Epidermal Growth Factor Inhibits Cytoskeleton-related Changes in the Surface of Parietal Cells

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ABSTRACT The effect of epidermal growth factor (EGF) on gastric acid secretion was correlated with the morphological changes of the apical pole of rat parietal cells studied by transmission electron microscopy.

Gastric acid secretion was stimulated by histamine, carbachol, pentagastrin, and insulin-induced hypoglycemia, and estimated by continuous recording of pH variations of gastric luminal perfusate. EGF inhibits acid secretion in these conditions.

The action of the hormone also results in the arrest or reversal of the changes in shape undergone by parietal cells as they go into secretion.

In view of the evidence involving cytoskeletal elements in the generation of these structural alterations, our observations suggest that the action of EGF on gastric acid secretion may be a consequence of a general effect of this hormone on cytoskeletal function.

Early effects produced by epidermal growth factor (EGF) include induction of changes in morphology of human epithelioid carcinoma cells in culture (8), stimulation of endocytosis (14), and enhancement of mobility of concanavalin A receptors in the plasma membrane of 3T3 cells (1). All these phenomena are known to involve the cytoskeleton.

Alongside these effects, it is interesting to note that EGF has been reported to inhibit gastric acid secretion (4, 12) and to be nearly indentical to urogastrone, a hormone for which a role in the regulation of hydrochloric acid secretion has been claimed (13). It appears of interest to establish whether this particular effect can be related to other known actions of EGF.

Previous work has shown that oxyntic cells undergo important changes in shape during acid secretion (17, 28), and we have presented evidence indicating that these changes are related to interactions between cytoskeletal elements and the plasma membrane (26, 27).

In this report, we show that EGF rapidly inhibits gastric acid secretion elicited in the rat by a variety of secretagogues and that this inhibition is accompanied by early and extensive changes in the morphology of the apical pole of parietal cells. These results constitute additional evidence for an effect of this hormone on cytoskeletal function and indicate that the inhibition of acid secretion may well be a manifestation of such an effect.

MATERIALS AND METHODS

Materials

Mouse EGF was purchased from Collaborative Research Inc., Waltham, Mass. Pentagastrin was obtained from Ayerst Laboratories Inc., New York. Crystalline insulin was from Novo Industri A/S, Copenhagen, Denmark. Histamine phosphate, carbachol, and atropine were obtained from Sigma Chemical Co., St. Louis, Mo. Metiamide was the generous gift of Dr. W. R. Brimblecombe of Smith, Klein and French Laboratories, Welwyn Garden City, England. Dextrostix were obtained from Ames Co., Division Miles Laboratories, Inc., Elkhart, Ind.

Gastric Acid Secretion Measurements

Male (290–310 g) rats of the Sprague-Dawley strain were fasted for 18–24 h in wire cages with free access to water; they were anesthetized by an intramuscular injection of 0.5–0.7 ml/100 g body weight of a 25% urethane solution. Continuous recording of acid secretion was performed by the method of Ghosh and Schild (11). Briefly, the lumen of the stomach was perfused by a dilute solution of NaOH (2.5×10^{-4} N) at a constant rate of 1 ml/min, driven by a peristaltic pump. The emerging fluid was passed over a combination pH electrode located 15 cm below the stomach level to avoid gastric distention. The pH was recorded graphically. Total dead space occupied by the tube leaving the stomach and the chamber containing the electrode was 1.4 ml. Variations in the perfusate volume were negligible. This method produces good quantitative results in the assay of inhibitors of gastric acid secretion (10) and also gives excellent qualitative information even in those cases in which its range of linearity (pH 7.5–4.5) is exceeded. All injections of single doses of secretagogues and EGF were given intravenously (i.v.) in 0.1–0.3 ml of physiological saline. For continuous infusion,

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a polyethylene catheter was placed in one of the iliac arteries. Arterial pressure was routinely recorded through a catheter in the carotid artery. This record showed that the variations in pressure elicited by some of the secretagogues had no significance in relation to the acid secretion process in our experimental conditions.

Hypoglycemia was produced by a single dose of insulin administered i.v. Glycemia levels were determined by the Dextrostix semiquantitative method. Some of the experiments were carried out after ligation of the renal vessels; this did not produce differences relevant to our results.

Electron Microscopy

The stomach was fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, containing 0.3 M sucrose, postfixed in 1% osmium tetroxide, block-stained with uranyl acetate and embedded in Epon. Sections were examined after staining with uranyl acetate and lead citrate.

