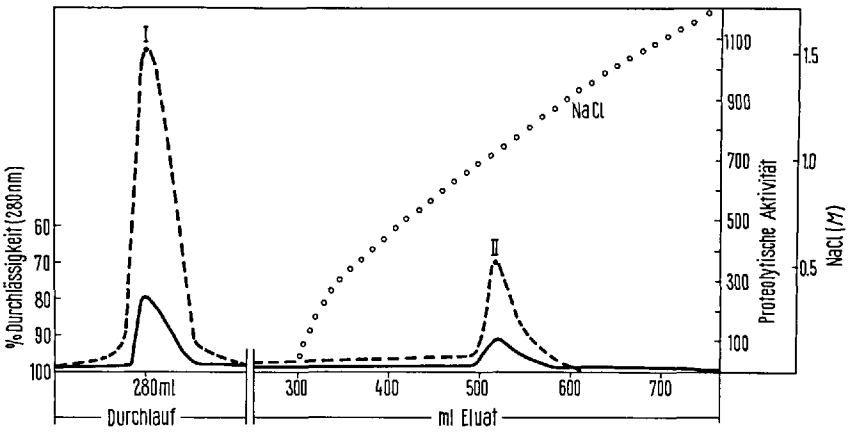

Reinigung lyophilisierter Mitteldärme von *Vespa orientalis* durch Anionen-Austauscher-Chromatographie über DEAE-Sephadex A-50. Säule: 3×53 cm, Puffer: *Tris*/HCl 0,01, pH 8,0, Gradient: 2 *M* NaCl kontinuierlich ansteigend, Mischgefäss: 500 ml. Die proteolytische Aktivität wurde in optischer Dichte mit Casein als Substrat ermittelt. I, ATEE-spaltende Fraktion; II, BAEE-spaltende Fraktion.

Protease nachweisen. Ob ihr Nichtvorhandensein im Zusammenhang mit der besonderen Ernährungsweise steht – Futtersaft («gelée royal») im Gegensatz zur Pollenernährung der Arbeiterinnen und Drohnen –, kann nur vermutet werden. Möglicherweise ist das Nichtvorhandensein einer BAEE-spaltenden Protease bei der Königin auf das Vorkommen von freiem Arginin und Lysin im Futtersaft zurückzuführen, während das Polleneiweiss diese Aminosäuren gebunden enthält[6]. Vergleichbare Ergebnisse sind jetzt auch von den Bienen bekanntgeworden[7]. Auch hier kann für die Königin keine BAEE- und BANA spaltende Protease nachgewiesen werden, während die Arbeiterinnen und Drohnen ein solches Enzym besitzen.

Betrachtet man die Hornissen-Proteasen in Abhängigkeit vom pH-Wert, so ergibt sich für beide ein Optimum im alkalischen Bereich, doch sollte man aus diesem Umstand keinen Anspruch auf Homologie mit den entsprechenden Säugerproteasen ableiten, wie es früher oft geschehen ist. Wahrscheinlich handelt es sich hier lediglich um einen notwendigen Milieufaktor, der aber für eine scharfe Kennzeichnung zu wenig aussagestark ist. Die Molekulargewichte weichen mit 12500 für die ATEE-spaltende Protease und mit 26000 für die BAEE-spaltende Protease mehr oder weniger erheblich von den entsprechenden Säuger-Proteasen ab. Hemmversuche mit Phenyl-methyl-sulphonyl-fluorid (PMSF) und Aminosäure-Chlormethan-Verbindungen (TLCK und TPCK) zeigen eine Ähnlichkeit der Hornissen-Proteasen im Hemmverhalten mit anderen Endopeptidasen, die zu ihrer katalytischen Wirkung ebenfalls einen Serin- und Histidinrest im aktiven Zentrum benötigen.

Im Hinblick auf eine mögliche Verwandtschaft zwischen den Proteasen erscheint die Spaltungsspezifität an der B-Kette des oxydierten Insulins am aussagestärksten. Die noch nicht endgültig abgeschlossenen Versuche zeigen durch die Zahl der gewonnenen Einzelpeptide und deren elektrophoretischer Beweglichkeit, dass neben identischen Spaltstellen zwischen den Hornissen-Proteasen und dem Chymotrypsin, nicht dem Trypsin der Säuger, auch neue Spaltstellen auftreten, die für eine etwas erweiterte Spaltungsspezifität gegenüber den Säugerproteasen sprechen. Die genaue Spezifität sowie die Bestimmung der Mol.-Gewichte mittels Ultrazentrifuge bedürfen noch der Auswertung.

Bei den Hornissen-Proteasen lassen sich neben der Hemmbarkeit auch in den katalytischen Eigenschaften Ähnlichkeiten feststellen, die Rückschlüsse auf den Bau des aktiven Zentrums zulassen. Dennoch kann die Frage, ob diese Ähnlichkeiten auf einer homologen oder einer konvergenten Entwicklung beruhen, heute noch nicht entschieden werden.

