liver in association with intense sympathetic nervous system activity, however induced. This in turn suggests that a DLSH reaction induced by a very large dose of insulin may be due to stressor activation of adrenal cortical and sympathetic nervous system mechanisms necessary to any DLSH reaction, rather than to a specific insulin action on liver NPSH.

Summary. A DLSH reaction (statistically significant decrease in liver NPSH) was secured in mice by administration of very large amounts of insulin, epinephrine or nor-epinephrine, or tourniquet traumatization. Trauma was as effective in inducing a DLSH reaction in alloxan diabetic or adrenal demedullated mice as in normal mice; hence the trauma induced DLSH reaction was not attributable to endogenous insulin or adrenal medullary hormones. The trauma induced DLSH reaction was practically abolished by either adrenalectomy or administration of the ganglion blocking drug chlorisondamine dimethochloride. This indicates that both adrenal cortical and sympathetic nervous system activities are required for the reaction.

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Failure of DL-Batyl Alcohol to Prevent Aplastic Anemia in Calves.* (24079)

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This work was prompted by the report of Evans *et al.*(1) that experimentally induced or field cases of Bracken poisoning in cattle could be successfully treated with subcutaneous or intravenous administration of DL-batyl alcohol and comprehensive antibiotic therapy. Hematological aspects and clinical course of Bracken poisoning in cattle are very similar(2) to aplastic anemia induced when certain specimens of trichloroethylene-extracted soybean oil meal (TCESOM) are fed to cattle or calves(3,4). In both instances the intoxication produces a blood dyscrasia characteristic of hypoplastic or, in more severe cases, of aplastic anemia, namely thrombocytopenia, leukopenia, granulocytopenia and a relative lymphocytosis, a moderate degree of anemia, a hemorrhagic syndrome, ter-

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TABLE I. Administration of DL-Batyl Alcohol to Calves with Incipient Aplastic Anemia.

Calf No.	TCESOM fed, lb*	Therapy with DL-batyl alcohol			Result
		Route†	mg, total‡	Started on	
1597	3/4	I.M.	1500	22nd day	Dead 32nd day
1599	3/4	"	"	22nd	" 28th
1623	1/2	"	2500	17th	" 27th
1624	1/2	"	"	14th	" 28th
1625	1/2	"	"	"	Survived§
1629	1/2	"	"	11th	Dead 31st day
1650	1/2	I.V.	2000	"	" 25th
1651	1/2	"	1500	"	" 24th
1680	1/2	I.P.	5000	"	" 26th
1681	1/2	"	"	"	" 28th
1695 & 1697	1/2	" ‖	none	"	" "

* Per day per 100 lb body wt for 10 days only.
† I.M. = intramuscular; I.V. = intravenous; I.P. = intraperitoneal.
‡ 500 mg/day/100 lb body wt on consecutive days except calves 1650 and 1651 which received this amount on the 11th, 12th, 14th, 16th day and on the 11th, 12th and 14th day respectively.
§ See text.
‖ Inj. with solution of Tween only.

minal pyrexia and death. A similar syndrome was recently produced by McKinney *et al.* (5) who administered to calves S-(dichlorovinyl)-L-cysteine, a compound which they synthesized in their search for the toxic factor in TCESOM.

These studies were also of interest in view of claims that batyl alcohol has a therapeutic effect in radiation injury in mice(6) and in patients suffering from irradiation leukopenia (7).

Procedure. Production of aplastic anemia. Aplastic anemia was induced in female Holstein calves, purchased in open market, weighing initially about 90 lb by feeding for 10 days only, ¾ lb or, in most instances, ½ lb/day/100 lb body weight of our standard specimen of TCESOM (referred to elsewhere (8) as TCESOM-6). This was suspended in milk and fed twice daily in equal amounts. The calves were fed milk and alfalfa hay throughout the experiment and during the pre-trial period of 10 to 14 days which was used to assure that the animals were clinically and hematologically normal. Blood counts were made by standard methods(9) at intervals of 2 to 3 days during 10 days of feeding TCESOM and daily thereafter. Specimens of bone marrow were removed for cytological examination by sternal biopsy at least 4 times from each calf and from the ribs when the calves were slaughtered in moribund condition. *Synthesis of* DL-*batyl alcohol.* Methyl- or ethyl-stearate was reduced(10) to l-octadecanol (M.P. 56.5°) and this was converted(11) to the p-toluene-sulfonate (M. P. 56.0°; sapon. equiv. 435.4). This ester was condensed with the sodium derivative of DL-O-isopropylideneglycerol(12) by the procedure(13) with which Baer *et al.* obtained elaidyl-D-O-isopropylideneglycerol(14). The DL-isopropylidene batyl alcohol was hydrolyzed(13) and the DL-batyl alcohol crystallized twice from acetone. The yield of the product (M.P. 71.0°) was as high as 65% based on the p-toluenesulfonate used. Elementary analysis: Found C = 73.0%, H = 12.87%, calculated for $C_{21}H_{44}O_3$: C = 73.2%, H = 12.87%. For injection, the batyl alcohol was solubilized in sterile 5% solution of Tween-80 or Tween-20 in 1% sodium chloride, as described by Evans *et al.* (1); for intramuscular injection of 2 calves, the batyl alcohol was finely ground in an agate mortar and suspended in distilled water. Control animals fed the toxic meal or only milk and alfalfa were also injected intraperitoneally with the Tween solutions to determine their effect on the blood picture.

