FINE STRUCTURE OF LIPID GRANULES IN PROXIMAL TUBULE CELLS OF MOUSE KIDNEY

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In electron microscopic investigations on the proximal tubule of the mouse kidney, large osmiophilic granules in the cytoplasm of the epithelial cells have been found by various authors (2, 13, 14, 20). Rhodin (13) and Clark (2) have pointed out that the granules contain concentric lamellae, but there have been no further investigations on the fine structure of these granules so far. Recently Miller (10) described granules which show a regular pattern of layered dense and light bands with a repeating period of about 80 to 90 A. In our electron microscopic investigations on the proximal tubule cells of the kidney,[1] we observed similar granules which show in some respects a different structure.

The granules which will be described were found in almost every epithelial cell of the proximal tubule, chiefly in the apical and intermediate zones of the cell. In one of three animals examined, every cell in one section contained 3 to 15 granules; in the other two animals there were only 1 to 2 granules in each cell. This difference did not appear to be related to any specific physiological condition. All granules seen were of the same type. The granules have a round, oval, or completely irregular profile (diameter 0.3 to 1.2 μ). They are surrounded by a one-layered membrane about 20 to 40 A thick and of medium density (Fig. 1), whereas Miller (10) described a membrane 60 A thick. Between this membrane and the outer lining of the granule itself there is a light space which is, on the average, 23 A wide. The granules regularly show at their outer periphery a thin rim of very dense granular material. In the interior of the granules two basic patterns can be discerned for descriptive purposes:

a) An "*ordered pattern*" (Figs. 1, 2 *a*): This consists of parallel, alternating dense and light bands, the center-to-center distance between two adjacent dense or light bands being 40 to 45 A (average 43 A). The dark bands appear somewhat narrower than the light ones and have an average width of 16 A, making the average width of the light bands about 27 A. Up to 33 parallel dense and light bands could be observed, forming a striped and concentrically arranged pattern.

This ordered pattern is found in most cases. In a few granules it appears to be not quite so regular, because the dense bands show a somewhat undulating course and therefore the distances between them vary and the concentric band pattern appears frayed.

b) A "*seemingly disordered pattern*" (Figs. 1, 2 *b*): Within a ground mass (1st component) having a medium density and a granular appearance, in which no particular order is evident, particles (2nd component) of very high density and varying size—diameter or width 20 to 50 A—are embedded. These particles, round or somewhat oblong in shape, are irregularly distributed, loosely scattered in some places and so densely packed in other places that the ground mass is completely covered by them.

The "seemingly disordered pattern" must not be confused with the "ordered pattern" in places where the plane of the layered lamellae is not exactly perpendicular to the plane of sectioning so as to give the characteristic ("ordered") band pattern. The pattern in these places also appears granular-like, but it is more regular and more finely granular than in the zones of the "seemingly disordered pattern." Also, the larger, irregularly distributed particles of high density (2nd component) mentioned above are absent.

[1] Kidney cortex of three normal mice (weight 15 to 20 gm) was fixed for 1 hour in 1 per cent buffered (pH 7.3), isotonic OsO_4 solution without additional contrasting. The specimens were embedded in Vestopal W (1 per cent initiator, 0.5 per cent activator) polymerized at 60°C (8). Thin sections were prepared with a Porter-Blum microtome. Electron micrographs (including focal series) were taken with the Siemens Elmiskop I at 80 kv.

