MORPHOGENESIS AND DEVELOPMENT OF MICROBODIES OF HEPATOCYTES OF RATS DURING PRE- AND POSTNATAL GROWTH

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ABSTRACT

In hepatocytes of fetal rats, cytoplasmic organelles identifiable as microbodies appeared, although only a few of them showed nucleoids and most of them generally had an electron-lucent appearance due to the low density of their matrices. Some of these microbodies, especially those lacking the nucleoid, showed a substantial connection with granular endoplasmic reticulum (ER), suggesting that microbodies might be formed from granular ER. Agranular tubular profiles projecting from the surface of microbodies were found with a high frequency in fetal and neonatal rats; however, this phenomenon may not provide crucial evidence suggestive of the derivation of microbodies from agranular ER. Growth and maturation of microbodies are considered to be brought about by an enlargement of these organelles, an increase in their matrices, an appearance and enlargement of the nucleoids, and an increase in the enzyme involved. The specific activity of urate oxidase in the isolated nucleoid fraction was significantly lower in the earlier stages of postnatal growth than later. Increases in the enzyme activity per nucleoid (maturation of the nucleoid), in the number of microbodies containing nucleoids (formation of the nucleoid), and in the size of nucleoids (growth of the nucleoid), may contribute to increases in the enzyme activity of the tissues.

INTRODUCTION

The independence of microbodies as a distinct class of cytoplasmic organelles in hepatocytes has almost been proven morphologically, and may well have certain substantiation biochemically. On the one hand, their electron microscopic features have been repeatedly reported in detail in a number of animal species, following the description in rat hepatocytes by Gansler and Rouiller (15) and Rouiller and Bernhard (28), and on the other hand, their enzymatic nature has been asserted by Baudhuin et al. (3, 4), de Duve and Baudhuin (8), and their coworkers who described them as containing urate oxidase, D-amino acid oxidase, alpha-hydroxy acid oxidase, and catalase. Among these enzymes, urate oxidase is considered, as suggested by Afzelius (1), Baudhuin et al. (3), Hruban et al. (18, 19), and Shnitka (30), to be associated with the nucleoids within microbodies. Further, this finding was confirmed in our laboratory from observations on isolated nucleoids (14, 33, 34).

However, several divergent hypotheses have been proposed to explain the morphogenesis of microbodies and the transformation of microbodies into other species of cytoplasmic organelles. Yet, as regards the morphogenesis and development of these organelles in hepatocytes of growing animals, only a few reports are available which are concerned with fetal and neonatal animals.

Since an increasing emphasis is being placed on

the significance of the function of microbodies in cell metabolism and since the investigations reported hitherto have had a limited scope, the present experiments were attempted for the purpose of elucidating the morphogenesis and development of hepatocyte microbodies in fetal and postnatal rats.

MATERIALS AND METHODS

Mk/Wistar strain rats, ranging in age between the 15th fetal and 60th postnatal day, were used. The fetal ages were estimated from the time of conception and were accurate to within 12 hr. The animals were kept at 22 ± 1°C. Suckling rats were weaned on the 20th day after birth, and thereafter were fed on a compressed diet (Oriental, No. NMF) and received drinking water *ad libitum*. Only male rats were used for the examinations on the animals of ages between the 10th and 60th postnatal day. All animals including fetuses were, then, killed by decapitation between 10:00 and 11:00 a.m. for the purpose of avoiding a possible diurnal fluctuation in morphology and enzyme activities. They were then exsanguinated. Their livers were quickly removed and dissected on a cold saline-moistened filter paper.

Homogenization of Liver and Preparation of Microbody-Nucleoid Fraction

10% homogenate (w/v) was prepared with an ice-cold 0.25 M sucrose solution (pH 7.6) containing 0.001 M ethylene diamine tetracetate or distilled water. We centrifuged the homogenate made in the sucrose solution at 700 *g* for 10 min to remove the nuclear fraction. The sediment was washed once with 0.25 M sucrose solution (pH 7.4) of half the volume of the original homogenate. The supernatant and the washing therefrom were combined and centrifuged at 25,000 *g* for 15 min. The sediment was washed twice with sucrose solution and submitted to Triton-treatment according to the procedure previously described (14, 34), the only exception being that in the present experiments a centrifugal force of 105,000 *g* for 30 min was chosen so that we could minimize a possible decrease of nucleoids that might occur during the treatment.

Electron Microscopic Examinations

Small tissue fragments were fixed in 2% osmium tetroxide in veronal-acetate buffer (pH 7.4), 0.25 M sucrose solution, at 4°C for 2 hr. After dehydration with a graded series of cold ethanol, the tissues were embedded in Epon 812. Sections were prepared and stained according to the double-staining method with uranyl acetate and lead citrate (27). Negative staining of the dispersed preparations of the Triton-treated specimens was carried out by the procedure previously described (34). The sizes of nucleoids studied were determined on micrographs with the aid of a Zeiss TGZ-3 particle analyzer, and were expressed in terms of the mean of the length of the major and minor axes of the particles.

