The 'early anaemia'; its relation to postnatal growth rate, milk feeding, and iron availability

Experimental study in rabbits

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Halvorsen, K., and Halvorsen, S. (1973). *Archives of Disease in Childhood*, **48**, 842. **The 'early anaemia'; its relation to postnatal growth rate, milk feeding, and iron availability: experimental study in rabbits.** In infants, the mechanism of the postnatal fall in haemoglobin to levels below those in adults, the 'early anaemia', is still unclear. In rabbits (as well as in other species) the 'early anaemia' develops during the suckling period, and Hb rapidly increases to adult levels after weaning. The fall in Hb and haematocrit is related to growth velocity.

The rabbit has large stores of iron in the liver at birth, and even at the time of declining Hb concentration there are considerable amounts of stored iron still present. In spite of this, serum iron decreases and total iron-binding capacity increases towards the end of the suckling period. Parenteral iron given from the 10th day of life completely prevents the anaemia, and even leads to increases of haematocrit and red cell volume above the normal levels of adult rabbits. The 'early anaemia' in rabbits is thus iron responsive. An iron-deficient type of erythropoiesis, at a time when depot iron is present suggests that iron is not mobilized from iron stores in amount sufficient to maintain the very rapid erythropoiesis. Thus it is concluded that unavailability of iron rather than lack of iron stores is mainly responsible for the 'early anaemia'.

The term 'physiological' applied to the 'early anaemia' may be misleading in some species because of lack of knowledge about the optimum supply of various essential nutrients for erythropoiesis. The same may also be true in premature infants who may be deficient in copper, folic acid, and vitamin E: in addition, unavailability of stored iron may contribute to the 'early anaemia'.

In the human term and premature infant, as in several other species, there is a postnatal drop in Hb to levels below the mean values of adults. The terms 'early' or 'physiological' have often been attached to this anaemia. The term 'physiological' implies that the anaemia regularly occurs in the species at a given age, and that it is independent of environmental factors such as nutrition.

The cause of these low Hb values is still unclear. A relatively increased plasma volume may account for some of the Hb reduction in man (Bratteby, 1968) and rat (Ganzoni, 1970), but does not account for the whole reduction. Rapid growth is accepted as an important factor, with the implication that the expansion of blood volume exceeds the capacity to

increase red cell volume, thus leading to 'anaemia', which is, in essence, a hypoplastic anaemia. The relation of the 'early anaemia' to the suckling period in certain animals has been known for a long time (Bunge, 1889, cited by Venn, McCance, and Widdowson, 1947; McCance and Widdowson, 1951; Lintzel, Rechenberger, and Schairer, 1944), suggesting that iron deficiency is a likely explanation, and in pigs iron therapy alleviates the anaemia (Venn *et al.*, 1947). Iron therapy has, on the other hand, not alleviated the 'early anaemia' in the premature infant (Hammond and Murphy, 1960) or in the rat (Contopoulos *et al.*, 1955).

Intraerythrocytic changes, particularly increased 2:3 diphosphoglycerate (2,3-DPG) has been found at the time of the lowest Hb levels. This suggests that the low Hb level is physiological in the sense

that adequate oxygenation of the tissues is maintained with the reduced peripheral Hb concentration, because of the change in the oxyhaemoglobin dissociation curve facilitating the delivery of oxygen to the tissues (Delivoria-Papadopoulos, Roncevic, and Oski, 1971). From the point of view of regulation of erythropoiesis, the 'early anaemia' could be due to an unresponsiveness of the erythropoietic regulatory system in this early period of life.

Previous papers on erythropoiesis in rabbits (Laird *et al.*, 1970) indicated that this animal might be a suitable model to study the regulation of erythropoiesis in the neonatal period, and the effect of rapid growth on erythropoiesis. The present paper reports investigations in rabbits to study the effect of rapid growth, the iron metabolism of young rabbits, and the effect of iron loading during the suckling period. Investigations of the regulation of erythropoiesis will be published elsewhere (Halvorsen and Halvorsen, 1973).

Material and methods

Albino rabbits were used throughout this study. The pregnant rabbits were admitted to the animal room at least one week before term. Litter size varied from 5 to 12 and was further reduced in order to get litters of varying numbers and thereby different weight gains in individual rabbits.

The mothers were fed pellets for rabbits containing 12.5 mg iron/100 g pellets. The mothers ate approximately 150 g/day. The litter was kept with the mother throughout the study, and it was noted when the young rabbits started to take the pellets by themselves.

