Investigating the hemoglobin of white rhinoceros (Cerathotherium simum simum) and possible implications for anesthesia.

Ray L Ball

Tampa's Lowry Park Zoo, 1101 W. Sligh Ave., Tampa, FL USA 33604

Introduction:

Respiratory depression is potentially the most significant complication surrounding the anesthesia of both free-ranging and captive white rhinos. Large mammals in general experience cardiopulmonary depression and perfusion-ventilation (V/Q) mismatching. Specific complications are hypoxemia, hypercapnia, and acidosis. In spite of these potential complications, morbidity and mortality related to anesthesia are rare and numerous examples of prolonged anesthesia in this species are reported in the literature and anecdotally. White rhino hemoglobin has a known higher affinity for oxygen as is expected in large mammals. This hemoglobin also has some unique beta chains that is are unique to mammals and more closely related to fish. We suggest that some of the unique properties may influence how routine blood gas analysis is interpreted and that white rhinos under anesthesia are not as severely compromised as routine measurements may suggest. This has profound impact as effects to correct these perceived problems may lead to other complications.

A series of anesthetic events in two seven year old captive born white rhinoceros males was performed at Busch Gardens Tampa, Florida. Both were anesthetized for electro-ejaculation five times and had blood gas sampling ate each event. Arterial blood was collected from an auricular artery on the inside of the ear using a heparinized 25-gauge butterfly set attached to a 3.0-ml heparinized plastic syringe. Blood gas and pH analyses on arterial blood samples were performed within 30 min of collection on a portable analyzer (AVL OPTI Critical Care Analyzer,AVL Scientific Corp., Roswell, Georgia30077, USA). Typical blood gas analysis from these procedures is given in Table 1. Overall hypoxia, academia, and hypercapnia were common and often considered life threatening. On some occasions, blood gas values were below 7.0. Supplemental oxygen was provided at each procedure and heart rate and respiratory rates were within acceptable parameters (Radcliffe and Morkel 2007). Pulse oximetry readings for oxygen saturation of hemoglobin ranged from 45-80%. In spite of these findings, each procedure resulted in a complete recovery without complications. Findings were similar to those reported in wild caught rhinos (Bush 2004) but even more extreme.

In order to better understand the disparity in the clinical outcome with the blood gas analysis, a clinical investigation was made with awake, standing rhinoceros. Blood gas values paired with pulse oximetry were collected from one adult male, two adult females, a 2 yr old juvenile and a 6month old female. Venous blood samples were collected due to the ease of collection and measured as the samples above. Pulse oximeter reading including pulse and oxygen saturation of hemoglobin was taken. A manual pulse rate was obtained either by a stethoscope auscultation of the heart or a digital pulse from the tail. These findings are summarized in Table 2 along with a comparison from the literature (Citino 2007). Whole blood in heparin was collected to isolate white rhinoceros hemoglobin as described by Bauer and Pacyna using gas chromatography. An adult female and the 6 month old calf from above were utilized in this assay. Carbon monoxide was used as a first step to generate absorbance spectra of white rhinoceros carboxyhemoglobin due to complications involved in using oxygen (Larson 2007, Wagman 2008). Absorbance spectra of HbCO in white rhinos was found to have a Sorbet band at 419nm . Normalized absorbance for titrated CO was higher in the adult female compared to the female calf.

Discussion and Conclusions:

