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The influence of fatty acids in vitro on mammalian cells from species differing in their fatty acyl desaturase capabilities.

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4.3 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM CAT AORTIC ENDOTHELIUM.

4.3.1 Effects of pseudo-Oils on Cell Viability.

The cultured cells were examined microscopically before, during and after each experiment and at no time were any morphological changes observed between control or dosed cells, except for cytoplasmic droplets observed with p-oil supplementation at high concentrations in some instances.

The effects of albumin, with final medium concentrations ranging from 0 to 250mg/l, were investigated, and found to have no significant effect, on cell viability (Fig. 4.3.1.1.), nor were any synergistic effects induced. Thus, the effects induced with the p-oils dosed were a result of the exogenous FA's and not the albumin used as FA carrier.

The number of control cells, seeded at $10x10^4$/ml, at the end of the 24 hour post-trypsinisation recovery period, was $10.5x10^4$/ml. This equated to 80% of the final control cell number at the end of the 48 hour incubation period, and represented the cytostatic number. Hence, only p-oil concentrations reducing cell viability to significantly below 80% were considered cytotoxic.

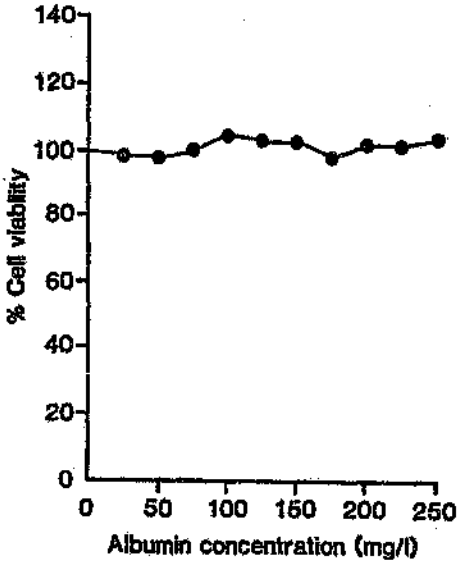
Figs. 4.3.1.2-4.3.1.8. show the effects of p-oil dosage

Legend to Figs. 4.3.1.1-4.3.1.9.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where "n" is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 4.3.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.3.1.2-4.3.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 4.3.1.9. shows the mean percent cell viability versus the albumin concentration (mg/l) for cells dosed with the amount of pseudo-oil which limits cell viability to 50% of the controls.

Fig. 4.3.1.1.

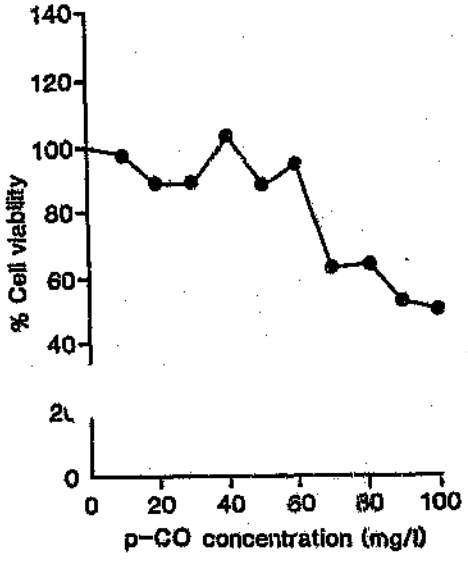
The percentage viability of cells derived from cat aortic endothelium incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.1	12
25	97.8	2.1	12
50	97.8	3.6	12
75	100.0	3.1	12
100	104.3	3.3	12
125	102.6	1.0	12
150	102.6	2.6	12
175	97.8	3.0	12
200	101.2	3.0	12
225	101.7	1.9	12
250	103.0	1.8	12

Fig. 4.3.1.2.

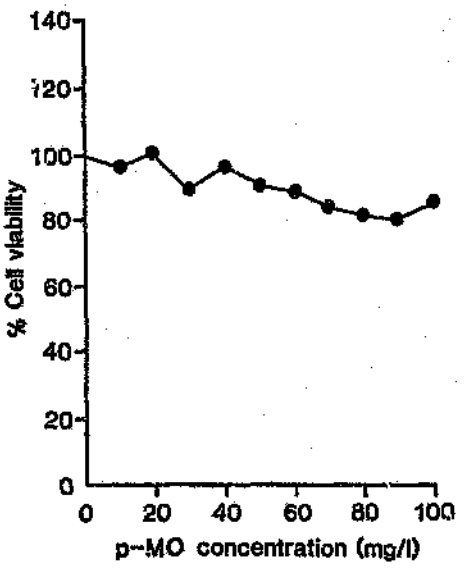
The percentage viability of cells derived from cat aortic endothelium incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	96.9	7.0	6
20	88.2	5.1	6
30	89.0	5.1	6
40	102.5	3.9	6
50	87.9	6.6	6
60	94.2	4.1	6
70	62.8	3.0	6
80	64.0	3.3	6
90	52.2	3.9	6
100	49.4	2.0	6

Fig. 4.3.1.3.

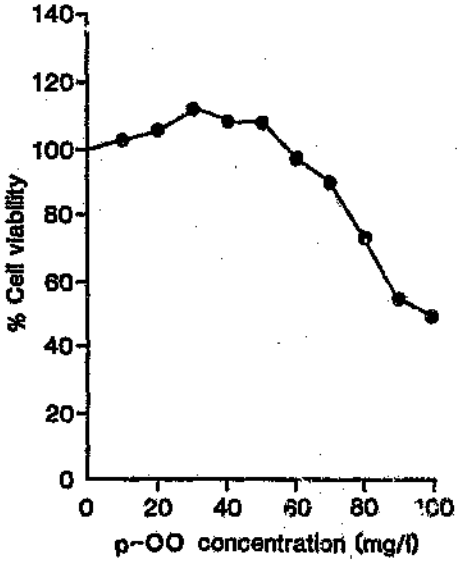
The percentage viability of cells derived from cat aortic endothelium incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.5	6
10	95.7	3.1	6
20	100.9	3.6	6
30	89.3	5.6	6
40	96.3	3.2	6
50	90.0	4.2	6
60	88.4	2.2	6
70	83.9	4.3	6
80	81.1	4.3	6
90	79.8	5.5	6
100	85.6	4.8	6

Fig. 4.3.1.4.

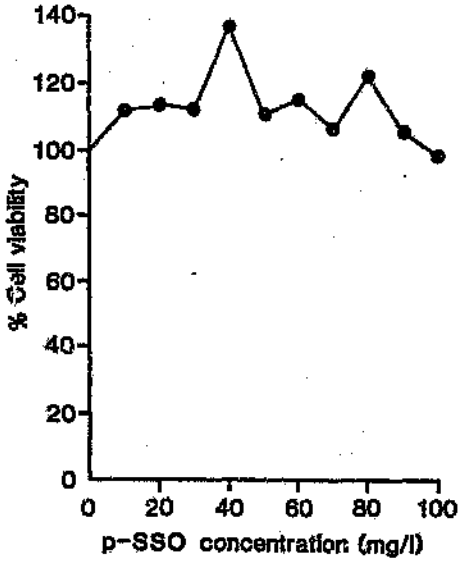
The percentage viability of cells derived from cat aortic endothelium incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	102.1	1.6	6
20	104.8	2.3	6
30	111.4	4.4	6
40	107.9	2.6	6
50	107.3	4.6	6
60	96.8	2.5	6
70	89.5	3.7	6
80	72.4	2.5	6
90	54.0	2.8	6
100	48.9	2.5	6

Fig. 4.3.1.5.

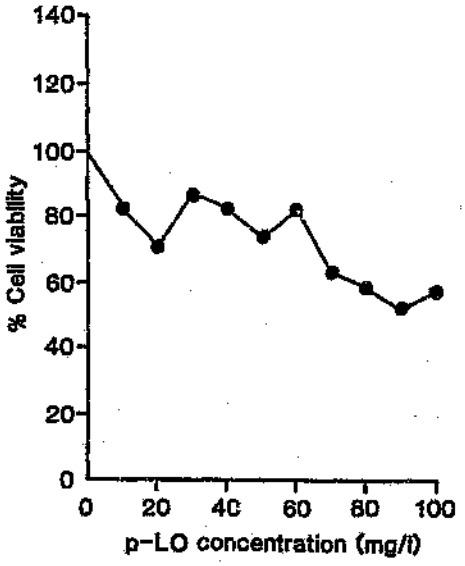
The percentage viability of cells derived from cat aortic endothelium incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	111.9	5.0	6
20	113.4	6.1	6
30	111.4	5.7	6
40	137.4	1.6	6
50	110.9	3.4	6
60	115.8	4.3	6
70	106.6	5.4	6
80	121.7	4.6	6
90	105.2	4.5	6
100	98.2	4.2	6

Fig. 4.3.1.6.

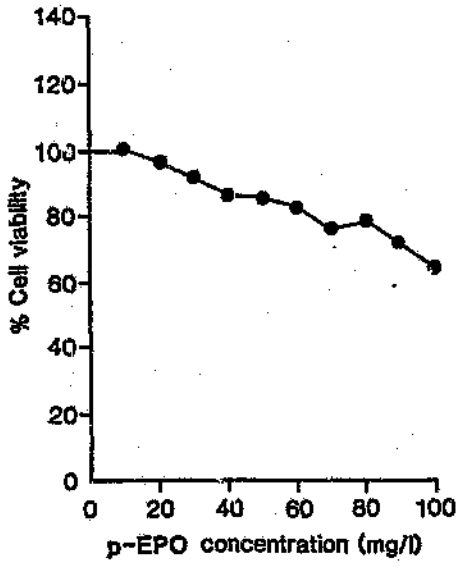
The percentage viability of cells derived from cat aortic endothelium incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.5	6
10	81.6	4.9	6
20	71.2	3.8	6
30	87.1	5.9	6
40	81.7	4.0	6
50	73.4	3.4	6
60	82.1	4.9	6
70	63.0	4.3	6
80	58.4	2.6	6
90	51.1	6.7	6
100	56.9	3.0	6

Fig. 4.3.1.7.

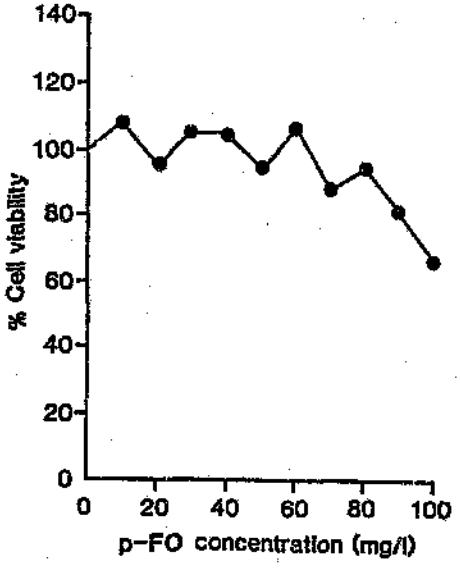
The percentage viability of cells derived from cat aortic endothelium incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.3	6
10	100.4	2.3	6
20	97.0	2.9	6
30	92.5	2.9	6
40	86.6	1.1	6
50	85.5	2.1	6
60	83.2	3.1	6
70	76.9	2.2	6
80	79.5	2.2	6
90	72.0	1.9	6
100	64.6	4.3	6

Fig. 4.3.1.8.

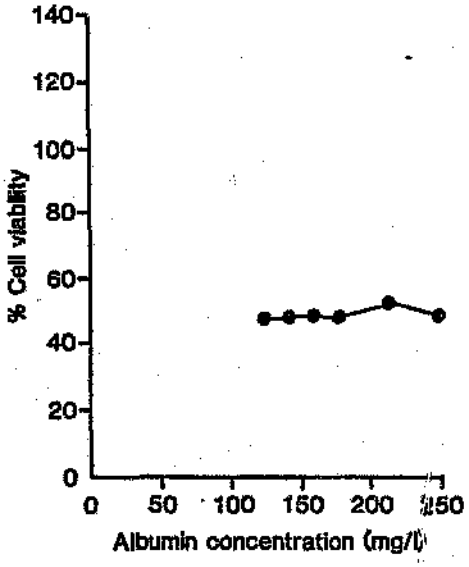
The percentage viability of cells derived from cat aortic endothelium incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.0	6
10	108.1	6.1	6
20	95.4	4.0	6
30	105.4	6.9	6
40	104.4	6.0	6
50	93.7	6.8	6
60	106.0	5.3	6
70	87.7	4.1	6
80	93.7	3.8	6
90	81.2	6.8	6
100	66.3	2.8	6

Fig. 4.3.1.9.

The percentage viability of cells derived from cat aortic endothelium incubated with albumin at the ID_{50} of p-OO.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.1	6
175	48.0	7.4	6
200	48.7	4.7	6
225	48.9	3.5	6
250	49.1	4.4	6
300	52.2	3.3	6
350	48.6	2.9	6

on cat endothelial cell viability. Growth limiting effects were generally induced, but some p-oils also stimulated and/or inhibited cell proliferation with particular concentrations. The magnitude of effects induced, however, were dependent on the p-oil and concentration dosed. p-SSO exhibited the greatest potential to stimulate cell proliferation, whereas p-CO, p-OO and p-LO were most growth inhibitory. ID_{50} values could, however, only be calculated for cell dosed with p-CO or p-OO (99mg/l each).

No major changes in cell viability occurred overall with 10 to 60mg/l p-CO dosage, while higher concentrations induced cytotoxicity such that 49.4% of cell were viable with 100mg/l (Fig. 4.3.1.2.).

Cell growth largely approximated to controls with up to 40mg/l p-MO incubation (Fig. 4.3.1.3.). Growth limiting effects were induced with higher amounts, in a general concentration dependent manner, to yield a minimum of 79.8% viable cells with 90mg/l p-MO. Cytostasis was induced with this concentration as the relative cell number approximated to the cytostatic number (80%). p-MO was ineffective in inhibiting cell growth.

10, 20 and 60mg/l p-OO had little effect on cell viability, but this was increased to 111.4%, 107.9% and 107.3% with 30, 40 and 50mg/l p-OO, respectively. (Table 4.3.1.4.). Cell growth was limited with 70mg/l

p-OO, while concentration dependent cytotoxicity was induced with concentrations of 80mg/l or greater such that 48.9% of cells were viable with 100mg/l p-OO.

Cell proliferation was enhanced with up to 90mg/l p-SSO supplementation (Fig. 4.3.1.5.). An increment of 5% to 15% was generally observed, although concentrations of 40 and 80mg/l p-SSO enhanced cell viability to 137.4% and 121.7%, respectively. 100mg/l p-SSO induced little effect on cell growth as viability was 98.2%.

p-LO concentrations up to 60mg/l largely induced cytostasis as relative cell viability approximated to the cytostatic number (80%). Higher concentrations resulted in toxicity, but cell viability was 51.1% and 56.9% even with 90 and 100mg/l p-LO, respectively (Fig. 4.3.1.6.).

p-EPO induced an overall concentration dependent growth limiting effect with concentrations up to 50mg/l, although cell growth was not significantly different from controls with concentrations up to 20mg/l (Fig. 4.3.1.7.). 60 to 80mg/l p-EPO induced cytostasis, while greater amounts were cytotoxic and progressively reduced cell viability to a minimum of 64.6% with 100mg/l p-EPO.

Cell growth generally approximated to that of controls with up to 60mg/l p-FO dosage, although 108.1% viability occurred with a concentration of 10mg/l (Fig. 4.3.1.8.). 70 and 80mg/l p-FO limited proliferation marginally, 90mg/l p-FO induced cytostasis, and cytotoxicity reduced

cell viability to 66.3% with 100mg/l p-FO.

To exclude the possibility that any of the effects induced with p-oil dosage were influenced by the amount of albumin used as FA carrier, cells were incubated with a fixed amount of p-OO (corresponding with its ID_{50}, about 99mg/l), but varying albumin concentrations. Six concentrations were chosen around which to vary the albumin amount, viz. 2 points above, 3 points below, and the ID_{50} of p-OO, and the appropriate amount of albumin added to cultures to give the desired concentrations. The results are shown in Fig. 4.3.1.9., which indicated that the amount of p-OO dosed reduced cell viability to approximately 50% of controls, and that such was not influenced by the albumin concentration present as FA carrier. Hence, synergistic effects of albumin could be excluded, and the effects induced with the p-oils were solely due to the exogenous FA's.

Cat aortic endothelial cells were subsequently plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient amounts for all qualitative and quantitative analyses to be carried out. Upon harvesting, cell viabilities were compared and found not to be statistically different from those in Figs. 4.3.1.1-4.3.1.8. All further biochemical assays were thus performed on these samples.

4.3.2 Effects of pseudo-Oils on Total Protein.

The total cellular protein concentrations determined with 0, 20 40 or 60mg/l p-oil dosage are shown in Table 4.3.2.1. expressed as µg total protein/10^6 cells seeded.

424.4µg protein per 10^6 control cells seeded was found. Cells supplemented with 20mg/l p-LO contained 396.1µg protein per 10^6 cells seeded, but all other dosed cells contained greater amounts of total protein than control cells, with the highest concentration measured being 656.3µg per 10^6 cells seeded, obtained with 40mg/l p-SSO supplementation. The total cellular protein content decreased with increasing concentrations of p-MO or p-EPO supplemented (554.3 to 454.0 and 515.5 to 455.8 µg/10^6 cells seeded, respectively), whereas the reverse was demonstrated for cells incubated with p-LO or p-FO (396.1 to 497.7 and 501.5 to 533.4µg/10^6 cells seeded, respectively). With p-CO, p-OO or p-SSO dosage, however, the protein increment was greatest with a concentration of 40mg/l (563.7, 524.3 and 656.3µg/10^6 cells seeded, respectively) and least with 20mg/l (444.7, 491.3 and 540.5µg/10^6 cells seeded, respectively).

Table 4.3.2.1.

The protein content of cells derived from cat aortic endothelium, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	424.4			
CO		444.7	563.7	475.6
MO		554.3	508.5	454.0
OO		491.3	524.3	506.3
SSO		540.5	656.3	602.5
LO		396.1	491.3	497.7
EPO		515.5	463.1	455.4
FO		501.5	524.3	533.4

4.3.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Cat Aortic Endothelium.

The FA spectra of cells incubated with 0, 20, 40 or 60mg/l of each of the p-oils is depicted in Table 4.3.3.1. Major FA's detected in control cells were 16:0 (19.9%), 18:0 (15.8%), 18:1ω9 (21.2%), 20:4ω6 (5.3%), 20:4ω3 (5.4%) and 22:4ω6 (15.5%). Varying amounts of these FA's were detected in dosed cells.

The levels of 16:0 and 18:0 were parallel to controls or decreased, whereas 16:1ω9 and 18:1ω9 levels were more varied. Dosed cells contained raised 18:2ω6 percentages, and significant increments in 20:3ω6 and 20:4ω6, but not 22:4ω6 and 22:5ω6, were noted with certain p-oils and concentrations. Dosed cells exhibited considerablly less 18:4ω3 and 20:4ω3 than controls, whereas 18:3ω3, 20:5ω3, 22:5ω3 and 22:6ω3 levels were parallel to controls or increased.

Supplementation with 20, 40 or 60mg/l p-CO induced no marked changes in 16:0 and 18:1ω9 percentages (21.7% to 24.3% and 20.5% to 25.4% vs 19.9% and 21.2% in controls, respectively), whereas 18:0 levels were decreased (10.8% to 12.8% vs 15.8% in controls). 16:1ω9 amounts were similar with 0 and 20mg/l p-CO, but enhanced with 40 and 60mg/l p-CO (2.1%, 1.8%, 3.0% and 3.5%, respectively). 20:3ω6 and 20:4ω6 levels were slightly increased (2.1%

Legend to Table 4.3.3.1.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where 'n' is the number of experiments. '[p-Oil]' refers to the pseudo-oil concentration used.

Table 4.3.3.1.

The fatty acid spectrum of cells derived from cat aortic endothelium.

FATTY ACID SPECTRUM (%)		CONTPOLS (n=3)	[p-COI] (mg/l) 20	40	60	[p-MOI] (mg/l) 20	40	60	[p-OOI] (mg/l) 20	40	60	[p-SSOI] (mg/l) 20	40	60	[p-LOI] (mg/l) 20	40	60	[p-EPOI] (mg/l) 20	40	60	[p-FOI] (mg/l) 20	40	60
SATURATES.	16:0	19.9±0.35	22.0	21.7	24.3	19.6	19.8	23.9	17.2	16.4	17.5	17.8	15.9	13.9	18.5	16.6	17.3	18.2	15.8	16.1	18.6	16.8	16.6
	18:0	15.8±0.40	12.8	11.8	10.8	12.6	13.1	13.7	9.0	8.0	6.9	11.5	10.3	8.5	12.0	10.7	9.6	12.0	10.5	9.5	11.4	9.2	6.8
	20:0	0.6±0.00	-	0.2	-	-	0.2	-	-	-	-	-	0.1	-	-	0.1	-	-	-	-	-	-	-
	22:0	0.9±0.00	0.1	0.1	0.2	0.1	0.2	0.1	-	0.2	-	-	0.1	-	-	-	0.1	-	0.1	0.1	-	0.1	0.1
	24:0	0.2±0.00	-	0.2	0.1	-	-	0.2	-	0.1	-	-	-	0.2	-	-	0.1	-	0.4	0.2	-	-	-
ω9 MONOS.	16:1	2.1±0.05	1.8	3.0	3.5	1.2	1.6	2.5	1.5	1.7	3.0	1.3	1.9	1.3	1.4	1.5	2.7	1.1	1.7	2.4	6.2	11.9	17.8
	18:1	21.2±0.30	22.6	20.5	25.4	22.3	21.5	23.5	33.3	42.8	45.2	21.2	20.2	21.2	21.4	18.2	17.9	18.3	15.5	14.8	20.1	18.1	18.9
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	-	-
ω6 POLYS.	18:2	3.0±0.05	4.8	3.2	4.5	6.0	6.3	9.3	6.9	9.1	10.0	15.9	25.1	35.7	7.7	8.2	10.4	17.1	26.2	32.7	5.6	5.6	8.0
	18:3	-	0.3	-	0.3	0.3	-	0.2	0.2	-	0.2	0.2	-	0.2	0.2	-	0.2	0.4	0.5	1.3	0.2	-	0.1
	20:2	-	0.1	0.1	0.2	0.2	0.3	0.3	0.7	1.7	1.8	0.5	1.3	1.7	0.2	0.2	0.2	0.6	1.0	1.2	0.2	0.5	0.6
	20:3	1.8±0.05	2.3	2.4	2.1	2.4	2.6	2.1	1.8	1.7	1.1	1.9	2.1	1.7	1.9	1.7	1.2	3.7	4.5	4.2	1.8	1.8	1.2
	20:4	5.3±0.15	8.9	8.6	7.2	10.0	10.2	9.2	6.6	5.5	3.6	6.1	5.8	4.3	7.9	7.3	5.6	6.2	5.5	3.9	6.7	5.4	4.1
	22:4	15.5±0.50	15.4	16.7	12.1	16.6	13.7	8.2	15.4	5.7	6.4	17.2	10.2	5.9	15.1	13.7	7.6	16.2	10.9	8.5	14.7	11.7	6.6
	22:5	1.9±0.09	-	0.5	0.1	0.1	0.4	0.1	-	0.4	0.1	-	0.3	0.1	0.1	0.4	0.1	0.2	0.4	0.1	0.1	0.3	-
ω3 POLYS.	18:3	0.4±0.00	0.5	0.6	0.6	0.6	0.6	0.5	0.6	0.8	0.7	0.4	0.4	0.4	6.9	14.4	22.1	0.3	0.3	0.4	0.5	0.5	0.6
	18:4	3.0±0.10	0.4	1.1	0.3	0.3	1.3	0.2	0.2	0.9	0.2	0.2	0.6	0.2	0.3	0.9	0.2	0.3	0.8	0.2	0.4	0.5	0.3
	20:4	5.4±0.05	1.3	1.6	1.2	1.6	1.9	0.4	1.6	0.5	0.5	1.4	1.0	0.4	1.6	1.6	0.8	2.1	1.3	0.7	2.8	2.3	1.3
	20:5	0.3±0.00	1.4	1.7	1.3	0.8	0.7	1.0	1.0	0.5	0.5	0.6	0.6	1.1	0.6	0.6	0.7	0.3	0.9	1.1	3.6	7.7	11.1
	22:5	1.5±0.05	3.0	2.9	2.6	3.0	2.9	2.3	2.2	2.0	1.3	2.2	2.1	1.6	2.5	2.0	1.6	1.9	1.9	1.2	5.1	5.7	4.1
	22:6	1.1±0.05	2.4	3.4	3.3	2.3	3.0	2.5	1.9	2.2	1.5	1.6	2.1	1.8	1.8	1.9	1.6	1.2	1.9	1.4	1.6	1.9	1.8

to 2.4% and 7.2% to 8.9% vs 1.8% and 5.3% in controls, respectively), but 22:4ω6 levels were parallel to controls (15.5%) or decreased. The percentages of 18:4ω3 and 20:4ω3 were significantly decreased in relation to controls (3.0% and 5.4%, respectively), although 20:5ω3, 22:5ω3 and 22:6ω3 amounts increased to a maximum of 1.7%, 3.9% and 3.4% compared to 0.3%, 1.5% and 1.1% in controls, respectively.

No significant changes in 16:0 and 18:1ω9 were found in cells dosed with p-MO, but 18:0 and 16:1ω9 levels were parallel to controls (15.8% and 2.1%, respectively), or decreased. 18:2ω6 incorporation increased 2 to 3 fold (6.0% to 9.3% vs 3.0% in controls), a small increase in 20:3ω6 was found (2.1% to 2.6% vs 1.8% in controls), and 20:4ω6 increased approximately 2 fold (10.0%, 10.2% and 9.2% with 20, 40 and 60mg/l, respectively vs 5.3% in controls). However, 22:4ω6 levels were not significantly increased, and only very small amounts of 22:5ω6 were found (<0.5% vs 1.9% in controls). p-MO decreased 18:4ω3 and 20:4ω3 levels, but 20:5ω3, 22:5ω3 and 22:6ω3 were significantly increased (0.8% to 1.0%, 2.3% to 3.0% and 2.3% to 3.0% vs 0.3%, 1.5% and 1.1% in controls, respectively).

18:1ω9 levels were increased with 20, 40 or 60mg/l p-OO supplementation (33.3%, 42.8% and 45.2%, respectively vs 21.2% in controls), whereas 16:0, 18:0 and 16:1ω9 were

decreased relative to controls (19.9%, 15.8% and 2.1%, respectively). 18:2ω6 percentages increased 2 to 4 fold (3.0% in controls), and a significant increase in 20:2ω6 was found, especially with 40 and 60mg/l p-OO (1.7% and 1.8%, respectively vs 0% in controls). Except for 6.6% 20:4ω6 found with 20mg/l p-MO (5.3% in controls), other ω6 PDFA percentages were decreased, particularly with increasing amounts of p-OO dosed. p-OO induced similar changes in the ω3 PUFA's as reported with p-CO and p-MO.

p-SSO incubation had little effect on cellular 18:1ω9 levels, induced a decrease in 16:0, 16:1ω9 and 18:0 (21.2%, 19.9%, 2.1% and 15.8% in controls, respectively) and resulted in a concentration dependent increase in 18:2ω6 (15.9%, 25.1% and 35.7% with 20, 40 and 60mg/l p-SSO, respectively vs 3.0% in controls). 20:2ω6 was significantly increased with 40 and 60mg/l p-SSO (1.3% and 1.7%, respectively vs 0% in controls), but this was not found for other ω6 PDFA's. The changes induced in ω3 PUFA's were as reported above.

Saturated and monounsaturated FA levels approximated to controls or were decreased with p-LO incubation. 18:2ω6 amounts increased 2 to 4 fold (3.0% in controls) and a small increase in 20:4ω6 was induced, but other ω6 PDFA levels were not significantly increased. A concentration dependent increase in 18:3ω3 was detected with 20, 40 or 60mg/l p-LO dosage (6.9%, 14.4% and 22.1%, respectively vs 0.4% in controls). 18:4ω3 and 20:4ω3 levels, however,

were at least 3 fold lower than controls (3.0% and 5.4%, respectively), but there was a 2 fold increase in 20:5ω3 over the range dosed (0.6% to 0.7% vs 0.3% in controls). Increased levels of 22:5ω3 and 22:6ω3 were also found (1.6% to 2.5% and 1.6% to 1.9% vs 1.5% and 1.1% in controls, respectively), but these increments were not greater than shown with other p-oils.

