THE INFLUENCE OF MATERNAL HYPOXIA UPON THE HYPOXIA TOLERANCE AND PROPERTIES OF BLOOD OF THE NURSING OFFSPRING

by

Robert E. Mammen

A THESIS

Presented to the Department of Physiology
and the Graduate Division
of the University of Oregon Medical School
in partial fulfillment
of the requirements for the degree of
Master of Science

May 1958

APPROVED:

[redacted]

(Professor in Charge of Thesis)

[redacted]

(Chairman, Graduate Council)

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

ACKNOWLEDGMENTS

The Author is indebted to Dr. B. B. Ross for his assistance in preparation of this work. Appreciation is expressed to Mrs. Edith Mammon for typing this thesis.

INTRODUCTION

The physiologic alterations which occur during altitude exposure have been the subject of intense investigation for nearly a century. Though the gross, readily observed changes have been studied repeatedly, little is known of the basic physiologic mechanisms involved in adaptation to hypoxia or high altitude.

From these studies, which will be described more fully in another section of this paper, there have developed two major concepts of the nature of this adaptation to altitude; the concept of cellular response to hypoxia and the "extra-cellular adaptation" or adaptive responses leading to alteration of the cellular environment such as respiratory alterations, blood changes and cardiovascular changes.

Many changes are here included in the category of extra-cellular response to hypoxia. Of immediate interest, are the changes in the blood of the individual adapting to hypoxia. The mechanism responsible for the increase in hematocrit, hemoglobin and red cell count is poorly understood and for lack of evidence, it has been considered that hypoxia acting directly on bone marrow is responsible for the change (1).

Recently Grant (2) reported certain observations which indicate erythropoietic stimulation without bone marrow hypoxia. He observed a rise in the red cell

count, hematocrit and oxygen capacity in the blood of nursing infants of hypoxic mothers (rats and mice). From this, he postulated a compound or hormone in the mothers' milk capable of inducing hemopoiesis in the normal offspring. From this observation many important questions are raised. If this compound were hemopoietically active, would it not also be possible that other alterations known to occur in altitude adaptation might be produced and studied in a similar experimental situation?

It was decided that this observation by Grant [2] could serve a useful purpose in the investigation of the adaptation to hypoxia and the possible role of hormonal stimuli in altitude acclimatization.

In making use of Grant's [2] observations and experimental design for the study of adaptation to hypoxia, it was first necessary to assume that his observations were valid and reproducible. The design of the experiments carried out in this program required that the validity of Grant's [2] observations and the possible effect of the postulated humoral agent could be established at the same time. Therefore, standard tests of hypoxia tolerance were used to determine the gross response of the infants to hypoxia and detailed studies were carried out to determine if any changes occurred in the blood which differed from the normal changes associated with growth and development.

It was decided that the acute hypoxia tolerance of the offspring of hypoxic and normal mothers would be used to evaluate the possible transference of hypoxia resistance. Hematocrit and hemoglobins were followed during the experimental procedure. Barker (3) has shown high oxygen affinity hemoglobin in the blood of animals exposed to hypoxia and the question of a different type hemoglobin occurring in altitude acclimatization has been raised. Thus, it was decided to evaluate the type of hemoglobins present in mother and offspring.

REVIEW OF THE LITERATURE

Adaptation to altitude hypoxia has been under investigation since the early 19th century. Paul Bert [4] in his observations on barometric pressure made the earliest description of the process of adaptation. He mentioned in his treatise "the respite from mountain sickness gained by climbers after a stay at altitude." He also recognized the necessity of oxygen in high altitude ascent.

It has become apparent that the limit of full adaptation to altitude is at an altitude of 17,500 feet for permanent residence (B=387, PO_2=81 mm Hg). Natives of the Aucanquilcha mining district in Chile, South America reside at 17,500 feet and ascend to 18,800 feet to work. Permanent residence above 17,500 feet quickly induces signs and symptoms of mountain sickness [5].

The latency of the various responses to altitude range over a period of a few hours to six months to one year. Within the first twenty four hours after arrival at altitudes above 10,000 feet, detectable changes in ventilation, cardiac output and heart rate appear. Associated with the increased ventilation are a reduction in PCO_2 and an increase in arterial pH (respiratory alkalosis) [6,7,8].

During the next few weeks at altitude there is an increase in red cell count, hemoglobin and hematocrit,

increased blood viscosity, reduction of the total alkali reserve, cardiac hypertrophy, and further alteration in blood pH. These are considered to be the compensatory responses induced by the earlier rapid responses (1,6,9).

The responses requiring six months to a year have been studied only recently, and include, return to a normal blood pH, normal total alkali reserve, increased standard work capacity, low oxygen debt after exercise and low blood levels of lactic and pyruvic acids during work. In addition, the oxygen dissociation curve is shifted to the right, indicating that there is less affinity for oxygen than hemoglobin of the normal sea level individual. These responses may be due to cellular adaptation (10).

In rapid ascent to high altitude the primary compensatory response is considered to be the increase in pulmonary ventilation. There is an abrupt increase at 12,000 feet and ventilation continues to increase with increasing altitude (7). This hyperpnea is considered to be a result of the hypoxic stimulation of the aortic and carotid bodies (11). The beneficial effect of hyperventilation depends on the effective elevation of alveolar PO_2 and also the reduction of alveolar and arterial PCO_2. The latter change enables the arterial blood to obtain a higher oxygen saturation by virtue of the Bohr effect (8). Carbon dioxide output is markedly increased during the initial phase of altitude hyperpnea, then

decreases slowly until the new steady state is reached (7,8). The loss of CO_2 is accompanied by an increase in pH of the body fluids, which is later compensated by excretion of cation, and the consequent reduction in alkali reserve. Riley, Otis and Houston (7) have stated that acclimatization to altitude is an adaptation to a low PO_2 and a low PCO_2.

Of the alterations which occur in blood at altitude, Viault is given credit for the initial observation of an increase in red cell count (12). Since Viault, numerous other investigators have confirmed repeatedly that polycythemia occurs at altitude. However, whether it is a necessary component of adaptation or not is still debated. There are many healthy individuals acclimatized to altitude who have no more hemoglobin than would be expected at sea level (5). It has been stated that polycythemia is not essential in the process of acclimatization (6). Altland and Highman (13) have shown that rats exposed to altitude adapt to their environment without developing polycythemia when folic acid antagonists are administered in order to inhibit red cell maturation in the bone marrow.

In Monges disease or high altitude disease, the person loses his tolerance to hypoxia, develops a tremendous polycythemia (9 million RBC/cmm), and still is obliged to move to lower altitudes. This occurs after years of residence at high altitude (17,000 feet) and

may indicate cellular aging as a mechanism in the disease. When polycythemia occurs without benefit to the patient, it may be argued that polycythemia is not a significant feature of adaptation to hypoxia.

The electrolyte shift occurring during acute exposure to altitude has been well documented. However, Hurtado (10a) has collected evidence indicating that the electrolyte balance after initial adaptation is not directly comparable to that of natives or permanent residents in the same environment. It has been demonstrated that for weeks after arrival at altitude, the newcomer is in a condition of alkalosis. This is slowly compensated by excretion of cation and reduction in alkali reserve (5,9,10a). Except for alteration in chloride and bicarbonate concentration, the total electrolyte balance of natives at altitude is similar to that occurring at sea level. The bicarbonate buffer system $\left[\frac{HCO_3^-}{H_2CO_3}\right]$ is reduced in the native and replaced by chloride. The pH of the blood is within the limits of the range accepted as normal (10).

It has become increasingly apparent that an adequate explanation of altitude acclimatization must be derived from study of cellular or hormonal responses to hypoxia. Workers in the field of hypoxic adaptation have just begun to present evidence in the last five to ten years concerning cellular activity and enzyme systems which become altered during the stress of hypoxia.

R.T. Clark, Jr. [14] found, in a study of iron deficient rats at altitude, an increase in the efficiency of oxygen utilization by observation of the succinoxidase and dehydrogenase systems. He also discovered during this study that survival at altitude was independent of hemoglobin concentration. H.G. Albaum [15] has reported that no alteration of cytochrome oxidase in rat brain occurs during or after acclimatization to altitude.

Hurtado [10] presents indirect evidence that adaptation occurs at the cellular level. In his study of residents at sea level (Lima, Peru) and at altitude (Morococha, Peru, 14,000 feet), the observations reported show maximal work tolerance at altitude was nearly twice as great as that at sea level. Further differences associated with the superior performance of an acclimatized native at altitude were higher ventilation, lower oxygen consumption, and a lower total oxygen debt with a slower pay off of the debt, when compared to his counterpart at sea level. Hurtado [10] calculated the acclimatized man's net gain in efficiency at 11.5% over man adapted to sea level conditions.

In the study of intact and adrenalectomized parabiotic rats, Hoelscher [16] has reported a polycythemia occurring in both animals when the intact partner was exposed to hypoxia. Unfortunately, exposure of the adrenalectomized partner was not included in the experimental procedure.

C. F. Merino (17) has presented evidence indicating the presence of a plasma erythropoietic factor in the serum of altitude acclimatized man. The injection of 300 cc of plasma from acclimatized individuals into normal sea level adapted man resulted in a 112% increase in reticulocytes in the peripheral blood. Injection of plasma from men adapted to sea level into normal men resulted in a 9% increase in reticulocytes.

Grant (2) in reporting an increase in red cell count, hematocrit and oxygen capacity in the blood of infants nursing hypoxic mothers, has added evidence supporting some humoral factor in erythopoietic stimulation. The hematocrits of his control animals ranged from 25% at the beginning of the study, dropped to 20% and returned to 25% at the completion of the study when individual animals showed no measurable difference. The test litters began with a hematocrit of about 31%, fell to 26% and returned to 28% hematocrits during the experimental period. The red cell count of the control animals dropped from 3.8 million to 3 million and finally rose to 4.2 million RBC/cmm. The test litters remained above 4.1 million RBC/cmm throughout the experimental procedure. The oxygen capacity of the control bloods remained at 10 volumes per cent throughout most of the experiment, but finally rose to 11 volumes per cent. The capacity of the test litter bloods rose from 12 volumes per cent to 13.5 volumes per cent and fell to 11-11.5 volumes per cent at

the termination of the experimental procedure. The paper presented a graphic description of the results of the test without data, means or variances which could be compared.

The study of hypoxia tolerance in animals requires an observable sign upon which a reliable estimate of adaptation to hypoxia can be fixed. An adequate and reliable test is the time required for an animal to loose its righting reflex (the ability to regain its footing) at a given barometric pressure.

M. J. Fregley (18) utilized an inclined plane in a decompression chamber and elevated the animal to 39,000 feet. The time from ascent until the animal rolled down the inclined plane is considered the "no righting time". During 14 daily exposures to altitude, the animals in Fregley's (18) study, tripled their "no righting time". This was an indication that adaptation occurred even during short term exposure. Thus, tolerance procedures performed even once upon an animal may cause some alteration of tolerance.