Biochemical Determinations

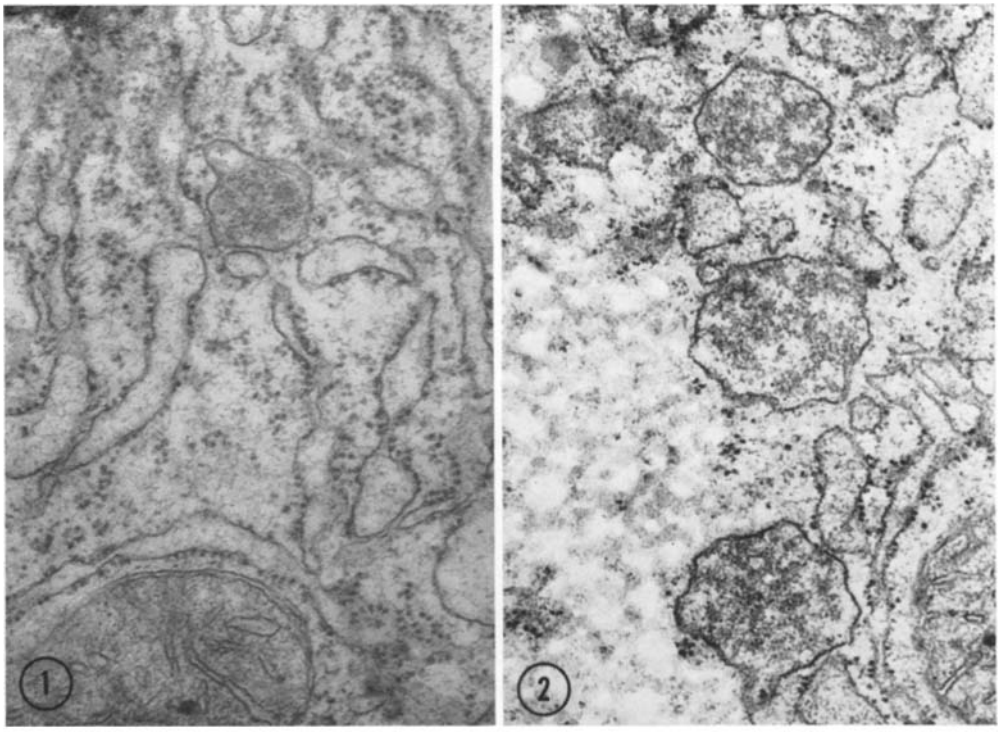
Urate oxidase activity was determined spectrophotometrically, according to the method of Praetorius (26) with a modification. The reaction mixture was composed of 0.5 ml of 0.005% lithium urate as substrate, 0.5 ml of 0.1 M borate buffer (pH 9.0), 0.5 ml of appropriately diluted homogenate or the fractionated specimens, and 1.5 ml of distilled water. After incubation at 37°C for 5 min, 1 ml of 20% ice-cold perchloric acid solution was poured into the reaction flasks, which were then cooled in ice for 5 min and centrifuged at 4,000 rpm for 5 min. The supernatant was spectrophotometrically assayed on uric acid disappearances at 285 mμ. Determinations of D-amino acid oxidase and catalase activities and of proteins were made according to the procedures described in our previous reports (14, 34).

RESULTS

Electron Microscopic Observations

In hepatocytes of fetal rats, a few single membrane-delimited organelles of irregularly round or corrugated shape and containing fine granules in variable numbers were found scattered randomly in the cytoplasm (Figs. 1 and 2). These organelles were distinctly smaller in size than mitochondria, and had no internal structures suggestive of mitochondria, multivesicular bodies, or lysosomes. The granules bore a strong resemblance to those of the matrices of microbodies in size, shape, and electron opacity. Furthermore, they appeared to be distinguishable from granules found in the interior of irregularly dilated ER, in that the latter granules were filamentous in shape or rather amorphous and much less electron opaque. This would suggest that the above-mentioned organelles might not be a portion of the ER tangentially sectioned. The granules in the matrices of these organelles were generally much fewer in number, and their distribution was far more irregular, as compared with the granules in hepatocyte microbodies in adult rats. On occasion, granules characteristic of the ER and granules characteristic of microbody matrices were found to exist concomitantly in one microbody-like organelle.

Occasionally, inside the above-mentioned organelles, an area was found which appeared to



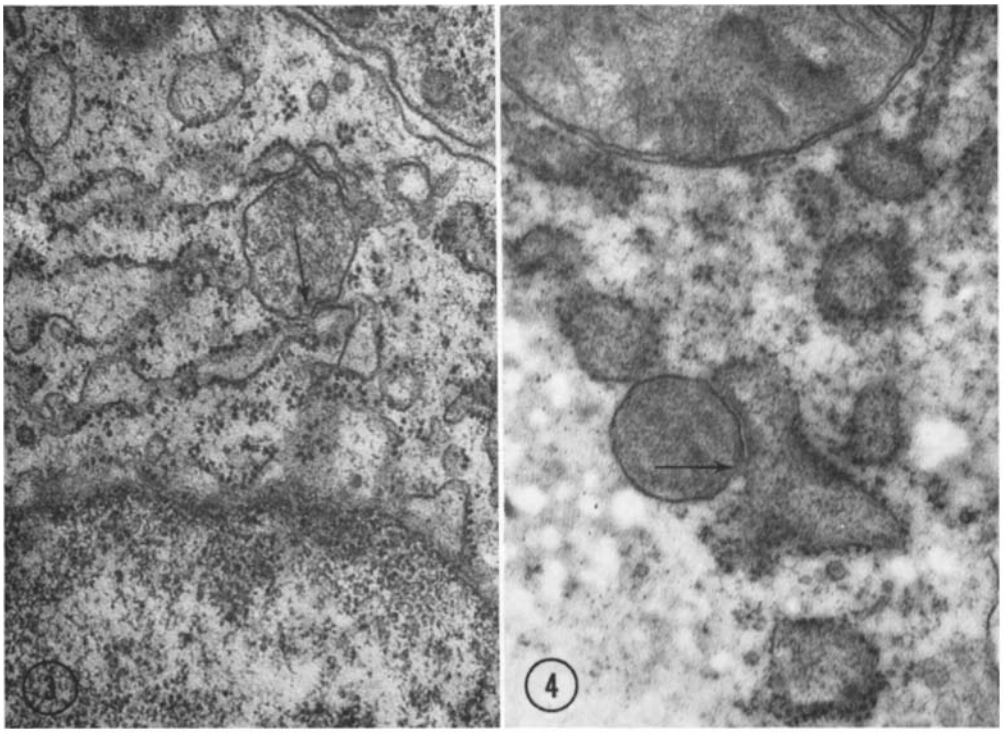
FIGURES. 1 and 2 Microbody-like organelles in hepatocytes of a 15-day-old fetus (Fig. 1) and of a 21-day-old fetus (Fig. 2). Single membrane–delimited microbody-like organelles are seen. These contain dense granules identical with those of microbody matrices, though smaller in number and more irregularly distributed as compared with those in microbodies of hepatocytes of adult rats. The nucleoids have not yet appeared. × 40,000.

