pareses in muscles of the extremities or the trunk and 2 developed convulsions after provocation by tail spinning.

The virus had to be of a mouse-brain-adapted strain in order to produce abnormal amounts of HVA. Virus strains adapted to chorioallantoic membranes of embryonated chicken eggs, gave no significant increase of HVA.

With the brain-adapted strain used, Syrian hamsters produced after i.c. inoculation (300 mouse LD_{50}) HVA concentrations (0.49 µg/g of brain tissue) equivalent to what was detectable in mice. The HVA activity on non-inoculated hamster brains was assayed to 0.06 µg/g of tissue.

Results of determinations of 5-HT and 5-HIAA in *H. simplex* infected mouse brains are listed in Table III. No effect on the 5-HT concentration was encountered by the herpetic infection and for non-infected brains essentially the same amounts of 5-HT and 5-HIAA were recorded. However, the virus encephalitis caused a significant increase in the 5-HIAA concentration, the mean-values being almost twice as high for infected as for non-infected brains.

It is known from previous studies that an increase of HVA or 5-HIAA with unchanged levels of DA or 5-HT might reflect an increased synthesis of DA and 5-HT in the monoaminergic neurons[7,8]. A retarded outflow of monoamines could also be possible. However, there are no obvious reasons why the herpetic encephalitis should cause a less effective outflow via the brain-blood barrier.

Table III. Contents of 5-HT and 5-HIAA in brains of mice infected with *H. simplex* virus and in non-infected mouse brains

Experiment	No. of mice	Non-infected 5-HT	Non-infected 5-HIAA	Infected 5-HT	Infected 5-HIAA
12	22	N.D.	0.45	N.D.	0.65
13	25	N.D.	0.28	N.D.	0.41
14	10	0.30	0.47	0.37	0.93
15	19	0.34	0.36	0.39	0.60
16	22	0.38	0.30	0.41	0.93
17	12	0.44	0.33	0.38	0.63
18	14	0.36	0.32	0.28	0.81
19	22	N.D.	0.39	N.D.	0.50
Means		0.36	0.36	0.37	0.68

The mice were inoculated i.c., each with 0.02 ml (30 LD_{50}) of the virus suspension. The brains were harvested on day 5 after the inoculation. The number of harvested brains are given. Brains of a matching number of non-inoculated mice were used as controls. 5-HT and 5-HIAA values are expressed in µg/g brain tissue. N.D. means not done.

On the contrary, an inflammatory condition will tend to increase the transport through the brain-blood barrier[9,10]. An increased monoamine synthesis seems, therefore, to be the most probable background for the findings of increased HVA and 5-HIAA concentrations in the *H. simplex* virus infected mouse brains.

For the mechanism behind a raised synthesis of monoamines in herpetic encephalitis, one possibility as a result of the viral infection would be that aminergic receptors were subjects to a blocking, an effect comparable to that evoked by chlorpromazin[7]. Such an explanation is, however, not compatible with the excitatory effect of the infection.

Another possibility which should be considered is that one or more of the synthesizing enzymes, e.g. the tyrosin hydroxylase which transfers tyrosin to DOPA, and which are rate limiting, are influenced by the infection. The synthesis of these enzymes may well be controlled by normally occurring inhibitory factor set out of action by the herpetic infection. It is tempting to suppose that the possible inhibitor has the effect of a repressor preventing the function of an operon and that, as a result of the virus synthesis, proteins are formed which combine with the repressor. As a consequence this would lead to an uncontrolled production of the monoamine synthesizing enzymes[11].

Zusammenfassung. Es wird nach i.c. Applikation von *Herpes-simplex*-Virus eine erhöhte Synthese von Monoaminen gefunden. Der Nachweis gelang auf Grund der erhöhten Konzentrationen der sauren Metabolite wie Homovanillinsäure und 5-Hydroxyindolessigsäure. Eine positive Korrelation zwischen der Synthese von infektiösem Virus und der Bildung von Homovanillinsäure wurde festgestellt.

E. Lycke and B.-E. Roos

Departments of Virology and Pharmacology, University of Göteborg (Sweden), 24 January 1968.

