

AGE VARIATIONS IN CORTICAL MEMBRANES OF ROTIFERS

ALBERT I. LANSING, Ph.D.

From the Department of Anatomy and Cell Biology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, and the Marine Biological Laboratory, Woods Hole, Massachusetts

ABSTRACT

Electron micrographs were made of rotifers of known ages embedded in Vestopal and stained with lead hydroxide. In epithelial cells lining the gut of the rotifer, anatomical continuity is seen between the cell membrane, the cortical membranes, and the rough endoplasmic reticulum at the basal region of the cells. At their luminal surface, these cells possess a terminal web, and colloidal gold is taken up via food vacuoles at this surface. Such pinocytosis does not occur on the peripheral or distal surface (pseudocoelomic) of these cells. Exposure of rotifers to adenine inhibits the formation of cortical infoldings of the cell membrane and the formation of the rough reticulum, whereas the ribosomes and the Golgi apparatus appear to be unaffected. The suggestion is made that the rough reticulum may be derived from modifications of the cell membrane and that the rate of formation of the rough reticulum in adult and elderly rotifers is lower than in the actively growing animal.

Cellular senescence constitutes one of nature's incongruities in that the biological system which has the capacity for self-synthesis, and hence should be able to maintain itself indefinitely, obviously does deteriorate or wear out to bring about ultimate death of the organism. As a first approximation, one might set up a working hypothesis that senescence involves a limitation of the process of self-synthesis which is triggered at the time of maturation of the biological system. This notion is supported, at least in part, by the observations I made a number of years ago that a transmissible, cumulative, and reversible factor exists in the rotifer ovum which accelerates aging and that this factor does not exist or express itself in adolescent rotifers but makes its appearance at the time of attainment of full growth (7).

The present study is the first of a series involving a comparative analysis of the fine structure of rotifers of known ages in a search for significant morphological differences among actively growing adolescent, adult, and elderly animals. The rotifer

is singularly well suited for studies on aging since it has a very short lifespan, it reproduces parthenogenetically so that genetically homozygous stocks may be produced from a single animal, it exhibits determinate development so that all cells in the body are of the same age, and it is readily maintained in isolation culture under standardized nutritive and environmental conditions to minimize the introduction of variables extraneous to that of main concern, senescence.

MATERIALS AND METHODS

1. ORGANISM: The organism used in these studies is the rotifer *Philodina citrina*. The rotifers were raised in isolation culture on pyrex glass depression slides and transferred daily to facilitate procurement of animals of known ages. Raised at 20°C in artificial pond water and fed the alga *Chlorella vulgaris*, the rotifer, which contains roughly one-thousand cells, lives about 30 days, reproduces parthenogenetically, and lays eggs which hatch in one day. Newly hatched rotifers grow rapidly by increase in cell volume and reach maximal size and sexual maturity between

days 5 and 6. A period of adult vigor extends from day 6 to days 10 to 12 and is followed by gradual decline and death between 24 to 30 days.

2. CULTURE MEDIA: To prepare the artificial pond water, 9 ml each of 10^{-1} M CaCl_2 , 10^{-1} M NaCl , and 10^{-3} M KCl were added to 873 ml of demineralized distilled H_2O and buffered to pH 8.6 with 9 ml of 0.06 M tris (hydroxymethyl) aminomethane buffer. Approximately 15 ml of this medium were added to an agar slant of *Chlorella* and the algae washed into

each, in styrene, and embedded in Vestopal W with initiator and activator as customary. The embedding was effected in small glass stender dishes which were lubricated to facilitate removal of the Vestopal disc containing the rotifers. Blocks containing single rotifers were then sawed free and oriented by cementing on suitable blocks for cross- or longitudinal sectioning. Sections were cut with the Porter-Blum microtome, stained with lead by the Karnovsky technique (5), and examined with the Philips EM-200.

4. SOLUTIONS: Colloidal gold (Matheson, Coleman, and Bell) was diluted 1:1 with culture medium, and, in two separate experiments using 6 animals per experiment, rotifers were exposed to this non-toxic medium for 2 hours, then fixed in osmium tetroxide as above.

Adenine in culture fluid at pH 8.6 is toxic and lethal in concentrations higher than 10^{-3} M. Fifteen experiments were done, using at least 6 rotifers per experiment, in which the animals were exposed to either tritiated or normal adenine, 0.0008 M or 0.0016 M, for times varying from 5 minutes to 24 hours, with fixation and processing as described above.

Since there is indication that adenine toxicity is reversed by pyrimidines (1), two experiments were done in which 12 rotifers were exposed to equimolar adenine and uridine (in culture fluid at pH 8.6) and then fixed and processed for electron microscopy.

RESULTS

The rotifer is one of the pseudocoelomate animals; its viscera are not covered with peritoneum and hence cell surfaces in the alimentary tube and associated glands are in direct contact with the fluid contents of the cavity. This is illustrated in Fig. 1

TABLE I
Numbers of Rotifers in the Various Age Groups
Sectioned and Studied and Total Number of
Grids Examined Containing at Least One
Pertinent Gastric Epithelial Cell

Material	Age in days			Mass culture
	1 to 4	5 to 7	8 to 21	
No. of rotifers	39	11	28	30
No. of pertinent grids	180	58	140	195

the solution by agitation. Three drops of this suspension were placed in a pyrex depression to support one rotifer for 24 hours.

The agar slants for culturing *Chlorella* under artificial light were prepared by adding 15 gm of agar to 1,000 ml of Bristol's inorganic salt solution containing 4 per cent soil extract. The autoclaved agar solution in test tubes was slanted and cooled for use.

