

Extraction solvent : acetone-chloroform Closed patch (48h)

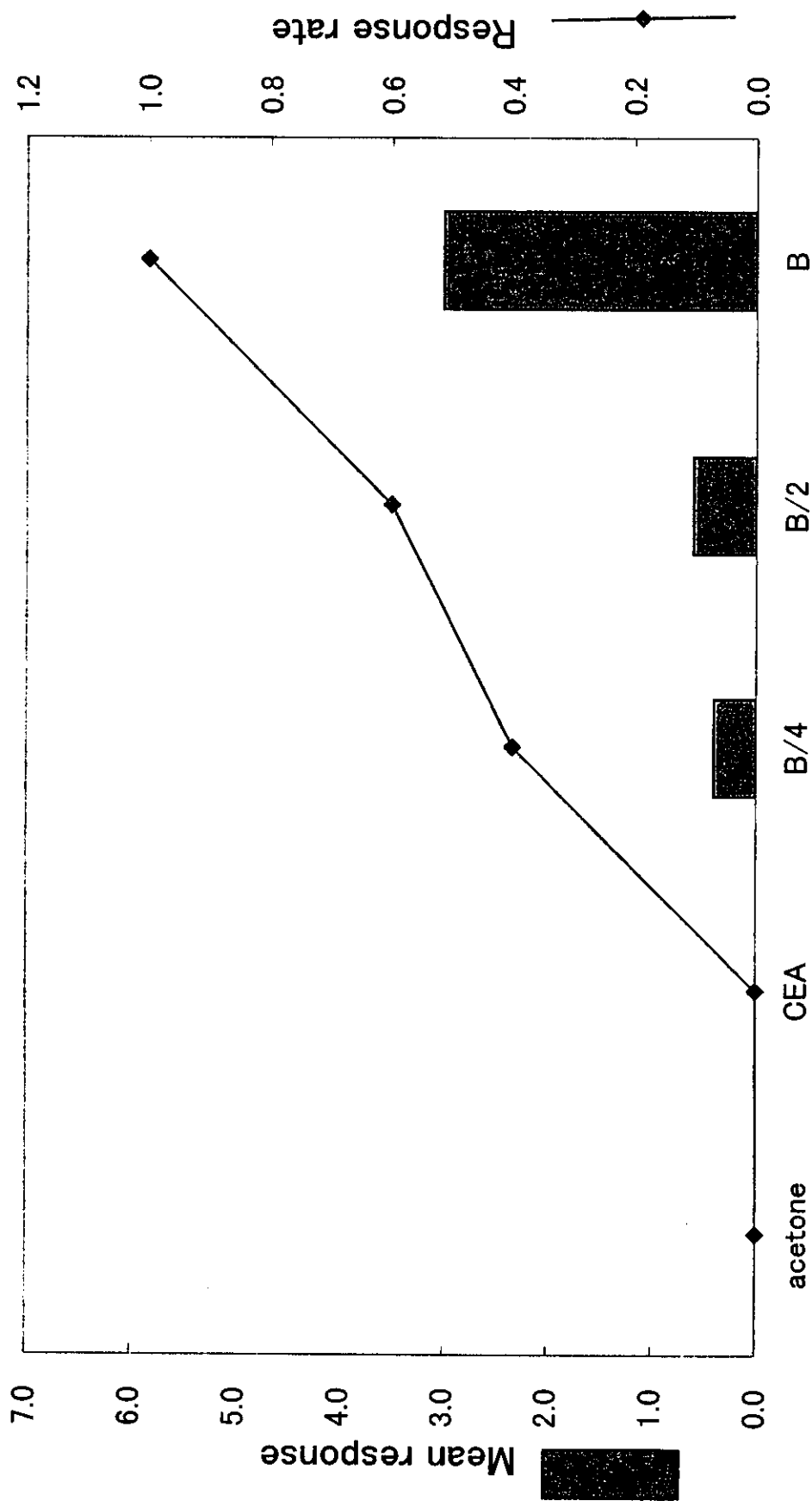


Fig 4. Comparison of the GPMT data among various induction concentrations prepared from material-B using organic solvent-extraction method under the closed patch condition.

Extraction solvent : acetone-chloroform Open patch (48h)

