安定濃縮同位体50Crを用いた犬の血液量の測定

誌名	Japanese journal of veterinary science
ISSN	00215295
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巻/号	51巻5号
掲載ページ	p. 855-860
発行年月	1989年10月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
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Determination of Blood Volume in Dogs using an Enriched Stable Isotope ^{50}Cr

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(Received 13 February 1989/Accepted 19 May 1989)

ABSTRACT. For the measurement of canine blood volume, various experimental conditions and techniques have been investigated using a non radioactive stable isotope ^{50}Cr. On the basis of the results in this preliminary work, erythrocytes were labeled using ^{50}Cr. Five μg of ^{50}Cr per 1 m*l* of blood was added and incubated for 60 min. The canine erythrocytes were tagged using ^{50}Cr and injected into vein of the same dogs. The blood samples collected at 60 min after the injection were irradiated by thermal neutron for 20 min at the reactor of the JAERI. Activities of ^{51}Cr (the ^{50}Cr concentration method) and $^{51}Cr/^{59}Fe$ radioactivity ratios (the $^{51}Cr/^{59}Fe$ ratio method) in the samples were measured. There was a very high correlation ($r=0.97$, $P<0.001$) between the blood volumes calculated by the ^{50}Cr concentration method and the $^{51}Cr/^{59}Fe$ ratio method. The latter method is less complicated than the former, because measurement of the sample weight and correction of thermal neutron flux are unnecessary. The mean blood volumes calculated by the ratio method and the Evans blue method were 89.8 ± 6.8 m*l*/kg B.W. (mean±SD) and 98.9 ± 10.6 m*l*/kg, respectively, showing a significant difference between them ($P<0.05$). However, these values are almost in accord by correction of venous blood PCV values with factor 0.97. As a detection limit of ^{50}Cr was approximately 0.1 ng per 1 m*l* of blood in this system, this method has been concluded to be applicable to the measurement of the blood volume of cattle.—KEY WORDS: activable tracer method, blood volume, dog, erythrocyte volume, ^{50}Cr

Jpn. J. Vet. Sci. 51(5): 855–860, 1989

To determine plasma and blood volumes, a variety of techniques have been used. In general, plasma volume determinations using the radio-iodinated (^{131}I) human serum albumin and the dye are familiar procedures [12]. As the dye, T-1824 (Evans blue) has widely been used to measure the plasma volume [2, 8, 11, 15].

When plasma volume is estimated using Evans blue, it is necessary to correct transudation of the dye from blood vessel [14]. To measure the blood volume, therefore, it is widely performed to label erythrocytes. The erythrocyte volume is generally determined by radioactive chromium (^{51}Cr) as a most reliable tracer [5, 7, 13, 16, 19, 20]. In 1950 Gray and Starling [10] reported that a ^{51}Cr was useful for labeling the erythrocytes *in vitro* and the radioactivity was retained without significant loss for about 24 hr. However, it is restricted to use radioactive materials in case of measuring blood volumes of animals in clinical use.

Recently, a few investigators have used a non-radioactive chromium (^{50}Cr) for tagging to erythrocytes, and determined the blood volume by neutron activation analysis. This method, which eliminates the irradiation from the patient, can be used in pregnant women and children. In 1968, Donaldson and his colleagues [3] applied the non-radioactive method to patients. Glomski *et al.* [9] published data using a lithium drifted germanium detector and neutron activation. But the method has disadvantages such as to require a large volume of blood for labeling and to be interfered with a small amount of ^{50}Cr existing in quartz ampoules [4, 6, 21]. Then, Yamabayashi *et al.* [22] improved these disadvantages, and developed an activable tracer method using ^{50}Cr to detect a change

of neonatal blood volume. In this study, authors calculated blood volume of dogs using ^{50}Cr to measure fundamental data for the blood volume of various animals.

MATERIALS AND METHODS

Preparation of $Na_2{}^{50}CrO_4$ solution: The enriched stable isotope ^{50}Cr (96%, natural abundance 4.3%) was obtained from Oak Ridge National Laboratory as a form of oxide ($^{50}Cr_2O_3$). The oxide was triturated in a platinum crucible with a 1:1:5 mixture of Cr_2O_3, $NaNO_3$, and Na_2CO_3, and then fused by heating to redness. The yellow melt was cooled and dissolved in normal saline solution, and the concentration was adjusted to 20 μg (^{50}Cr)/m*l*.

