

An Historical Perspective: IRON OVERLOAD DISEASE IN BROWSER RHINOCEROSES

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“When the situation was manageable it was neglected, and now that it is thorough out of hand we apply too late the remedies which then might have effected a cure.”



CLINICAL PROBLEMS IN CAPTIVE RHINOSCEROSSES

| | |
|-------------------------------|-------------------------|
| REPRODUCTION: | White, Indian, Sumatran |
| INFECTIOUS DISEASES: | Black, Sumatran |
| HEPATIC/G.I: | Black, White, Sumatran |
| SUDDEN DEATH: | Black, White, Sumatran |
| HOOOF DISORDERS: | Indian, Black |
| RENAL/NEOPLASTIC: | White, Black, Indian |
| OBESITY, BODY DECONDITIONING: | (All) |
| ANEMIA, IRON OVERLOAD | Black, Sumatran |

RBC "ABNORMALITIES"

(All species variably affected)



Enzyme "deficiencies"

Very low metabolic energy (2-5% ATP)

Impaired antioxidant capacity (Heinz bodies)

Reflect other tissues ?

ACUTE HEMOLYTIC ANEMIA

Most frequent cause of death in captive black rhinos before preventative measures (75%)

Oxidant stress → premature RBC death → **Hemoglobin** release & degradation → tissue deposits of iron pigments (**Hemosiderin**)

ISD called "Hemosiderosis"

NECROPSY REPORTS

HEMOLYTIC ANEMIA

or

IRON STORAGE DISEASE

???



Joseph E. Smith, DVM, PhD (1938 -1998)

“SILENT” DISORDERS

Hypertension

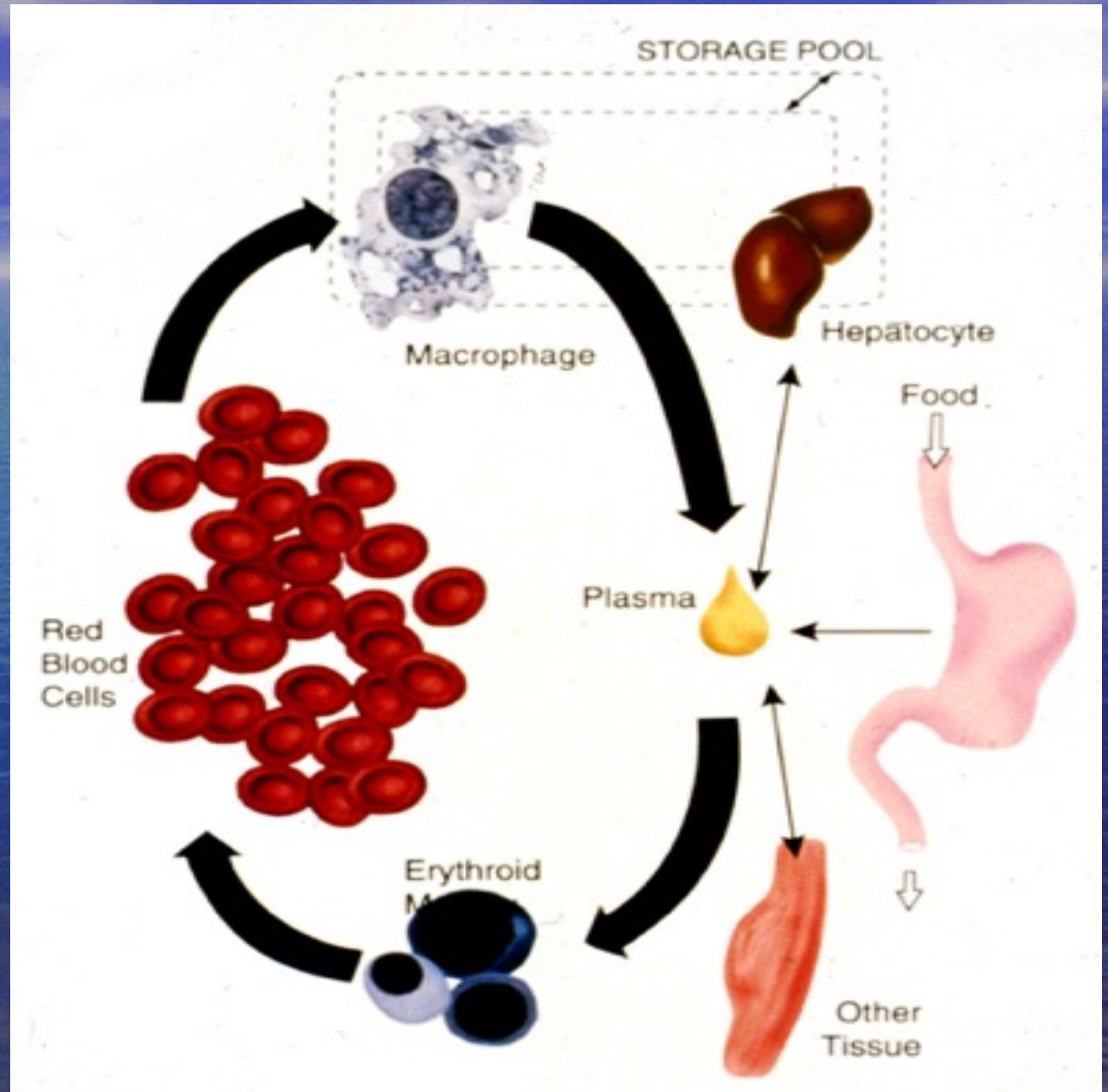
Arteriosclerosis

Diabetes

Alzheimer’s Disease

Iron Storage Disease

IRON CYCLING



IRON: The 'Goldilocks' Metal

BIOLOGICALLY ESSENTIAL

Hemoglobin, myoglobin, enzymes

DEFICIENCY

Most common anemia worldwide

HEREDITARY OVERLOAD

Hemochromatosis (HFE mutations)

Most common U.S. genetic disorder

HIGHLY TOXIC IN FREE FORM

IRON STORAGE DISEASE

All African black & Sumatran rhinos born or brought into captivity are affected

Total body iron loads correlate with time in captivity, reaching tenfold in 3-5 yrs

Massive ($>1,000x$) overloads are common among long-term captives

EVIDENCE FOR IRON OVERLOAD

BLOOD STUDIES (>250 rhinos, 4 sp.)

