

RESPONSE OF HEPATIC MICROBODIES TO A HYPOLIPIDEMIC AGENT, ETHYL CHLOROPHENOXYISOBUTYRATE (CPIB)

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INTRODUCTION

Current knowledge regarding the origin of microbodies, their function in cell metabolism, the manner in which they are destroyed or disposed of by the cell, and their responses to pathological conditions is limited, but emerging information concerning their chemical composition (1, 2), the structure and content of their nucleoid (1, 3-5), and their possible relationship to other cell organelles (6, 7) provides a stimulating and useful background for further investigation.

Though a limited range of alteration in the number or structure of microbodies has been described, these organelles, compared to other cell constituents, appear relatively indolent and unresponsive to most experimental manipulations. Accordingly, new information relating their morphologic and biochemical responses to specific metabolic stimuli may prove useful for further studies.

Current experiments in our laboratories have revealed conspicuous numerical and ultrastructural changes in microbodies following administration of a hypolipidemic agent (ethyl chlorophenoxyisobutyrate, CPIB) capable of lowering serum lipid levels in man (8, 9) and in experimental animals (10). The observations, though preliminary, provide strong support for the proposal of Novikoff and Shin (7) that microbodies are related to lipid metabolism because the structural changes are accompanied by a decrease in liver cholesterol and triglyceride levels, and an increase in liver catalase activity and weight. Concomitantly, a slight increase in liver protein and phospholipid occurs (11, 12).

Two electron microscope studies of rat liver following CPIB treatment have been reported. Paget (13) described an increase in size and number of mitochondria and the formation of "giant lysosomes" of uncertain identity. The second report (14) related the effects of CPIB treatment to numerical changes in microbodies and to enzyme activity in rat liver, but neither alterations of the matrix nor other changes in fine structure of microbodies were described.

