Hemopoietic Effects of Glyceryl Ethers. III. Inactivity of Selachyl Alcohol.* (25959)

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Prior to isolation of batyl alcohol from bovine yellow bone marrow by Holmes and associates(1), this ether of stearyl alcohol and glycerol had been thought to exist only in certain marine organisms. It has since been found in a number of mammals including man(2). Sandler(3) reported that batyl alcohol stimulated erythropoiesis in normal and benzene-poisoned rats. Two years later, Arturson and Lindbäck(4) described erythrocytosis and reticulocytosis in mice injected with this glyceryl ether. It has also been implicated in leukopoiesis(5,6) and thrombopoiesis (7). Our recent studies(8,9) confirmed the erythropoietic, thrombopoietic, and granulopoietic stimulatory activity of batyl alcohol. Although the exact physiologic significance of this compound is not known, the chemical and physiologic attributes common to batyl alcohol and the thermostable, ether-soluble plasma erythropoietic factor suggest that they may be closely related(10). However, many questions remain unanswered. Foremost among these is the apparent inconsistency between amount of batyl alcohol required to alter myelopoiesis in normal rats, *i.e.*, 25-50 mg per day(9), and the smaller quantities of a substance possessing specific biologic activity which would be expected to exert a readily detectable effect. Possible explanations include: 1) use of synthetic, racemic preparations rather than optically active, natural material or 2) close relationship to some other more active substance, *e.g.*, selachyl alcohol, $CH_3(CH_2)_7CH{:}CH(CH_2)_7CH_2O \cdot CH_2 \cdot CHOH \cdot CH_2OH$. The latter, which is the glyceryl ether found most abundantly in nature(11), differs only slightly in chemical structure from batyl alcohol, $CH_3(CH_2)_{16}CH_2O \cdot CH_2 \cdot CHOH \cdot CH_2OH$. However, selachyl alcohol is a liquid at room temperature, whereas batyl alcohol is a crystalline solid. This report describes experimental observations which bear directly on the above two possibilities.

Materials and methods. Forty normal female Wistar strain rats weighing 140-160 g were divided into 4 equal groups. The animals in each group received *via* gastric intubation 10 daily doses over a 2 week period (Saturdays and Sundays excepted) of one of the following test materials†: 1) 50 mg of synthetic, racemic selachyl alcohol; 2) 50 mg of optically active, natural selachyl alcohol; 3) 50 mg of optically active batyl alcohol prepared by hydrogenation of the natural selachyl alcohol and administered as a suspension in 0.5 ml of distilled water; or 4) 0.5 ml of distilled water. Baseline studies included hemoglobins determined by the cyanmethemoglobin technic, microhematocrits, hemacytometer erythrocyte and leukocyte counts, reticulocytes per 1000 erythrocytes enumerated on dried brilliant cresyl blue coverslip films counterstained with Wright's stain, and thrombocytes counted by phase microscopy. These measurements were repeated weekly. At end of treatment period, 5 rats in each group were killed and femoral marrow examined utilizing a technic previously described (12). Observations on the remaining animals provided information in respect to restoration of normal hemic equilibrium.

Results. The recipients of selachyl alcohol did not manifest evidence of altered hemopoiesis. Their peripheral erythroid values (Fig. 1) and thrombocyte and leukocyte counts (Fig. 2) remained stable. Marrow nucleated cell counts in these animals were comparable to those in the control group given water

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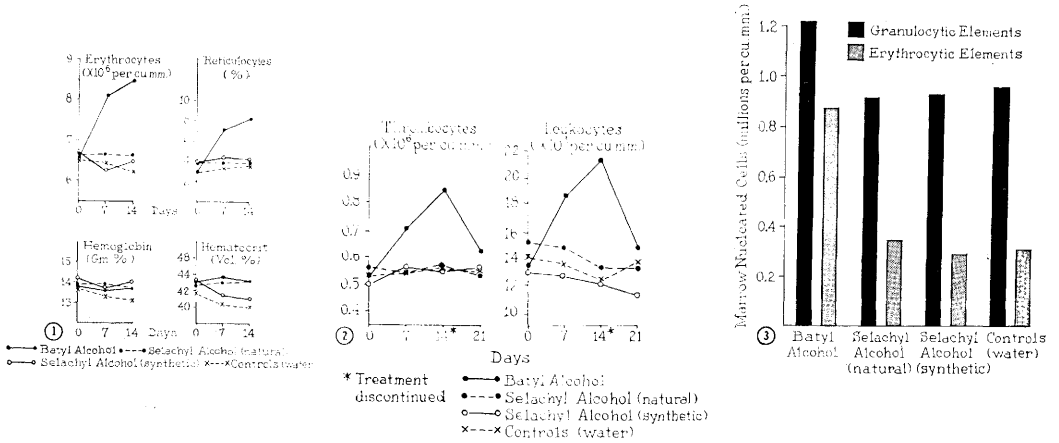

