Liver Cell Necrosis in Rats After Prolonged Ethanol Ingestion Under the Influence of an Alcohol-Dehydrogenase Inhibitor

Incidence and type of liver damage in a homogeneous group of 526 male alcoholics showed a significant correlation with duration and degree of alcohol abuse[1-4]. Precirrhotic and cirrhotic lesions predominantly occurred when mean daily ethanol intake had for many years continuously exhausted the limited capacity for alcohol metabolism in man (100 ± 30 mg per kg/h, range 59 to 180 mg per kg/h[5,6]). In the rat, however, the rate of ethanol oxidation is almost thrice as high. Experimentally, even incorporation of ethanol into totally liquid nutritious diets to the extent of 36–50% of total calories, though resulting in fatty changes, ultrastructural hepatic alterations and alcoholic hyaline[7-9], did not lead to severe hepatic damage as seen in the human alcoholic. Therefore, instead of trying to further increase ethanol intake, it seemed logical to investigate attempts to decrease the capacity for ethanol metabolism in rats by partially inhibiting the rate-limiting enzyme alcohol-dehydrogenase (ADH). Competitive, dose-dependent and specific inhibition of ADH in vitro and in vivo by pyrazole was reported by THEORELL et al.[10,11]. However, no further information on the pharmacology of this compound was available.

Material and method. Commercially available male Wistar-rats were each housed in individual cages and fed a commercial standard solid diet (Altromin-R®) ad libitum with free access to tap-water. Pyrazole, easily soluble in water and alcohol (purchased from Messrs. D. Schuchardt, Munich), was used as 1% and 5% w/v aqueous solutions. Ethanol was either fed as sole source of drinking fluid (15% v/v) or given by stomach tube (30% v/v). LD_{50} was determined according to WILCOXON and LITCHFIELD[12]. Blood alcohol concentration (BAC) in samples obtained by heart puncture was determined as means of triplicate analyses by WIDMARK's micromethod and the enzymatic method. Post-mortem examination was done on each animal; samples of liver, kidney, lung, heart, spleen, and adrenals were fixed in 10% formalin. Paraffin-embedded sections were stained with hematoxylin-eosin and Van Gieson's stain; frozen sections were stained for fat with Sudan-III.

Results. Acute oral toxicity (LD_{50}) of pyrazole, determined after a 24-hour fast with free access to tap-water in 2 groups of 40 rats each, weighing 205–220 g, was 1010 mg/kg (c.l.: 840–1214 mg/kg); minimal lethal dose was 850 mg/kg. After single doses larger than 1000 mg/kg leading to a comatose state with death occurring within 12–36 h, no liver cell alterations were seen microscopically; livers of rats dying 6–11 days after 850–1000 mg/kg showed extensive centrolobular necrosis with inflammatory reactions and appearance of fat in surviving liver cells.

In chronic experiments, pyrazole given daily by stomach tube in doses of 37–38 mg/kg ($^1/_{25}$ of minimal lethal dose) for 8 weeks together with sucrose in amounts isocaloric with 7 g/kg ethanol resulted in a retardation of growth but not in any microscopical liver damage. Even after 23 weeks, daily doses of 35 mg/kg pyrazole only led to the appearance of a few fat droplets in single scattered liver cells and some multinucleated hepatocytes.

In vivo inhibition of ethanol oxidation by pyrazole was studied in 4 groups (I–IV) of 11 non-fasted male rats. BAC was determined precisely 24 h after a single oral dose of 6.4 g/kg ethanol alone (I) or together with 32, 50 or 70 mg/kg pyrazole (II–IV). In group I mean BAC was 2 mg/100 ml (0–5), but in the pyrazole-treated groups it was still significantly elevated at this time (II: 28 mg/100 ml (3–49); III: 39 mg/100 ml (16–84); IV: 72 mg/100 ml (35–170)). Inhibition thus seemed to be dose-dependent.

