Effect of Acute Alcohol Intoxication on Granulocyte Mobilization and Kinetics
By Stephen J. Gluckman and Rob Roy MacGregor

Granulocyte mobilization into skin abrasions in human volunteers was significantly inhibited by acute alcohol intoxication (45,000 cells in 8 hr versus 353,000 in normal controls). Alcohol applied locally did not inhibit granulocyte delivery, and protection of the abrasion against heat loss did not reduce the inhibited delivery in intoxicated volunteers. Intoxication inhibited granulocyte adherence and local mobilization in parallel. Alcohol administration to rabbits shifted granulocytes from marginal to circulating pool in a manner similar to epinephrine. Mobilization of bone marrow granulocytes by glucocorticoid or endotoxin administration was not inhibited by intoxication, nor did it prevent the endotoxin-induced shift of granulocytes from circulating to marginal pool.

ACUTE ETHANOL INTOXICATION inhibits the movement of granulocytes into areas of experimental infection in animals.1,2 Nonintoxicated control animals inoculated with bacteria develop an acute inflammatory exudate that controls the infection locally. In contrast, intoxicated animals deliver few granulocytes to the infected focus, resulting in spread of the bacteria to the bloodstream and death from overwhelming infection. When a modified skin window technique was used to assess granulocyte delivery to sterile inflammatory sites in acutely intoxicated human volunteers, similar inhibition of granulocyte mobilization was found.3

We repeated these volunteer experiments to confirm the previous results and to evaluate possible mechanisms for the alcohol suppression of granulocyte exudation. In addition, we examined the effect of acute intoxication on granulocyte kinetics in three other conditions known to alter kinetics: (1) granulocyte demargination induced by epinephrine, (2) marrow mobilization following glucocorticoid administration, and (3) margination and marrow mobilization following endotoxin.

MATERIALS AND METHODS

Human studies. After giving informed consent, healthy nonalcoholic volunteers, ages 20 40 yr, drank 2 mg/kg 100% ethanol over 15-30 min in a mixer of their choice (equivalent to five or six mixed drinks). At the same time 1-cm² abrasions were made bilaterally with a scalpel blade on the volar surfaces of the volunteer’s forearms. The abrasions were covered with 5-ml glass cups (kindly supplied by Dr. Donald Louria, New Jersey Medical College, Newark, N.J.) filled through rubber ports with Hanks’ balanced salt solution (HBSS) containing 200 U/ml streptokinase/streptodornase. Fluid was changed every 2 hr, and the total cumulative granulocytes in the fluid were determined for an 8-hr period. This procedure is the Perillie modification of the Rebuck skin window technique,4 the same as that used in the earlier human study.5 In experiments evaluating the local effect of alcohol on inflam-
mation, HBSS containing 200 mg/dl ethanol was added to the chamber on one arm of nonintoxicated volunteers and the contralateral (control) chamber was filled with HBSS only. The cumulative 8-hr granulocyte counts for the two chambers were compared. To evaluate the possibility that decreased exudate into skin chambers of intoxicated subjects could result from lowered skin temperature caused by vasodilation and heat loss,5 volunteers were intoxicated as above and skin chambers were applied to each forearm. One arm was covered with a bulky insulating dressing and the other arm was left unwrapped. Cumulative cell counts were made for both chambers and the totals compared.

Granulocyte adherence was measured in nylon fiber columns6 using 5 ml heparinized venous whole blood drawn before and 1, 2, 4, and 6 hr after intoxication. Results were expressed as the percentage of normal adherence, defined as the preintoxication value for each subject.

Animal granulocyte kinetic studies. Male 3-kg New Zealand white rabbits were intoxicated by intravenous administration over 5 min of 35 ml of a 25% ethanol solution in normal saline. The animals developed nystagmus and ataxia, but all recovered by 8 hr. The mean peak blood alcohol level was 295 ± 15.3 (SE) mg/dl. Fifteen minutes after ethanol administration, the animals received one of the three medications discussed below, and blood granulocyte counts were measured at various intervals.

