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31. Received for publication March 7, 1983.

32. Accepted for publication September 19, 1983.

Erythrocyte 2,3-Diphosphoglycerate, Po₂50%, and Available Oxygen in Young Rabbits with and without Postnatal Fall in Hemoglobin

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Summary

We determined erythrocyte 2,3-diphosphoglycerate (2,3-DPG), Po₂50%, whole blood hemoglobin concentration, and available O₂ from the 12th to the 30th d after birth in two matched groups of young rabbits. One group received iron parenterally on the 12th, 15th, and 18th d and the other received no iron supplement. In the untreated group there was a marked fall in hemoglobin concentration from the 12th to the 22nd d and thereafter a marked increase to the initial level on the 30th d whereas the iron-treated animals showed a marked rise in hemoglobin concentration from the 12th to the 22nd d, and a subsequent, slight decline from the 22nd to the 30th d. The average values of Po₂50% and 2,3-DPG, and the changes in Po₂50% and 2,3-DPG were virtually identical for both groups. During the first period (12–22 d) there was a marked rise in both 2,3-DPG and Po₂50% whereas in the second period (22–30 d) there was a somewhat smaller rise in 2,3-DPG and only a slight tendency toward a further rise in Po₂50%. In the untreated animals "available O₂," reflecting the O₂ delivery capacity of the blood, remained unchanged during the period of fall in hemoglobin concentration and showed a rise during the second period. In the iron-treated animals "available O₂" rose markedly during the first period, with iron-treatment, and remained unchanged during the second period. We conclude that the marked postnatal rise in 2,3-DPG and Po₂50% in the rabbit seems to be independent of the changes in the hemoglobin concentration.

Abbreviations

2,3-DPG, 2,3-diphosphoglycerate

Hb, hemoglobin concentration

Hct, hematocrit

MCHC, mean corpuscular hemoglobin concentration

Po₂50%, Po₂ of blood at a hemoglobin O₂ saturation of 50%, corrected to pH 7.40 and Pco₂ 5.3 kPa.

So₂, hemoglobin O₂ saturation

Previous studies indicate that the postnatal fall in blood he-moglobin concentration of young rabbits, 1–10-d-old, is nearly perfectly matched by a shift to the right of the hemoglobin O_2 dissociation curve, which results in complete maintenance of the O_2 delivery capacity of the blood (8). The close correlation between the rise in $Po_250\%$ and erythrocyte 2,3-DPG and the fall in Hb, together with the fact that the O_2 affinity of pure hemoglobin remains the same throughout the postnatal period (9, 12) suggest that the O_2 delivery capacity of the blood is preserved by the same mechanisms that counter hypoxia in other types of anemia (2, 7, 19, 20). The possibility also exists that an initial rise in 2,3-DPG and $Po_250\%$ together with the increase in arterial Po_2 following the change from intrauterine to extrauter-ine life so improve the delivery of O_2 to the tissues that the hematopoietic stimuli are reduced and lead to a decline in Hb. There is support for this possibility in the fact that the postnatal fall in Hb does not seem to be accompanied by a rise in serum erythropoietin (4). The rise in 2,3-DPG and $Po_250\%$ and the decrease in Hb may also represent independent, unrelated proc-esses, the correlation being coincidental (8).

Halvorsen and Halvorsen (3) demonstrated that the marked fall in rabbit Hb occurring from the 10th to the 20th d postnatally may be avoided by parenteral iron supplements. This makes it possible to study whether or not there is a causal relationship between the rise in 2,3-DPG and $Po_250\%$ and the decrease in Hb.

The purpose of this work was to establish whether or not a causal relationship existed between the rise in 2,3-DPG and $Po_250\%$ and the fall in Hb during the postnatal period of young rabbits. To accomplish this, we determined erythrocyte 2,3-DPG, $Po_250\%$, and whole blood Hb from the 12th to the 30th day after birth in two matched groups of young rabbits. One group received iron parenterally and thus avoided the postnatal fall in Hb. The other group received no supplement and thus demon-strated ordinary development. In order to give a quantitative measure of the O_2 delivery capacity of the blood throughout the study, including the influence of changes both in Hb and the hemoglobin O_2 dissociation curve, we estimated "available O_2," in accordance with Jones *et al.* (11), as the O_2 release from hemoglobin accompanying the reduction of Po_2 from 13.3 to 2.7 kPa.

