PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Initiation of Salt and Water Transport in Mammalian Salivary Glands by Acetylcholine

O. H. Petersen

Phil. Trans. R. Soc. Lond. B 1971 **262**, 307-314
doi: 10.1098/rstb.1971.0096

Phil. Trans. Roy. Soc. Lond. B. **262**, 307–314 (1971) [307]
Printed in Great Britain

Initiation of salt and water transport in mammalian salivary glands by acetylcholine

By O. H. Petersen
Institute of Medical Physiology C, University of Copenhagen, Denmark

The major fluid production in the salivary glands occurs in the acinar cells. In many glands (e.g. the cat submandibular gland used in the present work) there is no secretion during resting conditions. Intra-arterial infusion of acetylcholine (ACh) to the gland evokes a vigorous secretion. The immediate action of ACh on the contraluminal acinar cell membrane is to increase the membrane potential. This hyperpolarization is due to a passive outward potassium (K) transport being only partly short-circuited by an inward sodium (Na) transport. Thus the intracellular Na concentration is enhanced by ACh stimulation. Secretion is probably initiated as a consequence of this increased Na concentration either directly activating a sodium chloride (NaCl) pump at the luminal membrane or indirectly activating the secretory mechanism by increasing the calcium influx. In addition to the NaCl pump, which can be inhibited by ethacrynic acid, there is a pump extruding Na and accumulating K, which can be inhibited by g-strophanthin.

Introduction

Most salivary glands only secrete significant volumes of fluid after stimulation of the secretory nerves. The major control of salivary secretion is exerted by the parasympathetic nervous system. The transmitter released from the post-ganglionic parasympathetic nerve endings is acetylcholine (ACh) (Emmelin 1967). The major site of the outward fluid transport in the salivary glands (i.e. from interstitial fluid to lumen) is the acinus (Burgen 1967). In the salivary glands producing hypotonic saliva the elaboration of the final secretory product is due to two separate processes. In the acinar cells a primary secretion with a composition, with respect to the concentrations of Na, K, Cl and H_2O, close to plasma is formed. In the duct system and particularly in the striated ducts a reabsorption of NaCl in excess of water occurs (Thaysen 1960; Young, Frömter, Schögel & Hamann 1967; Martinez 1969). It is generally assumed that the salivary secretory rate is equal to the fluid formation rate in the acini, as the water permeability of the duct system probably is very low (Young *et al.* 1967). In the present work the relation between the effects of different experimental procedures on the salivary secretory rate, K transport and acinar membrane potential has been investigated in an attempt at elucidating what happens in the acinus after stimulation with ACh.

Potassium transport

Burgen (1956) showed that when salivary glands were stimulated to secrete after a longer resting period, the gland cells lost K to both the saliva and the blood perfusing the gland. Burgen concluded that the mechanism of the K release over both faces of the cells after stimulation probably was the same. Burgen described the stimulation-induced K release in three salivary glands: the dog submandibular and parotid gland and the cat submandibular gland. Previously Thaysen, Thorn & Schwartz (1954) had shown, in the human parotid gland, that the K concentration in the first salivary samples taken after start of stimulation was greater than in salivary samples collected during steady state conditions. The same finding was made in the human lacrimal gland (Thaysen & Thorn 1954). Öbrink & Waller (1966) showed that

the K concentration in the gastric juice was transiently increased when the secretory rate was enhanced by stimulating more intensely with histamine. In the cat pancreas Case, Harper & Scratcherd (1969) showed that intra-arterial injection of secretin induced a release of K from the pancreas to the blood perfusing the gland. Burgen's original investigation on the K release phenomenon only showed that there was a net release of K from the gland cells after stimulation, but it was not known whether this was due to an enhanced K efflux or to a reduced influx. Schneyer (1967), however, demonstrated that the loss of ^{42}K from rat submandibular gland slices was greatly enhanced after addition of ACh to the bathing medium.