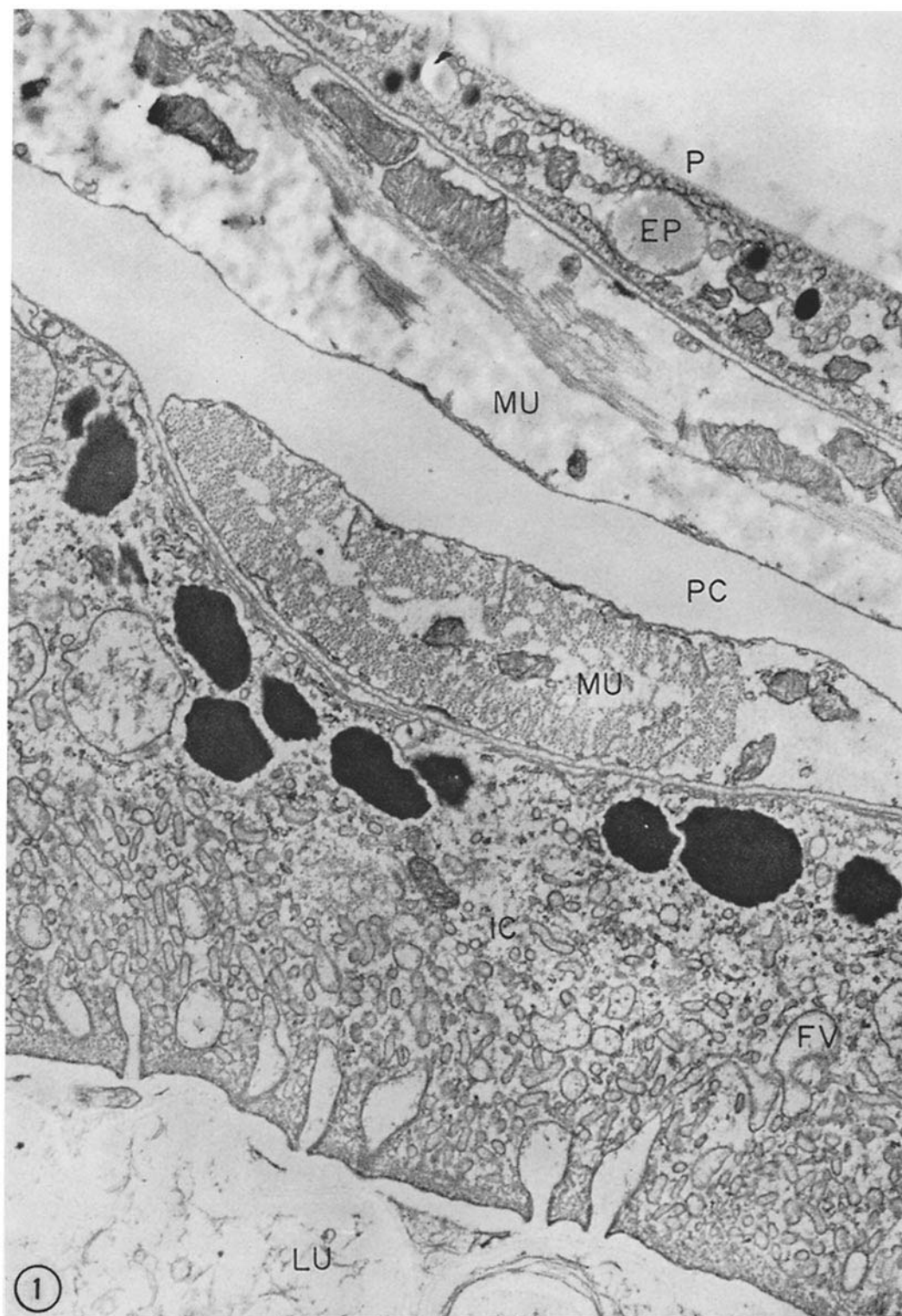
3. ELECTRON MICROSCOPY: Rotifers were fixed for 15 minutes in cold, unbuffered 2 per cent osmium tetroxide, rinsed in distilled water, dehydrated in ethanol, rinsed three times, 30 minutes

Abbreviations

BM, basement membrane
EP, epithelium
FV, food vacuole
G, Golgi apparatus
IC, intestinal (stomach) cell
LU, lumen of gut
MU, muscle cell
N, nucleus

NU, nucleolus
P, pellicle
PC, pseudocoelom
PG, pigment granule
R, ribosome
RR, rough reticulum
SR, smooth reticulum
TW, terminal web

FIGURE 1 Cross-section through 21-day-old rotifer at level of stomach, showing relationships of body layers. The outer circular layer of muscle appears to be rich in glycogen seen here as amorphous material. Note the paucity of ribosomes and rough reticulum in the stomach cell bordering the lumen of the gut. $\times 24,000$.



which is a low magnification electron micrograph of a cross-section approximately at the mid-level of the body. Also shown in this micrograph are most of the cell types encountered at mid-level: from the exterior of the rotifer inward are shown the syncytial epithelium with its characteristic pellicle, a circular muscle fiber rich in glycogen, the pseudocoelom, a longitudinal muscle fiber, a stomach cell, and the lumen of the stomach.

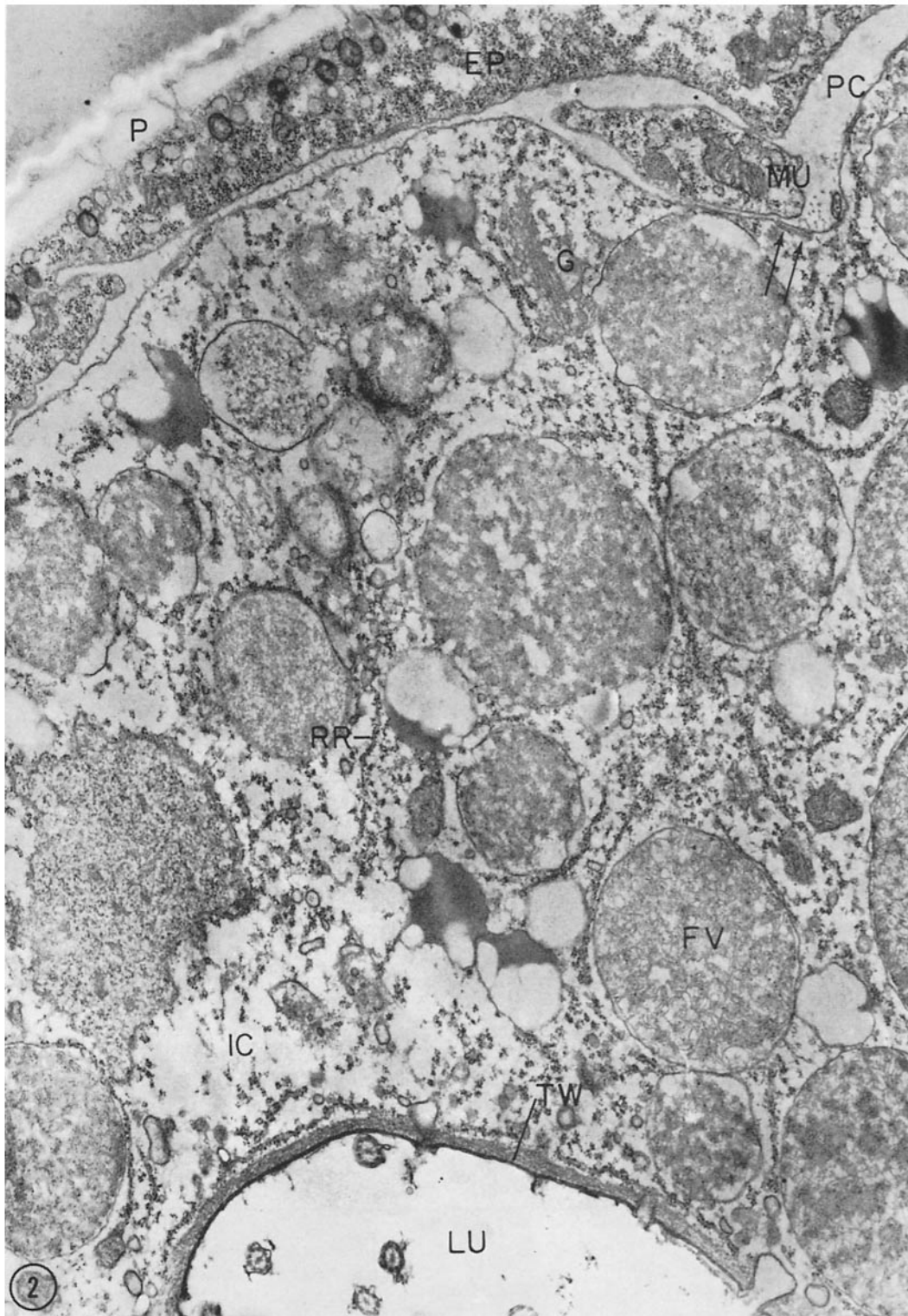
Figs. 1 and 2 illustrate a significant difference in the amount of free ribosomes and rough reticulum in the stomach cells of young and old rotifers. A young (2-day) rotifer is shown in Fig. 2; ribosomes, single or in clusters, are scattered freely throughout the cytoplasm of the stomach cell, and, in addition, a small amount of the rough reticulum is distributed irregularly throughout the cell. Stomach cells of rotifers 1 to 6 days of age contain as many (or more) ribosomes and elements of the rough reticulum as the specimen illustrated here. This micrograph also shows several dispersed lipoidal bodies which appear scalloped. Presumably, some material has been dissolved out of these bodies during the embedding procedure. In contrast, Fig. 1 shows the typical appearance of the stomach cell of an old (21-day) rotifer. Free ribosomes and the rough reticulum are very sparsely distributed, and the lipoidal bodies or granules present manifest a much greater electron opacity than those encountered in young cells of comparable section thickness.

