saliva readily in these experiments, but could be made to do so by injection of 100 µg pilocarpine nitrate in 1 µl saline. The secretion induced in this way also lacked detectable peroxidase.

When *Eumecopus* was injected with pilocarpine and 100 µg horseradish peroxidase (Koch-Light, U.K.), watery saliva collected 1–2 min after the injection gave a strong reaction for the enzyme; and, on dissection, a short length of the long, tubular, accessory salivary gland[1] stained deeply with the benzidine reagent. Sheath material secreted within 12 h of injection of horseradish peroxidase, either with or without pilocarpine, also reacted with the reagent. These reactions were inhibited by 0.005 *M* KCN or phenylthiourea. The injections were made in such a way[1] that accidental injury of the salivary apparatus would not have occurred, and experiments with 50 individuals gave consistent results. Insects injected with bovine blood albumin instead of horseradish peroxidase showed no reaction for the enzyme in haemolymph, salivary secretions, or salivary glands.

Plant-bugs, including *Eumecopus*, secrete a polyphenol oxidase in their saliva, and tests were made using DOPA as a substrate[5] to determine whether this enzyme had interfered in tests for peroxidase. A positive reaction for polyphenol oxidase was found in the haemolymph, sheath material, and parts of the accessory salivary gland of *Eumecopus* irrespective of the reaction for peroxidase, which occurred as indicated above only after injection of horseradish peroxidase.

These experiments did not indicate how the horseradish peroxidase passed into the salivary glands. It is conceivable that the apoenzyme and prosthetic group[6] could enter independently and recombine in the saliva; but, in the absence of evidence that the apoenzyme can be reduced in size and still retain its activity, it may be assumed that protein molecules of about 40,000 mol. wt. are transferable via the accessory gland directly from the haemolymph to saliva of *Eumecopus*. The insect belongs to a family that is not known to transmit virus diseases to plants; but there are sufficient similarities in the physiology of feeding and salivation throughout the Heteroptera: Pentatomorpha and Homoptera to permit analogies to be drawn[5]. The results presented here indicate that relatively large colloidal particles may pass unchanged directly through cellular barriers in these insects.

Zusammenfassung. Es wird nachgewiesen, dass grosse Proteinmoleküle mit einem Molekulargewicht von 40000 unverändert aus der Hämolymphe von *Eumecopus* in deren Speichel gelangen und damit ausgeschieden werden.

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[6] K. G. PAUL, in *The Enzymes* (Eds. P. D. BOYER, H. LARDY and K. MYRBÄCK; Academic Press, New York 1963), vol. 8, p. 227.

Transmembrane Secretory Potentials in the Cat Submandibular Gland During Perfusion with Potassium-Free and Low Sodium Locke Solutions

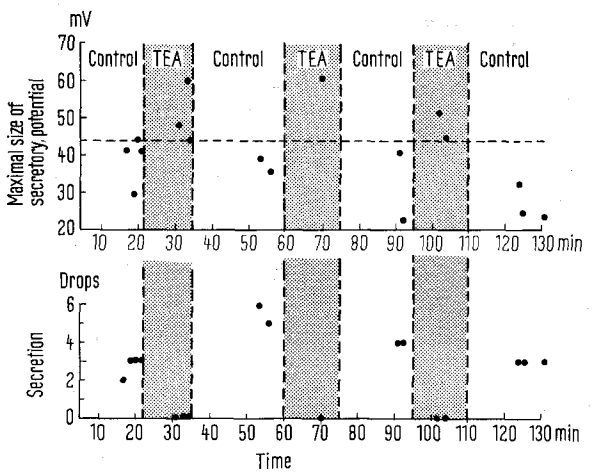
It was recently shown that the type I secretory potential, i.e. the hyperpolarization of the basal acinar cell membrane occurring during stimulation of the cat submandibular gland, was unaffected when all extracellular chloride was substituted by sulphate[1,2]. It is therefore likely that the ion transport producing the secretory potential is a cation transport from the acinar cells to the extracellular fluid. The potassium equilibrium potential across the basal acinar cell membrane is greater than the membrane potential during stimulation of the gland. The sodium equilibrium potential is, however, much smaller than the resting membrane potential[3]. An outward potassium current being partly short-circuited by an inward sodium current could thus explain the secretory potential on the basis of passive movements of ions down their electrochemical gradients. If this were true, the size of the secretory potential should increase during perfusion with potassium-free solutions and solutions in which most of the sodium was substituted by a bulky non-permeating cation. The experimental results were in accordance with this expectation.

Methods. Cats (2.4–3.6 kg) anaesthetized with chloralose (80 mg/kg i.p.) were used. The experiments were carried out as described previously[1,4], with the exception that the impalement of the acinar cells with the microelectrode was done by using a stepping motor micromanipulator remotely controlled (Transvertex, Sweden) and that the potentials were recorded on an UV-recorder (S.E. 3006). The glands were stimulated by close intra-arterial injections of 1 µg acetylcholine. Each time a secretory potential had been recorded, the number of drops of saliva secreted was counted. The control Locke solution contained (m*M*): 140 NaCl, 4.0 KCl, 2.4 Na_2HPO_4, 0.6 NaH_2PO_4, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 5.5. glucose. In the potassium-free solution all KCl was substituted by NaCl. In the tetraethylammonium (TEA) solution all NaCl was substituted by TEACl. The TEA Locke solution was prepared freshly for each experiment. The perfusion fluids were equilibrated with pure oxygen. After each shift of perfusion fluid at least 5 min passed before stimulating the gland.

Results. Perfusion with TEA Locke solution. In the Figure the results from 1 experiment are shown. It is seen that the salivary secretion was abolished during perfusion with TEA solution, while the sizes of the secretory potentials were enhanced. Table I shows the data from all experiments.

