The Relation between Cholecystokinin and Substance P

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The fact that a hormonal mechanism is responsible for contraction and emptying of the gallbladder was first demonstrated by Ivy (1928) [1], who named the hormone cholecystokinin. The methods for biological determinations of the hormone have varied since then, from pressure measurements on anaesthetized animals to intestinal bath experiments on isolated gallbladder. In experiments on isolated organs, cholecystokinin preparations have proved to contract not only the gallbladder, but also the isolated intestine of the guinea-pig. As late as 1953 and 1954, Gershbein *et al.*[2,3] stated that the guinea-pig intestine is as well suited as the gallbladder for a study of the cholecystokinin effect. The relation to substance P, studied by von Euler and Gaddum [4] and by Pernow [5], has been discussed, and the question whether these two substances are identical has been raised.

In experiments on the purification of cholecystokinin preparations, it was found that the intestinal effect could be entirely removed (Hultman 1955) [6], and that the factor in the crude preparations which produced intestinal contractions *in vitro* is, in all probability, substance P. Purified cholecystokinin preparations proved to have an effect *in vitro* on the guinea-pig gallbladder but not on the intestine, whereas substance P preparations were active on the guinea-pig intestine but not on the gallbladder (Table 1).

Table 1. Intestinal bath experiment (10 ml intestinal cuvettes, readings in mm on a scale).

		Intestine	Gallbladder
Substance P	3.3 units	10	0
H 289	0.05 mg	0	10
Substance P	3.3 units	9	0
H 289	0.05 mg	0	10
Substance P	6.6 units	10	0
H 282	0.07 mg	10	0
Substance P	1.2 units	2	0

Substance P obtained from Dr. Pernow. H. 282: a fraction obtained from the crude cholecystokinin. H 289: purified cholecystokinin (after removal of H 282).

The strongest preparations of cholecystokinin produced maximal contraction of the isolated guinea-pig gallbladder in doses of 1 μg/ml of fluid in the bath. On some sensitive gallbladders, a good effect was obtained with 0.5—0.1 μg/ml.

Table 2. Intestinal bath experiments as above (10 ml cuvettes).

		Gallbladder
P.S. 117	0.05 mg	10
H 271	0.005 mg	10
H 272	0.025 mg	12
Acetylcholine	0.001 mg	20

P.S. 117, H. 271, H 272: different purified cholecystokinin preparations.

Intravenous administration of the preparations produced a powerful secretion of bile in the anaesthetized cat.

In paper electrophoresis the purest substance showed an isoelectric area around pH 7. The preparations have, in addition, a strong effect on the secretion of pancreatic enzymes.

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On the Rate of Excretion of Bile Acids in the Rat

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Rats were given [24-^{14}C]-labelled bile acids intraperitoneally. The excretion of activity in the faeces was followed. Practically all of the isotope appeared in the faeces [1]. From the rate of excretion the half life of a bile acid molecule was estimated to be 2—3 days.

A certain amount of bile acid is thus lost during each enterohepatic circulation. One of the factors influencing the magnitude of this loss might be the intestinal microorganisms.

In order to study this, the rate of excretion was followed in rats in which the intestinal flora had been reduced by treatment with terramycin and phthalylsulfonazonum. In these rats the rate of excretion was much slower than in normal animals.

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Demonstration of a Thermostable Cofactor of Oxidative Phosphorylation in Rat Liver Mitochondria

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It has previously been reported that addition of adenosine triphosphate (ATP) and Mn^{++} to mitochondrial suspensions pretreated in the presence of 0.5 m*M* Ca^{++} and 4 m*M* Mg^{++} for 5 minutes at 30° C induces a reconstruction of the phosphorylations accompanying the aerobic oxidation of succinate [1]. Subsequently, these effects could be studied in greater detail with mitochondrial systems aged in Mg^{++} — and Ca^{++} — free media [2]. The yield of reconstructed phosphorylation in these systems shows an inverse relationship to the time of aging, and its fall can be greatly enhanced by the presence of Ca^{++} in the Mg^{++}-free preincubation medium. Recentrifugation studies of the aged suspensions have revealed that, during aging, a factor is released from the mitochondria, which is involved in the phosphorylative mechanism [3]. This factor has now been shown to be stabilized by heating the preincubation medium, obtained after recentrifugation, to 100° C for 2 minutes. Furthermore, the presence of the factor could be demonstrated in boiled extracts of freshly prepared mitochondria. Experimental data bearing on this are shown in Table 1.

Mitochondria from one rat liver were suspended in 30 ml of a buffer solution (pH 7.5) containing KCl, orthophosphate, adenylic acid, glucose and sucrose (in concentrations specified previously [2]). After removal of a sample serving as control (incubated immediately in the presence of succinate, hexokinase and Mg^{++}), the suspension was warmed to 30° C and supplemented with hexokinase and 0.5 m*M* Ca^{++}. After 5 minutes of preincubation under shaking, the suspension was cooled to 0° C, divided into four parts and each part recentrifuged for 5 minutes at 5 000 *g* in the high-speed attachement of an International Refrigerated Centrifuge. The four mitochondrial pellets (*a*—*d*) were resuspended, to a final volume equal to that before centrifugation, in *a*) a new incubation mixture of the same composition as before; *b*) its own supernatant; *c*) the supernatant heated for 2 minutes in a boiling water bath (the slight precipitate formed on heating was removed by centrifugation); and *d*) in an extract of freshly prepared mitochondria, obtained by heating a suspension (of the same composition as the original one, but twice as concentrated with respect to mitochondria) for 2 minutes in a boiling water bath, and centrifugation as above. From each of the four suspensions two aliquots of 1.5 ml were incubated in Warburg vessels, one with succinate alone, and a second one with succinate, ATP and Mn^{++}. Each vessel was furthermore supplemented with Mg^{++} and a suitable excess of hexokinase. Respiration and phosphorylation were measured for a period of 20 minutes.

Table 1. Oxydative phosphorylation in recentrifuged mitochondria aged in the presence of Ca^{++}.

Each Warburg vessel contained: KCl, 150 μmoles; orthophosphate, 50 μmoles; adenylic acid, 4.3 μmoles; glucose, 60 μmoles; sucrose, 125 μmoles; succinate, 30 μmoles; Mg^{++}, 4 μmoles; hexokinase, *ca.* 10 fold excess[2]; Ca^{++} (in pretreated systems), 1 μmole. Additions (where indicated): ATP 1.5 μmoles; Mn, 1 μmole. Final volume, 2.0 ml. Gas phase, air. Temp, 30° C. Time of incubation, 20 min.

	Additions	Respiration (μatoms oxygen)	Phosphorylation (μmoles phosphate)
Untreated system	—	14.9	27.0
Pretreated system resuspended in:			
a) new incubation mixture	—	18.0	1.0
	ATP, Mn^{++}	16.7	0.5
b) supernatant	—	16.6	2.1
	ATP, Mn^{++}	14.0	1.3
c) boiled supernatant	—	18.3	1.0
	ATP, Mn^{++}	15.9	20.0
d) boiled extract of fresh mitochondria	—	14.5	1.4
	ATP, Mn^{++}	18.0	23.2