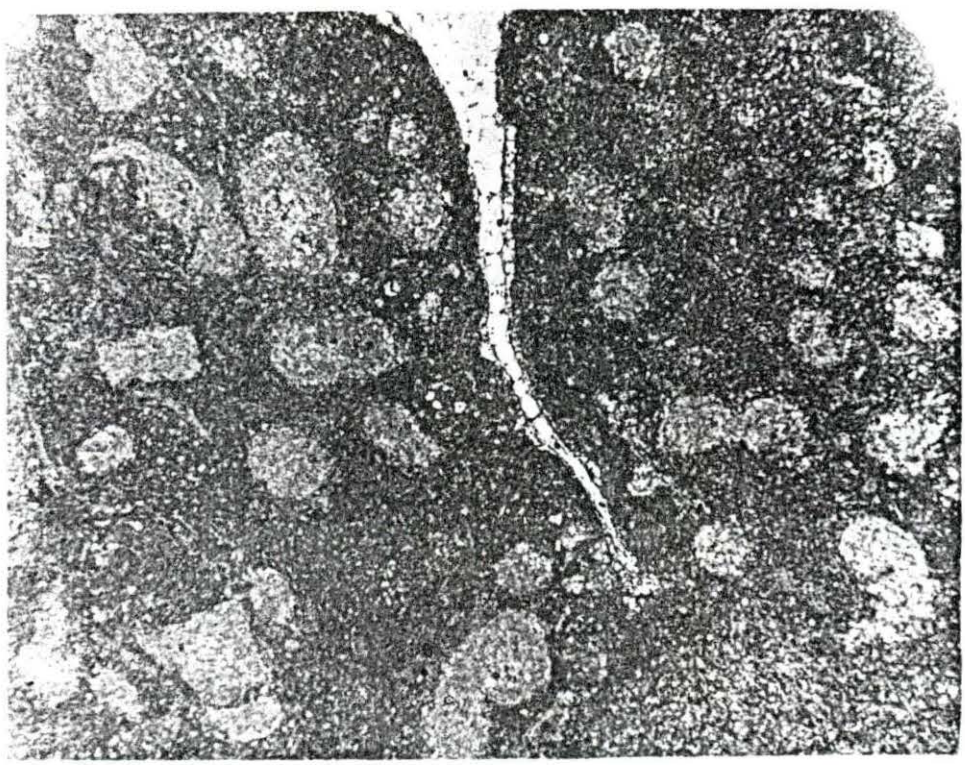
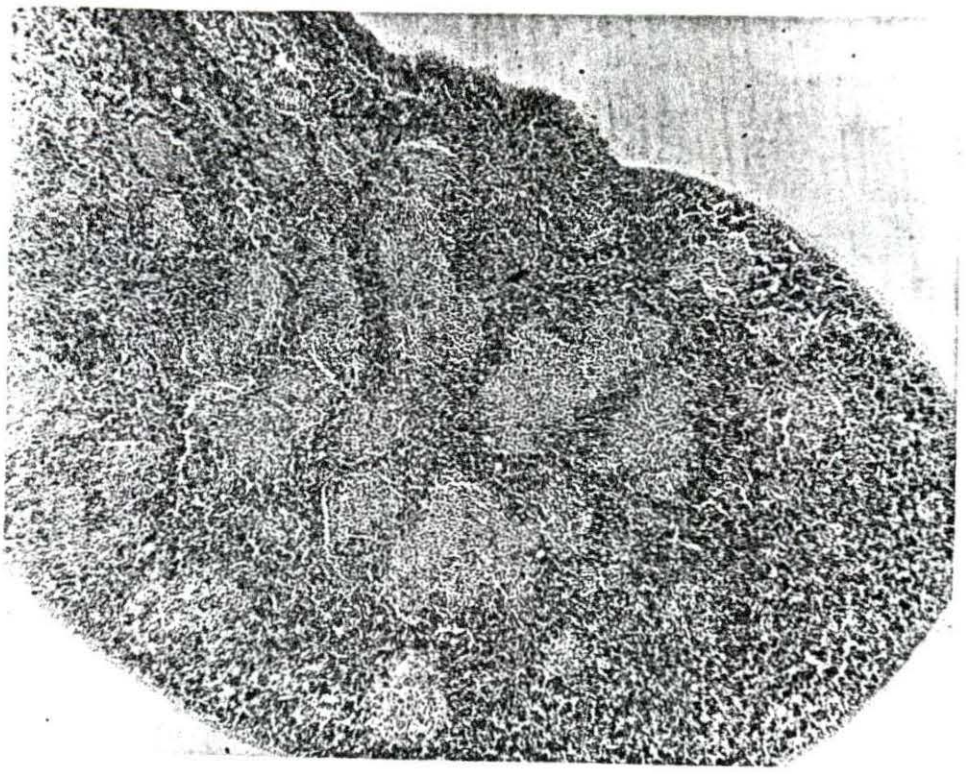


Figure 47. Deep inguinal lymph node 210 days after intraperitoneal inoculation with living M. paratuberculosis. Ziehl-Neelsen method. x60.

Figure 48. Distinct foci of reticuloendothelial tissue hyperplasia containing large Schaumann's bodies in an anterior mesenteric lymph node at 266 days after intraperitoneal inoculation with living M. paratuberculosis. Compare with Figures 21 and 37. Ziehl-Neelsen method. x60.



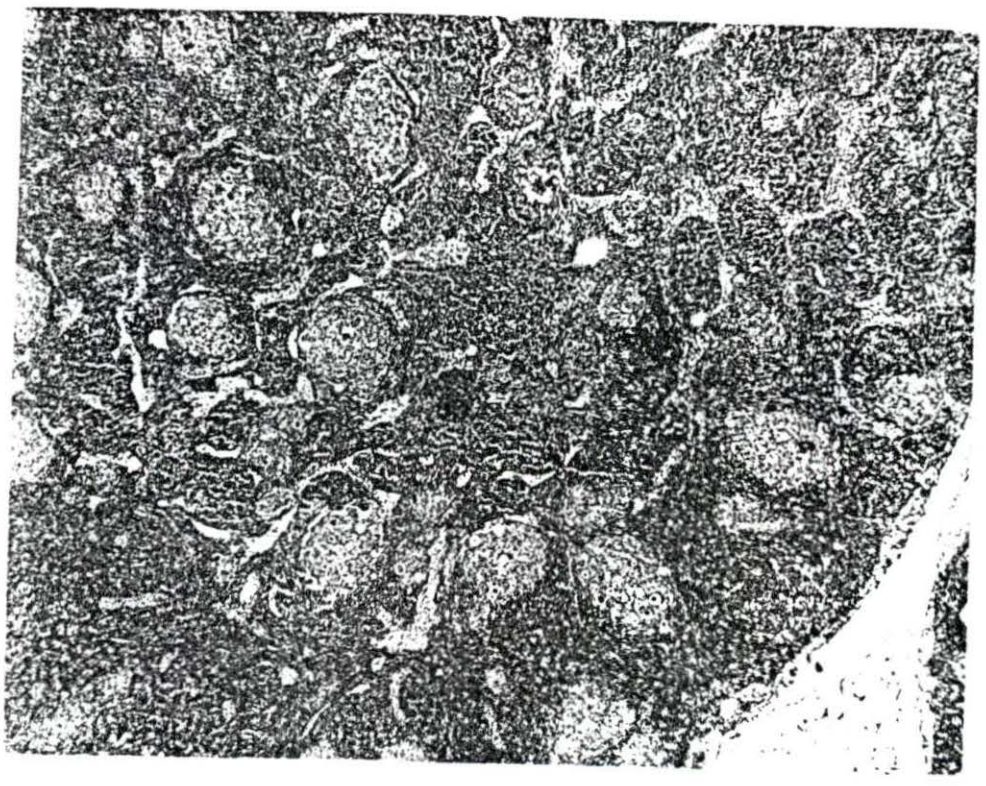
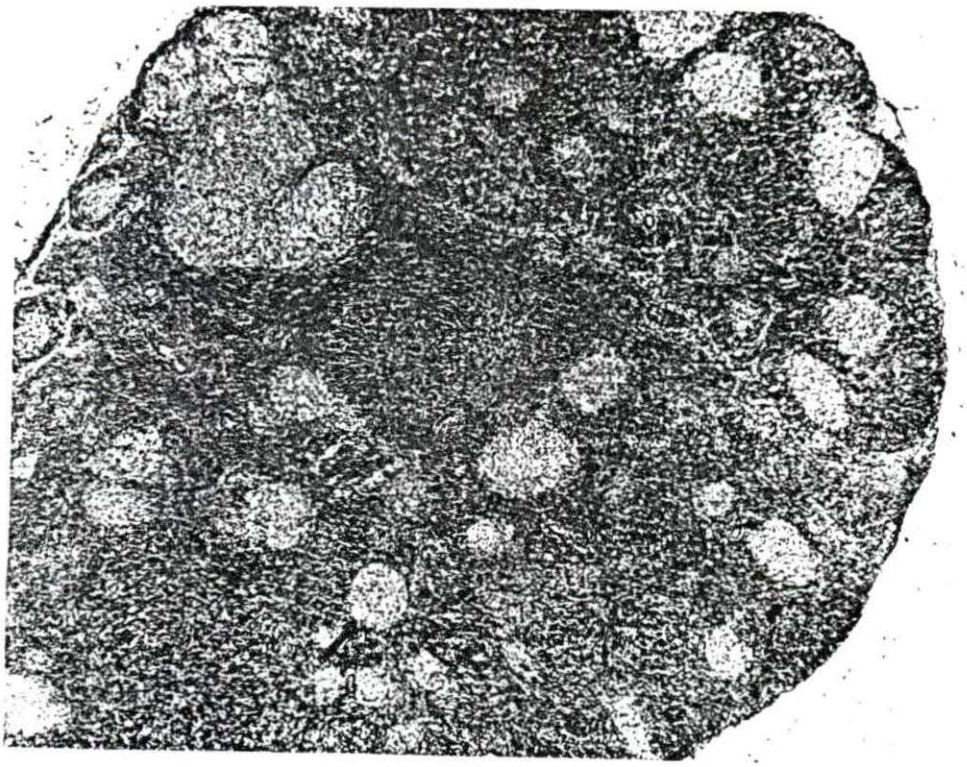


Figure 49. Prefemoral lymph node 266 days after intraperitoneal inoculation with living M. paratuberculosis. Harris' hematoxylin and eosin stain. x60.

Figure 50. Higher magnification of the foci of reticuloendothelia tissue hyperplasia indicated by arrow in Figure 49. Note the absence of lymphocytes in the area of hyperplasia, the small Schaumann's bodies, and the distinct border between the normal lymphoid tissue and the area of hyperplasia. Compare with Figures 14, 17, and 32. Harris' hematoxylin and eosin stain. x600.



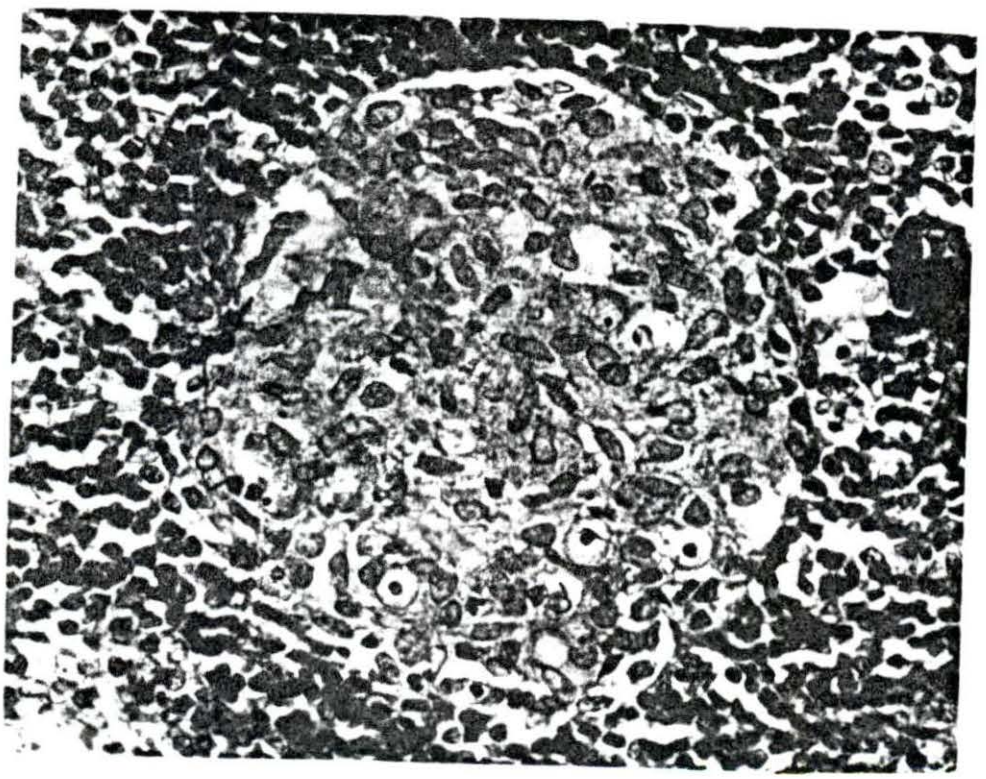
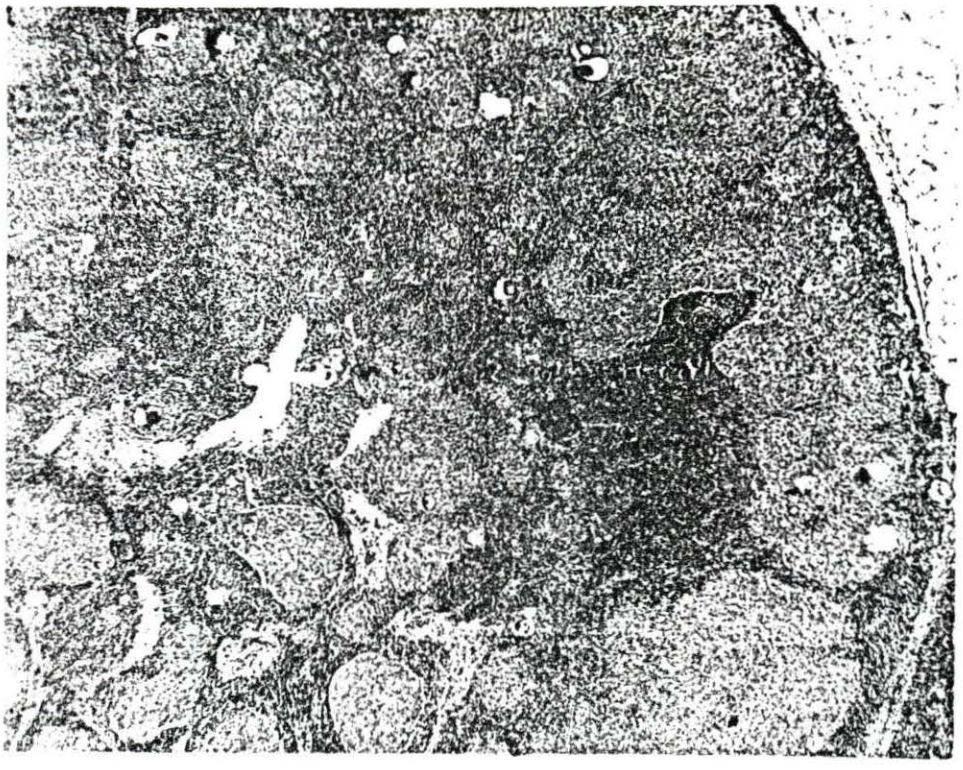
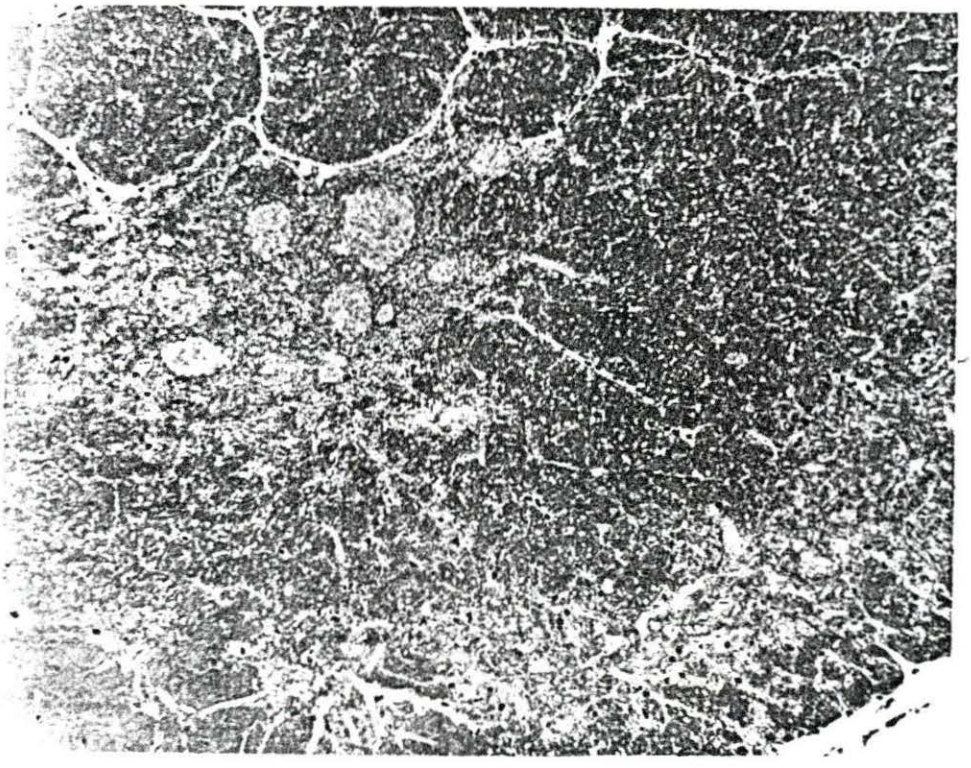


Figure 51. Extensive reticuloendothelial tissue hyperplasia in the ileocecal lymph node 329 days after intraperitoneal inoculation with living M. paratuberculosis. Note the large Schaumann's bodies and compare with Figure 23. Ziehl-Neelsen method. x60.

Figure 52. Thymus 154 days following intraperitoneal inoculation with living M. paratuberculosis. Compare with Figures 27 and 28. The small dark objects scattered throughout the thymus are mast cells. Ziehl-Neelsen method. x60.





lymphoid tissue in the sections of the mandibular and prefemoral lymph nodes examined at 154 days (Figure 46), of the prefemoral and axillary lymph nodes examined at 266 days (Figures 49 and 50), and of the prefemoral, axillary, parotid, and mandibular lymph nodes from the hamster examined at 329 days. The degree of hyperplasia reached its peak around 98 days and persisted at this level for the remainder of the experiment for both the visceral and regional lymph nodes. The reticuloendothelial tissue hyperplasia was extensive for the number of acid-fast bacilli present and the acid-fast bacilli were smaller and more sparsely distributed in the areas of hyperplasia at 266 and 329 days.

The character of the reticuloendothelial tissue hyperplasia is essentially the same as described in the hamsters inoculated intragastrically with living organisms. A more distinct border existed between the hyperplastic tissue and the normal lymphoid tissue in several of the lymph nodes from hamsters at 266 (Figures 49 and 50) and 329 days (Figure 51) that were inoculated intraperitoneally with living organisms than in hamsters at 266 and 329 days that were inoculated intragastrically with living organisms.

Schaumann's bodies were first observed at 98 days in both the visceral and regional lymph nodes (Figures 42 and 44). They increased in size and persisted throughout the remainder of the experiment. They were usually, but not always, confined to the area of reticuloendothelial tissue hyperplasia and, consequently, more were present in the visceral than in the regional lymph nodes. In the hamster examined at 329 days, suppurative foci surrounded a few of the Schaumann's bodies in the

mesenteric and deep inguinal lymph nodes and a few of the Schaumann's bodies were within foreign body giant cells of the Langhan type.

Post-capillary venules were found in most of the lymph nodes from hamsters included in this group. These venules were more accentuated in the regional than in the visceral lymph nodes and were most accentuated in those lymph nodes examined 98 to 154 days following inoculation.

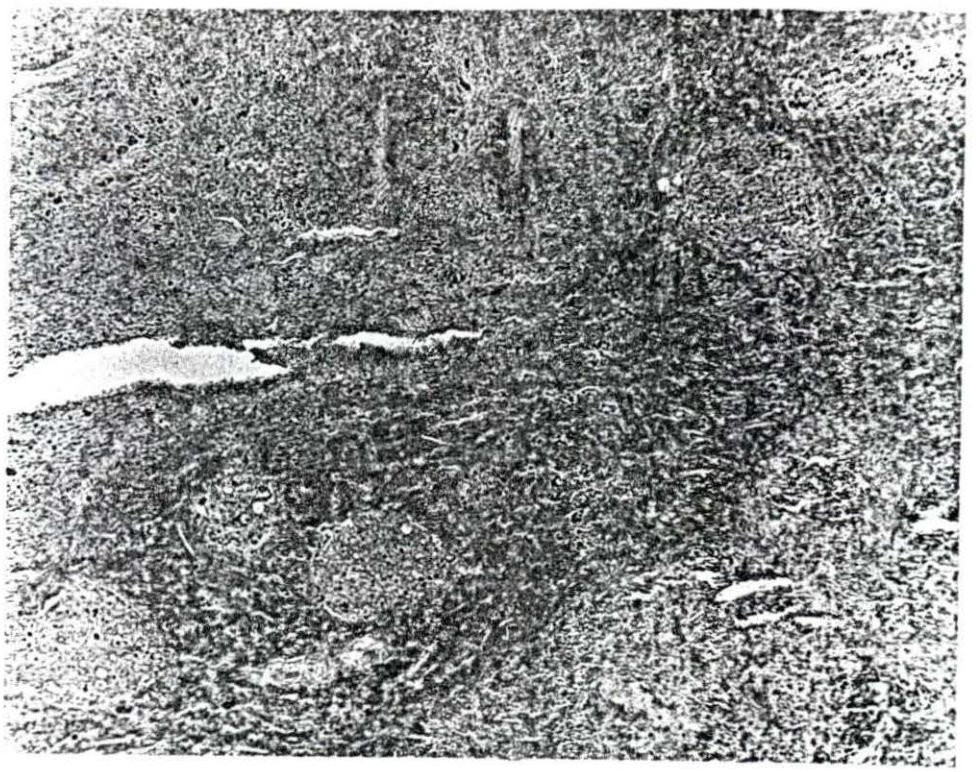
Foreign body giant cells were first observed in areas of reticuloendothelial tissue hyperplasia in visceral lymph nodes at 154 days and in regional lymph nodes at 266 days. They were prevalent in visceral lymph nodes and only occasionally encountered in regional lymph nodes.

No lesions of paratuberculosis or acid-fast bacilli were present in the thymus during the first 28 days. However, 2 minute suppurative foci were found in 1 lobule of the thymus at 28 days. Acid-fast bacilli were present within the cytoplasm of reticuloendothelial cells located in small foci of hyperplasia in the thymus from all of the hamsters examined at 56 through 329 days. The reticuloendothelial tissue hyperplasia was never as extensive as in the lymph nodes (Figure 52). About 5% of the thymic tissue was hyperplastic at 98 days and less was involved at other periods of examinations. Schaumann's bodies were found in the thymus only at 266 days and no foreign body giant cells were found during any of the intervals of examination.

Acid-fast bacilli were first observed in the spleen at 14 days. Both acid-fast bacilli and reticuloendothelial tissue hyperplasia were present at 56, 98 (Figures 53 and 54), 154, 210 (Figure 55), 266,

Figure 53. Spleen at 98 days after intraperitoneal inoculation with M. paratuberculosis. Note the areas of reticuloendothelial tissue hyperplasia within the lymph nodules. Compare with Figure 29. Ziehl-Neelsen method. x150.

Figure 54. Higher magnification of an area of reticuloendothelial tissue hyperplasia in the spleen in Figure 53. Note the small Schaumann's bodies and the phagocytized hemosiderin. Ziehl-Neelsen method. x600.



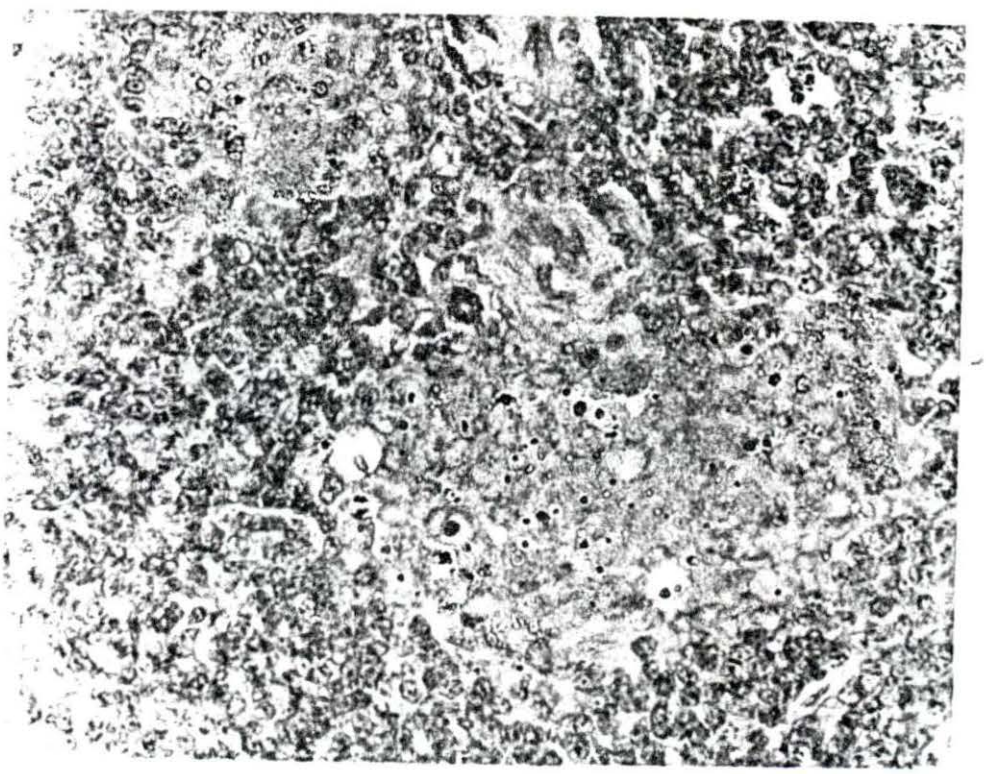
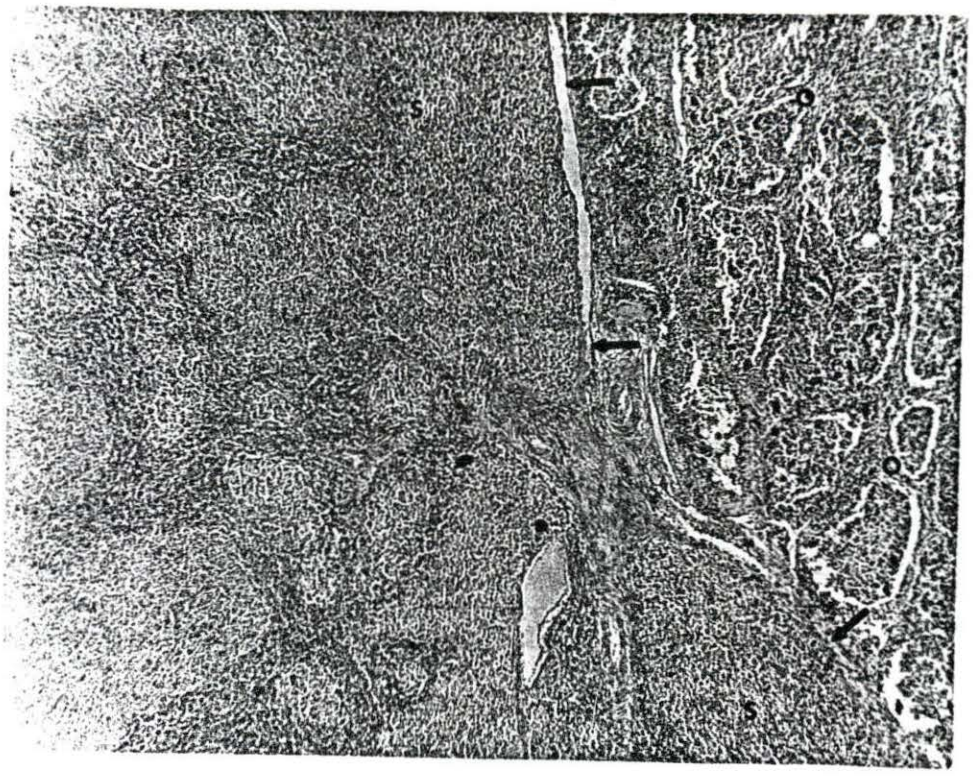
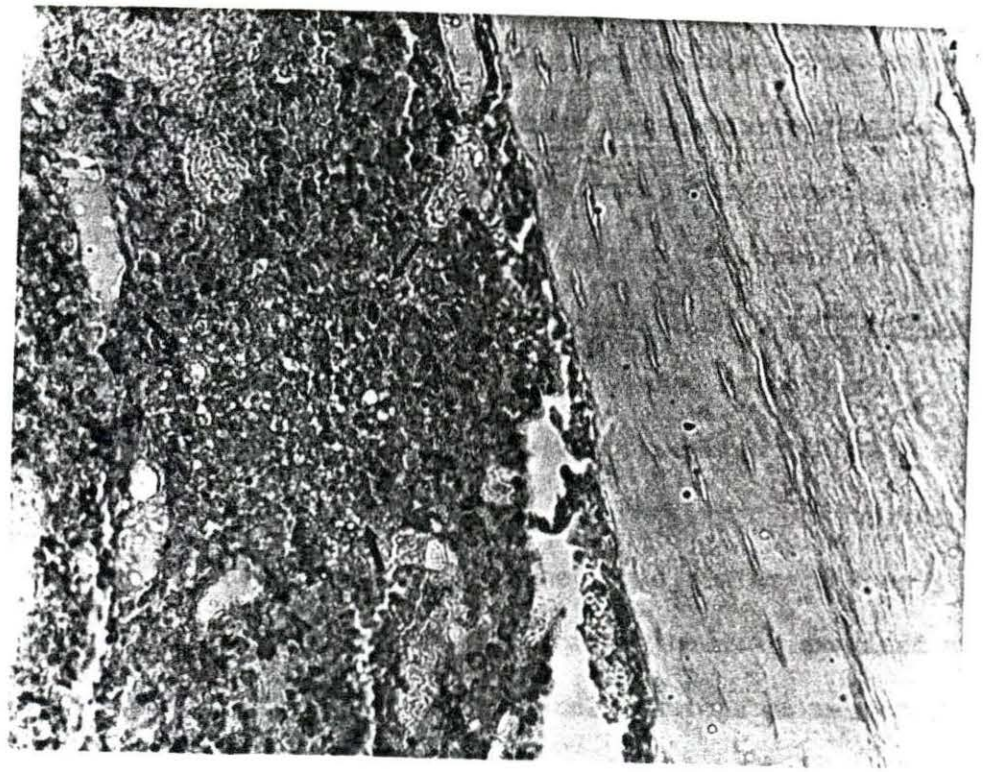


Figure 55. Portion of the omentum (O) adjacent to the hilus of the spleen (S) at 210 days following intraperitoneal inoculation with living M. paratuberculosis. Arrows point to the border of the spleen. Note the extensive granulomatous masses, which consisted mostly of macrophages, in the omentum. Also note the foci of reticuloendothelial tissue hyperplasia in the lymph nodules of the spleen. Ziehl-Neelsen method. x60.