FIG. 1. Erythrocytosis and reticulocytosis without associated increases in hemoglobin or hematocrit levels in normal rats given 50 mg of batyl alcohol daily via gastric intubation. Recipients of comparable amounts of selachyl alcohol failed to manifest evidence of erythropoietic stimulation. Avg determinations of 10 animals receiving each of the above described test materials.

FIG. 2. Thrombocytosis and leukocytosis in normal rats given batyl alcohol. Normal values were restored in those animals not killed for marrow studies one wk after treatment was stopped. Thrombocyte and leukocyte counts remained constant in recipients of selachyl alcohol and were comparable to those obtained in the control group. Avg counts of 10 animals in each group.

FIG. 3. Myeloid erythrocytic and granulocytic hyperplasia in normal rats given 10 daily 50 mg doses of batyl alcohol *per os*. Megakaryocytes were also increased in these animals. Marrow nucleated cell counts in recipients of selachyl alcohol were similar to those obtained in the control group. Avg counts of 5 animals receiving each of the above described materials.

(Fig. 3). However, the batyl alcohol was fully active. The rats given this material developed erythrocytosis and reticulocytosis without associated increases in hemoglobin or hematocrit levels (Fig. 1), thrombocytosis and leukocytosis (Fig. 2), and increased numbers of both erythrocytic and granulocytic precursors in their marrows (Fig. 3). Type and magnitude of the responses were similar to those observed in other animals given racemic batyl alcohol(9). The microcytes responsible for the erythrocytosis were readily discernible and demonstrable by Price-Jones measurements. The leukocytosis was chiefly due to a relative and absolute neutrophilia. In those animals not killed for marrow studies, thrombocyte and leukocyte counts had returned to normal baseline levels one week after the treatment was stopped (Fig. 2). All animals remained healthy throughout experimental period, gained weight normally, and exhibited no adverse reactions to the test materials or repeated gastric intubations.

Batyl alcohol is active orally, and experimental data indicate that the glyceryl ethers can be absorbed intact from the gastrointestinal tract(13). However, the above experiment was repeated using a parenteral route of administration, *i.e.*, subcutaneous, to exclude with certainty any possibility of gastrointestinal inactivation. As before, each of the parameters selected to assess hemopoiesis remained unchanged in the recipients of selachyl alcohol.

Discussion. Despite the chemical relationship between batyl alcohol ($C_{21}H_{44}O_3$) and selachyl alcohol ($C_{21}H_{42}O_3$), the physiologic characteristics of these 2 substances differ markedly. Whereas selachyl alcohol was lacking in stimulatory activity under the above experimental conditions, comparable amounts of batyl alcohol, prepared by hydrogenation of the former, induced a clear-cut hemopoietic response in normal rats. These findings contribute greatly to the specificity of biologic activity of batyl alcohol. If the hematologic phenomena ascribed to batyl alcohol were nonspecific, *e.g.*, the result of an "irritative" or unphysiologic effect rather than primary myelopoietic stimulation, selachyl alcohol might be expected to possess similar properties. The possibility that selachyl alco-

hol may be an inactive precursor of batyl alcohol or some other substance has not yet been excluded.

It should be emphasized that the precise role which batyl alcohol may play in regulation of hemopoiesis is, as yet, conjectural. However, several points of interest have emerged from studies on hemopoietic effects of the glyceryl ethers and humoral control of blood cell production. Current data indicate that 2 humoral agents govern erythropoiesis (10). One substance, which seems to be a mucoprotein(14), stimulates hemoglobin formation most likely by augmenting erythrocytic differentiation of the pluripotential myeloid reticulum cells. Although maturation of erythrocytic precursors and synthesis of hemoglobin apparently proceed at fixed rates unaffected by the humoral stimulus, mitotic or proliferative activity of these marrow elements appears to be subject to a second humoral factor(10). The latter is thermostable, ether-soluble, and probably a lipid.

