TOXOPLASMOSIS OF CAPTIVE WILD BIRDS AND MAMMALS *

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Toxoplasmosis is best known as a human disease affecting infants and children, and occurring much less frequently in adults. In the young it nearly always manifests itself as a meningo-encephalitis which usually ends fatally within a few weeks.[1-5] In adults, the symptoms of the few recorded cases have suggested Rocky Mountain tick fever, and evidence of generalized infection was found by post-mortem examination.[6,7] A number of species of animals are known to be susceptible to the naturally occurring form of this infection. In them the central nervous system of both young and adults is predominantly involved [8-13]; less frequently, lesions have been found also in lymph nodes, liver, and lungs.[14-17] When the disease is induced experimentally, either in mammals or birds, the central nervous system likewise is the site most commonly attacked, even though the organisms are introduced into other regions.

In contrast to the predilection for the nervous system in most hitherto reported cases of toxoplasmosis of either man or lower animals, we have found involvement of various other organs to be the dominant feature in 27 consecutive fatal examples of toxoplasmosis encountered during the past 10 years at the Philadelphia Zoological Garden. This paper deals with the epidemiology, the distribution and appearance of the disease processes, and the morphologic character of the parasites in these animals.

MATERIAL AND METHODS

The present series comprises 13 mammals and 14 birds which died during the period 1940 to 1950. All of the birds and 2 of the mammals were newly imported adults; none of them lived more than 4 months after arrival. Nine other mammals were adults, and had been exhibited for periods ranging from 10 months to 5 years. Two mammals, which were immature, were born in the Garden. In the majority of these animals the disease progressed to fatal termination without clinical signs of illness having become obvious. In fact, the nature of the disease usually was not recognized until tissues were examined microscopically. Since sections, as a rule, were made only from organs which were macroscopically abnormal, the distribution of the lesions,

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listed for this series in Table I, must be considered somewhat incomplete.

All tissues were fixed in either formaldehyde or Bouin's solution, and embedded in paraffin. Sections were cut at 3 to 4 μ and stained with hematoxylin and eosin, and by methods suggested by Perrin [18] for differentiating Toxoplasma from Encephalitozoon in tissues. Smears of tissues were stained by Giemsa's method.

Epidemiologic Survey

This series of animals has been arranged, in Table I, according to the chronologic sequence of their deaths. The first column gives the genus and species, and its common name. Exhibition periods, stated in the third column, are the intervals between arrival or birth, and death. Duration of illness, in the fourth column, is the interval between the recognition of illness and death. Organs listed as containing lesions are those in which definite necrosis and inflammation were associated with Toxoplasma infestation. Organs containing the parasites in cytoplasmic cysts within parenchymal cells, but exhibiting no other signs of tissue damage, are not included in this table.

The first animal of this series, a snow leopard, which died 2 years before the second animal, represents an isolated example of the disease both in its exhibition area and taxonomic group. In it, also, the disease apparently had progressed less rapidly than in any of the others.

Animals 2, 3, and 4 had all lived in one building, the Small Mammal House; all died of toxoplasmosis within a period of 4 months. Two, both squirrel monkeys (cases 2 and 3 in Table I), had been cage mates.

Four years later, in 1946, within a period of about 3 months, 5 additional deaths from toxoplasmosis occurred. One of these (case 6) also came from the Small Mammal House. The 4 penguins, *Spheniscus humboldti* (cases 5, 7, 8, and 9), had never been in contact with any of the other animals in the series. They were members of a group of 9 penguins, all of which had been received from a dealer at the same time, and which had been exhibited together in a shaded outdoor enclosure containing a large pool. The remaining penguins of the group died within 1 year after arrival, but no evidence of toxoplasmosis was found in any of them.