Figure 56. Bone marrow in a sternebra at 98 days after intraperitoneal inoculation with living M. paratuberculosis. The small focus of reticuloendothelial tissue hyperplasia (arrows) contained acid-fast bacilli. Ziehl-Neelsen method. x375.


S
O
O
S



and 329 days. The foci of reticuloendothelial tissue hyperplasia were confined to lymph nodules of the spleen and were found in almost every lymph nodule contained in the sections. The percentage of lymphoid tissue of the lymph nodules occupied by hyperplasia was usually less than that observed in most lymph nodes.

Considerable quantities of hemosiderin were confined to the foci of hyperplasia in contrast to very little that could be demonstrated in adjacent tissue of the spleen from the hamsters examined at 98, 154, 210, 266, and 329 days. Schaumann's bodies were associated with the hyperplastic foci at 98, 154, 210, and 266 days. No foreign body giant cells were observed in the spleen from any of the hamsters inoculated intraperitoneally with living M. paratuberculosis.

No acid-fast bacilli, lesions, or lymphoid tissue were found in any of the histologic sections made of the tissues adjacent to or in the root of the tongue from hamsters included in this group.

Alimentary system No gross lesions were observed in the buccal pouches, mandibular and parotid salivary glands, forestomach, stomach, duodenum, jejunum, spiral and terminal colon, and pancreas of the hamsters inoculated intraperitoneally with living M. paratuberculosis. The Peyer's patches of the jejunum and ileum (when normal) were essentially as described in the hamsters that received living M. paratuberculosis intragastrically.

The terminal ileum was observed to be rigid, enlarged some 3 times, and intussuscepted into the cecum of the hamster examined at 8 days. The ileum was enlarged approximately 2 times normal at 14 days

and 4 times at 28 days. The ileum of the hamster examined at 56 days was small and whitish, and a constriction of the lumen was present. Several whitish subserosal foci about 1 mm. in diameter were observed in the ileum, some being within Peyer's patches, in each instance of ileal enlargement. The diameter of the lumen was increased and the ileal wall was thickened in enlargements of the ileum, but the lumen was stenotic while the wall remained thickened in the ileum that was smaller than normal. Adhesions between the ileal serosa and adjacent parietal and visceral peritoneum were present in the more severe cases. The ileum was grossly normal at 2, 4, 98, 154, 210, 266, and 329 days following intraperitoneal inoculation with living M. paratuberculosis.

The cecum was grossly normal in all of the hamsters with the possible exception of an over-all thickening of the wall of the cecum from the hamster examined at 266 days.

Grossly, the liver was normal in all of the hamsters examined from this group. However, fibrinous adhesions were present between the visceral peritoneum on the diaphragmatic surface of the liver and the parietal peritoneum of the diaphragm during the early part of the experiment.

The omentum from the hamsters examined at 2, 4, 8, and 14 days PI contained small nodules 1 to 3 mm. in size. The nodules were largest at 2 days, gradually diminished in size, and were not observed after 14 days.

Histologically (Table 16), acid-fast bacilli or lesions of paratuberculosis were not observed in the buccal pouches, mandibular

and parotid salivary glands, forestomach, stomach, and duodenum.

The frequency of Peyer's patches in histologic preparations is summarized in Table 17. No histologic lesions were noted until 14 days following inoculation. At this time, there was a generalized increase in neutrophils and a couple of indistinct foci of reticuloendothelial tissue hyperplasia were present. However, no acid-fast bacilli were found. No acid-fast bacilli or lesions were present in the Peyer's patches from the hamster examined at 28 days.

The first acid-fast bacilli were found within reticuloendothelial cells located in small and medium size foci of reticuloendothelial tissue hyperplasia that occupied 3 to 5% of the lymphoid tissue in the Peyer's patches at 56 days. Acid-fast bacilli and typical lesions of paratuberculosis were present at 98, 154, 210, and 329 days PI. Hyperplasia never exceeded 5% of the lymphoid tissue in any Peyer's patch and was present in all but 2 of the 40 Peyer's patches examined at 56, 98, 154, 210, 266, and 329 days. One of the Peyer's patches without hyperplasia was found at 154 days and the other at 266 days. The amount of hyperplasia was fairly constant in all of the Peyer's patches that were examined from 56 through 329 days. However, there was a decline in the number of acid-fast bacilli present at 266 days and only a very occasional bacillus could be found at 329 days. Acid-fast bacilli could only be found associated with reticuloendothelial tissue hyperplasia. The distinctiveness of the foci of reticuloendothelial tissue hyperplasia was reduced at 266 and 329 days due to infiltration of lymphocytes and plasma cells. Small and medium size

Table 17. The frequency of encountering lymphoid tissue in the intestinal tract of hamsters inoculated intraperitoneally with living M. paratuberculosis

Duration following inoculation[a] (days)	Organ examined				
	Duodenum[b]	Peyer's patches[c]	Cecum[b]	Spiral colon[b]	Terminal colon[b]
2	0/5	9/9	2/1	0/5	1/2
4	0/6	6/8	4/1	0/4	1/3
8	1/6	7/7	4/1	0/6	0/2
14	0/5	10/10	3/1	1/6	0/3
28	0/5	6/7	2/1	1/8	0/2
56	0/6	6/8	2/1	0/6	1/3
98	0/5	6/6	1/1	1/8	0/4
154	0/4	7/10	0/1	0/6	1/4
210	0/4	8/10	3/1	1/6	0/4
266	0/8	6/8	7/2	2/10	0/3
329	0/5	7/9	1/1	0/6	0/3

[a]One hamster was examined at each time interval.

[b]Number of lymph follicles demonstrated/number of cross-sections examined from specified segment of intestinal tract.

[c]Number of Peyer's patches histologically demonstrated/number of Peyer's patches that were observed grossly and embedded for sectioning.

Schaumann's bodies were present at 154, 210, 266, and 329 days, but foreign body giant cells of the Langhan type were present only at 210 days.

The frequency of lymphoid nodules in sections of the cecum is summarized in Table 17. Small clumps of acid-fast bacilli were found within isolated reticuloendothelial cells in 1 of the 4 lymph nodules found in the hamster at 8 days and in very small foci of reticuloendothelial tissue hyperplasia in 1 of 2 lymph nodules found in the hamster examined at 56 days. Although a small amount of reticuloendothelial tissue hyperplasia was present in the lymph nodule at 98 days, the only acid-fast bacilli present were within macrophages in the submucosa outside of the lymph nodule. No acid-fast bacilli, lesions or lymph nodules were demonstrated at 154 days. Schaumann's bodies and a small amount of reticuloendothelial tissue hyperplasia were present at 210 days, but no acid-fast bacilli were found.

Acid-fast bacilli were present in the cytoplasm of reticuloendothelial cells of both lymph nodules of the submucosa and mucosa of the cecum from the hamster examined at 266 days. There was diffuse infiltration of lymphocytes and reticuloendothelial cells throughout the mucosa. Few Schaumann's bodies were present and no foreign body giant cells were found. No lesions or acid-fast bacilli were present in the cecum of the hamster examined at 329 days.

Frequency of lymph nodules in sections of the spiral and terminal colons is summarized in Table 17. The only acid-fast bacilli demonstrated in the spiral colon from any of the hamsters were 4 bacilli

within the cytoplasm of reticuloendothelial cells in 1 of the 2 lymph nodules examined 266 days following inoculation. Very little reticuloendothelial tissue hyperplasia was present. Only 1 acid-fast bacillus was found within the cytoplasm of a reticuloendothelial cell that was present in 1 of few indistinct foci of reticuloendothelial tissue hyperplasia of the lymph nodule from the terminal colon in the hamster examined at 154 days.

One small clump of acid-fast bacilli was demonstrated in a Kupffer cell of the liver at 2 and 8 days following inoculation. There was a moderate periportal lymphocytic infiltration around most of the hepatic triads and a slight proliferation of bile ducts in the liver from the hamster examined at 14 days. Several acid-fast bacilli were observed in the reticuloendothelial cells in the areas of lymphocytic infiltration and occasionally in the Kupffer cells lining the sinusoids. Many acid-fast bacilli were also found in macrophages located in fibrinous masses between the lobes and diaphragmatic surface of the liver. The degree of periportal lymphocytic infiltration at 56 days was about the same as at 14 days, but small indistinct foci of reticuloendothelial tissue hyperplasia were present in the areas of lymphocytic infiltration. The cytoplasm of the reticuloendothelial cells in those foci contained several acid-fast bacilli. Also, many acid-fast bacilli were observed within macrophages located in a granulomatous mass on the diaphragmatic surface of the liver. Several Schaumann's bodies were also present.

At 98 days, almost every hepatic triad was surrounded by, or had

adjacent to it, a focus of lymphocytes that was often almost obliterated or displaced by reticuloendothelial tissue hyperplasia. Acid-fast bacilli were very sparse and only 1 bacillus could be demonstrated for about every 10 foci of reticuloendothelial tissue hyperplasia. No Schaumann's bodies or giant cells were present.

At 154 and 210 days PI, about 25% of the hepatic triads were associated with lymphocytic infiltration, and each focus of lymphocytes contained a focus of reticuloendothelial tissue hyperplasia. A few acid-fast bacilli were demonstrated in the reticuloendothelial cells at 154 days, but none could be found at 210 days. Schaumann's bodies and foreign body giant cells were present at 154 days, but only Schaumann's bodies were present at 210 days.

At 266 and 329 days, several small lymphocytic foci were present and a few of these foci contained a small focus of reticuloendothelial tissue hyperplasia. Only an occasional acid-fast bacillus was demonstrated in these reticuloendothelial cells. No Schaumann's bodies or foreign body giant cells were present.

All of the Schaumann's bodies observed in the liver from hamsters in this group were confined to the areas of reticuloendothelial tissue hyperplasia. Considerable quantities of phagocytized hemosiderin were present in the foci of reticuloendothelial tissue hyperplasia at 329 days.

Many acid-fast bacilli were observed inter- and intracellularly in portions of the omentum adjacent to the stomach and spleen from the hamster examined at 2 days. The cellular response consisted of a diffuse

infiltration of neutrophils and macrophages. Many intercellular and few intracellular acid-fast bacilli, in the absence of any demonstrable cellular response, were still present in omentum adjacent to the stomach at 4 days. The 8-day examination revealed that most of the acid-fast bacilli were in small clumps within numerous macrophages that were forming small foci of reticuloendothelial tissue hyperplasia. By 14 days, the cellular reaction of the omentum was extensive and formed large foci of large pale eosinophilic staining cells that were indistinguishable from the reticuloendothelial tissue hyperplasia of the lymph nodes. However, these cells contained many more acid-fast bacilli than any of those found in the lymph nodes, and the foci of hyperplasia contained more lymphocytes.

Reticuloendothelial hyperplasia of the omentum from hamsters examined at 56 and 98 days was more extensive than at 14 days and occupied 75 to 80%. A decrease in the number of acid-fast bacilli per unit area was noted at 98 days. The hyperplasia was still extensive at 154 and 210 days (Figure 55), but acid-fast bacilli were very sparse.

No lesions or acid-fast bacilli were found in the omentum from hamsters examined at 28, 266, and 329 days following intraperitoneal inoculation with living M. paratuberculosis.

Few small Schaumann's bodies were present at 14 and 56 days PI. Several small and medium size Schaumann's bodies were present at 98 and 154 days and the first foreign body giant cell of the Langhan type was found in the omentum at 154 days. Many small, medium, and large size Schaumann's bodies along with many foreign body giant cells were present at 210 days.

Cardiovascular and hemopoietic system No gross lesions were observed in the heart or bone marrow of the lumbar vertebrae and sternebrae.

No microscopic lesions or acid-fast bacilli were found in the heart from the hamsters included in this group.

No microscopic lesions or acid-fast bacilli were found in the bone marrow of the hamsters examined at 2, 4, 8, 14, 28, and 154 days PI. Several small acid-fast bacilli were located within the cytoplasm of reticuloendothelial cells that were confined to small foci of hyperplasia in the bone marrow of both the sternebrae and lumbar vertebrae examined at 56, 98 (Figure 56), and 210 days PI. No bone marrow was included in sections of sternebrae of hamsters examined at 266 and 329 days, but lesions and a few acid-fast bacilli were present in the bone marrow of the lumbar vertebrae. Very small foci of reticuloendothelial tissue hyperplasia containing 2 or 3 acid-fast bacilli were present in the bone marrow of the hyoid bones in the root of the tongue examined 98, 266, and 329 days.

Hyperplasia of bone marrow was the least extensive of any tissue examined that contained acid-fast bacilli and reticuloendothelial tissue hyperplasia. Hyperplasia occasionally occupied as much as 3% of the bone marrow but never exceeded 5%. No Schaumann's bodies or foreign body giant cells were present in the bone marrow included in sections from hamsters of this group.

Nervous system No acid-fast bacilli or lesions or paratuberculosis were present in the spinal cord, cerebrum, cerebellum or meninges of the hamsters inoculated intraperitoneally with living M. paratuberculosis.

Respiratory system The lungs from all hamsters in this group were grossly normal.

Microscopically, no acid-fast bacilli were found in any of the lungs from hamsters included in this group. The only lesions were slight edema and a marked increase in neutrophils in the capillaries of the lungs from the hamster examined at 14 days and marked edema and hyperplasia of bronchial epithelium in the lungs from the hamster examined at 210 days.

Urogenital system No gross or microscopic lesions were present in kidneys from any hamsters included in this group. No acid-fast bacilli were found.

The urinary bladder of all hamsters in this group was grossly normal and contained 1 to 3 cc. of yellowish viscous fluid. Histologically, the only lesions were present on the serosal surface of the urinary bladder. Few acid-fast bacilli, located within macrophages, were in a small granulomatous mass on the serosa of the bladder from the hamster at 2 days. Two suppurative foci, 1 of which contained several acid-fast bacilli within macrophages, were present in the peritoneum of the bladder from the hamster at 56 days. Granulomatous foci containing only Schaumann's bodies were present on the serosa of the urinary bladder from the hamster at 98 and 210 days.

The uterus was grossly normal in hamsters in this group. The only acid-fast bacilli that were found were in macrophages located in the suspensory ligaments of the uterus from the hamster at 14 days. Schaumann's bodies were present in the suspensory ligaments at 210 days.

Skeletal system No lesions or acid-fast bacilli were observed in the osseous tissue of the lumbar vertebrae, hyoid bones, or sternebrae of any of the hamsters inoculated intraperitoneally with living M. paratuberculosis.

Special sense organs The eyes from hamsters in this group were grossly and histologically normal. No acid-fast bacilli were found.

The infraorbital lacrimal glands were grossly normal. Histologically, a few to several corpora amylacia were present in the ducts of those glands from all but 2 hamsters. No corpora amylacia were present in the infraorbital lacrimal glands from hamsters examined at 4 and 266 days.

No lesions or acid-fast bacilli were observed in the exorbital lacrimal glands from the hamsters inoculated intraperitoneally with living M. paratuberculosis.

Heat-Killed Organisms Administered Intraperitoneally

Clinical findings

The hamsters examined at 2, 4, 56, 98, 154, 210, 266, and 329 days PI were clinically normal.

The hamsters examined at 8 and 14 days PI had a watery diarrhea at 3 and 10 days respectively prior to postmortem examination. In the hamster examined at 8 days, the diarrhea terminated with an eversion of the rectum just prior to postmortem examination. The hamster examined at 28 days had no diarrhea but did show signs of illness such as lethargy, rough hair coat, shivering, and became comatose for a period of about 6 hours prior to postmortem examination. The diarrhea was not attributed to M. paratuberculosis. No clinical signs of paratuberculosis were observed in the hamsters included in this group.

Necropsy findings

Lymphatic system All lymph nodes from hamsters examined at 2, 4, 8, 56, 154, 266, and 329 days PI were grossly normal. The anterior mediastinal lymph nodes were slightly enlarged in the hamster examined at 14 days and the ileocecal lymph node from the hamster at 28 days was enlarged 2 to 3 times normal size. In the hamster examined at 98 days, the anterior mediastinal lymph nodes were enlarged 3 times normal size and the anterior mesenteric and ileocecal lymph nodes were 1 to 2 times larger than normal.

The thymus from all hamsters were normal.

No gross lesions were found in the spleen or the tissues adjacent to or in the root of the tongue from the hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis.

Histologically (Table 18), several small acid-fast bacilli were present in small clumps of 2 or 3 in the cytoplasm of isolated reticuloendothelial cells that were evenly distributed throughout each of the

Table 18. Distribution of organisms and lesions in hamsters inoculated intraperitoneally with 1 mg. of heat killed M. paratuberculosis

Organ	Time (days)										
	2	4	8	14	28	56	98	154	210	266	329
Ant. mediastinal lymph nodes	+	+	++	+	+	+++	+++	+++	++	o	-
Ant. mesenteric lymph node	-	+	+	+	+	+++	+++	++	-	-	o
Mesenteric lymph nodes, other	-	++	+	+	+	++	++	++	-	++	o
Medial iliac lymph nodes	-	-	+	-	-	-	-	-	-	-	-
Deep inguinal lymph nodes	-	-	+	-	-	-	-	-	-	-	-
Prefemoral lymph nodes	-	-	-	+	-	-	-	-	-	-	-
Axillary lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Mandibular lymph nodes	-	-	+	-	-	-	-	o	-	-	-
Parotid lymph nodes	-	-	-	-	-	-	-	-	-	-	-
Spleen	+	-	+	-	+	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	-	-	-	-	-
Base of tongue	-	-	-	-	-	-	-	-	-	-	-
Buccal pouches	-	-	-	-	-	-	-	-	-	-	-
Mandibular salivary glands	-	-	-	-	-	-	-	-	-	-	-
Parotid salivary glands	-	-	-	-	-	-	-	-	-	-	-
Fore stomach	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-
Duodenum (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Peyer's patches	-	-	-	-	-	-	-	-	-	-	-
Cecum (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Spiral colon (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Terminal colon (lymph nodules)	-	-	-	-	-	-	-	-	-	-	-
Liver	-	+	+	-	+	++	++	++	++	o	o
Pancreas	-	-	-	-	-	-	-	-	-	-	-
Omentum	+	++	++	+++	+++	+++	+++	+++	+++	+++	++
Heart	-	-	-	-	-	-	-	-	-	-	-
Sternal bone marrow	-	-	-	-	-	-	-	-	-	-	-
Vertebral bone marrow (lumbar)	-	-	-	-	+	-	-	-	-	-	-
Brain	-	-	-	-	-	-	-	-	-	-	-
Spinal cord (lumbar)	-	-	-	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	-	-	-	-
Kidneys	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	-	-	-	-
Eye	-	-	-	-	-	-	-	-	-	-	-
Infraorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-
Exorbital lacrimal gland	-	-	-	-	-	-	-	-	-	-	-

- = No organisms or tissue reaction
+ = Organisms within individually located macrophages
++ = Tissue reactions occupying less than 3% of the normal tissue
+++ = Tissue reactions occupying more than 3% of the normal tissue
o = Old healing lesion without organisms

3 anterior mediastinal lymph nodes from hamster examined at 2 days following inoculation. Approximately the same number of acid-fast bacilli with a similar distribution was present in the anterior mediastinal and anterior mesenteric lymph nodes from the hamster at 4 days following inoculation. The ileocecal lymph nodes from this hamster contained several small clumps of acid-fast bacilli within reticuloendothelial cells that were located in indistinct foci of hyperplasia.

The anterior mediastinal lymph nodes from the hamster examined at 8 days contained many acid-fast bacilli occurring singly and in small clumps of 2 or 3 within the cytoplasm of reticuloendothelial cells in small but distinct foci of hyperplasia. However, the anterior mesenteric, ileocecal, medial iliac, deep inguinal and mandibular lymph nodes contained only a few acid-fast bacilli within the cytoplasm of isolated reticuloendothelial cells that were evenly distributed throughout each of the lymph nodes.

No reticuloendothelial tissue hyperplasia was present in the lymph nodes from the hamster examined at 14 days, but a few acid-fast bacilli were present in isolated reticuloendothelial cells of the anterior mediastinal, anterior mesenteric, ileocecal, and prefemoral lymph nodes. About the same number and distribution of acid-fast bacilli were present in the anterior mediastinal, anterior mesenteric, and ileocecal lymph nodes from the hamster examined at 28 days as were found in the hamster examined at 14 days.

The anterior mediastinal, anterior mesenteric and ileocecal lymph nodes examined at 56, 98, and 154 days had about the same number and

distribution of lesions and acid-fast bacilli. The number of acid-fast bacilli per unit area was about the same as in the hamsters from this group that were examined earlier and there were more bacilli in the anterior mediastinal than in either the anterior mesenteric or ileocecal lymph nodes. Also, more acid-fast bacilli were present in lymph nodes examined at 56 days than at 154 days. The degree of hyperplasia involved anywhere from 10 to 50% of the lymphoid tissue in the anterior mediastinal lymph nodes and from 10 to 40% in the anterior mesenteric lymph nodes. Few small Schaumann's bodies and occasional foreign body giant cells were present and usually associated with the areas of hyperplasia. A considerable quantity of hemosiderin was present within the reticuloendothelial cells in the anterior mesenteric lymph node at 154 days. Few indistinct foci of reticuloendothelial tissue hyperplasia, containing several lymphocytes, were also present in the mandibular lymph nodes at 154 days following inoculation.

Only the anterior mediastinal lymph nodes from the hamster at 210 days and the ileocecal lymph nodes at 266 days contained acid-fast bacilli or reticuloendothelial tissue hyperplasia. Acid-fast bacilli were very sparse. Few small Schaumann's bodies and occasional foreign body giant cells were present. Some of the Schaumann's bodies appeared to be disintegrated. No acid-fast bacilli or reticuloendothelial tissue hyperplasia were present in the anterior mediastinal lymph node at 266 days, but a foreign body giant cell was found. Some hemosiderin was present in the reticuloendothelial cells of the lymph nodes from both hamsters.

No acid-fast bacilli, Schaumann's bodies, or foreign body giant cells were present in any of the lymph nodes from the 3 hamsters examined at 329 days following inoculation. However, indistinct foci of reticuloendothelial tissue hyperplasia, infiltrated with a few lymphocytes, were present in the anterior mesenteric and mesenteric lymph nodes (Figures 57 and 58).

Post-capillary venules were present in the lymph nodes from hamsters included in this group. They were most accentuated in the lymph nodes of hamster examined at 154 days and were more prominent in the regional than in the visceral lymph nodes.

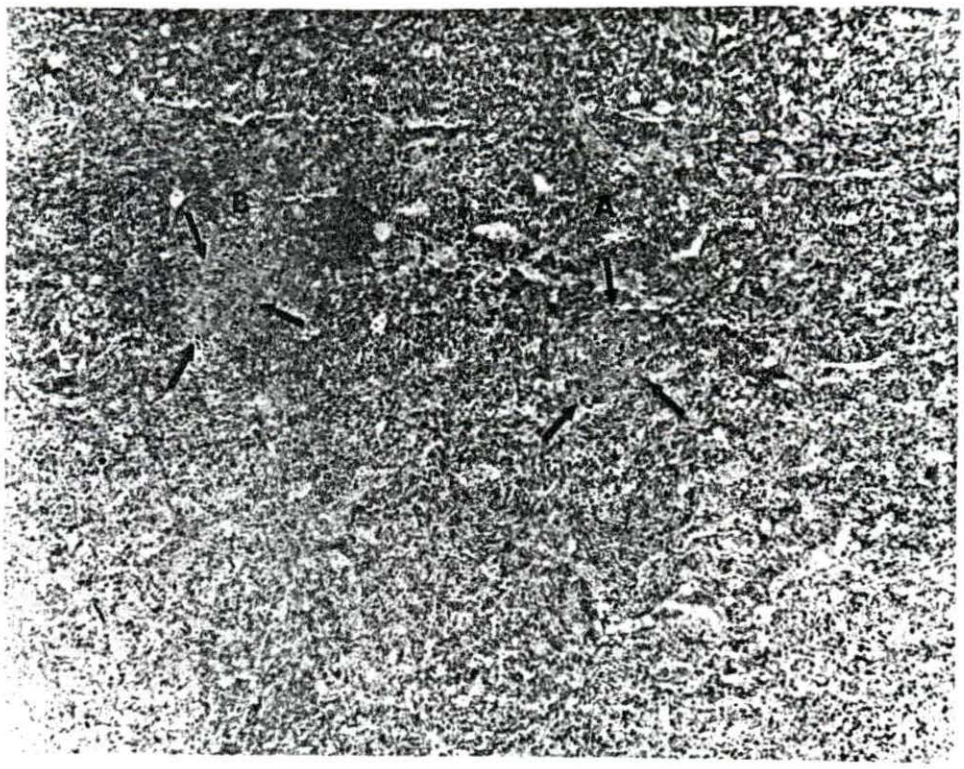
No acid-fast bacilli or lesions of paratuberculosis were present in the thymus from any of the hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis.