consist of dense granules arranged in such a pattern as to suggest that they were preparing to form small tubules resembling the unit tubules of nucleoids (34, 35). However, the possibility may not be excluded that the small aggregation of the unit tubules of nucleoids corresponds merely to a very peripheral portion of the completed nucleoid appearing on thin sections.

It was not a rare finding in fetal hepatocytes that the limiting membranes as well as the matrices of these microbody-like organelles, especially those lacking nucleoids and containing scanty granular matrices, showed a continuity into granular ER profiles (Figs. 3 and 4). The connection between the microbody-like organelle and the ER appeared to be a stem formed by constriction of a focal protrusion of the ER. Ribosomes attached to the surface of the ER were found to decrease in number in an irregular fashion in the vicinity where balloon-like protrusions and the ER were connected with each other. Furthermore, almost none or very few of the ribosomes were seen on the surface of the protrusion; the occurrence of organelles still bearing a considerable number of ribosomes (Figs. 5 and 6) was rare.

In hepatocytes of 21-day-old fetuses, a small number of microbodies, especially those exhibiting nucleoids, were shown to be replete with granular matrices, though the majority was akin to those in hepatocytes of 15- and 18-day-old fetuses with respect to their electron-lucent appearances.

In hepatocytes of 1-day-old rats, a considerable number of microbodies were enriched in granular matrices, in contrast to the predominance of electron-lucent microbodies in fetal hepatocytes. Findings indicating a correlation between microbodies and granular ER were also encountered at this postnatal stage. However, hook- or ringlike agranular tubular segments were occasionally observed projecting from the surface of micro-



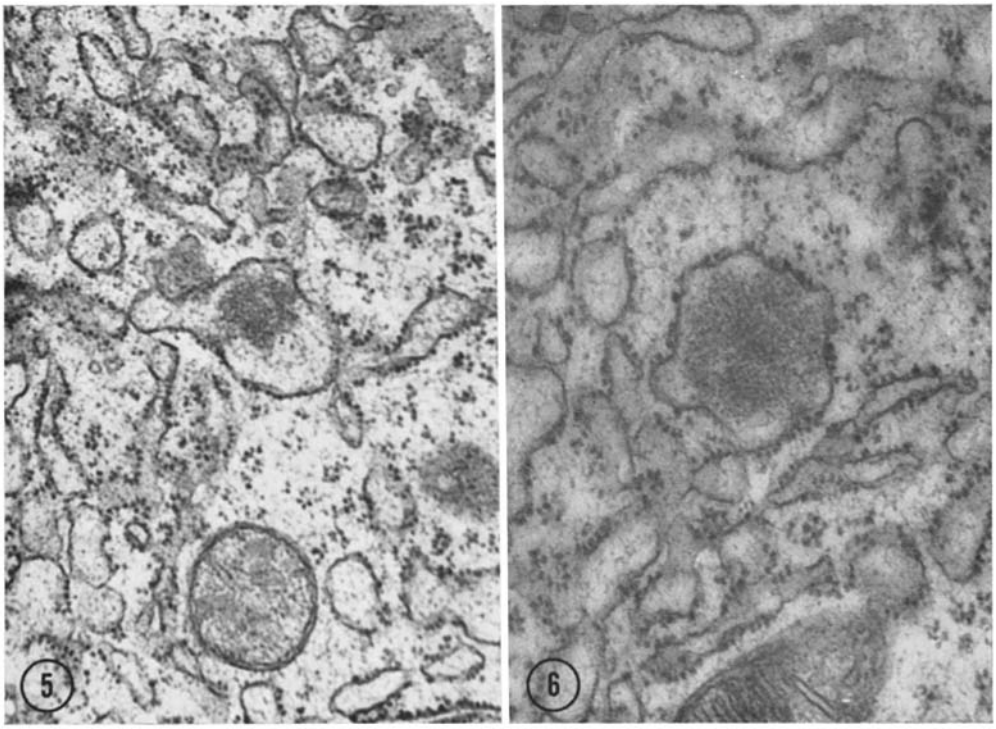
FIGURES 3 and 4 Microbody-like organelles in hepatocytes of an 18-day-old fetus (Fig. 3) and of a 21-day-old fetus (Fig. 4). These show substantial connections with granular ER profiles by narrow stems as indicated by arrows. × 40,000.

bodies (Figs. 7 and 8). For the most part, only one projection was seen for one microbody; however, two or three projections were occasionally recognized on one microbody. As shown in Table I, the incidence of microbodies bearing such projections was highest in 1- and 5-day-old rats and decreased as the animals grew older; there were almost no microbodies bearing projections in 40- and 60-day-old rats. In fetal livers, the incidence of these projections was somewhat less in 21-day-old fetuses than in 1-day-old neonatal rats. In livers of 15- and 18-day-old fetuses, it was difficult to distinguish the projections from the occasionally prominent corrugations of the limiting membranes of microbodies.

In hepatocytes of 10-day-old rats and in those of older rats, microbodies were frequently found surrounded by a cuff of closely applied agranular ER. However, we saw nothing to imply a substantial connection between microbodies and granular ER in these postnatal ages.

