Posttransfusional Recovery of Defective Respiratory Function of Stored Blood in Dogs

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Abstract Changes in the oxygenation properties and the relevant biochemical parameters of canine blood were followed during its storage in acid-citrate-dextrose (ACD) medium (4°C) and after transfusion of the stored blood. Reductions of the blood $P_{50}$ (pH 7.40, $P_{CO_2}$ 40 Torr, 37°C) and 2,3-diphosphoglycerate (DPG) during storage was much slower than in human blood. Half-decay time for DPG was 21 and 5 days for canine and human blood, respectively. The DPG decay could be extensively accelerated in the presence of bisulfite in ACD. The stored and DPG-depleted red cells required a considerably long period of time for the complete posttransfusional restoration of the DPG and $P_{50}$. In five dogs whose blood were exchanged by 84% in average with the stored blood of mean DPG depletion of 88%, mean extents of the DPG and $P_{50}$ restoration were 74 and 89% of the normal values after 24 h. After 5 days both the parameters were within the normal ranges. The red cell extracellular pH fell immediately after the transfusion, and restored to the normal level after 24 h. From these results, correlations between $P_{50}$ or red cell transmembrane pH gradient at the extracellular pH of 7.40 ($\Delta p$H) and red cell DPG/hemoglobin molar ratio ($x$) were derived as $P_{50} = 10.92x + 14.2$ ($r = 0.91$) or $\Delta p$H = 0.060$x$ + 0.135 ($r = 0.70$).

Key words: stored blood, oxygen, 2,3-diphosphoglycerate, transfusion, dog.

Blood transfusions have remarkably increased in modern medical practices. Blood for this purpose is now mostly the one stored in a preservation medium such as acid-citrate-dextrose (ACD) or citrate-phosphate-dextrose (CPD) solution. It was found, however, by Valtis and Kennedy (1954) that the storage might result in a rapid and possibly serious functional deterioration of blood. The discovery that a red cell glycolytic intermediate, 2,3-diphosphoglycerate (DPG), exerts a potent regulating effect upon the oxygen affinity of blood (Benesch and Benesch, 1967;
CHANUTIN and CURNISH, 1967), led promptly to the elucidation that the functional deterioration might be mainly caused by the red cell DPG reduction during the blood preservation (BUNN et al., 1969). This finding further prompted a number of basic and applied studies, which resulted in some practical improvements concerning blood storage (GREENWALT and JAMIESON, 1973).

So far, however, relatively few studies have been reported on the post-transfusional changes of the stored and functionally modified blood (BEUTLER and WOOD, 1969; VALERI and HIRSCU, 1969; SUGERMAN et al., 1970). In view of these circumstances, we report here an experimental approach to the problems concerning blood storage and transfusion. The results obtained also furnish us with valuable informations about the blood oxygenation properties and the relevant factors in dogs, a commonly used animal species for cardiopulmonary and organ blood flow studies.

MATERIALS AND METHODS

Storage of blood. Mongrel dogs of both sexes weighing 25 kg or more were anesthetized with intraperitoneal administration of pentobarbital sodium (30 mg/kg). Blood was obtained from the femoral artery and put into a sterilized vessel containing a determined amount of ACD (NIH-Formula A). After being well mixed, blood was immediately divided into three portions for preservation at 4°C with no addition (A) and with additions of final metabisulfite concentration of 5 mM (B) and 10 mM (C). Each of the three kinds of blood was further divided into several sterilized test tubes. After various lapse of time from 1 to 25 days at 4°C, the contents were examined for the changes in oxygen dissociation curve (ODC) and DPG. For the ODC examination, blood was washed three times with cold physiological saline, resuspended in canine plasma to attain the hematocrit (Ht) of 45% and then the base excess (BE) was corrected to zero with 1 M NaHCO₃. Exchange transfusion experiments were conducted using the blood stored under the condition (C) for 8 to 12 days.

