Evaluation of Plasma Erythropoietin Levels in Normal Adult Dogs by In vivo Bioassay using Concentrated Plasma

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ABSTRACT. Measurement of plasma erythropoietin level in normal dogs by in vivo bioassay has been considered to be impossible so far. In the present study, we successfully determined it by using concentrated plasma 60 times which allowed the lower limit to 2.7 mU/ml. This normal plasma erythropoietin level was the first to be determined as an in vivo bioactivity and was 9.14±7.81 mU/ml in 75 normal adult dogs. This value was sufficiently reliable in terms of accuracy of determination and considered to be meaningful as the low level in vivo bioactivity that hasn't been known to date. Furthermore, erythropoietin levels in normal plasma were within a certain lower range and showed neither difference in plasma erythropoietin level between males and females or among breeds nor correlation between erythropoietin and hemoglobin level.—KEY WORDS: bioassay, dog, erythropoietin, plasma concentration method, polycytemic mouse method.


It is extremely important to evaluate plasma erythropoietin (EPO) level in hemopoietic disorders. Since the quantitative unit of EPO is based on bioactivity [5], the EPO level determined by in vivo bioassay is supposed to directly indicate EPO activity. In recent years, an immunoassay which can provide determination with a small amount of sample has been developed for human, and has been spread as a routine test since correlation with measurements by in vivo bioassay was confirmed [13, 16]. This immunoassay is expected to be further spread even as a determination method for canine EPO in the future, and have already applied to determination of EPO level in dogs [19, 20, 22, 25, 26]. However, as thorough research hasn’t been done on cross immunity between canine EPO and human EPO or on comparison between immunoassay and in vivo bioassay, it is the question whether the canine EPO level determined by immunoassay using antibodies against human EPO could reflect substantial bioactivity or not. Particularly, plasma EPO activity in normal dogs is too low to detect [24, 33] and no data have been reported on its measurement by in vivo bioassay. Plasma EPO level in normal dogs hasn’t been defined yet as an in vivo-bioactivity. On the other hand, in order to determine such low EPO levels in plasma, a heat-concentration method of a large amount of plasma was devised in 1979, and the normal human plasma EPO level with high reliability was measured by in vivo bioassay for the first time [3, 11].

In the present study, we attempted to measure plasma EPO levels in normal adult dogs by in vivo bioassay using concentrated plasma. As the first step, we evaluated the accuracy of measurements of this method. Furthermore, we assessed differences in the obtained EPO levels and in red blood cell parameters between males and females or among breeds, as well as correlation between plasma EPO level and hemoglobin level in order to elucidate the relation between EPO and red blood cell parameters.

MATERIALS AND METHODS

In this study, in order to examine if accuracy of in vivo bioassay would be differ between plasma and concentrated plasma sample, its recovery rate was determined in each sample added with a certain amount of standard EPO, first of all. Furthermore, since EPO loss occurs during heat treatment and concentration processes of plasma, recovery rate in the heat-concentration procedure was also estimated after a certain amount of standard EPO was added to plasma. Plasma EPO levels in normal adult dogs were determined according to these determination accuracy.

Animals: Ninety-five dogs (54 males and 41 females, 1–7 years of age, weighing 7–42 kg) used for the experiments were clinically proved to be
healthy. These 10 mongrel dogs were used for the experiment of EPO recovery rate by in vivo bioassay, 10 mongrel dogs for the experiment of EPO recovery rate by plasma concentration method, and another 75 dogs consisting of 25 German Shepherds, 25 Beagles, and 25 mongrel dogs were used for measurement of plasma EPO levels as normal adult dogs.