Blood samples were withdrawn from ear veins by prick. Heart punctures were performed in some rabbits to study the central haematocrit, Hb, serum iron, total iron binding capacity (TIBC), and plasma erythropoietin.

Hb was determined by the cyanmethaemoglobin method; haematocrit was measured in duplicates in microhaematocrit tubes; reticulocytes were stained with brilliant cresyl blue and 2000 reticulocytes were counted. Reticulocyte counts were corrected for variations in haematocrit. Red blood cells were counted in an automatic counter. Serum iron and TIBC were measured by the method of Askeveld and Vellar (1967).

Red cell volume was determined with the ^{51}Cr method as described previously (Halvorsen, 1963). Donor blood was withdrawn from the mother for labelling of the erythrocytes, except for determinations in adult rabbits when autologous blood was used.

In a pilot experiment at the start of the study, iron dextran (Imferon, Astra) was given intramuscularly in doses of 0.05 ml. Later, iron sorbitol (Jectofer, Astra) was given intramuscularly at the time when the Hb started to decline, usually at 10 days. A dose of 0.2 ml iron sorbitol supplying 10 mg Fe^{+++} was given and was

repeated every third day depending upon growth rate, so the total amount given approximated 6 to 8 mg Fe^{+++} /100 g body weight at the age of 20 days.

Stainable iron in the bone marrow and liver was at first studied in sections stained with the Perl stain as described by Pearse (1960); at the same time, smears of the marrow and liver were also made and stained with potassium ferrocyanide and safranin. As the findings in smears and sections were similar, only smears were made latterly. Bone marrow smears were graded from 0 to 4, while the liver smears were graded from 0 to 6 because of the large content of iron in the newborn livers.

Results

Fig. 1 illustrates the mean haematocrit values in peripheral blood in rabbits weighing between 300 and 450 g at the age of 20 days. Peripheral blood

FIG. 1.—Haematocrit (Ht) and corrected reticulocyte (Ret.) curves during the first 40 days in rabbits weighing between 300 and 450 g at the age of 20 days.

values remained at the same level during the first 8 to 10 days, after which they fell to the lowest point between 21 and 23 days. Central blood values followed the same pattern, indicating that the observed changes in peripheral blood were real and not due to technical difficulties in blood sampling at the youngest age. From the age of 23 days there was a rapid increase in haematocrit, reaching the adult levels within 10 to 14 days. Hb values paralleled the haematocrit values.

It was apparent that the Hb and haematocrit depended upon the rate of weight gain. In order to magnify variations in weight gain, the litter size was regulated so that litters of from 1 to 12 rabbits were studied. Fig. 2 illustrates 4 individual rabbits with marked variations in haematocrit curves and with

FIG. 2.—Haematocrit curves of individual rabbits with great variations in weight gain. The weight curves of these rabbits (1, 2, 5, 12) are shown in Fig. 3.

great variations in weight gain due to differences in litter size. Fig. 3 shows the individual weight curves of these rabbits: the weight at 20 days of 172 g in a rabbit from a 12-rabbit litter with no anaemia differs from 750 g in a rabbit from a single rabbit litter with marked anaemia. In the rabbits with weight increases intermediate between these

FIG. 3.—Weight curves of 4 individual rabbits from litters of varying sizes. The number for each curve denotes the litter size.

extremes there were also intermediate degrees of anaemia. Fig. 4 depicts the lowest haematocrit observed plotted against the weight at 20 days, and showing an inverse relation between weight increase and haematocrit.

All litters stayed with their mothers during the weaning period. They started to take pellets between 20 and 24 days of age. The rabbits in small-sized litters (1–2) started later than those in larger litters. The time of increase in Hb and Ht ratio coincided with the weaning time.

Fig. 1 also shows the mean corrected reticulocyte curve of the rabbits weighing between 300 and 450 g at 20 days. The percentage of reticulocytes fell from about 10 during the first 10 days to below 5 at the age of 20. Coincident with weaning time there

FIG. 4.—Lowest haematocrit (Ht) related to weight at age 20 days.

was a marked increase in reticulocyte levels to about 20%, after which the reticulocytes fell within 2 weeks to 5%, and thereafter more slowly to adult levels. In the slowest growing animals with Hb levels close to those of adults, the post-weaning reticulocyte increase was only moderate, while in the most rapidly growing animals it was conspicuous.

