Erythropoietic Stimulating Activity of Urine from Anemic Human Subjects.* (23685)

JOHN WINKERT, ALBERT S. GORDON, PAUL T. MEDICI, SAM J. PILIERO, A. LEONARD LUHBY AND MYRON TANNENBAUM

Departments of Biology, Graduate School of Arts and Science, N. Y. University, St. John's University, Brooklyn, and Departments of Anatomy and Pediatrics, N. Y. Medical College

The erythropoietic stimulating activity of plasma from anemic human subjects has been repeatedly demonstrated(1-3). There are also indications that urine from anemic patients will induce erythroid marrow hyperplasia(1), elevate peripheral erythrocytic parameters(4) and increase the % incorporation of Fe^{59} into the red cells of recipient rats(3, and Mirand, personal communication).

The present studies were undertaken to determine the frequency with which erythropoietic stimulating activity appears in anemic human urine and to relate the occurrence of this activity to that of plasma.

Materials and methods. Seventeen 24-hour urine samples were collected from 6 children and 1 adult with hemoglobin levels ranging from 4 to 7 g%. Four additional samples were obtained from children with normal hemoglobin levels. All of the urines were preserved at 5°C with 5 ml of toluene added per liter. Five of the urine samples from patients with Cooley's anemia and 1 from a normal child were subjected to the acidification and boiling procedure described previously(4). The remainder of the urine samples were tested without modification. Normal mature female rats of a modified Long-Evans strain, weighing 150-200 g and maintained on a diet of Purina Chow, were used for testing the urines. Four or 5 rats were used in each assay. Each rat received 3 ml of urine injected subcutaneously once daily for 10 to 20 days.

Reticulocyte, red cell, hemoglobin and hematocrit determinations were performed prior to the injection period employing methods previously described(5). Reticulocyte counts were again made on the 6th day and red cell, hemoglobin and hematocrit estimations were repeated on the day following the last injection. These are the values indicated in Table I. The red cell counts for each animal were taken as the mean of the 4 chamber counts drawn from 2 separate pipettes. The same pipettes were used before and after treatment for each rat. In 4 assays, bone marrow smears were prepared after the last injection following the method already reported(6). In 1 assay, blood volume determinations were performed according to a modification of the method of Wang and Hegsted(7,8).

Results. Of the 5 urine samples taken from the patients with Cooley's anemia and subjected to the acidification-boiling procedure, only the sample listed in the first row of Table I possessed erythropoietic stimulating activity. The other 4 inactive samples are not listed in the table. Similarly treated urine obtained from the normal boy was inactive.

The remaining 12 urine samples which were obtained from the anemic children and the adult with a benign thymoma, were not acidified and boiled. Eight of these evoked increases in some of the erythrocytic values (Table I). These included 2 of 4 samples obtained from Cooley's patients, 3 of 5 secured from the hypoplastic anemia subject and 1 each from the myelogenous leukemia, benign thymoma, and sickle cell anemia patients. However, only 4 of the 8 positive responses involved more than 2 erythrocytic parameters. The 4 inactive samples are not included in the table.

No erythropoietic stimulating activity was noted with a Cooley patient's urine after a transfusion had brought his hemoglobin level to 12 g%. Similarly, the urine from the myeloblastic leukemia patient in remission was inactive as was the urine obtained from the normal girl.

The 2 samples of urine collected from the

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TABLE I. Effects of Urine from Anemic Patients on Peripheral Blood of the Rat.

Status of urine donor				Effects in recipient rats (mean ± S.E.)			
Age (yr)		Diagnosis	Hemoglobin (g %)	RBC (mill/mm³)	Hemoglobin (g %)	Hematocrit (%)	Retics (%)
6	♂	Cooley's anemia	5.4 B§	9.3 ± .2	16.0 ± .5	47.2 ± 1.0	2.1 ± .2
			A	12.0 ± .4†	18.4 ± .8*	54.6 ± 2.2*	4.6 ± .8*
			5.5 B	8.7 ± .4	15.3 ± .4	46.1 ± 1.3	2.5 ± .9
			A	9.7 ± .2	17.7 ± .5*	51.7 ± 2.6	7.7 ± .8†
			4.3 B	9.0 ± .2	14.9 ± .2	46.9 ± 1.9	3.9 ± .7
			A	11.1 ± .6*	18.3 ± .9*	57.7 ± 2.3*	8.3 ±1.3*
7	♂	Hypoplastic anemia	5.5 B	8.5 ± .1	14.7 ± .2	45.6 ± 1.2	3.0 ± .4
			A	11.0 ± .1†	18.1 ± .7†	55.6 ± 1.4†	5.2 ± .4†
			4.5 B	8.7 ± .3	15.3 ± .6	46.1 ± 1.2	3.9 ± .4
			A	10.3 ± .3†	17.1 ± .3*	51.0 ± .7†	7.7 ± .9†
			6.0 B	9.0 ± .2	15.9 ± .3	49.8 ± .9	2.9 ± .8
			A	10.0 ± .2*	16.0 ± .7	50.3 ± .7	3.7 ± .7
3	♂	Myeloblastic leukemia	6.5 B	9.3 ± .2	14.5 ± .3	54.5 ± .3	3.1 ± .6
			A	11.6 ± .2†	16.5 ± .2†	57.0 ± 2.1	6.0 ± .4†
3	♀	Sickle cell anemia	6.5 B	9.0 ± .2	14.9 ± .2	49.5 ± .5	2.0 ± .4
			A	10.2 ± .3*	16.5 ± .5*	52.0 ± 1.4	2.7 ± .9
68	♀	Benign thymoma	7.0 B	9.1 ± .1	15.6 ± .4	50.4 ± 1.3	2.8 ± .7
			A	10.2 ± .1*	16.7 ± .3	51.9 ± 1.1	4.6 ± .6
3	♂	Myeloblastic leukemia in remission	11.7 B	8.3 ± .1	15.6 ± .9	45.0 ± 4.3	2.9 ±1.3
			A	8.6 ± .3	14.9 ± .6	42.0 ± 1.0	3.2 ± .9
6	♂	Cooley's (transfused)	12.0 B	9.5 ± .1	17.6 ± .3	50.0 ± 1.7	2.9 ± .3
			A	9.0 ± .4	14.8 ± .2	44.0 ± 1.0	3.3 ± .4
6	♂	Normal	13.5 B	9.2 ± .2	16.6 ± .6	49.6 ± 1.2	2.4 ± .3
			A	8.9 ± .2	14.6 ± .6‡	45.4 ± 2.2	4.7 ± .7*
3	♀	"	13.0 B	8.3 ± .1	15.6 ± .3	49.6 ± .9	3.0 ± .8
			A	8.2 ± .2	14.9 ± .7	46.5 ± 1.1	3.2 ± .4