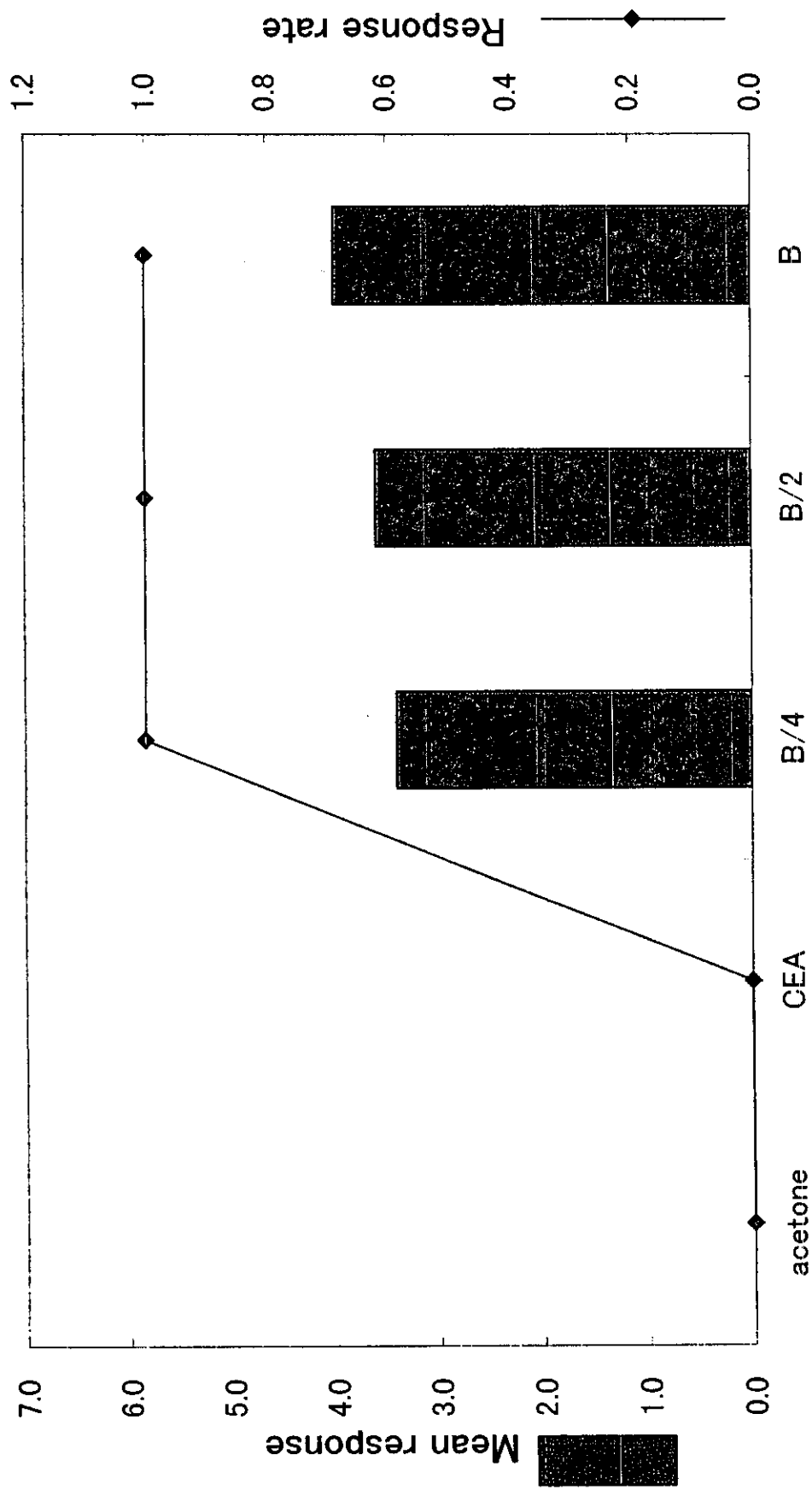
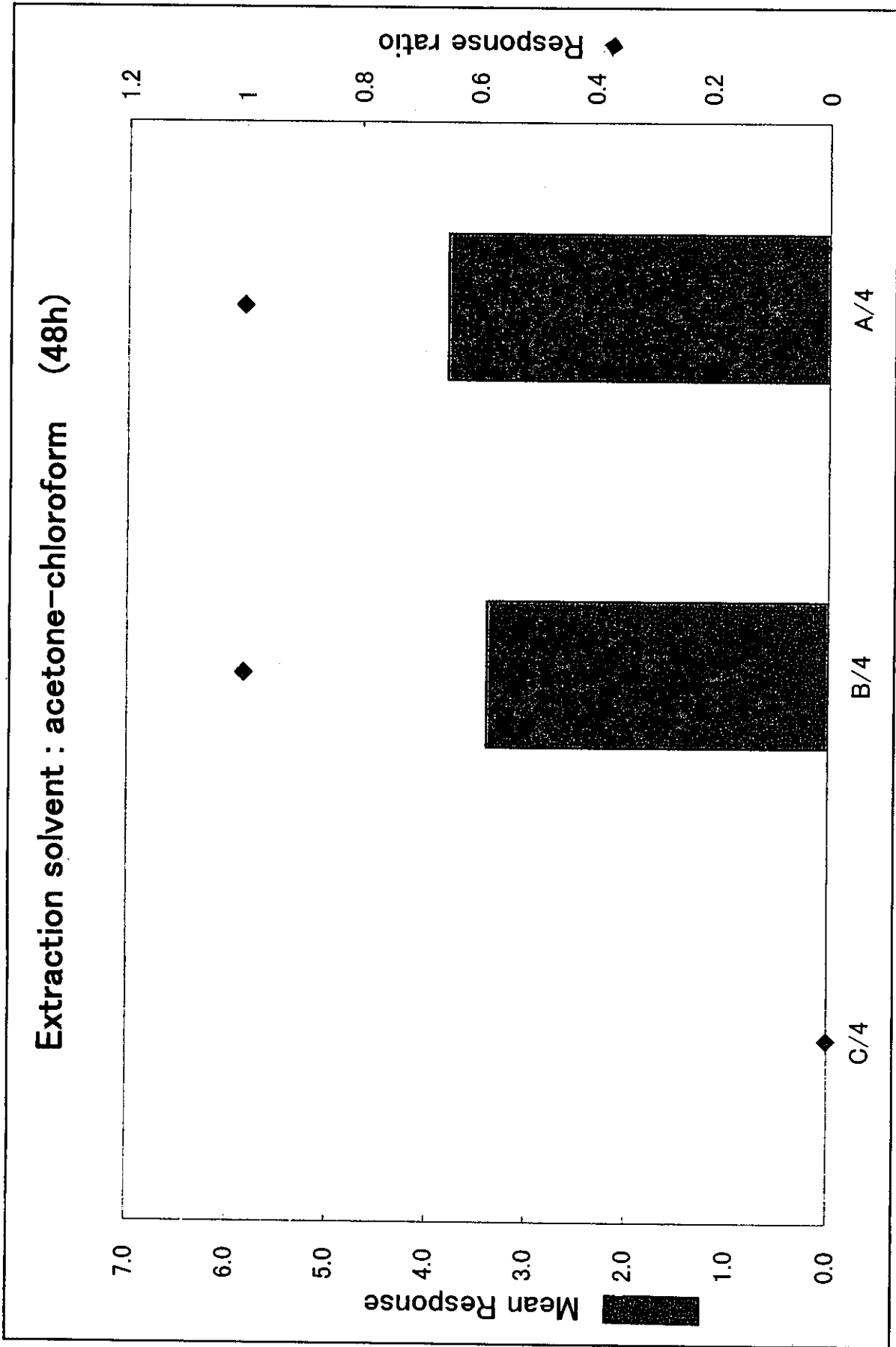


Fig 5. Comparison of the GPMT data among various induction concentrations prepared from material-B using organic solvent-extraction method under the open patch condition.



primary irritation : MR=0

Fig 6. Comparison of the GPM T data among three materials (A, B, C) using organic solvers extraction method under the open patch condition at four-folds diluted concent ratios of the extractants involved in materials.



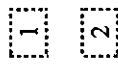
photograph 9

animal ID No. 41, 24 hours after removal of dressing

extractant: saline

induction : extract of test material A

challenge skin site	challenge	erythema/edema
1	saline	0/0
2	extract of test material A in saline	0/0



challenge skin site



photograph 10

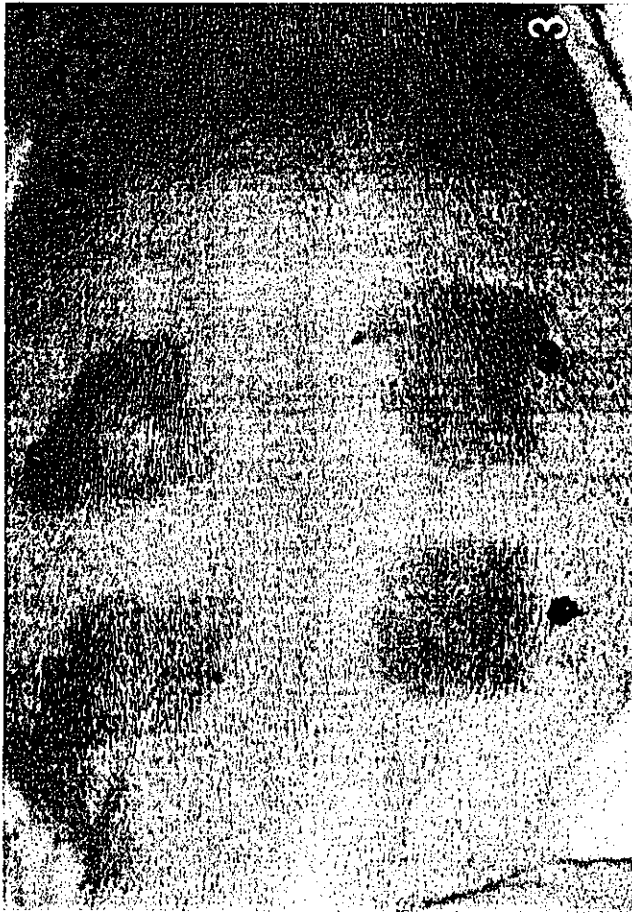
animal ID No. 47, 24 hours after removal of dressing

extractant: cottonseed oil

induction : extract of test material A

challenge skin site	challenge	erythema/edema
1	cottonseed oil	0/0
2	extract of test material A in cottonseed oil	1/0

Fig 7. Dermal reaction 48h after exposure with saline-(left) and cotton seed oil-(right) extracts prepared from material-A.