During the following year, 1947, 8 animals died of toxoplasmosis. The first, in chronologic order, was an infant sea lion (case 10), 10 days of age. Its mother was one of a group kept in a large outdoor pool; the number of its inhabitants has varied over the past 10 years with new arrivals, births, and deaths. Thus far, 43 sea lions have died

and have been examined post mortem, but no other examples of toxoplasmosis have been encountered. The spider monkey (case 11) was one of a group of 6, all of which were purchased at the same time and exhibited in one cage. Its fellows have remained healthy. The next animal (case 12) was one of a group of 14 penguins of various species, all received in May, 1947. The 13 remaining members of this group have since died and have been examined post mortem, but none was found to have been infected by Toxoplasma. The crowned pigeon (case 13) was one of a pair which were received and exhibited together. Its cage mate is living and apparently healthy at the present time.

In the above 4 fatal cases of toxoplasmosis no common source of infection was traceable, but in the following 4 cases it is highly probable that the disease was directly or indirectly transmitted. Three animals, two tree porcupines and a hyrax (cases 14, 16, and 17), were exhibited in the Small Mammal House where 4 of the earlier infections had developed. The wallaby (case 15), while not maintained in this building, was fed several times each week by an attendant who also worked in the Small Mammal House.

During 1948, the 2 fatal infections also probably were related; a second wallaby (case 18) was fed by a keeper also on duty in the Small Mammal House in which the woodchuck (case 19) was exhibited.

A period of almost 11 months then elapsed before the next examples of the disease were encountered. Then, within a few days, 8 penguins died of toxoplasmosis. These are listed as cases 20 to 27 in Table I. Cases 20 to 26, inclusive, were black-footed penguins, all of which appeared to be healthy upon arrival in the Zoological Garden on July 5, 1949. They continued to be well for only 1 week; then one of the birds refused food and became inactive. It was isolated and hand fed, but died 3 days later. In rapid succession others of this group also failed to eat, became inactive, and died 2 to 4 days thereafter, the last, 21 days after arrival. These birds were kept on a small pond where the other penguins listed in Table I had been maintained. At the time of their arrival in July, 2 penguins of other species, *S. magellanicus* and *S. humboldti,* were present in this enclosure. The first of these had been on exhibition since April 19, 1949, during which time it had behaved normally and fed regularly. It became ill on August 23, 1949, and died 3 days later, that is, the same day on which the last of the black-footed penguins succumbed. The other penguin, *S. humboldti,* a resident of this pond for about 2 years, has continued in good condition.

TABLE I

Mammals and Birds in Which Toxoplasmosis Has Been Recognized at the Philadelphia Zoological Garden, Arranged in the Sequence of Their Deaths, with Exhibition Periods, Duration of Recognizable Illness, and Distribution of the Lesions

Animals	Date of death	Exhibition period	Duration of illness	Distribution of lesions					
				Heart	Lungs	Liver	Spleen	Abdominal lymph nodes	Other organs
		months							
1. Snow leopard, *Felis uncia*	2-26-40	21	2 weeks		+	+		+	Adrenals, serous surfaces
2. Squirrel monkey, *Saimiri sciurea*	9-29-42	47	None	+	+	+	+	+	Hemorrhagic necrosis of adrenal cortex
3. Squirrel monkey	11-3-42	58	None		+	+			
4. Viscacha, *Viscacha viscacha*	2-2-43	17	None	+	+	+			
5. Humboldt's penguin, *Spheniscus humboldti*	8-9-46	0.3	None			+	+		
6. Mountain chinchilla, *Lagidium peruanum*	9-10-46	0.2	None	+	+	+	+		
7. Humboldt's penguin	9-11-46	1	2 days		+	+	+		
8. Humboldt's penguin	11-19-46	3	None	+	+	+	+		
9. Humboldt's penguin	11-29-46	3	6 days		+	+	+		
10. Sea lion *Zalophus californianus*	6-9-47 (newborn)	0.3	None	+	+	+		+	
11. Spider monkey, *Ateles geoffroyi*	8-2-47	10	3 days			+		+	Granuloma of the pancreas

