with granulated tubule cells[9] or striated duct cells[2]. It is interesting that the mean membrane potential in the mouse parotid gland, where there are no granulated tubules, was even higher than in the mouse submaxillary gland. The demonstration for the first time of a reversal potential for the initial phase of the secretory potential is consistent with the hypothesis previously put forward by PETERSEN[4] that ACh increases the permeability to K^+ and Na^+. Recently NISHIYAMA and KAGAYAMA[7] have reported that the magnitude of the initial depolarizing phase of the biphasic secretory potential in the cat submaxillary gland is dependent on the resting membrane potential in such a way that it increases with increasing resting potential. The slow and delayed hyperpolarization, reported in this paper, may be caused by a delayed increase in K^+ permeability, but it seems more likely that it is caused by an electrogenic pump, since it is abolished by Strophanthin-G. Such an electrogenic pump could be the Na^+-K^+ exchange pump of the acinar cells or the Na^+ pump directly responsible for the formation of the saliva[10]. More work is clearly needed to define the nature of the delayed hyperpolarization.

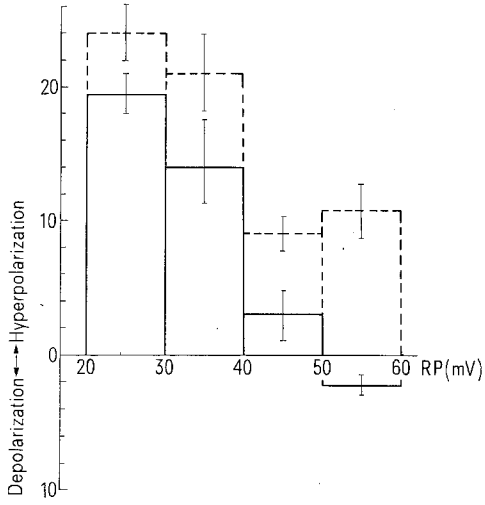

Fig. 2. Mouse submaxillary gland: Histogram showing the amplitude and polarity of the ACh-induced potential changes at different resting membrane potentials (RP). The fully drawn columns represent the first phase of the secretory potential while the columns outlined by the broken lines represent the second or delayed phase of the secretory potential.

Zusammenfassung. Acetylcholin erzeugt auf das Membranpotential von Azinuszellen der Glandula submandibularis der Maus eine diphasische Veränderung des Membranpotentials (rasche Hyperpolarisation und langsame, verzögerte Depolarisation oder rasche Depolarisation und langsame Hyperpolarisation). Hinweise sprechen dafür, dass Acetylcholin die Permeabilität für Na^+- und K^+-Ionen erhöht.

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[12] I am grateful to Dr. E. K. MATTHEWS, who placed all necessary facilities in his laboratory at my disposal, for his great interest in this project and for many useful discussions.
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Biphasic Secretory Potentials in Cat and Rabbit Submaxillary Glands

LUNDBERG[1] was the first to record transmembrane potentials in salivary glands. Stimulation of the secretory nerves to the submaxillary gland in the cat produced three types of potential changes (secretory potentials). The type I response to parasympathetic or sympathetic stimulation was a membrane hyperpolarization. LUNDBERG[2] suggested that the type I response was derived from the acinar cells and proposed that it was caused by an active transport of chloride ions through the contraluminal membrane from the interstitial fluid to the cytoplasm. Recently, YOSHIMURA and IMAI[3] and PETERSEN[4] have shown that the type I secretory potentials obtained from submaxillary glands in cat or dog are of normal size during perfusion with chloride-free solutions. PETERSEN[5–7] has suggested that the mechanism of action of acetylcholine on the contraluminal acinar cell membrane is to increase the permeability to both potassium and sodium ions. In the following some new and unexpected features of salivary gland electrophysiology will be reported.

Methods. Cats and rabbits anesthetized by chloralose (60–80 mg/kg) and urethane (1.5 g/kg), respectively, were employed. Membrane potentials were measured in the exposed submaxillary glands in vivo by using glass microelectrodes filled with $3M$ KCl, having resistances of $20–50\,M\Omega$. An indifferent electrode was placed under the neck skin opposite to the gland under study. The preganglionic lingual nerve fibres (parasympathetic) were stimulated at 0.2 or 20 c/sec through conventional stimulating electrodes.