Summary. Two proteinases from the digestive tract of the oriental hornet larva *Vespa orientalis* were investigated. One hydrolyzes the chymotrypsin substrates ATEE and APNE, the other one the trypsin substrates BAEE and BANA. They belong to the 'serine proteases', and split B-chain of oxidized insulin in a way similar to that of vertebrates.

H. E. Hagenmaier[8]

Zoologisches Institut I der Universität Münster, Hindenburgplatz 55, D-44 Münster (Deutschland), 5. März 1971.

[1] G. Braunitzer, J. Cell. Physiol. *67*, 1 (1966).
[2] E. Margoliash, Proc. natn. Acad. Sci. *50*, 672 (1963).
[3] H. Neurath, K. Walsh und W. Winter, Science *158*, 1638 (1967).
[4] G. Pfleiderer, R. Zwilling und H.-H. Sonneborn, Hoppe-Seyler's Z. physiol. Chem. *348*, 1319 (1967).
[5] R. Zwilling, G. Pfleiderer, H.-H. Sonneborn, V. Kraft und I. Stucky, Comp. Biochem. Physiol. *28*, 1275 (1969).
[6] A. P. de Groot, Physiologia comp. Oecol. *3*, 2 (1953).
[7] W. Giebel, R. Zwilling und G. Pfleiderer, Comp. Biochem. Physiol. *38*, 197 (1971).
[8] Der Deutschen Forschungsgemeinschaft sei für finanzielle Unterstützung gedankt.

Glycopeptides from Normal and Ulcered Gastric Mucosae of Pig

Some anti-inflammatory drugs, such as phenylbutazone, salycilate, cortisone and hydrocortisone, are known to induce gastric ulcers. For such drugs, besides such action, an inhibition of $^{35}SO_4^{2-}$ incorporation has been observed for the tissues of the stomach of hypophysectomized rats[1], for the gastric mucous of rats[2], for the mucopolysaccharides of cartilage and cornea[3] and for a mucoprotein fraction of sheep colonic mucosa[4]. Such inhibiton has also been reported for rat stomach after fasting[2]. In the present paper the isolation and the chemical characterization of glycopeptides have been carried out, after papain digestion of gastric mucosae of pigs 'Göttinger Zwerg-

schwein': the choice of this animal species was due to the fact that its gastrointestinal system is very similar to the human one[5]. Our experiments were carried out on gastric mucosae of normal and phenylbutazone treated animals and on a spontaneously ulcered pig mucosa.

Materials and methods. Our experiments were carried out on 5 animals. 4 of them were selected from a breed of pigs showing statistically no presence of ulcers in gastric mucosa; they were arbitrarily divided in 2 two-animals groups. 2 pigs from the 4 selected animals were treated with phenylbutazone after a 48 h fasting: the animal we named T1 received 100 mg/kg p.o. and the other one, T2, 100 mg/kg p.o. plus 100 mg/kg i.p.; they were sacrified 24 h after the phenylbutazone treatment. The 2 remaining pigs of the stock, C1 and C2, received no treatment and were used as a control. The 5th animal, D, obtained from another source, received no treatment and presented a spontaneously ulcered gastric mucosa. The following technique was employed to investigate the gastric glycopeptides from these animals. After the death, the whole stomach was preserved in 95% ethanol at −20 °C. The scraped mucosa was digested by papain (Sigma) and the glycopeptides were recovered as Na salts as previously described[6] and fractionated by chromatography on a 1.5 × 50 cm column of Dowex 1 × 2, 200–400 mesh, Cl^- form. The column was eluted with 300 ml of distilled water and then with 300 ml solutions of NaCl of increasing molarity (0.5, 1.0, 1.5, 2.0 *M*), collecting 15 ml fractions. The anthrone reaction[7] was performed on each fraction and the positive tubes for each eluate were pooled and dialyzed against water; the glycopeptides were then precipitated as Na salts[6] from each elution sample. The precipitates were tested for fucose[8], hexoses[7], sialic acid[9], uronic acids[10] and sulphates[11]. Amino acids and hexosamines were tested by Hitachi-Perkin Elmer 034 Liquid Chromatograph instrument, after hydrolysis with 6 *N* HCl (105 °C–24 h) and with 4 *N* HCl (105 °C–7 h) respectively. Neutral sugars were tested by gas-chromatography technique: the samples were hydrolyzed at 105 °C for 90 min with 1 *N* HCl, dried under vacuum and passed through columns (0.9 × 10 cm) of Dowex 50 × 8 (H^+) and subsequently of Dowex 1 × 2 (HCO_3^-), washing each column with water (150 ml) and collecting the aqueous eluate. The TMS derivates of the samples were then analyzed by a Fractovap Mod GV instrument (Carlo Erba, Italy) as reported in a previous paper[12].

