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# THE EFFECTS OF THE INFUSION OF STROMA-FREE HEMOGLOBIN INTO DOGS WITH SPLEENS

BY

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In the study reported here, the intravascular retention of crosslinked (bis 3,5-dibromosalicyl fumarate) human hemoglobin (DBBF) and unmodified

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bovine hemoglobin (BSFH) and BSFH modified by glutaraldehyde crosslinking were measured in dogs with spleens. The effects on hemodynamics, hematological parameters, coagulation system, and renal function were assessed.

The initial hemoglobin levels following infusion ranged from 900-2700 mg/dl with an intravascular recovery of 30-61%. The circulation of DBBF was longer than either modified or unmodified BSFH. Thirty-eight percent of infused DBBF remained circulating at 24 hours, whereas only 16 to 24% of the BSFH solutions circulated at 6 hours. Hemoglobinuria was not detectable after DBBF infusion. However, hemoglobinuria was present within 30 minutes after infusion of both unmodified and modified BSFH, indicating that the crosslinking of BSFH did not diminish urinary excretion.

All three solutions were associated with increases in total peripheral resistance and mean arterial pressure following infusion, indicating the presence of vasoconstrictor activity. There were no indications of renal toxicity or disseminated intravascular coagulation associated with any of the three hemoglobin solutions.

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## INTRODUCTION

Stroma-free hemoglobin (SFH) solutions have oncotic properties and the capacity to transport oxygen and have a potential use as a resuscitation fluid. Concerns in the use of a SFH solution as a blood substitute include vasoconstrictor effects[1-6], kidney toxicity, sterility and endotoxin content, methemoglobin levels, and the potential to precipitate disseminated intravascular coagulation.

When a large amount of free hemoglobin is present in plasma, the haptoglobin binding capacity is saturated, and the excess free tetrameric hemoglobin molecules dissociate into dimers and monomers, which are excreted through renal glomerular filtration into the urine[7-8]. In order to reduce renal excretion and improve intravascular retention, intramolecular and intermolecular crosslinking of stroma free hemoglobin using chemical modification has been developed to decrease the dissociation of the tetramers[8-9].

We have assessed the effects of the infusion of crosslinked (3,4 bis dibromosalicyl fumarate) human stroma-free hemoglobin and unmodified bovine stroma-free hemoglobin and crosslinked (glutaraldehyde) bovine stroma-free hemglobin on hemodynamics, hematological parameters, the coagulation system, and renal function. The intravascular retentions of the stroma-free hemoglobin solutions were measured in dogs with spleens.

## OBJECTIVE

Assessment of the effects of infusion of human diasprinated (3,5 bis dibromosalicyl fumarate) stroma-free hemoglobin (DBBF) and modified and unmodified bovine stroma-free hemoglobin (BSFH) on hemodynamics, hematological parameters, coagulation system and renal function.

The clearances of DBBF produced by Baxter Laboratories, Deerfield, IL. and unmodified BSFH, Oxypure, and modified BSFH, Hemopure, produced by Biopure Company, Boston, MA., were measured in dogs with spleens.

## METHODS

Six nonsplenectomized dogs weighing between 20 and 24 kilograms were studied. Two dogs were infused with a solution containing 39g of human DBBF stroma free hemoglobin (SFH), two dogs received 40g unmodified BSFH, and two received 42g of modified BSFH.

### Experimental Design

On the morning of the study the dogs were sedated with approximately 3.8 mg/kg of acepromazine given orally or with 2.8mg/kg given im. General anaesthesia was maintained with sodium pentathol throughout the study, and muscle relaxation was ensured with pancuronium. Catheterization of a femoral artery with a 20 gauge catheter was performed to permit direct measurement of mean arterial pressure and sampling of arterial blood. Catheterization of a femoral vein with a 16 gauge catheter was also performed to permit withdrawals of blood samples and administration of fluids and drugs. All dogs were intubated 1 hr prior to each experiment and were mechanically ventilated on room air throughout the study.

Measurements were made of the mean arterial pressure (MAP), central venous pressure (CVP), mean pulmonary arterial pressure (MPAP), cardiac output (CO), and heart rate, and blood samples were obtained before infusion of SFH, and at 10, 20, and 40 minutes and 1,2,4 and 6 hours following the infusion for measurement of arterial and

venous oxygen content, arterial ph, arterial blood gases, hemoglobin, hematocrit, white blood cell count, platelet count, creatinine, urea nitrogen (BUN), plasma hemoglobin, plasma methemoglobin, fibrinogen, factor VIII, and fibrin degradation products . In addition, some blood sample measurements were performed at 24, 48, 72 hours and 8 days following transfusion. Urine volumes were recorded, and urea and creatinine levels were measured and clearances calculated.

Cardiac ouput was measured by the thermal dilution method[10] using a cardiac output computer (Instrumentation Laboratories, Lexington, MA). The mean of triplicate measurements of body weight was used to calculate the cardiac index (CI). Total peripheral resistance was calculated from the difference in pressure divided by the flow.

Blood gas and pH measurements were made with standard electrodes and were corrected for body temperature.[10] Hemoglobin and percent saturation were measured by spectrophotometry (IL2, CO-Oximeter). Oxygen content was calculated from the oxygen tension and saturation. Oxygen consumption ($V_{O2}$) was calculated from the arterial and mixed venous oxygen content and the CI using the Fick equation, and CO2 production was calculated from arterial and venous CO2 content and the CI. The Berggren formula was used to calculate the physiologic shunt $Q_s/Q_t$ during 50% oxygen

breathing. The physiologic dead space fraction $V_D/V_t$ was calculated from arterial and mixed expired CO2 tensions.

The hemoglobin levels in the post infusion plasma samples were measured by the cyanmethemoglobin method modified by decreasing the sample dilution (Boehringer Mannheim Diagnostics, Houston, TX). When plasma hemoglobin concentrations were below 500 mg/dl, the measurements were repeated using a dual beam spectrophotometric method[9]. Methemoglobin measurements were made using the Co-Oximeter (Instrumentation Laboratories, Lexington, MA, Model 282).

Factor VIII and fibrinogen levels were determined using the Coag-A-Mate automated clotting instrument (Organon Teknika, Morris Plains, NJ). Fibrin degradation products were estimated using a Staph Clumping Test (Sigma Chemical, St Louis, MI).

The clearance of the SFH was calculated from the total infused hemoglobin, the measured plasma hemoglobin values following transfusion, and the plasma volume of the recipient dog estimated from weight and previous 51CR blood volume measurements. Changes in the dog's plasma volume due to fluid infused during the study were estimated from changes in the hematocrit values.

## RESULTS

The mean 1 hour intravascular recovery of human DBBF SFH was 51% of the total infused and the halftime of this component was approximately 30 hours. The intrasvascular retention of the total infused DBBF SFH at 24 hours measured in one dog was 38% (Figure 1, Table 1a.). There was no detectable hemoglobin excreted in the urine during the 24 hours following the infusion of the DBBF SFH (Table 1d).

There was 30% methhemoglobin present in the plasma during the first 30 minutes following the infusion of the DBBF SFH, which reflected the 25% present in the infused SFH (Figure 4, Table 1a).

The initial 1 hour intravascular recovery of unmodified BSFH was 39-62% of the total infused and the halftime of this component was 4 to 6 hours. The intravascular clearance of modified BSFH was similar to the unmodified BSFH, with a 1 hour recovery of 29-56%, and a halftime of 4 to 6 hours for this component (Figures 2, and 3, Tables 2a and 3a). The intravascular retention of unmodified and modified BSFH over the ensuing 24 hours was much lower than that of the DBBF.

The circulating methhemoglobin was 2-3% following the infusion of unmodified BSFH, and 2-4% following modified BSFH (Figures 5 and 6, Tables 2a and 3a).

In contrast to the DBBF experiments, excretion of the SFH began within 30 minutes after the infusion of either

unmodified or modified BSFH (Tables 2d and 3d). Hemoglobin was present in the urine 24 hours after the infusion of modified BSFH, but none was detectable 24 hours after the infusion of unmodified BSFH.

Serum BUN and creatinine levels decreased immediately following the infusion of DBBF SFH and unmodified and modified BSFH, reflecting hemodilution in the dogs as shown by the hematocrit values (Figures 7 - 15, Tables 1b,2b and 3b). The serum BUN and creatinine levels increased again at 24 hours and 48 hours after infusion. The increases in BUN ranged from 25 to 50% higher than the pre-transfusion levels. The increased levels were explained by separate in-vitro experiments which showed that a 1000-2000 mg/dl concentration of serum hemoglobin increased the measured BUN level by up to 41%.

Reduced concentrations of both BUN and creatinine were noted in the urine during the first 6 hours following the infusions of DBBF SFH and BSFH (Tables 1d, 2d and 3d). Renal function measured using BUN and creatinine clearance were variable, with no apparent correlation to the transfusions.

The arterial-venous differences in oxygen content, oxygen extraction ratios, and oxygen consumption were well maintained following infusion of DBBF SFH and unmodified and modified BSFH. Hemodynamically, all the dogs except one responded to SFH infusion with increases in total peripheral resistance and mean arterial pressure, and reductions in cardiac index and heart rate (Tables 1e, 1f, 1g, 2e, 2f, 2g,

3e, 3f and 3g). The exception was a dog infused with unmodified SFH, and in this dog, the mean arterial pressure dropped by 60% to 81 mmHg at 6 hours following infusion.

Decreases in plasma fibrinogen levels and factor VIII activity were observed after each of the SFH infusions, with subseguent increases to levels exceeding those at baseline. Fibrin degradation products increased slightly at 24 hours (Tables 1c, 2c and 3c).

## DISCUSSION

The initial plasma hemoglobin levels in dogs infused with human DBBF SFH solution and unmodified and modified BSFH solutions ranged from 900-2700 mg/dl.

The initial intravascular recovery of the three stroma-free hemoglobin solutions during the first hour following infusion were similar and range from 30-61%. During the subsequent 6 hours, the circulating levels of the DBBF SFH remained stable at approximately 50%, but the unmodified and modified BSFH were cleared faster with 16 to 24% circulating at 6 hours. Twenty-four hours following infusion, 38% of the DBBF SFH remained circulating, and during the 24 to 72 hour period following infusion, more of the DBBF SFH remained circulating than either the unmodified or modified BSFH.

There was no detectable hemoglobinurina following the infusion of DBBF crosslinked SFH, however, crosslinking of the modified BSFH did not prevent rapid urinary excretion. Hemoglobinuria was present within 30 minutes following infusion of both the unmodified and modified BSFH.

The 25% methemoglobin present in the infused DBBF SFH was reflected in a high post infusion methemoglobin level which was cleared along with the oxyhemoglobin.

The increased total peripheral resistance and mean arterial pressure that occured following the infusion of the DBBF SFH and BSFH indicated the presence of vasoconstrictor activity as described by other investigators[1-6]. The

infusion of the hemoglobin solutions did not produce any renal toxicity that could be detected from the BUN and creatinine levels in plasma and urine, and assays of the coagulation system determined that disseminated intravascular coagulation was not present.

The experiments in the dog have provided information concerning the relative retention and excretion of modified and unmodified hemoglobin solutions, their vasoconstrictor properties, and their effects on the coagulation system and the kidneys.

