Paw irritancy (oedema) and gastric irritancy (mucus effusion, gastric swelling) of Cu, Zn and Ni ions in rats: Correlation with free Cu(II)[a] applied in stomach after fasting or in extragastric tissue

Compound	pCu at		Paw irritancy[b]	Gastric irritancy[c]	
	pH 4.5	pH 7.4		Mucus effusion	Swelling
$CuCl_2$	1.7	1.7	+++	3.0	3.0
$Cu(imidazole)_2Cl_2$	2.3	4.7	+++	3.0	3.0
Cu(II)-carnosine	2.0	7.5	+++	2.7	3.0
Cu(II)-salicylate$_2$	2.6	5.4	++	1.0	0.4
Cu(II)-histidine$_2$	8.2	12.6	+	1.3	1.3
Cu(II)-TRIEN	6.8	14.7	0	0.3	0.5
Cu(II)-Gly · His · Gly	3.7	10.3	0	0.4	0.2
Cu(II)-Gly$_2$ · His · Gly$_2$	2.7	12.5	0	0.2	0.1
Cu(II)-NTA	7.0	9.9	0	0	0
Cu(I)Cl[d]			++++	1.0	2.5
$Cu(thiourea)_3Cl$[d]			++++	3.0	3.0
$Cu(CH_3CN)_4ClO_4$[d]			++	3.0	3.0
Cu(I)-thiomalate, Na$_2$			++	1.0	1.0
Cu(I)-penicillamine			0	0	0.3
10% thiodiglycol			0	0	0
Cu° (metal powder)			0	0	0
$ZnCl_2$			+++	0.3	0
$NiCl_2$			+	2.3	2.3

TRIEN, triethylenetretramine; NTA, nitrilotriacetic acid.

[a] pCu(II) = negative logarithm$_{10}$ of the Cu(II) concentration (cf. pH), calculated from pK_a's and stability constants[10,11] of the Cu-ligands, for total Cu = 20 m*M* using SPECON 18, a variant of the programme COMICS[12].

[b] Paw irritancy scored by increase in paw thickness: <1 mm = 0; 1–2 mm = +; 2–3 mm = ++; 3–4 mm = +++; >4 mm = ++++, 2 h after injecting 2 µmoles metal ion in isotonic saline; pH metal complexes adjusted to 6–6.5.

[c] 2 h after 100 µmoles/kg p.o. in starved rats.

[d] Cuprous compounds stabilized in aqueous solution with 10% (v/v) thiodiglycol.

2. Aspirin (100 mg/kg) causes considerable gastric lesions – even in unstressed rats within 2 h[5,9]. Pretreating a group of animals with oral $CuCl_2$ (50 µmoles/kg) 60 min before administering this dose of aspirin (= 550 µmoles/kg) induced sufficient effusion of mucus and watery exudate to wholly protect the mucosa from the deleterious effects of the aspirin. [Lesion index with no pretreatment = 17.2; with $CuCl_2$ pretreatment = 0]. Removing the available i.g. Cu(II) by subsequent administration of TRIEN or NTA (55 µmoles/kg) or L-histidine (110 µmoles/kg) 10 min before giving aspirin did not block the anti-ulcerant effect (i.e. lesion index was still zero). This showed that it was probably mucus released in response to Cu(II) that afforded protection from the aspirin since a) Cu-TRIEN and Cu-NTA are not protective irritants in the stomachs of fasted rats (Table), and b) this quantity of Cu(II) could 'neutralize' only 18% of the aspirin applied. (However, even Cu(II)-aspirin$_2$ is ulcerogenic[3], being decomposed by the normal gastric acidity to liberate free aspirin).

Cupric chelates have been reported to have anti-ulcer activity in the Shay rat ulcer model[13,14]. Our findings suggest that it is probably uncomplexed Cu(II), rather than the chelated Cu in the stomach, that has the ulcer-protectant activity.

Effect of Sulfated and Non-Sulfated Gastrin and Octapeptide-Cholecystokinin on Cat Gall Bladder in vitro[1]

J. R. Chowdhury[2], J. M. Berkowitz, M. Praissman and J. W. Fara[3]

Departments of Physiology and Biophysics, and Medicine, State University of New York at Stony Brook, Stony Brook (N.Y. 11794, USA); and Division of Gastroenterology, Nassau County Medical Center, East Meadow (N.Y. 11554, USA), 9 February 1976.

Summary. This study demonstrates that for the isolated cat gall bladder a smaller molar dose of the sulfated form of OP-CCK and gastrin is required to produce contraction as compared to the respective non-sulfated forms. For OP the D_{50} for the sulfated form versus the non-sulfated form was 1.94. For gastrin it was 1.10.