12. Penguin, *Spheniscus magellanicus*	8–18–47	3	None		+	+			
13. Crowned pigeon, *Gura victoria*	10–11–47	4	None		+	+	+		
14. Tree porcupine, *Coendou prehensilis*	10–13–47	61	2 weeks		+	+	+		
15. Wallaby, *Macropus bennetti*	10–14–47	14	None	+	+		+	+	
16. Cape hyrax, *Procavia capensis*	10–18–47	2	None		+	+	+		Mononuclear cells increased in meninges
17. Tree porcupine	10–24–47	61	2 weeks		+	+	+		
18. Wallaby	8–19–48	6	None	+	+				
19. Woodchuck, *Marmota monax*	8–24–48	24	None	+	+				
20-26. 7 Black-footed penguins, *Sphenicus demersus*	7–15–49 to 7–26–49	0.2 to 0.5	2–4 days		+	+	+		Combined with lesions of acute hepatitis
27. Penguin, *Sphenicus magellanicus*	7–26–49	4	4 days		+	+	+		Combined with lesions of acute hepatitis

In brief, 22 of the 27 deaths in this series could be accounted for by five separate outbreaks of the disease. In each of these outbreaks there was a high degree of probability that the infection had spread from a common source. However, this cannot be established definitely. Among these probable examples of group infection the susceptibility of penguins has been outstanding, one group of 7 having been exterminated within 3 weeks and, in another, 4 of 9 dying during a period of 3 months. Among the mammals, 2 squirrel monkeys and 2 tree porcupines were cage mates, and 5 others were closely associated or were fed by the same attendants. By contrast, evidence of a common source of infection was lacking for 5 animals; moreover their cage mates and close associates remained free of demonstrable infection.

Description of the Disease Processes

At necropsy the macroscopic appearance of the viscera was not distinctive in any of the animals. The changes most commonly encountered were dilatation of the heart, often associated with focal necrosis of the myocardium; pronounced edema of the lungs; intense congestion of the liver, sometimes with widely distributed necrotic foci; and hyperplasia of the spleen. Occasionally the mesenteric lymph nodes were enlarged, but otherwise unchanged. The brains of 8 mammals and 3 birds were examined carefully without finding evidence of infection; in the remaining 16 animals of the series no examination of the brain was made.

The one exception to this disease pattern was encountered in the snow leopard (case 1). In this animal the infection was associated with acute pleuritis and peritonitis, by enormous enlargement and caseation of mesenteric and retroperitoneal lymph nodes, and by fibrosis and caseation of the adrenals. The lungs were subcrepitant and contained poorly defined, soft nodules. The spleen was large and firm, the liver was congested, and the heart was dilated. The appearance of the organs suggested tuberculosis, but bacilli could not be found.

Microscopically, the changes in the tissues lacked variety. This was especially true of the lesions in the lungs. These were present to a greater or less degree in all animals, and consisted of patchy, or widely spread thickening of the alveolar walls and interlobular septa due to edema and infiltration of mononuclear leukocytes. The vessels usually were intensely injected (Figs. 1, 2, and 3). Interstitial inflammation in the lungs was not proportional to the number of Toxoplasma found in the sections. The parasites were never as abundant as in other organs, and in some animals they could not be demonstrated readily in sec-

tions. Fortunately, in 2 such cases contact smears of the cut surfaces were available for comparison; in them the parasites were seen readily, usually within cells.

In the heart, liver, spleen, and lymph nodes of the animals listed as cases 1 through 19, the lesions were closely similar. In the more acute stages they consisted mainly of foci of necrosis, varying in diameter from about 200 μ to 2 to 3 cm.; they were distributed at random throughout the organs. The smaller lesions were composed of compact, eosin-staining tissue remnants and nuclear fragments, about which had accumulated sparse mononuclear and polymorphonuclear leukocytes. Larger foci were expansions of smaller ones, with the central necrotic mass proportionately larger, and surrounded by a loosely arranged zone of leukocytes and macrophages, often imperfectly preserved and mixed with degenerated tissue (Fig. 4). In the more chronic lesions, the necrotic foci had been replaced by fibroblasts and macrophages.

