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**ISOLATED LIMB PERFUSION FOR MALIGNANT MELANOMA:
CLINICAL AND LABORATORY STUDIES**

Volume II of 2 volumes

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Submitted for the degree of M.D. to
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Group, Belvidere Hospital, Glasgow.

Submitted October 1989

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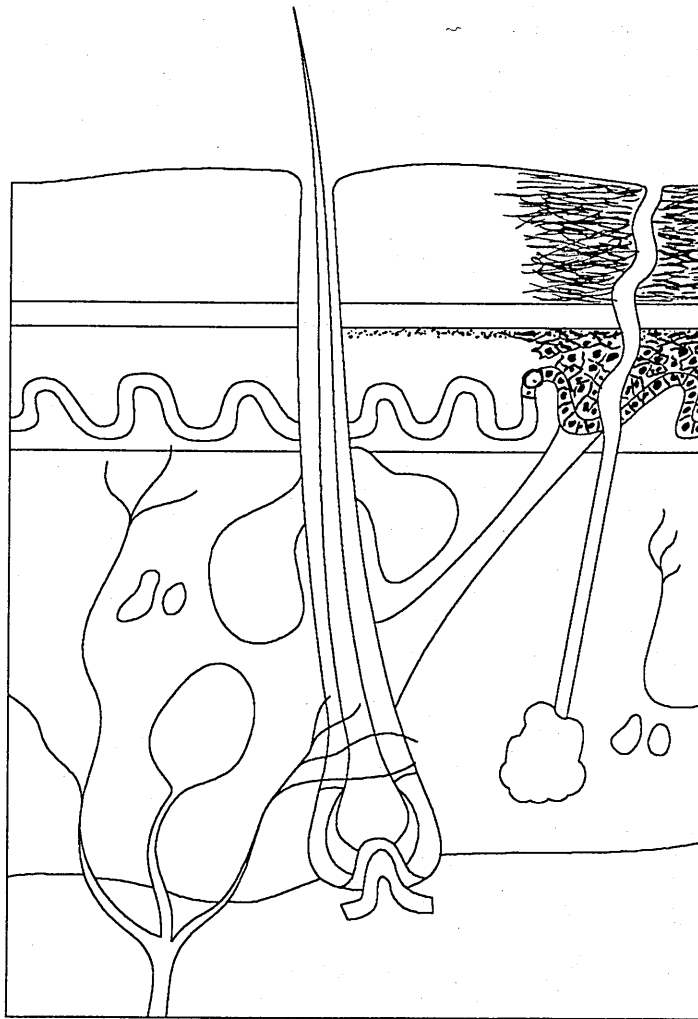
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46A-C	Experiment 10	144 & 5
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54A-C	Experiment 18	160 & 1
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56A-C	Experiment 20	163 & 4
57A-C	Experiment 21	165 & 6
58A-C	Experiment 22	167 & 8
59A-C	Experiment 23	169 & 70
60A-C	Experiment 24	171 & 2
61A-C	Experiment 25	173 & 4
62A-C	Experiment 26	175 & 6

ILLUSTRATIONS



Cornified layer

Lucid layer

Granular layer

Spiny cell layer

Basal cell layer

Dermis

Figure 1

Diagrammatic representation of skin structure

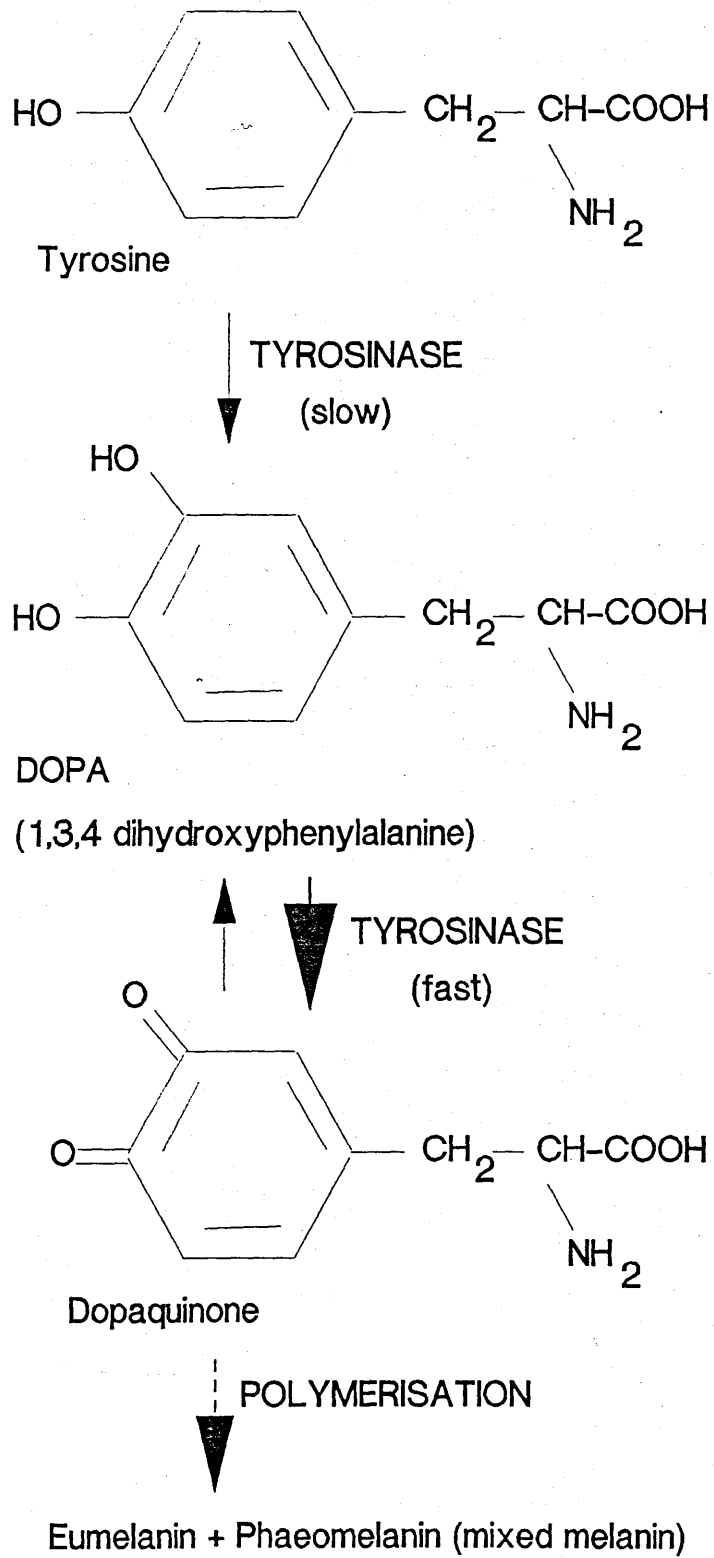


Fig. 2 MELANIN SYNTHESIS

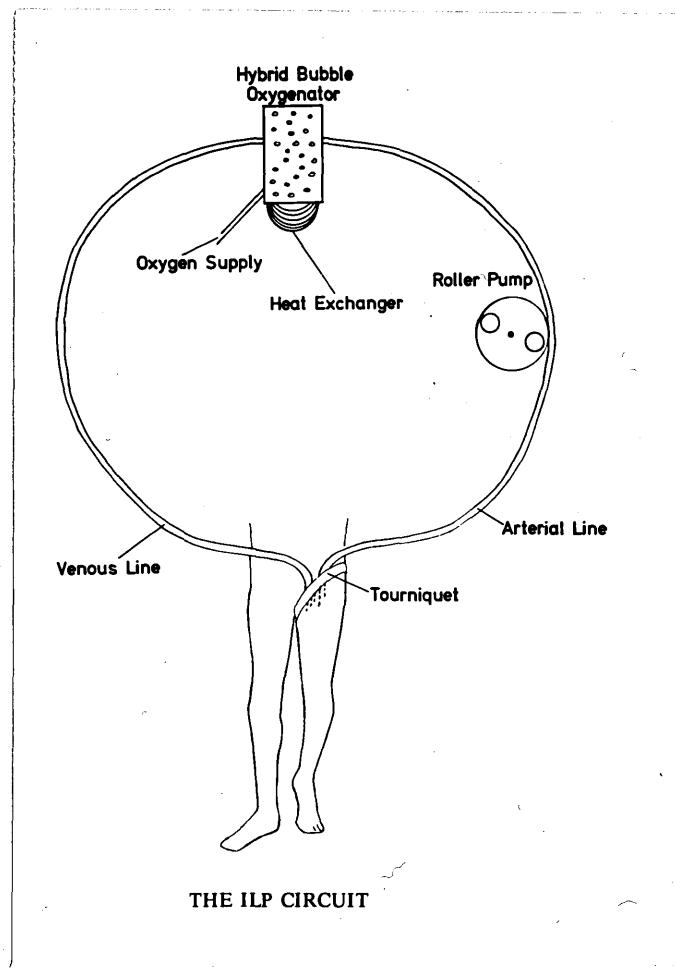


Figure 3

The essential components of the isolated limb perfusion circuit are shown diagrammatically.



Figure 4

From the foot of the operating table, with the patient supine, the right leg (to be treated) is seen wrapped in a heated water blanket. Wires from thermistor probes attached to the limb are seen emerging from beneath the blanket at the bottom of the illustration.

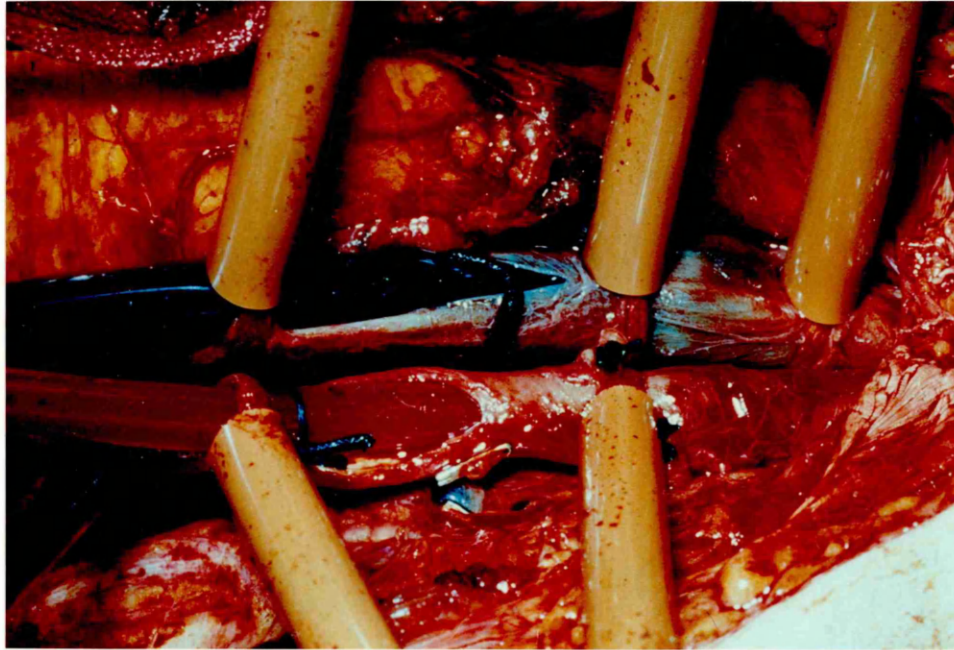


Figure 5

View of the operative exposure of the right external iliac vessels, after retroperitoneal dissection, seen from the patient's right. The tips of the cannulae are passed distally (left to right) and lie below the level of the inguinal ligament. Satisfactory positions have been achieved and the cannulae are secured with cotton snares.

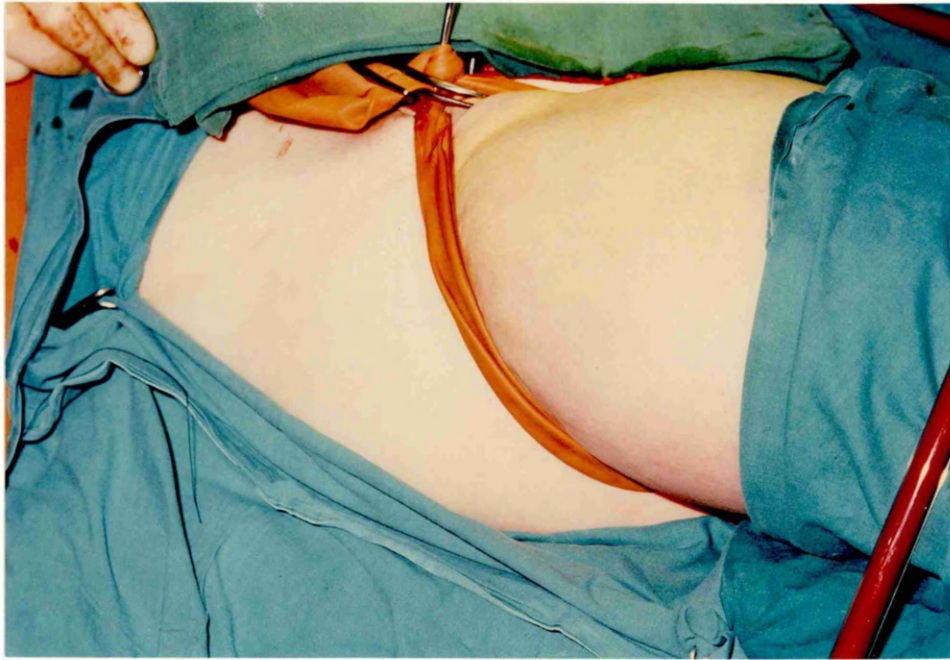


Figure 6

View of the lateral part of the right buttock and upper thigh during isolated limb perfusion (patient's feet to right). The Esmarch tourniquet is anchored by a Steinmann pin (top, middle) driven into the anterior superior iliac spine. Distal to the tourniquet the skin of the upper anterior thigh is stained with fluorescein.

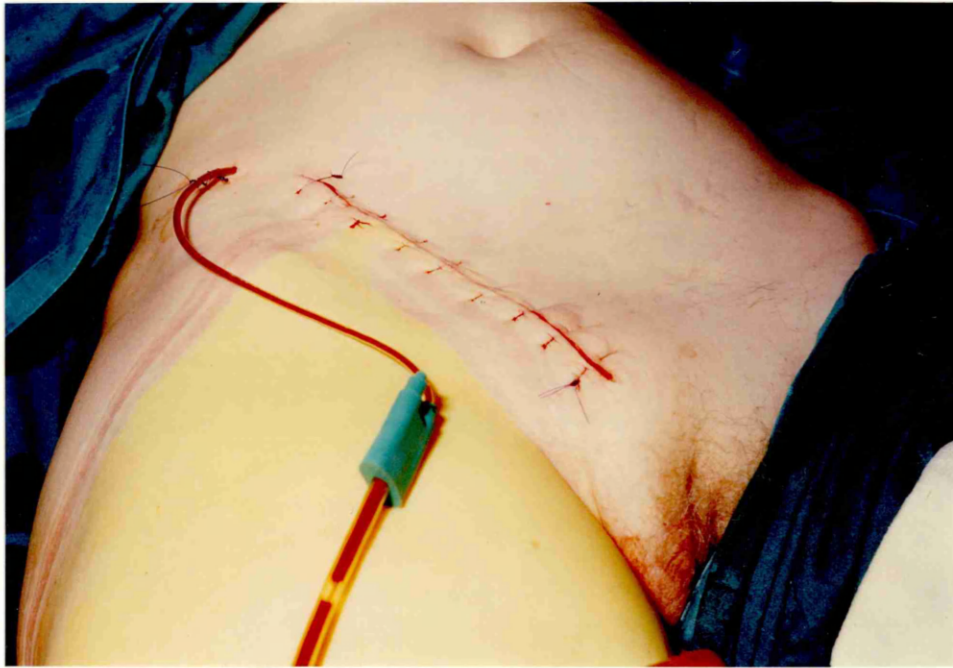
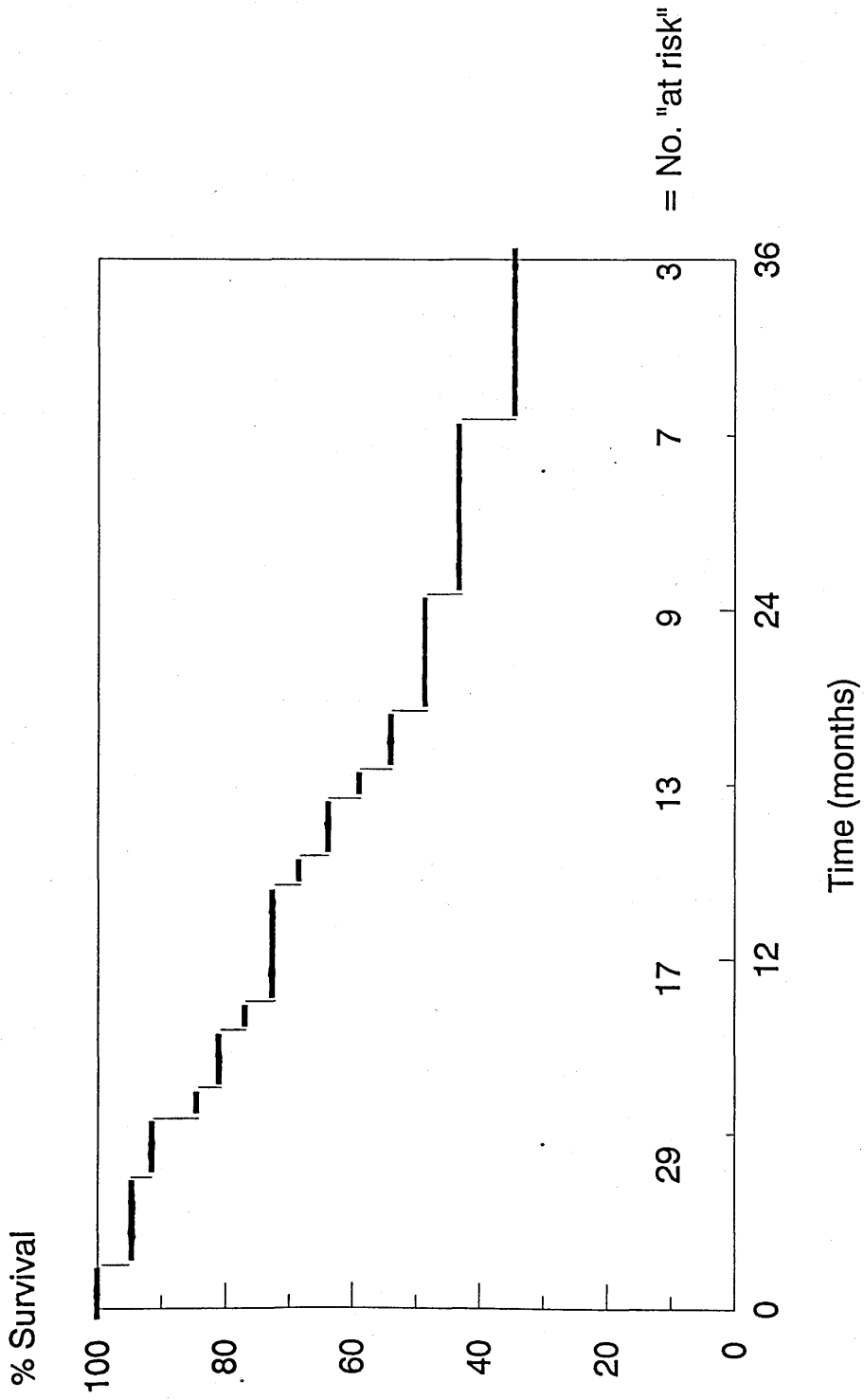


Figure 7

View of the right iliac fossa and upper anterior thigh immediately after isolated limb perfusion. The skin wound has been closed with interrupted nylon over a fine bore suction drain. Note that the skin is stained with fluorescein up to the marks of the tourniquet, but there is no proximal staining.

FIGURE 8 Survival after therapeutic isolated limb perfusion
Life-table analysis (n=38, initially)



A



B

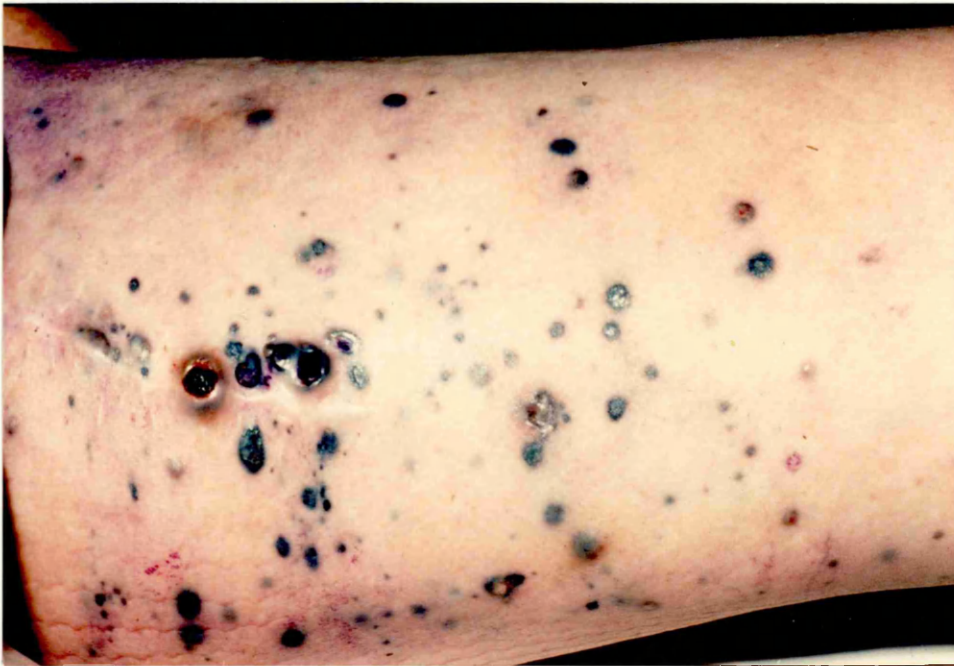


Figure 9

Response of our first patient (M.B., female) treated with isolated limb perfusion.

A Before isolated limb perfusion.

B Two weeks after isolated limb perfusion.



Figure 10A

Patient F.B. with satellitosis and in transit recurrent melanoma - note the pigmentation of prior radiotherapy. These lesions had progressed despite repeated surgery, local radiotherapy and systemic chemotherapy.



Figure 10B

Five days after isolated limb perfusion - the lesions are necrotic (cf. Fig. 10C)

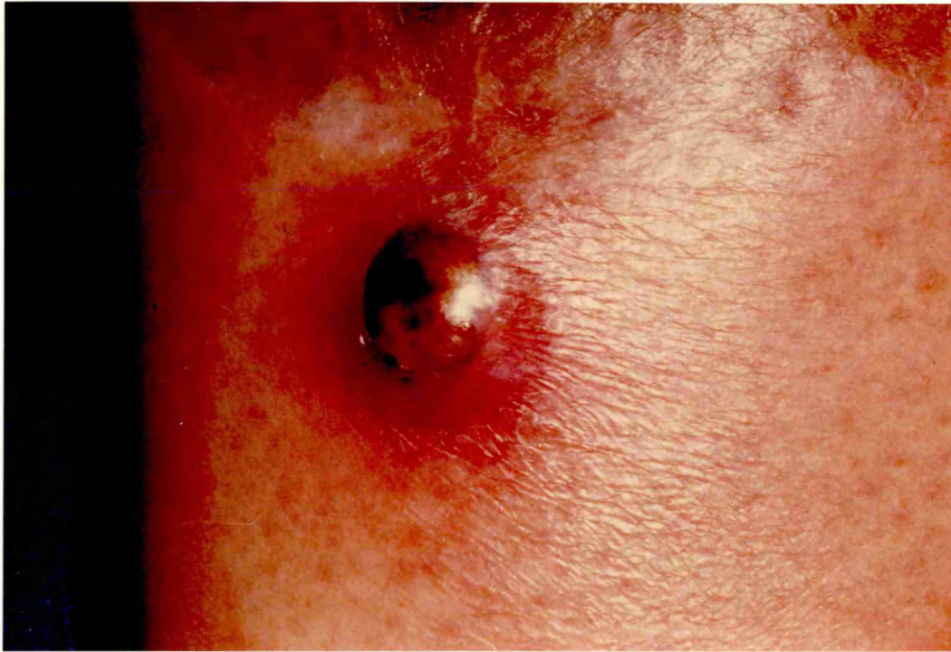


Figure 10C

Close-up view of necrotic melanoma nodule at five days after isolated limb perfusion (lesion below skin graft on Fig. 10B).



Figure 10D

The limb of patient F.B. one month after isolated limb perfusion - the lesions have become flattened and crusted over (cf. 10A & B)



Figure 11

The right leg of this patient (C.M.) was treated according to the M.R.C. protocol (2mg/kg by divided dose). At 14 days after isolated limb perfusion the limb is painful and stiff (Wieberdink grade IV reaction).

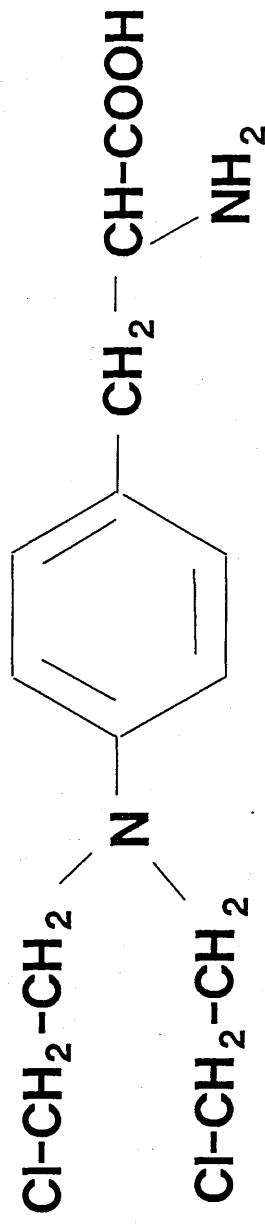


Figure 12 Chemical structure of MELPHALAN

Molecular mass: base, 305.2; hydrochloride, 340.7

FIGURE 13 MELPHALAN PHARMACOKINETICS IN ILP

Group A (1.5mg/kg) vs. Group B (1.75mg/kg)

[melphalan] micg/ml

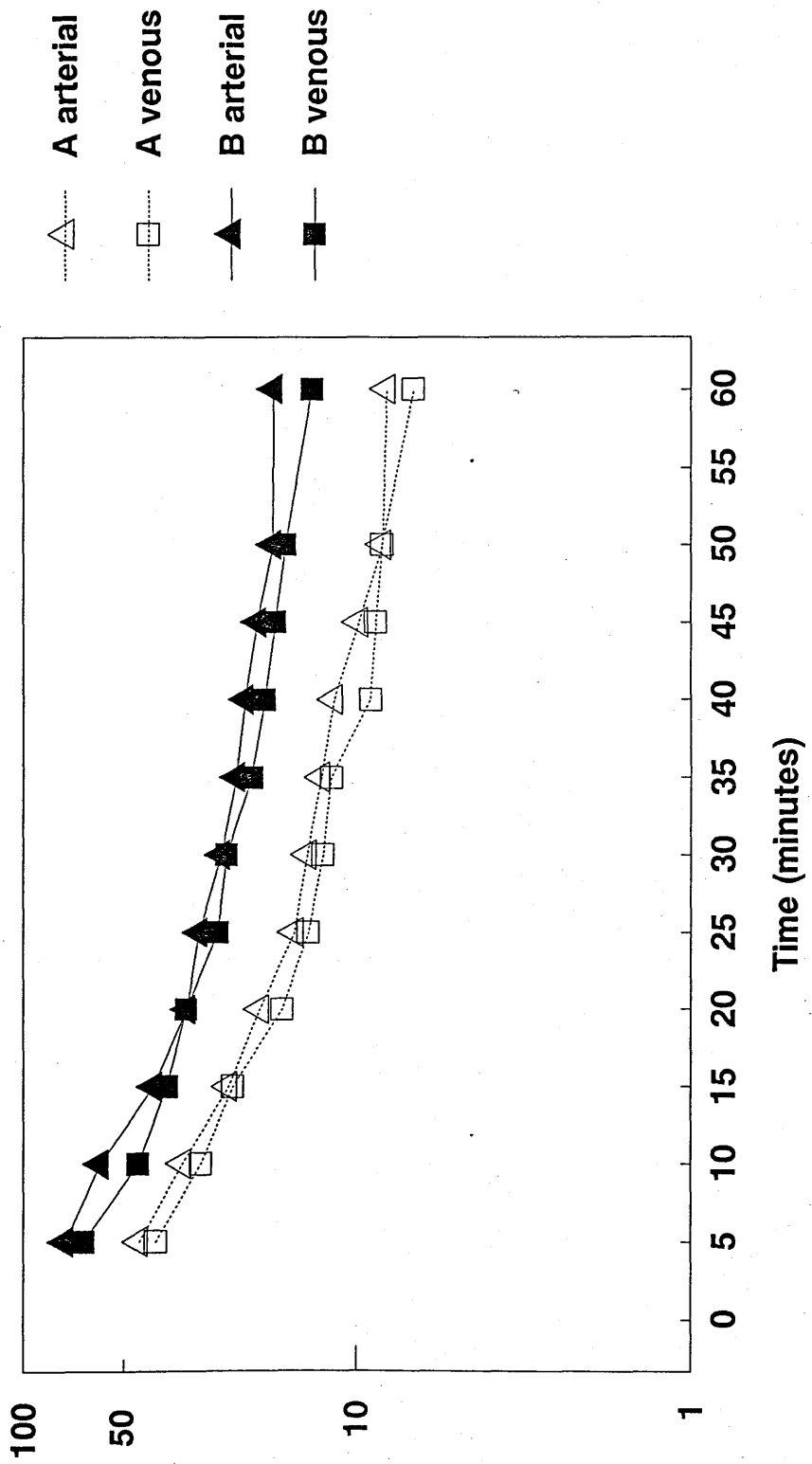


FIGURE 14 MELPHALAN PHARMACOKINETICS IN ILP
Best-fit lines for Groups A and B (cf. Fig. 13)

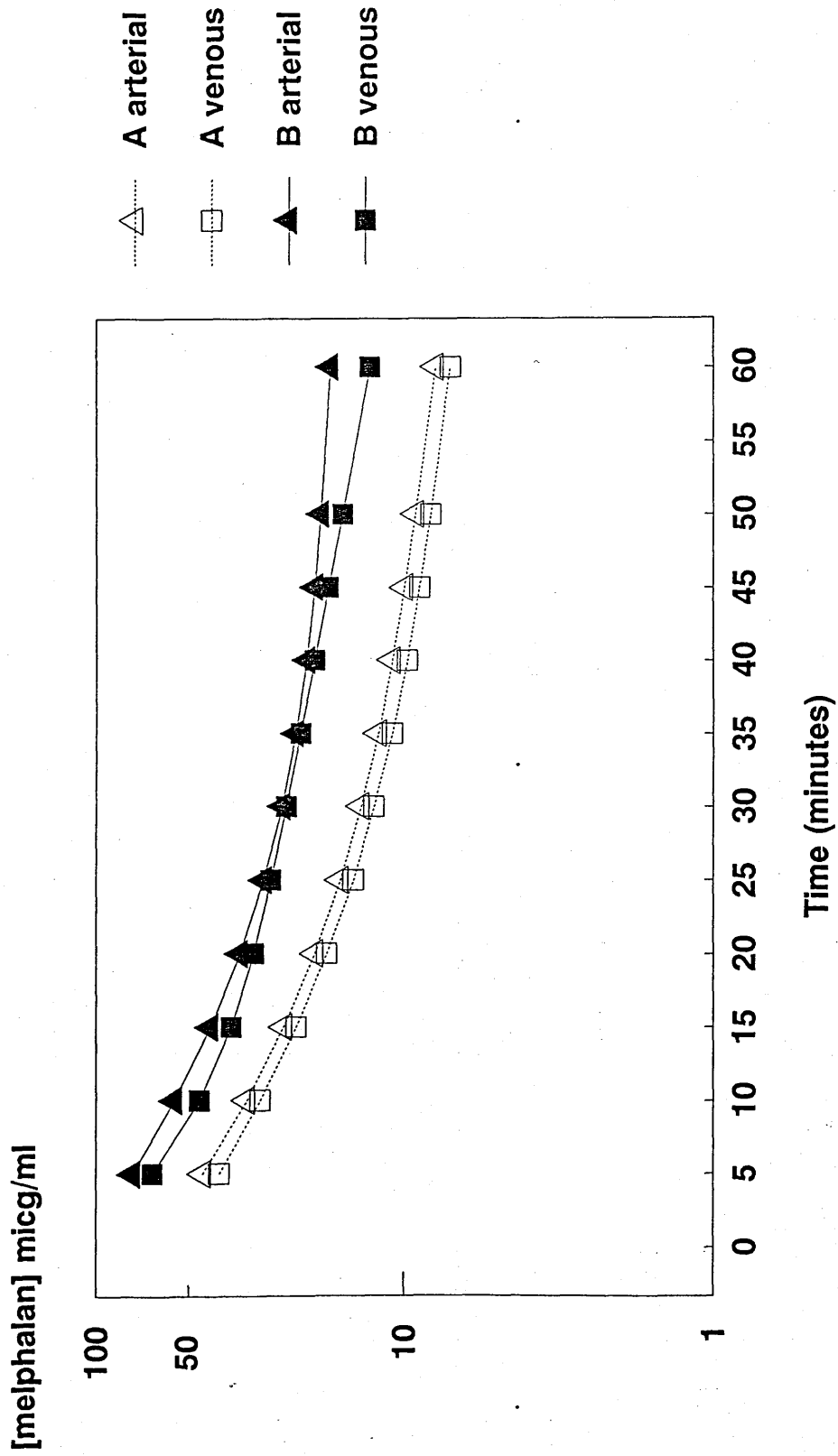


FIGURE 15 MELPHALAN PHARMACOKINETICS IN "MOCK" ILP

100mg melphalan injected, 37degC (semi-log plot)

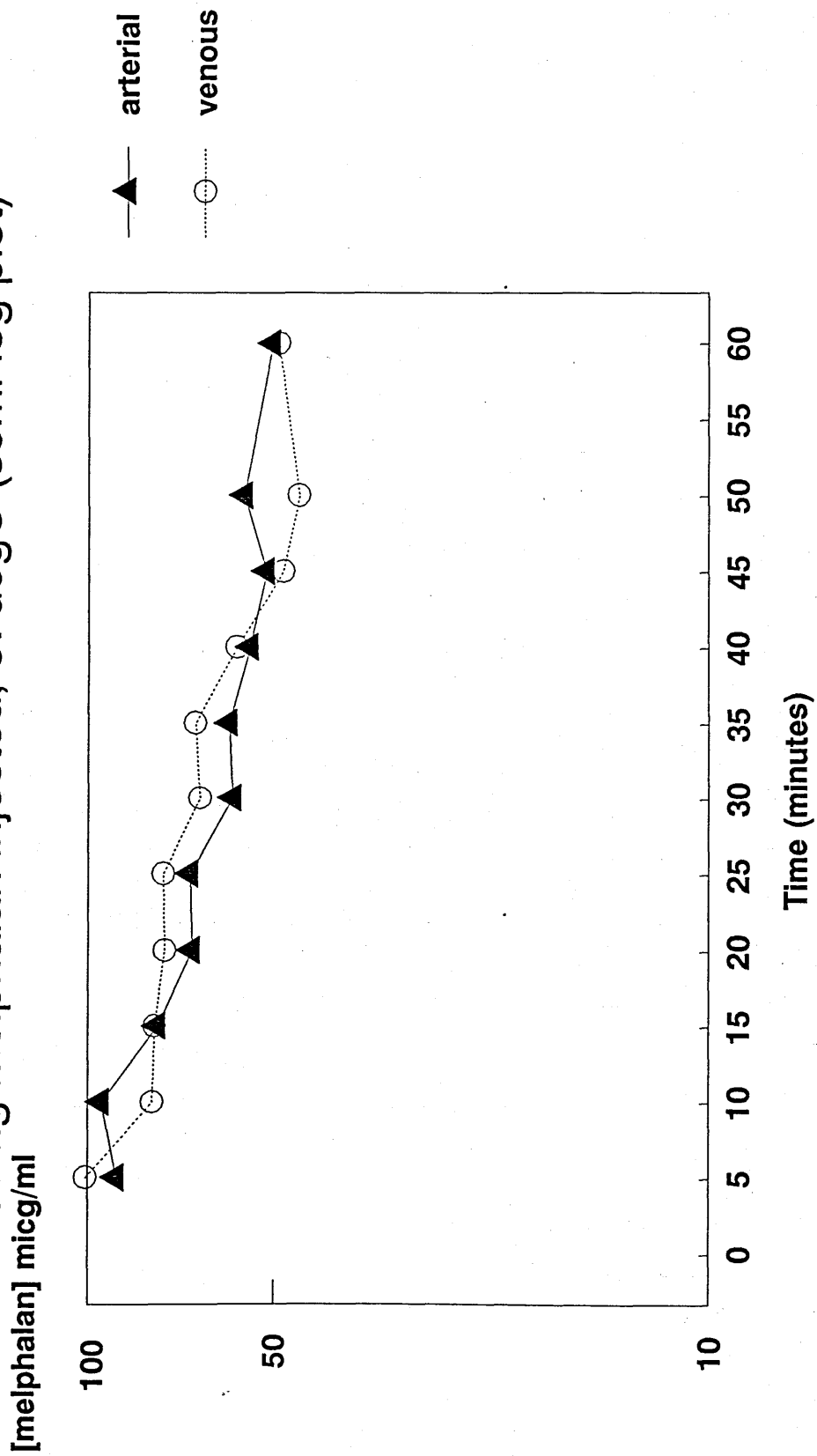


FIGURE 16 MELPHALAN PHARMACOKINETICS IN "MOCK" ILP
100mg melphalan injected, 39degC (semi-log plot)

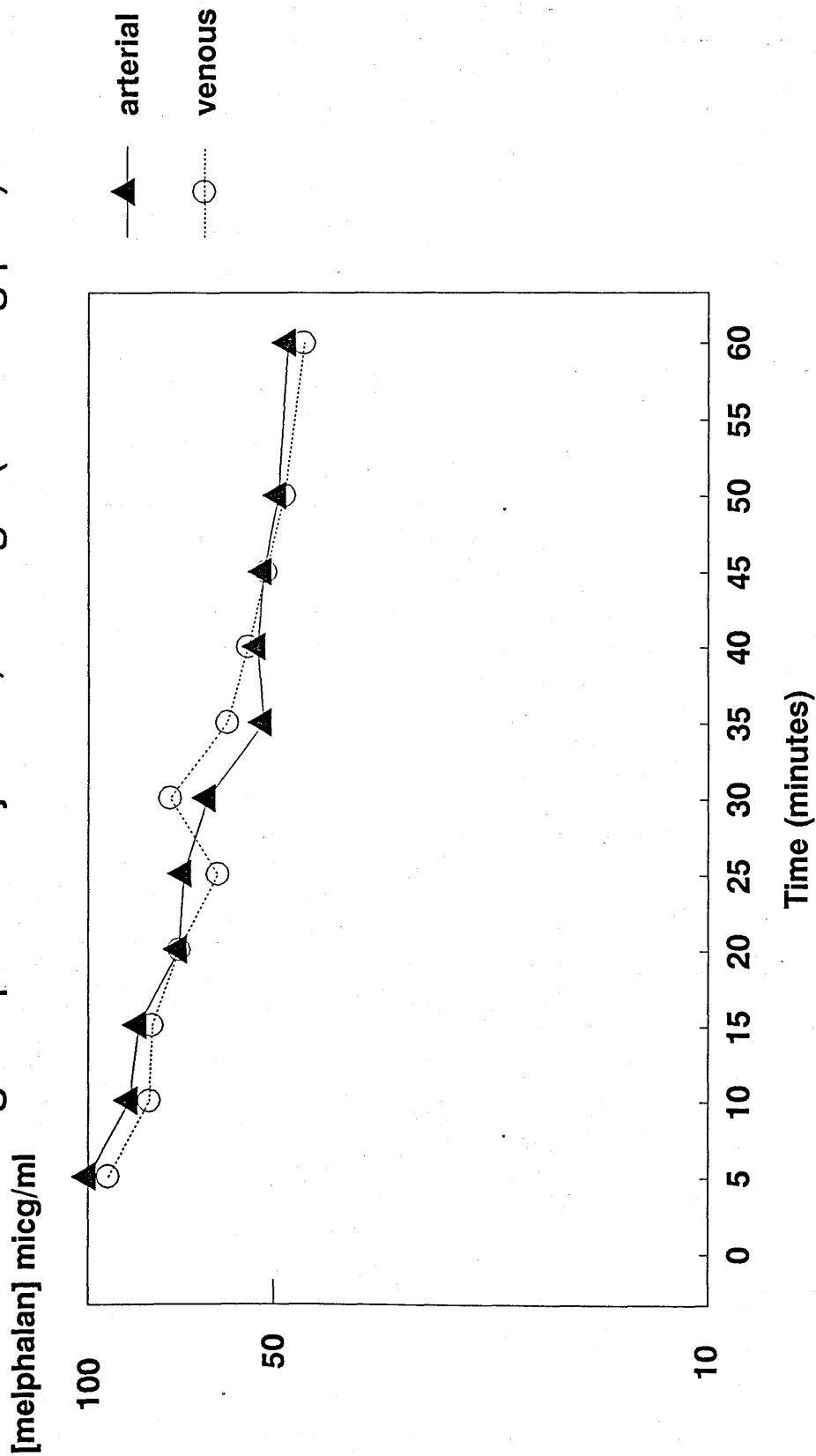


FIGURE 17 % melphalan remaining in perfusate:

Derived from AUC₀₋₃₀ & AUC₃₀₋₆₀ data (Figs. 13, 16)

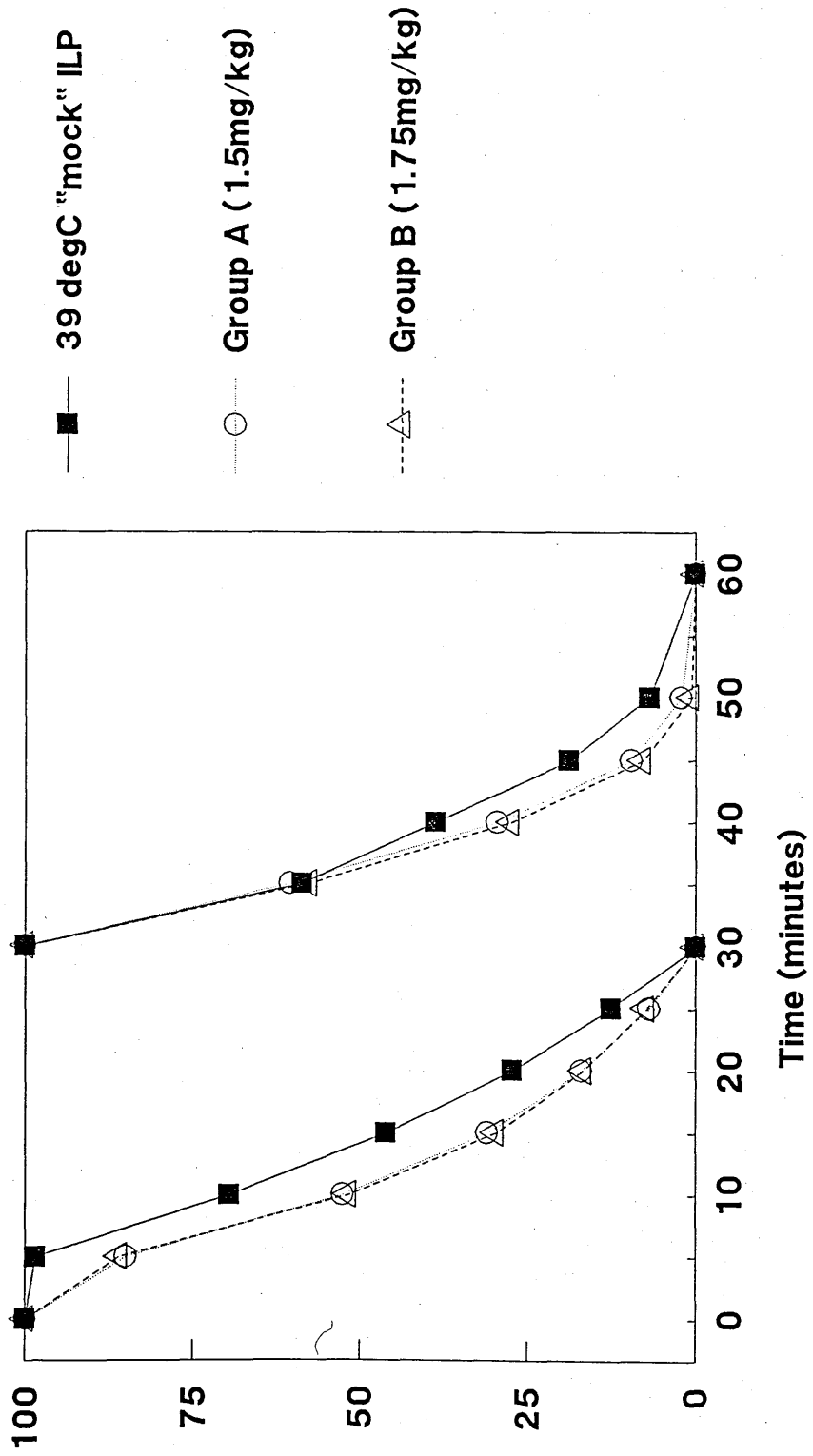


FIGURE 18 MELPHALAN PHARMACOKINETICS IN ILP

Group A: Perfusate vs. Systemic (semi-log plot)

