

# BIOMEDEA III

Stuttgart, 22 – 25 September 2005

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International Symposium on  
Patient Safety

## Increase in Patient Safety by Blood Cryopreservation Technology

Günter Rau

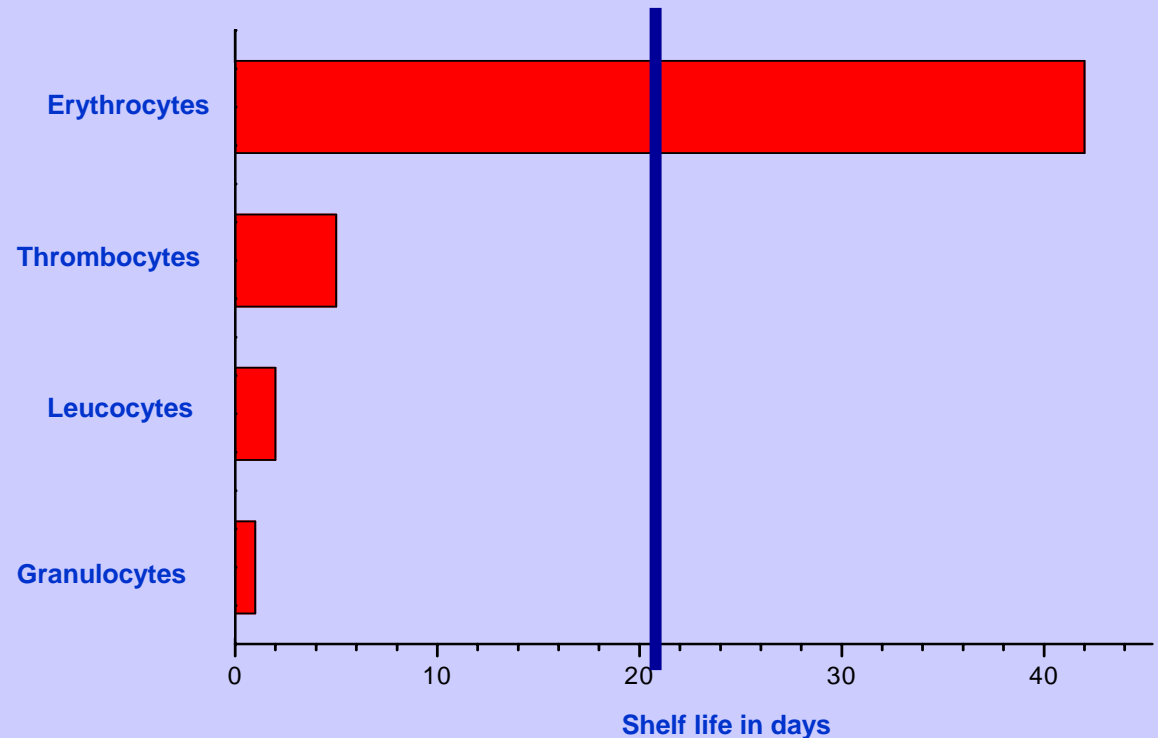
# Cryopreservation: Why?

- Transfusion medicine:

Biomaterial: Blood Cells in Suspension (Erythrocytes, Lymphocytes, Thrombocytes)

- Aims:
- Safe homologous RBCs
  - Accumulating autologous Red Blood Cells
  - Depots of stored blood
  - Rare blood types (10%, very rare 2 %)

Storage life in vitro at 4°C:



# What are the problems today?

- Shortage of blood (Summer vacation, hazards,...)
- Shortage of “safe” blood donors (no infection, no “travelling”)
- Rare/very rare blood types: Availability?
- Autologous blood supply: storage duration too short
- Infected blood:
  - Expensive screening
  - Diagnostic Window **6 months or more**
  - **not for all potential infections screened**
- Bacterial contamination
- Immunological reactions
- **WHO: Each country has to be self-contained in blood supply**
- **10% of blood bags are discarded !!!**

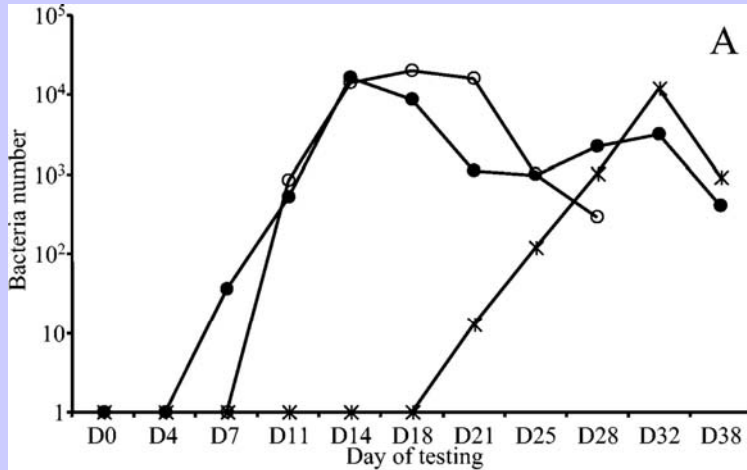
## Impact of Donor Travel Deferrals

- **BSE/vCJD Deferrals – May and October ‘02**
  - Projected loss 5.0% nationwide
  - Actual loss not directly measurable
  - Major component of industry more restrictive
- **Disproportionate impacts of travel deferrals**
  - Coastal cities: 50% ↑ donor loss (e.g. NYC and SF)
  - Rural US: 50% ↓ impact
  - New York area “Euroblood” lost by pan-European deferral
  - Military bases
  - TSEAC requested supply monitoring and assessment

# Morbidity and Mortality?

Increase of Morbidity and Mortality:  $\longrightarrow$  Bacterial Growth

$\longrightarrow$  Storage Lesion



Storage lesion:

“RBCs undergo time-dependent metabolic, biochemical, and molecular changes”

Clinically:

“age of blood has been associated with

multiple organ failure, postoperative pneumonia and wound infection (sepsis)”

# Transfusion Risks: Infections

<b>Hepatitis A:</b>	<b>No check in US</b>	
<b>Hepatitis B:</b>	<b>1 : 66,000</b>	<b>( 200 cases )</b>
<b>Hepatitis C:</b>	<b>1 : 121,000</b>	<b>( 100 cases)</b>
<b>HIV</b>	<b>1 : 1,000,000</b>	<b>(12 to 15 cases)</b>
<b>HTLV-1:</b>	<b>1 : 641,000</b>	
<b>Hepatitis G:</b>	<b>1 : 100</b>	<b>not dangerous at present (West African Children: 15%)</b>

**Herpes Virus: ca. 40% are infected**

**Total number of Transfusions RBCs: ca. 12 Mio/Year  
in USA**

**The next virus will come for sure  
And: we do not know him!!!**

# Blood Shortage: Depots

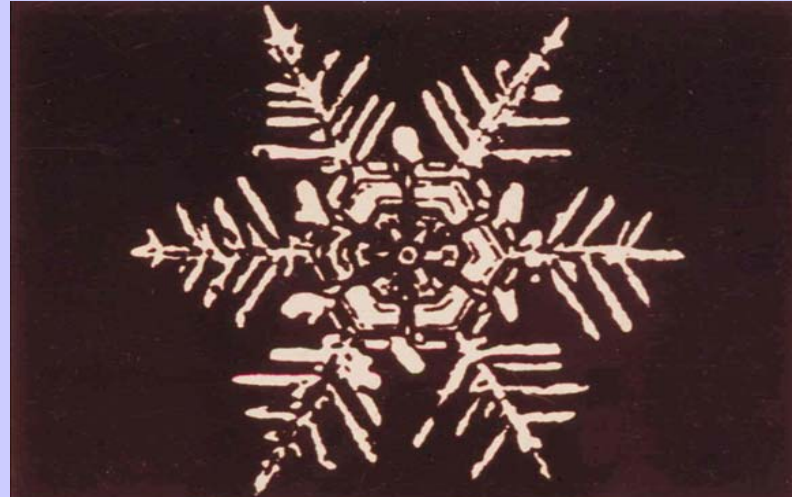
We can cryopreserve fresh frozen blood and store it at any volume numbers of blood bags

We can provide autologous blood supply without any time constraints (the best!!)

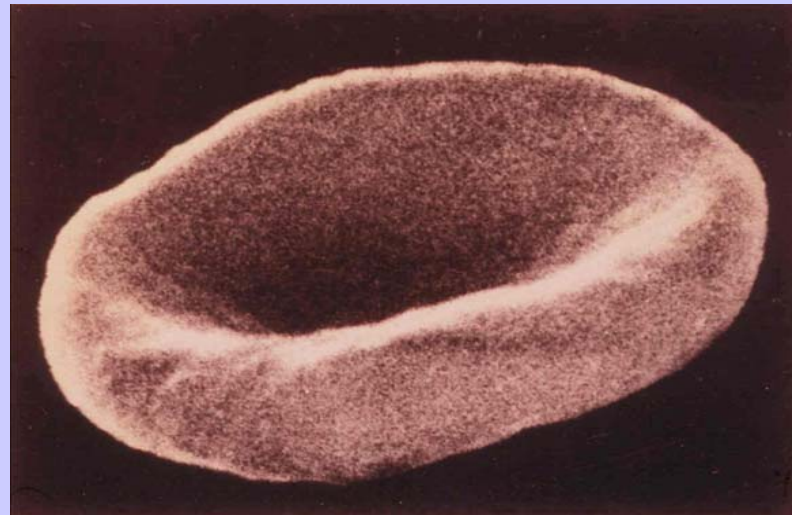
We can considerably reduce the risk of infection even with new and unknown virus types

We can “close the diagnostic window” of 6 months and we can detect antibodies  
without “knowing” the virus