[7] N.-E. Andén, B.-E. Roos and B. Werdinius, Life Sci. *3*, 149 (1964).
[8] B.-E. Roos, N.-E. Andén and B. Werdinius, Int. J. Neuropharmac. *3*, 117 (1964).
[9] M. Vulpe, A. Hawkins and B. Rozdilsky, Neurology *10*, 171 (1960).
[10] L. Bakay, J. Neuropath. *21*, 424 (1962).
[11] We are indebted to Mrs. Gun Lundblad, Miss Gun Alfredsson and Miss Inger Karlsson for skilful technical assistance and to the Swedish Medical Research Council, grants Nos. K68-16X-594-04 and K67-21X-165-03 for economical support.

Stimulatory Action of Secretin on Gastric Pepsin Secretion

Since Greenlee's demonstration[1] that i.v. secretin markedly diminished gastric acid secretion from the Heidenhain pouch, the inhibitory effect of secretin on acid secretion has been extensively studied in dogs and man. There is no study, however, on the action of secretin on pepsin secretion in either species.

Recently Jorpes and Mutt prepared highly purified porcine secretin[2] and reported the amino acid composition and primary sequence for its 27 amino acid residues[3].

We have found that the i.v. administration of 3 units Jorpes secretin/min during continuous histamine stimulation (1 mg/h) caused a sharp rise in pepsin output from the Heidenhain pouch to a level 8 times that obtained during the control period.

[1] H. B. Greenlee, E. H. Longhi, J. D. Guerrero, T. S. Nelson, A. L. El Bedri and L. R. Dragstedt, Am. J. Physiol. *190*, 396 (1957).
[2] J. E. Jorpes and V. Mutt, Biochem. biophys. Res. Commun. *9*, 275 (1962).
[3] V. Mutt, S. Magnusson, J. E. Jorpes and E. Dahl, Biochemistry *4*, 2358 (1965).

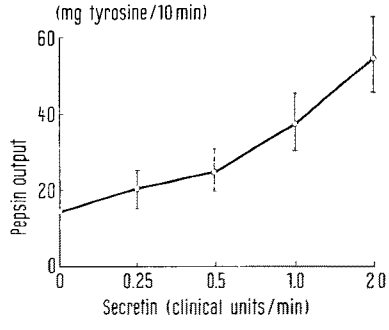
The effect of JORPES secretin (lot number 16771) on the output of pepsin has been investigated on 6 adult fasting dogs, each with a Heidenhain fundic pouch. In constructing the dose response curve, doses were changed every 30 min and the mean output of the last two 10-min-collection periods at each dose level was calculated as the response to each dose.

The Heidenhain pouch was filled with 25 ml of 0.9% saline adjusted with HCl to pH 2. This was collected and the pouch washed through with a further 25 ml of saline at the end of each 10-min-collection period. Peptic activity was determined by NORTHROP's modification[4] of the hemoglobin substrate method of ANSON[5].

The output of pepsin in response to graded doses of i.v. secretin increased progressively as the dose of secretin was increased (Figure).

BABKIN and KOMAROV[6] reported that in dogs HCl or food introduced into the intestine often stimulated pepsin secretion, whereas JANOWITZ et al.[7] showed that in man intraduodenal instillation of a variety of protein secretagogues did not augment pepsin secretion.

It is interesting to note that BUCHER and GREENGARD[8] and BABKIN and KOMAROV[6] prepared secretin which was free of pepsin stimulant although they could not isolate a pepsin stimulant itself. BABKIN and KOMAROV[6] reported that the substance or substances responsible for the pepsigogue effect were undoubtedly of a protein nature and had many properties in common with secretin. They concluded that the pepsigogue effect of crude preparations of secretin is due not to the hormone secretin but to some other substance or substances extracted from the intestinal mucosa along with it.



Dose response curve for pepsin output from the Heidenhain pouch in response to continuous i.v. secretin. Each point is the mean of 6 experiments in 6 dogs, the vertical bars represent the standard error of the mean.

In the present study it was found that the highly purified pancreozymin of JORPES and MUTT[9] (lot number 26731 and 26761), even in large doses up to 16 Crick, Harper and Raper units/min, did not stimulate pepsin secretion from the Heidenhain pouch.