To provide quantitative support for some of the observations, parietal cells were photographed at × 5,000 and the negatives projected with a photographic enlarger at × 15,000. The outline of the cell and of the areas occupied by tubulovesicular system were traced on paper and subsequently cut out and weighed in a precision scale.

RESULTS

Effect of EGF on Gastric Acid Secretion

EGF inhibits gastric acid secretion elicited in three different manners: by single intravenous doses of histamine, carbachol, or pentagastrin, by insulin-induced hypoglycemia, and by a continuous infusion of histamine.

After single i.v. doses of either histamine (300 μg), carbachol (1 μg), or pentagastrin (10 μg) are administered to anesthetized rats, a corresponding stimulatory effect on acid secretion begins to be detected in 2–6 min, reaches a maximal level of ~0.5 μeq/min for carbachol and histamine, and ~2 μeq/min for pentagastrin, and finally returns to its basal level after periods which vary according to the drug employed. Those responses are abolished by one single i.v. dose of EGF (10 μg) injected shortly before the application of the secretagogue; the responsiveness to new injections of these compounds is recovered after 60 min (Fig. 1 *a* and *b*).

The highest levels of acid secretion were obtained by insulin-induced hypoglycemia (Fig. 2 *a*). The stimulatory effect begins to appear at glycemia values of 0.45–0.25 mg/ml, and is preceded by a lag time of ~30–40 min. This effect is blocked by atropine and vagal nerve section but not by metiamide (not shown), indicating a participation of muscarinic receptors for acetylcholine and a probable postsynaptic liberation of this neurotransmitter by vagal stimulus. The sharp decrease of the perfusion liquid pH is changed abruptly by the administration of EGF (10 μg) and the basal level of secretion is restored by a second injection (5 μg) of the hormone (Fig. 2 *b*).

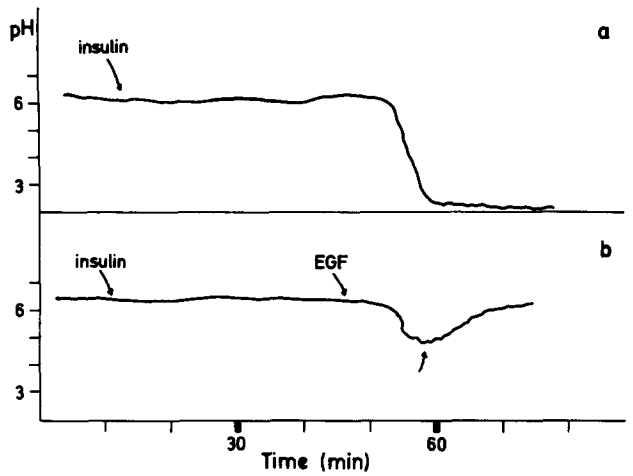

FIGURE 2 Inhibitory effect of EGF on gastric acid secretion stimulated by insulin-induced hypoglycemia; pH recording of gastric lumen perfusate. (*a*) Stimulatory effect produced by a rapid i.v. injection of insulin (1.5 U). Initial glycemia was ~1.3 mg/ml. The glycemia at the lowest point of the curve was ~0.25 mg/ml, and acid output 24 μeq/min. (*b*) Treatment as in *a*, but EGF was injected i.v. (10 μg) 35 min after insulin administration; an additional 5 μg were injected i.v. at the point indicated by the arrow. The glycemia was similar to that in *a* and was not altered by EGF.

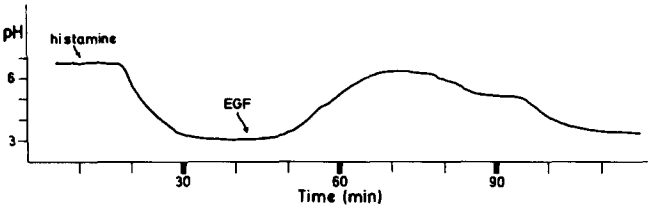

FIGURE 3 Inhibitory effect of EGF on gastric acid secretion stimulated by a continuous infusion of histamine. pH recording of gastric lumen perfusate. At the point indicated, histamine (25 μg/min) was infused i.v. at a rate volume of 50 μl/min and maintained for the duration of the experiment. EGF (10 μg) was given i.v. once the stimulation of acid secretion was maximal for this rat (1 μeq/min).

