

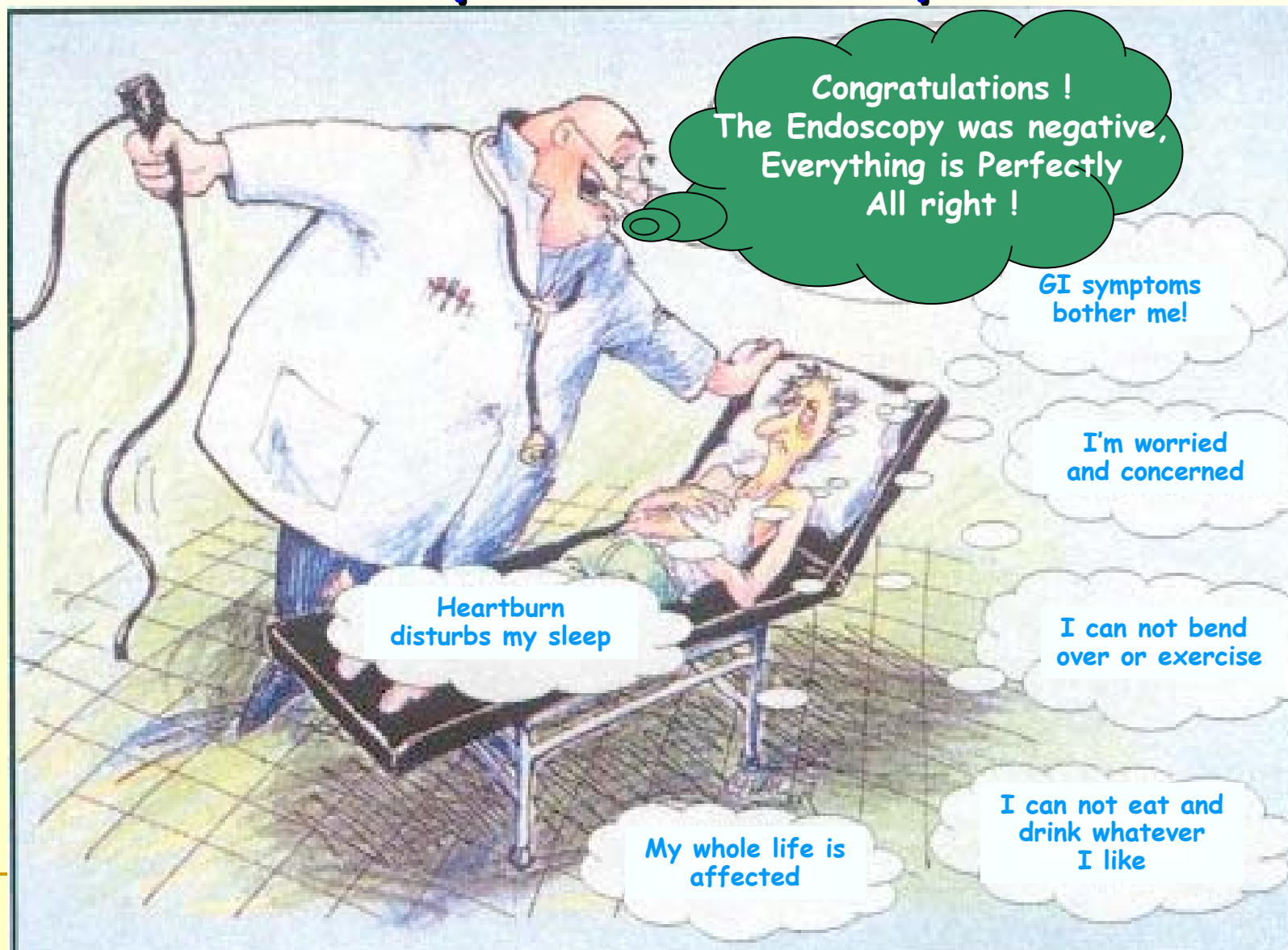
# 培養細胞系を用いたin vitro薬物動態研究

## Pharmacokinetic Study Using *In Vitro* Culture Cell Methods

株式会社 大塚製薬工場 研究開発センター  
内藤 真策

**Shinsaku Naito**  
**Research and Development Center**  
**Otsuka Pharmaceutical Factory, Inc.**

# What's important to patients?

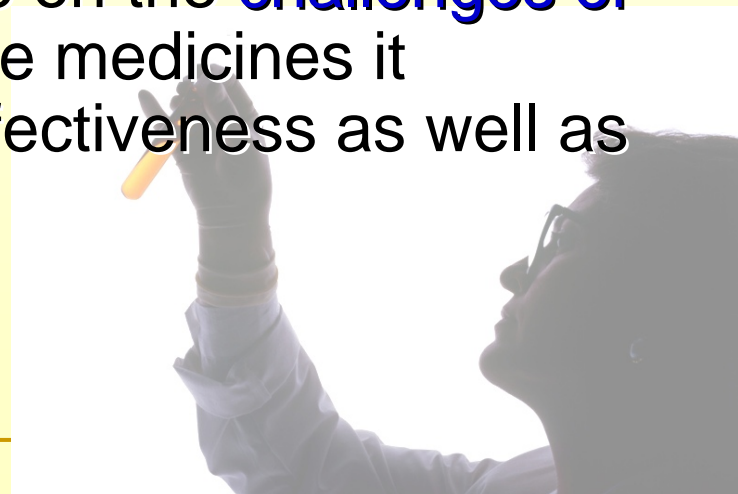


# The mission of pharmaceutical industry

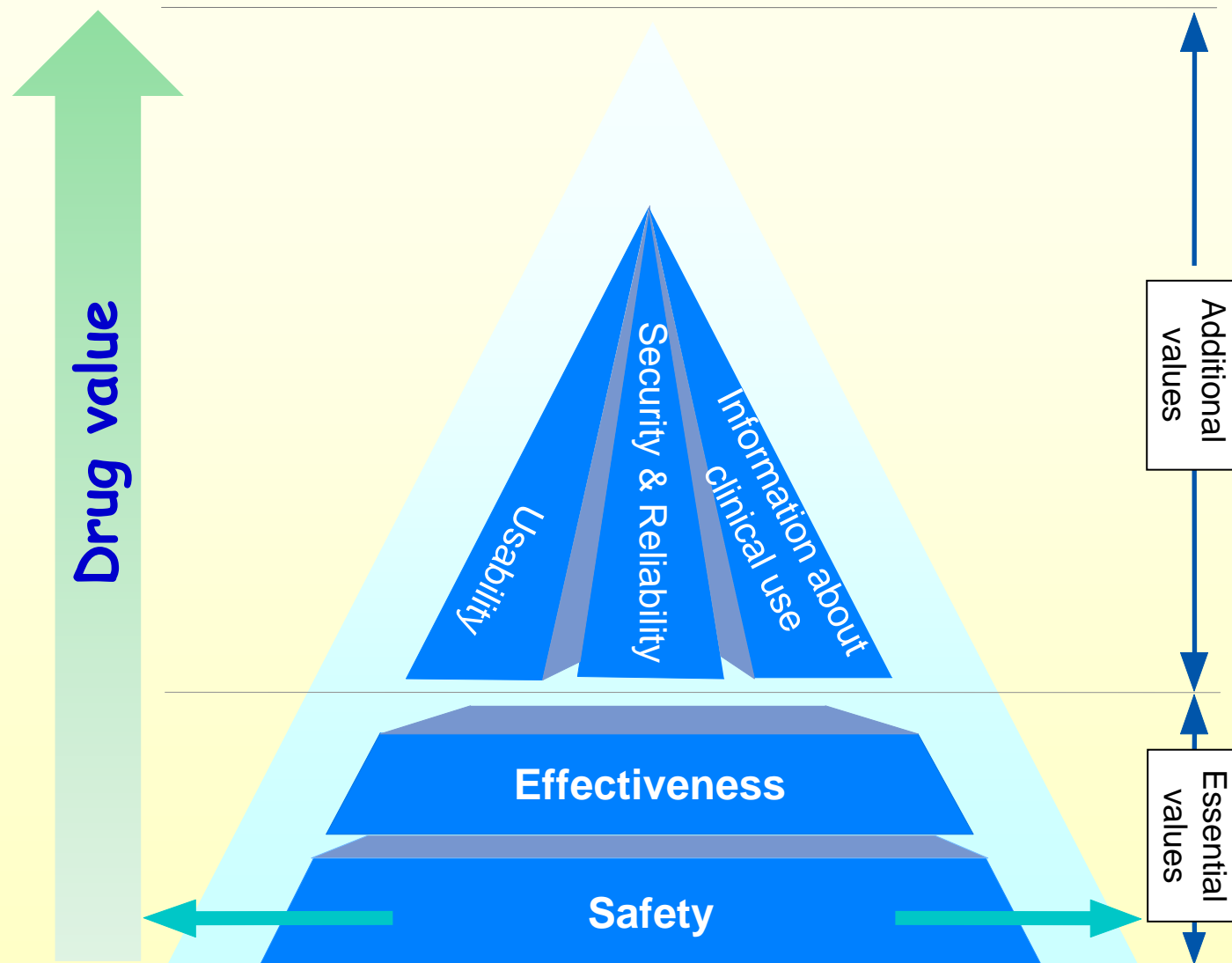


- The R&D-based pharmaceutical industry has the mission to continue to provide **effective and innovative medicines to patients** suffering from various diseases all over the world.

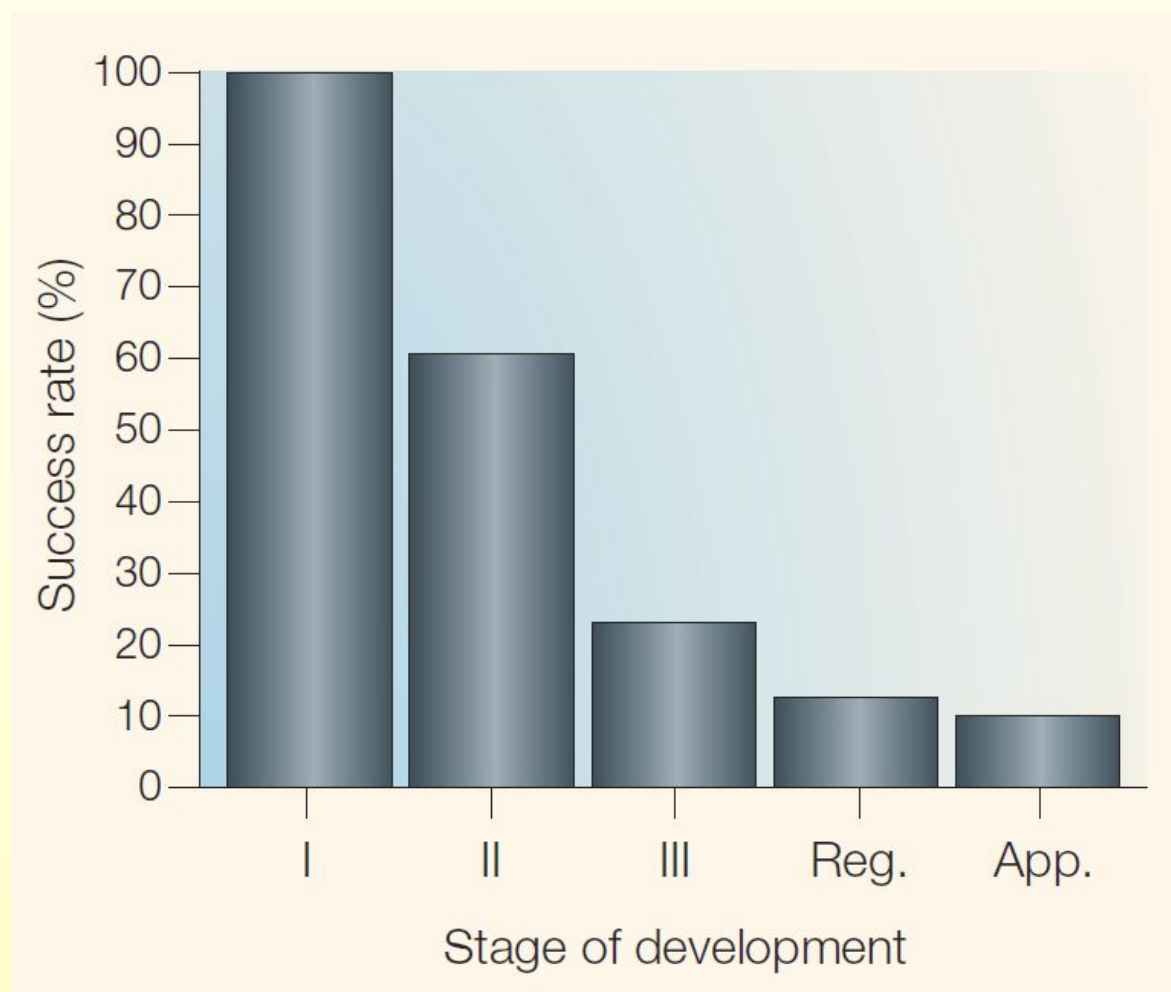
“In order to fulfill this mission, the pharmaceutical industry must continue to take on the **challenges of R&D**, and the evaluation of the medicines it creates should reflect their effectiveness as well as innovativeness.”