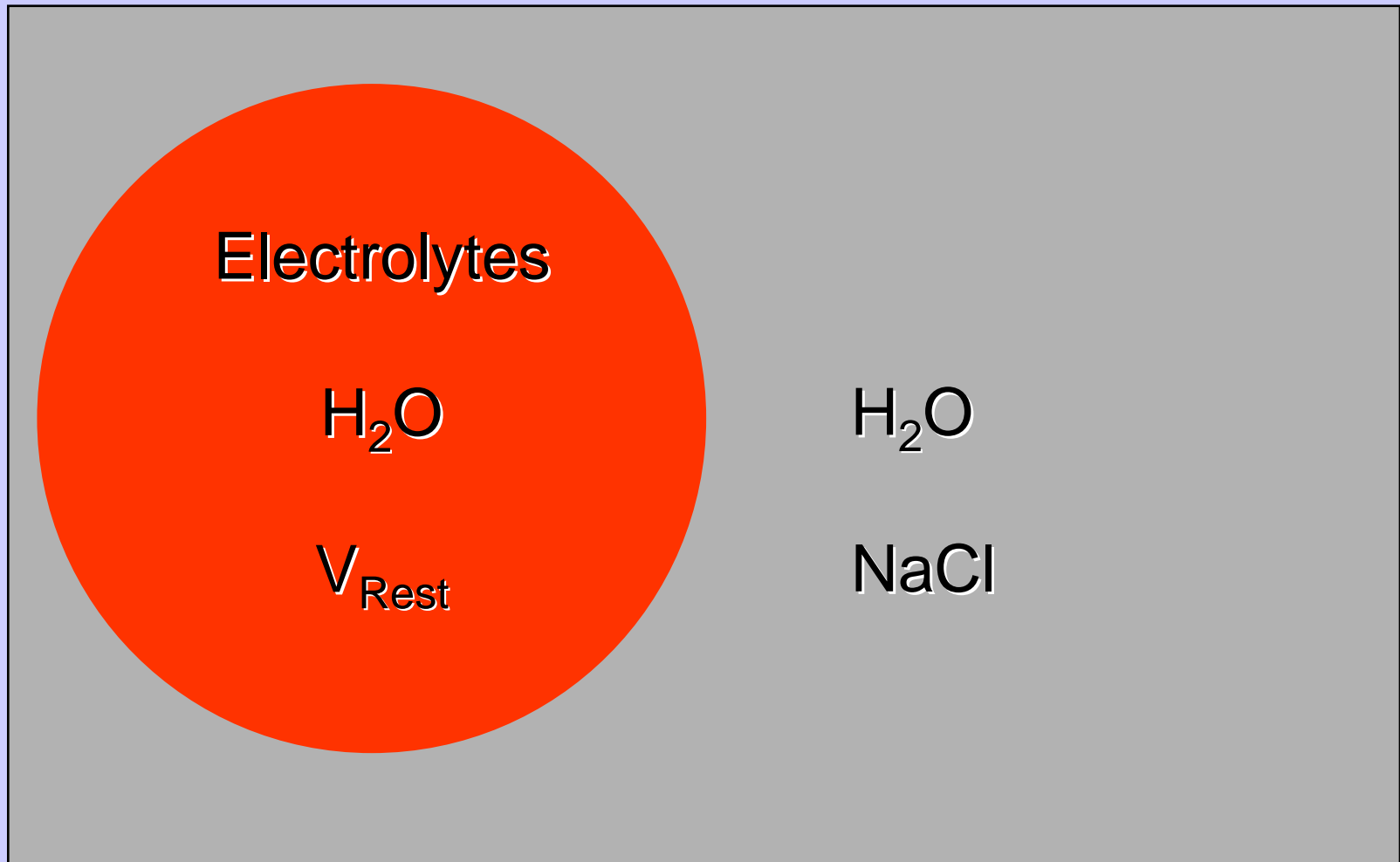
## Crystal Formation



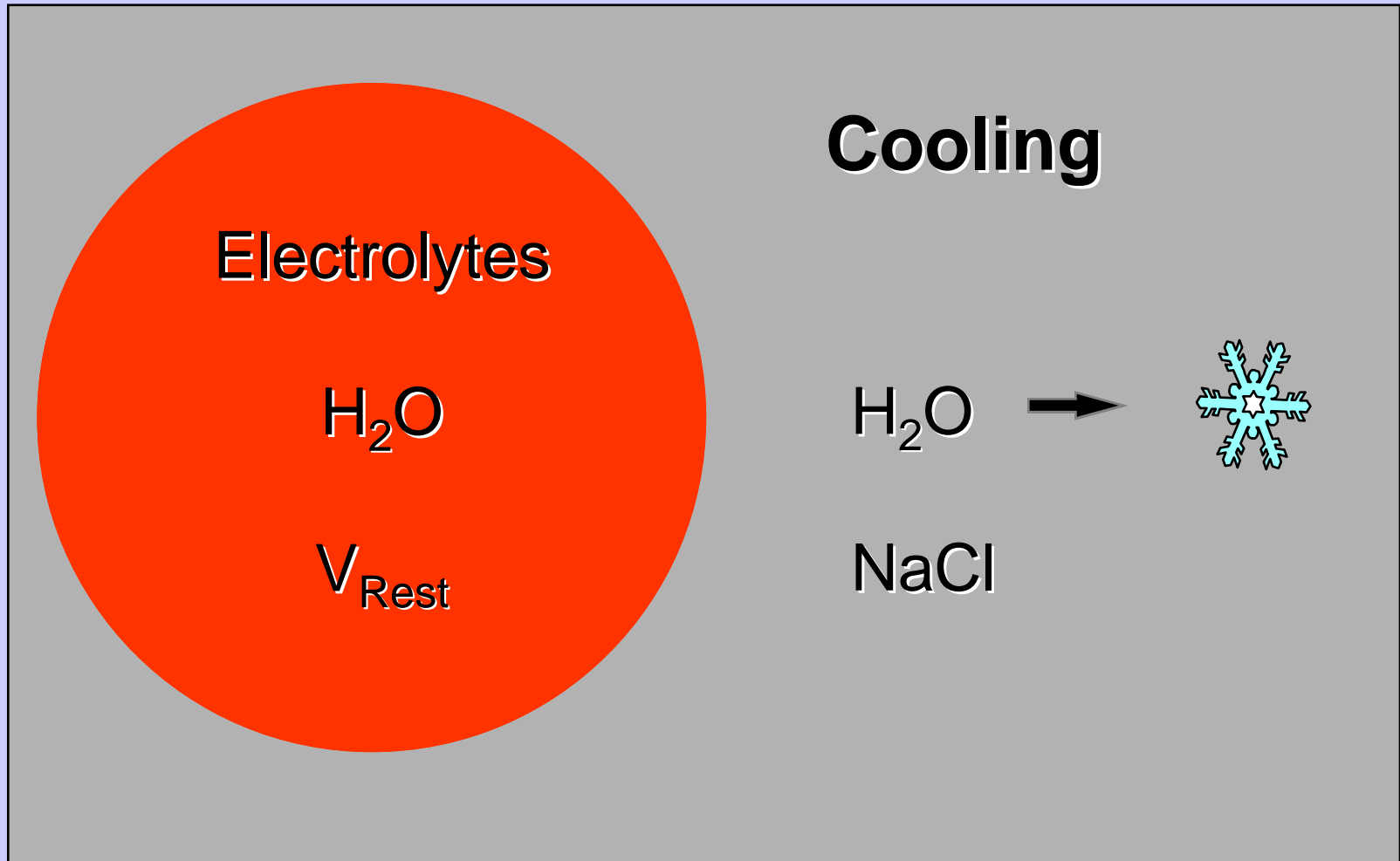
What happens  
with the Cell??