Animals: Nineteen normal dogs were used ranging in weight from 4 to 11.5 kg in this study.

Evans blue method: A blood sample was collected from a juglar vein for preparing blank plasma sample and measuring microhematocrit as packed cell volume (PCV) prior to injection of the dye. The 0.5% solution of Evans blue (1 m*l*) was injected intravenously and then blood samples were collected at 10, 15, 20, 25, 30, 60, 120, 180, 240, and 300 min later. These samples were centrifuged at 3000 rpm for 15 min, and the plasma samples were read at 620 nm against a blank plasma sample on a spectrophotometer. And the optical density of the Evans blue solution diluted 1:500 in blank plasma was read similarly (Es). The initial dye concentration expressed as optical density were derived by extrapolation to the zero time (Em_0) of disappearance curve. The plasma volume was calculated by the equation;

$$\text{Plasma volume } (\mathrm{m}l) = \frac{\mathrm{Es} \times 500}{\mathrm{Em}_0}$$

The blood volume was calculated from the PCV. Equation for calculation of blood volume was the following.

$$\text{Blood volume } (\mathrm{m}l) = \frac{\text{Plasma volume}}{100 - \mathrm{PCV}(\%)} \times 100$$

^{50}Cr method: The method employed is originated from Yamabayashi *et al.* [22]. The procedure is shown in Fig. 1. A blood (9 m*l*) was collected from juglar vein, and then the blood (1 m*l*) was centrifuged and the erythrocyte portion obtained was freeze-dried (0St). The rest of the blood (8 m*l*) was incubated with 40 μg of ^{50}Cr (2 m*l*) for 60 min, and the mixture was incubated for 10 min with 60 mg of ascorbic acid (0.6 m*l*). A 2.6 m*l* of the ^{50}Cr tagged blood was used to measure PCV (H0), and the erythrocyte portion was freeze-dried (C0, R0). The tagged blood (8 m*l*) was injected intravenously. One mililiter of blood was collected at 30 min after injection of the tagged blood. The blood sample used to measure PCV (Hx) was centrifuged and freeze-dried (Ct, Rt).

Thermal neutron irradiation and gamma

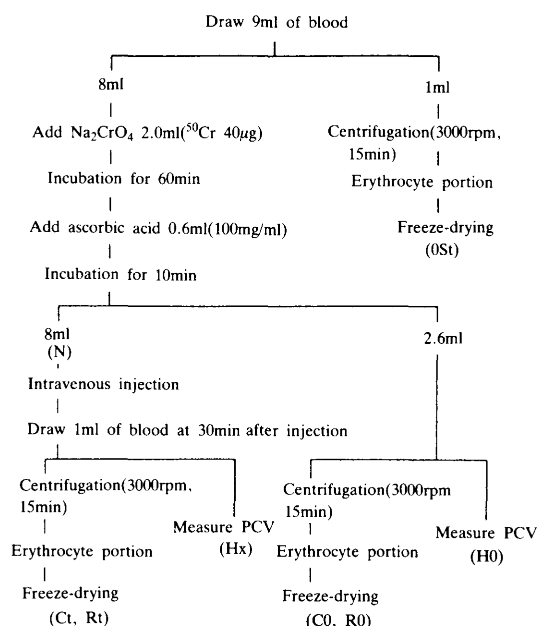

Fig. 1. Flowchart of ^{50}Cr method.

