developed considerable increases in temperature after enterotoxin, and the recipients of their plasma responded with fevers having the characteristics ascribed to endogenous pyrogen. (Similar fevers were also elicited by transfer of sera from non-tolerant donors to tolerant recipients.) The recipients' temperatures typically began to rise 10–15 min after the transfer, and the responses were usually over after 2–3 h. These pyrogenic responses differed significantly from the responses to plasma from enterotoxin-tolerant donors ($P < 0.02$, Mann-Whitney U test[7]). Repeated injections of pyrogenic plasma did not result in tolerance development by the recipients.

Discussion. In accord with current concepts of the pathogenesis of fever[5], the most likely explanation for the fevers seen in these experiments is that they were due to transfer of endogenous pyrogen which was released into the plasma of the donor during enterotoxin-induced fever. The responses were typical of endogenous pyrogen when released by a variety of other agents and in other species. Circulating endogenous pyrogen has also been reported after enterotoxin administration in rabbits[8]. The results, however, do not eliminate the possibility that a metabolically altered form of enterotoxin is responsible for the fever. Such an explanation is perhaps favored by our inability to demonstrate an enhanced release of pyrogen from granulocytes (the most likely source of endogenous pyrogen in most experimental fevers) by in vitro incubation with enterotoxin.

The enterotoxin originally given the donors cannot account for recipient responses since the recipients were tolerant to much larger amounts of enterotoxin than can possibly be transferred in the 5-h plasma. Studies in a number of species, including the cat, indicate that enterotoxin is very rapidly removed from blood[9,10]. Neither can contamination with extraneous pyrogens such as bacterial endotoxin account for the results since the response pattern, latency, etc. were different than those expected for endotoxin; no tolerance could be demonstrated; no bacterial contamination was noted in sterility tests, and fevers were not caused by plasma from enterotoxin-tolerant donors handled similarly to the pyrogenic plasma from non-tolerant donors[11].

Conclusion. The presence of a pyrogen with the physiological characteristics of endogenous pyrogen was demonstrated in plasma (and serum) taken from cats 5 h after i.v. injection of staphylococcal enterotoxin B.

Résumé. On a montré la présence d'une substance pyrogène qui avait les caractéristiques physiologiques endogènes dans le plasma et dans le sérum sanguin de félin, pris 5 h après l'injection i.v. d'entérotoxine staphylococcique B.

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Cholecystokinetic Activity of a New Synthetic Caerulein-Like Heptapeptide in Man

It has recently been shown (ANASTASI et al.[1]) that the biological activity of caerulein and caerulein-like peptides is greatly reduced by de-sulphation. Since the tyrosyl-*O*-sulphate bond is rather unstable, particularly at pH < 7, the synthesis of analogues in which the tyrosyl sulphate residue is replaced by the stable *p*-sulphonylphenylalanine residue was undertaken. It was hoped to obtain peptides still retaining the biological activities of caerulein and which would be more stable and possibly longer acting.

In the present note, the cholecystokinetic activity of the new heptapeptide *p*-Phe(SO_3Na)-Thr-Gly-Trp-Met-Asp-Phe-NH_2 is reported. Experiments were carried out on 25 normal volunteers of both sexes, aged 20–60 years. The biliary system was filled by oral (15 subjects) or endovenous contrast medium (10 subjects). Iodopanoic or ioglycamic acid, respectively, were employed. However, no difference in the spasmogenic effect of the peptide was noted by changing the contrast medium or the route of administration.

The new synthetic peptide, supplied by Farmitalia Research Laboratories (Milan), (molecular weight 1004) in a 10% methanolic solution, was administered after removal of the solvent under air stream in a boiling water-bath and the remaining aqueous liquid was brought to the desired volume with physiological saline solution. Doses of 0.5, 1, 10 μg/kg body weight by i.m. injection and of 10 μg/kg by nasal insufflation were used. These doses were approximately 10-fold as high as doses used in the same experimental conditions in previous investigations (ORLANDINI and AGOSTI[2]). Indeed unpublished experiments performed in this Institute of Pharmacology (BERTACCINI, personal communication) showed that this heptapeptide has in different laboratory animals approximately $^1/_{10}$th of the activity of caerulein.

Radiographic serial exposures were obtained at 15, 30, 45, 60, 90 and 120 min after the beginning of the administration. The quantitative evaluation of gall bladder emptying was calculated either by measuring the largest transverse diameter according to BRODÉN[3] or by calculating the whole area of the gall bladder on the roentgenogram with a Salmoiraghi planimeter.

Results obtained are shown in Figure 1. Data represent reduction of volume of the gall bladder after synthetic peptide and caerulein administration in comparison with basal (pre-drug) values considered as 100. It is evident from the figure that the action of the synthetic peptide is more prompt and less lasting than that of caerulein. The 2 peak effects were practically equal identical.

The peptide produced an active cholecystic contraction and the effect on the evacuation was marked by a reduction in the gall bladder size and by filling of the biliary ducts.

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The threshold spasmogenic dose by intramuscular route was 0.5–1 μg/kg. With this dose reduction of gall bladder size never exceeded 10% and lasted about 15 to 20 min. With the dose of 10 μg/kg there was a potent cholecystokinetic effect and the cholecystis decrease to about 70–80% of the initial pre-drug size, the gall bladder contraction began 5–10 min after the administration and lasted more than 90–120 min.