The number of Toxoplasma found in the necrotic lesions in the heart, liver, spleen, and lymph nodes was roughly proportional to the size of the necrotic foci. The smaller foci usually contained relatively few organisms, and these lay within the degenerated tissue elements, singly or in small groups. In the larger foci, the organisms usually were abundant, and also often numerous in parenchymal cells and macrophages adjacent to the necrotic foci (Fig. 4). In tissue invasion of this type, the organism did not form cytoplasmic cysts. In the foci where macrophages and fibroblasts had replaced the necrotic tissue more or less completely, the parasites were present only in small numbers (Fig. 5).

The lesions in the 8 penguins listed as cases 20 through 27 were somewhat different from those of other members of the series. Obvious necrosis of the liver, spleen, or myocardium was not present. Instead, the vascular bed of the liver and spleen was packed with phagocytic cells, many of which contained incompletely or completely segmented masses of organisms which corresponded, in their staining reactions, to Toxoplasma. The hepatic and myocardial cells were not invaded by the parasite (Figs. 6 and 7). The reactions in the lungs, however, closely resembled those described above. It seems likely that in this group of birds the infection was more fulminant than in the other members of the series.

The Parasite

The organisms identified as Toxoplasma have been studied in sections of tissues from all of the 27 animals, and in contact smears from

one or more organs of 2 mammals and 8 birds. Except for the greater ease with which organisms were found in the smears, there was no essential difference between their appearance in smears and in sections from the corresponding organs. There were, however, considerable differences in the shape and size of the organisms from animal to animal. On the basis of morphology there appeared to be three distinct types or stages of Toxoplasma. The most common form was of relatively large size, 5 to 8 μ in length and about 3 μ in width. The second form was similar in shape, but distinctly smaller, 4 to 5 μ in length and about 1 μ in width. The third appeared as masses of incompletely segmented cytoplasm in which imperfectly formed, small crescents could be seen, or as masses of unsegmented cytoplasm containing large numbers of tiny nuclei.

The smaller crescents were especially numerous in smears and sections of tissue from the crowned pigeon (case 13), a wallaby (case 15), and a tree porcupine (case 17). However, in these animals, as well as in occasional others of the series, the small crescents occurred together with the larger ones, but intermediate stages were never encountered, nor did the two forms ever exist within the same parasitized cells.

The plasmodial type varied in size from 10 to 100 μ in diameter. The cytoplasm stained well with basic dyes; the nuclei appeared as solidly stained, darker masses about 1 μ in diameter. In one animal, the tree porcupine (case 17), plasmodia were associated with small crescentic parasites. In the penguins (cases 20 to 27) they were greatly predominant, although both large and small crescents also were present.

It is not yet possible to express an opinion concerning the relationship of these three forms; they may be unrelated species, or they may be different stages of the same parasite.

Discussion

Organisms of the genus Toxoplasma are known to be widely distributed and relatively common parasites of many species of mammals and birds.[5] Active toxoplasmosis, however, appears to be an uncommon disease, and usually has developed as a subacute or chronic process. With one exception the disease in this series of animals was acute, and the lesions corresponded closely to toxoplasmosis of adult human beings.

The conditions under which Toxoplasma become virulent parasites are not known. One may postulate that the organisms initiate disease only when their hosts are weakened by malnutrition or concurrent infection and consequent interference with the immune mechanism.

This supposition is supported by some reports in the literature,[6,9,10] but, more often, factors that might have reduced the health of the host have not been obvious.[5] In the present series, there were some animals in which toxoplasmosis was clearly associated with another disease. The crowned pigeon (case 13) and the penguins (cases 20 through 27) apparently had acquired infection by the virus of avian hepatitis. But in all other animals careful consideration leaves us unable to suggest factors which could have contributed to susceptibility toward the organism. It is possible that more than a single type of organism was encountered, although this is still conjectural. A better understanding of toxoplasmosis must await more complete knowledge of the life history of the parasite, and of the conditions under which it becomes virulent.