Structure-activity relationships of several gastrointestinal hormones and their analogues have been studied on a variety of tissues. Of particular interest has been the importance of sulfation of the tyrosine residue in the C-terminal 7th position in cholecystokinin (CCK) and its analogues, and in the 6th position in gastrin. The present

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[2] Present address: Dept. of Medicine, Division of Gastroenterology, Albert Einstein College of Medicine, Bronx, N.Y., USA.

[3] Address reprint requests to JWF, Dept. of Physiology and Biophysics, Health Sciences Center, State University of New York, Stony Brook, N.Y. 11794, USA.

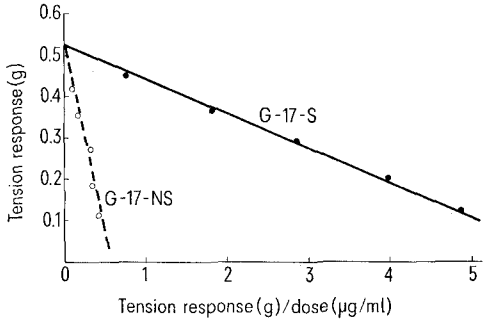
study was done to compare isomeric tension responses to sulfated (G-17-S) and non-sulfated (G-17-NS) porcine gastrin and sulfated (OP-S) and non-sulfated (OP-NS) synthetic C-terminal octapeptide of cholecystokinin on strips of cat gall bladder.

Methods. Gall bladders were removed from cats anesthetized with ketamine (30 mg/kg) and pentobarbital (20 mg/kg). Each gall bladder was cut into 15 mm × 3 mm strips which were then mounted in a Krebs solution bath[4], between a stationary hook and an isometric strain gauge (Grass FT-03 or Statham UC-2). Temperature of the bath was maintained at 37 °C and aerated and stirred with a gas mixture of 95% O_2 and 5% CO_2. Tension was recorded on a Grass 7C polygraph.

To begin each experiment, a 1200 mg passive tension was applied and 30–45 min allowed before drugs or hormones were tested. Following this period, the viability of the preparation was tested by adding acetylcholine (1 µg/ml) to the bath. Individual tissue strips gave reproducible results over 6–8 h.

Hormones and drugs used: non-sulfated synthetic human gastrin I (HSG-NS), purchased from Imperial Chemical Industries, Ltd., Cheshire, England; sulfated porcine gastrin II (G-17-S) and non-sulfated porcine gastrin I (G-17-NS), prepared by Professor R. A. GREGORY and Dr. H. J. TRACY, University of Liverpool, and kindly given to us by Dr. M. I. GROSSMAN; sulfated (OP-S) and non-sulfated (OP-NS) octapeptide-cholecystokinin, kindly provided by Dr. M. ONDETTI, Squibb Institute, Princeton, N.J.; and acetylcholine chloride (Sigma). Doses are expressed as molar concentrations in the bath.

Dose-response curves were obtained by randomly introducing a dose of one hormone and waiting until the maximal response to that dose was reached (usually 10–15 min). The bath was then rinsed 3 times and 15 min allowed before a new dose was tested. In some experiments, cumulative dose-response curves were also obtained. Both methods gave essentially the same curves. To compare responses between two hormones, they were tested on the same tissues in a randomized sequence. The range of effective concentrations of OP-S and OP-NS were studied on 7 tissues obtained from 4 cats. G-17-S, G-17-NS, and HSG-NS were tested on 10 tissues from 6 different cats. The maximal response to each hormone and the dose required to produce half the maximal response (D_{50}) were obtained both by direct observation and by DOWD-RIGGS linear transformation of Michaelis-Menten constants[5]. Plotting the response on the ordinate and the response/dose ratio on the abscissa, the y-intercept gives the calculated maximal response (CMR), and the slope of the curve, with change of sign, gives the D_{50} (Figure). Only the linear portion of the curves were used to calculate CMR and D_{50}.



DOWD-RIGGS transformation of a typical dose-response curve of G-17-NS, dashed line ($y = -0.9x + 0.515$; $r = 0.90$) and G-17-S, solid line ($y = -0.82x + 0.525$; $r = 0.89$). Isometric tension response (g) is plotted on the ordinate, and tension response (g)/dose of gastrin (µg/ml) on the abscissa. The y-intercept gives the calculated maximal response, and the slopes of the curves the respective D_{50}'s.

