PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **175**, 219–225 (1984)

Mechanism of Inhibition of Granulopoiesis by Ethanol[1] (41792)

P. S. IMPERIA, G. CHIKKAPPA, AND P. G. PHILLIPS

Medical and Research Services, Veterans Administration Medical Center and the Department of Medicine, Albany Medical College, Albany, New York 12208

Abstract. The mechanism of inhibition of neutrophilic granulopoiesis by ethanol has not been well characterized. Possible mechanisms investigated include: (a) a direct toxic effect on the granulocyte–macrophage cluster-colony forming unit (CFU-GM), and/or (b) inhibition of production of CFU-GM proliferation stimulating factor activity (CSA) from T lymphocytes (T cells). Addition of as much as 600 mg% ethanol to T-cell- and monocyte-depleted light-density marrow (TMoDLDBM) cells from humans in soft agar cultures which were stimulated with an exogenous source of CSA did not inhibit the CFU-GM proliferation, suggesting that ethanol has no direct toxic effect on the CFU-GM. T cells obtained from the blood of normal humans were cultured in the presence of phytohemagglutinin with 100 to 600 mg% ethanol. Cell-free conditioned media (CM) from these cultures were tested for CSA concentration by their capacity to stimulate proliferation of CFU-GM from human TMoDLDBM or rat whole bone marrow cells. The results indicated that ethanol at a concentration >100 mg% inhibited CSA production from T cells. There was no evidence for production of an inhibitor of CFU-GM proliferation from T cells in the presence of ethanol. These results suggest that the neutropenia which occurs in relation to alcohol abuse may in part be related to decreased CSA production from T cells.

Ethanol ingestion with or without nutritional deficiency has been associated with neutropenia in man (1–5). The exact mechanism for development of such neutropenia is not known (1–5). The possible mechanisms include: (a) direct inhibition of proliferation of the neutrophil progenitor, the granulocyte–macrophage cluster-colony forming unit (CFU-GM),[2] and/or (b) decreased production of CFU-GM proliferation stimulating factor activity (CSA) from the T lymphocytes (T cells), monocytes (Mo), and other cells known to produce CSA. Tisman and Herbert (6) showed that ethanol, in concentrations commonly found in the blood of ethanol abusers, inhibits lymphocyte transformation and CFU-GM proliferation *in vitro* despite the addition of sufficient quantities of folic acid, pyridoxine HCl, pyridoxal HCl, and vitamin B_{12}. These authors suggested that the decreased CFU-GM proliferation may have been due to the inhibition of CSA production by ethanol in the leukocyte feeder layers used as a source of CSA in their bone marrow cultures. Recently Meagher *et al.* (7) showed that inhibition of CFU-GM proliferation by ethanol does not occur in the presence of an exogenous source of CSA, which suggests that ethanol acts on CSA-producing cells. The purpose of the study reported here was to characterize ethanol's inhibition of granulopoiesis by studying its effects *in vitro* on CFU-GM proliferation and on CSA production from normal human blood T cells and Mo. The results indicate that ethanol inhibits CSA production from T cells and not from Mo. The reduced CSA production may contribute to the neutropenia seen in some ethanol abusers. As has been shown by others (7), ethanol has no direct inhibitory effect on CFU-GM proliferation.

Materials and Methods. *Subjects.* Blood and/or bone marrow (BM) samples were collected in syringes containing preservative-free heparin from normal 25- to 45-year-old males and females. Informed written consent was

---

[1] This work was presented in part at the 1983 National Student Forum by P.S.I. who was a IV-year Albany Medical College student. It was awarded "The University of Texas Medical Branch Cancer Award" for oncology–immunology research.

[2] Abbreviations used: BM, bone marrow; CFU-GM, granulocyte–macrophage cluster-colony forming unit; CM, conditioned medium; CSA, colony-stimulating factor activity; GCM, giant cell-conditioned medium; HPM, human placental-conditioned medium; LCM, L-cell-conditioned medium; MNC, mononuclear cells; MNC-Mo, MNC depleted of monocytes; Mo, monocytes; PHA, phytohemagglutinin-M; T cell, T lymphocytes; TMoDLDBM, T-cell- and Mo-depleted light-density bone marrow cells.

0037-9727/84 $1.50

obtained, as required by the Institutional Human Studies Committee.