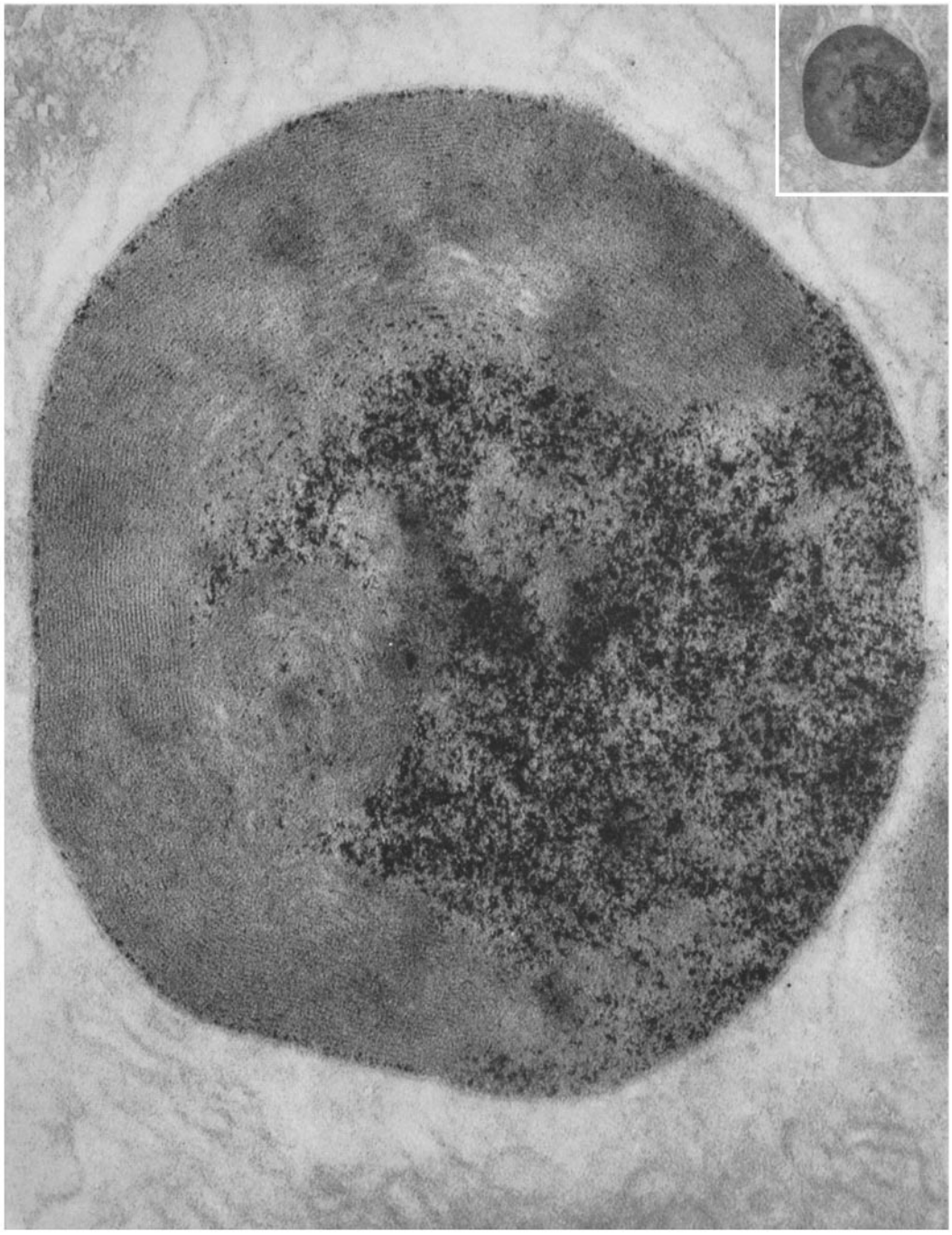

FIGURE 1

Large osmiophilic granule in epithelial cell of proximal tubule. At high magnification, two patterns are visible within the granule: a concentric, regular pattern of alternating dense and less dense bands (left and upper regions), and a pattern of disorderly arranged, more or less dense particles (middle and right regions). The granule is bounded by a one-layered membrane. × 160,000; inset, × 24,000.

Both the "ordered" and the "seemingly disordered" patterns are present in the majority of the granules to a varying extent. Often they merge into each other: the band pattern of the "ordered" zone gradually merges with the granular pattern of the "disordered" zone, or the very dense material in the "disordered" zone is incorporated in the band pattern of the "ordered" zone, *e.g.* as if twisted into a spiral (see Fig. 1).

Granules with systems of layered membranes have been observed in many other tissues (1, 3–7, 9, 12, 15–17), but a band pattern with one regular, invariable, repeating period has been described in only a few cases (spleen (17), adrenal cortex (3), cultured nerve cells (4), embryonal liver (7)). Duncan and Hild (4) give a picture which corresponds strictly to our Fig. 2 *a*, including the center-to-center distance and the width of the dark lines in the band pattern (according to measurements which we made because the authors did not give measurements in their text).

