SPECULATIONS BASED ON THE MORPHOLOGY OF THE GOLGI SYSTEMS IN SEVERAL TYPES OF PROTEIN-SECRETING CELLS

R. F. ZEIGEL, Ph.D., and A. J. DALTON, Ph.D.

From the Laboratory of Viral Oncology, Virus Oncology Section, National Cancer Institute, National Institutes of Health, United States Department of Health, Education, and Welfare, Bethesda, Maryland

ABSTRACT

Electron microscopical observations on the relationship of the Golgi region to other intracellular organelles in certain protein-secreting cells have substantiated and extended existing hypotheses. In micrographs of several cell types, the juxtanuclear Golgi regions were observed to be closely associated with nuclear "pores." The "transition elements" of the ergastoplasmic membranes possess "blebs" which may represent a transport process facilitating the movement of intracisternal contents into the Golgi zone. A "blebbing" process of this nature may be one source of the small variety of Golgi vesicles. Zymogen granules of different densities were observed and their significance was postulated. Light Golgi vacuoles were observed. It is suggested that these vacuoles represent accumulations of relatively fluid material segregated from the secretory product in these cell types. These hypotheses from inferential evidence are discussed and extended.

Morphological and biochemical studies have demonstrated a basic pattern associated with cells that elaborate proteins destined for secretion. Recently Hirsch (1) summarized current concepts of the structural and biochemical aspects of cells, and compared these with his observations from phase contrast and light microscopic studies on living systems. Morphological evidence suggests that the Golgi system plays an important role in various secretory phenomena; however, this role has not been clearly defined despite much careful work on the subject (2–5).

The cloud of mystery shrouding the Golgi system has, however, recently been penetrated. Certain ideas perpetuating an old concept that the Golgi system is an artifact of fixation, even in cells containing relatively large amounts of phospholipid, have been discounted (4). Caro (5) correlated biochemical and morphological observations which strongly support the idea that the Golgi system functions in secretory cells as a segregation and concentration center, a concept which previously was supported largely by inferential evidence.

One of the many unanswered questions concerns the method by which the intracisternal contents gain access to, and are processed by, the Golgi system. This problem constitutes the "missing link" of Hirsch (1). Several theories have been proposed concerning the origin and function of the Golgi vesicles, vacuoles, and lamellae, but morphological evidence, even if inferential, supporting these ideas is relatively sparse. It is the purpose of this report to present micrographs indicating spatial relationships of the Golgi system to other cellular organelles in several different cell types.

MATERIALS AND METHODS

The material presented here has been selected from a necessarily large number of micrographs of

various tissues, all associated with the secretion of various kinds of proteins. The chick pancreas produces proteinaceous enzyme precursors known as zymogens. The glands of the mid-portion of the hen oviduct secrete albumin (6). The goblet cells of rat intestinal epithelium secrete mucus (mucoproteins) (3). The cells of Merwin plasma cell tumor (MPC-2) (7, 8) secrete a complex of specific globulins, and human multiple myeloma cells probably elaborate abnormal globulins (9). All tissues were prepared in the following manner. The tissue was fixed in chrome-osmium (10) for 1 hour at 5°C, postfixed for 1 hour in 10 per cent neutral formalin at 5°C, dehydrated with increasing concentrations of ethanol (70 per cent, 80 per cent, 90 per cent, 95 per cent, absolute), and infiltrated with, and embedded in, a prepolymerized 3:1 mixture of *n*-butyl and methyl methacrylate at 80°C containing 0.25 per cent benzoyl-peroxide as the catalyst. Sections were cut from the blocks on Porter-Blum microtomes (Servall) with Venezuelan diamond knives. The sections were picked up on formvar- or collodion-coated, 200-mesh copper grids from the surface of a 25 to 35 per cent aqueous acetone solution. The thin sections were routinely "stained" with various heavy metallic salts (11). Electron micrographs were taken with an RCA EMU-2C or a Siemens Elmiskop I electron microscope.

