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Fatal Infection of Hepatozoon-like Organisms in the Young Captive Cranes (*Grus monacha*)

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Introduction

Nabezuru (*Grus monacha*) is one of the special national monuments, wintering in Izumi plain, Kagoshima Prefecture and a small number of them have been kept in the Hirakawa Zoological Park, Kagoshima City for exhibition. Having been studying diseases of Nabezuru and Manazuru (*Grus vipio*) in Izumi for these several years, we happened to find young cranes heavily infected with avian malaria-like organisms[7] and *Tetrameres grusi*[8] as reported previously. In relation to these studies we could detect fatal infection of Hepatozoon-like parasites in 3 young Nabezuru in the Hirakawa Zoological Park as described in this paper.

Materials and Methods

Three young female cranes hatched and bred in the Hirakawa Zoological Park, Kagoshima City, died suddenly at the age of 2, 4 and 4.4 months, on August 11, and October 21, in 1983 and October 19, 1984, respectively. All were autopsied in the Laboratory of Veterinary Pathology, Faculty of Agriculture, Kagoshima University for pathological examinations. Main organs were fixed in 10 % phosphate buffered formalin solution, embedded in paraffin, and sections 3-4μm thick were stained with hematoxylin and eosin (H-E), Giemsa and periodic acid-Schiff (PAS) for microscopy. Smears of fresh tissues of the liver, spleen, bone marrow and blood films were stained with May-Gruenwald Giemsa for hematological and parasitological examinations. Parts of the most fresh liver and spleen were fixed with 2.5 % glutaraldehyde in 0.1 M cacodylate buffer solution, post-fixed with 2 % osmium tetroxide solution in the same buffer, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and were observed with H-600 electron microscope, Hitachi, Japan.

Results

Gross appearances of these 3 cranes were normal and well nourished with good weight gains (Fig. 1). However, in the opened body cavity were seen markedly enlarged livers densely studded with white spots 1-4 mm in diameter both on the capsular and cut surfaces, showing a mosaic appearance intermixed with congested parenchyma (Fig. 2). The body

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weight ratio of the livers increased 2-4 times more than usual. Splenomegaly was evident but no remarkable changes were found in the other organs except for occasional hemorrhagic spots in the lungs.

The blood films and smears of the organs revealed innumerable round or comma-shaped protozoa (Fig. 5), the former in the cytoplasm of mononuclear leucocytes (Fig. 3) or cells of reticuloendothelial system and the latter mostly free in the blood. The sizes of the parasites measured on the blood films were $14.0-22.0\times7.0-10.0\ \mu m$ (average $16.9\times8.5\ \mu m$) for the extracellular type and $11.0-17.0\times6.0-11.0\ \mu m$ (average $14.9\times8.9\ \mu m$) for the intracellular type. Smaller immature types were also observed in the cytoplasm of the host cells. In general, each host cell contained a parasite but occasionally 2 parasites. The parasite had cytoplasm stained dark blue with Giemsa and a large round nucleus with a distinct pink nucleolus. The nucleus was located in the center of the cell body of the round type, while in the comma-shaped type it was located a little nearer to the thick end of the cell body. The thick end was reddish purple in color with a blunt conic shape. A darker ovoid spot was found between the cone and the nucleus and a black ball was noticed at the thin end (Fig. 4). These cell bodies showed weak positivity to PAS. A serial growth from the small intracytoplasmic parasite to the larger round type and further to the comma-shaped type was observed, and then going out of the host cell it took a little longer comma-shape, the extracellular form or gametocytes. In the blood of the youngest crane were recognized a few large mononuclear cells containing 8-10 small parasites in the cytoplasm, each having a staining property similar to those described above (Fig. 6). This may be the stage of schizogony, each small parasite being a merozoite.

