CYTOCHEMICAL DIFFERENCES IN KIDNEYS FROM WINTER-HIBERNATING AND AROUSED BATS (*MYOTIS LUCIFUGUS*), WITH PARTICULAR REFERENCE TO THE GOLGI ZONE

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ABSTRACT

Kidneys from winter bats (*Myotis lucifugus*) were removed and fixed in cold formalin-calcium while the animals were in the following states: (*a*) natural hibernation; (*b*) arousal from hibernation for 24 hours; (*c*) laboratory maintained hibernation; and (*d*) no hibernation since the previous winter. With fixed frozen sections, the lead salt method of Wachstein and Meisel with adenosine triphosphate as substrate (pH 7.2) showed enzymic activity localized in large vacuoles and smaller vesicles or droplets in the Golgi region of distal and proximal tubular epithelial cells of kidneys from hibernating bats. No ATPase activity was detected in the basal lamellae of tubular epithelium from hibernating bats. ATPase activity in the Golgi region was not seen in cells from kidney tubules of bats aroused from hibernation 24 hours previously or of animals that had not hibernated, whereas activity for ATPase was present in the basal infoldings of tubular epithelium from these animals. Inosine di- and triphosphatase and calcium activated ATPase activities were also detected in the Golgi region of hibernating bats but were not present in the basal infoldings of tubular epithelium from active animals. There was little or no activity toward the mono- and diphosphates of adenine, thiamine pyrophosphate, and the di- or triphosphates of guanidine, cytidine, or deoxyadenosine. The loss of enzymic activity from the Golgi region of the tubular epithelium from hibernating bats and its increase in the region of the basal infoldings of tubular epithelium in aroused bats suggests that the Golgi region plays a role in the synthesis of enzymic protein usually identified with the external cell membrane.

Differences in organization of the basal lamellae or infoldings of the proximal convoluted tubular epithelium between cold-stored and active summer bats (*Myotis lucifugus*) have been reported (Melman and Rosenbaum, 1963). In those studies, the method of Wachstein and Meisel (1957) for visualization of adenosine triphosphatase (ATPase) activity was employed to detect changes in the height as well as differences in enzymic activity of the basal infoldings between the variously treated animals. While the several criteria employed (Melman and Rosenbaum, 1963) suggested that the cold-stored animals had attained hibernation, it was not deemed possible to know to what extent the cold-stored animals had reached a state approximating that of natural winter hibernation. Accordingly, kidneys from naturally hibernating winter bats were studied to detect whether any of the differences described between cold-stored and active summer animals could be seen in winter-hibernating bats.

Although originally these experiments were

intended to compare kidneys from summer cold-stored and winter-hibernating bats using identical cytochemical methods, a different and apparently unusual localization of ATPase activity pointed to possible histophysiological differences between animals exposed to the two different situations. In the present study we considered the activity of several kidney phosphatases with particular reference to their intracellular localization during winter hibernation and immediately following awakening from the hibernating condition.

MATERIALS AND METHODS

Seventeen male and female *Myotis lucifugus* captured during December and January in caves in the vicinity of Rochester, New York, were employed for the present investigation. Six animals were sacrificed in the caves, while in a state of deep hibernation, by means of cervical fracture, and the kidneys fixed immediately. Five bats were removed from the cave and maintained in deep hibernation in a laboratory hibernaculum. Three of these animals were killed after 24 hours, two were killed 2½ months later. Four animals had never hibernated and were summer bats maintained in the laboratory at room temperature throughout the winter. Two animals were winter hibernators returned to the laboratory and maintained at room temperature for 24 hours. They were very active just prior to sacrifice but had no access to food.

Kidney segments were fixed in cold (4°C) calcium-formalin (Baker, 1946) for 24 hours, sectioned on a freezing microtome at approximately 5μ, washed briefly in cold distilled water, and stained for enzymic activity by several methods.

Acid phosphatase activity was visualized by means of the metal-salt method of Gomori (1952), with β-glycerophosphate as substrate at pH 5.2. Incubation took place for up to 40 minutes at 37°C. ATPase activity was demonstrated with the method of Wachstein and Meisel (1957) employing adenosine triphosphate ($2Na:3H_2O$) as substrate and 2 per cent $MgSO_4$ as activator. Incubation took place in Tris-maleate buffer (pH 7.2) at 37°C for 20 to 30 minutes. Adenosine di- and monophosphates (sodium salts) were substituted in the lead-salt substrate incubation mixtures for demonstration of activity of the corresponding enzymes. For demonstration of sites of false-positive localization of reaction product, sections of kidney were exposed to boiling water for 30 minutes and then incubated in the substrate medium. Other sections of kidney were incubated in a medium containing ATP which had previously been incubated (45 minutes at 37°C) with apyrase (Sigma Chemical Company, St. Louis, Missouri; potato source) in the presence of 0.002 M calcium chloride at pH 7.0. Subsequent addition of lead nitrate at pH 5.2 produced lead phosphate which would thus serve as a test for adsorption onto or affinity of this reaction product for specific tissue sites. Some calcium-formalin-fixed kidney slices sectioned in a cryostat were stained by the calcium method of Padykula and Herman (1955) at pH 9.4 using incubation times of 3 minutes to 1 hour.

