

# Formation of Actin Clusters in Rat Liver Parenchymal Cells on Phalloidin Poisoning as Visualized by a Fluorescent Phallotoxin

Werner Jahn, Heinz Faulstich, Axel Deboben, and Theodor Wieland

Max-Planck-Institut für medizinische Forschung, Abteilung Naturstoff-Chemie, Jahnstraße 29, D-6900 Heidelberg

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By staining of cryo-sections of rat liver with a fluorescent phallotoxin, the distribution of filamentous actin in liver cells could be demonstrated by fluorescence microscopy.

While in untreated livers filamentous actin forms an almost continuous layer at the cell periphery, the poisoning by phalloidin leads to the formation of actin clusters, preferentially located near the cell membrane.

Recently a fluorescent phallotoxin was synthesized, which binds to F-actin and allows the identification of filamentous actin by fluorescence microscopy [1]. Using this method we investigated the distribution of actin in rat liver tissue before and after phalloidin poisoning.

## Methods

The livers of male Wistar rats were used for the experiments. Phalloidin poisoning was induced *in vivo* by i. v. injection of phalloidin (4 mg/kg) 20 min prior to fixation, or *in vitro* by perfusion of the isolated liver at 27 °C for 1 to 4 h in the presence of phalloidin (0.5 mg/50 ml perfusion medium) as described elsewhere [2]. The perfusion medium usually contained 2% albumin. The isolated livers or the livers *in situ* were fixed by perfusion via the portal vein for 2 min with about 100 ml 4% formaldehyde in perfusion buffer (NaCl 120 mM; KCl 4 mM; CaCl<sub>2</sub> 1.4 mM; MgCl<sub>2</sub> 0.5 mM; NaH<sub>2</sub>PO<sub>4</sub> 0.4 mM; NaHCO<sub>3</sub> 30 mM). Samples from the great liver lobes were further fixed in the same solution for 2 to 4 h. Tissue samples from phalloidin poisoned livers and parts of the samples from control livers were incubated overnight in 50% dimethylsulfoxide (V/V) in KCl buffer (KCl 0.1 M; MgCl<sub>2</sub> 2 mM; Tris 50 mM; pH 7.4). Before sectioning all samples were suspended in 50% sucrose (w/w) in KCl buffer for at least 3 h. Sections 1 to 2 µm thick were cut from the frozen tissue on a LKB Ultratome III equipped with the cryo kit, at a specimen temperature of –80 to –40 °C and a

chamber temperature between –20 and –40 °C. Sections were taken from the dry glass knife by use of a small droplet of water placed at the spherical end of a glass rod. The sections were collected on a water drop on a microscopic slide and were allowed to dry. For staining the sections were incubated for 10 min with a drop of fluorescent phallotoxin (2 to 5 µg/ml) in 0.5 M ethanolamine buffer (pH 9.3), washed with this buffer within 30 sec and then embedded in 50% sucrose (w/w) in the same buffer. The sections were examined in a photomicroscope III (Zeiss, Oberkochen) equipped with a fluorescence attachment. Photographs were taken on HP5 films (Ilford).

## Results

When sections of rat liver were treated with the fluorescent phallotoxin, the borders of liver parenchymal cells exclusively were stained (Fig. 1a). A pronounced fluorescence was seen around the bile canaliculi. Endothelial cells (Fig. 1b, En) seem to contribute very little to this staining pattern.

The staining was completely prevented by previous incubation of the sections with phalloidin (0.5 mg/ml for 10 min), indicating the specificity of the staining.

Samples of rat liver poisoned *in vivo* or *in vitro* by phalloidin were treated with 50% dimethylsulfoxide for extraction of the phalloidin bound during poisoning. Staining of sections after this treatment produced a different pattern of the fluorescent label. There was only a very thin layer of stain at the cell borders which sometimes appeared to be disrupted, while instead bright fluorescent spots were visible

