Fungicides, Burgess, Minneapolis, 1961, 208.

10. Cochrane, V. W., *Physiology of Fungi,* Wiley, New York, 1958, 481.

11. Blackwood, V. B., Naber, E. C., *Poultry Sci.,* 1963, v42, 782.

12. Cochrane, V. W., *ibid.,* 39-41.

13. Norton, T., Dasler, W., *Experientia,* 1963, v19, 627.

14. Bachhuber, T. E., Lalich, J. J., Angevine, D. M., Schilling, E. D., Strong, F. M., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 294.

15. Ehrhart, L. A., Lipton, S. H., Strong, F. M., *Biochemistry,* 1963, v2, 300.

16. Cochrane, V. W., *ibid.,* 441.

17. ———, *ibid.,* 10.

Received January 15, 1964. P.S.E.B.M., 1964, v116.

Effect of Necrosis on Hemoglobin, Serum Protein Profile and Erythroagglutination Reaction in Golden Hamsters.* (29160)

ANTHONY BETTS, ROBERT TANGUAY AND GILBERT H. FRIEDELL
(Introduced by Shields Warren)
Regional Memorial Hospital, Brunswick, Maine, and Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.

The results of our previous studies have emphasized the importance of tissue necrosis in the pathogenesis of the anemia of malignancy and the anemia accompanying chronic inflammation(1-4). It was further noted that in hamsters a positive erythroagglutination reaction (EAR) developed before onset of anemia(5,6). This abnormal agglutinability of red cells by rabbit antihamster serum *in vitro* is attributed to a protein "coating" on the test erythrocytes. Since the rabbit antihamster serum is prepared with normal hamster serum as the antigen, presumably such a "coating" represents a protein normally present in the hamster serum. It was therefore decided to explore further the effect of tissue necrosis on development of the erythroagglutination reaction, on the serum protein electrophoretic pattern, and on the development of anemia.

Materials and methods. Forty-seven mature golden hamsters were divided into 7 groups, 5 experimental and 2 smaller control groups. They were all maintained on Purina chow and water *ad lib.* Blood samples were obtained by cardiac puncture immediately prior to the experimental procedure, and on days 3, 6 and 8, or as otherwise indicated in Fig. 1, during the experiment. Animals were anesthetized with 0.15 cc of intraperitoneal sodium pentobarbital (Nembutal, Abbott, 50 mg per ml) before cardiac puncture. The following determinations were made on the samples: hemoglobin, hematocrit, serum electrophoretic pattern and erythroagglutination reaction.

Hemoglobins were measured by the cyanohemoglobin technique (normal = 16.3 ± 1.3 g%). Hematocrit was determined by micro-method (normal = 53-56%). Serum electrophoresis was carried out in the Shandon Electrophoresis apparatus on cellulose acetate (Oxoid) strips, 2.5 × 0.2 cm in Barbitone acetate buffer pH 8.6, at 125 v-0.4 ma per cm width. Strips were stained with Ponceau S 0.2% in 3% tricholoracetic acid. Bands were then eluted with chloroform and absolute ethanol (90%/.10%) and the percent transmission of the eluate was read in a Coleman Jr. spectrophotometer. Hamster normals: albumin 65-71%, alpha$_1$ globulin 8.0-10.5%, alpha$_2$ globulin 5.0-7.0%, gamma globulin 9.5-12.5%. Erythroagglutination reactions were performed by the technique reported previously. The rabbit antihamster serum was absorbed with normal hamster red cells before being used. Surgery was performed under aseptic technique after intraperitoneal sodium pentobarbital anesthesia. Animals were weighed daily.

* This investigation was supported by U. S. Public Health Service grants and by U. S. Atomic Energy Commission Contract with New England Deaconess Hospital.

Group I. Seven hamsters received an intramuscular injection of 0.2 ml of sterile turpentine into the left thigh. In approximately 3 days an abscess developed which broke down and discharged after another 2-3 days.

Group II. Eight animals had the right kidney excised and placed freely in the peritoneal cavity. The abdominal incision was closed with chromic catgut for the peritoneum and muscle, and with silk for approximating skin edges.

Group III. Nine animals had the right kidney excised and placed freely in the subcutaneous tissue in the region of the operative incision. The incision was closed as above.

Group IV. Seven hamsters had one kidney exposed. Two thin nickel-plated wires were introduced through the renal cortex approximately 1 cm apart, an electric current (150 v-10-20 ma for 10-15 sec) was passed, and a localized burn was produced in the region of the medulla. The wires were withdrawn and the wound was closed with chromic catgut and silk sutures in successive layers.

Group V. Eight animals received an injection of 0.5 cc Freund's complete adjuvant (Difco) intraperitoneally. Such an injection had previously been shown to produce a positive erythroagglutination reaction(4).

Group VI. Four control animals for Groups I and V (those receiving injections) were anesthetized and bled at the same intervals as the other animals.

Group VII. Four control hamsters for Groups II, III and IV (those with renal surgery) were anesthetized, the right kidney was removed from each animal, and the wound was closed as above. These animals were then bled at the same intervals as the experimental animals.

