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Changes in membrane surface areas in mouse parietal cells in relation to high levels of acid secretion

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INTRODUCTION

Many reports have now shown that the tubulovesicular compartment of parietal cells during gastric acid secretion becomes strikingly depleted and that this change occurs contemporaneously with an increase in membrane at the free (luminal) surface of the cells (Adkins, Ende & Gobbel, 1967; Frexinos, Carballido, Luis & Ribet, 1971; Helander & Hirschowitz, 1972; Hubner, Klein & Eder, 1969; Ito & Schofield, 1974; Rosa, 1963; Sedar, 1962). The mechanisms underlying the virtual disappearance of one parietal cell membrane compartment and a rapid increment in another are uncertain. If the two membrane systems are interconnected then their transposition is easily explained but, so far, it has not been possible to establish morphological continuity between them (Ito & Schofield, 1974). The extreme lability of the tubulovesicular membrane during acid secretion, and the lack of appropriate techniques for effective labelling of this membrane prior to its breakdown or dissemination, are major difficulties encountered by investigators who have attempted to relate changes in parietal cell membranes to levels of gastric acid secretion.

Stereological methods have been employed to determine whether the loss in tubulovesicular membrane during gastric acid secretion is matched by the increment in free surface membrane in parietal cells (Helander & Hirschowitz, 1972; Helander, Sanders, Rehm & Hirschowitz, 1972; Ito & Schofield, 1974). Estimates for the surface area of tubulovesicular membrane in parietal cells of dogs treated with histamine have shown a decrease to less than half the control level, a change accompanied by a tenfold increase in the membrane at the free surface of the cells (Helander & Hirschowitz, 1972: Helander *et al.* 1972). Corresponding estimates for the two membrane systems are not yet available for other animal species, but similar if less pronounced effects, measured as changes in the relative volume occupied by the tubulovesicular and microvillous compartments of parietal cells, have been recorded for man following pentagastrin treatment (Frexinos *et al.* 1971) and for the mouse following insulin treatment (Ito & Schofield, 1974). It seems reasonable to postulate that the changes seen in the tubulovesicular and microvillous compartments of stimulated parietal cells during acid secretion are related and that, taken together, they represent the most significant morphological correlate of hydrogen ion release. This premise has been basic to the design of the present study.

In previous studies on parietal cells the extent to which levels of hydrogen ion

secretion by the gastric mucosa are related to the changes in the tubulovesicular and the plasma membrane compartments has been largely neglected.

Previous reports on levels of gastric acid secretion by anaesthetized or unaesthetized mice following administration of secretagogues have shown maximal values not exceeding 7 μequiv./15 minutes (Berenblum & Fogel-Kaufman, 1957; Ito & Schofield, 1974) and similar values have been obtained in excised mouse stomachs maintained under hyperbaric oxygen (Davenport & Chavré, 1950). Although these values may be maximal for a given set of experimental conditions they may not represent the maximal capacity of the mouse stomach to release hydrogen ions. The present study attempts to determine which set of experimental procedures elicits the most pronounced and consistent release of gastric acid in the mouse, so that the morphology of parietal cells during and after what is assumed to be a maximal secretory response can be compared.

MATERIALS AND METHODS

Animals

Male mice (16–35 g) were used in some 600 experiments and, except where indicated, were fasted overnight in wire bottom cages with free access to water and sucrose crystals. The animals were bred and maintained under routine laboratory conditions (Swiss-Webster and CSL, Commonwealth Serum Laboratory, Melbourne Australia). CSL mice bred and maintained under specific pathogen or parasite-free conditions were also used, in each case within 72 hours of removal from the protected environment.

Perfusion studies

Anaesthesia induced for surgical preparation was maintained throughout gastric perfusion except in conscious mice where perfusion was through a gastrostomy tube inserted two or more days prior to the experiment. Acid secretion in response to administration of secretagogues was obtained with whatever anaesthetic employed, but the response to an individual secretagogue was consistently higher when sodium pentobarbitone (repeated 0·2 ml doses of a 5 mg/ml solution) dissolved in pyrogen-free water was used; this was therefore used routinely for both induction and maintenance of anaesthesia.

Gastric perfusates used to collect the secretory responses included distilled water, unbuffered 154 mM sodium chloride, unbuffered 103 mM calcium chloride, Ringer's solution, and other buffered physiological saline solutions. The rate of perfusion appeared to be significant, highest acid secretion values being recorded with perfusion rates of 22·5 ml/hour using sterile pyrogen-free Ringer's solution. To maintain body temperature at normal level, the mice and perfusion fluids were warmed with heating pads and warm air heaters.

When animals given free access to food were used, values for acid secreted during the experiments were often misleading, owing presumably to trapping of hydrogen ion by the food and subsequent unpredictable release of the ion during gastric perfusion.

Secretagogues used alone or in combinations included insulin (5 u./kg, I.P.), pentagastrin (Pentavlon, 10–500 μg/kg, I.P.; or 10–100 μg/ml/hour, subcutaneously) and carbachol administered in the gastric perfusate in concentrations of 0·05–5 mg/ml.

In studies on conscious mice a polythene tube was introduced through an abdominal incision into the non-glandular part of the stomach and the external opening of the tube sealed. After 2–8 days the mice were placed in restraining cages and the stomach perfused using a fine polythene tube introduced into the stomach through the existing tube which also served as a drain. The volume of the perfusate recovered suggested that little if any fluid was lost by passage into the duodenum. In studies on anaesthetized mice, the following procedure resulted in the most pronounced and consistent secretory response and was used routinely in most of the experiments. The animals were anaesthetized with sodium pentobarbitone and prepared for gastric perfusion using a modification of the method of Gosh & Schild (1958) as described previously (Ito & Schofield, 1974). Gastric perfusion using sterile pyrogen-free Ringer's solution was carried out at a constant rate of 22·5 ml/hour usually until acid levels of less than 2 μequiv. were recorded in at least two consecutive 15 minute collections. Then histamine (0·23 mg free base/ml/hour) was infused subcutaneously for 15–45 minutes prior to, and then concurrently with, gastric perfusion with Ringer's solution containing carbachol (0·5 mg/ml) at a constant rate of 22·5 ml/hour. Assessment of the acid content of gastric perfusates collected at 15 minute intervals was by titration (Ito & Schofield, 1974). The procedure adopted enabled rapid monitoring of acid secretion so that tissues could be fixed within 2 minutes after the completion of a 15 minute collection.

Morphology

Preparation of tissues taken for electron microscopy was as described previously (Ito & Schofield, 1974). Stereological methods were used to estimate membrane surface areas in parietal cells from perfused, but unstimulated, control animals showing minimal acid secretion, and from perfused stimulated animals showing the highest level of acid release recorded in the present study and fixed at the time of the peak secretory response.

Sampling

Blocks from each animal were sectioned oblique to the surface so that the whole thickness of the mucosa was available for study in each section. Sections were mounted on 300 mesh grids and 20–24 micrographs taken at instrument magnifications of 1500 and 7300 in each of two blocks with subsequent photographic enlargement of $\times 4{\cdot}1$. At the lower magnification most of the area occupying grid squares was included in the micrograph; at the higher magnification a specified corner of each grid was sampled systematically whenever it contained parietal cells. Cells in the superficial, intermediate and deep zones of the gastric mucosa were represented in micrographs from the sections studied. During photographic enlargement, a test system, containing 45 lines intersecting at 13·33 mm intervals, was superimposed on each print using a transparent overlay. The micrographs were calibrated by reference to micrographs of tropomyosin crystals (period 39·5 nm) taken at the same instrument magnification and subjected to the same photographic enlargement.

Quantitative procedures

In estimating the surface area of membranes, stereological surface densities relate the surface area of a membrane compartment to a standard unit of reference volume, usually μm^3 or cm^3. Given this characteristic, surface densities as such

provide information only about the membrane within the standard unit of reference volume.

Because we were interested in describing membrane changes in parietal cells, rather than in a unit volume containing an unknown number of cells, it became necessary to know the amount of a specific type of membrane in an 'average' parietal cell at a given time. Stereological methods exist for obtaining such information (Loud, 1968; Weibel, Stäubli, Gnagi & Hess, 1969), but for all practical purposes they are restricted to uninucleate cells which contain spheroidal nuclei. Parietal cells with their pleomorphic nuclei are not well suited to this type of analysis. The problem was to find a way of comparing membrane surface areas in parietal cells from different animals without the requirement of finding the average cell volume or the number of cells per cm^3 in each animal. A satisfactory solution was obtained with the surface area ratio method. An average cell, at any given time, has a specific amount of membrane surface area distributed among various morphologically recognizable compartments (organelles). Changes in membrane compartments, including those induced experimentally, are produced by adding or subtracting membrane metabolically, or by moving it from one compartment to another. If, however, a specific organelle maintains a state of dynamic equilibrium, then its surface area may be used as a reliable reference for those surfaces which are undergoing changes. By determining the membrane changes with both methods, namely surface density and surface ratio, experimental changes can then be interpreted in the absence of estimates for average cell volumes.

Surface densities. The surface density (S_{V_i}) of a component i was determined by applying a test system (Bolender, 1974) to electron micrographs of parietal cells and then counting intersections between the test lines and the membrane traces below. A surface density is calculated by dividing twice the number of intersections (I_i) by the total length of the test line (L_T), as described by Tomkeieff (1945):

$$S_{V_i} = \frac{21}{L_T}. \tag{1}$$

Surface ratio. The surface ratio method uses the surface area of an appropriate membrane compartment as the reference, and is entirely independent of the ambiguities associated with surface density reference volumes. A requirement is that the surface area of the reference membrane remains constant for the control and experimental data. The validity of the reference assumption can be checked, as shown in Figure 8.

Control surface ratio

$$SR_{i,\mathrm{ref}}[C] = \frac{S_{V_i}[C]}{S_{V_{\mathrm{ref}}}[C]} = \frac{I_i[C]}{I_{\mathrm{ref}}[C]}. \tag{a}$$

The ratio (SR) of the surface of a membrane (i) to the surface of reference membrane (ref) of the control (C) is calculated simply from intersection counts (I_i and I_{ref}) taken from the same electron micrographs. The resulting ratio indicates the surface area of membrane (i) relative to that of the reference membrane.

