1. Augustinsson, K-B., *Nature,* 1958, v181, 1786.
2. ———, *Acta Chem. Scand.,* 1959, v13, 571.
3. ———, *ibid.,* 1959, v13, 1081.
4. ———, *ibid.,* 1959, v13, 1097.
5. Lundblad, G., Paulsson, J-E., von Zeipel, E., Björklund, B., *Acta Path. Microbiol. Scand.,* in press.
6. Lundblad, G., *Acta Chem. Scand.,* 1961, v15, 212.
7. Moore, S., Stein, W. H., *J. Biol. Chem.,* 1954, v211, 907.

Received April 16, 1962. P.S.E.B.M., 1962, v110.

Thrombocytosis-Promoting Activity of Normal Plasma.* (27550)

JAMES W. LINMAN AND ROBERT V. PIERRE
Department of Medicine, Northwestern University Medical School and Veterans Administration Research Hospital, Chicago, Ill.

Data from many laboratories have confirmed the existence of a humoral erythropoietic regulatory mechanism(1-3). Recent observations suggest that other aspects of hemopoiesis may be similarly influenced. The thermostable fractions of plasmas from patients with polycythemia vera(4) and from rabbits with acute phenylhydrazine-induced anemia(5) evoke thrombocytosis and leukocytosis in normal rats in addition to erythrocytosis and reticulocytosis. Thrombocytosis has also been reported in rats injected with plasma or serum from thrombocytopenic donors(6-8). These findings are in accord with the hypothesis that thrombopoiesis is subject to humoral homeostatic control; however, the concept of such a physiologic regulator demands that the responsible substance (or substances) also be demonstrable in normal blood. The experiments described herein bear directly on this problem.

In the majority of our studies on the humoral control of hemopoiesis, we have used, as measures of hemopoietic stimulation, changes in the peripheral erythroid values, reticulocyte, leukocyte, and thrombocyte counts, and marrow cytology in normal rats given multiple daily doses of test plasmas. For purposes of uniformity, individual doses usually have been calculated on the basis of 2 ml of the original plasma per 100 g of the recipients' body weights. These quantities of normal plasma are erythropoietically and leukopoietically inactive when assayed as just described, although erythropoietic activity has been detected in normal plasma with other technics(9) and is demonstrable in concentrates thereof(2,10). In contrast, however, such doses of normal human or rabbit plasma have consistently induced thrombocyte increases in normal rats(4,5). An example of the thrombocyte responses in recipients of normal human plasma extracts by gastric tube is shown in Table I. Two weeks after the treatment was stopped, normal baseline counts had been restored. In this report are described observations on the thrombopoietic effects of lesser amounts of normal plasma.

Materials and methods. Fresh normal human plasma was procured from the blood bank, pooled, adjusted to a pH of 5.5 by addition of 1N HCl, and boiled over a direct flame for 30 minutes. The material was filtered and the coagulum discarded. The filtrate was divided into 3 equal portions and reconstituted with distilled water so that 0.5 ml of the final extracts represented, respectively, 0.5, 1.0, or 2.0 ml of the unmodified plasma. Forty normal female Wistar strain rats were divided into 4 equal groups and given, over a period of 2 weeks (Saturdays and Sundays excepted), 10 doses of test materials by gastric tube. Three groups received normal plasma extracts in doses equivalent to either 0.5, 1.0, or 2 ml of the origi-

* Supported in part by research grant from N.I.H., U.S.P.H.S. The technical assistance of Marlene Maguire, Norma Rosenberg, and Marilyn Grayhack is gratefully acknowledged.

TABLE I. Thrombocytosis in Normal Rats Given the Thermostable Fraction of Normal Human Plasma by Gastric Tube. Mean counts and stand. dev. ($\times 10^3$/mm^3) of 45 animals treated with the plasma extract and 30 receiving comparable amounts of water.

