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Physicochemical Changes of the Blood in Experimental Thrombopenic Purpura.*

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A broader understanding of the mechanism of hemostasis requires, among other things, a systematic investigation of the physical and chemical constants of the blood during disturbances of that function. The present report summarizes observations on the venous pressure and viscosity of the blood, the colloid osmotic pressure and proteins of the plasma at various stages of thrombopenic purpura produced in dogs experimentally with antiplatelet serum.

The type of dog, their diet and living conditions, the method for preparation and standardization of the serum, counting platelets, measuring clot retraction, the mean bleeding time and petechial reaction of the skin have been described elsewhere.[1, 2, 3] In the present group the bleeding time was done on at least 3 locations in the skin of the abdomen and thorax and discontinued if it exceeded 900 seconds. Venous pressure was measured by the direct method with a 22 gauge needle connected by a 2-way stop-cock to a manometer filled with salt solution. Pressures were taken, with a minimum of trauma, in the arm and leg vein each time, with the animals lying horizontally and the extremity held at the approximate level of the heart; at least 10 seconds were allowed for stabilization of the pressure. Viscosity of the blood and plasma were measured in a Hess viscosimeter at 20°C. (±0.5°), critical negative pressures of 50 mm. Hg. for plasma and 100 mm. Hg. for blood being used to move the columns of liquid. The average of at least 5 readings was taken each time. Colloid osmotic pressure of the plasma was measured in duplicate, in Wells' micro osmometer,[4] with collodion membranes having permeability numbers between 15×10^{-8} and 30×10^{-8}, and adhering in all details to Wells' technique. Plasma proteins were analyzed in duplicate by the macro Kjeldahl method. Globulin was precipitated at 38°C. with 22.5% sodium sulphate.[5]

* Aided by a grant from the J. Ewing Mears Research Fund. Miss Katherine Pierpoint rendered technical assistance.

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Fibrinogen was analyzed as fibrin by the method of Cullen and Van Slyke.[6] Nitrogen values were converted into protein values by multiplying by the factor 6.25. Non-protein N was determined in the plasma filtrate after precipitation of the protein with 10% trichloracetic acid. Each group of determinations was done in a single sample of blood collected without stasis from the jugular vein of the fasting animal into a syringe containing a small amount of a 25% solution of potassium oxalate. This introduces a dilution of a little less than 2%. Studies were carried out before, during and after the thrombopenia induced by a slow intravenous injection of a moderate dose of antiplatelet serum (0.1 cc. per kilo body weight). Eight experiments were performed on 5 animals. Three of the animals received 2 injections, not more than 4 days

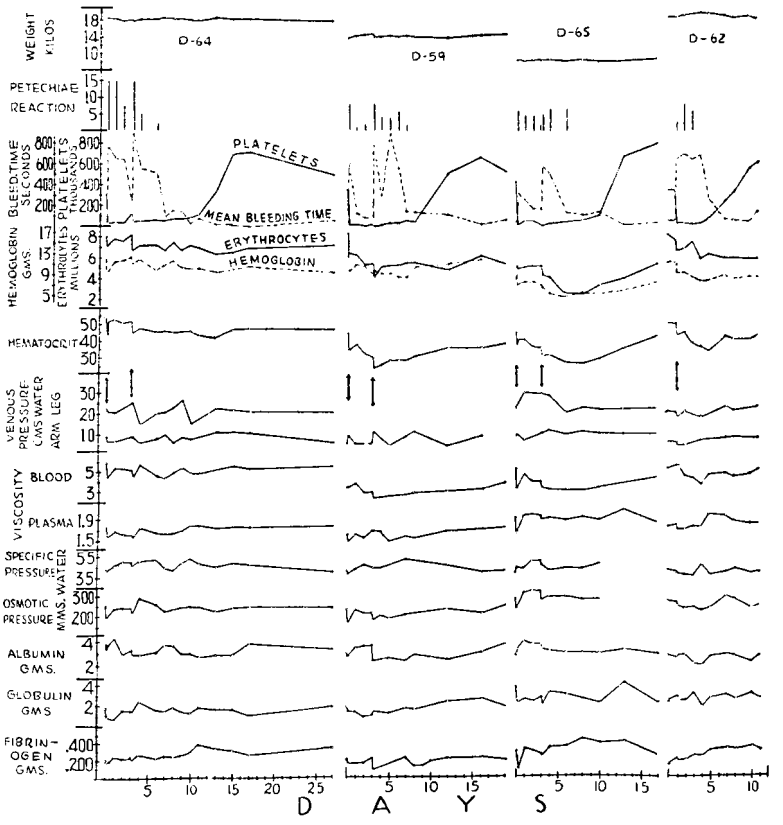

FIG. 1.

Effect of the injection of antiplatelet serum (indicated by arrows) on 4 dogs. The first determination after each injection was performed 2 hours after the serum was administered.

[6] Cullen, G. E., and Van Slyke, D. D., *J. Biol. Chem.*, 1920, **41**, 587.

apart; the remaining 2 received a single injection each. A figure was considered significant when it differed from the mean normal for that determination by more than 2½ times the standard deviation. Each correlation coefficient was calculated from groups of 76 to 107 pairs of variables and was considered significant if it exceeded 6 times its probable error. Results in 4 dogs are illustrated in Fig. 1.

