centration of Components B, A and f.

Summary. Hemolysates of aged red cells were analyzed by electrophoresis before and after incubation with a number of phosphorylated metabolic intermediates. There was a marked increase in concentrations of one of the components (B) and a reduction in the amount of 2 components (A and f) in presence of added 2,3 diphosphoglycerate and adenosine triphosphate. Small increases in Component B concentration were observed in the adenosine diphosphate and 3-phosphoglyceric acid groups. A new electrophoretic component appeared after adding fructose 1,6-diphosphate.

1. Bartlett, G. R., Barnet, H. N., *J. Clin. Invest.*, 1960, v39, 56.

2. Berry, E. R., Chanutin, A., *ibid.*, 1958, v37, 974.

3. Fiske, C. H., Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

4. Longsworth, L. G., *Chem. Rev.*, 1942, v30, 323.

Received August 30, 1962. P.S.E.B.M., 1963, v112.

Normal Response to Erythropoietin or Hypoxia in Rats Made Anemic with Turpentine Abscess.* (27954)

A. GUTNISKY AND D. VAN DYKE

Universidad Nacional del Noreste, Argentina, and Donner Laboratory of Medical Physics, Lawrence Radiation Laboratory, University of California, Berkeley

It is apparent that the anemia which develops in the presence of inflammation has a complex etiology. Disturbances in iron metabolism result in a markedly lowered plasma iron concentration and shunting of iron to storage pools(1,2,3,4). Wintrobe(5) has shown that the anemia of inflammation can be abolished by administration of cobalt, the action of which is assumed to be through stimulation of erythropoietin production(6). Yet in a preliminary study, Erslev(7) obtained evidence suggesting that both the production and effectiveness of erythropoietin were defective in the presence of inflammation. The present study was designed to clarify the role of erythropoietin in the etiology of the anemia which follows the production of a sterile turpentine abscess by testing the ability to respond to both exogenous erythropoietin and endogenous erythropoietin (hypoxia).

Materials and methods. Specific pathogen free, adult, female rats of the Sprague-Dawley strain weighing approximately 200 g and fed *ad libitum* on Diet 1† were used throughout the study except that rats of the Long-Evans strain were used to make the erythropoietin dose-response curve(8). Turpentine abscesses were produced by injection of N.F. oil of turpentine into the gluteal muscle (0.125 ml on days 1 and 2 and 0.250 ml on day 7). On day 15, the rats were weighed, and total circulating red cell volume and hemoglobin were determined by the Fe^{59} labeled red cell dilution method(9). Leucocytes and reticulocytes were counted. Bone marrow smears were made from femoral marrow diluted with rat serum. Weight of the spleen was recorded. Determination of plasma iron and total iron binding capacity of the plasma was done on plasma pooled from rats of the same group(10).

Erythropoietin was obtained by the collodion adsorption method(11) from the urine of a patient who had complete red cell aplasia. It had been assayed by a variety of methods(8). One-half or 2.5 C.S.‡ units

* Supported in part by Consejo Nacional de Investigaciones Cientificas y Tecnicas, Argentina, and by U. S. Atomic Energy Commission.

† The diet, obtained from Simonsen Laboratories, Gilroy, Calif., consisted of 59.0% wheat, 11.7% skim milk, 11.2% casein, 11.2% rice bran, 3.3% vegetable oil, 1.3% $CaCO_3$, 0.7% NaCl, and vitamin and mineral mixtures to 100%.

‡ Comparative Standard(12).

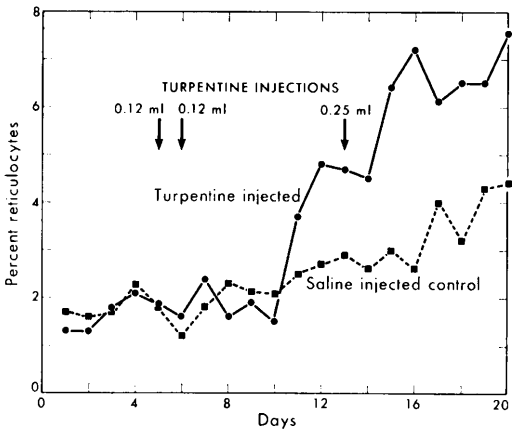

FIG. 1. Per cent reticulocytes in peripheral blood in normal rats and in rats following production of a turpentine abscess. The gradual increase in reticulocyte count in normal controls is assumed to be the result of blood loss through daily sampling.

were given daily subcutaneously for 14 days starting the day of the first turpentine injection. The controls were given saline.