Other nucleoside phosphates employed as substrates included di- and triphosphates of uridine, inosine, guanidine, cytidine, and deoxyadenosine. We used the methods described by Novikoff and Goldfischer (1961) for localization of nucleoside diphosphatase activity in the Golgi region. Tris-maleate buffer (pH 7.2) in combination with 0.025 M $MgCl_2$ was employed in the incubation medium. The ability to hydrolyze thiamine pyrophosphate was also studied using the method described by Novikoff and Goldfischer (1961). Control tissues consisted of rat seminal vesicle, epididymis, and kidney fixed in cold calcium-formalin and incubated together with the test tissues.

Classical staining methods for the Golgi apparatus were employed on kidney sections from six additional animals, consisting of two each of deeply hibernating and recently awakened winter bats, and animals that had not hibernated over the previous winter. Tissues were fixed overnight in 10 per cent formalin prepared with M/23 cadmium chloride ($CdCl_2 \cdot 2\frac{1}{2} H_2O$), embedded in paraffin, and stained with the Da Fano silver method (Lillie, 1954) and Elftman's modification (1950) of the silver method of Aoyama.

RESULTS

Localization of Adenosinetriphosphatase Activity in Kidneys of Winter-Hibernating Bats

With the incubation times we employed, magnesium activated-ATPase activity was localized in the Golgi region of cells from both distal and proximal tubules of kidneys from the hibernating bat (Fig. 1). All of the animals killed while in hibernation showed enzymic activity in the Golgi zone but the appearance of this activity was different in the two kinds of tubular epithelium. In distal tubules, multivesicular or large vacuolar structures were present about the nucleus, frequently filling the cytoplasm and even extending to the basement membrane (Figs. 2 and 5). Smaller stained vesicular bodies could be detected around these larger structures. In proximal tubules, the brush border showed enzymic activity (Fig. 3). In cells from the proximal tubules, the

Golgi region did not have a vacuolar appearance (Fig. 3). With slight over-incubations of 40 minutes, staining of the Golgi region for ATPase activity frequently appeared to merge with the heavily stained brush border (Figs. 3 and 4) and there appeared to be diffusion of reaction product as well. Smaller stained droplets with ATPase activity appeared below the Golgi regions (Fig. 4). A few distal and proximal tubules from hibernating bats' kidneys did not stain for ATPase activity with the methods we employed (Figs. 1 and 4). There was no staining of the glomerulus in any of the tissues we studied (Figs. 1 and 9). Even with extended incubation times of up to 60 minutes, we could detect no staining of the infolded membranes comprising the basal lamellae of hibernated bat-kidneys.

In some tubules, most often proximal ones, the contents of the lumen were stained (Figs. 3, 4, 6, 7, and 9). With absence of ATP as substrate, with boiled and subsequently incubated tissue or with incubation media containing ATP substrate previously treated with apyrase, such luminal contents continued to show deposition of the lead sulfide reaction so that, in our hands, this represented false-positive staining. In all cases, the brush border of proximal convolutions were stained and could be distinguished from the stained luminal contents to varying degrees (compare Figs. 3 and 4).

Localization of ATPase Activity in Kidneys of Winter Bats Aroused from Hibernation or Which had Never Hibernated

Localization of Golgi region-associated ATPase activity in cells of the proximal convolutions became altered after arousal from natural winter hibernation. Tissues for this group of observations came from several sources. Some animals were in a hibernaculum but not in deep hibernation. In kidneys from these partially awake bats, staining of the Golgi zone was evident but there was "smudging" and often less vacuolar staining (Fig. 6). It appeared that there was a shift in position of the region of ATPase activity toward the luminal portion of the proximal epithelium. In most cases, "droplets" or smaller vesicles were also stained (Fig. 9). The brush border showed enzymic activity, but no staining of basal lamellae of the proximal tubular epithelium could be detected (Figs. 6 and 7).

Two bats that were caught in caves while in deep hibernation and returned to the laboratory were fully awake 24 hours later. Kidneys from these animals showed no enzymic staining of the Golgi region with ATP as substrate (Figs. 7 and 8). As previously described for cold-stored summer bats (Melman and Rosenbaum, 1963), intense staining of the basal lamellae of the proximal tubular epithelium could be seen (Fig. 8). Staining of the brush border was also intense.

Four bats were maintained in the laboratory without hibernating for at least 12 months. Enzymic staining of the Golgi region of both the distal and proximal tubular epithelia could not be detected in any of these animals. However, basal infoldings of the proximal epithelium stained for ATPase activity.

ATPase activity was not detected in the Golgi region of any of the control tissues including rat kidney and epididymis.

Enzymic activity employing other phosphate substrates

The staining reactions obtained with several other phosphate substrates are summarized in Table I. Acid phosphomonoesterase activity as revealed by the Gomori lead salt method was present only in proximal kidney tubules of animals that had not hibernated for at least 12 months. Enzymic activity appeared as "droplets," some of which were in the Golgi zone but most of which were distributed throughout the cytoplasm of the proximal tubular epithelium and were distinguishable from those of the Golgi zone (Novikoff and Goldfischer, 1961). Staining for acid phosphatase activity in these tissues was identical with that previously described (Melman and Rosenbaum, 1963) in kidney tubules from active, feeding summer bats. No or very little acid phosphomonoesterase activity was detected in kidneys from hibernating or very recently aroused, unfed winter bats. We made no observations relative to the appearance of acid phosphatase activity in kidney tubules once the aroused winter bats had begun to feed.