The main purpose was to study the effect of prolonged ethanol intake on rat liver under the influence of inhibitory threshold doses of pyrazole. After 5 weeks on a 15% ethanol solution as sole source of drinking fluid 5 male rats were then given 31 mg/kg pyrazole daily by stomach tube. Activity seemed not impaired but spontaneous ethanol intake was reduced and animals died 5–19 days later. Livers of all 5 animals showed a greyish-yellow mottling; microscopically there was patchy centrolobular to extensive massive acidophilic liver cell necrosis with inflammatory cell reaction and fatty degeneration of surviving parenchyma.

In another group of 95 rats with free access to tap-water and the standard diet, ethanol was administered once daily by stomach tube in doses of 6.0–7.7 g/kg together with a mean dose of 36.6 mg/kg pyrazole (range 25–50 mg/kg). Acute intoxication was generally noted during the first 8 h of each day after which animals usually began to regain fairly normal activity. 40 rats of this group died within the first week of this treatment, 23 survived up to 2 weeks, 16 up to 3 weeks, and 16 longer than 3 weeks (maximum 68 days). BAC determined at different times (13th–25th day) in 5 rats 24 h after the last preceding application ranged from 26 to 307 mg/100 ml.

Livers of more than half of the animals were brown-yellow to yellowish-red with fine to coarse greyish mottling. Microscopically, only 9 rats showed no hepatocellular damage. In 27 rats there were mild to distinct mostly centrolobular necrobiotic lesions, such as cytoplasmic hyalinization, hydropic degeneration, pyknosis and karyorrhexis and considerable fatty change. In 59 rats, however, there was frank acidophilic necrosis ranging from patchy centrolobular distribution to massive destruction of large irregular areas accompanied by varying degrees of inflammatory cell reaction (Figures 1a, b). In a few animals attempts at repair with proliferation of fibrous tissue, distorsion of lobular architecture and formation of giant cells and new bile ductules were found (Figures 1c, d).

Discussion. Prolonged ethanol administration under the influence of partial inhibition of ADH by threshold