During the course of fetal and postnatal growth of rats, there was no evidence to indicate transformation between microbodies and other cytoplasmic organelles such as mitochondria, multivesicular bodies, or Golgi apparatus. Also, no preferential intracytoplasmic localization of microbodies was recognized.

Changes in the number of microbodies and the incidence of microbodies exhibiting nucleoids during pre- and postnatal growth are shown in Table I. The total number of microbodies evidently increased during the 15th and 18th fetal day, and also during the 21st fetal and 5th postnatal day. For the subsequent periods of growth, the curve of the numerical changes formed an approximate plateau; however, in 60-day-old rats the number was somewhat smaller than in 40-day-old rats. The number of microbodies exhibiting nucleoids, on the other hand, was found to increase continuously up to the 40th day after birth, at a more rapid pace in younger, and at a slower pace in older animals.



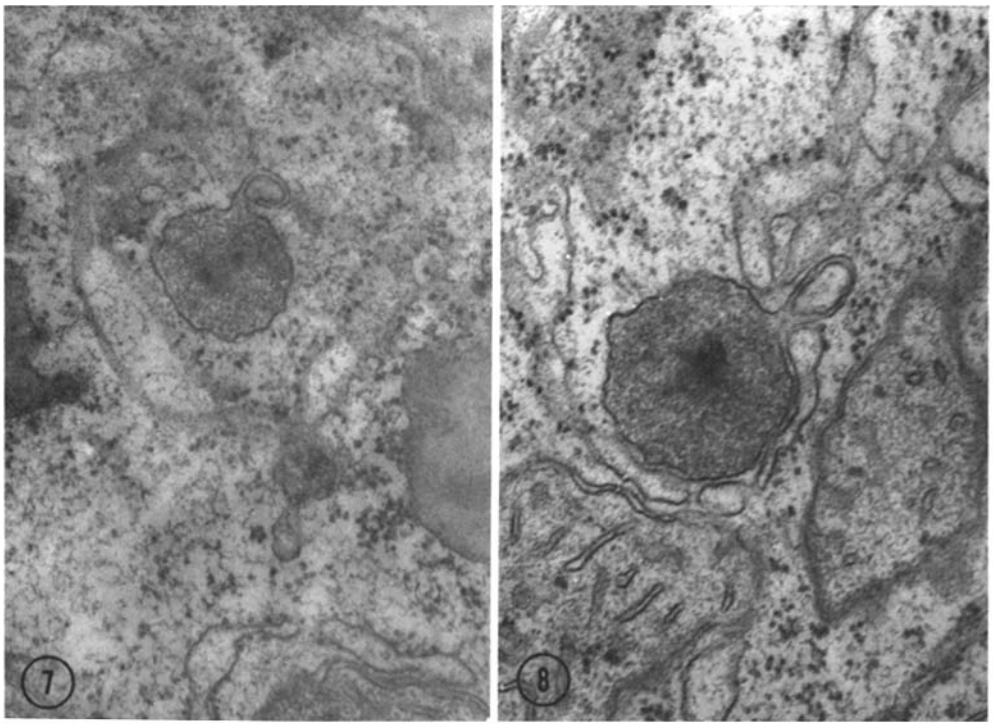
FIGURES 5 and 6 Microbody-like organelles in hepatocytes of an 18-day-old fetus (Fig. 5) and of a 21-day-old fetus (Fig. 6). The organelles show ribosomes on the surface of their limiting membranes, though in a smaller number and in an irregular fashion. The organelle seen in Fig. 5 contains filamentous granules characteristic of ER in addition to granules characteristic of microbody-matrices. × 40,000.

It was difficult to assess the sizes of microbodies, since estimations were, of necessity, made from profile dimensions in micrographs of thin sections. However, a trend was seen in which the sizes increased in the following order; 5- and 10-day-old rats, 60-day-old rats, and 40-day-old rats (Figs. 9–11). It was also noted that the sizes of microbodies in fetal hepatocytes and in the hepatocytes of 1-day-old rats varied. But, in general, they appeared to be somewhat greater than those in 5- and 10-day-old rats.

As shown in Table I and Figs. 12–16, the sizes of nucleoids were found to gradually decrease up to the 10th day after birth, and then to increase up to the 40th day. In 60-day-old rats, the nucleoid sizes were smaller than in 40-day-old rats. It was also noted (Fig. 12) that the distribution of the sizes was wider in 40- and 60-day-old rats than in rats of other ages. In addition, virtually no difference in electron microscopic structure was recognized between large and small nucleoids, as clearly seen from Figs. 13–16.

Biochemical Results

The pattern of changes in activities of urate oxidase, D-amino acid oxidase, and catalase in the liver during fetal and postnatal growth is shown in Table II. Urate oxidase activity was at first measurable in 18-day-old fetuses, and then increased up to the 1st day after birth. In 5- and 10-day-old rats, on the other hand, the activity was slightly decreased, though the decrease is statistically significant. This was followed by an increase again up to the 40th day, at which time the maximum value was obtained. Catalase activity, in contrast to urate oxidase activity, was found to increase continuously as rats grew, reaching the maximum level in 60-day-old rats. D-Amino acid oxidase activity was at first measurable in 21-day-old fetuses, and it increased up to the 20th day after birth.



FIGURES 7 and 8 Microbodies in hepatocytes of 1-day-old rats. These microbodies show hook- or ring-like agranular projecting segments of their limiting membranes. Nucleoids are seen at the center of microbodies. × 40,000.

Table III shows the changes in urate oxidase activity and protein content of the isolated nucleoid fraction during the postnatal period. The specific activity of urate oxidase of the nucleoid fraction was found to increase until the 40th day. The increase was gradual betwen the 1st and 10th day, and became considerably more rapid during the successive 10 days. The maximum value was obtained in 40-day-old rats, in which the maximum enzyme activity in the liver tissues, the maximum size of nucleoids, and also the maximum incidence of microbodies exhibiting nucleoids were found.

