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ULTRASTRUCTURAL ALTERATIONS IN HUMAN HEPATOCYTES FOLLOWING INGESTION OF ETHANOL WITH ADEQUATE DIETS

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There have been numerous reports of ultrastructural changes in the human hepatocyte after acute and chronic ingestion of alcoholic beverages.[1-5] The accompanying dietary conditions, however, were not controlled, nor were biopsies made prior to the ingestion of alcohol to ascertain whether or not pre-existing derangement was present. The study reported here included histologic and ultrastructural examination of sequential needle biopsy specimens from livers of volunteers ingesting a measured amount of pure ethanol under controlled dietary conditions.[6,7] The fine structure of hepatocytes in biopsy specimens obtained before and after alcohol ingestion were compared and consistent changes in the extent and organization of agranular endoplasmic reticulum were noted.

MATERIAL AND METHODS

Four subjects were admitted to the metabolic unit of the Cornell Medical Division, Bellevue Hospital, and given a balanced alcohol-free diet for 2 to 4 months. This diet contained 16 per cent total calories as protein, 48 per cent as carbohydrate and 36 per cent as fat. Although all patients gave a history of previous alcoholism, Menghini needle biopsy specimens of the liver obtained at the end of the control period did not show cirrhosis, nor were they fatty or otherwise abnormal by light microscopy.

After this control biopsy was obtained, alcohol (diluted 95 per cent ethanol) was given for 16 to 18 days in increasing amounts, up to 46 per cent of total calories, isocalorically replacing carbohydrates. (Ethanol and alcohol are used synonymously in this paper.) Depending on the caloric intake of the individual, ethanol ingestion

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ranged from 98 to 138 gm per day. Three individuals received ethanol with a diet of normal fat content (36 per cent of calories), while one was given ethanol in a high carbohydrate, low fat diet (5 per cent of calories). On the morning following the last day of alcohol ingestion another liver biopsy was performed. Each patient was then returned to a normal balanced alcohol-free diet and 6 to 8 weeks later a third biopsy was made, representing a second control. Three patients were then again given the same amount of alcohol for the same period of time, but the 2 who had first received ethanol with a fat-containing diet were now given the low fat diet, while the third, fed a low fat regimen at first, now received the fat-containing diet. Another needle biopsy was performed in each patient on completion of the second period of alcohol intake. In 2 cases, additional biopsies were made at 1 and 6 weeks following the last period of alcohol ingestion.

A part of each biopsy specimen was examined by light microscopy, while another part was prepared for electron microscopy. All specimens for light microscopy were fixed in formalin and stained with hematoxylin and eosin, Masson's trichrome, and Best's carmine stains. The specimens for electron microscopy were fixed in 4 per cent phosphate-buffered glutaraldehyde at pH 7.2, followed by 1 per cent unbuffered osmium tetroxide. The tissues were then dehydrated in a graded series of alcohols and embedded in Epon 812. Sections cut with an LKB Ultrotome were stained with solutions of lead salts and examined with an RCA EMU 2B electron microscope. In order to reduce bias, the specimens were coded by one investigator and the tissue examined and interpreted by the other investigator prior to decoding.

Results

Electron Microscopy

The appearance of hepatocytes in biopsy tissue obtained from each patient after the initial control diet period conformed essentially to prior descriptions of normal cells.[8,9] Because the specimens were small segments of a narrow needle biopsy, however, the zonal position of the cells examined was not always determined. Although some variability was noted in any specimen, the general architecture was similar in many cells, permitting characterization of the specimen in terms of the appearance of the majority examined. Moreover, variations in minute structural details were fewer when comparing cells from the same specimen, or from two control specimens from the same subject, than when comparing typical cells in control specimens from different patients. These minor but distinctive differences between hepatocytes in control biopsy specimens from different patients included differences in the size of endoplasmic reticulum arrays, the relationships between lamellae in the stacks of granular endoplasmic reticulum, the position of the nucleus relative to the granular endoplasmic reticulum and the distribution of the areas of glycogen accumulation.

