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Erythropoietic Stimulating Effects of Plasma Extracts from Anemic Human Subjects. (22738)

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It is now a well documented finding that various types of anoxia evoke the production, in laboratory animals, of circulatory factors capable of stimulating erythropoiesis(1-11). These observations raise the question as to whether such activity is also demonstrable in the blood of patients with various forms of anemia. Few studies have attempted to assess the erythropoietic (EP) properties of body fluids of human subjects. Some of the experiments have been performed on umbilical cord blood of normal newborn babies and have indicated EP activity in such material when tested in rodents and man(12-16). EP activity is also stated to be present in the serum of a man sojourning at altitude for 8 days (12), in the plasma of patients with chronic heart disease(1), in human subjects with anemia following hemorrhage(17) and in infants with erythroblastosis fetalis(16). Plasma from subjects with congenital spherocytosis or pernicious anemia in reticulocyte crisis was ineffective(18). These studies with human material fail to evaluate the response of the blood-forming organs in the test animals. The criteria used by the investigators, namely red cell (RBC) counts and/or reticulocyte response, cannot always be depended upon as reflecting the degree of EP stimulation(19,20).

The present studies are concerned with the effects exerted by extracts of plasma and one of urine from patients with certain types of anemia. Since previous experiments(5-8,10) indicate that more of the EP factor is recoverable in hemolytic than in other types of experimentally induced anemias, possibly because the hemolytic varieties are usually more severe, it was felt that an examination of the effects of body fluids secured from patients with Cooley's and sickle cell anemia might be rewarding.

Materials and methods. Plasma was obtained from 7 authenticated cases of Cooley's anemia (6 children and 1 adult) and 2 patients with sickle cell anemia. One case of chronic hypoplastic anemia in a child is also included. Four normal subjects served as donors for control plasma. A 24 hour sample of urine from one of the Cooley's subjects was also tested. In Table I are indicated the hemoglobin concentration, reticulocyte percentage and nucleated RBC numbers in the peripheral blood of each of these individuals at the time of blood withdrawal.

Because of the effectiveness of an acidification-boiling procedure in revealing the EP factor in the blood and urine of anoxic rodents (5-7,10,11,21), it was decided to utilize this extraction technic for the plasmas of the anemic patients. Blood collected from each of the patients was heparinized (10 mg/50 ml blood). The plasmas were acidified to pH 5.5 with lN HCl and boiled for 10 minutes. Following filtration, the coagulated residues were discarded and the filtrates neu-

TABLE I. Effects of Plasma from Anemic Patients upon Erythropoiesis in the Intact Rat.