OBSERVATIONS AND DISCUSSION

A record of the activities within cultured osteoblasts was made by Rose, utilizing phase contrast cinematography (12, 13). These pictures demonstrated a close spatial relationship between the cell nuclei and the Golgi regions. Observations from light microscopy, other than Rose's, indicate that in various types of cells, such as intestinal epithelial cells, plasma cells, pancreatic acinar cells, and cells of epididymal epithelium, the Golgi region is closely associated with the nucleus. The connections and relationship of the nucleus to the ergastoplasm have been frequently and thoroughly documented, but because of the limited number of cells observed, and the thinness of the sections required for electron microscopy, the topographical relationship of the nucleus to the Golgi region is infrequently noted. However, in this survey, Golgi elements were observed adjacent to the nuclei in cells of the chick pancreas (Figs. 2 and 3) and cells of plasma cell tumors (MPC-2) (Figs. 9 and 10). In these cases, open nuclear pores are seen close to the Golgi region (Figs. 2 and 3). Reasons for this proximity are not known, although Rose suggested a possible "synthesis" relationship. If nuclear contents move into the cytoplasm (or *vice versa*!), it is conceivable that the juxtanuclear location of the Golgi material might have functional significance.

It has been established that the ribosomes of the ergastoplasm play a major role in the synthesis of proteinaceous secretory products of cells (14, 15). The secretory product may be stored in, or

FIGURE 1

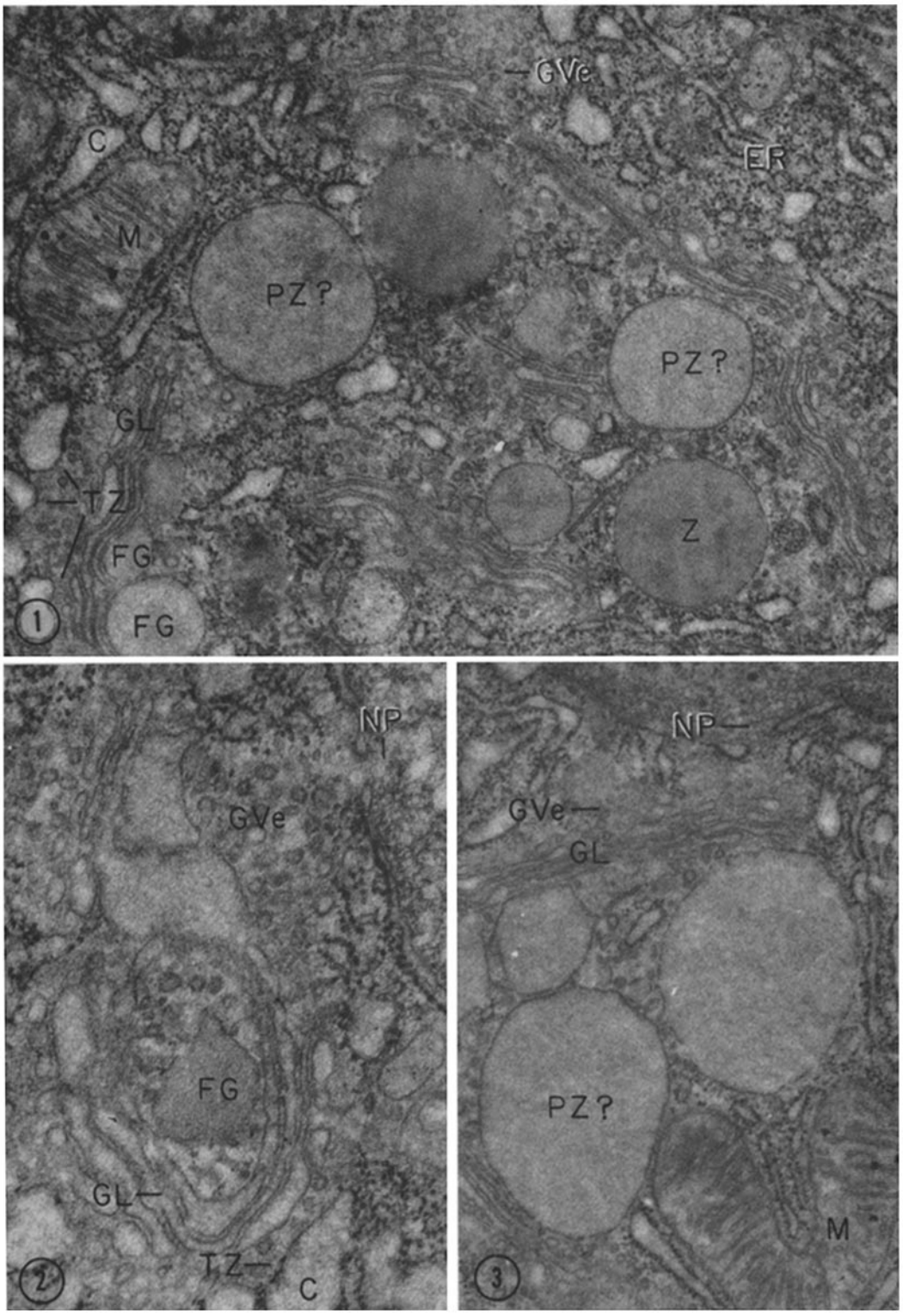
Illustrates a Golgi region in a chick pancreatic acinar cell. The classical Golgi elements, the small vesicles (*GVe*) and lamellar membranes (*GL*), are present. Several "transition zones" (*TZ*) are present. Small formative granules (*FG*) may be observed in close association with the lamellar membranes. Several large, relatively less dense granules (*PZ?*) are evident. A large zymogen granule (*Z*) has approximately the same density as apical zymogen granules observed in this tissue. *C*, cisterna; *ER*, endoplasmic reticulum; *M*, mitochondrion. Stained with aqueous 1 per cent uranyl nitrate. × 38,000.