In one subject, a portion of the second control biopsy specimen used for electron microscopy exhibited an increase in portal fibrous tissue. This, however, did not appear to reflect a diffuse change, since the adjacent portion used for light microscopy appeared normal. There was also an increase in the depth of the interhepatocyte recesses in the space

of Disse with loss of the usual close approximation of plasma membranes of adjacent cells. This ultrastructural alteration has been reported to be characteristic of regenerating nodules.[10] The specimen was therefore not included as a control in the evaluation of changes induced by alcohol ingestion.

In all of the specimens obtained following a period of alcohol ingestion, the hepatocytes demonstrated a prominent change in the appearance of the agranular endoplasmic reticulum (ER) in the glycogen storage zones. Anastomosing thin tubular channels coursing between aggregates of glycogen rosettes (Figs. 1 and 2) were replaced by numerous vesicular profiles scattered throughout the glycogen-rich areas of cytoplasm (Figs. 3 and 4). These vesicular forms of agranular ER were observed in all cells in all specimens obtained immediately after the period of alcohol intake, but were noted to be reduced in number after the administration of a low fat diet during one of the two periods of alcohol ingestion. Furthermore, cells in an additional specimen obtained a week after cessation of alcohol intake showed vesicular profiles of agranular ER among the glycogen rosettes, as well as a re-establishment of the tubular anastomosing channels (Fig. 5). Vesicular forms of agranular ER were noted in a small proportion of cells in control tissues as well, but were usually far less extensive.

In addition to this consistent change in the appearance of the agranular ER, alterations in the structure of the mitochondria in a large percentage of cells were noted after ingestion of alcohol. In any plane of section, 25 per cent or more exhibited this change. Giant mitochondria with bizarre shapes, crystalline inclusions (Figs. 6 and 7) and disturbances in the configuration and orientation of cristae were observed, but within any given cell, a majority of mitochondria retained normal structure. Similarly, dilatation of the granular ER was noted in most cells in tissues obtained following alcohol administration, but was not uniform and occurred, to a lesser extent, in some of the control specimens.

Light Microscopy

None of the biopsy specimens exhibited an increase in fibrous tissue when stained with hematoxylin and eosin or the Masson trichrome stain, nor was gross glycogen depletion noted when Best's carmine stain was employed. Fatty changes ranging in degree from moderate to marked were present in the post-alcoholic specimens with less steatosis in those on low fat than on the fat-containing diets, as described previously.[7] No "alcoholic hyalin" of Mallory was seen. Rare fat vacuoles measuring 6 to 8 μ were also present in some of the control tissues. Since no more than 3 or 4 cells in any section contained a vacuole, this was not considered to represent a pathologic feature.

Discussion

Although previous reports of the ultrastructure in human hepatic parenchymal cells following acute or chronic ingestion of alcoholic beverages have included changes in the endoplasmic reticulum [1,4] and mitochondria [3–5] similar to those described here, the dietary conditions during the period of alcohol intake and the state of the liver prior to the period of ethanol intake have not been controlled. The reported changes have therefore been based on a comparison with a general norm but may have been present before the alcohol intake. Since persons imbibing excesses of alcohol often reduce their food intake, eat deficient diets, or ingest toxic substances other than ethanol, the effect of these factors on fine structure must also be considered before ascribing the observed changes to alcohol toxicity.

The multiple sequential needle biopsy specimens obtained from each subject in this study have provided a direct comparison of the deviations from a defined "normal" state to that induced by the ingestion of pure ethanol during a period of controlled adequate dietary conditions. The two specimens obtained from each subject after a period of 3 months of well balanced diet were found to be very similar. This similarity in the fine structure of cells in samples from the same patient obtained months apart suggests that although liver cell structure may change dramatically in response to metabolic alteration, under stable standard conditions it may return to very much its original state.

In spite of the minute but distinctive features which allowed identification of specimens from different patients, all the specimens taken immediately following periods of alcohol ingestion exhibited a similar change when compared to their respective controls. The predominantly tubular anastomosing channels of agranular ER around aggregates of glycogen rosettes were replaced by large numbers of dilated vesicular profiles scattered among glycogen rosettes. A reduction of dietary fat to 5 per cent of total calories during alcohol ingestion diminished this effect, but did not qualitatively alter it. This suggests that the ethanol-induced change in the agranular ER becomes exaggerated when the liver is called upon to function under a combined ethanol and lipid load. This observation parallels the observation that after ingestion of the same amount of alcohol hepatic steatosis is more pronounced in subjects on fat-containing than in those on low fat diets, in both the rat [11] and man.[7] The slow restoration after alcohol is eliminated from the diet may indicate that the changes noted are not dependent directly upon the presence of alcohol, but are responses to other subtle but enduring effects.

