formation, but that an unidentified component of the infusion is utilized for this purpose.

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Ethionine-Induced Depression of Plasma Antihemophilic Globulin in the Rat. (21283)

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To date there have been few studies relevant to the origin of antihemophilic globulin (AHF). Boldyreff(1) reported some work with dogs from which he concluded that the pancreas produced AHF. In these studies, either pancreatic fistula or complete pancreatectomy was followed by a prolongation of the whole blood coagulation time; however, no demonstration of the nature of the clotting defect was made. Graham and associates(2) produced acute liver injury in dogs by chloroform intoxication. In these animals AHF was well preserved despite a degree of liver damage which caused almost complete depletion of prothrombin and fibrinogen. Moreover, it was found that dicumarol was unable to depress AHF even at a dosage sufficient to produce severe hypoprothrombinemia. These studies would seem to exclude the liver as the source of AHF, in contrast to its role in the synthesis of most clotting factors. This same group of investigators showed that AHF was unaffected by heavy total body X-irradiation(3), indicating that the cells which produce AHF must be relatively radio-resistant.

Ethionine, the ethyl analog of methionine, is a metabolic blocking agent which interferes with the synthesis of certain proteins and produces striking histological changes in some tissues(4-6). In the studies to be reported, it was found that this drug produced a profound depression of plasma AHF levels in rats. The tissues which exhibited damage were interpreted as suggested sites for further and more definitive investigation of a possible role in AHF production.

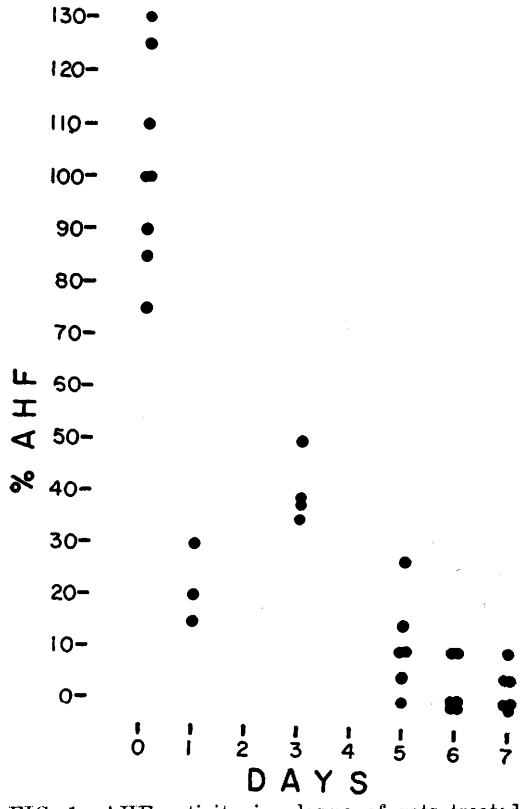

FIG. 1. AHF activity in plasma of rats treated with ethionine. Each dot represents one animal.

Methods and materials. Male albino rats

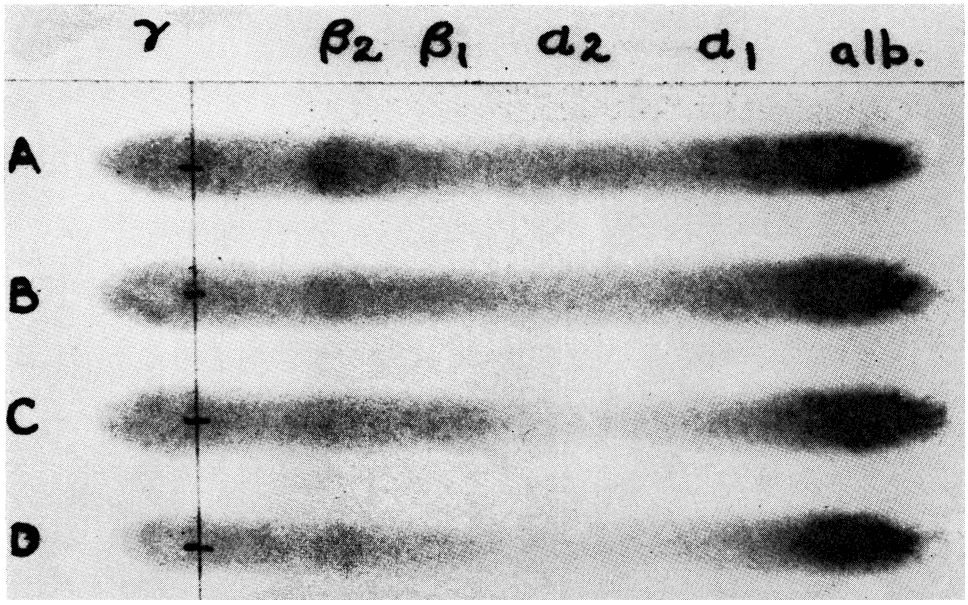

FIG. 2. Paper electrophoretic patterns of rat sera. A. Normal control. B-D. Animals after 3 days of ethionine injections.