J. N. Barker (3) was especially interested in high affinity hemoglobin* and its association with hypoxia tolerance. She has presented evidence which shows a consistent positive correlation between the presence of high affinity hemoglobin and hypoxia tolerance. Infants

* High affinity hemoglobin: The affinity has been defined as that partial pressure of oxygen at which 50% of the hemoglobin is saturated with oxygen or the P_{50} saturation value.

exposed to hypoxia retained high affinity hemoglobin and tolerance during exposure and the disappearance of the two ran a parallel time course. In the study of the hemoglobin affinity, high affinity was found in all cases of exposure to altitude, though there was a range of affinities ranging from the control values of 32 mm Hg PO_2 to 25 mm PO_2 for ½ saturation values. The affinities were determined at pH 6.80 to avoid the Bohr effect on hemoglobin affinity. Barker (3) decided that there was a factor in determination of hypoxia tolerance in the modification of affinity of hemoglobin for oxygen or modification of hemoglobin type. Since Barcroft's (9) report of high affinity hemoglobin occurring in altitude acclimatization and later, his report of high affinity hemoglobin in fetal blood (which lead to his classic title "Everest in Utero") the presence of high affinity hemoglobin has been studied frequently.

Hall (19) was unable to detect any change in affinity in the hemoglobins of the bloods of the members of an expedition to altitude. He also made his determinations at a constant pH. Dill (5) reports that in Colorado, his observations indicated a decrease in oxygen affinity. This agrees with Hurtado's (10) results in Morococha, Peru, where two hundred blood samples were analyzed. The O_2 dissociation curves had a ½ saturation value at a PO_2 of 27.2 mm Hg. At sea level the ½ saturation value was 24.75 mm Hg PO_2 in the blood samples of sea level adapted

man. The difference was statistically significant. It must be remembered that with the exception of the new born and the Morococha study, most studies have been performed as short term experimental situations and the results may not be comparable.

In Barker's (3) study of hypoxia treated infants, it might be possible that the presence of fetal type high affinity hemoglobin is prolonged by the hypoxic stimulus.

METHODOLOGY

The premise for the experiments to be described stem from Grant's (2) observations concerning the changes observed in the blood of infant rats and mice nursed by mothers exposed to hypoxia. Accordingly, newly delivered litters and the parent females constituted the experimental population. The mothers were exposed intermittently to a simulated altitude in an altitude chamber of similar design to that used by M. J. Fregley (18). The influence of hypoxia was followed up to a period of 49 days after birth. The changes in infant hematocrit, hemoglobin, acute hypoxia tolerance and alkali resistance of the hemoglobin were followed. Normal changes associated with infant maturation and growth were observed in a control group. The specific tests will be discussed in the following section.

The exposure of the nursing mothers to hypoxia was begun four or five days postpartum. They were exposed four hours daily to a PO_2 ranging from 65 to 85 mm Hg, until the infants stopped nursing. The mothers were tested for hypoxia tolerance and their blood examined for alterations in hemoglobin, hematocrit and alkali resistance. The infants were tested at intervals for their acute hypoxia tolerance and changes in hemoglobin, hematocrit and alkali resistant hemoglobin.

Production of Hypoxia

The chamber was constructed to produce hypoxia without alteration in barometric pressure. A 2' x 2' x 4' plywood box was built with a removable door. A shelf for animal cages was positioned three inches above the floor of the chamber. The chamber below this shelf housed trays for baralyme CO_2 absorbers, a ventilating fan and shaft connected to the main chamber, and the entrance gas port. The exit gas port was located in the upper chamber and entered by a length of rubber tubing. The reduction in oxygen concentration was accomplished by flushing the sealed chamber with nitrogen. The PO_2 in the effluent gas was continuously analyzed in a Beckman Oxygen Analyzer. When the partial pressure of oxygen reached the range 65-85 mm Hg, the nitrogen was turned off and compressed air was introduced at a flow of 1.2 to 1.5 liters per minute. Carbon dioxide was measured intermittently with a Cambridge CO_2 Meter. When PCO_2 values rose above 1%, trays of new baralyme were placed in the chamber. The nursing mothers were placed inside the chamber in animal cages and exposure for four hours undertaken. Animal cages were used primarily to keep the animals quiet during exposure. When the infants no longer appeared to be nursing, exposures were discontinued.

Treatment of Adult Rabbits

Within the week following the last exposure to hypoxia, the adult animals were exposed to decreasing oxygen concentration until loss of righting reflexes occurred. Blood was obtained by cardiac puncture. Hematocrits, hemoglobins and alkali denaturations were done.

Treatment of Infant Rabbits

The infant rabbits were exposed to acute hypoxia, to test righting reflexes, in a decompression chamber adapted from a dessicator jar. Alkali denaturation, hematocrit, and hemoglobin, determinations were done on blood samples from the infants.

Treatment of Adult Rats

The adult rats were exposed to decompression to determine acute hypoxia tolerance and loss of righting reflexes.

Adult rats were decompressed to B = 200 mm Hg and exposed for thirty minutes to each 10 mm Hg decrement to B = 140 mm Hg, or until all animals were dead. Animals were counted (living and dead) at each change in barometric pressure, to determine the pressure at which 50% of the animals were dead. Blood was obtained from living adults, by cardiac puncture, or by cutting off the tip of the tail. Hematocrits and hemoglobins were determined.

Treatment of Infant Rats

Infant rats were decompressed in the dessicator adapted for decompression, to determine their righting reflex changes occurring with acute exposure to hypoxia. Hematocrit and hemoglobin determinations were done on blood from infants ranging from 17-49 days of age. The infants were exposed to decompression, staying at each 10 mm Hg decrement for 30 minutes to determine the pressure at which 50% of the animals were dead.

Procedures

Loss of Righting Reflex

A dessicator was connected in series with a vacuum pump and a barometer. A regulator needle valve was placed in the rubber stopper at the top of the dessicator. The animal was placed in the dessicator and rapidly decompressed to a barometric pressure of 250 mm Hg. The jar was tilted on its side and rolled while the pressure was reduced at 10 mm Hg decrements every three minutes. The pressure at which the animal was no longer capable of regaining its footing was considered the critical pressure and recorded. PO_2 was calculated from the observed pressure. Example: The animal lost its righting reflex at B = 147 mm Hg.

Then $PO_2 = (B - PH_2O) (.209)$

$PO_2 = (147 - 47) (.209)$

$PO_2 = 20.9$

Hemoglobin Determinations

For simplicity, Heilmeyer's (20) alkaline hemoglobin method was chosen for hemoglobin determinations. Blood was drawn by cardiac puncture. A 0.10 ml. sample was pipetted into 9.90 ml. of 0.4% NH_4OH. The optical density of the solutions at 480 mu was recorded, using a Beckman Spectrophotometer, Model B.

A graphic relationship of the optical density of the alkaline hemoglobin versus grams of hemoglobin had previously been constructed (Fig. 4). The Heilmeyer (20) method was compared against the grams of hemoglobin in a blood sample, as determined by the method of Wong (21), utilizing a standard iron solution at 480 mu. In figure 4, the optical density of a sample of alkaline hemoglobin was then directly converted to grams of hemoglobin.

Hematocrits

The hematocrits were done by a capillary tube microhematocrit technique adapted from the description by McInroy (22). Thin walled, three inch long 0.8 mm I.D. capillary tubes were heparinized and dried. The blood sample obtained by cardiac puncture was discharged into a heparinized tube and gently shaken to insure adequate mixing. The capillary tube was introduced into the blood sample and allowed to fill by capillary

attraction to a height of 1 cm. from the clean end. The clean tip was then fired, sealed and flattened. The tubes were centrifuged at 2000 g for thirty minutes. The error when compared to Wintrobe hematocrit tube determinations was within ±1% of the Wintrobe hematocrit.

Alkali Denaturation

The remaining blood was centrifuged. The red cells were washed with saline three times and hemolyzed. The solution was centrifuged, the clear layer removed with a clean needle and syringe and adjusted to a 10% ± 1% hemoglobin solution. For ease, simplicity, and sensitivity the timed alkaline denaturation method of Singer et. al. (23) was employed.

In using Singer's (23) method, a denaturation constant must be obtained. The ½ denaturation time is defined as the time required for 50% of the initial concentation of hemoglobin to be denatured. The ½ denaturation time is also species specific (24). It must be determined by following the continuous alkaline denaturation of the hemoglobin of the species in question, until the curve reaches a base line asymptotic minimal optical density on the spectrophotometer.

Required for the determination is a solution of N/12 KOH or NaOH, pH 12.7, either freshly prepared, or stored in refrigerated paraffin lined bottles. A

precipitating reagent is prepared by adding to 800 ml. of 50% saturated $(NH_4)_2SO_4$ a 2 ml. portion of 10 N HCl. This is used to remove chromogens from the solution.

To 1.6 ml. of N/12 KOH is added 0.10 ml. of 10% hemoglobin. A stop watch is started, the tube shaken gently for 10 seconds, and exactly 15 minutes later, 3.4 ml. of precipitating reagent is added. The tube is shaken six times and filtered thru a double layer of filter paper. The optical density at 540 mμ is obtained from the spectrophotometer and recorded. A blank is prepared using 1.6 ml. N/12 KOH and 3.4 ml. of precipitating reagent. The optical density of the total hemoglobin present is determined by adding 0.10 ml. of the 10% hemoglobin solution to 5 ml. of distilled water. The percent resistant hemoglobin present is then calculated and extrapolated back to zero time.

RESULTS

Acute Hypoxia Tolerance of Infant Rabbits

Figure 1 is the graphic representation of the PO_2 at which loss of righting reflexes occurred during acute hypoxia of the test litters at different ages. It can be seen that there is a wide variation in response between litters, as well as in the same litter at different ages (table 1).

Figure 2 is the illustration of the PO_2 at which loss of righting reflexes occurred during acute hypoxic exposure of control litters at different ages. It is readily apparent that there is a wide range of variation in the response of a litter as maturation occurs. The response of the separate litters is also widely dispersed (table 2).

Figure 3 is a composite graph, comparing PO_2 at loss of righting reflexes and age, of both the control and test rabbit litters. By inspection of the extreme variation of the test and control litters, it can be seen that a demonstration of a difference between test and control animals cannot be made.

