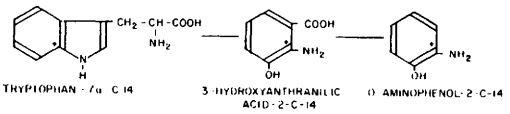

FIG. 1

of 3-hydroxyanthranilic acid, formed in the metabolism of tryptophan (Fig. 1).

Additional evidence for this pathway has been obtained in this laboratory in studies of the metabolism of some impure 3-hydroxyanthranilic acid-1-C^{14} in mammary tumor mice. The urines of such mice receiving impure 3-hydroxyanthranilic acid-1-C^{14} has been shown to contain approximately 0.15% of the administered dose in the form of *o*-aminophenol.

Summary. The metabolic fate of DL-Tryptophan-7a-C^{14} in a human with multiple myeloma was studied. Only 5% of the administered C^{14} was excreted as respired $C^{14}O_2$ and 14% in the urine in the first 24 hours. *O*-aminophenol-2-C^{14} was isolated from the urine.

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Response to Erythropoietin.* (27537)

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Alpen and Cranmore(1) and Erslev(2) have concluded that erythropoietin increases red cell output by accelerating the rate of stem cell differentiation into erythroid precursors. Observations on erythropoiesis in polycythemic mice by Gurney, *et al.*(3) have supported this conclusion. However, observations by several investigators(4-7) seem to indicate that the action of the erythropoietic humoral factor(s) is not limited to regulation of stem cell differentiation. Experiments reported here tested the effect of erythropoietin on later phases of erythroid formation and the results suggest an action here as well as in the early stem cell phase.

Methods. Female Sprague-Dawley rats, weighing 180 to 200 g, were made polycythemic by one or several transfusions of packed homologous red blood cells. Each transfusion equaled 50% of the animal's original red cell mass. At various intervals following the initial transfusion the polycythemic rats were given a single injection of human urine erythropoietin(8) or purified sheep erythropoietin (9).‡ Subsequent changes in erythropoiesis were assessed by reticulocyte counts(10) and measurement of the 18-hour erythrocyte radio-iron incorporation(8). Erythroid elements per 1000 nucleated cells were enumerated in smears of marrow from control polycythemic animals.

In 3 experiments, polycythemic rats were given single intraperitoneal injections of 2 ml of urine concentrate on either the 5th, 15th or 31st day after their first transfusion. Con-

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TABLE I. Red Cell Radio-Iron Incorporation, Reticulocyte Count and Hematocrit of Normal and Polycythemic Rats.

Type	Days after transfusion	No. of rats	18-hr red cell Fe^{59} incorporation	Reticulocyte count	Packed cell vol
			%		
Normal	—	25	19.6 ± 5.33†	2.2 ±1.33†	44.4 ± 2.06†
Transfused	1	10	14.5 ± 4.32	.84 ± .33	54.4 ± 3.13
	2	14	14.3 ± 4.34	1.03 ± .51	55.8 ± 2.12
	3	19	11.1 ± 3.27	.49 ± .30	56.3 ± 2.37
	4	12	5.5 ± 1.10	.21 ± .28	52.9 ± 2.96
	5	12	3.3 ± .76	.12 ± .12	54.0 ± 2.52
	5–	4*	2.4 ± .50	.13 ± .16	63.5 ± 2.22
	6–8	11	3.7 ± .59	.13 ± .10	55.1 ± 3.50
	6–9	12*	2.8 ± .60	.09 ± .09	59.1 ± 2.48
	10–12	12*	3.9 ± 1.50	.03 ± .05	56.4 ± 1.86
	14–19	10*	3.3 ± .13	.07 ± .08	55.6 ± 1.97

* Rats transfused on 1st and 4th day. † Stand. dev. of mean.

trol polycythemic rats were injected with saline. At the end of each successive 24-hour period following erythropoietin or saline injection, representative animals from each group were killed and the reticulocyte counts, hematocrits and erythrocyte radio-iron incorporation were determined. The radio-iron was injected 18 hours before the rats were killed. All groups given urine concentrate included at least 5 rats.

