We also succeeded in coordinating by chir-optical methods the chiral sulfoxide centre of amatoxins to the sulfoxides prepared by oxydation of phalloidin. The ORD curves of the 2 diastereomeric phalloidin sulfoxides are mirror images[6] (Figure 4a and b). This confirms the observations of HENSON and MISLOW[7], who found that the sign of the ORD curves of sulfoxides is determined predominantly by the chirality at the sulfur atom. Only the phalloidin sulfoxide, having positive Cotton effects in the region of 290–360 nm (Figure 4a) possess the toxicity of phalloidin[8].

Amanin is an amatoxin without a phenolic hydroxyl group and therefore possesses the same chromophoric system as the sulfoxides of phalloidin[8]. It has an ORD curve (Figure 4c) similar to the toxic phalloidin-sulfoxide. Comparison of amanitin sulfoxide was achieved by acetylation of the phenolic group[9] in position 6, which shifts the absorption of amanitins 10 nm to shorter wavelengths, so that it corresponds to those of amanin and phalloidin sulfoxides. The ORD-curve of *O*-acetyl-γ-amanitin (Figure 4d) resembles also that of the toxic phalloidin sulfoxide. From this it must be concluded that the configuration of the sulfoxides in amanin and in the amanitins is identical with that in the toxic phalloidin sulfoxide. The absolute configuration, however, of this centre of chirality still remains to be elucidated.

Zusammenfassung. Die Cottoneffekte der Amanitine konnten dem Indolteil bzw. den Amidgruppen des Molekühls zugeordnet werden. Das untoxische Amanullin besitzt die gleiche Konformation wie die toxischen Peptide, das untoxische Aldoamanitin dagegen eine andere. Die absolute Konfiguration der Sulfoxidgruppe der Amatoxine ist identisch mit der des toxischen Phalloidinsulfoxids.

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Acquisition of an Embryonal Biochemical Feature in the Rat Liver after Portacaval Shunt

The end-to-side portacaval shunt (PCS) leads to a marked alteration in quantity and composition of the blood reaching the liver. Consequently, shunt-induced alterations in metabolic stimulation might, in a general way, explain the various morphological and functional changes of the liver[1–4]. To our knowledge the specific hypothesis has not been tested whether some of the hepatic consequences of a PCS might be regarded as a regression of the hepatocytes to a more immature or embryonal state. A study of biochemical features which differ significantly in fetal and adult animals might shed light on this question.

In the mature rat liver, the enzyme γ-glutamyl transpeptidase (GGTP, γ-glutamyl transferase, EC 2.3.2.1) is barely measurable[5], whereas in the newborn rat it exhibits 10 to 20 times more activity[6]. Derepression of this enzyme has also been reported in transplantable chemically induced rat hepatomas[7]. Hepatic GGTP was, therefore, measured in rats with a PCS and in appropriate controls.

The experimental details of our animal model have been described previously[1,3]. Adult male Sprague-Dawley rats were examined for liver GGTP activity 10, 20 and 30 days after an end-to-side portacaval shunt. Non-operated control animals from the same batch were sacrified simultaneously. Enzyme assays were carried out on 10% (w/v) liver homogenates in 0.9% saline solution, using γ-glutamyl-*p*-nitroanilid as substrate[5].

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γ-glutamyl transpeptidase activity after end to side portacaval anastomasis ($\bar{x} \pm$ SD)

Experimental procedure	n	Liver weight (g/100 g)	Enzyme activity (μmoles/min/g wet wt.
Unoperated controls	6	3.66 ± 0.33	0.006 ± 0.012
Portacaval anastomosis			
after 10 days	7	2.38 ± 0.41	0.060 ± 0.018[a]
after 20 days	5	2.16 ± 0.27	0.067 ± 0.027[a]
after 30 days	5	2.35 ± 0.5	0.104 ± 0.043[a]

[a] Significantly different from unoperated controls ($p < 0.001$).

In all animals subjected to PCS, the liver weight decreased markedly (Table). This liver 'atrophy' corresponded to the general experience in this and other laboratories[1-4] and implied a well functioning PCS. 10 days after PCS, GGTP activity had increased tenfold and remained at this level throughout the period of observation. In the control group, the activity was almost undectable (Table).