Serum Iron

Transferrin Saturation

Ferritin



NECROPSY STUDIES (>60 rhinos, 5 sp.)

Histopathology (iron stains)

Quantitative tissue iron analyses

SERUM IRON ANALYTES

IRON

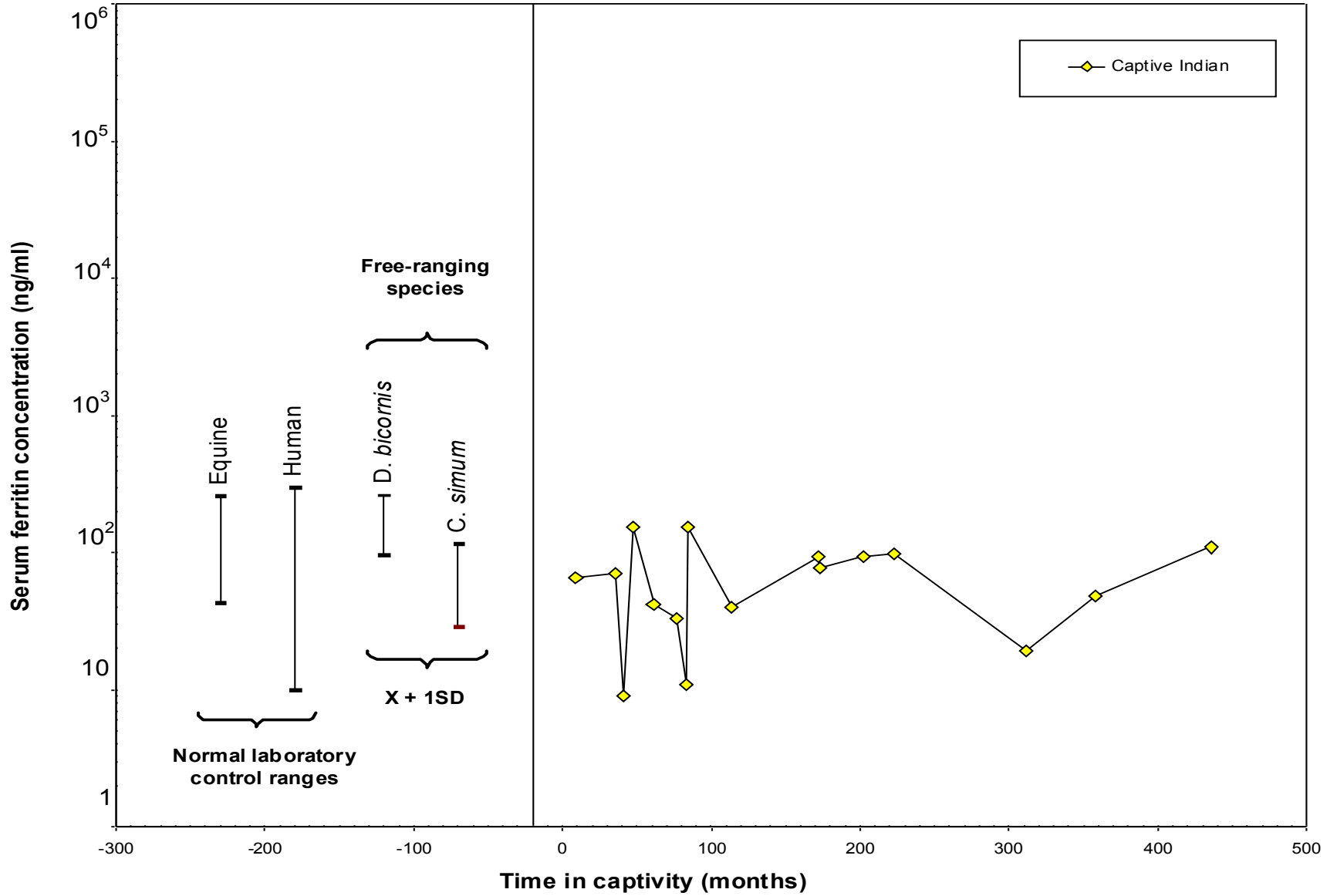
**TRANSFERRIN (transport protein)
(Total Iron Binding Capacity)**

TRANSFERRIN SATURATION (%)