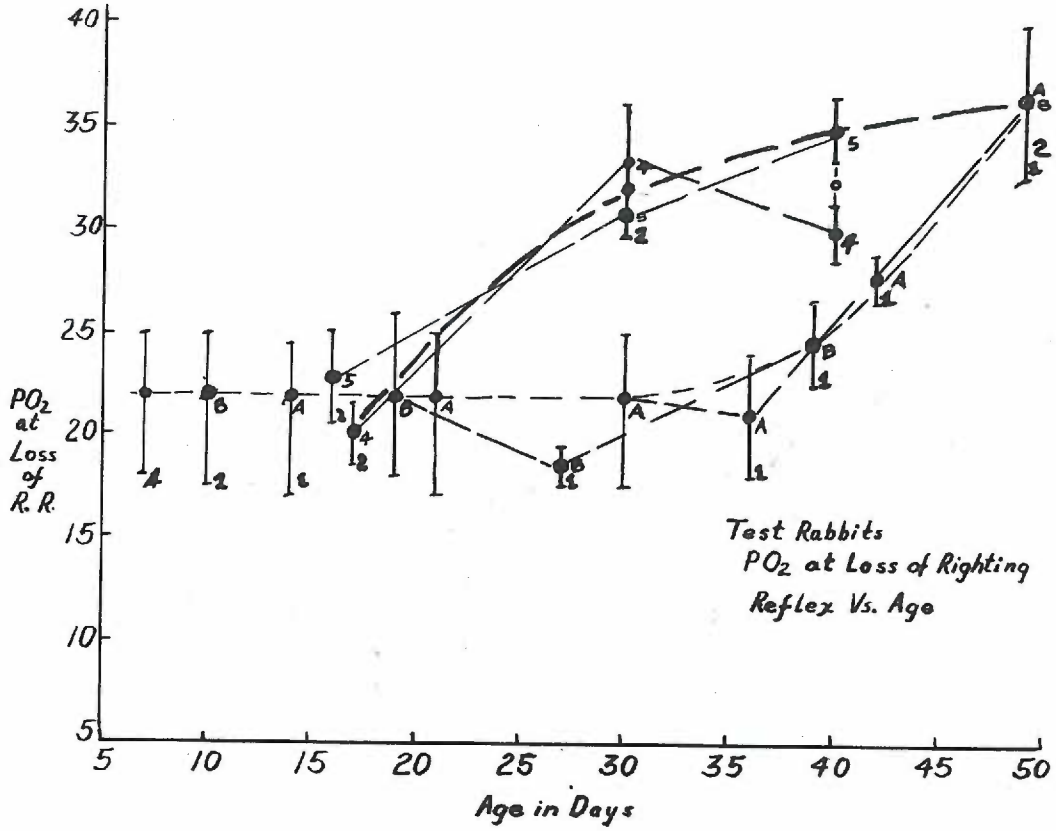
The dessicator air flow during decompression was considered to be adequate to avoid the possibility of the animal rebreathing his own respiratory gases with the concomitant increase in PCO_2 and decrease in PO_2. Table 3 lists the calculated air flow at various reductions in barometric pressure.

Figure 1 (above)

Acute hypoxia tolerance of infants of hypoxic rabbits. The PO_2 at which loss of righting reflexes occurred in the infants of hypoxic mothers from 7-49 days of age. Individual litters are referred to by the letters A, B, numerals 4 and 5. Numeral 1, first group; numeral 3, second group; vertical lines, 95% confidence limits of the means.

Figure 2 (below)

Acute hypoxia tolerance of control infant rabbits. The PO_2 at which loss of righting reflexes occurred in the control litters as they matured (7-49 days of age). Numeral 1, first group control; numeral 2, second group control; vertical lines, the 95% confidence limits of the means.


40
35
30
25
20
15
10
5
PO_2 at Loss of R. R.
5
10
15
20
25
30
35
40
45
50
Age in Days
Test Rabbits
PO_2 at Loss of Righting Reflex Vs. Age

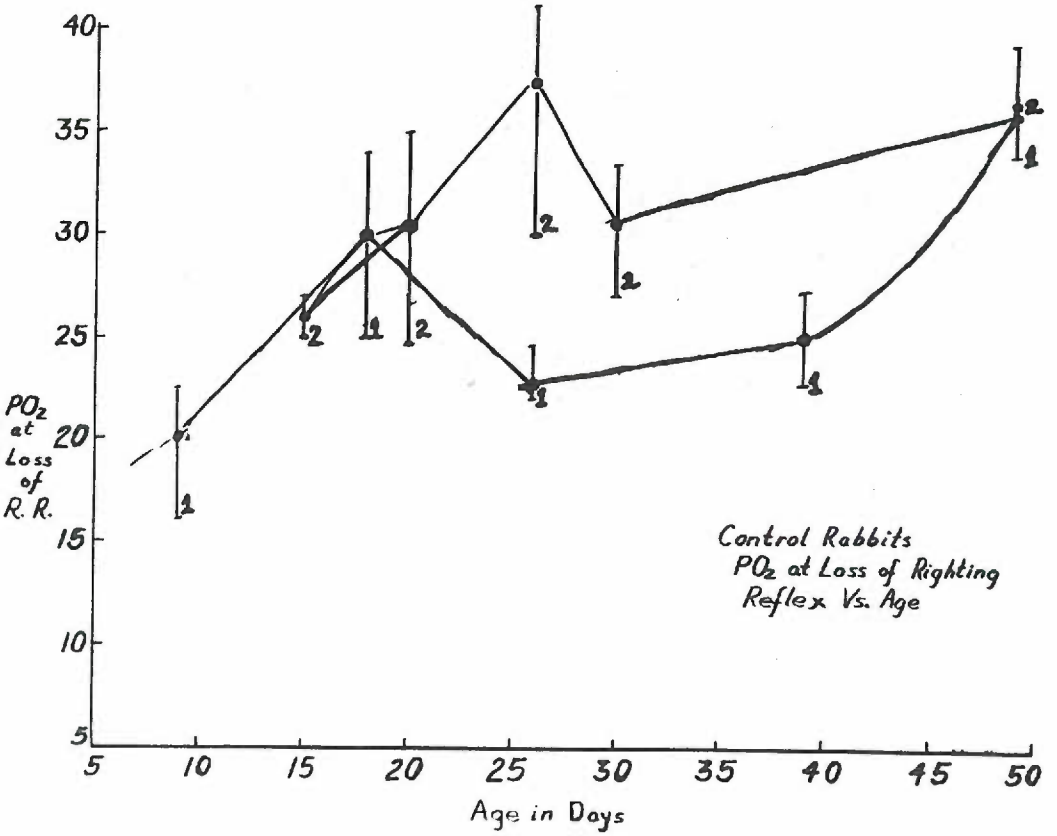
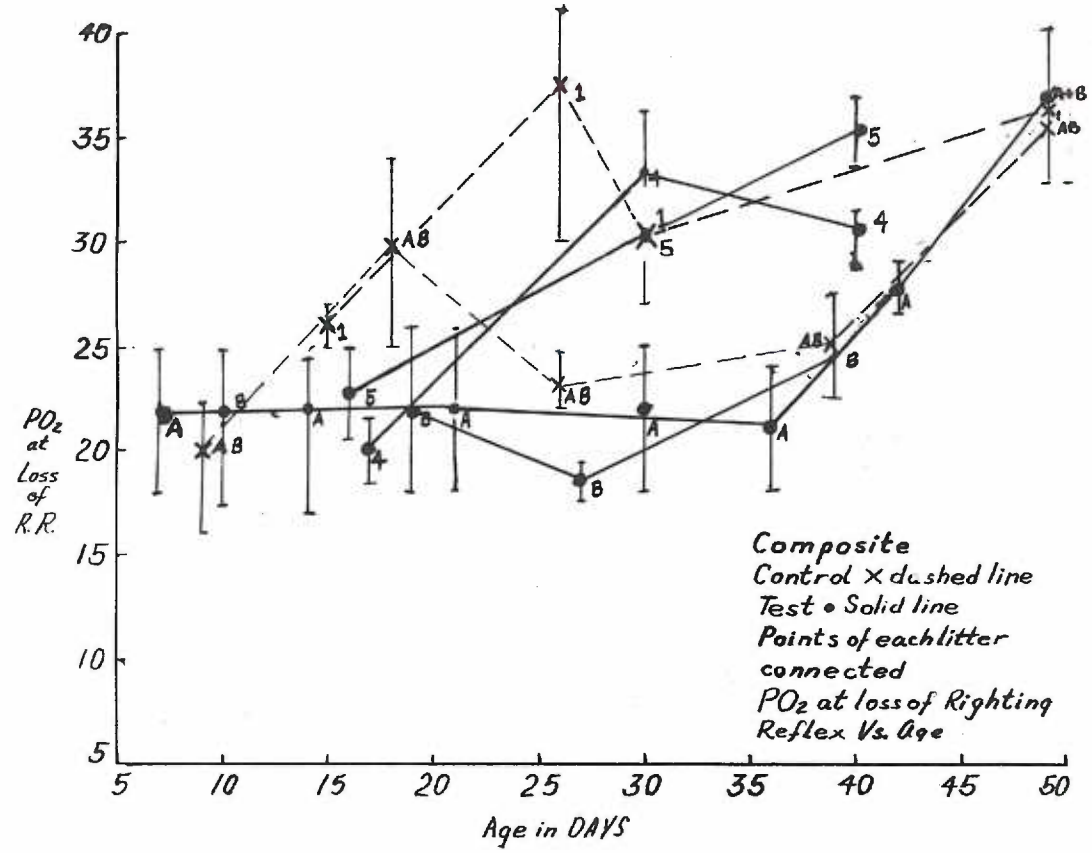

40
35
30
25
20
15
10
5
PO_2 at Loss of R. R.
5
10
15
20
25
30
35
40
45
50
Age in Days
Control Rabbits
PO_2 at Loss of Righting Reflex Vs. Age

Figure 3 (above)

The composite of the acute hypoxia tolerance of control and test infant rabbits (age 7-49 days). The PO_2 at which loss of righting reflex occurred. Letter AB, first group control; letters A and B, test litters. Numeral 1, second group control; numerals 4 and 5, test litters. Control animals x dashed lines. Test animals . solid lines. Vertical lines, 95% confidence limits of the mean.

Figure 4 (below)

Optical density of the Heilmeyer alkaline hemoglobin versus grams hemoglobin/100 cc. as determined by the Wong Standard Iron Method.


