that the leukemic virus was transmitted through the milk of nursing females; the latter, however, had leukemia at least part of the time they were nursing. It remains to be determined whether healthy, but virus-carrying, females could also transmit the virus in their milk. 5. Under our experimental conditions, foster-nursing by healthy C3H females of offspring born to virus-injected C3H mothers, reduced the incidence, but did not entirely prevent, the development of leukemia.

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Received January 16, 1962. P.S.E.B.M., 1962, v109.

Erythropoietin Assays Using Iron^{59} Incorporation into Blood and Spleen of the Polycythemic Mouse. (27351)

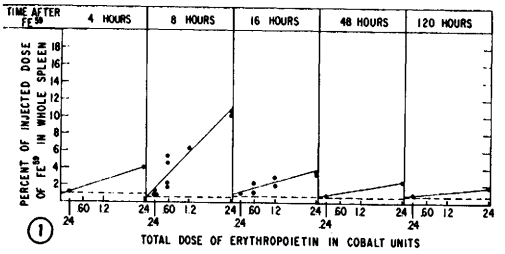
WENDELL F. ROSSE, THOMAS A. WALDMANN AND DELORES E. HOUSTON
(Introduced by N. I. Berlin)
Metabolism Service, National Cancer Institute, Nat. Inst. of Health, Bethesda, Md.

The presence of a specific humoral factor stimulating erythropoiesis (erythropoietin, erythropoiesis stimulating factor, ESF) has been confirmed and measured by a number of bioassay technics(1). These have usually involved suppression of erythropoiesis in the assay animal by hypophysectomy, starvation, dehydration or induced polycythemia(1,2). Transfusion-induced polycythemia has the advantages that it is more physiologic than other means and that suppression can be maintained indefinitely. Jacobson *et al.*(3) have used reticulocyte response in the transfused mouse as a semi-quantitative assay technic. In an effort to find a sensitive assay system for ESF requiring small amounts of test material, we have investigated the erythropoietic response of the transfused polycythemic mouse to injections of ESF-rich serum as measured by the incorporation of iron^{59} into the blood and spleen, a normal hematopoietic organ in the mouse.

Methods. Female NIH strain mice weighing 18-22 g were injected intraperitoneally on Day 1 with 0.7 ml of an 80% suspension of homologous red blood cells which had been collected in acid citrate-dextrose solution and concentrated by centrifugation. On Day 3 they were injected with a further 0.4-0.8 ml of 80% RBC suspension and distributed into groups of 8 animals. Each animal was injected subcutaneously with 0.5 ml doses of test material on Day 3 and Day 4. Saline dilutions of the serum of a patient with aplastic anemia were used as stimulus for dose- and time-response data. This serum was found by comparison with a distributed standard* to contain 12 cobalt units of ESF as defined by Schlueter *et al.*(4) per ml. Animals injected with normal saline were the negative controls.

On day 5, 0.5 μc of Fe^{59} citrate in 0.1 ml of normal saline was injected intraperitone-

* Distributed by Hematology Study Section, Division of Research Grants, Nat. Inst. Health.



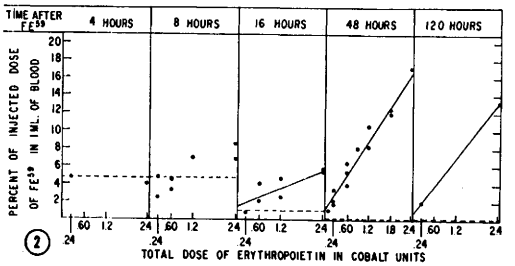


FIG. 1. Response of the polycythemic mouse to graded doses of erythropoietin as measured by incorporation of Fe^{59} into the spleen at intervals after inj. of the isotope. Each point represents the mean of 8 animals. Broken lines (– – –) represent means of unstimulated controls. Solid lines represent dose-response curves calculated by the least squares method.

FIG. 2. Response of the polycythemic mouse to graded doses of erythropoietin as measured by incorporation of Fe^{59} into the peripheral blood at intervals after inj. of the isotope. Each point repsesents the mean of 8 animals. Broken lines (– – –) represent means of unstimulated controls. Solid lines represent dose-response curves calculated by the least squares method.

ally with a microliter syringe. At specific time intervals ranging from 4 to 120 hours, the animals were killed by decapitation and bled into tared bottles which were reweighed to determine the amount of blood. Duplicate microhematocrits were determined for each animal. Spleens were removed, weighed on a torsion balance, and placed in vials for counting. The percent of injected radioactivity present in blood, plasma, and spleens was determined using a well-type scintillation counter with a single channel spectrometer. The data from those animals whose hematocrits were below 52% at time of bleeding were eliminated, since the percent incorporation of Fe^{59} into the peripheral RBC and spleen of saline injected mice was seen to increase markedly when the hematocrit was below this.