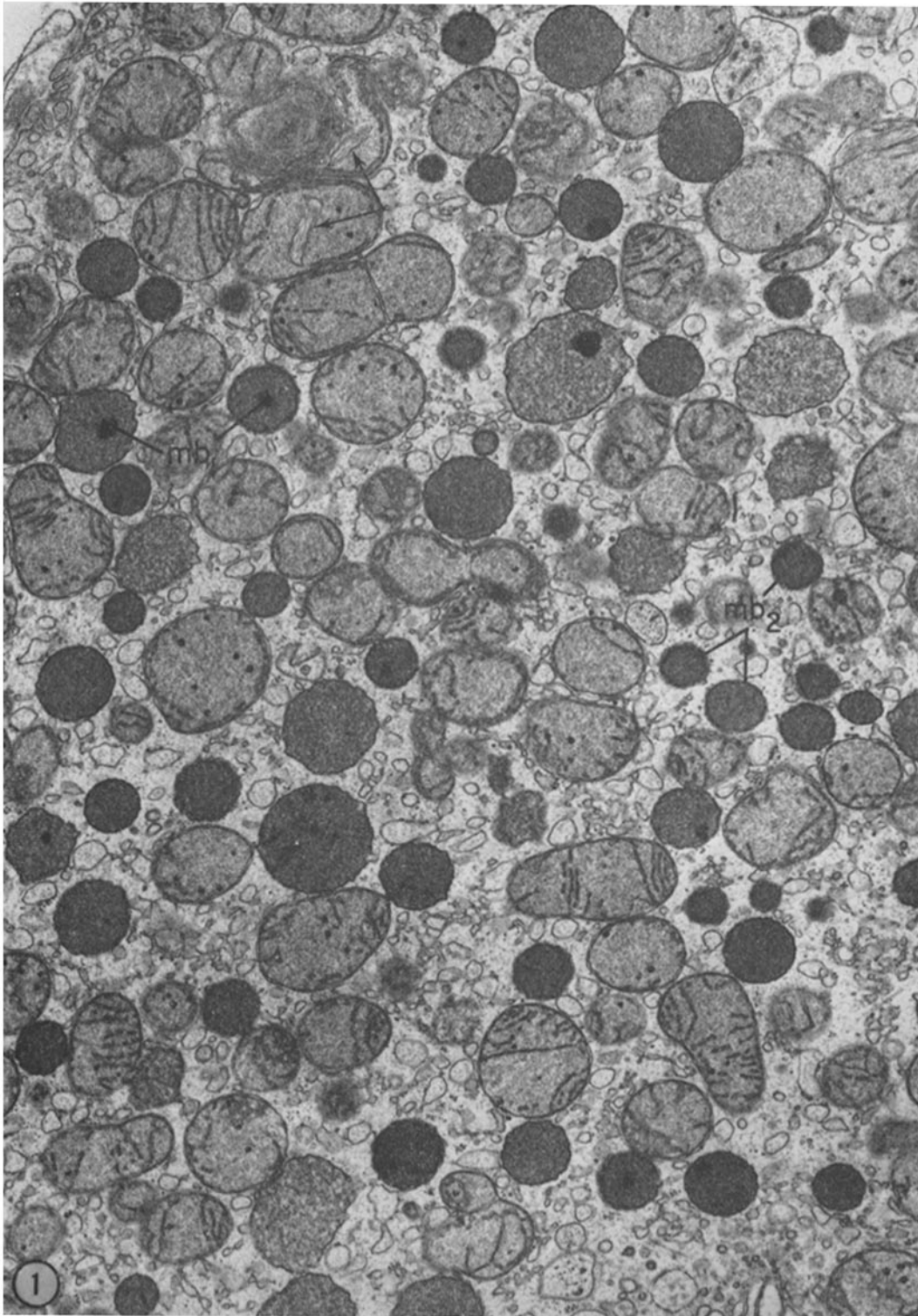
MATERIAL AND METHODS

Eight male CFE and F-344 rats, weighing approximately 120 grams, were given 0.25 to 0.5% CPIB in chow diet and sacrificed at 3, 14, and 30 days. Male C3H and Swiss Webster mice were treated similarly. The animals were opened under light ether anesthesia. Small portions of liver were removed, minced into blocks of 1 mm³ or less, fixed for 1 hr in 2% osmium tetroxide buffered with *s*-collidine, dehydrated in alcohol, and embedded in epoxy resin. Thin sections were cut on an LKB 8800A ultramicrotome equipped with a DuPont diamond knife, stained with lead (15), and examined with an RCA 3G electron microscope employing a 35 to 40 μ objective aperture and an accelerating voltage of 50 kv. In all, approximately 40 blocks of tissue were examined for each time interval, and 30 to 60 sections from each block were studied. Catalase activity was determined by the spectrophotometric method described by Lück (16). Uricase activity was assayed according to the procedure of Loudon and Hudson (17).

RESULTS

A marked and uniform increase in the number of microbodies in all hepatocytes (Fig. 1) and a proliferation of the smooth endoplasmic reticulum were the first alterations present as early as 3 days after treatment and persisting at 14 and 30 days. While many microbodies were of normal size and had typical nucleoids, others were identified by their density, matrix striations, or their circular, single membrane-limited configuration. Many microbodies lacking characteristic nucleoids with a crystalline substructure had a flocculent matrix (Figs. 2 and 5). Such flocculent structures frequently possessed small matrix densities resembling immature or noncrystalline nucleoids and were attached to membranes of the endoplasmic reticulum (Figs. 5, 6, 8, 9, and 11). By 3 days, changes were also present in mitochondria. Many mitochondria had membrane-limited inclusions containing wavy or beaded fibrils (Fig. 3); others were elongated and flattened, with close apposition of their limiting membranes (Fig. 4).

By 30 days of treatment, many microbodies were bizarre in shape and size and contained in their matrix several linear "striations" or lamellae



All figures are of sections of rat liver stained with lead.

