Proliferation of Microbodies and Synthesis of Catalase in Rat Liver

Induction in Tumor-Bearing Host by CPIB

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STUDIES ON THE SYSTEMIC EFFECTS of spontaneous, induced or transplanted neoplasms on the tumor-bearing host have yielded significant information concerning the biologic and functional adaptation of host organs.[1] Of particular interest is the finding that the level of catalase activity in the liver and kidney is reduced significantly in tumor-bearing animals,[1-5] in which a clear-cut relationship between the size of tumor and extent of depression of catalase appears to exist. Extirpation of tumor is known to result in a prompt recovery of the enzyme activity to normal levels.[2] Depression of catalase in tumor-bearing hosts does not appear to be limited to laboratory animals, however, since reduction in the enzyme activity of catalase may also occur with advanced malignant tumors in man.[6] Further, it is to be emphasized that the depression of catalase activity in liver and kidney should not be considered to exist exclusively in tumor-bearing hosts since other factors such as radiation [7] administration of homogenates of certain tissues [8] and injection of several chemicals [4,5,8,9] have been shown to affect catalase activity in nontumor-bearing animals.

The phenomenon of the depression of catalase in tumor-bearing animals and in man has been the subject of several investigations.[4,5] The precise mechanism by which a tumor distant from these organs depresses their catalase activity is not understood although it is conceded that nutritional, metabolic, toxic, humoral or other factors, singly or in combination, may exert their influence in lowering catalase activity.[4,5] It is of considerable interest to recall the studies of Nakahara and Fukuoka,[10] who isolated a substance from tumors that inhibits catalase and designated it as toxohormone.[11] In spite of extensive studies dealing with its purification, characterization and biologic effects, a great deal of controversy persists regarding the specific role of toxohormone in the

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depression of catalase activity in tumor-bearing animals.[4,11,12] Regardless of the underlying mechanism of depression of catalase activity in liver and kidney, the tumor-bearing animal appears to be a useful biologic model in which to study the effect of various enzyme inducers, particularly those that induce the synthesis of catalase.

Previous studies [13–15] have demonstrated that administering CPIB (ethyl-α-*p*-chlorophenoxyisobutyrate, clofibrate) to male rats results in a significant increase in the number of hepatic microbodies associated with a significant rise in liver catalase activity,[14] resulting from enhanced synthesis of catalase enzyme protein.[16] The effect of this drug on the induction of catalase in liver, therefore, can be assessed reliably by morphologic as well as biochemical studies. Since there is a marked depression in the activity of catalase in liver and kidney of tumor-bearing animals, presumably due to decreased synthesis affected by an unknown mechanism(s), it is of significance to study the inducibility by CPIB of catalase and of microbodies in the livers of tumor-bearing rats.

In this paper, we report the results of administering CPIB on the proliferation of hepatic microbodies and levels of catalase in male rats bearing subcutaneously transplanted tumors. These studies demonstrate that, in tumor-bearing animals (1) the content and activity of catalase enzyme protein in liver is reduced significantly, (2) administering CPIB causes a significant increase in the number of microbodies in hepatic parenchymal cells and (3) CPIB also significantly elevates the content and activity of catalase protein in the liver. Since catalase enzyme and proliferation of microbodies are readily induced in the liver of tumor-bearing animals by CPIB, the role, if any, of toxohormone and of other factors in the depression of catalase activity requires reevaluation.

Materials and Methods

Animals and Diet. Inbred F-344 rats obtained from A. R. Schmidt Co, Madison Wisconsin, were used in these experiments. Male rats were used exclusively in these experiments since CPIB-induced proliferation of microbodies and synthesis of catalase were shown to be related to testosterone in our previous studies.[15] CPIB in a concentration of 0.25% was administered in ground Purina Chow.[13,14]

Tumor Transplants. Weanling male rats weighing between 40–60 g each were used for tumor transplantation. In our laboratory, two lines of transplantable hepatomas [17,18] (aflatoxin-induced hepatic tumor in 38th transfer and ethionine-induced hepatic tumor in 40th transfer) and one of mesothelioma [19] (actinomycin-induced tumor in 29th transfer) are available. The tumors were transplanted subcutaneously into the left groin (Table 1). When the tumors reached the desired size, generally within 4–8 weeks, CPIB was started and the animals killed 4 weeks later.

