An Effect of Prostaglandin E_1 on the Acinar Cell of the Rat Parotid Gland

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The effects of *in vivo* administration of prostaglandin E_1 (PGE_1) on rat parotid gland acinar cells were studied and compared with glands removed from animals which had been either fasted or stimulated to discharge stored secretory granules by an injection of isoproterenol (IPR). Depletion of tissue amylase, increased plasma amylase, and alterations in secretory cell ultrastructure were used to assess the effects of PGE_1. Initially following PGE_1 administration, the secretory granules enlarged their matrices and lost electron opacity, and numerous fusions between secretory granules occurred. Concomitant with the above events, material having an appearance identical to that within the secretory granules was observed in the acinar lumina *and* the intercellular spaces. The PGE_1-induced release of stored secretory materials from storage granules was transient. Five hours after the onset of hourly PGE_1 injections, the biochemical and structural features of the gland were "normal." The effects of PGE_1 on the rat parotid gland are discussed. A theory of PGE_1 action based on the translocation of ionic calcium is proposed.

Accumulating evidence indicates that prostaglandins may play an active role in the function of the gastrointestinal tract (*12*). Of the organs comprising this system, the effects of prostaglandins have been studied in the stomach, exocrine pancreas, and intestine. Prostaglandins are released from these organs after neural and hormonal stimulation, and they affect both vascular and visceral smooth muscle in addition to secretory processes. It is the effect of prostaglandins upon exocrine secretion that will be further explored in the present study.

While there are differing opinions as to the site of action of prostaglandins in the stomach, there is general agreement that prostaglandin E_1 (PGE_1), PGE_2, and some of their analogs inhibit gastric acid secretion (*11*, *12*, *14*) and, according to some, enzyme release (*23*). Prostaglandins in the pancreas appear to have a dual effect on the final secretory product; they decrease fluid volume and electrolyte content and increase the secretion of enzymes (*24*). The mechanism of prostaglandin action has been related to an equally ubiquitously distributed enzyme, adenyl cyclase. Horton

(*9*) and earlier Ramwell and Shaw (*19*) proposed that prostaglandins were formed consequent to neural or hormonal stimulation of adenyl cyclase and that, in the intact cell, prostaglandins act as a negative feedback mechanism limiting the activity of adenyl cyclase. Intriguing as this proposal seems, it unfortunately lacks universal application. Butcher et al. (*4*) among others have demonstrated that PG's stimulate cAMP formation in many rat tissues and organs.

The parotid gland of the rat was chosen for this study of prostaglandin effects on exocrine secretion for the following reasons: (1) the gland is a totally serous salivary gland having sufficient size to provide adequate quantities of tissue for biochemical analysis; (2) amylase, the major secretory protein of the gland, is relatively stable and its activity is easily assayed; and (3) the release of stored amylase from the gland has been established to be dependent upon, among other factors, the elevation of cyclic AMP (*1*, *26*). This last observation indicating that though prostaglandins have not been isolated from the gland, an enzyme system, adenyl cyclase, upon which this substance has been proposed to act, is present and integral to the secretory process.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats weighing 250 ± 15 g were used. The animals were fed pelleted rat chow ad libitum and housed under controlled light conditions (6 a.m. to 8 p.m.) for 4 days prior to beginning experiments in an attempt to standardize secretory cycles. After acclimatization food was withheld for 15 hours prior to beginning the experiment. Experiments were initiated between 8 and 9: 00 a.m. (time 0), when animals were injected subcutaneously with a freshly prepared salt of PGE_1 (supplied by the Upjohn Company, Kalamazoo) in a dose of 25 μg/100 g b.w. Crystalline PGE_1 (1 mg) was first dissolved in 95% ethanol (0.2 ml). While stirring this solution, 1.8 mM sodium carbonate in water (1.8 ml) was then rapidly added to give a final solution with a pH in the range of 6–7.5. To circumvent the rapid metabolic breakdown reported for PGE_1, hourly injections were given. This dose and regimen were based on previous studies of gastric mucosa in which a similar injection regimen was shown to effectively block secretion (*23*). For the purpose of further comparison, the effect of the drug on secretion was contrasted not only to fasted controls, but also to isoproterenol (IPR)-treated animals. This drug, dissolved in saline, was administered intraperitoneally as a single injection of 0.8 mg/100 g b.w. at time 0 noted above. At the end of an experimental time period, animals were treated by one of two methods depending upon the nature of the particular experiment: (1) killed by a blow to the head prior to biochemical studies or (2) anesthetized with chloral hydrate (i.p. injection of 7% chloral hydrate, 0.5 ml/100 g b.w.) in preparation for morphological observations.