**In vivo bioassay:** The polycythemic mouse method of Takaku et al. [31] was followed for in vivo bioassay as shown in Fig. 1. Seven-week-old dd-strain female mice were made up polycythemia through two-time-blood transfusion (1.0 ml of 50% erythrocytes suspension/mouse, i.v. at the coccygeal vein) from donors of the same strain conducted on days 1 and 3. Three days after the last transfusion, 4 mice of each group were subcutaneously injected with 1.0 ml of canine plasma (or concentrated plasma), standard EPO (Green Cross Co., purified from human urine, Osaka), or saline solution as a control. On day 8, 0.5 μCi $^{59}$FeCl$_3$ (NEZ-037, New England Nuclear) in 1.0 ml of saline was intraperitoneally injected to each mouse, and then 1.0 ml of blood were collected by cardiac puncture 48 hrs. later for the determination of radioactivity by using Gamma Counter (Auto Well Gammaysystem, ARC-500, Aloka Co., Tokyo). The $^{59}$Fe incorporation rate to hemoglobin per mouse was calculated according to the formula shown in Fig. 1, and EPO activities of samples were calculated from the ln dose-ln response curve between standard EPO (0.1, 0.2, 0.5 U/ml) and $^{59}$Fe incorporation rate. The lower limit of this assay [15] was determined by $^{59}$Fe incorporation rate of the control group injected with saline solution.

**Plasma concentration:** The method of Caro et al. [3] and Ersev et al. [11] was followed for plasma concentration as shown in Fig. 2. Blood samples were collected from jugular vein of each dog in the amount of 550–600 ml and were mixed with ACD solution (acid-citrate dextrose, Terumo, Tokyo) as much as 14% of the total volume. Then 300 ml of this plasma-ACD mixture was obtained by centrifugation at 3,000 rpm for 10 min. at 4°C. After the pH was adjusted to 5.5 by addition of acetic acid, the plasma mixture was distributed into ten polypropylene test tubes of 50 ml and they were placed in a boiling water bath for exactly 5 min. for heat treatment. The tubes were rapidly cooled in an ice bath and spun for 30 min. at 3,000 rpm at 4°C to obtain supernatant. After saline was added to the precipitates and mixed thoroughly, the tubes were spun again in the same manner. The combined supernatant was readjusted pH to 7.4 by addition of sodium hydroxide and poured into a dialysis bag (Cellophane Tubing Sheamless, Union Carbide Co., U.S.A.), which was placed in dry Carbowax (Polyethylene glycol 20,000, Wako Pure Chemical

![Fig. 1. The schedule of in vivo bioassay by the polycythemic mouse method and the calculation of $^{59}$Fe incorporation rate from the murine blood radioactivity.](image)

![Fig. 2. Schematics of the plasma concentration method.](image)
Industries, Tokyo) for 18 hrs. at 4°C for concentration. The concentrated sample was made up to 5.0 ml with saline solution and 4.0 ml aliquots were used for in vivo bioassay. The results of the bioassay were expressed as mU per ml of the original sample by the following equation.

\[
\text{mU/ml (plasma)} = \frac{\text{mU/ml (concentrated plasma)} \times 5}{\text{recovery rate (\%)} \times \text{plasma volume (ml)}}
\]

The lower limit of in vivo bioassay by utilizing plasma concentration method was calculated by substituting blank value obtained with saline for concentrated plasma and the value obtained from normal canine hematocrit (45%) [29] for plasma volume in this equation.

**EPO recovery rate in in vivo bioassay:** For the determination of EPO recovery rate in in vivo bioassay, plasma samples from 5 phlebotomized dogs and concentrated plasma samples from 5 normal dogs were used. In 5 phlebotomized dogs, blood corresponding to 2% of body weight was removed three times at intervals of 12 hrs, and plasma samples were collected 48 hrs after the last phlebotomy. Concentrated plasma samples were obtained from 5 normal dogs by the method mentioned above. Each of ten samples was divided into two portions; one containing 4.9 ml sample and 0.1 ml standard EPO (1.0 U/0.1 ml saline), the other used 0.1 ml saline in place of standard EPO. EPO levels in these samples were determined by in vivo bioassay, and recovery rate was calculated from the difference in EPO level between the standard EPO-added (0.2 U addition per 1.0 ml) and the saline-added samples.

**EPO recovery rate in plasma concentration:** For the experiment of EPO recovery rate in plasma concentration method, blood samples were obtained from 10 normal dogs. Plasma obtained from two individuals was used as one sample, and five samples of plasma-ACD solution were prepared. One ml standard EPO (2.5 U/1.0 ml saline) or saline was added to 299 ml plasma-ACD solution. These samples were concentrated 60 times by the plasma concentration method. The concentrated plasma samples were subjected to recovery test of EPO by in vivo bioassay. The recovery rate was calculated by subtracting the EPO level of the saline-added concentrated plasma sample from that of standard one.