16:0, 16:1ω9, 18:0 and 18:1ω9 amounts were parallel to controls (19.9%, 2.1%, 15.8% and 21.2%, respectively) or decreased in cells dosed with p-EPO. On the other hand, 18:2ω6 levels increased markedly (3.0%, 17.1%, 26.2% and 32.7% with 0, 20, 40 and 60mg/l p-EPO, respectively), while smaller increments were found for 20:2ω6 and 18:3ω6 (0.6%, 1.0% and 1.2%, and 0.4%, 0.5% and 1.3%, respectively vs 0% each in controls). 20:3ω6 percentages increased 2 to 3 fold (3.7%, 4.5% and 4.2% with 20, 40 and 60mg/l p-EPO, respectively vs 1.8% in controls), but concentration dependent decreases in 20:4ω6 and 22:5ω6 were found (6.2%, 5.5% and 3.9%, and 16.2%, 10.9% and 8.5% vs 5.3% and 15.5% in controls, respectively). p-EPO decreased 18:4ω3 and 20:4ω3 levels, but induced small increments in 20:5ω3, 22:5ω3 and 22:6ω3 with certain concentrations.

16:0, 18:0 and 18:1ω9 levels were parallel to controls or decreased when cells were dosed with 20, 40 or 60mg/l p-FO relative to controls (19.9%, 15.8% and 21.2%,

respectively), but a concentration related increment in 16:1ω9 was found (6.2%, 11.9% and 17.8%, respectively vs 2.1% in controls). 18:2ω6 incorporation was less marked (5.6% to 8.0% vs 3.0% in controls), and PDFA levels were parallel to controls or decreased. Significant increases in 20:5ω3 and 22:5ω3 were detected with 20, 40 and 60mg/l p-FO (3.6%, 7.7% and 11.1%, and 5.1%, 5.7% and 4.1% vs 0.3% and 1.5% in controls, respectively), but the increment in 22:6ω3 was smaller (1.6%, 1.9% and 1.8%, respectively vs 1.1% in controls).

4.3.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides measured in control and dosed cells and their their respective spent culture media are shown in Table 4.3.4.1. The concentrations are expressed as nmoles MDA/10^6 cells, but in the case of the spent media this represent the nmoles of MDA in the volume of medium from which 1x10^6 cells were obtained.

Lipoperoxide amounts generated by dosed cells ranged from a low of 0.9nmoles MDA/10^6 cells (obtained with 20mg/l p-SSO, 20mg/l p-EPO or 60mg/l p-CO dosage) to a high of 2.4nmoles MDA/10^6 cells (induced with 60mg/l p-LO), compared to 0.8nmoles MDA/10^6 control cells. p-CO had little overall effect on lipoperoxide formation over the range dosed (0.9 to 1.1nmoles MDA/10^6 cells), while 20, 40 and 60mg/l p-MO induced greater amounts (2.0, 1.5 and 1.2nmoles MDA/10^6 cells, respectively). Dosage with 20, 40 or 60mg/l p-LO or p-FO generated similar levels of lipoperoxides (1.3, 1.7 and 2.4 vs 1.7, 1.6 and 2.1 nmoles MDA/10^6 cells, respectively), whereas smaller amounts were induced with p-SSO (0.9, 1.3 and 1.1nmoles MDA/10^6 cells, respectively), p-EPO (0.9, 0.9 and 1.6 nmoles MDA/10^6 cells, respectively) and p-OO (1.1, 1.0 and 1.7nmoles MDA/10^6 cells, respectively).

Lipoperoxides were absent in spent control mediu while concentrations were greatest for medium dosed with

Table 4.3.4.1.

Lipoperoxide formation by cat aortic endothelial cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS 0	20	40	60
Control	0.8			
CO		1.0	1.1	0.9
MO		2.0	1.5	1.2
OO		1.1	1.0	1.7
SSO		0.9	1.3	1.1
LO		1.3	1.7	2.4
EPO		0.9	0.9	1.6
FO		1.7	1.6	2.1
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM 0	20	40	60
Control	-			
CO		-	-	0.1
MO		-	-	0.3
OO		-	0.1	0.1
SSO		-	0.4	-
LO		0.1	0.4	1.1
EPO		-	0.1	0.2
FO		0.3	0.5	1.2

20, 40 or 60 p-FO (0.3, 0.5 and 1.2nmoles MDA/10^6 cells, respectively) or p-LO (0.1, 0.4 and 1.1nmoles MDA/10^6 cells, respectively). Spent media from all other dosed cultures, however, contained fewer lipoperoxides ($\leq$0.4 nmoles MDA/10^6 cells), or none at all.

4.3.5 Discussion.

Dosing primary cultures of cat endothelial cells with p-oils modulated the viability of such cells, although the effects induced were not consistent with the degree of p-oil unsaturation (Figs. 4.3.1.2-4.3.1.8.). Even p-oils of similar FA compositions induced different effects, suggesting that the particular p-oil FA composition (Table 2.3.3.2.) was important in the modulation of the effects reported. It was apparent that the presence of GLA only in p-EPO induced growth limiting and inhibitory effects, whereas its absence in p-SSO modulated extensive cell proliferation which could be largely related to an abundance of LA. GLA was thus a potent agent preventing the proliferation of these cells, unlike LA which promoted cell growth. ALA seemed less effective than GLA in this regard as the extent of growth inhibition induced with p-LO was not significantly greater than with p-EPO incubation, despite more ALA in p-LO than GLA in p-EPO (about 63% vs 9%, respectively). Such differences may relate to the different double bond positions in each trienoic FA and the fact that GLA is a PDFA, unlike ALA which requires prior delta-6-desaturation to become biologically active in the cat (Frankel et al 1978). On the other hand, counteraction between inhibitory and proliferative effects modulated between different FA's in p-FO could account for the overall absence of marked cell viability

changes induced with supplementation of this p-oil. This may relate to the more even balance of saturated, monounsaturated and polyunsaturated FA's in p-FO. The involvement of p-oil FA synergism/antagonism could also partly account for the cell viability changes other p-oils induced. This phenomemon could explain the enhancement of cell proliferation with low, and inhibition thereof with high, p-OO concentrations as a result of interations between OA with other FA's, as well as the particularly extensive capability of 40mg/l p-SSO to stimulate cell growth. It was nevertheless apparent from comparison of the p-oil FA spectra (Table 2.3.3.2.) with the cell viability changes the p-oils induced (Figs. 4.3.1.2-4.3.1.8.) that different FA's, or combinations thereof, had the capability to stimulate, limit and/or inhibit cell proliferation to a greater or lesser extent.

The incorporation of p-oils and modification of the FA profiles of dosed cells in relation to controls (Table 4.3.3.1.) could have modulated membrane fluidity and be an important mechanism whereby the p-oils induced cell viability changes. The greater complement of saturated FA's in p-CO than in p-MO, for example, may have shifted membrane fluidity to a less stable state by causing rigidification when dosed at high concentrations, thus inducing cell death. The presence of PUFA's and a greater complement of monoenoic FA's in p-MO, however,

may have been able to maintain membrane fluidity, and therefore cell viability, more effectively than p-CO. Despite the possible mechanisms by which cell viability was mediated, none of the p-oils and concentrations supplemented induced marked cytotoxicity (Figs. 4.3.1.2-4.3.1.8.). The ability of these cells to tolerate relatively large amounts of p-oil FA's possibly relates to fact that the endothelium in vivo is continuously exposed to FA's in the circulation and may therefore be relatively resistant to the effects thereof.

Supplementation of cultures with p-oils resulted in total cellular protein changes (Table 4.3.2.1.) which generally reflected the cell viability changes induced (Figs. 4.3.1.2-4.3.1.8.). However, the finding that cell numbers alone were unable to quantitatively explain the increased protein amounts found when cell proliferation was enhanced or limited implied FA involvement in the stimulation of cellular protein biosynthesis. The extent of protein stimulation varied with supplementation of identical concentrations of different p-oils, even when this yielded similar cell numbers, suggesting that FA's of different chain lengths and degrees of unsaturation vary in their capability to modulate protein synthesis. It was also likely that synergistic/antagonistic interactions between p-oil FA's played a role in this process. Alterations in the absolute amount of protein per cell could nevertheless reflect appropriate changes

in the expression of lipid metabolising enzymes as a result p-oil FA incorporation.

Increased PDFA formation in dosed cat endothelial cells compared to controls (Table 4.3.3.1.) suggested the capability for desaturation and elongation. However, the low 18:3ω6 and 18:4ω3 amounts formed, despite pronounced uptake of 18:2ω6 with p-SSO or p-EPO and 18:3ω3 with p-LO dosage, indicated impaired Δ6D capability in these cells. It was suggested therefore that 20:3ω6 formation could have related to limited 18:2ω6 elongation and Δ8-desaturation, although direct elongation of 18:3ω6 incorporated with p-EPO dosage could also account for the 20:3ω6 increment found with this p-oil. Formation of small amounts of 20:4ω6 was nevertheless consistent with limited Δ5D activity. This enzyme was also expressed with ω3 PUFA's, although the inability to efficiently desaturate 18:3ω3 incorporated with LA dosage implied utilisation of endogenous 18:4ω3 and 20:4ω3 as Δ5D substrates. This would indeed explain the significantly decreased 18:4ω3 and 20:4ω3 levels found in dosed cells. ω3 PUFA profiles supported the potential for significant 20:5ω3 elongation and Δ4D expression, whereas the inability to efficiently elongate 20:4ω6 could account for the lack of 22:5ω6 formation via Δ4D. This supported the greater activity of desaturase cascade enzymes with ω3 than ω6 PUFA's (Brenner et al 1966, Mead et al 1976 and Kanau et al 1977) and the potential of ω3 PDFA's to

suppress/inhibit further ω6 PDFA formation (eg. Garcia et al 1965 and Sprecher 1981).

The above suggested a mechanism whereby cat endothelial cells could metabolise circulating PDFA's, but not EFA's. This was consistent with the <u>in vivo</u> studies of Rivers et al (1975a) and Frankel et al (1978) who demonstrated that the cat lacks the ability for Δ6-desaturation, but not elongation, of FA's. Other <u>in vivo</u> studies also showed that the cat exhibits Δ5D capability (Sinclair 1979 and Davidson et al 1989, 1990a), although the presence or absence of Δ4D was not confirmed. None of the studies, however, investigated the potential for desaturation and elongation in cat endothelium. As the endothelium plays a central role in eicosanoid biosynthesis for vascular homeostasis (Moncada et al 1976a, 1976b, 1984), its capability to desaturate and elongate PDFA's to generate eicosanoid precursors may partially explain the results we obtained. No consistent evidence to support desaturation or elongation of saturated and monounsaturated FA's was found, however, and this could reflect a lack/suppression of enzyme expression, or competitive inhibition by cellular polyenoic FA's which has been demonstrated in EFA/PDFA-sufficient states (eg. Brenner et al 1965a, 1965b, 19866, 1969, Brenner 1977, Sprecher 1981 and Holman 1986a, 1986b).

The cell viability changes induced with p-oil dosage

(Figs. 4.3.1.2-4.3.1.8.) had no significant effect on the desaturase cascade enzyme capability of these cells (Table 4.3.3.1.), and it was more likely that the relative amounts of cellular FA's, both of the same and different families, modulated enzyme expression by competitive interactions which have been well documented (eg. Mohrhauer et al 1963a, 1963b, Garcia et al 1965, Brenner et al 1966, Brenner 1974, Sprecher 1981, de Schriver et al 1982 and Nassar et al 1986). Evidence suggests that dietary FA's alter the lipid composition of microsomal membranes and that such is involved in the modulation of desaturase enzyme activity (Nervi et al 1968, Kurata et al 1980, Garda et al 1984, 1985 and Garg et al 1988a, 1988b, 1988c). This probably occurred when p-oil incorporation modified the FA profiles of the dosed cells in relation to controls (Table 4.3.3.1.). It was theoretically possible that incubation of cells with saturated FA-rich p-oils induced a relative EFA/PDFA deficiency which enhanced desaturation and elongation of endogenous PDFA's in an attempt to restore membrane fluidity. This could well explain the greater 22:6ω3 amounts formed with supplementation of p-CO or p-MO than with p-oils abundant in unsaturated FA's.

MDA detection in cat endothelial cells both in the presence and absence of dosed p-oils (Table 4.3.4.1.) supported the ability of these cells to oxidise FA's enzymatically and/or via auto-oxidation when the

mechanisms of protection against free radical attack were saturated. Control cell lipid peroxides implied oxidation of endogenous unsaturated FA's released during the continuous cycling of PGL's, whereas enhanced lipid peroxide measurement in dosed cells additionally reflected oxidation of unsaturated p-oil FA's. This related to the p-oil concentration incubated, p-oil FA composition (Table 2.3.3.2.), and FA availability and susceptibility to oxidation.

The finding that cellular lipoperoxide formation with p-CO supplementation approximated to that of controls reflected the overall dearth of double bonds in the FA's comprising this p-oil. Quantitation of small cellular lipoperoxide amounts also with dosage of p-SSO, 20 or 40mg/l p-OO and 20 or 40mg/l p-EPO, despite these p-oils containing significant levels of unsaturated FA's which were incorporated (Table 4.3.3.1.), however, implied that the substrates for peroxidation were largely lipid-bound and stable, although oxidation of free LA and OA probably accounted for the significant increment in lipoperoxide formation found with 60mg/l p-EPO or p-OO dosage. The fact that cells incubated with 20 or 40mg/l p-MO exhibited the greatest AA levels (Table 4.3.3.1.) and induced similar or greater cellular lipoperoxide amounts than other p-oils at these concentrations could have related to the utilisation of free AA as a direct endoperoxide substrate. The reason why lipoperoxide

production decreased with increasing concentrations of p-MO supplemented was unclear, but may relate to the availability of unsaturated FA's for peroxidation. The amount and susceptibility of ALA in p-LO and EPA in p-FO could largely account for the fact that these p-oils induced the highest lipoperoxide concentrations overall, although the smaller amount of EPA in p-FO compared to ALA in p-LO (about 18% and 63%, respectively) supported the greater susceptibility of the former to peroxidation due to its greater chain length and number of double bonds.

The abundance of saturated FA's in new growth medium (Table 2.3.3.3.), the capability of these cells to incorporate exogenous FA's (Table 4.3.3.1.), and the lack of p-oil-induced cytotoxicity with concentrations of 20, 40 or 60mg/l (Figs. 4.3.1.2-4.3.1.8.) supported the likelyhood that the spent medium lipoperoxides (Table 4.3.4.1.) originated intracellularly and were transfered into the incubation medium through the plasma membrane. This correlated with studies which implicated lipoperoxides in increasing membrane permeability (Chio et al 1969, Mead 1976, Tappel 1980, Gavino et al 1981b, Morisaki et al 1982a and Frankel 1984). It was also possible that these spent medium lipoperoxides reflected released cellular FFA's which were oxidised extracellularly by released cellular peroxidising enzymes.

No correlation between lipoperoxide formation and growth

inhibition could be established due to the lack of cytotoxicity induced with 20, 40 or 60mg/l p-oil (Figs. 4.3.1.2-4.3.1.8.), although these compounds should not be overlooked as a mechanism involved in the modulation of cell death at higher concentrations. Quantitative changes in lipoperoxide production (Table 4.3.4.1.) did not always reflect the alterations in cell viability induced with p-oil dosage (Figs. 4.3.1.2-4.3.1.8.), and membrane fluidity changes were proposed as playing a larger role in the modulation of cat endothelial cell proliferation.

4.4 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM CAT SKELETAL MUSCLE.

4.4.1 Effects of pseudo-Oils on Cell Viability.

Control cells were examined microscopically before, during and after each experiment and no morphological changes were seen at any time. Cells dosed with p-oils were morphologically similar to controls, although cytoplasmic droplets were observed with p-oil incubation at high concentrations in some instances.

Final medium albumin concentrations ranging from 0 to 250mg/l were found to induce no significant effect on cell viability (Fig. 4.4.1.1.), nor were any synergistic effects demonstrated. The effects induced with the dosed p-oils were therefore solely due to the exogenous FA's and not of the albumin used as FA carrier.

The number of control cells, seeded at $10x10^4$/ml, at the end of the 24 hour post-trypsinisation recovery period, was $11.5x10^4$/ml. This equated to approximately 67% of the final control cell number at the end of the 48 hour incubation period and represented the cytostatic number. Only p-oil concentrations reducing cell viability to significantly below 67% were thus considered cytotoxic.

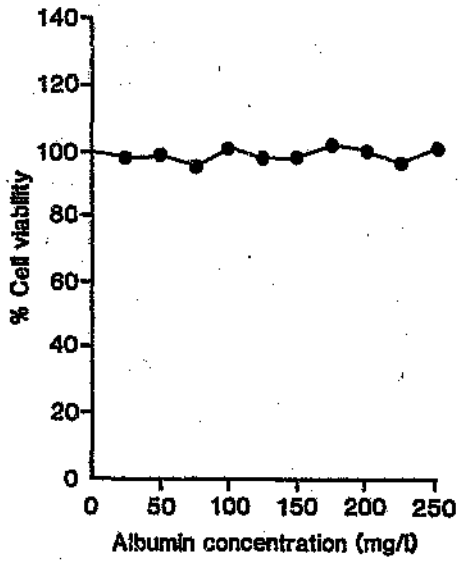
The results of the incubation of cells with p-oils are shown in Figs. 4.4.1.2-4.4.1.8. Low concentrations of

Legend to Figs. 4.4.1.1-4.4.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where "n" is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 4.4.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.4.1.2-4.4.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 4.4.1.1.

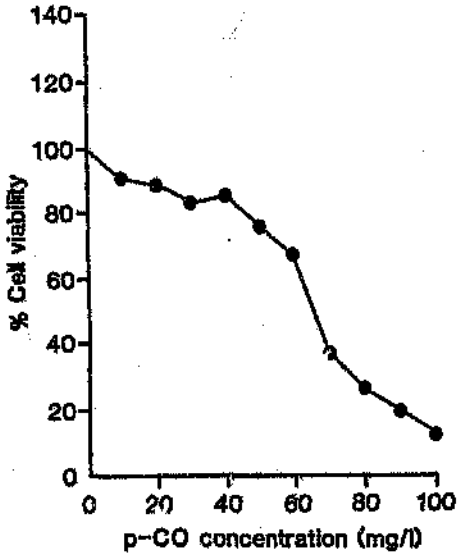
The percentage viability of cells derived from cat skeletal muscle incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.5	12
25	97.4	3.3	12
50	98.8	3.9	12
75	95.2	4.4	12
100	100.4	[illegible]	12
125	97.5	3.0	12
150	97.6	3.9	12
175	101.1	3.5	12
200	99.3	5.5	12
225	96.3	4.4	12
250	100.4	3.9	12

Fig. 4.4.1.2.

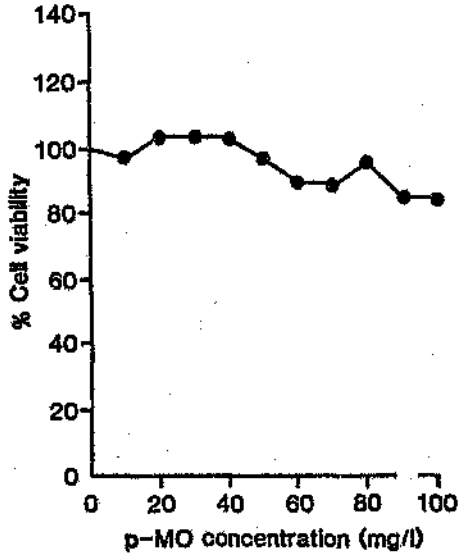
The percentage viability of cells derived from rat skeletal muscle incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.8	6
10	90.7	3.1	6
20	88.9	2.4	6
30	82.8	2.2	6
40	84.9	2.0	6
50	75.1	2.3	6
60	66.3	3.8	6
70	36.3	3.1	6
80	25.2	1.7	6
90	19.1	1.4	6
100	11.7	1.6	6

Fig. 4.4.1.3.

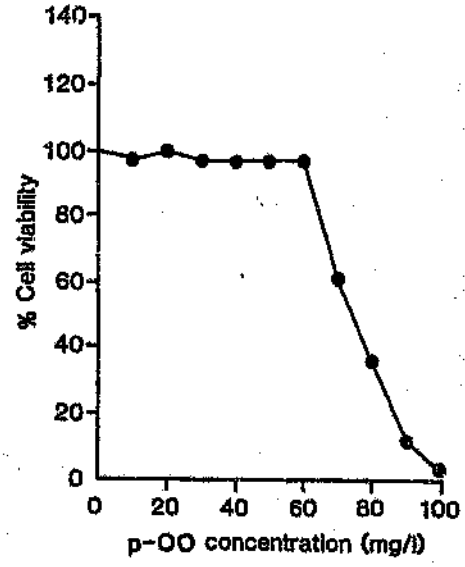
The percentage viability of cells derived from cat skeletal muscle incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.6	6
10	97.1	2.1	6
20	103.4	2.1	6
30	103.7	2.7	6
40	102.4	2.8	6
50	96.8	2.3	6
60	89.8	3.7	6
70	88.6	3.2	6
80	95.1	3.1	6
90	84.9	3.0	6
100	84.2	3.2	6

Fig. 4.4.1.4.

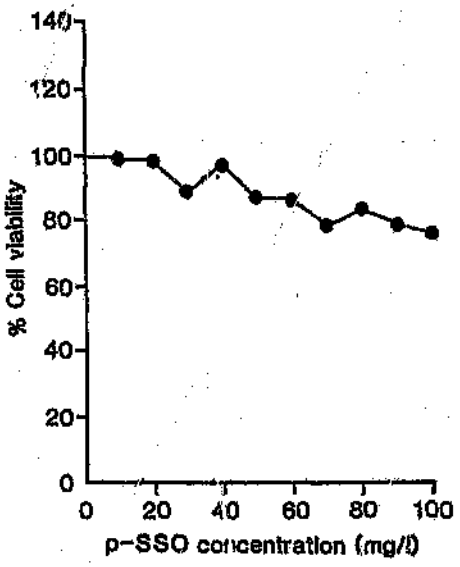
The percentage viability of cells derived from cat skeletal muscle incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.7	6
10	96.5	1.9	6
20	99.5	2.5	6
30	97.3	2.4	6
40	96.8	3.0	6
50	97.0	2.5	6
60	96.5	1.5	6
70	60.8	2.4	6
80	36.0	3.0	6
90	12.0	1.7	6
100	2.5	0.5	6

Fig. 4.4.1.5.

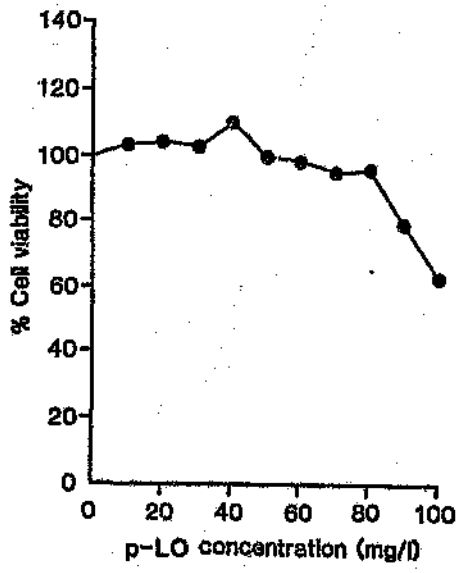
The percentage viability of cells derived from cat skeletal muscle incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	98.9	2.5	6
20	97.9	3.6	6
30	88.1	3.6	6
40	96.6	1.7	6
50	87.1	1.5	6
60	85.5	3.1	6
70	78.1	4.1	6
80	82.9	1.9	6
90	78.6	4.0	6
100	75.2	2.0	6

Fig. 4.4.1.6.

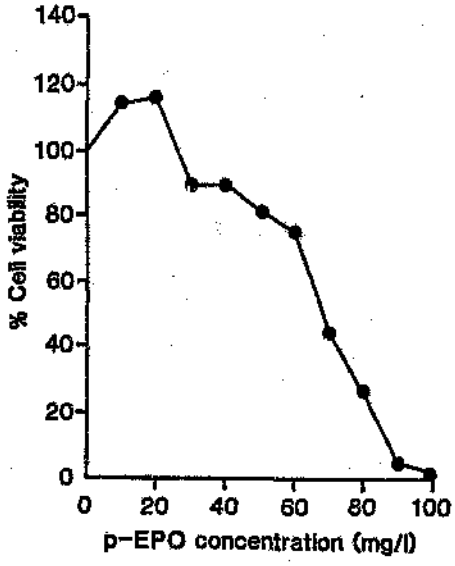
The percentage viability of cells derived from cat skeletal muscle incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.5	6
10	103.4	4.1	6
20	104.5	2.5	6
30	102.4	2.8	6
40	109.9	3.4	6
50	99.3	2.8	6
60	98.0	3.6	6
70	95.2	2.5	6
80	95.9	4.3	6
90	79.1	2.7	6
100	63.0	2.6	6

Fig. 4.4.1.7.

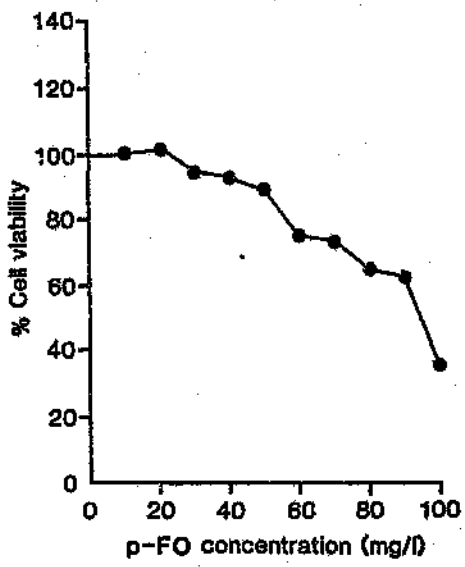
The percentage viability of cells derived from cat skeletal muscle incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.6	6
10	114.7	3.7	6
20	116.0	3.2	6
30	89.6	3.7	6
40	89.6	4.6	6
50	81.3	5.4	6
60	74.8	6.0	6
70	44.5	3.4	6
80	26.3	2.3	6
90	4.6	1.9	6
100	0.8	0.5	6

Fig. 4.4.1.8.

The percentage viability of cells derived from cat skeletal muscle incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.6	6
10	100.6	3.6	6
20	101.7	3.5	6
30	95.1	3.1	6
40	93.5	3.6	6
50	89.5	4.6	6
60	75.6	4.2	6
70	73.9	2.3	6
80	65.3	3.3	6
90	62.5	3.6	6
100	35.8	2.6	6

p-EPO and p-LO enhanced cell proliferation, whereas all p-oils were growth limiting, although this potential varied greatly in magnitude, depending both on the p-oil and concentration dosed. High concentrations of p-EPO, p-OO, p-CO and p-FO, however, were cytotoxic. p-EPO induced the most dramatic overall changes in cell growth, while p-MO and p-SSO were the least effective.

p-CO supplementation induced growth limitation with concentrations up to 50mg/l and cytostasis with 60mg/l (Fig. 4.4.1.2.). Approximately 65mg/l p-CO reduced cell viability by half (ID_{50}), whereas higher concentrations became progressively more cytotoxic such that 88.3% of cells took up Trypan blue with 100mg/l p-CO.

Dosage with up to 50mg/l p-MO had little effect on cell proliferation (96.8% to 103.7% viability), and greater concentrations induced some growth limitation (Fig. 4.4.1.3.). No ID_{50} could be calculated, however, since even with 100mg/l p-MO, 84.2% of cells were viable.

Supplementation with up to 60mg/l p-OO also had little effect on cell viability (Fig. 4.4.1.4.). Concentrations of 70mg/l p-OO or greater, however, induced extensive cytotoxicity and reduced the number of viable cells in a concentration dependent fashion to 50% with 75mg/l p-OO (ID_{50}) and only 2.5% with 100mg/l p-OO.

No significant changes in cell proliferation were found

concentrations limited cell growth slightly to a minimum of 75.2% with 100mg/l (Fig. 4.4.1.5.). p-SSO did not induce cytostasis or cytotoxicity with any concentration dosed, thus no ID_{50} could be calculated.

Dosage with up to 80mg/l p-LO induced no marked changes in cell proliferation, although such was enhanced to 109.9% with 40mg/l p-LO (Fig. 4. .1.6.). Cell growth was limited to 79.1% with 90mg/l p-LO and 63.0% with 100mg/l p-LO, hence no ID_{50} could be determined.

Cell proliferation was enhanced to 114.7% and 116.0% with 10 and 20mg/l p-EPO, respectively, whereas 30 and 40mg/l p-EPO both limited cell growth to 89.6%, compared to 74.8% with 60mg/l p-EPO and 50% with approximately 68mg/l p-EPO (ID_{50}) (Fig. 4.4.1.7.). Cytotoxicity was induced with higher concentrations, which reduced cell viability progressively to 4.6% with 90mg/l p-EPO and practically 100% of cells took up the Trypan blue with 100mg/l p-EPO.

