Proc. Nat. Acad. Sci. USA
Vol. 72, No. 3, pp. 1137–1141, March 1975

Experimental Alcohol-Induced Hepatic Necrosis: Suppression by Propylthiouracil

(liver damage/thyroid hormones/hypermetabolic state/hypoxia/alcoholism)

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Communicated by Charles H. Best, December 23, 1974

ABSTRACT **We have previously reported that a hypermetabolic state, resembling that produced by thyroid hormones, exists in the livers of animals treated chronically with ethanol. We propose that this alteration produces a relative hypoxia in the centrilobular zone of the liver which, if severe enough, leads to cellular death and to the production of hepatitis.**

Rats consuming ethanol for 30 days, given with a nutritionally adequate diet, and exposed to reduced oxygen tensions for only 6 hr, developed histological and biochemical evidence of hepatocellular necrosis and inflammatory lesions confined to the centrilobular zone. The severity was proportional to the degree of hypoxia. Pair-fed (nonalcohol) controls showed no such lesions. Treatment of the animals with propylthiouracil for 3–10 days abolished the hypermetabolic state of the liver in ethanol-consuming animals, and drastically reduced the histological and biochemical effects of hypoxia in them. These findings may have implications for pathogenesis and treatment of alcoholic hepatitis in man.

Livers of rats chronically treated with ethanol utilize oxygen at higher rates than control livers (1–5). The hypermetabolic state results from increased utilization of ATP by the cell membrane (Na+K)-ATPase (ATP phosphohydrolase, EC 3.6.1.3), associated with an increase in the active transport of monovalent cations across the membrane (2, 6). We have also reported that the hypermetabolic state can be completely abolished by ouabain, an inhibitor of the (Na+K)-ATPase (2, 3).

Similar changes in (Na+K)-ATPase activity, cation transport, and oxygen consumption occur in livers of animals treated with thyroid hormones, and can also be completely abolished by ouabain (3, 4, 7–10). We have also pointed out other similarities between the livers of animals chronically treated with ethanol and those treated with thyroid hormones (3, 4, 7). These similarities suggested that a *functional* hyperthyroid state exists in the livers of alcohol-treated animals (3, 7).

In human alcoholic hepatitis (11, 12), hepatocellular necrosis is most commonly seen in the central region of the hepatic lobule (zone 3 of Rappaport). Since the livers of ethanol-treated animals consume oxygen at higher rates (1–5), the possibility exists that the gradient of decreasing oxygen tension from the portal to the central venous end of the sinusoid is accentuated. We propose that, when the availability of oxygen is reduced, it is this accentuation of centrilobular hypoxia which leads to necrosis.

Abbreviations: PTU, 6-propyl-2-thiouracil; SGOT, serum glutamic-oxalacetic transaminase [aspartate transferase]; SOCT, serum ornithine carbamyltransferase.

To test this hypothesis we have exposed rats briefly to atmospheres with reduced oxygen tensions (5–11%). In animals treated chronically with ethanol, but not in controls, this period of hypoxia resulted in various degrees of centrilobular damage ranging from focal necrosis to massive confluent necrosis with accompanying inflammatory reaction. We have also found that a short pretreatment with the antithyroid drug, 6-propyl-2-thiouracil (PTU), abolishes the hypermetabolic state and the ouabain-sensitive respiration in the livers of ethanol-treated animals, and markedly suppresses the tissue damage provoked by hypoxia.

In hypoxia experiments, male Wistar rats (High Oak Farms, Ontario) were pair-fed nutritionally adequate liquid diets providing 35% of the calories as ethanol or as sucrose (controls) for a period of 30 days (13, 14). Liquid diets were replaced overnight (16 hr) by water before the experiments. Next morning, capillary blood (200 μl) was taken from the cut tip of the tail for baseline measurement of serum enzyme activities. Animals were then placed individually in cylindrical glass chambers (40 × 20 cm) and were exposed for 6 hr to oxygen at the selected concentrations (11, 7.5, or 5%, the remainder being pure nitrogen) at atmospheric pressure. The gas flowed continuously through the chambers at a rate of 1.5–2 liters/min. After exposure to hypoxia, animals were anaesthetized with ether and 6–8 ml of blood was drawn from the abdominal aorta.