The dose of peptides required to produce half the maximal response (D_{50}), and the relative potency of different peptides in relation to OP-S on the basis of D_{50}

Peptide[a]	Mean D_{50} (M)	D_{50} relative to OP-S[b]
OP-S	8.36×10^{-10}	1
OP-NS	7.92×10^{-7}	943
G-17-S	3.71×10^{-8}	44
G-17-NS	4.07×10^{-7}	485
HSG-NS	4.29×10^{-7}	513

[a] OP-S and OP-NS were each tested one or more times on 7 tissues obtained from 4 cats. G-17-S, G-17-NS, and HSG-NS were tested on 10 tissues from 6 cats. [b] Number of molecules needed to produce a similar response. Because the CMRs for gastrin averaged 70% that of OP-S, for gastrin preparations to produce an effect equal to that of D_{50} OP-S, the dose required would be the D_{50} of gastrin times 1.43.

Results. The minimally effective dose of OP-S was 2.2×10^{-10} M. The maximal response was obtained with 5.3×10^{-9} M. Values for OP-NS were 2.2×10^{-7} M and 7.0×10^{-6} M, respectively. There was no significant difference between the maximal isomeric tension responses obtained with OP-S and OP-NS.

The effective dose range for G-17-S was 3.5×10^{-9} to 1.1×10^{-7} M. Values for G-17-NS were 5.7×10^{-8} to 2.7×10^{-6} M, and for HSG-NS were 1.1×10^{-7} to 1.8×10^{-6} M. There was no significant difference in maximal responses obtained with G-17-S, G-17-NS, and HSG-NS, and the maximal response obtained with each of the three gastrins averaged 70% that obtained with OP-S.

On comparison of respective D_{50}'s (Table), OP-S was nearly 1000-times more potent than OP-NS, while G-17-S was about 10-times more potent than G-17-NS and HSG-NS.

Discussion. Effects of sulfated versus non-sulfated gastrin and octapeptide-CCK have not been previously compared on cat gall bladder, although such studies have been done in other animals and on other tissues. The large difference in potency currently observed between OP-S and OP-NS is consistent with effects on guinea-pig gall bladder in vitro[6] and on rat pancreatic secretion[7], and emphasizes the importance of sulfation of the 7th position tyrosine.

For gastrin, which shares the same C-terminal pentapeptide group with OP-CCK but which has a sulfated or non-sulfated tyrosine residue in the 6th position, the effect of sulfation is not as critical for determining biologic potency. On cat gall bladder, G-17-NS was about 10-times less potent than G-17-S. By contrast, G-17-S and G-17-NS are reportedly equipotent on dog gall bladder in vivo[8]. In other in vitro preparations of rabbit and guinea-pig gall bladder, sulfated gastrin is slightly more

[4] J. R. CHOWDHURY, J. M. BERKOWITZ, M. PRAISSMAN and J. W. FARA, Am. J. Physiol. *229*, 1311 (1975).
[5] J. E. DOWD and D. S. RIGGS, J. biol. Chem. *240*, 863 (1965).
[6] A. ANASTASI, L. BERNARDI, G. BERTACCINI, G. BOSISIO, R. DE CASTIGLIONE, V. ERSPAMER, O. GOFFREDO and M. IMPICCIATORE, Experientia *24*, 771 (1968).
[7] G. J. DOCKRAY, Q. Jl exp. Physiol. *58*, 163 (1973).
[8] M. VAGNE and M. I. GROSSMAN, Am. J. Physiol. *215*, 881 (1968).

potent than the non-sulfated form[9]. Therefore, it appears that for the in vitro gall bladder G-17-S is generally more potent than G-17-NS; however, for other target tissues one cannot generalize. Thus, G-17-S and G-17-NS are equipotent in stimulating rat gastric secretion in vivo and in contracting hamster stomach and rat colon in vitro[10]. They are also equipotent in stimulating cat gastric acid secretion (Dr. M. GROSSMAN, personal communication). But as a secretogogue of bullfrog gastric mucosa in vitro, G-17-S is 10-times more potent than G-17-NS[11].

There are no reports wherein the sulfated form of gastrin, CCK, or their analogues has been found to be less potent than the non-sulfated form.

The molar concentrations of OP-S which were currently found to be effective in eliciting a response on cat gall bladder, i.e. 2.2×10^{-10} to 5.3×10^{-9}, approximate fasted human plasma levels of CCK[12,13]. On the other hand, the concentrations of OP-NS and the 3 gastrin preparations needed to elicit responses probably would not be reached in any physiological situation.