The stomach cell is interesting in that the surface bordering the lumen is highly differentiated and different from the cell surface bordering the pseudocoelom. The cell surface at the lumen is sharply outlined by the usual trilaminar membrane, approximately 60 Å wide, underlying which is a peculiar reticular layer of material reminiscent of the terminal web of the intestinal epithelium (Puchtler and Leblond, 14, and Palay and Karlin, 12). At irregular intervals along the length of the luminal surface of the stomach cell of the rotifer are well developed goblet-shaped pockets from which the food vacuoles appear to form (Fig. 3).

Rotifers exposed to colloidal gold for 1 to 2 hours prior to fixation show gold particles in the goblet-like pockets and food vacuoles of the gastric epithelium. It would appear that the gold enters the cell through the goblet-like pocket and thence specifically into the food vacuoles (Fig. 4). The cell surface bordering the pseudocoelom is much less differentiated and is bounded only by a unit membrane approximately 60 Å thick (Figs. 5, 8, and 9). Close examination of this cortical region reveals a striking difference between young and adult cells which may be recognized in Figs. 1 and 2. Even at low magnification, the cortex of the old cell (Fig. 1) shows a high frequency of cortical membranes coursing roughly parallel to the cell surface and arranged in approximately parallel array. Some of these cortical membranes appear to be associated with ribosomes and some not. In the very young rotifer the pseudocoelomic surface of the stomach cell is quite different from the preceding. At 1 to 3 days of age, single (as opposed to stacked) cortical membranes (Figs. 2, 6, and 7) are frequently found immediately subjacent to the cell membrane. With very rare exceptions, these membranes appear to be associated with ribosomes. The double arrows in Figs. 2 and 6 call attention to such exceptions; in each of these illustrations a cortical membrane devoid of ribosomes extends into the cortex of the cell. This cortical membrane is continuous with the cell membrane (Fig. 2), and it would appear that this is an infolding of the cell membrane comparable to the β -cytomembrane of Sjöstrand (16).

Between 3 and 6 days of age, during which time the rotifer is growing from adolescence into adulthood, there is a progressive increase in the frequency of occurrence of smooth-surfaced and stacked cortical membranes in the periphery of the cell and a corresponding decrease in frequency of occurrence of the long single lengths of cortical membranes paralleling the cell surface and possessing variable numbers of ribosomes. After the attainment of adulthood or maximal size at 6 days, there is a reversal of the progression described for

FIGURE 2 Cross-section through 2-day-old rotifer at level of stomach. Ribosomes and rough reticulum are scattered throughout the stomach cell. Double arrow calls attention to smooth cortical membrane usually found in adult rather than very young rotifers. $\times 24,000$.



the actively growing animals. From 7 through 21 days of age, there is a steady increase in the frequency of occurrence of the stacked, smooth, trilaminar cortical membranes described for the 21-day-old rotifer and a steady decrease and virtual disappearance of the single cortical membranes associated with ribosomes which are characteristic of the 1- to 3-day-old rotifers.

Unlike the luminal surface which takes up colloidal gold into food vacuoles, the pseudocoelomic surface, despite the frequent invaginations of the cell membrane into the cell cortex, does not show passage of colloidal gold particles from the pseudocoelom into the cell although the colloidal gold does enter the pseudocoelom as shown in Fig. 5. All of the colloidal gold is contained in the food vacuoles, and in no instance was it found free in the cytoplasm or in the lumina of the rough reticulum or the smooth reticulum, or in the Golgi apparatus of stomach cells of rotifers of various ages.

The fine structure of cortical membranes in the periphery of adult and old cells is more complex than in young cells. The cell membrane bordering the pseudocoelom in old animals, as in the young, is approximately 60 Å thick and has frequent invaginations. Since the invaginations of the cell membrane give rise to flattened sac-like spaces or sinuses which are relatively isolated from the general extracellular milieu (Figs. 8 to 10), and since the cell membrane is coated with an amorphous material, reminiscent of a basement membrane (Fig. 10), which is continuous over the opening of the sac-like space and further isolates this space, the latter may be referred to as a cisterna. Very commonly the infoldings of the cell membrane (to be referred to as cortical membranes) stack up in approximately parallel arrays two to four deep. The thickness of all of these trilaminar cortical membranes is uniformly about 60 Å.

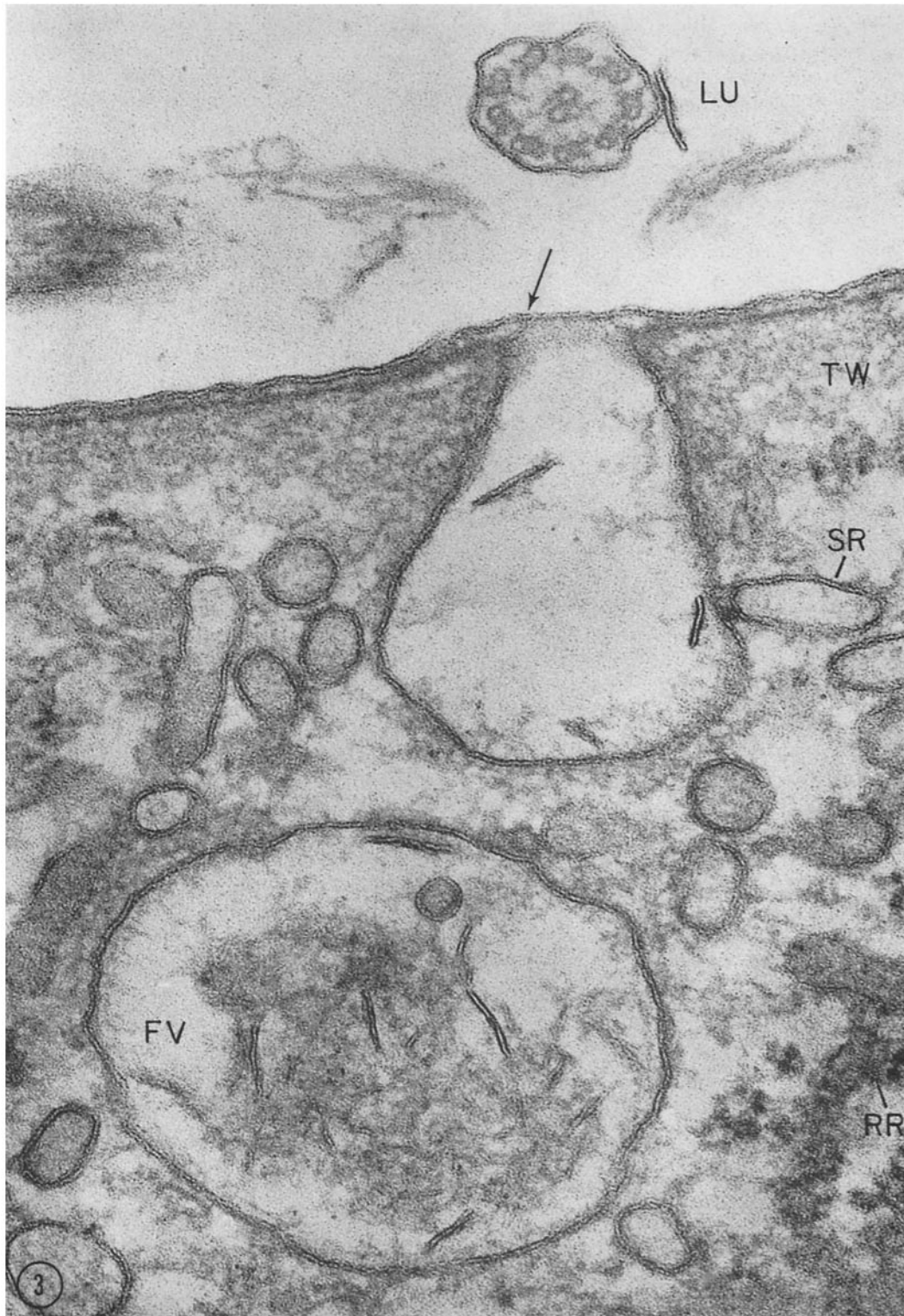
Subjacent to these stacked cortical membranes there is often a membrane system which is atypical. This cortical membrane, which usually parallels the closely overlying stacked cortical membranes,