Transfusion experiments with stored blood. The stored blood was centrifuged to discard the plasma portion immediately prior to use. After they were washed twice with an excess of saline, the packed red cells were mixed with a roughly equal volume of the plasma. The plasma had been separated from fresh ACD-blood which was obtained from another donor dog(s) the day before. Recipient mongrel dogs of relatively small size (average body weight of 10.5 ± 1.4 kg) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.). A femoral artery and opposite femoral vein were cannulated and polyethylene tubings were connected. The tubings were led subcutaneously to be fixed on the back, filled with heparin solution in saline and closed with a plastic cap. Transfusion of the stored blood via the vein and simultaneous withdrawal of the recipient’s blood through the artery were carried out at a roughly comparable rate. Respiration was artificially controlled (tidal volume ≈ 100 ml, 20 cycle/min) throughout the exchange transfusion with an AICA
respirator (model R/60) and the animals were treated with a muscle relaxant (Suxamethonium-HCl, 20–40 mg) if necessary. The abdominal region of the dogs was covered with a water (39°C)-circulated heating pad (Hamilton, M-11) in order to maintain the normal body temperature. When the transfusion was completed, 500 ml each of an isotonic electrolyte mixture (Ohtsuka, KN-3B solution), physiological saline and 5% glucose solution were consecutively and continuously infused through the venous cannula. After 24 h when the recipient dogs were fully restored, the cannulae were removed under light pentobarbital anesthesia and the incisions were closed. The blood was drawn through the arterial and venous cannula from time to time during and after the transfusion. Subcutaneous veins of the foreleg were used to obtain the blood 5 days after the transfusion.

Analytical methods.

Oxygen dissociation curve (ODC): A gasometric procedure previously reported (Enoki, 1959) in which the blood oxygen saturation ($S_{O_2}$) by Roughton-Scholander syringe was referred to gas phase $P_{O_2}$ by Haldane's analyzer was generally followed, except that $S_{O_2}$ was determined by a spectrophotometric method (Siggard-Andersen, 1963) using a Radiometer oxygen saturation meter (OSM 1). The sample blood, preserved in a mercury-sealed syringe in ice-water, was transferred into a microhematocrit tube (Thermo, 1.5 x 75 mm) under minimal contact with air, spun with a microhematocrit centrifuge (Kubota, KH-120) at 12,000 rpm for 5 min, frozen at $-20^\circ$C and then thawed by warming with fingers. After repeating the freezing-thawing procedure twice, the red cell lysate portion was cut off and its content was carefully introduced into a OSM cuvette of 0.100 mm path length (Radiometer, 921-211). Mean value of the duplicate or triplicate determinations was calculated for $S_{O_2}$. Standard $P_{50}$ (pH 7.40, $P_{CO_2}$ 40 Torr, 37°C) was derived to express the blood oxygen affinity by using the Bohr factor of $-0.498$ for canine blood (Reeves et al., 1982).

pH: Blood pH was measured with a blood gas analyzer (Radiometer, BSM-Mk 2) at 37 or $5^\circ$C. The whole system was circulated with a Komatsu-Yamato cooling unit (CTE-310) thermostated at the desired temperatures. Red cell intracellular pH was determined by a modification of the procedure as previously described (Enoki et al., 1972).

DPG, Ht, and hemoglobin (Hb) concentration: Red cell DPG was determined by an enzymatic (Maeda et al., 1971) or a chromatographic procedure (Bartlett, 1968; Ochiai and Enoki, 1974). Ht and Hb concentration were measured by a routine micro-Ht method using a micro-Ht centrifuge (Kubota, KH-120) and a cyanomet-Hb procedure, respectively.

Fractionation and quantitation of red cell acid-soluble phosphorus compounds: Five to 10 ml sample of blood was washed twice with 4 volumes of ice-chilled physiological saline after removal of plasma by centrifugation at 3,000 rpm for 5 min. The packed red cells were extracted three times with cold trichloroacetic acid, first 10% and then 5% twice. Trichloroacetic acid was removed by shaking with ether, which in turn was dissipated by bubbling with $N_2$. The
resultant extract was neutralized with 10% ammonia water to be frozen to preserve until analysis (−20°C). Fractionation and quantitation of the acid soluble phosphorus compounds were performed by an anion exchange chromatographic procedure of BARTLETT (1968) as slightly modified by us (OCHIAI and ENOKI, 1974). The eluents were monitored by the absorbance at 260 nm and also by the phosphorus content (BARTLETT, 1959).