FIGURE 1 After 3 days of CPIB treatment. Marked increase in number and variation in size of microbodies were apparent. While some microbodies had nucleoids (*mb*₁), most did not (*mb*₂). The nucleoid, when present, was usually small, eccentric, and lacked the normal crystalloid substructure. Intramitochondrial bodies containing fibrils are apparent at the unmarked arrows. $\times 17,000$.

approximately 500 to 600 Å apart and 200 to 300 Å wide (Figs. 6 to 8, and 10). At this time interval also, several densities were present within cisterns of the endoplasmic reticulum. Such densities were identifiable as microbodies or as material of similar structure, even in the absence of a nucleoid, by the presence of the matrix striations (Figs. 7 and 8). Connections of newly formed atypical microbodies with membranes of the smooth endoplasmic reticulum remained prominent. At this time interval, catalase activity values in homogenates were significantly higher than control values, while uricase levels were unchanged or slightly decreased.

By 9 to 11 days, there was a similar increase in number of microbodies in the mouse, but the increase was not uniform from cell to cell and matrix striations were not present.

While many microbodies had diameters within the normal range of 0.3 to 0.6 μ , there was an obvious increase in the proportion of these organelles that had a diameter at the smaller end of the range, 0.2 to 0.3 μ . In contrast, those microbodies with a flocculent rather than compact matrix usually had a diameter greater than 0.6 μ .

Many microbodies possessed elongated, strap-shaped extensions (Fig. 10) that measured 0.7 to 1 μ .

DISCUSSION

The main effects of CPIB treatment are hypocholesterolemia and hepatomegaly (12). The cause of hepatomegaly is unclear at present, but it is evidently not related to the hypolipidemic effect of

the drug because, in some species, decrease in serum cholesterol occurs in the absence of hepatomegaly (18). Similarly, hepatomegaly is not due to increase in the number of microbodies since by 2 wk after withdrawal of CPIB the liver weight returns to normal but the increased number of microbodies persists (14).

The observation that microbodies increase remarkably in number and undergo prominent changes in ultrastructure simultaneously with hypolipidemia due to CPIB treatment suggests a relationship, although indirect, between microbodies and lipid metabolism. Striations or formed elements similar to those related to CPIB treatment were noted by Hruban and Swift (5) after administration of acetylsalicylic acid, tetracycline, azaserine, and thioacetamide. While we have observed that microbodies show an increase in number and a variability in size after thioacetamide treatment, we have not detected matrix striations within them. With another hepatocarcinogen, *N,N'*-2:7-fluorenylenebisacetamide, however, extensive clusters of microbodies with bleblike evaginations of their limiting membrane were conspicuous (19) (Fig. 12).