Determination of amylase in the tissues and plasma. Tissue amylase was extracted by homogenization of the tissue in distilled water. After centrifugation, the supernatant was assayed for its amylase activity. Alpha amylase activity was determined by hydrolysis of Cibachrome Blue F_3 GA-amylose substrate as described in a previous paper (*13*). Parallel aliquots were precipitated with 0.5 N PCA and the DNA extracted from the precipitate

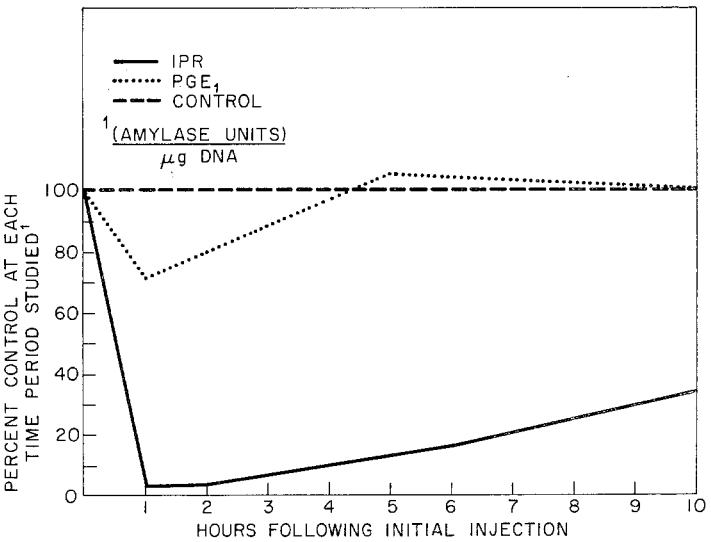

FIG. 1. Restitution of parotid gland amylase following PGE_1 and IPR-induced release is shown over the 10-hour period studied.

by acid hydrolysis at 70°C. DNA content of the hydrolyzate was determined using the diphenylamine reaction (*3*) and comparing it to known concentrations of calf thymus DNA. Comparison between experimental (PGE_1 treated), IPR stimulated, and control (fasted) animals were made 1, 5, and 10 hours after the initial (PGE_1, IPR, or sham) injection and based on their DNA content in order to avoid errors caused by hydration in the use of wet tissue weight or by the choice of a total protein index in a secretory model in which 50% of all the protein of the gland is exportable (*25*).

Plasma amylase activity 1 hour after the initial injection was determined by the same assay procedure used for tissue amylase, but groups of animals were compared on the basis of microliters of heparinized plasma. Blood for these studies was removed from the left ventricle prior to perfusion of the animal for electron microscopic studies.

Electron microscopy. For electron microscopy, anesthetized animals were perfused via left ventricular injection with prewarmed (37°C) 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2. (*10*). Approximately 70 ml were perfused over a 10-minute period. After removal and dicing the gland tissue, fixation in the perfusate was contined for 1 hour. The tissues were then rinsed in 0.1 M phosphate buffer containing 4.5% sucrose prior to postfixation in 0.1 M phosphate buffered 1% osmium tetroxide. Following fixation, tissues were dehydrated through graded alcohols and propylene oxide, then embedded in Araldite:Epon (*17*). To assess the quality of fixation and the variation in morphology between individual lobules, sections 1 μm in thickness were stained with 1% toluidine blue (pH 9.3) and surveyed prior to thin sectioning. Thin sections were stained with uranyl acetate and lead citrate. Ultrastructural studies of the tissues were made at 1, 5, and 10 hours following the initial injection at time 0.

