Fine Structure of the Surface of Mouse Hepatic Cells

TREVOR HEATH AND STEVEN L. WISSIG
Department of Veterinary Preclinical Sciences, University of Melbourne, Parkville, Victoria, Australia, and Department of Anatomy, University of California, San Francisco Medical Center, San Francisco, California

ABSTRACT Mouse hepatic cells appear in thin sections as polygons with six or more sides. The plasma membrane covering these sides may contact either bile canaliculi, the narrow intercellular space, the space of Disse or extensions of the space of Disse between adjacent cells. The plasma membrane covering microvilli in bile canaliculi and the space of Disse is thicker than that in contact with the narrow intercellular space. Bile canaliculi, which contact about 6% of the perimeter of each cell, are each separated by tight junctions from the narrow intercellular space. This space contacts more than one-half the perimeter of each cell and is about 220 Å in width. It is continuous around the occasional studlike junctions which occur, but is interrupted at frequent intervals by circumscribed tight junctions, and occasionally by desmosomes. The narrow intercellular space is in free communication through the space of Disse with the plasma space. An interstitial fluid space, separate from the plasma space, does not occur in the liver lobule. Protein molecules from plasma enter hepatic cells in both coated and pinocytotic vesicles. These vesicles are derived from invaginations of the plasma membrane that borders the narrow intercellular space and the spaces between microvilli in the space of Disse. Pinocytotic vesicles may also incorporate fat droplets into hepatic cells.

Segments of the perimeter of parenchymal cells located in the interior of a hepatic lobule border one of three types of extracellular space: a lumen of a bile canaliculus, a perisinusoidal space of Disse or a narrow intercellular space separating adjacent hepatic cells. The plasma membrane and adjacent cytoplasm along each type of space have specific morphologic characteristics peculiar to that region, and descriptions of some of these have already appeared in the literature (Fawcett, '55; David, '61; Biava, '64).

Bile canaliculi in the mouse, rat, guinea pig and man are most often limited by segments of the plasma membrane of two, and less often of three or more hepatic cells (David, '61; Biava, '64). The plasma membrane which limits the canaliculi, at least in man, is 100–110 Å thick but the thickness and density can vary depending on the procedures used to prepare the specimens for examination (Biava, '64). The cytoplasm which borders each canaliculus contains a fine filamentous meshwork, and strands from this meshwork extend into microvilli which project into the lumen (Wood, '61). Minute tubules, 70–90 Å in diameter, have also been described in canalicular microvilli in the rabbit liver (David, '61).

There is some disagreement about the degree of continuity between the lumen of the canaliculi and the remainder of the extracellular space. In an early series of reports, Rouiller ('54, '56) stated that bile canaliculi communicate freely with the space of Disse. As this statement has not been substantiated, and as it cannot be reconciled with the known facts of liver physiology, it seems likely that the author confused some extensions of the space of Disse for bile canaliculi. Later, Ashworth and Sanders ('60) concluded that a cleft about 100 Å wide separated adjacent hepatic cells at the margins of canaliculi. They believed that this was just wide enough to allow water, ions and small molecules to pass from the space of Disse into canaliculi, but was too narrow to allow the passage of proteins. More recently, Farquhar and Palade ('63) showed that the plasma membranes of adjacent hepatic cells in the rat and guinea pig fuse to form an apparently impermeable tight junction where they meet at the margins of a canaliculus. These tight junctions exist as a continuous band (*zonula occlu-*

dens) along the length of the margins of all canaliculi and should effectively seal off the canalicular lumen from the adjacent intercellular space. Farquhar and Palade ('63) described two other components of a junctional complex at the lateral margins of the canaliculi. These components, the intermediate junction (*zonula adhaerens*) and the desmosome (*macula adhaerens*), were believed to act as intercellular attachment devices. The junctional complex at the margins of human bile canaliculi consists of essentially the same components (Biava, '64).

The segments of the hepatic cell surface which face the space of Disse and its extensions are covered by numerous microvilli (Rouiller, '54). The cytoplasm immediately adjacent to the space of Disse contains many small vesicles, and some of these vesicles appear to be connected to the space of Disse by tubular invaginations of the plasma membrane (Rouiller, '54; Novikoff and Essner, '60; Bruni and Porter, '65). It has been suggested that these vesicles are pinocytotic in nature and that they are important in the uptake of materials by hepatic cells (Roth and Porter, '62; Bruni and Porter, '65). The observation of Hampton ('58) that colloidal particles introduced into the space of Disse are incorporated into these vesicles seems to support this view.

Over much of their perimeter, neighboring hepatic cells face each other across a narrow intercellular space. This space varies in width, and is interrupted at intervals by various types of junctions between the adjacent cells (Fawcett, '55; Ashworth and Sanders, '60; Hampton, 60, '64; David, '61). Small dense droplets have been observed in the space (Cossel, '62), and Hampton ('64) described small vesicles in the adjacent cytoplasm.

The extracellular spaces in other tissues are believed to contain interstitial fluid which drains to the bloodstream in the lymphatics. Although the liver produces relatively large volumes of lymph, little is known of its origin. Terminal lymphatics have not been described within the hepatic lobule, and the relative contributions made by lobules and by periportal areas to the hepatic lymph have not been determined (Brauer, '63). In this connection it would be interesting to determine whether a true extravascular interstitial fluid space exists within the hepatic lobule and serves as a reservoir from which lymph drains in a manner analogous to its drainage from other tissues.

Although such topics as the differences in structure between different regions of the surface of hepatic cells, the nature of the junctions between neighboring hepatic cells, and the existence of a true interstitial fluid space have been treated incidentally and sporadically by several papers in the literature, they have not been systematically investigated for their own sake. The present paper presents detailed quantitative and morphologic data concerning the entire surface of parenchymal cells of the mouse liver, and also contains a description of the structure and location of various types of intercellular junctions along their perimeter. In addition, ferritin was introduced into the circulating blood to serve as a tracer so that the accessibility of extravascular spaces within the lobule to constituents of plasma could be examined.

MATERIALS AND METHODS

Mice of either the Cal A or the C3H strain were allowed free access to food until the experiments were started in the mornings. The mice were anesthetized with pentobarbitone sodium. A midline incision was made through the abdominal wall and thin strips of liver were removed into a drop of chilled fixative. The strips were cut into small blocks which were immersed in fixative. In some experiments, fixation was initiated by dripping chilled fixative onto the surface of the liver for ten minutes before a thin strip of fixed tissue was removed, cut into blocks and immersed in fixative.

Some mice received an intravenous or intraperitoneal injection of a 10% ferritin solution before the liver specimens were collected. Prior to injection, the solution of ferritin was dialyzed against sodium versenate to remove traces of cadmium (Farquhar, Wissig and Palade, '61). Specimens of liver were removed up to 80 minutes after 0.5 ml of ferritin solution was injected into the tail vein of unanesthetized mice or into the external jugular

vein of anesthetized mice or 150 minutes after 1.0 ml was injected into the peritoneal cavity of unanesthetized mice.

The blocks of liver which were collected in each experiment were all fixed for 1–2 hours in buffered solutions of either osmium tetroxide or glutaraldehyde. One or 2% solutions of osmium tetroxide were buffered to a pH of 7.5 with either acetate-veronal (Palade, '52), phosphate (Millonig, '62) or *s*-collidine (Bennett and Luft, '59). Redistilled glutaraldehyde was buffered to pH 7.5 with either cacodylate or phosphate (Sabatini, Bensch and Barrnett, '63).

The blocks were subsequently dehydrated in acetone and embedded in either Araldite or Epon (Luft, '61). Some of the blocks were immersed in a solution of 1% potassium permanganate in acetone for five minutes during dehydration (Parsons, '61).

Thin sections were mounted on grids covered with formvar that had been stabilized with carbon, and were stained with both uranyl acetate (Watson, '58) and a lead salt (Millonig, '61; Reynolds, '63). The sections were examined with a Siemens Elmiskop 1.

Quantitative data

In the first series, measurements were made of the total perimeter of hepatic parenchymal cells, and of the percentage of the perimeter which bordered bile canaliculi, the space of Disse and its extensions, and the narrow intercellular space. A flexible ruler was used to make these measurements on cells magnified 6,400 × that had been sectioned through the approximate center of their nuclei. It is possible that some of the cells contained more than one nucleus, and in this case the plane of section may not have passed through the equator of the cell. In segments of the surface coated with microvilli, the total length of the plasma membrane could not be accurately measured at this magnification. For this reason, the linear extent of the surface bordering bile canaliculi was measured as the distance between the luminal extremities of the tight junctions at the canalicular margins (line 'A', fig. 1a), and that bordering the space of Disse and its extensions was measured across the bases of the microvilli (line 'A', fig. 1b). Hepatic cells appeared polygonal in outline when viewed in equatorial section, and the number of sides forming their perimeter, as well as the number of bile canaliculi, desmosomes and stud-like processes at their surface was recorded (figs. 2–4). These measurements were all made on 22 cells from various regions of hepatic lobules from four mice.

In the second series, the increase in length of the plasma membrane resulting from the presence of microvilli along segments of the cell surface was estimated on micrographs with a final magnification of 56,000–84,000 ×. Within bile canaliculi, the total length of the plasma membrane covering microvilli, isolated profiles of microvilli and intermicrovillous spaces

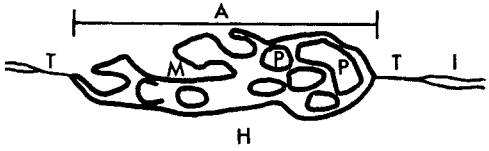

Fig. 1a Diagram of bile canaliculus showing method used to estimate the increase in length of plasma membrane resulting from the presence of microvilli. The total length of the plasma membrane within the canaliculus (thick line) was compared with the length of the long axis of the canaliculus (line 'A'). *Abbreviations: H,* hepatic cell; *M,* microvillus; *T,* tight junction at margin of canaliculus; *I,* intermediate junction; *P,* isolated profile of microvillus.

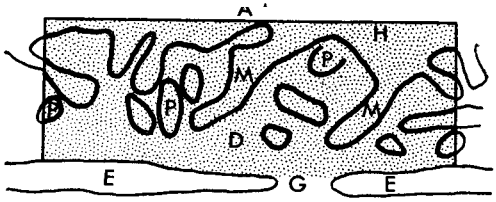

Fig. 1b Diagram of hepatic cell surface facing the space of Disse showing method used to estimate the increase in length of the plasma membrane resulting from the presence of microvilli. The total length of hepatic plasma membrane within the stippled area (thick line) was compared with the linear extent of the cell surface upon which the microvilli are located (line 'A'). *Abbreviations: H,* hepatic cell; *M,* microvilli; *P,* isolated profiles of microvilli; *E,* sinusoidal lining cell; *G,* gap between sinusoidal lining cells.