RESULTS

Physiological and biochemical changes in canine blood when stored in ACD media (4°C)

Figure 1 shows time course of pH changes actually measured at 5°C of normal human blood during storage in ACD for 25 days (4°C). An almost linear decrease of the pH was observed, attaining the value of 6.9 at the current preservation time limit for blood of 21 days. The initial Ht values (41.5 ± 3.1%, n = 10) were only slightly altered to 40.5 ± 2.8% after 20 days of the preservation.

DPG content in human red cells, as determined enzymatically, showed a rapid exponential decrease (Fig. 2). Mode of the change in canine red cells was considerably different in that the reduction was much slower and almost linear (Fig. 2). When compared at the 21st day of storage, reduction in the DPG was 89% in man and 52% in dog, respectively. The situations were well reflected in the chromatographic elution profiles (Fig. 3a, b). It should be noted that the storage induced, to some extent, reduction of ATP and hexose diphosphates and a conspicuous increase in inorganic phosphate fraction. ADP fraction underwent no remarkable alteration.

Fig. 1. pH changes of human blood during storage in ACD (4°C). pH was actually measured at 5°C. Results were mean ± S.D. (n = 10).
Physiological and biochemical changes in canine blood when stored in ACD media containing bisulfite (4°C)

As stated above, canine red cells showed a much slower decrease of DPG when stored in ACD, which would be of insufficient extent to follow the posttransfusional changes of the organic phosphates and the oxygen affinity. The sufficient reduction could, of course, be achieved by longer preservation of the blood, which, however, might result in other unfavourable outcomes such as hemolysis or reduced viability of red cells. We therefore tried the procedure of Parker (1969), who showed a marked acceleration by bisulfite of DPG decomposition in human red cells. Bisulfite proved the considerable accelerating effect in canine red cells, too. After 7 days' preservation, the DPG was reduced by 18.8, 55.8, and 71.0% in the absence and presence of 5 and 10 mM sodium bisulfite, respectively (Fig. 2). Since no unfavourable effect of bisulfite was found on the viability and functions of the red cells, DPG depletion in the following experiments was performed by the preservation in ACD containing 10 mM bisulfite for 8 to 12 days (4°C). The accelerated reduction in DPG, accompanied by a marked elevation in inorganic phosphate, was also represented in the chromatographic elution profiles (Fig. 3). Some accelerations in reduction were also observed in such phosphate compounds as ATP, ADP, and hexose diphosphates. The DPG reduction was accompanied by a proportional elevation of the blood oxygen affinity (Fig. 4).

Physiological and biochemical changes in recipient dog blood during exchange transfusion with DPG-depleted blood

Exchange transfusion experiments were carried out in five dogs. Blood volumes of the recipients were 808 ± 106 ml based on their body weight, and 1,540 ± 344 ml of the DPG-depleted blood (DPG/Hb: 0.16 ± 0.04) were exchange-transfused in 2 to 10 days. The hemoglobin concentration in the recipient dogs was 205 ± 10 g/l. The change in the blood DPG content was monitored during the exchange transfusion.
Fig. 3. Chromatographic profiles of acid-soluble phosphorus compounds from red cells of fresh and ACD-stored canine blood. Fresh blood (a) and stored blood for 7 days in ACD (b) and in ACD with 5 (c) or 10 mM bisulfite (d) at 4°C. Elution was performed through Dowex 1-X8 (200-400 mesh, 1 × 25 cm, formate form) by concentration gradient of formate. --- P in mmol/L RBC, ---- absorbance at 260 nm. Figures attached to the elution peaks were their concentrations as expressed by P content. \( P_i \), inorganic phosphate; ADP, adenosine diphosphate; HDP, hexose diphosphates.
Fig. 4. Leftward shift in ODC (pH 7.40, $P_{CO_2}$ 40 Torr, 37°C) of canine blood during its storage in ACD with 10 mM bisulfite (4°C). Storage days and DPG/Hb molar ratio were: ▲ (0 and 1.4), △ (5 and 1.0), ● (12 and 0.4), and ○ (21 and 0.1).

Fig. 5. Changes in several blood parameters of a dog during exchange transfusion with ACD-stored and DPG-depleted blood (DPG/Hb: 0.18).
3 h period. Average % exchange of blood was estimated as 84% (80–88%) by the method of DIAMOND (1947).