In the present work the K transport in the perfused cat submandibular gland was investigated by measuring the K concentration in the perfusion fluid leaving the gland vein during different experimental conditions. During perfusion with an ordinary Locke's solution, a close intra-arterial injection of ACh was followed by a short-lasting increase in the venous K concentration (K release) followed by a period in which the K concentration fell below the arterial level (K accumulation). About 10 min after an injection of 10 μg ACh the K concentration in the perfusion fluid leaving the gland was again equal to the arterial K concentration. An intra-arterial injection of adrenaline gave a similar response (Petersen 1970*a*). Petersen (1970*a*, 1971*b*) discussed from which cellular elements the K release originated and concluded that probably the acinar cells contributed most. During resting conditions the acinar membrane potential in the perfused cat submandibular gland was about -20 mV. The intracellular K concentration was probably about 115 mmol/l (Burgen & Emmelin 1961). As the perfusions of the cat submandibular gland were carried out at about 20 °C the K equilibrium potential (E_K) across the basal acinar cell membrane was about -80 mV. Thus it is seen that during resting conditions a large electrochemical gradient favouring K loss exists. The K uptake occurring after the stimulation-induced K release must therefore be due to an active transport mechanism. It is not very surprising, therefore, that 2,4-dinitrophenol (10^{-4} mol/l) nearly abolished K uptake in the perfused gland (Petersen 1970*a*, *c*). The cardiac glycoside g-strophanthin which is thought to be a specific inhibitor of the Na–K activated ATPase (Glynn 1957) also abolished K uptake in a concentration of 10^{-5} mol/l (Petersen 1970*a*, 1971*b*). An Na–K activated ATPase has been found and studied in the salivary glands (Schwartz, Laseter & Krainz 1963, Schwartz & Moore 1968). It is therefore very likely that it is the Na–K activated ATPase that is responsible for K accumulation in the salivary glands as is the case in most cells (Skou 1965). As discussed below it is likely that stimulation-induced K release is coupled with Na uptake. The K accumulation occurring after a stimulation-induced K release is therefore probably induced by the increased intracellular Na concentration stimulating the internal site of the Na–K activated ATPase (Skou 1965). The K accumulation is not activated by ACh stimulation. During perfusion with a Na-free lithium (Li) solution no K uptake occurred; immediately after reintroduction of control (Na) solution K uptake started, although more than 5 min had passed since the last injection of ACh was given (Petersen 1970*a*). The presence of calcium and bicarbonate in the perfusion fluid was not needed for K accumulation (Petersen 1971*b*). Replacement of external Na by Li or tetraethylammonium (TEA) abolished K uptake, and replacement of external Cl by NO_3 or SO_4 also abolished K uptake. These inhibitions of the K uptake were readily reversible after returning to control (NaCl) solution (Petersen 1970*a*; Petersen & Poulsen 1969). Ethacrynic acid in a concentration effective in inhibiting salivary secretion had only little influence on K uptake. Acetazolamide (10^{-3} mol/l) did not affect K uptake (Petersen 1971*b*).

While the K uptake undoubtedly is due to an active transport mechanism, the same cannot be postulated for the K release. As stated above there is a large electrochemical gradient favouring K release through the contraluminal acinar cell membrane during resting conditions. After an injection of ACh (1 μg) the acinar membrane potential was about -45 mV during perfusion with an ordinary Locke's solution (Petersen 1970*b*). Even under these conditions there was a considerable electro-chemical gradient favouring K release. There is some evidence indicating that the stimulation-induced K release is actually due to passive transport processes. During perfusion with a dinitrophenol (10^{-4} mol/l) containing solution, at a time when both salivary secretion and K uptake were severely inhibited, K release was not affected (Petersen 1970*a*). Replacement of external Na by Li did not primarily inhibit stimulation-induced K release whereas this was the case when Na was replaced by TEA (Petersen 1970*a*). The inhibition of K release by TEA perfusion could be explained by an inhibition of K permeability increase as has been described in many other cells (Ito, Kuriyama & Sakamoto 1970), however, this is not the case in the acinar cells as will be shown in the section on the mechanism of the secretory transmembrane potential. The most ready explanation for this inhibition is that K release is normally followed by Na uptake but that the TEA ion is unable to permeate through the acinar cell membrane easily. This will lead to an increase in membrane potential with a subsequent reduction in the driving force for the K release. The Na uptake probably occurring concomitantly with the K release takes place along a steep electrochemical gradient, as the E_{Na} across the contraluminal acinar cell membrane is about $+74$ mV (Burgen 1967). K release can be evoked during conditions under which salivary secretion has been abolished or severely inhibited whereas no experimental procedures inhibiting K release without affecting the secretory process are known (Petersen 1970*a*, 1971*b*).

