

## Brief Report

## TRANSFUSIONS OF POLYMERIZED BOVINE HEMOGLOBIN IN A PATIENT WITH SEVERE AUTOIMMUNE HEMOLYTIC ANEMIA

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**H**EMOGLOBIN solutions have several potential advantages as substitutes for erythrocytes for transfusion. Hemoglobin solutions have a prolonged shelf life, are associated with a lower risk of transfusion reactions, and provide faster uptake of oxygen.<sup>1</sup> Hemoglobin-based oxygen carriers (HBOC) have been studied primarily in patients with hemorrhage, but the absence of cell-surface antigens in these solutions suggests that they may have a role in the treatment of autoimmune hemolytic anemias. We report the use of a polymerized bovine hemoglobin, HBOC-201 (Hemopure, Biopure, Cambridge, Mass.), in a woman with severe autoimmune hemolytic anemia.

### CASE REPORT

A 21-year-old woman with a petechial rash and gingival bleeding (weight, 67 kg) was referred to our institution. Her medical, family, and social history was unremarkable, and her only medication was an oral contraceptive. Physical examination revealed multiple petechiae; no hepatosplenomegaly was noted. Table 1 shows the results of laboratory testing on admission. Bone marrow biopsy showed adequate cellularity, a predominance of erythroid cells, and a leftward shift in all cell counts. A presumptive diagnosis of idiopathic thrombocytopenic purpura was made. Treatment with prednisone was initiated at a dose of 1 mg per kilogram of body weight per day, with initial improvement in the platelet count to 83,000 per cubic millimeter.

Twelve days after her initial presentation, the patient was readmitted, reporting weakness, dyspnea, and fever (temperature, up to 39°C). Table 1 shows the results of laboratory testing at the time of readmission. Within 12 hours after readmission, the hematocrit had declined to 7.5 percent, and tachycardia, chest pressure, dyspnea, and electrocardiographic changes indicative of ischemia had developed. The symptoms and electrocardiographic changes resolved as the hematocrit rose to 12.4 percent (hemoglobin level, 4.2 g per deciliter [2.6 mmol per liter]) after the transfusion of 2 units of packed red cells. Methylprednisolone (pulses of 1 g per day) and intravenous immune globulin (total dose, 5 g per

kilogram over a 10-day period) were administered, with transient improvement of the anemia.

Over the next 45 days, hemolysis increased, and the anemia was found to be refractory to treatment with high doses of glucocorticoids, plasmapheresis, splenectomy, and a second course of intravenous immune globulin (Fig. 1). The peripheral blood smear showed marked spherocytosis and as many as 128 nucleated red cells per 100 white cells. The reticulocyte count peaked at 37 percent. Antibodies in the patient's serum reacted with erythrocytes from all available donors. To maintain a hematocrit greater than 12 percent and a hemoglobin level greater than 4.0 g per deciliter (2.5 mmol per liter), approximately the level previously associated with correction of the ischemic changes in the electrocardiogram, as many as 8 units of packed red cells per day were needed. The patient began to have fever, nausea, and back pain during the erythrocyte transfusions; these symptoms were attributed to acute hemolysis. At this time, therapy with cyclophosphamide (1000 mg per square meter of body-surface area) was begun.

Since conventional transfusions were ineffective, we obtained the patient's written informed consent to administer HBOC-201 as an alternative oxygen-carrying solution until the autoimmune hemolytic anemia abated. Additional approval for compassionate use of this product was obtained from our institutional review board, the U.S. Army Medical Command, and the Food and Drug Administration.

### METHODS

HBOC-201 is a sterile solution of glutaraldehyde-polymerized bovine hemoglobin buffered in lactated Ringer's solution. Each unit contains 30 g of polymerized hemoglobin, equivalent to approximately half the hemoglobin contained in 1 unit of human packed red cells.<sup>2</sup> Table 2 lists the physical properties of HBOC-201. Since HBOC-201 is a solution, it does not contribute to the measured hematocrit. The level of hemoglobin resulting from the administration of HBOC-201 is estimated as the total hemoglobin level minus one third of the hematocrit.