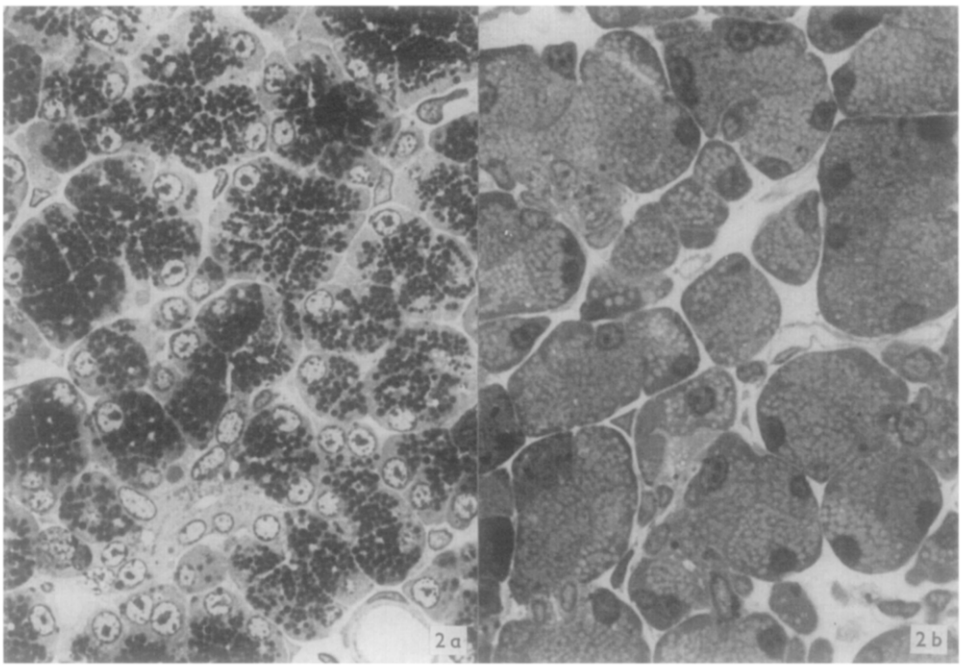

FIG. 2. Light micrographs of parotid glands demonstrating: (a) morphology of a fasted, control gland with normally appearing secretory granules in contrast to (b) a PGE_1 treated gland where the secretory granules are much enlarged and more lightly stained. (a) × 650; (b) × 700.

RESULTS

Amylase levels of parotid gland and plasma

Amylase levels in the gland following PGE_1 and IPR treatment are compared to the fasted control in Fig. 1. Administration of PGE_1 led to an initial release of 28 % of the tissue amylase within 1 hour when compared with this enzyme's level in the fasted control gland ($P=0.01$). Though administration of PGE_1 was continued, the activity of amylase in the tissue returned to and slightly surpassed control levels ($P=0.78$) 5 hours after the first injection. In contrast to PGE_1-treated animals, a single injection of IPR resulted in the release of approximately 98 % of tissue amylase within 1 hour. Subsequently during the 9 hours following this discharge, IPR-treated glands resynthesized 38 % of the amylase found in fasted control glands.

Plasma amylase 1 hour following either PGE_1 or IPR treatment was elevated above control levels. These elevations reflected rather closely the effectiveness of the two agents in releasing amylase from tissue stores. Plasma from PGE_1-treated animals

averaged 1.26 times more activity than the control while their IPR counterparts had average plasma amylase activity values 1.97 times the control value.