There was little difference between the findings in animals that were given 2 injections and those that received a single one, although in the former the period of thrombopenia was twice as long as in the latter. Changes in the blood 2 hours after an injection were less marked after the second injection. With exception of the blood viscosity and non-protein nitrogen, most changes during and after the phase of purpura were unimportant and could be attributed chiefly to variations in concentration of the blood as a result of circulatory disturbances induced by injection of the antiserum. For example, 2 hours after an injection nearly all measurements showed a decrease of approximately equal magnitude (averaging about 20%). In the absence of information on fluctuations of the blood and plasma volume at various periods of the experiment, it is not possible to state how much these changes were due to dilution or concentration of blood. The experiments were intended primarily, however, to find out whether any characteristic changes in the physicochemical properties of the blood occurred when the tendency to prolonged bleeding existed; therefore, the prevailing values, whether they were the result of blood dilution or concentration, should answer this point. Since nearly all values decreased 2 hours after an injection, the regularly occurring increase in non-protein N, although small (+19.8%), must have some significance. Because this N was not fractionated, it is not possible to state in what fraction the increase occurred. In dogs that lost a moderate amount of blood the decrease in total cell volume led to a significant diminution in blood viscosity. The average percent decrease in percent cell volume and in blood viscosity in the animals given 2 injections was about the same (−34.2% and −33.7% respectively) and occurred about 4 days after the second injection. In those that received a single injection the decreases were similar in magnitude (−29.9% for the percent cell volume and −33.6% for the blood viscosity) and appeared about the 3d day after the injection. There was a high direct correlation between the percent volume of cells in the blood and blood viscosity (+0.803±0.027). Although the correlation between the blood viscosity and mean bleeding time was not significant (−0.319±0.069), the decrease in blood viscosity during an at-

tack of purpura might influence bleeding, not by affecting its duration, but by allowing a greater volume output of blood per unit time from the wound. An increase in mean output of blood per second from wounds is often found in clinical and experimental thrombopenias.[3]

There were several significant correlations between the physicochemical variables themselves, which will be taken up elsewhere. Besides those already stated, there were few significant zero or first order correlations between the mean bleeding time, number of platelets, hematocrit, clot retraction, petechiæ reaction and each of the physicochemical variables. One of the highest correlations found was between the amount of fibrinogen and mean bleeding time (-0.413 ± 0.059), thus reinforcing clinical knowledge of the occasional association, causal or otherwise, between fibrinopenia and prolonged bleeding from wounds. Within the range of values observed in this study there was no significant correlation between the degree of clot retraction and the fibrinogen content of the blood ($+0.036 \pm 0.73$) thus supporting the fact that qualitative changes in the fibrin play a more important part in determining that property of the clot, other things being equal, than quantitative changes.[7] There was a moderately high, direct correlation between the number of blood platelets and the total protein content of the plasma ($+0.403 \pm 0.063$). Changes in globulin were perhaps largely responsible for this significant correlation, since disintegration of platelets is said to increase the globulin content of the medium[8] and, of the proteins studied, the globulin fraction showed the greatest correlation with the number of platelets ($+0.304 \pm 0.064$). A decrease in globulin is observed in clinical and experimental thrombopenia,[9] an increase following a phase of thrombocytosis.[10]

The physicochemical forces studied do not apparently play a dominant part in the mechanism of disturbed hemostasis. Investigation of these forces was carried out because it was felt that the number of blood platelets alone did not adequately explain the disturbance of this mechanism in thrombopenic purpura.[2] Subsequent experiments have indicated that any correlation between platelets and bleeding must be established from counts on arterial blood[11] and that a volume increase in platelets may, at times, compensate partially for a deficiency in numbers.

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Summary. The blood of dogs with thrombopenic purpura induced by antiplatelet serum shows a moderate decrease in blood viscosity (directly correlated with a decrease in total cell volume) and a transient increase in non-protein nitrogen. The venous pressure, plasma viscosity, total and specific colloid osmotic pressure and plasma proteins do not undergo significant changes.

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Hyperalimentation in Normal Animals Produced by Protamine Insulin.

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Beginning some years ago there were clinical reports[1-5] that undernourished, non-diabetic patients gained weight under the influence of insulin. The latter is regarded as stimulating the appetite, leading to a higher caloric intake of food. It is now used for this purpose by many although good proof that it is efficacious is still lacking. The reason for this is because of the many factors involved in such clinical observations. Experiments on animals have been disappointing, such as the negative results recorded for rabbits.[6] In experiments on normal rats carried out some seven years ago with ordinary insulin we were unable to influence either the food intake or body weight. In an attempt to duplicate experimentally with protamine insulin the occurrence of fatty livers, which has been attributed to chronic hypoglycemia in patients,[7] we were surprised by the marked influence on alimentation. A typical experiment is presented in Fig. 1. Each group of rats was composed of 3 adult males of about the same weight. They were on a diet supplied *ad lib.* and containing casein 25, starch 40, butter fat 15, lard 10, brewers yeast 5 and standard salt mixture 5. Protamine zinc insulin* was given subcutaneously in doses of 8 units (0.2 cc.) per

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* We are indebted to Eli Lilly & Company of Indianapolis for the supply of Protamine zinc insulin used in this investigation.