# The Value of Medicine

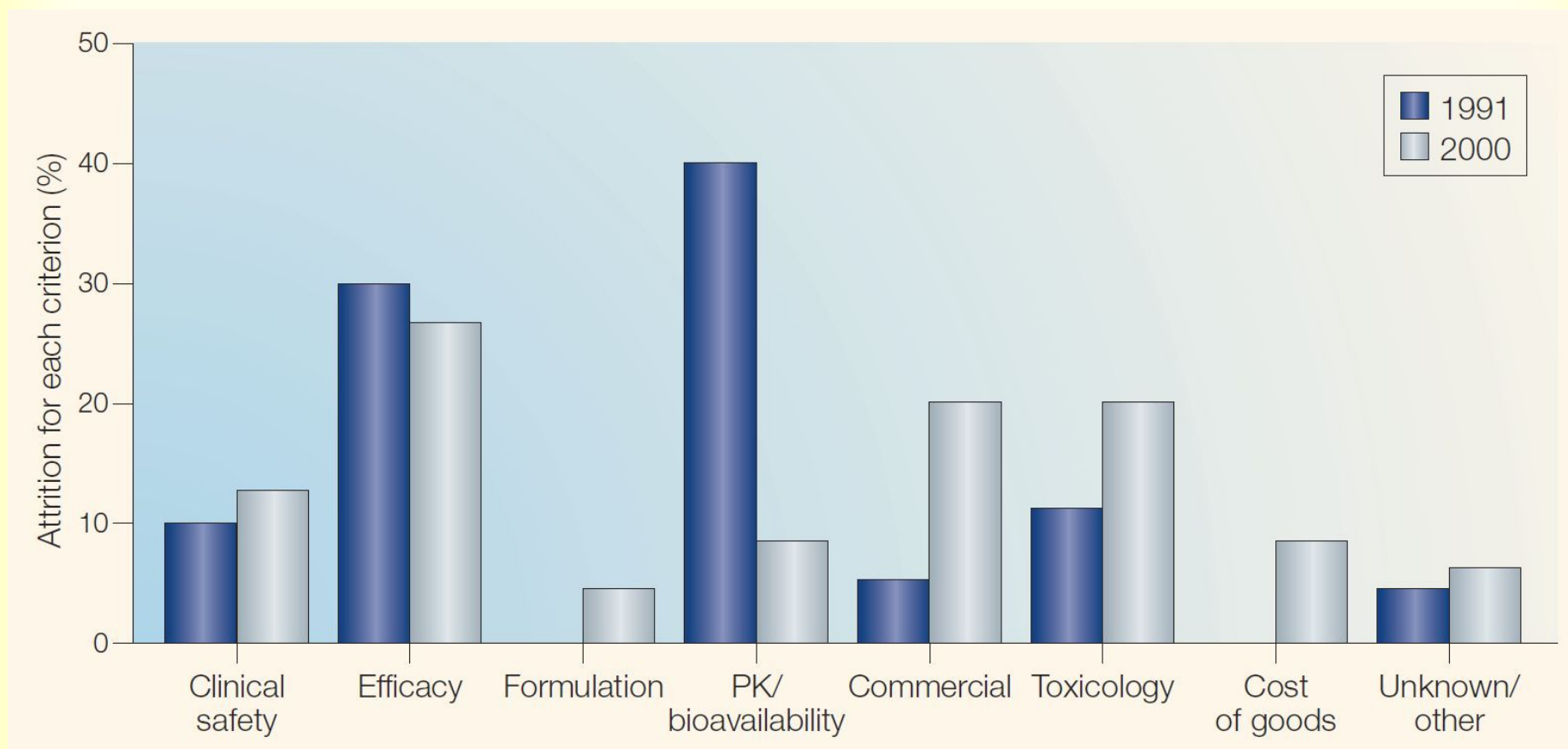


# Success rate by stage of development

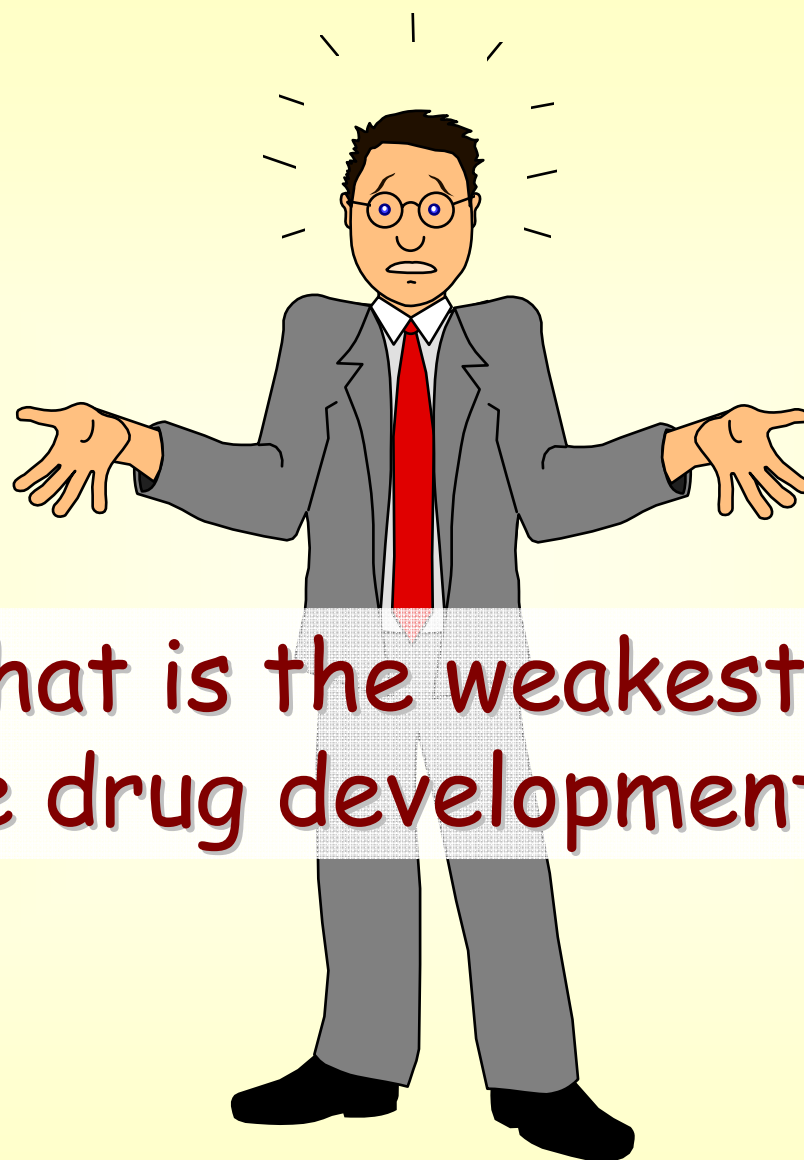


The percentage rate of success of compounds entering first in man that progress to subsequent development phase. App, approval; Reg, registration.

# Reasons for attrition (1991–2000)



PK, pharmacokinetics.



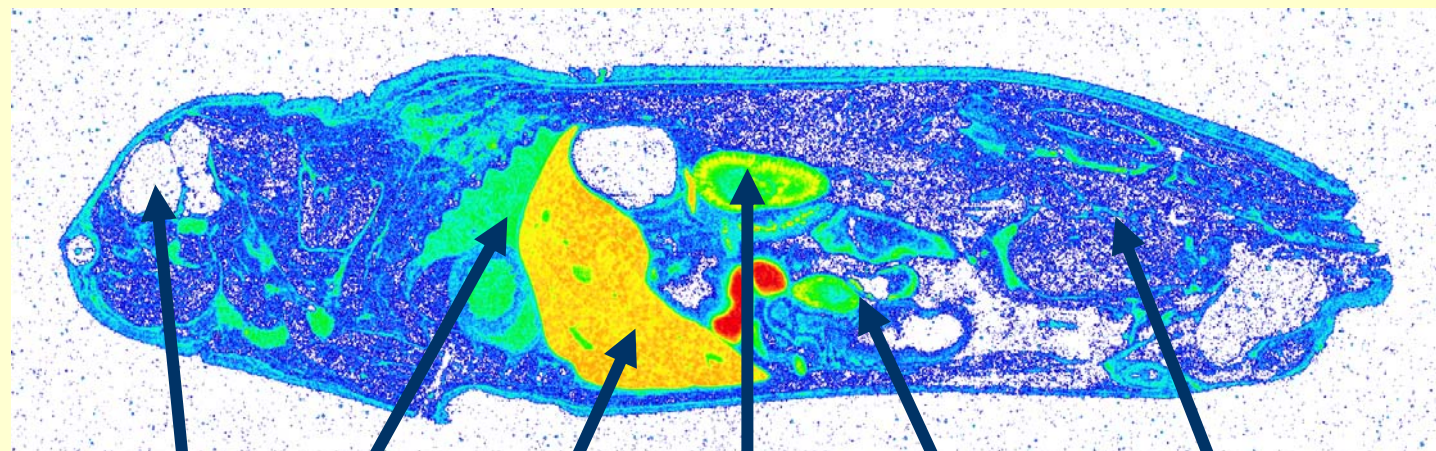
What is the weakest link  
in the drug development chain?



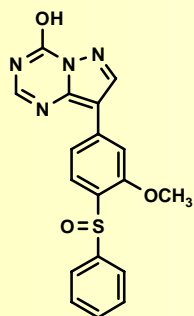
# ARG after iv administration

<sup>14</sup>C-labelled racemic BOF-4272 administered in rats at 5 mg/kg

15 min

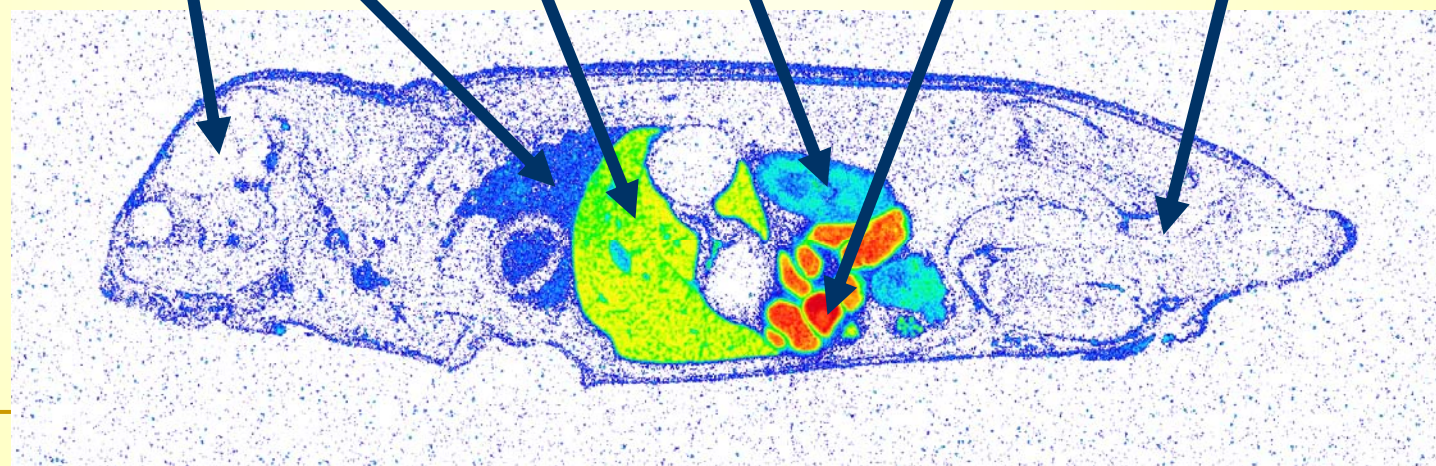


BOF-4272



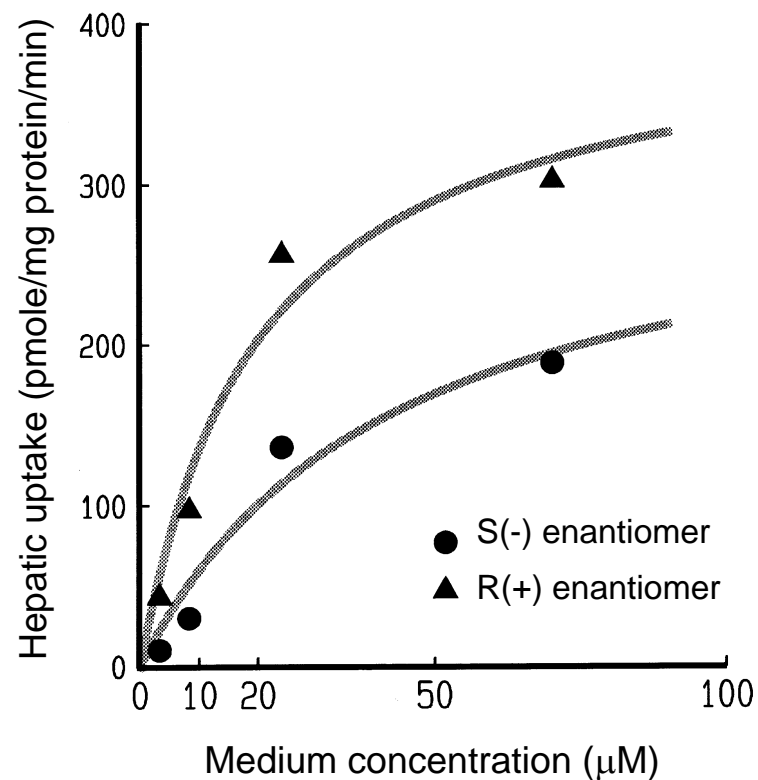
Brain    Lung    Liver    Kidney    Intestinal contents    Muscle

60 min





## Effect of initial substrate concentration on temperature-dependent uptake of BOF-4272 enantiomers by isolated rat hepatocytes

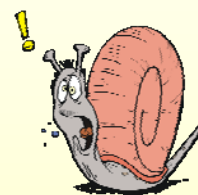


Kinetic parameters of BOF- 4272 in rat hepatocytes

	$K_m$ ( $\mu\text{M}$ )	$V_{max}$ (pmol/mg/min)
S(-) enantiomer	$59.3 \pm 23.7$	$350 \pm 192$
R(+) enantiomer	$25.7 \pm 13.0^*$	$384 \pm 180$

Data are mean  $\pm$  SD of four separate experiments.