Morphologic Studies. Small pieces of liver from animals with subcutaneously transplanted tumors were fixed for 1–2 hours in 2% osmium tetroxide, and then were processed for electron microscopy.

For cytochemical demonstration of peroxidase activity, samples of liver were fixed in 3% glutaraldehyde, buffered with 0.1 M sodium cacodylate (pH 7.4) [20] for

Table 1. Catalase Activity in Male F-344 Rats Bearing Transplanted Tumors and Treated with CPIB*

Treatment	No. of animals	Age of tumor (weeks)†	Catalase activity (units/mg protein) (mean ± SE)
Normal rats			
Control	5	—	40 ± 2.3
CPIB-treated	6	—	85 ± 4.2
Transplanted tumors (subcutaneous)			
Ethionine hepatoma	5	8	19 ± 4.7
Ethionine hepatoma + CPIB	8	10	63 ± 6.1
Aflatoxin hepatoma	5	9	16 ± 3.9
Aflatoxin hepatoma + CPIB	4	12	59 ± 4.1
Actinomycin tumor (mesothelioma)	4	13	13 ± 5.1
Actinomycin tumor + CPIB	6	14	70 ± 5.6

*CPIB was administered in diet for 4 weeks before sacrifice.
†Age of tumor at sacrifice.

4 hours at 4 C. After the tissues were fixed, they were rinsed for 16 hours in 0.1 M cacodylate buffer (pH 7.4) containing 0.22 M sucrose. The tissues were incubated for peroxidase activity at pH 9.0, in the diaminobenzidine (Sigma Chemical Co, St Louis, Missouri) reaction medium of Novikoff and Goldfischer,[21] modified from Graham and Karnovsky.[22]

Biochemical Studies. Catalase activity was determined by the spectrophotometric method described by Luck.[23] Total proteins were determined by the method of Lowry *et al.*[24]

Immunochemical Procedures. Catalase from rat liver was purified according to the method of Price *et al* [25] and anti-catalase antibody was prepared by injecting, into the footpads of rabbits, 12 mg of catalase from rat liver emulsified in an equal volume of complete Freund's adjuvant (Difco). Quantitating catalase protein in livers of normal rats and rats bearing subcutaneous transplants of ethionine-induced hepatoma with and without CPIB treatment was carried out by the immunoprecipitation method outlined by Ganschow and Schimke,[26] using deoxycholate-treated 5% liver homogenates centrifuged at 105,000 *g* for 60 minutes in a Spinco model L ultracentrifuge.

Results

General. The tumors generally reached the desired size between 8–12 weeks after they were transplanted. The weight of the liver in tumor-bearing animals given CPIB for 4 weeks was increased.

Electron Microscopy. Dilatation of the cisternae of endoplasmic reticulum with focal disaggregation of polyribosomes, depletion of glycogen and a moderate increase in the number of lysosomes were the prominent changes in hepatic parenchymal cells of rats bearing subcutaneously transplanted tumors. Although the number of microbodies in the livers of tumor-bearing control rats appeared unchanged, the density of the microbody matrix, the structure of the nucleoid and the

limiting membrane were altered conspicuously. The density of the matrical proteins of microbodies varied significantly. Some of the microbodies appeared to contain a residual nucleoid within an irregular, at times partially dissolved, limiting membrane.

The number of microbodies in liver cells of animals bearing subcutaneously transplanted tumors increased significantly after CPIB was administered. The size or the type of the transplanted tumor or the nutritional state of the tumor-bearing host did not appear to influence the proliferative response of microbodies in host liver after CPIB. Cytochemical studies for endogenous peroxidase activity revealed a significant increase in the number of organelles with positive peroxidase activity in the livers of tumor-bearing animals given CPIB (Fig 1), compared with tumor-bearing controls (Fig 2).

Catalase Activity. The catalase activity in the livers of rats bearing transplanted tumors was significantly lower than that of normal rats (Table 1). The depression of catalase activity in livers was evident with all three transplanted tumors. When CPIB was administered to these animals, the catalase activity of the liver increased considerably; the increase appeared to parallel closely the increase in the number of hepatic microbodies.