has one surface devoid of ribosomes and an opposing surface studded with ribosomes as seen in Figs. 9, 12, 13: invariably the surface adjacent to the *closely* overlying stacked cortical membranes is smooth, whereas the surface exposed to the deeper portions of the cytoplasm is rough or studded with ribosomes. A variation of this architecture is encountered near the cell membrane (Figs. 8 and 11). Here, a trilaminar cortical membrane lying just subjacent to the cell membrane is anatomically continuous with a cortical membrane studded with several or many ribosomes. Again, the cortical membrane surface closest to the cell membrane is virtually devoid of ribosomes; the surface facing away from the cell membrane and exposed to the deeper region of the cytoplasm is the one that is rich in ribosomes.

It is of interest to note that, on occasion, the angle of cut is such that a trilaminar structure is discernible in some portions of the cortical membranes containing ribosomes (Figs. 12 and 13); this trilaminar membrane appears to measure approximately 40 Å rather than the usual 60 Å. It is only in the region of the cell periphery that cortical membranes associated with ribosomes exhibit the 40-Å-thick trilaminar structure. The rough reticulum deep in the cytoplasm or medulla of the cell has not been observed to exhibit a trilaminar membrane structure at magnifications of up to 158,000 times.

Fig. 10 is an electron micrograph of the pertinent region of the stomach cell of a 12-day-old rotifer. This rotifer had been exposed to adenine for 1 hour and then returned to normal culture fluid for 1 day, at which time it was fixed. Although the animal had been exposed to experimental manipulation, the fine structure of the cortex of the cell is germane to this problem. This electron micrograph (magnification $\times 132,000$), shows the basement membrane-like material continuous over the cisterna formed by invagination of the cell membrane which is trilaminar at the surface as well as in the invaginated portion. The trilaminar membrane on the right side of the cortical membrane

FIGURE 3 Section of stomach cell of 6-day-old rotifer, showing surface bordering the lumen of gut. Two food vacuoles and a number of smooth-surfaced vesicles are present. Note that trilaminar membrane of food vacuole at surface abuts on cell membrane (arrow). $\times 132,000$.



system makes a sharp hairpin bend at the point where it appears to be anatomically continuous with the rough reticulum.

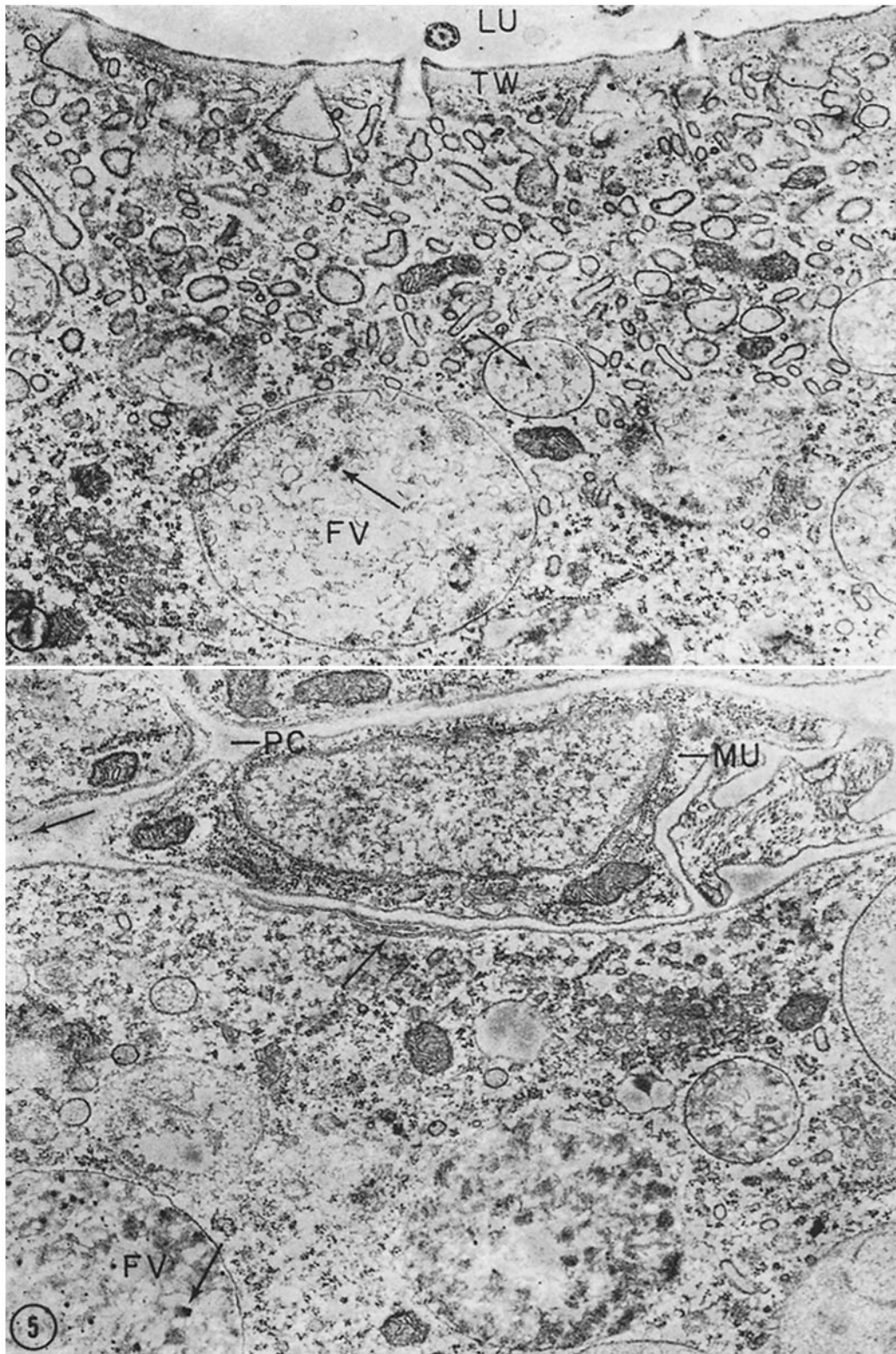
Close inspection of the trilaminar membranes such as cell membranes and their intracytoplasmic infoldings, the cortically located rough reticulum, and the smooth-membraned vesicles strongly suggests that there is a usual, but not invariable character to the electron opacity of the dense layers of the unit membrane. Bearing in mind that all of the material in this study has been stained with lead and that much of the contrast in the membranes depends upon this lead staining, one can only refer to the degree of lead staining in attempting to evaluate variations in density. In normal sections of the cell membrane that are cut suitably thin, it is generally observed that the degree of lead staining of the dense layer that is proximal to the cytoplasm is considerably greater than that of the dense layer facing the external milieu (Figs. 8 to 12). This pattern also applies to the extensions of the cell membrane into the cell cortex as seen in Fig. 10. The short stretches of trilaminar cortical membrane that can be identified as being in association with the rough reticulum, as in Figs. 10, 12, and 13, not only have an over-all thickness of about 40 A, but also differ from trilaminar cell membranes in that the two bounding dense laminae both reveal a low and equal level of lead staining. Smooth-membraned vesicles, as illustrated in Figs. 6, 10, and 13, generally exhibit a high degree of lead staining in the dense layer bordering the lumen of the vesicle and a significantly lower degree of staining in the dense layer contacting the cytoplasm. The problem of unit membrane thickness and asymmetry of the components of unit membranes has been discussed by Sjöstrand (17, 18) and Millington (9).