Secretory potentials

Lundberg (1955) first described membrane potential measurements in salivary glands. In the cat submandibular gland three different microelectrode responses were found. In the present work only the type I response originating from the acinar cells (Imai 1965; Henriques & Sperling 1966; Fritz & Botelho 1969*a*) will be considered. During resting conditions the potential difference across the basal cell membrane is about -20 mV. Electrical stimulation of the parasympathetic or sympathetic nerves, or intra-arterial injection of ACh, adrenaline or pilocarpine results in an increase in membrane potential to about -50 mV. The increase in membrane potential after stimulation is called the secretory potential. Lundberg (1958) described secretory potentials in both the cat submandibular and sublingual gland. Secretory potentials have been studied in the cat parotid gland (Fritz & Botelho 1969*b*), the dog submandibular gland (Yoshimura & Imai 1967) and the rat submandibular gland (Schneyer 1969). Secretory potentials have also been described in the lacrimal gland (Hisado & Botelho 1968), the exocrine pancreas (Dean & Matthews 1968) and the gastric mucosa (Sachs 1971). In the salivary glands the secretory potentials have the following characteristics: (1) a long latency—about 300 ms (this has been discussed in detail by Creed & Wilson (1969)); (2) the rate of hyperpolarization is relatively low—about 150 to 200 mV/s; (3) the amplitude of the secretory potential depends on the voltage of the electrical stimulus exciting the secretory nerve and the frequency (graded response), and (4) the membrane potential only returns to the resting

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

level after stimulation has been stopped. The mechanism of the secretory potential was investigated in the perfused cat submandibular gland. The effect of ionic omissions or replacements on the size of the secretory potentials was tested. Lundberg (1957*a*, *b*) postulated that the secretory potential in the cat sublingual gland, which is very similar to that described in the submandibular gland, was due to an active transport of chloride ions through the contraluminal cell membrane from the interstitial fluid to the cytoplasm. During perfusion with a chloride and bicarbonate free isotonic sucrose solution normal secretory potentials could be

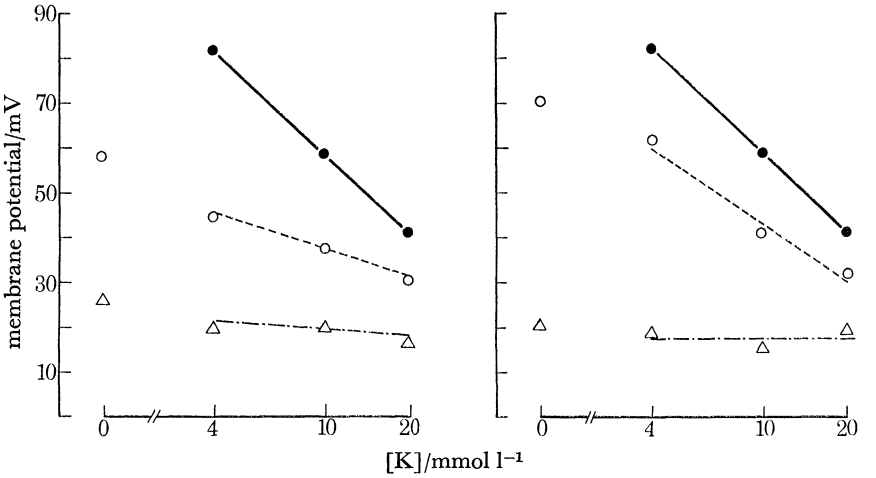

FIGURE 1. FIGURE 2.

