Blood clotting, serum halo rings, and the bloodstains on the Shroud of Turin







#### Whole Blood



#### Whole Blood

# **Plasma Composition**



Water 90% Proteins 7-9% Salts 0.9% Sugar 0.1% Urea 0.03 %

#### Difference between Plasma and Serum





# Blood clotting



# Clot retraction Serum exuded at edges of clot

# Blood clotting



#### "Serum halo"

# Visible under ultraviolet light

# Blood clotting



## "Serum halo"

# Visible under ultraviolet light



#### Serum halo rings indicate that clotted blood was transferred to the cloth



# Natural (White) Light



# Ultraviolet Light



#### White Light

Whole Blood

Cells

Plasma

Serum (after clotting)



#### White Light UV



#### **Blood serum**



#### **Blood serum**





Miller and Pellicori, "Ultraviolet fluorescence Photography of the Shroud of Turin", J. Biological Photography, 49: 3, 1981

#### Camera Filter (L-42e)



Lamp –

Filter (C7-54e)









#### White

UV



#### White

UV

Serum

Halo



## Serum halo ring formation



0 min 10 min 20 min 30 min 40 min

Filter paper added to sample @ time indicated

# Blood on skin transferred after clotting

# White





# UV





#### Blood transferred directly

#### White





## Direct transfer



Q-tip





Control (Clot)

## Blood transferred directly

#### White

JV

Variation in angles Variation in clotting time

## Direct transfer

#### Post-clot (vigorous stirring)



Dried blood (ground, rehydrated)





Control (Clot)



#### Serum halo rings were only observed when clotted (whole) blood was transferred

#### Serum halo rings were not observed when blood was directly transferred

# Skin simulant (ballistics gel)







#### Blood transfer



#### Gelatin (collagen)

#### Connective tissue (ground, hydrated)

#### Bone (ground, hydrated)










## **Blood clotting**



# EDTA Sodium Citrate ( $Na_3C_6H_5O_7$ )



# **Blood clotting**



# Many fruits contain Citric Acid $(C_6H_8O_7)$



## **Ultraviolet light**





# **Ultraviolet light**



## **Ultraviolet light**









## White







### Blood + honey/alcohol mixture

## White

## UV



## Blood + lemon juice

## Bilirubin and UV fluorescence

Human Serum plus Bilirubin

50x



# Serum (UV) WT rat Gunn rat





## WT mice hUGT1 mice







"One cannot simply say that the blood images were painted on afterwards. One would need a constant supply of fresh clot exudates from a traumatically wounded human... and then finally paint a serum contraction ring about every wound. Logic suggests that this is not something a forger or artisan before the present century would not only know how to do, but even know that it was required."

Alan Adler

## Sources of Bilirubin

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Plants Make Bilirubin, Too

Scientists discover that the colorful tetrapyrrole-based pigment derived from heme in animals also occurs in colorful plant seeds

By Elizabeth K. Wilson

### SCIENCE & TECHNOLOGY Concentrates

Plants Make Bilirubin, Too

Nerve Receptor Binds Hallucinogenic Ligand

Magnetic Doping Speeds Up Protein NMR

Designer Surfactant For Micellar Catalysis

Hemoglohin Helner Aids

Scientists have discovered that the pigment responsible for the brilliant orange seed arils of the bird-ofparadise tree is bilirubin, a molecule thought to exist only in animals (*J. Am. Chem. Soc.*, DOI: **10.1021/ja809065g**).



J Am Chem Soc. 2009 Mar 4;131(8):2830. doi: 10.1021/ja809065g

#### Animal pigment bilirubin discovered in plants.

Pirone C<sup>1</sup>, Quirke JM, Priestap HA, Lee DW.

Author information

#### Abstract

The bile pigment bilirubin-IXalpha is the degradative product of heme, distributed among mammals and some other vertebrates. It can be recognized as the pigment responsible for the yellow color of jaundice and healing bruises. In this paper we present the first example of the isolation of bilirubin in plants. The compound was isolated from the brilliant orange-colored arils of Strelitzia nicolai, the white bird of paradise tree, and characterized by HPLC-ESMS, UV-visible, (1)H NMR, and (13)C NMR spectroscopy, as well as comparison with an authentic standard. This discovery indicates that plant cyclic tetrapyrroles may undergo degradation by a previously unknown pathway. Preliminary analyses of related plants, including S. reginae, the bird of paradise, also revealed bilirubin in the arils and flowers, indicating that the occurrence of bilirubin is not limited to a single species or tissue type.



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#### MARCH 11, 2009

## First discovery of 'animals-only' pigment bilirubin in plants

In a first-of-its-kind discovery that overturns conventional wisdom, scientists in Florida are reporting that certain plants — including the exotic "White Bird of Paradise Tree" -- make bilirubin. Until now, scientists thought that pigment existed only in animals. The finding may change scientific understanding of how the ability to make bilirubin evolved, they say in a report in the *Journal of the American Chemical Society*.

## Bird of paradise



## Sources of Bilirubin



<u>AoB Plants</u>. 2010; 2010: plq020. Published online 2010 Oct 28. doi: <u>10.1093/aobpla/plq020</u> PMCID: PMC3000704 PMID: <u>22476078</u>

### Bilirubin present in diverse angiosperms

Cary Pirone,<sup>1,\*</sup> Jodie V. Johnson,<sup>2</sup> J. Martin E. Quirke,<sup>3</sup> Horacio A. Priestap,<sup>1</sup> and David Lee<sup>1</sup>

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### Abstract

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### Background and aims

Bilirubin is an orange-yellow tetrapyrrole produced from the breakdown of heme by mammals and some other vertebrates. Plants, algae and cyanobacteria synthesize molecules similar to bilirubin, including the protein-bound bilins and phytochromobilin which harvest or sense light. Recently, we discovered bilirubin in the arils of *Strelitzia nicolai*, the White Bird of Paradise Tree, which was the first example of this molecule in a higher plant. Subsequently, we identified bilirubin in both the arils and the flowers of *Strelitzia reginae*, the Bird of Paradise Flower. In the arils of both species, bilirubin is present as the primary pigment, and thus functions to produce colour. Previously, no tetrapyrroles were known to generate display colour in plants. We were therefore interested in determining whether bilirubin is broadly distributed in the plant kingdom and whether it contributes to colour in other species.

## Sources of Bilirubin





Hedychium coronarium



Costus lucanusianus



Heliconia collinsiana



Strelitzia reginae



AoB Plants. 2010; 2010: plq020. Published online 2010 Oct 28. doi: 10.1093/aobpla/plq020 PMCID: PMC3000704 PMID: 22476078

#### Bilirubin present in diverse angiosperms

Cary Pirone, 1,\* Jodie V. Johnson, 2 J. Martin E. Quirke, 3 Horacio A. Priestap, 1 and David Lee



### Non-image

### Image

## Bloodstain in Image area













## Blood 1<sup>st</sup> Image 2nd

## **Image Formation**



## **Image Formation**









### What if?

### Blood + x

## x is an oxidizing/reducing agent









### Summary

The presence of serum "halos" could be observed without uv light, but visibility was certainly enhanced by it Serum halos were observed only when clotted blood was transferred Serum halos were not observed when blood was directly transferred\* \*Halos were seen in direct transfer when blood + additives was used



### Summary cont.

Increased bilirubin in serum is associated with enhanced uv fluorescence

> It was noted that blood is not the only source of bilirubin

The action of an oxidizing/reducing agent in the interpretation of protease treatment experiments was suggested for further thought