THE OCCURRENCE AND STRUCTURE OF MICROBODIES

A Comparative Study

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ABSTRACT

Livers from chickens, rats, mongrel dogs, Dalmatian dogs, and man have been examined in the electron microscope in order to compare the microbodies with the known content of uricase. It is concluded that microbodies *with inclusion* are present in rats, mongrel dogs, and, although the inclusion generally is smaller, in Dalmatian dogs. The inclusion has a characteristic structural appearance. These species (rat, dog) have uricase. Chickens and man lack both the enzyme uricase and the microbody inclusion. This evidence and that from previously published electron micrographs in the literature on microbodies support the notion of a positive correlation between uricase and microbodies with an inclusion. It is recommended that the term "uricosome" be used for such microbodies that have an inclusion of the appearance here described.

Microbodies are usually defined as cytoplasmic particles surrounded by a single membrane and having a size of 0.2 to 0.6 μ. The term was introduced by Rhodin to denote certain particles in the tubule cells of the mouse kidney (1). According to Novikoff "the particles are best identified by the presence of an inner lamellated (crystalline?) body" (2). Such an inner body is not always present, however, and is absent from the material presented by Rhodin. Recently it has been shown that microbodies in the rat kidney have a crystalline inclusion, whereas those in mouse kidney do not (3). Microbodies with or without inclusions have been described from mammalian liver, hepatoma, and kidney.

A suggestion as to their function comes from the work by de Duve and coworkers (4) who found that, during density centrifugation, uricase, catalase, and D-amino acid oxidase distribute themselves differently from both the lysosomal and the mitochondrial enzymes, and might thus belong to a separate class of cytoplasmic particles. The three enzymes do not become identically distributed, however; according to de Duve, this means that they "may either belong to separate particles showing very similar properties, or they are associated together in varying proportions with a single group of particles" (4). In subsequent electron microscope investigations, Novikoff (2) and Baudhuin and Beaufay (5) came to the conclusion that the particles containing "uricase and related activities" are the microbodies.

This hypothesis may be tested by comparing livers from animals in which uricase is known to be present with those from animals in which uricase is absent with respect to the presence or absence of microbodies. The distribution of uricase is relatively well known among vertebrates and can be correlated, in turn, to both the phylogenetic relationships and the metabolic needs of the vertebrate classes. For a recent account of these interesting correlations the reader is referred to Baldwin's treatise (6).

Uricase is known from fishes, amphibians, and

most mam.mals Uricotelic vertebrates (birds and most reptiles), on the other hand, have uric acid as the principal end product in nitrogen metabolism and lack uricase. Man and higher apes similarly lack uricase and may excrete milligram amounts of uric acid daily. Uric acid is also excreted by the Dalmatian dog but not by other dog breeds examined (7). The liver of the Dalmatian dog is, however, reported to contain uricase, as does that of other dogs (8). There might be a quantitative difference, since uric acid injected into the bloodstream of the Dalmatian dog breaks down to allantoin less readily and completely than in the non-Dalmatian. The presence of uric acid in the urine of the Dalmatian dog is due to an abnormally low renal threshold for this substance rather than to a lack of catabolic pathways for it (9).

This investigation is a comparative study testing the hypothesis that microbodies might contain uricase.

MATERIAL AND METHODS

Liver fine structure was examined in the following species: young Leghorn chickens, young Wistar rats, young mongrel dogs, 5-month old Dalmatian dogs, and human adults.

Small pieces of liver were taken during operations (dogs, men), or after sacrificing the animals (rats, chickens). Samples were fixed in 1 per cent osmium tetroxide in phosphate buffer (10), or in 3 per cent glutaraldehyde in cacodylate buffer (11), followed by a buffer wash and an osmium tetroxide postfixation. Dehydration and Epon embedding were performed as prescribed by Luft (12). Thin sections were cut with an LKB Ultrotome and examined in a Siemens Elmiskop I. In some cases the sections were stained with lead citrate (13).

RESULTS

CHICKEN: Particles having the appropriate size of a microbody and a single limiting membrane are found in liver cells of chicken but they invariably lack the dense inclusion. These particles are most often found in close apposition to the Golgi apparatus. They appear quite infrequently.

MAN: No microbodies with inclusions can be found in human liver cells. The microbodies are of the type shown in Fig. 1. They are homogeneous bodies ranging in size from about 0.3 to 0.7 μ. They occur quite frequently in the cytoplasm of the parenchymatous cells and are evidently without preferential orientation within the cytoplasm.