FIGURE 2

A Golgi zone is located near the nucleus in a chick pancreatic acinar cell. A nuclear "pore" (*NP*) is evident. Small Golgi vesicles (*GVe*) are close to the "pore." Elements of the ergastoplasm are continuous with the "transition zone" (*TZ*) adjacent to the Golgi region. *GL*, Golgi lamellae; *FG*, formation granule; *C*, cisterna. Stained with lead acetate and treated with ammonium hydroxide vapors. × 58,000.

FIGURE 3

A Golgi region in a chick pancreatic cell is demonstrated near the nucleus and a nuclear "pore" (*NP*). Golgi vesicles (*GVe*), Golgi lamellae (*GL*), and a mitochondrion (*M*) are seen. Large, relatively less dense granules (*PZ?*) are located near the Golgi lamellae (*GL*). Mature granules of typical density are not present in this micrograph. Uranyl nitrate staining. × 38,000.


GVe
C
ER
M
PZ?
PZ?
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FG
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C
2
NP
GVe
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PZ?
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3

transported via, the intracisternal pathways. In cell types in which the Golgi system plays an active role in modifying the morphological form of the secretory product (if not the biochemical form), the process by which the secretory product enters the Golgi system from the cisternae of the endoplasmic reticulum is unclear. Palade (16, 17) was the first to suggest that small Golgi vesicles "bud" from the elements of the endoplasmic reticulum adjacent to the Golgi zone. Evidence for a similar situation in the green alga has been provided (18). Dalton (4) stated that various Golgi vesicles may fuse together or may be incorporated into either the Golgi lamellae or the larger Golgi vesicles. The inferential evidence presented here supports these recent observations and hypotheses. Regions of the ergastoplasmic membranes (endoplasmic reticulum) adjacent to the Golgi zone occasionally do not have ribosomes on their "outer surface." These agranular areas may be termed "transition elements" and represent regions of transfer between the ergastoplasmic membranes and the Golgi system. We suspect these "transition elements" to be a possible source of the small Golgi vesicles. In the chick pancreas (Figs. 4 and 5), several small (40 to 50 mμ) blebs in the "transition elements" may be observed, which are of the same diameter as the typical small variety of Golgi vesicles. In Fig. 7, similar regions may be observed in a goblet cell in rat intestinal epithelium. Figs. 8, 9, and 10, of mouse plasma cells, illustrate similar small bleb-like membranous structures. Note that in Fig. 10 a "bleb" apparently originates from the external nuclear membrane. Fig. 11 illustrates similar structures in the "transition elements" of a human multiple myeloma cell. The difficulty of interpretation is to attach an "arrow," figuratively speaking, to the small bleb-like structures, if in reality they are involved in a directional process. Do they "bud" into the Golgi zone and fuse with the classical Golgi membrane components, or do they originate from the Golgi components and join or fuse with the "transition elements" of the ergastoplasm?

Hirsch (1) described the fate of what are presumably the "Mankowski" or intracisternal granules (19) in the guinea pig pancreas; they apparently "dissolve" or disappear at the periphery of the Golgi zone, after migrating to it from other regions (primarily basal) of the cell. Current concepts regarding the direction of flow of the secretory products suggest that the vesicles may indeed originate or "bud" from the "transition elements" of the endoplasmic reticulum into the