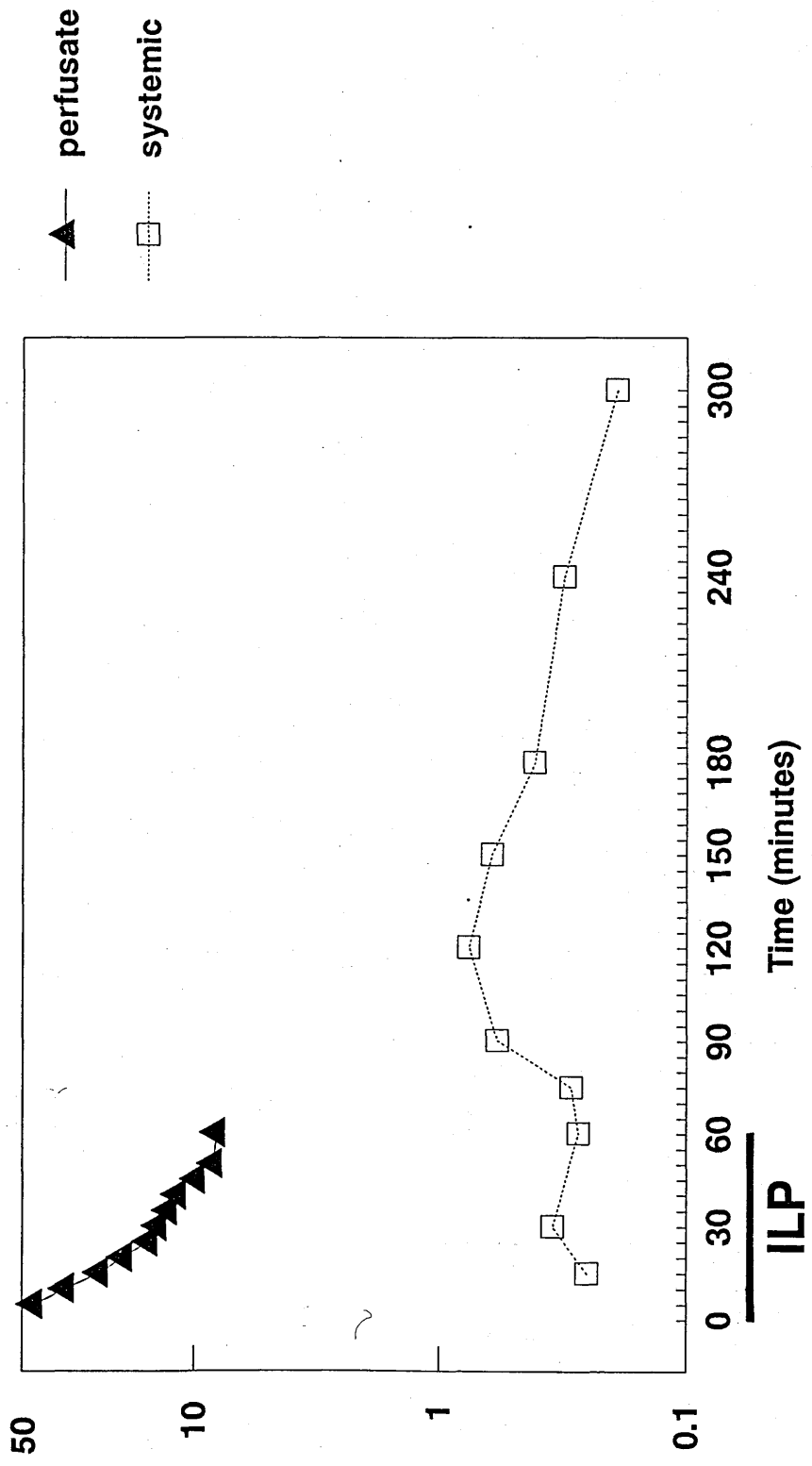


FIGURE 19 MELPHALAN PHARMACOKINETICS IN "MOCK" ILP
Best-fit curves for 37degC and 39degC (cf. Figs. 15 & 16)

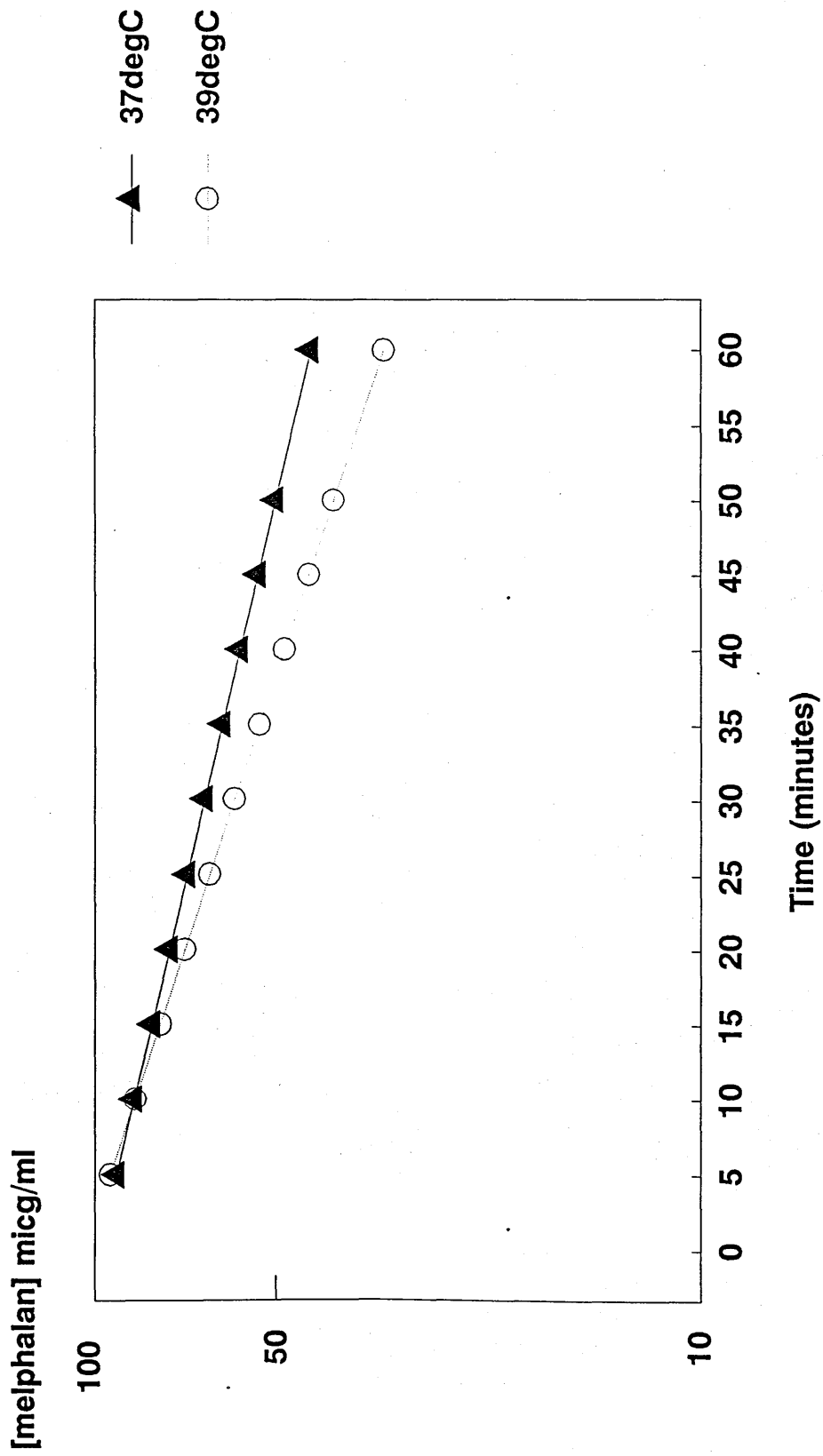


FIGURE 20 MELPHALAN PHARMACOKINETICS IN ILP
Group B vs. Group C (bolus vs. divided dose)

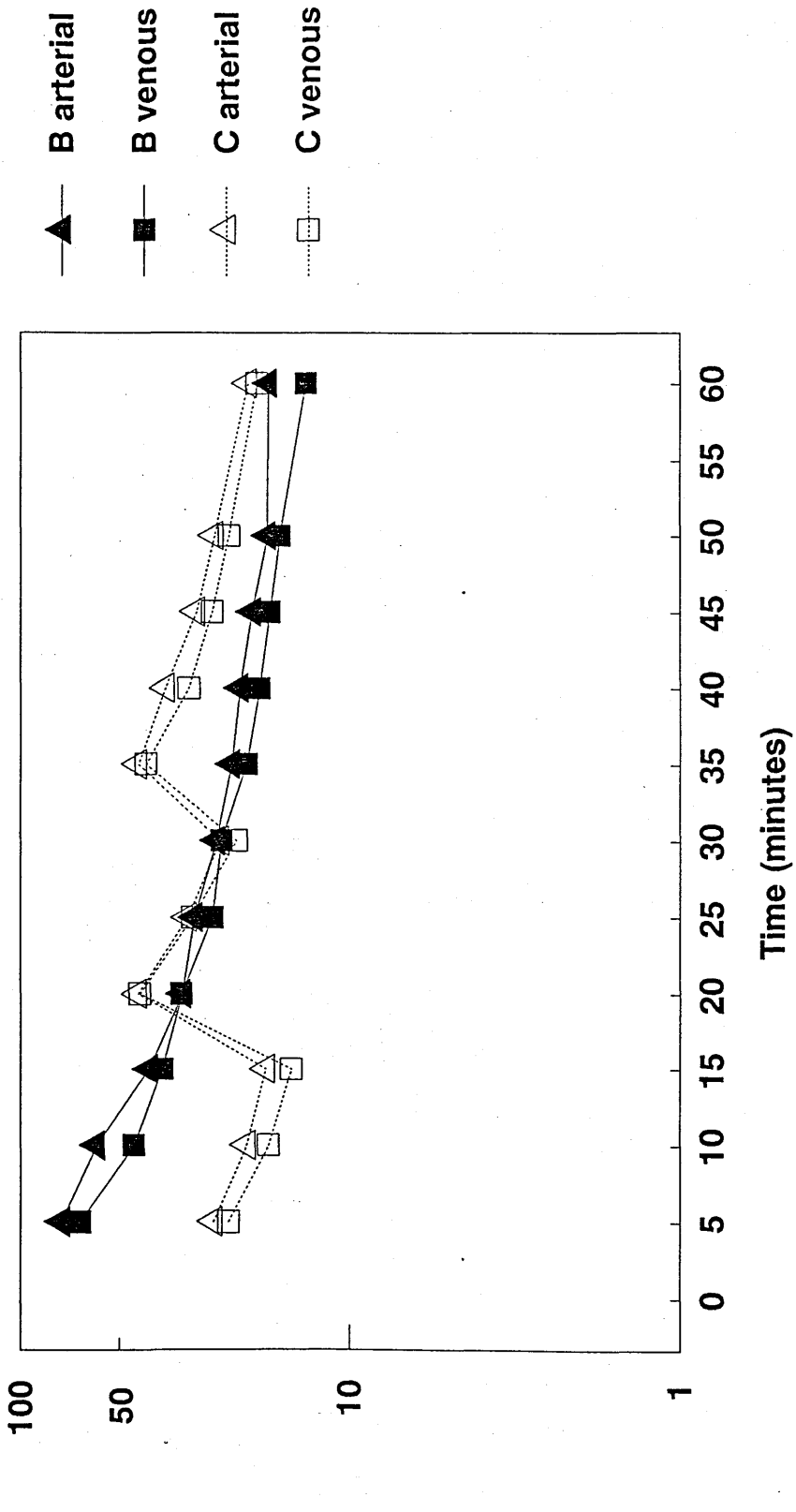


FIGURE 21 MELPHALAN DEGRADATION IN VITRO

Incubation in human plasma: semi-log plot

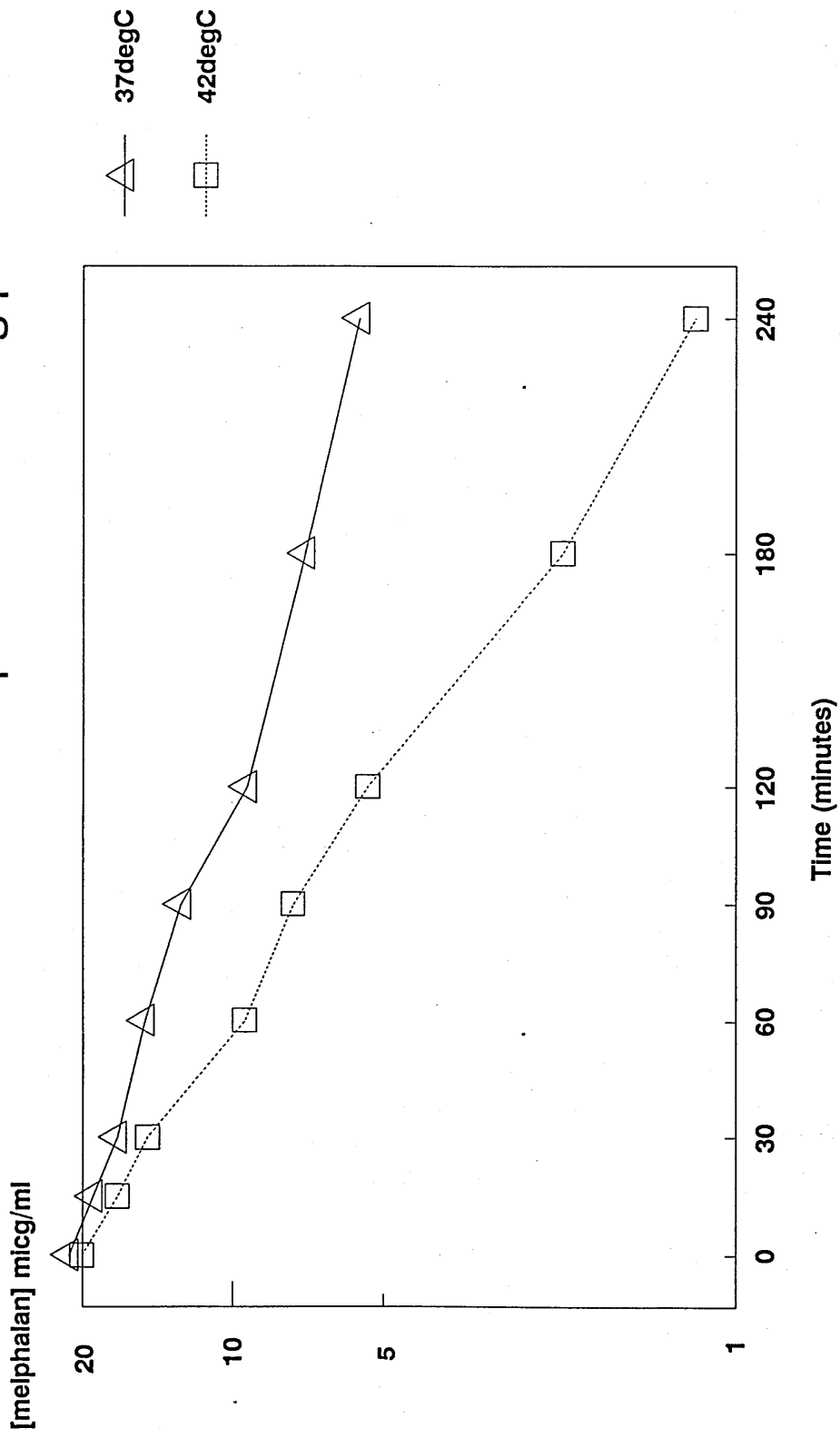
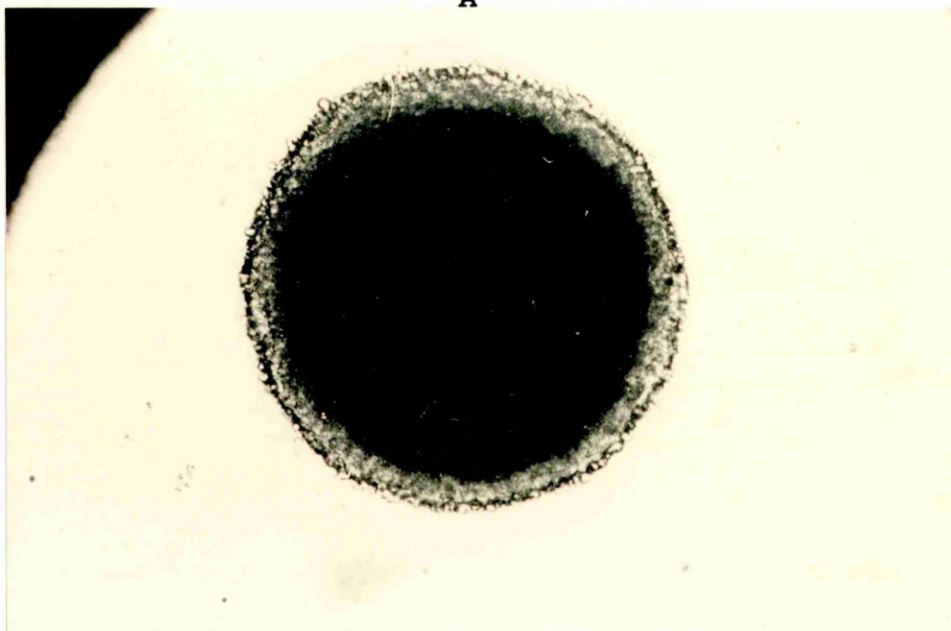




Figure 22

The right leg of this patient (T.T.) was treated according to the protocol of Group C in our pharmacokinetic studies (1.75mg/kg by divided dose). The painful limb is oedematous; the skin is erythematous with exfoliation and blistering over the dorsum of the foot (Wieberdink grade IV reaction).

A



B

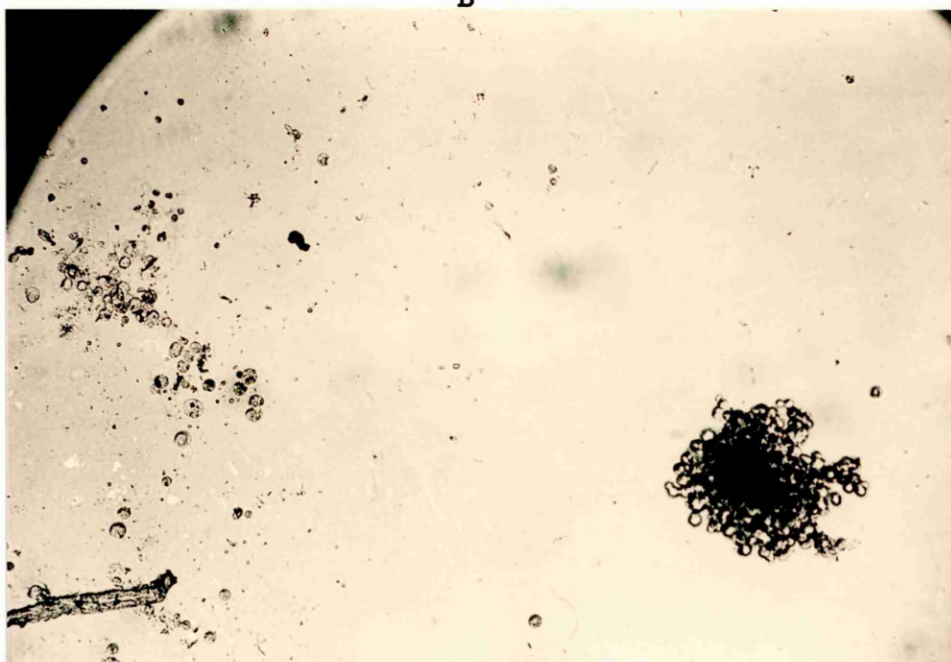


Figure 23

- A** Untreated control B0008 human melanoma spheroid regrowing normally at 11 days (800 μ m diameter)
- B** Treated B0008 spheroid which is just beginning to regrow at 11 days (250 μ m diameter)

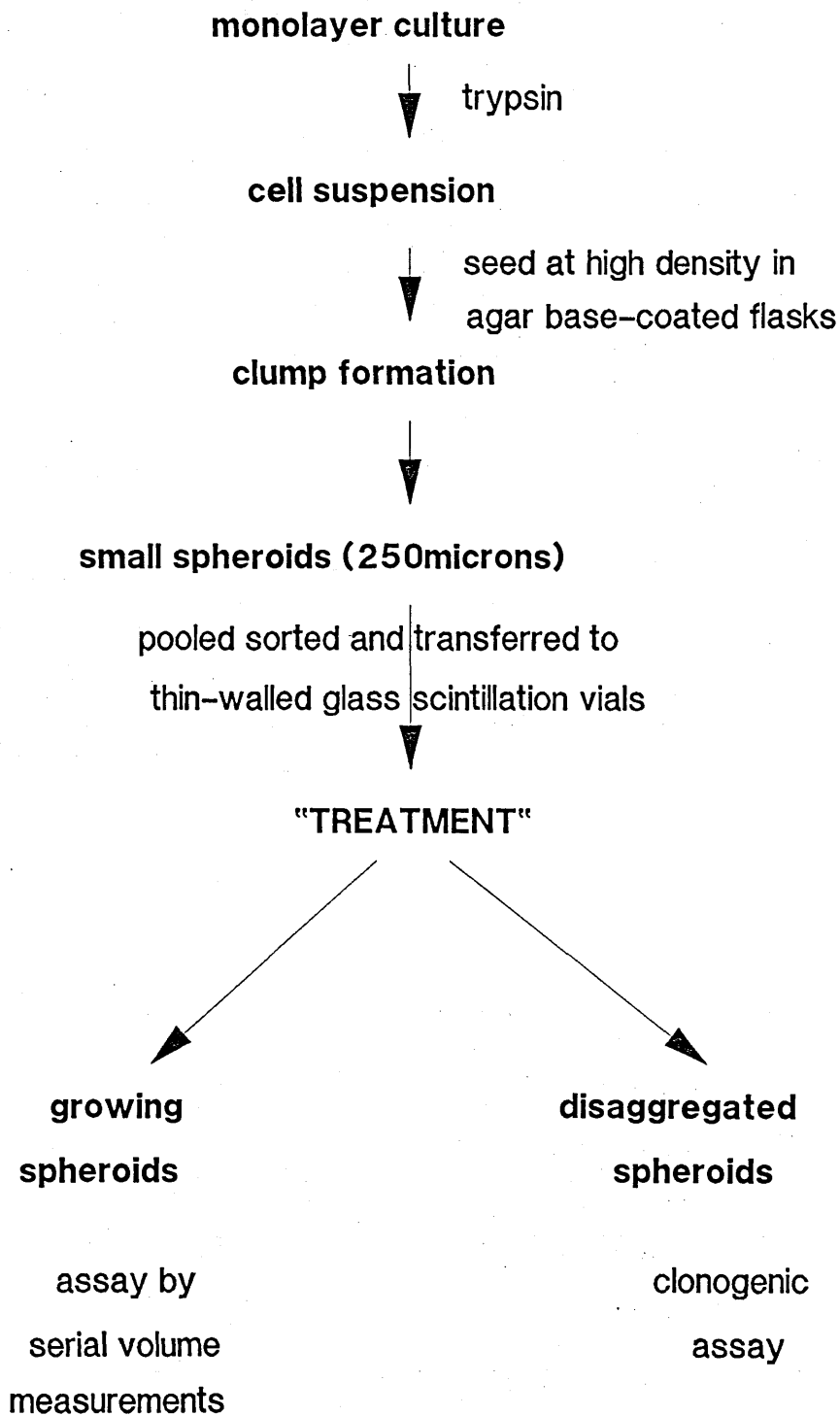


Figure 24 EXPERIMENTAL PROTOCOL

FIGURE 25 MELPHALAN DEGRADATION IN VITRO
Incubation in Eagle's Medium: semi-log plot

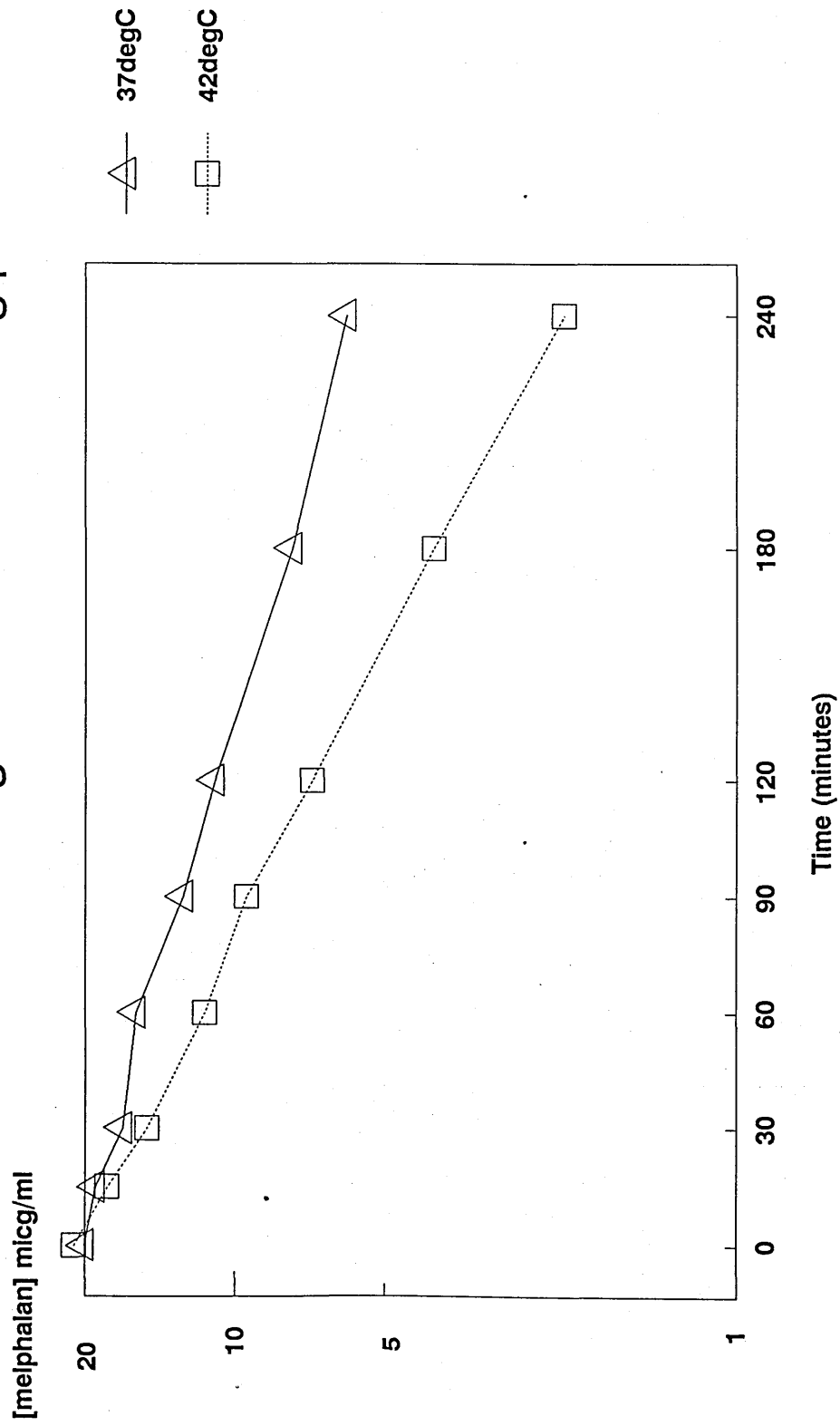


FIGURE 26 The effect of pH on melphalan degradation
Incubations in Eagle's MEM at 37degC

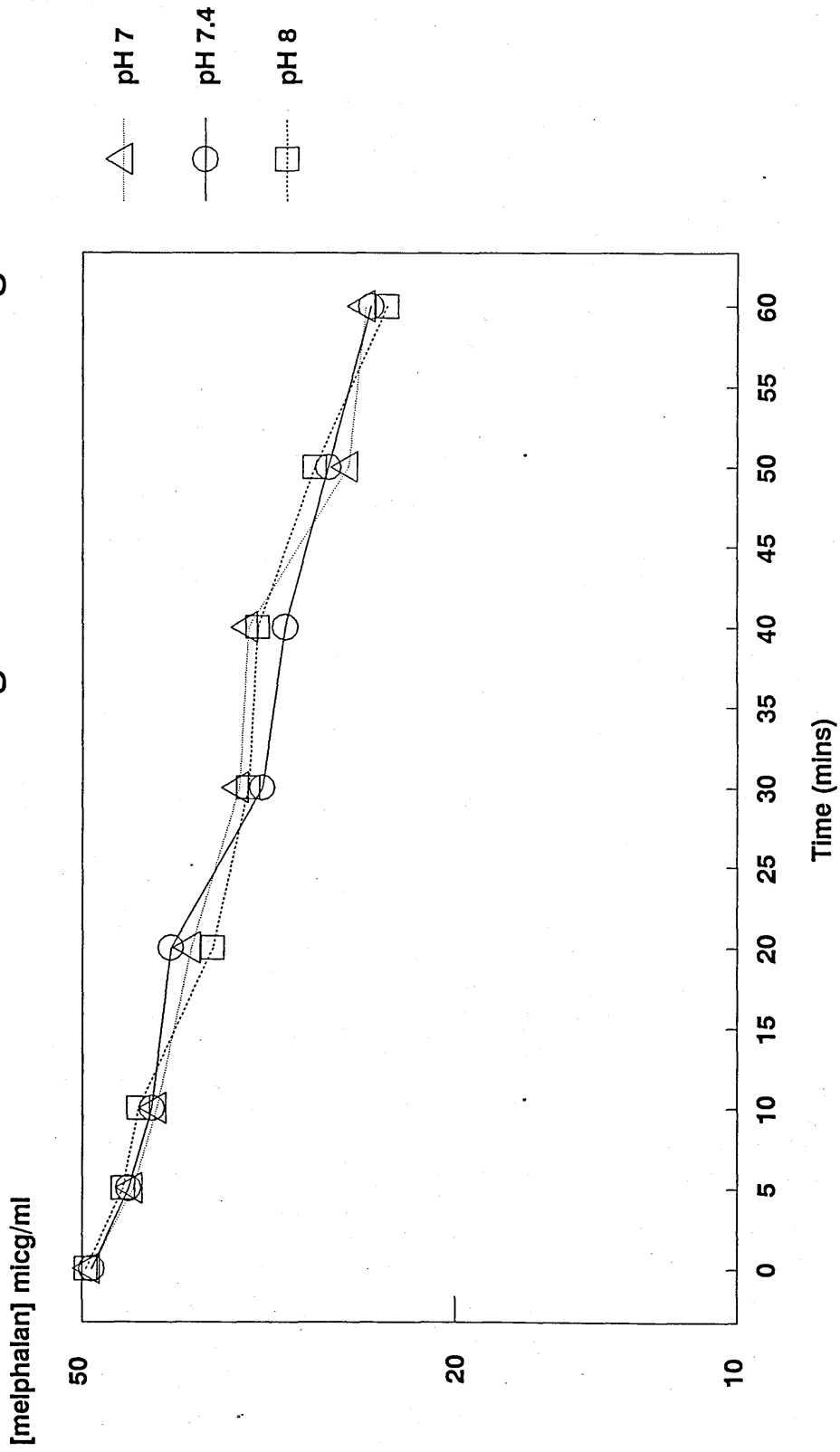


FIGURE 27 Derivation of $\log V_E$ from regrowth curve of treated spheroids which return to same growth pattern as controls

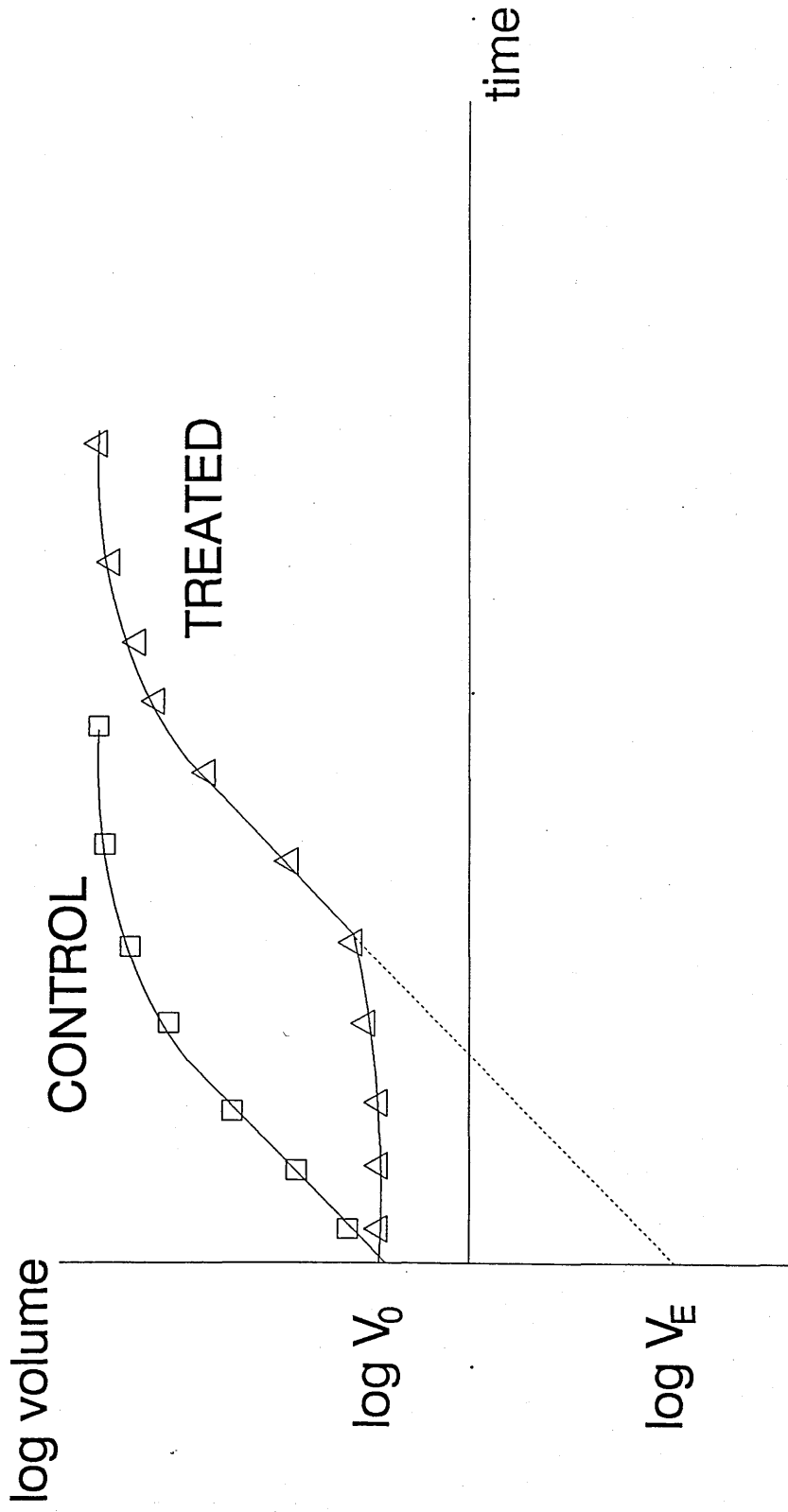


FIGURE 28A EXPERIMENT 1: Melphalan cytotoxicity to B0008 spheroids in MEM with solvent +/- diluent controls

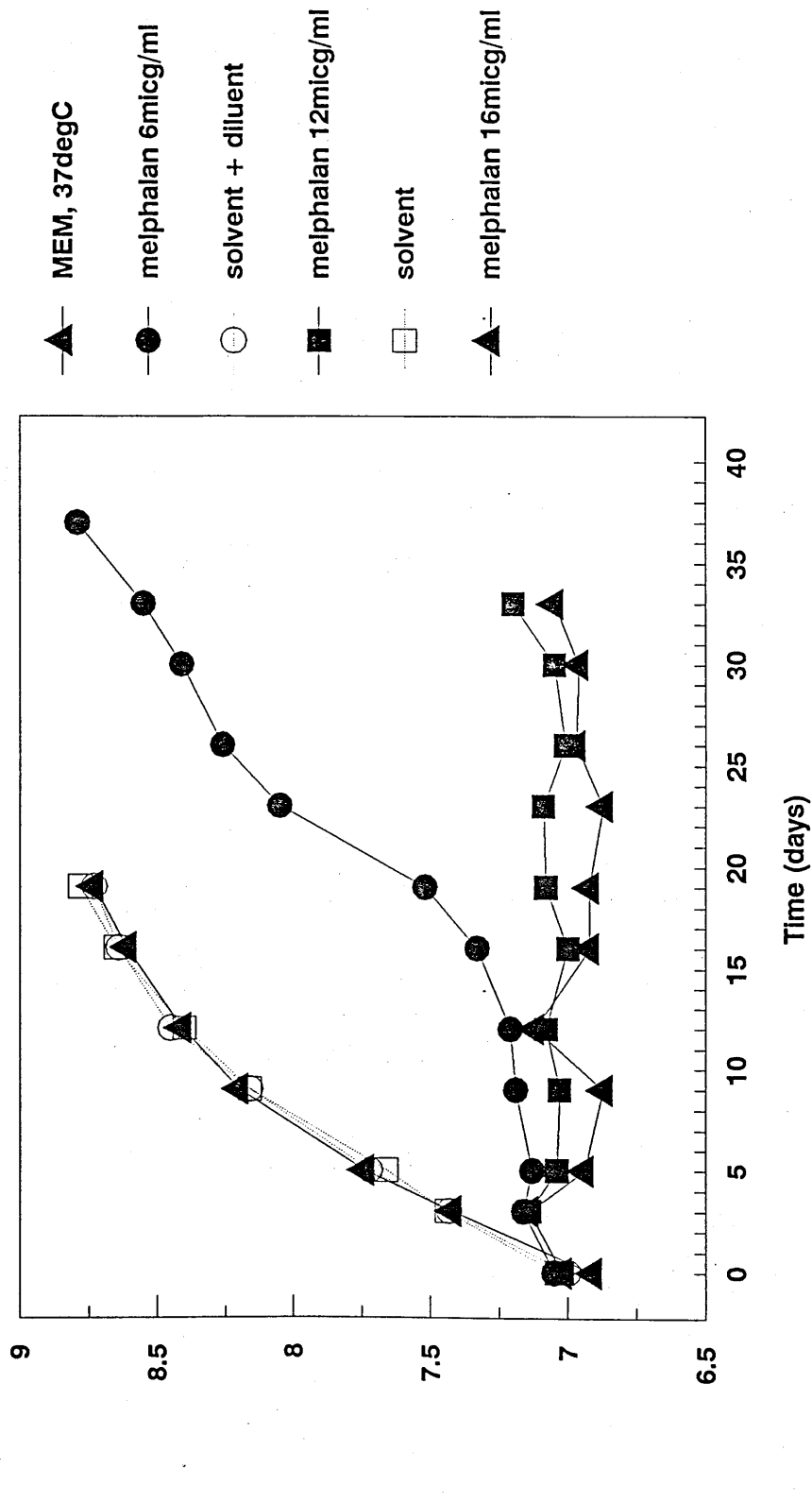


FIGURE 28B EXPERIMENT 1 B0008 human melanoma spheroids:
Regrowth delay after treatment with solvent/diluent +/- melphalan

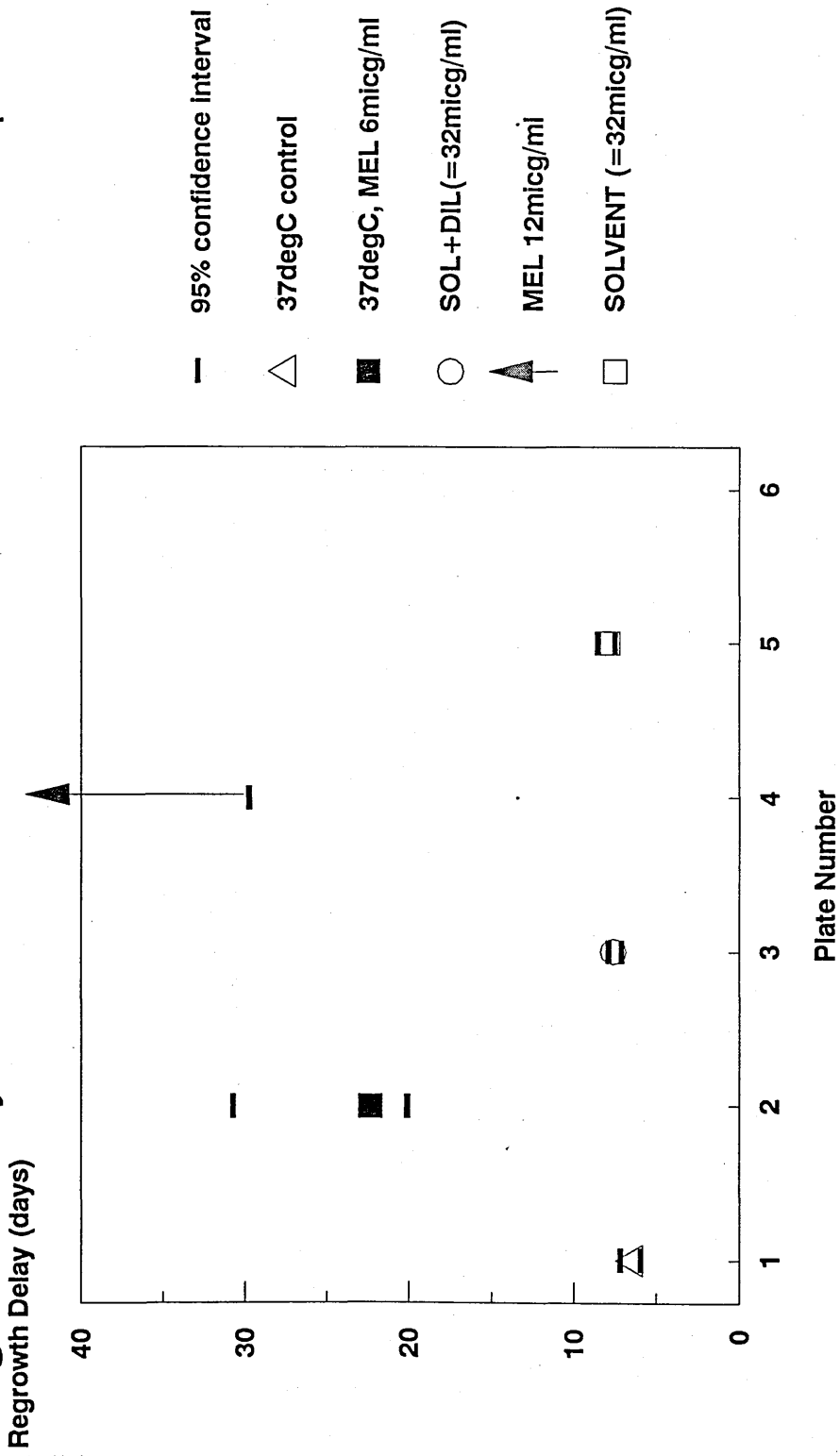


FIGURE 29A EXPERIMENT 2: Melphalan (mel) cytotoxicity to B0008 spheroids in MEM +/- amphotericin-B (FZ)

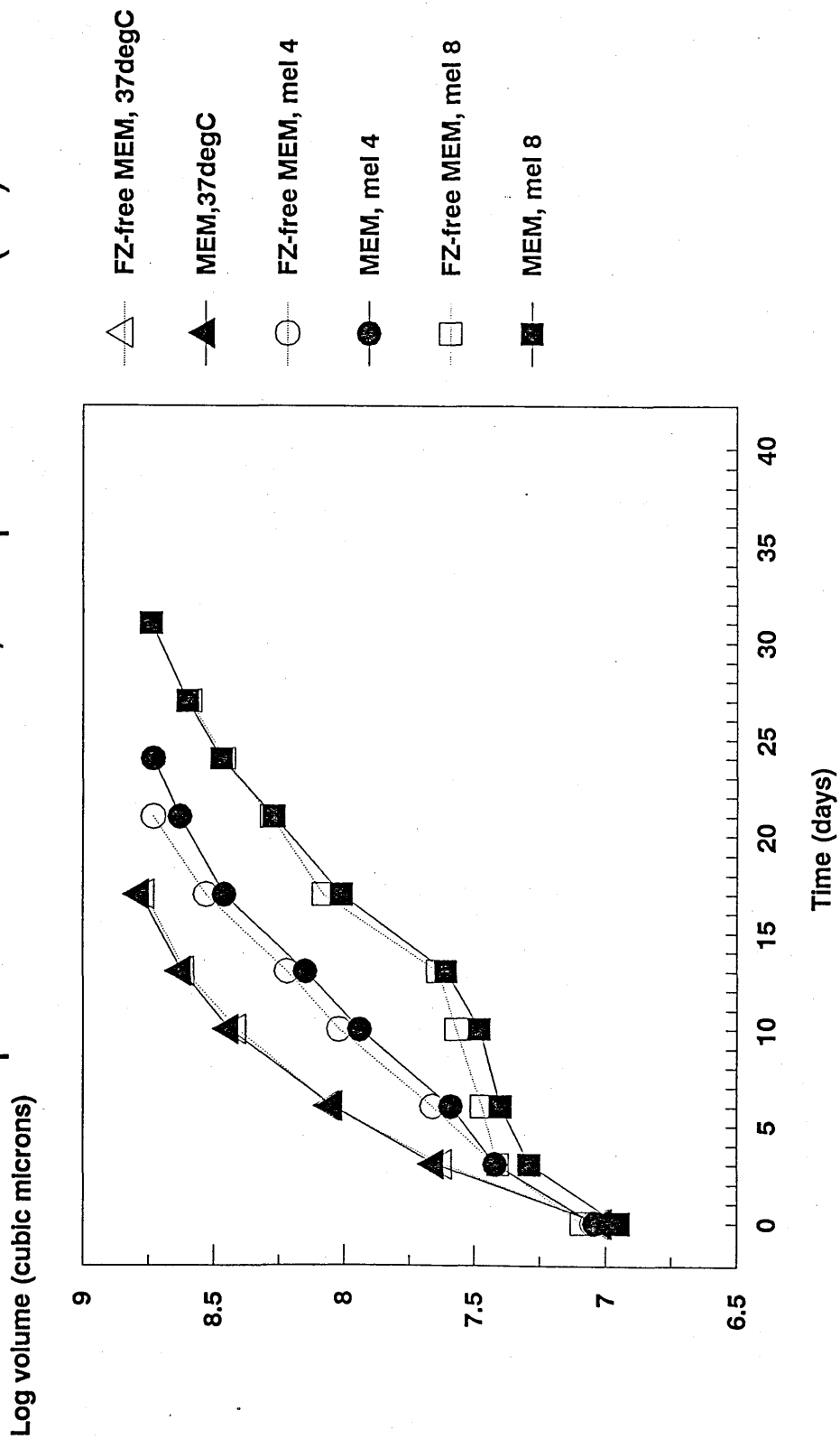


FIGURE 29B EXPERIMENT 2 Regrowth delay of B0008 spheroids treated with melphalan (MEL) in Eagle's MEM +/- amphotericin-B (FZ)