First, to determine the effect of intoxication on epinephrine-induced shift of granulocytes from the marginal to the circulating granulocyte pool, six intoxicated rabbits received 0.1 ml of a 1:10,000 epinephrine solution subcutaneously, and blood granulocyte counts were counted 5, 10, 15, 30, 45, and 60 min later. Six control animals received the same dose of epinephrine without intoxication. To control for the possible effects of ethanol intoxication itself on granulocyte counts, six other animals received ethanol alone, and their granulocyte counts were followed serially.

In the second study, hydrocortisone 10 mg was given intravenously to six intoxicated animals and peripheral blood granulocytes were counted at 2-hr intervals for 8 hr; six controls received hydrocortisone alone. Because of the findings in the epinephrine experiments detailed below, granulocyte counts were expressed in cells/mm³ rather than as a percentage of pretreatment values.

In the third study, endotoxin (Escherichia coli 0127:B8 lipopolysaccharide B, Difco, Detroit, Mich.), 0.1 µg/kg body weight, was administered intravenously to six animals 15 min after acute intoxication. Blood was drawn for granulocyte counts 15, 30, and 60 min and 2, 4, and 7 hr later. These counts were expressed as the percentage change from preintoxication (baseline) values and compared to those in controls receiving endotoxin in the nonintoxicated state.

RESULTS

Human studies. All six volunteers intoxicated acutely developed slurred speech and sleepiness. Peak blood alcohol levels developed 1-2 hr after ingestion (range 102-239 mg/dl, mean of 157 mg/dl). (Intoxication is defined legally in most states as concentrations greater than 150 mg/dl). The effect of acute intoxication on granulocyte mobilization into the skin chambers is shown in Fig. 1. Mobilization was most strikingly inhibited during the first 4 hr of intoxication but was significantly below normal values at all intervals (p < 0.01, Student's t test). After 8 hr the mean cumulative granulocyte delivery was 45,800 cells in the six intoxicated volunteers, compared to 353,000 in ten normal controls. The effect of local ethanol administration was assessed in four nonintoxicated volunteers, with the abrasion in their contralateral arm serving as control (Fig. 2). Equivalent numbers of granulocytes were mobilized into alcohol-containing and control chambers. Insulating the skin against heat loss did not improve granulocyte mobilization in acutely intoxicated volunteers (Table 1). Differences in mean values were not significant at any time intervals.

Mean granulocyte adherence values in the six intoxicated volunteers are shown in Fig. 3. Adherence was only 67.7% of prealcohol values 1 hr after alcohol administration, 70.5% at 2 hr, 74.9% at 4 hr, and 88.4% at 6 hr. Inhibition at 1, 2, and 4 hr was statistically significant (p < 0.001 at 1 and 2 hr, p < 0.02 at 4 hr, paired sample t test). Thus the inhibition of adherence decreased with time after intoxication, as did the inhibition of granulocyte mobilization into skin chambers.
Animal kinetic studies. Epinephrine administration alone more than doubled the peripheral blood granulocyte count within 10 min, with a gradual return to preadministration level by 1 hr (Fig. 4). This granulocytosis has been shown to represent a shift of cells from the marginal to the circulating granulocyte pool. Animals given ethanol intravenously 15 min before receiving epinephrine failed to develop granulocytosis after epinephrine administration (Fig. 4). Thus acute intoxication prevented the epinephrine-induced demargination granulocytosis. This
Table 1. Effect of Skin Heat Loss on Granulocyte Mobilization in Intoxicated Human Volunteers.

<table>
<thead>
<tr>
<th>Skin Condition</th>
<th>Time After Alcohol Ingestion</th>
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<tbody>
<tr>
<td></td>
<td>2 hr</td>
</tr>
<tr>
<td>Insulated</td>
<td>1802</td>
</tr>
<tr>
<td>Uncovered</td>
<td>3110</td>
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could result from an inhibition of movement from marginal to circulating granulocyte pool or from demargination caused by ethanol itself, leaving no cells in the marginal pool to respond to the epinephrine stimulus. To evaluate the latter possibility, six animals were given ethanol alone and granulocyte counts were measured sequentially (Fig. 4, middle curve). By 15 min after ethanol administration granulocyte counts had increased to the same degree seen 5–15 min after subcutaneous epinephrine administration. Thereafter, the counts gradually fell to normal by 1 hr, as seen with epinephrine administration. In Fig. 5 the mean maximal granulocyte counts in animals receiving epinephrine alone are compared with those in animals receiving ethanol 15 min before epinephrine. For both groups the original granulocyte counts were similar, as were the maximal increases. However, in the alcohol-treated animals most of the increase was accomplished by the alcohol, leaving little potential for additional granulocytosis by epinephrine demargination.