The light microscopy revealed extensive infiltration of mononuclear cells in the liver, possibly starting from the portal area and most of them presented intracytoplasmic parasitism of the protozoa, showing a growth from pale, smaller, immature forms (Figs. 7 and 10) to darker mature ones (Fig. 9). The parasites invade even to the hepatocellular cord, resulting in shrinkage and disappearance of the hepatocytes. In the sinusoids and the blood vessels were seen many parasites either intracytoplasmic or free in the lumina. These features somewhat resembled those of leukemic liver (Fig. 11) but essentially they were granulomas with little necrosis. Larger cell bodies resembling multinucleated giant cells (Fig. 8) which might be at the schizogonic stage were observed in the liver of the youngest crane, but they were rather rare in the other cranes. The spleen had many focal growths of the parasite-containing cells (Fig. 12) in the red pulp and free comma-shaped parasites in the blood spaces. Blood vessels in every organ harbored the protozoa, showing leukemia-like focal lesions in the lung (Fig. 13), kidney (Fig. 14) and bone marrow (Fig. 15). Lamina propria of the intestininal mucosa had this kind of mononuclear infiltration and some of the parasites were seen in the collapsing villous epithelia (Fig. 16). We could not find any parasites that might be assumed to be micro- or macrogametocytes. As to complications, we could observe many necrotic foci in the lung of the youngest crane, in which a mucor-like fungus was detected by PAS stain.

By electron microscopy the protozoa, both intracellular and extracellular forms, proved to have cytoplasm rich in ribosomes, many mitochondria, some rough endoplasmic reticula and vacuoles with or without an electron dense inclusion body (Fig. 17). In the thick end of the comma-shaped parasite were observed organella characteristic to the subphylum Api-

complexa[4], such as micronemes and conoid-like and polar ring-like structures (Fig. 18). The spherical structure at the tip of the thin end contained an electron dense mass of considerable size.

Discussion

The morphological features we described above showed that these cranes were infected with a protozoon that belongs to the subphylum Apicomplexa[4], causing massive granuloma in the liver and spleen related with intracytoplasmic proliferation and growth of the parasite. On the ground that there was no evidence of sexual reproduction in the host and only the stage of schizogony and formation of gametocytes were observable in the blood and cells of the reticuloendothelial system, especially in the liver and sleen, we suspected this parasite to be very closely related to *Hepatozoon*[3]. Before making decisive diagnosis of hepatozoonosis of the crane, additional researches into its vector and sexual generation will be required.

Hepatozoon is a genus of sporozoan parasites of the order Eucoccidiorida, whose schizogony occurs in the endothelial cells of the liver and gametocytes may be found in the erythrocytes or leucocytes of vertebrate hosts. Sporogony occurs in ticks and other blood-sucking arthropods[3,5]. In reptiles many reports on parasitism of genus *Hepatozoon* have been made but only a few in birds and none in cranes[6]. Anemia and focal necrotizing granulomas are known to be the main lesions in *Hepatozoon* infections[3]. which seems not to be inconsistent with those in the present cranes. Disseminated visceral coccidiosis has been reported in sandhill cranes (*Grus canadensis*)[1] and whooping cranes (*Grus americana*)[2] but gross appearance of multiple nodules on the visceral surfaces and microscopic detection of gametogony and oocysts are quite different from the cranes infected with the Hepatozoon-like organisms.

Retrospective examination of the specimens of a young crane which died in 1973 in the same Zoological Park revealed the same lesions and parasites, whose gross diagnosis had been leukosis. Another young crane of 1 month of age which died in July, 1986 showed as many parasites in schizogony in the liver and blood as those seen in the 2 month-old crane, suggesting early prevalence of schizogony. Most probable source of infection may be their parents in the reservoir state, which had close contact with the young birds. Not all young cranes died of this protozoonosis but one surviving crane is still alive to this date in the Zoological Park. Some predisposition or complications may contribute to the occurrence of heavy infection of this protozoon. Infection of wild young cranes in Izumi plain has been found in our recent study, the lesions starting from focal growths in the liver and spleen up to the fatal systemic case (data unpublished). The adult may become resistant, as we have never encountered one severely infected with this parasite.

Further studies will be required for elucidation of the vector and the sexual generation of the parasite so as to complete the life cycle.

Summary

Three young cranes, *Grus monacha*, died suddenly, showing hepatomegaly and splenomegaly with disseminated white spots. Blood film and smears of main organs stained with May-Gruenwald Giemsa revealed many large round parasites, each growing up in the

cytoplasm of a monounclear cell to be liberated free in the blood as a comma-shaped organism which has a large nucleus with a pink nucleous and dark blue cytoplasm. This was presumed to be in a stage of gametogony. In the blood of the youngest crane and in the section of the liver were found a few larger mononuclear cells that contain 8-10 small parasites (merozoites) in the cytoplasm, which were assumed to be in the stage of schizogony. Massive proliferation of the mononuclear cells of reticuloendothelial system associated with the intracytoplasmic growth of the parasite was seen in the liver and other organs, causing leukemia-like lesions. Electron microscopy showed that the gametocyte-like bodies, comma-shaped organisms, have micronemes and polar ring-like and conoid-like structures characteristic to the subphylum Apicomplexa at their thick ends. Judging from the above-mentioned morphological features, this protozoon is presumed to belong to *Hepatozoon* or the closely related genus.