Quantity of Catalase Protein. The content of catalase protein in livers of rats bearing ethionine-induced transplantable hepatoma and the effect of CPIB on the catalase protein content in these animals was determined by the immunoprecipitation method.[26] It was found that approximately 0.06 ml of anti-catalase serum precipitated completely the catalase activity from 1 ml of 5% liver extract from a tumor-bearing rat, whereas 0.17 ml of anti-catalase serum was needed to precipitate the catalase activity from the same amount of liver extract from a tumor-bearing rat treated with CPIB. Further, to precipitate the catalase activity from 1 ml of 5% liver extract from normal rats, approximately 0.1 ml of antiserum was needed, whereas 0.21 ml of antiserum was needed to precipitate completely the catalase activity from 1 ml of extract from normal rats in CPIB steady state. Thus, it is evident from these experiments that the content of catalase protein in the liver extracts of normal rats and of rats bearing ethionine hepatoma transplants increased significantly when these animals were given CPIB.

Discussion

Despite numerous studies establishing that the catalase activity of liver and kidney is depressed in tumor-bearing animals, much uncertainty prevails at present about the mechanism and significance of

catalase depression in these animals.[4,5] Toxohormone, poor nutritional status (including lack of specific amino acids or proteins), hormonal imbalance, infection and a wide variety of other factors are mentioned frequently as being responsible for the depression of catalase activity in tumor-bearing animals.[4,5] It is known, however, that the depression of catalase activity of a tumor-bearing host is not a consequence of enzyme inhibition or structural change in the catalase molecule, but is believed to be due to reduction in the rate of synthesis of this enzyme in these animals.[4,27] In addition to depression of catalase activity, other microbody enzymes such as D-amino acid oxidase and urate oxidase are also reported to be reduced in the liver and kidney of tumor-bearing animals.[4,28] Ultrastructural studies of liver in tumor-bearing animals [29] indicate an appreciable increase in the number of hepatocellular lysosomes and a questionable reduction in microbodies but these morphologic changes do not account sufficiently for the reduction in catalase activity in the liver of these animals.

It was shown in our previous studies that CPIB is a very effective inducer of hepatic synthesis of catalase associated with microbody proliferation in male rats.[14,15] In the present studies, proliferation of microbodies was noted in the liver cells of rats bearing subcutaneously transplanted hepatomas. Proliferation of microbodies was also evident in the liver cells of uninvolved liver parenchyma in rats bearing intrahepatic primary tumors, but not in the tumor cells.[30] Hepatic catalase activity in rats bearing subcutaneously transplanted tumors showed a significant increase after treatment with CPIB. The content of catalase protein in the liver, as estimated by the immunoprecipitation method was very low in rats bearing large transplanted tumors (ethionine-induced hepatoma). When CPIB was given to these tumor-bearing animals, the content of catalase protein increased approximately threefold. These results indicate that, despite low levels of catalase enzyme in the liver of a tumor-bearing host, which is presumably due to tumor-related inhibition, this enzyme can be induced effectively in the liver of these animals by administering CPIB.

The mechanism of the action of CPIB in initiating the proliferation of microbodies and the synthesis of catalase in male rat liver is not understood.[31,32] It has been shown, however, that the elevation of catalase activity and of the content of catalase protein in the rat liver is due to an increased rate of synthesis and not to alterations in the rate of its degradation.[16] Accordingly, CPIB appears to be useful as an inducer of catalase and of microbodies in male rats and therefore, the inducibility of this enzyme can be tested adequately in a wide variety of experi-