When adenosine monophosphate was substituted as substrate, no staining of the Golgi zone in any of the bat kidneys was obtained. Adenosine diphosphate as substrate was hydrolyzed only slightly by the Golgi region in tubular epithelium of naturally hibernating animals, while no reaction

was detected in kidney sections from awakened bats.

With thiamine diphosphate (thiamine pyrophosphate) as substrate, the Golgi region of distal and proximal tubules from hibernating bats stained only slightly, while no enzymic activity could be detected in kidney tubules from hibernators awake for 24 hours or animals that had not hibernated for over 12 months (Table I).

When the diphosphate and triphosphate of inosine were employed as substrates, intense staining of the Golgi region in cells of the proximal and distal convolutions from winter-hibernating bats was observed (Fig. 10). Inosine diphosphatase activity in the Golgi zone was also intense in the kidney of previously hibernating animals which had been aroused 24 hours, while only slight activity could be detected in kidney tubules from animals that had never hibernated. Inosine triphosphatase activity in the Golgi region from awakened bat kidneys followed the pattern observed when the diphosphate was used as substrate, except that staining was less intense. Although several other nucleoside di- and triphosphates were employed as substrates, our results with these were either highly variable or negative. We obtained no staining of any renal tubular epithelium with the di- or triphosphate of guanidine, cytidine, or deoxyadenosine as substrate. Slight staining of droplets in the Golgi region was obtained with di- and triphosphates of uridine at the incubation times we employed.

Attempts at Localization of Calcium-Stimulated ATPase Activity in the Golgi Region from Kidneys of Winter-Hibernating Bats

With the calcium salt method for ATPase activity (Padykula and Herman, 1955) applied to cold calcium-formalin–fixed sections cut on a cryostat, there was considerable staining of the brush border of the proximal tubular epithelium even with short (30-minute) incubation times. This was presumably due to non-specific alkaline phosphatase activity (Fig. 11). With incubations longer than 30 minutes, some staining of the Golgi region of the proximal convolution could be de-

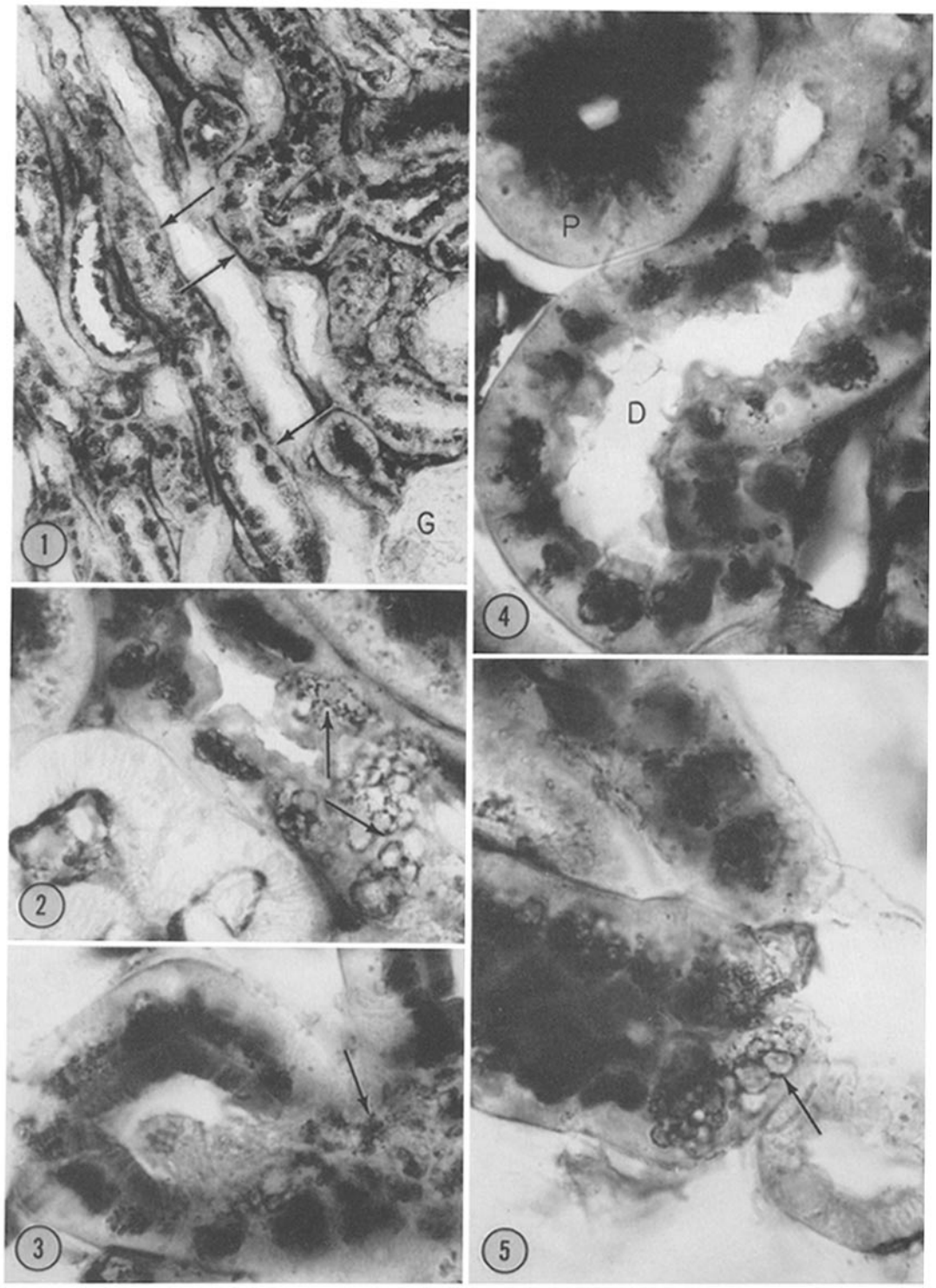
FIGURE 1 Section of a kidney from a hibernating-winter bat stained for ATPase activity by the method of Wachstein and Meisel (pH 7.2) with a 30 minute incubation. Enzymatic activity is present in the Golgi region (arrows) of many distal and proximal convoluted tubules. Especially in proximal convolutions, debris in the lumen shows false-positive staining. The glomerulus (*G*) and some tubules remain unstained. × 260.

