KINETICS OF IRON METABOLISM IN EQUINE INFECTIOUS ANEMIA

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In equine infectious anemia, disturbances of iron metabolism are revealed. They include a decrease of serum iron and a certain increase of plasma-iron-binding capacity at the paroxysm, which lead to the decrease of the saturation percentage, a reverse relationship between the storage iron content of the spleen and that of the liver as compared with those in the non-infected animal, diminution of the circulating red cell volume¹⁴, and shortening of the erythrocyte life span¹⁵.

No conclusion has been drawn from any morphological investigation concerning the bone-marrow activity in this disease. Some referred to the hypoplasia and others to the hyperplasia of the bone marrow^{5,6}. ISHII and coworkers¹² observed that the ratio of erythroid cells to total nucleated cells in the bone marrow decreased after the paroxysm of the disease. This paper deals with the kinetics of iron metabolism in infected horses studied especially to know red cell production.

METHODS

1. Procedure^{8,11,16}: Spin heparinized blood to separate plasma from it. Add saline dilution of 20-70 μ c of $^{59}\text{FeCl}_3$ to the plasma and incubate it at 37°C for 30 minutes. Care should be taken not to add metallic iron beyond the iron-binding capacity of the plasma. Inject about 50 ml of ^{59}Fe -bound plasma into the jugular vein and remove 6-8 ml each of oxalated blood for collecting plasma from the opposite side of the vein 10, 20, 30, and 60 minutes after injection, then 2 to 4 times at one hour's intervals and on the next day. Thereafter, draw 5 or 6 ml blood every day for 2-3 weeks. Put 2 ml each of the plasma or the blood into 2 plastic tubes and count the activities with a well-type scintillation counter. Examine hematocrit, serum iron, hemoglobin, blood cell count, and siderocytes which are defined, in this case, as white blood cells containing Prussian blue positive iron granules. Dilute an adequate amount of the plasma used for injection with water in a mess flask and reserve it as standard.

2. Calculation: Calculation was made in such a usual manner as indicated in Table 1^(4,11). For plasma clearance, the activity of the plasma was plotted on a semilogarithmic paper against time and extrapolated to time zero by least square. When the clearance was curvature, the half time of plasma-iron clearance ($T_{1/2}$) was gained by making a tangent at time zero to the curve⁹. When there were two components, the $T_{1/2}$ was determined from the second component¹. For ^{59}Fe uptake of erythrocytes, the blood activity was corrected for the hematocrit and plotted on a semilogarithmic paper. In case when the experiment was repeated at intervals of 2-3 weeks, the rate of ^{59}Fe incorporation into red cells was calculated by subtracting the activity still remaining, which was reserved on the initial day of plasma-iron clearance, from the activity which appeared after correction was made with the hematocrit determined.

3. Animals: Two healthy horses, 11 months old, and three infected horses, 6 and 21 months old, were used. Two of infected horses were newly inoculated with virus immediately after the first performance of clearance. Thereafter, the same experiment was repeated in

Table 1. Calculation of Ferrokinetic Data

$PV = \frac{TAi}{a_0}$	PV=plasma volume in milliliters TAi=total activity injected a_0 =plasma activity at time 0
$BV = \frac{100 PV}{100\% - PCV}$	BV=blood volume in milliliters PCV=packed-cell volume
$RBCI = Hb \cdot \frac{BV}{100} \cdot 3.4$	RBCI=total erythrocyte iron in mg Hb=Hemoglobin expressed in grams per 100 ml. 3.4 is for the milligrams of iron per gram of Hb
$PITR = \frac{0.693 \cdot PI \cdot PV \cdot 24}{T \ 1/2}$	PITR=plasma-iron turnover rate in mg per day PI=plasma iron in mg per milliliter T 1/2=time in hours in which one-half of the injected dose disappeared from the plasma 24 is the number of hours per day
$Fe_c^{59} = 100 \cdot \frac{BV_{max} \cdot A_{max}}{TAi}$	Fe_c^{59} =percentage of injected radioactive iron incorporated into erythrocyte BV _{max} =blood volume on the day when the radioactivity in the erythrocyte reached maximum A _{max} =radioactivity per milliliter of blood at this time
$RBCIR = PITR \cdot Fe_c^{59}$	RBCIR=red blood cell iron incorporation rate in mg per day
If there is no change in blood volume, this will be equal to the red blood cell iron turnover rate (RBCITR) in mg per day.	
$RBCIRD = \frac{RBCITR}{RBCI}$	RBCIRD=fraction of red blood cell iron renewed per day Reciprocal of this figure expresses the erythrocyte life span

those horses at adequate stages of the disease. In these cases, the ^{59}Fe uptake of red cells before the first paroxysm was regarded as normal and compared with rates determined later. The other infected horse, 21 months old was at the remission stage with a rapid recovery of red cell count.