mental conditions.[14,31,32] However, it is to be noted that we have previously demonstrated that CPIB-induced microbody proliferation can occur in the absence of significant synthesis of catalase in male rat liver.[32] Accordingly, the proliferation of microbodies observed on routine electron microscopic examination after administering CPIB may not indicate enhanced catalase activity under certain experimental conditions. Therefore, to ascertain the increase in catalase activity when proliferation of microbodies is obvious, catalase activity must be determined biochemically at the same time. Alternatively, cytochemical demonstration of peroxidase activity in these microbodies might indicate reasonably accurately the presence of catalase.[33,34] Since biochemical and cytochemical studies in the present investigation demonstrated an unequivocal increase of the catalase activity and peroxidase-positive cytoplasmic organelles in the liver of tumor-bearing animals after CPIB was administered, the possibility that proliferation of microbodies occurred in the absence of catalase synthesis can be ruled out. It is significant, therefore, that proliferation of microbodies and an increase in the content and activity of hepatic catalase enzyme occurs after CPIB is administered in tumor-bearing animals despite the initial low level of activity. Accordingly, the possibility that tumor metabolites and nutritional factors play a role in depressing liver catalase activity in the liver of a tumor-bearing animal appears doubtful. Preliminary studies [35] in this laboratory utilizing purified samples of toxohormone [12] indicated that this substance does not inhibit the CPIB-induced proliferation of microbodies or synthesis of catalase in male rat liver. However, it can be argued that CPIB somehow interferes with the production or action of toxohormone, thereby elevating catalase activity. Likewise, proliferation of microbodies was noted in the liver after CPIB was injected intraperitoneally into acutely starved male rats (starved for 5 days).[35] Therefore, the depression of catalase activity in the liver and kidney of tumor-bearing animals may be a reflection of adaptation of certain biological functions of the host.

Summary

Despite initially low levels of hepatic catalase activity in livers of tumor-bearing rats, proliferation of microbodies and an increase in the content and activity of hepatic catalase occurs in these animals after CPIB is administered.

References

1. Begg RW: Tumor-host relations. Advances Cancer Res 5:1–54, 1958
2. Greenstein JP, Andervont HB: The liver catalase activity of tumor-bearing

mice and the effect of spontaneous regression and of removal of certain tumors. J Nat Cancer Inst 2:345–355, 1942