The oxygen saturation derived by blood gas analysis was substantially different than that derived by pulse oximetry in the adult awake rhinoceros but there is good correlation between the calf for these values. The 2 year old juvenile lies in between these groups. It is possible that the pulse oximetry readings reflect the differing hemoglobin affinity for oxygen in the different size classes of these rhinoceros with the calf hemoglobin most closely fitting that of humans or domestic horses. The differences in oxygen saturation in this group of rhinoceros compared to Citino are difficult to explain but may be due to differences in sampling. PaO2 was shown to increase over time within 5 minutes of collection in iced water samples (Deane et al. 2004; Picandet et al. 2007) and to increase (Picandet et al. 2007) or decrease in samples kept at room temperature (Deane et al. 2004), so perhaps handling can explain these differences. The reported values of the anesthetized rhinoceros during electro-ejaculation are also not sensible in that that prolonged hypoxia and acidosis of this magnitude would be expected to result in longer term complication and potentially even death in the worst cases. Interestingly, SpO2 readings of 88–100% were obtained on several occasions on a white rhinoceros in dorsal recumbency for abdominal laporatomy, although readings of less than 94% predominated (Valverde 2010). All of these clinical findings and contradictions add support to the fact that differences in white rhinoceros hemoglobin have been documented (Baumann 1984). All of the values reported on the blood gas analyzer used are calculated from the algorithms of hemoglobin reflectance based on human oxygen dissociation curves (ODC) and hence the need for more specific ODC for large mammals and especially one with such a unique hemoglobin. White rhinoceros hemoglobin does not appear to be responsive to organic anions (2, 3 DPG or ATP) nor to CO2 (Baumann 1984). It is speculated that the ODC for white rhinoceros may closely resemble that of humans without any 2,3 DPG added. The development of a carboxyhemoglobin spectrum is the first technical step towards developing this ODC for white rhinoceros and future efforts will try to finalize this. Future work will now focus on determining an equilibrium binding constant.

References:

1. Bauer, C. and B. Pacyna (1975). The conversion of trivalent to divalent iron in hemoglobin of various species. Anal Bischem., 65:445-448.
2. Baumann R, Mazur G, Braunitzer G (1984) Oxygen binding properties of hemoglobin from the white rhinoceros (b2-GLU) and the tapir. Respir Physiol 56, 1–9.
3. Bush,M. ,J P Raath, D Grobler, and L Klein. Severe hypoxaemia in field-anaesthetised white rhinoceros (Ceratotherium simum) and effects of using tracheal insufflation of oxygen. Jl S.Afr.vet.Ass. (2004) 75(2): 79–84
4. Citino, S and M Bush. Reference cardiopulmonary physiologic parameters for standings, unrestrained white rhinoceros. (*CERATOTHERIUM SIMUM*) Journal of Zoo and Wildlife Medicine 38(3): 375–379, 2007

5. Deane JC, Dagleish MP, Benamou AEM et al. (2004) Effects of syringe material and temperature and duration of storage on the stability of equine arterial blood gas variables. Vet Anaesth Analg 31, 250– 257.
6. Larson W Randy, Jaroslava Miksovska. Time resolved thermodynamic of ligand binding to heme proteins. Coordination Chemistry Reviews Volume 251, Issues 9-10, May 2007, P 1101-1127.
7. Radcliff, R. and P Morkel. Rhinoceros In: Zoo Animal and Wildlife Immobilization and Anesthesia. Blackwell Publishing. 2007.
8. Picandet V, Jeanneret S, Lavoie JP (2007) Effects of syringe type and storage temperature on results of blood gas analysis in arterial blood of horses. J Vet Intern Med 21, 476–481.
9. Wagman, J., R. Larson, C. Reimink, R. Ball. CO binding properties of managed white rhinoceros. Senior Honors Thesis, University of South Florida 2008.
10. Valverde ,A., G. Crawshaw, et al. Anesthetic management of a white rhinoceros (Ceratotherium simum) undergoing an emergency exploratory celiotomy for colic. Veterinary Anaesthesia and Analgesia, 2010, 37, 280–285

