regard to note that nystatin activity is reduced by cysteine and that cysteine reactivates triosephosphate dehydrogenase which has been inactivated by oxidation. These latter observations may have a causal relationship.

The manometric studies reported here have been concerned only with fermentation, in spite of the fact that it has been previously shown that respiratory reactions are also affected. Therefore the effects of nystatin reported herein are but one of several possible manifestations. It is premature on the basis of these data to assert that the primary mode of action of nystatin concerns oxidative phosphorylation. Certainly this is an important site and the correlation between manometric findings and growth studies constitutes circumstantial evidence that this may be the case.

Summary. Glucose fermentation by *Candida stellatoidea* was inhibited by sodium arsenate and by the fungistatic antibiotic, nystatin. Mutants selected for resistance to one inhibitor simultaneously developed increased resistance to the other inhibitor. The inhibitory effects of arsenate and nystatin on glucose fermentation by the sensitive parental strain were reversed by appropriate concentrations of phosphate.

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Studies on Erythropoiesis IX. Mechanism of Decreased Erythropoiesis In Experimental Polycythemia. (24187)

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That increased erythropoiesis following production of acute anemia is mediated on a hormonal basis was first suggested by Carnot and Deflandre(1). In recent years, numerous investigators have demonstrated the appearance of a humoral erythropoietic-stimulating factor in anemic animals(2-9). Fried, Plzak, Jacobson, and Goldwasser(10) have presented evidence supporting the hypothesis that the rate of erythropoiesis is determined by the amount of erythropoietin in plasma at all times. It has long been known that experimentally-induced polycythemia depresses erythropoiesis(11-15). That decreased concentrations of erythropoietin are responsible for depression of erythropoiesis in transfusion-induced polycythemia was first suggested by Fried *et al.*(10) and Jacobson *et al.*(16). Demonstration of decreased concentrations of erythropoietin in plasma of transfusion-induced plethoric animals and human beings is as important in establishing validity of the concept of a humoral control of erythropoiesis as is demonstration of increased erythropoietin concentrations in plasma obtained from anemic donors. In this paper, the decline in reticulocyte count and incorporation of Fe^{59} into newly-formed red cells of polycythemic rats, and degree of decline in relation to severity of polycythemia are reported. In addition, response of these polycythemic animals to anemic plasma and cobaltous chloride is described.

Methods and materials. Rats of the Sprague-Dawley strain were used in this study. Animals in last experiment were hypophysectomized by Hormone Assay Laboratories of Chicago. Blood was removed from donor animals by cardiac puncture, using heparin as anticoagulant. Intravenous trans-

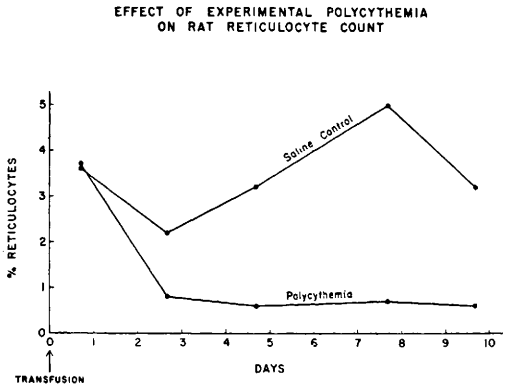

FIG. 1. Effect of experimentally-induced polycythemia on No. of reticulocytes in the rat.

fusions consisted of whole blood or 50% red cells resuspended in saline. Intraperitoneal injections consisted of 80% red cells suspended in saline. In the last 3 experiments, periodic hemoglobin and reticulocyte counts were determined on blood obtained from tail vein. In all other studies, blood was obtained by cardiac aspiration at time of sacrifice. Hemoglobin values were determined by measurement of oxyhemoglobin as described by Evelyn(17). Reticulocytes were counted by direct smear method, using brilliant cresyl blue stain without counter-stain. Rate of erythropoiesis was then determined as follows(18): Fe^{59} citrate (1 to 2 μc) was injected into tail vein of animals, and 16 hours thereafter 1 ml of blood was obtained by cardiac puncture. Standards were prepared at time the isotope was administered to animals. Activity of standards and the blood was determined by counting in a well-type scintillation counter. The amount of radioactivity in the entire circulating red cell mass was calculated by multiplying the activity per ml of blood by the blood volume and was expressed as per cent of injected dose of Fe^{59} in peripheral red cells. Blood volumes of rats with varying degrees of polycythemia were determined in preliminary experiments by the method of Berlin *et al.*(19). Plasma referred to as "anemic" rat plasma was obtained from rats whose hematocrit had been reduced to 25% by 3 daily cardiac aspirations. Human plasma was obtained from normal donor and a donor with idiopathic aplastic anemia, using heparin as anticoagulant. Extracts of human plasma were prepared by the method of Borsook *et al.*(20).