Structural observations

Structural changes in the parotid gland of the experimental animals were most marked 1 hour after the initial injection. The light micrographs of the fasted control (Fig. 2a) and PGE_1 treated (Fig. 2b) glands demonstrated these initial changes in secretory granule structure and size. The granules were less dense and increased in size causing the acinar cells to appear enlarged and excessively filled with secretory granules. Ultrastructurally (Fig. 3), many of the enlarged granules were observed to have fused with adjacent granules. The electron opacity of stored secretory material was markedly reduced in PGE_1 treated animals and was similar in appearance to the condensing vacuoles observed in fasted control glands. Flocculent material similar in appearance to the altered secretory granules could be found not only within the acinar lumen and intercellular canaliculi, but also in the intercellular spaces separating the basal portions of the adjoining acinar cells. Material located in this later space was still contained within the acinar cell cluster by an intact basal lamina and was not found in the connective tissue stroma of the gland. Based on the structural changes observed above, the effect of PGE_1 during this period appeared to be limited to the secretory granules and apical and lateral plasma membranes. The morphology of cell organelles such as the nucleus, mitochondria, and rough endoplasmic reticulum appeared similar to those observed in control glands. The morphology of parotid glands removed from fasted, control, and IPR-treated animals using identical fixation techniques has been treated in detail elsewhere (*13*). Five and 10 hours after the onset of PGE_1 injections most of the membrane changes that typified the earlier time period were reduced or absent (Fig. 4). Secretory granules while still less dense than control granules were considerably more dense than those described for the 1-hour time period. While adjoining surfaces of adjacent granules were flattened, there were fewer examples of granule fusion. There was also a consistent lack of flocculent material in any of the previously discussed extra- or intercellular spaces.