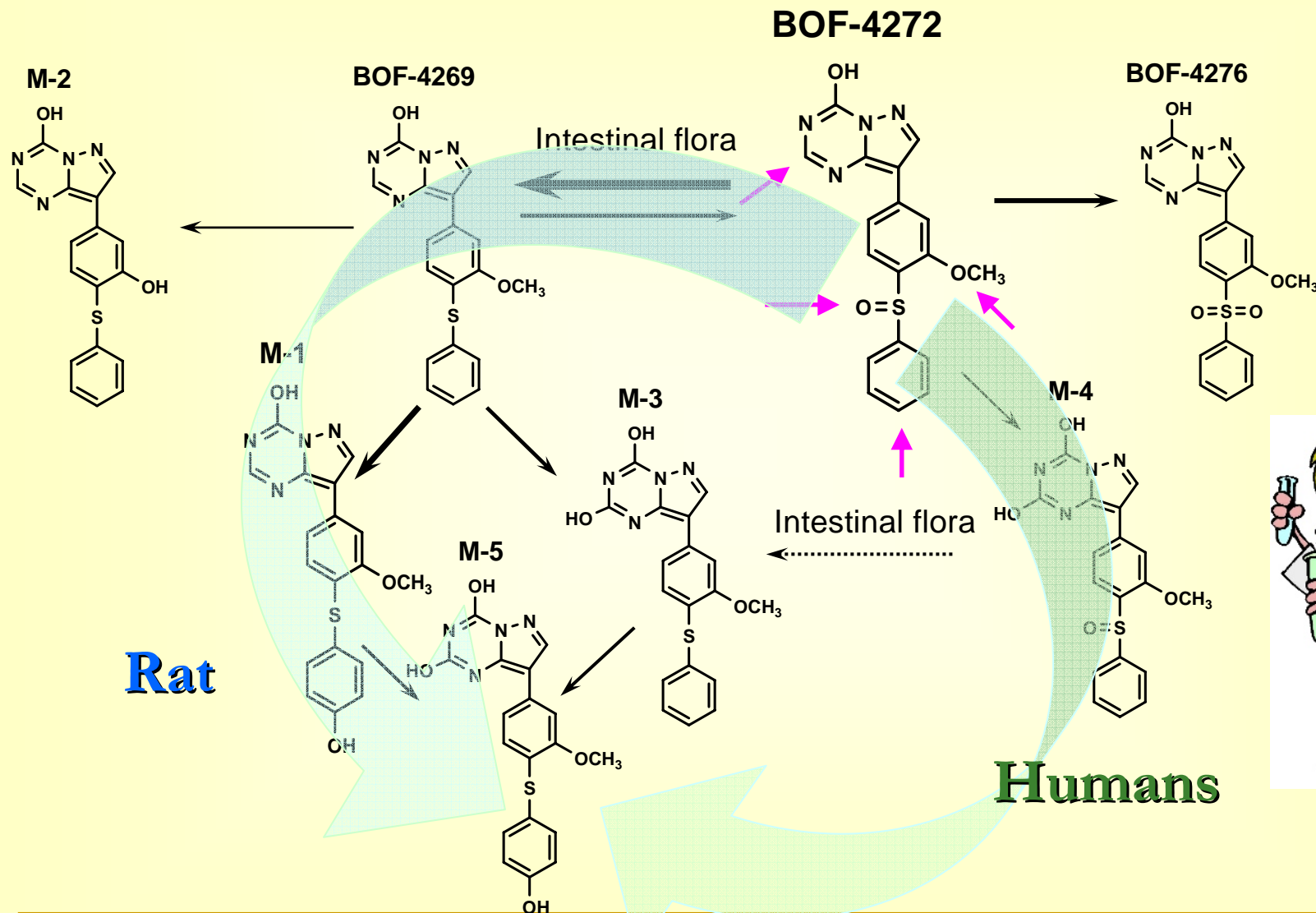
\* $p < 0.05$  vs. S(-) enantiomer.



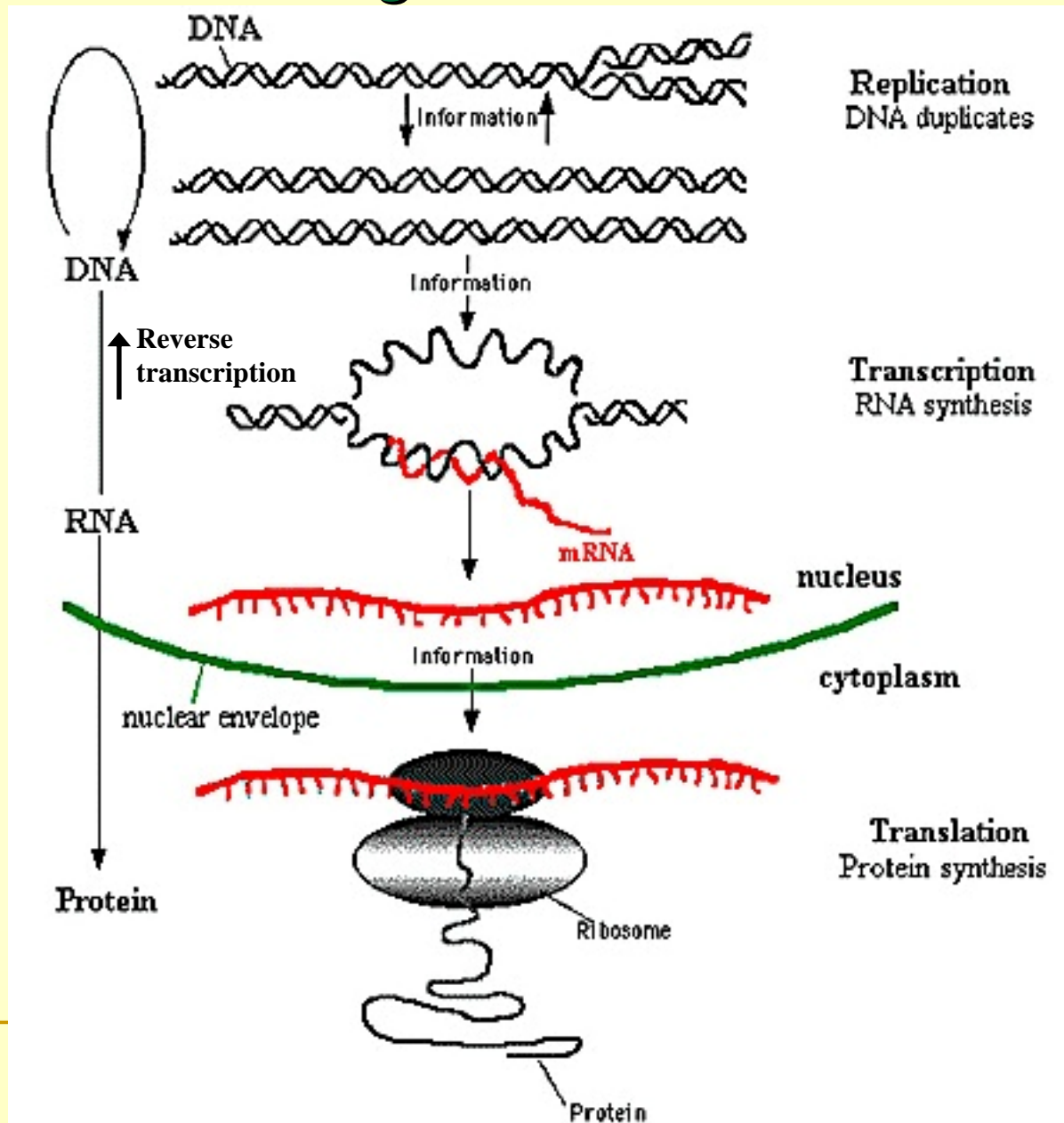
$$v = \frac{V_{max}}{1 + \frac{K_m}{[S]}}$$

Experiments were performed in duplicate. Data show typical saturation kinetics obtained in four separate experiments.

# Species comparison of metabolite profile *in vivo* & *in vitro* studies



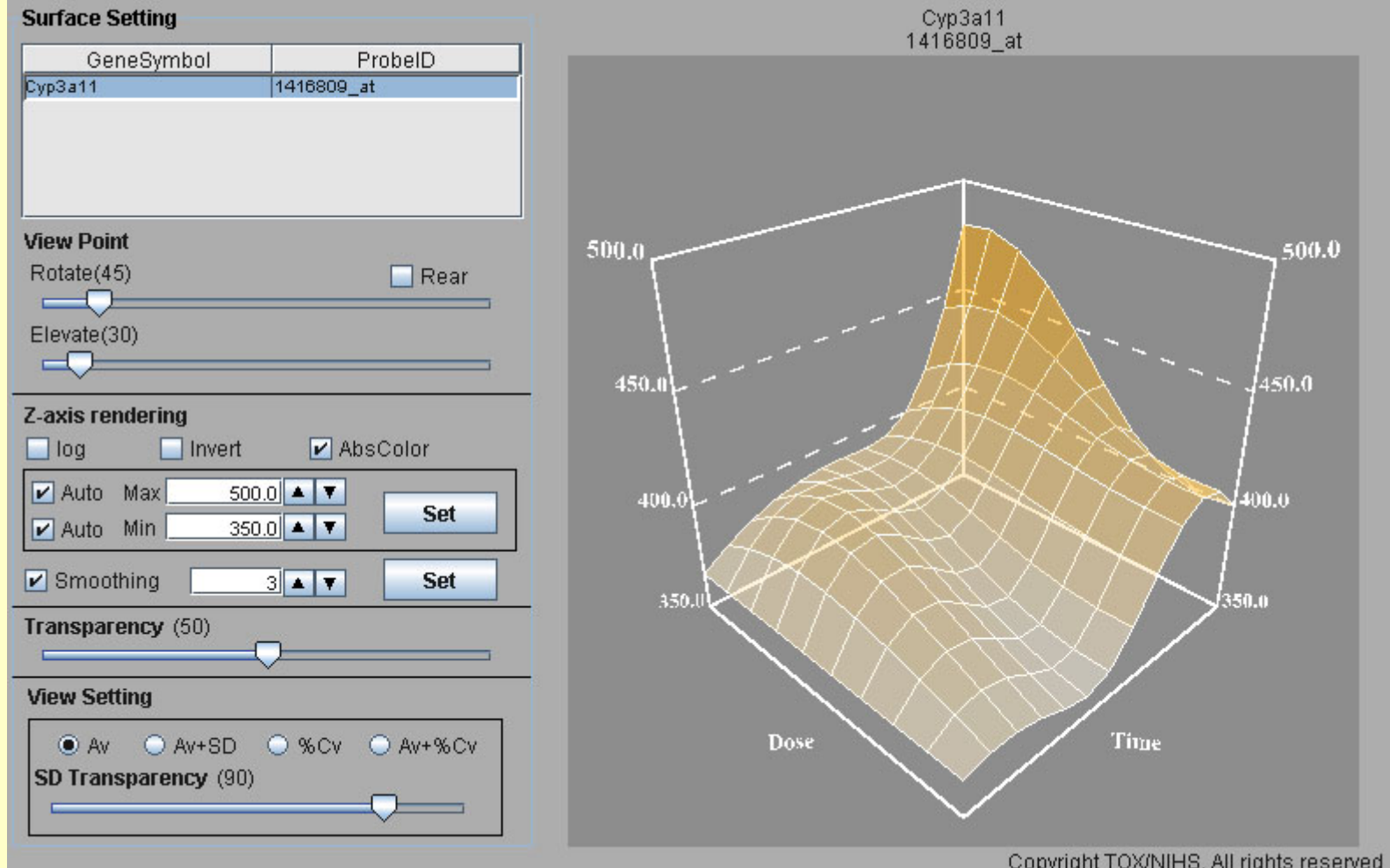
# The Central Dogma of molecular biology



# Precellome project - ToxicOmics database



Phenobarbital // 0.5%CMC // Single // Gavage // Mouse // C57BL/6 // Male // 12wo // 2,4,8,24hr // 0,15,50,150mg/kg // liver



# mRNA analysis

## One-Step RT-PCR assay

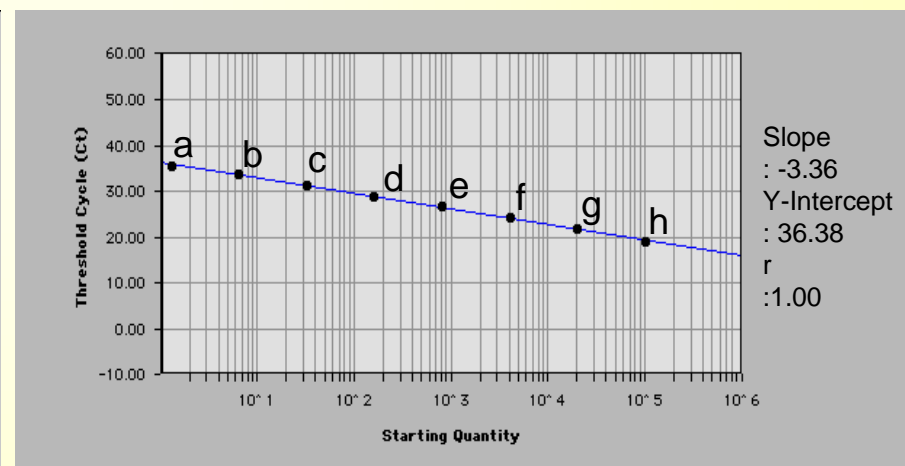
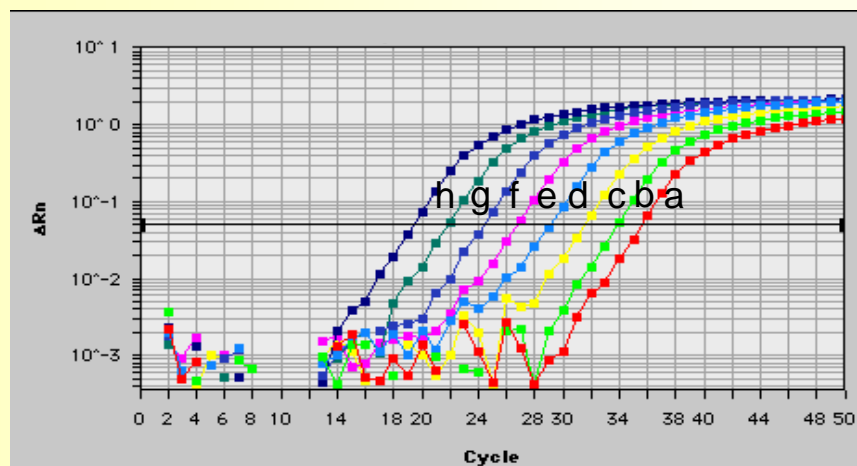
Design of primers and probes: Primer Express software (Applied Biosystems)