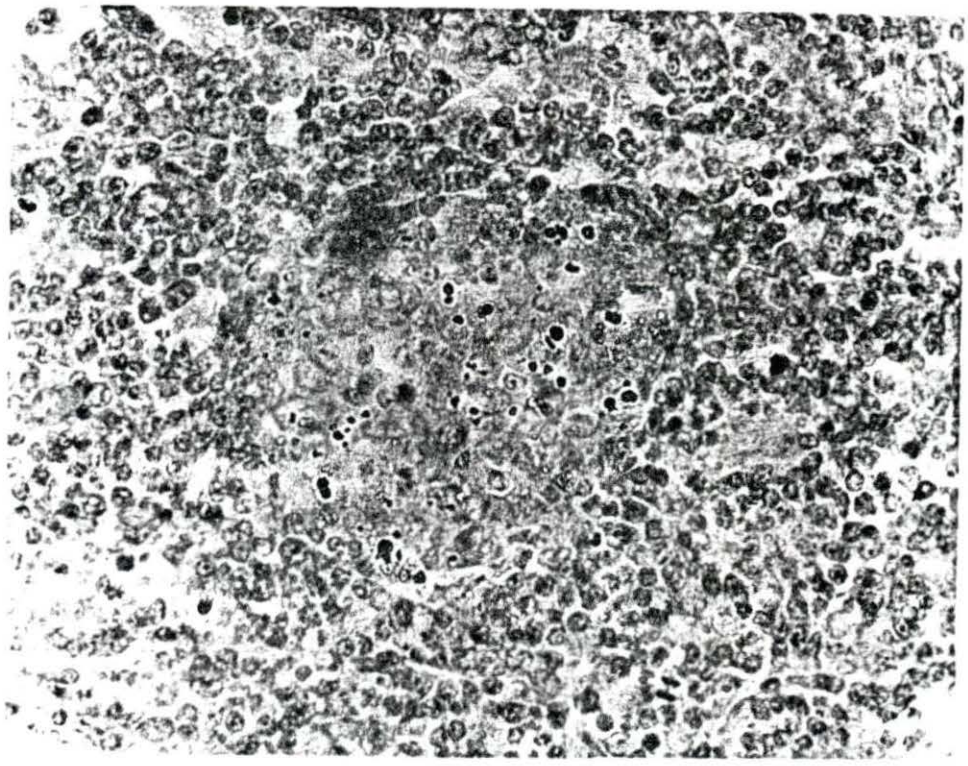
Small clumps of 2 or 3 acid-fast bacilli were present in the cytoplasm of isolated reticuloendothelial cells lining the sinusoids of the red pulp of the spleen from the hamster examined at 2 days. A few acid-fast bacilli were present within isolated reticuloendothelial cells in the cells in the lymph nodules of the spleen from hamsters at 8 and 28 days. No lesions of paratuberculosis or acid-fast bacilli were present in the spleen from any other hamsters included in this group. Considerable quantities of hemosiderin were present in reticuloendothelial cells of the spleen in hamsters examined at 154, 266, and 329 days.

No acid-fast bacilli, lesions, or lymphoid tissue were found in any of the histologic sections of tissues adjacent to or included in the root of the tongue of hamsters from this group.

Figure 57. Indistinct foci (arrows) of reticuloendothelial tissue hyperplasia in the anterior mesenteric lymph node at 329 days after intraperitoneal inoculation with heat-killed M. paratuberculosis. Compare with Figures 23, 38, and 51. Ziehl-Neelsen method. x150.

Figure 58. Higher magnification of a regressing lesion (A) in Figure 57. Note the absence of Schaumann's bodies and foreign body giant cells. Phagocytized hemosiderin pigments were present but no acid-fast bacilli were found. Ziehl-Neelsen method. x600.


B
A



Alimentary system No gross lesions were observed in the buccal pouches, mandibular and parotid salivary glands, forestomach, stomach, duodenum, jejunum, cecum, spiral and terminal colons, and pancreas of the hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis. The Peyer's patches were essentially the same as described above for the hamsters inoculated intragastrically with living M. paratuberculosis.

The ileum was rigid and enlarged about 3 times normal size in the hamsters examined at 14 and 28 days following inoculation. There were adhesions between the serosa of adjacent organs and the parietal peritoneum. Several small whitish foci about 1 to 3 mm. in diameter were present in the subserosa. The wall was 3 to 4 times thicker than normal while the lumen was dilated about 2 times normal size. The ileum examined at 56 days was whitish, smaller, the wall was thicker than normal, and the lumen was constricted. The ileum was normal in all other hamsters of this group.

There was an antemortem intussusception involving the complete terminal colon of the hamster examined at 8 days.

The liver was grossly normal in all except 1 hamster included in this group. An hepatic cyst was present in the liver of 1 of the 3 hamsters examined at 329 days.

Mesenteries and omentums were cloudy and a fibrinous peritonitis was present in the hamster examined at 2 days following inoculation.

Histologically (Table 18), no acid-fast bacilli or lesions of paratuberculosis were observed in the buccal pouches, mandibular and

parotid salivary glands, forestomach, stomach, Peyer's patches, cecum, spiral and terminal colons, or pancreas of any of the hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis.

The frequency of Peyer's patches, both grossly and histologically, is summarized in Table 19 for each hamster of this group. Table 19 also summarizes the frequency that lymphoid tissue was demonstrated in the submucosa of the duodenum, cecum, and spiral and terminal colons.

Occasional periportal foci of lymphocytes were present in the liver from hamsters examined at 4, 8, and 28 days. An occasional acid-fast bacillus was demonstrated in isolated reticuloendothelial cells in these foci. Small foci of reticuloendothelial tissue hyperplasia that contained an occasional acid-fast bacillus were present in the area of lymphocytic infiltration in the liver from hamsters examined at 56 and 98 days. The amount of reticuloendothelial tissue hyperplasia, that contained Schaumann's bodies and foreign body giant cells, was about the same at 154 and 210 days as at 56 and 98 days and no acid-fast bacilli could be found. The amount of periportal lymphocytic infiltration remained relatively constant and never involved over 10% of the hepatic triads of the livers examined at 4, 8, 28, 56, 98, 154, and 210 days. Schaumann's bodies were present at 98 days and thereafter. Occasional periportal foci of lymphocytes were present at 266 and 329 days. Slight reticuloendothelial tissue hyperplasia, an occasional Schaumann's body, and foreign body giant cell were present. However, no acid-fast bacilli were found. No granulomatous lesions, as often found with living organisms inoculated intraperitoneally, were found on the diaphragmatic surface of any livers.

Table 19. The frequency of encountering lymphoid tissue in the intestinal tract of hamsters inoculated intraperitoneally with heat killed M. paratuberculosis

Duration following inoculation[a] (days)	Organ examined				
	Duodenum[b]	Peyer's patches[c]	Cecum[b]	Spiral colon[b]	Terminal colon[b]
2	0/6	8/9	0/1	0/5	0/3
4	0/6	8/8	0/1	1/4	0/5
8	0/4	8/9	1/1	1/3	2/3
14	0/6	6/7	2/1	1/3	0/5
28	0/3	5/6	1/1	0/6	1/5
56	0/5	7/8	2/1	2/4	0/3
98	0/6	8/8	2/1	2/8	0/4
154	0/4	7/11	1/1	1/6	1/4
210	0/5	8/9	1/1	0/6	1/4
266	0/5	5/6	3/2	2/8	0/3
329	0/5	8/8	1/2	0/8	2/6
329	0/5	10/10	1/2	1/8	1/4
329	0/4	9/9	1/2	2/6	0/4

[a]One hamster was examined at each time interval.

[b]Number of lymph follicles demonstrated/number of cross-sections examined from specified segment of intestinal tract.

[c]Number of Peyer's patches histologically demonstrated/number of Peyer's patches that could be observed grossly and were embedded for sectioning.

Many small clumps of acid-fast bacilli were present, both inter- and intra-cellularly in the cytoplasm of macrophages, in the omentum of the hamster examined at 2 days and a diffuse infiltration of neutrophils was present. Four and 8 days following inoculation, distinct foci of macrophages, many containing small clumps of acid-fast bacilli, were found in the omentum adjacent to the stomach. The omentum of the hamsters examined at 14, 28, 56, 98, and 154 days contained many acid-fast bacilli within reticuloendothelial cells that formed confluent foci of hyperplasia which sometimes occupied as much as 80 to 90% of the omental tissue included in the sections.

The reticuloendothelial tissue hyperplasia was still as extensive at 210 and 266 days but very few acid-fast bacilli were present. The omentum of the hamsters examined at 329 days did not contain any acid-fast bacilli but did have several large foci of reticuloendothelial tissue hyperplasia. The hyperplastic foci had indistinct borders and contained foreign body giant cells that enclosed several large Schaumann's bodies.

Several small clumps of acid-fast bacilli were found within isolated macrophages in the mesentery of the duodenum, jejunum, cecum, and colon of the hamsters examined at 2, 4, 8, 14, 28, 56, 98, and 154 days.

Cardiovascular and hematopoietic system No gross lesions were found in the heart or the bone marrow of the lumbar vertebrae or sternebrae of any hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis.

No microscopic lesions or acid-fast bacilli were found in the heart from any of the hamsters included in this group. A clump of 3 acid-fast bacilli were demonstrated within a reticuloendothelial cell in the bone marrow of a hyoid bone and 1 clump of 6 acid-fast bacilli were found within a reticuloendothelial cell in the bone marrow of a lumbar vertebrae from the hamster examined at 28 days. No acid-fast bacilli or lesions were found in the bone marrow of any other hamsters included in this group.

Nervous system No lesions were found in the lumbar spinal cord, cerebrum, cerebellum, and meninges.

Respiratory system Lungs from all hamsters in this group were grossly normal.

Microscopically, no acid-fast bacilli were found in lungs from hamsters included in this group. The only lesions noted were a moderate edema in 1 lung from the hamster examined at 14 days and a marked increase in the number of neutrophils in the capillaries of the lung from the hamster examined at 28 days. A large quantity of hemosiderin was present within macrophages in the lungs of 1 of the hamsters at 329 days.

Urogenital system No lesions or acid-fast bacilli were present in the kidneys of any hamsters.

The urinary bladder of all hamsters in this group were grossly normal and contained from 1 to 3 cc. of yellowish thick fluid. Histologically, all acid-fast bacilli and lesions were present in the peritoneum. Several acid-fast bacilli were present within isolated macrophages in granulomatous lesions of the peritoneum at 14 days.

No acid-fast bacilli were found but reticuloendothelial tissue hyperplasia, Schaumann's bodies, and foreign body giant cells were present in the peritoneum at 98 days. No acid-fast bacilli or reticuloendothelial tissue hyperplasia were present but a few Schaumann's bodies were found in the peritoneum at 154 days.

The uterus was normal in hamsters in this group.

Skeletal system No lesions or acid-fast bacilli were observed in the osseous tissue of the lumbar vertebrae, sternebrae, or hyoid bones of any of the hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis.

Special sense organs The eyes from hamsters in this group were normal. No acid-fast bacilli were observed.

Infraorbital lacrimal glands of hamsters in this group were grossly normal. Histologically, no acid-fast bacilli or lesions of paratuberculosis were present. Several corpora amylacia were present in the ducts of the infraorbital lacrimal glands from all but 3 hamsters. No corpora amylacia were found in the infraorbital lacrimal glands from the hamsters examined at 2, 154 days, and 1 of 3 hamsters at 329 days.

No lesions or acid-fast bacilli were found in the exorbital lacrimal glands from hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis.

Electron Microscopy of Hamsters Inoculated Intraperitoneally with Living M. paratuberculosis

The ultrastructure of longitudinal and cross sections of M. paratuberculosis is illustrated in Figures 59 and 60.

Hamster examined 42 days postinoculation

Light microscopy of a mid-saggital section from the anterior mediastinal lymph node revealed that 10% of the normal lymphoid tissue was displaced by reticuloendothelial cells and several of these cells contained acid-fast bacilli. This involvement was predominantly around the periphery of the lymph node.

With electron microscopy, no bacilli were demonstrated in the tissue samples. Although no bacilli were demonstrated, many macrophages were present and usually contained only very few, if any, lysosomes (Figure 61). Plasma cells were prominent, many exhibited a distention of the cisternae of the rough endoplasmic reticulum (Figures 62 and 63). Eosinophils were prevalent in both the light and electron microscopic examinations (Figure 62). The lymphocytes had large nuclei with a scanty but rather electron dense cytoplasms that contained mostly free ribosomes and a few small mitochondria (Figure 62). The macrophages were easily differentiated from the lymphocytes on the basis of having a large and irregular shaped nucleus, a prominent golgi complex, lysosomes, and many more mitochondria.

Figure 59. An electron micrograph of a thin section of M. paratuberculosis that had been digested from the intestinal mucosa of a steer with clinical signs of paratuberculosis and used to inoculate the hamsters in this study. The round polyphosphate bodies (P) are rich in metaphosphate and are considered to be the fuel cell of the organism. Note the unexplainable arrangement of very electron dense particles (D) and vacuoles (V). Osmium tetroxide fixation, Epon 812 embedment, and lead citrate staining. x106,600.

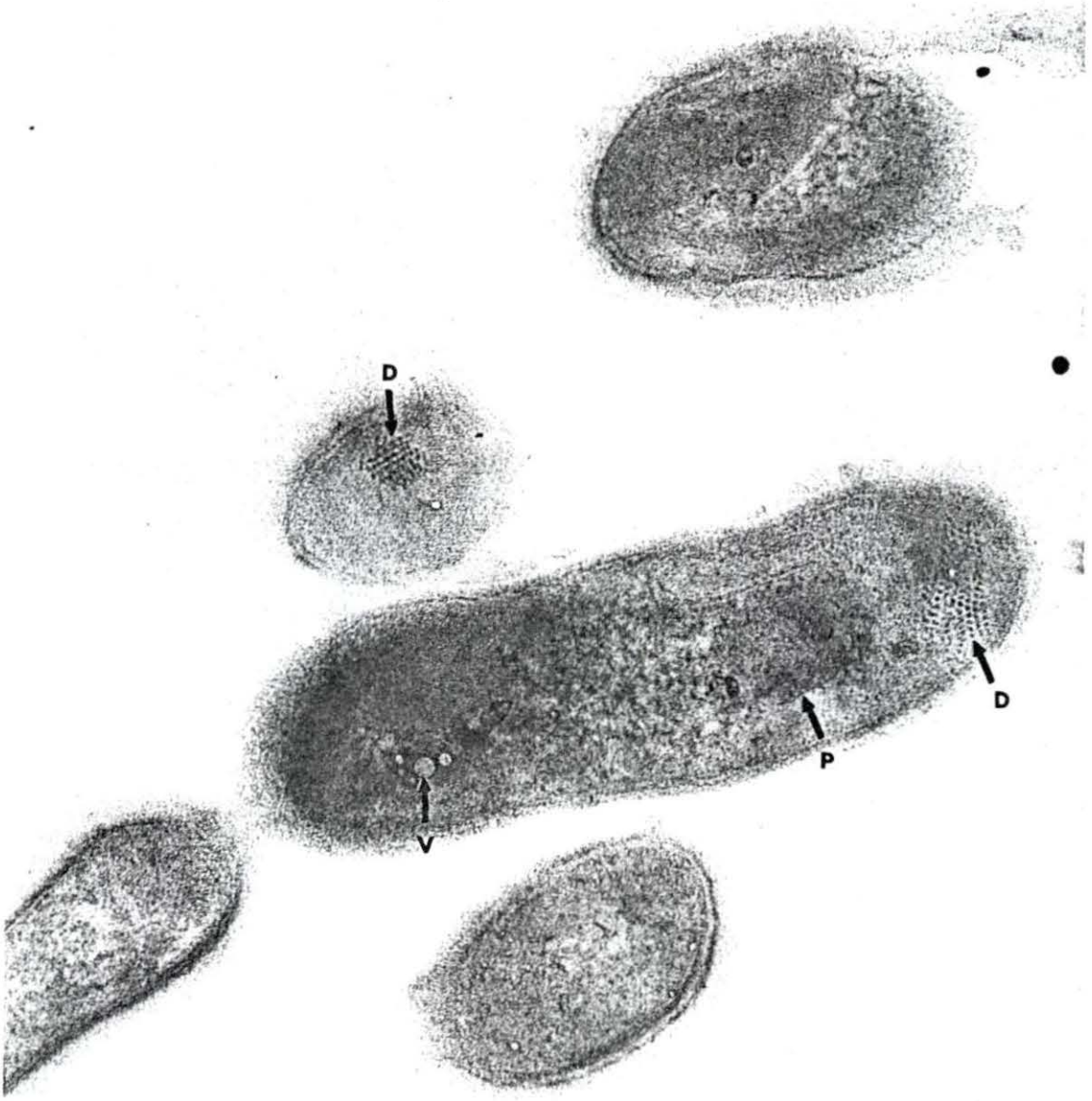

D
D
P
V

Figure 60. A cross section of a bacillus of M. paratuberculosis that illustrates the cell wall (W) and the double layered plasma membrane (M) in which the outer layer usually remains attached to the cell wall while the inner layer retracts with the cell cytoplasm. The nucleoid material (N) occupies a large part of the bacillus in this region and represents the DNA. Osmium tetroxide fixation, Epon 812 embedment, and lead citrate staining. x244,400.

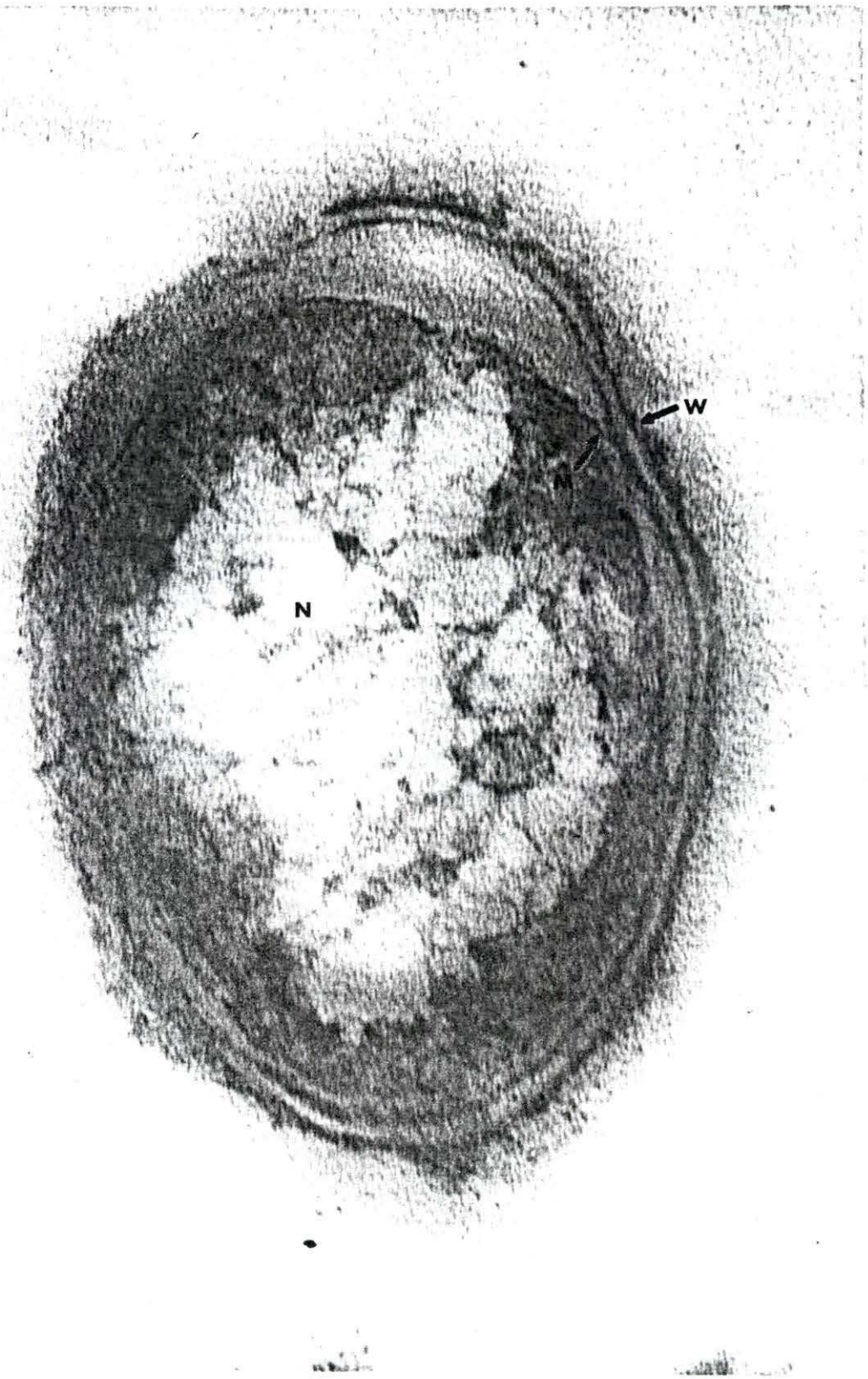

W
M
N

Figure 61. Cortex of the anterior mediastinal lymph node examined at 42 days. Note the nonphagocytic reticuloendothelial cells (RE) with large, oval or elongated nuclei and low electron dense cytoplasm. The more electron dense cytoplasm (C) that contained many small vesicles and mitochondria (M) is probably that of a phagocytic reticuloendothelial cell (macrophage) whose nucleus is in a different plane than that of this section. Note the longitudinal and cross sections of cytoplasmic processes (P) of the nonphagocytic reticuloendothelial cells that are protruding into the cytoplasm of the macrophage and illustrating the intricate "interdigitation" of the plasma membranes of different cells. Osmium tetroxide fixation, Epon 812 embedment, and lead citrate staining. x5,200.

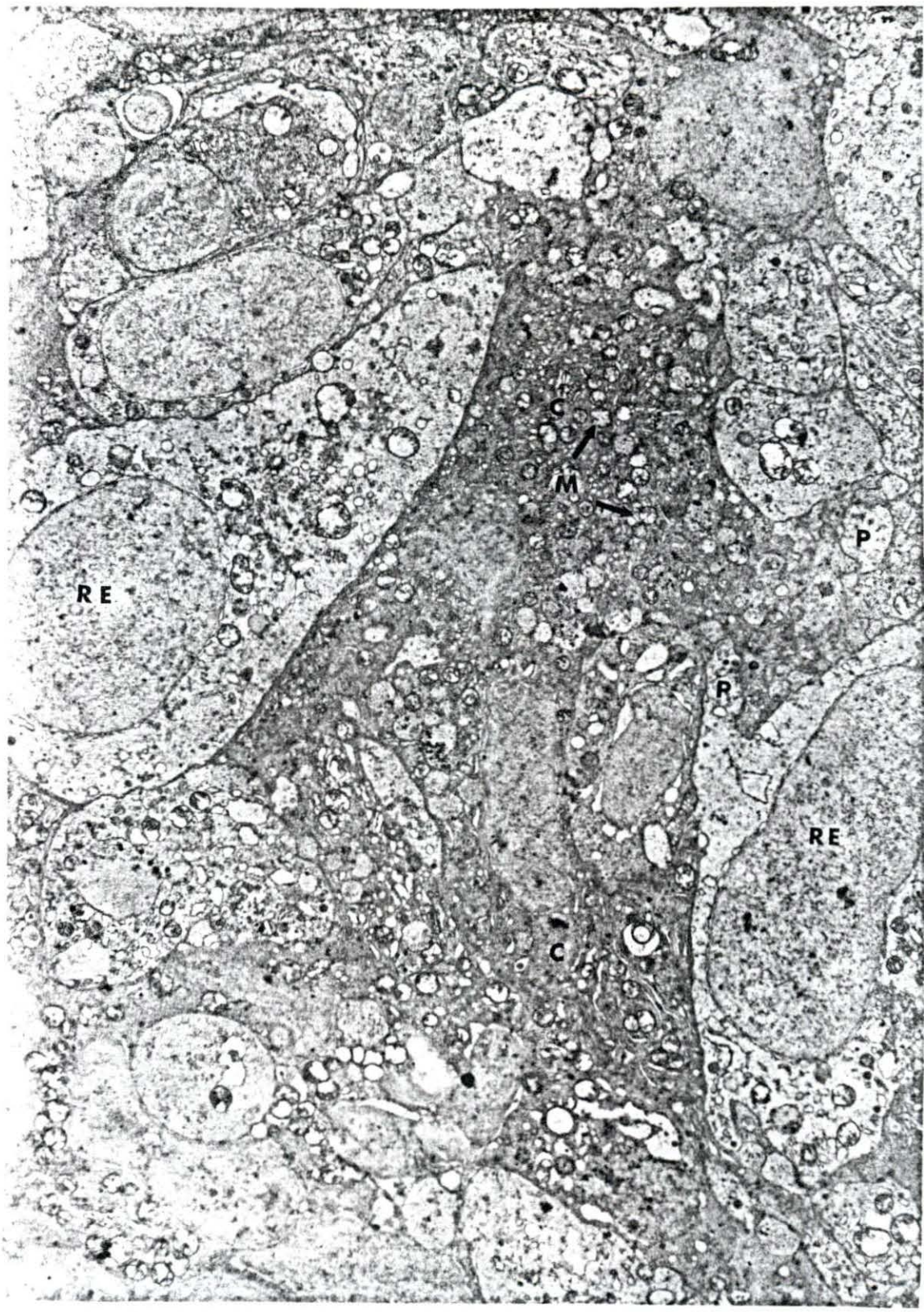

C
M
P
RE
R
RE
C

Figure 62. Electron micrograph of the anterior mediastinal lymph node examined at 42 days that illustrates 3 plasma cells (P) adjacent to each other and an eosinophil (E). Note the markedly distended cisternae of the rough endoplasmic reticulum of the plasma cells. Lymphocytes (L) are in the tissue surrounding the plasma cells. Osmium tetroxide fixation, Epon 812 embedment, and lead citrate staining. x9,000.