FIGURE 2 Section of a distal tubule of kidney from a winter-hibernating bat stained by the method of Wachstein and Meisel for localization of ATPase activity. There is staining of structures (Golgi apparatus *sensu strictu*?) in the Golgi zone reminiscent of the shape of the classical reticular apparatus. Smaller droplets near this region are also stained. This is especially apparent where the section has cut through three or four epithelial cells (arrows). The basement membrane is stained. × 480.

FIGURE 3 Section of a proximal convoluted tubule from kidney of a winter-hibernating bat stained for ATPase activity by the method of Wachstein and Meisel. There is intense staining of the Golgi region as well as staining of "droplets" within the region. The brush border shows enzymic activity. Debris within the lumen is unstained. A cross-section through the Golgi zone of several cells is seen at the right (arrow). Basal infoldings are unstained. × 480.

FIGURE 4 ATPase activity (method of Wachstein and Meisel) in several kidney tubules from a winter-hibernating bat. The Golgi region is stained in nearly all cells of the distal tubule (*D*). Droplets and brush border are stained in cells of the proximal convolution (*P*). × 480.

FIGURE 5 Thick-frozen section (12μ) from the kidney of a winter-hibernating bat stained for ATPase activity by the method of Wachstein and Meisel. Large Golgi vacuoles (arrow) and small droplets in the Golgi zone are stained. × 540.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tected (Fig. 11), while staining of a similar region in the distal tubule was absent or spotty. Variable staining of the basal region of the proximal tubular epithelium suggestive of mitochondrion-associated enzymic activity was observed (Wachstein *et al.*, 1962). We made no observations relative to the possible biochemical inhibition of calcium-activated ATPase.

Enzymic Staining of Control Sections of Rat Kidney, Epididymis, and Seminal Vesicle

No activity for either calcium- or magnesium-activated ATPase was detected in the Golgi region of rat kidney, epididymis, or seminal vesicle. Enzymic activity in these tissues was generally restricted to cell membranes and capillaries. With use of nucleoside diphosphates (inosine, uridine) and thiamine pyrophosphate, intense staining occurred in the Golgi region of seminal vesicle and epididymis.

Classical Methods for Visualization of the Golgi Apparatus

Intense silver staining of the Golgi region was obtained in cells from the distal tubules of deeply hibernating bats (Fig. 14). The Golgi region of proximal tubular cells also stained but less intensely than the distal tubular cells (Fig. 13). There was virtually no Golgi staining of proximal or distal tubules in animals that had been aroused from hibernation or had never hibernated (Fig. 12).

DISCUSSION

The observations presented here show that ATPase activity, revealed by the method of Wachstein and Meisel, cannot be demonstrated in basal infoldings or lamellae of the proximal convolutions of kidneys from winter-hibernating bats until the hibernating animals have been awake for at least 24 hours. With this method, however, the Golgi region of both proximal and distal tubules from kidneys of hibernating bats stains intensely. The presence of an apparently well developed Golgi region of the proximal and distal convoluted tubular epithelium was not anticipated and the intense staining for ATPase activity in this region was very surprising.

