(LAP) activity during third trimester of pregnancy was significantly increased in 11 patients with documented twin pregnancy, when compared with 25 patients with a single fetus of same gestational age.

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Biological Assay of Human Urinary Erythropoietic Stimulating Factor (ESF)* (25963)

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Previous studies have demonstrated the presence of ESF in urine of patients with a variety of anemias(1-4). Because of the ready availability of Thalassemia patients with predictably low levels of hemoglobin our efforts have been restricted to these subjects. An attempt has been made to correlate concentration of ESF in the urine with hemoglobin level in the blood of donor subjects at the time urine was collected. Although the data obtained previously were insufficient to indicate any association between these factors(4), the evidence now available is sufficient to suggest that concentration of ESF in urine does relate to severity of anemia. This information is useful as a guide in selection of patients as donors of ESF. Based upon the hematocrit and reticulocyte responses to ESF, a bioassay has been developed which is probably more reliable than existing ones which depend upon the use of only one parameter in evaluating erythropoietic responses.

Materials and methods. Thirty-four samples of urine were collected from 13 patients with Thalassemia major at hemoglobin levels ranging from 2.5% to 8.0%. The samples were frozen immediately and subsequently thawed, filtered, and frozen in sterile injection bottles until assayed. Mature 170 to 230 g Long-Evans female rats, maintained upon a definite surplus supply of water and Purina Laboratory Chow for at least 2 weeks after removal from the stock cages, were used for assessment of erythropoietic effects. The size of the groups used varied from 3 to 5 animals depending upon the precision required and availability of material. Each rat received 5 daily 3 ml subcutaneous injections of urine or extract. Microcapillary hematocrit and reticulocyte values were determined prior to assay, and upon the day after last injection. Reticulocytes were stained with new methylene blue according to the procedure of Brecher *et al.*(5). With the aid of an ocular counting disk, one-fourth of the erythrocytes in a microscopic field were counted while all reticulocytes in the field were enumerated and divided by 4. The percent reticulocytes was calculated after counting 1000 cells.

Results. In Fig. 1 and 2, reticulocyte and hematocrit responses are plotted separately as functions of hemoglobin levels of donor patients. Two equations were derived from the analysis of these relations by the method of least squares(6). The first equation describes the regression of reticulocyte responses in recipient rats (y) upon hemoglobin levels of donor patients (x).

$$(a)\quad y = -2.1x + 13.2$$

The second equation represents the regression of hematocrit responses in recipients (z) upon donor hemoglobin levels (x).

$$(b)\quad z = -1.8x + 11.1$$

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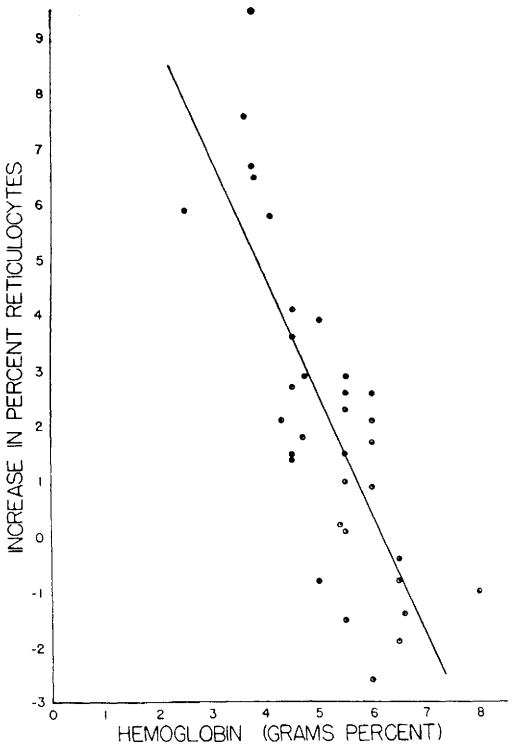

FIG. 1. Reticulocyte responses in ESF treated rats as a function of hemoglobin values of donor patients.

It is clear from the negative slopes of these regression lines that ESF concentration in urine increases with decreasing hemoglobin levels in the donor patients. When the 2 equations are solved simultaneously, an expression for y as a function of z is obtained:

$$(c)\quad y = -1.16z + .03$$

Since the y intercept in relation (c) is small, it may be disregarded, and because the slope 1.16 within the limits of scatter of the data is approximately equal to 1, equation (c) may be simplified to $y = z$. With this convenient approximation, hematocrit and reticulocyte changes (obtained on sixth day after initiation of the injections only) may be regarded as equivalent and can therefore be averaged or added. The value obtained by addition of the rise in hematocrit percentage to the increase in reticulocyte percentage is referred to as the H-R unit. Potency of an ESF preparation is thus expressed in terms of response in H-R units obtained by injecting a stated daily dose in mg or ml for 5 days.