**Measurement of plasma EPO level in normal adult dogs:** Plasma EPO levels were determined by in vivo bioassay by using concentrated plasma of 75 dogs, 25 German Shepherds, 25 Beagles, and 25 mongrel dogs. They were examined for differences between gender or among breeds. Student t test was used for analysis of significant difference between mean values.

**Red blood cell parameters in normal adult dogs:** All the 75 dogs, prior to the measurement of plasma EPO levels, were subjected to determination of red blood cell parameters. Red blood cell count (RBC) was measured by using Micro Cell Counter (CC-130A, Sysmex, Kobe), hematocrit value (Ht) was measured by the microhematocrit method, and hemoglobin concentration (Hb) was measured by the cyanmethemoglobin method. The Wintrobe erythrocyte indexes, mean corpuscular volume (MCV: Ht/RBC), mean corpuscular hemoglobin (MCH: Hb/RBC), and mean corpuscular hemoglobin concentration (MCHC: Hb/Ht) were calculated from RBC, Ht, and Hb. Each value of red blood cell parameters was examined for their difference by gender or breed.

**Correlation between plasma EPO and Hb Level:** Correlation between plasma EPO and Hb level was evaluated in 75 normal adult dogs. Statistical analysis of significance in correlation coefficient was done by using F test.

**RESULTS**

The present study involved 21 assays in total and the dose-response curve was drawn for each assay. The slope, intercept, and correlation coefficient of the obtained curves were 1.274±0.339, 0.932±0.394 and 0.989±0.010 (Mean±S.D.), respectively. Mean \(^{59}\)Fe incorporation rate obtained from saline administration as the blank value corresponded to 51.0 mU/ml of EPO level, and this value was set as the lower limit of in vivo bioassay.

**EPO recovery rate in in vivo bioassay:** The EPO levels in the plasma from phlebotomized dogs and in the concentrated plasma from normal dogs were 0.179–2.165 U/ml and 0.079–0.664 U/ml, respectively. On the other hand, the EPO levels in the EPO-added plasma from phlebotomized dogs and in the EPO-added concentrated plasma from normal dogs were 0.364–1.449 U/ml and 0.279–0.843 U/ml, respectively. The EPO recovery rate was 100.4±8.0% in plasma from phlebotomized dogs.
Table 1. The recovery rate of additional erythropoietin (EPO) in bioassay using the plasma samples (a) from 5 phlebotomized dogs and the concentrated plasma samples (b) from 5 normal dogs.

(a) Plasma Samples

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Added EPO (U/sample)</th>
<th>Original level (U/ml)</th>
<th>Additional level (U/ml)</th>
<th>Recovered EPO (U/sample)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.614</td>
<td>0.811</td>
<td>0.197</td>
<td>98.5</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.179</td>
<td>0.364</td>
<td>0.188</td>
<td>94.0</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>1.265</td>
<td>1.449</td>
<td>0.184</td>
<td>92.0</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>1.008</td>
<td>1.226</td>
<td>0.218</td>
<td>109.0</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.832</td>
<td>1.049</td>
<td>0.217</td>
<td>108.5</td>
</tr>
</tbody>
</table>

(Mean±S.D.) 100.4±8.0

(b) Concentrated plasma samples

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Added EPO (U/sample)</th>
<th>Original level (U/ml)</th>
<th>Additional level (U/ml)</th>
<th>Recovered EPO (U/sample)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2</td>
<td>0.079</td>
<td>0.291</td>
<td>0.212</td>
<td>106.0</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.105</td>
<td>0.279</td>
<td>0.174</td>
<td>87.6</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.664</td>
<td>0.843</td>
<td>0.179</td>
<td>89.0</td>
</tr>
<tr>
<td>4</td>
<td>0.2</td>
<td>0.321</td>
<td>0.560</td>
<td>0.239</td>
<td>119.5</td>
</tr>
<tr>
<td>5</td>
<td>0.2</td>
<td>0.172</td>
<td>0.358</td>
<td>0.186</td>
<td>93.0</td>
</tr>
</tbody>
</table>

(Mean±S.D.) 99.0±13.6

Table 2. The recovery rate of additional erythropoietin (EPO) in the plasma concentration methods

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Added EPO (U/sample)</th>
<th>Recovered EPO (U/ml of conc. plasma)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5</td>
<td>1.083</td>
<td>43.3</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>0.920</td>
<td>36.8</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>1.025</td>
<td>41.0</td>
</tr>
<tr>
<td>4</td>
<td>2.5</td>
<td>1.072</td>
<td>42.9</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>0.928</td>
<td>37.1</td>
</tr>
</tbody>
</table>

(Mean±S.D.) 40.2±3.1

and 99.0±13.6% in concentrated plasma from normal dogs (Table 1).