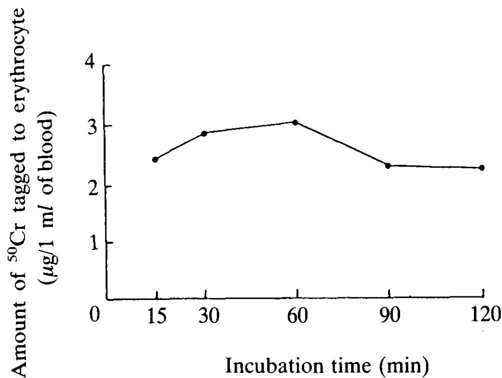

Fig. 2. Incubation time and amount of ^{50}Cr tagged to erythrocyte.

ray measurement: All freeze-dried samples were sealed into polyethylene sheet bags from which ^{50}Cr was excluded. Neutron irradiation was performed at the reactor (JRR-4) of the Japan Atomic Energy Research Institute. The neutron flux was $8\times10^{13}\text{cm}^{-2}\cdot\text{s}^{-1}$ and the irradiation time was 20 min. A standard sample containing a known amount of ^{50}Cr was simultaneously irradiated with blood samples in every irradiation capsule. Approximately 20 days after the neutron irradiation, the radioactivities of samples were measured by a high-pure germanium detector (relative efficiency 30%, CANBERRA) and a 4096 channels pulse-height analyzer (Series 35 Plus, CANBERRA) for gamma-ray spectrometry. The concentration of ^{50}Cr (C0, Cx) and ^{51}Cr/^{59}Fe counts ratio (R0, Rt) were calculated by photopeak area of gamma-ray. The blood volume was calculated by the following equation.

^{50}Cr concentration method:

$$\text{Blood volume (m}l\text{)}=\frac{\text{C0}\cdot\text{H0}}{\text{Ct}\cdot\text{Hx}}\times 8$$

^{51}Cr/^{59}Fe ratio method:

$$\text{Blood volume (m}l\text{)}=\frac{\text{R0}\cdot\text{H0}}{\text{Rt}\cdot\text{Hx}}\times 8$$

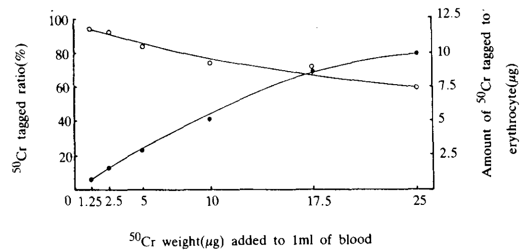

Fig. 3. The effect of additional amount of ^{50}Cr on tagged ratio of ^{50}Cr ; ○ and on amount of ^{50}Cr tagged to erythrocyte ; ●.

RESULT AND DISCUSSION

Fig. 2 shows the relationship between incubation period and ^{50}Cr tagged ratio. The ^{50}Cr tagged ratio gradually increased with the incubation period within 60 min. However, when the incubation period exceeded 90 min, it decreased slightly. This suggests that appropriate incubation period may be approximately 60 min. A slight hemolysis which appeared at 90 and 120 min was considered to cause decrease in ^{50}Cr tagged ratio.

Fig. 3 shows the effect of ^{50}Cr addition on ^{50}Cr tagged ratio and amount of ^{50}Cr tagged to erythrocyte. When the least amount (1.25 μg) of ^{50}Cr was added to 1 m*l* of blood, the highest tagged ratio (about 94%) was observed. The amount of ^{50}Cr tagged to erythrocyte increased and ^{50}Cr tagged ratio decreased with increasing added amount to 1 m*l* blood. In case an additional amount of ^{50}Cr was 17.5 μg and above, hemolysis was observed. It is considered that the most suitable added amount of the ^{50}Cr is below 10 μg per 1 m*l* of blood.

A total of 19 dogs were used to measure blood volume and erythrocyte volume using ^{50}Cr. The mean blood volumes calculated by ^{50}Cr concentration method and ^{51}Cr/^{59}Fe ratio method were 86.7±6.2 m*l*/kg B.W. (mean ±SD) and 89.8±6.8 m*l*/kg B.W. (mean±SD), respectively, showing a very high correlation ($r=0.97$, $P<0.001$) each

other (Fig. 4). This proves that as the iron concentration of erythrocyte was kept constant for short period, the dilution rates calculated by two different method almost agreed. The $^{51}Cr/^{59}Fe$ ratio method is less complicated than the ^{50}Cr concentration method, because it is unnecessary to measure sample weights and to correct variations of thermal neutron flux during irradiation.