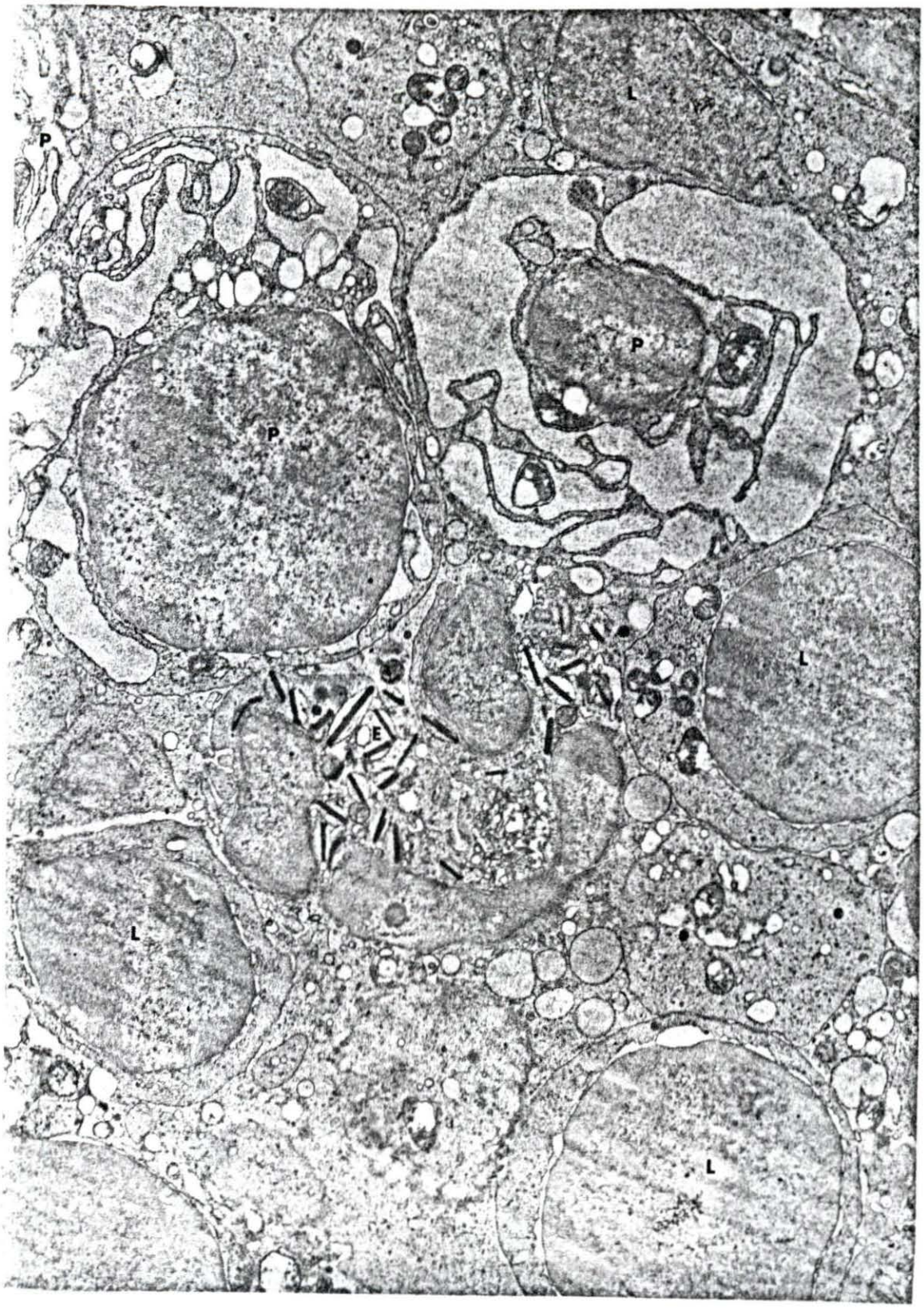
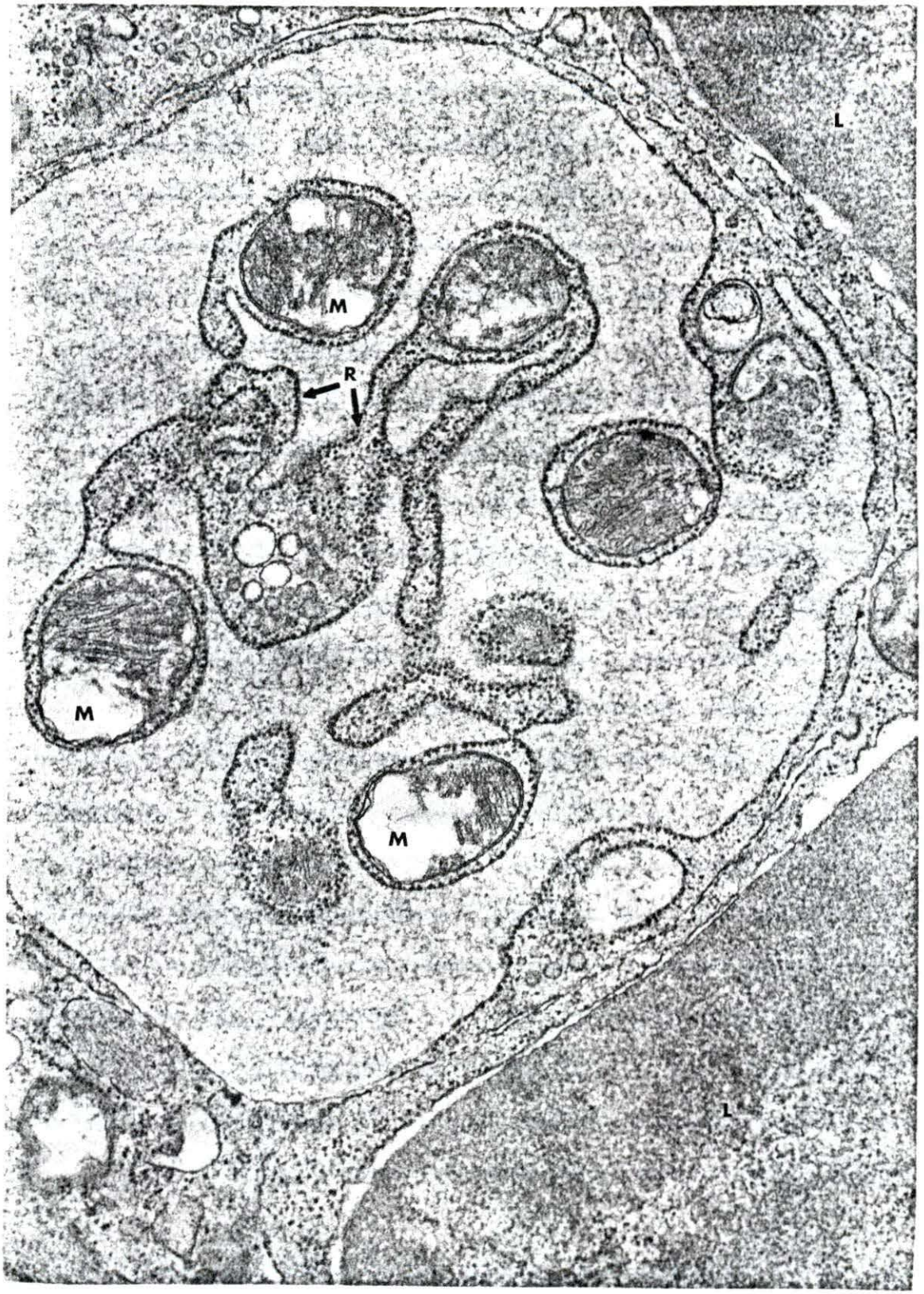

P
L
P
P
L
E
L
L

Figure 63. A tangential section through the cytoplasm of a plasma cell extending between lymphocytes (L) in the anterior mediastinal lymph node examined at 42 days. The arrangement and location of the ribosomes (R) indicates the large, low electron dense area to be the cisternae of the rough endoplasmic reticulum. Note the position of the mitochondria (M) between the rough endoplasmic reticulum. The clear spaces in the mitochondria are artifacts due to fixation. Osmium tetroxide fixation, Epon 812 embedment, and lead citrate staining. x25,000.


L
M
R
M
M
L

Hamster examined 90 days postinoculation

Examination by light microscopy of a mid-saggital section from the anterior mesenteric lymph node revealed that about 30% of the tissue had alterations. Reticuloendothelial cells containing acid-fast bacilli were more frequently demonstrated than at 42 days.

With electron microscopy, many phagocytic reticuloendothelial cells that contained bacilli were demonstrated (Figure 64). There was also an increase in the number of lysosomes in the phagocytic reticuloendothelial cells and frequently bacilli were present within these lysosomes, thus they become phagosomes (Figures 65 and 66). Many more plasma cells were present at 90 days than at 42 days. The cisternal space of the rough endoplasmic reticulum of the plasma cells was in various stages of distention but none were as markedly distended at 90 days as at 42 days (Figure 67). No difference could be observed between the lymphocytes at 42 and 90 days.

Necrotic intracellular foci were found in several of the reticulo-endothelial cells examined at 90 days and often a bacillus was present in these foci (Figures 68 and 69). A few reticuloendothelial cells were sometimes found that contained intracellular laminated structures that were about the same size or slightly larger than the necrotic intracellular foci (Figures 70 and 71). Occasionally, these contained what resembled a bacillus-like structure (Figure 69). An increase in pinocytosis was noted in the endothelium of 1 of the small capillaries that was located in the normal lymphoid tissue (Figure 77).

Figure 64. An area of phagocytic reticuloendothelial cells containing several acid-fast bacilli in the anterior mesenteric lymph node examined at 90 days. Most of the bacilli (B) show cytoplasmic condensation indicating degradation. Note the 1 bacillus within a lysosome (L). Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x9,000.

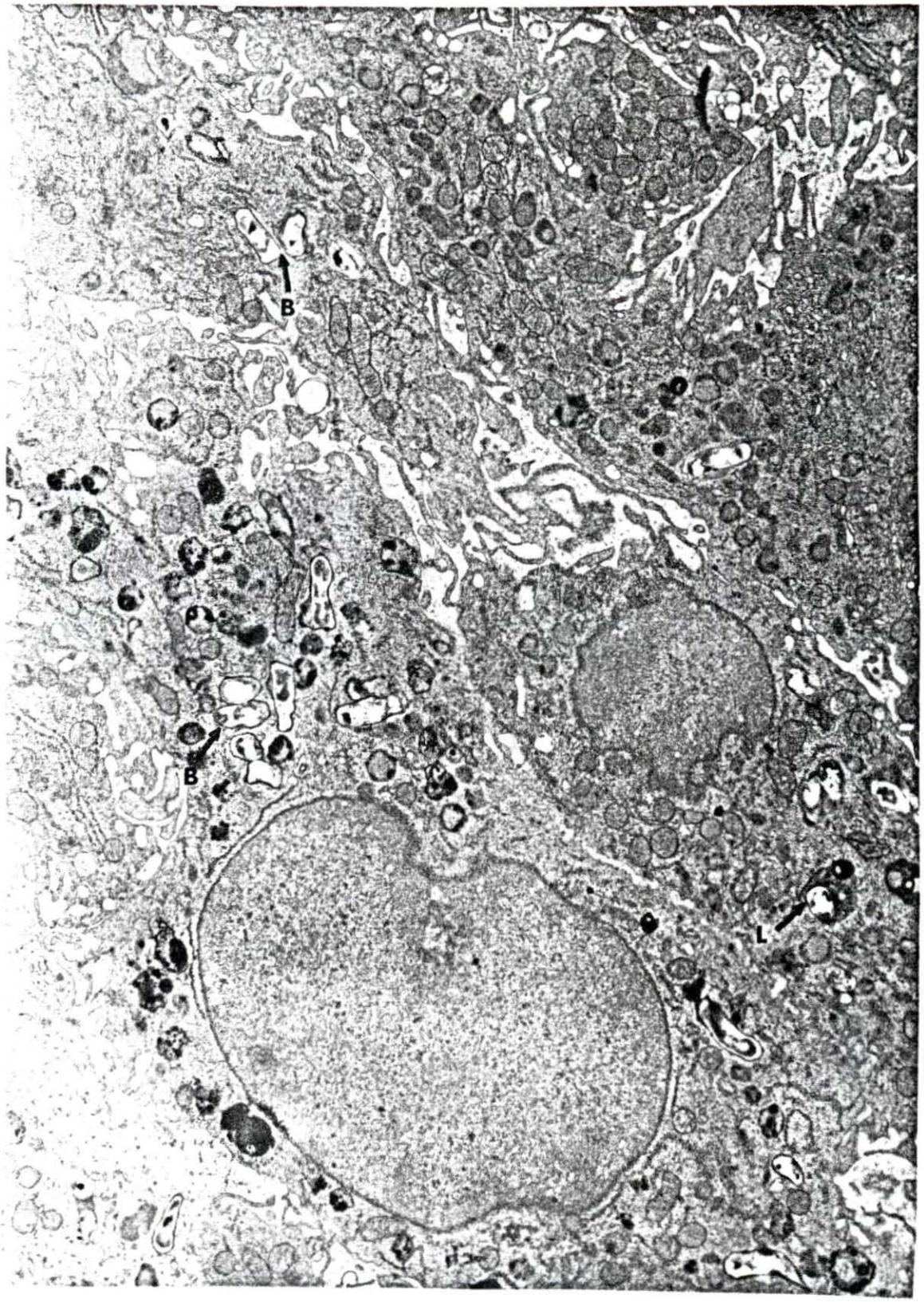

B
B
L

Figure 65. Large phagocytic reticuloendothelial cell (macrophage) in the anterior mesenteric lymph node examined at 90 days. It contains large lysosomes (L) that enclose bacilli (B). Note the irregular eccentric nucleus (N), many small vesicles (V), and the golgi complex (G). Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate fixation. x19,800.

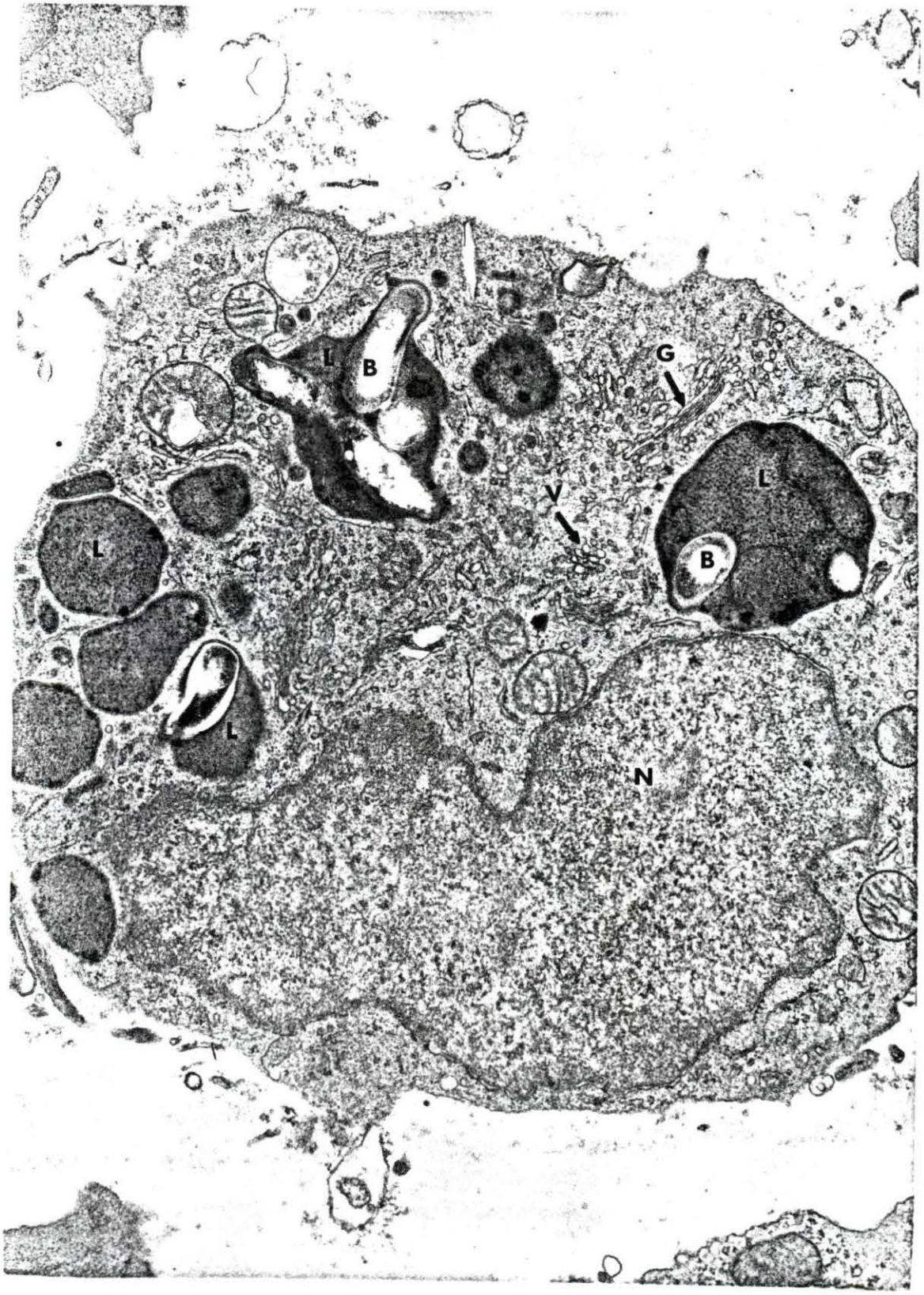

L
B
G
L
V
L
B
L
N

Figure 66. Large lysosomes in a phagocytic reticuloendothelial cell cytoplasm (C) from the anterior mesenteric lymph node examined at 90 days. Note the bacilli (B) in the lysosome. Portions of the cytoplasm of 3 adjacent plasma cells (P) are present. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x25,000.

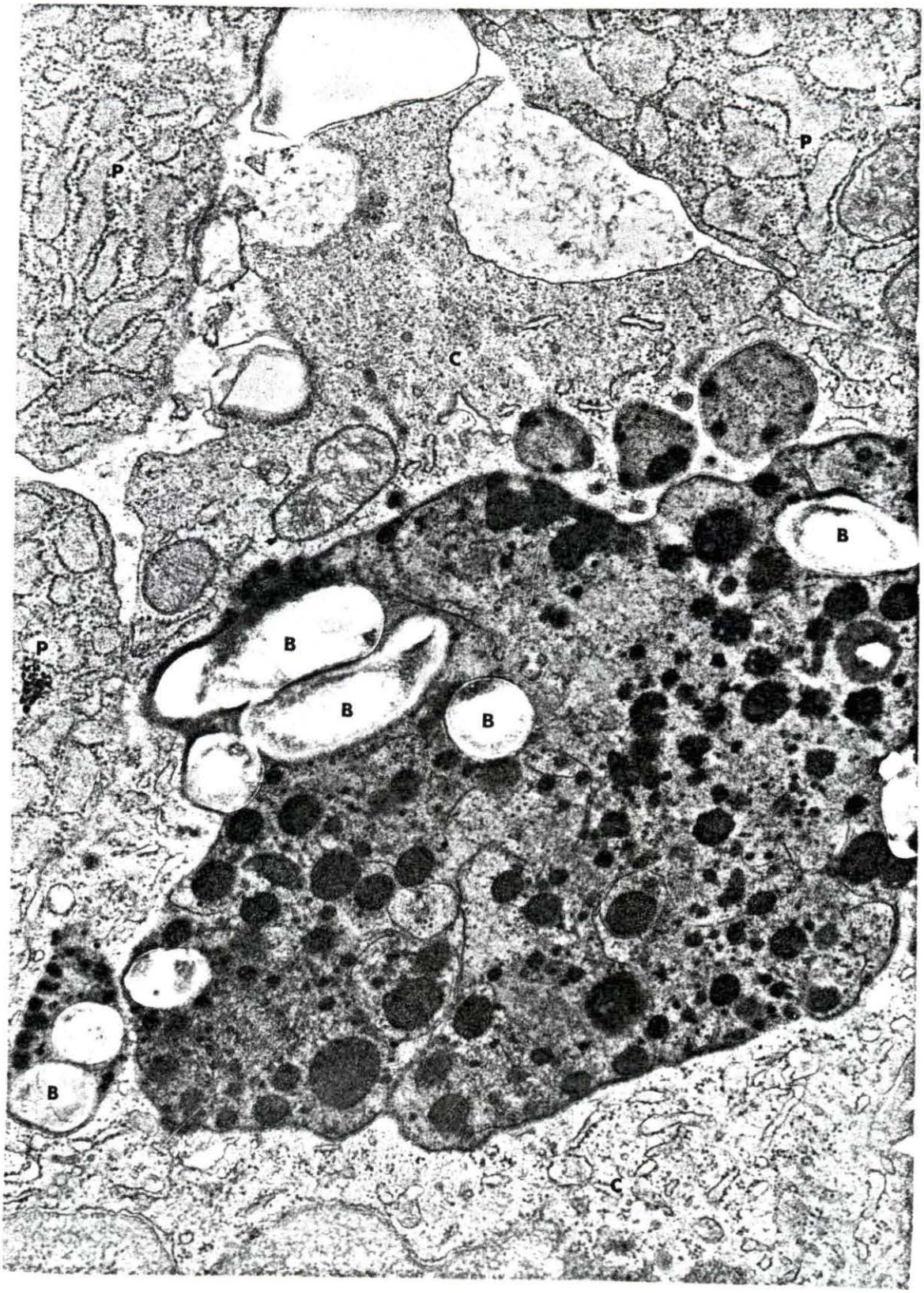

P
P
C
B
P
B
B
B
B
C

Figure 67. An electron micrograph from the anterior mesenteric lymph node examined at 90 days that illustrates parts of 1 lymphocyte (L) and 3 plasma cells (P1, P2, and P3). Note the difference in the distention of the cisternal spaces in the 3 different plasma cells. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x25,000.

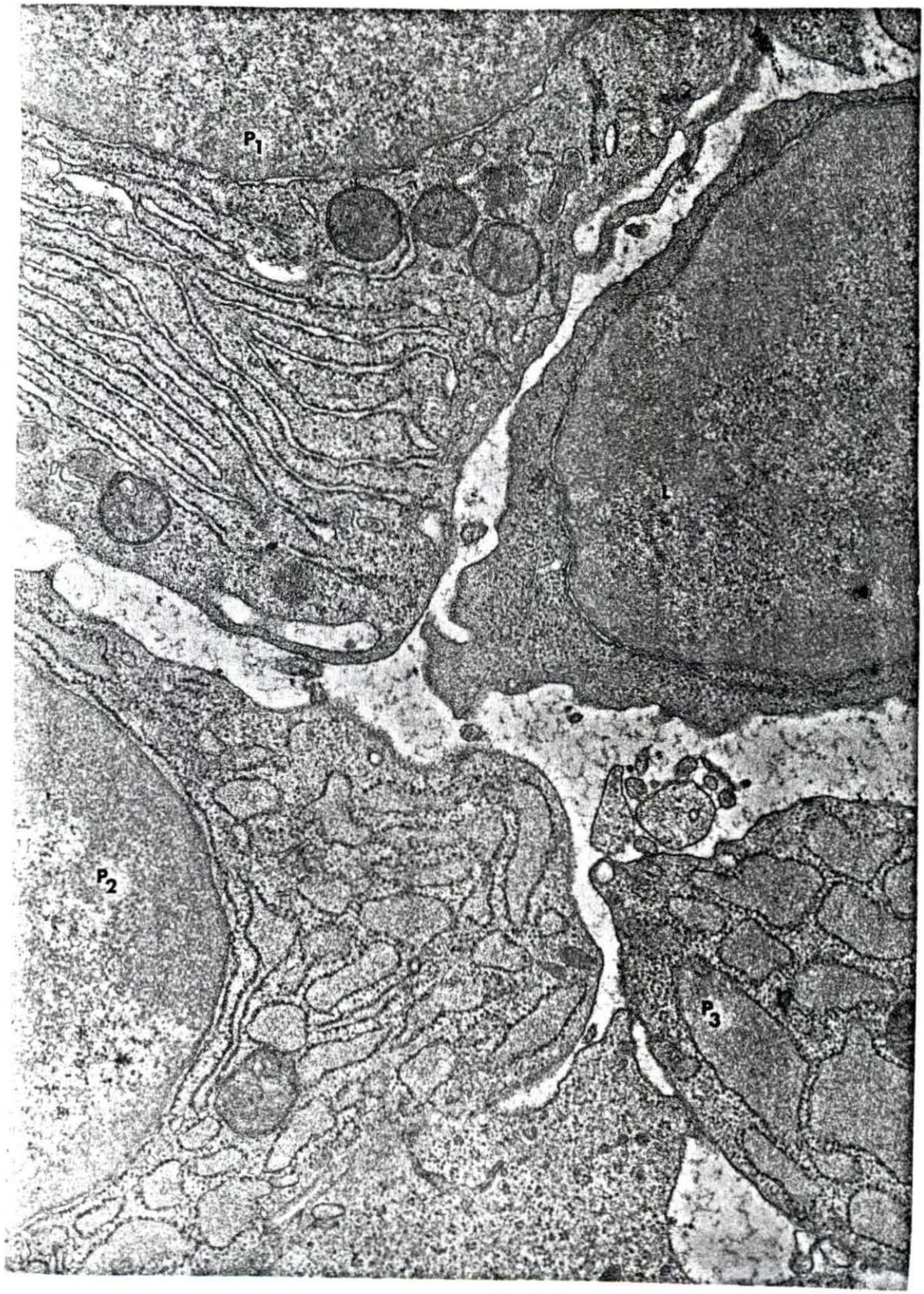

P1
L
P2
P3

Figure 68. Necrotic intracellular focus in the cytoplasm of a reticuloendothelial cell in the anterior mesenteric lymph node examined 90 days postinoculation. Note the cross section of a bacillus (B) in the edge of the necrotic focus and the presence of many lysosomes (L). One of the lysosomes also contains a bacillus. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x14,750.

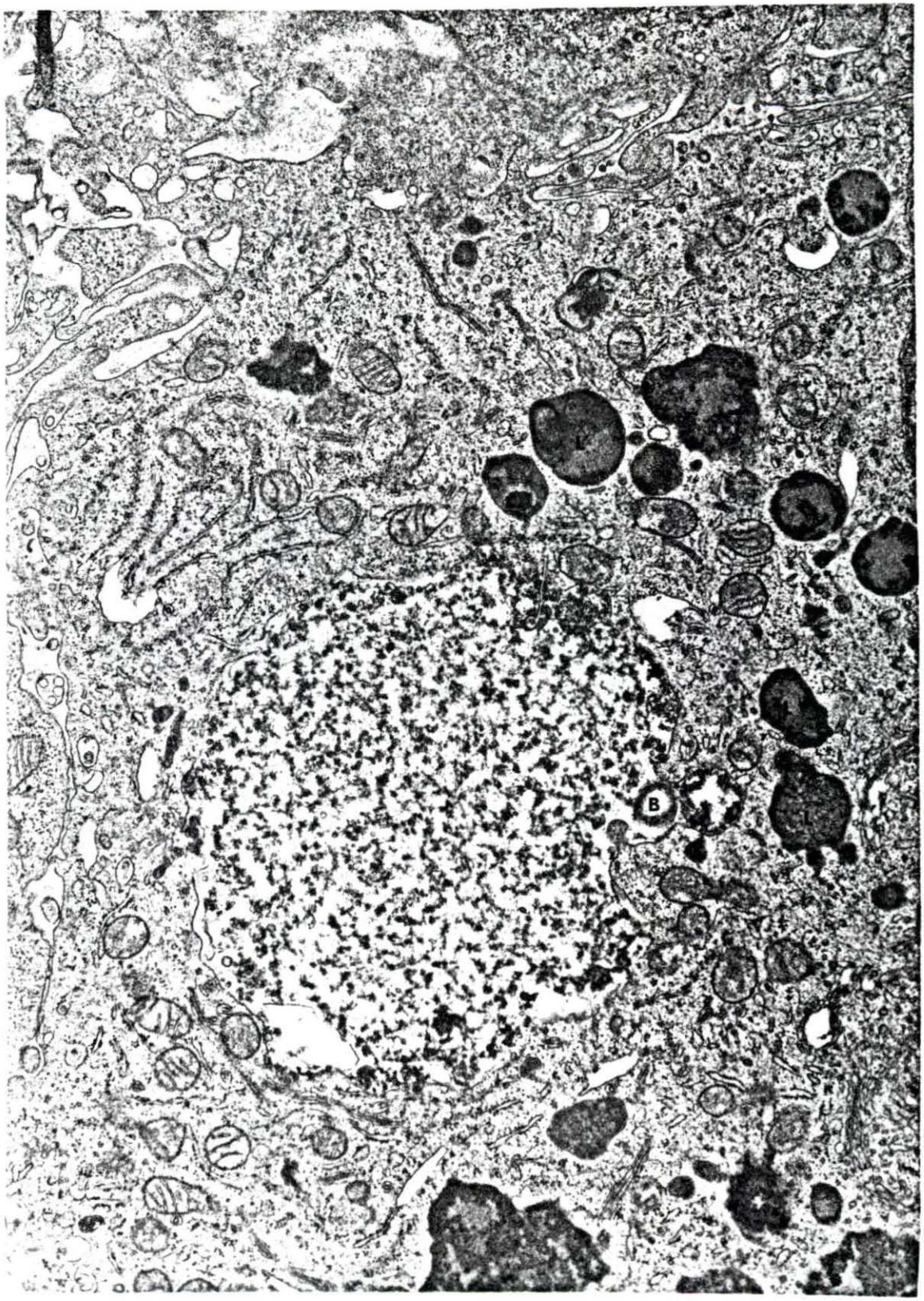

B

Figure 69. Necrotic intracellular focus in the cytoplasm of a phagocytic reticuloendothelial cell in the anterior mesenteric lymph node examined at 90 days. Note the cross section of a bacillus (B) in the necrotic focus and the well developed golgi complex (G) at the opposite end of the cell. Glutaraldehyde fixation, Osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x11,800.

