Phalloidin poisoning of isolated hepatocytes: Inhibition of protein synthesis[1]

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Summary. Phalloidin added to isolated hepatocytes inhibits amino acid incorporation into protein. Inhibition is very strong with 20 μg/ml phalloidin and occurs after a lag period of about 30 min. After this period, poisoned hepatocytes also show morphological changes in their plasma membrane.

The early damage induced by Amanita phalloides in the liver of several animals is due to phalloidin that seems to disaggregate the cellular membrane, leading to cell swelling and to formation of large vacuoles in the hepatocyte[3]. A number of alterations have been described in the liver of phalloidin-poisoned animals[4]. Previous work from these laboratories[5] has shown that phalloidin, given to rats, induces a strong dissociation of liver polyribosomes. This change seems to be dependent on an inhibition of the polysomal cycle at the level of chain initiation. In vitro, phalloidin does not inhibit chain initiation in a liver cell-free system. This toxin, therefore, does not appear to be a direct inhibitor of protein synthesis. In preliminary experiments[5], we were not able to show any effect of phalloidin on hepatocytes in single cell suspension. By improving the method of preparation of hepatocytes, we have found that phalloidin, added in vitro at very low concentration, inhibited amino acid incorporation into protein of isolated hepatocytes. In this report, we describe the results of these studies.

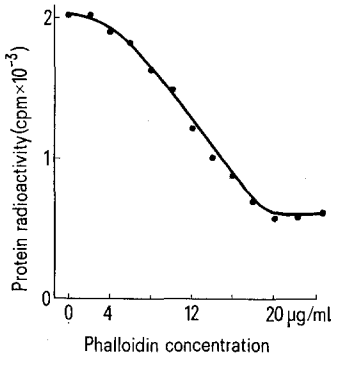

Fig. 1. Effect of phalloidin on amino acid incorporation into protein of isolated hepatocytes. Phalloidin was added 30 min before the labelled amino acids. Results are given as cpm/10^6 cells/30 min incubation.

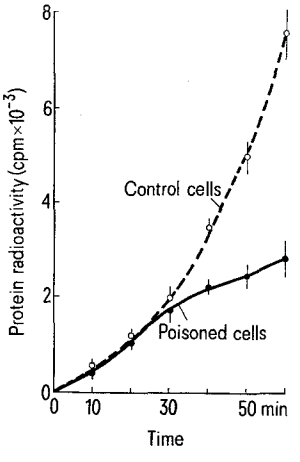

Fig. 2. Time course of amino acid incorporation into protein of isolated hepatocytes. Phalloidin (20 μg/ml) was added at the zero time together with the labelled amino acids. Results are given as cpm/10^6 cells. Each point represents mean of 3 experiments ± SD.

Materials and methods. Rat hepatocyte suspensions were prepared, according to the method of Berry and Friend[6], as modified by Jeejeebhoy et al.[7]. Hepatocytes were suspended in Hanks' solution containing 1% bovine serum albumin (Sigma, fraction V, fatty acid free), 3 mM phosphate buffer pH 7.2 and 0.1% penicillin G. After counting, the hepatocyte suspension was diluted with the same medium to 2.5×10^6 cells/ml. Aliquots of the final cell suspension were placed into 15 ml glass vials (flat bottom, 15 mm diameter) and incubated at 37 °C and 100 oscillations/min. The incubation mixture contained: 0.4 ml hepatocytes, 0.025 ml water or phalloidin solution and 0.025 ml amino acid mixture. The final concentration of amino acids was the following: 0.1 mM glycine and other amino acids in the relative proportions described by Waymouth[8]. The ^{14}C-amino acid incorporation was initiated by adding 0.025 ml (0.5 μCi) of protein hydrolysate ^{14}C(U) (Radiochemical Centre, Amersham; specific activity 57 mCi/mAtom carbon). After incubation cell protein was precipitated with 5% trichloroacetic acid (TCA) and then rinsed 4 times again with 5% TCA. Radioactivity was measured in digested protein (Soluene-350, Packard Instrument Co.) with a liquid-scintillation counter.

Results and discussion. The viability of the isolated hepatocytes was 90–95% as measured by the Trypan blue exclusion test. Morphological changes of the hepatocytes exposed to phalloidin were observed under light microscopy. As early as 30 min after poisoning with 10 μg/ml of the toxin, almost all hepatocytes presented cytoplasmic bulges outwards from various regions of the cell surface. This feature of the in vitro poisoned hepatocytes has been reported[9]. We found that the observed morphological alterations were not followed, for a period of at least 2 h, by a decrease in the number of Trypan blue excluding cells.