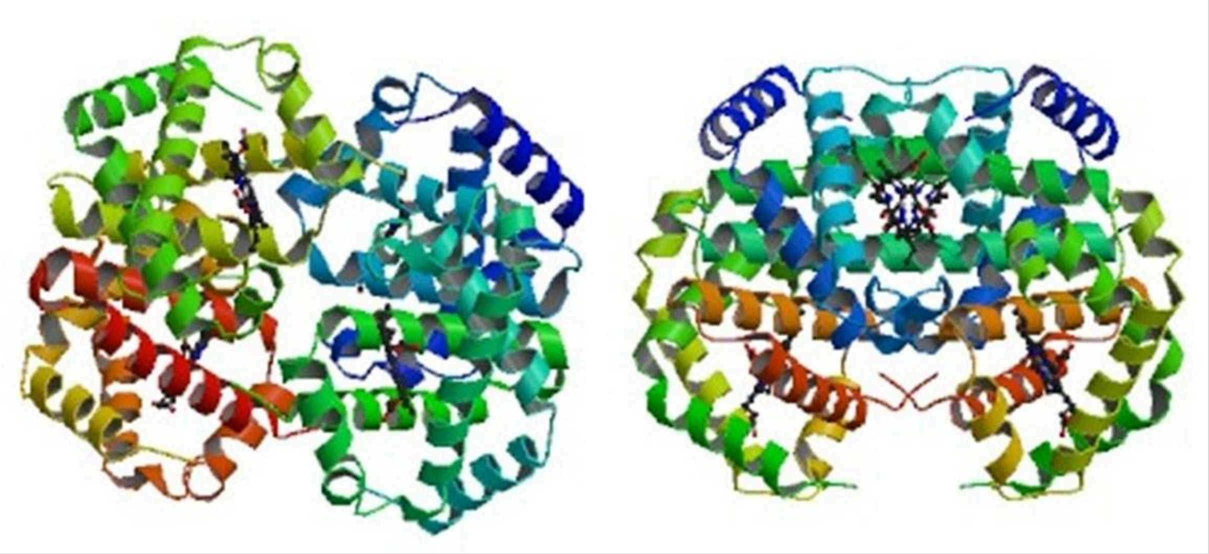
Table 1. Examples of blood gas values from white rhinoceros anesthetized for electroejaculation.

White rhinoceros #1				
Time from first sample	pH	pCO2 mmHg	pO2 mmHg	sO2 %
T0	7.333	64.6	47.4	82.3
T15	7.32	59.7	41.8	76.6
T30	7.345	64	41.1	79.9
T45	7.404	49.9	43	78.4
White rhinoceros #2				
	pH	pCO2 mmHg	pO2 mmHg	sO2 %
T0	7.21	92.9	28.9	43.9
T15	7.089	83.3	45.4	75
T30	7.154	85.3	35.3	59.2
T45	7.235	79.2	33.4	58.5

Table 2. Pulse oximetry readings and venous blood gas analysis of standing, awake white rhinoceros and comparison to published values.

	Adult rhinoceros			2yr old juvenile			6 month old calf			Citino 2007 (Arterial)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Pulse Ox %O2	68.8	3.839	15	75.6	0.5477	5	92	1.224	5			
Pulse Ox Pulse (bpm)	60.133	11.25	15	79.2	1.095	5	46	2	5			
Digital pulse (bpm)	59.53	10.09	15	78	1.412	5	47.2	1.095	5	39	0.8	66
Blood gas (V)												
pH	7.361	0.024	15	7.346	0.0127	5	7.361	0.0031	5	7.391	0.007	61
pCO2 mmHg	53.833	1.084	15	51.98	0.526	5	57.32	0.7918	5	49	0.9	61
pO2 mmHg	76.38	6.68	15	51.52	0.462	5	86.88	0.925	5	98.2	1.4	61
sO2 %	93.74	1.182	15	86.74	0.416	5	96.36	0.5319	5	97.2	0.1	61

Investigating the hemoglobin of white rhinoceros (Cerathotherium simum simum) and possible implications for anesthesia.