Dosage with up to 30mg/l p-FO induced little effect on cell viability, but this was progressively limited from 93.5% to 73.9% with 40 to 70mg/l p-EPO, respectively (Fig. 4.4.1.8.). 80mg/l p-FO induced cytostasis as cell viability (65.3%) approximated to the cytostatic number (about 67%), but higher concentrations were cytotoxic. The number of viable cells yielded with 90mg/l p-FO was

was reduced markedly to 35.8% with 100mg/l p-FO.

Subsequent to these studies, cells derived from cat skeletal muscle were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient quantities for all the quantitative and qualitative analyses to be performed. Cell viabilities were compared upon harvesting and found to be statistically similar to those in Figs. 4.4.1.1-4.4.1.8. All further biochemical assays were therefore performed on these samples.

4.4.2 Effects of pseudo-Oils on Total Protein.

The results in Table 4.4.2.1. show the total protein concentrations determined for dosed cells compared to controls.

Total protein concentrations in dosed cells ranged from 271.6 to 418.9µg/10^6 cells seeded (obtained with 60mg/l p-CO and 20mg/l p-EPO incubation, respectively) compared to 300.1µg/10^6 control cells seeded. Cells supplemented with 60mg/l p-CO or p-FO contained slightly less protein than controls, but all other dosed cells exhibited more protein than controls. Protein concentrations decreased across the range dosed with p-CO, p-MO, p-EPO and p-FO (330.4 to 271.6, 383.1 to 348.5, 418.9 to 314.6 and 376.5 to 285.8µg/10^6 cells seeded, respectively), but increased slightly with p-SSO supplementation (348.2 to 353.8µg/10^6 cells seeded). p-LO induced more protein with a concentration of 40 than 20 or 60mg/l (377.5, 368.1 and 363.2µg/10^6 cells seeded), while the converse occurred with p-OO dosage (372.1, 383.6 and 383.3µg/10^6 cells seeded).

Table 4.4.2.1.

The protein content of cells derived from cat skeletal muscle, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	300.1			
CO		330.4	319.9	271.6
MO		383.1	352.8	348.5
OO		383.6	372.1	383.3
SSO		348.2	350.6	353.8
LO		368.1	377.5	362.4
EPO		418.9	351.1	314.6
FO		376.5	348.6	285.8

4.4.3 Effects of pseudo-p on the Fatty Acid Spectrum of Cells Derived From Cat Skeletal Muscle.

Table 4.4.3.1. shows the FA spectra of cells incubated with 20, 40 or 60mg/l of each of the p-oils in relation to controls.

The greatest proportion of any single FA in control cells was 18:1ω9 (21.1%). Significant amounts of 16:0, 18:0 and 22:4ω6 were also found (15.3%, 14.5% and 17.1%, respectively), whereas smaller percentages of 16:1ω9 and 20:4ω6 were detected (5.8% and 8.2%, respectively).

The relative amounts of 16:0, 16:1ω9 and 18:0 were generally lower in dosed cells than in controls. The converse was true for 18:1ω9 and 18:2ω6, yet PDFA levels were generally lower in dosed cells. Similarly, ω3 PUFA levels were generally parallel to controls or decreased.

Cellular 16:0 and 18:1ω9 levels increased with 20, 40 or 60mg/l p-CO supplementation (20.3%, 24.6% and 25.7%, and 23.9%, 25.4% and 27.5% vs 15.3% and 21.1% in controls, respectively), 18:0 decreased over the same range (13.7%, 12.5% and 11.2%, respectively vs 14.5% in controls), and an increase in 16:1ω9 was detected only with 60mg/l p-CO (8.5% vs 5.8% in controls). PUFA levels were parallel to controls or decreased, although 22:6ω3 was slightly increased (3.6%, 3.2% and 2.9% with 20, 40

Legend to Table 4.4.3.1.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where 'n' is the number of experiments. '[p-Oil]' refers to the pseudo-oil concentration used.

Table 4

The fatty acid spectrum of cells derived from cat skeletal muscle.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	15.3±0.30	20.3	24.6	25.7	20.1	21.0	22.8	11.7	13.3	14.6	7.4	12.6	13.5	13.4	12.7	13.6	5.9	13.0	13.3	7.7	15.9	11.2
	18:0	14.5±0.29	13.7	12.5	11.2	16.1	14.4	12.1	20.2	6.0	5.5	29.6	9.1	8.7	13.3	9.6	8.4	31.3	9.7	9.3	29.5	8.4	14.3
	20:0	0.3±0.00	–	0.1	–	–	0.1	–	–	–	–	–	–	–	–	0.1	–	–	–	–	–	–	0.4
	22:0	0.1±0.00	0.3	0.2	0.2	0.1	0.2	0.2	–	–	–	–	0.1	–	0.1	0.1	0.1	–	0.2	–	–	0.1	–
	24:0	0.3±0.00	0.1	0.4	–	–	–	0.1	–	–	–	–	0.3	–	–	0.1	0.1	–	0.5	0.4	–	–	–
ω9 MONOS.	16:1	5.8±0.06	3.9	5.1	8.5	1.6	1.9	3.6	0.8	1.5	2.1	0.4	1.6	2.0	1.7	1.4	2.4	0.3	1.6	1.5	4.3	17.8	14.3
	18:1	21.1±0.10	23.9	25.4	27.5	24.5	24.6	26.4	41.9	49.9	55.1	25.9	20.9	22.6	20.5	17.8	16.9	23.9	14.7	14.9	30.7	19.8	22.2
	24:1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ω6 POLYS.	18:2	3.3±0.10	4.8	2.6	3.6	7.6	8.0	10.7	6.4	9.2	10.4	20.0	32.7	37.4	10.4	11.0	12.1	18.8	34.5	41.1	12.3	6.9	11.5
	18:3	–	0.4	0.1	0.4	0.3	0.1	0.3	0.1	0.1	0.3	1.9	0.2	0.9	0.7	0.1	0.3	5.7	1.2	2.3	–	0.1	1.2
	20:2	0.2±0.00	0.1	–	–	0.3	0.6	0.3	2.9	1.8	1.8	1.0	1.6	1.4	0.2	0.3	0.2	1.1	1.4	1.2	–	0.5	3.5
	20:3	2.9±0.05	2.3	2.1	1.8	2.5	2.6	2.2	1.5	1.6	1.1	1.3	2.5	2.0	1.7	1.5	1.0	2.1	5.9	5.1	0.9	1.5	1.6
	20:4	8.2±0.10	7.8	7.2	5.9	9.1	9.5	8.5	4.0	3.6	2.6	2.8	4.4	2.9	7.3	6.9	4.9	2.4	4.5	3.0	2.5	4.7	2.3
	22:4	17.1±0.30	12.1	9.0	7.0	8.8	6.7	6.9	5.1	7.1	3.0	4.3	7.9	5.0	12.3	6.7	4.9	4.5	6.7	3.7	4.0	6.3	2.5
	22:5	0.5±0.00	–	0.5	–	0.1	0.7	–	0.1	0.2	–	0.1	0.3	–	–	0.3	–	0.1	0.4	0.1	0.1	0.4	0.1
ω3 POLYS.	18:3	0.8±0.03	0.9	0.7	0.8	1.0	0.7	0.4	0.7	0.8	0.7	1.9	0.5	0.4	11.4	24.5	30.6	–	0.3	0.4	1.4	0.5	2.7
	18:4	0.7±0.05	0.5	0.9	0.5	0.5	1.1	0.3	–	0.4	–	0.5	0.3	0.2	0.3	0.5	0.2	1.0	0.4	0.2	0.1	0.2	–
	20:4	2.1±0.00	1.2	1.7	0.6	0.9	2.5	0.4	1.3	1.3	0.3	0.9	0.9	0.5	1.4	2.3	1.2	1.2	0.9	0.6	1.1	0.8	0.9
	20:5	1.2±0.05	1.0	1.0	1.1	0.6	0.4	0.6	0.2	0.4	0.3	0.1	0.7	0.3	0.6	0.5	0.5	0.1	0.7	0.4	2.4	8.5	7.2
	22:5	3.2±0.00	3.1	2.7	2.4	3.0	2.5	2.0	1.7	1.4	1.0	1.1	1.7	1.2	2.9	1.9	1.3	0.9	1.7	1.2	2.5	6.0	2.9
	22:6	2.4±0.10	3.6	3.2	2.9	3.0	2.5	2.2	1.6	1.6	1.2	0.9	1.7	1.3	2.0	1.8	1.4	0.7	1.7	1.3	0.7	1.9	1.3

and 60mg/l p-CO, respectively vs 2.4% in controls).

Incubation with 20, 40 or 60mg/l p-MO enhanced 16:0 and 18:1ω9 levels (20.1%, 21.0% and 22.8%, and 24.5%, 24.6% and 26.4% vs 15.3% and 21.1% in controls, respectively), had little overall effect on 18:0, but induced a marked reduction in 16:1ω9 (1.6%, 1.9% and 3.6%, respectively vs 5.8% in controls). 18:2ω6 increased significantly (7.6%, 8.0% and 10.7%, respectively vs 3.3% in controls) and little change occurred in 20:4ω6 (9.1%, 9.5% and 8.5%, respectively vs 8.2% in controls), whereas other ω6 PDFA levels were parallel to controls or decreased. Dosed cells generally contained lower ω3 PUFA amounts than controls, although 22:6ω3 was slightly increased with 20mg/l p-MO (3.0% relative to 2.4% in controls).

18:1ω9 was incorporated in a concentration dependent manner when cells were dosed with 20, 40 or 60mg/l p-OO (41.9%, 49.9% and 55.1%, respectively vs 21.1% in controls). 16:0, 18:0 and 16:1ω9 levels were lower than controls (15.3%, 14.5% and 5.8%, respectively), although 18:0 was slightly increased with 20mg/l p-OO (20.2%). A concentration dependent increase in 18:2ω6 was also demonstrated over the range dosed (6.4%, 9.2% and 10.4%, respectively vs 3.3% in controls), and there was a significant increase in 20:2ω6 (2.9%, 1.8% and 1.8%, respectively vs 0.2% in controls). However, the levels of 20:3ω6, 20:4ω6, 22:4ω6 and 22:5ω6 were lower than controls (2.9%, 8.2%, 17.1% and 0.5%, respectively), as

were those of ω3 PUFA's.

16:0 and 16:1ω9 percentages were decreased with 20, 40 or 60mg/l p-SSO incubation (7.4% to 13.5% and 0.4% to 2.0% vs 15.3% and 5.8% in controls, respectively), 18:0 was increased only with 20mg/l p-SSO (29.6% vs 14.5% in controls); while little change occurred in 18:1ω9 levels (21.1% in controls). 18:2ω6 amounts increased in a concentration dependent fashion (3.3%, 20.0%, 32.7% and 37.4% with 0, 20, 40 and 60mg/l p-SSO, respectively), a significant amount of 18:3ω6 was found with 20mg/l p-SSO (1.9% vs 0% in controls), and 20:2ω6 levels increased significantly over the range dosed (1.0%, 1.6% and 1.4%, respectively vs 0.2% in controls). All other ω6 PDFA levels, however, were decreased with p-SSO dosage, and this was also demonstrated with ω3 PDFA's.

Cells supplemented with p-LO exhibited decreased amounts of 16:0 (12.7% to 13.6% vs 15.3% in controls), 16:1ω9 (1.4% to 2.4% vs 5.8% in controls), 18:0 (8.4% to 13.3% vs 15.4% in controls), and 18:1ω9 (16.9% to 20.5% vs 21.1% in controls). 18:2ω6 levels increased 3 to 4 fold (10.4% to 12.1% vs 3.3% in controls) and 18:3ω3 was markedly enhanced (11.4%, 24.5% and 30.6% with 20, 40 and 60mg/l p-LO, respectively vs 0.8% in controls), but PDFA levels were not significantly raised.

p-EPO dosage decreased cellular 16:0, 16:1ω9 and 18:0 levels in relation to controls (15.3%, 5.8% and 14.5%,

respectively), although 18:0 increased approximately 2 fold with 20mg/l p-EPO (31.3%). 18:1ω9 percentages approximated to controls (21.1%) with 20mg/l p-EPO (23.9%), but were decreased with 40 and 60mg/l p-SSO (14.7% and 14.9%, respectively. A concentration related increment in 18:2ω6 was demonstrated (3.3%, 18.8%, 34.5% and 41.1% with 0, 20, 40 and 60mg/l p-EPO, respectively vs 3.3% in controls), and 20:2ω6 increased significantly (1.1% to 1.4% vs 0.2% in controls), as did 18:3ω6 (5.7%, 1.2% and 2.3%, respectively vs 0% in controls). Despite more 20:3ω6 detected with 40 and 60mg/l p-EPO than in controls (5.9%, 5.1% and 2.9%, respectively), 20:4ω6 percentages decreased significantly (2.4%, 4.5% and 3.0%, respectively vs 8.2% in controls) as did those of 22:4ω6 (4.5%, 6.7% and 3.7%, respectively vs 17.1% in controls). ω3 PUFA levels approximated to, or were below, their respective control levels.

Cells enriched with p-FO exhibited 16:0 levels parallel to, or lower than, controls (15.3%). Increments in 18:0 and 18:1ω9 were detected with 20mg/l p-FO (29.5% and 30.7%, respectively), while percentages were parallel to or lower than controls (14.5% and 21.1%, respectively) with 40 and 60mg/l p-FO. 16:1ω9 proportions, however, increased significantly with 40 and 60mg/l p-FO (17.8% and 14.3%, respectively vs 5.8% in controls). 2 to 4 fold more 18:2ω6 was found (6.9% to 12.3% vs 3.3% in controls), and 20:2ω6 was significantly increased with

60mg/l p-FO (3.5% vs 0.2% in controls), but this did not induce an enhancement in ω6 PDFA levels. p-FO dosage resulted in 20:5ω3 incorporation (2.4% with 20mg/l, 8.5% with 40mg/l and 7.2% with 60mg/l vs 1.2% in controls), yet 22:5ω3 was increased only with 40mg/l p-FO (6.0% vs 3.2% in controls), and 22:6ω3 levels were decreased with all concentrations (0.7% [illegible].9% vs 2.4% in controls).

4.4.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 4.4.4.1. shows the lipoperoxides measured both in control and dosed cells, as well as their respective spent growth media. The results are shown as nmoles MDA/10^6 cells, but in the case of the spent incubation medium, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained.

Lipoperoxide production ranged from 0.5 to 1.8nmoles MDA/10^6 dosed cells (0.6nmoles MDA/10^6 control cells), and increased in an overall concentration dependent manner. Relatively little lipid peroxidation was induced when cells were supplemented with 20, 40 or 60mg/l p-CO, p-MO or p-OO (0.5 to 1.1nmoles MDA/10^6 cells). This was also found with p-SSO or p-EPO dosage (0.5, 0.7 and 1.0, and 0.7, 0.8 and 1.1nmoles MDA/10^6 cells, respectively), whereas greater amounts were induced with 40 and 60mg/l p-LO (1.3 and 1.8nmoles MDA/10^6 cells, respectively) and p-FO (1.1 and 1.8nmoles MDA/10^6 cells, respectively).

Lipoperoxides were absent in spent control medium, while concentrations were greatest for medium incubated with 40 or 60mg/l p-LO (0.5 and 1.0nmoles MDA/10^6 cells) or p-FO (1.1 and 1.2nmoles MDA/10^6 cells). Spent media from all other dosed cultures, however, contained fewer lipoperoxides ($\leq$0.4nmoles MDA/10^6 cells), or none at all.

Table 4.4.4.1.

Lipoperoxide formation by cat skeletal muscle cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.6			
CO		0.6	0.6	0.7
MO		0.6	0.8	0.9
OO		0.6	0.6	0.9
SSO		0.5	0.7	1.0
LO		0.5	1.3	1.8
EPO		0.7	0.8	1.1
FO		0.5	1.1	1.8
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		0.2	0.2	0.2
MO		-	-	-
OO		-	-	0.1
SSO		-	-	0.4
LO		0.2	0.5	1.0
EPO		-	-	0.1
FO		0.1	1.1	1.2

4.4.5 Discussion.

Supplementation of cat skeletal muscle primary cultures with p-oils modulated the viability of those cells in a p-oil-specific manner (Figs. 4.4.1.2-4.4.1.8.), dependent on p-oil FA composition (Table 2.3.3.2.) and concentration dosed rather than the degree of p-oil unsaturation or p-oil EFA/PDFA levels. The cell viability changes p-OO, p-SSO and p-LO induced related primarily to the high OA, LA and ALA contents of these p-oils, respectively, and it was apparent that ALA was more effective than LA in reducing cell viability only at very high concentrations in the range dosed, although OA was more effective. The pronounced cell viability differences mediated with p-EPO or p-SSO dosage, which exhibited similar FA compositions, probably related to the presence of small amounts of GLA (about 9%) in the former only. This FA appeared to be involved in the stimulation of cell proliferation with low, growth limitation with intermediate, and cytotoxicity with high, p-EPO concentrations in the range dosed. However, the finding that cell viability was unchanged or merely limited in the absence of GLA with p-SSO incubation implied that LA had no stimulatory or inhibitory effect on the proliferation of these cells. Comparison of the FA spectra of p-EPO and p-LO with the cell viability changes these p-oils mediated upon dosage also indicated that GLA (in p-EPO) exhibited greater capability to

modulate cell proliferation than ALA (in p-LO). These differences could relate to the double bond positions in these trienoic C18 FA's. The fact that cell growth was significantly enhanced only with p-EPO or p-LO supplementation nevertheless suggested that the presence of 3 double bonds in an 18 carbon FA exhibited greater potential to stimulate the proliferation of these cells than FA's with other structures. Although the cell viability changes the dosed p-oils induced reflected the presence of particular exogenous FA's, it was also likely that synergistic and antagonistic interactions between p-oil FA's amplified or deminished certain effects. This could explain, for example, the dramatic cell viability changes p-EPO induced, the absence of marked alterations in cell viability overall with p-MO supplementation, as well as the capability of OA in p-OO to alter cell viability only at high concentrations. The cell viability changes observed could nevertheless be the result of alterations in membrane fluidity induced as a result of differential p-oil FA incorporation (Table 4.4.3.1.). Rigidification of the plasma membrane could account for the progressive reduction in cell viability found with dosage of increasing amounts of p-CO, whereas the converse was suggested with p-FO, p-OO and p-EPO.

p-Oil dosage induced alterations in total cellular protein (Table 4.4.2.1.) which generally reflected the

cell viability changes found (Figs. 4.4.1.2-4.4.1.8.). The measurement of increased cellular protein with 20mg/l p-EPO incubation correlated with the greatest stimulation of cell viability reported, and possibly represented increased cell number with constant, or increased, protein per cell. The latter seemed more likely as consistent, but increased, protein levels were measured in cells dosed with p-OO, despite the fact that cell viability was parallel to controls. Cells incubated with other p-oils which did not alter cell viability significantly in relation to controls, eg. 20mg/l p-MO, p-SSO, p-LO and p-FO, exhibited numerically different protein levels, but such were also increased in relation to controls. Increased cellular protein was even found when p-oils induced growth limitation, eg. 40mg/l p-CO, p-EPO and p-FO. On the other hand, the decreased protein found with 60mg/l p-CO or p-FO dosage implied decreased cell number with constant or decreased protein per cell. It was apparent from these findings that exogenous FA's have the capability to modulate protein synthesis in cat skeletal muscle cells. Such varied with different p-oils and this may relate to differences in FA structure and the mediation of synergistic and antagonistic effects between p-oil FA's. Absolute changes in cellular protein could nevertheless reflect alterations in the production of enzymes required for the metabolism of incorporated p-oil FA's.

The literature does not describe the desaturation capability of cultured cat skeletal muscle cells. In vivo studies in our laboratory, however, showed that feeding cats diets either sufficient in all PDFA's, or deficient in ω3, ω6, or all PDFA's, induced changes in the FA spectra of their skeletal muscle (Davidson et al 1989, 1990a). Such indicated that Δ6D activity was absent, while evidence for Δ5D activity was found. As the diets were made more PDFA deficient, the proportion of 18:2ω6 increased and a concommitant decrease was generally found for both ω3 and ω6 post-Δ5D FA's. The presence or absence of Δ4D, however, could not be proven (Davidson et al 1989, 1990a). The present study showed that the overall desaturation and elongation capability of cultured cat skeletal muscle cells was also poor, despite significant incorporation of the p-oil FA's supplemented (Table 4.4.3.1.). This implied that the increased cellular protein levels reported with p-oil incubation (Table 4.4.2.1.) did not reflect enhanced expression of desaturase cascade enzymes.

The finding that 16:0 percentages were significantly decreased and 18:0 increased only with 20mg/l p-SSO, p-EPO or p-FO dosage suggested the capability for 16:0 elongation. However, 18:0 desaturation via Δ9D seemed unlikely since 18:1ω9 levels were not significantly enhanced, except with 20mg/l p-FO incubation. This could have related to elongation of 16:1ω7 incorporated with

20mg/l FO dosage, although such activity was impaired with higher p-FO concentrations, despite cellular uptake of large amounts of this moiety from the medium. Little significant Δ6D capability was shown with incubation of 18:2ω6-rich p-oils (p-SSO or p-EPO). 20:2ω6 formation thus resulted primarily from a build up of 18:2ω6, some of which was elongated due to the lack, or suppression, of Δ6D activity. On the other hand, the small 18:3ω6 levels detected could have reflected extramicrosomal retroconversion of endogenous 20:3ω6, which could account for the decreased levels of this moiety. 18:3ω6 incorporated with p-EPO dosage was elongated to 20:3ω6, but Δ5- or Δ4-desaturation of ω6 PDFA's was not found in cells enriched with any p-oil. Elongation of 20:5ω3 occurred with 40mg/l p-FO supplementation, but no evidence of Δ4-desaturation to 22:6ω3 was found, nor was 18:3ω3 incorporated with p-LO dosage significantly desaturated or elongated to PDFA's. Evidence to support desaturation and elongation of ω3 PDFA's, however, was found with p-CO incubation. This p-oil did not contain PUFA's (Table 2.3.3.2.), thus the elevated 22:6ω3 levels detected must represent desaturation/elongation of cellular ω3 PUFA's. The finding that 18:3ω3 and 18:4ω3 levels were similar to controls, whereas 20:4ω3 amounts were decreased, suggested desaturation and elongation of endogenous 20:4ω3 to 22:6ω3. This supported the absence of Δ6D, but not of Δ5D, elongase and Δ4D, expression.

It was possible that supplemention of cells with p-CO increased the requirement for PDFA's, and this may have enhanced 22:6ω3 production in a rather poor attempt to re-establish a more even balance between saturated and unsaturated FA's in the membranes. However, the level of 16:0 in the cells could still have been too high to maintain normal membrane fluidity, and the known inhibitory/suppressive effects of saturated FA's on Δ6D, Δ5D and Δ4D activities (eg. Brenner et al 1966) may also have limited the extent to which the desaturases were stimulated. Such factors could explain the decreased cell viability shown with p-CO incubation. In contrast, cells dosed with p-oils abundant in unsaturated FA's may already have achieved an optimum FA balance for maintaining membrane fluidity and other cell functions. Suppression of further desaturation would thus be consistent with maintenance of the existing FA balance, and such has been reported to occur via complex mechanisms, including feedback inhibition (Brenner 1965a, 1965b, Holman et al 1964, Garcia et al 1965, Brenner et al 1966, 1967, 1969 and Actis Dato et al 1970). Such control mechanisms may explain the lack of 22:5ω6 and 22:6ω3 formation with p-MO or p-FO dosage in particular.

The poor desaturase/elongase capability reported overall in these cells (Table 4.4.3.1.) did not appear to relate to the cell viability changes induced with p-oil

supplementation (Figs. 4.4.1.2-4.4.1.8.) as poor enzyme activity was found even when cell viability was parallel to, or greater than, controls. The overall FA balance in the cells, as well as competitive interactions between cellular FA's appeared to be more important factors explaining the desaturase cascade capability we reported for these cells.

The results obtained from MDA measurement following the 48 hour incubation period showed that even in the absence of any exogenously added p-oil, control cells produced lipoperoxides (Table 4.4.4.1.). This was not surprising, since the FA spectra (Table 4.4.3.1.) showed FA's that were potential candidates to act as substrates for the formation of lipoperoxides. This could have occurred enzymatically and/or or via auto-oxidation of endogenous unsaturated FA's released during PGL cycling once the anti-oxidant defenses were saturated. Enhanced MDA formation with p-oil dosage additionally reflected oxidation of unsaturated p-oil FA's. The quantitative variations in MDA levels measured, however, related to differences in p-oil FA composition (Table 2.3.3.2.) and concentration supplemented, as well as FA availability and susceptibility to oxidation. The overall lack of double bonds in the FA's comprising p-CO could indeed explain why cellular lipoperoxide formation approximated to that of controls with incubation of this p-oil. On the other hand, the finding that p-MO, p-OO, p-SSO or

p-EPO dosage induced small, or no increments in cellular lipoperoxides, despite significant levels of unsaturated FA's in these p-oils which were incorporated (Tables 2.3.3.2. and 4.3.3.1.), implied that the substrates for peroxidation were largely lipid-bound and stable. In contrast, the greatest lipoperoxide amounts measured overall with p-LO or p-FO dosage both in the cells and spent medium reflected the amount and susceptibility of ALA in p-LO and EPA in p-FO to oxidation, dependent not only on the number a position of double bonds in such FA's, but also on the availability of these moieties in the free form. However, the abundance of saturated FA's in new culture medium (Table 2.3.3.3.), the ability of these cells to incorporate p-oil FA's (Table 4.4.3.1.), the lack of cytotoxicity induced with 20, 40 or 60mg/l p-oil dosage (Figs. 4.4.1.2-4.4.1.8.), and the ability of lipoperoxides to increase membrane permeability (Chio et al 1969, Mead 1976, Pryor 1976, Tappel 1975, Gavino et al 1981c, Morisaki et al 1982b and Frankel 1984) suggested that most spent media lipoperoxides originated intracellularly via passage through the plasma membrane.

Supplementation of cat skeletal muscle cells with 20, 40 or 60mg/l p-oil stimulated and/or limited, but never inhibited, cell viability (Figs. 4.4.1.2-4.4.1.8), thus no correlation between lipoperoxide formation (Table 4.4.4.1.) and cytotoxicity could be established; these compounds should, however, be considered as a possible

mechanism involved in the modulation of cell death with higher p-oil concentrations. On the other hand, the inverse correlation found between MDA levels and cell viability with p-SSO, p-EPO or p-FO dosage suggested lipoperoxide involvement in the modulation of growth limitation. The finding that the MDA levels found did not always correlate quantitatively with the degree of growth limitation the p-oils induced, however, indicated that lipoperoxides were only partly responsible for modulating the viability of these cells, and it was proposed that alterations in membrane fluidity were additionally involved.

4.5 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM CAT BRAIN.

4.5.1 Effects of pseudo-Oils on Cell Viability.

Microscopic examination of control cells before, during and after each experiment showed no changes in cell morphology at any time, and p-oil dosed cells were morphologically similar to controls, except for cytoplasmic droplets observed with p-oil supplementation at high concentrations in some instances.

The effects of 0 to 250mg albumin/l culture medium were investigated on cell viability and shown to have no significant effect (Fig. 4.5.1.1.), nor were synergistic effects induced. Hence, the effects induced with the dosed p-oils were a result of the exogenous FA's and not the albumin utilised as FA carrier.

The number of control cells, seeded at $10x10^4$/ml, at the end of the 24 hour post-trypsinisation recovery period, was $10.2x10^4$/ml. This equated to about 88% of the final control cell number at the end of the 48 hour incubation period, and represented the cytostatic number. Only p-oil concentrations limiting cell viability to significantly below 88% were thus considered cytotoxic.

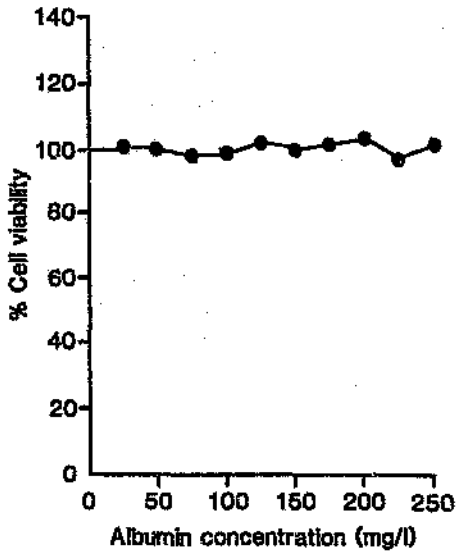
The results of the incubation of cells with p-oils are depicted in Figs. 4.5.1.2-4.5.1.8. Cell proliferation

Legend to Figs. 4.5.1.1-4.5.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where 'n' is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 4.5.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.5.1.2-4.5.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 4.5.1.1.

