Table II. Preformed plasma clot dissolving versus fibrinolytic activity. Inefficiency of streptokinase and urokinase as compared to Niflumic acid plus N_2H_4

Hanging clot in plasma containing	Lysis of hanging plasma clot	Fibrin plate Lysis area mm^2
Streptokinase (100 U/ml)	none	156
Urokinase (250 CTA U/ml)	none	280
12 m*M* Niflumic acid + 2.5 m*M* N_2H_4	complete	139

For readings, see text.

Small concentrations of N_2H_4 enhance the fibrinolytic activity induced by synthetic compounds, in part by a further reduction of antiplasmin activity[13]. There are 2 types of synthetic compound-induced fibrinolytic activities: in vitro or 'chemically' induced[8] and in vivo or 'pharmacologically' induced[14]. The vasoactive drug, nicotinic acid, is an example of this 'pharmacological' type of compounds which is inactive in vitro. Niflumic acid, a trifluoro-methylphenyl amino derivative of nicotinic acid as shown in this communication, induces fibrinolytic activity by both the 'chemical'[15] and the 'pharmacological' pathway, thus demonstrating the possibility to design drugs inducing fibrinolysis by both mechanisms. Niflumic acid enhances the fibrinolytic activity of both streptokinase and urokinase. It is also of interest that fibrinolytically inactive concentrations of a congener of Niflumic acid, flufenamic acid, enhance very markedly in vitro the endogenous fibrinolytic activity of pig plasma, when fibrinolytic activity is generated in vivo by a liver bypass[16]. Furthermore, as shown with the model of hanging clot in plasma, Niflumic acid is a much more effective agent for dissolving preformed clots than streptokinase or urokinase. This difference appears to be due to the small molecular size of Niflumic acid, as compared to the large size of streptokinase and urokinase. This enables it to diffuse quickly into the clot, thus inducing fibrinolytic activity from within[17]. The fact that Niflumic acid possesses, in addition to fibrinolytic properties mentioned above, the ability to prevent platelet and also erythrocyte aggregation which play a role in thrombus formation[18], makes it an attractive prototype to be used as a stepping stone for the development of optimal multiaction antithrombotic agents.

Zusammenfassung. Niflumsäure, Prototyp eines mehrfach wirkenden antithrombotischen Medikamentes, induziert Fibrinolyse in vitro und in vivo, hemmt Erythrozyten- und Thrombozytenaggregation und induziert eine stärkere Fibrinolyse vorgebildeter Gerinnsel als Strepto- und Urokinase.

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[13] K.N. VON KAULLA, in *Synthetic Fibrinolytic/Thrombolytic Agents* (Eds. K.N. VON KAULLA and J.F. DAVIDSON; C.C. Thomas, Springfield/Ill.), in press.
[14] G.R. FEARNLEY, in *Synthetic Fibrinolytic/Trombolytic Agents* (Eds. K.N. VON KAULLA and J.F. DAVIDSON; C.C. Thomas, Springfield/Ill.), in press.
[15] J.-R. BOISSIER, J.-M. LWOFF and F. HERTZ, Thérapie *25*, 43 (1970).
[16] P. OSTENDORF and K.N. VON KAULLA, in preparation.
[17] K.N. VON KAULLA, in preparation.
[18] H.I. BICHER, *Blood Cell Aggregation in Thrombotic Process* (C.C. Thomas, Springfield/Ill. 1972), pp. 5 and 19.

Possible Cellular Localization of Cholecystokinin-Pancreozymin

A number of electron-microscopic studies were carried out on the duodenal mucosa, and a total of 7 types of endocrine cells have been identified. According to the latest classification[1], the cell types in question are the EC, S, EG, I, D, D_1 and G types. However, the hormonal product is known only with some of these types of endocrine cells. The enterochromaffin (EC) cells product is the 5-hydroxytryptamine[2], the G type cells product is the gastrin[3–5], the S type cells product is the secretin[6,7], the EG type cells produce the enteroglucagon[8] and the D_1 type cells produce the gastric inhibitory polypeptide[9]. In all the cell types enumerated, with the exception of the EC cells, the presence of the hormone in the cell was proved by means of specific antisera, via immunofluorescence.

