Uricase: Localization in Hepatic Microbodies

Abstract. *Microbodies of rat hepatic cells and hepatomas contain a crystalloid with the same structural characteristics as crystals of purified uricase, when viewed by electron microscopy. We conclude that crystalline uricase is located within hepatic microbodies.*

The microbody is a characteristic inclusion in the cytoplasm of vertebrate liver parenchymal cells (*1*), hepatomas (*2*), renal tubular cells (*3*), and possibly other tissues as well. In most cases it may be described as slightly smaller than mitochondria, spherical or ellipsoidal in shape (in rat liver, about 0.2 to 0.6 μ in diameter), bounded by a single smooth membrane, and containing a moderately dense uniform matrix. Within the matrix area is a central region of higher density, often with a crystalloid structure.

The function of the microbody has long been a puzzle. Rouiller (*1*) suggested it was a stage in the formation of mitochondria. Novikoff (*4*) considered it to be lysosome-like in nature. More recently DeDuve (*5*) has pointed out, on the basis of gradient centrifugation of liver homogenates, that three enzymes show an unusual density distribution, being lighter than mitochondria and lysosomes, and heavier than the glycogen and microsomal fraction.

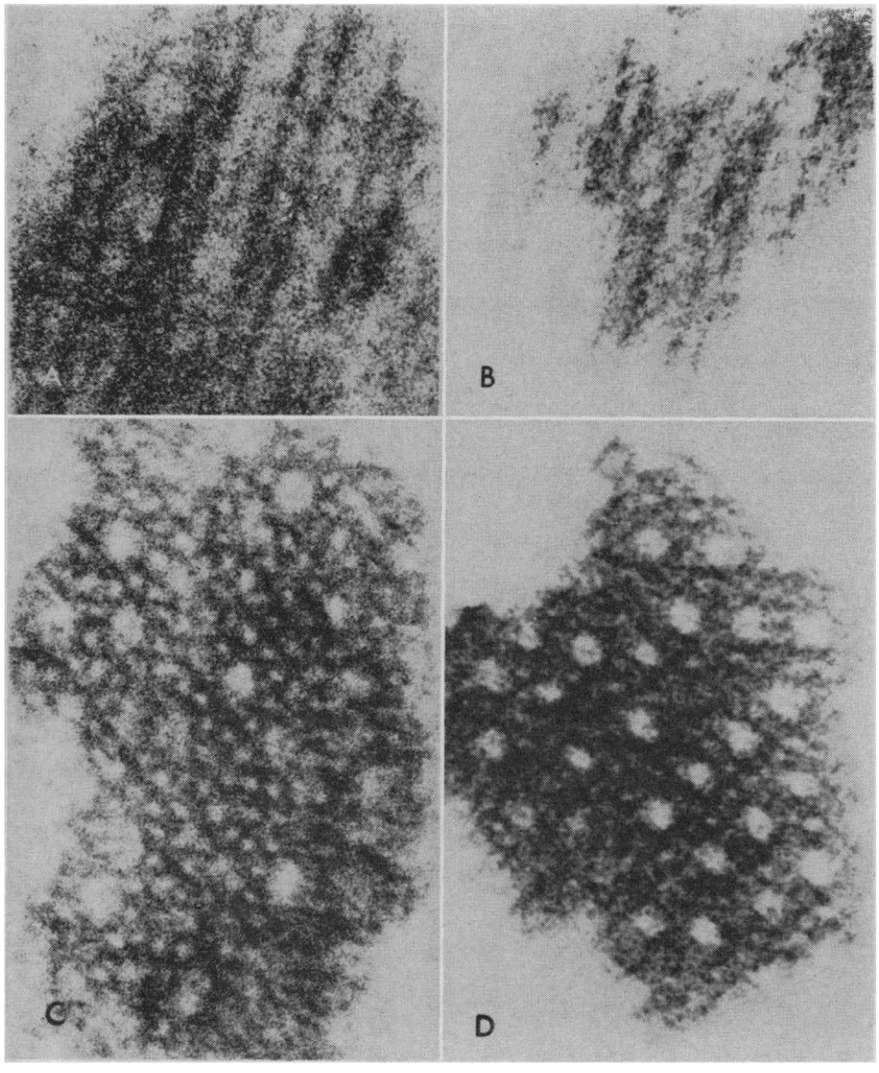

Fig. 1. Comparison of the fine structure of the crystalloid of microbodies and of crystalline uricase (Sigma). (× 430,000). *A*, Longitudinal section of a crystalloid from a cell of Reuber H-35 hepatoma (*14*). *B*, Longitudinal section through a crystal of uricase. *C*, Cross section through a crystalloid of a microbody from liver of rat treated with azaserine. Large electron lucid circles are surrounded in some areas by ten smaller circles. *D*, Crystal of uricase in cross section.