Acknowledgments

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Explanation of Figures

Fig. 1. Gross appearance of a 4 month-old crane weighing 2.75 kg.

Fig. 2. Macroscopic lesions in the swollen liver. Note disseminated white spots on the capsular surface.

Fig. 3. Intracellular round parasites in the blood film of a crane. May-Gruenwald Giemsa stain (M-G) × 750

Fig. 4. An extracellular comma-shaped parasite in the blood film. M-G. × 1125

Fig. 5. Smears of the spleen of the youngest crane. Note many comma-shaped parasites. M-G × 300

Fig. 6. Blood film of the youngest crane. Note a large mononuclear cell containing 8 small parasites (merozoites) in the cytoplasm, which was presumed to be at the stage of schizogony. M-G. × 750

Fig. 7. Marked proliferation of mononuclear cells in the liver, each containing a round pale parasite in the cytoplasm. Giemsa stain (Giemsa). × 750

Fig. 8. Photomicrograph of the liver of the youngest crane, showing a mononuclear cell with 10 small parasites (merozoites) contained in the cytopasm. These parasites are presumed to be at the stage of schizogony. Giemsa. × 750

Fig. 9. The border of a focal proliferation of mononuclear cells in the liver. Each cell contains a dark-staining parasite in the cytoplasm. Some parasites are invading the hepatic cell cord. Giemsa. × 400

Fig. 10. The border of a focal proliferation of mononuclear cells. Each cell contains a pale parasite in the cytoplasm which was assumed to be at the younger stage than the dark parasite shown in Fig. 9. Giemsa. × 400

Fig. 11. Lower magnification of the liver. Focal proliferation of the parasite-containing cells, starting from the portal area (above). Giemsa. × 200

Fig. 12. A focus of parasite-containing cells in the spleen, some invading the travecula. Giemsa. × 200

Fig. 13. Parasite-containing cells accumulated in the respiratory bronchioles of the lung. Parabronchus (above). Giemsa. × 200

Fig. 14. Focal interstitial infiltration of parasite-containing cells in the kidney. Giemsa. × 200

Fig. 15. A focus of parasite-containing cells in the bone marrow. Giemsa. × 400

Fig. 16. The parasites invading collapsing villous epithelia in the intestine. Giemsa. × 200

Fig. 17. Electron micrograph of an intracytoplasmic parasite in the liver, which has a prominent nucleus and cytoplasm containing ribosomes, mitochondria and vacuoles with and without a dense core. × 14000

Fig. 18. Electron micrograph of a comma-shaped extracellular parasite in the liver. Note the micronemes, conoid-like and polar ring-like structures at the thick end. × 14000

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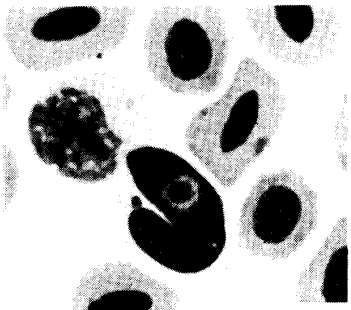

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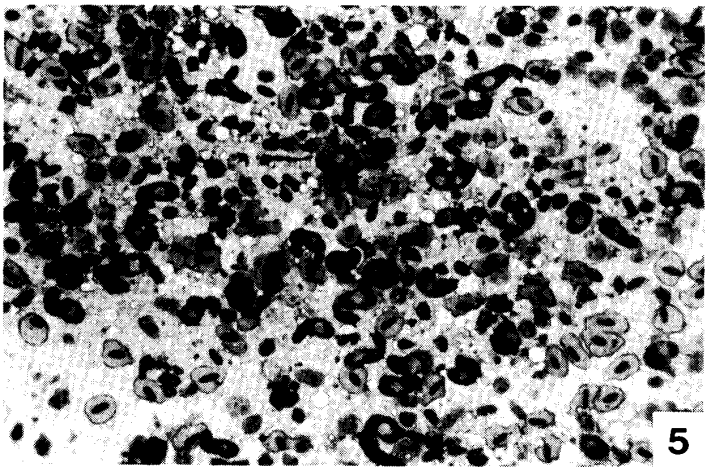