At the end of the experiment all animals were killed by an overdose of intraperitoneal sodium pentobarbital. Gross and microscopic autopsy examinations were performed.

Results. The findings in the various groups of animals are seen in Fig. 1. In almost all of the animals a positive EAR followed induction of sterile necrosis. Freund's adjuvant produced a positive EAR in all of the animals.

Three animals in the turpentine group had hemoglobins below 13 g%. None of the animals in other experimental or control groups became anemic. Weight loss occurred in some animals from all groups of operated animals, but there was no consistent pattern noted. Nonoperated controls maintained their weight.

In almost all of the experimental animals there was a relative increase in alpha and gamma globulins during the experiment, but only those increases exceeding 2 standard deviations were considered significant. Serum protein changes of this magnitude are represented in Fig. 1, together with the erythroagglutination reactions. Reference to the graphs reveals no correlation between serum protein changes and the EAR.

On microscopic examination there appeared to be more marked necrosis of the kidney returned to the peritoneal cavity than of the one removed and placed subcutaneously, and the exudative and proliferative cellular response was also greater around the former. In the electrocoagulation group an irregular zone of coagulation necrosis was present in the medulla of each kidney, but the amount of necrosis was generally small.

Microscopic sections of the spleens from all animals in the experimental groups showed some degree of congestion, reticuloendothelial cell hyperplasia, plasmacytosis and extramedullary hematopoiesis. In most instances the latter was limited to erythropoiesis. These changes were most prominent in animals given turpentine abscesses, but there was moderate variation within any single group and differences between animals in the different experimental groups did not appear to be significant. No characteristic changes were found in the other organs examined.

Discussion. A positive erythroagglutination reaction appears to be independent of the electrophoretic pattern of the serum proteins, and is not dependent on the elevation of any single globulin fraction. In the animals injected with turpentine, for instance, there was in all animals an elevation of gamma globulin greater than 2 standard deviations from the mean within 3 days after the injection. The EAR, however, although positive in all animals by day 6, was only positive in

one animal on day 3. On the other hand, the injection of Freund's adjuvant evoked a positive EAR in a majority of animals on day 4, and in all of the animals by day 6, but significant changes in serum protein levels were noted in only 2 animals on day 4 and in only 1 animal on day 6.

Nephrectomy alone produced a positive EAR in only one of 4 animals, and in this animal it was positive on days 3 and 6, but absent on day 8. In this animal the gamma globulin was elevated more than 2 standard deviations above the mean on day 3. In none of the bled but nonoperated control animals