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FIGURE 1

**The intravascular retention of human DBBF stroma-free hemoglobin.**

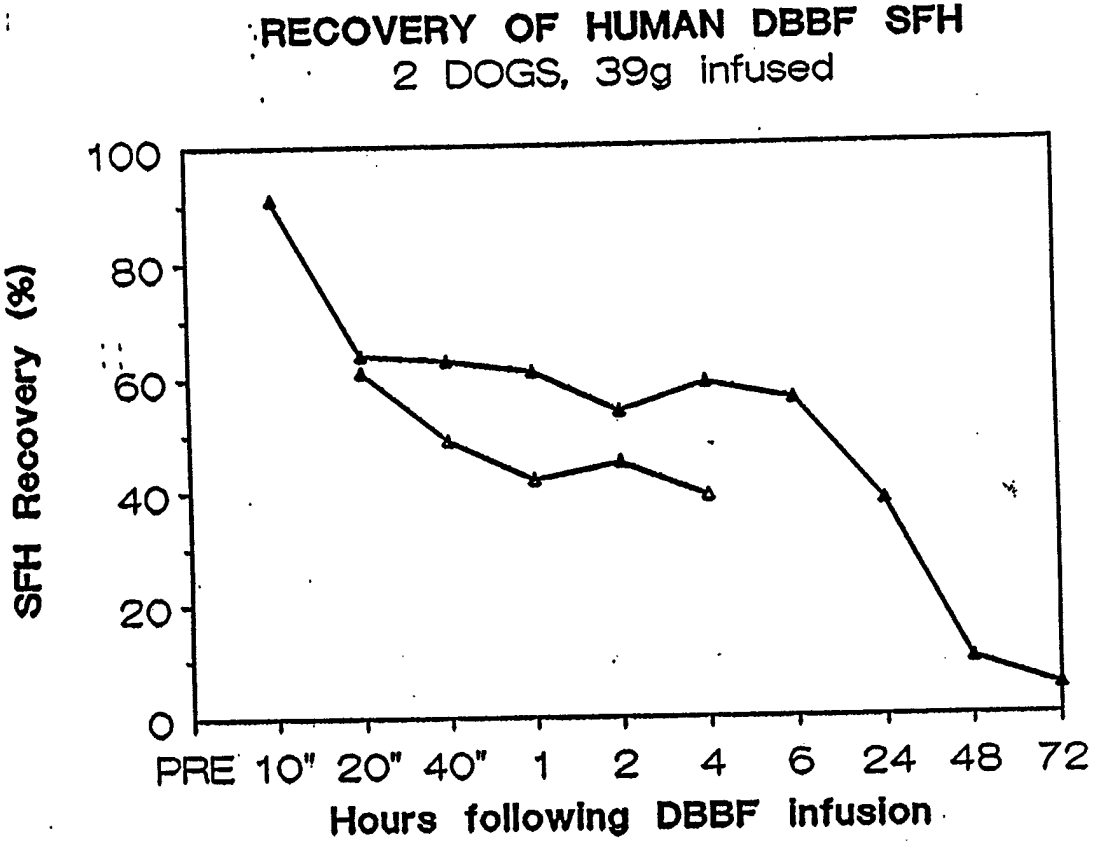
RECOVERY OF HUMAN DBBF SFH
2 DOGS, 39g infused
SFH Recovery (%)
100
80
60
40
20
0
PRE 10" 20" 40" 1 2 4 6 24 48 72
Hours following DBBF infusion

FIGURE 1

FIGURE 2

The intravascular retention of unmodified bovine stroma-free hemoglobin.

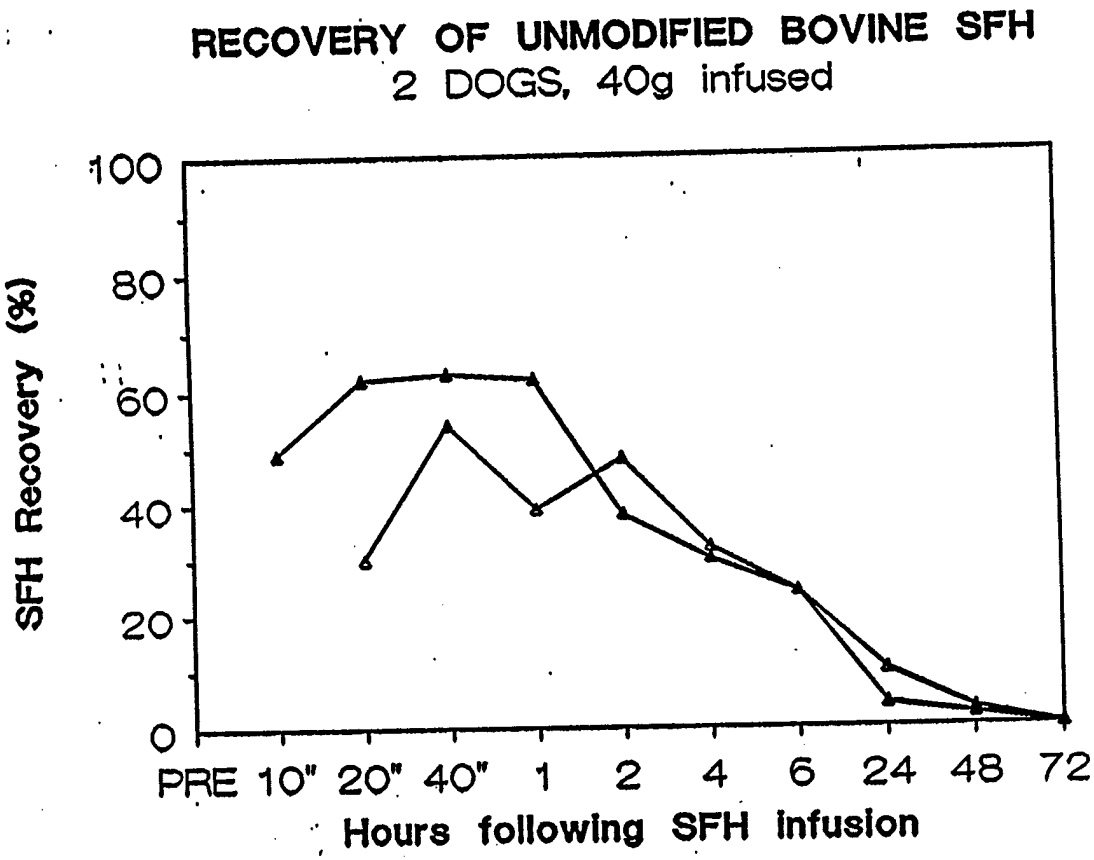
RECOVERY OF UNMODIFIED BOVINE SFH
2 DOGS, 40g infused
SFH Recovery (%)
100
80
60
40
20
0
PRE 10" 20" 40" 1 2 4 6 24 48 72
Hours following SFH infusion

FIGURE 2

FIGURE 3

The intravascular retention of modified bovine stroma-free hemoglobin.

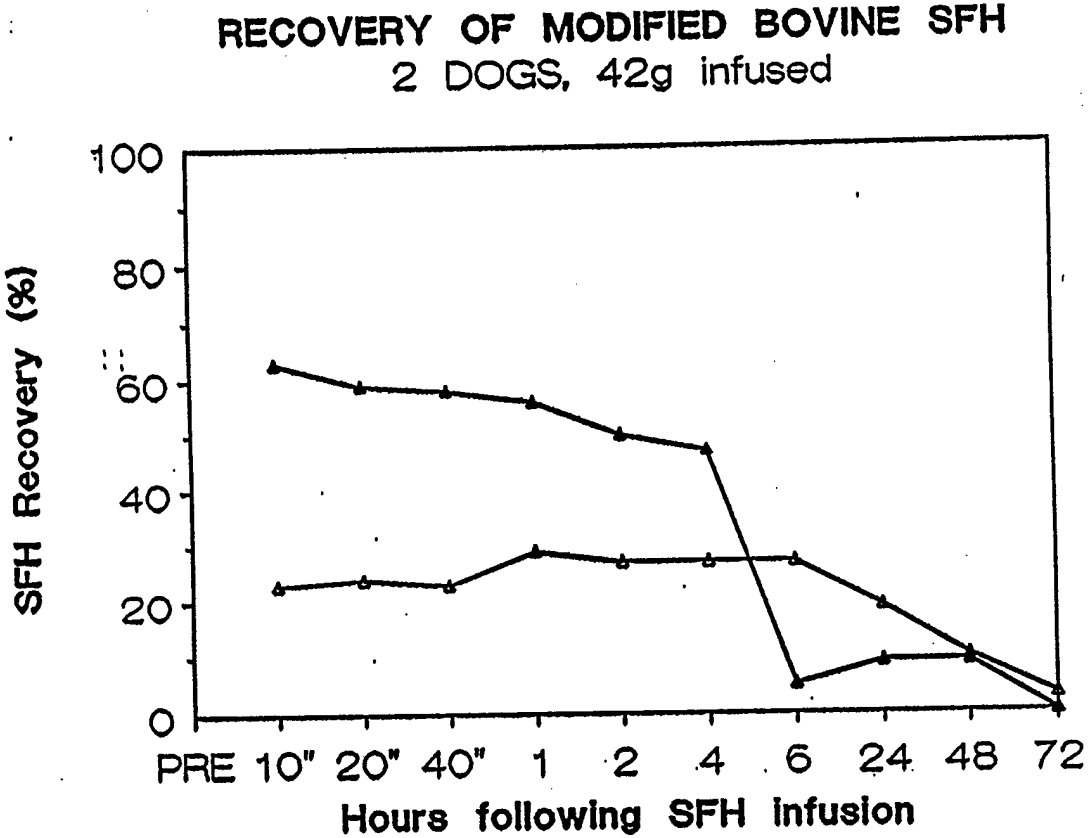
RECOVERY OF MODIFIED BOVINE SFH
2 DOGS, 42g infused
SFH Recovery (%)
100
80
60
40
20
0
PRE 10" 20" 40" 1 2 4 6 24 48 72
Hours following SFH infusion

FIGURE 3

FIGURE 4

Intravascular methemoglobin levels following infusion of human DBBF stroma-free hemoglobin.

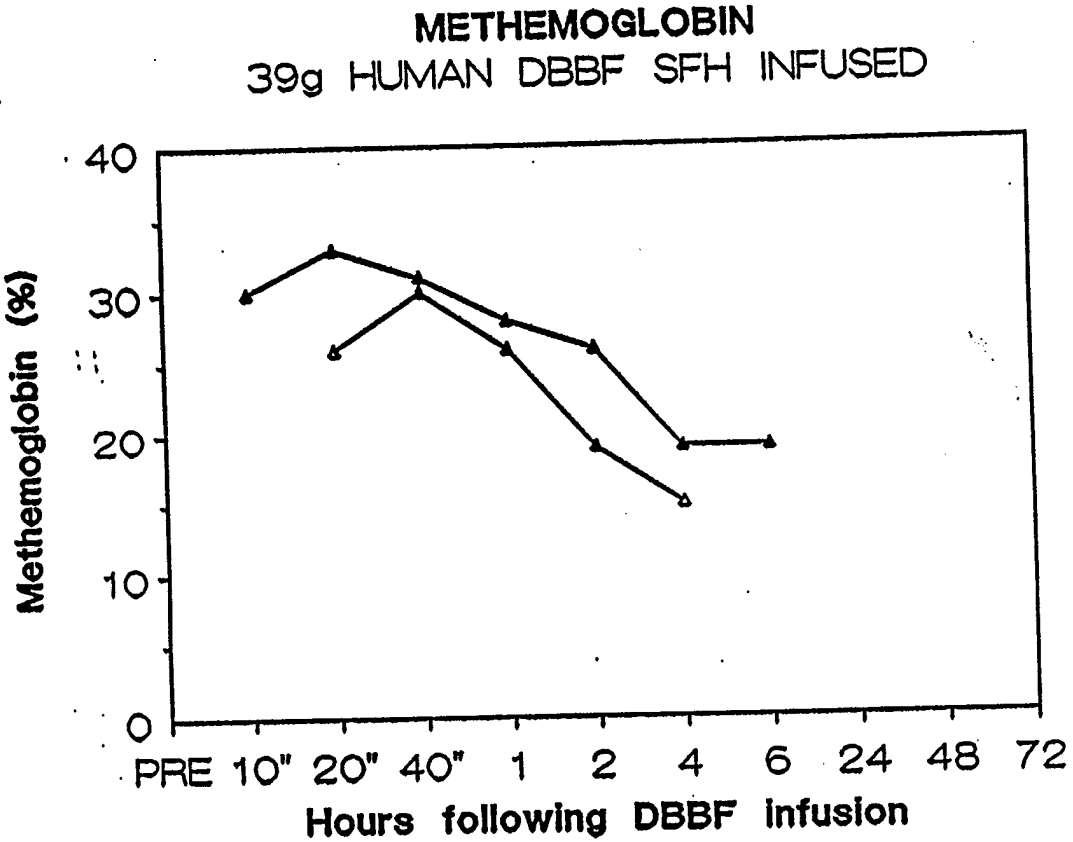
METHEMOGLOBIN
39g HUMAN DBBF SFH INFUSED
Methemoglobin (%)
40
30
20
10
0
PRE 10" 20" 40" 1 2 4 6 24 48 72
Hours following DBBF infusion

FIGURE 4

FIGURE 5

Intravascular methemoglobin level following infusion of unmodified bovine stroma-free hemoglobin.

