Histochemistry 47, 89–99 (1976)

Histochemistry

The Localization of Thiamine Pyrophosphatase Activity in the Acinar Cells of Stimulated and Non-Stimulated Sublingual Glands of the Rat

S.K. Kim, S.S. Han, and C.E. Nasjleti

Veterans Administration Hospital, Ann Arbor, Michigan 48105, USA,
and Departments of Anatomy and Oral Biology The University of Michigan

Summary. The distribution of thiamine pyrophosphatase (TPPase) activity in the acinar cells of the rat sublingual gland has been studied at various stages of the secretory cycle following stimulated secretion. The rats were stimulated to secrete by an intraperitoneal injection of isoproterenol and pilocarpine. In non-stimulated glands, TPPase activity is detected mainly in 3–4 cisternae at the inner concave side of the Golgi complex and in some adjacent condensing vacuoles as in other cells. In the acinar cells 1 to 2 h after stimulation, however, reaction product for the same enzyme activity is detected in the cisternae at the outer aspect, as well as the inner aspect, of the Golgi complex and even in the cisternae of the endoplasmic reticulum (ER). About 4 h after stimulation, TPPase activity becomes concentrated in 3–4 cisternae at the inner concave side of the Golgi complex as in the acinar cells under non-stimulated conditions. Morphological observations of the acinar cells 1 to 2 h after the stimulation have indicated that the reorganization of the Golgi complex and ER is a major event which occurs at this stage. It is possible that this cellular event is related to the occurrence of TPPase activity in those sites which normally show negative reaction in non-stimulated state.

Introduction

Thiamine pyrophosphatase (TPPase) activity has been demonstrated cytochemically in the Golgi complex of a wide variety of cells (Allen and Slater, 1961; Novikoff and Goldfischer, 1961; Novikoff et al., 1962; Osinchak, 1964; Holtzman and Dominitz, 1968; Friend, 1969; Cheetham et al., 1971; Goldfischer et al., 1971; Novikoff et al., 1971; Farquhar et al., 1974), including mucous and serous cells of the salivary glands (Hand, 1971). The enzyme activity is localized in Golgi cisternae and TPPase is considered to be useful marker enzyme for Golgi complexes (Novikoff and Goldfischer, 1961; Novikoff et al., 1962).

Previous cytochemical studies at the electron microscope level have shown that the reaction product for TPPase is localized in the inner cisternae, usually 2 to 3, at the concave side of the Golgi stack in many cells (Friend, 1969; Cheetham et al., 1971; Goldfischer et al., 1971; Hand, 1971; Novikoff et al., 1971; Farquhar et al., 1974). The reaction product has been observed also in some vesicles around the Golgi cisternae (Friend, 1969), but never in the cisternae which are at the outer convex side of the Golgi stack. These outer cisternae have been shown to be sites for osmium impregnation (Fawcett, 1967; Friend, 1969; Novikoff et al., 1971). The cisternae at the inner and outer aspects of the Golgi complex correspond to those which are described as maturing and forming faces (Mollenhauer and Whaley, 1963) or trans- and cis-Golgi cisternae (Farquhar et al., 1974), respectively.

In many of the above mentioned cells, the Golgi complex is the only organelle which reveals cytochemically detectable TTPase activity. It has been reported that TPPase activity is detectable in the cisternae of the endoplasmic reticulum (ER) of some cells, such as, cells of the liver, kidney, small intestine and hepatomas (Novikoff et al., 1962). However, with the exception of the hepatocytes the enzyme activity in the ER has not been demonstrated convincingly. In hepatocytes (Cheetham et al., 1971; Goldfischer et al., 1971; Farquhar et al., 1974) both ER and the plasma membrane facing the bile canaliculi reveal TPPase activity.

The present study is an attempt to illustrate the distribution of TPPase activity in the acinar cells of the rat sublingual gland following stimulated secretion. The study shows that TPPase activity is detectable in more than 2–3 cisternae at the inner, concave side of the Golgi complex and even in ER cisternae in acinar cells following stimulated secretion before new granules form.