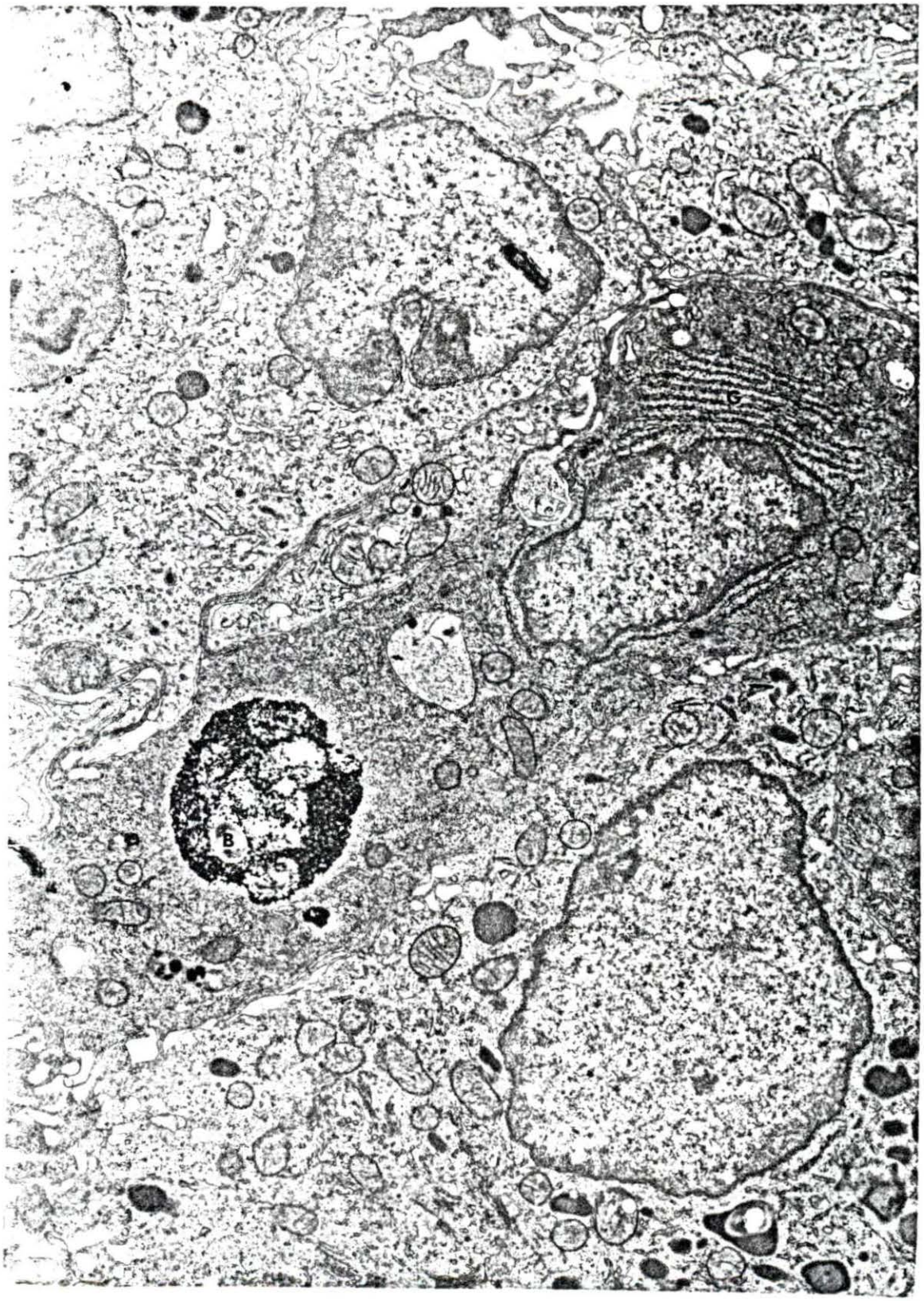

B

Figure 70. Large necrotic intracellular focus in the cytoplasm of a reticuloendothelial cell in the anterior mesenteric lymph node examined at 90 days. Note the 2 concentric lamellae (L) of crystal-like needles around what appears to be a disintegrating bacillus (B). Also note the amorphous material between the outer lamella and a limiting membrane (M). Glutaraldehyde fixation, Osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x25,000.

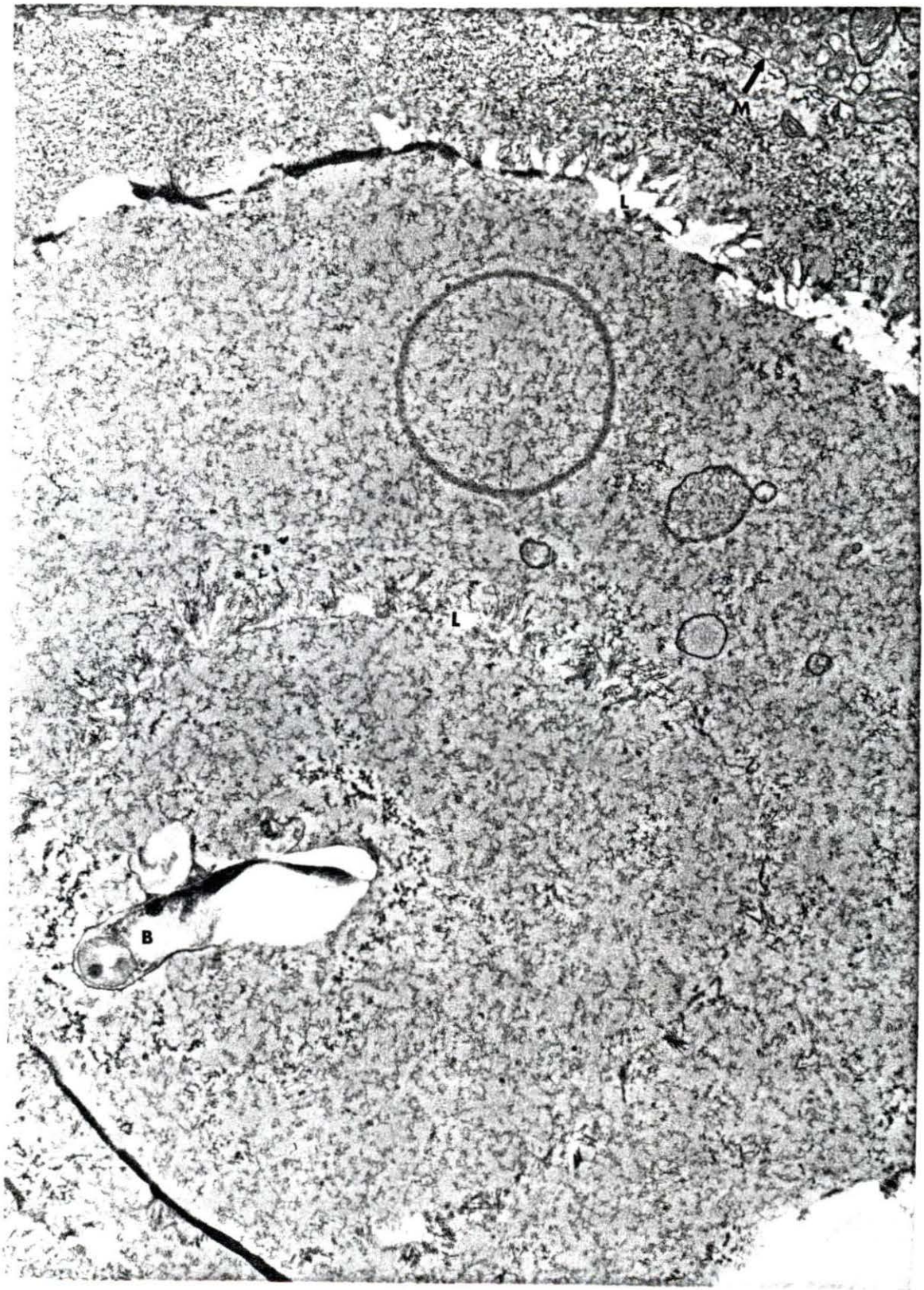
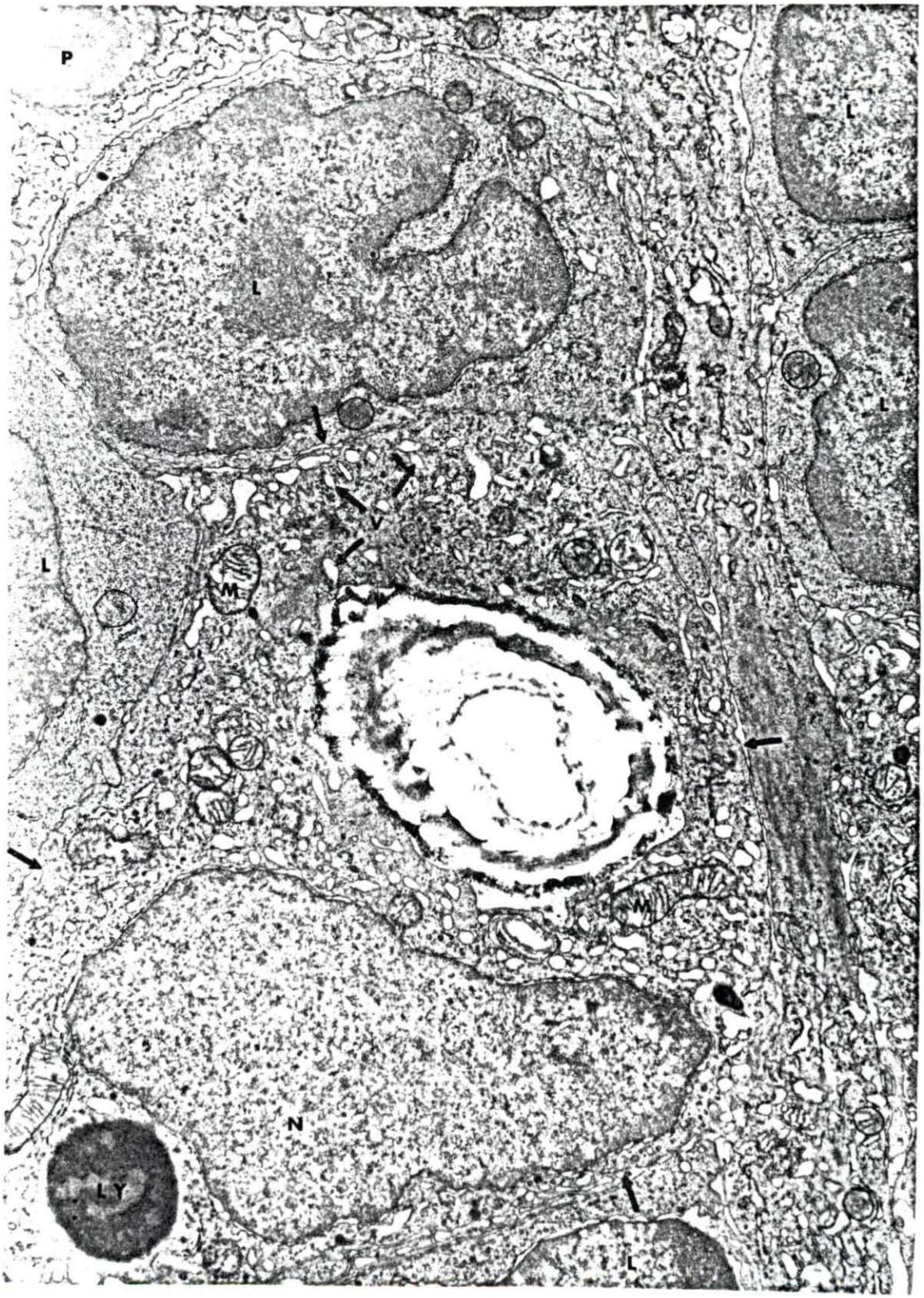

M
L
L
B

Figure 71. An early stage of what appears to be an intracytoplasmic Schaumann's body in the cytoplasm of a reticuloendothelial cell of the anterior mesenteric lymph node that was examined at 90 days. Note the eccentric nucleus (N) and many small vesicles (V) in the cell cytoplasm. The plasma membrane (arrows) surrounds the cytoplasm of the reticuloendothelial cell which contains a large lysosome (LY) and several large mitochondria (M). Lymphocytes (L) and a plasma cell (P) are surrounding the reticuloendothelial cell. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x11,800.


P
L
L
L
L
V
M
M
N
LY
L

Hamster examined 168 days postinoculation

Light microscopy of a mid-saggital section from the ileocecal lymph node revealed less than 10% tissue alterations and no acid-fast bacilli. Therefore, due to the improbability of obtaining a section of the lesion, electron microscopy was not attempted.

Hamster examined 175 days postinoculation

Light microscopy of a mid-saggital section from the anterior mesenteric lymph node from a hamster that was inoculated at 175 and 98 days prior to examination revealed extensive reticuloendothelial tissue hyperplasia that occupied between 30 to 40% of the normal lymphoid tissue. Many intracellular acid-fast bacilli and several small Schaumann's bodies were present.

Electron microscopy demonstrated a more definite organization in the intracytoplasmic laminated bodies (Schaumann's bodies) when compared with those examined at 90 days (Figures 72 and 73).

A distinct border was observed between the foci of reticuloendothelial tissue hyperplasia and the normal lymphoid tissue. A partial barrier was formed by a single cell layer of fibroblasts (Figure 74). Although a few lymphocytes were present on the hyperplastic side of this partial barrier, it formed the outer limits for the cytoplasm of the hyperplastic reticuloendothelial cells. Several of the reticuloendothelial cells were multinucleated and contained several bacilli in their cytoplasm (Figures 72, 74, and 75). In 1 instance, the nucleus of a reticuloendothelial cell was observed to contain a double membrane enclosed vacuole whose internal components were cell

Figure 72. A Schaumann's body in the cytoplasm of a reticuloendothelial cell located in a focus of reticuloendothelial tissue hyperplasia in the anterior mesenteric lymph node examined at 175 days. This electron micrograph also contains a multinucleated reticuloendothelial cell (MN) and 2 lymphocytes (L). Note the small bacilli (B) in the cytoplasm of the reticuloendothelial cells. Also note the size of the Schaumann's body in relation to the size of the nuclei of the reticuloendothelial cells and compare it with the Schaumann's bodies in Figures 6, 14, 25, 26, 42, 44, and 50. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x5,200.

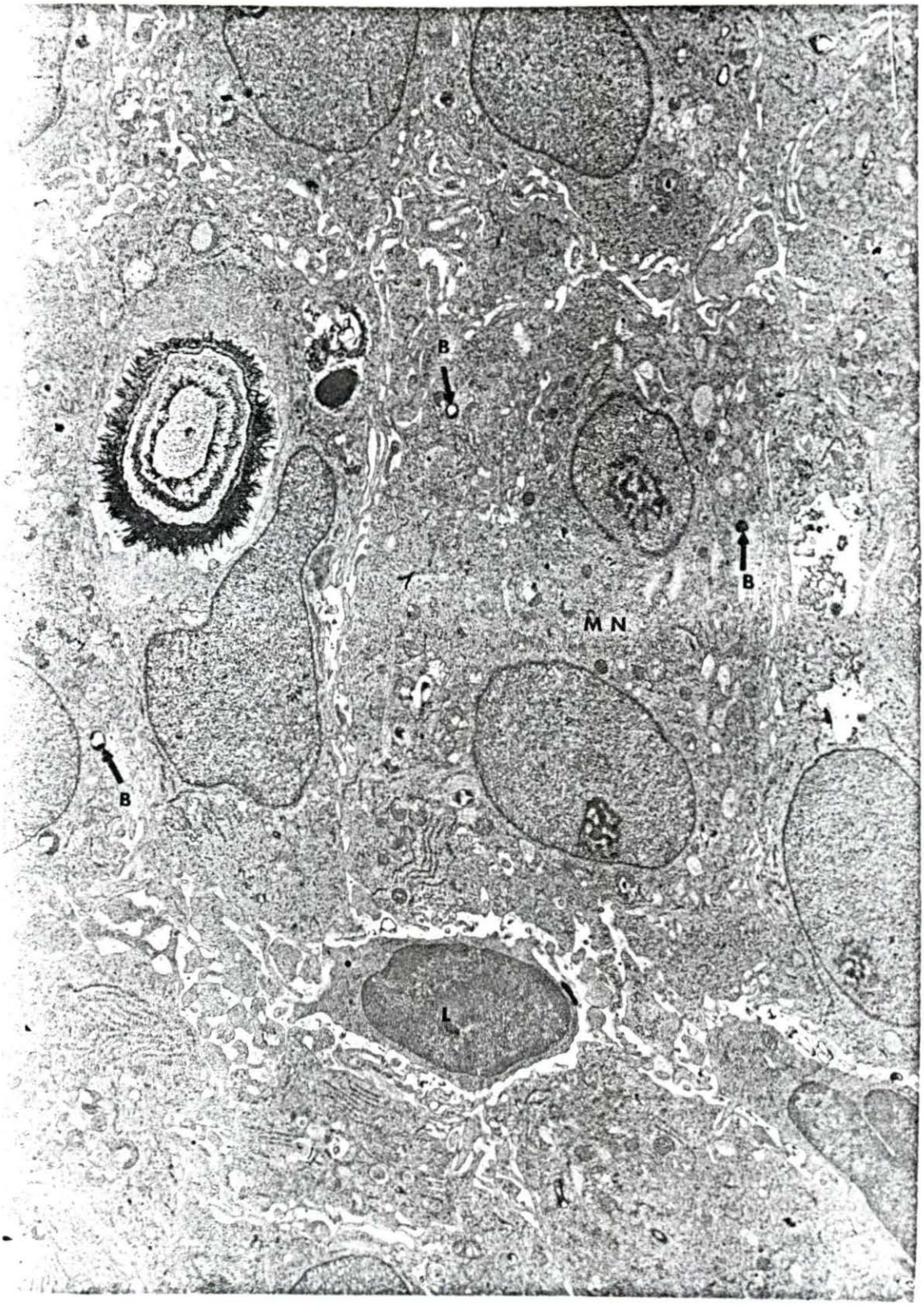

B
B
B
MN
L

Figure 73. Higher magnification of the intracytoplasmic Schaumann's body in Figure 71. Note that a limiting membrane surrounds the body and that a space exists between this limiting membrane and the outermost lamella of needle-like crystals. x25,000.

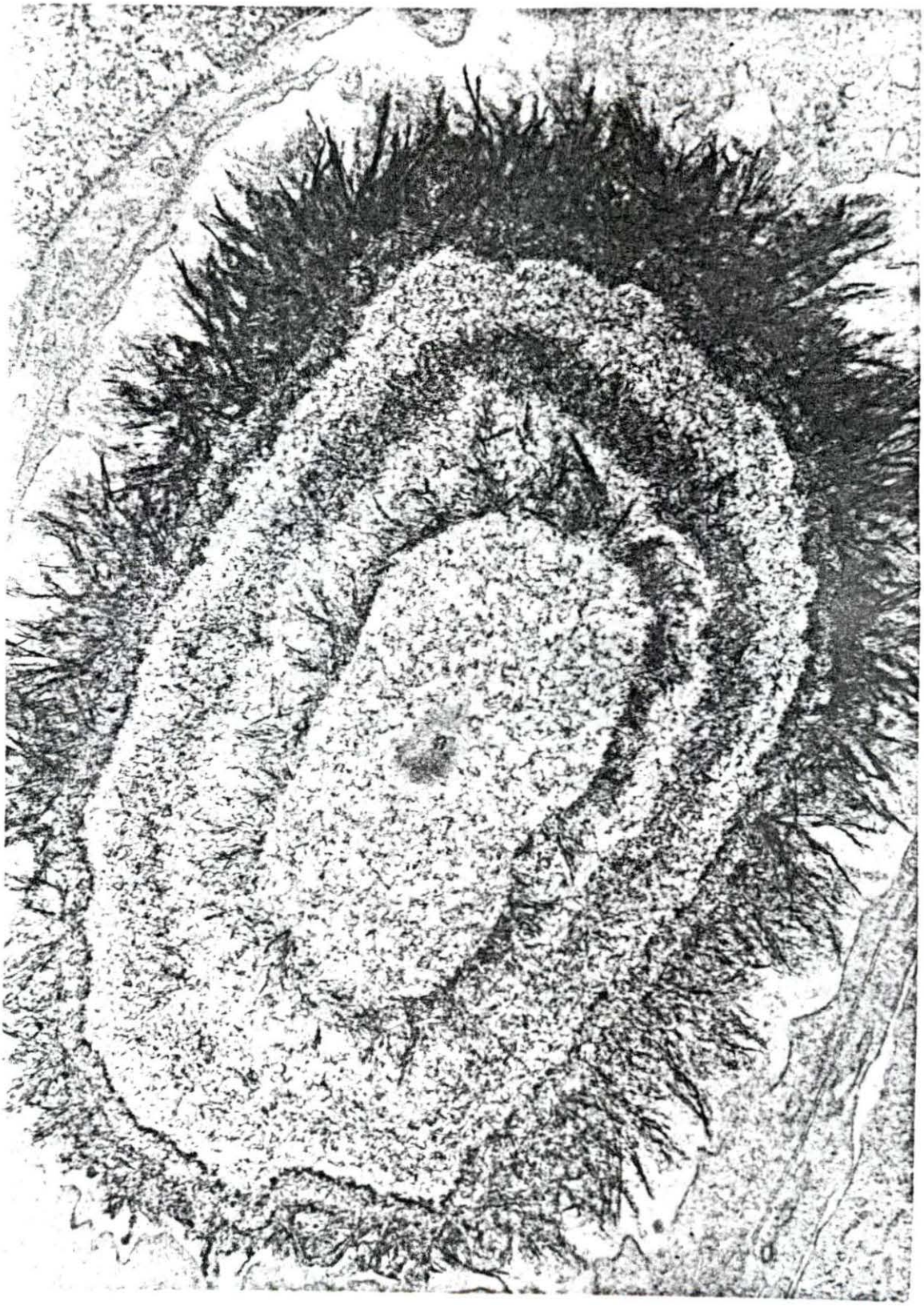


Figure 74. This electron micrograph reveals the boundary between a focus of reticuloendothelial tissue hyperplasia and the normal lymphoid tissue of the anterior mesenteric lymph node examined at 175 days. The cytoplasm (arrows) of a fibroblast (F) extends along the junction of the hyperplastic tissue and the normal lymphoid tissue. Note the large round nuclei of the reticuloendothelial cells (R) and their extensive cytoplasm that contains several bacilli. Compare the distinctiveness of the border of the hyperplastic tissue with that observed in Figures 6, 13, 14, 16, 18, 32, 42, and 50. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x2,780.

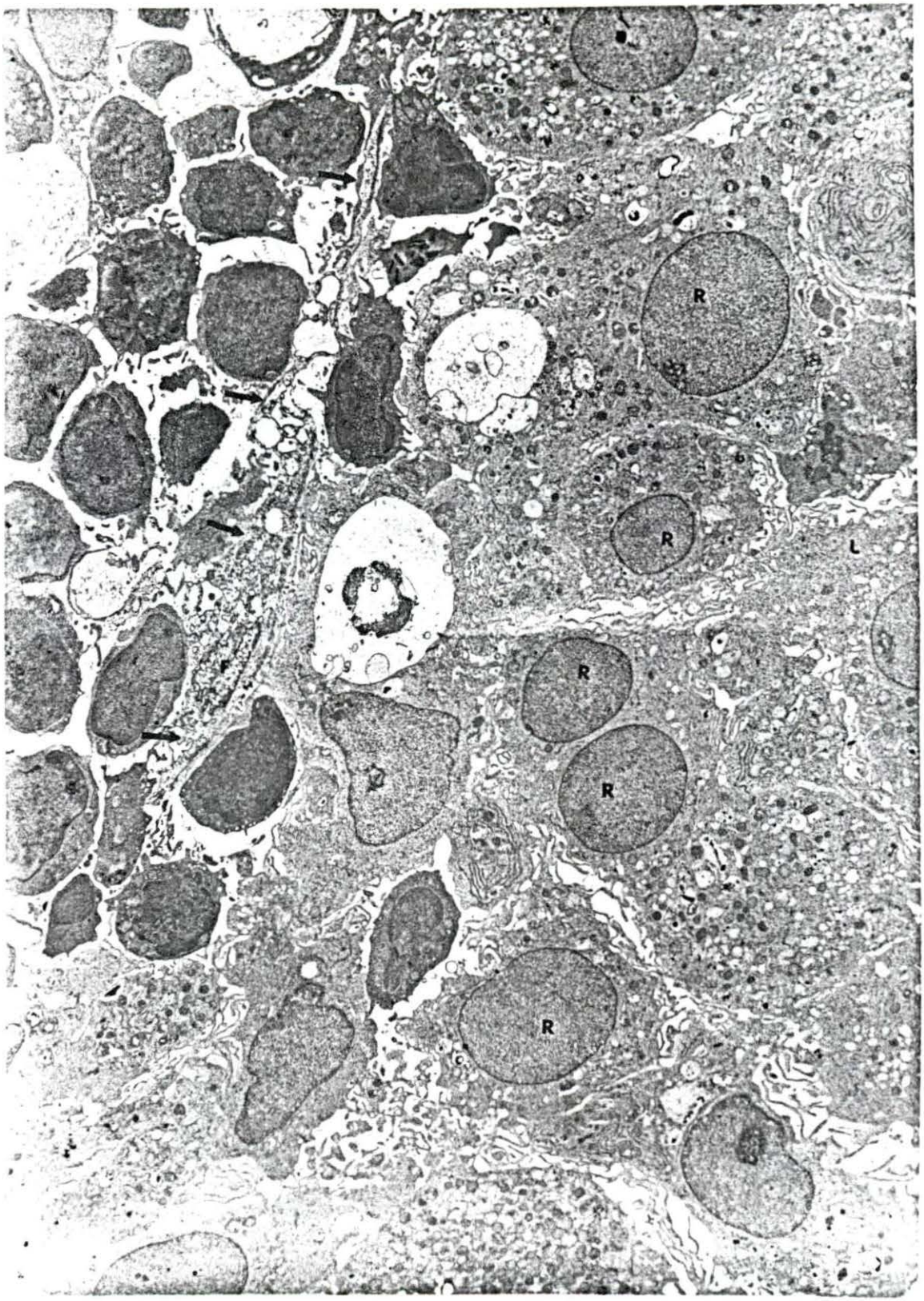

R
R
L
R
R
R

Figure 75. Electron micrograph at the edge of a focus of reticuloendothelial tissue hyperplasia from the anterior mesenteric lymph node examined at 175 days. Note the 3 multinucleated reticuloendothelial cells that contain several bacilli in their cytoplasm. The large round hole (H) was the location of a Schaumann's body that was lost in the process of ultrasectioning. Compare with Figure 26. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x2,780.

