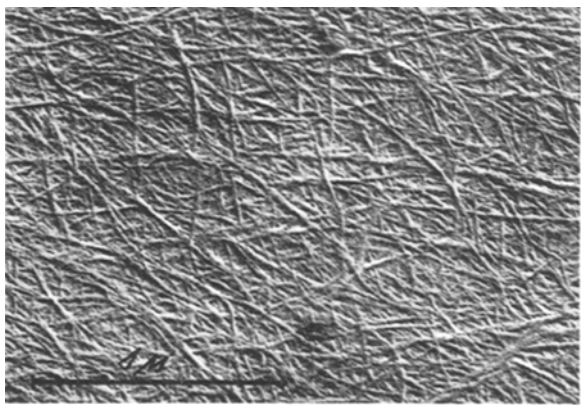
amount of plankton being filtered as to how the band is transported through the intestine. Perhaps coils are formed, when the secretion of peritrophic membranes cannot follow the rapid intake of food.

The peritrophic membranes did not dissolve even after prolonged treatment with 40% potassium hydroxide at 80 °C. As they did not stain with chlorinezinciodine and as they did not dissolve in copper oxide-ammonia (Schweizer's reagent), it seems unlikely that they contain cellulose. But they gave a very distinct chitosan reaction, which shows that chitin is an essential element in the construction of these peritrophic membranes.

An investigation of their fine structure confirmed this view. There are microfibrils in these membranes, showing a texture that resembles the texture of microfibrils found in plants[3,4], insects[7,8], and other animals[9]. It is an irregular, felt-like texture. Frequently the microfibrils are not interwoven in one level, but pass through several levels (see also [7]). Sometimes a number of microfibrils are united to form coarser fibres, which are strewn irregularly over the surface of the ground texture or form a flat band. The single microfibrils show notches, suggesting that these chitin-containing microfibrils of tunicates as well as the cellulose-containing microfibrils of plants[10-12] (and tunicates[1,2]) consist of smaller units[13].



Phallusia mammillata: Irregular network of microfibrils containing chitin. Shadowed with platinum. × 33,600.

Zusammenfassung. Peritrophische Membranen kommen nicht nur bei Arthropoden vor, sondern sind im Tierreich weit verbreitet. Chitin enthaltende peritrophische Membranen werden auch von den bisher daraufhin untersuchten Ascidien gebildet. Die frühere Auffassung, nach der Chitin bei Deuterostomiern nicht auftritt, muss daher endgültig aufgegeben werden.

W. Peters

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Inhibition of Secretion and Secretory Potentials in the Submandibular Gland of the Cat by Acetazolamide

Lundberg[1-4] recorded transmembrane potentials in the acinar cells of the submandibular and sublingual glands of the cat. He showed that stimulation of the secretory nerve increased the intracellular negativity, and he was able to demonstrate that secretory potentials were due to an active inward transport of anions, largely chloride, through the outer acinar cell membrane.

It is well known that the potent carbonic anhydrase inhibitor acetazolamide (diamox) is able to inhibit a number of secretory processes[5]. Among others Maren and Robinson[6] suggested that the diamox-inhibition of cerebrospinal fluid formation is due to an inhibition of a chloride pump in the chorioid plexus.

Carbonic anhydrase has been found in salivary glands of many species, and recently Morris and Swayne[7] showed that most of this carbonic anhydrase is located at the acini.

In the present work it was shown that diamox is able to inhibit salivary flow rate as well as secretory potentials.

Methods. Young cats (2–3 kg) anaesthetized with chloralose (70–90 mg/kg i.p.) were used. Salivary flow rate was measured by collecting the saliva obtained from the cannulated submandibular duct in a tuberculin syringe. Secretory rate was measured in 10 successive periods, of which the first 2 lasted $^1/_2$ min each, the rest having a duration of 1 min each. The salivary flow rate decreased during the first periods but remained relatively constant in the last 4 periods. The flow rate measured in the last 1 min period was taken as a 'steady state'. Salivary secretion was elicited by electrical stimulation of the chorda tympani (10 c/sec, 10 V).

Transmembrane potentials were measured by the technique of Lundberg[1] with the only difference that the potentials were recorded from the gland in situ. Therefore, only a small part of the gland, needed for the micropuncture, was exposed. The potentials were recorded with a Mingograf writer. Secretory potentials were obtained after stimulation of the chorda tympani (15 c/sec, 10 V).

