Nonheme Iron Mobilization from the Liver in Piglets

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(Received for publication November 20, 1973)

Abstract. An experiment was conducted with 38 piglets to clarify relationships of hepatic xanthine oxidase and plasma ferroxidase to storage iron mobilization. In conjunction with the result of hypotransferremia, hepatic xanthine oxidase and plasma ferroxidase were extremely low at birth. Accordingly, it seems likely that due to these enzymatic and protein deficits, the rate of iron storage cells to plasma iron transfer may be quite limited, if at all. When there were sudden increases in plasma transferrin, ferroxidase activity and hepatic xanthine oxidase after piglets had utilized colostrum, a corresponding increase in iron release from the liver was observed. It is possible that the ferrous to ferric cycles in the liver and plasma are activated by the increase in iron-releasing enzymes and plasma transferrin, and that thereafter the rate of transport of iron from storage cell to erythropoietic organ is accelerated still more.

Eventually, as a result of storage iron depletion, plasma iron and hemoglobin levels decreased and overt iron deficiency appeared markedly from 3 days of age onward. Milk ferroxidase and transferrin showed a high level at farrowing and decreased progressively to 10 days postpartum. Although plasma ferroxidase increased intensively after piglets had absorbed colostrum, the amount of colostrum ferroxidase absorbed by piglets was very small, as compared with that derived from endogenous sources after the cessation of the absorption of colostrum protein by the piglets.

The iron mobilization from iron storage cells to plasma transferrin has been the subject of considerably many experiments. It is generally believed that xanthine oxidase oxidizes xanthine, hypoxanthine, or inosine into uric acid and, at the same time, reduces Fe^{+++} -ferritin to Fe^{++} -ferritin. The reduced ferritin iron is less tightly bound to the protein than is the original ferritin iron and appears on the cell surface in the reduced form [9-10, 15-16]. Ferrous iron is oxidized into ferric iron by catalysis of plasma ferroxidase. After that the ferric iron joins the carrier-protein, apotransferrin, for delivery to the site of hemoglobin synthesis [12, 14, 18-22, 24-26].

Mazur and Carleton [17] reported that

an inverse relationship existed between ferritin iron content and xanthine oxidase activity in the liver of the developing rat. The author's previous study [8] on ferrokinetics using ^{59}Fe -citrate in newborn piglets also indicates that the release response of nonheme iron in the liver into the circulatory system is closely associated with the increase in plasma ceruloplasmin and transferrin. Only limited data are available on the process of iron inflow to the plasma from storage organs in the neonatal period.

The purpose of the present experiment is to clarify relationships of hepatic xanthine oxidase and plasma ferroxidase to storage iron mobilization in piglets.

Materials and Methods

Milk ferroxidase: Seven sows of the Yorkshire, Landrace, and Hampshire breed were reared on concrete-floored pens for a 10-day experimental period. They were fed a diet with water twice a day. The composition of the diet is the same as previously described [5]. Milk samples were collected at 0 hour (never after nursing), 12 hours and 3 and 10 days after parturition. Colostral and milk samples were collected by manual milking from the functional teats and pooled. The milk fat was removed from all the samples by centrifugation at 15,000–25,000 g for 30–45 minutes at 4°C. Decaseination of milk was accomplished by centrifugation at 45,000 g for 1 hour at 4°C, the casein settling to the bottom of the tube.

Hepatic xanthine oxidase and plasma ferroxidase: Thirty-eight piglets of seven litters belonging to the Yorkshire and Landrace breed and crossbreed of Yorkshire × Landrace were used for the study. They were housed with their dams on concrete-floored pens for a 10-day experimental period. Exogenous iron contamination was avoided insofar as possible with rust-inhibiting paint, although the piglets had access to the sows' feed and feces. The pens were cleaned daily. Creep feed was not provided. Additional details of sow feeding were done as described above. Piglets were killed by bleeding at birth and 24–30 and 72–84 hours after the first nursing. At 3 days of age, the piglets were randomly divided into iron-dextran injected (6 pigs) and uninjected (7 pigs) groups within the litters. The injected group received 1 ml of iron-dextran intramuscularly (100 mg of iron). Pigs from each group were killed by bleeding at 10 days of age. Immediately before killing, blood samples were obtained from the anterior vena cava. The liver was removed, weighed, washed, and stored frozen at

–20°C for 3 days, when xanthine oxidase inhibitor diminished [1].

Hepatic xanthine oxidase was determined by the method of Van Pilsum [27] using 10% homogenate of hepatic tissue in 0.66 M phosphate buffer (pH 7.4). Milk or plasma ferroxidase activity was measured spectrophotometrically by the method of Johnson et al. [11]. Plasma ceruloplasmin ferroxidase activity, plasma and milk transferrin, plasma iron, plasma copper, hemoglobin and nonheme iron in the liver were estimated as previously described [4–6, 8].