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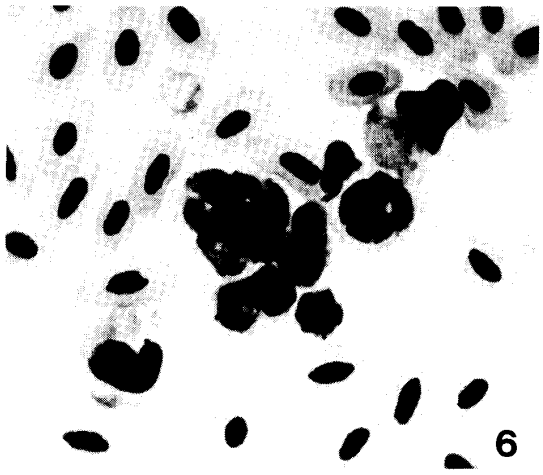

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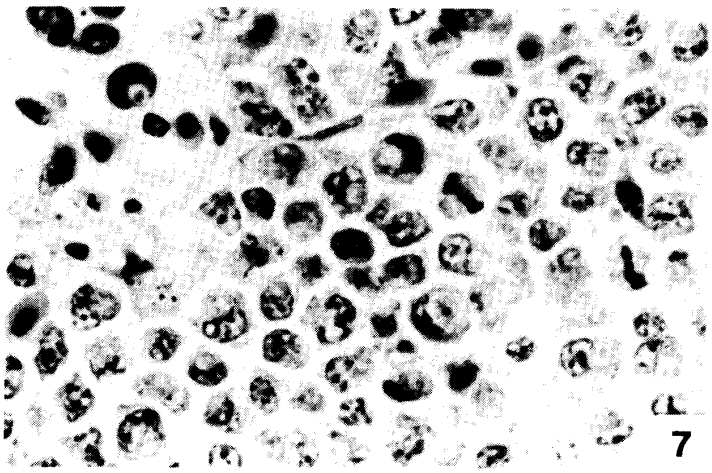

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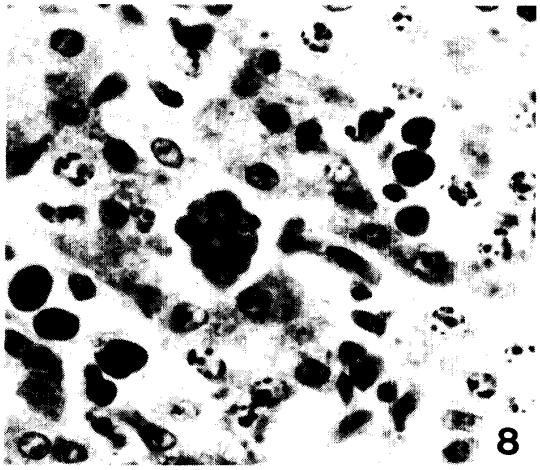

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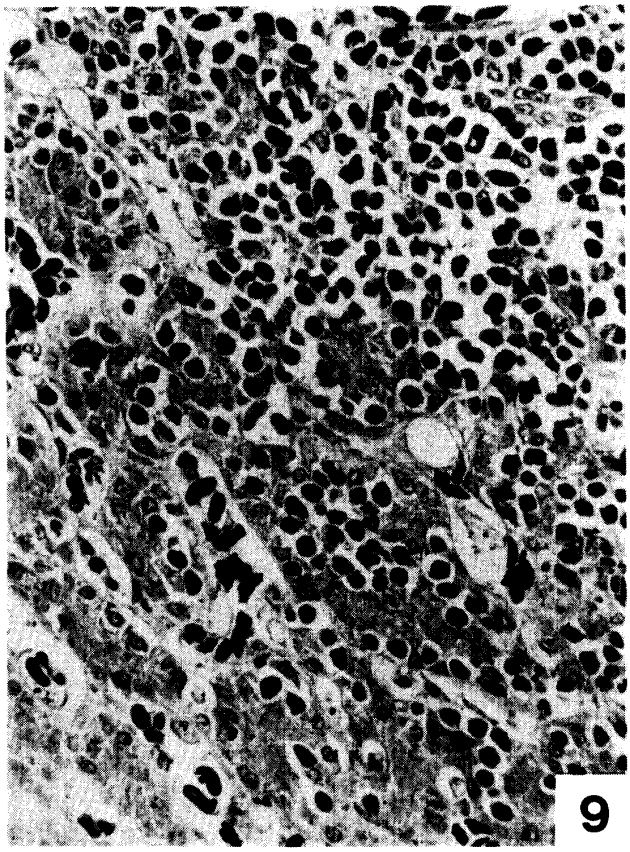

