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Ingestion of Ethanol Increases the Hematotoxicity of Inhaled Benzene in C57BL Mice

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Pathological conditions caused by occupational exposure to chemicals may not be caused by the toxicity of the chemicals alone but may reflect the combined effects of the chemicals and personal habits. One of these habits, ethanol ingestion, has come under increasing scrutiny as a factor in the pathogenesis of work-related disease. Ingested ethanol has been found to increase the activity of a number of enzymes including: hepatic cytochrome P-450 and epoxide hydrase (HIETANEN et al. 1980), intestinal cytochrome P-450 (SEITZ et al. 1978), hepatic and kidney 7-ethoxycoumarin-O-deethylase (SAVOLAINEN et al. 1978), and hepatic dimethylnitrosamine demethylase (GARRO et al. 1981). The heightened enzyme activations induced by ethanol have been found to increase the mutagenic potency of the carcinogens dimethylnitrosamine (GARRO et al. 1981) and benzo(a)pyrene (SEITZ et al. 1978). It is widely believed that benzene is metabolized to hematotoxic agents by mixed function oxidase (GONASUN et al. 1973). Ingested ethanol could therefore affect the hematotoxic potency of inhaled benzene. Studies have been undertaken in this laboratory to investigate this possibility.

MATERIALS AND METHODS

Male, six week old C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were quarantined and observed for anomalous behavior for two weeks and then randomly distributed into six groups of 20 mice each. Each group was assigned to one of the following combined inhalation and ingestion treatments: air + water, air + 5% ethanol, air + 15% ethanol, 300 ppm benzene + water, 300 ppm benzene + 5% ethanol, 300 ppm benzene + 15% ethanol. When not undergoing benzene exposure, mice were housed, five to a box, over wood chip bedding in polycarbonate boxes fitted with stainless steel tops.

Benzene exposures were conducted in a 1 m^3 stainless steel and glass dynamic exposure chamber (DREW & LASKIN 1973). Benzene vapor was generated by passing an air stream across the surface of liquid benzene and feeding this benzene-laden air into the chamber. Exposures were performed for 6 h/day, 5 days/wk (Monday through Friday)

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for 18 weeks. Exposure concentrations were monitored at one hour intervals by an ultraviolet technique that has been previously described (SNYDER et al. 1978). Air exposed animals were treated with filtered, conditioned air in a duplicate chamber using the same experimental regime as benzene exposed animals. Neither food (Purina Lab chow) nor water was provided when animals were in the exposure chambers.

Ethanol was provided ad libitum 4 days/wk (Monday through Thursday) in the drinking water at concentrations of 15% or 5% w/w. All animals received water ad libitum Friday through Sunday. Fluid consumption was measured during those periods when ethanol was supplied.

Free flowing venous tail blood was obtained for peripheral blood cell counts. For a given ethanol treatment, benzene exposed and air exposed mice were bled within 30 minutes to minimize differences due to handling, circadian rhythms, etc. Red and white cell counts were determined on a Coulter Model ZB-I blood cell counter (Coulter Electronics, Hialeah, FL). Differential blood cell counts were performed manually using Wright-Geimsa stained blood smears. Hematocrits were determined in duplicate using Strumia microhematocrit tubes.

Animal weights were determined weekly for the first four weeks of the study and biweekly thereafter.

RESULTS

The animals received 85 exposures over 124 days (18 weeks) at a mean ± S.D. benzene concentration of 300.4 ± 7.8 ppm.

Fluid consumption was found to be more a function of ethanol concentration than benzene exposure. The four groups of mice ingesting either water or 5% ethanol consumed similar amounts of fluid averaging 0.185 g/g body weight x day. The two groups of mice ingesting 15% ethanol consumed similar but smaller amounts of fluid averaging 0.105 g/g body weight x day.