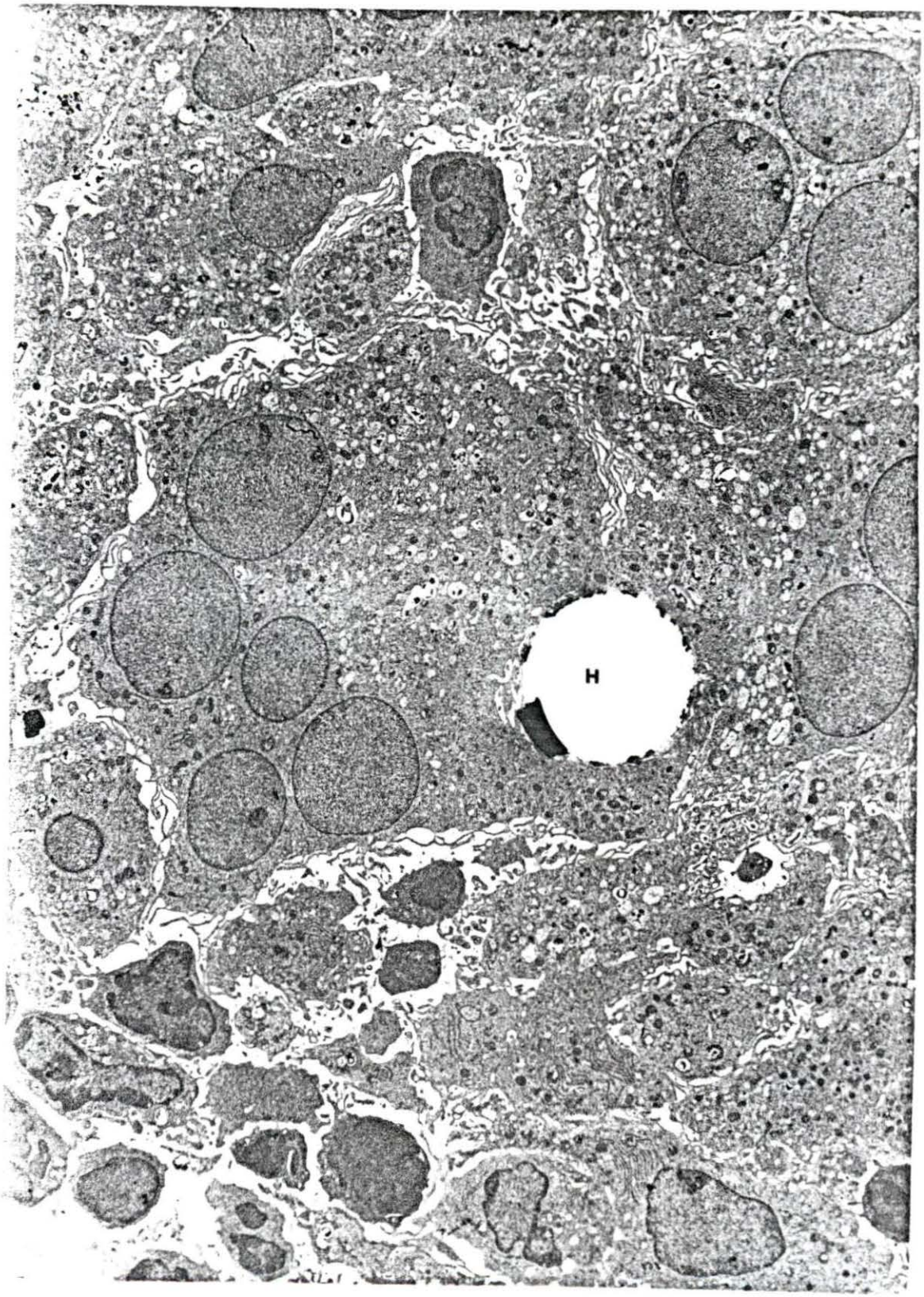
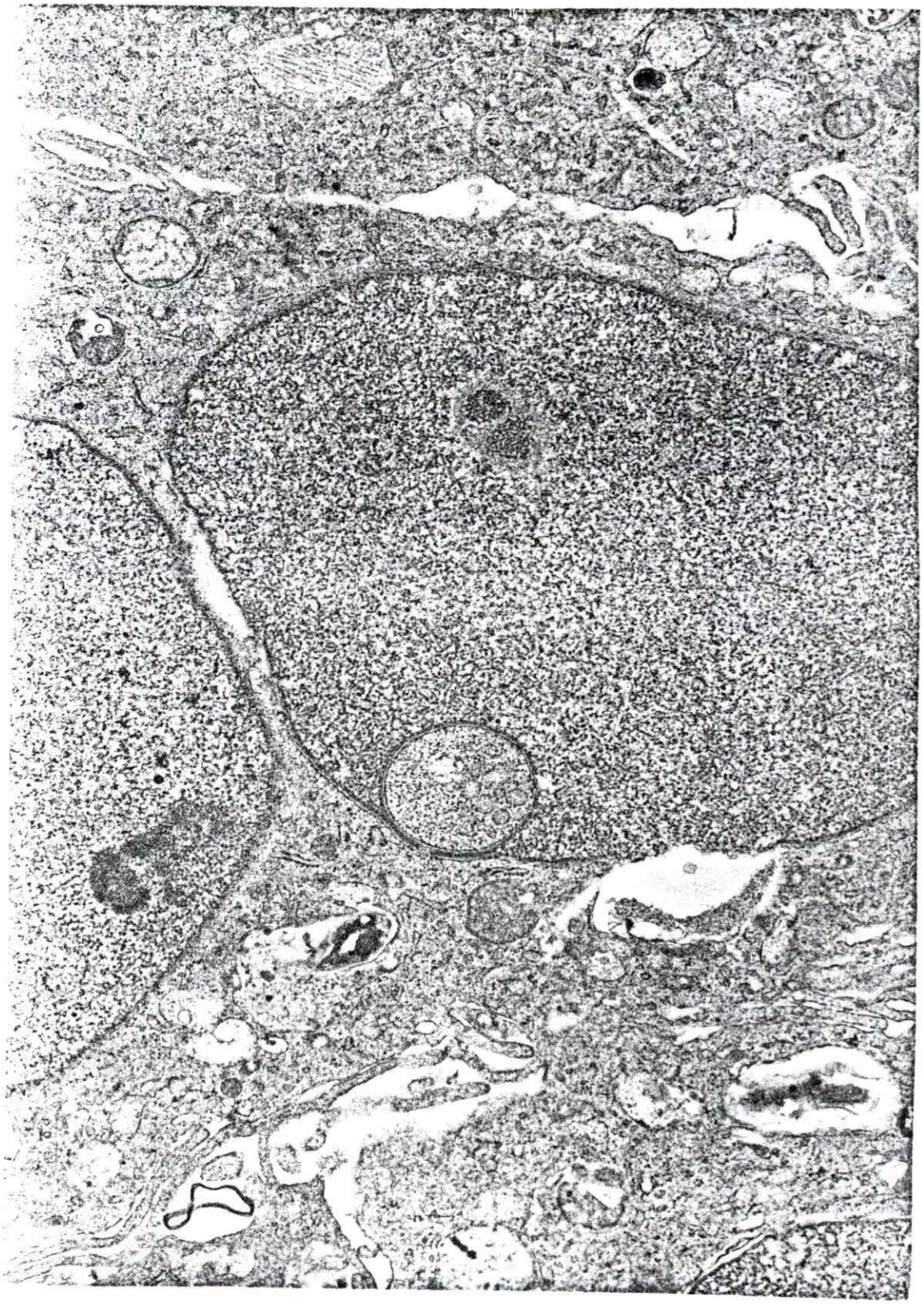

H

Figure 76. Electron micrograph of the nucleus of a reticuloendothelial cell that contains a double membrane enclosed vacuole which in turn contains cell organelles normally present only in the cytoplasm. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x9,750.



organelles normally limited to the cytoplasm (Figure 76).

The post-capillary venules that were observed by light microscopy and illustrated in Figures 17, 18, and 24 were examined by electron microscopy (Figure 78). The increase in thickness was due to lymphocytes that were located between the single layer of endothelial cells and the basement membrane of the capillary.

A very marked increase in the acid phosphatase activity was observed in areas of reticuloendothelial tissue hyperplasia stained by Gomori's method and observed by light microscopy (Figures 79 and 80).

The increase in acid-phosphatase activity was observed to be within lysosomes in the reticuloendothelial cells of the foci of hyperplasia. The tissue was treated by Gomori's method for acid-phosphatase and examined by electron microscopy (Figure 81).

Figure 77. Electron micrograph demonstrating a stage of active pinocytosis in a normal capillary of the anterior mesenteric lymph node examined at 90 days postinoculation. Note that the pinocytotic vesicles (V) in the cytoplasm (C) of the endothelial cell are present on both the lumen (L) side as well as on the basement membrane (B) side of the cell whose nucleus is in a different plane than that of this section. The nuclear portion of another endothelial cell (E) is included in this section. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x25,000.

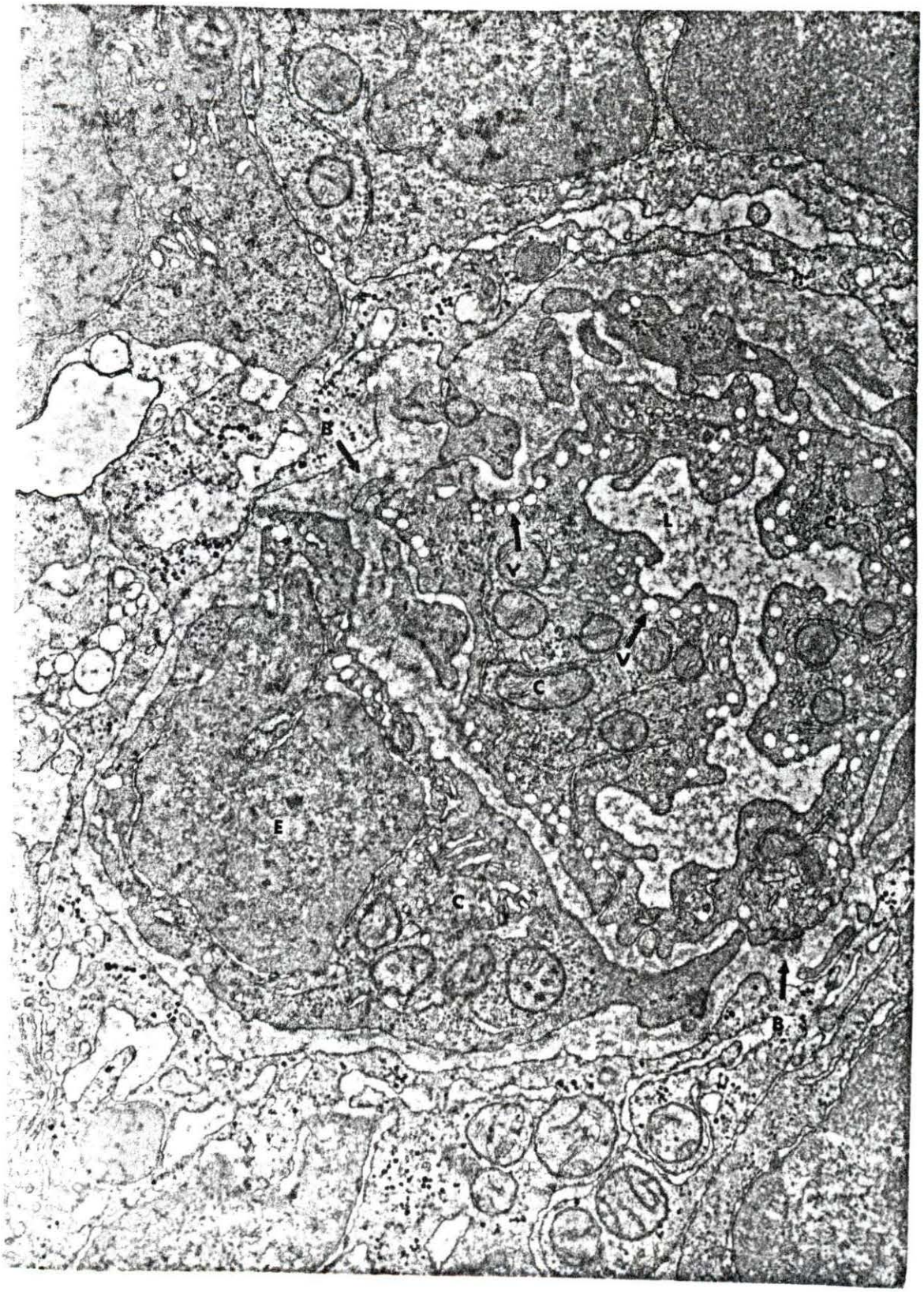

B
V
V
L
C
C
E
C
B

Figure 78. Electron micrograph of a capillary that contained several lymphocytes (L) located between the basement membrane (B) and the endothelial cells (E) that lined the lumen of the capillary. Pericytes (P) surround the capillary. Compare with Figures 17, 18, 24, and 77. Glutaraldehyde fixation, osmium tetroxide post-fixation, Epon 812 embedment, and lead citrate staining. x5,200.

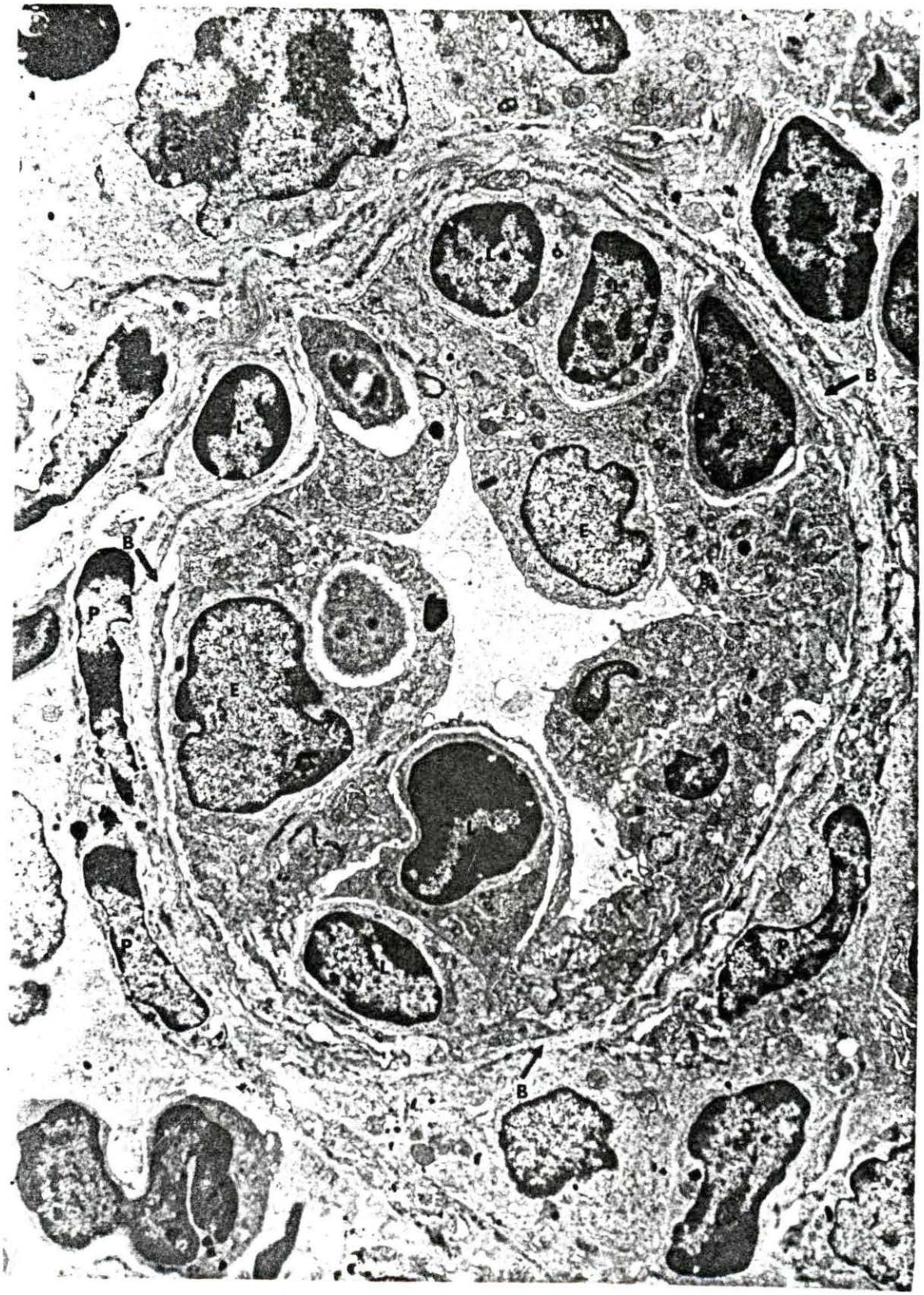
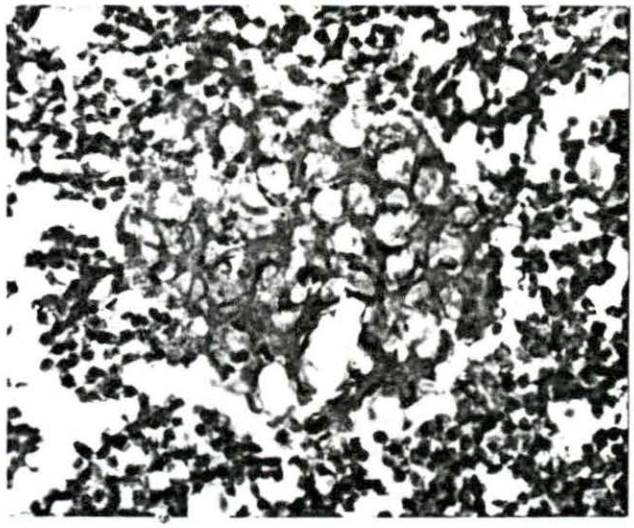

L
L
B
E
B
P
E
L
P
L
B

Figure 79. Frozen section from the anterior mesenteric lymph node of the hamster that was examined at 175 days. Note the focus of reticuloendothelial tissue hyperplasia. Glutaraldehyde fixation. Harris' hematoxylin and eosin stain. x600.

Figure 80. Serial section of the same area shown in Figure 79 that was treated by Gomori's method for acid phosphatase. Note that the yellowish brown stain is confined to the area of reticuloendothelial tissue hyperplasia and indicates acid phosphatase activity. Glutaraldehyde fixation. x600.



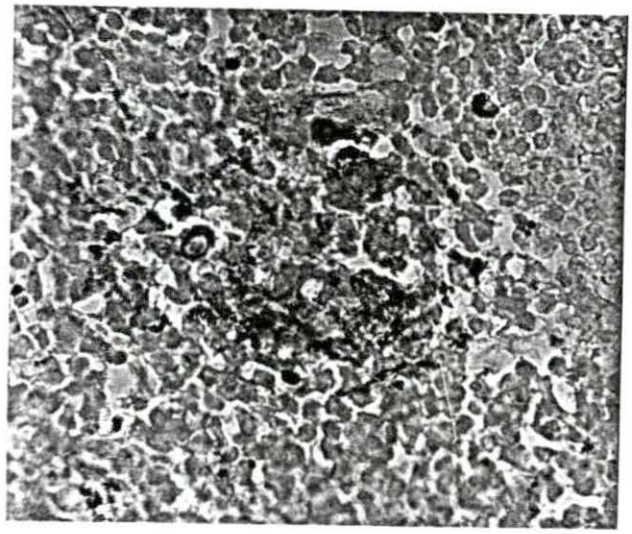
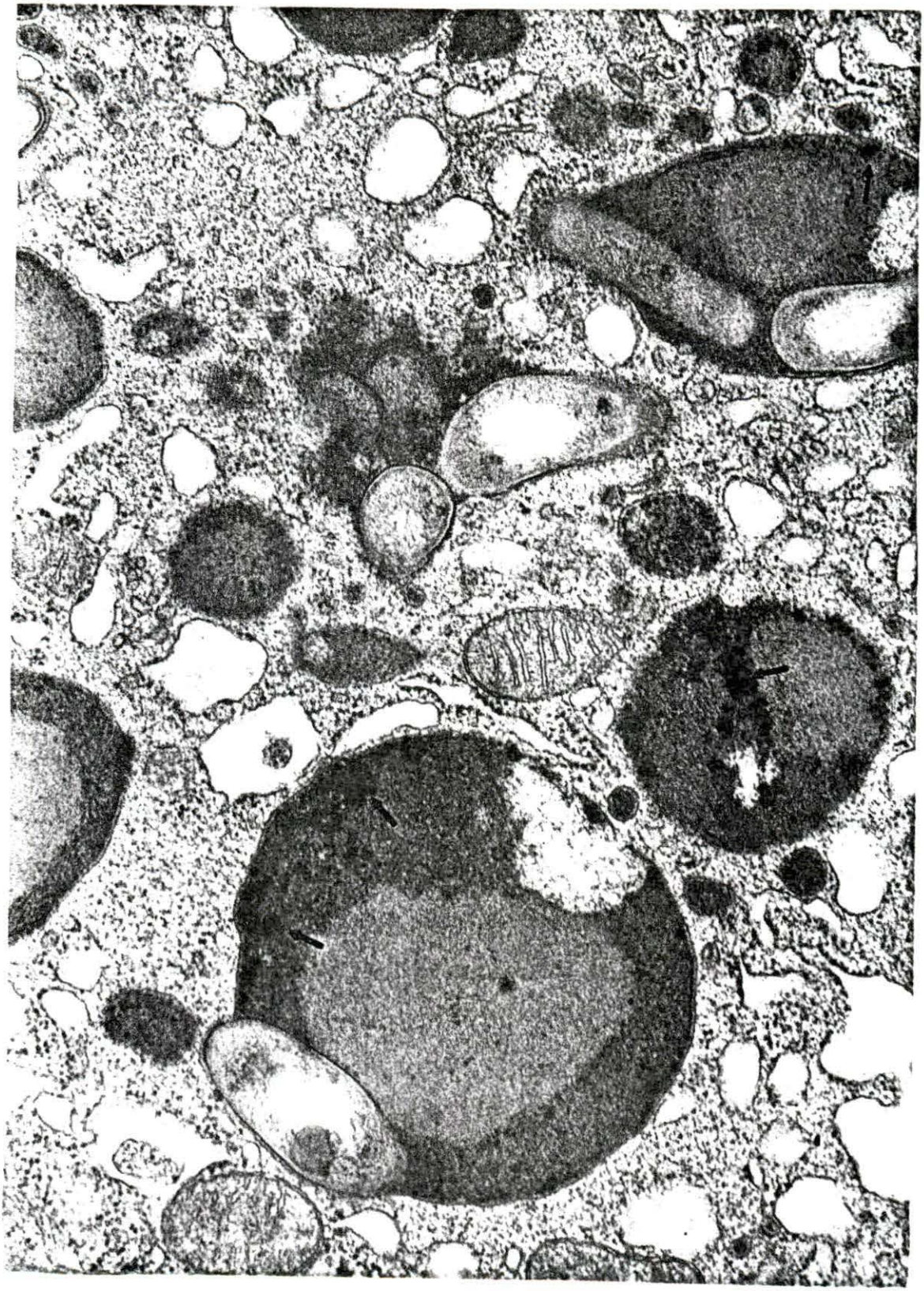


Figure 81. Electron micrograph of the cytoplasm of a phagocytic reticuloendothelial cell from the anterior mesenteric lymph node that was treated by Gomori's method. Acid phosphatase activity is indicated by the presence of dark electron dense areas (arrows) in the lysosomes (phagosomes). Note the intact polyphosphate bodies that still exist in a bacillus in 1 of the lysosomes. Glutaraldehyde fixation, incubation in Gomori's medium, osmium tetroxide post-fixation, Epon 812 embedment and lead citrate staining. x34,000.



DISCUSSION

Source of Inoculum

Segal and Bloch (93) have shown that M. tuberculosis is more virulent for mice when grown in vivo than when grown in vitro. They also demonstrated a different metabolic and biochemical activity between in vivo and in vitro grown M. tuberculosis and that a greater degree of immunity was induced by M. tuberculosis grown in vitro (92). Mycobacterium paratuberculosis will change from smooth colonies to rough colonies when grown, over a period of time, in vitro. Further, M. paratuberculosis requires mycobactin for initial isolation, but after continuous culturing in vitro, it will eventually develop the capability to grow without mycobactin. Therefore, if the metabolic activity of M. paratuberculosis is changed by culturing on artificial medium, its pathogenicity for laboratory animals may also be altered. Since previous researchers (33, 34, 47, 51) have had difficulty in establishing M. paratuberculosis in laboratory animals, it was deemed desirable to use the least altered organisms obtainable. Therefore in vivo grown M. paratuberculosis, used in inoculating the hamsters in this study, was obtained from the intestinal mucosa of a clinical case of bovine paratuberculosis.

Size of Dose

Chandler (21, 23, 24, 25) inoculated 4-week-old mice intraperitoneally with 0.0001, 0.01, 0.2, 2, 8, 10, and 24 mg. of M.

paratuberculosis and concluded that doses of 0.2 mg. and below were not effective in producing intestinal lesions. He observed that intraperitoneal inoculations of 0.02 and 2 mg. in rats produced no progressive lesions (22). Further, Francis (34) stated that results from hamsters inoculated intraperitoneally with 5 mg. were less consistent than in mice. Hirch (47) also gave oral doses of 0.6 to 1.5 mg. of moist organisms to hamsters and obtained little success. In view of the results of previous workers, it was concluded that a minimum of 1 mg. (based on dry weight) dose of M. paratuberculosis would be used in the various routes of inoculation of hamsters included in this study.

The number of organisms per mg. was determined in the following manner. The size of the individual organism has been described as being a cylinder, 1 to 2 μ long and 0.5 μ in diameter (66). If 1.5 μ is accepted as the average length, then the volume or the displacement capacity of M. paratuberculosis would be 0.3 μ^3 per bacillus ($1/4 \times 3.14 \times 0.5^2 \times 1.5 = 0.3\ \mu^3$). There are $10^9\ \mu^3$ in a cc. Therefore, the number of μ^3 per cc. divided by the number of μ^3 per bacillus would give the total number of M. paratuberculosis per cc. ($10^9 \div 0.3 = 3.3 \times 10^9$). It may be assumed that its specific gravity is similar to that of M. tuberculosis or 1.045.[1] If the specific gravity is 1.045, then there would be 1045 mg. of M. paratuberculosis per cc. Thus, there would be $3.3 \times 10^9 \div 1.045 \times 10^3 = 3.16 \times 10^6$ organisms in 1 mg. of wet

---

[1]Larsen, A. B., Ames, Iowa. Data on M. paratuberculosis. Private communication. 1963.

M. paratuberculosis. Wet packed bacilli (centrifuged at 3,000 r.p.m. for 30 min.) consist of about 85% moisture.[1] Then, 0.15 mg. of dry M. paratuberculosis would contain 3.16×10^6 bacilli or 1 mg. would contain 2.1×10^7 bacilli. According to this calculation, each hamster in this study was inoculated with approximately 21 million organisms. This was a large dose for the hamsters that were inoculated intraperitoneally because it was noted that the heat-killed organisms also produce extensive lesions. This dose was more realistic for the intragastric inoculations as, after mixing with the ingesta, many of the bacilli probably never made contact with the intestinal wall or crossed the "absorptive zone."

Brotherston et al. (20) reported that 2 mg. wet sample of M. paratuberculosis contained about 10^8 to 10^9 bacilli. If this were corrected for 85% moisture, then there would be 3.3×10^8 to 3.3×10^9 bacilli per mg. dry weight. This would be 16 to 158 times more organisms per mg. than that calculated above. Lominski et al. (60) have reported that a bacillus of M. paratuberculosis weighs 2×10^{-10} mg. or that there are 5×10^9 organisms per mg. This was about 238 times more bacilli per mg. than calculated for the inoculum used in this study. If the specific gravity and moisture content were correct in the above calculations, then it would seem inconceivable to think 16 times more organisms could be packed into a cc. than had been obtained by centrifugation at 3,000 r.p.m., let alone 158 or 238 times more

---

[1] Larsen, A. B., Ames, Iowa. Data on M. paratuberculosis. Private communication. 1963.

bacilli. If calculated on the basis that the bacillus was only 0.5 μ long (an unrealistic figure), there still would only be 6.4×10^7 bacilli per mg. dry weight or only about 3 times more than that calculated for the dose used in this study. Although the inoculum was digested from tissue, it was estimated to be about 98% pure bacilli of M. paratuberculosis.

Route of Inoculation

Payne and Rankin (80) have reported that the tonsil is a common portal of entry for M. paratuberculosis in calves inoculated by the oral route. Also, Dunkin (51) isolated M. paratuberculosis from only the submaxillary and suprapharyngeal lymph nodes of 2 cows, again indicating that the tonsil may be the portal of entry. Therefore, to avoid entry via the tonsil, the intragastric route was chosen instead of oral dosing for two groups of hamsters in this study. Other factors for using the intragastric route were to avoid possible inhalation of the organisms, to decrease the chance of regurgitation, to maintain a more persistent dosage, and to avoid mechanical damage to the esophagus that was encountered in preliminary work on passing a stomach tube.

One of the purposes of this study was to find a laboratory animal with an incubation period for M. paratuberculosis that was shorter than that in ruminants. Therefore, intraperitoneal inoculations with living M. paratuberculosis were made in one group of hamsters and the rate of spread of organisms and the development of lesions were

compared with the hamsters that received the living organisms intragastrically. Also, since dead acid-fast bacilli are known to produce lesions when inoculated intraperitoneally (42, 43, 54, 104), heat-killed M. paratuberculosis was inoculated intraperitoneally for assessment of tissue reactions to non-metabolic foreign material of the organisms. Thus, an evaluation was made of the distribution and comparison of the lesions produced by living and heat-killed M. paratuberculosis.

