3 strains producing different alkaloids can incorporate into the molecule of the synthesized alkaloids, amino acid analogues replacing the natural by occurring amino acids thus producing the corresponding alkaloid analogues.
It can therefore be concluded that the enzyme which is at the base of this biosynthetic reaction is not strictly specific. With the same strain and the same analogue, the incorporation is higher in the mutant requiring for growth the very amino acid that will be replaced in the alkaloid (table 2). These results indicate that the biosynthesis of the peptide moiety of the ergot alkaloids is, at least for the part here considered, controlled by the relative concentration of amino acids in the internal pool, and point to the possibility of obtaining new and potentially pharmacologically interesting alkaloids.

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Difference in resistance of subunits A and B of *Vibrio cholerae* toxin (choleragen) to treatment with pronase

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Summary. Disc electrophoresis in sodium dodecyl sulphate, performed on choleragen after incubation with pronase, only showed the band corresponding to the B subunit, while the A subunit was lost. When examined in immunodiffusion, the digested choleragen was still able to precipitate with specific antibodies. On the other hand, toxicity was considerably reduced.

In previous papers, we reported that the exotoxin produced by *Vibrio cholerae* (choleragen) maintained its electrophoretic pattern, toxicity and immunological reactivity when incubated in vitro, under non-denaturing conditions, with trypsin, chymotrypsin[1], elastase or papain[2].

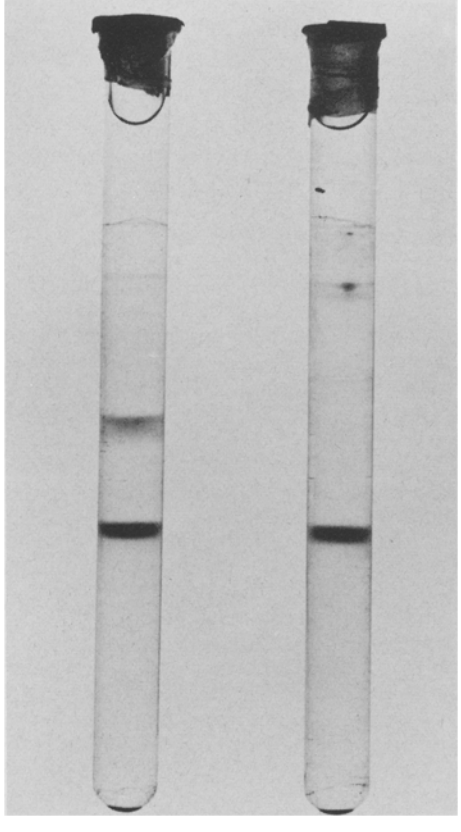

Fig. 1. Sodium dodecylsulphate-polyacrylamide gel electrophoresis of choleragen before (left) and after (right) treatment with pronase.

This remarkable resistance to enzymes showing different types of narrow specificity can be explained on the assumption that choleragen has a native structure in which the peptide bonds, specifically cleaved by the enzymes studied, are not easily accessible.
In order to confirm this hypothesis 'a contrariis', we investigated the effect of incubating choleragen with a mixture of exo- and endo-peptidases with broad specificity, such as pronase, which can attack the protein molecule simultaneously in several different points.
Materials and methods. Highly purified cholera toxin was prepared according to Saletti et al.[3]. The toxin was characterized by physico-chemical, immunological and biological methods: polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS)[4] showed bands corresponding to the A and B subunits, while immunodiffusion on agar plates[5] against a specific antiserum, showed a single band.
The toxic activity, as determined by the skin test in the rabbit[6] was 700,000 blueing doses/mg of protein. Proteins were determined according to Lowry et al.[7]. Pronase Grade B (Calbiochem, USA) was used. The enzymatic activity was determined, according to Narahashi[8], and found to be 690 PU/mg.

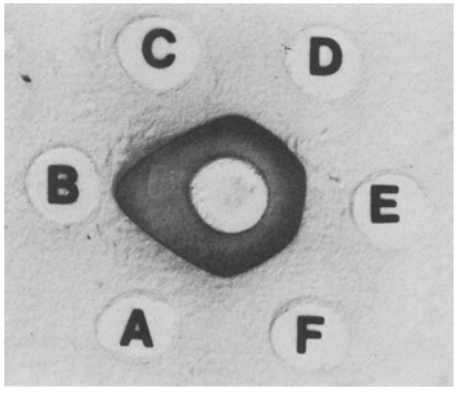

Fig. 2. Agar immunodiffusion of choleragen against the specific antiserum (centre well). A,C,E,F: choleragen; B: pronase; D: choleragen after treatment with pronase.

Choleragen (0.4 mg/ml) was incubated in 0.1 M $NaBO_3$ buffer pH 7.5 containing 5 mmoles of $CaCl_2$/l, and pronase at an enzyme/toxin ratio of 1/200, at 37 °C for 180 min.