Diamox was administered intravenously according to Birnbaum and Hollander[8] in some experiments, in

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Inhibition of secretory rate by diamox

Experiment No.	Dose of diamox mg/kg	Mode of administration	Secretory rate before diamox µl/min	Secretory rate during maximal diamox inhibition µl/min	Latency for maximal inhibition h	Maximal inhibition %	Secretory rate 1 h after max. inhibition µl/min
39	60	i.v.	490	280	$5^1/_2$	43	
40	90	i.v.	380	75	5	80	320
42	100	i.v.	510	15	5	97	290
43	100	i.v.	510	150	6	71	
44	100	i.v.	270	110	8	59	130
45	100	i.a.	360	30	2	92	55
46	90	i.a.	290	90	$^3/_4$	69	210
48	65	i.a.	400	330	$1^3/_4$	18	340
48	110	i.a.	310	80	$^1/_4$	74	120
49	155	i.a.	450	120	$1^1/_2$	73	260

others it was injected retrogradely into the cannulated lingual artery. In these experiments diamox was dissolved in 0.8–0.9 ml 1 *N* NaOH to obtain a pH of 7.4, and was infused with a constant flow rate over a period of 15–17 min. As a control a similar volume of isotonic saline was injected in the same way before the diamox injection.

Results. Inhibition of salivary flow rate. The results are summarized in the Table. The greatest difference between the 2 modes of diamox administration is the temporal course of the inhibition. In the experiments with intra-arterial administration, the inhibition is marked immediately after the injection has been finished, whereas in the experiments with intravenous administration only a slight inhibition is noticed 1 h after the injection. No change of the secretory rate was observed after the control injection of saline.

Inhibition of secretory potentials. All the secretory potentials in the present work belong to LUNDBERG's[1] type I group originating from the acinar cells. As seen in Figures 1 and 2 there is a marked difference between the size of the secretory potentials recorded before the diamox injection and the size of those recorded at the time of maximal diamox-inhibition. In one experiment the secretory potentials were recorded in every interval between the measurements of the secretory rate. The result is shown in Figure 3.

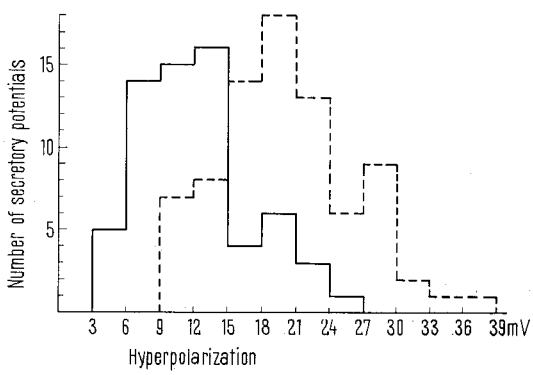

Fig. 1. Secretory potentials plotted as a function of hyperpolarization. 2 different groups of secretory potentials are shown, one being a control group of normal secretory potentials, the other secretory potentials recorded at the time of maximal diamox-inhibition. - - - - = without diamox; ——— = with diamox.

Discussion. A correlation was shown between the secretory rate and the size of the secretory potentials, which according to LUNDBERG[3] is a measure of the active transport of anions through the outer acinar cell membrane. From this it would appear that at least part of the diamox-inhibition of the secretory rate is due to an inhibition of this transport process.