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The data presented in figure 1 show the effect of various concentrations of phalloidin on protein synthesis in isolated hepatocytes. The ^{14}C-amino acid incorporation was initiated 30 min after the addition of the toxin and was continued for 30 min. It is evident that the incorporation is inhibited by phalloidin concentrations lower than 6 µg/ml. The maximum point of inhibition takes place at phalloidin concentration of 20 µg/ml. In the experiments reported in figure 2, the labelled amino acids were added soon after phalloidin and the incorporation was studied as a function of incubation time. It is evident that under these conditions incorporation follows a parallel pattern in experimental and in control hepatocytes for about 30 min, and thereafter the inhibiting effect of phalloidin begins. The existence of this lag period is in accordance with the onset of the polysome dissociation observed after in vivo poisoning[5]. In our previous paper[5], we have shown that phalloidin does not inhibit protein synthesis in a liver cell-free system. In the latter, there is a saturating level of amino acids and ATP, as well as an optimum concentration of ions required for protein synthesis. It is therefore possible that phalloidin acts by decreasing some factor that is present in limiting amount within the intact cell. We observed in some experiments (the data are not included in this report) that the phalloidin-induced inhibition of amino acid incorporation into protein of isolated hepatocytes was not dependent on a decreased level of amino acid inside the cell. A decrease of ATP levels does not occur in the first hours after phalloidin poisoning[10]. It has been reported that hepatocytes exposed to phalloidin lose potassium ions[11,12]. The possibility of a role of this alteration in the impairment of protein synthesis is obvious and requires further investigation.

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Die Rudimentation des Rumpfkanals bei kavernikolen Populationen des Astyanax (Characidae, Pisces)

The rudimentation of the lateral canal of cavernicolous Astyanax populations (Characidae, Pisces)

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Summary. The lateral canal of the eye- and pigment-reduced cave populations of Astyanax mexicanus shows fragmentations. This is in contrast with the epigean ancestor and is attributed to the loss of its biological function.

Als einziger Characidae ist ein Vertreter der in Südamerika weitverbreiteten Gattung Astyanax nach dem Entstehen einer Landbrücke zwischen den beiden Amerikas weit nordwärts vorgedrungen[1]. Die Form ist heute in den Flussystemen der pazifischen und der atlantischen Seite Mexikos häufig zu finden und hat ihre Nordgrenze über den Rio Grande bis nach Texas vorgeschoben. Sie wurde in mehreren vom taxonomischen Standpunkte revisionsbedürftigen Arten und Unterarten beschrieben (*A. mexicanus* [Filippi 1853], *A. fasciatus fasciatus* [Cuvier 1819], *A. fasciatus macrophthalmus* Regan 1908 und andere mehr).

Als Lebensraum dieses Fisches können im typischen Falle rasch bis turbulent fliessende, klare, sauerstoffreiche und häufig relativ flache Gewässer gelten. Auf Grund seiner euryöken Natur konnte er zudem – wenn auch seltener – stagnierende Gewässer besiedeln. So wurde die Form sowohl an der Nord- wie auch an der Ostküste Yucatáns in dolinenartigen Einbrüchen, Cenotes, die praktisch keine Strömung aufweisen, isoliert. Auch im Catemaco-See (Bundesstaat Veracruz) ist sie vertreten. In einem räumlich begrenzten Bereich Zentralmexikos (Bundesstaat San Luis Potosi) konnten darüber hinaus Stillgewässer besiedelt werden, die zudem noch als Höhlen durch Lichtlosigkeit charakterisiert sind[2–4].

Auf Grund des durch das Fehlen des Lichts bedingten Verlusts ihrer biologischen Funktion sind Augen und Melaninpigmente dieser Populationen reduziert[5–9]. Die Folgen des weitgehenden Fortfalls eines weiteren für den ursprünglichen Biotop charakteristischen Umweltfaktors, starker Strömung, in den Höhlen fand dagegen bislang in keinen Untersuchungen Berücksichtigung. Schemmel[5] hob hervor, dass bereits der oberirdische Fisch über ein gut entwickeltes System der freien Neuromasten und der Kopf- und Rumpfkanäle verfüge und die räumliche Orientierung ihm daher auch bei Fehlen des Lichtes keine Schwierigkeiten bereite. Er wies allerdings auf das Auftreten von Fragmentationen des Rumpfkanals bei Originaltieren der cavernicolen Sabinos-Population hin, die er bei Labornachzuchten der Höhlenform nicht feststellen konnte.

Die Untersuchung des Rumpfkanals weiterer Originalfänge aus ober- und unterirdischen Biotopen konnte diese Beobachtungen bestätigen: Von insgesamt 30 aus drei kavernikolen Populationen stammenden Individuen wiesen ganz wenige einen vollständig ausgebildeten Rumpfkanal auf. Bei der Mehrzahl der Tiere war dieses Organ partiell nicht mehr vorhanden. So zeigte sich, dass der Kanal geschlossen nur in der ersten Hälfte bzw. dem ersten Drittel des Körpers entwickelt war. In den anschliessenden Regionen war die Ausbildung lückenhaft

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