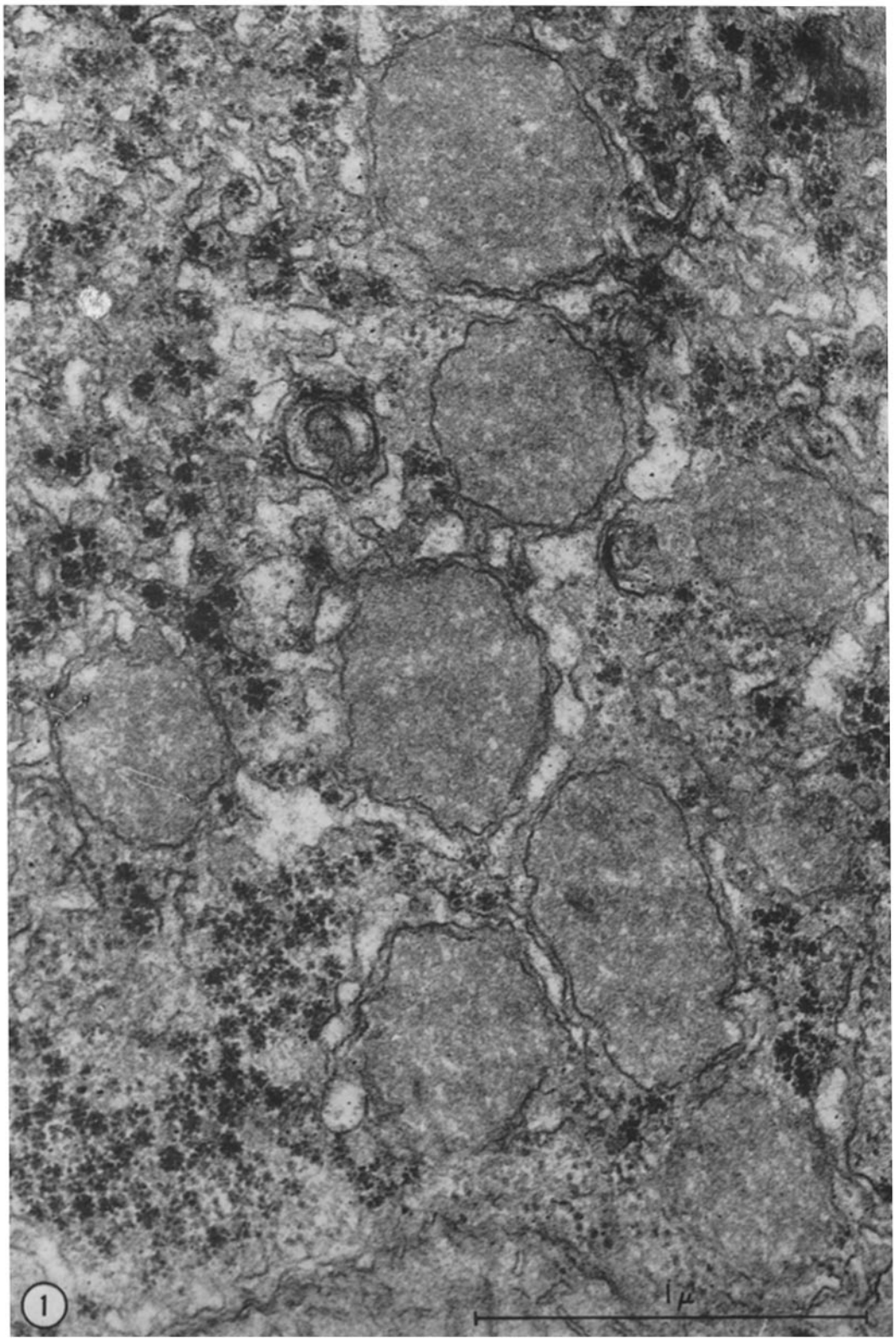
RAT: Typical microbodies with inclusions are common in rat liver cells. Depending on the orientation within the section, the inclusion may appear rounded or rectangular or it may not be visible. In cases where an inclusion is not visible, it is of course impossible to know whether the inclusion is really absent or simply outside the plane of the section. From a consideration of probability, it appears that a certain proportion of the sectioned microbodies could lack the inclusion.

