

# Measurement of Erythrocyte Volumes in Splenectomized Horses and Sham-Operated Horses at Rest and during Maximal Exercise

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**ABSTRACT.** Erythrocyte volumes of thoroughbred horses were measured. The volumes of splenectomized horses and sham-operated horses 2 hr after injection of <sup>50</sup>Cr- tagged erythrocytes (at rest) and during maximal exercise were measured using the non-radioactive isotope <sup>50</sup>Cr. Because splenic erythrocytes are released into circulation during exercise, it was estimated that the erythrocyte volumes of the sham-operated horses during maximal exercise are larger than those of the horses at rest. However, the erythrocyte volumes of the sham-operated horses at rest were about equal to those during maximal exercise. In the splenectomized horses, furthermore, erythrocyte volumes at rest and those at exercise were nearly equal. From these results, blood stored in the equine spleen is gradually mixed with circulating blood, and it was clarified that the phenomenon was completed within 2 hr. Although it is basically impossible to measure the circulating erythrocyte volume at rest using the erythrocyte tagged method, we observed that it is possible to measure the total erythrocyte volume using the <sup>50</sup>Cr method. Also, the plasma volumes of the splenectomized horses during maximal exercise were found to be slightly smaller than those at rest. On the other hand, in the sham-operated horses, the plasma was decreased by a large quantity after maximal exercise. Therefore, it was suggested that the spleen participates in the phenomenon involving the disappearance of plasma from circulation due to exercise. — **KEY WORDS:** chromium-50, equine, erythrocyte volume, plasma volume, spleen.

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It is well known that the equine spleen stores blood in large quantities and it is contracted by a slight stimulation such as excitation and exercise [3]. The reserved blood of the spleen is then released into circulation. However, at rest when the spleen does not contract, it is not clear whether the reserved blood in the spleen is exchanged with the blood of circulation during a short time. On the assumption that injected <sup>50</sup>Cr-tagged erythrocytes are not miscible with the reserved blood in the spleen in a short time in the case of measuring circulating blood volumes excluding splenic blood, the circulating blood volumes of horses were measured using <sup>50</sup>Cr [5]. If the <sup>50</sup>Cr-tagged erythrocytes are miscible with the reserved splenic blood during rest, it is difficult to measure accurately the circulating erythrocyte volume excluding splenic erythrocyte volume.

In the horse, it is well-known that exercise cause an increase in packed cell volume (PCV). This phenomenon has been explained by mobilization of erythrocytes stored in the spleen [12]. On the other hand, there is a report that the rate of decrease in plasma volume due to exercise (% $\Delta$ PCV) of an intact horse and that of an splenectomized horse agreed [11]. From these results, it is estimated that the increase in erythrocytes and the decrease in plasma participates in the increase in PCV due to exercise.

In the present study, to clarify whether the reserved blood in the spleen is exchanged with the blood of circulation during a short time, the erythrocyte volumes of horses at rest and during maximal exercise were measured using <sup>50</sup>Cr. Further, erythrocyte volumes, PCV, plasma proteins (PP) and the % $\Delta$ PCV of splenectomized horses were measured.

## MATERIALS AND METHODS

**Horses:** Six thoroughbred horses (4 stallions, 2 mares) ranging in weight from 384 to 478 kg were used in this study. Two stallions and one mare (all 3 years) were splenectomized (SPH), and the other two stallions (3 years and 10 years) and one mare (3 years) had a sham operation (SOH) performed. The splenectomy was carried out by the method of Roberts and Groenendyk [13]. The detailed method of the splenectomy has been reported in another paper [6]. The mean weight of the removed spleens was 11 kg. Erythrocyte volumes of all horses during exercise were measured before the operation (in August). Further, the erythrocyte volumes of the horses at rest and during exercise were measured 2 months after the operations (in November). During the experiments, we did not give food and drinking water to the horses.

**Preparation of Na<sub>2</sub>CrO<sub>4</sub> solution:** The stable isotope <sup>50</sup>Cr (94.6%, natural abundance 4.3%) was obtained from Oak Ridge National Laboratory in the form of the oxide (<sup>50</sup>CrO<sub>3</sub>). The oxide was mixed in a platinum crucible with a 1:1:5 mixture of Cr<sub>2</sub>O<sub>3</sub>, NaNO<sub>3</sub>, and Na<sub>2</sub>CO<sub>3</sub> and then fused by strong heating. The <sup>50</sup>Cr concentration of the solution was adjusted to 100  $\mu$ g/ml.

