Variations in Paper Electrophoretic Patterns of Dog Sera and Plasmas Prepared by Different Technics.* (22987)

WILLIAM H. GOLDWATER AND CECIL ENTENMAN
Biological and Medical Sciences Division, U. S. Naval Radiological Defense Laboratory, San Francisco, Calif.

Recent paper electrophoresis studies on the sera of X-irradiated dogs afforded the occasion to compare the results obtained with those of other investigators on the electrophoretic patterns of normal dog plasma(1-3) and serum(4-8). Apart from the less precise designations for the protein fractions used by some of these workers, a distinct, but previously unreported difference between serum and plasma electrophoresis patterns was apparent, in that the alpha-4 globulin seemed to predominate over the alpha-3 component in dog sera, while the reverse tended to hold for dog plasmas.

A distinct exception to these findings has been reported(5). However, the present work describes how the patterns obtained may depend on technics of collection for different types of serum or different types of plasma, thus accounting for this apparent exception. This communication describes further the nature of these "new" protein differences between dog sera and plasmas.

Methods. Normal female mongrel dogs weighing 15-35 lb were maintained and postabsorptive blood samples were drawn as described earlier(9). Samples designated *"standard serum"* were obtained by our customary technic, allowing blood to clot one hour at room temperature and then in the refrigerator for another hour, after which centrifugation yielded the "standard serum." *"Sedimented serum"* was obtained similarly, except that the whole blood was centrifuged immediately upon collection, so that clotting took place after the blood cells had been sedimented to the bottom of the tube. Clotting at room temperature, refrigeration, and centrifugation, as above, then yielded the "sedimented serum." *Oxalated plasma* was obtained from blood samples collected in tubes containing 1.2 mg ammonium oxalate and 0.8 mg potassium oxalate per ml blood(10), and *heparinized plasma* was obtained from blood collected in heparinized syringes. All samples were finally obtained by centrifugation at 2000 rpm (about 1000 x g), and were handled together at room temperature and during refrigeration and centrifugation. Paper electrophoresis was performed as described previously, using horizontal migration in a closed, refrigerated cabinet, on Whatman 3 MM filter paper and barbital buffer at pH 8.6 and ionic strength 0.05. Five microliter samples of serum or plasma were pipetted to spots on the starting line, midway between the buffer vessels, and constant voltage was applied for 6½ hours at 500 volts, or 16½ hours at 175 volts. At the end of the run the paper was dried for 20 minutes in a forced-draft oven at 60°C. The position of a small tan spot was marked at the edge of the paper. This location is the same as that of the alpha-4 globulin in the subsequently developed pattern from normal dog serum, although it is absent or, at most, very faint after runs on dog plasma. Protein patterns were stained with bromphenol blue according to Piantanida and Muic(11), lipids with Sudan Black B by the method of Swahn(12), and polysaccharides following the technic of Köiw and Grönwall(13).

Results. In Fig. 1 and 2 are shown the patterns obtained by staining proteins and polysaccharides, respectively, on samples of "standard serum," "sedimented serum," oxalated plasma, and heparinized plasma from the blood of a normal dog. The results shown are typical of those obtained in similar comparison runs on over 15 dogs, sampled in the normal, fasting state.

* This work was supported, in part, by funds provided by Bureau of Medicine and Surgery, U. S. Navy Department.

The opinions or assertions contained herein are private ones of the authors and are not to be construed as official, or reflecting the views of the Navy Department.

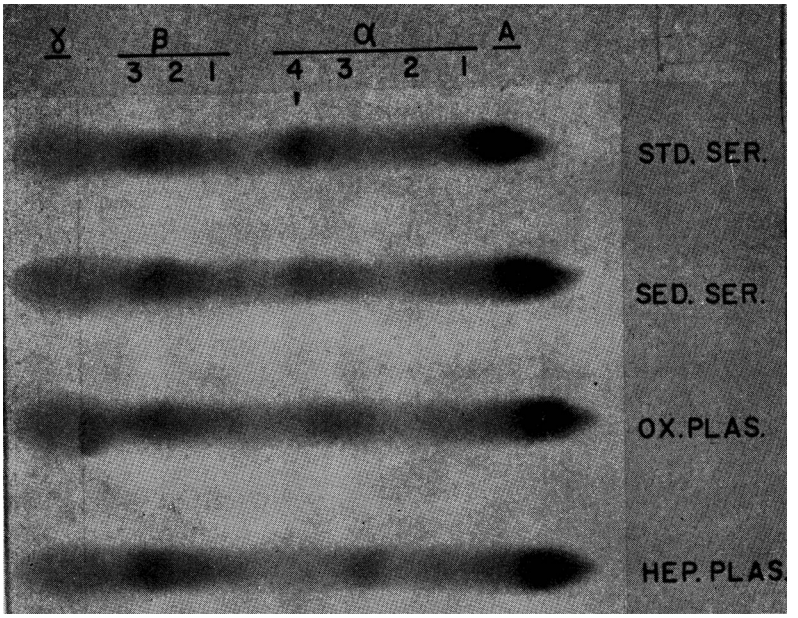

FIG. 1. Protein patterns, stained with bromphenol blue, from paper electrophoresis of samples of normal dog "standard serum," "sedimented serum," oxalated plasma, and heparinized plasma, reading from top to bottom. Starting line toward left, migration is from left to right. Electrophoretic components labeled at top are albumin, α-1-2-3-4, β-1-2-3, and γ-globulin. Pencil mark, locating "small tan spot" described in text, is directly under "4" in α-globulin region.

