Microcirculation and its Application

-Evaluation of EMF Exposure Effects in Animals-

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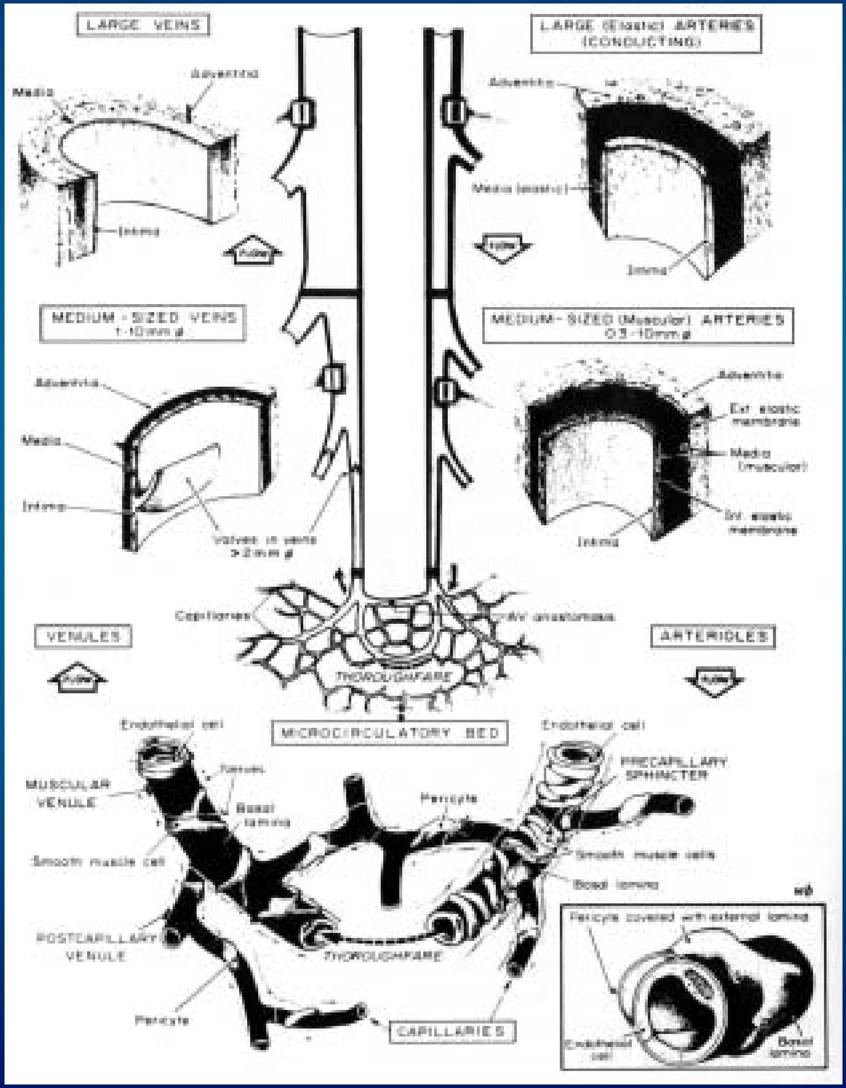
Microcirculation and its Application

1. Microcirculation and Intravital Microscopy
2. Pharmacological Evaluation
3. Hypertension Model
4. Atherosclerosis Model
5. Cigarette Smoking Model
6. Evaluation of EMF

Microcirculation



Microcirculatory Unit

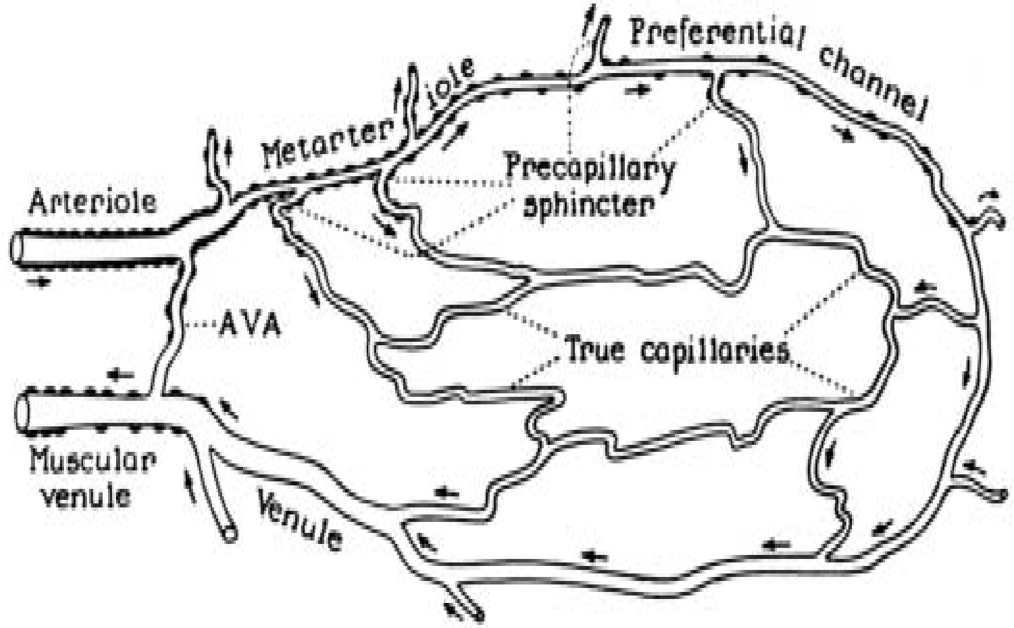
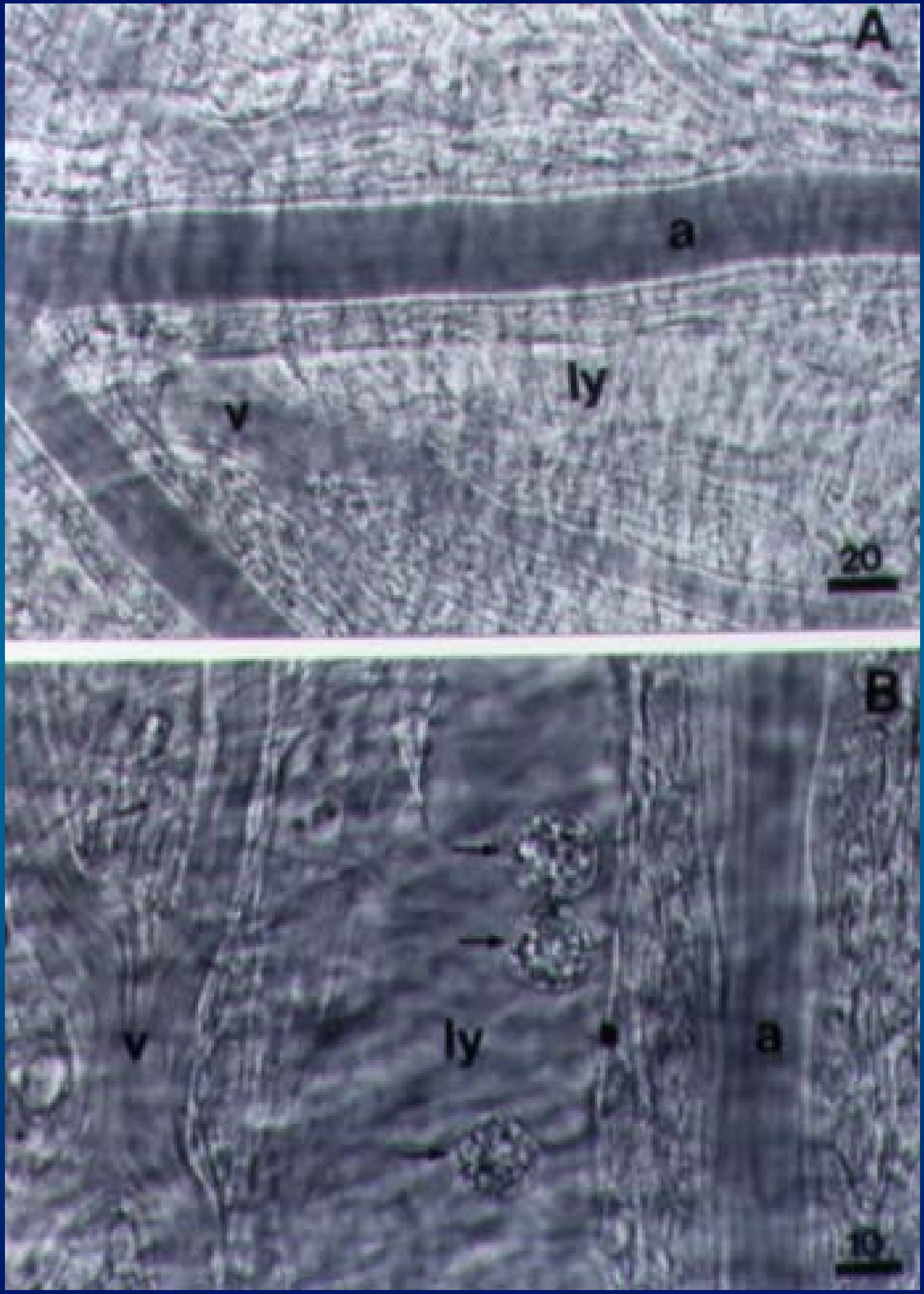


Fig. 2.4. A schematic representation of the structural pattern of the capillary bed. (From Zweifach, 1949.)



a: arteriole

v: venule

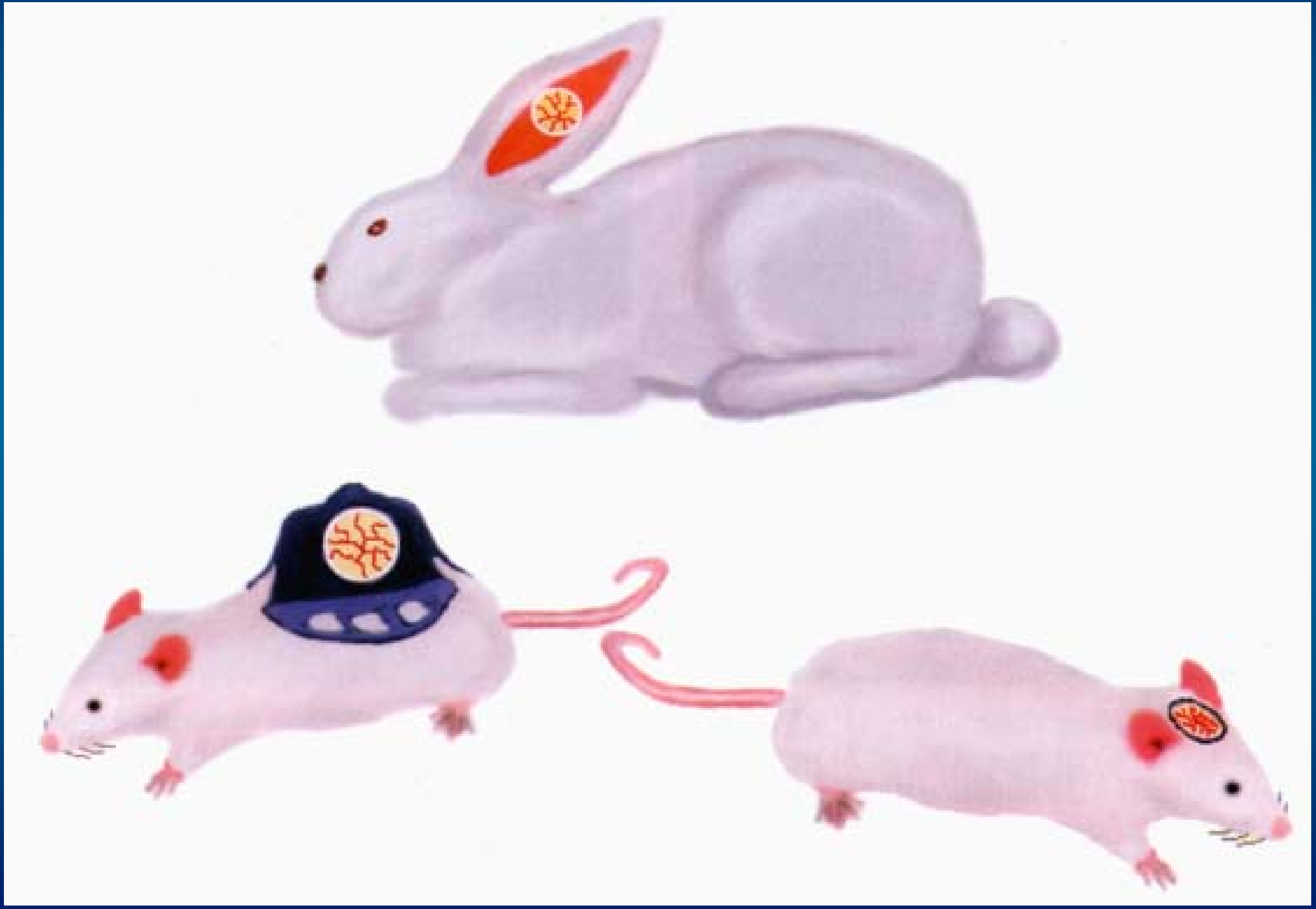
ly: lymphatic capillary

Methods for in vivo evaluation of microcirculation

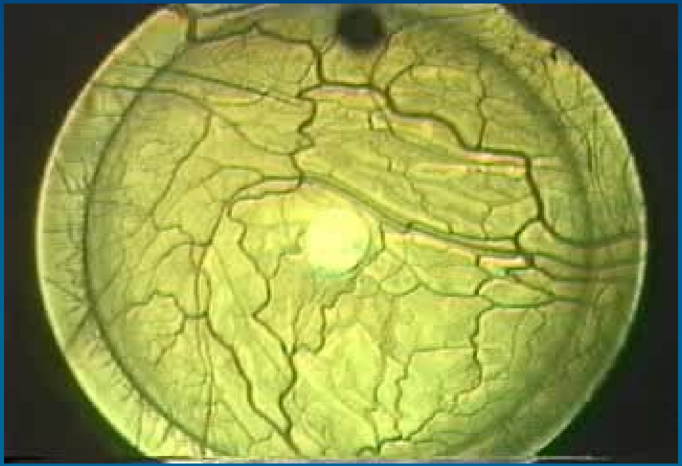
Purpose？
Period？
Animal？
Tissue/organs？
.. ..

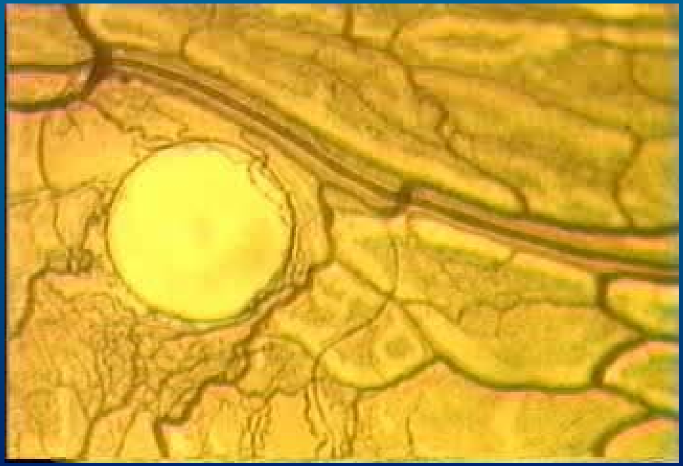
mesentery
hamster check pouch
cremaster muscle
cranial window
rabbit ear chamber
dorsal skinfold chamber
nail bed
....

Chamber Methods



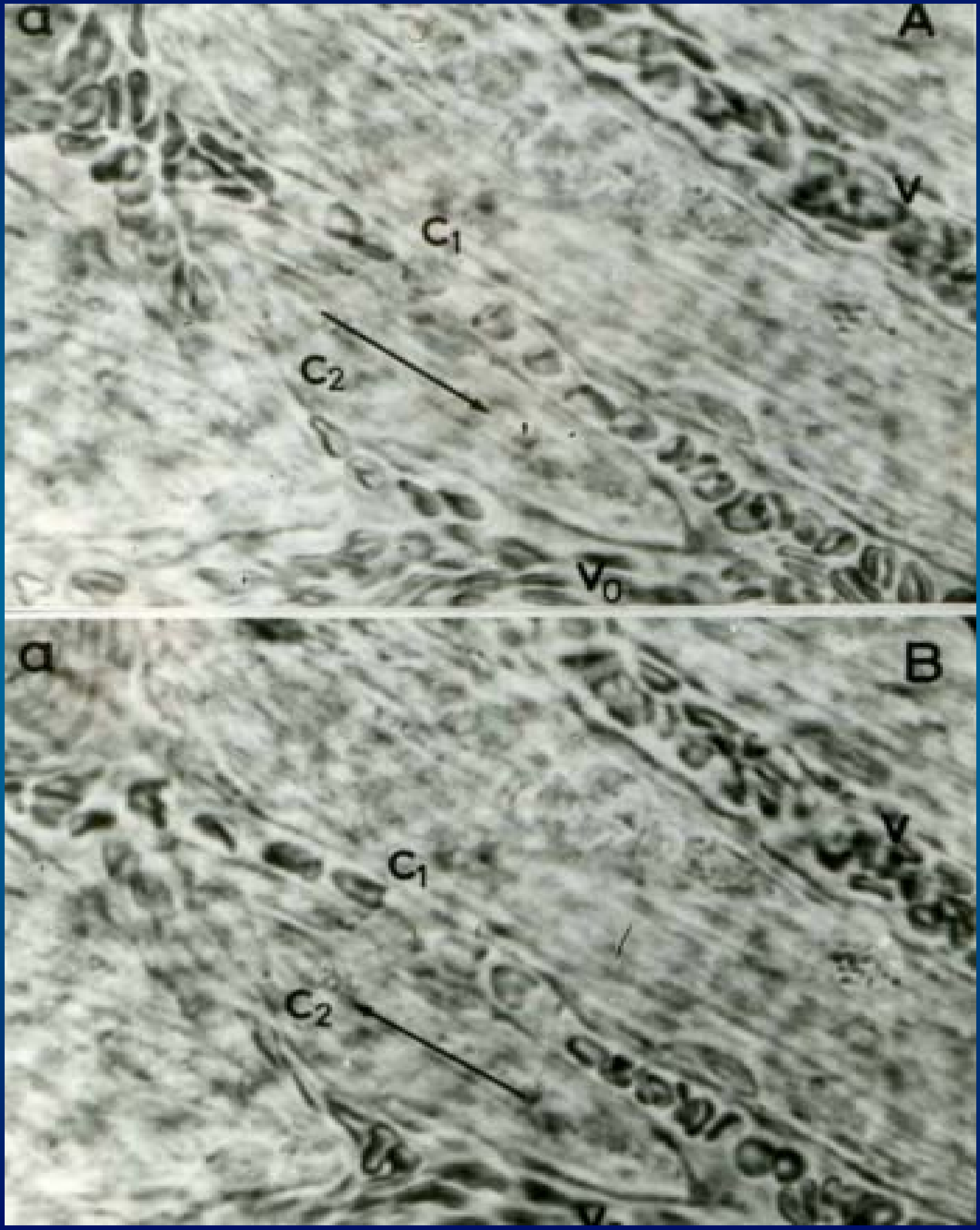
Rabbit Ear Chamber Method



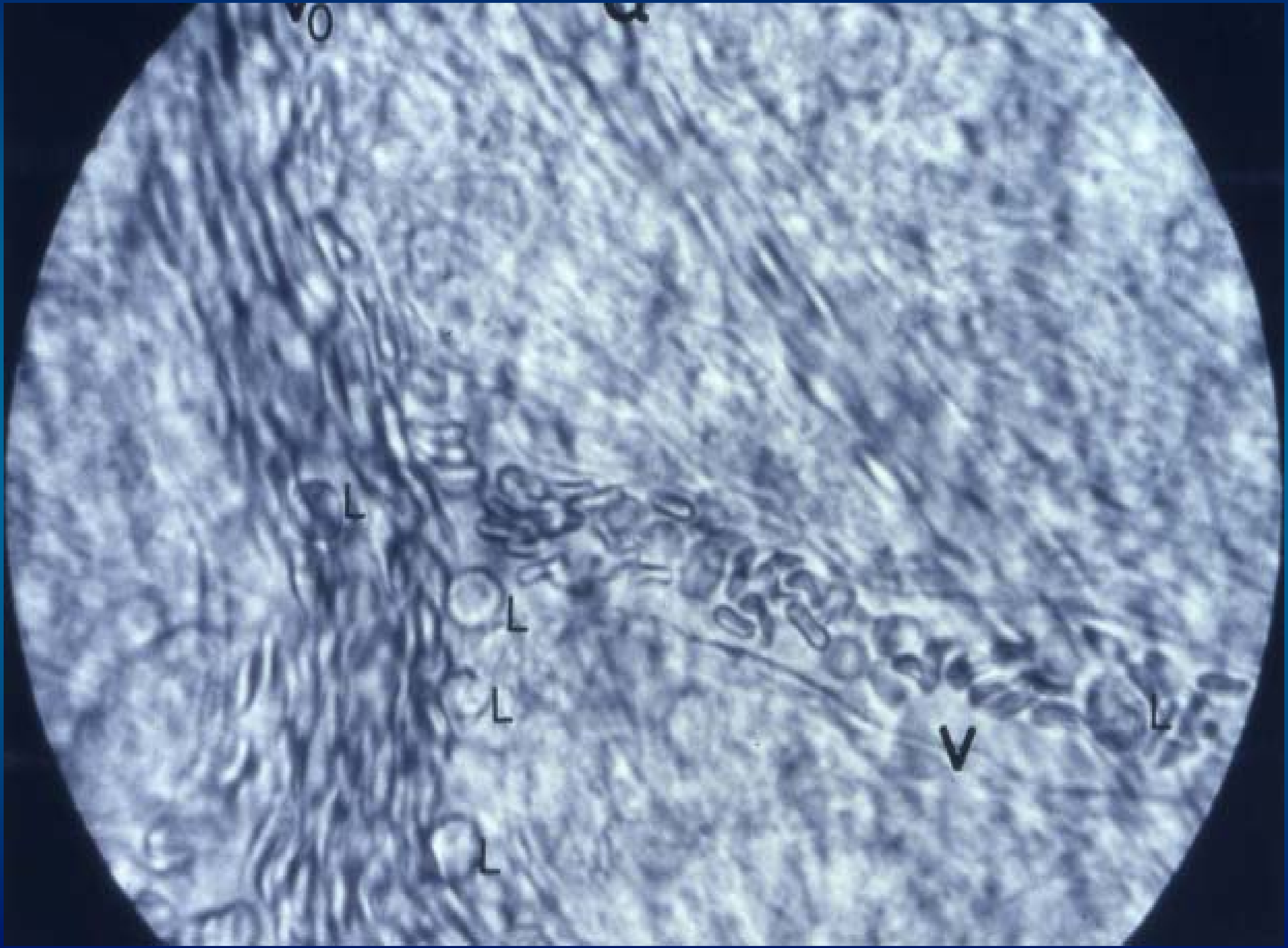


Circulatory Regulation

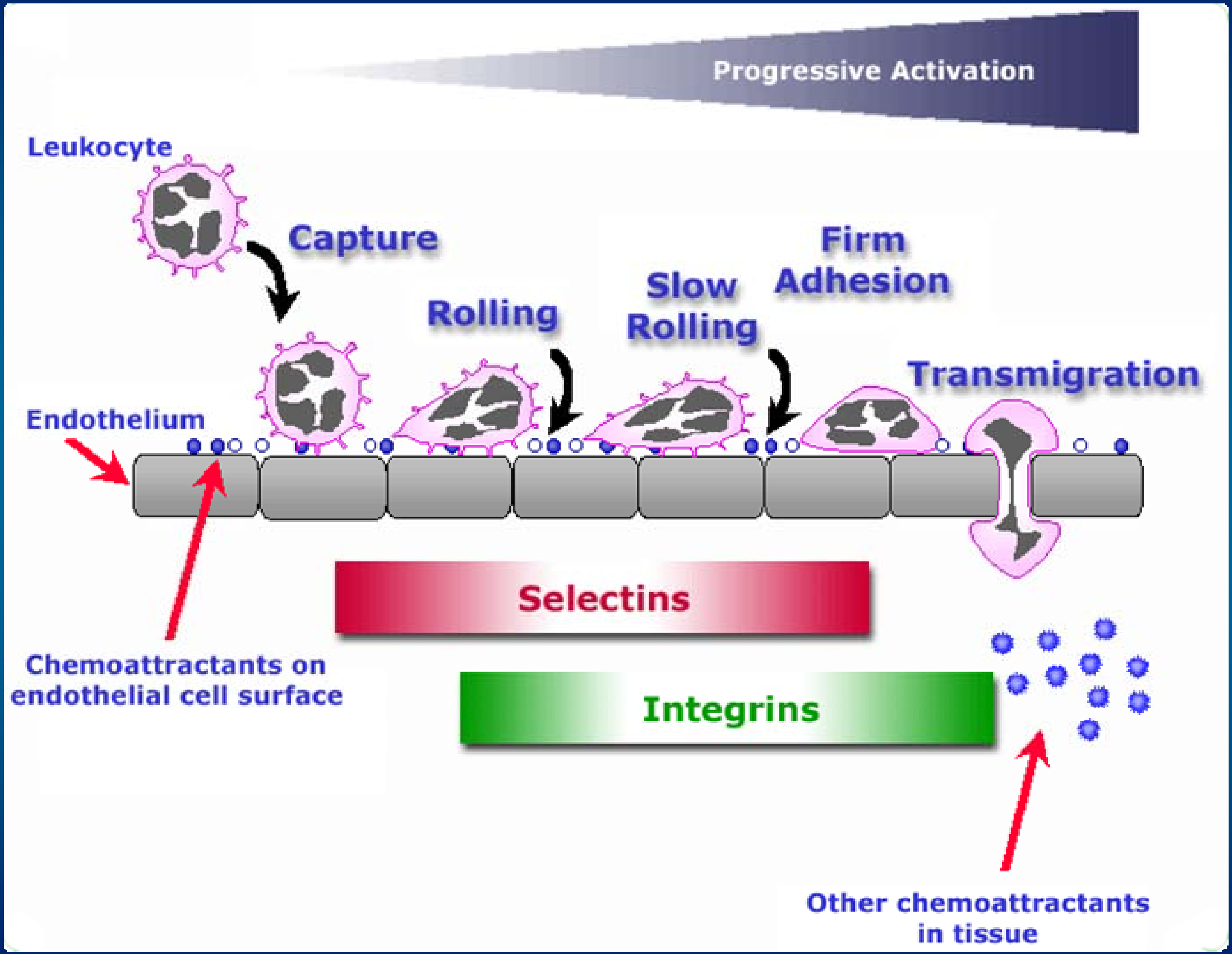
RBCs



WBCs



Inflammation: The Leukocyte Adhesion Cascade



(cited from http://bme.virginia.edu/ley/)