Ray L Ball, Tampa's Lowry Park Zoo,
1101 W. Sligh Ave., Tampa, FL USA
33604

White rhinoceros anesthesia

- Ultra-potent narcotics
  - Etorphine
  - Carfentanil
- Sedatives
  - Alpha 2
  - Azaperone
- Detomidine/Butorphanol
- Medetomidine/Butorphanol

White rhinoceros anesthesia complications

- Hypoxia
- Acidosis
- Hypercapnea
- Tachycardia
- Hypertenson

- Mortality uncommon in NA
- Prolonged procedures

Anesthetic management of a white rhinoceros (*Ceratotherium simum*) undergoing an emergency exploratory celiotomy for colic

Alexander Valverde*, Graham J Crawshaw†, Nicola Cribb*, Maria Bellei*, Giacomo Gianotti*, Luis Arroyo*, Judith Koenig*, Maya Kummrow*,† & Maria Carolina Costa*
*Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada
†Toronto Zoo, Scarborough, ON, Canada

Series of immobilizations for ejaculation

- Etorphine induction
  - ~4mg
- Alpha 2 (xylazine)
- Supplemental oxygen
- Electro-ejaculation
- NSAIDs
- Blood gas via auricular artery

Immobilization for ejaculation

- HR 50-72 at rest
  - 100 when stimulated
- RR 6-24
- SpO2 45-80%
- Various drugs used to try and improve blood gas findings (n=4)
  - Dopram
  - Naloxone
  - $NaHCO_3$
- All recovered well
- Rhinos given stimulants all constipated for 24-36 hrs post-recovery

Typical blood gas analysis

Rhino #1

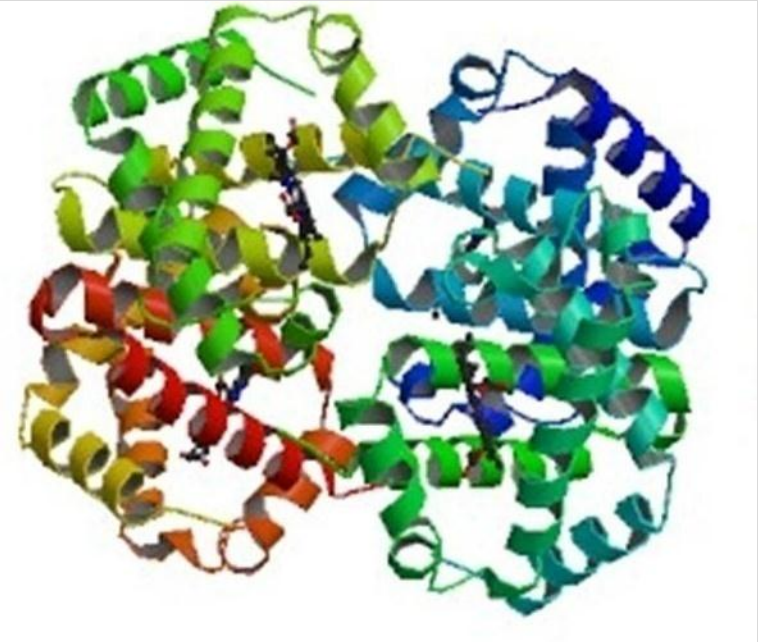
	pH	pCO2 mmHg	pO2 mmHg	SpO2 %
T0	7.333	64.6	47.4	82.3
T15	7.32	59.7	41.8	76.6
T30	7.345	64	41.1	79.9
T45	7.404	49.9	43	78.4

Rhino #2

	pH	pCO2 mmHg	pO2 mmHg	SpO2 %
T0	7.21	92.9	28.9	43.9
T15	7.089	83.3	45.4	75
T30	7.154	85.3	35.3	59.2
T45	7.235	79.2	33.4	58.5

How did these rhinos with severe hypoxia and acidosis survive??

- Induction?
- Methods of ABG collection?
- Effect of electro-ejaculation?
- White rhinoceros hemoglobin and instrumentation?



Ribbon representative of white rhinoceros de-oxyhemoglobin. Wagman 2007.

