THE FINE STRUCTURAL LOCALIZATION OF ADENOSINE TRIPHOSPHATASE IN THE SMALL INTESTINE, KIDNEY, AND LIVER OF THE RAT

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ABSTRACT

The distribution of the reaction product of a staining method for adenosine triphosphatase (ATPase) in rat small intestine, kidney, and liver was studied with electron microscopy. Several procedures were tried but the best results were obtained from tissue that had been quenched in liquid nitrogen, sectioned at 25 μ in a cryostat, fixed for 30 to 90 minutes at 4°C in formalin-sucrose buffered to pH 7.2, incubated with substrate, and then osmicated and prepared for electron microscopy in the usual way. This procedure enabled the localization of mitochondrial ATPase to be studied. In tissue fixed in small blocks in osmium tetroxide for 3 minutes prior to incubation with substrate, good preservation was noted, and the reaction product for ATPase was localized on the cell membrane and nuclei. The reaction product was present in abundant amount in the nuclei, and particularly within nucleoli, of all tissues studied. Because the histochemical localization of nuclear enzymes poses numerous interpretative problems at the present time, the significance of this nuclear localization is uncertain. Cell (plasma) membranes were the site of localization, especially at areas where it has been proposed that active transport mechanisms may occur, namely, on the microvilli of intestinal epithelium, endothelial lining of capillaries, glomerular epithelial cell membranes, basal infoldings of the cell membrane of renal tubules, on the microvilli of bile canaliculi, and on the microvilli of proximal convoluted tubular epithelial cells. ATPase localization on the cristae mitochondriales was also demonstrated.

Since adenosine triphosphatase (ATPase) promotes the splitting of adenosine triphosphate (ATP) with the release of energy, the cellular localization of this enzyme in terms of fine structure should contribute to the understanding of the mechanisms of performance of cell activities. The presence of ATPase can be demonstrated readily with light microscopy by the method of Wachstein and Meisel (32), as well as by other procedures (22, 23). These observers and others (21, 29) have demonstrated several characteristics of the localization of ATPase in various tissue sites with light microscopy.

Essner and others (8) were successful in the demonstration of the reaction product for ATPase and 5'-nucleotidase in an electron microscopic study of the liver. They showed that the reaction products for these enzymes were localized in the plasma membranes of the microvilli protruding in the bile canaliculi and in the space of Disse, and they emphasized the possible role of phosphatases in membrane activity and transport. Nelson (17) demonstrated the localization of ATPase in the filaments of the tails of spermatozoa, and pointed out the potential activity of the enzyme in energy-forming reactions related to con-

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tractile cellular mechanisms. The localization of ATPase with electron microscopy in renal tubules and glomeruli has also been reported (20). The intracellular localization at the electron microscopic level of this specific phosphatase in small intestinal absorptive epithelium, however, has not been described to our knowledge. Nor has mitochondrial localization of ATPase been demonstrated by electron microscopy, although recently Wachstein and others (31) using light microscopy have shown the localization of ATPase in mitochondria.

The localization of enzymes with electron microscopy presents certain technical problems and difficulties of interpretation. Diffusion and displacement artifacts are magnified and of more critical significance than at the light microscopic level. These problems have been discussed by Essner and others (8). Even with these difficulties several seemingly valid and pertinent observations have been made, not only upon the localization of ATPase, as already cited, but also upon the localization of succinic dehydrogenase (18, 3), acid phosphatase (8, 13), alkaline phosphatase (4, 16), 5'-nucleotidase (8), and acetylcholinesterase (2).

The present study is an effort to confirm and extend the observations which others have made heretofore upon the cellular and intracellular localization of the reaction product for ATPase in the kidney and liver of the rat, and to describe the localization in the rat small intestine.

METHODS

Adult male and female rats of the Sprague-Dawley strain averaging 250 gm were used. Tissue was obtained under light ether anesthesia from the upper jejunum, the liver and kidney.

In the preparation of tissue the following procedures were employed:

1. Blocks of tissue 1 cu mm in size were fixed for 3 minutes in 1 per cent osmium tetroxide containing 7.5 per cent sucrose buffered to pH 7.2 with veronal (osmium-sucrose) (6). These blocks were incubated according to the method of Wachstein and Meisel (32) for 15 minutes. In some instances, after washing in water, the tissue was placed in 1 per cent ammonium sulfide for 1 minute. In others, the ammonium sulfide step was omitted. The tissue blocks were then fixed additionally in osmium-sucrose for 2 hours, dehydrated in alcohol, and embedded in methacrylate. Thin sections were obtained from the outermost portion of the block for electron microscopy, and ob-

served on formvar films with an RCA EMU-3 microscope.

2. Slices of tissue 1 to 2 mm thick were obtained with a razor blade and quenched in liquid nitrogen, after which 25 μ sections were cut in a cryostat. Some of these cryostat sections were fixed in 10 per cent formalin with 4.9 per cent sucrose buffered to pH 7.2 (formalin-sucrose) at 4°C for 30 to 90 minutes (procedure 2A). Earlier, neutral buffered formalin without sucrose was used but was replaced by buffered formalin-sucrose after Holt and Hicks (12) reported that better tissue preservation was obtained with the latter fixative. Other cryostat sections were placed in osmium-sucrose for 2 minutes (procedure 2B). The sections were then incubated in the Wachstein and Meisel medium for 15 minutes. In still another group, the 25 μ cryostat sections were incubated in the substrate for 10 minutes without any prior fixation (procedure 2C). As in procedure 1, ammonium sulfide treatment was omitted in some experiments. Osmication and methacrylate embedding were accomplished as in procedure 1, using small blocks from the outer portion of the sections.

3. Slices of tissue 1 to 2 mm thick were fixed in formalin-sucrose for 90 minutes. From some of the formalin-fixed tissue slices, sections 25 μ thick were obtained with a freezing microtome and then incubated (procedure 3A). In others, the 1 to 2 mm thick slices were incubated as such (procedure 3B). Osmication and methacrylate embedding were then carried out on small pieces obtained from the outer portions of the tissue, as in procedure 1. The ammonium sulfide step was omitted in some instances.

4. Control specimens were prepared by following each of the procedures as outlined above, with the exception that ATP was omitted from the incubation mixture.

5. ATPase localization in intestine, kidney, and liver, using light microscopy, was also carried out according to the procedure of Wachstein and Meisel (32).

The various steps employed in the procedures outlined in 1, 2, and 3, up through the stage of incubation with substrate, are summarized in Table I.

RESULTS

Treatment of incubated tissue in ammonium sulfide provided the opportunity to select small portions of the areas darkened with lead sulfide for osmium tetroxide fixation and ultrathin sectioning. This proved to be advantageous in that a more abundant reaction product, indicating the presence of the enzyme, was found in this way. When ammonium sulfide was omitted, the reaction product of lead phosphate was, nevertheless, usually readily demonstrable with electron micros-

TABLE I

Procedures for Tissue Preparation

In some experiments of each procedure, after washing in distilled water the tissue was passed through 1 per cent ammonium sulfide for 1 minute, while in other experiments this step was omitted. Control experiments were carried out for each group by omitting ATP from the Wachstein-Meisel incubation medium.