The starved rat assay used was a modification of that of Fried *et al.*(5). The rats were starved on day 1 and injected subcutaneously with 2.0 ml of test material on Day 2 and Day 3 respectively. On Day 4, 1 μc of Fe^{59} citrate was injected intraperitoneally, and blood was removed 16 hours later by cardiac puncture. The radioactivity due to Fe^{59} and the percent incorporation into peripheral RBC was calculated using a diluted standard and assuming that total blood volume was 5% of body weight.

Results and discussion. Metabolism of iron^{59}. The time course of incorporation of Fe^{59} into a hematopoietic organ, the spleen, and into the peripheral blood of transfused polycythemic mice which were injected with saline or graded amounts of ESF is shown in Fig. 1 and 2. These represent dose-response curves at intervals after injection of the radioiron. Incorporation of Fe^{59} into either blood or spleen was maximal when 2.4 cobalt units were given.

The Fe^{59} incorporated into the spleens of the saline injected animals remained between 0.7 and 1.2% of the administered doses throughout the studies. There was rapid uptake of Fe^{59} into the spleen of the ESF injected animals reaching a peak of 10.5% in the maximally stimulated animals at 8 hours after administration of the isotope. Radioactivity in the spleen of the ESF-stimulated animals decreased progressively from that time. This decrease was coincident with the appearance of labelled cells in the circulating blood.

Four and 8 hours after injection of Fe^{59}, 4.5% of the administered dose was in 1 ml of peripheral blood of saline injected animals. All of this activity was contained in the serum. After 8 hours, the radioactivity remaining in the blood rapidly fell as the serum was cleared of Fe^{59}. By 48 and 120 hours, only 0.15-0.20% of administered radioactivity was present per ml of blood. The majority of the Fe^{59} was present in the RBC and represents the result of erythropoiesis which continues at a very much reduced rate even when the physiologic stimulus is removed by transfusion(3).

By 8 hours, there was a significantly greater incorporation of Fe^{59} into the circulating red cells of mice given 2.4 cobalt units

TABLE I. Characteristics of Polycythemic Mouse and Starved Rat Assays.

Assay	Hr after inj. of Fe^{59}	Dose-response line: Coefficient of: Correlation	Regression	Minimal detectable dose (in cobalt units)*	Index of precision*
Transfusion polycythemic mouse spleen	4	—	1.28	Between .24 and 2.4	—
	8	.949	4.19	" .24 " .60	.252
	16	.899	1.05	" .24 " .60	.630
	48	—	—	" .24 " 2.4	—
	120	—	—	" .24 " 2.4	—
Transfusion polycythemic mouse blood	4	—	—	None detectable	—
	8	—	—	2.4	—
	16	.840	1.76	Between .24 and .60	.693
	48	.982	6.60	Less than .03	.364
	120	—	5.21	" " .24	.288
Starved rat blood	16	.990	1.18	Between .96 and 2.4	3.96

* See text.

of ESF than of the saline injected animals. The amount of radioactivity in the blood of the stimulated animals progressively increased reaching a maximum at 48 hours after injection of the isotope.

Comparison of assays. The studies of the metabolism of Fe^{59} in the transfused polycythemic mouse suggested that radioiron incorporation into the blood and spleen of these animals might be used in quantitation of ESF in biologic materials. Dose-response measurements in the transfused polycythemic mouse at intervals after injection of the isotope and in the starved rat are characterized in Table I by 4 parameters: 1) the coefficient of correlation of the dose-response line derived by the least squares method considering the means of each group of 8 animals as a single point; 2) the coefficient of regression of the dose-response line (the slope of that line); 3) the minimal detectable dose of ESF, defined as the smallest dose tested which caused incorporation of Fe^{59} in stimulated animals greater than the incorporation of unstimulated controls so that the p value of the standard difference of the means of each group was less than 0.01; 4) the index of precision, defined by Gaddum(6) as the ratio of slope to variance; the lower the value of this index, the more precise the assay.