**Erythrocyte labeling:** Approximately 200 ml of blood was collected with a transfusion bag (containing 28 ml of CPD solution) from the jugular vein. The blood was incubated with 1.5 mg of <sup>50</sup>Cr (15 ml) for 30 min, and the mixture was then incubated for 10 min with 700 mg of ascorbic acid (7 ml). Five ml of the <sup>50</sup>Cr tagged blood was used to measure

the PCV, and the rest of the blood was then centrifuged and the erythrocyte portion obtained was freeze-dried. The rest of the tagged blood in the bag was injected intravenously. A 10-ml sample of blood was collected 2 hr after injection of the tagged blood. The blood sample was used to measure the PCV and the PP sample was centrifuged and freeze-dried. The measurement of PCV was performed by the microhematocrit method (12,000 rpm, 5 min) twice, and the mean value was calculated. The PP was measured using a refractometer.

**Exercise:** To exercise the horses, a treadmill (Mustang 2200, Kagra, Swiss) was used. The horses ran on the treadmill set at the speed of 4 m/s and 0% grade for 5 min (warm-up), and then they were allowed to rest for 5 min. Then they ran up a 7% (6.3°) grade on the treadmill set at an initial speed of 1.5 m/s. Speed was increased gradually (1.5 4.4 6.0 7.4 8.6 9.4 m/s); the duration of the speed was 1 min in each case. When the horses were not able to keep up with the speed of the treadmill because of fatigue, the exercise was stopped. The blood was collected from the jugular vein at maximal speed (maximal exercise) and 5 min after termination of exercise; the PCV and PP of the collected blood were then measured. Further, the rest of the blood in maximal exercise was centrifuged and freeze-dried.

**Thermal neutron irradiation and  $\gamma$ -ray spectrometry:** All freeze-dried erythrocyte samples, weighing about 80 mg, were individually doubly sealed in polyethylene bags. To determine the amount of  $^{50}\text{Cr}$ , 50  $\mu\text{l}$  of  $\text{Na}_2\text{CrO}_4$  solution (5  $\mu\text{g}$  of  $^{50}\text{Cr}$ ) was absorbed in a piece of filter paper and dried. The filter papers were sealed in polyethylene bags and contained in polyethylene irradiation capsules with other samples. Neutron irradiation was performed with the reactor (JRR-4, maximal power 3.5 MW) at the Japan Atomic Energy Research Institute. Neutron flux was  $8 \times 10^{13} \text{ cm}^{-2} \cdot \text{s}^{-1}$  and the irradiation time was 20 min. After the irradiation, the capsule was cooled for about 20 days to allow the short half life of the radioisotope to decay. The  $\gamma$ -rays from the irradiation samples were measured by a high purity germanium detector (relative efficiency 30%, CANBERRA) connected to a 4096 channel pulse height analyzer (Series 35 Plus, CANBERRA) for  $\gamma$ -ray spectrometry. Erythrocyte volumes at rest and during exercise were calculated by the  $^{51}\text{Cr}/^{59}\text{Fe}$  ratio method [8].

**Calculation of the rate of decrease in plasma volume due to exercise (% $\Delta$ PPV):** There is a report [15] that the % $\Delta$ PPV in humans was calculated by the following equation.

$$\% \Delta \text{PPV} = [100 / (100 - \text{PCV}_{\text{Pre}})] \times [100 \times (\text{PCV}_{\text{Pre}} - \text{PCV}_{\text{Post}}) / \text{PCV}_{\text{Post}}]$$

where  $\text{PCV}_{\text{Pre}}$  is the PCV of circulating blood at rest (%),  $\text{PCV}_{\text{Post}}$  is the PCV of circulating blood during maximal exercise (%).

In the calculation method, however, the effect of splenic blood is neglected. Therefore, Mckeever *et al.* [11] calculated the % $\Delta$ PPV of a horse before and after exercise by

the method below. Because the splenic erythrocyte reserve is mobilized after exercise, they suggested that the hemodynamics reach a plateau within the first 1–2 min of exercise. Therefore, they subtracted the PCV of circulating blood at rest from that 2 min after termination of exercise, then subtracted the calculated value from the PCV of circulating blood during maximal exercise. It was assumed that the obtained value is  $\text{PCV}_{\text{Post}}$ . In this study, we calculated the % $\Delta$ PPV by the method described above.

**Statistical analysis:** Because the data were small numbers, the distribution patterns of erythrocyte volumes were unclear. Therefore, the Mann-Whitney's U-test was performed to analyze the mean values between the two groups.