Animals. Suckling Dutch Belted rabbits were used for the study.

EXPERIMENTAL PROCEDURE

General. To secure a definite fall in Hb, relatively small litters with rapidly growing animals were used (3). To be able to withdraw 1–2 ml blood repeatedly without seriously affecting the animals and influencing the Hb concentration adversely, we started the experiment when the animals were 12-d-old and weighed about 170 g. Anesthesia and blood sampling were per-formed as previously described (8).

Determination of the relationship between Hb concentration, erythrocyte 2,3-DPG, $Po_250\%$ and available O_2 in iron-treated and untreated young rabbits. Four litters, each consisting of six animals, were used for the study. At the start of the experiment, all 12-d-old animals were weighed and blood samples were withdrawn for analysis of Hb, 2,3-DPG, and $Po_250\%$ and cal-culation of available O_2. Thereafter three animals from each litter were chosen at random and given intramuscular injections of 10 mg of Fe^{3+} three times, on the 12th, 15th, and 18th d after birth. Weighing and blood sampling were repeated when the animals were 22- and 30-d-old. The young rabbits tolerated the blood sampling and the injections very well.

ANALYTICAL PROCEDURES

Standard methods. Hb was determined wtih the cyan-methe-moglobin method, reading the optical density at 540 nm on a Linson Junior Photometer. Hct was determined by centrifuga-tion at 3000 r/min for 3 min in a Ljungberg Cellokrit centrifuge.

Pco_2, Po_2, and pH were determined at 37°C by means of an Instrumentation Laboratory Blood Gas Analyser (IL 613).

Determination of erythrocyte 2,3-DPG. We determined 2,3-DPG with the enzymatic method described in Sigma Technical Bulletin No. 35-UV (12-74), modified for blood samples of 250 μl, using a Beckman DBGD spectrophotometer.

Determination of So_2. So_2 was determined spectrophotomet-rically using the procedure described by Refsum (16, 17) and modified by Holter et al. (8) for microsamples of blood with rapid fall in So_2 after hemolysis (8, 17). Separated erythrocytes were hemolyzed by freezing in a Radiometer Hemolyzer Type Hem 1 and transferred to the microcuvette of Klungsøyr and Støa (13). The optical density was read at 576 and 560 nm on a Beckman DBGD spectrophotometer.

Determination of $Po_250\%$. $Po_250\%$ was determined by a mix-ing technique described by Holter et al. (8). Equal amounts of blood, equilibrated with a humidified CO_2-O_2-N_2 gas mixture in an Astrup Micro Tonometer to give about 0 and 100% So_2, were mixed, and the mixture analyzed for So_2, Pco_2, Po_2, and pH (see above). Po_2 was corrected for deviations of So_2 from 50% by means of the slope of hemoglobin O_2 dissociation curve at So_2 = 50% (5, 18) and deviations of pH from 7.40 by means of the fixed acid Bohr effect of young rabbits (8).

Estimation of available O_2. Available O_2 of the blood was estimated in accordance with Jones *et al.* (11) as Hb × 1.39 × (Sao_2 - Svo_2), at Pao_2 = 13.3 kPa and at Pvo_2 = 2.7 kPa. The Blood Gas Calculator (18) was set at the observed $Po_250\%$, and the So_2 values were read directly at the above Po_2 values. For the actual problems the physically dissolved O_2 was not taken into consideration.
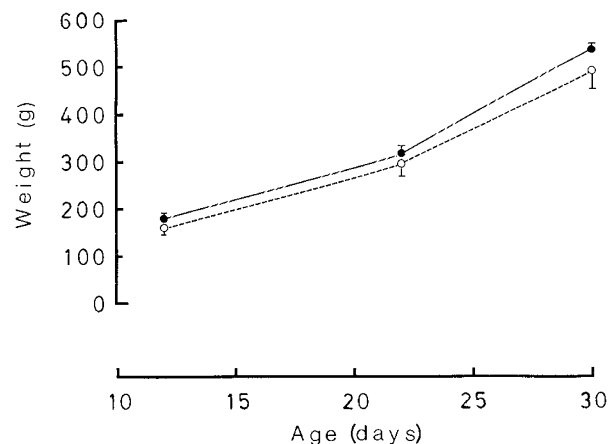