Acute glucocorticoid administration causes a release of marrow granulocytes and has been used as a test of marrow granulocyte reserve. The granulocytosis that followed intravenous hydrocortisone administration in six rabbits is shown in Fig. 6. When the animals were intoxicated 15 min before receiving hydrocortisone, their ultimate granulocyte count augmentation was similar to that in the nonintoxicated animals, and the granulocytosis was of equal duration. The difference in granulocyte counts between the two groups was not statistically significant at any time interval (p > 0.1, Student’s t test). To determine if continued high-degree intoxication throughout the 8-hr test might cause a late change in the granulocytosis curve, several animals were given a second dose of ethanol (20 ml of a 25% solution) 4 hr after their first. Results with these animals were the same as in those given a single alcohol dose.

Endotoxin administration to normal rabbits caused a profound granulocytopenia as the cells marginated (Table 2). Granulocytosis developed by 7 hr as cells entered circulation from the bone marrow. Animals intoxicated 15 min before
PMN KINETICS DURING ALCOHOL INGESTION

Fig. 4. Peripheral blood granulocyte counts following epinephrine administration alone, epinephrine following alcohol, or alcohol alone. Points, mean for six rabbits; brackets, ± SEM. For epinephrine and alcohol group, pretreatment granulocyte count was determined 15 min after alcohol administration, immediately before epinephrine administration.

receiving endotoxin took slightly longer to reach the nadir in their granulocyte count but reached a level as low as in the nonintoxicated controls; recovery, and the development of granulocytosis occurred at the same rate in both groups.

DISCUSSION

In 1938 Pickrell's classic studies showed the antiinflammatory effect of alcohol intoxication.1 Rabbits injected intracutaneously with type-1 pneumococcus developed a brisk inflammatory response that showed many granulocytes on biopsy. The animals survived their infection without becoming bacteremic. Biopsies in intoxicated animals showed swarms of subcutaneous bacteria and few granulocytes; these animals developed bacteremia and died. Of interest, granulocytes that did reach the site of infection were able to phagocytize and kill normally despite the high alcohol concentration; this lack of interference with phagocytosis and killing has been confirmed by others.1,9

It was not until 1970 that granulocyte mobilization was investigated in in-
toxicated humans. Louria’s group showed that granulocyte delivery into sterile inflammatory sites was inhibited in man just as in experimental animals. The present study, using the same technique and actual chambers as in Louria’s work, confirms the fact that acute intoxication suppresses the inflammatory response in man. In addition, we explored the mechanism of the antiinflammatory effect. Our results show that alcohol does not act locally to suppress inflammation and that local skin temperature does not play a role in the poor mobilization. Other evidence also suggests that local temperature fluctuations do not account for the antiinflammatory effect: Pickrell found that infected nonintoxicated animals whose body temperatures were rendered subnormal by exposure had the same granulocyte delivery as those with normal temperatures. Moreover, intoxication suppresses mobilization of granulocytes into the inflammed peritoneum, a site relatively well insulated against heat loss.

The inhibition of granulocyte adherence noted in our volunteers is a reasonable mechanism by which to explain their poor granulocyte delivery. Cells must adhere to the endothelium before diapedesis into the tissues, thus inhibition of adherence could be anticipated to block delivery of granulocytes into inflammatory areas. In fact, we have shown that inhibition of granulocyte adherence is a general property of antiinflammatory drugs and that drugs that correct the adherence defect in vivo improve deficient granulocyte mobilization as well.