Results. Single shock stimulation in both the cat and the rabbit resulted in either biphasic secretory potentials (type IB) (depolarization followed by hyperpolarization) or monophasic hyperpolarizing secretory potentials (type IM) (Figure 1).

In the cat submaxillary gland the mean latency of the type IB response was 176 msec $\pm$ 17 (80–300 msec) and that of the type IM was 291 msec $\pm$ 11 (100–500 msec). The resting membrane potentials for the cells displaying type IB responses were higher (-40.4 mV $\pm$ 0.4) than for those displaying type IM responses (-31.0 mV $\pm$ 0.8). Each histogram showing the frequency distribution of the resting potentials for these 2 groups exhibited

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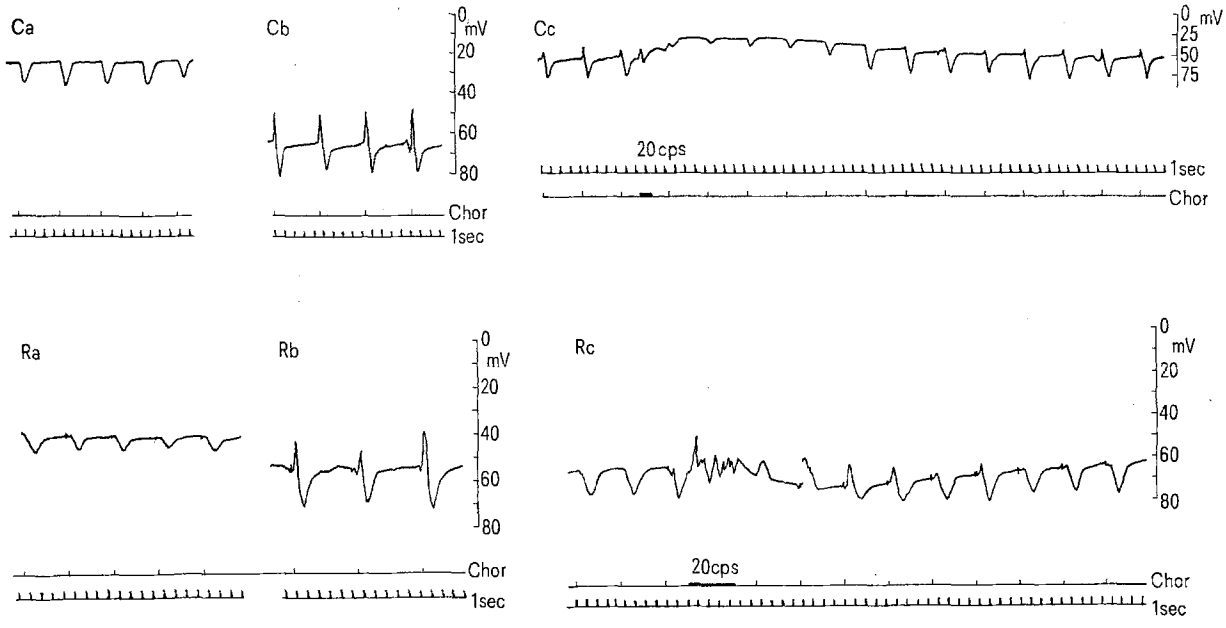

Fig. 1. Transmembrane secretory potentials in cat (C) and rabbit (R) submaxillary glands obtained by single or repetitive chorda-lingual (Chor) nerve stimulations. a) and b) are examples of monophasic hyperpolarizing and biphasic secretory potentials, repectively, obtained by single stimulation, c) represent examples of secretory potentials obtained by repetitive stimulation.

asymmetry (Figure 2). However, the histogram showing the frequency distribution of all the resting potentials (type IM + IB) showed a fairly good symmetrical distribution. The mean resting potential of all the cells was -35.0 mV $\pm$ 0.7. The amplitude of the initial depolarization of the type IB response was roughly proportional to the size of the resting potential. The membrane resistance in the type IM cells decreased during lingual nerve stimulation. Single or repetitive stimulations decreased membrane resistance from $2.9 M\Omega \pm 0.2$ to $2.5 M\Omega \pm 0.2$ and $2.1 M\Omega \pm 0.2$, respectively.