Figure 5 shows a typical example of changes in several blood parameters during the exchange transfusion. In this case, the recipient blood, its assessed initial volume and initial DPG/Hb molar ratio being 770 ml and 1.38, was replaced with 1,400 ml of the DPG-depleted blood (DPG/Hb: 0.18). The final DPG/Hb molar ratio of 0.30 gave an estimated blood replacement of 90%, which was fairly comparable with that by the Diamond procedure (84%). The Ht and Hb concentration showed that the recipient blood resumed its original state after a transient and light hemoconcentration.

Posttransfusional changes in physiological and biochemical parameters in recipient dog blood

Posttransfusional changes in blood oxygen affinity and the relevant parameters were followed in the five recipient dogs (Table 1 and Fig. 6). The recipients’ blood was replaced by 84.4 ± 2.9% with the stored blood whose DPG were depleted by 88.6 ± 7.2%. A transient hemoconcentration followed by a hemodilution was observed in the early posttransfusional period, and the dogs maintained a steady state from 8 h to 5 days. The blood pH was considerably low immediately after the transfusion, which would be a natural consequence of the large-scale replacement of the recipient blood with stored blood of lowered pH. The pH maintained a lowered level after a rapid recovery to some extent, and it was only after 24 h to reach the lowest margin of the normal range. The red cell intracellular pH showed a parallel trend of the change, but was a little lower than normal, even after 24 h.

The red cell DPG showed the lowest value immediately after the transfusion. Although a relatively rapid recovery was observed, the level was still considerably out of the normal range after 24 h and was ultimately restored to normal after 5 days. The oxygen affinity, as reciprocally expressed by \( P_{50} \), the oxygen tension for

<table>
<thead>
<tr>
<th>Time after transfusion</th>
<th>Ht (%)</th>
<th>pH(_i)</th>
<th>( \Delta ) pH(^a)</th>
<th>DPG/Hb (molar ratio)</th>
<th>( P_{50} ) (^b) (Torr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>34 ± 4</td>
<td>7.08 ± 0.05</td>
<td>0.11 ± 0.05</td>
<td>0.42 ± 0.11</td>
<td>18.5 ± 2.6</td>
</tr>
<tr>
<td>3</td>
<td>36 ± 8</td>
<td>7.03 ± 0.06</td>
<td>0.13 ± 0.03</td>
<td>0.51 ± 0.15</td>
<td>20.1 ± 3.1</td>
</tr>
<tr>
<td>8</td>
<td>32 ± 2</td>
<td>7.11 ± 0.07</td>
<td>0.15 ± 0.03</td>
<td>0.79 ± 0.23</td>
<td>21.6 ± 1.8</td>
</tr>
<tr>
<td>15</td>
<td>31 ± 1</td>
<td>7.06 ± 0.06</td>
<td>0.18 ± 0.01</td>
<td>0.95 ± 0.19</td>
<td>23.8 ± 1.5</td>
</tr>
<tr>
<td>24</td>
<td>28 ± 6</td>
<td>7.12 ± 0.04</td>
<td>0.18 ± 0.02</td>
<td>1.04 ± 0.15</td>
<td>25.7 ± 0.8</td>
</tr>
<tr>
<td>5 days</td>
<td>28 ± 3</td>
<td>7.11 ± 0.03</td>
<td>0.19 ± 0.02</td>
<td>1.40 ± 0.15</td>
<td>28.9 ± 0.8</td>
</tr>
</tbody>
</table>

\(^a\) Red cell transmembrane pH gradient. \(^b\) \( P_{50} \) for half-oxygenation of blood at pH\(_i\) 7.40, \( P_{CO2} \) 40 Torr at 37°C. Results were means ± S.D. (\( n = 5 \)).
Fig. 6. Summarized representation of changes in the oxygen affinity ($P_{50}$ at pH 7.40, $P_{CO_2}$ 40 Torr, 37°C) and the related parameters in blood of dogs after exchange transfusion with ACD-stored and DPG-depleted blood. Normal ranges for each parameter are shown by shaded zones. $pHe$, extracellular pH (blood pH); $pHi$, intracellular pH.

