Short papers submitted expressly for this section, reporting original and significant findings of immediate interest and judged to be acceptable without major revision, will be published within approximately three months. See inside back cover for details.

LYSOSOMES IN TYPE II GLYCOGENOSIS

Changes During Administration of Extract from *Aspergillus niger*

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Type II glycogenosis is a fatal disease of infants (1) characterized by increased concentration of tissue glycogen and deficient activity of lysosomal acid α glucosidase (α glucosidase) (2, 3). In hepatic electron photomicrographs, Baudhuin et al. observed vacuoles filled with glycogen which they interpreted as abnormal lysosomes, i.e., as the morphological correlate of the biochemical deficiency (4). They injected intramuscularly an extract of the fungus *Aspergillus niger* which Pazur and Ando had shown to contain enzymes with α amylase, amyloglucosidase, and maltase activity (5). The treatment did not produce appreciable changes of the abnormal lysosomes. In our patient such changes were observed after prolonged, intravenous administrations of an identically prepared fungal extract.

PATIENT AND METHODS

An alcohol precipitate of *Aspergillus niger* served as the starting material. 20 g of the powder were extracted with 600 cc of distilled water filtered through a Buchner funnel, lyophilized, dissolved in 60 cc of 0.9% NaCl, sterilized with passages through Millipore filters, and kept in the refrigerator until used. The extract had a protein concentration of 25 mg/cc. It catalyzed the essentially complete conversion of glycogen to glucose at the rate of 22 μmoles of glucose formed per milligram of protein per minute at pH 4 and 37°C.

The liver was biopsied with the Menghini needle (6). The concentration of glycogen and the activity of α glucosidase in the biopsy specimens were determined according to standard procedures (2) and as reported previously (7). For ultrastructural examination the tissue specimens were immersed immediately after the biopsy in cold (0–5°C), buffered 3% glutaraldehyde (pH 7.3; 0.1 M phosphate buffer), cut into small pieces, kept in the refrigerator for 24 hr, and washed overnight in cold buffer. Postfixation was in cold (0–5°C), buffered 1% OsO_4 (pH 7.3; 0.1 M phosphate buffer) for 90 min. After dehydration in graded ethanol, the tissue was embedded in Epon 812. Thin sections were cut with glass knives on a Reichert OMU2 ultramicrotome, were stained on uncoated grids with uranyl acetate (5 min) followed by lead citrate (5 min), and were examined in a Zeiss EM 9 electron microscope.

The patient was a 3 month old negro girl with the typical features of Type II glycogenosis (1). After the liver biopsy, the extract of *Aspergillus niger* was infused intravenously in a daily, single dose of 7 cc. The activity of α glucosidase in the serum had a half-life of 110 min with a peak level immediately after the infusion of 20 μmoles/cc/min. The treatment was carried out for 18 days. 24 hr after the last infusion, α glucosidase activity was not detect-

able in the serum, and the liver biopsy was repeated. Thereafter, treatment with the extract was continued, encouraged by slight improvement in the child's condition and in her electrocardiographic tracings.

RESULTS AND COMMENTS

In the preinfusion specimen of the liver, the glycogen concentration was increased and the activity of α glucosidase was barely measurable. In the postinfusion specimen, the concentration of hepatic glycogen was normal and the α glucosidase activity was more than twice the highest value obtained in 24 "normal" biopsy specimens of human liver (Table I).

TABLE I

Biochemical Analysis of Liver Biopsy Specimens

Tissue	Glycogen*	α glucosidase‡
Normal	2.0–6.0	0.183–0.679§
Patient		
Before treatment	7.8	0.004
After treatment	2.9	1.62

* Expressed as per cent of wet tissue weight.
‡ Expressed as μmoles of glucose liberated from glycogen/min/g of tissue.
§ Range of 24 biopsies.

