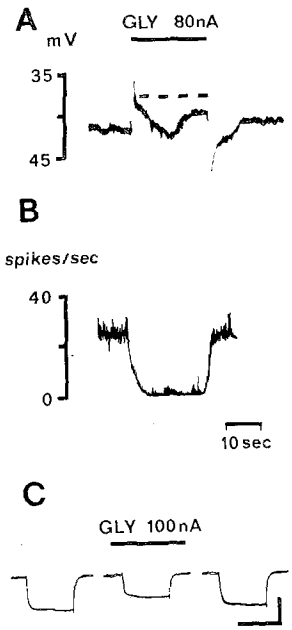
palement with the microelectrode. However, in a small number of cells it was possible to record reasonably stable membrane potentials (–40 to –65 mV) for several min. One neurone with a spike of 65 mV was held in good conditions for approximately 45 min. In the present study only cells with membrane potentials over –40 mV have been included (13 cells). Glycine ejected with currents of 60–100 nA hyperpolarized the membrane (2–8 mV) with a time course similar to that observed in spinal and in Deiters' neurones[2, 3, 8, 10]. Figure A illustrates a hyperpolarization by glycine (80 nA) of a medullary neurone which was firing at a rate of approximately 25 spikes/sec. The hyperpolarization was accompanied by complete depression of firing of this neurone (Figure B).



Action of glycine on membrane potential (A), firing rate (B) and membrane conductance (C) of two different neurones of the medulla oblongata. A) Membrane potential (–41 mV, initially –50 mV) recorded with a K-citrate electrode on a rectilinear ink recorder. Glycine (GLY) was ejected with a current of 80 nA from one barrel of an extracellular 4 barrel micropipette. Duration of glycine ejection is indicated by bar above trace. The horizontal broken line indicates the coupling artifact (4 mV) that was estimated after withdrawal of the intracellular microelectrode from the cell. B) Ratemeter record of the firing rate (spikes/sec) of the same neurone as A). Time 10 sec for A) and B). C) Measurements of membrane conductance of another neurone by the injection of hyperpolarizing current pulses (2 nA) before, during and after administration of glycine (100 nA) for approximately 10 sec. Calibrations: 10 mV, time 20 msec.

The action of glycine on the membrane conductance was studied in 5 cells. Glycine reversibly reduced the amplitude of the potential change produced by a hyperpolarizing current pulse passed through the recording electrode (Figure C). Glycine also blocked the action potential evoked by an intracellular depolarizing pulse in two cells. The administration of glycine depolarized 3 cells impaled with KCl electrodes probably due to the diffusion of chloride ions from the recording electrode.

Our results demonstrating that glycine causes a hyperpolarization and an increase in membrane conductance support the hypothesis that glycine is an inhibitory transmitter substance in the medulla oblongata. However, it has yet to be shown that postsynaptic inhibition of brain stem neurones is blocked by strychnine in the same manner as the hyperpolarization by glycine.

Zusammenfassung. Mikroelektrophoretisch verabreichtes Glycin erzeugt eine Hyperpolarisation und eine Zunahme der Leitfähigkeit der Zellmembran von Neuronen der Medulla oblongata der Katze. Diese Ergebnisse unterstützen die Hypothese, dass Glycin die Funktion einer hemmenden Überträgersubstanz im Hirnstamm hat.

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Mechanism of Allyl Alcohol-Induced Hepatic Necrosis

Although allyl formate and its metabolite, allyl alcohol, were shown to cause extensive periportal necrosis of the rat liver more than half a century ago[1], the biochemical mechanism of their hepatotoxic action remains controversial[2–5]. Recently, REES and TARLOW[6] reported that inhibition of mitochondrial respiration in vitro and blockade of protein synthesis by allyl formate depend upon the nonmicrosomal enzyme alcohol dehydrogenase and can be mimicked by the metabolite acrolein. REES and TARLOW[6] also confirmed that alcohol dehydrogenase is localized in the periportal regions of the hepatic lobule[7] and postulated that the periportal distribution of allyl formate-induced hepatic necrosis results from the alkylation of macromolecules by a metabolite, acrolein, produced within periportal hepatocytes. The present studies provide the first direct evidence in vivo that allyl alcohol causes periportal necrosis through the covalent binding of a metabolite to periportal hepatocytes.