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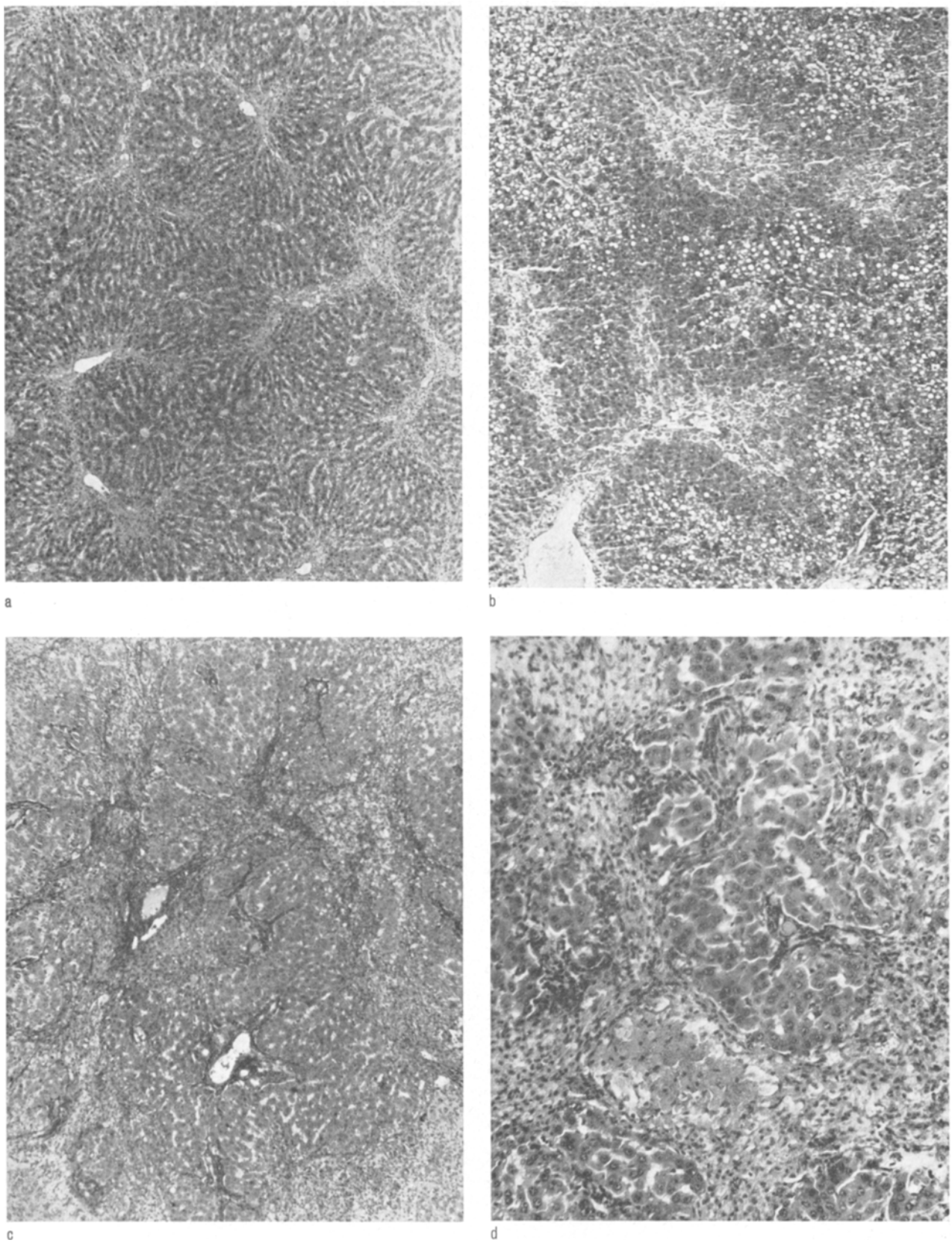

(a) Network-like hepatocellular necrosis. 6.2 g/kg ethanol + 50 mg/kg pyrazole daily, death after 10 days. Hematoxylin-Eosin. (b) Patchy centrolobular necrosis with fatty infiltration of remaining parenchyma. 6.8 g/kg ethanol + 30 mg/kg pyrazole daily, death after 44 days. Hematoxylin-Eosin. (c and d) Attempts at repair with proliferation of fibrous tissue, production of bile ductules and distorsion of lobular architecture. 6.7 g/kg ethanol + 35 mg/kg pyrazole daily, death after 19 days. Van Gieson's stain.

doses of pyrazole had an effect on livers of rats similar to that of frank hepatotoxic agents. Massive liver cell necrosis developing without symptoms of acute intoxication, when animals were kept on ethanol solutions as sole source of drinking fluid during pyrazole-induced inhibition of ADH, seems to argue against the assumption that necrosis was merely due to hypoxia or shock. At present it could not be decided whether ethanol only potentiated pyrazole toxicity. However, it seemed more likely that pyrazole-induced inhibition of ethanol metabolism increased ethanol toxicity resulting in liver cell damage of a severity hitherto not reported with conventional feeding techniques. This raises the question whether any hypothetical hepatotoxic action of ethanol may be distinct from its metabolic effect. In rats, with their high rate of ethanol oxidation, the use of a competitive ADH-inhibitor may be instrumental in producing liver lesions resembling those of 'acute alcoholic hepatitis' in man. This experimental design may prove to be of value in studying possible hepatotoxic properties of ethanol.

Zusammenfassung. Alkoholzufuhr bei partieller Hemmung der Alkoholdehydrogenase durch Pyrazol führte bei Ratten mit ihrem relativ hohen Alkoholumsatz in kurzer Zeit zu ausgedehnten Leberzellnekrosen, bei einigen Tieren auch zu bindegewebigen Reparations- und Umbauvorgängen.

