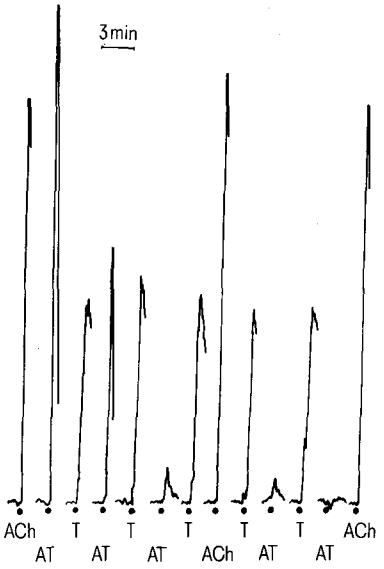
(Reid's 'thrombocytin'), Zucker's smooth muscle contractile substance, $K+$ [1-3], taurine, homotaurine, cysteine sulfinic acid, Zn^{2+} ions or prostaglandins[4].

Having in mind that anaphylatoxins (AT) might be formed when APS is incubated with platelets and fresh guinea-pig serum as a source of complement, some experiments were carried out on guinea-pig ileum preparations stimulated by T and rat AT. Platelets were separated from citrated horse blood and used both to raise antibodies in rabbits and produce T. The anaphylatoxin was obtained from rat serum made IM in relation to ε amino-caproic acid and activated by inulin at 37 °C for 1 h[5]. Although inulin, like zymosan, endotoxin, agar, carrageenin, yeast cells, activate complement by the alternative pathway by-passing Cl, the AT released is indistinguishable from that produced by IgG immunocomplex via the classical pathway (C142 mechanism).



Guinea-pig ileum isolated preparation bathed (10 ml) in Krebs-bicarbonate solution gassed with 5% CO_2 in O_2 at 37 °C. ACh = 2.5×10^{-8} M acetylcholine; AT = anaphylatoxin solution (5 µl/ml); T = 'thrombocytolysin' from horse platelets (2 µl/ml).

When successive doses of AT were applied at short intervals to a guinea-pig ileum preparation bathed in Krebs-bicarbonate physiological solution, tachyphylaxis rapidly developed, the tissue being partially or completely desensitized after a few such applications. A typical experiment is summarized in the figure. Although desensitized after 5 successive additions of AT the preparation still responded regularly not only to T but to calibrated doses of acetylcholine as well. As one of the most important features of the anaphylatoxic activities on smooth muscle preparations is a cross-desensitization, it seems that an AT can be ruled out as the major active component in partially purified T preparations.

1 H. Moussatché and W.O. Cruz, Archs. int. Pharmacodyn. Thér. *91*, 224 (1952).
2 H. Moussatché, Experientia *15*, 309 (1954).
3 H. Moussatché, Ann. Fac. Med. Montevideo *44*, 379 (1959).
4 F.B. Ubatuba, unpublished results.
5 T.E. Hugli, E.H. Vallota and H.J. Müller-Eberhard, J. biol. Chem. *250*, 1472 (1975).

Cytochalasin D is able to mimic the effects of phalloidin on the rat liver

W. Jahn[1]

Max-Planck-Institut für medizinische Forschung, Abteilung Naturstoff-Chemie, Jahnstrasse 29, D-6900 Heidelberg (Federal Republic of Germany), 27 February 1979

Summary. Cytochalasin D induces a strong potassium release in the isolated perfused rat liver and leads to vacuolization of the liver parenchymal cells. These effects are similar to the action of phalloidin on the rat liver. Since phalloidin and cytochalasin act in a different way on microfilaments, it is suggested that any disturbance of the function of microfilaments can induce these effects.

Phalloidin, a toxin, acting specifically on actin filaments in liver parenchymal cells[10], induces vacuole formation and potassium release in the isolated perfused rat liver[3]. It is not clear, however, in which way the molecular effect of phalloidin causes the pharmacological phenomena. Therefore we studied the effect of cytochalasin D, another drug interfering with the function of microfilaments.