Results. I. Decrease of reticulocyte count and red cell incorporation of Fe^{59} following intraperitoneal injection of blood. Rats weighing 250-280 g were made polycythemic by single intraperitoneal injection of packed cells equal to 4% body weight, and control animals were given the same volume of normal saline. Reticulocyte counts and red cell Fe^{59} incorporation were determined in separate groups of 5 animals at the times indicated in Fig. 1 and 2. No change in reticulocyte count was noted 17 hours after administration of blood, but at 2 days and thereafter a marked reduction in number of reticulocytes was noted (Fig. 1). Similarly, incorporation of Fe^{59} was reduced at 4 days and fell steadily, reaching its low value of 5% when the tracer was given 9 days after the animals were made polycythemic (Fig. 2).

II. Effect of varying degrees of polycythemia on number of reticulocytes and incorporation of Fe^{59}. Rats weighing 150 to 200 g were given intraperitoneal injections as indicated in Table I. Fe^{59} was administered 8 days later, and the following morning incorporation of iron and number of reticulocytes was obtained. Only a modest rise in hemoglobin was obtained following administration

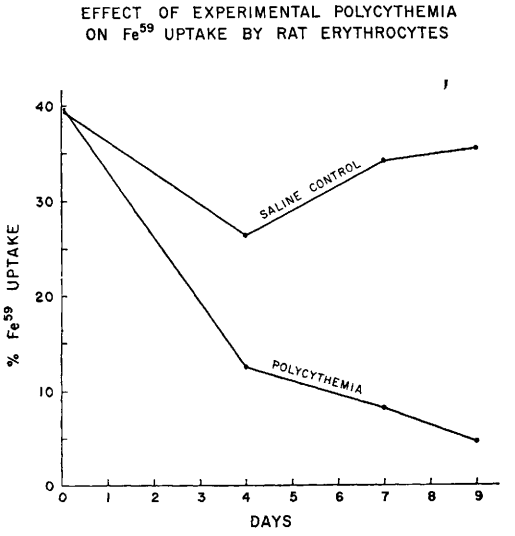

FIG. 2. Effect of experimentally-induced polycythemia on incorporation of Fe^{59} into newly-formed red blood cells in the rat.

TABLE I. Depression of Erythropoiesis following Experimental Polycythemia.

Vol inj.*	Packed cells 2%	Saline 2%	Packed cells 4%	Saline 4%	Packed cells 8%	Saline 8%
No. of animals	5	5	5	4	5	4
Hemoglobin	15.6	14.6	16.7	13.6	21.2	14.8
Reticulocyte (%)	1.1	5.6	.2	6.3	.1	5.1
Fe^{59} uptake (%)	19	48	8	48	6	48

* Vol inj. in ml is numerically equal to 2, 4, or 8% of animals' body wt in g.

of smallest amount of blood, but a definite reduction in number of reticulocytes and incorporation of Fe^{59} was obtained (Table I). It appears that packed cells in 4% of animal's body weight, elevating the hemoglobin to only 16.7 g %, are almost as efficacious in suppressing reticulocyte production and red cell Fe^{59} incorporation as twice this volume of packed cells, an amount producing a far more dramatic elevation of hemoglobin.