Activities of glutamic-oxalacetic transaminase (SGOT) [L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1] and of ornithine carbamyltransferase (SOCT) [carbamylphosphate:L-ornithine carbamyltransferase, EC 2.1.3.3] were determined in sera before and after exposure to low oxygen tensions. These activities were determined in 5–20 μl of serum by microadaptations of the techniques of Bergmeyer and Bernt (15) and Reichard and Reichard (16) respectively. For SOCT, this required the reduction of [^{14}C]ornithine in the incubation mixture to a final concentration of 0.11 mM. Both activities were linear with respect to time and amount of serum added.

A sample of each liver was fixed in buffered 10% formalin solution and sections were stained with hematoxylin-eosin for light microscopy. Liver necrosis and other abnormalities detectable by light microscopy were assessed by one of us (JMP) in histological preparations previously coded in order to avoid bias. Liver necrosis was scored as follows: 0, no necrosis; 1+, focal necrosis of one to two cells per lesion; 2+, focal necrosis of more than two cells per lesion; 3+, massive confluent necrosis; and 4+, zonal massive necrosis plus

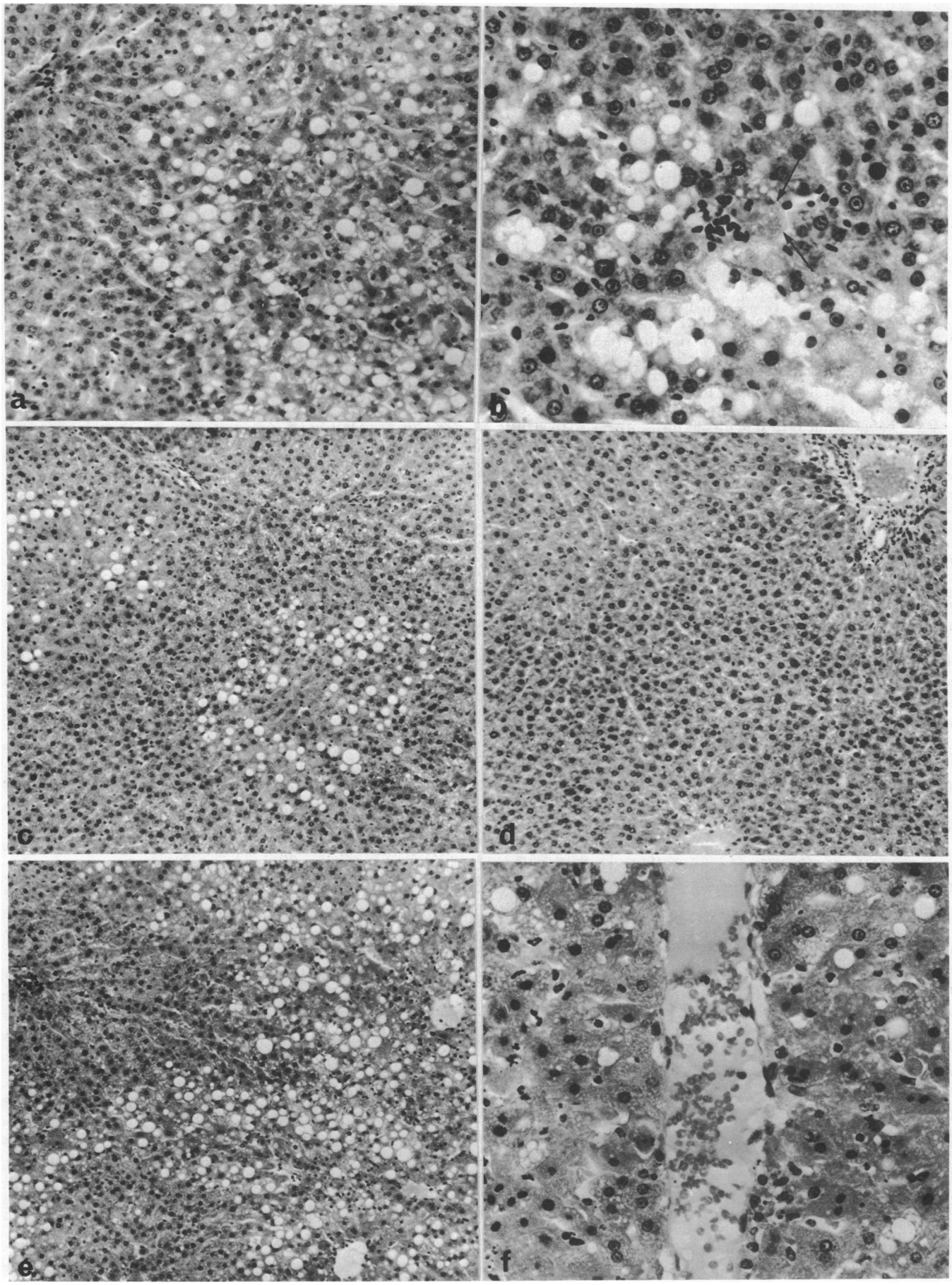

FIG. 1. (*Legend at bottom of next page.*)

TABLE 1. *Effect of chronic ethanol treatment and hypoxia on liver histology and serum glutamic-oxalacetic transaminase (SGOT)*

Group (chronic treatment)	Serum glutamic-oxalacetic transaminase (units/ml)				Liver necrosis (relative units)
I	*Air*	*11% O_2*	Δ	*P*	
Ethanol	129.8 ± 17.2(6)	188.5 ± 21.5(6)	58.7 ± 15.5(6)	<0.01	0.40 ± 0.8(5)