(thick line, fig. 1a), was measured with a flexible ruler and compared with the linear extent of the surface bordering the canaliculi (line 'A', fig. 1a). Similar measurements were made within the space of Disse. A line was drawn across the bases of the microvilli and at either end a perpendicular line was drawn to intersect the plasma membrane of the endothelial cell lining the sinusoid. The total length of the hepatic plasma membrane within the roughly rectangular area defined by these three lines and the plasma membrane of the endothelial cell (stippled area, fig. 1b) was measured and compared with the linear extent of the surface (line 'A', fig. 1b).

In the third series, the percentage of the narrow intercellular space occluded by tight junctions was estimated on a set of micrographs with a final magnification of 56,000–84,000 ×. The tight junctions at the margins of bile canaliculi were not included in these estimations.

No attempt was made to relate these linear measurements to the surface of the intact cell because of the complexity and lack of uniformity of the hepatic cell surface.

In the final series, the thickness of different segments of the hepatic plasma membrane was measured by a method similar to that described by Millington ('64). Electron micrographs taken at an initial magnification of 40,000 × were enlarged photographically to 300,000 × and the membrane was measured with a handlens containing a scale calibrated to 0.1 mm. To minimize errors arising from the effects of fresnel fringes, all micrographs used for these measurements were taken by one microscopist.

OBSERVATIONS

Mouse hepatic cells sectioned through the center of their nuclei appear polygonal in outline (fig. 2). A typical hepatic cell has four sides facing its neighbors across a narrow intercellular space and two sides facing the space of Disse surrounding visible sinusoids (figs. 2, 3). The narrow intercellular space is continuous with the space of Disse and its extensions between adjacent hepatic cells, but is interrupted at intervals by bile canaliculi (figs. 2–4).

The wall of a bile canaliculus is generally formed by two hepatic cells, although as many as one-quarter are limited by three cells. Each canaliculus formed by two cells is roughly elliptical in outline and its long axis, which coincides with a line joining the luminal extremities of the tight junctions at its margins, is often of the order of 1 μ in length. The long axes of canaliculi comprise about 6% of the perimeter of hepatic cells (table 1), but, because of the presence of microvilli, the length of plasma membrane within the canaliculi represents a considerably higher percentage of the total plasma membrane of the cell. For example, in one series of measurements the length of the plasma membrane within each canaliculus was 7.8 ± 0.4 times greater than the long axis of the elliptical cross section of the canaliculus. Although occasionally a canaliculus and part of the space of Disse are located in close proximity, they are usually separated by a segment of narrow intercellular space several microns in length. This intercellular space is always separated from the lumen of the canaliculus by a tight junction (Farquhar and Palade, '63). The constituents of junctional complexes at the margins of canaliculi well be considered in more detail in a subsequent section.

The space of Disse surrounds each sinusoid, following its general contour, but it also extends for considerable distances between hepatic cells adjacent to the sinusoid (figs. 8, 15, 16). These extensions of

Fig. 2 Tracing of the principle features in a montage of electron micrographs of part of a mouse hepatic lobule. The plane of section did not pass through the nuclei of most hepatic cells, but did pass through the approximate center of the nuclei (*HCN*) of three cells near the middle of the picture. These cells each have six sides, of which either one or two contact the space of Disse (stippled) surrounding visible sinusoids (*S*). Most of the remaining sides are in contact, along part of their length, with extensions of the space of Disse. These are shown as stippled areas between adjacent hepatic cells. The space of Disse is continuous with the narrow intercellular space which is interrupted at intervals by bile canaliculi (arrowed), tight junctions and desmosomes (not shown), but is generally continuous around studlike junctions (*J*). A white blood cell in the space of Disse (*WBC*) and a nucleus of a sinusoid lining cell (*ECN*) are also shown.

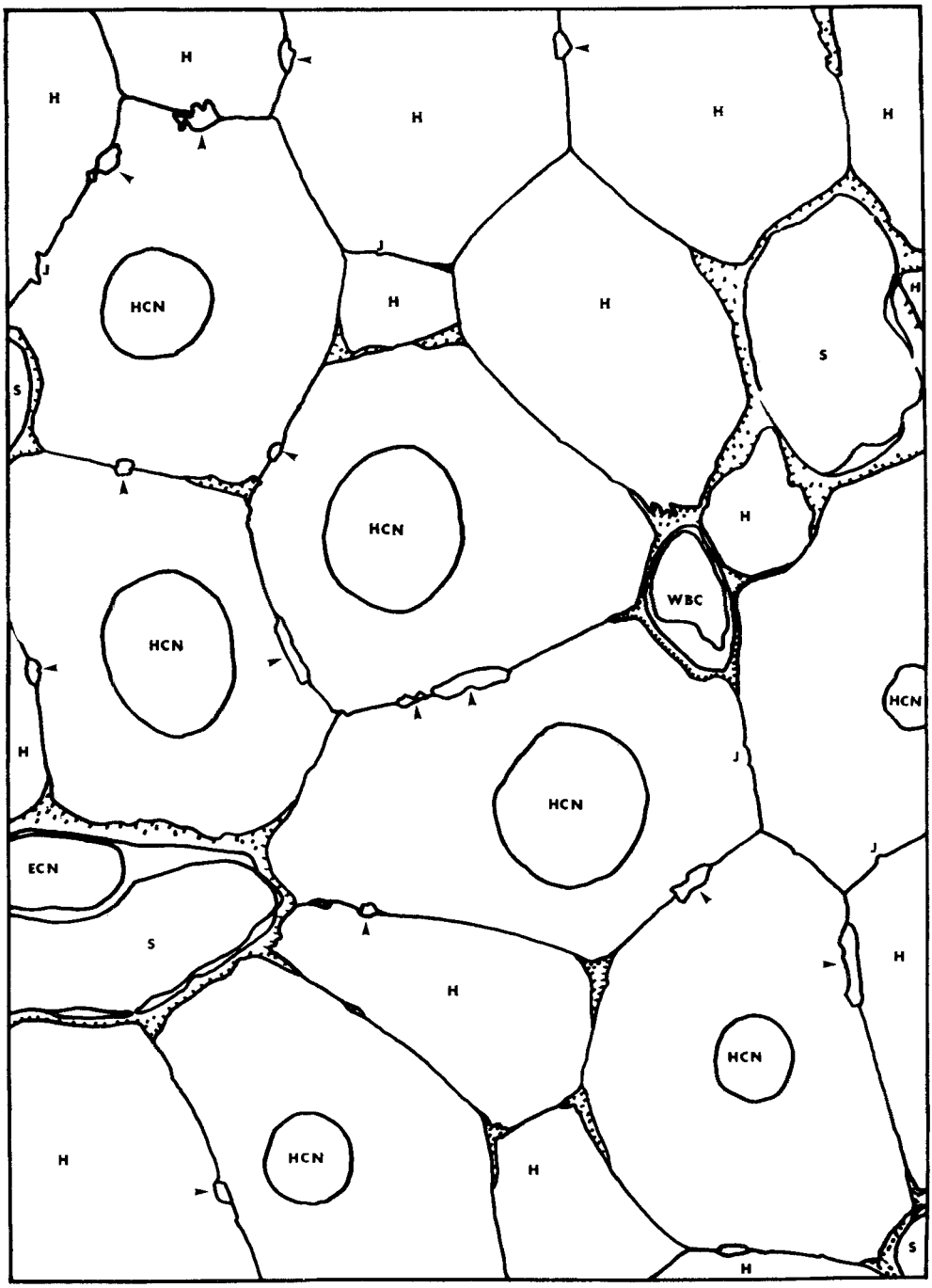
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Figure 2

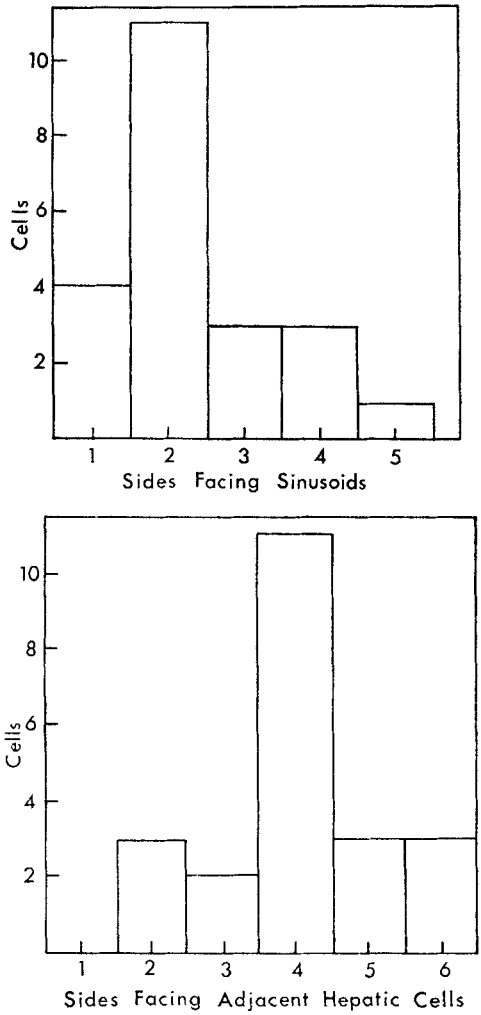

Fig. 3 Number of sides of each hepatic cell which face sinusoids (top) and adjacent hepatic cells (bottom). Measurements were made on 22 cells sectioned through the center of the nucleus.

the space of Disse often appear in sections as isolated dilations of the intercellular space which may bear superficial resemblance to bile canaliculi (fig. 9). The criteria used for distinguishing them will be discussed later. Although about one-third of the sides of hepatic cells face the space of Disse surrounding visible sinusoids (fig. 3), more than 80% of the remaining sides contact at least one extension of the space of Disse and about 10% are in contact with an extension of the space of Disse along their entire

