Erythropoietic Stimulating Activity During the First Ninety Days of Life. (29800)

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The hypothesis that erythropoiesis is influenced by a humoral substance, erythropoietin, rests on multiple experimental and clinical observations(1,2). Its unequivocal demonstration in normal plasma has not been accomplished and the significance of erythropoietin in the physiological control of erythropoiesis is uncertain(2). In contrast definite elevations of plasma erythropoietin have been demonstrated in certain pathologic states manifested by major erythropoietic deviations(1,2). Conclusions regarding the physiologic role of erythropoietin based on the foregoing observations are, at best, indirect.

The alterations of erythropoiesis which occur during the first months of life provide a unique opportunity to explore the possible physiologic effects of erythropoietin. This report details serial observations made on plasma erythropoietin levels of infants during the first 90 days of life. Results indicate that erythropoietin may exert physiologic control of erythropoiesis in the newborn period.

Methods and materials. Twenty-one newborn infants weighing greater than 2.5 kg and with no family history of blood dyscrasias were studied. Cord blood was obtained by needle aspiration after pulsation had ceased. Subsequent blood samples were obtained by femoral vena puncture at 4, 30, 60 and 90 days following birth. Blood was anticoagulated with EDTA, centrifuged, and the plasma removed and frozen until bioassay. The usual feeding regimen without vitamin supplementation was followed. If illness was found or reported, the infant was eliminated from the study, and 7 of the initial infants were followed to completion.

Erythropoietin assay was carried out by a modification of a method previously described by Cotes and Bangham(3). Six-week-old CF #1 virgin female mice weighing 15-28 g were maintained on a diet of Purina Laboratory Chow with tap water allowed *ad libitum*. Erythrocythemia was produced by exposure to discontinuous hypobaric hypoxia (0.5 atmospheres) for a 2-week period after which the hypoxic stimulus was withdrawn. The assay was carried out during the 96-hour period following hypoxic stimulation, while the animal was at ambient pressure. At 24 and 48 hours 0.2 ml of plasma or 0.9% saline solution was administered I.P. Seventy-eight hours following the end of hypoxic stimulation 0.2 μc Fe^{59}/0.2 μg Fe^{56} citrate was administered I.V. *via* the tail vein, and red blood cell radioiron incorporation determined 18 hours later(4).

Results. Hemoglobin and hematocrit determinations are presented in Table I. The initial hematocrit and hemoglobin determinations in cord blood were normal. By day 4 there had been an increase, by day 30 hematocrit and hemoglobin had diminished below the levels found in cord blood. There was a further decrease by day 60, but hematocrit and hemoglobin then stabilized. Erythropoietic stimulating activity determined by the method described above was slightly but significantly ($p>0.01$) elevated in cord blood (Table I). Plasma from 4-day-old and 30-day-old infants did not demonstrate significant erythropoietic stimulating activity whereas at day 60 and day 90 there was a significant ($p<0.001$) increase. The relationship between erythropoietin activity and hemoglobin levels is presented in Figure 1. As hemoglobin level diminished erythropoietin activity increased.

Comment. Studies of erythrocyte production utilizing reticulocyte counts, marrow erythroid cell counts and radioiron erythrocyte incorporation(5,6) have demonstrated accelerated erythropoiesis at birth with a decrease to lower levels within a few days. Diminished erythropoiesis is reflected in hemo-

TABLE I. Results of Erythropoietin Assay, Hematocrit and Hemoglobin Levels in the Neonatal Period.

Age of infants	No. infants	Hb, g % ± S.D.M.	Hct, vol % ± S.D.M.	18 hr Fe^{59} RBC incorporation		Probability
				Saline control	Neonatal plasma	
Cord blood	7	14.8 ± 1.1	49 ± 2.8	3.6 ± 1.2	5.2 ± 1.8	.01
4 Day	7	17.4 ± 1.2	57 ± 4.5	3.5 ± 1.2	3.2 ± 1.5	NS
30 "	7	12.9 ± 1.6	40 ± 4.8	2.5 ± 1.6	3.6 ± 1.3	NS
60 "	7	9.9 ± .7	32 ± 2.4	2.5 ± .6	5.8 ± 1.8	.001
90 "	7	10.0 ± .8	32 ± 2.5	3.5 ± .8	7.0 ± 1.3	.001

globin and hematocrit levels in the neonatal period. Suggested explanations of these observations are that there is a primary failure of the marrow, or alternatively that the diminution in erythrocyte production following birth is a temporary adjustment to the environmental increase in oxygen with a reduced hemoglobin need(5,6).

Erythropoietin levels in the plasma and urine of patients with anemia and bone marrow failure have been invariably elevated(1, 2). However, remission(7) or transfusion(8) produces reciprocal diminution of erythropoietin titers as the packed cell volume increases, and indeed elevated erythropoietin titers do not occur in bone marrow failure until the hematocrit has diminished below 30 volumes percent(9). In the present study, hemoglobin and hematocrit values were not diminished to the levels in which increased erythropoietin titers are observed. Therefore, conclusions as to the role of bone marrow failure in the diminished erythropoiesis of the neonatal period are unwarranted.