Materials and Methods

Male Sprague-Dawley rats, weighing about 150 to 200 g were used for this study. Procedures used in stimulating the rats to secrete and preparing the tissues for electron microscopy were described in detail elsewhere (Kim et al., 1972). Briefly, the rats were stimulated to secrete by an intraperitoneal injection of isoproterenol (50 mg/kg) and pilocarpine (50 mg/kg), sacrificed 1, 2, and 4 h after the injection, the sublingual glands of these rats were perfusion fixed and prepared for incubation. The fixative used for perfusion contained 1% paraformaldehyde and 1.5% glutaraldehyde buffered with 0.1 M sodium cacodylate to pH 7.4 as described by Karnovsky (1965). Following the perfusion, the glands were removed, sliced and fixed by immersion in the same fixative at room temperature for 1 h. Tissues were rinsed several times with 0.1 M cacodylate buffer containing 7.5% sucrose and stored in the refrigerator overnight. Tissues were further sliced into smaller pieces and incubated in the medium for 60 min with one change at the end of a 30-min-period. The incubation medium was prepared as described by Novikoff et al., (1971), but the amount of thiamine pyrophosphate (obtained from Sigma) was doubled. The incubation was carried out at 37° C. For control, some tissues were placed in the same incubation medium without the substrate or with the substrate in the presence of 0.1 M NaF.

Following the incubation, the tissues were rinsed and fixed in 1% OsO_4 and embedded in Epon. Sections were examined after staining with lead citrate (Venable and Coggeshall, 1965) in a Hitachi 11C operated at either 50 KV or 75 KV.

Results

Detailed descriptions of the histological and cytological structure of the sublingual gland of stimulated and non-stimulated rats can be found elsewhere (Kim et al., 1972). The present study is concerned primarily with the Golgi complex of the acinar cells. The mucous acinar cells are the major cell type found in secretory acini. Serous demilune cells are located at the terminal portions of the acini but are few in number.

Non-Stimulated Acinar Cells. Most acinar cells in non-stimulated glands are filled with a large number of mucous granules which are often fused. In these

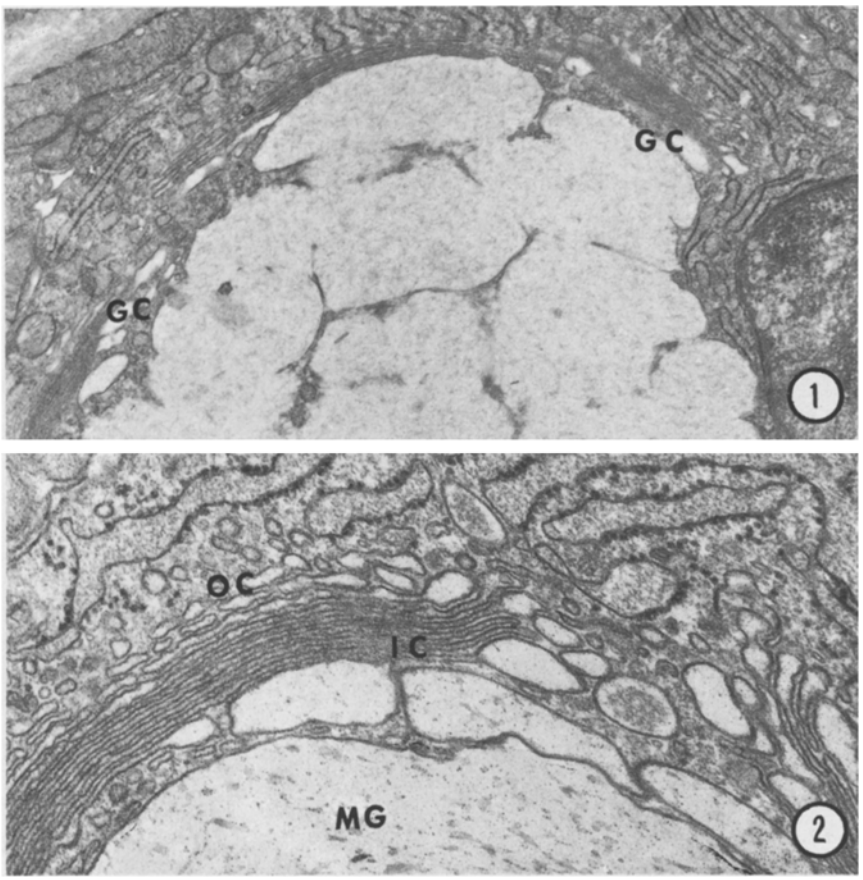
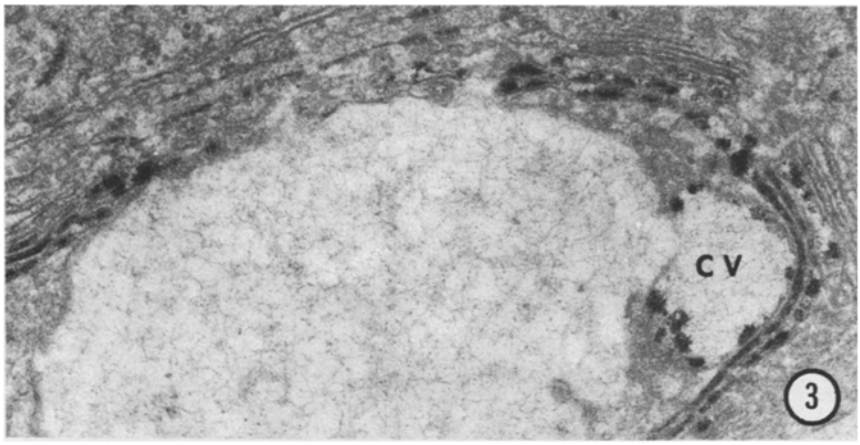


