Summary. Twenty-five per cent of a group of Osborne-Mendel rats thymectomized within 24 hours of birth and injected with Moloney virus at 48 hours of age developed neoplasms, 8% malignant lymphoma, 17% reticulum cell sarcoma, while 95% of the intact group developed malignant lymphoma over the period of the study. All of the thymectomized rats tested, however, were producing virus. Similar deviations from normal were found in the serum protein patterns of both thymectomized and intact virus-inoculated rats.

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Regulation of Erythropoiesis XIX. Effect of Hypoxia on Erythropoiesis in the Newborn Animal.* (30854)

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During late fetal life erythropoiesis in the rat is almost entirely hepatic and splenic. Erythropoiesis is first seen in the bone marrow at birth. In the next few days of life there is an explosive increase in red cell production so that by the 7th day of life, approximately 60% of the marrow elements are erythroid precursors(1). The peripheral blood at birth is characterized by the presence of hypochromic macrocytes which are short-lived. During the first 7-10 days of life, the cell production continues to be hypochromic and macrocytic in character; but the degree of macrocytosis is constantly decreasing so that there is a gradual shift in the indices toward those seen in adult rats. By the 40th to 60th days after birth, normocytic-normochromic cells are present in the peripheral blood and macrocytes are no longer seen.

The hepatic and early myeloid phase of erythropoiesis in the rat is characterized by the presence of large cells(1) frequently appearing in syncytial network with a leptochromatic nucleus with a diameter of about 15-20 μ; the nucleus stains a light pink and has a rather spongy appearance. The cytoplasm is slightly basophilic with fine vacuolization. When the cell membranes are discrete the cell diameter measures about 30-40 μ. This type of cell comprises about 5-10% of the nucleated elements of the bone marrow at birth. During the first 10-20 days of neonatal life, the frequency of this cell begins to decrease and after the 30th to 40th day

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only occasional cells of this type are seen.

In adult life macrocytosis invariably precedes hypochromia(2,3); the sole exception is the unusual case of sideroachrestic anemias in man. Thus the appearance of hypochromic macrocytes in the newborn animal is distinctly unusual and suggests that perhaps the factors governing cell size in the newborn animal differ from those in the adult. The temporal correlation of the presence of the previously described syncytial cell and the production of hypochromic macrocytes also suggests that fetal red cell production may be regulated differently than that of adult animals. Further support for a different regulatory mechanism was provided by studies in which rats were nephrectomized at intervals after birth. In adult rats nephrectomy produced a substantial suppression of erythropoiesis within 48 hours; only occasional late normoblasts were seen after 48 hours and the percentage of erythroid elements at that time had decreased from the normal value of 20-30% to values of 1-7%. In striking contrast, erythropoiesis in the newborn was little affected by bilateral nephrectomy(1). Suppression of erythropoietin production in the mother by nephrectomy or hypertransfusion did not affect red cell production in the nursing newborn, indicating that maternal transfer of erythropoietin did not account for the continued red cell production in the newborn nephrectomized rat. After the 15th day of life there was a gradual transition from the neonatal to the adult type of response to nephrectomy. It is clear from these studies that control of red cell formation in the newborn rat is in large measure independent of renally produced erythropoietin. Finally, the degree of erythroid response seen in newborn rats appears to be out of proportion to the mild anemia present suggesting that hypoxia does not play the dominant role in regulation of red cell production.

The data available on the relationship of hypoxia and erythropoietin to red cell production during the neonatal phase of life in the rodent is limited and is somewhat in conflict. Garcia(4) reported that hypoxia produced by exposure to 9% oxygen for 6 hours a day for a period of 2 weeks did not significantly affect erythropoiesis as judged from the measurement of red cell mass until after the 50th day of life. There was, however, a slight though not statistically significant increase in the red cell mass of animals exposed between the 20th and 44th day of life. In these studies values for the parameters reflecting more acute changes in red cell production, *e.g.*, reticulocytes, bone marrow erythroid values, and iron incorporation were not reported. Van Dyke and Garcia(5) also reported that the rat after the 15th day of life was capable of responding to erythropoietin. Grant(6) reported data that erythropoietin in lactating mothers affected red cell production in nursing newborns, but Garcia was unable to confirm this observation. There are no data on direct measurements of erythropoietin production during early neonatal life. In view of the apparent differences in the regulatory mechanism of the neonatal rat, as judged by the studies on the effect of nephrectomy, and the questions raised in previous studies in respect to the relative importance of hypoxia and erythropoietin, the present evaluation of the relationship of hypoxia to red cell production in the newborn animal was carried out.

Materials and methods. Sprague-Dawley (NIH) rats were mated after 3-4 months of age. Fetuses were delivered spontaneously. Blood samples were collected by cardiac puncture into potassium versenate. Red cell values were measured with standard technics. Bone marrow preparations were made with a brush technic from the split femur and stained with Wrights and Giemsa. Fe^{59} incorporation was measured after the intravenous injection of plasma bound radioiron and based on direct measurement of the blood volume with $chromium^{51}$. The animals were exposed to 0.4 atmosphere in a decompression chamber for 6 hours/day over a period of 7 days. Nursing animals were returned to their mother during the remainder of the day and nursing controls were removed from the mother for a period of 6 hours/day throughout the study.