of the Slonaker strain, weighing 175-225 g were used. Experimental animals were given intraperitoneal injections of ethionine.* The daily dose was 250 mg: 10 cc of a 2.5% solution of crystalline ethionine in distilled water. *Blood specimens* were obtained either from the internal jugular vein or directly from the heart, depending on whether subsequent specimens were required. In either case, the site of puncture was surgically exposed, and blood was obtained without contamination by tissue fluid. As the anticoagulant, one part of 0.1 molar potassium oxalate was used for nine parts of blood. Generally one cc of blood was removed. *AHF* was assayed in a manner similar to that used by Spaet and Kinsell(7). In this method, the activity of the test plasma is measured by its ability to improve prothrombin consumption in the blood of a known hemophiliac. Several different hemophiliacs provided assay blood for this study, and a curve was constructed for each patient in which serial dilutions of plasma obtained from a pool of five normal rats were tested against each patient's blood. The plasmas of the experimental and control animals were diluted 2:3 with saline and 0.1 cc of these dilutions was added to 1.9 cc of hemophilic blood, giving a final ratio of 2 parts test plasma to 98 parts hemophilic blood. The serum prothrombin was determined 4 hours after mixing, and the AHF content of the specimen estimated by comparison with the control curve. True prothrombin and proconvertin were measured by the method of Owren and Aas(8), and labile factor was assayed by the Quick technic(9). Tissues were fixed in Bouin's solution, and stained with hematoxylin and eosin. Liver fat was demonstrated by Sudan IV staining of frozen sections. Bone marrow specimens were made by smearing fresh material obtained from the femur. Marrow slides were stained with Wright's and counterstained with Giemsa. Paper electrophoresis of serum proteins was accomplished by a modification of the method of Kunkel and Tiselius(10).

Results. Fig. 1 shows the effect of ethionine on plasma AHF. A significant reduction in activity is seen after the first dose of the drug, and this depressed activity is maintained so long as ethionine administration is continued. When the drug was discontinued, there was a gradual restoration of plasma AHF starting within three days. Ethionine did not inactivate rat AHF *in vitro,* as shown by the follow-

* Purchased from U. S. Industrial Chemicals Co., San Francisco.

