from phenylbutazone treated animals, D animal and controls was observed in the F2 fractions, for which a 50% decrease in SO_4^{2-} ions content was found for the glycopeptides from ulcered mucosae: the average values for the total hexosamines/SO_4^{2-} ratios, reported in the table, are 2.4 for the controls and 5.0 for the ulcered animals. A second variation has been observed in the D-F2 fraction, which showed a lack of sialic acid, while this component was found in similar amounts in the F2 fractions from phenylbutazone treated and control animals.

On the basis of the ratio total carbohydrates/total protein for F1 and F2 fractions, we observed that the glycopeptides richer in sialic acid and sulphate ions also contain a higher amount of amino acids, and it seems possible to formulate the hypothesis that the acid glycoproteins are less digested by papain than the neutral ones. In spite of these differences, the amount of glycopeptides F1 and F2 isolated from controls and ulcerated animals seems to be unchanged.

Conclusions. The aim of our investigation was to analyze and to compare the glycopeptides from gastric mucosae of normal and ulcered pigs. 2 glycopeptide fractions (F1 and F2) obtained by means of chromatography on Dowex 1×2 from the crude extract of each mucosa were studied. The F2 fraction is characterized by the presence of both SO_4^{2-} ions and sialic acid, in addition to hexosamines, hexoses and fucose, and this distinctive feature makes it similar to the glycopeptides from dog gastric mucosa[13] and dog submaxillary glands[14]. This F2 fraction is the only one showing a significant difference among the 3 samples (C, T and D), the SO_4^{2-} ions content in ulcered animals being about 50% less than in control ones. The result reported in the present paper could support the hypothesis of MARTIN et al.[15] and of BERARD et al.[16], who underline the importance of the SO_4^{2-} ions in gastric mucosubstances in protecting mucosa; these ions binding to protein substrate cover and protect gastric mucosa from the injury of pepsin and HCl. Concerning the sialic acid content, we did not find any difference between phenylbutazone treated and control animals; but this component is completely lacking in the spontaneously ulcered animal[17,18].

Zusammenfassung. Die Zusammensetzung der aus normalen und ulzerösen Magenschleimhäuten von Zwergschweinen isolierten Glykopeptide ergibt 50% Sulfat-Ionen weniger als aus denjenigen gesunder Magenschleimhäute.

G. PALLAVICINI, G. CETTA,
A. QUARONI and A. A. CASTELLANI

Istituto di Chimica Biologica dell'Università, Piazza Botta 1, I-27100 Pavia (Italy), 5 November 1970.

[13] T. PAMER G. B. J. GLASS and M. I. HOROWITZ, Biochemistry *7*, 3821 (1968).
[14] C. BIGNARDI, G. AURELI, C. BALDUINI and A. A. CASTELLANI, Biochem. biophys. Res. Commun. *17*, 310 (1964).
[15] R. MARTIN A. BERARD, A. JOVENCEAUX and R. LAMBERT, C. r. Soc. Biol., Paris *161*, 848 (1967).
[16] A. BERARD, R. MARTIN and R. LAMBERT, Gastroenterologia *108*, 209 (1967).
[17] Acknowledgement. Thanks are due to Dr. W. MURMANN from Selvi Laboratories, Milan, who provided the animals for these experiments.
[18] This work was supported by the grant No. 70.00079/03 115.1927 from Consiglio Nazionale delle Ricerche (C.N.R.), Italy.

The Effect of Denervation on ATP Dependent Calcium Uptake by Microsomes of the Submaxillary Gland of Rats

ATP- dependent calcium uptake by muscle and nervous tissues microsomes has been clearly demonstrated and related to physiological events[1,2]. The occurrence of similar phenomena in microsomes from rat salivary glands has recently been shown [3,4]. These results are interpreted as an active transport across the microsomal membrane. Further, calcium plays an important role in stimulus-secretion coupling in salivary glands[5]. In the present investigation the effects of authonomic denervation upon ATP-dependent calcium uptake by microsomes from rat submaxillary gland were studied. This seemed to be of particular interest since denervation of salivary glands are known to produce marked morphological and functional changes[6].