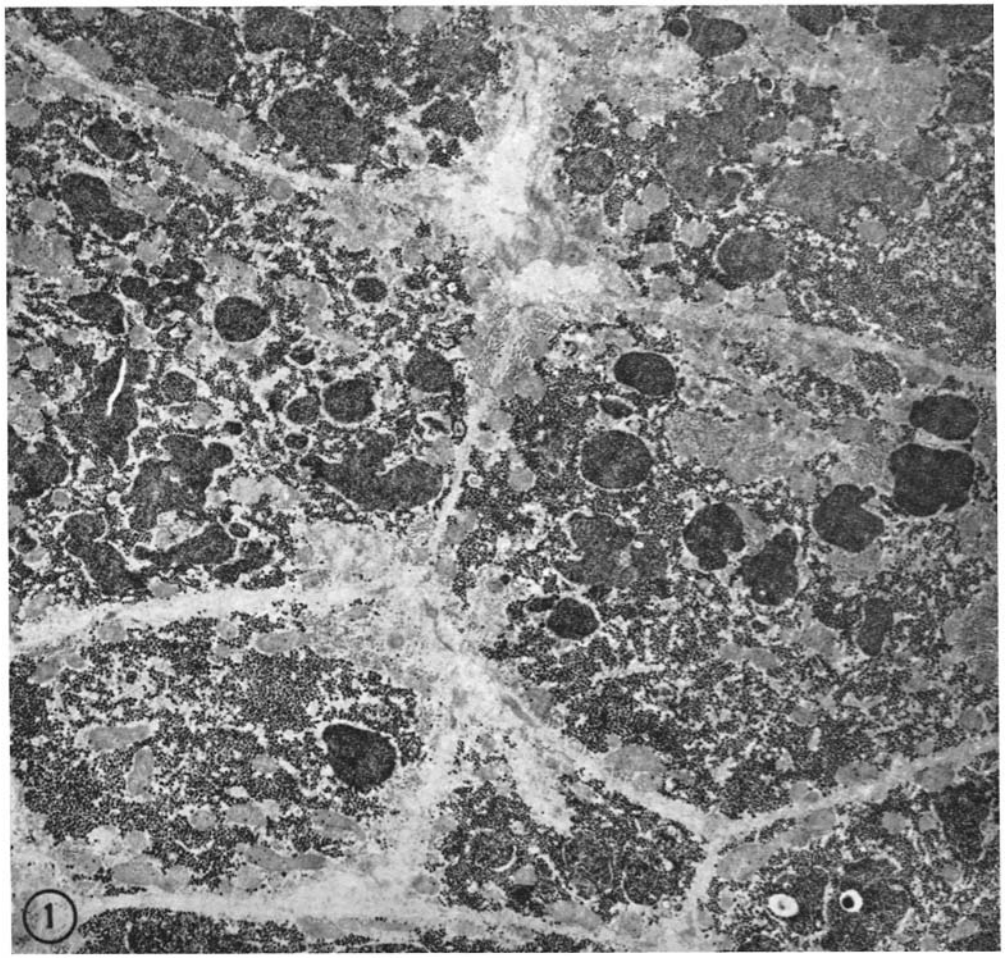
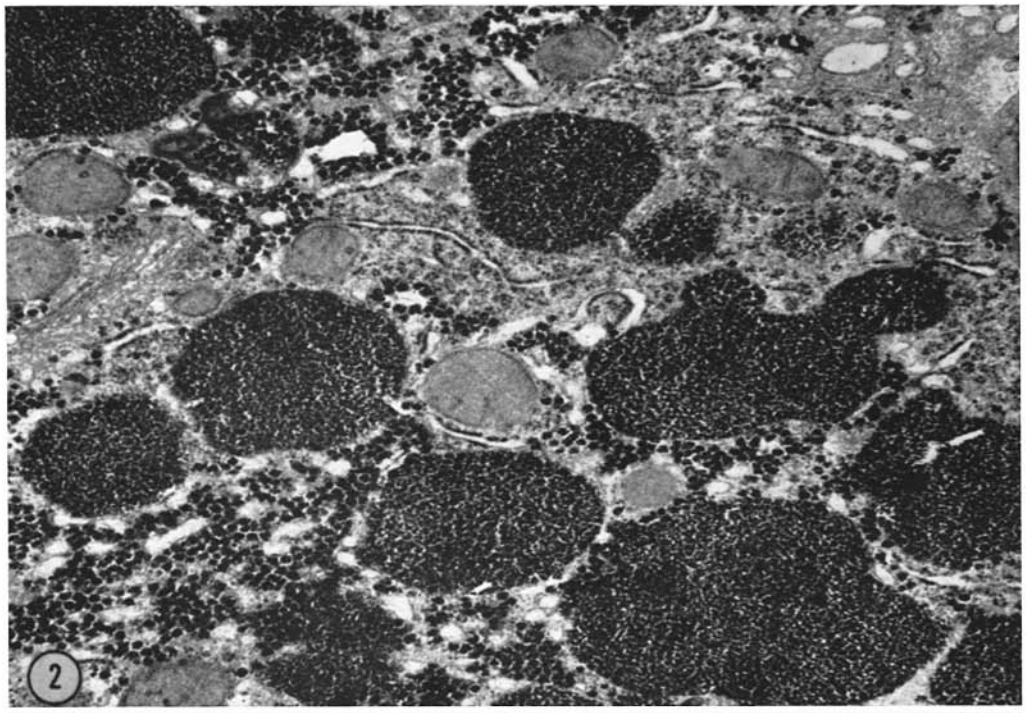