Control	69.1 ± 8.9(6)	87.1 ± 12.1(6)	18.0 ± 8.2(6)	<0.05	0.0 ± 0 (5)
II	*Air*	*7.5% O_2*	Δ	*P*	
Ethanol	137.1 ± 17.1(6)	203.2 ± 28.5(6)	66.1 ± 12.8(6)	<0.005	0.90 ± 0.3(6)
Control	123.5 ± 20.1(6)	96.1 ± 11.5(6)	−27.4 ± 9.3(6)	N.S.	0.0 ± 0 (6)
III	*Air*	*5% O_2*	Δ	*P*	
Ethanol	197.5 ± 19.4(6)	1197 ± 217(6)	1001 ± 215(6)	<0.005	3.25 ± 0.48(5)
Control	204.3 ± 28.9(6)	243 ± 88(6)	49.7 ± 73(6)	N.S.	0.0 (2)

See *text* for details of treatments. Values represent the mean ± SEM, with N for each group shown in parentheses. Δ values represent the changes in SGOT activity produced by exposure to the low oxygen tension. Histologically detectable lesions did not occur in ethanol or control (sucrose) animals in air. N.S., not significant.

necrotic bridging between central veins (terminal hepatic veins).

Exposure to hypoxia caused an increase in SGOT level, of marginal significance, in only one of the three control groups (Table 1). In contrast, there were highly significant increases in all alcohol-treated groups, which became more pronounced as the oxygen was reduced. The 6-hr period of hypoxia also produced liver necrosis exclusively in the alcohol-treated animals. No necrosis was found in the alcohol-treated or the control animals exposed to air. Increased fat, of centrilobular distribution, found in all the ethanol-treated animals, occurred independently of exposure to hypoxia, and of the presence or absence of necrosis. Necrosis, when it occurred, was limited to the centrilobular zone, and was accompanied by centrilobular swelling of cells, polymorphonuclear leukocytic infiltration, and cell degeneration.