We administered HBOC-201 if there was clinical evidence of end-organ ischemia (acidosis or base excess) or hemodynamic decline or if the whole-blood hemoglobin level decreased to 4 g per deciliter or less. The first unit was administered at a rate of 0.25 g per minute to assess tolerance. Subsequent units were administered at 0.50 g per minute, a rate previously tolerated by patients with sickle cell disease.<sup>3</sup> The toxicity of HBOC-201 was assessed by monitoring the patient's vital signs, the results of laboratory tests, and symptoms. Certain laboratory tests were not performed, because HBOC-201 interferes with the colorimetric assays on which those tests are based<sup>4,5</sup> (Table 2). Hemodynamic monitoring included measurements of blood pressure by radial-artery catheter and, during periods of hypotension, measurements of cardiac output, central venous pressure, and pulmonary-artery pressure with use of a thermodilution pulmonary-artery catheter. Urine output was measured hourly.

### RESULTS

A total of 11 units of HBOC-201 (330 g [4.9 g per kilogram]) were administered as one 90-g, two 60-g, and four 30-g doses over a seven-day period. A peak plasma HBOC-201 level of 3.36 g per deciliter (2.1 mmol per liter) was attained after the administration of the ninth unit. No adverse effects attributable to HBOC-201 were identified. Five units were administered in response to clinical evidence of ischemia (units 1, 2, 7, 10, and 11), three as part of volume resuscitation during an episode of septic shock (units 4, 5, and 6), and three (units 3, 8, and 9) to maintain the total hemoglobin level above 4 g per deciliter. The average total hemoglobin level during

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TABLE 1. HEMATOLOGIC LABORATORY FINDINGS.\*

VARIABLE	FIRST ADMISSION	SECOND ADMISSION
White-cell count (per mm <sup>3</sup> )	3500	3,300
Differential count (%)		
Neutrophils	45.9	12.5
Lymphocytes	41.5	67.0
Monocytes	9.0	17.0
Eosinophils	2.3	0.0
Band forms	1.3	2.0
Metamyelocytes	0.0	1.0
Myelocytes	0.0	0.5
Hemoglobin (g/dl)	13.7	4.8
Hematocrit (%)	39.0	13.8
Reticulocyte count (%)	—	22.8
Platelet count (per mm <sup>3</sup> )	7000	12,000
Antiglobulin test (direct)	Negative	Warm antibody (IgG) positive, C3 complement negative
Prothrombin time (sec)†	13.2	13.3
Activated partial-thromboplastin time	Normal	Normal
Serologic test for human immunodeficiency virus	Negative	—
Test for antinuclear antibody	Negative	—
Serum C-reactive protein (mg/dl)	<0.4	—
Serum haptoglobin (mg/dl)	—	<6
Serum bilirubin (mg/dl)		
Total	0.5	3.0
Unconjugated	—	2.8
Serum lactate dehydrogenase (U/liter)	—	1,308
Nucleated red cells (per 100 white cells)	—	1
Blood-smear findings	—	Slight anisocytosis, polychromasia, basophilic stippling, and spherocytosis; marked thrombocytopenia and leukopenia

\*To convert the values for hemoglobin to millimoles per liter, multiply by 0.6206. To convert the values for bilirubin to micromoles per liter, multiply by 17.1.

†The normal range is 10.8 to 12.8 seconds.