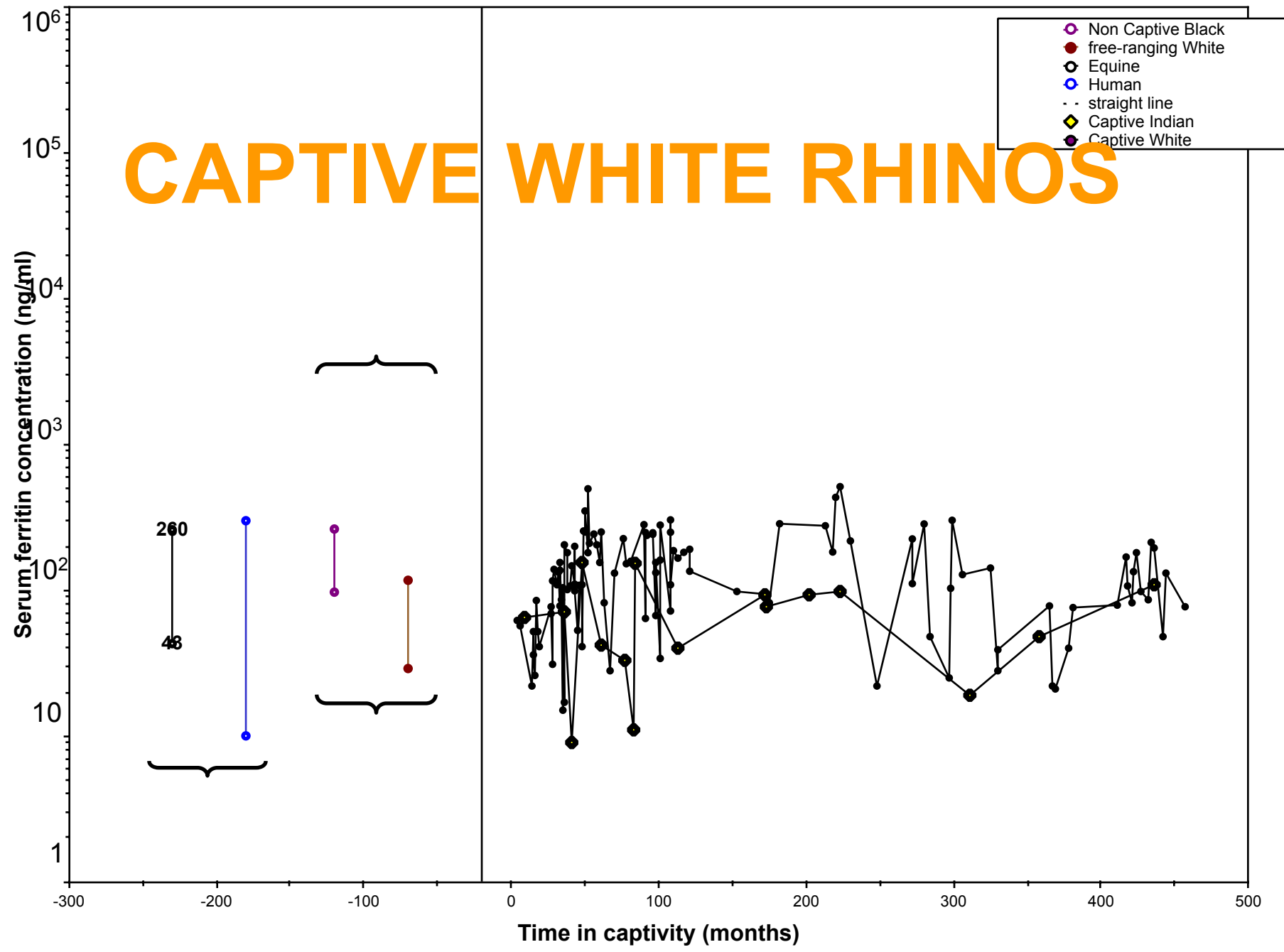
FERRITIN (storage-protein complex)