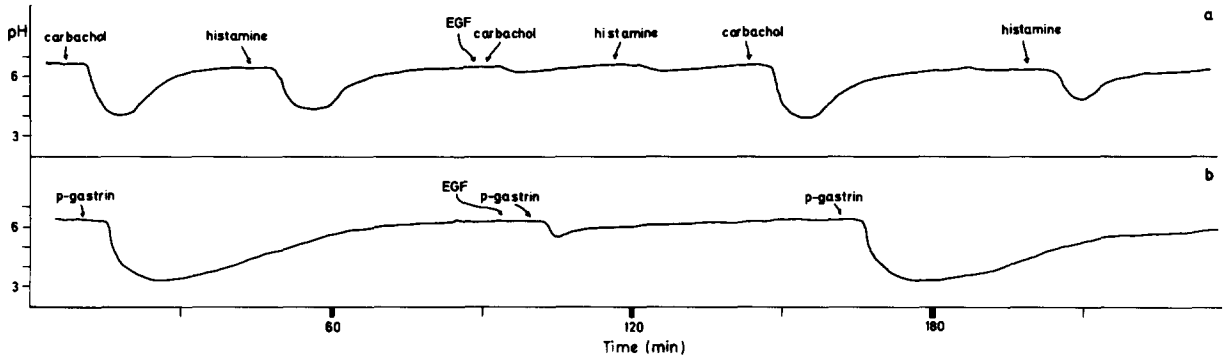

FIGURE 1 Gastric acid secretion detected by pH variations of the lumen perfusate. Inhibitory effect of EGF on the responses induced by single doses of secretagogues. (*a*) Carbachol (1 μg), histamine (300 μg) and EGF (10 μg) were injected i.v. EGF practically eliminated the response to carbachol and histamine injected 2 and 25 min later, respectively. A new dose of carbachol given 60 min after EGF was as effective as the first one in stimulating acid secretion, whereas 2 h after EGF, histamine evoked only 57% of its initial response. (*b*) Pentagastrin (p-gastrin) (10 μg) and EGF (10 μg) were injected i.v. as shown. The response to p-gastrin was 90% inhibited by EGF given 6 min before the secretagogue. The inhibitory action was finished 70 min later.