Cell separation. T cell and Mo were isolated by the method of Mangan and Desforges (8). Blood was centrifuged through an Isolymph (Gallard-Schlesinger Chemical Corp., Carl Place N.Y.), Mo were removed from MNC by plastic adherence. Adherent Mo were retrieved with 12 m*M* lidocaine solution, as described by Rabinovitch and DeStefano (9). Sheep erythrocytes were added to the nonadherent MNC-Mo fraction to rosette the T cells. The rosetted T cells were pelleted by centrifugation through an Isolymph gradient, and the sheep erythrocytes were lysed with Tris–NH_4Cl to free the T cells. All cells were washed twice with McCoy's 5A complete medium; contents described by Pike and Robinson (10). The lymphocyte purity was 90 ± 3% and the monocyte purity was 92 ± 5% as determined by rerosetting the T cells and staining the Mo with α-naphthylacetate for cytoplasmic esterase (11, 12).

T-cell- and Mo-conditioned medium. The T cells and Mo were suspended separately in 2 to 3 ml of McCoy's 5A complete medium at (0.5 to 1.0) × 10^6 cells/ml in 10 × 35-mm plastic petri dishes and incubated at 37°C with 5% CO_2 flow. The T-cell cultures contained 1% phytohemagglutinin-M (PHA) (GIBCO, Grand Island, N.Y.), and the Mo cultures contained 1% BCG (NIH, Bethesda, Md.). Control T-cell and Mo cultures were also set up without PHA or BCG. Ethanol (Publicker Industries, Linfield, Pa.) was added at time zero or 24 hr to T-cell cultures, and at time zero to Mo cultures and to cell-free medium to final concentrations of 100, 300, and 600 mg%. Cohort control cultures lacked ethanol. Ethanol concentrations in these cultures were determined at intervals with ACA Analytical Test Packs (Clinical Systems, Dupont, Wilmington, Del.). In one study ethanol was added at 24-hr intervals to replenish the measured losses. The T-cell cultures were harvested at 96 hr and the Mo cultures at 48 hr. At the time of harvesting, cell counts were performed, and cell-free media (conditioned media, CM) were collected after centrifugation at 400*g* for 20 min. The CM were stored frozen at −20°C until the day of testing their CSA concentrations.

Comparison of the effect of ethanol with that of puromycin on CSA production from T cells. Puromycin dihydrochloride (Sigma, St. Louis, Mo.), which inhibits protein synthesis by acting on ribosomal RNA to terminate peptide chain synthesis prematurely, was used to compare its effect with ethanol's on CSA production from T cells. The T-cell cultures were set up, and all contained PHA. Except in the case of the control cultures, additions were made: (a) of ethanol alone to 50, 100, or 300 mg%, (b) of puromycin alone to 10 μg/ml, or (c) both. The cultures were terminated at 96 hr, at which time cell counts were performed and CM were prepared. The CM were dialyzed in distilled water for 3 days at 4°C to remove residual ethanol and puromycin to avoid possible toxicity in the CSA assay. They were then filtered (Millipore filters, 0.22 μm pore size) and stored frozen until analyzed for CSA.

Assay for CSA. Various CM were tested for CSA by their capacity to stimulate CFU-GM proliferation from Mo- and T-cell-depleted light-density marrow cells (TMoDLDBM) from normal humans and/or unseparated bone marrow cells from 4- to 6-week-old male Sprague–Dawley rats. The TMoDLDBM were prepared exactly as described before (13). Rat cells were used in some studies because they are easier and less expensive to obtain than human cells, and this did not compromise the results since our earlier studies with these two types of cells as CFU-GM targets showed comparable patterns of CSA levels in CM samples. The rat cells were tibial marrow cells obtained from at least two rats after flushing their marrow cavity with McCoy's 5A complete medium. The target cells were suspended in 0.3% agar medium to a final concentration of 0.5 × 10^5 human cells or 2.0 × 10^5 rat cells/ml. One-milliliter aliquots of the suspension were pipetted into 35 × 10-mm plastic petri dishes containing 0.1 ml of CM. Separate control dishes were also set up with human placental conditioned medium (HPCM) or L-cell-conditioned medium (LCM), for human and rat cells, respectively, as a CSA source to test the plating efficiency of the target cells. In all studies for each test condition, at least two culture dishes were prepared. The dishes were incubated at 37°C with 5% CO_2 flow for 7 to 9 days. They were processed to prepare permanent slides, which were stained (12) with alum hematoxylin (Harleco, Gibbston, N.J.). The CFU-GM aggregates were scored as clusters (8 to 32 cells) and colonies (>32 cells). Since the cluster to colony ratio was similar (3- to 4:1) in all studies, the data were combined for presentation of results. The cellular composition of the CFU-GM aggregates was typed by staining the cultures with a triple-stain technique, as previously described (12). Such staining facilitates simultaneous recognition of neutrophil, eosinophil, macrophage, and mixed cellular aggregates from each agar culture disc.