The severity of the histological lesion increased as the oxygen tension was reduced. After exposure to 5% oxygen, massive centrilobular necrosis and bridging were observed. The periportal and midzonal areas did not show necrotic foci or other abnormalities. No lesions were found in the sucrose control animals exposed to 5% oxygen; their livers did not differ from those of control and naive animals (stock) exposed to air. Thus, depending on the degree of hypoxia, a continuum exists between the absence of necrosis and a massive centrilobular necrosis with bridging. A focal lesion resembling an alcoholic hepatitis without Mallory bodies is an intermediate stage in this continuum (Fig. 1, a and b). Mallory bodies are not essential for the diagnosis of alcoholic hepatitis in man (11, 17–20).

The 5% oxygen concentration was used for a more detailed study of the effects of hypoxia on SGOT, SOCT, and liver histology in alcohol-treated and control animals pretreated with PTU. Rats were pair-fed the ethanol or equicaloric sucrose diets for 30 days. During the last 10 days, half of each group received a solution of PTU (5 mg/100 g daily) by gastric intubation, while the other half received water.

Exposure to 5% oxygen for 6 hr again markedly increased the SGOT activity in the alcohol-treated animals but not in the sucrose controls (Table 2). The SOCT values increased dramatically (60-fold) in the alcohol group. The change in SOCT activity in the sucrose controls, though statistically significant, was only 5–6% of that found in the alcohol group. An average necrosis score of 2.69+ was found in ethanol-treated animals, whereas sucrose controls had only 0.25+, due entirely to one animal with a 2+ lesion. Treatment with PTU reduced the histological rating of necrosis in the ethanol-treated animals by about 80%, and prevented statistically significant increases in SGOT and SOCT levels. Representative liver histology of the ethanol-treated and sucrose animals with or without pretreatment with PTU is shown in Fig. 1c–f.

The effect of PTU administration on the alcohol-induced hypermetabolic states was determined in separate experiments. Rats were fed liquid diets containing 35% of the total calories as ethanol or as isocaloric sucrose for 28–49 days, as described by Videla *et al.* (1). PTU (5 mg/100 g daily) was given by stomach tube for 3–21 days, starting after 28 days on the ethanol or sucrose diets. Controls for the PTU experiments received only the vehicle. The rates of total and ouabain-sensitive oxygen consumption by liver slices from these animals were then measured as previously described (4).

The extra respiration seen in livers of ethanol-treated animals was suppressed by PTU treatment (Fig. 2), in keeping with the marked protection exerted by PTU against liver

FIG. 1. Micrographs (on preceding page). (a) Liver of a rat fed ethanol for 30 days, then exposed to 7.5% oxygen for 6 hr. Portal and periportal regions are normal. Note the fatty change, focal necrosis, and focal inflammatory infiltrates in midzonal and centrilobular regions. (Hematoxylin and eosin ×210). (b) Same liver as in (a). Higher magnification of centrilobular region showing fatty change, focal liver cell degeneration and necrosis (arrows), and focal inflammatory cell infiltration. (Hematoxylin and eosin ×310). (c) Liver histology in PTU-treated animals fed ethanol chronically and subjected to hypoxia (5% oxygen) for 6 hr. There is mild fatty change in the centrilobular region; otherwise, the hepatic structure is normal. (Hematoxylin and eosin ×180). (d) Liver histology in PTU-treated animals fed the sucrose control diet and subjected to hypoxia (5% oxygen) for 6 hr. The hepatic structure is entirely normal. (Hematoxylin and eosin ×180). (e) Liver histology in animals chronically fed ethanol and subjected to hypoxia (5% oxygen) for 6 hr. The portal region, on the left, is normal. Note the extensive necrosis, fatty change, and degeneration of hepatocytes in midzonal and centrilobular regions. Bridging necrosis from one central vein to another is shown. (Hematoxylin and eosin ×180). (f) Same liver as in (e). Higher power of magnification of centrilobular region shows hepatocellular necrosis, with pyknotic liver cell nuclei and condensed cytoplasm. Leukocytic infiltration can also be seen. (Hematoxylin and eosin ×310).