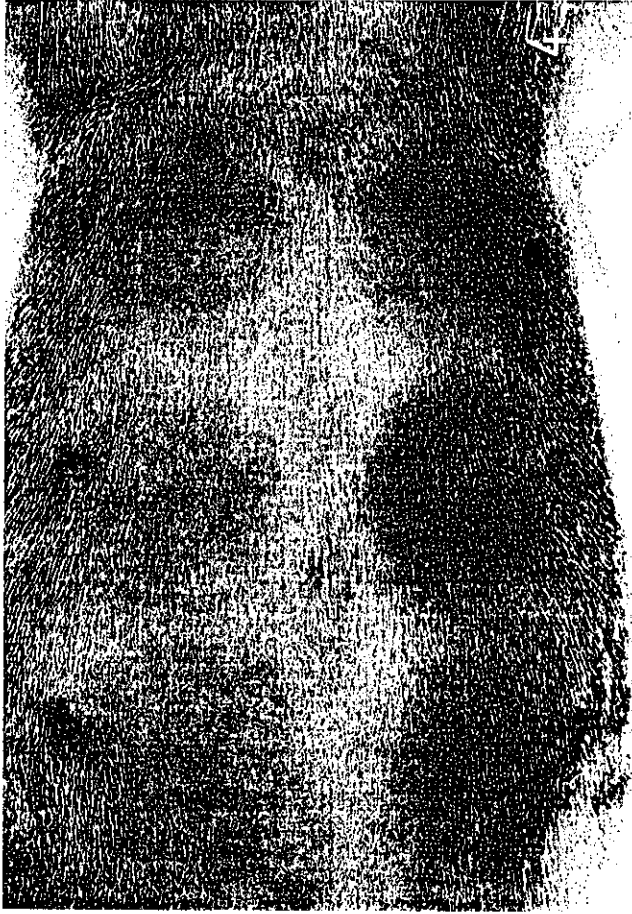


photograph 3

animal ID No. 11, 24 hours after application
 extractant: mixture of acetone and chloroform
 induction : extract of test material B

challenge skin site	challenge	erythema/edema
1	acetone	0/0
2	condensate of extractant in acetone	0/0
3	0.96% extract of test material B in acetone	3/1
4	3.85% extract of test material B in acetone	3/1
5	1.93% extract of test material B in acetone	3/1
6	7.7% extract of test material B in acetone	3/1

5 3 1
 6 4 2
 challenge skin site



photograph 4

animal ID No. 19, 24 hours after removal of dressing
 extractant: mixture of acetone and chloroform
 induction : extract of test material B

challenge skin site	challenge	erythema/edema
1	vaseline	0/0
2	condensate of extractant in vaseline	0/0
3	0.96% extract of test material B in vaseline	0/0
4	3.85% extract of test material B in vaseline	2/1
5	1.93% extract of test material B in vaseline	1/0
6	7.7% extract of test material B in vaseline	2/1

Fig 8. Dermal reaction 48h after exposure with organic solvent extracts prepared from material-B under the open patch (left) and closed patch (right) conditions.

Extraction solvent : acetone-chloroform Open patch (72h)