In preliminary experiments samples of pancreas were also removed and processed

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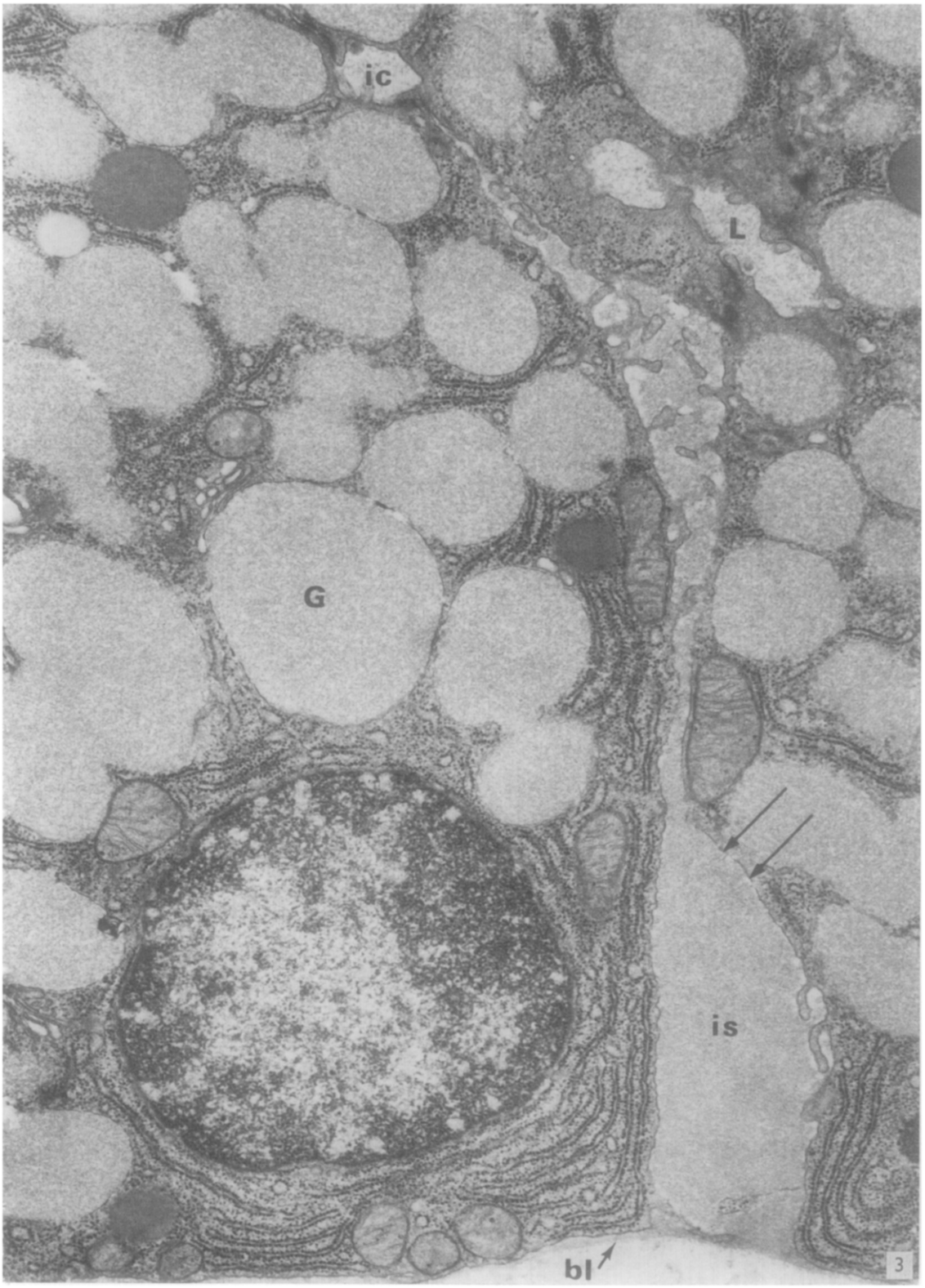
FIG. 3. A parotid gland acinar cell 1 hour after PGE_1 administration showing enlarged secretory granules (*G*). Breaks in the plasma membrane indicated by the arrows are probably artifacts and do not reflect the mode of release of secretory product into the intercellular space. Discharged secretory material is present in the acinar lumen (*L*), intercellular canaliculus (*ic*) and the intercellular space (*is*), although no secretory material appears to have crossed the basal lamina (*bl*) of the acinus. ×18 600.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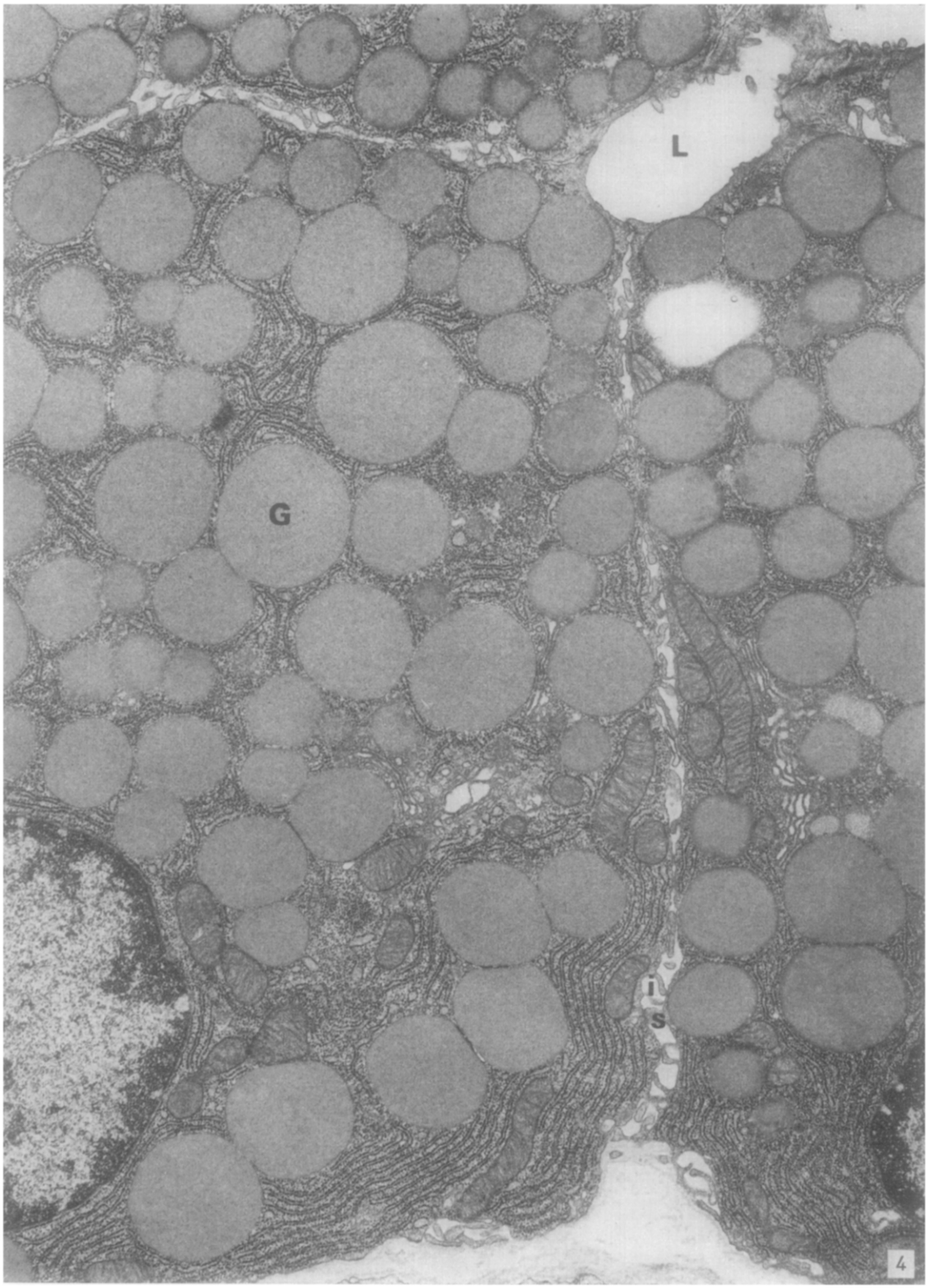
for structural evaluation. No effects of PGE_1 or IPR were observed in the exocrine cells with the dosages and regimen used in this study; therefore further observations were not made on the pancreas.