DISCUSSION

Previous hypotheses concerning the morphogenesis of hepatocyte microbodies may be classified into the following possibilities: (*a*) derivation from agranular ER (12, 16, 20–24, 31, 32, 35); (*b*) derivation from multivesicular bodies or Golgi apparatus (5, 9–11, 29, 36–38); and (*c*) derivation from granular ER (13).

The main grounds on which the first possibility may stand might be the findings that tubular images of agranular ER were connected directly with the limiting membranes of microbodies, or that short agranular tubular segments of the limiting membranes were found to project from the surface of microbodies. In our previous paper (35), an assumption was tentatively made that a morphogenetic relation seemed to be present between microbodies and agranular ER. However, in the present experiments, no crucial evidence was obtained to endorse specifically the concept that microbodies are derived from agranular ER; it would be difficult to regard the short tubular segments of the limiting membranes of microbodies, sectioned as they were, as a part of the agranular ER. This seems true, inasmuch as the observations were made on approximately 130 microbodies bearing the projections. These microbodies were frequently enriched in granular matrices and contained nucleoids; in other words,

TABLE I

Number of Microbodies, Incidence of Agranular Tubular Projections of Microbody-Membranes, and Size of Microbody-Nucleoids in Rat Hepatocytes

Ages of rats	No. of microbodies		Incidence of agranular projections	Size of nucleoids
	Total No.	No. of microbodies containing nucleoids		
Fetuses (days of gestation)				
15 (12*)	2.29 ± 0.224‡	0.21 ± 0.014‡		
18 (10)	4.56 ± 0.412	1.06 ± 0.109		
21 (10)	4.08 ± 0.390	2.21 ± 0.276	4.32§ (16/371‖)	
Postnatal rats (days after birth)				
1 (7)	6.41 ± 0.553	2.63 ± 0.238	7.45 (42/564)	234.8 ± 1.00¶
5 (7)	8.22 ± 0.606	3.18 ± 0.334	6.07 (36/597)	226.6 ± 8.08
10 (5)	8.51 ± 0.520	4.10 ± 0.396	3.30 (14/425)	210.4 ± 6.16
20 (5)	8.63 ± 0.470	4.75 ± 0.217	1.50 (6/405)	255.7 ± 8.84
40 (5)	8.76 ± 0.803	5.62 ± 0.216	0 (0/309)	332.2 ± 9.42
60 (5)	7.11 ± 0.624	3.75 ± 0.286	0.14 (1/387)	274.0 ± 2.40

* Figures in parentheses are the number of determinations.

‡ Mean ± standard error in 100 μ^2-cytoplasmic area of hepatocytes. Determinations were made on each approximately 2,000 μ^2-cytoplasmic areas.

§ Percentage.

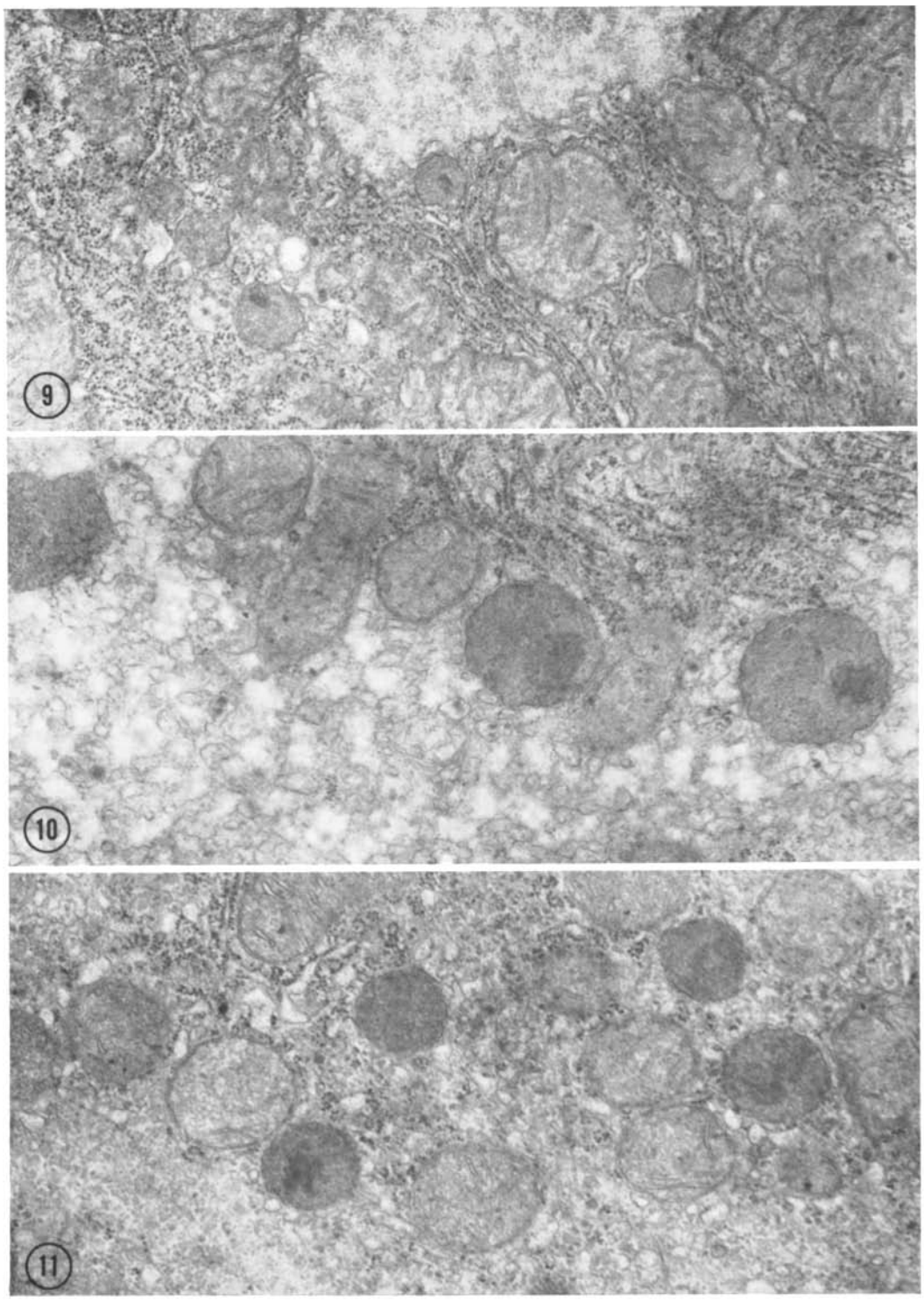
‖ Figures in parentheses: numerators are the number of microbodies bearing agranular projections, and denominators are the number of total microbodies.