## RESULTS AND DISCUSSION

For all horses in this study, the maximal speed of the treadmill was above 8.6 m/s. In the horses, the pulse during maximal exercise was from 220 to 240 beats per minute. There is a report [9] that the maximal heart rate is approximately 230 beats per minute. In the exercise, therefore, it is considered that maximal exercise was applied to the horses.

The erythrocyte volumes of the SOH were  $22,400 \pm 1,300 \text{ ml}$  (Mean  $\pm$  S.D.,  $n=3$ ) at rest and  $23,300 \pm 2,500 \text{ ml}$  (Mean  $\pm$  S.D.,  $n=3$ ) during maximal exercise, and there was no significant difference in the erythrocyte volumes (Fig. 1). In the horse, it is estimated that the spleen stores blood in large quantities. Therefore, if  $^{50}\text{Cr}$ -tagged erythrocytes are not mixable with the splenic blood during rest (within 2 hr after injection of  $^{50}\text{Cr}$ -tagged erythrocytes into the body), it must be calculated that the erythrocyte volume at rest is smaller than that during exercise. However, both volumes almost agreed. In the SPH, further, erythrocyte volumes were  $13,100 \pm 100 \text{ ml}$  (Mean  $\pm$  S.D.,  $n=3$ ) at rest and  $12,500 \pm 800 \text{ ml}$  (Mean  $\pm$  S.D.,  $n=3$ ) during maximal exercise, and there was no significant difference in these two values (Fig. 1).

In the previous paper [5], we reported that the  $^{51}\text{Cr}/^{59}\text{Fe}$  ratio gradually decreased within 60 min after injection and then was maintained at a stationary level. In the paper, we reported that the injected  $^{50}\text{Cr}$ -tagged erythrocytes mixed completely with circulating blood in about 60 min at rest. Because the circulating blood of the equine spleen was gradually exchanged with the blood of circulation at rest, it is clear that most of the splenic blood was exchanged within 60 min after injection of  $^{50}\text{Cr}$ -tagged erythrocytes. From these observations, in the horse, it is basically impossible to measure the circulating erythrocyte volume excluding splenic blood using the erythrocyte labeled method; however, it can be used to measure the total erythrocyte volume using the  $^{50}\text{Cr}$  method.

To support the previous results, the splenic erythrocyte volume of the horse used in this study was estimated. The mean weight of the removed spleen from thoroughbred horses ( $n=3$ , all 3 years, mean body weight is 468 kg) was

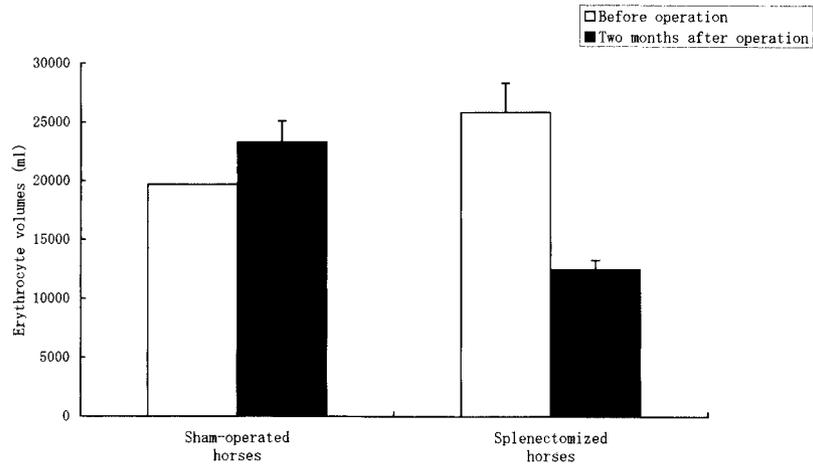


Fig. 1. Erythrocyte volumes of sham-operated horses and splenectomized horses during maximal exercise before splenectomy and two months after splenectomy. Error bars show standard deviation ( $1\sigma$ ). Erythrocyte volumes of sham-operated horse before splenectomy are based on the data for one head.

11,000 g. It is reported that the mean splenic weight of the removed blood is approximately 2,000 g [2]. There is no report on the specific gravity of equine splenic blood. Therefore, the specific gravity of whole blood of a 3-year-old thoroughbred horse (1.055) [1] was used in the calculation. Although there is no report on the measured value in the PCV of equine splenic blood at rest, it is estimated that the value is approximately 80% [11]. There are reports that the PCV in the splenic blood of a dog is about 78% [14], and that of a cat is 80% [4]. When we calculated using these values, it was determined that the splenic erythrocyte volume of the horse in this study was approximately 6,800 ml.