Fig. 1. A portion of a non-stimulated acinar cell. Most of the cytoplasm is occupied by mucous granules, many of which are fused. Golgi complexes (*GC*) occur at the periphery of the mass of mucous granules. ×30,000

Fig. 2. The Golgi complex in a non-stimulated acinar cell. It consists of cisternae, vesicles and some vacuoles comparable to condensing vacuoles in other exocrine cells. The terminal portions of many cisternae are diluted. The outer cisternae (*OC*) near the ER are short, dilated and electron translucent. The ER in this region is partially studded with ribosomes. The inner cisternae (*IC*) are long, of regular thickness and form a stack. A mucous granule (*MG*) in the Golgi region is indicated. ×115,000



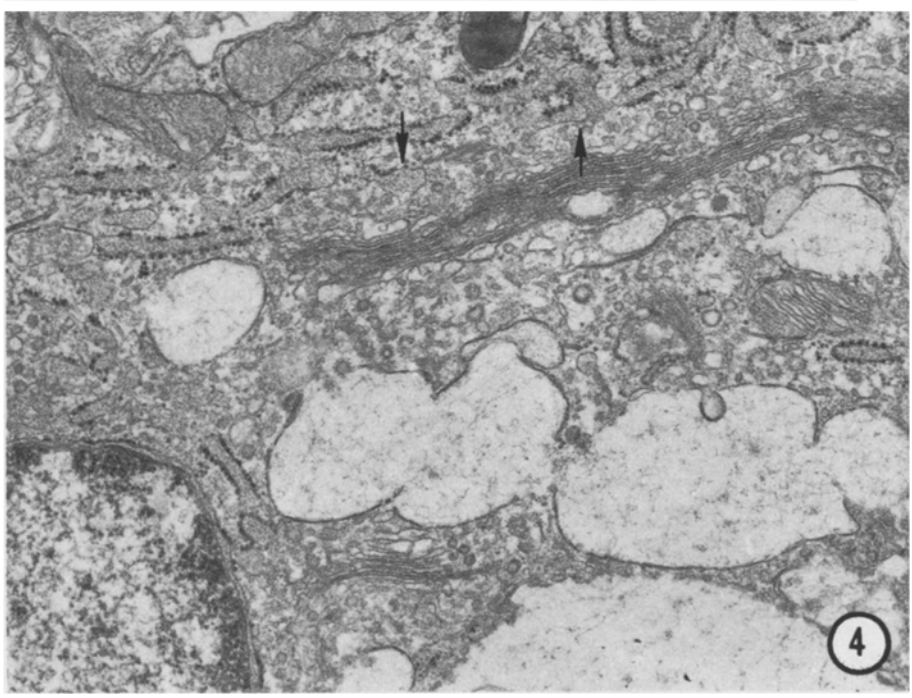


Fig. 3. A portion of a non-stimulated acinar cell incubated for the demonstration of TPPase activity. The reaction product is localized in the inner 2–3 cisternae, dilated terminal portions of some of the cisternae, and in a condensing vacuole (*CV*). However, the reaction product is absent in the outer cisternae of the Golgi and in the ER. ×90,000

Fig. 4. Portion of an acinar cell about 2 h after stimulation. The Golgi complex region is characterized by a large number of vesicles, somewhat irregularly stacked cisternae and vacuoles. The ER near the Golgi complex appears segmented and some of the segments are partially studded with ribosomes (arrows). ×31,000

cells, Golgi complexes are seen at the periphery of the mass of mucous granules which are located apical to the nucleus (Fig. 1).