A significant correlation ($r=0.77$, $P<0.001$) was observed between erythrocyte volume calculated by the ^{50}Cr method ($^{51}Cr/^{59}Fe$ ratio method) and body weight, and the relationship was recurred by the equation;
$y=26.4+36.1x$ (y: erythrocyte volume; m*l*, x: body weight; kg)$\cdots\cdots$(I).

A significant correlation ($r=0.83$, $P<0.001$) was recognized between plasma volume determined by the Evans blue method and body weight, and a regression equation; $y=78.3+42.5x$ (y:plasma volume; m*l*, x: body weight; kg)$\cdots\cdots$(II) was obtained. Furthermore, although the highest correlation coefficient ($r=0.9$) was obtained when these relation was recurred by the equation;
$1/y=4.73\times10^{-4}+0.015\ 1/x$ (y:plasma volume; m*l*, x: body weight; kg) $\cdots\cdots$ (III), this hyperbolic curve (III) is close to the above straight line (II) within normal weight range. Therefore the plasma volume is considered to be more directly proportional than erythrocyte volume to the body weight.

There was a significant correlation ($r=0.76$) between blood volume using the ^{50}Cr method and body weight, and the relationship was recurred by the equation; $y=103.2+75.9x$ (y:blood volume; m*l*, x: body weight; kg)$\cdots\cdots$ (IV). The relationship between blood volume using the Evans blue method and body weight was recurred by the equation;
$y=76.6+88.6x$ ($r=0.89$) (y:blood volume; m*l*, x: body weight; kg)$\cdots\cdots$ (V).

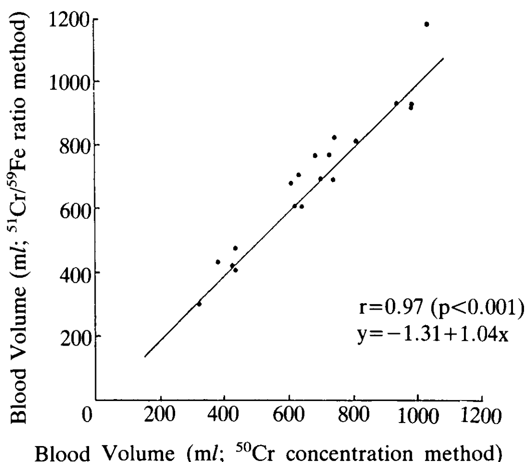

Fig. 4. Correlation between blood volume calculated by ^{50}Cr concentration method and that by $^{51}Cr/^{59}Fe$ ratio method.

The highest correlation coefficient ($r=0.93$) was obtained when the relation was recurred by the equation;
$1/y=2.36\times10^{-4}+0.0084\ 1/x$ (y:blood volume; m*l*, x: body weight; kg) $\cdots\cdots$ (VI).
Sluiter *et al.* [18] reported that the relationship between blood volume calculated by ^{51}Cr and body weight for CBA female mice could be described by the following equation;
$y=0.0972x-0.00187x^2$ (y:blood volume; m*l*, x: body weight; g) $\cdots\cdots$ (VII).

These equations (VI, VII) show a tendency that the blood volume increases monotonously with increasing body weight. And it is shown that an increase rate of blood volume gradually decreases with an increase of body weight. The decrease of the rate might be related to increasing in fat deposit with body weight.

The blood volume of 15 dogs was measured synchronously by the Evans blue method and ^{50}Cr method. The mean blood volume calculated by the Evans blue method was 98.9 ± 10.6 m*l*/kg B.W. (mean$\pm$SD), which was higher than that measured by the ^{50}Cr method with a significant difference ($P<0.05$). And the blood

volumes calculated by two methods have low correlation coefficient (r=0.48) (Fig. 5). The blood volumes calculated by ^{50}Cr were estimated from erythrocyte volumes and PCV values, and those by Evans blue were estimated from plasma volumes and PCV values. In this study, as the PCV values used for the blood volume measurement were obtained from venous blood hematocrit values, they did not indicate whole blood PCV values. As the venous blood hematocrit values of juglar vein was used, it is necessary to correct the PCV values for the calculation of blood volume. In general, it is known that the venous PCV value is higher than the whole blood PCV value. Reeve *et al.* [17] used a correction factor of 0.96 in their studies on blood volume in the dog. Dalton and Fisher [1] applied a correction factor of 0.94 to blood volume determinations in the cow using the Evans blue. When PCV values multiplied by 0.97 were used, the mean blood volumes using two different methods almost agreed. Therefore it is concluded the correction factor in our experiment is 0.97.

