weight of dry tissue and total tissue water. ECS expressed as % of tissue water was then calculated from the following formula

$$\text{ECS } \% = \frac{\text{Supernatant total cpm}}{\text{Perfusion media cpm/ml} \cdot \text{Tissue water}} \cdot 100$$

In a few experiments, ECS has been determined by means of inulin ^{14}C in isolated midguts of Bombyx mori perfused for 60 min, following the described procedure.

Supernatants were also assayed for Na^+, K^+, Mg^{++}, Ca^{++} and Cl^-. For Na^+, K^+ and Cl^- determinations, supernatants were previously diluted 1:1 with $HClO_4$ 0.6 N and centrifuged to precipitate the proteins. Na^+ and K^+ were determined by means of a flame photometer (Beckman DU-2). Mg^{++} and Ca^{++} were assayed colorimetrically after preparation of samples following Magnesium Merckotest (Art. No. 3338) and Calcium Kit (Clinton) indications. Samples were then read by C.E. 343 Single Sample Spectrometer Cecil Instrument Ltd, Cambridge. Chlorides were determined by means of mercurimetric tritration according to Chloride Merckotest (Art. No. 3311).

Results and discussion. In midguts of Philosamia cynthia mounted as tubes, a stable total ECS value of about 42% tissue water is reached with sucrose after 60 min (figure). Experiments not reported here, performed with sulphate ^{35}S, give after 10 min an ECS value of 40.5 ± 0.7% tissue water (4 experiments): this value remains constant for 60 min, but in the second h it slowly increases with time, presumably because sulphate enters the cells or somehow interferes with the charges of epithelial membranes. Total extracellular space has also been determined with sucrose in Bombyx mori midgut, where a constant ECS value of about 45% is reached after 40 min (figure). In this tissue the inulin ECS after 60 min of incubation is 35.1 ± 0.9% (4 experiments): this lower value can easily be explained by the larger molecular weight of the marker. It is known that sucrose is not a good marker for intestinal tissue of vertebrates since it is metabolized: on the other hand, the constant value found in the midgut of both Lepidoptera suggest that the entity of metabolization, if any, is negligible in these species. Furthermore, Bombyx mori midgut seems to be lacking in α-glucosidase[9].

A very small ECS is reported by Harvey et al.[10] for the midgut of another Lepidopterum, Hyalophora cecropia; this value has been questioned by Zerahn[11]. This author determined on the same larva a sucrose ECS value very similar to those reported in this paper (45–48% tissue water). The particular morphological features of this epithelium, characterized by deep plications and invaginations of the plasma membrane[6] may provide the reason for the large ECS. Moreover it should be emphasized that the midgut of these larvae cannot be scraped, so that the ECS determination is performed on the entire tissue. Besides, the non-epithelial part of the midgut is a minor fraction of the tissue[6]. Large ECS are not unusual in vertebrates intestines too[12,13].

The tissue concentrations of Na^+, K^+, Mg^{++}, Ca^{++} and Cl^- have been determined in midguts perfused for 60 min as tubes. The intracellular values were then calculated, correcting for the proper ECS value (table). Na ion concentration is very low, not very far from that of the perfusion fluid; divalent cations are also present in very small amount, even if Mg concentration is quite high in the extracellular fluids, being in Philosamia the most concentrated cation (74 mEq/l). Potassium concentration, on the contrary, is very high in both animals, being 197.2 ± 8.4 in Philosamia and 180.9 ± 6.8 mEq/l cellular water in Bombyx. These K^+ cellular concentrations are considerably higher than those found in the midgut of Hyalophora by Zerahn who reported a cellular concentration value of 137 ± 8 mEq/l[2].

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Effect of vinblastine on pancreatic enzymes secretion induced by cyclic nucleotide derivatives[1]

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Summary. Vinblastine did not affect the basal secretion of enzymes from the rat pancreas, but it potentiates the secretory response to dibutyryl cyclic AMP. This potentiation is confirmed by the observation of numerous pictures of exocytosis at the apical part of the acinar cell. Dibutyryl cyclic GMP by itself, or associated with vinblastine, failed to modify the spontaneous release of enzymes or the secretion induced by dibutyryl cyclic AMP.

The presence of microtubules in the acinar cell of the exocrine pancreas has been recognized by several authors[2,3]. They have been involved in the secretory response of the pancreas to cholinergics[2] and digestive hormones[3]. Both types of secretagogues enhance the level of cGMP[4] in the acinar cell[5] but fail to affect the level of cAMP[4–6]. Recent evidence suggests that cAMP[7,8] and cGMP[8] may play a role in the regulation of the structure and function of microtubules and tubulin. It is generally admitted that cAMP and its dibutyryl derivative (DbcAMP[4]) stimulate enzyme release in the pancreas of many species[9–12]. However, the effect of DbcGMP[4] is still disputed[5,12,13]. In order to investigate the possible participation of microtubules in the secretory response of the pancreas to both cyclic nucleotide derivatives, we have examined the effects of a mitotic spindle-inhibitor (vinblastine[4,14]) upon both the function and the ultrastructure of the acinar cell.