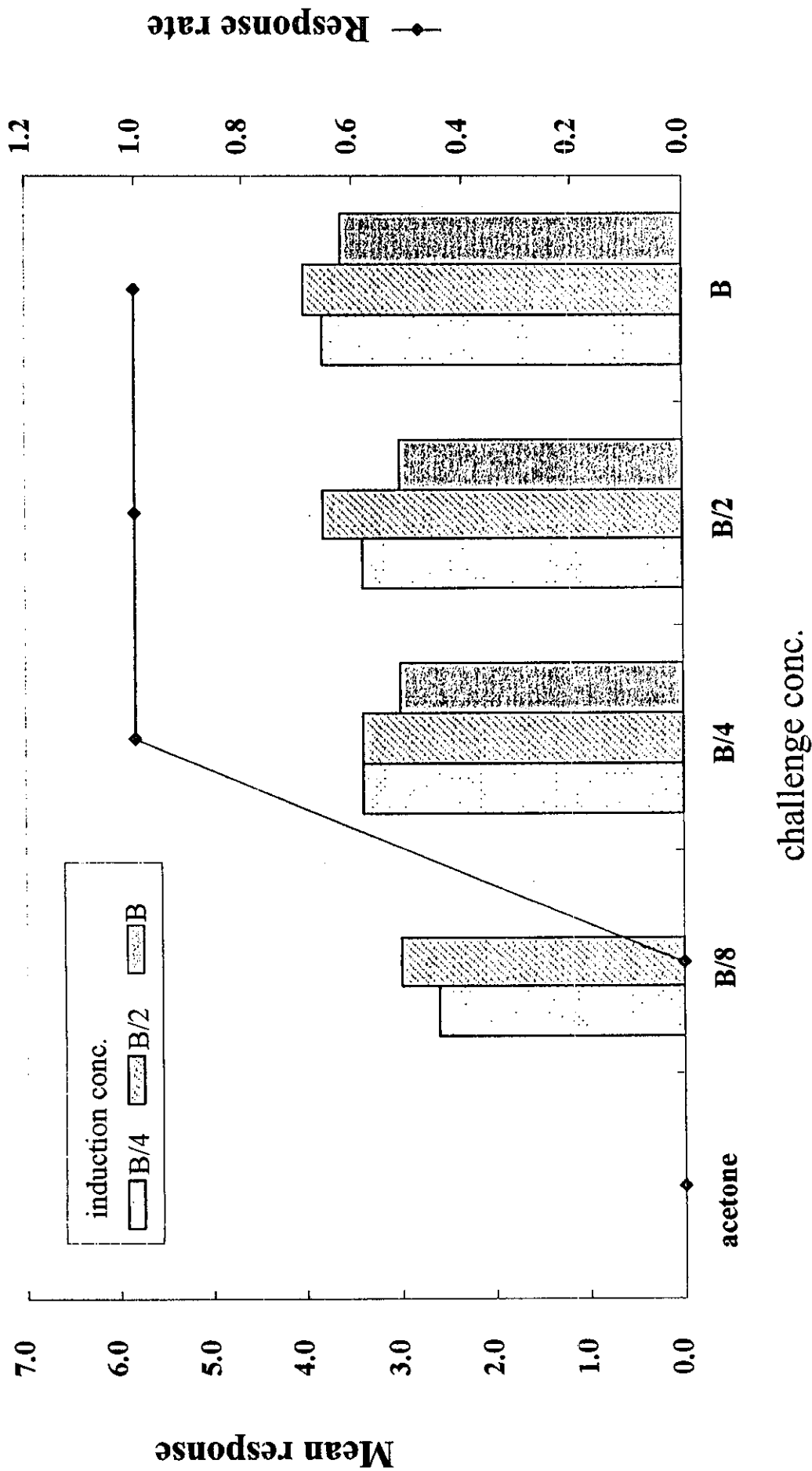
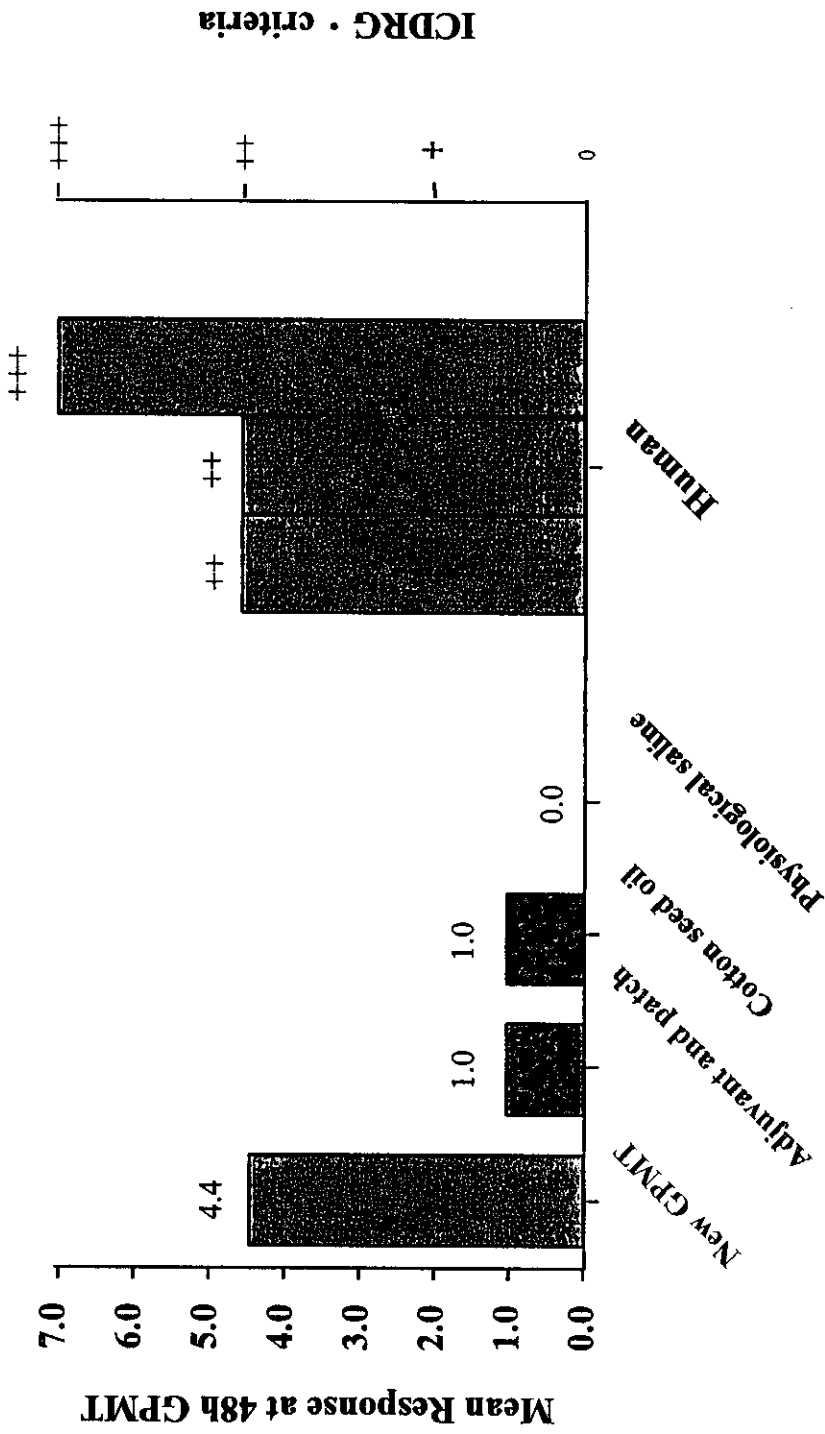


Fig 9. Comparison of GPMT data with various challenge concentrations (B/8, B/4, B/2, B) among three groups treated with different induction concentrations (B/4, B/2, B).

Material-B



Materials amounts : New GPMT < Cotton seed oil + physiological saline
 $1.75g < 3.2g = 1.6g + 1.6g$

Fig 10. Data of human, adjuvant and patch test and GPMT obtained from three kinds of extraction method (New GPMT=organic solvent, cotton seed oil, physiological saline).