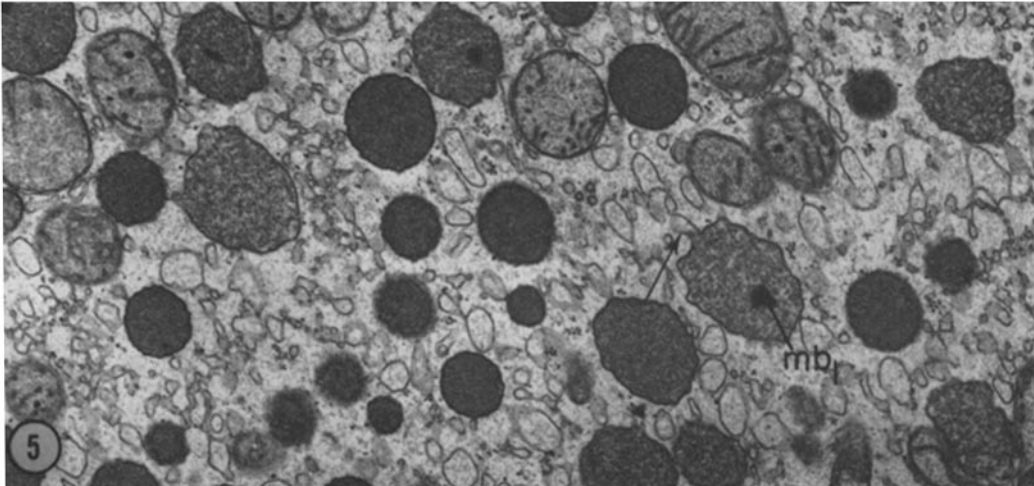
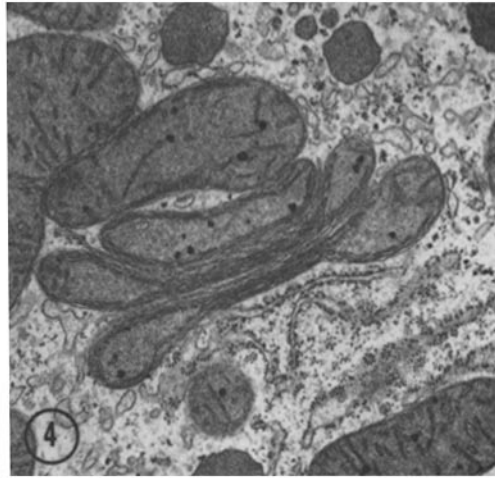
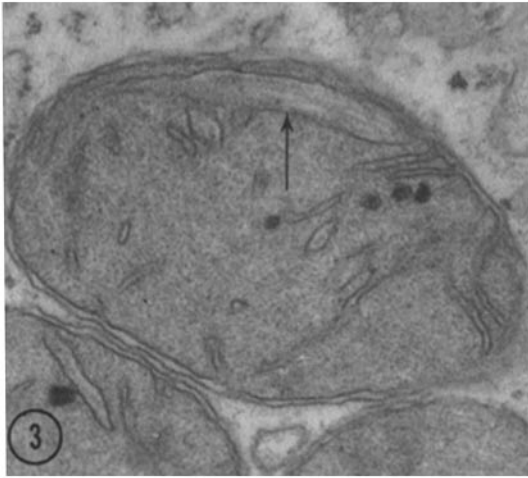
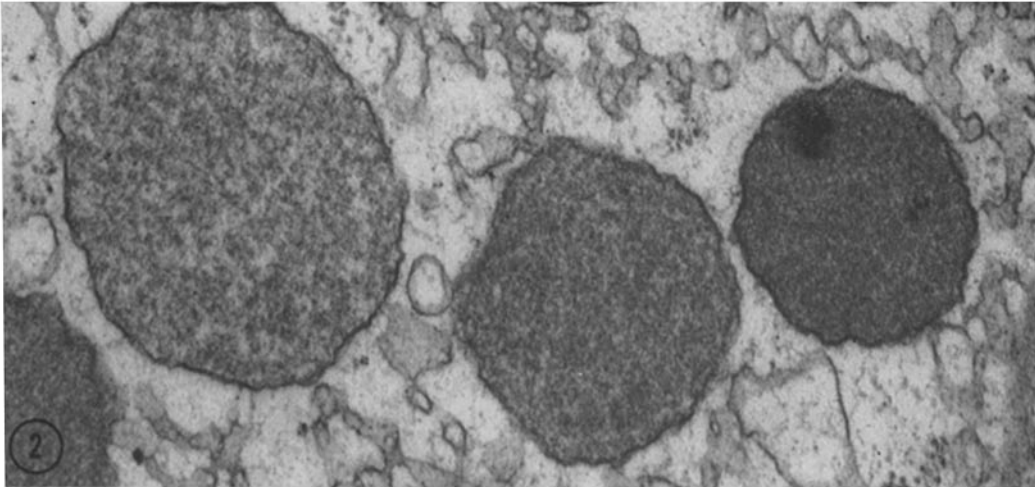
Differences in the number of microbodies within zones of hepatic lobules and their increase after partial hepatectomy were noted by Novikoff and Shin (7). Continuity of the single limiting membrane of microbodies to smooth endoplasmic reticulum was demonstrated in rat livers after partial hepatectomy and dimethylaminoazobenzene administration (7), in rat hepatomas (20),

FIGURE 2 After 3 days of CPIB treatment. The matrix of the microbodies was of variable density and remained so at 14 and 30 days. The microbody on the right is of normal density and has a small, eccentric nucleoid. Those in the center and on the left have a flocculent, less dense matrix. $\times 29,000$.