Correlates with total body burden

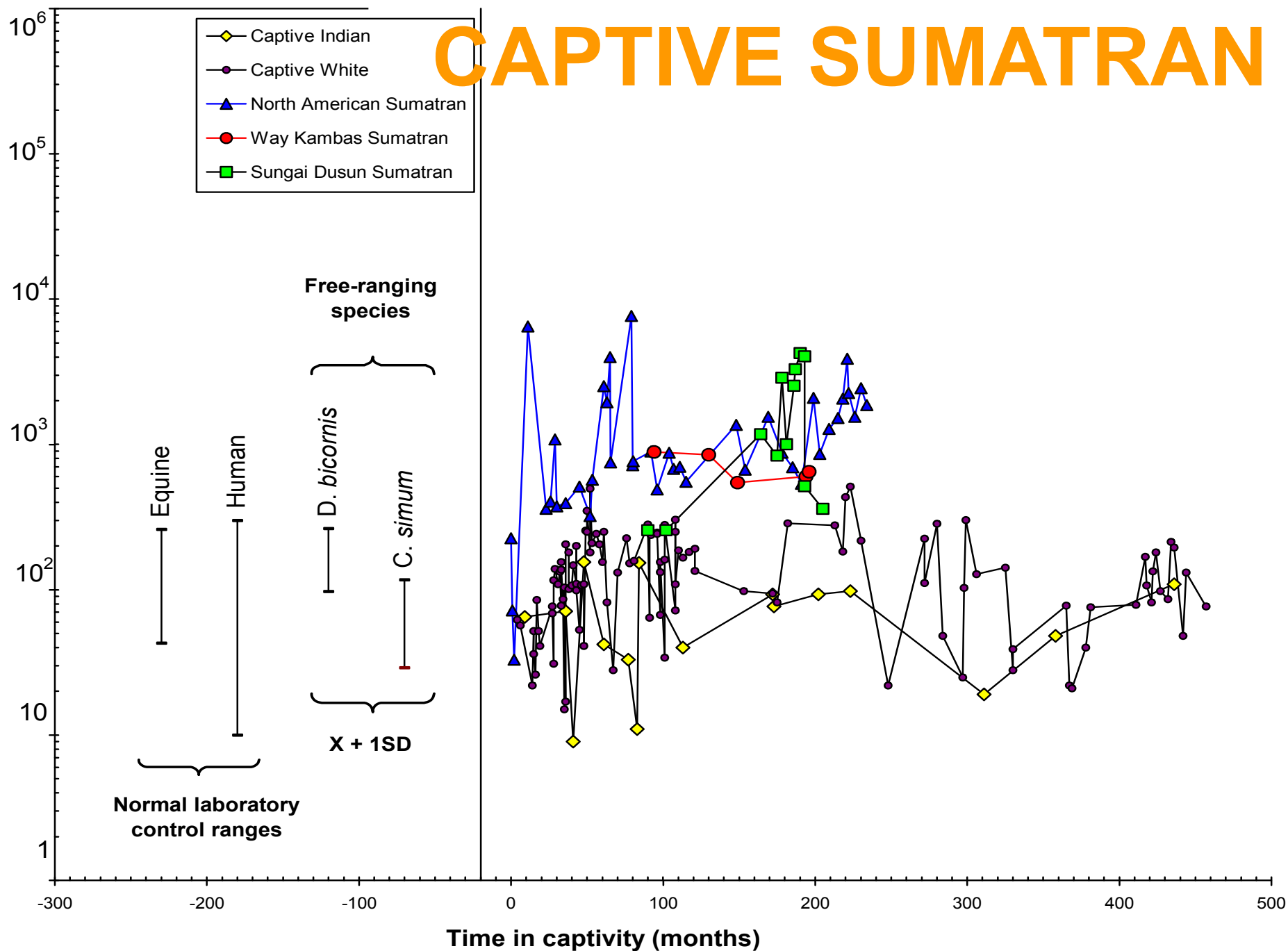
CAPTIVE INDIAN RHINOS

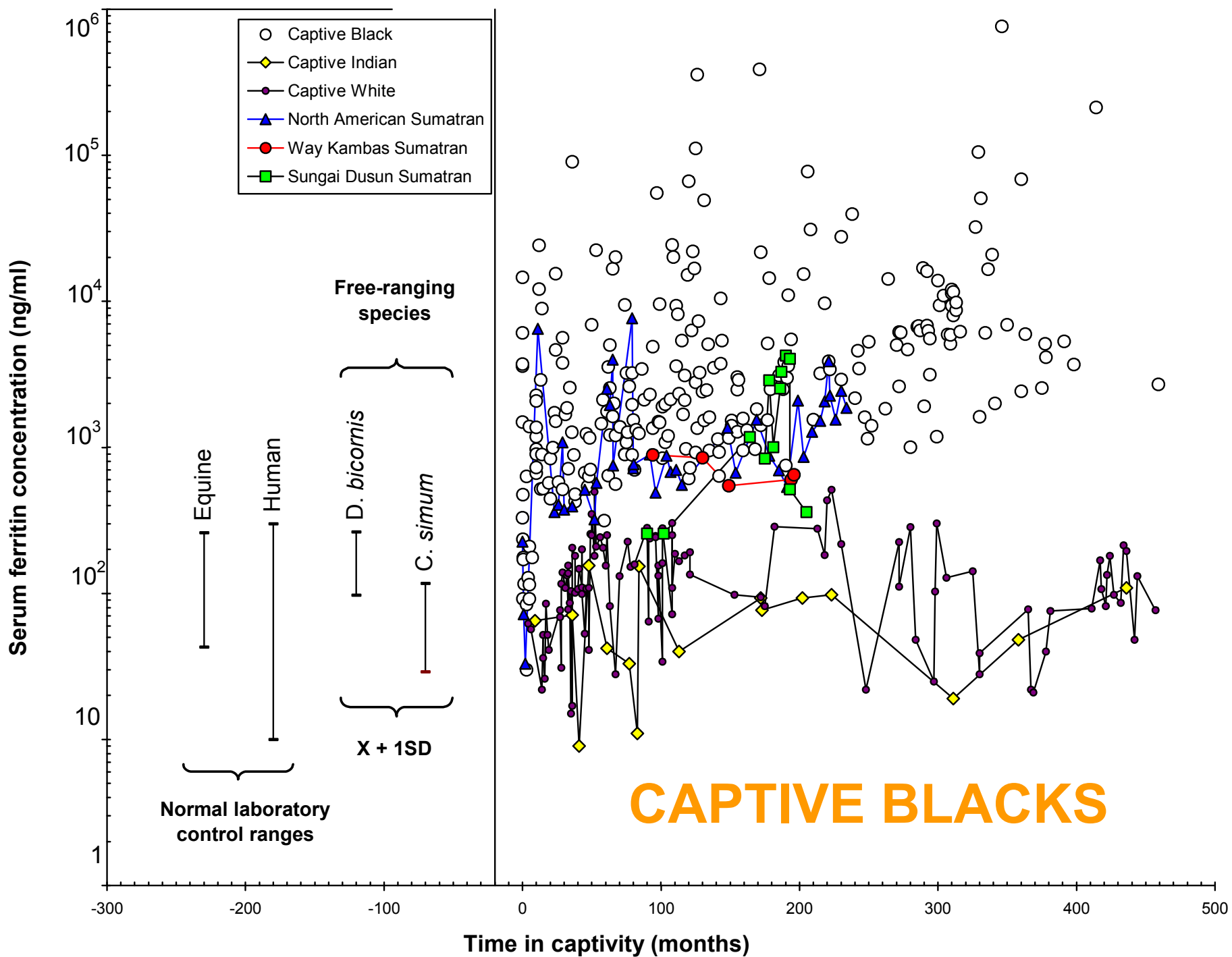


CAPTIVE WHITE RHINOS



CAPTIVE SUMATRAN

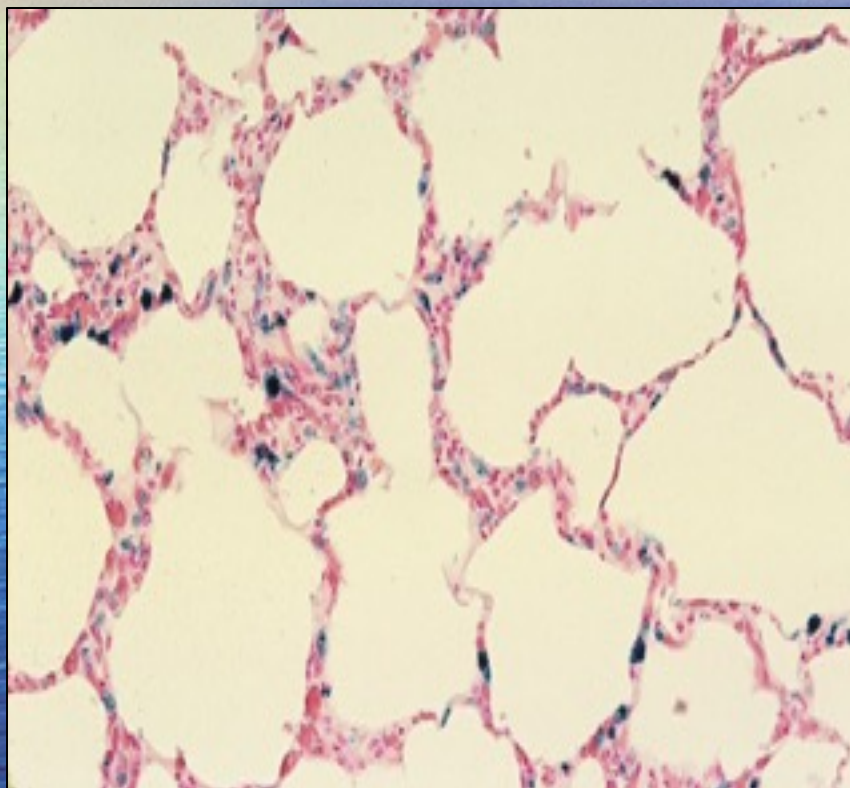