This reproducible alteration in a particular organelle has been previously noted in the liver cells of rats ingesting alcohol chronically.[12-14] It is, however, not to be considered specific for alcohol toxicity since proliferation of the agranular ER has been seen after the administration of a number of toxic agents whose particular actions are different.[15-26] This suggests that it is a common response to a range of chemical stimuli. In fact, since the enzymes which function in detoxification of some drugs have been demonstrated to reside in the agranular ER,[27] the changes observed may reflect the induction of alcohol-metabolizing enzymes. The exact relationship between the morphologic alterations described and the multiple metabolic abnormalities produced by ethanol[28] is not known, although a number of the enzymes of intermediary metabolism have also been localized in the agranular ER.[29,30]

The morphologic changes observed in mitochondria are possibly more directly due to the damaging effects of alcohol, but they do not serve well as indices of toxicity because they appear less uniformly in the affected cells. In addition, scattered bizarre mitochondria are encountered in almost all normal liver specimens[31-33] and may be present in large numbers in a great variety of pathologic conditions.[23,32,34,35] The most striking feature of the mitochondrial alteration is the variety of forms seen, even in a single specimen. Some mitochondria are enlarged and misshapen, others show derangement of the cristal pattern and others exhibit crystalline inclusions. The significance of this diversity is unclear, but since a similar range of alterations is seen in widely differing types of pathologic states, it seems likely that the propensity to change in different ways is a property of mitochondria rather than the reflection of specific damage induced by alcohol.

Mitochondrial alterations have been described as the most prominent feature in hepatocytes in rats following a prolonged period of alcohol ingestion.[12-14] In these studies, it was noted that the changes became extensive and uniform only after many weeks of ethanol intake. The relative brevity of exposure in the present study may account for the less uniform mitochondrial alterations.

Summary

Four human subjects, continuously receiving a normal balanced diet under metabolic ward conditions for 4 to 6 months, were given a measured amount of pure ethanol during 2 widely spaced 16- to 18-day periods. Liver specimens were obtained by percutaneous Menghini needle biopsy before and after each course of alcohol and the multiple specimens from each patient were compared. In addition to steatosis, all tissues obtained after alcohol ingestion exhibited vesiculation and in-

crease in extent of the agranular endoplasmic reticulum. Less uniform alterations in mitochondrial structure were also noted. The study indicates that a reproducible alteration occurs in the human liver cell in response to alcohol ingestion under normal dietary conditions.

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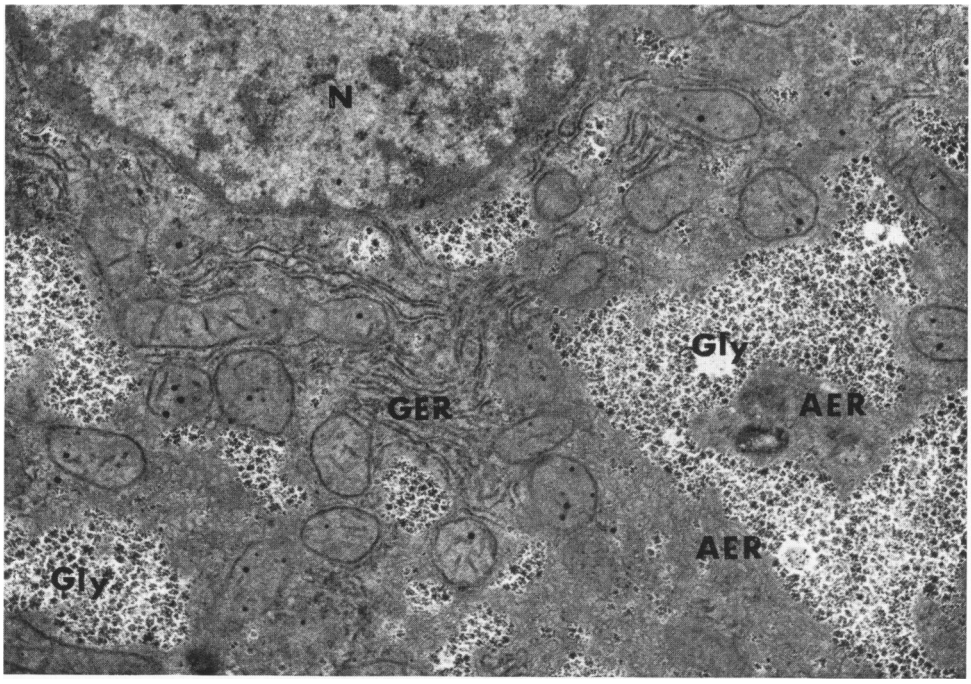
[*Illustrations follow*]