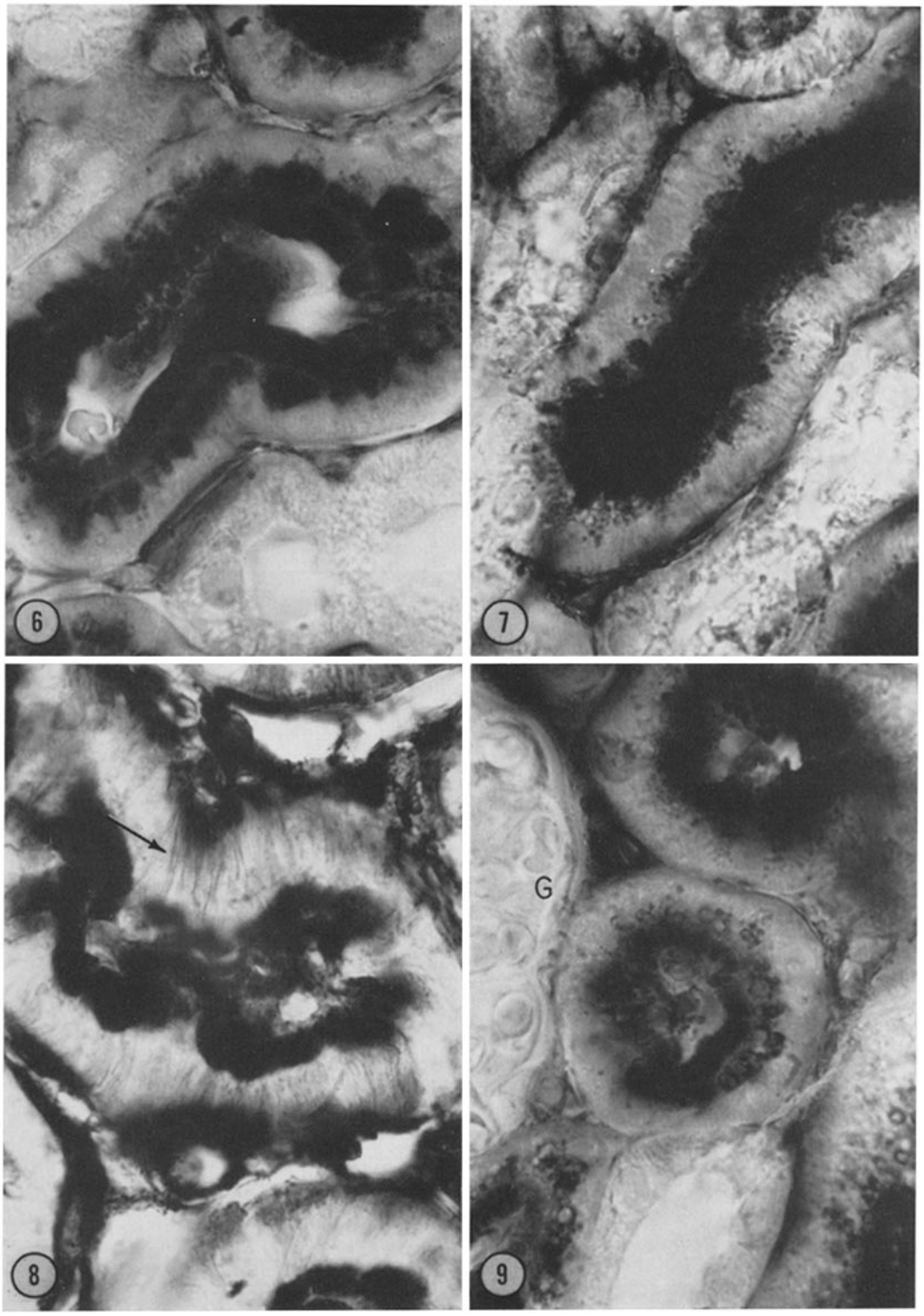
Aside from possible calcium-activated ATPase activity in the Golgi bodies of rat cerebellum (Tewari and Bourne, 1963) and enzymic activity visualized with a modified Wachstein and Meisel

FIGURE 6 Proximal convoluted tubule from a kidney of a winter-hibernating bat maintained in a hibernaculum for 21 days and stained for ATPase activity with a 20 minute incubation in the lead medium of Wachstein and Meisel. Staining of the Golgi region appears smudged, although localization of enzymic activity in droplets is evident. Such diffuse staining was variably encountered in tissues from different animals and does not appear due to over-incubation but rather to relative degrees of solubility of enzyme. The brush border is stained. There are no stained basal infoldings. × 480.

FIGURE 7 ATPase activity in proximal convoluted tubule of kidney from a winter-hibernating bat awake for 24 hours. Staining of a definitive Golgi region is minimal (compare with Figs. 3 and 6), but some stained vacuoles and droplets are evident toward the luminal side of the cells. There is slight staining of basal infoldings. The brush border is heavily stained but is obscured by staining of the luminal contents which was shown to be false-positive. × 480.

FIGURE 8 Proximal convoluted tubule of kidney from a winter-hibernating bat awake for 24 hours and stained for ATPase activity. There is staining of basal infoldings (arrow) and the brush border. No Golgi region is evident. × 480.

FIGURE 9 Cross-section of proximal and distal convoluted tubules of kidney from a winter-hibernating bat. ATPase activity in the Golgi region of the center proximal tubule is confined to distinct vacuoles which appear toward the luminal border of the cells. Staining of the brush border is present. The proximal tubule in the upper portion of the figure stains very diffusely for ATPase activity, obscuring details of brush border staining. The distal tubule in the lower portion of the figure and the glomerulus (*G*) show no enzymic activity. × 480.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incubation medium in rat cerebellum and a mouse plasma cell tumor (Novikoff and Goldfischer, 1961), adenosinetriphosphatase has not been reported in the Golgi apparatus histochemically. While earlier biochemical studies of isolated Golgi fractions of rat epididymis indicated ability to hydrolyze adenosine triphosphate in the presence of magnesium ions (Schneider and Kuff, 1954), improved isolation technipues showed that such enzymic activity does not reside in the Golgi fraction proper (Kuff and Dalton, 1959). Our own control sections of rat epididymis confirm this with visualization methods.

times we employed, however, only inosine diphosphate was hydrolyzed at a rate equivalent to that observed for the triphosphates of adenosine and inosine.

