LIFE SPAN OF $^{51}$Cr-LABELED ERYTHROCYTES IN EQUINE INFECTIOUS ANEMIA

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Erythrocytes labeled with chromium-51 have been used to determine the apparent life span of red cells and the red-cell destruction in various hematological disorders$^{5,7,13}$. This method is originally employed to evaluate the viability of preserved blood for transfusion and used ordinarily in such a condition that the production and destruction of erythrocytes are steady, that is, when the blood volume is constant$^{11}$. Therefore, the condition in which the blood volume is changing might cause an error in the evaluation of the result. This method, however, has advantages of getting results rather promptly and using the same animal for repeated experiments at appropriate intervals$^9$. The apparent half life of $^{51}$Cr-labeled erythrocytes in various animals is known to be 28.4 to 32.8 days in man$^{4,10}$, 17 days in pigs$^8$, 21-30 days in dogs$^{10}$, 18.4 days in rats$^6$, and 12 days in rabbits$^5$. No data are available on horses. This paper deals with the determination of the life span of the red cell in healthy horses and the comparison of the life span between healthy and infected horses.

METHODS

1. Labeling and counting$^5$: To heparinized blood, saline dilution of about 500 μc of Na$_2^{51}$CrO$_4$ was added so that the amount of metallic chromium might not be over 5 μg per ml of red cells. The blood was then incubated at room temperature for 40-60 minutes, being shaken gently, and an adequate quantity of vitamin C was added to reduce $^{51}$Cr not combined with erythrocytes. The blood was spun at 2-3,000 rpm for 20 minutes in the refrigerated centrifuge at 4°C. Plasma was siphoned off from it and it was spun again at 3,000 rpm for 20-30 minutes in the centrifuge after addition of saline cooled at 0°C and amounting to three times the volume of packed red cells. The supernatant was discarded. About 50 ml of red cells were injected into the jugular vein and recorded. Oxalated blood was drawn from the opposite side of the vein three time within an hour to estimate the blood volume. For 3 weeks, 5-6 ml of blood were removed every day and placed in a couple of plastic tubes in 2 ml amounts to count the activity with a well-type scintillation counter (manufactured by the Rikagaku Kenkyusho, Ltd.). As a standard, 1-2 ml of red cells used for injection were diluted with water and preserved in a mess flask to correct the decay of radioactivity.

2. Animals: Four healthy and two infected horses were used. Two of each group were tested again at 20 days' intervals when the activity of the blood was diminished to about one-tenth of the initial. Hematological examination was conducted twice a week.

3. Calculation: The activity of the blood was plotted against the time on a semilogarithmic paper after corrected with hematocrit and extrapolated to time zero by least square. As it was usually noticed that the initial part of the curve was different from the following, that part was excluded. When hematocrit was progressively decreased in the infected horses, the activities both corrected and not corrected with hematocrit were plotted.
on the paper. No correction was made for the bleeding which amounted to 50-150 ml in total.

RESULTS

The survival time of erythrocytes in the healthy horses was indicated in Fig. 1. The apparent half life, as shown in 6 experiments, was 14.96 (in a range of 12.6-18.5) days. In the infected horses, body temperature, hematological changes, and the survival time of erythrocytes were as given in Figs. 2 and 3. The first paroxysm appeared 16 days after virus inoculation in Horse No. 240, which suffered from two more attacks until death which occurred 37 days after inoculation. Red cell count showed a gradual decrease to 2 millions at the final stage. Hematocrit decreased to below 10% at the moribund stage. Siderocytes, which are defined, in this case, as white blood cells containing Prussian blue positive iron granules, were found at a rate of 35% of the white blood cells in the peripheral blood. The half life of erythrocytes was 15 days in the first performance and about 10 days in the second. When no correction of the activity was made for the hematocrit, the half life became 11 days in the first and 5 days in the second performance. Horse No. 258 suffered from the first paroxysm 11 days after virus inoculation and from two more fever attacks during the experiment. A decrease in red cell count and hematocrit was remarkable in this horse as in No. 240. Siderocytes were numerous. The half life of erythrocytes was 10 days in the first and 8.8 days in the second performance. When the activity was not corrected for the hematocrit, the half life became 7 and 6 days, respectively.