Procedure number	Preliminary tissue preparation	Immediate fixation	25 μ Sections in cryostat with freezing microtome	Additional Pre-incubation fixation	Incubation in Wachstein-Meisel medium (32)	Tissue preservation	Localization of reaction product on cell membrane	Localization in mitochondria
1	1 cu mm tissue blocks	Osmium-sucrose 3 min.	0	0	Blocks incubated 15 min.	Good	+	0
2A	2 mm thick slices quenched in liquid nitrogen	0	yes	Osmium-sucrose	25 μ sections incubated 15 min.	Modt.	+	+
2B	Same as 2A	0	yes	Buffered formalin-sucrose pH 7.2	25 μ sections incubated 15 min	Modt.	+	+
2C	Same as 2A	0	yes	0	25 μ sections incubated 10 min.	Poor	0	0
3A	2 mm slices of tissue	Buffered formalin-sucrose	yes	0	25 μ sections incubated 15 min.	Modt.	+	0
3B	Same as 3A	Buffered formalin-sucrose	0	0	2 mm slices incubated 15 min.	Modt.	+	0

copy. In tissue prefixed in osmium tetroxide before incubation, the darkening due to reaction product could not be appreciated and it was necessary to use random blocks for ultrathin sectioning.

The comparative results of the different procedures are indicated in the table. Tissue that was block-fixed in osmium-sucrose for 3 minutes and then incubated as a block with the substrate prior to further osmium fixation was better preserved by this procedure than by any other. Localization of the reaction product on the outer cell membranes was well demonstrated by this method, but the mitochondria were not the site of localization.

The most consistent and abundant deposition of reaction product for ATPase in these studies was obtained from tissue that first had been quenched in liquid nitrogen, sectioned in the cryostat at 25 μ, fixed for 30 to 90 minutes in buffered formalin sucrose at 4°C, incubated in Wachstein-Meisel substrate for 15 minutes, and then treated with ammonium sulfide. It was only in tissues treated in this manner that any localization of the reaction product in the mitochondria was accomplished. Tissue preservation was not so satisfactory with this procedure as it was with the procedure in which the tissue was first fixed as a block in osmium tetroxide.

Tissue that had been quenched in liquid nitrogen, sectioned at 25 μ in the cryostat, and incubated without any prior fixation showed very poor preservation. When this procedure was used, there was an erratic distribution of the reaction product, and the precipitate failed to localize in areas where it is known from light microscopy to occur. This was interpreted to indicate an artifactual deposition or dislocation of the reaction product.

Frozen sections of formalin-sucrose fixed tissue, which had been incubated with substrate, gave a more widespread and uniform distribution of the ATPase localization product in electron microscopy than did block-fixed and block-incubated tissue. Even so, there was considerable variation of results among different experiments using the same procedure, and within the same tissue of a given experiment. As pointed out by Holt and Hicks (12), some of this variability can be accounted for by imperfect penetration in tissue of varying thickness. In some preparations, there were erratic interstitial and cytoplasmic deposits of reaction product, bearing no apparent relationship to specific fine structural components.

FIGURE 1

Electron micrograph of control tissue incubated in the Wachstein-Meisel medium without ATP, followed by treatment with dilute ammonium sulfide. Prior to incubation, the tissue was quenched with liquid nitrogen, sectioned at 25 μ in a cryostat, and fixed in formalin-sucrose. Osmication, dehydration, and methacrylate embeddings were then carried out. The section is from intestinal epithelium and shows microvilli (MV), mitochondria (M), and cell membrane (CM) between two adjacent cells. A few black particles of metal precipitate are noted (X) between the microvilli, but they are sparse and are not distributed according to any pattern. Magnification, 14,500.

FIGURE 2

Electron micrograph of control tissue prepared as in Fig. 1. The deep portions of adjacent intestinal epithelial cells are shown. Nuclei (N) are present and a nucleolus (NU) is present in one nucleus. No metallic precipitate is visible. Magnification, 11,500.

FIGURE 3

Electron micrograph of glomerulus from kidney tissue treated as a control. This specimen was fixed as a block, 1 cu mm. in size, in formalin-sucrose, and incubated in the Wachstein-Meisel medium from which ATP was omitted. The tissue was treated with ammonium sulfide after incubation. Glomerular capillaries (C) are shown, and the basement membrane (B) is present. The endothelial lining (EN) can be identified, and the foot processes of the glomerular epithelial cells (F) are apparent. There is no recognizable metallic precipitate in the glomerulus. Magnification, 14,500.
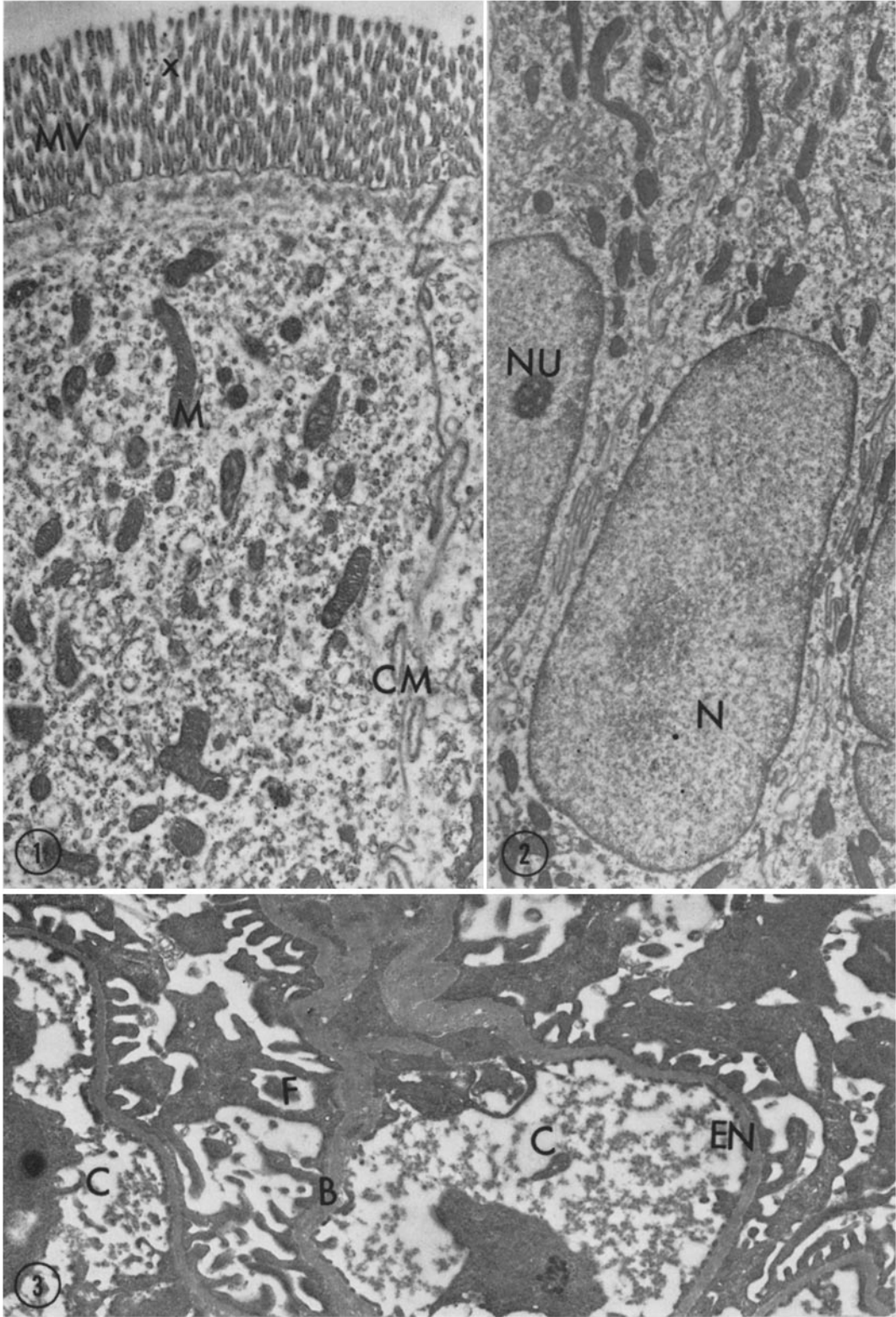
These were interpreted as being due to diffusion artifacts. Nevertheless, consistencies of localization were found that are believed to be indicative of a certain distribution pattern. In the presentation of material, most illustrations were deliberately chosen to reveal these more consistent patterns.