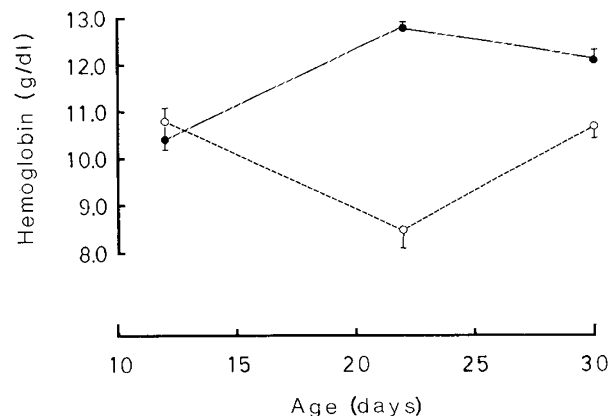Fig. 1. Body weight and age in young rabbits with (●) and without (○) extra iron (mean ± SEM).

Fig. 2. Blood hemoglobin concentration and age in young rabbits with(●) and without (○) extra iron (mean ± SEM).

The level of significance of differences between means was determined in accordance with Student's t test (for paired observations within groups and four unpaired observations between groups).

RESULTS

The average weight and weight gain were the same for both groups of rabbits (Fig. 1). In the untreated group of rabbits (Fig. 2), the fall in Hb from the 12th to the 22nd d followed by an increase to about the initial level on the 30th d thus demonstrated the usual development of young rabbits during the transition from suckling to pellet feeding (3). In contrast, the iron-treated group showed a marked rise in Hb from the 12th to the 22nd d

and a subsequent, slight decline from the 22nd to the 30th d. Even 12 d after the last iron injection, Hb was about 1.5 g/dl higher in the iron-treated than in the untreated group.

The average $Po_2 50\%$ and the change in $Po_2 50\%$ (Fig. 3) were the same for both groups. During the first period, from the 12th to the 22nd d, there was a marked rise in $Po_2 50\%$ whereas during the second period, from the 22nd to the 30th d, there was only a slight tendency toward a further increase. Both groups showed virtually identical, marked rises in 2,3-DPG (Fig. 4), the rise being slightly greater during the first period than during the second; however, at the end of the observation period 2,3-DPG was slightly higher in the iron-treated than in the untreated group ($P < 0.005$). Both groups demonstrated essentially the same relationship between $Po_2 50\%$ and 2,3-DPG (Fig. 5): a high $\Delta Po_2 50\%/\Delta$ 2,3-DPG ratio from the 12th to the 22nd d and a very low ratio during the second period of observation.

Hct and 2,3-DPG/Hct (Table 1) followed essentially the same patterns of change as Hb (Fig. 2) and 2,3-DPG/Hb (Fig. 4), with 2,3-DPG/Hct definitely lower in the untreated group than in the iron-treated ($P < 0.001$) on the 30th d. MCHC (Table 1) was the same in both groups on the 12th and 22nd d but was lower in the untreated group on the 30th d ($P < 0.01$).