DISCUSSION

The apparent half life of erythrocytes in healthy horses was about 15 days in the present study. This value is close to
that in pigs\(^2\). That the initial curve was very steep in this experiment is perhaps due to the influence of the procedures of labeling, washing or centrifuging.

In the infected horses, the half-life was about 11 days on the average. The value was expressed as an activity per unit volume of red cells. Therefore, if the production of erythrocytes diminished, the life span would be prolonged, and if the destruction overcame the production, the life span would be shortened. It was found in another ferrokinetic experiment on infected horses, which will be reported later\(^3\), that erythropoiesis diminished at the paroxysm of the disease. Accordingly, the shortening of the half life of erythrocytes indicates the predominance of erythrocyte destruction over erythropoiesis. When the activity of the blood was not corrected with hematocrit\(^3,10\), the half life was distinctly shortened, being 7 days on the average. Although it was reported that hematocrit changed even in healthy animals according to circumstances\(^5\), the remarkable decrease in hematocrit and red cell count observed in this experiment was considered to show the decrease of red cell volume. Furthermore, since no reason was found in those horses for the increase of red cell production, it may well be that the results obtained indicate the increase of red cell destruction. Any actual cause of the increased red cell destruction in the infected horses remains to be studied.

SUMMARY

The survival time of erythrocytes labeled with \(^{51}\)Cr was determined in healthy and infected horses. In healthy horses, the apparent half life was 14.96±1.98 days, being in the range of 12.6-18.5 days. In infected horses, the half life was 11 days when the activity was corrected with hematocrit, or 7 days when it was not. It was, therefore, concluded that red cell destruction was promoted actively in infected horses.

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REFERENCES

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馬伝染性貧血における $^{51}$Cr 標識赤血球の寿命について

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種々の血液疾患の場合に、$^{51}$Cr で標識した赤血球の寿命を測定比較することによって、赤血球破壊の状態を知る方法が用いられている。各種の動物における $^{51}$Cr 標識赤血球の見かけの半寿命は、人で 28～33 日、豚で 17 日、犬で 21～30 日、ラットで 18 日、ウサギで 12 日とされるが、馬での成績はない。今回の、伝貧馬の発熱時を中心として赤血球寿命を測定し、健康馬のそれと比較した。

本法は、元来輸血用保存血液の活力をみるために工夫されたもので、原則として赤血球の生産と破壊が平衡状態にあり、血液が恒常に保たれている個体に適用される。従って測定期間中、これらの条件が変化する状態では、結果の判定に慎重を要するところである。一般に、標識赤血球注射後の血液の放射活性は、ヘマトクリット値（以下 Het と略す）で補正して、一定赤血球容積当たりの、すなわち測定当初の赤血球量に直した値としてあらわす。このときの活性は、非標識赤血球に対する標識赤血球の割合として出てくるから、もし赤血球生産がおとろえていれば、寿命は延長してあらわれ、またその逆の場合も成り立つ。一方、測定期間中に Het また赤血球が急激に減少した場合、実際に赤血球量そのものが減少していれば、活性を Het で補正しないほうが、より真実性がある。かかる点を考慮して、伝貧馬の場合の活性を Het 無補正のもと二様に計算して、片対数表にプロットした。その結果、伝貧馬の赤血球の見かけの半寿命は 11 日、Het 無補正値は 7 日で、健康馬の半寿命 14.96±1.98 日（範囲 12.6～18.5）に比べて明らかにその短縮を認めた。

伝貧馬の貧血機転については多数の見解があるが、その多くは形態学的な所見によるもので、明確さを欠いている。本実験によって、伝貧馬の貧血は、少なくとも赤血球寿命の短縮、すなわち溶血性貧血の範疇に属する現象によっておこることが明らかとなった。骨髄造血能の測定結果については後報する。