* Mean significantly greater than pretreatment level, P <.05.
† *Idem* P <.01.
‡ Mean significantly lower than pretreatment level, P <.05.
§ B = Before treatment; A = After treatment.

boy with hypoplastic anemia which elicited the marked increases in all 4 peripheral parameters listed in Table I (Hb 5.5 and 4.5 g%) also induced erythroid marrow hyperplasia (Table II). Blood volume values, determined in the animals receiving the first of these 2 active urines, were also significantly elevated (Table III). Nucleated red cell percentages were increased in the bone marrow of rats receiving the urine of the normal boy (Table II). This was probably not a true erythropoietic stimulating effect but most likely was a secondary consequence of the fall in hemoglobin induced in the recipient rats by this urine sample (Table I).

TABLE II. Effect of Urine from Anemic Patients on Bone Marrow of the Rat.

Status of urine donor				Marrow response after treatment (mean ± S.E.)
Age		Diagnosis	Hb. (g %)	Nucleated RBC (% of total nucleated cells)
6	♂	Cooley's anemia	5.4	36.6 ± 3.4
			5.5	42.2 ± 6.9*
7	♂	Hypoplastic anemia	5.5	45.8 ± 1.9†
			4.5	44.2 ± 4.0†
3	♀	Sickle cell anemia	6.5	27.2 ± 7.0
6	♂	Normal	13.5	41.0 ± 1.5*
		50 untreated control rats		32.4 ± 2.4

* Mean significantly greater than untreated controls, P <.05.
† *Idem*, P <.01.

Discussion. It is clear that some samples of urine obtained from anemic human subjects markedly augment peripheral red cell parameters in the rat. These rises, most likely, cannot be attributed to hemoconcentration since, as has been shown with 1 active sample, there was a concomitant rise in blood volume. Furthermore, it is probable that this

TABLE III. Effect of Urine from an Anemic Patient on Blood Volume of the Rat.

Donor	Blood vol (cc/100 g body wt)
Hypoplastic anemia (Hb. 5.5 g %)	10.1 ± .3*
Untreated controls	8.3 ± .4

* Mean significantly greater than untreated controls, $P < .01$.

increase in the total numbers of circulating erythrocytes is the result of enhanced production since there was an associated increase in the numbers of peripheral reticulocytes and an augmentation in the % of nucleated erythrocytes in the bone marrow.

Only one-fourth of the urine samples examined, however, elicited an increase in at least 3 erythrocytic parameters. Obviously, with such a low incidence of activity in anemic human urine, more than 4 samples are required to determine whether unconcentrated urine of normal subjects is consistently inactive.

There was no remarkable difference in the occurrence of activity between urine subjected to the acidification-boiling procedure and that not so treated. The presence of activity in at least 1 sample after boiling suggests that the factor in urine is heat stable. In this respect, it is similar to the factor obtained previously from the plasma of these same patients(1). When compared to anemic human plasma(1), anemic human urine is a much less constant source of erythropoietically active material. It is probably more dilute, since more injections are required to evoke detectable responses. As a source for the isolation of the factor, urine has the advantage, however, of being readily available in large quantities relatively free from plasma proteins.

It is not certain why urine is less frequently active than plasma. Examination of the clinical records indicates no correlation between the presence of activity in urine and the general status of the patient, the severity of the anemia, or the degree of concentration (specific gravity) of the urine. It is of interest that the most frequently active urine has come from the patient with hypoplastic anemia whose plasma, at least on one occasion, has been shown to be devoid of erythropoietic activity(1).

More work is required to establish the frequency of occurrence of erythropoietic activity in normal human urine and to elucidate its variable presence in the urine of anemic subjects. In this regard, the question of normal and pathologic variations in renal threshold should be studied.

Summary. Of 17 unconcentrated urine samples taken from 7 anemic humans, approximately 25% displayed erythropoietic stimulating properties. Blood volume and bone marrow studies confirmed the erythropoietic nature of the responses obtained. Urine from 4 children with normal hemoglobin levels showed no ability to stimulate erythropoiesis. When compared to anemic human plasma, anemic human urine is a more dilute and less constant source of erythropoietically active material.

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