It has been demonstrated that the synthetic gastrin pentapeptide is not a significant stimulant of pepsin secretion[10]. Natural hog gastrin, on the other hand, has been shown to have potent pepsigogic action in large doses[11]. It seems, therefore, that secretin may contribute to the maintenance of the pepsin secretion which continues after the cephalic phase of gastric secretion is over[12].

Zusammenfassung. Der Einfluss von Sekretin und Pankreozymin auf die Sekretion von Pepsin wurde in Hunden mit Heidenhain-Tasche untersucht. Die Hormone wurden ohne Narkose i.v. eingegeben. In allen Tieren verursachte Sekretin eine Steigerung der Pepsin-Sekretion im Magen; aber Pankreozymin hatte keinen Effekt.

D. F. MAGEE and S. NAKAJIMA

Department of Physiology and Pharmacology, Creighton University School of Medicine, Omaha (Nebraska 68131, USA), 4 April 1968.

[4] J. H. NORTHROP, M. KUNITZ and R. HERRIOTT, *Crystalline Enzymes* (Columbia University Press, New York 1948).
[5] M. L. ANSON, J. gen. Physiol. *22*, 79 (1938).
[6] B. P. BABKIN and S. A. KOMAROV, Revue can. Biol. *3*, 344 (1944).
[7] H. D. JANOWITZ, F. HOLLANDER and A. WINKELSTEIN, Clin. Res. Proc. *1*, 98 (1953).
[8] G. R. BUCHER and H. GREENGARD, Fedn Proc. Fedn Am. Socs exp. Biol. *1*, 11 (1942).
[9] J. E. JORPES, V. MUTT and K. TOCZKO, Acta. chem. scand. *18*, 2408 (1964).
[10] S. NAKAJIMA and D. F. MAGEE, Gastroenterology *54*, 134 (1968).
[11] R. A. GREGORY and H. J. TRACY, Gut *5*, 103 (1964).
[12] Acknowledgment. The expert technical assistance of Mr. G. RICE and the generosity of Prof. J. E. JORPES for supplies of highly purified secretin and pancreozymin are gratefully acknowledged.

A Complex Synaptic Apparatus in Spinal Cords of Cats

GRAY[1] described axo-axonic contacts in the spinal cord of cats as the morphological basis of presynaptic inhibition. Such contacts have since been found by many authors[2]. A more complicated synaptic apparatus is described in this report.

Normal adult cats anaesthetized with nembutal were perfused with 3% glutaraldehyde in a phosphate buffer (pH 7.4) using the technique of PALAY et al.[3]. After perfusion the seventh lumbar segment was identified and transferred to the phosphate buffer. The grey matter of the ventral horn was dissected out and cut into small pieces. After washing thoroughly in the phosphate buffer, the material was transferred for 2 h to 2% osmic acid in the buffer kept at 4 °C. The tissue was dehydrated in an ascending series of alcohols and embedded in Maraglas[4]. Thin sections were stained with a lead salt[5].

During the course of an investigation of axo-axonic contacts, a complex synaptic apparatus was found in the ventral horn of the lumbar region of the spinal cord. The

[1] E. G. GRAY, Nature *193*, 82 (1962); J. Anat. *97*, 101 (1963).
[2] M. KIDD, J. Anat. *96*, 179 (1962). – F. WALBERG, Expl Neurol. *8*, 112 (1963); Expl Neurol. *13*, 218 (1965). – E. G. GRAY and J. Z. YOUNG, J. Cell Biol. *21*, 87 (1964). – N. I. ARTYUKHINA, Dokl. Akad. Nauk. SSSR *170*, 715 (1966). – A. PETERS and S. L. PALAY, J. Anat. *100*, 451 (1966). – L. E. WESTRUM, Nature *210*, 1289 (1966).
[3] S. L. PALAY, S. M. MCGEE RUSSELL, S. GORDON and M. A. GRILLO, J. Cell Biol. *12*, 385 (1962).
[4] J. A. FREEMAN and B. O. SPURLOCK, J. Cell Biol. *13*, 437 (1962).
[5] G. A. MILLONIG, J. Biophys. Biochem. Cytol. *11*, 736 (1961).