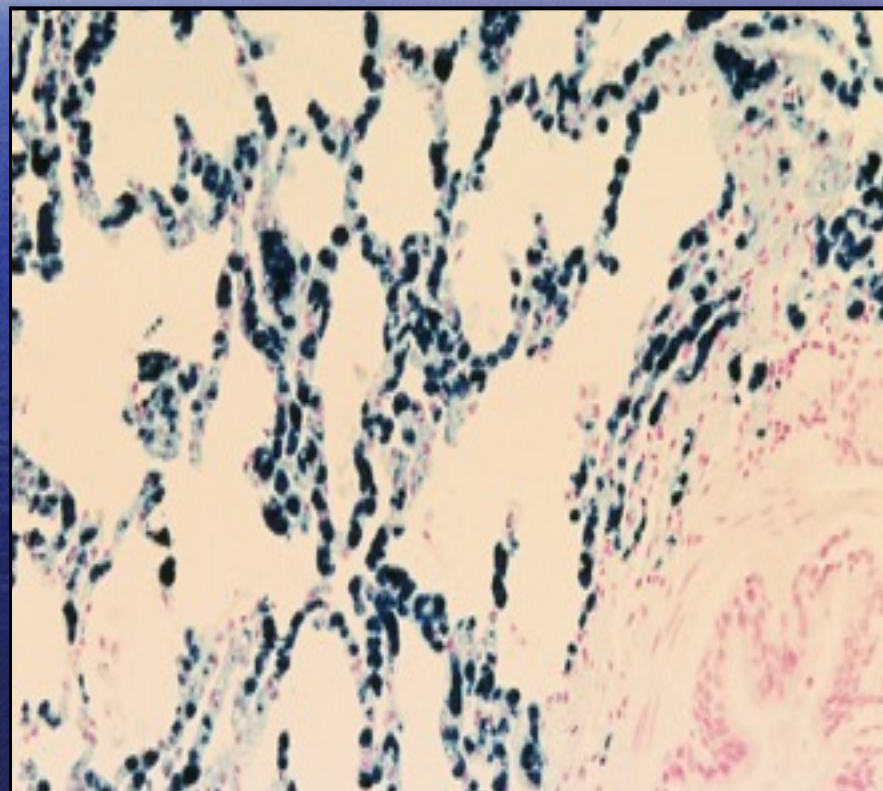
SERUM FERRITIN IN BLACK RHINO CALVES



**NECROPSY PATHOLOGY
(LUNG)**

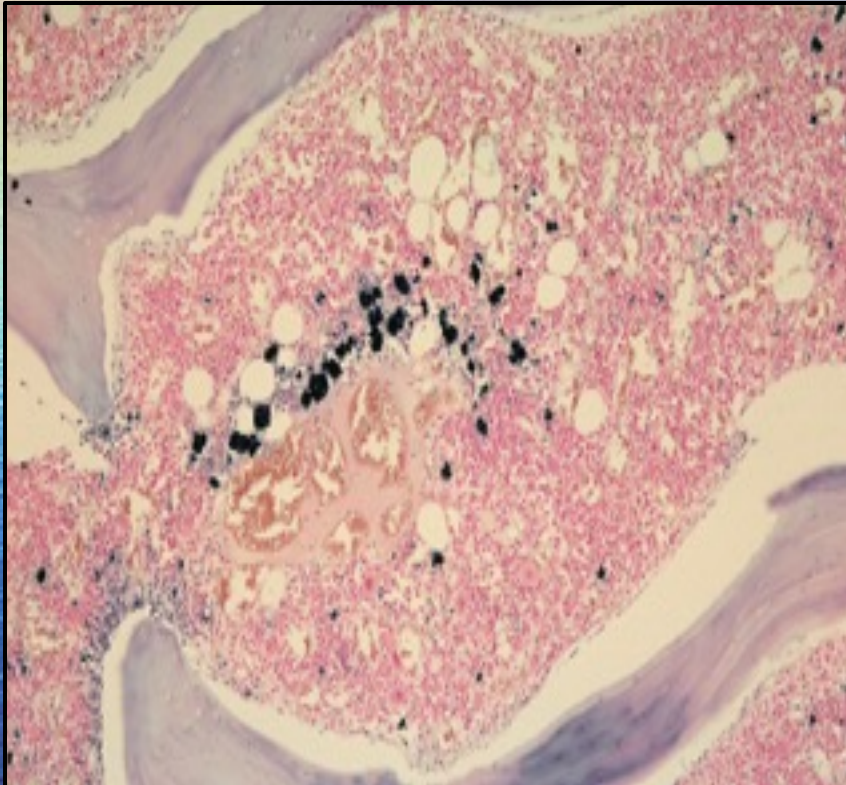


WHITE RHINO

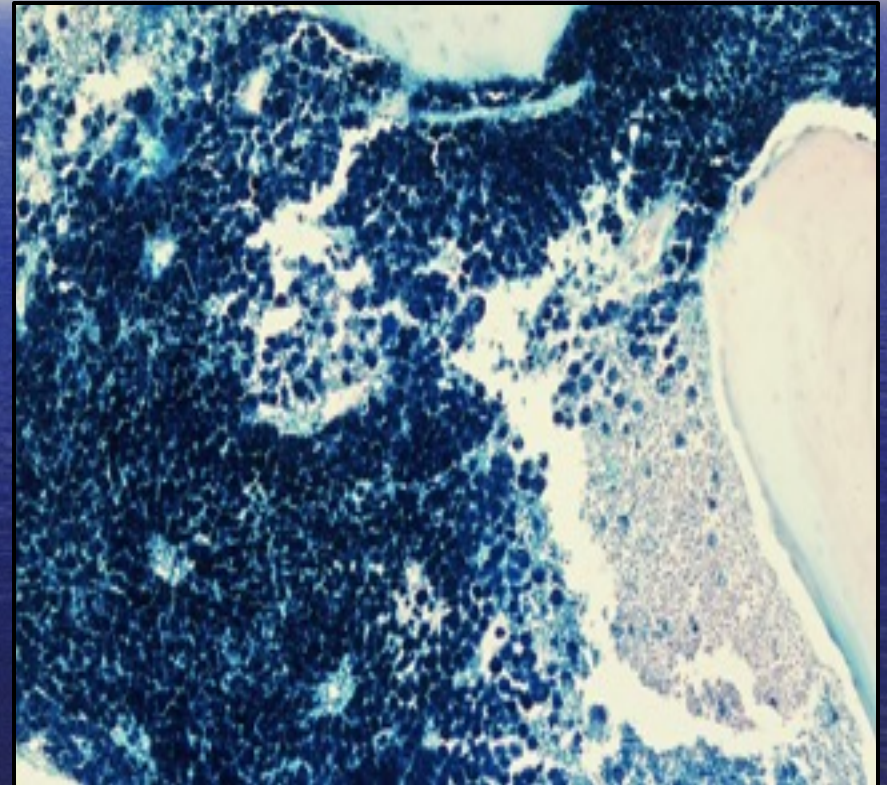