In the rabbit submaxillary gland, the mean resting potential was -54.0 mV $\pm$ 3.1. The latency of the type IB response was 168 msec $\pm$ 34 (100–300 msec), while that of the type IM response was 550 msec $\pm$ 130 (400–1000 msec). The secretory potentials in the rabbit submaxillary gland were more complex than those in the cat. Repetitive stimulation changed the configuration of the secretory potentials caused by single shock stimulation. For a short time following repetitive stimulation, the initial depolarization had a larger amplitude and the secondary hyperpolarization was reduced in a number of type IB cells. Sometimes the type IM response of one cell could be changed into a type IB response, concomitantly herewith the latency was considerably shortened (Figure 1 Rc). In the type IM cells, single shock stimulation decreased membrane resistance from $8.7 M\Omega \pm 1.4$ to $7.2 M\Omega \pm 1.0$ while repetitive stimulation reduced the resistance from $7.4 M\Omega \pm 1.0$ to $4.5 M\Omega \pm 0.6$. The decrease in membrane resistance slightly preceded the potential change (Figure 3 upper tracings). In the type IB cells the membrane resistance decreased concomitantly with the potential change (Figure 3 lower tracing). Single shock stimulation decreased the membrane resistance from $6.0 M\Omega \pm 1.8$ to $3.2 M\Omega \pm 0.7$ while repetitive stimulation caused a reduction from $6.6 M\Omega \pm 1.1$ to $1.9 M\Omega \pm 0.7$.

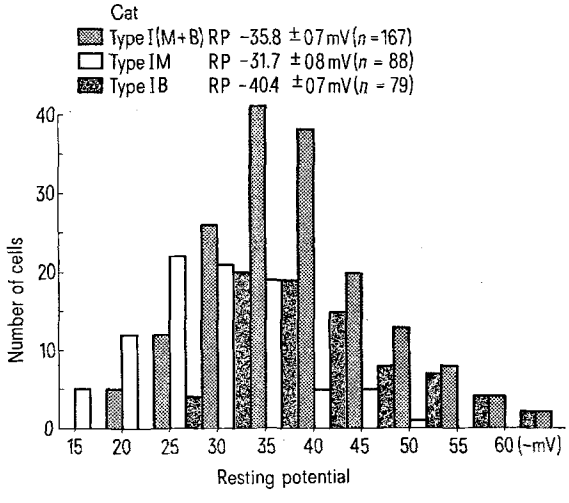

Fig. 2. Cat submaxillary gland: Frequency distribution of the resting membrane potentials from the cells displaying monophasic hyperpolarizing (type IM) and biphasic (type IB) secretory potentials, and of the entire cell population (type IM + IB).

Discussion. SCHNEYER and YOSHIDA[8] showed the existence of biphasic secretory potentials in the rat submaxillary gland. In all previous studies of membrane potentials in cat submaxillary glands[1,2,4–6,9] it has been shown that the contraluminal membrane of the acinar cells hyperpolarizes when the gland is stimulated to secrete. The present work shows clearly that biphasic secretory potentials also exist in the cat submaxillary gland and indeed in the rabbit submaxillary gland. The initial depolarization has not been observed previously, probably because only low resting membrane potentials (20–30 mV) have been recorded. The facts that only the

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frequency distribution of the entire cell population (type IM + IB) was symmetrical and that monophasic and biphasic secretory potentials could be found in the same cells (Figure 1) suggest that all cells studied in the present work are acinar. The finding that the latency for the stimulation-induced potential change was considerably longer for the type IM cells than for the type IB cells is of considerable interest especially in view of the finding that the decrease in membrane resistance preceded the type IM response but appeared concomitantly with the type IB response. The very marked decrease in membrane resistance seen during nerve stimulation leaves little doubt that ACh, which is the transmitter being released[10], increases the membrane permeability to ions. The fact that the secretory potential is often biphasic suggests that at least two ionic channels are opened by ACh. The results are thus consistent with the hypothesis of PETERSEN[5–7] that ACh acts by increasing the permeability to K^+ and Na^+.