In the SOH, we compared the total erythrocyte volumes before operation and those after operation. The total erythrocyte volumes of the SOH were 19,700 ml ( $n=1$ ) before the sham operation and  $23,300 \pm 2,500$  ml (mean  $\pm$  S.D.,  $n=3$ ) 2 months after the sham operation (Fig. 2). The total erythrocyte volumes 2 months after the operation were

larger than those before the operation. However, the total erythrocyte volume before the operation is based on the data for one horse. In the horse, the total erythrocyte volume before the operation was 20,800 ml. The value was similar to that after the operation. Therefore, we thought that there is no change in the erythrocyte volume before and after the operation.

In the SPH, further, we compared the total erythrocyte volumes before operation and those after operation. The total erythrocyte volumes of the SPH were  $25,850 \pm 1,800$  ml (Mean  $\pm$  S.D.  $n=2$ ) before the splenectomy and  $12,500 \pm 800$  ml (Mean  $\pm$  S.D.  $n=3$ ) 2 months after the splenectomy (Fig. 2). The difference between these two values was 13,350 ml, and it is seemed that the difference corresponded to the erythrocyte volume of the spleen. As described above in this paper, however, it was estimated that the erythrocyte volume of the spleen calculated from the weight of the removed spleen was approximately 6,800 ml. The estimated splenic erythrocyte volume was smaller than the difference

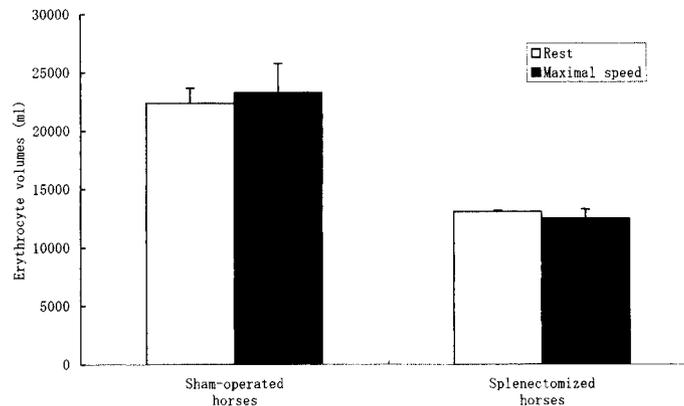


Fig. 2. Erythrocyte volumes of sham-operated horses and splenectomized horses at rest and during maximal exercise. Error bars show standard deviation ( $1\sigma$ ).

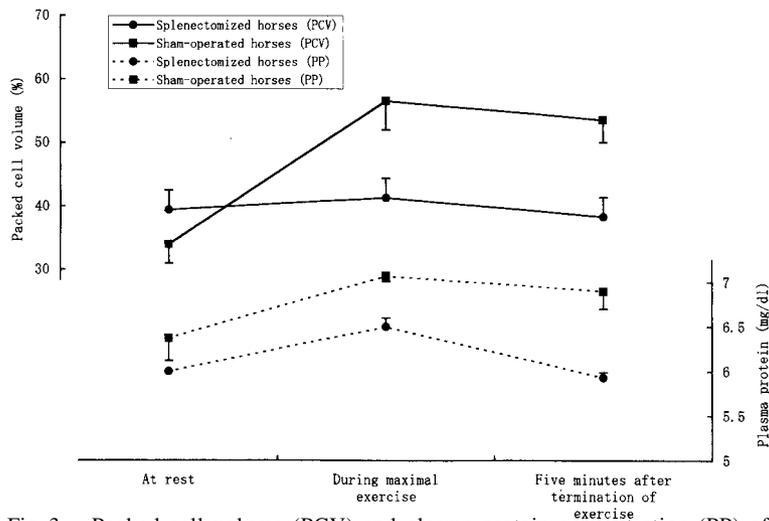


Fig. 3. Packed cell volume (PCV) and plasma protein concentration (PP) of splenectomized horses and sham-operated horses at rest, during and after exercise. Error bars show standard deviation ( $1\sigma$ ).

Table 1. The  $\% \Delta PV$  of the horses

Horse	$\% \Delta PV$
Splenectomized horses	$-7.7 \pm 0.6$
Sham-operated horses	$-34.3 \pm 15.5$

Mean  $\pm$  S.D. (n=3)

in the total erythrocyte volumes before and after splenectomy. The difference between these two volumes was approximately 6,500 ml.

As previously described, the total erythrocyte volumes of the SOH before and after the sham operation were unchanged. In all horses used in this experiment, further, hemorrhage during the operation was slight, and the postoperation course was uneventful. There is a report [16] that production of erythrocytes in a splenectomized dog decreases, and then the number of circulatory erythrocytes in the dog decreases. Therefore, it is considered that the number of erythrocytes was decreased by splenectomy.