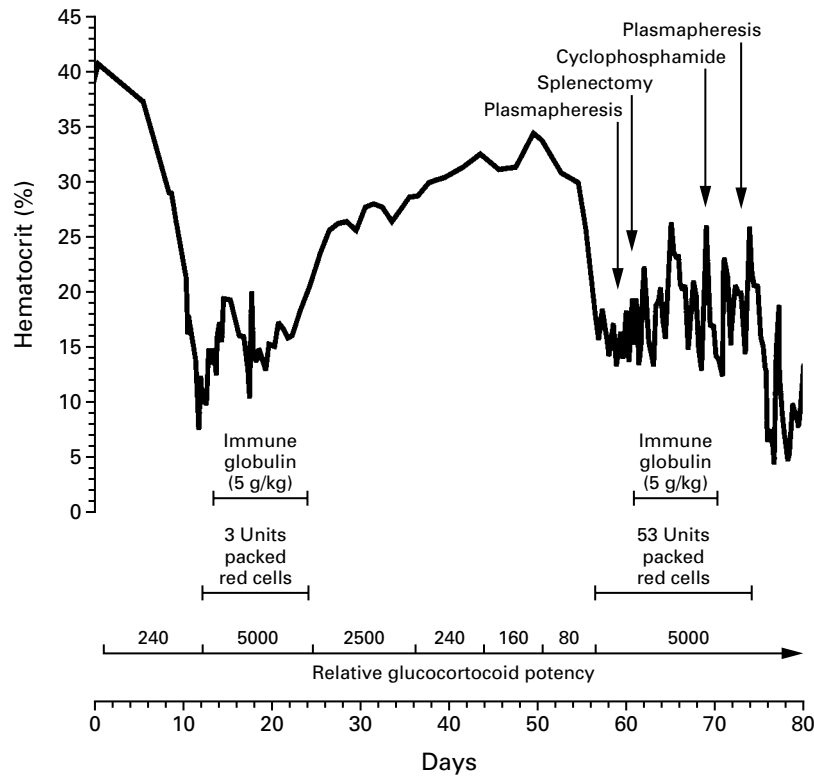
the course of HBOC-201 therapy was 5.5 g per deciliter (3.4 mmol per liter), with a corresponding average hematocrit of 9.5 percent. In some instances the hemoglobin level and the hematocrit were nearly equal (Fig. 2), suggesting that most of the oxygen-carrying capacity of the blood was attributable to soluble hemoglobin.

#### Relief of Ischemia

The initial 60-g dose of HBOC-201 (units 1 and 2) was administered in response to accelerated hemolysis, 75 days after the patient's initial presentation. The hematocrit had fallen from 22 percent to 13.8 percent over the course of 36 hours, with resultant tachycardia (heart rate, 130 beats per minute) and elevation of the serum lactic acid level to 2.2 mmol per liter. During this four-hour HBOC-201 infusion, the hematocrit declined further, to 6.4 percent, and the total hemoglobin level fell to 3.7 g per deciliter (2.3 mmol per liter), of which approximately 1.6 g per deciliter (1.0 mmol per liter) was HBOC-201. De-

spite this, the patient's heart rate decreased to 70 beats per minute and subsequently ranged between 70 and 90 beats per minute, she remained hemodynamically stable, and there was no electrocardiographic evidence of ischemia.

Units 4, 5, and 6 of HBOC-201 were administered 48 hours after the initial dose as part of volume expansion for profound hypotension (mean arterial pressure, 40 mm Hg) during an episode of neutropenic septic shock. A norepinephrine infusion was also begun. Unit 7 was administered 16 hours later in response to a drop in the arterial pH from 7.45 to 7.22, with an accompanying decrease in base excess from -5.1 to -17.8 mmol per liter. Improvement in the pH to 7.31 and in the base excess to -13.2 mmol per liter occurred within 10 minutes after the HBOC-201 infusion was started, with subsequent improvement in the pH to 7.37 and in the base excess to -11.8 mmol per liter during the first hour. There was no change in the rate of norepinephrine infusion during this interval.



**Figure 1.** Treatment before the Initiation of HBOC-201 Therapy.

Day 0 is the day of initial presentation. HBOC-201 therapy was initiated on day 75. Relative glucocorticoid potency indicates the daily antiinflammatory effect of glucocorticoid treatment relative to that of hydrocortisone (on a scale where 1 is equivalent to 1 mg of hydrocortisone); in this case it remained 5000 after 80 days.

Unit 10 of HBOC-201 was administered on day 6 after the initial dose in response to declines in the arterial pH from 7.35 to 7.20 and in base excess from  $-8.2$  to  $-10.8$  mmol per liter over a six-hour period. Increases in the arterial pH to 7.34 and in base excess to  $-9.2$  were noted over the course of the one-hour infusion.

The last unit of HBOC-201 was administered the next day in response to a stable base excess of  $-3.7$  mmol per liter, with improvement noted to 1.0 mmol per liter within the first hour of the infusion. The minimal hematocrit supported during HBOC-201 therapy was 4.4 percent (total hemoglobin level, 3.5 g per deciliter [2.2 mmol per liter], of which 2.03 g per deciliter [1.3 mmol per liter] was HBOC-201), measured on the second day after the start of therapy. An electrocardiogram obtained at this time showed no important abnormalities (Fig. 3).