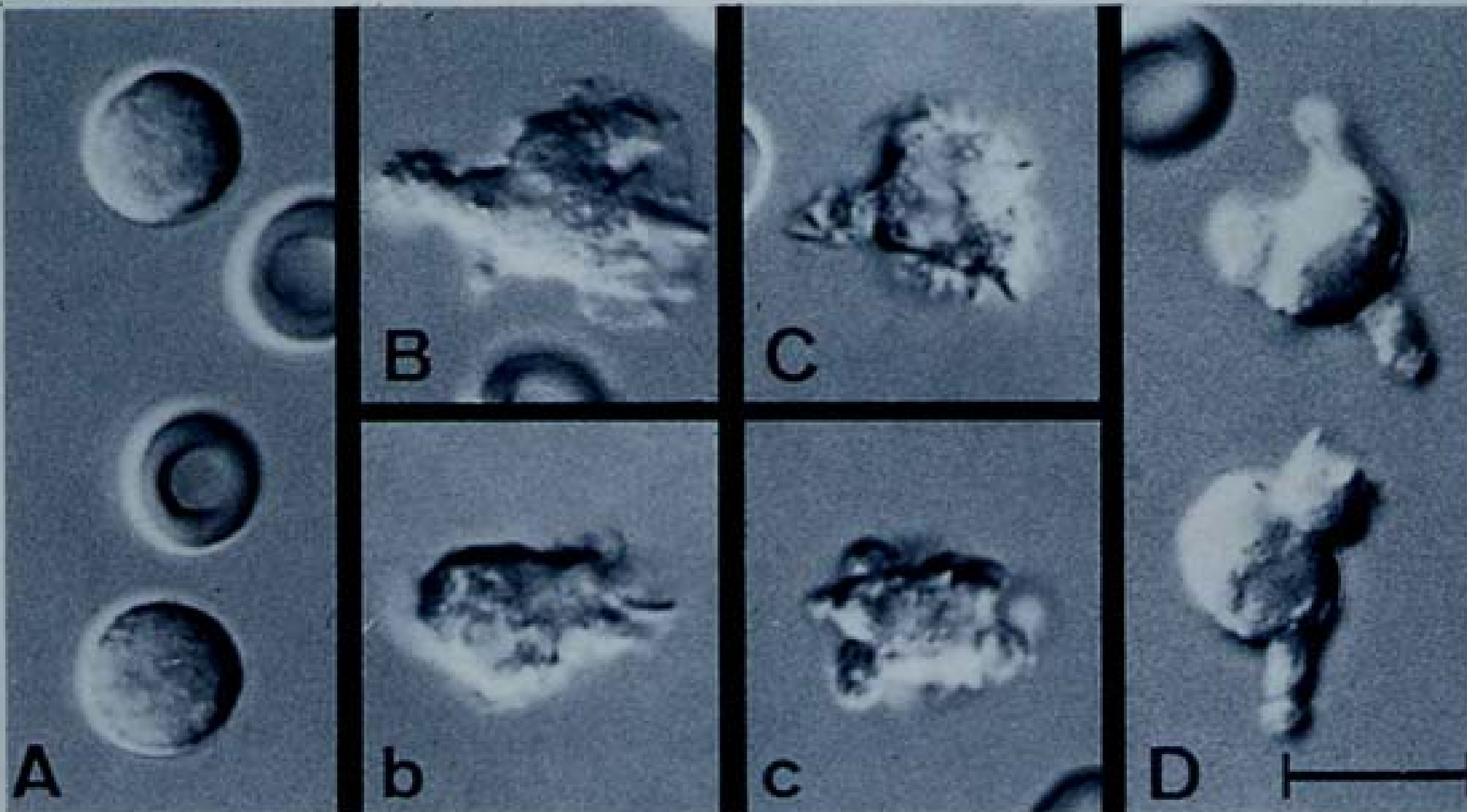
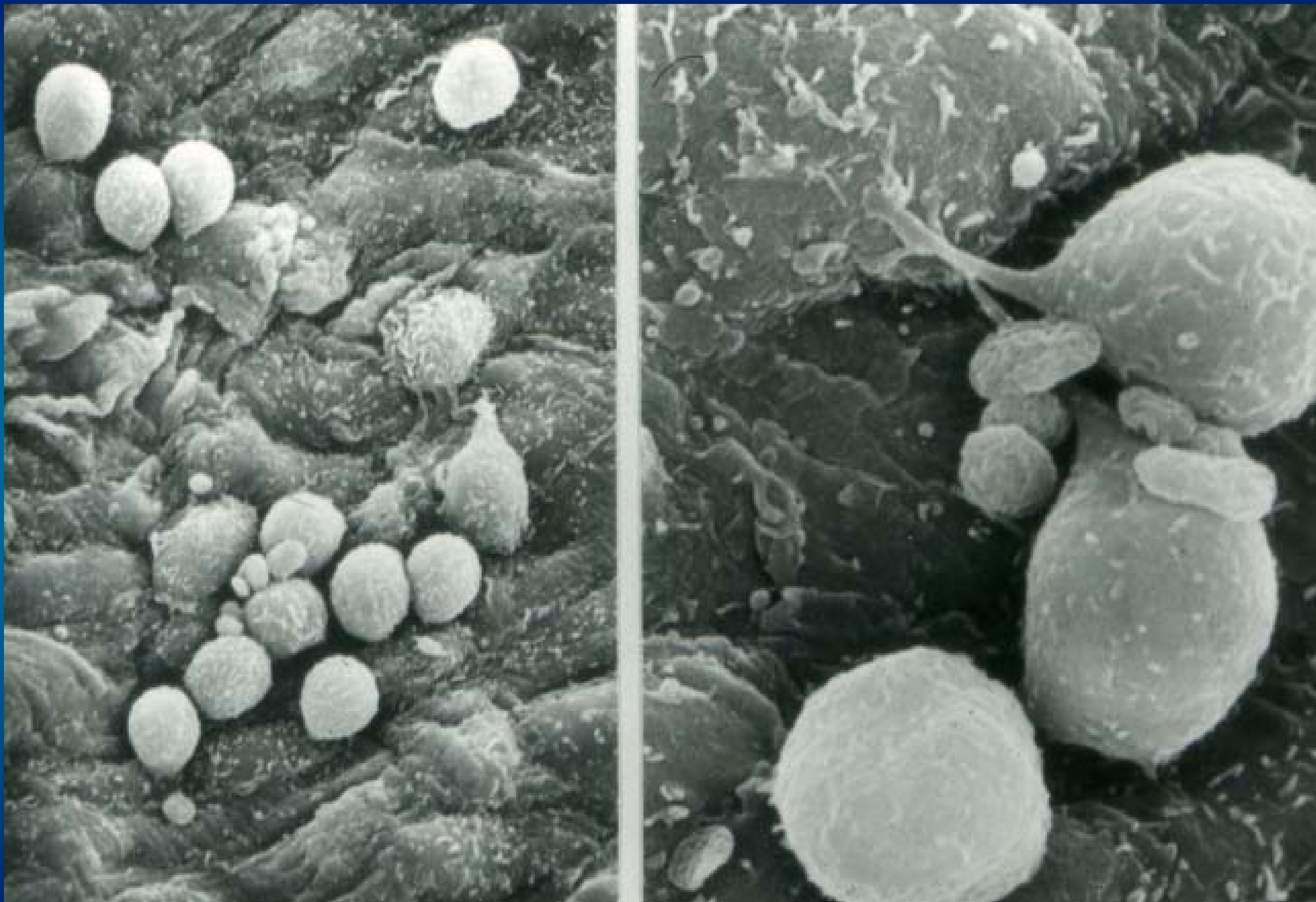
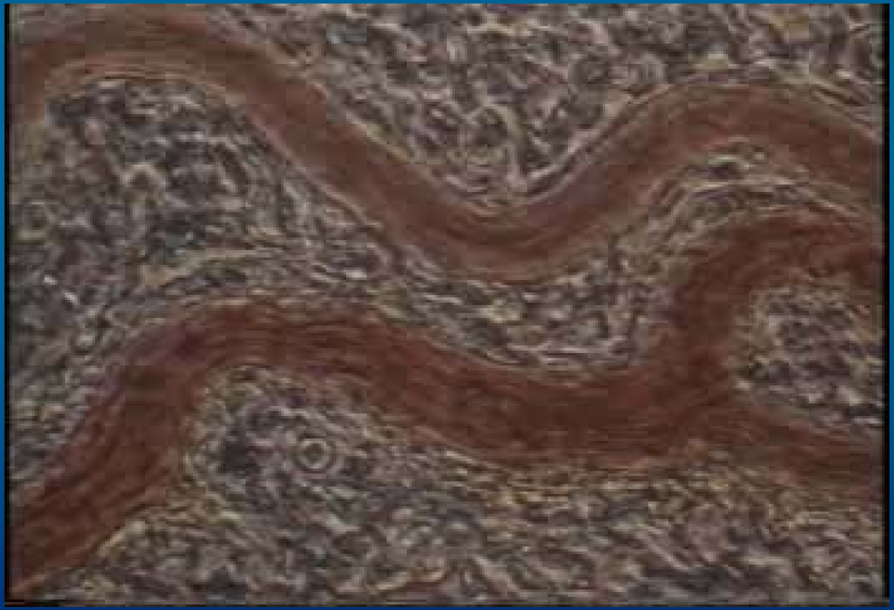
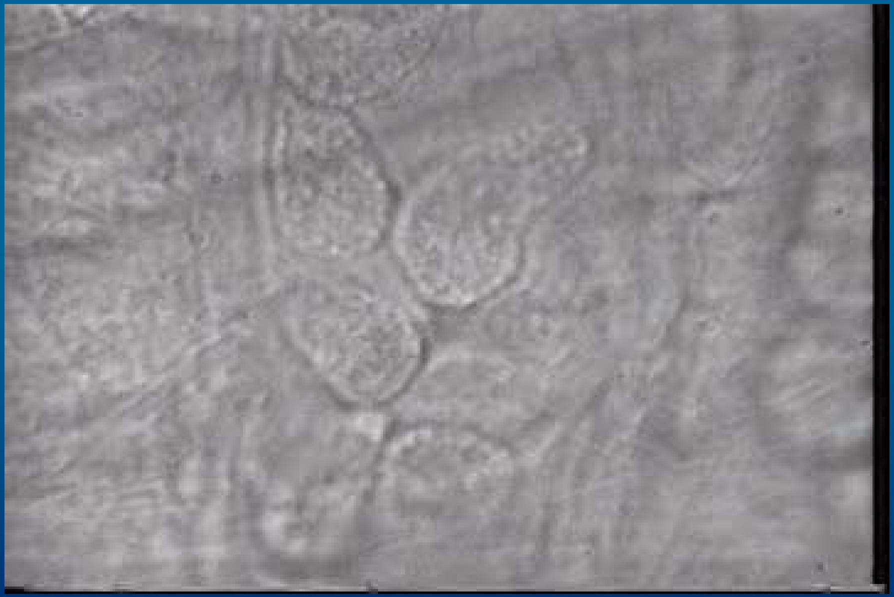


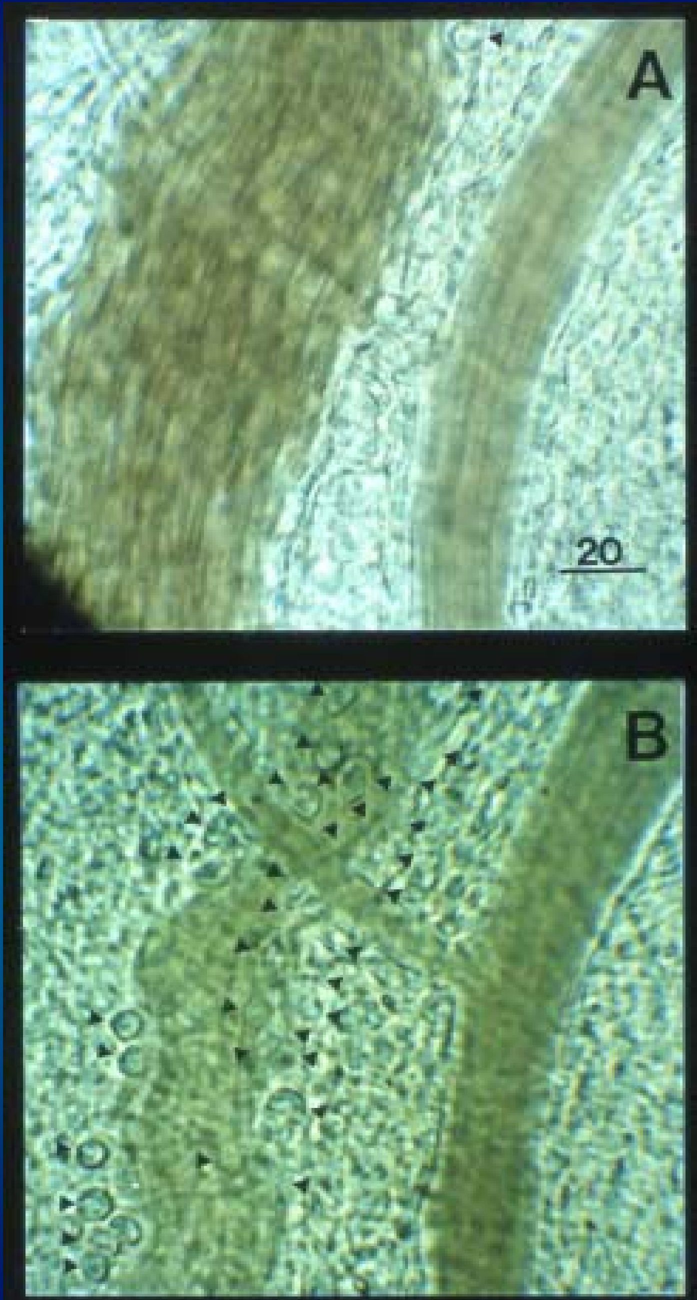
Fig. 1. Relationship between neutrophil shape, motility and locomotion: Spherical cells (*A*) are non-motile. Polarized cells with (*B*) and without (*b*) tail exhibit crawling-like movements in suspension and are capable of locomotion under conditions of limited adhesion. Neutrophils with a fuzzy irregular outline (*C*) or blebs (*c*) are motile but not locomoting. Neutrophils treated with microtubule-disassembling drugs exhibit blebs rather than ruffles at the leading front. They may or may not locomote on the substratum. Control cells (A), cells treated with 10^{-8} M (*B*, *b*) or 10^{-5} M (*C*, *c*), fMLP or 10^{-6} M nocodazole (*D*) were fixed in suspension and photographed with differential interference contrast (Nomarsky) microscopy. Scale bar: 10 µm (for details see [11, 16]).



WBC Activation & Pathophysiology







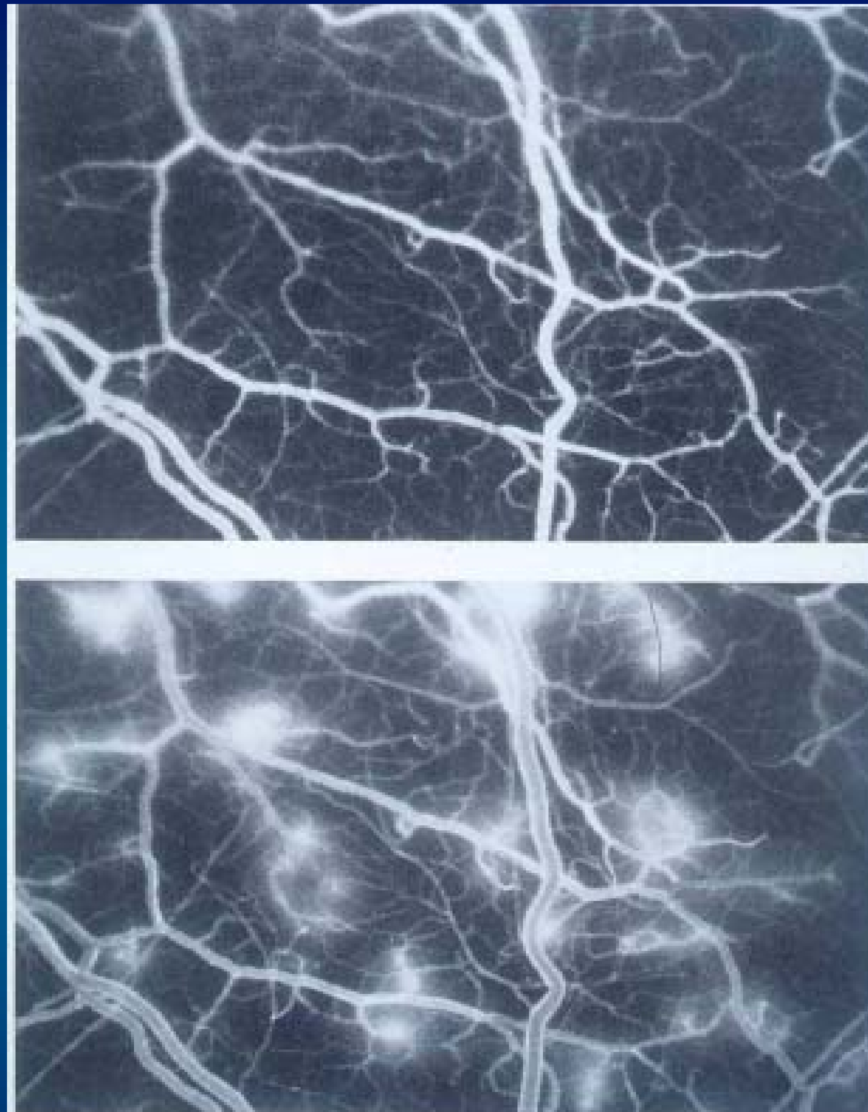
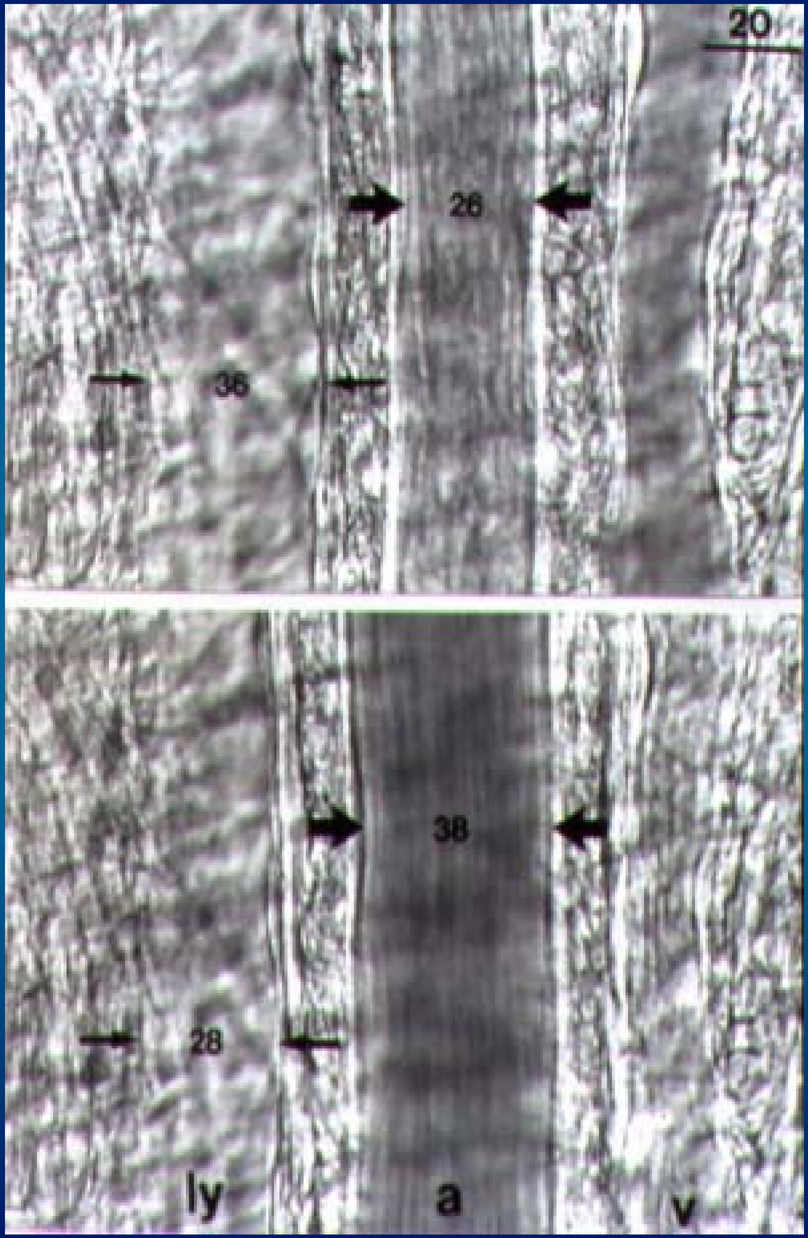
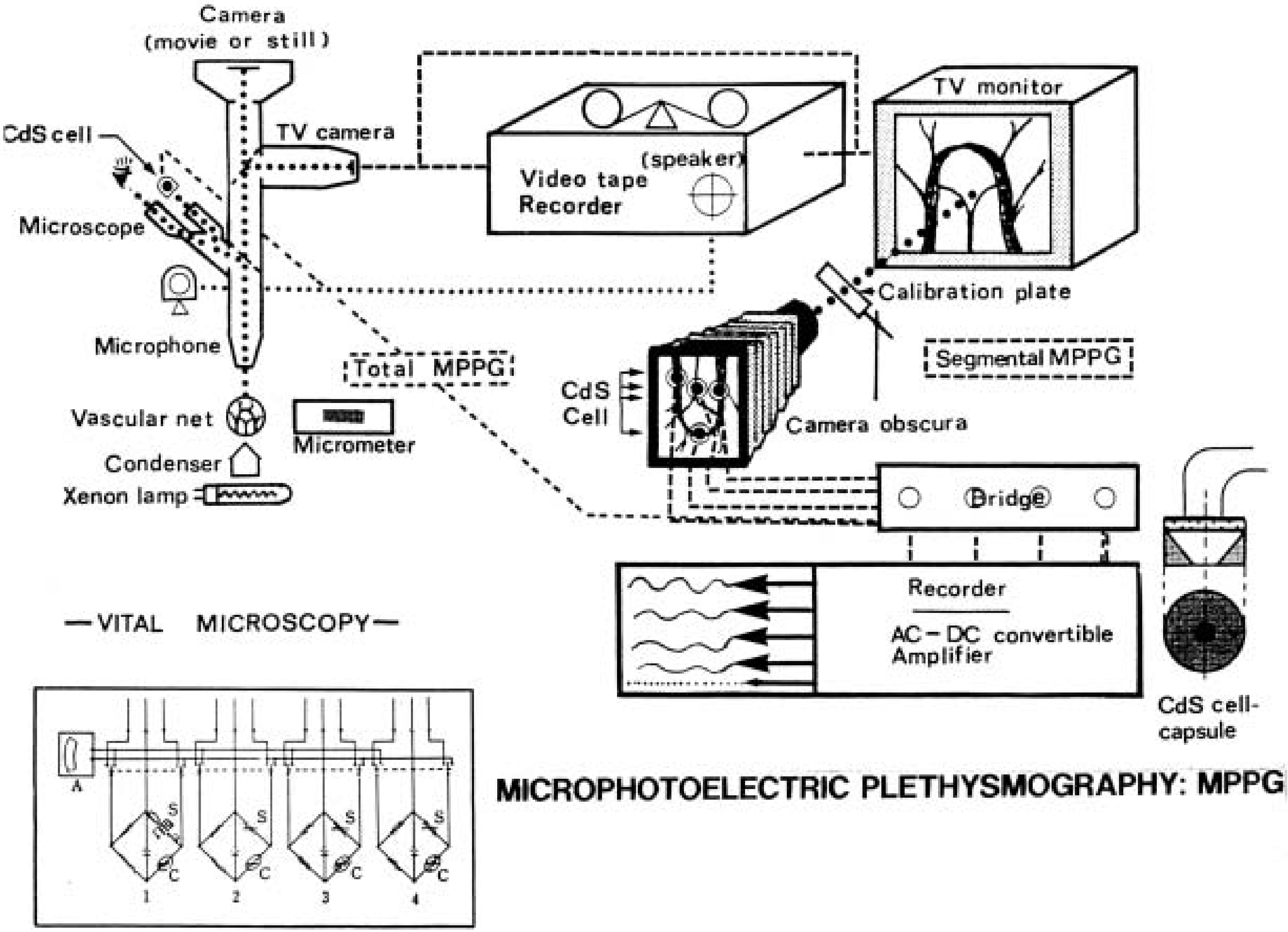


Fig. 1. Fluorescent micrographs of the hamster cheek pouch microvasculature. *a* control situation; *b* 5 min after the application of 4 nM leukotriene C_4. Note increased vascular leakage only at postcapillary venules.