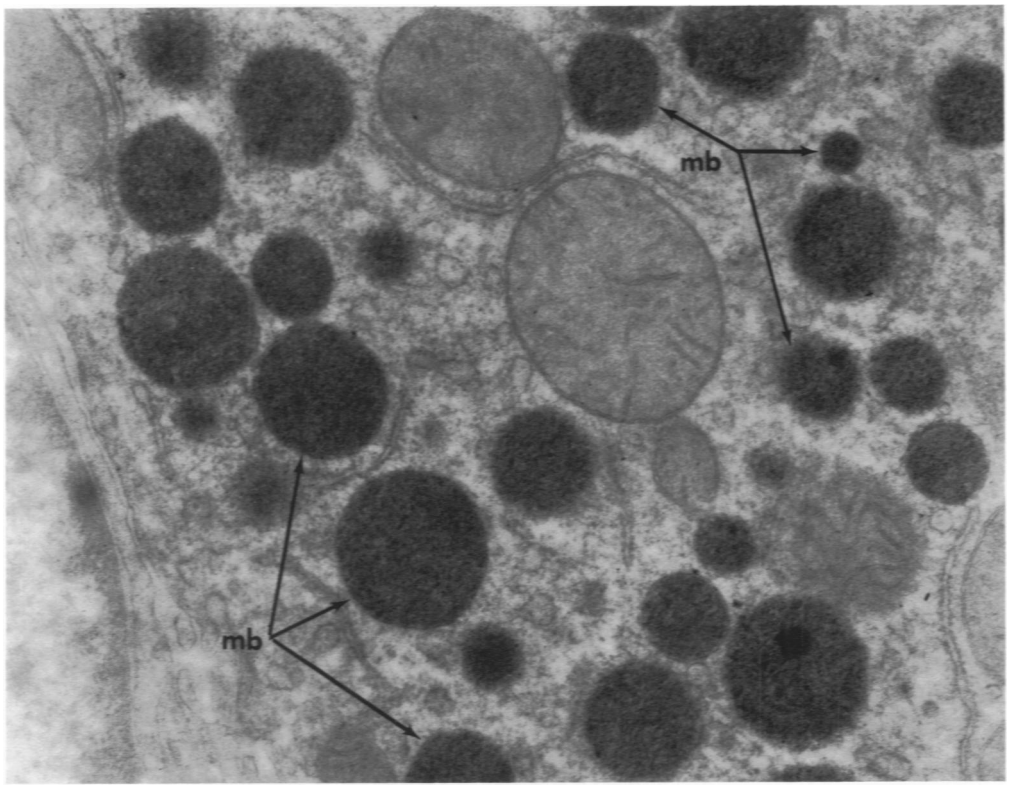
3. Greenstein JP, Andervont HB, Thompson JW: Kidney and blood catalase activity of tumor-bearing animals. J Nat Cancer Inst 2:589–594, 1942
4. Hruban Z, Rechcigl M Jr: Microbodies and related particles: morphology, biochemistry and physiology. Int Rev Cytol: Suppl I:173–178, 1969
5. Kampschmidt RF: Mechanism of liver catalase depression in tumor-bearing animals: a review. Cancer Res 25:34–45, 1965
6. Ohnuma T, Maldia G, Holland JF: Hepatic catalase activity in advanced human cancer. Cancer Res 26:1806–1818, 1966
7. Feinstein RN, Butler CL, Hendley DD: Effect of whole body X-radiation and of intraperitoneal hydrogen peroxide on mouse liver catalase. Science 111:149–150, 1950
8. Day ED, Gabrielson FC, Lipkind JB: Depressions in the activity of liver catalase in mice injected with homogenates of normal mouse spleen. J Nat Cancer Inst 15:239–252, 1954
9. Nishimura ET, Rosenheim S, Klein L: Depression of hepatic catalase in mice after subcutaneous injury. Lab Invest 12:415–421, 1963
10. Nakahara W, Fukuoka FA: A toxic cancer tissue constituent as evidenced by its effect on liver catalase activity. Japan Med J 1:271–277, 1948
11. Nakahara W: Toxohormone, Methods in Cancer Research. Vol 2. Edited by H Busch. New York, Academic Press, Inc, p 203
12. Hozumi M, Ohashi M, Ono T: Fractionation of toxohormone with calcium phosphate gel. Gann 56:313–315, 1965
13. Svoboda D, Azarnoff D: Response of hepatic microbodies to a hypolipidemic agent, ethyl chlorophenoxyisobutyrate (CPIB). J Cell Biol 30:442–450, 1966
14. Svoboda D, Grady H, Azarnoff D: Microbodies in experimentally altered cells. J Cell Biol 35:127–152, 1967
15. Svoboda D, Azarnoff D, Reddy J: Microbodies in experimentally altered cells. II. The relationship of microbody proliferation to endocrine glands. J Cell Biol 40:734–746, 1969
16. Reddy J, Chiga M, Bunyaratvej S, Svoboda D: The relationship of microbody proliferation to catalase synthesis. Amer J Path 59:83a, 1970, abstr
17. Svoboda D, Higginson J: A comparison of ultrastructural changes in rat liver due to chemical carcinogens. Cancer Res 28:1703–1733, 1968
18. Svoboda D, Grady H, Higginson J: Aflatoxin B_1 injury in rat and monkey liver. Amer J Path 49:1023–1051, 1966
19. Svoboda D, Reddy J, Harris C: Invasive tumors induced in rats with actinomycin D. Cancer Res 30:2271–2279, 1970
20. Sabatini DD, Bensch K, Barrnett RJ: The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol 17:19–58, 1963
21. Novikoff AB, Goldfischer S: Visualization of microbodies for light and electron microscopy. J Histochem Cytochem 16:507, 1968
22. Graham RC, Karnovsky MJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302,
23. Lück H: Catalase, Methods of Enzymatic Analysis. Edited by H Bergmeyer. New York, Academic Press, Inc, 1963, p 885