Fifty-four female rats bred at this Department were used. 4–5 month-old animals, weighing about 170 g, were studied. The glands were either normally innervated on both sides ('control glands') parasympathetically or sympathetically denervated on one or both sides and normally innervated on the other side ('contralateral glands'). In all cases litters of 4–8 rats were used and 2 rats of each litter were taken as controls. A preganglionic parasympathetic denervation was achieved by section of the chorda-lingual nerve and a post-ganglionic sympathetic denervation by excision of the superior cervical ganglion. The operations were aseptically performed under ether anaesthesia. The animals were killed 3–4 weeks later by cervical dislocation. The submaxillary glands were carefully removed, cleaned, dried between filter paper and weighed (wet wt.). They were immediately homogenized in glass homogenizers containing 125 m*M* KCl and 5 m*M* histidine buffer (ph 6.5). After centrifugation at 10,000 *g* for 30 min the supernatant was decanted and recentrifugated at 26,000 *g* for 1 h. The resulting pellet was dissolved in the same solution. All procedures were performed at 0–4 °C.

For calcium uptake determinations the microsomes were incubated in the presence of 5 m*M* $MgCl_2$, 45 m*M* *Tris* buffer (pH 7.4) 100 m*M* KCl, 4 m*M* *Tris*-ATP and 0.05 m*M* $^{45}CaCl$ for 5 min in a total volume of 2 ml. From it, 1.5 ml was run through a millipore filter (0.45 μm average pore) which was under negative pressure. The filter containing the microsomes was then washed with 10 ml of 100 m*M* $CaCl_2$ and immediately dissolved in

[1] W. HASSELBACH, Progr. Biophys. molec. Biol. *14*, 169 (1964).
[2] M. OTSUKA, I. OHTSUKI and S. EBASHI, J. Biochem. *58*, 188 (1965).
[3] G. L. ALONSO, P. M. BAZERQUE and D. ARRIGO, Int. Ass. dent. Res., Buenos Aires *23* (1969).
[4] S. SELINGER, E. NAIM and M. LASSER, Biochim. Biophys. Acta. *203*, 326 (1970).
[5] W. V. DOUGLAS and A. M. POISNER, J. Physiol., Lond. *165*, 528 (1963).
[6] A. S. V. BURGEN and N. G. EMMELIN, *Physiology of the Salivary Glands* (Edward Arnold Ltd., London 1961).

ATP dependent Ca uptake by microsomes (mean ± S.E.M.) in unilateral and bilateral parasympathetically or sympathetically denervated submaxillary glands of the rat, in contralateral normally innervated and in the corresponding control glands

	No. of glands	Gland weight (g)	ATP dependent Ca uptake (nMoles/mg protein)	Ca uptake ratio with ATP/without ATP
Control	36	129 ± 3.13	7.7 ± 1.30	10.9 ± 1.63
Contralateral	36	130 ± 2.91	6.5 ± 0.62	10.1 ± 1.66
Parasympathetically denervated	22	93 ± 4.72[a]	6.7 ± 1.43	9.3 ± 1.44
Sympathetically denervated	14	117 ± 4.29[b]	2.8 ± 0.92[a]	5.1 ± 1.28[a]

[a] $P < 0.001$, [b] $P < 0.01$ when compared with control or contralateral.

10 ml of a scintillation solution (acetone-methanol-PPO-POPOP-toluene). Radioactivity was measured in a liquid scintillation counter. In all experiments blanks without microsomes were measured and subtracted from all other values. Protein concentration was measured by the method of LOWRY et al.[7]. All experimental determinations were performed on a pool of 2–6 glands from animals of the same litter.