Experimental surface ratio

$$SR_{i,\mathrm{ref}}[E] = \frac{S_{V_i}[E]}{S_{V_{\mathrm{ref}}}[E]} = \frac{I_i[E]}{I_{\mathrm{ref}}[E]}. \tag{b}$$

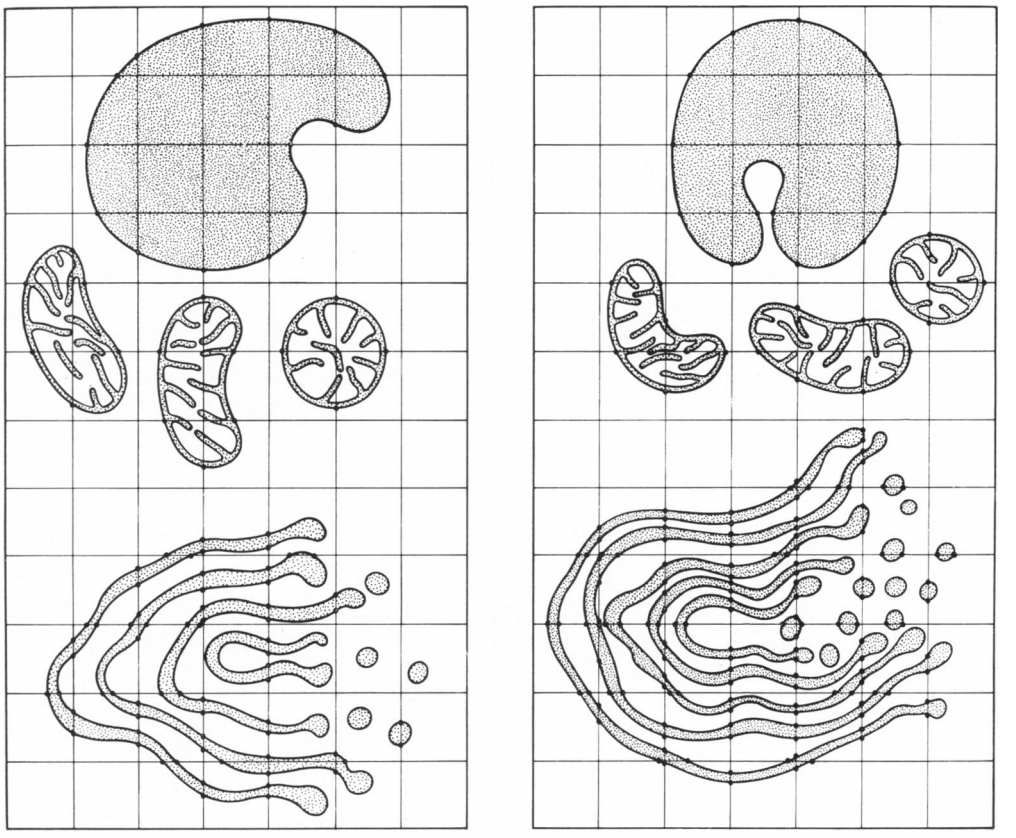


Fig. 1. Sample calculation for surface ratio method. Here, the surface areas of the Golgi membranes (*GA*) are related to those of the nuclear membrane reference, which is assumed to remain constant. By forming a surface area ratio (*SR*) between the experimental [*E*] and control [*C*] values, the extent of the change can be detected as a percentage of the control where: $E/C = 1$ represents no change; $E/C > 1$ represents an increase; and $E/C < 1$ represents a decrease. Control:

$$SR_{ga,n}[C] = \frac{S_{V_{ga}}[C]}{S_{V_n}[C]} = \frac{I_{ga}[C]}{I_n[C]} = \frac{66}{14} = 4{\cdot}71.$$

This calculation indicates that for each unit (e.g. 1 cm²) of nuclear membrane in the control there are 4·71 units of Golgi membrane.

Experimental:

$$SR_{ga,n}[E] = \frac{S_{V_{ga}}[E]}{S_{V_n}[E]} = \frac{I_{ga}[E]}{I_n[E]} = \frac{134}{14} = 9{\cdot}57.$$

Here there are 9·57 units of Golgi membrane surface for each nuclear unit, which represents an 103 % increase when compared with the control: $(SR_{ga,n}[E]/SR_{ga,n}[C] = 9{\cdot}57/4{\cdot}71 = 2{\cdot}03)$.

This ratio indicates the surface area of a membrane (*i*) relative to the area of the reference surface under experimental conditions (*E*). The percentage change of membrane (*i*) associated with the experimental situation is obtained by comparing the two ratios. Equations (*a*) and (*b*) can be combined to give a single expression.

$$\frac{SR_{i,\,\mathrm{ref}}[E]}{SR_{i,\,\mathrm{ref}}[C]} = \frac{I_i[E]}{I_{\mathrm{ref}}[E]} \times \frac{I_{\mathrm{ref}}[C]}{I_i[C]}. \qquad (2)$$

A result equal to 1 represents no change, greater than 1 an increase, and less than 1 a decrease in the surface area of membrane (*i*). An example of the method is given in Figure 1.

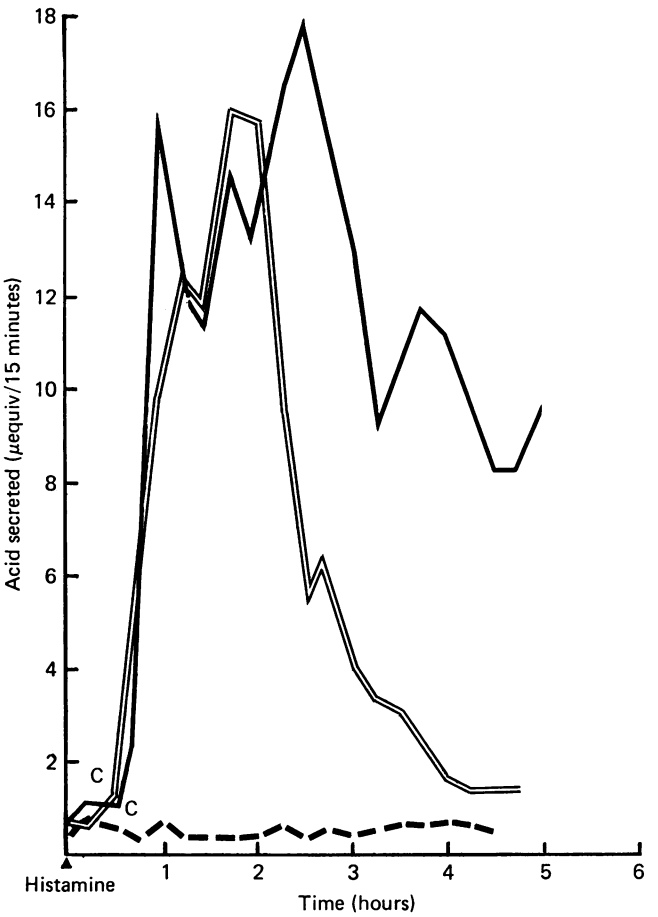


Fig. 2. Acid secretion in two perfused mice treated with histamine (0·23 mg free base/ml/hour) and carbachol (C; 0·5 mg/ml in the gastric perfusate). Levels in a perfused but untreated control mouse (––) are also shown. In the stimulated animals treatment was continued throughout the experiment. One animal showed a spontaneous fall in acid secretion to near the control level, and in the other secretion was maintained at about half the peak level.

RESULTS

Perfusion studies

Acid secretion in control mice

Acid present in the stomach at the beginning of the experiments and removed by the initial gastric washout ranged from 0·25 μequiv. to as high as 21·25 μequiv. In anaesthetized animals, acid in the perfusate fell to usually less than 1 μequiv./15 minutes within the first hour of perfusion, and was maintained at this level throughout the experiments (Figs. 2, 3). In anaesthetized control mice, perfused for periods of 3–6 hours, the mean level of acid secreted was 0·60 ± 0·018 S.D. μequiv./15 minutes. The mean acid release for all anaesthetized control mice perfused for 1 hour or longer was 0·03 ± 0·01/S.D. μequiv./g body wt/15 minutes.

In conscious mice the range of acid levels in the initial gastric washout was similar to that in anaesthetized mice, but the levels of acid released during subsequent perfusion without administration of secretagogues were higher and more variable.

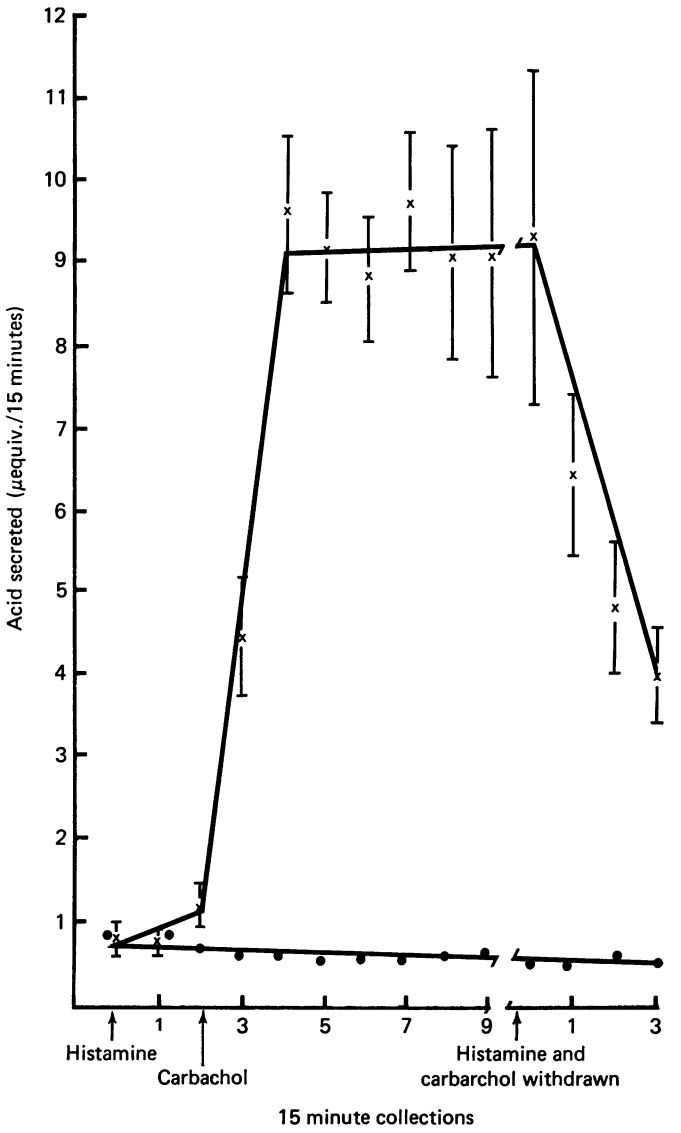


Fig. 3. Pooled secretory responses in 20 perfused mice treated with histamine and carbachol (—), and in 5 perfused untreated mice (●). Introduction of carbachol (0·5 mg/m) into the gastric perfusate after pre-treatment with histamine leads to a rapid release of acid. A less rapid fall in acid secretion follows withdrawal of both secretagogues.