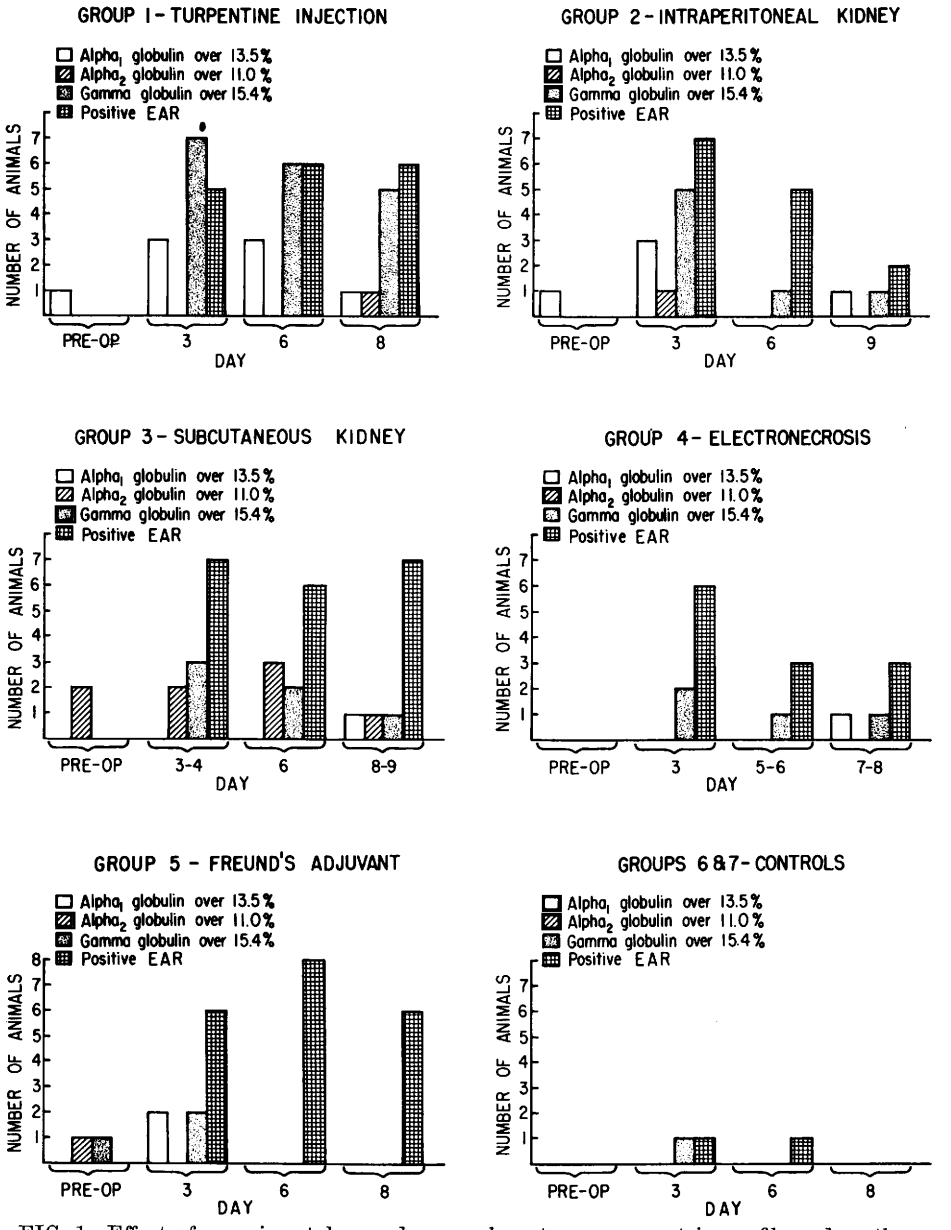

FIG. 1. Effect of experimental procedures on hamster serum protein profile and erythroagglutination reaction (EAR). Globulin percentages in these charts represent mean values in normal animals plus 2 standard deviations. Groups 6 and 7 represent 4 animals with unilateral nephrectomy and 4 injected with saline.

was the EAR positive or was there a significant deviation from the normal electrophoretic pattern.

Summary. The production of tissue necrosis by various aseptic techniques induced a positive erythroagglutination reaction in non-tumor-bearing golden hamsters within 6 days. The rapidity of onset and the duration of the EAR varied somewhat, depending on the procedure, but there was no associated characteristic change in the serum protein profile, and anemia appeared in only 2 animals. Freund's adjuvant induced a positive EAR in all animals within 6 days, but very little change in the serum protein profiles.

1. Sherman, J. D., *Blood,* 1959, v14, 1223.
2. Friedell, G. H., Sherman, J. D., Sommers, S. C., *Arch. Path.,* 1960, v70, 363.
3. Sherman, J. D., Friedell, G. H., *Arch. Int. Med.,* 1962, v109, 33.
4. Rigby, P. G., Betts, A., Hackett, R. L., Emerson, C. P., Friedell, G. H., *J. Lab. and Clin. Med.,* 1963, v62, 216.
5. Betts, A., Rigby, P. G., Emerson, C. P., Friedell, G. H., *ibid.,* 1961, v58, 652.
6. Sherman, J. D., Rigby, P. G., Hackett, R. L., Friedell, G. H., *Cancer Res.,* 1963, v23, 1689.

Received January 16, 1964. P.S.E.B.M., 1964, v116.

Production of Members of the Blood Coagulation and Fibrinolysin Systems by the Isolated Perfused Liver.* (29161)

R. MATTII,† J. L. AMBRUS, J. E. SOKAL AND I. MINK
Roswell Park Memorial Institute and State University of N. Y. at Buffalo, N. Y.

Whipple and Madden(1,2) reviewed the clinical and experimental evidence indicating that most plasma proteins, including fibrinogen, are produced by the liver. Direct demonstration was accomplished only later(3-9) using radioisotopes and immunologic methods in experiments with isolated livers or liver slices. More detailed study of individual clotting factors were undertaken by Pool and Robinson(10) who found that rat liver slices *in vitro* produce factor VII and to a lesser extent, prothrombin. Barnhart(11) identified the liver as the source of prothrombin by immunofluorescent methods. The purpose of this study was to investigate the production of members of the blood coagulation and fibrinolysin systems by the isolated liver.

Materials and methods. The apparatus and procedure to maintain isolated perfused rat livers was that of Miller *et al*(6,7) as modified by Aydin and Sokal(12). All glassware was siliconized. Sprague-Dawley rats of about 250 g body weight were given 10 mg vitamin K intramuscularly; 24 hours later they were anesthetized with ether and the isolated liver perfusion established. The bile duct was cannulated and bile flow was used as one of the criteria of viability of the preparation. About 25 ml of perfusing medium was allowed to pass through the liver and the effluent discarded before recirculation of the medium was established. A blood sample taken at this time was used to establish baseline values of the blood coagulation and fibrinolysin systems. The perfusing medium was prepared by bleeding 15-20 Sprague-Dawley rats under ether anesthesia through the aorta, using a 19-gauge Fenwall hemorepellent needle and a 10 ml siliconized glass syringe containing 1 ml of 3.8% sodium citrate solution. The pooled blood (200 ml) was centrifuged at 1500 rpm in a refrigerated centrifuge (4°C) for 10 minutes, the platelet rich plasma and buffy coat were discarded and the red cells were washed 3 times with citrated saline (100 ml of 3.8% sodium citrate, 500 ml of 0.85% sodium chloride) solution. The red cells were then suspended in an equal volume of medium made up as follows: 100 ml Tissue Culture Medium 199

* Supported in part by grants from United Health Foundation of Western N. Y. and Nat. Inst. Health, U.S.P.H.S.

† Lilly Fellow in Hematology.