BLACK RHINO

**NECROPSY PATHOLOGY
(BONE MARROW)**

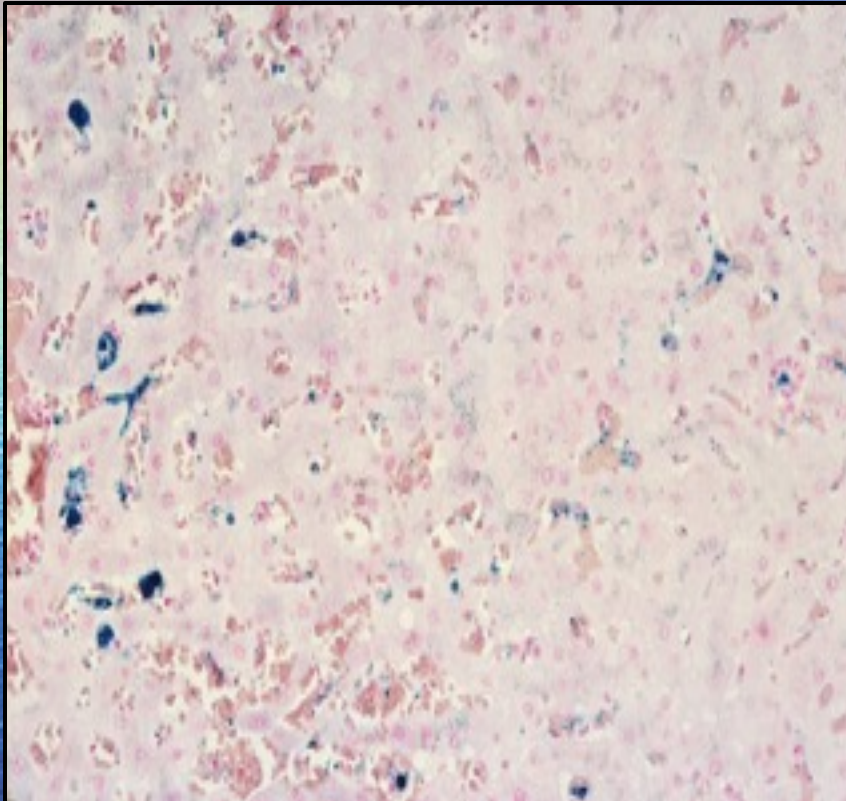


WHITE RHINO

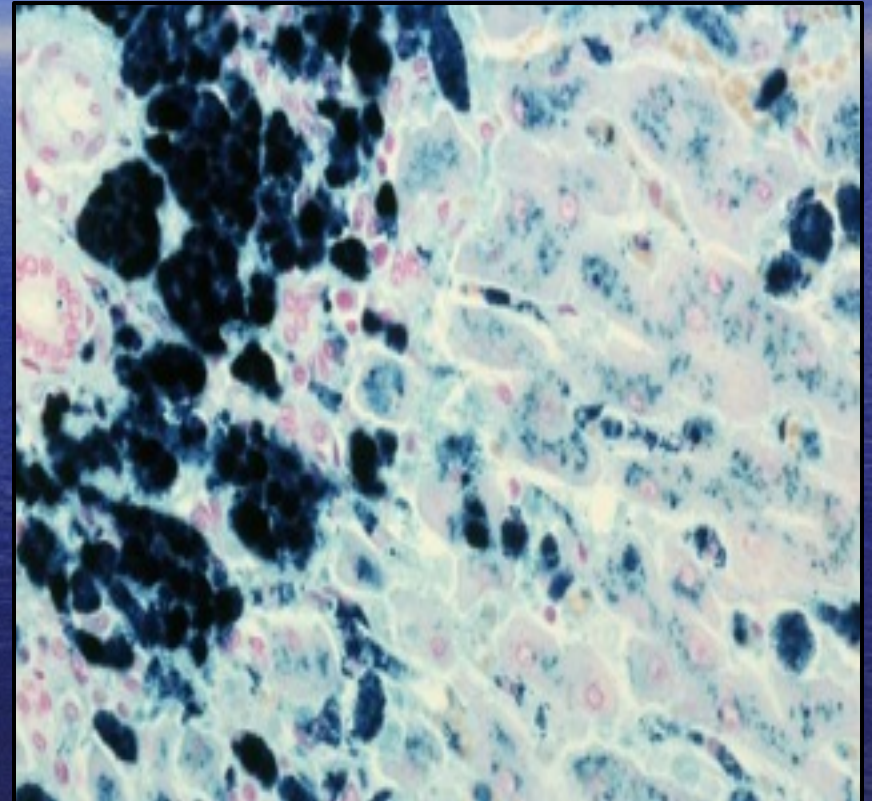


BLACK RHINO

NECROPSY PATHOLOGY (LIVER)