In control specimens (Figs. 1 to 3) incubated as blocks, as slices 1 to 2 mm thick, or as frozen sections in medium from which ATP was omitted, metal precipitates were usually entirely absent. Sparse deposits were found in some areas, but these bore no constant relationship to cellular components, with the possible exception of nuclei. In a few of the control specimens, occasional nuclei were partially overlaid by clumps of metal precipitate. When present, these deposits were unrelated to nucleoli or other specific nuclear constituents.

The patterns of localization of the ATPase reaction product in intestine, kidney, and liver seen with light microscopy are shown in Figs. 4 to 7 for correlation with the finer localization revealed by electron microscopy.

INTESTINE: Most consistently, as light microscopic study has amply revealed (24), the enzyme localization was found in the microvillous area. It is apparent that the reaction product is located at the outer surface of the microvilli (Fig. 8). No evidence was found of any significant localization within the substance nor on the fine longitudinal filaments of the microvilli. The precipitate seemed to encrust the microvilli, and the deposit was too heavy to permit accurate determination of its relationship to the cell membrane. In certain areas, however, the linear deposits of reaction product appeared to coincide with the cell membranes of the microvilli (Fig. 9).

At areas of cellular apposition, the plasma membranes were the site of localization. In some examples, the cells were strikingly demarcated by this deposition (Fig. 10). Usually, there was no deposition of the reaction product at sites of desmosomes, although it was present frequently at the terminal bar.

Localization of reaction product in the terminal web was highly variable, and, in no case, was it marked. Usually a few granular deposits were found in this area, occurring in about the same amount as in the ground cytoplasm elsewhere in the epithelial cells.

Mitochondrial localization of the reaction product was demonstrated only in those experiments where tissue had been quenched and then sectioned in the cryostat at 25 μ. In these experi-

FIGURE 4

Photomicrograph of small intestinal mucosa prepared for ATPase by the method of Wachstein and Meisel. Microvillous localization of the reaction product is apparent. It is not possible to discern a definite pattern of reaction within the cytoplasm, but the nuclei and nucleoli give a faint reaction. Magnification, 160.

FIGURE 5

Photomicrograph of kidney tubules. The dark tubule in the center is a distal convoluted tubule. The reaction product is deposited in the basal part of the cytoplasm in a faintly striated pattern. The adjacent light-staining proximal convoluted tubules show a faint reaction on the brush border and in the nucleoli. Magnification, 800.

FIGURE 6

Photomicrograph of ATPase reaction in kidney. Glomeruli are the seat of marked localization. Although there is no heavy deposit on the brush border of the proximal convoluted tubules, some darkening of this area indicates a slight reaction. The fine dots in the tubular epithelial cells are nucleoli showing localization of the metallic precipitate. Magnification, 270.

FIGURE 7

Photomicrograph of ATPase reaction in liver. Most of the visible deposits mark the bile canaliculi. Magnification, 200.
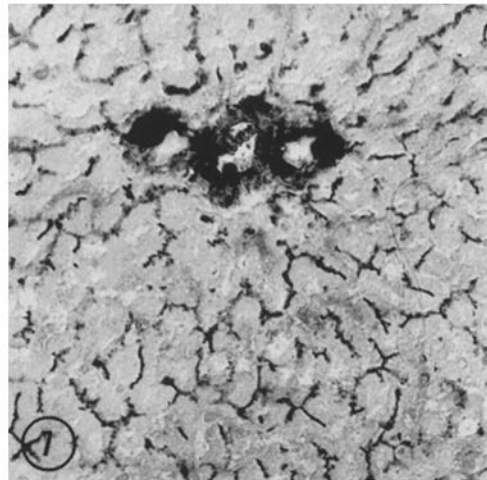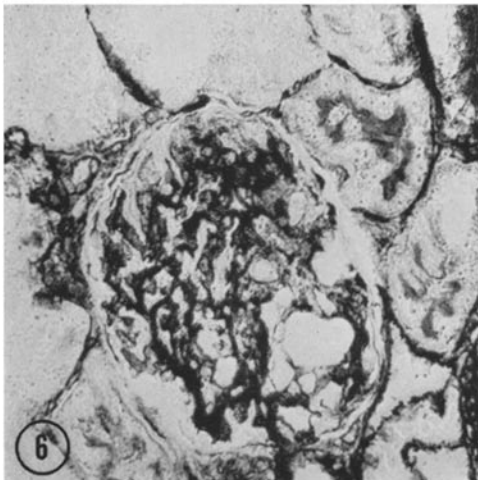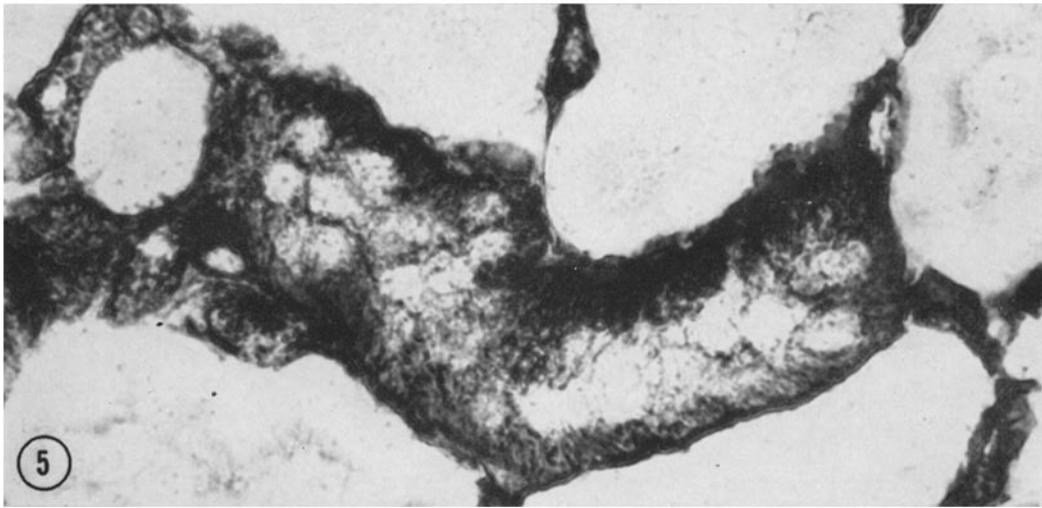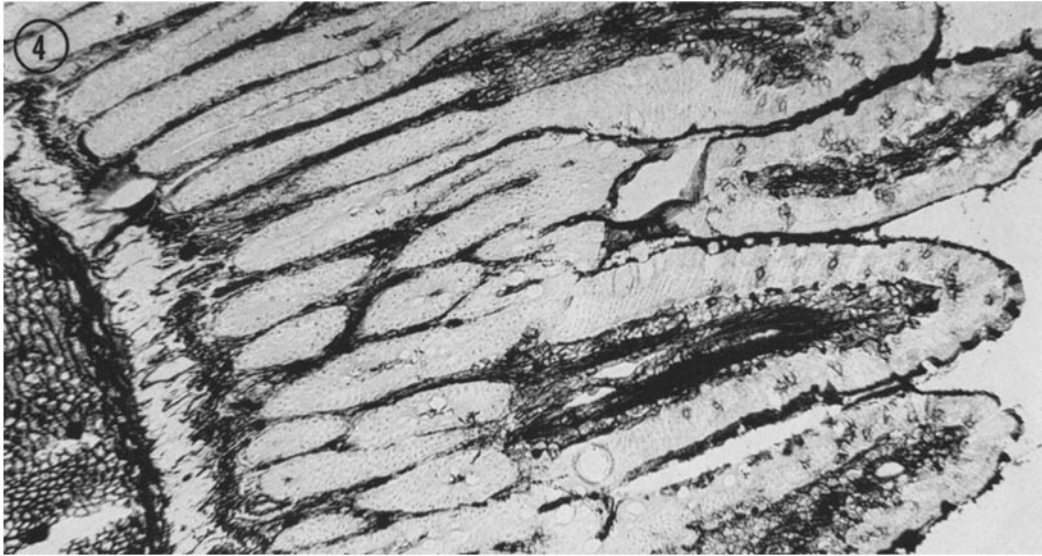
ments, the outer mitochondrial membrane and the cristae mitochondriales were blackened by the deposits (Figs. 11 and 12). In some mitochondrial deposits the reaction product was no thicker than the cristae themselves, but in most instances the cristae were moderately thickened by the encrustment.