Results. Production of aplastic anemia by feeding of TCESOM for short periods. Two calves were fed ¾ lb TCESOM/day/100 lb for 7 days only; one was slaughtered in moribund condition on the 32nd day with typical

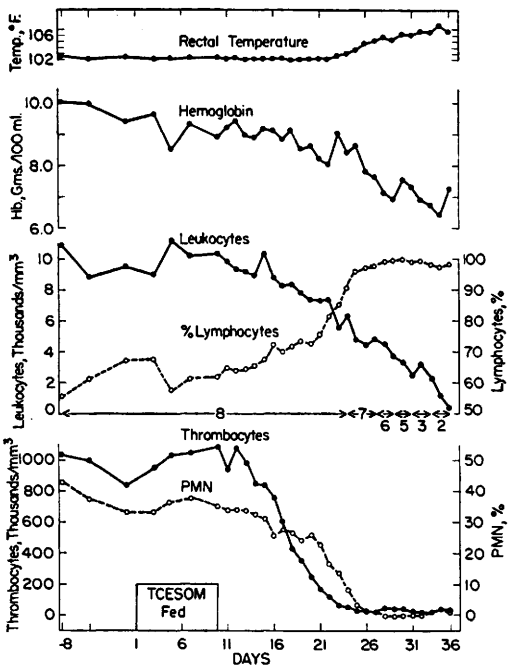

FIG. 1. Changes in cell counts, hemoglobin and rectal temperature during development of aplastic anemia in calves. Aplastic anemia was induced by feeding ½ lb of TCESOM/day/100 lb for 10 days. Each point is the mean of values obtained from the number of calves shown on horizontal line of figure. Decrease in number of calves was due to their death; all calves died.

signs of aplastic anemia, while the other, after temporary severe thrombocytopenia and granulocytopenia, made apparently a complete hematologic recovery and was slaughtered on the 151st day. Feeding of essentially the same total quantity of TCESOM-6 during a 10 day period produced a much higher incidence of fatal aplastic anemia as shown in Fig. 1 and Table I. All calves were clinically normal in every respect until about the 20th day after the start of feeding TCESOM. They consumed their rations eagerly and gained weight. However, as in the case of continuous feeding of the same daily dosage of TCESOM-6(8), after about the 16th day there occurred a rapid decrease of thrombocytes followed by a more gradual decrease of leukocytes. The differential cell counts showed a rapid development of granulocytopenia after the 21st day until virtually all circulating white cells were lymphocytes. Towards the terminal stages there was also a decrease of erythrocytes and of hemoglobin and development of a marked pyrexia. The time sequence of occurrence and the extent of these changes are illustrated in Fig. 1. The mean thrombocyte count of this group of calves, through the 13th day of trial was for unknown reasons higher than usually encountered with calves of this age. This, however, did not delay the onset of severe thrombocytopenia. One calf was slaughtered in moribund condition on the 24th day after feeding of TCESOM had started, whereas 2 survived until the 35th day. There was no distinct difference with respect to onset and course of the blood dyscrasia between calves fed ½ or ¾ lb TCESOM/day/100 lbs.

Microscopic examination of bone marrow biopsy specimens removed prior to about the 18th day after the start of feeding TCESOM failed to reveal morphological abnormalities in the nucleated cells of the erythroid or myeloid series. After that time, however, there occurred a rapidly progressing depletion of all bone marrow cells. In megakaryocytes hyalinization of the cytoplasm was observed as early in the 10th day, followed by progressive disappearance of these cells.

Post mortem examination of calves usually revealed the presence of hemorrhages, primarily in the thoracic cage and the small intestine. In general, however, these were not as extensive, massive and numerous as those found in calves to which the same daily amount of the toxic agent is fed continuously. No external hemorrhages were observed. In this series there also occurred several cases of hemorrhagic consolidation of the apical portion of one lobe of the lungs, with pleural adhesions. This may be related to the respiratory distress experienced by some calves after intravenous injections of batyl alcohol or Tween.

The sequence of events which occurs in development of aplastic anemia when calves are managed as described above, should be particularly favorable for testing the effect of potential therapeutic agents because these can be administered after the toxic compound has been removed from the diet and before the blood dyscrasia has become pronounced.