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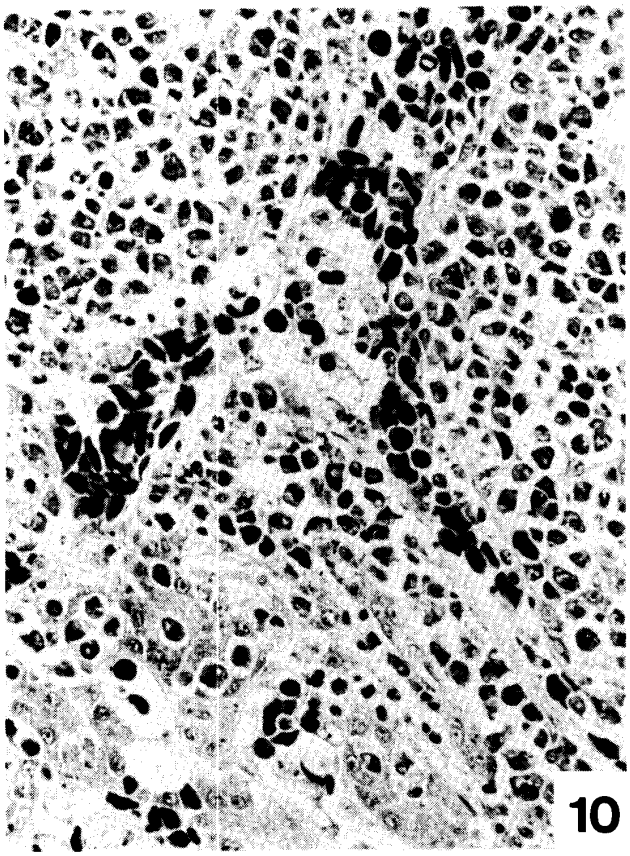

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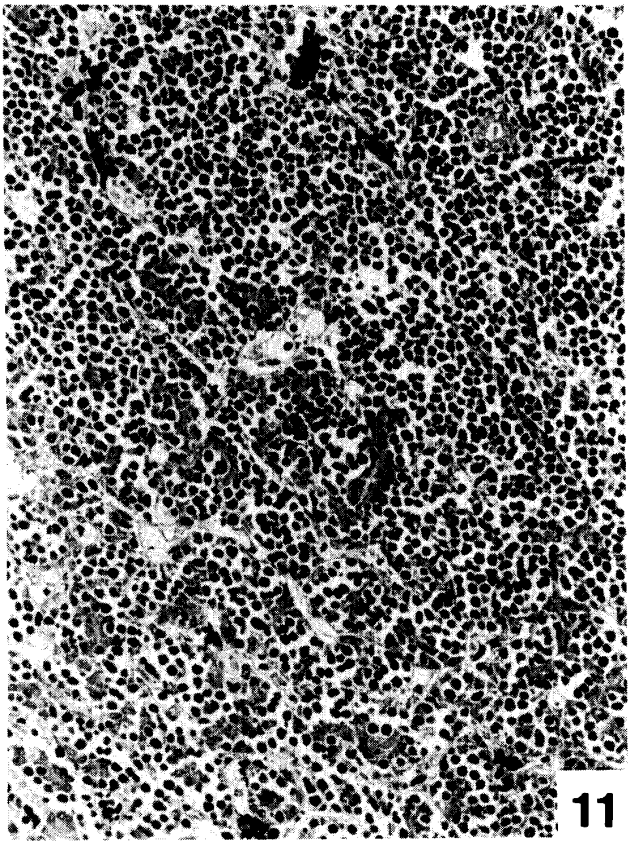