Fig. 5 depicts the mean haematocrit values for the rabbits treated with iron sorbitol, compared with weight-matched controls. In the iron-treated rabbits there was an increase in haematocrit during the suckling period to levels higher than in adult animals. In the post-weaning period after iron was stopped, haematocrit values declined to adult levels. The changes in Hb levels paralleled the haematocrit levels. Iron sorbitol thus completely alleviated the anaemia during the suckling period, even in the most rapidly growing rabbits. The reticulocyte curve in

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FIG. 5.—The mean haematocrit (Ht) curve of 8 rabbits given intramuscular iron sorbitol from the 10th day of life compared with weight-matched, nontreated controls.

iron-treated rabbits showed a steady decline from about 10% to levels of about 4% at 32 days of life, with no post-weaning increase.

Fig. 6 gives the red cell volume (RCV/kg body weight) of rabbits treated with iron sorbitol and of controls. In the control rabbits the RCV/kg was lower at age 20 days than in the 32-day-old or adult rabbits. In the iron-treated rabbits the RCV/kg was higher at age 20 days than in the controls at any age. A similar amount of iron sorbitol per kg body weight for 10 days produced no increase in RCV/kg in adult rabbits. Iron sorbitol given to suckling rabbits thus increased RCV/kg above the

FIG. 6.—RCV/kg body weight at age 20 and 32 days and in adult rabbits with and without supplement of iron sorbitol. The number of rabbits in each group and SEM are shown.

normal level for adults, though when given to adults it did not increase RCV/kg.

The livers were heavily loaded with stainable iron at birth and during the first 8 to 10 days (Fig. 7). Between 10 and 20 days the amount of stainable iron in the liver decreased, but up to 18 to 20 days areas with considerable amounts of stainable iron were still found. Relatively large amounts of iron were thus present in the liver at a time when the rabbits responded to iron sorbitol with increased erythropoiesis. The content of stainable iron in the bone marrow was much less pronounced quite shortly after birth, and though stainable iron was still to be found during the whole suckling period, it was only present in islands of connective tissue during the latter part of this period. From these studies it was apparent that bone marrow iron was

FIG. 7.—Stainable iron in the liver and bone marrow of young rabbits without iron supplement. The haematocrit (Ht) curve of weight-matched controls is shown.

used extensively while liver iron was not totally utilized, even in a period when parenteral iron treatment was capable of increasing erythropoiesis. After weaning, the amount of stainable iron in the bone marrow increased, while that in the liver was unchanged.

Serum iron decreased between 10 and 20 days while TIBC increased (Fig. 8). After weaning, serum iron again rose and TIBC decreased. The fall in serum iron was apparent at a time when large amounts of stainable iron were present in the liver.

Comparative data. The Table lists data from previous studies in several species in which an 'early anaemia' has been found during the initial phase of

FIG. 8.—Serum iron ($\mu\text{g}/100\text{ ml}$) (Δ) and total iron binding capacity ($\mu\text{g}/100\text{ ml}$) ($\circ$) in young rabbits showing mean and range of the values.

extrauterine life. The data further indicate that the degree of anaemia in rats varies from study to study, so that external factors or differences between strains may be important. The development of the early anaemia is, as previously pointed out, related to the period of milk feeding, with the lowest values towards the end of this period. In most species the anaemia is rapidly corrected after weaning.

In man the period of milk feeding generally does

not have so definite an end point as in most other species. The 'early anaemia' of prematurity develops during a period when the infant is almost exclusively milk fed, and the recovery, which is very gradual, occurs during a period of 'mixed' (milk and solid food) feeding.

Because of the great differences in life span of the species listed, it is appropriate to compare the weight increase up to the time when Hb is at its lowest point (McCance and Widdowson, 1951), which in most species coincides with weaning time. The weight increase is expressed as the number of times the birthweight has increased. The goat and the premature infant double the birthweight during this period, while the rabbit has a four- to tenfold and the pig a ten- to twelvefold increase. There is some indication of a correlation between growth velocity and the degree of anaemia in different species. The Table also lists a few trials of alleviating the anaemia with iron supplement. In the rat, Contopoulos *et al.* (1955) failed to observe any such effect. In the most rapidly growing animal listed in the Table, the pig, Venn *et al.* (1947) and Talbot and Swenson (1970) completely prevented the anaemia by means of additional iron. This fits nicely with our own observations in rabbits that iron supplement completely abolishes the postnatal fall in Hb concentration, even in the most rapidly growing animals which otherwise would have developed a severe anaemia. In premature infants some studies