Primer and probe conditions:

Forward primer: 300 nM, Reverse primer: 900 nM, TaqMan probe: 200 nM

Real-time RT-PCR: ABI PRISM 7700 Sequence Detector system (Applied Biosystems)

## Calibration curve for CYP3A4



***In vitro* evaluation of drug induction of human drug-metabolizing enzyme mRNA**

## Specificity of RT-PCR at CYP1As and CYP3As

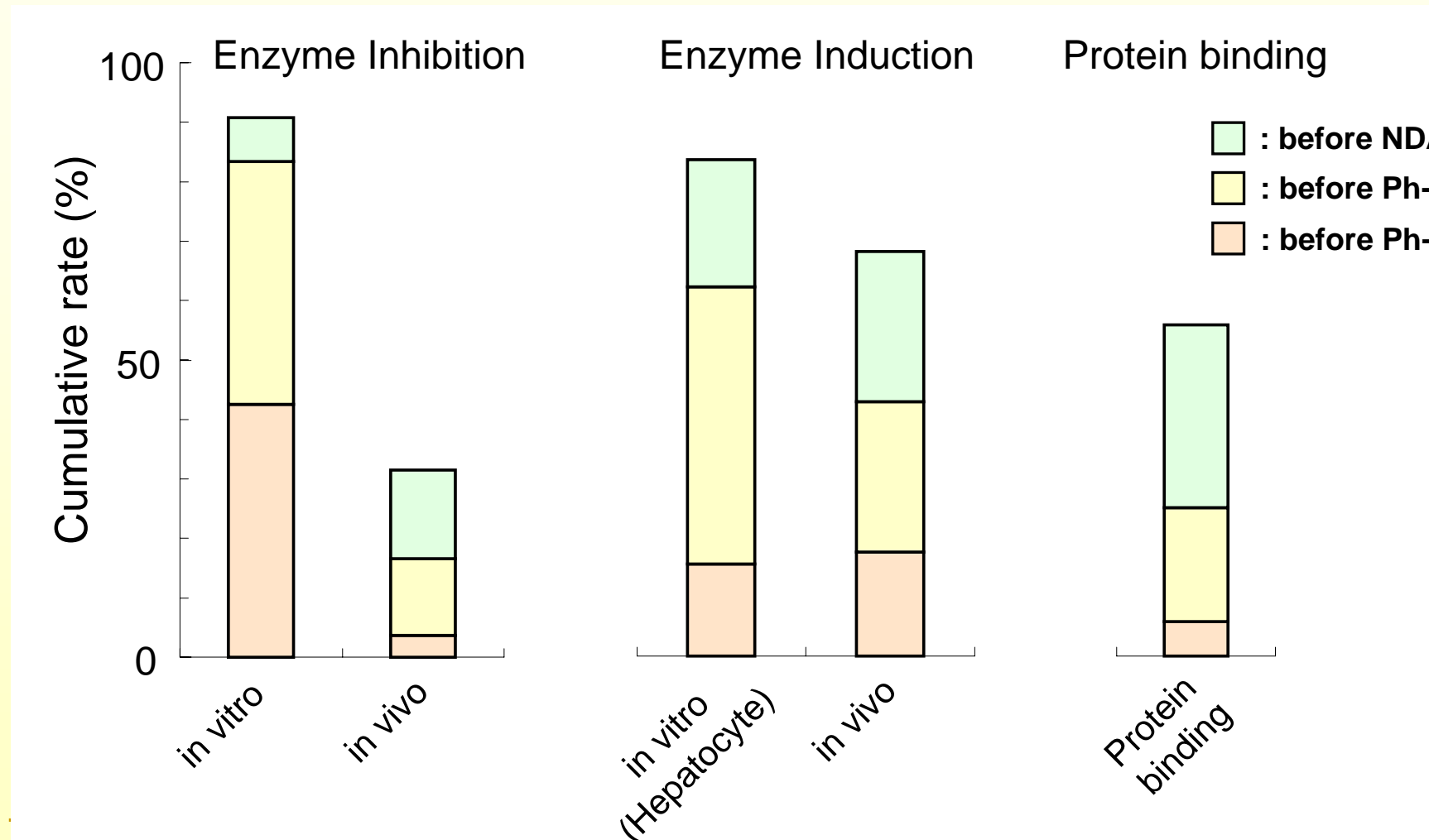
Total RNA was extracted from *Escherichia coli* transfected with human CYP1As or CYP3As (Kamataki T, unpublished observation)

Total RNA	Specific primer sets and probes	
	CYP1A1	CYP1A2
CYP1A1 mRNA gene expression	1	$< 3.0 \times 10^{-8}$
CYP1A2 mRNA gene expression	$< 3.0 \times 10^{-8}$	1

Total RNA	Specific primer sets and probes	
	CYP3A4	CYP3A5
CYP3A4 mRNA gene expression	1	$< 4.8 \times 10^{-7}$
CYP3A5 mRNA gene expression	$< 6.0 \times 10^{-8}$	1



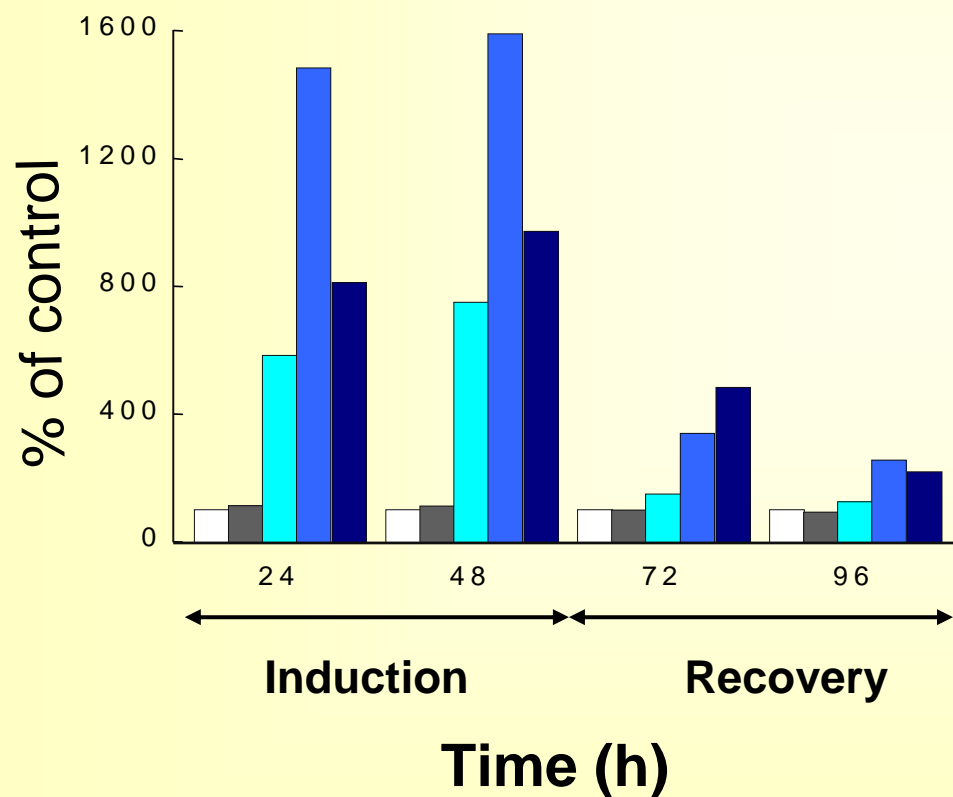
# Investigation on the timing of ADME studies — Drug-drug interaction —



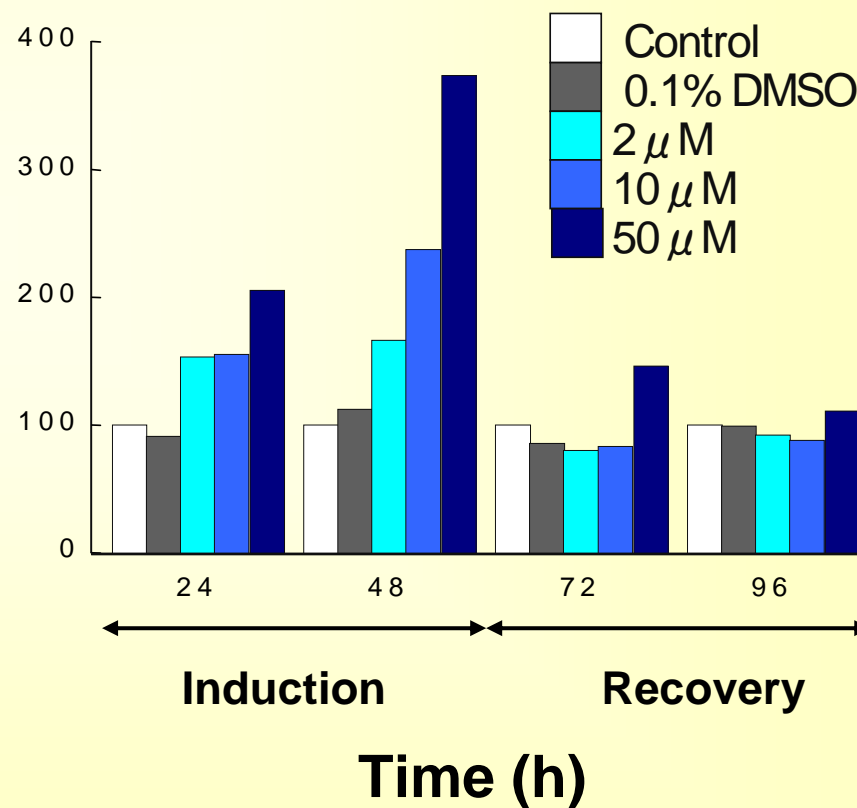
# Time-course of mRNA expression after rifampicin exposure

Human hepatocytes were treated with probe drug.

## CYP3A4



## CYP3A5



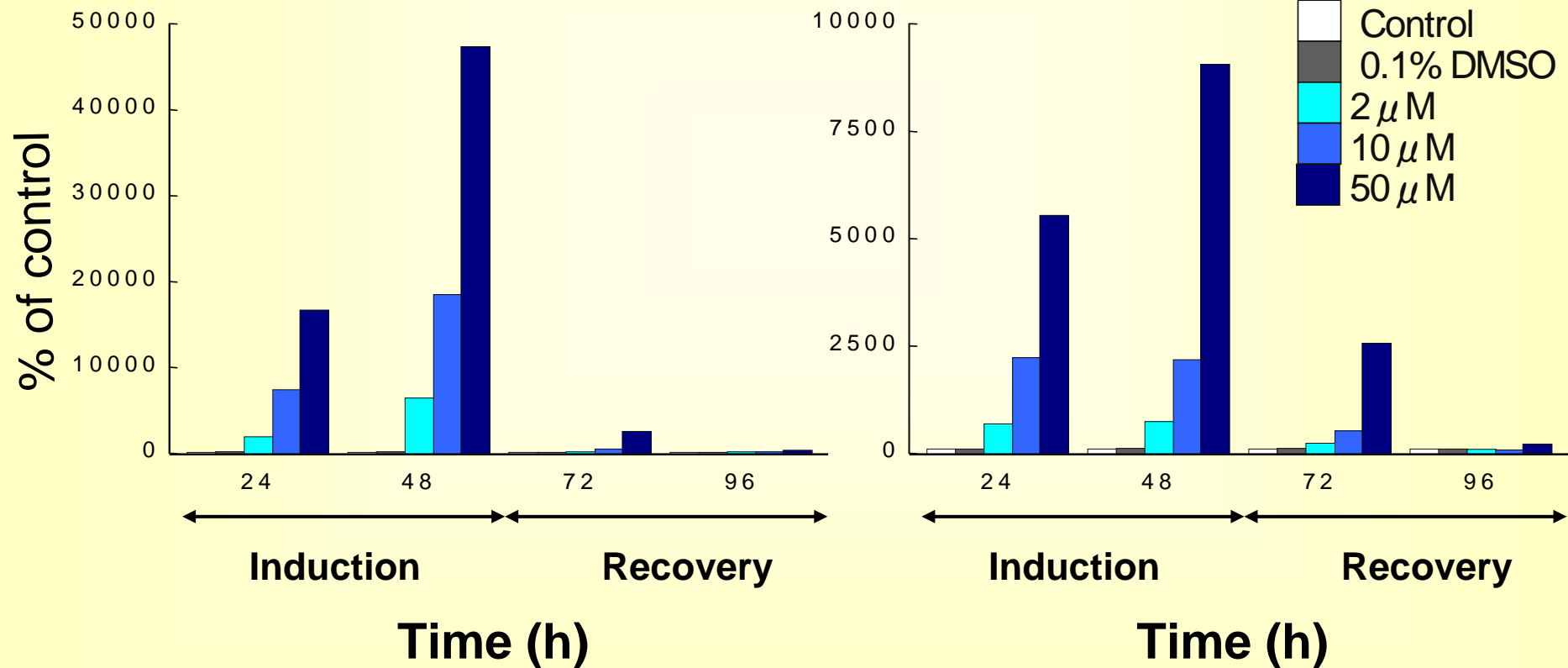
# Time-course of mRNA expression after omeprazole exposure

Human hepatocytes were treated with probe drug.