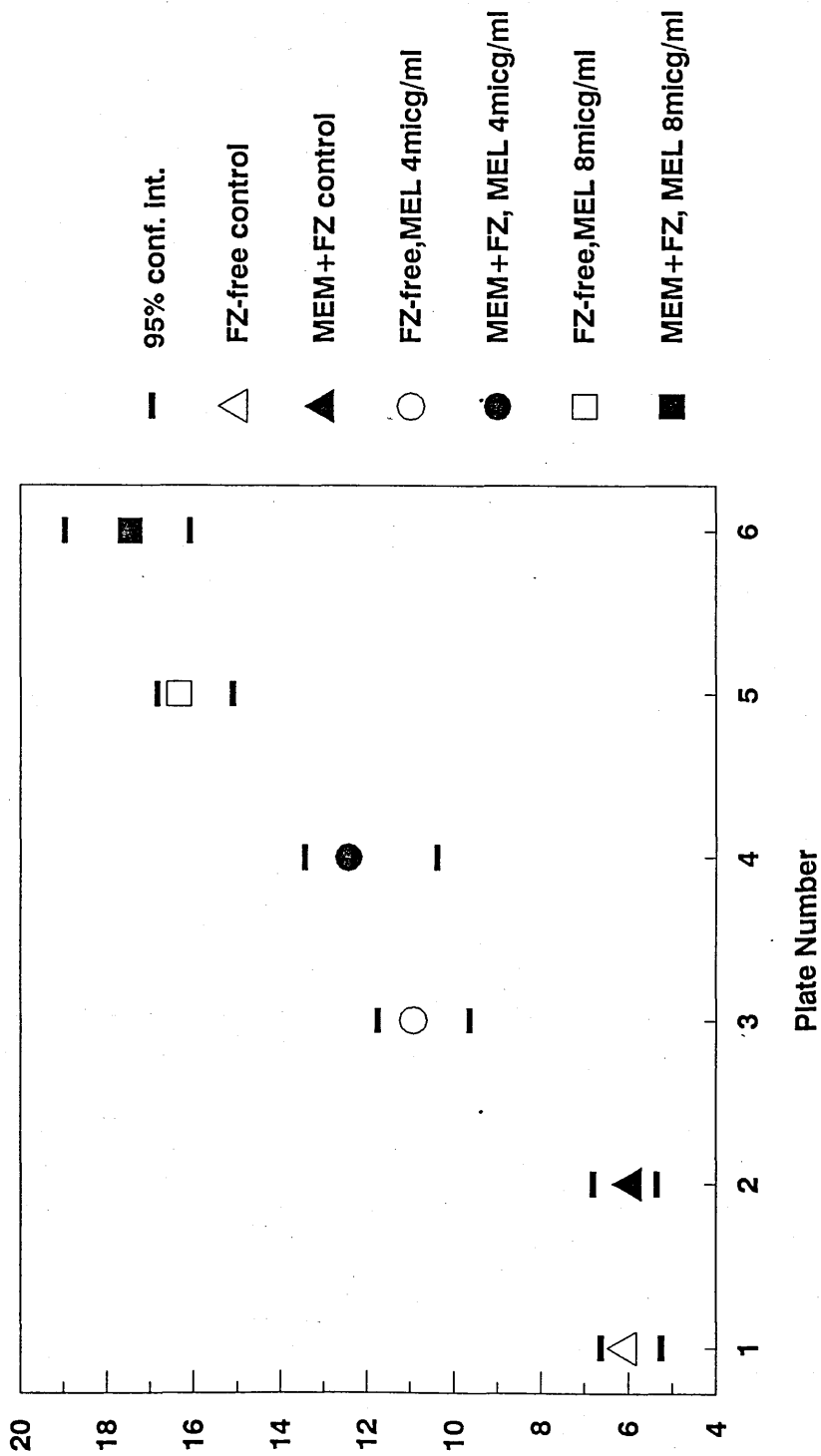


FIGURE 30A EXPERIMENT 3: Temperature, melphalan solvent (solv) and amphotericin-B (FZ); regrowth of B0008 spheroids

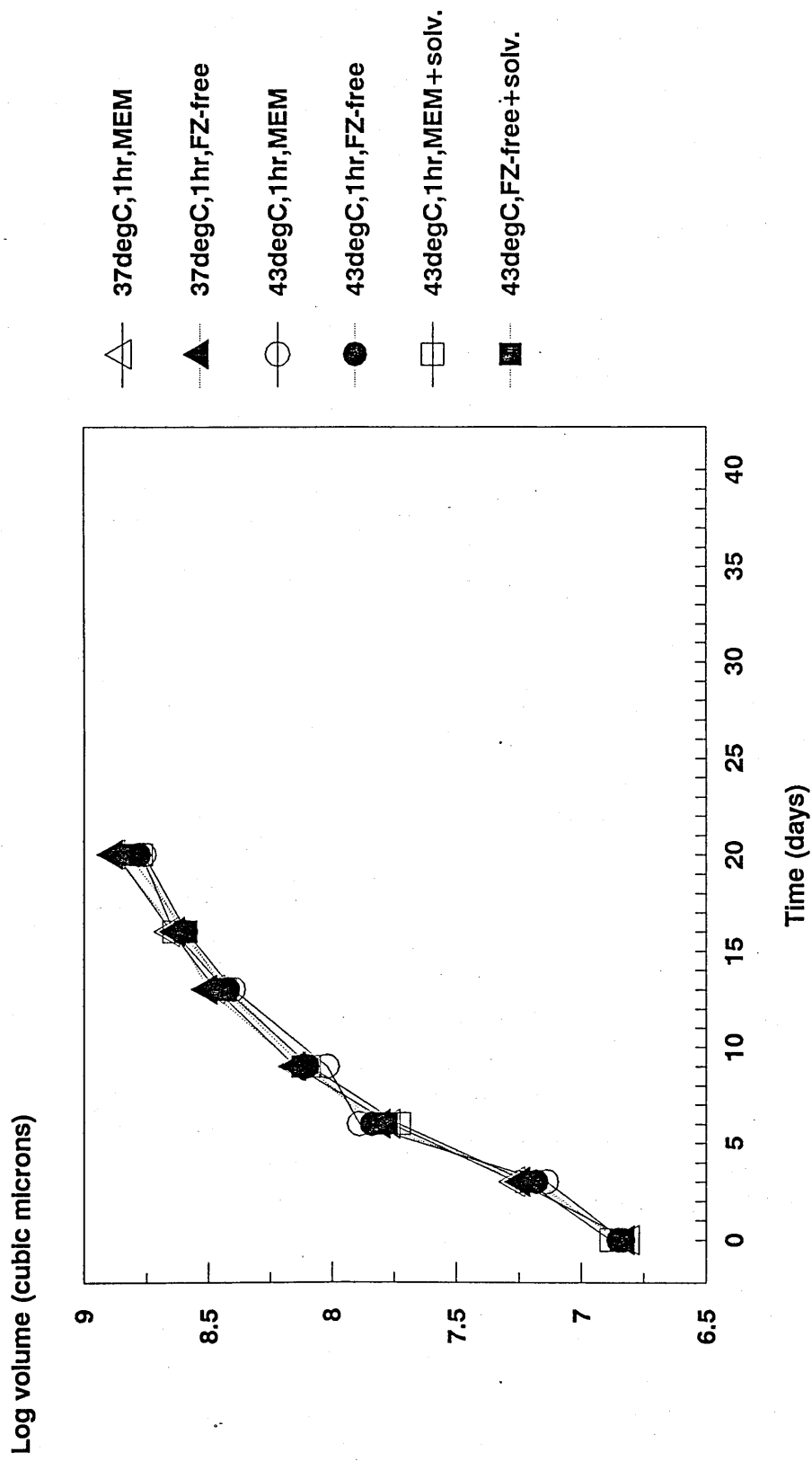


FIGURE 31 EXPERIMENT 4 Regrowth of B0010 spheroids after treatment with melphalan at 37degC

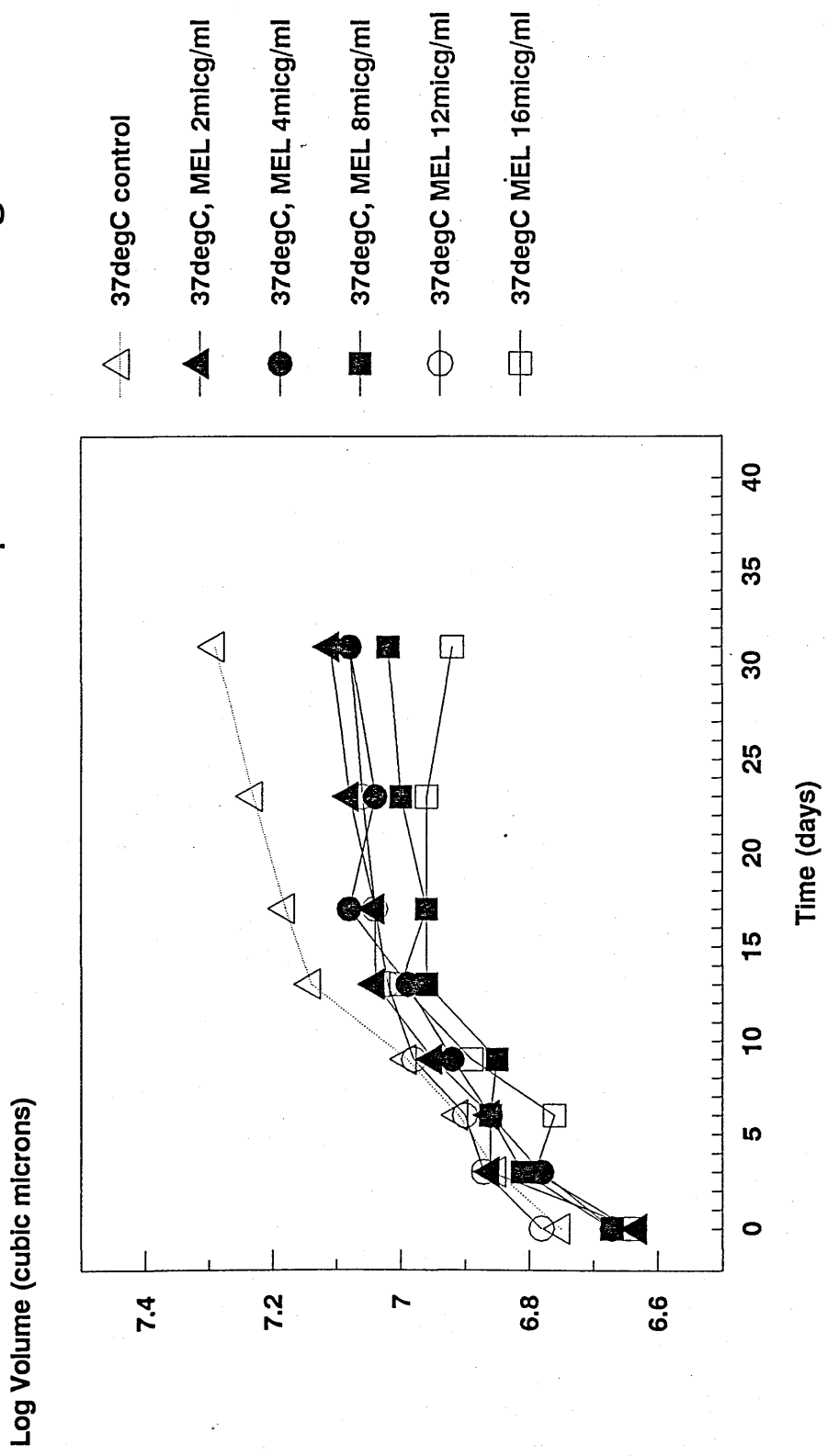


FIGURE 32A EXPERIMENT 5 Regrowth of MEL57 spheroids after treatment with melphalan at 37degC

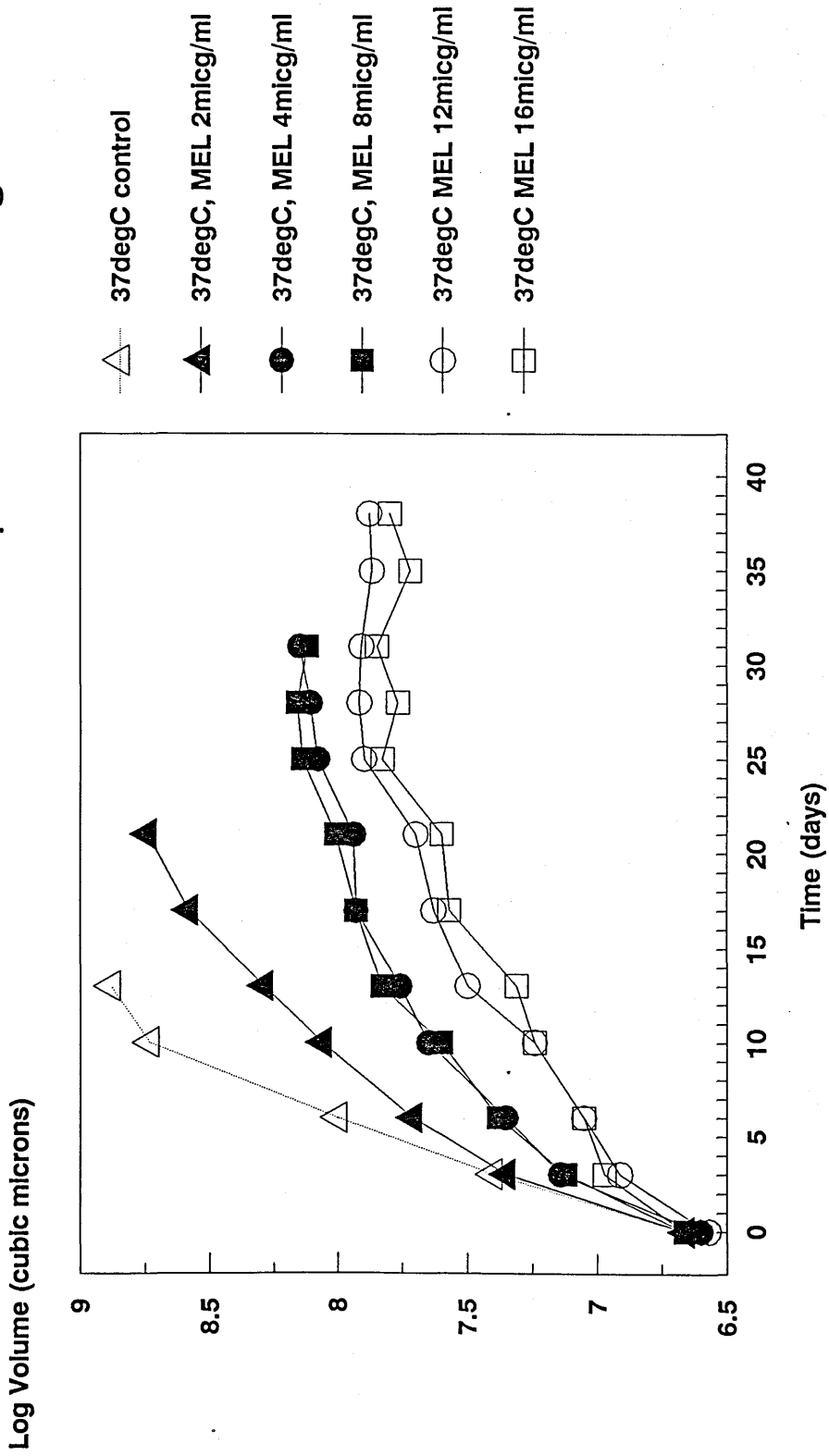


FIGURE 32B EXPERIMENT 5 Regrowth delay of MEL57 human melanoma spheroids after treatment with melphalan at 37degC

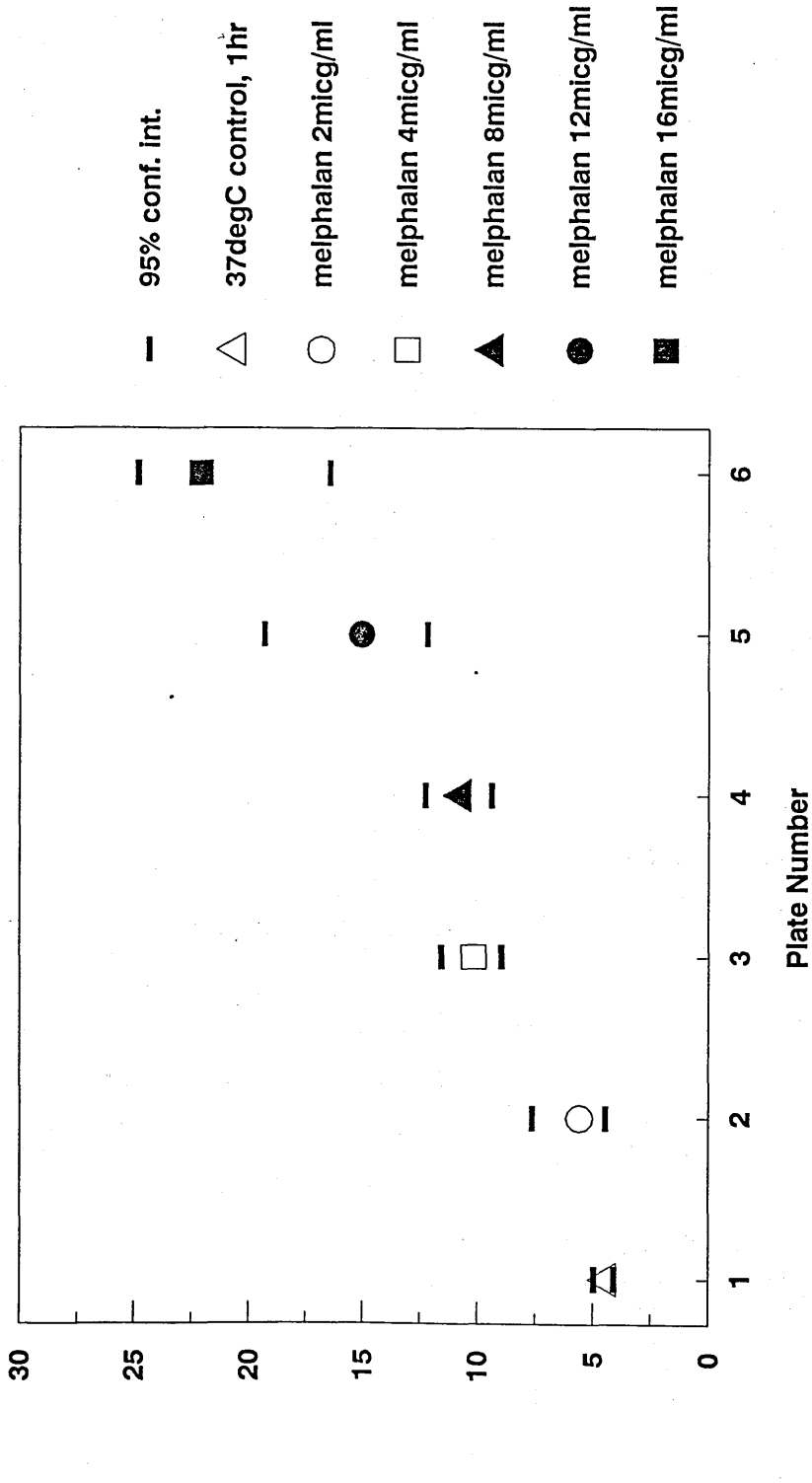


FIGURE 33A EXPERIMENT 6 Regrowth of B0010 spheroids
after treatment with melphalan at 41 degC

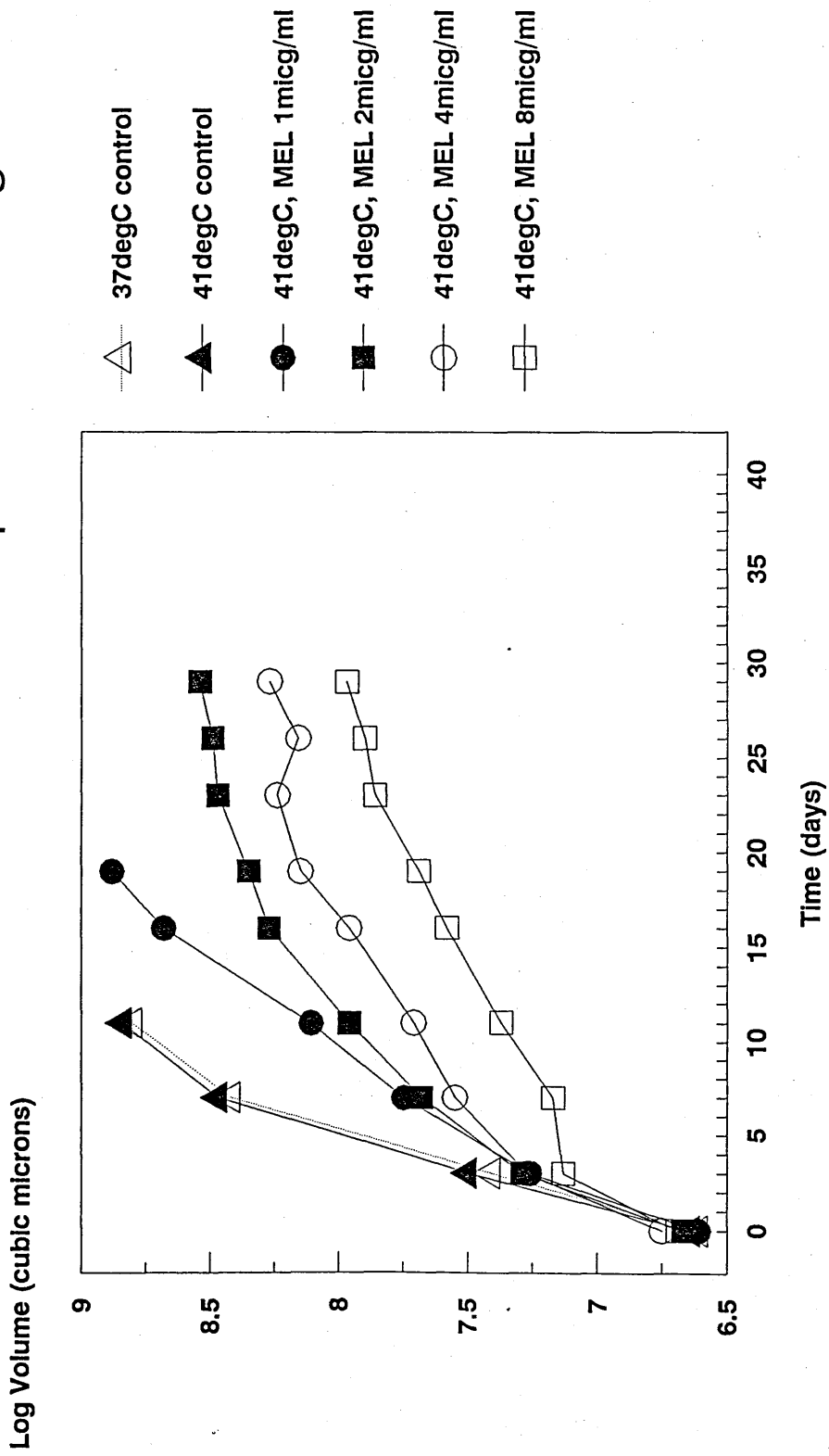


FIGURE 33B EXPERIMENT 6 Regrowth delay of MEL57 spheroids after treatment with melphalan at 41degC

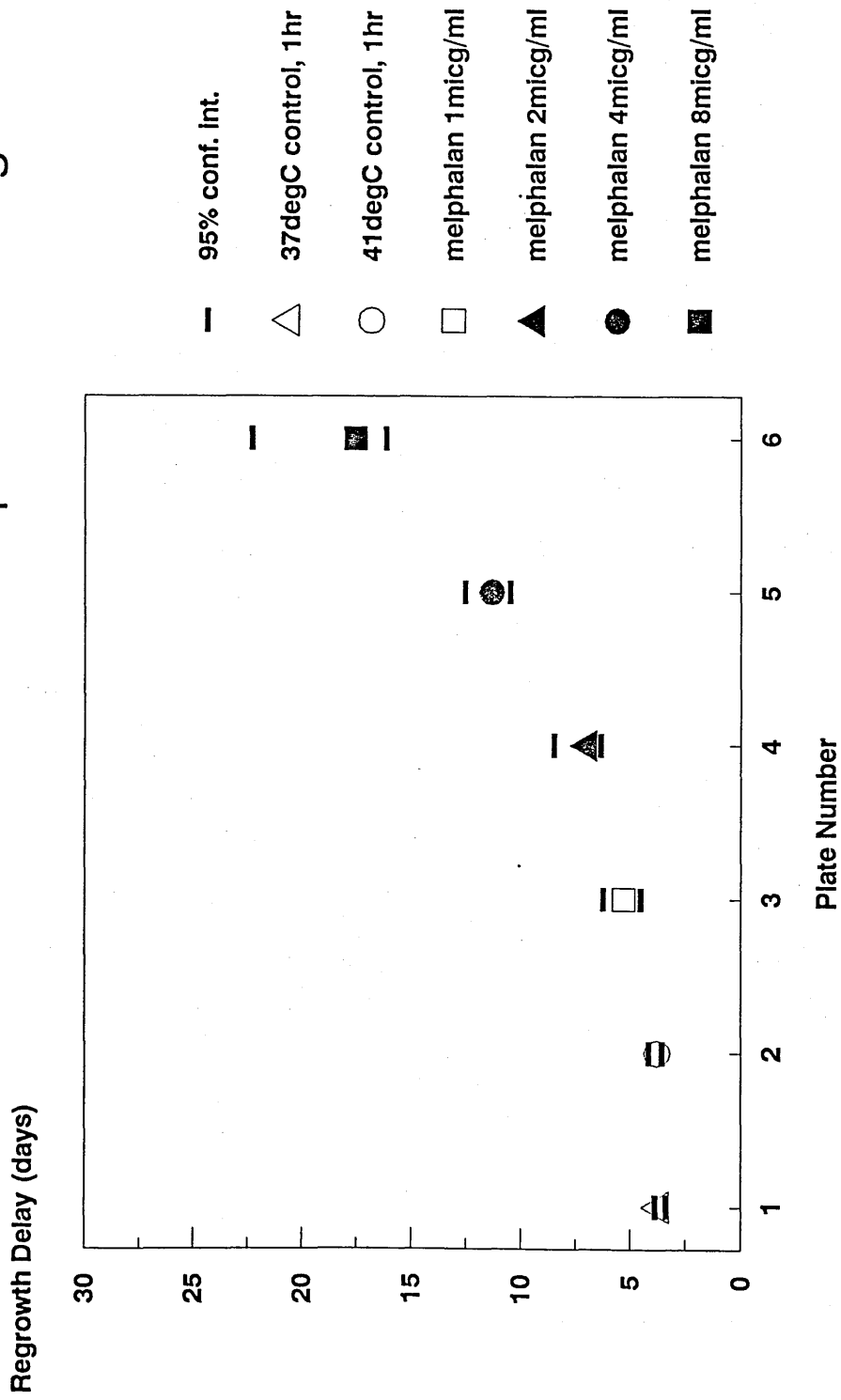


FIGURE 34A EXPERIMENT 7 Regrowth of B0008 spheroids
after treatment with melphalan at 31degC

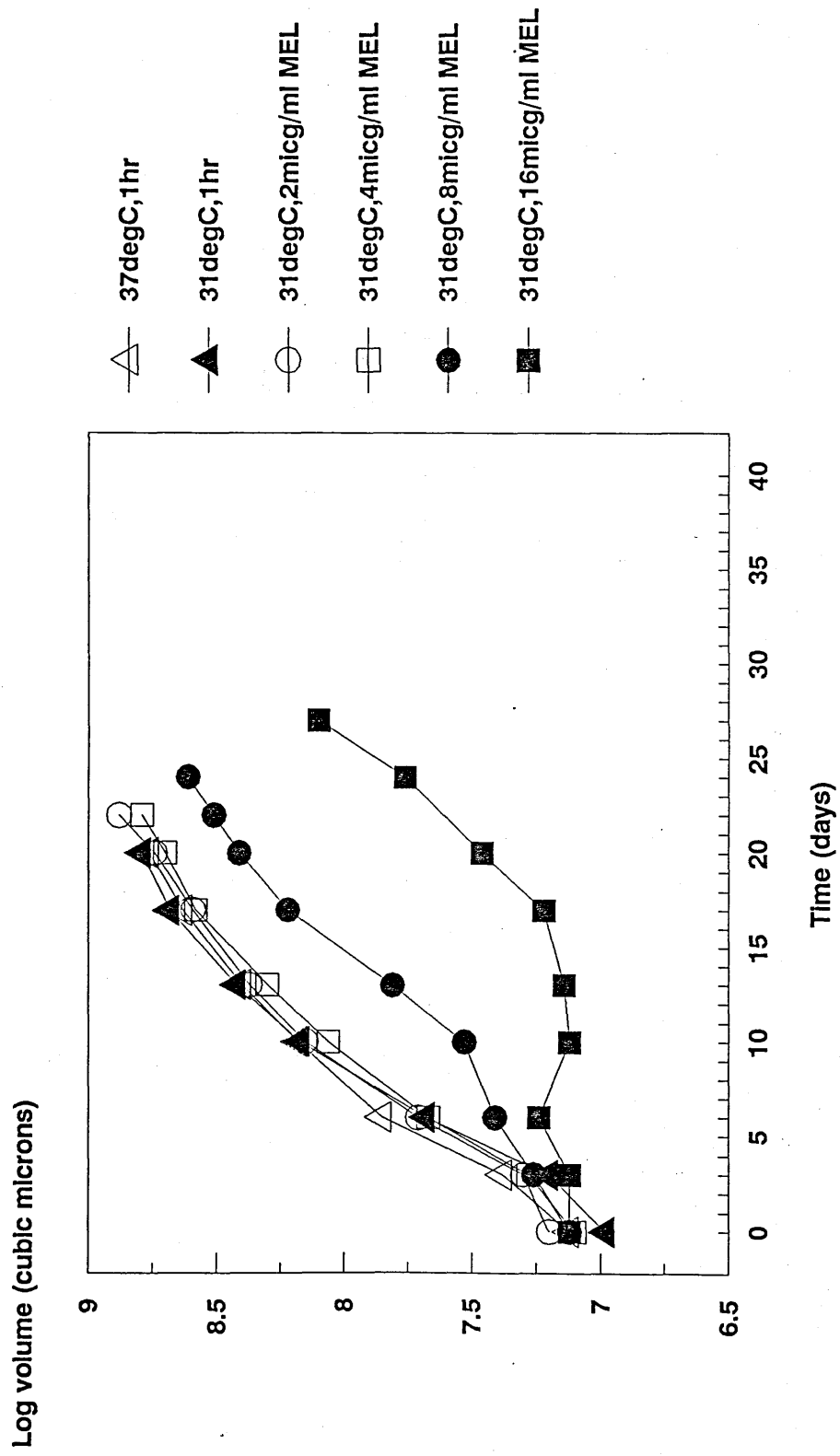


FIGURE 34B EXPERIMENT 7 Regrowth delay of B0008 spheroids after treatment with melphalan at 31degC

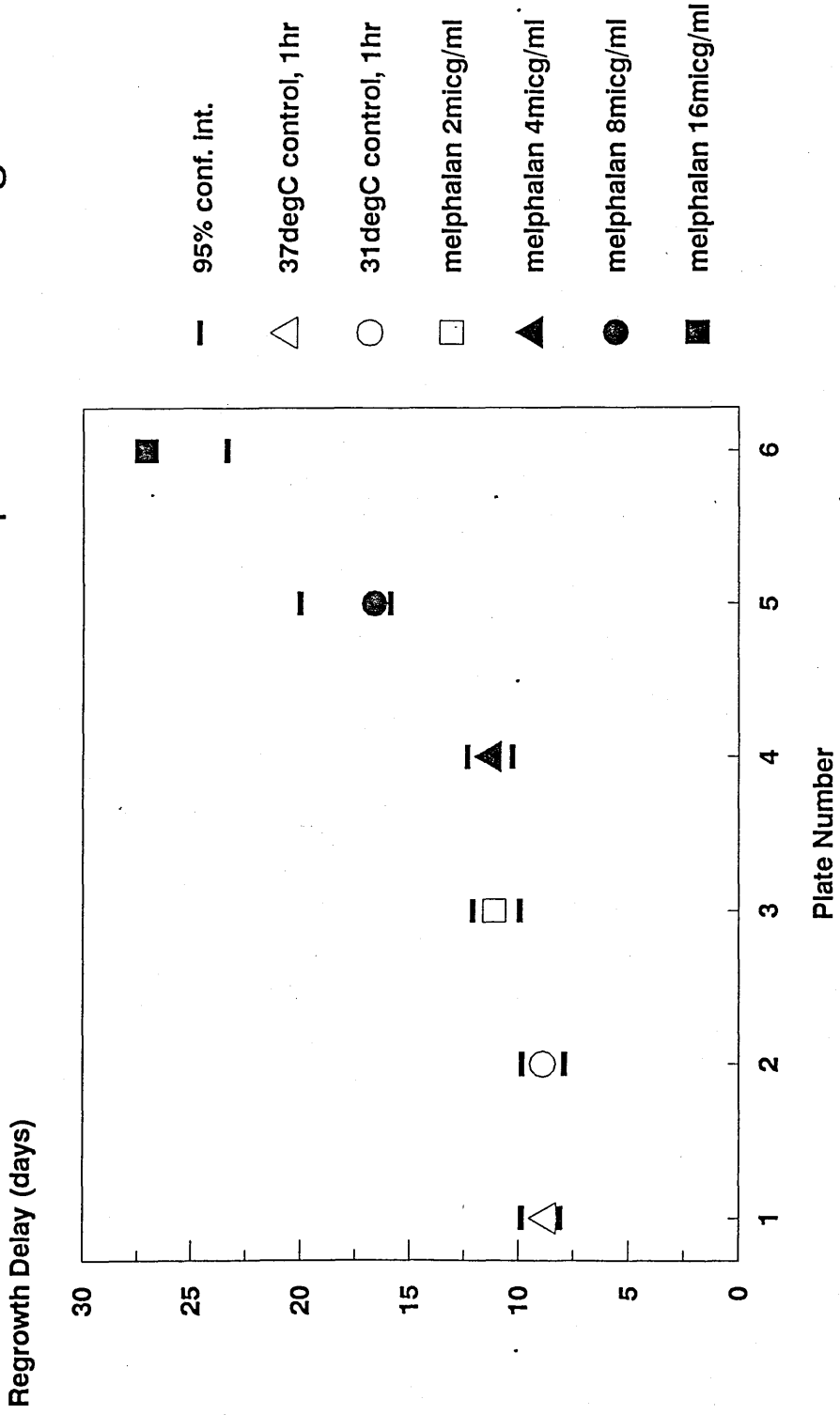


FIGURE 35A EXPERIMENT 8 Regrowth of B0008 spheroids after treatment with melphalan at 31degC

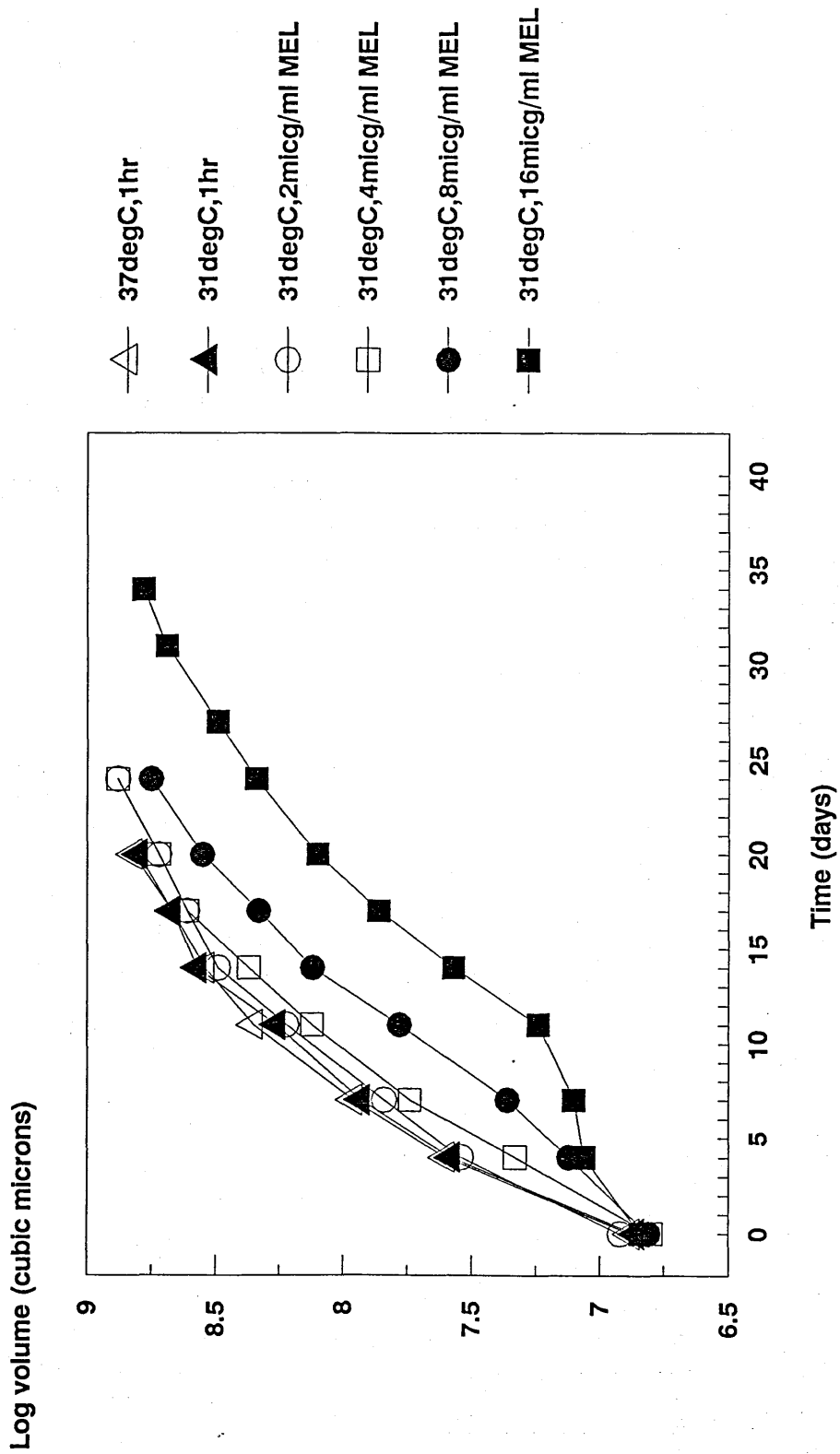


FIGURE 35B EXPERIMENT 8 Regrowth delay of B0008 spheroids after treatment with melphalan at 31degC

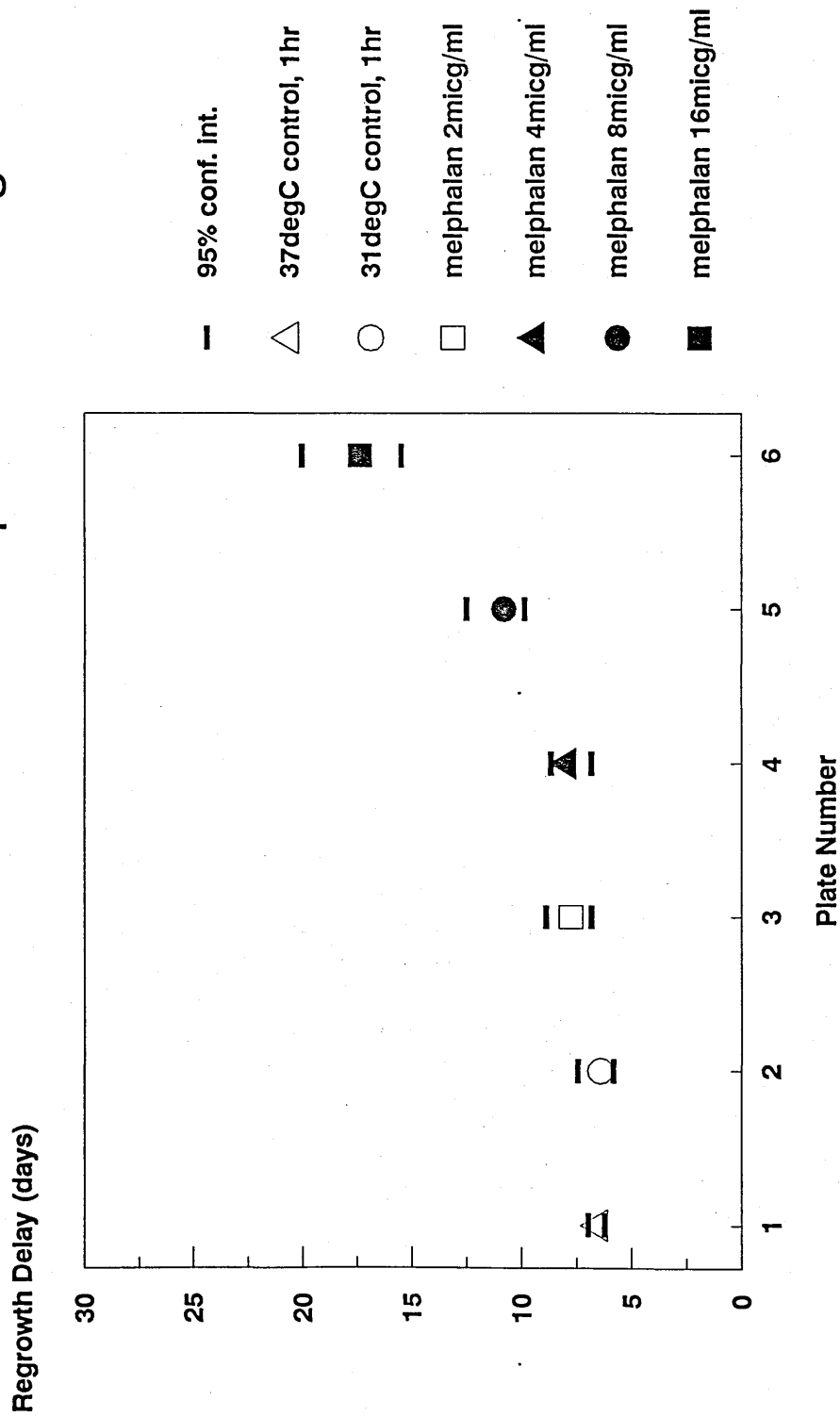


FIGURE 36A EXPERIMENT 9 Regrowth of B0008 spheroids
after treatment with melphalan at 35degC