Methods. Male Sprague-Dawley rats (200 g) were pretreated either with saline (0.5 ml i.p.) or with pyrazole (375 mg/kg i.p.) an inhibitor of hepatic alcohol dehydro-

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Binding of ^{14}C-allyl alcohol: effect of pyrazole or phenobarbital treatment

Treatment (*N*)	Time of killing (h)	Concentration of bound radioactivity (pmoles/mg protein)		
		Liver	Lung	Kidney
Control (5)	08.00	119.5 ± 36.7	16.1 ± 3.6	10.7 ± 2.3
Pyrazole (2)	08.00	21.6 ± 0.6[a]	11.2 ± 1.7	4.2 ± 0.8
Control (2)	24.00	80.2 ± 10.2	10.5 ± 0.9	3.6 ± 0.9
Pyrazole (2)	24.00	12.9 ± 0.4[a]	4.9 ± 0.5[a]	2.4 ± 0.3
Control (3)	06.00	80.2 ± 7.9	12.3 ± 0.8	13.6 ± 1.5
Phenobarbital (3)	06.00	57.2 ± 2.8	9.7 ± 0.6	12.4 ± 1.0

[a] Significantly different from controls at 5% level. Number of rats per group (N) given in parentheses.

genase[8–10]. 2 hours later all rats were given ^{14}C-allyl alcohol (0.05 ml/kg i.p.; specific activity 2.46 mCi/mmole), and were killed by cervical fracture 8 or 24 h thereafter. In another experiment rats were pretreated with phenobarbital (80 mg/kg i.p.) 72, 48 and 24 h before ^{14}C-allyl alcohol administration to induce microsomal cytochrome P-450 enzymes in the liver[11,12]. For autoradiographic studies, paraffin sections of liver, lung, and kidneys[13] were coated with Kodak NTB-2 emulsion, developed 4 weeks later and stained with hematoxylin and eosin[14]. Since no precautions were taken to prevent the extraction of unbound radiolabeled material into the organic solvents used to prepare the paraffin sections, it is assumed that most of the exposed grains of the emulsion indicate radioactive molecules covalently bound to the tissue sections.

To quantitate the amount of ^{14}C-allyl alcohol bound in liver, lung or kidney the tissues were homogenized in 4 volumes of water. Proteins and nucleic acids were precipitated by the addition of an equal volume of cold 20% trichloroacetic acid. After 5 extractions of the precipitate with 10 ml of hot methanol (60 °C) no significant amounts of radioactivity could be removed from the pellet by further methanol extractions. Therefore, it is assumed that after 5 methanol extractions most of the radioactivity associated with the pellet was covalently bound to macromolecules. The pellet was dissolved in NaOH (1.0 *N*) and aliquots were taken for liquid scintillation counting and protein determination[15].

Results. Within 24 h after administration of ^{14}C-allyl alcohol the livers showed extensive periportal necrosis (Figure 1a) as reported by other investigators[1–6]. In contrast, no necrosis was observed in the kidneys and