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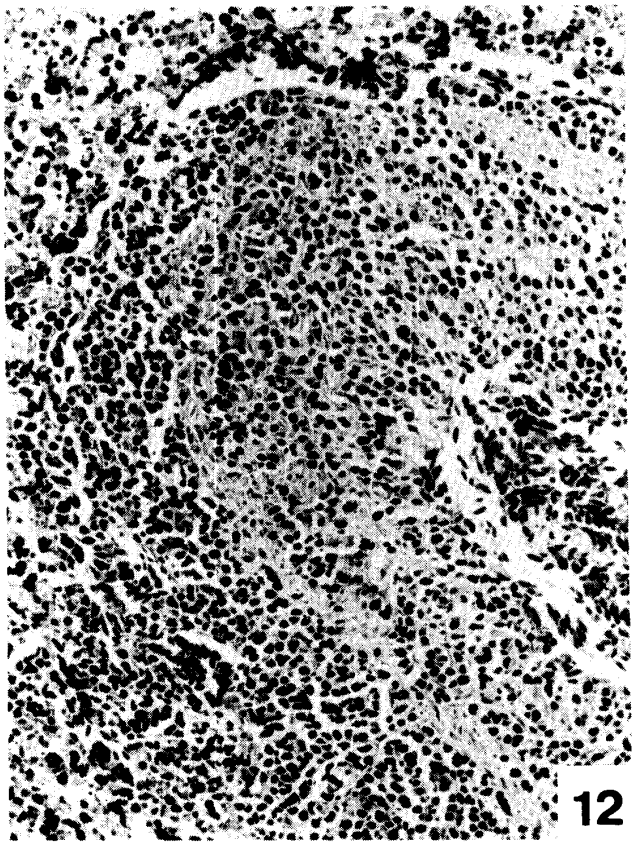

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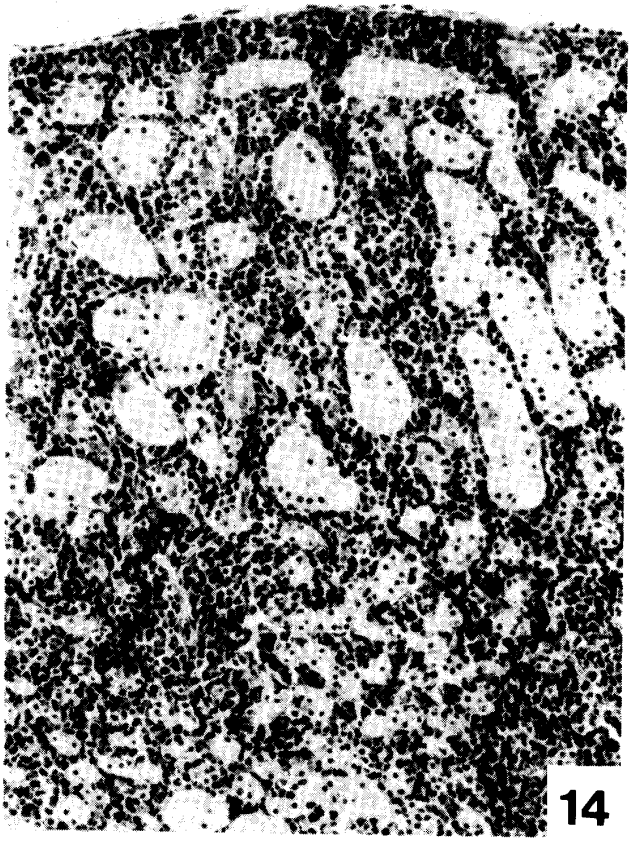

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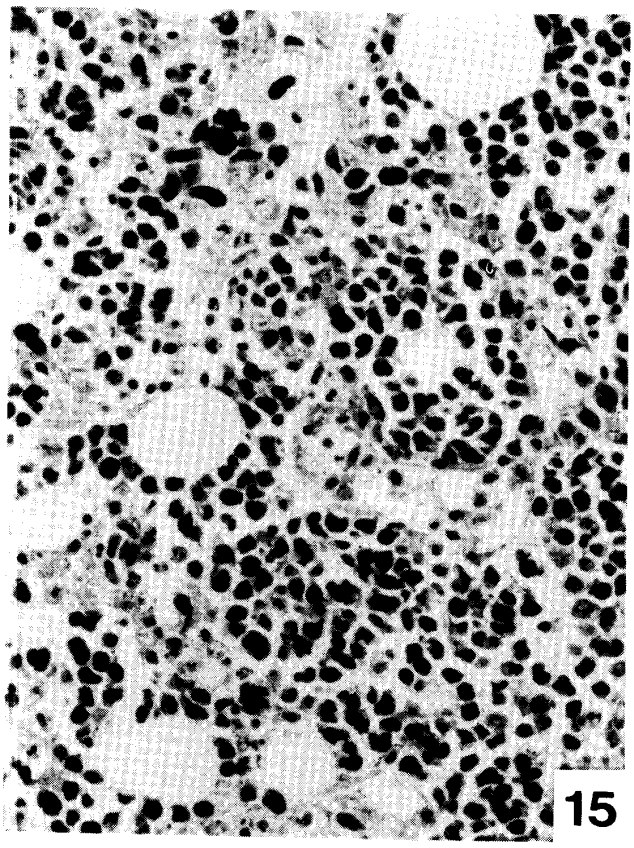