LEGENDS FOR FIGURES

FIG. 1. Hepatocyte in a specimen obtained after 3 months of balanced adequate diet. Glycogen-containing areas (Gly) situated between aggregates of granular endoplasmic reticulum (GER) occupy a large part of the cytoplasm. Fine tubular channels of agranular endoplasmic reticulum (AER) coursing between aggregates of glycogen rosettes appear as vague densities at this magnification since their full diameters are often far less than the thickness of the section. Nucleus (N). × 6,500.

FIG. 2. A portion of the cytoplasm of a typical hepatocyte in a specimen obtained from another subject following a period of controlled adequate diet. The tubular channels of the agranular endoplasmic reticulum (AER) are seen to pass around aggregates of glycogen (Gly). Even at this higher magnification, the outlines of these thin structures are obscured by superposition (arrows) within the section. Granular endoplasmic reticulum (GER); mitochondria (M). × 18,000.



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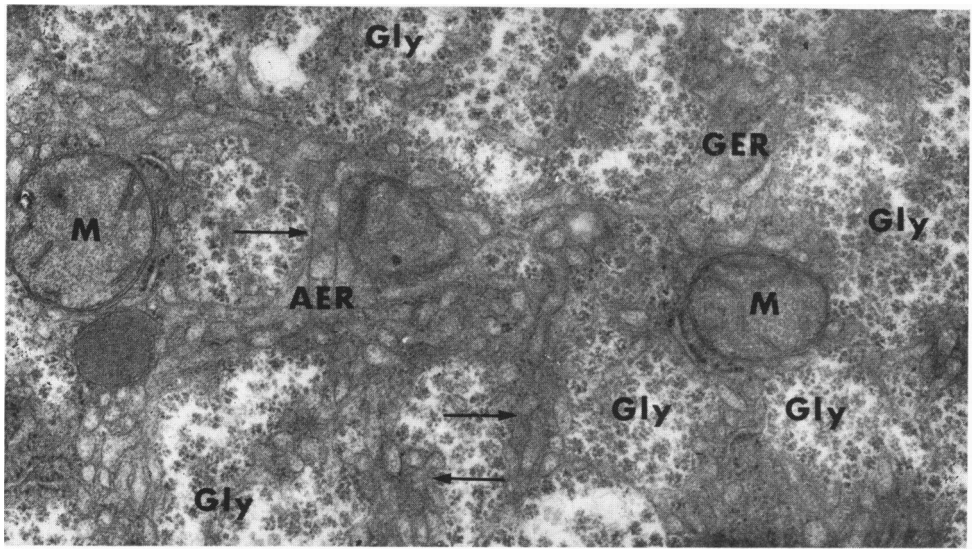