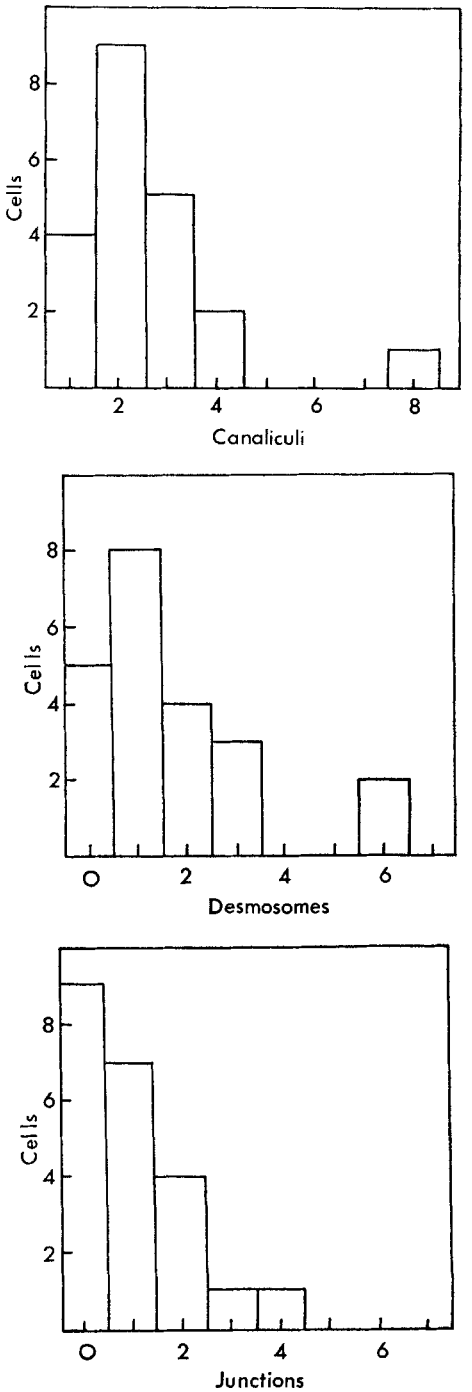

Fig. 4 Number of bile canaliculi (top), desmosomes (middle) and studlike junctions (bottom) at the perimeter of each of 22 cells sectioned through the center of the nucleus.

TABLE 1

Extracellular spaces in contact with the perimeter of hepatic cells

Average total perimeter [1]	76 ±3.4 μ
Percentage of perimeter facing:	
Bile canaliculi [2]	6.3 ± 1.3%
Space of Disse [3,4]	17 ±2.1%
Extension of space of Disse [4]	25 ±2.2%
Narrow intercellular space	54 ±4.5%

[1] Mean perimeter of 22 cells (from four mice) sectioned through the approximate center of their nuclei.
[2] Long axes of canaliculi measured between luminal extremities of tight junctions at their margins (i.e., line "A," fig. 1a).
[3] Those portions of the surface facing space of Disse surrounding visible sinusoids.
[4] Linear extent of cell surface, measured across bases of microvilli (i.e., line "A," fig. 1b).

length. In fact, an average of more than 40% of the total linear extent of the perimeter of each hepatic cell is in contact with a space of Disse or its extensions (table 1). Because of the presence of microvilli in this region, however, a higher percentage of the total length of the plasma membrane is in contact with the space of Disse. In one series of measurements, the length of plasma membrane covering microvilli and intermicrovillous spaces was 6.0 ± 0.8 times greater than the linear extent of the cell surface bordering this space.

Surfaces bordering bile canaliculi. The microvilli in bile canaliculi project in more or less random directions from the surface of the hepatic cells, and those which are not oriented parallel to the plane of section appear as isolated profiles in the canalicular lumen (figs. 9–11). The microvilli contain fine filaments which are more numerous near the limiting membrane and which appear continuous with a cytoplasmic feltwork of fine filaments which surrounds each canaliculus (figs. 9–11). We did not observe the more complex structure of microvilli described by David ('61) in canaliculi and by McNabb and Sanborn ('64), Graney ('64), and Rostgaard and Barrnett ('65) in the intestinal mucosa.

Vesicular invaginations were absent from the limiting membranes of canaliculi, and no vesicles were present in the adjacent cytoplasm. These observations suggest that pinocytosis is not a prominent phenomenon at this surface, but they provide no information about the mechanism by which bile constituents leave hepatic cells.

In sections that have been fixed in osmium tetroxide and stained with uranium and lead salts, the plasma membrane limiting the canaliculi and the space of Disse is thicker than that along the narrow intercellular space (table 2). These membranes all appear slightly thicker in Epon-embedded than in Araldite-embedded tissues. In all cases, the inner and outer leaflets stain with similar intensity (fig. 10), and all of the three leaflets are of approximately equal thickness (20–30 Å).

The outer, dense leaflets of the plasma membranes of adjacent hepatic cells fuse to form the central leaflet of a tight junction at each lateral margin of a canaliculus (figs. 9–11). These tight junctions, which vary in length from 0.1–0.3 μ (0.16 ± 0.01 μ), are often continuous with intermediate junctions (Farquhar and Palade, '63). These intermediate junctions are much less clearly defined than tight junctions and vary in length from 0.1–0.25 μ. They differ from tight junctions in that the apposed plasma membranes do not fuse, but are separated by a space 130–220 Å in width. The cytoplasm adjacent to tight junctions and intermediate junctions is relatively dense, especially in tissues fixed in osmium tetroxide, and it contains a feltwork of fine filaments continuous with that which surrounds each canaliculus (figs. 10, 11). Intermediate junctions occur only near the bile canaliculi and are not present at any other points on the cell perimeter. At the distal end of an intermediate junction a desmosome or a second tight junction is occasionally present (figs. 9–11), but in most cases the space between the plasma membranes is continuous with the narrow intercellular space.

Surfaces bordering the space of Disse. The microvilli which extend in various directions from this surface of the hepatic cell are curved and occasionally branched, and in thin sections many appear as isolated profiles in the space of Disse (figs. 6, 8, 12–14). These microvilli are of relatively uniform width (0.07–0.12 μ) but are often slightly constricted near their base (figs. 12, 14). They do not contain either vesicles or surface invaginations and do not appear to have any internal structure apart from a fine filamentous feltwork

TABLE 2

Thickness of the plasma membrane of hepatic cells [1]

Cell	Embedding medium	Location of membrane		
		Microvilli of bile canaliculi	Microvilli in space of Disse	Facing narrow intercellular space
		Å	Å	Å
1	Araldite	73	68	63
2	Araldite	75	70	66
3	Araldite	76	79	64
4	Araldite	79	72	66
5	Epon	88	82	75
6	Epon	88	80	66

[1] Means of ten measurements made along each of three segments of the plasma membrane are shown for six cells. These cells were from specimens fixed in osmium tetroxide and stained with both uranium and lead salts. In addition, the specimens containing cells 1 and 2 were treated with potassium permanganate during dehydration.

(figs. 13, 14). This feltwork, which extends into the microvilli from the adjacent cytoplasm, can often be seen more clearly in specimens fixed in glutaraldehyde and post-fixed in osmium tetroxide than in specimens fixed in buffered osmium tetroxide alone. The ends of almost all the microvilli in our preparations were free in the space of Disse, and no microvilli were closely attached to sinusoidal lining cells in the manner described by Wasserman ('58).

A finely fibrillar material extends throughout the space of Disse and is continuous with the plasma through gaps in the walls of the sinusoids. The fibrillar material in the space of Disse has a similar density and texture to that in the lumen of the sinusoids (figs. 6, 12), and is thought to represent the image of fixed plasma protein. Small fat droplets, 0.1–0.15 μ in diameter, may occur in the space of Disse and in the lumen of the sinusoids (figs. 12, 15, 16), but their concentration is often higher in the space of Disse than within the sinusoids.

Two types of vesicular invaginations of the plasma membrane occur between the bases of the microvilli which project into the space of Disse. The first type, the "coated" invagination, is about 0.1 μ wide and may be sharply constricted at its opening into the space of Disse (figs. 13, 14). These invaginations contain material of the same density and structure as that in the space of Disse, but do not appear to incorporate fat droplets. They are thought to represent stages in the formation of "coated" vesicles which occur in the hepatic cell cytoplasm near the space of Disse (fig. 13). Coated vesicles are also about 0.1 μ in diameter and are limited by a membrane of the same density and thickness as the plasma membrane. Each coated vesicle and coated invagination is bordered by a narrow band of increased cytoplasmic density which contains periodic dense striations oriented at right angles to the limiting membrane (figs. 13, 14). Coated vesicles do not appear to contain fat droplets, but are filled with a fine filamentous material of slightly greater density than that in the space of Disse. Vesicles of this type have been described previously in a number of tissues including the rat liver and have been variously termed "coated," "complex" and "alveolate" vesicles (Farquhar, Wissig and Palade, '61; Gray, '61; Roth and Porter, '62, '64; Palay, '63; Rosenbluth and Wissig, '63; Bruni and Porter, '65).

The second type of invagination is also about 0.1 μ wide and may be constricted at its opening into the space of Disse (figs. 12, 15, 16). Pinocytotic vesicles limited by a membrane which appears identical to the plasma membrane occur in the adjacent cytoplasm (fig. 12), and are thought to be derived from these invaginations. Pinocytotic vesicles and invaginations differ from the coated structures in that the cytoplasmic leaflet of the limiting membrane lacks any form of specialization. They contain a finely filamentous material, but fat droplets, which completely fill some vesicles, may also be

present (fig. 12). Pinocytotic vesicles are generally larger and more irregular than coated vesicles, and vary in diameter from about 0.1–0.5 μ.

A third type of vesicle, the small dense vesicle, is also present in the hepatic cell cytoplasm near the space of Disse, and is more obvious in specimens fixed in glutaraldehyde (fig. 15). These vesicles are all about 0.1 μ in diameter and are filled with a moderately dense, homogenous material. They do not resemble either of the types of surface invaginations described above, but may contain fat droplets that could be derived from the space of Disse.

In addition to these three types of vesicles, large (up to 0.5 μ) and relatively irregular "vesicles" may occur in the hepatic cell cytoplasm very near the space of Disse in regions where the plasma membrane is tortuous (fig. 8). These "vesicles", which are limited by a membrane identical to the plasma membrane, contain material which appears the same as that in the space of Disse. They are thought to represent cross sections of indentations of the space of Disse into the surface of the hepatic cell. We did not observe the "granule-containing vacuoles, with a dense matrix," which were described by Bruni and Porter ('65) in the cytoplasm near the space of Disse in rat liver.