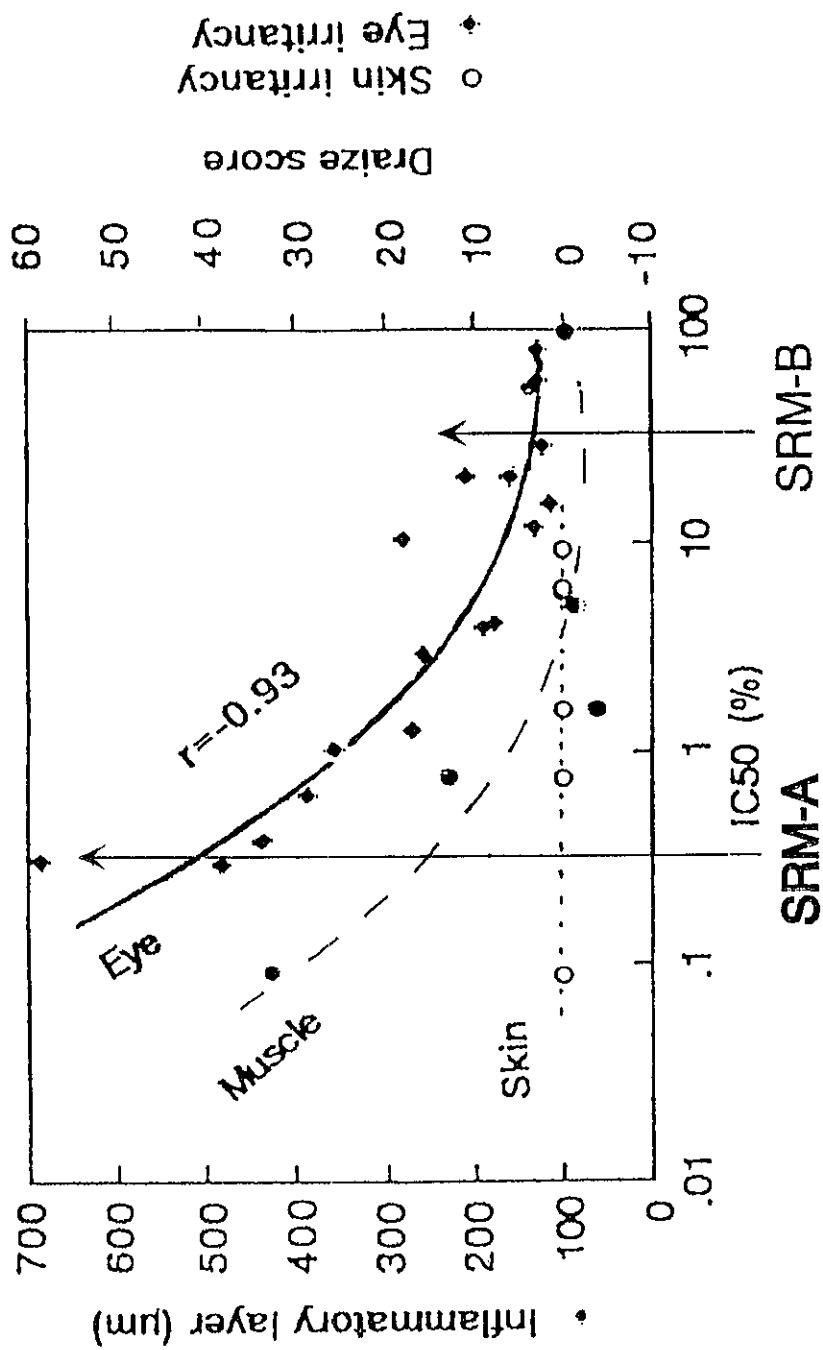


Fig.11 Comparison of cytotoxic potentials with the potentials of *in vivo* responses of muscle inflammation, eye, and skin irritancy.

SRM-A (48h culture) 5%FCS-MEM

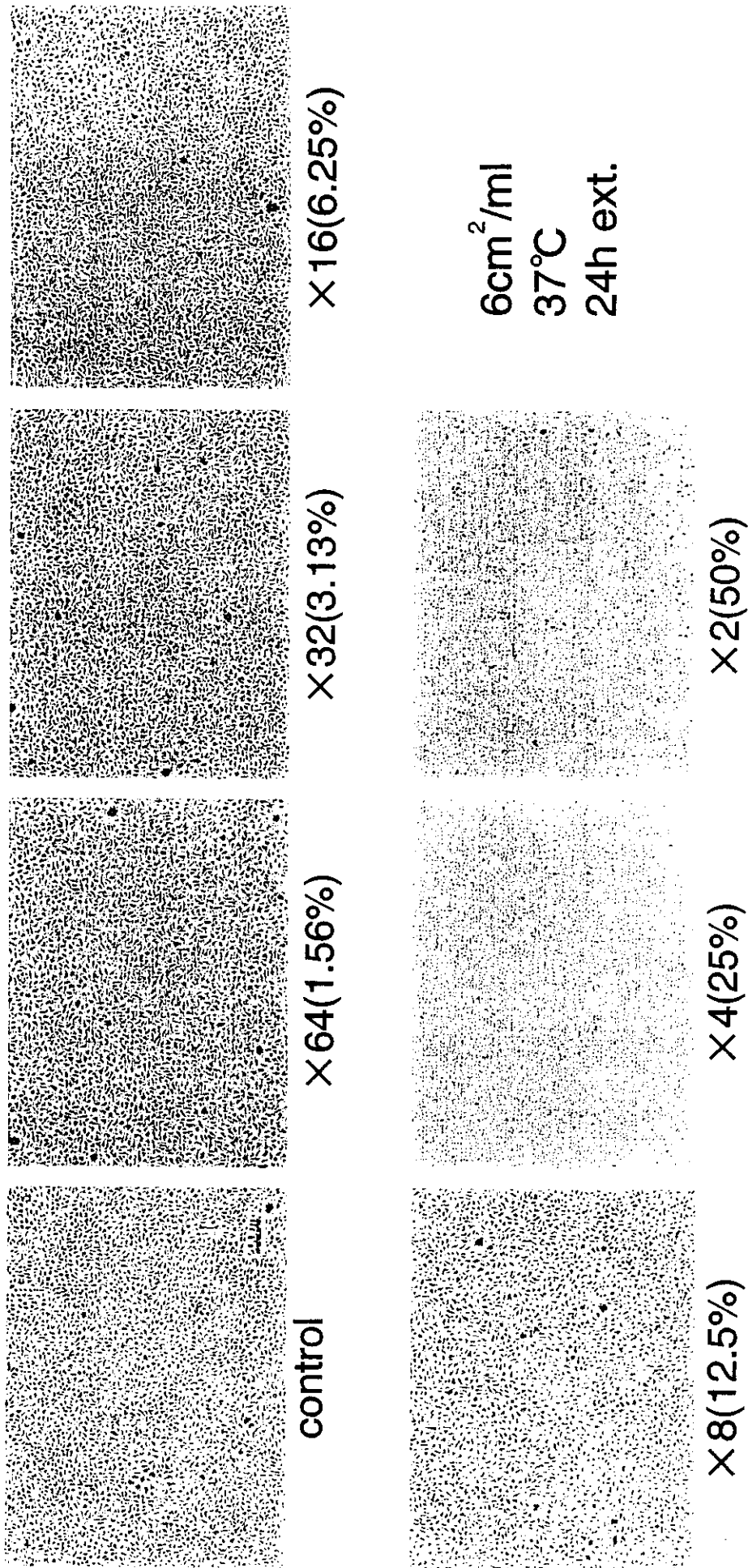


Fig. 12 Optical microphotographs of L929 cells incubated for 48h with the extracts prepared from SRM-A using 5% FCS-MEM.

SRM-B (48h culture) 5%FCS-MEM 6cm²/ml, 37°C 24h ext.