Results

Milk ferroxidase

As shown in Fig. 1, milk ferroxidase decreased drastically from 0.85 to 0.33 IU/ml in the first 10 postpartum days. Postpartum changes in milk ceruloplasmin were almost similar to those in milk ferroxidase. The concentration of milk transferrin decreased markedly from 139 to 9 mg/100 ml in the first 10 postpartum days (Fig. 2). Hepatic xanthine oxidase and plasma ferroxidase

The mean body and liver weights of the iron-dextran-injected and uninjected groups are shown in Table 1. They increased as the piglets became older in both supplemented and unsupplemented groups. Results of determination of hemoglobin, nonheme iron in the liver, plasma iron, and plasma copper, as changing with the advance in age, are presented in Table 2.

Table 1. Body and liver weights from birth to 10 days of age

Days of age	No. of animals	Body weight (kg)	Liver weight (g)
Birth	8	1.4±0.0*	41±2
1	8	1.5±0.1	38±4
3	9	1.9±0.1	60±5
10			
No supplemental iron	7	3.5±0.3	98±6
Supplemental iron**	6	3.9±0.3	110±9

Remarks.

* Mean ± standard error.

** 1 ml of iron-dextran (100 mg of iron) injected intramuscularly at 3 days of age.

Fig. 1. Ferroxidase and ceruloplasmin in sow's milk from farrowing to 10 days postpartum

Fig. 2. Transferrin in sow's milk from farrowing to 10 days postpartum

Remarks.

The value is given as mean $\pm$ standard error.

This remark is applied to all the following figures.

Table 2. Hematological and tissue parameters in piglets from birth to 10 days of age

Days of age	Hemoglobin, g/100ml	Nonheme iron per g of liver, μ g	Plasma iron, μ g/100 ml	Plasma copper, μ g/100 ml
Birth	10.4 $\pm$ 0.5*	176 $\pm$ 25 (7.3 $\pm$ 1.2)**	48 $\pm$ 9	44 $\pm$ 5
1	9.2 $\pm$ 0.3	191 $\pm$ 63 (6.5 $\pm$ 2.0)	61 $\pm$ 10	78 $\pm$ 5
3	8.1 $\pm$ 0.4	32 $\pm$ 7 (1.7 $\pm$ 0.2)	46 $\pm$ 6	128 $\pm$ 5
10				
No supplemental iron	7.8 $\pm$ 0.5	11 $\pm$ 1 (1.1 $\pm$ 0.1)	46 $\pm$ 9	214 $\pm$ 7
Supplemental iron***	10.1 $\pm$ 0.4†	264 $\pm$ 57† (29.8 $\pm$ 6.9)	††	213 $\pm$ 14

Remarks.

* Mean $\pm$ standard error.

** mg per liver.

*** 1 ml of iron-dextran (100 mg of iron) injected intramuscularly at 3 days of age.

† Significantly greater than any other value within the experiment ($P < .01$).

†† It was impossible to assay plasma iron of the iron-dextran group exactly by the present method.

Fig. 3. Hepatic xanthine oxidase in piglets from birth to 10 days of age

Fig. 5. Plasma transferrin from birth to 10 days of Age

Fig. 4. Plasma ferrooxidase and ceruloplasmin from birth to 10 days of age

At 3 days of age, hemoglobin concentration decreased to 79% of the value at birth. After that, it decreased slightly up to 10 days of age. With the iron supplement, hemoglobin concentration was significantly high at 10 days of age, as compared with that in the unsupplemented group ($P < 0.01$). Nonheme iron concentration in the liver was maintained at a high level at birth and 1 day of age. It began to be mobilized markedly at 3 days of age and was almost depleted at 10 days of age. With the iron supplement, nonheme iron concentration in the liver was significantly high at 10 days of age. In the unsupplemented group, plasma iron level was consistently low until 10 days of age. There was no difference in plasma copper level increased with the advance in age between the supplemented and unsupplemented pigs.

Developmental changes in the level of hepatic xanthine oxidase are shown in Fig.

3. Hepatic xanthine oxidase was extremely low in level at birth and 1 day of age. It began to increase markedly at 3 days of age, reaching a level several times as high as the value at birth at the end of 10 days of nursing. There was no significant difference in level of hepatic xanthine oxidase between the supplemented and unsupplemented pigs.

Developmental changes in the levels of plasma ferroxidase and ceruloplasmin are presented in Fig. 4. Plasma ferroxidase was very low at birth, but became two-fold the value at birth at 1 day of age and was elevated linearly until 10 days of age, showing no difference between the supplemented and unsupplemented pigs. At birth ceruloplasmin ferroxidase was 0.3% of the value at 10 days of age, and thereafter increased in parallel with ferroxidase throughout the experimental period. Ceruloplasmin and plasma copper paralleled each other quite well. Plasma transferrin value was very low at birth. At 1 day of age, it increased by 25% of the value at birth (Fig. 5). It increased abruptly at 3 days of age and persisted until 10 days of age. With the iron supplement, plasma transferrin was suppressed, as compared that in the unsupplemented group at 10 days of age.