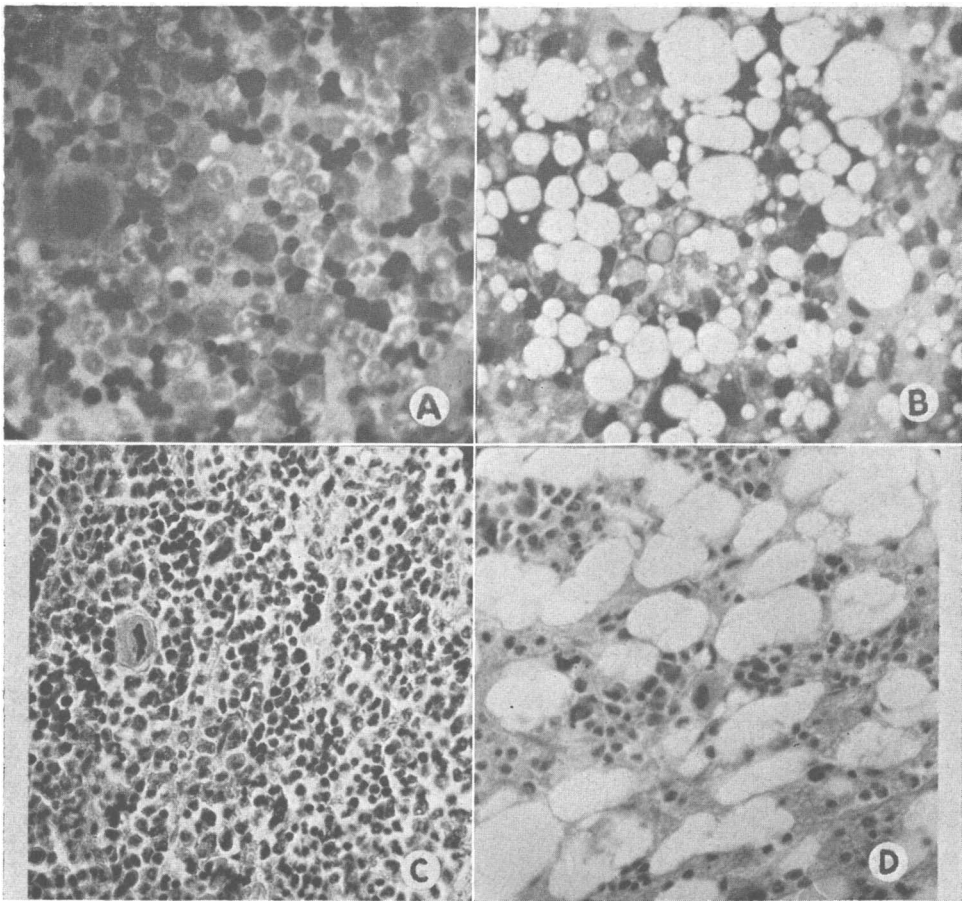

FIG. 3. Effect of ethionine on bone marrow. A. Normal rat, smear preparation, Wright-Giemsa stain. B. Ethionine-treated rat, same technic. C. Normal rat, marrow section, hematoxylin-eosin stain. D. Ethionine-treated rat, same technic as C.

ing experiment: serial dilutions of ethionine were added to oxalated rat plasma, and these mixtures were incubated at 37° C for thirty minutes. No plasma so treated showed any loss of AHF activity. These findings indicate that the ethionine-induced depression of AHF represented a true metabolic effect.

In the ethionine treated animals there was a prompt reduction of true prothrombin, proconvertin, and labile factor, confirming the studies of Witte(11). However, fibrinogen was little affected. As with AHF these coagulation changes persisted as long as the drug was continued. Electrophoretic patterns of the experimental animals showed consistent changes starting on the second day of treatment, as seen in Fig. 2. Alpha and beta globulins were markedly depressed, but albumin and gamma globulin were unaffected, even after ten days of treatment. These results confirm the selectivity of ethionine action on protein synthesis.

The following results were obtained from histological surveys of the tissues obtained from ethionine treated animals: in contrast to previous reports(12,13), no changes were seen in our animals following a single dose of the drug despite the striking changes found in the clotting factors. It would appear that the male Slonaker rats used were less susceptible to tissue damage than the males of other species used by other investigators. After three days of high dosage injections the pancreas did show typical changes consisting of loss of basophilia, zymogen granules, and acinar structure. The liver displayed nuclear changes and in-

creased fat deposition. A hitherto undescribed finding was that of severe bone marrow depression. The marrow changes are shown in Fig. 3, and consist of diminished cellularity with replacement of the normal cells by fat, hemorrhagic areas, and vacuolization of many of the reticulo-endothelial cells. Sections of the kidney, gut, spleen, muscle, salivary glands, and lymph nodes showed no significant changes. Further evidence of the resistance of the Slonaker strain to ethionine was seen in the prolonged survival of these animals on these huge daily doses of the drug. The treated animals lived from 6 to 12 days despite a dose reported fatal to other strains within 72 hours.

None of the control animals had any consistent clotting disorder or tissue changes.

Because of the damage inflicted by ethionine on the pancreas and liver, further studies were performed on these organs. Attempts were made to destroy the pancreas in the rat by total surgical extirpation and by ligation of the common bile duct. Since neither of these measures gave satisfactory pancreatic destruction, use was made of dogs with complete surgical pancreatectomy. These animals were 2 months postoperative, and had been given only insulin treatment for 2 weeks prior to testing.† Four pancreatectomized dogs were studied, and none showed reduction in plasma AHF as compared to normal dogs.

Ten rats were given 0.2 cc of carbon tetrachloride by intraperitoneal injection. Twenty-four hours later they were sacrificed, and their plasma studied for blood clotting factors. The livers of these animals showed severe damage by gross and histological examination, and there was corresponding depression of prothrombin (less than 30%) and proconvertin (less than 1%). However, in conformity with the findings of Graham *et al.*(2), AHF was intact in all animals.

† Kindly supplied by Dr. Leslie L. Bennett and Mr. James Sutherland, University of California, Berkeley.

Summary and conclusions. Parenteral administration of ethionine markedly depressed the AHF content of the blood in rats. This effect probably represents interference with synthesis of the protein rather than increased destruction. Although other coagulation factors were affected, the ethionine action did not involve all serum proteins, as shown by paper electrophoresis. Extensive tissue damage in ethionine-treated animals was confined to the pancreas, liver and bone marrow. Neither total pancreatectomy in dogs, nor carbon tetrachloride-induced liver damage in rats had any effect on plasma AHF levels. Studies are in progress to determine a possible role of the reticulo-endothelial system.

We wish to thank Dr. Lelland J. Rather for his evaluation of pathological specimens.

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