Direct effect of ethanol on CFU-GM. To test the possibility that ethanol has an effect on CFU-GM, it was added to dishes to final concentrations of 0, 200, or 300 mg%. The dishes also contained HPCM (0.1 ml per dish). Human TMoDLDBM cells (0.5×10^5/ml) were mixed in 0.3% agar medium and pipetted in 1-ml volumes into these dishes. Similar culture studies were done using rat BM cells (2.0×10^5 per dish), and LCM was used as a source of CSA in these cultures.

Inhibitors of CFU-GM. To test the CM that were prepared from T-cell cultures which contained ethanol (300 mg%), and ethanol (300 mg%) plus puromycin (10 μg/ml), for the presence of inhibitors of CFU-GM proliferation, separate dishes were prepared each containing 0.1 ml of HPCM or CM, and also dishes each with 0.1 ml of HPCM plus 0.1 ml of CM. Agar medium (0.3%) containing human TMoDLDBM was pipetted into these dishes. The numbers of aggregates formed from cultures containing CM plus HPCM were compared with those from cultures containing HPCM alone.

Statistics. A paired or two-sample t test was applied to determine the significance (P value) of differences between the results of cohort samples. A value of $P < 0.05$ is considered significant. All these statistics were obtained with Hewlett–Packard Model 85 programs.

Results. The pattern of ethanol loss from culture dishes with and without cells is shown in Fig. 1. A loss rate of about 1% per hour (r = 0.99) was observed, regardless of the cell type and presence or absence of cells, indicating that the cells were not responsible for the observed losses of ethanol from the dishes. Similar patterns of ethanol losses were observed from dishes containing 100 and 300 mg%.

The direct effect of ethanol on CFU-GM proliferation from normal human TMoDLDBM cells containing HPCM as an exogenous source of CSA is shown in Fig. 2. In accordance with other reports (7), as much as 600 mg% ethanol did not inhibit the CFU-GM proliferation. Similar results were ob-

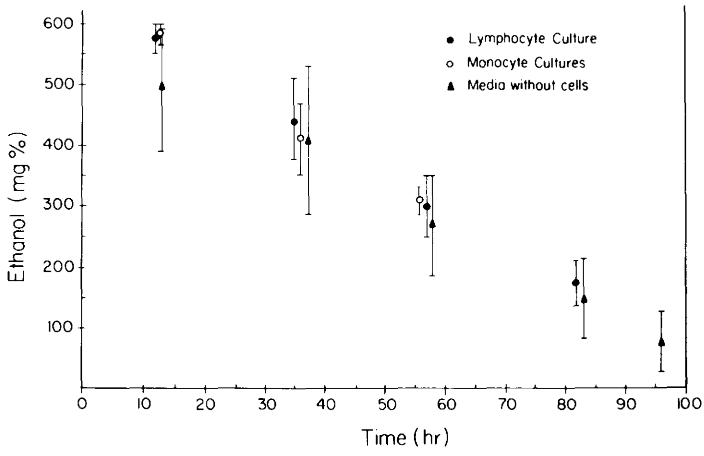

FIG. 1. Pattern of ethanol loss from dishes containing no cells, T lymphocytes, or monocytes in McCoy's 5A complete medium. The original ethanol concentration in all these dishes was 600 mg%. Values are pooled from replicate samples and expressed as means ± 1 SD.