White rhinoceros hemoglobin

Baumann 1984

- Glutamic acid at Beta 2 position
- **Little effect from CO_2, ATP and 2,3 DPG**
- Similar to fish Hb
- High affinity for oxygen
  - P50 = 17mmHg (2.67kPa)
- Trend for larger mammal to have high O_2 affinity

- *Can a clinically useful algorithm be developed for $SpO_2$and ABG?*
- *Can a ODC for white rhinos be developed?*
- *Does myoglobin have a role??*

Measure awake rhinoceros

- 1.2 adults (n=15)
- 0.1 2 year old (n=5)
- 0.1 6 month old (n=5)
- Venous BG samples
- Heart rates
- Pulse oximetry readings

Journal of Zoo and Wildlife Medicine 38(3): 375–379, 2007

REFERENCE CARDIOPULMONARY PHYSIOLOGIC PARAMETERS FOR STANDING, UNRESTRAINED WHITE RHINOCEROSES (*CERATOTHERIUM SIMUM*)

Scott B. Citino, D.V.M., Dipl. A.C.Z.M. and Mitchell Bush, D.V.M., Dipl. A.C.Z.M.

VBG and SpO2 from standing awake rhinoceros

	Adult rhinoceros			2yr old juvenile			6 month old calf			Citino 2007 (Arterial)		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
SpO2 %	68.8	3.84	15	75.6	0.548	5	92	1.224	5			
pH	7.361	0.02	15	7.35	0.013	5	7.361	0.003	5	7.391	0.007	61
pCO2 mmHg	53.833	1.08	15	52	0.526	5	57.32	0.792	5	49	0.9	61
pO2 mmHg	76.38	6.68	15	51.5	0.462	5	86.88	0.925	5	98.2	1.4	61
SaO2 %	93.74	1.18	15	86.7	0.416	5	96.36	0.532	5	97.2	0.1	61

VBG and SpO_2 from standing awake rhinoceros

- SpO_2
  - Differences larger in adults
  - Calf similar to horse/human
- SaO_2 close to the same
  - pH calculated from this
- pO_2/pCO_2 venous vs. arterial
- pCO_2 higher than horses

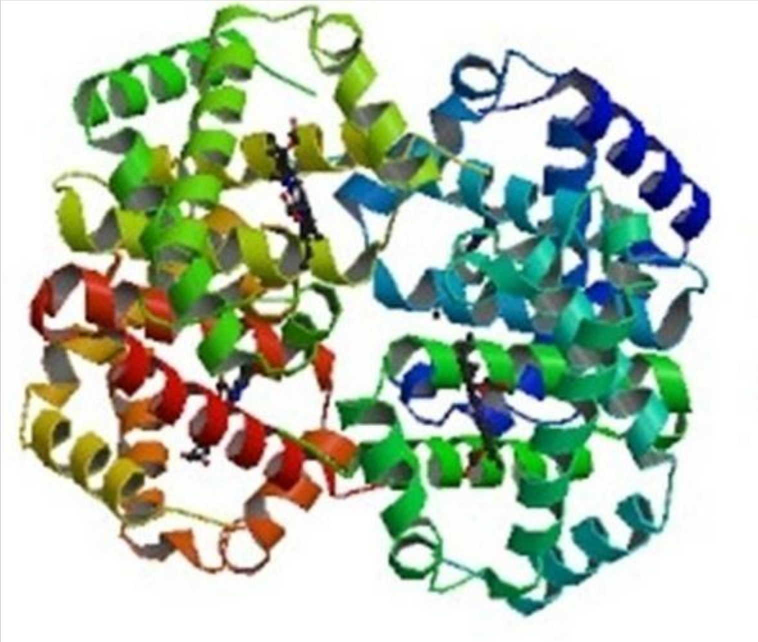
- **pH 7.35-7.45**
- **pCO_2 35-45 mmHg**
- **pO_2 >79 mmHg**
- **CO_2 23-30 mmHg**
- **SO_2 >94%**
- **pH 7.31-7.41**
- **pCO_2 41-51 mmHg**
- **pO_2 30-40 mmHg**
- **CO_2 23-30 mmHg**
- **SO_2 75%**