Test materials	Baseline Mean	Baseline S.D.	2 wk Mean	2 wk S.D.
Normal plasma extract*	.662	.064	.857	.133
Water	.635	.059	.629	.077

* Daily doses = 2 ml of original plasma/100 g body wt.

nal plasma per 100 g body weight. Using the appropriate extract reconstituted as described above, each animal received similar quantities of test materials. The fourth group was given comparable amounts of water. Baseline studies included hemoglobins determined by the cyanmethemoglobin technic, microhematocrits, hemacytometer erythrocyte and leukocyte counts, reticulocytes per 1000 red cells counted on brilliant cresyl blue coverslip films counterstained with Wright's stain, and thrombocyte counts. The latter were enumerated by phase microscopy; 1% ammonium oxalate was used as the diluent.† All measurements were repeated weekly × 4. The animals were fed a regular diet and given water *ad libitum*.

Results. Peripheral erythroid values and leukocyte counts remained constant in each group (Table II). Thrombocytosis was observed in recipients of the heat-denatured filtrate of normal plasma in daily doses equivalent to 2 ml of the original plasma per 100 g body weight (Fig. 1, Table III). Two weeks after treatment was stopped, thrombocyte counts had returned to normal. Serial measurements in rats given either one-half or one-fourth this daily dose of the thermostable fraction of normal plasma did not differ significantly from their baseline values or from determinations in recipients of water (Fig. 1). All animals remained healthy and exhibited no adverse effects attributable to the treatment or repeated gastric intubations. Subsequent experiments have corroborated these findings.

Discussion. The 30% increase in thrombocyte counts observed in this study confirms the thrombocytosis-promoting activity of normal plasma previously described. The substance(s) responsible for this phenomenon has not been identified but is markedly thermostable and active orally. The dose-response relationship and restoration of normal values 2 weeks after treatment was stopped contribute to the specificity of this stimulus as does the oral route of administration. The latter tends to negate the possibility that the thrombocytosis might reflect a nonspecific or irritative effect, an important consideration since Odell and his associates(11) have described platelet increases in normal rats in-

† With this technic, we found the average thrombocyte count and standard deviation therefrom of 865 normal female Wistar strain rats to be .666 ± .088 × 10^6 per cu mm.

TABLE II. Stability of Peripheral Erythroid Values and Leukocyte Counts in Normal Rats Given Normal Human Plasma Extracts or Water by Gastric Tube. Average determinations of 10 animals in each group.

Measurement	Test materials*	Baseline	1 wk	2 wk
RBC × 10^6 (per mm^3)	1	6.89	7.03	7.06
	2	6.89	6.94	7.03
	3	6.92	7.02	6.90
Retics (%)	1	3.4	4.2	4.5
	2	3.7	3.7	4.0
	3	3.7	3.6	3.7
Hemoglobin (%)	1	13.8	14.0	13.8
	2	14.2	13.9	13.7
	3	14.7	15.0	14.5
Hematocrit (%)	1	44.9	45.3	44.0
	2	45.5	44.8	44.1
	3	44.8	44.6	43.8
WBC × 10^3 (per mm^3)	1	14.2	14.2	14.7
	2	13.9	15.2	14.5
	3	14.7	15.0	14.5

* 1—Normal plasma (2 ml/100 g/day); 2—Normal plasma (1 ml/100 g/day); 3—Water.

TABLE III. Standard Deviations of Mean Thrombocyte Counts ($\times 10^6$/mm^3) in Groups of 10 Normal Rats Given the Thermostable Fraction of Normal Human Plasma or Water by Gastric Tube.

