Reserpine-Induced Depletion of Gastric Vitamin B_{12}-Binding Proteins in the Rat

The cellular storage site of the antipernicious principle (intrinsic factor, IF) is unknown. Current opinion seems to locate IF to different exocrine gland cells in different species[1-6]. We have suggested that IF originates from one of the several types of endocrine cells[7-9], which occur in great number in the gastric mucosa and which belong to the cell system responsible for the formation of gastrointestinal polypeptide hormones (cf. ref.[10-14]). This concept is based on the strikingly similar regional and topographical distribution of some of these cells and of the gastric vitamin B_{12}-binding proteins in several mammals, and on the parallel development of these cells and the amount of gastric vitamin B_{12}-binding proteins in young rats. The endocrine cells of the gastric mucosa belong to either of two categories: argyrophil, argentaffin cells (also referred to as enterochromaffin) or argyrophil, nonargentaffin cells (also referred to as enterochromaffin-like)[11]. Among these two main categories several subtypes of cells can be recognized on the basis of differences in their ultrastructure[12-14]. One feature that all these endocrine cells have in common is their capacity to produce and store amines such as histamine and arylethylamines (cf. ref.[10,11]). The amine stores of some of the gastric endocrine cell types can be depleted by reserpine, whereas those of others are unaffected. This difference has been used to distinguish between otherwise histochemically indistinguishable gastric endocrine cells[9-11].

In the mouse, rat and hamster, the regional and topographical distribution of vitamin B_{12}-binding proteins, associated with IF activity, coincides with the distribution of the enterochromaffin-like cells[7,8], which are particularly numerous in the oxyntic gland area. In these species the enterochromaffin cells are almost exclusively found in the pyloric gland area. Also in the guinea-pig, the distribution of the vitamin B_{12}-binding proteins is well correlated with that of the enterochromaffin-like cells[8], which in this species predominate in the pyloric gland area and in the adjacent portion of the oxyntic gland area. The rabbit, however, is different in that the regional and topographical distribution of the vitamin B_{12}-binding proteins corresponds more closely with a rich population of 5-HT-containing enterochromaffin cells in the oxyntic gland area[8]; these enterochromaffin cells differ from those of the pyloric gland area in that they are conspicuously resistant to the 5-HT-depleting effect of reserpine[10,11].

Recently, reserpine was found to deplete the rat stomach of its vitamin B_{12}-binding proteins[9], while at the same time reducing the gastric histamine content by half[9] (cf. ref.[15,16]). From histochemical evidence it appeared that reserpine caused total depletion of histamine in about 50% of the enterochromaffin-like cells; the histamine stores of the remaining cells were unaffected[9]. This suggested that IF in the rat is contained exclusively in the reserpine-sensitive enterochromaffin-like cells of the oxyntic gland area[9].

Vagal denervation is known to prevent the reserpine-induced mobilization of gastric histamine in the rat[15]. In the present study, the effect of vagal denervation on both the gastric histamine content and the gastric vitamin B_{12}-binding proteins was investigated. In addition, the effect of reserpine on the gastric stores of vitamin B_{12}-binding proteins in some other mammals was studied.

Experimental. Reserpine was given i.p. in a dose of 5 mg/kg to mice, rats, hamsters, guinea-pigs and rabbits. Rats were denervated by cutting both vagal trunks just below the diaphragm. At the same time a pyloroplasty was performed in order to prevent gastric dilation. The rats were allowed to recuperate from the operation for at least 2 weeks. After receiving either 0.9% saline (controls) or reserpine, all animals were fasted until sacrifice, 24 h later. The B_{12}-binders were extracted from the mucosa of the oxyntic gland area of the mouse, rat, hamster and rabbit stomach, and from the entire gastric mucosa of the guinea-pig. The content of vitamin B_{12}-binding proteins was assayed by a semi-quantitative

Table I. Effect of reserpine on the gastric content of vitamin B_{12}-binding proteins expressed as amount of ^{57}Co-cyanocobalamin (cpm) bound to proteins extracted from 100 µg tissue (wet weight)

Species	Treatment Saline		Reserpine (5 mg/kg)	
Mouse	14,360 ± 1,320	(4)	15,640 ± 880	(6)
Rat	8,000 ± 640	(6)	1,800 ± 560	(9) [a]
Hamster	6,000 ± 680	(8)	6,200 ± 760	(17)
Guinea-pig	3,360 ± 800	(7)	2,700 ± 480	(11)
Rabbit	8,400 ± 1,960	(5)	9,200 ± 1,920	(8)

Mean ± S.E.M. [a] $P < 0.001$. Student's t-test for differences between saline-injected and reserpine-injected animals.

Table II. Failure of reserpine to mobilize gastric histamine and B_{12}-binding proteins after vagotomy

Treatment	Histamine (µg/g)		B_{12}-binding proteins (cpm/100 µg)	
Controls	70 ± 4	(4)	7,160 ± 670	(8)
Reserpine	45 ± 4	(4) [b]	900 ± 160	(12) [a]
Vagotomy	77 ± 2	(4)	8,060 ± 200	(8)
Vagotomy + Reserpine	70 ± 7	(4)	7,010 ± 820	(12)

Mean ± S.E.M. (n). [a] $P < 0.001$; [b] $0.001 < P < 0.01$. Student's t-test for differences between untreated and reserpine-treated animals.