Valverde 2010

- SpO2 between 88% and 100% saturation
- Values for blood gases were probably not accurate for PaO2 due to handling temp
  - Still reflect progressive hypoxia, acidosis, hypercapnea
- SpO2 values overestimated SaO2
  - Other reports SpO2 underestimates SaO2
- Conflicts about SpO2 and ABG reliability

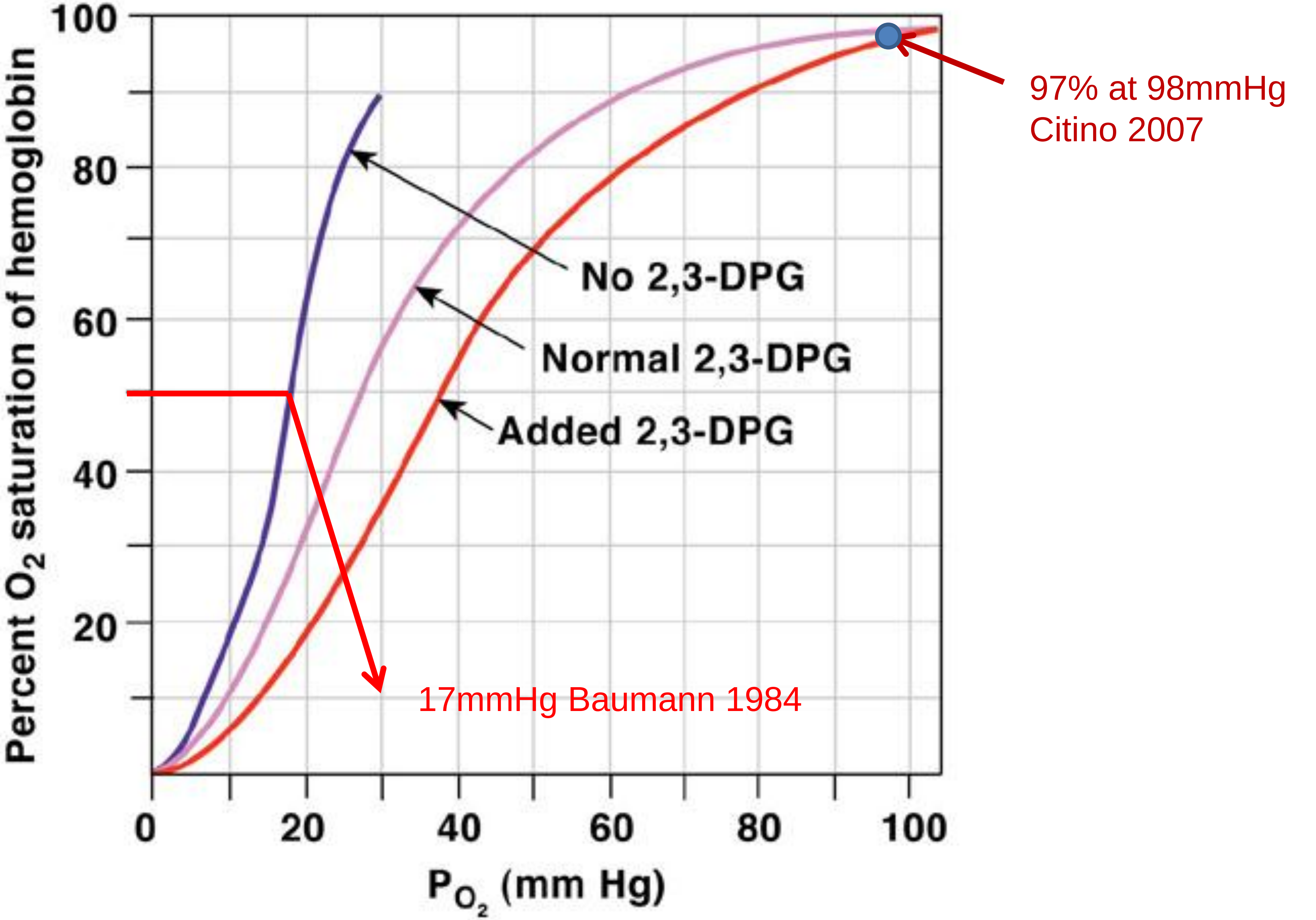
CO binding properties of white rhinoceros.