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Respiratory Responses of the Conscious Dog to Severe Heat Stress

When the conscious sheep[1] and ox[2] are subjected to severe heat stress, body temperature, respiratory frequency and respiratory dead space ventilation all increase. Respiratory frequency ultimately reaches a maximum and then declines; during this second phase respiratory minute volume and alveolar ventilation further increase and respiratory alkalosis develops. During subsequent cooling there is a reversion to rapid shallow panting before respiration slowly returns to normal. The pattern is quite different in the anaesthetized dog[3,4]: there is no increase in respiratory frequency until there has been a considerable rise in body temperature (T_r) and the second phase is associated with a decrease in respiratory minute volume and alveolar ventilation which is not reversed by subsequent cooling; death from cardiovascular failure usually occurs[5]. Higgins and Iampietro[6] showed that alkalosis of venous blood can occur in the absence of hyperthermia in the conscious dog, but this study does not permit the complete resolution of the question whether the results obtained on the anaesthetized dog are wholly attributable to the effects of anaesthesia, or whether there are real species differences in the respiratory responses to heat stress. Further studies on the conscious dog which can be compared directly with those made on the sheep[1] and ox[2] have therefore been made.

Methods. 3 mongrel dogs weighing 15.3–16.7 kg were used. The right common carotid artery was brought to a subcutaneous position by previous surgery[1]. A polyethylene catheter (bore 1.0 mm, wall 0.25 mm) was introduced into the artery through a 13 s.w.g. hypodermic needle, after local infiltration with procaine. The animal then stood or lay quietly in a room at approximately 17 °C dry bulb/12 °C wet bulb temperature for about 1 h before entering an environment of 40/26 °C. After 20 min, humidity was rapidly raised to give a wet bulb temperature of 38 °C. The animal remained in this environment until T_r reached 42 °C, after which it was returned to the cool environment.

Rectal temperature, at a depth of 10 cm, and respiratory frequency were recorded throughout each experiment. Samples of arterial blood were taken at intervals, and a blood gas and pH electrode system (Radiometer, Copenhagen) was used to determine blood P_{CO_2} with an accuracy of $\pm$ 1 mm Hg, P_{O_2} with an accuracy of $\pm$ 2 mm Hg and pH with an accuracy of $\pm$ 0.005 units. Blood oxygen saturation (S_{O_2}) was measured to within 0.5% on an oximeter (American Optical Company) which had been calibrated with a standard spectrophotometric technique[7].

In an additional experiment on one dog tidal volume and respiratory minute volume were determined under the same experimental conditions using a pneumotachometer[8], but no blood samples were taken. In another experiment a dog was anaesthetized with sodium pentobarbitone (30 mg/kg i.v. + 1.5 mg/kg at 30 min intervals commencing 2 h after the initial dose) and was heated beneath an electric blanket while rectal temperature and respiratory frequency were recorded.

Results and discussion. The 3 conscious dogs responded similarly. The effects of severe heat stress on respiratory frequency, T_r, blood gases and blood pH are shown in Figure 1. Immediately upon exposure to the hot dry environment respiratory frequency increased 18-fold from 18 breaths/min to 340 breaths/min. When ambient humidity was raised there was a further increase in respiratory frequency which rose, within 10 min, to a peak rate of 410 breaths/min, before decreasing to 260 breaths/min.

From the record of thoracic movements and visual observations it was evident that when respiratory frequency rose, the depth of breathing decreased. However, when respiratory frequency fell at the onset of the second phase, the depth of breathing increased. This was confirmed by respiratory ventilation measurements on one of the dogs (Figure 2). Tidal volume decreased from 215 ml to 30 ml, and then increased to 203 ml. The result was a gradual increase in respiratory minute volume to 3 times the pre-exposure value at the peak of rapid shallow panting, and then a further increase to 11 times the pre-exposure level with slower deeper breathing. These changes are very similar to those seen in the

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