The highest spontaneous release of acid (10·4 μequiv./15 minutes) was obtained 1 hour after the commencement of perfusion in a fasted mouse, in which the initial gastric washout contained a relatively small amount of acid (2·5 μequiv.); a still higher induced level (14·2 μequiv. corresponding to 0·47 μequiv./g body wt/15 minutes) was recorded 1¾ hours later, shortly after this animal was offered and had eaten 10 mg of food pellet. These observations provided a guide in subsequent experiments on secretagogue-treated mice directed at establishing levels of acid secretion which could be interpreted as maximal.

Acid secretion in secretagogue-treated mice

In conscious mice secretagogues employed included subcutaneous histamine infusions, subcutaneous Histalog, intraperitoneal or subcutaneous injections of carbachol, insulin or pentagastrin, carbachol administered in the perfusate, or these drugs given in various combinations. Some mice were subjected to repeated experiments at intervals of 2 or 3 days. Highest acid values obtained were 7·3 μequiv./15 minutes after subcutaneous administration of carbachol (4 μg/ml/hour) and 6·4 μequiv/15 minutes after subcutaneous administration of histamine (0·23 mg free base/ml/hour).

In anaesthetized mice the most reproducible secretory response was obtained in specific pathogen-free and parasite-free animals anaesthetized with sodium pentobarbitone and subjected to subcutaneous infusion with histamine (0·23 mg free base/ml/hour), followed 15–45 minutes later by the inclusion of carbachol (0·5 mg/ml) in the gastric perfusate. The perfusate used was sterile, pyrogen-free Ringer's solution administered at a rate of 22·5 ml/hour. The duration of gastric perfusion in the presence of the secretagogues ranged from three quarters of an hour to 5 hours.

Examples of individual responses by anaesthetized Agouti C_3H mice treated with carbachol and histamine, and a typical control level, are illustrated in Figure 2. Pooled values for acid secretion in a group of 20 mice, and showing the effect of withdrawal of the secretagogues, are given in Figure 3. In mice showing a secretory response, acid release usually reached a peak within 45 minutes after the onset of carbachol perfusion and persisted for as long as 2¼ hours in the presence of secretagogue administration; peak values for acid secretion ranged from 8·2 to 30·6 μequiv./15 minutes. In some mice acid secretion showed a spontaneous fall between 1 and 3¾ hours after the onset of the response; peak values for animals showing this attenuated response ranged from 9·6 to 15·9 μequiv./15 minutes.

Of the 118 animals studied in this series 33 failed to respond to treatment with histamine–carbachol, and gave acid values similar to those obtained in perfused, untreated animals; results from these experiments are not included in Figure 3. In 12 mice maximal acid values recorded ranged from 18·0 to 30·6 μequiv./15 minutes, with a mean peak value of $0{\cdot}89 \pm 0{\cdot}206$ S.D./μequiv./g body wt/15 minutes. This response represents a 30-fold increase over resting levels obtained in anaesthetized unstimulated mice, including those used for stereology, and is approximately double the highest value obtained in a conscious mouse following the physiological stimulus of food intake. Response in three animals fixed at the time of peak acid secretion were 1·02, 0·88, and 0·98 μequiv./g body wt/15 minutes. Parietal cells from these animals were selected for detailed stereological analysis on the assumption that they would approximate most closely to, and thus best represent, cells involved in maximal acid secretion in the mouse.

Parietal cell structure

The structure of major cell compartments of parietal cells was examined in three groups of mice subjected to gastric perfusion following sodium pentabarbitone anaesthesia. These groups comprised untreated control mice, including animals fixed after perfusion for periods of up to 6 hours; the three secretagogue-treated mice showing a maximal secretory response and fixed at the time of peak acid secretion; and mice fixed at various times during a progressive fall in acid secretion after withdrawal of secretagogue and after a peak response of at least 0·5 μequiv./g body wt/15 minutes.

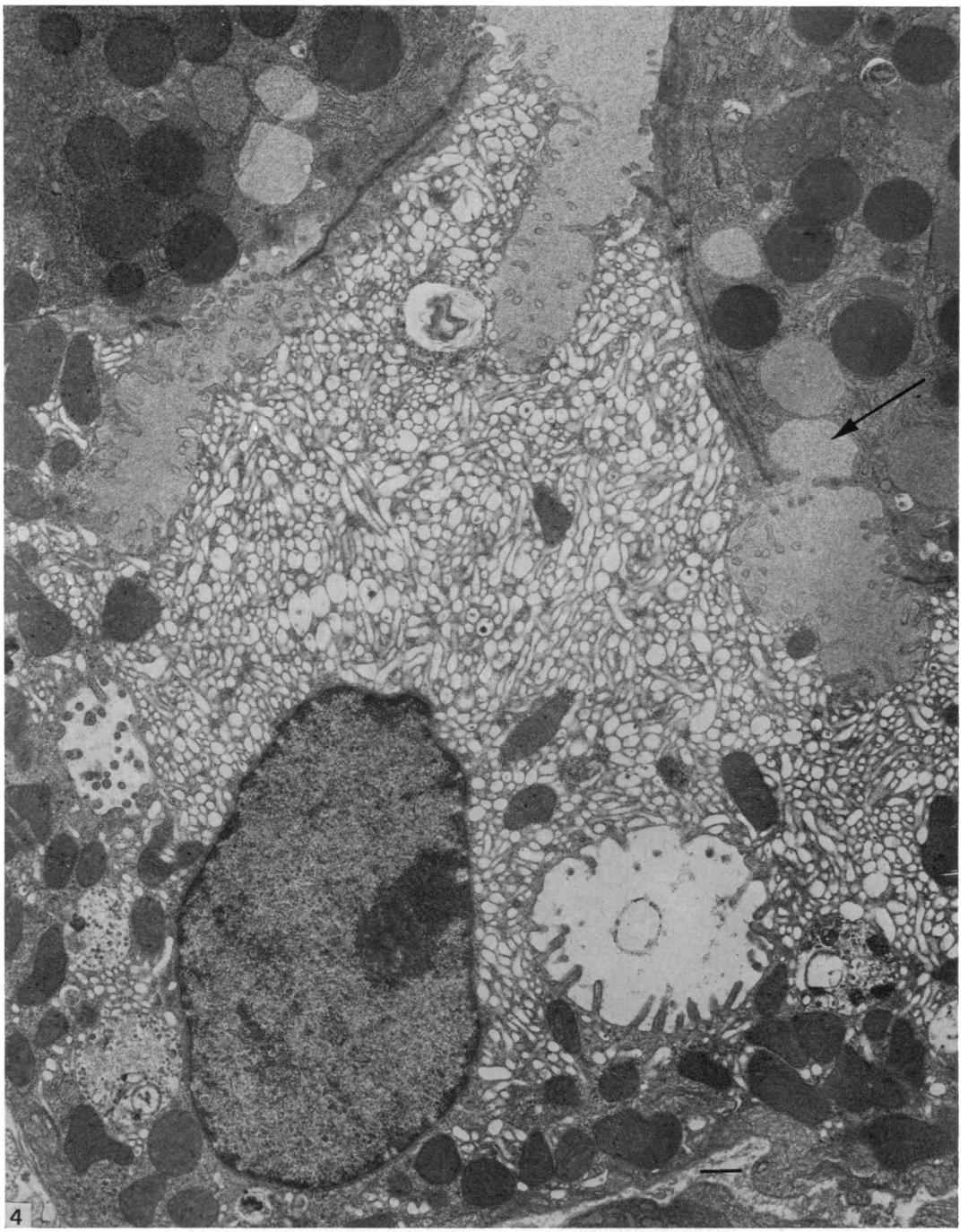


Fig. 4. An electron micrograph of a parietal cell from a mouse perfused for 3½ hours in the absence of secretagogues. Acid secretion in this animal averaged 0·25 μequiv./g body wt/15 minutes. Surface density estimates are included in Table 1 (S407). The tubulovesicular compartment is prominent and, even at low magnification, microvesicles can be identified in the lumen of the component tubules and vesicles. The lumen of the gastric gland contains material similar in density to that of granular profiles seen in adjacent chief cells, and one of the profiles (arrow) is open to the gland lumen. This natural marker substance is not present in either of the secretory canaliculi shown here. Microvilli lining the canaliculi and present at the apical membrane of the parietal cell are few in number and show a relatively dense core. × 6600. Bar, 1 μm.