Test materials	Baseline Mean	Baseline S.D.	2 wk Mean	2 wk S.D.
Normal plasma				
2 ml/100 g/day	.650	.044	.849	.045
1 ml/100 g/day	.668	.031	.684	.026
Water	.660	.040	.685	.034

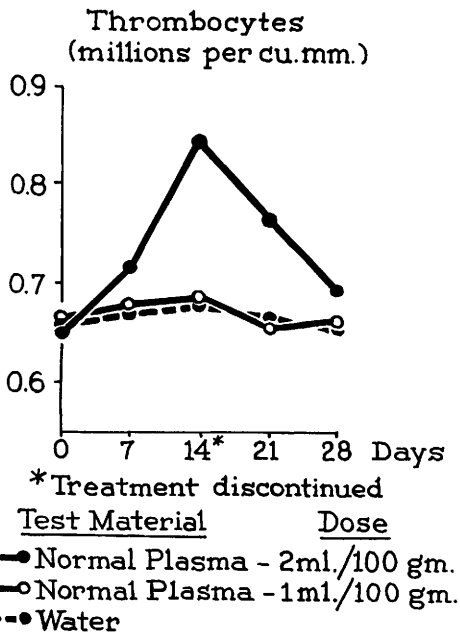

FIG. 1. Thrombocyte responses in normal rats given 10 daily doses of the thermostable fraction of normal human plasma or water by gastric tube. Counts did not change in recipients of water or normal plasma extracts in doses equivalent to 0.5 (not shown) or 1.0 ml of the original plasma per 100 g body wt. Avg determinations of 10 animals in each group.

jected with foreign materials such as solutions of egg albumin or a suspension of finely ground glass.

Our findings are in agreement with the observations of Schulman and his associates (12) on a child with chronic thrombocytopenia. Normal thrombocyte counts have been repeatedly but temporarily restored in their patient by administration of normal plasma. These investigators have concluded that this patient is deficient in an essential thrombopoietic substance which is contained in normal plasma. More recently, they have also described thrombocyte increases in rats given multiple injections of the boiled filtrate of normal human plasma(13). Steinberg and coworkers(14) have also reported thrombopoietic activity in normal human plasma as have Odell *et al.*(11). The latter investigators did not note thrombocytosis in rats given 4 injections of normal rat plasma(8). Using rabbits as donors and recipients, Spector(7) concluded that normal plasma does not induce thrombocytosis; however, he employed a single injection of plasma. Such short-term experiments would not appear to exclude the presence of minimal thrombopoietic activity.

The demonstration of a thrombopoietic stimulus in normal human plasma lends additional credence to the thesis that thrombopoiesis is subject to humoral regulatory control. It also points out the need for caution in interpretation of experimental observations on humoral control of thrombopoiesis. The ability to quantify, at least to a certain extent, the thrombocytosis-promoting activity of normal plasma (Fig. 1) contributes to the significance of the higher thrombocyte counts (about 70% increases) observed in recipients of "polycythemic" or "anemic" plasmas(4,5). On the basis of available data, therefore, it is proposed that a humoral regulator does exert physiologic control over thrombopoiesis and that it may contribute to the increased thrombocyte production accompanying acute blood loss anemia, in polycythemia vera, and after production of acute thrombocytopenia by such measures as antiplatelet sera.

The preliminary nature of these speculations and the voids in existent knowledge must be emphasized. The possible mode of action of this thrombopoietic factor is purely conjectural. It would appear to stimulate the production of thrombocytes but could accomplish this in any of several ways, *e.g.*, by increasing megakaryocytic differentiation from the primitive pluripotential marrow cells, by stimulating proliferation of megakaryocytic precursors, by enhancing thrombocyte formation by individual megakaryocytes, etc. In addition, the possibility of more than one humoral factor with thrombopoietic stimulatory effects or of activator-inhibitor complexes must be excluded. Consequently, it would seem desirable to defer use of specific terms, *e.g.*, "thrombopoietin", for humoral agents with thrombopoietic activity pending clarification of their nature and mode of action.