The results are summarized in the Table. The ATP dependent calcium uptake by gland microsomes is expressed in nMoles/mg protein and the calcium uptake relation in presence of ATP/without ATP. The weight of the submaxillary gland was found to be significantly decreased by about 30% after section of the chorda-lingual nerve and by 10% after sympathetic denervation. After sympathetic denervation both the ATP dependent calcium uptake as well as the ratio with ATP/without ATP were markedly decreased, by 61% and 51% respectively. No significant changes were observed in both parameters after preganglionic parasympathetic denervation. The values of parasympathetically and sympathetically unilateral or bilateral denervated glands are presented together since no differences were observed in the results obtained. It should be pointed out that the total amount of microsomal protein was similar in all experimental groups, around 5 mg/g wet glandular tissue.

The role played by calcium ions in the secretory mechanisms in salivary glands should be located as occurring after the liberation of the neuronal transmitter and likely at intracellular level. This hypothesis is supported by the conclusions of DOUGLAS and POISNER[5] obtained on the cat's submaxillary gland. Further studies on amilase liberation from slices of rat parotid gland suggest that the site of action of calcium ions occurs at the step point of extrusion of the secretory products[4]. Our results obtained on a microsomal fraction from the rat's submaxillary gland support the existence of an intracellular site for the action of calcium which can be altered by the depletion of catecholamines following sympathetic postganglionic denervation[8]. The ATP- dependent microsomal calcium uptake was not affected by preganglionic parasympathetic denervation. It should be noticed, however, that even after parasympathetic decentralization there is a continuous leakage of neurotransmitter from the endings of the remaining postganglionic fibers[9].

Zusammenfassung. Nachweis, dass in einer Mikrosomen-Fraktion von Speicheldrüsen ein ATP-abhängiges Calciumtransportsystem existiert und dass dieses von der sympathischen Innervierung der Drüsen, nicht jedoch von der parasympathischen Innervierung abhängig ist.

C. J. PEREC[10,11] and G. L. ALONSO[10,11]

Cátedra de Fisiologia, Facultad de Odontologia, Universidad de Buenos Aires, Charcas 2142, Buenos Aires (Argentina), 5 February 1971.

[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and J. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[8] U. S. v. EULER and G. RYD, Acta physiol. scand. *59*, 62 (1963).
[9] N. ASSARSON and N. EMMELIN, Br. J. Pharmac. *22*, 119 (1964).
[10] Members of the Carrera del Investigador Científico del Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.
[11] This work was supported in part by grants No. 3211/68 and 3155/67 from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

Zur Reaktionsweise eines Zeisigs (*Carduelis spinus* (L.)) auf gefilterte Kontaktrufe

Unsere Erfahrungen mit modifizierten akustischen Signalen und über deren Effekt für den natürlichen Rezipienten stehen noch am Anfang. In Vorversuchen zu umfassenden Experimenten über die bidirektionale Kommunikation von Zeisigen (*Carduelis spinus* (L.)) zeichnete sich ein bemerkenswertes Ergebnis ab.

In einer speziell entwickelten Anlage (Sch.) wurden einem Zeisig, der in einer schallisolierten Kammer lebte und mit einem Artgenossen nur über Mikrophone und Lautsprecher kommunizieren konnte, gefilterte Kontaktrufe vorgespielt. Über 5 min wurde dem einen Kommunikanten (♂ A) der sich ständig wiederholende Ruf «tetterett» (Figur 1) gefiltert mit einem Hoch- bzw. Tiefpass (KFl, Messelektronik Berlin, Sperrdämpfung für Hochpass bei 0,7facher Durchlassfrequenz 4,5 Np = 39 dB, für Tiefpass bei 1,4facher Durchlassfrequenz 4,5 Np = 39 dB), dem anderen (♀ C) ungefiltert geboten. Währenddessen war die gegenseitige Kommunikation unterbrochen und zwischen den einzelnen Versuchen dann jeweils für mindestens 8 min wieder hergestellt worden. Nach 4 Filterversuchen wurde eine Kontrolle mit ungefiltertem Abspiel für ♂ A vorgenommen. Das Reaktionsmittel von 2 eine Serie von 4 Versuchen begrenzenden Kontrollen wurde mit 100% bewertet. Das ausgewertete Resultat bilden auf elektronischem Wege erhaltene Zählungen der akustischen Einzelimpulse von ♂ A als