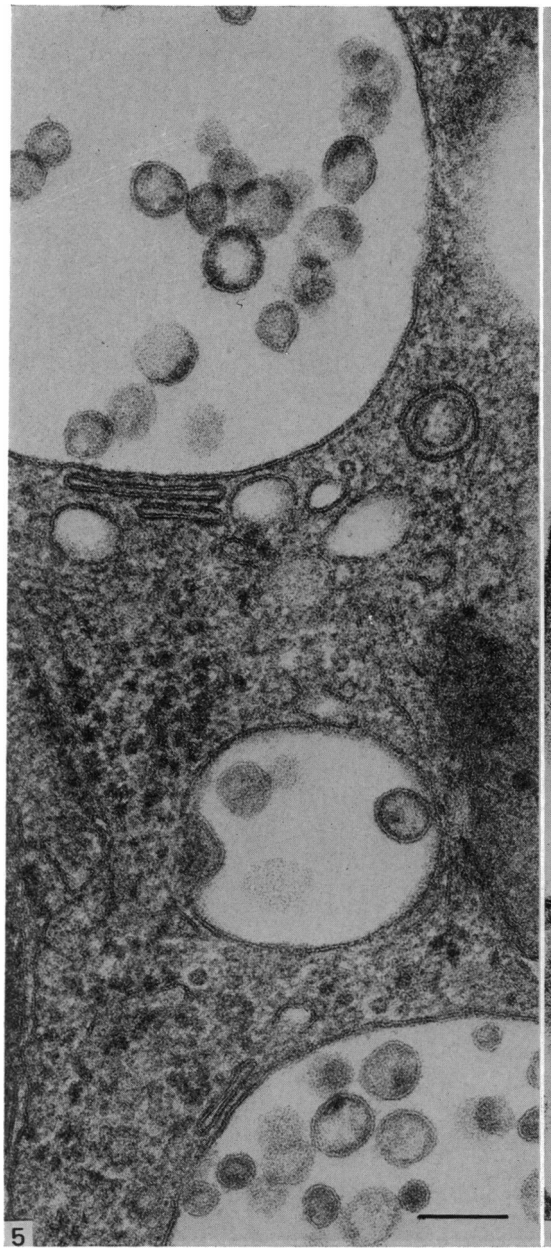
In control animals distinct and characteristic structures in the parietal cells of unstimulated control mice were the intracellular or secretory canaliculi which followed a meandering course, or appeared as rounded profiles. In either case they contained relatively few short microvilli projecting into a conspicuous lumen (Fig. 4). The secretory canaliculi were probably interconnected as one system which extends from the glandular lumen deep into the infranuclear cytoplasm. In most parietal cells there was reason to believe that all regions of the canaliculus were open to the gland lumen. In the non-secreting state the lumen of gastric glands may be filled with a substance resembling secreted pepsinogen (Fig. 4). This natural marker may also extend into the lumen of secretory canaliculi of parietal cells, and thus provides evidence of continuity between glandular and canalicular lumen. However, in control animals perfused for periods of 2–3 hours, parietal cells adjacent to marker-filled gland lumen contained secretory canaliculi in which the marker was absent (Fig. 4). It seems likely, therefore, that segments of canaliculi may become internalized through loss of continuity with the remainder of the canalicular system.

The tubulovesicular compartment in mice perfused for periods of 2–6 hours was strikingly obvious in all parietal cells, and consisted of cytoplasmic tubules averaging 130 nm in diameter and often arranged in groups which followed a sinuous course between larger vesicles (Fig. 4). Continuity between tubules and vesicles and other bulbous enlargements was seen commonly. Other smaller vesicular profiles or microvesicles were also frequent within the lumen of both of these components of the tubulovesicular compartment. Favourable sections showed that, in some cases, the microvesicles were probably pedunculated invaginations of the tubular or vesicular membrane.

Other membrane-limited cytoplasmic elements included spherical and crescentic coated vesicles at or near the free surface and the lateral and basal membrane and in association with the Golgi apparatus. Our attention was drawn to two types of unusual vesicular structures not previously described in parietal cells. One structure appeared as a concentric membrane profile, comprising in its most common form an outer circle 80–100 nm in diameter enclosing a smaller circle 40–50 nm in diameter. The other structure was a flattened vesicle 25–35 nm in width and 80–150 nm in length (Fig. 5).

Another feature not previously noted in control animals was the occasional presence of unusually elongated and enlarged microvilli containing an extension of the tubulovesicular system from the subjacent cytoplasm (Fig. 6). Other parietal cell components in control animals perfused for periods of up to 6 hours were similar to those described previously for control animals (Ito & Schofield, 1974).

During maximal acid secretion, parietal cells in mice showing the highest levels of acid release recorded in the present study and fixed at the time of peak secretion were strikingly uniform in appearance (Fig. 7). Microvilli occupying the canalicular lumen were more elongated and numerous than those in control animals. The lumen of the secretory canaliculi formed little more than narrow intervals between the closely packed microvilli. The flocculent material seen in the canaliculi and gland lumen of control animals was now replaced by a clear matrix, and continuity between canaliculi and the gland lumen was obvious. In many cell profiles the canaliculi formed a horsehoe-shaped investment to the central nucleus with mitochondria arranged in palisade fashion at the margins of the canaliculi. Coated and other vesicles of similar size were similar in frequency and distribution to those seen in control animals. However, cytoplasmic tubulovesicular elements were virtually



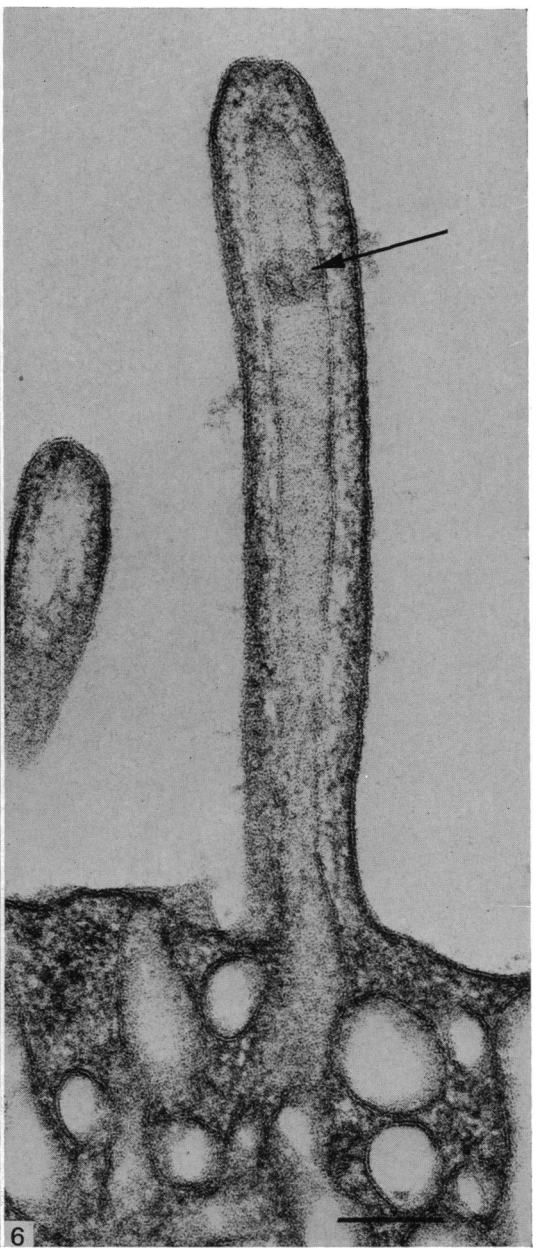


Fig. 5. From the parietal cell of a mouse perfused for 3¾ hours in the absence of secretagogue stimulation. Acid secretion in this animal averaged 0·02 μequiv./g body wt/15 minutes. A concentric membrane profile, and two flattened vesicles, both seen only infrequently in control animals, are shown adjacent to a multivesicular body. Vesicular elements with this appearance were a strikingly common feature in parietal cells from mice showing a fall in acid secretion (see Figs. 13, 14). ×115000. Bar, 100 nm.

Fig. 6. From the same animal as in Fig. 5. The microvillus shown here is unusual for a control mouse in that it is elongated and is invaginated by a cytoplasmic tubule containing a microvesicle (arrow). Other cytoplasmic tubules and vesicles show close apposition with intermicrovillous regions of the canalicular membrane. Other parietal cell microvilli seen elsewhere in this mouse resembled those illustrated in Fig. 4. ×130000. Bar, 100 μm.