Results. Two glycopeptide fractions were obtained from each of the 5 gastric mucosa extracts by means of chromatography on Dowex: fraction 1 (F1) was eluted by water and fraction 2 (F2) by 0.5 *M* NaCl; the dried weight ratio F1/F2 being approximatively 5:1. The fractions were analyzed and the results are shown in the Table.

The F1 fractions are very similar: galactose, fucose and hexosamines of the carbohydrate moiety were present in similar amounts. The gas-chromatography analysis did not detect any other neutral sugar, except for traces of glucose. Sialic acid and sulphate ions are completely absent. The ratios threonine + serine/total protein and aspartic + glutamic acids/total protein of the F1 fractions showed no substantial differences in the protein moiety of the glycopeptides isolated from normal and ulcered mucosae. In spite of some small differences among F1 fractions, it is possible to conclude that the F1 glycopeptides are almost identical in the mucosae studied. However, they largely differ from F2 fractions in both protein and carbohydrate moieties: the protein moiety of the F2 fractions is characterized by the aspartic + glutamic acids/total protein ratio that is generally higher than the threonine + serine/total protein ratio in the F2, while it is lower in the F1 fractions (Table). As for the carbohydrate moiety of the F2 fractions, the total hexosamines content is lower than that found in the F1 fractions, and in addition to the other components, sialic acid and SO_4^{2-} ions are also present; gas-chromatography analysis shows similar amounts of mannose and traces of glucose in addition to fucose and galactose, and for the D animal shows also 2 peaks having the same retention time of standard xylose and rhamnose. The most important variation among the glycopeptides

[1] C. W. DENKO, J. Lab. clin. Med., *51*, 174 (1968).
[2] E. EZER and L. SZPORNY, J. Pharm. Pharmac. *22*, 143 (1970).
[3] M. W. WHITEHOUSE and H. BOSTRÖM, *Biochemical Pharmacology* (Pergamon Press Ltd., London 1962), vol. 11, p. 1175.
[4] P. W. KENT and A. ALLEN, Biochem. J. *101*, 43 (1966).
[5] W. MURMANN, A. GAMBA and E. PACEI, Boll. chim.-farm. *108*, 108 (1969).
[6] A. A. CASTELLANI, L. ZONTA, C. BALDUINI and G. PALLAVICINI, It. J. Biochem. *14*, 680 (1965).
[7] W. E. TREVELYAN and J. S. HARRISON, Biochem. J. *50*, 298 (1952).
[8] Z. DISCHE, B. LANDRUM and A. SHETTLES, J. biol. Chem. *175*, 595 (1948).
[9] L. SVENNERHOLM, Acta chem. scand. *12*, 547 (1958)
[10] T. BITTER and H. MUIR, Analyt. Biochem. *4*, 330 (1962).
[11] H. MUIR and S. JACOBS, Biochem. J. *103*, 367 (1967).
[12] C. BALDUINI, A. BROVELLI and A. A. CASTELLANI, It. J. Biochem. *17*, 257 (1968).

Composition of glycopeptides from pig gastric muscosae (% values)

	C1		C2		T1		T2		D	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
Hexoses	32.0	23.4	28.3	24.0	28.7	28.3	25.1	25.5	26.6	24.0
Fucose	10.0	7.8	10.4	8.0	9.8	9.2	9.5	8.3	11.6	11.0
Sialic acid	0.0	3.3	0.0	3.3	0.0	2.8	0.0	3.4	0.0	0.0
N-Ac-Glucosamine	13.0	12.7	14.9	13.3	15.8	15.6	15.1	12.1	20.8	15.6
N-Ac-Galactosamine	12.3	7.7	10.9	5.9	9.8	6.0	10.2	6.9	9.1	6.3
Total Hexosamines	25.3	20.4	25.8	19.2	25.6	21.6	25.3	19.0	29.9	21.9
SO_4^{2-} ions	0.0	8.8	0.0	7.5	0.0	4.4	0.0	4.3	0.0	3.9
Total protein	8.6	22.9	11.1	21.0	11.4	11.1	10.3	23.3	16.0	13.1
Total carbohydrates/total protein	7.88	2.39	5.84	2.59	5.65	5.61	5.84	2.41	4.28	4.34
Threonine + serine/total protein	0.31	0.11	0.25	0.11	0.42	0.27	0.32	0.12	0.37	0.26
Aspartic + glutamic acids/total protein	0.09	0.60	0.12	0.55	0.08	0.34	0.12	0.52	0.12	0.26
Total hexosamines/SO_4^{2-} ions	–	2.31	–	2.56	–	4.90	–	4.41	–	5.61

C1 and C2, controls; T1 and T2, phenylbutazone treated animals; D, spontaneously ulcered animal.

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).