Subsequently, rats were given a single transfusion and in contrast to the foregoing experiments, the time between production of polycythemia and erythropoietin injection was shortened to one day. On the day following transfusion, control polycythemic rats were injected intraperitoneally with saline while the test animals received either 2 ml of urine concentrate or 18 units(11) of sheep erythropoietin. All groups given erythropoietin included at least 5 rats and serial assessment of erythropoiesis was undertaken as described above.

Results. Production of polycythemia resulted in diminished red cell radio-iron incorporation and reticulocyte counts (Table I) although absolute reticulocytopenia did not occur. The percentage of marrow erythroid precursors likewise decreased (Table II) but erythroid cells did not entirely disappear from the marrow. Injection of erythropoietin produced a temporary increase in erythropoiesis in all the polycythemic rats.

The response of transfused rats to erythropoietin is shown in the figures. When urine concentrate was administered 15 or 31 days after the production of polycythemia (Fig. 1 and 2) no increases in either reticulocyte counts or erythrocyte radio-iron incorporation were noted during the first 24 hours after erythropoietin injection. Maximum increases in iron incorporation and reticulocyte levels did not occur until 48 hours or more after injection; both had returned to control levels by 144 hours.

Urine concentrate injected on the 5th day after production of polycythemia (Fig. 3) produced a somewhat different effect in that iron incorporation and reticulocytes appeared to be increased slightly 24 hours after erythropoietin injection. Further shortening of the interval between transfusion and injection of urine concentrate or purified sheep erythropoietin (Fig. 4 and 5) resulted in definite in-

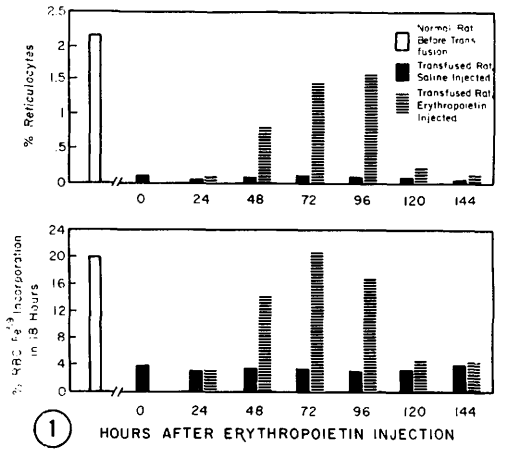
TABLE II. Erythroid Cells in Marrow of Normal and Polycythemic Rats.

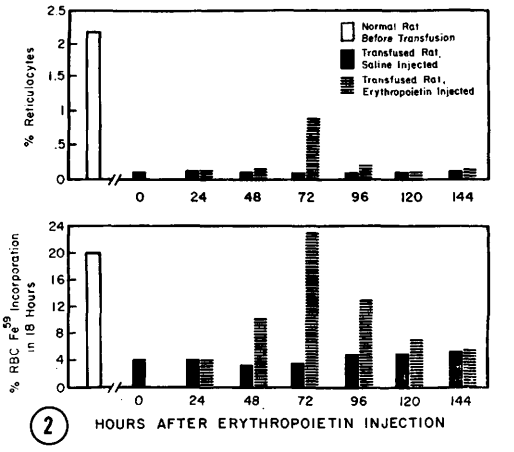
Type	Day after transfusion	No. of rats	% erythroid marrow cells	Comment
Normal	—	16	15.0 ± 1.10†	Erythroblasts and normoblasts present
Transfused	1	9	9.1 ± 3.89	*Idem*
	5	11	5.6 ± 1.86	Occasional erythroblast
	10*	10	3.2 ± 1.56	Normoblasts only

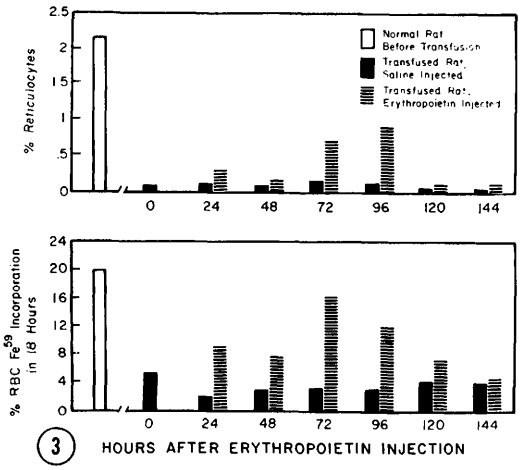
* Rate transfused on 1st and 4th day. † Stand. dev. of mean.