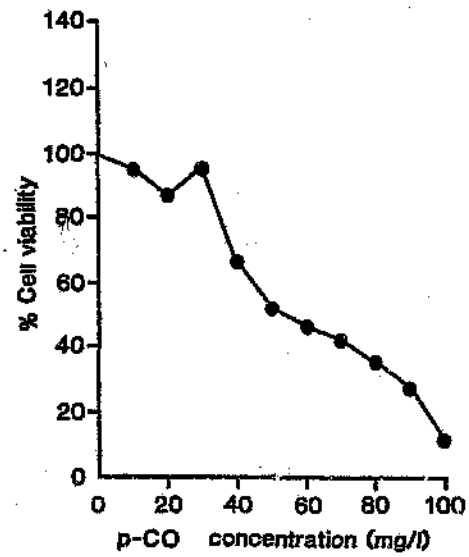
The percentage viability of cells derived from cat brain incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.3	12
25	100.3	5.2	12
50	99.4	4.7	12
75	98.0	3.4	12
100	99.2	4.0	12
125	101.6	3.6	12
150	99.4	5.0	12
175	101.6	4.8	12
200	103.0	6.0	12
225	96.5	3.6	12
250	101.6	5.0	12

Fig. 4.5.1.2.

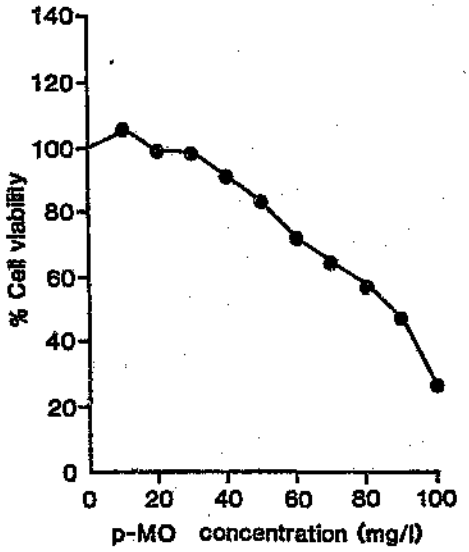
The percentage viability of cells derived from cat brain incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.0	6
10	94.6	5.0	6
20	87.0	3.2	6
30	94.6	5.7	6
40	66.5	4.3	6
50	52.2	2.7	6
60	47.0	3.8	6
70	42.4	4.2	6
80	35.9	5.0	6
90	27.2	4.6	6
100	12.0	3.9	6

Fig. 4.5.1.3.

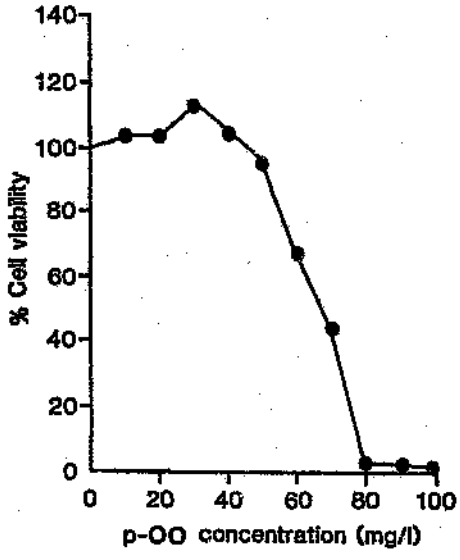
The percentage viability of cells derived from cat brain incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.3	6
10	105.0	4.7	6
20	99.2	4.4	6
30	97.5	4.2	6
40	90.1	6.3	6
50	83.3	4.8	6
60	71.9	3.8	6
70	64.5	4.1	6
80	57.0	4.9	6
90	47.1	3.2	6
100	26.5	3.3	6

Fig. 4.5.1.4.

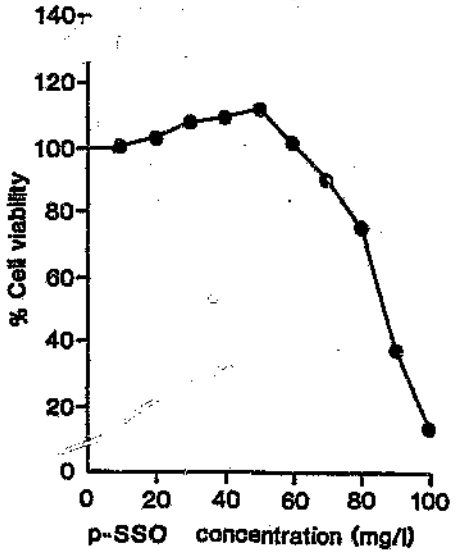
The percentage viability of cells derived from cat brain incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.1	6
10	103.3	4.0	6
20	103.3	1.8	6
30	113.1	2.3	6
40	104.1	3.7	6
50	95.0	4.1	6
60	67.3	1.5	6
70	44.1	1.6	6
80	2.5	1.0	6
90	1.8	1.0	6
100	0.7	0.7	6

Fig. 4.5.1.5.

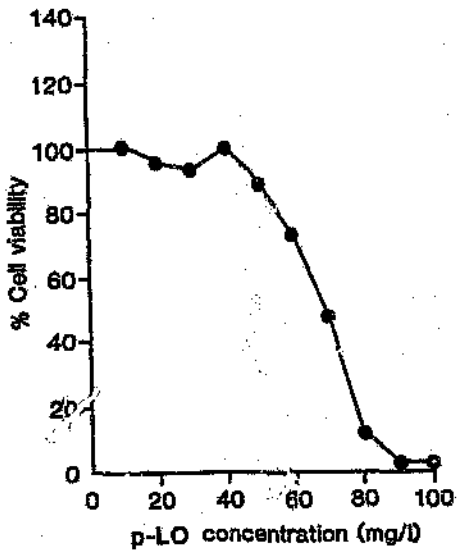
The percentage viability of cells derived from cat brain incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.7	6
10	100.8	3.4	6
20	103.8	3.3	6
30	108.3	7.8	6
40	109.8	4.0	6
50	111.3	6.0	6
60	101.5	5.7	6
70	90.2	7.8	6
80	75.2	4.3	6
90	37.6	5.4	6
100	13.5	2.6	6

Fig. 4.5.1.6.

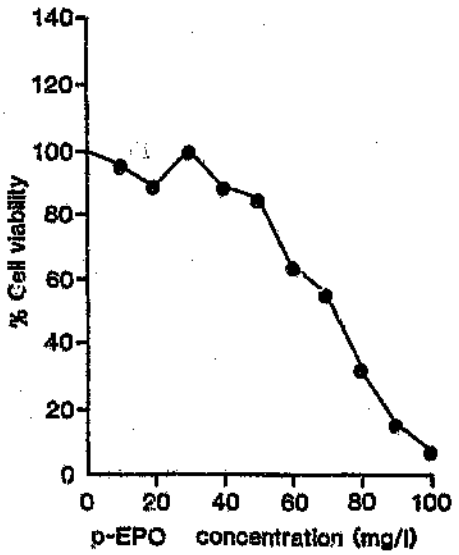
The percentage viability of cells derived from cat brain incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.3	6
10	100.3	3.2	6
20	95.5	2.1	6
30	93.1	1.7	6
40	99.7	2.8	6
50	88.1	2.6	6
60	72.6	2.1	6
70	47.3	3.2	6
80	11.9	1.7	6
90	2.5	1.4	6
100	2.5	1.2	6

Fig. 4.5.1.7.

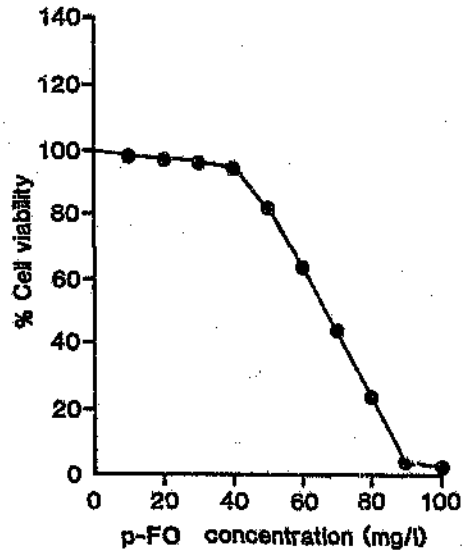
The percentage viability of cells derived from cat brain incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.4	6
10	95.0	4.1	6
20	89.0	3.2	6
30	98.3	5.5	6
40	89.0	4.9	6
50	84.2	3.8	6
60	63.6	4.4	6
70	54.8	4.5	6
80	31.6	2.8	6
90	15.2	2.1	6
100	6.6	2.6	6

Fig. 4.5.1.8.

The percentage viability of cells derived from cat brain incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.9	6
10	97.9	5.3	6
20	96.5	3.4	6
30	95.8	5.4	6
40	94.0	5.5	6
50	81.6	5.3	6
60	63.4	4.3	6
70	43.8	4.9	6
80	23.5	3.2	6
90	3.5	1.3	6
100	0.4	0.4	6

was significantly enhanced with certain concentrations of p-OO and p-SSO, but all p-oils were growth inhibitory with the cytotoxic potential varying in magnitude dependent on the nature of the p-oil and concentration dosed. Overall, p-CO was most, and p-SSO was least, effective in reducing the viability of these cells.

10 and 30mg/l p-CO had little effect on cell growth (Fig. 4.5.1.2.), but cytostasis was induced with 20mg/l p-CO as relative cell viability (87.0%) approximated to the cytostatic number (about 88%). Higher p-CO amounts induced concentration dependent cytotoxicity, and cell viability was reduced to 50% with 55mg/l p-CO (ID_{50}) and to 12.0% with 100mg/l p-CO.

Cell proliferation was not significantly different from controls with up to 30mg/l p-MO dosage, cytostasis was induced with 40mg/l p-MO, but greater amounts of p-MO were increasingly cytotoxic in an almost linear manner (Fig. 4.5.1.3.). Cell viability was 50% of controls with about 88mg/l p-MO (ID_{50}), and this was reduced to 26.5% with 100mg/l p-MO.

Supplementation of cells with p-OO induced a sigmoidal-like response (Fig. 4.5.1.4.). 10, 20, 40 and 50mg/l p-OO had little effect on cell viability, but such was enhanced to 113.1% with 30mg/l p-OO. Concentrations greater than 50mg/l p-OO were increasingly cytotoxic, and approximately 68mg/l p-OO reduced cell viability to

50% of controls (ID_{50}). Only 2.5% of cells were viable with 80mg/l p-OO, and practically all cells incorporated Trypan blue with 100mg/l p-OO dosage.

10, 20 and 60mg/l p-SSO had little effect on cell growth but such was enhanced to 108.3%, 109.8% and 111.3% with 30, 40 and 50mg/l p-SSO, respectively (Fig. 4.5.1.5.). 70mg/l p-SSO induced cytostasis, whereas cytotoxicity reduced cell viability to 50% with 87mg/l p-SSO (ID_{50}) and only 13.5% of the cells survived after incubation with 100mg/l p-SSO.

Supplementation with up to 40mg/l p-LO did not affect cell growth markedly (Fig. 4.5.1.6.), and cytostasis was induced with 50mg/l p-LO as relative cell viability (88.1%) and the cytostatic number (88%) were alike. Higher p-LO concentrations progressively inhibited cell growth to 50% with 69mg/l p-LO (ID_{50}), 11.9% with 80mg/l p-LO and 2.5% with both 90 and 100mg/l p-LO.

10 and 30mg/l p-EPO had little effect on cell viability, but this was reduced to 89.0% with both 20 and 40mg/l p-EPO such that cytostasis was induced (Fig. 4.5.1.7.). Concentrations of 50mg/l p-EPO, or greater, induced concentration dependent cytotoxicity, reducing cell viability almost linearly to 50% of controls with about 74mg/l (ID_{50}) and to 6.6% with 100mg/l.

Cell proliferation was not significantly altered with supplementation of up to 40mg/l p-FO (Fig. 4.5.1.8.).

Higher concentrations, however, were increasingly toxic, and linearly reduced cell viability to 3.5% with 90mg/l p-FO and almost completely with 100mg/l p-FO. The ID_{50} for p-FO, p-OO and p-LO were similar (approximately 67mg/l, 68mg/l and 69mg/l, respectively), although the effects p-FO induced resembled those modulated by p-LO more closely than those for p-OO.

Cells derived from cat brain were subsequently plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l growth medium in sufficient amounts for all quantitative and qualitative analyses to be carried out. Cell numbers were compared upon harvesting with controls and found to be statistically similar to those obtained in Figs. 4.5.1.1-5.5.1.8., thus all further biochemical assays were performed on these samples.

4.5.2 Effects of pseudo-Oils on Total Protein.

Table 4.5.2.1. shows the total cellular protein contents determined at each of the 3 p-oil concentrations dosed, compared to controls.

The amount of total protein found in dosed cells ranged from 106.4 to 220.7µg/10^6 cells seeded (obtained with 60mg/l p-CO and 40mg/l p-SSO dosage, respectively) in relation to 175.1µg/10^6 control cells seeded. Cellular protein levels were parallel to controls or increased with supplementation of 20 or 40mg/l p-oil, but the converse was generally true with 60mg/l p-oil. Protein concentrations decreased across the range dosed with p-CO (205.7 to 106.4µg), p-MO (216.1 to 164.2µg), p-OO (219.5 to 142.7µg), p-EPO (198.1 to 139.8µg) and p-FO (209.6 to 153.9µg), but p-SSO and p-LO induced more protein with a concentration of 40 than 20 or 60mg/l (220.7, 186.1 and 216.9, and 216.1, 205.3 and 189.8µg per 10^6 cells seeded, respectively).

Table 4.5.2.1.

The protein content of cells derived from cat brain, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	175.1			
CO		205.7	172.4	106.4
MO		216.1	201.8	164.2
OO		219.5	215.3	142.7
SSO		186.1	220.7	216.9
LO		205.3	216.1	189.4
EPO		198.1	187.1	139.8
FO		209.6	182.9	153.9

4.5.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Cat Brain.

Table 4.5.3.1. shows the FA spectra for cells incubated with 0, 20, 40 or 60mg/l p-oil.

The greatest proportion of any single FA in controls was 18:1ω9 (33.7%), but significant levels of 16:0 (20.4%), 18:0 (11.5%), 18:2ω6 (7.2%) and 22:4ω6 (10.0%) were also found. Dosed cells contained varying proportions of these FA's.

16:0 and 16:1ω9 percentages were generally lower in dosed cells than in controls, while the converse was found for 18:0. 18:1ω9 and 18:2ω6 levels were more variable, but more 20:3ω6 was generally found in dosed cells, unlike 20:4ω6, 22:4ω6 and 22:5ω6. In most instances, however, dosed cells contained lower amounts of ω3 PUFA's than controls.

Cellular 16:0 and 18:0 levels were decreased with dosage of 20 or 40mg/l p-CO, but increased with 60mg/l p-CO (9.8%, 2.2% and 32.3%, and 0%, 0.4% and 17.7% vs 20.4% and 11.5% in controls, respectively). A significant increment in 22:0 was detected with 60mg/l p-CO (1.2% vs 0.5% in controls), 16:1ω9 was considerably decreased with all concentrations dosed (0.2% to 1.3% vs 4.8% in controls), whereas 18:1ω9 was greatly increased with 20 and 40mg/l p-CO, but decreased with 60mg/l p-CO (77.5%,

Legend to Table 4.5.3.1.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where 'n' is the number of experiments. '[p-Oil]' refers to the pseudo-oil concentration used.

Table 4.5.3.1.

The fatty acid spectrum of cells derived from cat brain.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	20.4±0.15	9.8	2.2	32.3	16.4	17.8	24.3	17.2	14.8	14.0	6.8	12.0	10.6	7.1	10.6	10.7	7.0	12.6	15.0	11.7	13.0	21.5
	18:0	11.5±0.30	-	0.4	17.7	24.7	31.3	15.8	4.5	1.4	-	-	17.3	20.7	13.5	21.1	21.2	12.7	20.7	34.4	38.5	25.9	15.8
	20:0	-	-	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:0	0.5±0.00	0.3	-	1.2	0.4	0.2	0.1	0.4	0.1	-	-	-	-	-	-	0.1	-	-	-	-	-	-
	24:0	0.1±0.00	-	-	-	0.3	1.0	-	0.1	-	-	-	-	-	-	-	-	0.1	0.3	1.7	-	-	-
ω9 MONOS.	16:1	4.8±0.10	0.8	0.2	1.3	0.1	0.3	0.6	2.9	-	-	0.4	-	-	1.1	-	-	0.8	-	-	4.3	10.8	17.4
	18:1	33.7±0.35	77.5	95.6	28.3	39.0	23.6	26.5	52.8	60.0	61.0	55.2	24.9	20.6	43.0	26.3	17.2	39.1	22.2	15.7	26.8	22.0	17.9
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	7.2±0.00	-	0.1	1.8	2.0	3.7	7.0	3.1	4.2	8.2	16.2	22.2	32.5	20.9	11.7	9.8	16.9	20.9	20.8	1.8	3.6	6.1
	18:3	-	-	-	-	-	-	0.3	0.3	0.5	0.6	2.3	2.1	1.1	-	0.1	-	-	2.2	1.4	-	-	-
	20:2	-	-	-	-	0.4	-	-	0.4	0.9	1.4	-	0.8	0.6	-	0.3	-	-	0.7	-	-	-	-
	20:3	1.1±0.10	-	-	1.4	2.6	2.9	4.2	2.5	3.5	2.6	4.7	9.0	3.7	0.4	1.3	0.4	10.5	10.6	1.4	1.5	1.9	0.9
	20:4	3.6±0.15	2.6	1.3	3.7	5.5	7.1	7.8	4.6	3.2	1.8	3.5	3.7	1.7	0.7	3.2	2.4	5.3	3.3	1.7	3.2	1.9	1.2
	22:4	10.0±0.65	4.4	0.2	7.3	3.4	6.7	8.7	6.0	7.3	7.2	5.8	4.4	5.8	1.0	4.1	4.6	3.4	4.3	5.2	5.1	8.4	7.4
	22:5	0.3±0.03	-	-	-	0.5	-	-	-	-	-	-	-	-	0.1	0.2	-	-	0.1	-	-	-	0.1
ω3 POLYS.	18:3	0.7±0.00	-	-	0.7	-	0.4	0.5	0.6	0.8	0.5	-	-	0.1	10.3	17.8	28.3	-	-	-	-	0.2	0.4
	18:4	1.1±0.10	1.1	-	0.7	0.2	1.1	1.0	1.5	1.3	0.7	0.6	0.4	0.3	0.1	-	1.1	0.7	0.1	0.6	0.7	0.4	0.5
	20:4	2.2±0.00	1.6	0.1	0.5	1.4	0.6	0.4	0.4	0.3	0.6	1.2	1.1	0.9	1.1	0.7	2.6	2.0	0.4	0.7	0.8	2.1	1.6
	20:5	0.1±0.00	-	-	0.1	0.2	0.7	0.1	0.3	0.1	-	-	-	-	0.1	0.3	-	-	-	-	1.2	4.5	6.7
	22:5	0.9±0.09	0.3	0.1	0.9	0.6	0.8	0.7	0.8	0.4	0.4	0.3	0.6	0.5	0.3	0.9	0.5	0.5	0.6	0.3	3.1	4.2	1.9
	22:6	2.2±0.09	1.5	0.2	2.4	2.0	2.1	2.2	1.6	1.2	1.1	1.0	1.5	1.0	0.2	1.4	1.1	1.1	1.1	1.2	1.5	1.2	0.7

95.6% and 28.3%, respectively vs 33.7% in controls). PUFA percentages, however, were generally parallel to, or significantly lower than, controls.

p-MO supplementation had no significant effect on 16:0 levels, but resulted in decreased 16:1ω9 (0.1% to 0.6% vs 4.8% in controls) and increased 18:0 (15.8% to 31.3% vs 11.5% in controls), detection. On the other hand, 18:1ω9 levels were greater than controls (33.7%) with 20mg/l p-MO, but were decreased with 40 and 60mg/l p-MO (39.0%, 23.6% and 26.5%, respectively). 18:2ω6 amounts were parallel to controls (7.2%) or decreased (≤7.0%), but 20:3ω6 was significantly increased (2.6%, 2.9% and 4.2% with 20, 40 and 60mg/l p-MO, respectively vs 1.1% in controls). Raised levels of 20:4ω6 were also found over the range dosed (5.5%, 7.1% and 7.8%, respectively vs 3.6% in controls), yet 22:4ω6 and 22:5ω6 percentages were parallel to controls (10.0% and 0.3%, respectively) or decreased. This was also found with ω3 PUFA's.

Incubation with 20, 40 or 60mg/l p-OO induced marked increases in cellular 18:1ω9 (52.8%, 60.0% and 61.0%, respectively vs 33.7% in controls), but 16:0, 16:1ω9 and 18:0 levels were decreased relative to controls (20.4%, 4.8% and 11.5%, respectively). 20:2ω6 was significantly increased with 60mg/l p-OO (1.4% vs 0% in controls) and 20:3ω6 percentages were raised at least 2 fold (2.5% to 3.5% vs 1.1% in controls), but no marked increments in 20:4ω6, 22:4ω6 or 22:5ω6 were found. ω3 PUFA percentages

were parallel to controls or decreased.

16:0 and 16:1ω9 levels were significantly decreased with p-SSO supplementation (≤12.0% and ≤0.4% vs 20.4% and 4.8% in controls, respectively). 18:0 levels increased over the range dosed (0%, 17.3% and 20.7%, respectively vs 11.5% in controls), but the converse was found for 18:1ω9 (55.2%, 24.9% and 20.6%, respectively vs 33.7% in controls). 18:2ω6 increased in a concentration dependent manner (16.2%, 22.2% and 32.5% with 20, 40 and 60mg/l p-SSO, respectively vs 7.2% in controls) and significant increases in 18:3ω6 and 20:3ω6 were found with 20, 40 and 60mg/l p-SSO (2.3%, 2.1% and 1.1%, and 4.7%, 9.0% and 3.7% vs 0% and 1.1% in controls, respectively). However, subsequent desaturase and elongase product levels were parallel to controls or decreased. In every instance, the proportion of ω3 PUFA's were decreased in dosed, compared to control, cells.

Considerably less 16:0 and 16:1ω9 were detected in cells supplemented with p-LO than in controls (20.4% and 4.8%, respectively), while 18:0 was increased (13.5% to 21.2% vs 11.5% in controls). 18:1ω9 increased significantly with 20mg/l p-LO dosage, but was decreased with 40 and 60mg/l p-LO (43.0%, 26.3% and 17.2%, respectively vs 33.7% in controls). 18:2ω6 levels were significantly raised with 20mg/l p-LO (20.9% vs 7.2% in controls), and 18:3ω3 increased in a concentration dependent manner

with 20, 40 or 60mg/l p-LO supplementation (10.3%, 17.8% and 28.3%, respectively vs 0.7% in controls), yet PDFA levels were not significantly raised.

p-EPO supplementation decreased cellular 16:0 and 16:1ω9 levels (7.0% to 15.0% and ≤0.8% vs 20.4% and 4.8% in controls, respectively), and 24:0 amounts were slightly increased with 60mg/l (1.7% vs 0.1% in controls). p-EPO increased 18:0 percentages in a concentration dependent manner (11.5%, 12.7%, 20.7% and 34.4% with 0, 20, 40 and 60mg/l, respectively), whereas the converse occurred for 18:1ω9 (33.7%, 39.1%, 22.2% and 15.7%, respectively). p-EPO dosage raised cellular 18:2ω6 levels (16.9%, 20.9% and 20.8% with 20, 40 and 60mg/l, respectively vs 7.2% in controls), and 18:3ω6 was significantly increased with 40 and 60mg/l p-EPO (2.2% and 1.4%, respectively vs 0% in controls). 20:3ω6 levels were raised about 10 fold with 20 and 40mg/l p-EPO (10.5% and 10.6%, respectively vs 1.1% in controls), and 20:4ω6 was only increased with 20mg/l p-EPO (5.3% vs 3.6% in controls), but 22:4ω6 and 22:5ω6 levels were decreased. All ω3 PUFA levels were decreased in dosed cells compared to controls.

p-FO supplementation decreased cellular 16:0 and 18:1ω9 percentages overall, but induced increases in 16:1ω9 (4.8%, 4.3%, 10.8% and 17.4% with 0, 20, 40 and 60mg/l, respectively) and 18:0 (11.5%, 38.5%, 25.9% and 15.8%, respectively). 20:3ω6 levels were nearly 2 fold greater than controls (1.1%) with 40mg/l p-FO (1.9%), but other

ω6 PUFA levels were not significantly increased. 20:5ω3 levels increased with addition of 20, 40 and 60mg/l p-FO (1.2%, 4.5% and 6.7%, respectively vs 0.1% in controls), and this was also found for 22:5ω3 (3.1%, 4.2% and 1.9%, respectively vs 0.9% in controls), yet 22:6ω3 levels were decreased (1.5%, 1.2% and 0.7%, respectively vs 2.2% in controls).

4.5.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides measured both in control and dosed cells, and in their respective growth media are shown in Table 4.5.4.1. as nmoles MDA/10^6 cells. In the case of the spent incubation media, however, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained. The MDA amounts measured varied with the p-oil dosed, but increased in an overall concentration dependent manner.

Lipoperoxide production in dosed cells ranged from 0.1 to 3.1nmoles MDA/10^6 cells compared to 0.1nmoles MDA/10^6 control cells. The p-oils abundant in saturated FA's (p-CO and p-MO) consistently induced small lipoperoxide amounts (0.1 to 0.2nmoles MDA/10^6 cells), which were parallel to controls, but slightly greater amounts were generated by cells incubated with p-OO, p-SSO or p-EPO (0.1 to 0.6nmoles MDA/10^6 cells), particularly with a concentration of 60mg/l. Small lipoperoxide amounts were induced by cells dosed with 20 or 40mg/l p-LO, but production was considerably greater with 60mg/l p-LO (0.2, 0.3 and 2.1nmoles MDA/10^6 cells, respectively). p-FO, however, induced the largest cellular lipoperoxide levels at every concentration dosed (0.4, 0.4, 3.1nmoles MDA/10^6 cells with 20, 40 and 60mg/l, respectively).

Spent incubation medium obtained from control cultures

Table 4.5.4.1.

Lipoperoxide formation by cat brain cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.1			
CO		0.1	0.2	0.2
MO		0.1	0.2	0.2
OO		0.2	0.3	0.6
SSO		0.1	0.3	0.4
LO		0.2	0.3	2.1
EPO		0.1	0.1	0.5
FO		0.4	0.4	3.1
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		0.2	0.3	0.5
MO		0.1	0.3	0.6
OO		-	0.3	0.7
SSO		0.2	0.4	1.6
LO		0.8	0.8	7.2
EPO		0.2	0.4	1.4
FO		1.1	3.2	14.5

contained no MDA, but this was measured in spent medium derived from dosed cultures. As with the cells, the smallest lipoperoxide amounts measured in spent media occurred with p-CO or p-MO incubation (0.1 to 0.6nmoles MDA/10^6 cells), although similar concentrations were measured in spent media with p-OO dosage. Larger amounts of spent medium lipoperoxides were measured when p-oils containing significant levels of PUFA's, especially of the ω3 series, were dosed. Lipoperoxide concentrations were similar in medium enriched with 20, 40 or 60mg/l p-SSO or p-EPO (0.2, 0.4 and 1.6 vs 0.2, 0.4 and 1.4 nmoles MDA/10^6 cells, respectively). Considerably larger lipoperoxide amounts were measured in spent medium dosed with 20, 40 or 60mg/l p-LO (0.8, 0.8 and 7.2nmoles MDA/10^6 cells, respectively), although p-FO induced the largest amounts (1.1, 3.2 and 14.5nmoles MDA/10^6 cells, respectively). Clearly, the pattern of lipoperoxides found in the cells and spent medium of dosed cultures were similar, despite numerical variations.

4.5.5 Discussion.

No reference has been found in the literature in which the effects of FA's on the growth of cat brain cells has been found. This study showed that primary cultures derived from cat brain were susceptible to alterations in cell viability as a result of p-oil supplementation. The results reported (Figs. 4.5.1.2-4.5.1.8.) indicated that exogenous FA's modulated the proliferation of these cells to a greater or lesser extent, dependent on p-oil FA composition (Table 2.3.3.2.) and concentration dosed. The large amounts of OA, LA and ALA present in p-OO, p-SSO and p-LO, respectively could probably account for the cell viability changes these p-oils induced. It was apparent that OA and ALA exhibited greater cytotoxic potential than LA, although the increased cell viability induced with low to intermediate concentrations of p-OO and p-SSO only also suggested the involvement of OA and LA in the stimulation of cell proliferation. On the other hand, the inability of p-EPO to stimulate cell proliferation, despite a similar FA composition to p-SSO, could have related to the presence GLA in p-EPO only. The presence of this moiety could also explain the more extensive growth inhibition p-EPO induced compared to p-SSO. This indicated that GLA exhibited no potential to stimulate cell proliferation, but was more effective than LA in mediating growth inhibition. Comparison of

with the amount of GLA in p-EPO and ALA in p-LO (about 9% and 63%, respectively) also implied that GLA was more potent than ALA in mediating cytotoxicity. The different effects OA, LA, GLA and ALA appeared to induce could have related to variations in the number and position of double bonds between these C18 FA's. While the data suggested that specific FA's within p-oils were largely responsible for certain cell viability changes, it was also possible that synergistic and antagonistic interactions between p-oil FA's enhanced or diminished particular effects of individual FA's. Such may explain, for example, the stimulation of cell proliferation only with certain concentrations of p-OO and p-SSO, or the similar cell viability changes p-LO and p-FO induced, despite considerable differences in the FA profiles of these p-oils.