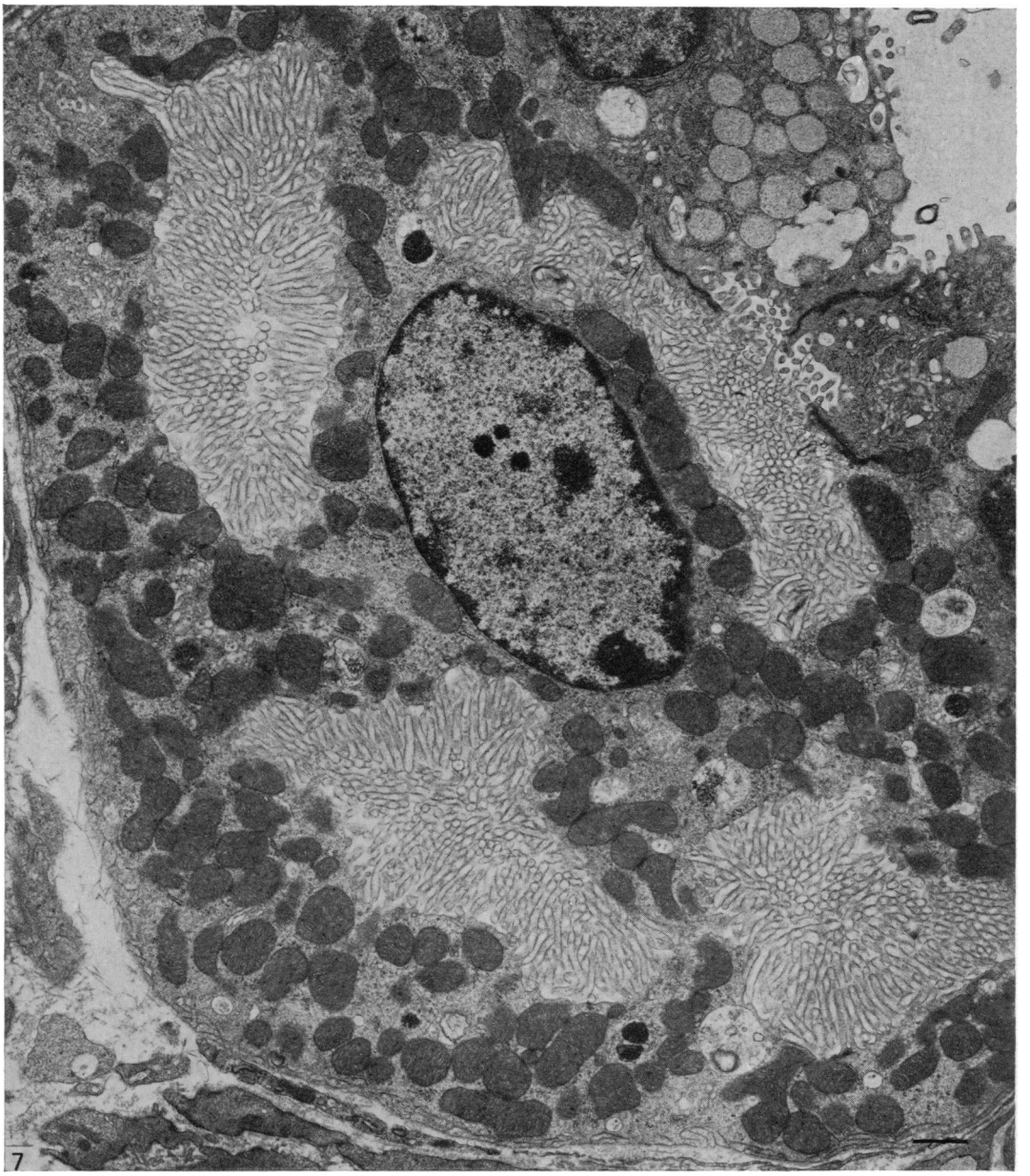


Fig. 7. A parietal cell from a perfused mouse fixed at the time of peak acid secretion (1·02 μequiv./g body wt/15 minutes) recorded 1 hour after the onset of secretagogue treatment. The duration of perfusion was 2¾ hours and acid secretion recorded prior to stimulation was 0·03 μequiv./g body wt/15 minutes. Stereological data for this animal are included in Table 1 (S351). The prominence of microvilli, and the absence of cytoplasmic tubulovesicular membranes, are characteristic features of mice showing high levels of gastric acid secretion. × 7200. Bar, 1 μm.

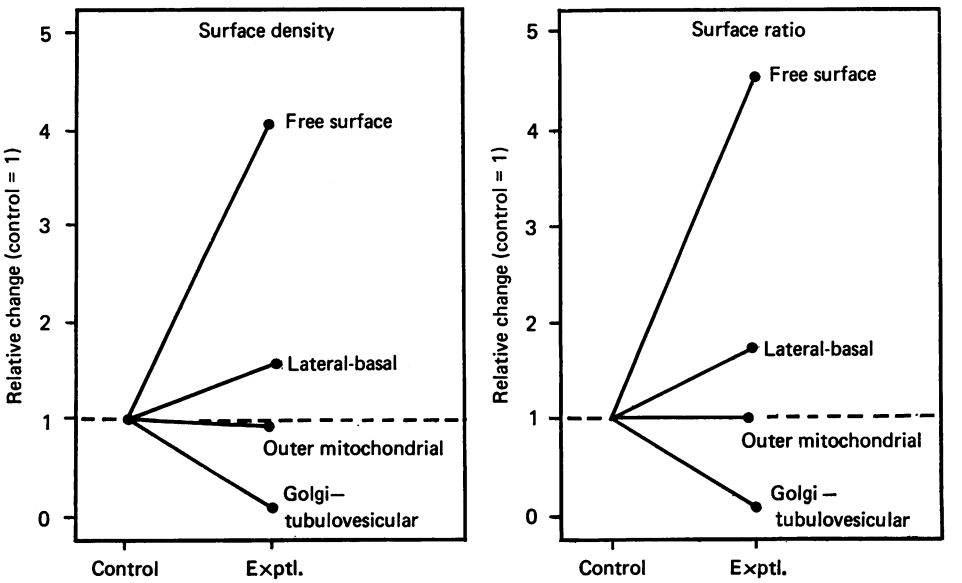


Fig. 8. Relative changes in parietal cell membranes determined with the surface density and surface ratio methods. Control membrane surface areas are compared with the membrane distributions seen in animals secreting H^+ at a maximal rate. Plasma membranes are described as 'free surface' when at the cell apex and lining secretory canaliculi, and 'lateral-basal' when at the sides and base of the cell. The dashed line assumes no change in the surface area of the outer mitochondrial membrane. See text for details.

absent so that both the canalicular areas and the mitochondria were silhouetted in the cytoplasmic matrix containing some ribosomes, granular endoplasmic reticulum, filaments and microtubules.

Concentric membrane profiles and profiles of flattened vessicles illustrated in Fig. 5) were as uncommon in animals killed during maximal acid secretion as they were in control animals.

Stereology

The highest level of gastric acid secretion recorded in this study approximated to 0·9 μequiv./g body wt/15 minutes. During the course of over 600 experiments this level of acid release was recorded in only 12 mice. In three animals the stomach was fixed within 2 minutes of the collection from which maximal secretion was recorded; parietal cells from these mice were selected for detailed stereological analysis on the assumption that membrane changes seen would best represent those occurring in the mouse under conditions of maximal acid secretion.

Figure 8 illustrates the *relative* changes in membrane surface areas calculated with both the density and ratio methods. If, for example, the number of cells per cm^3 in both the control and experimental animals is the same, then the relative changes estimated by the two different methods should be identical. Figure 8 indicates that, in fact, the surface density curves closely resemble those of the surface ratio. The slight difference seen is that, for free surface and lateral and basal membranes, the relative increase in the surface densities is about 10 % less than the relative increase in the surface ratios. This observation suggests that in the experimental animals parietal cells have undergone some swelling, but that this volume change

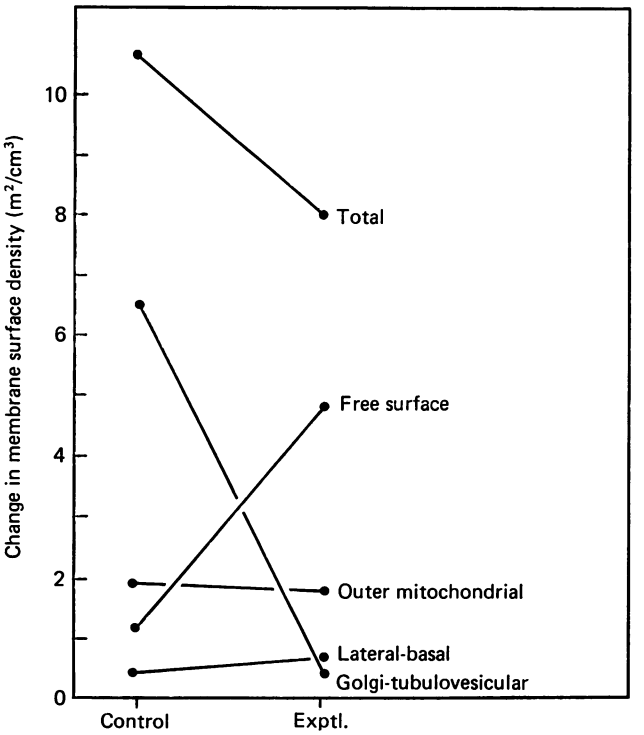


Fig. 9. Changes in membrane surface areas associated with maximal H^+ secretion. The surface densities are used to describe changes in an average cell, using the arguments of Fig. 1. The loss of membrane from the tubulovesicular compartment appears to be partly accounted for by the membrane added to the free surface, implying a transfer from one membrane compartment to another, but possibly is also partly accounted for by loss of membrane from the cell as a whole.

Table 1. *Surface densities of parietal cell membranes* (m^2/cm^3)

	N	OMi	FS	GTV	LaBa	Ly	Total
			Control				
S407	0·103	1·90	0·693	6·74	0·629	0·411	
S410	0·0611	1·78	1·11	5·66	0·225	0·561	
S416	0·113	2·14	1·78	7·37	0·507	0·232	
$\bar{\chi}$	0·0924	1·94	1·20	6·59	0·454	0·401	10·7
S.E.	0·0159	0·805	0·318	0·498	0·120	0·0950	
Sv (%)	0·84	17·6	14·0	59·8	4·11	3·64	100
			Experimental				
S331	0·064	1·71	5·78	0·861	0·273	0·124	
S343	0·0808	1·74	3·98	0·433	0·555	0·177	
S351	0·0707	1·87	4·57	0·131	1·29	0·250	
$\bar{\chi}$	0·0718	1·77	4·78	0·475	0·707	0·184	7·99
S.E.	0·00488	0·0479	0·530	0·212	0·304	0·0365	
Sv (%)	0·90	22·2	59·8	5·95	8·85	2·30	100

Surface densities of parietal cell membranes at × 29000 in control mice and mice fixed during maximal acid secretion (experimental). Membrane compartments are nuclear (N), outer mitochondrial (OMi), free or luminal surface (FS), Golgi–tubulovesicular (GTV), lateral and basal (LaBa), and lysosomal (Ly). The standard errors (S.E.) for each set of data are listed below the mean values ($\bar{\chi}$). Surface densities (Sv) for each compartment are also expressed as a percentage of the total density estimates for each group of animals. Comparison of results for control and experimental animals indicates a significant difference ($P < 0·05$) for the free surface ($P = 0·014$) and for the Golgi–tubulovesicular ($P = 0·002$) membranes.

would result in only a slight decrease in the number of cells per cm^3. These findings indicate that, in this case, surface densities may be used to describe changes within cells in addition to changes within a cubic centimetre of cells. The suitability of the outer mitochondrial membrane as a reference for the ratio method is confirmed by the density data in Figure 8, as the surface densities of the reference membranes are essentially the same for both control and experimental points.

Given the arguments outlined above, surface densities were used to describe membrane changes occurring in cells. Figure 9 illustrates that three experimental membrane compartments showed notable changes: the free surface increased by 3·6 m^2/cm^3, while at the same time the Golgi–tubulovesicular membranes were decreased by (5·1 m^2/cm^3), and the total membranes by (2·7 m^2/cm^3). These membrane changes are interpreted as the principal morphological correlates of the most pronounced of the secretory responses recorded in this study. Estimates for membrane compartments in the animals studied are given in Table 1.