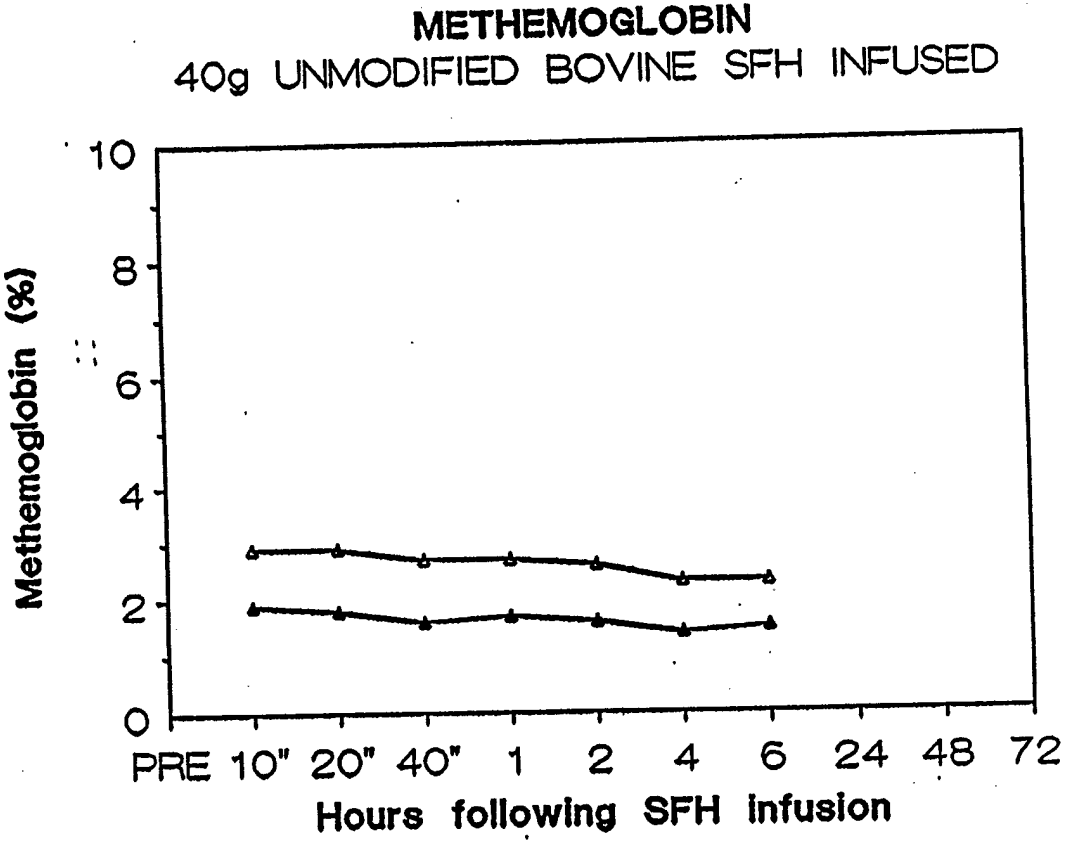
METHEMOGLOBIN
40g UNMODIFIED BOVINE SFH INFUSED
Methemoglobin (%)
10
8
6
4
2
0
PRE 10" 20" 40" 1 2 4 6 24 48 72
Hours following SFH infusion

FIGURE 5

FIGURE 6

Intravascular methemoglobin levels following infusion of modified bovine stroma-free hemoglobin.

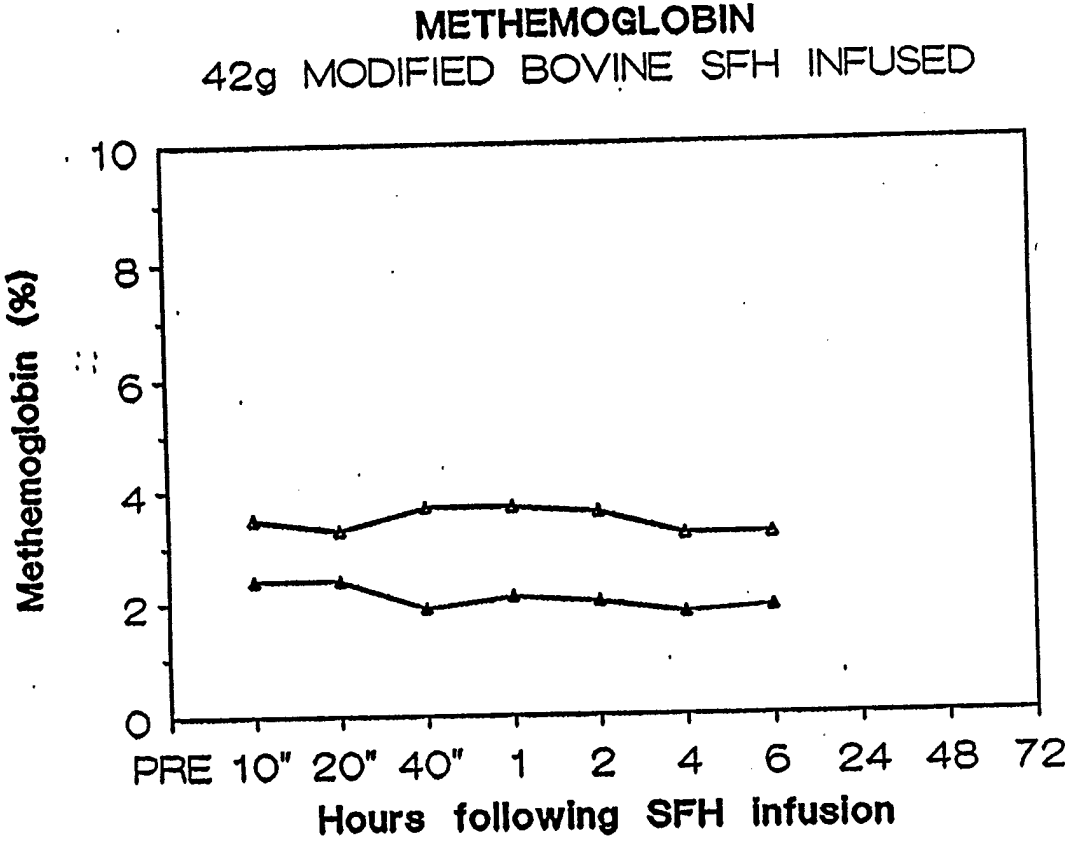
METHEMOGLOBIN
42g MODIFIED BOVINE SFH INFUSED
Methemoglobin (%)
10
8
6
4
2
0
PRE 10" 20" 40" 1 2 4 6 24 48 72
Hours following SFH infusion

FIGURE 6

FIGURE 7

Peripheral venous hematocrit following infusion of human DBBF stroma-free hemoglobin.

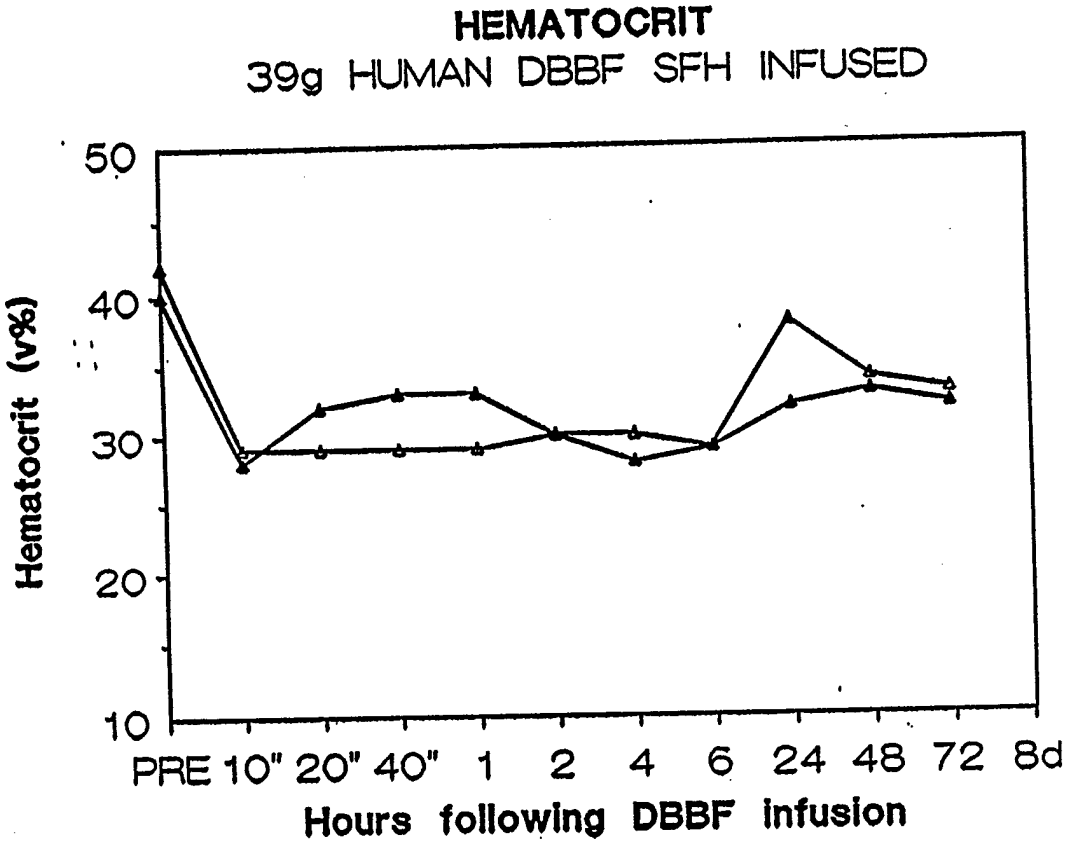
HEMATOCRIT
39g HUMAN DBBF SFH INFUSED
Hematocrit (v%)
50
40
30
20
10
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following DBBF infusion

FIGURE 7

FIGURE 8

Peripheral venous hematocrit following infusion of unmodified bovine stroma-free hemoglobin.

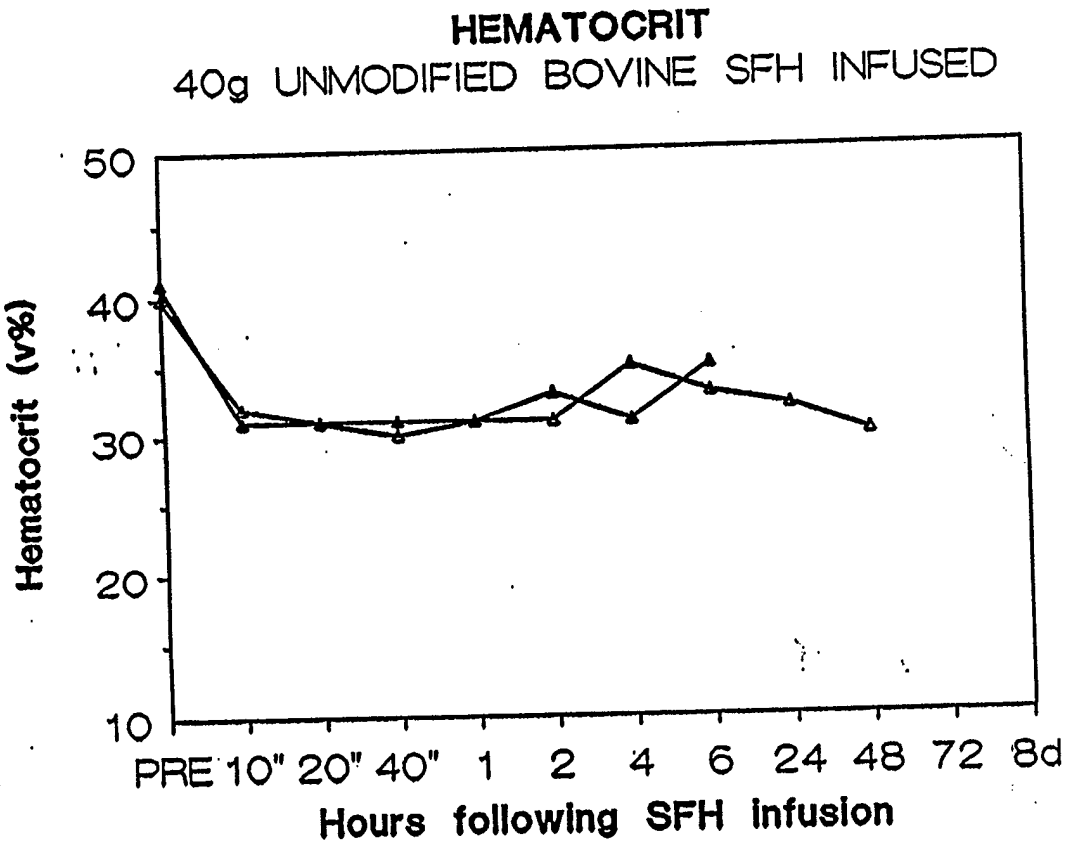
HEMATOCRIT
40g UNMODIFIED BOVINE SFH INFUSED
Hematocrit (v%)
50
40
30
20
10
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following SFH infusion

FIGURE 8

FIGURE 9

Peripheral venous hematocrit following infusion of modified bovine stroma-free hemoglobin.