III. Effect of rat plasma and heat-denatured extract of human plasma on incorporation of Fe^{59} into erythrocytes of the polycythemic rat. Rats weighing 75 to 125 g were given packed red cells, in milliliters equal to 4% of body weight in g. On 11th and 12th days after infusion, these animals received 2 ml of plasma or plasma extract given subcutaneously (Fig. 3). Radioiron uptake by the erythrocytes was then determined, the tracer dose being administered on the 13th day after establishment of polycythemia. Approximately 6-fold increases in Fe^{59} incorporation resulted from administration of anemic rat plasma or the heat-denatured extract of plasma from an anemic human patient.

IV. Effect of cobalt on number of reticulocytes and uptake of Fe^{59} in polycythemic rat. Rats weighing 140 to 170 g were made polycythemic by intraperitoneal injection of packed cells in the volume in ml equal to 4% of their body weight in g. On the third day after injection, the animals were divided into 2 groups by random selection. One group received cobaltous chloride subcutaneously and the other group received normal saline. On the 12th day, Fe^{59} was administered and its incorporation was determined 16 hours later. Reticulocyte and hemoglobin values are recorded in Table II. Although the animals were fortuitously divided in such a manner that the saline control group on all occasions had slightly lower average hemoglobin values than the animals receiving cobalt, reticulocyte averages were higher in the animals receiving cobalt. This difference became more striking with the passage of time. Further, incorporation of Fe^{59} into newly-formed red cells in polycythemic animals was increased from an average value of 5% in the rats receiving saline to an average value of 32% in the rats receiving cobalt.

V. Effect of multiple transfusions on reticulocyte count in the polycythemic rat. Four rats weighing 150 to 185 g were made and maintained polycythemic for 48 days by multiple transfusions in an effort to obtain com-

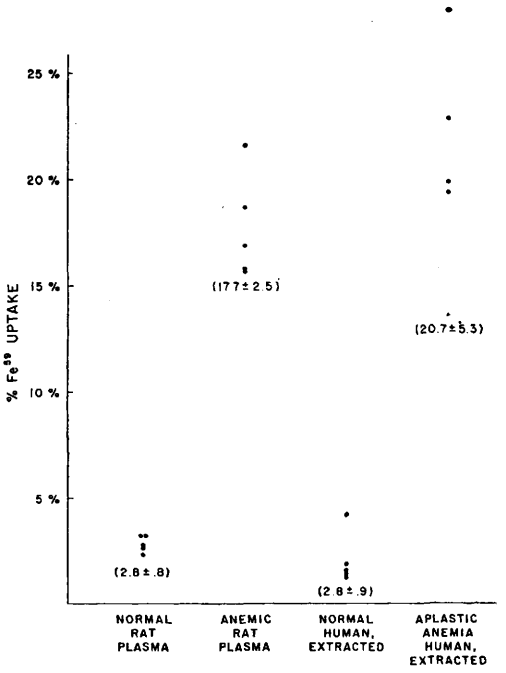

FIG. 3. Effect of normal and anemic rat plasma and normal and anemic human plasma (heat-denatured extract) on incorporation of Fe^{59} into newly-formed red blood cells in polycythemic rats. Numbers enclosed in parentheses are avg values ± one stand. dev.

TABLE II. Reticulocyte Response following Co^{++} in the Polycythemic Rat.

	Control (5 rats)			Cobalt (4 rats)		
Day	Hb	Retic.	Injection	Hb	Retic.	Injection
3			Saline, 1 cc/day			Cobalt, 5 μM/day
6	17.8	.3		18.9	.7	
8	17.9	.3		18.5	1.6	
10	17.1	.1	Saline, 1 cc B.I.D.	18.0	1.7	Cobalt, 5 μM B.I.D.
12	17.2	.3		18.2	2.5	

plete suppression of erythropoiesis as indicated by a complete disappearance of reticulocytes from the peripheral blood. At least 4 days elapsed after each transfusion before counts were repeated in order to permit maturation of transfused reticulocytes. A 50% suspension of red cells in saline was used after the first week in an effort to eliminate stimulation of erythropoiesis by erythropoietin present in normal plasma. Every reticulocyte value given in Table III was determined by examining 10,000 cells after the polycythemia was established. It was not possible to rid the rats completely of reticulocytes, although while the hemoglobin was maintained above 20 g. it frequently was necessary to count 10,000 red cells on a smear to find a single reticulocyte (Table III).