Vasomotion

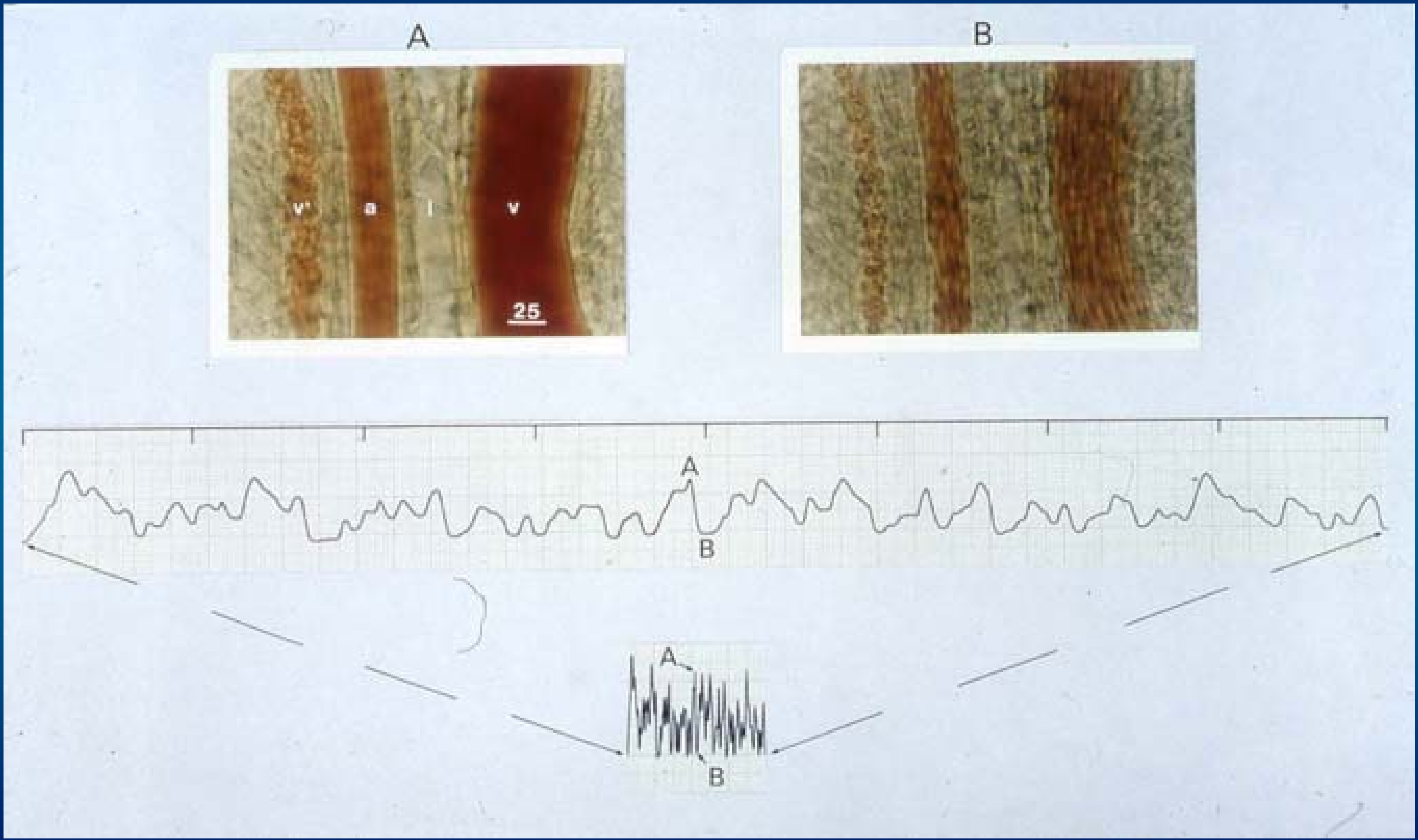


Vasomotion and Lymphatic Flow



MICROPHOTOELECTRIC PLETHYSMOGRAPHY: MPPG

Vasomotion and MPPG



Effect of Warming (Hair Dryer)

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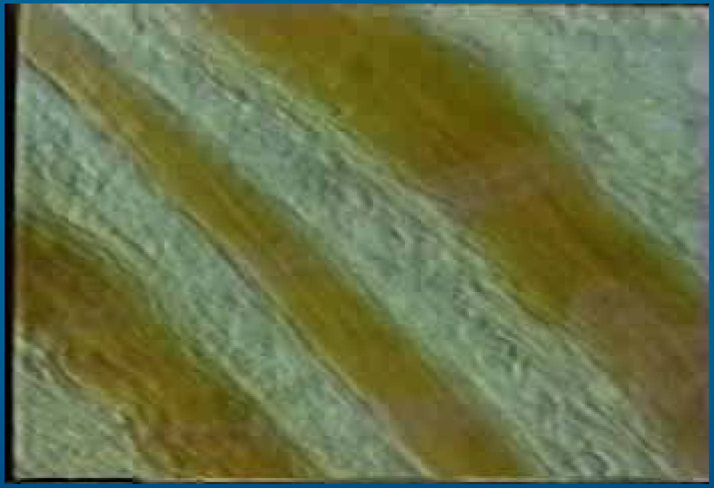
Vasodilator and Vasoconstrictor

Nor-Adrenaline

Acetylcholine



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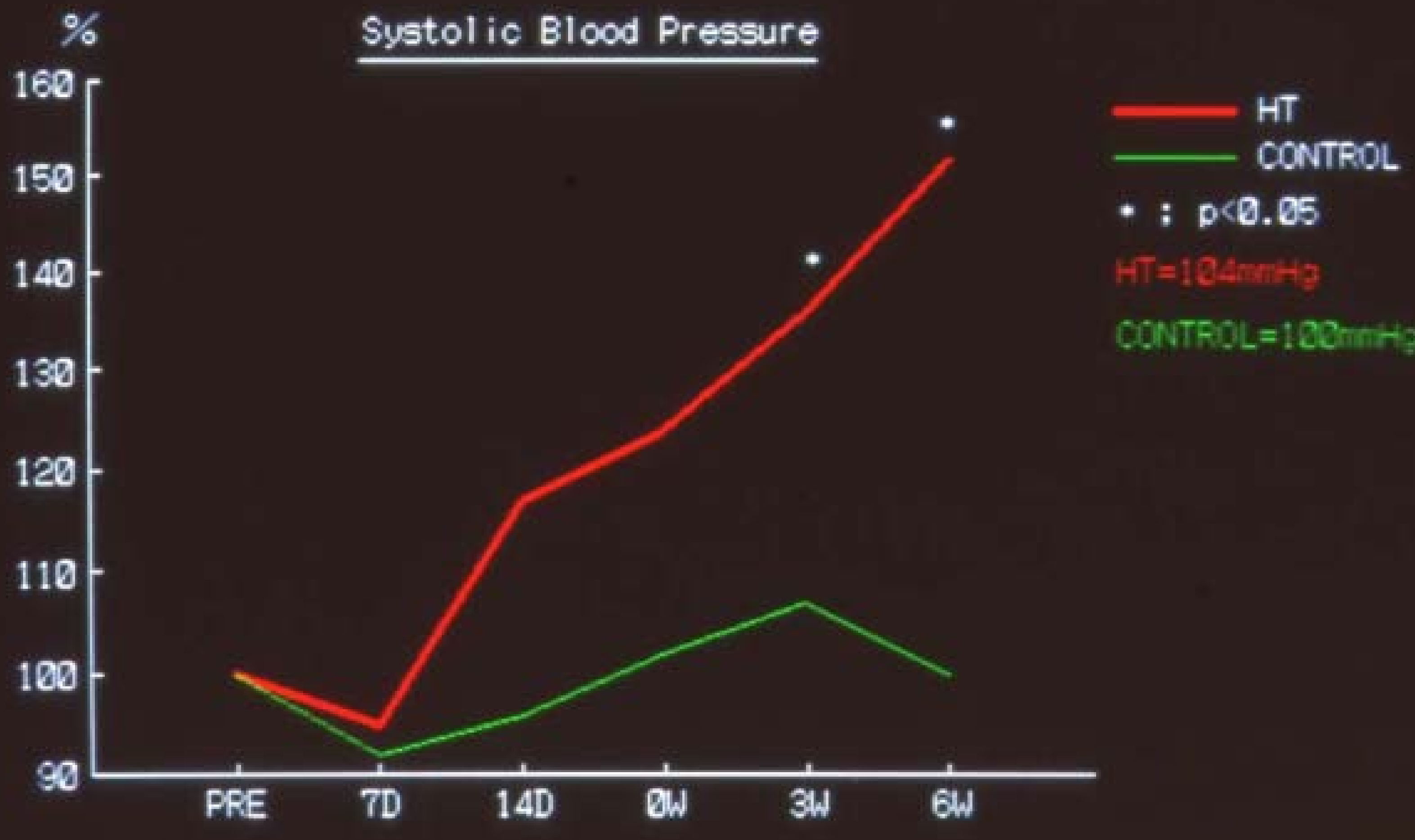
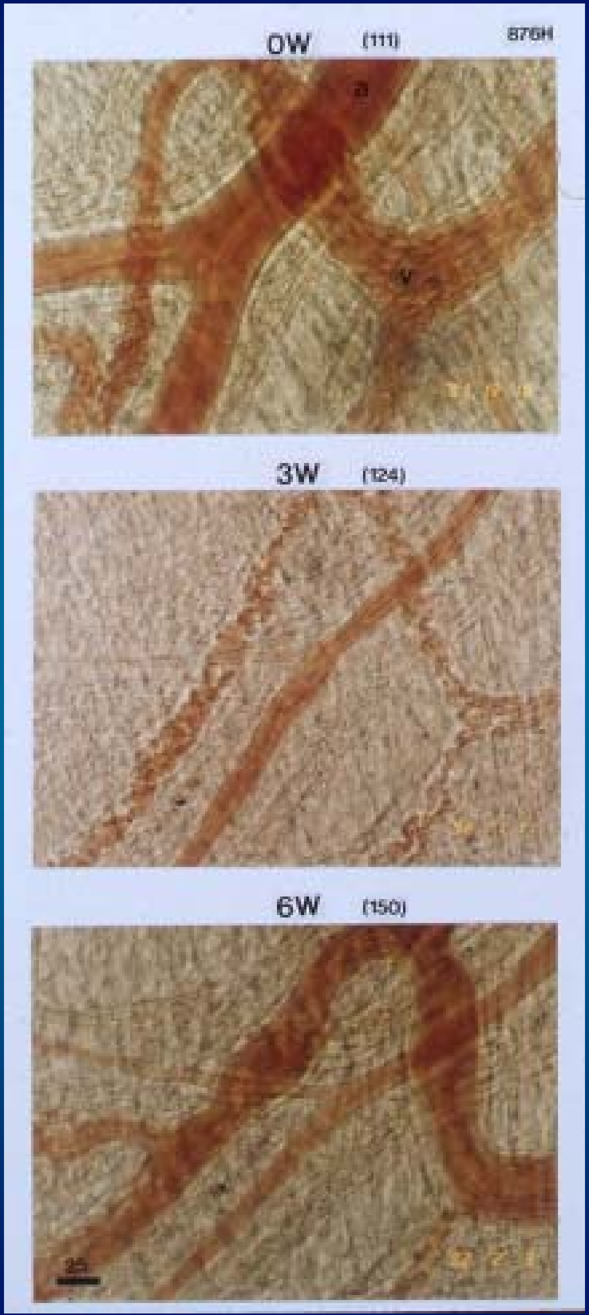
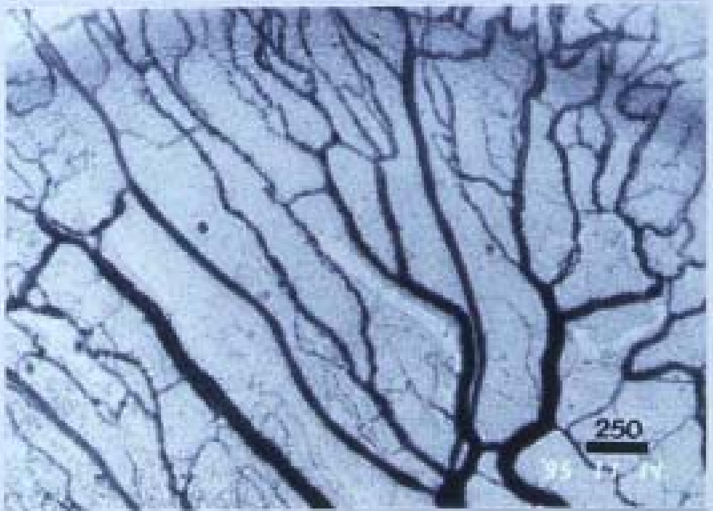


Systolic Blood Pressure
%
160
150
140
130
120
110
100
90
PRE
7D
14D
0W
3W
6W
HT
CONTROL
* : p<0.05
HT=104mmHg
CONTROL=100mmHg



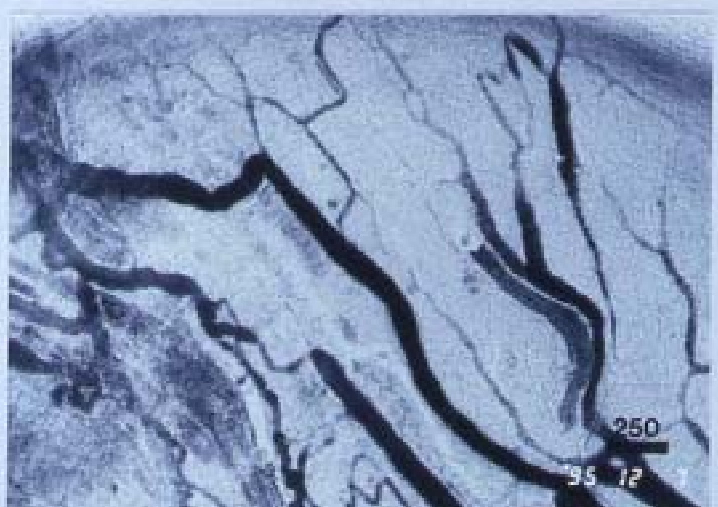
0W
(111)
876H
a
v
3W
(124)
6W
(150)
25

#1280



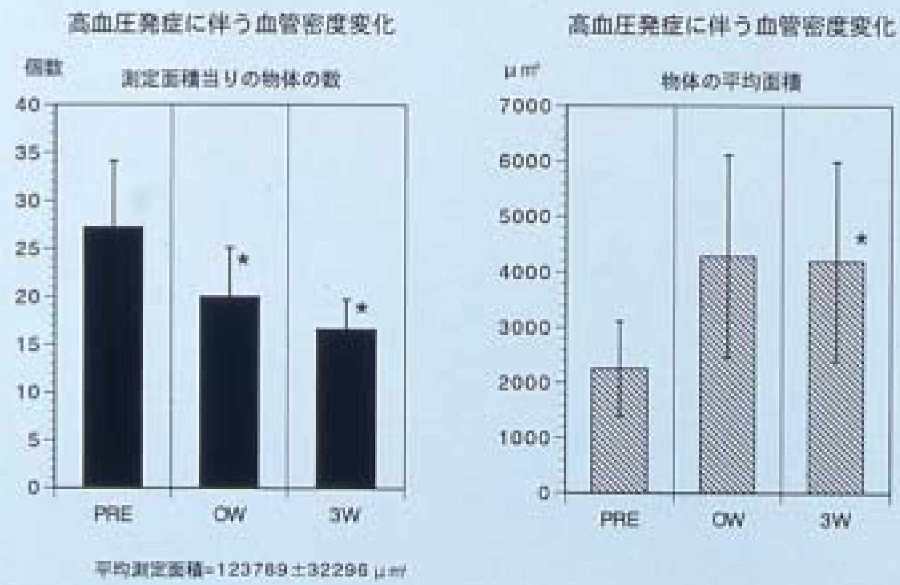
250
'95

Pre



250
'95 12

0W



高血圧発症に伴う血管密度変化
個数
測定面積当りの物体の数
40
35
30
25
20
15
10
5
0
PRE
0W
3W
*
*
高血圧発症に伴う血管密度変化
μm²
物体の平均面積
7000
6000
5000
4000
3000
2000
1000
0
PRE
0W
3W
*
平均測定面積=123789±32296 μm²