24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951
25. Price VE, Sterling WR, Tarantola VA, Hartley RW Jr, Rechcigl M Jr: The kinetics of catalase synthesis and destruction *in vivo*. J Biol Chem 237:3468–3475, 1962
26. Ganschow RE, Schimke RT: Independent genetic control of the catalytic activity and the rate of degradation of catalase in mice. J Biol Chem 244: 4649–4658, 1969
27. Nishimura ET, Kobara TY, Kaltenbach JP, Wartman WB: Immunological evidence of repressed catalase synthesis in livers of tumor-bearing mice. Arch Biochem Biophys 97:589–595, 1962
28. Lan TH: The α-amino acid oxidase, uricase and choline oxidase in two transplanted rat tumors and in isolated nuclei of tumor cells. Cancer Res 4:37, 1944
29. Parry EW, Ghadially FN: Ultrastructure of the livers of rats bearing transplanted tumors. J Path Bact 93:295–299, 1967
30. Svoboda D: Unpublished observations, 1969
31. Reddy J, Bunyaratvej S, Svoboda D: Microbodies in experimentally altered cells. IV. Acatalasemic (Cs^b) mice treated with CPIB. J Cell Biol 42:587–596, 1969
32. Reddy J, Chiga M, Bunyaratvej S, Svoboda D: Microbodies in experimentally altered cells. VII. CPIB-induced hepatic microbody proliferation in the absence of significant catalase synthesis. J Cell Biol 44:226–234, 1970
33. DeDuve C, Baudhuin P: Peroxisomes (microbodies and related particles). Physiol Rev 46:323–357, 1966
34. Reddy J, Bunyaratvej S, Svoboda D: Microbodies in experimentally altered cells. V. Histochemical and cytochemical studies on the livers of rats and acatalasemic mice treated with CPIB. Amer J Path 56:351–370, 1969
35. Reddy J, Svoboda D: Unpublished observations, 1970

Legends for Figures

Fig 1.—Liver from rat bearing subcutaneously transplanted aflatoxin hepatoma, given CPIB, and stained for endogenous peroxidase activity. Numerous microbodies *(mb)* stain for peroxidase (not counterstained, × 30,000).

Fig 2.—Liver from control tumor-bearing host, stained for peroxidase. Number of peroxidase-positive organelles *(mb)* are few in comparison to that in CPIB-treated group (not counterstained, × 30,000).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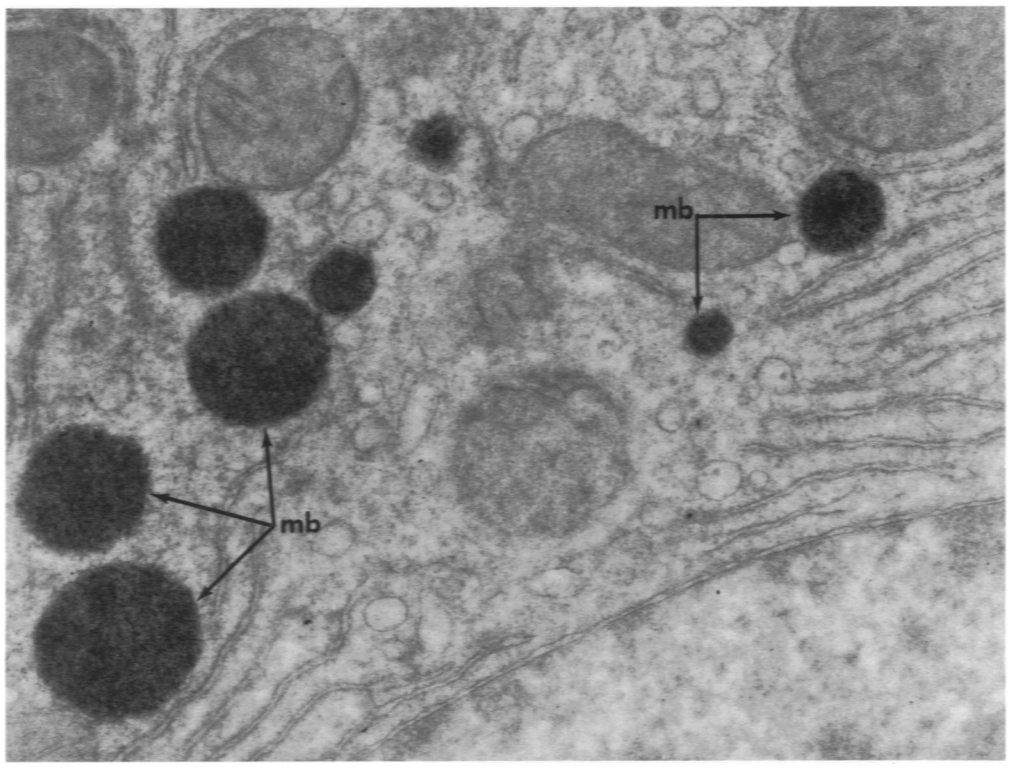
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