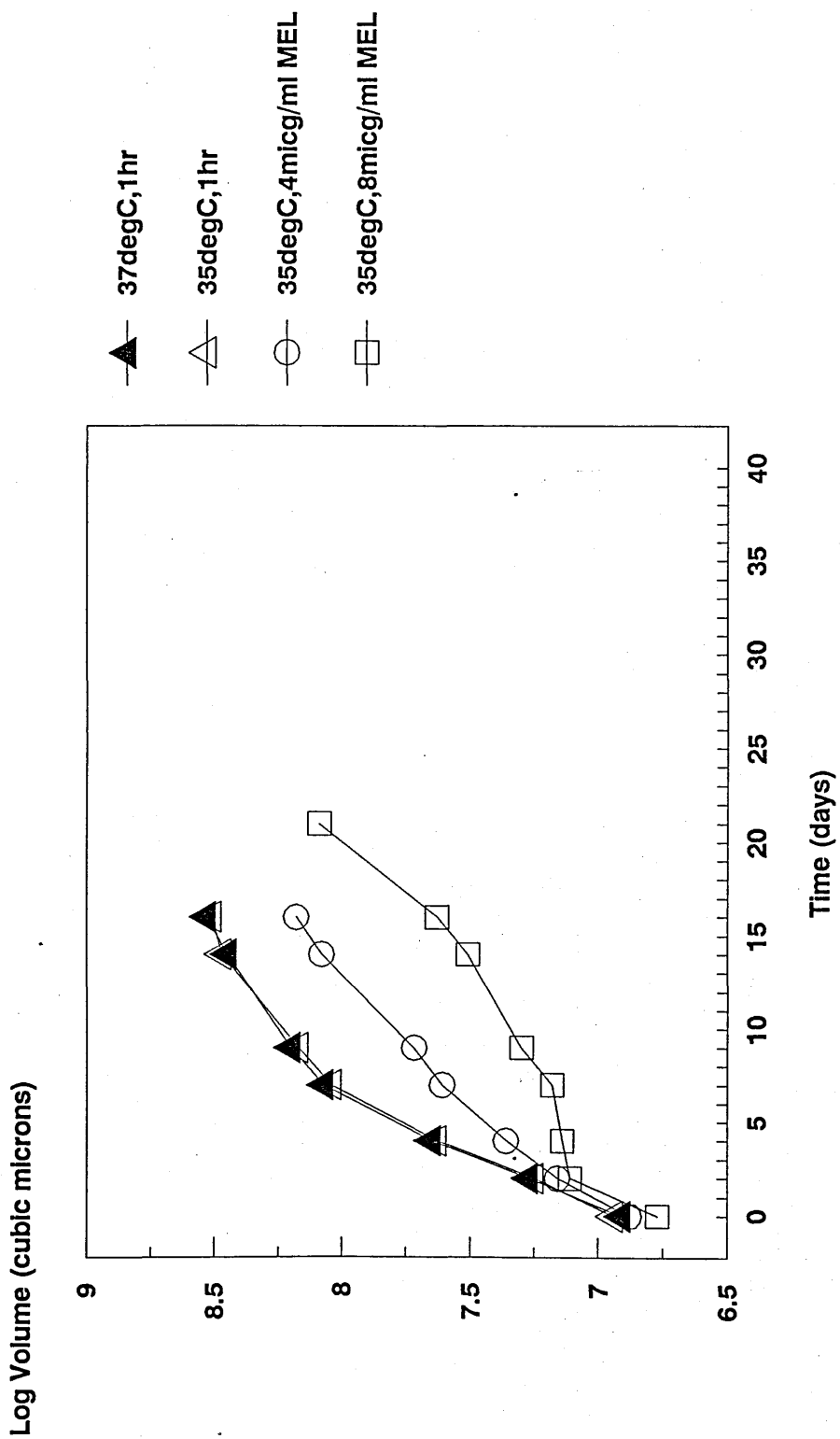


FIGURE 36B EXPERIMENT 9 Regrowth delay of B0008 spheroids after treatment with melphalan at 35degC

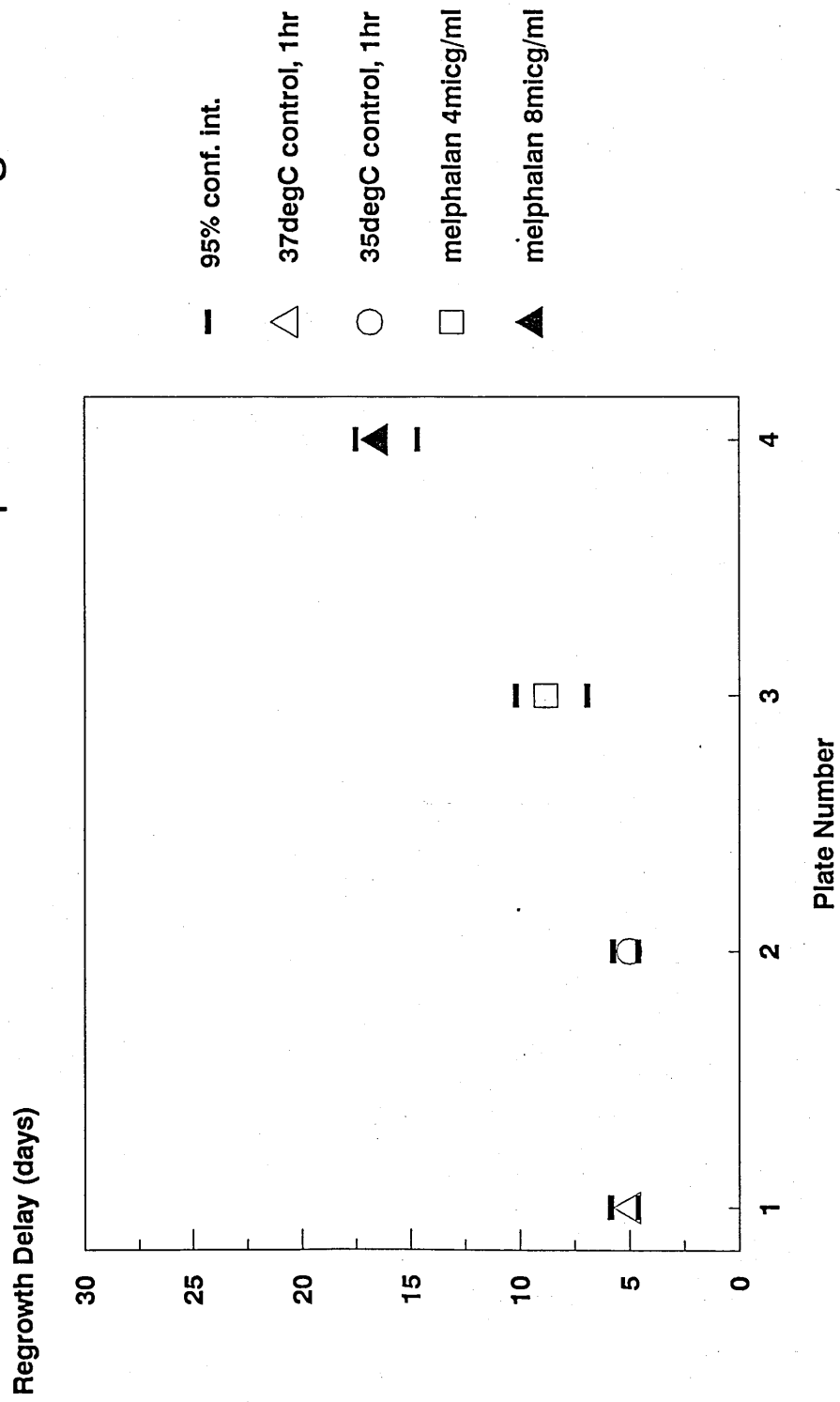


FIGURE 37A EXPERIMENT 10 Regrowth of B0008 spheroids
after treatment with melphalan at 37degC

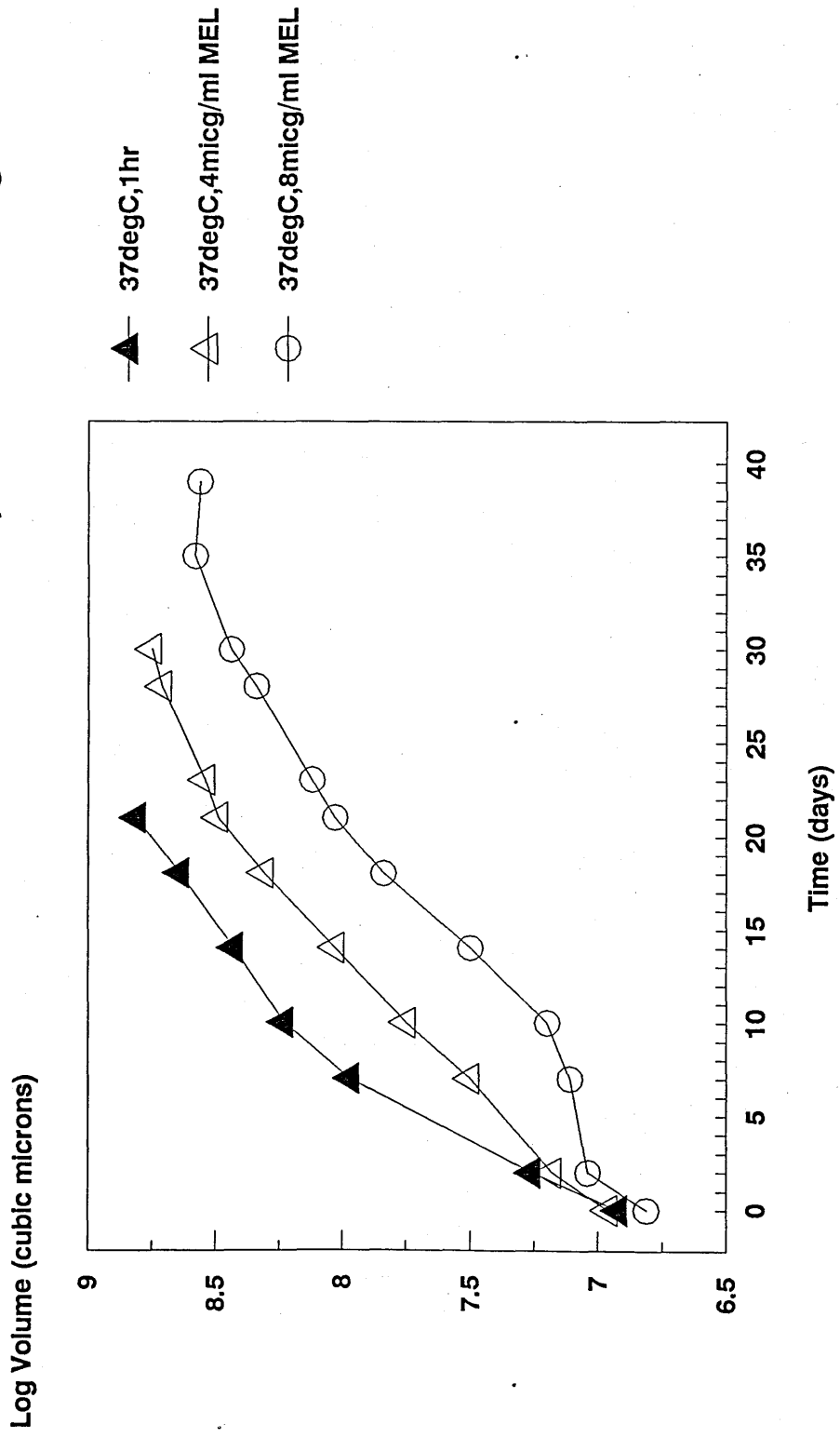


FIGURE 37B EXPERIMENT 10 Regrowth delay of B0008 spheroids after treatment with melphalan at 37degC

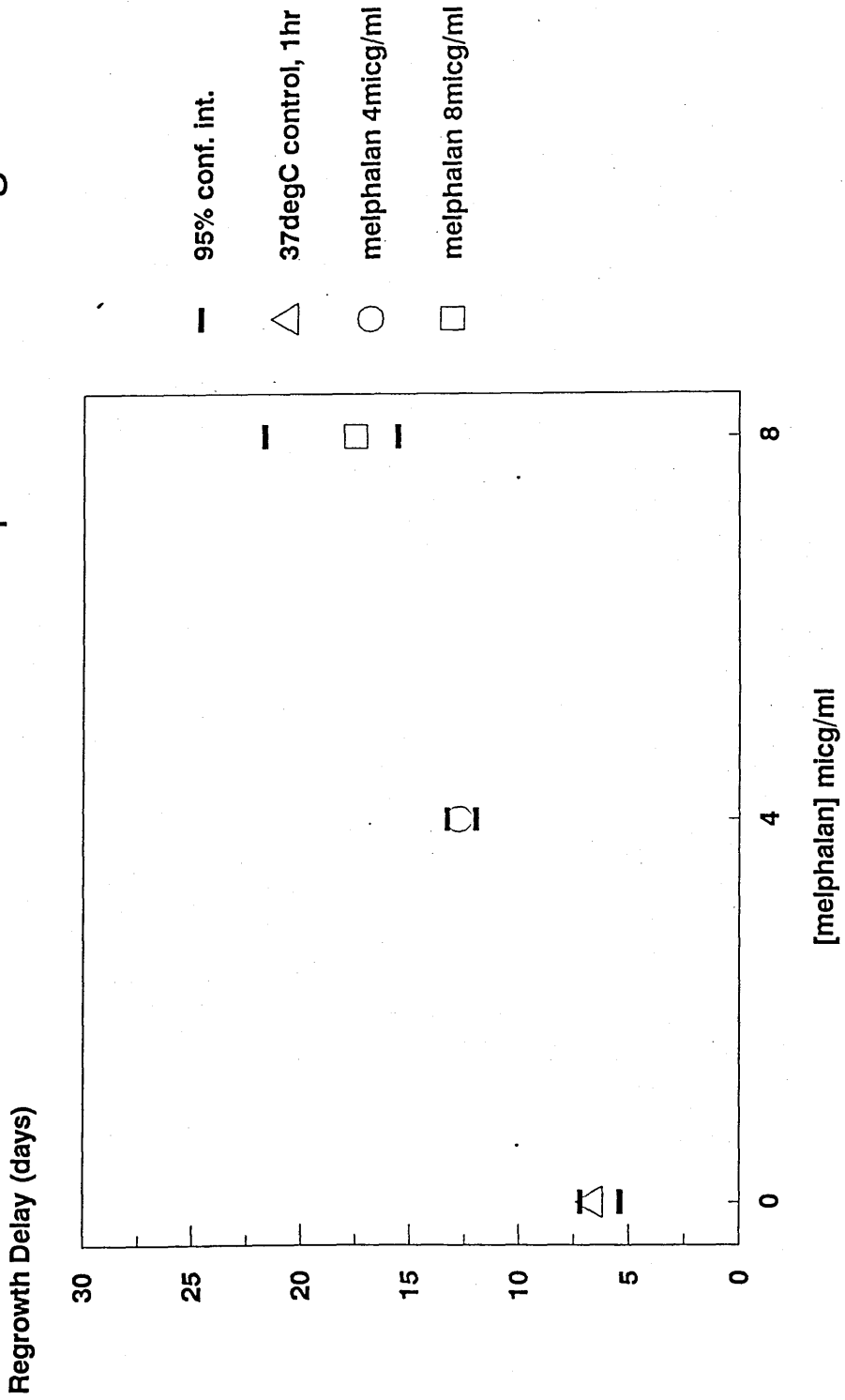


FIGURE 38A EXPERIMENT 11 Regrowth of B0008 spheroids after treatment with melphalan at 37degC

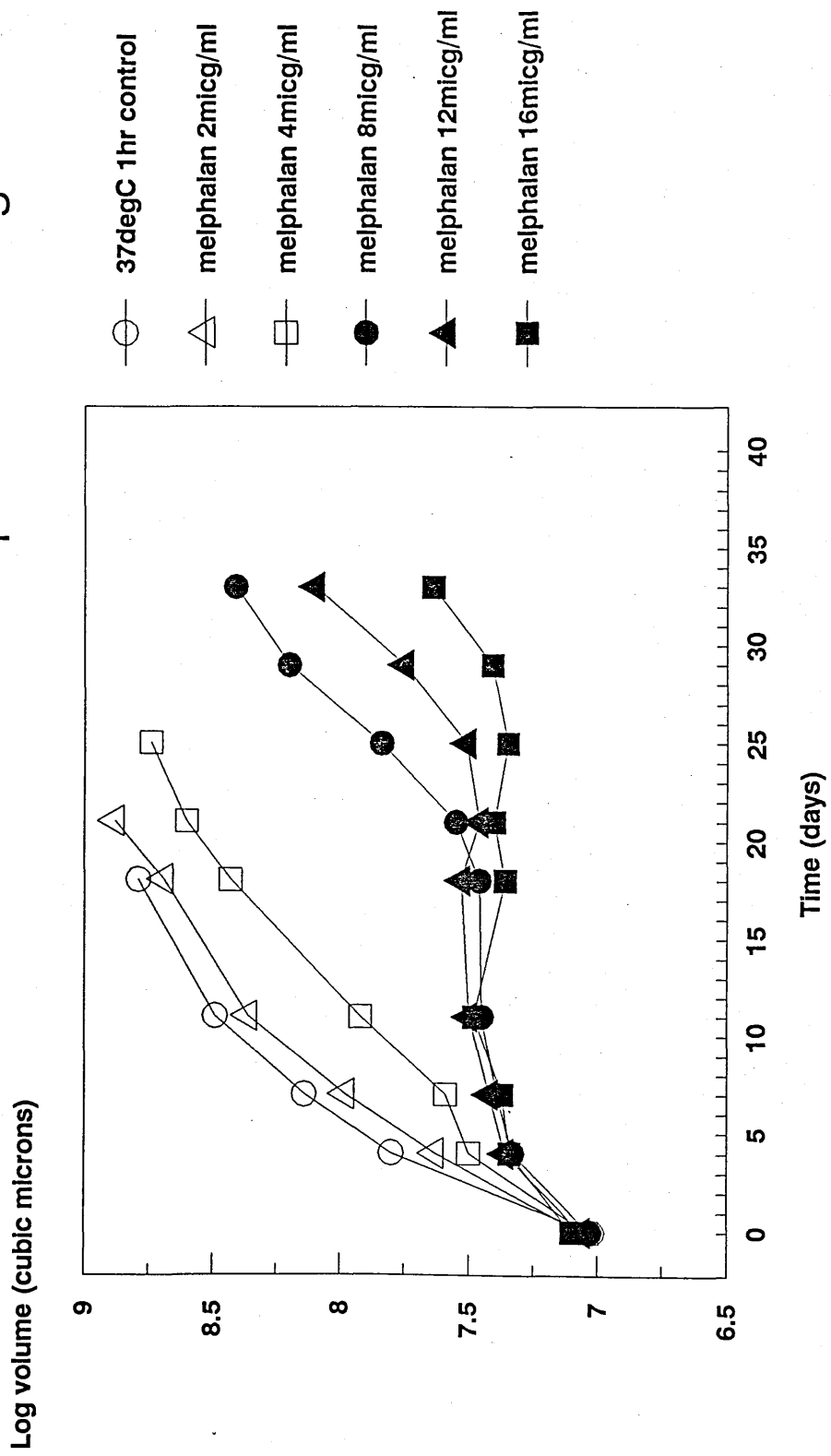


FIGURE 38B EXPERIMENT 11 Regrowth delay of B0008 spheroids after treatment with melphalan at 37degC

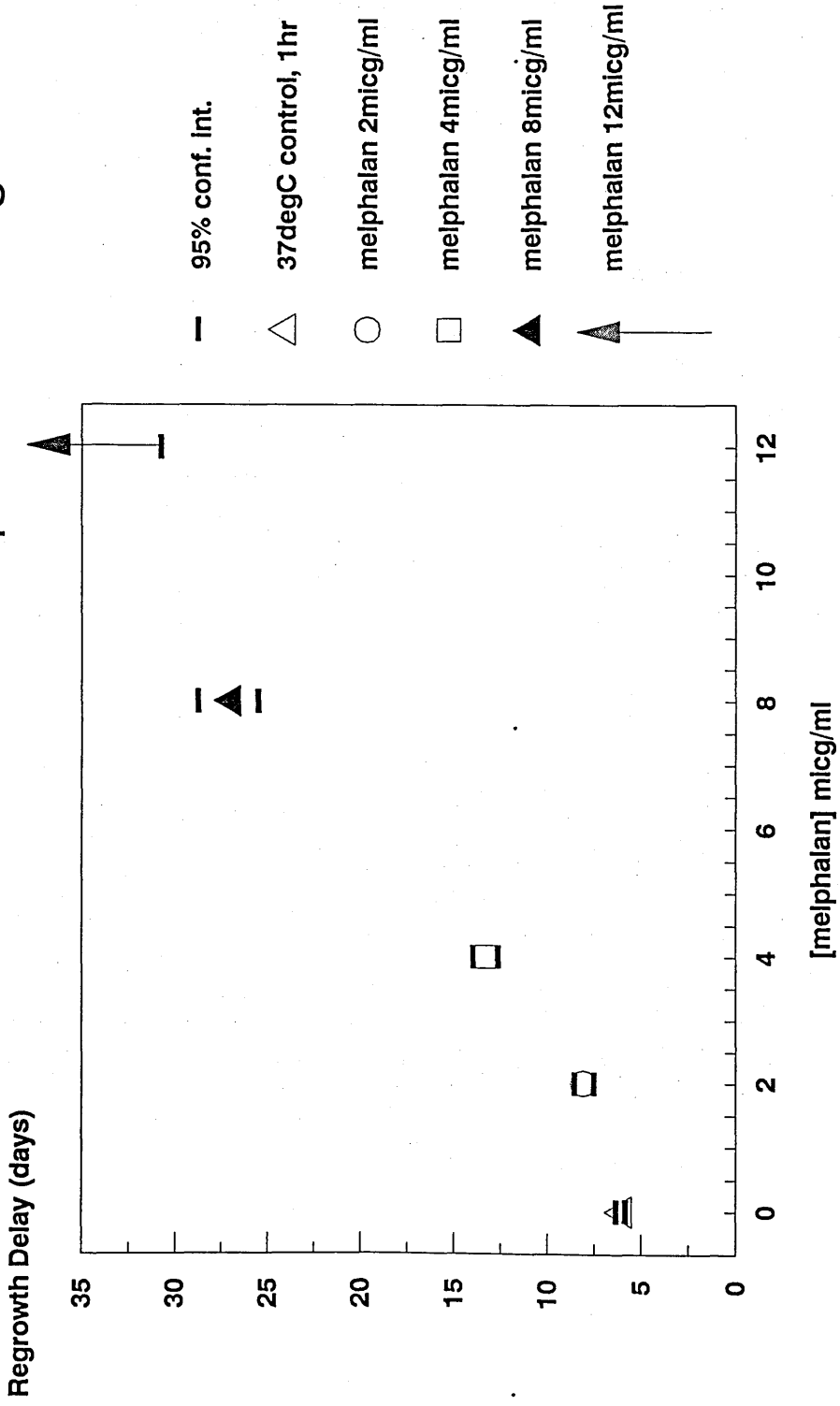


FIGURE 39A EXPERIMENT 12 Regrowth of B0008 spheroids after treatment with melphalan at 37degC

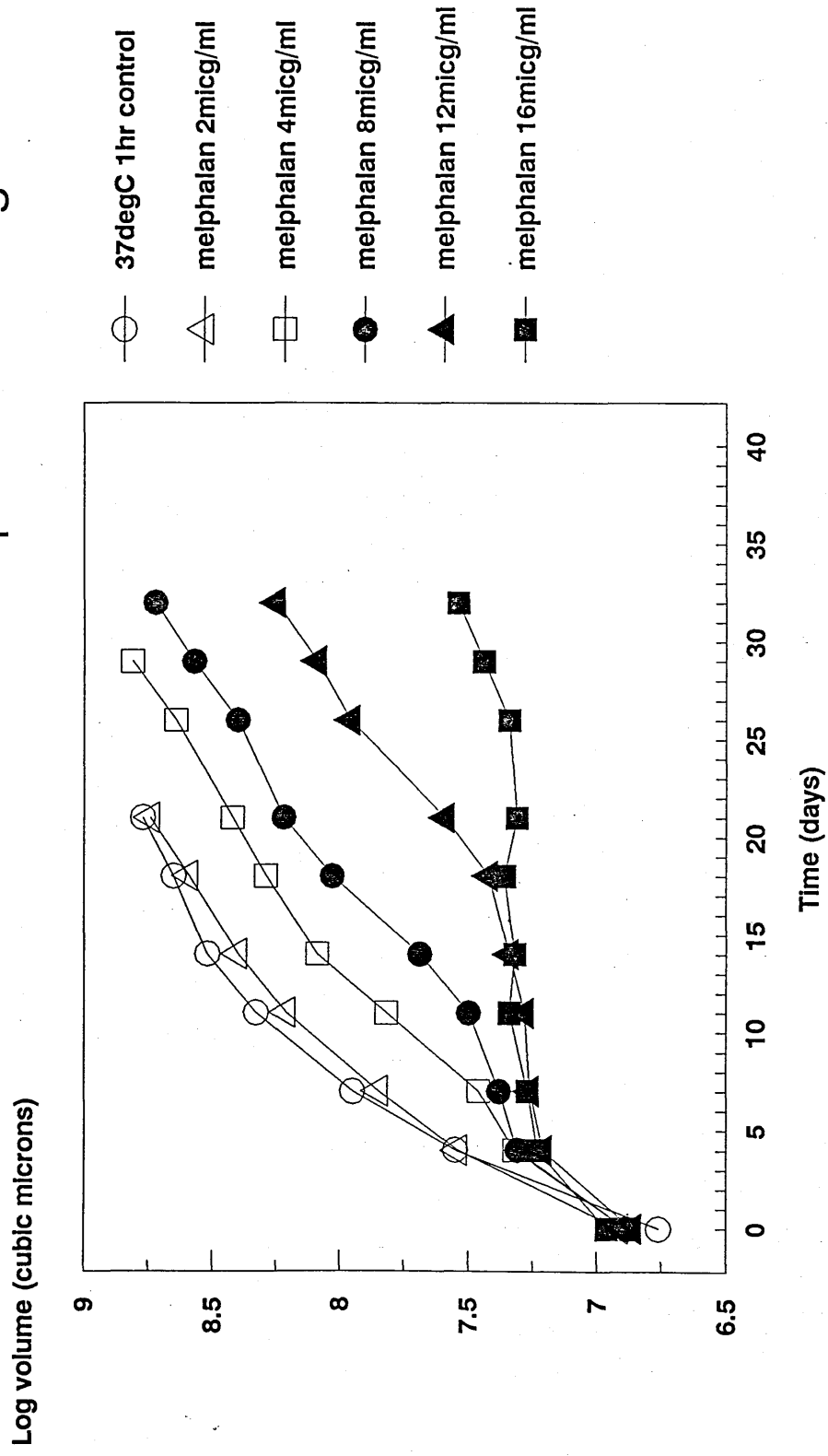


FIGURE 39B EXPERIMENT 12 Regrowth delay of B0008 spheroids
 after treatment with melphalan at 37degC

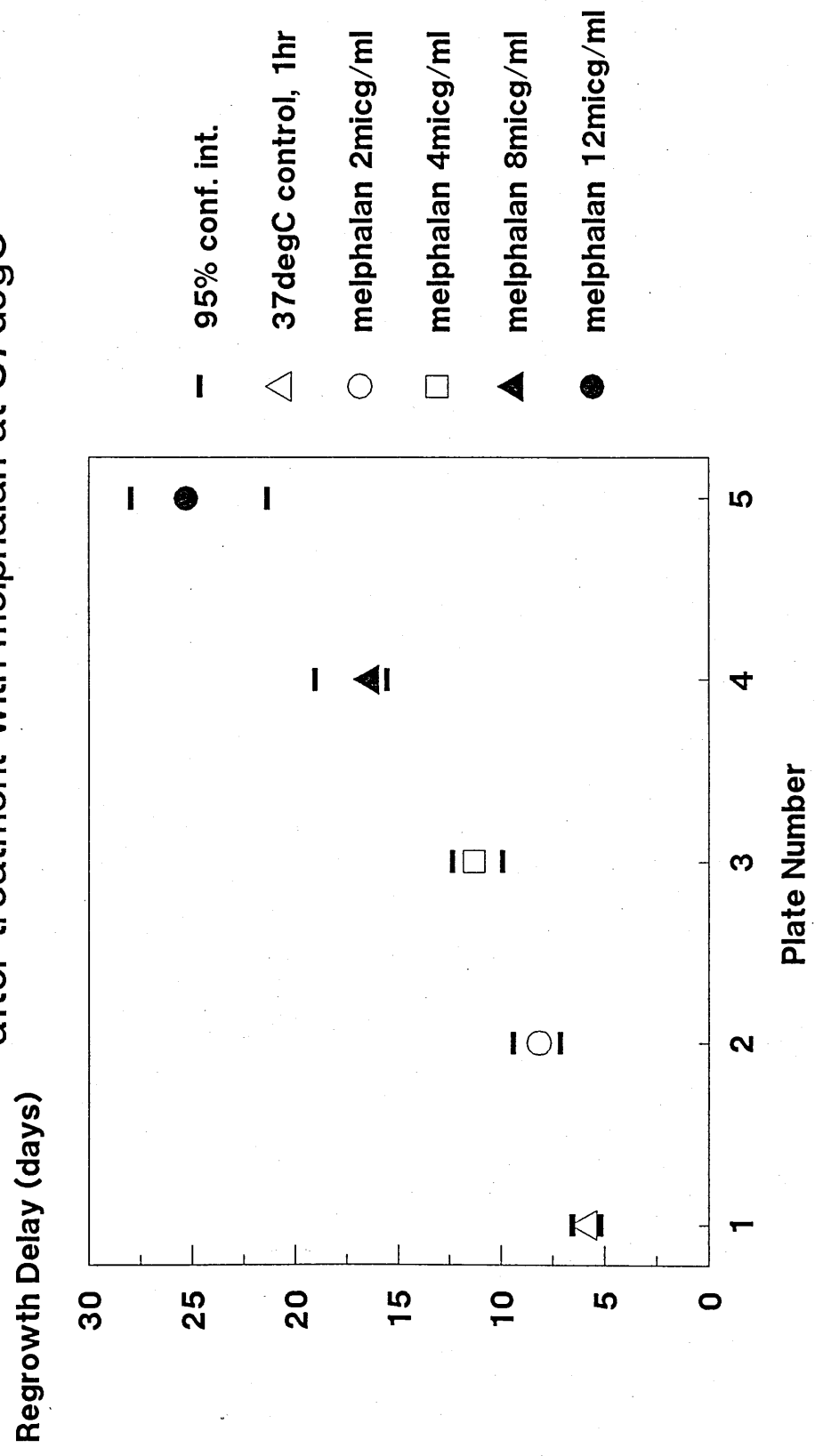


FIGURE 40A EXPERIMENT 13 Regrowth of B0008 spheroids after treatment with melphalan at 39degC

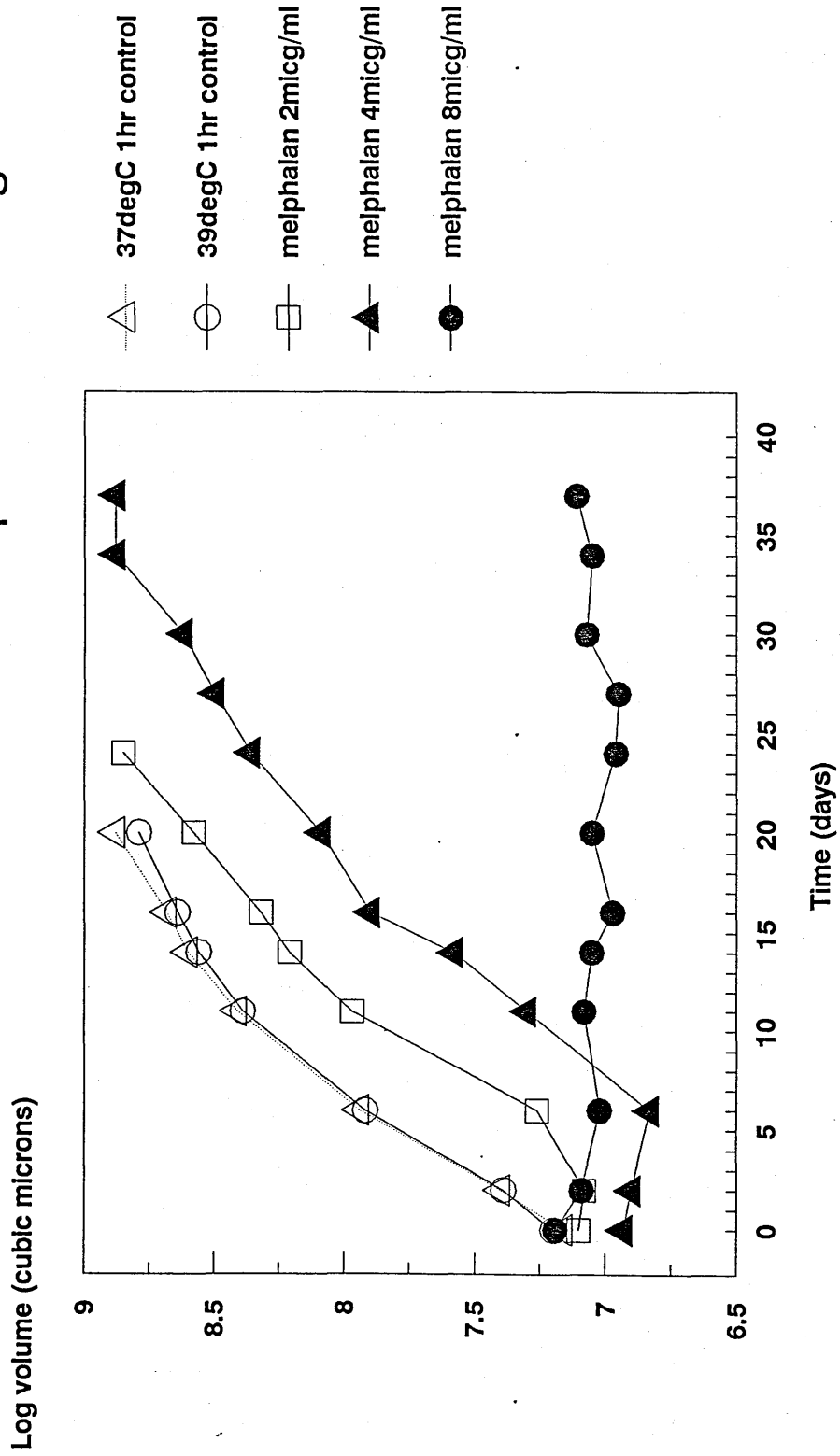


FIGURE 40B EXPERIMENT 13 Regrowth delay of B0008 spheroids after treatment with melphalan at 39degC

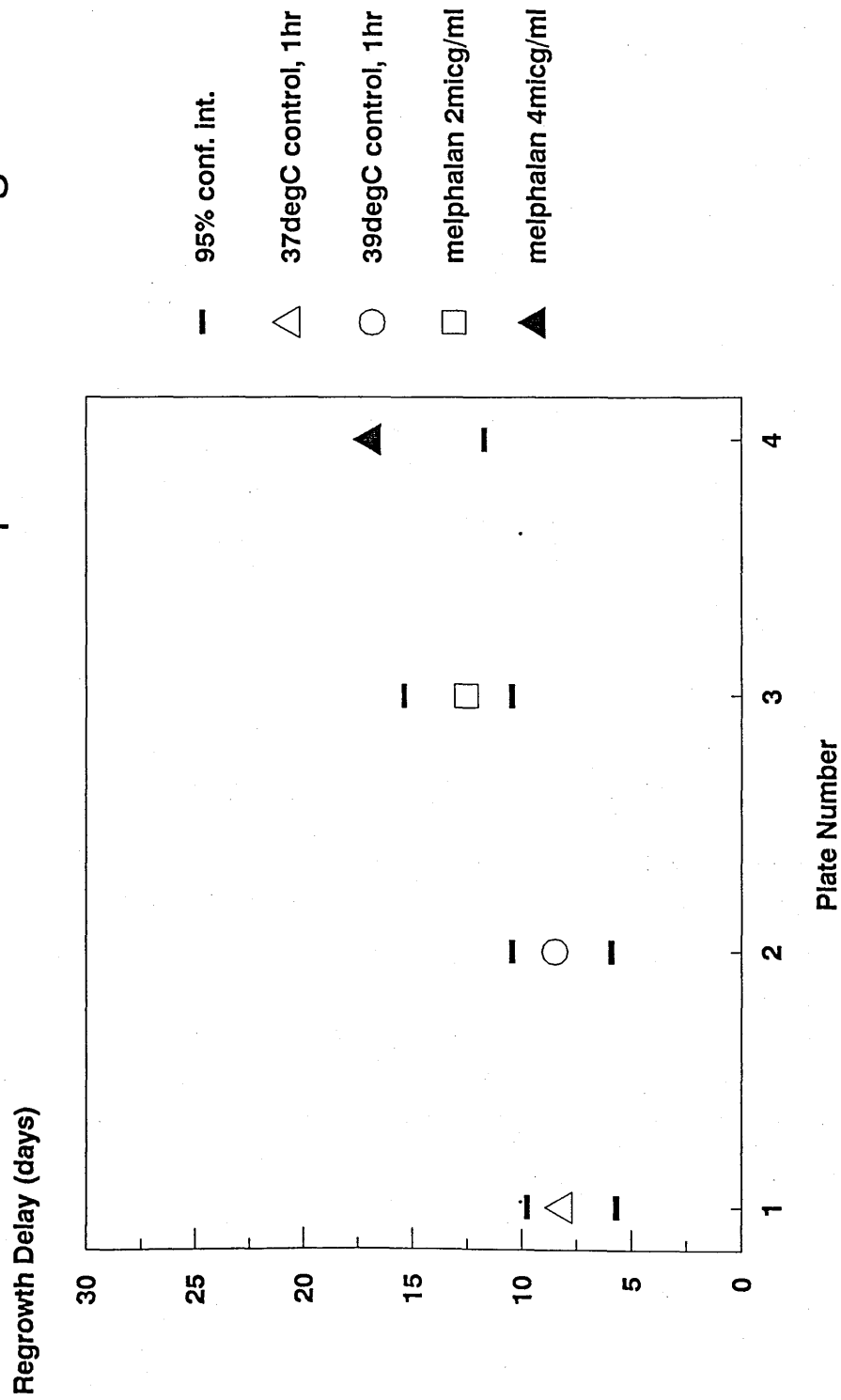


FIGURE 41A EXPERIMENT 14 Regrowth of B0008 spheroids after treatment with melphalan at 39degC

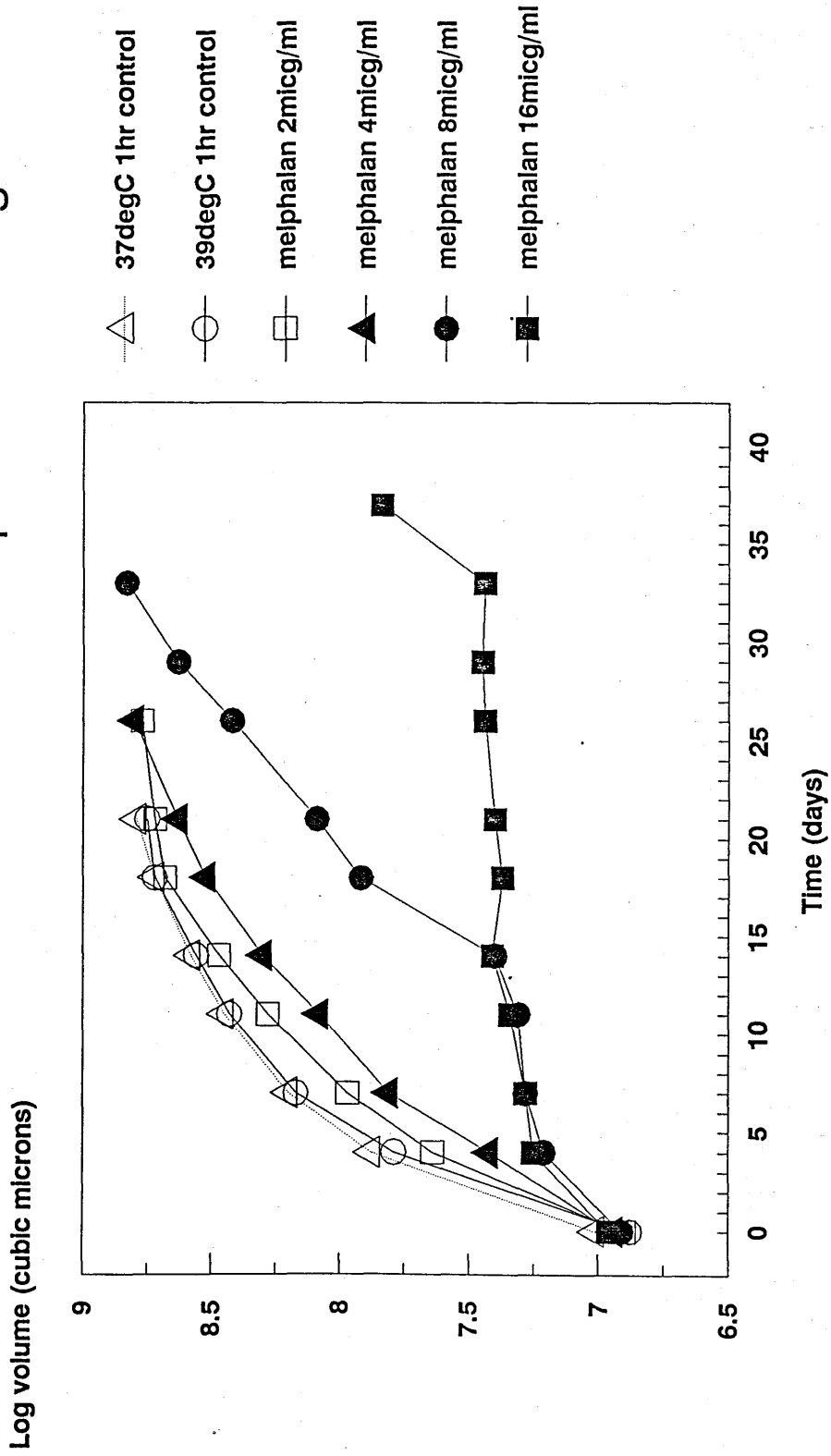


FIGURE 41B EXPERIMENT 14 Regrowth delay of B0008 spheroids after treatment with melphalan at 39degC

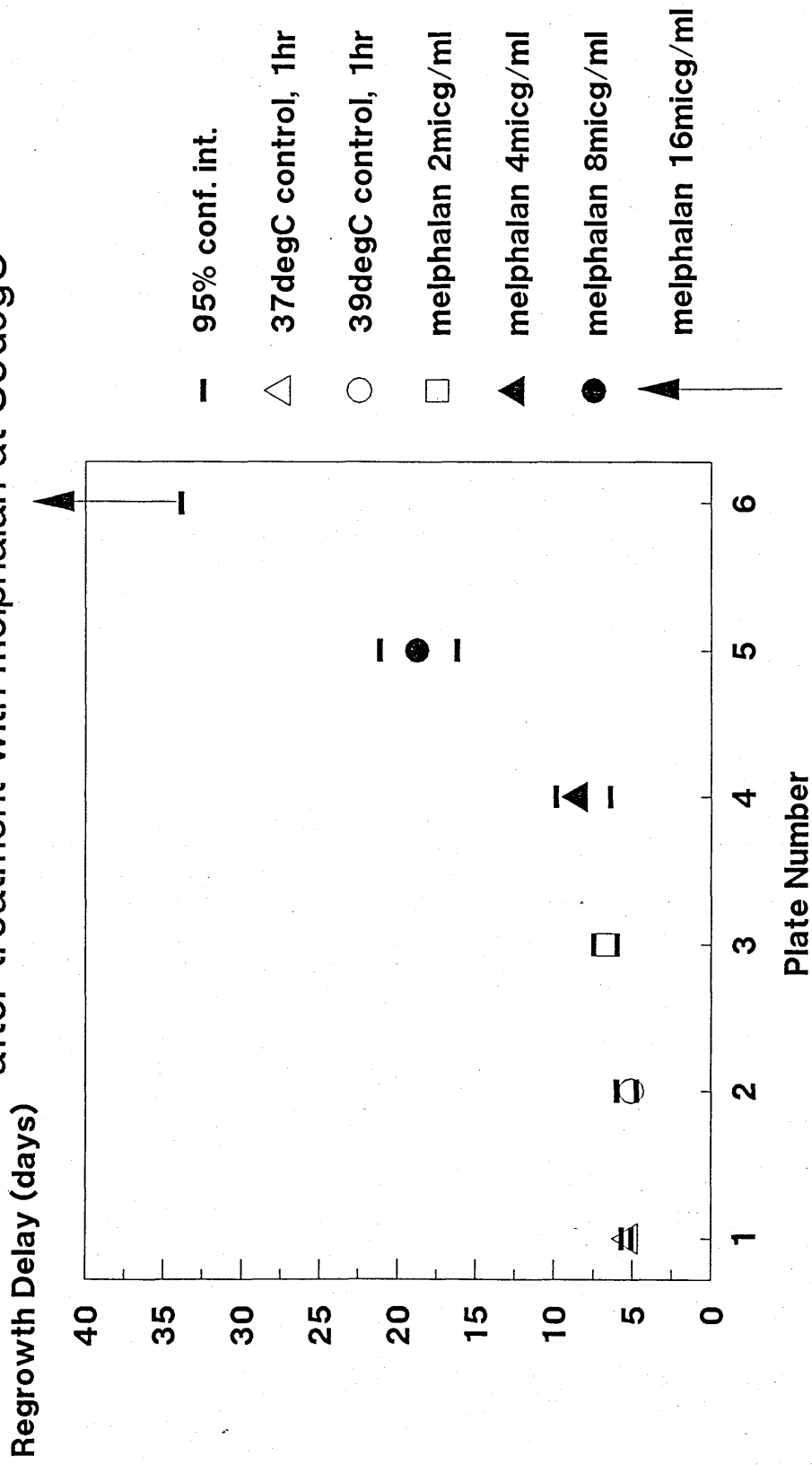


FIGURE 42A EXPERIMENT 15 Regrowth of B0008 spheroids
 after treatment with melphalan at 41degC