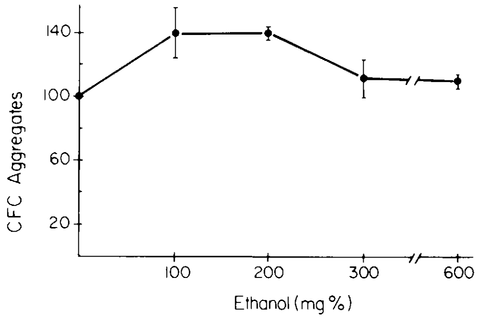

FIG. 2. Direct effect of ethanol on granulocyte–macrophage cluster-colony forming unit (CFU-GM) proliferation from T lymphocyte- and monocyte-depleted light-density bone marow cells from humans in agar culture medium containing human placental conditioned medium as an exogenous source of CFU-GM proliferation stimulating factor activity (CSA). Values are pooled from two separate studies and expressed as percentage of the mean ± 1 SD of controls.

tained from rat BM cell cultures stimulated with LCM, indicating that ethanol has no direct inhibitory effect on CFU-GM proliferation and did not interfere with the stimulatory effect of exogenous CSA.

The CSA concentration in CM of T-cells cultures with PHA was higher (142 ± 30 aggregates per 0.1 ml CM per 25 × 10^3 TMoDLDBM) than in CM of T cells cultured without PHA (126 ± 6 aggregates per 0.1 ml CM per 25 × 10^3 TMoDLDBM), and the latter was higher than that proliferated in the absence of an exogenous CSA (62 ± 2 aggregates per 25 × 10^3 TMoDLDBM). In subsequent studies the CM were prepared from T-cell cultures with PHA.

The effect of ethanol on CSA production from T cells and Mo is shown in Fig. 3. Ethanol at >100 mg% inhibited CSA production from T cells in a dose-related fashion ($r = 0.932$). In contrast, as much as 600 mg% ethanol had no effect on CSA production from Mo. The results were the same regardless of the time of initial ethanol addition (0 to 24 hr) and whether or not the measured losses were replenished daily. The degree of inhibition from T-cell CM varied among subjects, especially at 100 mg% ethanol concentration, but the variation was not significant. The pattern was the same whether the human or rat marrow cells were used as CFU-GM targets for CM CSA studies.

The observations of cell type in CFU-GM aggregates (Table I) indicated that the CFU-Gm reduction in cultures with CM obtained from T-cell cultures with 100 and 300 mg% ethanol, compared with cultures with control CM, occurred to a comparable extent in neutrophil, macrophage, and eosinophil aggregates.

Table II shows the effect of ethanol on CFU-GM proliferation from human BM cells cultured on feeder layers containing T cells, Mo, or GCM. Ethanol inhibited the CFU-GM in a dose-dependent manner from cultures with T cells in feeder layers. It did not inhibit the CFU-GM proliferation from cultures with Mo or GCM in feeder layers. These results again indicate that ethanol inhibits CSA production from T cells.

The influence of ethanol and puromycin on CSA production from T cells is shown in Fig. 4. Puromycin at the concentration used in this study has been shown to inhibit CSA production from T cells (14). Ethanol (at 100 and 300 mg%) and puromycin (10 μg/ml) independently inhibited CSA production.

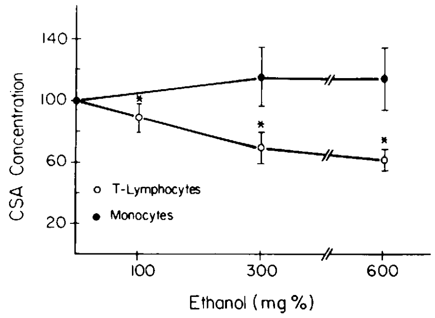

FIG. 3. Effect of ethanol on CSA production from T lymphocytes and monocytes in suspension cultures. CSA concentrations were determined from cell-free media (conditioned media, CM) of these suspension cultures by incorporating them into CFU-GM clonogenic assays containing human TMoDLDBM cells or unseparated rat BM cells. The values are pooled from five studies (human cells in two studies and rat cells in three) and expressed as percentage of the mean ± 1 SD of controls. The control represents the CFU-GM cultures stimulated with CM of T-cell cultures without ethanol. Asterisks denote that the value is different ($P < 0.05$) from the cohort control. See legend to Fig. 2 for abbreviations.