Comparison of FA spectra between control and dosed cells (Table 4.5.3.1.) supported the ability of cat brain cells in culture to incorporate exogenous FA's. A possible mechanism whereby the p-oils modulated cell viability could thus have related to differential uptake of exogenous FA's from the growth medium and alterations in membrane fluidity. Substancial evidence indeed exists showing that dietary FA supplementation induces changes in membrane physical properties (eg. King et al 1971, Ginsberg et al 1981, Simon et al 1982 and Stubbs et al 1984). The greatest cytotoxicity induced with p-SO

incubation could thus have related to the abundance of saturated FA's and lack of PUFA's in this p-oil. Such would have decreased the membrane unsaturation index to a greater extent than with dosage of other p-oils, thus increasing membrane rigidification and the susceptiblity to cell lysis. The low PUFA levels found in cells dosed with p-CO (Table 4.5.3.1.) would indeed support this. On the other hand, the greater unsaturated FA content of p-MO may have been able to maintain membrane fluidity more effectively than with p-CO dosage, accounting for the lower higher ID_{50} calculated with p-MO than p-CO. It was also possible that incorporation of large amounts of unsaturated and polyunsaturated FA's from other p-oils, particularly at high concentrations dosed, increased membrane fluidity and instability to such an extent that cell viability could not continue.

Supplementation of cultures with p-oils induced changes in the amount of cellular protein (Table 4.5.2.1.) which generally paralleled changes mediated in cell viability (Figs. 4.5.1.2-4.5.1.8.). When p-oils stimulated cell proliferation, a concommitant increase in total protein was found, which indicated increased cell numbers with constant, or increased, protein per cell. The latter possibility seemed more likely, however, as increased protein was also found when cell viability was limited with incubation of certain p-oils and concentrations.

protein levels, although the protein amounts found were higher than expected considering the number of viable cells reported; this was also consistent with increased protein per cell. These findings implied the involvement of p-oil FA's in the stimulation of protein synthesis, and the numerical variations in such even when different p-oils yielded similar cell numbers could relate to differences in FA structure. An absolute increment in cellular protein could nevertheless reflect enhanced expression of lipid metabolising enzymes induced as a result of p-oil FA incorporation.

p-Oil incorporation by cultured brain cells (Table 4.5.3.1.) significantly modified the FA profiles of the dosed cells compared to controls, dependent upon p-oil FA composition. In vivo studies have also reported changes in the spectra of brain FA's when cats were fed diets either sufficient in all PDFA's, or deficient in ω3, ω6, or all PDFA's (Davidson et al 1989, 1990a). Generally, the changes in saturated and monounsaturated FA's were slight, but more striking differences were apparent in the PUFA profiles between dietary groups. The data nevertheless supported the absence of Δ6D activity, and presence of an active Δ5D, but could not prove whether Δ4D was present or not. However, it confirmed the earlier in vivo findings of other workers (Sinclair 1979, Sinclair et al 1981) showing that 18:2ω6

cat brain, despite incorporation, thus indicating a lack or suppression of Δ6D expression. This was confirmed in the present study (Table 4.5.3.1) since ω3 PUFA profiles indicated that cultured cat brain cells exhibited no significant capability for Δ6-desaturation. The small increment in cellular 18:3ω6 with p-SSO dosage could thus reflect retroconversion of 20:3ω6 formed via 18:2ω6 elongation and Δ8-desaturation. This sequence could account for the increased 20:3ω6 levels found in most p-oil dosed cells, although the large increment in 20:3ω6 with p-EPO incubation could also have related to elongation of incorporated 18:3ω6. However, the finding that 20:4ω6 levels were slightly increased only with 20mg/l p-OO or p-EPO incubation implied very limited or suppressed Δ5D capability, which ω3 PDFA profiles supported. In addition, no 20:4ω6 elongation occurred, but the potential to elongate 20:5ω3 was demonstrated with p-FO dosage. However, it was apparent that Δ4D was not expressed.

The FA spectra of dosed cells were consistent with the lack or suppression of 16:0 desaturation and 16:1ω9 elongation (Table 4.5.3.1.). The finding that the levels of 16:0 were generally lower and 18:0 higher in dosed than in control cells supported elongation of the former. The presence or absence of Δ9D, however, could not be proven as the FA profiles of cells dosed with

while elevated 18:1ω9 levels with p-CO incubation suggested the opposite. The formation of 18:1ω9 by cells fed p-CO, however, could have reflected enhancement of Δ9D expression in an attempt to maintain membrane fluidity altered with incorporation of saturated FA's.

The findings we presented clearly indicated that the overall capability for desaturase cascade enzyme expression was poor in cat brain cells (Table 4.5.3.1.). The possibility that such enzyme expression was suppressed by the growth limiting and/or cytotoxic effects the p-oil FA's induced (Figs. 4.5.1.2-4.5.1.8.) seemed unlikely, as desaturase cascade enzyme activity was not improved even when p-oils had little effect on, or stimulated, cell proliferation. It was more likely that the overall FA balance in the cells was more important in modulating enzyme expression by competitive interations which have been documented (eg. Garcia et al 1965, Brenner et al 1966, Brenner 1974, Ullman et al 1971a, de Schriver et al 1982 and Nassar 1986), although the poor capability for desaturase cascade enzyme expression may also be a characteristic of these cells. This would imply a requirement for preformed PDFA's in the diet to maintain normal cell physiology.

MDA detection in cat brain cultures both in the presence and absence of dosed p-oils (Table 4.5.4.1.) supported the ability of these cells to oxidise FFA's by enzymic

and/or non-enzymic mechanisms. However, measurement of only small lipoperoxide amounts in control cells, despite detection of potential FA substrates for the formation of these compounds in such (Table 4.5.3.1.), implied efficient mechanisms of protection against free radical formation, eg. peroxidase, superoxide dismutase and/or vitamin E. It was also possible that most cellular unsaturated FA's in control cells were present as components of complex lipids, eg. membrane PGL's, which would thus not be readily accessible to oxidation. Enhanced lipoperoxide formation as a result of p-oil dosage, however, reflected saturation of the cellular anti-oxidant mechanisms due to exogenous unsaturated FA incorporation and/or increased cellular unsaturated FA availability for oxidation. This supported the increased concentrations of lipoperoxides found overall with the amount of p-oil supplemented (Table 4.5.4.1.).

The ability of these cells to incorporate exogenous FA's (Table 4.5.3.1.), the presence of albumin as FA carrier and abundance of saturated FA's in culture medium (Table 2.3.3.3.), supported the probability that most lipid peroxides found in the spent media of dosed cultures originated intracellularly. The capability of cellular lipoperoxides to increase membrane permeability and even to disrupt biological membranes (Chio et al 1969, Mead 1976, Tappel 1975, 1980, Gavino et al 1981b, Morisaki et al 1982b and Frankel 1984) implied their leakage through

the plasma membrane of intact cells when p-oil dosage did not induce cell lysis at the concentrations studied, or as a direct result of p-oil-induced cytotoxicity. Cellular peroxidising enzymes and FFA's could well have been released into the culture medium in this process, particularly since superoxide radicals have been shown to mediate an increase in the permeability of the cat brain to albumin (Wei et al 1986). Thus, the possiblity that further FFA oxidation occurred extracellularly, and contributed to the lipoperoxides measured in the spent medium, could not be overlooked. Such may well account for the greater lipoperoxide amounts found in the spent medium of cultures enriched with p-CO, p-SSO, p-EPO, p-LO, p-FO, 60mg/l p-MO or 60mg/l p-OO than in the cells themselves. In vivo studies by Kontos et al (1985) found that the metabolism of exogenous or endogenous AA in cat brains resulted in the appearance of superoxide anions in the extracellular space. Their studies suggested that these superoxides emerged from the interior of undamaged cells via existing membrane channels. Such a mechanism could also explain the higher levels of medium than cellular lipoperoxides measured in our study when dosed p-oils did not induce cytotoxicity.

The quantitative variations in MDA levels reported with p-oil supplementation at a particular concentration reflected differences in p-oil FA composition (Table 2.3.3.2.). This related not only to the amount of

unsaturated FA's present in any p-oil, but also to their susceptibility to oxidation, dependent on the number and position of double bonds in a FFA. The lowest lipid peroxide amounts induced with p-CO or p-MO incubation reflected the abundance of saturated FA's in these p-oils, and probably related to oxidation of their small amounts of unsaturated FA's and/or FFA's released during PGL turnover. On the other hand, the small lipoperoxide amounts measured with p-OO dosage, despite the abundance of OA in the cells (Table 4.5.3.1.) and the capability of OA to undergo oxidation (Frankel 1984), implied that such was largely lipid-bound. This could also explain the small cellular lipoperoxide concentrations measured with p-SSO or p-EPO supplementation, although such could partly relate to lipoperoxide release into the medium, which was significantly increased. The fact that p-EPO and p-SSO induced similar MDA amounts nevertheless reflected their similar FA compositions; the lipid peroxides formed probably related mainly to oxidation of free LA. On the other hand, the availability and susceptibility of free ALA and EPA could explain the large lipoperoxide concentrations measured with p-LO or p-FO dosage, respectively. The greater susceptibility of EPA to oxidation than ALA was consistent with the greatest MDA levels measured with p-FO dosage, despite more ALA in p-LO than EPA in p-FO (approximately 63% and 18%, respectively). Such could also reflect a faster

cellular pool from membrane lipids with p-FO incubation. EPA incorporated with p-FO dosage could also have been utilised directly as an endoperoxide substrate, whereas ALA could not, particularly since these cells did not express Δ6D (Table 4.5.3.1.).

Lipoperoxides have been implicated in affecting vital cell functions by damaging proteins, membranes, and even inhibiting cell division, amoungst others (Chio et al 1969, Mead 1976, Miller at al 1980, Tappel 1980, Frankel 1984 and Beppu et al 1987). Furthermore, oxygen radicals have been shown to damage cerebro-vascular tissue in cat brain (Christman et al 1984). In the light of this, and the finding that the MDA concentrations reported both in the dosed cells and corresponding spent culture medium (Table 4.5.4.1.) were generally inversely related to the cell viability changes induced with p-oil dosage (Figs. 4.5.1.2-4.5.1.8.), lipoperoxide involvement in the modulation of cell proliferation was possible. The fact that the lipoperoxide concentrations did not always correlate quantitatively the with cell numbers, however, indicated that other mechanisms were also involved in the modulation of cell viability, such as alterations in membrane fluidity.

4.6 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM CAT LUNG.

4.6.1 Effects of pseudo-Oils on Cell Viability.

Microscopic examination of cells before, during and after each experiment showed no morphological changes between control or p-oil dosed cells at any time, although cytoplasmic droplets were observed with p-oil incubation at high concentrations in some instances.

Investigation of the effects of albumin with final medium concentrations ranging from 0 to 250mg/l showed no significant effect on cell viability (Fig. 4.6.1.1.). The effects induced with the dosed p-oils were therefore a direct result of the exogenous FA's and not the albumin used as FA carrier.

The control cell number, seeded at $10x10^4$/ml, at the end of the 24 hour post-trypsinisation recovery period, was $12.3x10^4$/ml. This equated to about 66% of the control cell number at the end of the 48 hour incubation period, and represented the cytostatic number. Only p-oil concentrations reducing cell viability to significantly below 66% were thus considered cytotoxic.

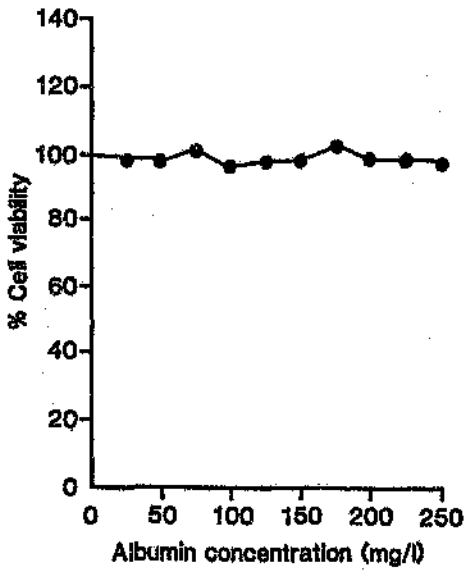
The results of the incubation of cells with p-oils are shown in Figs. 4.6.1.2-4.6.1.8. p-MO, p-LO, p-FO and p-OO enhanced cell proliferation with low concentrations

Legend to Figs. 4.6.1.1-4.6.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where "n" is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 4.6.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.6.1.2-4.6.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 4.6.1.1.

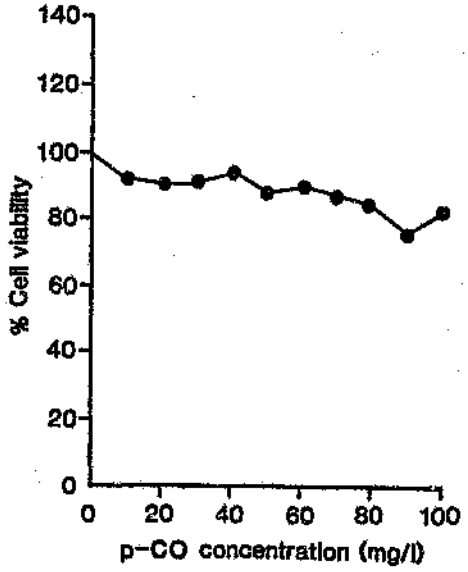
The percentage viability of cells derived from cat lung incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.0	12
25	99.3	6.3	12
50	98.4	4.2	12
75	102.8	5.4	12
100	96.9	5.0	12
125	98.3	3.8	12
150	99.2	4.8	12
175	103.3	3.9	12
200	98.8	3.4	12
225	99.2	5.4	12
250	98.2	3.8	12

Fig. 4.6.1.2.

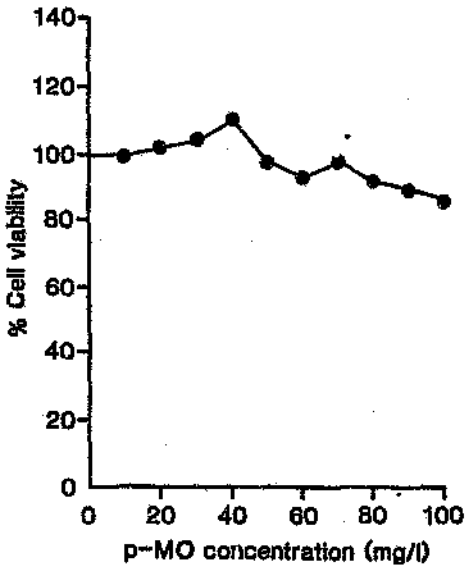
The percentage viability of cells derived from cat lung incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	92.1	3.0	6
20	90.6	1.5	6
30	91.0	1.5	6
40	92.9	2.8	6
50	88.7	2.2	6
60	90.5	2.5	6
70	87.1	1.9	6
80	84.7	2.0	6
90	76.8	2.7	6
100	82.6	4.0	6

Fig. 4.6.1.3.

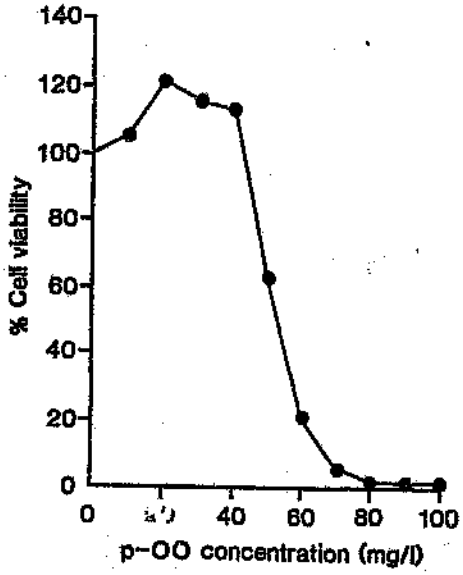
The percentage viability of cells derived from cat lung incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.2	6
10	99.0	3.6	6
20	102.9	2.8	6
30	103.9	2.7	6
40	109.9	1.8	6
50	97.4	3.7	6
60	93.6	3.2	6
70	97.4	3.3	6
80	91.7	2.6	6
90	89.1	1.7	6
100	86.2	2.0	6

Fig. 4.6.1.4.

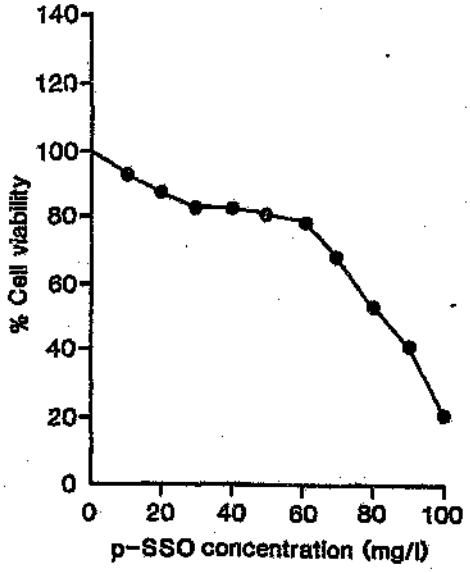
The percentage viability of cells derived from cat lung incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.6	6
10	104.8	3.3	6
20	121.3	3.2	6
30	115.6	4.0	6
40	113.1	1.4	6
50	61.4	2.6	6
60	20.2	4.0	6
70	5.5	1.4	6
80	0.8	0.3	6
90	0.0	0.0	6
100	0.0	0.0	6

Fig. 4.6.1.5.

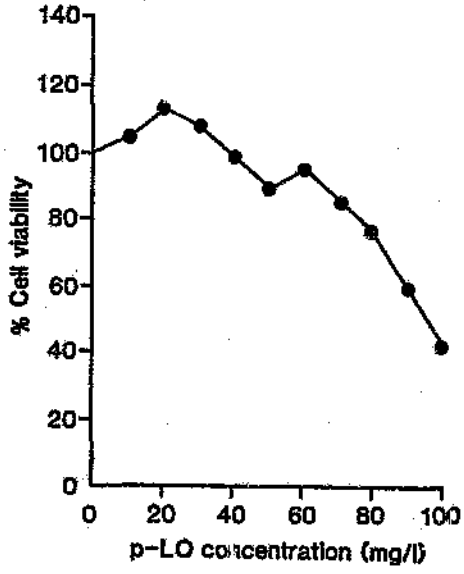
The percentage viability of cells derived from cat lung incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.9	6
10	93.2	2.0	6
20	86.7	2.7	6
30	83.4	3.2	6
40	82.4	4.1	6
50	81.5	2.5	6
60	78.0	2.4	6
70	68.4	1.8	6
80	53.2	1.4	6
90	40.8	2.4	6
100	21.3	1.8	6

Fig. 4.6.1.6.

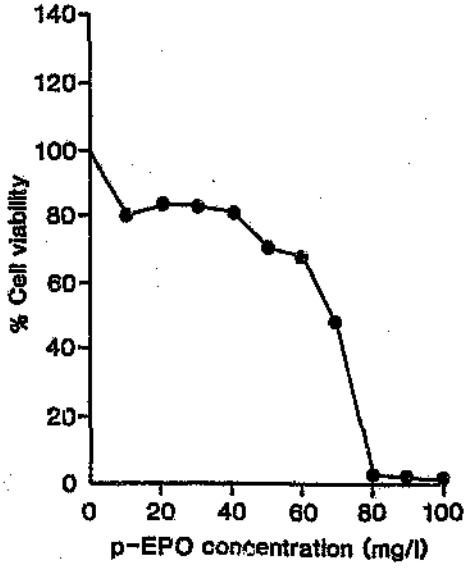
The percentage viability of cells derived from cat lung incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.9	6
10	104.1	2.8	6
20	112.3	3.9	6
30	107.9	5.2	6
40	98.8	3.6	6
50	89.2	3.3	6
60	94.8	4.8	6
70	86.1	4.4	6
80	76.8	2.6	6
90	60.2	3.0	6
100	42.0	2.0	6

Fig. 4.6.1.7.

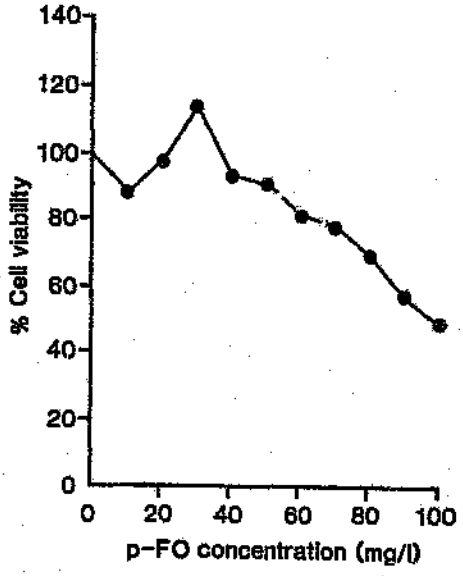
The percentage viability of cells derived from cat lung incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	79.8	2.3	6
20	83.5	2.8	6
30	82.7	2.0	6
40	81.3	2.5	6
50	71.1	3.1	6
60	68.3	2.4	6
70	47.0	2.9	6
80	2.4	1.1	6
90	0.2	0.2	6
100	0.0	0.0	6

Fig. 4.6.1.8.

The percentage viability of cells derived from cat lung incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.3	6
10	87.3	5.8	6
20	97.0	1.5	6
30	113.7	4.2	6
40	92.9	1.5	6
50	90.6	4.6	6
60	81.3	2.9	6
70	77.3	3.5	6
80	68.7	3.7	6
90	56.8	0.8	6
100	48.9	2.4	6

in the range dosed, all p-oils caused growth limitation, whereas cytotoxicity was only duced with p-oils rich in monoenoic and/or polyenoic FA's. The effects induced, however, varied in magnitude dependent on the p-oil and concentration dosed.

Cell viability was not adversely affected when dosed with p-CO or p-MO (Figs. 4.6.1.2. and 4.6.1.3.) as these p-oils were at most only marginally growth limiting, and 40mg/l p-MO enhanced cell proliferation to 109.9%. The minimum yield of viable cells found was 76.8% and 86.2% with 90mg/l p-CO and 100mg/l p-MO, respectively, thus no ID_{50} could be determined.

p-OO induced the most dramatic cell viability changes, manifested by a sigmoidal-type growth curve (Fig. 4.6.1.4.). Cell viability was enhanced to 121.3%, 115.6% and 113.1% with 20, 30 and 40mg/l p-OO, respectively, but higher concentrations were cytotoxic. Half the cells were killed with 53mg/l p-OO, representing the lowest ID_{50} found. 60mg/l p-OO reduced cell viability to 20.2%, and all cells practically took up Trypan blue with p-OO concentrations of 80mg/l or greater.

Cell proliferation was progressively limited with dosage of up to 70mg/l p-SSO, at which concentration cytostasis was induced (Fig. 4.6.1.5.). Higher amounts dosed caused cytotoxicity, yielding 21.3% viable cells with 100mg/l p-SSO. Cell growth was limited by approximately

20% with 10 to 40mg/l p-EPO and 30% with 50mg/l p-EPO, whereas 60mg/l p-EPO induced cytostasis (Fig. 4.6.1.7.). Higher concentrations were cytotoxic, and reduced cell viability to 47.0% with 70mg/l p-EPO, while practically 100% Trypan blue uptake was observed with p-EPO amounts of 80mg/l or greater. p-EPO was thus more effective in reducing cell growth than p-SSO, and this was supported by the ID_{50} values (68mg/l and 83mg/l, respectively).

p-LO and p-FO elicited similar effects on cell growth (Figs. 4.6.1.6. and 4.6.1.8.). Cell proliferation was enhanced to 112.3% and 107.9% with 20 and 30mg/l p-LO, respectively, compared to 113.7% with 30mg/l p-FO. Higher p-oil concentrations progressively limited cell growth such that 76.8% viable cells were found with 80mg/l p-LO and 68.7% with 80mg/l p-FO. Cytotoxicity was induced with 90mg/l p-LO or p-FO, but 42.0% and 48.9% of cells were still viable 100mg/l p-LO or p-FO incubation, respectively, and similar ID_{50} values were calculated (95mg/l and 98mg/l, respectively).

Subsequent to these studies, cells derived from cat lung were plated and dosed appropriately with 0, 20, 40 or 60 mg p-oil/l culture medium in sufficient quantities for all quantitative and qualitative analyses to be carried out. Upon harvesting, cell viabilities were compared and found not to be statistically different from those obtained in Figs. 4.6.1.1-4.6.1.8., thus all further

4.6.2 Effects of pseudo-Oils on Total Protein.

The total cellular protein concentrations determined at each of the 3 p-oil concentrations dosed are shown in Table 4.6.2.1. in relation to controls.

Cells dosed with 60mg/l p-OO exhibited significantly less total protein than controls (169.2 vs 230.4µg/10^6 cells seeded). With this exception, total protein levels in p-oil dosed cells were parallel to controls or significantly increased, and the greatest increment was induced with 20mg/l p-OO incubation (337.2µg protein/10^6 cells seeded). Cellular protein levels were greater in cells enriched with 20 than with 60mg/l p-oil, and concentrations decreased across the range dosed with p-OO (337.2 to 169.2µg), p-SSO (240.1 to 236.5µg) and p-LO (317.3 to 261.3µg). On the other hand, more protein was found in cells dosed with 40 than with 20 or 60mg/l p-CO (263.4, 243.6 and 233.6µg, respectively), p-MO (304.4, 286.0 and 262.2µg, respectively), p-EPO (255.5, 245.3 and 228.3µg, respectively) or p-FO (283.8, 271.1 and 242.7µg, respectively).

Table 4.6.2.1.

The protein content of cells derived from cat lung, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	230.4			
CO		243.6	263.4	233.6
MO		286.0	304.4	262.2
OO		337.2	315.8	169.2
SSO		240.1	237.0	236.5
LO		317.3	281.5	261.3
EPO		245.3	255.5	228.3
FO		271.1	283.8	242.7

3.6.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Cat Lung.

FA spectra of cells incubated with 0, 20, 40 or 60mg/l of each of the p-oils is shown in Table 4.6.3.1.

Control cells exhibited a FA spectrum in which 18:1ω9 comprised 21.7% of all FA's, compared to 14.4% 16:0, 18.6% 18:0, 8.1% 20:4ω6 and 14.1% 22:4ω6, whereas other FA's were detected in smaller proportions.

Dosed cells generally contained less 18:0 and 16:1ω9 than controls, while 16:0 and 18:1ω9 levels approximated to controls, or were increased. 18:2ω6 percentages were generally greater in dosed than in control cells, while the converse was true for 20:3ω6, 20:4ω6, 22:4ω6 and 22:5ω6. Furthermore, 20:3ω6, 20:4ω6 and 22:4ω6 amounts generally occurred in progressively smaller proportions with increments in p-oil concentration dosed. 18:3ω3 levels were similar between control and dosed cells, but ω3 PDFA levels were generally decreased in dosed cells.

Cells supplemented with 20, 40 or 60mg/l p-CO exhibited increased levels of 16:0 (21.4%, 25.3% and 30.4%, respectively vs 14.4% in controls), 16:1ω9 (4.2%, 8.1% and 3.1%, respectively vs 2.5% in controls) and 18:1ω9 (24.2%, 27.8% and 29.2%, respectively vs 21.7% in controls), but 18:0 was decreased (16.0%, 13.1% and 11.7%, respectively vs 21.7% in controls). 22:6ω3 was

Legend to Table 4.6.3.1.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where "n" is the number of experiments. "[p-Oil]" refers to the pseudo-oil concentration used.

Table 4.6.3.1.