Inclusions with a rectangular outline have striations running parallel with the longer axis. Inclusions with a rounded contour sometimes show several ring-shaped profiles each with a diameter of 100 to 130 A. Evidently these two views represent different planes of sectioning through a formation made up of parallel cylinders. The fine structure of each individual cylinder can sometimes be seen as a ring of still smaller cylinders (about 40 A). Although the inclusion may occupy a large portion of the microbody and have the appearance of a straight rod, it has never been found to deform the outline of the organelle.

DALMATIAN DOG: Microbodies are present in the liver cells of the Dalmatian dog (Figs. 2 and 3). In Fig. 2 the "cylinders" of the inclusion can be seen both in an end-on view (microbody at upper left) and cut lengthwise. The inclusion within the microbodies is usually smaller than that found in microbodies in livers from rats or other dogs.

MONGREL DOG: With respect to both their ultrastructure and their distribution in the cytoplasm, the microbodies in mongrel dog liver resemble the microbodies in rat liver. In Fig. 4 a microbody with a cross-cut inclusion is seen. The subunits of the walls of the cylinder are discernible. Since there is also such a high proportion of

FIGURE 1 A portion of the cytoplasm of a human hepatocyte. In this field several microbodies can be seen, each of which is characterized by a homogeneous interior and a simple limiting membrane. The microbodies have no crystalline inclusion. The ground cytoplasm outside the microbodies contains glycogen, ribosomes, and ferritin particles. In the glycogen areas the agranular endoplasmic reticulum is abundant. Lead citrate staining. × 60,000.


1
1 μ

particles without any sign of an inclusion, one is lead to the conclusion that the mongrel dog liver has many inclusion-free microbodies as well. In this respect there seems to be a variation from one cell to another.

DISCUSSION

The outcome of this investigation reveals that in the species examined there is a positive correlation between uricase and microbodies *with an inclusion.* The suggestion that microbodies carry uricase and related activities can thus be supported only if the addition "with inclusion" is made. On these grounds it is felt that there is a distinction between microbodies with and without inclusion. It is thus recommended that the term "uricosome" be used for such microbodies which have an inclusion resembling that described under Results. The term "microbody" might be retained for any single-membrane-limited particle with a size of 0.1 to 1.5 μ (commonly 0.3 to 0.6 μ) and a matrix substance which is homogeneous or contains an inclusion.

The study supports the suggestion of Novikoff (2) and Baudhuin and Beaufay (5) that microbodies contain uricase, whereas some older hypotheses regarding the functional significance of the microbodies (14, 15) remain unsupported.

It is to be noted that not every sectioned uricosome will reveal the inclusion. Some particles might have the inclusion outside the plane of the section. It is thus necessary to have a field of a dozen of these particles in order to be able to tell whether a cell contains uricosomes. The criteria for definition of a uricosome are strict enough to be of diagnostic value. The definition of a microbody is so unspecific that any of a large number of secretory granules or other cell inclusions might fit it.

In order to make the material of comparison broader, the present author has scanned the electron microscopy literature with respect to the structure of the microbody in different species. Such publications, dealing with the human liver, have exceeded one hundred in number during the last five years (15–120). Quite consistently, the illustrations show the presence of homogeneous microbodies rather than of uricosomes (24, 28, 35, 37, 41, 45, 47, 58, 63, 65, 75, 76, 79, 92, 94, 95, 97, 107, 111, 118). In only a few cases (47, 76, 112) is it also mentioned in the text that the microbodies appear homogeneous, but this feature has not been previously correlated with the known lack of uricase in the human liver. Higher apes have not been studied in this respect, and the electron micrographs obtained from three monkey species are not informative on this point (121, 122). The livers of birds (45, 123–132), of lizards (133, 134), and of snakes (45) also contain no uricosomes but sometimes show bodies with a homogeneous appearance.

The livers of dogs (45, 103, 110, 135–139), like those of other mammalian species (excepting man and possibly the Syrian hamster) (David's review, reference 45, is particularly useful), have microbodies with an appearance like that in rat liver.

When the observations in this paper had been completed, two reports appeared in the literature, one by Hruban (140) and the other by Hruban and Swift (138), which showed that crystalline uricase has the same morphological characteristics as the microbody inclusion. These authors conclude that the inclusion consists of a crystal of this enzyme. This feature would in itself be of great biochemical interest. It is difficult to understand the advantage of compacting the enzyme in an organelle into a crystalline state, unless it is done for the purpose of storage and export from the cell. It is of interest, in this context, that Schneider and Hogeboom (141) demonstrated that the activity of uricase in cellular fractions is independent of treatments which disrupt the membranes.