FIGURE 4

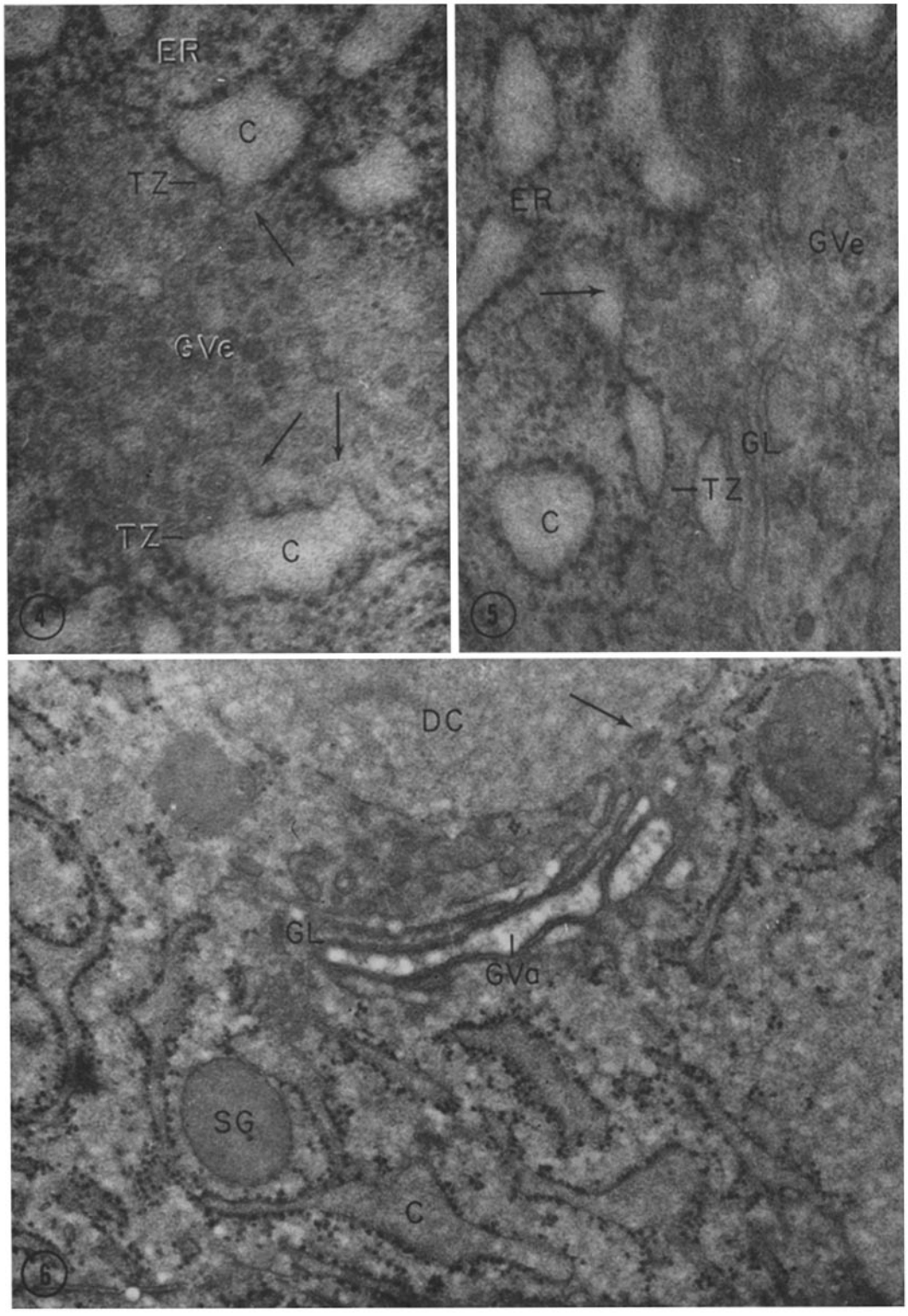
Chick pancreatic cells demonstrate small convex profiles ("blebs"), indicated by arrows, continuous with the "transition zones" (*TZ*) of the ergastoplasm (*ER*) adjacent to the Golgi region. Small Golgi vesicles (*GVe*) have approximately the same diameter as the "blebs" would have if the latter were detached and circular. *C*, cisternae. No stain. × 92,000.

FIGURE 5

A chick pancreatic acinar cell illustrates a "transition element" (*TZ*) with several small evaginations (?) (arrow) into the Golgi region. Golgi lamellae (*GL*), Golgi vesicles (*GVe*), and ergastoplasm (*ER*) are present. The intracisternal contents (*C*) are relatively light and diffuse in the near Golgi regions. Uranyl nitrate staining. × 84,000.