FIGURE 3 A mitochondrion after 3 days of CPIB treatment. A membrane-limited area containing wavy or beaded fibrils, similar to those found in protein-deficient rat liver (30) and corpus striatum of the rat (31), is apparent at the arrow. $\times 66,000$.

FIGURE 4 Several mitochondria, after 3 days of CPIB treatment, were elongated and flattened, with close apposition of their limiting membranes. $\times 17,000$.

FIGURE 5 After 14 days CPIB treatment, the number of microbodies remained increased. Those with a flocculent matrix could be identified as microbodies because of their possession of a nucleoid (*mb₁*). Continuity of the limiting membrane with smooth endoplasmic reticulum was frequently seen (arrow). $\times 9600$.



after thienylalanine (21), and in normal liver (1). Dalton (22), in a study of hepatomas, concluded that the size and complexity of microbodies was inversely proportional to the growth rate of the tumors. He described tubules of 100 μ diameter in the matrix of microbodies. In our studies cross-sections of microbodies did not disclose tubular structures; instead, the formed elements were consistently lamellar.

Presumably, the increased number of microbodies that are smaller than normal represents a true proliferation because the increase was present in all zones of the hepatic lobule and was accompanied by a simultaneous increase in catalase activity in liver homogenates (18, 23).

Recent studies have suggested that the nucleoid of microbodies in several species contains uricase (1, 4), and Afzelius (3) has pointed out that those species whose microbodies lack nucleoids also lack uricase. While the relationship appears generally true, it is apparent that the presence of nucleoids is not an all-or-none phenomenon in a single species (Fig. 1). Though, in the rat, nucleoids are uniformly present in microbodies under normal conditions, our studies demonstrate that, after CPIB treatment, many newly formed microbodies lack nucleoids. Accordingly, under the present experimental conditions, a mixed population of microbodies exists in this species. While it might be argued that the absence of a nucleoid in most microbodies is a result of a plane of section failing to include the nucleoid, numerical studies of normal rat liver indicate that a central section through the hepatic cell contains an average of 10 microbodies (range, 3 to 18), with 6 (range, 2 to 11) possessing a nucleoid. The ratio of microbodies to mito-