The fatty acid spectrum of cells derived from cat lung.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	14.4±0.05	21.4	25.3	30.4	19.9	20.8	21.7	13.2	13.0	16.3	12.2	11.4	14.3	9.3	10.6	11.8	10.1	10.8	14.7	15.2	14.8	36.1
	18:0	18.6±0.25	16.0	13.1	11.7	16.2	14.2	12.3	8.7	6.0	6.0	12.7	9.8	9.1	24.9	9.2	24.4	26.7	10.1	10.9	12.5	9.9	10.2
	20:0	0.4±0.00	-	0.2	-	-	0.1	-	-	-	0.7	-	-	-	-	0.1	-	-	-	-	-	-	-
	22:0	0.1±0.00	0.4	0.3	0.2	0.1	0.2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
	24:0	0.4±0.00	0.2	0.2	-	-	0.3	0.1	0.1	0.2	-	-	0.1	0.6	-	0.9		0.2	0.3	0.5	-	-	-
ω9 MONOS.	16:1	2.5±0.10	4.2	8.1	3.1	1.3	1.5	2.9	1.5	1.4	2.1	1.0	1.3	1.2	0.6	1.0	0.5	0.4	1.0	1.8	10.8	17.5	15.4
	18:1	21.7±0.05	24.2	27.8	29.2	23.5	25.1	27.7	46.1	53.2	56.0	21.0	20.1	21.7	21.8	15.8	25.3	18.1	13.4	14.8	20.4	19.6	19.1
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	2.9±0.07	3.6	2.4	2.9	8.1	10.6	13.6	9.3	11.4	10.3	28.1	39.1	40.6	11.0	12.0	.2	23.6	42.0	40.6	6.4	7.9	5.6
	18:3	-	0.1	-	0.1	0.2	0.2	0.3	0.1	0.1	-	0.1	0.1	0.3	0.8	0.	-	0.4	1.4	2.8	0.1	0.1	0.1
	20:2	-	-	-	-	0.3	0.5	0.4	1.5	2.0	1.5	1.7	1.8	0.9	0.3	0.4	-	1.2	1.8	0.4	0.6	1.0	0.7
	20:3	2.6±0.03	2.2	1.8	1.5	2.5	2.4	2.0	1.6	1.5	0.8	2.7	2.2	1.2	1.1	1.1	0.9	4.6	6.3	2.9	1.7	1.4	0.5
	20:4	8.1±0.20	8.2	5.7	5.3	9.9	8.5	8.4	4.6	3.3	1.6	5.3	3.2	2.1	5.5	6.6	1.1	4.2	3.3	2.2	5.2	3.2	1.3
	22:4	14.1±0.20	9.5	6.1	7.3	9.4	7.0	5.1	6.8	3.0	2.2	9.0	5.8	4.4	4.6	4.0	3.5	5.5	4.8	4.8	8.2	4.3	4.6
	22:5	1.0±0.00	-	0.4	-	-	0.4	0.1	-	0.1	-	-	0.2	-	0.1	0.1	0.1	0.2	0.2	0.1	-	0.3	0.1
ω3 POLYS.	18:3	0.6±0.00	0.7	0.6	0.6	0.8	0.6	0.4	0.9	0.8	0.3	0.5	0.4	0.3	13.6	32.8	11.0	0.2	0.3	0.3	0.7	0.6	0.5
	18:4	2.1±0.05	0.6	1.1	0.5	0.3	0.7	0.2	0.2	0.4	0.3	0.2	0.4	0.2	0.8	0.4	4.5	0.4	0.3	0.2	0.2	0.8	0.3
	20:4	3.3±0.12	0.9	1.3	1.2	0.8	1.3	0.5	0.3	0.7	0.4	0.5	0.9	0.8	2.2	1.7	0.8	0.8	0.8	1.0	0.9	1.4	0.7
	20:5	1.3±0.10	1.2	1.1	1.1	0.5	0.8	0.9	0.9	0.3	0.1	0.4	0.3	0.4	0.3	0.3	-	0.2	0.3	0.3	6.7	10.3	3.3
	22:5	3.3±0.03	3.1	2.1	2.2	3.0	2.2	1.7	2.0	1.3	0.7	2.3	1.4	0.9	1.7	1.5	0.5	1.7	1.3	[illegible]	7.7	5.1	1.0
	22:6	2.6±0.03	3.7	2.7	2.7	3.4	2.6	2.0	2.2	1.5	0.8	2.3	1.6	1.1	1.6	1.5	0.4	1.8	1.6	1.1	2.5	1.9	0.7

slightly increased with 20mg/l p-CO dosage (3.7% vs 2.6% in controls), but no significant increment in other PUFA's were detected.

16:0 and 18:1ω9 percentages increased slightly with 20, 40 or 60mg/l p-MO dosage (19.9%, 20.8% and 21.7%, and 23.5%, 25.1% and 27.7% vs 14.4% and 21.7% in controls, respectively), whereas 16:1ω9 and 18:0 were parallel to controls (2.5% and 18.6%, respectively) or decreased. A concentration dependent increase in 18:2ω6 was detected (8.1%, 10.6% and 13.6% with 20, 40 and 60mg/l p-MO, respectively vs 2.9% in controls), and 20:4ω6 was slightly increased only with 20mg/l p-MO (9.9% vs 8.1% in controls), yet 22:4ω6 and 22:5ω6 levels were lower than those of controls (14.1% and 1.0%, respectively).

Dosage of cells with 20, 40 or 60mg/l p-OO had little effect on 16:0 levels (13.0% to 16.3% vs 14.4% in controls), but decreased 18:0 and 16:1ω9 percentages (6.0% to 8.7% and 1.4% to 2.1% vs 18.6% and 2.5% in controls, respectively). On the other hand, increased amounts of 18:1ω9 (46.1%, 53.2% and 56.0%, respectively vs 21.7% in controls) and 18:2ω6 (9.3%, 11.4% and 10.3%, respectively vs 2.9% in controls) were detected, as well as a significant increment in 20:2ω6 (1.5% to 2.0% vs 0% in controls). However, ω6 PDFA proportions were parallel to controls or significantly decreased.

Enrichment of cells with p-SSO induced little change in

16:0 and 18:1ω9 levels, but 18:0 and 16:1ω9 were decreased (9.1% to 12.7% and 1.0% to 1.3% vs 18.6% and 2.5% in controls, respectively). A marked increment in 18:2ω6 was detected (28.1%, 39.1% and 40.6% with 20, 40 and 60mg/l p-SSO, respectively vs 2.9% in controls), and 20:2ω6 increased slightly over the same range (1.7%, 1.8% and 0.9%, respectively vs 0% in controls). However, ω6 and ω3 PDFA levels were parallel to controls or significantly decreased.

16:0 and 16:1ω9 percentages were decreased with 20, 40 or 60mg/l p-LO dosage (9.3% to 11.8% and 0.5% to 1.0% vs 14.4% and [illegible]% in controls), whereas 18:0 and 18:1ω9 levels were more variable (24.9%, 9.2% and 24.4%, and 21.8%, 15.8% and 25.3% vs 18.6% and 21.7% in controls, respectively). p-LO induced an increment in 18:2ω6 (11.0% to 15.2% vs 2.9% in controls), and 18:3ω3 (13.6%, 32.8% and 11.0% with 20, 40 and 60mg/l, respectively vs 0.6% in controls), and 18:4ω3 increased approximately 2 fold with 60mg/l p-LO (4.5% vs 2.1% in controls). No significant increments in other ω3, and all ω6, PDFA's were detected, however.

p-EPO incubation had little effect on 16:0, increased 18:0 only with 20mg/l (26.7% vs 18.6% in controls), but decreased 16:1ω9, 18:1ω9 and all ω3 PUFA s levels. A marked increase in 18:2ω6 was induced (23.6%, 42.0% and 40.6% with 20, 40 and 60mg/l p-EPO, respectively vs 2.9% in controls). 18:3ω6 was significantly increased with 40

and 60mg/l p-EPO (1.4% and 2.8%, respectively vs 0% in controls), and such was also found for 20:2ω6 and 20:3ω6 with 20 and 40mg/l p-EPO (1.2% and 1.8%, and 1.8% and 6.3% vs 0% and 2.6% in controls, respectively). However, lower amounts of 20:4ω6, 22:4ω6 and 22:5ω6 were detected in dosed cells.

Supplementation of cells with p-FO increased 16:0 levels only with 60mg/l (36.1% vs 14.4% in controls), decreased 18:0 levels, had little effect on 18:1ω9 percentages, but increased 16:1ω9 amounts significantly (10.8%, 17.5% and 15.4% with 20, 40 and 60mg/l, respectively vs 2.5% in controls). No significant increment in ω6 PDFA's were detected, despite approximately 2 fold more 18:2ω6 than in controls (2.9%). p-FO dosage induced an increase in 20:5ω3 (6.7%, 10.3% and 3.3% with 20, 40 and 60mg/l, respectively vs 1.3% in controls) and 22:5ω3 (7.7% and 5.1% with 20 and 40mg/l, respectively vs 3.3% in controls), but not in 22:6ω3 (2.5%, 1.9% and 0.7% with 20, 40 and 60mg/l, respectively vs 2.6% in controls).

4.6.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Cat Lung.

Table 4.6.4.1. shows the radioactivity recovered from cultures incubated with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6, or [^{14}C]-18:3ω3.

The total counts recovered in the spent incubation media ranged from 2.4 to 2.9x10^{6}cpm. Only 0.3 to 0.4x10^{6}cpm were recovered from the pooled buffers after washing the cells, and this accounted for non-specific binding. For both [^{14}C]-18:1ω9 and [^{14}C]-18:3ω3, the equivalent of 4.3x10^{6}cpm were dosed to cultures, and total recovery of each was approximately 84% and 86%, respectively. For [^{14}C]-18:2ω6 the equivalent of 4.8x10^{6}cpm were dosed to the incubation medium, and total recovery of this radioisotope amounted to about 85%. However, the total amount of each of these radioisotopes incorporated into the cells approximated to 18% for [^{14}C]-18:1ω9, 18% for [^{14}C]-18:2ω6 and 15% for [^{14}C]-18:3ω3.

Table 4.6.4.2. shows the percentage of each incorporated radiolabelled C18 FA converted to ω9, ω6 or ω3 series products after incubation with cat lung fibroblasts.

28.3% of the total [^{14}C]-18:1ω9 counts derived upon GLC analysis (1034cpm) remained unchanged, while the proportions of 20:1ω9, 22:1ω9 and 24:1ω9 detected were 33.6%, 29.9% and 8.2%, respectively.

Table 4.6.4.1.

The radioactivity (cpm) recovered from cat lung cultures incubated with radiolabelled C18 fatty acids, expressed as mean (±s.e.m.), n=3.

SPENT MEDIA (M)	POOLED WASHINGS (W)	TOTAL (M+W)	CELLS	TOTAL $\times 10^6$
Total 18:1 counts dosed to incubation medium = 4.3×10^6				
2350080 (±3340)	427440 (±6060)	2777520	69494 (±1308)	3.6
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
2866720 (±7200)	387300 (±6300)	3254020	862419 (±1728)	4.1
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
2817320 (±11320)	278700 (±4440)	3096020	644634 (±1428)	3.7

Table 4.6.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from cat lung, expressed as a percentage of the total counts recovered upon GLC analysis.

ω9 MONOS.	[^{14}C]-18:1ω9	ω6 POLYS.	[^{14}C]-18:2ω6	ω3 POLYS.	[^{14}C]-18:3ω3
18:1	28.3	18:2	23.4	18:3	27.1
20:1	33.6	18:3	8.1	18:4	10.0
22:1	29.9	20:2	36.1	20:3	24.6
24:1	8.2	20:3	14.3	20:4	19.6
		20:4	5.7	20:5	7.4
		22:4	6.9	22:5	8.1
		22:5	5.5	22:6	3.3
TOTAL CPM	1034	TOTAL CPM	1998	TOTAL CPM	1484

23.4% of the total [^{14}C]-18:2ω6-derived counts measured (1998cpm) remained unchanged, and 36.1% 20:2ω6 was detected. 8.1% and 14.3% of total counts recovered were found as 20:2ω6 and 20:3ω6, respectively, compared to 5.7% 20:4ω6, 6.9% 22:4ω6 and 5.5% 22:5ω6.

The total counts derived following GLC analysis of cells incubated with [^{14}C]-18:3ω3 were 1484cpm, of which 27.1% was detected for 18:3ω3, 10.0% for 18:4ω3, 24.6% for 20:3ω3 and 19.6% for 20:4ω3. Smaller proportions of post-Δ5D FA's were found, amounting to 7.4% 20:5ω3, 8.1% 22:5ω3, and only 3.3% 22:6ω3.

Δ6D represents the first enzyme in the desaturase enzyme cascade, thus total activity of this enzyme is reflected by the sum of all subsequent desaturase and elongase products.

4.6.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides quantified in dosed cells and their respective growth media are presented in Table 4.6.5.1. in relation to controls. Values are reported as nmoles MDA/10^6 cells, but in the case of the spent incubation media, this represents the nmoles of MDA in the volume of medium from which 1x10^6 cells were obtained. The lipoperoxide amounts measured in dosed cultures varied with p-oil, but increased in an overall concentration dependent manner.

Cat lung fibroblasts dosed with p-oils contained 0.3 to 6.9nmoles MDA/10^6 cells, compared to 0.3nmoles MDA/10^6 control cells. p-CO and p-MO induced the smallest lipid peroxide amounts over the range dosed (0.3 to 0.4nmoles MDA/10^6 cells), and p-OO generated larger concentrations only with 60mg/l (1.3nmoles MDA/10^6 cells), compared to increments with 40 and 60mg/l p-FO (0.9 and 1.0nmoles MDA/10^6 cells). p-SSO and p-EPO induced similar lipid peroxide levels with concentrations of 20 and 40mg/l (0.4 to 0.5nmoles MDA/10^6 cells), but larger amounts were formed with 60mg/l p-EPO than p-SSO (2.8 vs 1.7 nmoles MDA/10^6 cells, respectively). However, cellular lipid peroxide levels were greatest with 40 and 60mg/l p-LO (1.2 and 6.9nmoles MDA/10^6 cells, respectively).

No MDA was measured in spent control medium or in that

Table 4.6.5.1.

Lipoperoxide formation by cat lung cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.3			
CO		0.3	0.4	0.3
MO		0.4	0.4	0.4
OO		0.3	0.3	1.3
SSO		0.5	0.4	1.7
LO		0.4	1.2	6.9
EPO		0.4	0.5	2.8
FO		0.5	0.9	1.0
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		0.2	0.1	0.2
MO		-	-	-
OO		-	-	0.6
SSO		0.1	0.2	1.6
LO		0.3	0.8	1.8
EPO		0.1	0.3	1.7
FO		0.3	0.6	1.0

derived from cultures incubated with p-MO. Small lipid peroxide amounts were detected in the spent medium with p-CO supplementation (0.1 to 0.2nmoles MDA/10^6 cells), and p-OO only induced an increment with a concentration of 60mg/l (0.6nmoles MDA/10^6 cells). Higher lipoperoxide levels were detected with addition of 20, 40 or 60mg/l p-FO to the incubation medium (0.3, 0.6 and 1.0nmoles MDA/10^6 cells, respectively). Spent media derived from cultures incubated with 20, 40 or 60mg/l p-EPO or p-SSO contained similar lipoperoxide levels (0.1, 0.3 and 1.7 vs 0.1, 0.2 and 1.6nmoles MDA/10^6 cells, respectively), although amounts were greatest overall with p-LO dosage (0.3, 0.8 and 1.8nmoles MDA/10^6 cells, respectively).

4.6.6 The Eicosanoid Profile of Cells Derived from Cat Lung.

The eicosanoids positively detected in control and dosed cat lung fibroblasts are presented in Table 4.6.6.1. as a percentage of the total area detected. However, an indication of the total eicosanoid amounts quantified is given for controls in "( )", expressed as pmoles/10^6 cells.

Total eicosanoid formation amounted to only 32.9pmoles per 10^6 control cells, of which 15.6% could be positively identified with the prostanoid standards available. Approximately half this proportion was composed of TXB_2 (7.3%), whereas PGF_{α} and PGI_2 comprised smaller amounts (5.4% and 2.9%, respectively). However, no PGE_1, PGE_2 or PGD_2 were found.

Total percentages for the prostanoids detected in dosed cells varied significantly with p-oil concentration, but levels were greater than in control cells (15.6%). The increment was least with dosage of 40mg/l p-oil (16.8% to 25.2%), but significantly greater both with 20mg/l p-oil (31.2% to 40.0%) or 60mg/l p-oil (33.1% to 40.5%). These differences were reflected by appropriate changes in the amount of individual prostanoids. PGI_2 levels were decreased with 20mg/l p-CO or p-LO dosage (2.1% and 1.5%, respectively), but enhanced with all other p-oils and concentrations supplemented (3.2% to 7.7% vs 2.9% in

Legend to Table 4.6.6.1.

Values are tabulated as relative percent of the total area detected, and as pmoles total eicosanoids/10^6 control cells.

Table 4.6.6.1.

The profile of the eicosanoids detected in extracts of cells derived from cat lung.

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	PGF_{α} (1+2)	PGE_2	PGE_1	PGD_2
CONTROL (32.9)		15.6	2.9	7.3	5.4	-	-	-
CO	20	34.1	2.1	17.2	14.8	0.3	-	-
	40	17.8	3.5	8.1	6.2	-	-	-
	60	40.5	7.7	18.1	14.2	0.5	-	-
MO	20	33.4	4.9	17.4	9.7	1.4	-	-
	40	21.6	5.5	11.6	4.5	-	-	-
	60	33.1	3.2	19.7	8.7	1.5	-	-
OO	20	36.6	4.1	20.3	11.1	1.1	-	-
	40	22.8	7.2	9.5	6.1	-	-	-
	60	39.1	5.9	21.0	11.0	1.2	-	-
SSO	20	40.0	5.7	19.9	12.5	-	1.9	-
	40	22.6	5.6	9.6	5.2	2.2	-	-
	60	39.9	6.3	20.7	10.5	0.8	1.4	0.2
LO	20	31.2	1.5	17.5	11.1	1.1	-	-
	40	16.8	4.5	8.2	4.1	-	-	-
	60	38.4	4.3	20.4	11.1	2.6	-	-
EPO	20	35.3	5.1	17.2	9.6	1.2	2.2	-
	40	25.2	4.6	10.1	7.3	3.2	-	-
	60	35.9	4.4	18.3	9.1	1.9	2.2	-
FO	20	39.4	5.9	19.5	12.4	0.8	0.5	0.3
	40	22.5	3.7	11.9	6.9	-	-	-
	60	33.5	4.8	18.0	9.5	1.2	-	-

controls). TXB_2 percentages were marginally greater than controls (7.3%) with 40mg/l p-oil incubation (8.1% to 11.9%), but these amounts increased 2 to 3 fold with 20 or 60mg/l p-oil supplementation (17.2% to 21.0). PGF_{α} levels were parallel to controls (5.4%) with dosage of 40mg/l p-oil (4.1% to 7.3%), but production was enhanced to a similar extent with 20 or 60mg/l p-oil incubation (8.7% to 14.8%). Dosed cells contained small amounts of both PGE_2 (0% to 3.2% vs 5% in controls) and PGE_1 (0% to 2.2% vs 0% in controls), but neither control nor dosed cells formed significant amounts of PGD_2.

4.6.7 Discussion.

The results obtained from the incubation of cultured cat lung fibroblasts with p-oils indicated that exogenous FA's have the capability to modulate the proliferation of these cells to a greater or lesser extent (Figs. 4.6.1.2-4.6.1.3.), dependent on p-oil FA composition (Table 2.3.3.2.) and concentration dosed. The finding that all p-oils, except p-CO, could inhibit and/or enhance cell proliferation implied that unsaturated rather than saturated FA's were primarily responsible for the cell viability changes the p-oils induced. The significantly greater amount of unsaturated FA's in p-MO than in p-CO (21.6% vs 5.3%, respectively) could well account for the increased cell growth p-MO induced. The fact that both saturated FA-rich p-oils, p-CO and p-MO, could not induce cytotoxicity at the concentrations dosed, unlike those p-oil abundant in monoenoic and/or polyenoic FA's, supported unsaturated FA involvement in the modulation of growth inhibition in these cells. Alternatively, saturated FA's may be more protective against growth inhibition than unsaturated FA's. The finding that the extent of growth inhibition induced did not always correlate with the unsaturated FA content of the p-oils, however, suggested that cytotoxic potential varied between unsaturated FA's. The abundance of OA, LA and ALA in p-OO, p-SSO and p-LO, respectively probably accounted for the cell viability changes these p-oils

induced, and it was apparent that OA exhibited greater cytotoxic potential than LA, whereas ALA was less effective. However, the increased cell viability induced with low concentrations of p-OO and p-LO also suggested OA and ALA involvement in the stimulation of cell proliferation. LA did not exhibit this capability since no increment in cell viability was observed with p-SSO or p-EPO incubation. The greater capability of p-EPO to limit and inhibit cell viability than p-SSO, despite similar FA compositions, could nevertheless have related to the presence of GLA in p-EPO only. This indicated that GLA was more effective than LA in mediating growth inhibition. Comparison of the extent of growth reduction p-EPO and p-LO induced with the amount of GLA in p-EPO and ALA in p-LO (approximately 9% and 63%, respectively) also indicated that GLA was more potent than ALA in mediating growth limitation and inhibition.

The different cell viability changes which p-oil FA's appeared to induce could have related to variations in FA structure between different moieties, although it was also likely that the effects induced by individual FA's were enhanced or diminished by synergistic and antagonistic interactions between p-oil FA's. Such could explain, for example, any non-linear relationships between cell viability and p-oil concentration dosed, the greater stimulation of cell proliferation with 40mg/l p-MO, 20mg/l p-OO, 20mg/l p-LO and 30mg/l p-FO

than with other concentrations, as well as the similar cell viability changes p-LO and p-FO induced, despite pronounced differences in p-oil FA spectra. The ability of these cultured cat lung fibroblasts to incorporate exogenous FA's (Table 4.6.3.1.) nevertheless suggested that the modulation of cell viability related to differential uptake of exogenous FA's from the growth medium and alterations in membrane fluidity.

p-Oil supplementation of cat lung fibroblast cultures influenced the total protein content of those cells (Table 4.6.2.1.), and this generally reflected the cell viability changes induced (Figs. 4.6.1.2-4.6.1.8.). The lowest and highest protein amounts measured with p-OO dosage indeed corresponded with the greatest cytotoxic and proliferative effects induced, respectively. Cell numbers alone, however, did not correlate quantitatively with the increments in total protein found when cell viability was enhanced or limited with p-oil dosage, and the 80% cytotoxicity which 60mg/l p-OO caused also could not justify the 30% reduction in total protein found. These findings indicated that while measurement of total protein may reflect cell growth in terms of actual cell numbers, p-oil FA's were involved in the stimulation of protein biosynthesis. The extent of protein stimulation varied numerically with incubation of identical amounts of different p-oils, even when similar cell numbers were yielded, and suggested that the capability to modulate

protein synthesis varied with FA structure; it was also possible, however, that FA synergism and antagonism played a role in this process. Increments in the amount of protein per cell could nevertheless reflect enhanced expression of enzymes to metabolise incorporated p-oil FA's.

The lung has been implicated a primary eicosanoid biosynthetic site (Mathe et al 1977, Hyman et al 1978 and Harper et al 1984), thus any ability of this tissue to desaturate and elongate PUFA's would be of major importance in the provision of eicosanoid precursors. Whether cat lung has desaturase cascade capability, however, has not been described in the literature. The results obtained from the supplementation of cat lung fibroblasts with p-oils showed that such cells exhibited very little potential to desaturate or elongate PUFA's (Table 4.6.3.1.). The findings were consistent with an overall lack or suppression of Δ6D, Δ5D and Δ4D expression, although certain elongation steps seemed possible. The presence of small 20:2ω6 amounts in most dosed cells reflected limited elongation of incorporated 18:2ω6 which could not be further metabolised via Δ6D. 20:3ω6 formation only in cells supplemented with p-EPO supported impaired Δ6D expression, but was consistent with elongation of incorporated 18:3ω6; the lack of further 20:3ω6 metabolism, however, supported impairment of Δ5D expression. Limited elongation of 20:5ω3

incorporated with p-FO dosage also occurred, but no desaturation to 22:6ω3 via Δ4D was found. On the other hand, the small increment in 18:4ω3 found only with 60mg/l p-LO dosage was consistent with extramicrosomal retroconversion of 20:4ω3. The data could not exclude the potential for 16:0 elongation (suggested with 20 or 60mg/l p-LO dosage) or 16:0 desaturation/18:1ω9 retroconversion (suggested with 20 or 40mg/l p-CO dosage), but 18:0, 16:1ω9 and 18:1ω9 were neither desaturated nor elongated. Nevertheless, it was unlikely that desaturase cascade enzyme activity was impaired by the growth limiting and/or cytotoxic effects mediated with p-oil dosage (Figs. 4.6.1.2-4.6.1.8.), as desaturation was not improved even when p-oils had little effect on, or stimulated, cell proliferation. This phenomenon may rather be a characteristic of this tissue type, implying a requirement of preformed PDFA's from the diet or other tissues from which to generate eicosanoids.

To assess whether the pattern of desaturation reported above was modulated by FA mixtures, p-oil desaturation (Table 4.6.3.1.) was compared with that of individual FA's (Table 4.6.4.2.). As with p-oil desaturation, cat lung fibroblasts were unable to desaturate [^{14}C]-18:1ω9. The pathway for [^{14}C]-18:1ω9 elongation was shown, however, and such was possibly suppressed with p-oil, or enhanced with [^{14}C]-18:1ω9, supplementation. On the other hand, evidence for [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6

desaturation and elongation were found. Detection of large proportions of 20:3ω3 and 20:2ω6, but only small percentages of 18:4ω3 and 18:3ω6, suggested direct elongation of [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6 as a result of impaired Δ6D expression. Thus it was possible that 20:4ω3 and 20:3ω6 were formed from 20:3ω3 and 20:2ω6, respectively via an active Δ8D. This pathway seemed likely as the cat has been shown to be Δ6D deficient (eg. Rivers et al 1975a and Frankel et al 1978). The small proportions of 18:3ω6 and 18:4ω3 formed with [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 dosage could either reflect stimulation/expression of Δ6D, or more likely retro-conversion of 20:4ω3 and 20:3ω6, respectively. Formation of 18.8% ω3, and 18.1% ω6, PDFA's supported Δ5D expression in these cells, but detection of small amounts of 22:6ω3 and 22:5ω6 indicated very limited capability to express Δ4D.

The finding that desaturase enzyme expression was more limited with p-oil compared to individual FA dosage (Tables 4.6.3.1. and 4.6.4.2.) supported the mediation of competitive interactions between p-oil FA's for these enzymes. However, as organisms consume mixtures rather than individual FA's in the diet, the desaturase capability shown with p-oils probably relates closer to the situation in cat lung <u>in vivo</u> than that suggested with single FA's.

MDA quantitation showed that cat lung fibroblasts exhibited the capability to produce lipoperoxides (Table 4.6.5.1.). This could occur either enzymatically or by auto-oxidation of monoenoic and/or polyenoic FA's when the preventative anti-oxidant mechanisms were saturated. Enhanced MDA measurement when cultures were supplemented with p-oils reflected increased unsaturated FA availability for oxidation, and this correlated with the increased lipoperoxide concentrations found overall with the amount of p-oil supplemented (Table 4.6.5.1.). The quantitative variations in MDA concentrations found between dosed cultures, however, reflected the different p-oil FA compositions (Table 2.3.3.2.). The abundance of saturated FA's in p-CO and p-MO could well explain why lipoperoxide formation approximated to that of controls with incubation of these p-oils. On the other hand, the finding that significant increments in lipid peroxide levels were induced only with dosage of 40 and/or 60mg/l of p-oils rich in monoenoic and/or polyenoic FA's correlated with the greater availability and susceptibily of the unsaturated FA's in such p-oils to oxidation. Lipoperoxide formation with 60mg/l p-OO incubation confirmed the capability of OA to undergo oxidation (Frankel 1984), whereas the abundance of LA in p-SSO and p-EPO could largely account for the lipid peroxides these p-oils induced. However, the small, or lack of, increment in cellular MDA levels induced with

20 or 40mg/l p-OO, p-SSO or p-EPO dosage, despite large amounts of OA in p-OO and LA both in p-SSO and p-EPO which were incorporated (Tables 2.3.3.2. and 4.6.3.1.), implied that these substrates for oxidation were largely lipid-bound and stable. Quantitation of similar MDA levels with p-SSO or p-EPO dosage nevertheless reflected the similar FA compositions of these p-oils, although the larger cellular lipoperoxide amounts 60mg/l p-EPO induced could have related to the presence of GLA only in p-EPO. ALA oxidation probably accounted largely for the greatest lipid peroxide concentrations detected overall with p-LO dosage, while the smaller amounts p-FO induced reflected the more even balance of saturated, monoenoic and polyenoic FA's in this p-oil.

