Studies on Thrombopoiesis. IV. Thrombocytosis-Promoting Activity of Normal Urine.* (28376)

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The authors have recently demonstrated a thermostable, orally active, thrombocytosis-inducing factor(s) in normal human plasma (1). Increased activity is found in the plasma of rabbits with phenylhydrazine-induced anemia(2) and in the plasma of patients with polycythemia vera or essential thrombocythemia(3). Studies on humoral thrombopoietic factors using long term animal assays have been limited by the nonspecific thrombocytosis that may follow parenteral administration of certain materials(4) and by the need to obtain large amounts of test plasmas. The former difficulty has been surmounted, at least in part, by the finding that the humoral agent(s) in plasma is active orally. When administered by gastric tube, urine from a patient with polycythemia vera induced thrombocytosis in normal rats comparable to that produced by the patient's plasma; normal urine exerted an effect similar to that of normal plasma (Fig. 1). These observations prompted us to study normal urine as a source of humoral thrombopoietic factor(s).

Materials and methods. Fresh human urine collected without preservative from 4 normal adult males was pooled and frozen. Just prior to testing, the urine was thawed and dialyzed against running tap water for 16 hours. The dialyzed urine was divided into 6 portions and concentrated by boiling over a direct flame for 30 minutes so that 0.5 ml of the material tested represented 0.5, 1.0, 2.0, 4.0, 6.0, and 12.0 ml, respectively, of the original urine. To keep the boiling time constant, it was necessary to add distilled water to the most dilute specimens and to prepare the more concentrated in several lots. For example, 1800 ml were divided into six 300 ml aliquots; each was reduced by boiling for 30 minutes to 12.5 ml and recombined to yield 75 ml of which 0.5 ml represented 12 ml of the original specimen. All test materials were processed on the same day and stored at 6°C in sterile containers during the treatment period.

Forty-two female Wistar strain rats weighing approximately 160 g were divided into 7 equal groups and given 10 doses of test materials by gastric tube over a period of 2 weeks (Saturdays and Sundays excepted). Six groups received the normal urine concentrates in doses equivalent to 0.5, 1.0, 2.0, 4.0, 6.0, and 12.0 ml, respectively, of the original pooled urine specimen per 100 g body weight. Using the appropriate concentrate as described above, each animal received a similar volume. The seventh group received a comparable amount of Ringer's solution.

Baseline hematologic determinations were performed by the following methods: hemoglobin, cyanmethemoglobin technique; hematocrit, microhematocrit; red blood cell and leukocyte counts, hemocytometer; reticulocytes, per cent of 1000 red cells on brilliant cresyl blue coverslip films counterstained with Wright's stain. Phase microscopy thrombocyte counts were done in duplicate using 2 pipettes and separate counting chambers. One per cent ammonium oxalate was used as the diluent. With this technic, the average thrombocyte count and standard deviation of 1000 normal female Wistar strain rats was $.670 \pm .088 \times 10^6$ per cu mm. All studies were repeated weekly for 4 weeks. The animals received a regular laboratory diet and water *ad libitum*. Experience has shown that the maximal thrombocyte increase obtainable with this assay is approximately 70% over baseline value which corresponds to counts of 1.0 to 1.1 million per cu mm.

Results. Normal urine in daily doses

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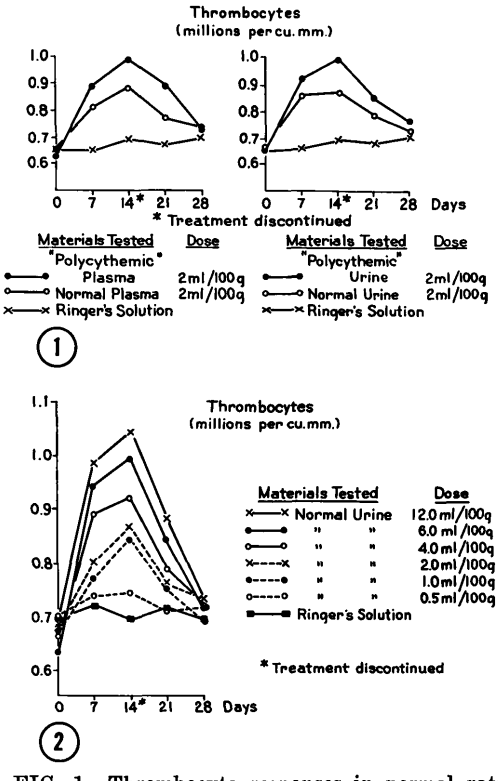

FIG. 1. Thrombocyte responses in normal rats given 10 daily doses of boiled filtrates of normal human urine or plasma and of "polycythemic" urine or plasma. Donor of the latter was a 69-yr-old male with untreated polycythemia vera. At the time plasma and urine were obtained, thrombocyte count was 639,000 per cu mm; hemoglobin was 20.5 g % and hematocrit 67%. Avg determinations of 5 animals in each group.

FIG. 2. Thrombocyte responses in normal rats given 10 daily doses of normal human urine or Ringer's solution by gastric tube. Avg determinations of 6 animals in each group.

TABLE I. Mean Thrombocyte Counts with Standard Deviations [$\times 10^6/mm^3$] in Groups of 6 Normal Rats Given Concentrates of Normal Human Urine or Ringer's Solution by Gastric Tube.