Exposure to hypoxia was accomplished by placing rats with or without a turpentine abscess in a decompression chamber at a simulated altitude of 20,000 feet for 14 days, starting the day of the first injection of turpentine.

Results. As shown in columns 1 and 2 of Tables I, II, and III, turpentine abscess caused a mild anemia, with reduction in total circulating red cell volume of 18% ($P < 0.001$§), in total circulating hemoglobin of 15% ($P < 0.001$) compared to the untreated controls, a significant increase in reticulocytes ($P < 0.001$), and a suggestion of a reduction in per cent erythroblasts in the marrow ($P < 0.02$). There was a steady rise in the per cent reticulocytes in the controls, which is assumed to be the result of stimulation of erythropoiesis due to blood loss from daily sampling. Following administration of turpentine, there was no increase in reticulocytes during the first few days, but after the 6th day the per cent reticulocytes increased to twice the number in controls (Fig. 1). Both large and small doses of erythropoietin increased the hemoglobin, hematocrit, total circulating red cell volume, and total circulating hemoglobin (Table I and II). At the lower dose of erythropoietin this resulted in repair of the anemia produced by turpentine, and at the higher dose in production of polycythemia in the presence of a turpentine abscess. For each dose, the percentage increases in total circulating red cell volume were essentially identical in rats with and without abscesses.

Both the normal and turpentine-treated rats showed a marked increase in hematocrit, hemoglobin, total circulating red cell volume, and total circulating hemoglobin in response to hypoxia (Table III). The increase in total circulating red cell volume was 70% in the rats having a turpentine abscess and 68% in the rats having no abscess. Total circulating hemoglobin increased in proportion to total circulating red cell volume.

Discussion. The production of a turpentine abscess was followed by development of a mild anemia characterized by a reduction in total circulating red cell volume and total circulating hemoglobin of approximately 16%. This degree of anemia is comparable to that reported by previous workers (Wintrobe *et al.*(5)).

Administration of either large or small doses of human urinary erythropoietin produced the same per cent increase in hemoglobin, hematocrit, and total circulating red cell volume in rats having a turpentine abscess as in normal rats, although the final values fell short of those obtained in the normal rats by the same difference as prevailed in the respective controls. Thus, although the rats with an abscess showed no deficiency in response to erythropoietin, the administration of erythropoietin did not abolish the defect produced by the turpentine abscess. Since there is a log-dose response relationship to erythropoietin(8), a large dose such as was used in this experiment would be expected to abolish any small differences in endogenous erythropoietin titer which might have existed in the rats if the etiology of the anemia were on the basis of inadequate endogenous erythropoietin levels. The fact that the defect persisted after large doses of erythropoietin indicates that it is based on

§ Fisher "t" test.

some factor other than an inadequate level of erythropoietin in the circulation.

Exposure to hypoxia (a simulated altitude of 20,000 feet for 14 days) resulted in the same per cent increase in hemoglobin, hematocrit, and total circulating red cell volume in the rats having a turpentine abscess as in the normal controls, although the values in rats with an abscess again fell short of those obtained in the normal rats by the same difference as prevailed in the respective controls. These results indicate that the rat having a turpentine abscess is capable of producing and utilizing erythropoietin normally when stimulated, suggesting that the anemia which accompanies a turpentine abscess is not the direct result of a defect in erythropoietin metabolism but that the mild anemia

TABLE I. Effect of a Small Dose of Erythropoietin on Hematologic Values, Body and Spleen Weight of Normal and Turpentine-Treated Rats.* (.5 C.S. units/day for 14 days.)