Cat lung fibroblasts incorporated exogenous FA's (Table 4.6.3.1.), thus it seemed unlikely that spent medium lipoperoxides (Table 4.6.5.1.) were primarily formed extracellularly via auto-oxidation, particularly since p-oil FA's were dosed bound to albumin. The lack of cytotoxicity found with p-CO, p-MO, p-SSO, p-LO, p-EPO or p-FO dosage, and the greater amounts of cellular than medium MDA measured, rather suggested that medium lipid peroxides resulted from cellular release. Such may have occurred as a result of increased membrane permeability induced by the formation of cellular lipid peroxides, which others have reported (Chio et al 1969, Tappel 1980 and Mead 1976). The similar medium lipoperoxide levels

found with p-EPO or p-SSO dosage could indeed relate to the similar extent to which cell viability was limited (Figs. 4.6.1.7. and 4.6.1.5., respectively). On the other hand, the finding of greater MDA amounts in the cells than spent medium with 60mg/l p-OO dosage, despite marked cytotoxicity induced (Fig 4.6.1.4.), suggested that most substrates for oxidation were membrane-bound.

p-CO and p-MO had little effect on MDA formation (Table 4.6.5.1.) and induced little growth limitation (Figs. 4.6.1.2-4.6.1.3.), whereas MDA generation and cell viability were inversely related with p-OO, p-SSO, p-LO, p-EPO or p-FO dosage (Figs. 4.6.1.4-4.6.1.8.). This suggested that lipid peroxide involvement in the modulation of cell growth was possible. The fact that the greatest cytotoxicity 60mg/l p-OO induced did not correlate with the highest lipoperoxide amounts found, however, indicated that lipoperoxides were only partly responsible for the modulation of cell viability, and alterations in membrane stability were postulated as also being involved.

The finding that total eicosanoid production by control cat lung fibroblasts only accounted for about 10% of all lipid peroxides measured (Tables 4.6.5.1. and 4.6.6.1.) could have related to the poor capability of such cells to supply eicosanoid precursors via desaturation. Thus lung may be reliant on an external supply of eicosanoid precursors. The observation that the prostanoids studied

comprised a fraction of the molar amount quantitated indicated that these cells were largely involved in the production of other eicosanoids, but nevertheless supported functional cyclooxygenase activity in cat lung fibroblasts. Mayeux et al (1989) similarly reported that microsomal fractions isolated from cat lung not only contained cyclooxygenase activity, but also prostacyclin synthetase, thromboxane synthetase and GSH-dependent PGE_2 isomerase activities. Under the experimental conditions employed in the present study, the enzymic pathways for TXB_2 and PGF_{α} synthesis were greatly favoured over those involved in PGI_2 production, and it was possible that PGH_2-PGE_2, PGH_1-PGE_1 and PGH_2-PGD_2, isomerases were suppressed/inhibited rather than absent as limited PGE_2, PGE_1 and PGD_2 formation occurred with p-oil dosage, respectively. Others showed the ability of cat lung to form large quantities of PGE_2, with considerably smaller amounts of PGI_2 and 6-keto-PGE_1 (Ferreira et al 1967 and Forstermann et al 1983). Cat lung, however, has also been implicated in the marked clearance of PGE_2, but not of PGI_2 (Forstermann et al 1983), and this could explain the absence of PGE_2 in control and low levels of such in dosed cells, as well as the significantly larger PGI_2 amounts both in control and dosed cells in the present study (Table 4.6.6.1.).

Supplementation of cat lung fibroblasts with p-oils stimulated total prostanoid production in every instance

by enhancing TXB_2, PGF_α, PGI_2, PGE_2, PGE_1 and/or PGD_2 synthesis (Table 4.6.6.1.). The extent of stimulation was both p-oil and concentration dependent, but did not relate to the degree of p-oil unsaturation (Table 2.3.3.2.) or ability of the p-oils to provide direct eicosanoid precursors. Waring et al (1988) suggested that TXA_2, PGI_2, and vasoconstrictor prostanoids ($PGF_{2\alpha}$, PGD_2 and PGE_2) were formed in response to exogenous 20:4ω6 administration in the cat lung. p-MO contained 20:4ω6 (Table 2.3.3.2.), the direct 2-series eicosanoid precursor, while the 1-series precursor, 20:3ω6, was formed from 18:3ω6 with p-EPO dosage (Table 4.6.3.1.), yet these p-oils induced no significant increase in prostanoid production in relation to other p-oils devoid of such moieties, dosed at identical concentrations. Even p-LO and p-FO yielded prostanoid profiles similar to those derived from cells incubated with corresponding amounts of other p-oils, despite the known inhibitory actions of 18:3ω3 and 20:5ω3 on 20:4ω6 metabolism via cyclooxygenase, and hence 2-series prostanoid production (Lands et al 1971, 1973, Dyerberg et al 1978, Culp et al 1979, Hwang et al 1980, Hamazaki et al 1982, Marshall et al 1982 and Nassar et al 1987). The lack of significant differences in PGI_2, TXB_2, PGF_α, PGE_2, PGE_1 and PGD_2 levels found with dosage of different p-oils at the same concentration implied that exogenous FA's <u>per se</u> induced little effect on the biosynthesis of the eicosanoids

studied, thus enhancement of prostanoid production related to utilisation of membrane 20:3ω6 and 20:4ω6, which were detected in cat lung fibroblasts (Table 4.6.3.1.). However, the finding that total prostanoid production was slightly stimulated with 40mg/l p-oil, but increased about 2 fold with 20 and 60mg/l p-oil, implied p-oil concentration involvement in prostanoid synthesis. This could have influenced eicosanoid precursor availability, or modulated the expression of enzymes involved in the formation of the prostanoids studied, particularly at the PGH level.

The present study could not establish any correlation between the eicosanoid profiles (Table 4.6.6.1.) and cell viability changes (Figs. 4.6.1.2-4.6.1.8.) induced with p-oil dosage. This implied that the endogenously biosynthesised prostanoids studied were not directly involved in the modulation of cell proliferation, despite alterations in overall prostanoid production or the balance between individual prostanoid groups. The involvement of lipoperoxides and alterations in membrane physical properties were proposed as likely mechanisms.

4.7 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM CAT SKIN.

4.7.1 Effects of pseudo-Oils on Cell Viability.

Cells were examined microscopically before, during and after each experiment, and at no time were changes seen in the morphology of control or p-oil dosed cells, except for cytoplasmic droplets observed with p-oil incubation at high concentrations in some instances.

Final medium albumin concentrations ranging from 0 to 250mg/l were shown to have no significant effect on cell viability (Fig. 4.7.1.1.), nor were any synergistic effects induced. The cell viability changes induced with the p-oils were therefore a result of the exogenous FA's alone, and not the albumin used as FA carrier.

The number of control cells, seeded at $10x10^4$/ml, at the end of the 24 hour-trypsinisation recovery period, was $11.7x10^4$/ml. This equated to approximately 63% of the final control cell number at the end of the 48 hour incubation period and represented the cytostatic number. Only p-oil concentrations limiting cell viability to significantly below 63% were thus considered cytotoxic.

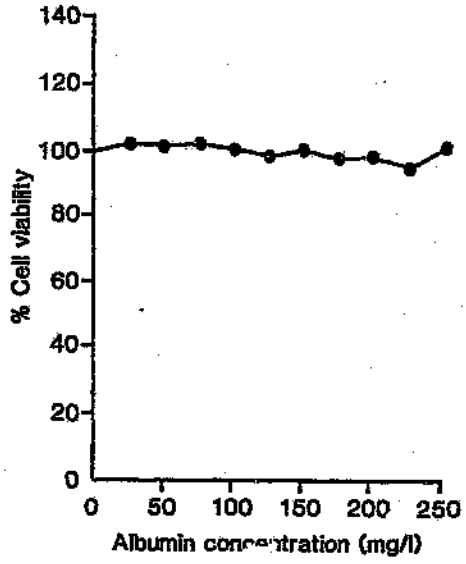
The results of the incubation of cells with p-oils are depicted in Figs. 4.7.1.2-4.7.1.8. Cell proliferation was enhanced with p-MO, p-OO, p-EPO or p-FO dosage, all

Legend to Figs. 4.7.1.1-4.7.1.9.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where 'n' is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 4.7.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.7.1.2-4.7.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 4.7.1.9. shows the mean percent cell viability versus the albumin concentration (mg/l) for cells dosed with the amount of pseudo-oil which limits cell viability to 50% of the controls.

Fig. 4.7.1.1.

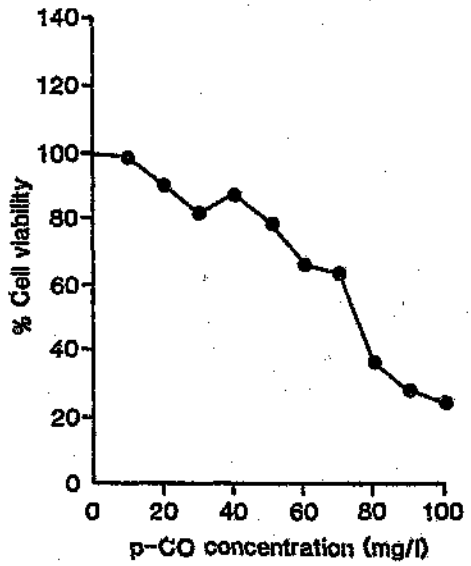
The percentage viability of cells derived from cat skin incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.1	12
25	102.7	5.3	12
50	101.6	5.4	12
75	102.9	3.3	12
100	100.9	7.3	12
125	98.6	2.8	12
150	100.7	5.4	12
175	97.9	5.1	12
200	98.8	4.3	12
225	95.4	4.0	12
250	101.3	2.7	12

Fig. 4.7.1.2.

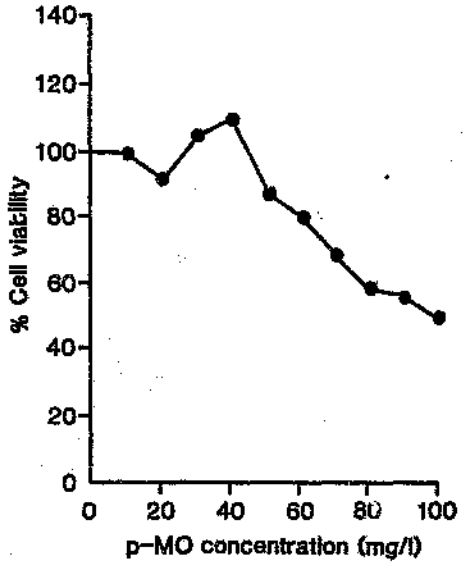
The percentage viability of cells derived from cat skin incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.0	6
10	99.0	2.5	6
20	90.0	2.5	6
30	81.8	2.3	6
40	87.2	1.2	6
50	79.7	2.7	6
60	66.7	2.4	6
70	63.4	2.6	6
80	36.2	2.0	6
90	28.7	2.1	6
100	24.3	1.4	6

Fig. 4.7.1.3.

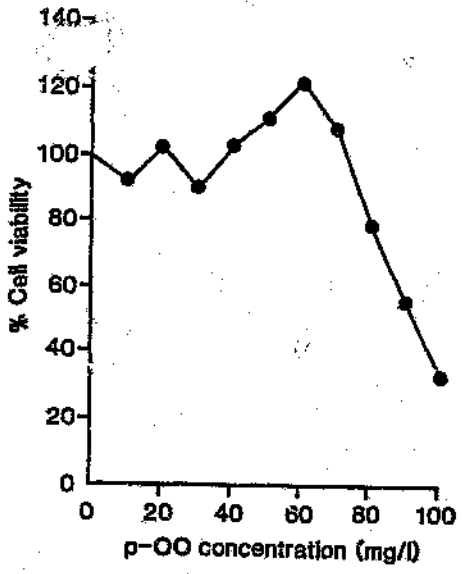
The percentage viability of cells derived from cat skin incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.7	6
10	101.0	3.4	6
20	92.5	5.2	6
30	105.1	4.5	6
40	110.7	4.1	6
50	88.1	4.6	6
60	80.9	1.9	6
70	69.4	2.2	6
80	58.6	3.9	6
90	55.7	2.2	6
100	49.2	2.4	6

Fig. 4.7.1.4.

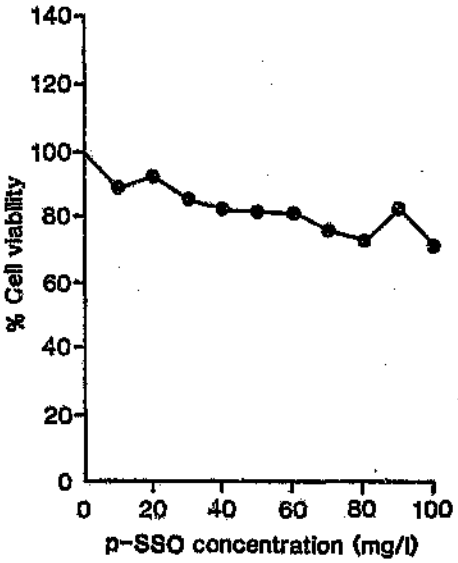
The percentage viability of cells derived from cat skin incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.8	6
10	92.5	5.0	6
20	102.7	5.5	6
30	90.7	4.1	6
40	103.3	3.8	6
50	112.0	1.4	6
60	122.3	6.5	6
70	107.6	4.3	6
80	79.4	4.9	6
90	55.7	5.8	6
100	33.2	5.5	6

Fig. 4.7.1.5.

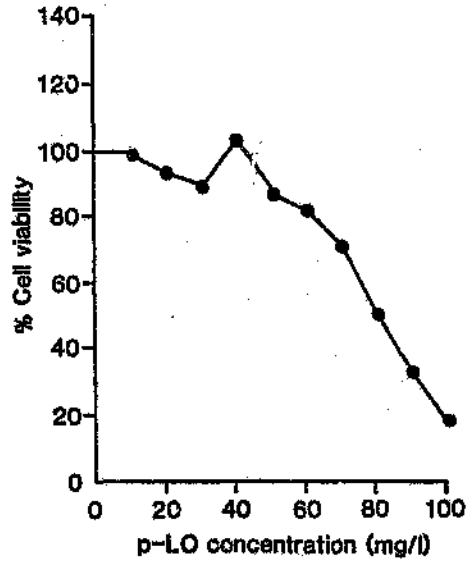
The percentage viability of cells derived from cat skir incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	88.1	1.8	6
20	92.4	2.2	6
30	84.7	3.0	6
40	82.5	2.1	6
50	80.8	2.6	6
60	80.7	3.8	6
70	75.2	2.9	6
80	72.4	2.7	6
90	81.9	2.6	6
100	70.6	5.0	6

Fig. 4.7.1.6.

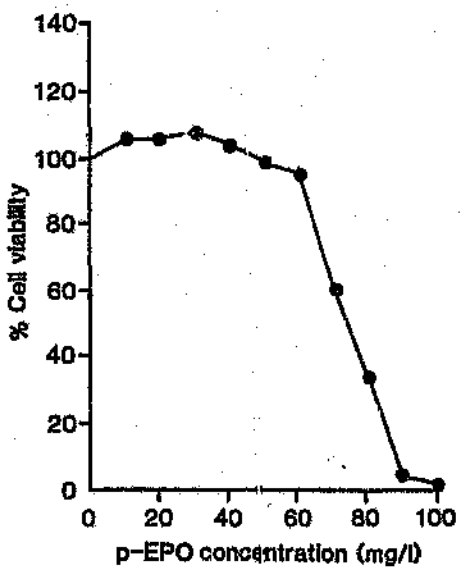
The percentage viability of cells derived from cat skin incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	99.2	4.4	6
20	93.2	3.2	6
30	89.5	5.3	6
40	103.6	4.7	6
50	86.7	4.3	6
60	81.9	2.7	6
70	70.6	5.4	6
80	50.4	3.7	6
90	32.7	3.2	6
100	17.7	3.8	6

Fig. 4.7.1.7.

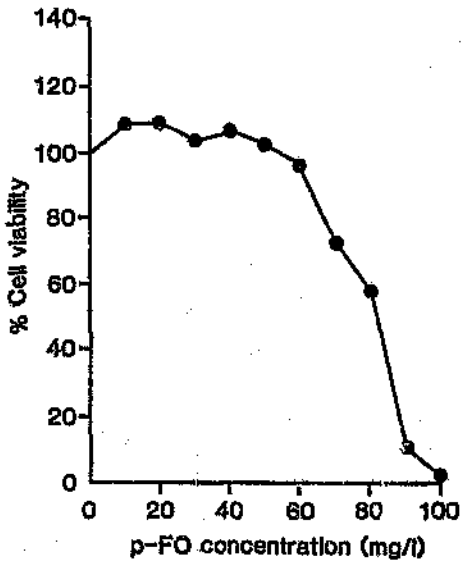
The percentage viability of cells derived from cat skin incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.1	6
10	106.2	2.8	6
20	105.1	1.5	6
30	108.2	2.3	6
40	104.7	3.9	6
50	98.4	4.3	6
60	94.6	3.9	6
70	61.1	2.1	6
80	32.7	3.8	6
90	3.5	1.5	6
100	0.4	0.4	6

Fig. 4.7.1.8.

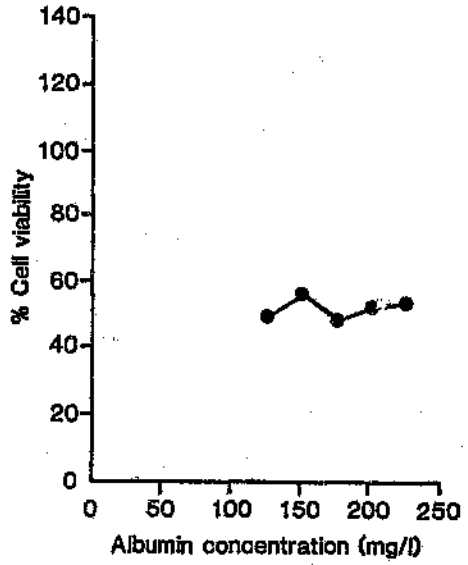
The percentage viability of cells derived from cat skin incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.6	6
10	109.1	2.3	6
20	109.1	3.2	6
30	103.4	2.6	6
40	106.0	4.1	6
50	102.3	3.5	6
60	96.3	3.7	6
70	72.4	3.4	6
80	57.6	3.3	6
90	10.5	1.8	6
100	1.4	0.8	6

Fig. 4.7.1.9.

The percentage viability of cells derived from cat skin incubated with albumin at the ID_{50} of p-EPO.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.8	6
125	49.4	5.2	6
150	56.7	4.4	6
175	49.1	4.2	6
200	52.7	4.3	6
225	54.1	3.3	6

p-oils were cytotoxic at high concentrations in the range dosed, although p-SSO only limited cell growth. The cell viability changes induced varied in magnitude dependent on the p-oil and concentration dosed, but p-OO caused the greatest enhancement of cell proliferation, whereas the overall pattern of growth inhibition was greatest with p-EPO or p-FO dosage.

10mg/l p-CO had little effect on cell proliferation, but higher amounts resulted in an overall concentration dependent reduction in cell viability such that cytostasis was induced with 70mg/l p-CO (Fig. 4.7.1.2.). 10mg/l p-MO similarly had no significant effect on cell growth, but this was enhanced with 30 and 40mg/l p-MO to 105.1% and 110.7%, respectively (Fig. 4.7.1.3.). p-MO increasingly limited the proliferative capability of the cells as greater concentrations were dosed, and induced cytotoxicity with concentrations of 80mg/l, or greater. Cell viability was 49.2% with 100mg/l p-MO, compared to 24.3% with 100mg/l p-CO, and the ID_{50} values supported the greater capability of p-CO to reduce cell viability than p-MO (about 75mg/l and 100mg/l, respectively).

Cell proliferation was not markedly influenced with incubation of up to 40mg/l p-OO, but was enhanced to 112.0%, 122.3% and 107.6% with 50, 60 and 70mg/l p-OO, respectively (Fig. 4.7.1.4.). Cell viability was limited to 79.4% with 80mg/l p-OO, but cytotoxicity reduced cell viability to 50% of controls with 93mg/l p-OO (ID_{50}) and

to 33.2% with 100mg/l p-OO.

Dosage with increasing amounts of p-SSO limited cell growth marginally in an overall concentration dependent fashion to a minimum of 70.6% with 100mg/l p-SSO (Fig. 4.7.1.5.). p-SSO was the only p-oil which did not induce cytotoxicity in these cells, thus no ID_{50} could be calculated.

10mg/l p-LO had little effect on cell proliferation (Fig. 4.7.1.6.), but was progressively growth limiting with 20 to 70mg/l p-LO, although cell growth was enhanced to 103.6% with 40mg/l p-LO. Cytotoxicity was induced with concentrations greater than 70mg/l, which linearly reduced cell viability to 50% with 80mg/l p-LO (ID_{50}) and 17.7% with 100mg/l p-LO.

Dosage with p-EPO or p-FO enhanced cell proliferation slightly with amounts less than 50mg/l, to a maximum of 108.2% with 30mg/l p-EPO, and 109.1% with 10 and 20mg/l p-FO (Figs. 4.7.1.7. and 4.7.1.8., respectively). Cytostasis was induced with 70mg/l p-EPO, and in the range 70 to 80mg/l p-FO, whereas cytotoxicity progressively reduced cell viability with higher concentrations to yield 0.4% and 1.4% viable cells with 100mg/l p-EPO and p-FO, respectively. p-EPO and p-FO thus induced similar cell viability changes, although p-EPO was slightly more growth limiting and cytotoxic, which the ID_{50} values supported (about 74mg/l and 82mg/l, respectively).

To exclude the possibility that any of the effects observed with p-oil dosage were influenced by the amount of albumin bound to the FA's, cells were incubated with a fixed amount of p-oil (74mg/l p-EPO, ID_{50}) and dosed with varying amounts of albumin. Five points were chosen around which to vary the albumin concentration, keeping the p-oil concentration fixed, viz. 2 points above, 2 points below, and the ID_{50} of p-EPO. The appropriate amount of albumin was added to these cultures to give the desired concentrations. The results shown in Fig. 4.7.1.9. indicated that the concentration of p-EPO dosed reduced cell viability to approximately 50% of controls, and that such was not influenced by different albumin concentrations. Thus, the effects induced with the p-oils could be attributed solely to the exogenous FA's, and synergistic effects of albumin could be excluded.

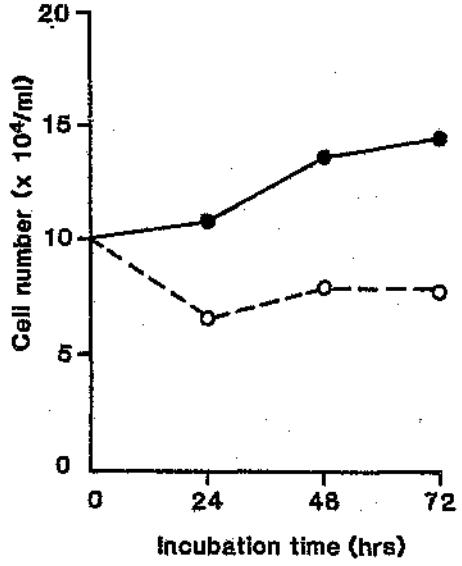
Fig. 4.7.1.10. depicts the growth of cells obtained from cat skin in culture medium supplemented with 10% serum derived from its own species compared to commercially available fetal calf serum (FCS) in order to extablish whether FCS modulated cell growth in any way. The cells grew from 10.0 to 14.5×10^4 over a 72 hour period when culture medium was supplemented with 10% FCS. Plating efficiency was low in the presence of 10% cat serum (CS), however, resulting in an initial decrease in the number of viable cells at 24 hours to 6.6×10^4. Cell growth increased marginally over the next 48 hours, but

Legend to Fig. 4.7.1.10.

The results are expressed as mean cell number±standard error of the mean (s.e.m.), where 'n' is the number of experiments. The cell numbers given are $x10^4$/ml growth medium. Fig. 4.7.1.10. shows mean cell numbers versus the incubation period (hours).

Fig. 4.7.1.10.

The growth of cells derived from cat skin in DMEM containing 10% foetal calf serum compared with 10% adult cat serum.



Incubation Time (hrs)	Mean Cell Number (x10^4/ml)	±s.e.m.	n
Foetal calf serum:			
24	10.8	0.3	9
48	13.6	0.4	9
72	14.5	0.3	9
Adult cat serum:			
24	6.6	0.3	9
48	7.9	0.3	9
72	7.7	0.2	9

only $7.7x10^4$ viable cells were found at the end of the incubation period. The routine use of 10% FCS thus did not limit cell growth compared to 10% CS, and promotion of growth limitation and cytotoxicity were attributed solely to the exogenous FA's incorporated.

Subsequent to these studies, cat skin fibroblasts were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium, in sufficient quantities for all quantitative and qualitative analyses to be carried out. Upon harvesting, cell viabilities were compared and found not to be statistically different from those in Figs. 4.7.1.1-4.7.1.8. All further biochemical assays were thus performed on these samples.

4.7.2 Effects of pseudo-Oils on Total Protein.

Table 4.7.2.1. shows the total amounts of cellular protein quantitated at each of the p-oil concentrations dosed compared to controls.

The lowest protein concentration found in dosed cells (304.3µg/10^6 cells seeded with 60mg/l p-FO) correlated with that of controls (304.5µg/10^6 cells seeded). All other dosed cells contained more protein than controls, although the amounts varied numerically with p-oil and concentration dosed, but was highest with 60mg/l p-OO incubation (467.7µg/10^6 cells seeded). More protein was found in cells supplemented with 40 than 20 or 60mg/l p-MO (345.0 to 420.1µg), p-LO (346.4 to 392.1µg), p-EPO (338.1 to 383.7µg) or p-FO (304.3 to 381.3µg). On the other hand, protein amounts decreased overall with increments in p-CO (347.1 to 318.9µg) or p-SSO (353.7 to 330.9µg) dosage, but the converse occurred with p-OO incubation (326.1 to 467.7µg).

The spent incubation media derived from these cultures were also analysed for total protein. The values for all samples were summed, and a mean protein concentration of 40.0±0.23mg/10^6 cells seeded obtained (n=22). However, statistical comparison of individual samples by analysis of variance showed no significant differences in the spent media protein levels between control and dosed cultures ($F=136.5$, $p=0.001$).

Table 4.7.2.1.

The protein content of cells derived from cat skin, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	304.5			
CO		347.1	347.2	318.9
MO		345.0	420.1	348.2
OO		326.1	357.3	467.7
SSO		353.7	339.3	330.9
LO		354.9	392.1	346.4
EPO		368.1	383.7	338.1
FO		372.7	381.3	304.3

4.7.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Cat Skin.

The FA spectra for both control and cells incubated with 20, 40 or 60mg/l p-oil are shown in Table 4.7.3.1.

The greatest proportion of any single FA in control cells was 21.6% 18:1ω9, compared to 15.6% 16:0, 14.5% 18:0, 8.2% 20:4ω6 and 16.6% 22:4ω6. Other FA's were detected in smaller amounts.

18:0 and 16:1ω9 proportions were generally lower in dosed than in control cells, while 16:0 and 18:1ω9 percentages were more varied. Dosed cells generally contained higher 18:2ω6 levels than controls, but the converse was found for ω6 PDFA's. Similarly, ω3 PUFA percentages were generally lower in dosed than in control cells.

A concentration dependent increase in 16:0 incorporation was shown when cells were incubated with 20, 40 or 60mg/l p-CO (21.2%, 25.2% and 28.8%, respectively vs 15.6% in controls). 18:1ω9 levels increased in a similar manner (23.5%, 25.0% and 28.0%, respectively vs 21.6% in controls), but 18:0 and 16:1ω9 were parallel to controls (14.5% and 4.0%, respectively) or decreased (11.0 to 16.3% and 2.5% to 4.3%, respectively). A small increment in 20:4ω6 was found with 20mg/l p-CO incubation (9.1% vs 8.2% in controls), whereas 20:5ω3 was significantly, and

Legend to Tables 4.7.3.1. and 4.7.3.2.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where 'n' is the number of experiments. '[p-Oil]' refers to the pseudo-oil concentration used.

Table 4.7.3.1.