Summary. Filtrates of normal human plasma that had been boiled for 30 minutes were administered by gastric tube to groups of normal rats. Ten daily doses each equiva-

lent to 2 ml of the original plasma per 100 g body weight induced a 30% increase in the recipients' thrombocyte counts. Smaller doses, *i.e.*, 0.5 or 1 ml per 100 g body weight, did not promote thrombocytosis. All other hematologic measurements in these animals remained stable. These findings provide additional support for the hypothesis that thrombopoiesis is subject to humoral regulatory control.

1. Gordon, A. S., *Physiol. Rev.*, 1959, v39, 1.
2. Linman, J. W., Bethell, F. H., *Factors Controlling Erythropoiesis*, Charles C Thomas, Springfield, Ill., 1960.
3. Jacobson, L. O., Gurney, C. W., Goldwasser, E., in *Advances in Internal Medicine*, Year Book Publishers, Chicago, Ill., 1960, v10, 297.
4. Linman, J. W., Bethell, F. H., Long, M. J., *Ann. Int. Med.*, 1959, v51, 1003.
5. Linman, J. W., *J. Lab. and Clin. Med.*, 1962, v59, 262.
6. Kelemen, E., Lehoczky, D., Perkedy, J., Cserháti, I., Rák, K., *Lancet*, 1960, v1, 1134.
7. Spector, B., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 146.
8. Odell, T. T., Jr., McDonald, T. P., Detwiler, T. C., *ibid.*, 1961, v108, 428.
9. Reichlin, M., Harrington, W. J., *Blood*, 1960, v16, 1298.
10. Gurney, C. W., Goldwasser, E., Pan, C., *J. Lab. and Clin. Med.*, 1957, v50, 534.
11. Odell, T. T., Jr., McDonald, T. P., Detwiler, T. C., Howsden, F.L., *Blood*, 1961, v18, 796.
12. Schulman, I., Pierce, M., Lukens, A., Currimbhoy, Z., *ibid.*, 1960, v16, 943.
13. Schulman, I., Currimbhoy, Z., Fort, E., Alcalde, V., *Am. J. Dis. Children*, 1960, v100, 747.
14. Steinberg, B., Dietz, A. A., Martin, R. A., *Acta Haemat.*, 1959, v21, 78.

Received April 12, 1962. P.S.E.B.M., 1962, v110.

Conditioning Effect of Certain Minerals upon Development of Nephrotoxic Serum Arthritis in Rabbits.* (27551)

CONSTANTIN V. TEODORU AND BRUNO W. VOLK
(With technical assistance of Alice Einhorn)
Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

It has been shown that repeated injections of small amounts of Duck Anti-Rabbit Kidney Serum (DARKS) into the joints of rabbits cause a progressive, self-perpetuating arthritis which develops after incubation periods averaging 10 weeks(1-3). Normal Duck Serum (NDS) given under similar circumstances fails to produce arthritis. Since the slow progress of the monoarthritis produced by DARKS is impractical for experimental purposes an attempt was made to accelerate its development by conditioning factors such as high intake of NaCl which had previously been shown to enhance the effects of DARKS in nephritis(4) and in arthritis (2).

The present paper describes the effect of high intake of NaCl and KCl upon the clinical and pathological evolution of the nephrotoxic serum arthritis.

* Supported by Nat. Inst. Health grant.

Material and methods. DARKS and NDS were prepared following the method previously described(1,5). Bacteriological cultures were frequently performed on all serum samples. Contaminated sera were eliminated. Duck serum globulins were isolated and concentrated from both DARKS and NDS following the alcohol precipitation method as described by Mellors(5).

Adult albino rabbits of either sex weighing 3-4 kg were separated into 3 main groups. A) One group of 26 animals was preconditioned with 0.9% NaCl given instead of drinking water. B) Another group of 18 rabbits was preconditioned with 1.14% potassium chloride given the same way. C) A third group of 26 rabbits drinking tap water, served as controls. Each group was divided into 3 equal subgroups which were treated as follows: 1) One-third of each group was injected with DARKS in the right knee. 2) The second