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Failure of Newborn Rat to Respond to Hypoxia with Increased Erythropoiesis.* (21210)

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It is known that the newborn young of many mammals have a normal or higher than normal erythrocyte count and hemoglobin(1) and that these values decrease immediately after birth, reaching minimal and subnormal levels within a matter of days or weeks depending on the species. One of the proposed explanations of this neonatal anemia is that in intra-uterine life the fetus exists under reduced oxygen tension, to which it responds by active erythropoiesis(2). After birth the newborn, having adequate oxygen, no longer requires the excess of red cells formed during intra-uterine life. Red cell production decreases somewhat, the physiological anemia of the newborn supervenes, and the count only gradually returns to a stable, normal level.

If the hypothesis were sound that hypoxia is the direct determining factor for the high count at birth and, therefore, indirectly responsible for the ensuing anemia, then continued hypoxia after birth should prevent the neonatal anemia or perhaps even result in polycythemia. It is the object of this paper to report the hematological changes found in the newborn rat when exposed to low oxygen tension, as compared with the well-known erythropoietic response of older rats to hypoxia.

Materials and methods. Rats of the Long-Evans strain were used throughout these experiments. Two litters of 6 male rats each were divided into 2 groups; 3 were chosen from each litter. One group, 6 rats, was exposed in a large decompression chamber to a simulated altitude of 15,000 feet for 6 hours a day from the 4th to the 18th days of age. At the end of each exposure, the young were returned to their own mothers. The remaining 6 rats, which served as controls, were also removed from their mothers over the same period daily for 6 hours. The temperature of the chamber was maintained at 25° ± 2°C. The relative humidity was in excess of 95% throughout the experiment. To further protect the young from chill and desiccation, they were placed in a small feeding can in a deep well of shavings, which simulated a nest and prevented their scattering. The controls, during the period when they were separated from their mothers, were similarly protected. The nursing mothers were maintained on a

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TABLE I. Comparison of the Erythropoietic Response of Newborn Male Rats Exposed 6 Hours Daily for 14 Days at 15000 Feet with that of Adult Male Rats Similarly Treated.

Age group, days	Altitude, ft (×1000)	No. of rats	Body wt, g	Hemoglobin, g/100 ml	Hemoglobin/100 g body wt, g	Hematocrit, %	Red blood cell vol/100 g body wt, ml
4– 18	S*	6	42 ± .7	6.7 ± .2†	.47 ± .01	24.1 ± .6	1.61 ± .06
4– 18	15	6	39.7± .7	7.3 ± .1	.51 ± .03	24.2 ± .4	1.67 ± .04
90–104	S	6	304 ±10.0	13.5 ± .3	.74 ± .04	44.4 ± .8	2.39 ± .10
90–104	15	5	301 ± 7.0	14.4 ± .2	.90 ± .03	47.7 ± .7	2.96 ± .05

* S = Sea level.

† Stand. error = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

stock diet known to be adequate for lactation.† The particular mothers chosen for the experiment had an excellent record for milk production. The newborn young, while still hairless, were checked daily, by direct inspection, for the presence of milk distended stomachs. Milk could still be seen in the stomach at the end of the 6-hour period in the chamber. Three unsuccessful attempts to perform this experiment were conducted before these necessary precautions were established. Records of body weight were kept for all the newborns and no significant changes in body weights were found between the animals exposed to the simulated high altitude and their respective controls (Table I, II). At the end of the experiment, determinations of red cell volume were made using the Fe^{59} tagged cell dilution method(3). Hemoglobin determinations were done using Turner's method(4). Parallel determinations of these values were made in adult (90-day) rats for comparison of the response to hypoxia. Other groups of newborn rats were exposed daily for 6 hours to a simulated altitude of 22000 feet from the 4th to the 16th day of life. This type of intermittent stimulation to hypoxia (corresponding to that at 15000 or 22000 feet altitude) was known to cause an erythropoietic response in normal rats similar to, or even higher than that resulting from continuous exposure to hypoxia(5).