The present study suggests elevated erythropoietin titers in the cord plasma at birth and a subsequent fall in the postnatal period with a rebound rise as the hemoglobin level reaches an undefined critical level. These alterations in erythropoiesis and erythropoietin levels may be due to changes in arterial oxygen tension. The fetus exists in an atmosphere of diminished oxygen tension and hemoglobin levels at birth presumably result from an oxygen debt *in utero*. Immediately following birth there is improvement in oxygenation of the blood and normal oxygen tensions are reached in about 3 hours(10). Compatible with this hypothesis is the finding of increased titers in the cord blood of infants and inability to detect erythroproietin in the plasma at 4 and 30 days when the hemoglobin level is in the normal adult range. These results are in agreement with those of Halvorsen(11) who demonstrated increased erythropoietic activity in cord blood and the blood from hypoxic anemic infants. A rise in the marrow erythroid cell count and reticulocytosis has been reported by Gardiner *et al*(5,6) 60 and 90 days following birth when the hemoglobin has fallen to the lowest level in the newborn period. The slight increase in plasma erythropoietin at these times demonstrated in the present study may be responsible for this augmentation of red blood cell production. These results are suggestive of variable erythropoietin levels and it is possible that this represents physiologic or near physiologic levels of erythropoietin. These relationships need further clarification before definite conclusions can be drawn regarding the part which erythropoietin plays in the control of erythropoiesis in the newborn period.

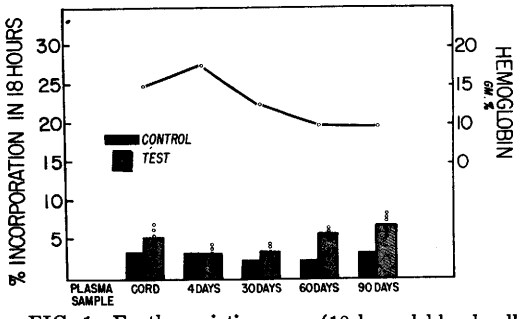

FIG. 1. Erythropoietin assay (18 hr red blood cell radioiron incorporation) and hemoglobin levels during first 90 days of life. Control animals were given saline, test animals neonatal plasma.

Summary. Serial observations were made on plasma erythropoietin levels of infants during the first 90 days of life. Results indicate variable erythropoietin titers and are

suggestive of a physiologic control of erythropoiesis during the newborn period by erythropoietin.

1. Stohlman, F., New Engl. J. Med., 1962, v267, 342.
2. ———, ibid., 1962, v267, 392.
3. Cotes, P. M., Bangham, D. R., Nature, 1961, v191, 1065.
4. Gallagher, N. I., Hagen, D. Q., McCarthy, J. M., Lange, R. D., Proc. Soc. Exp. Biol. and Med., 1961, v106, 127.
5. Gardiner, D., Marks, J., Roscoe, J. D., Arch. Dis. Child., 1952, v27, 128.
6. ———, ibid., 1952, v27, 214.
7. Lange, R. D., McCarthy, J. M., Gallagher, N. I., Arch. Int. Med., 1961, v108, 850.
8. Medici, P. T., Gordon, A. S., Piliero, S. J., Luhby, A. L., Yuceoglu, P., ACTA Haemat., Basel, 1957, v18, 325.
9. Jacobson, L. O., Goldwasser, E., Gurney, C. W., Kinetics of Cellular Proliferation, Stohlman, F., Ed., Grune & Stratton, Inc., New York, 1959, p344.
10. Smith, C. A., Kaplan, E., Am. J. Dis. Child., 1942, v64, 843.
11. Halvorsen, S., Acta Paed., 1963, v52, 425.

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Comparative Study of *in vivo* and *in vitro* Grown *Mycobacterium tuberculosis*. IV. Immunogenic Differentiation.* (29801)

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In a comparative study of the biochemistry, pathogenicity and immunogenicity of *in vivo* and *in vitro* grown *Mycobacterium tuberculosis*, strain H37Rv, significant differences were found to exist(1,2). Whereas the *in vivo* grown bacillus exhibited double the virulence for mice as measured by mean survival time, its immunizing capacity using phenol-killed vaccines was lower. This latter observation was true regardless of whether the immunized mice were challenged with tubercle bacilli grown *in vivo* or *in vitro*. The following report involves an attempt to extend this finding of differential immunogenicity of *in vivo* and *in vitro* cultured *M. tuberculosis*, and thereby to gain some insight into the mechanism involved.

Methods. The strain of *in vitro* tubercle bacilli used in this study was H37Rv, grown on the surface of Kirchner's synthetic liquid medium. The growth of the *in vivo* bacilli was carried out in mice (strain CF1) which were initially infected with strain H37Rv grown *in vitro*, but thereafter by successive transfers from mouse to mouse with the *in vivo* grown bacilli. The separation by differential centrifugation of the *in vivo* grown tubercle bacilli (LRv†) from tuberculous mouse lung homogenates was carried out according to the procedure previously described (1). It has been found that there is no significant difference, with regard to biochemical, pathogenic and immunogenic characteristics, in the LRv separated after one mouse passage and the LRv separated after 25 serial mouse passages.

Degree of immunity was determined by recording individual survival times of vaccinated mice (strain CF1, 6 weeks old, female) after challenge infection, with confirmation by necropsy that the cause of death was tuberculosis. The mice were divided into 8 vaccinated groups of 15 mice each, in addition to control groups of non-vaccinated mice which were injected with phosphate buffer or adjuvant. Each group was vaccinated twice, at 1-week intervals, subcutaneously with 0.1 ml volumes of various vaccine preparations. Four groups received different vac-

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† LRv is the designation used throughout for the *in vivo* grown tubercle bacilli, separated by differential centrifugation from lung homogenates of tuberculous mice initially infected with *in vitro* grown strain H37Rv, and maintained thereafter by successive transfers from mouse to mouse.