FIGURE 1 Preinfusion specimen. Abnormal lysosomes filled with glycogen are present in every hepatocyte. × 5,000.



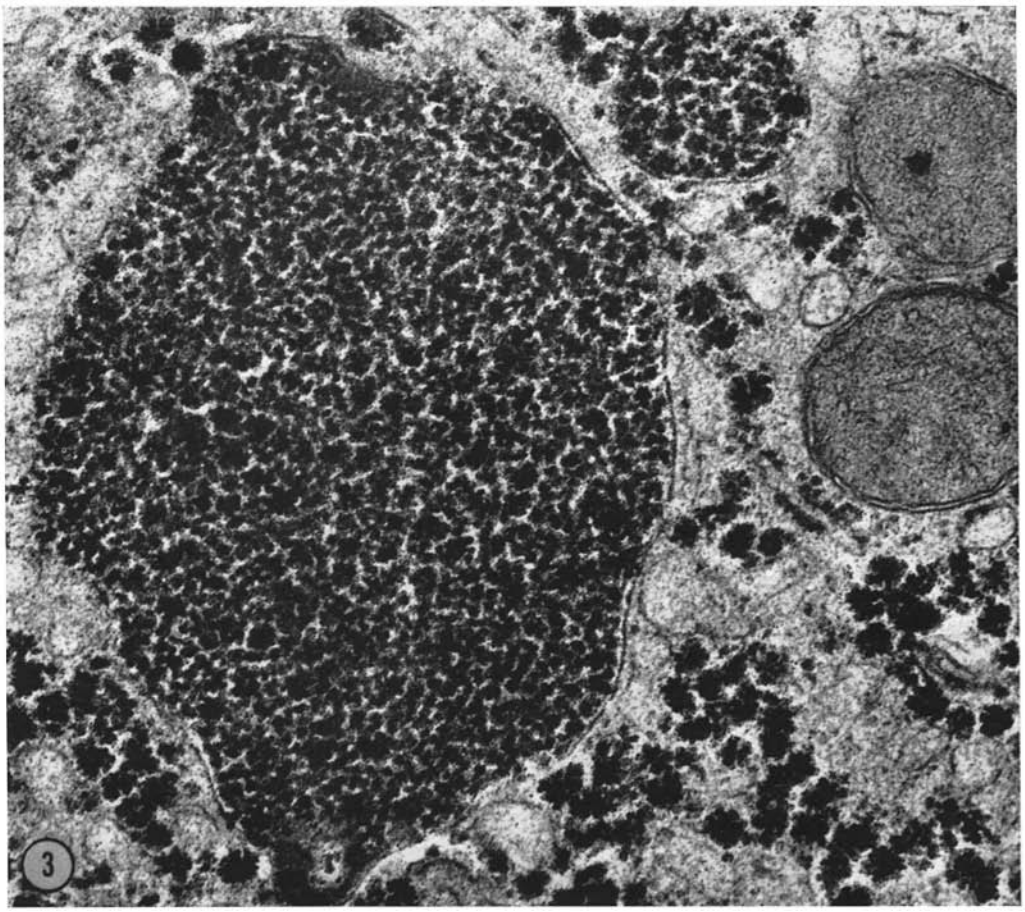


FIGURE 2 Preinfusion specimen. The picture is dominated by the abnormal lysosomes filled with lysosomal glycogen. The cytoplasmic glycogen appears not abnormal. × 21,000.

FIGURE 3 Preinfusion specimen. There are two abnormal lysosomes of small to moderate size. The lysosomal glycogen is surrounded by a membrane. × 60,000.