Materials and methods. Pancreata were taken from $2^1/_2$ months old albino rats fasted for 12 h. They were trimmed from lymphatic ganglia and epiploic fat and cut into 8 to 10 pieces. The incubation medium, enriched by D-glucose

(10 mM) and the L-amino acid mixture of Campagne and Gruber, as previously described[15], was buffered with bicarbonate (25 mM) at pH 7.4. The gas phase was 95% O_2, 5% CO_2. DbcAMP and DbcGMP were added to the incubation medium 30 min after VB[4]. Secretion of digestive enzymes was monitored by the determination of the activity of amylase[16] and lipase[17] on aliquots from the incubation medium. Electron microscope examination was performed on pancreatic fragments fixed in 2% buffered glutaraldehyde, postfixed in 1% buffered osmium tetroxide, dehydrated in alcohols and embedded in araldite. Thin sections, doubly stained with uranyl acetate and lead citrate, were examined with a Philips EM 300 electron microscope.

Results. VB 5×10^{-5} M induces the disappearance of microtubules accompanied by the appearance of paracrystalline deposits in the cytoplasm of the acinar cells. This structural alteration requires a lag time of 20–30 min. It also depends on the concentration of the vinca alcaloid: above 5×10^{-7} M, VB completely disrupts microtubules and potentiates the secretory response to DbcAMP. The potentiation of the secretion of at least 2 enzymes:

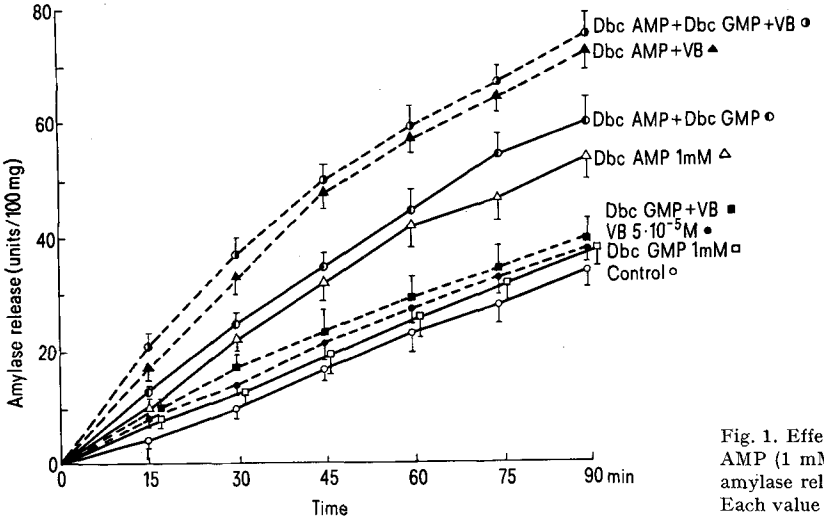

Fig. 1. Effects of vinblastine (5×10^{-5} M) on dibutyryl cyclic AMP (1 mM) and dibutyryl cyclic GMP (1 mM) induced amylase release from the rat pancreas in function of time. Each value is the mean ± SEM of 11 experiments.

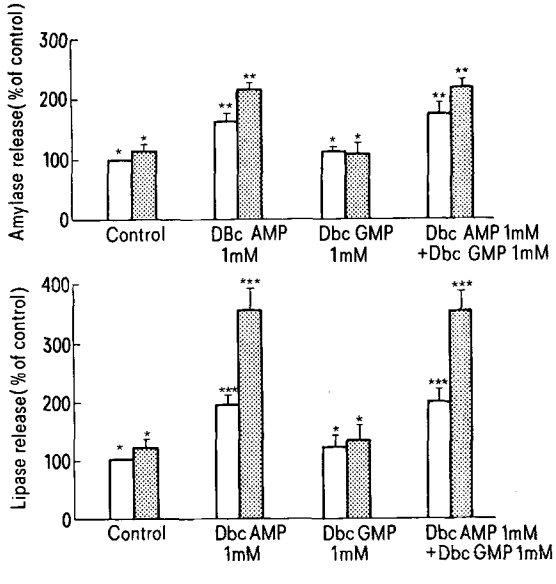

Fig. 2. Effects of vinblastine on dibutyryl cyclic AMP and dibutyryl cyclic GMP induced release of amylase (upper part) and lipase (lower part) from rat pancreas incubated for 90 min. Results are expressed in percentage of control. Control amylase: 100% = 33.5 ± 3.8 units/100 mg/90 min. Control lipase: 100% = 19.0 ± 1.8 μEq/100 mg/90 min. Each column represents the mean ± SEM of 7 experiments. Open columns: without vinblastine. Hatched columns: with vinblastine 5×10^{-5} M. The statistical significance was calculated (paired tests) for cyclic nucleotide derivatives treated tissues versus the non-treated control; VB-treated tissues in presence or in absence of nucleotide derivatives (hatched columns) were compared with their non-VB treated respective controls (open columns). * $p > 0.05$; ** $p < 0.01$; *** $p < 0.005$.