TABLE 2. *Effect of chronic ethanol treatment, hypoxia, and propylthiouracil on serum enzymes and liver histology*

Chronic Treatment	Air	5% O_2	Δ	P^c
	Serum glutamic-oxalacetic transaminase (units/ml)			
Ethanol	110.6 ± 12.8(6)	496.5 ± 93.3(6)	394.3 ± 76.3	<0.002
Control	111.9 ± 13.1(6)	136.7 ± 14.9(6)	24.8 ± 17.8	N.S.
Ethanol + PTU	102.5 ± 17.4(6)	190.6 ± 58.3(6)	88.1 ± 58.8[a]	N.S.
Control + PTU	128.6 ± 21.6(6)	94.3 ± 9.0(6)	−34.2 ± 19.1	N.S.
	Serum ornithine carbamyl transferase (pmol/hr per ml)			
Ethanol	90.5 ± 35.5(6)	6151 ± 1096(6)	6060 ± 1105	<0.002
Control	41.8 ± 5.9(6)	390 ± 131(6)	356 ± 131	<0.02
Ethanol + PTU	55.2 ± 20.3(6)	2514 ± 1248(6)	2459 ± 1248[b]	N.S.
Control + PTU	143.5 ± 60.2(6)	161.4 ± 70.7(6)	18.2 ± 24.5	N.S.
	Necrosis (relative units)			
Ethanol	—	2.69 ± 0.54(8)	—	—
Control	—	0.25 ± 0.25(8)[a]	—	—
Ethanol + PTU	—	0.56 ± 0.20(8)[a]	—	—
Control + PTU	—	0.12 ± 0.04(8)	—	—

See *text* for details of treatments. Values represent the means ± SEM, with N for each group shown in parentheses. Δ is same as for Table 1.

[a] *P* value with respect to ethanol without PTU (unpaired data) <0.01.

[b] *P* value with respect to ethanol without PTU (unpaired data) <0.05.

[c] *P* value shown for the effect of 5% oxygen (paired data).

necrosis. Similarly, PTU reduced the ouabain-sensitive respiration to control values. Since the effects of PTU on oxygen consumption seemed to be exerted very early, they were studied in a larger group after 3 and 6 days of treatment. Maximal inhibitory effects were found after only 3 days of PTU treatment (Table 3).

PTU is known to act on the thyroid gland by inhibiting synthesis of thyroxine, and also to act as a powerful inhibitor of peripheral deiodination of thyroxine into the more active form, triiodothyronine (21–23). The latter effect is exerted very quickly. In man, PTU sharply reduces circulating triiodothyronine to new steady-state levels in about 3 days, while only marginally reducing circulating thyroxine (24). The rapidity of action of PTU in reducing the alcohol-induced hypermetabolic state in the liver, suggests that this hypermetabolic state is more likely related to triiodothyronine rather than to thyroxine. The present results, however, do not distinguish between a hypermetabolic state produced by increased levels or activity of thyroid hormones, and a permissive role for thyroid hormone.

Since PTU reduced both the hypermetabolic state of the liver and susceptibility of the liver to damage by hypoxia in animals treated chronically with ethanol, the hypermetabolic state may be responsible for the supersensitivity to hypoxia.

TABLE 3. *Effect of propylthiouracil on the rate of oxygen consumption by liver slices of chronically ethanol-treated and control rats*