To study the effects of ethanol intoxication on granulocyte kinetics further, we
chose the rabbit peritonitis model to avoid any risk to our human volunteers. Our experiments indicate that ethanol shifts granulocytes from the marginal to the circulating granulocyte pool in a manner similar to epinephrine-induced demargination. The maximum increase in granulocyte count occurs 15 min after intravenous ethanol administration, a bit later than with epinephrine, but it resolves by 1 hr in both cases. The granulocytosis after intoxication could represent granulocyte release from the marrow rather than demargination, but the latter appears more likely for the following reasons: (1) Following alcohol-induced leukocytosis, epinephrine fails to augment the granulocyte count to the degree seen when it is given to unintoxicated animals (500-cell increase in intoxicated animals versus 2000-cell increase in controls). This suggests that the marginal pool has been emptied by alcohol administration, leaving few cells to be mobilized into the circulating pool by epinephrine. (2) The rapid increase in granulocyte count and the subsequent rapid fall to normal are characteristic of epinephrine-induced demargination but they occurred much faster than the changes seen when granulocytes are mobilized from the marrow. (3) Epinephrine decreases granulocyte adherence concomitant to its induction of granulocytosis by demargination. As found in the present study and previously, alcohol also decreases adherence as it induces granulocytosis. Thus it appears that the degree of granulocyte adherence at least partially determines the distribution of cells between the marginal and circulating granulocyte pools.

Granulocytosis induced by glucocorticoid administration results from mobilization of cells from the marrow and from decreased rate of granulocyte egress from the intravascular pool. In addition, Dale et al. showed that a shift from the marginal to the circulating granulocyte pool occurs with prednisone therapy. Our data suggest a similar demargination in rabbits given hydrocortisone: in animals intoxicated before steroid administration, the rate of increase in granulocyte count in the first 2 hr following hydrocortisone was less than in nonintoxicated controls, but they started with a higher presteroid granulocyte count because of their alcohol-induced demargination. The flattened kinetic curve in the first 2 hr in intoxicated animals suggests that demarginating cells contribute to the degree of granulocytosis observed in that period in the nonintoxicated animals. A mechanism for demargination in steroid-treated animals could be the depression in granulocyte adherence described previously.

Release of granulocytes from the marrow reserve does not appear to be inhibited by intoxication. Increases in circulating granulocyte counts were equivalent in intoxicated and control animals given either hydrocortisone or endotoxin.

The profound granulocytopenia that develops within 15 min of endotoxin administration results from a shift of cells from the circulating to the marginal granulocyte pool, with cells becoming sequestered in the pulmonary capillaries. We have found previously that this margination is associated with a marked increase in granulocyte adherence, which could cause the cells to leave the circulating pool and adhere to the pulmonary capillary endothelium. Endotoxin administration has been shown to activate the complement system, which in turn augments granulocyte adherence. Hemodialysis and continuous flow filtration leukapheresis also activate complement and cause transient granulocytopenia, apparently by increasing granulocyte adherence. Thus it appears that
complement activation leading to increased granulocyte adherence is the common mechanism for the margination granulocytopenia in all three of these instances. Of interest, O’Flaherty et al. were able to prevent hemodialysis-induced augmentation of granulocyte adherence and the transient granulocytopenia by alcohol administration; our failure to prevent endotoxin-induced granulocytopenia by intoxicating the animals indicates that the endotoxin stimulus for margination was much stronger than the alcohol stimulus for demargination. This suggests that endotoxin-induced pulmonary sequestration may involve more than complement activation and increased granulocyte adherence.

Our studies indicate that ethanol intoxication has profound effects on granulocyte kinetics. Most significant is the inhibition of granulocyte mobilization to sites of inflammation, which appears to result from a depression of granulocyte adherence. Intoxication also causes a shift of granulocytes from the marginal to the circulating pool, most likely through the same mechanism of inhibited adherence. Movement of cells from the marrow into circulation does not appear to be affected by ethanol. Therapy directed toward correction of the adherence defect might enable intoxicated subjects to respond more effectively to infection, although the defect does not persist in the experimental model once sobriety is achieved.

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