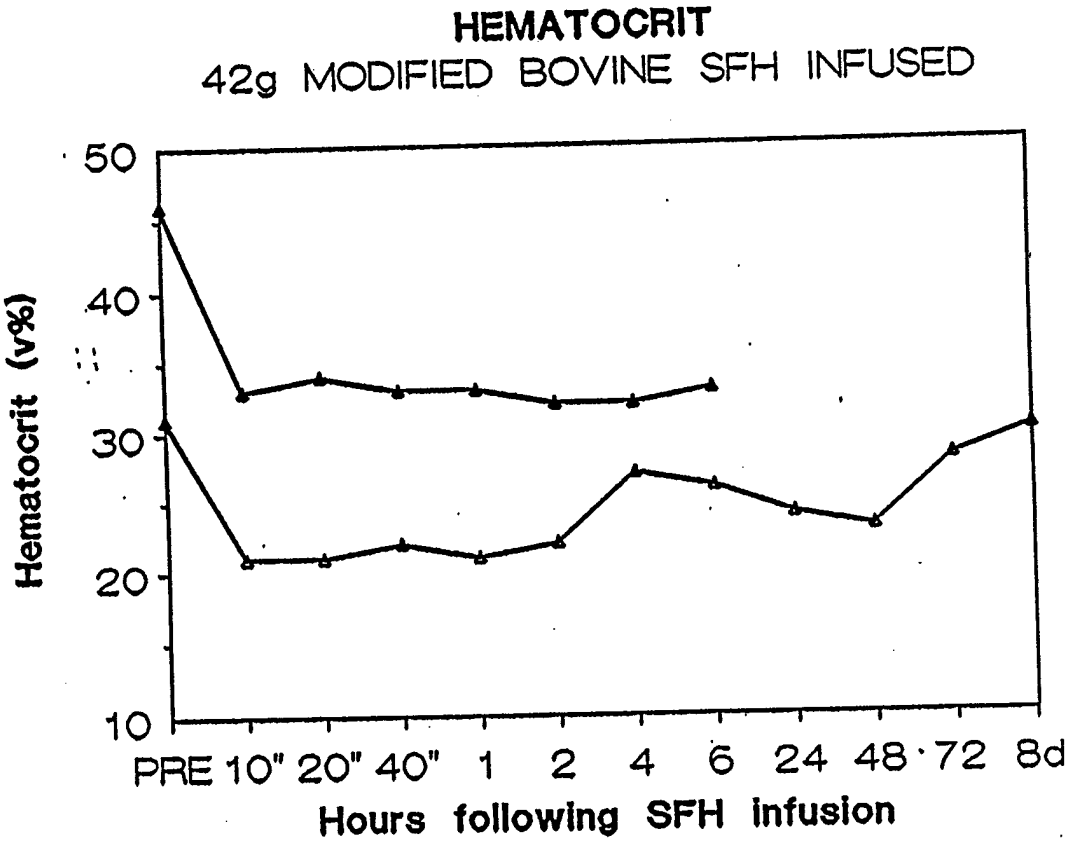
HEMATOCRIT
42g MODIFIED BOVINE SFH INFUSED
Hematocrit (v%)
50
40
30
20
10
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following SFH infusion

FIGURE 9

FIGURE 10

Plasma BUN following infusion of human DBBF stroma-free hemoglobin.

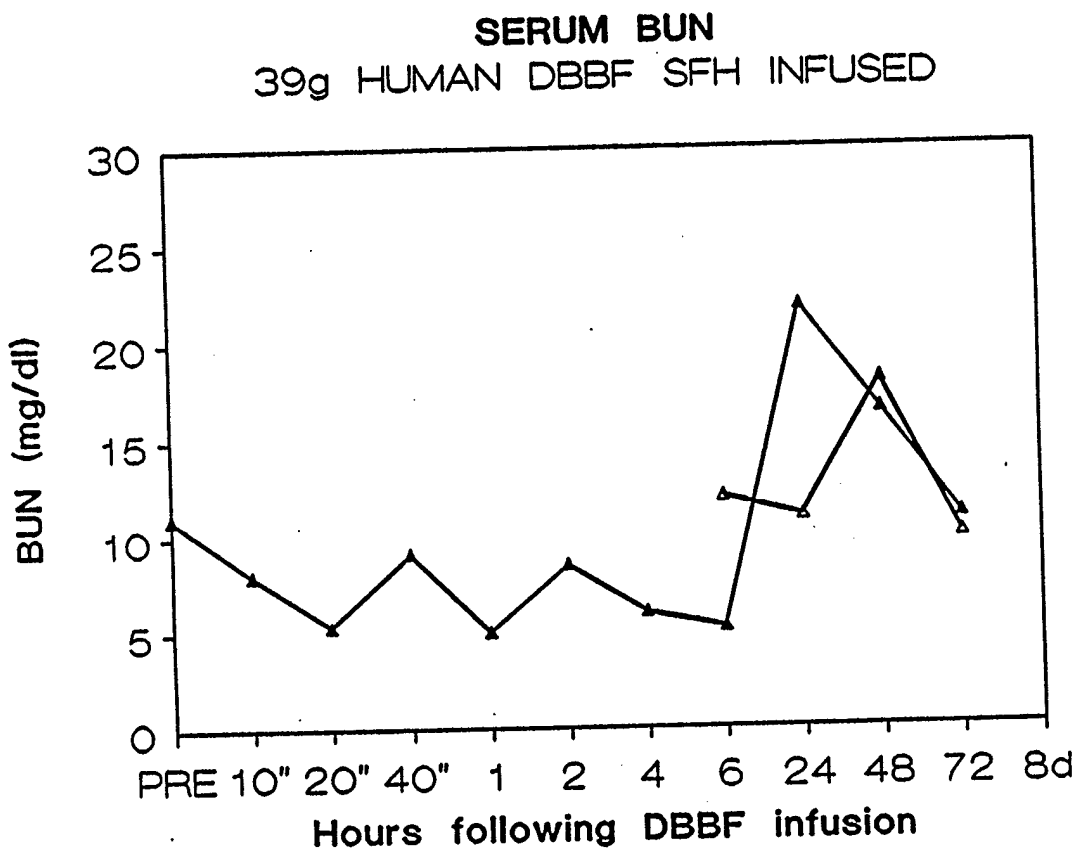
SERUM BUN
39g HUMAN DBBF SFH INFUSED
BUN (mg/dl)
30
25
20
15
10
5
0
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following DBBF infusion

FIGURE 10

FIGURE 11

Plasma BUN following infusion of unmodified bovine stroma-free hemoglobin.

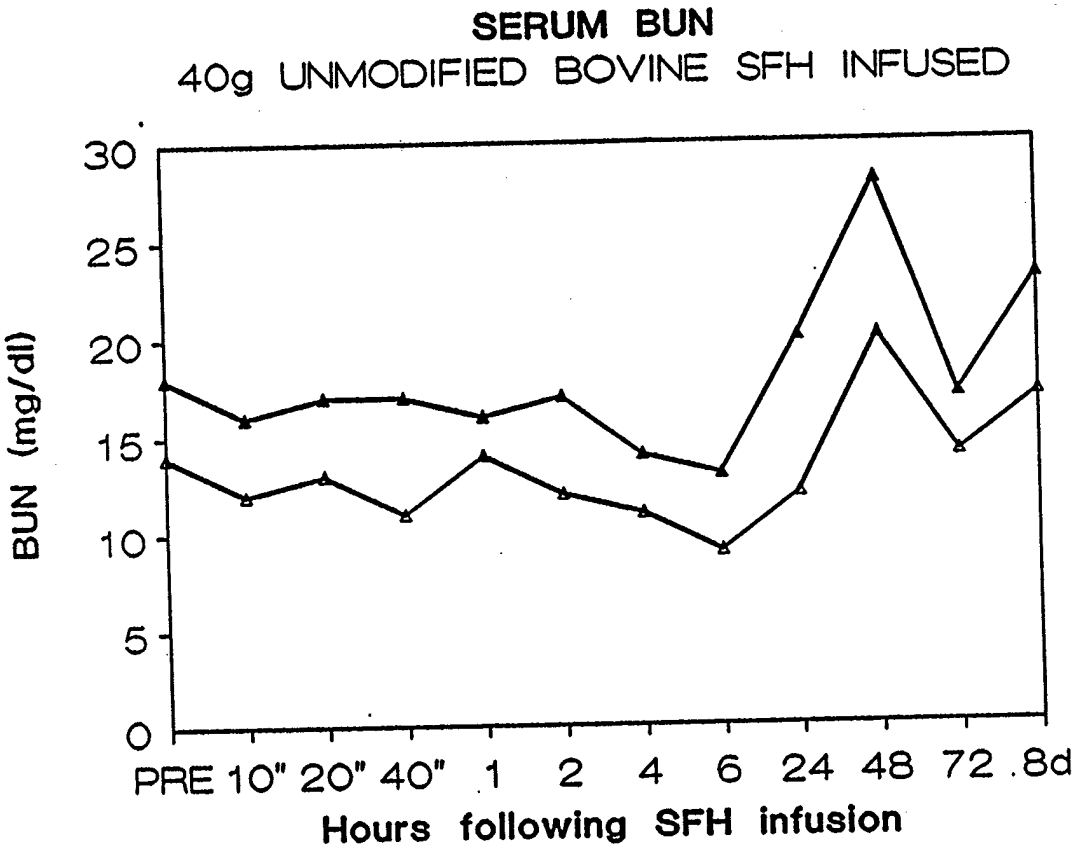
SERUM BUN
40g UNMODIFIED BOVINE SFH INFUSED
BUN (mg/dl)
30
25
20
15
10
5
0
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following SFH infusion

FIGURE 11

FIGURE 12

Plasma BUN following infusion of modified bovine stroma-free hemoglobin.

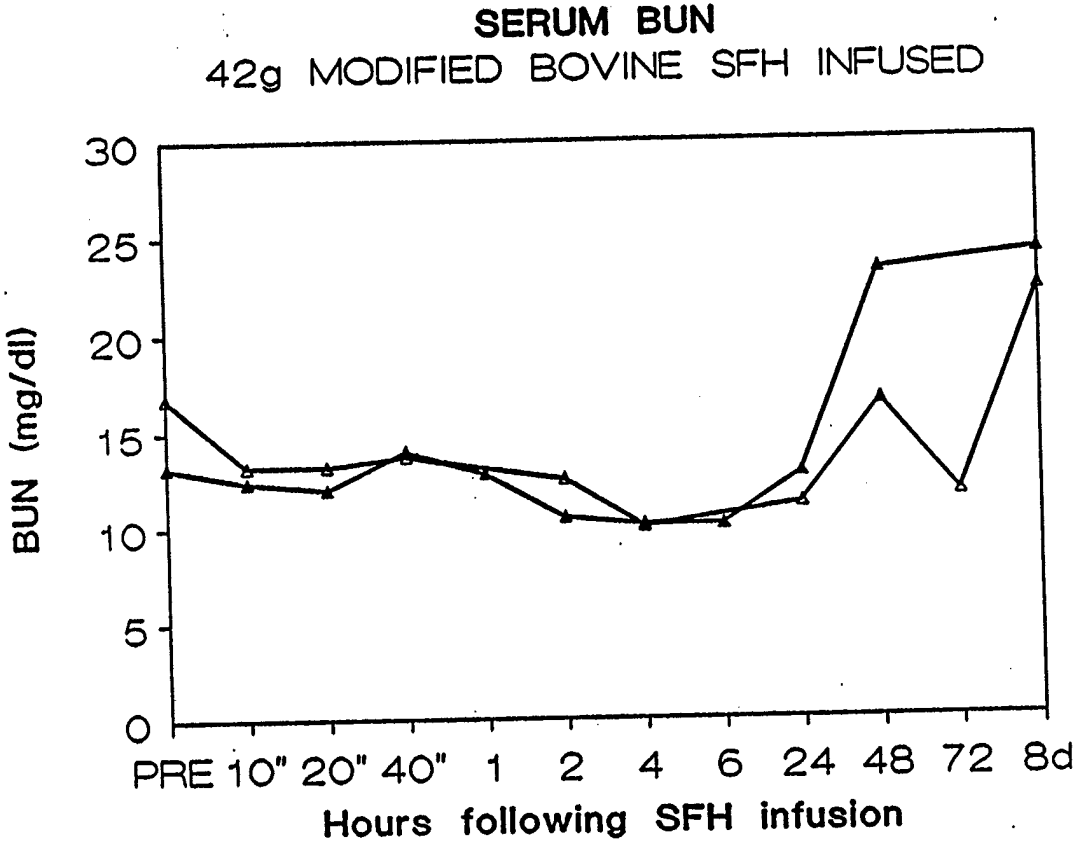
SERUM BUN
42g MODIFIED BOVINE SFH INFUSED
BUN (mg/dl)
30
25
20
15
10
5
0
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following SFH infusion

FIGURE 12

FIGURE 13

Plasma creatinine following infusion of human DBBF stroma-free hemoglobin.