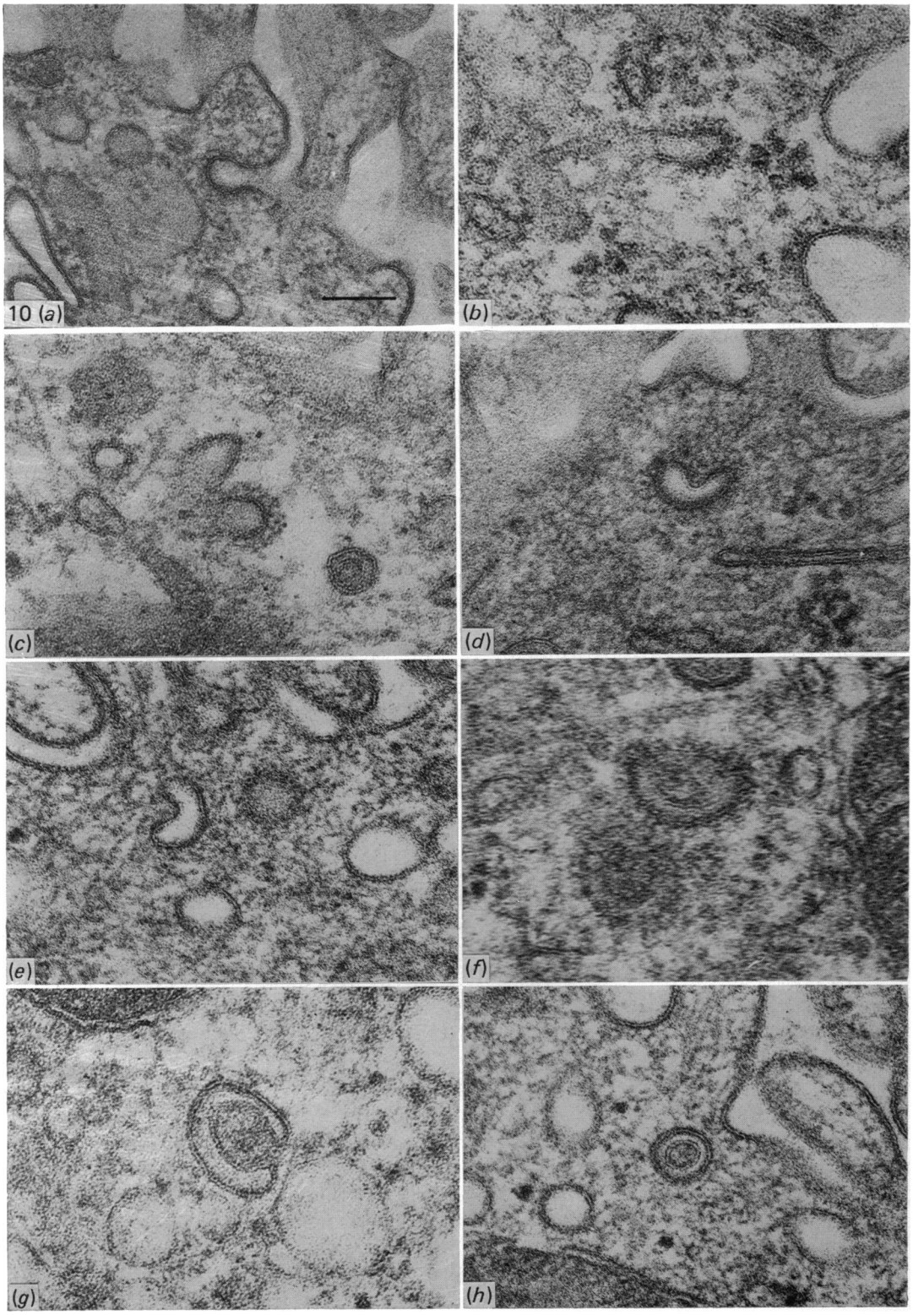
Reconstitution of tubulovesicular elements after the peak of a secretory response

Animals selected for study all showed a peak secretion of at least 0·5 μequiv./g body wt/15 minutes, followed usually by a rapid and progressive fall in acid release.

Vesicular elements aggregated in focal cytoplasmic areas of parietal cells were seen 15 minutes after the onset of a fall in acid secretion. With more prolonged post-peak survival periods there was a progressive accumulation of cytoplasmic tubulovesicular elements, but reconstitution of the tubulovesicular compartment to an extent matching that found in perfused control animals was not seen at any stage studied. There also appeared to be a progressive reduction in the size of microvilli, and in the area of cell profiles occupied by secretory canaliculi. No evidence of direct membrane continuity between the surface membrane and the expanding tubulovesicular compartment of the cells was seen.

Animals fixed 15 minutes after the peak of a secretory response showed a fall in acid secretion to levels ranging between 13 and 50 % of the peak levels. Parietal cells in these mice all showed a similar variety of vesicular elements aggregated in focal cytoplasmic areas adjacent to the canalicular membrane or the lateral and basal membrane. Apart from these aggregates the cells were indistinguishable from those in animals fixed at the time of peak secretion. Numerous coated vesicles were seen (Fig. 10*a–d*). Other elements seen in large numbers included uncoated vesicles similar to the coated vesicles in size, position and shape, and small flattened vesicles. Coated and uncoated vesicles, ranging in size, and showing varying degrees of indentation and partially enveloping small areas of relatively dense cytoplasm were also present (Fig. 10*e–g*). The most ubiquitous vesicular elements were concentric profiles. Mostly commonly the outer membrane profile was 80–100 nm in diameter and the inner 40–50 nm in diameter (Fig. 10*h*); the inner profile usually enclosed material greater in density than that of the neighbouring cytoplasm. Smaller and larger concentric membranes were also seen, and the diameter of these structures ranged from that of smaller coated vesicles to that of the smaller vesicles found in the tubulovesicular compartment of control animals.

Animals fixed $\frac{3}{4}$–3 hours after the peak of a secretory response showed levels of acid secretion ranging from 63 % of the peak response to levels recorded prior to stimulation. Reconstitution of the tubulovesicular compartment was advanced in some cells (Fig. 11), but most cells contained extensive areas of cytoplasm devoid of tubulovesicular elements. However, focal areas of cytoplasm containing vesicular


10 (a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)

elements ranging from coated vesicles to concentric membrane profiles were present throughout all parietal cells, and frequently these areas also contained Golgi elements and small multivesicular bodies (Figs. 12, 13).

During reconstitution of the tubulovesicular compartment the appearance of tubular elements followed that of the vesicular components. The possibility that coalescence of adjacent vesicles is one mechanism of tubule formation was suggested by the presence of adjacent vesicles showing membrane apposition and tubular profiles showing a central constriction (Fig. 12). There was no evidence to suggest a transformation of flattened vesicles into tubules. The flattened vesicles often formed quite elongated profiles (Fig. 10*d*) sometimes aggregated as cisternal stacks.

DISCUSSION

Changes in parietal cell compartments associated with high rates of acid secretion consisted essentially of a depletion of the tubulovesicular compartment accompanied by an increment in the microvillous (free surface) compartment. Measurement of membrane surface areas in these compartments using the surface ratio method showed that maximal hydrogen ion release was associated with at least a fourfold increase in free surface membrane and a reduction in tubulovesicular membrane to less than 10 % of that found in parietal cells of perfused control animals. These results support the view that tubulovesicular membrane or constituents of tubulovesicular membrane assume a position at the free surface of the cells following stimulation. A reduction in Golgi–tubulovesicular membrane from 6·6 m^2/cm^3 in control animals to 0·5 m^2/cm^3 in maximally stimulated mice, and an increase in free surface membrane from 1·2 m^2/cm^3 to 4·8 m^2/cm^3 occurred within 1 hour after the onset of a secretory response. The average maximal rate of increment of free surface membrane in parietal cells of maximally stimulated mice thus appears to be in the region of 0·06 m^2/cm^3/minute.

In estimating membrane changes in parietal cells consideration was given to the artefacts associated with stereological analysis of sectioned material. Section thickness examined using the fold method (Small, 1968) was found to be about 45 nm. Since the movement of membranes did not involve a major change in configuration, correction for section thickness was not applied. Section compression determined from measurement of the axes of chief cell zymogen granules indicated an over-estimate for surface densities of about 8 % in both control and experimental animals (Bolender, Paumgartner, Losa, Muellener & Weibel, 1978).

It may be assumed that in control mice as compared with maximally stimulated animals, the free surface of parietal cells is virtually inactive in respect of hydrogen ion release. Acid secretion by parietal cells appears to involve the formation of a

Fig. 10. Vesicular elements present in large numbers in parietal cells during the post-peak stage of an induced secretory response. Similar structures are also present in appreciably smaller numbers in both control and maximally stimulated mice. Indentations of canalicular membrane (*a*), spherical and other forms of coated vesicles (*b*, *c*, *e*), and indented or crescentric vesicles of varying size, are represented (*d–g*). In Fig. 10(*g*) the cytoplasm partially enveloped by the inner vesicular membrane shows greater density than the adjacent cytoplasm. Structures with this appearance are usually smaller in diameter than in the vesicle illustrated here. Fig. 10(*h*) shows a concentric membrane profile in which the inner and outer membranes are apparently discontinuous. Similar structures are illustrated in Figs. 13, 14. All Figures are printed at ×112000. Bar, 100 nm.