As shown in Table 1, rates of weight gain were found to be a function primarily of benzene exposure but also of

TABLE 1
Initial and Final Body Weights (Mean ± S.E.)
of Mice Treated with Benzene and Ethanol

Weeks After First Exposure	Treatment		
	Air	Air + 5% Ethanol	Air + 15% Ethanol
0	25.6 ± 0.7	26.1 ± 0.7	26.2 ± 0.6
18	31.2 ± 0.8	31.8 ± 0.6	30.8 ± 0.5
	Benzene	Benzene + 5% Ethanol	Benzene + 15% Ethanol
0	24.9 ± 0.5	24.9 ± 0.5	26.1 ± 0.5
18	26.4 ± 0.7	26.6 ± 0.7	24.4 ± 0.6

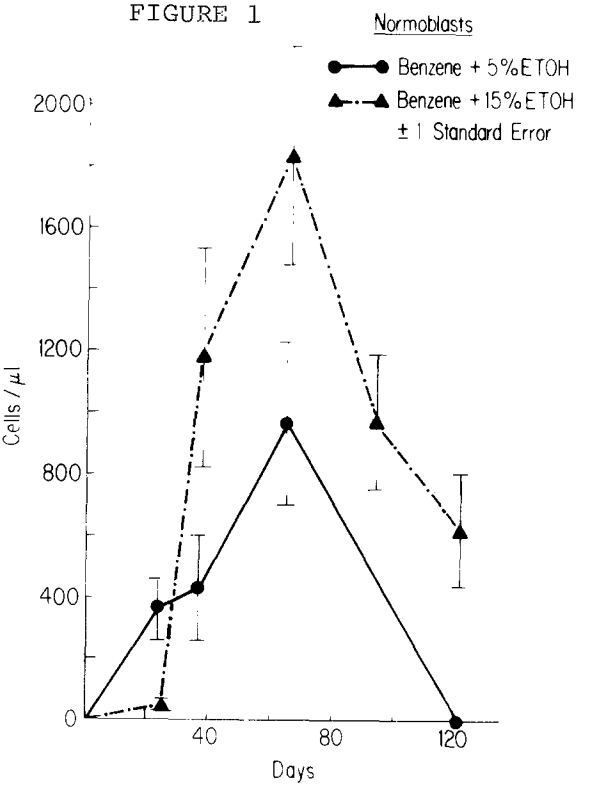
ethanol consumption. The three groups exposed to air had similar rates of weight gain regardless of the amount of ethanol ingested. The three groups exposed to benzene had similar weights which were lighter than those groups exposed to air. The group receiving 15% ethanol and exposed to benzene was unique in showing a weight loss.

Table 2 shows that peripheral red cells and lymphocyte levels declined as a function of both benzene exposure and ethanol consumption. All benzene exposed groups showed depressions in these peripheral cell counts relative to corresponding air exposed groups. Moreover the two groups exposed to benzene and consuming ethanol exhibited greater depressions in cell counts than the group exposed to benzene alone. There also appears to be a dose response effect relative to ethanol consumption as the group treated with benzene and 15% ethanol generally showed the most severe decline in these cells.

By the fourth exposure week increased numbers of nucleated red cells (normoblasts) began to appear in the peripheral blood of both groups treated with benzene and ethanol. The concentration of these cells peaked at about nine weeks of exposure and declined thereafter (Fig. 1). An average of only 2 normoblasts per µl was seen in the peripheral blood of the other groups.

Hematocrit values (not shown) for all groups paralleled red cell counts. In addition there appeared to be no consistent differences between the neutrophil counts of all six groups.

FIGURE 1



DISCUSSION

Based on observed consumption and average body weights, the average daily amounts of ethanol consumed for the benzene + ethanol treated groups was 0.24 g/mouse for the 5% ethanol treated group and 0.4 g/mouse for the 15% ethanol treated groups. WANG et al. (1976) demonstrated that rats consuming 20% ethanol in water over a