Although extensions of the space of Disse may bear superficial resemblance to bile canaliculi, they differ in a number of morphologic characteristics. For instance, a finely fibrillar material extends throughout the space of Disse, but canaliculi are relatively empty except for sparse finely fibrillar or granular material (figs. 9–11). The lumen of each canaliculus is always separated from the adjacent narrow intercellular space by tight junctions, but tight junctions do not generally occur at the confluence of the space of Disse with the narrow intercellular space (figs. 8–11, 15). In sections from the livers of mice which have received exogenous ferritin, ferritin molecules are present throughout the space of Disse, but are absent from bile canaliculi (see later section). In addition, vesicles and surface invaginations occur along the borders of the space of Disse (figs. 12–16), but are absent from the borders of canaliculi.

Surfaces bordering the narrow intercellular space. A narrow intercellular space contacts a large proportion of the perimeter of hepatic cells (table 1). This space is relatively uniform in width (220 ± 14 Å), and the limiting membranes, which are thinner than those covering other segments of the cell surface (table 2), are generally smooth or slightly curved (figs. 5, 7, 8, 17). They may be more convoluted in localized regions and may even separate to form small dilatations of the space. These dilated segments often contain small droplets of fat (figs. 17, 18).

The plasma membranes of the apposed cells are modified at various points to form junctions across the narrow intercellular space. The outer leaflets of the apposed membranes often fuse to form the central leaflet of a tight junction (figs. 9, 18, 19). These central leaflets are always thinner than either of the outer leaflets from which they are derived, and may be barely visible even at high magnification (figs. 18, 19). The tight junctions along the narrow intercellular space resemble those at the margins of bile canaliculi, but the adjacent cytoplasm does not contain any evidence of filaments or of increased density (fig. 19). The tight junctions are generally straight (fig. 18), but irregular and tortuous froms may be present. In some cases, a series of tight junctions may occur in which the individual junctions are separated by short segments of narrow intercellular space. The distance between the cytoplasmic margins of tight junctions remote from bile canaliculi is 100–150 Å, and the junctions vary in length from 0.5–2.0 μ (1.0 ± 0.1 μ). They occupy about one-fifth (20 ± 2.0%) of the length of cell sides which border the narrow intercellular space.

Desmosomes, which vary in length from 0.1–0.2 μ (0.13 ± 0.01 μ), may also occur along the narrow intercellular space (fig. 11). The structure of hepatic desmosomes will not be considered as they have already been described in detail in the literature (Farquhar and Palade, '63).

Studlike processes that project from the surface of one hepatic cell into a concavity in the surface of an adjacent cell (Fawcett, '55) are the least common form of junction. In this junction, the apposed

plasma membranes are of the same thickness and staining intensity as the membranes that border the adjacent narrow intercellular space (figs. 17, 19). This space is generally continuous around each studlike process (fig. 19), but occasionally the apposed plasma membranes fuse to form a tight junction within the studlike junction.

Compound vesicular structures may occur in the hepatic cell cytoplasm near the narrow intercellular space, and these are thought to represent transverse sections of studlike junctions (fig. 5). The structures are 0.2–0.4 μ in diameter and contain a membrane-bounded core which has the same density and texture as the surrounding cytoplasm. This core is separated from the external limiting membrane by a narrow (100–200 Å) ring of low density which presumably represents a segment of narrow intercellular space within the studlike junction. Both the external membrane and the membrane surrounding the core have the same thickness and structure as the plasma membrane bordering the narrow intercellular space.

The desmosomes, studlike junctions and tight junctions remote from bile canaliculi are distributed more or less at random along the narrow intercellular space. A few cell sides lack cell junctions except for those at the margins of the canaliculi, and an occasional cell side lacks both cell junctions and bile canaliculi.

Coated and pinocytotic vesicles and invaginations occur in the region of the narrow intercellular space, but are much less common than near the space of Disse. Neither small dense vesicles nor vesicular structures believed to be cross sections of pockets of the space of Disse have been observed in the hepatic cell cytoplasm near the narrow intercellular space.

Distribution of exogenous ferritin. A high concentration of ferritin molecules was present in the narrow intercellular space, the space of Disse and the hepatic sinusoids of all mice which had received injections of ferritin (figs. 12, 13, 15, 16, 18, 19). We were not able to determine whether consistent variations existed in the concentration of ferritin molecules between these areas.

At the margins of bile canaliculi, ferritin molecules were occasionally present between the apposed plasma membranes of intermediate junctions, but did not appear either between the leaflets of tight junctions or in the lumens of canaliculi. Ferritin molecules were not found between the leaflets of tight junctions elsewhere on the plasma membrane although they did appear within short segments of narrow intercellular space separating adjacent tight junctions (fig. 18). From these results, we conclude that tight junctions are impermeable to ferritin and that the narrow intercellular space is continuous around circumscribed segments of the tight junctions remote from bile canaliculi. Ferritin molecules were present between the apposed plasma membranes within studlike junctions (fig. 19), verifying the continuity of the narrow intercellular space around these junctions.

Ferritin molecules were also present within coated and pinocytotic invaginations and vesicles in the region of the space of Disse and the narrow intercellular space (figs. 12, 13). Some of the pinocytotic vesicles contained both fat droplets and ferritin molecules (fig. 12). The concentration of ferritin molecules in the vesicular structures which are thought to represent cross sections of small pockets of the space of Disse was the same as the concentration in the adjacent areas of the space of Disse.

DISCUSSION

In conventional histologic sections of mammalian liver, the lobule seems to consist of more or less straight cords, one parenchymal cell in thickness, that converge from the periphery of the lobule to terminate at the central vein. After analysing such preparations Elias ('49) concluded that the radial cords of parenchymal cells in fact represent sections through relatively flat plates of cells. These plates are separated from one another by sinusoids of relatively uniform diameter which converge towards the central vein into which they ultimately empty.

When thin sections of liver are examined with the electron microscope, most sinusoids appear circular in outline. The appearance of the sinusoids in thin sec-

tions can best be reconciled with their appearance in the considerably thicker conventional histologic sections if we assume that they pursue a relatively serpentine course through fenestrations in the plates of liver cells. Their lateral digressions may not be sufficiently great to carry them out of the plane of a thick histologic section, but could cause them to repeatedly leave and re-enter the plane of thin sections.

If the sinusoids do pursue a serpentine course, we should expect that the plane of thin sections would sometimes transect the space of Disse surrounding a sinusoid which was out of the plane of section (fig. 6). These tangential sections of the space may resemble transverse sections of radial extensions of the space of Disse. Thus some of the "extensions of the space of Disse" which we observed may have represented tangential sections through the roughly annular space surrounding the sinusoids. However, most appeared to be sectioned normally and are thought to represent radial extensions of the space of Disse between hepatic cells adjacent to the sinusoids.

The extracellular spaces. Bile canaliculi extend throughout the liver lobule, but are separated from the remainder of the extracellular spaces by tight junctions. Although Rouiller ('56) and Ashworth and Sanders ('60) described open connections between the canalicular lumen and the space of Disse, a number of authors have provided evidence to the contrary, (Fawcett, '55; Hampton, '58; Novikoff and Essner, '60; Daems, '61; Farquhar and Palade '63). Farquhar and Palade ('63) demonstrated that tight junctions exist at the margins of canaliculi and concluded that these tight junctions are impermeable to protein molecules. In our studies, tight junctions were always present at each canalicular margin, and these appeared to prevent the entry of ferritin molecules into the canaliculi. Farquhar and Palade ('63) also provided evidence which suggests that tight junctions may be impermeable to smaller molecules and possibly to water. If this is true, all the biliary constituents from the blood must pass through hepatic cells in order to reach the canaliculi.

The narrow intercellular space was wider in our studies than in those of other authors (Fawcett, '55; Ashworth and Sanders, '60; Daems, '61) but these variations may reflect different methods of specimen preparation (c.f. Hampton, '60). Major changes in the width of the space may result from relatively minor changes in the volume of the adjacent cells during fixation and embedding. The width of the narrow intercellular space may also very depending on the species (c.f. David, '61) and on the physiological condition of the animal. For example, the width of the space between epithelial cells in the intestine apparently varies in different nutritional conditions (Sjostrand, '63).

Several types of modifications of the cell surfaces occur along the narrow intercellular spaces. In tissues embedded in methacrylate, Fawcett ('55) and Daems ('61) described stud-like processes which extend from one hepatic cell into a corresponding recess on the surface of an adjacent cell. However, when Hampton ('60) examined liver specimens embedded in Araldite, he was unable to demonstrate these processes and concluded that they were alterations due to distortion during embedding or sectioning. We have found these processes in specimens embedded both in Araldite and in Epon and believe that they probably do occur in the living animal. However, because they are relatively uncommon, and because the narrow intercellular space is continuous around the processes, we believe that they do not exert any significant function in cell cohesion.

Desmosomes are present along the narrow intercellular space but the apposed plasma membranes do not appear to be joined by any type of cement substance, and the narrow intercellular space appears to continue without interruption between the two membranes. The apposed plasma membranes with their associated fibril-containing areas of increased cytoplasmic density may act as focal points of structural rigidity. However, as desmosomes are not common in the liver lobule, and as each desmosome covers a very small proportion of the total cell surface, their importance is probably slight.

It seems likely that the main intercellular attachment devices in the liver lobule are the tight junctions which occur at the margins of the canaliculi and at frequent intervals along the narrow intercellular space. These tight junctions appear to be essentially similar to the "external compound membrane" of Robertson ('58) and the "quintuple-layered cell interconnections" of Karrer ('60). Those which occur along the narrow intercellular space remote from canaliculi are not constant in position with reference to any landmark of the hepatic cell, and they are not present along all the cell sides which face the narrow intercellular space. For this reason, we believe that these junctions do not form a belt of any length around the cells, but that they are relatively circumscribed areas of fusion of the plasma membranes. The conclusion is supported by the results of experiments in which ferritin molecules introduced into the circulation were found along the length of the narrow intercellular space and between adjacent tight junctions. Although it is possible that ferritin molecules could have reached some parts of the narrow intercellular space by vesicular transport through hepatic cells, the amount of ferritin present in vesicles in these experiments did not appear sufficient to warrant serious consideration of this alternative explanation.

Transport of materials between hepatic cells and extracellular spaces. It has been suggested that some substances which are removed from the blood by cells lining the sinusoids are subsequently transferred to hepatic cells (Wasserman, '58). However, we were unable to find either microvilli closely attached to lining cells or any evidence to suggest that exogenous ferritin molecules could be transferred directly from the lining cells to microvilli of hepatic cells. These observations concur with those of Hampton ('58) and seem to indicate that plasma constituents must enter the space of Disse before they can be absorbed by hepatic cells.