In the light of a previous observation of Lansing

and Rosenthal (8) and current unpublished pilot data of Lansing supporting the possibility that RNA is a component of cell membranes, an attempt was made to trace the sequence of events in the invagination of the cell membrane to form cortical membranes by labeling rotifer stomach cells with tritiated adenine for autoradiographic analysis. It was quickly found that adenine, tritiated or not, is highly toxic in concentrations greater than 10^{-3} M. At adenine concentration of 10^{-4} M, rotifers were viable but sluggish in their movements even with exposures of no more than 1 to 2 hours. Ultrathin sections of 2-day-old rotifers exposed to 0.0008 or 0.0016 M adenine for from 5 minutes to as much as 24 hours revealed an interesting and apparently related series of changes. After exposure to adenine for 5 minutes, there appears beneath the cell membrane a zone approximately 1 μ thick which is relatively devoid of granules as well as smooth reticulum and rough reticulum. After exposures of 1 hour, smooth membranes become very scarce and it is necessary to scan the surfaces of several cells before finding a single cortical membrane in the region of the cell periphery. After 18 to 24 hours, not only are the cortical membranes absent, but also the rough reticulum of the cells is inconspicuous (Figs. 17 and 18), whereas the Golgi apparatus does not appear to be seriously affected. When the rough reticulum is found in the cell, it is irregular in form and has relatively few ribosomes associated with it. At the same time, free ribosomes, single or in clusters, abound in a random manner throughout the cytoplasm. Curiously enough, the typical nuclear envelope is made conspicuous by a rich profusion of ribosomes adherent to its external layer, i.e. that which is adjacent to the cytoplasm (Fig. 16). The appearance of the rough reticulum in control material is illustrated in Fig. 15. The addition of

FIGURE 4 Absorptive surface of stomach cell of 2-day-old rotifer, showing terminal web, pockets at the surface from which food vacuoles appear to form, and specific localization of colloidal gold particles (arrows) in food vacuoles. $\times 24,000$.

FIGURE 5 Distal surface of stomach cell of 2-day-old rotifer, showing pseudocoelom and small portion of a muscle cell. Two colloidal gold particles are seen to be present in the pseudocoelom (arrow upper left) but none is present in cisternae of the cortical membranes or the rough reticulum. At lower left (arrow) gold particles can be seen to be concentrated in food vacuole. 2-day-old rotifer; $\times 26,000$.



equimolar uridine significantly inhibits the above effects on both the cortical membranes and the rough reticulum.

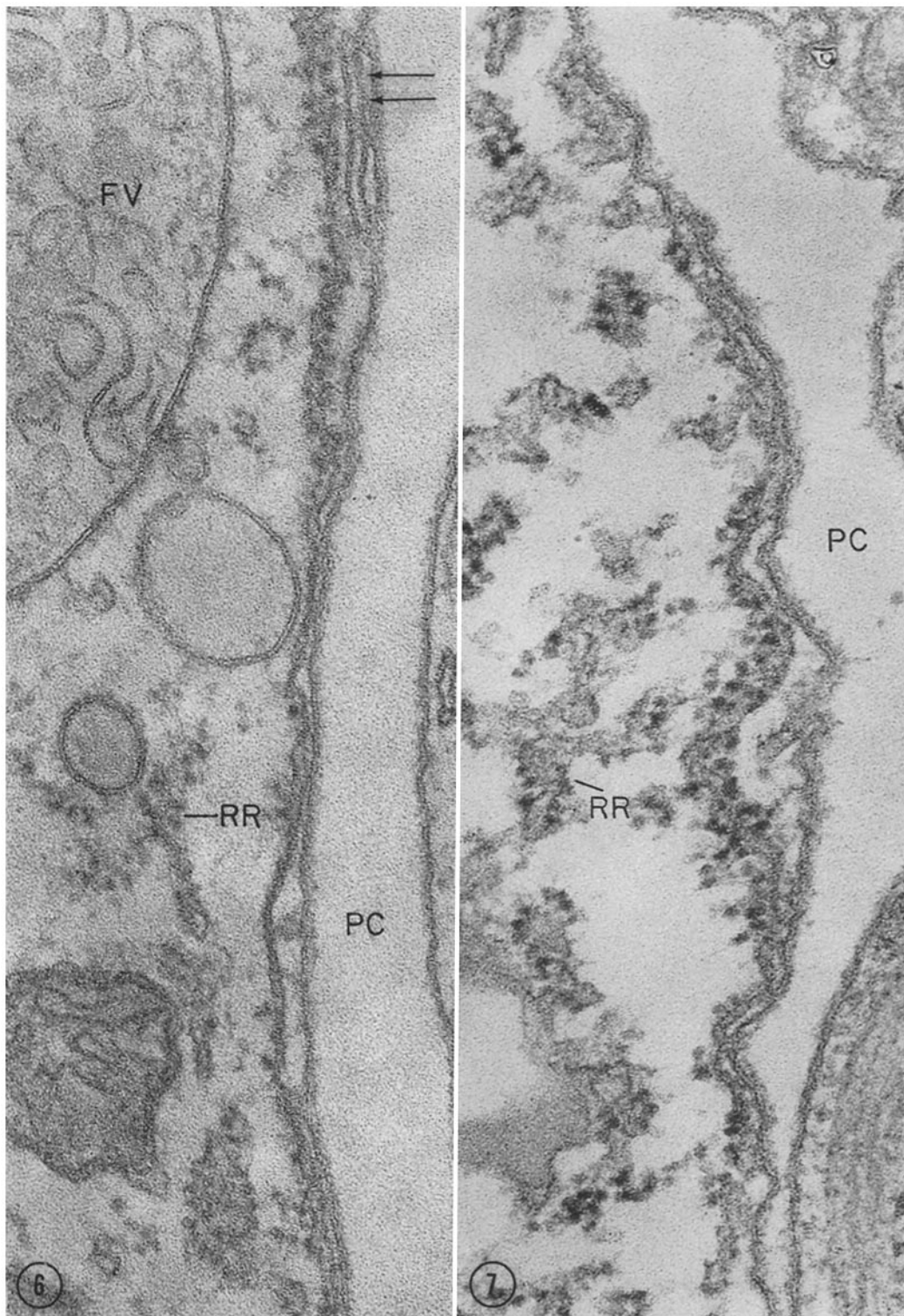
DISCUSSION

Porter's (13) review in 1961 encompasses most of the data pertinent to the identification, structure, and possible functions of the endoplasmic reticulum. The extensions of the cell membrane into the cortical cytoplasm of rotifer gut cells do not fit into the strict definition of the endoplasmic reticulum. For want of a better term, I have referred to these as cortical membranes which are continuous with the cell membrane. That these cortical membranes are anatomically continuous with the cell membrane is unequivocally demonstrated (see Figs. 5, 8 to 10), and it also seems clear that, as illustrated in adult rotifers, the smooth-surfaced, trilaminar membranes stacked in approximately parallel arrays are identical structures and hence originate from invaginations of the cell membrane bordering the pseudocoelom. These infoldings of the cell membrane appear to be similar to the basal folds of the gastric parietal cell described by Ito and Winchester (4). The fact that colloidal gold does not appear in the lumina created by these infoldings of the cell membrane, even though the colloidal gold does find its way into the pseudocoelom (although in limited amount), suggests that pinocytosis is not occurring in these areas. One possible explanation is that the basement membrane is continuous over the cisternae (sac-like invaginations of the cell membrane) and constitutes an impediment to the movement of particulate material from the pseudocoelom into the cisternae. It also is evident that colloidal gold particles ingested *via* the food vacuoles do not leave the cell through the cell membrane infoldings.