RESULTS

The data from which ferrokinetic values were calculated are presented in Table 2. The ferrokinetic data are given in Table 3.

1. Healthy horses: The plasma-iron clearance and the ^{59}Fe uptake of red cells are shown in Fig. 1. The T 1/2 was 1.48 hours on the average. The plasma-iron turnover rate (PITR) was 132 mg per day, or the plasma-iron turned over 10–13 times in a day. The highest activity of the blood appeared on the 11th day after injection of ^{59}Fe -bound plasma, when the incorporation rate (Fe_c^{59}) was 76% of the injected dose. The red blood cell iron incorporation

Table 2. Data for Calculation of Ferrokinetics

Horse No.	Age in months	Body weight kg	Plasma volume ml	Blood volume ml	Packed-cell volume %	Hemoglobin g/dl	Plasma iron μ /dl	RBC iron g	Plasma iron mg
228	11	237	11516	17317	33.5	12.5	100	7.36	11.5
230	11	245	11078	16172	31.5	11.6	104	6.38	11.5
Mean		241	11297	16745	32.5	12.1	102	6.87	11.5
	6	185	9697	14366	32.5	12.3	74	6.01	7.2
259	6	186	11088	14589	24.0	9.6	72	4.76	8.0
	6.5	194	10417	13706	24.0	10.8	62	5.03	6.5
	6	200	11080	15828	30.0	11.2	57	6.03	6.3
261	6	186	11252	15414	27.0	10.8	38	5.66	4.3
	6.5	192	11065	14370	23.0	9.8	65	4.79	7.2
	8	216	12630	16840	25.0	11.6	42	6.63	5.3
232	21	300	15879	19294	17.7	8.6	105	4.90	16.7

Table 3. Ferrokinetic Data

Horse No.	$T_{1/2}$ in hours	PITR mg/day	Fe_c^{59} %	RBCIIR (RBCITR) mg/day	RBCIRD %	PITR mg/kg/day	RBCIIR mg/kg/day	Condition
228	1.25	153	77	121.5	1.651	0.646	0.513	Healthy
230	1.72	111	74	84.1	1.318	0.453	0.343	
Mean	1.48	132	76	102.8	1.485	0.550	0.428	
259	0.820	145	82	119	1.98	0.783	0.643	First fever attack
	0.665	200	47	94	1.971	1.08	0.505	Second " "
	0.664	162	40	65	1.28	0.835	0.332	Second " "
261	0.895	105	72	76	1.26	0.525	0.380	First fever attack
	0.597	119	27	32	0.569	0.62	0.167	Remission
	0.824	145	61	89	1.85	0.755	0.461	Second fever attack
	0.620	142	57	81	1.22	0.66	0.375	Second fever attack
232	0.850	326	78	254	5.18	1.09	0.846	Remission

Note: $T_{1/2}$ =time in which one-half of the injected dose disappeared from the plasma; PITR=plasma-iron turnover rate; Fe_c^{59} =percentage of injected Fe^{59} incorporated into erythrocyte; RBCIIR=red blood cell iron incorporation rate; RBCIRD=fraction of red blood cell iron renewed per day

rate (RBCIIR) was 102.8 mg per day, or 1.49% of total hemoglobin iron. The reciprocal of this value indicates the red cell life span, which was calculated to be 69 (61 and 76) days. PITR and RBCIIR were 0.55 and 0.428 mg per kg of body weight per day, respectively.

2. Infected horses: Body temperature, blood picture, plasma-iron

Fig. 1. Plasma Iron Clearance and ^{59}Fe Incorporation into Red Cells in Healthy Horses