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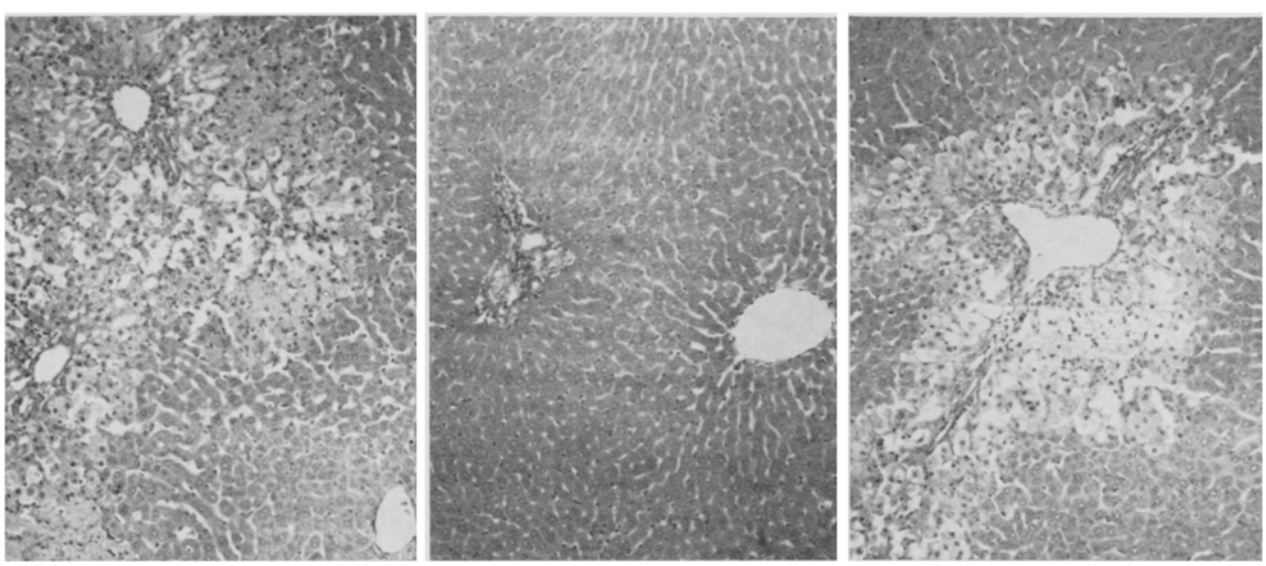

a) b) c)

Fig. 1. Photomicrograph of paraffin sections from livers of rats killed 24 h after administration of allyl alcohol (0.05 ml/kg). Doses for prior treatments with saline, pyrazole or phenobarbital are given in text. Sections were stained with PAS and hematoxylin. × 80. a) Saline pretreatment. Necrotic hepatocytes surround 2 portal triated on left; normal cells surround centrolobular vein in lower right corner. b) Pyrazole pretreatment. No necrosis is evident. c) Phenobarbital pretreatment. Periportal necrosis is similar to that in saline pretreated rats.