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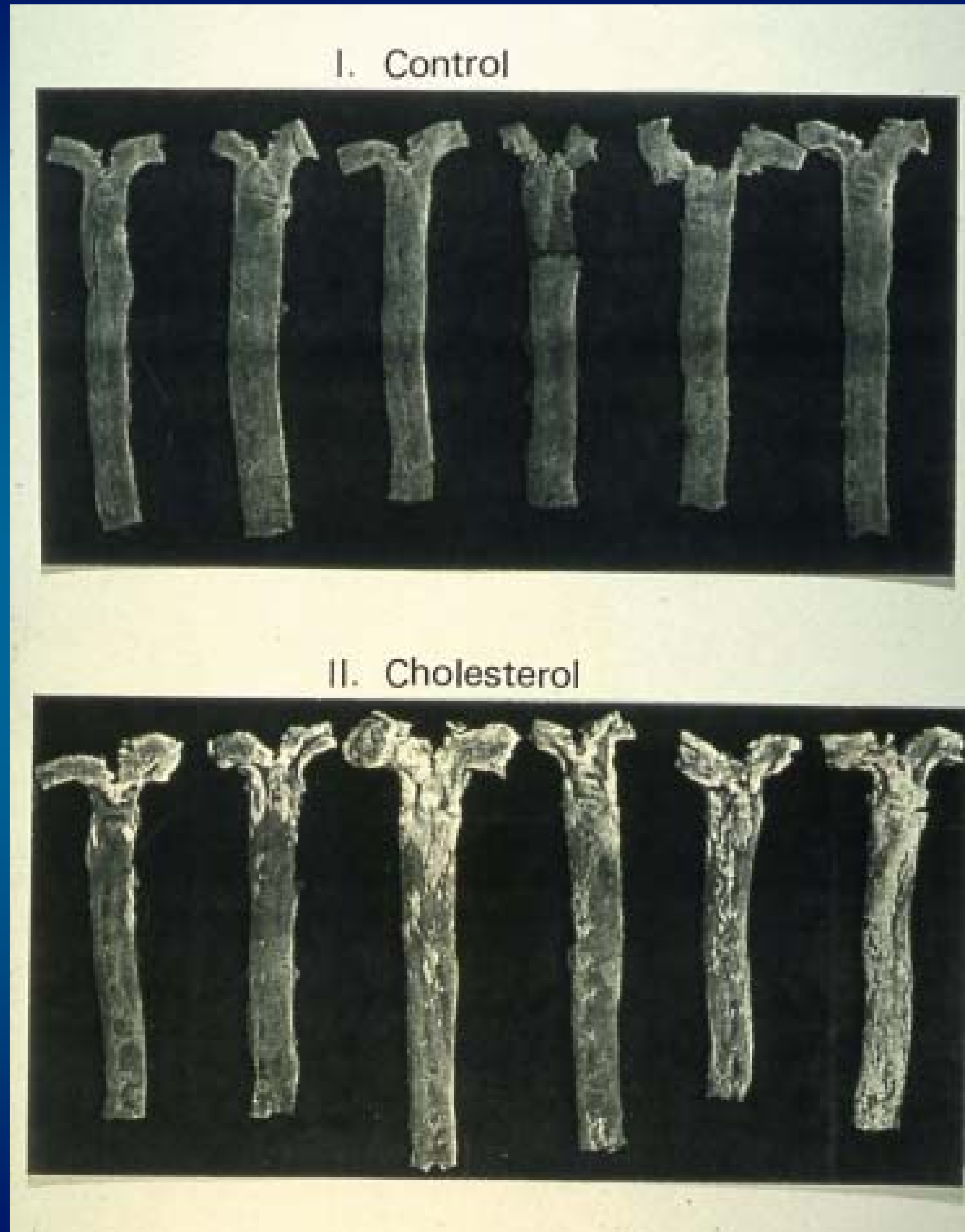
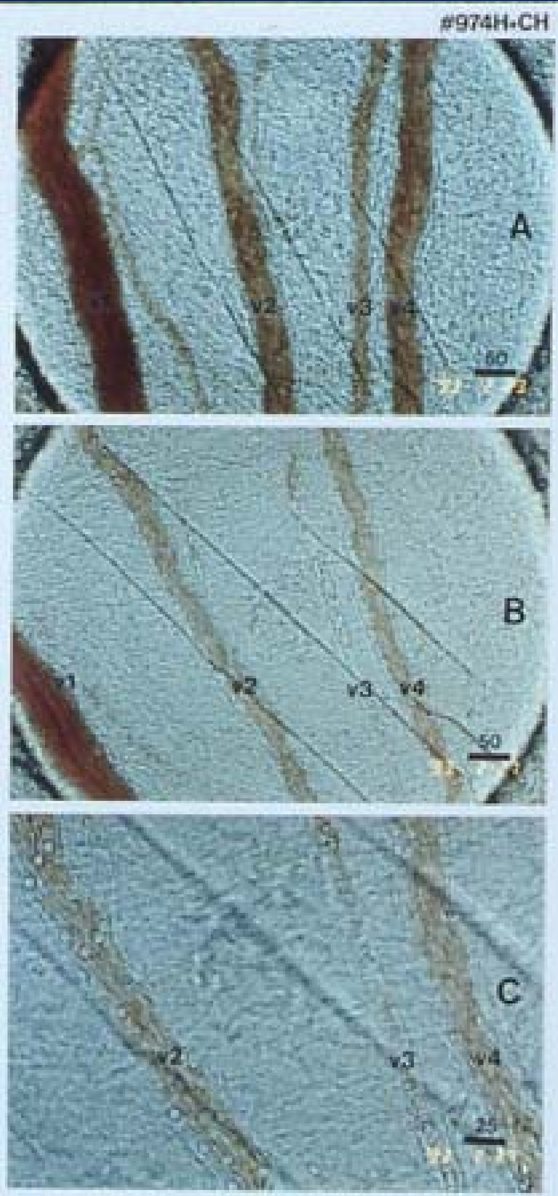
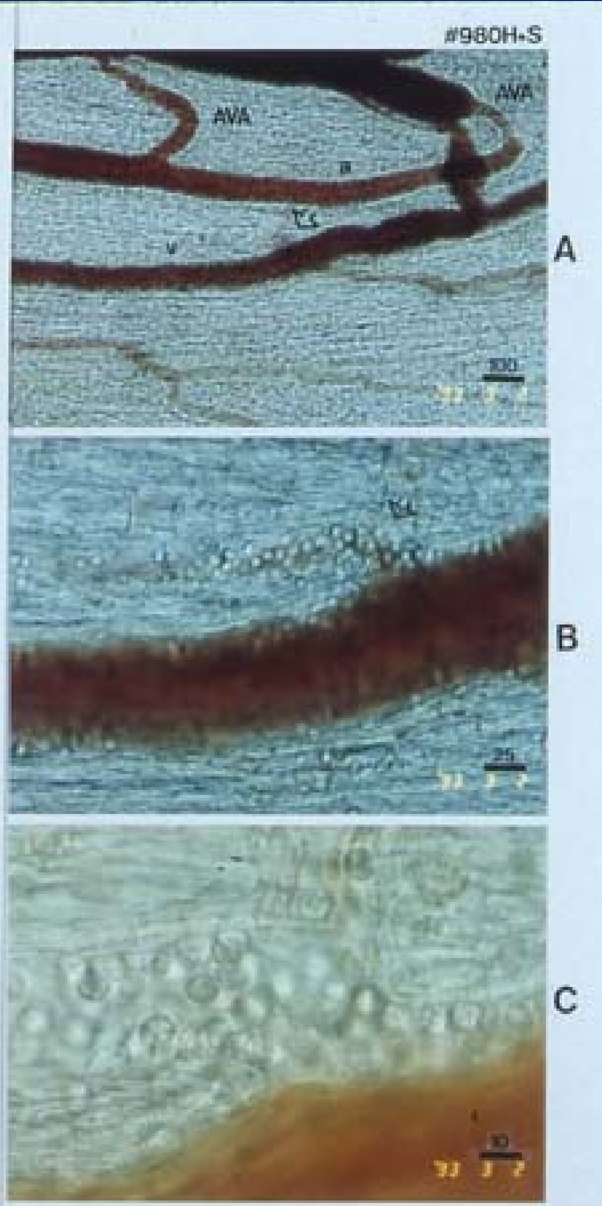
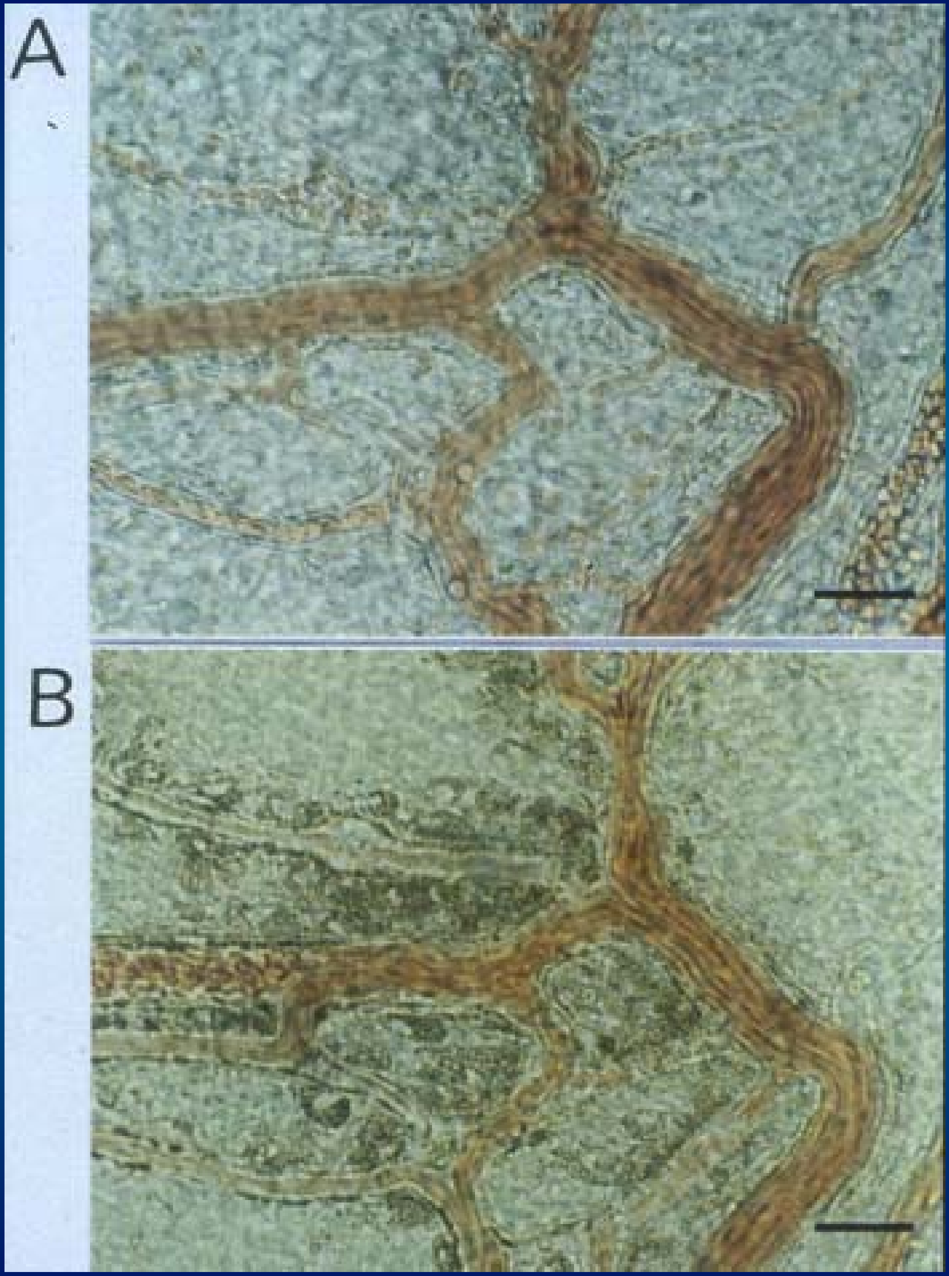
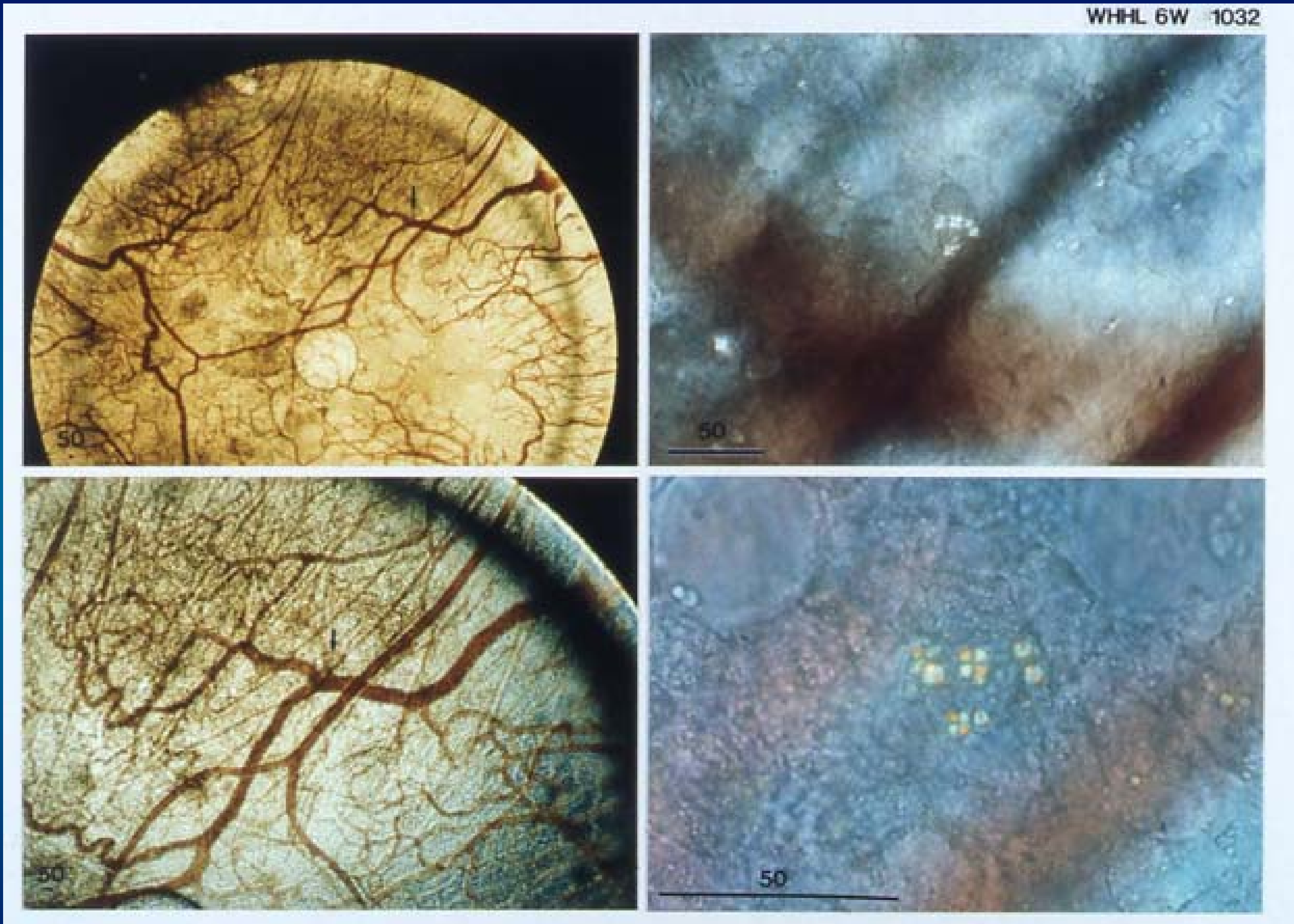

I. Control
II. Cholesterol


#974H-CH
A
v2
v3
v4
50
B
v1
v2
v3
v4
50
C
v2
v3
v4
25


#980H-S
AVA
AVA
A
300
B
25
C
30


A
B


WHHL 6W 1032
50
50
50
50

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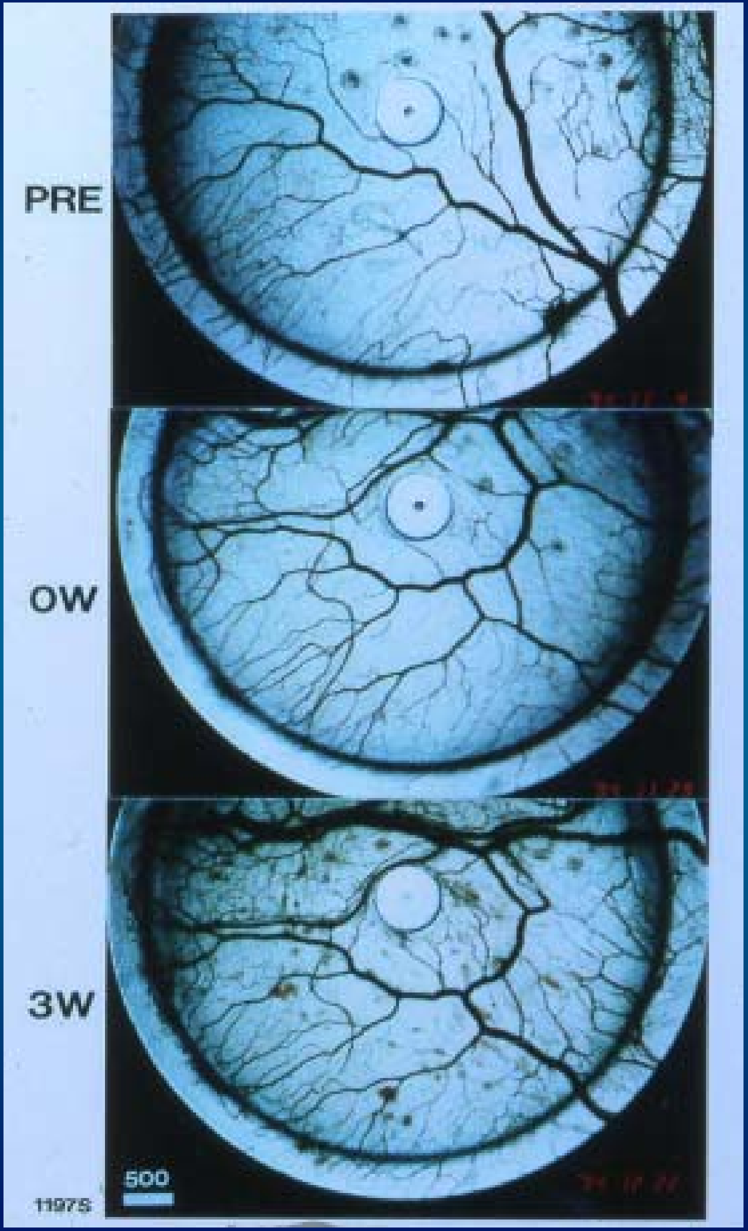
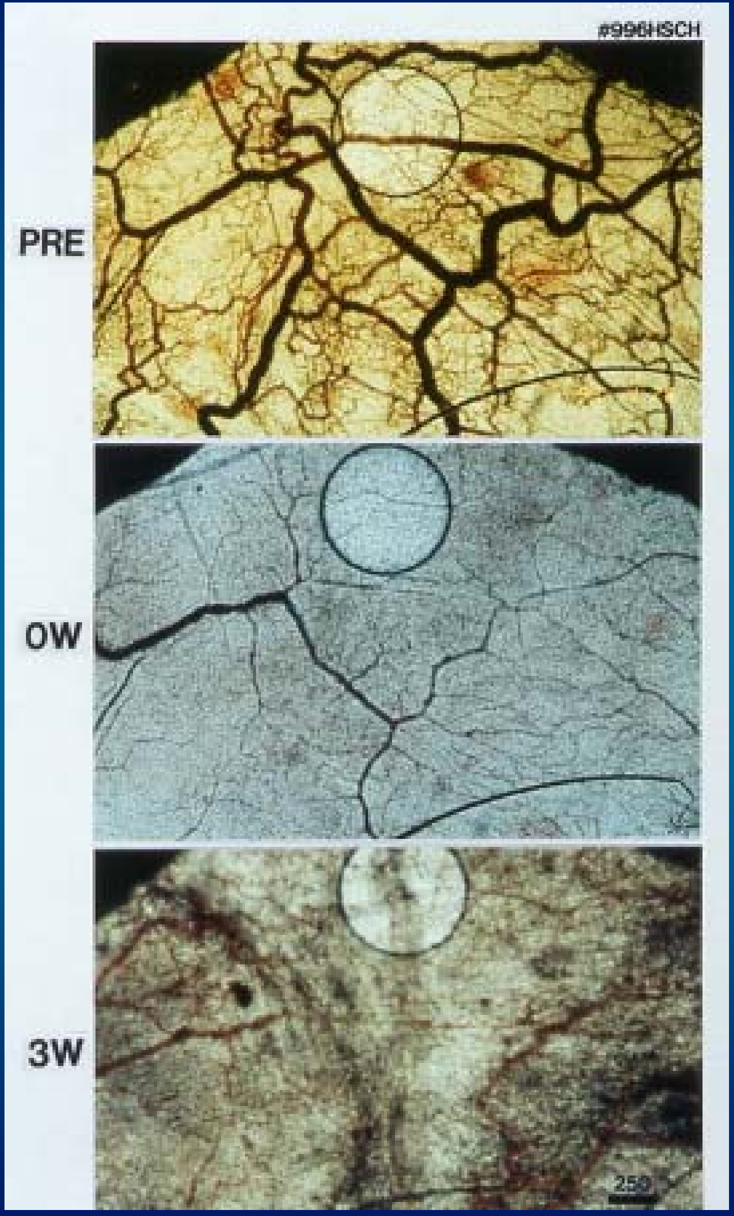

PRE
OW
3W
500
1197S


#996HSCH
PRE
OW
3W
250

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1. Power Frequency EMF

Effects of continuous whole-body exposure to 50 Hz electromagnetic fields on the intramicrovascular leukocyte behavior in mice

Dorsal Skinfold Chamber

Left: titanium Right: Resin (Duracon)

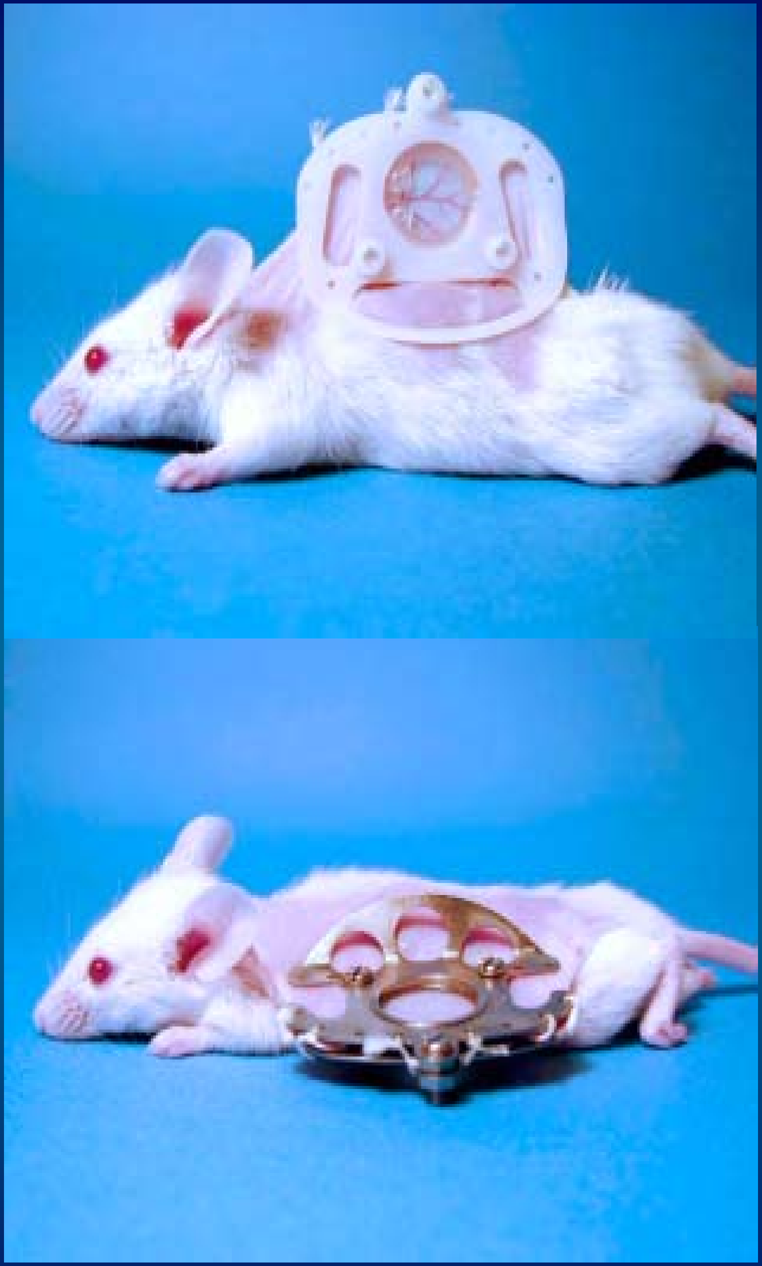
Frame weight comparison

Resin frame 1.6ｇ

Titanium frame 4.1ｇ

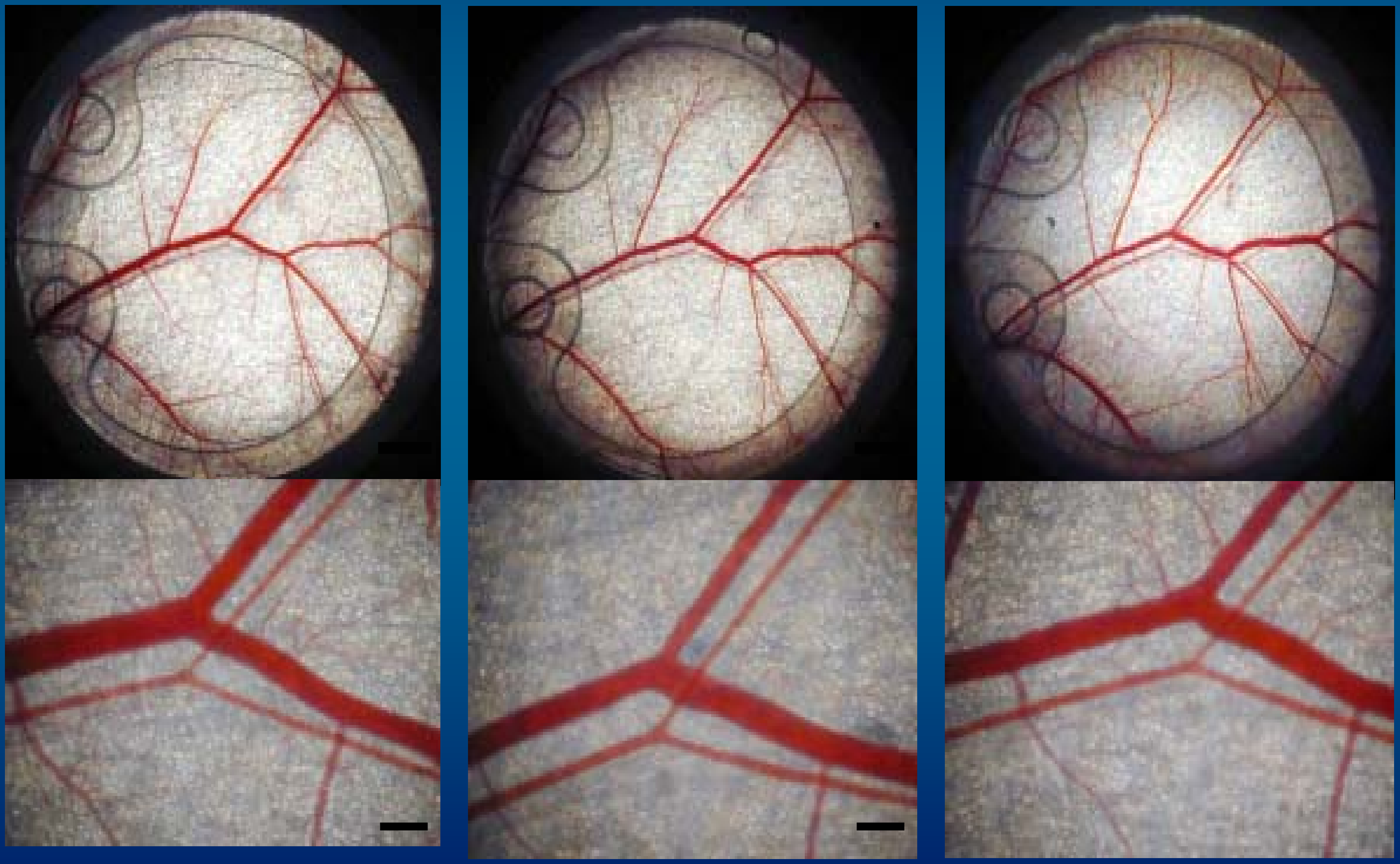
↓

Relieve of weight



10 days after installation

Intra-vital microscopic overviews of DSC



(scale 1mm)