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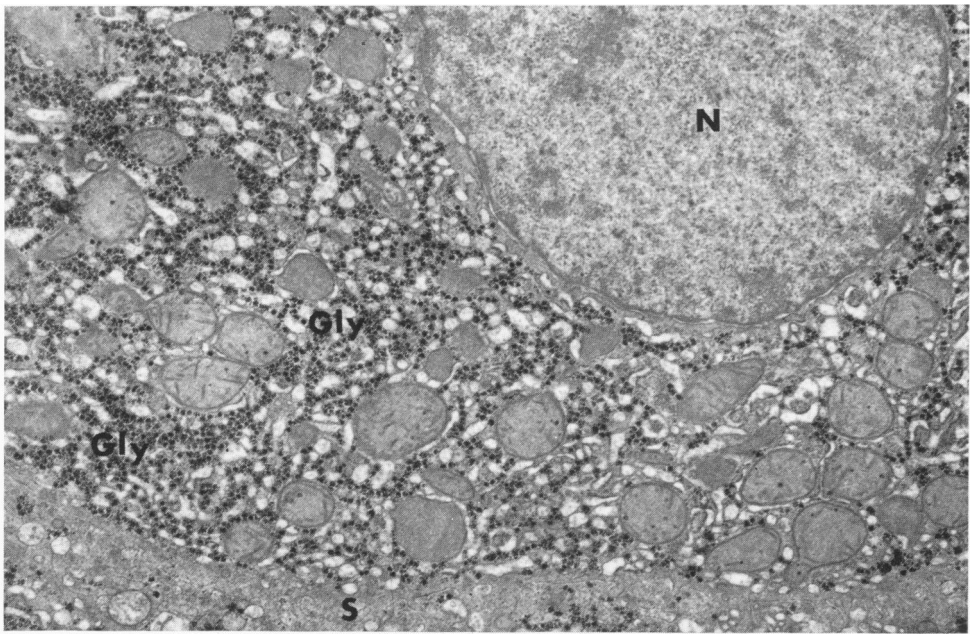

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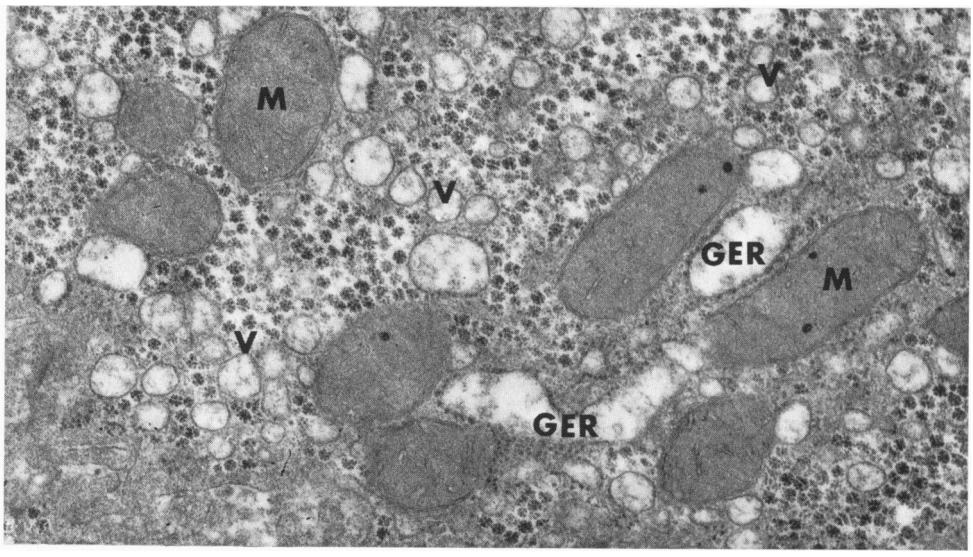