The hormonal product of the rest of the cells (the D type cells and the I type cells) is not known. Theoretically probable are, beside other substances, first of all the motilin[10] and the cholecystokinin-pancreozymin (CCK-PZ)[11], it was on the duodenal mucosa that the 2 substances have been reliably determined by biochemical analysis. Application of antisera to identify the CCK-PZ in the cells carries with it a danger of non-specific reactions, as is indicated in works of VANNORDEN and PEARSE[12], who

[1] E. SOLCIA, A.G.E. PEARSE, D. GRUBE, S. KOBAYASHI, G. BUSSOLATI, W. CREUTZFELD and W. GEPTS, Rc. Gastroenterol. *5*, 13 (1973).
[2] V. ERSPAMER and B. ASERO, Nature, Lond. *169*, 800 (1952).
[3] J.E. MCGUIGAN, Gastroenterology *55*, 315 (1968).
[4] W.G. FORSSMANN and L. ORCI, Z. Zellforsch. mikrosk. Anat. *101*, 419 (1969).
[5] G. BUSSOLATI and A.G.E. PEARSE, Histochemie *21*, 1 (1970).
[6] J.M. POLAK, S. BLOOM, I. COULLING and A.G.E. PEARSE, Gut *12*, 605 (1971).
[7] G. BUSSOLATI, C. CAPELLA, E. SOLCIA, G. VASSALLO and P. VEZZADINI, Histochemie *26*, 218 (1971).
[8] J.M. POLAK, S. BLOOM, I. COULLING and A.G.E. PEARSE, Gut *12*, 311 (1971).
[9] J.M. POLAK, S.R. BLOOM, M. KUZIO, J.C. BROWN and A.G.E. PEARSE, Gut *14*, 284 (1973).
[10] J.C. BROWN, V. MUTT and J.R. DRYBURGH, Can. J. Physiol. *49*, 399 (1971).
[11] A.A. HARPER, H.S. RAPER, J. Physiol., Lond. *102*, 115 (1943).
[12] S. VAN NOORDEN and A.G.E. PEARSE, Internat. Res. Commun. Syst. *73–3*, 13-1-1 (1973).

observed the cross-reacting property of the antisera exhibited in caerulein with the CCK-PZ and the gastrin. The caerulein, the CCK-PZ and the gastrin possess a highly similar terminal octapeptide. Therefore, we have decided to resort to physiological reactions to obtain at least an approximate localization of the CCK-PZ. In digesting the proteins in the stomach, the pepsin hydrolyses the bonds between the phenylalanine and another amino acid, thus partially digesting the proteins which change into polypeptides[13]. The polypeptides which come to the duodenum therefore have, as a rule, phenylalanine at their chain ends. These polypeptides are probably the principal stimulators of CCK-PZ secretion.

MEYER, SPINGOLA and GROSSMAN[14] proved that, following introduction of L-phenylalanine solution into the duodenum, a mass-scale release of endogenous CCK-PZ takes place, whereas the secretin production is not stimulated simultaneously. The release of the CCK-PZ after introducing the L-phenylalanine into the duodenum became the crucial point of our study, which was carried out in an effort to identify the cellular type releasing secretory granules following stimulation with L-phenylalanine. A similar method was used by FUJITA and KOBAYASHI[15] in proving the release of the hormone from D type cells of pyloric antrum of a dog, following stimulation with 0.1 *N* HCl. On the basis of their findings, these 2 authors have formed a hypothesis that the pyloric D type cells produce a hormone, such as secretin, for instance, which inhibits the HCl secretion in the stomach.

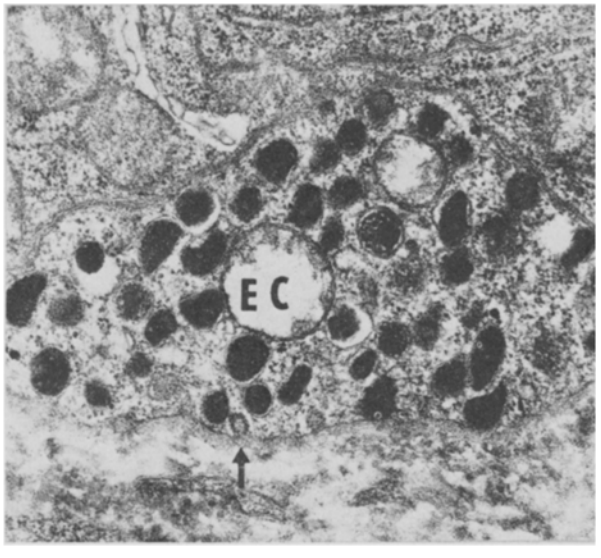

Fig. 1. Enterochromaffin cell (EC). Emiocytotic release of basal secretory granule in dog given L-phenylalanine in the duodenum. ×28,000.