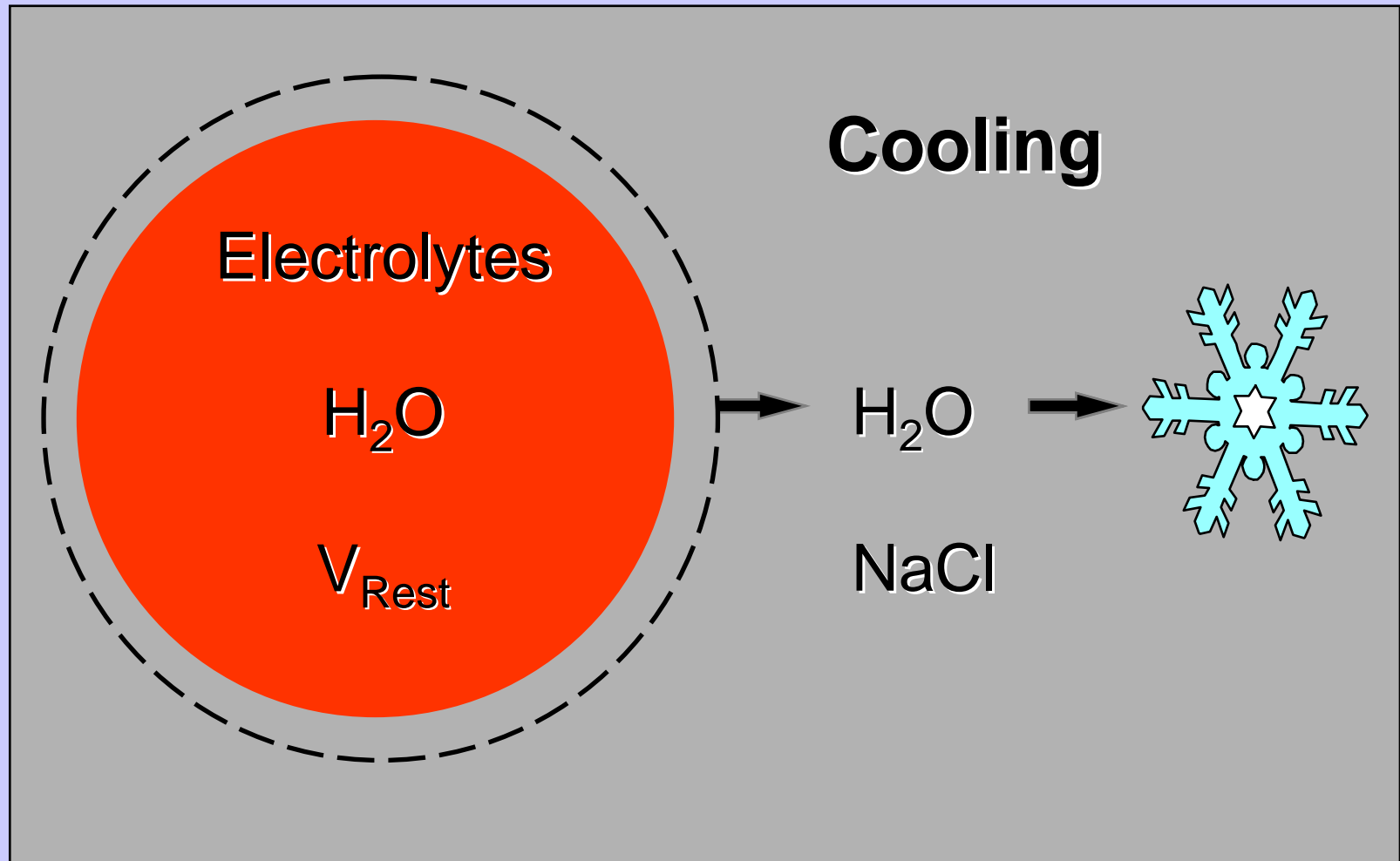
# Osmotic Shrinkage During Freezing I



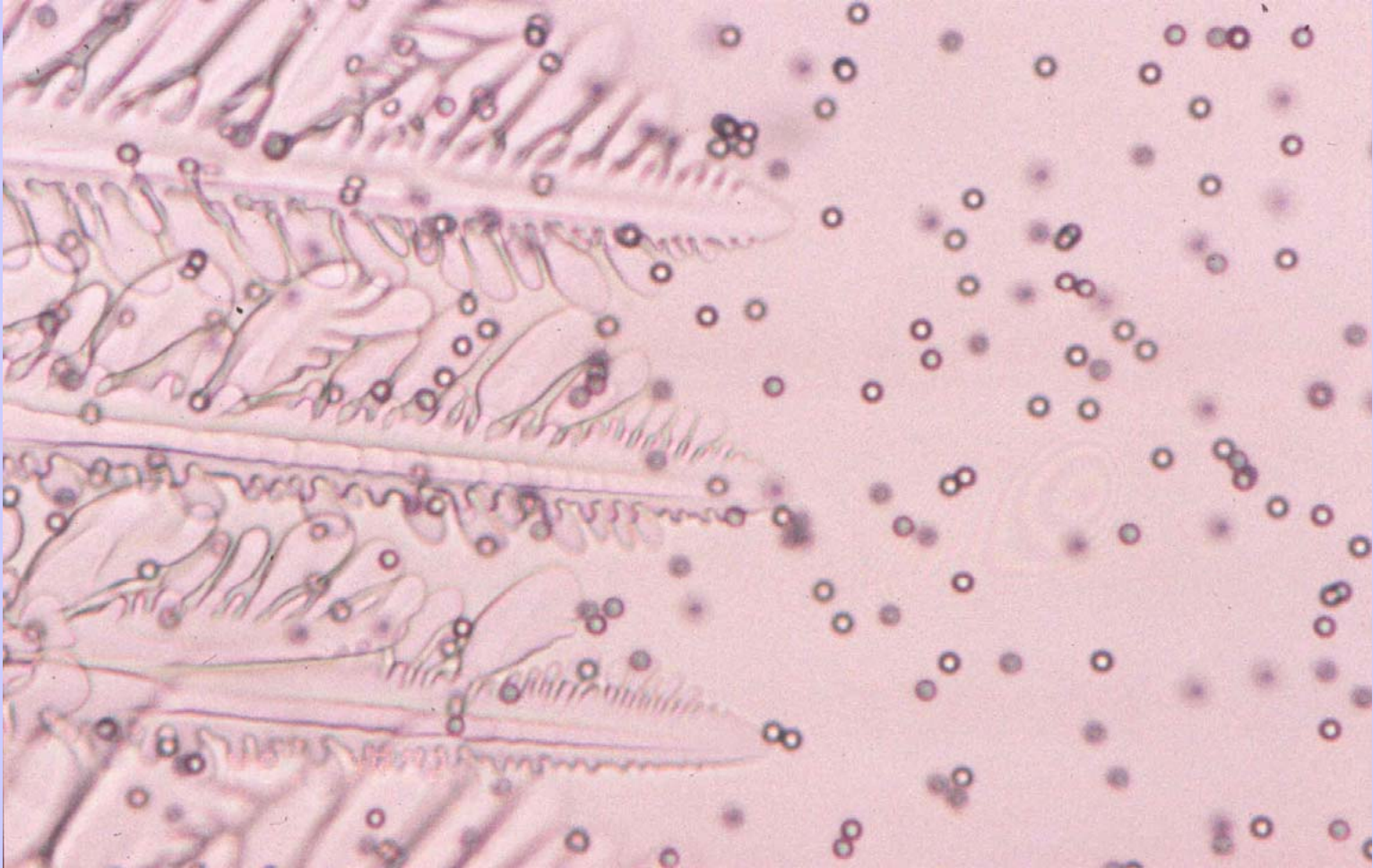
# Osmotic Shrinkage During Freezing II



# Osmostic Shrinkage During Freezing III



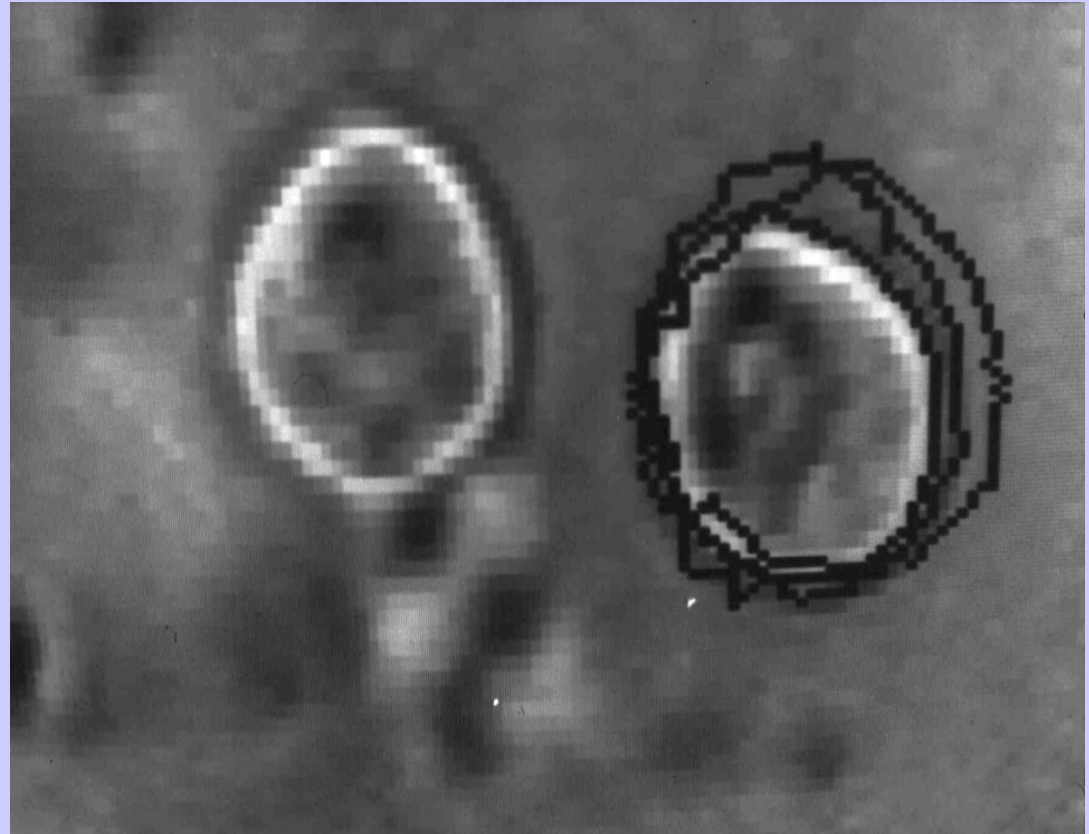
# Erythrocytes under osmotic stress



# Cell shrinkage during Freezing Process

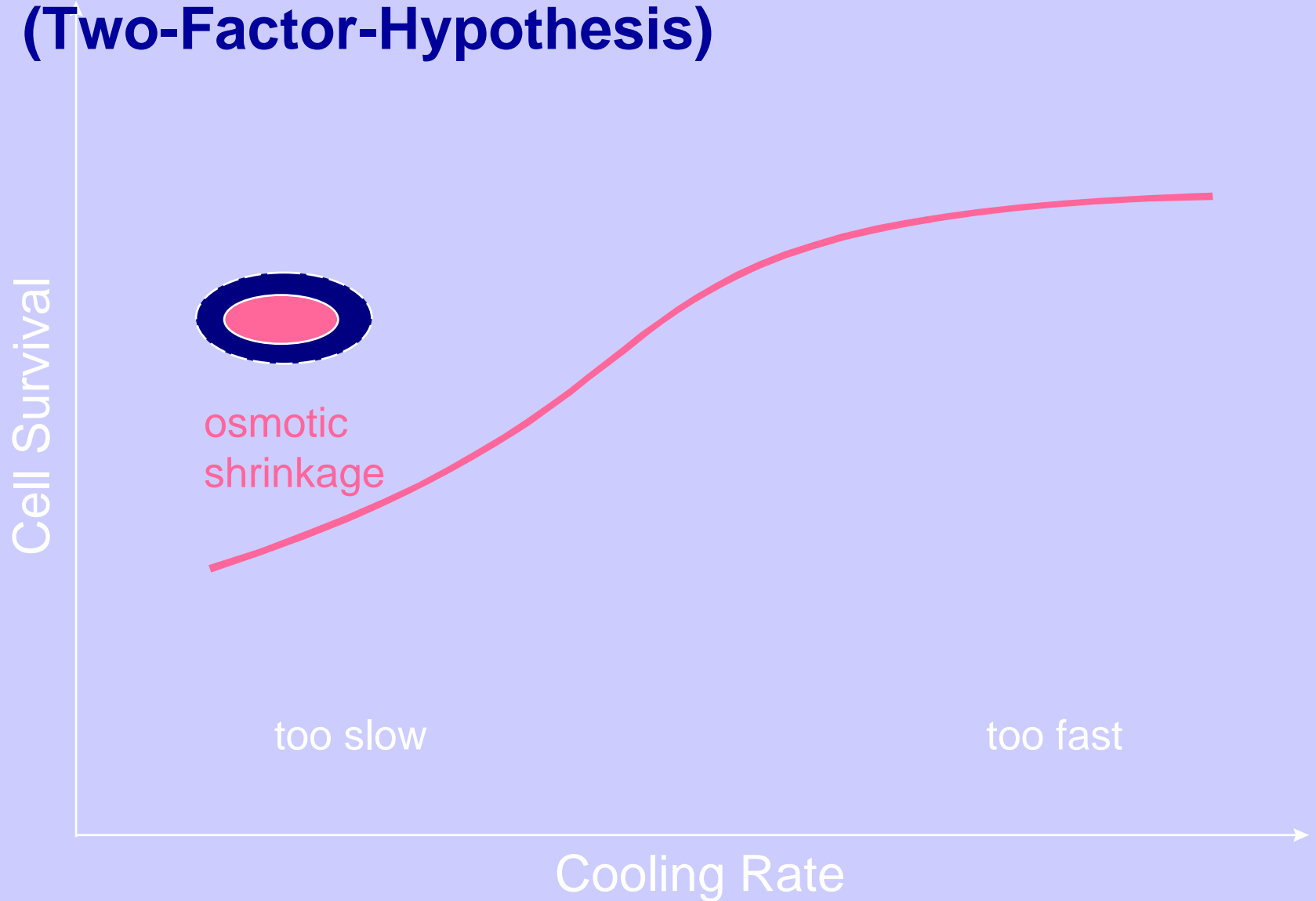
## Microscopic Measurement in the Volume Decrease