TABLE
Hb or haematocrit values of different species at birth, at lowest level, and at adulthood; and growth velocity and effect of iron supplement*

Species	Hb (g/100 ml) or Ht (%) values			Age at lowest values (dy)	Weight increase ($\times$ birthweight)	Effect of iron supplement
	At birth	Lowest	Adult			
Rat (Garcia, 1957)	11	5	13	15-20	6-8	—
Rat (Contopoulos <i>et al.</i> , 1955)	12.5	6.9	12.5	18	6-8	No effect
Rat (Masters, Leslie, and Kaldor, 1972)	(38)	(31)	(41)	18	—	—
Guinea pig (Constable, 1963)	13.2	11	16	21	—	—
Goat (Riegel, Hilpert, and Bartels, 1961)	11.5	8.5	12.2	21	2	—
Pig (Talbot and Swenson, 1970)	11.8	6.3	13.1	30-40	?10	No anaemia
Rabbit (Laird <i>et al.</i> , 1970)	13.5	9.5	14.2	20	—	—
Rabbit (present data)	(50)	(23)	(40)	21-23	4-10	No anaemia
Dog (beagles) (Deavers, Smith, and Huggins, 1971)	12.2	7.6	16.4	30-35	4-5	—
Cat (Mott, 1968)	16.0	7.2	10.6	42	—	—

*Growth velocity indicated by the number of times the animal has increased its birthweight up to the time of the lowest Hb or haematocrit level.

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with iron supplement have failed to alleviate the early anaemia (Hammond and Murphy, 1960; Gorten and Cross, 1964), while in others (Gairdner, Marks, and Roscoe, 1955; Sisson and Whalen, 1958) the infants given iron supplements did develop a lesser degree of 'early anaemia' than controls.

Discussion

The data from the present study in rabbits support previous findings in other species and allow the following conclusion. The 'early anaemia' occurs in the first weeks or months of life when growth velocity is maximal. It occurs in the period when the animals are exclusively or almost exclusively milk fed. The lowest Hb and haematocrit values usually occur towards the end of the suckling period, but regain normal Hb values after weaning. Variations from studies in different sets of animals indicate other possible environmental factors affecting the development of the anaemia.

Rapid growth is an important factor in the development of the 'early anaemia'. In the present study the degree and duration of the anaemia was clearly related to weight increase during the first 3 weeks of life. The anaemia was, however, alleviated by iron supplement even in the most rapidly growing animals, where birthweight was increased by a factor of 10. The same observation has been made in pigs with a ten- to twelvefold increase in birthweight during the suckling period. In rabbits and pigs rapid growth can therefore only be a pathogenetic factor in the development of the early anaemia by increasing the need for essential nutrients, particularly iron. In these species the regulatory system of erythropoiesis and the erythrocyte-producing organs have the capacity to maintain Hb concentration and RCV/kg within the limits of adult animals (Halvorsen and Halvorsen, 1973). The 'early anaemia' in these species is thus an iron-responsive anaemia.

The growth rate in the human infant is not comparable to that of the pig and rabbit. While these animals may increase their weight from birth to the end of the suckling period by tenfold, the premature baby only doubles its birthweight up to the time when Hb is at its lowest, and the term baby only increases its birthweight by a factor of 1.5. Though such differences in species may occur, it seems most unlikely that the relatively slow-growing human infant, premature or term, lacks the capacity to increase erythropoiesis sufficiently to increase its red cell volume in parallel with its modest rate of body growth. This is further supported by the finding that premature infants given cobalt

(Gairdner *et al.*, 1955) and infants with cyanotic heart disease may maintain Hb values within the adult range. It therefore seems unlikely that rapid growth contributes to the development of the 'early anaemia' of infants other than perhaps by increasing the need for essential nutrients.

The serum iron in rabbits is higher than in man. This difference may be of importance regarding the maximum rate of Hb synthesis (Jacobs and Finch, 1971).

What is remarkable in the rabbit is, however, that iron therapy increases erythropoiesis at a time when iron is still present in the depots. Presumably, iron in the depots is not mobilized as rapidly as is needed; in other words the iron in the liver is unavailable for Hb synthesis in bone marrow. The data on the serum iron fit well into this hypothesis.