¶ mμ, mean ± standard error. Approximately 200 nucleoids were examined in each determination.

they were in a completed form and not in a forming stage. It may be presumed that the projections have been formed from microbodies to serve as a passageway for the transportation of substances necessary for the function or for the growth and maturation of the organelles. In older animals, the cuff of closely applied agranular ER may be responsible for the transportation system, as suggested by Novikoff and Shin (23) and others.

The evidence suggesting the second possibility is considered to be a topographical relation of microbodies to multivesicular bodies or Golgi apparatus. The exception seems to be the observations of Bruni and Porter (5) and Dvořák et al. (9, 10), in which the presence of a direct transformation from multivesicular bodies or Golgi apparatus to microbodies was emphasized. However, Daems (6), Novikoff and Shin (23), Essner and Masin (13), and the present authors failed to demonstrate any transitional forms between microbodies and multivesicular bodies. Essner and Masin (13) agreed on a spatial relation between microbodies and Golgi apparatus, but the present observations indicate no preferential intracytoplasmic localization of microbodies. If it is assumed that a spatial relation between the two does exist, there still might remain a possibility that this represents merely a functional, but not a morphogenetic relationship.

During the preparation of the present paper, a report by Essner and Masin (13) was published which supports the third possibility for microbody formation. Our observations on fetal and neonatal rats might also support this assumption, in that the first step of microbody formation is a focal protrusion of a dilated region of granular ER. The connection between microbodies and granular ER profiles was also shown in the text-figure of a report by Dallner et al. (7), although it was not explained in the written text. As to the site of microbody formation from the ER, Essner and Masin emphasized a cisternal dilatation at the terminal region of the ER. Yet, in the pesent experiments, it seems probable that the protrusion leading to microbody formation might not be restricted to the terminal region of the ER, but may be formed anywhere over the total length of the ER where the ER is



FIGURES 9–11 Portions of hepatocyte cytoplasms of a 10-day-old rat (Fig. 9), a 40-day-old rat (Fig. 10), and a 60-day-old rat (Fig. 11). It is to be noted that the size of microbodies increases in the following order: 10-, 60-, and 40-day-old rats. × 20,000.