Fig. 2. Relations among Body Temperature, Hematological Change, Plasma Iron Clearance, and ^{59}Fe Incorporation Rate into Red Blood Cells in an Infected Horse

clearance, and Fe_e^{59} are presented in Figs. 2 and 3. Horse No. 259 had the first paroxysm 12 days after virus inoculation and suffered from three more fever attacks before sacrifice at the moribund stage. Its red cell count diminished gradually and reached 3 millions at the end of the course of disease, showing a very rapid decrease after the third fever attack. In horse No. 261, the first paroxysm took place 15 days after virus inoculation and the second 43 days after that. Its red cell count decreased gradually but not so remarkably. In horse No. 259, $T_{1/2}$ and PITS were 0.82 hour and 145 mg before virus inoculation, 0.67 hour and 200 mg at the first fever attack and 0.66 hour and 162 mg at the second paroxysm. In horse No. 261, those values were 0.89 and 105 before virus inoculation, 0.59 and 119 at the first paroxysm, 0.82 and 145 at the remission stage, and 0.62 and 142 at the second paroxysm. Fe_e^{59} showed 82 %, 47 % and 40 %, respectively, in horse No. 259 and 72, 27, 61 and 57 %, respectively, in horse No. 261, according to different stages of infection. At the paroxysm, PITS increased, but the Fe_e^{59} uptake of erythrocytes decreased, likely relating to the severity of the disease. PITS and RBCIIR

values per Kg of body weight per day also indicated the similar changes. At the remission stage, these values seemed to approach to the normal. In another infected horse, horse No. 232 (Fig. 4), which had suffered from three fever attacks in the past 3 months and the red cell count of which rapidly returned from 3 to 5 millions during the experiment, a shortened $T_{1/2}$ and an increased PITS were observed. The Fe_e^{59} was 78% and the initial slope was normal or steeper than normal. In this case, the activity of blood was not corrected for the hematocrit because of the rapid increase of red cell count.

Fig. 3. Relations among Body Temperature, Hematological Change, Plasma Iron Clearance, and ^{59}Fe Incorporation Rate into Red Blood Cells in an Infected Horse

Fig. 4. Plasma Iron Clearance and ^{59}Fe Incorporation Rate into Red Blood Cells in an Infected Horse Showing Rapid Recovery of Red Cell Count

DISCUSSION

There have been no kinetic data on iron metabolism in the horse. Ferrokinetic data

in various animals show that $T_{1/2}$ and Pitr are 1.82 hours and 0.81 mg/kg/day, respectively, in cattle¹¹, 1.57 and 0.56 in lambs¹¹, 1.19 and 1.11 in pigs⁴³, and 1.7 and 0.54 in man¹⁰. In this experiment, $T_{1/2}$ and Pitr in horses were found to be 0.86 hour and 0.65 mg/kg/day, respectively, at 6 months of age and 1.48 and 0.55 at 11 months of age. The plasma-iron clearance was more rapid and Pitr larger in younger horses than in older ones. Fe_c^{59} was about 76% and reached the maximum about 10 day after injection, this period being nearly the same as that seen in other animals. In this connection, for the determination of ferrokinetics in infected horses, the experiment was repeated several times on the same horse at various stages of infection as was described in the method. In infected horses, shortening of $T_{1/2}$ and increase of Pitr were observed at the paroxysmal stage. However, these values approached to those of the healthy state at the remission stage. On the contrary, the Fe_c^{59} and RBCIIR decreased markedly to about one-half or below of the normal value around the period of pyrexia. This phenomenon of low uptake of erythrocytes must be considered in the relation to the reduction of the red cell life span which was indicated by ^{51}Cr experiment¹⁵. However, this could not be denied, even when the presumed red cell destruction at this time was taken into consideration^{8,71}, from the appearance of the ^{59}Fe incorporation. In a horse which was at the remission stage and the red cell count of which was rapidly recovering, the Fe_c^{59} and RBCIIR were normal or above normal.

It has been generally noticed that Pitr increases under the hyperfunction and decreases under the hypofunction of the bone marrow^{2,8,13}. The results obtained from this experiment, however, did not always show the case, but the increase of Pitr was recognized in spite of the hypofunction of the bone marrow shown around the febrile stage⁹. Moreover, the experiment on the bone marrow uptake of plasma-bound ^{59}Fe , which will be reported elsewhere, showed that the ^{59}Fe cleared from the plasma did not shunt to other organs, such as the liver and spleen in an infected horse at the febrile stage as well as in a healthy one. There might be a possibility of ineffective erythropoiesis.

SUMMARY

The kinetics of iron metabolism was studied in 2 healthy horses, 11 months old, and 3 infected horses two of which were 6 months and the other 21 months old. The former two of infected horses, were inoculated with virus just after the plasma-iron clearance was performed and received repetition of the same experiment at various stages of infection. These horses suffered from 2 or 3 paroxysms during the experimental period. The ^{59}Fe uptake of red cells in them before the first febrile attack was regarded as normal and was compared with the values obtained later. The other one was at the remission stage with a rapid recovery of red cell count.

1. Healthy horses: The average value of $T_{1/2}$ was 1.48 hours, Pitr was 0.55 mg/kg/day, and the rate of ^{59}Fe incorporated into red cells was 76%, from which the life span of red cells was calculated to be 69 days.