Comparison of the Four Groups of Hamsters Inoculated with M. paratuberculosis

Previous histopathology has been done on a very limited number of tissues of hamsters inoculated with M. paratuberculosis by Francis (34), Harding (45), Gilmour and Brotherston (36), and Gilmour et al. (37).

Living organisms administered intragastrically

In the hamsters in this study that received living M. paratuberculosis intragastrically (Table 12), only 2 acid-fast bacilli were observed in a macrophage in the lamina propria of the duodenum at 2 days PI. No other acid-fast bacilli were observed until 14 and 28 days PI. An increase in distribution of acid-fast bacilli and 2-plus lesions (Figures 4, 9, 10, and 11) were noted at 56 days PI. After 98 days PI, the acid-fast bacilli and lesions were widely distributed in the lymphoid tissue. By 154 days PI, the distribution of bacilli and lesions had reached its peak. Also, by 154 days, there were distinct foci of reticuloendothelial tissue hyperplasia in all of the lymph nodes examined

(Figures 12, 13, 15, 16, and 17). The most notable difference after 154 days PI was the increase in area occupied by the lesions of reticuloendothelial tissue hyperplasia (Figures 19, 20, and 23) and the development of Schaumann's bodies and foreign body giant cells (Figures 21, 23, 25, and 26).

The failure to demonstrate acid-fast bacilli at 4 and 8 days PI was attributed more to the "dilution effect" as the organisms were taken into the various tissues of the host than to destruction of the organisms by the host as suggested by Gilmour et al. (37). The acid-fast bacilli appeared in the mesenteric lymph nodes, Peyer's patches and liver about the same time and their persistence in these organs suggested that they became established simultaneously in these locations of the host.

By 56 days PI, the appearance of the acid-fast bacilli in the regional lymph nodes indicates that they have been carried by the lymphatic system into the bloodstream. From here, they were disseminated to all parts of the body and again collected by the regional lymph nodes. Greater emphasis of this point was indicated by the reticuloendothelial tissue hyperplasia in the tissue examined at 98 and 154 days PI.

These results were similar to those of Gilmour and Brotherston (36) as they demonstrated no lesions in the livers or mesenteric lymph nodes of hamsters examined 2 mo. PI after oral inoculation with 10^7 bacilli (about 1/2 as many bacilli as in the dose used in this study), but they demonstrated lesions in the mesenteric lymph nodes of 1 of 6 hamsters from each group examined at 1 mo. PI and in all 6 hamsters in each group

examined 2 mo. PI after oral inoculation with 10^8 or 10^9 bacilli (5 to 50 times more bacilli than in the dose used in this study). However, they did not demonstrate any liver lesions in any of the hamsters they examined. Lesions, containing acid-fast bacilli, were present in the livers in this study.

Gilmour et al. (37), using 6×10^7 organisms of cultural origin in oral inoculation of hamsters (about 3 times larger dose than used in this study), produced no lesions in the liver until 8 mo. PI or in the small intestine until 11 mo. PI. Lesions were obtained much earlier in this study in both the liver and in the Peyer's patches of the small intestine. The fact that intestinal lesions were confined to lymphoid tissue of the intestine in this study and that the only lesion that Gilmour et al. (37) demonstrated was in a Peyer's patch, which they admitted difficulty in including in sections, may explain the absence of intestinal lesions in their examinations of the intestine at earlier time intervals PI. Only one-third the number of organisms were inoculated in this study and lesions appeared earlier (98 and 154 days) in both the liver and the Peyer's patches. This would indicate that the organisms used were more virulent than those used by Gilmour et al. (37). The increased virulence may be attributed to the fact that the organisms had never been cultured in vitro.

With the exception of the duodenum, the intestinal lymph nodules were fairly numerous in the intestinal tract (Table 13). The Peyer's patches, which could be observed grossly, were easily demonstrated histologically and acid-fast bacilli and/or lesions were found in over

90% of those examined at or after 56 days.

Heat-killed organisms administered intragastrically

No acid-fast bacilli or lesions (Figures 33, 34, 35, 36, 37, and 38) were found in the tissues from any hamsters that were intragastrically inoculated with heat-killed M. paratuberculosis (Table 14). Intestinal lymph nodules were observed as frequent in this group (Table 15) as in any of the other 3 groups examined (Tables 13, 17, and 19). The absence of acid-fast bacilli in the tissues from this group may be explained on the basis that either the dead bacilli were not capable of crossing the intestinal barrier or if they did cross the intestinal barrier, and since no multiplication took place, their number was not great enough to be detected by histopathologic examinations. This group not only served as a control group for the other 3 groups included in the study but also as a control for the hamsters that were inoculated intragastrically with living organisms. This comparison emphasized that the organisms did multiply and a progressive infection was established in hamsters receiving living M. paratuberculosis intragastrically. This would be in agreement with the viable unit count examination of M. paratuberculosis in hamsters done by Gilmour and Brotherston (36) and by Gilmour et al. (37).

Living organisms administered intraperitoneally

Several comparative differences were made between the groups receiving living M. paratuberculosis intraperitoneally or intragastrically (Tables 16 and 12). More acid-fast bacilli were present

in the tissues of hamsters that were inoculated intraperitoneally and examined at 2, 4, 8, 14, 56, and 98 days PI (Figures 39, 40, 41, and 43) than in the hamsters that received living organisms intragastrically. Widespread distribution of 3-plus lesions were present by 56 days compared with 154 days in the hamsters receiving living organisms intragastrically. Little difference was noted in the distribution of lesions in the 2 groups after 56 days. However, the reticulo-endothelial tissue hyperplasia became more extensive in the lymphoid tissue with duration of time following intraperitoneal inoculation with living M. paratuberculosis (Figures 45, 46, 47, 48, 49, 50, and 51).

The absence of either bacilli or lesions in the tissues of the hamster inoculated intraperitoneally and examined at 28 days may be explained on the basis that either the hamster was never inoculated or that the organisms were inoculated into the urinary bladder.

The later appearance of lesions produced by the living organisms inoculated intragastrically, when compared with living organisms inoculated intraperitoneally, may be due to the necessary time for multiplication of M. paratuberculosis in the host tissues in quantities sufficient to produce lesions.

Heat-killed organisms administered intraperitoneally

The hamsters inoculated intraperitoneally with heat-killed M. paratuberculosis served as a control for the hamsters that received intraperitoneally living M. paratuberculosis (Table 18). It is interesting to note that the presence and distribution of the acid-fast bacilli

at 2, 4, 8, 14, and 28 days PI were similar to that observed in the group receiving living organisms intraperitoneally. Although 3-plus lesions did develop at about the same time as the lesions in hamsters receiving living organisms intraperitoneally, their distribution was never as extensive. Also, after 266 days PI, the lesions were regressing. It was interesting to note that bacilli and lesions persisted longer in the omentum than in other tissues. Schaumann's bodies and foreign body giant cells were present.

A comparison of the results from the 4 groups of hamsters studied are summarized in Table 20. It may be noted here that heat-killed M. paratuberculosis, if taken into the tissues of the host, was capable of producing lesions that were indistinguishable from the lesions produced by living M. paratuberculosis. Three-plus lesions, that may occupy 40 to 50% of some of the involved lymph nodes, were produced by heat-killed organisms. However, their distribution in the lymphoid tissue was never as extensive as the lesions produced by living organisms. These lesions eventually regressed.

Schaumann's bodies, first observed at 98 days in either of the 3 groups with lesions, and foreign body giant cells were prevalent and more correlated with the degree of hyperplasia than with the route of inoculation. None were found in hamsters that were inoculated intragastrically with heat-killed M. paratuberculosis.

Table 20. Incidence of lesions in hamsters inoculated with 1 mg. of M. paratuberculosis

Duration following inoculation (days)	Route of inoculation			
	Intragastrically		Intraperitoneally	
	Living organisms	Heat killed organisms	Living organisms	Heat killed organisms
2	-	-	+	+
4	-	-	+	+
8	-	-	+	+
14	+	-	++	+
28	+	-	-	+
56	++	-	+++	+++
98	++	-	+++	+++
154	+++	-	+++	++
210	+++	-	+++	++
266	+++	-	+++	o
329	+++	-	+++	o

- = No organisms or tissue reaction
\+ = Organisms within individually located macrophages
++ = Tissue reactions occupying less than 3% of the normal tissue
+++ = Tissue reactions occupying more than 3% of the normal tissue
o = Old healing lesions without organisms

Character of the Lesions

The lesion producing factor of M. paratuberculosis does not reside entirely in the active metabolic state of the organisms. There exists in the lipids of the bovine tubercle bacillus a certain fatty acid (phthioic acid) which, after injected intraperitoneally in animals in a pure form, will cause lesions resembling those produced by the activity of the living organisms (6, 87). Although this fatty acid has not been isolated from the paratuberculosis bacillus, there is no doubt but that it does exist in this bacillus and is probably responsible for the lesions described in this study in the hamsters receiving heat-killed organisms intraperitoneally.[1]

Lesions of paratuberculosis in the peritoneum of guinea pigs as described by Hagan and Mansfield (43) consisted principally of masses of monocytes and epithelioid cells generally surrounded by a few lymphocytes. Harding (45) described the lesions of paratuberculosis in the Peyer's patches of the hamster as being round discrete foci of epithelioid cells with abundant pale, eosinophilic cytoplasm. Runnells et al. (86) describes the lesions in the bovine mesenteric lymph nodes as having no necrosis and consisting of macrophages that are often filled with acid-fast bacteria. Twort and Craig (104) considered M. paratuberculosis to be one of the least toxic of the acid-fast bacteria and attributes this factor to the capability of

---

[1]Merkal, R. S., Ames, Iowa. Data on fractionation of M. paratuberculosis. Private communication. 1967.

the organism to survive and multiply in cells without killing the host cell.

Histologically, the inflammatory response to M. paratuberculosis in this study was strictly granulomatous. Initially, when many organisms were present, as in the omentum of the hamsters inoculated intraperitoneally, there was an infiltration of neutrophils and lymphocytes that persisted for about 4 days. By 8 days, most of the neutrophils had disappeared and lymphocytes and macrophages predominated.

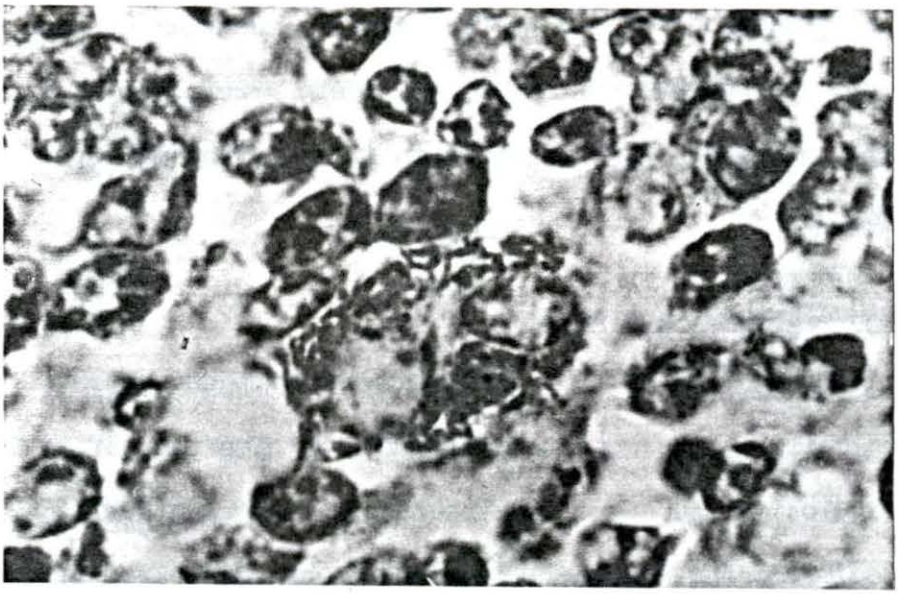
Very little neutrophilic response was observed in the lymph nodes of any of the hamsters in the 4 groups. The first lesions were individual reticuloendothelial cells containing acid-fast bacilli (Figure 3) which eventually developed into distinct foci of reticuloendothelial tissue hyperplasia (Figures 6, 14, 16, 17, 32, 42, and 50). The lesions were essentially similar in all of the lymphoid tissue. Except in the omentum and peritoneum, the lesions were distributed throughout the body and confined primarily to the lymphoid tissue.

There was a very distinct border between the reticuloendothelial tissue hyperplasia and the adjacent normal lymphoid tissue (Figures 6, 14, 17, and 32). Electron microscopic examination revealed that a single layer of fibroblasts were often present between the normal lymphoid tissue and the hyperplastic reticuloendothelial cells (Figure 74). These cells probably represent a barrier around the lesions and were considered responsible for the distinct border.

Figures 82 and 83 show the differences in the organisms in macrophages from the bovine and from the hamster. The macrophage in

Figure 82. Acid-fast bacilli in a distended macrophage of the bovine intestinal mucosa used as a source of M. paratuberculosis for inoculation of hamsters. Ziehl-Neelsen method. x1,250.

Figure 83. Acid-fast bacilli in a macrophage of the anterior mesenteric lymph node of hamster. Note number and size of bacilli and compare with those in Figure 82. Ziehl-Neelsen method. x1,250.



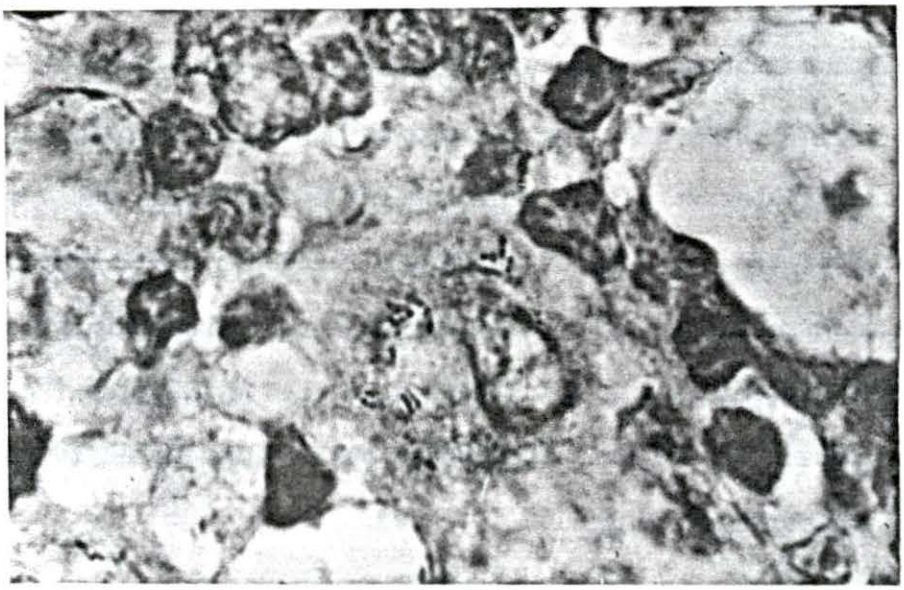


Figure 82 was from the bovine intestinal mucosa that was used as a source of M. paratuberculosis for inoculating hamsters in this study. The acid-fast bacilli in the bovine were much larger than those observed in the hamster. Further, there were never as many acid-fast bacilli per macrophage in the hamster as in the macrophage from the bovine.

Multinucleated giant cells

Simpson (94) in 1966 described the fine structure of Langhans' giant cells in the mesenteric lymph node of a case of bovine paratuberculosis. He supported the theory that giant cells arise from fusion of mononuclear cell macrophages and described irregularly shaped nuclei, many cytoplasmic vesicles, and desmosome-like structures along the plasma membrane.

Multinucleated foreign body giant cells were often present in the more advanced lesions in this study (Figures 72, 74, 75, and 76) and usually contained bacilli and/or Schaumann's bodies (Figures 26 and 75). The absence of the desmosome-like structures and large cytoplasmic vesicles, the presence of uniformly rounded nuclei, many mitochondria, and electron dense cytoplasm indicated that these giant cells may be of a different origin than those described by Simpson (94). No evidence was present to indicate that these cells were formed by fusion of mononuclear macrophages.

Plasma cells

Plasma cells have been described as secretory cells that produce

and secrete gamma globulins with specific affinities for particular antigens (44). Their cytoplasm shows a great specialization for the production of protein secretion, and are copiously supplied with rough-surfaced endoplasmic reticulum (Figures 62, 63, and 67). The rough endoplasmic reticulum in the figures shows various stages of dilatation that indicates various stages of protein production (9). The cisternae contain a fine floccular substance and according to Movat and Fernando (72), they may also contain small dense bodies (Russell's bodies) and crystals. The ribosomes manufacture the protein and it is passed across the membrane into the cisternal space where it is transferred to the golgi complex and "packaged for export" from the cell. If this holds true, it can be theorized that the plasma cells in Figures 62, 63, and 67 are in various stages of protein production, possible antibodies against M. paratuberculosis.

The plasma cells and eosinophils were most numerous around 42 days PI in the hamsters inoculated intraperitoneally for electron micrographic studies (Figure 62). The cisternae were usually most distended at 42 days (Figures 62 and 63). This cellular reaction to M. paratuberculosis would probably be the "secondary response" of inflammation as described by Speirs (96).

Nuclear vacuoles

The double membrane enclosed vacuole in 1 of the nuclei of a multinucleated reticular cell (Figure 76) has been described in bovine leukemia (53), Burkitt's lymphoma (1), and malignant lymphoma in children (29). Other than this study, these vacuoles have only been

described in the above leukemias. This vacuole contains cytoplasmic components and must be regarded as of cytoplasmic origin. It is thought to be a cross section of an invagination of the cytoplasm into the nucleus. Most likely, it is a characteristic of very hyperplastic cells and not associated with any one particular disease or condition.

Thymus

The absence of lymph nodules and germinal centers in the thymus has been attributed to the theory that antigens were not gaining access to the thymic lymphoid tissue. After transfusions of tritiated labeled lymphocytes into the thoracic duct of adult rats, Gowans and Knight (40) were unable to demonstrate any such cells in the thymus although the labeled cells were present in the lymph nodes, lymph nodules of the spleen, and Peyer's patches. Weiss (110) has demonstrated that many of the thymic vessels have small luminal diameters of about 1 μ and thought that such vessels may carry plasma preferentially. Weiss also demonstrated that their endothelium is without aperatures, their basement membrane broad, and their walls further increased by adventitial cells and extracellular tissue. However, Weiss did demonstrate that thorium dioxide could cross into the basement membrane and associated extravascular tissue.

Reticuloendothelial tissue hyperplasia was very limited in the thymus in this study. It rarely exceeded 3% of the thymic tissue in the sections studied. The vascular barrier in the thymus may explain why only a few acid-fast bacilli and foci of hyperplasia were found in

those hamsters inoculated with living M. paratuberculosis (Figures 27 and 28). The fact that acid-fast bacilli did gain access to the lymphoid tissue and that reactive centers of hyperplasia did occur would indicate that large particles can cross this barrier. This would be contrary to Metcalf's belief (67) that macrophages and other cell complexes, which are necessary for antigen handling and for the framework of reactive centers, are absent. Since the thymus has no afferent lymphatic like the lymph nodes, it must depend on the blood supply for any antigens that it may receive. Conclusion is made here that the blood barrier system is probably the most important factor for the lack of lesions in the thymus in this study.

Pathogenesis of M. paratuberculosis in the Hamster

The method by which M. paratuberculosis crosses the epithelial barrier of the intestinal mucosa and enters the lymphatic system has not been determined. Since M. paratuberculosis contains a lot of lipid in its cell wall, it may be theorized that the bacillus may follow a similar route as that of lipid droplets. It is known that particles as large as 1 μ can cross the epithelial cell of the intestine. Since M. paratuberculosis does cross the intestinal barrier in some manner and that it is not much larger than this, it would be somewhat realistic to think it may follow the same route as lipid absorption.

Palay and Karlin (79) delineated the pathway of fat absorption. They found that fat droplets were taken into pinocytotic vesicles

in the intermicrovillous spaces of the striated border of the epithelial cells. These vesicles join or empty into the endoplasmic reticulum, in the lumen of which the droplets pass toward the lateral surface of the epithelial cell at a position below the terminal bars. Here, by reversal of pinocytosis, they are released into the intercellular spaces of the epithelium. The droplets were found to traverse the basement membrane of the epithelium into the interstitial spaces of the lamina propria, penetrate the basement membrane of the central lacteal, and finally slip between the overlapping endothelial cells to enter the lymphatic.

Once an acid-fast bacillus passed around the terminal bars of the epithelial cells, it would not be difficult to visualize its crossing the basement membrane into the interstitial spaces of the lamina propria. The first bacilli observed in this study were within a macrophage in the lamina propria. Once in the afferent lymphatics, the intracellular or extracellular bacilli would be carried to the mesenteric lymph node where they would be exposed to the phagocytic cells in the subcapsular sinus. Some of the first bacilli observed in this study were within the reticuloendothelial cells adjacent to the subcapsular sinus. Reticuloendothelial tissue hyperplasia could be observed to develop around the large phagocytic reticular cells (Figure 9) which were so prevalent in the normal lymph nodes of the hamsters (Figures 7, 8, 35, and 36). With multiplication of the intracellular bacilli and the resulting death of the cell, the free bacilli, along with those in phagocytes, were carried by the efferent lymphatics to the thoracic duct.

From here, they were emptied into the bloodstream and disseminated to all parts of the body. The bacilli were again picked up by the lymphatics and phagocytized by the reticuloendothelial cells of the regional lymph nodes.

Structure of M. paratuberculosis

No distinct enveloping capsule was detected in thin sections of M. paratuberculosis by electron microscopy (Figures 59 and 60). Chapman et al. (26) have described a microcapsule within an enclosing membrane that appeared to delineate the bacterial cell and its possible capsular material from the host cytoplasm. However, Chapman interpreted that these membranes were probably formed by tissue cells rather than the bacteria. It has been hypothesized that the high lipid content of the capsule and non-polar wetting properties of the cell wall are related to the high resistance of mycobacteria to acids, alkalies, and other chemical substances and to their slow rate of metabolism and growth (75). Reaction of mycobacteria with specific immune sera will reduce their resistance and result in increase cohesiveness and agglutination and greater ease of being phagocytized (73, 74).

When a slight shrinkage occurs in fixation, a space occurs between the protoplast and the cell wall as shown in Figure 60. This artifact elucidates what has been described as a plasma membrane consisting of 2 dense layers, each about 30 Å in width (49).

The polyphosphate bodies observed in M. paratuberculosis in Figure 59 have been shown to increase in size and number under conditions

favoring accumulation of metaphosphate in mycobacterial cells. The stored polyphosphate has been shown in P^{32} tracer studies to be utilized in nucleic acid synthesis (77). The polyphosphate bodies of mycobacteria are also considered to be homologous with the metaphosphate granules of Corynebacterium diphtheriae (90). Mudd et al. (76) have induced vacuoles in the high energy-yielding electron dense areas of mycobacteria by intense electron bombardment. This may explain the presence of vacuoles in a few of the bacilli in Figure 59. No description was found in the literature that could be related to the very small dense particles also observed in some of the bacilli in Figure 59.

The very faint "lamellar structures" that can be observed in certain areas of the bacilli in Figures 59 and 60 were considered to be folded extensions of the cytoplasmic membrane into the cytoplasm. Extensive enzymic activity has been demonstrated on these "lamellar structures," thus indicating it plays an important role in the metabolism of the bacteria (55). The extension and concentration of the cytoplasmic membrane into a lamellar-like structure would serve in intensifying the metabolic activity of the bacillus. The lesion producing capability of living organisms may be enhanced with an increase in metabolic activity.

Electron Microscopy of M. paratuberculosis in the Tissue

Electron micrography has been made on M. lepraemurium in tissue of mice by Allen et al. (4) who described peri-bacillary spaces and

peri-bacillary bodies. Imaeda and Convit (49) made an extensive ultrastructural study of M. leprae in human biopsies. They described plasma membranes, polyphosphate bodies, nuclear apparatus, and an intracytoplasmic membrane system. Simpson (94) described M. paratuberculosis in a membrane enclosed area of low electron density in macrophages from the mesenteric lymph node of a cow.

The development of phagosomes from lysosomes and endocytic invaginations containing particulate matter has been described by De Duve (28). Cohn and Wiener (27) demonstrated that lysosomal hydrolase was released into the phagocytic vacuoles (phagosomes) and presumably aids in the digestion of engulfed particles.

In contrast to the results of Allen et al. (4), Imaeda and Convit (49), and Simpson (94), no peri-bacillary bodies or peri-bacillary spaces were present around the phagocytized bacilli observed in this electron microscopic study of M. paratuberculosis (Figures 64, 65, 66, and 81). Instead the bacilli were often contained within electron dense masses, the phagosomes (Figures 65, 66, and 81). De Duve stated that the enzymes of the phagosome digest its contents and the products diffuse into the cytoplasm. Only remnants that prove to be refractory to attacks by the enzyme are left behind to form a residual body. The accumulation of residual bodies plays a part in the aging of such phagocytic cells.

Polyphosphate bodies could be demonstrated in the bacilli present in large lysosomes stained for acid phosphatase (Figure 81). Since the acid phosphatase study in this experiment was made at 98 days after the

last injection of M. paratuberculosis, this would indicate that either the host cell was fairly tolerant to the bacillus or that the hamster produced no antibody to M. paratuberculosis. The presence of activated plasma cells indicates that the hamster did produce antibodies. Further, little alteration was noted in the cell since the mitochondria, that are sensitive to intracellular environmental changes, appeared normal. Therefore, the bacillus in Figure 81 must not be very toxic to the host tissue.

Post-Capillary Venules

It has been shown that the main flow of lymphocytes from blood to lymph lies within the lymph nodes and that small lymphocytes enter by crossing the walls of a specialized set of blood vessels, the post-capillary venules. In rats, Gowans and Knight (40) traced tritiated adenosine labeled lymphocytes from the thoracic duct, via the blood-stream, to the lymph nodes where they observed the lymphocytes as they passed through the endothelium of the post-capillary venules into the cortex. In an electron microscopic study, Marchesi and Gowans (63) demonstrated many small lymphocytes between the endothelium and the periendothelial sheath of the post-capillary venules in lymph nodes of rats. They noted, in serial section, that the lymphocytes traversed the cytoplasm of the endothelial cells instead of passing through the intercellular junctions.

Small thick-walled vessels, called post-capillary venules, were described in all 4 groups of hamsters in this study (Figures 17, 18,

and 24). Although the post-capillary venules were present in large numbers per amount of tissue examined, their frequency was considered to be within normal limits since they were present in the lymph nodes of hamsters that received both living and killed M. paratuberculosis, they were present in most lymph nodes of any particular hamster and they were more prevalent in the growing hamster than in the mature adult. An electron microscopic study revealed that most of the cells in the wall were lymphocytes and that the vessels were post-capillary venules (Figure 78) as described by Gowans and Knight (40) and by Marchesi and Gowans (63). The lymphocytes were located between the endothelial cells and the pericytes forming the periendothelial sheath. Lymphocytes were also noted within the cytoplasm of endothelial cells. This is in contrast to polymorphonuclear leukocytes and monocytes which migrate from the blood by passing between the endothelial cells of inflamed venules of lymph nodes and other tissues (40).

Schaumann's Bodies

Schaumann's bodies may occur in a variety of granulomatous lesions, especially in the golden hamster.

Schaumann (91) described bodies, to which is affixed his name, in lymphogranulomatosis benigna and noted that other elements (elastic fibers, connective fibers, hair, tubercle bacilli, and fungus elements of streptothrix) had been found in the interior or in relation to the bodies by other workers. Frenkel (35) described the bodies in hamsters inoculated with photochromogenic mycobacteria and Binford (10) found

many such bodies around acid-fast bacteria in golden hamsters that had been inoculated with tissue that contained M. leprae. Okudaira et al. (78) found Schaumann's bodies in hamsters that had been inoculated with Histoplasma sp., Blastomyces sp., M. tuberculosis, or Escherichia coli. Rasmussen and Caulfield (85) reported that Schaumann's bodies, around photochromogenic mycobacteria in golden hamsters, were composed of an apatite (hydroxyapatite) and iron.