Material and methods. The liver of male Wistar rats (mean animal weight 200 g) was perfused at 27 °C with a recirculating medium using a membrane oxygenator. The perfusion medium (vol. 50 ml) contained 8% dextran but no red cells. The perfusion rate was 3–4 ml/g/min. Details of the method have been described[4]. For light and electron microscopic investigation, livers were fixed by perfusion with 2.5% glutardialdehyde in the buffer solution used for preparing the perfusion medium, postfixed with 1% OsO_4 and embedded in ERL (Serva, Heidelberg). Cytochalasins (Aldrich, Milwaukee) were dissolved in dimethylsulfoxide (20 mg/ml). For i.v. application, additional DMSO (about 100 µl/kg) and Cremophor EL® (about 100 µl/kg; BASF, Ludwigshafen) were added. The solvents alone had no effect on the parameter investigated.

Results and discussion. When cytochalasin D (1 mg) was applied to the isolated perfused rat liver a potassium release of about 45 µEq/g liver could be observed within 60 min (figure 1, a). Between 60 and 120 min, a small amount of the potassium previously released was taken up again by the liver. This potassium uptake was not observed after a dose of 2 mg cytochalasin D (2 experiments, not shown). In contrast, cytochalasin B[4], even after a dose of 4 mg, induced only a small potassium release (up to 15 µEq/g) which was completely reversible within 60–70 min (2 experiments, not shown). The bile flow ceased after application of cytochalasin D within 6–10 min. The potassium release from the cytochalasin D treated liver was prevented by a high Mg^{2+} concentration (40 mM) in the perfusion medium (figure 1, a). Under the same conditions, the potassium release after phalloidin poisoning was also inhibited[5].

Application of hexobendine or 4,7-phenanthroline (15–25 mg) to the perfusion medium, 60 or 120 min after cytochalasin D led to a nearly complete re-uptake of the potassium previously released (figure 1, b). These drugs were similarly effective in the phalloidin-poisoned liver[6].

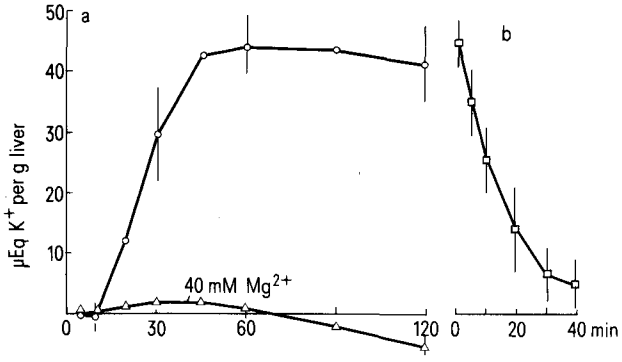


Fig. 1. *a* Potassium release of the isolated perfused rat liver after application of 1 mg cytochalasin D (upper curve). The potassium release was prevented by a high Mg^{2+} concentration (40 mM; standard concentration 0.5 mM) of the perfusion medium (lower curve). Each curve represents 4 experiments. The potassium release of the liver in control experiments was lower than 3 µEq/g liver within 120 min. *b* Application of 15 to 25 mg hexobendine (3 experiments) or 4.7-phenanthroline (2 experiments) 60 or 120 min after cytochalasin D caused a nearly complete re-uptake of the potassium previously released within 40 min.

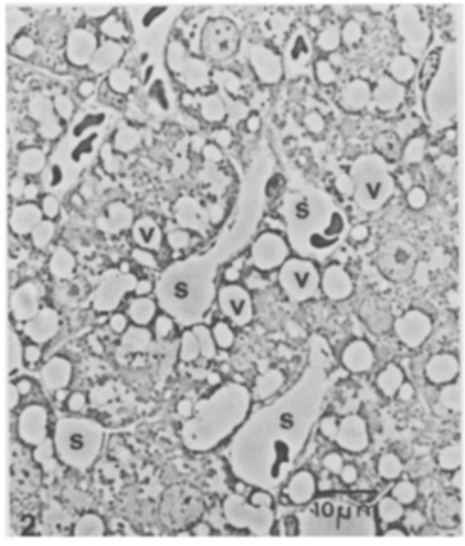


Fig. 2. Semi-thin section of a rat liver 3 min after i.v. application of 2 mg/kg cytochalasin D (phase contrast). Liver parenchymal cells contain large vacuoles (v) preferentially located adjacent to the sinus (s).

The weight of the cytochalasin D-treated liver increased during the experiments by 70–80%, while the corresponding values of the control liver and of the phalloidin treated liver were about 20% and 90–110% respectively[5].