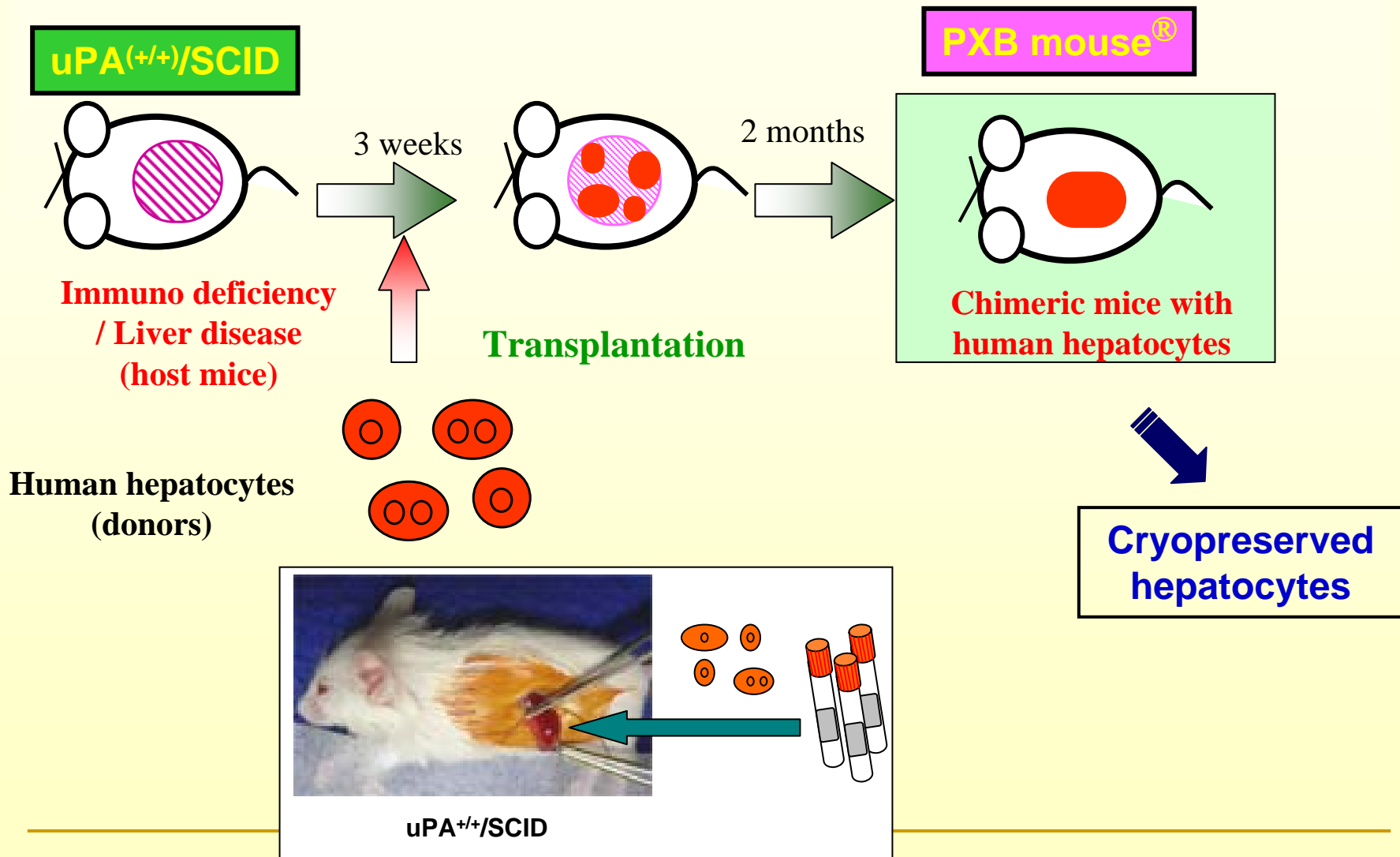


## CYP1A1

## CYP1A2

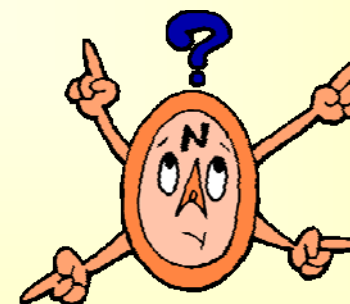
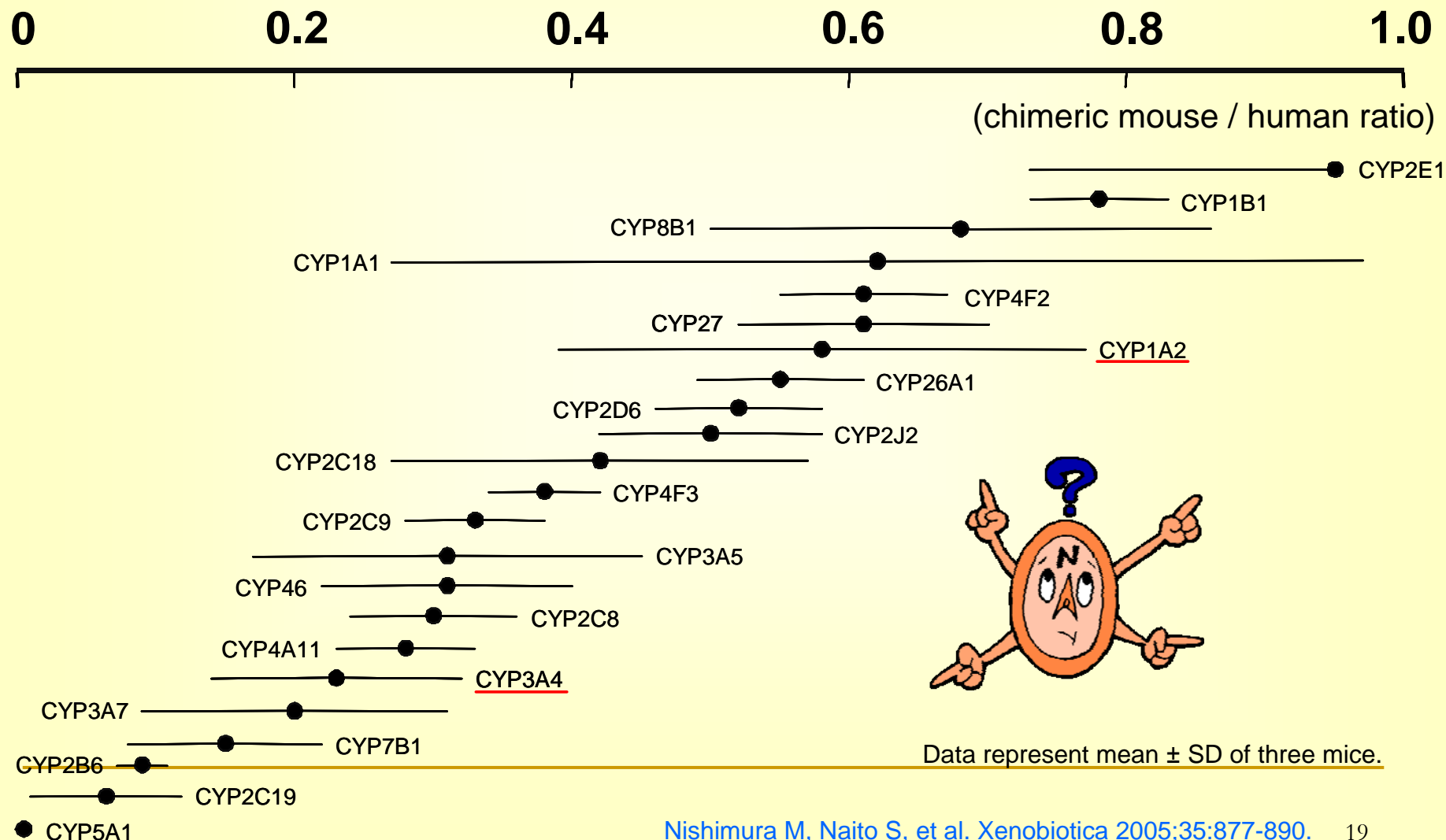


# Generation of chimeric mice with humanized Liver



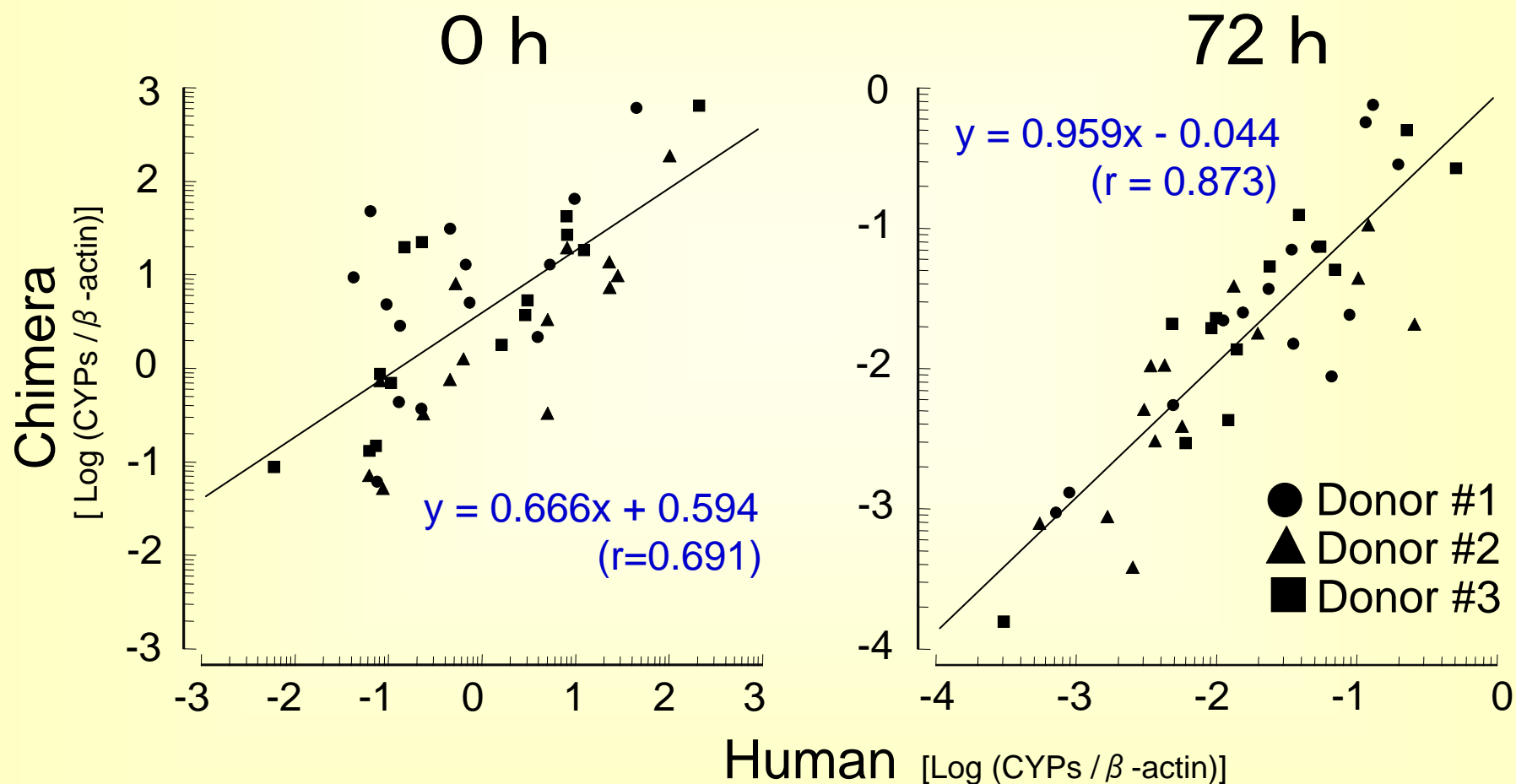
# mRNA expression profile of human cytochrome P450 enzymes

Data are expressed as the ratio of target mRNA in the chimera mouse liver to target mRNA in the human liver of the donor.



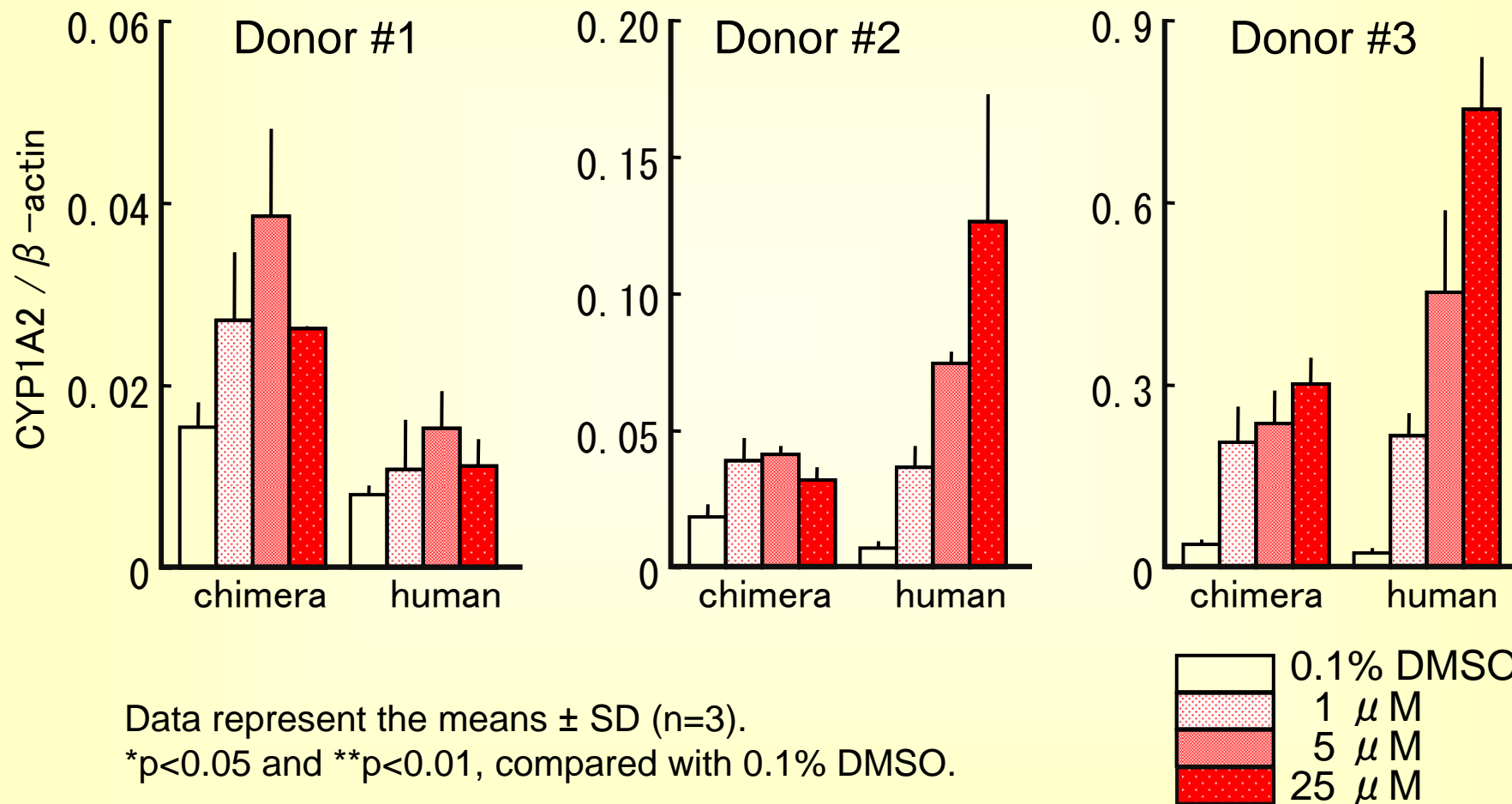
# Correlation between mRNA levels of CYPs expressed in chimeric and human hepatocytes

