concerned. However, the correlation between appearance and percentage of radiochromium release and of interdigitations is much stricter in PHA-induced than in antibody-mediated cytotoxicity. The different kinetics of the two cytotoxic phenomena may be explained by the fact that the effector cells are not the same in the two systems. In PHA-induced cytotoxicity, the effector cells are considered to be T cells, whereas in antibody-mediated cytotoxicity the effector cells, which have receptors for the Fc fragment of immunoglobulins, may belong to a subpopulation of B cells lacking surface immunoglobulins, or to an entirely different population of lymphocytes. The former type of cytotoxicity implies cooperation between several cell types which is probably not necessary in the latter[3-6]. These functional differences are not correlated with specific ultrastructural characteristics which might allow a distinction between these different cell types on morphological grounds. It seems likely that in PHA-induced cytotoxicity, the activation of effector cells and their contact with and consequent damage to target cells is slower and more progressive, presumably because of the more complex mechanisms of cell cooperation mentioned above. The interdigitation phenomenon appears identical in the two types of cytotoxicity. The relatively frequent occurrence of interdigitations between a single lymphocyte and several Ab-ChRBC is in agreement with the observation that presumably each effector cell has the capacity of destroying several target cells[6] and, according to our findings, also at the same time.

The observation of interdigitations, also in those cytotoxicity assays in which Ab-ChRBC had not been labelled with radiochromium, seems to exclude any relevance of the tracer in the induction of the phenomenon. Once established, as it is in our opinion, that interdigitation is the morphological evidence of the cytotoxic effect of lymphocytes on target cells, the intimate mechanisms by which this phenomenon occurs remain to be elucidated. Studies are now in progress to investigate the changes occurring in the electric charges at the surface of cells during the phenomenon in order to establish whether or not they may play a major role in it.

Summary. In the course of antibody-mediated lymphocyte cytotoxicity, ultrastructural studies show interaction between effector and target cells characterized by interdigitations. The significance of the observation is discussed.

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Production of Antibodies to Amanitins as the Basis for Their Radioimmunoassay

Amanitins[1,2] are the main toxins of the toadstools *Amanita phalloides* (Death Cap) and *Amanita verna* (Destroying Angel) which are responsible for some hundreds of poisonings, with many deaths, every year[3]. There has been no method, until now, for measuring the concentrations of these toxins in biological fluids. We describe here a radioimmunoassay which allows detection of as little as 0.5 ng of amanitins per ml of serum.

Since the first attempt of Calmette in 1897, several efforts were made in the past to obtain an antiserum to the toxins of *Amanita phalloides* by injecting animals with extracts of the toadstool[4]. The low molecular weight of the toxins (about 900) accounts for the failure of these attempts[5]. More recently β-amanitin was made antigenic by conjugating it to proteins[6-8]. The conjugates proved to be very toxic for those cells which display a high protein uptake, such as liver sinusoidal cells[9], proximal tubule cells of kidney[10], and macrophages[11]. As a consequence of this toxicity they could not be administered to rabbits and mice in immunogenic doses[7].

In the experiments reported here, we succeeded in obtaining antibodies against amanitins by injecting immunogenic doses of an amanitin-albumin conjugate into the rat, which is several times more resistant to amanitins[12], as well as to amanitin-protein conjugates[13], than rabbits and mice.

For the preparation of the conjugate, β-amanitin (16 mg) was allowed to react with 30 mg of rabbit serum albumin (RSA) and 13 mg of 1 cyclohexyl-3-(2-morpholinyl-(4)ethyl)carbodiimide (Morpho-CDI) in 1.5 ml of water at 22 °C for 24 h. The conjugate (amanitin-RSA) was separated from free β-amanitin and from unreacted Morpho-CDI by gel filtration on a 1.2 × 100 cm column of Sephadex G-75 equilibrated and eluted with 0.9% NaCl solution. The molar ratio of amanitin to albumin in amanitin-RSA, calculated according to Derenzini et al.[9], was found to be 1.4. The conjugate was brought to a protein concentration of 4 mg/ml by vacuum dialysis against 0.9% NaCl and was emulsified with an equal volume of complete Freund's adjuvant.

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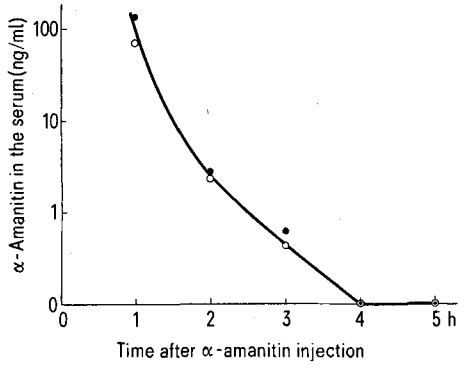
Four male Wistar rats, weighing about 250 g, received s.c. 1.5 ml of the emulsion (= 3 mg amanitin-RSA) administered in 3 different sites, 0.5 ml being injected in each site. After 1 month, the rats received a subcutaneous booster dose of 1.5 mg of amanitin-RSA in 0.75 ml of 0.9% NaCl. A week later,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