In Fig. 3, response to a single preparation is plotted as a function of urine dose in ml. The response is neither a linear nor a logarithmic function of the dose. Nevertheless, comparisons may be made between preparations diluted to an activity of about 10 H-R units. In some cases, approximate comparisons may be made between preparations assaying from 0-10 H-R units with the assumption that the response is nearly linear in that range. By comparison of the response in terms of H-R units to a standard preparation (CS-1) supplied by Dr. Henry Borsook with the response to this preparation estimated by the method of Fried *et al.*(8) and White *et al.*(9), it has been tentatively established that a daily dose which produces a 1.3 cobalt unit response by the latter method will elicit an H-R response of 1 unit.

Discussion. It is established that urinary concentration of ESF increases with severity of anemia in Thalassemia subjects. The existence of this relation serves as additional evidence favoring the view that ESF is a feed-

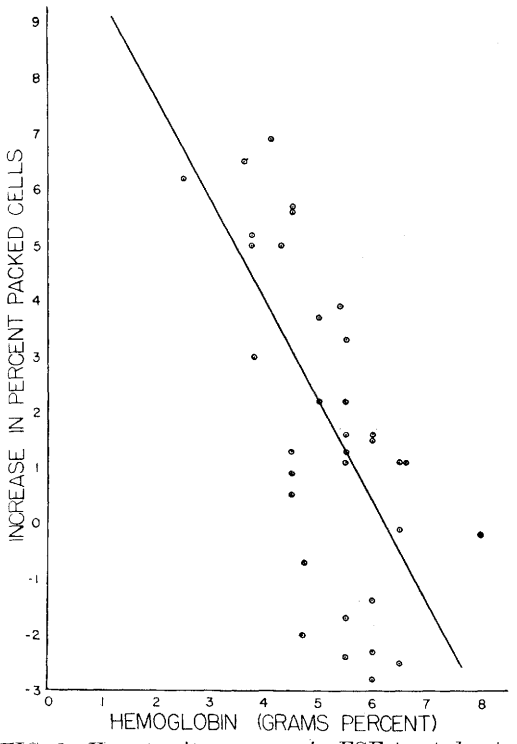

FIG. 2. Hematocrit responses in ESF treated rats as a function of hemoglobin values of donor patients.

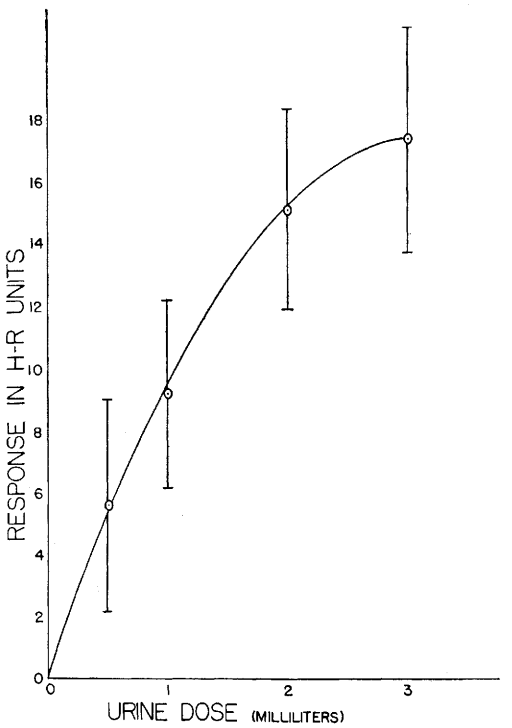

FIG. 3. Responses of rats in H-R units as a function of daily doses of ESF expressed in ml of urine. The line is drawn through the mean values for the difference between increases in H-R units observed in 3 treated and 3 untreated control rats. Height of the vertical lines above and below mean values indicates stand. errors of differences in means.

back agent which is present in greater concentration when there is a greater demand for increased erythropoiesis. From the curves in Fig. 1 and 2 it may be noted that a high potency urine from Thalassemia subjects can reasonably be expected when their hemoglobin levels fall below 4 g %. The cut-off point for appearance of ESF in urine is approximately at hemoglobin levels of 6 g %. It is not certain whether this cut-off point occurs at the renal threshold, or is merely a fall in ESF concentration to levels which are not detectable by the assay. A similar drop in ESF in plasma to undetectable levels has been noted by Lowy *et al.* working with rabbits at equivalent low hemoglobin values(7). It may be that severe anemia enhances the discrepancy between rate of production and rate of utilization of ESF such that the factor accumulates to demonstrable levels.