Our observations utilizing different nucleoside phosphates as substrate minimize the possibility that high levels of adenosine triphosphatase activity in blood vessels or serum could hydrolyze substrate ATP thereby producing the corresponding nucleoside diphosphate which, in turn, could act as substrate for any adenosine diphosphatase activity residing in the Golgi region or elsewhere within the cell. In our material, adenosine diphosphate as

TABLE I

Differences in Intensity of Enzymic Activity in the Golgi Zone of the Distal and Proximal Convoluted Kidney Tubules from Winter Bats in Various States as Revealed by Ability to Hydrolyze Several Nucleoside Phosphates

Substrate	Relative intensity of enzymic activity			
	Incubation time	Hibernators (11 animals*)	Hibernators awake 24 hrs. (2 animals)	Active throughout winter (4 animals)
	minutes			
β-glycerophosphate (pH 5.2)	40	0	0	+
adenosine monophosphate (pH 7.2)	20	0	0	0
adenosine diphosphate (pH 7.2)	30	0–+	0	0
adenosine triphosphate (pH 7.2)	20–30	+++	++	+–0
inosine diphosphate (pH 7.2)	30	++++	+++	+–0
inosine triphosphate (pH 7.2)	20–30	+++	++	+–0
thiamine pyrophosphate (pH 7.2)	30	+	0	0

* 6 animals killed while in winter hibernation in caves; 5 animals killed while in winter hibernation in the hibernaculum. Of these five animals, 2 were not in complete hibernation.

The recent cytochemical localization of one kind of nucleoside diphosphatase activity in the Golgi region (Allen, 1961; Allen and Slater, 1961) and the modification and refinement of these methods to the staining of the Golgi apparatus *per se* (Novikoff and Goldfischer, 1961; Novikoff *et al.*, 1962) has provided a fresh means for studying the morphology as well as the histophysiology of this region. Novikoff and Goldfischer (1961) and Novikoff *et al.*, (1962) have reported that primarily nucleoside diphosphatase activity appears to be localized in the Golgi lamellae of most cells studied to date. As reported here, the presence of ATPase activity in the Golgi region of the tubular epithelium of the winter-hibernating bat appears to be a special case. Further, this region is also capable of hydrolyzing inosine triphosphate and, to a lesser degree, uridine triphosphate. At the incubation

substrate was poorly hydrolyzed in kidney tubular epithelium of hibernators. On the other hand, inosine di- and triphosphatase activities were both intense, especially in tissue sections from hibernators, and we cannot rule out the possibility that sufficient amounts of IDP could be produced by "inosine" triphosphatase or even adenosine triphosphatase activity so as to yield high levels of substrate. While ultimately we must consider the question of specificity with respect to various nucleoside phosphates, we cannot yet answer whether high levels of ATPase activity in our material can yield a nucleoside diphosphate capable of being split by "IDPase" activity. (See Note added in Proof.)

Currently, based on electron microscope observations, the Golgi complex is interpreted as being composed of smooth-surfaced lamellae, large

vacuoles, and small vacuoles or granules (see review of Pollister and Pollister, 1957). At the level of light microscopy alone, it seems futile to speak of localization of enzymic activity in any one or more of the structural components. Not only limits of optical resolution but errors induced by diffusion and through direct effects on precipitation of final reaction product make interpretation with the metal-salt methods quite difficult. We cannot feel certain whether what we describe in the present investigation as "vacuoles" are not, in fact, lamellar and hence represent the Golgi apparatus *in sensu strictu*. Similarly, we cannot be certain whether what we describe as smaller "vesicles" or "droplets" are really part of the heterogeneous structure of the Golgi complex. These droplets, possessing ATPase activity, are not similar in location to other droplets having acid phosphomonoesterase activity and visualized in the present kidney tissue as well as tissues from summer bats (Melman and Rosenbaum, 1963). Droplets possessing acid phosphomonoesterase activity have been distinguished from the nucleoside diphosphatase activity of the Golgi lamellae *per se* by Novikoff and Goldfischer (1961). Cytochemically demonstrable nucleoside phosphatase activity and problems of identifying it with specific Golgi components have recently been considered by Allen (1963).

Investigation as to possible functions of the Golgi region in kidney tubular cells extends back to the classical studies of Jasswoin (1925) on amphibian kidneys and of Nassonov (1926) on kidneys from axolotl, mouse, and cat, with vital stains in combination with silver impregnation techniques for visualization of the Golgi apparatus. Bowen (1929) interpreted the formation of droplets of accumulated dye within the Golgi zone as indicative of secretory activity within this region. Until recently, however, little further study on the presence or function of Golgi material in kidney tubular epithelium has been attempted, presumably since the cells did not come under the category of glandular secretion.