Ice formation causes increase of the extracellular saline concentration

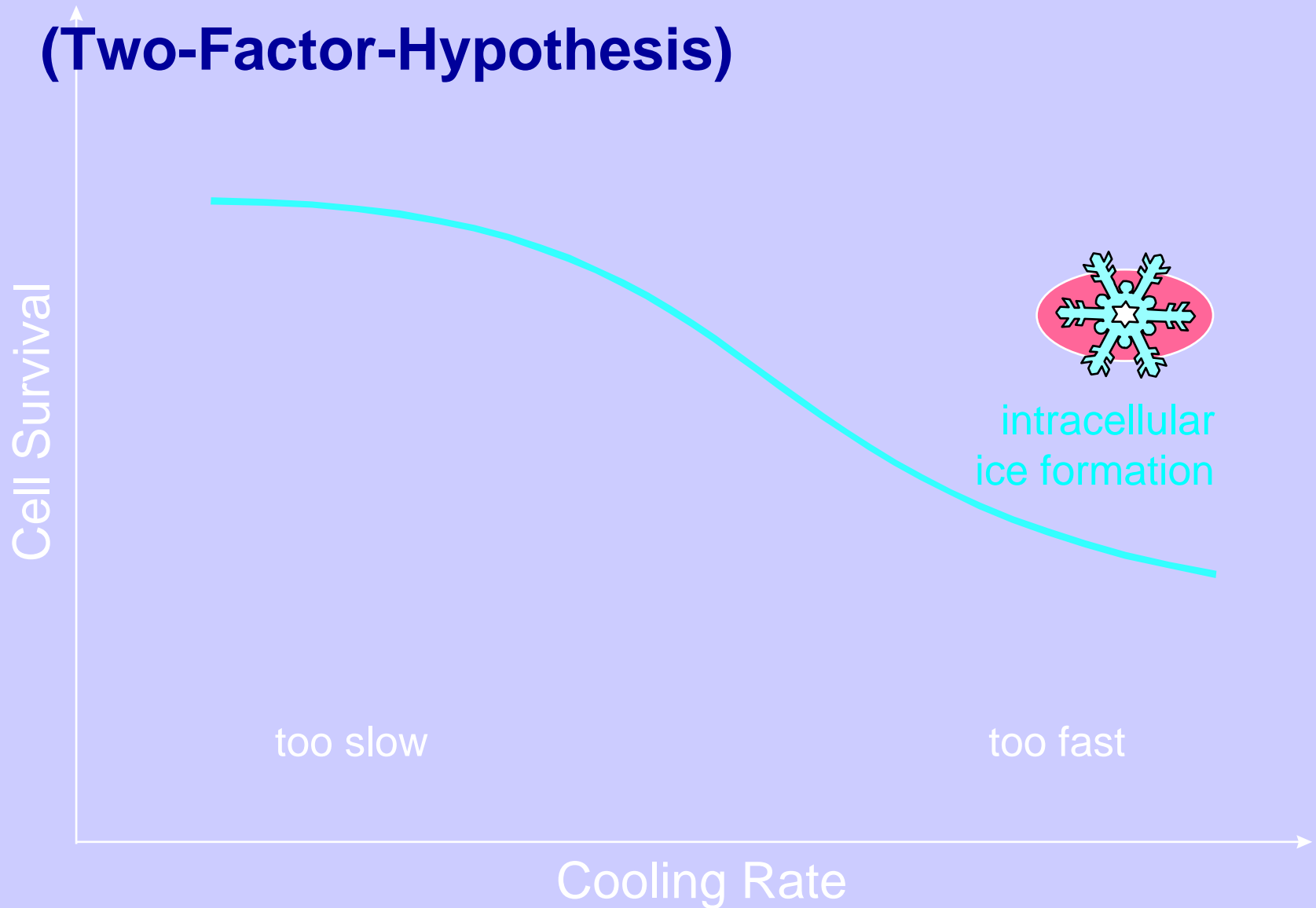


# Cell Damage during Freezing I

(Two-Factor-Hypothesis)