Coated and pinocytotic vesicles are both concerned with uptake of these plasma constituents by hepatic cells, but show different degrees of selectivity in the nature of the materials they will ingest. Our observations on the uptake of exogenous ferritin molecules by coated vesicles would seem to confirm that the role of coated vesicles in protein uptake in the liver resembles that in other tissues, (Farquhar, Wissig and Palade, '61; Rosenbluth and Wissig, '63; Roth and Porter, '64; Bruni and Porter, '65). However, coated vesicles in liver cells do not appear to concentrate ferritin preferentially as they do in the cells of toad spinal ganglia (Rosenbluth and Wissig, '63).

Pinocytotic vesicles also incorporate ferritin molecules, but these vesicles are predominantly concerned with the uptake of fat droplets from the space of Disse and the narrow intercellular space. Fat droplets have been described previously in both the space of Disse and adjacent cytoplasmic vesicles, but their mode of entry into the hepatic cells has remained obscure (c.f. Ashworth, Stembridge and Sanders, '60; French, '63). Our observations appear to confirm the suggestion of Trotter ('65) that pinocytotic invaginations and vesicles play an important part in this process. However, we were unable to confirm the observation of Schlesinger and Essner ('65) that coated invaginations and vesicles may incorporate fat droplets into hepatic cells.

The fat droplets in our preparations were all between 0.1 μ and 0.14 μ in diameter, and we were unable to determine whether they were chylomicrons synthesized in the gut wall from exogenous fat, or lipoproteins derived from endogenous sources. The very low density lipoproteins vary in size up to 0.14 μ, and closely resemble small chylomicrons (c.f. Courtice and Garlick, '62; French, '63). In addition, physiologic experiments have revealed that both chylomicrons and lipoproteins are removed from the blood in the liver (French and Morris, '58; Bragdon and Gordon, '58; Stein and Shapiro, '60; Rodbell, Scow and Chernick, '64). If the fat droplets present in our preparations were chylomicrons, their uniform small size may indicate that only smaller chylomicrons can pass through the gaps in the sinusoidal walls. It is also possible that some of the droplets may have been lipoproteins which had been discharged from hepatic cells and were passing through the space of Disse to the bloodstream.

Although microvilli cover a large proportion of the surface of hepatic cells, their role in the transfer of materials between the hepatic cell and the extracellular spaces is unknown. Vesicles and surface invaginations do not occur in microvilli but appear to be confined to the intermicrovillous spaces. In this respect, microvilli of hepatic cells resemble those of epithelial cells of the intestinal wall (c.f. Palay and Karlin, '59; Lacy and Taylor, '62). However, fat droplets may be absorbed into intestinal microvilli (Rostgaard and Barrnett, '65) but do not appear to enter microvilli of hepatic cells.

Structure of the plasma membrane. On the basis of studies of tissues fixed in potassium permanganate, Robertson ('59, '61) postulated that cellular membranes consist of a central lipid leaflet bordered on both surfaces by layers of nonlipid material. Subsequent studies have revealed that considerable variations may occur within the framework of this simple unit membrane model. In tissues fixed in osmium tetroxide, the structure of plasma membranes may vary between different cells and between different parts of the same cell (Wissig, '62; Sjostrand, '63; Farquhar and Palade, '63; Millington, '64). For instance, the plasma membrane covering the microvilli of intestinal epithelial cells is substantially thicker than that covering the lateral and basal surfaces of the cell (Sjostrand, '63; Millington, '64) in the intestine. These differences may indicate that variations in the physiologic characteristics of the cell are reflected in slight changes in the structure of the membranes.

Variations in the methods used during preparation of specimens for examination may also cause alterations in the structure of membranes. In many epithelial cells which have been stained with either uranium or lead salts, the outer leaflet of the plasma membrane is thinner and less dense than the inner leaflet. If the cells are treated with potassium permanganate during dehydration, or if they are stained with both uranium and lead salts, the outer leaflet can be visualized more readily (Farquhar and Palade, '63). In our study, no differences were apparent between the inner and outer leaflets of the plasma membranes of hepatic cells stained with both uranium and lead salts.

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Abbreviations

c, bile canaliculus	*e*, extension of the space of Disse
d, space of Disse	*f*, fine filaments
dv, dense vesicle	*z*, zonula occludens (tight junction)

The mice from which the specimens shown in figures 8, 12, 13, 15, 16, 18 and 19 were obtained recevied an intraperitoneal or intravenous injection of ferritin solution prior to sacrifice.

PLATE 1

EXPLANATION OF FIGURE

5 Hepatic cells of the mouse liver are shown at relatively low magnification. The borders of sinusoids appear at the upper and lower margins of the figure. The hepatic cells are roughly polygonal in outline. Two bile canaliculi (*c*) interrupt the narrow space between hepatic cells. A cross section of a studlike junction (arrow) is shown near the center of the figure. × 10,000.

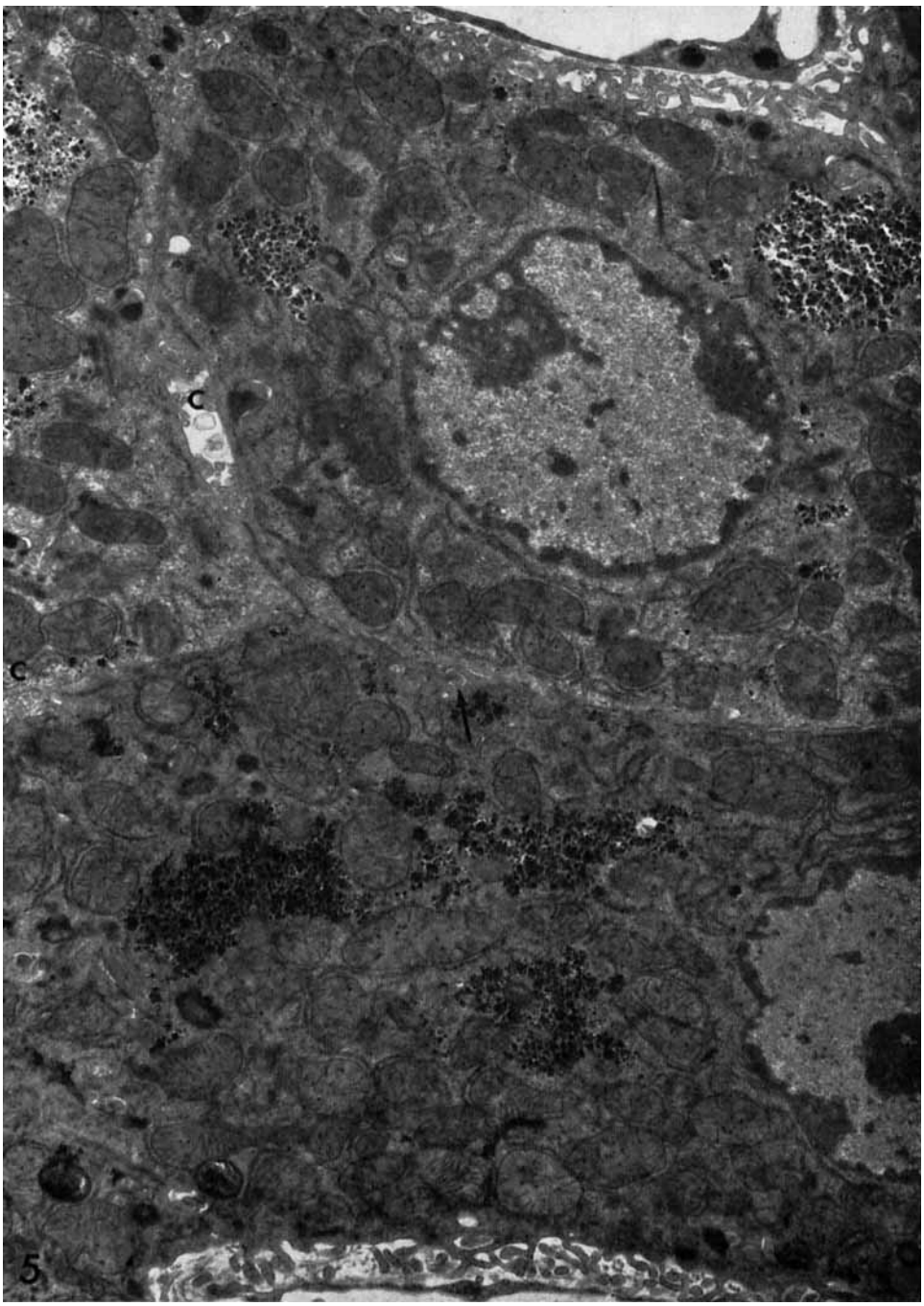

C
C
5

PLATE 2

EXPLANATION OF FIGURES

The figures illustrate the diversity of structure of intercellular spaces between hepatic cells. The intercellular space extends between spaces of Disse situated at the top and bottom of each figure.

6 The widely separated surfaces of the hepatic cells are coated with microvilli, and the space between them is broadly continuous with the spaces of Disse at the top and bottom of the figure. The lining cells of both sinusoids are sectioned obliquely. This field probably contains a single sinusoid sectioned at a point where it makes a serpentine bend. In the center of the figure, the sinusoid passes out of the plane of section which intercepts only its bordering space of Disse. × 27,000.

7 A narrow space separates the hepatic cells in this field. A bile canaliculus (*c*) with cross sections of microvilli filling its lumen interrupts the space midway along its length. A zonula occludens, poorly visualized at this low magnification, occurs at each lateral margin of the canaliculus. × 15,000.

8 A bile canaliculus (*c*) is situated close to a space of Disse (*d*) in the narrow space between hepatic cells. Its lumen, filled with microvillous profiles, is separated from the space of Disse above and the narrow intercellular space below by zonulae occludentes. A lumen (*e*) containing a few microvillous profiles dilates the narrow space near the sinusoid at the lower border of the figure. It is identified as an extension of the space of Disse because ferritin molecules can be detected in its lumen in the original micrograph and it lacks zonulae occludentes in its lateral margins (see text). A vesicular structure (arrow), believed to represent a cross section of an invagination of the space of Disse, is present in the lower part of the figure. × 25,000.