In the intestinal epithelium, as in all other tissues studied, localization of the reaction product in the nuclei was a striking and consistent finding. This occurred in tissue sectioned in the cryostat as well as in block-fixed tissues (Fig. 13). The reaction product was usually most abundant in the nucleoli, where it appeared to occupy the sites of the coarse (probably ribonucleoprotein) granules. It was also concentrated in the nuclear material just within the nuclear membrane, and in clumped areas throughout the remainder of the nucleus. These nuclear deposits differed from those occurring infrequently in control specimens incubated with lead salts in the absence of ATP by the greater amount of opaque deposits, the more uniform distribution, and by the finer, more diffuse deposition in the nuclear substance and in the nucleoli.

In the lamina propria the reaction product occurred in the nuclei of lymphocytes, plasma cells, fibrocytes, and endothelial cells, where its distribution was similar to that in the intestinal epithelial cell nuclei. Also, abundant deposits were found in the endothelial cytoplasm of capillaries and lymphatics. In the smooth muscle cells of the muscularis mucosae and muscularis, irregular deposits were also observed in the cytoplasm. The bundles of myofibrils were not specifically stained, however. Heavy, clumped deposits of the reaction product were noted frequently in mitochondria of the muscle cells. These deposits did not localize on the cristae or outer membranes of the mitochondria as clearly as they did in the mitochondria of intestinal epithelial cells.

KIDNEY: The glomeruli were regularly the seat of abundant deposition (Fig. 14). Some of the reaction product was located in the thin endothelial cytoplasmic lining of the glomerular capillaries, but was more abundant in the epithelial cell foot processes. A heavy encrustment was found on or in the cell membrane of the epithelial cytoplasmic trabeculae and along the site of attachment of the foot processes to the outer part

FIGURE 8

Electron micrograph of small intestine mucosa after ATPase reaction using small block osmium-sucrose fixation and block incubation (procedure 1 of Methods). Ammonium sulfide treatment was carried out in this experiment. The reaction product can be seen as a heavy coating on the microvilli (MV). Some of the cell membranes (CM) and the nucleus (N) also react in this area. Mitochondria (M) show no reaction in this preparation. A fine granular deposit can be seen throughout the ground cytoplasm. Magnification, 10,000.

FIGURE 9

Electron micrograph of small intestine mucosa in area where the reaction product for ATPase in the obliquely sectioned microvilli is relatively slight. In some areas (X) the linear deposits are of approximately the same thickness as the tangentially sectioned cell membranes of the microvilli. Reaction product in the terminal web (TW) is sparse and erratic. Tissue preparation by quenching in liquid nitrogen, fixation of 25 μ thick cryostat sections in formalin-sucrose prior to incubation (procedure 2A of Methods). Ammonium sulfide was used. Magnification, 45,000.