#### Hemodynamic Response

Hemoglobin-based solutions have been reported to raise both systemic and pulmonary arterial pressures.<sup>6</sup> In this patient, there was no immediate pressor

effect, although there was an overall trend toward higher blood pressures at the end of therapy. The patient's average mean arterial pressure during the five days before the initiation of HBOC-201 therapy was 93.6 mm Hg, and the average during the five days after the completion of therapy was 119.7 mm Hg. The average mean arterial pressure during the course of HBOC-201 therapy was 104.7 mm Hg, when values measured during volume expansion in response to septic shock are excluded. The mean arterial pressure did not change during or immediately after the administration of any of the units of HBOC-201. Invasive hemodynamic monitoring showed an initial decrease in the cardiac index during the period of septic shock; this index later improved in parallel with the improvement in mean arterial pressure as the shock resolved. Pulmonary arterial systolic and diastolic pressures increased only slightly with HBOC-201 therapy.

#### Clinical Outcome

While receiving HBOC-201 during the period of profound neutropenia (absolute neutrophil count, 78 per cubic millimeter) caused by cyclophosphamide,

**TABLE 2.** PHYSICAL PROPERTIES OF POLYMERIZED BOVINE HEMOGLOBIN AND HUMAN WHOLE BLOOD.\*

VARIABLE	POLYMERIZED BOVINE HEMOGLOBIN†	HUMAN WHOLE BLOOD
Hemoglobin (g/dl)	11.0–15.0	11.5–15.5
Colloidal osmotic pressure (mm Hg)	25	25
Osmolality (mOsm/kg)	290–310	275–295
pH	7.6–7.9	7.35–7.45
P <sub>50</sub> (mm Hg)‡	36–38	26
Viscosity at 37°C (centipoise)	1.3	3.5
Sodium (mmol/liter)	145–160	137–145
Potassium (mmol/liter)	3.5–5.5	3.8–5.2
Chloride (mmol/liter)	105–120	98–107

\*Values for polymerized bovine hemoglobin are ranges or means. Values for human whole blood are normal ranges or means. To convert the values for hemoglobin to millimoles per liter, multiply by 0.6206.

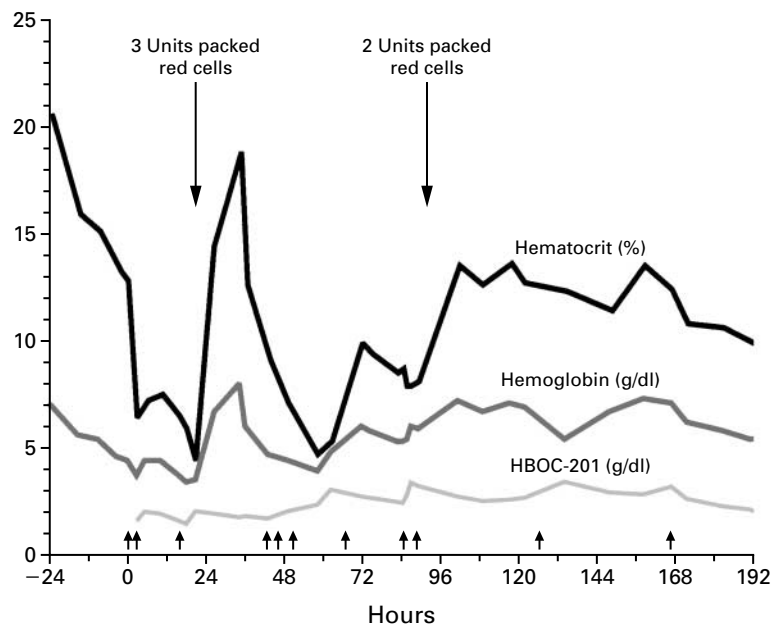
†Components of serum that cannot be measured in the presence of polymerized bovine hemoglobin because of interference with the standard colorimetric assays include calcium, albumin, bilirubin, cholesterol, creatine kinase, phosphorus, amylase, lipase, lactate dehydrogenase, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and  $\gamma$ -glutamyltransferase. Levels of the antibiotics gentamicin and vancomycin also cannot be measured.