Results. It can be seen from Table I that exposure of the suckling rat to hypoxia from the 4th to the 18th day of life, at 15000 feet, did not result in stimulation of erythropoiesis as measured by an increase in the hemoglobin, hematocrit or red cell volume. The adequacy of this stimulus to erythropoiesis in older animals is also shown in Table I. Similarly, exposure to a simulated altitude of 22000 feet from day 4 to day 16 did not stimulate erythropoiesis (Table II). It should be noted that these degrees of hypoxia were not even sufficient to prevent the development of neonatal anemia. At the 16th day of life (or the 18th, when the neonatal anemia is most severe) the decrease in the hematological values of hypoxic rats was the same as those of their normal age controls.

When it was found that rats did not respond to hypoxia in this neonatal period, other groups of young, growing rats were exposed to hypoxia for similar periods. Table II shows the hematological data from rats in which hypoxia was instituted at progressively older ages, from the 18th day to the 68th day of life. Rats in this age range all responded with significant increase in hematocrit, hemoglobin and red cell volume (Table II). In fact, the increase when hypoxia was instituted at 18 days of age was as great as in adults under the same stimulus and resulted in polycythemia.

Discussion. The failure of the rat, in the earlier neonatal period, to respond to hypoxia with an increased erythropoiesis makes it improbable that hypoxia is the direct stimulus to red cell production which leads to the high values found at birth. It is evident that termination of the hypoxia at birth could not, therefore, explain the post-natal anemia or the subsequent gradual recovery from this anemia.

† Diet I consists of 67.5% wheat, 15% casein, 7.5% skim milk powder, 6.75% hydrogenated vegetable oil, 1.0% fish oil, 0.75% NaCl, 1.5% $CaCO_3$, KI added (analysis 1 μg iodine per g diet).

TABLE II. Erythropoietic Response of Male Rats of Different Ages Exposed Daily to an Altitude of 22000 Feet for 6 Hours.

Age group, days	Altitude, ft (×1000)	No. of rats	Body wt, g	Hemoglobin, g/100 ml	Hemoglobin/100 g body wt, g	Hematocrit, %	Red blood cell vol/100 g body wt, ml
4–16	S*	5	31 ± 1.0	8.8 ± .1†	.53 ± .02	29.0 ± 1.4	1.67 ± .04
	22	6	33 ± 0.9	8.3 ± .4	.52 ± .01	29.4 ± .6	1.75 ± .04
18–30	S	4	84 ± 2.0	12.2 ± .3	.81 ± .01	39.3 ± 1.3	2.59 ± .10
	22	6	72 ± 2.0	14.1 ± .7	1.07 ± .01	46.9 ± 1.0	3.38 ± .10
24–36	S	5	95 ± 2.0	11.6 ± .4	.74 ± .01	39.1 ± 1.2	2.41 ± .05
	22	5	91 ± 3.0	15.8 ± .3	1.10 ± .03	44.8 ± 1.4	3.10 ± .20
40–52	S	5	144 ± 3.0	14.8 ± .1	.78 ± .01	46.9 ± .5	2.44 ± .08
	22	5	145 ± 5.0	15.4 ± .1	1.08 ± .03	53.0 ± 1.5	3.28 ± .20
68–80	S	4	249 ± 3.0	13.6 ± .2	.66 ± .02	44.1 ± 1.5	2.25 ± .10
	22	5	275 ± 2.0	15.7 ± .3	.95 ± .03	49.9 ± 1.6	3.02 ± .10

* S = Sea level.

† Stand. error = $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$

Hypoxia before birth might be a contributing factor, but additional assumptions would still be necessary, such as: a substance from the mother passes the placenta and stimulates the fetal marrow; further, that this substance is not produced by the fetus itself immediately after birth in adequate amounts to maintain erythropoiesis at the birth level.

A pituitary fraction has recently been described which stimulated erythropoiesis in the hypophysectomized, adrenalectomized and normal rat(6-10) which is also able to prevent the development of neonatal anemia when injected into the newborn rat(11,12). Since it is known that the pituitary does not produce or release some of the known trophic hormones in the early period of life, it is not impossible that lack of production or release of the erythropoietic factor, during the neonatal period, results in the development of neonatal anemia and in the failure of the newborn rat to respond to hypoxia. The implication that the pituitary is at least in part responsible for the erythropoietic response to hypoxia is supported by the fact that hypophysectomized rats give an impaired response to hypoxia as compared with normal animals(5).

Summary. Suckling rats, between the age of 4 to 16 days, do not respond to hypoxia with an increased erythropoiesis as judged by increased hematocrit, hemoglobin and red cell volume.

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