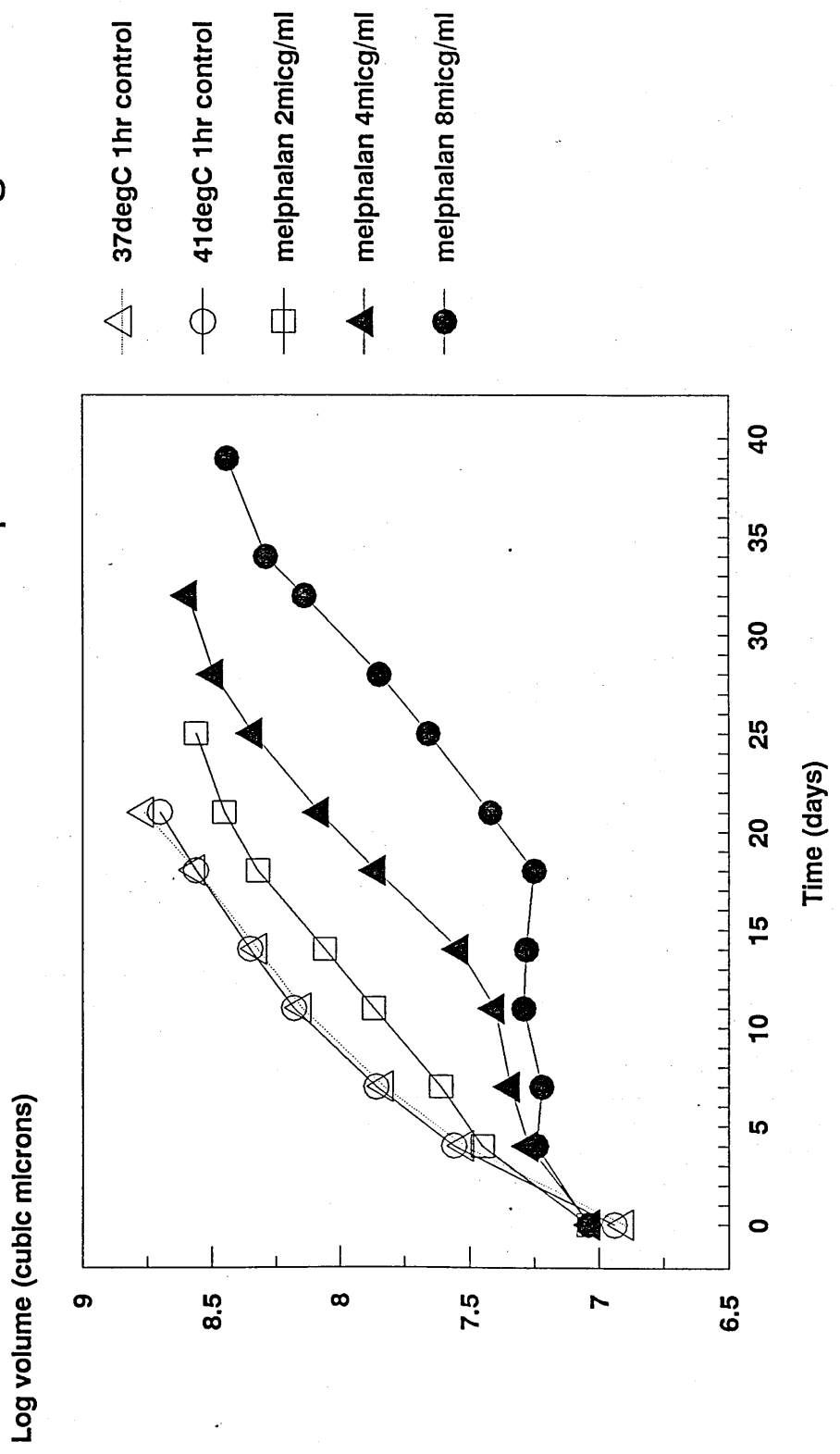


FIGURE 42B EXPERIMENT 15 Regrowth delay of B0008 spheroids after treatment with melphalan at 41degC

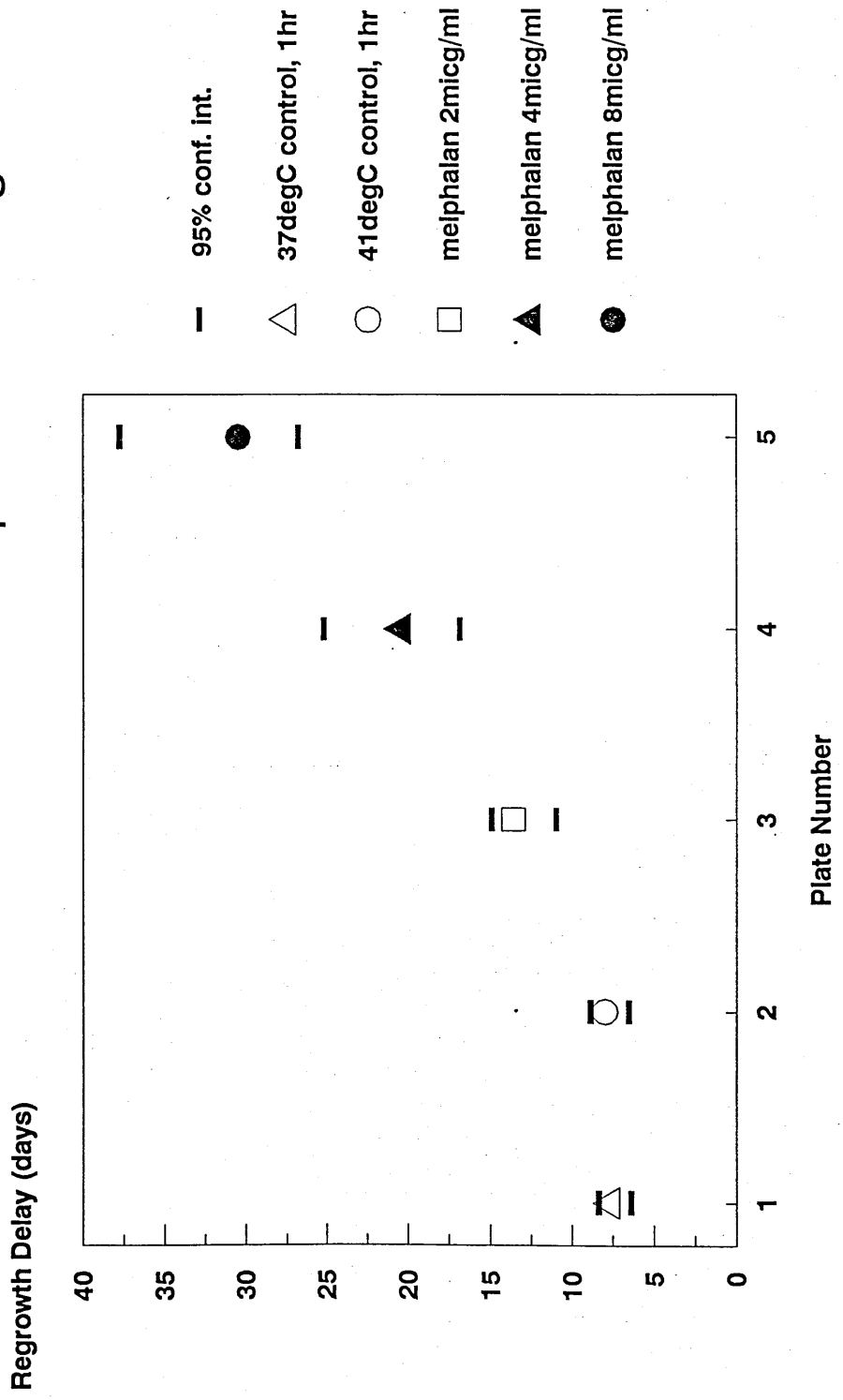


FIGURE 43A EXPERIMENT 16 Regrowth of B0008 spheroids after treatment with melphalan at 42.5degC

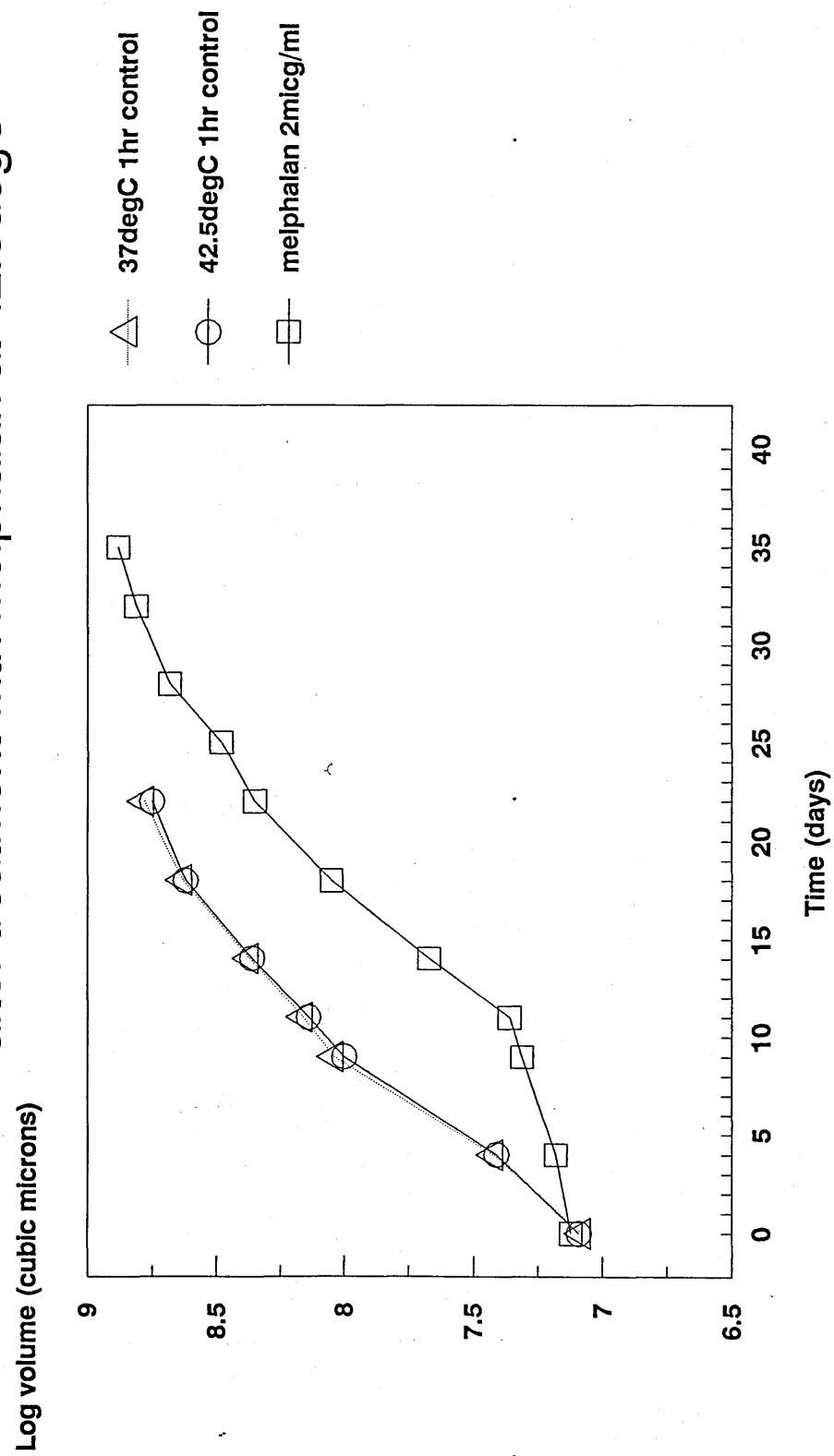


FIGURE 43B EXPERIMENT 16 Regrowth delay of B0008 spheroids after treatment with melphalan at 42.5 degC

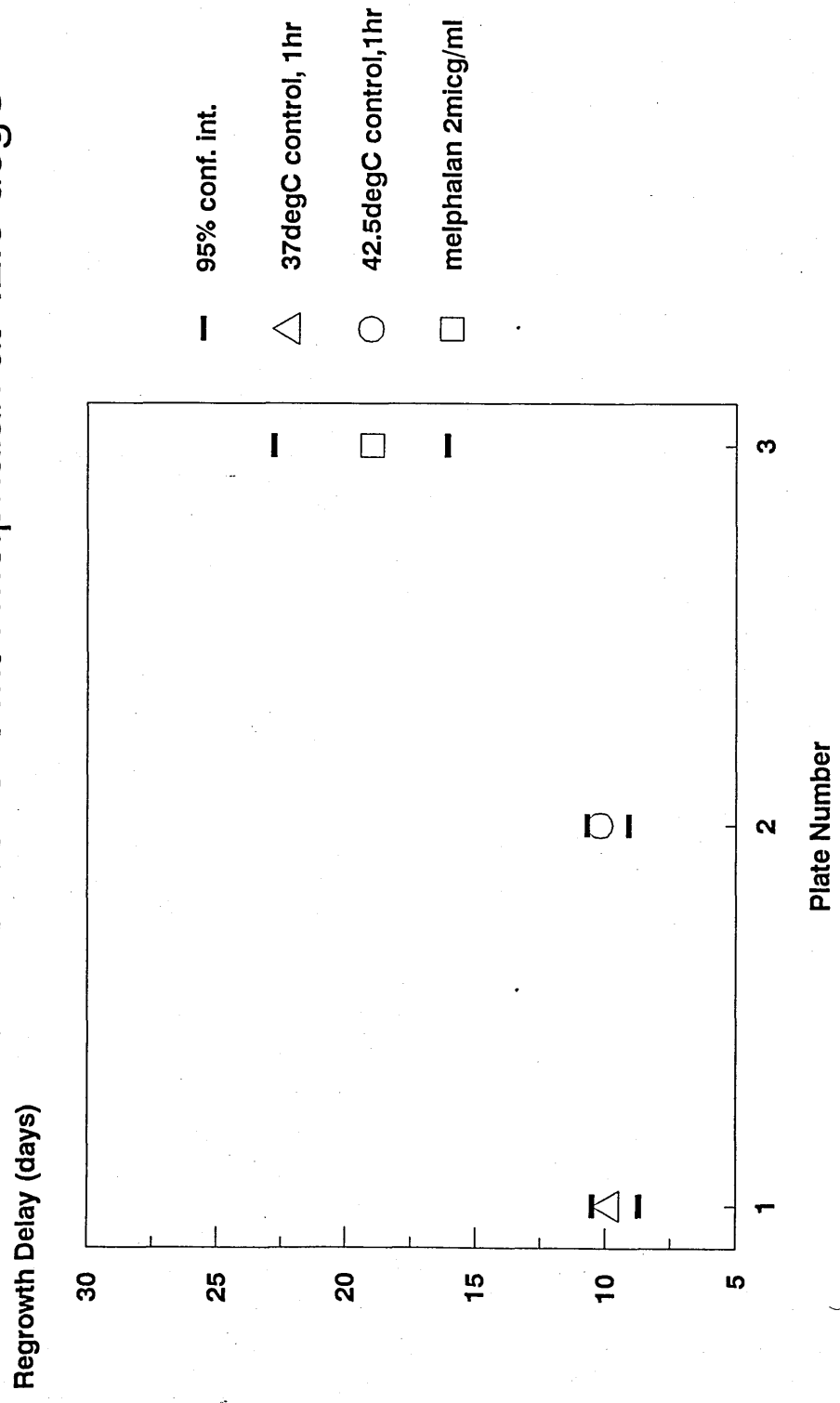


FIGURE 44A EXPERIMENT 17 Regrowth of B0008 spheroids
after treatment with melphalan at 42.5degC

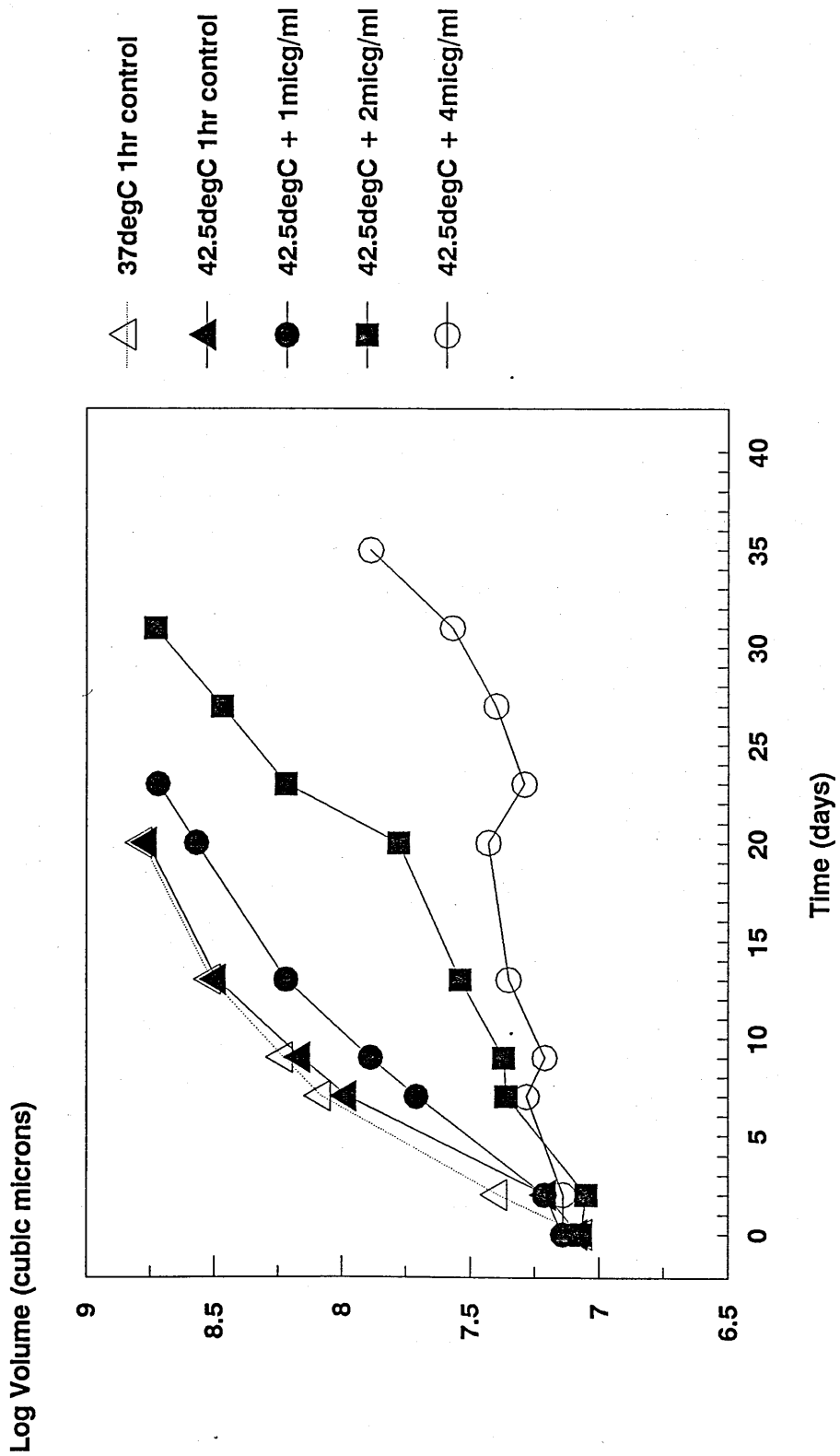


FIGURE 44B EXPERIMENT 17 Regrowth delay of B0008 spheroids after treatment with melphalan at 42.5degC

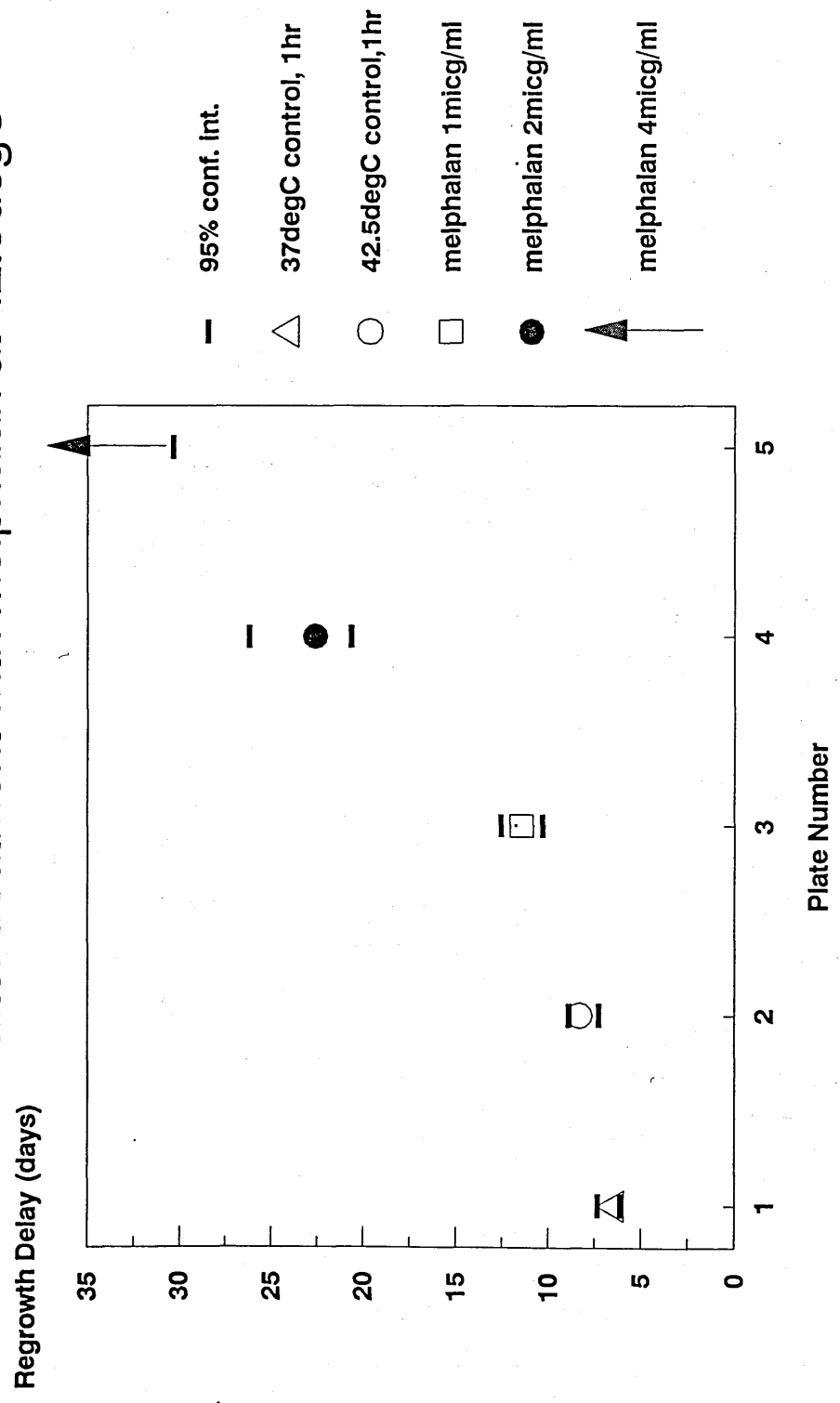


FIGURE 45B EXPERIMENT 18 Regrowth delay of B0008 spheroids after treatment with melphalan at 42.5degC

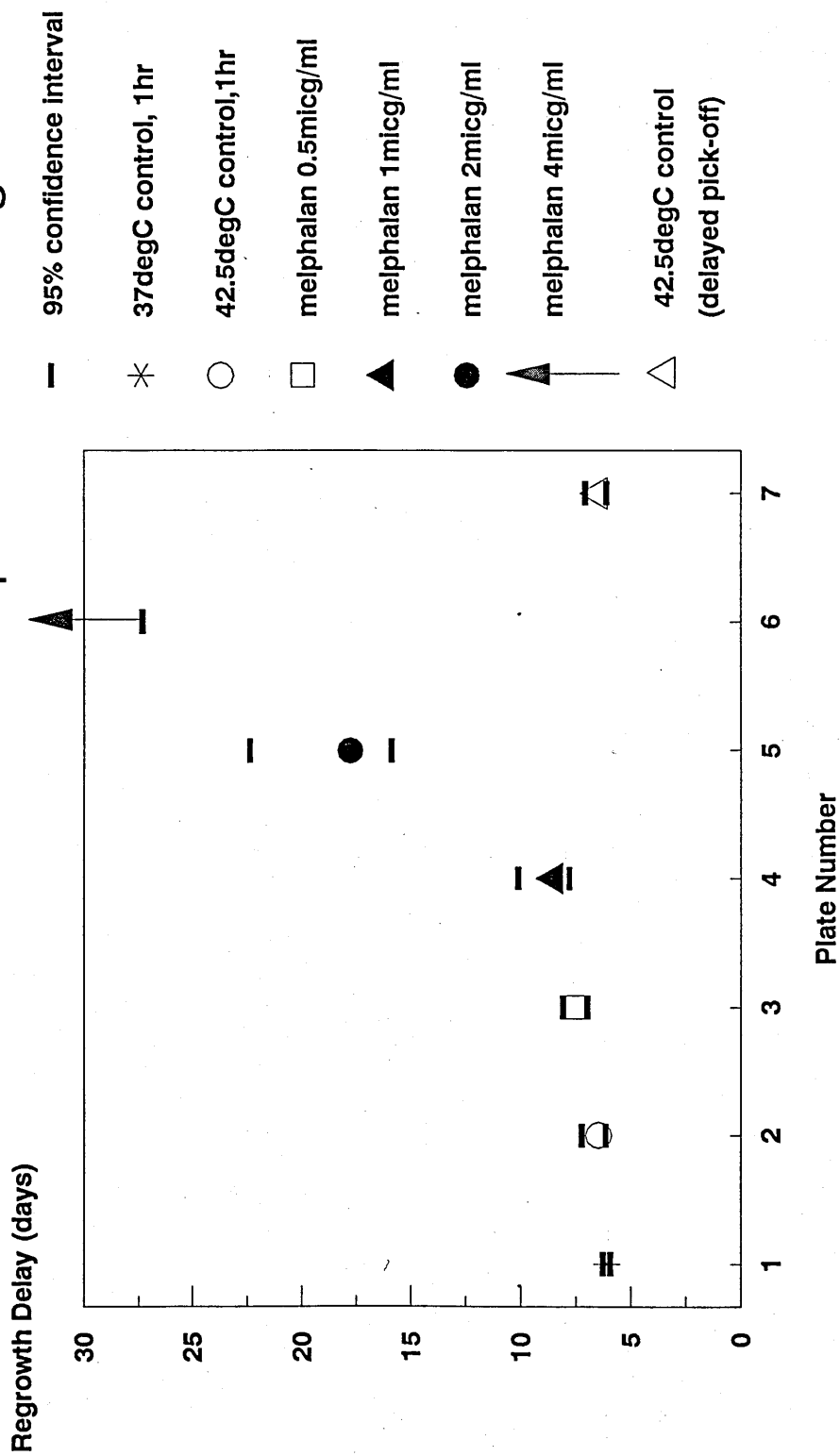


FIGURE 46B EXPERIMENT 20 Regrowth delay of B0008 spheroids after treatment with melphalan at 37degC for 15-60mins

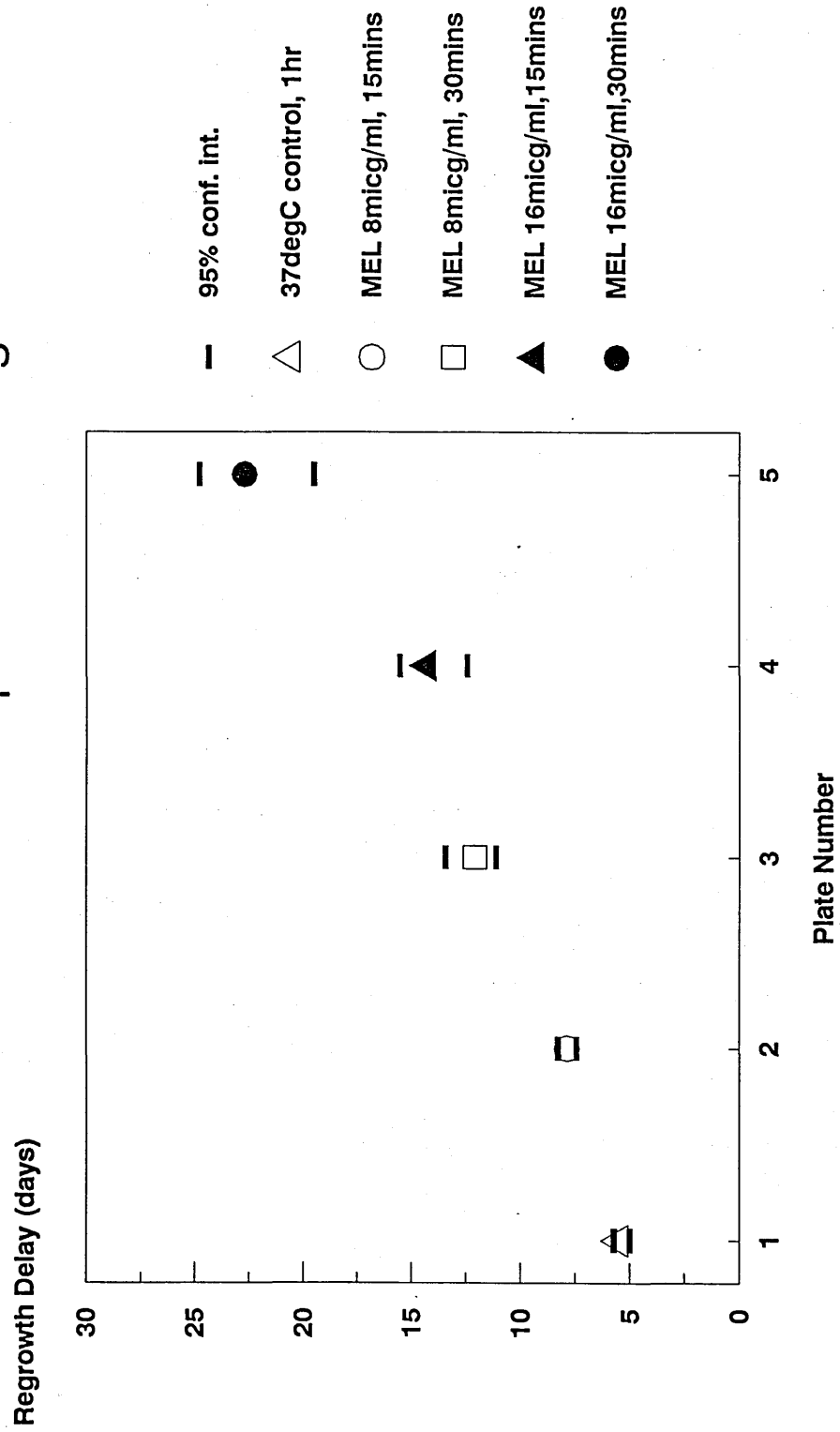


FIGURE 47A EXPERIMENT 21 Regrowth of B0008 spheroids

after treatment with melphalan at 37degC, 15-120mins

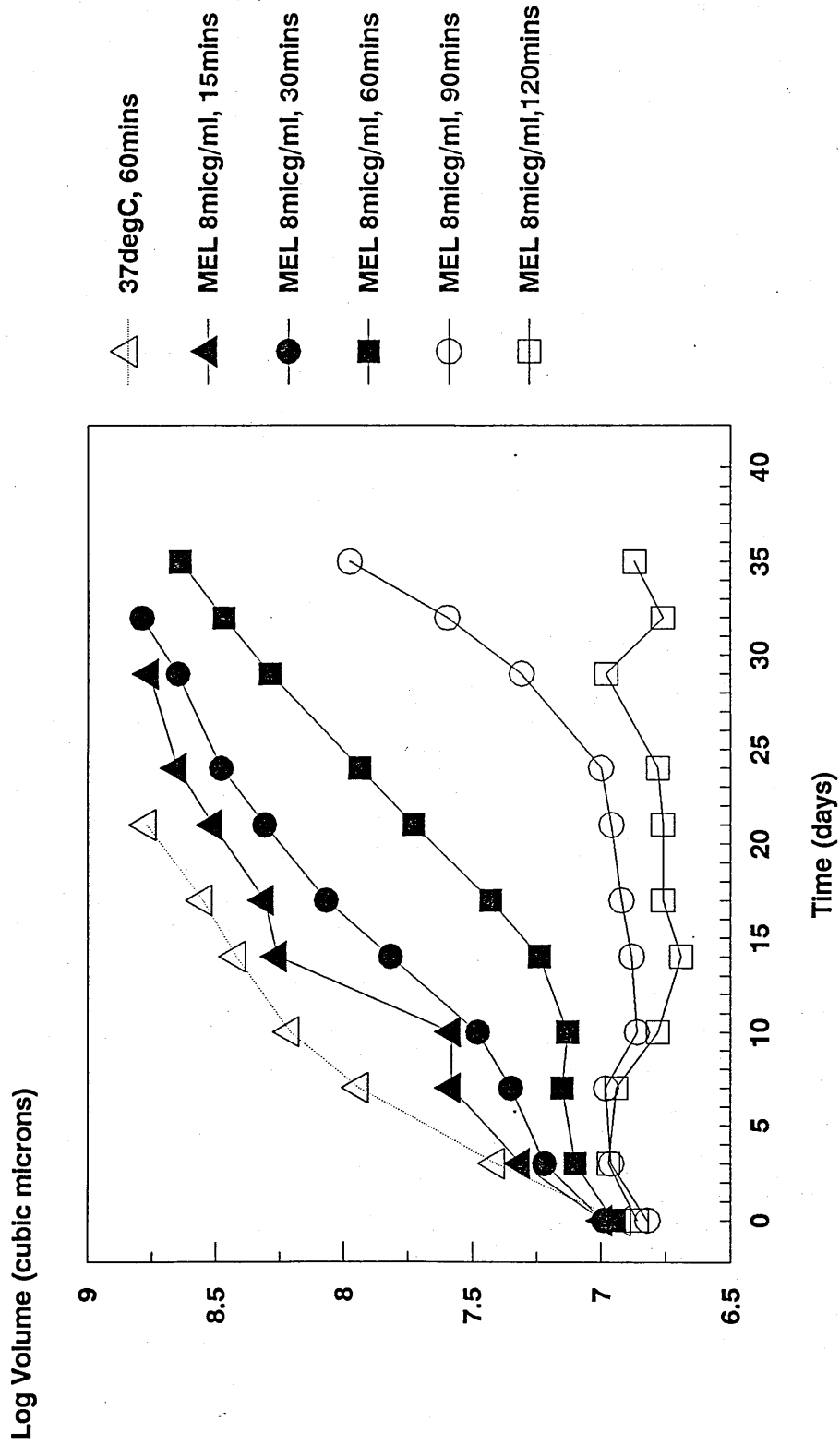


FIGURE 47B EXPERIMENT 21 Regrowth delay of B0008 spheroids after treatment with melphalan at 37degC for 15-60mins

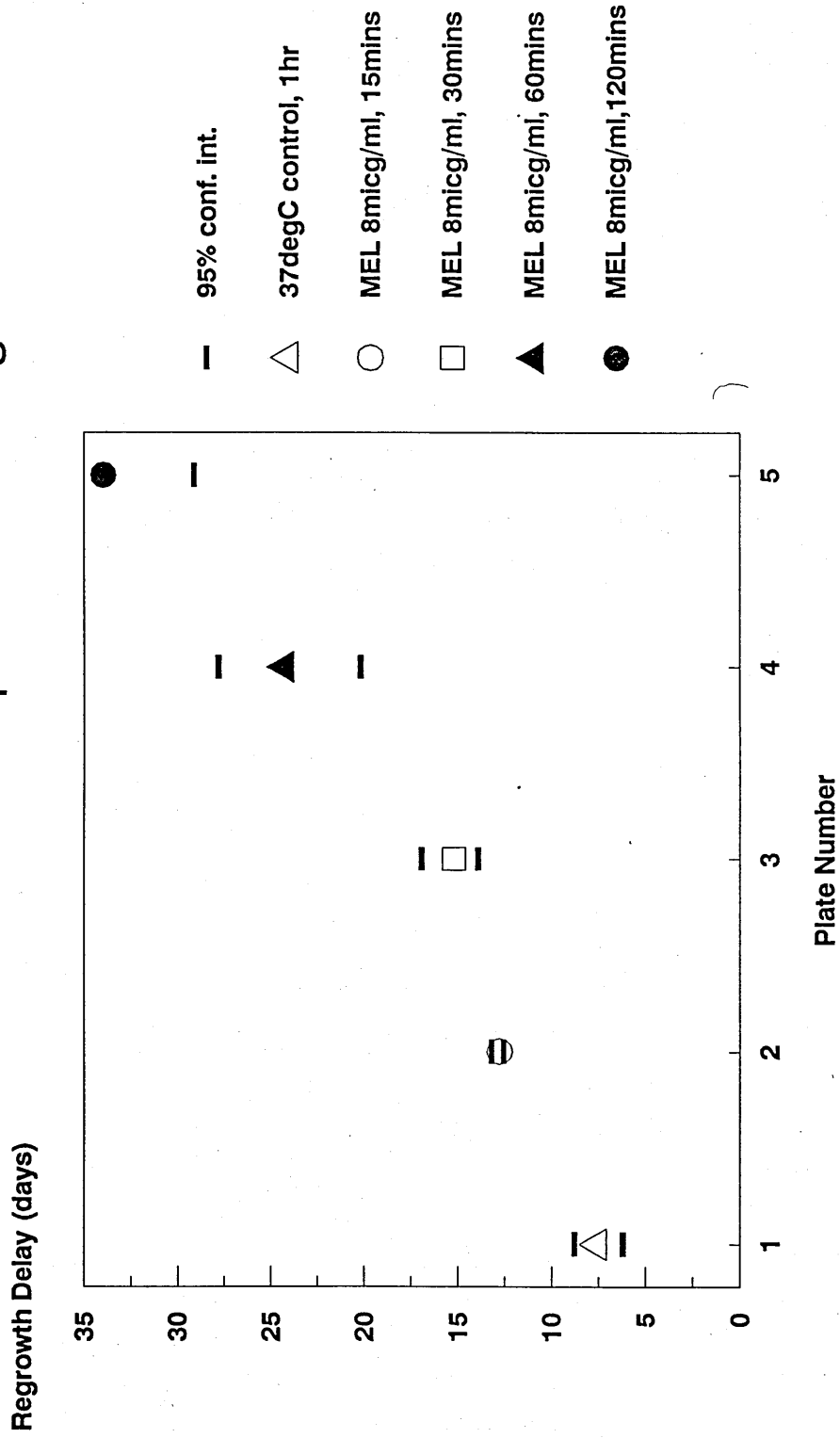


FIGURE 48A EXPERIMENT 22 Regrowth of B0008 spheroids after melphalan treatment at 31degC, for 30mins

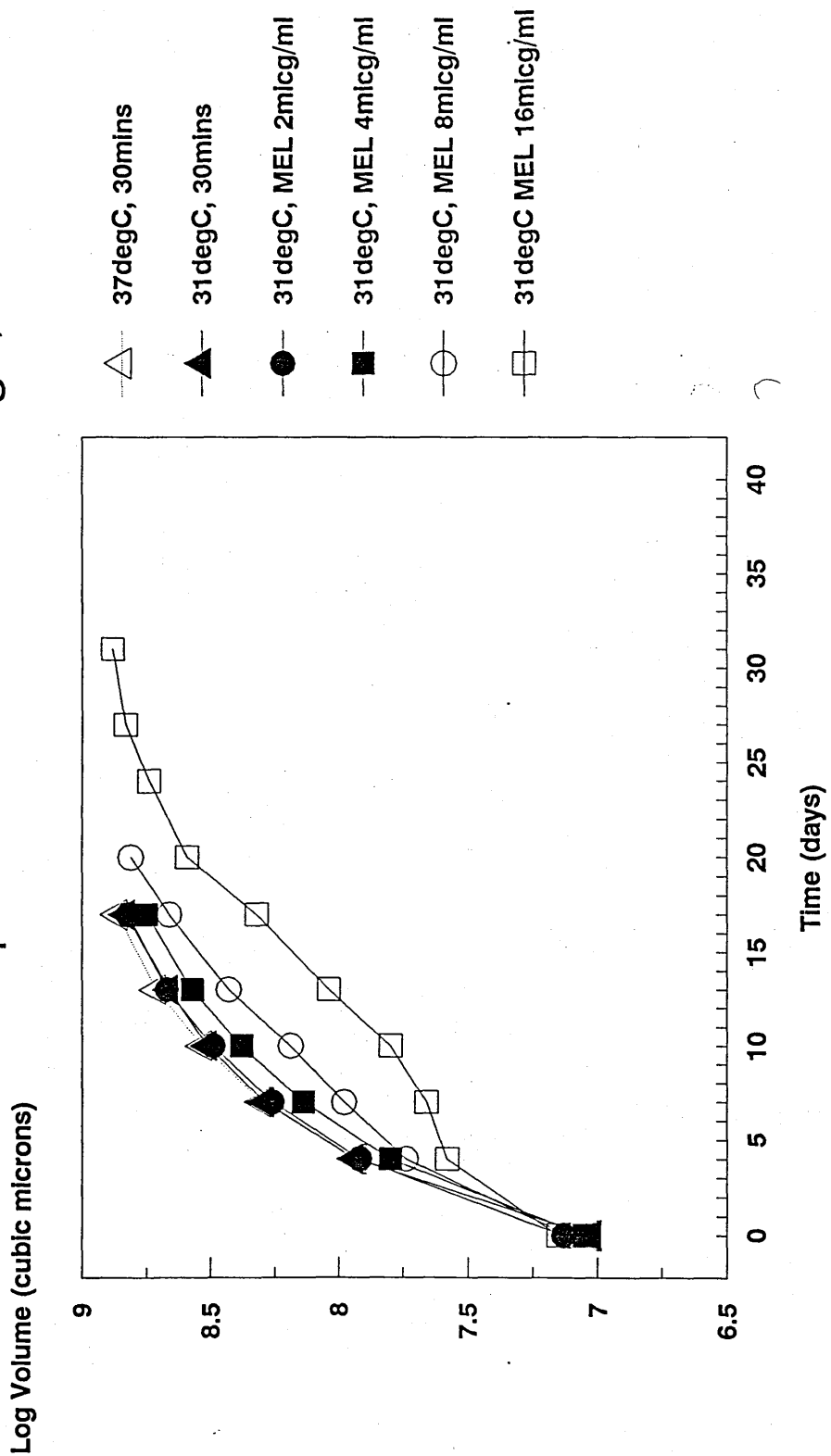


FIGURE 48B EXPERIMENT 22 Regrowth delay of B0008 spheroids after treatment with melphalan at 31degC for 30mins

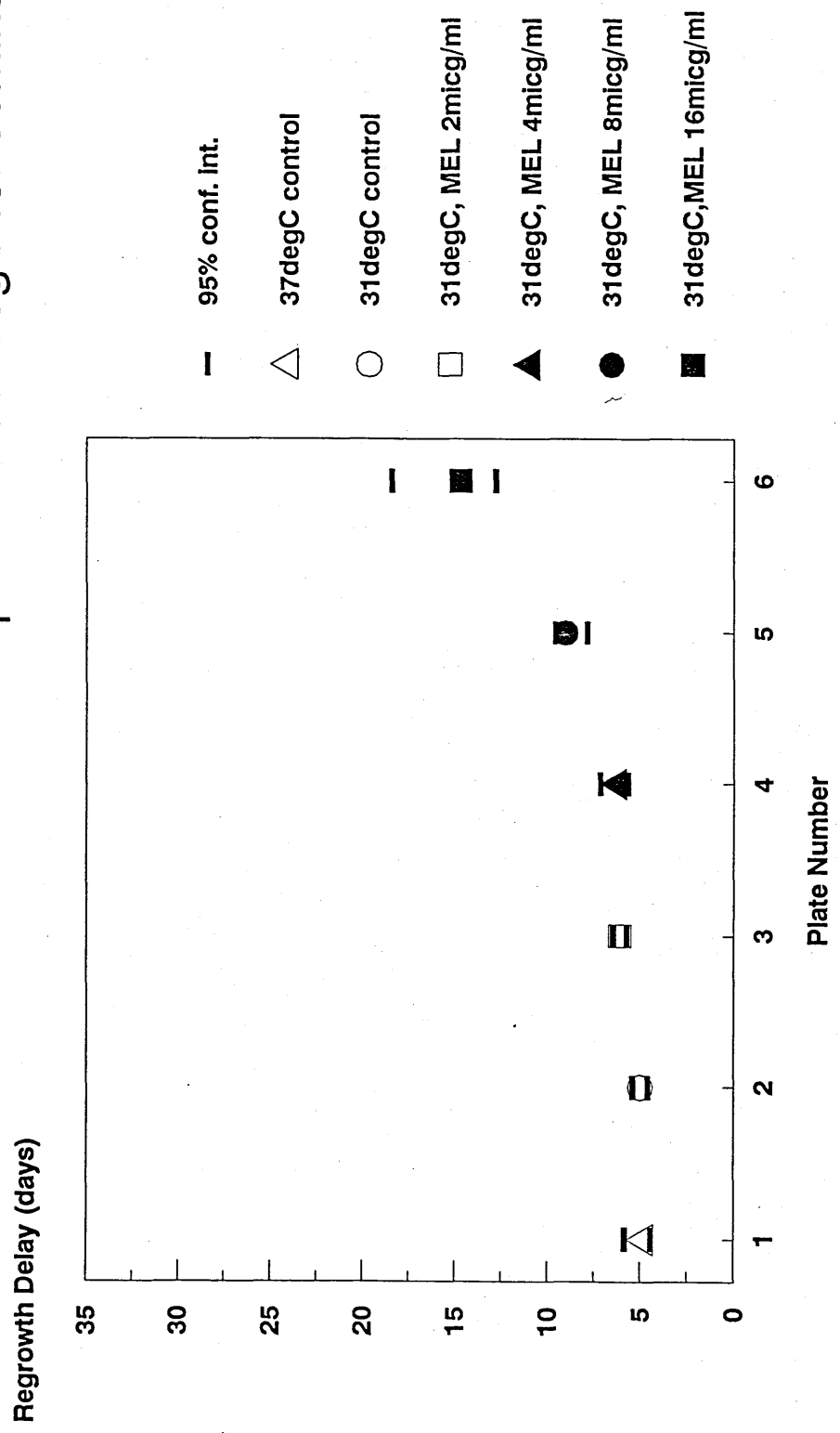


FIGURE 49A EXPERIMENT 23 Regrowth of B0008 spheroids after treatment with melphalan +/- verapamil at 37degC