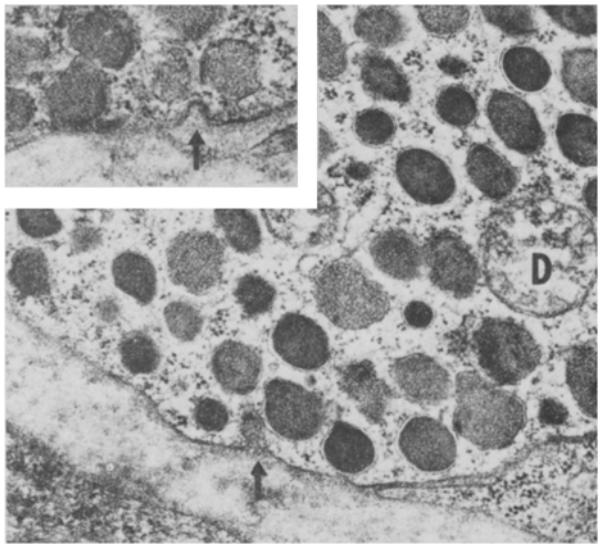

Fig. 2. Release of the D cell secretory granules in dog given L-phenylalanine. ×28,000.

Materials and method. Intraperitoneal injection of Thiopental was made into 3 anaesthetized adult dogs, and 15 ml of 127 m*M* solution (pH 7) of L-phenylalanine was introduced into the duodenum of these dogs. Samples of duodenal mucosa were taken from the point at 5 cm distance from the pylorus after 15 min in 2 dogs, and after 20 min in 1 dog. In 4 control-group dogs the duodenal mucosa samples were taken following anaesthetization only without duodenum stimulation. The samples of the tissue were fixed in 5% glutaraldehyde buffered at pH 7.4, were postfixed with 1% OsO_4 and were embedded in Vestopal W. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under Tesla BS 242 E electron microscope.

Results and discussion. All the 7 known types of endocrine cells (EC, S, EG, I, D, D_1, G) were found in dogs whose duodenal mucosa had been treated with the L-phenylalanine solution and in the duodenum of the controlgroup dogs. Following application of L-phenylalanine solution, the content of the secretion granules is released by emiocytosis into the intercellular space in the case of 2 of the cellular named types. In the basal part of the cell, some of the sacs of the secretion granules open into the intercellular space, after the fusion with plasma membrane of the cell, making Ω-shaped invaginations. We have observed this process in enterochromaffin (EC) cells (Figure 1) and in duodenal D type cells (Figure 2). No release of secretion granules was observed in any type of endocrine cells of the control-group dogs.

The enterochromaffin cells (EC) occur throughout the whole gastrointestinal mucosa of the dogs, from the cardia to the large intestine. These cells have elongated secretion granules, irregular in shape, with a highly osmiophilic core. The size of the secretion granules is within 120–480 nm range. The second cell type which exhibits the release of the secret, following application of L-phenylalanine stimulus, are the D type cells. These occur in the gastrointestinal mucosa, from the fundus, where they are sparse, to the opening of the small intestine, with maximum at the upper part of the duodenum. Their secretion granules are rounded, ranging between 160 and 400 nm in size, these granules possess medium electron density granular core.

The hormonal product is known in only one of these cell types, i.e. the EC type. The cells of this type produce 5-hydroxytryptamine[2]. Even if the EC cells did produce another substance, in addition to the 5-hydroxytryptamine, their occurrence along the whole gastrointestinal

[13] S. WRIGHT, *Applied Physiology* (Oxford University Press, London, New York, Toronto 1965), p. 455.
[14] J.H. MEYER, L.J. SPINGOLA and M.I. GROSSMAN, Am. J. Physiol. *221*, 742 (1971).
[15] T. FUJITA and S. KOBAYASHI, Z. Zellforsch. mikrosk. Anat. *116*, 52 (1971).

tract corresponds neither to the CCK-PZ localization[11,16] nor to the localization of motilin[10,17].

The hormonal product of the D type cells has not yet been identified, the localization of these cells which have their maximum in the duodenum, corresponds to the CC-PZ[11,16] as well as to the motilin[10,17]. Our findings, after the L-phenylalanine stimulation, rather testify to the presence of the CCK-PZ in the D type cells. Following introduction of L-phenylalanine, a mass-scale release of the CCK-PZ takes place, without affecting the secretin, as has been proved by MEYER, SPINGOLA and GROSSMAN[14]. Following the same stimulation, we observed the release of the secretion granules, beside the above-mentioned EC cells, only in D type cells.

FUJITA and KOBAYASHI[15] have documented the release of hormones from the D cells of the pyloric antrum after stimulation with 0.1 *N* HCl. Solutions of extremely low pH value were conductive to a strong release of the CCK-PZ[18–20]. The FUJITA and KOBAYASHI hypothesis, stating that the D cells produce an inhibitor of the secretion of the stomach acid, may be correct, although the inhibitor is not the secretin but the CCK-PZ. The CCK-PZ, similarly to secretin, acts as an inhibitor of gastrin-stimulated secretion of the stomach acid in man and in dog[16,21–23]. The other substance, which we considered to be the possible product of the D cells, is the motilin. The motilin is released, however, after alkalinization of the duodenum[17], i.e. in conditions just contrary to those in which the secret of the D type cells is released. Consequently, it is probable that a cell type other than the D type cells is responsible for the production of motilin.