FIGURE 1. Acinar membrane potential plotted as a function of the perfusion fluid K concentration. △–·–△, measured during resting conditions, ○–––○, measured at the time where the maximal size of the secretory potential had been reached after intra-arterial injection of ACh (1 μg). For comparison is inserted, ●—●, the calculated values for the K equilibrium potential (E_K) across the acinar cell membrane. The values for the measured membrane potentials were obtained from Petersen (1970*b*). The perfusion solution employed in these experiments was an ordinary Locke's solution. When the K concentration was altered corresponding alterations in the Na concentration were undertaken to ensure constant osmolarity.

FIGURE 2. Acinar membrane potential plotted as a function of the perfusion fluid K concentration. The symbols have the same meaning as in figure 1. The values for the measured membrane potentials (Petersen 1970*b*) were obtained in experiments in which the perfusion fluid used was a Locke's solution with all NaCl replaced by TEACl. When the K concentration was altered corresponding alterations in the TEA concentration ensured constant osmolarity.

evoked after injection of ACh. No decline in the size of the secretory potentials was observed even after 20 min of perfusion with the sucrose solution (Petersen 1971*a*). This finding is in agreement with previous results (Petersen & Poulsen 1969) showing that normal secretory potentials could be elicited during perfusion with a sulphate solution not containing chloride and bicarbonate. These results show that the secretory potential cannot be generated by an inward chloride transport. An attempt was made to decide whether the secretory potential was generated by some electrogenic pump mechanism or by a change in membrane permeability for one or more ions. 15 min after introduction of a DNP (10^{-4} mol/l) containing perfusion solution salivary secretion and active K uptake were abolished. Nevertheless, secretory potentials of normal size could be evoked after intra-arterial injection of ACh (Petersen 1970*c*). Therefore it is probable that the secretory potential is due to passive ion transport as a consequence of permeability changes induced by ACh. Figure 1 shows the dependence of the resting membrane potential and the membrane potential during stimulation with ACh on the

perfusion fluid K concentration ($[K]_0$). It is seen that the relationship even during ACh stimulation was far from that expected for a K selective electrode, although the stimulated membrane potential is sensitive to variations in $[K]_0$. Figure 2 shows the same relationship, but this time after replacement of all NaCl in the perfusion solution by TEACl. It is seen that the behaviour of the stimulated membrane now was closer to that expected for a K selective electrode. It is also seen that the stimulated membrane potential at a $[K]_0$ of 4 mmol/l was greater during perfusion with TEA solution than during perfusion with control solution. These results can most easily be interpreted by assuming that the action of ACh on the basal acinar cell membrane is to increase the permeability to both K and Na with resulting K efflux and Na influx. The K efflux must dominate since the change in membrane potential is a hyperpolarization. In the rat submandibular gland (Schneyer 1969) the change in membrane potential after stimulation may be either a small hyperpolarization or a small depolarization, it is thus possible that in the rat, sodium and potassium currents evoked by stimulation are of about equal size. Secretory potentials could be elicited under many different conditions where salivary secretion was abolished (during perfusion with: sucrose, Li, TEA, SO_4, Ca-free and DNP solution), but no experimental conditions are known to inhibit the secretory potentials without inhibiting salivary secretion (Petersen 1970*b*, *c*, 1971*a*; Petersen & Poulson 1969).

Isotonic water transport

In the rat submandibular and parotid glands micropuncture studies have shown that the osmolarity and the concentration of Na, K and Cl of fluid collected from the intercalated ducts are not very different from the plasma levels (Martinez, Holzgreve & Frick 1966; Young & Schögel 1966; Mangos, Braun & Hamann 1966). The composition of the intercalated duct fluid was the same during resting and stimulated conditions (Young & Schögel 1966; Mangos *et al.* 1966). This indicates that in the rat salivary glands the acinar cells perform an isotonic water transport. The relationship between Na and K concentration in the cat submandibular saliva and the secretory rate is most easily accounted for by assuming that the same is true of the acinar cells in this gland (Petersen & Poulsen 1967).