FIGURE 10

ATPase reaction in small intestinal mucosa prepared by procedure 1 of Methods (ammonium sulfide was used). The reaction product outlines obliquely sectioned microvilli (MV). The cell membranes (CM) are clearly demarcated by deposits of the reaction product. At (X), desmosomes of the cell membranes are seen in which the reaction product is absent, but at the terminal bars (T) it is present. Mitochondrial (M) localization is negative in this type of preparation. Magnification, 10,000.
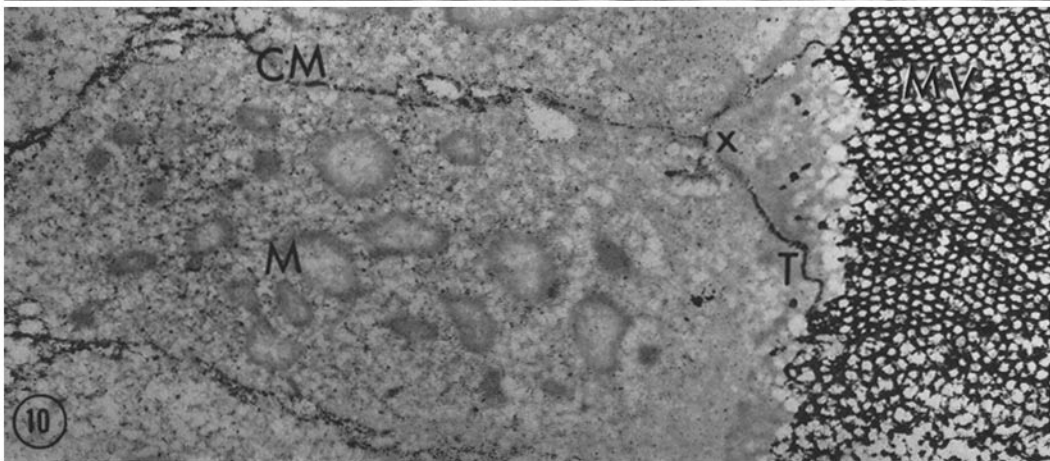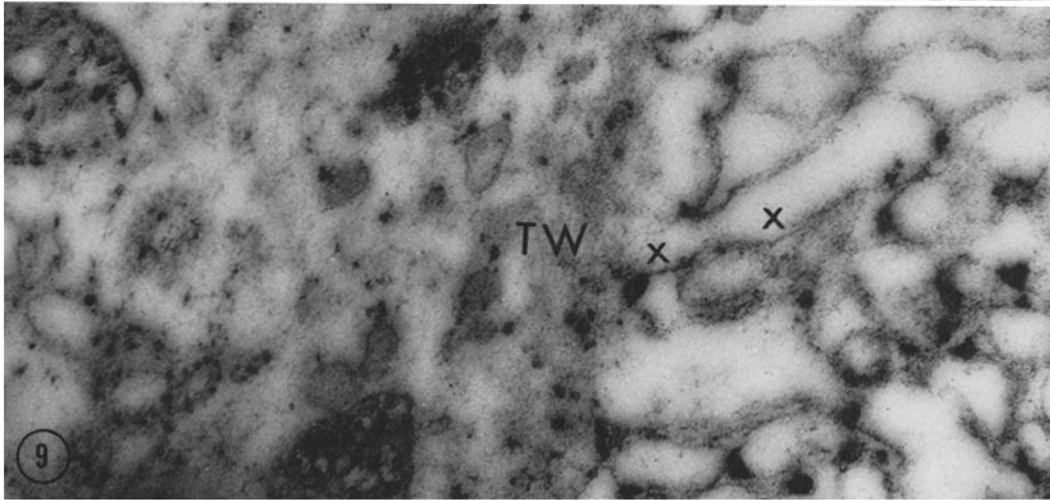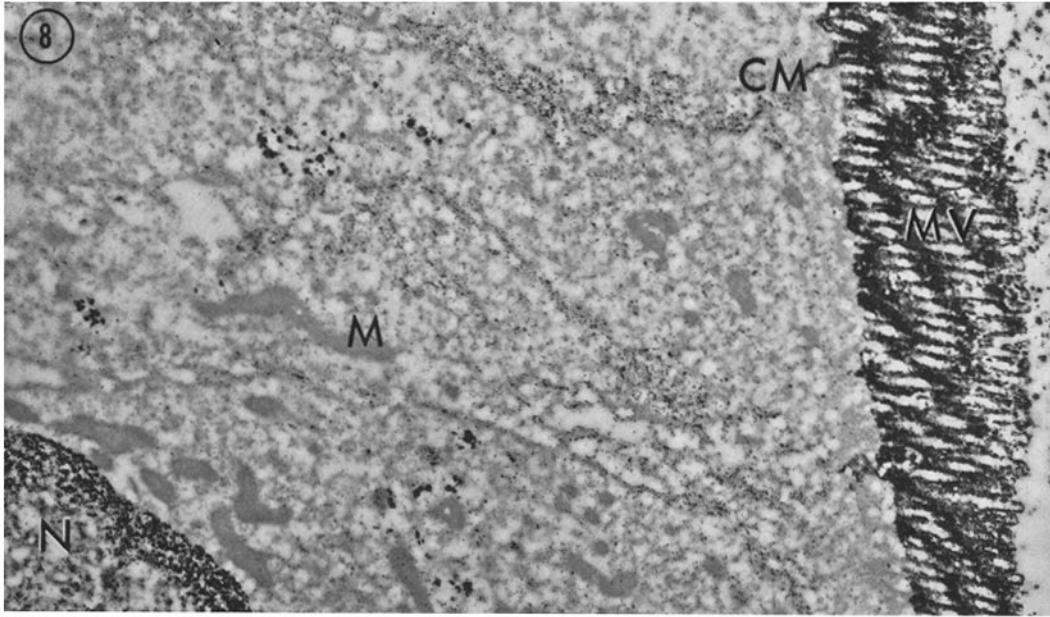
of the basement membrane. Deposits of the reaction product also occurred focally and variably within the basement membrane. These were seen in block-fixed as well as in cryostat-sectioned tissue, and their significance is unknown. Nuclei of the epithelial and endothelial cells in the glomeruli were involved as noted previously in other cells.

The proximal and distal convoluted tubules were stained more intensely than the other segments of the renal tubules. Although deposits were formed on and between the microvilli of the proximal convoluted tubules (Fig. 15), they were not nearly so marked as on the intestinal microvilli. This corresponds with the observation of much less ATPase localization with light microscopy in proximal convoluted tubules than on intestinal epithelium, although very abundant alkaline phosphatase is known to occur at the brush border (30). The cell membrane of the smaller and sparser microvilli of the distal convoluted tubules was frequently encrusted with the reaction product.

The other site of localization in the proximal and distal convoluted tubules was at the base of the cells (Fig. 16). In some areas the deposit occurred on the basal infoldings of the cell membrane. It was more pronounced in the distal convoluted tubules and ascending limbs of Henle where basal cytoplasmic membrane infoldings are more abundant and extend deeper than in the proximal convoluted tubules (Fig. 17). Irregular deposits were frequently seen also in the adjacent basement membrane. In tubules in which abundant precipitate was found in the basement membrane, the deposit on the cell membrane infoldings was usually minimal or absent. In many renal tubules, mitochondrial localization was apparent (Fig. 18). These deposits sometimes conformed to the mitochondrial membranes as they did in intestinal epithelium, but, in other instances, coarse clumps of the reaction product were ir-

regularly distributed in the mitochondrial matrix. Nuclei of all renal tubular epithelial cells contained the reaction product. The cytoplasm of peritubular capillary endothelial cells was also the seat of localization of the reaction product.

LIVER: There was greater variability in ATPase localization and less satisfactory preservation in liver cells than in intestine or kidney. The precipitate was found on and in the microvillous projections of the bile canaliculi in almost all experiments (Fig. 19). It also extended along the cell membrane where liver cells were in close apposition, and into the hepatic cell membrane fronting on the space of Disse. The sheets of cytoplasm of the sinusoidal endothelial cells were frequently the site of localization (Fig. 20). In a few experiments, it was possible to demonstrate deposition of the reaction product in the cristae and outer membranes of mitochondria of the parenchymal liver cells. Round or oval dense bodies near the bile canaliculi (lysosomes?) usually contained abundant reaction product. Lipid droplets within the liver cells were often surrounded by a distinct ring of the reaction product. The most consistent site of deposition in the liver was the nuclei. The pattern of distribution here coincided with that seen in the nuclei of other sites studied, being more heavily concentrated in the nucleoli.

DISCUSSION

These studies reveal that the best localization of ATPase reaction product, as studied with electron microscopy, is obtained when tissue has been quenched with liquid nitrogen, sectioned at 25 μ in a cryostat or with a freezing microtome, fixed in formalin-sucrose, and then incubated with the substrate. This procedure makes it possible to demonstrate localization of the reaction product on mitochondria as well as on the plasma membrane. Our findings are similar to those of Holt and Hicks (12) in indicating that formalin-sucrose

