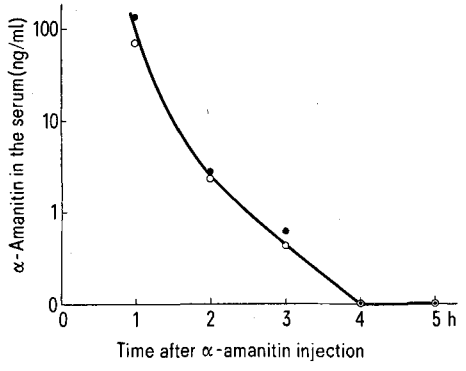
Four male Wistar rats, weighing about 250 g, received s.c. 1.5 ml of the emulsion (= 3 mg amanitin-RSA) administered in 3 different sites, 0.5 ml being injected in each site. After 1 month, the rats received a subcutaneous booster dose of 1.5 mg of amanitin-RSA in 0.75 ml of 0.9% NaCl. A week later, the rats were bled from the retro-orbital plexus.

The amanitin-binding capacities of the 4 sera, measured by the ammonium sulphate method according to Farr[14], resulted to range from 1.13 to 1.63 nmoles of amanitin per ml of serum.

For the radioimmunoassay, 1 µl of immune serum, various amounts of α- or β-amanitin (0.2–1 ng), 100 µl of *Tris*-HCl buffered saline pH 7.8, and 400 µl of normal human serum were mixed and incubated at 2°–4°C for 6 h. After addition of 1 pmole of [$^3$H] *O*-methyl-demethyl-γ-amanitin[15] (2.4 Ci/mmole), the mixture was again incubated at 2–4°C for 12–14 h and then precipitated with 500 µl of a neutral, saturated ammonium sulphate solution. The precipitate sedimented by centrifugation at 6000 *g* at 4°C was dissolved in 5 ml of the buffered saline and precipitated again 3 times with an equal volume of the ammonium sulphate solution. The final precipitate was dissolved in 2.5 ml of Soluene-350 at 60°C. After addition of 5 ml methoxyethanol and 10 ml of toluene-scintillation fluid, the radioactivity was counted.

The DPM precipitated (means of 4 values ± SE) in the absence of α-amanitin and in the presence of 0.2, 0.4, 0.6, 0.8, 1 ng of the toxin were respectively 2087 ± 24, 1775 ± 33, 1622 ± 38, 1513 ± 16, 1335 ± 28 and 1032 ± 23. In the absence of antiserum, 94 ± 8 DPM were precipitated. Therefore the inhibition of binding of radioactive γ-amanitin by 0.2 and 1 ng α-amanitin was 15% and 50% respectively. Equal results were obtained with unlabelled β-amanitin. The limit of sensitivity of this assay is 0.5 ng of α- or β-amanitin per ml of serum.




Disappearance of α-amanitin from the blood of poisoned mice; 36 male Swiss mice weighing 24 to 28 g received i.p. 350 ng of α-amanitin per 1 g body weight. The toxin was dissolved in 0.9% NaCl solution and administered in a volume of 10 µl per 1 g body weight. For each time interval, 6 mice were bled from the retro-orbital plexus, the blood from 3 mice was pooled and the sera were analyzed for α-amanitin. Each point represents the mean of 3 determinations.

By this procedure, the rate of disappearance of α-amanitin was determined from the blood of mice i.p. injected with 1 $LD_{100}$ of the toxin. The concentrations of α-amanitin in the sera were measured 1, 2, 3, 4 and 5 h after the injection of the toxin. The results reported in the Figure show that clearance of α-amanitin from mouse serum is rapid; by 4 h, no more toxin is detectable by our assay. This finding is in agreement with that of FAULSTICH and FAUSER[16], who failed to detect the toxin in the serum of dogs 5 h after the injection of labelled amanitin.

In human poisoning by *Amanita phalloides* or *verna*, the first symptoms appear after 10–15 h. Hospitalization does not generally take place before 15–30 h. Some physicians think that after such a long time it is improbable that amanitins are still present in the blood, and therefore they reject the use of immediate hemodialysis or exsanguinotransfusion which are recommended by other physicians. By the radioimmunoassay described here, it will be possible to know how long the amanitins remain in the blood of poisoned patients and consequently whether prompt action to remove them should be considered necessary.

*Summary.* The production of antibodies against amanitins is described. By means of these antibodies, a radioimmunoassay was developed which allows detection of as little as 0.5 ng of amanitins in 1 ml of serum. By this method, the clearance of α-amanitin from the blood of poisoned mice was measured.

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## Possible Effect of Caerulein on Calcitonin Secretion in Man

Recently, factors other than plasma calcium ions have been reported to elicit calcitonin (CT) release in experimental animals and in man. Among these factors, some gastrointestinal hormones, namely enteroglucagone[1], gastrin, and the complex CCK-PZ[2], seem particularly active in this respect.