The "standard serum" protein pattern (Fig. 1) is seen to include on the right hand side the prominent albumin spot, on the trailing side of which a slight tail represents the alpha-1 globulin. Following this are faint alpha-2 and -3 globulins, and then a more prominent alpha-4 globulin spot. Three beta globulin spots may be distinguished, after which comes the broad gamma globulin spot, which has migrated backwards due to electröosmosis(14).

The *"sedimented serum"* pattern differs slightly in having a somewhat fainter alpha-4 spot and a stronger alpha-3 globulin. Similar patterns were obtained with *oxalated plasma*. However, with *heparinized plasma* hardly any stain appears in the alpha-4 position, but a pronounced spot is apparent in the alpha-3 globulin. As was to be expected, both oxalated and heparinized plasmas show staining of the fibrinogen fraction at the starting line, although this component was rather weak in the case of the heparinized plasma shown here in Fig. 1.

Patterns treated with the polysaccharide stain are shown in Fig. 2. The *"standard serum"* pattern shows very faint staining in the albumin region, no stain in the alpha-1 globulin, faint spots in the alpha-2 and -3 positions, a more prominent alpha-4 component, and additional faint spots in the beta globulin regions. As in the case of the protein patterns, the *"sedimented serum"* and oxalated plasma patterns show a more even distribution of stain between the alpha-3 and alpha-4 positions. The *heparinized plasma* again shows the stain concentrated in the alpha-3 globulin.

Similar differences in lipid patterns from corresponding sera and plasmas have not been observed. However even if such differences were present, they would be difficult to detect due to the faint staining of the small quantities of lipids in the alpha-2-4 region of the electrophoretic patterns of normal dog serum or plasma.

Discussion. The results described here show that a distinct difference exists between the electrophoretic patterns obtained from dog serum and plasma, apart from the an-

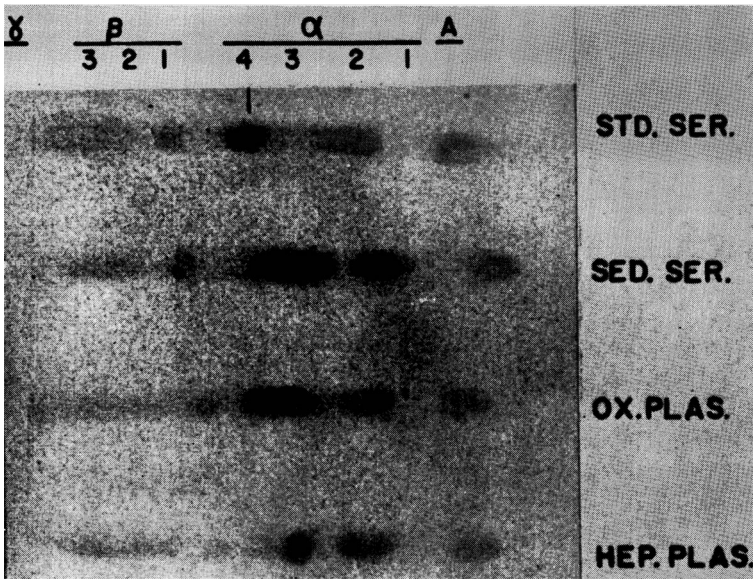

FIG. 2. Polysaccharide patterns, stained with periodic acid-fuchsin sulfite, from paper electrophoresis of samples of normal dog serum, "sedimented serum," oxalated plasma, and heparinized plasma, from top to bottom. Other details as in Fig. 1.

ticipated presence of fibrinogen in the latter. An additional observation has been that technics of obtaining different types of sera or different types of plasmas can also produce changes in the electrophoretic patterns. Of special significance in the interpretation of either free or paper electrophoresis patterns from the dog is the finding that the pattern shifts observed are a function of polysaccharide components as well as of proteins, thus implicating glycoproteins in the phenomenon. The two extremes of this situation are represented by the "standard serum," with the prominent alpha-4 globulin in protein and polysaccharide patterns, and the heparinized plasma, with the alpha-4 globulin greatly diminished and the alpha-3 component predominant in both protein and polysaccharide patterns.

Although the shift to a faster mobility resembles that seen in proteins of stored human plasma(15) the freshness and equal age of all samples compared in the present study eliminate ageing as an explanation for this phenomenon. Nor is the effect explainable solely as an *in vitro* heparin effect(16,17), since it is seen to a similar degree in oxalated plasma and "sedimented serum," the latter containing no anticoagulant whatsoever. Comparison of results in these two cases, one unclotted, the other clotted, eliminates the blood clotting mechanism as the sole cause for the shift from alpha-3 to alpha-4.