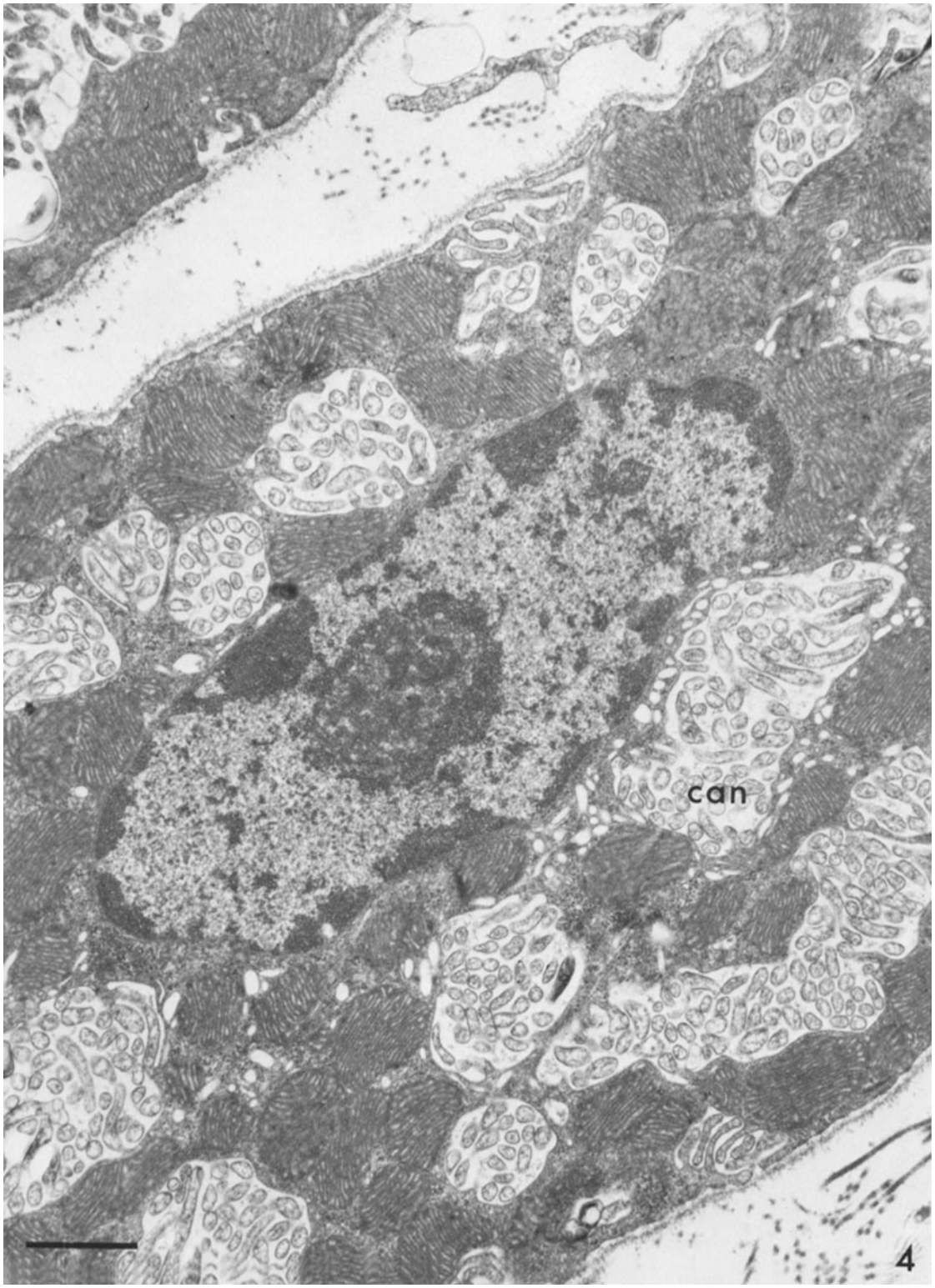

FIGURE 4 Parietal cell from a gastric mucosa stimulated by insulin-induced hypoglycemia; *can*, intracellular canaliculus. Bar, 1 μm. × 17,500.

The time-course of EGF inhibitory effect is seen more clearly in rats that are maintained at a plateau level of acid secretion by a continuous i.v. infusion of histamine. In the experiment of Fig. 3, acid secretion was stabilized by this procedure at a rate of 1 μeq/min. This effect is mediated by histamine H_2 receptors, as demonstrated by its complete inhibiton by metiamide, a specific H_2 receptor antagonist (2). As shown in Fig. 3, the inhibitory effect of EGF is detected after 5 min, begins to decline at ~30 min, and has an overall duration of ~70 min. Even though variations were observed in the maximal levels of acid secretion elicited by histamine, the magnitude of the inhibition produced by EGF did not vary accordingly.