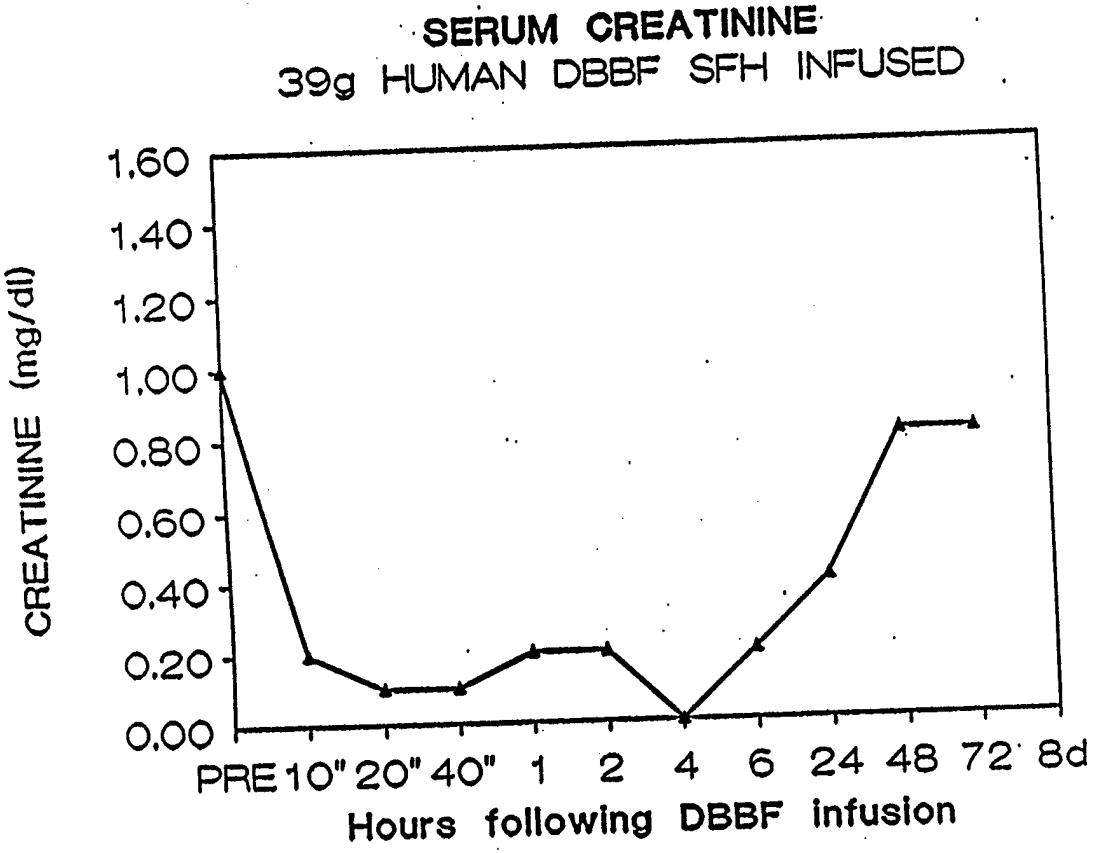
SERUM CREATININE
39g HUMAN DBBF SFH INFUSED
CREATININE (mg/dl)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following DBBF infusion

FIGURE 13

FIGURE 14

Plasma creatinine following infusion of unmodified bovine stroma-free hemoglobin.

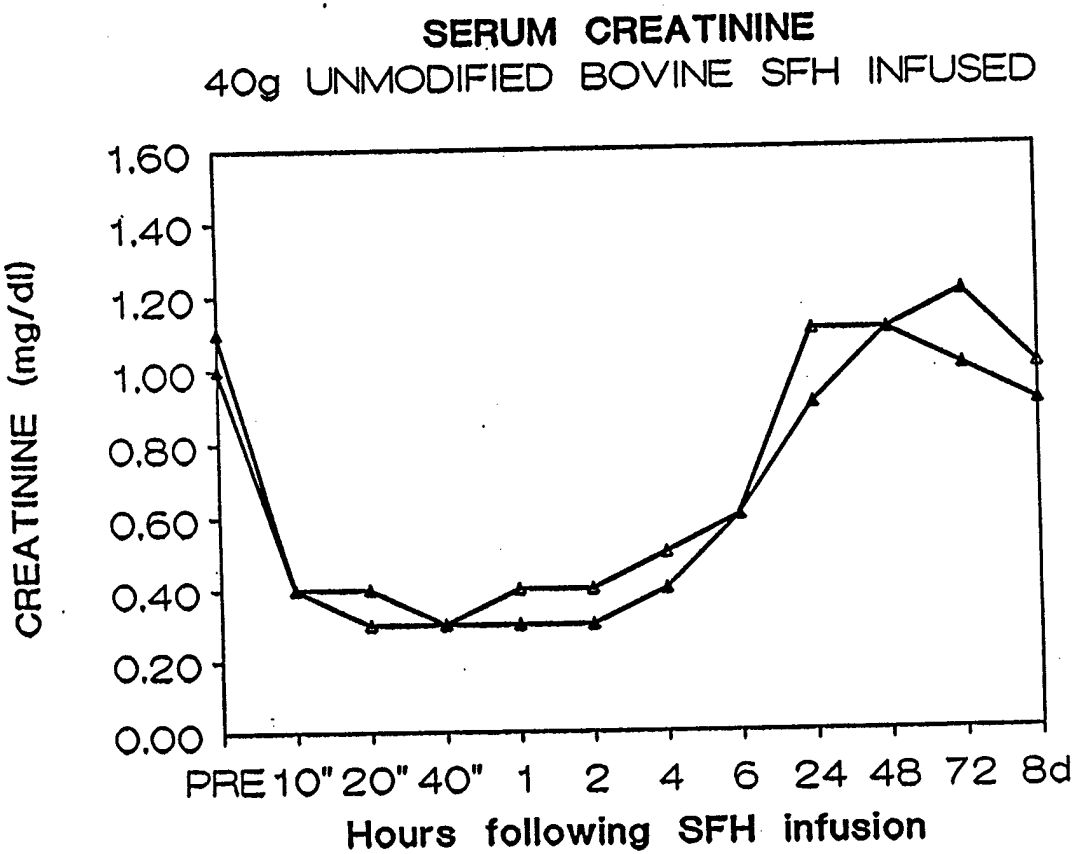
SERUM CREATININE
40g UNMODIFIED BOVINE SFH INFUSED
CREATININE (mg/dl)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following SFH infusion

FIGURE 14

FIGURE 15

**Plasma creatinine following infusion of modified bovine stroma-free hemoglobin.**

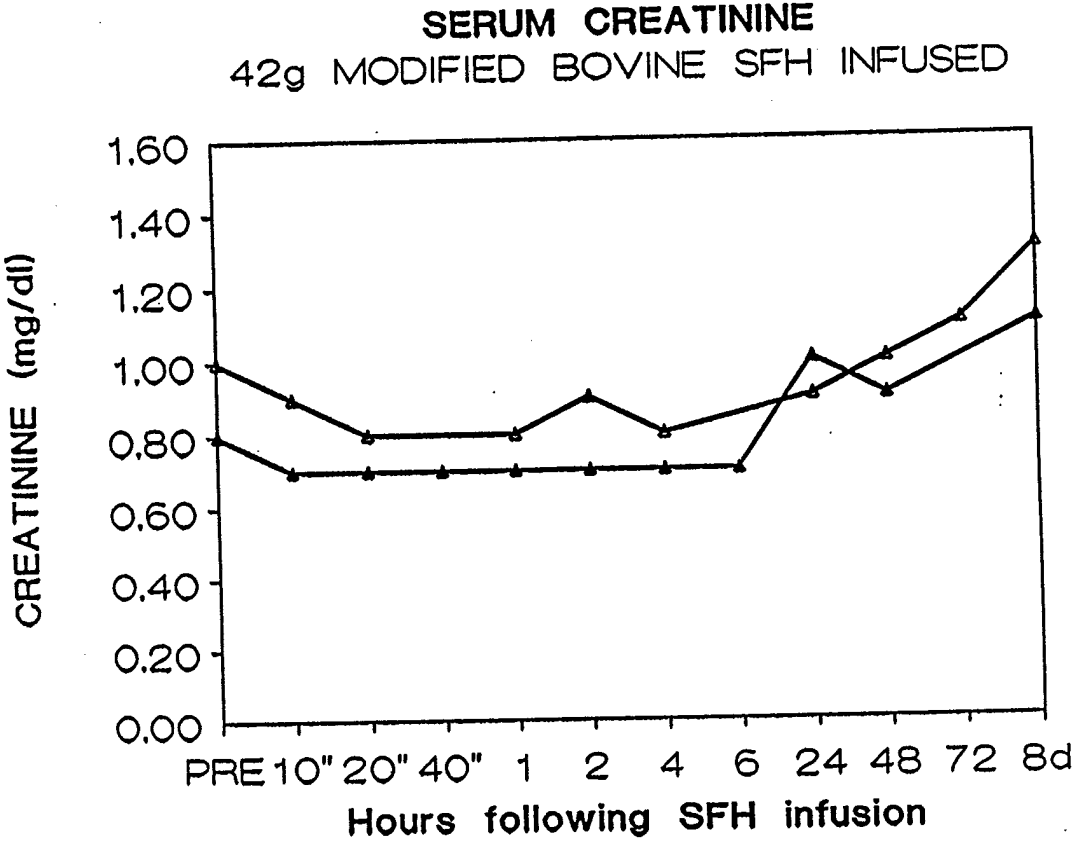
SERUM CREATININE
42g MODIFIED BOVINE SFH INFUSED
CREATININE (mg/dl)
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
PRE 10" 20" 40" 1 2 4 6 24 48 72 8d
Hours following SFH infusion

FIGURE 15

TABLE 1a

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR |
| Plasma hgb (mg/dl) | 1. | 35 | 35 | 2747 | 2324 | 2512 | 2567 | 2068 | 2197 | 2317 | 1748 | 502 | 219 |
| | 2. | 20 | 15 | ---- | 1736 | 1435 | 1267 | 1633 | 1367 | ---- | ---- | --- | --- |
| | Mean: | 28 | 25 | 2747 | 2030 | 1917 | 1867 | 1851 | 1782 | 2317 | 1748 | 502 | 219 |
| SFH recovery (%) | 1. | -- | -- | 90.9 | 64.1 | 63.3 | 61.0 | 54.0 | 59.0 | 56.4 | 37.9 | 10.3 | 5.0 |
| | 2. | -- | -- | ---- | 61.0 | 48.5 | 41.5 | 45.0 | 39.2 | ---- | ---- | ---- | --- |
| | Mean: | -- | -- | 90.9 | 62.6 | 55.9 | 51.3 | 49.5 | 49.1 | 56.4 | 37.9 | 10.3 | 5.0 |
| Plasma MetHb (%)* | 1. | -- | -- | 30 | 33 | 31 | 28 | 26 | 19 | 19 | -- | -- | -- |
| | 2. | -- | -- | -- | 26 | 30 | 26 | 19 | 15 | -- | -- | -- | -- |
| | Mean: | -- | -- | 30 | 30 | 31 | 27 | 23 | 17 | 19 | -- | -- | -- |

*25% Methb infused

Table 1b

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR |
| Hgb (%) | 1. | 16.0 | 14.3 | 13.8 | 13.3 | 14.3 | 14.0 | 13.2 | 12.2 | 13.1 | 13.5 | 13.5 | 11.8 |
| | 2. | 17.6 | 14.8 | 12.5 | 12.2 | 12.4 | 11.9 | 12.0 | 12.1 | 12.3 | 14.5 | 12.5 | 12.1 |
| | Mean: | 16.8 | 14.5 | 13.2 | 12:8 | 13.4 | 13.0 | 12.6 | 12.2 | 12.7 | 14.0 | 13.0 | 12.0 |
| Hct (%) | 1. | 42.0 | 37.3 | 28.3 | 31.5 | 32.5 | 32.8 | 30.0 | 28.0 | 28.5 | 31.5 | 33.0 | 31.8 |
| | 2. | 47.0 | 37.0 | 29.0 | 29.0 | 29.0 | 28.7 | 29.5 | 29.7 | 29.2 | 38.2 | 33.5 | 32.5 |
| | Mean: | 45.0 | 37.1 | 28.7 | 30.5 | 30.8 | 30.8 | 29.8 | 28.9 | 28.9 | 34.9 | 33.3 | 32.2 |
| Serum BUN (mg/dl) | 1. | 11.0 | 11.0 | 8.0 | 5.3 | 9.1 | 5.0 | 8.5 | 6.0 | 5.2 | 21.8 | 16.5 | 11.0 |
| | 2. | ---- | ---- | --- | --- | --- | --- | --- | --- | 12.1 | 10.8 | 18.4 | 10.0 |
| | Mean: | 11.0 | 11.0 | 8.0 | 5.3 | 9.1 | 5.0 | 8.5 | 6.0 | 8.7 | 16.3 | 17.5 | 10.5 |
| Serum creatinine (mg/dl) | 1. | 1.1 | 0.90 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.0 | 0.2 | 0.4 | 0.8 | 0.8 |
| | 2. | .5 | 10.5 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | Mean: | 1.1 | 0.90 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.0 | 0.4 | 0.4 | 0.8 | 0.8 |