Fite (32) reported that the Schaumann's body began as an intracellular protein condensate in the cytoplasm and consisted of a central core surrounded by a clear halo. A second condensate formed around the central core and calcification occurred as a secondary phenomenon, although calcification was not essential to the development of the initial body. Fite (32) noted that secondary condensates formed a third or fourth layer and, with extrusion from the parent cell, the body provoked a foreign tissue reaction. Fite (32) concluded that the presence of both bacteria and Schaumann's bodies in a single cell were not essential and that an initial antigen-antibody reaction was the inciting cause.

Many Schaumann's bodies were present in this study (Figures 12, 14, 21, 23, 25, 26, 30, 42, 43, 44, 45, 46, 48, 50, 51, 71, 72, and 73). The bodies were first observed at 98 days in the ileocecal lymph node of hamsters inoculated intragastrically with living organisms, at 98 days in both visceral and regional lymph nodes from hamsters inoculated intraperitoneally with living organisms, and at 98 days in the liver of hamsters inoculated intraperitoneally with heat-killed organisms.

They were also found at and after 90 days in the anterior mesenteric lymph nodes of the hamsters used in electron microscopy (Figures 71, 72, and 73).

The precise sequence of intracellular changes involved in Schaumann's body formation is obscure. Fite (32) considered that an organism was not essential for the central core of the Schaumann's body. In this study, early stages of what was presumably Schaumann's body formation (Figures 68, 69, and 70) were associated with a bacillus. Rasmussen and Caulfield (85) assumed that the lamella capsules originated by bacterial products diffusing into the surrounding cytoplasm where calcium and phosphates were precipitated into an apatite crystal. The presence of mineralization was demonstrated in this study by the von Kossa stain (Figure 26). However, if the inciting factor for Schaumann's body formation came from the bacteria, it would be difficult to explain the formation of the second or third lamella once the first was formed or after the bacillus had disintegrated. Also, if the "inciting factor" comes from the bacteria, it must be a degradation product as Schaumann's bodies were present in this study after inoculation with heat-killed M. paratuberculosis.

Okudaira et al. (78) suggested the lamellar capsule formed around the organism as the result of cellular or cytoplasmic reaction of phagocytes to the intracellular and degenerated organism or to calcified material. This was a very non-specific suggestion. It was demonstrated in this study (Figure 70) and by Rasmussen and Caulfield (85) that the bacteria were degraded. Mineralization was considered as secondary and

not essential by Fite and Rasmussen and Caulfield. No evidence was found in this study that mineralization triggered the initial intracellular reaction, although mineralization was noted later (Figures 26, 71, 72, and 73).

No report was noted which mentioned any possible role that the lysosome may be involved in Schaumann's body formation. It was observed that the body did form around a bacillus. Since many bacilli were observed to be phagocytized and partially degraded, it was concluded that these bacterial products had a very minor, if any, role in Schaumann's body formation.

Since only a few Schaumann's bodies were found in comparison to the number of bacilli observed, it was concluded that all bacilli did not participate in Schaumann's body formation. Therefore, some factor other than the bacillus must be involved.

A large focus of intracellular necrosis is shown in Figures 68 and 69. In the necrotic area, a bacillus was found. The granular material in the membrane-enclosed area stains very dense with lead citrate as do the lysosomes in the same cell. This would indicate that a protein substance is concentrated in the area of necrosis, possibly that of hydrolytic enzymes. Since it is known that hydrolytic enzymes are present in the lysosome and that rupture of this organelle will lead to cytoplasmic damage or even death of the cell, it was theorized that the site of Schaumann's body formation was initially a damaged lysosome.

The Algerian gerbil (Meriones sp.) is a rodent closely related to the hamster. Its reaction to photochromogenic and other mycobacteria

was reported to be essentially similar in pattern to that of the hamster, with the production of numerous Schaumann's bodies (85).

Since many bacilli could be demonstrated in lysosomes, it was obvious that not all bacilli-laden lysosomes formed Schaumann's bodies. Since it is known that the bodies can be regularly produced by the same organisms in hamsters and rarely in rats and mice (78), it was concluded that the lysosome of the phagocytic reticuloendothelial cell of the hamster may genetically be more sensitive or easily damaged by a lipid containing factor of several granulomatous disease-producing agents.

Therefore, it was concluded that the Schaumann's body was the result of a lysosomal accident when the phagocytic vacuole and the lysosome merged to form the phagosome. The lamellae probably represent periods of repeated rupture of an already weakened membrane of the lysosome (phagosome). Figures 68, 69, 70, 71, 72, and 73 represent stages of Schaumann's body formation.

Ileitis

The enlargements or constrictions of the ileum that were encountered at 14, 28, and 156 days following intragastric inoculation with living bacilli, at 8, 14, 28, and 56 days following intragastric inoculation with heat-killed bacilli, at 8, 14, 28, and 56 days following intraperitoneal inoculation with living bacilli, and at 14, 28, and 56 days following intraperitoneal inoculation with heat-killed bacilli were gross lesions of hamster ileitis described by Boothe and Cheville (11). Histologic lesions confirmed this diagnosis. The condition was not

considered to be in any way associated with M. paratuberculosis since it occurred in all 4 groups in about equal numbers at similar time intervals. Further, hamster ileitis has also been observed in uninoculated hamsters.

SUMMARY AND CONCLUSIONS

1. Sixty hamsters were inoculated with 1 mg. of M. paratuberculosis as follows:

Fifteen hamsters were inoculated intragastrically with living M. paratuberculosis.

Fifteen hamsters were inoculated intragastrically with heat-killed M. paratuberculosis.

Fifteen hamsters were inoculated intraperitoneally with living M. paratuberculosis.

Fifteen hamsters were inoculated intraperitoneally with heat-killed M. paratuberculosis.

Postmortem examination was done on 1 hamster from each group at 2, 4, 8, 14, 28, 56, 98, 154, 210, and 329 days following inoculation.

2. No clinical signs of paratuberculosis were produced in the hamster.

3. Regardless of the route of inoculation, the regional as well as the visceral lymph nodes became equally involved.

4. There was a very extensive reticuloendothelial tissue hyperplasia for the number of acid-fast bacilli present in the lymphoid tissue.

5. The foci of reticuloendothelial tissue hyperplasia in the lymphoid tissue of the hamster were very distinct. With electron microscopy, a partial barrier of fibroblasts were present.

6. In the intestinal tract, the acid-fast bacilli and lesions of hamster paratuberculosis were confined to the Peyer's patches and lymph nodules.

7. The bacilli of heat-killed M. paratuberculosis were not demonstrated to have crossed the epithelial barrier of the intestinal tract.

8. The macrophage of the hamster may contain several acid-fast bacilli but they never become distended with organisms.

9. The bacilli of M. paratuberculosis in the macrophage of the hamster were smaller than has been described for other species of animals.

10. Lesions of paratuberculosis were extensive at 56 days following intraperitoneal or 154 days following intragastric inoculation with living M. paratuberculosis.

11. Comparison of the distribution of acid-fast bacilli and of lesions in hamsters receiving living and heat-killed M. paratuberculosis indicated that a definite multiplication of M. paratuberculosis occurred and progressive lesions were produced in the lymphoid tissues.

12. Identical lesions were produced with heat-killed or living M. paratuberculosis when inoculated intraperitoneally.

13. Lymphoid tissue was rare in the duodenum but was prevalent in the remainder of the intestinal tract.

14. No linguinal or palatine tonsils were found in the hamster.

15. Schaumann's bodies were produced in the hamster with M. paratuberculosis.

16. This study indicated that the hamster is not a suitable diagnostic animal since more organisms are needed for the establishment of

a progressive infection than that found in the usual specimen submitted for diagnosis. However, in carefully controlled experiments, the hamster might be useful as an assay animal in testing drugs or vaccines for paratuberculosis.

LITERATURE CITED

1. Achong, B. G. and Epstein, M. A. Fine structure of the Burkitt tumor. Journal of the National Cancer Institute 36: 877-897. 1966.

2. Adler, S. Origin of the golden hamster, Cricetus auratus, as a laboratory animal. Nature (London) 162: 256-257. 1948.

3. Alikaeva, A. P. Eksperimental'nyĭ paratuberkulez krolikov (Experimental Johne's disease in rabbits). Veterinariya, Moscow No. 2: 26-32. 1940. Original not available; abstracted in the Veterinary Bulletin 14: 149. 1944.

4. Allen, J. M., Brieger, E. M. and Rees, R. J. W. Electron microscopy of the host-cell parasite relation in murine leprosy. The Journal of Pathology and Bacteriology 89: 301-306. 1965.

5. Andersen, C. W. Übertragung von Paratuberkulose auf Versuchstiere. Archiv für Wissenschaftliche und Praktische Tierheilkunde 47: 77-84. 1921.

6. Anderson, R. J. The separation of lipoid fractions from tubercle bacilli. Journal of Biological Chemistry 74: 525-535. 1927.

7. Bang, B. Chronische pseudotuberkulöse Darmentzündung beim Rinde. Berliner Tierärztliche Wochenschrift 42: 759-763. 1906.

8. Beebe, W. L. Johne's disease in cattle. American Veterinary Review 33: 709-716. 1908.

9. Berenbaum, M. C. Antibody content of single cells. Journal of Clinical Pathology 11: 543-547. 1958.

10. Binford, C. H. Studies on a mycobacterium obtained from the golden hamster (Cricetus auratus) after inoculation with lepromatous tissue. Laboratory Investigation 11: 942-955. 1962.

11. Boothe, A. D. and Cheville, N. F. The pathology of proliferative ileitis of the golden Syrian hamster. Pathologia Veterinaria 4: 31-44. 1967.

12. Boquet, A. Sur les propriétés pathogénes du bacille de Johne (entérite hypertrophiante des bovidés). A l'égard du rat blanc et de la souris blanche. Comptes Rendus, Société de Biologie 93: 219-220. 1925.

13. Boquet, A., Nègre, L. and Valtis, J. Infection et surinfection du cobaye et du lapin par le bacille paratuberculeux de la fléole. Comptes Rendus, Société de Biologie 102: 838-841. 1929.

14. Boquet, A., Nègre, L. and Valtis, J. Sur la dispersion des bacilles paratuberculeux de la fléole inoculés au cobaye par la voie sous-cutanée. Comptes Rendus, Société de Biologie 101: 903-905. 1929.

15. Boquet, A., Nègre, L. and Valtis, J. Sur la dispersion des bacilles paratuberculeux de la fléole inoculés au cobaye par la voie trachéale. Comptes Rendus, Société de Biologie 103: 1225-1227. 1930.

16. Boquet, A. and Saenz, A. Sur la perméabilité de la muqueuse digestive du cobaye au bacille paratuberculeux de la fléole. Comptes Rendus, Société de Biologie 105: 260-263. 1930.

17. Boquet, A. and Valtis, J. Sur la surinfection du cobaye par le bacille paratuberculeux de la fléole. Comptes Rendus, Société de Biologie 104: 1182-1184. 1930.

18. Brosseau, J. Anatomie descriptive et topographique des organes post-diaphragmatiques du Hamster doré (Foie, estomac, pancréas, rate). Unpublished Veterinary Thesis. Library a'Cholet, France, (Maine-et-Loire). 1936.

19. Brotherston, J. G. and Gilmour, N. J. L. A reappraisal of the pathogenesis of an immunity to Mycobacterium johnei in sheep, calves, hamsters and mice using quantitative bacteriological techniques. World Veterinary Congress No. 17, Hannover Proceedings 1: 487-493. 1963.

20. Brotherston, J. G., Gilmour, N. J. L. and Samuel, J. McA. Quantitative studies of Mycobacterium johnei in the tissues of sheep. I. Routes of infection and assay of viable M. johnei. Journal of Comparative Pathology and Therapeutics 71: 286-299. 1961.

21. Chandler, R. L. Infection of laboratory animals with Mycobacterium johnei. I. Infection in Swiss white mice and its modification by suramin and cortisone. Journal of Comparative Pathology and Therapeutics 71: 118-130, 1961.

22. Chandler, R. L. Infection of laboratory animals with Mycobacterium johnei. II. Infection in white rats; effect of cortisone treatment. Journal of Comparative Pathology and Therapeutics 71: 131-134. 1961.

23. Chandler, R. L. Infection of laboratory animals with Mycobacterium johnei. III. Liver and spleen infections in vaccinated and unvaccinated Swiss white mice. Journal of Comparative Pathology and Therapeutics 71: 135-139. 1961.

24. Chandler, R. L. Infection of laboratory animals with Mycobacterium johnei. IV. Comparative susceptibility to infection of C.57, C.B.A. and Swiss white mice. Journal of Comparative Pathology and Therapeutics 71: 233-242. 1961.

25. Chandler, R. L. Infection of laboratory animals with Mycobacterium johnei. V. Further studies on the comparative susceptibility of C_{57} black mice. Journal of Comparative Pathology and Therapeutics 72: 198-213. 1962.

26. Chapman, G. B., Hanks, J. H. and Wallace, J. H. An electron microscope study of the disposition and fine structure of Mycobacterium lepraemurium in mouse spleen. Journal of Bacteriology 77: 205-211. 1959.

27. Cohn, Z. A. and Wiener, E. The particulate hydrolases of macrophages. II. Biochemical and morphological response to particle ingestion. Journal of Experimental Medicine 118: 1009-1020. 1963.

28. De Duve, C. The lysosome. Scientific American 208: 64-72. 1963.

29. Dorfman, R. F. The fine structure of a malignant lymphoma in a child from St. Louis, Missouri. Journal of the National Cancer Institute 38: 491-504. 1967.

30. Eveleth, D. F. and Gifford, A. B. Johne's disease in farm animals. Journal of the American Veterinary Medical Association 102: 27-34. 1943.

31. Eveleth, M. W. and Eveleth, D. F. Johne's disease of goats. Veterinary Medicine 38: 258-261. 1943.

32. Fite, G. L. The Schaumann body. Review of the Second Conference on Progress and Potentials in Leprosy Investigations. United States Department of Health, Education, and Welfare. Public Health Service Publication No. 641. 1958.

33. Francis, J. Infection of laboratory animals with Johne's disease and leprosy. Nature (London) 152: 250-251. 1943.

34. Francis, J. Infection of laboratory animals with Mycobacterium johnei. Journal of Comparative Pathology and Therapeutics 53: 140-150. 1943.

35. Frenkel, J. K. Hormonal effects on a sarcoid-like response with Schaumann bodies and amyloid in golden hamsters infected with photochromogenic mycobacteria. American Journal of Pathology 34: 586-587. 1958.

36. Gilmour, N. J. L. and Brotherston, J. G. Susceptibility of hamsters to the oral administration of Mycobacterium johnei. Nature (London) 192: 1202-1203. 1961.

37. Gilmour, N. J. L., Campbell, J. and Brotherston, J. G. The pathogenesis of Mycobacterium johnei orally dosed hamsters. Journal of Comparative Pathology and Therapeutics 73: 98-106. 1963.

38. Glover, R. E. The standardization of Johnin. I. The suitability of the guinea-pig as a test animal. The Veterinary Journal 97: 3-15. 1941.

39. Glover, R. E. The standardization of Johnin. II. Purification of Johnin and its comparison with an unpurified product. The Veterinary Journal 97: 179-193. 1941.

40. Gowans, J. L. and Knight, E. J. The route of re-circulation of lymphocytes in the rat. Proceedings of the Royal Society of London, Series B. Biological Sciences 159: 257-282. 1964.

41. Gomori, G. An improved histochemical technic for acid phosphatase. Stain Technology 25: 81-85. 1950.

42. Hagan, W. A. and Levine, P. The pathogenicity of the saprophytic acid-fast bacilli. Journal of the American Veterinary Medical Association 81: 723-733. 1932.

43. Hagan, W. A. and Mansfield, H. L. The lesions produced by the Johne bacillus of disease in the peritoneal cavity of the guinea pig. Journal of the American Veterinary Medical Association 76: 182-190. 1930.

44. Ham, A. W. Histology. 5th ed. Philadelphia, Pennsylvania, J. B. Lippincott Company. 1967.

45. Harding, H. P. The histopathology of Mycobacterium johnei infection in small laboratory animals. Journal of Pathology and Bacteriology 78: 157-169. 1959.

46. Heelsbergen, T. van. Are hens susceptible to infection with the bacillus of Johne's disease (para-tuberculosis)? Journal of Comparative Pathology and Therapeutics 44: 213-215. 1931.

47. Hirch, A. Infection of hamsters and rabbits with Mycobacterium johnei. Journal of Comparative Pathology and Therapeutics 66: 260-269. 1956.

48. Holth, H. Reinzüchtung des Bacillus der spezifischen chronischen Darmentzündung des Rindes (Paratuberkelbacillus). Zeitschrift fur Infektionskrankheiten, Parasitäre Krankheiten und Hygiene der Haustiere 11: 378-387. 1912.

49. Imaeda, T. and Convit, J. Electron microscope study of Mycobacterium leprae and its environment in a vesicular leprous lesion. Journal of Bacteriology 83: 43-52. 1962.

50. Jöhne, H. A. and Frothingham, L. Ein eigentümlicher Fall von Tuberkulose beim Rind. Deutsche Zeitschrift für Tiermedizin und vergleichende Pathologie 21: 438-454. 1895.

51. Johne's disease [Discussion of]. Imperial Veterinary Conference No. 1, London Proceedings 1938: 68-77. 1938.

52. Johnson, E. P. and Pratt, A. D. Johne's disease and Johnin reactions. American Journal of Veterinary Research 5: 117-124. 1944.

53. Knocke, K. W. Electronemikroskopische Befunde an Lymphozyten and Lymphoidzellen des peripherischen Blutes bei der Leukose des Rindes. Zentral blatt für Veterinärmedizin 11: 1-10. 1964.

54. Koch, R. Fortsetzung der mittheilungen über ein heilmittel gegen tuberkulose. Deutsche Medizinische Wochenschrift 17: 101-102. 1891.

55. Koike, M. and Takeya, K. Fine structures of intracytoplasmic organelles of mycobacteria. The Journal of Biophysical and Biochemical Cytology 9: 597-608. 1961.

56. Konst, H. and Watson, E. A. Studies on Johne's disease: Reactions to Johnin in sensitized guinea pigs. American Journal of Veterinary Research 4: 34-44. 1943.

57. Larsen, A. B. and Merkal, R. S. A technique for concentrating Mycobacterium paratuberculosis present in intestinal mucosa. American Journal of Veterinary Research 22: 1074-1076. 1961.

58. Levi, M. L. Correspondence. The Veterinary Journal 97: 138-141. 1941.

59. Levi, M. L. The susceptibility of voles to Mycobacterium johnei. Journal of Comparative Pathology and Therapeutics 60: 10-16. 1950.

60. Lominski, I., Cameron, J. and Roberts, G. B. S. Experimental Johne's disease in mice. Journal of Pathology and Bacteriology 71: 211-222. 1956.

61. Luft, J. H. Improvements in epoxy resin embedding methods. The Journal of Biophysical and Biochemical Cytology 9: 409-414. 1961.

62. Mangold, E. Handbuch der Ernahrung und des Stoffwechsels der Landwirtschaftlichen Nutztiere. Volume 2, Berlin, Springer. 321-322. 1929.

63. Marchesi, V. T. and Gowans, J. L. The migration of lymphocytes through the endothelium of venules in lymph nodes: an electron microscope study. Proceedings of the Royal Society of London, Series B, 159: 283-290. 1964.

64. Markus, H. Ein specifische Darmentzündung des Rindes, wahrscheinlich tuberculöser Natur. Zeitschrift für Thiermedicin 8: 68-78. 1904.

65. Matsumoto, T. Nutritive value of urea as a substitute for feed protein. I. Utilization of urea by the golden hamster. Tohoku Journal of Agricultural Research 6: 127-131. 1955.

66. Merchant, I. A. and Packer, R. A. Veterinary bacteriology and virology. 6th ed. Ames, Iowa, Iowa State College Press. 1961.

67. Metcalf, D. The thymus. Its role in immune responses, leukaemia development and carcinogenesis. Heidelberg, Germany, Springer-Verlag. 1966.

68. Meyer, K. F. The specific paratuberculous enteritis of cattle in America. The Journal of Medical Research 29: 147-189. 1913.

69. Meyn, A. and Weiske, G. Das Huhnals Versuchstier für Paratuberkalose Deutsche tierärztliche Wochenschrift 51: 62-63. 1943.

70. Michel, G. Beitrag zur Anatomie und Topographie der Lymphknoten des Syr. Goldhamsters (Mesocricetus auratus W.). Zentralblatt für Veterinärmedizin 3: 705-714. 1956.

71. Mohler, W. H. Johne's disease infection of laboratory animals with aid of paraffin oil. Journal of the American Veterinary Medical Association 94: 590-594. 1939.

72. Movat, H. Z. and Fernando, N. V. P. The fine structure of connective tissue. II. The plasma cell. Experimental and Molecular Pathology 1: 535-553. 1962.

73. Mudd, S., McCutcheon, M. and Lucke, B. Phagocytosis. Physiological Reviews 14: 210-275. 1934.

74. Mudd, S. and Mudd, E. B. H. On the mechanism of the serum sensitization of acid-fast bacteria. Journal of Experimental Medicine 46: 173-195. 1927.

75. Mudd, S. and Mudd, E. B. H. The surface composition of the tubercle bacillus and other acid-fast bacteria. Journal of Experimental Medicine 46: 167-172. 1927.

76. Mudd, S., Takeya, K. and Henderson, H. J. Electron-scattering granules and reducing sites in mycobacteria. Journal of Bacteriology 72: 767-783. 1956.

77. Mudd, S., Yoshida, A. and Koike, M. Polyphosphate as accumulator of phosphorus and energy. Journal of Bacteriology 75: 224-235. 1958.

78. Okudaira, M., Schwarz, J. and Adriano, S. M. Experimental production of Schaumann bodies by heterogeneous microbial agents in the golden hamster. Laboratory Investigation 10: 968-982. 1961.

79. Palay, S. L. and Karlin, L. J. An electron microscopic study of the intestinal villus. II. The pathway of fat absorption. The Journal of Biophysical and Biochemical Cytology 5: 373-384. 1959.

80. Payne, J. M. and Rankin, J. D. The pathogenesis of experimental Johne's disease in calves. Research in Veterinary Science 2: 167-174. 1961.

81. Pearson, L. A note on the occurrence in America of chronic bacterial dysentery of cattle. American Veterinary Review 32: 602-605. 1908.

82. Pitcock, J. A. and Gisler, D. B. Paratuberculosis (Johne's disease) in the monkey (Macaca mulatta). School of Aerospace Medicine, USAF Aerospace Medical Center, Brooks Air Force Base, Texas Document 61-86. 1961.

83. Popp, P. Züchtungs-und Infektionsversuche mit Paratuberkelbakterien (Culture and infection trials with Mycobacterium johnei). Inaugural Dissertation, Leipzig. 1940. Original not available; abstracted in The Veterinary Bulletin 12: 526. 1942.

84. Rankin, J. D. The experimental production of Johne's disease in laboratory rabbits. Journal of Pathology and Bacteriology 75: 363-366. 1958.

85. Rasmussen, P. and Caulfield, J. B. The ultrastructure of Schaumann bodies in the golden hamster. Laboratory Investigation 9: 330-338. 1960.

86. Runnells, R. A., Monlux, W. S. and Monlux, A. W. Principles of veterinary pathology. 7th ed. Ames, Iowa, Iowa State University Press. 1965.

87. Sabin, F. R. and Doan, C. A. The biological reactions in rabbits to the protein and phosphatide fractions from the chemical analysis of human tubercle bacilli. Journal of Experimental Medicine 46: 645-670. 1927.

88. Sahai, L. Johne's disease infection of laboratory animals. The Veterinary Journal 96: 407-414. 1940.

89. Sahai, L. Johne's disease infection of laboratory animals. The Veterinary Journal 97: 101-104. 1941.

90. Sall, T., Mudd, S. and Takagi, A. Polyphosphate accumulation and utilization as related to synchronized cell division of Corynebacterium diphtheriae. Journal of Bacteriology 76: 640-645. 1958.

91. Schaumann, J. On the nature of certain peculiar corpuscles present in the tissue of lymphogranulomatosis benigna. Acta Medica Scandinavica 106: 239-253. 1941.

92. Segal, W. and Bloch, H. Biochemical differentiation of Mycobacterium tuberculosis grown in vivo and in vitro. Journal of Bacteriology 72: 132-141. 1956.

93. Segal, W. and Bloch, H. Pathogenic and immunogenic differentiation of Mycobacterium tuberculosis grown in vitro and in vivo. The American Review of Tuberculosis and Pulmonary Diseases 75: 495-500. 1957.

94. Simpson, C. F. Genesis and morphologic characteristics of macrophages and Langhans' giant cells. American Journal of Veterinary Research 27: 533-540. 1966.

95. Smith, H. W. The isolation of mycobacteria from the mesenteric lymph nodes of domestic animals. Journal of Pathology and Bacteriology 68: 367-372. 1954.

96. Speirs, R. S. How cells attack antigens. Scientific American 210: 58-64. 1964.

97. Stavitsky, A. B. and Beck, J. D. Reaction of the developing chicken embryo and the chorioallantoic membrane to inoculation with various strains of Mycobacterium paratuberculosis (Johne's bacillus). American Journal of Veterinary Research 7: 470-476. 1946.

98. Taylor, A. W. The results of the inoculation of guinea-pigs with M. paratuberculosis (Ovine type) in liquid paraffin. The Veterinary Journal 96: 415-417. 1940.

99. Taylor, A. W. Varieties of Mycobacterium johnei isolated from sheep. Journal of Pathology and Bacteriology 63: 333-336. 1951.

100. Trautmann, A. and Fiebiger, J. Fundamentals of the histology of domestic animals (Translated and revised from the 8th and 9th German ed., 1949.) Ithaca, New York, Comstock Publishing Associates. 1952.

101. Twort, C. C. The agglutination and complement fixation reactions in animals experimentally inoculated with Johne's bacillus, with special reference to the relation of this bacillus to the other acid-fast bacilli. Centralblatt für Bakteriologie und Parasitenkunde, Abt. 1, Originale 66: 317-320. 1912.

102. Twort, C. C. The experimental production of Johne's disease in some of the laboratory animals. The Veterinary News 11: 79-81. 1914.

103. Twort, C. C. Jöhne's disease in rabbits and mice. The Veterinary News 10: 272. 1913.

104. Twort, C. C. and Craig, T. The pathogenicity of Johne's bacillus compared with that of other acid-fast bacilli for some of the laboratory animals. Centralblatt für Bakteriologie und Parasitenkunde, Abt. 1, Originale 68: 455-470. 1913.

105. Twort, F. W. and Ingram, G. L. Johne's disease. London, England, Baillière, Tindall and Cox. 1913.

106. Twort, F. W. and Ingram, G. L. Y. A method for isolating and cultivating the mycobacterian enteritidis chronicae pseudotuberculararae bovis, Jöhne, and some experiments on the preparation of a diagnostic vaccine for pseudo-tuberculous enteritis of bovines. Royal Society of London Proceedings 84: 517-542. 1912.

107. Vallée, H., Rinjard, P. and Vallée, M. Sur la prémunisation de l'entérite paratuberculeuse due au bacille de Johne. Bulletin Academie de Medicine, Paris 125: 195-198. 1941.

108. Venable, J. H. and Coggeshall, R. A simplified lead citrate stain for use in electron microscopy. The Journal of Cell Biology 25: 407-408. 1965.

109. Verlinde, J. D. and Bekker, J. H. Besmetting van de cavia met paratuberkelbacillen. Tijdscrift voor Diergeneeskunde 70: 329-333. 1945.

110. Weiss, L. Electron microscopic observations on the vascular barrier in the cortex of the thymus of the mouse. The Anatomical Record 145: 413-438. 1963.

ACKNOWLEDGMENTS

This project was conducted under the United States Government Employees' Training Act Public Law 85507.

The author wishes to thank Drs. W. S. Monlux and A. B. Larsen for their assistance and guidance throughout the project. Appreciation is also expressed to Drs. F. K. Ramsey and W. S. Monlux for their counsel and editorial suggestions.

The author is indebted to Dr. W. S. Monlux and Mr. R. M. Glazier for the photographs that appear in the thesis.