FIGURE 11

Small intestinal mucosa revealing mitochondrial (M) localization of the ATPase reaction product. Tissue preparation by procedure 2A of Methods, using ammonium sulfide. The cristae reveal dense deposits, and in some areas (X) the outer mitochondrial membranes are the seat of deposition. While most of these deposits are thicker than the width of the unstained cristae, others are of approximately the same width, suggesting that the reaction product is located either between the two unit membranes of the cristae, or within the two unit membranes. Magnification, 24,000.
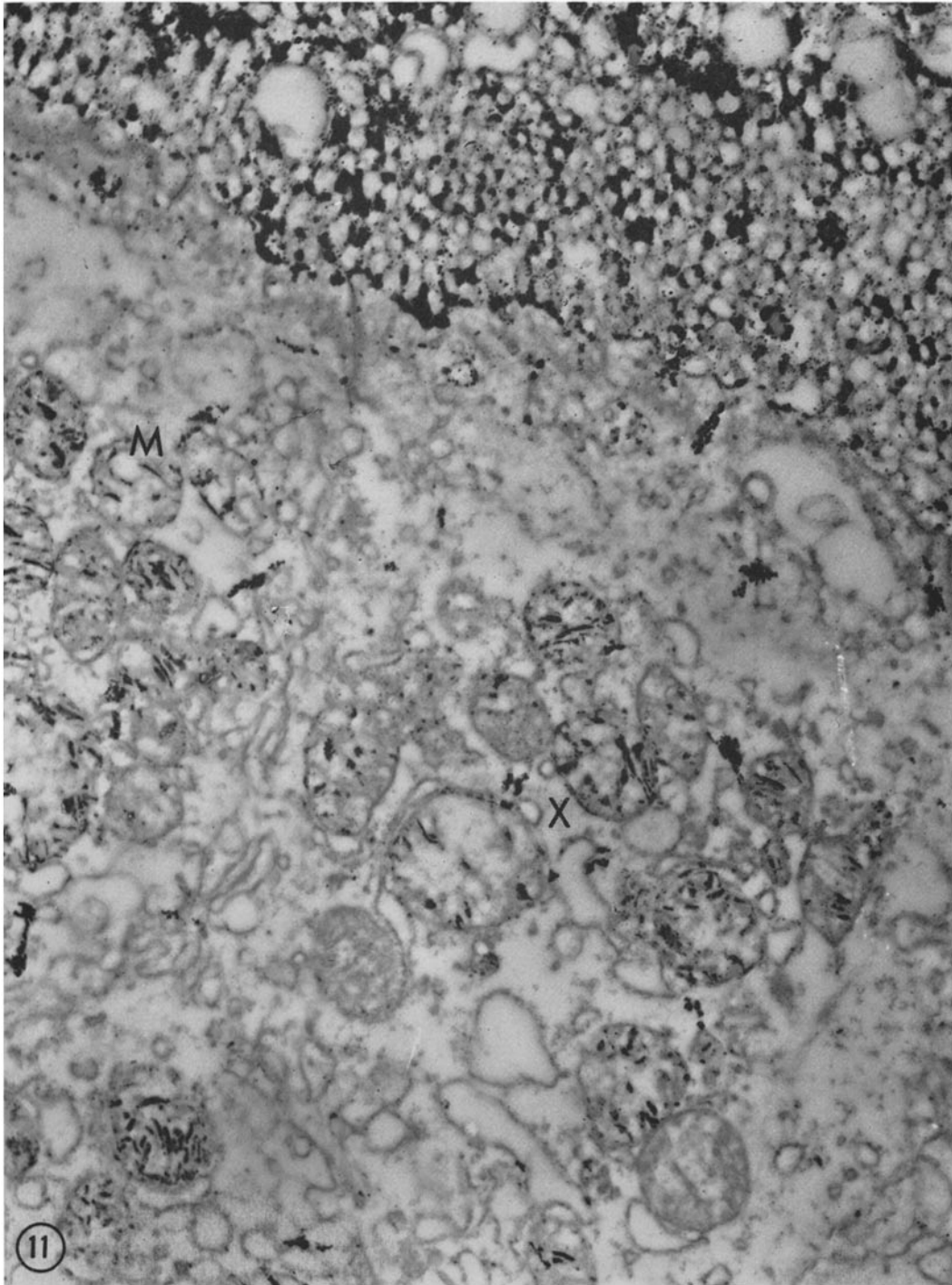
buffered to pH 7.2 is a useful tissue preservative for localization of the ATPase reaction product by electron microscopy. Brief preliminary fixation of small blocks in osmium tetroxide also gave good results for cell membrane localization, and the best tissue preservation was obtained by this procedure. Mitochondrial localization, however, was not accomplished when this procedure was employed.

Most of the existing data on the intracellular localization of enzymes are based upon studies in which chemical enzyme determinations were performed on various cell fractions obtained by ultracentrifugation. These studies have contributed greatly to the understanding of cell metabolism and have resulted in the establishment of a spectrum of patterns of intracellular enzyme distribution. Nevertheless, this method of study presents certain well known obstacles. The known impurity of cell fractions has been one of the sources of difficulty. As an example, although de Duve (5) formulated the concept of lysosomes several years previously, mitochondria at first were separated along with lysosomes and, until fractionation procedures were improved, the differences in enzyme content in these two organelles were not revealed by direct chemical analyses. The localization of acid phosphatase on lysosomes rather than on mitochondria with electron microscopy (7) aided in the clarification of this problem. In the

FIGURE 12

Electron micrograph of basal portion of intestinal epithelial cell. Tissue preparation by procedure 2A (without ammonium sulfide). Mitochondria (M) in this location also are the seat of localization of the reaction product of the ATPase procedure. Magnification, 45,000.

FIGURE 13

Electron micrograph showing localization of the reaction product in nuclei (N) of intestinal epithelial cells. The deposits are somewhat more concentrated in the nuclear material near the nuclear membrane and in the nucleoli (NU). Tissue prepared by formalin-sucrose fixation of 1 mm thick slice, followed by incubation of 25 μ frozen sections (procedure 3A of Methods, without ammonium sulfide). Magnification, 9,000.

FIGURE 14

Electron micrograph of portion of glomerulus after ATPase reaction. Tissue preparation by procedure 1, using ammonium sulfide. Most of the deposit outlines the cytoplasmic processes and pedicels (P) of the epithelial cells. The basement membrane (B) itself is almost entirely devoid of any reaction product. At (X) a very slight deposit is seen in the thin endothelial lining of glomerular capillaries. The membrane-lined structures (V) within capillaries which are the seat of marked deposit of the reaction product probably represent endothelial cytoplasmic blebs. Magnification, 8,000.

FIGURE 15

Electron micrograph of portion of cell of proximal convoluted tubule. Tissue preparation same as in Fig. 14. A moderately heavy deposit of reaction product can be seen on the microvilli (MV) where it is partially localized on the outer membrane. Mitochondria fail to show localization of the reaction product in this procedure of tissue preparation. Magnification, 18,000.