Table 1c

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR |
| Fibrinogen (mg/dl) | 1. | 127 | 120 | 49 | 79 | 80 | 82 | 80 | 240 | 85 | 230 | 550 | 440 |
| | 2. | 125 | 110 | 70 | 71 | 74 | 68 | 71 | 74 | 80 | --- | 540 | 530 |
| Mean: | | 126 | 115 | 60 | 75 | 77 | 74 | 76 | 157 | 83 | 230 | 545 | 485 |
| Factor 8 (%) | 1. | 424 | 298 | 242 | 226 | 200 | 214 | 190 | 192 | 186 | 632 | 632 | 544 |
| | 2. | 500 | 304 | 191 | 174 | 162 | 171 | 181 | 167 | 154 | --- | 412 | 472 |
| Mean: | | 462 | 301 | 217 | 200 | 181 | 193 | 186 | 180 | 170 | 632 | 522 | 508 |
| Platelet ($10^3/mm^3$) | 1. | 238 | 235 | 198 | 187 | 157 | 158 | 142 | 117 | 115 | 123 | 145 | 129 |
| | 2. | 142 | 132 | 113 | 120 | 118 | 149 | 139 | 179 | 154 | 221 | 200 | 328 |
| Mean: | | 190 | 184 | 156 | 154 | 138 | 154 | 141 | 148 | 135 | 172 | 173 | 229 |
| WBC ($10^3/mm^3$) | 1. | 7.3 | 7.2 | 8.3 | 7.4 | 9.0 | 9.6 | 8.6 | 7.5 | 10.3 | 17.1 | 12.9 | 11.3 |
| | 2. | 8.4 | 5.3 | 4.5 | 5.9 | 5.9 | 6.5 | 6.9 | 7.1 | 7.0 | 28.7 | 16.9 | 11.7 |
| Mean: | | 7.9 | 6.3 | 6.4 | 6.7 | 7.5 | 8.1 | 7.8 | 7.3 | 8.7 | 22.9 | 14.9 | 11.5 |

Table 1d

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | 10 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR |
| Urine Hb (mg%) | 1. | 33 | 8 | -- | 8 | 0 | -- | 0 | 0 |
| | 2. | 41 | -- | -- | -- | 8 | 16 | 0 | 33 |
| Urine Volume (ml) | 1. | -- | 450 | 480 | 200 | -- | 275 | 450 | -- |
| | 2. | 20 | -- | 40 | -- | 32 | 450 | 80 | 150 |
| Urine Total Hb (mg) | 1. | -- | 36 | 38 | 0 | -- | 0 | 0 | -- |
| | 2. | -- | -- | -- | -- | 3 | 72 | 0 | 50 |
| Urine BUN (mg/dl) | 1. | 1371 | 23 | 60 | 59 | -- | 214 | 331 | -- |
| | 2. | -- | -- | -- | -- | 1159 | 452 | 245 | -- |
| Urine Creatinine (mg/dl) | 1. | 202 | 41 | 7 | 6 | -- | 26 | 52 | -- |
| | 2. | -- | -- | -- | -- | 116 | 52 | 25 | -- |
| Creatinine Clearance (ml/min) | 1. | | | | | | | | |
| | 2. | | | | | 165 (avg of 6 hrs) | | | |
| BUN Clearance (ml/min) | 1. | -- | 1294 | 79 | 107 | -- | 177 | 248 | -- |
| | 2. | -- | -- | -- | -- | -- | 60 | -- | -- |

Table 1e

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| MAP | 1. | 146 | 154 | 178 | 175 | 179 | 176 | 162 | 152 | 154 |
| (mmHg) | 2. | 110 | 125 | 155 | 158 | 164 | 166 | 176 | 166 | 159 |
| Mean: | | 128 | 140 | 167 | 167 | 172 | 171 | 169 | 159 | 156 |
| apO2 | 1. | 99 | 103 | 105 | 105 | 104 | 110 | 110 | 110 | 110 |
| (mmHg) | 2. | 103 | 92 | --- | 91 | 96 | 113 | 111 | 103 | 90 |
| Mean: | | 101 | 98 | 105 | 98 | 100 | 112 | 111 | 107 | 100 |
| apCO2 | 1. | 34 | 33 | 28 | 28 | 27 | 26 | 25 | 24 | 24 |
| (mmHg) | 2. | 37 | 36 | 36 | 40 | 39 | 37 | 30 | 24 | 26 |
| Mean: | | 36 | 35 | 32 | 34 | 33 | 32 | 28 | 24 | 25 |
| CVP | 1. | 1 | 1 | 1 | 2 | 1 | 0 | 1 | 0 | 1 |
| (mmHg) | 2. | 7 | 15 | 12 | 12 | 10 | 8 | 9 | 9 | 5 |
| Mean: | | 4 | 8 | 7 | 7 | 6 | 4 | 5 | 5 | 3 |
| MPAP | 1. | 4 | 4 | 3 | 4 | 4 | 2 | 1 | 1 | 1 |
| (mmHg) | 2. | 17 | 22 | 25 | 11 | 20 | 20 | 20 | 25 | 16 |
| Mean: | | 11 | 14 | 14 | 7 | 11 | 11 | 11 | 13 | 9 |

Table 1f

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| A-V Difference (ml/dl) | 1. | 3.2 | 2.0 | 2.7 | 3.1 | 4.3 | 5.1 | 6.2 | 5.9 | 5.7 |
| | 2. | 3.0 | 2.7 | 2.2 | 2.5 | 3.0 | 3.4 | 4.6 | 5.7 | 5.8 |
| Mean: | | 3.1 | 2.4 | 2.5 | 2.8 | 3.7 | 4.3 | 5.4 | 5.8 | 5.8 |
| O2 extra-tion (%) | 1. | 15.2 | 14.3 | 15.4 | 17.1 | 22.4 | 25.8 | 33.3 | 33.6 | 29.9 |
| | 2. | 12.2 | 12.7 | 12.7 | 14.4 | 17.2 | 19.9 | 27.1 | 33.2 | 33.2 |
| Mean: | | 13.7 | 13.5 | 14.1 | 15.8 | 19.8 | 22.9 | 30.2 | 33.4 | 31.6 |
| CI L/min/kg | 1. | 0.15 | 0.15 | 0.13 | 0.14 | 0.11 | 0.08 | 0.08 | 0.08 | 0.09 |
| | 2. | 0.29 | 0.26 | 0.27 | 0.25 | 0.19 | 0.16 | 0.12 | 0.10 | 0.09 |
| Mean: | | 0.22 | 0.21 | 0.20 | 0.20 | 0.15 | 0.12 | 0.10 | 0.09 | 0.09 |
| TPR (units) | 1. | 2.6 | 1.8 | 3.6 | 3.5 | 4.5 | 6.4 | 5.9 | 5.2 | 4.8 |
| | 2. | 1.1 | 1.3 | 1.6 | 1.8 | 2.5 | 3.1 | 4.2 | 4.8 | 5.5 |
| Mean: | | 1.9 | 1.6 | 2.6 | 2.7 | 3.5 | 4.7 | 5.1 | 5.0 | 5.2 |
| HR beats/min | 1. | 243 | 198 | 141 | 137 | 104 | 87 | 78 | 70 | 72 |
| | 2. | 249 | 238 | 188 | 187 | 169 | 151 | 224 | 154 | 148 |
| Mean: | | 246 | 219 | 165 | 162 | 137 | 119 | 150 | 112 | 110 |

Table 1g

INFUSION OF 39G HUMAN STROMA-FREE HEMOGLOBIN (DBBF) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| O2 Cons (ml/min/kg) | 1. | 3.9 | 3.3 | 3.1 | 3.5 | 3.8 | 3.2 | 3.8 | 3.8 | 4.1 |
| | 2. | 7.3 | 5.7 | 4.9 | 5.2 | 4.9 | 4.4 | 4.7 | 4.8 | 4.2 |
| Mean: | | 5.6 | 4.5 | 4.0 | 4.4 | 4.4 | 3.8 | 4.3 | 4.3 | 4.1 |
| CO2 Prod (ml/min/kg) | 1. | 3.5 | 2.9 | 3.2 | -0.7 | 2.6 | 2.6 | 2.2 | 2.6 | 2.8 |
| | 2. | 4.6 | 3.0 | -0.3 | 0.0 | 3.3 | 3.4 | 3.4 | 2.7 | 2.2 |
| Mean: | | 4.1 | 3.0 | 1.5 | -0.4 | 3.0 | 3.0 | 2.8 | 2.7 | 2.5 |
| Deadspace (%) | 1. | 41 | 43 | 28 | 112 | 44 | 41 | 46 | 38 | 35 |
| | 2. | 45 | 53 | 101 | --- | 41 | 42 | 54 | 57 | 61 |
| Mean: | | 43 | 48 | 65 | 112 | 43 | 42 | 50 | 48 | 48 |
| Shunt (%) | 1. | 21 | 21 | 36 | 33 | 26 | 22 | 16 | 16 | 17 |
| | 2. | 22 | 25 | 45 | 35 | 30 | 23 | 16 | 13 | 14 |
| Mean: | | 22 | 23 | 41 | 34 | 28 | 23 | 16 | 15 | 16 |

Table 2a

INFUSION OF 40G OF NON-MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (OXYPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR | 8 DAY |
| Plasma Hb (mg/dl) | 1. | 13 | 102 | 1618 | 2044 | 2075 | 2033 | 1248 | 981 | 803 | 136 | 56 | 7 | -- |
| | 2. | 17 | -- | -- | 897 | 1616 | 1169 | 1438 | 968 | 716 | 312 | 79 | 17 | 5 |
| Mean: | | 15 | | | 1470 | 1850 | 1597 | 1343 | 975 | 760 | 224 | 68 | 12 | 5 |
| SFH recovery (%) | 1. | -- | -- | 49 | 62 | 63 | 62 | 38 | 30 | 24 | 4 | 2 | -- | -- |
| | 2. | -- | -- | -- | 30 | 54 | 39 | 48 | 32 | 24 | 10 | 3 | -- | -- |
| Mean: | | -- | -- | 49 | 46 | 59 | 41 | 43 | 31 | 24 | 7 | 3 | -- | -- |
| MetHb (%) | 1. | 1.5 | 1.7 | 1.9 | 1.8 | 1.6 | 1.7 | 1.6 | 1.4 | 1.5 | -- | -- | -- | -- |
| | 2. | 2.5 | 2.5 | 2.9 | 2.9 | 2.7 | 2.7 | 2.6 | 2.3 | 2.3 | -- | -- | -- | -- |
| Mean: | | 2.0 | 2.1 | 2.4 | 2.4 | 2.2 | 2.2 | 2.1 | 1.9 | 1.9 | | | | |

Table 2b

INFUSION OF 40G OF NON-MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (OXYPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR | 8 DAY |
| Hb (g%) | 1. | 14.0 | 13.7 | 12.2 | 12.4 | 12.3 | 12.0 | 12.5 | 12.4 | 12.7 | | | | |
| | 2. | 14.6 | -- | 13.0 | 13.0 | 13.0 | 12.0 | 12.0 | 13.0 | 12.0 | 12.0 | 11.0 | -- | -- |
| Mean: | | 14.3 | 13.7 | 12.6 | 12.7 | 12.7 | 12.0 | 12.3 | 12.7 | 12.4 | 12.0 | 11.0 | -- | -- |
| Hct (%) | 1. | 41 | 38 | 31 | 31 | 31 | 31 | 33 | 31 | 35 | -- | -- | -- | -- |
| | 2. | 40 | -- | 32 | 31 | 30 | 31 | 31 | 35 | 33 | 32 | 30 | -- | -- |
| Mean: | | 41 | 38 | 32 | 31 | 31 | 31 | 32 | 33 | 34 | 32 | 30 | -- | -- |
| Serum BUN (mg/dl) | 1. | 19 | 18 | 16 | 17 | 17 | 16 | 17 | 14 | 13 | 20 | 28 | 17 | 23 |
| | 2. | 14 | -- | 12 | 13 | 11 | 14 | 12 | 11 | 9 | 12 | 20 | 14 | 17 |
| Mean: | | 17 | 18 | 14 | 15 | 14 | 15 | 15 | 13 | 11 | 16 | 24 | 16 | 20 |
| Serum Creatinine (mg/dl) | 1. | 1.1 | 0.9 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.6 | 0.9 | 1.1 | 1.0 | 0.9 |
| | 2. | 1.1 | -- | 0.4 | 0.3 | 0.3 | 0.4 | 0.4 | 0.5 | 0.6 | 1.1 | 1.1 | 1.2 | 1.0 |
| Mean: | | 1.1 | 0.9 | 0.4 | 0.4 | 0.3 | 0.4 | 0.4 | 0.5 | 0.6 | 1.0 | 1.1 | 1.1 | 1.0 |