SUMMARY

During a period of the past 10 years, 13 mammals and 14 birds developed fatal toxoplasmosis at the Philadelphia Zoological Garden. The disease is relatively rare, constituting less than 1 per cent of all deaths.

With one exception, the form of toxoplasmosis encountered was acute, and in all instances involved organs of both the thoracic and abdominal cavities. Clinical signs of localization of the organisms in the central nervous system were lacking. The brains of 8 mammals and 3 birds of the series were closely examined at necropsy, and no lesions were found.

The predominant tissue changes were foci of necrosis in liver, spleen, heart, lymph nodes, and lungs. The more acute lesions were accompanied by infiltration of monocytes. In less acute lesions, monocytes and fibroblasts largely replaced the necrotic tissue. In the lungs, diffuse interstitial infiltration of monocytes and edema usually characterized the disease, whether or not focal necrosis of the tissue had developed.

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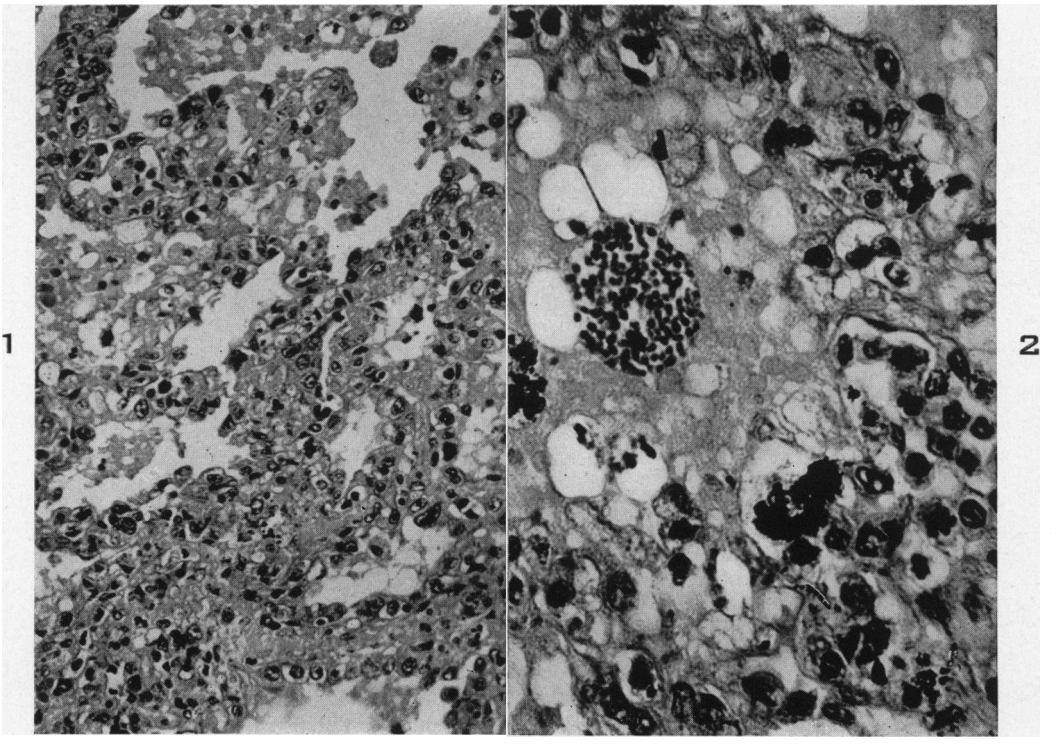
DESCRIPTION OF PLATES

PLATE 116

FIG. 1. Lung, tree porcupine (*Coendou prehensilis*). This field illustrates the thickened alveolar walls and the increased numbers of alveolar macrophages which seemed to be part of the reaction of many animals of this series to infection by Toxoplasma. × 200.

FIG. 2. Lung, tree porcupine (*Coendou prehensilis*). This field includes a part of an alveolar wall, the surface of which is covered by macrophages, some of which contain carbon particles. Toxoplasma are scattered through these cells, and in the upper part of the field a large mass of these organisms lies within the outlines of a distended cell. × 800.