FIGURE 16

The basal portion of the same cell shown in Fig. 15, from the proximal convoluted tubule. The basal cytoplasmic membrane infoldings (I) are sharply defined by the reaction product. There is a heavy deposit in the basement membrane (B), while mitochondria (M) are negative. Magnification, 18,000.
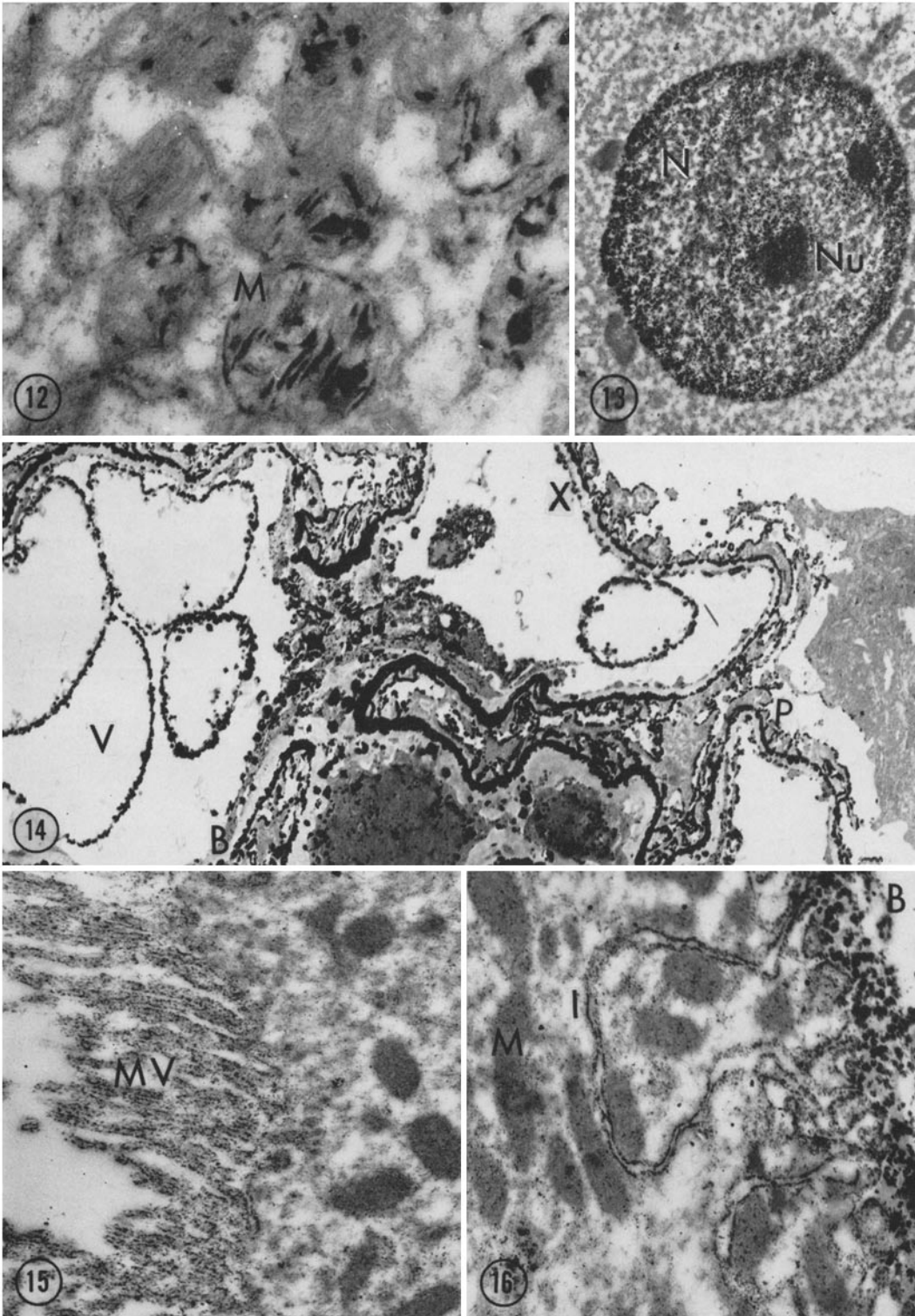
separation of cell constituents by centrifugation of homogenates, the cell (plasma) membrane has not been successfully isolated for chemical enzyme analysis but is probably separated out with the microsomal fraction, along with other cytomembranes. Another factor which affects the interpretation of enzyme determinations on cell fractions is the mixture of different cell types existing in mammalian tissues. The enzymic content of different cell types undoubtedly varies considerably. These and other difficulties in chemical enzyme analyses are thoroughly appreciated, and have been carefully weighed by most investigators. It is evident, from these and other considerations, that electron microscopic studies will shed additional light on the enzyme content and localization in specific organelles and in the various types of cell membranes, some of which have not yet been successfully separated by ultracentrifugation techniques.

Previous reports already cited have demonstrated the localization and discussed the possible biochemical significance of ATPase on microvilli of intestinal epithelium, in the bile canaliculi and cell membranes of hepatic cells, and at the basal portion of proximal convoluted tubules of the kidney. Novikoff (20) also depicted the localization of the reaction product for ATPase in the glomeruli. His studies showed the deposit to occur on the endothelial lining of the glomerular capillaries. Our results indicate that the reaction occurs even more abundantly on the cell membrane of the pedicels of the glomerular epithelial cells. In his preparations, tissue had been fixed in formal-calcium, while formalin-sucrose was used in our studies. This difference in tissue preparation possibly might account for the different results.

Chemical studies on cell fractions have indicated that ATPase is present predominantly in mitochondria (21, 28) and in nuclei (9). A small amount also has been found in the microsomal fraction but none in the supernatant fluid. Our observations with electron microscopy substantiate and extend these findings, revealing localization of the reaction product for the enzyme on the mitochondrial cristae, on plasma membranes, and in the nuclei of renal, hepatic, and small intestinal epithelial cells.

In a study of the localization of acid phosphatase in intestinal epithelium, Sheldon and others (2⁷

FIGURE 17

Area of lining epithelium of Henle's loop. Reaction product is localized on the cell membrane (CM), on the cytoplasmic membrane infoldings (I), and in the nucleus (N). Tissue preparation as in Fig. 16. Magnification, 18,000.

FIGURE 18

Electron micrograph of portion of distal convoluted tubule showing localization of reaction product in mitochondria (M). The deposit is located on the cristae. Some of the deposits do not exceed the total thickness of the cristae. A clear space between the lines of deposit on a few cristae or on the outer mitochondrial membrane can be seen (X). Procedure 2A without ammonium sulfide. Magnification, 45,000.

FIGURE 19

Electron micrograph of liver showing localization on the microvilli (MV) of the bile canaliculus. (C) Small dense bodies (D) containing reaction product are observed around the bile canaliculus. Procedure 1 with ammonium sulfide. Magnification, 14,500.