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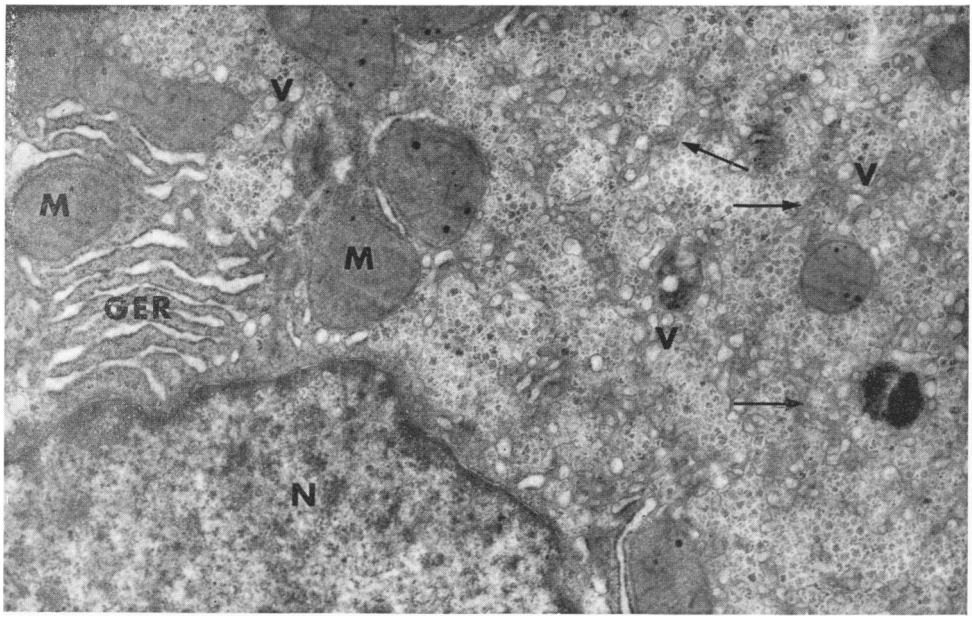
FIG. 3. A hepatocyte from a specimen obtained after 18 days of a diet in which 46 per cent of calories consisted of ethanol. The glycogen-rich areas of cytoplasm (Gly) include large numbers of round electron-lucent profiles scattered among rosettes. Nucleus (N); sinusoid (S). × 6,000.

FIG. 4. A portion of a hepatocyte in a specimen obtained from a patient following a course of alcohol ingestion accompanied by a balanced adequate diet. Numerous smooth membrane-bound vesicles (V) several times the diameter of the fine tubules of agranular ER in control biopsies are distributed among the glycogen rosettes. Since superposition and curvature of membranes are reduced by this increase in dimensions, the outlines of these membranous structures are clear. Granular endoplasmic reticulum (GER); mitochondria (M). × 17,000.



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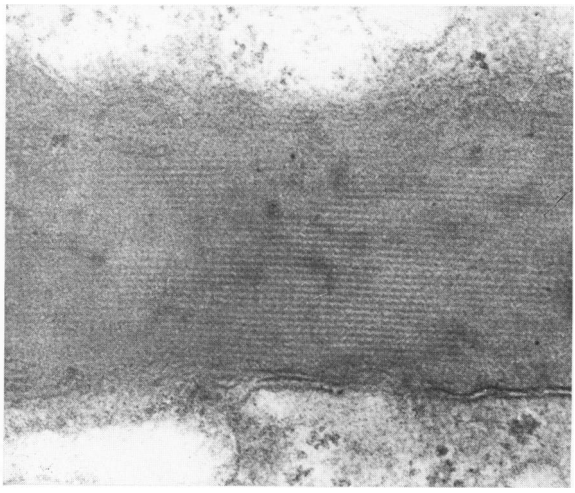

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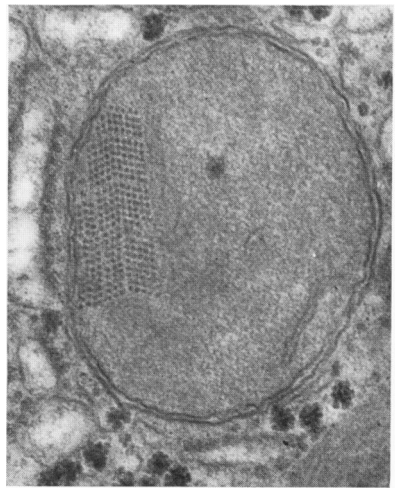

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FIG. 5. A portion of a hepatocyte in a specimen obtained 1 week after cessation of alcohol administration. The glycogen-rich cytoplasm contains both the anastomosing fine tubular channels of agranular ER (arrows) noted in controls and the dilated vesicular forms (V) observed in tissues taken during periods of alcohol ingestion. Nucleus (N); granular endoplasmic reticulum (GER); mitochondria (M). × 11,000.

FIG. 6. A portion of a mitochondrion in a specimen obtained at the conclusion of a period of alcohol ingestion. This small segment of a mitochondrion which measured 30 micra in total length exhibits a large crystalline matrix inclusion × 35,000.

FIG. 7. A similar mitochondrial inclusion cut in another plane. The crystalline nature is apparent in this geometric array. × 35,000.