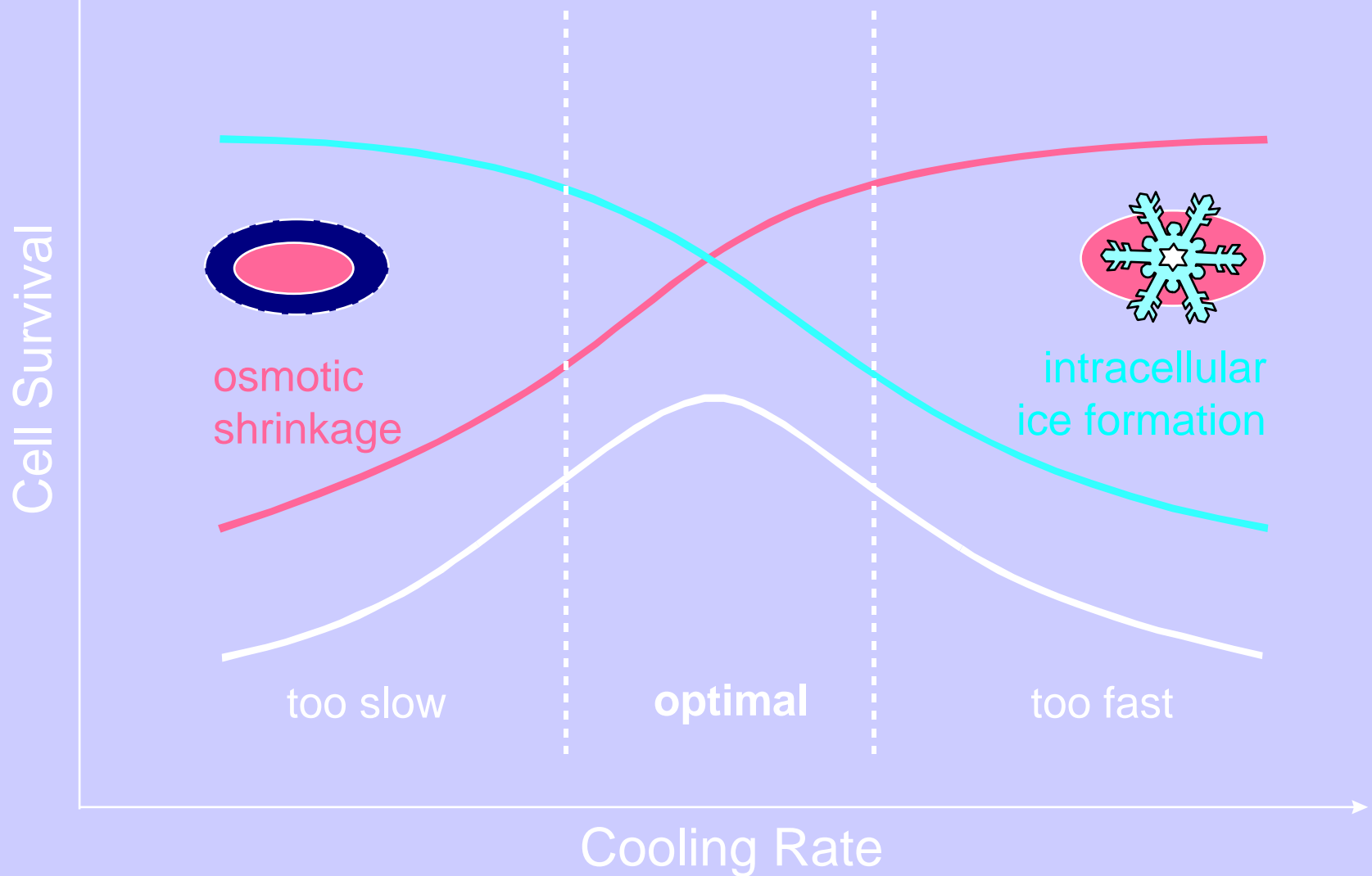


# Cell Damage during Freezing II

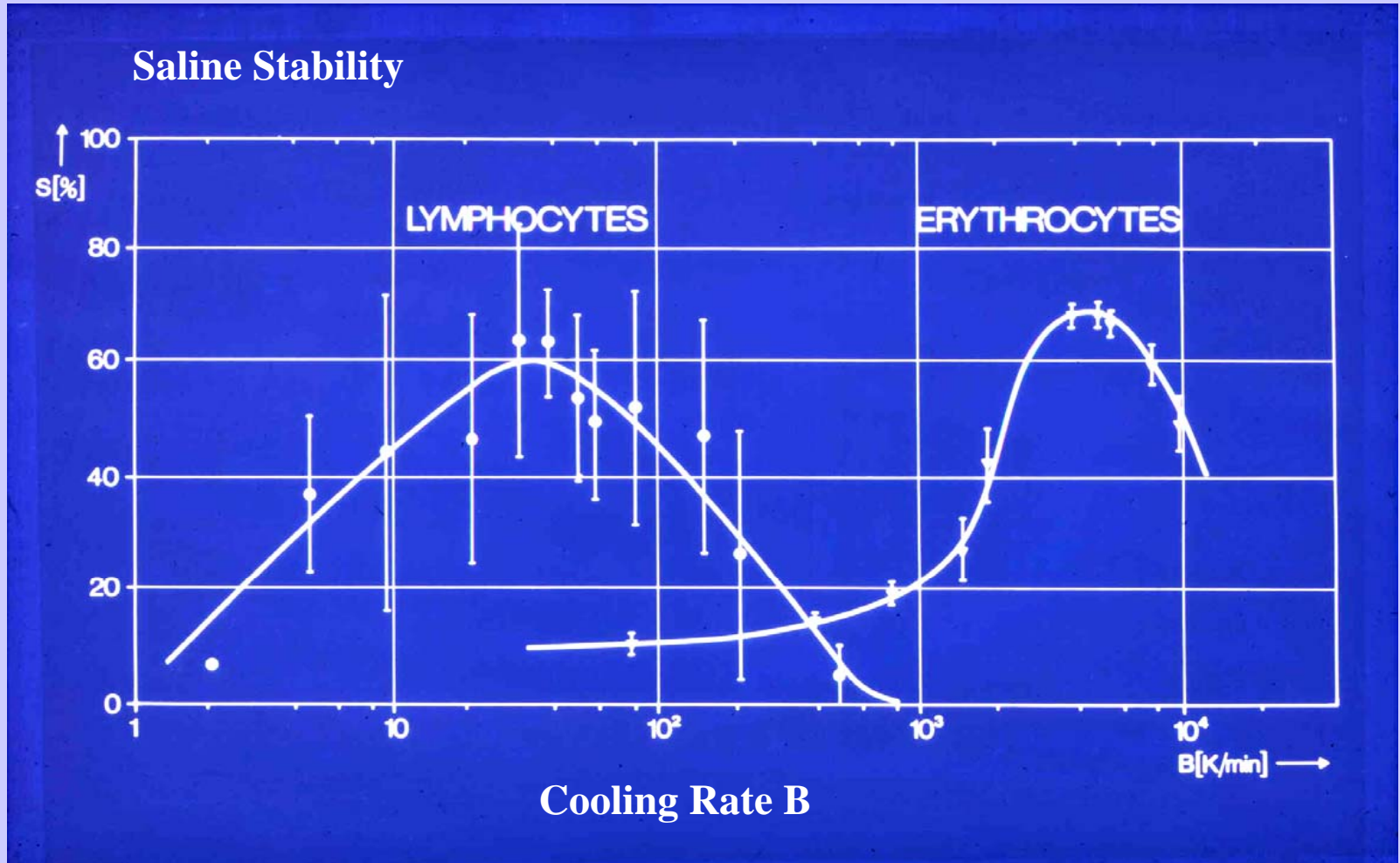


# Cell Damage during Freezing III

## (Two-Factor-Hypothesis)

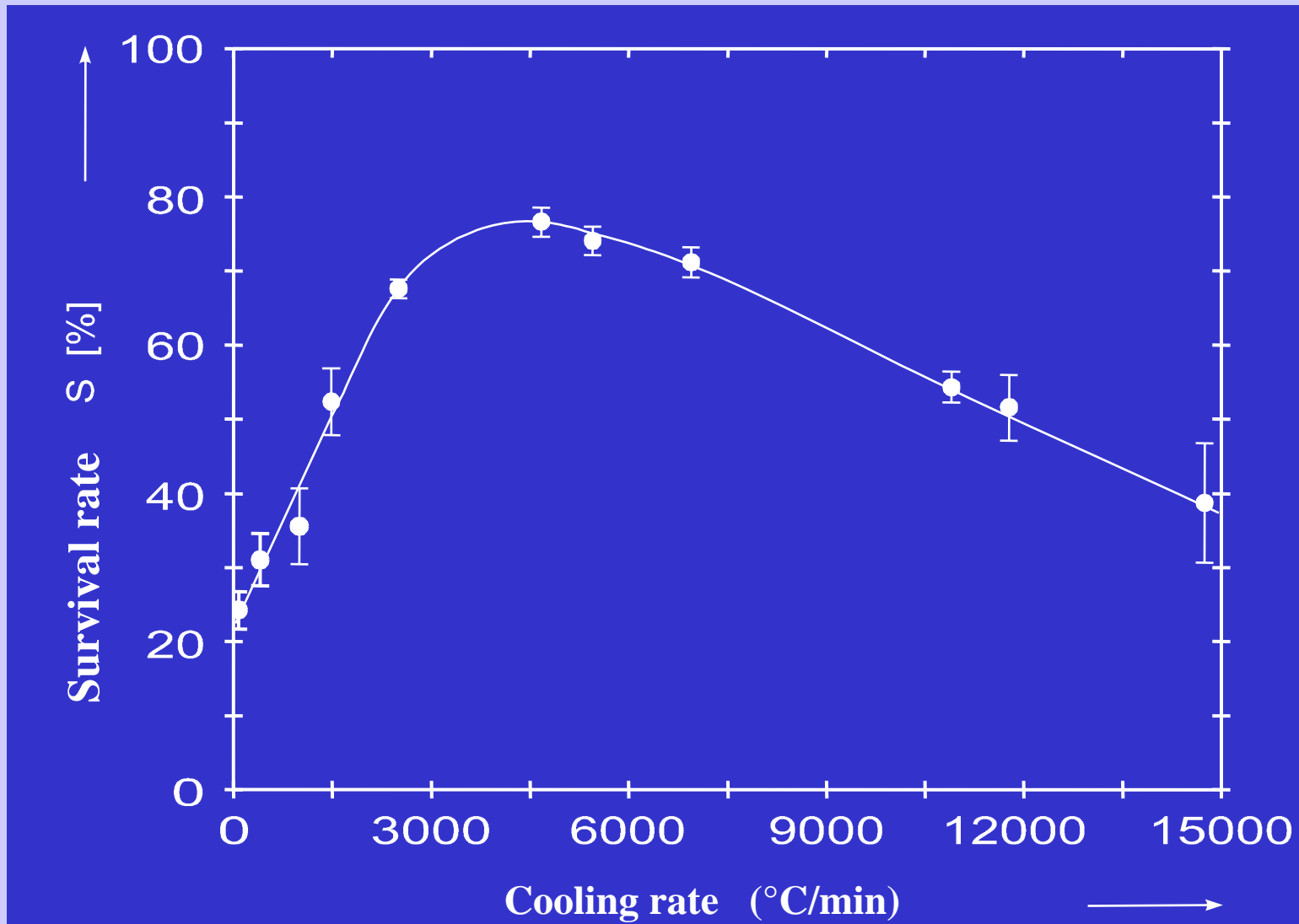


# „Survival Rate“ (Saline Stability)

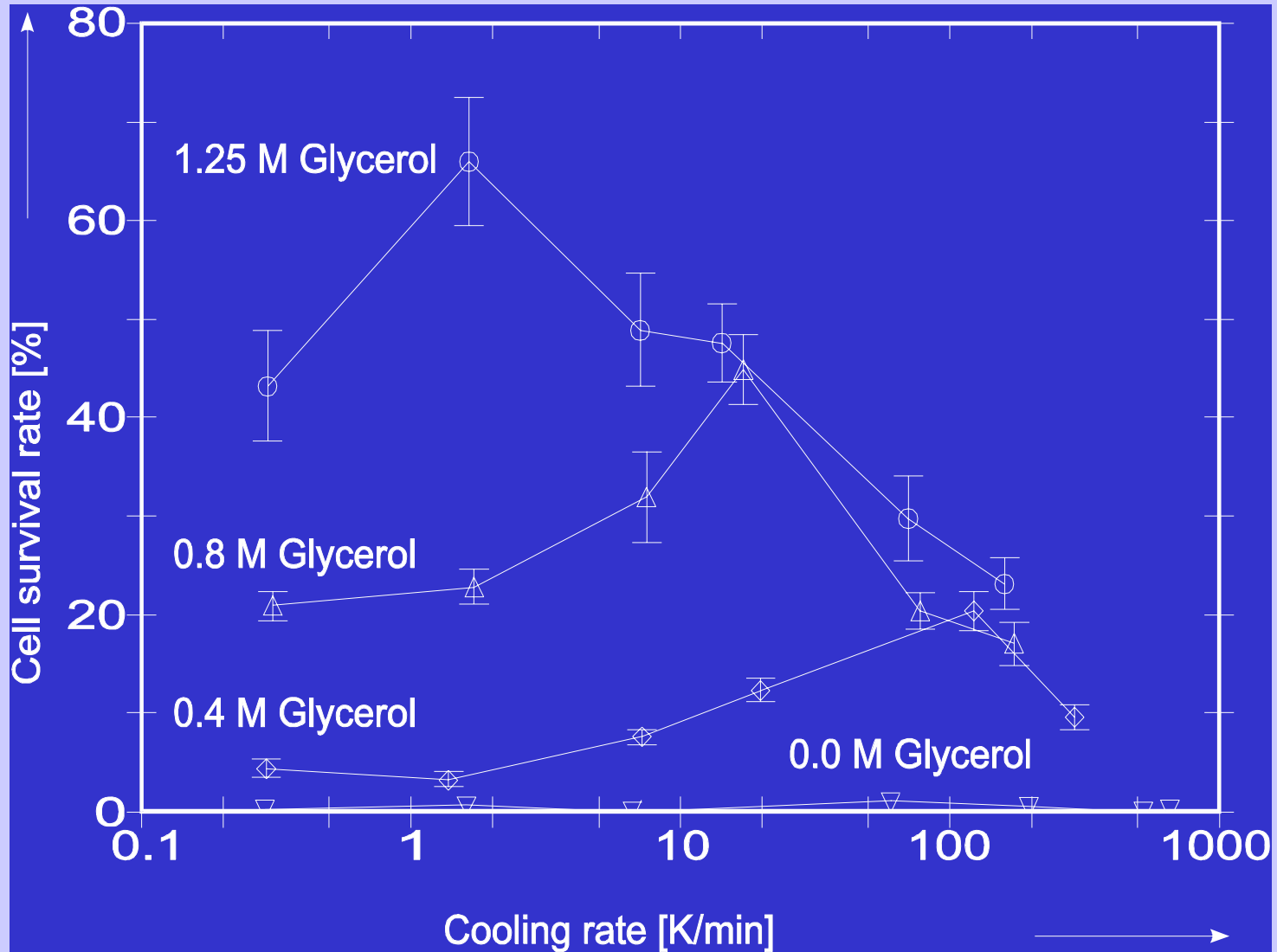


# Survival Rate of Erythrocytes with Cooling Rate

## 0.9 % Saline



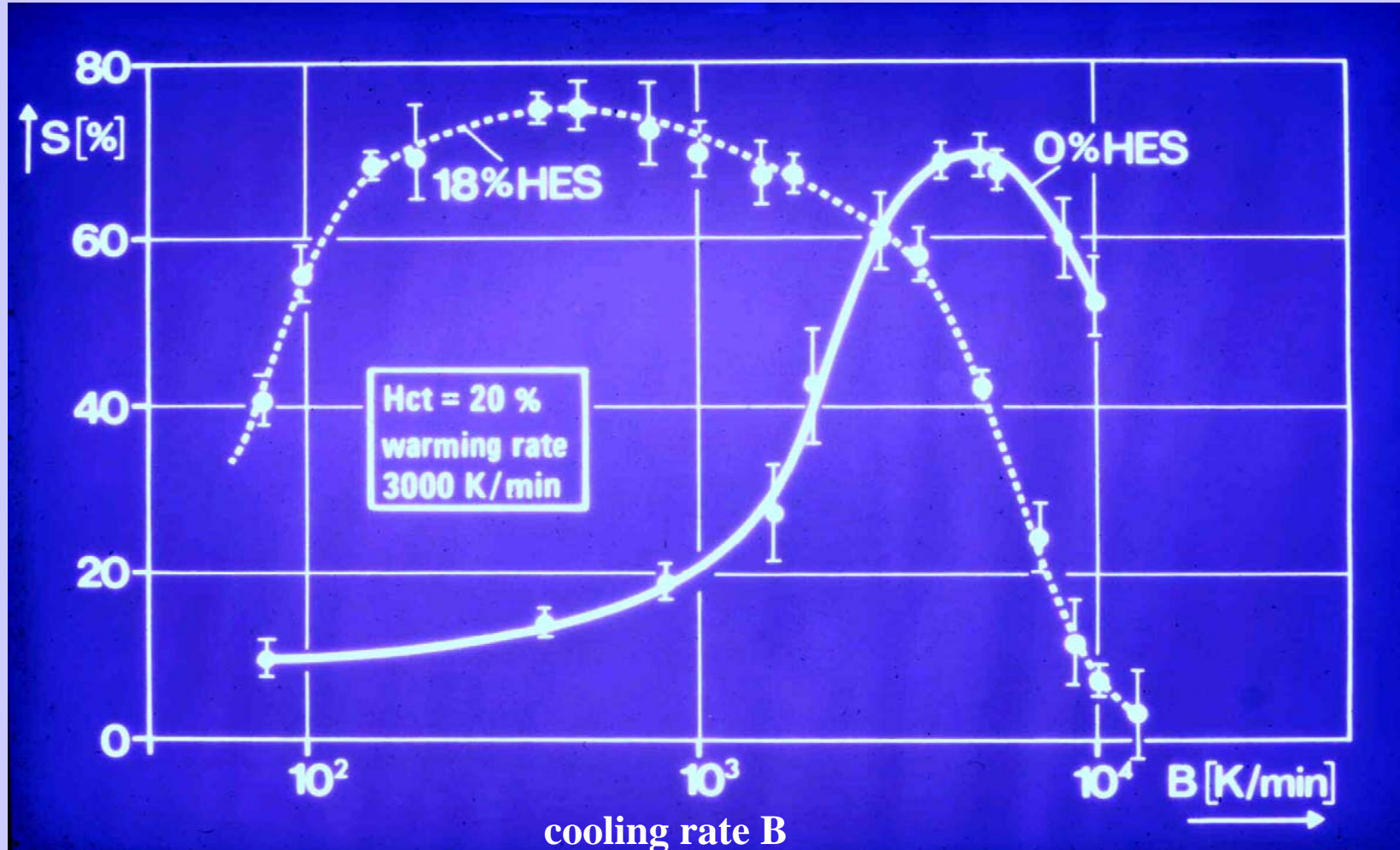