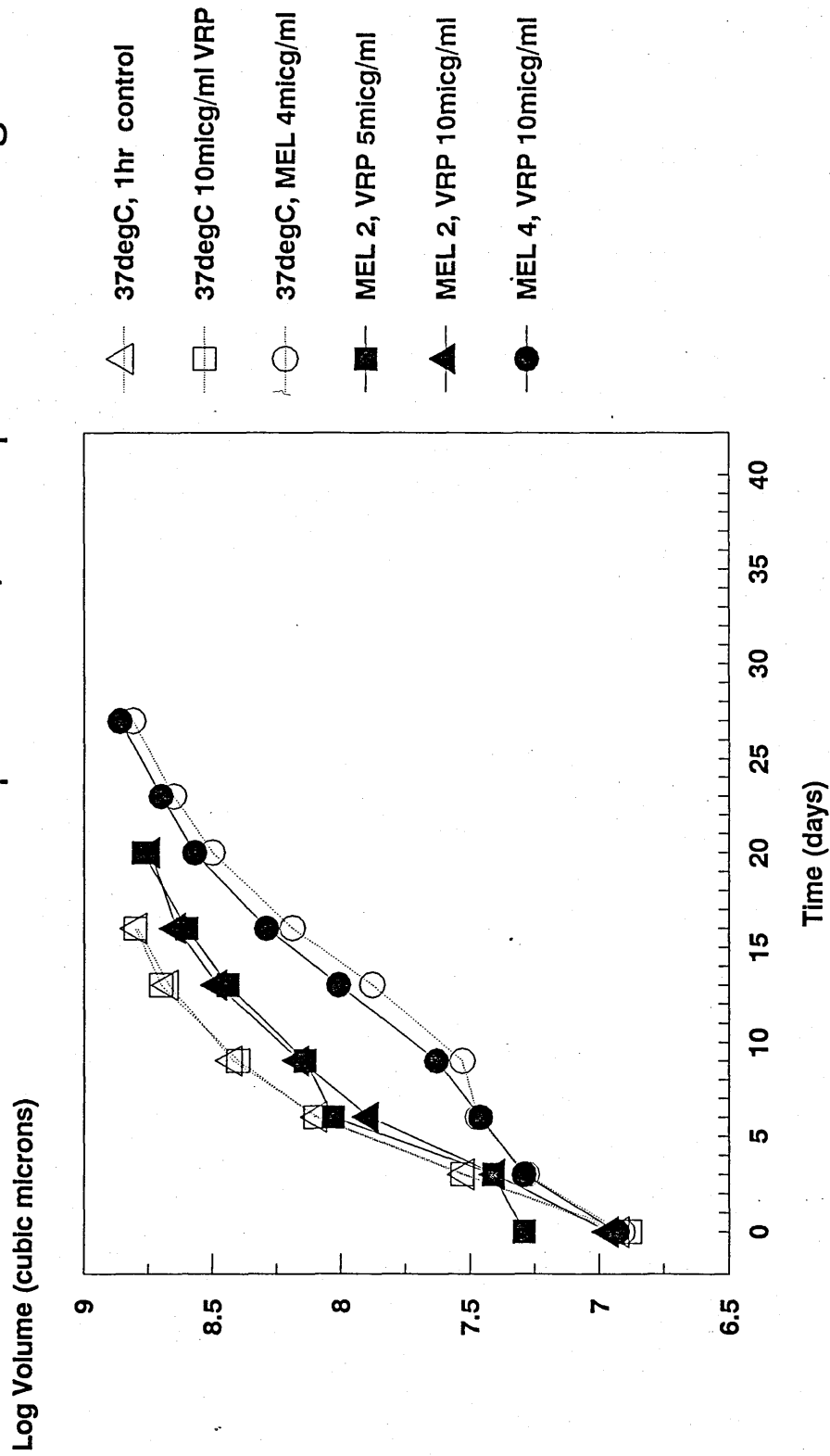


FIGURE 49B EXPERIMENT 23 Regrowth delay of B0008 spheroids treated with melphalan +/- verapamil

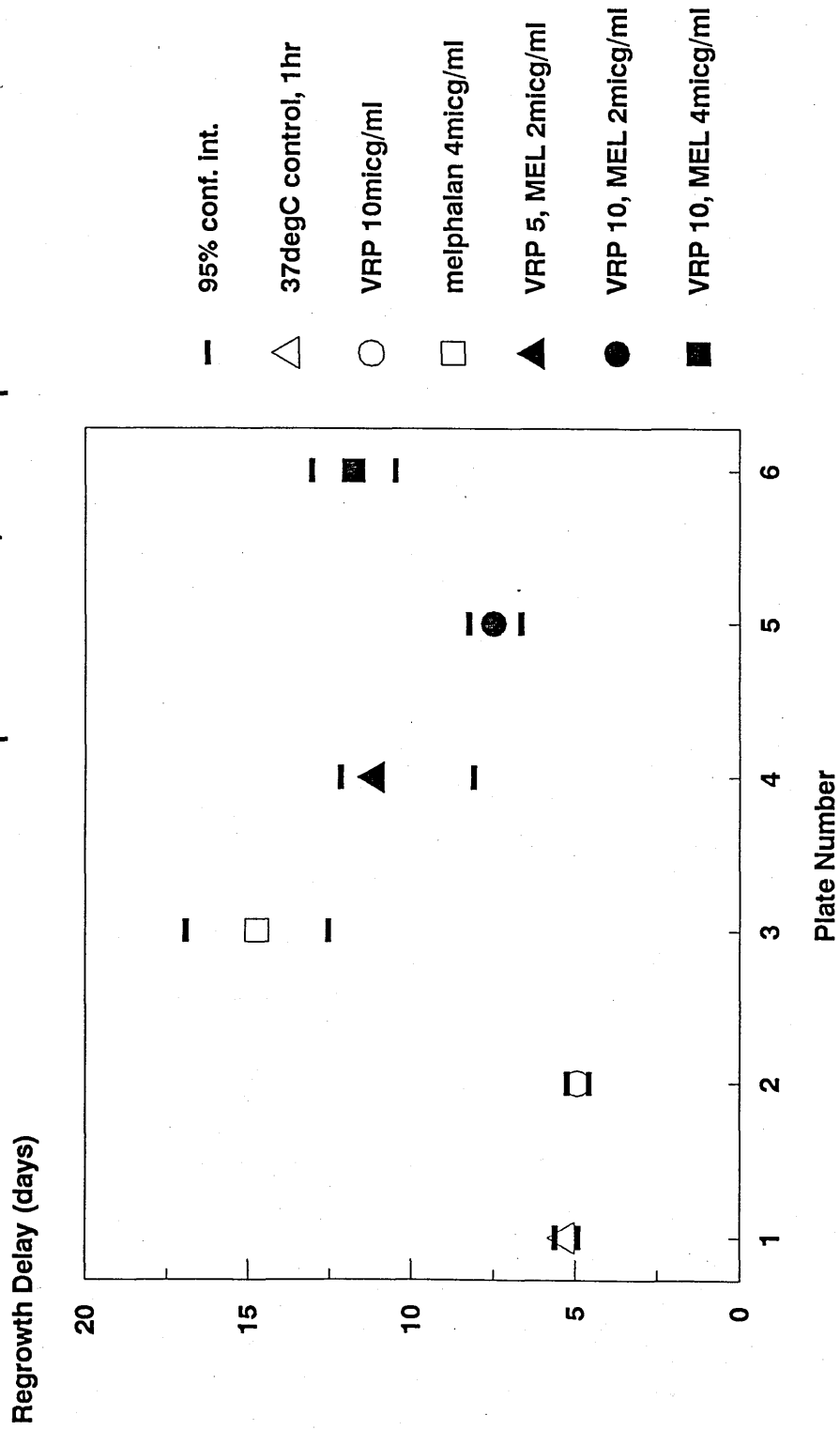


FIGURE 50A EXPERIMENT 24 Regrowth of B0008 spheroids after treatment with melphalan +/- verapamil at 37degC

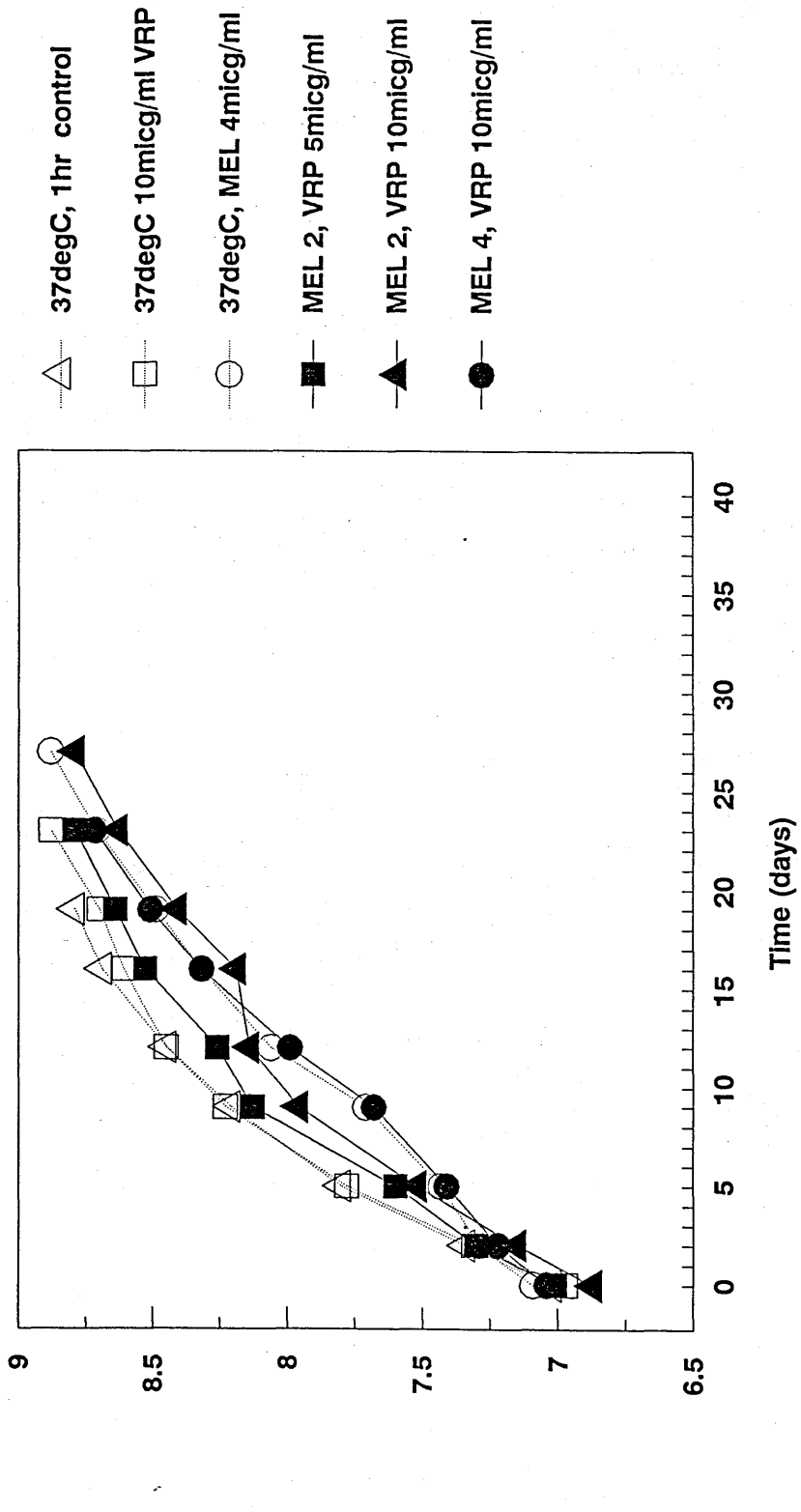


FIGURE 50B EXPERIMENT 24 Regrowth delay of B0008 spheroids after treatment with melphalan (MEL) +/- verapamil (VRP)

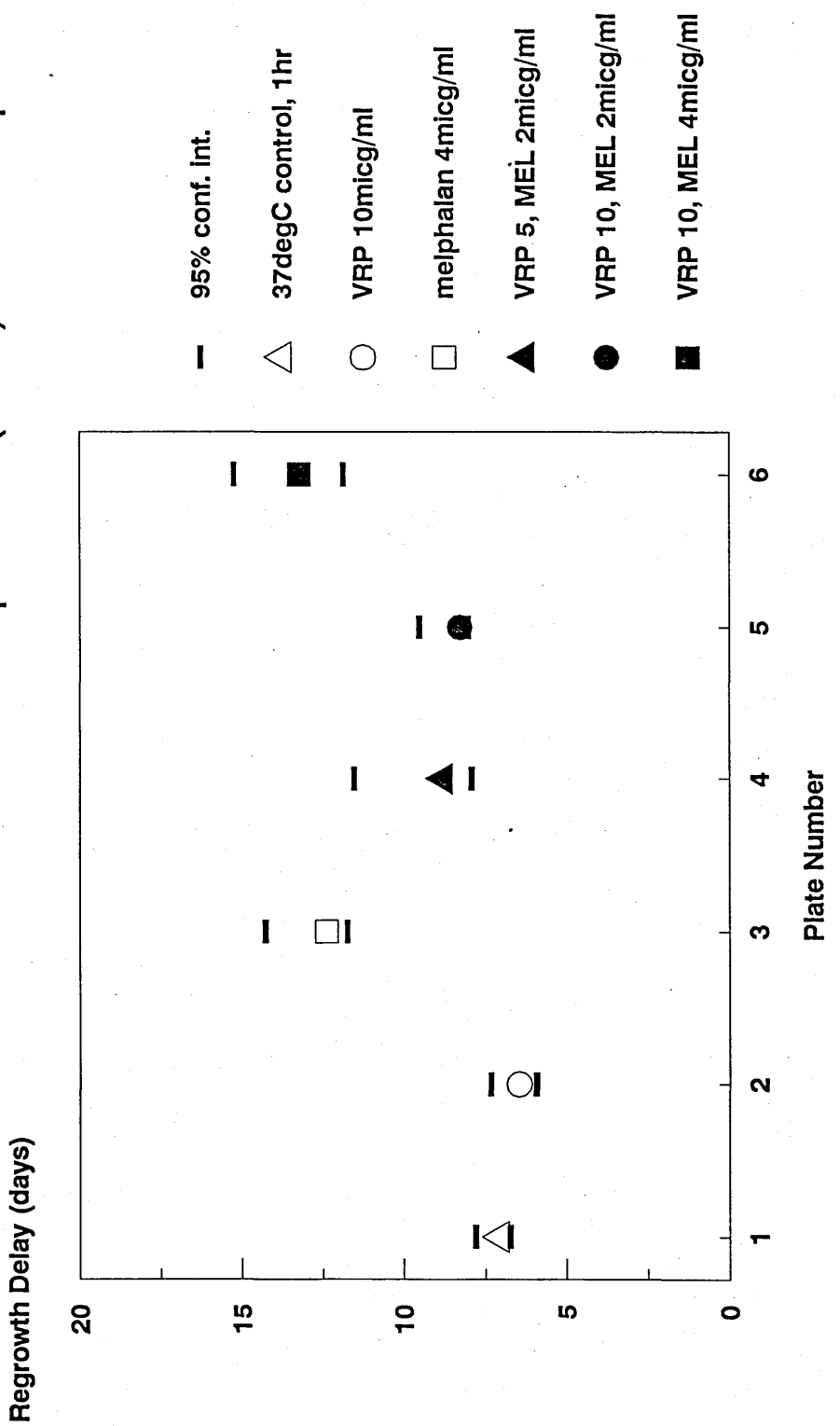


FIGURE 51A EXPERIMENT 25 pH and the cytotoxic effect of melphalan on large B0008 spheroids

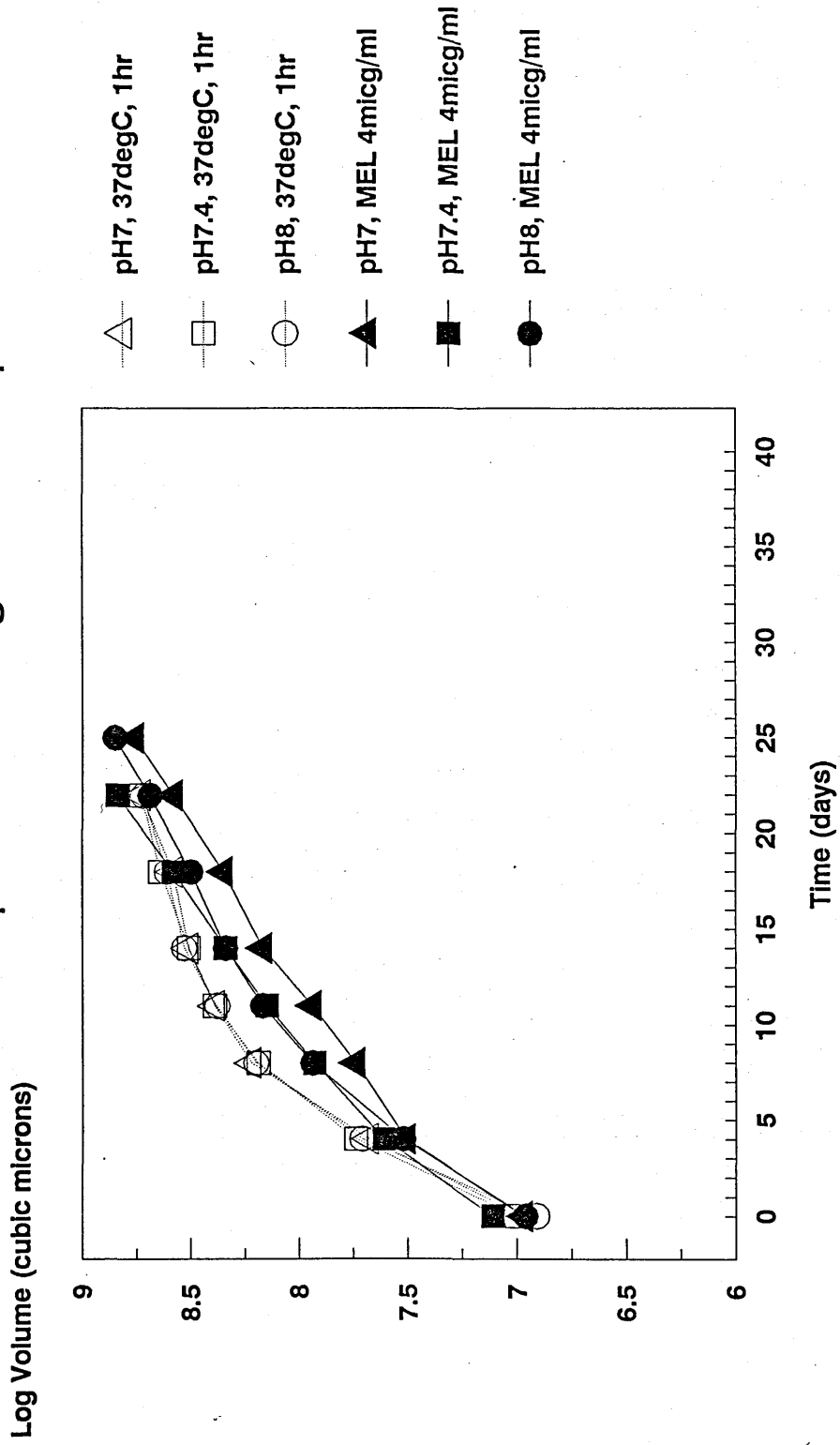


FIGURE 51B EXPERIMENT 25 pH and melphalan cytotoxicity:
LARGE B0008 melanoma spheroids (cf. Fig. 52B, Experiment 26)

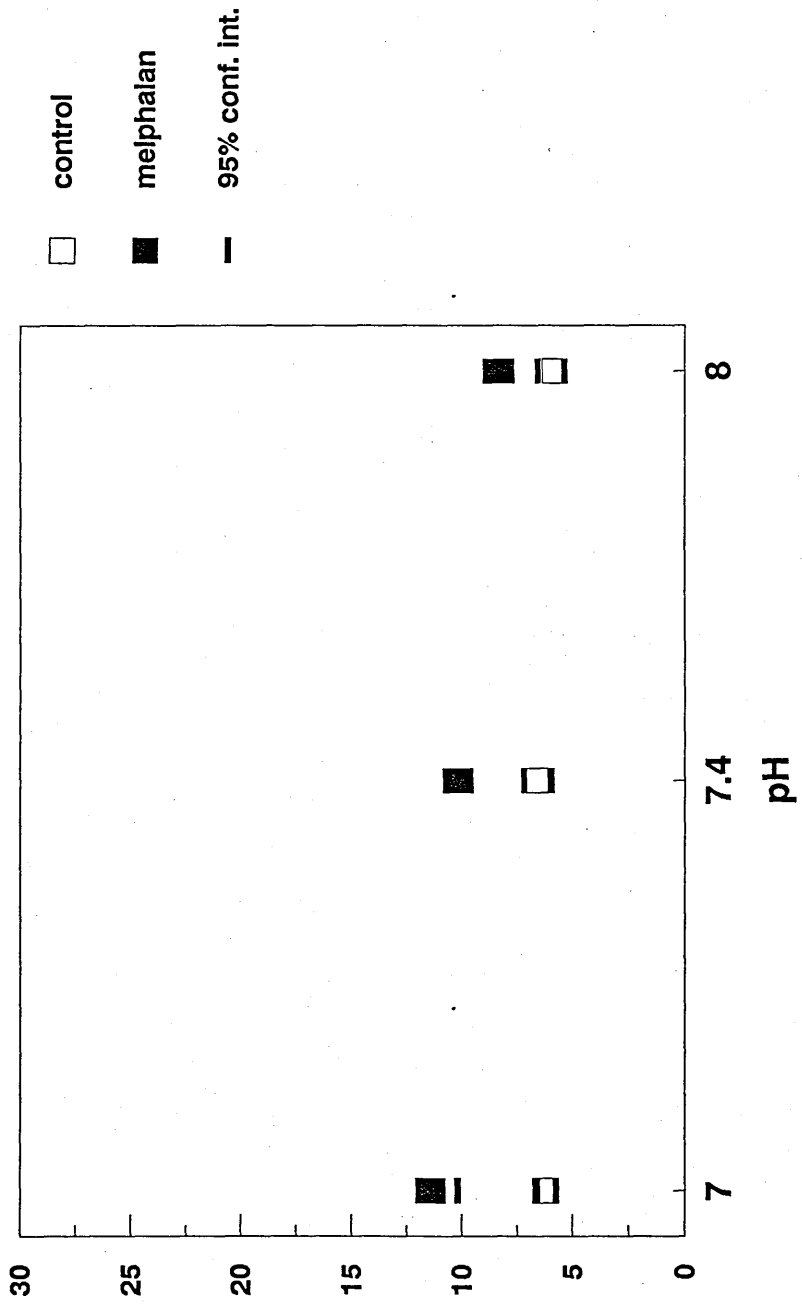


FIGURE 52A EXPERIMENT 26 pH and the cytotoxic effect of melphalan on small B0008 spheroids

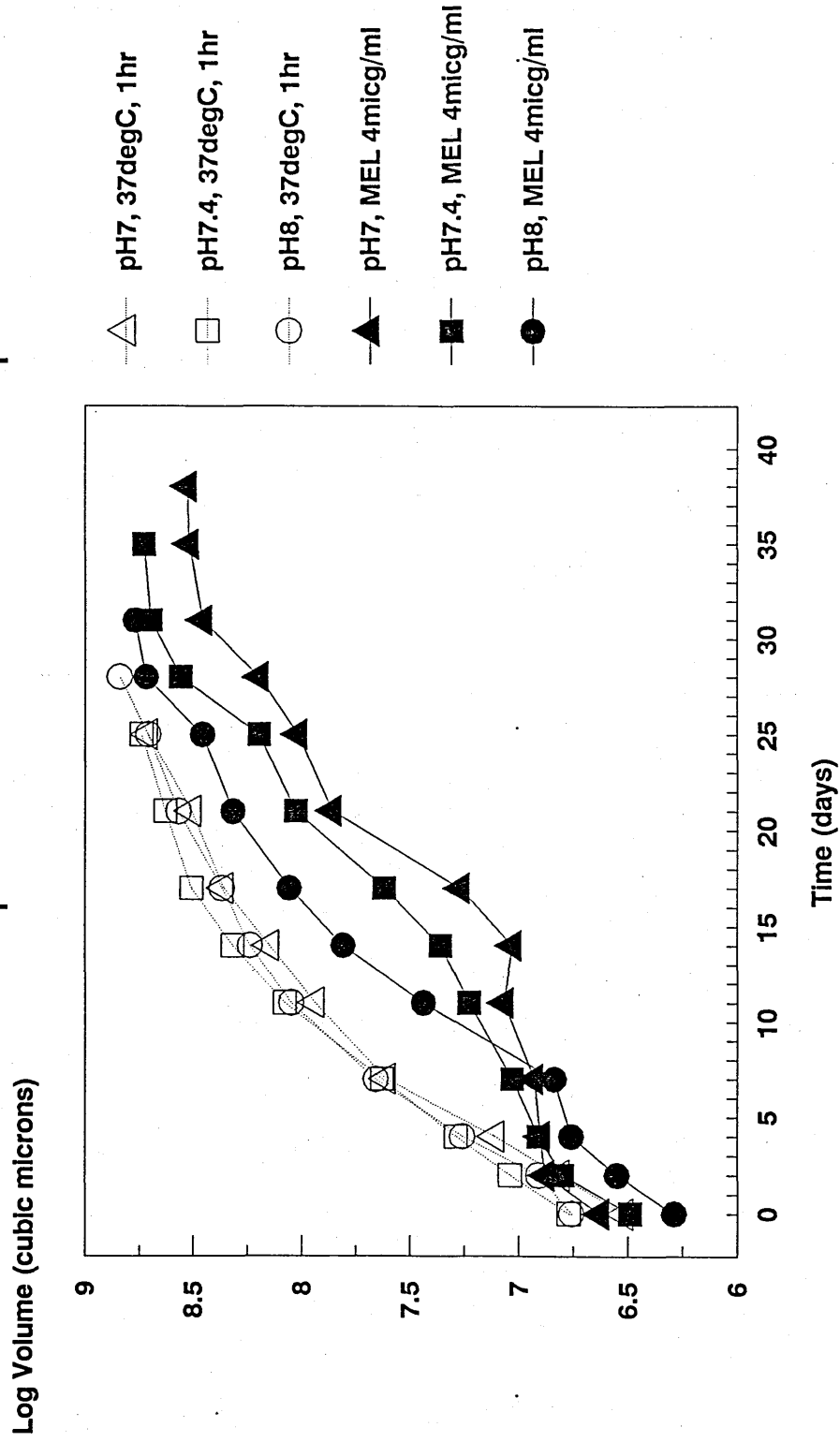


FIGURE 52B EXPERIMENT 26 pH and melphalan cytotoxicity:
SMALL B0008 melanoma spheroids (cf. Fig.51B, Experiment 25)

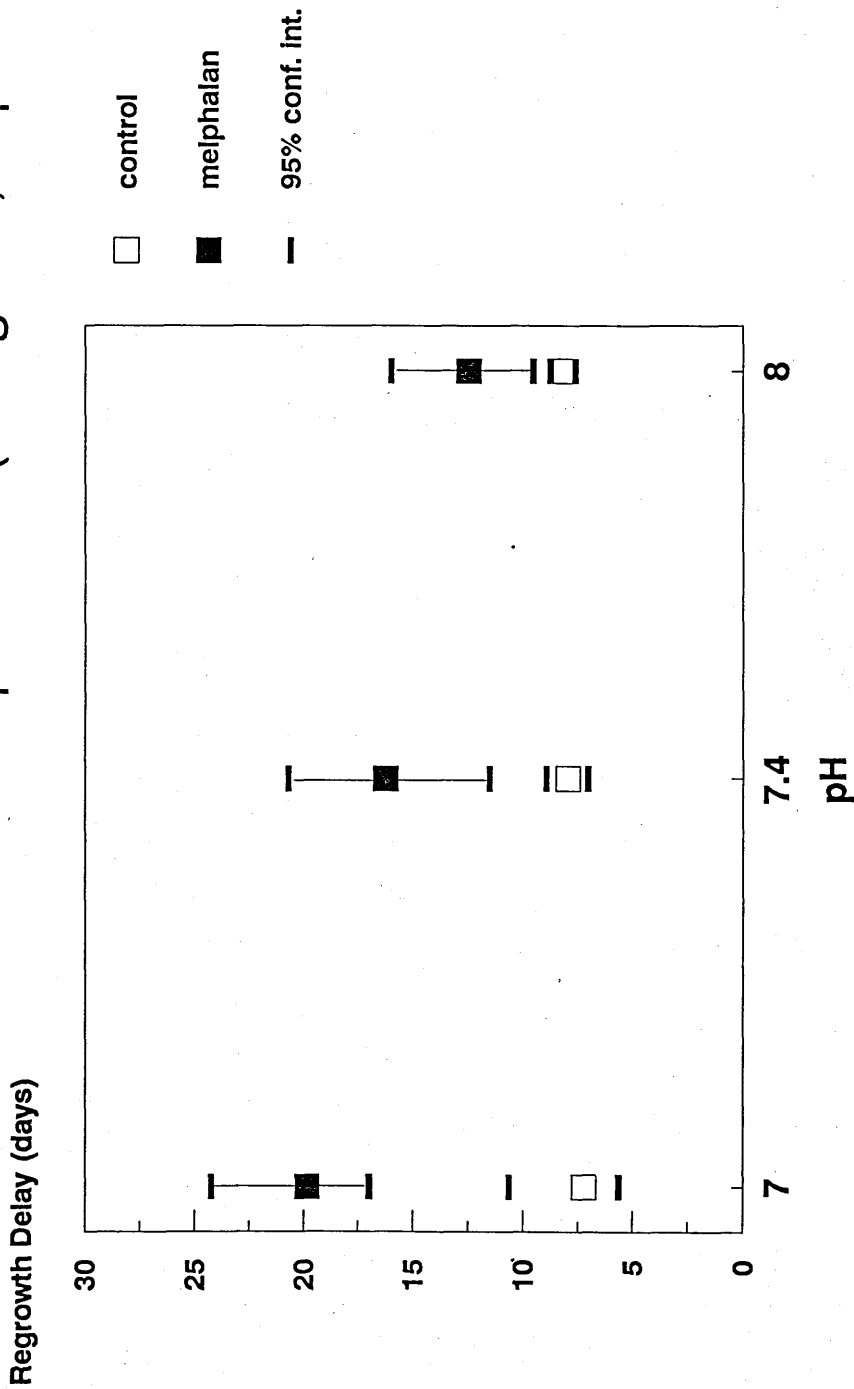


FIGURE 53 Percentage of B0008 spheroids "cured" by melphalan at 31-44.5degC in Experiments 7-19

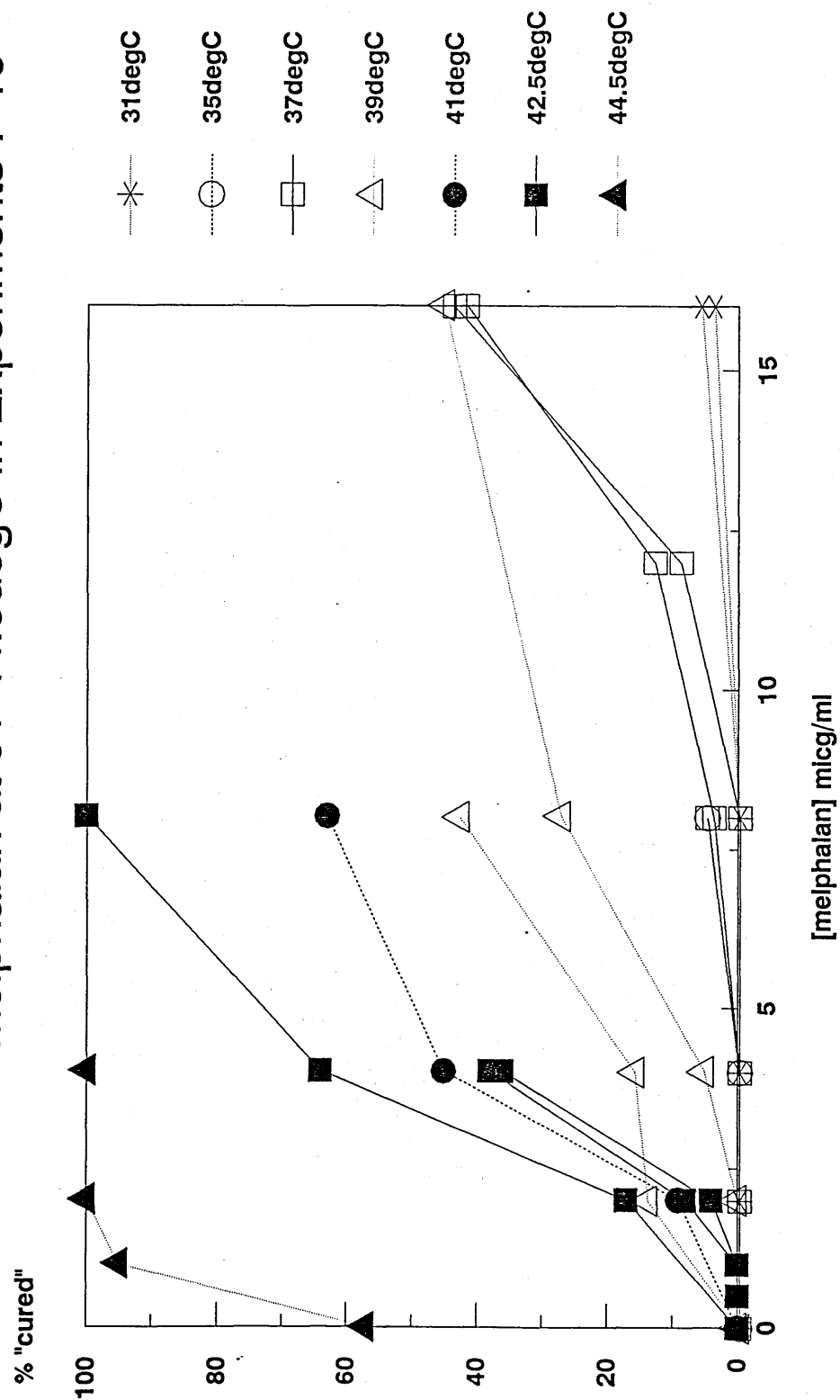


FIGURE 54 Dose/survival curve from clonogenic assay of disaggregated B0008 spheroids (Experiments 7-18)

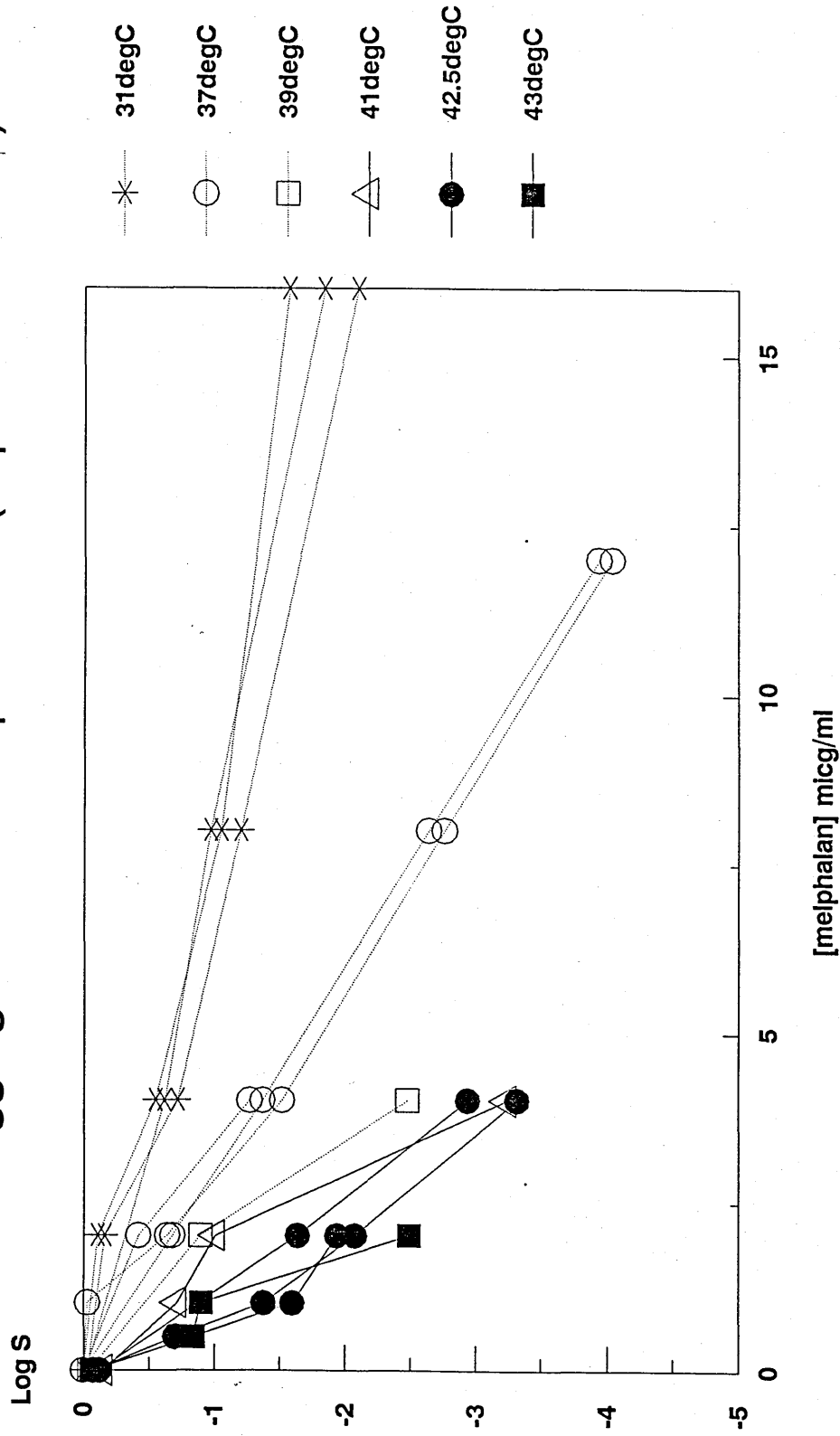
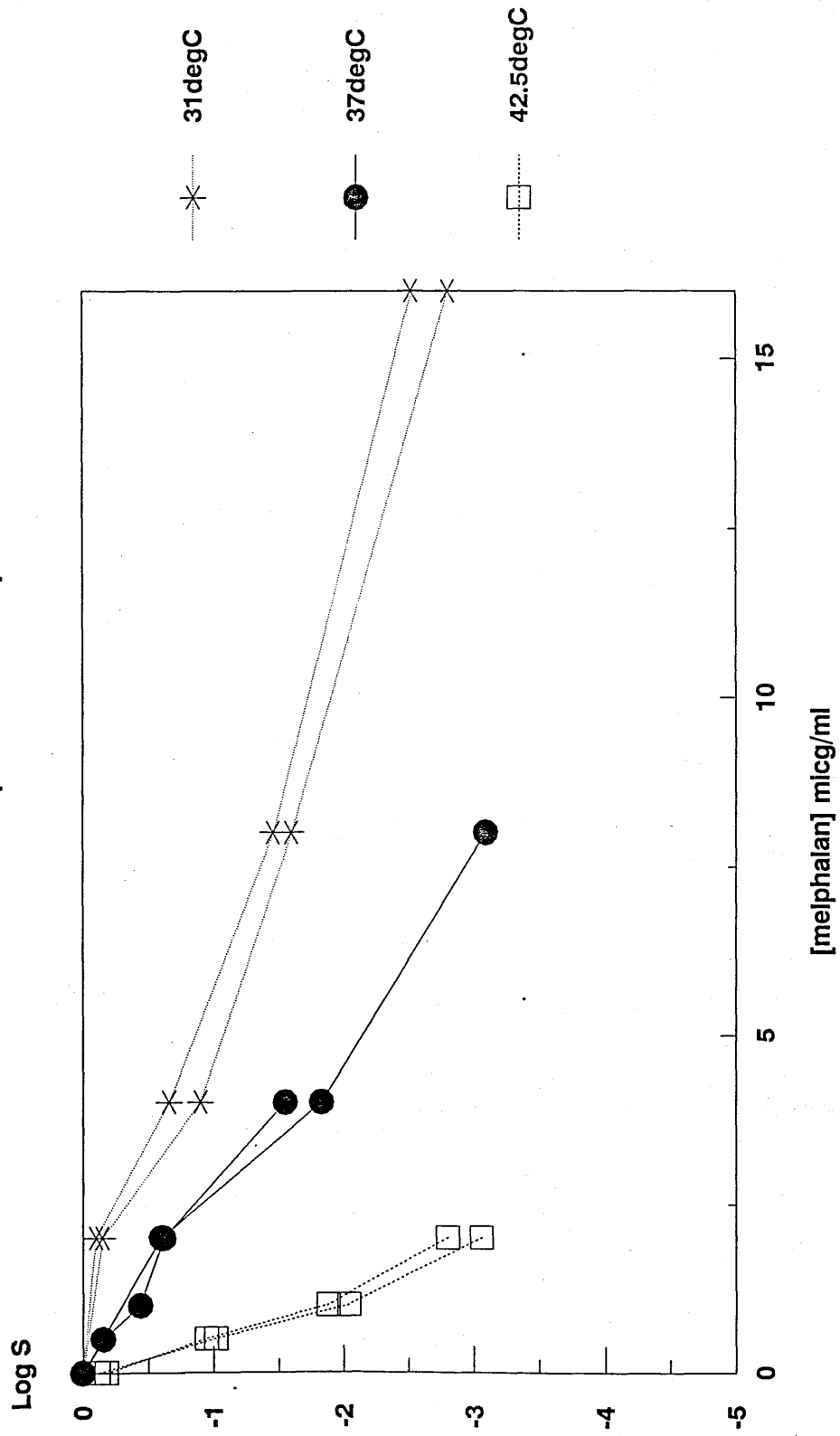


FIGURE 56 Dose/survival curves for B0008 monolayers treated in exponential phase



TABLES

Table 1

Classification of patients with dysplastic naevi proposed
by Kraemer and colleagues³⁶

Type A	-	Sporadic, no family history
Type B	-	Dysplastic naevi in family
Type C	-	Dysplastic naevi plus melanoma, but no family history of dysplastic naevi
Type D-1	-	One family member with melanoma and dysplastic naevi
Type D-2	-	2 or more family members with melanoma and dysplastic naevi

Table 2

Original three stage clinical staging system¹⁰⁷ for
cutaneous malignant melanoma

<u>Stage</u>	<u>Criteria</u>
I	Localised primary malignant melanoma or "local recurrence"
II	Metastases to regional lymph nodes or in transit metastases
III	Disseminated malignant melanoma, beyond draining lymph nodes

Table 3

M.D. Anderson clinical staging system¹⁰⁸ for cutaneous malignant melanoma*

<u>Stage</u>	<u>Criteria</u>
I	Localised primary melanoma A - Primary intact B - Primary locally excised C - Multiple primaries
II	Local recurrence or primary with local peripheral nodules <3cm
III	Regional metastases A - in transit B - lymph nodes AB - in transit plus lymph nodes
IV	Systemic metastases

* The original M.D. Anderson staging system distinguished between stage 0, "superficial melanoma" and stage I, "more deeply invasive melanoma"¹⁰⁸. Stage 0 has now been abandoned¹⁷¹ and all invasive melanoma is included in stage I.

Table 4

Clark levels of invasion in primary cutaneous malignant melanoma¹²⁷

<u>Level</u>	<u>Criteria</u>
I	Confined to the epidermis (in situ)
II	Invasion of the papillary dermis
III	Filling and distortion of the papillary dermis
IV	Invasion of the reticular dermis
V	Invasion of the sub-cutaneous fat

Table 5

Summary of study quoted as evidence in favour of "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors:	Milton, Shaw et al, 1982 ²²¹
Type of study:	Single centre, retrospective Non-randomised
Length of follow-up:	2-32 years, median not given
No. patients:	1319, Clinical Stage I,
Sites:	Axial and limb
No. Excision alone:	939
No. ELND:	380
No. ELND with involved nodes:	19 (5%)
Subgroups benefiting from ELND:	Males with limb lesions of Breslow 1.6-3mm
Morbidity of ELND:	"acceptably low"
Mortality associated with ELND:	2 deaths

Table 6

Summary of study quoted as evidence in favour of "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors:	Balch, Soong et al, 1982 ¹⁶⁸
Type of study:	Two centre, Sydney And Alabama Part prospective, non-randomised
Length of follow-up:	2-25 years, median not given
No. patients:	Alabama, 676 Clinical Stage I Sydney, 1110 Clinical Stage I
Sites:	Axial and limb
No. Excision alone:	Alabama 162 Sydney 703
No. ELND:	Alabama 163 Sydney 328
No. ELND with involved nodes:	Not given
Subgroups benefiting from ELND:	All Breslow 1.5-3.99mm Males Breslow 0.76-1.5mm
Morbidity of ELND:	No comment
Mortality associated with ELND:	No comment

Table 7

Summary of study quoted as evidence in favour of "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors:	McCarthy et al, 1985 ²³⁸
Type of study:	Single centre Part prospective, non-randomised
Length of follow-up:	10 year
No. patients:	2347 Clinical stage I
Sites:	trunk and limb
No. Excision alone:	1719
No. ELND:	628
No. ELND with involved nodes:	27 (4%)
? Benefit from ELND:	1.6-3mm Breslow primaries Men > women
Morbidity of ELND:	No comment
Mortality associated with ELND:	No comment

Table 8

Summary of study quoted as evidence in favour of "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors: Reintgen, Cox et al, 1983²²²

Type of study: Regional referral centre
(Surgical treatment before referral)
Retrospective

Length of follow-up: 2-10 years, median not given

No. patients: 613 with "complete staging"
Breslow 0.76-4mm, Clinical Stage I

Sites: Trunk and limb

No. Excision alone: 426

No. ELND: 187

No. ELND with involved nodes: Not given

? Benefit from ELND: Overall borderline statistical
significance, significant benefit in
intermediate thickness group

Morbidity of ELND: No comment

Mortality associated with ELND: No comment

Table 9

Summary of study quoted as evidence in favour of "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors: Fortner et al, 1977²³⁹

Type of study: Regional referral centre
Retrospective

Length of follow-up: 5-10 years

No. patients: 404 Clinical Stage I
No Breslow data

Sites: Trunk and limb

No. Excision alone: 145

No. ELND: 259

No. ELND with involved nodes: 40 (15%)

? Benefit from ELND: 9 patients had only micro-
metastases and 6/9 ("67%") survived 10 years

Morbidity of ELND: No comment

Mortality associated with ELND: No comment

Table 10

Summary of study quoted as evidence in favour of "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors: Wanebo et al, 1975¹³²

Type of study: Retrospective

Length of follow-up: 5-9 years

No. patients: 151 Clinical Stage I

Sites: Extremities

No. Excision alone: 38

No. ELND: 113

No. ELND with involved nodes: 14 (12.4%)

? Benefit from ELND: ? for Clark level III

Morbidity of ELND: No comment

Mortality associated with ELND: No comment

Table 11

Summary of study quoted as evidence against routine "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors: Veronesi, Adamus et al,1977²²⁵

Veronesi, Adamus et al,1982²²⁶

Type of study: Multi-centre Prospectively Randomised

Length of follow-up: updated to average 10.4 years²¹⁷

No. patients: 553

Stage I, inc. satellites within 5cm

Site: distal 2/3 of limb

No. Excision alone: 286

No. ELND: 267

No. ELND with involved nodes: 19.7%

? benefit from ELND: None

Morbidity of ELND: No comment

Mortality associated with ELND: No comment

Table 12

Summary of study quoted as evidence against routine "elective lymph node dissection" (ELND) in the management of cutaneous malignant melanoma.