- Wagman, J., R. Larson, C. Reimink, R. Ball.
  - Senior Honors Thesis, University of South Florida 2008.
  - Preliminary work shows a higher absorbance for CO in adult rhinos
    - Suggest higher affinity for O2
  - Adult hemoglobin differs from juvenile rhinoceros



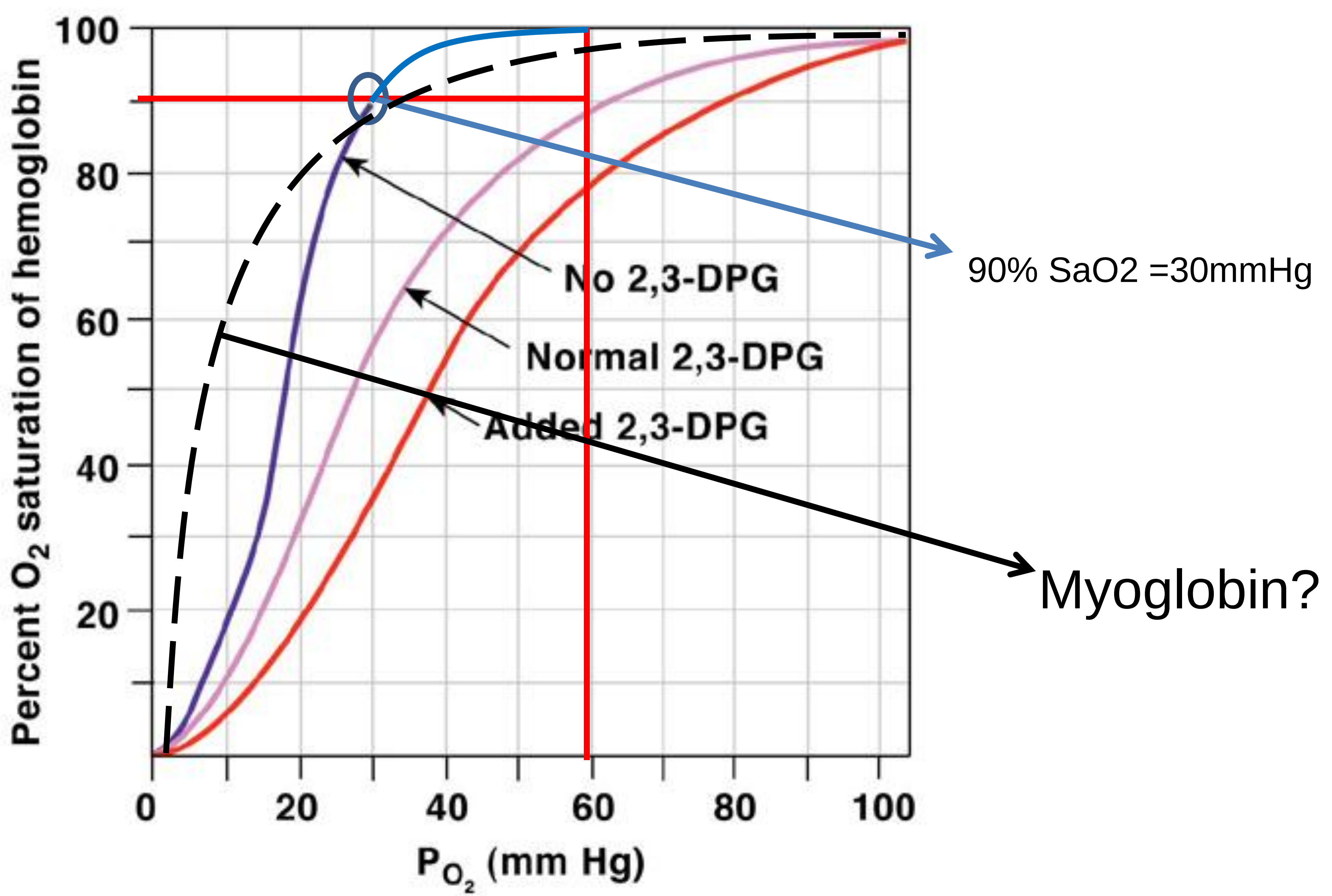
Significance

- PaO_2 <60mmHg hypoxemia in horses
  - SaO_2 93%
- Apply to white rhinoceros??
- Redefine hypoxia in white rhinoceros
  - Prevent unnecessary treatment
  - Opioids and constipation



100
80
60
40
20
Percent O2 saturation of hemoglobin
0
20
40
60
80
100
PO2 (mm Hg)
No 2,3-DPG
Normal 2,3-DPG
Added 2,3-DPG
17mmHg Baumann 1984
97% at 98mmHg
Citino 2007

 Fig. 18-11



Percent O_2 saturation of hemoglobin
100
80
60
40
20
0
20
40
60
80
100
P_{O_2} (mm Hg)
No 2,3-DPG
Normal 2,3-DPG
Added 2,3-DPG
90% SaO2 =30mmHg
Myoglobin?

 Fig. 18-11

Summary

- White rhino ODC will be shifted to the left
  - P50 17-20mgHg
- Organic anions have little affect on oxygen binding