Measurement	Normal untreated	Turpentine only	Normal + ESF	Turpentine + ESF
Total circulating red cell vol, ml	5.48 ± .33†	4.51 ± .24	6.62 ± .48	5.02 ± .36
Hematocrit, %	41.4 ± 1.66	38.0 ± 2.01	49.1 ± 2.17	42.7 ± 2.37
Hemoglobin, g/100 ml	12.5 ± .59	11.3 ± .57	14.2 ± .83	12.4 ± .70
Reticulocytes, %	1.8 ± .52	7.9 ± 2.34	4.8 ± .42	6.6 ± 2.04
Spleen wt, mg	570 ±91	852 ±102	595 ±104	903 ±225
Body wt, initial, g	194 ± 9.6	194 ± 8.9	193 ± 11.4	194 ± 9.2
" " final, g	238 ±11.7	221 ± 10.1	234 ± 10.4	217 ± 14.0

* 6 female rats of Sprague-Dawley strain in each group. † Standard deviation.

TABLE II. Effect of Large Dose of Erythropoietin on Hematologic Values, Body and Spleen Weight of Normal and Turpentine-Treated Rats. (2.5 C.S. units/day for 14 days.)

Measurement	Normal untreated (6)*	Turpentine only (5)	Normal + ESF (6)	Turpentine + ESF (4)
Total circulating red cell vol, ml	5.44 ± .54†	4.21 ± .63	9.41 ± 1.17	7.71 ± 1.73
Hematocrit, %	41.5 ± 2.15	36.1 ± 3.16	55.4 ± 3.16	51.0 ± 5.65
Hemoglobin, g/100 ml	13.4 ± .60	12.6 ± .38	17.1 ± .93	16.0 ± .83
Plasma iron, μg/100 ml	358	262	72	127
Total iron binding capacity, μg/100 ml	785	704	584	657
Reticulocytes, %	2.8 ± .93	6.9 ± 2.08	7.9 ± 1.08	9.7 ± 2.44
Marrow erythroblasts, %	18.3 ± 9.5	13.1 ± 8.3	28.8 ± 8.3	23.2 ± 3.4
Leucocytes/mm³ × 10^3	12.60 ± 4.12	12.08 ± 3.31	12.05 ± 2.11	13.96 ± 2.52
Spleen wt, mg	666 ±276	716 ±232	1,064 ±238	1,182 ±150
Body wt, initial, g	204 ± 6.63	204 ± 14.6	204 ± 10.7	200 ± 13.4
" " final, g	242 ± 14.7	225 ± 27.8	247 ± 11.0	225 ± 17.1

* No. of female rats of the Sprague-Dawley strain in each group is indicated in parentheses.
† Standard deviation.

TABLE III. Effect of Hypoxia on Hematologic Values and Body Weight of Normal and Turpentine-Treated Rats. (20,000 feet for 14 days.)

Measurement	Normal untreated (6)*	Turpentine only (3)	Normal + hypoxia (5)	Turpentine + hypoxia (6)
Total circulating red cell vol, ml	5.31 ± .37†	4.61 ± .45	8.10 ± .63	7.64 ± 1.11
Hematocrit, %	42.7 ± 1.66	38.3 ± 1.83	63.0 ± 3.16	56.8 ± 2.46
Hemoglobin, g/100 ml	13.4 ± .55	12.2 ± .50	18.7 ± .83	16.7 ± 1.60
Reticulocytes, %	1.5 ± .50	2.8 ± 1.57	4.4 ± 1.72	4.7 ± 2.50
Marrow erythroblasts, %	20.7 ±10.9	8.6 ± 2.97	29.7 ±11.8	22.9 ± 4.8
Leucocytes/mm³ × 10^3	12.19 ± 1.88	11.14 ± 1.11	10.45 ± 1.93	12.05 ± 1.33
Body wt, initial, g	197 ±13.2	198 ± 7.7	197 ±13.3	201 ±14.5
" " final, g	243 ±26.5	215 ±15.9	202 ±11.1	200 ±17.5

* No. of female rats of Sprague-Dawley strain indicated in parentheses. † Standard deviation.

does not serve as an adequate stimulus to increased erythropoietin production. A turpentine abscess was chosen as an easily standardized method of producing an inflammatory lesion with the realization that inflammatory lesions from bacterial(13), antigenic(14) or other agents may give quite different results.