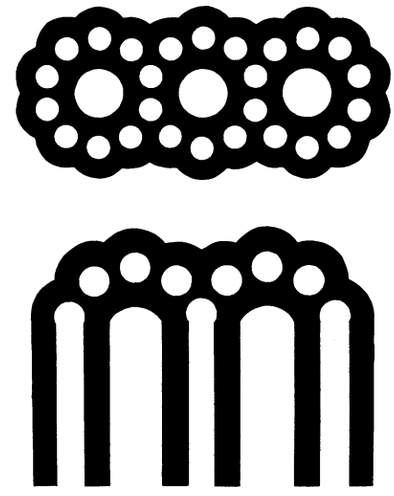

Fig. 2. Suggested structure for microbody crystalloid. The lower diagram represents a cross section at the top, and a longitudinal section at the bottom (upper diagram × 660,000; lower diagram × 880,000).

Beaufey has suggested that these enzymes, uricase, catalase, and D-amino acid oxidase may be localized in microbodies (*6*).

While studying the fine structure of rat hepatomas (*2*), we were impressed by the unusually large crystalloid in the microbodies of the Reuber H-35 tumor (*7*). Although qualitatively similar to the crystalloid in normal hepatocytes, its size and common occurrence facilitated structural studies. Along one plane of section, the crystalloid consisted of parallel dense lines about 45 Å wide. In most cases these were separated by less dense spaces of 45 Å. A characteristic feature, pointed out by Rouiller and Bernhard (*1*), was the presence of occasional wider spacings, approximately 90 to 115 Å (Fig. 1*A*). In cross section the crystalloid contained a pattern of less dense areas of two sizes, either 45 or 95 to 115 Å in diameter, arranged so that each larger area was surrounded by a ring of ten of the smaller regions (Fig. 1*C*). It is thus probable that the crystalloid has the structure shown in Fig. 2, with a bundle of cylinders of two different sizes. The pattern of the crystalloid in dog hepatocytes is identical with that in normal rat hepatocytes or in rat hepatoma cells, but the crystalloid from mouse hepatocytes is less regular.

The structure of this crystalloid seemed to possess enough unique features to make possible its physical identification. Consequently, we ob-

tained the purified enzymes suspected by DeDuve and Beaufey of being associated with microbodies. These were crystallized, fixed in 2 percent osmic acid, embedded in methacrylate, and sectioned for electron microscopy. Samples were also placed on grids and negatively stained with sodium phosphotungstate (*8*).

Particles of *d*-amino acid oxidase were needle-shaped and tactoid structures, as seen in negatively stained preparations. Similar structures have not been seen in microbodies. Negatively stained crystals of catalase, particularly when dissolving, showed regular particles, each about 65 Å in diameter and 45 Å thick, packed into rows, opposed along their longer dimension (Fig. 3). Particles of approximately these dimensions occur in the matrix of microbodies but certainly cannot be positively identified with the enzyme.

When crystals of uricase were fixed in OsO_4 (in saturated ammonium sulfate to keep them from dissolving), sectioned for the electron microscope, and stained with 2 percent $KMnO_4$ (*9*), they matched quite precisely the structure of the microbody crystalloid (Fig. 1, *B* and *D*). These structures tended to possess a greater degree of regularity than the microbody crystalloid, but showed cylinders of the same two dimensions and with the same relationship of ten small tubules surrounding one large tubule. The electron dense cylinders showed some indication of a particulate substructure of approximately 30 Å. There was a suggestion that the large cylinder may contain a central core. The similarity seems striking enough to justify the identification of the microbody crystalloid as consisting of uricase.

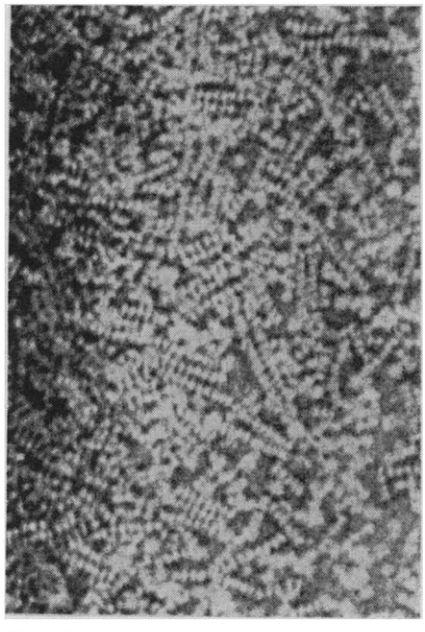

Fig. 3. Dissolving crystal of catalase, negatively stained with phosphotungstate (× 220,000).