TABLE 2

RED BLOOD CELL COUNTS x 10^6

Weeks After First Exp.	Air + H_2O	Air + 5% ETOH	Air + 15% ETOH	Benzene + H_2O	Benzene + 5% ETOH	Benzene + 15% ETOH
0	10.1 ± 0.2	9.8 ± 0.4	10.0 ± 0.2	9.8 ± 0.2	10.0 ± 0.2	9.9 ± 0.2
2	9.6 ± 0.2	8.5 ± 0.2	9.3 ± 0.2	7.8 ± 0.2[a]	7.6 ± 0.1[a]	7.2 ± 0.1[a,b,c]
4	8.8 ± 0.1	8.2 ± 0.2	8.5 ± 0.2	5.9 ± 0.1[a]	5.8 ± 0.1[a]	6.3 ± 0.2[a]
6	8.2 ± 0.3	8.0 ± 0.2	9.0 ± 0.1	5.5 ± 0.1[a]	4.4 ± 0.1[a,b]	4.7 ± 0.2[a,b]
10	9.2 ± 0.3	8.6 ± 0.2	9.3 ± 0.5	5.3 ± 0.1[a]	4.6 ± 0.2[a,b]	3.7 ± 0.3[a,b,c]
14	8.4 ± 0.2	7.1 ± 0.2	8.1 ± 0.2	5.2 ± 0.2[a]	4.2 ± 0.2[a,b]	3.8 ± 0.4[a,b]
18	9.7 ± 0.2	9.1 ± 0.2	10.1 ± 0.2	6.8 ± 0.1[a]	5.4 ± 0.2[a,b]	4.0 ± 0.5[a,b,c]
			LYMPHOCYTE COUNTS x 10^3			
0	11.3 ± 1.1	9.4 ± 1.1	12.2 ± 0.5	12.5 ± 0.7	8.1 ± 0.8	10.8 ± 0.9
2	14.0 ± 1.4	10.4 ± 1.0	13.0 ± 0.8	3.9 ± 0.3[a]	2.0 ± 0.2[a,b]	1.8 ± 0.1[a,b]
4	9.7 ± 0.9	11.2 ± 0.8	7.0 ± 0.9	1.5 ± 0.2[a]	1.3 ± 0.1[a]	1.4 ± 0.1[a]
6	8.1 ± 0.7	2.3 ± 0.3	6.1 ± 0.7	0.9 ± 0.1[a]	1.6 ± 0.2	1.2 ± 0.2[a]
10	9.1 ± 1.0	10.7 ± 1.0	7.3 ± 0.9	0.9 ± 0.1[a]	1.0 ± 0.1[a]	0.4 ± 0.1[a,b,c]
14	7.8 ± 1.1	9.5 ± 1.1	8.4 ± 1.1	0.8 ± 0.1[a]	------	0.3 ± 0.1[a,b]
18	9.9 ± 1.1	8.1 ± 1.4	7.9 ± 1.1	1.4 ± 0.2[a]	1.4 ± 0.3[a]	0.7 ± 0.2[a,b]

Values are reported as mean ± S.E.

[a] denotes statistically less than corresponding control at $p < 0.05$

[b] denotes statistically less than benzene and water values at $p < 0.05$

[c] denotes statistically less than benzene and 5% ETOH values at $p < 0.05$

22-week period, maintained a total caloric intake equal to rats consuming water. The average daily caloric intake for C57BL/6J mice is about 21.3 cal. (Jackson Laboratories, personal communication). The accepted biological caloric content of ethanol is 7 cal/g (HOENSCH 1971). Therefore on those days when ethanol was provided, the percentage of caloric intake supplied by ethanol was 7.8% for the 5% ethanol + benzene treated group and 13.1% for the 15% ethanol + benzene treated group. Since ethanol was only supplied 4 d/wk, the weekly caloric intake due to ethanol was 4/7 of the daily intake or 4.5% for the 5% ethanol + benzene treated group and 7.5% for the 15% ethanol + benzene treated group. Since only a small percentage of caloric intake was due to ethanol, it seems highly unlikely that the severe hematotoxic responses observed in the ethanol + benzene treated groups was due to the non-nutritive calories of ethanol.

The appearance of large numbers of nucleated red cells in the peripheral blood is indicative of an unusual and severe erythropoietic stress. This may be due to an attempt by the hematopoietic organs to overcome decreased red cell production and survival induced by the treatments. It is well known that benzene exposure interferes with red cell production (GOLDSTEIN 1977). The initial rates of decline of peripheral red cell levels in the three benzene treated groups indicate that hemolysis of mature red cells has also occurred. These initial rates of decline correspond to a red cell survival half-life of between 32-36 days. The accepted half-life for rodent red blood cells is about 53 days (RUSSELL 1970).

These results indicate a true potentiation of the toxic effects of benzene by ethanol. There appears to be no hematotoxic response in those groups treated with ethanol alone but the hematotoxic responses of those groups treated with both benzene and ethanol were greater than the response of the group treated with benzene alone.

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