40
35
30
25
20
15
10
5
PO2 at Loss of R.R.
5
10
15
20
25
30
35
40
45
50
Age in DAYS
Composite
Control × dashed line
Test • Solid line
Points of each litter connected
PO2 at loss of Righting Reflex Vs. Age

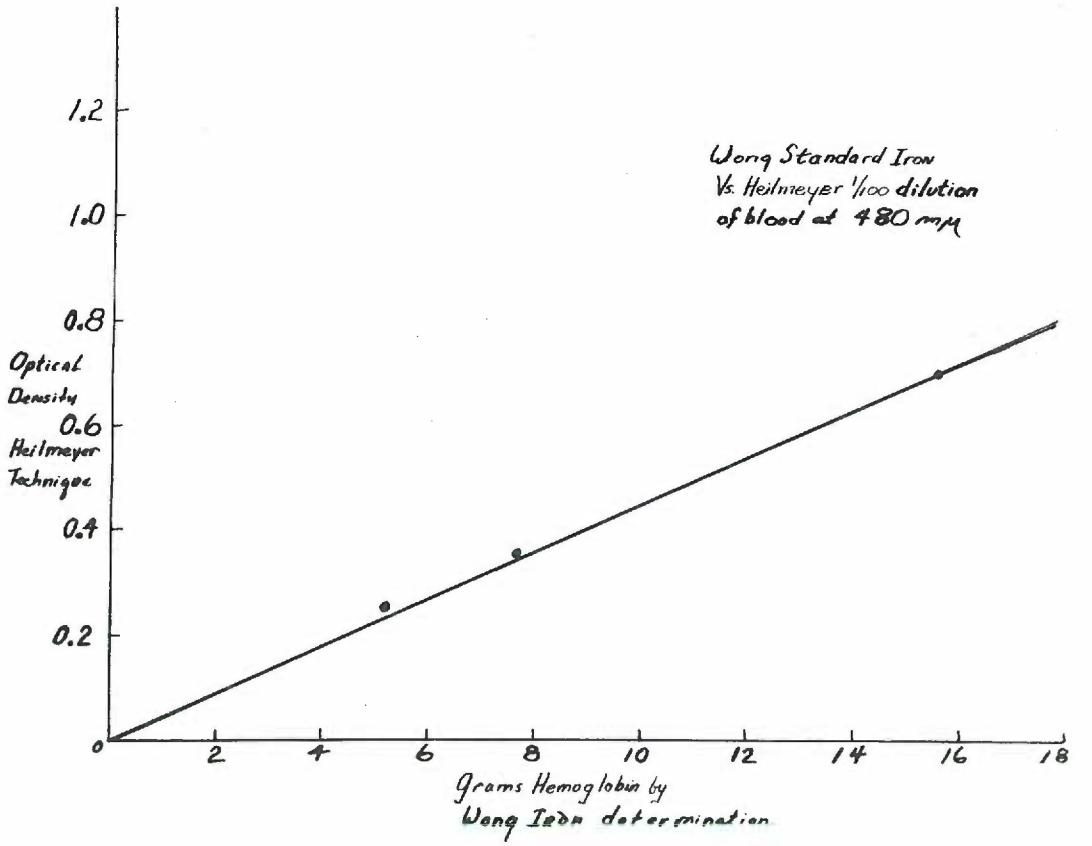

1.2
1.0
0.8
0.6
0.4
0.2
0
Optical Density Heilmeyer Technique
2
4
6
8
10
12
14
16
18
Grams Hemoglobin by Wong Iron determination
Wong Standard Iron Vs. Heilmeyer 1/100 dilution of blood at 480 mμ

Figure 5 is the representation of the change in hemoglobin and hematocrit of the test and control litters from seven to forty-nine days of age. The illustation clearly shows the alteration normally associated with the physiologic anemia of the neonatal period. No other alteration, which could be ascribed to the test procedure, is demonstrated in this figure (table 4).

Adult Rabbits

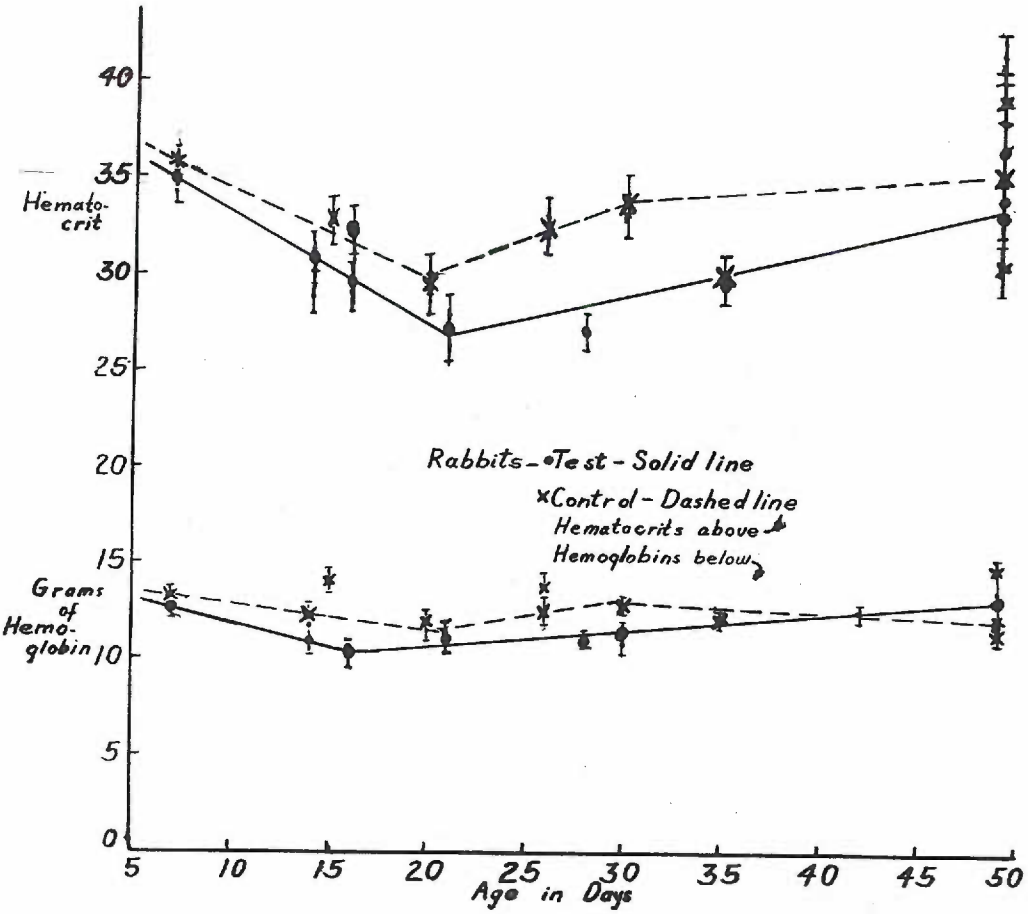
Table 5 represents the acute hypoxia tolerance of the adult animals, control and hypoxia exposed. The adult rabbits were exposed to acute hypoxia by reduction in oxygen concentration in a lucite chamber. Their size interfered with the use of the decompression chamber. The response of the rabbits who had been exposed to hypoxia was dramatically different from the response of the rabbits who had never been exposed. The three control animals lost consciousness within three minutes after arrival at a PO_2 of 32-30 mm Hg on the scale of the Beckman Oxygen Analyzer. The four adults who had been exposed to hypoxia at 65-85 mm Hg PO_2 were able to maintain their equilibrium and consciousness at a PO_2 of 20-22 mm Hg for ten minutes. The scale of the Beckman Oxygen Analyzer had to be extrapolated to obtain the approximate PO_2 for the tolerance of the chronic hypoxia exposed animals (table 5). The flow of nitrogen-oxygen mixture was 1.2-1.5 liters per minute.

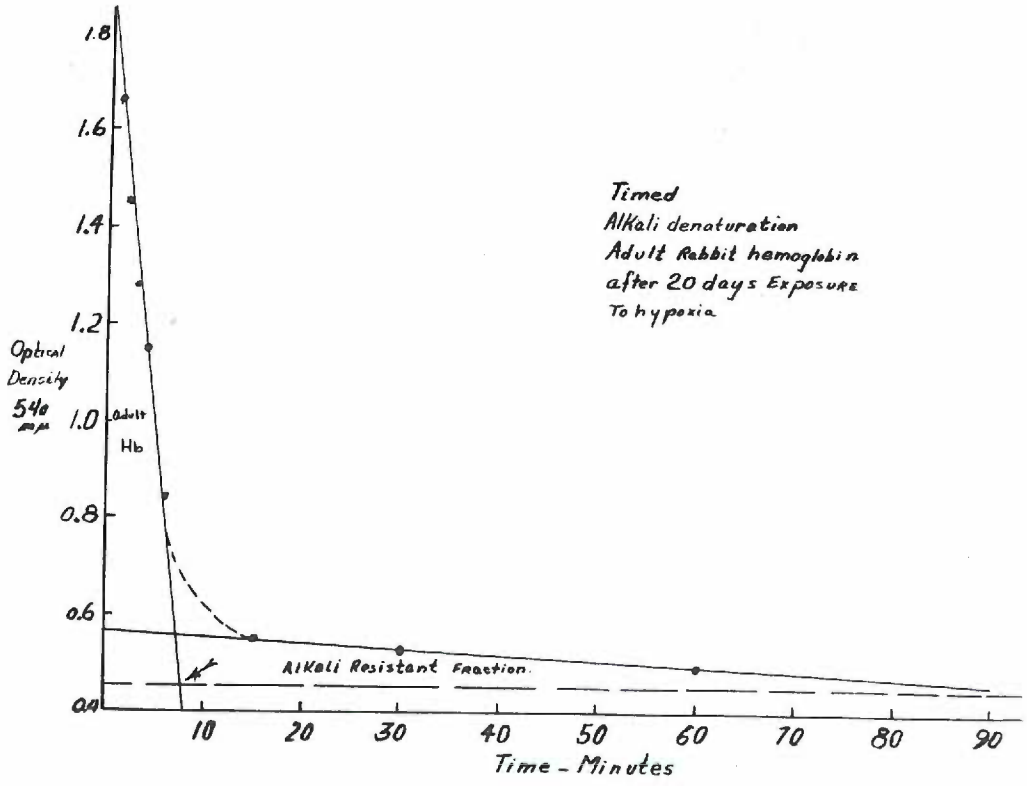
Figure 5 (above)

Alterations in hematocrit and hemoglobin concentrations occurring in the blood of infants of control and hypoxic rabbits during the first 49 days of life. Control litters x dashed lines. Test litters • solid lines. Vertical lines, standard deviations of the means.

Figure 6 (below)

Timed alkali denaturation of an adult rabbit hemoglobin after 20 days exposure to hypoxia. The steeply descending portion is the denaturation of adult hemoglobin; the slowly descending portion is the denaturation of resistant hemoglobin. Arrow indicates point in time at which extrapolation of steeply descending portion of curve intersects the minimal optical density; and represents 100% denaturation of adult hemoglobin (8 minutes). Slowly descending portion intersects minimal optical density at 100 minutes.


Hemato-crit
Grams of Hemo-globin
Rabbits - •Test - Solid line
×Control - Dashed line
Hematocrits above
Hemoglobins below
Age in Days


Optical Density 540 mμ
Adult Hb
Alkali Resistant Fraction
Time - Minutes
Timed
Alkali denaturation
Adult Rabbit hemoglobin
after 20 days Exposure
To hypoxia

The hemoglobin and hematocrit values of the hypoxia exposed mothers were, on the average, slightly higher than those of unexposed mothers. These values are listed in table 5.

Alkali Denaturation

The alkali denaturation of a hemoglobin solution obtained from a hypoxic mother revealed the presence of an alkaline resistant fraction. Figure 6 represents the timed alkaline denaturation curve of that blood and is the curve from which the ½ denaturation constants for the adult and resistant rabbit hemoglobins were determined. In figure 6, the steeply descending portion of the curve represents the denaturation of the adult type hemoglobin. The slowly descending portion of the curve from time 15 minutes to 100 minutes is the denaturation rate of the alkaline resistant hemoglobin. At 100 minutes the optical density reached a minimum, with no further change in density during the next 24 hours. The steeply descending portion is extrapolated to an intersection with the minimal optical density. The time required to reach this optical density is considered the 100% denaturation time of the adult hemoglobin and is read from the time scale on the abscissa. The extrapolation to the minimum intersects at T=8 minutes. For denaturation to be 99.4% complete, seven equal time intervals are required. Thus, $(\frac{8 \times 60'}{7})$ 68.5 seconds is the ½ denaturation time of the

adult hemoglobin. The alkaline resistant hemoglobin reaches minimal optical density in 100 minutes. The ½ denaturation time is then 857 seconds ($\frac{100 \times 60''}{7}$).

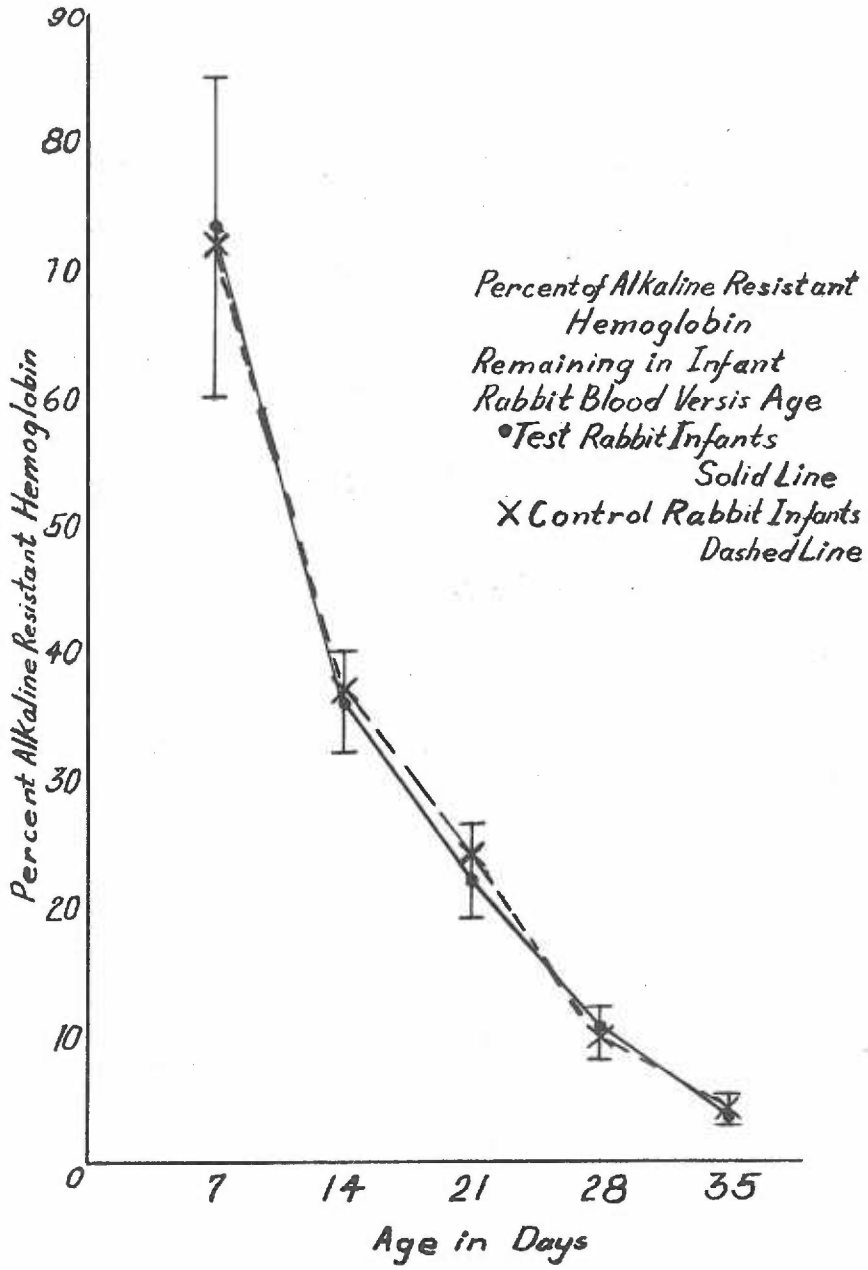
Table 6 tabulates the values obtained by the alkali denaturation of the adult rabbit hemoglobins. The adult animals exposed to hypoxia, using these denaturation constants, had 9% or more alkaline resistant hemoglobin. The adults not exposed to hypoxia, had less than 3% alkaline resistant hemoglobin in their blood. Though the number of animals is small, the difference between the two groups is great enough to justify the assumption that chronic exposure to hypoxia does increase the resistant fraction of hemoglobin.

Alkali Denaturation of Infant Rabbit Hemoglobin

Figure 7 represents the serial alkali denaturation values obtained from the infant bloods, test and control, from seven days of age to thirty-five days of age. The illustration shows the parallel time course of the disappearance of resistant hemoglobin from the blood of both test and control animals. It is evident from the illustration, that there is no demonstrable difference in the rate of loss, nor any prolongation of the presence of resistant hemoglobin in the test litters. Table 7 is the tabulation of data derived from alkali denaturation of the infants' hemoglobins.

Figure 7

Percent alkaline resistant hemoglobin remaining in the blood of infant rabbits of control and hypoxia exposed adults from 7-35 days of age. Control x and dashed line. Test litters • and solid line. Vertical lines, standard deviation of the means.


Percent of Alkaline Resistant Hemoglobin Remaining in Infant Rabbit Blood Versis Age
•Test Rabbit Infants Solid Line
X Control Rabbit Infants Dashed Line
Percent Alkaline Resistant Hemoglobin
90
80
70
60
50
40
30
20
10
0
7
14
21
28
35
Age in Days

The increase in resistant hemoglobin in the adult hypoxic animals, without alteration of the disappearance rate of resistant hemoglobin in the test offspring leads to three plausible interpretations. First, if there were a humoral agent in the milk, its stimulus was so slight that its effect was unnoticeable in the maturing infants of the hypoxic mothers. Second, if there were a humoral factor involved in the development of alkaline resistant hemoglobin in the hypoxic adult, it was not transmitted to the offspring in the mother's milk. Third, it can be interpreted to indicate that there is no humoral agent involved, and each animal must be directly exposed to hypoxia to develop resistance.

Rats

Figure 8 represents the PO_2 at loss of righting reflexes as a function of increasing age in the litters of rats exposed to hypoxia. It can be seen that there is, with some variation, a decreasing hypoxia tolerance to thirty days of age. The animals then stabilize, but a wide variation in hypoxia tolerance is present (table 8).

Figure 9 is the graphic illustration of the PO_2 at loss of righting reflexes in control rat litters. It can be seen that the trend of the control litters is extremely similar to that of the test litters. There is, also apparent, the similar wide variation in hypoxia tolerance after thirty days of age (table 9).

Figure 10 is the composite of the control and test rat litters' PO_2 at loss of righting reflex as a function of age. Inspection of the variations of the test and control litters can lead visually to the fact that there is no detectable difference between test and control animals. Close inspection of the points, x control, • test, in the composite shows the intimate mixing of test and control groups throughout all determinations.

Figure 11 is the illustration of the PO_2 at which 50% of a group of animals, exposed to that PO_2 for 30 minutes, were dead. This is the estimated LD 50 for that PO_2. The control animals and the test animals follow the same LD 50 with increasing age, so closely as to be indistinguishable. Thus, it can be seen, that between test and control animals, there is no demonstrable difference.(table 10).

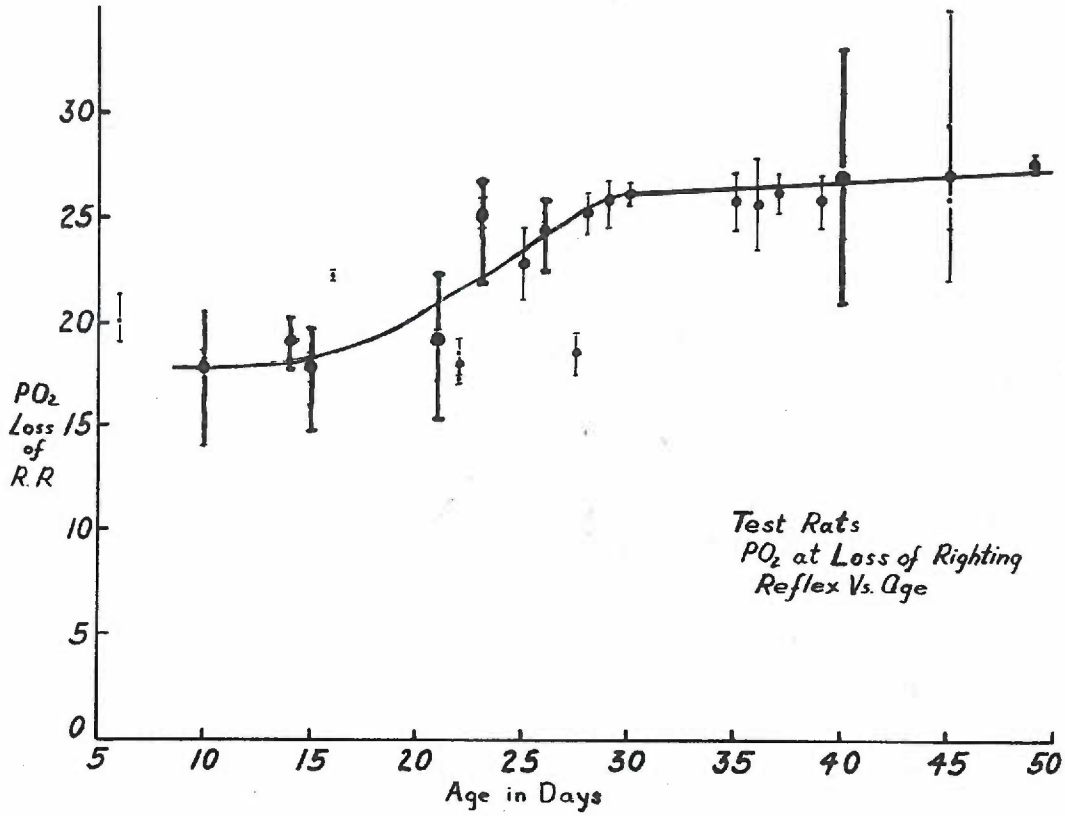
Table 10 lists the hemoglobin concentrations and hematocrit values of the control and test litters. It is readily observed that the hematocrits and hemoglobins of both groups of animals reflect clearly the physiologic anemia of the new born. However, there are no alterations which can be attributed to a difference between the two groups as result of the experimental procedure. Table 11 also lists the hematocrit and hemoglobin concentration found in seven control and ten test adult animals. It is evident that hypoxia did cause an increase in the hemoglobin and hematocrit in the animals exposed to hypoxia,

Figure 8 (above)

Acute hypoxia tolerance of infant rats nursing hypoxic mothers. The P_{O_2} at which loss of righting reflexes occurred as a function of increasing age. Vertical lines, 95% confidence limits of the means.

Figure 9 (below)

Acute hypoxia tolerance of infant rats nursing control mothers. The P_{O_2} at which loss of righting reflexes occurred as a function of increasing age. Vertical lines, 95% confidence limits of the means.


30
25
20
PO_2 Loss of R.R
15
10
5
0
5
10
15
20
25
30
35
40
45
50
Age in Days
Test Rats
PO_2 at Loss of Righting Reflex Vs. Age

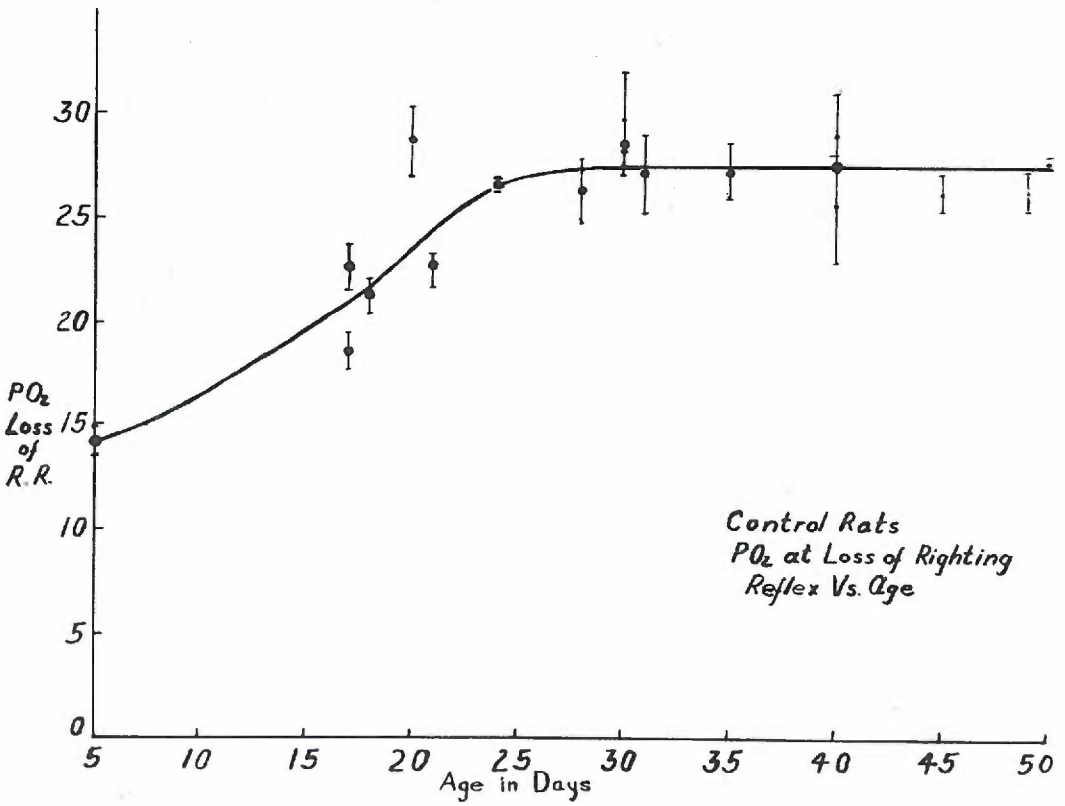
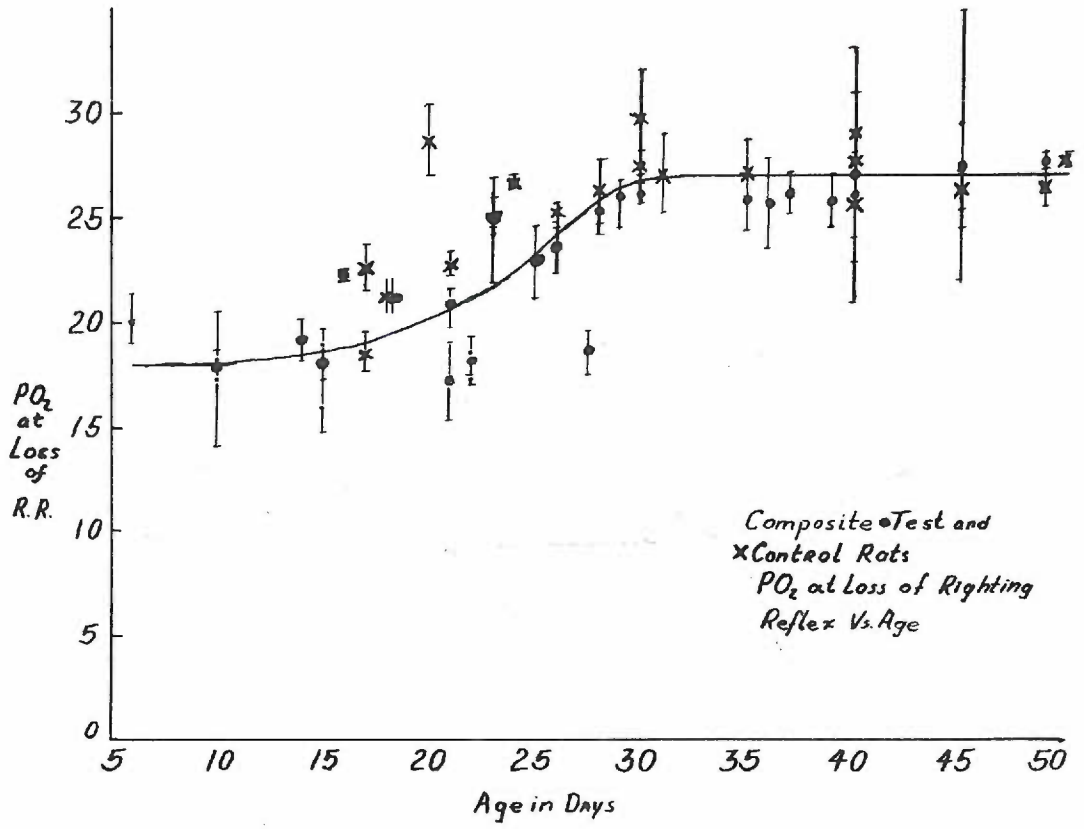

30
25
20
PO_2 Loss of R.R.
15
10
5
0
5
10
15
20
25
30
35
40
45
50
Age in Days
Control Rats
PO_2 at Loss of Righting Reflex Vs. Age

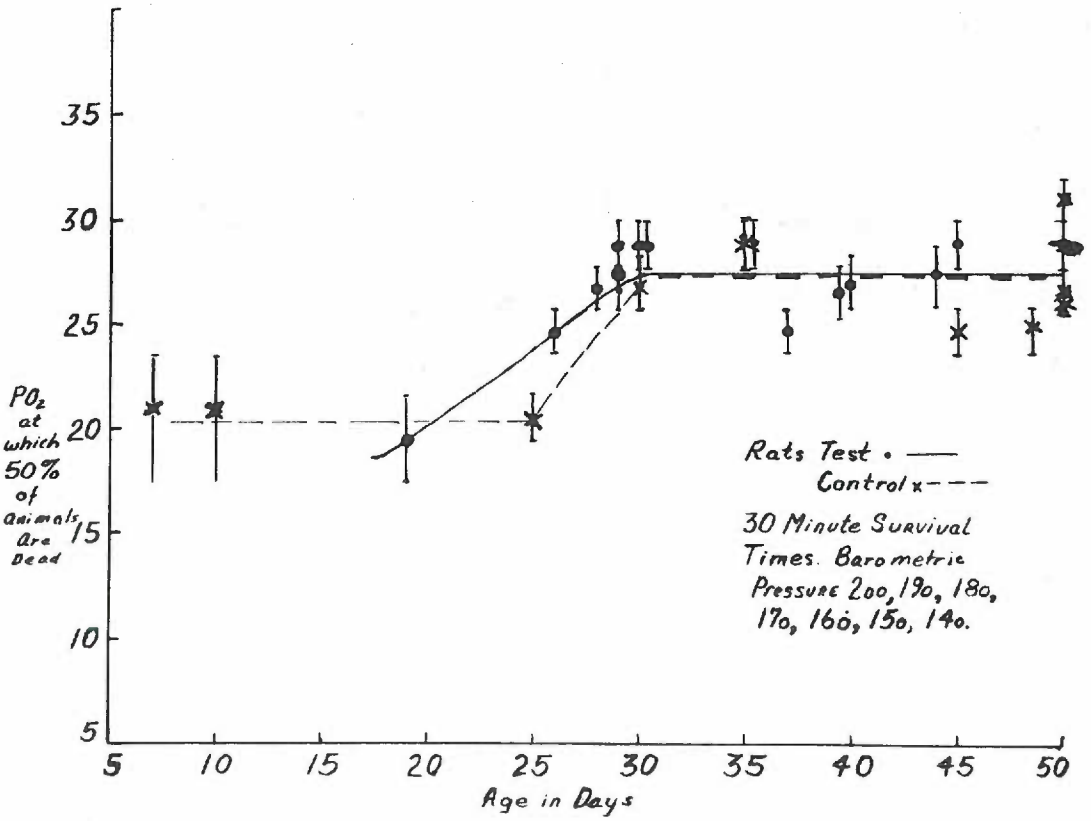
Figure 10 (above)

Composite of the acute hypoxia tolerance of the infants of control and hypoxia exposed mothers. The PO_2 at which loss of righting reflexes occurred as a function of increasing age. Control infant rats x dashed lines. Test infant rats • solid lines. Vertical lines, 95% confidence limits of means.

Figure 11 (below)

The 30 minute survival times of infants of control and hypoxia exposed mothers. Control infants x, dashed lines. Test infants •, solid lines. Vertical line, LD_{50} range of PO_2 exposure to which 50% of the animals were dead.


30
25
20
15
10
5
0
PO_2 at Loss of R.R.
5
10
15
20
25
30
35
40
45
50
Age in Days
Composite •Test and
×Control Rats
PO_2 at Loss of Righting
Reflex Vs. Age


35
30
25
20
15
10
5
PO_2 at which 50% of animals are Dead
5
10
15
20
25
30
35
40
45
50
Age in Days
Rats Test • ——
Control × - - -
30 Minute Survival
Times. Barometric
Pressure 200, 190, 180,
170, 160, 150, 140.

DISCUSSION

Evidence has been cited (2,16,17) which gave credence to the assumption that hypoxia produces a humoral factor capable of inducing polycythemia. Grant's (2) report of a polycythemia in infant rats and mice nursing hypoxic mothers was considered to be useful in the study of the mechanism of adaptation to hypoxia. It was assumed that a humoral agent might be involved in the acclimatization process, if it could be shown that hypoxia tolerance could be transmitted from mother to offspring.

The changes anticipated in the nursing offspring of hypoxic mothers were a prolongation of the high resistance to hypoxia which the new born naturally possesses, and an increase in hematocrit and hemoglobin concentration. Barker (3) reported the persistence of high affinity hemoglobin in infant rats directly exposed to hypoxia. This lead to the assumption that her rats might have continued to produce fetal hemoglobin. It follows that the infants of hypoxic animals might also continue to produce a larger than normal amount of alkaline resistant fetal type hemoglobin.

The control litters were utilized to establish the natural loss of hypoxia tolerance, the natural hematologic alterations of the neonatal period and the degree of anemia and the disappearance rate of alkaline resistant hemoglobin.

It might be pointed out that Fregley's (18,18a) work indicates that a single exposure to decompression stimulates an adaptive response which influences the resistance to hypoxia for a period of six to ten days. Thus, intermittent exposure to hypoxia has some effect on the total response. It was for this reason that exposures to hypoxia were limited to seven to ten day intervals between exposures.

It might also be pointed out that Grant (2) reported a control oxygen capacity of 10 volumes per cent or $\left(\frac{10 \text{ vols.}\%}{1.34 \text{cc} \cdot .1 \text{ gram}}\right)$ 7.43 grams/100 cc. of hemoglobin. His highest hematocrit reported was 31%. In this study, the infant rats reached a low mean hematocrit of 28% with a hemoglobin of 10 .5 grams/100 cc. In the study by Hoelscher (16), the hematocrit and hemoglobin concentration of his control and hypoxia exposed animals agreed fairly well with those observed in this study (42 , 15.4 ±.9 grams/100 cc.). Barker (3) observed in adult rats a hemoglobin of 14 grams/100 cc. The infant rats in her study were found to have a neonatal anemia of 10±1 grams per 100 cc of hemoglobin.

Thus, Grant's (2) hemoglobin of 7.4 grams/100cc. as calculated from oxygen capacity indicates he was using either an aberrant population, an anemic species of rat, or, the determinations of oxygen capacity and the hemoglobin are suspect. None the less, it might well be that

only anemic species are able to show the type of response reported by Grant (2). He utilized decompression to B=300-300 mm Hg while in this study B=760 mm Hg remained constant, with a reduction in oxygen concentration being employed to produce hypoxia. Thus, there might be a difference in the magnitude of stimulus in the two situations.

The data permit one major conclusion: There is not a transference of hypoxia tolerance from the hypoxic mother to the offspring by the milk.

The investigation has lead to the following conclusions:

1. By the methods used, maternal hypoxia has no demonstrable influence on the hypoxia tolerance of the nursing offspring.
2. The assumption of a humoral factor occurring in the milk is not valid.
3. Maternal hypoxia does cause an increase in the alkaline resistant fraction of hemoglobin in the hypoxic animal but not in the infants.
4. Whether the alkaline resistance fraction occurring in the hypoxic animals is produced by hypoxia directly or by a hormonal stimulus cannot be answered through the present method of investigation.

BIBLIOGRAPHY

1. Grant, W. C. and Root, W. S. The Fundemental Stimulus for Erythropoiesis. Physiol. Rev., 32:449-486, 1952

2. Grant, W. C. The Influence of Hypoxia of Lactating Rats and Mice on the Blood of Their Normal Offspring. Air Univ. School of Avn. Med., Project No. 21-2301-0005, Report #3.

3. Barker, J. N. Role of Hemoglobin Affinity and Concentration in Determining Hypoxia Tolerance of Mammals During Infancy, Hypoxia, Hyperoxia and Irradiation. A. J. P., 189(2):281-289, 1957

4. Bert, Paul, Barometric Pressure., The English Translation., Hitchcock, M. A. and Hitchcock, F. A., 1st Ed., College Book Co., 1943

5. Dill, D. B. Life, Heat, and Altitude, 1st Ed., Harvard Univ. Press, 1938

6. Wiggers, C. J. Physiology in Health and Disease, 5th Ed., Lea and Febiger Co., 1949

7. Riley, R. L., Otis, A. B. and Houston, C. S. Handbook of Respiratory Physiology. Air Univ. School of Avn. Med., Project No. 21-2301-0003, Page 143-157, 1954

8. Rahn, H. and Fenn, W. O. A Graphic Analysis of the Respiratory Gas Exchange. 2nd printing, Am. Physiologic Soc., 1956

9. Barcroft, The Respiratory Function of the Blood. 1st Ed., Cambridge Univ. Press, 1914

10. Hurtado, A. Mechanisms of Natural Acclimatization Studies on the Native Resident of Morococha, Peru at an Altitude of 14,900 Feet. Air Univ. School of Avn. Med., Report 56-1, 1956

10a. Hurtado, A. Blood Gas Transport and Acid-Base Balance at Sea Level and at High Altitudes. Air Univ. School of Avn. Med., Report 56-104, 1956

11. Daly, M. DeB. Observations on the Volume of Blood Flow and Oxygen Utilization of the Carotid Body in the Cat. J. Physiol., 125:67-87, 1954

Bibliography continued

12. Drabkin, D. L. Symposium on Molecular Heterogeneity of Hemoglobin. Introduction: Heredity and Environment in Structure of Hemoglobin. Fed. Proc. Fam. Soc. Exp. Biol., 16(3):740-747, 1957

13. Altland, P. D. and Highman, B. Effect of a Folic Antogonist (9-Methyl P G A) Upon Rats Exposed to High Altitudes. A. J. P., 176:1-5, 1954

14. Clark, R. T. Jr. Cellular Adaptation During Exposure to Hypoxia. Air Univ. School of Avn. Med., Project No. 21-1201-0013, Report # 5, 1954

15. Albaum, H. G. Enzyme Studies in Acclimatization to Altitude. I. Brain Metabolism During Altitude Acclimatization. Air Univ. School of Avn. Med., Project No. 21-1209-0009, Report # 1., 1953

16. Hoelscher, B. Mechanisms of Altitude Adaptation. Comparative Study of Hypoxia and Physical Exercise in Intact-Adrenalectomized Parabiotic Rats. Air Univ. School Avn. Med., Project No. 21-1201-0001, Report #6, 1954

17. Merino, C. F. The Plasma Erythropoietic Factor in the Polycythemia of High Altitudes. Air Univ. School of Avn. Med., No. 56-103, 1956

18. Fregley, M. J. Cross-Acclimatization Between Cold and Altitude in Rats. A. J. P., 176:267-273, 1954

18a. Fregley, M. J. Minimal Exposures Needed to Acclimatize Rats to Cold. A. J. P., 173:393-402, 1953

19. Hall, F. G. The Effect of Altitude on the Affinity of Hemoglobin for Oxygen. J. Biol. Chem., 115:485-490, 1936

20. Heilmeyer, L. Spectrophotometry in Medicine. English Translation, Jordan, A. and Tippell, T. L., 1st Ed., Adam Hilger LTD., 1943

21. Hawk, Oser and Summerson, Practical Physiological Chemistry, 12th Ed., Blakistrom Co., 1949

22. McInroy, R. A. A Micro-Haematocrit for Determining the Packed Cell Volume and Haemoglobin Concentration on Capillary Blood. Jour. Clin. Path., 7:32-35, 1954

Bibliography continued

23. Singer, K. et al. Studies on Abnormal Hemoglobins. I. Their Demonstration in Sickle Cell Anemia and Other Hematological Disorders by Means of Alkali Denaturation. Blood, Jour. Hematology. 6:413, 1951

24. Beaver, G. H. and White, J. C. A Review of the Varieties of Human Haemoglobin in Health and Disease. Jour. Clin. Path., 7:175-193, 1954

TABLE I

The PO_2 at which loss of righting reflexes occurs in infants of hypoxic rabbits as a function of increasing age.

No.	Age	$\bar{B}_L$	$\bar{B}$-47	$\bar{X}PO_2$	σ	95% Confidence of the mean
A	7	152	105	22.0	± 2.0	20-24
A	14	152	105	22.0	± 2.0	18-26
A	21	152	105	22.0	± 2.0	18-26
A	30	152	105	22.0	± 2.0	18-26
A	36	147	100	21	± 1.4	17-25
A	42	177.6	130.6	27.8	± .8	26.8-28.8
A	49	221.0	174.0	36.5	± 3.3	32.6-40.3
B	10	152	105	22.0	± 2.0	19-25
B	19	152	105	22.0	± 2.0	19-25
B	27	134	87	18.2	± 1	17.4-19.0
B	39	164.2	117.2	24.5	± 2.4	22.6-26.6
B	49	219	172	36.0	± 1.8	34.5-37.5
4	17	144.4	97.4	20.1	± 1.6	18.5-21.7
4	30	206.8	159.8	33.5	± 2.7	30.8-36.2
4	40	189.7	142.7	29.9	± .7	28.6-31.2
5	16	156.0	109	22.8	± 2.5	20.6-25.1
5	30	196.3	149.3	30.9	± 1.3	29.7-32.1
5	40	214.7	167.7	35.1	± .3	33.4-36.7

Symbols: B_L is the mean barometric pressure at which loss of righting reflexes occurred; B-47 is the correction of the mean barometric pressure B_L for alveolar PHOH; XPO_2 is the calculated mean alveolar PO_2; σ is the standard deviation of the mean.

TABLE 2

The PO_2 at which loss of righting reflexes occurs in the litters of control rabbits.

No.	Age	$\bar{B}_L$	$\bar{B}$-47	$\bar{X}PO_2$	σ	95% confidence of the mean
AB	9	142.5	95.5	20	± 2.4	17-23.0
AB	18	190.3	143.3	30	± 3.3	26-34.0
AB	26	158.8	111.8	23.4	± 1.3	22.0-24.8
AB	39	166.6	119.6	25.1	± 2.0	22.7-27.3
AB	49	214.6	167.6	35.1	± 3.0	31.7-38.5
1-3	16	171.3	124.3	26.0	± 1.2	25.0-27.0
1-3	20	192.5	145.5	30.4	± 1.5	26.1-34.7
1-3	26	227.3	180.3	37.7	± 3.8	30.7-44.7
1-3	30	192.7	145.7	30.4	± 1.6	27.4-33.4
1-3	49	222.5	175.5	36.7	± 2.9	34.1-39.3

TABLE 3

Volume airflow through dessicator decompression chamber of various reductions in barometric pressure.

B.	Flow Rater Reading	Volume
145	5.3	3400 cc.
165	6.4	4500 cc.
185	6.8	4900 cc.
205	7.3	5300 cc.
225	8.2	6200 cc.
245	8.6	6600 cc.
265	9.6	7600 cc.
425	14.7	12,000 cc.

TABLE 4

Hematocrit and hemoglobin values of test and control rabbit infants during the first forty nine days of life.

Test Animal	Age	Hematocrit	Grams Hemoglobin
A	7	34.5±1.0	14.1±0.2
A	14	28±1.0	11.0±0.2
A	21	26.0±1.0	11.0±0.2
A	28	27.0±1.0	11.0±0.2
A	42	30±1.0	12.5±0.2
A	49	33.5±1.0	12.8±0.2
B	7	36.0±1.0	14.6±0.2
B	14	30.7±1.0	11.8±0.2
B	28	27.3±1.0	11.0±0.2
B	49	33.2±1.0	12.8±0.2
4	16	31±1.0	10.6±0.2
4	30	39±1.0	11.5±0.2
4	40	33.6±1.0	11.8±0.2
5	16	29.5±1.0	10.9±0.2
5	30	41.0±1.0	11.4±0.2
5	40	33.4±1.0	12.2±0.2
Control			
AB	7	35.9±1.0	13.2±0.2
AB	14	31.5±1.0	12.2±0.2
AB	26	28.0±1.0	11.5±0.2
AB	39	30.1±1.0	12.2±0.2
AB	49	30.5±1.0	12.3±0.2

continued

Table 4 continued

Test Animal	Age	Hematocrit	Grams Hemoglobin
1-3	16	32.8±1.0	14.2±0.2
1-3	20	30.0±1.0	12.0±0.2
1-3	26	32.0±1.0	12.5±0.2
1-3	30	33.0±1.0	12.8±0.2
1-3	49	38.0±2.0	14.8±0.2

TABLE 5

Adult rabbits, acute hypoxia resistant, hemoglobin concentration and hematocrit.

Test	(PO_2)	Loss Righting Time	Time at PO_2	Hemoglobin	Hematocrit
A	22-20	no	10'	15.8±0.2	39.3±1.0
B	22-20	no	10'	15.6±0.2	39.7±1.0
4	22-20	no	10'	16.4±0.2	44.7±1.0
5	22-20	no	10'	15.6±0.2	43.0±1.0
Control					
AB	32-30	yes	3'	14.8±0.2	34.9±1.0
1	30	yes	4'	14.8±0.2	34.5±1.0
3	32-30	yes	3'	15.0±0.2	38.2±1.0

TABLE 6

Timed 15 minute alkaline denaturations of adult rabbit hemoglobins.

Test	Optical Density at 0'	15'	100'	Resistant % Hb Remaining	"0" Time
A	1.9	0.55	0.45	6.89	13.2%
B	1.23	0.48	0.43	6.25	11.9%
4	0.51	0.03	0.00	5.66	10.7%
5	0.61	0.03	0.00	4.92	9.4%
Control					
Ab	1.50	0.65	0.64	1.1	2.1%
1	1.67	0.67	0.66	.99	2.0%
3	1.58	0.50	0.50	0	0

TABLE 7

The alkaline denaturation of infant rabbit hemoglobin during the first 35 days of life. Per cent resistant hemoglobin present at time "0" calculated from the 15' alkaline denaturation values.

Age	Control Infants of Litter AB and 1-3 AB	1-3	$\bar{X}$ σ
7	78 ± 5 %	67±5 %	72.3±2.5%
14	36.3±2.5%	-------	36 ± 2.5%
21	---------	25±2.5%	25 ± 2.5%
28	10 ± 2 %	-------	10 ± 2 %
35	3.9 ± 1.5%	4 ±1.5%	4 ± 1 %

Age	Test Infants of Litter A, B, and 4 A	B	4	$\bar{X}$ σ
7	85±5 %	63.5±5 %	72.3±5 %	73.3±14%
14	40±2.5%	32 ± 2.5%	---------	36 ± 4%
21	-------	19 ± 2.5%	25 ±2.5%	22 ± 3%
28	9.5±2 %	12 ± 2 %	---------	10.8 ± 1%
35	2.5±1.5%	3.5 ± 1.5%	5.1 ± 1.5%	3.7 ± 1%

TABLE 8

The PO_2 at which the loss of righting reflexes occurs in infants of hypoxic rats as a function of increasing age.

No.	Age Days	$\tilde{B}_L$	$\tilde{B}$-47	$\bar{X}PO_2$	σ	95% Confidence Limit of Mean
1	21	129.0	82.0	17.2	± 1.5	15.2-19.2
1	27	135.6	88.6	18.5	± .9	17.4-19.5
1	36	170	123.0	25.7	± 1.6	23.5-27.9
2	22	129.3	82.3	17.3	± .2	17.0-17.4
2	26	160	113	23.7	± 1.0	22.4-24.8
2	35	173.0	126	26.4	± .4	25.6-27.2
3	15	123.0	76	15.9	± 1.0	14.7-17.1
3	23	171.6	124.6	26.1	± .5	25.5-26.7
3	39	170.8	123.8	25.9	± 1.2	24.5-27.3
4	16	153.0	106.0	22.2	± .2	21.8-22.6
4	23	166.6	119.6	25.0	± .8	24.1-25.9
4	31	177.3	130.3	27.3	± .6	26.0-28.6
5	15	137.5	90.5	18.9	± .6	18.3-19.6
5	23	135.0	88.0	18.4	± .8	17.5-19.3
5	32	172.0	125.0	26.2	± .3	25.6-26.8
6	14	138.3	91.3	19.1	± .9	18.1-20.1
6	21	147.6	100.6	21.0	± 1.1	19.7-22.3
6	37	172.3	125.3	26.2	± .8	25.2-27.2
7	15	133.5	86.5	18.1	± .3	17.7-18.5
7	26	167.5	120.5	25.2	± .5	24.6-25.8
7	49	180	133	27.8	± .1	27.6-28.2

continued

Table 8 continued

No.	Age Days	$\bar{B}_L$	$\bar{B}$-47	$\bar{X}P0_2$	σ	95% Confidence Limit of Mean
8	10	130	83	17.3	± 2.0	14.0-20.3
8	29	172.3	125.3	26.7	± 1.0	24.6-26.7
8	40	180	133	27.8	± .2	27.4-28.2
9	10	134	87	18.3	± .8	17.4-19.2
9	28	167.6	120.6	25.2	± .3	24.3-26.2
9	40	177.5	140.5	27.3	± .5	26.3-28.3
10	7	142.5	95.5	20.2	± 1.0	19.0-21.4
10	23	162.2	115.2	24.1	± 1.7	21.7-26.5
10	45	170.8	123.8	25.9	± 1.0	24.5-27.3
11	25	156.7	109.7	22.9	± .9	21.2-24.6
11	45	198.3	151.3	29.5	± 2.0	25.4-33.6
12	23	168.6	121.6	25.4	± 1.0	24.3-26.5
12	35	170.7	123.7	25.9	± .8	24.4-27.4
12	49	177.8	130.8	27.4	± 1.0	26.0-28.8

TABLE 9

The FO_2 at which the loss of righting reflexes occurs in the infants of control rats.

No.	Age Days	$\bar{B}_t$	$\bar{B}$-47	$\bar{X}FO_2$	σ	95% Confidence Limit of Mean
1	18	150.6	103.6	21.3	± .6	20.53-22.07
1	31	176.2	129.2	27.1	± 1.4	25.2 -29.0
1	40	187.3	140.3	29.4	± .6	27.7 -31.0
2	17	136.3	89.3	18.6	± .75	17.7 -19.4
2	24	174.0	127.0	26.8	± .10	26.5 -27.3
2	30	189.6	142.6	29.9	± .70	27.8 -32.0
3	20	184.4	137.4	28.7	± 1.20	27.1 -30.3
3	28	172.5	125.5	26.3	± .9	24.7 -27.9
3	35	177.3	130.3	27.3	± .7	26.0 -28.6
4	5	115.3	68.8	14.4	± .3	13.9 -14.9
4	20	146.3	99.3	20.8	± .60	19.6 -22.0
4	40	167.0	120.0	25.6	± 1.4	22.9 -28.3
5	17	154.8	107.8	22.6	± .8	21.5 -23.7
5	40	172.5	125.5	26.3	± .5	25.4 -27.2
5	60	180	133	27.8	± 1.8	25.7 -30.9
6	21	156.2	109.2	22.9	± .9	21.6 -23.1
6	28	168.6	121.6	25.5	± .3	24.5 -26.5
7	10	127.0	80.0	16.7	± .5	15.4 -18.0
7	30	178.6	131.6	27.6	± .2	27.1 -28.1

TABLE 10

The LD_{50} PO_2 determined by 30' survival at each 10mm Hg decrement of pressure beginning at B 200mm Hg, for test and control infant rats.

Rat	Age	No.	LD50 PO_2
1-6	50	42	32-30
7	37	6	27.8-25.3
8,	37	6	25.7-23.6
8	44	4	28.7-25.7 (LD_{100})
9	35	6	30-27.8
10	30	4	30-27.8
10	35	6	30-27.8
11	29	6	30-27.8
12	29	6	27.9-25.8
7,8,9	49	6	25.8
10,11, 12	40	6	27.8-25.7
10,11, 12	49	4	25.75 (LD_{100})
13	18	6	21
13	26	4	25.7-23.6
13	36	5	27.8-25.7 (LD_{100})
14	10	4	21
14	30	4	27.8-25.7
14	50	6	27.8-25.7
15	7	4	21
15	45	8	25.7-23.6

continued

Table 10 continued

Rat	Age	No.	LD_{50} PO_2
16	30	4	27.8-(LD_{50}) 25.75-(LD_{100})
17	45	6	30-27.85
18	28	6	27.8-25.7
18	35	4	27.8-25.7 (LD_{100})
19	25	6	21.6
Adult	365	9	25.75

TABLE 11

Hematocrit and hemoglobin determinations of adult and infant rats. Included are the control adults and their litters; and the hypoxia exposed animals and their litters.

Control Infants			Test Infants		
Age	Hematocrit X	Hemoglobin X	Age	Hematocrit X	Hemoglobin X
10	33.4±1.0	11.3±.5	10	30.4±1.5	10.9±.5
17	29.6±1.7	10.5±.5	20	29.3±1.8	10.8±.5
23	28.3±1.5	10 ± .5	23	30.6±1.2	10.5±.5
25,26	31.5±1.5	10.8±.5	30	33 ± 1.5	11.2±.5
35	38 ± 2	12.3±.5	36	35 ± 1	11.2±.5
40	42.1±1.3	14.4±.5	40	44 ± 1.8	14.6±.5
			45	45 ± 1	15 ± .5
Control Adults			Test Adults		
365	41.5±1.7	14.3±.3	365	50.9	16.8±.4