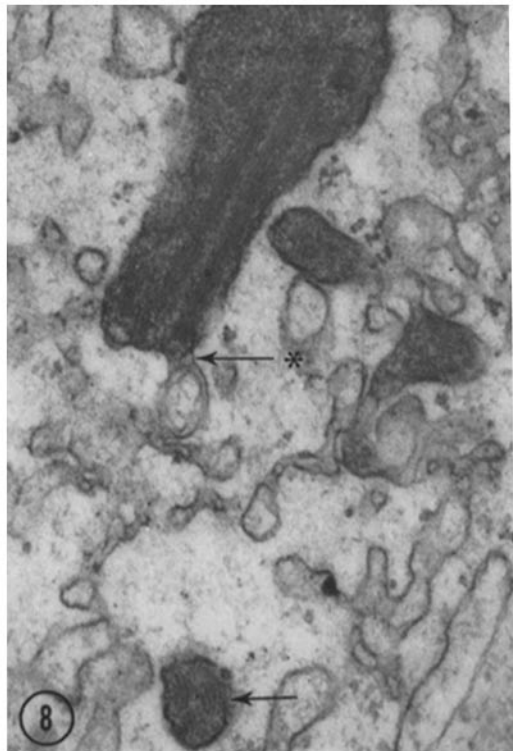
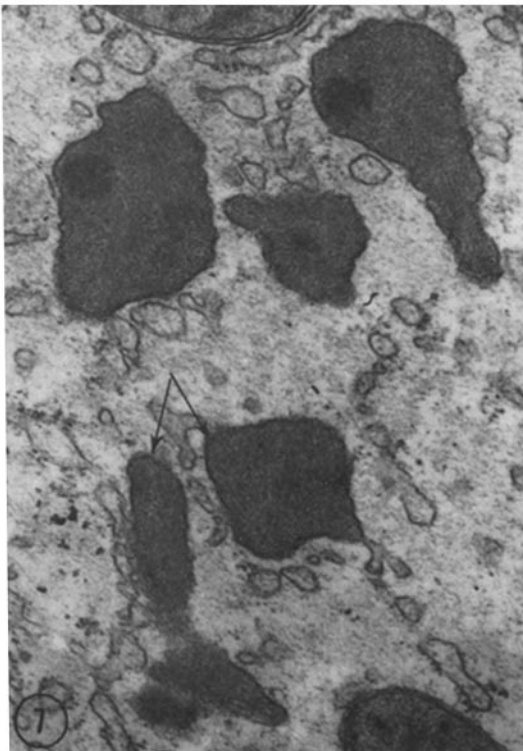
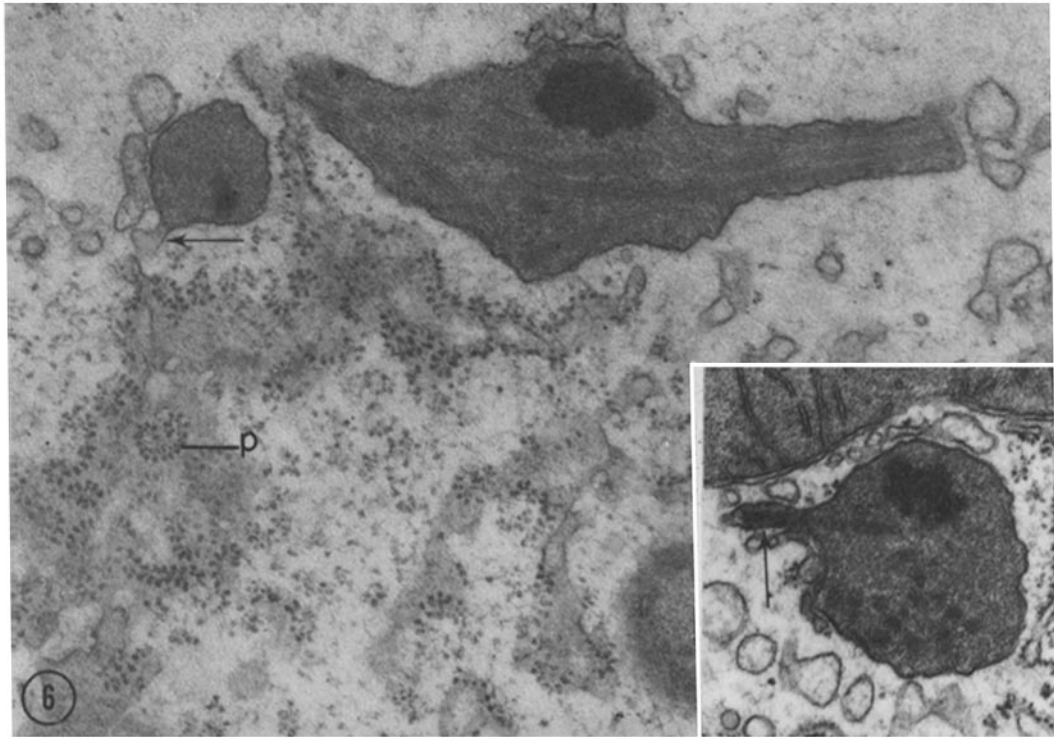
chondria in a normal rat liver cell is approximately 1:8 (1), after CPIB treatment the number of microbodies per cell increases tenfold, but the number containing nucleoids is not proportionately increased. Considering that numerous random sections in control and experimental animals were examined in this study, it would appear that the absence of a nucleoid in a high percentage of microbodies is not just an accident of sectioning, but instead represents a proliferation of new microbodies which, for reasons not yet clarified, lack nucleoids. Indeed, uricase levels after CPIB treatment may be unchanged or slightly decreased at an interval when microbodies lacking a nucleoid are prominent and catalase activity is increased. It would appear, therefore, that under selected experimental conditions the enzymic constituents and their usual morphological equivalents may become dissociated. Studies to determine this point are currently in progress.