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amylase (figures 1 and 2) and lipase (figure 2) is immediate and sustained (figure 1) after a 30 min preincubation of the pancreatic fragments in presence of VB 5×10^{-5} M. Increased enzyme release by glands treated with DbcAMP 1 mM alone or in association with VB 5×10^{-5} M is confirmed by the observation of numerous pictures of exocytosis at the apex of the acinar cells as well as the presence of a dense secretory material in the acinar lumina.

DbcGMP 1 mM and VB 5×10^{-5} M, combined or not, do not significantly affect the spontaneous release of enzymes from pancreas in vitro (figures 1 and 2). In addition, DbcGMP associated with VB or alone does not modify the secretory response to DbcAMP (figures 1 and 2). These data are confirmed by the failure of DbcGMP to induce, by itself, any ultrastructural modifications.

Discussion. In a previous report[18], it was suggested that DbcAMP stimulates enzyme release from the pancreas through a direct interaction with the acinar cell. The present findings support and strenghten this view and imply the participation of microtubules in the secretory process induced by the nucleotide derivative. Indeed, the disappearance of microtubules in the acinar cell, depending on the concentration and the time of exposure to the vinca alcaloid, is accompanied by an increase of enzyme secretion in response to DbcAMP. However, the intracellular mechanism of action remains unclear. Considering the findings of Haymovits and Scheele[5] that the cellular level of cGMP is enhanced by DbcAMP, it may be speculated that cGMP plays a major role in modulating the dynamic equilibrium between microtubules and their subunits. But the microtubule-inhibitor does not affect the secretion of enzymes in presence of the dibutyryl derivative of cGMP. Moreover, the latter is completely inert on the structure and function of the exocrine pancreas, as already noted by Heisler and Grondin[13]. While the impermeability of cell membranes to this nucleotide derivative cannot be disregarded, further investigations may be necessary to clarify the interaction between cyclic nucleotides and microtubules in the pancreatic acinar cell.

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'High-affinity' binding sites for glycine in synaptosomal-mitochondrial fractions of rat CNS regions[1]

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Summary. 'High-affinity' binding mechanisms for glycine exist in synaptosome-enriched preparations of various regions of rat CNS. Such mechanisms may represent interactions of glycine with its synaptic receptors.

Glycine may be a post-synaptic inhibitory neurotransmitter in the vertebrate CNS[2-6]. 'High-affinity' mechanisms for the binding and uptake of glycine to slices and subcellular particles of various CNS regions have been demonstrated[7-10]; but such mechanisms do not appear to involve an association of glycine with synaptic receptors. However, Young and Snyder[11] have shown that ^{3}H-strychnine is bound to crude CNS membrane preparations by a 'high-affinity' mechanism ($K_D \cong 3 \times 10^{-9}$ M) which might represent an interaction of this drug with glycine-receptors. The present study reveals that 'high-affinity' glycine-binding mechanisms, which could be related to synaptic receptors, exist in 'synaptosomal-mitochondrial' fractions of several regions of rat CNS.

Materials and methods. Wistar rats weighing 175–360 g (males for data of figures 1 and 2 and females for data of the table) were decapitated, and the following CNS regions were rapidly excised and pooled: cerebral cortex (2 rats); whole cerebellum (3 rats); cervico-thoracic spinal

Amino acid competition for specific ^{3}H-glycine binding sites

Amino acid (10^{-3} M)	^{3}H-Glycine bound (pmole/g P_2) Total	Specific	Decrease in specific ^{3}H-glycine binding (%)
None	179.3 ± 14.2	159.8	–
Glycine	19.5 ± 1.9	–	100
β-Alanine	112.8 ± 10.4	93.3	41.6
L-α-alanine	120.8 ± 10.6	101.3	36.6
DL-β-aminoisobutyric acid	131.8 ± 14.4	112.3	29.7
Taurine	157.3 ± 16.8	137.8	13.8
GABA	160.2 ± 14.8	140.7	12.0
L-Glutamate	163.2 ± 17.2	143.7	10.1

^{3}H-glycine concentration was 2.4×10^{-8} M; pellets were corrected for trapped supernatant fluid using sucrose distribution ratios[12]. Specific ^{3}H-glycine binding was obtained by correcting values for 'nonspecific' binding which occurred in the presence of 10^{-3} M unlabelled glycine. Means ± SEM or mean values; 6 samples in all cases.

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