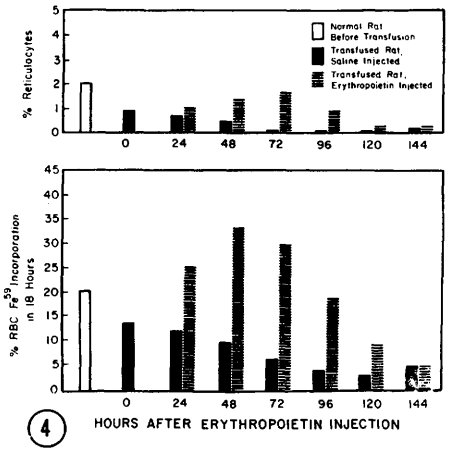
creases in both reticulocyte counts and red cell radio-iron incorporation 24 hours after erythropoietin injection.

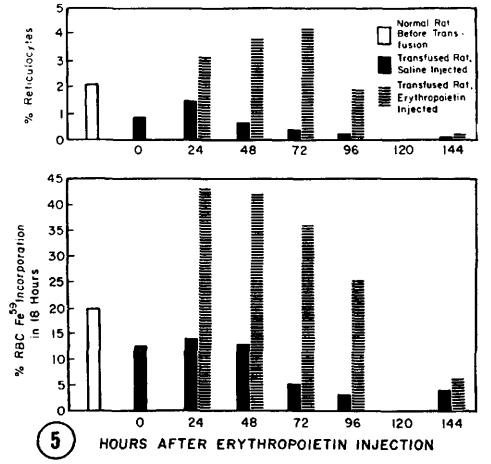
Discussion. Jacobson, *et al.*(12) demon-











Each bar represents mean value for 5 or more animals in test group or 3 or more saline inj. control rats.

FIG. 1. Response of polycythemic rats to human urinary erythropoietin. These rats were transfused once weekly and the polycythemic state maintained for 14 days prior to inj. of 2 ml of urine concentrate on 15th day.

FIG. 2. Response of polycythemic rats to human urinary erythropoietin. These rats were transfused once weekly and the polycythemic state maintained for 30 days prior to inj. of 2 ml of urine concentrate on 31st day.

FIG. 3. Response of polycythemic rats to human urinary erythropoietin. These rats were transfused twice during a 4-day period. Two ml of urine concentrate were inj. on 5th day.

FIG. 4. Response of polycythemic rats to human urinary erythropoietin. These rats were given a single transfusion and on the following day 2 ml of urine concentrate were inj.

FIG. 5. Response of polycythemic rats to purified sheep erythropoietin. These rats were given a single transfusion and on the following day 18 units of erythropoietin were inj.

strated a reduction in erythropoiesis and an enhanced responsiveness to exogenous erythropoietin in animals made polycythemic by hypertransfusion. They postulated that these changes resulted from a decreased elaboration of endogenous erythropoietin. In the present experiments production of polycythemia in rats was followed by a decreased rate of red cell erythrocyte radio-iron incorporation and percentage of reticulocytes in the peripheral blood (Table I) of the hypertransfused animals. This diminution in erythropoiesis persisted while the polycythemic state was maintained.

Injection of erythropoietin produced a temporary increase in erythropoiesis which terminated in all experiments by 120 to 144 hours, when reticulocyte counts and erythrocyte radio-iron incorporation were the same in the test and saline injected control animals. However, the changes in the peripheral blood indicating enhanced erythropoiesis differed in time of onset in the various groups. This difference appeared to be related to the interval between production of polycythemia and injection of erythropoietin.

When polycythemia had been sustained for 2 or more weeks (Fig. 1 and 2) increases in radio-iron incorporation and reticulocytes resulting from stimulation by erythropoietin did not occur until at least 24 hours had elapsed following injection. The marrows of these rats appeared to be devoid of erythroblasts (Table II) at the time erythropoietin was injected. Thus this delayed change in erythropoiesis suggested that the new erythroid cells which entered the peripheral blood may have resulted from the stem cell effect of erythropoietin as proposed by Erslev(2), and by Alpen and Cranmore(1).