A major question brought up by some of the fine structural details observed in this study is whether or not anatomical continuity exists between the basal infoldings of the cell membrane

and the rough reticulum. (1) In a number of instances, patterns, as illustrated in Fig. 14, have been observed in which a basal infolding, clearly bounded by smooth trilaminar membranes, shows an extension from it of electron-opaque material which appears to be continuous with a well defined rough reticulum. This may be interpreted as a fortuitous juxtaposition of the three materials in question, or it may be interpreted as anatomical continuity between the smooth-membraned basal infoldings and the rough reticulum, with the region of connection not being in a normal plane of section. That such anatomical continuity may occur is supported by Fig. 10. In this electron micrograph there is apparent continuity between the cell membrane and the smooth-membraned extension of it into the cortex of the cell; the trilaminar wall of the invaginated cell membrane makes a sharp hairpin turn to the right where it becomes continuous with the rough reticulum. (2) As shown in Figs. 6 to 8, the rough reticulum is continuous with the smooth cortical membranes. A significant variation of this pattern is illustrated in Figs. 9 and 12. In both of these electron micrographs, which are typical of a frequently encountered situation, several smooth cortical membranes are stacked in parallel array. In one membrane system, the cortical membrane is essentially parallel to the cell membrane and to the overlying stacked cortical membranes and possesses one wall which is a smooth membrane; this smooth wall invariably is the distal surface, *i.e.* the surface toward the cell membrane. The opposing membrane, *i.e.* that which impinges on the deeper regions of the cytoplasm, is studded with ribosomes. This suggests, but by no means establishes, that the surface of the cortical membrane system which is freely exposed to ribosomes is the surface which attaches ribosomes. (3) As illustrated in Figs. 8 to 10, 12, and 13, the rough reticulum in the cortex of the basal portion of the cell is trilaminar for at least short distances. Measurements where feasible indicate that the trilaminar membranes of the rough reticulum are approxi-

FIGURES 6 AND 7 Sections through surface of stomach cell bordering the pseudocoelom of 2-day-old rotifers, showing low level of lead staining in membranes of young rotifer material. The cortical membranes coursing parallel to the cell surface are both smooth and rough. Double arrow in Fig. 6 calls attention to smooth cortical membrane usually found in adult rotifers and rarely in actively growing animals. $\times 137,000$.



mately 40 Å in over-all thickness rather than 60 Å as found in the cell membranes. Thus far, it has been possible to resolve the trilaminar membrane in the rough reticulum only in that reticulum located in the cortex of the cell at the pseudocoelomic surface. All of the rough reticulum in the remainder of the cell appears to possess single line membranes about 40 Å thick.

The detailed structure of the limiting membrane of the rough reticulum has not been clearly defined. Robertson (15) is frequently cited as having established the trilaminar nature of the membranes of the rough reticulum. In his review article on the ultrastructure of cell membranes, Robertson states "The membranes of components of the ER show the same ~75 Å unit structure seen in cell surfaces (Fig. 17, Plate IV)." However, the figure cited is an electron micrograph illustrating the cell membranes of two adjacent smooth muscle cells and does not illustrate the structure of the membranes of the rough reticulum. Both Smith (19), observing the sarcoplasmic reticulum of insects, and Karrer (6), studying the reticulum in mouse lung, indicate that the sarcoplasmic or rough endoplasmic reticulum possesses single line membranes measuring about 50 Å, whereas the cell membranes are 75-Å-thick trilaminar membranes. Finck (3), on the other hand, noted that the membranes of the rough reticulum in mouse pancreas appeared to be trilaminar. Yamamoto (20) made measurements of KMnO_4 -fixed membranes in bullfrog ganglion cells. He noted that the membranes of the rough reticulum are about 20 per cent thinner than the cell membranes and that they are trilaminar. The one pertinent electron micrograph in his paper does show three cisternae of the reticulum bounded by trilaminar membranes; however, since the material was fixed in

KMnO_4 , the ribosomes are not apparent and it is difficult to establish that the rough reticulum is indeed being analyzed.

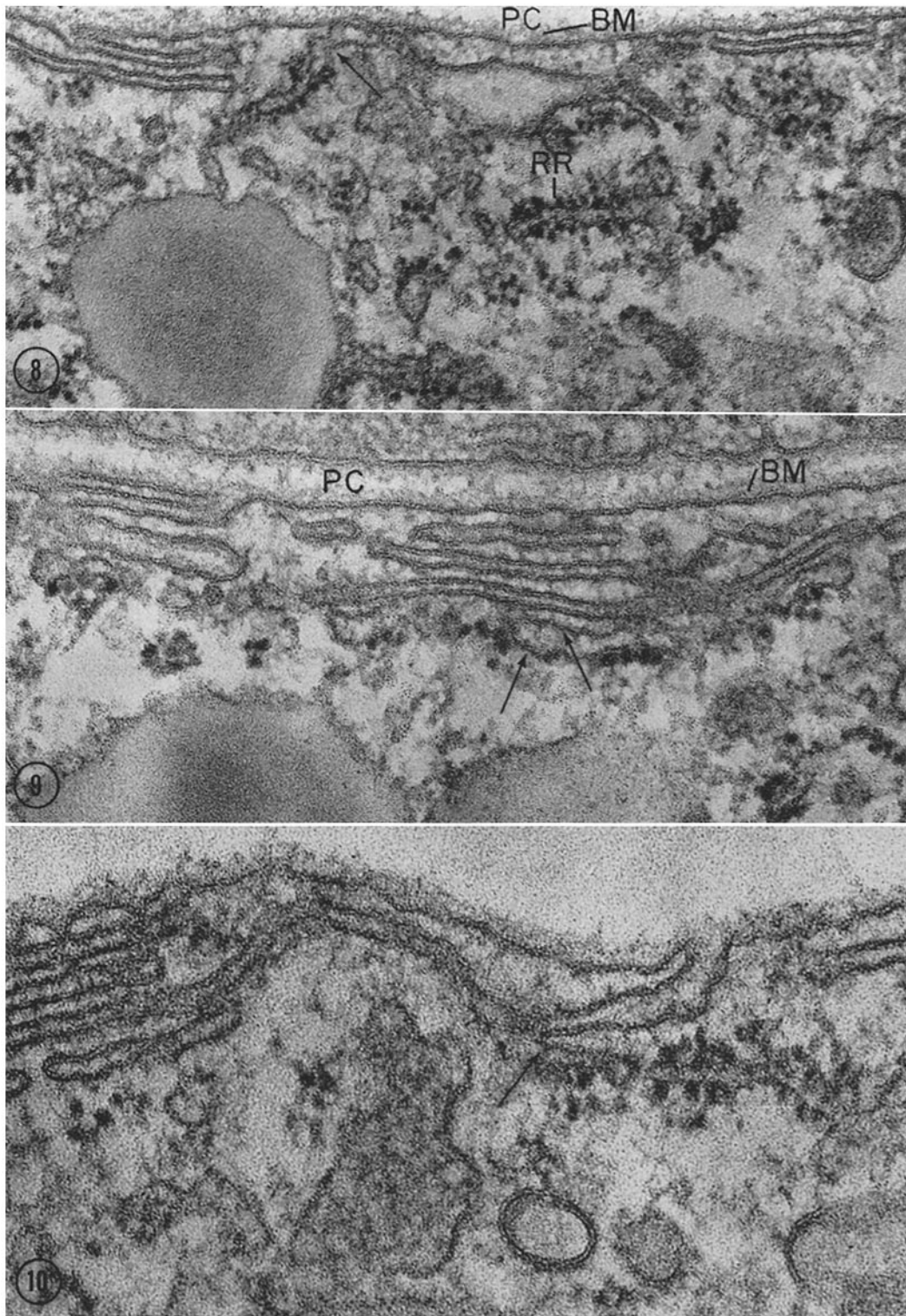
Continuity of the rough reticulum and the cell membranes, at least in some situations, has been suggested by Palade (10, 11) and also by Epstein (2). The continuity between the cell membrane and the rough reticulum described in the latter report has been claimed by Robertson (15) as not being clearly established, in that unequivocal continuity cannot be traced. The observations in the present study support the point of view of both Palade and Epstein. The frequent occurrence of cortical membranes with a smooth distal wall and a rough proximal wall (distal and proximal with respect to the cell interior) warrants consideration of the possibility that the rough reticulum is not only continuous with the cell membrane, but also may be derived from appropriately modified cell membranes. This interpretation has been reached with full awareness of the studies, summarized by Porter (13), which establish that continuity exists between the nuclear envelope and the rough reticulum. These two points of view are not mutually exclusive. Further analytical studies will be required to clarify the functional significance of the continuous canalicular system between the cell membrane and the nuclear envelope.