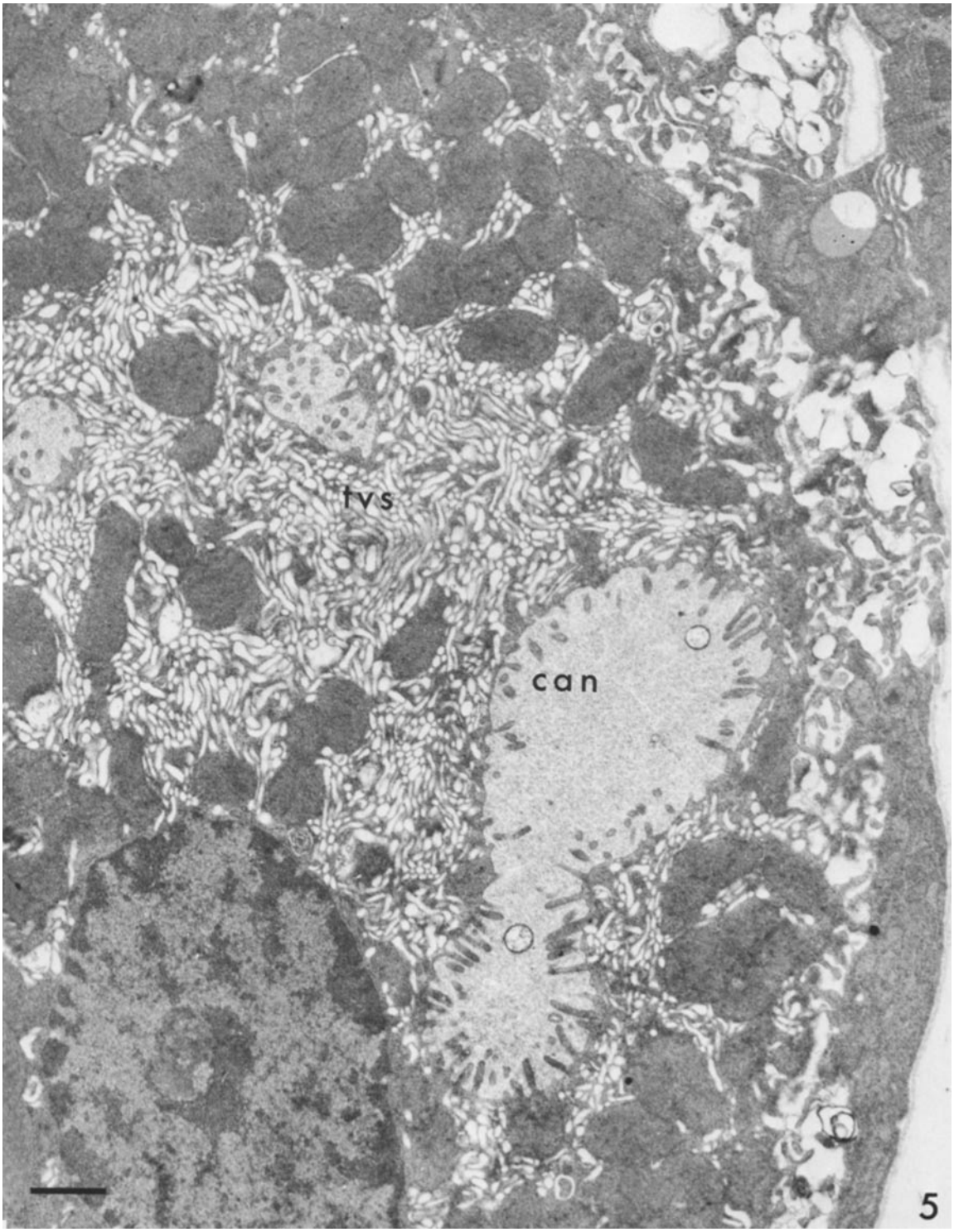

FIGURE 5 Parietal cell from a mucosa stimulated by insulin-induced hypoglycemia and treated with EGF in the sequence indicated in Fig. 2 *b*. In the lumina of the canalicular profiles, a finely granular material of undetermined nature is seen. *can*, Intracellular canaliculus; *tvs*, tubulovesicular system. Bar, 1 μm. × 11,800.

Effect of EGF on Parietal Cell Morphology

Insulin administration proved to be an extremely effective manner of obtaining parietal cells with images characteristic of active secretion. Fig. 4 shows a cell taken from a mucosa whose acid secretion curve is illustrated in Fig. 2*a*. The branched and tortuous intracellular canaliculus penetrates to all portions of the cell. The canalicular wall is folded into abundant villous processes that occupy the lumen. The cytoplasm is filled mainly with densely packed mitochondria, with very few membranous elements belonging to the tubulovesicular system. The administration of EGF generates a completely different picture (Fig. 5). The great majority of the parietal cells exhibit an image

typical of the nonsecreting state, which shows large areas of cytoplasm occupied by closely packed tubulovesicular elements, and comparatively smooth intracellular canaliculi provided with scanty villous processes. We frequently found cells whose canalicular walls appeared smooth to an extent seen only exceptionally in the stomach of the fasting rat. Even though some cells showed an image more typical of the nonsecreting state than others, the overall appearance was not at all uncertain, and a simple inspection of the sections at × 10,000–15,000 was enough to determine whether the animal had received only insulin or insulin followed by EGF.

Quantitative support for these observations was obtained by taking advantage of the well-known fact that the non-secreting parietal cell has a large amount of tubulovesicular system that occupies well-defined zones of the intracellular compartment. Parietal cells identified at low magnification were chosen at random, with the only condition that at least one of the diameters of the profile be >10 μm, so as to avoid grazing sections through one of the cell poles. 38 cells obtained from three randomly chosen insulin-plus-EGF-treated blocks were compared with an equal number of cells obtained from three randomly chosen insulin-treated blocks. Total cell section areas and the corresponding tubulovesicular system areas were determined as described under Materials and Methods. The results are shown in Table I. It is seen that the treatment with EGF has brought about a fourfold increase in the area of cell section occupied by tubulovesicular system.

TABLE I

Effect of EGF on Tubulovesicular System Area in Rat Parietal Cells

Condition	Total cell area	Total tubulovesicular system area	Tubulovesicular system area per 100 μm^2 cell area
	μm^2	μm^2	
Insulin-treated	2,535	66	2.6
Insulin + EGF-treated	2,952	318	10.8

The cortex of the canalicular wall in the insulin-treated stomachs exhibits a finely granular texture, and shows filaments of the 6- to 8-nm variety, most of which are randomly oriented; some of them, however, form small straight bundles. The processes that fill the canalicular lumen contain abundant straight microfilaments oriented along their longitudinal axes (Fig. 6*b*). In their most frequent disposition, these filaments tend to become arranged in a single plane (Fig. 6*a*) underlying the plasma membrane; they are interconnected by a fuzzy material of moderate electron density that is also occasionally seen between the filaments and the membrane. In some cases, however, the filaments seem to be uniformly distributed within the process; only very exceptionally do they appear to integrate into a central core bundle within a villus. A comparison of these images with those of stomachs whose secretion has been elicited by refeeding instead of insulin administration reveals no significant differences.