	Rate of oxygen consumption (μmol/g of liver per min)		
	No additions	Ouabain (1 mM)	Ouabain-sensitive respiration
A. *Chronic ethanol treatment*			
(a) Control	0.96 ± 0.03(9)	0.94 ± 0.03(9)	0.02 ± 0.03(9)
(b) Ethanol	1.37 ± 0.04(9)	0.95 ± 0.03(9)	0.42 ± 0.03(9)
B. *Chronic ethanol treatment—3 days PTU administration*			
(c) Control	0.97 ± 0.02(6)	0.93 ± 0.03(6)	0.04 ± 0.02(6)
(d) Control + PTU	1.02 ± 0.04(6)	0.89 ± 0.02(6)	0.13 ± 0.03(6)
(e) Ethanol	1.28 ± 0.03(6)	0.88 ± 0.03(6)	0.40 ± 0.04(6)
(f) Ethanol + PTU	0.95 ± 0.05(6)	0.92 ± 0.04(6)	0.03 ± 0.02(6)
C. *Chronic ethanol treatment—6 days PTU administration*			
(g) Control	0.87 ± 0.04(5)	0.82 ± 0.05(5)	0.05 ± 0.03(5)
(h) Control + PTU	0.86 ± 0.03(4)	0.82 ± 0.05(4)	0.04 ± 0.04(4)
(i) Ethanol	1.16 ± 0.04(5)	0.85 ± 0.04(5)	0.31 ± 0.06(5)
(j) Ethanol + PTU	0.91 ± 0.04(5)	0.85 ± 0.02(5)	0.06 ± 0.03(5)

All the animals received liquid diets containing 35% of the calories as ethanol or as isocaloric sucrose (controls) for 28–34 days. For the last 3 days (group B) or 6 days (group C), the animals received a daily dose of 6-propyl 2-thiouracil (PTU) by intubation (5 mg/100 g per day). Controls for the PTU experiments received the saline vehicle. Animals in group A received neither PTU nor vehicle. Values represent the means ± SEM, with the number of animals per group shown in parentheses.

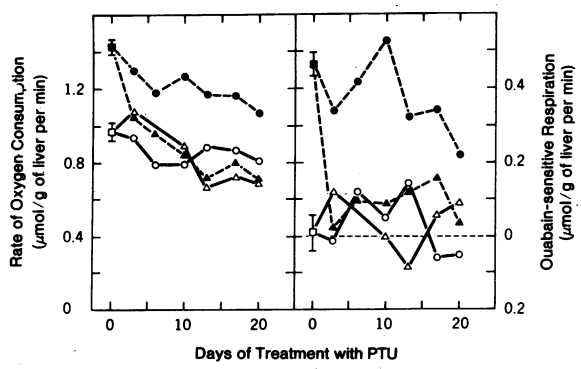

FIG. 2. Effect of propylthiouracil on the rate of oxygen consumption and ouabain-sensitive respiration of liver slices of chronically ethanol-treated and sucrose control rats. After 28 days of liquid diet administration (preceding day zero), the animals received PTU (5 mg/100 g per day) or saline vehicle by intubation. The animals continued to receive liquid diets containing ethanol or isocaloric sucrose for up to a total of 49 days (21 days of PTU treatment). ●, Ethanol + saline; ○, sucrose + saline; ▲, Ethanol + PTU; △, sucrose + PTU.

If a hypermetabolic state also occurs in the liver of the alcoholic patient, as suggested by the increased rate of alcohol metabolism observed in such patients (25–27), it is conceivable that a reduction in the availability of oxygen to the liver, to levels below those required to maintain this hypermetabolic state, could explain the production of alcoholic hepatitis. Such reductions could be caused by the respiratory depression that occurs in heavy alcohol intoxication, by respiratory diseases such as pneumonia and emphysema, or by two other conditions known to occur in alcoholics, namely, anemia (28) and a marked reduction in respiratory capacity (29). If our hypothesis is correct, PTU should also be therapeutically effective in the treatment of alcoholic hepatitis in man. In principle, PTU should also have protective value in hepatocellular damage associated with other pathological states which cause hypoxia, and in some types of liver necrosis in which cell swelling reduces sinusoidal blood flow.

In conclusion, we have shown that the livers of rats chronically treated with ethanol are supersensitive to short exposure to reduced oxygen tensions that do not affect the livers of control animals. Under these conditions, centrilobular lesions occur only in the animals treated with ethanol. Pretreatment of animals with propylthiouracil abolishes the hypermetabolic state that occurs in the liver of these animals and markedly suppresses the production of the liver lesions elicited by the low oxygen tensions.

The skillful technical assistance of Mrs. Sarala Kareti, Mrs. Federica Araneda, and Mr. Terrance Ruddy is gratefully acknowledged.

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