Zusammenfassung. Nach intraduodenaler Stimulation der Cholecystokinin-Pancreozymin Sekretion (CCK-PZ) beim Hund durch L-Phenylalanin finden sich Anhaltspunkte für die Ausschleusung von Sekretgranula bei 2 Typen von endokrinen Zellen. Es handelt sich einerseits um enterochromaffine Zellen (EC), die 5-Hydroxytryptamin produzieren und deren Verteilung im Gastrointestinaltrakt nicht dem CCK-PZ entspricht, andererseits um D-Zellen, deren Hormonalprodukt noch nicht identifiziert ist. Es wird angenommen, dass CCK-PZ von den D-Zellen des Gastrointestinaltraktes produziert wird.

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11 March 1974.

[16] S. KONTUREK, J. TASLER and W. OBTULOWICZ, Am. J. Physiol. *222*, 16 (1972).
[17] J.C. BROWN, L.P. JOHNSON and D.F. MAGEE, Gastroenterology *50*, 333 (1966).
[18] S. NAKAJIMA and D.F. MAGEE, Am. J. Physiol. *218*, 545 (1970).
[19] H. BERRY and R.J. FLOWER, Gastroenterology *66*, 409 (1971).
[20] A.C. IVY, Physiol. Rev. *14*, 1 (1934).
[21] I.E. GILLESPIE and M.I. GROSSMAN, Gut *5*, 342 (1964).
[22] L.R. JOHNSON and M.I. GROSSMAN, Am. J. Physiol. *218*, 550 (1970).
[23] A.M. BROOKS and M.I. GROSSMAN, Gastroenterology *59*, 114 (1970).

Compensatory Spawning Response After Unilateral Ovariectomy in the Skipper Frog, *Rana cyanophlyctis* (Schn.)

VIJAYAKUMAR[1] tried subtotal ovariectomy as an indirect method of determining the occurrence of compensatory hypertrophy in newly spawned toads. The reported increase in weight of the ovarian piece left after subtotal ovariectomy can be accepted only with some reservations, because the difference in weight between the two ovaries is not regular in frogs and toads. So an attempt was made to demonstrate compensatory hypertrophy through spawning induction.

Gravid female skipper frogs weighing 25–40 g, collected around Mysore City (India), were distributed into 5 groups as mentioned in the Table. Each group contained 6 frogs.

All the frogs were induced to spawn with a homogenate of 4 toad pituitaries in 1 ml of distilled water injected i.p. and were maintained in individual aerated aquaria containing 1 to 2 cm of spring water, at room temperature 25 ± 1 °C. 24 h after induction the number of eggs spawned per frog was counted and calculated results are given in the Table.

Group No.	Treatment	Eggs spawned/frog
1	Fresh intact controls induced on day 1	857.8 ± 94.0[a]
2	Unilaterally ovariectomized and induced on day 1	413.3 ± 73.6
3	Unilaterally ovariectomized maintained for 20 days and induced on day 21	644.3 ± 67.5
4	Unilaterally ovariectomized on day 21 and induced on the same day	446.5 ± 68.1
5	Fresh intact controls induced on day 21	803.0 ± 99.4

[a] Arithmetic mean ± standard error.

It is interesting to note that Group 1 and 5 intact control frogs spawned nearly 858 and 803 eggs respectively and the unilaterally ovariectomized frogs induced on day 1 or day 21 (Group 2 and 4) spawned almost half the number of eggs, namely 413 and 446 respectively. These results are in agreement with our previous report[2]. However, hemispayed frogs maintained in the laboratory for 20 days and induced on the 21st day to spawn (Group 3) showed an increase in the spawning rate by 55.9 and 44.3 % when compared to the controls of Groups 2 and 4 respectively, the percent increase being significant.

Hemispaying results in increased ovulation by the contralateral ovary in mammals[3–6]. VIJAYAKUMAR[1] reports

[1] S. VIJAYAKUMAR, Gen. comp. Endocr. *13*, 538 (1969).
[2] M. SUVARNALATHA and H. B. D. SARKAR, Biol. Reprod. *6*, 234 (1972).
[3] C. G. HARTMAN, Am. J. Anat. *35*, 1 (1925).
[4] G. S. GREENWALD, Endocrinology *66*, 89 (1960).
[5] G. S. GREENWALD, J. Reprod. Fert. *2*, 351 (1961).
[6] A. MCLAREN, Proc. R. Soc. B. *166*, 316 (1966).