(scale 100µm)

Day 1 Day 5 Day 10

Set up of intravital microscopy

Trans-illumination
Fluorescent illumination
(epi-illumination，conforcal laser)

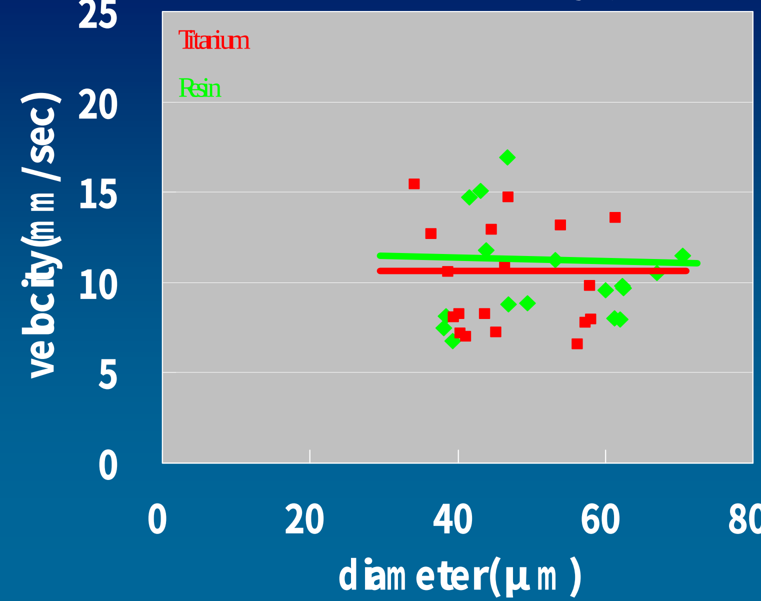
- blood velocity
- blood flow volume
- leukocyte behavior
- vessel density(tumor grouth)

Arteriolar blood flow rate

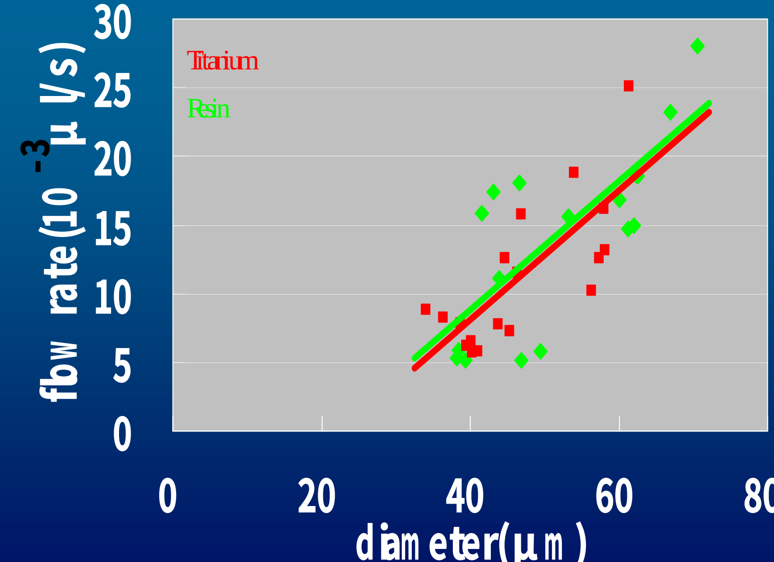
Strobe epi-illumination method

(Strobe 200Hz = 5msec/flash)

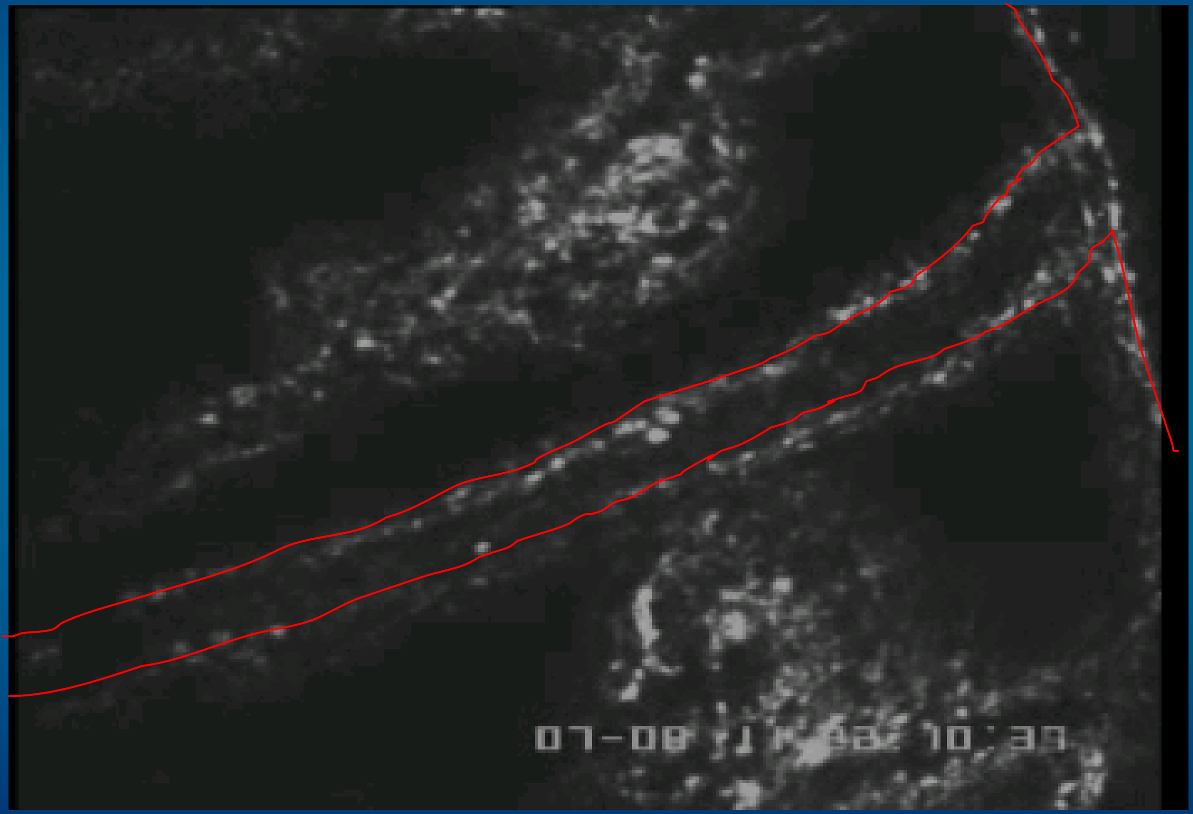
Max. blood velocity(Vmax)



Blood flow rate



Visualized Leukocytes under Intravital confocal Microscopy



$$\text{Rolling count (\%)} = \frac{\text{rolling or adherent cell}}{\text{total cell}} \times 100$$

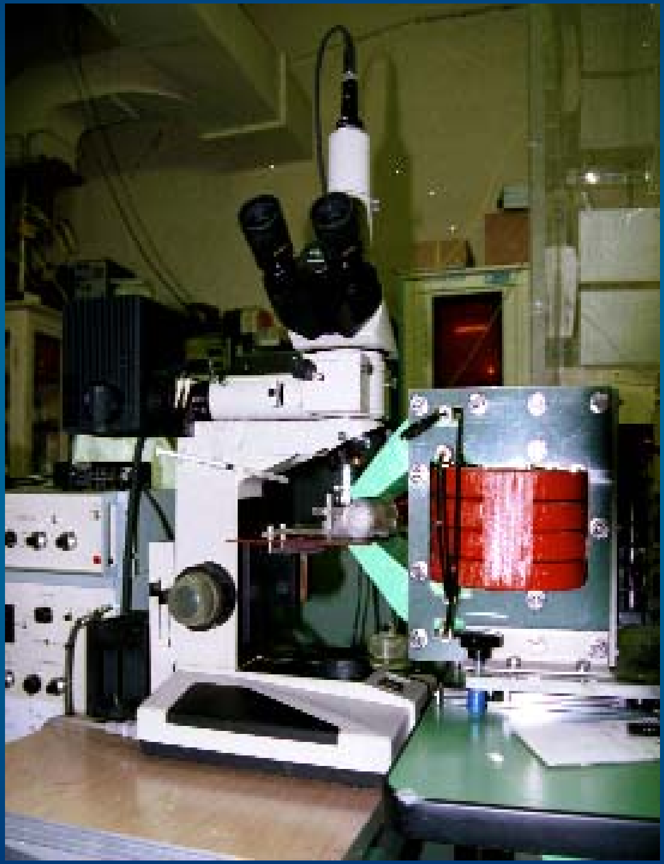
1) Acute exposure experiment

Exposure conditions

50 Hz EMF

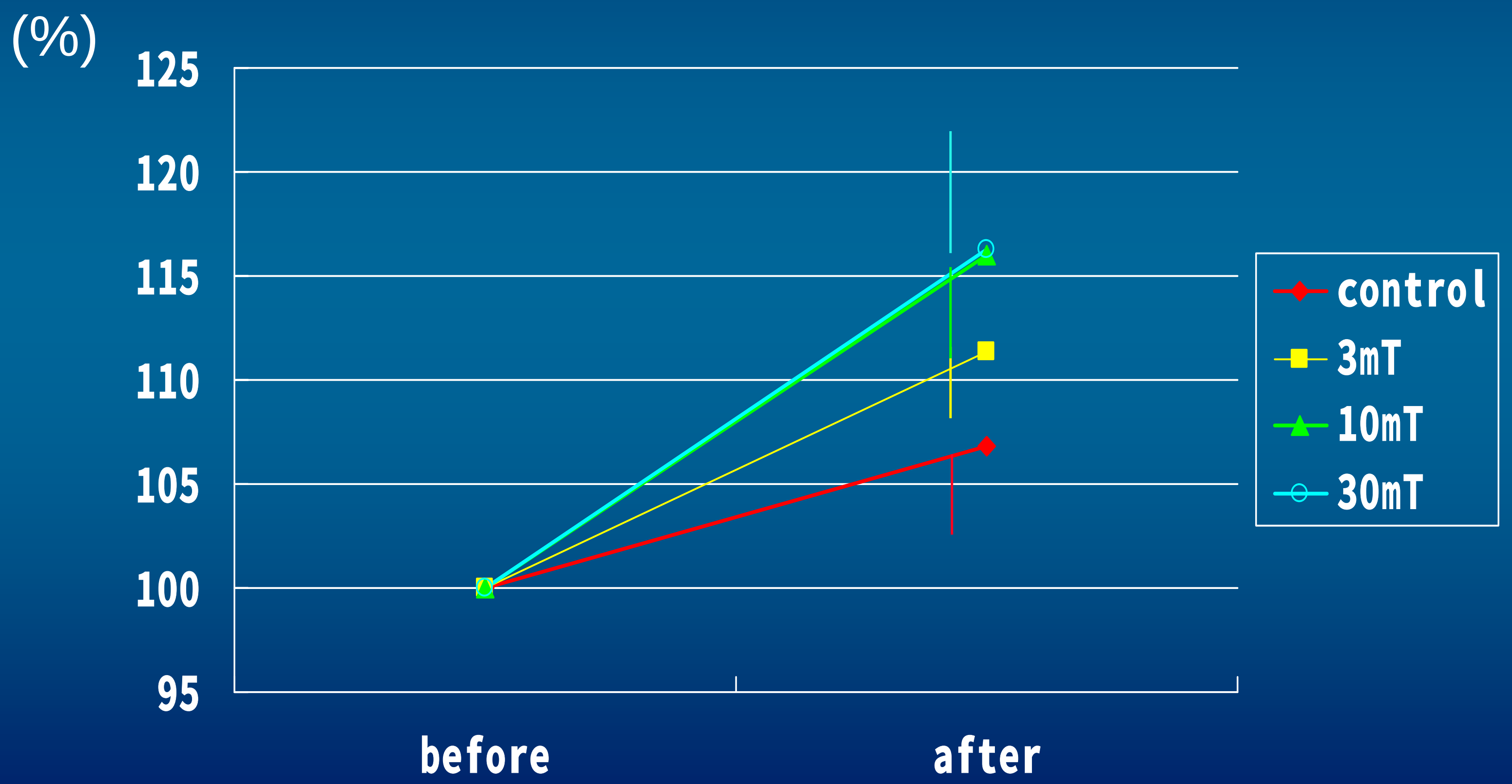
Intensity: 3、10、30 mT

Period：30 minutes

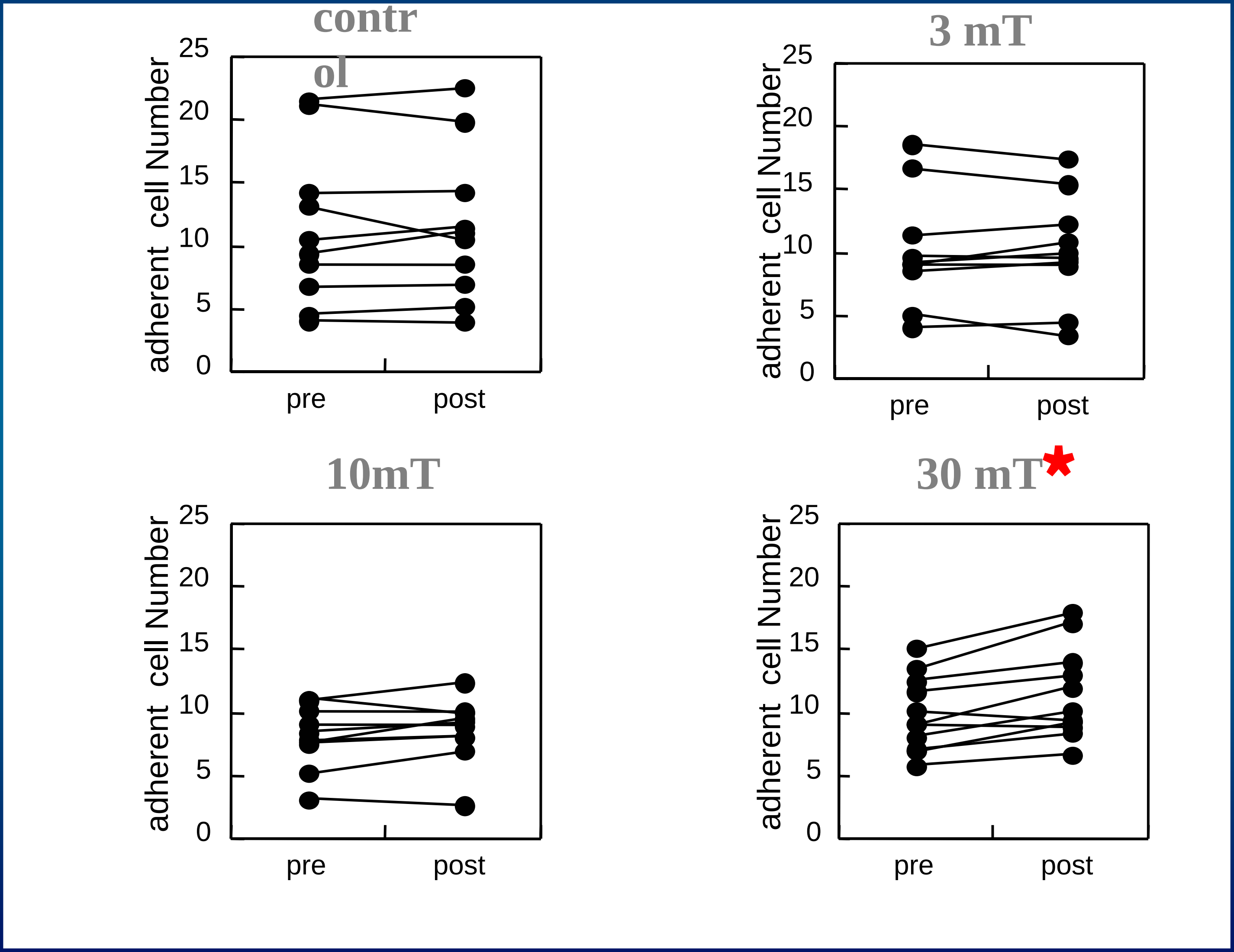


Venular diameter at before and after ELF exposure

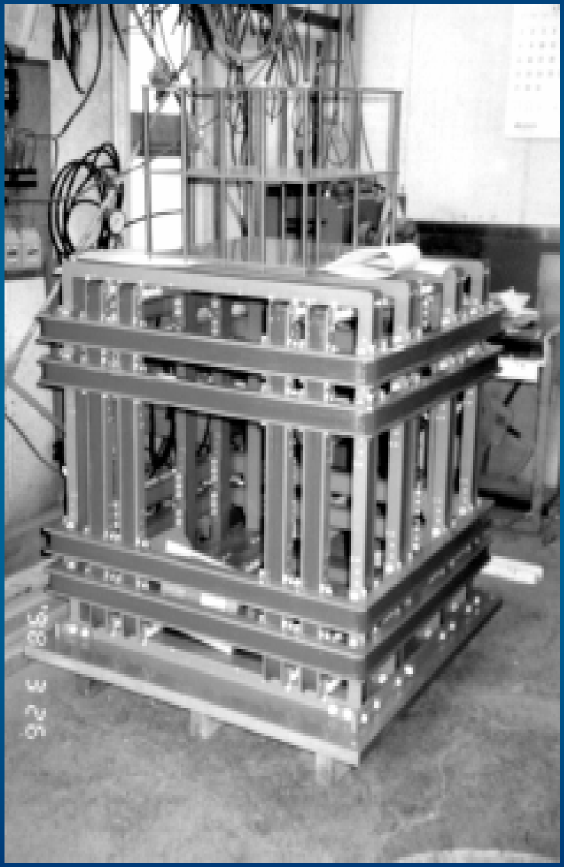
Venular blood flow rate at before and after ELF exposure

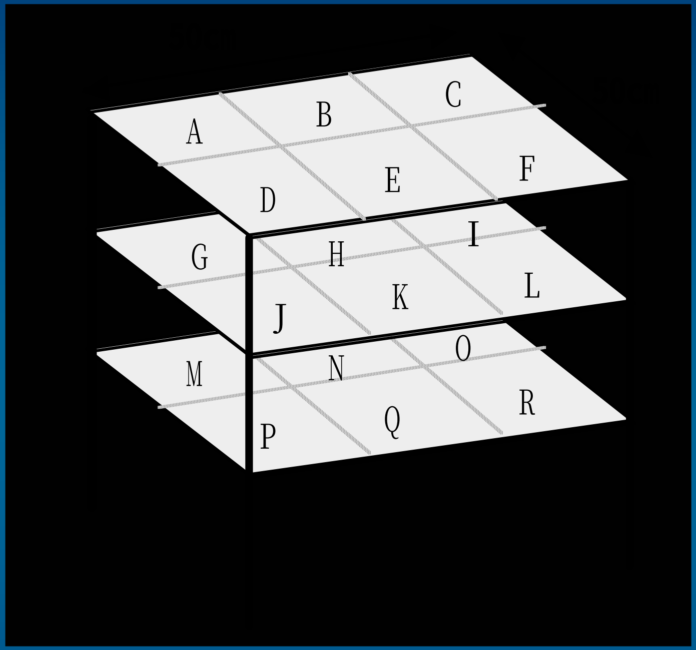


Adherent leukocytes at before and after ELF exposure



2) Subchronic exposure experiment





frequency：50 Hz

magnetic density：3 mT

Protocol

-5 dorsal skinfold chamber implantation to BALB/c mice (male, 8w)

0 mammary tumor cell implantation to the chamber (MMT060562 , 1x10^5 cell/mice)

beginning of ELF exposure **(22 hr/day)**

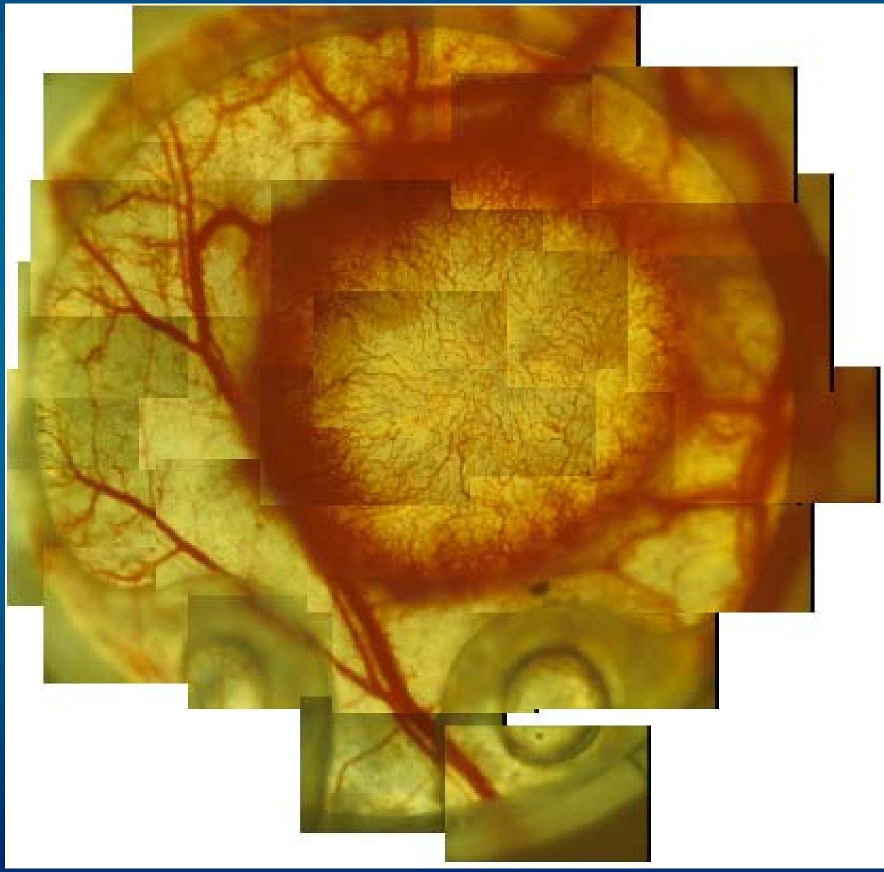
Day 0 & day 15

observation and analysis of microcirculatory parameters, tumor growth, and serum cytokine level