Administration of batyl alcohol. Table I summarizes the results obtained when 500 mg of DL-batyl alcohol/day/100 lb of body weight was administered by various routes to calves with incipient aplastic anemia. Treatment was started at various intervals after the beginning of feeding TCESOM. In all 10 calves thus treated and in 2 control calves which received only injections of Tween the hematological changes, with respect to time and severity, were essentially those shown in Fig. 1. Eleven of these 12 animals developed fatal aplastic anemia and became moribund between the 24th and 32nd day after the start of feeding TCESOM. One calf (No. 1625, Table I) made a spontaneous recovery from the blood dyscrasia after her blood counts, between the 28th and 31st day had dropped to 20000 thrombocytes/cmm, 1300 leukocytes/cmm and 0% polymorphonuclear leukocytes (for 3 consecutive days) and her rectal temperature had reached 106.5°F. She was kept under observation until the 110th day after the start of feeding TCESOM. By the 50th day the total and differential leukocyte counts had attained normal values; the thrombocyte count reached 700000/cmm between the 59th and 63rd days but subsequently fell again to become stabilized at about 350000/cmm. Since this work has been completed, similar spontaneous recoveries after a near-fatal hematologic crisis between the 26th and 30th day have also been observed with calves injected intravenously, for 10 days only, with 10 mg of S-(dichlorovinyl)-L-cysteine/100 lb/day (unpublished). The amount of TCESOM fed was apparently not quite sufficient to cause 100% mortality. In the other calves treated with batyl alcohol, development and course of the blood dyscrasia were in no way affected by the treatment and no evidence could be found, either from blood counts or from examination of the bone marrow, that hematopoietic stimulation had occurred at any time. Calf 1625 was one out of 12 cases in which the same level of feeding TCESOM was not lethal; her recovery cannot be ascribed to treatment with batyl alcohol.

Two calves which were fed only milk and alfalfa hay were also injected intraperitoneally with Tween-20 for 10 consecutive days; their blood counts remained in the normal range throughout this time and for the following 24 days, after which they were slaughtered.

Intravenous injection of the Tween solutions, with or without batyl alcohol, was not well tolerated by these young calves. Even though injections into a jugular vein were made very slowly, hyperpnea was induced rapidly, followed in several instances by collapse and a period of about 2 minutes when the animals were comatose. Within about 2 hours the animals became essentially normal again. Some calves reacted more severely than others. Because of the severity of the reaction, intravenous injections were not made in the later stages of the intoxication with TCESOM.

Our failure to obtain a curative response with DL-batyl alcohol in TCESOM poisoning must be reconciled with the observations of Evans *et al.*(1) who reported that this compound, together with treatment with antibiotics, had a therapeutic effect in cattle whose blood dyscrasia from Bracken poisoning had reached an acute clinical stage, with associated hemorrhages and pyrexia. Our animals were treated while they were still clinically normal, before such signs appeared. No antibiotics were given to our calves during therapy with batyl alcohol because there was no pyrexia or other evidence of a complicating infection at that time. The total and daily amounts of batyl alcohol administered were presumably at least as large as those (1 g/day to bullocks whose weight was not stated) used by Evans *et al.*(1). It is possible that the toxic agent in TCESOM interferes with different biochemical reactions and at an earlier stage of the development of the affected cell types in the hematopoietic system than the poison in Bracken fern. This cannot be decided until both compounds have been isolated. It is also possible that a relatively large dose of the toxic factor from TCESOM or the induced biochemical defect persist in the animal after consumption has ceased or that an irreversible inhibition of the hematopoietic system is not overcome by therapeutic measures.

Summary. Feeding of ¾ or ½ lb trichlo-

roethylene-extracted soybean oil meal/day/100 lb to young calves for 10 days induced aplastic anemia and caused death 14-25 days after feeding of the toxic material had ceased. DL-batyl alcohol injected by various routes after feeding of the toxic agent had ceased, but before appearance of clinical symptoms, failed to prevent development of aplastic anemia.

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Influence of Cobaltous Chloride on Aortic Atheromatosis and Plasma Lipid Pattern in Cholesterol-Fed Chickens. (24080)

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Several investigators have studied the influence of various metallic elements on cholesterol metabolism and experimental atheromatosis in animals(1-8). The action of cobaltous chloride is of particular interest because of its damaging effect on pancreatic α-cells (9). Caren and Carbo(8) found that cobaltous chloride given intravenously causes an increase in plasma cholesterol concentration of rabbits lasting 1 or 2 weeks.

The present paper reports the influence of cobaltous chloride, given in the diet and by subcutaneous injection, on aortic atheromatosis and plasma lipid pattern in cholesterol-fed chickens.

Methods used have been described in detail previously(10). Eight-week-old White Leghorn cockerels were set out in groups of 10 in wire batteries and fed a diet containing 2% cholesterol and 5% cottonseed oil until they were 16 weeks old. Experiments were then terminated. Food was withheld overnight, 4 ml of blood were drawn from alar vein and mixed with citrate solution, the birds were decapitated, and thoracic aortas and brachiocephalic arteries were removed and graded for degree of atheromatosis, using a scale of 0 to 4. Plasma aliquots were analyzed for total cholesterol(11) and lipid phosphorus(12,13), and for cholesterol in lipoprotein fractions separated by Cohn's Method 10(14,15).

Results. $CoCl_2 \cdot 6H_2O$ was given initially in the diet at concentration of 0.5%. It was toxic and accordingly was fed only for the second half of the 8-week experimental period. The cobalt-fed birds in 5 experiments (Table I) had less atheromatosis and lower blood cholesterol concentrations than did the controls. There was no effect on lipid phosphorus concentrations. The treated birds