**EPO recovery rate in plasma concentration:** By using five samples of plasma-ACD solution, recovery rate of 2.5 U EPO in plasma concentration method was calculated from the difference in EPO levels between saline- and EPO-added concentrated plasma. The EPO yield was 0.920~1.083 U in the 5 samples, resulting in 40.2±3.1% recovery rate (Table 2). From this recovery rate, 40.2%, and lower limit of in vivo bioassay, 51.0 mU/ml, the lower limit of in vivo bioassay using concentrated plasma was calculated to be 2.69 mU/ml.

**Plasma EPO level in normal adult dogs:** Plasma EPO level was 9.44±8.38 mU/ml in German Shepherds, 8.90±7.13 mU/ml in Beagles, and 9.09±7.81 mU/ml in mongrel dogs (Fig. 3, Table 3). Mean plasma EPO level for all the 75 dogs was

![Fig. 3. Plasma erythropoietin levels in normal mongrels, German Shepherds, and Beagles measured by in vivo bioassay using the concentrated plasma: The lower limit in this bioassay system was 2.7 mU/ml.](image-url)
PLASMA ERYTHROPOIETIN IN NORMAL DOGS

Table 3. Values of the plasma erythropoietin (EPO) and erythrocyte parameters in normal adult dogs: 75 dogs consisting of three breeds (25 mongrels, 25 German Shepherds, and 25 Beagles), and of 45 males and 30 females

<table>
<thead>
<tr>
<th>Dog group</th>
<th>Number</th>
<th>EPO (mU/ml)</th>
<th>RBC (x10^6/mm³)</th>
<th>Hb (g/dl)</th>
<th>Ht (%)</th>
<th>MCH (pg)</th>
<th>MCV (μm³)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All dogs</td>
<td>75</td>
<td>9.14±7.81</td>
<td>645.5±52.8</td>
<td>13.7±0.5</td>
<td>44.1±2.0</td>
<td>21.3±1.5</td>
<td>68.7±4.1</td>
<td>31.1±0.9</td>
</tr>
<tr>
<td>Mongrels</td>
<td>25</td>
<td>9.09±8.17</td>
<td>622.7±48.0</td>
<td>13.8±0.6</td>
<td>43.4±1.8</td>
<td>22.3±1.7</td>
<td>70.0±5.5</td>
<td>31.9±0.5</td>
</tr>
<tr>
<td>Shepherds</td>
<td>25</td>
<td>9.44±8.38</td>
<td>664.2±51.5*</td>
<td>13.7±0.4</td>
<td>44.6±1.8</td>
<td>20.7±1.0*</td>
<td>67.4±3.4</td>
<td>30.8±0.7*</td>
</tr>
<tr>
<td>Beagles</td>
<td>25</td>
<td>8.90±7.13</td>
<td>649.2±52.2</td>
<td>13.6±0.4</td>
<td>44.5±2.8</td>
<td>20.9±1.1*</td>
<td>67.8±2.8</td>
<td>30.5±0.4*</td>
</tr>
<tr>
<td>♂ Mongrels</td>
<td>4</td>
<td>9.27±8.25</td>
<td>647.0±53.0</td>
<td>13.7±0.5</td>
<td>44.2±2.0</td>
<td>21.3±1.6</td>
<td>68.6±4.6</td>
<td>31.1±0.9</td>
</tr>
<tr>
<td>♀ Mongrels</td>
<td>3</td>
<td>8.95±7.23</td>
<td>643.0±53.4</td>
<td>13.7±0.5</td>
<td>44.1±2.2</td>
<td>21.4±1.3</td>
<td>68.9±3.5</td>
<td>31.1±0.9</td>
</tr>
<tr>
<td>♂ Shepherds</td>
<td>16</td>
<td>8.89±8.03</td>
<td>629.9±56.6</td>
<td>13.8±0.6</td>
<td>43.4±1.9</td>
<td>22.1±2.1</td>
<td>69.4±6.4</td>
<td>31.8±0.5</td>
</tr>
<tr>
<td>♀ Shepherds</td>
<td>9</td>
<td>9.45±8.89</td>
<td>609.9±25.1</td>
<td>13.8±0.5</td>
<td>43.2±1.7</td>
<td>22.6±1.0</td>
<td>70.9±3.5</td>
<td>31.9±0.3</td>
</tr>
<tr>
<td>♂ Beagles</td>
<td>15</td>
<td>10.42±9.34</td>
<td>668.2±56.3</td>
<td>13.8±0.5</td>
<td>44.7±2.0</td>
<td>20.7±1.1</td>
<td>67.2±3.5</td>
<td>30.8±0.7</td>
</tr>
<tr>
<td>♀ Beagles</td>
<td>10</td>
<td>7.96±6.91</td>
<td>658.1±45.5</td>
<td>13.7±0.4</td>
<td>44.4±1.5</td>
<td>20.9±0.8</td>
<td>67.7±3.3</td>
<td>30.8±0.7</td>
</tr>
<tr>
<td>♂ Beagles</td>
<td>14</td>
<td>8.48±7.71</td>
<td>643.7±38.8</td>
<td>13.5±0.3</td>
<td>44.4±1.9</td>
<td>21.0±0.9</td>
<td>69.0±2.6</td>
<td>30.4±0.8</td>
</tr>
<tr>
<td>♀ Beagles</td>
<td>11</td>
<td>9.44±6.63</td>
<td>656.3±66.9</td>
<td>13.6±0.5</td>
<td>44.6±2.8</td>
<td>20.9±1.3</td>
<td>68.3±3.2</td>
<td>30.6±1.0</td>
</tr>
</tbody>
</table>