Our findings of very low activity of GGTP in normal adult rat livers are in agreement with earlier observations[5,8-10]. In these animals GGTP activity may be found histochemically in the endothelial cells of periportal vessels, in bile ducts and in Kupffer cells, whereas virtually no activity can be detected in hepatocytes[11]. The high enzyme activity in certain rat hepatomas which contain glycogen, produce bile and have a high level of glucose-6-phosphatase[7], suggests that GGTP may be activated in parenchymatous liver cells during the carcinogenic process. This has been interpreted as a re-acquirement of a biochemical feature which predominates in the fetus, but is repressed in the adult liver[7].

The microscopical anatomy of the liver of shunted rats looks grossly normal[2,12]. Measurements of DNA content have suggested that the initial loss of liver mass is due more to a reduction in cell size rather than in cell number[13]. Some observers noted a slight increase in the number of Kupffer cells[14]. Even though we have no information about the histochemical distribution of GGTP activity in shunted rats, the generally recognized minor morphological alterations do not appear sufficient to explain the tenfold increase in enzyme activity after PCS. The impressive elevation in hepatic GGTP-content could therefore be interpreted more reasonably in the context of the similar findings in neonatal liver and in chemically induced rat hepatomas. According to this view, the shunt consequences might be associated with derepression of an enzyme, normally present only in the embryonal liver. Further studies are required to demonstrate whether such a mechanism is limited to a few specific enzymes or represents a form of generalized hepatic immaturity after PCS.

Zusammenfassung. In Leberhomogenaten von Ratten mit einem portocavalen «Shunt» wurde gegenüber unbehandelten Kontrolltieren eine zehnfache Vermehrung der Aktivität der γ-Glutamyltranspeptidase festgestellt. Dieser Befund könnte als Derepression dieses Enzyms gedeutet werden, da ähnlich gesteigerte Enzymaktivitäten bisher nur bei embryonalen Lebern und bei chemisch induzierten Hepatomen gemessen worden sind.

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The Use of Alfalfa Residual Juice for Production of Single-Cell Protein

One of the most serious problems facing the world nowadays is the provision of an adequate diet for a rapidly increasing population. This can be especially observed in Latin America, where the protein shortage constitutes the main nutritional deficiency[1]. Under these circumstances, the developing of processes for conversion of waste materials into edible microbial foods is of paramount importance.

Several processes for the obtention of leaf protein concentrate have been reported in the literature[2-4]. During these processes, alfalfa residual juice is obtained as a byproduct and the feasibility of its use for biomass production was the aim of the present investigation. Earlier results have been published elsewhere[5,6].

Materials and methods. The culture selected for its highest growth rate was *Candida sp.*[5]. The medium used in fermentation contained 0.5 g of K_2HPO_4/l of alfalfa residual juice. Fermentation was conducted at 30 °C and at a controlled pH of 5.0. The fermentor used throughout this study was a 14 l total capacity (Fermentation Design Inc.) with a working volume of 9 l. Stirrer speed was of 400 rpm. Simple on-off control was used, arranged so that the dissolved oxygen partial pressure did not fall below 0.10 atm. Inoculum was grown in 250 ml Erlenmeyer flasks with 50 ml of medium, and incubation was for 1 day. Dry weight was determined gravimetrically after drying to constant weight the cell material at 100 °C.

Approximately 33 mg of dried biomass was subjected to acid hydrolysis with 6*N* HCl at 110 °C for 48 h. The hydrolysate was evaporated under vaccum to dryness and then resuspended in 20 ml of distilled water. This step was repeated 3 times and then the hydrolysate was taken up in a pH 3.1 sodium acetate-acetic acid buffer. The analysis for amino acid was carried out upon a 0.5 ml sample using a Hitachi Perkin-Elmer amino acid analyzer (Model KLA-3B). Tryptophane was determined by a colorimetric method[7]. The amino acid content of soybean meal was also determined by the procedures mentioned above.

Results and discussion. The stationary phase was practically attained after the 50th h of cultivation, as is shown in Figure 1a. The maximum productivity p was

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