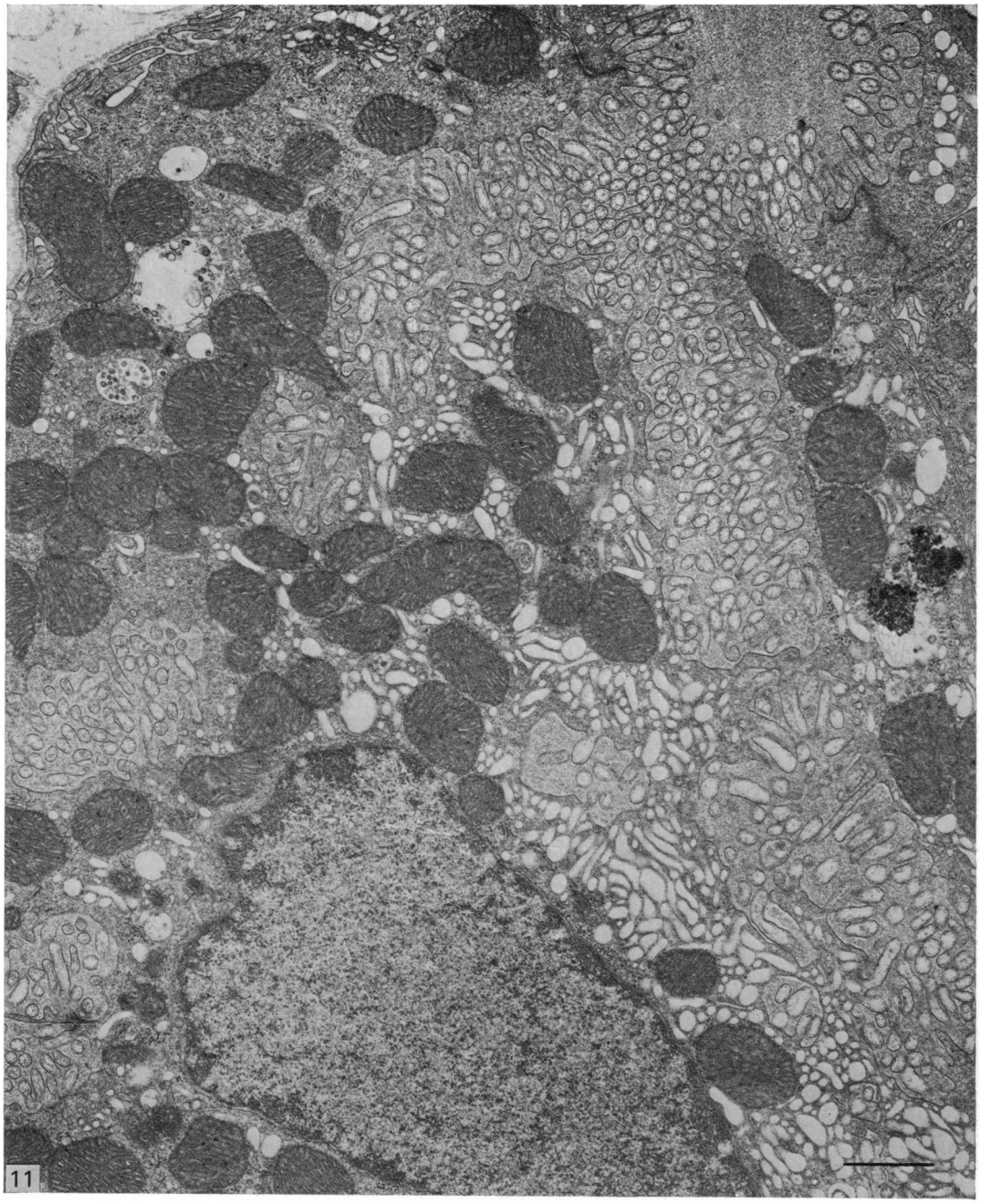
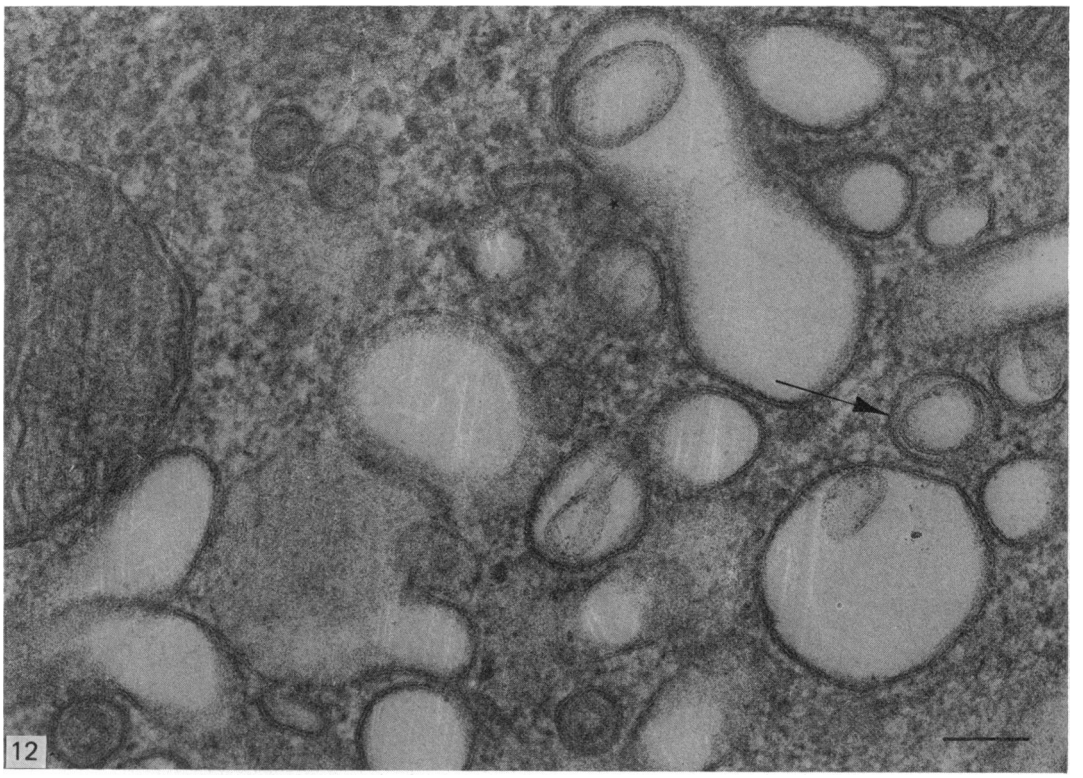


Fig. 11. A parietal cell from a stimulated mouse in which acid secretion had fallen to 44% of the recorded peak level (0·56 μequiv./g body wt/15 minutes) over a period of 45 minutes. Induced acid secretion above control levels was maintained for 2¾ hours prior to the peak. Duration of perfusion was 5 hours. In this cell the tubulovesicular compartment is prominent. Secretory canaliculi are open to the gland lumen, and both lumina contain material similar to that of the chief cell granular profile shown in Fig. 4. Microvilli are still numerous and enlarged. ×13000. Bar, 1 μm.

composite free surface membrane consistent with mingling of constituents derived from the tubulovesicular compartment and constituents which are intrinsic to the free surface. Evidence of continuity between tubulovesicular and free surface membrane was not apparent in any preparation, but a previous study suggested that some at least of the membrane is brought into proximity with the free surface by migration of cytoplasmic tubules into the core of elongating microvilli (Ito & Schofield, 1974). Profiles of parietal cells containing microvilli resembling that illustrated in Figure 6 are not uncommon during the early stages of a secretory response. However, they are not seen during high levels of acid secretion when the microvillous cores are characteristically electron-lucent, nor are they seen during a fall in acid secretion following withdrawal of secretagogue in animals which have shown a maximal secretory response, although they are present after a submaximal response. One interpretation is that early during an induced secretory response cytoplasmic tubules invaginate the microvilli and that, in some amicroscopic way, their membrane constituents are incorporated into and expand the adjacent surface membrane. It is possible, also, that the continuous release of minimal amounts of acid in control animals is associated with spontaneous migration of cytoplasmic tubules, perhaps in restricted areas as represented in Figure 6.

Reconstitution of the tubulovesicular compartment is due presumably to withdrawal of membrane constituents from the free surface and their reassembly to form the cytoplasmic tubules and vesicles characteristic of parietal cells. Vesicular elements present in parietal cells during the post-peak stage of secretory activity provide a possible way of transporting membrane from the free surface to the expanding tubulovesicular compartment. These include flattened vesicles and concentric membrane profiles of varying size which, because of the striking increase in their numbers, are clearly associated with the period during which reconstitution of the tubulovesicular compartment occurs. Coated vesicles also appeared to be most numerous during a fall in acid secretion. They were found in association with canalicular and other surface membranes and were also present as expanded and indented forms in the subjacent cytoplasm. Concentric membrane profiles of parietal cells possibly correspond to the double walled vacuoles found in kidney tubule cells involved in a continuous turnover of membrane glycoprotein (Haddad, Bennett & Leblond, 1977). One explanation of the way in which they are formed may be found in the type of vesicular structure illustrated in Figure 10(*g*). This complex vesicle consists of an inner membrane invaginated by cytoplasm and continuous about the isthmus of the invagination with an outer membrane enclosing an incompletely spherical space. It is possible to regard this structure as an intermediate between a coated or uncoated vesicle in its indented or crescentic form, and a concentric membrane profile, if it is assumed that in most concentric membrane profiles continuity between the inner and outer membranes is not included in the plane of section.

There was little difficulty in identifying a range of vesicular elements intermediate in form between the most common variety of concentric membrane profile, with an outer diameter of 80–100 nm, and vesicles of the tubulovesicular compartment containing microvesicles. The presence of microvesicles within both the tubules and vesicles of this compartment has long been difficult to account for and it seems reasonable to reach a provisional conclusion that the microvesicles of the tubulovesicular compartment are derived from the inner membrane of the concentric membrane profiles. The involvement of coated vesicles and concentric membrane