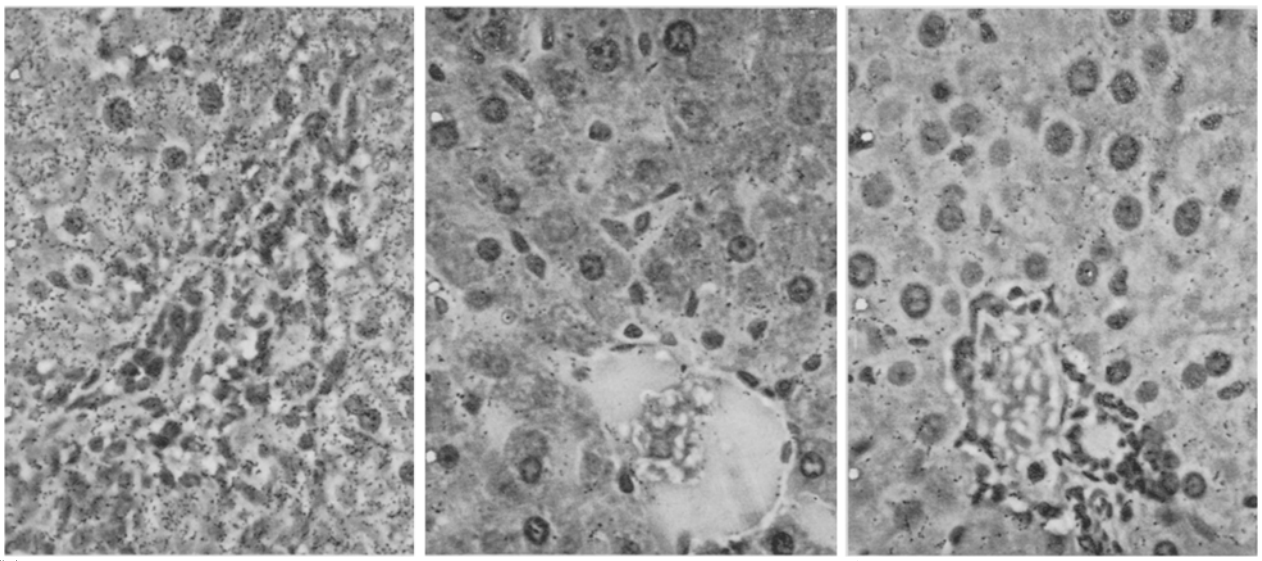

Fig. 2. Autoradiograms of livers of rats killed 6 h after administration of ^{14}C-allyl alcohol (0.05 ml/kg). Doses for prior treatments with saline or pyrazole are given in text. Paraffin sections were stained with hematoxylin and eosin. ×630. a) Saline pretreatment. Numerous exposed grains of emulsion representing tissue-bound ^{14}C-allyl alcohol are concentrated in hepatocytes surrounding a portal triad. b) Saline pretreatment. Relatively little radioactivity is observed in hepatocytes surrounding a centrolobular vein. c) Pyrazole pretreatment. Compared with Figure 2a, the amount of radioactivity bound to periportal hepatocytes is markedly reduced.

lungs after ^{14}C-allyl alcohol administration. ^{14}C-allyl alcohol was bound in liver to the extent of 178 pmoles/mg protein at 8 h, whereas about half that amount was bound at 24 h (Table). In contrast, very little binding was observed in lung and kidney. Autoradiograms demonstrated that most of the binding occurred in the periportal zone of the liver (Figure 2a), whereas relatively little radioactive material was localized in the centrolobular zone (Figure 2b).

Prior inhibition of the nonmicrosomal enzyme alcohol dehydrogenase[16] by pyrazole completely blocked the hepatic necrosis induced by ^{14}C-allyl alcohol (Figure 1b), and reduced by over 80% the amount of radiolabeled material bound in the liver (Table). Autoradiograms confirmed that pyrazole administration markedly reduced the binding of ^{14}C-allyl alcohol in periportal hepatocytes (Figure 2c). Pyrazole administration also decreased the binding in the lung at 24 h but did not significantly affect renal binding of ^{14}C-allyl alcohol (Table). In contrast, as previously reported[17,18], induction of microsomal enzymes by administration of phenobarbital did not affect the hepatotoxicity of ^{14}C-allyl alcohol (Figure 1c), and did not significantly alter the binding of the toxin in liver, lung or kidney (Table).

Discussion. Although the lung and kidney can metabolize ^{14}C-alcohol to $^{14}CO_2$ *in vitro*[19], initial oxidation of alcohols in vivo occurs mainly in the liver[20]. These facts provide an explanation for the much smaller amounts of ^{14}C-allyl alcohol bound in lung and kidney than in the liver. This observation in turn suggests that tissue necrosis may be determined by the extent of covalent binding, since no histopathology was observed in the lungs or kidneys. The studies with pyrazole indicate that both the binding of ^{14}C-allyl alcohol to liver macromolecules and the subsequent periportal hepatic necrosis are mediated by a metabolite of the alcohol. This conclusion is compatible with the hypothesis that the toxic metabolite is the aldehyde, acrolein[4,6].

Some experimental hepatoxins produce centrolobular instead of periportal hepatic necrosis[21]. Centrolobular necrosis induced by ^{14}C-bromobenzene administration is believed to be caused by the covalent binding of a chemically reactive metabolite of bromobenzene to proteins in centrolobular hepatocytes[22–25]. Both the binding and the necrosis are blocked by prior administration of SKF 525-A (diethylaminoethyl diphenylpropyl acetate) and enhanced by phenobarbital[22,25,26], compounds which block or induce, respectively, the activities of microsomal cytochrome P-450 enzymes[11,12,27] localized mainly in the centrolobular zone of the liver lobule[28–31]. Thus, with

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either allyl alcohol or bromobenzene, chemically reactive metabolites may produce hepatic necrosis by alkylating tissue macromolecules, and the distribution of the binding and necrosis appears to be determined by the localization within the liver lobule of the enzymes which produce the toxic metabolites. These studies may be relevant to the mechanisms by which therapeutic agents occasionally produce liver necrosis and other tissue lesions in man.