FIGURE 6

Illustrates a limited portion of the Golgi system in an albumin-secreting cell of the hen oviduct. A distend.d cisterna (*DC*) presumably contains the albuminous secretory product. The membranes of these distended cisternae may occasionally be observed to be continuous with other cisternae (*C*) and are occasionally associated with ribosomes on the extracisternal or cytoplasmic surface. Golgi lamellae (*GL*) and small Golgi vesicles (arrow) associated with the distended cisterna are demonstrated. Clear or less dense Golgi vacuoles (*GVa*) are typical of these cells. Several Golgi regions may be observed associated with distended cisternae in a single cell. *SG*, secretory granule. Stained with lead subacetate. × 51,000.


ER
C
TZ
GVe
TZ
C
4
ER
GVe
GL
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C
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DC
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GVa
SG
C
6

Golgi region, taking with them small quantities of the proteinaceous substances and enzyme precursors thought to accumulate in the intracisternal cavities.

The fate of the small Golgi vesicles is as uncertain as their origin. As previously mentioned, Dalton (4) suggested that the vesicles may fuse together or join the Golgi membranes. Our present view is that the small Golgi vesicles probably fuse to, or join with, the Golgi lamellae or vacuolar regions. Fig. 7 (arrow 4) indicates a profile suggestive of a small vesicle joining a Golgi vacuole.

The Golgi lamellar membranes are perhaps the most consistent morphological form of Golgi component. In certain inactive secretory cells and in embryonic secretory cells (20), the lamellar Golgi structures predominate. The movement of secretory material via the small Golgi vesicles to the Golgi lamellar membranes and the associated concentration process in these membranes would result in the appearance of (*a*) light vacuoles, the segregated highly fluid material, and (*b*) the distinct secretory masses as demonstrated in Figs. 1, 2, 7, and 8. These ideas are in agreement with the concept that there is no direct and permanent continuity in the form of membrane-lined channels between the cavities of the ergastoplasm and the Golgi lamellae. In this view, the small Golgi vesicles provide a means of transporting intracisternal contents to the Golgi region, first disconnecting from the "transition elements," then joining the Golgi lamellar membranes. This lack of direct and permanent continuity of the Golgi lamellae with the endoplasmic reticulum is compatible with the hypothesis that the Golgi complex is a discrete, morphologically isolated, cellular organelle, and not merely an extension of other closely associated cellular components.

Micrographs of chick pancreatic acinar cells exhibit granules of varying density within the Golgi region (Figs. 1, 2, and 3), in addition to the typical Golgi vesicles, Golgi vacuoles, and Golgi lamellar membranes (11, 20). The relationship between the large, less dense granules (prozymogen granules or immature zymogen granules) and the relatively more dense granules (mature zymogen granules) is not clear. Frequently the prozymogen granules are larger than the mature zymogen granules at their equatorial zone (Fig. 1). Occasionally (Fig. 3) the population of granules in the Golgi region is comprised only of the larger, less dense granules. There are several possible interpretations. One possibility is that the larger, less dense granules do indeed represent "prozymogen granules" or accumulations of large quantities of relatively more fluid secretory aggregates, similar in density to the intracisternal contents. Water may be removed from them, and the contents may be subsequently concentrated, resulting in the typical, denser "mature zymogen granules."