Three subjects, treated with 10 μg/kg of the synthetic peptide, experienced nausea, facial flush, mild tachycardia and profuse perspiration. These untoward reactions, however, lasted only a few minutes after the injection.

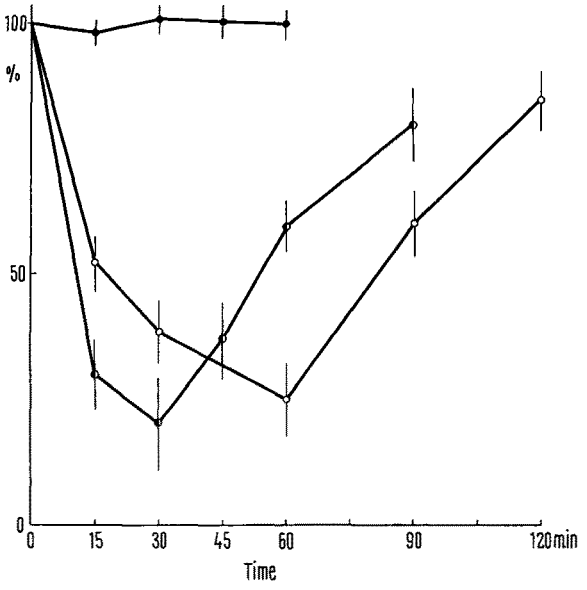

Fig. 1. Percentage of reduction of the largest transverse diameter of the gall bladder obtained after administration of 10 μg/kg i.m. of the heptapeptide (◐) or 1 μg/kg i.m. of caerulein (○). ●, controls which received only physiological saline. Each point represents the mean of the values obtained from 5 individuals. Vertical bars are S.E. of the mean.

Results obtained after nasal administration were approximately overlapping to those reached after i.m. injection (10 μg/kg) and this confirmed the observations of one of us (AGOSTI and BERTACCINI[4]) in the case of caerulein (Figure 2).

In the present investigation it has been shown that the new synthetic caerulein-like possesses on human cholecystis a striking spasmogenic activity, which was more prompt than that elicited by 1 μg/kg of caerulein. In fact gall bladder peak reduction (70–80%) was reached 15–30 min after synthetic peptide and 60 min after caerulein. The ratio of activity between caerulein and this peptide was, on weight basis, about 1:10.

These results seem to be of interest also for possible clinical use of this new synthetic peptide in the radiological routine examination of the biliary apparatus as well as caerulein, the advantages of which have been previously reported (BERTACCINI, BRAIBANTI and UVA[5]; ORLANDINI and AGOSTI[2]).

Riassunto. Su un gruppo di soggetti normali è stata studiata, in corso di colecistografia, l'azione colecistocinetica di un nuovo eptapeptide caeruleinosimile, che iniettato per via intramuscolare alla dose di 10 μg/kg ha provocato riduzioni del volume della colecisti in media del 70–80%.

A. AGOSTI, I. ORLANDINI and R. DE CASTIGLIONE[6]

Istituto di Farmacologia dell'Università di Parma, Reparto Radiologico, Ospedale Carlo Poma, Mantova, and Istituto di Ricerche, Farmitalia S.A., Via dei Gracchi 35, I-20146 Milano (Italy),
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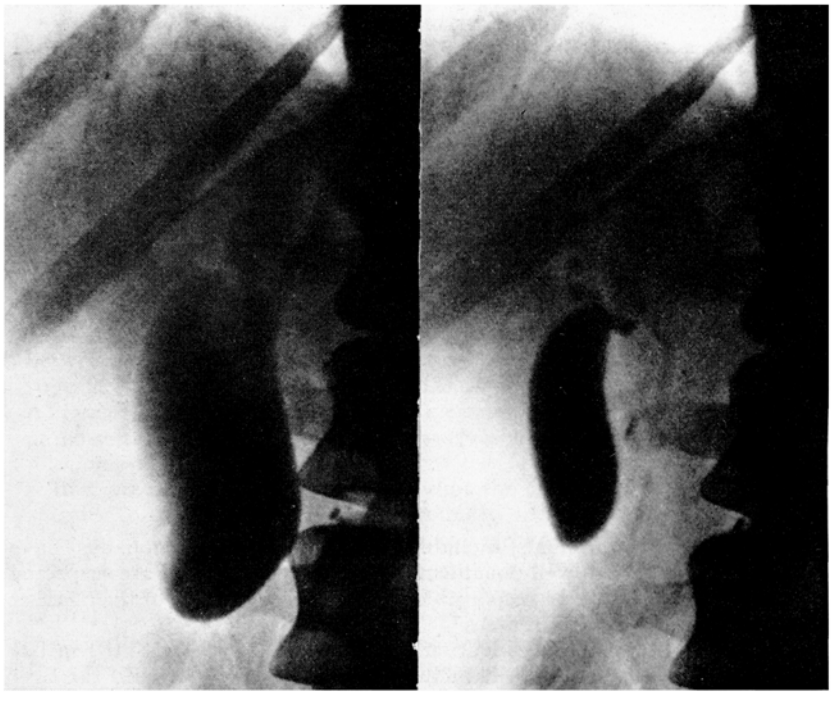

Fig. 2. Contraction of the gall bladder of a volunteer elicited by 10 μg/kg of the heptapeptide administered by nasal insufflation. On the left basal roentgenogram; on the right 30 min after the administration of the peptide.