The rabbit is unique in the sense that it has a large iron depot in the liver at birth. Rabbit milk has a low iron content and the young rabbit draws upon its iron depots in the liver for Hb synthesis during the suckling period. The data on the stainable iron in the rabbit liver presented in this paper fit well with the quantitative data presented by McCance and Widdowson (1951). In their study the *total* liver iron content at birth and on day 15 was the same though its *concentration* had decreased. (However, the rabbits in their study had gained less weight than those in the present study.) In the study by Lintzel *et al.* (1944), on the other hand, one rabbit studied at day 19 had very little iron in the liver, and this animal had gained weight faster than the mean rate in our study. In the present study only stainable iron was investigated, but McCance and Widdowson's data, together with our finding that the rabbits' livers increased in size while still containing iron, support our main conclusion that the rabbits had considerable iron stores at a time when the anaemia was iron reponsive.

The rate of erythropoiesis is, among other things, dependent upon the supply of iron to the erythropoietic organs. When there is a reduction in total body iron or iron deficiency as defined by Bainton and Finch (1964), iron deficiency classical anaemia develops. Iron deficient erythropoiesis may also occur, however, when the rate of erythropoiesis exceeds the normal rate without a proportionate increase in iron supply. Hillman and Henderson (1969) found that the rate of erythropoiesis after a standardized bleeding in an individual with normal or even increased iron stores could be increased when iron was given orally or parenterally as well, and particularly so if the test subject was given nonviable red cells. The rate of erythropoiesis is thus dependent, among other

factors, upon the amount of iron immediately available.

The young rabbit is apparently in a similar situation, with a rate of erythropoiesis far exceeding the steady state of erythropoiesis of the adult animal. Iron responsiveness implies that the young rabbit lacks available iron for this rate of erythropoiesis, though it is not iron deficient in the sense that the depots are depleted.

Whether iron unavailability occurs in other species during this period of life is unknown, but it is a tempting hypothesis. In premature infants of birthweights below 1500 g there is a marked increase in erythropoiesis about 6 to 8 weeks of age (Seip and Halvorsen, 1956). A relative lack of iron in the bone marrow for this degree of erythropoiesis is possible. The data of Gairdner *et al.* (1955) and Sisson and Whalen (1958), that early iron supplement tended to mitigate the early anaemia, support such a hypothesis. The present study in rabbits does not, however, allow any conclusion regarding the situation in the human infant. The doses of iron used in the present study may also be dangerous in the human infant.

Iron unavailability is not the only deficiency that may occur in premature infants during the period of early anaemia. Melhorn and Gross (1971) studied vitamin E dependent anaemia in the low birthweight premature infant and made the important observation that iron medication to infants deficient in vitamin E increased haemolysis and lowered Hb. Hypocupraemia may also occur in premature infants and may lead to anaemia (Ashkenazi *et al.*, 1972). The recent observations that caeruloplasmin is important for iron mobilization may link hypocupraemia to the lack of complete mobilization of iron from the stores (Frieden, 1970). Folic acid deficiency is also common in premature infants (Hoffbrand, 1970). Multiple factors may thus contribute to the 'early anaemia' of prematurity and acceptance of the 'early anaemia' as 'physiological' is premature until there is more complete knowledge about the optimum concentration at this age period of all the factors essential for erythropoiesis. Since premature infants show no obvious handicap from a moderately reduced Hb level, heroic therapeutic trials should be avoided and may be dangerous (Melhorn and Gross, 1971).

Much interest has recently focused on 2,3-DPG and its compensatory effects on tissue oxygenation by changing the oxyhaemoglobin dissociation curve (Delivoria-Papadopoulos *et al.*, 1971). However, since increased intracellular 2,3-DPG occurs in most types of anaemia (Hjelm, 1969), the increase during the period of 'early anaemia' may be no more

than a secondary phenomenon. It is possible, however, that the high phosphate intake and high serum phosphorus levels may contribute to the increased 2,3-DPG levels in this age period.

Iron dextran was used in the pilot study, and when given to 2- to 3-day-old rabbits did not influence the Hb. However, the doses tolerated by the newborn rabbit were much smaller than the doses of iron sorbitol used between 10 and 20 days. Any comparison then between iron dextran and iron sorbitol is impossible. Iron sorbitol gives a rapid increase in serum iron, and it is possible that this may contribute to the increased erythropoiesis, though iron sorbitol has not been found more effective in increasing Hb synthesis than peroral iron when iron absorption is normal (Olsson, 1972). This was not tested in the present study.

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