In stomachs treated with insulin followed by EGF, the number of processes is greatly diminished so that relatively long stretches of smooth canalicular wall can be observed. The cortex presents tightly packed granular and filamentous elements, the latter occasionally being arranged in bundles that course parallel to the canalicular surface. The shorter processes, which are typical of quiescent canaliculi, do not show any form of subplasmalemmal arrangement of the filaments, which instead tend to form a loose bundle (Fig. 7).

The matrix between the tubulovesicular elements presents a different aspect in those places that are nearer to the canalicular

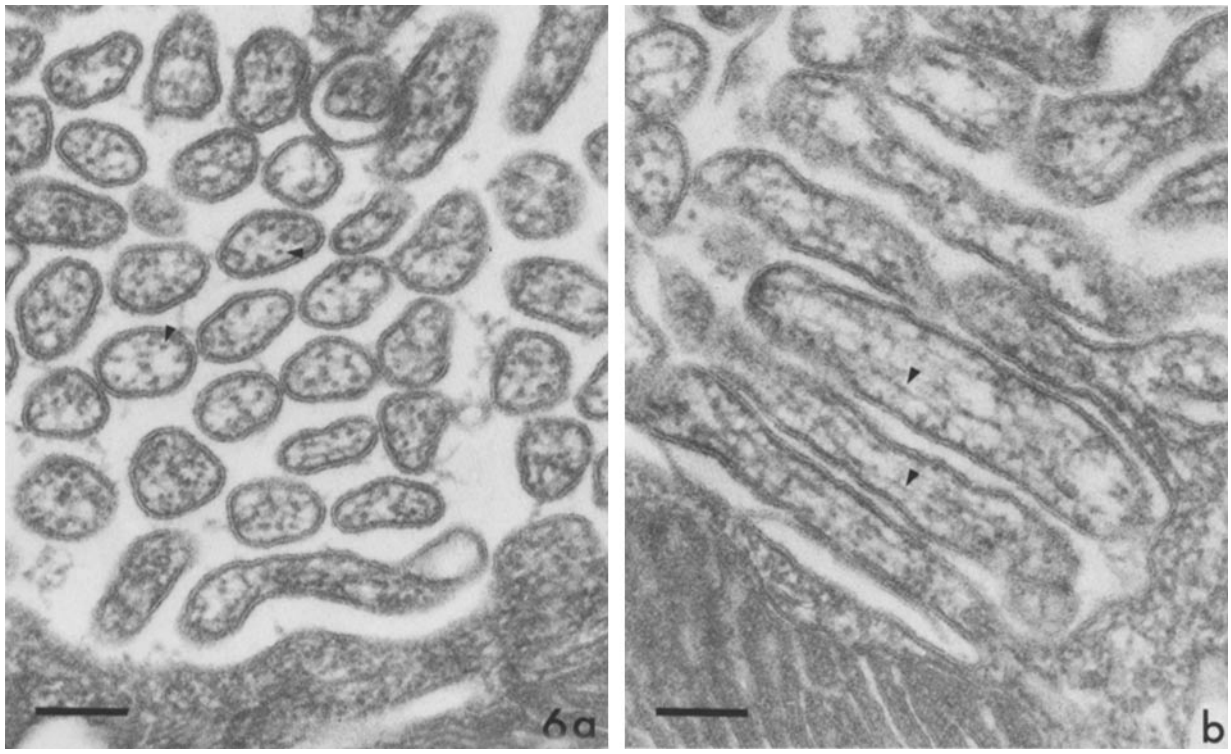

FIGURE 6 Sections through canalicular wall in two parietal cells after treatment with insulin. Transverse and longitudinal sections of processes that illustrate the arrangement of microfilaments (arrowheads) are shown. Bars, 0.1 μ. × 120,000.

wall, in comparison with those that are far removed from it. In the neighborhood of the canaliculus, the membranous elements are separated by a dense granular matrix, whereas in other places only an extremely thin translucent space without any discernible structure is apparent. As reported in other studies on oxyntic cells, no microfilaments can be demonstrated between the tubular cytomembrane elements (26).