Items: CYP1A1, 1B1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5, 3A7

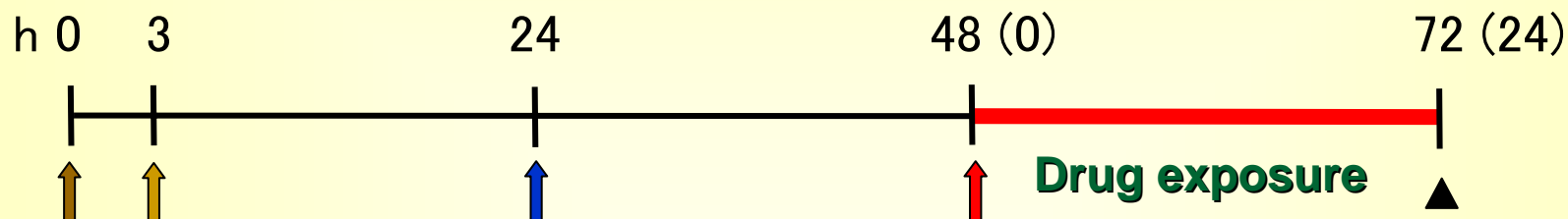




# Changes in CYP1A2 mRNA expression following exposure to $\beta$ -naphthoflavone for 24 h



# Evaluation of gene induction of drug-metabolizing enzymes in human and **cynomolgus monkey** hepatocytes after exposure to drugs for 24 h

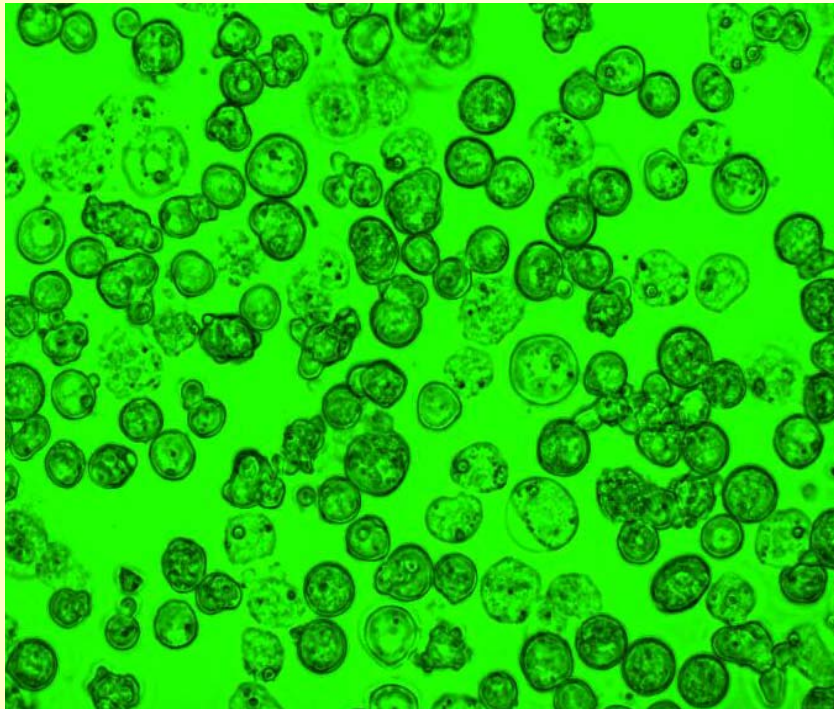


- ↑ : Inoculation ( $1 \times 10^5$  viable cells/0.4 mL/well [2 cm<sup>2</sup>])
- ↑ : Medium change
- ↑ : Medium change (without Hydrocortisone, hEGF, or antibiotic)
- ↑ : **Drug exposure** (without Hydrocortisone, hEGF, or antibiotic and with 0.1% DMSO)
- ▲ : Total RNA extraction (Rneasy Mini Kit [Qiagen])

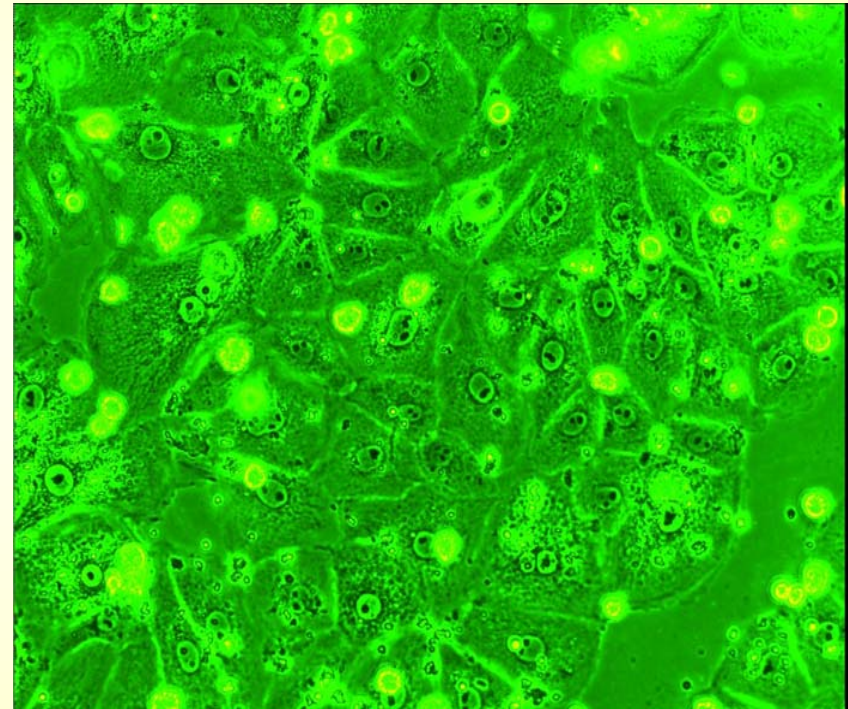
Medium: Hepatocyte Culture Medium Bullet Kit  
(Cambrex Bio Science Walkersville, Inc.)

# Cynomolgus monkey hepatocytes

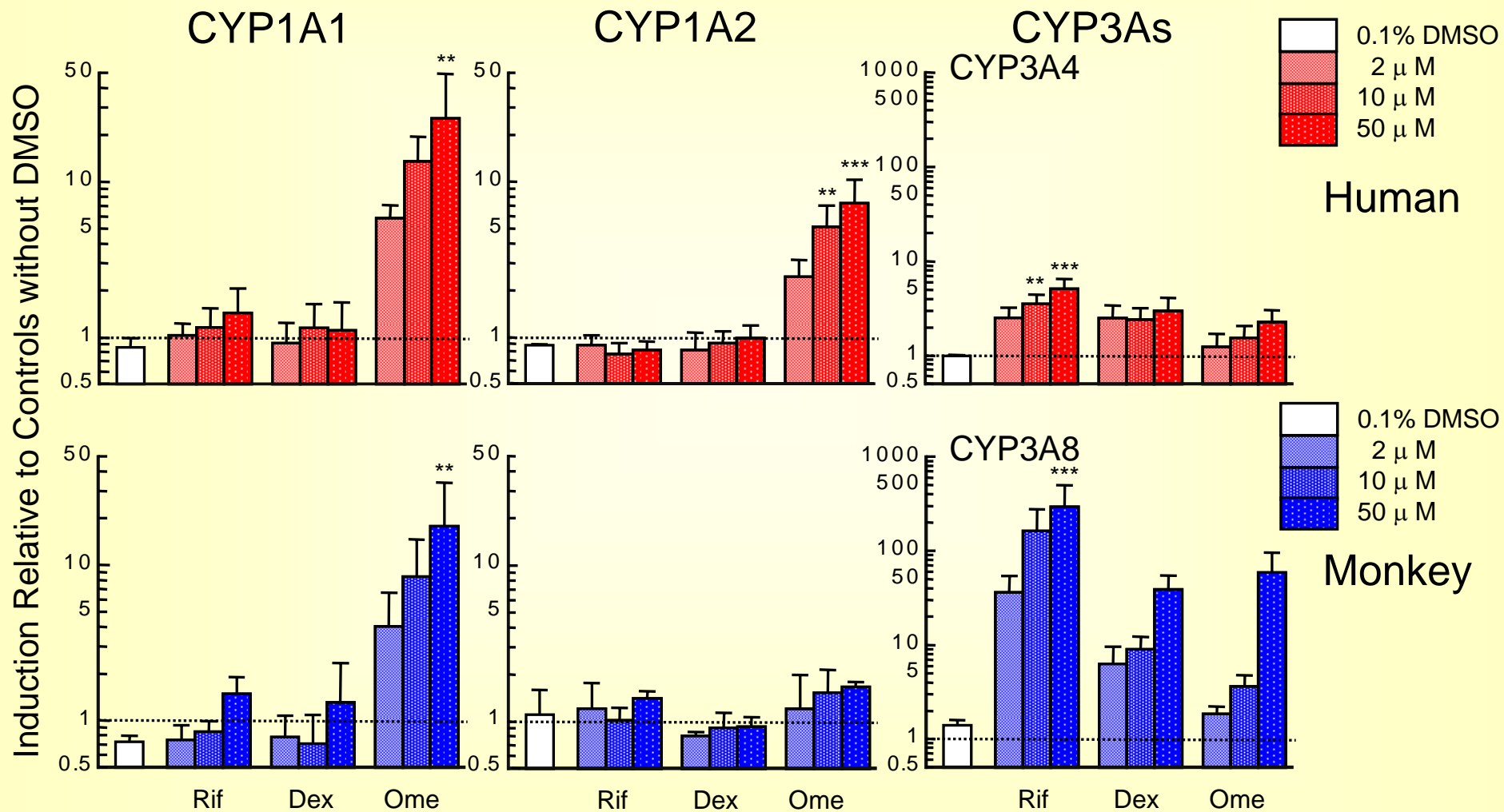
**3 h after inoculation**



**72 h after inoculation**



# Effect of exposure to inducers (24 h) on CYP1As and CYP3As mRNA expression in hepatocytes



Values are mean ± SD (n=3).

Significantly different from 0.1% DMSO (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

# Infant rat TPN\* model with hepatobiliary dysfunction

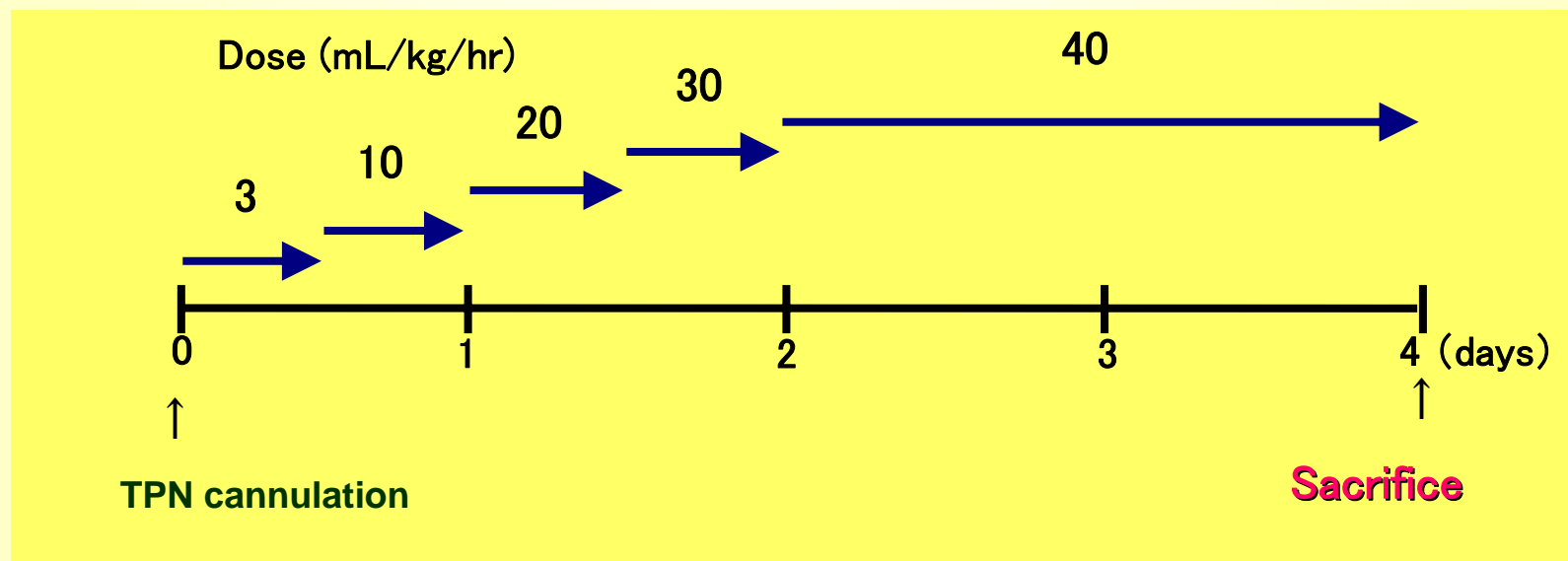
Animal: Infant male SD rats (3 wks, 60–70 g)

Group: Fat-free TPN\*, Fat-containing TPN\*, and Oral diet

Analysis: Biochemical analysis, Morphological study, PGx study

Dose: Continuous infusion via jugular vein catheter

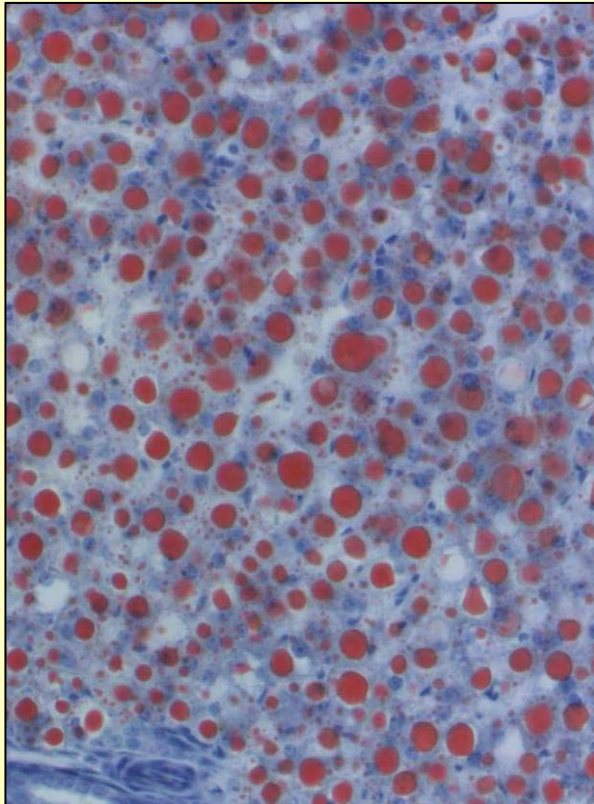
\* Total Parenteral Nutrition.