Electron and light microscopic investigation revealed an extensive vacuolization of the liver parenchymal cells. In early stages of treatment, some vacuoles had a connection to the extracellular space, indicating their endocytotic origin. In the presence of 40 mM Mg^{2+}, vacuolization occurred to the same extent.

Cytochalasin D also induced vacuolization of liver parenchymal cells in vivo (figure 2), but doses which led to a strong vacuolization (1–2 mg/kg i.v.) were usually lethal within a few minutes. Obviously death was due not only to the action of cytochalasin on the liver.

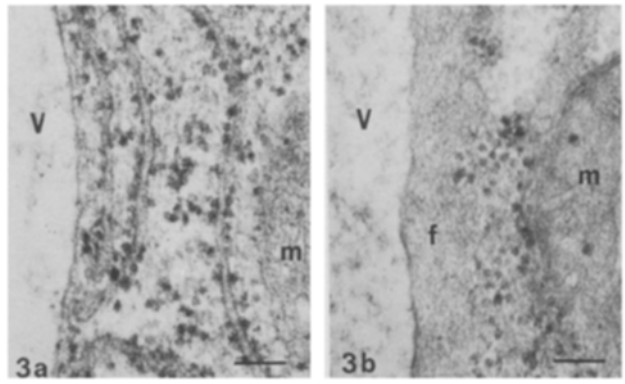


Fig. 3. *a* Electron micrograph of a vacuole (V) formed in a liver parenchymal cell in vivo 14 min after i.v. application of 1 mg/kg cytochalasin D. The membrane of the vacuole is not surrounded by a filamentous web. *b* Vacuole (V) induced by exerting a negative pressure (−14 mm water for 60 sec) on the liver surface. The membrane of the vacuole is surrounded by a well marked filamentous web (f). m, mitochondrion; bar = 1 µm.

Endocytotic vacuoles formed by cytochalasin D in vivo or in vitro were not surrounded by a filamentous web (figure 3, a). An exception was that, in the experiments in vivo, traces of filamentous material could be detected beneath the membrane of 20–40% of the vacuoles. In contrast, corresponding vacuoles induced by mechanical distension of the liver tissue in vivo[7] or in vitro[8] were surrounded by a well marked filamentous web (figure 3, b). An increase of the filamentous areas around bile canaliculi as was observed in the phalloidin poisoned rat liver[2], could not be detected after cytochalasin treatment.

The inhibition of formation of a filamentous web underneath the membrane of endocytotic vacuoles indicates a disturbance of the function of microfilaments by cytochalasin D. From the striking similarity between the pharmacological effects of cytochalasin D and phalloidin it may be concluded that the interaction with microfilaments is a common pharmacological basis of the action of the 2 drugs, but cytochalasin D may also exert additional effects on the rat liver. Since phalloidin and cytochalasins interact with actin filaments in a different way[10,11], it can be assumed that the phenomena investigated do not reflect the specific interaction of phalloidin or cytochalasin with microfilaments, but any disturbance of the function of microfilaments may induce these effects.

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2 B. Agostini, Z. Naturforsch. *30*, 793 (1975).
3 M. Frimmer, J. Gries, D. Hegner and B. Schnorr, Naunyn-Schmiedebergs Arch. Pharmak. *286*, 157 (1967).
4 W. Jahn, in: Membrane Alterations as Basis of Liver Injury, p. 267. Ed. H. Popper, L. Bianchi and W. Reutter. MTP Press, Lancaster 1977.
5 W. Jahn, Naunyn-Schmiedebergs Arch. Pharmak. *293*, 197 (1976).
6 W. Jahn, Naunyn-Schmiedebergs Arch. Pharmak. *272*, 182 (1972).
7 W. Jahn, Cytobiologie *18*, 188 (1978).
8 W. Jahn, Cytobiologie *15*, 452 (1977).
9 V.M. Govindan, H. Faulstich, Th. Wieland, B. Agostini and W. Hasselbach, Naturwissenschaften *59*, 521 (1972).
10 A.M. Lengsfeld, I. Löw, Th. Wieland, P. Dancker and W. Hasselbach, Proc. nat. Acad. Sci. *71*, 2803 (1974).
11 J.A. Spudich, Cold Spring Harb. Symp. quant. Biol. *37*, 585 (1973).