Age, yr		Peripheral blood values of patient: Hb (g %)	Retics (%)	Nuc. RBC (per 100 WBC)		Effects in recipient rats (means ± S.E.): RBC (mill/mm³)	Hb (g %)	Hemat. (%)	Retics (%)	Marrow nuc. RBC (%)
I. Cooley's anemia										
5	♂	5.5	2.1	4	B*	8.82 ± .50	17.57 ± .87	46.6 ± 2.7	1.1 ± .30	
					A*	9.77 ± .74	18.03 ± .98	54.4 ± 2.0	6.7 ± .55	46.8 ± .2
10	♂	7.5	.6	3	B	8.90 ± .53	16.51 ± .15	47.5 ± .50	1.2 ± .35	
					A	10.30 ± .17	17.40 ± .21	49.0 ± 1.0	1.4 ± .40	45.5 ± 7.5
5	♀	5.0	1.1	0	B	8.05 ± .90	16.56 ± .90	47.7 ± .90	2.2 ± .30	
					A	10.24 ± .21	19.26 ± .43	59.0 ± 1.1	11.3 ±1.40	51.5 ± 3.5
5	♀	7.0	2.5	4	B	8.11 ± .57	17.75 ± .28	49.6 ± .40	1.8 ± .50	
					A	9.17 ± .23	18.52 ± .35	52.5 ± 2.50	1.9 ± .40	32.5 ±17.5
4	♀	6.0	3.5	13	B	8.41 ± .90	18.51 ± .35	52.8 ± 2.30	1.5 ± .60	
					A	9.85 ± .25	18.94 ± .08	60.5 ± 2.53	5.2 ± .70	44.5 ± 2.5
12	♂	8.0	7.2	240	B	8.79 ± .24	18.45 ± .56	51.1 ± .10	2.1 ± .50	
					A	9.06 ± .68	18.93 ± .76	57.2 ± 1.80	3.0 ±1.10	45.5 ± 2.5
32	♀	9.5	6.0	1	B	8.42 ± .30	17.56 ± .65	49.6 ± 1.90	2.5 ± .19	
					A	9.51 ± .22	18.80 ± .31	52.5 ± 1.10	4.7 ± .64	43.6 ± 1.2
II. Sickle cell anemia										
11	♂	8.0	6.4	0	B				1.8 ± .26	
					A				5.6 ± .56	52.3 ± 1.2
15	♂	7.5	1.0	2	B	8.94 ± .28	16.36 ± .15	49.0 ± 2.01	1.9 ± .38	
					A	9.13 ± .34	17.38 ± .47	51.6 ± 2.06	2.4 ± .06	28.0 ± 2.7
III. Hypoplastic anemia										
7	♂	5.0	0	0	B	9.34 ± .22	18.22 ± .80	51.5 ± 1.34	2.2 ± .15	
					A	9.54 ± .47	18.86 ± .24	52.3 ± .93	3.8 ± .59	34.3 ± 4.1
IV. Controls										
12	♂	12.5	1.0	0	B	8.44 ± .70	17.15 ±1.52	47.5 ± 1.11	2.6 ± .12	
					A	8.36 ± .36	15.93 ± .14	43.5 ± .95	3.6 ±1.23	32.0 ± 1.0
12	♂	13.0	.4	0	B	8.61 ± .29	16.76 ± .24	50.6 ± 1.36	1.7 ± .62	
					A	8.77 ± .54	16.78 ± .02	49.8 ± 1.24	1.2 ± .03	27.5 ± 3.5
5	♂	12.0	.2	0	B	9.04 ± .09	18.27 ± .39	49.6 ± 2.17	2.0 ± .85	
					A	8.40 ± .28	17.26 ± .90	49.1 ± 1.19	1.8 ± .13	27.0 ± 3.0
6	♂	10.0	.4	0	B	8.75 ± .16	18.20 ± .15	48.8 ± .36	1.9 ± .21	
					A	8.65 ± .04	16.70 ± .34	46.6 ± .65	1.4 ± .15	33.5 ± 1.5
V. Cooley's anemia Urine extract										
(See above)		5.5	2.1	4	B				1.5 ± .23	
					A				3.6 ± .61	45.2 ± 1.1

* B—Before treatment; A—After 5 injections.

tralized with lN NaOH. In some cases saline was added during or after the boiling procedure in order to achieve a volume close to that of the original plasma. A similar acidification and boiling technique was used for the specimen of urine obtained from one of the patients with Cooley's anemia.

In view of the relatively small amounts of blood available, and to avoid the obviously inadequate procedure of pooling the plasmas of the different patients, no more than 2 to 3 recipient rats (young adult females of a modified Long-Evans strain) could be used in assessing EP activity in the plasma extract of each subject. Each rat received subcutaneous injections, daily for 5 days, of 1.8 to 3.0 ml of filtrate, equivalent in all cases to approximately 3.0 ml of original plasma. Five rats were employed to test the EP properties of the extract prepared from the urine of one of the Cooley's patients. Each rat received daily subcutaneous injections for 5 days of 1.5 ml of the urine extract, equivalent to 3.0 ml of the original urine. All rats were killed on the 6th day for erythrocytic studies of the peripheral blood and bone marrow as described

previously(7,22).

Results. It will be observed from Table I that in all cases, increases in the RBC count, hemoglobin and hematocrit values occurred in the rats receiving the plasma filtrates of patients with Cooley's anemia. Marked reticulocytosis was evident in Cases 1, 3 and 5, and strong stimulation of marrow erythropoiesis was noted with all extracts except that obtained from Case 4. With the latter extract, one marrow possessed 50% but the other only 15% nucleated RBC. Previous studies(11) have shown that marrows from 40 untreated rats of the same strain and sex possess 35.1 $\pm$ 1.9% nucleated RBC. A similar degree of EP stimulating activity, as judged by the reticulocyte and marrow response (only parameters examined), was revealed in the extract of plasma from one of the 2 cases (Case 1) of sickle cell anemia. The plasma extract from the second patient proved ineffective. None of the extracts obtained from the control donor subjects stimulated erythropoiesis in the recipient rats.