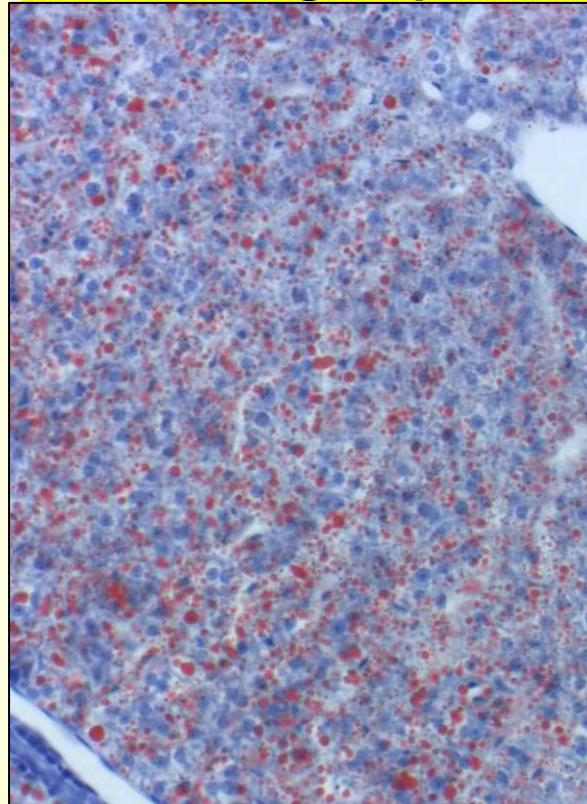


# Hepatic changes: oil red O staining

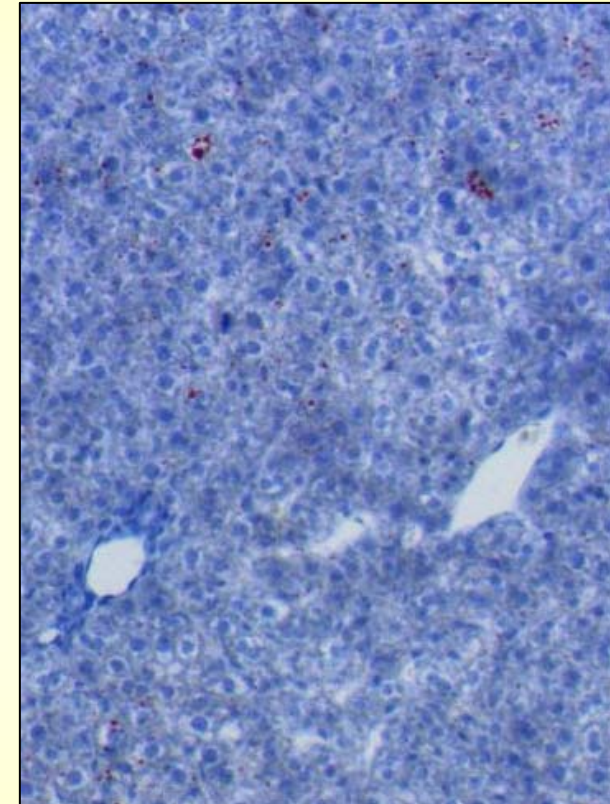
Fat-free TPN group



20% Fat-containing TPN group



Oral diet group



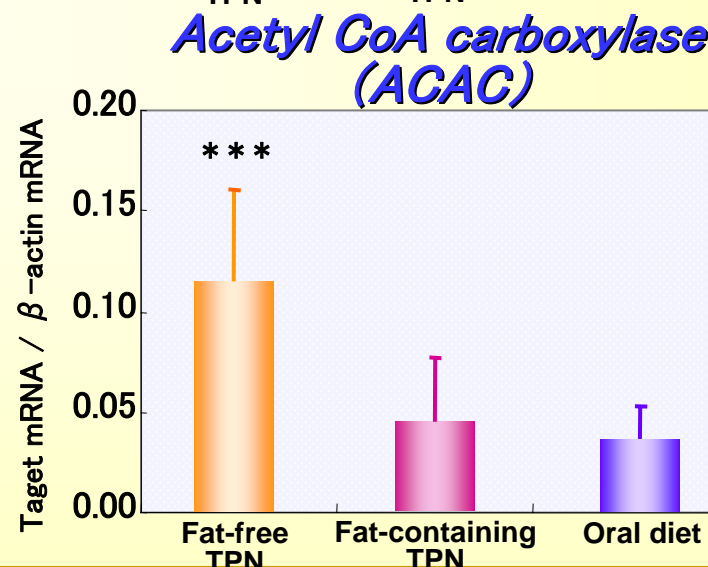
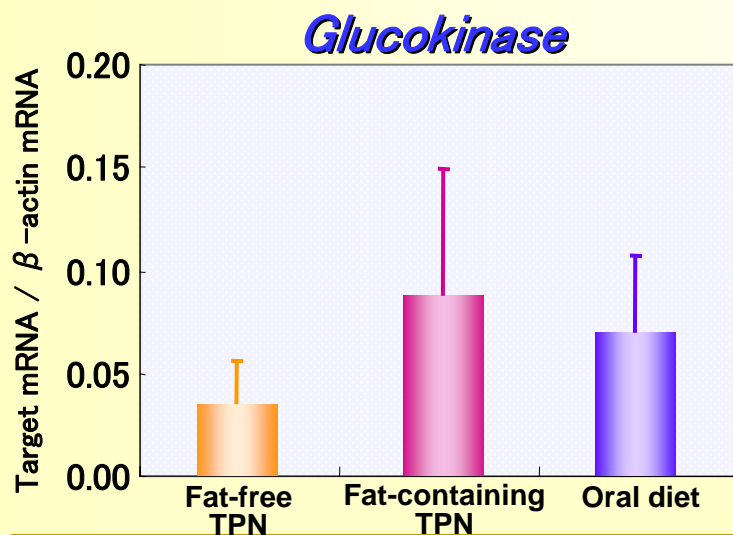
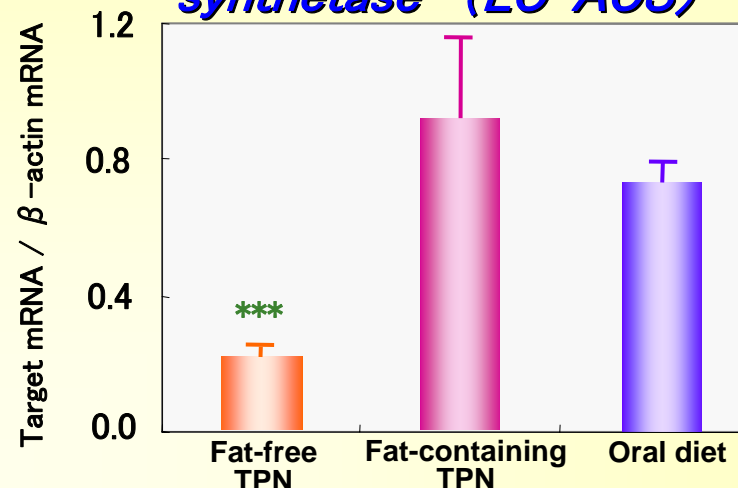
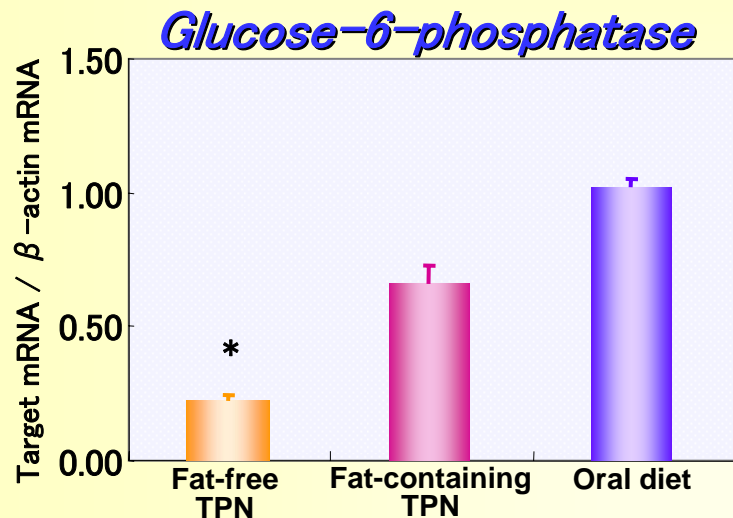
In the fat-free TPN group, the hepatocytes are found to have **large lipid droplets** in all hepatocytes, showing hypertrophy with eosinophilic granular cytoplasm.



# Changes in mRNA expression in infant rats

## Enzymes of glucose and lipid metabolism

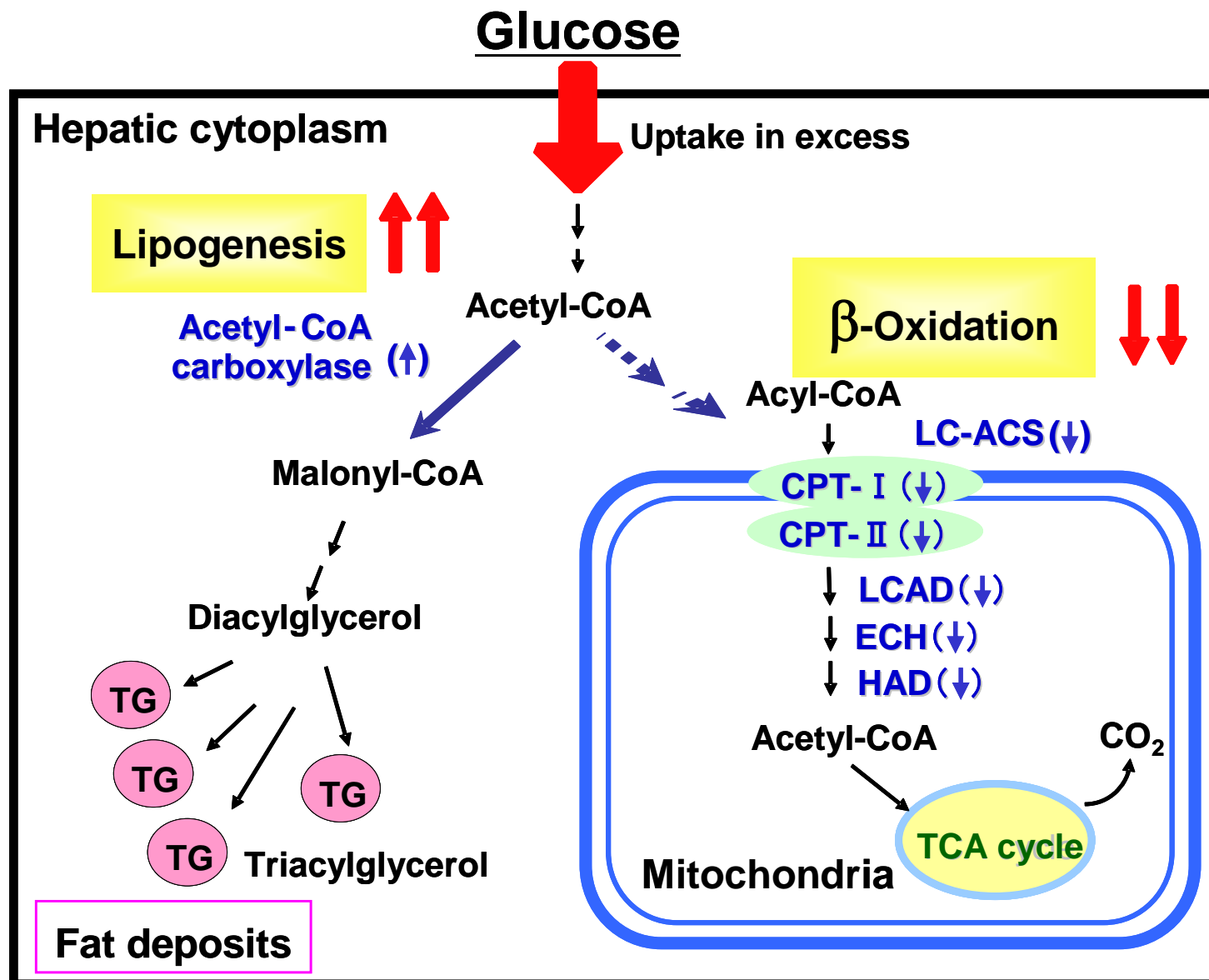
### Long-chain acyl-Coenzyme A synthetase (LC-ACS)