Fig. 7. Correlation between red cell DPG content and oxygen affinity ($P_{50}$ at pH 7.40, $P_{CO_2}$ 40 Torr, 37°C) of canine blood. The data were taken from the fresh (△), ACD-stored (○), and posttransfusional (●) blood.
half-oxygenation of blood at pH 7.40, $P_{CO_2}$ 40 Torr, 37°C, was highest immediately after the transfusion ($P_{50}$: 18.5 Torr), and subsequently showed a rapid but insufficient recovery to $P_{50}$ of 25.7 Torr after 24 h. The normal range was attained after 5 days. Mode of the posttransfusional changes was well in parallel to that in the DPG.

As shown in Fig. 7 and Eq. 1, a close correlation was present between the $P_{50}$ and DPG content throughout the fresh, stored, and posttransfusional canine blood;

$$y = 10.92x + 14.2 \quad (r = 0.91),$$

where $y$ and $x$ were $P_{50}$ (Torr) and DPG/Hb molar ratio, respectively. Correlation was also found between the pH gradient across the red cell membrane ($\Delta p$H = $p$H$_{e}$ - $p$H$_{i}$) and DPG content (Fig. 8);

$$y = 0.060x + 0.135 \quad (r = 0.70),$$

where $y$ and $x$ denoted $\Delta p$H at $p$H$_{e}$ = 7.40 and DPG/Hb molar ratio, respectively.

Figure 9 represents a chromatographic follow-up of the posttransfusional changes in the red cell acid soluble phosphorus compounds. As readily anticipated from the situation in the stored blood, the recipient blood just after completion of the exchange transfusion was characterized by a marked reduction in DPG and a marked elevation in inorganic phosphate. The phosphorus compounds other than ATP also showed a decrease. Although a rapid recovery to normal was observed, the DPG and inorganic phosphate still remained outside the normal range after 24 h.

Fig. 8. Dependence of red cell transmembrane pH gradient upon the red cell DPG content of posttransfusional blood in dogs. Ordinate: pH difference across red cell membrane at the extracellular pH 7.40. Abscissa: DPG/Hb molar ratio.
DISCUSSION

An improved method for whole blood ODC. ODC provides us with important information concerning the respiratory function of blood; but for its construction, experienced skills and a specialized and time-consuming technique are required. These facts have hampered the routine use in both basic and clinical respiratory physiology. The present procedure, using a spectrophotometric determination of the $S_{O_2}$, is intended for a partial solution of these problems and this aim appears to be achieved fairly well (Table 2). Later the present method has been further modified by the use of the Clark electrode for blood $P_{O_2}$ measurement, and the new version enabled us to perform a more rapid and simple determination of whole blood ODC (KOHZUKI et al., 1983).

Fig. 9. Posttransfusional changes in acid soluble phosphorus compounds of red cells in a dog exchange-transfused with DPG-depleted red cells (DPG/Hb: 0.16). The determinations were made 0, 8, and 24 h after the transfusion. Refer to explanation for Fig. 3.
Physiological and biochemical changes of canine blood during storage in ACD. It has been generally considered that the rapid DPG decrease and the consequent functional deterioration of blood in ACD is mainly due to a low pH condition during the storage (BARTLETT, 1973), but the actual and quantitative proof has been rather scanty. The present result offers clear evidence demonstrating a steady decrease of blood pH under the storage condition (5°C) (Fig. 1). The lowered pH may induce the lowered DPG through its inhibition of the red cell phosphofructokinase and 2,3-DPG mutase and also its activation of the 2,3-DPG phosphatase (BARTLETT, 1973).

Although complete explanation for the much slower DPG decay in canine red cells (Fig. 2) is not feasible, a possible mechanism may be a combined outcome of the higher phosphofructokinase and lower pyruvate kinase activities as compared with those in human red cells (RAPOPORT, 1968). Acceleration by bisulfite of the DPG degradation, previously reported for human red cells (PARKER, 1969), is now confirmed for the canine red cells, too (Fig. 2). The principal mechanism of the action may be an enhancement of the 2,3-DPG phosphatase activity by this sulfur-containing anion (MANYAI and VARADY, 1956).