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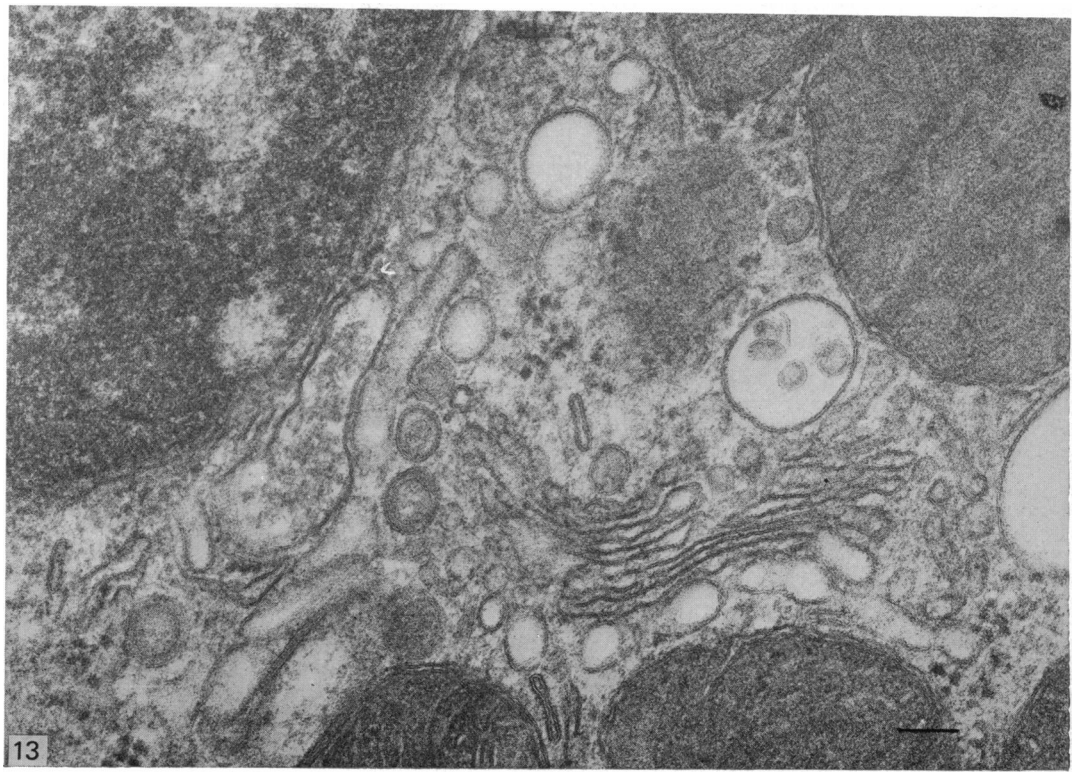

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profiles in the transport of membrane from the free surface of parietal cells to the tubulovesicular compartment would account for the absence in the mouse of evidence of internalization of extensive areas of canalicular membrane such as Helander & Hirschowitz (1972) have suggested occurs in the dog during the reconstitution of this compartment.

There is a marked difference between surface density estimates for membrane compartments in parietal cells as reported by Helander & Hirschowitz (1972) for the dog and those reported here. Following stimulation a tenfold increase in free surface was recorded for the dog (compared with a fourfold increase in the mouse). It is probable that this lack of accord represents differences in sampling methods employed, or differences in the physiological status of the respective groups of animals studied, rather than a species difference. Nevertheless, both studies showed a fall in total surface density estimates in stimulated animals (see Fig. 9 for the mouse) attributable either to enlargement of the cells following secretagogue treatment, or loss of membrane during the secretory response.

Current views of the mechanism of acid release envisage the asymmetric liberation of hydrogen and hydroxyl or bicarbonate ions (Forte & Solberg, 1973), functions which are likely to be attributable to different membrane components in the cells. The observation that the surface area of lateral and basal membranes in animals showing maximal secretion is 50 % greater than that in control animals would seem to be an important one, for an increment at this site may indicate a role for these membranes which is no less significant in terms of ionic exchange than that for membrane at the free surface. Cytoplasmic areas containing vesicles ranging from coated varieties to concentric membrane profiles were certainly found in association with the lateral and basal membranes. These membranes also need to be considered as a source for reconstitution of the tubulovesicular compartment, and possibly contribute membrane constituents with a specific ion exchange role.

The total volume of the parietal cell mass, and the number of cells it contains has not yet been estimated for the mouse. However, if the surface density estimate for free surface of parietal cells in control animals (1·26 m^2/cm^3) is taken as an arbitrary unit of membrane area, an approximation to the relationship between area of free surface membrane and release of hydrogen ion can be made for maximally stimulated animals. Such a relationship does not assume that free surface membranes in parietal cells from control and experimental animals are identical in composition, but indicates that for each 1 m^2 increment of free surface/cm^3 gained during maximal stimulation an additional $1{\cdot}084 \times 10^{19}$ H^+/g body wt/15 minutes is released. This value corresponds to a secretory capacity of approximately $1{\cdot}8 \times 10^7$ $H^+/\mu m^2$ free surface/minute in a mouse secreting acid at a maximal rate.

Fig. 12. Vesicular elements in a parietal cell from a mouse showing a fall in acid secretion to 63 % of the recorded peak value (0·75 μequiv./g body wt/15 minutes). Microvesicles, some of which appear to be pedunculated, can be seen in vesicles of the tubulovesicular compartment. Several concentric membrane profiles are shown, and one (arrow) is similar in diameter to vesicles containing pedunculated microvesicles. × 110000. Bar, 100 nm.

Fig. 13. Concentric membrane profiles and flattened vesicles associated with Golgi apparatus and a small multivesicular body. From a mouse showing a fall in acid secretion to 21 % of the peak value over a period of 2¼ hours. × 84000. Bar, 100 nm.

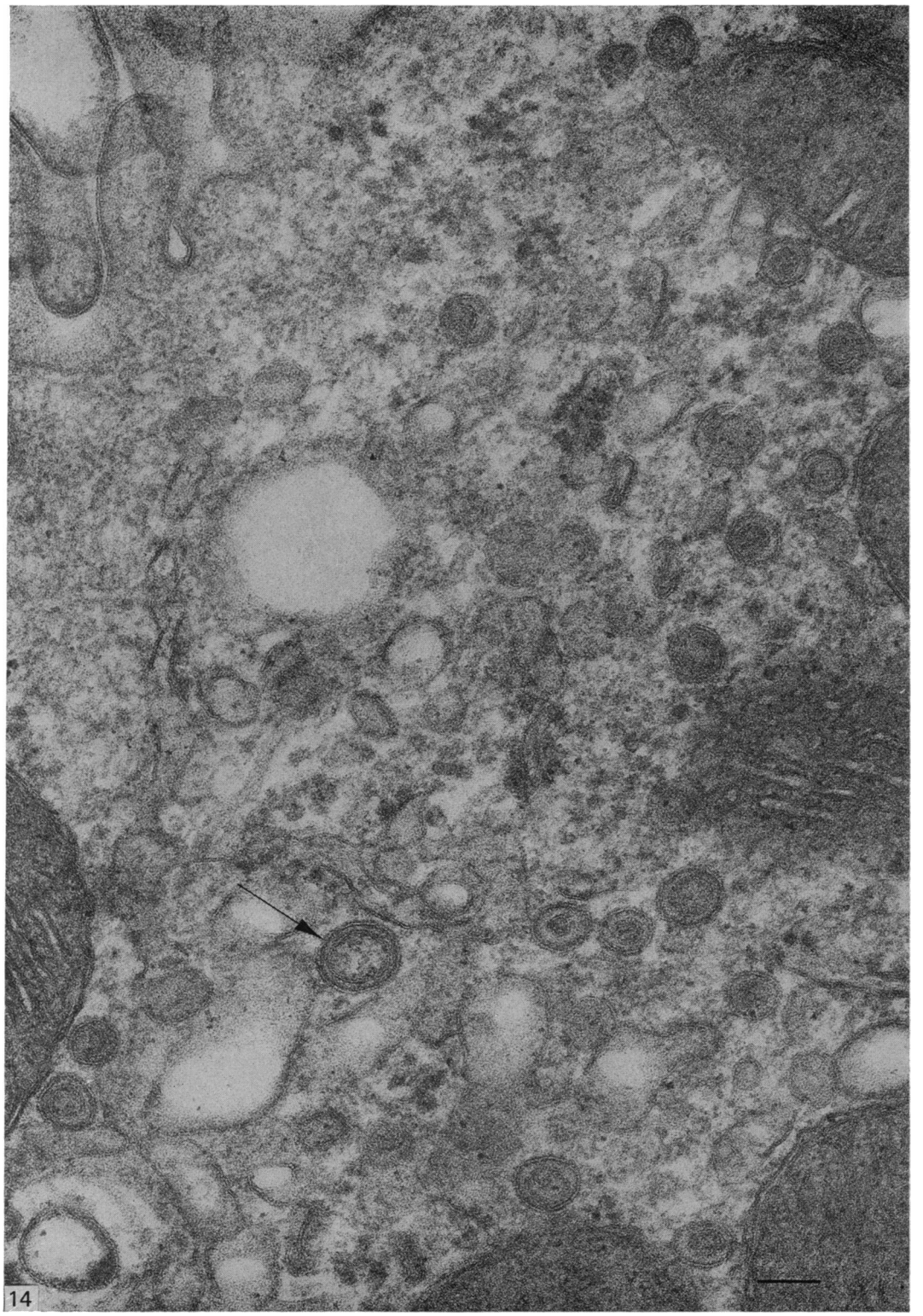

Fig. 14. A parietal cell from a mouse showing a fall in acid secretion to 14% of the recorded peak level (0·5 μequiv./g body wt/15 minutes) over a period of 2½ hours. Numerous concentric membrane profiles can be seen. In the largest profile (arrow) the cytoplasm enclosed by the inner membrane is less homogeneous than in other profiles shown. This profile is similar in diameter to the smaller of the vesicles of the tubulovesicular compartment. × 100000. Bar, 100 nm.

SUMMARY

Levels of gastric acid secretion which may be maximal for the mouse were recorded following treatment with histamine and carbachol. A 30-fold increase over control levels was obtained in perfused animals, corresponding to a fourfold increase over highest levels recorded previously for stimulated mice. Stereological methods were used to estimate surface areas of membrane compartments of parietal cells in control and stimulated animals. Estimates of relative changes in membrane surface areas using a surface ratio method in this case substantiated changes detected by calculating surface densities. Main changes in membrane compartments of parietal cells from animals showing maximal acid secretion were a fourfold increase in free (luminal) surface, a 50 % increase approximately in lateral and basal membrane, and a 90 % reduction approximately in the tubulovesicular membrane compartment.

Following withdrawal of secretagogues, acid secretion usually returned to control levels within 3 hours, but complete reconstitution of the tubulovesicular compartment was not seen within any survival period up to 5 hours. Reappearance of tubulovesicular elements first occurred shortly after the peak of a secretory response in focal cytoplasmic areas containing spherical and indented coated vesicles, and also numerous concentric membrane profiles not previously described in parietal cells. The way in which movement of membrane from the tubulovesicular compartment to the free surface occurs is not yet clear. However, reconstitution of the tubulovesicular compartment during a fall in acid secretion appears to involve movement of membrane from the free surface through coated vesicles, and their progression through indented forms and concentric membrane profiles to vesicles of the tubulovesicular compartment.

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