‡P<sub>50</sub> denotes the partial pressure of arterial oxygen at which 50 percent of hemoglobin is saturated under standardized conditions.

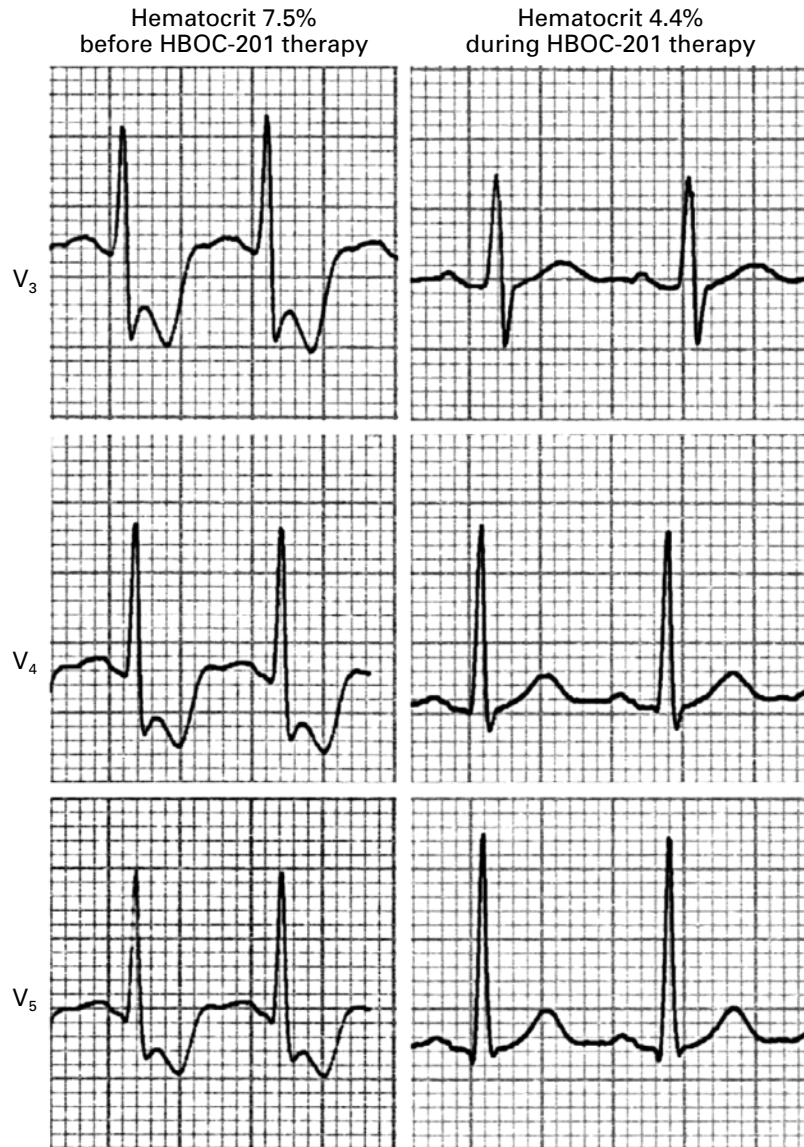
the patient had gram-negative septic shock and, later, gram-negative pelvic osteomyelitis. Treatment required two surgical débridements of the right anterior ilium and long-term administration of antibiotics. Although the cyclophosphamide induced a brief remission in hemolysis in the patient, further treatment with this drug was not attempted, because of the complications. A second trial of plasmapheresis and intravenous immune globulin again failed to induce a sustained response, so immunosuppressive therapy with cyclosporine was initiated. There was a sustained response to cyclosporine, with no further need for transfusions. The patient was discharged to her home in good condition 100 days after the completion of HBOC-201 therapy. Eight months after discharge, the patient remained well, with a hematocrit consistently greater than 35 percent.

## DISCUSSION

Interest in the development of a safe, effective substitute for human erythrocytes as a transfusable medium for oxygen transport has increased substantially in the past decade. A number of products are under investigation.<sup>7,8</sup> Polymerized forms of bovine hemoglobin, such as HBOC-201, show particular promise. They have a molecular structure similar to that of human hemoglobin but have lower concentrations of organic phosphates, resulting in more pronounced oxygen unloading in ischemic tissue (the acid Bohr



**Figure 2.** Hematocrit, Hemoglobin Levels, and Calculated HBOC-201 Levels during HBOC-201 Therapy. Hour 0 indicates the initiation of HBOC-201 therapy. Each small arrow indicates the transfusion of 1 unit of HBOC-201. To convert values for hemoglobin and HBOC-201 to millimoles per liter, multiply by 0.6206.