The biological assay for ESF in terms of a single parameter such as the reticulocyte response alone, or Fe^{59} uptake by red cells(8,9) is hazardous since any agent which produces a hemolytic anemia would be likely to produce a positive response. Use of 2 parameters, such as the reticulocyte and hematocrit responses tends to remove this difficulty but it would appear cumbersome to base a unit on 2 separate assay parameters. Nevertheless, it has been found possible to add reticulocyte and hematocrit increments (in 6-day assays only) and thus obtain a single H-R unit. This unit has the advantage that hemolytic effects frequently observed in animals responding to crude urine are likely to be cancelled out. Once the potency of a crude urine sample is established in terms of H-R units, fractionation of ESF is more economically followed by the method of Fried *et al.*(8) and White *et al.*(9) since their assay, which is based on the Fe^{59} incorporation into the erythrocytes of starved rats, requires injection of less ESF. On the other hand, for screening large numbers of samples of urine for ESF activity under circumstances where only a few samples are likely to be potent, the H-R unit assay is the more economical one, for it eliminates the costs involved in use and disposal of Fe^{59}.

Summary. Concentration of ESF in the urine of Thalassemia patients increases with severity of anemia. Subjects with hemoglobin levels below 4 g % may reasonably be expected to provide urine with a high titre of human ESF. A biological assay depending upon the single unit obtained by the additive changes in hematocrit and reticulocyte values of rats has been developed which produces a cheaper and more reliable method of screening samples for ESF activity than the starved rat red cell Fe^{59} uptake procedure. The latter method, however, is more sensitive and therefore more useful in following the purification of ESF preparations of established potency.

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Effect of Dietary Lipids on Growth of Transplantable Neoplasms in Mice.* (25964)

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The effects of total restriction of diet on incidence and growth of neoplasms in experimental animals have been summarized by Tannenbaum(1) and by Cowdry(2). Neoplasms inhibited by dietary restriction are spontaneous mammary tumors, induced skin tumors, induced sarcomas, primary lung tumors, and leukemias. Spontaneous tumors are affected more than are artificially induced tumors.

Diets with a high content of fat have enhanced development of certain spontaneous and artificially induced neoplasms in rats and in mice(3), including stilbesterol-induced mammary carcinoma in rats, chemically induced hepatic carcinoma in rats, and carcinoma of the skin in mice produced by tars and polycyclic hydrocarbons. The fatty acid composition and degree of saturation of the fats used in most of these experiments varied and showed no consistent effect, but varied with individual neoplasms.

This report deals with a comparison of the effects of diets containing various lipids upon incidence and rate of growth of L1210 lymphatic leukemia in DBA/2 mice and the adenocarcinoma 755 in C57 Bl/6 mice(4). These transplantable tumors were selected because their characteristics are consistent and predictable. The dietary fats were selected to compare the effects of fats with a high content of unsaturated fatty acids with those with a lower content of unsaturated fatty acids.

Materials and methods. L1210 leukemia in DBA/2 mice. Two hundred fifty adult DBA/2 mice, 125 males and 125 females, were divided into 5 dietary groups of 50 mice each, (25 males and 25 females). They were approximately 4 months of age when special diets were substituted for *Purina Laboratory Chow*. The artificial diets were identical in composition except for fat content (Table I). The basic material, "fat-free" diet powder†, consisted of vitamin-free casein (21.1%), alphacel "cellulose" (16.45%), sucrose (58.45%), salt mixture (4%), and vitamins. Diet A (group A mice) consisted of the basic powder, Vit. B_{12} (3 μg/100 g diet), folic acid (0.2 mg/100 g diet), and ascorbic acid (0.1 g/100 g diet). Diets B, C, and D (groups B, C, and D) consisted of the basic diet powder and added vitamins plus 11% (by weight) of corn oil,‡ hydrogenated vegetable oils,§ or lard.‖ The characteristics of these various fats are shown in Table II.

The mice were maintained on the special

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† Nutritional Biochemicals Corp., Cleveland.

‡ Mazola, Corn Products Co., Argo, Ill. furnished through the courtesy of Mr. Neal E. Artz.

§ Shortening J (similar to Crisco), Procter and Gamble Co., Cincinnati, furnished through the courtesy of Mr. W. H. Meyer.

‖ Reliable Packing Co., Chicago, Ill., through the courtesy of Mr. Hugo E. Wistreich, who also supplied a chemical assay of this product.