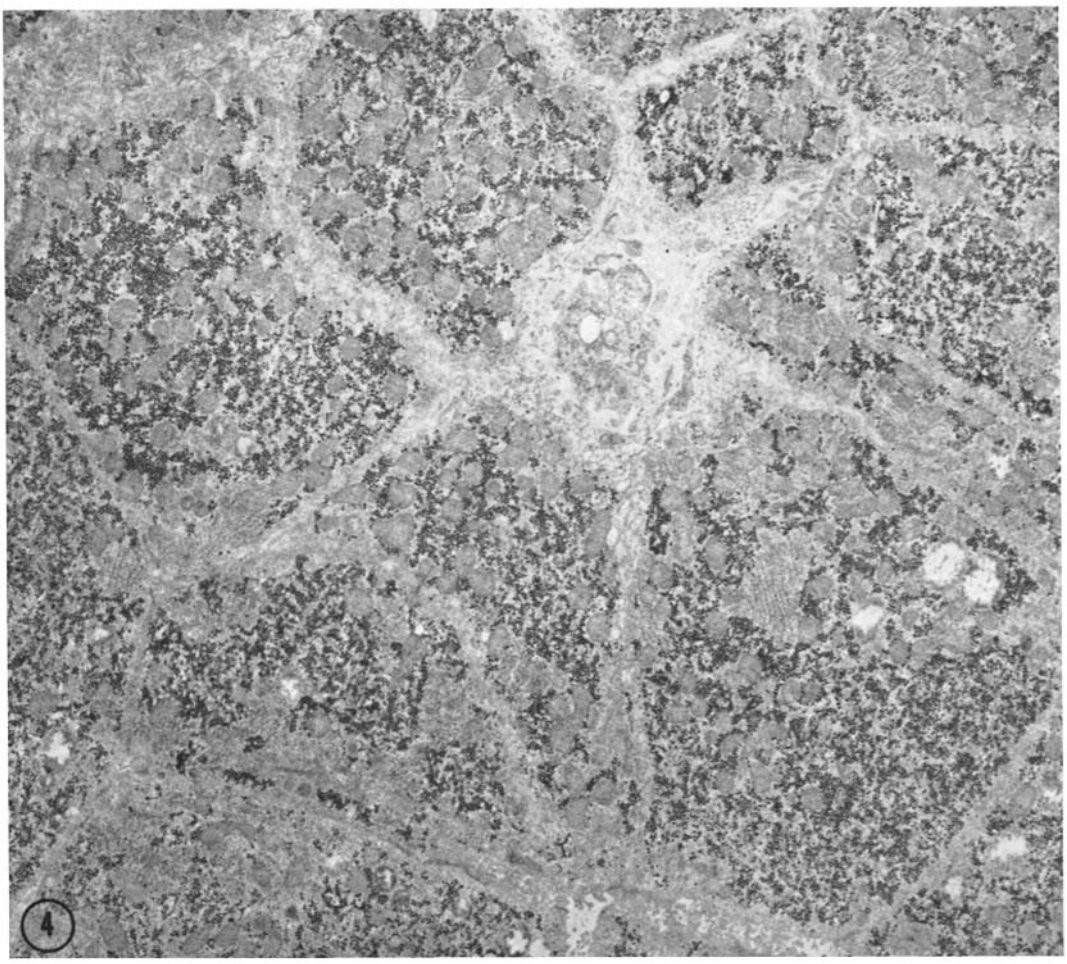


FIGURE 4 Postinfusion specimen. The glycogen-filled lysosomes of the preinfusion specimen have disappeared. × 5,000.

Electron photomicrographs of the preinfusion specimen showed the characteristic abnormal lysosomes packed with lysosomal glycogen. Notable was their abundance in every hepatocyte. In addition, there was cytoplasmic glycogen predominantly of the multigranular variety (α particles) Otherwise the cytoplasmic glycogen was similar to that seen in normal liver (Figs. 1–3). After 18 days of enzyme administration, the cytoplasmic glycogen appeared unchanged. However, the glycogen-filled lysosomes had disappeared. Instead there were structures smaller than the abnormal lysosomes of the preinfusion specimen and occurring about one-fifth as frequently. Usually they were empty or contained some cellular debris, feathery material, and fine granules. Some contained a few glycogen particles, mostly of the multigranular variety. These structures had a tendency to be located in the vicinity of cell borders and bile canaliculi (Figs. 4–6). On light microscopic examination, the postinfusion specimen could not be distinguished from normal human liver, whereas hepatic tissue of untreated Type II glycogenosis had the characteristic abnormalities (8).