While the origin of microbodies cannot unequivocally be answered by the present studies, Figs. 7 and 8 support Novikoff and Shin's suggestion that electron-opaque material may be deposited in the cavities of dilated smooth endoplasmic reticulum before the cavities separate from the main reticulum (7). Similarly, Higashi and Peters (24, 25), from biochemical data, have suggested that catalase is first synthesized in the endoplasmic reticulum and later transferred to a particulate fraction.

Since the increase in number of microbodies is temporally associated with elevated catalase levels, it appears that their formation is an expression of synthesis of enzyme protein for intracellular use. Alternatively, it has been suggested that micro-

FIGURE 6 After thirty days of CPIB treatment, the microbodies frequently assumed abnormal shapes with elongated extensions. Matrix striations were conspicuous. Note the polyribosomes (*p*) and the attachment of a microbody to a vesicle of the smooth endoplasmic reticulum (arrow). The inset at lower right illustrates a microbody (CPIB, 30 days) with a protrusion (arrow) and with matrix containing short, crescentic striations. $\times 66,000$.

FIGURES 7 and 8 After 30 days of CPIB treatment. Cisterns of the smooth endoplasmic reticulum contained dense material with striations (arrows, Fig. 7, unmarked arrow, Fig. 8). Though the transitional forms were considerably smaller than normal microbodies, the density and the striations were indistinguishable from those of the matrix of typical microbodies after CPIB, and suggested origin from the dense material in the cisternae. Attachment to smooth endoplasmic reticulum is present at arrow (*). Fig. 7, $\times 66,000$; Fig. 8, $\times 72,000$.



body enzymes perform important catalytic functions in gluconeogenesis (26), in the destruction of cell metabolites (27), or in protection against random oxidative destruction of cell constituents by hydrogen peroxide (28). That the microbodies represent a secretory granule for export outside the parent cell, similar to zymogen granules in the pancreas (5), seems unlikely since there is little cytochemical or morphological evidence to support this analogy.

Crystalline bovine hepatic catalase (Caperase, Laboratorio P.E.V.Y.A., Barcelona, Spain) administered parenterally to humans has been reported (29) to lower serum cholesterol and uric acid levels. Possibly, the endogenous increase of liver catalase is associated with the hypolipidemic effects of CPIB and the administered catalase may produce its effect by a similar mechanism.

The manner in which microbodies are disposed of by the cell is not clear (6, 7). Bruni and Porter (6) reported that microbodies fuse with lysosomes to form bodies with the structural features of both. No evidence for such fusion or transition forms was evident in our experiments.