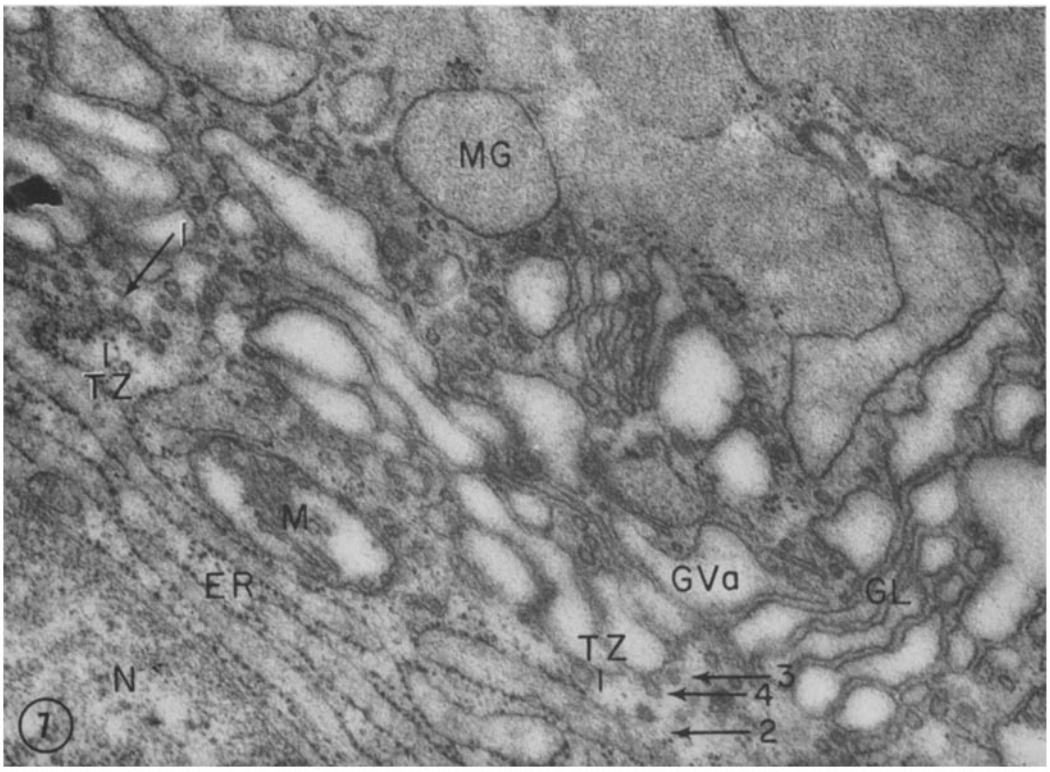
A second possibility is that the Golgi region may not function equally efficiently in concentrating all zymogen granules; *i.e.*, the efficiency may vary during different phases of the secretory stimulus and cycle, and hence some granules may be more fluid than others. The heterogeneous granules in the chick pancreas (20) and in the mouse pancreas

FIGURE 7

A goblet or mucus secretory cell of rat intestinal epithelium is illustrated. The extensive Golgi region contains mucus granules (*MG*) which perhaps are being concentrated. Golgi lamellae (*GL*), and less dense Golgi vacuoles (*GVa*). A portion of the cell nucleus (*N*) and elements of the ergastoplasm (*ER*) are present. It is suggested that the small vesicles, arrows 1 and 2, "bud" from "transition elements" (*TZ*) into the Golgi region and may fuse with other Golgi elements as shown at arrows 3 and 4. *M*, mitochondrion. Lead acetate stain treated with ammonium hydroxide vapors. Micrograph by Dr. J. S. Trier. × 52,000.

FIGURE 8

A Golgi zone in a murine Plasma cell neoplasm (MPC-2) possesses Golgi vesicles (*GVe*), Golgi lamellae (*GL*), and less dense Golgi vacuoles (*GVa*). The arrows indicate small convex profiles in the "transition elements." It is suggested that these represent the morphological basis of a "budding" phenomenon, or a source of the small Golgi vesicles. *M*, mitochondrion. Lead subacetate stain. × 60,000.


MG
1
TZ
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GVa
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3
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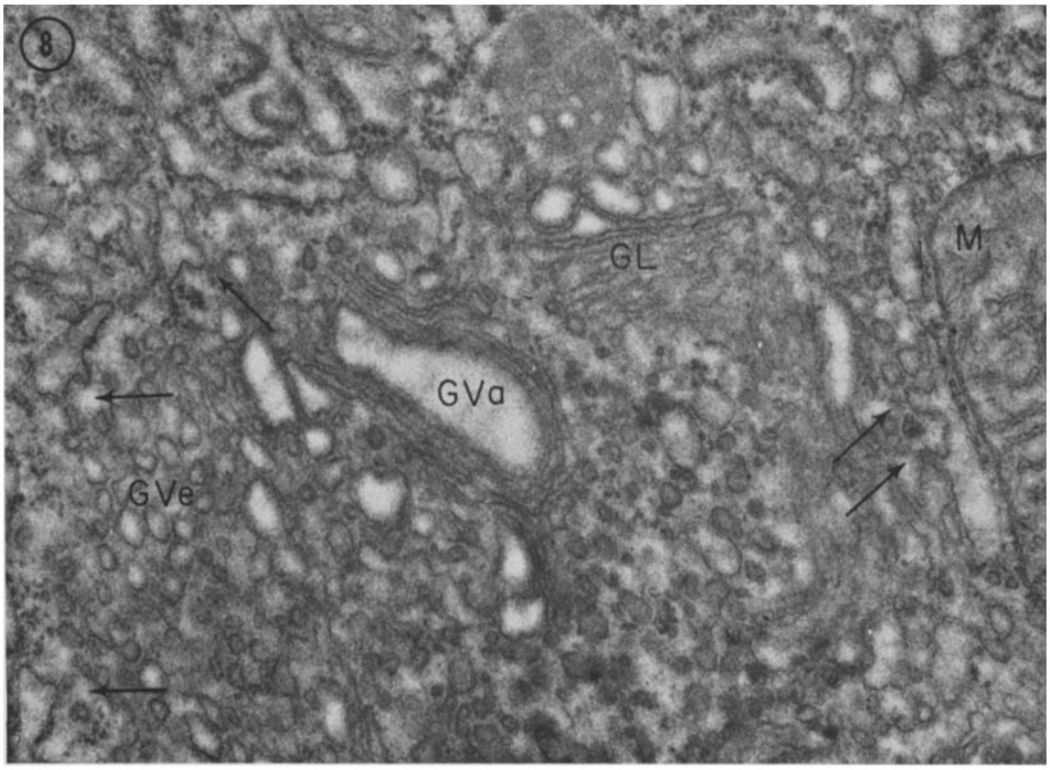

8
GL
M
GVa
GVe

(21) may indicate variations in fluid control in these particular cells. They may represent granules only partially concentrated in the Golgi region, or perhaps previously concentrated granules which have taken up fluid after their release from the Golgi system. It is suggested that these heterogeneous zymogen granules are not technical artifacts, but reflect a real difference in composition between individual zymogen granules.

A third possibility must also be considered. Two species of granules might be assembled in the Golgi region: the predominant type, the typical homogeneous dense zymogen granules which convey the proteinaceous product to the external environment, and the less dense, or larger, lighter granules which may represent accumulations of a different material perhaps containing more water.

A different cell system, the albumin-secreting cells of the hen oviduct, apparently possesses a specialized method of concentrating, storing, and secreting copious amounts of protein. The albuminous material may accumulate and be released into the glandular lumina from distended ergastoplasmic cisternae directly, without being first concentrated into "secretory granules" in the Golgi region.

The albumin cells do not have a topographically localized Golgi system. The various Golgi elements appear in thin sections to be distributed throughout the cell in small patches of cytoplasm located between, and along the perimeters of the distended albuminous sacs. The topographical relationships of the dilated cisternae and the light Golgi vacuoles are illustrated in Fig. 6. It is suggested that the less dense Golgi vacuoles represent an accumulation of fluid (water?) which has been removed from the cisternal sacs. This suggests that the dispersed Golgi material may function in a condensing capacity, by the removal of fluid (water?) from the larger albuminous sacs (distended cisternae). The hypothesis that one of the functions of the Golgi region is to concentrate secretory products, by the removal of water from them, is not new. It has previously been postulated that the Golgi system is a phylogenetic modification of the contractile vacuole of unicellular and less highly specialized organisms (4).

To reiterate, our morphologic observations of the various cell types support these postulated functions of the Golgi region. Under similar fixation and embedding procedures, several of these cell types, *i.e.*, albumin-secreting cells of the hen oviduct (Fig. 6), goblet cells of intestinal epithelium (Fig. 7), and cells of plasma cell neoplasms (Fig. 8), exhibit light Golgi vacuoles. It is suggested that these vacuoles represent accumulations of less dense substances which have been segregated from the secretory material and have not been redistributed or excreted.

The members of the mammalian and avian groups presented here are land animals and face

FIGURE 9

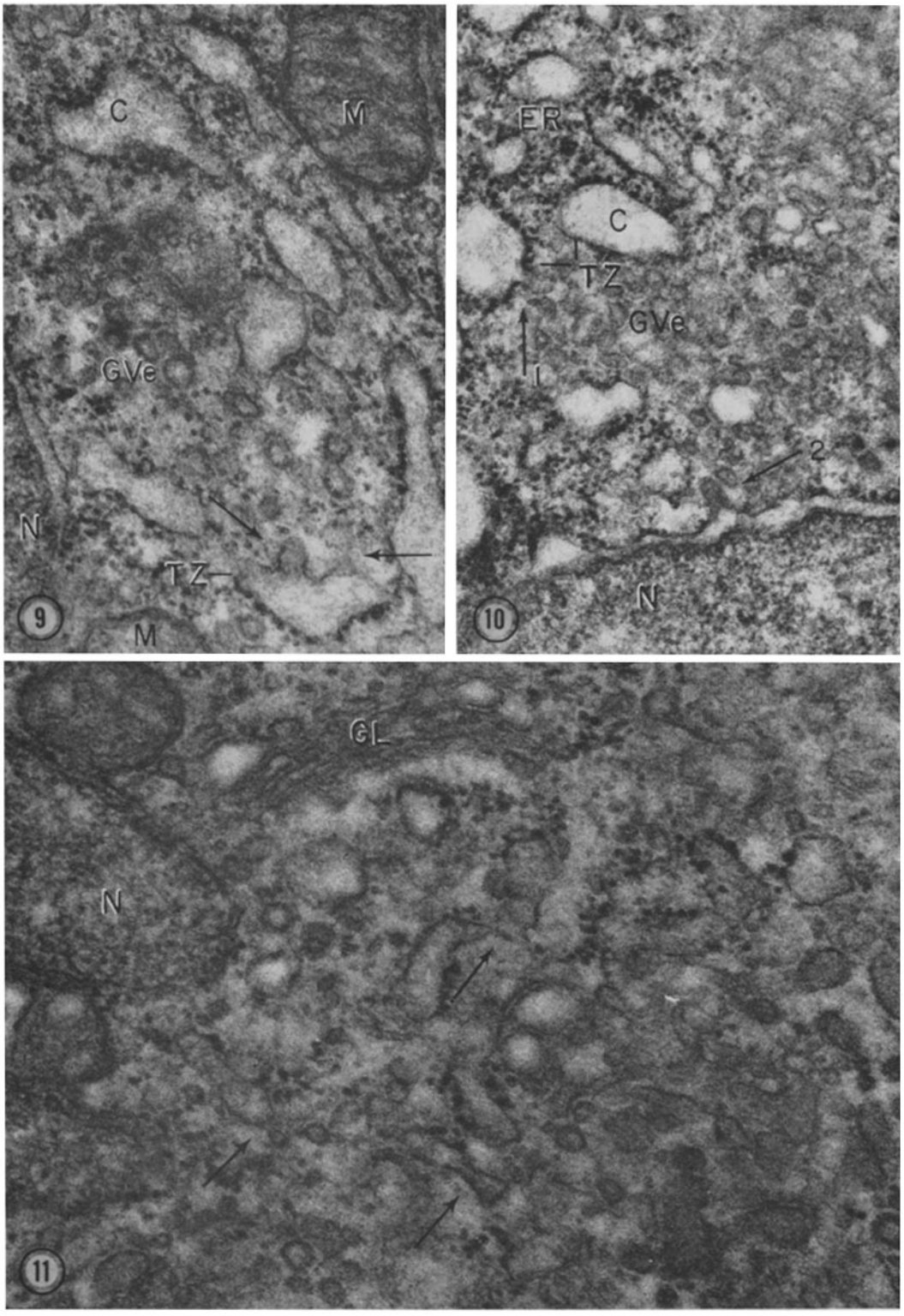
A region of a murine pla ma cell (MPC-2) illustrates several other small convex profiles (arrows) continuous with the "transition elements" (*TZ*) of the modified ergastoplasmic membranes. The nucleus (*N*), mitochondria (*M*), and cisternae (*C*) of the endoplasmic reticulum are indicated. *GVe*, Golgi vesicles. Lead subacetate stain. × 84,000.

FIGURE 10

A portion of the Golgi zone in a murine plasma cell (MPC-2) contains Golgi vesicles (*GVe*). A portion of a nucleus (*N*) and ergastoplasmic membranes (*ER*) are indicated. Arrow 1 indicates a convex membrane profile of the type previously described. Arrow 2 indicates a convex profile similar in size and density and adjacent to the Golgi region but continuous with the "inner cytoplasmic membrane" (outer nuclear membrane). *TZ*, transition zones. Lead subacetate stain. × 62,000.

FIGURE 11

A human multiple myeloma cell contains Golgi lamellae (*GL*) and various "transition elements" (arrows) which are associated with small, convex membrane profiles similar in size and density to the small Golgi vesicles. *N*, nucleus. Lead subacetate stain. × 80,000.


C
M
GVe
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TZ
9
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11

the problem of desiccation. Although the kidney, intestine, and other organs are remarkably efficient in the process of conserving water, the total organism may benefit if the various secretory systems conserve watery fluids associated with the manufacture of secretory products. It would be interesting to know the function of the Golgi region in cells of fresh water animals, for example, where the problem of constant "bailing" exists rather than that of the conservation of water. Further study may well show that the role of the Golgi complex in cells of a secretory nature is relatively more easily demonstrated. However, since the Golgi region is apparently a universal cellular organelle, its functional significance in non-secretory cells may prove to be even more interesting.

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