DISCUSSION

Our results confirm previous reports on the effect of EGF on gastric acid secretion (4, 12). Furthermore, they show that at the doses employed the initiation of its action is very rapid and that the effect is maintained for periods ranging from 40 min to 1 h. The possibility that EGF might act through the stimulation of the secretion of gastric inhibitory polypeptide (21), the only other known agent that exhibits somewhat comparable properties, is ruled out by the persistence of the effect of EGF after excision of the entire intestine (22). Thus, a direct interaction between parietal cells and EGF seems most likely. Cultured cells that respond in some way to EGF are able to bind it specifically at their surface (7); the hormone is subsequently internalized and finally degraded in the lysosomes (6, 7). The possibility that a similar sequence may occur in rat parietal cells is strengthened by the finding of receptors for EGF on the surface of a wide variety of fibroblastic (6, 9, 23) and epithelioid (8, 9) cells and in membrane fractions from tissues of several species including the rat (19).

Parietal cells are known to possess receptors for histamine, gastrin, and acetylcholine (24). The intracellular biochemical signals that mediate the response to the last two secretagogues are thought to be different from the one intervening in the histamine effect, which is known to be cyclic AMP (24). The fact that EGF is an effective inhibitor of the secretion induced by all three of these substances renders direct competition for receptors unsatisfactory as an explanation of the action observed. We cannot rule out, however, that alterations in membrane properties induced by the hormone could determine the removal, masking, or inactivation of all the secretagogue receptors of the cell.

Parietal cells possess quite distinct configurations according to their secretory state (17, 28). Resting cells show relatively smooth intracellular canaliculi and a large amount of peculiar apical cytomembranes known as the tubulovesicular system. When cells are actively secreting, the intracellular canaliculi become extensively developed and present a highly irregular surface covered with cytoplasmic processes; at the same time, the tubulovesicular system diminishes to the point of disappearance. Advantage was taken of this structural change to obtain a quantitative estimation of the effect of EGF on the morphology of the insulin-stimulated parietal cell. The fourfold increase of the tubulovesicular system area corresponds very well with the qualitative impression that the majority of parietal cells in the EGF-treated stomachs are in the nonsecreting state. The general aspect of a stomach treated with insulin followed by EGF was entirely comparable to the stomach of a fasting