VI. Effect of polycythemia on reticulocyte count of hypophysectomized rat. Six days after hypophysectomy, the reticulocyte count of 9-week-old 120-140 g rats averaged .06% and average hemoglobin was 18.9 g %. None of 7 rats was completely free of reticulocytes, however. Two daily intravenous injections of blood, totaling 14 ml per rat, were given and 8 days later the average hemoglobin was 23.4 g %. The average reticulocyte count was .006%, and in 4 of the 7 rats, the count was 0.00%, no reticulocytes being seen in 10,000 cells.

Discussion. The present investigation has been directed toward the accumulation of evidence that decreased erythrocyte formation in transfusion-induced polycythemia is a result of lowered erythropoietin titers. In this regard, it is of interest to note that the high titer of erythropoietin associated with some anemias in human beings declines when, as a consequence of transfusions, the hemoglobin increases(21,22).

Slight increases in circulating levels of hemoglobin reduce but do not completely inhibit erythrocyte production since reticulocyte production and iron uptake were reduced but not completely inhibited 8 days after transfusion. This reduction was greater when associated with increased plethora, but it is of interest to note that the reduction in erythropoiesis, as measured by the number of reticulocytes, appeared to be of about the same magnitude when the hemoglobin was 16.7 g % as it was when the value was 21.2 g %. Since none of the animals in the latter group was free of reticulocytes, we then attempted to eliminate the reticulocyte count in the rat with multiple transfusions, as has been accomplished in mice(16). This reticulocyte count was easily depressed to 0.00% in several hypophysectomized rats made polycythemic. Although marked depression of the reticulocyte count was accomplished in the normal rat made polycythemic by transfusion, in no instance was a complete absence of reticulocytes found. It appears likely to us that the poly-

TABLE III. Reticulocyte Suppression in Prolonged Transfusion-Induced Polycythemia.

	1		2		3		4	
Day	Hb	Retic., %	Hb	Retic., %	Hb	Retic., %	Hb	Retic., %
0	11.6	5.7	9.6	6.4	10.4	5.2	12.3	6.9
15	21.6		21.6	.03	20.9	.12	21.4	.08
25	22.7	.03	24.0	.02	22.8	.03	23.4	.10
29	21.4	.02	24.8	.01	22.7	.02	20.8	.01
48	21.2	.02	19.4	.04	19.4	.04	21.4	.03

Transfusions given on days 0, 4, 7, 11, 18, 19, 20, 29, 40, 41.

cythemic rats do not deliver enough oxygen to the site of production of erythropoietin to completely suppress its formation, but after hypophysectomy the metabolic rates and oxygen requirements are depressed, hence in the polycythemic hypophysectomized rat the additional oxygen made available after transfusion is sufficient to accomplish this purpose.

The increased uptake of Fe^{59} in the polycythemic rats following 2 injections of anemic, but not normal, rat plasma suggests that reduction of erythropoiesis in the plethoric rat is mediated through reduction of erythropoietin titers. This concept is fortified by the finding that the extract of plasma obtained from an anemic human patient following removal of the bulk of the protein by heat-denaturation also increases the incorporation of iron in plethoric rats, while a similarly prepared extract of normal human plasma fails to do so. Further evidence for this concept is found in the cobalt-induced reversal of the depression of erythropoiesis in the polycythemic rat, since it has been demonstrated by Goldwasser and his co-workers(23) that cobalt-induced polycythemia appears to be a consequence of increased erythropoietin production.

Summary. 1) Reduction of erythropoiesis in rats rendered polycythemic by intraperitoneal infusion of packed red cells bears a relation to the degree of plethora produced. Plasma from anemic rats, a heat-denatured plasma extract from a patient with aplastic anemia, and subcutaneous cobaltous chloride all stimulate erythropoiesis in the polycythemic rat. These observations are consistent with the theory that decreased erythropoiesis in the polycythemic rat is the immediate consequence of a decreased titer of erythropoietin in these animals, and constitute further support for the theory of the humoral regulation of erythropoiesis. 2) In these experiments it was not possible to suppress erythropoiesis completely, as measured by the reticulocyte count, in normal rat by transfusion, although complete suppression was readily accomplished in transfused hypophysectomized rat.

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