The incorporation of Fe^{59} into the blood of polycythemic mice killed 4 or 8 hours after injection of the isotope cannot be used in measurement of ESF since an insufficient amount of Fe^{59} is incorporated into peripheral RBC's to permit differentiation of stimulated from unstimulated animals (Fig. 2). Since no greater incorporation of Fe^{59} into the blood in stimulated mice or greater decrease in blood radioactivity in the unstimulated animals was seen at 120 hours as compared to 48 hours, and since the added time necessitated a third injection of blood to maintain sufficient polycythemia, the 120-hour blood assay was not investigated further. The 48-hour blood assay is superior to the 16-hour blood assay since it has much greater sensitivity as judged by the smaller minimal detectable dose and greater coefficient of regression.

Among those assays using the splenic incorporation of Fe^{59} as the measurement of erythropoiesis, those in which the animals are killed 48 and 120 hours after injection of the isotope are insensitive since the amount of radioiron present in the spleen of the stimulated animals has been reduced by release of labelled cells into the peripheral blood. The 4-hour splenic assay is not optimal since insufficient splenic incorporation of Fe^{59} has taken place in the stimulated animals (Fig. 1). The minimal detectable dose of the 8-hour and the 16-hour splenic assays is the same but the coefficient of correlation, coefficient of regression and the index of precision are better for the 8-hour splenic assay as compared to the 16-hour splenic assay.

The 48-hour assay using Fe^{59} incorporation into the blood is for most purposes the best technic for determination of ESF using the polycythemic mouse. It is the most sensitive assay both in terms of minimal detectable

dose and in increase in incorporation of iron per unit of stimulating material. However, in certain instances, the shorter time between injection of the isotope and sacrifice of the animals in the 8-hour splenic assay may be advantageous.

Uptake of iron^{59} into peripheral RBC's in the starved rat is directly proportional to the injected dose of ESF in the range between 2.4 and 48 cobalt units.

The minimal amount of ESF needed to increase significantly Fe^{59} incorporation above saline controls in this assay is 80 times that needed in the 48-hour blood assay using the polycythemic mouse. The greater sensitivity of the 48-hour mouse blood assay is also seen in the greater coefficient of regression of the dose-response curve. This heightened sensitivity is of most importance when the test material is limited in activity or amount. The upper limit of the linear portion of the dose-response curve in the 48-hour mouse blood assay is 2.4 cobalt units which coincides with the minimal detectable dose of the starved rat assay. These 2 assays are thus complementary and the rat asay, which does not require laborious preparation of the assay animal, may be used more conveniently when very active material is to be assayed.

Summary. Incorporation of Fe^{59} with blood and spleen of transfused polycythemic mice at various intervals has been used to find sensitive assay systems for erythropoietin. Transfusion polycythemia markedly reduces erythropoiesis as judged by low levels of Fe^{59} uptake into the spleen and into the blood after 16 hours. In polycythemic mice receiving injections of erythropoietin, incorporation of Fe^{59} is maximal in the spleen at 8 hours and in the blood at 48 hours after injection of the isotope. Incorporation of Fe^{59} into the blood of the polycythemic mouse 48 hours after giving the isotope was found to be the most sensitive assay when compared to the starved rat assay and other polycythemic mouse assays.

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Received January 16, 1962. P.S.E.B.M., 1962, v109.

Effect of Cortisol on Liver Phosphorylase Activity.* (27352)

ALBERT B. EISENSTEIN

Department of Medicine, Jewish Hospital and Departments of Medicine and Preventive Medicine, Washington University, St. Louis, Mo.

Phosphorylase activity in several organs and tissues is altered by various hormones. Epinephrine and glucagon cause a rapid increase in liver phosphorylase activity under *in vivo* and *in vitro* conditions(1,2,3). ACTH specifically increases adrenal phosphorylase but has no effect on enzyme activity in other tissues(4). Skeletal and cardiac muscle phosphorylases are subject to hormonal influence, epinephrine producing a rise in activity of both enzymes, whereas glucagon has greater effect on cardiac muscle phosphorylase(5,6). Adrenal cortical hormones are known to stimulate liver phosphorylase(7,8) although effects of these hormones on the enzyme have not been thoroughly investigated. The time required for phosphorylase activity to rise after corticosteroid administration and the duration of increased enzyme activity have not been established. Furthermore, it is not known whether continued hormone administration will have greater effect on liver

* Supported by grants from Nat. Inst. of Arthritis and Metab. Dis. and from Nat. Vitamin Foundation.