Results. The results are presented in Table I. Animals exposed at the 16th day of life had no significant change in peripheral

blood parameters although there was a slight increase in the erythroid elements in the bone marrow. Those exposed from the 17th to the 24th day of life had an increase in both peripheral blood and bone marrow values, but the magnitude of the response was relatively small. There was no change in cell size. In animals exposed between the 25th and 32nd day of life there was a substantial increase in erythropoiesis in the bone marrow as judged by differential together with a significant reticulocytosis and increase in hematocrit. This response was macrocytic in character. The response to hypoxia in the latter group approached that seen in the adult in that there was a change in all erythroid parameters together with the production of macrocytic cells.

Discussion. The pattern of response to hypoxia observed in the newborn animals bears striking resemblance to that seen in the nephrectomized animal(1). As in the case of nephrectomy there was little change in the rate of red cell production when animals were exposed prior to the 15th day of life. Thereafter, there was a gradual transition to the adult type of response. In the present studies we observed, as has been previously reported (7), that the erythroid response of the adult to hypoxia is associated with the production of macrocytic cells. This type of response as well as a significant elevation in the hematocrit was first seen in animals exposed between the 25th and 32nd day of life.

The failure to elicit an erythropoietic response to hypoxia during early neonatal life might be explained by the inability of the newborn animal to produce erythropoietin, a relative insensitivity of the bone marrow to erythropoietin or both. As yet there are no data available on the ability of the newborn animal to produce erythropoietin in response to hypoxia. Van Dyke and Garcia(5), however, have adduced evidence to indicate that rats after the 15th day of life will respond to erythropoietin; the magnitude of the response, however, appears to be less than from similar doses in adult animals. There are no data, however, on the response to erythropoietin prior to the 15th day of life. Until such data become available a decision cannot

TABLE I. Effect of Hypoxia in the Newborn Rat.

	Age											
	16 days		24 days		32 days		Adult					
							Male			Female		
	Cont	Exp	Cont	Exp	Cont	Exp	Cont	Exp		Cont	Exp	
Number of rats	5	12	7	14	8	12	6	7		6	6	
Hct (%)	30 ± 1.2	30 ± .6	36 ± .9	39 ± 1.3	38 ± 1.1	50 ± .6	45 ± .9	55 ± .5		42 ± .9	53 ± .4	
Reticulocyte (%)	14 ± .3	17 ± .7	16 ± 1.6	23 ± 3.3	8 ± .6	14 ± .7	3.9 ± .6	14.9 ± .7		2.2 ± .2	12.6 ± .7	
Erythroid precursors (%)	40 ± 4.5	47 ± 1.8	41 ± 1.8	45 ± 1.6	31 ± .9	45 ± 1.4	38 ± 3.2	74 ± 5.1		20 ± 1.8	71 ± 2.7	
MCV (μ^3)	63	64	62	62	58	73	51 ± .9	56 ± 2.0		52 ± 1.7	59 ± .6	
Fe^{59} Inc (%)			58 ± 3.3	71 ± 2.7	64 ± 1.9	79 ± 4.9	23 ± 1.0	31 ± 1.9		22 ± 1.9	31 ± 2.4	

Rats were exposed to 0.4 atmosphere for 6 hr a day for 7 days; days refer to day of sacrifice. Values given are mean ± 1 S.E. Hct—hematocrit; MCV—mean corpuscular volume; Fe^{59} Inc—20 hr iron incorporation value.

be made between the alternative of an inability to produce erythropoietin and/or insensitivity of the bone marrow to erythropoietin as the explanation for the failure of the rat to respond to hypoxia during the early neonatal period. Whatever the explanation, however, it would appear that hypoxia is not the dominant regulatory mechanism in the newborn rat.

It is of some interest that the change to an adult type of response to hypoxia is temporally correlated with the disappearance of the previously described(1) immature syncytial cells in the marrow. It is tempting therefore, to speculate that this cell serves as a fetal erythroid stem cell and in adult life is replaced by a more differentiated cell which is perhaps more responsive to erythropoietin. A similar cell has been described during the hepatic and early myeloid phase of erythropoiesis in the human fetus(8). The newborn rat is relatively immature at birth and in fact is at a stage of development perhaps analogous to the 6th month of human gestation. A parallel might be drawn, then, between erythropoiesis in late intrauterine life in the human being and that observed during the neonatal period in the rat.

Summary. The erythropoietic response of the newborn rat exposed to 0.4 atmosphere 6 hours/day for a period of 7 days was measured. Exposure prior to the 16th day of life failed to influence the rate of erythropoiesis. Thereafter there was a gradual transition to the adult type of response in which polycythemia is induced and is characterized by the production of macrocytic red cells.

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