The Golgi complex (Fig. 2) consists of several stacked cisternae, vesicles and vacuoles. The cisternae at the outer or convex side are often dilated, shorter and less electron dense than those which are at the inner, concave side near the mucous granules. The terminal portions of some cisternae are dilated, and the material contained in these dilated portions appears similar in density and texture to that in vacuoles and mucous granules in this region. The vacuoles are probably immature granules comparable to the condensing vacuoles in serous exocrine cells.

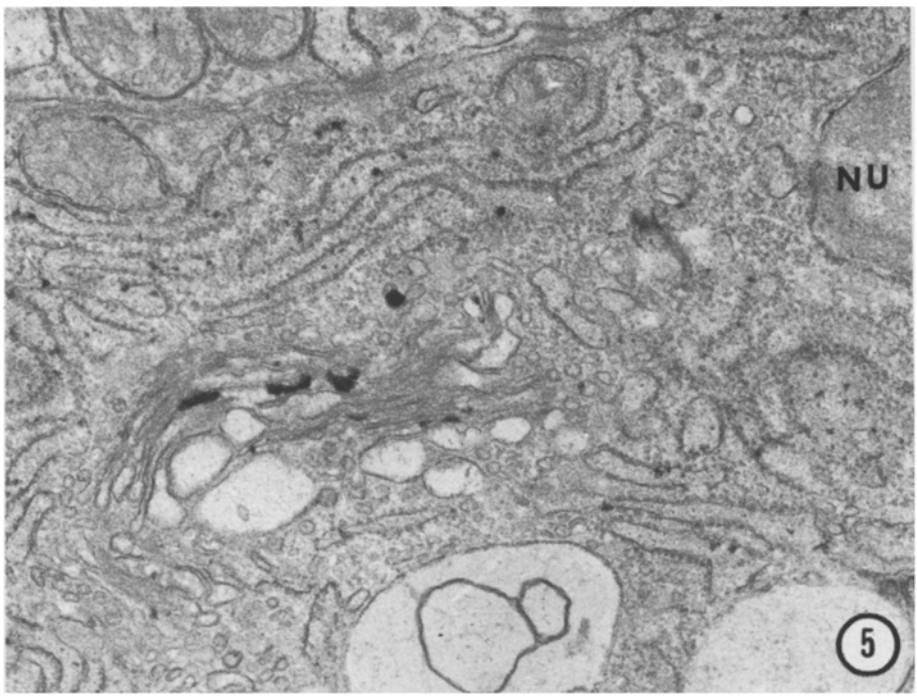
Observations of the non-stimulated gland incubated for TTPase activity indicate that the enzyme activity occurs in the cisternae at the inner concave side of the Golgi complexes. As can be seen in Figure 3, the reaction product is localized mainly in 2 or 3 cisternae, dilated portions of some of these cisternae and condensing vacuoles. However, the reaction product is not detected in cisternae at the outer, convex side of the Golgi complexes or in any other organelles.