Response to human urinary erythropoietin administered on the fifth day (Fig. 3) after production of polycythemia did not differ markedly from that following erythropoietin injection either on the 15th (Fig. 1) or 31st (Fig. 2) post-transfusion day. Nevertheless, in the animals that had been polycythemic for the shorter period, slight increases in reticulocytes and iron incorporation were noted during the first 24 hours after stimulation. This early increase in erythropoiesis, although not striking, suggested a possible extra stem cell effect of erythropoietin which required appraisal.

If this limited early increase in erythropoiesis had been related to the shorter duration of polycythemia which obtained prior to stimulation, then this early effect should have been potentiated by further decreasing the interval between production of polycythemia and stimulation with erythropoietin. This conjecture was substantiated by the increase in reticulocytes and radio-iron incorporation seen within the first 24 hours in rats that were polycythemic for only one day prior to injection of erythropoietin (Fig. 4, 5).

The early augmentation of erythropoiesis during the first 24 hours after erythropoietin injection does not appear to be directly related to its action on stem cells. As shown in the first 2 experiments in this series and by Gurney, *et al.*(3) in their studies in polycythemic mice, peripheral blood changes resulting from a stem cell action by erythropoietin do not occur until at least 24 or more hours after injection. The nature of the earlier response to erythropoietin is not clear but presumably is related to some effect on the later phases of erythroid cell formation. Gordon, *et al.*,(6) recently reported that purified sheep erythropoietin effected a prompt release of reticulocytes and also normoblasts from the marrow of isolated, perfused rat femur. Such an action presupposes the existence of later marrow erythroid precursors which were shown to be present in the marrow of the acutely transfused rat. Further assessment of the action of erythropoietin is under way.

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The Lymphocytosis Stimulating Factor (L.S.F.) (27538)

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Metcalf(1) has described a factor which can be isolated from the thymus of normal mice and is able to induce lymphocytosis when injected into newborn mice. In leukemic mice, a factor is found in the plasma which has the same effect as the thymic factor. The first has been named the lymphocytosis stimulating factor (L.S.F.); the second, the lymphocytosis stimulating hormone (L.S.H.); but there is no clear evidence that they differ from one another.

The main interest in this problem is the possible function of LSF and LSH in either spontaneous or radiation-induced leukemogenesis. It could be that endogenous leukemia-inducing viruses are able to promote leukemia only if LSF is present in excess. On the other hand, it is likely that injection of a large excess of virus can induce leukemia even in animals in which LSF is limited. In many respects the mechanism of leukemogenesis would therefore be similar to the one admitted for carcinogenesis of the mammary gland.

It seems necessary then to repeat the experiment of Metcalf which seems to deal with the most fundamental aspects of leukemogenesis.

Materials and methods. Extracts were tested by being injected into newborn ICR mice; they belong to the Wistar breeding colony and are not inbred. The C57Bl, DBA/2, BRVR and AkR mice used were from inbred lines. Lewis rats were also used as a source of material. *Tissue culture.* Two strains of cells grown *in vitro* have been used: mouse fibroblast, strain L; and mouse lymphoblast, strain L-5178Y(2). *Preparation of extract.* Extracts were made according to Metcalf's procedure. Organs or cells grown *in vitro* were ground in the cold in a mortar with sterile sand, phosphate buffered saline (PBS) with penicillin added to obtain a 10% suspension (instead of a 5% suspension as used by Metcalf). The suspension was centrifuged in cold for 15 minutes (3000 rpm), the supernatant fluid harvested and stored at -60°C.

Heparinized plasma was prepared from blood obtained by heart or eye puncture. The plasma obtained after centrifugation was injected undiluted.

Testing of extract. Newborn ICR less than 48 hours old were injected intracerebrally with 0.03 ml of extract or plasma. Six days later the tail was cut and the first drop of blood used to make a smear. After staining by May-Grunwald-Giesma the ratio of lymphocyte to polymorphonuclear leucocyte (L/P) was determined. In our first experiments control smears had been obtained from untreated litters of the same age; later we thought it more accurate to split each litter, inject one-half with the extract and keep the other half either untreated or injected with PBS.

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