[32] I thank Mr. JOHN GEORGE, Miss KATHY LALUSH and Mrs. MARY ALICE LARSON for their expert technical assistance.

Résumé. Ces recherches indiquent que la répartition des lésions nécrotiques produites chimiquement peut être déterminée par la localisation intrahépatique d'enzymes métabolisant les drogues, enzymes qui synthétisent, dans les cellules où ils sont produits, les métabolites chimiquement actifs capables d'alkylation des macromolécules.

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Protein Synthesis Stimulation in Rat Liver by Chloroquine

Chloroquine[1], a powerful antimalarial drug, produces a large number of biological effects, which reveals a diversity of activities. Evidence has been obtained that CQ acts on several cell sites, by binding to RNA, DNA and proteins[2–4] and by inhibiting many hydrolytic and respiratory enzymes[5]. As a consequence of its large spectrum of interactions, an inhibition on cell replication and macromolecular biosynthesis has been reported in bacterial and tissue cultures[6,7]. That CQ behaves as a cytostatic agent has been confirmed in our experiments on Yoshida ascite cells, but we have also observed that, soon after CQ injection into normal rats, the incorporation of amino acids into liver proteins is enhanced.

In fact, 4 h after an i.p. injection of 30 mg/kg body wt. of CQ in male albino Wistar rats (weighing about 150 g) the in vivo incorporation of leucine ^{14}C into liver proteins increases about 40% compared with controls. This stimulation is also observed in the liver post-mitochondrial fractions tested in a cell-free assay system.

A close dose-response relationship is present in a concentration range of 15–45 mg/kg body wt.; doses above 60 mg give frequent rise to toxic accidents. In time experiments this stimulation is appreciated within 60 min after the injection of the drug; it reaches its maximum effect after 4 h, and thereafter it slightly decreases.

In separate experiments we observed that both cytosol and pH 5-fraction prepared from CQ-treated rats are not able to stimulate the amino acid incorporation in the in vitro assay. Polysomes, however, are shown to be more active than those of the controls when tested for their endogenous mRNA (Table I). Polyuridylic acid-directed polyphenylalanine synthesis was found to be lower in polysomes from treated rats and the ratio of the incorporation values in absence and in presence of artificial messenger demonstrates that less endogenous mRNA-free sites are available in CQ-polysomes. These results also correspond to the zonal sedimentation profiles, which show a larger fraction of heavier aggregates in CQ-treated rat liver polysomes.

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Table I. Incorporation of ^{14}C phenylalanine into proteins by the postmitochondrial fractions and polysomes isolated from rat liver at various time intervals after a single i.p. injection of 30 mg/kg body wt. of CQ

	Postmitochondrial supernatant		Polysomes		
Time after injection	Counts per min / mg of proteins		Counts per min / mg of RNA		Incorporation ratio
			− poly U	+ poly U	+ poly U / − poly U
Control (h)	2,380	100	26,150	58,150	2.24
1	2,860	120	68,200	83,400	1.23
4	3,420	144	113,750	121,800	1.07
18	3,160	134	98,600	114,300	1.16
24	2,730	114	81,000	92,500	1.14

The complete system contained in 1 ml:
Tris-HCl pH 7.6 50 μmole; $MgCl_2$ 5 μmole; KCl 25 μmole; β-mercaptoethanol 5 μmole; ATP 1 μmole; GTP 0.4 μmole; phosphoenolpyruvate 10 μmole; phosphoenolpyruvatekinase 10 μg; aminoacids ^{12}C 6.10^{-2} μmole each; ^{14}C phenylalanine (457 mCi/mmole) 1,6 μCi; cell sap 330 μl; polysomes 10 O.D.; polyuridylic acid 1000 μg. In the experiments performed with the postmitochondrial supernatant the cell sap was omitted and 650 μl of the supernatant were used (total proteins 3 mg). After incubation for 30 min at 37 °C, aliquots were absorbed onto paper disks and processed according to MANS and NOVELLI[12].