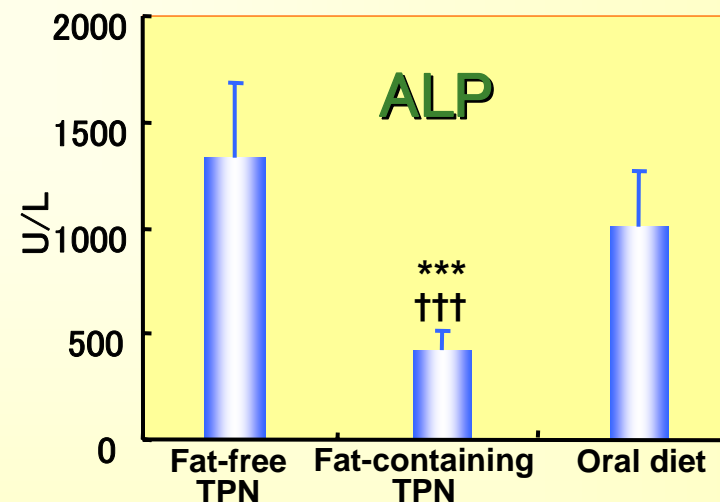
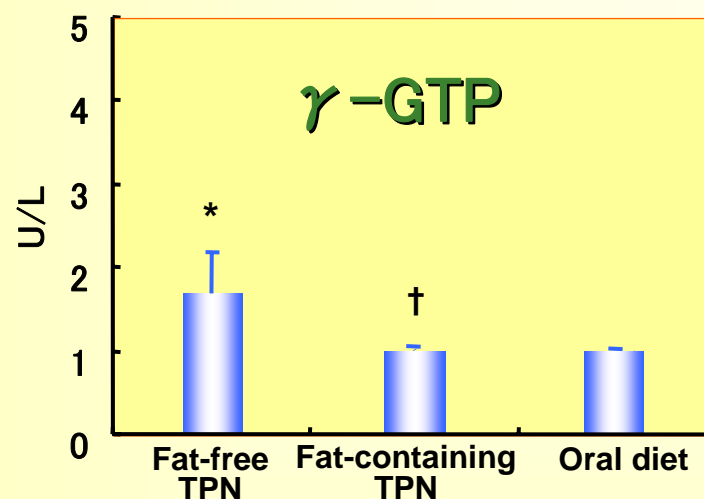
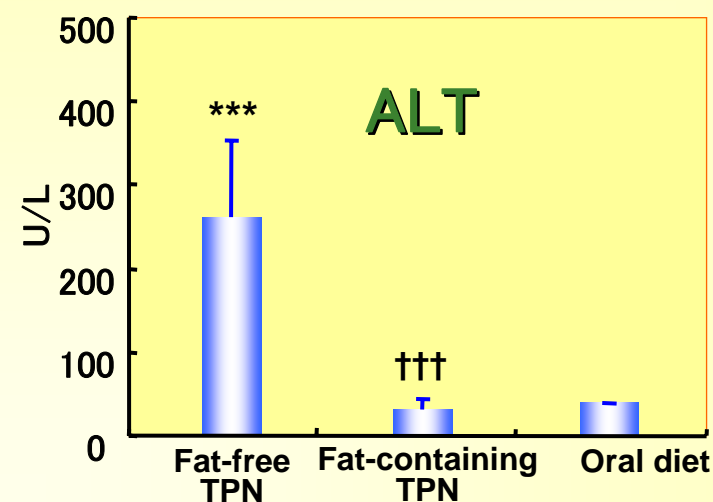
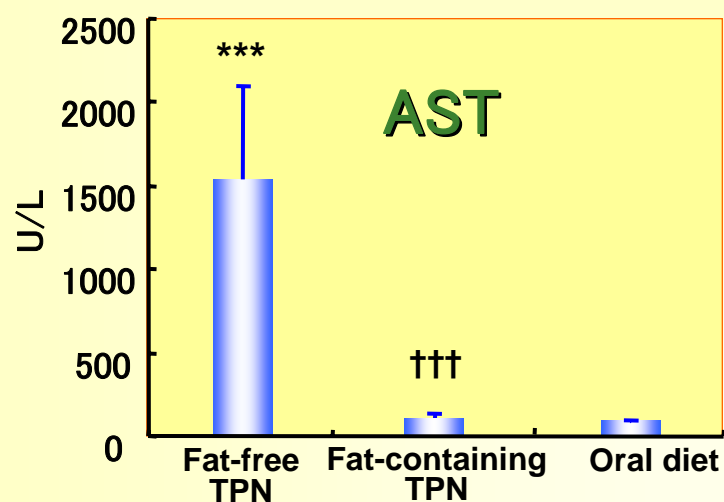
\* $p < 0.05$  and \*\*\* $p < 0.001$ , compared with Oral diet.

Mean  $\pm$  SD, n=7 - 8

# Mechanism of fat-free TPN-induced hepatic steatosis



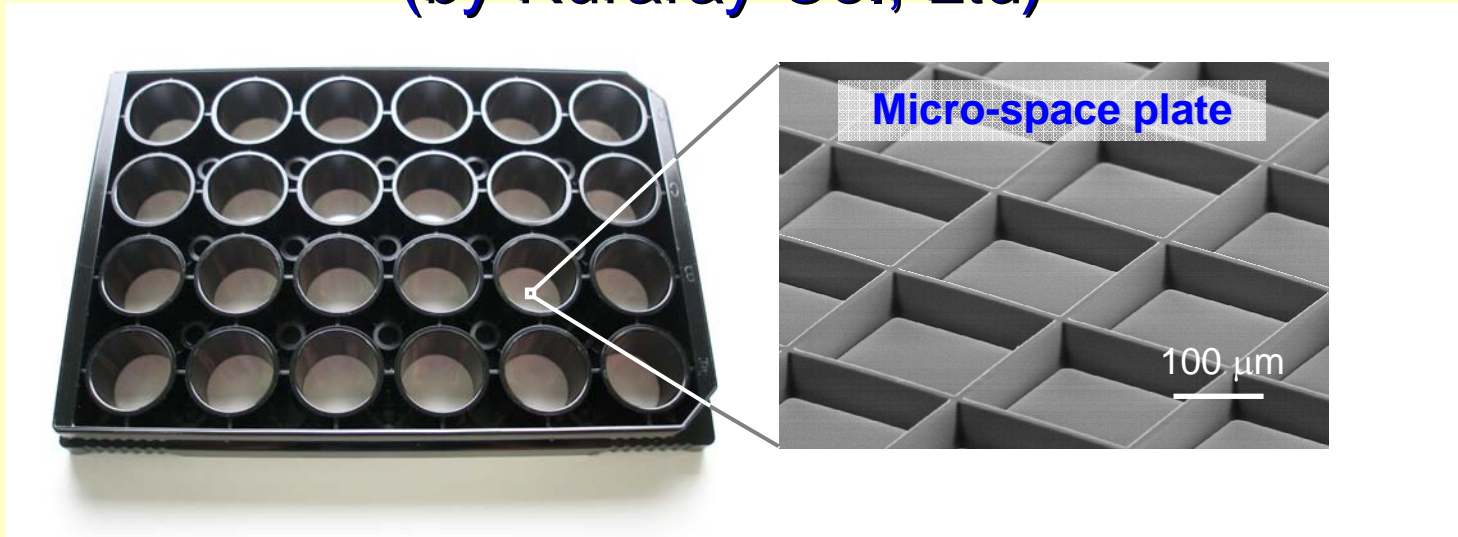
## Serum biochemical analysis in infant rats



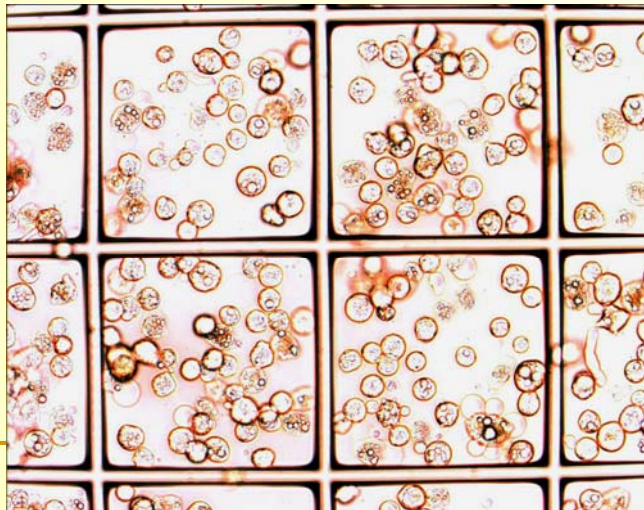
\* $p < 0.05$  and \*\*\* $p < 0.001$ , compared with Oral diet. † $p < 0.05$  and ††† $p < 0.001$ , compared with Fat-free TPN.

AST; aspartate aminotransferase (GOT), ALT; alanine aminotransferase (GPT), Mean  $\pm$  SD, n=7 - 8  
 ALP; alkaline phosphatase,  $\gamma$ -GTP ;  $\gamma$ -Glutamyltranspeptidase

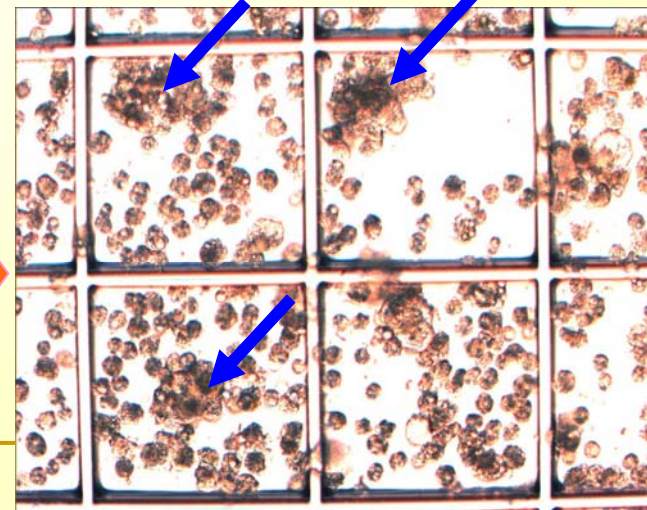
# Three-dimensional culture system for human hepatocytes (by Kuraray Co., Ltd)



3 h



120 h

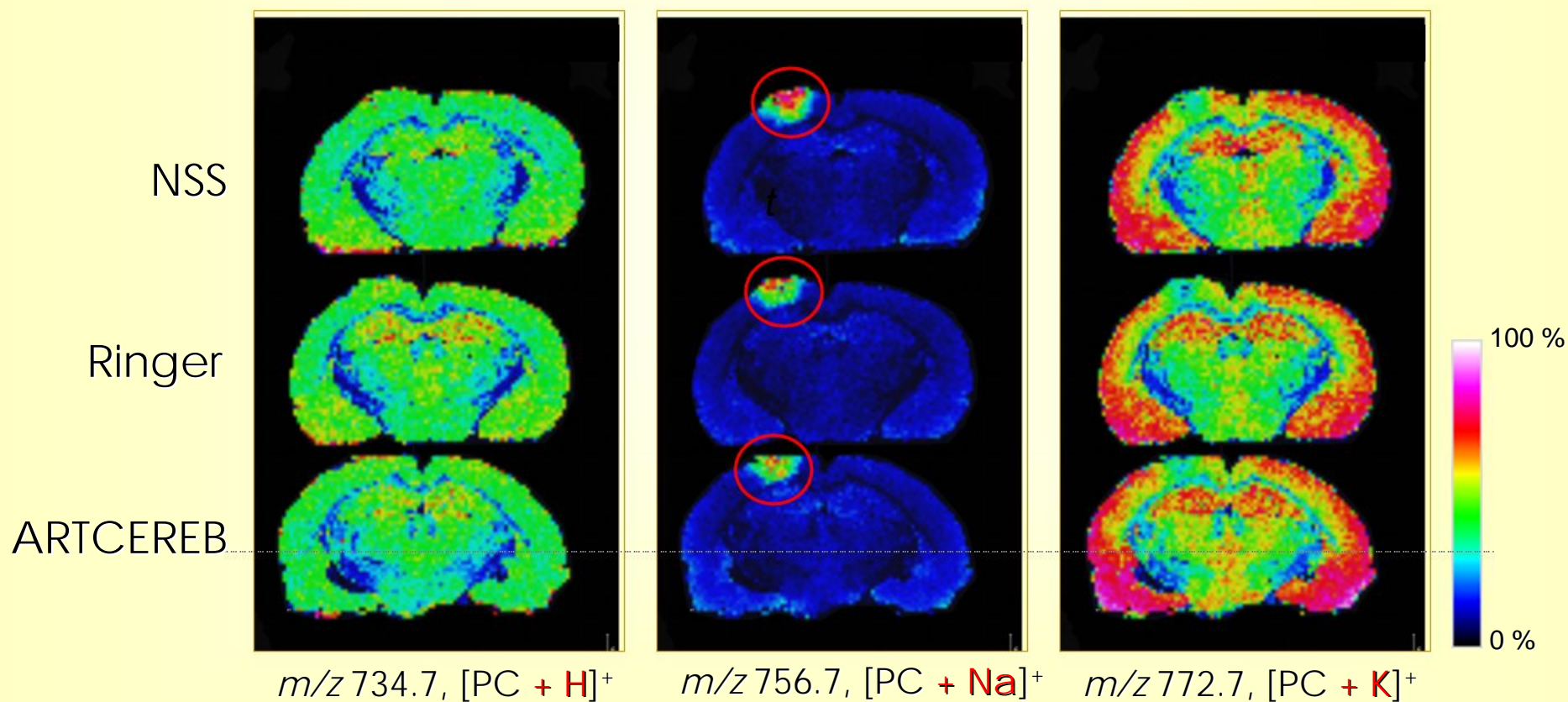




# Visualization of the effects of various irrigation fluids during experimental neurosurgery in rats by imaging mass spectrometry (by Setou M, Hamamatsu University School of Medicine)

## Phosphatidylcholine (16:0 / 16:0)

○: Injured area



# Acknowledgments

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- All other research members