In Figure 6, available O_2 was estimated as the amount of O_2 released from the hemoglobin when Po_2 is reduced from 13.3 to 2.7 kPa (11). In the untreated animals, available O_2 remained unchanged from the 12th to the 22nd d (the period of marked fall in Hb), but showed a definite rise during the second part of the observation period ($P < 0.001$). In the iron-treated animals, however, available O_2 rose markedly during the first period with iron-treatment ($P < 0.001$) but remained unchanged during the second period. For comparison, the O_2 delivery capacity was also calculated as the O_2 release accompanying reduction of Po_2 from 13.3 to 5.3 kPa. The pattern of changes was essentially the same except that the untreated animals showed a slight increase from the 12th to the 22nd d.
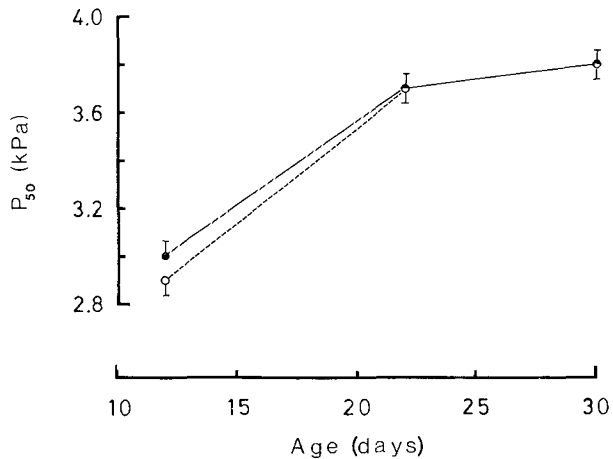

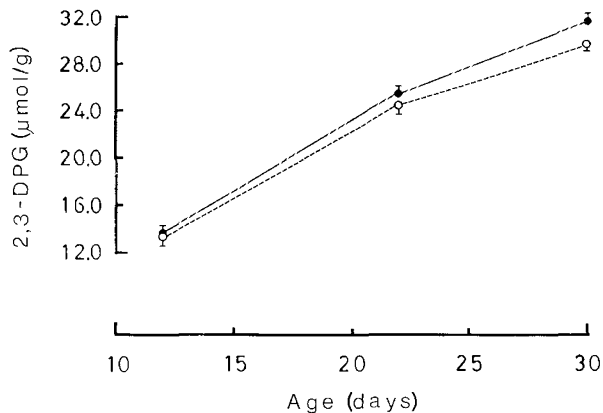Fig. 3. $Po_2 50\%$ and age in young rabbits with (●) and without (○) extra iron (mean ± SEM).

Fig. 4. Erythrocyte 2,3-DPG and age in young rabbits with (●) and without (○) extra iron (mean ± SEM).
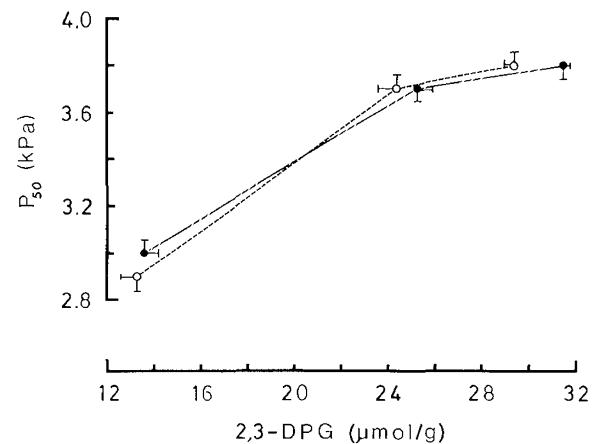

Fig. 5. Relationship between $Po_2 50\%$ and erythrocyte 2,3-DPG in young rabbits with (●) and without (○) extra iron (mean ± SEM).

Table 1. *Hematocrit (Hct), erythrocyte 2,3-DPG*/Hct and mean corpuscular hemoglobin concentration (MCHC) in two groups of young rabbits, one without iron supplementation and one given iron intramuscularly on the 12th, 15th, and 18th d postnatally (mean ± SD)*

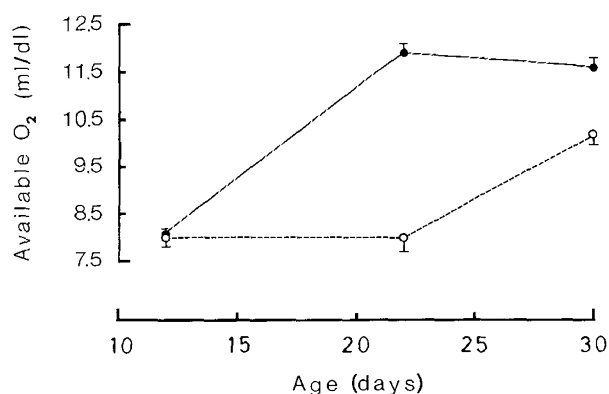| Age (d)	Untreated			Iron-treated		
	Hct (%)	MCHC (g/dl)	2,3-DPG/Hct (mmol/l)	Hct (%)	MCHC (g/dl)	2,3-DPG/Hct (mmol/l)
12	40.0 ± 2.8	27.1 ± 1.2	3.6 ± 0.6	38.5 ± 3.8	27.2 ± 1.1	3.7 ± 0.4
22	30.1 ± 3.9	28.3 ± 1.1	6.9 ± 0.8	45.2 ± 1.6	28.2 ± 0.7	7.1 ± 0.8
30	39.2 ± 2.3	27.3 ± 1.2	8.0 ± 0.4	41.9 ± 2.5	28.9 ± 0.9	9.1 ± 0.4