Results and discussion. Pronase is able to digest fully the subunit A of choleragen, leaving the subunit B apparently unaffected. In fact, as shown in figure 1, the electrophoretic band corresponding to subunit A is no longer present in the sample deriving from the incubation mixture, while the subunit B shows the same pattern as that found in the sample of untreated toxin.

The ability to bind specific antibodies also remains unaltered by the treatment with pronase. As shown in figure 2, a reaction of complete identity is obtained in agar gel immunodiffusion between native and treated toxin. On the other hand, the toxicity of choleragen is reduced to only 15,000 blueing doses/mg after incubation with pronase.

These findings are consistent with the observation that the subunit A is a poor antigen[9], since the ability to bind antibodes raised against the whole toxin is not lost, although the subunit A is destroyed in our experiments. It has also been reported that the subunit A is essential for the toxicity, as it consitutes the effector moiety of choleragen[10]. This is in agreement with the reduced toxicity found after pronase incubation. The resistance of subunit B is not, generally speaking, a surprising finding. In fact many proteins have a core or a specific subunit that, in its native conformation, is not split by proteases. In our experiment this effect seems particularly pronounced.

Consequently, the limited proteolysis of choleragen with pronase could be of practical value for the preparation of the choleragen B subunit, to be used as an agonist to prevent the binding of choleragen to its receptors, or the provoke antitoxic immunity by including it in a cholera vaccine.

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Histamine H_1- and H_2-receptor-mediated gastric microcirculatory effects in the aetiology of stress ulceration in the rat stomach

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Summary. Stress produced severe mucosal ulcers, increased mucosal microcirculation and lowered mast cell counts in the glandular wall of rat stomachs. Mepyramine i.m. or metiamide i.p. effectively prevented both ulceration and microcirculatory changes but not stress-reduced mast cell counts.

Stomach wall mast cell degranulation with resulting mucosal microcirculatory changes are considered to play a dominant role in stress-induced gastric ulceration in rats[2-11]. Histamine H_1- or H_2-receptor antagonists decrease ulceration[2,3]. However, the mechanism of their anti-ulcer actions remains unclear. This study now examines the effects of histamine H_1- or H_2-receptor antagonism on gastric mucosal microcirculation and changes in mast cell counts in stressed rats.

Materials and methods. Male Sprague-Dawley rats (150–200 g), starved for 48 h beforehand, had free access to sucrose 8% in NaCl 0.2% w/v which was removed 1 h before starting experiments. Mepyramine maleate (M&B) Ltd) 6.3 or 25 mg/kg i.m. (expressed as the salt) or metiamide (SK&F Labs) 25 or 100 mg/kg i.p. were given, either separately or together, 0.5 h before stress. Similar volumes (2 ml/kg) of NaCl 0.9% w/v (saline) were given by the appropriate routes to the controls.

Animals to be stressed were put into close-fitting tubular restraint cages of wire mesh and exposed to 4 °C; controls were left in their starvation cages at room temperature (22±1 °C). After 2 h, all were killed by a sharp blow on the head. Stomachs, opened along the greater curvature, were examined for mucosal lesions with an illuminated magnifier (×3). Lesions were measured (mm) and their lengths expressed as an ulcer index[6]. The glandular segment of each stomach was processed for mast cell counts[6,9,12] and studies on mucosal microcirculation[7,9]. The number of metachromatically stained mast cells in 40 oil immersion fields (magnification × 1000) was counted in the mucosal, submucosal and muscle layers[6,7]. Microcirculatory volumes were determined by measuring the density of stained haemoglobin in the muscularis propia which acted as the tissue blank (TB), the upper third of the mucosa (SM) and the lower third of the mucosa (DM)[7,9]; differences between mean density readings (15 readings per area) indicated the relative blood volumes in the superficial (TB–SM) or deep (TB–DM) mucosa. Data were analysed using Student's t-test.

Results. All nonstressed groups showed low ulcer indices as occasional petechiae were found only in the glandular mucosa (table 1, A). Stress markedly increased the glandular ulcer index in saline-pretreated rats (table 1, B); the lesions appeared as haemorrhagic ulcers. Pretreatment with graded doses of mepyramine or metiamide progressively lowered the ulcer index in stressed animals. Combined lower doses tended to lessen ulcer severity further.

Stress significantly increased the microcirculatory blood volume only in the superficial gastric glandular mucosa of saline-pretreated rats (table 1, B). Mepyramine or metiamide pretreatment also reversed dose-dependently these stress-induced microcirculatory changes in the superficial mucosa. The relative blood volumes in the deep mucosa of nonstressed (table 1, A) or stressed (table 1, B) animals