# Protective Effect of Cryoprotectants



# Effect of HES as a Cryoprotectant

- Hydroxyethyl starch:    - non toxic  
                                 - not penetrating the membrane

1981



# Freezing Bag (leakage resistant)

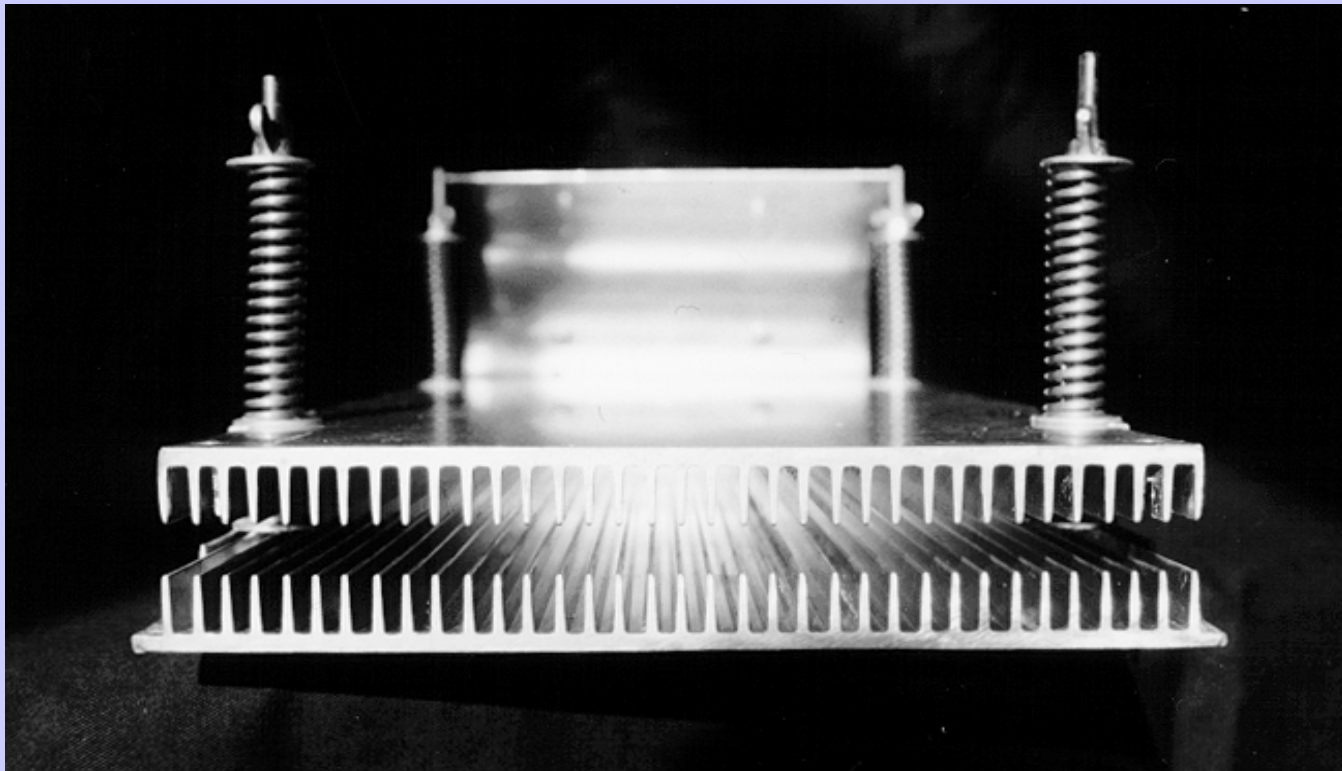
Filled with RBC blood (liquid)



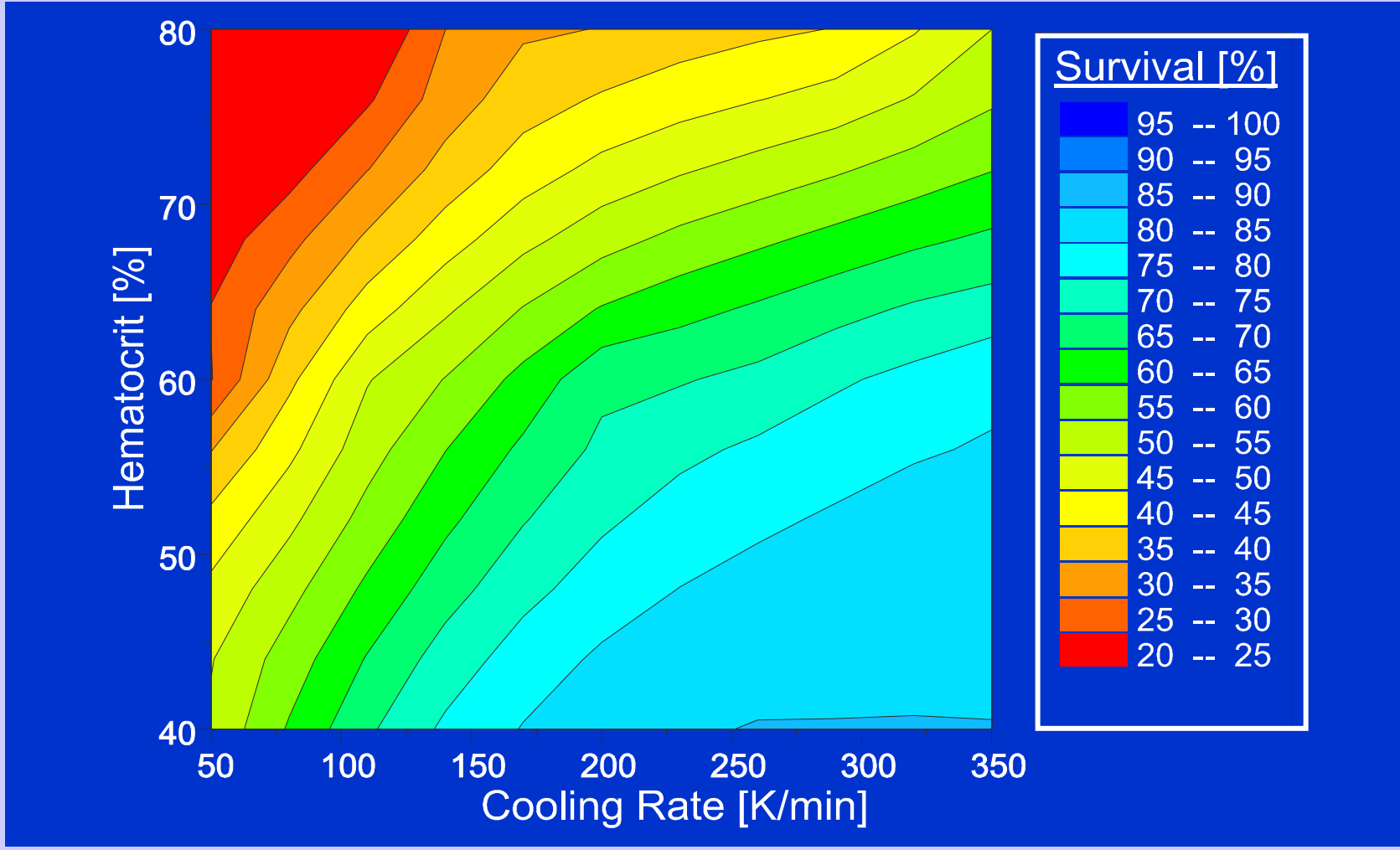
# Freezing Container for RBC Blood Bags

Optimized for blood bag with defined volume:

- high cooling rates
- defined geometry (  $d < 5 \text{ mm}$  )