FIG. 3. Lung, penguin (*Spheniscus demersus*). This field includes the wall of a secondary bronchus, recognizable here by three bits of smooth muscle at the top of the field. These bands of smooth muscle surround passages through which air moves into alveolar spaces in the bird. These passages and the alveolar spaces are filled by mononuclear leukocytes. These cells also are abundant in the capillaries and larger blood vessels in this field. This view illustrates the most severe form of pneumonitis associated with toxoplasmosis of birds of this series. × 200.



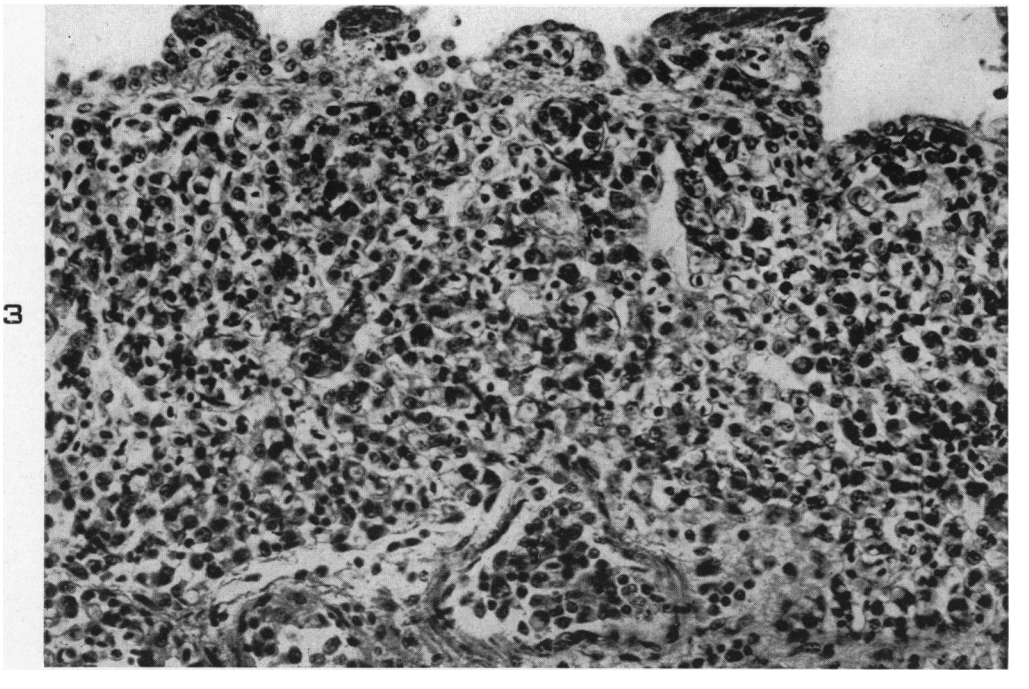


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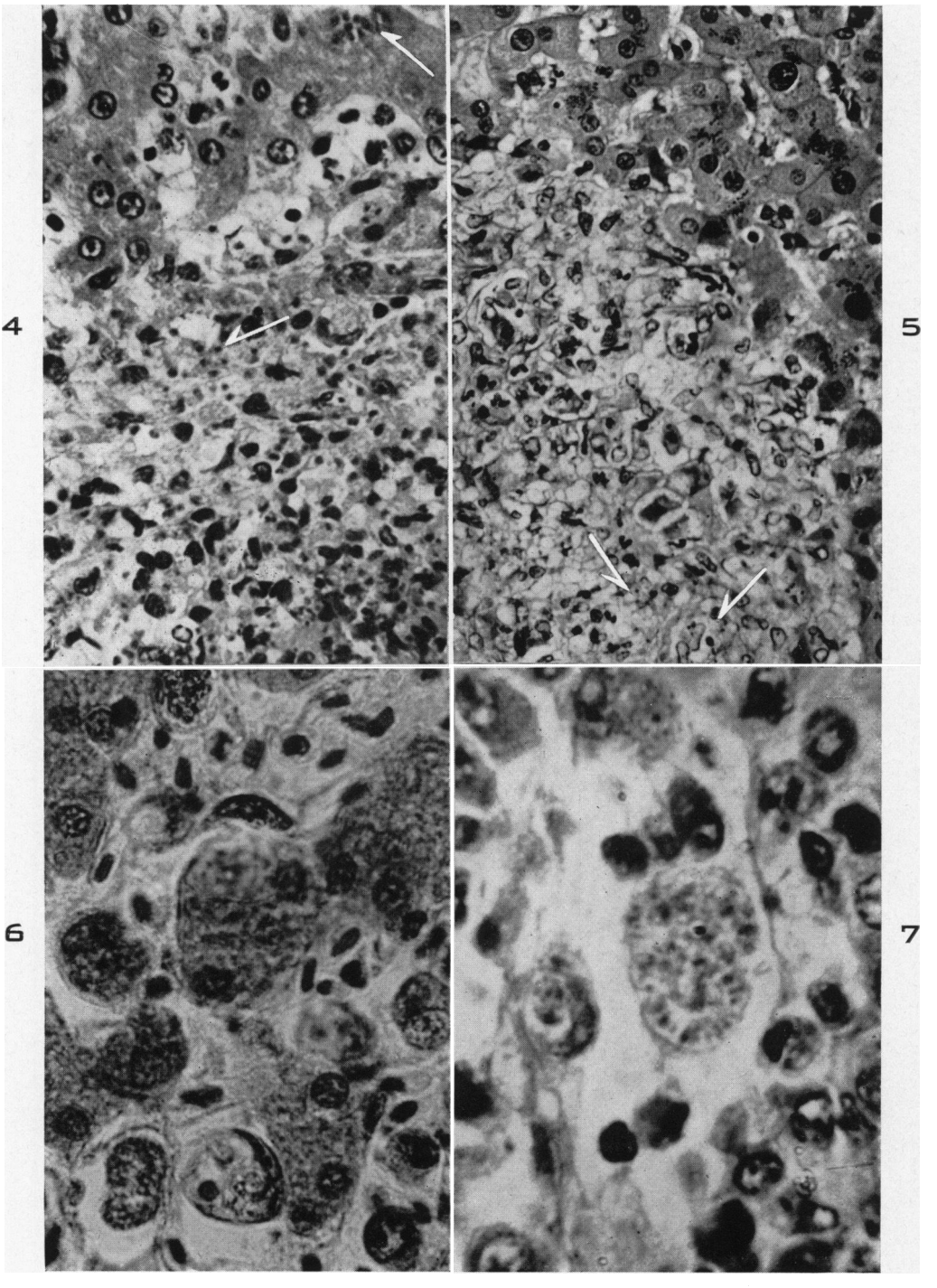
PLATE 117

FIG. 4. Liver, penguin (*Spheniscus humboldti*). This field includes part of a necrotic focus and its border of liver cells. Arrows point to Toxoplasma, which are numerous among the tissue fragments of the lesions, and to one small group of organisms which may be seen in the cytoplasm of a cell near the upper right border of the field. × 600.

FIG. 5. Liver, wallaby (*Macropus bennetti*). This field includes part of a lesion in which macrophages and fibroblasts replaced the parenchyma. Arrows point to small numbers of Toxoplasma in clear spaces within these cells. × 800.

FIG. 6. Liver, penguin (*Spheniscus demersus*). This photomicrograph illustrates the hyperplasia of Kupffer cells in the sinusoids of the livers of penguins no. 20 to 26 of Table I. Six of the Kupffer cells in this field contain various forms of the organism. × 1000.

FIG. 7. Liver, penguin (*Spheniscus demersus*). A mass of the small, crescent-shaped forms of Toxoplasma, such as is shown in the lower left field of Figure 6, is photographed here from a section cut at 2 μ and stained by iron hematoxylin. In this cell the small crescents were arranged radially about a jumbled mass of material. × 1700.



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