Cell membranes must undergo major modifications if they are to become the rough reticulum. The cell membrane is about 60 Å thick; the internal (facing the cytoplasm) dark line of the trilaminar unit membrane, after staining with lead, appears denser than the external dark line; the inner or proximal surface of the cell membrane exhibits no propensity for attachment of ribosomes. In contrast, the membrane of the rough reticulum is about 40 Å thick, and is trilaminar

FIGURE 8 Pseudocoelomic surface of stomach cell of 18-day-old rotifer, showing continuity (at arrow) between smooth cortical membrane and the rough reticulum. $\times 120,000$.

FIGURE 9 Pseudocoelomic surface of stomach cell of 18-day-old rotifer, showing trilaminar cortical membranes in periphery of basilar region of cell and one cortical membrane which has a smooth surface (toward cell membrane, upper arrow) and a rough surface (toward cytoplasm, lower arrow). $\times 132,000$.

FIGURE 10 Pseudocoelomic surface of stomach cell of 12-day-old rotifer, showing basement membrane, continuity of cell membrane with trilaminar smooth cortical membrane, and continuity of the latter (arrow) with the rough reticulum. $\times 132,000$.



only in short stretches; both dark lines of the unit membrane stain poorly with lead, and the surface of the rough reticulum has the ability to attach ribosomes.

In this study, the observations on the effect of adenine on cortical membranes lend indirect support to the possibility that, at least in the rotifer, the rough reticulum may be derived from cell membranes. Brief exposure to adenine results in the disappearance of the smooth-surfaced membranes located in the cortex of the basal portion of the stomach cell. After exposure of the rotifers to adenine for 18 to 24 hours, most of the rough reticulum in the cell disappears along with the cortical membranes. Ribosomes, single and clustered, persist, as does the Golgi apparatus, which does not appear to be altered significantly. The addition of equimolar uridine to the adenine completely vitiates the above effects. In this connection, it is interesting to note that adenine has been shown to interfere with protein synthesis and that pyrimidines inhibit this effect of adenine (1).

Much of the discussion of cortical membranes is based upon observations on adult and elderly rotifers. The very young and actively growing rotifers exhibit a much less elaborate pattern of cortical membranes than do adults. As illustrated in Figs. 6 and 7, one frequently encounters, in the periphery of the basal portion of the stomach cell, long cortical membranes coursing essentially parallel and very close to the cell membrane. These cortical membranes are smooth for long stretches and continue for further long stretches as the rough reticulum. In the young rotifer the cortical membranes consistently stain very poorly with lead, and it is rare that one observes a system of well defined, smooth-surfaced and trilaminar cortical membranes as is most common in the adult.

The interpretation of the cortical architecture of the very young cell would be exceedingly difficult in the absence of the information available on the adult cell; the latter makes possible a working hypothesis which should be amenable to analysis. In the actively growing young rotifer, in which protein synthesis presumably is going on at a rapid rate, the infoldings of the cell membrane rapidly undergo conversion into the rough reticulum so that only single (as opposed to stacked) cortical membranes are found subjacent to and parallel to the cell membrane; these cortical membranes rapidly attach ribosomes. In the adult and elderly rotifer, protein synthesis no doubt goes on at a lower rate than in the young. The infoldings of the cell membrane occur, but the transition to the rough reticulum may go on at a slow rate. Thus, the smooth cortical membranes pile up and only the most internally located cortical membranes (and presumably the earliest formed) becoming associated with ribosomes. It may be either that the formation of the rough reticulum in the adult rotifer is influenced by a slower rate of modification of the membrane for receptivity to ribosome attachment or that fewer ribosomes are available in the adult than in the growing rotifer.

The preceding discussion develops a hypothesis that appears justifiable in that it establishes a framework for experimentation on the mechanisms of cellular aging. Decline of the adult organism into senescence must involve inadequacy of cellular synthetic processes. In this study, evidence is offered which establishes age-dependent alterations in organelles of the cell which may be linked to protein synthesis.