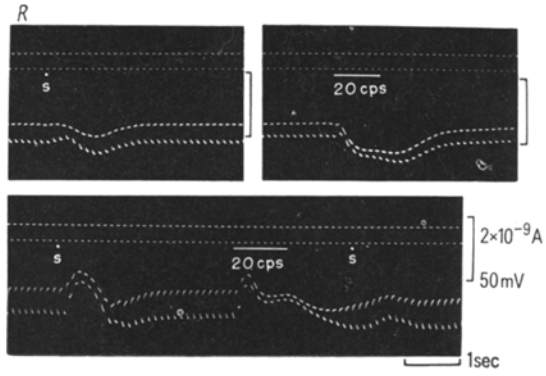

Fig. 3. Rabbit submaxillary gland: Measurement of membrane resistance during the secretory potential by application of hyperpolarizing current pulses through the intracellular recording microelectrode. s: single shock stimulation, 20 cps: repetitive stimulation.

Zusammenfassung. Die Membranpotentiale, in Azinuszellen der Submanibularisdrüse der Katze und des Kaninchens gemessen, waren höher als die in der früheren Literatur festgestellten. Die Stimulation erzeugte biphasische sekretorische Potentiale (Depolarisation-Hyperpolarisation), begleitet von einem Nachlassen des Membranwiderstandes. Es wird vermutet, dass Acetylcholin die Membranpermeabilität der Azinuszellen für zwei verschiedene Ionenarten erhöht.

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Reactivity of Myoglobin in Heart, Striated Muscle and Uterus in a Methaemoglobinaemia

Pathological processes during an anemic hypoxia of the methaemoglobin type can be characterized by the reactivity of two systems: 1. The detoxicating functions, i.e. the chemical transformation of methaemoglobin-forming agents, excretion and enzymatic reversibility of methaemoglobin formation; 2. The compensating capacity of the cardiovascular system: increase of cardiac output and reactivity of blood vessels. Whereas the complex of questions mentioned under 1. were thoroughly investigated during recent years, many physiological and biochemical problems connected with 2. remained without a clear pathophysiological answer. The results obtained from analyses of the rat heart proved that the changes of the percentages of the inactivated myoglobin components play an important role[1,2]; with respect to the microanatomical properties of various muscles, it was necessary to determine the extent of myoglobin inactivation under the same physiological conditions parallel in 3 preparations: the heart, the striated muscle and the uterus.

Materials and methods. 22 female rabbits were used for the experiments: 12 animals served as controls, and 10 were given a single injection of $NaNO_2$ solution. The animals were sacrificed 40, resp. 80 min after application of $NaNO_2$, and venous blood samples for haemoglobin and methaemoglobin determination[3] were taken. The concentration of myoglobin in the left heart ventricle, the triceps muscle and the uterus was estimated by the method of REYNAFARJE[4], and the myoglobin components (CO-myoglobin, NO-myoglobin and metmyoglobin) by the method previously described[1].

Results. In various types of muscles a differentiated reaction of myoglobin was observed. The amount of total myoglobin was not significantly changed in the $NaNO_2$-treated groups (Figure). A differentiated reactivity was observed in the determination of metmyoglobin formation. It is evident that in different types of muscles a different limiting rate can be observed. While in the heart the maximum amount was reached with 150 mEq $NaNO_2$ after 40 min, the maximum average value in the striated muscle was reached at the same time, but it was higher than in the heart. The highest rate of metmyoglobin formation was observed in the uterus, where also the speed rate was different: the maximum amount was found with 75 mEq $NaNO_2$ after 40 min.

Another characteristic can be seen during the myoglobin inactivation by forming NO-myoglobin. It could be proved that the higher dose and the longer treatment period increase the rate of NO-myoglobin formation. Therefore the total amount of inactivated myoglobin is after 75 mEq in 40 min greater than the inactivated part of haemoglobin. However, after 150 mEq in 40 min the amount of inactivated components remains in the same degree as that of inactivated haemoglobin in the erythrocytes.

Discussion. Myoglobin and its physiological properties remained, compared with haemoglobin, until today relati-

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