Admission of the infused enzymes into the hepatocyte might be by pinocytosis, which would provide vesicles surrounded by membranes. The membranes would separate the enzymes from the cytoplasmic glycogen thus preventing its degrada-

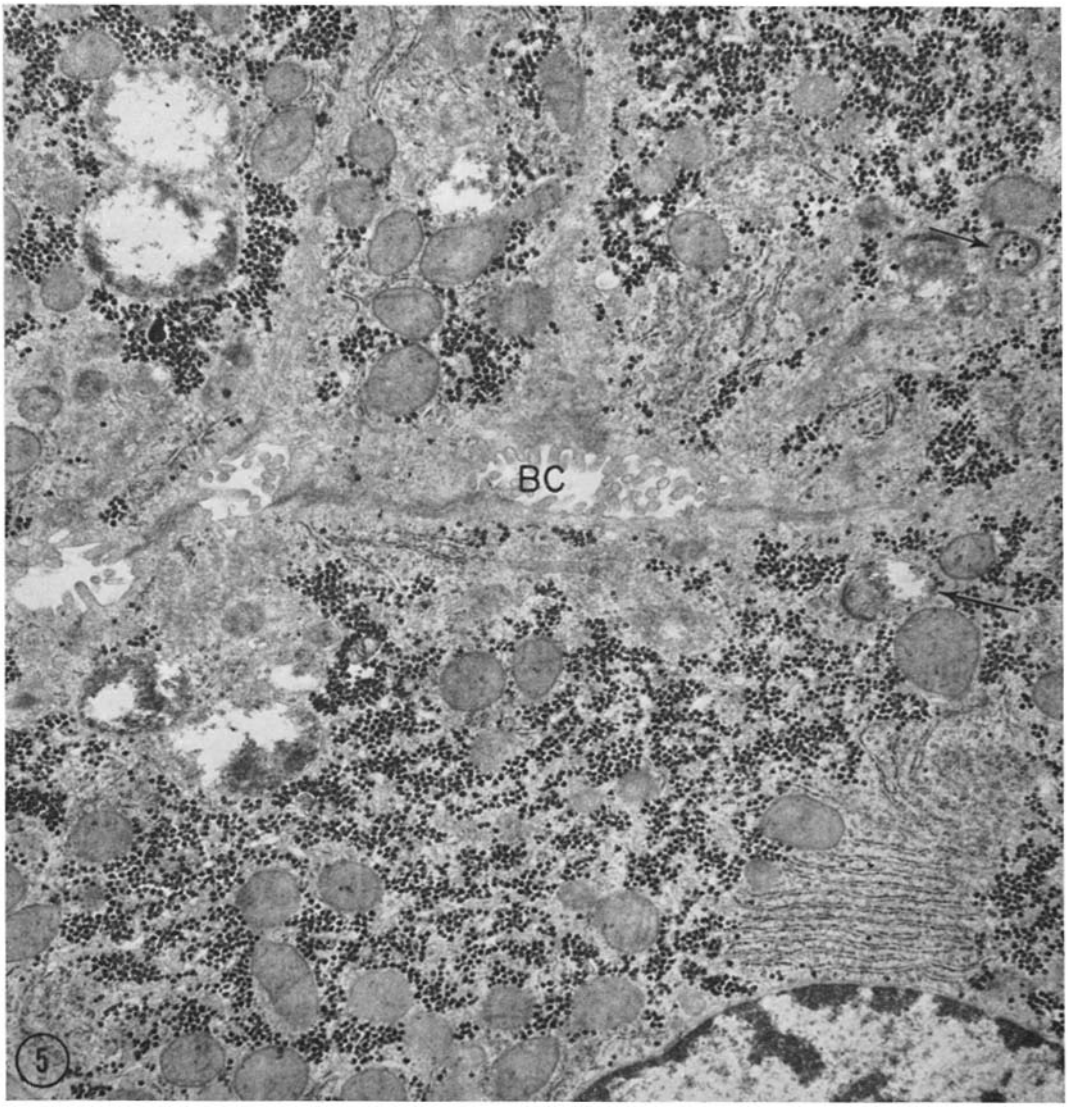


FIGURE 5 Postinfusion specimen. There are a few nearly empty vacuoles usually located close to cell borders or bile canaliculi (*BC*). Rarely, they contain some glycogen particles (arrows). × 15,000.

tion. The pinocytotic vesicles might then fuse with the abnormal lysosomes allowing the *Aspergillus niger* enzymes to degrade the lysosomal glycogen. Presumably lysosomes would continue to be formed during the enzyme administration; however, the partially empty structures of the postinfusion specimen cannot be adequately explained with the available information.