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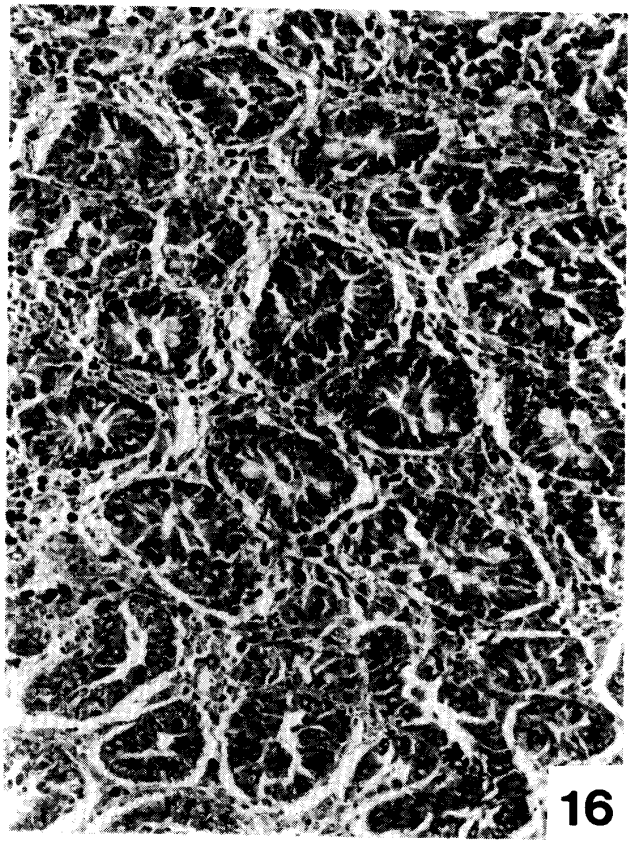

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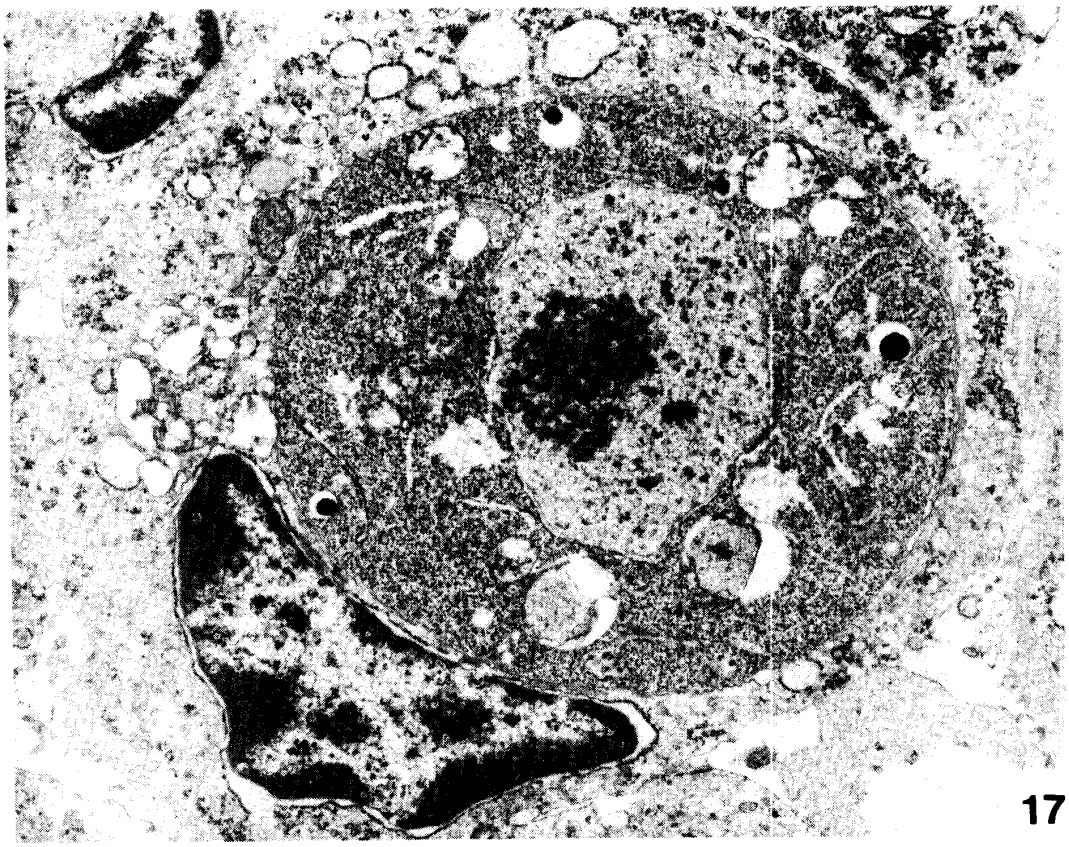