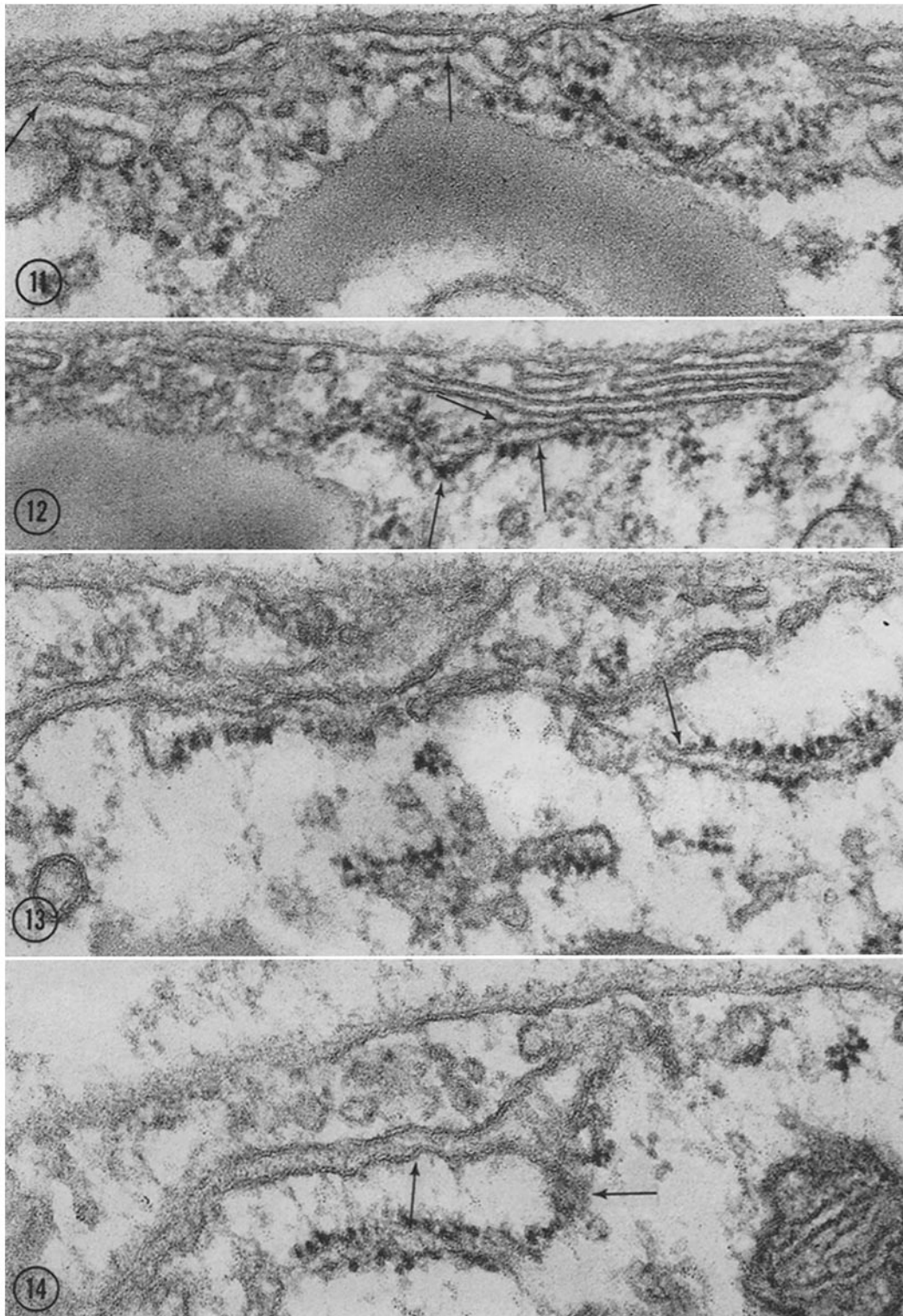
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FIGURE 11 Stomach cell of 18-day-old rotifer, showing continuity of smooth and ribosome-studded membranes in cortex of cell. Trilaminar structure of membranes appears evident at arrows. $\times 70,000$.

FIGURE 12 Stomach cell of 18-day-old rotifer, showing relationship of cortical membranes. The trilaminar rough reticulum may be seen at arrows. $\times 120,000$.

FIGURES 13 AND 14 Similar to Fig. 12, showing the trilaminar membrane in the rough reticulum measuring about 40 Å (Fig. 13, arrow), apparent continuity between cortical membrane and rough reticulum (Fig. 14, arrows). $\times 120,000$.

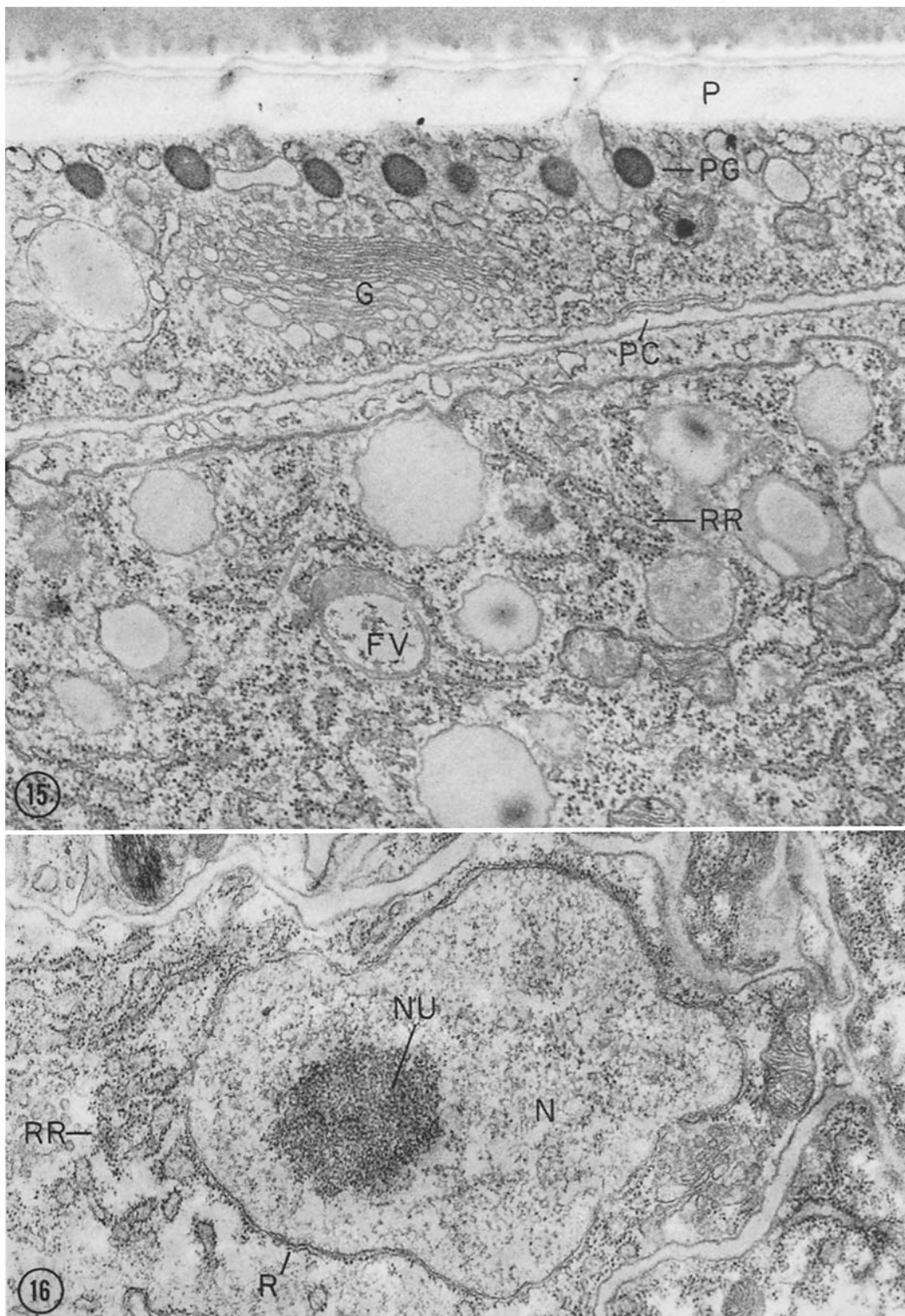


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FIGURE 15 Stomach cell of 4-day-old rotifer, illustrating the amount of the rough reticulum normally apparent in the cytoplasm of cell of young rotifer. $\times 29,000$.

FIGURE 16 Stomach cell of 2-day-old rotifer, showing nucleus and surrounding cytoplasm after exposure to adenine for 20 hours. Note the outlining of the nuclear envelope by ribosomes and the small patch of rough reticulum at the left which has not been affected by the adenine. $\times 23,000$.



FIGURES 17 AND 18 Stomach cells of 2-day-old rotifers after exposure to adenine for 20 hours. The cortical membranes and the rough reticulum are entirely absent, but single and clustered ribosomes are profusely present. $\times 24,000$.