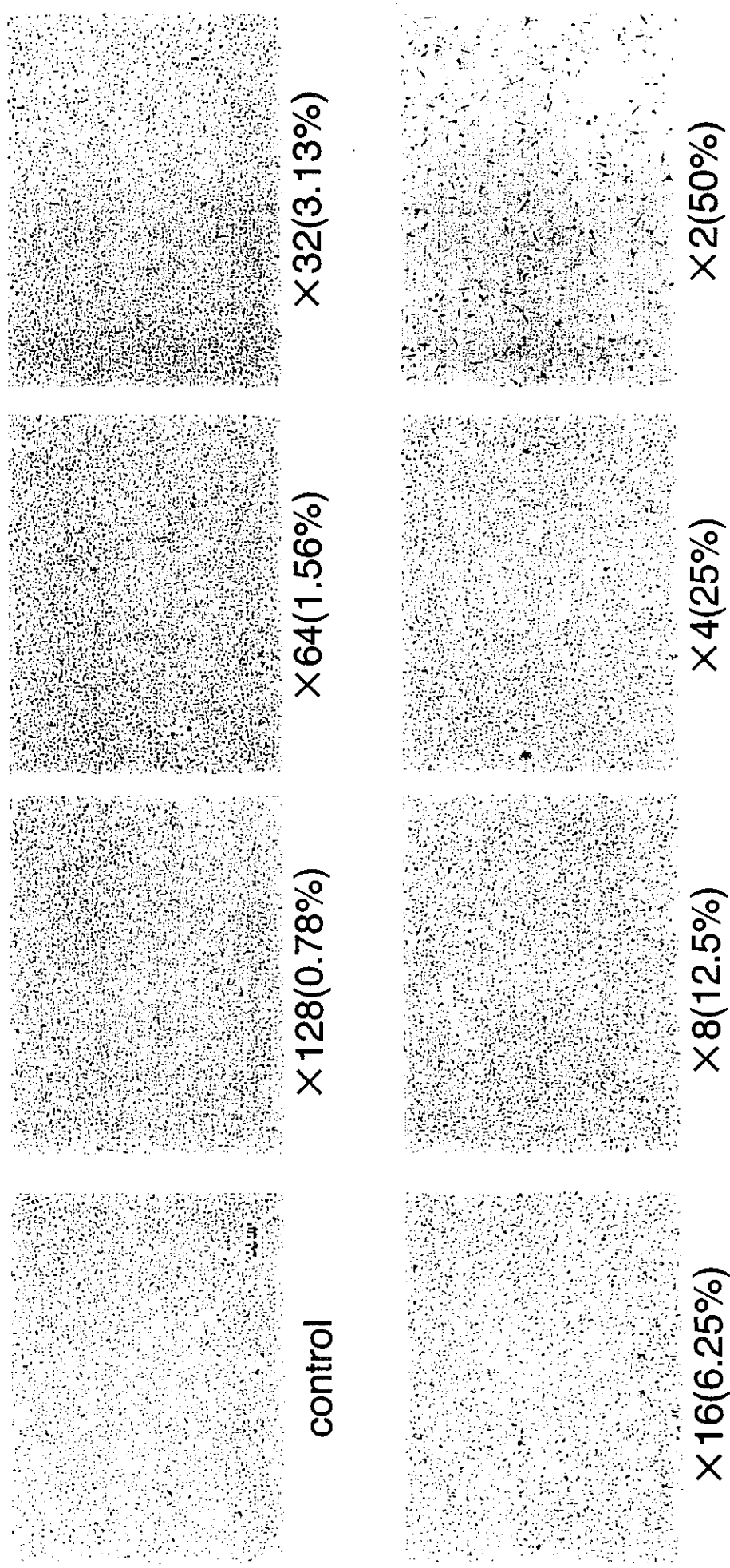
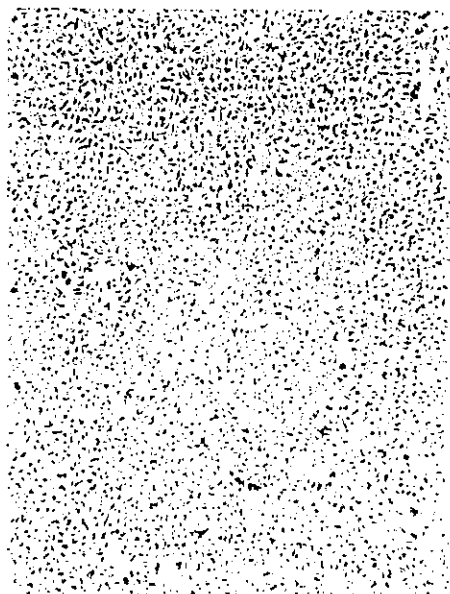
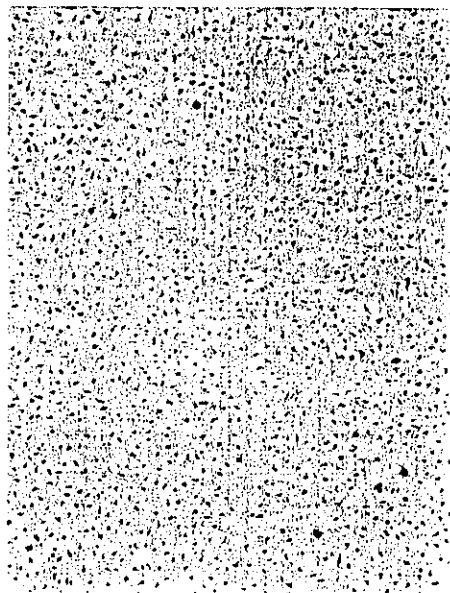


Fig. 13 Optical microphotographs of L929 cells incubated for 48h with the extracts prepared from SRM-B using 5% FCS-MEM.

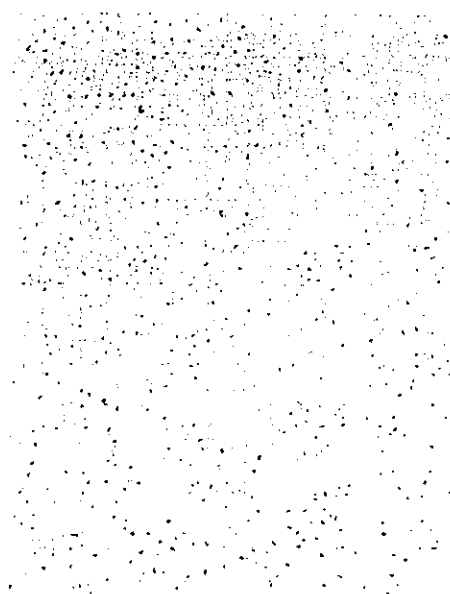
SRM-A 6cm²/ml 37°C, 24h ext. (X175)



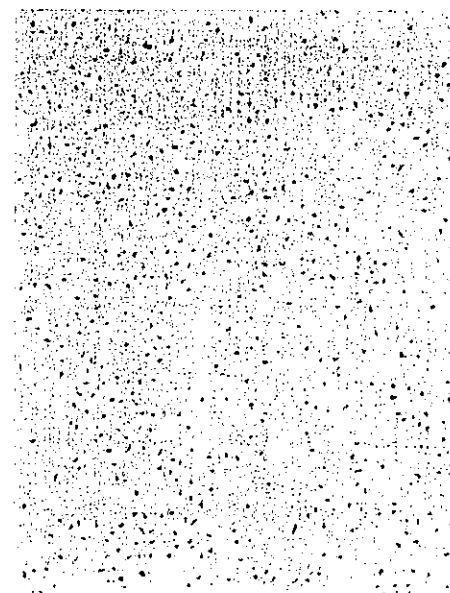
control



0% FCS-MEM ext.



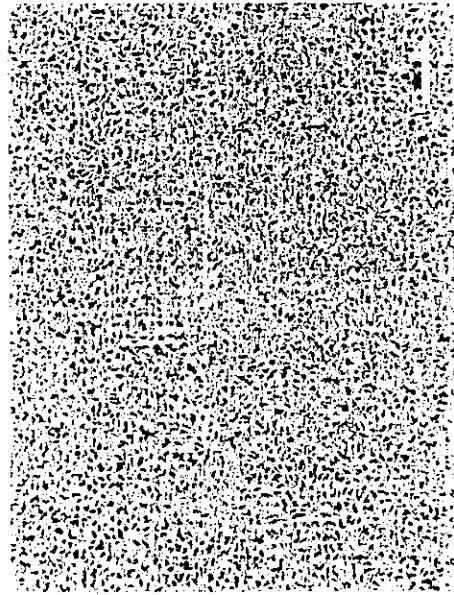
5% FCS-MEM ext.