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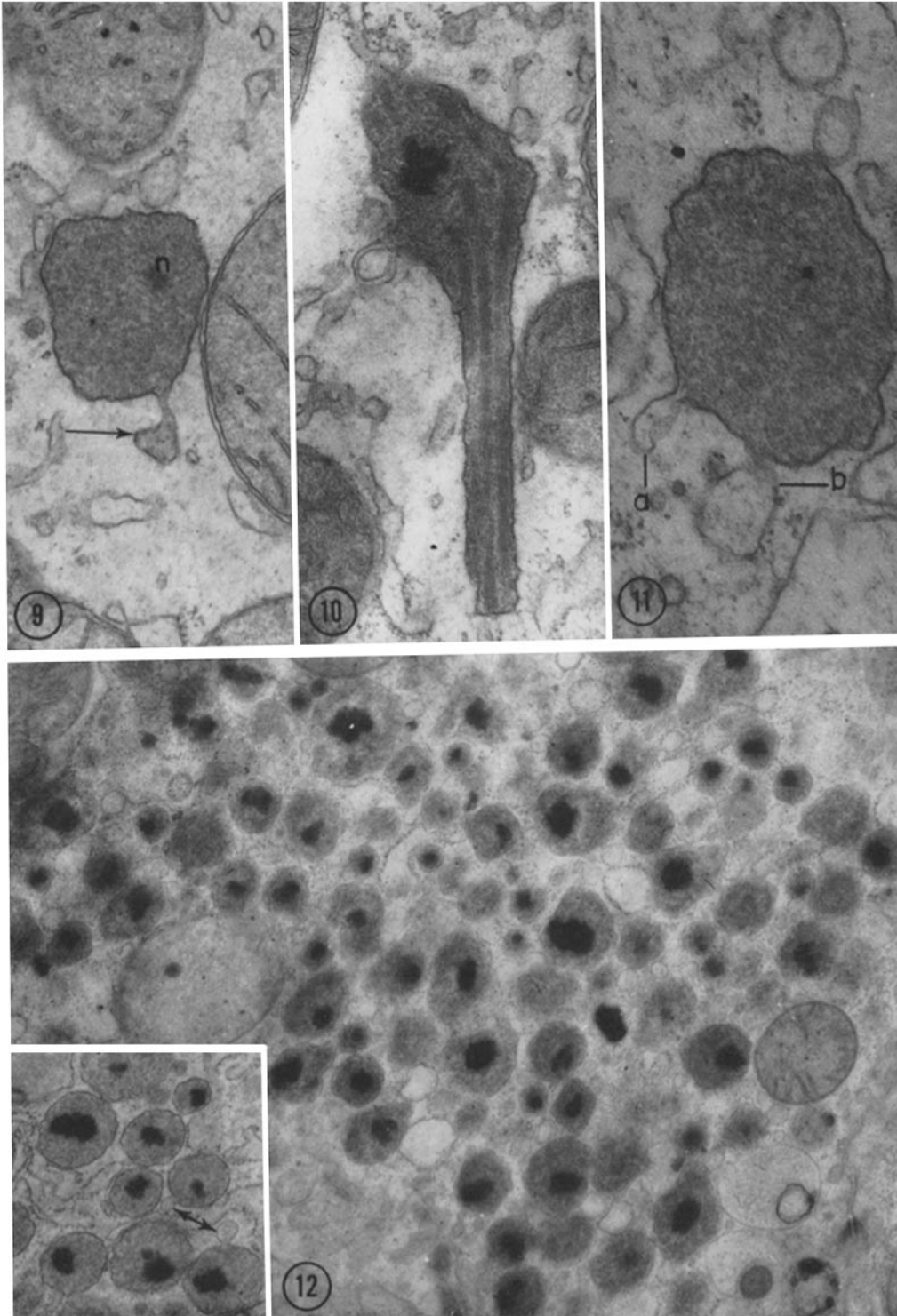
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FIGURE 9 After 30 days of CPIB. A microbody with matrix of less-than-normal density is continuous, through a narrow channel with a secondary density (arrow). A small nucleoid is present (*n*). $\times 66,000$.

FIGURE 10 A microbody contains an elongated, straplike extension containing matrix striations. $\times 29,000$.

FIGURE 11 After 30 days of CPIB treatment. Continuity of a microbody with smooth endoplasmic reticulum is present at *a*. A similar connection is suggested at *b*. $\times 72,000$.

FIGURE 12 Rat liver after 60 days of treatment with *N,N'*-2,7-fluorenylenebisacetamide. A cluster of microbodies is present in a liver cell. Most of the bodies contain nucleoids. The inset at lower left is from the same specimen and shows bleblike evaginations of the limiting membrane (arrows). $\times 17,000$.



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