RBC : Red blood cell count. EPO : Mean corpuscular hemoglobin. MCHC : Mean corpuscular volume.
Hb : Hemoglobin concentration. MCH : Mean corpuscular hemoglobin.
Ht : Hematocrit value. MCV : Mean corpuscular hemoglobin concentration.

* Significant (P<0.05) to mongrel’s mean value.

9.14±7.81 mU/ml (Table 3). Thus, no difference was observed in plasma EPO level of normal adult dogs between males and females or among breeds.

Red blood cell parameter in normal adult dogs: All the red blood cell parameters (RBC, Ht, Hb, MCV, MCH, MCHC) were confined to within the normal range in 75 normal adult dogs. Although no difference was recognized in any parameter between males and females, significant differences were observed in RBC, Ht, MCH, and MCHC between mongrel dogs and German Shepherds while in MCH and MCHC between mongrel dogs and Beagles (Table 3). No significant difference was observed between German Shepherds and Beagles.

Correlation between plasma EPO and Hb level: Examining correlation between plasma EPO and Hb level, correlation coefficient was 0.18 in German Shepherds, -0.32 in Beagles, and -0.07 in mongrel dogs. In addition, that for all the dogs was -0.05. No significance was observed between plasma EPO and Hb level (Fig. 4).

DISCUSSION

In the present study, before the determination of plasma EPO levels in normal adult dogs, we evaluated the accuracy of the bioassay. It is said that in vivo bioassay of EPO, favorable dose-response curve can be obtained because radio-iron is specifically incorporated into hemoglobin [4]. The stan-
standard curves also showed suitable reproducibility as well as an approximately 100% recovery rate of EPO added to samples. This supported reliability of measurements in this bioassay. On the other hand, a stable EPO recovery rate, 40.2%, was obtained in plasma concentration method. Therefore, it was considered that EPO loss during heat treatment [11] was rather relatively invariable and would not cause any remarkable error in conversion to plasma EPO level from concentrated plasma EPO level. On the basis of these data, the 60 times plasma concentration method improved the assay sensitivity from the detectable limit of 51.0 mU/ml to 2.69 mU/ml. This plasma concentration method for in vivo bioassay was devised by Caro et al. [3] and Erslev et al. [11] in 1979, and they reported that the EPO recovery rate in the 60 times concentration method was 42% in normal human plasma and the lower limit of in vivo bioassay was 2.8 mU/ml. Their data on EPO level are still considered to be highly reliable ones as in vivo bioactivities [30, 32], although no further study has followed their method so far. We applied their method to dogs in this study and obtained the results similar to their data. This fact supported that their method is a fully reliable assay.