DISCUSSION

While the author recognizes that secretory granules of the parotid gland are notoriously difficult to preserve, the structural changes reported here are felt not to merely represent artifacts of fixation. During the time that secretory granules show maximum structural alteration and expelled secretory material can be shown within various extracellular and intercellular spaces, tissue amylase activity as studied in animals *not* processed for morphological study (which can be regarded as an independent control) was significantly decreased while plasma amylase was elevated. *Both* structural and biochemical parameters simultaneously return to control values as the PGE_1 injections continued. The elevation of plasma amylase during secretion which occurred in this study following PGE_1 and IPR injections has not, to my knowledge, been previously reported in the literature; however, the phenomenon has been observed by Barka in his studies using IPR (*2*). Any explanation of how such an elevation of plasma amylase activity occurs is, at this point speculative, but reabsorption of the intact protein across the mucosa of the gastrointestinal tract can probably be ruled out on temporal grounds alone. Release of stored material into a nonluminal compartment with subsequent absorption into the capillaries of the gland would seem a most reasonable explanation at least in the case of PGE_1 treated animals.

The most plausible explanation for the changes observed following PGE_1 treatment can probably be related to the drug's translocation of the calcium ion. Prostaglandins by direct displacement of membrane-bound calcium have been found to affect major fluxes of Ca^{2+} across both the plasma membrane (*20*) and the membranes of certain intracellular organelles (*5*, *28*). While fluxes in Ca^{2+} have also been reported to be integral in exocrine secretion following the activation of adenyl cyclase (*6*, *8*, *16*, *28*), the action of prostaglandins in inducing these ionic shifts has been reported to be independent of adenyl cyclase activity (*5*, *20*, *28*). Calcium has also been shown to be important not only in the maintenance of membrane structure (*22*), but also in the integrity of the secretory granule matrix (*18*). Pletcher et al. have shown Ca^{2+} and ATP in storage granules interact with secretory proteins to form "dynamic" complexes which osmotically stabilize the granule and allow concentration of secretory materials (*18*). While this later study was made in chromaffin granules of

FIG. 4. A parotid gland acinar cell 5 hours after the onset of the PGE_1 regimen. Secretory granule membranes are intact and the granule matrix demonstrates increased electron opacity. The acinar lumen (*L*) and intercellular space (*is*) do not contain secretory material. × 14 000.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the adrenal medulla, it has been found that secretory granules of the parotid gland contain large concentrations of Ca^{2+} (*27*).

With these facts and theories, the observed ultrastructural changes in the parotid following PGE_1 treatment could be related to changes in levels or translocation of Ca^{2+} displacement from a previously osmotically stable enzyme complex.