**Figure 3.** Representative Portions of Recordings from Electrocardiographic Leads  $V_3$ ,  $V_4$ , and  $V_5$  before and during HBOC-201 Therapy.

The left-hand panels show recordings from leads  $V_3$ ,  $V_4$ , and  $V_5$  of the patient's electrocardiogram while her hematocrit was 7.5 percent before HBOC-201 therapy. The right-hand panels show recordings from these electrocardiographic leads while her hematocrit was 4.4 percent with a calculated HBOC-201 level of 2.03 g per deciliter (1.3 mmol per liter).

effect) and increased hemoglobin binding of carbon dioxide in the deoxygenated state (the Haldane effect).<sup>9</sup> The affinity of bovine hemoglobin for oxygen is also partially regulated by serum chloride ions, whereas the affinity of human hemoglobin for oxygen is influenced by 2,3-diphosphoglycerate.<sup>10</sup> These features result in excellent oxygen-transport properties.

Bovine hemoglobin has been shown to maintain tissue oxygenation<sup>11</sup> and to permit survival for more than one month with hematocrits as low as 2.4 percent in an ovine model.<sup>12</sup> HBOC-201 has been found to be safe and well tolerated in normal adults<sup>2,13</sup> and in patients with sickle cell disease<sup>3</sup> and has been studied in patients undergoing elective abdominal aortic

surgery.<sup>14</sup> To our knowledge, this is the first report of the use of HBOC-201 to support oxygen delivery in a patient with severe autoimmune hemolytic anemia. Our patient received a larger dose (330 g) of HBOC-201 than previously administered to a human subject and also attained a higher plasma HBOC-201 level (3.36 g per deciliter) than any reported previously.

A vasoconstrictive response manifested by elevations in systemic and pulmonary arterial pressures typically occurs with the administration of hemoglobin-based solutions, an effect that has been attributed to the binding of nitric oxide by the hemoglobin moiety. This response is associated with a decreased cardiac output and, in several studies, has been associated with impaired oxygen delivery.<sup>2,14</sup> We did not perform invasive hemodynamic monitoring at the time therapy was initiated, but we did not observe a substantial vasoconstrictive response to HBOC-201. The absence of a hypertensive response has likewise been noted with administration of HBOC-201 to patients with sickle cell disease.<sup>3</sup> In addition, concurrent sepsis may have blunted any hypertensive effect, as has been demonstrated in an ovine model,<sup>15</sup> or higher doses of HBOC-201 may cause less vasoconstriction, as has been observed in patients undergoing abdominal aortic surgery.<sup>14</sup> In addition, the cardiac index, once measured, varied in parallel with rather than inversely with the mean arterial pressure, and metabolic acidosis as a reflection of ischemia improved predictably with the administration of HBOC-201.

Our patient had an episode of life-threatening gram-negative sepsis during HBOC-201 therapy and had profound ill effects from gram-negative osteomyelitis. It has been speculated that cell-free hemoglobin substances may support bacterial virulence by offering a ready supply of iron, thus sustaining bacterial replication and inhibiting neutrophil function.<sup>16,17</sup> There is additional evidence that increased hemolysis itself increases the risk of infection.<sup>18</sup> It is unclear what role, if any, HBOC-201 may have had in promoting our patient's infectious complications, given her neutropenia and preexisting hemolytic anemia.

In summary, the use of HBOC-201 as an alternative medium for oxygen delivery appears to have been a lifesaving intervention in a patient with refractory autoimmune hemolytic anemia. HBOC-201 supported the patient at a hematocrit of 4.4 percent without immediate or long-term evidence of ischemic injury.

The absence of cell-surface antigens in HBOC-201 may make it a useful agent to support oxygen delivery in patients with severe autoimmune hemolytic anemia.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

*We are indebted to Biopure for the donation of test product HBOC-201 for compassionate use in this case.*

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