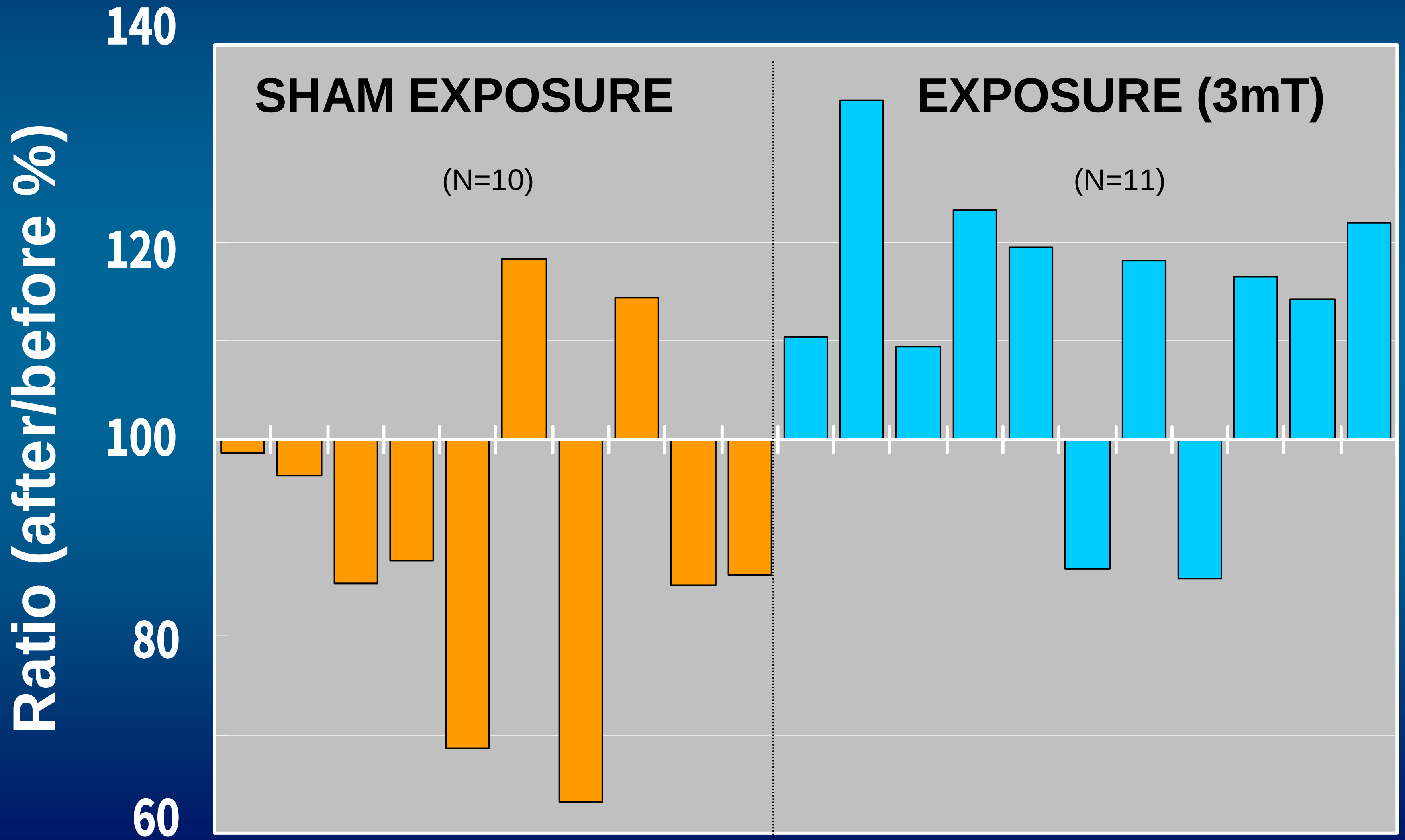
15 end of ELF exposure

Tumor and Angiogenesis

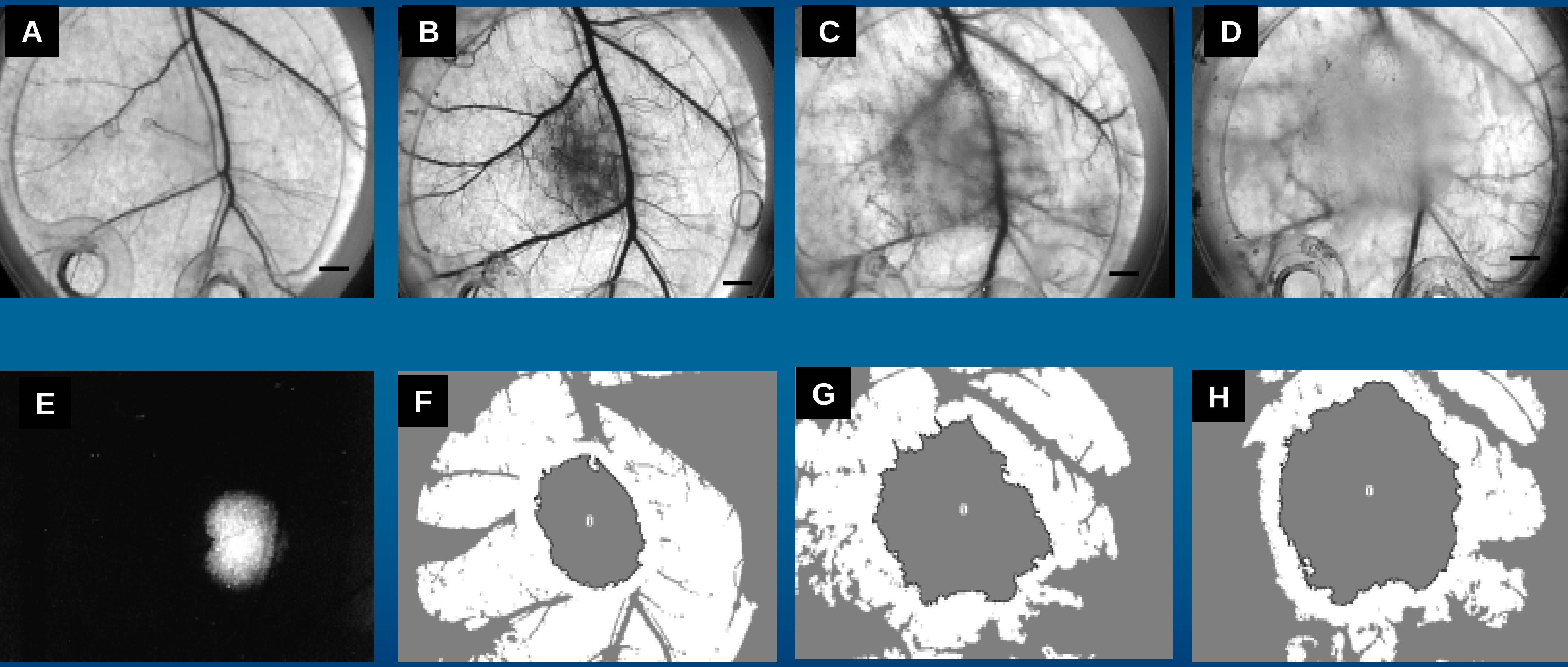
- Angiogenesis is the recruitment of new blood vessel.
- Angiogenesis in tumor tissue is an essential process of the tumor growth.



Adherent leukocytes at before and after ELF exposure

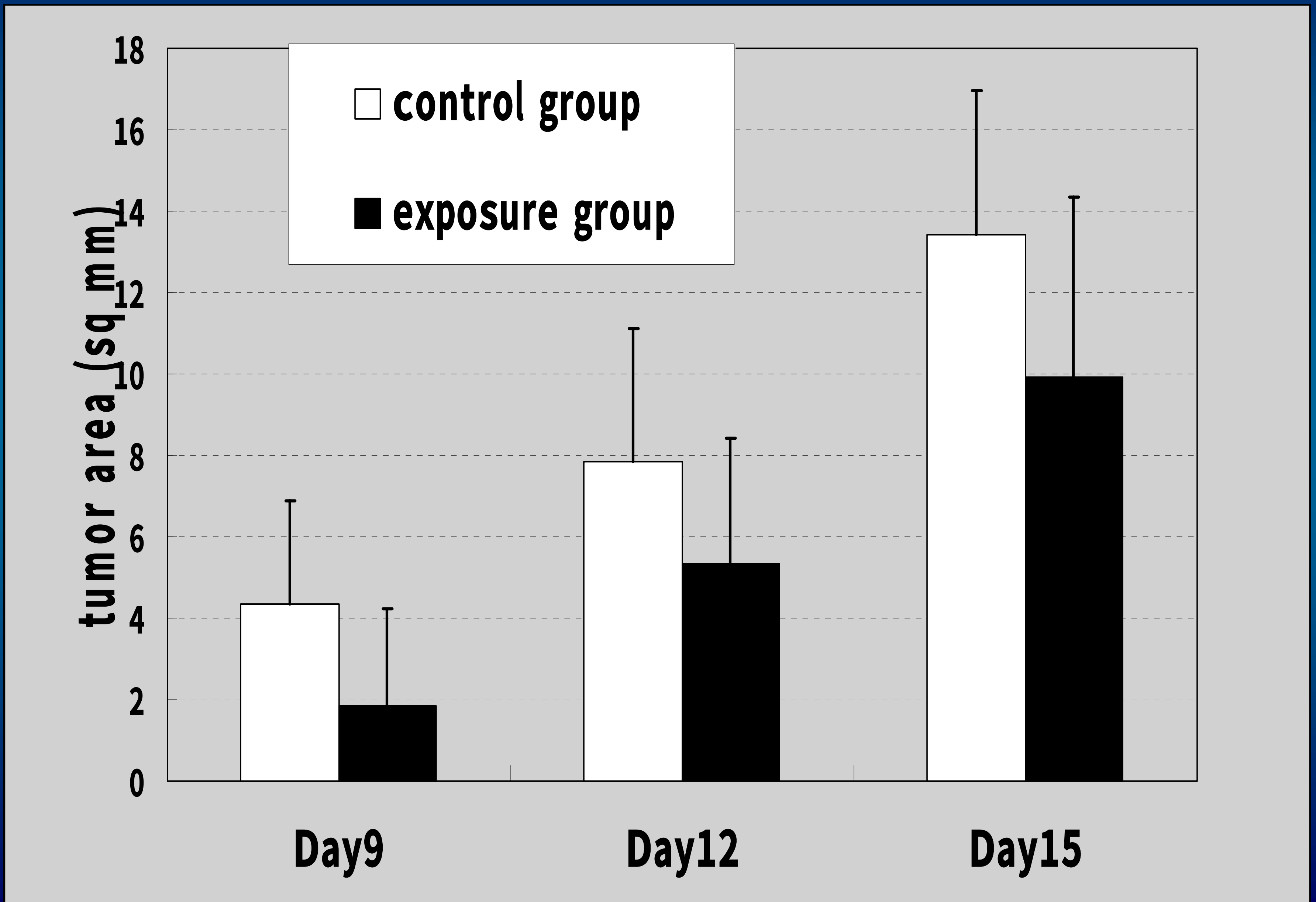


Estimation of tumor size within DSC



(Images were analyzed by NIH image)

Tumor size within DSC



Conclusion: Power Frequency EMF

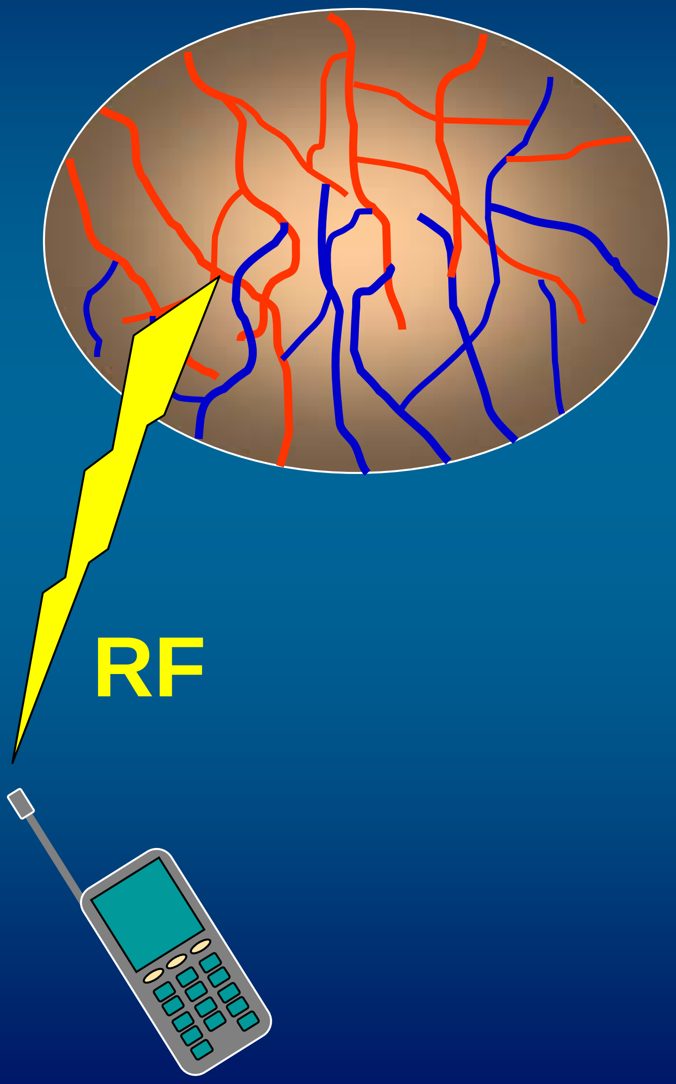
Power frequency EMF with mT levels may influence cell to cell interaction between endothelial cells and leukocytes. However, no effects on mammary tumor growth rate were recognized in tumor grafting mice.

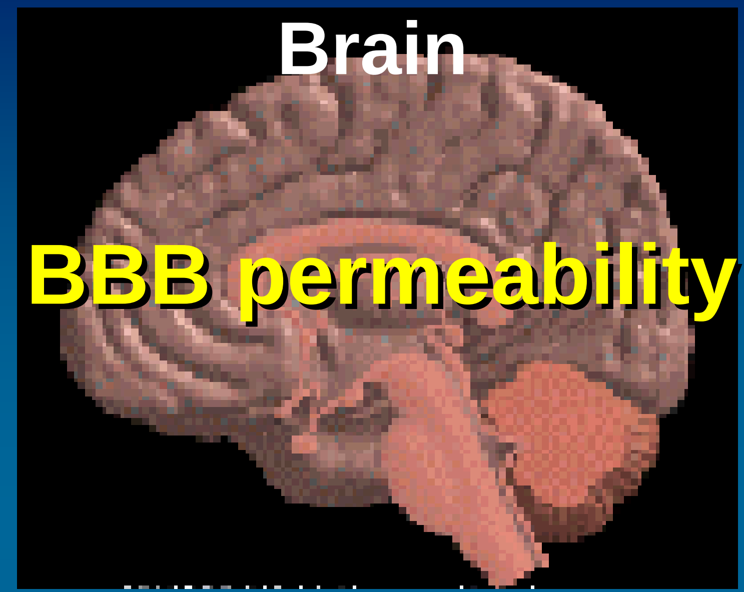
2. Radio Frequency EMF

Intra-vital microscopic evaluation of acute effects on the brain by local exposure to radio-frequency electromagnetic fields in rats

Effects of RF on the cerebral microcirculation

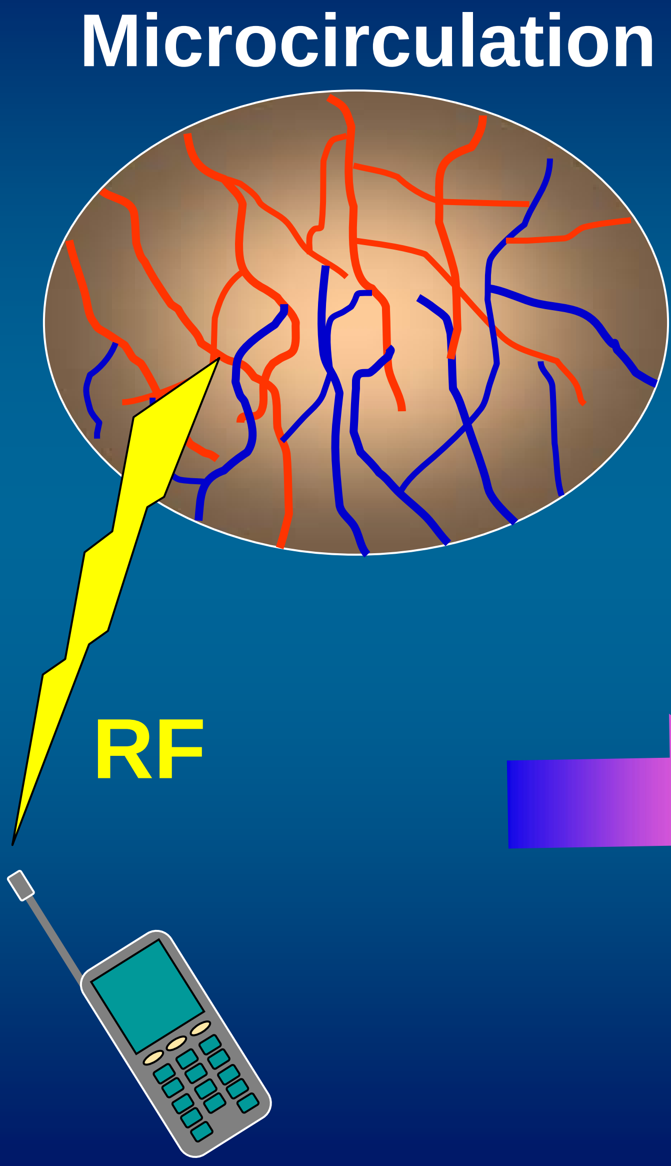
Microcirculation

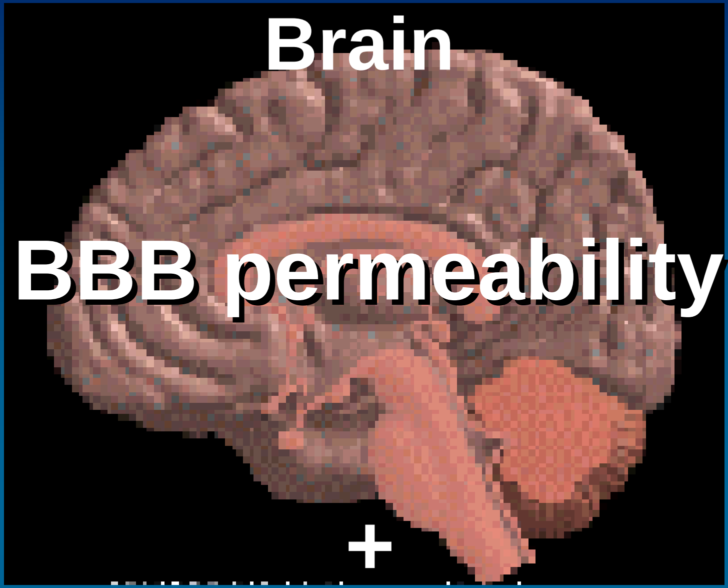




Other parameters ?

Effects of RF on the cerebral microcirculation





Leukocyte behavior
Plasma velocity
Vessel diameter
Blood viscosity
⋮

Previous approach

Items	Histological approach
Animal	Postmortem
Observable region	Whole brain
BBB permeability	Yes High sensitivity
Protein expression	Yes
Leukocyte behavior	No
Blood flow	No
Vessel diameter	No
Plasma viscosity	No
PO_2 level	No
Others	Easy to compare with previous results

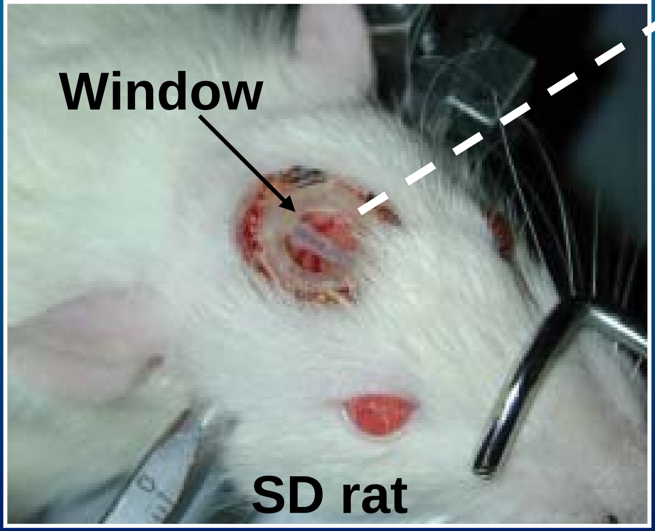
Our combination approach

Intravital microscopic approach	Items	Histological approach
Live	Animal	Postmortem
Pia mater (<200μm depth)	Observable region	Whole brain
Yes Real time	BBB permeability	Yes High sensitivity
No / (Yes with GFP)	Protein expression	Yes
Yes	Leukocyte behavior	No
Yes	Blood flow	No
Yes	Vessel diameter	No
Yes	Plasma viscosity	No
Yes	PO_2 level	No
Possible to observe dynamic changes	Others	Easy to compare with previous results

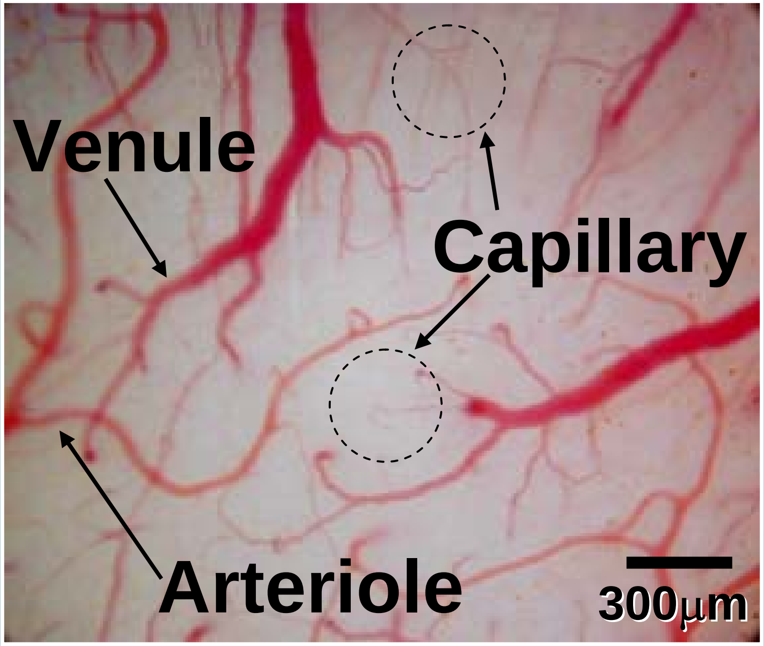
Modified cranial window method

Long-term observation
of the same cerebral region

RF exposure
with cranial window



Pial microcirculation



Fluorescence microscopy

Pial microcirculation

Venule

Venule

Arteriol

Na^+-fluorescein injection

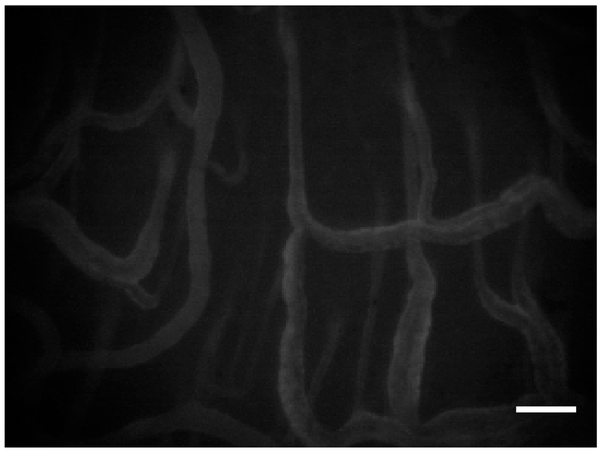
Evaluated parameters

1. BBB permeability
2. Leukocyte behavior
3. Plasma velocity
4. Vessel diameter

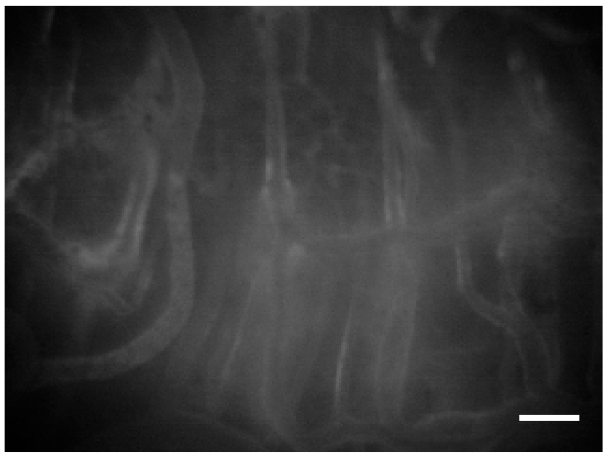
1. BBB permeability (1)