TABLE I. CELL TYPE OF CFU-GM[a] AGGREGATES IN TMoDLDBM CELL CULTURES STIMULATED WITH CONDITIONED MEDIA (CM) OF T-CELL CULTURES WITHOUT ETHANOL (CONTROL) AND WITH 100 AND 300 MG% ETHANOL (ETHANOL)

Type of CM added to CFU-GM culture	Cell type of aggregates (% of total)			
	Neutrophil	Macrophage	Eosinophil	Mixed
Control CM	43 ± 3	49 ± 4	7 ± 3	1 ± 2
Ethanol CM (100 mg%)	48 ± 4	47 ± 4	5 ± 2	0.3 ± 0.6
Ethanol CM (300 mg%)	44 ± 9	50 ± 6	4 ± 2	2 ± 1

[a] See Footnote 2 for abbreviations.

Ethanol (100 mg%) plus puromycin inhibited CSA production in a synergistic fashion, and 300 mg% ethanol plus puromycin showed additive inhibition. Total cell counts of ethanol- and/or puromycin-containing T-cell cultures remained essentially similar to those of controls which were without either agent.

Addition of CM, which were prepared from ethanol- and/or puromycin-containing T-cell cultures, to BM cultures stimulated with an exogenous source of CSA (HPCM) did not decrease CFU-GM proliferation (Table III).

TABLE II. EFFECT OF ETHANOL ON CFU-GM PROLIFERATION FROM TMoDLDBM CELLS IN DISHES WITH FEEDER LAYERS CONTAINING GIANT CELL-CONDITIONED MEDIUM (GCM), T CELLS, AND Mo AS SOURCES OF CSA

Source of CSA in feeder lyers	Ethanol (mg%)	CFU-GM per dish (% of control)
T cells	0	100
	100	61 ± 5[a]
	300	39 ± 1[a]
Mo	0	100
	100	105 ± 6
	300	124 ± 13
GCM	0	100
	100	92 ± 11
	300	106 ± 10

Note. Values are expressed as percentage of mean ± 1 SD of controls which did not contain ethanol but had one of the sources of CSA. See Footnote 2 for abbreviations.

[a] Inhibition is significant, $P < 0.05$, compared with control.

These results indicate that these CM did not contain an inhibitor of CFU-GM.

Discussion. Our studies indicate that ethanol has an effect on CFU-GM proliferation through inhibition of CSA production from T cells. The concentrations (100 and 300 mg%) of ethanol that inhibit CSA pro-

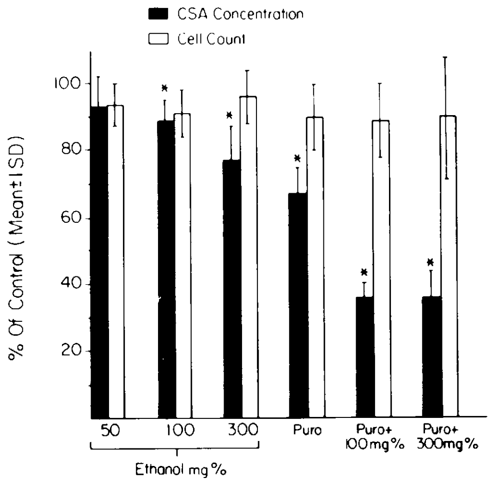

FIG. 4. Effect of ethanol compared with that of puromycin (puro) on CSA production from T cells and the number of cells in each culture dish. In these studies T-cell CM of a constant volume (0.1 ml per dish) were incorporated into CFU-GM clonogenic cultures containing human TMoDLDBM cells. Solid bars represent CSA concentration in CM and open bars, cell count in the respective cultures. Values are expressed as percentage mean ± 1 SD of control cultures (which were stimulated with CM prepared from T-cell cultures without ethanol or puro). An asterisk denotes that the value is different ($P < 0.05$) from the cohort control.

TABLE III. EVALUATION OF CFU-GM INHIBITORS IN CM PREPARED FROM T-CELL CULTURES WITH 300 mg% ETHANOL OR 300 mg% ETHANOL PLUS 10 μg/ml PUROMYCIN

Additives to CFU-GM cultures[a]	CFU-GM per dish (% of control)
0.1 ml HPCM	100 ± 6
0.1 ml HPCM + 0.1 ml CM of T-cell cultures with ethanol	100 ± 4
0.1 ml HPCM + 0.1 ml CM of T-cell cultures with ethanol and puromycin	97 ± 17

Note. The values are expressed as percentage mean ± 1 SD of the control, which was obtained from CFU-GM cultures stimulated with a standard source of CSA, human placental-conditioned medium (HPCM).