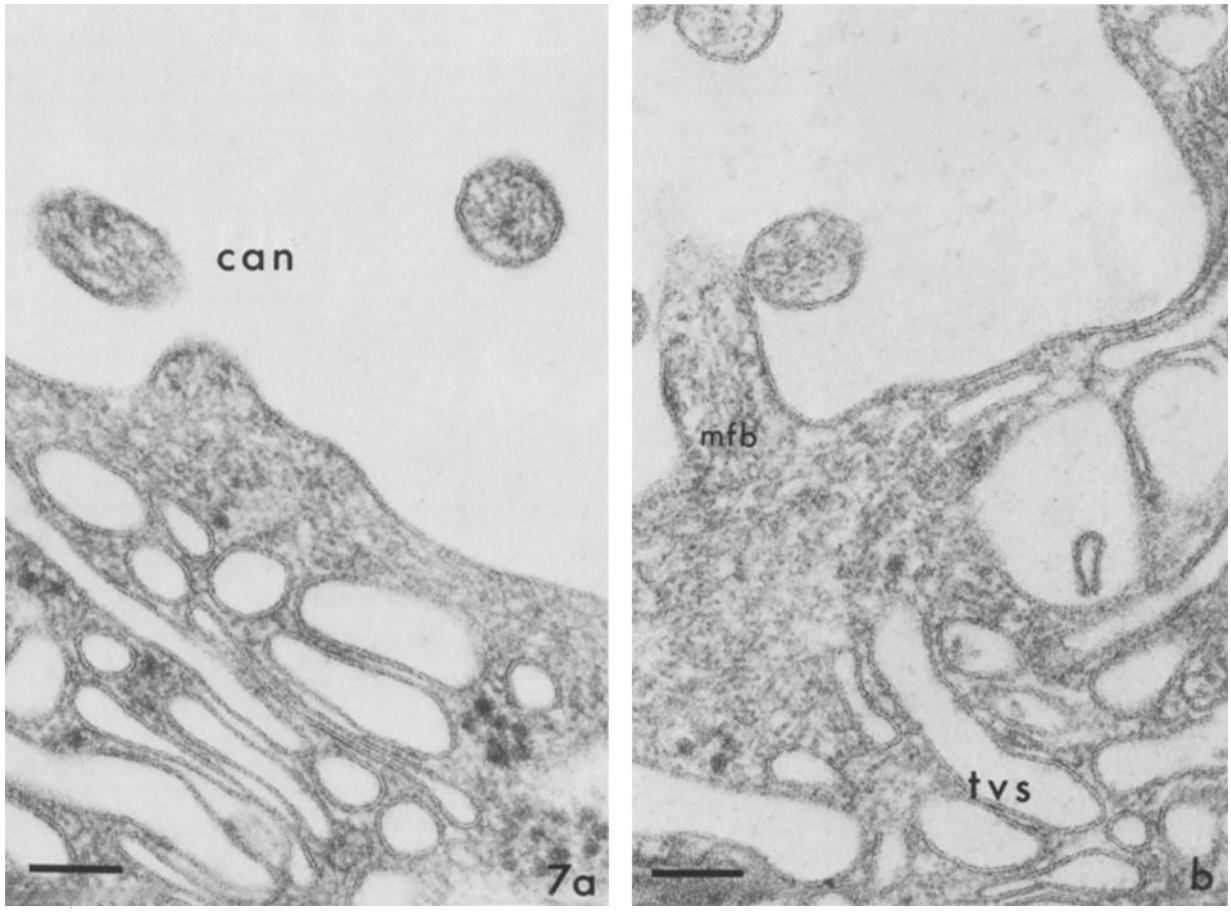

FIGURE 7 Sections through canalicular wall in two parietal cells after treatment with insulin followed by EGF. Canalicular cortex, processes, and arrangement of microfilaments are demonstrated. *can*, Intracellular canaliculus; *tvs*, tubulovesicular system; *mfb*, microfilament bundle. Bars, 0.1 μm. × 120,000.

rat, except for the relatively high incidence of cells whose canaliculi showed extremely smooth contours.

The results reported show that the administration of EGF to stomachs stimulated by insulin-induced hypoglycemia resulted in a reorganization of the secretory poles of parietal cells involving its two most prominent components, namely the cytomembranes and the microfilaments. It has been proposed that the elaboration of the canalicular membrane during secretion, which entails a considerable increase in area, is carried out through incorporation of tubulovesicular membrane to the secretory surface. There is also evidence indicating that the changes in apical surface topography and the translocation of cytomembranes are expressions of the motility of these cells that are probably mediated by cytoskeletal elements (26). The presence of actin and myosin has been demonstrated biochemically in oxyntic cells (18, 27). Actin filaments are associated with the plasma membrane at the secretory pole in a highly distinctive pattern, but they are not associated with the membranes of the tubulovesicular system (26). Finally, cytochalasin B, known to interfere with cytoskeletal action, inhibits both the passage to a secretory configuration and the secretion of HCl (3).

After the administration of the secretagogues employed, EGF prevented the development of the ruffled surface characteristic of the secretory state or forced its reversal in the cases in which it had become established. Because the different chains of events set in motion by these secretory stimuli must finally converge on the mechanism that generates the topological changes, it is reasonable to believe that the action of EGF on surface configuration and cytomembrane translocation may be exerted through interference with cytoskeletal function. Such a possibility is rendered more likely by the results reported by Chinkers et al. (8) on the early action of EFG on the morphology of cultured human carcinoma cells. These authors showed that when these cells are exposed to EGF, rapid and transient ruffling activity is first produced and is followed within 1 h by an extraordinary smoothing of the cell surface. The time-course of these morphological changes is in good agreement with our observations on the effect of EGF on acid secretion. The results reported here differ from others reported for EGF (5, 8, 14) in one important aspect, namely that the contact with the hormone presumably takes place at the basal aspect of the cell, whereas the recorded effect is seen at the transcellular secretory pole.

EGF, a hormone that elicits a variety of functional responses (7) is present in the plasma and in several tissue extracts from normal human subjects (15, 25). Our results, taken together with the other reported effects of EGF involving different manifestations of cellular motility (1, 5, 8, 14), indicate that the action of this hormone may be concerned with the regulation of cytoskeletal function and/or with the relation of cytoskeletal elements and the plasma membrane at different sites. This hormonal action may be completely unrelated to the action of EGF on DNA synthesis, this last effect being known to exhibit a dissimilar time-course (16); however, because drugs that disrupt the cytoskeleton enhance the stimulatory effect of growth factors on DNA replication (20), EGF action on the cytoskeleton might turn out to be one step in a sequence of events leading to hormonal regulation of mitogenesis.

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