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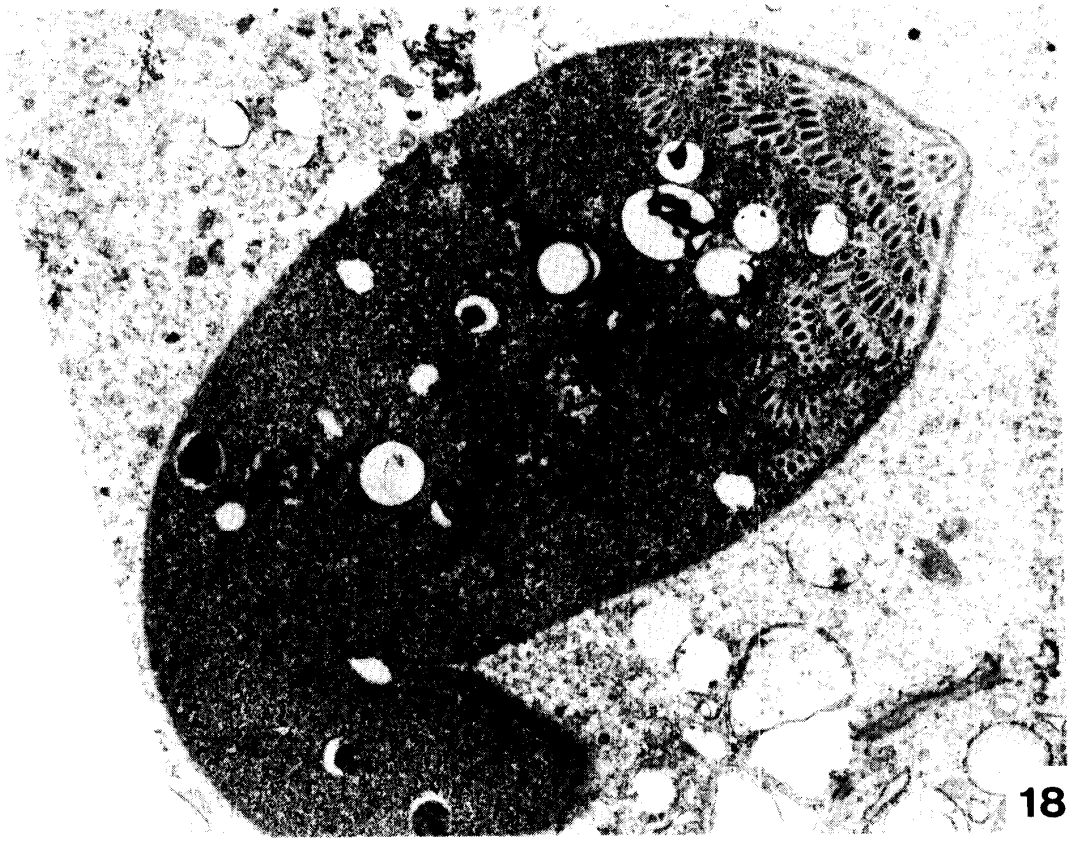

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