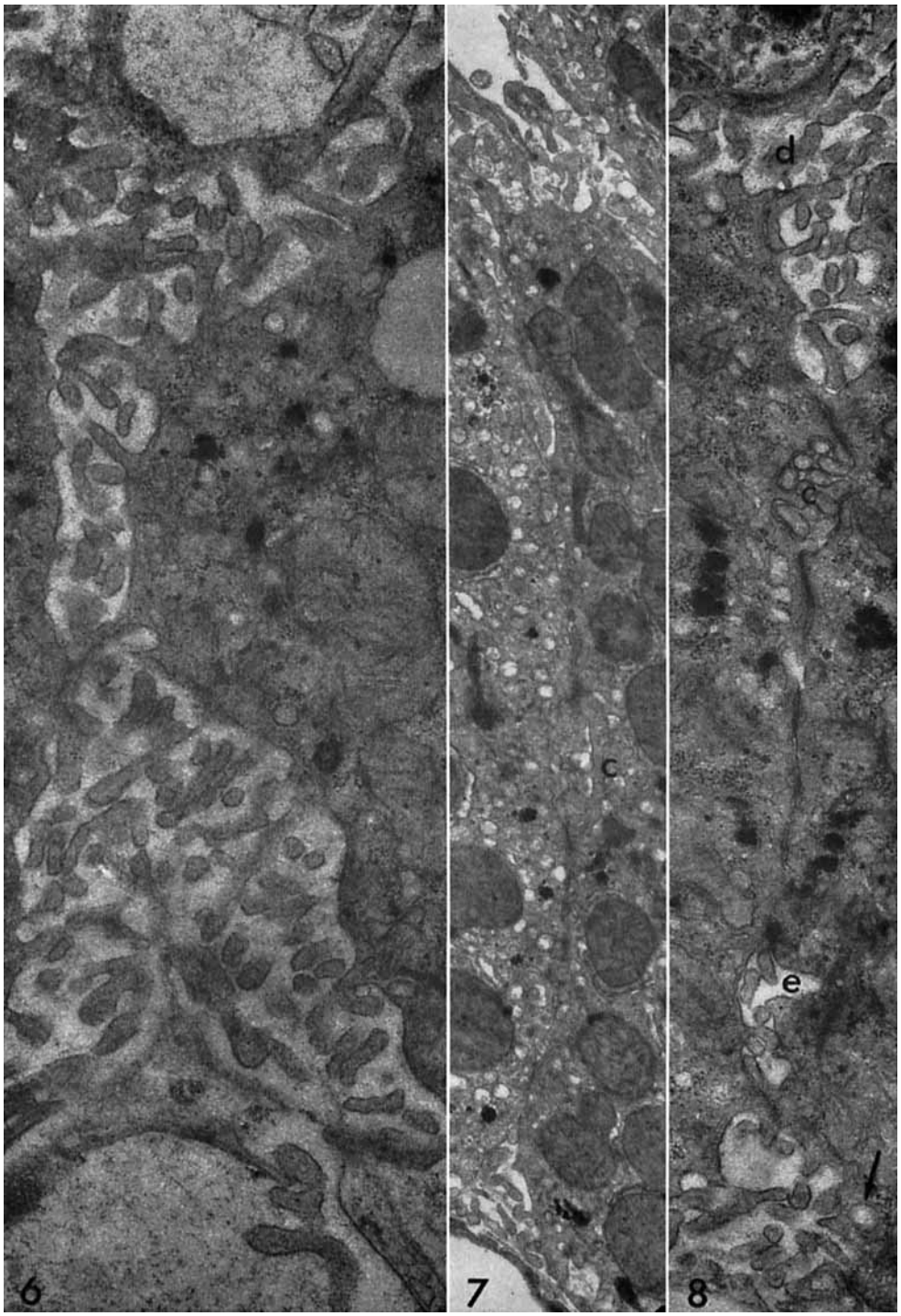

6
7
c
8
d
c
e

PLATE 3

EXPLANATION OF FIGURE

9 A bile canaliculus in a specimen fixed in buffered osmium tetroxide solution. The numerous microvillous profiles in its lumen are limited by distinct unit membranes, and sparse filamentous or granular material is seen in their cores. Fine filaments (*f*) appear in the bordering cytoplasmic matrix. Zonulae occludentes (*z*) occur at the lateral margins of the canaliculus and are bordered by fine filaments. A short distance from one zonula occludens, the narrow intercellular space is obliterated (arrow) where the plasma membranes of the hepatic cells are fused together. The lumen of the canaliculus appears empty, and there is no sign of vesicles either forming or emptying along its border. × 88,000.

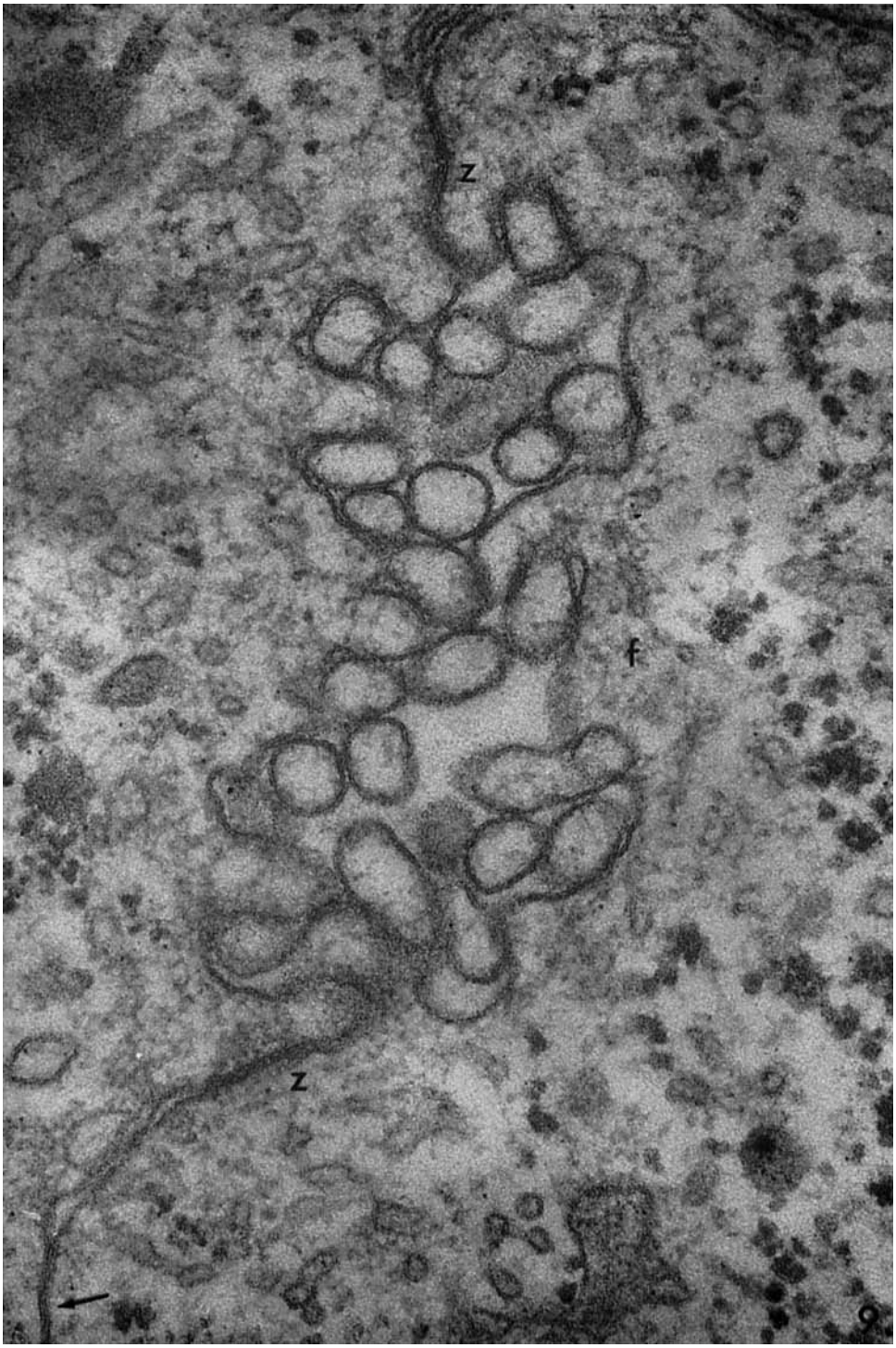

z
f
z

PLATE 4

EXPLANATION OF FIGURES

10 A zonula occludens (z) bordered by fine filaments is situated at a lateral margin of a bile canaliculus. Below it the plasma membranes are sectioned obliquely, and their relationship to each other cannot be discerned. There are abundant fine filaments in the adjacent cytoplasm. The membranes again appear sectioned perpendicularly near the lower margin figure (arrow), and here they are fused together. × 144,000.

11 At the lateral margin of a bile canaliculus the plasma membranes of adjacent hepatic cells fuse to form a zonula occludens (z) near its lumen. A desmosome (macula adhaerens) can be recognized in the lower portion of the figure. Between these two elements of a junctional complex, the plasma membranes are sectioned obliquely for most of their length, but they can be seen to be clearly separated at two points (arrows). It is not possible to decide with certainty whether an intermediate junction (zonula adhaerens) occurs in this region. A mesh of fine filaments stretching from the zonula occludens to the desmosome lines the cytoplasmic surface of the plasma membranes. × 120,000.

