The acid chloride of IX was cyclized in high dilution (benzene) in the presence of triethylamine.

X has been shown to be identical in melting point, IR-spectrum, optical rotation, chromatographic behaviour and microbiological activity[7] (Staph. aureus ATCC 6538-P, *in vitro*) with naturally occurring Enniatin B.

[7] We are very much indebted to Prof. B. FUST and Dr. ERIKA BÖHNI from our Microbiological Department for this bioassay.

Zusammenfassung. Die Struktur von Enniatin B, eines antimikrobiellen Wirkstoffes aus Fusarienstämmen (ETH, Zürich), wird durch Synthese als die eines Cyclohexapeptolids bewiesen (vgl. X).

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The Stability of Purified Preparations of Substance P

Crude preparations of the pharmacologically active polypeptide known as Substance P are reasonably stable in solution, but various workers have found that preparations containing 600 U/mg or more, lose activity when the solutions are diluted for testing (STERN[1]).

Experiments were undertaken to discover the cause of this instability and means of overcoming it. 1 mg of a highly purified preparation of Substance P was supplied by Dr. W. Haefely of Hoffmann-La Roche & Co. Ltd., Basle. Solutions of this preparation were compared with a standard preparation of Substance P (75 U/mg) by their action on isolated guinea-pig ileum. When the purified preparation was dissolved in Tyrodes solution and added to the bath in less than 1 min, the activity appeared to be about 800 U/mg and this fell to 350 U/mg 30 min later.

The presence of proteins in the solution had two effects on the responses to Substance P.

(1) The effect of the standard preparation was potentiated. In one experiment, for example, the presence of human plasma albumin (10^{-5} g/ml in the solution added in the bath) increased the potency of the standard 3.5 times. Beef γ-globulin (10^{-5} g/ml) or gelatin (5%) had a smaller effect (1.6 times). This effect is like that described by GRANT, HOOD, and RAMWELL[2] who found that certain proteins increased the action of acetylcholine on frog rectus abdominis. In order to avoid complications in later experiments, the standard was dissolved and diluted in the same solutions as the preparations compared with it.

(2) The stability of the purified preparation was increased. In one series of experiments 120 µg of the purified preparation was weighed out, dissolved in 1 ml of Tyrode solution containing beef γ-globulin (10^{-3} g/ml), and diluted 10 times for the test. The activity of the original solution was estimated as 4200 U/mg of the purified preparation, and it appeared to be stable for a week at 4°. On dilution with 10^{-3} globulin the activity fell 30–60% in 1 h. The loss was not abolished by using polythene vessels, and was increased when the glass vessel was treated with silicone. When the diluting fluid contained 5% glycerol no loss was detected in 1 h.

In three experiments in which 10^{-3} γ-globulin was used as the diluting fluid, the glass surface was increased by adding small glass particles (ballotini) and the loss was 84, 86 and 94% in less than 40 min. This increase in the rate of disappearance of Substance P suggested that it was being adsorbed on the surface of the glass, and it was found that over 50% of the activity could be recovered by washing the ballotini with $N/50$ HCl.

In another series of experiments 120 µg of the preparation was dissolved in 1 ml of 0.9% saline containing 5% gelatin. This solution was diluted with 5% gelatin 100 times for testing, and estimated to contain 6700 U/mg of the preparation. The activity fell to 5000 in 90 min and to 4700 in 24 h. These strong solutions of gelatin thus gave better protection than γ-globulin.

A similar phenomenon has been studied by HILL[3,4] who found that insulin was adsorbed on glass, and that 5% gelatin prevented this adsorption and could be used to elute insulin from glass. Others have made similar observations[5]. Presumably the gelatin competes with the active substances for receptors in the glass, though it is possible that the active substances form a stable but still active compound with gelatin. STOUFFER and LIPSCOMBE[6] have studied a similar phenomenon in experiments which showed that highly active A.C.T.H. preparations were adsorbed on glass and could be eluted with acid.

Other workers have also described the adsorption of small quantities of active substances on glass. For example, MARSHALL[7] found that organic bases were adsorbed on glass unless the glass was cleaned by soaking in concentrated nitric acid.

VEALL and VETTER[8] state that albumin labelled with radioiodine is lost on glass, but that this can be prevented if the total concentration of albumin is kept over 1%. HJORTH[9] found that pollen extracts lost their potency as antigens in glass vials, and that this loss was prevented by adding 0.01% of the nonionic detergent known as Tween 80; in this case the loss depended on the presence of air in the vial.