The most thoroughly investigated tissues carrying out an isotonic water transport are the gall-bladder and the proximal convolution of the kidney. It seems to be characteristic for these tissues that they possess long intercellular channels closed at one end (Tormey & Diamond 1967), that the transepithelial resistance is low, about 5 Ω cm^2 in the mammalian proximal kidney convolution and 30 Ω cm^2 in the rabbit gall-bladder (Hegel, Frömter & Wick 1967; Diamond 1971) and that there is only a very small transepithelial potential difference (with the exception of the necturus proximal tubule having a transcellular p.d. of 20 mV) (Frömter & Hegel 1966; Diamond 1962; Machen & Diamond 1969; Giebisch 1961). The acinar cells of salivary glands possess intercellular channels (Rutberg 1961; Yoshimura, Inoue, Imai & Yoshimura 1962; Leeson 1967). Yoshimura *et al.* (1962) have demonstrated that the intercellular spaces of the dog submandibular gland, communicating with the acinar lumen, are distended after stimulation with pilocarpine but are nearly collapsed during resting conditions. In the cat sublingual gland Lundberg (1957*a*) found that the resistance of the tubule wall (the tubular cells in the sublingual gland correspond to the acinar cells of the submandibular gland) was 16 Ω cm^2 during resting conditions and 11 Ω cm^2 during stimulation. In the cat sublingual gland the p.d. across the tubule wall during secretion is about 20 to 30 mV, the lumen being negative

with respect to the interstitium. This p.d. however, is a consequence of the secretory potential at the basal membrane. As may be understood from the section on the secretory potential, this potential change after stimulation is not due to the isotonic water transport. It may thus be postulated that isotonic water transport in itself only generates a very small transepithelial p.d.

Salivary secretion at maximal rate required the presence in the perfusion fluid of both Na and Cl. In contrast to both the gall-bladder and the proximal kidney tubule the presence of CO_2/HCO_3 in the perfusion fluid was not needed for maximal secretory ability (Diamond 1964; Rumrich & Ullrich 1968; Petersen 1971*b*). Carbonic anhydrase inhibition, again in contrast to what had been found in the gall-bladder (Wheeler, Ross & King 1969), did not influence the secretory rate (Petersen 1971*b*). Replacement of all Cl in the perfusion fluid by SO_4 abolished secretion. Replacement of all Cl by NO_3 severely reduced the secretory rate. When 50 % of the perfusion fluid Cl was replaced by either SO_4 or NO_3 the secretory rate was hardly affected (Petersen & Poulsen 1969, Petersen 1970*a*). The dependence of the secretory rate on the perfusion fluid Na concentration was much more marked than the dependence on the Cl concentration. Thus the secretory rate was halved after replacement of 50 % of the perfusion fluid NaCl by an equiosmolar amount of sucrose or after replacement of 50 % of the perfusion fluid Na by Li (Petersen 1970*d*; Petersen & Poulsen 1969). K in the perfusion fluid was not needed for the secretory process (Petersen 1970*d*) and g-strophanthin (10^{-5} mol/l) in a concentration sufficient to abolish K uptake into the gland did not primarily affect the secretory rate. On the other hand ethacrynic acid (10^{-4} mol/l) reduced the secretory ability of the gland without seriously affecting K uptake (Petersen 1971*b*). These results are in agreement with the findings of Whittembury & Fishman (1969) and Whittembury & Proverbio (1970) that in the proximal kidney tubule there exists two modes of Na extrusion, one coupled to K uptake which can be inhibited by g-strophanthin, and one coupled to Cl extrusion being blocked by ethacrynic acid. It is thus probable that the primary secretion in the acinar cells is being formed by a NaCl transport into the acinar lumen or into the intercellular spaces. Whether it is an electrogenic Na pump electrically coupled to Cl as is probably the case in the necturus proximal tubule (Whittembury 1971) or an electroneutral NaCl pump as it has been proposed in the gall-bladder (Diamond 1962) cannot yet be answered.