In previous studies, no ATPase activity was detected in the Golgi region of either active or cold-stored summer bats (Melman and Rosenbaum, 1963). The results of the present investigation suggest that lack of Golgi staining in the summer cold-stored bat could indicate that a state of deep hibernation had not been established. Positive enzymic activity in the Golgi region in kidney tubular epithelium studied in the present research was observed only when the animals were killed in an undisturbed state of deep hibernation. Such differences in localization of enzymic activity in kidneys between summer cold-stored bats and winter-hibernating bats could reflect the possibility that the cold-stored bats studied earlier had attained a hypothermic state only. Kayser (1960) has speculated as to the possible biochemical alterations in tissues of hibernating hibernators made hypothermic in summer. Significant biochemical studies at the cellular level are currently lacking, as is information on the morphological alterations in the kidney that accompany the entrance into or the arousal from deep hibernation (Riedesel, 1960).

In our earlier study (Melman and Rosenbaum, 1963) on kidneys from active and cold-stored summer bats, we employed several different cytochemical techniques to observe apparent renal adaptations to the states of relative dehydration (Hong, 1958; Kallen, 1960) and decreased renal circulation (Hong, 1958) accompanying hibernation. Patterns of staining for ATPase activity in the basal lamellae of proximal convolutions from active summer bats suggested swelling of the extracellular compartments such as were visualized more definitively in the ischemic rat kidney by electron microscopy (Ruska *et al.*, 1957). In the present investigation a pattern showing narrow basal lamellae, possibly identifying unswollen interlamellar compartments, was detected in winter bats aroused from hibernation for 24 hours. A similar appearance of the basal lamellae was observed in cold-stored bat kidneys (Melman and Rosenbaum, 1963). Final conclusions as to the state of the interlamellar compartments will require study at the level of the electron microscope.

The disappearance of a pronounced Golgi region showing high activity for some nucleoside triphosphatases as well as diphosphatases as the naturally winter-hibernating bat awakens corresponds to an increase in ATPase activity associated with the basal lamellae or infoldings in tubular epithelium of the proximal convolutions. Perhaps, in this case, the Golgi region is involved in a reorganization of the enzymic activity of the basal lamellae which in turn serve as the locus of filtration at the cellular level. In the aroused bat, such a reorganization could serve to control increased active transport through the cell. There is

evidence that the Golgi lamellae are continuous with the channels of the endoplasmic reticulum (Palade, 1956; De Robertis *et al.*, 1960; Scharrer and Brown, 1961). Viral protein may move to the exterior of the cell from the Golgi system via channels of the endoplasmic reticulum (Marcus, 1962). It is tempting to consider that enzymic protein may also follow such an intracellular course fed perhaps through a membrane flow scheme such as suggested by Bennett (1956). Indeed, Palade (1959), describing secretion of zymogen granules in the guinea pig pancreas, has suggested that the piles of cisternae in the centrosphere region represent the membrane depot of the cell. In this way, movement of membranes from the Golgi region to the cell surface would occur with concomitant movement from the cell surface to the Golgi cisternae. As Palade (1959) has pointed out, any unidirectional movement of the cell membrane system would result in considerable enlargement of the cisternal lumen and eventual exhaustion of the intracellular membrane system. Perhaps the large vacuolar structures in the Golgi region of kidney tubular epithelium from hibernating bats represent such a membrane flow temporarily in one direction. One would then suppose that arousal from hibernation restores this scheme to a cyclic balance, resulting in diminution or disappearance of extraordinary, large vacuoles. The possibility of a direct contribution of triphosphatase activity originating from the Golgi apparatus to the external cell membrane could involve such a dynamic event. In maize root epidermis undergoing division, the Golgi apparatus produces a secretory vesicle which apparently contributes to a new plasma membrane developed in the region of cell plate formation (Whaley and Mollenhauer, 1963). As yet, no electron micrographs of the Golgi region of our present material are available and we have no information as to the configuration or degree of complexity of the Golgi zone in the tubular epithelium of hibernating bats. It is possible that further study of the present material at the level