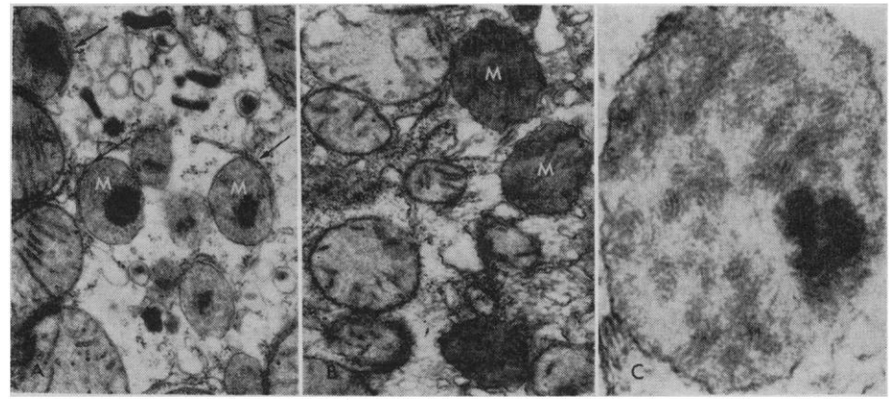

Fig. 4. *A*, Typical microbodies (*M*) from a section of rat hepatocyte. Arrows indicate close association with smooth membranes. (Structures appear typical for normal rats, although the animal was treated briefly with thioacetamide.) (× 15,500). *B*, Enlarged microbodies (*M*) from liver of a rat fed 1 percent acetylsalicylic acid for 12 days. Electron density of the matrix is increased (× 17,500). *C*, Enlarged microbody from a rat treated with acetylsalicylic acid. The matrix is filled with irregular, formed elements. The crystalloid is seen in cross section on the right (× 67,500).

Although this observation strongly supports the suggestion of Beaufey and DeDuve that microbodies contain uricase and, by inference, catalase and *d*-amino acid oxidase as well, the problems as to the origin and function of microbodies still remain. Microbodies are frequently closely associated with the endoplasmic reticulum (Fig. 4*A*). In some cases, the limiting microbody membrane appears continuous with short sequences of smooth membranes, which may have derived from the endoplasmic reticulum (*10*). Microbodies are often localized in the Golgi region (*11*). The functional significance of these associations, however, is not clear. Also of interest are the variations in microbody structure encountered in different hepatomas (*2*) or in normal liver after various treatments (*12*). Microbodies are, as mentioned above, usually large in the Reuber H-35 hepatoma, and are absent entirely from the Morris 3683 and Novikoff hepatomas (*2*). The Novikoff hepatoma is known to lack uricase (*13*). The crystalloid is enlarged in livers of rats treated with azaserine. In rat livers, after prolonged administration to the rat of tetracycline, azaserine, thioacetamide, and particularly acetylsalicylic acid, the matrix areas become filled with formed elements (Fig. 4, *B* and *C*). Such observations suggest that the microbody, like the zymogen granule of the pancreas, may represent the secretory granule of the liver, containing a collection of hepatic enzymes secreted in various patterns in response to altered physiological conditions. Whether these enzymes act within the cytoplasm of the hepatocyte or are secreted to act elsewhere is at present not clear.

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14. The samples of livers for electron microscopic studies were fixed in cold 1 percent OsO_4 in *s*-collidine buffer, *p*H 7.4, and embedded in methacrylate. The sections were stained by the Karnofsky lead method A. More intensive staining of the crystalloid is obtained by Lawn's $KMnO_4$ method.
15 Investigation was supported by PHS grants CA-05310 and CA-03544 and by an Advanced Clinical Fellowship to Z.H. from the American Cancer Society. Azaserine was supplied by Cancer Chemotherapy National Service Center. Hepatomas were supplied by Dr. M. Rechcigl.

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Catnip: Its Raison d'Être

Abstract. *Catnip (nepetalactone) is closely related chemically to certain cyclopentanoid monoterpenes recently isolated from insects, and it shares with some of these terpenes an ability to repel insects. It is suggested that the adaptive function of catnip is to protect the plants that produce it against phytophagous insects.*

Catnip (nepetalactone, Fig. 1, IV), a cyclopentanoid monoterpene produced by certain plants of the mint family, has always been the source of much interest because of its peculiar ability to excite cats and their feline relatives. The true adaptive significance of this substance, like that of so many others extracted for one purpose or another from plants, has remained a mystery. Surely, a mint plant derives no benefit from an ability to stimulate cats!