The nature of the PGE_1 effect cannot in any way be considered a "normal" secretory process (*7*, *15*). In fact, the only similarity between the normal secretory process and the events that occur after PGE_1 administration is the intracellular fusion of secretory granules. While fusion of the secretory granule membrane with the apical plasma membrane and the subsequent expulsion of the secretory product into the lumen ("normal release") has been observed after PGE_1 administration. I have not observed a similar process on the lateral aspects of the cell. Therefore, the mode of secretory product access to the intercellular space is not clear. To my knowledge, however, the presence of secretory material within the intercellular space has not been observed in glands stimulated to release their secretory product by either feeding (*15*) or IPR (*13*). It is therefore proposed that the action of PGE_1 on the parotid gland is due to a direct effect on intracellular Ca^{2+} rather than cyclase or cAMP which has been shown to participate in the normal parotid secretory event.

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REFERENCES

1. AMSTERDAM, A., OHAD, I. and SCHRAMM, M., *J. Cell Biol.* **41**, 753 (1969).
2. BARKA, T., Personal communication.
3. BURTON, K., *Methods Enzymol.* **12B**, 163 (1968).
4. BUTCHER, R. W., BAIRD, C. E. and SUTHERLAND, E. W., *J. Biol. Chem.* **243**, 1705 (1968).
5. CARAFOLI, E. and CROVETTI, F., *Arch. Biochem. Biophys.* **154**, 40 (1973).
6. FEINSTEIN, H. and SCHRAMM, M., *Eur. J. Biochem.* **13**, 158 (1970).
7. HAND, A., *Amer. J. Anat.* **135**, 71 (1972).
8. HEISLER, S., FAST, D. and TENENHOUSE, A., *Biochim. Biophys. Acta* **279**, 561 (1972).
9. HORTON, E. W., *Physiol. Rev.* **49**, 122 (1969).
10. KARNOVSKY, M. J., *J. Cell Biol.* **27**, 137A (1965).
11. LEE, Y. H., CHANG, W. D., BINACHI, R. B., MOLLISON, K. and HANSEN, J., *J. Prostaglandins* **3**, 29 (1973).
12. LEVINE, A., *Gastroenterology* **59**, 280 (1970).
13. LILLIE, J. H. and HAN, S. S., *J. Cell Biol.* **59**, 708 (1973).
14. LIPPMAN, J., *Pharm. Pharmacol.* **22**, 65 (1970).

15. MACCALLUM, D. and LILLIE, J., in preparation.
16. ORLOFF, J., HANDLER, J. S. and BERGSTRÖM, S., *Nature (London)* **205**, 397 (1965).
17. PALEVITZ, B. A. and NEWCOMB, E. H., *J. Cell Biol.* **45**, 383 (1970).
18. PLETSCHER, A., DAPRADA, M., BERNEIS, K. H. and STEFFEN, H., Proceedings of Symposium on Cytopharmacology of Secretion, Raven, New York. In press.
19. RAMWELL, P. W. and SHAW, J. E., *in* BERGSTRÖM, S. and SAMUELSON, B. (Eds.) 2nd Nobel Symp., p. 283. Interscience, New York, 1967.
20. —— *Recent Progr. Horm. Res.* **26**, 139 (1970).
21. RASMUSSEN, H., *Science* **170**, 404 (1970).
22. RASMUSSEN, H. and TENENHOUSE, A., *Proc. Nat. Acad. Sci., U.S.* **59**, 1364 (1968).
23. ROBERT, A., NEZAMIS, J. and PHILLIPS, J., *Gastroenterology*. **55**, 481 (1968).
24. RUDICK, J., GONDA, M., DREILING, D. and JANOWITZ, D., *Gastroenterology* **60**, 272 (1971).
25. SCHRAMM, M., *Biochim. Biophys. Acta* **165**, 546 (1968).
26. SCHRAMM, M., and NAIM, E., *J. Biol. Chem.* **245**, 3225 (1970).
27. SELINGER, Z., personal communication.
28. STJÄRNE, L., *Acta Physiol. Scand.* **87**, 428 (1973).