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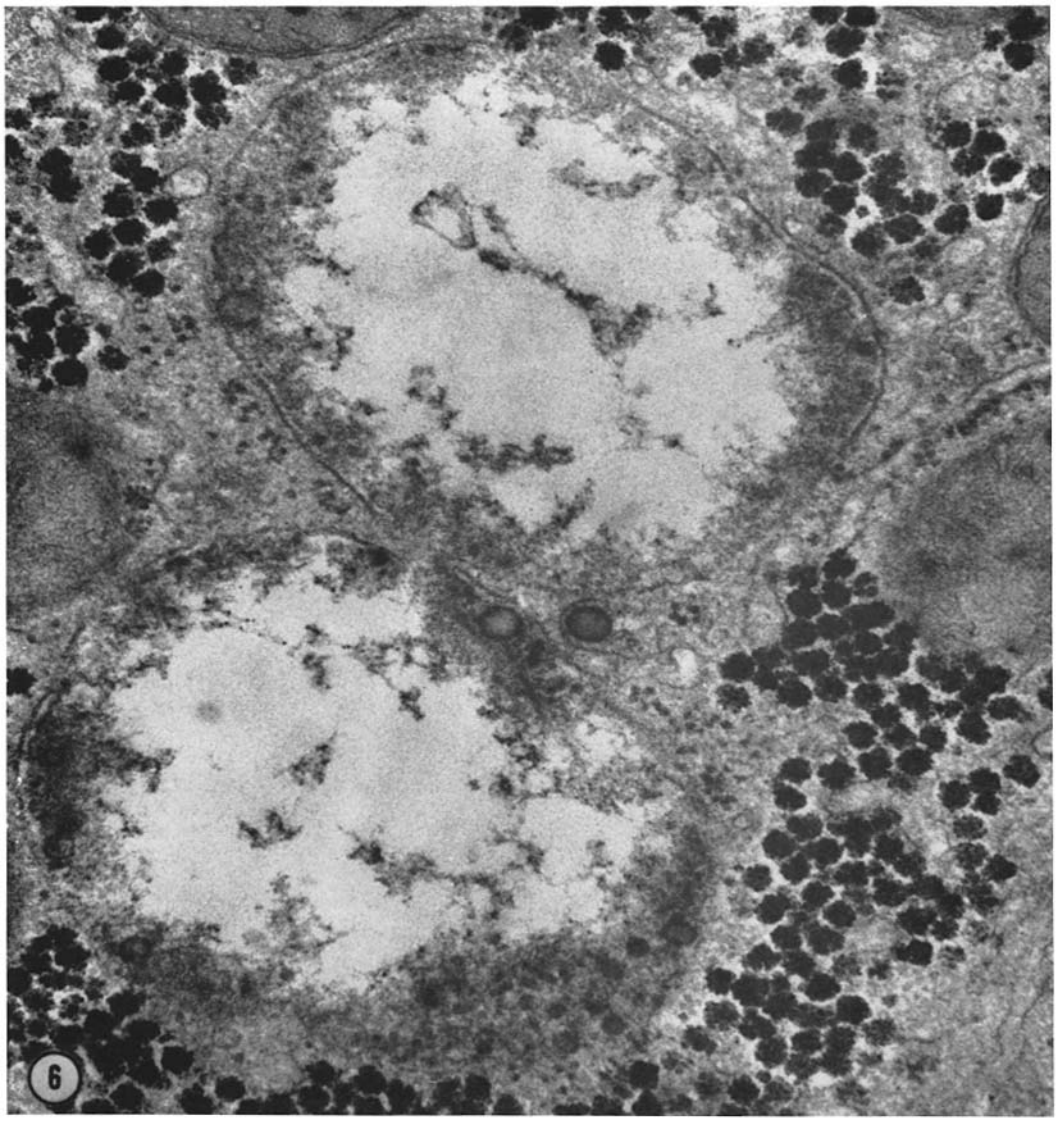

FIGURE 6 Postinfusion specimen. There are two large-sized vacuoles which are surrounded by membranes and do not contain glycogen. These structures are usually about the size of mitochondria and seem delineated occasionally by double membranes. Their identity is uncertain. The cytoplasmic glycogen is mostly of the α variety. × 60,000.

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