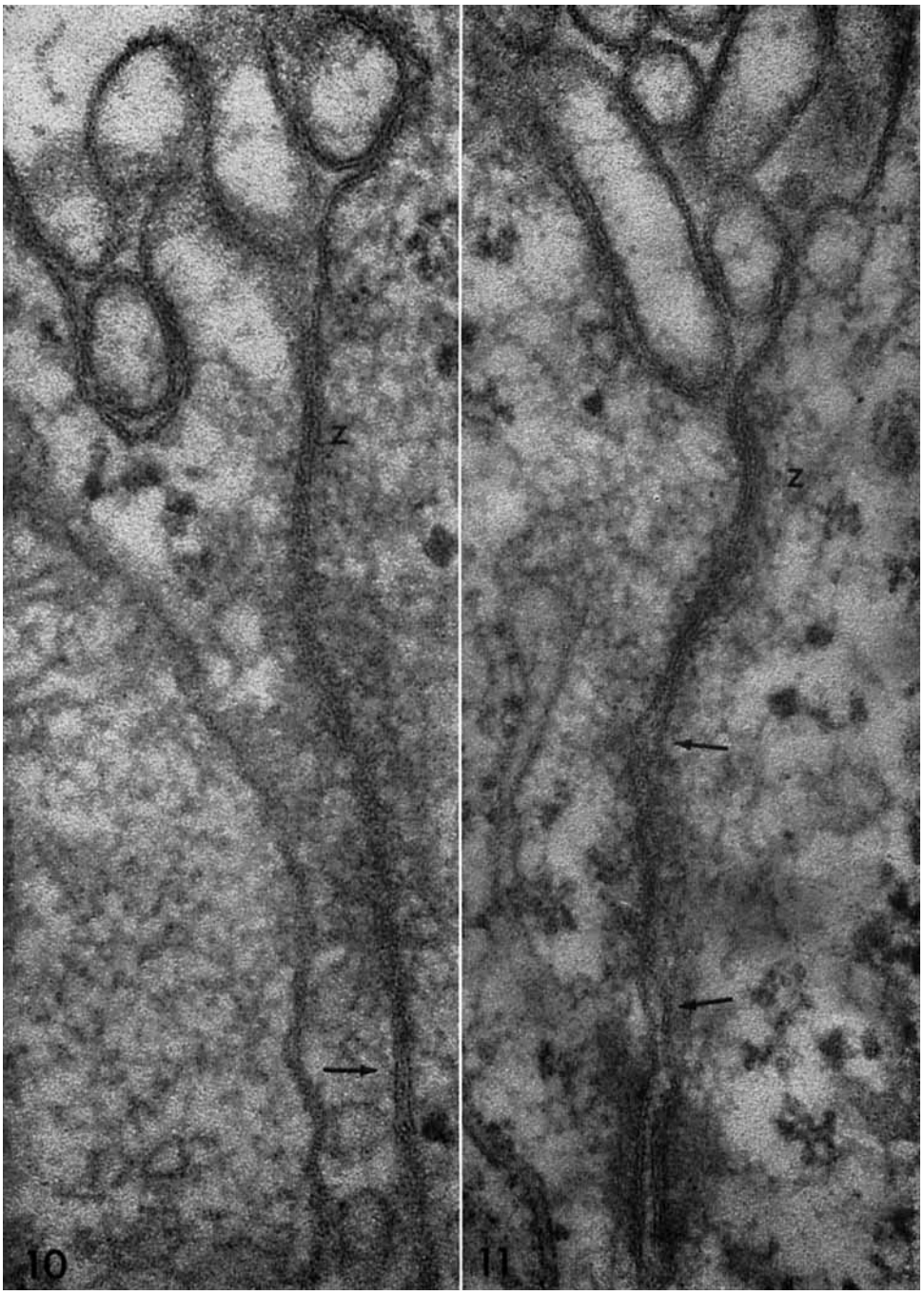

Z
Z
10
11

PLATE 5

EXPLANATION OF FIGURE

12 A dense concentration of ferritin molecules is seen in the sinusoidal lumen in the upper portion of the figure. Small droplets of moderate density (arrows) within it are identified as either chylomicrons or very low density lipoprotein particles. Several vesicles in the lining cell bordering the lumen contain numerous ferritin molecules. A few contain, in addition, a dense homogenous substance. Tortuous microvilli of the subjacent hepatic cell extend into the space of Disse which also contains a dense concentration of ferritin. The surface of the hepatic cell is deeply invaginated at one point (*1*) between the bases of adjacent microvilli. A pinocytotic vesicle containing ferritin molecules and faintly outlined lipid particles (2) appears free in the cytoplasm near the surface of the hepatic cell. × 91,000.

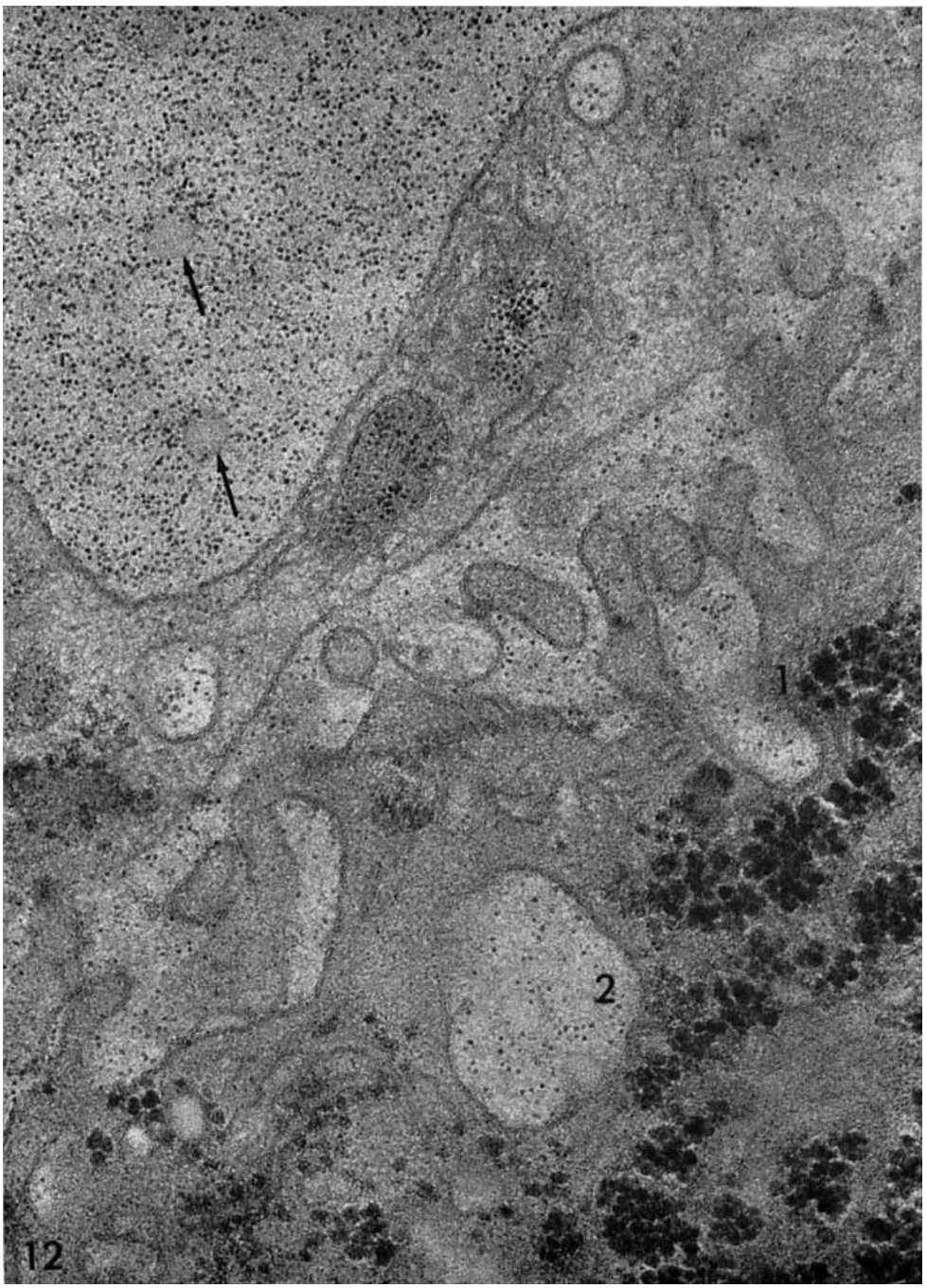

1
2
12

PLATE 6

EXPLANATION OF FIGURES

13 Arrows indicate coated vesicles containing ferritin in the sinusoidal lining cell (upper part of the figure) and hepatic cell (lower part of the figure). A vesicle containing only homogenous dense material (*dv*) appears near the surface of the hepatic cell. × 67,000.

14 Two coated invaginations (arrows) appear along the surface of a hepatic cell. A vesicle with dense homogenous content (*dv*) appears to the left of one of the invaginations. × 128,000.