[a] Human TMoDLDBM cells were cultured.

duction are well within the levels attained in the blood of humans drinking alcohol. It is not likely that residual ethanol in the T-cell CM affected the results, since addition of ethanol directly to BM cultures containing an exogenous source of CSA did not inhibit CFU-GM proliferation. The lower CSA levels in CM obtained from T-cell cultures with ethanol are not due to death of T cells in cultures, since the viable cell numbers in them were similar to those in controls without added ethanol. The observation that ethanol replenishment at intervals after its initial addition failed to alter the pattern of CSA production from T-cell cultures suggests that the dose-related inhibition occurs in accordance with the initial concentration, whether or not it is maintained throughout the culture period.

Feeder layer studies provide further confirmatory evidence for ethanol's dose-dependent inhibition of CSA production from T cells. The greater degree of inhibition by ethanol in the feeder studies than in the CM studies is likely to be related to the differences in the culture conditions. Use of CM involves initial addition of CSA derived from equivalent of (0.5 to 2.0) × 10^5 T cells (0.1 ml of CM added per dish; the CM was obtained from cultures of (5.0 to 20.0) × 10^5 T cells per ml, whereas CSA from T cells (5.0 × 10^5 per dish) in feeder layers is elaborated over 8 to 9 days, the period of culture. Furthermore, these feeder layer studies performed without the use of PHA provide a control for the studies of T-cell CM which were prepared from T-cell cultures with PHA and ethanol. This indicates that the ethanol's effect of inhibition of CSA production from the T cells is not peculiar to PHA-stimulated cells.

CSA consists of a family of glycoproteins (15). Predictably, our results and those of others (14) have shown inhibition of CSA production by puromycin. Ethanol has been shown to inhibit protein synthesis in a variety of cells in cultures (16). The synergistic effect of ethanol and puromycin in the inhibition of CSA production suggests that, although ethanol may share puromycin's effect on ribosomal RNA, i.e., premature termination of peptide chain synthesis, it also acts at other sites of T-cell CSA production. The inhibition may be related to ethanol's ability to increase the levels in lymphocytes (17) of cAMP, a substance that has been shown to inhibit CSA production from endothelial cells (18). Yet another possibility is that ethanol may impair CSA secretion from the lymphocytes by altering the integrity of the microtubules that are essential for normal protein secretion. Such an effect has been thought to be responsible for inhibition of protein secretion from the hepatic cells in association with ethanol (19).

The reduced CSA detected in CM of T-cell cultures with ethanol is not due to inhibitors of CFU-GM proliferation, because addition of such CM to BM cultures stimulated with an exogenous source of CSA failed to inhibit the number of CFU-GM produced compared with control cultures which contained the exogenous CSA only. Furthermore, dialysis of the CM, which removes low-molecular-weight inhibitors, did not alter their CFU-GM stimulatory capacity.

The reason for the lack of inhibition of CSA production from Mo by ethanol is not clear. However, it may be related to data showing that Mo, unlike T cells, produce CFU-GM proliferation promotors (CSA) as well as inhibitors (prostaglandin E and acidic isoferritin) (20, 21). We speculate that ethanol may have affected the balance of the production of CFU-GM proliferation stimulators and inhibitors from Mo in such a way that their resultant net effect was unchanged.

On the basis of our data, we speculate that the neutropenia seen in some alcohol abusers may be due to an inhibition of CSA production from T cells. However, *in vivo* many other cells, e.g., marrow "stromal cells," monocytes–macrophages, vascular endothelial cells, etc., seem to be involved in CSA production (14, 15, 21–24). The overall rate of *in vivo* granulopoiesis promotion probably depends on the sum of the effects of the CSA produced from these cells. To what extent ethanol affects the CSA production from these cells and thus contributes to neutropenia is not known.