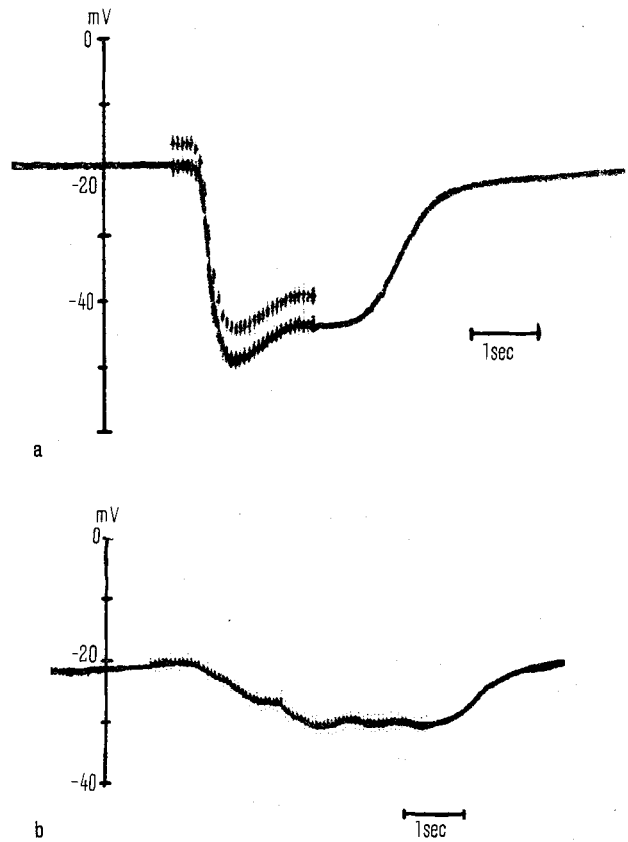

Fig. 2. (a) An example of an uninhibited type I secretory potential. (b) A diamox-inhibited type I secretory potential. Both secretory potentials were recorded after chorda stimulation (15 c/sec, 10 V). The stimulus artefact indicates the period of stimulation.

EMRICH and ULLRICH[9] showed that diamox inhibited human sweat flow rate. They suggested that it was the transport process responsible for the production of the 'primary sweat' that was inhibited. SCHULTZ et al.[10] and MARTINEZ et al.[11] found from micropuncture studies of human sweat glands and rat submandibular glands that the 'primary secretion' formed in the glomerulum and the acini respectively had a plasma-like composition with chloride as the dominating anion.

The marked inhibition of the secretory rate found in the present work can thus not be explained by a mere inhibition of bicarbonate formation in the acini, but could be explained by an inhibition of LUNDBERG's[4] 'chloride pump'.

Previous unsuccessful attempts by others[12] to inhibit salivary secretion with diamox have probably been due to the employment of too small a dose, and to the fact that the maximal inhibition does not occur until about 5 h after intravenous administration of diamox.

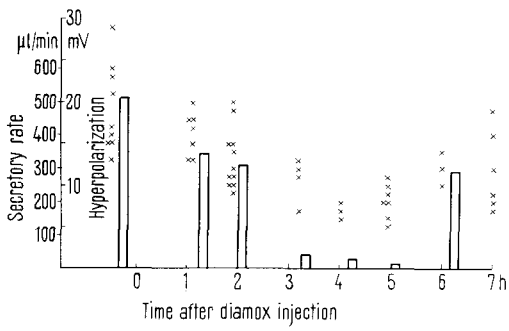

Fig. 3. The time course of inhibition in experiment No. 42 of both secretory potentials and secretory rate. Each cross represents one secretory potential. × = secretory potential; ⌐¬ = salivary flow rate.

Zusammenfassung. Es wird eine Hemmung der Speichelflussrate in der Submandibularisdrüse der Katze durch den Kohlensäureanhydratasehemmstoff Acetazolamid hervorgerufen und gleichzeitig eine Hemmung der sekretorischen Potentialunterschiede über Azinuszellmembrane gemessen. Die Sekretionshemmung liesse sich durch die Hemmung der sekretorischen Potentiale, die ein Mass für einen aktiven Anionentransport ist, erklären.

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Effect of Actinomycin D on Mice Infected with the Lactate Dehydrogenase Virus[1]

Following infection of mice with the lactate dehydrogenase (LDH) virus[2], there is a viremia and an elevation in plasma LDH activity which persists for weeks or months until death[3,4]. Although studies have been reported to indicate that the increase in enzyme activity is due to an impaired rate of plasma LDH clearance, related perhaps to an effect of the virus on the host's reticuloendothelial system[5–7], there is recent evidence to suggest that the mechanism of enzyme elevation may also involve an increase in the influx of endogenous LDH into the plasma[8,9]. In an attempt to obtain additional information relevant to these possibilities, experiments were initiated to determine the effect(s) of certain metabolic inhibitors on mice infected with the LDH virus. This paper will describe results obtained in studies with actinomycin D.

Materials and methods. Adult C57BL/Fg mice, weighing 18–20 g, received an intraperitoneal injection (0.1 ml/mouse) of $10^{7.0}$ ID_{50}/ml of virus. 1 h after infection, the animals were injected i.p. with actinomycin D (10 µg/mouse) dissolved in equal parts of ethyl alcohol and phosphate-buffered saline (PBS), pH 7.2. For controls, 28 infected mice received an i.p. injection of the solvent (0.1 ml/mouse). Blood was collected by tail bleeding from experimental and control animals at intervals of from 24 h to 3 weeks after treatment; the plasma LDH activity of each sample was determined as described previously[10]. Plasma samples were then pooled (4 specimens/pool), according to treatment and time after infection, and frozen at – 30 °C. After thawing, serial tenfold dilutions of the plasma pools were prepared in cold PBS and inoculated i.p. (0.1 ml/mouse) into recipient animals. Test mice were bled 1 week after inoculation and

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