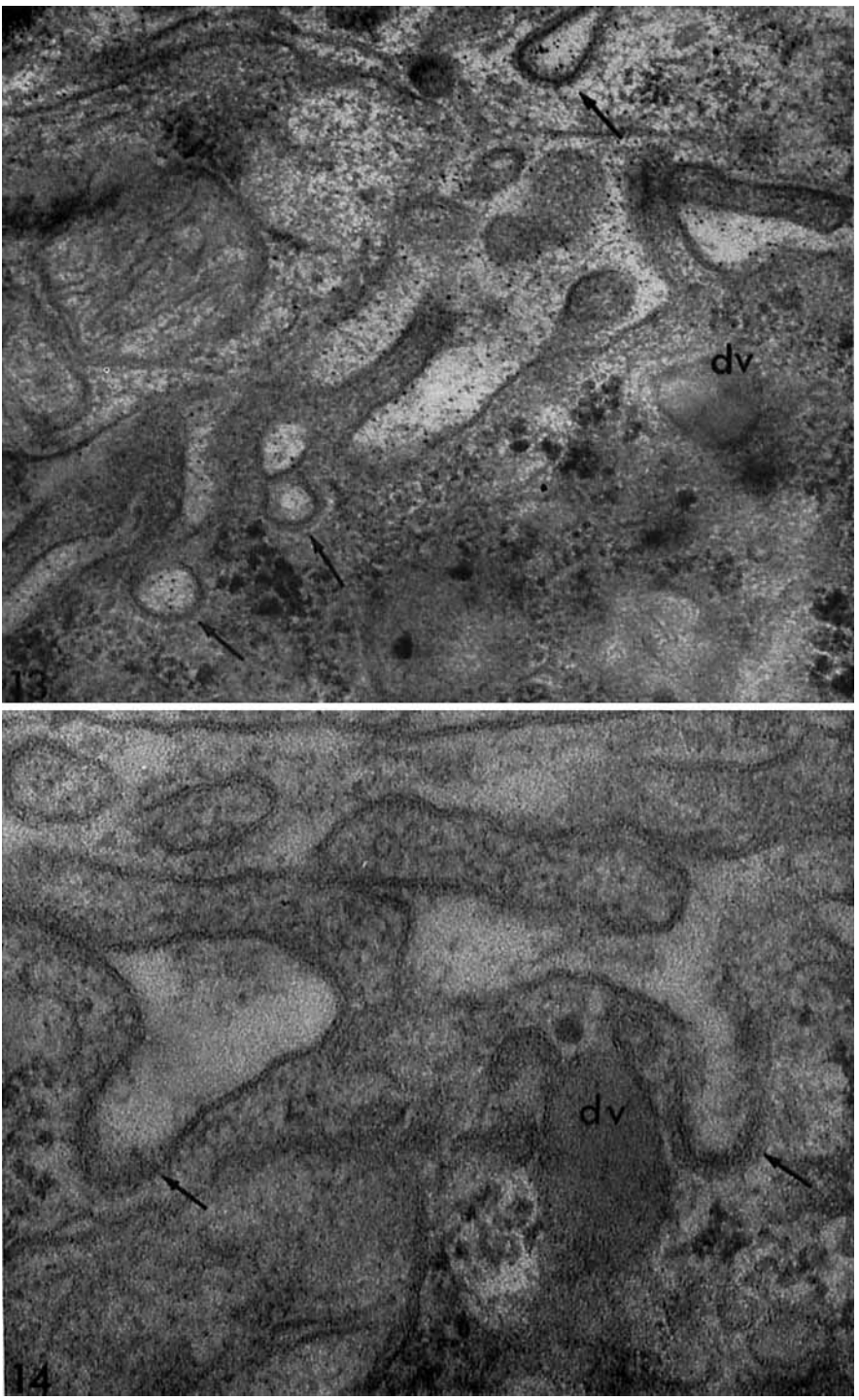

dv
13
dv
14

PLATE 7

EXPLANATION OF FIGURES

15–16 Both figures show portions of the space of Disse that extend some distance away from a sinusoid and dilate the space between hepatic cells. Numerous microvilli protrude into the extension of the space which contains many ferritin molecules and spherical particles, presumably lipid, which are not easily visualized because of their lack of density. The cytoplasm bordering the extensions contains numerous small dense vesicles (x) with a content that matches in diameter and density the spherical particles in the lumen. Some of the vesicles also contain ferritin. Two of them (arrows) are seen opening at the surface of hepatic cells. $\times$ 48,000 (fig. 15), $\times$ 62,000 (fig. 16).

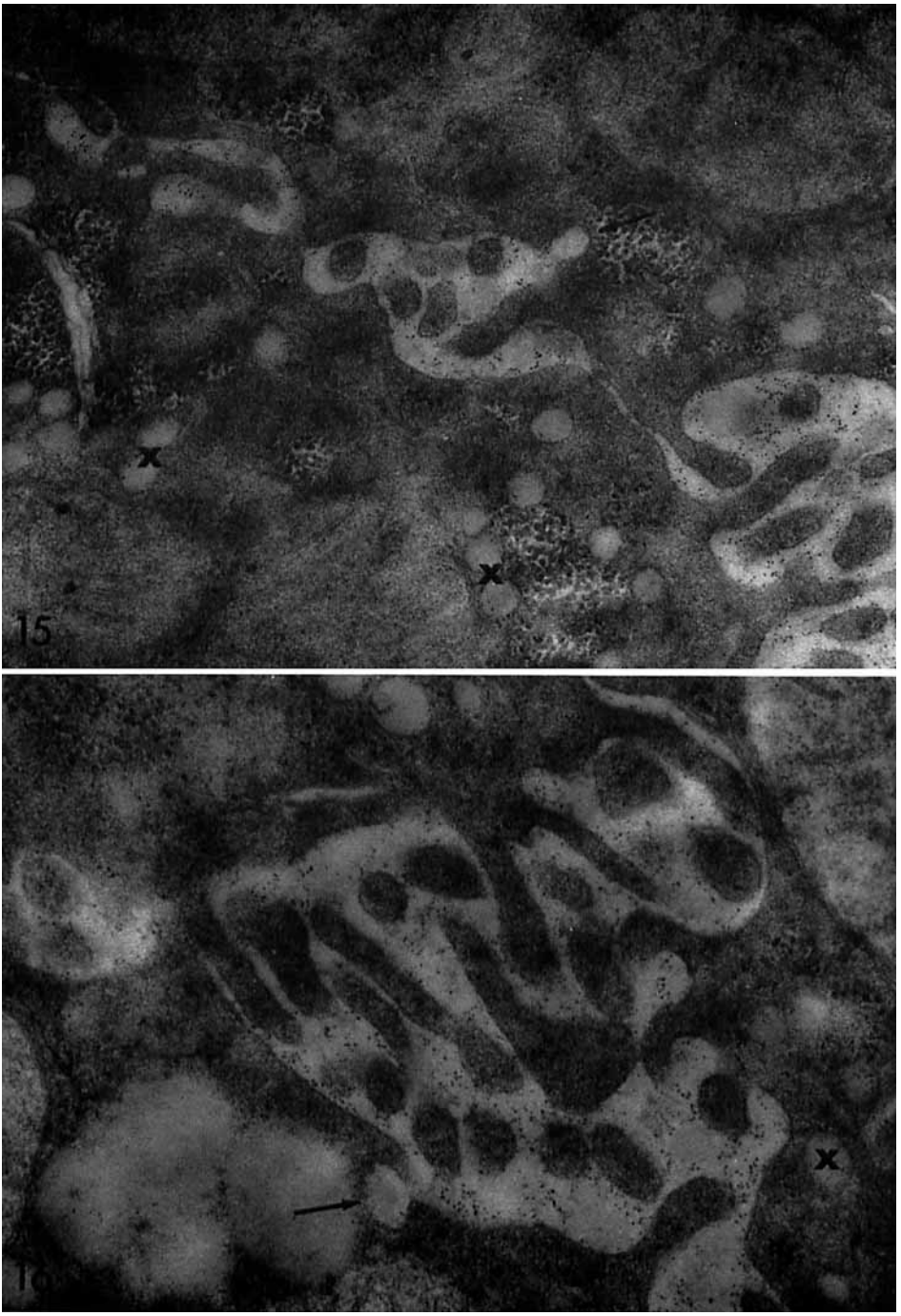

15
16

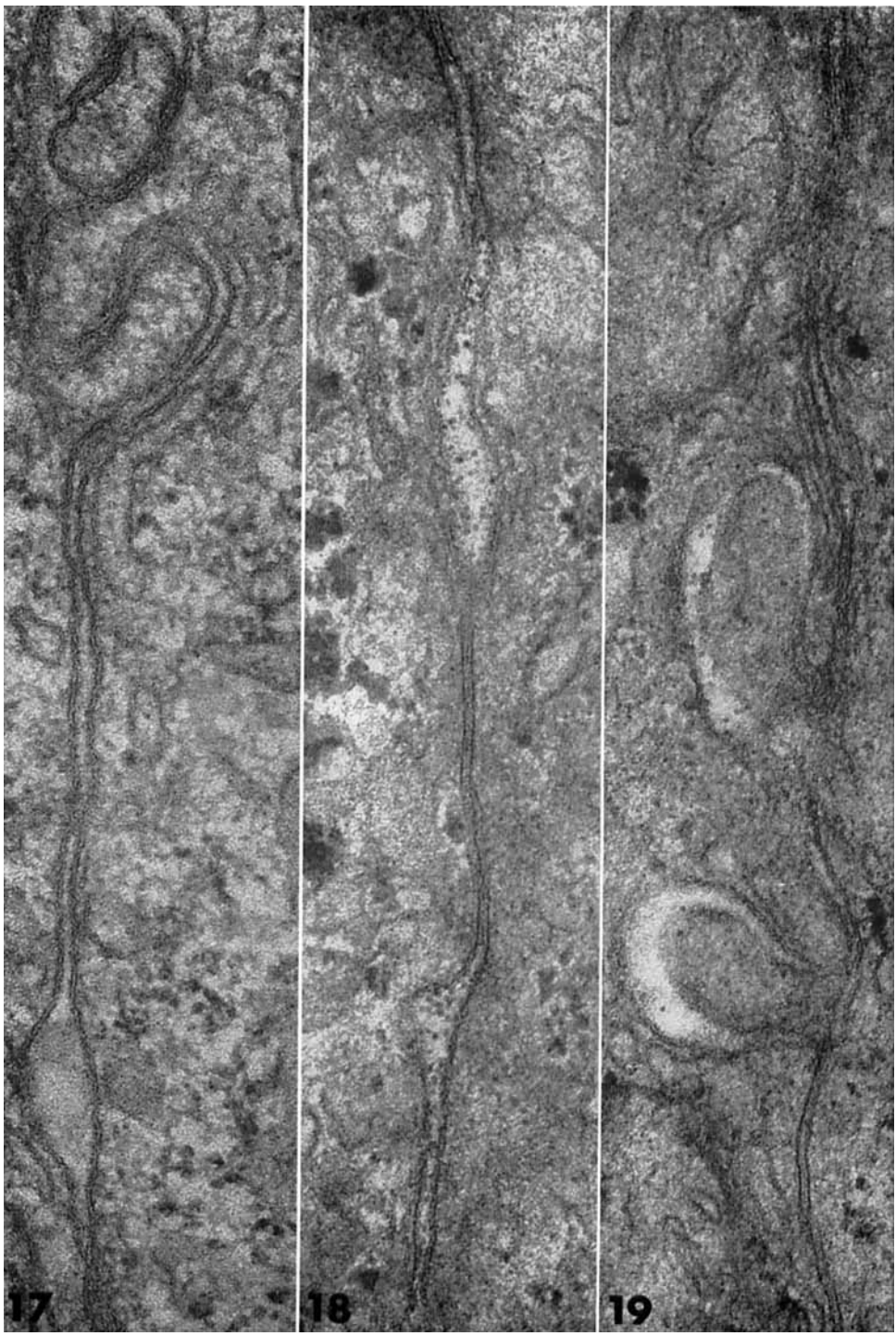
PLATE 8

EXPLANATION OF FIGURES

17 Two studlike intercellular junctions appear in the upper part of the figure. The upper one is cut in cross section, the lower one in longitudinal section. Within both junctions the hepatic cells are separated by a distinct intercellular space. In the lower part of the figure a cluster of lipid droplets are seen in a localized dilation of the narrow intercellular space. × 102,000.

18 At the top margin and in the center of the figure the plasma membranes of neighboring hepatic cells are fused together obliterating the narrow intercellular space. Between the zones of fusion, a distinct narrow space containing numerous ferritin molecules is present. × 122,000.

19 Studlike junctions appear in the central and lower portions of the figure. Within each junction the adjacent hepatic cells are separated by a distinct space which contains ferritin. The plasma membranes of the hepatic cells are fused together in the lower part of the field. × 111,000.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