Summary and conclusions. Rats with a turpentine abscess developed characteristic mild anemia. Such rats showed an erythropoietic response comparable to that of normal controls following treatment with human urinary erythropoietin or exposure to hypoxia. Therefore, the rat having a turpentine abscess is capable of producing erythropoietin and the marrow is capable of responding when stimulated, suggesting that the mild anemia which accompanies a turpentine abscess does not serve as an adequate stimulus for increased red cell production.

1. Cartwright, G. E., Lauritsen, M. A., Jones, P. J., Merrill, I. M., Wintrobe, M. M., *J. Clin. Invest.*, 1946, v25, 65.

2. Cartwright, G. E., Lauritsen, M. A., Humphreys, S., Jones, P. J., Merrill, I. M., Wintrobe, M. M., *ibid.*, 1946, v25, 81.

3. Greenberg, G. R., Ashenbrucker, H., Lauritsen, M., Wintrobe, M. M., *ibid.*, 1947, v26, 114.

4. Freireich, E. J., Miller, A., Emerson, C. P., Ross, J. F., *Blood,* 1957, v12, 972.

5. Wintrobe, M. M., Grinstein, M., Dubach, G. G., Humphreys, S. R., Ashenbrucker, H., Worth, W., *ibid.*, 1947, v2, 323.

6. Goldwasser, E., Jacobson, L. O., Fried, W., Plzak, L. F., *ibid.*, 1958, v13, 55.

7. Erslev, A. J., Lavietes, P. H., *Clin. Res.*, 1954, v2, 54.

8. Van Dyke, D. C., Pollycove, M., *Erythropoiesis,* Grune and Stratton, New York, 1962, p340.

9. Garcia, J. F., Van Dyke, D. C., *J. Appl. Physiol.*, 1959, v14, 233.

10. Peters, T., Giovaniello, T. J., Apt, L., Ross, J. F., *J. Lab. Clin. Invest.*, 1956, v48, 274.

11. Van Dyke, D. C., *Ciba Found. Symp. on Haemopoiesis,* Churchill, London, 1960, p397.

12. Keighley, G., Lowy, P. H., Borsook, H., Goldwasser, E., Gordon, A. S., Prentice, T. C., Rambach, W. A., Stohlman, F., Jr., Van Dyke, D. C., *Blood,* 1960, v16, 1424.

13. Freireich, E. J., Ross, J. F., Bayles, T. B., Emerson, C. P., Finch, S. C., *Clin. Res. Proc.*, 1954, v2, 54.

14. Cartwright, G. E., Wintrobe, M. M., *Advances in Internal Medicine,* Year Book Publishers, Chicago, 1952, v5, 165.

Received September 4, 1962. P.S.E.B.M., 1963, v112.

Effect of Endotoxins on Phagocytic Activity of the Reticuloendothelial System of the Rat. (27955)

MARY I. ARREDONDO AND RALPH F. KAMPSCHMIDT

Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, Okla.

Intravenous or intraperitoneal injection of lipopolysaccharides from gram-negative bacteria into mice and rabbits results in an early injury to the phagocytic cells of the liver and spleen(1,2). These injured cells show a deficiency in ability to clear and metabolize injected colloidal material from the blood. These effects were dose-dependent, and were followed after recovery by a period of increased phagocytic activity of the Reticuloendothelial System (RES) which might have been due to new cell formation.

Interest in the RES in our laboratory has been focused on its effects on iron metabolism, particularly iron transport in the rat(3, 4,5). Blocking of the RES in both dog and rabbit has been shown to result in an increase of plasma iron concentration and to prevent the decrease in plasma iron which normally follows production of a sterile inflammation (6,7). Our inability to demonstrate a similar increase in plasma iron concentration after attempted blockade of the RES in the rat (8) has led us to postulate that the response of the RES to injected materials may be different in this species. The present experiments on phagocytic activity of the RES after injections of endotoxin would tend to