In the kidney, such granules as are described here were previously unknown. They differ from the granule which Miller (10) recently observed in the same part of the nephron: the repeating period of the band pattern (43 A) is about one-half the period (78 A) measured by Miller (10). There are also differences in the granules of the cortical collecting tubule described by the same author (11): (*a*) The center-to-center distance between the dense or light bands of the "ordered pattern" (corresponding to pattern No. 2 of Miller) and also the width of the dense bands are considerably smaller (center-to-center distance 43 A as compared with 96 A, width of dark bands 16 A as compared with 48 A). (*b*) Thread-like elements in a regular hexagonal or square array (pattern No. 1 of Miller) could not be detected. (*c*) The limiting membrane toward the cytoplasmic matrix is one-layered, as a rule, and is less dense and slightly more blurred in the picture.

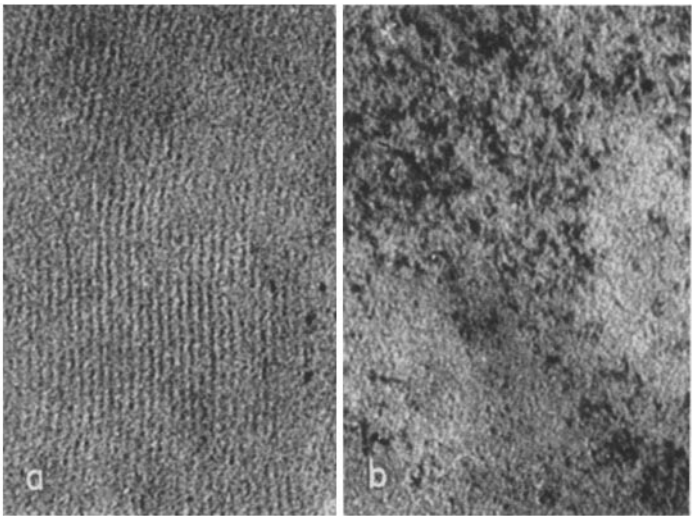

FIGURE 2

The two patterns of the lipid granule at higher magnification. × 320,000.

a. "Ordered pattern" of alternating dense and less dense bands (myelin figure). Mean center-to-center distance between the dense or light bands is 43 A.

b. "Seemingly disordered pattern" of particles of varying density. The densest particles (2nd component) frequently take the shape of oblong profiles (threads?).

The band pattern which we found in the granules of the cells of the proximal tubule epithelia can be compared with the myelin figures which were prepared from phospholipid mixtures by Stoeckenius (18, 19). There is surprisingly close agreement between the data given by Stoeckenius (center-to-center distance between the dark or light bands about 40 A, width of the dark bands about 17 A) and our own data (43 A and

16 A, respectively). Because of this close agreement we assume that the layered structures established in our granules are myelin figures. On the basis of his data and histochemical findings, Miller (11) comes to the conclusion that there is crystallized *lipoprotein* in the granules which he described. On the contrary, in line with the findings of Stoeckenius (18, 19), it appears as though the "ordered pattern" of the granules which we investigated consists only of *lipids*.

There are probably several substances involved in the formation of the "seemingly disordered pattern". It cannot be established from the micrograph whether the particles described (1st and 2nd components) are granular or thread-like elements. It is possible that a part of the particles are lipid complexes in which the molecules have no determinate orientation with respect to one another. The factors are yet unknown that determine whether and to what extent the lipid molecules assume the state of order of the myelin figures during granule formation. It should be mentioned, however, that the very dense particles (2nd component) represent particles of a completely different nature, for example iron-containing particles, although we have not found typical tetrad figures of ferritin.

The appearance of the "seemingly disordered pattern" resembles in many places that of the crescents of the so-called "vacuolated bodies" (11) occurring in the same cell type. It is possible that it may be the same structure, but further investigations on this subject must be carried out.

When one considers the one-layered limiting membrane of the granule toward the cytoplasmic matrix, the similarity between it and the surface membrane of the myelin figures which were prepared in the protein solution becomes apparent (18, 19). However, the membrane at the surface of the granule is not so dense as the surface membrane of the myelin figures prepared in the protein solution. Nevertheless, it is possible that the surface membrane of the granules is a limiting membrane formed by the cytoplasm and possibly contains protein and lipid.

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