Authors: Sim, Taylor et al, 1978²²³
Sim, Taylor et al, 1986²²⁷

Type of study: Prospective randomised,
single centre

Length of follow-up: Updated to 13 years (median 4.5)

No. patients: 171 Clinical Stage I

Site: 34 trunk, 137 limb

No. excision alone: 62

No. ELND: 55 delayed,
54 immediate

?benefit from ELND: None

Morbidity of ELND: lymphoedema 32/109
seroma 15/109
delayed healing 7/109

Mortality associated with ELND: Nil

Table 13

Objective response rates after isolated limb perfusion for
loco-regional advanced and recurrent malignant melanoma

<u>Reference</u>	<u>Number of pts</u>			<u>%</u>
	<u>total</u>	<u>CR</u>	<u>PR</u>	
Name(date) ^{No}				CR+PR [#]
Bulman (1980) ³³⁸	29	?	?	48
Rosin (1980) ²¹¹	80	21	29	62
Jonsson (1983) ³⁶⁰	15	1	10	73
Lejeune (1983) ³³⁹	23	15	6	91
Vaglioni (1985) ³⁴¹	32	18	8	81
Storm (1985) ³⁴⁰	26	21	0	81
Kroon (1987) ³⁴⁴	18	7	8	83

#CR = Complete response)
PR = Partial response) U.I.C.C. Criteria (see I.5.4)

Table 14

Survival after isolated limb perfusion for loco-regional advanced and recurrent malignant melanoma

<u>Reference</u> Name(date) ^{No}	<u>No.patients</u>	<u>Stage</u>	<u>Survival</u>
Krementz (1986) ³¹⁸	39	II	68% 5YS
Stehlin (1979) ³⁵²	27	II-IIIAB	52.5% 5YS
Shingleton (1975) ³³⁴	43	II-IIIAB	28% 5YS
Rosin (1980) ²¹¹	130	II-IIIAB	34% 3YS
Stehlin (1979) ³⁵²	30*	IIIA	74% 5YS
Krementz (1986) ³¹⁸	70	IIIA	29% 5YS
Storm (1985) ³⁴⁰	26	IIIA-IIIAB	25% 3YS
Krementz (1986) ³¹⁸	96	IIIAB	31% 5YS
Hartley# (1987) ³⁴⁷	22	II-IIIA	58% 5YS
	26	IIIB	70.5% 5YS
	17	IIIAB	29% 5YS
Baas (1988) ³⁴⁸	36	II	74% 5YS
	45	IIIA	67% 5YS
	28	IIIAB	40% 5YS

*Subgroup treated by "hyperthermic" limb perfusion

#For comparison data converted according to M.D. Anderson staging system

Table 15

Survival after surgery plus adjuvant isolated limb perfusion for primary melanoma

<u>Reference</u> ^{No} <u>(date)</u>	<u>No.</u> <u>Pts.</u>	<u>Criteria</u> <u>for ILP</u>	<u>Controls</u>	<u>Survival</u> [#]
Sugarbaker ³⁵⁷ (1976)	92	>Clark III	Yes (Historical	83% 10YS 57% 10YS)
Tonak ³⁶⁵ (1979)	51	Stage I	Yes (Historical	98% 3YS 72% 3YS)
Koops ³⁶² (1981)	110	>1.5mm*	None	78% 5YS
Janoff ³⁶¹ (1982)	122	Stage I	None	**81% 5YS
Rege ³⁶⁴ (1983)	39	Stage I	Yes (Concurrent	86% 10YS 55% 10YS)
Martijn ³³¹ (1986)	91 (84)	>1.5mm*	Groningen (v. Sydney	75% 10YS 59% 10YS)
Krementz ³¹⁸ (1986)	381	Stage I	None	77% 10YS
Franklin ³⁶⁷ (1988)	227 (238)	Stage I	Groningen (v. Westphalia	75% 10YS N.S.)

* Clark IV or V

** disease-free

YS = years survival,

Control results in (....)

Table 16

Clinical details of 42 candidates for therapeutic isolated limb perfusion

SEX

10 men 32 women

AGE (whole group)

mean 59.5 years (S.D. 12.4), range 33-79

CLINICAL STAGE (M.D. Anderson)

Stage	No. pts.
II	3
IIIA	12
II-III A	13
IIIAB	12
IIIB	1
IV	1

TIME SINCE TREATMENT OF PRIMARY MELANOMA

median 18 months, (range 1-156 months)

FURTHER TREATMENT SINCE DEFINITIVE SURGERY FOR PRIMARY

a) Surgery:

	<u>No. procedures since primary surgery</u>							
	0	1	2	3	4	5	7	18
No. pts.	7	16	8	6	2	1	1	1

b) Chemotherapy: Six patients, (none given melphalan)

c) Radiotherapy: One patient

Table 17

Clinical details of 15 patients treated by adjuvant
isolated limb perfusion

SEX

4 men

11 women

AGE

mean 48.8 years (S.D. 12.9), range 24-69

SITE OF PRIMARY

	thigh	leg	foot	subungual
No. Pts.	2	8	3	2

BRESLOW THICKNESS

mean 4.3mm (S.D. 1.74), range 2.3-7.5

Table 18

Response rates to therapeutic isolated limb perfusion

a) in all 21 cases with measurable tumour treated by therapeutic perfusion (including 4 repeat perfusions)

	#CR	PR	NR	Total
No. pts. (%)	9 (43)	11 (52)	1 (5)	21 (100)

b) in the 17 cases where ILP was performed for the first time

	#CR	PR	NR	Total
No. pts. (%)	9 (52)	8 (48)	0	17 (100)

#KEY

CR = Complete response)
 PR = Partial response) U.I.C.C. Criteria (see I.5.4)

NR = No or minimal Response

Table 19

Pattern of recurrent disease in 38 patients treated by
therapeutic isolated limb perfusion

<u>Site of Subsequent Recurrence</u>	<u>No.Pts.</u>	<u>Time in months median (range)</u>
None	16	8 (4-33)
Limb only	3	8 (5-8)
Limb+node	1	8
Limb + systemic	5	8 (6-10) + 10 (8-24)
Limb + node + systemic	2	2+7+7, 4+4+10
Node + systemic	2	6+10, 7+9
Systemic	9	10 (1-24)

Table 20

Recurrence and metastasis after adjuvant isolated limb perfusion in 15 patients (cf. Table 15)

<u>Site of recurrence</u>	<u>No.Pts.</u>	<u>Time in months</u> median (range)
None, disease-free	11	29 (6-37)
Nodes	3	8 (3-26)
Limb	1	9

Table 21

Physiology of isolated limb perfusion

a) Mean flow rate during the hour of perfusion (57 perfusions).

	ml/min
Overall mean	368
Std. Deviation	136
Range	98-700

b) Tissue temperatures before and during isolated limb perfusion.

i) Calf muscle versus skin temperatures AUCs (11 patients)

<u>Patient No.</u>	<u>Skin Temp. AUC</u> (°C.min)	<u>Muscle Temp. AUC</u> (°C.min)
2	3621.75	3562.5
4	3273.75	3262.5
5	3346.5	3411.75
6	3225.75	3450
7	3189	3186
8	3317.25	3342.75
9	3576.75	3462.75
10	3575.25	3459.75
11	3248.25	3229.5
12	3288	3250.5
13	3288.75	3289.5

Wilcoxon signed rank test, value 25, N.S.

ii) Limb surface temperatures (60 perfusions)

	<u>mean temperature °C (Std.dev.)</u>		
	Pre-ILP	Start ILP	End ILP
Calf	32.9 (1.6)	36.9 (1.2)	38.4 (1.4)
Thigh	32.8 (1.8)	36.7 (1.6)	38 (1.2)

Table 22

Physiology of isolated limb perfusion (continued from Table 21)

c) Gas tensions and acid/base status in the perfusate

i) Mean ARTERIAL values (Std. dev.) n = 22 perfusions

	<u>Time in minutes</u>		
	<u>15</u>	<u>30</u>	<u>55</u>
P _{O2} mmHg	408 (50)	409 (58)	421 (62)
P _{CO2} mmHg	21 (6.2)	18.6 (6.3)	16.4 (5.9)
pH	7.49 (0.06)	7.55 (0.1)	7.61 (0.1)
Base excess	-5 (2.1)	-3 (1.9)	-1.3 (2.1)

ii) Mean VENOUS values (Std. dev.) n = 22 perfusions

	<u>Time in minutes</u>		
	<u>15</u>	<u>30</u>	<u>55</u>
P _{O2} mmHg	49.4 (8.5)	44.9 (7.5)	40.3 (10)
P _{CO2} mmHg	26.5 (5.7)	23.5 (6.2)	19.9 (5.7)
pH	7.42 (0.05)	7.49 (0.1)	7.56 (0.1)
Base excess	-4.8 (2)	-3.1 (1.9)	-1 (2)

Table 23

 Complications and toxicity in 61 isolated limb perfusions

a) General Complications

Problem	No. Patients	Comments
Deep venous thrombosis	2	one week and two months after ILP
Bleeding	1	wound re-explored immediately post-op
Angina and postural hypotension	1	woman history of myocardial infarct <12 months prev.
Post-operative confusional state	1	patient died two months later, brain secondaries

b) Regional Toxicity

Problem	No. Patients	Comments
Severe cellulitis and desquamation	2	1 with interdigital sepsis
Nerve symptoms	3	1 arm pain 1 dysaesthesiae 1 femoral nerve palsy -> temporary quadriceps paresis
Muscle symptoms	1	severe pain, swelling, calf tenderness and late temporary foot drop
Thermal burns	1	due to mal-function of thermistor probe

c) Systemic Toxicity

Problem	No. Patients	Comments
Leucopenia (<3x10 ⁹ /litre)	6	usually delayed 1 with late alopecia
Thrombocytopenia	1	<100x10 ⁹ /litre
Pancytopenia	1	Haemoglobin 8.6g/dl, Platelets 23, WBC 1

Table 24

Scottish Melanoma Group 5-year survival rates for patients registered in 1979⁷³.

<u>Breslow thickness</u>	<u>No. patients</u>	<u>5yr. survival</u>
0-1.49mm	92	93%
1.5-3.49mm	78	67%
3.5mm and over	81	37%
ALL patients	251	62%

Table 25

Wieberdink grading system for description of the reaction of normal tissues[#] to isolated limb perfusion with melphalan⁴⁰⁰

Grade	Description of reaction
I	No subjective or objective evidence of reaction
II	Slight erythema and /or oedema
III	Considerable erythema and or oedema with some blistering; slightly disturbed motility possible
IV	Extensive epidermolysis and/or obvious damage to the deep tissues, causing definite functional disturbances; threatening or manifest compartment syndromes
V	Reaction which may necessitate amputation

[#]Peak reaction determines grading

Table 26

MELPHALAN PHARMACOKINETICS IN ISOLATED LIMB PERFUSION:

Group A results, 11 patients treated with melphalan 1.5mg/kg body weight, given as a single bolus (see also Fig.13, 14 & 18).

Tabulated values are in $\mu\text{g/ml}$.

Time of Sample (mins.)	LIMB PERFUSATE			
	ARTERIAL		VENOUS	
	[melphalan] mean (std.dev.)	S.E.M.	[melphalan] mean (std.dev.)	S.E.M.
2	54.2 (35.6)	11.3	74.5 (30.3)	9.6
5	45 (14.9)	4.5	40 (12.3)	3.7
10	33.3 (8.9)	2.7	29.3 (9.1)	2.7
15	24.2 (10)	3	23.5 (11)	3.3
20	19.4 (7.1)	2.1	16.7 (6.6)	1.98
25	15.3 (5.1)	1.53	13.9 (3.6)	1.09
30	14 (5.1)	1.61	12.6 (4.3)	1.3
35	12.7 (4.8)	1.45	11.9 (4.3)	1.29
40	11.7 (3.7)	1.12	9.1 (2.7)	0.83
45	9.81 (3.3)	0.98	8.7 (2)	0.6
50	8.35 (3.8)	1.14	8.37 (4.35)	1.31
60	8.1 (4.1)	1.24	6.75 (2.7)	0.81

Time of Sample (mins)	SYSTEMIC BLOOD	
	[melphalan] mean (std.dev.)	S.E.M.
0	0	
15	0.25 (0.37)	0.11
30	0.35 (0.47)	0.14
60	0.27 (0.39)	0.12
75	0.29 (0.33)	0.1
90	0.58 (0.58)	0.17
120	0.76 (0.93)	0.28
150	0.61 (0.67)	0.2
180	0.4 (0.63)	0.19
240	0.31 (0.55)	0.17
300	0.19 (0.42)	0.13

Table 27

MELPHALAN PHARMACOKINETICS IN ISOLATED LIMB PERFUSION:
 Group B results, 9 patients treated with melphalan
 1.75mg/kg body weight, given as a single bolus (see also
 Figs. 13& 14).

Tabulated values are in $\mu\text{g/ml}$.

Time of Sample (mins.)	LIMB PERFUSATE			
	ARTERIAL		VENOUS	
	[melphalan] mean (std.dev.)	S.E.M.	[melphalan] mean (std.dev.)	S.E.M.
2	156.2 (128)	42.6	91.6 (58.9)	19.6
5	75.3 (44.4)	14.8	66.1 (33.5)	11.2
10	58.9 (40.5)	13.5	45.3 (20.2)	6.7
15	40.7 (17.7)	5.9	37.1 (15.5)	5.2
20	32.3 (14.2)	4.7	32.4 (13.1)	4.37
25	29.7 (15.5)	5.17	26.1 (11.8)	3.93
30	25.4 (13.8)	4.6	24.5 (12.9)	4.3
35	22.9 (10.7)	3.57	20.6 (8.11)	2.7
40	21.6 (10.7)	3.57	18.8 (9)	3
45	19.8 (8.61)	2.87	17.5 (7.5)	2.5
50	17.8 (10.3)	3.43	16.3 (8.3)	2.77
60	17.7 (9.88)	3.29	13.6 (7.1)	2.37

Time of Sample (mins)	SYSTEMIC BLOOD	
	[melphalan] mean (std.dev.)	S.E.M.
0	0	
15	0.02 (0.06)	0.02
30	0.02 (0.05)	0.02
60	0.03 (0.08)	0.03
75	0.05 (0.08)	0.03
90	0.5 (0.28)	0.09
120	0.53 (0.37)	0.12
150	0.43 (0.57)	0.19
180	0.39 (0.54)	0.18
240	0.37 (0.64)	0.21
300	0.4 (0.93)	0.31

Table 28

MELPHALAN PHARMACOKINETICS IN ISOLATED LIMB PERFUSION:
 Group C results, 6 patients treated with melphalan given
 in three aliquots at 0,15, and 30 minutes, to total dose
 of 1.75mg/kg body weight.(see also Fig. 20).

Tabulated values are in $\mu\text{g/ml}$.

Time of Sample (mins.)	LIMB PERFUSATE			
	ARTERIAL		VENOUS	
	[melphalan] mean (std.dev.)	S.E.M.	[melphalan] mean (std.dev.)	S.E.M.
2	41.8 (23.1)	9.4	29.1 (19.5)	7.96
5	25.9 (8.4)	3.43	23.3 (7.3)	2.98
10	20.5 (7.8)	3.18	17.5 (7)	2.86
15	17.9 (6.8)	2.78	15 (5)	2.04
20	44 (22.8)	9.3	43.4 (24.6)	10.04
25	31 (10.9)	4.45	30 (11.7)	4.78
30	24.7 (8.1)	3.31	22.1 (7.4)	3
35	44 (16)	6.5	41.6 (13.9)	5.68
40	36.3 (11)	4.49	30.9 (10)	4.08
45	29.3 (9)	3.67	26.1 (8)	3.27
50	25.8 (7.27)	2.97	23.3 (7.2)	2.94
60	20.4 (6.49)	2.65	19.2 (6.1)	2.49

Time of Sample (mins)	SYSTEMIC BLOOD	
	[melphalan] mean (std.dev.)	S.E.M.
0	0	
15	0	
30	0	
60	0.1 (0.15)	0.06
75	0.1 (0.19)	0.08
90	0.4 (0.25)	0.1
120	0.31 (0.21)	0.09
150	0.17 (0.19)	0.08
180	0.1 (0.15)	0.06
240	0.03 (0.06)	0.02
300	0	

Table 29

MELPHALAN PHARMACOKINETICS IN ISOLATED LIMB PERFUSION:
Dose of melphalan, perfusate AUC_a and AUC_v data for individual patients in Groups A, B and C compared with the $AUC_{0-\infty}$ of systemic concentrations of melphalan (AUC_s).

The unit for AUC is the $\mu\text{g}\cdot\text{min}/\text{ml}$

<u>Group A</u> (1.5mg/kg bolus)				
	Dose (mg)	AUC_a	AUC_v	AUC_s
	100	597	504	26.5
	75	780	932	48.9
	100	919	848	37.5
	125	1140	957	30
	150	806	748	246
	70	1011	579	287
	75	858	869	402
	100	1324	1360	92.3
	125	1229	1187	98.2
	130	1158	1087	9.5
	100	1508	1105	29.4
<u>median</u>	100	1011	932	48.9
<u>Group B</u> (1.75mg/kg bolus)				
	90	1204	984	361
	165	1912 *	1680	231
	140	1649 *	2083	26.4
	70	1144	900	9.5
	135	4011	3056	74.3
	140	2720	2030	70.1
	105	1758	1570	34.6
	90	1019	963	27.2
	120	1275	1425	24.9
<u>median</u>	120	1649	1570	34.6
<u>Group C</u> (1.75mg/kg divided dose)				
	175	2116 **	1903	62.8
	84	1443 *	1326	56.4
	100	1129	1018	8.6
	100	988	902	9.7
	120	2128	2024	25.2
	89	2180	1950	35.7
<u>median</u>	100	1779	1614	30.5

Wieberdink Toxicity:
(cf. Table 25)

* Grade III
** Grade IV

Table 30

a) Summary of the values obtained for the best-fit lines describing concentration time curves for melphalan in arterial and venous perfusate in Groups A and B (Fig.14 and see III.6.2.)

	Parameter			
	A	α	B	β
<u>Group A</u>				
Arterial	48.9	0.1	16.8	0.01
Venous	42.8	0.09	13.7	0.01
<u>Group B</u>				
Arterial	86.5	0.09	21.4	0.003
Venous	66.5	0.19	44.3	0.02

b) Values for half-life of alpha and beta phases of the fitted lines ($t_{1/2}\alpha$ and $t_{1/2}\beta$) for arterial and venous perfusate in Groups A and B (Fig.14).

	$t_{1/2}\alpha$ (minutes)	$t_{1/2}\beta$ (minutes)
<u>Group A</u>		
Arterial	6.85	54.2
Venous	7.58	60.4
<u>Group B</u>		
Arterial	7.72	180
Venous	3.68	33.7

Table 31

Regional toxicity in patients treated by isolated limb perfusion in pharmacokinetics studies (cf. Tables 23 & 29)

Wieberdink ⁴⁰⁰ Toxicity Grade	No. Patients		
	Group A (1.5mg/kg)	Group B* (1.75mg/kg)	Group C# (1.75mg/kg)
I	1	0	0
II	10	7	4
III	0	2	1
IV	0	0	1
V	0	0	0

KEY

* bolus dose

divided dose

Table 32

MELPHALAN PHARMACOKINETICS IN ISOLATED LIMB PERFUSION:

Calculated uptake of melphalan by tissues of the leg according to Formula (2): $MEL_{ex} = (AUC_a - AUC_v) \times Q$ (see III.5.6. for derivation). In the clinical groups, mean values for AUC and flow are used.

Group A (n=11)

$$MEL_{ex} = (1030 - 925) \times 443 = 46.5\text{mg}$$
$$= 44\% \text{ of mean dose}^*$$

Group B (n=9)

$$MEL_{ex} = (1855 - 1633) \times 316 = 70\text{mg}$$
$$= 60\% \text{ of mean dose}^*$$

Group C (n=6)

$$MEL_{ex} = (1664 - 1520) \times 331 = 47.6\text{mg}$$
$$= 43\% \text{ of mean dose}^*$$

* Each of these values appears to be an overestimate (III.7.5), unless one proposes that there is very avid uptake of melphalan by the tissues of the leg. Hence the same calculation is applied to the 39°C mock perfusion data:

$$MEL_{ex} = (3761 - 3705) \times 400 = 22\text{mg}$$
$$= 22\% \text{ of dose, which is the proportion "lost" to the cellular components of perfusate and to the perfusion circuit.}$$

Table 33

MELPHALAN PHARMACOKINETICS IN ISOLATED LIMB PERFUSION:
Calculation of melphalan extraction from the perfusate,
according to the AUC_{0-30} and AUC_{30-60} , used in Equation
(2).

Group A

0-30 minutes $MEL_{ex} = (722 - 648) \times 443 = 32.8mg^{\#}$

30-60 minutes $MEL_{ex} = (308 - 277) \times 443 = 13.7mg^{\#}$

Group B

0-30 minutes $MEL_{ex} = (1248 - 1097) \times 316 = 47.7mg^{\#}$

30-60 minutes $MEL_{ex} = (607 - 536) \times 316 = 22.4mg^{\#}$

Group C

0-30 minutes $MEL_{ex} = (759 - 701) \times 331 = 19.2mg^{\#}$

30-60 minutes $MEL_{ex} = (905 - 819) \times 331 = 28.5mg^{\#}$

These data are uncorrected (cf. Table 32)

Table 34

Tissue concentrations of melphalan at 55minutes during isolated limb perfusion.

Patient	[melphalan] $\mu\text{g/g}$		
	Tumour	Fat	Skin
a)	4.83	1.02	5.36
b)	2.29	0.885	2.2
c)	3.76	0.48	4.95
d)	0.66	1	1.43
e)	1.68	1.045	-
f)	7.85	2.11	5.4
g)	2	1.19	-
h)	3.04	1.56	-
i)	4.64	0.87	2.82
j)	3.2	1.43	1.9
k)	2.8	0.96	3.19
l)	0.74	3.4	4.5
m)	6.125	-	3.25
n)	6.5	3.5	7.16
o)	0.88	1.3	3.78

Statistical comparisons by Wilcoxon Signed Rank Test:

Tumour versus fat, $p < 0.01$

Tumour versus skin, not significant

Table 35

Studies of experimental chemotherapy with single agents or combinations, in which multicellular tumour spheroids of human origin have been used.

<u>Drug</u>	<u>Cell line</u>	<u>Reference</u> (<u>First author, year</u>)
Adriamycin	* Various including melanoma xenografts	Jones, 1982 ⁵¹⁸
	Bladder carcinoma	Erlichman, 1984 ⁵¹⁹
	# Melanoma and squamous lung cancer	Kohno, 1987 ⁵²⁰
	(+ 4'deoxyrubicin) L-DAN (lung tumour)	Kerr, 1986 ⁴⁸³
CCNU	*	Jones, 1982 ⁵¹⁸
Cis-platinum	*	Jones, 1982 ⁵¹⁸
	#	Kohno, 1987 ⁵²⁰
5-fluorouracil	U118 (glioma) Hth-7 (thyroid ca.)	Nederman, 1984 ⁵²¹
Melphalan	*	Jones, 1982 ⁵¹⁸
Methotrexate	osteo-sarcoma	West, 1980 ⁵²²
NY 3170 (1-propargyl-5-chloro-pyrimidin-2-one)	NHIK 3025 (cervical cancer)	Wibe, 1981 ⁵²³
Vinca alkaloids	NHIK 3025	Wibe, 1980 ⁵²⁴ & 1981 ⁵²³ .
	U118, Hth-7	Nederman, 1981 ⁴⁶⁹ & 1984 ⁵²¹
	*	Jones, 1982 ⁵¹⁸
	#	Kohno, 1987 ⁵²⁰

Table 36

Supplemented Eagle's MEM, as used for routine cell culture
in all experiments.

Ingredient [#]	Quantity
Eagle's MEM (Hepes 25mM)	450ml
Foetal Calf Serum	50ml
L-glutamine (200mM)	5ml
Penicillin/Streptomycin Solution (penicillin 10,000iu/ml + streptomycin 10,000µg/ml)	5ml
Amphotericin B in water (250µg/ml)	5ml

[#]All ingredients supplied by Gibco, Paisley, Scotland

Table 37A

Growth of B0008 melanoma spheroids in Experiment 1

Plate No. 'Treatment': 60 minutes at 37°C in...

1	MEM
2	MEM + melphalan 6 μ g/ml
3	MEM + solvent/diluent (= 32 μ g/ml)
4	MEM + melphalan 12 μ g/ml
5	MEM + solvent (=32 μ g/ml)
6	MEM + melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.91 (6.76-7.06)	7.05 (6.9-7.2)
3	7.42 (7.26-7.58)	7.16 (7.02-7.32)
5	7.74 (7.66-7.89)	7.13 (7.06-7.34)
9	8.2 (8.13-8.29)	7.19 (7-7.34)
12	8.41 (8.38-8.5)	7.21 (7.08-7.5)
16	8.61 (8.59-8.68)	7.33 (6.96-7.92)
19	8.73 (8.71-8.76)	7.52 (7-8.18)
23		8.05 (7.21-8.48)
26		8.26 (7.59-8.56)
30		8.41 (7.99-8.75)
33		8.55 (8.19-8.88)
37		8.79 (8.39-8.88)
	PLATE 3	PLATE 4
0	7 (6.87-7.12)	7.02 (6.96-7.12)
3	7.43 (7.3-7.56)	7.14 (7.03-7.25)
5	7.72 (7.62-7.8)	7.04 (6.88-7.22)
9	8.16 (8.07-8.21)	7.03 (6.94-7.2)
12	8.45 (8.42-8.51)	7.08 (6.94-7.18)
16	8.64 (8.59-8.66)	7 (6.84-7.13)
19	8.73 (8.71-8.77)	7.08 (6.92-7.21)
23		7.09 (6.88-7.51)
26		7.01 (6.9-7.86)
30		7.05 (6.87-8.1)
33		7.2 (6.91-8.24)
	PLATE 5	PLATE 6
0	7.04 (6.95-7.13)	7.02 (6.75-7.27)
3	7.44 (7.29-7.55)	7.14 (6.86-7.27)
5	7.66 (7.56-7.86)	6.94 (6.71-7.19)
9	8.16 (8.02-8.28)	6.87 (6.65-7.16)
12	8.4 (8.3-8.53)	7.12 (6.61-7.17)
16	8.65 (8.54-8.68)	6.92 (6.57-7.21)
19	8.78 (8.7-8.81)	6.92 (6.65-7.25)
23		6.87 (6.67-7.14)
26		6.97 (6.63-7.2)
30		6.96 (6.61-7.65)
33		7.05 (6.55-7.89)

Table 37B

Regrowth delay of spheroids in Experiment 1

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.37	(5.94-7.17)
2	22.38	(20.13-30.75)
3	7.61	(7.11-7.89)
4	1000	(29.77-1000)
5	7.92	(7.51-8.56)

Table 37C

Proportion of spheroids "cured" in Experiment 1

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/22	0
2	3/24	13
3	0/22	0
4	13/23	57
5	0/23	0
6	14/21	67

Table 38A

Growth of B0008 melanoma spheroids in Experiment 2

Plate No. 'Treatment': 60 minutes at 37°C in...

1	MEM without amphotericin-B
2	MEM
3	as 1, with melphalan 4 μ g/ml
4	as 2, with melphalan 4 μ g/ml
5	as 1, with melphalan 8 μ g/ml
6	as 2, with melphalan 8 μ g/ml

MEDIAN LOG VOLUME (\geq 95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.02 (6.88-7.13)	7.01 (6.91-7.10)
3	7.62 (7.51-7.71)	7.65 (7.55-7.68)
6	8.05 (7.96-8.13)	8.04 (7.98-8.12)
10	8.41 (8.37-8.49)	8.44 (8.39-8.49)
13	8.61 (8.53-8.64)	8.62 (8.53-8.66)
17	8.76 (8.73-8.8)	8.78 (8.74-8.81)

	PLATE 3	PLATE 4
0	7.05 (6.92-7.17)	7.04 (6.96-7.09)
3	7.42 (7.3-7.46)	7.42 (7.32-7.46)
6	7.66 (7.5-7.73)	7.59 (7.52-7.71)
10	8.02 (7.93-8.11)	7.94 (7.82-8.05)
13	8.22 (8.06-8.36)	8.15 (8.01-8.27)
17	8.53 (8.36-8.62)	8.46 (8.35-8.59)
21	8.73 (8.64-8.81)	8.63 (8.54-8.72)
24		8.73 (8.73-8.88)

	PLATE 5	PLATE 6
0	7.09 (7.05-7.23)	6.95 (6.84-7.03)
3	7.41 (7.34-7.52)	7.29 (7.17-7.38)
6	7.47 (7.43-7.64)	7.4 (7.31-7.53)
10	7.57 (7.57-7.74)	7.48 (7.35-7.59)
13	7.64 (7.64-7.94)	7.61 (7.42-7.69)
17	8.08 (8.08-8.32)	8.01 (7.72-8.13)
21	8.28 (8.28-8.55)	8.27 (8.13-8.37)
24	8.46 (8.46-8.74)	8.47 (8.37-8.56)
27	8.59 (8.59-8.88)	8.6 (8.56-8.71)
31		8.74 (8.7-8.88)

Table 38B

Regrowth delay of B0008 melanoma spheroids in Experiment 2

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.04	(5.25-6.63)
2	5.92	(5.35-6.81)
3	10.94	(9.64-11.76)
4	12.44	(10.39-13.44)
5	16.34	(15.11-16.84)
6	17.47	(16.09-18.97)

Table 38C

Proportion of spheroids "cured" in Experiment 2

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/21	0
3	0/21	0
4	0/22	0
5	0/24	0
6	0/23	0

Table 39A

Growth of B0008 melanoma spheroids in Experiment 3

Plate No.	'Treatment': 60 minutes ...
1	at 37°C in MEM
2	at 37°C in FZ-free MEM
3	at 43°C in MEM
4	at 43°C in FZ-free MEM
5	at 43°C in MEM + solvent (=32µg/ml)
6	at 43°C in FZ-free MEM + solvent (as 5)

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.8 (6.75-6.87)	6.82 (6.76-6.89)
3	7.26 (7.2-7.38)	7.24 (7.18-7.34)
6	7.76 (7.69-7.85)	7.8 (7.7-7.92)
9	8.15 (8.09-8.24)	8.14 (8.11-8.18)
13	8.47 (8.44-8.53)	8.5 (8.46-8.51)
16	8.65 (8.6-8.72)	8.63 (8.58-8.7)
20	8.87 (8.79-8.88)	8.88 (8.88-8.88)
	PLATE 3	PLATE 4
0	6.83 (6.73-6.9)	6.85 (6.74-6.88)
3	7.14 (7.06-7.24)	7.18 (7.08-7.28)
6	7.89 (7.62-8.37)	7.84 (7.61-7.94)
9	8.02 (7.96-8.07)	8.1 (8-8.12)
13	8.4 (8.34-8.48)	8.42 (8.37-8.45)
16	8.59 (8.55-8.65)	8.61 (8.54-8.69)
20	8.76 (8.73-8.82)	8.78 (8.75-8.84)
	PLATE 5	PLATE 6
0	6.88 (6.78-7)	6.84 (6.76-6.89)
3	7.22 (7.17-7.32)	7.2 (7.14-7.28)
6	7.73 (7.68-7.77)	7.78 (7.67-7.79)
9	8.09 (8.07-8.15)	8.12 (8.04-8.17)
13	8.43 (8.4-8.47)	8.44 (8.39-8.47)
16	8.64 (8.59-8.69)	8.59 (8.57-8.64)
20	8.77 (8.73-8.82)	8.82 (8.76-8.88)

Table 39B

Regrowth delay of B0008 melanoma spheroids in Experiment 3

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.3	(5.7-6.9)
2	6.2	(5.5-6.92)
3	5.68	(5.14-7.9)
4	6.1	(5.6-7.84)
5	7.31	(6.23-7.87)
6	6.6	(6.4-7.4)

Table 39C

Proportion of spheroids "cured" in Experiment 3.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/22	0
2	0/24	0
3	0/24	0
4	0/24	0
5	0/23	0
6	0/24	0

Table 40A

Growth of B0010 melanoma spheroids in Experiment 4.

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM
2	melphalan 2 μ g/ml
3	melphalan 4 μ g/ml
4	melphalan 8 μ g/ml
5	melphalan 12 μ g/ml
6	melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.75 (6.69-6.91)	6.63 (6.59-6.76)
3	6.85 (6.85-6.91)	6.86 (6.78-6.9)
6	6.91 (6.84-6.99)	6.86 (6.83-6.91)
9	6.99 (6.88-7.04)	6.95 (6.9-6.99)
13	7.14 (7.02-7.23)	7.04 (6.97-7.09)
17	7.18 (7.11-7.22)	7.04 (7-7.11)
23	7.23 (7.13-7.32)	7.08 (7.05-7.17)
31	7.29 (7.15-7.36)	7.11 (7.08-7.14)
	PLATE 3	PLATE 4
0	6.67 (6.59-6.9)	6.67 (6.59-6.84)
3	6.78 (6.71-6.97)	6.81 (6.65-6.84)
6	6.86 (6.78-6.94)	6.86 (6.73-6.94)
9	6.92 (6.83-6.99)	6.85 (6.8-7)
13	6.99 (6.95-7.09)	6.96 (6.87-7.05)
17	7.08 (6.99-7.12)	6.96 (6.9-7.09)
23	7.04 (6.99-7.12)	7 (6.9-7.1)
31	7.08 (7-7.18)	7.02 (6.92-7.14)
	PLATE 5	PLATE 6
0	6.78 (6.63-7)	6.64 (6.45-6.83)
3	6.87 (6.73-6.95)	6.79 (6.59-6.86)
6	6.9 (6.75-6.96)	6.76 (6.59-6.81)
9	6.98 (6.86-7.1)	6.89 (6.78-7.02)
13	7.02 (6.95-7.1)	7 (6.86-7.08)
17	7.04 (6.94-7.09)	6.96 (6.86-7.09)
23	7.06 (6.95-7.09)	6.96 (6.86-7.04)
31	7.08 (7.03-7.16)	6.92 (6.86-7.04)

Table 40B

The B0010 melanoma spheroids failed to increase volume by ten-fold during the period of observation (see Table 42A and Fig. 31), hence regrowth delays cannot be calculated.

Table 40C

Proportion of B0010 spheroids "cured" in Experiment 4.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	6/18	33
2	13/23	57
3	18/21	86
4	22/24	92
5	19/19	100
6	22/22	100

Table 41A

Growth of MEL57 melanoma spheroids in Experiment 5.

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM
2	melphalan 2 μ g/ml
3	melphalan 4 μ g/ml
4	melphalan 8 μ g/ml
5	melphalan 12 μ g/ml
6	melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.66 (6.61-6.73)	6.66 (6.47-6.76)
3	7.4 (7.32-7.47)	7.35 (7.23-7.49)
6	8 (7.87-8.13)	7.71 (7.61-7.82)
10	8.73 (8.56-8.91)	8.06 (7.87-8.15)
13	8.88 (8.88-8.88)	8.29 (8.13-8.42)
17		8.58 (8.29-8.68)
21		8.74 (8.48-8.88)
	PLATE 3	PLATE 4
0	6.6 (6.47-6.73)	6.66 (6.59-6.69)
3	7.14 (7.04-7.22)	7.12 (6.96-7.16)
6	7.35 (7.26-7.52)	7.38 (7.21-7.41)
10	7.65 (7.52-7.74)	7.6 (7.53-7.67)
13	7.76 (7.66-7.88)	7.83 (7.68-7.93)
17	7.93 (7.81-8.16)	7.93 (7.84-8.11)
21	7.94 (7.88-8.15)	8.01 (7.88-8.11)
25	8.08 (7.96-8.25)	8.14 (8.05-8.26)
28	8.11 (7.99-8.27)	8.16 (8.01-8.29)
31	8.15 (8.04-8.25)	8.12 (7.98-8.24)
	PLATE 5	PLATE 6
0	6.57 (6.45-6.61)	6.66 (6.5-6.78)
3	6.91 (6.8-7.08)	6.97 (6.87-7.11)
6	7.05 (6.91-7.17)	7.05 (6.96-7.15)
10	7.24 (7.08-7.46)	7.24 (7.12-7.32)
13	7.5 (7.23-7.62)	7.31 (7.25-7.44)
17	7.63 (7.48-7.82)	7.57 (7.44-7.63)
21	7.7 (7.41-7.87)	7.6 (7.48-7.65)
25	7.9 (7.73-8.08)	7.83 (7.6-7.9)
28	7.92 (7.82-8.04)	7.77 (7.69-7.94)
31	7.91 (7.73-8.04)	7.85 (7.62-8.02)
35	7.87 (7.59-8.08)	7.72 (7.62-7.92)
38	7.88 (7.67-8.16)	7.8 (7.63-7.97)

Table 41B

Regrowth delay of MEL57 melanoma spheroids
in Experiment 5.

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	4.41	(4.08-4.96)
2	5.6	(4.45-7.65)
3	10.19	(8.98-11.59)
4	10.71	(9.4-12.29)
5	15.07	(12.21-19.33)
6	22.1	(16.46-24.83)

Table 41C

Proportion of spheroids "cured" in Experiment 5.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/23	0
2	0/22	0
3	0/23	0
4	0/23	0
5	0/24	0
6	0/23	0

Table 42A

Growth of MEL57 melanoma spheroids in Experiment 6.

Plate No. 'Treatment': 60 minutes in

1	at 37°C in MEM
2	at 41°C in MEM
3	at 41°C in melphalan 1µg/ml
4	at 41°C in melphalan 2µg/ml
5	at 41°C in melphalan 4µg/ml
6	at 41°C in melphalan 8µg/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.61 (6.5-6.69)	6.64 (6.55-6.76)
3	7.41 (7.32-7.51)	7.5 (7.44-7.6)
7	8.42 (8.3-8.47)	8.47 (8.36-8.57)
11	8.8 (8.73-8.83)	8.84 (8.76-8.88)
	PLATE 3	PLATE 4
0	6.61 (6.45-6.71)	6.67 (6.52-6.76)
3	7.26 (6.97-7.4)	7.29 (7.12-7.4)
7	7.75 (7.52-7.97)	7.68 (7.55-7.88)
11	8.11 (7.84-8.36)	7.96 (7.82-8.03)
16	8.68 (8.45-8.88)	8.27 (8.14-8.38)
19	8.88 (8.88-8.88)	8.35 (8.17-8.48)
23		8.47 (8.36-8.65)
26		8.49 (8.36-8.76)
29		8.54 (8.35-8.88)
	PLATE 5	PLATE 6
0	6.75 (6.67-6.81)	6.69 (6.67-6.86)
3	7.27 (7.14-7.37)	7.13 (7.01-7.2)
7	7.55 (7.5-7.69)	7.17 (7.01-7.32)
11	7.71 (7.6-7.79)	7.37 (7.26-7.55)
16	7.96 (7.91-8.17)	7.58 (7.47-7.71)
19	8.15 (8.06-8.23)	7.69 (7.61-7.77)
23	8.24 (8.14-8.42)	7.86 (7.69-7.92)
26	8.16 (7.99-8.34)	7.9 (7.67-8.01)
29	8.27 (8.16-8.42)	7.97 (7.78-8.04)

Table 42B

Regrowth delay of MEL57 melanoma spheroids
in Experiment 6

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	3.66	(3.38-3.86)
2	3.78	(3.56-4.15)
3	5.31	(4.52-6.24)
4	6.94	(6.36-8.5)
5	11.34	(10.5-12.56)
6	17.6	(16.19-22.34)

Table 42C

Proportion of spheroids "cured" in Experiment 6.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/24	0
3	0/24	0
4	0/23	0
5	1/21	5
6	1/23	4

Table 43A

Growth of B0008 melanoma spheroids in Experiment 7

Plate No.	'Treatment': 60 minutes ...
1	at 37°C in MEM
2	at 31°C in MEM
3	at 31°C in MEM with melphalan 2 μ g/ml
4	at 31°C in MEM with melphalan 4 μ g/ml
5	at 31°C in MEM with melphalan 8 μ g/ml
6	at 31°C in MEM with melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.12 (7-7.24)	6.98 (6.87-7.05)
3	7.38 (7.2-7.55)	7.2 (7.1-7.34)
6	7.85 (7.66-7.95)	7.68 (7.55-7.81)
10	8.18 (8.06-8.31)	8.17 (8.05-8.22)
13	8.4 (8.34-8.51)	8.42 (8.34-8.49)
17	8.63 (8.59-8.73)	8.68 (8.62-8.71)
20	8.76 (8.71-8.88)	8.79 (8.76-8.85)
	PLATE 3	PLATE 4
0	7.2 (7.02-7.3)	7.1 (7.08-7.27)
3	7.3 (7.21-7.43)	7.28 (7.25-7.48)
6	7.71 (7.5-7.91)	7.67 (7.61-7.79)
10	8.15 (7.88-8.25)	8.06 (7.94-8.17)
13	8.37 (8.18-8.45)	8.3 (8.22-8.37)
17	8.59 (8.44-8.68)	8.58 (8.51-8.62)
20	8.74 (8.68-8.81)	8.7 (8.64-8.77)
22	8.88 (8.73-8.88)	8.79 (8.76-8.88)
	PLATE 5	PLATE 6
0	7.12 (7.02-7.32)	7.12 (7.02-7.28)
3	7.26 (7.1-7.37)	7.12 (7.02-7.35)
6	7.41 (7.21-7.46)	7.24 (7.04-7.36)
10	7.53 (7.14-7.6)	7.12 (6.95-7.37)
13	7.81 (7.2-7.85)	7.14 (7.02-7.37)
17	8.22 (7.59-8.28)	7.22 (7.06-7.52)
20	8.41 (7.98-8.48)	7.46 (6.97-7.79)
22	8.51 (8.19-8.58)	
24	8.61 (8.27-8.7)	7.76 (6.86-8.17)
27		8.1 (6.91-8.34)

Table 43B

Regrowth delay of B0008 melanoma spheroids in Experiment 7

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	8.72	(8.12-9.88)
2	8.91	(7.92-9.87)
3	11.13	(9.96-12.13)
4	11.24	(10.31-12.37)
5	16.65	(15.92-20.06)
6	27.1	(23.4-1000)

Table 43C

Proportion of spheroids "cured" in Experiment 7

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/23	0
2	0/24	0
3	0/22	0
4	0/24	0
5	0/21	0
6	1/23	4

Table 44A

Growth of B0008 melanoma spheroids in Experiment 8

Plate No. 'Treatment': 60 minutes in MEM at..