It is possible that the apparent increase of activity, when proteins were added to the standard preparation, as described above, was due to the fact that in the absence of added protein there was some loss on the walls of the bath in which the assay was made. However, the fact that the standard preparation seemed to be stable casts doubt on this explanation.

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Equal volumes of a solution of purified Substance P in gelatin were measured into a series of ampoules and freeze-dried. The amount of Substance P in one ampoule was estimated by comparison with the standard preparation (75 U/mg) by four different methods. The results are shown in the Table.

Method	Units per Ampoule
Rat uterus	< 1
Guinea-pig intestine	11.2, 13.3
Fowl rectal caecum	10
Goldfish intestine	12.2

The purified preparation had no action on the rat uterus in the concentrations used. This confirms the view (GADDUM[10]) that the action of the standard preparation on this tissue is not due to Substance P itself but to an impurity.

The results obtained by the other three methods agreed with one another within the errors of the assays. It seems likely that the action of the standard in these tests is due to Substance P itself. The results with fowl rectal caecum and guinea-pig ileum confirm similar observations by FRANZ, BOISSONNAS, and STÜRMER[11], who also found that these tests agreed with tests on rabbit blood pressure. The result with the goldfish intestine confirms the view that this tissue provides a sensitive test for Substance P (GADDUM and SZERB[12]).

Zusammenfassung. Hochgereinigte Präparate von Substanz P sind in verdünnter Lösung nicht stabil, da die aktive Substanz an Glas adsorbiert wird. Der Verlust an Wirksamkeit konnte durch Zusatz von Protein zur Lösung weitgehend verhindert werden. Die wirksame Substanz kontrahierte den Darm von Meerschweinchen, Huhn und Goldfisch, war aber am Rattenuterus fast wirkungslos.

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Histochemical Adenosine Triphosphatase in the Subcommissural Organ[1]

The subcommissural organ (S.C.O.) is a specialized part of the posterior wall of the third cerebral ventricle. No fully positive notion of the function of this organ exists, though numerous facts speak in favour of its secretory activity. Granular material considered as secretion can be demonstrated by selective histological methods in the ependymal cells of the S.C.O.

A strong activity of many histochemically demonstrable enzymes, e.g. that of acid and alkaline phosphatase and non-specific esterases, occurs in the S.C.O.[2]. These findings are taken to support the view that intense metabolic processes take place in the organ.

The purpose of our study was to investigate the eventual activity of adenosine triphosphatase in the S.C.O. and to describe its localization in this organ. The rat and the cow were taken as objects of investigation because the structure of their S.C.O. exhibits the two basic patterns of the organ. The S.C.O. of the rat is made up only of an ependymal and a hypendymal layer. In the cow, as in the other ruminants, the ependymal cells are partly located in the hypendymal region, forming hypendymal rosettes and ducts there (Figure 1).

Method. For the demonstration of adenosine triphosphatase, the method of PADYKULA and HERMAN[3] was used. Unfixed cryostat sections cut at 5–40 µ and attached to cover slips were immersed for 5–30 min at 37°C in the incubating medium with the pH 9.4. The sections were mounted in glycerine jelly.

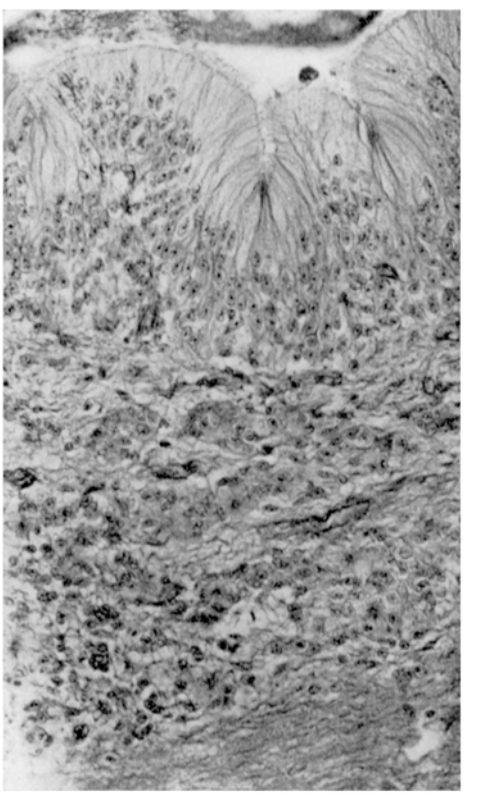

Fig. 1. Section from the subcommissural organ of the cow. The superficial ependyma is made up of tall columnar cells and the hypendyma consists of a reticular tissue and of ependymal cells forming rosettes and ducts. Below, the posterior commissure. Haematoxylin-eosin. 260 ×.

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