In recent years, certain compounds chemically allied to catnip have been isolated from insects (Fig. 1). Two of these (I, II) stem from ants (*1*); the other (III) is known from both ants (dolichodial) (*2*) and a walkingstick (anisomorphal) (*3*). There can be no doubt, at least as regards iridomyrmecin and anisomorphal, that these substances have a defensive role. Iridomyrmecin has considerable insecticidal activity (*4*), and anisomorphal, which is ejected by the walkingstick as a spray against predators, has been shown to be strongly repellent to ants, beetles, spiders, birds, and even ourselves (*5*). Also of interest is the fact that two cyclopentanoid monoterpenes (V, VI) other than nepetalactone, but both with catnip-like activity, have recently been identified from a Japanese plant; one of these (V) is identical to iridomyrmecin (see *6*).

The preceding data strongly suggest that catnip may itself be a defensive substance, perhaps protecting the plant against phytophagous insects. This possibility was investigated by a series of simple experiments (*7*).

One of these consisted in observing the response of a variety of insects to the vapors emanating from the tip of a fine capillary tube filled with pure liquid nepetalactone, and pointed to their bodies from a few millimeters away. The insects tested (Table 1) were a mixed assortment that had come to rest at night on an illuminated surface. The majority (part *A*) showed a distinct avoidance response, which varied somewhat with the particular species. The caddis-flies flew away. The alleculid beetles fell to the ground (as do many beetles when disturbed). The remainder simply turned away from the capillary and walked off. As they escaped, complete control could be exercised over the course of their locomotion by maintaining the capillary at close range, pointed at them from various directions; their every move was in distinct avoidance of the vapors. Capillaries filled with water had no comparable effect.

Only relatively few species remained undisturbed by catnip (part *B*). In the pentatomids and reduviids, this indifference might be taken to reflect an insensitivity to catnip, since these same insects showed strong avoidance of two other compounds that were tested (methacrylic acid, *p*-benzoquinone) (*8*). The chironomid, as well as the pyralid and arctiid moths, remained undisturbed even by these substances, indicating perhaps that under the conditions of the experiment (that is, while the insects are illuminated and at rest) they are generally unresponsive to noxious vapors.

Insects that responded to the vapors of nepetalactone also reacted characteristically to direct contact with the liquid. When mouthparts or antennae were touched with the wet tip of the capillary, they fled instantly and quickly, pausing occasionally to cleanse antennae with their front legs, or to wipe mouthparts against the substrate.

Additional tests were made with ants foraging along trails. The results were similar for the two species tested (*Solenopsis germinata, Monomorium pharaonis*). When a droplet of nepetalactone was placed on the trail, the ants stopped abruptly, building up in numbers around the droplet, but with not a single one venturing to within about 5 centimeters from it. When a circle was drawn around a group of ants with a glass rod dipped in nepetalactone, the ants similarly refused to cross the "line" and remained temporarily trapped within the circle. An ant (*S. germinata*) that was carrying a small live curculionid beetle, dropped its prey instantly when a droplet of nepetalactone was applied to the beetle, and began intensive cleansing activities. Other workers that made casual contact with this ant were themselves induced to cleanse.

Figure 2 illustrates yet another experiment, done with the ant *Pogonomyrmex badius*, an especially aggressive species. Two freshly killed cockroaches, one with a droplet of nepetalactone on its abdomen, the other an untreated control, were placed near the

Table 1. Species of insects exposed to vapors of nepetalactone. Families are numbered.

A. Repelled by catnip

1. Fulgoridae
   Acanalonia sp.
2. Cercopidae
   Monecophora bicincta
3. Formicidae
   Camponotus floridanus (winged queen)
4. Leptoceridae
   Leptocella sp.
   Oecetis inconspicua
5. Dytiscidae
   Coptotomus interrogatus
6. Lampyridae
   Photinus sp.
7. Cicindelidae
   Cicindela trifasciata
8. Staphylinidae
   Two unidentified small species
9. Scarabaeidae
   Ataenius sp.
10. Chrysomelidae
    Disonycha conjugata
11. Tenebrionidae
    Leichenum canaliculatum
12. Alleculidae
    Hymenorus sp.
13. Curculionidae
    Derelomus sp.
    Conotrachelus sp.
    An unidentified small species

B. Indifferent to catnip

14. Pentatomidae
    Thyanta sp.
    Euschistus sp.
15. Reduviidae
    Oncocephalus geniculatus
    Pnirontis sp.
16. Pyralidae
    Paraponyx allionealis
17. Arctiidae
    Afrida ytadodes
18. Chironomidae
    Chironomus sp.