1	37°C
2	31°C
3	31°C with melphalan 2 μ g/ml
4	31°C with melphalan 4 μ g/ml
5	31°C with melphalan 8 μ g/ml
6	31°C with melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.88 (6.83-6.96)	6.86 (6.65-6.99)
4	7.6 (7.52-7.71)	7.58 (7.43-7.69)
7	7.96 (7.9-8.05)	7.93 (7.8-8.04)
11	8.35 (8.24-8.38)	8.26 (8.23-8.4)
14	8.54 (8.49-8.6)	8.57 (8.44-8.62)
17	8.67 (8.63-8.72)	8.67 (8.55-8.76)
20	8.82 (8.75-8.88)	8.8 (8.76-8.88)
	PLATE 3	PLATE 4
0	6.92 (6.73-7.02)	6.8 (6.71-7.09)
4	7.54 (7.39-7.62)	7.33 (7.25-7.55)
7	7.84 (7.63-7.95)	7.74 (7.64-7.94)
11	8.22 (8.17-8.28)	8.12 (8.05-8.27)
14	8.49 (8.4-8.54)	8.37 (8.28-8.52)
17	8.61 (8.56-8.65)	8.61 (8.48-8.71)
20	8.72 (8.67-8.8)	8.72 (8.58-8.84)
24	8.88 (8.78-8.88)	8.88 (8.74-8.88)
	PLATE 5	PLATE 6
0	6.81 (6.65-6.95)	6.84 (6.8-6.87)
4	7.12 (7.04-7.25)	7.06 (7-7.16)
7	7.36 (7.21-7.52)	7.1 (6.97-7.22)
11	7.78 (7.65-7.97)	7.24 (7.02-7.4)
14	8.12 (7.92-8.25)	7.57 (7.29-7.73)
17	8.33 (8.1-8.45)	7.86 (7.62-8.02)
20	8.55 (8.33-8.65)	8.1 (7.84-8.3)
24	8.75 (8.55-8.84)	8.34 (8.23-8.51)
27		8.49 (8.43-8.69)
31		8.69 (8.54-8.75)
34		8.78 (8.67-8.88)

Table 44B

Regrowth delay of B0008 melanoma spheroids in Experiment 8

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.52	(6.22-6.98)
2	6.42	(5.82-7.44)
3	7.76	(6.83-8.89)
4	7.93	(6.83-8.68)
5	10.82	(9.87-12.52)
6	17.39	(15.5-20.06)

Table 44C

Proportion of spheroids "cured" in Experiment 8

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/21	0
2	0/20	0
3	0/23	0
4	0/22	0
5	0/23	0
6	1/18	6

Table 45A

 Growth of B0008 melanoma spheroids in Experiment 9

Plate No. 'Treatment': 60 minutes in MEM...

1	at 37°C
2	at 35°C
3	at 35°C with melphalan 4µg/ml
4	at 35°C with melphalan 8µg/ml

MEDIAN LOG VOLUME (≥95% confidence intervals)

	PLATE 1	PLATE 2
0	6.91 (6.74-7.00)	6.94 (6.76-7.04)
2	7.27 (7.13-7.37)	7.25 (7.14-7.37)
4	7.65 (7.48-7.68)	7.63 (7.49-7.69)
7	8.07 (7.92-8.11)	8.04 (7.95-8.06)
9	8.2 (8.07-8.23)	8.17 (8.1-8.21)
14	8.45 (8.37-8.52)	8.47 (8.41-8.5)
16	8.53 (8.46-8.6)	8.51 (8.49-8.55)
DAY	PLATE 3	PLATE 4
0	6.88 (6.71-6.94)	6.77 (6.67-6.97)
2	7.16 (7.01-7.21)	7.11 (6.97-7.26)
4	7.36 (7.21-7.45)	7.14 (7-7.32)
7	7.61 (7.44-7.7)	7.18 (7.06-7.38)
9	7.72 (7.62-7.88)	7.3 (7.22-7.47)
14	8.08 (7.99-8.19)	7.51 (7.44-7.6)
16	8.18 (8.06-8.27)	7.63 (7.53-7.77)
21		8.09 (8.01-8.19)

Table 45B

Regrowth delay of B0008 melanoma spheroids in Experiment 9

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	4.91	(4.6-5.76)
2	5	(4.62-5.87)
3	8.84	(6.93-10.2)
4	16.5	(14.7-17.54)

Table 45C

Proportion of spheroids "cured" in Experiment 9

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/20	0
3	0/24	0
4	1/22	5

Table 46A

Growth of B0008 melanoma spheroids in Experiment 10

Plate No. 'Treatment': 60mins at 37°C in...

1	MEM
2	MEM with melphalan 4 μ g/ml
3	MEM with melphalan 8 μ g/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.92 (6.36-7.73)	6.96 (6.81-7.12)
2	7.26 (6.52-7.92)	7.18 (7.02-7.36)
7	7.97 (7.21-8.28)	7.5 (7.38-7.66)
10	8.23 (7.66-8.49)	7.75 (7.61-7.8)
14	8.43 (8.07-8.66)	8.03 (7.94-8.12)
18	8.64 (8.29-8.84)	8.31 (8.19-8.36)
21	8.81 (8.58-8.88)	8.49 (8.34-8.57)
23		8.54 (8.29-8.61)
28		8.71 (8.57-8.75)
30		8.75 (8.66-8.88)

PLATE 3

0	6.81 (6.67-6.94)
2	7.04 (6.91-7.17)
7	7.11 (6.91-7.25)
10	7.2 (7.09-7.34)
14	7.5 (7.26-7.7)
18	7.84 (7.44-8.04)
21	8.03 (7.75-8.23)
23	8.12 (7.93-8.27)
28	8.34 (8.25-8.48)
30	8.44 (8.35-8.55)
35	8.58 (8.46-8.7)
39	8.56 (8.44-8.67)

Table 46B

Regrowth delay of B0008 melanoma spheroids
in Experiment 10

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.6	(5.4-7.2)
2	12.76	(12-13.3)
3	17.54	(15.6-21.7)

Table 46C

Proportion of spheroids "cured" in Experiment 10

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/23	0
2	0/21	0
3	1/20	5

Table 47A

Growth of B0008 melanoma spheroids in Experiment 11

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM
2	MEM with melphalan 2 μ g/ml
3	MEM with melphalan 4 μ g/ml
4	MEM with melphalan 8 μ g/ml
5	MEM with melphalan 12 μ g/ml
6	MEM with melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.02 (6.94-7.1)	7.06 (7-7.16)
4	7.8 (7.69-7.84)	7.63 (7.6-7.73)
7	8.14 (8.1-8.17)	7.98 (7.88-8.03)
11	8.49 (8.47-8.52)	8.36 (8.28-8.38)
18	8.79 (8.77-8.8)	8.69 (8.67-8.74)
21		8.88 (8.82-8.88)

	PLATE 3	PLATE 4
0	7.04 (6.92-7.15)	7.03 (6.96-7.09)
4	7.5 (7.41-7.54)	7.33 (7.27-7.41)
7	7.59 (7.52-7.67)	7.39 (7.29-7.44)
11	7.92 (7.82-8.02)	7.45 (7.41-7.56)
18	8.43 (8.22-8.48)	7.46 (7.37-7.57)
21	8.6 (8.42-8.66)	7.55 (7.47-7.77)
25	8.74 (8.63-8.81)	7.84 (7.75-8.03)
29		8.2 (8.01-8.33)
33		8.41 (8.27-8.64)

	PLATE 5	PLATE 6
0	7.06 (6.99-7.2)	7.1 (7.01-7.19)
4	7.36 (7.26-7.44)	7.34 (7.29-7.47)
7	7.42 (7.34-7.47)	7.37 (7.29-7.47)
11	7.5 (7.43-7.62)	7.48 (7.44-7.53)
18	7.53 (7.36-7.6)	7.36 (7.31-7.52)
21	7.46 (7.34-7.57)	7.4 (7.25-7.44)
25	7.51 (7.41-7.71)	7.35 (7.24-7.57)
29	7.75 (7.53-8.02)	7.41 (7.35-7.56)
33	8.1 (7.88-8.33)	7.64 (7.36-8)

Table 47B

Regrowth delay of B0008 melanoma spheroids
in Experiment 11

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	5.99	(5.85-6.35)
2	8.11	(7.53-8.56)
3	13.37	(12.65-13.99)
4	26.97	(25.54-28.76)
5	1000	(30.75-1000)
6	1000	(1000-1000)

Table 47C

Proportion of spheroids "cured" in Experiment 11

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/22	0
3	0/24	0
4	1/24	4
5	3/24	13
6	10/24	42

Table 48A

 Growth of B0008 melanoma spheroids in Experiment 12

Plate No. 'Treatment': 60 minutes at 37°C in...

1	MEM
2	MEM with melphalan 2 μ g/ml
3	MEM with melphalan 4 μ g/ml
4	MEM with melphalan 8 μ g/ml
5	MEM with melphalan 12 μ g/ml
6	MEM with melphalan 16 μ g/ml

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.76 (6.71-6.88)	6.91 (6.73-7.1)
4	7.55 (7.47-7.63)	7.54 (7.39-7.61)
7	7.95 (7.86-7.99)	7.85 (7.66-7.94)
11	8.33 (8.23-8.42)	8.21 (8.08-8.29)
14	8.52 (8.39-8.59)	8.4 (8.27-8.44)
18	8.65 (8.58-8.73)	8.59 (8.49-8.69)
21	8.77 (8.71-8.85)	8.74 (8.62-8.81)
	PLATE 3	PLATE 4
0	6.88 (6.76-6.96)	6.88 (6.83-6.97)
4	7.32 (7.23-7.44)	7.31 (7.21-7.37)
7	7.46 (7.36-7.58)	7.38 (7.23-7.43)
11	7.82 (7.69-7.94)	7.5 (7.43-7.57)
14	8.09 (7.92-8.2)	7.69 (7.56-7.79)
18	8.29 (8.21-8.43)	8.03 (7.88-8.16)
21	8.42 (8.33-8.6)	8.22 (8.07-8.3)
26	8.64 (8.6-8.73)	8.4 (8.3-8.51)
29	8.81 (8.75-8.88)	8.57 (8.47-8.64)
32		8.72 (8.53-8.77)
	PLATE 5	PLATE 6
0	6.86 (6.78-6.95)	6.96 (6.84-7.1)
4	7.21 (7.12-7.26)	7.23 (7.18-7.41)
7	7.26 (7.18-7.32)	7.27 (7.22-7.46)
11	7.28 (7.21-7.39)	7.34 (7.25-7.48)
14	7.34 (7.25-7.47)	7.32 (7.21-7.46)
18	7.42 (7.3-7.55)	7.36 (7.28-7.51)
21	7.59 (7.32-7.78)	7.31 (7.21-7.54)
26	7.96 (7.67-8.17)	7.34 (7.22-7.57)
29	8.09 (7.91-8.21)	7.44 (7.21-7.67)
32	8.25 (8.11-8.34)	7.54 (7.27-7.78)

Table 48B

Regrowth delay of B0008 melanoma spheroids
in Experiment 12

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	5.8	(5.21-6.57)
2	8.17	(7.15-9.43)
3	11.32	(9.95-12.38)
4	16.37	(15.57-19.06)
5	25.31	(21.38-27.38)
6	1000	(1000-1000)

Table 48C

Proportion of spheroids "cured" in Experiment 12.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/22	0
2	0/23	0
3	0/24	0
4	0/24	0
5	2/23	9
6	10/23	44

Table 49A

Growth of B0008 melanoma spheroids in Experiment 13

Plate No. 'Treatment': 60 minutes in MEM..

1	at 37°C
2	at 39°C
3	at 39°C with melphalan 2 μ g/ml
4	at 39°C with melphalan 4 μ g/ml
5	at 39°C with melphalan 8 μ g/ml

MEDIAN LOG VOLUME (\geq 95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.16 (7.08-7.4)	7.2 (7.06-7.56)
2	7.4 (7.16-7.92)	7.39 (7.12-7.89)
6	7.94 (7.69-8.32)	7.92 (7.67-8.29)
11	8.41 (8.3-8.64)	8.39 (8.28-8.6)
14	8.6 (8.48-8.74)	8.56 (8.46-8.74)
16	8.68 (8.57-8.74)	8.64 (8.56-8.79)
20	8.88 (8.74-8.88)	8.79 (8.74-8.88)

PLATE 3

0	7.1 (6.91-7.24)
2	7.08 (6.94-7.25)
6	7.26 (6.94-7.6)
11	7.97 (7.65-8.16)
14	8.21 (7.99-8.37)
16	8.32 (8.15-8.51)
20	8.58 (8.48-8.66)
24	8.85 (8.71-8.88)

PLATE 4

0	6.93 (6.81-7.06)
2	6.9 (6.8-7.08)
6	6.83 (6.67-7.17)
11	7.3 (6.9-8.06)
14	7.58 (6.75-8.21)
16	7.9 (6.86-8.45)
20	8.09 (6.87-8.5)
24	8.36 (6.87-8.5)
27	8.5 (6.88-8.88)
30	8.62 (6.84-8.88)
34	8.88 (6.87-8.88)
37	8.88 (6.86-8.88)
41	8.88 (6.88-8.88)

PLATE 5

0	7.19 (6.94-7.3)
2	7.09 (6.83-7.34)
6	7.02 (6.91-7.21)
11	7.08 (6.92-7.38)
14	7.05 (6.81-7.61)
16	6.97 (6.8-7.85)
20	7.05 (6.84-8.29)
24	6.96 (6.81-8.55)
27	6.95 (6.87-8.83)
30	7.07 (6.95-8.88)
34	7.05 (6.86-8.88)
37	7.11 (6.52-8.88)
41	7.13 (6.94-8.88)

Table 49B

Regrowth delay of B0008 melanoma spheroids
in Experiment 13

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	8.14	(5.71-9.78)
2	8.5	(5.92-10.46)
3	12.57	(10.49-15.42)
4	16.9	(11.74-1000)

Table 49C

Proportion of spheroids "cured" in Experiment 13.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/22	0
3	3/22	14
4	3/19	16
5	9/21	43

Table 50A

Growth of B0008 melanoma spheroids in Experiment 14

Plate No. 'Treatment': 60 minutes in MEM...

1	at 37°C
2	at 39°C
3	at 39°C with melphalan 2 μ g/ml
4	at 39°C with melphalan 4 μ g/ml
5	at 39°C with melphalan 8 μ g/ml
6	at 39°C with melphalan 16 μ g/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.01 (6.95-7.04)	6.88 (6.83-7.01)
4	7.88 (7.8-7.91)	7.79 (7.73-7.89)
7	8.2 (8.14-8.26)	8.17 (8.13-8.2)
11	8.45 (8.41-8.51)	8.43 (8.42-8.44)
14	8.58 (8.51-8.61)	8.56 (8.53-8.58)
18	8.72 (8.65-8.76)	8.72 (8.68-8.73)
21	8.79 (8.67-8.88)	8.75 (8.74-8.77)

DAY	PLATE 3	PLATE 4
0	6.9 (6.86-7.04)	6.94 (6.76-6.99)
4	7.64 (7.6-7.69)	7.42 (7.31-7.5)
7	7.97 (7.92-8.04)	7.81 (7.67-8.09)
11	8.28 (8.26-8.32)	8.08 (8.01-8.13)
14	8.47 (8.43-8.53)	8.3 (8.23-8.34)
18	8.68 (8.64-8.7)	8.52 (8.5-8.58)
21	8.72 (8.69-8.75)	8.63 (8.58-8.71)
26	8.77 (8.72-8.84)	8.8 (8.79-8.88)

DAY	PLATE 5	PLATE 6
0	6.91 (6.84-6.97)	6.96 (6.84-7.06)
4	7.21 (7.17-7.28)	7.25 (7.12-7.4)
7	7.28 (7.19-7.36)	7.28 (7.12-7.46)
11	7.31 (7.22-7.39)	7.34 (7.17-7.47)
14	7.4 (7.29-7.55)	7.41 (7.29-7.5)
18	7.92 (7.72-8.09)	7.37 (7.21-7.51)
21	8.09 (7.92-8.23)	7.4 (7.23-7.48)
26	8.42 (8.34-8.54)	7.44 (7.26-7.63)
29	8.63 (8.46-8.7)	7.45 (7.32-7.9)
33	8.83 (8.72-8.88)	7.44 (7.1-8.04)
37		7.84 (7.3-8.29)

Table 50B

Regrowth delay of B0008 melanoma spheroids
in Experiment 14

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	5.21	(5.06-5.69)
2	5.06	(4.74-6.01)
3	6.74	(5.98-7.48)
4	8.45	(6.43-9.88)
5	18.79	(16.23-21.17)
6	1000	(33.9-1000)

Table 50C

Proportion of spheroids "cured" in Experiment 14

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/23	0
2	0/21	0
3	0/20	0
4	1/22	5
5	6/22	27
6	10/22	45

Table 51A

Growth of B0008 melanoma spheroids in Experiment 15

Plate No. 'Treatment': 60 minutes at

1	at 37°C in MEM
2	at 41°C in MEM
3	at 41°C in 2 μ g/ml melphalan
4	at 41°C in 4 μ g/ml melphalan
5	at 41°C in 8 μ g/ml melphalan

MEDIAN LOG VOLUME ($\geq 95\%$ confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.9 (6.76-7.14)	6.94 (6.77-7.2)
4	7.52 (7.44-7.7)	7.56 (7.47-7.74)
7	7.83 (7.8-7.94)	7.86 (7.79-7.99)
11	8.15 (8.12-8.24)	8.18 (8.1-8.23)
14	8.32 (8.29-8.41)	8.35 (8.32-8.43)
18	8.56 (8.54-8.62)	8.56 (8.55-8.64)
21	8.72 (8.7-8.74)	8.7 (8.7-8.7)
	PLATE 3	
0	7.04 (6.99-7.18)	
4	7.45 (7.25-7.56)	
7	7.61 (7.45-7.77)	
11	7.87 (7.75-8.04)	
14	8.06 (7.98-8.19)	
18	8.32 (8.23-8.37)	
21	8.45 (8.37-8.49)	
25	8.56 (8.55-8.6)	
	PLATE 4	PLATE 5
0	7.03 (6.81-7.15)	7.04 (6.96-7.12)
4	7.27 (7.03-7.39)	7.24 (7.16-7.33)
7	7.34 (7.14-7.49)	7.22 (7.16-7.36)
11	7.4 (7.11-7.63)	7.29 (7.2-7.37)
14	7.54 (7.07-7.81)	7.28 (7.22-7.37)
18	7.86 (7.31-8.12)	7.25 (7.13-7.41)
21	8.08 (7.6-8.35)	7.42 (7.35-7.59)
25	8.34 (7.96-8.52)	7.66 (7.32-7.86)
28	8.49 (8.39-8.59)	7.85 (7.43-8.15)
32	8.59 (8.45-8.7)	8.14 (7.76-8.42)
34		8.29 (7.76-8.42)
39		8.44 (8.1-8.7)
42		8.63 (8.29-8.7)

Table 51B

Regrowth delay of B0008 melanoma spheroids
in Experiment 15

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	7.64	(6.4-8.37)
2	8	(6.54-8.89)
3	13.62	(11-14.94)
4	20.47	(16.89-25.24)
5	30.52	(26.8-37.76)

Table 51C

Proportion of spheroids "cured" in Experiment 15.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/23	0
2	0/23	0
3	2/23	9
4	11/23	45
5	12/19	63

Table 52A

Growth of B0008 melanoma spheroids in Experiment 16

Plate No. 'Treatment': 60 minutes at

1	37°C in MEM
2	42.5°C in MEM
3	42.5°C in melphalan 2µg/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.08 (6.92-7.2)	7.09 (6.94-7.21)
4	7.42 (7.34-7.52)	7.41 (7.32-7.5)
9	8.04 (7.92-8.12)	8 (7.94-8.08)
11	8.16 (8.14-8.21)	8.14 (8.1-8.19)
14	8.37 (8.31-8.43)	8.36 (8.31-8.44)
18	8.63 (8.55-8.7)	8.62 (8.54-8.69)
22	8.78 (8.73-8.82)	8.75 (8.71-8.81)

PLATE 3

0	7.12 (6.96-7.16)
4	7.18 (7.05-7.32)
9	7.31 (7.05-7.55)
11	7.36 (7.16-7.66)
14	7.67 (7.17-7.99)
18	8.05 (7.32-8.28)
22	8.35 (7.58-8.58)
25	8.48 (8.4-8.69)
28	8.68 (8.11-8.88)
32	8.81 (8.73-8.88)
35	8.88 (8.68-8.88)

Table 52B

Regrowth delay of B0008 melanoma spheroids
in Experiment 16

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	9.8	(8.7-10.5)
2	10.2	(9.1-10.7)
3	19	(16.1-22.8)

Table 52C

Proportion of spheroids "cured" in Experiment 16.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/22	0
3	4/24	17
4	14/22	64
5	23/23	100

Table 53A

Growth of B0008 melanoma spheroids in Experiment 17

Plate No.	'Treatment'.....
1	60 minutes at 37°C in MEM
2	60 minutes at 42.5°C in MEM
3	60 minutes at 42.5°C in melphalan 1µg/ml
4	60 minutes at 42.5°C in melphalan 2µg/ml
5	60 minutes at 42.5°C in melphalan 4µg/ml
6	<u>20 minutes</u> at 42.5°C in melphalan 4µg/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.06 (6.88-7.14)	7.08 (6.9-7.21)
2	7.39 (7.3-7.5)	7.2 (7.12-7.34)
7	8.08 (8.01-8.17)	7.98 (7.95-8.06)
9	8.23 (8.14-8.31)	8.16 (8.11-8.24)
13	8.51 (8.48-8.58)	8.49 (8.44-8.57)
20	8.78 (8.73-8.82)	8.76 (8.71-8.88)
	PLATE 3	PLATE 4
0	7.14 (7.04-7.18)	7.07 (6.96-7.14)
2	7.21 (7.12-7.32)	7.05 (6.99-7.21)
7	7.71 (7.57-7.83)	7.36 (7.22-7.45)
9	7.89 (7.77-8.03)	7.37 (7.24-7.46)
13	8.22 (8.16-8.32)	7.54 (7.44-7.78)
20	8.57 (8.4-8.74)	7.78 (7.52-7.96)
23	8.72 (8.61-8.88)	8.22 (7.79-8.5)
27		8.47 (8.09-8.64)
31		8.73 (8.47-8.86)
	PLATE 5	PLATE 6
0	7.14 (7.03-7.22)	7.17 (7.05-7.25)
2	7.14 (7.01-7.21)	7.28 (7.2-7.34)
7	7.28 (7.16-7.4)	7.69 (7.55-7.8)
9	7.21 (7.1-7.39)	7.78 (7.7-7.89)
13	7.35 (7.18-7.59)	8.11 (8-8.2)
20	7.43 (7.06-7.64)	8.42 (8.33-8.55)
23	7.29 (7.09-7.63)	8.62 (8.49-8.79)
27	7.4 (6.95-7.9)	8.83 (8.68-8.88)
31	7.57 (7.08-8.25)	

Table 53B

Regrowth delay of B0008 melanoma spheroids
in Experiment 17

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.41	(6.11-7.35)
2	8.31	(7.28-8.89)
3	11.46	(10.32-12.58)
4	22.66	(20.69-26.32)
5	1000	(30.33-1000)
6(20min. exposure)	14.89	(11.98-18.01)

Table 53C

Proportion of spheroids "cured" in Experiment 17

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/24	0
3	0/22	0
4	2/24	8
5	8/21	38
6(20min. exposure)	1/24	4

Table 54A

Growth of B0008 melanoma spheroids in Experiment 18

Plate No. 'Treatment': 60 minutes

1	at 37°C in MEM
2 *	at 42.5°C in MEM (IMMEDIATE PICK-OFF)
3	at 42.5°C in melphalan 0.5 µg/ml
4	at 42.5°C in melphalan 1 µg/ml
5	at 42.5°C in melphalan 2 µg/ml
6	at 42.5°C in melphalan 4 µg/ml
7 *	at 42.5°C in MEM (2 hour delayed PICK-OFF)

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.83 (6.75-6.91)	6.86 (6.76-6.92)
2	7.06 (6.97-7.18)	7.05 (6.94-7.19)
6	7.8 (7.68-7.91)	7.79 (7.67-7.92) **
9	8.08 (7.99-8.17)	8.08 (7.97-8.16)
13	8.42 (8.35-8.5)	8.41 (8.33-8.49)
16	8.67 (8.54-8.71)	8.64 (8.52-8.68)
21	8.77 (8.74-8.88)	8.75 (8.72-8.82)
	PLATE 3	PLATE 4
0	6.75 (6.65-6.84)	6.88 (6.78-6.96)
2	7.01 (6.88-7.09)	7.05 (6.94-7.16)
6	7.6 (7.55-7.68)	7.5 (7.47-7.57)
9	7.95 (7.87-7.99)	7.82 (7.76-8.17)
13	8.24 (8.2-8.32)	8.15 (8.08-8.2)
16	8.55 (8.44-8.59)	8.26 (8.17-8.32)
21	8.73 (8.64-8.77)	8.62 (8.55-8.69)
24		8.7 (8.58-8.78)
27		8.85 (8.71-8.88)
	PLATE 5	PLATE 6
0	6.85 (6.76-6.91)	6.96 (6.81-7.05)
2	6.99 (6.88-7.09)	7.11 (6.96-7.19)
6	7.24 (7.15-7.32)	7.23 (7.11-7.34)
9	7.29 (7.21-7.4)	7.26 (7.09-7.34)
13	7.44 (7.18-7.6)	7.18 (6.97-7.28)
16	7.7 (7.29-7.91)	7.27 (6.96-7.43)
21	8.05 (7.59-8.18)	7.12 (6.91-7.46)
24	8.28 (7.89-8.37)	7.37 (6.95-7.73)
27	8.37 (8.21-8.55)	7.42 (6.91-7.93)
30	8.59 (8.35-8.65)	7.76 (6.94-8.16)
34	8.73 (8.57-8.83)	7.99 (7-8.31)
	PLATE 7	
0	6.8 (6.75-6.9)	
2	7.08 (7.01-7.17)	
6	7.79 (7.69-7.87)	
9	8.09 (8-8.15)	
13	8.41 (8.32-8.44)	
16	8.69 (8.52-8.72)	
21	8.8 (8.76-8.88)	

Table 54B

Regrowth delay of B0008 melanoma spheroids
in Experiment 18

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.09	(5.95-6.28)
2	6.61	(6.15-7.24)
3	7.61	(7-8.1)
4	8.51	(7.81-10.12)
5	17.81	(15.93-22.4)
6	515.07	(27.33-1000)
7	6.48	(6.11-7.08)

Table 56C

Proportion of spheroids "cured" in Experiment 18

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/22	0
3	0/21	0
4	0/23	0
5	1/24	4
6	8/22	36
7	0/24	0

Table 55C

B0008 melanoma spheroids in Experiment 19

Plate No. 'Treatment': 60 minutes

1	at 37°C in MEM
2 *	at 44.5°C in MEM
3	at 44.5°C in melphalan 1 μ g/ml
4	at 44.5°C in melphalan 2 μ g/ml
5	at 44.5°C in melphalan 4 μ g/ml

Proportion of spheroids "cured" in Experiment 19

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	13/23	57
3	18/19	95
4	22/22	100
5	22/22	100

Table 56A

 Growth of B0008 melanoma spheroids in Experiment 20

Plate No.	'Treatment'
1	60 minutes at 37°C in MEM
2	15 minutes at 37°C in melphalan 8µg/ml
3	30 minutes at 37°C in melphalan 8µg/ml
4	15 minutes at 37°C in melphalan 16µg/ml
5	30 minutes at 37°C in melphalan 16µg/ml
6	60 minutes at 37°C in melphalan 16µg/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.95 (6.86-7.03)	6.96 (6.88-7.05)
2	7.34 (7.28-7.4)	7.31 (7.21-7.39)
6	8.04 (8.03-8.11)	7.8 (7.71-7.88)
9	8.33 (8.28-8.35)	8.06 (8.04-8.13)
13	8.6 (8.56-8.65)	8.39 (8.36-8.43)
16	8.76 (8.73-8.82)	8.59 (8.53-8.66)
21		8.83 (8.74-8.87)
	PLATE 3	PLATE 4
0	6.88 (6.8-6.92)	6.94 (6.88-7.02)
2	7.14 (7.11-7.26)	7.23 (7.15-7.31)
6	7.39 (7.31-7.52)	7.43 (7.37-7.57)
9	7.55 (7.44-7.7)	7.55 (7.44-7.67)
13	7.9 (7.8-8.1)	7.87 (7.71-7.96)
16	8.17 (7.95-8.29)	8.08 (7.98-8.16)
21	8.56 (8.47-8.62)	8.46 (8.4-8.54)
23	8.65 (8.56-8.71)	8.55 (8.5-8.64)
27	8.8 (8.71-8.85)	8.77 (8.72-8.83)
	PLATE 5	PLATE 6
0	6.91 (6.86-7.03)	6.94 (6.91-7.02)
2	7.22 (7.14-7.33)	7.21 (7.11-7.29)
6	7.34 (7.25-7.44)	7.29 (7.23-7.34)
9	7.32 (7.27-7.49)	7.3 (7.18-7.34)
13	7.46 (7.33-7.57)	7.39 (7.24-7.43)
16	7.48 (7.32-7.64)	7.25 (7.21-7.28)
21	7.86 (7.66-8.23)	7.33 (7.25-7.4)
23	8.03 (7.88-8.36)	7.32 (7.2-7.36)
27	8.37 (8.15-8.55)	7.36 (7.29-7.55)
34	8.64 (8.54-8.81)	7.47 (7.27-7.7)

Table 56B

Regrowth delay of B0008 melanoma spheroids
in Experiment 20

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	5.49	(5-5.73)
2	7.88	(7.44-8.28)
3	12.1	(11.14-13.45)
4	14.31	(12.45-15.55)
5	22.71	(19.53-24.8)
6	1000	(1000-1000)

Table 56C

Proportion of spheroids "cured" in Experiment 20

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/20	0
2	0/22	0
3	0/22	0
4	0/24	0
5	0/21	0
6	10/24	42

Table 57A

 Growth of B0008 melanoma spheroids in Experiment 21

Plate No.	'Treatment'
1	60 minutes at 37°C in MEM
2	15 minutes at 37°C in melphalan 8µg/ml
3	30 minutes at 37°C in melphalan 8µg/ml
4	60 minutes at 37°C in melphalan 8µg/ml
5	90 minutes at 37°C in melphalan 8µg/ml
6	120 minutes at 37°C in melphalan 8µg/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.92 (6.8-7.22)	6.99 (6.92-7.06)
3	7.41 (7.33-7.6)	7.31 (7.19-7.46)
7	7.94 (7.81-8.03)	7.58 (7.44-7.66)
10	8.21 (8.18-8.29)	7.58 (7.38-7.68)
14	8.42 (8.37-8.57)	8.26 (8.13-8.35)
17	8.55 (8.5-8.62)	8.31 (8.24-8.36)
21	8.77 (8.63-8.85)	8.51 (8.42-8.56)
24		8.65 (8.54-8.71)
29		8.76 (8.63-8.79)
	PLATE 3	PLATE 4
0	6.99 (6.86-7.11)	6.95 (6.86-7.04)
3	7.22 (7.15-7.31)	7.1 (7.01-7.25)
7	7.35 (7.23-7.45)	7.15 (7.04-7.3)
10	7.48 (7.35-7.58)	7.13 (7.01-7.33)
14	7.82 (7.75-7.88)	7.24 (6.96-7.48)
17	8.07 (8.01-8.13)	7.43 (6.95-7.67)
21	8.31 (8.22-8.38)	7.73 (7.38-8.03)
24	8.48 (8.43-8.52)	7.94 (7.7-8.2)
29	8.65 (8.56-8.76)	8.29 (7.9-8.49)
32	8.79 (8.72-8.88)	8.47 (8.23-8.57)
35		8.64 (8.39-8.75)
	PLATE 5	PLATE 6
0	6.82 (6.71-6.92)	6.86 (6.75-6.94)
3	6.96 (6.9-7.02)	6.97 (6.87-7.06)
7	6.98 (6.95-7.12)	6.94 (6.86-7.03)
10	6.86 (6.67-6.97)	6.78 (6.69-6.88)
14	6.88 (6.83-7.02)	6.69 (6.61-6.9)
17	6.92 (6.81-7.01)	6.76 (6.65-6.84)
21	6.96 (6.76-7.21)	6.76 (6.61-6.95)
24	7 (6.81-7.26)	6.78 (6.57-7)
29	7.31 (6.96-7.82)	6.98 (6.81-7.12)
32	7.6 (6.95-8.05)	6.76 (6.61-7.34)
35	7.98 (7.27-8.41)	6.87 (6.65-7.62)

Table 57B

Regrowth delay of B0008 melanoma spheroids
in Experiment 21

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	7.58	(6.2-8.79)
2	12.78	(12.58-13.21)
3	15.23	(13.93-16.94)
4	24.28	(20.22-27.81)
5	33.96	(29.12-1000)
6	1000	(1000-1000)

Table 57C

Proportion of spheroids "cured" in Experiment 21

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/21	0
2	0/22	0
3	0/22	0
4	0/23	0
5	7/21	33
6	13/21	62

Table 58A

Growth of B0008 melanoma spheroids in Experiment 22

Plate No. 'Treatment': 30 minutes at

1	37°C in MEM
2	31°C in MEM
3	31°C in melphalan 2µg/ml
4	31°C in melphalan 4µg/ml
5	31°C in melphalan 8µg/ml
6	31°C in melphalan 16µg/ml

MEDIAN LOG VOLUME (≥95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.03 (6.87-7.14)	7.02 (6.88-7.18)
4	7.93 (7.81-8.01)	7.94 (7.79-8.01)
7	8.3 (8.25-8.33)	8.29 (8.2-8.38)
10	8.53 (8.49-8.56)	8.51 (8.48-8.6)
13	8.71 (8.65-8.76)	8.66 (8.62-8.76)
17	8.86 (8.83-8.88)	8.83 (8.78-8.88)
	PLATE 3	PLATE 4
0	7.13 (7.04-7.23)	7.05 (6.97-7.15)
4	7.92 (7.82-7.97)	7.8 (7.71-7.89)
7	8.26 (8.21-8.3)	8.14 (8.09-8.21)
10	8.49 (8.45-8.54)	8.38 (8.31-8.4)
13	8.67 (8.61-8.71)	8.57 (8.46-8.6)
17	8.82 (8.76-8.86)	8.75 (8.63-8.83)
	PLATE 5	PLATE 6
0	7.11 (7.01-7.2)	7.15 (6.96-7.47)
4	7.74 (7.5-7.85)	7.58 (7.41-7.89)
7	7.98 (7.82-8.07)	7.66 (7.38-8.01)
10	8.19 (8.04-8.31)	7.8 (7.52-8.16)
13	8.43 (8.24-8.49)	8.04 (7.83-8.35)
17	8.66 (8.47-8.69)	8.32 (8.26-8.61)
20	8.81 (8.67-8.86)	8.59 (8.4-8.71)
24		8.74 (8.48-8.88)
27		8.83 (8.56-8.88)
31		8.88 (8.88-8.88)

Table 58B

Regrowth delay of B0008 melanoma spheroids
in Experiment 22

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	4.76	(4.42-5.84)
2	5	(4.58-5.4)
3	6.1	(5.73-6.47)
4	6.22	(5.65-7.15)
5	9.04	(7.85-9.59)
6	14.72	(12.81-18.46)

Table 58C

Proportion of spheroids "cured" in Experiment 22.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/22	0
2	0/23	0
3	0/18	0
4	0/24	0
5	0/23	0
6	0/21	0

Table 59A

Growth of B0008 melanoma spheroids in Experiment 23.

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM
2	verapamil 10µg/ml
3	melphalan 4µg/ml
4	verapamil 5µg/ml + melphalan 2µg/ml
5	verapamil 10µg/ml + melphalan 2µg/ml
6	verapamil 10µg/ml + melphalan 4µg/ml

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.92 (6.87-7.05)	6.88 (6.78-6.97)
3	7.52 (7.5-7.6)	7.53 (7.48-7.59)
6	8.09 (8.05-8.13)	8.1 (8.06-8.13)
9	8.42 (8.38-8.67)	8.4 (8.39-8.43)
13	8.67 (8.64-8.69)	8.7 (8.67-8.73)
16	8.79 (8.76-8.81)	8.8 (8.78-8.82)
	PLATE 3	PLATE 4
0	6.91 (6.84-7.05)	7.29 (7.03-7.41)
3	7.28 (7.17-7.34)	7.41 (7.31-7.5)
6	7.47 (7.31-7.59)	8.03 (7.9-8.14)
9	7.53 (7.3-7.65)	8.14 (8.05-8.2)
13	7.88 (7.4-7.97)	8.44 (8.41-8.49)
16	8.19 (7.7-8.26)	8.6 (8.55-8.65)
20	8.5 (8.19-8.57)	8.77 (8.74-8.87)
23	8.65 (8.38-8.7)	
27	8.81 (8.58-8.88)	
	PLATE 5	PLATE 6
0	6.96 (6.9-7.09)	6.93 (6.73-7.08)
3	7.4 (7.29-7.51)	7.29 (7.21-7.43)
6	7.89 (7.72-7.92)	7.46 (7.39-7.64)
9	8.16 (8.02-8.23)	7.63 (7.48-7.83)
13	8.48 (8.39-8.52)	8.01 (7.91-8.19)
16	8.64 (8.57-8.68)	8.29 (8.17-8.42)
20	8.74 (8.72-8.81)	8.57 (8.47-8.62)
23		8.7 (8.61-8.78)
27		8.86 (8.78-8.88)

Table 59B

Regrowth delay of B0008 melanoma spheroids
in Experiment 23

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	5.29	(4.91-5.61)
2	4.94	(4.58-5.25)
3	14.74	(12.55-16.92)
4	11.12	(8.11-12.18)
5	7.5	(6.66-8.25)
6	11.78	(10.5-13.05)

Table 59C

Proportion of spheroids "cured" in Experiment 23.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/21	0
2	0/17	0
3	0/16	0
4	0/19	0
5	0/20	0
6	0/18	0

Table 60A

Growth of B0008 melanoma spheroids in Experiment 24.

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM
2	verapamil 10 μ g/ml
3	melphalan 4 μ g/ml
4	verapamil 5 μ g/ml + melphalan 2 μ g/ml
5	verapamil 10 μ g/ml + melphalan 2 μ g/ml
6	verapamil 10 μ g/ml + melphalan 4 μ g/ml

MEDIAN LOG VOLUME (\geq 95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	7.02 (6.91-7.12)	6.97 (6.8-7.1)
2	7.34 (7.27-7.44)	7.31 (7.19-7.4)
5	7.8 (7.73-7.87)	7.78 (7.7-7.88)
9	8.21 (8.17-8.3)	8.23 (8.15-8.28)
12	8.45 (8.42-8.49)	8.45 (8.41-8.49)
16	8.69 (8.62-8.73)	8.61 (8.51-8.64)
19	8.79 (8.76-8.85)	8.7 (8.67-8.77)
23		8.88 (8.88-8.88)
	PLATE 3	PLATE 4
0	7.09 (6.92-7.12)	7.01 (6.91-7.16)
2	7.29 (7.2-7.33)	7.3 (7.17-7.37)
5	7.43 (7.3-7.53)	7.6 (7.46-7.7)
9	7.71 (7.51-7.83)	8.13 (7.91-8.28)
12	8.06 (7.76-8.13)	8.26 (8.17-8.34)
16	8.32 (8.22-8.42)	8.53 (8.43-8.61)
19	8.49 (8.35-8.58)	8.64 (8.57-8.72)
23	8.71 (8.63-8.84)	8.79 (8.73-8.85)
27	8.88 (8.78-8.88)	
	PLATE 5	PLATE 6
0	6.87 (6.83-7.01)	7.04 (6.92-7.17)
2	7.15 (7.1-7.26)	7.22 (7.11-7.34)
5	7.52 (7.45-7.57)	7.41 (7.31-7.51)
9	7.96 (7.85-8.03)	7.68 (7.55-7.77)
12	8.14 (8.03-8.19)	7.99 (7.82-8.04)
16	8.19 (8.01-8.35)	8.32 (8.18-8.38)
19	8.41 (8.21-8.51)	8.51 (8.31-8.6)
23	8.63 (8.56-8.73)	8.72 (8.61-8.79)
27	8.79 (8.72-8.88)	

Table 60B

Regrowth delay of B0008 melanoma spheroids
in Experiment 24

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	7.06	(6.73-7.79)
2	6.46	(5.93-7.33)
3	12.39	(11.74-14.28)
4	8.79	(7.92-11.54)
5	8.29	(8.03-9.54)
6	13.24	(11.88-15.25)

Table 60C

Proportion of spheroids "cured" in Experiment 24.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/23	0
2	0/22	0
3	0/23	0
4	0/22	0
5	0/23	0
6	0/21	0

Table 61A

Growth of large B0008 melanoma spheroids in Experiment 25.

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM at pH7
2	MEM at pH7.4
3	MEM at pH8
4	melphalan 4μg/ml at pH7
5	melphalan 4μg/ml at pH7.4
6	melphalan 4μg/ml at pH8

MEDIAN LOG VOLUME (≥95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.97 (6.91-7.06)	7 (6.9-7.15)
4	7.68 (7.63-7.74)	7.74 (7.61-7.78)
8	8.22 (8.19-8.24)	8.19 (8.17-8.24)
11	8.39 (8.35-8.4)	8.39 (8.35-8.41)
14	8.51 (8.49-8.53)	8.51 (8.49-8.56)
18	8.58 (8.57-8.6)	8.64 (8.58-8.65)
22	8.73 (8.67-8.75)	8.73 (8.71-8.77)
	PLATE 3	PLATE 4
0	6.91 (6.86-7.09)	6.97 (6.87-7.05)
4	7.71 (7.65-7.78)	7.51 (7.4-7.6)
8	8.2 (8.18-8.25)	7.74 (7.67-7.79)
11	8.38 (8.36-8.43)	7.94 (7.87-8.02)
14	8.53 (8.48-8.56)	8.17 (8.11-8.22)
18	8.61 (8.57-8.63)	8.35 (8.31-8.42)
22	8.72 (8.7-8.75)	8.58 (8.48-8.65)
25		8.76 (8.69-8.81)
	PLATE 5	PLATE 6
0	7.11 (6.97-7.22)	6.96 (6.88-7.02)
4	7.61 (7.59-7.71)	7.52 (7.46-7.63)
8	7.93 (7.86-7.97)	7.94 (7.86-8.04)
11	8.15 (8.07-8.19)	8.17 (8.08-8.24)
14	8.34 (8.28-8.4)	8.34 (8.23-8.41)
18	8.58 (8.46-8.7)	8.5 (8.46-8.63)
22	8.84 (8.74-8.88)	8.69 (8.55-8.75)
25		8.85 (8.74-8.88)

Table 61B

Regrowth delay of B0008 melanoma spheroids
in Experiment 25

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	6.25	(5.76-6.61)
2	6.68	(5.98-7.2)
3	5.85	(5.4-6.59)
4	11.32	(10.19-11.95)
5	10.09	(9.64-10.74)
6	8.41	(7.8-8.96)

Table 61C

Proportion of spheroids "cured" in Experiment 25.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/24	0
2	0/23	0
3	0/21	0
4	0/22	0
5	0/17	0
6	0/20	0

Table 62A

Growth of small B0008 melanoma spheroids in Experiment 26.

Plate No. 'Treatment': 60 minutes at 37°C in..

1	MEM at pH7
2	MEM at pH7.4
3	MEM at pH8
4	melphalan 4 μ g/ml at pH7
5	melphalan 4 μ g/ml at pH7.4
6	melphalan 4 μ g/ml at pH8

MEDIAN LOG VOLUME (>95% confidence intervals)

DAY	PLATE 1	PLATE 2
0	6.52 (6.39-6.73)	6.77 (6.59-6.86)
2	6.81 (6.71-6.97)	7.04 (6.95-7.21)
4	7.11 (6.83-7.16)	7.29 (7.16-7.45)
7	7.61 (7.34-7.99)	7.63 (7.55-7.77)
11	7.94 (7.77-8.07)	8.08 (7.96-8.19)
14	8.15 (7.94-8.24)	8.32 (8.26-8.41)
17	8.36 (8.26-8.42)	8.51 (8.4-8.63)
21	8.51 (8.4-8.55)	8.63 (8.52-8.68)
25	8.71 (8.61-8.77)	8.74 (8.69-8.83)
	PLATE 3	PLATE 4
0	6.76 (6.52-6.84)	6.63 (6.55-6.75)
2	6.91 (6.83-7.05)	6.88 (6.73-6.95)
4	7.26 (7-7.32)	6.9 (6.8-7.01)
7	7.66 (7.32-7.73)	6.93 (6.65-7.05)
11	8.05 (7.89-8.18)	7.07 (6.67-7.28)
14	8.24 (8.06-8.35)	7.03 (6.76-7.62)
17	8.37 (8.25-8.45)	7.27 (6.96-7.9)
21	8.57 (8.44-8.63)	7.86 (7.32-8.18)
25	8.71 (8.66-8.72)	8.02 (7.7-8.44)
28	8.84 (8.76-8.88)	8.2 (7.94-8.59)
31		8.46 (8.07-8.69)
35		8.52 (8.07-8.73)
38		8.53 (8.14-8.88)
	PLATE 5	PLATE 6
0	6.49 (6.36-6.8)	6.29 (6.18-6.39)
2	6.8 (6.69-6.91)	6.55 (6.45-6.67)
4	6.91 (6.76-7.04)	6.76 (6.52-6.87)
7	7.03 (6.75-7.19)	6.84 (6.63-7.1)
11	7.23 (7.03-7.43)	7.44 (6.87-7.52)
14	7.36 (7.09-7.71)	7.81 (7.01-7.91)
17	7.62 (7.46-7.98)	8.06 (7.33-8.17)
21	8.03 (7.82-8.36)	8.32 (7.58-8.46)
25	8.2 (8.04-8.54)	8.46 (7.93-8.57)
28	8.56 (8.32-8.66)	8.72 (8.39-8.76)
31	8.7 (8.57-8.8)	8.77 (8.42-8.88)
35	8.73 (8.64-8.88)	

Table 62B

Regrowth delay of B0008 melanoma spheroids
in Experiment 26

PLATE No.	Median Regrowth Delay	≥95% confidence intervals
1	7.23	(5.64-10.63)
2	7.95	(7.04-8.95)
3	8.16	(7.62-8.75)
4	19.84	(17-24.24)
5	16.27	(11.54-20.72)
6	12.48	(9.54-16.02)

Table 62C

Proportion of spheroids "cured" in Experiment 26.

PLATE No.	Proportion of spheroids "cured"	% SPHEROIDS "CURED"
1	0/22	0
2	0/22	0
3	0/19	0
4	2/24	8
5	1/24	4
6	0/22	0

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