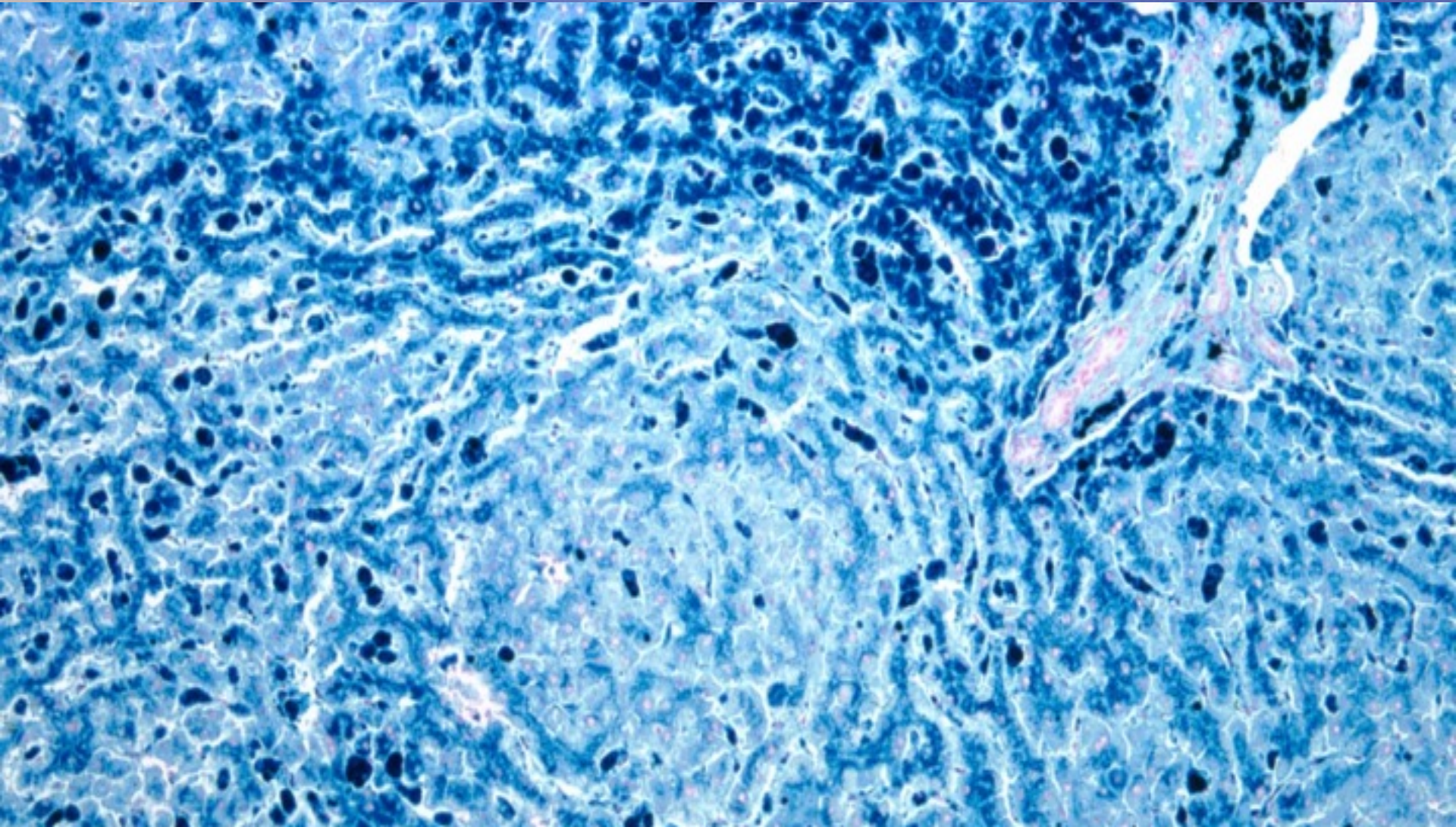


WHITE RHINO



BLACK RHINO

LIVER CARCINOMA IN A BLACK RHINO



9000

6750

4500

2250

0

1-1.5

6.1-10.1

23.1-28.1



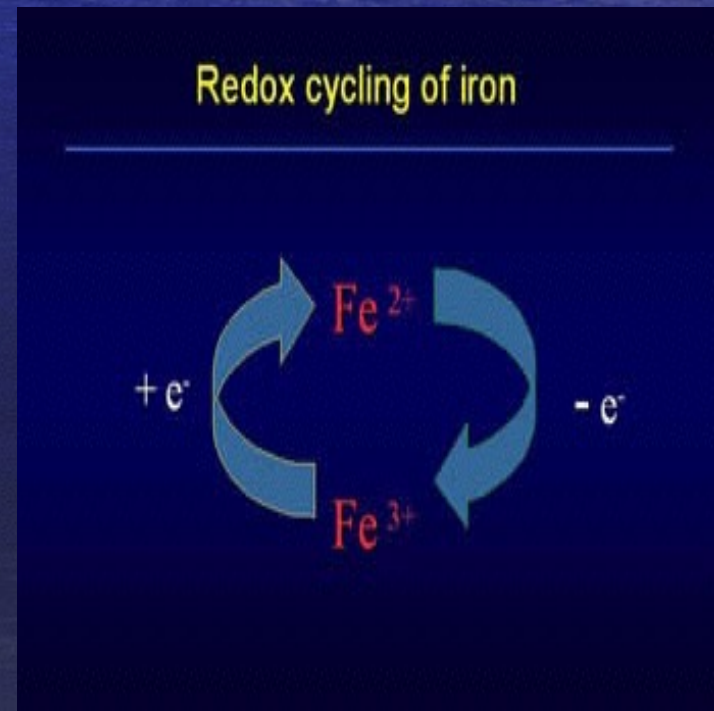
BIOCHEMICAL EFFECTS OF EXCESS FREE IRON

Catalytic production of “Reactive Oxygen Species”

Hydroxyl free radicals,
superoxide, peroxides

Oxidative damage

Molecular, organelle,
cellular, organ



CLINICAL CONSEQUENCES OF IRON OVERLOAD

Cellular damage in multiple
organs → dysfunction, failure
Increased vulnerability to

INFECTIOUS DISEASES

ROLE OF IRON OVERLOAD IN INFECTIOUS DISEASES

**Iron available to invading organisms
(loss of “nutritional immunity”)**

Increased virulence of microbes (TB)

Susceptibility to exotic organisms

Impaired white cell function

CAPTIVITY-INDUCED IRON STORAGE DISEASE

**An anthropogenic pandemic
across multiple taxa....**

Causes & Correctives....?

ETIOLOGIC POSSIBILITIES

NUTRITIONAL FACTORS

Browsers vs. grazers:

**Tannin, fiber, phytates, phenols,
phosphate, L-mimosine, DHBA ?**

GENETIC PREDISPOSITIONS

Molecular regulators of iron:

**Hepcidin, ferroportin,
hemojuvelin, HFE, TfR-2**

MOLECULAR REGULATION OF IRON BALANCE

FERROPORTIN:

Channel for intracellular iron → plasma

HEPCIDIN:

Blocks ferroportin channel

HEPCIDIN DEFICIENCY

LOSS OF FERROPORTIN MODULATION

→ Unrestricted flow of dietary
and storage iron into plasma

→ IRON OVERLOAD DISORDERS
(Hemochromatosis)

EVOLUTIONARY ASPECTS

- 5 B.y.a: Primordial atmosphere=volcanic gases
- 4 B.y.a: H₂O condensation → hydrosphere
- 3 B.y.a: Anaerobic (reducing) atmosphere
- 2 B.y.a: Bacteria & eukaryotes arise
- 1 B.y.a: Blue-green algae & photosynthesis → oxygen, ozone → terrestrial life forms
- 350-230 M.y.a: Oxygen = 35% → 15-21%
- 100 M.y.a: Modern mammals & birds arise
- 50-60 M.y.a: Browser rhinos appear
- 25 M.y.a: Savannah grasslands → grazers arise

PREVENTION & THERAPY OF IRON OVERLOAD

THERAPY

Chelating drugs

PREVENTION

Phlebotomies



PHLEBOTOMY RATIONALE

Blood contains measurable
hemoglobin iron ($\sim 0.5\text{g/L}$)