In adult animals, normal level of circulating red blood cell is maintained thorough erythropoiesis in bone marrow which is principally regulated by EPO [23]. Therefore, plasma EPO level in normal dogs would reflect the erythropoietic state in bone marrow. The EPO level gives meaningful information associated with dyshemopoiesis. EPO value is expressed as international unit based on its bioactivity [5], which can be directly determined by in vivo bioassay. However, as plasma EPO level in normal dogs is too low to determine by using in vivo bioassay, there have appeared no reports on canine plasma EPO level. In the present study, plasma EPO level in 75 normal adult dogs by in vivo bioassay combining with the plasma concentration method was 9.14±7.81 mU/ml. There was no difference in plasma EPO level between gender or among breeds. No correlation was recognized between plasma EPO and Hb levels. According to Caro et al. [3] and Erslev et al. [11], EPO level in normal human plasma was 3.9~15.4 mU/ml (mean 7.8 mU/ml) without any difference by gender or any correlation with Hb level. Their results were closely akin to our findings on dogs. It is well known that human plasma EPO level becomes higher with lower Hb level in anemic condition, being marked negative correlation between them [10, 12]. In normal condition, however, such clear correlation is not observed and plasma EPO seems to keep constant level even if Hb varies within the normal range. As for red blood cell quantity in the normal beagle, some reports say that there is some difference by gender [1, 21] while others do not [2]. There is also a little difference in red blood cell parameters among breeds [7, 17, 27]. In the present study, although no difference in gender was observed, differences among breeds were recognized in some red blood cell parameters. From these results, the difference in red blood cell parameter within normal range among breeds may be attributed to the factors other than EPO.

Serum EPO level in normal dogs has been determined so far by two kinds of in vitro bioassay based on different principle, indicating 0.91±0.07 U/ml [8] and 94.9±28.7 mU/ml [18]. The former is about 10 times higher than the latter, and both are much higher than our result obtained by in vivo bioassay. Such high EPO level could be measured by in vivo bioassay without plasma concentration method. In any case, those EPO levels reported by in vitro bioassay are greatly different from that by the in vivo bioassay using concentrated plasma. Human EPO level by in vitro bioassay is 54±31 mU/ml [28], which is much higher than 7.8 mU/ml by the in vivo bioassay [3, 11]. Presumably cause for this difference between in vitro and in vivo bioassay levels is presence of asialo EPO, which shows its bioactivity in vitro but no in vivo [6, 9, 14]. There is an opinion that in vitro bioassay, having problems in its quantitative analysis, should be used for rather qualitative means to trace EPO [23]. In vitro bioassay for quantitative analysis of EPO must be compared with in vivo bioassay as a standard method. Recently, serum EPO values in normal dogs have been determined by immunoassay using antibodies against human EPO [20, 26]. However, as even cross immunity between canine EPO and human EPO hasn’t been examined in the present state, the question still remains whether the EPO determined by the immunoassay could reflect substantial bioactivity or not. Immunoassay has been routinely used in human medicine currently and has been already compared with in vivo bioassay, revealing correlation between bioactivity and immunoactivity [15]. The normal value of human serum EPO determined by immunoassay was about 20 mU/ml [15], which was close to normal plasma
EPO level obtained by in vivo bioassay [3, 11]. However, it is not yet known whether asialo EPO implicates in this immunoassay or not.

At all events, it was considered that the plasma EPO level obtained by in vivo bioassay in this study at least could be some help to solve the problems for the future, such as difference in bioactivity between in vivo and in vitro bioassay, difference between bioactivity and immunoactivity, and cross immunity between canine EPO and human EPO. The present study not only revealed plasma EPO level in normal dogs but examined differences of plasma EPO level in gender and breed, and correlation between plasma EPO and Hb levels. Further study will have to be done to clarify the involvement of plasma EPO level corresponding EPO production in kidney.

REFERENCES