- Define hypoxia for WR at SaO2 ~30mmHg
- Juvenile rhinos may have ODC similar to humans and horses

Anesthetic management

- Always provide oxygen
- Do not worry about CO2
- Interpret ABG values with caution
- Usefulness of ABG ??

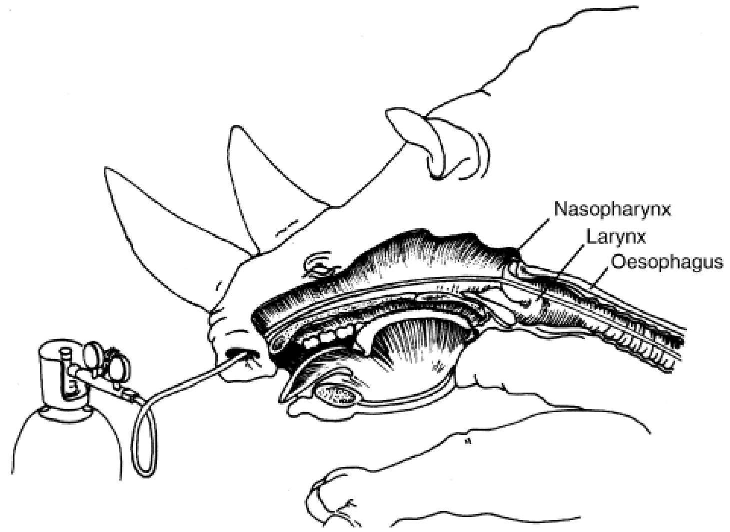


Fig. 1: **Drawing of a recumbent rhinoceros showing the placement of the nasotracheal tube passing through the larynx into the trachea, with the tube attached to an oxygen tank with a pressure regulator and a flow meter to control the O_2 flow.**

Bush M, Raath J P, Grobler D, Klein L **Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen.** *Journal of the South African Veterinary Association* (2004) 75(2): 79–84 (En.). Conservation and Research Center, Smithsonian National Zoological Park, 1500 Remount Road, Front Royal, Virginia 22630, USA.

Acknowledgments

- Rhino Staff @ Busch Gardens
- Justine O'Brien & Todd Robeck SeaWorld/Busch Gardens
- Terri Roth & Monica Stoops, CREW, Cincinnati
- Lowry Park Zoo