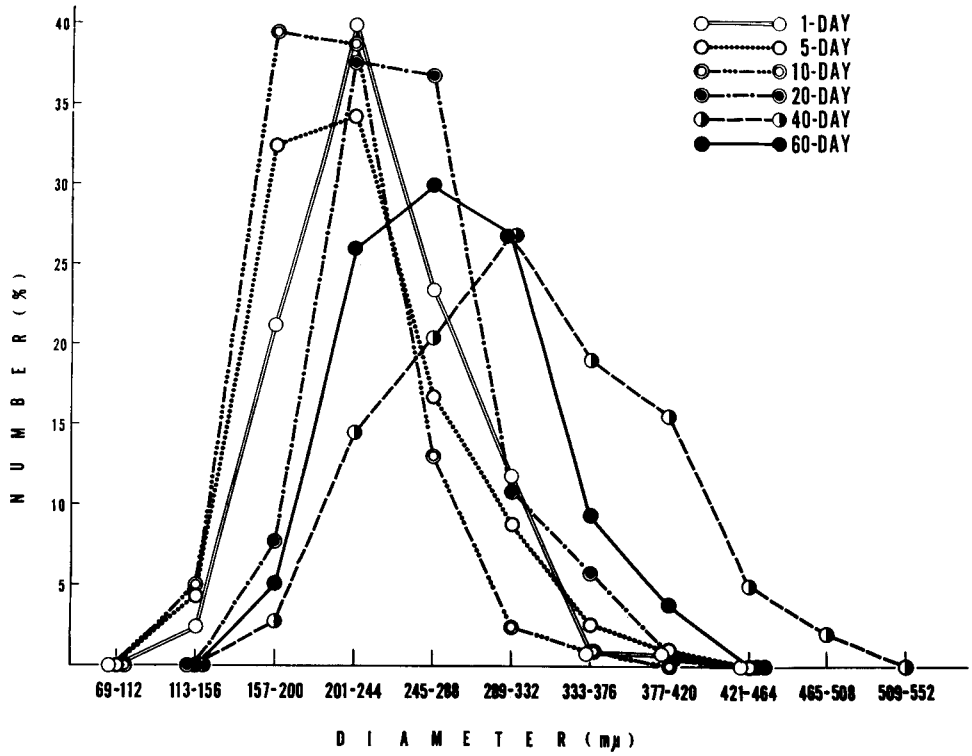
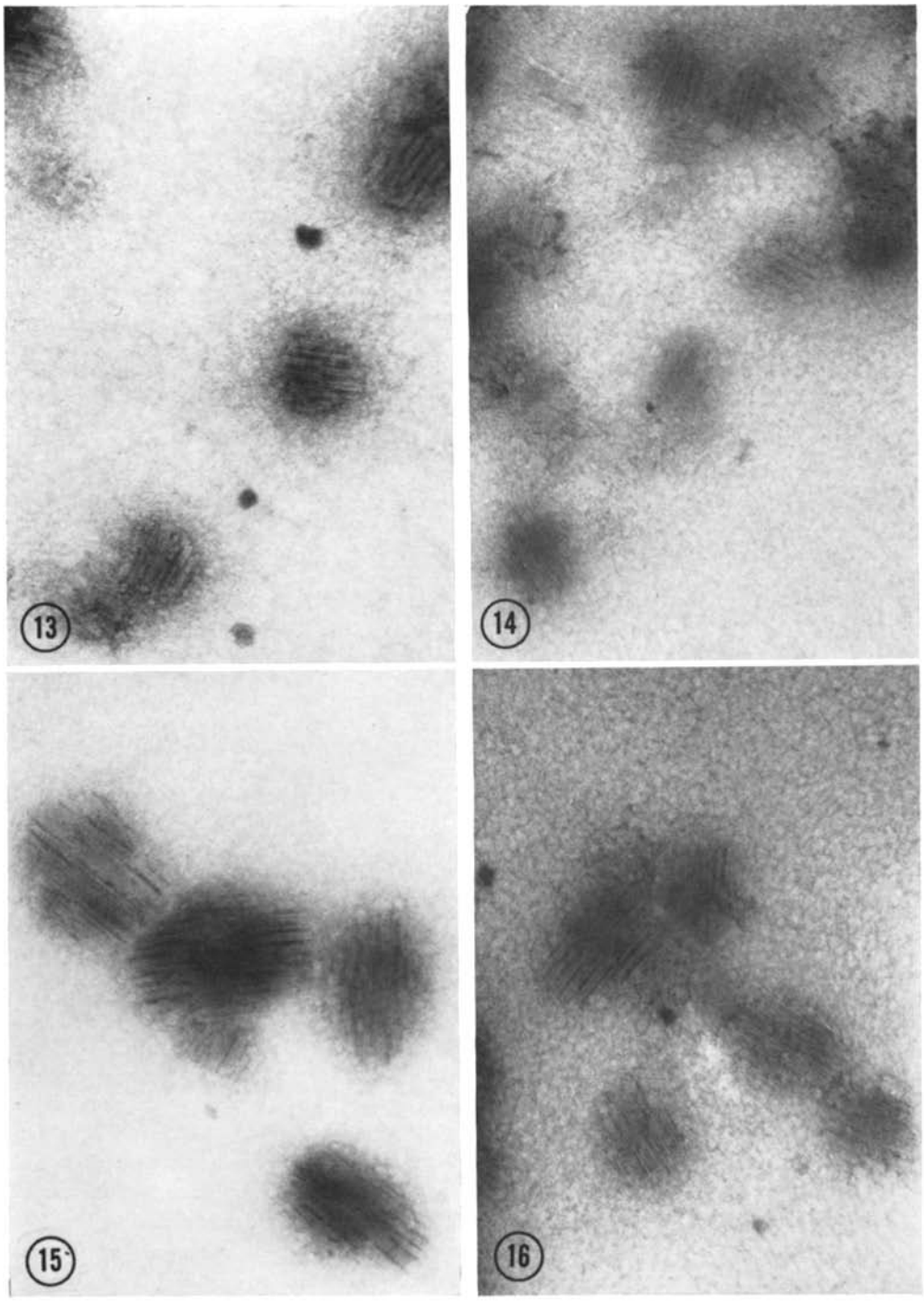


FIGURE 12 Distribution of the sizes of microbody-nucleoids. Determinations were made on negatively stained dispersed preparations of isolated nucleoids.

irregularly dilated. Although ER ribosomes disappear rapidly from the surface of the protrusion, they still remain, on rare occasions, attached to the surface of microbodies, even after the matrices have increased considerably in mass. Now, this would seem also to support the concept that microbodies are formed from granular ER.

As to the numerical changes in microbodies during fetal and postnatal periods, Wood (37, 38), Palade (24), and Dvořák et al. (9, 10) reported a rapid increase during later periods of gestation and neonatal stages. Phillip et al. (25) also reported that microbodies were numerous between the 1st and 12th hour after birth, while Jézéquel et al. (22) found an increase after the second postnatal day. In the present quantitative estimation of the numerical changes, the number was found to increase during pre- and postnatal periods up to the 5th day after birth. The exception was the period between the 18th and 21st fetal day, during which proliferation and hypertrophy of hepatocytes may occur in parallel with the formation of microbodies. As to the sizes of microbodies, Dvořák et al. (9, 10) reported that they increased after birth and that on the 14th day the size reached was comparable to that in adult rats. In the present experiments, on the other hand, the sizes were found to be quite small in 5- and 10-day-old rats, suggesting that these microbodies are in a growing stage.

As to the morphogenesis of nucleoids, Novikoff and Shin (23) described electron-opaque granules in microbodies in which they suspected that the nucleoid might develop. Although it might be fairly difficult to trace such morphogenesis on thin sections, the initial unit tubules of the nucleoid might be formed suddenly within an area of the matrices. Immediately afterwards or, seemingly, at the same time as the formation of the unit tubules, a unit configuration (34) or a minute nucleoid might develop, probably in such a fashion that an appositional formation of new tubules takes place on the preformed tubules in a successive manner. Inasmuch as only one nucleoid is found



FIGURES 13–16 Isolated microbody-nucleoids. Negatively stained, dispersed preparations of isolated nucleoids in these figures were obtained from 5-day-old rats (Fig. 13), 10-day-old rats (Fig. 14), 40-day-old rats (Fig. 15), and 60-day-old rats (Fig. 16), respectively. It is noted that the sizes of the nucleoids increase in the following order: 10-, 5-, 60-, and 40-day-old animals. No difference in the fine structures is recognized among these nucleoids. × 50,000.