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FIGURE 2 A region of a liver cell from a Dalmatian dog. In this cell the inclusion, which characterizes the microbody, is prominent in many of these organelles (arrows), but it is less evident in others, or may be outside the plane of the section. It may well be that some of the bodies lack the inclusion. The microbody in the upper left corner has the inclusion cross-cut. Lead citrate staining. × 48,000.

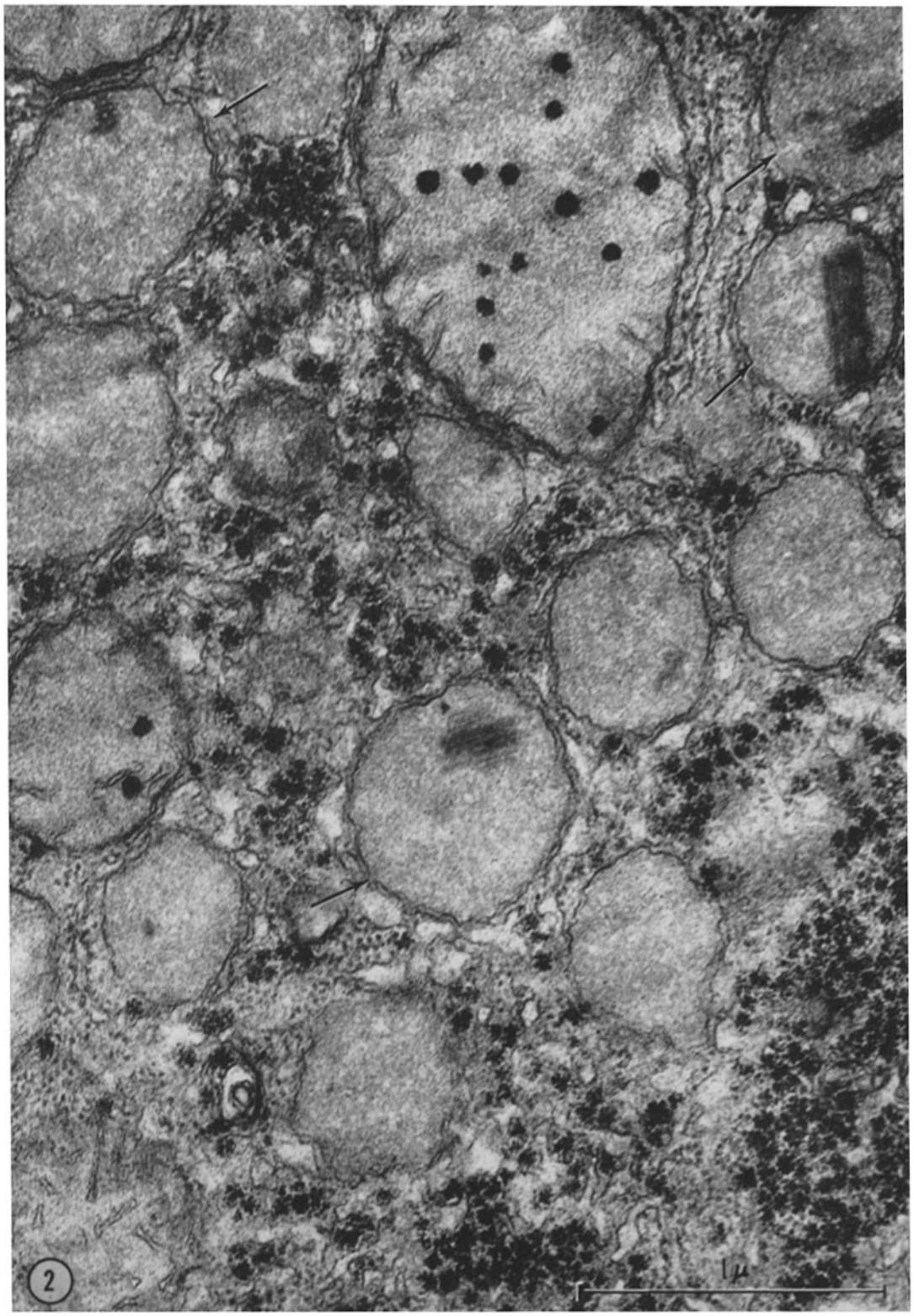
2
1 μ

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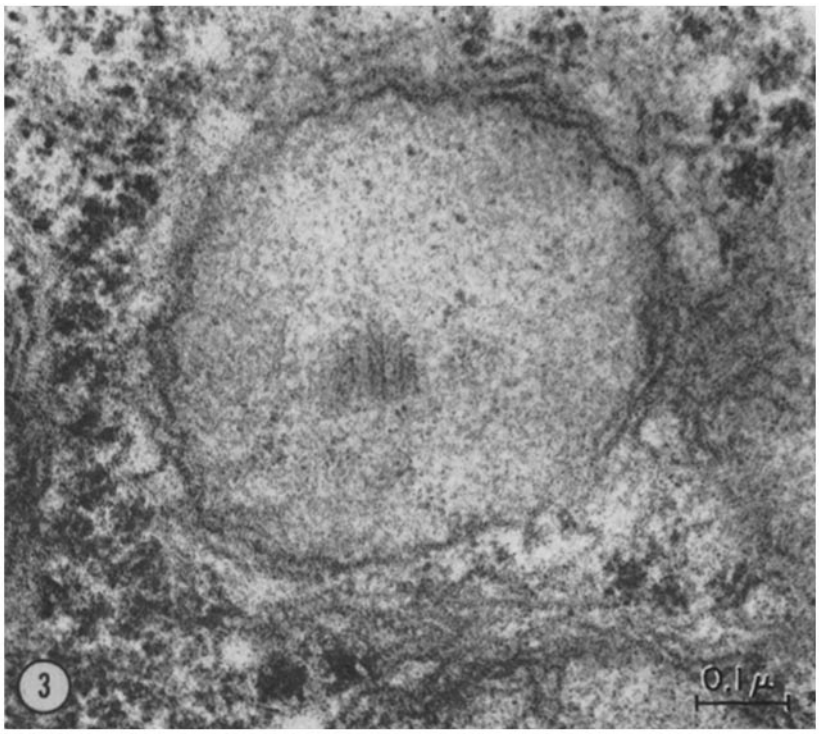