Extravasation of FITC-Dx from pial vessels

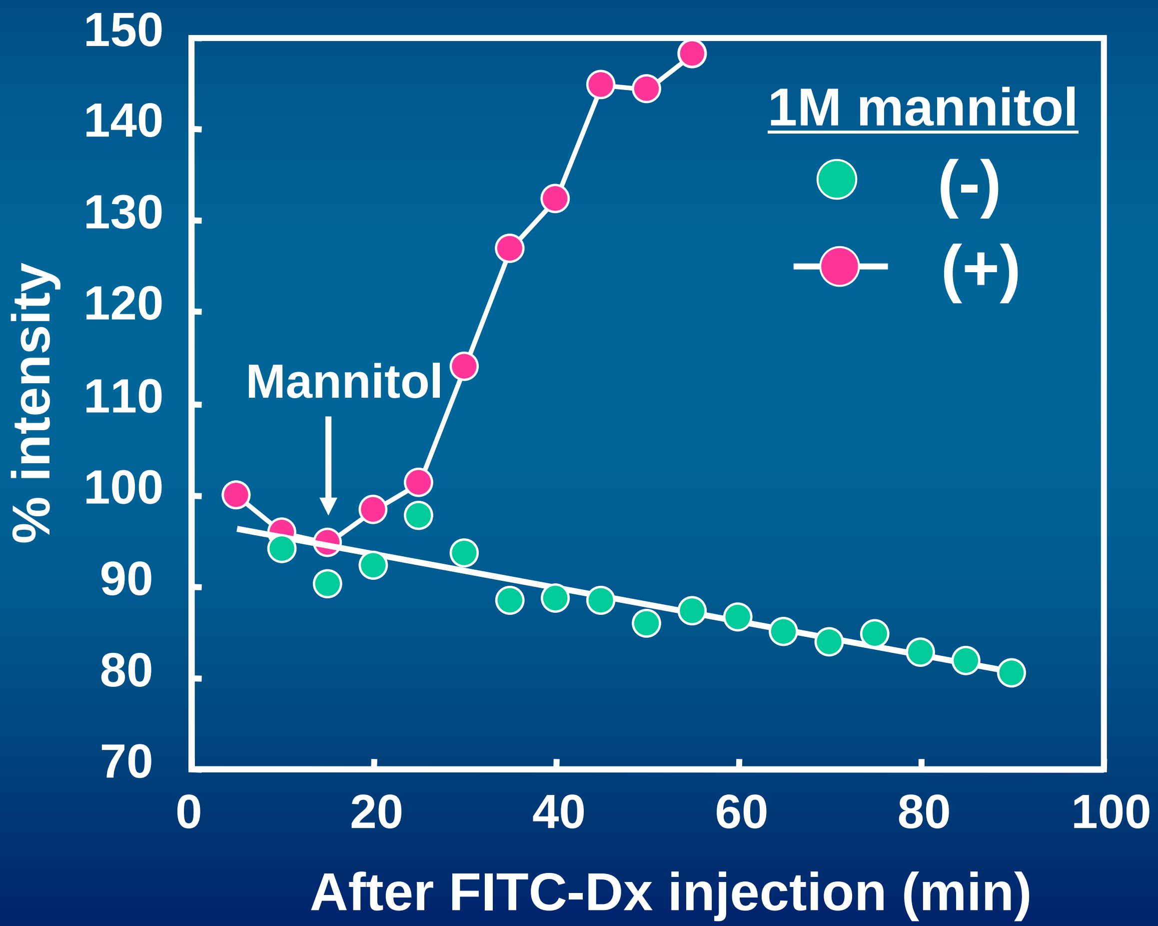
Normal condition



1M mannitol

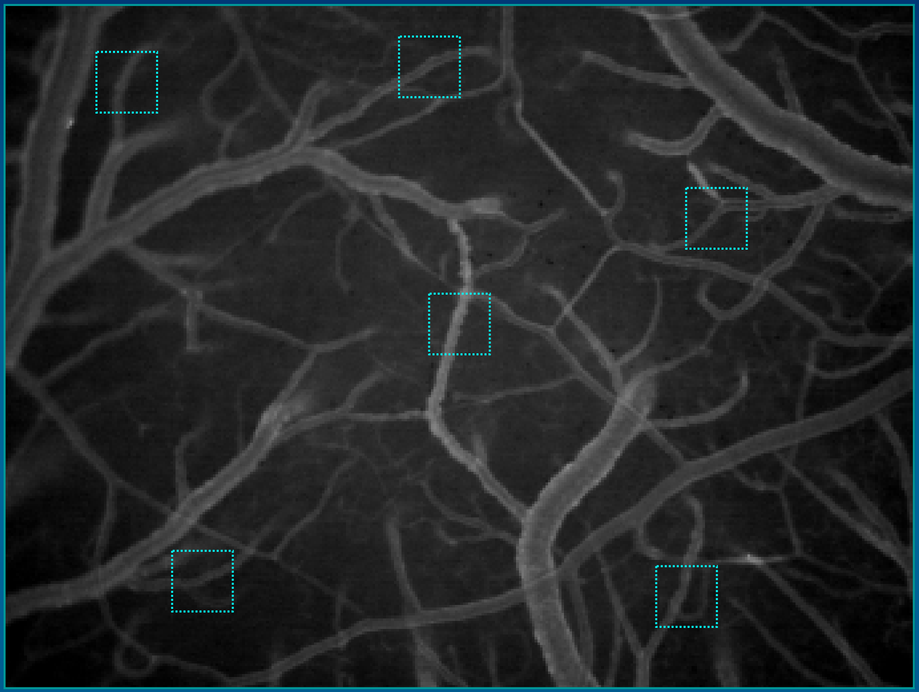


Bar : 100μm



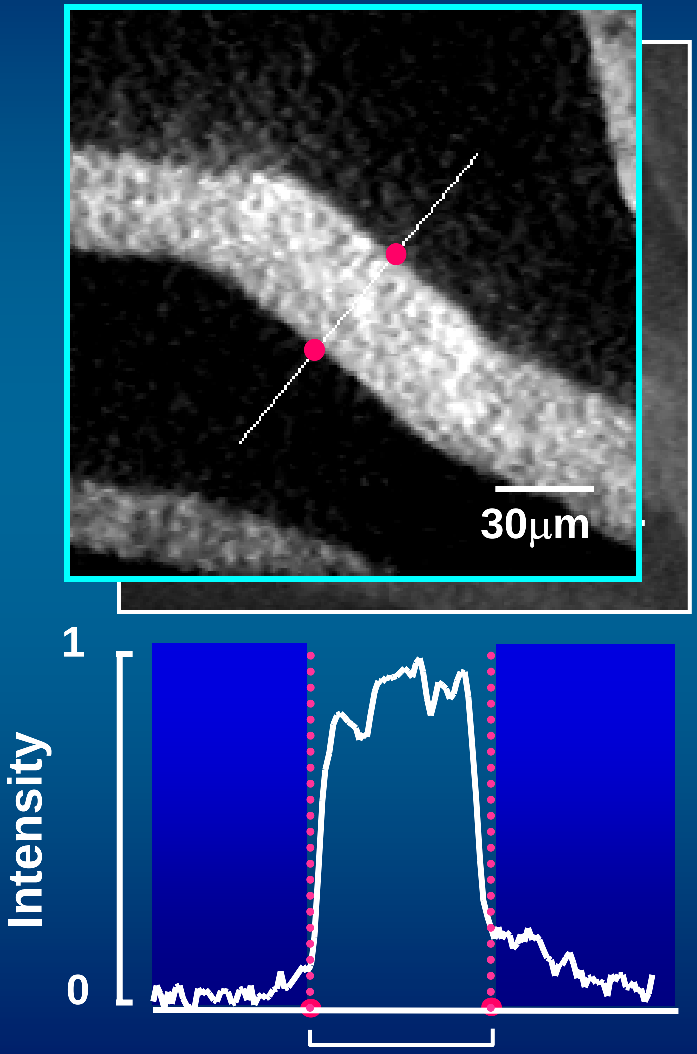
1. BBB permeability (2)

Pial venules

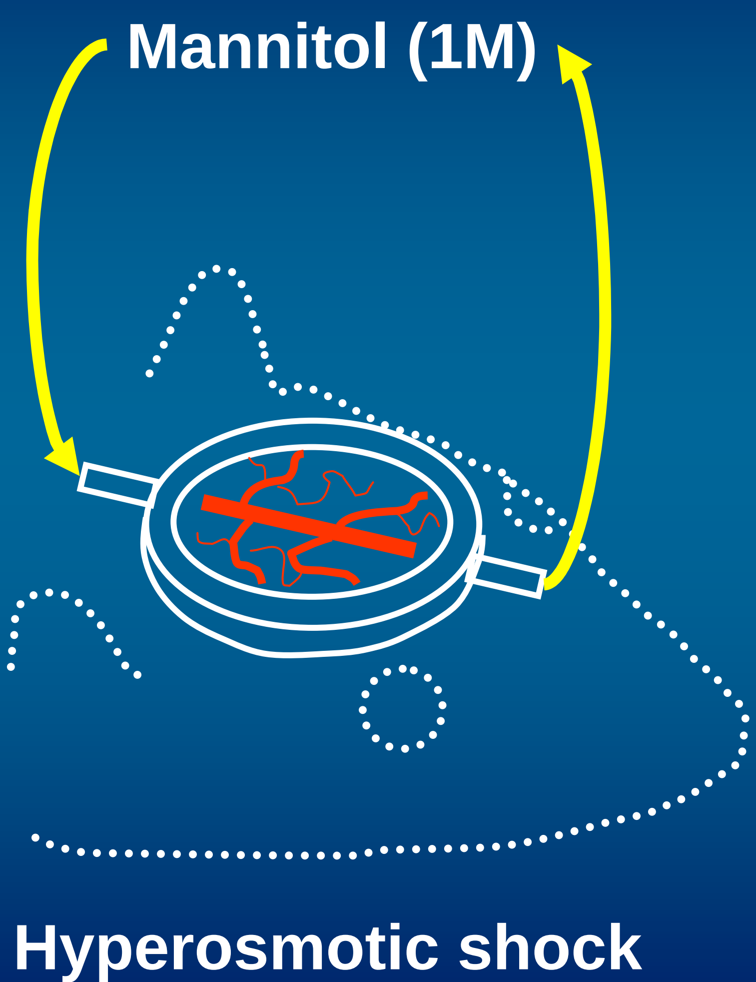


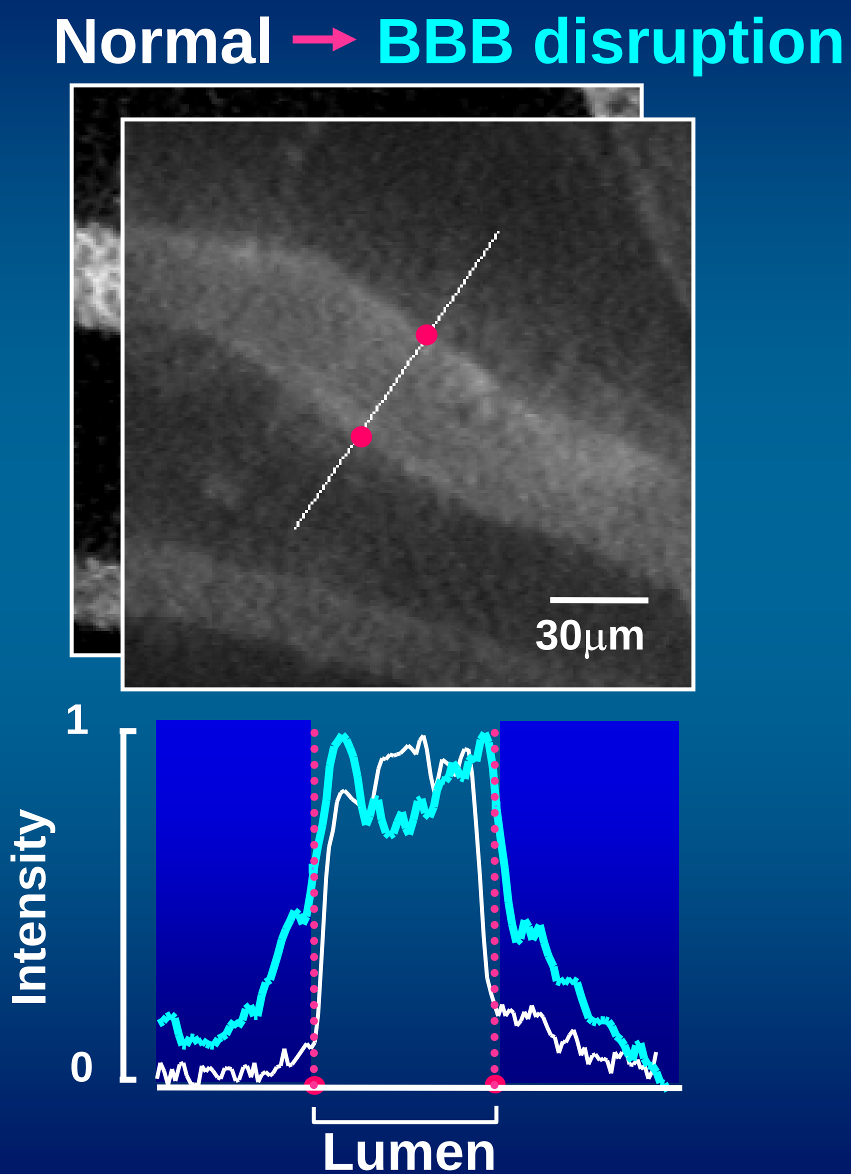
After Na^+-fluorescein injection

Normal



1. BBB permeability (2)

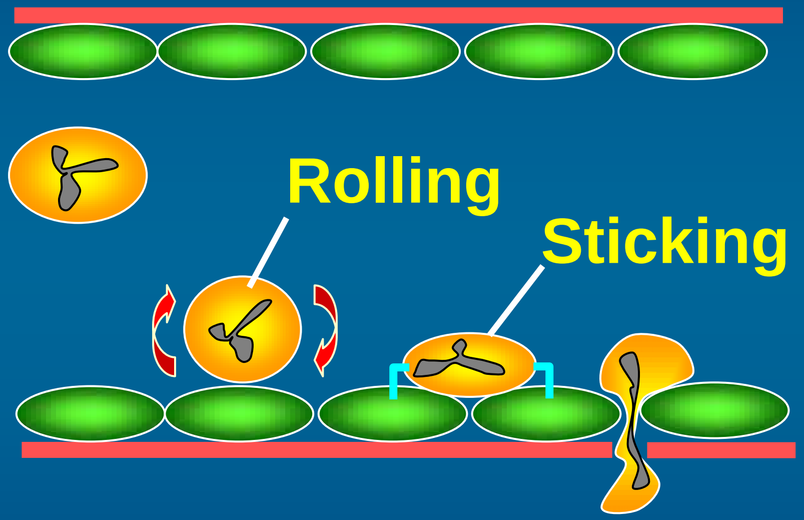




2. Leukocyte behavior

Stained leukocytes

Stained with Rhodamine 6G

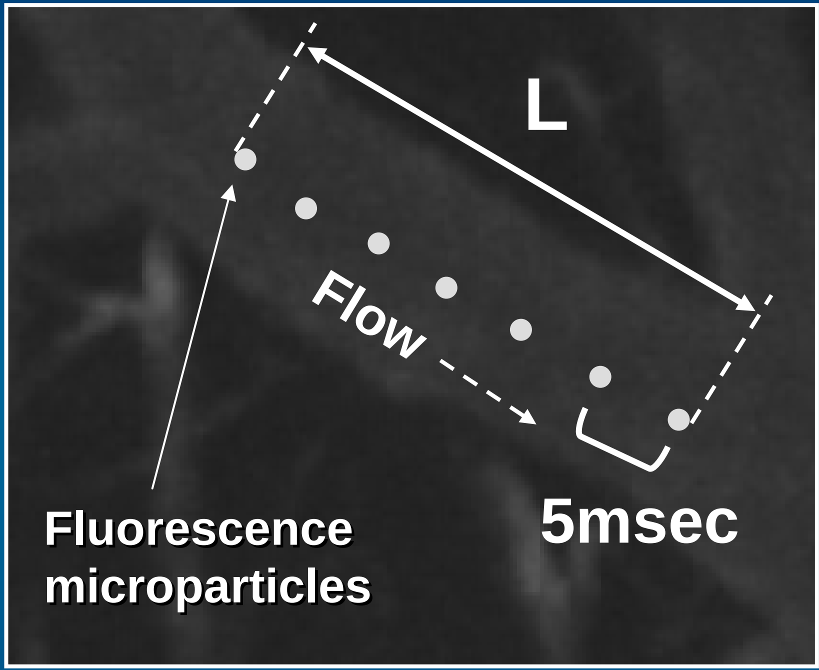


3. Plasma velocity

After fluorescent microparticles injection

3. Plasma velocity

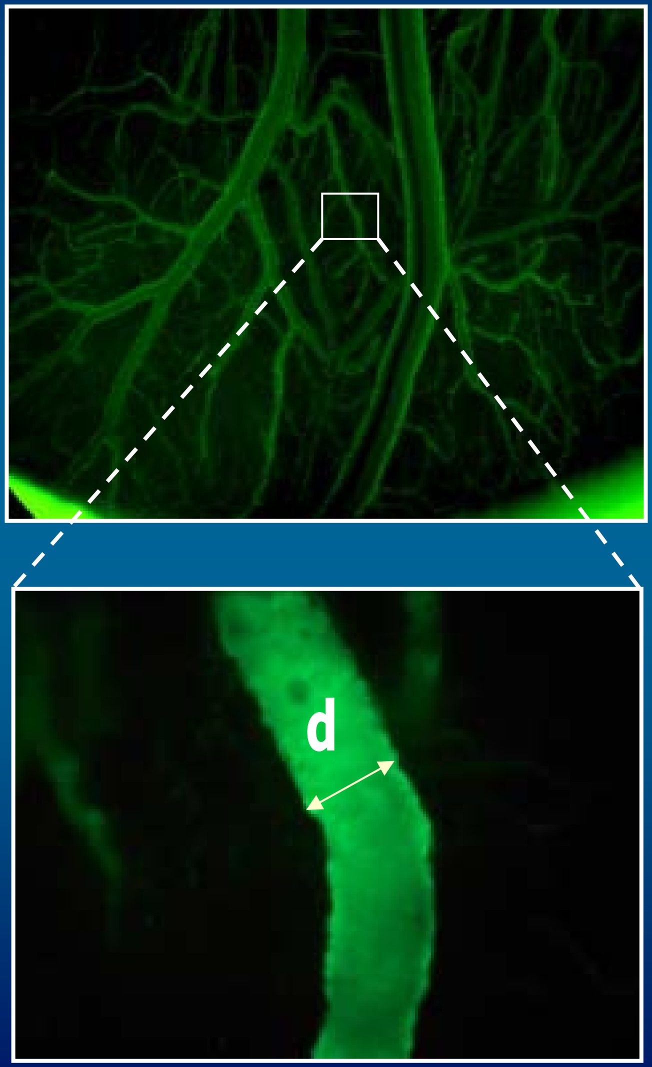
One frame under 200Hz-flashlight



$$\text{Velocity} = \frac{L}{6 \text{ intervals} \times 5 \text{ ms}}$$

4. Vessel diameter

After Na^{+}-fluorescein injection

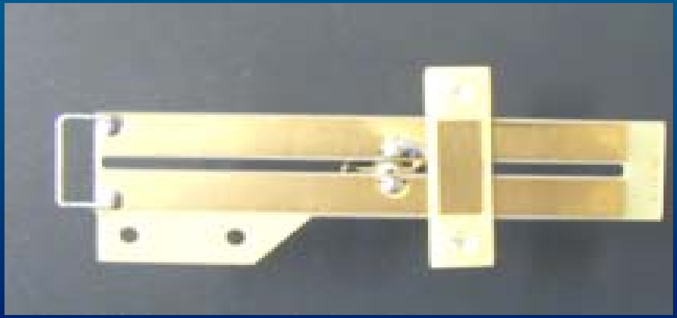


Loop antenna and SAR values

(W/kg)

Brain	Average	0.18	1.80	6.84
	Peak	0.27	2.96	11.30
Whole body	Average	$\leq$0.009	$\leq$0.09	$\leq$0.342

The threshold of thermal effects : about 1 to 4 W/kg (the average SAR of the whole body)



$$\text{Whole body} \leq \frac{1}{20}\ \text{Brain}$$

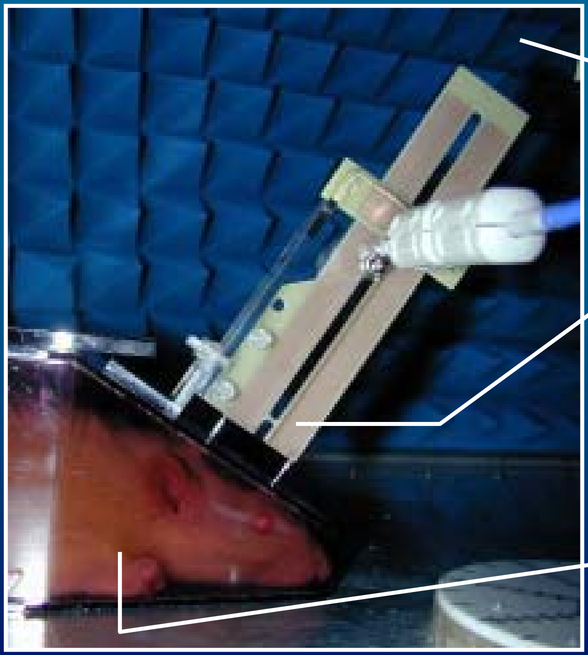
Exposure protocol

Pre Measurements → **10 min RF exposure** → **Post Measurements**

with anesthesia

Brain average SAR

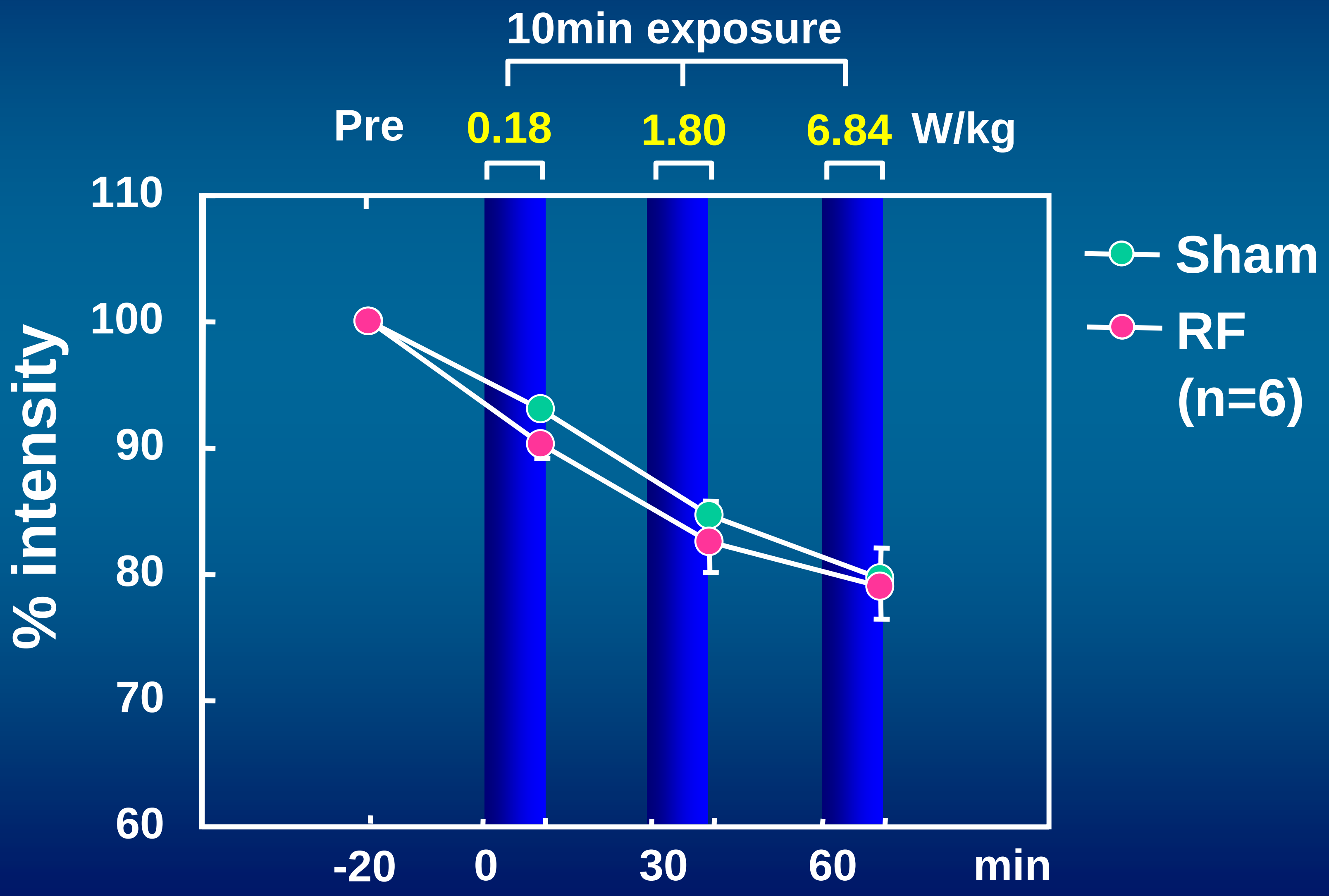
- 0.18 W/kg
- 1.80 W/kg
- 6.84 W/kg



- **Small anechoic chamber**
- **Loop antenna**
  - Frequency : 1439 MHz
  - Signal type : TDMA (PDC)
- **SD rat with cranial window**