Phlebotomy induces **slight anemia**

Anemia **mobilizes storage iron**

for new red cell production

Can be quantitatively monitored

RHINO ISD

HISTORICAL CHRONOLOGY

1993: White Oak Conference

1995: Smith et al JZWM

1999: St. Louis Int'l Conference, AAZV

2000: AAZV (Tapirs)

2003: IRKA (Phlebotomy protocol)

2004: AAZV (Phlebotomy protocol)

2005/6: IRKA

2011: Orlando Conference

2012: JZWM Special Supplement

SUMATRAN RHINO CAPTIVE BREEDING PROGRAM

"Emi"

Los Angeles Zoo



PHLEBOTOMIES: COST/BENEFIT ANALYSIS

Potential Costs:

Restraining chute (?)

Staff time: training, performance

Venesection supplies

Laboratory monitoring

“TIPPING POINTS”

POPULATION SUSTAINABILITY

BIRTH/DEATH RATIOS:

> 1 → POPULATION GROWTH

< 1 → EXTINCTION

AFRICAN RHINOS = 1.0 ± 2-6%

HUMAN ISD (HEREDITARY HEMOCHROMATOSIS)

SYMPTOM ONSET = 30-50 yrs

**UNTREATED → LIVER CIRRHOSIS →
CANCER (>60 yrs old = 50%)**

**PHLEBOTOMIES in PRECIRRHOTICS →
NORMAL LIFE SPANS**

PHLEBOTOMIES: POTENTIAL BENEFITS

Long-Term Captives:

Decrease toxic iron overburdens

Increase quality of life

**Avoid euthanasia for organ
dysfunction/failure**

Extend longevity (?)

PHLEBOTOMIES: POTENTIAL BENEFITS

Juveniles & Newly Captive:

Prevent iron accumulation → **ISD**

Increase high quality of life

Extend life expectancy 20-30%

Extend reproduction 1-2 cycles

Alter tipping point → sustainability

SUMMARY

Captivity induces pathological iron overloads (**ISD**) in browsers

Iron toxicity causes cell & organ dysfunction and increases virulence of microorganisms

Periodic phlebotomies can reduce iron loads &/or prevent ISD in young or newly captured animals

MAX PLANCK
1858-1947



“A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather its opponents eventually die, and a new generation grows up that is familiar with it.”

Max Planck

With gratitude to.....

IRKA

International Rhino Foundation

SOS Rhino

C.I.E.S. (Fulbright Foundation)

Morris Animal Foundation

LB Research & Education Foundation

Disney Animal Programs

Multiple Zoos & Conservation Centers