Table 2c

INFUSION OF 40G OF UNMODIFIED BOVINE STROMA-FREE HEMOGLOBIN INTO DOGS WITH SPLEENS

TIME AFTER SFH INFUSION

| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fibrinogen (mg/dl) | 1. | 130 | 100 | 70 | 74 | 75 | 76 | 85 | 83 | 110 | 430 | 450 | 300 |
| | 2. | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Factor VIII (%) | 1. | 568 | 484 | 256 | 274 | 242 | 256 | 218 | 249 | 287 | 672 | 896 | 588 |
| | 2. | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| FDP (ug/ml) | 1. | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | 2. | 26 | -- | 6 | 6 | 26 | 6 | 26 | 13 | 26 | 103 | 102 | 102 |
| Platelet ($10^3/mm^3$) | 1. | 229 | -- | 88 | 89 | 83 | 83 | 207 | 329 | 210 | 196 | 199 | -- |
| | 2. | 191 | 151 | 54 | 64 | 103 | 110 | 139 | 136 | 121 | -- | -- | -- |
| Mean: | | 210 | 151 | 71 | 77 | 93 | 97 | 173 | 233 | 166 | 196 | 199 | -- |
| WBC ($10^3/mm^3$) | 1. | 11.3 | -- | 13.1 | 13.6 | 14.5 | 14.2 | 12.5 | 12.0 | 11.7 | 21.4 | 23.5 | -- |
| | 2. | 4.5 | 5.3 | 9.0 | 8.4 | 9.1 | 9.1 | 9.5 | 10.9 | 14.0 | -- | -- | -- |
| Mean: | | 7.9 | 5.3 | 11.1 | 11.0 | 11.8 | 11.7 | 11.0 | 11.5 | 12.9 | 21.4 | 23.5 | -- |

Table 2d

INFUSION OF 40G OF NON-MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (OXYPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR | 8 DAY |
| Urine Hb (mg%) | 1. | -- | 0 | -- | -- | -- | 335 | 446 | 567 | 223 | 2 | 0 | 0 | |
| | 2. | 33 | 65 | -- | 569 | 537 | 700 | 732 | 553 | 455 | -- | 0 | -- | |
| Urine Vol (ml) | 1. | -- | -- | -- | -- | -- | 55 | 360 | 220 | 600 | -- | -- | -- | |
| | 2. | -- | -- | 10 | 10 | 10 | 100 | 600 | 500 | 800 | -- | 250 | -- | |
| Urine Total Hb (mg) | 1. | -- | -- | -- | -- | -- | 184 | 1606 | 1247 | 1338 | -- | -- | -- | |
| | 2. | -- | -- | -- | 57 | 54 | 700 | 4392 | 2765 | 3640 | -- | -- | -- | |
| Urine BUN (mg/dl) | 1. | 895 | -- | -- | -- | -- | 305 | 274 | 252 | 333 | 249 | 922 | 771 | 901 |
| | 2. | 2798 | 2398 | 3572 | 1659 | 933 | 865 | 681 | 148 | 124 | -- | 1612 | -- | -- |
| Urine Creatinine (mg/dl) | 1. | 92 | -- | -- | -- | -- | 25 | 22 | 18 | 28 | 14 | 98 | 81 | 98 |
| | 2. | 267 | 217 | 308 | 186 | 106 | 79 | 65 | 15 | 13 | -- | 163 | -- | -- |
| Creatinine Clearance (ml/min) | 1. | -- | -- | -- | -- | -- | 11 | 98 | 33 | 130 | -- | -- | -- | -- |
| | 2. | -- | -- | -- | -- | -- | 693 | 1667 | 136 | 142 | -- | -- | -- | -- |
| BUN Clearance (ml/min) | 1. | -- | -- | -- | -- | -- | 44 | 403 | 74 | 216 | -- | -- | -- | -- |
| | 2. | -- | -- | -- | -- | -- | 210 | 588 | 55 | 96 | | | | |

Table 2e

INFUSION OF 40G OF NON-MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (OXYPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| MAP (mmHg) | 1. | 153 | 154 | 183 | 184 | 181 | 177 | 175 | 172 | 156 |
| | 2. | 140 | 130 | 193 | 197 | 206 | 212 | 207 | 192 | 81 |
| Mean: | | 157 | 142 | 188 | 190 | 193 | 195 | 191 | 182 | 119 |
| apO2 (mmHg) | 1. | 100 | 102 | 97 | 108 | 97 | 100 | 97 | 95 | 93 |
| | 2. | 95 | 95 | 93 | 93 | 90 | 97 | 98 | 86 | 100 |
| Mean: | | 98 | 99 | 95 | 101 | 94 | 99 | 98 | 91 | 97 |
| apCO2 (mmHg) | 1. | 37 | 36 | 34 | 33 | 33 | 34 | 34 | 34 | 36 |
| | 2. | 43 | 40 | 41 | 40 | 41 | 41 | 39 | 37 | 30 |
| Mean: | | 40 | 38 | 38 | 37 | 37 | 38 | 37 | 36 | 33 |
| CVP (mmHg) | | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| MPAP (mmHg) | | -- | -- | -- | -- | -- | -- | -- | -- | -- |

Table 2f

INFUSION OF 40G OF NON-MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (OXYPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| A-V | 1. | 3.6 | 3.0 | 4.1 | 4.5 | 4.8 | 5.9 | 6.4 | 6.8 | 8.9 |
| | 2. | 2.8 | 2.5 | 2.6 | 2.9 | 3.5 | 3.9 | 4.3 | -- | 6.2 |
| Mean: | | 3.2 | 2.8 | 3.4 | 3.7 | 4.2 | 4.9 | 5.4 | 6.8 | 7.6 |
| O2 Extra- | 1. | | 16.0 | 24.2 | 26.9 | 29.3 | 34.9 | 37.4 | 41.1 | 50.9 |
| ction | 2. | 14.6 | 13.6 | 15.0 | 17.0 | 24.0 | 23.2 | 25.1 | -- | 16.4 |
| Mean: | | 14.6 | 14.8 | 19.6 | 22.0 | 24.9 | 29.1 | 31.3 | 41.1 | 33.7 |
| CI | 1. | .11 | .14 | .12 | .10 | .09 | .09 | .07 | .09 | .10 |
| L/min/kg | 2. | .23 | .31 | .27 | .24 | .21 | .16 | .13 | .10 | .09 |
| Mean: | | .17 | .22 | .20 | .17 | .15 | .13 | .10 | .10 | .10 |
| TPR | 1. | 4.0 | 3.1 | 4.2 | 5.3 | 5.9 | 5.4 | 6.8 | 5.5 | 4.7 |
| units | 2. | 1.7 | 1.2 | 2.0 | 2.2 | 2.7 | 3.7 | 4.3 | 5.4 | 2.3 |
| Mean: | | 2.9 | 2.2 | 3.1 | 3.7 | 4.3 | 4.6 | 5.6 | 5.5 | 3.5 |
| HR | 1. | 123 | 116 | 107 | 100 | 88 | 97 | 96 | 149 | 167 |
| beats/min | 2. | 185 | 192 | 255 | 201 | 151 | 123 | 112 | 107 | 91 |
| Mean: | | 154 | 154 | 181 | 150 | 120 | 110 | 104 | 128 | 129 |

Table 2g

INFUSION OF 40G OF NON-MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (OXYPURE) INTO DOGS WITH SPLEENS

| | | | TIME AFTER SFH INFUSION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| O2 Cons | 1. | 3.4 | 3.6 | 4.3 | 0.2 | 3.6 | 4.6 | 3.9 | 5.0 | 7.1 |
| ml/min/kg | 2. | 5.4 | 6.4 | 5.8 | 5.8 | 6.0 | 5.0 | 4.7 | --- | 4.8 |
| Mean: | | 4.4 | 5.0 | 5.1 | 3.0 | 4.8 | 4.8 | 4.3 | 5.0 | 6.0 |
| CO2 Pro- | 1. | 1.9 | 0.3 | 2.1 | 0.1 | 2.5 | 1.8 | 2.1 | 4.1 | 6.2 |
| duction | 2. | 5.0 | 4.7 | 4.4 | 0 | 3.9 | 3.6 | 3.6 | 3.5 | 4.2 |
| ml/min/kg | | | | | | | | | | |
| Mean: | | 3.5 | 2.5 | 3.3 | 0.1 | 3.2 | 5.4 | 2.9 | 3.8 | 5.2 |
| Deadspace | | 49.1 | 46.5 | 44.4 | 2.7 | 52.1 | 49.2 | 45.5 | 45 | 42 |
| Shunt | 1. | 18.4 | -2.7 | 13.4 | 14.4 | 12.5 | 11.5 | 9.1 | 10.7 | 11.5 |
| (%) | 2. | 52.6 | 52.1 | 48.1 | 46.1 | 50.0 | 41.9 | 36.6 | -- | 23 |
| Mean: | | 35.5 | 24.7 | 30.8 | 30.3 | 31.3 | 26.7 | 22.9 | 10.7 | 17.3 |

Table 3a

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR | 8DAY |
| Plasma Hb (mg/dl) | 1. | 14 | 11 | 2049 | 1914 | 1947 | 1960 | 1732 | 1579 | 237 | 298 | 330 | --- | 28 |
| | 2. | -- | -- | 860 | 885 | 859 | 910 | 865 | 863 | 862 | 610 | 322 | 84 | -- |
| Mean: | | 14 | 11 | 1450 | 1400 | 2806 | 1435 | 1299 | 1221 | 550 | 298 | 330 | 84 | 28 |
| SFH Recovery (%) | 1. | -- | -- | 63 | 59 | 58 | 56 | 50 | 47 | 5 | 9 | 9 | --- | -- |
| | 2. | -- | -- | 23 | 24 | 23 | 29 | 27 | 27 | 27 | 19 | 10 | 3 | -- |
| Mean: | | -- | -- | 43 | 42 | 41 | 44 | 39 | 37 | 16 | 14 | 10 | 3 | -- |
| Met Hb (%) | 1. | 1.6 | 1.5 | 2.4 | 2.4 | 1.9 | 2.1 | 2.0 | 1.8 | 1.9 | --- | --- | --- | |
| | 2. | 1.6 | 1.6 | 3.5 | 3.3 | 3.7 | 3.7 | 3.7 | 3.6 | 3.2 | 3.2 | --- | --- | |
| Mean: | | 1.6 | 1.6 | 3.1 | 2.9 | 2.8 | 2.9 | 2.8 | 2.5 | 2.6 | --- | --- | --- | |

Table 3b

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR | 8DAY |
| Hb (g%) | 1. | 16.7 | 15.1 | 13.1 | 13.4 | 12.9 | 13.3 | 13.1 | 13.1 | 12.7 | | --- | --- | -- |
| | 2. | 11.8 | --- | 9.6 | 10.0 | 9.9 | 9.6 | 9.9 | 11.3 | 10.6 | 9.6 | 8.9 | 10.9 | 12.0 |
| Mean: | | 12.8 | 15.1 | 11.4 | 11.7 | 11.4 | 11.5 | 11.4 | 12.2 | 11.7 | 9.6 | 8.9 | 10.9 | 12.0 |
| Hct (%) | 1. | 46 | 43 | 33 | 34 | 33 | 33 | 32 | 32 | 33 | --- | --- | --- | --- |
| | 2. | 31 | -- | 21 | 21 | 22 | 21 | 22 | 27 | 26 | 24 | 23 | 28 | 30 |
| Mean: | | 39 | 43 | 27 | 28 | 28 | 27 | 27 | 30 | 30 | 24 | 23 | 28 | 30 |
| Serum BUN (mg/dl) | 1. | 15.4 | 13.2 | 12.4 | 12.0 | 14.0 | 12.8 | 10.5 | 10.1 | 10.1 | 12.8 | 23.2 | -- | 24.1 |
| | 2. | 16.8 | -- | 13.2 | 13.2 | 13.7 | 3.8 | 12.5 | 10.0 | 2.1 | 11.2 | 16.5 | 11.7 | 22.2 |
| Mean: | | 16.1 | 13.2 | 12.8 | 12.6 | 13.9 | 8.3 | 11.5 | 10.1 | 6.1 | 12.0 | 20.0 | 11.7 | 23.2 |
| Serum Creat-inine (mg/dl) | 1. | 0.9 | 0.8 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 0.7 | 1.0 | 0.9 | -- | 1.1 |
| | 2. | 1.0 | -- | 0.9 | 0.8 | 10.0 | 0.8 | 0.9 | 0.8 | 0.1 | 0.9 | 1.0 | 1.1 | 1.3 |
| Mean: | | 1.0 | 0.8 | 0.8 | 0.8 | 0.9 | 0.8 | 0.8 | 0.8 | 0.5 | 1.0 | 1.0 | 1.1 | 1.2 |