TABLE II

Activities of Urate Oxidase, Catalase, and D-Amino Acid Oxidase of Rat Liver

Ages of rats	Urate oxidase	Catalase	D-Amino acid oxidase
Fetuses (days of gestation)			
18	0.53 ± 0.086*	7.53 ± 0.840	0
21	1.21 ± 0.180	18.60 ± 0.620	0.30 ± 0.021
Postnatal rats (days after birth)			
1	6.80 ± 0.320	33.7 ± 1.60	0.81 ± 0.034
5	4.95 ± 0.230	35.1 ± 0.90	1.28 ± 0.153
10	6.00 ± 0.126	37.8 ± 1.52	1.87 ± 0.070
20	15.80 ± 0.555	47.1 ± 0.85	5.64 ± 0.460
40	19.15 ± 0.450	92.8 ± 3.66	6.12 ± 0.668
60	16.75 ± 0.310	109.8 ± 1.40	5.65 ± 0.260

*Mean ± standard error. Values were obtained from each seven determinations. Urate oxidase activity: uric acid oxidized, μg/5 min/mg dry tissues. Catalase activity: k/min/100 mg dry tissues. D-Amino acid oxidase activity: pyruvic acid produced, μg/20 min/mg dry tissues.

in one microbody, a nucleoid increases its size by appositional crystallization of structural proteins which leads to the formation of unit tubules at the peripheral portion of the nucleoid. It is relevantly suggested that the nucleoids in early stages after formation might be immature and that they might bear less urate oxidase than those in later stages after formation, though no electron microscopic changes occur except for an increase in their size in later stages. The ratio of the formation to the growth and maturation of nucleoids might vary according to the varying ages of the animals. During the 1st and 10th day after birth, formation seems to be in the forefront, while growth and maturation may be predominant in the successive growing periods of rats.

In hepatocytes of rats administered ethyl chlorophenoxyisobutyrate, the number of microbodies was reported to increase outstandingly (2, 17, 31, 32). Hess et al. (17) reported that catalase activity of the liver of the treated rats was increased whereas urate oxidase activity was decreased. They suggested that the decrease in the latter activity was due to an increase in microbodies lacking nucleoids and to a dilution phenomenon resulting from an enlargement of the liver, as well. As judged from the present experiments, the low urate oxidase activity might also be ascribed to an appearance of immature nucleoids.

As to the changes in activities of microbody-enzymes while the number of microbodies is increasing, Azarnoff and Svoboda (2) also found that in the liver of rats administered the above-mentioned drug the catalase activity was increased whereas D-amino acid oxidase activity was decreased. Although the mechanism of the dissociation of the two has not as yet been clarified, an explanation is tentatively made. It may be that a hydrogen peroxide-destroying mechanism is more essential and develops earlier than the hydrogen peroxide-producing system. This, also, was observed in the present experiments on rats during earlier periods of growth. Furthermore, it is assumed that the difference in the changes between urate oxidase and D-amino acid oxidase activities during the first 10 days after birth is related to a possible overproduction of uric acid in neonatal animals.

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TABLE III

Urate Oxidase Activity and Protein Content of Homogenate, Cytoplasmic Fraction, 700–25,000 g–Sedimentable Fraction, and Microbody-Nucleoid Fraction of Rat Liver

Ages of rats (Days after birth)	1	5	10	20	40	60
Urate oxidase activity*						
Homogenate	31.8 ± 1.12‖	25.1 ± 2.15	28.5 ± 2.11	81.3 ± 5.31	122.8 ± 4.11	97.4 ± 5.14
Cytoplasm	30.8 ± 2.50	25.0 ± 2.14	28.2 ± 0.58	80.4 ± 7.20	116.0 ± 3.40	90.3 ± 2.53
700-25,000 *g*-fraction	24.2 ± 1.40	18.5 ± 1.48	23.1 ± 0.80	78.0 ± 7.20	115.0 ± 3.40	90.8 ± 4.90
Nucleoids	8.5 ± 1.60	12.1 ± 1.39	20.4 ± 0.53	77.7 ± 5.31	104.8 ± 6.31	77.0 ± 6.34
Protein‡						
Homogenate	17.8 ± 1.18	19.0 ± 0.52	21.9 ± 0.41	21.2 ± 0.24	22.5 ± 0.89	23.8 ± 0.29
Cytoplasm	14.0 ± 0.32	15.5 ± 0.41	17.8 ± 0.60	18.6 ± 0.15	19.9 ± 0.78	21.5 ± 0.24
700-25,000 *g*-fraction	3.70 ± 0.200	5.14 ± 0.259	6.18 ± 0.300	6.85 ± 0.230	6.73 ± 0.208	7.10 ± 0.183
Nucleoids	0.10 ± 0.014	0.11 ± 0.010	0.11 ± 0.001	0.15 ± 0.003	0.16 ± 0.019	0.13 ± 0.008
Specific activity§						
Homogenate	1.8	1.3	1.3	3.8	5.5	4.1
Cytoplasm	2.2	1.6	1.6	4.3	5.8	4.2
700-25,000 *g*-fraction	6.5	3.6	3.7	11.4	17.1	12.8
Nucleoids	85.0	110.0	185.4	518.6	655.0	592.3

* μg uric acid oxidized/100 mg wet tissue or fractions equivalent to 100 mg tissue.

‡ μg protein/100 mg wet tissue or fractions equivalent to 100 mg tissue.

§ Urate oxidase activity/protein.

‖ Mean ± standard error. Values were obtained each from five determinations.

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