Relation between the ACh evoked membrane currents and secretion

The two first sections of this paper have been used to describe that ACh when injected into the artery of the gland acts on the basal acinar cell membrane by increasing the permeability to both K and Na with a subsequent K efflux and Na influx. It would be important to know whether these currents are of importance for the initiation of the secretory mechanism. There is some fragmentary evidence in favour of such a hypothesis. It has been stated above that no experimental conditions are known in which the secretory potentials are abolished and secretion proceeds. The idea that the inward Na current could be important in the initiation of the secretory mechanism was supported by the above mentioned finding that the secretory rate was much more dependent on the extracellular Na concentration than on the Cl concentration. The size of the inward Na current is determined by the concentration difference for Na across the basal membrane and by the membrane potential. To test whether variations in membrane potential, influencing the size of the inward Na current, would affect the secretory rate,

perfusions with different K concentrations in the extracellular fluid were carried out. Augmentation of the perfusion fluid K concentration, reducing the membrane potential during ACh stimulation (figure 1) and consequently reducing the inward Na flux after stimulation, reduced the salivary secretory rate. Omission of K from the perfusion fluid, increasing the membrane potential during stimulation (figure 1) and consequently enhancing the Na influx after ACh stimulation, augmented salivary secretion (Petersen 1970*d*). It would be relevant to ask how an increase in intracellular Na concentration could initiate the secretory mechanism. One possibility would be that the enzyme system responsible for the NaCl extrusion at the luminal membrane could directly be activated by an increased intracellular Na concentration. However, it is possible that such a hypothesis is too simple. Douglas & Poisner (1963) showed that Ca in the perfusion fluid was needed for the secretory process. Omission of Ca from the perfusion fluid resulted in a gradual decline of the secretory ability which was finally abolished. Augmentation of the perfusion fluid Ca concentration enhanced salivary secretion when this was submaximal, i.e. evoked by a submaximal ACh stimulus. Augmentation of the perfusion fluid Mg concentration slightly inhibited salivary secretion. Extracellular Ca was neither needed for the generation of secretory potentials (Petersen, Poulsen & Thorn 1967) nor for the stimulation-induced K loss (Petersen 1971*b*). It is thus possible that the entry of Ca to the cytoplasm could be of critical importance for the secretory mechanism. Dreisbach (1964) showed that ACh evoked an enhanced ^{45}Ca influx to rat parotid gland slices. Selinger, Naim & Lasser (1970) have shown that the rat submandibular and parotid glands possess powerful ATP dependent Ca accumulating microsomal fractions. This Ca accumulating mechanism could maintain a low Ca concentration in the cytoplasm which could be raised by Ca influx evoked by ACh stimulation. The question then arises how ACh could elicit Ca influx. One possibility would be that ACh in addition to its effects on Na and K permeability also enhanced the Ca permeability of the basal membrane. Another hypothesis can, however, be suggested on the basis of the findings made by Baker, Blaustein, Hodgkin & Steinhardt (1969) and Glitsch, Reuter & Scholz (1970). These authors showed that Ca influx to the squid axon and the guinea-pig atria was dependent on the intracellular Na concentration, in such a way that an enhanced intracellular Na concentration augmented Ca influx. The calculated increase in intracellular Na concentration after stimulating the cat submandibular gland maximally by ACh is such that if it had taken place in the guinea pig atria it would double Ca influx (Petersen 1971*c*). Still it cannot be excluded that the only importance of extracellular Ca is to secure normal permeability conditions in the acinus, but the finding in the proximal convolution of the rat kidney that the isotonic water reabsorption was unaffected during peritubular capillary perfusion with a Ca-free solution in spite of a greatly enhanced transtubular permeability (Ullrich, Baldamus, Uhlich & Rumrich 1969) may indicate that this is not the only role for Ca in the salivary glands.