Table 3c

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | BASE-LINE | PRE-INFUSION | TIME AFTER SFH INFUSION 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fibrinogen | 1. | 120 | 120 | 62 | 71 | 70 | 70 | 69 | 72 | 85 | 340 | 410 | 210 |
| (mg/dl) | 2. | 215 | -- | 120 | 120 | 115 | 110 | 105 | 130 | 140 | 300 | 360 | 600 |
| Mean: | | 168 | | 91 | 96 | 93 | 90 | 87 | 101 | 113 | 320 | 385 | 405 |
| Factor VIII | 1. | 304 | 408 | 238 | 242 | 264 | 244 | 196 | 184 | 162 | 500 | 478 | 624 |
| (%) | 2. | 796 | -- | 170 | 178 | 182 | 212 | 178 | 212 | 262 | 376 | 472 | 688 |
| Mean: | | 550 | | 204 | 210 | 223 | 228 | 187 | 198 | 212 | 438 | 475 | 656 |
| FDP | 1. | 13 | 8 | 13 | 3 | 3 | -1 | 2 | 13 | -- | 51 | 102 | 26 |
| (ug/ml) | 2. | 205 | -- | 102 | 51 | 102 | 102 | -- | 102 | 102 | 205 | 410 | -- |
| Platelet ($10^3/mm^3$) | 1. | 180 | 158 | 72 | 85 | 92 | 139 | 155 | 153 | 159 | -- | -- | -- |
| | 2. | 293 | -- | 91 | 153 | 153 | 133 | 195 | 196 | 218 | 153 | 153 | 236 |
| Mean: | | 237 | 158 | 82 | 119 | 123 | 136 | 175 | 175 | 189 | 153 | 153 | 236 |
| WBC ($10^3/mm^3$) | 1. | 4.9 | 5.4 | 5.3 | 5.7 | 6.0 | 6.8 | 6.7 | 6.9 | 8.0 | -- | -- | -- |
| | 2. | 6.4 | -- | 3.8 | 4.5 | 5.0 | 5.3 | 5.8 | 6.1 | 7.1 | 10.2 | 8.5 | 10.5 |
| Mean: | | 5.7 | 5.4 | 4.6 | 5.1 | 5.5 | 6.1 | 6.3 | 6.5 | 7.6 | 10.2 | 8.5 | 10.5 |

Table 3d

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR | 24 HR | 48 HR | 72 HR | 8 DAY |
| Urine Hb (mg%) | 1. | -- | -- | 676 | 708 | 830 | 708 | 1204 | -- | 732 | 2440 | -- | -- | 8 |
| | 2. | 41 | -- | -- | -- | -- | 766 | -- | 407 | 350 | 8 | 16 | 16 | -- |
| Urine Vol (ml) | 1. | -- | -- | 17 | 14 | 17 | 15 | 150 | -- | 150 | 240 | -- | -- | -- |
| | 2. | -- | -- | -- | -- | -- | 75 | -- | 800 | 130 | 350 | 100 | 150 | -- |
| Urine Total Hb (mg) | 1. | -- | -- | 115 | 99 | 141 | 106 | 1860 | -- | 1098 | -- | -- | -- | -- |
| | 2. | -- | -- | -- | -- | -- | 575 | -- | 3526 | 455 | 28 | 16 | -- | -- |
| Urine BUN (mg/dl) | 1. | -- | -- | 2339 | 1865 | 1331 | 1478 | 1637 | -- | 1228 | 533 | -- | -- | 628 |
| | 2. | -- | 1715 | 1218 | -- | -- | -- | -- | 279 | 279 | 380 | 1066 | 1072 | -- |
| Urine Creatinine (mg/dl) | 1. | -- | -- | 168 | 116 | 106 | 103 | 122 | -- | 110 | 37 | -- | -- | 46 |
| | 2. | -- | 184 | 110 | -- | -- | -- | -- | 21 | 24 | 43 | 82 | 105 | -- |
| Creatinine Clearance (ml/min) | 1. | -- | -- | 130 | -- | -- | 113 | 442 | -- | 103 | -- | -- | -- | -- |
| | 2. | -- | -- | -- | -- | -- | -- | -- | 117 | 527 | -- | -- | -- | -- |
| BUN Clearance (ml/min) | 1. | -- | -- | 106 | -- | -- | 92 | 390 | -- | 77 | -- | -- | -- | -- |
| | 2. | -- | -- | -- | -- | -- | -- | -- | 124 | 144 | -- | -- | -- | -- |

Table 3e

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | | TIME AFTER SFH INFUSION | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| MAP | 1. | 120 | 127 | 163 | 159 | 155 | 154 | 156 | 158 | 150 |
| (mmHg) | 2. | 147 | 148 | 154 | 164 | 174 | 71 | 168 | 158 | 156 |
| Mean: | | 133 | 138 | 159 | 162 | 165 | 113 | 162 | 158 | 153 |
| apO2 | 1. | 89 | 83 | 97 | 101 | 92 | 93 | 90 | 93 | 91 |
| (mmHg) | 2. | 98 | 102 | 95 | 95 | 94 | 93 | 91 | 92 | 87 |
| Mean: | | 95 | 93 | 96 | 98 | 93 | 93 | 91 | 92 | 89 |
| apCO2 | 1. | 41 | 41 | 40 | 40 | 38 | 39 | 40 | 35 | 35 |
| (mmHg) | 2. | 30 | 28 | 29 | 31 | 28 | 29 | 30 | 28 | 28 |
| Mean: | | 36 | 35 | 34 | 36 | 33 | 34 | 35 | 32 | 32 |
| CVP | 1. | 5 | 5 | 5 | 6 | 5 | 6 | 4 | 5 | 5 |
| (mmHg) | 2. | 10 | 8 | 12 | 13 | 13 | 12 | 11 | 2 | 5 |
| Mean: | | 8 | 7 | 8 | 9 | 9 | 9 | 8 | 4 | 5 |
| MPAP | 1. | 17 | 10 | 16 | 12 | 10 | 8 | 6 | 8 | 7 |
| (mmHg) | 2. | 18 | 22 | 21 | 21 | 21 | 20 | 15 | 6 | 12 |
| Mean: | | 18 | 16 | 19 | 17 | 16 | 14 | 11 | 7 | 10 |

Table 3f

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | TIME AFTER SFH INFUSION | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
| A-V Difference (ml/dl) | 1. | 3.8 | 3.6 | 2.0 | 2.1 | 2.6 | 3.3 | 3.9 | 5.0 | 5.1 |
| | 2. | 1.1 | 0.7 | 4.2 | 4.0 | 2.1 | 2.6 | 2.1 | 5.2 | 5.5 |
| Mean: | | 2.5 | 2.2 | 3.1 | 3.1 | 2.4 | 3.0 | 3.0 | 5.1 | 5.3 |
| O2 Extraction(%) | 1. | 16.0 | 16.8 | 10.9 | 11.5 | 14.6 | 18.4 | 21.6 | 28.3 | 29.9 |
| | 2. | 22.6 | 18.1 | 33.0 | 29.2 | 31.6 | 31.9 | 29.1 | 38.2 | 38.8 |
| Mean: | | 19.3 | 17.5 | 22.0 | 20.4 | 23.1 | 25.2 | 25.4 | 33.2 | 34.4 |
| CI L/min/kg | 1. | .26 | .26 | .32 | .25 | .19 | .14 | .12 | .10 | .10 |
| | 2. | .11 | .17 | .13 | .10 | .11 | .11 | .07 | .05 | .08 |
| Mean: | | .19 | .22 | .23 | .18 | .15 | .13 | .10 | .08 | .09 |
| TPR units | 1. | 2.0 | 1.1 | 1.2 | 1.4 | 1.9 | 2.6 | 3.1 | 3.5 | 3.3 |
| | 2. | 3.0 | 2.1 | 2.7 | 3.7 | 3.7 | 1.4 | 5.6 | 8.3 | 4.6 |
| Mean: | | 2.0 | 1.6 | 2.0 | 2.6 | 2.8 | 2.0 | 4.4 | 5.9 | 4.0 |
| HR beats/min | 1. | 215 | 236 | 171 | 157 | 110 | 110 | 73 | 63 | 62 |
| | 2. | 160 | 161 | 152 | 117 | 115 | 126 | 167 | 173 | 177 |
| Mean: | | 188 | 199 | 162 | 137 | 113 | 118 | 120 | 118 | 120 |

Table 3g

INFUSION OF 42G MODIFIED BOVINE STROMA-FREE HEMOGLOBIN (HEMOPURE) INTO DOGS WITH SPLEENS

| | | BASE-LINE | PRE-INFUSION | 10 MIN | 20 MIN | 40 MIN | 1 HR | 2 HR | 4 HR | 6 HR |
|---|---|---|---|---|---|---|---|---|---|---|
| O2 Cons | 1. | 8.4 | 7.7 | 5.2 | 4.4 | 4.0 | 3.8 | 3.8 | 4.3 | 4.5 |
| ml/min/kg | 2. | 3.2 | 3.2 | 4.6 | 2.9 | 3.7 | 3.8 | 2.2 | 1.9 | 3.6 |
| Mean: | | 5.8 | 5.5 | 5.9 | 3.7 | 3.9 | 3.8 | 3.0 | 3.1 | 4.1 |
| CO2 Prod | 1. | 9.4 | 7.0 | 7.8 | 0 | 4.8 | 1.7 | 2.1 | 2.5 | 2.8 |
| ml/min/kg | 2. | -- | -- | -- | - | -- | -- | -- | -- | -- |
| Mean: | | | | | | | | | | |
| Deadspace | 1. | 108.1 | -- | 31.3 | 2.8 | 36.9 | 2.9 | 56.4 | 53.1 | 53.1 |
| (%) | 2. | 110.4 | 112.0 | 3.9 | 3.7 | 65.0 | 54.9 | 54.7 | 57.5 | 52.8 |
| Mean: | | 109.0 | 112.0 | 17.1 | 3.3 | 50.9 | 29.0 | 55.6 | 55.3 | 53.0 |
| Shunt | 1. | 24.2 | 28.3 | 35.8 | 26.6 | 25.6 | 21.8 | 22.2 | 15.1 | 14.4 |
| (%) | 2. | 12.1 | 14.8 | 17.5 | 17.2 | 17.6 | 18.0 | 19.6 | 14.9 | 15.2 |
| Mean: | | 18.2 | 21.6 | 26.7 | 21.9 | 21.6 | 19.9 | 20.9 | 15.0 | 14.8 |

Table 4

INCREASE IN BUN LEVEL AND THE PLASMA HEMOGLOBIN CONCENTRATION 24 TO 48 HOURS FOLLOWING SFH INFUSION

| Hemoglobin concentration Infused | Serum BUN mg/dl Pre | 48 hr | % Increase 48 hr | Plasma Hemoglobin (mg/dl) 24 hr | 48 hr |
|---|---|---|---|---|---|
| DBBF | 11 | 17 | 50 | 1742 | 502 |
| Mod BSFH | 16 | 20 | 25 | 298 | 330 |
| Unmod BSFH | 17 | 24 | 29 | 224 | 68 |

Table 5

IN VITRO EFFECT OF THE PRESENCE OF HEMOGLOBIN ON THE MEASUREMENT OF BUN IN DOG SERUM

BUN measurments in 5 aliquots of a serum samples containing increasing hemoglobin concentrations in a constant volume of 1.2 ml

| Aliquot | Hemoglobin (mg/dl) | Serum BUN (mg/dl) | Serum BUN (% increase above aliquot 1) |
|---|---|---|---|
| Experiment 1. | | | |
| 1 | 0 | 12.5 | -- |
| 2 | 207 | 12.6 | 0.8 |
| 3 | 517 | 13.6 | 8.8 |
| 4 | 1034 | 13.8 | 10.4 |
| 5 | 2067 | 13.2 | 5.6 |
| Experiment 2. | | | |
| 1 | 0 | 6.5 | -- |
| 2 | 210 | 6.7 | 3.7 |
| 3 | 525 | 6.7 | 4.0 |
| 4 | 1050 | 7.9 | 21.7 |
| 5 | 2100 | 9.1 | 41.7 |