DPG, being a non-penetrating polyanion, exerts a profound influence upon the $H^+$ distribution across red cell membrane via the Gibbs-Donnan mechanism. In addition to the previous findings which were obtained for the red cells with an artificially elevated DPG content (DUHM, 1971), the present result offers firm evidence for the similar dependence of the pH gradient upon the DPG of subnormal concentration (Fig. 8).

In vivo physiological and biochemical changes of stored canine blood after transfusion. There has been mounting evidence which demonstrates that an elevated oxygen affinity of blood is disadvantageous in oxygen transport under

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**Table 2. Comparison of oxygenation characteristics of normal human adult blood as determined by the present method with those by the standard gasometric method (37°C).**

<table>
<thead>
<tr>
<th>Blood pH</th>
<th>$P_{50}$ (Torr)$^a$</th>
<th>$n$$^b$</th>
<th>Bohr factor$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present result</td>
<td>Bartels$^d$ et al.</td>
<td>Present result</td>
</tr>
<tr>
<td>7.60</td>
<td>21.8</td>
<td>21.5</td>
<td>2.72</td>
</tr>
<tr>
<td>7.40</td>
<td>26.9</td>
<td>26.8</td>
<td>2.65</td>
</tr>
<tr>
<td>7.20</td>
<td>33.8</td>
<td>33.4</td>
<td>2.60</td>
</tr>
<tr>
<td>7.00</td>
<td>41.2</td>
<td></td>
<td>2.56</td>
</tr>
</tbody>
</table>

$^a$Corrected for the Bohr shift by using the Bohr factor of $-0.48$. $^b$Hill's exponent. $^c$A log $P_{50}$/$\Delta$H for the pH ranges (i) 7.60–7.40, (ii) 7.40–7.20, and (iii) 7.20–7.00. $^d$BARTLETS et al. (1961).
normoxic state (Valtis and Kennedy, 1954; Bunn et al., 1969; Yhap et al., 1975; Ochiai and Enoki, 1976). Living organisms, however, respond to this dysfunction of blood in a variety of compensatory mechanisms, e.g. increased cardiac output. As for the transfusion of stored blood with elevated oxygen affinity, it should be also stressed that blood itself tends to gradually recover its normal function. To date, however, the mode of posttransfusional restoration has been scarcely and incompletely investigated (Beutler and Wood, 1969; Valeri and Hirsch, 1969; Sugerman et al., 1970).

Since the time course of the posttransfusional restoration could be modified by a number of physiological factors, the reported time needed for the complete recovery varies largely from 6 to 24 h (Valtis and Kennedy, 1954; Beutler and Wood, 1969; Valeri and Hirsch, 1969; Sugerman et al., 1970). Naturally, one important factor responsible for these differences is the transfused blood volume or % exchange of blood. In the present results the functional and biochemical parameters do not attain the initial and normal level even after 24 h (Fig. 6 and Table 1). On the contrary, only 16 h are required for the complete recovery in several human surgical cases, in which the recovery rate is almost similar to that in dogs (Watanabe et al., unpublished). The distinction between both the cases may be explained mainly by the difference in % exchange of blood, i.e. 84 ± 3% in canine vs. 53 ± 11% in human. Another relevant factor is the metabolic states including acid-base status in the recipients, influencing the DPG metabolism in red cells which, in turn, modifies the oxygen affinity of blood. It has been well known that red cell DPG metabolism is strongly affected by such pH-sensitive glycolytic enzymes as phosphofructokinase, 2,3-DPG mutase, and 2,3-DPG phosphatase in red cells (Bartlett, 1973).

Concerning the oxygen transport function of blood, one thing should be noted here. Blood pH and the intracellular pH remains subnormal to a considerable extent and for a considerable duration after a large scale transfusion of stored blood (Fig. 6 and Table 1). Through the Bohr effect, these changes may possibly counteract the disadvantageous change of the enhanced oxygen affinity due to the DPG depletion.

To conclude, although compensatory and restoring changes can occur, stored blood of young shelf-life or fresh blood, if possible, would be more desirable for severe clinical cases in which a large scale blood transfusion is necessary. The notion is still valid for blood stored in CPD, since the red cell DPG and consequently the oxygen affinity suffers from a rather rapid decay in the later two-thirds of the shelf-life (21 days) (Ochiai and Enoki, 1976).

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REFERENCES