# Survival Rate for Erythrocytes (bulk volume)



Any portion of the sample must be „blue“



# Freezing of Erythrocytes in Blood Bag (LN2)

Freezing Container with Blood Bag

Optimized for RBC Preservation Bags

Immersed in LN2



# Cryo-Blood Bag with Erythrocyte (concentrated)

**Bulk volumes:**

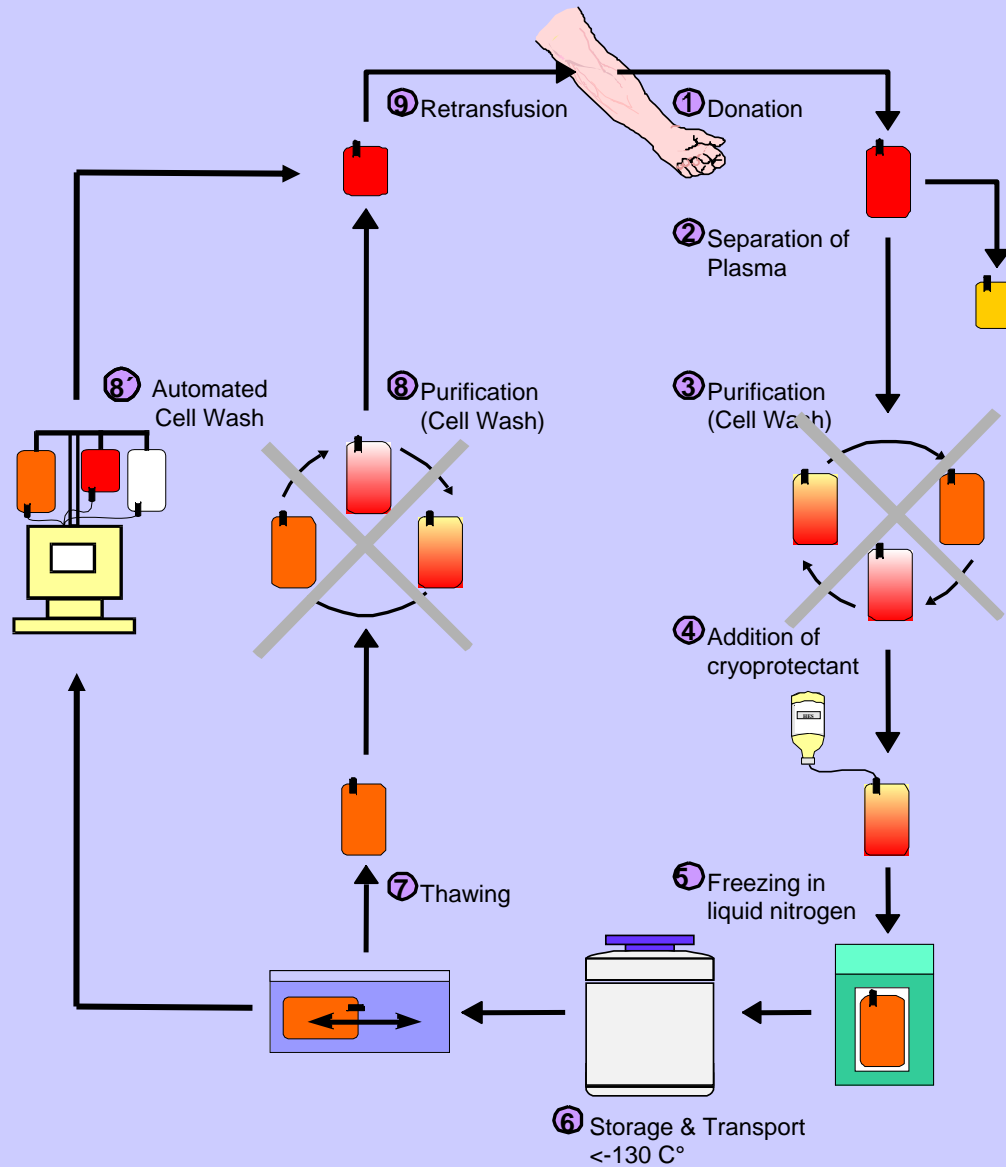
- Defined geometry
- thickness: 2-5 mm



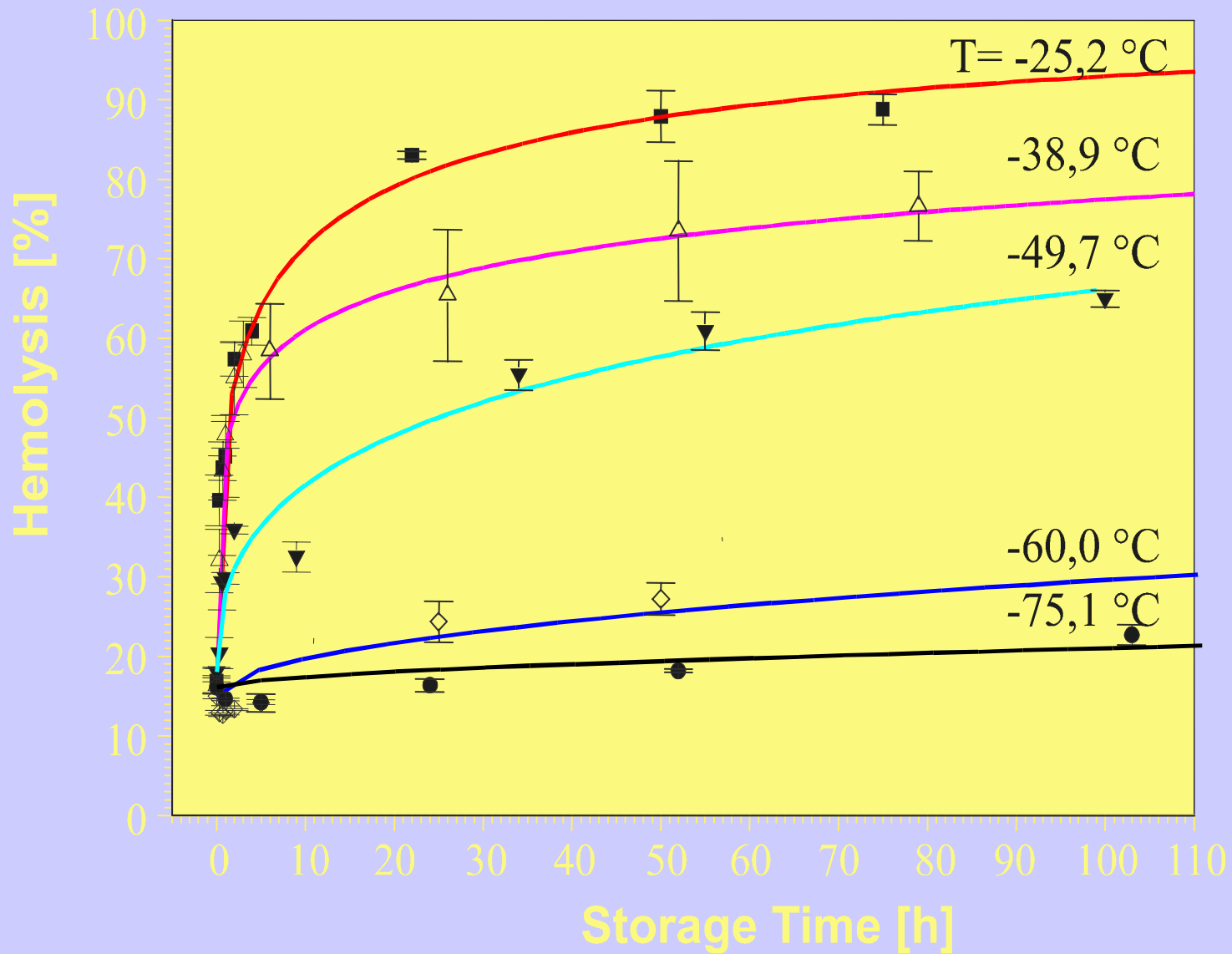
erythrocytes can not be  
cultured

(no re-duplication!)