Discussion

Recently, the oxidation of Fe^{++} by some serum component other than ceruloplasmin (ferroxidase—I) has been reported. Lee et al. [13] have proposed citrate as an alternative source of catalytic activity in converting Fe^{++} to Fe^{+++} in low ceruloplasmin plasma. Topham and Frieden [26] isolated a ferrous iron-oxidizing enzyme (ferroxidase—II) and reported that ferroxidase-II activity in Wilson's disease serum represented a much higher percentage of the

total ferroxidase activity than that in normal serum. In neonatal piglets, however, despite their low level of ceruloplasmin plasma, changes in plasma ferroxidase activity are almost the same as those in ceruloplasmin ferroxidase activity. Therefore, it is verified that ceruloplasmin ferroxidase plays a major part in the movement of iron from cell to plasma in the neonatal period. In newborn piglets, ceruloplasmin ferroxidase activity in plasma was extremely low or virtually absent, whereas colostral ceruloplasmin was relatively high in level, as compared with plasma ceruloplasmin. Accordingly, it seems likely that an intense and immediate elevation may occur to plasma ferroxidase activity after nursing as a result of intact absorption of colostral ceruloplasmin. It is, thereby, postulated that ceruloplasmin derived from colostrum may play an important role in increasing the rate of iron transport for intact absorption of colostrum. This is not consistent with the results of the previous study which indicate that colostrum transferrin contributes only a little to the increase of low serum transferrin [6]. However, the amount of intact absorption of colostral ceruloplasmin is much smaller than that of ceruloplasmin derived from endogenous sources after the cessation of absorption of intact colostral protein from the gastrointestinal tract in the piglets.

In the present experiment, newborn piglets showed an extremely low value in hepatic xanthine oxidase activity. This observation, in conjunction with the occurrence of hypotransferrinemia and hypoceruloplasminemia, accounts for the results described in previous reports [4, 5, 8], in which erythropoietic activity was extensive and iron storage in the liver and spleen high, but plasma iron levels were

low in newborn piglets. Accordingly, it seems likely that, due to these enzymatic and protein deficits, the rate of iron storage cells to plasma iron transfer may be quite limited, if at all. The low levels of plasma ceruloplasmin and transferrin and active erythropoiesis in the neonatal period are also suggestive of a greater contribution of that enzyme to the one-way iron transfer from mother to fetus. It remains obscure, however, why iron saturation to plasma transferrin was relatively high at birth [4], despite extreme low levels of ceruloplasmin. It seems that there may be a specific mechanism of iron transport from the mother's placenta to the fetus.

When there were sudden increases in plasma transferrin, ceruloplasmin, and hepatic xanthine oxidase in piglets after the utilization of colostrum, a corresponding increase occurred to the iron release. From their experiment with developing rats, Mazur and Carleton [17] reported that a significant increase in the level of hepatic xanthine oxidase had coincided with a marked decrease in the hepatic ferritin level. In pigs, the amount of iron accumulated in the liver during fetal development cannot meet even the daily iron requirement of a piglet [5]. Besides, the iron content of sow's milk is remarkably small [23]. Accordingly, due to the increases of the iron-releasing enzymes and plasma transferrin, the ferrous-to-ferric cycles in the liver and plasma are activated and the rate of transport of iron from storage cells to the erythropoietic organ is accelerated still more. Eventually, as a result of storage iron depletion, hemoglobin synthesis slows down and overt iron deficiency anemia appears. In the present study, the marked symptom of iron deficiency as clarified by reduced values of liver iron and plasma iron appeared at 3 days of age. Despite a

great increase of plasma ceruloplasmin, however, copper did not show the same rapid mobilization from the liver in neonatal period iron did [7].

Although anemia in neonatal piglets is due to hemodilution caused by the increase in plasma volume resultant from the ingestion of maternal colostrum into the vascular system [2, 3], the absorbed colostrum constituents, in turn, accelerate the production of the iron-releasing enzymes and plasma transferrin. Therefore, in growing piglets, an iron-utilizing ability to meet the great iron demand in the nursing period develops rapidly in the early stage of growth. After this stage, iron administration is very effective for arresting the anemia. This fact also indicates that in the neonatal piglet iron metabolism changes rapidly from the fetal pattern to the adult one.

Acknowledgements: The author expresses his appreciation to Professor Kazuya Usui of the University of Tokyo, for his valuable advice and review of this manuscript.

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