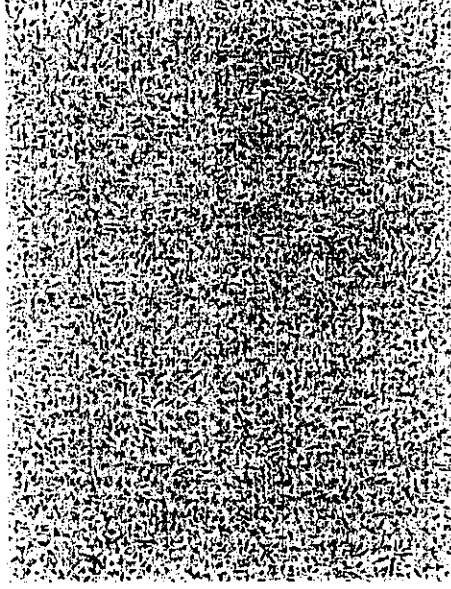
10% FCS-MEM ext.

Fig. 14 Optical micrographs of L929 cells incubated for 48h with the extracts prepared from SRM-A using different FCS concentration media.

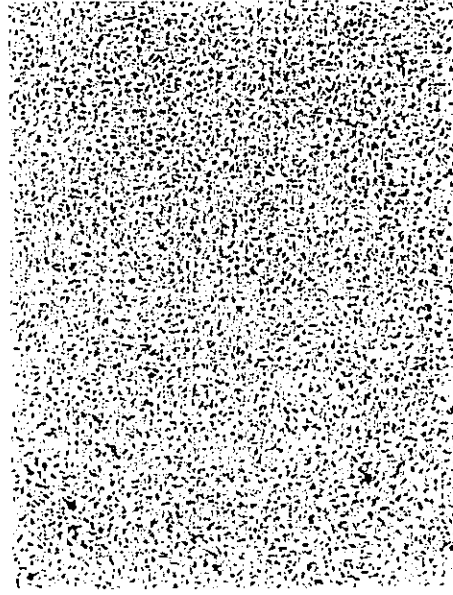
Negative-SRM 6cm²/ml 37°C, 24h ext. (×175)



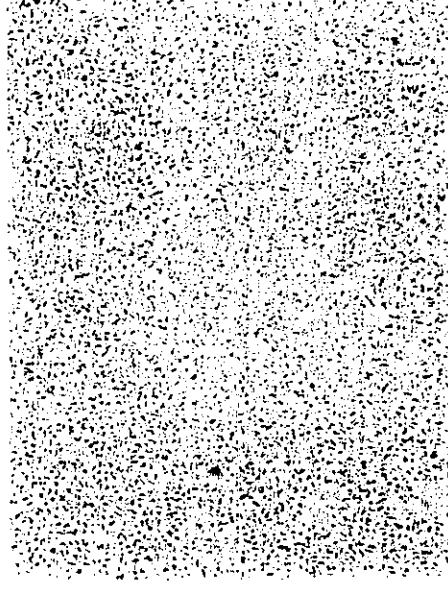
control



0% FCS-MEM ext.



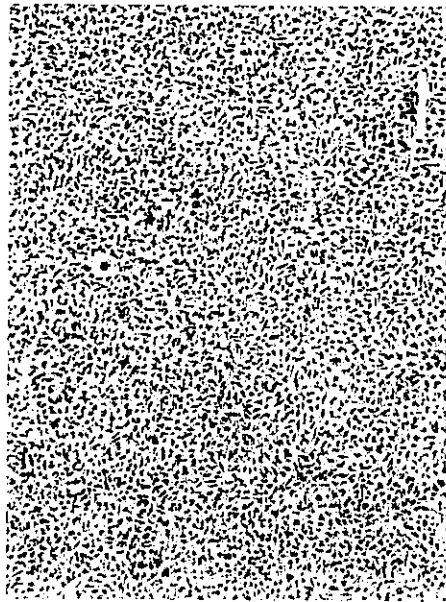
5% FCS-MEM ext.



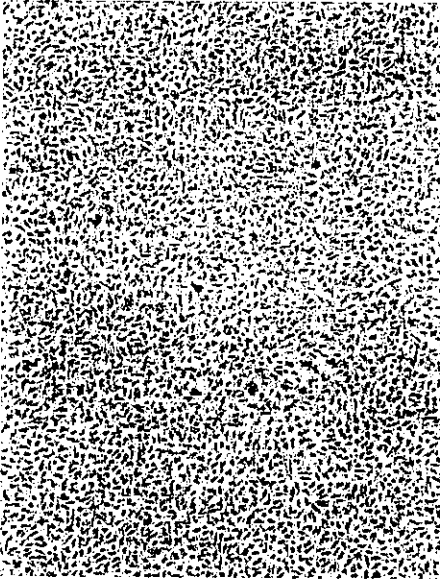
10% FCS-MEM ext.

Fig. 15 Optical micrographs of L929 cells incubated for 48h with the extracts prepared from Negative-SRM using different FCS concentration media.

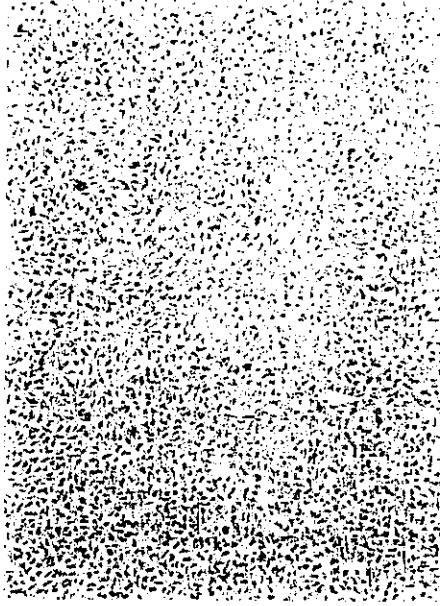
SRM-A 6cm²/ml d-H₂O ext. (×175)