* 2,3-DPG, 2,3-diphosphoglycerate.

Fig. 6. Available O_2 in blood and age in young rabbits with (●) and without (○) extra iron (mean ± SEM).

DISCUSSION

The course of the Hb and Hct in the untreated young rabbits agreed with previously reported findings during the same period (3, 14). The fact that iron-treatment prevented anemia and even led to a marked rise in Hb supports the assumption that the postnatal fall in Hb in young rabbits is in some way due to a deficiency of available iron (3). The fact that MCHC on the 22nd d was the same in untreated animals with low Hb as in iron-treated animals with high Hb is in accordance with other observations that the availability of iron is important not only for the synthesis of hemoglobin, but also for erythropoiesis as such (6). The fact that there was a very close relationship between $Po_250\%$ and 2,3-DPG in both iron-treated and untreated animals throughout the study supports previous observations (1, 8, 10) which indicate that the rise in $Po_250\%$ is primarily due to an increase in 2,3-DPG.

With regard to the mechanisms behind the rise in 2,3-DPG and $Po_250\%$ from the 12th to the 30th d, it should be noted that the changes were essentially the same for both the iron-treated and untreated animals both during the first period, when Hb and Hct decreased in the untreated animals and rose markedly in the iron-treated group, and during the second period, when Hb and Hct increased in the untreated animals and showed a slight decrease in the iron-treated group. This demonstrates that the rise in 2,3-DPG and $Po_250\%$ is independent of variations in Hb and Hct and vice versa.

The data gave no evidence as to why 2,3-DPG and MCHC were lower in the untreated than in the iron-treated animals on the 30th d; however, under other circumstances young erythrocytes have shown lower MCHC and 2,3-DPG than older ones (15). The differences are probably due to a higher erythropoietic activity and a younger erythrocyte population in the untreated than in the iron-treated animals at this moment.

The fact that available O_2 remained unchanged during the fall of Hb and Hct in the untreated animals from the 12th to the 22nd d is in accordance with the pattern observed during the first 10 postnatal d (8). As an isolated finding this might indicate that the increase in 2,3-DPG synthesis and the rise in $Po_250\%$ are parts of the ordinary anti-hypoxia mechanisms observed during other types of ordinary anemia (2, 7, 19, 20). But the complete lack of corresponding relationship between 2,3-DPG, $Po_250\%$, Hb, and available O_2 in the iron-treated animals demonstrated that no such causal relationship exists. The same

conclusion was also reached when O_2 delivery capacity was expressed as the O_2 release between 13.3 and 5.3 kPa.

This study indicates that the fall in Hb does not induce the rise in 2,3-DPG and $Po_250\%$, and that the rise in $Po_250\%$ does not depress the hematopoiesis. The postnatal fall in Hb and the rise in 2,3-DPG and $Po_250\%$ in the rabbit seem to be due to parallel, independent pre-programmed processes most probably related to the growth and maturation of the animals.

The present study evaluated the O_2 delivery capacity of the blood only and did not take into consideration the possible changes in cardiac output and its distribution. As far as the O_2 delivery capacity of the blood is concerned, we conclude from the untreated animals data that the marked postnatal fall in Hb did not lead to a deterioration of the oxygen supply to the tissues.

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21. This study was supported by the Norwegian Research Council for Science and the Humanities, and Carl Sembs Foundation for Medical Research.
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23. Received for publication June 3, 1982.
24. Accepted for publication April 29, 1983.