Summary. The alpha globulin portions of the electrophoretic patterns of dog sera and plasma have been shown to be affected by the technics of preparation. While "standard serum" patterns show a predominance of alpha-4 over alpha-3 protein and polysaccharide, "sedimented serum" and oxalated or heparinized plasma show greater proportions of alpha-3 components.

1. Zeldis, L. J., Alling, E. L., McCoord, A. B., and Kulka, J. P., *J. Exp. Med.*, 1945, v82, 411.

2. Muntz, J. A., Barron, E. S. G., and Prosser, C. L., *Arch. Biochem.*, 1949, v23, 434.

3. Rappaport, A. M., Clarke, D. W., and Stewart, M., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 585.

4. Gjessing, E. C., Ludewig, S., and Chanutin, A., *J. Biol. Chem.*, 1947, v170, 551.

5. Boguth, W., *Naturwissenschaften*, 1953, v40, 22.

6. Fasoli, A., Glassman, M. D., Magid, E. B., and Foa, P. P., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 298.

7. Adlersberg, D., Bossak, E. T., Sher, I. H., and Sobotka, H., *Clin. Chem.*, 1955, v1, 18.

8. Sohar, E., Bossak, E. T., and Adlersberg, D.,

PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 305.

9. Goldwater, W. H., and Entenman, C., *Radiation Res.*, 1956, v4, 243.

10. Heller, V. G., and Paul, H., *J. Lab. Clin. Med.*, 1934, v19, 777.

11. Piantanida, M., and Muic, N., *Arch. Biochem. Biophys.*, 1953, v46, 110.

12. Swahn, B., *Scand. J. Clin. Lab. Invest.*, 1952, v4, 98.

13. Köiw, E., and Grönwall, A., *ibid.*, 1952, v4, 244.

14. Kunkel, H. G., and Tiselius, A., *J. Gen. Physiol.*, 1951, v35, 89.

15. Hoch, H., and Chanutin, A., *J. Biol. Chem.*, 1954, v209, 661.

16. ———, *ibid.*, 1952, v197, 503.

17. Blasius, R., and Seitz, W., *Klin. Wochschr.*, 1952, v30, 905.

Received October 1, 1956. P.S.E.B.M., 1957, v94.

Myelograms Relating to Anemia and Hematopoiesis Following Thermal Injury in Rats*. (22988)

MILES D. MCCARTHY AND JOHN ODELL
Department of Zoology, Pomona College, Claremont, Calif.

Anemias following thermal injury in man and laboratory animals have been repeatedly observed(1-6) and have been shown in part to result from increased rate of hemolysis(1, 2,5) and disturbed liver function(4). Suppressed medullary erythropoiesis has also been suggested as contributing to the anemia by James and co-workers(2) while Moore and his co-workers(1) and Wintrobe and his co-workers(7) have presented data from radioiron uptake studies that indicate direct medullary inhibition. However, the work of Davis, Alpen and Davis(5) and Davis, Alpen and Sheline(6) showed an increase in rate of radioiron uptake which they interpreted as evidence for increased extramedullary and medullary erythropoiesis.

In this investigation we have studied the quantitative changes in femoral marrow of control rats, bled rats and thermally injured rats. We have attempted to discover from the data (a) whether suppression of the medullary hematopoiesis exists following thermal injury and (b) whether there is any quantitative difference in medullary hematopoiesis between moderately injured animals and severely injured animals.

Materials and methods. Unshaved male Wistar rats weighing between 200 and 210 g were used. Rats were housed individually in wire mesh cages at room temperature varying between 72 and 78°F and relative humidity varying between 20 and 40%. Except for the immediate 10 hour period following injury, all rats received Purina Laboratory Chow *ad lib.* This diet was supplemented once a week with carrots. Groups A and B received thermal injuries of 50 ± 2% and 20 ± 2% of the body surface respectively. Group H was bled and infused. Group C was the uninjured control. Thermal injury was effected under ether anesthesia in water at 90°C for 35 seconds. Prior to experiment a polyethylene tube was inserted into the posterior facial vein of each rat. Immediately following the thermal injury each rat was infused with 4% body weight of 1/6 M sodium lactate solution and 14% body weight of 1.4% sodium chloride solution. The details of these procedures have been previously reported(8). Animals in control group H were operated, bled by cardiac puncture and infused as described above. Three cc of blood were withdrawn to approximate immediate blood loss from thermal injury. This volume was determined from our Fe^{59} studies. In each of the thermally injured groups A and B and in the bled group, H, 3 animals were sacrificed for femoral bone marrow studies at 24, 48, 96, 168 hours and 2 and 4 weeks following injury or bleeding re-

* This investigation was conducted under contract from Office of Surgeon General, Department of Army.