---

1. Sullivan LW, Herbert V. Suppression of hematopoiesis by ethanol. J Clin Invest **43**:2048–2062, 1964.
2. Eichner ER, Hillman RS. The evolution of anemia in alcoholic patients. Amer J Med **50**:218–232, 1971.
3. Liu YK. Leukopenia in alcoholics. Amer J Med **54**:605–610, 1973.
4. Liu YK. Effects of alcohol on granulocytes and lymphocytes. Semin Hematol **17**:130–136, 1980.
5. McFarland W, Libre EP. Abnormal leukocyte response in alcoholism. Ann Intern Med **59**:865–877, 1963.
6. Tisman G, Herbert V. In vitro myelosuppression and immunosuppression by ethanol. J Clin Invest **52**:1410–1414, 1973.
7. Meagher RC, Sieber F, Spivak JL. Suppression of hematopoietic-progenitor-cell proliferation by ethanol and acetaldehyde. N Engl J Med **307**:845–849, 1982.
8. Mangan KF, Desforges JF. The role of T lymphocytes and monocytes in the regulation of human erthropoietic peripheral blood burst forming units. Exp Hematol **8**:717–727, 1980.
9. Rabinovitch M, DeStefano MJ. Use of the local anesthetic lidocaine for cell harvesting and subcultivation. In Vitro **11**:379–381, 1975.
10. Pike BL, Robinson WA. Human bone marrow colony growth in agar-gel. J Cell Physiol **76**:77–84, 1970.
11. Knowles DM, Hoffman T, Ferrarini M, Kunkel HG. The demonstration of acid α-napthylacetate esterase activity in human lymphocytes: Usefulness as a T cell marker. Cell Immunol **35**:112–123, 1978.
12. Phillips PG, Chikkappa G, Brinson P. A triple stain technique to evaluate monocyte, neutrophil and eosinophil proliferation in soft agar cultures. Exp Hematol **11**:10–17, 1983.
13. Chikkappa G, Phillips PG, Brinson P. Differences in the sensitivity of normal human peripheral blood and bone marrow granulocytic macrophagic and eosinophilic colony forming cells (CFC) to a source of colony stimulating factor. Exp Hematol **10**:852–858, 1982.
14. Ruscetti FW, Chervenick PA. Regulation of the release of colony-stimulating activity from mitogen-stimulated lymphocytes. J Immunol **114**:1513–1517, 1975.
15. Parker JW, Metcalf D. Production of colony-stimulating factor in mitogenstimulated lymphocyte cultures. J Immunol **112**:502–510, 1974.
16. Syapin PJ, Noble EP. Studies on ethanol's effects on cells in culture. In: Majchrowicz E, Noble EP, eds. Biochemistry and Pharmacology of Ethanol. New York, Plenum, Vol 1:p5s1, 1979.
17. Hynie S, Lanefelt F, Fredholm BB. Effects of ethanol on human lymphocyte levels of cyclic AMP. In Vitro: Potentiation of response to isoproterenol, PGE_2 or adenosine stimulation. Acta Pharmacol Toxicol **47**:58–65, 1980.
18. Ramsey R, Hays EF. Factors promoting colony stimulating activity (CSA) production in macrophages and epithelial cells. Exp Hematol **7**:245–254, 1979.
19. Lieber CS. Alcohol, protein metabolism and liver injury. Gastroenterology **79**:373–390, 1980.
20. Kurland JI, Bockman RS, Broxmeyer HE, Moore MAS. Limitation of excessive mylopoiesis by the intrinsic modulation of macrophage-derived prostaglandin E. Science **199**:552–555, 1978.
21. Broxmeyer HE, Bognacki J, Ralph R, Dorner MH, Lu L, Castro-Malaspina H. Monocyte-Macrophage-Derived acidic isoferritins: Normal feedback regulators of granulocyte-macrophage progenitor cells in vitro. Blood **60**:595–607, 1982.
22. Knudtzon S, Mortesen BT. Growth stimulation of human bone marrow cells in agar culture by vascular cells. Blood **46**:937–943, 1975.
23. Schwartz RS, Greenberg PL. Stromal colony-stimulating activity production and myeloid colony-forming cells in human hemopoietic and nonhemopoietic bone marrow. Blood **57**:771–780, 1981.
24. Sheridan JW, Stanley ER. Tissue sources of bone marrow colony stimulating factor. J Cell Physiol **78**:451–460, 1971.

Received September 6, 1983. P.S.E.B.M. 1984, Vol. 175.
Accepted October 24, 1983.