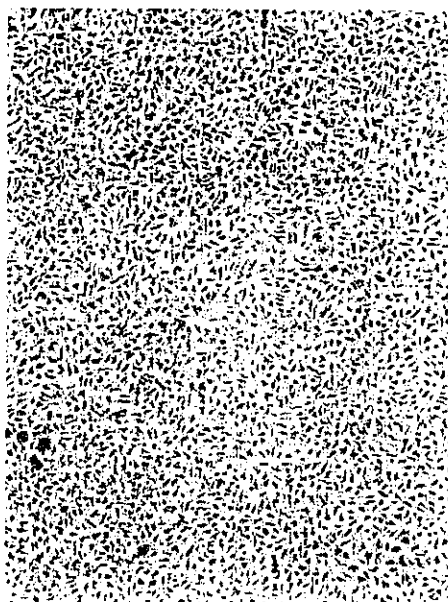
5% fcs-MEM control



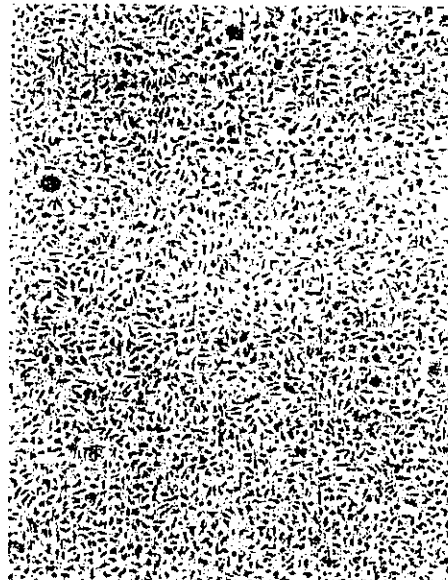
37°C/24h



50°C/72h



70°C/24h



121°C/h

Test solution:

mixture of 1vol. of d-H₂O ext.

+

1vol. of 10% fcs-MEM

Fig. 16 Optical micrographs of L929 cells incubated for 48h with the distilled water extracts of SRM-A under the four kinds of extraction temperature / hours.

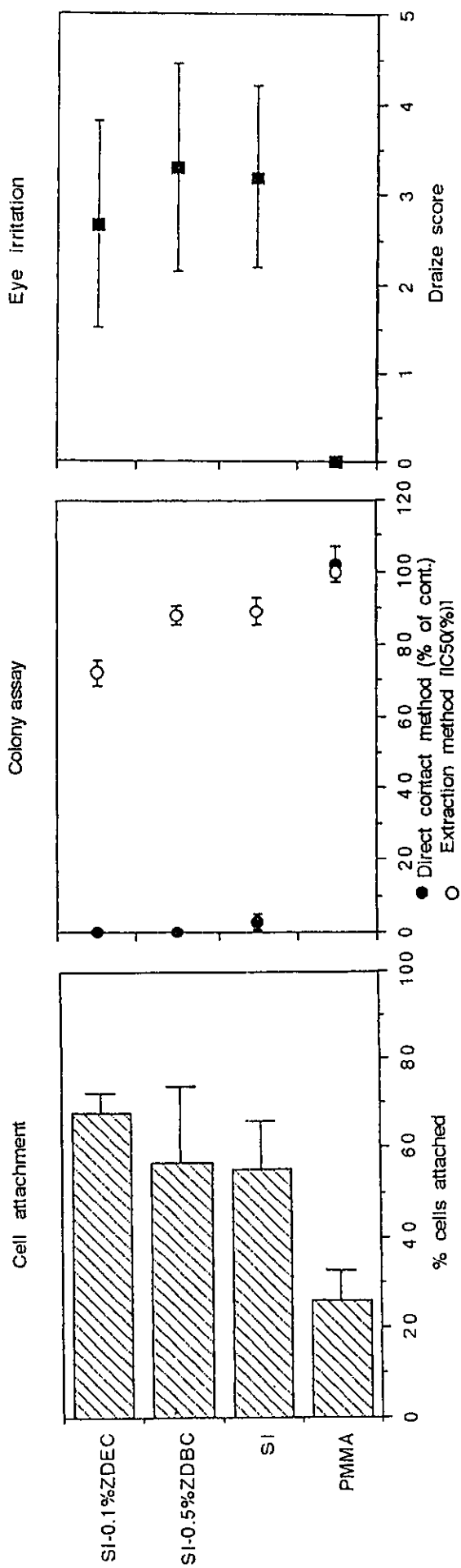


Fig. 17. Comparison between cell attachment, cytotoxicity and Draize score using lens materials. Cell attachment was tested twice using cells incubated for 16h after seeding, and colony formation was assayed by extraction twice using the 72 h extracts prepared from lens materials. Each value is the mean and bars indicate SD.

Table 1. Comparison of human data with GPMT data obtained from the various extraction conditions.

GPMT (Mean Response)		Human (ICDRG-criteria)
(acetone-chloroform extracts)	cotton- seed oil	(Prof. Andersen's data)
open	physio- logical saline	(numbers)
Material-A 3.8 (3.0)	1.2 0	+++ (2), ++ (1)
Material-B 3.6 (2.2)	0.8 0	+++ (1), ++ (2)
Material-C 0.4 (0)	0 0	- (4)

T.Tsuchiya NIHS

72hr

Table 2. Comparison of various amounts of the materials required for obtaining the GPMT data (Mean Response and primary irritation) under the open patch conditions.

Material amounts	tested conc.	Mean Response (primary irritation)		
		24h	48h	72h
7g	B	4.0(1.0)	4.0(0.6)	3.6(0.2)
3.5g	B/2	4.4(0.2)	4.8(0.2)	3.8(0)
1.75g	B/4	4.0(0.2)	4.4(0.2)	3.4(0)

extraction, evaporator adjusted to

B : 7g
 1ml+4ml+1ml
 B/2 : 3.5g
 1ml+4ml+1ml
 B/4 : 1.75g
 1ml+4ml+1ml

GPMT(induction and challenge)
Test group : 10animals
Control group : 10animals

Table 3. The IC50(%) values of SRM-A and SRM-B obtained from V79 colony assay using 5% FCS-MEM(MO5).

Colony assay (V79 cells)	
SRM-A	SRM-B
IC50 (%)	0.48
	54.1
0.1g/ml (MO5 : 5%fcs-MEM)	37°C, 24h-extraction

Table 4. Reactivity Grades for Elution Test

Grade	Reactivity	Conditions of all Cultures
0	<i>None</i>	Discrete intracytoplasmic granules; no cell lysis
1	<i>Slight</i>	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	<i>Mild</i>	Not more than 50% of the cells are round, and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	<i>Moderate</i>	Not more than 70% of the cell layers contain rounded cells or are lysed
4	<i>Severe</i>	Nearly complete destruction of the cell layers

Table 5. Reactivity grades of SRM-A and SRM-B obtained from L929 Elution test using 5% FCS-MEM.

Grade	
Extraction conc.(%)	SRM-A SRM-B
100	4 4
50	4 4
25	4 0
12.5	0 0
6cm ² /ml (5%fcs-MEM) 37°C,24h-extraction	

Table 6. Reactivity grades of SRM-A, SRM-B and Negative-SRM obtained from L929 Elution test using the distilled water and three, different FCS concentration media.

Ext. med.	Grade					
	SRM-A		SRM-B		Negative-SRM	
	24h	48h	24h	48h	24h	48h
d-H ₂ O	0	0	0	0	0	0
0%fcs-MEM	3	3	1	2	0	0
5%fcs-MEM	4	4	4	4	0	0
10%fcs-MEM	4	4	4	4	0	0

37°C, 24h-extraction

(L929 Cells)