The singular type of erythropoietic response observed in normal rats given batyl alcohol is identical with that induced by the thermostable, ether-soluble plasma erythropoietic factor. It consists of erythromicrocytosis, reticulocytosis, and myeloid erythrocytic hyperplasia without associated increases in hemoglobins, hematocrits, or iron-59 uptake(9). In addition to this erythropoietic effect, the thermostable or ether-soluble fractions of plasmas from patients with polycythemia vera also induce, as does batyl alcohol, thrombocytosis and granulocytosis in normal recipient rats(15). Thus, there exist rather striking similarities between the chemical and physiologic properties of batyl alcohol and the thermostable, ether-soluble plasma factor which has been studied. Although persuasive of a close relationship, unequivocal support for this conclusion is not yet available.

The existent evidence is indirect and incomplete but is in accord with the thesis that batyl alcohol or some similar substance may play an important role in fundamental control of hemopoiesis. However, the answers to many problems must await further experimentation. For example, our studies fail to explain the need for the large amounts of batyl alcohol required to stimulate hemopoiesis in the normal rat. It may be simply a reflection of poor absorption and vastly different endogenous and exogenous requirements. It is hoped that additional observations on hemopoietic effects of the glyceryl ethers now in progress may help to elucidate the remarkable homeostatic mechanisms which govern hemopoiesis.

Summary. 1. Natural and synthetic selachyl alcohol is devoid of hemopoietic stimulatory activity in normal rats. 2. Batyl alcohol, derived from hydrogenation of selachyl alcohol, enhances proliferation of all myeloid elements but does not stimulate synthesis of hemoglobin. This effect is manifested in normal rat recipients by erythromicrocytosis, reticulocytosis, thrombocytosis, granulocytosis, and increased numbers of both erythrocytic and granulocytic elements in their marrows. 3. Batyl alcohol is active both orally and parenterally. The hemopoietic properties of this substance are not dependent on optical activity. 4. It is suggested, although not proved, that batyl alcohol may play an important role in regulatory control of hemopoiesis.

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Production of Acute Glomerulonephritis in Mice with Soluble Antigen-Antibody Complexes Prepared from Homologous Antibody.* (25960)

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Soluble antigen-antibody complexes, injected intravenously in antigen excess, are capable of producing the characteristic lesions of experimental serum sickness within a short period of time. When large amounts of either rabbit antiovalbumin-ovalbumin or rabbit anti-BSA-BSA (bovine serum albumin) complexes are injected into mice or rats a high incidence of acute glomerulonephritis results. In addition a focal arteritis and a valvulitis may be seen on occasion(1-4). Fluorescent labeled antibody technics have shown both the antigen and the antibody to be present at the site of the lesion. In the present study these observations have been extended to production of lesions with soluble complexes prepared from homologous mouse antibody. Antiovalbumin was produced in mouse ascitic fluid according to the method of Munoz(5) and compared with the corresponding rabbit antibody with regard to its ability to elicit glomerular lesions when administered to mice as soluble antigen-antibody complexes. The incidence and severity of anaphylaxis in both systems was studied. The effect of varying the antigen to antibody ratio in preparation of these complexes was also explored.

Materials and methods. Animals: Swiss Webster male and female mice weighing 20 to 30 g. *Antigens*: Hen ovalbumin, 3 times recrystallized, from Worthington Biochemical Co., Freehold, N. J. *Immunization*: Rabbit antisera against hen ovalbumin were prepared as previously described(3). Mice were immunized according to the method of Munoz. An emulsion containing 1 mg ovalbumin and 1 mg *Mycobacterium butyricum* per ml was prepared with Difco complete adjuvant. One ml was injected into the peritoneal cavity of each mouse on days 1, 18, and 38. Between 50 and 80% of the mice became ascitic, and between 1.0 and 15.0 cc of fluid was obtained from each animal. These collections were pooled. The various pools of antibody were analyzed for antibody protein content and antigen-antibody ratio at equivalence by the quantitative precipitin technic(6). Precipitates were analyzed for antibody protein by UV absorption at 280 mu in the case of rabbit antibody(7), and by the microKjeldahl method in the case of mouse antibody(6). Rabbit antiovalbumin. Pool I which contained 4.2 mg antibody protein/ml. Pool II which contained 4 mg antibody protein/ml. Mouse antiovalbumin Pool I which contained 8 mg antibody protein/ml. Pool II which contained 6.2 mg antibody protein/ml.

Preparation and injection of soluble complexes. Soluble complexes for injection into mice for the experiments on glomerulonephritis were prepared by direct addition of excess antigen to either the rabbit antisera or mouse ascitic fluid at room temperature. Volume was adjusted to 0.5 ml per injection and injected into the tail vein within 5 minutes. For the studies on anaphylaxis soluble complexes were prepared as previously described, by dissolving the washed immune precipitate in antigen excess(3). *Preparation of histolo-*

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