[9] M. S. AMER, Endocrinology *84*, 1277 (1968).
[10] E. MIKOS and J. R. VANE, Nature, Lond. *214*, 105 (1967).
[11] L. W. WAY and R. P. DURBIN, Gastroenterology *56*, 1266 (1969).
[12] R. F. HARVEY, M. HARTOG, L. DOWSETT and A. E. READ, Lancet *2*, 826 (1973).
[13] D. A. REEDER, H. D. BECKER, N. J. SMITH, P. L. RAYFORD and J. C. THOMPSON, Ann. Surg. *178*, 304 (1973).

Loss of Bioluminescence in *Anomalops katoptron* due to Starvation[1]

V. B. MEYER-ROCHOW

Department of Biological Sciences, University of Waikato, Hamilton (New Zealand), 1 March 1976.

Summary. After 3 weeks of starvation the bacterial light-organs of the bioluminescent shallow-water fish *Anomalops katoptron* cease to produce light. Because of a reduction of the number of symbionts in the cells of the light organ, it is concluded that the fish supplies its luminescent bacteria with nutrients out of its own metabolism. As a result of starving the fish, the luminescent bacteria decrease in number and finally cease to emit light.

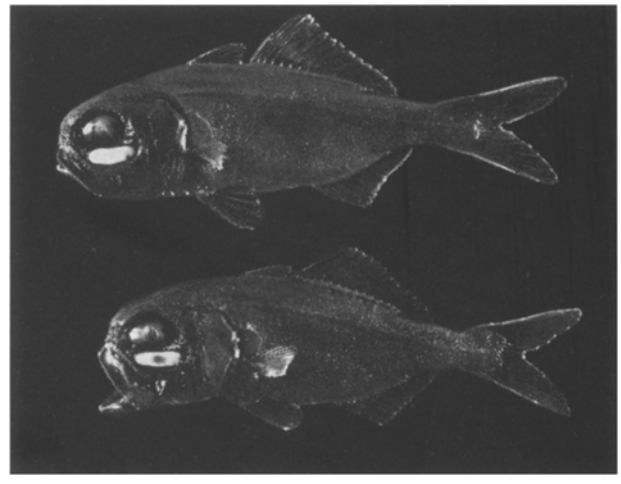
Anomalops katoptron is a bioluminescent shallow-water fish, which is indigenous to the Banda Islands in the South Moluccan Sea. It emits a greenish light from a pair of bean-shaped organs below the eyes. The light-emitting structures contain luminous bacteria[2]. STECHE[3] studied the anatomy and histology of the light organ in detail.

The side of the organ which emits light continuously, is creamcoloured, while the opposite face is black owing to a pigmented cell layer. During the day the light is extinguished by rotation of the entire light organ along its long axis, so that the luminous side is turned towards the body, presenting the black pigmented face to the outside. At night the luminous side is turned to the outside, and the light is presumably used to aid in navigation, find food and help keeping the school together.

During the 1975 Alpha Helix South East Asia Bioluminescence Expedition, I kept 7 *Anomalops* in a $73 \times 32 \times 30$ cm glass aquarium, the sides of which were covered with dark-red cellophane. The water was oxygenated and hiding places for the fish, made from dead coral blocks and rocks, existed. The fish were caught during moonless nights by local fishermen. *Anomalops* can be easily herded by the beams of 2 torches into shallow water, where they are caught with hand nets.

Normally the fish were hiding under stones during the day and had their lights extinguished by the method described above. Coinciding with sunset they regularly came out and swam all night, displaying a bright luminescence. At dawn they retreated again to their resting places. Although the light would be displayed when the fish were moved into the dark, even during the day, the activity rhythm appeared to be independent of whether the animals were kept in continuous light or darkness.

After 1 week of starvation the light intensity emitted by the light organs of all *Anomalops* became noticeably weaker. After 2 weeks starvation light production in the central part of the light organ had dropped to a level which made the light organ seem to have a grey spot when studied at night (Figure). After 3 weeks of starvation the light organs, to the human eye, did not appear to emit any light any longer; they were just of a white creamy colour. Yet, all the fish behaved perfectly normal and continued their precise circadian activity rhythm. After 4 weeks of starvation, (the fish still did not show any signs of malnutrition) some of the non-luminescent light organs were fixed in seawater/collidine-s buffered 2% OsO_4 solution, and later examined in the electron microscope.



Two *Anomalops*, the lower individual starved for 2 weeks. Photograph slightly retouched to demonstrate how the light organ of the starved fish gradually turned non-luminescent, starting in the centre.

[1] Supported by a Queen Elizabeth II Fellowship in Marine Science, and carried out during the 1975 'Alpha Helix' South East Asia Bioluminescence Expedition.
[2] Y. HANEDA and F. I. TSUJI, Science *173*, 143 (1971).
[3] O. STECHE, Z. wiss. Zool. *93*, 349 (1909).