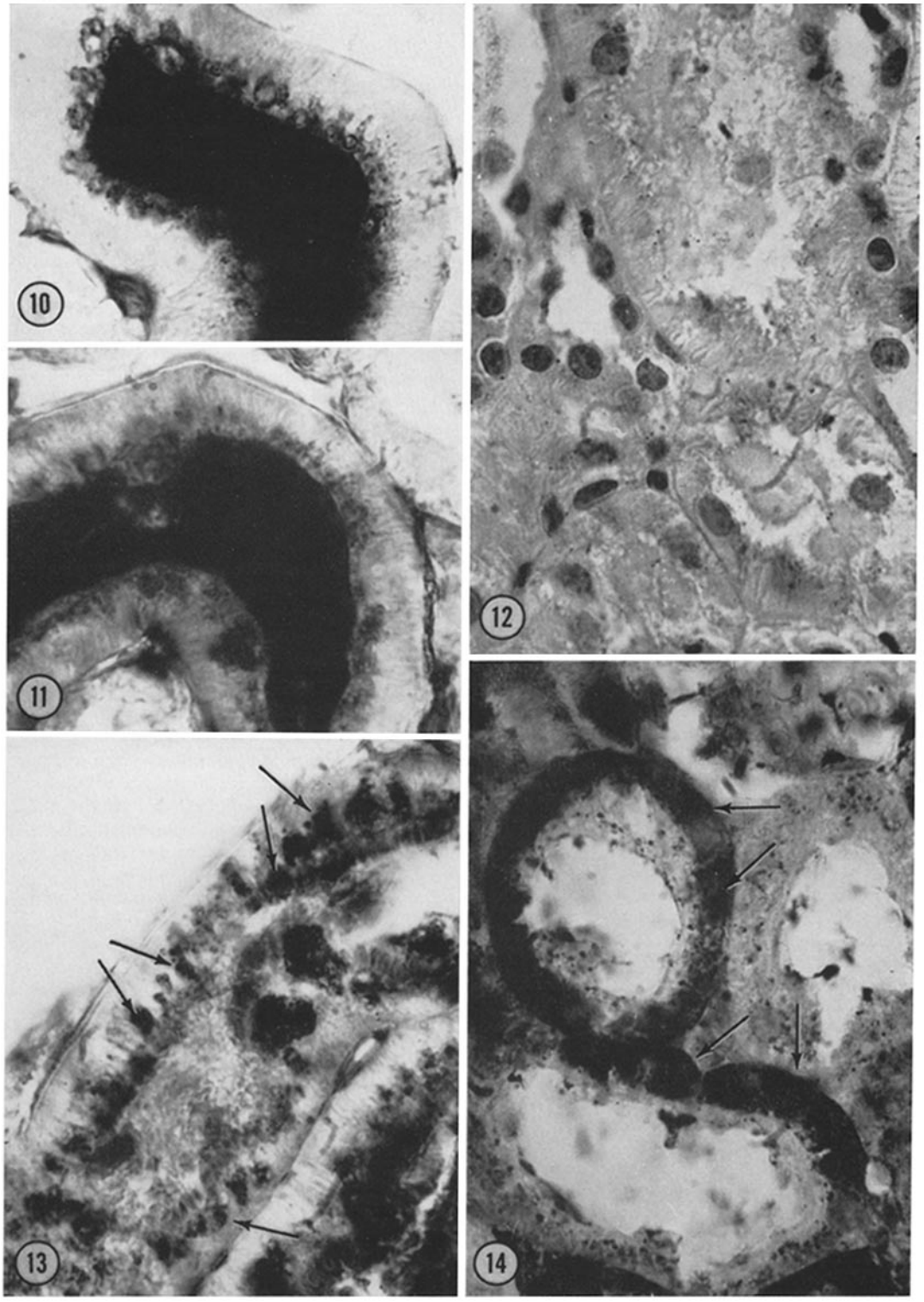
FIGURE 10 Proximal tubule from kidney of a winter-hibernating bat stained with a lead-salt method employing inosine diphosphate as substrate, as described by Novikoff and Goldfischer (1961). Staining of the Golgi region near the luminal surface of the tubular epithelium can be seen. The reaction is similar to that obtained with ATP as substrate (Figs. 3 and 9). The brush border is stained but obscured due to the intense false-positive reaction of the luminal contents. × 480.

FIGURE 11 Proximal tubule of kidney from a hibernating bat fixed in cold calcium-formalin and stained by the method of Padykula and Herman, with ATP as substrate at pH 9.0. There is less distinct staining of the Golgi region than in Figs. 6 and 9. The basement membrane and brush border are stained. Irregular staining of the region of basal infoldings is suggestive of mitochondrion-associated enzymic activity. × 480.

FIGURE 12 Section of kidney from a bat that had not hibernated since the previous winter fixed in cadmium-formalin fixative, stained by Elftman's modification of the silver method of Aoyama for demonstration of the Golgi apparatus and counterstained with dilute Harris' hematoxylin. There is little or no staining of Golgi elements in either proximal or distal convoluted tubules. Nuclei are stained by hematoxylin. × 480.

FIGURE 13 Section of a proximal tubule from a kidney of a winter-hibernating bat fixed and stained as in Fig. 12. Deposits of reduced silver (arrows) occur in areas of Golgi zones. As when staining for enzymic activity, the Golgi region in the proximal tubule is distinctive, consisting of densely packed granules located toward the luminal portion of the cell. Dilute Harris' hematoxylin counterstain. × 480.

FIGURE 14 Section of a distal tubule of kidney from winter-hibernating bat stained as in Fig. 12. Staining of the Golgi region appears as a heavy deposition of reduced silver filling much of the cytoplasm of the epithelial cell (arrows). Such appearance is distinct from that seen in the proximal tubule and is similar to the appearance of the Golgi region visualized in distal tubular epithelium by enzymic activity (Figs. 2, 4, and 5). Dilute Harris' hematoxylin counterstain. × 480.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of subcellular morphology may reveal some direct contribution to the functioning of the basal infoldings from the Golgi lamellae *per se*.

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Note added in proof: Since the completion of this paper, a report by Goldfischer *et al.* (*J. Histochem. and Cytochem.*, 1964, **12,** 72) has appeared dealing with some pitfalls in staining for nucleoside phosphatase activities, especially at the level of the electron microscope.

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