2. Infected horses: $T_{1/2}$ and Pitr which were obtained before virus inoculation were 0.86 hour and 0.65 mg/kg/day and the ^{59}Fe incorporation rate which was observed before the first febrile attack was 77% on the average. Values of $T_{1/2}$, Pitr/kg/day and Fe_c^{59} obtained according to the stages of disease were 0.67 hour, 1.08 mg and 47% at the first fever attack, and 0.66 hour, 0.84 mg and 40% at the second pyrexia in one patient horse, and 0.59 hour, 0.62 mg and 27% at the first fever attack, 0.82 hour, 0.76 mg and 61% at the remission and 0.62 hour, 0.66 mg and 57% at the second pyrexia in the other one. Shortening of $T_{1/2}$, increase of Pitr and decrease of ^{59}Fe utilization by red cells were observed at the paroxysmal stage. At the remission stage between fever attacks, the

values of T 1/2, Pitr, and incorporation rate approached to the normal. In a horse in which anemia was recovering rapidly, the incorporation rate was 78%, which was normal or above normal.

It is concluded, therefore, that the red cell production of the infected horse decreases around the period of pyrexia and fluctuates according to the severity or the course of disease. The function comes to a normal or more active state than normal at the remission stage in which the animal has tolerated severe anemia, showing a rapid recovery from it.

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馬 伝 染 性 貧 血 の Ferrokinetics に つ い て

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私たちは、馬伝染性貧血の発熱時には、血清鉄が減少するが、総血清鉄結合能は変わらないこと、肝・脾の貯蔵鉄量が健康時とは逆になって、肝の鉄量が脾の鉄量より多くなること、循環赤血球量が減少すること、赤血球の寿命が短縮することなどを認め、特に貧血が赤血球破壊の増加に原因することを報告した。しかし、貧血は赤血球の産生と破壊の両面から考えられるべきである。今回、骨髓造血能を知る目的で、放射性鉄を用い、血清鉄ならびに赤血球鉄の代謝回転を測定した。

この実験では、*in vitro* で血漿に結合させた ^{59}Fe の一定量を静脈内に注射して、その放射活性の半分が循環血漿中から消失する時間 ($T_{1/2}$) を測定することによって、24 時間に血漿中に入出する鉄量、すなわち血清鉄の代謝回転率 (PITR) を求めた。次にこの消失した ^{59}Fe が、骨髓で赤血球中のヘモグロビン鉄に生合成され、ふたたび流血中にあらわれる活性を測定して、赤血球の ^{59}Fe 利用率および赤血球鉄の代謝回転率を求めることによって、定量的に骨髓造血能を判定した。

従来種々の動物で測定された $T_{1/2}$ および PITR の値を示すと、牛では 1.82 時間で 0.81 mg/kg/day (以下、単位を省略する)、緬羊では 1.57 時間で 0.56、豚では 1.19 時間で 1.11、人では 1.7 時間で 0.54 である。馬での成績はない。この実験での健康値は、生後 6 カ月の馬で 0.86 時間と 0.65、生

後 11 カ月の馬で 1.48 時間と 0.55 であった。

赤血球の ^{59}Fe 利用率は 76% で、約 10 日でその極大値に達し、他の動物と同様な成績を示した。ヘモグロビン鉄の 1 日代謝量は、総ヘモグロビン鉄の 1.49% で、この逆数、すなわち赤血球寿命は約 70 日であった。

伝染馬では、種々の病的時期に測定した。発熱時には血清鉄の $T_{1/2}$ は短縮し、その代謝回転率は増加した。しかし赤血球の鉄利用率は 27%, 40% 等と減少し、赤血球鉄の代謝回転も $\frac{1}{2}$ に減少した。このことは明かに骨髓造血能の減退を意味している。

一方、無熱時には、これら $T_{1/2}$ 、PITR および赤血球の鉄利用は、健康値に近い値を示した。また無熱時で赤血球数が急速に回復しつつあった 1 例では、赤血球の鉄利用は、健康値あるいはそれ以上を示した。

伝染馬骨髓の形態学的な研究は数多くあるが、貧血との関係について明確な結論はなされていない。石井らは、穿刺骨髓液で有核赤血球数の総有核細胞数に対する比が、発熱時減少することを観察している。本実験によって、伝染馬の赤血球産生は、少なくとも熱発作期に減退し、貧血は一部この骨髓造血機能の低下に基づくことを確認し得たものと考えられる。