Reprint requests to Dr. Werner Jahn.  
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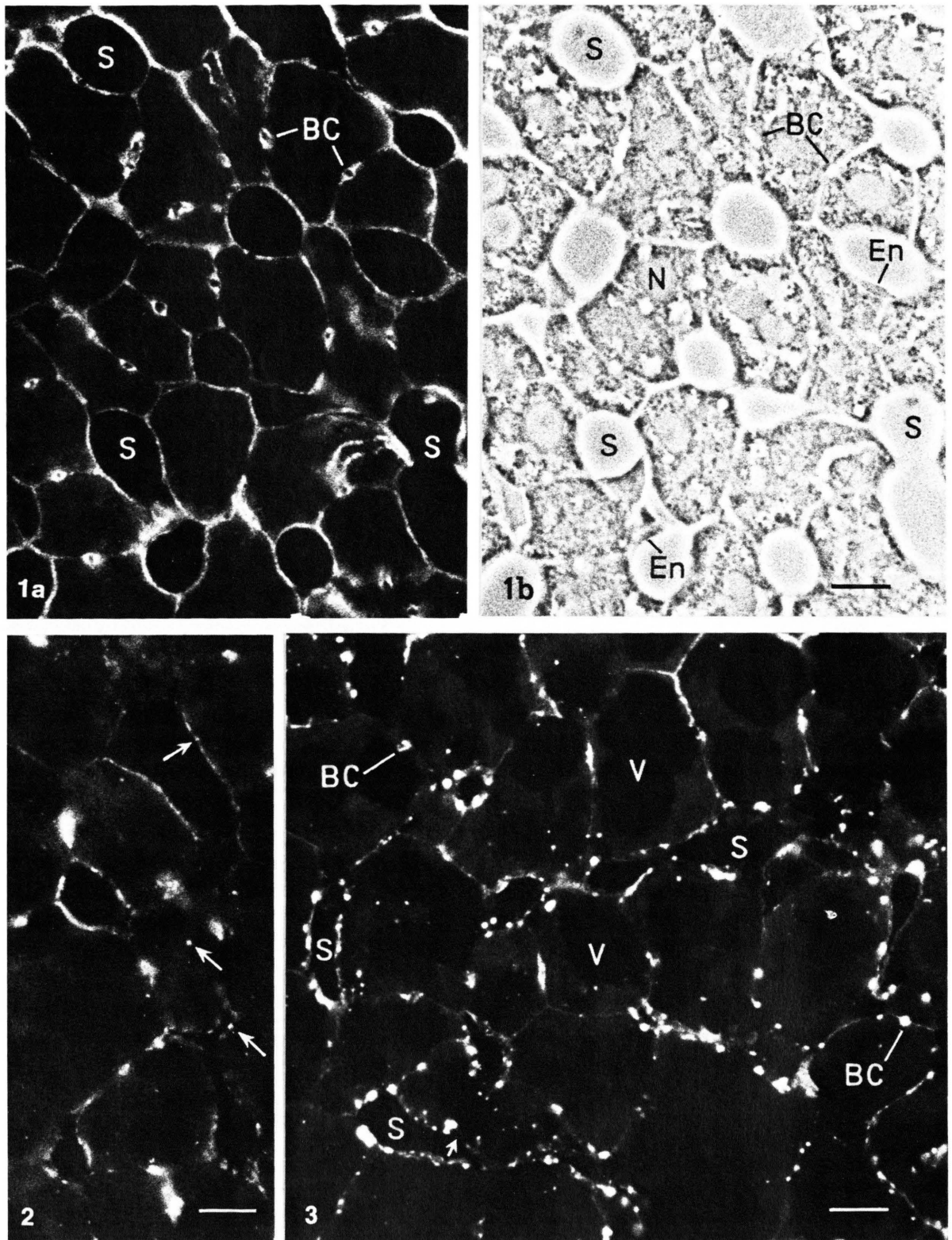


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mostly at the cell periphery and to a lesser degree also in the cytoplasm. These events could be demonstrated as soon as 25 min after poisoning *in vivo* (Fig. 2), but were pronounced in the liver perfused for 1 or 4 h in the presence of phalloidin (Fig. 3). An identical staining pattern was found when samples of phalloidin poisoned liver were, instead of extraction of the phalloidin by dimethylsulfoxide, incubated overnight in a solution of the fluorescent phallotoxin (20 µg/ml) in KCl buffer. By this method the bound phalloidin was exchanged by the fluorescent phallotoxin, however, this procedure usually led to a high background staining. Dimethylsulfoxide did not produce any changes of the staining pattern of the samples as compared with controls.

## Discussion

From earlier studies it was determined, that the fluorescent phallotoxin binds to F-actin and stains cells other than those of the liver in a way similar to actin antibodies [1]. Indeed, the staining pattern of liver cells obtained by use of the novel fluorescent label for actin corresponds well with the analogous staining patterns of liver cell sections obtained by others with anti-actin antibodies [3, 4]. However, staining with the fluorescent phallotoxin exhibits much more details. This advantage may be due to the easy accessibility to actin filaments in the tissue by the small fluorescent dye.

A prerequisite for the visualization of actin filaments in phalloidin poisoned livers was the extraction of the phalloidin taken up by the liver during poisoning. This could be done by treatment of the tissue samples with 50% dimethylsulfoxide.

The remarkable feature of the phalloidin poisoned liver cells was the presence of bright spots at the cell borders and in the cytoplasm. From biochemical investigations it is well known that phalloidin shifts the equilibrium between F and G-actin toward F-actin which binds phalloidin forming Ph-actin, a species stabilized against depolymerisation. Obviously, the bright spots are clusters of Ph-actin, resulting from the process mentioned. Filamentous areas which may correspond to these actin clusters are known from electron microscopic investigations [3, 5].

The fluorescent layer at the cell borders found in control livers is diminished and sometimes disrupted after phalloidin poisoning. This indicates that actin from the submembranous filamentous web may be transferred to the actin clusters. Probably, this process is one of the factors giving rise to the forming of invaginations of the cell membrane in the phalloidin poisoned liver or of protrusions in isolated liver cells after phalloidin poisoning, respectively.

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Fig. 1 a and b. Fluorescence micrograph of a cryo-section of rat liver stained with fluorescent phallotoxin (a) and the corresponding phase contrast micrograph (b). A fluorescent layer is seen at the cell borders and around the bile canaliculi (BC). Endothelial cells (EN) contribute very little to this staining pattern. N = nucleus, S = sinus. Bar = 10 µm; × 1000.

Fig. 2. Fluorescence micrograph of a rat liver after poisoning with phalloidin *in vivo* for 20 min. Numerous bright spots are seen at the cell borders and in the cytoplasm (arrows). Bar = 10 µm; × 1000.

Fig. 3. Phalloidin poisoned isolated rat liver, perfused for 4 h. The presence of bright spots is striking, while the fluorescent layer at the cell borders appears very thin or is disrupted (arrows). V = vacuole, BC = bile canaliculi, S = sinus; Bar = 10 µm, × 1000.