The PCV and PP of the SPH and SOH before and after exercise are shown in Fig. 3. In the SPH, the PCV during maximal exercise was slightly higher than that at rest. However, the PCV 5 min after exercise was similar to that at rest. Changes in the PP of the SPH were synchronized with those in the PCV of the SPH. Because the SPH does not have a spleen with a large reserve volume of erythrocytes, it is improbable that the erythrocytes are mobilized into circulation. Further, the increase in PP during exercise shows the concentration of plasma. In this study, the sweat volumes of the horses during exercise were not measured. However, because the exercise was finished in a short time (within 10 min), it is considered that the sweat volume was small. From these observations, it is thought that the temporary increase in the PCV and PP of the SPH due to exercise is caused by temporarily moving

plasma water to the extravascular space. In the SOH, on the other hand, the PCV during maximal exercise increased about 66% in comparison with the PCV at rest (Fig. 3). Further, the rate of increase in the PP in the SOH was higher than that in the SPH. The phenomenon is considered to be caused by mobilization of erythrocytes from the spleen into circulation and the temporary movement of plasma. We calculated the  $\% \Delta PV$  of the SPH and SOH and compared the volumes.

Table 1 shows the  $\% \Delta PV$  in the SPH and SOH. The  $\% \Delta PV$  values were  $-7.7 \pm 0.6\%$  (Mean  $\pm$  S.D., n=3) in the SPH and  $-34.4 \pm 15.5\%$  (Mean  $\pm$  S.D., n=3) in the SOH, and the rate of decrease in the SOH was higher than that in the SPH ( $P < 0.05$ ).

The calculation formula for  $\% \Delta PV$  has been used to calculate the  $\% \Delta PV$  in humans before and after exercise [15]. In this method, the rate of change in plasma is calculated from only the change in the PCV before and after exercise. Because little blood is stored in the human spleen, in the method of calculating the  $\% \Delta PV$ , the effect of mobilization of the splenic blood into circulation has been neglected. However, the horse has a spleen which stores a lot of blood, and the stored blood is released into the circulation during exercise. There is a report [3] that the mobilization of the splenic blood into the circulation reaches a plateau within 2 min after exercise. Therefore, Mckeever *et al.* [11] calculated the  $\% \Delta PV$  in horses according to the following method. They subtracted the PCV of circulating blood at rest from that 2 min after exercise, then subtracted the obtained value from the PCV during maximal exercise. They considered the calculated value as the PCV after exercise. In this study, we calculated the  $\% \Delta PV$  according to their method. As the result of the comparison of the  $\% \Delta PV$  in the SOH and SPH calculated by the method, the  $\% \Delta PV$  in the SOH showed a value up to four times that of SPH.

Mckeever *et al.* [11] reported that the  $\% \Delta PV$  in both an intact horse and a splenectomized horse was - 18%. The  $\% \Delta PV$  in the SPH in this study was below half the result of Mckeever *et al.* Regarding the reason, there is a possibility of a difference in the strength of the exercise. However, they performed according to the experimental conditions (inclination of treadmill, 6°; maximal speed, 7 m/s). The strength of exercise in this study was hard compared to that in Mckeever's. Therefore, it is difficult to believe that the strength of exercise in this study leads to the difference. Certainly, the  $\% \Delta PV$  in the SOH in this study was higher than that in Mckeever's. The cause of the lower  $\% \Delta PV$  of the SPH in this study compared to that in the study by Mckeever *et al.* is unclear.

Mckeever *et al.* [10] reported that exercise caused significant increases in plasma protein concentration and plasma  $K^+$  concentration in intact and splenectomized horses and that the plasma  $Na^+$  concentration did not increase significantly in either horse. Therefore, they suggested that a decrease in plasma volume with exercise is due to an isotonic shift of fluid into the interstitial spaces. In this study, it is considered that the mechanism of the decrease in the plasma volume in the SOH and SPH is similar to that of Mckeever's. However, the rate of decrease in plasma volume in the SOH was up to four times that in the SPH. In the horse, therefore, it was suggested that the spleen participates in the phenomenon of the disappearance of plasma from circulation with exercise.

Recently, there is a report [7] that the spleen of the rat has a role in the decrease in plasma volume. In this paper, it is reported that hypervolemia causes an increase in the intrasplenic filtration of cell-free fluid from the circulation.

Based on this report and our observation results, the possibility exists that the spleen of the horse has a role in the decrease in plasma during exercise.

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