References (Petersen)

Baker, P. F., Blaustein, M. P., Hodgkin, A. L. & Steinhardt, A. 1969 *J. Physiol., Lond.* **200**, 431–458.
Burgen, A. S. V. 1956 *J. Physiol., Lond.* **132**, 20–39.
Burgen, A. S. V. 1967 *Handbook of physiology* § 6, vol. 2, *Secretion*, pp. 561–579. Washington: American Physiological Society.
Burgen, A. S. V. & Emmelin, N. G. 1961 *Physiology of the salivary glands*, p. 205. London: Arnold.
Case, R. M., Harper, A. A. & Scratcherd, T. 1969 *Exocrine glands*, pp. 39–56. Philadelphia: University of Pennsylvania Press.
Creed, K. E. & Wilson, A. F. 1969 *Austr. J. exp. Biol. med. Sci.* **47**, 135–144.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Dean, P. M. & Matthews, E. K. 1968 *J. Physiol., Lond.* **198**, 90–91*P*.
Diamond, J. M. 1962 *J. Physiol., Lond.* **161**, 474–502.
Diamond, J. M. 1964 *J. gen. Physiol.* **48**, 1–14.
Diamond, J. M. 1971 *Electrophysiology of epithelial cells* Stuttgart-New York: Schattauer Verlag (in the Press).
Douglas, W. W. & Poisner, A. M. 1963 *J. Physiol., Lond.* **165**, 528–541.
Dreisbach, R. H. 1964 *Salivary glands and their secretions*, pp. 237–249. Oxford: Pergamon Press.
Emmelin, N. 1967 *Handbook of physiology* § 6, vol. 2. *Secretion*, pp. 595–632. Washington: American Physiological Society.
Fritz, M. E. & Botelho, S. Y. 1969*a* *Am. J. Physiol.* **216**, 1180–1183.
Fritz, M. E. & Botelho, S. Y. 1969*b* *Am. J. Physiol.* **216**, 1392–1398.
Frömter, E. & Hegel, U. 1966 *Pflügers Arch. ges. Physiol.* **291**, 107–120.
Giebisch, G. 1961 *J. gen. Physiol.* **44**, 659–678.
Glitsch, H. G., Reuter, H. & Scholz, H. 1970 *J. Physiol., Lond.* **209**, 25–43.
Glynn, I. M. 1957 *J. Physiol., Lond.* **136**, 148–173.
Henriques, B. L. & Sperling, A. L. 1966 *J. appl. Physiol.* **21**, 1247–1250.
Hegel, U., Frömter, E. & Wick, T. 1967 *Pflügers Arch. ges. Physiol.* **294**, 274–290.
Hisado, M. & Botelho, S. Y. 1968 *Am. J. Physiol.* **214**, 1262–1267.
Imai, Y. 1965 *J. Physiol. Soc. Jap.* **27**, 304–312. (In Japanese.)
Ito, Y., Kuriyama, H. & Sakamoto, Y. 1970 *J. Physiol., Lond.* **211**, 445–460.
Leeson, C. R. 1967 *Handbook of physiology* § 6, vol. 2. *Secretion*, pp. 463–495. Washington: American Physiological Society.
Lundberg, A. 1955 *Acta physiol. scand.* **35**, 1–25.
Lundberg, A. 1957*a* *Acta physiol. scand.* **40**, 35–58.
Lundberg, A. 1957*b* *Acta physiol. scand.* **40**, 101–112.
Lundberg, A. 1958 *Physiol. Rev.* **38**, 21–39.
Machen, D. W. & Diamond, J. M. 1969 *J. Membr. Biol.* **1**, 194–213.
Mangos, J. A., Braun, G. & Hamann, K. F. 1966 *Pflügers Arch. ges. Physiol.* **291**, 99–106.
Martinez, J. R. 1969 *Exocrine glands*, pp. 20–30. Philadelphia: University of Pennsylvania press.
Martinez, J. R., Holzgreve, H. & Frick, A. 1966 *Pflügers Arch. ges. Physiol.* **290**, 124–133.