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method[7]. The gastric mucosa was homogenized in 0.1 *M* phosphate buffer, pH 8.0. Final concentration: 20 mg (wet weight) per ml. After centrifugation at 10,000 × *g* for 10 min at 0 °C, aliquots (5–20 µl) were added to 0.5 ml of 0.1 *M* acetate buffer, pH 3.5, and mixed with ^{57}Co-labelled cyanocobalamin (approx. 20,000 cpm). 0.2 ml of the mixture was passed through a Sephadex G 25 column (length 200 mm, inner diameter 8 mm). The column was washed with 0.1 *M* phosphate buffer, pH 7.0. Protein-bound vitamin B_{12} was excluded from the gel and appeared in the first 3 ml fraction after the void volume. This fraction was collected and quantitated by γ-spectrometry. Blank values were obtained by running a parallel separation of identical amounts of free vitamin B_{12} and collecting the same fraction for quantitation.

Results and comments. In the rat, reserpine was found to mobilize gastric vitamin B_{12}-binding proteins. In the mouse, hamster, guinea-pig and rabbit, however, reserpine was without effect (Table I).

Total truncal vagotomy of the rat did not significantly affect the concentration of gastric histamine or vitamin B_{12}-binding proteins. Vagal denervation did, however, cause a total inhibition of the capacity of reserpine to mobilize both gastric histamine and vitamin B_{12}-binding proteins (Table II). The mechanism behind the effect of vagotomy on the action of reserpine is unknown. Interestingly, gastrin, which is believed to originate from a similar type of gastric endocrine cell in the pyloric gland area[17–19], is mobilized by reserpine; this gastrin-mobilizing effect of reserpine is also abolished by vagal denervation[20].

While reserpine fails to deplete the gastric stores of vitamin B_{12}-binding proteins in the mouse, hamster, guinea-pig and rabbit, the B_{12}-binders of the rat reside in a reserpine-sensitive storage pool. This seems to agree with the existence of amine-storing gastric endocrine cells, which are markedly different as regards their sensitivity to the amine-releasing action of reserpine[9–11]. In the rat, reserpine mobilizes both histamine and vitamin B_{12}-binding proteins from the gastric mucosa. After vagal denervation, reserpine fails to affect the gastric content of histamine as well as of B_{12}-binding proteins. Together these observations support the concept that locates IF to some cell type within the system of gastric endocrine cells[21].

Zusammenfassung. Reserpin mobilisiert Histamin und den «Intrinsic factor» aus dem Rattenmagen. Eine trunkuläre Vagotomie hebt diese Wirkung von Reserpin auf. Reserpin hat keinen Effekt auf den «Intrinsic factor» des Magens von Maus, Hamster, Meerschweinchen und Kaninchen.

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The Herbicide Eptam® 6-E: A Selective Female Chemosterilant for the Egyptian Cotton Leafworm, *Spodoptera littoralis*

Several adult Lepidoptera have been successfully sterilized in the laboratory through the use of the most common insect chemosterilants[1]. But for practical interest, the lack of specific food and generally the ignorance of effective attractants require their pre-emergence chemosterilization. As many other lepidopterous pests[2,3], the larval treatment of the Egyptian cotton leafworm *Spodoptera littoralis* Boisduval (Noctuidae); the most voracious pest in UAR, with aziridines[4,5] or antimetabolites[5] that mostly have been proved effective when administered to imagoes resulted in a partial (if any) sterility in the resulting adults even at toxic doses[4,5].

Therefore, compounds with apparently new mode of action were sought for and investigations were initiated in our laboratory to find effective agents that can be used as larval chemosterilants in an efficient and practically integrated program of eradication for this insect pest.

This paper reports the effects of the larval feeding with the herbicidal thiolcarbamate Eptam® 6-E (S-ethyl-dipropylthiolcarbamate (75.5% active ingredient)) on the growth rate, adult emergence, number of eggs laid and hatch. The effectiveness of other compounds against this pest will be reported elsewhere.

The rearing and the feeding techniques, as well as the assessment of the sterilant activity, were previously described[6]. The exploratory dosage-mortality tests of this study, which are not presented, showed that the optimum rate tolerated at 24 h was 250 µg active ingredient/larva where no kill observed. Each test contained at least 75 Eptam-fed last instar larvae along with about an equal number for the check group. Experiments, including untreated checks, were replicated 4 times. The Table summarizes the data obtained.

When fed to larvae, the herbicide Eptam apparently inhibited egg laying in the ensuing females that were mated to untreated males. Although in some replicates (No. 2 and 4) very few eggs were deposited in a single patch, no sign of hatch was detected. Untreated females mated to males that developed from treated larvae laid fewer eggs which were less viable than untreated checks. The average reduction in the biotic potential of these females was found to be 23.3%. The herbicide had also high selectivity for inducing a deteriorating development in females. At immature stages, the late mortality and teratogenesis were so conspicuous among females that

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