FIGURE 3 A microbody from a Dalmatian dog liver, shown at a higher magnification. The section passes lengthwise through the tubules and gives the appearance of striation. Lead citrate staining. × 120,000.

FIGURE 4 A microbody from a mongrel dog liver, showing the inclusion cross-cut. This particular inclusion appears as four cylinders. In the wall of each cylinder some ten subunits are discerned. × 90,000.


3
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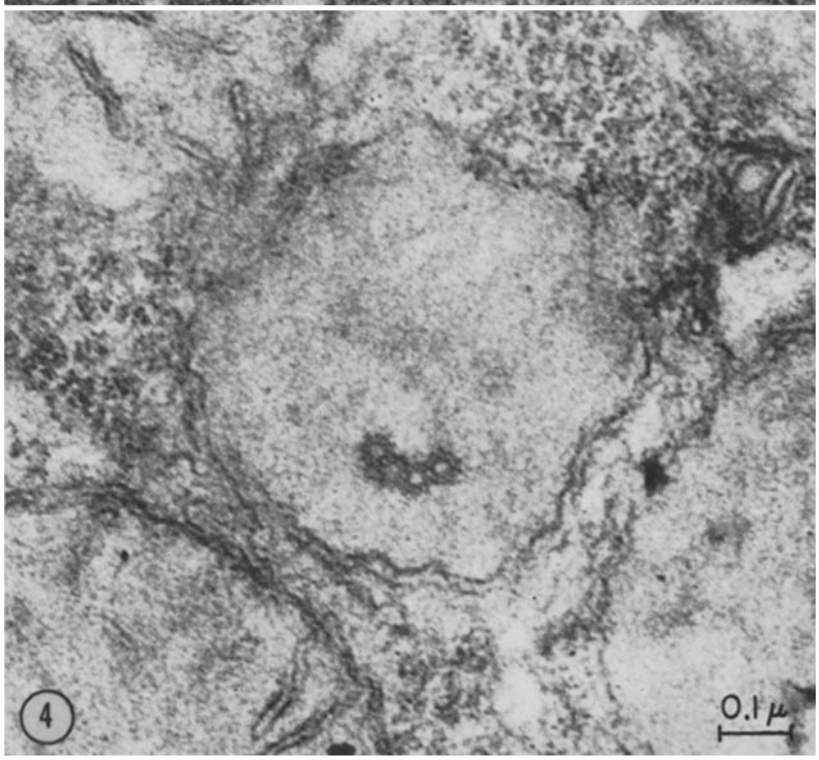
4
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