The extract of urine obtained from a child with Cooley's anemia (Case 1) also stimulated erythropoiesis, as noted from the significant reticulocytosis and the increased percentages of nucleated RBC in the marrows of the recipient rats (Table I). Unfortunately, in this case, the other peripheral erythrocytic parameters of the test rats were not ascertained.

The case of chronic hypoplastic anemia examined was a boy possessing this condition since birth. His RBC count, at the time of obtaining the peripheral blood sample was 1.8 mill/mm^3 and sternal biopsy indicated only 2% nucleated RBC within his bone marrow. Despite the intensity of anemia in this subject, no EP activity in the plasma extract was demonstrable in recipient rats (Table I).

Discussion. It seems clear that considerable EP stimulating activity exists in the blood of patients with Cooley's anemia. Activity was also present in the plasma of one of 2 subjects with sickle cell anemia. The EP factor detected here is heat stable and can be recovered in boiled filtrates of the plasma and urine. In these respects, it resembles the material present in the plasma of rabbits and rats subjected to hemolytic or hemorrhagic anemia(5,6,10,21). The activity of human circulating "erythropoietin" as seen in some of the patients (*e.g.* Cooley case 3), is actually greater than that detectable in any of the bled or phenylhydrazine-treated animals examined by us up to date, considering that the material of human origin was administered for only 5 days. It would thus seem that either human "erythropoietin" is somewhat different in chemical structure from the animal type or, what is more likely, it is the same substance but exists in larger concentration in the blood of the anemic patients studied. It was of interest that the extract of urine from one of the patients with Cooley's anemia possessed EP properties. Considering that the urine was concentrated to no more than 50% of its original volume, the reticulocyte and marrow response in the recipient rats represent a considerable degree of EP activity. Others(21) have detected EP activity in extracts of urine from rabbits subjected to bleedings. It is also apparent from the data that no correlation can be drawn between severity of the anemia in the patient at the time of blood withdrawal and intensity of the EP response to the plasma extracts in the recipient rats. Nor can it be related to the duration of the condition in the patient. Evidently the quantity of "erythropoietin" circulating in the blood is a variable one, dependent undoubtedly not only on the rate of production of the factor but also upon its rate of utilization or destruction. Any of these processes could conceivably change from time to time.

The results indicate that erythromicrocytosis, which is sometimes characteristic of the response to the plasma EP factor(10,11) was not apparent in the rats receiving the plasma extracts from the Cooley cases 1, 3, 5 and 6 (*i.e.* the percentage increase in the hematocrit values matched the percentage rise in RBC numbers). In most instances, hemoglobin production was not stimulated to the same extent as RBC proliferation, thus resulting in a lowered corpuscular hemoglobin content.

Since the blood of the subjects with Cooley's anemia displays strong EP stimulating

qualities and their marrows reveal marked erythroid hyperplasia, it would seem unlikely that a contributory factor to this condition is a reduction in the production or utilization of the EP factor. Undoubtedly, the more basic etiology centers about the abnormal hemoglobin and hemolytic mechanisms characteristic of these disease states(23-25). A significant finding was the absence of EP activity in the plasma extract of the patient with severe chronic hypoplastic anemia. It is possible that there is an impaired capacity of such subjects to elaborate the EP principle. Should this prove to be the case in more detailed studies of hypoplastic anemia, the administration to these subjects of purified "erythropoietin," preferably that obtained from human sources, would constitute a justifiable therapeutic measure.

Summary. Boiled filtrates of acidified plasma obtained from 7 patients with Cooley's anemia and one of 2 patients with sickle cell anemia stimulated erythropoiesis in intact rats. A similarly prepared extract of urine from one of the Cooley's subjects was also active. Plasma filtrates from normal subjects and one patient with chronic hypoplastic anemia were inactive. The implications of these findings are discussed.

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