If the accurate blood volume is expected to be derived, the simultaneous measurement of erythrocyte volume and plasma one is required. As in the Evans blue method it is necessary to withdraw blood frequently to obtain the early plasma concentration of dye it is undesirable to apply to the animal with a small quantity of blood. As plasma volume changes easily under the various physiological conditions, the blood volume should be calculated from erythrocyte volume.

A detection limit of ^{50}Cr was about 0.1 ng per 1 m*l* of blood on the measurement system in this study. When additional ^{50}Cr amount per 1 m*l* of blood is 5 μg and an injection amount of ^{50}Cr tagged blood is 50 m*l* per 500 kg B.W., measuring the blood volumes of cattle and horses is considered to be possible. It is fit to use the non-radioactive isotope ^{50}Cr in the field work.

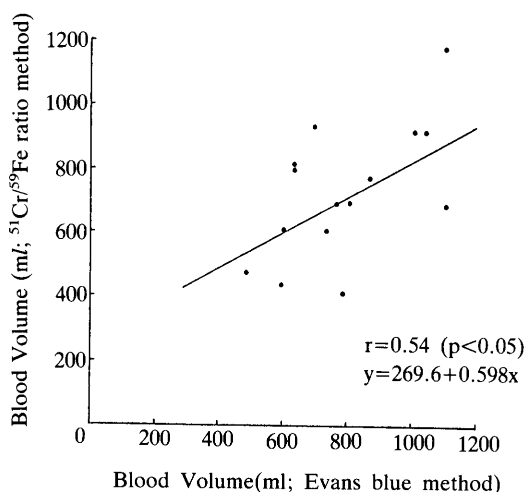

Fig. 5. Correlation between blood volume calculated by $^{51}Cr/^{59}Fe$ ratio method and that by Evans blue method.

Therefore, it may be possible to apply the blood volume measurement using the ^{50}Cr to wild animals and many kinds of animals in zoo.

ACKNOWLEDGEMENTS. We are indebted to Dr. F. Akiha and Mr. T. Takano for their technical support of the neutron irradiation. This work was partly supported by the Inter-University Joint Research Program Using the JAERI Facilities and by Kitasato University School of Veterinary Medicine and Animal Sciences research grant (No. 6109).

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要　約

安定濃縮同位体^{50}Crを用いた犬の血液量の測定：椚山　巌・伊藤伸彦・古川義宣（北里大学獣医畜産学部獣医放射線学教室）——野外実験に使用可能な非放射性の安定同位体^{50}Crを赤血球標識のマーカーとして用い，中性子放射化分析法を利用して犬の血液量の測定に必要な諸条件および手技を検討した．犬血液を標識する場合，今回の実験で得られた結果から，血液1 mlあたり^{50}Crを5 μg添加し，60分間インキュベーションした．^{50}Crで標識した血液を体内に注入し，60分後に採血して注入前後の血液中^{50}Cr濃度（濃度法）および^{51}Cr/^{59}Fe放射能比率（比率法）を中性子放射化法で測定し，希釈割合から血液量を算定した．両方法で求めた血液量は高い相関（r=0.97）を示し，ほぼ一致した測定値が得られた．比率法は試料重量の測定，照射中の熱中性子束密度の変動の補正が不要であるため，濃度法より簡便であると考えられた．また^{50}Cr法と従来臨床領域で用いられてきたエバンスブルー法で求めた単位体重あたりの平均血液量は，それぞれ89.8±6.8ml/kg, 98.9±10.6ml/kgであり，この差は有意であった．しかし，血液量値の計算に用いたヘマトクリット値を0.97の係数で補正した結果，両方法で求めた値は，ほぼ一致した．今回使用した実験系では，^{50}Crの検出限界は血液1 mlあたり0.1ngであった．^{50}Crの添加量を血液1 mlあたり5 μgとし，^{50}Cr標識血液注入量を体重500kgあたり50mlとすれば，大動物の血液量測定にも適用可能であることが明らかにされた．