Perfusion with potassium-free Locke solution. Table II shows the data from all the experiments. The sizes of the secretory potentials recorded during perfusion with

[1] O. H. PETERSEN and J. H. POULSEN, Experientia *24*, 919 (1968).
[2] O. H. PETERSEN and J. H. POULSEN, in *Exocrine Glands* (Eds. S. Y. BOTELHO, F. P. BROOKS and W. B. SHELLEY; University of Pennsylvania Press, Philadelphia 1969)., p. 34.
[3] A. S. V. BURGEN, in *Handbook of Physiology*, Section 6 (American Physiological Society, Washington 1967), vol. II, p. 574.
[4] O. H. PETERSEN and J. H. POULSEN, Acta physiol. scand. *70*, 293 (1967).



Maximal sizes of secretory potentials (the difference between the maximal level of the membrane potential after stimulation and the resting membrane potential) and secretion as a function of time after start of perfusion in experiment No. 39.

Table I. Maximal sizes of secretory potentials in mV during perfusion with control and TEA Locke solutions

Experiment No.	Control (*n*)	TEA (*n*)	*t*-test
28	37.6 (3)	39.2 (5)	–
37b	23.2 (6)	31.6 (3)	–
38b	31.6 (5)	44.6 (5)	–
39	34.2 (11)	51.6 (6)	–
Total	31.4 ± 1.6 (25)	43.3 ± 2.5 (19)	$p < 0.001$

Table II. Maximal sizes of secretory potentials in mV during perfusion with control and potassium-free Locke solutions

Experiment No.	Control (*n*)	Potassium-free (*n*)	*t*-test
29	27.2 (4)	28.0 (7)	–
35	23.8 (7)	31.0 (7)	–
36	23.0 (4)	29.0 (4)	–
37a	21.7 (3)	32.5 (6)	–
38a	28.3 (4)	42.0 (4)	–
Total	24.8 ± 1.0 (22)	32.2 ± 1.3 (28)	$p < 0.001$

potassium-free solution were significantly greater than the sizes of those recorded during perfusion with control Locke solution.

Discussion. During perfusion with a 10 m*M*[5] or a 20 m*M*[4] potassium Locke solution, the sizes of the secretory potentials were significantly diminished compared with those recorded during perfusion with control Locke solution. Thus the size of the secretory potential seems to depend on the size of the potassium equilibrium potential across the basal acinar cell membrane. The results obtained with the low sodium TEA solution suggest that the outward potassium current is normally followed by an inward sodium current. In the rat submandibular gland, the acinar secretory potential may either consist in a hyper- or depolarization[6]. The difference between these secretory potentials and those found in the cat[1,7,8] and dog[4] may only be due to different sizes of the sodium and potassium currents, the sodium current being the most important in the depolarizing cells and the potassium current dominating in the hyperpolarizing acinar cells. This concept agrees well with the recent finding[9] that dinitrophenol in a concentration sufficient to inhibit salivary secretion and active uptake of potassium into the gland has no effect on the size of the secretory potentials if the gland has not lost too much potassium.

Zusammenfassung. Während der Perfusion der Submandibulardrüse der Katze mit kaliumfreier Lockelösung oder mit einer Lockelösung, in der das meiste Kochsalz durch Tetraethylammoniumchlorid ersetzt wurde, sind die sekretorischen Potentialdifferenzen der basalen Azinuszellmembran vergrössert. Ein Kaliumstrom aus der Azinuszelle, der teilweise von einem Natriumstrom in die Zelle kurzgeschlossen wird, könnte die sekretorischen Potentialdifferenzen erklären.

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[5] H. Yoshimura and Y. Imai, Jap. J. Physiol. *17*, 280 (1967).
[6] L. H. Schneyer and Y. Yoshida, Proc. Soc. exp. Biol. Med. *130*, 192 (1969).
[7] A. Lundberg, Acta physiol. scand. *35*, 1 (1955).
[8] M. E. Fritz and S. Y. Botelho, Am. J. Physiol. *216*, 1392 (1969).
[9] O. H. Petersen, Acta physiol. scand., in press.
[10] With the technical assistance of G. Pedersen.

A Histamine-Dependent Increase of 5-Hydroxytryptamine in the Rat Brain in vivo

In a previous report[1] we have produced evidence suggesting that the cerebral acetylcholine, released during central nervous system stimulation, is triggering a mechanism to induce an enhancement of the rate of synthesis of histamine in the rat brain. The present data show that the increase of cerebral histamine during central nervous system stimulation is in turn inducing an increase in cerebral 5-hydroxytryptamine.

Material and method. Albino rats of either sex, 70–120 g of body weight, were placed in individual wooden cages (9 × 4 × 5 inches) provided with a copper wired grid bottom connected to an electronic stimulator. Electrical stimulation was applied to the paws of the rat over a period of 5 min, 8 c/sec, 20 msec duration, and over a period of 30 min, 4 c/sec, 20 msec duration. A voltage was employed high enough to cause a discrete continuous jumping of the animal. At the end of the stimulation period, the rats were sacrificed by decapitation, the brains were removed as soon as possible (less than 3 min) and the cerebral hemispheres and brain stem homogenized in ice-cold acid ethanol. After extraction, histamine and 5-hydroxytryptamine were separated by descending paper chromatography using Whatman paper No. 1 and a solvent system of isopropanol–0.1 *N* HCl: 7:3. Chromatograms were run for 18 h at room temperature and the eluates assayed in the guinea-pig ileum and the rat stomach respectively. Specific antagonists were employed.

[1] H. A. Campos and H. Jurupe, Experientia, in press.