Öbrink, K. J. & Waller, M. 1966 *Acta physiol. scand.* **68**, suppl. 277, p. 234.
Petersen, O. H. 1970*a* *J. Physiol., Lond.* **208**, 431–447.
Petersen, O. H. 1970*b* *J. Physiol., Lond.* **210**, 205–215.
Petersen, O. H. 1970*c* *Acta physiol. scand.* **80**, 117–121.
Petersen, O. H. 1970*d* *Experientia* **26**, 1103–1104.
Petersen, O. H. 1971*a* *Pflügers Arch. ges. Physiol.* **323**, 91–95.
Petersen, O. H. 1971*b* *J. Physiol., Lond.* (in the Press).
Petersen, O. H. 1971*c* *Electrophysiology of epithelial cells* Stuttgart-New York: Schattauer Verlag (in the Press).
Petersen, O. H. & Poulsen, J. H. 1967 *Acta physiol. scand.* **70**, 158–167.
Petersen, O. H. & Poulsen, J. H. 1969 *Exocrine glands*, pp. 3–20. Philadelphia: University of Pennsylvania Press.
Petersen, O. H., Poulsen, J. H. & Thorn, N. A. 1967 *Acta physiol. scand.* **71**, 203–210.
Rumrich, G. & Ullrich, K. J. 1968 *J. Physiol., Lond.* **197**, 69–70*P*.
Rutberg, U. 1961 *Acta odont. scand.* **19**, suppl. 13, 7–69.
Sachs, G. 1971 *Electrophysiology of epithelial cells*. Stuttgart-New York: Schattauer Verlag (in the Press).
Schneyer, L. H. 1967 *Secretory mechanisms of salivary glands*, pp. 32–55. New York–London: Academic Press.
Schneyer, L. H. 1969 *Proc. Soc. exp. Biol. Med.* **130**, 192–196.
Selinger, Z., Naim, E. & Lasser, M. 1970 *Biochim. biophys. Acta* **203**, 326–334.
Schwartz, A., Laseter, A. H. & Krainz, E. 1963 *J. cell. comp. physiol.* **62**, 193–205.
Schwartz, A. & Moore, C. A. 1968 *Am. J. Physiol.* **214**, 1163–1167.
Skou, J. C. 1965 *Physiol. Rev.* **45**, 596–619.
Thaysen, J. H. 1960 *Hanb. exp. Pharmak. Erganzungswerk Bd.* **13**, 424–438.
Thaysen, J. H. & Thorn, N. A. 1954 *Am. J. Physiol.* **178**, 160–164.
Thaysen, J. H., Thorn, N. A. & Schwartz, I. L. 1954 *Am. J. Physiol.* **178**, 155–159.
Tormey, J. McD. & Diamond, J. M. 1967 *J. gen. Physiol.* **50**, 2031–2060.
Ullrich, K. J., Baldamus, C. A., Uhlich, E. & Rumrich, G. 1969 *Pflügers Arch. ges. Physiol.* **310**, 369–376.
Wheeler, H. O., Ross, E. D. & King, K. K. 1969 *Am. J. Physiol.* **216**, 175–178.
Whittembury, G. 1971 *Electrophysiology of epithelial cells* Stuttgart–New York: Schattauer Verlag (in the Press).
Whittembury, G. & Fishman, J. 1969 *Pflügers Arch. ges. Physiol.* **307**, 138–153.
Whittembury, G. & Proverbio, F. 1970 *Pflügers Arch. ges. Physiol.* **316**, 1–25.
Yoshimura, H. & Imai, Y. 1967 *Jap. J. Physiol.* **17**, 280–293.
Yoshimura, H., Inoue, T., Imai, Y. & Yoshimura, F. 1962 *Jap. J. Physiol.* **12**, 1–17.
Young, J. A., Frömter, E., Schögel, E. & Hamann, K. F. 1967 *Secretory mechanisms of salivary glands*, pp. 11–31. New York–London: Academic Press.
Young, J. A. & Schögel, E. 1966 *Pflügers Arch. ges. Physiol.* **291**, 85–98.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES