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Hormonal stimulus for erythropoiesis

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A HORMONAL STIMULUS FOR ERYTHROPOIESIS

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INTRODUCTION

The red blood cell has long served as a favorite subject for the study of cellular physiology and as an index of pathological changes within the organism. Most investigators who have worked with blood have noted the remarkable constancy both of red cell concentration and of red cell volume or mass. Observations of this sort have given rise to the conception that the body must possess some regulatory control of the rate of red cell production and destruction. It has been known for many years that in the adult mammal, red blood cells are formed under ordinary circumstances in the bone marrow. The secondary formation of red blood cells in other organs, such as the liver, spleen, and portions of the reticulo-endothelial system, has been designated as extra medullary hematopoiesis.

The erythroid precursor cells present in the marrow and the circulating red cell mass have been designated collectively as the erythron (1). Hypertrophy of the erythron is universally believed to occur under conditions of anoxic anoxia. For this

reason, anoxia is regarded as the fundamental or primary stimulus for erythropoiesis. This theory was first advanced by Miescher in 1893 (2). This theory has stood the test of almost a half century of criticism. However, it seems possible and even probable that anoxia is only the primary stimulus in a chain of events leading to ultimate hypertrophy of the erythron.

Investigations (1) during the last ten years have shown that the probability of a hormonal factor present in the serum of adult mammals may indeed be the key link in this chain of events. It must also be kept in mind that several factors working independently of each other may act as a stimulus for erythropoiesis. Most of the earlier studies yield information concerning only the number of erythrocytes per cubic millimeter of whole blood. Reports of this type of investigation, using blood cell concentration as the sole criterion of erythropoietic stimulation, are rapidly disappearing from the literature. This is the result of an increasing awareness of the factors at work in the normal physiology of the human body. Several of these factors are: hemodilution and hemoconcentration resulting from fluid retention or loss of plasma from the blood, and from splenic release or the withdrawal

of circulating red cells. Ideal studies of the erythron would entail a qualitative description as well as a quantitative measure of both the circulating red blood cell volume and of the volume of active erythroid marrow. At present, it is possible to measure accurately the former. The latter value can be estimated only roughly (3). Since the total erythron can not accurately be measured, the usual criterion for study of erythropoietic stimulation combines evidence of increased red cell concentration or total volume with signs of precursor activity in the erythroid marrow, such as reticulocytosis or marrow hyperplasia. The increase in the number of circulating reticulocytes is the most widely used indirect criterion of erythroid marrow stimulation. The functional implication and practical interpretation of an increase in the number of these immature erythrocytes is well established and supported.

The increase in reticulocytes accompanies a stimulated erythropoietic organ in many conditions. A few of these are: anoxic anoxia, during regeneration of cells after hemorrhagic anemia, in iron treatment of hypochromic anemia, during recovery from pernicious anemia, following blood destruction by chemical or parasitic agents, and during cobalt administration. Three to eleven days are required

for the development of reticulocytosis after the application of an adequate stimulus (3). More difficult to interpret, at present, are the many instances of reticulocytosis occurring several days after the administration of various substances, but with no evidence of increased erythrocyte levels in the circulating blood. Some of the substances giving this type of reaction are: nucleic acids, yeast extractions or Congo Red, and normal human urine. A third type of reticulocyte response may be seen following the intravenous injection of epinephrin or sodium carbonate, after central nervous system trauma, and after a large hemorrhage. These reactions require only fifteen to thirty minutes to reach maximal values, and they subside as rapidly. The most reasonable explanation for this is that of depot exhaustion of pre-formed reticulocytes without any erythroid marrow stimulation (4). The use of reticulocyte determinations as an index of erythroid activity has become the most widely used investigating tool when correlated with hematocrit and hemoglobin levels.

MECHANISMS CONCERNED WITH ERYTHROPOIETIC STIMULATION

Stimulation of red cell formation occurs under conditions of anoxia, irrespective of whether the functional state is one of anoxic or anemic anoxia.

Only one exception to this has been found. This is the effect of cobalt as a means of producing marrow hyperplasia. Various states or conditions may also bring about a stimulation of erythropoiesis. The first of these is altitude. Bert (5) wrote the original paper discussing his observation that the concentration of hemoglobin and red blood cells in the blood of an individual living at high altitude is greater than at sea level. Altitude, then, should be looked upon as a condition of continuous anoxia. Transient exposure to high altitudes, or discontinuous anoxia, does not produce a similar increase in reticulocyte percentages or marrow hyperplasia.

Certain pathological changes also may lead to erythroid hyperplasia and thus result in polycythemia. Foremost of these are congenital malformations of the heart and great vessels which prevent effective circulation of blood to the lungs, or which permit contamination of arterial with venous blood. These conditions produce arterial anoxia with its attendant cyanosis and polycythemia (6). The arterial oxygen unsaturation in the tetrology of Fallot may be extreme with values as low as ten percent oxygen saturation being reported. Values as high as a red blood cell count of ten million, hemoglobin of twenty-two grams, and a hematocrit percentage

of eighty are attendant laboratory findings in this pathological state (7). Changes in red cell mass are also found in patients with chronic pulmonary diseases in which a certain degree of arterial oxygen unsaturation is present. Emphysema, pulmonary fibrosis, Ayerza's disease, and pneumothorax are well known clinical entities which may produce an increase in red cell mass.

The commonest form of anemic anoxia is that resulting from the loss of erythrocytes by dissolution or removal from the body. In this condition, regeneration of the red cell mass begins immediately and ends with the resumption of the control value with no real evidence of polycythemia. One research technique of investigating anemic anoxia is to study animals in a carbon monoxide atmosphere. This lowers the effective red cell concentration, although the mass is unaltered. Extensive investigations have shown to date that it is apparent that lower animals develop a striking polycythemia as a result of partial saturation of hemoglobin with carbon monoxide. This response is not as marked in the human subject (8).

The common use of cobalt as a clinical means of producing polycythemia suggests that it may in some way be concerned with the regulatory mechanism of erythropoiesis (7). Marston (9) has presented

evidence that cobalt is present in a concentration of four percent in the vitamin B_{12} complex, and thus may be an agent of physiological importance. The site of action of cobalt seems to be erythropoietic tissue alone and not the myeloid tissue. Although cobalt is regarded as a potent erythropoietic agent, there seems to be no good reason for explaining its action in local anoxia. Warren et al (10) concluded from the results of extensive investigation that the effect of cobalt was not mediated by way of the peripheral nervous system. Although the presence of cobalt in vitamin B_{12} complex appears to place this element in a physiological role, it is fairly obvious that further investigations of the mechanism of cobalt action are necessary.

Various mechanisms have been proposed as being the primary stimulus of erythropoiesis. The first of these is marfow anoxia. This mechanism was first proposed in 1893 by Miescher (2). He postulated this following the demonstration of polycythemic response to altitude by Bert (5). Miescher believed that a relative degree of anoxia existed at all times in the bone marrow, thus providing a condition of constant erythroid activity and insuring a steady supply of erythrocytes to replace those destroyed daily. He supposed that at altitude an increased degree of anoxia occurred and that erythropoiesis

was more intensely stimulated, leading eventually to polycythemia. The evidence offered for the anoxic marrow mechanism is indirect and is derived from the association of accelerated erythropoiesis with the low arterial pO_2 present at high altitudes. Miescher was attempting to establish a unitarian concept of erythropoietic stimulation with his hypothesis. Grant and Root (11,12) were unable to substantiate Miescher's theory in their investigations determining oxygen saturation and tension of bone marrow in dogs, which were either bled excessively or maintained at constant anemia levels. Berk et al (13) applied the same method of determining bone marrow oxygen saturation used by Grant and Root to the human subject and was also unable to provide evidence in support of Miescher's theory. The in vitro studies of Magnussen (14) studying the response of rabbit marrow samples to varying pO_2 have also failed to corroborate Miescher's theory. Other investigators using the same techniques have arrived at similar results. Reusch (7) was unable to stimulate localized marrow hyperplasia by means of anoxia.

Experiments to support the concept of carbon dioxide tension acting locally in bone marrow as the fundamental erythropoietic stimulus, advanced

by Jordan and Speidel (15) in 1924, also failed to demonstrate that carbon dioxide per se acted in this capacity.

Extensive studies (7), carried on mostly in European research centers, appear to have adequately shown that the nervous system does not regulate erythropoiesis. The literature covering this subject is exhaustive and will not be reviewed in this paper. Since the nervous system does not appear to regulate erythropoiesis, an alternate possibility is that one or more of the hormones, produced by the endocrine glands, is the fundamental stimulus for erythrocytosis. However, the part played by the endocrine glands and the known hormones is one of modification of existing processes rather than initiation or absolute regulation. Of these glands, the pituitary appears to be the most significant, although it has been shown that the hypophysectomized rat is capable of regenerating lost red blood cells and, given a sufficient anoxic stimulus, becomes polycythemic.

Probably the most interesting view advanced as the possible stimulus for erythropoiesis is that of Carnot and Deflandre (16), who in 1906 postulated the presence of an erythropoietic stimulating substance in the circulating blood. Carnot introduced the term "hemopoietine" to designate the unknown

stimulating substance in the serum. These investigators injected the serum of a rabbit bled twenty hours previously into normal rabbits. An increase of 1.5 million red blood cells occurred within on to three days. The bone marrow of the recipient was hyperplastic, an increased percentage of normoblasts being present, and many small erythrocytes were noted in the circulating blood.

This original observation stimulated a large series of investigations, generally following this same approach. The serum of animals subjected to a variety of proven or questionable erythropoietic stimuli was injected into various test organisms through the efforts of subsequent investigators. Carnot's original observation of the stimulating influence of anemic serum on the normal animal has been confirmed and extended, although some investigators have noted only a reticulocytosis with no increase in red blood cells after a single injection of serum. Carnot also reported that the serum from rabbits which had been subject to frequent bleedings showed no erythropoietic properties. This observation was interpreted as evidence of the depletion of the supply of hemopoietine. However, Forster and Kiss (17) found no evidence of depletion in long continued anemia. When anemic serum was injected into another anemic animal, the rate of red cell

regeneration was reported to be increased (18). One investigator who was unable to confirm the original observation of Carnot, denied the presence of hemopoietine in the serum of a bled animal (19).

IN VIVO STUDIES

The presence of hemopoietine in the sera of animals exposed to low oxygen tensions was first described by Forster (20) using a normal animal a test object. The serum of a man at altitude taken before acclimitization had occurred (eight days) produced an increase in the red blood count of a normal rabbit. The influence of stagnant anoxia was examined by injecting plasma obtained from patients with conjestive heart failure into rabbits which have been previously immunized against normal human plasma (21). A slight but statistically significant increase in the red blood cell count and reticulocytes was reported to occur in the first two days, whereas a depression in these values followed the injection of normal human plasma.

The active erythropoiesis which occurs in the newborn has been utilized in a variety of attempts to demonstrate the existence and nature of hemopoietine. Various investigators have reported a significant elevation of the red blood count and

reticulocyte percentage within three days after the injection of plasma obtained from human umbilical cord blood at delivery, into the peritineum of rabbits. The active principle was found only in plasma and not in the red blood cells and was said to be stable at 67° centigrade, whereas, Carnot's original principle in anemic rabbits' serum was destroyed at 56° centigrade.

A unique method was employed by Bonsdorff in an attempt to locate the site of formation of hemopoietine. Whole blood obtained from normal rabbits was exposed for four hours to barometric pressures of from two hundred to four meters hemoglobin. After separation, the plasma was injected into normal rabbits and increases up to 0.8 million red blood cells and six to seven percent reticulocytes lasting two to four days were found in the recipient animal. The same blood at sea level pressure for four hours or plasma alone exposed to low pressure produced no effects when injected. Other investigators were unable to verify these results. There is no other systematic investifation of the probable site or sites of formation of hemopoietine found in the literature. Carnot reported an increase in the red blood cell count of a normal animal after an injection of bone marrow extract from an anemic animal, but found no response

following administration of normal marrow. However, it has been found that marrow extracts from normal animals do stimulate erythropoiesis.

All work with hemopoietine previously described involved usually a single injection of serum or plasma from a stimulated animal into a normal animal. The injection volumes amounted to from two to ten percent of the plasma volume of the recipient and were administered by either the intraperitoneal or intravenous route. It is apparent that if hemopoietine exists in the serum of the stimulated animal it will suffer a rather large dilution upon entering the blood stream of the recipient. Any influence of the injected serum would probably be only transitory. In order to overcome certain of these obstacles to a clear demonstration of the existence of hemopoietine, one group of investigators injected normal rabbits intravenously with plasma obtained from anemic rabbits. The injections were made daily for eighteen days and a group of control animals were similarly injected with normal plasma. Reticulocytes, hemoglobin, and red blood cell values of the rabbits receiving anemic plasma exceeded those of the controls during the period of injection and for several days thereafter. The differences were statistically significant.

An ingenious approach to the problem was made by Reissmann (23) who utilized the parabiotic rat preparation. One member of the pair was maintained in an eight to ten percent oxygen mixture while the other breathed room air. A polycythemic response was noted in both members during the several weeks of exposure. The appearance of polycythemia in the control animal does not itself indicate the existance of hemopoietine since the free interchange of erythrocytes as well as plasma from one member of a chronic parabiotic pair to the other has been demonstrated. However, the percentage of erythroid elements in the bone marrow of both animals increased with the degree of polycythemia. Unless a stimulating substance was formed in the anoxic rat and was passed to his partner, the erythroid marrow hyperplasia of the normally oxygenated rat is difficult to explain.

Using a somewhat different approach, Grant (24) utilized the anoxic lactating rat and mouse. Mothers were placed in low pressure chamber for six hours a day while their litters remained at sea level pressure. After a week or two of this routine it was found that the circulating red cell concentration and total body hemoglobin of the babies nursed by the intermittently anoxic mothers were greater than those of babies nursed by the control mothers who were maintained at sea level pressure.

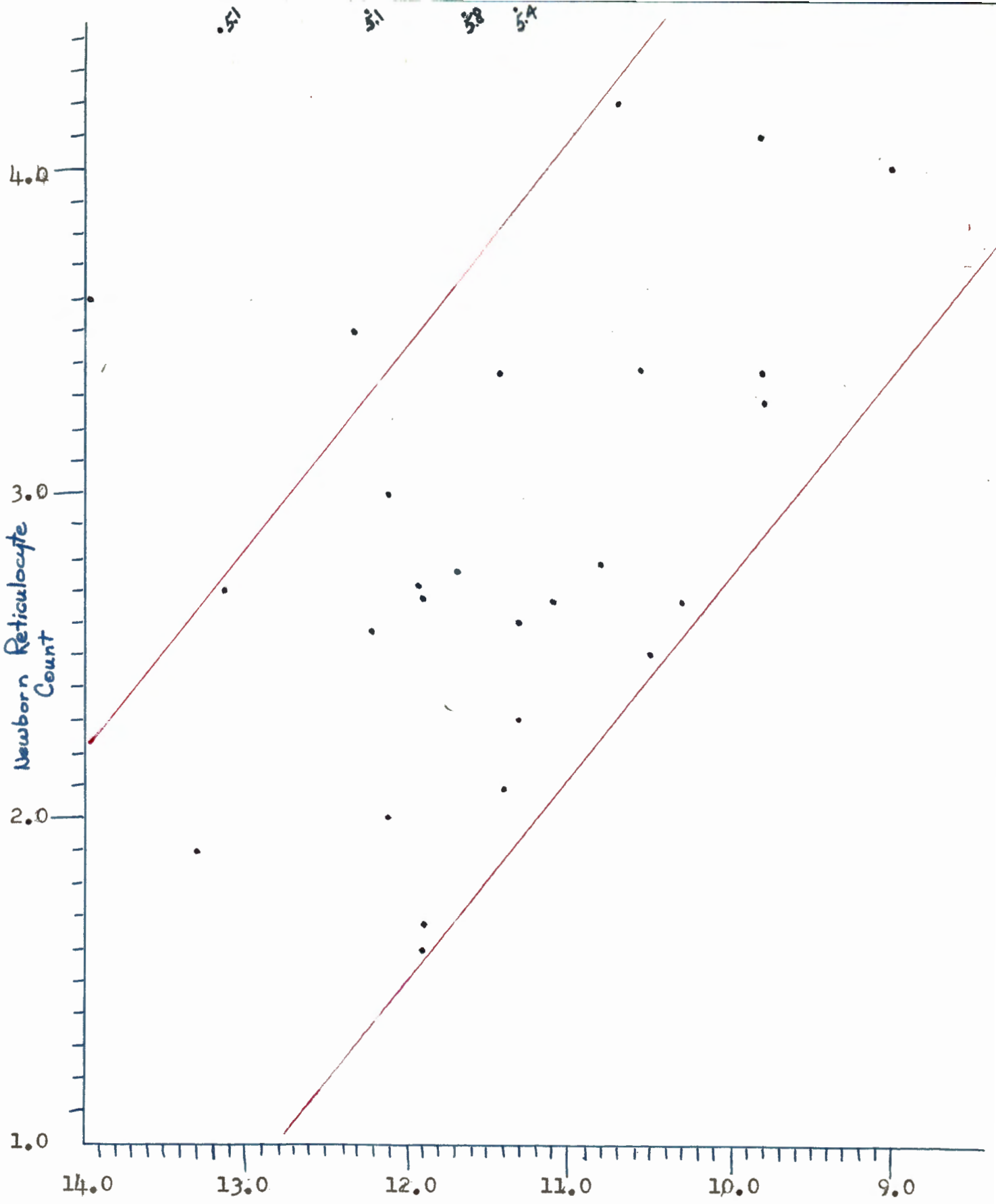
CLINICAL STUDY

The purpose of this study was an attempt to verify and add substantiative evidence to the hemopoietine theory of erythropoietic stimulation. It was postulated that if a circulating hormonal plasma factor was present in the plasma of anemic pregnant mothers, it might also pass the placental barrier and act in some way on the erythropoietic mechanisms of the fetus. An attempt was made to demonstrate this by comparing the maternal hemoglobin with the hemoglobin, reticulocyte count, and packed cell volume of the newborn. Thus, if the hemopoietine substance does exist, and if it passes the placental barrier, an inverse reciprocal relationship would be evident. This study was dependent on the hypothesis that the more anemic the mother, the more hormonal stimulating substance would be present in the plasma of the mother and the fetus. Also, the more anemic the mother, the more the erythropoietic mechanisms of the fetus would be stimulated. This would be reflected by an increase in the newborn's reticulocyte count, hemoglobin, and packed cell volume comparable to the decrease in the maternal hemoglobin.

Fifty-seven obstetrical cases, picked at random, were analyzed in an attempt to correlate various aspects of the maternal blood picture with comparable

portions of their respective newborns. Of the original fifty-seven cases studied, thirty were used in the final analysis. Twenty-two cases were ruled out either because the maternal hemoglobin was taken before twenty-eight weeks of gestation or because no hemoglobin was obtained until the postpartum period. Five others were ruled out because the newborn blood work was done on the first or second day of life. It was realized that to have the newborn stabilized as much as possible, the blood work had to be done no sooner than the fourth or fifth day. Of the remaining thirty cases, the maternal hemoglobins were taken between the twenty-eighth and fortieth week of gestation, while the newborn blood work was done on the fourth or fifth day.

The average maternal hemoglobin was approximately 11.5 grams, the range being from 9.0 grams to 14.0 grams hemoglobin. The newborn hemoglobins ranged from 16.7 grams to 23.0 grams hemoglobin, the average being 19.32 grams hemoglobin. Fifteen mothers had hemoglobin values ranging below 11.5 grams. The hemoglobins of their respective offspring averaged 19.066 grams hemoglobin. The remaining mothers had hemoglobin values ranging 11.5 grams or above. The hemoglobins of their respective offspring averaged 19.580 grams hemoglovin. These figures fail to show any inverse reciprocal relation between the hemoglobin


.51
5.1
5.8
5.4
4.0
3.0
2.0
1.0
Newborn Reticulocyte Count
14.0
13.0
12.0
11.0
10.0
9.0
Maternal Hemoglobin

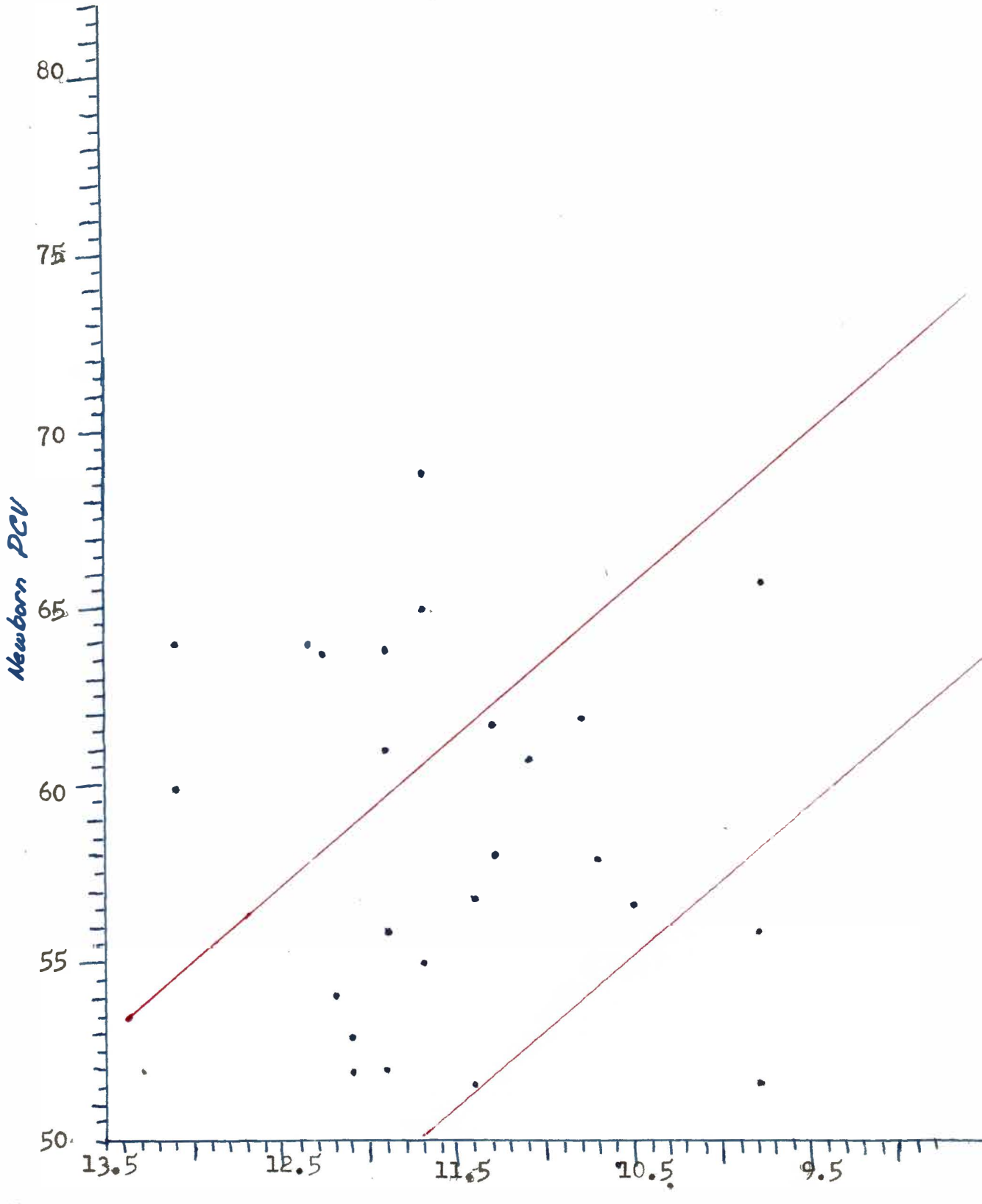
Graph I

values of the mothers and offsprings. In truth, the direct opposite was obtained. That is, a direct reciprocal relationship. This can be more accurately realized if one refers to Graphs II and III, and notes the scattergram patterns. No correlation is found to support the humoral concept.

When the maternal hemoglobins are plotted against the newborn reticulocyte counts as shown in Graph I, the results are more gratifying. Here, the results are rather suggestive of an inverse relationship between the maternal hemoglobins and the newborn reticulocyte counts. In analyzing Graph I, one can see that twenty-four out of the thirty cases fall within the expected range to support the humoral concept. Four of the six cases that were outside the expected range showed no correlation at all. The histories of these mothers were reviewed in hopes that some other factor might be found that could account for the lack of correlation. No factor or factors were found. Another possibility that might explain the lack of correlation is technical error. This must be considered in any study which relies on laboratory results.

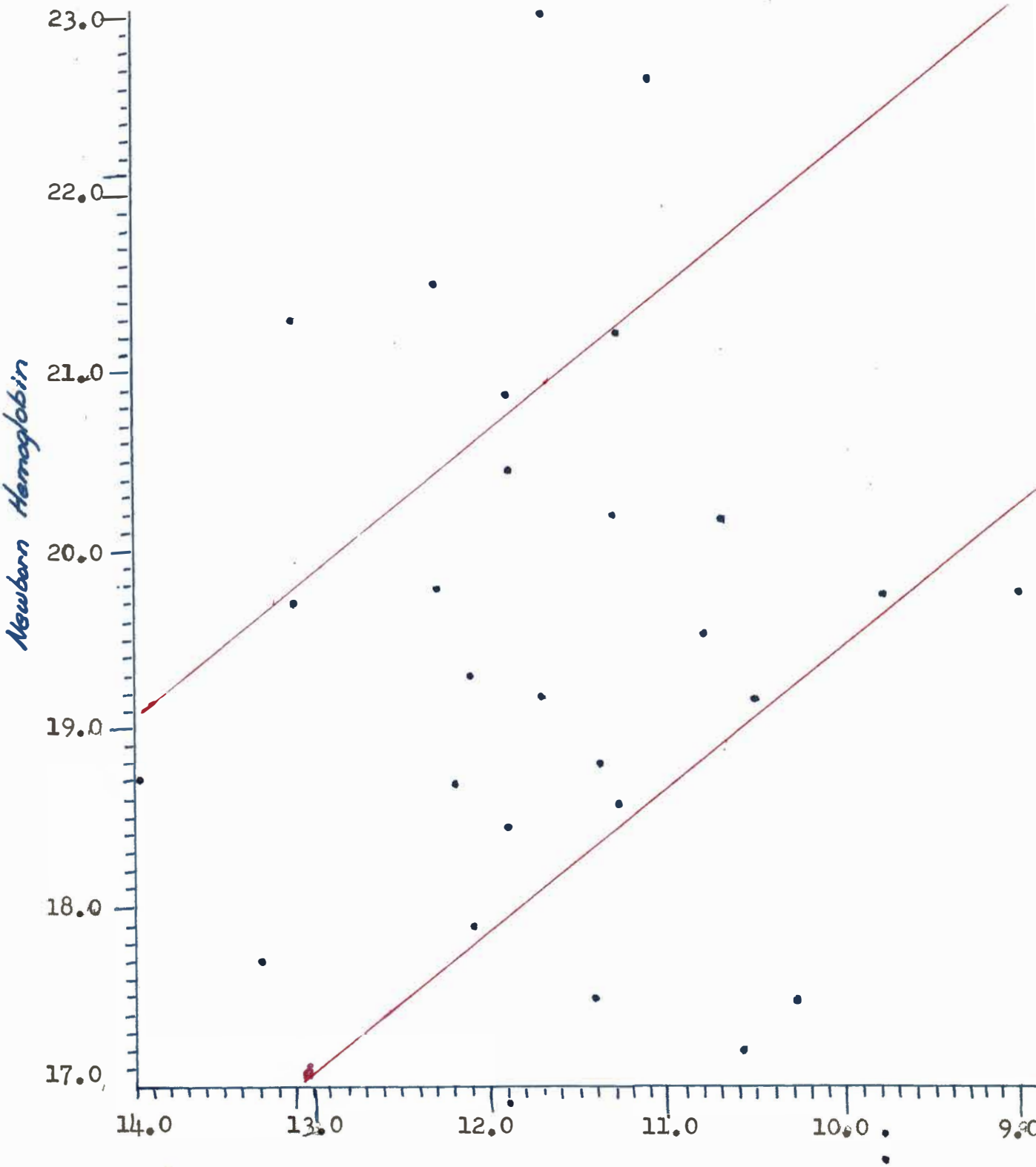
COMMENT

No evidence was found to support the humoral concept of erythropoietic stimulation when the maternal hemoglobin with respect to the newborn hemoglobin



Maternal Hemoglobin

Graph II


Newborn Hemoglobin
23.0
22.0
21.0
20.0
19.0
18.0
17.0
14.0
13.0
12.0
11.0
10.0
9.0
Maternal Hemoglobin

Graph III

and packed cell volume was analyzed. It was noted, however, that the results of maternal hemoglobin plotted against newborn hemoglobin and maternal hemoglobin plotted against newborn packed cell volume were quite similar. The author believes this to be fairly good, indicative evidence that laboratory error was minimal in this study.

A plausible explanation was sought to explain the correlation between maternal hemoglobin and newborn reticulocyte count and yet the lack of correlation between maternal hemoglobin and newborn hemoglobin. It is possible that several factors control erythropoietic peoduction in the mother and fetus during pregnancy. One of these, the humoral factor, may be a stimulus. Other factors may act as depressants. Inutero, the fetus is under the same control as the mother with balance between stimulation and depression. This may account for the parallel hemoglobin values noted when the maternal and newborn hemoglobins were analyzed---- (refer to Graph II). After birth, the depressant factors associated with pregnancy are no longer active in the infant. This allows the humoral factor to manifest itself and thus stimulate the newborn erythropoietic mechanisms. Since the reticulocyte count is used as an index of whether the red cell

mass is being replaced or increased, an increased reticulocyte count following birth may well be the first effect noted of the humoral action on the newborn erythropoietic mechanisms. This concept could adequately explain the results obtained in this study.

It is realized that inherent technical difficulties are involved in a study of this type. Anticipating further study of this interesting concept, using the same basic procedure outlined in the report, several factors should be mentioned. In order to standardize the maternal hemoglobin as much as possible, they should all be taken during the last month of pregnancy. Also the maternal cardio-pulmonary status should be reviewed in each case, along with the arterial oxygen saturation, to rule out any anoxic condition in the mother that might produce more of the humoral factor than would result from pregnancy alone. Whether the mother received iron therapy during the gestation period may be important and especially whether it was intramuscular iron therapy or oral iron therapy. The physiological hemolysis of red cells in the newborn may be another important factor.

From the results of this study, the author concludes that suggestive evidence was demonstrated in support of the hemopoietine theory, although

the number of cases in this study was too small. The inverse reciprocal relationship noted between maternal hemoglobin and newborn reticulocyte counts in this study gives suggestive support to the hemopoietine concept proposed by Carnot and Deflandre (16). The original concept of anoxia being the primary stimulus for erythropoiesis has not been supported in recent studies (11,12,13,13,7). Conversely, the hemopoietine or humoral concept has gained much favor through the experimental efforts of Carnot (11,12), Forster and Kiss (17), Bonsdorff (22), and others (7).

SUMMARY

True erythropoietic stimulation can be achieved experimentally by only three conditions: anoxic anoxia, anemic anoxia and excessive cobalt. The mechanism or mechanisms of action of the fundamental stimulus for erythropoiesis are not known. Direct anoxic stimulation, nervous system stimulation, and endocrine control of the bone marrow have been advanced as the primary stimulus. They have received little support, however, in recent years. The hemopoietine concept proposes that in either anemic or anoxic anoxia, a blood-borne substance stimulated the erythroid bone marrow. Neither the site of formation nor the nature of this substance is known. Unequivocal

evidence is lacking, but enough suggestive evidence has accumulated to make the humoral concept the most useful working hypothesis for further investigations of the mechanism of action of the fundamental erythropoietic stimulus.

FINIS

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