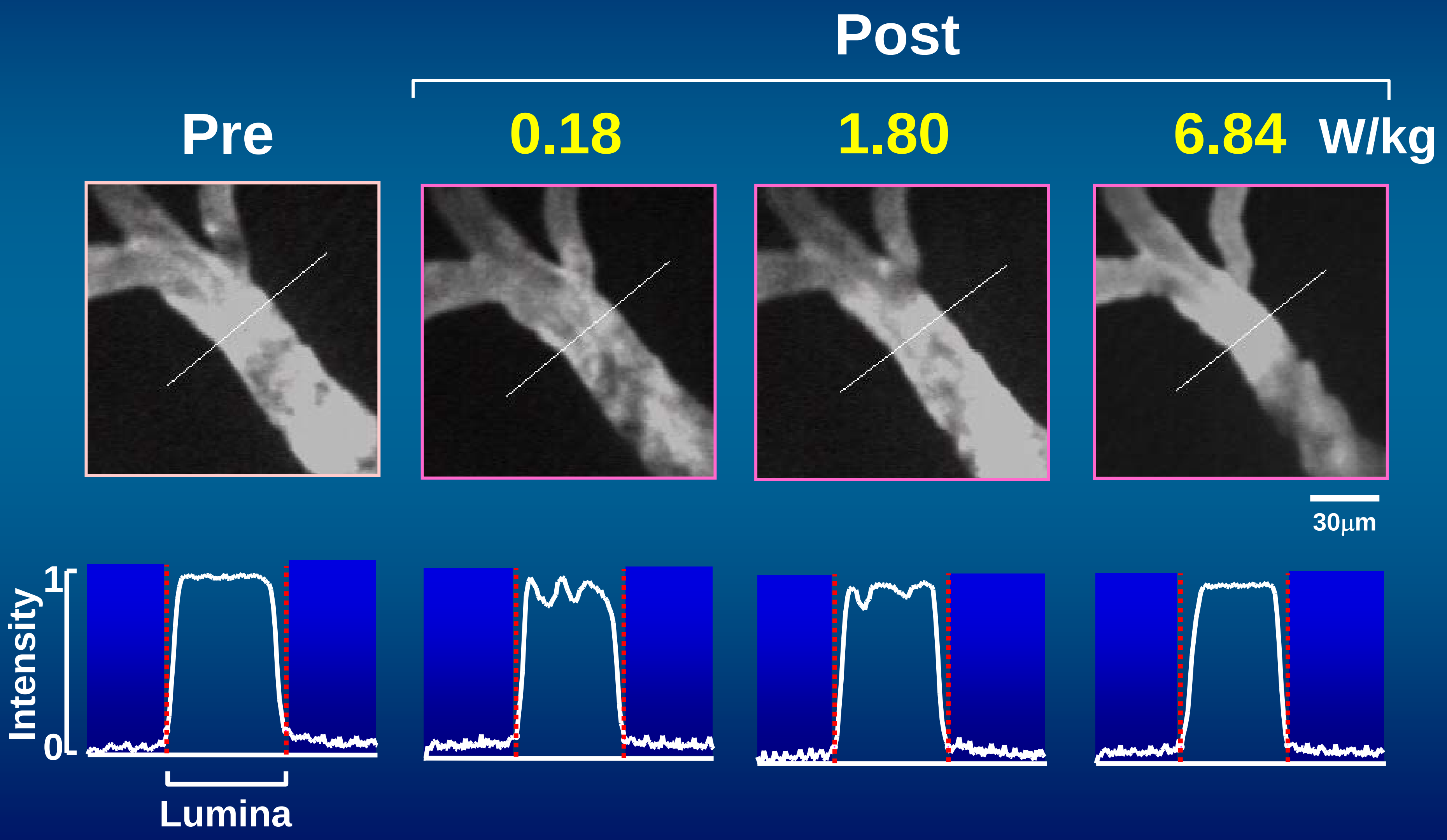
BBB permeability (1)

Extravasation of FITC-Dx from pial vessels



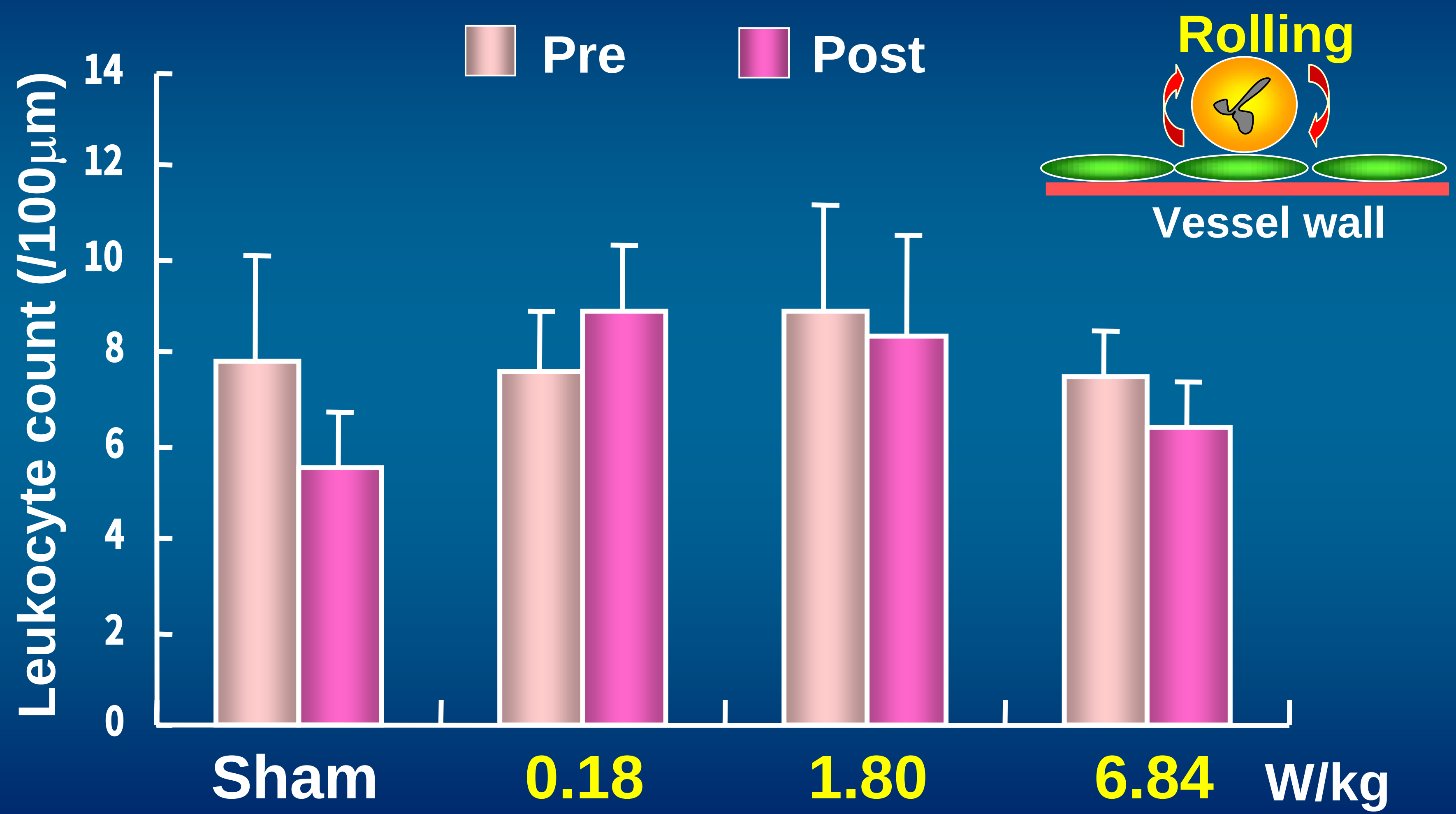
BBB permeability (2)

Extravasation of Na^+-fluorescein from pial venule



Leukocyte behavior (1)

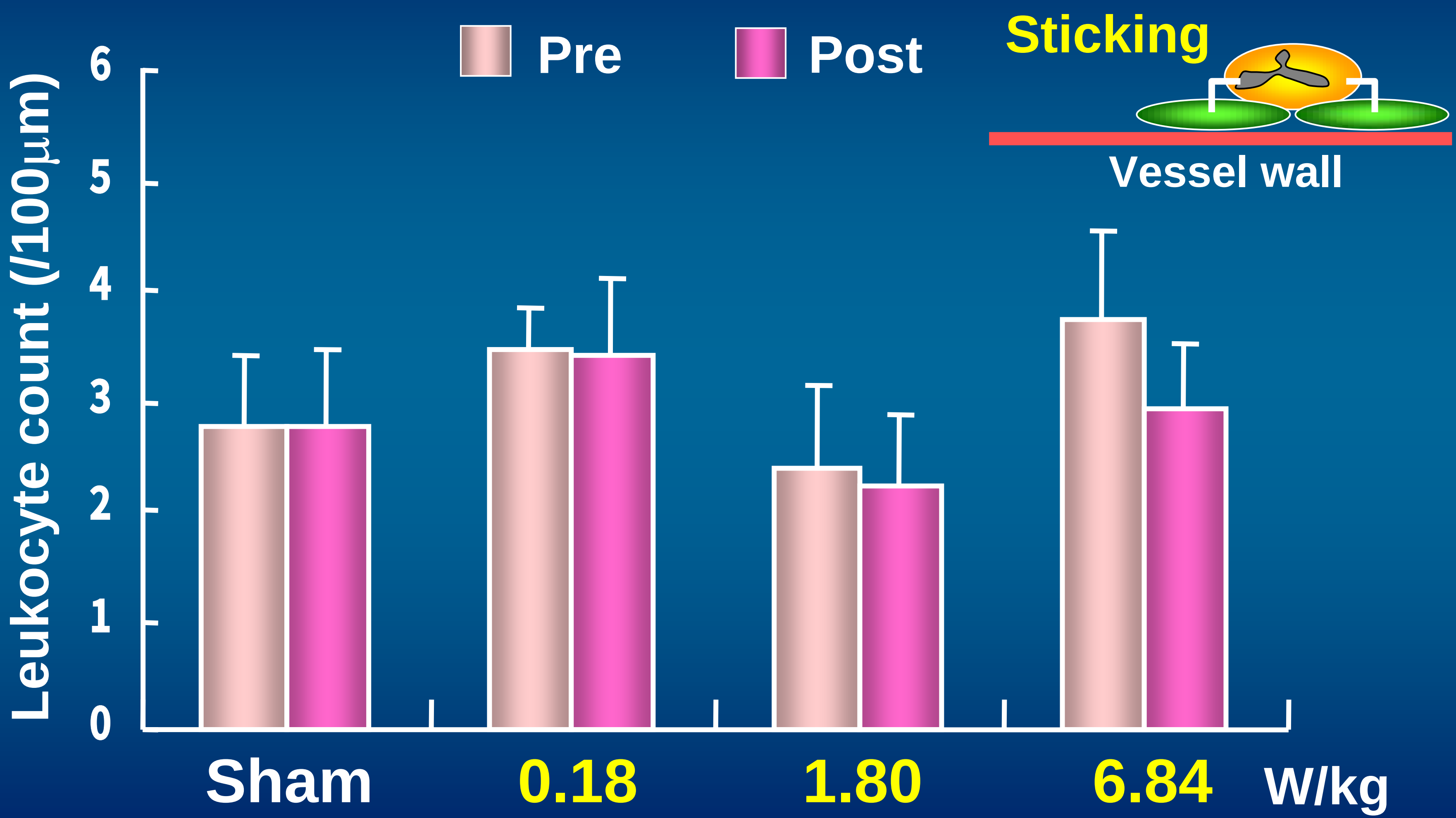
Rolling-leukocyte counts in the pial venule



(venule: 8-30μm, n = 7-8)

Leukocyte behavior (2)

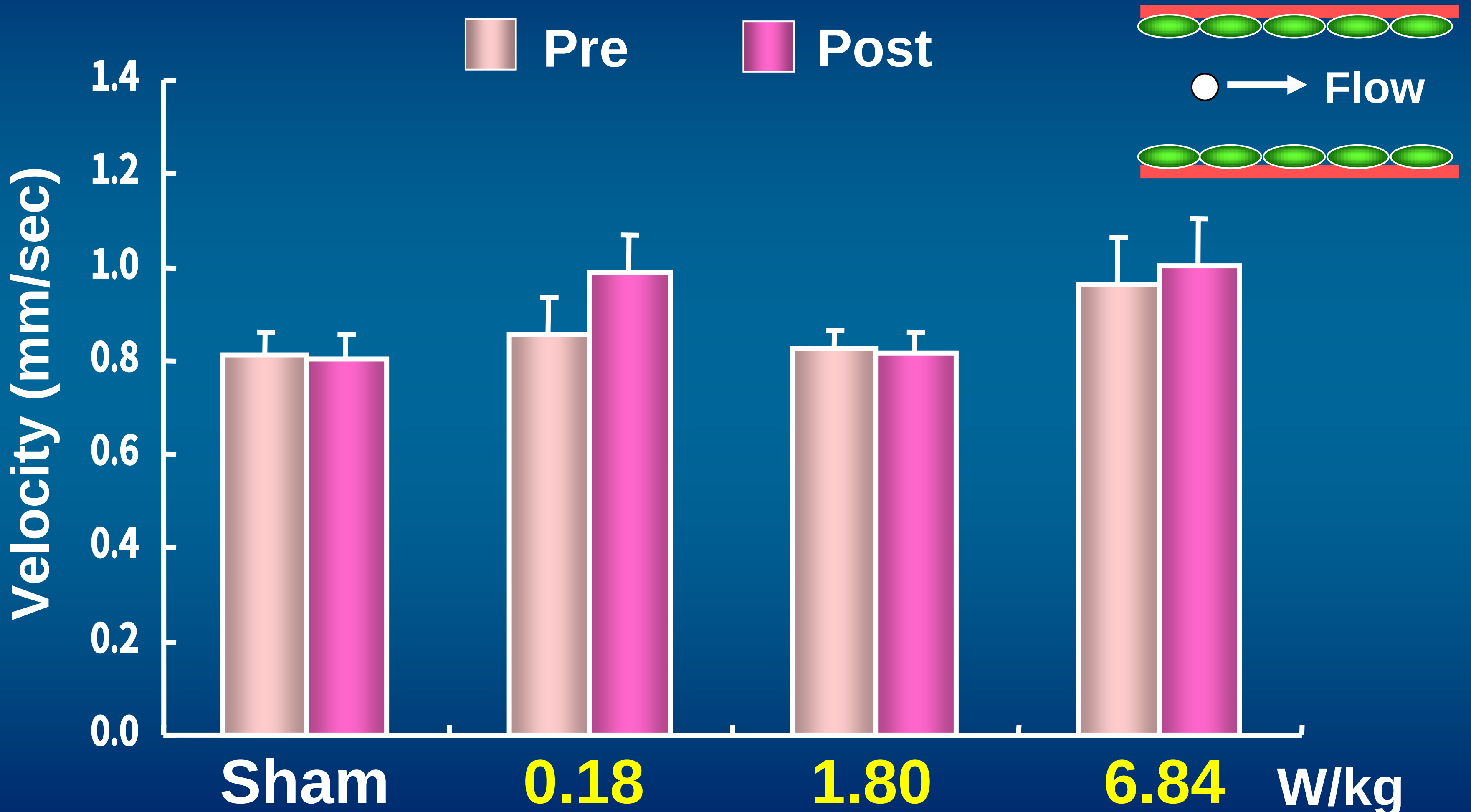
Sticking-leukocyte counts in the pial venule



(venule: 8-30μm, n = 7-8)

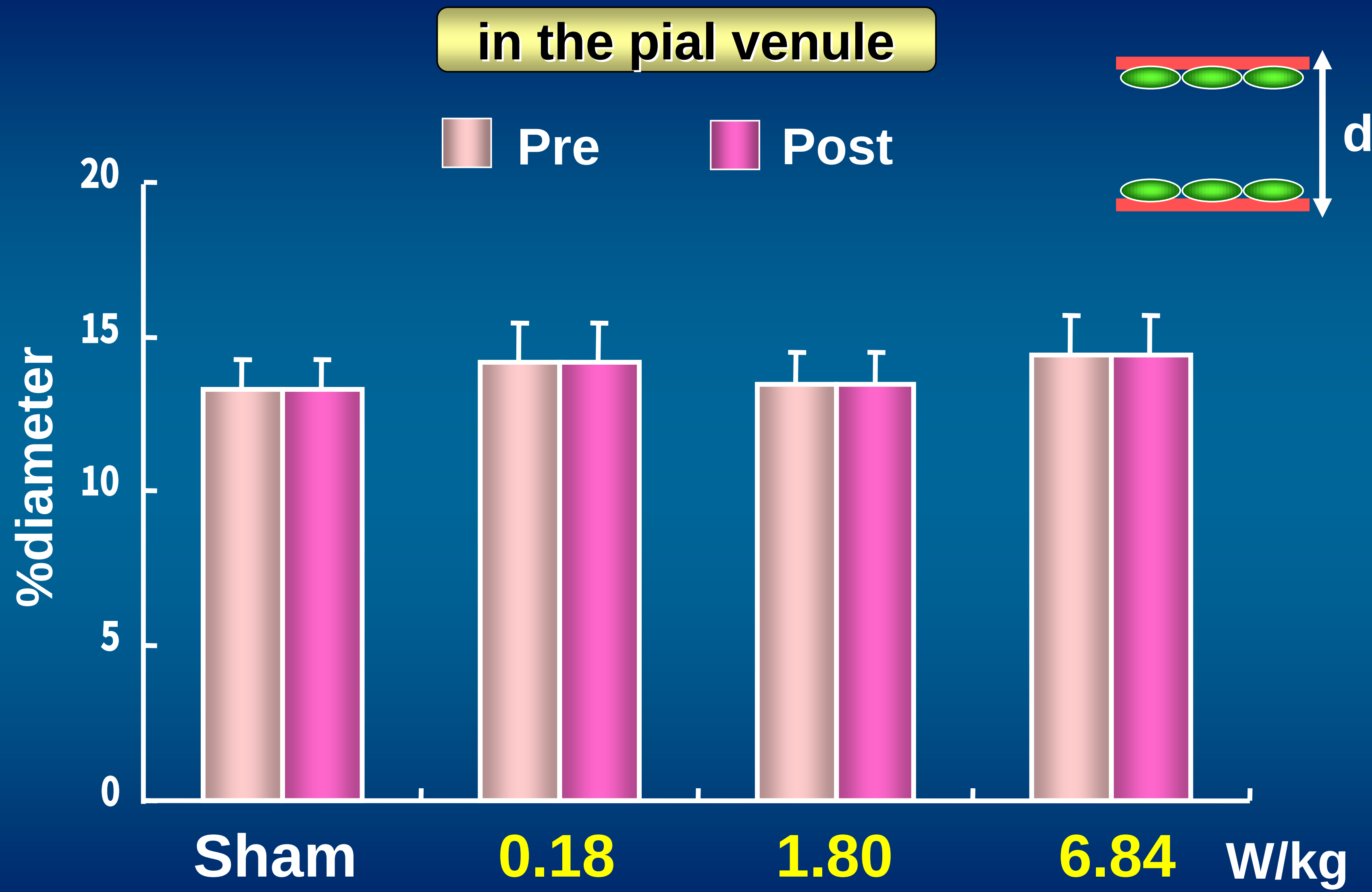
Plasma velocity changes

Microparticle velocity in the pial venule



(venule: 8-30μm, n = 11-14)

Vessel diameter changes



(venule: 8-30μm, n = 11-14)

Acute effect of RF exposure

RF
1439MHz
Loop

→

BSAR
0.18 W/kg
1.80 W/kg
6.84 W/kg

→

Period
10 min

- BBB permeability
- Leukocyte behavior
- Plasma velocity
- Vessel diameter

Support

No significant changes

Conclusion: Radio Frequency EMF

We evaluated acute effects of RF local exposure whose intenisties are more than permissible exposure limits due to ICNIRP guidelines by use of loop antenna on cerebral microcirculation within cranial window using intra-vital microscopy in rats . No noticeable changes occurred due to our exposure conditions for the four microcirculatory parameters including BBB function.

3. Static MF

Modulatory effects of static magnetic fields with mT levels on circulatory system in experimental animals.

Publications related with Static MF

1) Ohkubo C, Xu S. 1997. Acute effects of static magnetic fields on cutaneous microcirculation in rabbits. In Vivo 11:221-225.

2) Xu S, Okano H, Ohkubo C. 1998. Subchronic effects of static magnetic fields on cutaneous microcirculation in rabbits. In Vivo 12:383-389.

3) Okano H, Gmitrov J, Ohkubo C. 1999. Biphasic effects of static magnetic fields on cutaneous microcirculation in rabbits. Bioelectromagnetics 20:161-171.

4) Xu S, Okano H, Ohkubo C. 2000. Acute effects of whole-body exposure to static magnetic fields and 50-Hz electromagnetic fields on muscle microcirculation in anesthetized mice. Bioelectrochemistry 53:127-135.

5) Okano H, Ohkubo C. 2001. Modulatory effects of static magnetic fields on blood pressure in rabbits. Bioelectromagnetics 22:408-418.

6) Gmitrov J, Ohkubo C. 2002a. Artificial static and geomagnetic field interrelated impact on cardiovascular regulation. Bioelectromagnetics 23:329-338.

Publications related with Static MF

7) Gmitrov J, Ohkubo C. 2002b. Verapamil protective effect on natural and artificial magnetic field cardiovascular impact. Bioelectromagnetics 23:531-541.

8) Gmitrov J, Ohkubo C, Okano H. 2002. Effect of 0.25 T static magnetic field on microcirculation in rabbits. Bioelectromagnetics 23:224-229.

9) Okano H, Ohkubo C. 2003a. Anti-pressor effects of whole-body exposure to static magnetic field on pharmacologically induced hypertension in conscious rabbits. Bioelectromagnetics 24: 139-147.

10) Okano H, Ohkubo C. 2003b. Effects of static magnetic fields on plasma levels of angiotensin II and aldosterone associated with arterial blood pressure in genetically hypertensive rats. Bioelectromagnetics 24:403-412.

11) Okano H, Masuda H, Ohkubo C. 2004a. Effects of 25 mT static magnetic field on blood pressure in reserpine-induced hypotensive Wistar-Kyoto rats. Bioelectromagnetics. in press.

12) Okano H, Masuda H, Ohkubo C. 2004b. Decreased plasma levels of nitric oxide metabolites, angiotensin II and aldosterone in spontaneously hypertensive rats exposed to 5 mT static magnetic field. Bioelectromagnetics. in press.

Conclusion: Static MF

The results suggest that Static MF with mT levels could modulate beneficially the micro- and macrocirculation and/or blood pressures in a drug-treated animal and a genetically hypertensive, and a drug-treated hypotensive animal. These effects would be used in possible explanation for the therapeutic effects on many diseases related to dysfunction in micro- and macrocirculation in human subjects. .

Overall Conclusion

Intra-vital microcirculatory measurement is one of the most rational and fundamental tools for evaluating the exposure effects of EMF in animals.

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