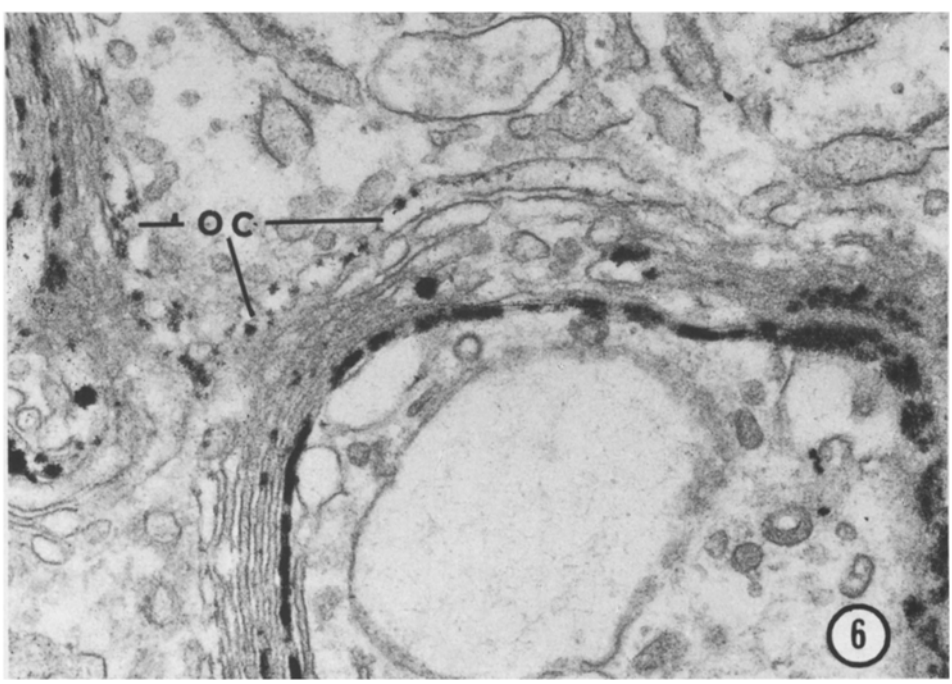
Stimulated Acinar Cells. Previous studies (Kim et al., 1972) have shown that the acinar cells of the rat sublingual gland can be stimulated to secrete by injecting the rat with isoproterenol and pilocarpine. The acinar cells begin to discharge secretory granules soon after the injection. The granule discharge is completed within 2 h post-stimulation, as evidenced by the absence of mucous granules in the acinar cells. At the completion of secretion, many acinar cells reveal vacuole-like spaces in the cytoplasm, which are created presumably by the evacuation of mucous granules. In these acinar cells, the reorganization of Golgi complexes and ER appears to be a major event which occurs prior to the formation of new secretory granules.

An acinar cell at the completion of secretion is shown in Figure 4. Distinct secretory granules are not present, although there are some membrane bound structures of irregular sizes and shapes. Golgi complexes are made up of cisternae, numerous vesicles and some vacuoles. The outer cisternae nearest to the fragments of ER are somewhat more dilated than the remainder of the cisternae and appear electron translucent. The ER around the Golgi region are segmented. Some of these ER segments are only partially studded with ribosomes.

The observations of the acinar cells in the stimulated glands have indicated that TPPase activity is much more widely distributed than in the acinar cells of the non-stimulated glands. TPPase activity is detected in more than 2 or 3 cisternae of the Golgi complex and even in some ER cisternae. The enzyme activity in the ER cisternae is more frequently detected in the acinar cells 1 h after stimulation than at the end of the 2-h period. As can be seen in Figure 5, the reaction product is clearly localized in the cisternae of the ER, although it is scattered diffusely. At 2-h post-stimulation, there are cells which do not reveal the enzyme activity in the ER cisternae.

The reaction product in the Golgi complex of these stimulated cells appears to be concentrated also in the cisternae at the inner, concave side (Fig. 6). However, deposits of the reaction product occur scattered in all Golgi cisternae


NU
5


OC
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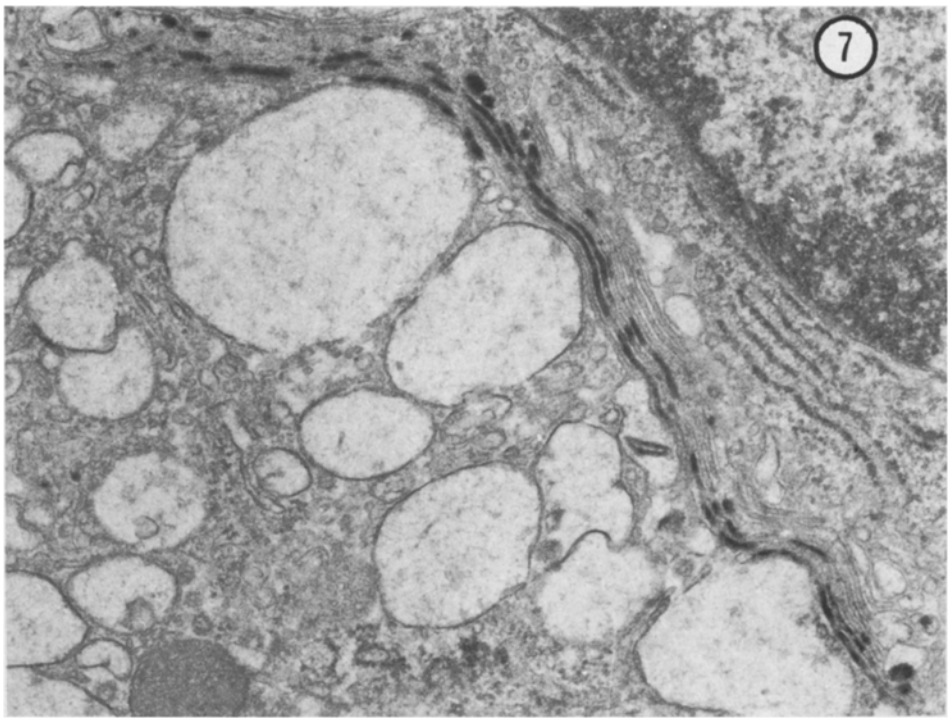

Fig. 7. Portion of an acinar cell 4 h after stimulation, incubated for TPPase activity. An increase in the number of immature mucous granules (condensing vacuoles) is obvious in the Golgi region. The reaction product is mainly detected in the inner cisternae of the Golgi complex and small vacuoles near the ends of the cisternae. However, the reaction product is not detectable in the outer cisternae of the Golge complex or in any other organelles. ×43,000

Figs. 5 and 6. The stimulated acinar cells incubated for TPPase activity, illustrating the intracellular localization of the enzyme activity 1–2 h post-stimulation. TPPase activity is detected in the cisternae of the Golgi complex and ER. The enzyme activity in the ER cisternae is detected largely in cells 1 h after stimulation but only in some cells 2 h post-stimulation

Fig. 5. Portion of an acinar cell 2 h after stimulation showing the reaction product in the ER cisternae. Deposits of reaction product are scattered diffusely in the cisternae of the ER in addition to those present in the Golgi cisternae. The nucleus (*NU*) and other cell organelles included in the figure show negative reaction. ×67,000

Fig. 6. Portion of an acinar cell 2 h after stimulation showing the deposits of reaction product in the Golgi complex. The deposits occur in the outer dilated cisternae (*OC*) as well as in the more inner cisternae. ×53,000

including the dilated cisternae at the outer convex side of the stack (Fig. 6). These dilated cisternae are located adjacent to the ER cisternae and might be those which are in transition from ER to Golgi cisternae.

The acinar cells begin to form new secretory granules at about 2 h after the stimulation as evidenced by the presence of a few granules in the Golgi region. The number of granules increases continuously in the acinar cells and reaches the prestimulation level by about 24 h after the stimulation. During this period, TPPase activity appears to become gradually concentrated in the cisternae at the concave side of the Golgi complex. This can be seen in an acinar cell 4 h after stimulation, shown in Figure 7. The reaction product for TPPase occurs mainly in 3–4 cisternae and some vacuoles which occur in the concave side of the Golgi complex. However, the reaction product is not detectable in the cisternae at the outer, convex side of the Golgi complexes or any other organelles, including the ER cisternae. In the control preparations of stimulated and non-stimulated glands, the reaction product was not detected.

Discussion

TPPase activity is detected in about 3 Golgi cisternae which are located at the inner, concave side near the forming mucous granules in the acinar cells of non-stimulated sublingual glands of the rat. This is a situation similar to that seen in the Golgi complexes of many different cells (Novikoff et al., 1962; Osinchak, 1964; Holtzman and Dominitz, 1968; Friend, 1969; Cheetham et al., 1971; Goldfischer et al., 1971; Novikoff et al., 1971; Farquhar et al., 1974) including the acinar cells of other salivary glands (Hand, 1971).

Recent cytochemical studies (Novikoff et al., 1971) using the method of tilting thick sections in the electron microscope, have shown that, in neurons, only one inner element of the Golgi complex displays TPPase activity, and that more than one TPPase rich cisterna observed in thin sections are twisted portions of this element. It is unknown whether or not the 3–4 cisternae which display TPPase in the acinar cells of the rat sublingual gland and in other cells are also portions of a single Golgi element.

The cisternae that are located at the outermost aspect of the Golgi stack adjacent to the ER in stimulated cells shows TPPase activity. It is unclear whether these cisternae are the same as the outer Golgi cisternae which stain with osmium in other cells (Fawcett, 1967; Friend, 1969; Novikoff et al., 1971). These TPPase positive outer cisternae are likely to be different from those located at the inner aspect of the stack, as they are dilated and similar in appearance to the ER cisternae without the attached ribosomes. These outer cisternae could be the ER cisternae which are in transition to Golgi elements and comparable to those which have been described as "transitional sheets" (Novikoff et al., 1971).

Previous studies have indicated that the Golgi membranes originate from the ER. The mechanisms proposed for such transformation include the direct conversion of the ER to Golgi membranes (Essner and Novikoff, 1962; Novikoff et al., 1966), or the formation of vesicles by ER which unite and form membranes

at the Golgi region (Mollenhauer and Whaley, 1963; Friend, 1965). Furthermore, it has been established that the transfer of secretory material occurs from ER to condensing vacuoles through the Golgi region (Caro and Palade, 1964; Jamieson and Palade, 1967a; 1967b). Structures, such as the segments of ER, partially studded with ribosomes, and small vesicles, observed near the Golgi region in this and other studies are generally considered to be those in transition from ER to Golgi membranes (Novikoff et al., 1962; Zeigel and Dalton, 1962; Claude, 1970; Novikoff et al., 1971).

If in fact membranes flow from one compartment to another within the cell, there must be an active transformation of membranes from the ER to the Golgi complex in the sublingual acinar cells following stimulated secretion. Such an active transformation might necessitate an elevated rate of TPPase synthesis in the ER cisternae and a temporary increase in the concentration of the enzyme, which becomes detectable cytochemically in the stimulated cells. It is unknown whether or not there is an increased rate of TPPase synthesis in the acinar cells following stimulated secretion. However, an increased rate of protein synthesis has been demonstrated in pancreas (Alfrey et al., 1953; Ferreira-Fernandes and Junqueira, 1955; Farber and Sidransky, 1956) and parotid gland (Grand and Gross, 1969) following the stimulation of secretory granule discharge in vivo.

Thus, the presence of TPPase activity in the ER cisternae and the outer elements of the Golgi complexes in the stimulated cells may be explained by assuming that membranes flow from one cellular compartment to another together with the secretory products. However, recent biochemical studies have shown that the different membranes which are involved in the secretory granule formation reveal considerable differences in the lipid and protein composition and in enzyme activities (Meldolesi et al., 1971a; 1971b; Meldolesi and Cova, 1971; Meldolesi, 1974). These results suggest that membranes do not mix randomly with one another during the transport of secretory products and contradict the membrane flow model (Meldolesi, 1974).

The presence of TPPase activity in ER cisternae has been documented previously in liver cells (Cheetham et al., 1971; Goldfischer et al., 1971; Farquhar et al., 1974). Goldfischer et al., found that TPPase in ER is most active at pH 8 unlike the TPPase in the Golgi complex which has optimum of pH 7. On the basis of their findings, they suggest that the lack of information on TPPase activity in ER of other cells might be due to suboptimal incubation at neutral pH. The presence of TPPase reaction product in the present study of those sites, which in non-stimulated cells usually show negative reaction, suggests that the detectability of TPPase activity is probably related to the cellular metabolic or secretory activity.

The possibility cannot be ruled out that the unusual localization of TPPase reaction product in stimulated cells is due to other factors, such as the changes in membrane permeability or physiological conditions, affecting the cells caused by the drugs used for stimulation. However, previous studies have also demonstrated parallel changes in the distribution of the enzymes associated with the Golgi complex related to the cellular metabolic or secretory activities. In plant cells (Dauwalder et al., 1969), the absence of detectable TPPase reactivity has

been noticed in cells with low metabolic activity. In mammotrophic hormone-producing cells of the rat adenohypophysis (Smith and Farquhar, 1970), the reaction product for another marker enzyme for the Golgi complex, nucleoside diphosphatase, is detected in as many as 6 Golgi cisternae during the period of the highest secretion of the hormone. In the same cells, however, the reaction product is absent or restricted to patchy deposits in a few cisternae during the period of suppressed hormone secretion. It has been also shown that, during the development of eosinophils (Bainton and Farquhar, 1970), deposits of reaction product for peroxidase and lysosomal enzymes are present in ER, Golgi vesicles and immature granules only during the periods of known protein synthesis and packaging. The last mentioned study involves the enzymes which are secretory products unlike TPPase or nucleoside diphosphatase which is presumably a membrane-bound enzyme. Nevertheless, these results, together with those of the other studies mentioned above, seem to support the suggestion that the detectability of TPPase activity as well as other enzyme activity is related to the cellular secretory activity.

Acknowledgements. This is Veterans Administration Research Project No. 4927:01. The authors are grateful to Virginia Hartog for her technical assistance and effort in the preparation of this manuscript.

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Received November 29, 1975