FIGURE 20

Electron micrograph of liver cells treated for ATPase as in Fig. 19. The reaction product is visible on the cell membrane (CM) at the sinusoidal surface (S). A small amount of endothelial cytoplasm (E) remains, which shows a slight deposit of reaction product. The intercellular spaces are delineated by a reaction on the cell membrane, and there is a marked reaction on the microvilli of the bile canaliculi (C). A dense line around lipid droplets (L) suggests a reaction at this site. No localization occurs on the mitochondria in this type of tissue preparation. Magnification, 14,500.
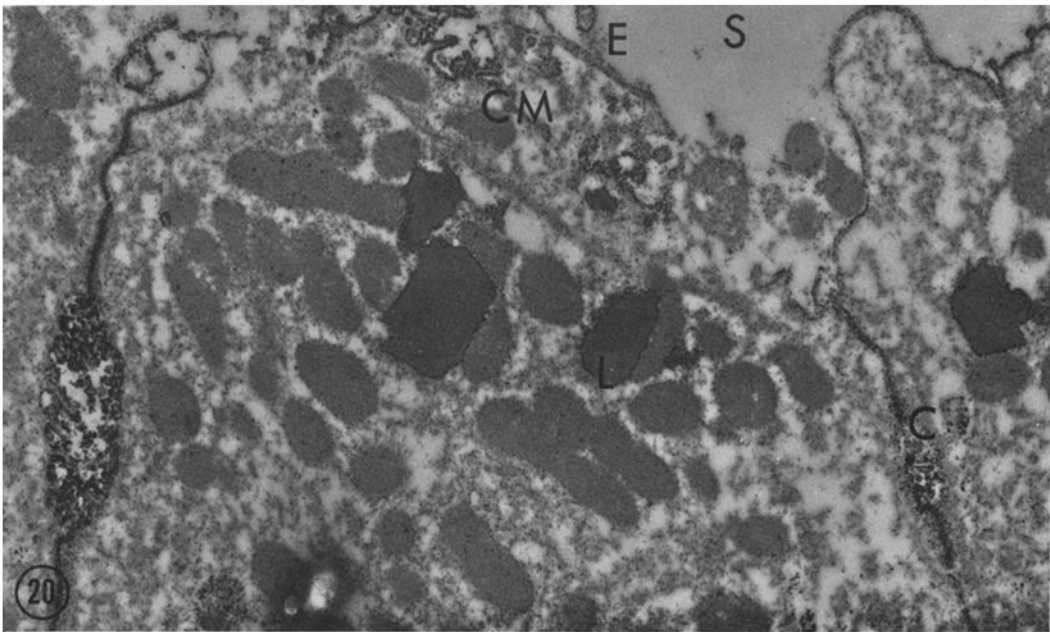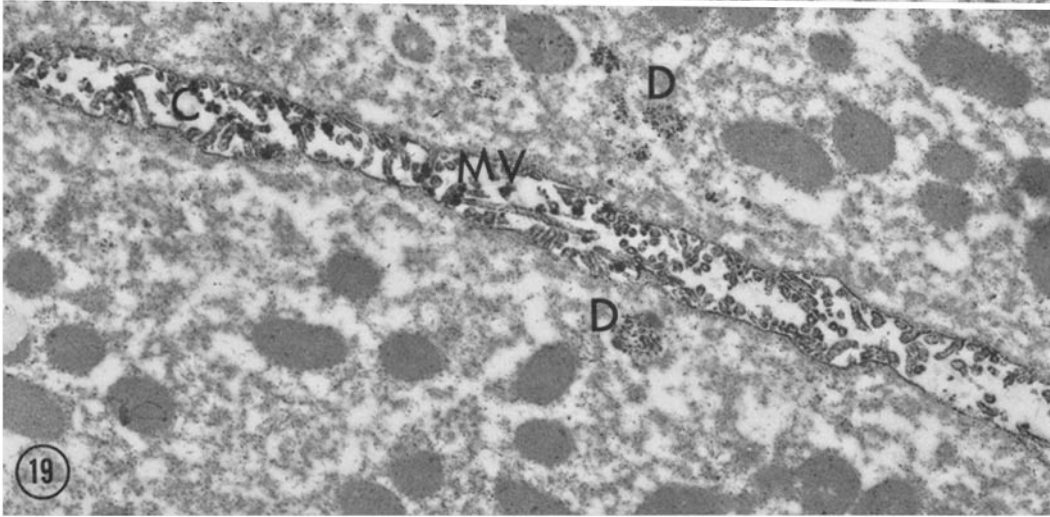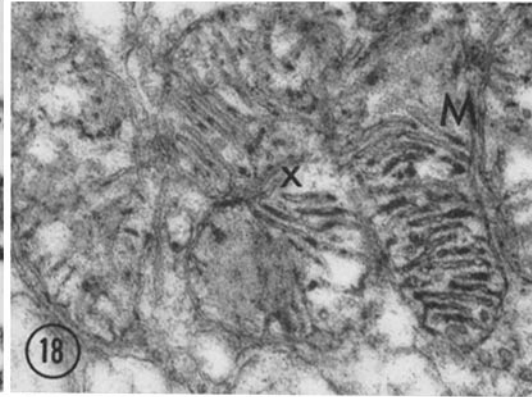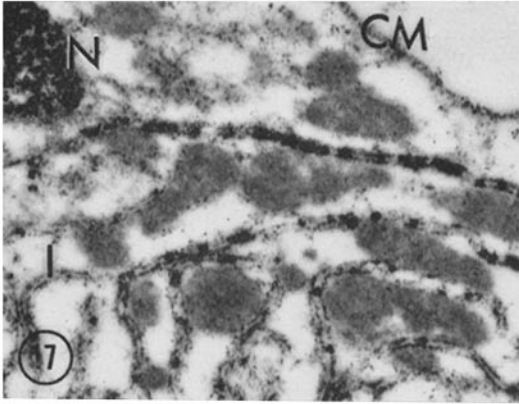
showed that the reaction occurred mainly in the intermicrovillous spaces, but its exact relationship to the microvilli was not readily apparent. In our material also, the precise localization of ATPase on the cell membrane has not been resolved adequately. Whether it is located actually within or on the "unit membrane" described by Robertson (26) cannot be ascertained since the deposits of the reaction product overlie and obscure the membrane. The unit membrane over the microvilli is known to be thicker than most other cell membranes, and this might be related to a large amount of phosphatases concentrated in or on this membrane.

The demonstration of ATPase in mitochondria by electron microscopy is of considerable interest, since this had not been accomplished until the recent work of Wachstein and others (31). Using a modification of their procedure, they showed with light microscopy that mitochondria may react for ATPase. Their studies indicated that sectioning in the cryostat may produce sufficient mitochondrial damage to allow the ATPase reaction to occur. Apparently, one of the difficulties is the fine degree of localization on the cristae which would be difficult or impossible to see with light microscopy. In some of our material, larger clumps of the reaction product occurred in the matrix rather than on the cristae of the mitochondria. This could be explained by the probable occurrence of marked alterations in the mitochondria during the processes of quenching in liquid nitrogen, cryostat sectioning, and incubation with the substrate. Such larger clumps might be visualized more readily with light microscopy than the more finely localized product on the cristae.

Localization of the reaction product for ATPase on the cristae and outer membranes of mitochondria is in accord with the chemical demonstration of the enzyme in mitochondrial fractions obtained by ultracentrifugation of cell homogenates (23). Such a localization of ATPase on mitochondrial cristae also can be reconciled with prevalent concepts of the localization on the cristae of other mitochondrial enzymes (10), especially those involved in the processes of biological oxidation. Siekevitz and others (28) have suggested a possible role for mitochondrial ATPase in oxidative phosphorylation. Pearse and Scarpelli (25) showed a pattern of distribution of succinic dehydrogenase in isolated mitochondria suggestive of localization of this enzyme on the mitochondrial cristae.

In light microscopic studies for ATPase a faint metal deposit can be seen in the nuclei, and especially in the nucleoli. Electron microscopy reveals this much more clearly because the metal precipitate is more electron opaque than and different in configuration from nuclear granules. Nevertheless, as discussed by Novikoff and others (21), the present status of nuclear enzymes is questionable. Partly, this is due to the difficulty in obtaining nuclei uncontaminated with other cell constituents. The tendency for any existing nuclear enzymes to be lost or inactivated during preparation (1) adds to the difficulty of interpretation of chemical analyses for nuclear enzymes. Although ATPase has been demonstrated by chemical studies (9) on nuclear fractions, this evidence cannot be construed as positive evidence that the enzyme is present in intact nuclei. Additionally, there is the problem, as discussed by Holt (11), that nuclei manifest a non-specific affinity for lead salts from lead-containing media. The activation of amino acids for protein synthesis within the nucleus requires the presence of ATP (14), but there is no evidence that this process requires ATPase. In view of these difficult and complex problems which beset the interpretation of nuclear enzyme localization, the observations upon nuclear localization of the metal precipitate in our experiments cannot be subjected to fruitful interpretation.

Although it is believed that most of the variations in content of ATPase from area to area in the cells studied could be explained by artifacts of various types, it is also possible that some enzymic heterogeneity exists among cells of the same type. Novikoff (19) indeed has demonstrated such a heterogeneity for ATPase, and other phosphatases within the hepatic lobule. To evaluate this possibility using electron microscopy, it would be necessary to develop the technical methods to a more reliable status.

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