# Freeze-Storage-Thawing Process



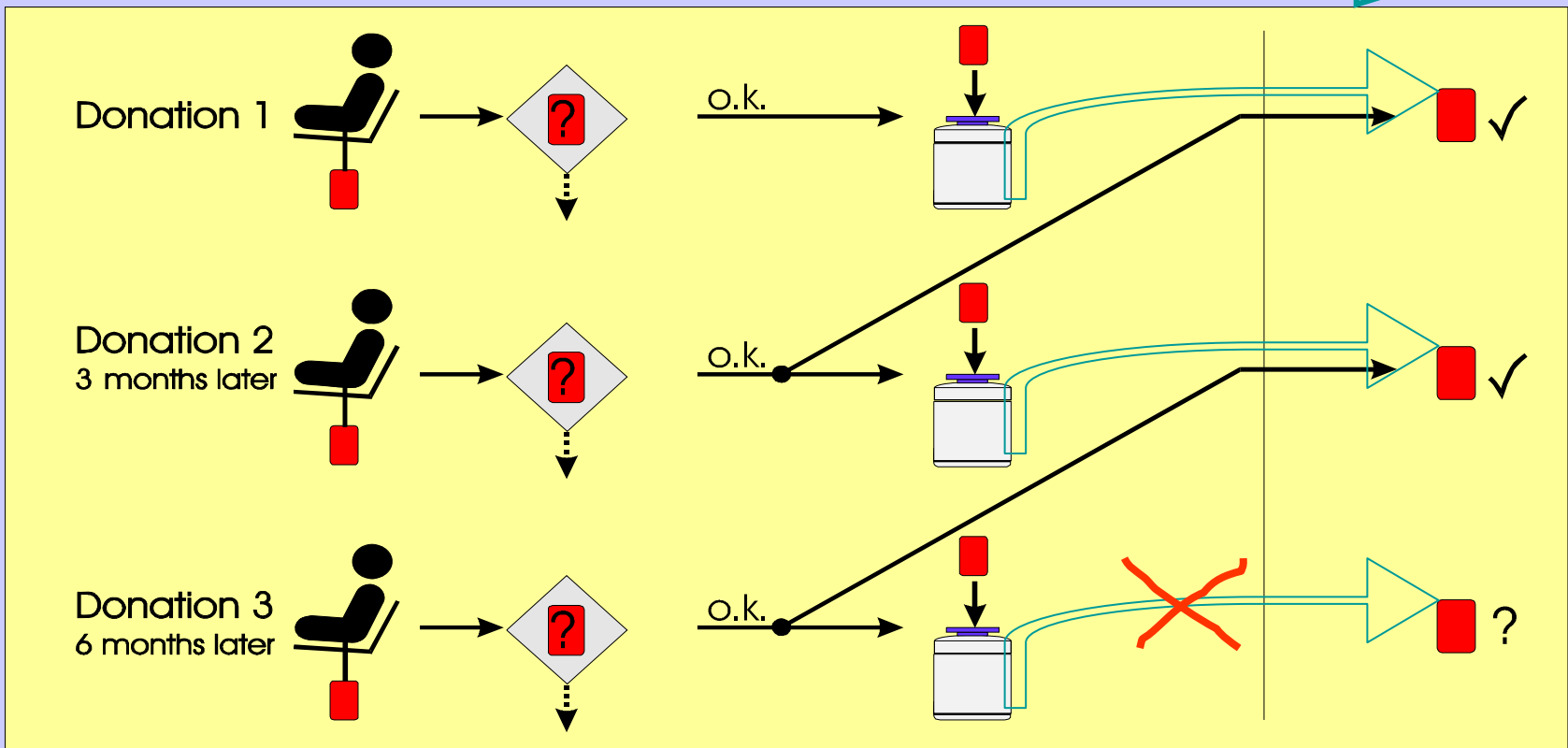
# Storage time of cryopreserved RBCs



# Cryopreservation of Blood

## Safe Blood Transfusion Red Blood Cells

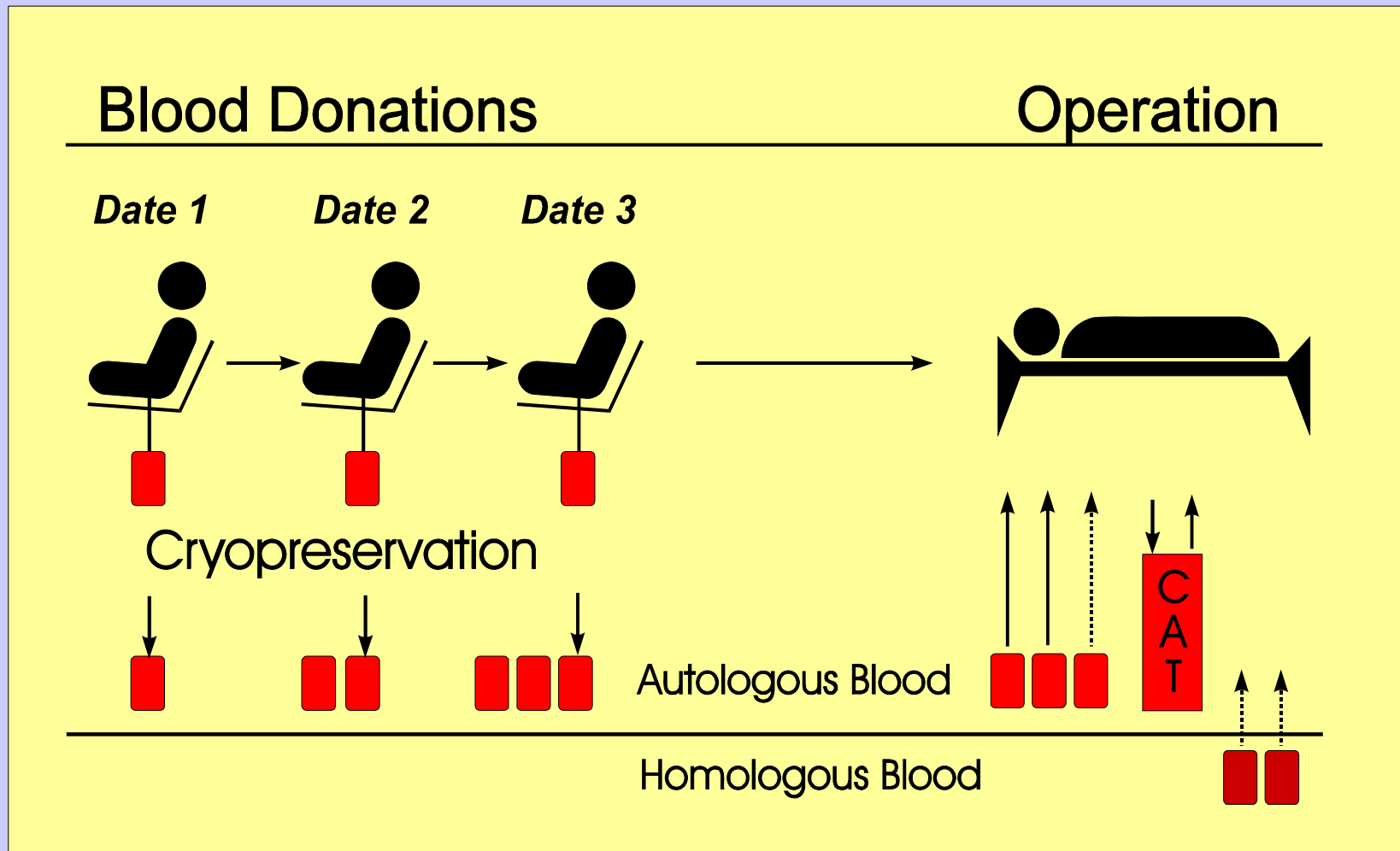
release 



Big Depots and Rare Blood Types: **No shortages**

# Cryopreservation of Blood

## Autologous Blood Supply with Erythrocytes



# What did we achieve?

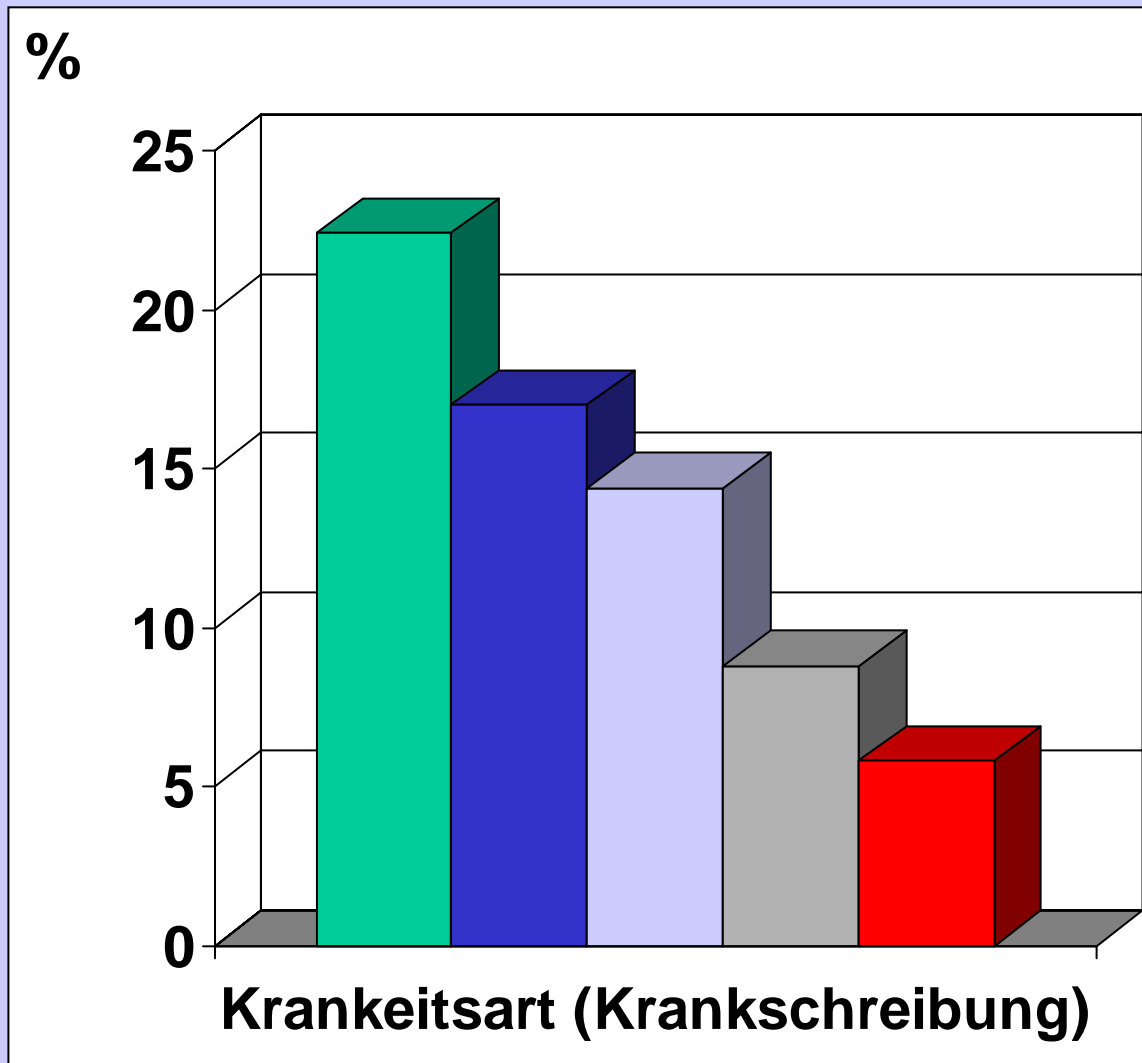
- We extended the shelf life to 20 years
  - Depots (vacation, hazards, OP planning...)
  - Autologous blood (elderly, elective OP...)
  - Storage of rare/very rare blood types
- No bacterial growth
- Utilize the “diagnostic window”: reduce infection
- Reduction of incompatibility: more time to check
- Probably: the only way for Developing Countries to get out of their high bacterial and viral infection rate:






**Some African Countries: 30 - 60% HIV infected**

Cryopreservation of Blood  
can contribute  
patient safety



# Die häufigsten Krankheitsarten (die zu Krankschreibungen führten)



-  Muskel-Skelett-System
-  Atmungssystem
-  Verletzungen
-  Psychische Störungen
-  Herz-Kreislauf

Quelle: DAK, Stand 2003

# Cryopreservation of Red Blood Cells

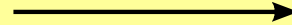
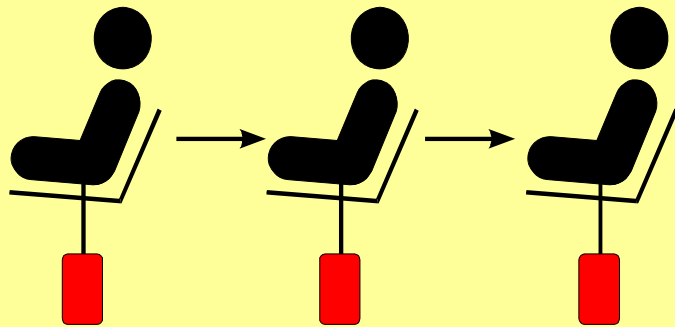
## Blood Donations

## Operation

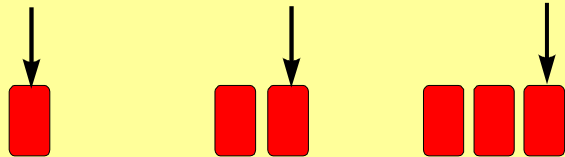
*Date 1*

*Date 2*

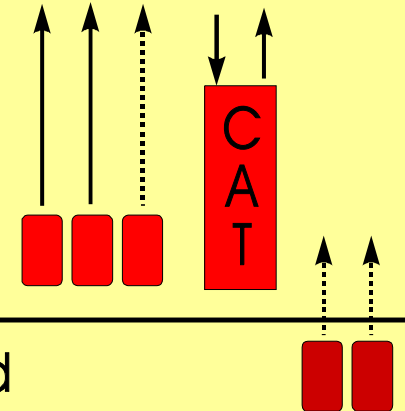
*Date 3*



Cryopreservation



Autologous Blood



Homologous Blood

# Toxicity of Cryoprotectants