Caerulein, a polypeptide similar in chemical structure to the C-terminal octapeptide of CCK-PZ[3], has been shown to stimulate CT secretion from pig thyroid in vitro[2]. The aim of the present study was to state if also in humans caerulein stimulates CT secretion. For this reason we have evaluated the pattern of serum Ca levels following i.v. infusion of caerulein. In addition, since calcitonin is able to reduce the disappearance rate of

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Effect of caerulein infusions on serum calcium and phosphorus levels

| Caerulein infusions | Time (min) | | | | | | | Nadir value |
|---|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 90 | 120 | |
| Calcium (60 min) | 10.1 ± 0.24 | 9.7 ± 0.24[c] | 9.4 ± 0.27[c] | 9.2 ± 0.27[c] | 9.3 ± 0.27[c] | 9.4 ± 0.22[c] | 9.5 ± 0.22[b] | 9.0 ± 0.24[c] |
| Phosphorus (60 min) | 3.0 ± 0.14 | 2.8 ± 0.18 | 2.8 ± 0.19 | 2.7 ± 0.21 | 2.7 ± 0.19 | 2.6 ± 0.20[b] | 2.8 ± 0.24 | 2.3 ± 0.15[b] |
| Calcium (15 min) | 9.8 ± 0.50 | 8.3 ± 0.38[c] | 8.2 ± 0.26[c] | 7.9 ± 0.20[c] | 7.7 ± 0.45[b] | 7.8 ± 0.26[b] | 8.3 ± 0.33[a] | 7.6 ± 0.28[c] |

Means ± S.E.M. are indicated. [a] $p < 0.05$; [b] $p < 0.02$; [c] $p < 0.005$ vs the 0 min value.

$^{47}Ca$ injected i.v.[4,5], we have also evaluated the effect of caerulein infusion on the disappearance rate of $^{47}Ca$.

*Materials and methods.* The experimental program consisted of two studies and was carried out on a total of 24 normal subjects, aged 59–85 years (mean 71.6). In the first study, 8 subjects underwent an i.v. caerulein infusion (4 ng/kg body wt./min for 60 min) and 7 subjects underwent a similar caerulein infusion (4 ng/kg body wt./min for 15 min). Blood samples were taken on both occasions at 0, 15, 30, 45, 60, 90 and 120 min and tested for serum Ca levels[6]. In the first 8 subjects, serum phosphorus levels were also determined[7]. In the second study, the effect of caerulein on radioactive Ca kinetics was investigated in 2 groups of subjects (4 subjects for each group) by infusing, respectively 60 min and 90 min after the injection of 50 mCi $^{47}CaCl_2$, caerulein at a dosage of 4 ng/kg body wt./min for 15 min. Venous blood samples were taken at 5, 20, 60, 65, 75, 85, 100 and 120 min in the first group and at 5, 45, 90, 95, 100, 105, 115, 130, 150 and 180 minutes in the second group, and evaluated for specific radioactivity by conventional scintillation counting techniques.

The tests were always begun at about 09.00 h after an overnight fast and at least 1 h of bed rest.

*Results.* The Table indicates the effect of the 2 caerulein infusions on serum Ca and P levels; the reduction of serum Ca levels was statistically significant, either after the 60 or the 15 min infusion, in every point of the curves. Following the first caerulein infusion, a slight decrease of serum P levels was also observed, significancy being however reached only at the 90 min point. The nadir value of serum Ca was reached at approximately 72 min and at 73 min respectively after the first and the second caerulein infusion; the nadir value of serum P levels was reached at the same time.

In the second study, caerulein infused 60 or 90 min after 50 mCi $^{47}CaCl_2$, increased the specific plasma radioactivity. Owing to the few data available, no statistical analysis was performed.

*Discussion.* In the present study we have shown that the administration of caerulein, a natural polypeptide similar in its chemical structure to the C-terminal octapeptide of CCK pancreozymin, is able to induce hypocalcemia in normal subjects. The degree of hypocalcemia detected in our patients is similar to that induced by calcitonin. This result, taken together with the findings of CARE et al.[2] of a stimulatory role of caerulein on calcitonin secretion from isolated guinea-pig thyroid in vitro, suggests that caerulein elicits calcitonin release also in man.

In addition, caerulein has been shown to affect the regression curve of $^{47}Ca$, in a manner similar to calcitonin, though with a little delay. This delay would indicate that caerulein does not act directly on calcium levels, but via a stimulation of calcitonin secretion.

Taken together, our results would indicate that caerulein stimulates in humans, as well as in the guinea-pig, calcitonin secretion. However, at present we cannot rule out the possibility that caerulein affects directly calcium metabolism, mimicking calcitonin effects.

In any case, the effect of caerulein on calcium metabolism is of great interest, in that, if our results will be confirmed, caerulein would be of advantage in those bone diseases in which calcitonin has proved helpful[8].

*Summary.* Caerulein, infused in normal subjects, significantly reduces serum Calcium levels; in addition, when infused 60 or 90 min after radioactive calcium, it increases the specific plasma radioactivity, in a manner similar to calcitonin. These results suggest that in man caerulein stimulates calcitonin release.

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## Chronic Treatment of Immature Male Rats with Synthetic LH-RH[1]

The neuroendocrine mechanisms which initiate the sexual maturation are not well known. It seems that the production and liberation of gonadotropin-releasing hormones by the hypothalamus, the secretion of gonadotropins by the pituitary and the production of sex hormones by the gonads are determinant factors[2]. Studies carried out in maturing animals[3] and prepubertal

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