Test materials	Baseline Mean	Baseline S.D.	1 wk Mean	1 wk S.D.	2 wk Mean	2 wk S.D.
Normal urine ml/100 g/day						
.5	.703	.043	.740	.027	.747	.044
1.0	.676	.052	.775	.038	.849	.035
2.0	.688	.043	.802	.035	.869	.032
4.0	.666	.043	.891	.046	.922	.041
6.0	.635	.041	.947	.038	.997	.031
12.0	.681	.043	.988	.046	1.047	.030
Ringer's solution	.696	.027	.723	.027	.699	.037

TABLE II. Stability of Peripheral Erythroid Values and Leukocyte Counts in Normal Rats Given Normal Human Urine Concentrates or Ringer's Solution by Gastric Tube. Average determinations of 6 animals in each group.

Measurement	Test* materials	Baseline	1 wk	2 wk
RBC $\times 10^6$ (per cu mm)	1	6.26	6.22	6.31
	2	6.36	6.30	6.32
	3	6.27	6.38	6.36
	4	6.32	6.46	6.43
	5	6.35	6.46	6.40
	6	6.38	6.43	6.51
	7	6.32	6.42	6.21
Reticulocytes (%)	1	3.2	3.5	3.9
	2	3.1	3.6	3.6
	3	3.7	3.9	4.2
	4	3.1	3.2	4.0
	5	3.0	3.9	4.2
	6	2.9	3.9	4.2
	7	3.9	3.8	4.0
Hemoglobin (%)	1	13.9	14.0	14.3
	2	13.5	13.7	14.0
	3	13.6	13.8	14.1
	4	14.3	14.1	14.4
	5	13.8	14.1	14.0
	6	14.1	14.4	14.1
	7	13.2	14.3	14.1
Hematocrit (%)	1	43.5	44.5	44.6
	2	42.2	43.5	43.3
	3	41.8	43.1	43.9
	4	43.5	43.4	42.9
	5	41.8	43.0	43.6
	6	41.7	42.8	43.6
	7	41.2	44.3	42.8
WBC $\times 10^3$ (per cu mm)	1	11.4	13.7	12.1
	2	11.4	12.2	10.5
	3	11.8	11.1	10.1
	4	12.4	11.4	11.6
	5	12.0	13.0	13.9
	6	12.1	11.4	11.9
	7	11.9	11.2	12.3

* 1–Normal urine (0.5 ml/100 g/day); 2–Normal urine (1.0 ml/100 g/day); 3–Normal urine (2.0 ml/100 g/day); 4–Normal urine (4.0 ml/100 g/day); 5–Normal urine (6.0 ml/100 g/day); 6–Normal urine (12.0 ml/100 g/day); 7–Ringer's solution (1.0 ml/day).

equivalent to 1.0 ml or more per 100 g body weight evoked clear-cut thrombocytosis in recipient animals (Fig. 2, Table I). The peripheral erythroid values and leukocyte counts did not vary significantly from baseline determinations (Table II). After treatment was discontinued, thrombocyte counts returned to normal levels. Thrombocyte counts of recipients of normal urine in daily doses equivalent to 0.5 ml per 100 g body weight did not differ significantly from their baseline values or from the control group.

Successive dose increases elicited a progressively greater thrombocyte response. The thrombocyte counts at 2 weeks exhibited a linear log-dose response relationship. All animals remained healthy, gained weight at a similar rate, and exhibited no adverse effects attributable to the treatment or repeated gastric intubations.

Discussion. These findings confirm the thrombocytosis-promoting activity of normal human urine. The slow rise in thrombocytes, the persistence of the response until therapy was discontinued, and the subsequent return to baseline levels suggest that the thrombocytosis was secondary to increased production. The absence of a response in recipients of small doses of urine or of Ringer's solution, the dose-response relationship, and the stability of the erythroid values and leukocyte counts testify to the specificity of the thrombocyte increases. The demonstration of a greater thrombocyte response to lesser amounts of "polycythemic" urine (Fig. 1 and 2) adds further support to the specificity of the thrombocytosis-promoting activity of normal urine.

The oral route of administration, together with the stability of the thrombocyte counts in the control animals subjected to daily gastric intubation, argues against the possibility that the thrombocytosis observed in the recipients of urine might be an irritative or nonspecific effect. Odell and his associates (4) have described increased thrombocyte counts in normal rats injected with foreign materials such as egg albumin or a suspension of finely ground glass.

The thrombocytosis-promoting activity of normal urine is comparable to that of normal plasma(1). The exact nature of the thrombopoietic factor in urine is unknown, but it is heat resistant, non-dialyzable, and not destroyed by the enzymes of the rat stomach or intestine. In these aspects, it is remarkably similar to the plasma thrombopoietic factor(s) that we have studied(1-3). The exact mode of action of the urine thrombopoietic factor is unknown. Although the findings reported above strongly suggest that production was increased, it has not been proved.

Van Dyke and associates(5) have also described thrombocytosis in human subjects and experimental animals injected with urine from an anemic, thrombocytopenic donor. These workers did not find activity in normal urine; however, the amount given and the duration of the treatment period were less than in our experiments. The demonstration of a thrombopoietic substance in urine greatly facilitates studies on the control of thrombopoiesis since large quantities of test material can be readily obtained without risk or discomfort to the donor. Additional studies are now in progress to establish the presence or absence of thrombocytosis-inducing activity in the urine of patients with a variety of abnormalities in thrombopoiesis, to quantify the activity therein, to correlate urine with plasma activity, and to elucidate the chemical and physical properties of this substance(s).

Summary. Ten daily doses of boiled normal human urine equivalent to 0.5, 1.0, 2.0, 4.0, 6.0, and 12.0 ml per 100 g body weight were administered by gastric tube to normal rats. Doses equal to 1% of the rats' weights evoked thrombocytosis. Larger doses induced progressively greater responses. The thrombocytosis-promoting activity of normal urine was comparable to that in similar amounts of normal plasma. Urine from a polycythemic subject appeared to contain increased activity. These studies are in accord with the thesis that thrombopoiesis is subject to humoral regulatory control.

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