The fatty acid spectrum of cells derived from cat skin.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	15.6±0.05	21.2	25.2	28.8	18.8	19.2	21.5	14.1	13.3	14.4	13.3	12.2	13.2	12.9	12.5	15.8	11.6	12.3	9.6	17.1	15.7	34.6
	18:0	14.5±0.07	16.3	11.9	11.0	13.1	12.1	11.1	10.0	5.3	4.4	11.0	8.6	8.1	11.5	8.5	9.4	22.6	8.8	13.7	11.7	7.7	9.7
	20:0	0.2±0.00	-	0.1	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:0	0.2±0.00	0.1	0.1	0.2	0.1	0.2	0.1	-	-	-	-	0.1	0.1	-	-	0.1	-	-	0.6	-	0.1	-
	24:0	0.1±0.00	0.1	-	-	-	3.0	-	-	-	-	-	0.2	-	0.1	0.1	0.1	0.1	0.3	-	-	-	-
ω9 MONOS.	16:1	4.0±0.25	2.5	4.3	3.3	1.6	2.1	2.2	1.8	2.2	1.9	2.2	1.8	2.6	2.3	1.4	1.0	1.0	1.6	0.6	12.2	18.9	16.6
	18:1	21.6±0.05	23.5	25.0	28.0	22.1	23.1	26.9	43.6	50.7	56.0	20.8	19.5	21.3	18.6	17.4	17.5	18.8	14.2	15.8	22.0	19.6	17.8
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	3.9±0.00	3.7	2.9	2.8	6.4	8.9	11.7	7.7	10.0	11.0	25.0	32.3	40.7	8.9	11.9	11.2	23.9	38.9	39.2	6.4	7.5	4.8
	18:3	-	-	-	-	-	0.2	0.2	-	0.1	0.1	0.1	0.1	0.1	-	0.1	0.1	0.4	1.5	3.5	-	-	-
	20:2	0.2±0.00	0.1	-	-	0.3	0.3	0.2	3.1	1.8	2.0	1.4	1.4	1.0	0.2	0.3	0.1	1.1	1.2	1.4	0.3	0.6	0.6
	20:3	3.6±0.07	3.0	2.4	1.9	2.9	2.7	2.3	1.9	1.5	1.0	2.8	2.2	1.6	2.0	1.5	1.1	4.6	5.4	4.9	2.1	1.5	0.7
	20:4	8.2±0.05	9.1	6.8	5.6	9.3	9.6	9.2	4.6	3.1	2.2	5.1	3.3	2.7	6.6	5.6	3.3	4.2	3.5	2.2	5.4	3.3	1.1
	22:4	16.6±0.55	10.0	8.1	7.8	14.9	9.3	8.1	7.0	6.9	3.9	12.3	11.9	4.8	14.7	4.5	7.3	6.2	7.0	3.7	8.9	6.6	8.2
	22:5	0.6±0.00	0.3	0.5	0.1	0.1	0.5	0.2	0.3	0.2	-	0.1	0.6	0.1	0.1	0.3	0.1	0.6	0.4	0.2	0.2	0.3	0.5
ω3 POLYS.	18:3	0.7±0.03	0.7	0.7	0.6	1.0	0.7	0.5	0.7	0.7	0.5	0.5	0.4	0.3	14.8	29.4	29.4	0.3	0.3	1.0	0.7	0.6	0.4
	18:4	1.3±0.00	0.7	0.8	0.6	0.4	0.6	0.3	-	0.2	-	0.2	0.3	0.1	0.3	0.5	0.2	0.5	0.4	0.7	0.3	0.4	0.2
	20:4	2.3±0.10	0.4	1.5	0.9	1.5	1.9	0.7	0.6	1.0	0.2	0.9	1.7	0.3	1.8	1.4	0.6	1.2	1.0	0.8	1.3	1.0	1.0
	20:5	0.7±0.05	1.7	3.8	1.2	1.8	0.7	0.5	0.8	0.4	0.5	0.3	0.8	0.6	1.1	1.4	0.7	0.2	0.6	0.2	4.7	10.0	2.6
	22:5	3.4±0.03	3.5	2.6	2.3	3.1	2.4	2.1	1.9	1.2	0.9	2.4	1.4	1.2	2.3	1.6	1.1	1.5	1.4	0.9	5.3	4.6	1.1
	22:6	2.3±0.00	3.1	3.4	3.0	2.7	2.6	2.5	1.9	1.5	1.1	1.8	1.5	1.3	1.8	1.8	1.1	1.4	1.5	1.0	1.6	1.5	0.4

22:6ω3 slightly, increased with p-CO supplementation (1.2% to 3.8% and 3.1% to 3.4% vs 0.7% and 2.3% in controls, respectively). Percentages for other PUFA's, however, were parallel to controls or decreased.

Cells supplemented with 20, 40 or 60mg/l p-MO exhibited slightly increased 16:0 and 18:1ω9 levels (18.8%, 19.2% and 21.5%, and 22.1%, 23.1% and 26.9%, vs 15.6% and 21.6% in controls, respectively), 24:0 was significantly increased with 40mg/l p-MO (3.0% vs 0.1% in controls), whereas 18:0 and 16:1ω9 levels were lower than controls (14.5% and 4.0%, respectively). 18:2ω6 was significantly and 20:4ω6 slightly increased with p-MO dosage (6.4% to 11.7% and 9.2% to 9.6% vs 3.9% and 8.2% in controls, respectively), but no significant increments in other ω6 PDFA's were found. 20:5ω3 increased approximately 2 fold with 20mg/l p-MO incubation (1.8% vs 0.7% in controls), and 22:6ω3 was slightly increased across the range dosed (2.5% to 2.7% vs 2.3% in controls), but other ω3 PUFA levels approximated to controls or were decreased.

18:1ω9 levels increased 2 to 3 fold when cells were fed with 20, 40 or 60mg/l p-OO (43.6%, 50.7% and 56.0%, respectively vs 21.6% in controls), whereas 16:0, 18:0 and 16:1ω9 were decreased elative to controls (15.6%, 14.5% and 4.0%, respectively). 18:2ω6 increased over the range dosed (7.7% to 11.0% vs 3.9% in controls), as did 20:2ω6 (3.1%, 1.8% and 2.0%, respectively vs 0.2% in controls), but ω6 and ω3 PDFA levels were parallel to

controls or decreased.

p-SSO supplementation had little effect on 18:1ω9, but decreased 16:0, 16:1ω9 and 18:0, percentages compared to controls (21.6%, 15.6%, 4.0% and 14.5%, respectively). 18:2ω6 increased markedly and 20:2ω6 significantly (25.0%, 32.3% and 40.7%, and 1.4%, 1.4% and 1.0% with 20, 40 and 60mg/l p-SSO vs 3.9% and 0.2% in controls, respectively), but no increments in any other ω6 or ω3 series PUFA's were induced.

16:0, 16:1ω9, 18:0 and 18:1ω9 levels were parallel to, or lower than, controls (15.6%, 4.0%, 14.5% and 21.6%, respectively) with 20, 40 or 60mg/l p-LO incubation, but 18:2ω6 and 18:3ω3 were increased (8.9%, 11.9% and 11.2%, 14.8%, 29.4% and 29.2% vs 3.9% and 0.7% in controls, respectively). However, the only significant increment in PDFA levels was detected for 20:5ω3 with 40mg/l p-LO (1.4% vs 0.7% in controls).

p-EPO incubation caused 16:0, 18:0, 16:1ω9 and 18:1ω9 levels to decrease, although 18:0 was increased with a concentration of 20mg/l (22.6% vs 14.5% in controls). p-EPO induced a concentration dependent increase in 18:2ω6 uptake (23.9% with 20mg/l, 38.9 with 40mg/l, and 39.2% with 60mg/l vs 3.9% in controls), and the levels of both 20:2ω6 and 18:3ω6 were raised (1.1% to 1.4% and 0.4% to 3.5% vs 0.2% and 0% in controls, respectively). An increment in 20:3ω6 was also induced (4.6%, 5.4% and

4.9% with 20, 40 or 60mg/l p-EPO, respectively vs 3.6% in controls), but 20:4ω6 levels decreased 2 to 4 fold compared to controls (8.2%), and no increments in 22:4ω6 or 22:5ω6 were found. Furthermore, all ω3 series PDFA's detected were lower in dosed cells than in controls.

Supplementation with 20, 40 or 60mg/l p-FO induced an increase in 16:1ω9 (12.2%, 18.9% and 16.6%, respectively vs 4.0% in controls), decreased 18:0 levels, but had little effect on 18:1ω9 and 16:0, although the latter increased about 2 fold with 60mg/l p-FO (34.6% vs 15.6% in controls). ω6 series PDFA's were not significantly raised, despite more 18:2ω6 in dosed than control cells (4.8% to 7.5% vs 3.9%, respectively). Raised amounts of 20:5ω3 were detected (4.7%, 10.0% and 2.6% with 20, 40 and 60mg/l p-FO, respectively vs 0.7% in controls), and 22:5ω3 was increased with 20 and 40mg/l p-FO (5.3% and 4.6%, respectively vs 3.4% in controls). However, no evidence to suggest increased 22:6ω3 was found.

The FA spectra of the spent growth medium removed from each of the above cultures is shown in Table 4.7.3.2. The medium in which control cells had been grown contained 97.4% 14:0, 1.4% 17:0 and less than 0.5% each of 16:0, 16:1ω9, 18:0, 18:1ω9 and 22:4ω6. Spent media obtained from cultures dosed with p-oils similarly contained at least 96% 14:0 and 1.0% to 2.0% 17:0, but only trace amounts of the major FA components present in

Table 4.7.3.2.

The fatty acid spectrum of the spent culture medium of cells derived from cat skin.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	12:0	-	0.2	0.2	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14:0	97.4±0.34	96.4	97.9	98.2	96.1	96.4	98.2	97.8	97.8	97.7	97.7	97.9	97.7	97.0	97.3	97.2	97.5	97.1	97.6	97.7	97.6	97.4
	16:0	0.3±0.07	0.8	0.1	0.1	0.9	1.3	0.1	0.2	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.1	0.1	0.1	0.1
	17:0	1.4±0.07	1.5	1.3	1.0	1.9	1.6	1.1	1.2	1.2	1.2	1.3	1.1	1.3	2.0	1.6	1.5	1.5	1.5	1.5	1.5	1.6	1.4
	18:0	0.1±0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
MONO.	16:1	0.1±0.05	0.3	-	-	0.3	-	-	-	-	-	-	-	-	-	-	-	-	0.3	-	0.1	0.2	0.3
	18:1	0.3±0.03	0.3	0.2	0.1	0.4	0.4	0.2	0.4	0.5	0.7	0.2	0.3	0.3	0.3	0.3	0.5	0.2	0.3	0.3	0.2	0.2	0.3
ω6 POLYS.	18:2	-	-	-	-	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.4	0.1	0.1	0.1	0.2	0.3	0.3	0.1	0.1	0.1
	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:4	0.3±0.03	0.3	0.3	0.2	0.2	0.2	0.2	0.3	0.2	0.1	0.3	0.3	0.2	0.2	0.2	0.1	0.3	0.2	0.2	0.3	0.2	0.1
	22:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω3 POLYS.	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.3	0.4	-	-	-	-	-	-
	18:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1
	22:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

the p-oils remained in the medium following the 48 hour incubation period.

4.7.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Cat Skin.

The radioactivity recovered from cultures incubated with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 is shown in Table 4.7.4.1.

For both [^{14}C]-18:1ω9 and [^{14}C]-18:3ω3, the equivalent of 4.3x10^{6}cpm were dosed to the cultures, and total recovery approximated to 81% and 95%, respectively. For [^{14}C]-18:2ω6 the equivalent of 4.8x10^{6}cpm were dosed to the growth medium, and the total recovery of this radiolabel after 48 hours incubation amounted 94%. The counts recovered from the spent incubation media ranged from 1.8 to 2.8x10^{6}cpm, whereas only 0.3 to 0.5x10^{6}cpm were recovered from the pooled buffers after washing the cells, which accounted for non-specific binding. The amount of each of these radioisotopes incorporated into the cells themselves equated to 31% for both [^{14}C]-18:1ω9 and [^{14}C]-18:2ω6, and 23% for [^{14}C]-18:3ω3.

The percentage conversion of incorporated [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 to ω9, ω6 and ω3 series products, respectively after 48 hours incubation with cat skin fibroblasts are shown in Table 4.7.4.2. As Δ6D represents the first and rate limiting enzyme in the desaturase cascade, total activity of this enzyme is reflected by the sum of all subsequent desaturase and elongase products.

Table 4.7.4.1.

The radioactivity (cpm) recovered from cat skin cultures incubated with radiolabelled C18 fatty acids, expressed as mean (±s.e.m.), n=3.

SPENT MEDIA (M)	POOLED WASHINGS (W)	TOTAL (M+W)	CELLS	TOTAL $\times 10^6$
Total 18:1 counts dosed to incubation medium = 4.3×10^6				
1745160 (±3400)	391380 (±3660)	2136540	1343979 (±1962)	3.5
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
2540660 (±10260)	491460 (±8760)	3032120	1463025 (±4281)	4.5
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
2826600 (±15300)	296880 (±4800)	3123480	969108 (±573)	4.1

Table 4.7.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from cat skin, expressed as a percentage of the total counts recovered upon GLC analysis.

ω9 MONOS.	[^{14}C]-18:1ω9	ω6 POLYS.	[^{14}C]-18:2ω6	ω3 POLYS.	[^{14}C]-18:3ω3
18:1	29.9	18:2	31.0	18:3	32.0
20:1	37.3	18:3	7.7	18:4	6.4
22:1	20.5	20:2	23.2	20:3	22.5
24:1	12.4	20:3	15.9	20:4	21.4
		20:4	8.5	20:5	7.3
		22:4	8.4	22:5	6.2
		22:5	5.2	22:6	4.3
TOTAL CPM	2808	TOTAL CPM	3524	TOTAL CPM	1639

29.9% of the total [^{14}C]-18:1ω9 counts derived upon GLC analysis (2808cpm) remained unchanged, while the amounts detected for the subsequent elongation products were 37.3% 20:1ω9, 20.5% 22:1ω9 and 12.4% 24:1ω9.

31.0% of the total [^{14}C]-18:2ω6-derived counts detected following GLC (3524cpm) remained unaltered, whereas 23.2% was found as 20:2ω6, but only 7.7% as 18:3ω6, 15.9% 20:3ω6 was detected, but 20:4ω6, 22:4ω6 and 22:5ω6 only comprised 8.5%, 8.4% and 5.2% of the total counts, respectively.

1639cpm were derived upon GLC analysis of cells dosed with [^{14}C]-18:3ω3, of which 32.0% remained as 18:3ω3, 22.5% was found as 20:3ω3, but only 6.4% as 18:4ω3. These amounts were similar to those detected for the corresponding products of [^{14}C]-18:2ω6 metabolism. 21.4% of the total [^{14}C]-18:3ω3-derived counts were present as 20:4ω3, whereas the percentages for 20:5ω3, 22:5ω3 and 22:6ω3 were 7.3%, 6.2% and 4 3%, respectively. These post-Δ5D FA proportions were slightly lower than those detected for equivalent products in the ω6 PUFA series.

4.7.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 4.7.5.1. shows the lipoperoxides quantified both in cultured cells and their respective growth media in the presence and absence of dosed p-oils. The values are shown as nmoles MDA/10^6 cells, but in the case of the spent media, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained.

MDA levels varied between dosed cells with p-oil, ranging from 0.1 to 5.8nmoles MDA/10^6 cells, compared to 0.4nmoles MDA/10^6 control cells. Lipoperoxide amounts were parallel to, or lower than, controls when cells were supplemented with 20mg/l p-oil, but increased in an overall concentration dependent manner as higher p-oil amounts were dosed. Lipoperoxide production was low with p-CO, p-MO or p-OO incubation (0.1 to 0.5nmoles MDA/10^6 cells). p-EPO and p-SSO induced similar amounts of lipid peroxides (0.3, 0.6 and 0.6 vs 0.4, 0.6 and 0.9nmoles MDA/10^6 cells with 20, 40 and 60mg/l respectively), and p-FO generated significantly greater amounts only with a concentration of 60mg/l (4.9nmoles MDA/10^6 cells). p-LO, however, induced the highest overall levels (0.3, 1.6 and 5.8nmoles MDA/10^6 cells with 20, 40 and 60mg/l, respectively).

The spent medium derived from cultures incubated with 60mg/l p-LO or p-FO contained small lipid peroxide

Table 4.7.5.1.

Lipoperoxide formation by cat skin cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.4			
CO		0.1	0.3	0.5
MO		0.3	0.1	0.5
OO		0.3	0.4	0.5
SSO		0.3	0.6	0.6
LO		0.3	1.6	5.8
EPO		0.4	0.6	0.9
FO		0.2	0.7	4.9
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		-	-	-
LO		-	-	0.1
EPO		-	-	-
FO		-	-	0.3

amounts (0.1 and 0.3nmoles MDA/10^6 cells, respectively), but these compounds were absent from the spent media of all other dosed cultures, as well as control medium.

4.7.6 The Eicosanoid Profile of Cells Derived from Cat Skin.

Table 4.7.6.1. shows the eicosanoids positively detected in control and dosed cat skin fibroblasts. All data is presented as a percentage of the total area quantitated, but an indication of the actual amount found is given for controls in "( )" as pmoles/10^6 control cells.

Total eicosanoid synthesis by control cells amounted to only 80.5pmoles/10^6 cells, of which 21.0% correlated with the retention times of the prostanoid standards used. This fraction was composed of 10.7% TXB_2, 6.9% PGF_α, 1.8% PGI2 and 1.6% PGE2. Varying proportions of these prostanoids were found in dosed cells, but neither control nor dosed cells formed significant amounts of PGE_1 or PGD2.

Total percentages for the prostanoids detected in dosed cells varied significantly with p-oil concentration as such approximated to controls (21.0%) with 40mg/l p-oil (15.5% to 24.9%), but were greatly increased both with 20mg/l (34.2% to 50.1%) and 60mg/l (32.9% to 41.8%) p-oil. These changes were reflected by appropriate alterations in individual prostanoid levels. PGI_2 biosynthesis was increased with incubation of 40mg/l p-oil (3.0% to 9.5% vs 1.8% in controls), but more varied with 20mg/l (0.8% to 6.0%) and 60mg/l (0.9% to 3.1%) p-oil. TXB_2 levels approximated to controls (10.7%) or were

Legend to Table 4.7.6.1.

Values are tabulated as relative percent of the total area detected, and as pmoles total eicosanoids/10^6 control cells.

Table 4.7.6.1.

The profile of the eicosanoids detected in extracts of cells derived from cat skin.

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	$PGF_{2\alpha}$ (1 ?)	PGE_2	PGE_1	PGD_2
CONTROL (80.5)		21.0	1.8	10.7	.9	1.6	-	-
CO	20	40.5	1.9	21.8	15.2	1.6	-	-
	40	24.9	4.4	11.5	9.0	-	-	-
	60	38.3	1.9	23.0	12.8	0.6	-	-
MO	20	50.1	2.9	24.1	21.3	1.8	-	-
	40	22.0	3.0	8.7	)	-	-	-
	60	41.8	2.5	24.1	.	0.3	-	-
OO	20	37.2	2.6	19.8	13.3	1.5	-	-
	40	23.7	7.9	8.5	7.3	-	-	-
	60	38.7	3.1	24.3	10.2	0.8	0.3	-
SSO	20	39.7	6.0	18.3	13.8	1.6	-	-
	40	20.0	9.5	5.8	4.7	-	-	-
	60	34.2	1.3	19.3	13.2	0.1	0.3	-
LO	20	38.8	2.9	21.2	13.1	1.6	-	-
	40	15.5	5.0	7.6	2.9	-	-	-
	60	38.9	1.0	24.5	12.9	0.5	-	-
EPO	20	34.2	0.8	17.2	14.5	1.7	-	-
	40	23.5	6.2	9.3	8.0	-	-	-
	60	32.9	1.5	17.2	13.9	0.3	-	-
FO	20	42.4	3.0	23.1	14.8	1.5	-	-
	40	16.6	4.7	8.9	3.0	-	-	-
	60	34.7	0.9	23.9	9.0	0.9	-	-

decreased with dosage of 40mg/l p-oil (5.8% to 11.5%), but increased approximately 2 fold with 20 or 60mg/l p-oil (17.2% to 24.5%). Similarly, the changes in PGF_{α} production were smallest with incubation of 40mg/l p-oil (2.9% to 10.3% vs 6.9% in controls), but were greater with 20mg/l (13.1% to 21.3%) and 60mg/l (9.0% to 14.9%) p-oil. PGE_2 production approximated to that of controls (1.6%) when cells were supplemented with 20mg/l p-oil (1.5% to 1.8%), but was suppressed in the presence of 60mg/l p-oil (0.1% to 0.9%) and inhibited with 40mg/l p-oil.

4.7.7 Discussion.

p-Oil supplementation of cultured cat skin fibroblasts induced effects ranging from stimulation to inhibition of cell proliferation, and the different cell viability changes reported (Figs. 4.7.1.2-4.7.1.8.) reflected the unique FA composition of each p-oil (Table 2.3.3.2.) and the concentration dosed. The pronounced differences in cell viability induced with p-OO, p-SSO or p-LO dosage probably related to the abundance of OA, LA and ALA in these p-oils, respectively. It was apparent that OA exhibited th potential to stimulate cell proliferation when dosed at intermediate concentrations in the range used, unlike LA and ALA, whereas the cytotoxic potential of ALA was greater than OA, followed by LA when these p-oils were dosed at high concentrations. The marked variations in cell viability induced with p-EPO or p-SSO dosage, despite similar FA compositions, could have related to the presence of GLA in p-EPO only. This FA appeared to stimulate cell growth with low, and inhibit such with high, concentrations of p-EPO dosed, compared only to growth limitation induced by LA with p-SSO feeding. It was also apparent that GLA (in p-EPO) was more effective in mediating cytotoxicity than ALA (in p-LO) at high concentrations, and such may relate to the fact that GLA is a PDFA. Indeed, PDFA involvement in the modulation of cytotoxicity seemed likely as dosage with high concentrations of p-EPO or p-FO, which

contained significant PDFA amounts, were most effective as cytotoxic agents, while their absence led to less effective cell killing. However, the enhancement of cell viability reported with supplementation of p-MO, p-OO, p-EPO or p-FO, but not with p-CO, p-SSO or p-LO, correlated with the presence of EFA desaturation and/or elongation products in the former p-oils only, and also suggested involvement of such FA's in the stimulation of cell growth with low and/or intermediate concentrations of p-oils. Dosage with p-OO or p-MO, each containing about 2% 20:2ω6 or 20:4ω6, respectively, enhanced cell viability to a greater extent than did p-EPO or p-FO, containing 9% 18:3ω6 and 18% 20:5ω3, respectively. Small amounts of exogenous EFA desaturation and/or elongation products thus seemed more effective in the stimulation of cell proliferation.

The different cell viability changes which the p-oil FA's appeared to induce could have related to structural variations between different moieties. It should be kept in mind, however, that synergistic or antagonistic interactions between different p-oil FA's could also have played a role in the modulation of cell viability. This may explain why cell viability changes did not always correlate linearly with the amount of p-oil supplemented.

The finding that only trace amounts of the major FA

components present in the p-oils remained in the spent medium following the 48 hour incubation period (Table 4.7.3.2.) indicated the capability of these cells to incorporate exogenous FA's, even in the presence of high 14:0 levels derived from the culture medium itself (Table 2.3.3.3.). The FA spectra of dosed cells (Table 4.7.3.1.) supported p-oil FA uptake from the culture medium. A mechanism whereby p-oils affected cell growth may therefore relate to differential exogenous FA uptake and alterations in membrane stability, dependent on p-oil FA composition and concentration dosed. Pronounced distortions of membrane fluidity would indeed support the limitation and inhibition of cell proliferation found when large quantities of saturated or unsaturated FA-rich p-oils were fed (Figs. 4.7.1.2-4.7.1.8.).

p-Oil supplementation of cat skin fibroblasts induced concentration changes in total cellular protein (Table 4.7.2.1.) which generally reflected the cell viability changes induced (Figs. 4.7.1.2-4.7.1.8.). The largest amount of total protein quantitated indeed corresponded to the greatest stimulation of cell viability found with 60mg/l p-OO dosage. However, the finding that protein increased about 50% compared to a 22% increment in cell viability indicated that such protein changes did not relate solely to increased cell numbers, particularly since increased protein was found in cells dosed with p-oils that caused growth limitation, eg. p-CO or p-SSO.

This indicated p-oil FA involvement in the stimulation of protein synthesis, which may reflect enhanced enzyme expression to metabolise the incorporated p-oil FA's. The fact that the extent of protein stimulation varied when supplementation with identical concentrations of different p-oils yielded similar cell numbers suggested that the capability to modulate protein biosynthesis related to differences in FA structure. Whether this related to specific FA's in any p-oil, or to synergistic effects between p-oil FA's, was unknown, although a combination of such effects seemed most likely. However, as the protein concentrations in spent media derived from dosed cultures were not statistically different from controls (section 4.7.2), medium protein itself probably did not contribute significantly to the changes demonstrated in cellular protein (Table 4.7.2.1.).

The literature does not describe the desaturation capability of cat skin either <u>in vivo</u> or <u>in vitro</u>. The results obtained from the incubation of cat skin fibroblasts with p-oils showed that such cells exhibited very little potential to desaturate or elongate FA's, despite significant p-oil FA uptake (Tables 4.7.3.1. and 4.7.3.2.). No significant 18:4ω3 or 18:3ω6 formation occurred, even with p-LO or p-SSO supplementation, respectively, implying impaired Δ6D capability. This was consistent with reports which have indicated an overall lack or suppression of Δ6D capability in the cat (eg.

Rivers et al 1975a, Frankel et al 1978, Sinclair 1979, Sinclair et al 1981 and Davidson et al 1990a). Thus 20:3ω6 formation only with p-EPO dosage more than likely related to elongation of incorporated 18:3ω6, while the presence of small 20:2ω6 amounts with p-SSO or p-EPO dosage reflected limited elongation of incorporated 18:2ω6 which could not be further desaturated. However, no evidence for 20:3ω6 desaturation via Δ5D was shown. Limited elongation of 20:5ω3 incorporated with p-FO incubation occurred, but this was not converted to 22:6ω3 via Δ4D. On the other hand, p-( ) dosage induced significantly increased cellular 20:5ω3 and 22:6ω3 levels. This p-oil was PUFA-deficient (Table 2.3.3.2.), thus it was possible that such enhanced desaturation and/or elongation of cellular PDFA's in an attempt to maintain membrane fluidity; this indeed correlated with decreased cellular 18:4ω3 and 20:4ω3 levels.

The overall lack or suppression of desaturase cascade enzyme capability in these cells (Table 4.7.3.1.) did not relate to the mediation of growth limitation induced with p-oil supplementation (Figs. 4.7.1.2-4.7.1.8.) as enzyme capability was not improved even when p-oils stimulated cell proliferation. Impairment of enzyme expression may rather be a characteristic of cat skin, implying a requirement for preformed PDFA's from the diet or other cat tissues, particularly for eicosanoid production.

p-Oil desaturation was compared with that of individual C18 FA's (Table 4.7.4.2.) to assess whether FA mixtures modulated the pattern of p-oil desaturation reported (Table 4.7.3.1.). Cat skin fibroblasts were unable to efficiently desaturate 18:1ω9 when such was supplemented with p-oils or individually. Elongation was induced with [^{14}C]-18:1ω9 feeding, however, and such was possibly suppressed with p-oil, or enhanced with [^{14}C]-18:1ω9, supplementation. On the other hand, the pattern of radioactivity derived from the incubation of cells with [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 was consistent with both desaturation and elongation of these moieties. However, PDFA formation from [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 via an active Δ6D was unlikely since expression of this enzyme is impaired in the cat (eg. Rivers et al 1975a and Frankel et al 1978). The data rather suggested that [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 were directly elongated to 20:2ω6 and 20:3ω3, followed by Δ8-desaturation to 20:3ω6 and 20:4ω3, respectively. The small 18:3ω6 and 18:4ω3 amounts detected may therefore reflect 20:3ω6 and 20:4ω3 retroconversion. The formation of about 20% post-Δ5D PUFA's nevertheless supported the capability for Δ5D expression. However, the apparent accumulation of 20:3ω6 and 20:4ω3 may have been an indication that the rate of their formation was greater than the rate of their conversion to post-Δ5D products and/or that Δ5D activity was not fully expressed. This could account for the

small amounts of radioactivity detected for 22:5ω6 and 22:6ω3, although it was also apparent that these cells exhibited very limited ΔD capability.

The findings above indicated that desaturase cascade enzyme expression was more limited in the presence of p-oils than individual FA's (Tables 4.7.3.1. and 4.7.4.2). This could have related to the mediation of competitive interactions between p-oil FA's for the desaturase and elongase enzymes by complex mechanisms, which are documented (Brenner et al 1966, Mohrhauer et al 1967, Ullman et al 1971a, de Schriver et al 1982, Holman 1986a, Nassar 1986 and Cook et al 1987). Similar effects probably occur <u>in vivo</u> when dietary oils are fed, thus p-oil desaturation may be a better indicator of such enzyme capability in cat skin <u>in vivo</u> than that suggested with individual FA's, particularly since FA's are presented to the organism as mixtures.

Cellular MDA detection both in the presence and absence of dosed p-oils (Table 4.7.5.1.) indicated that cat skin fibroblasts exhibit enzymic and/or non-enzymic pathways for the oxidation of monoenoic and/or polyenoic FA's. MDA detection in control cells was not surprising, since these cells contained FA's which were potential candidates to act as substrates for the formation of lipoperoxides (Table 4.7.3.1.). Measurement of small, or no, increments in MDA concentration with p-CO or p-MO dosage reflected the abundance of saturated FA's in

these p-oils (Table 2.3.3.2.). However, the formation of relatively small cellular lipoperoxide amounts also with p-OO, p-SSO or p-EPO dosage implied that the substrates for lipoperoxidation were not so readily accessible to oxidation, possibly because they were present as components of complex membrane lipids, eg. PGL's. This could also explain the lower MDA levels measured in some dosed cells than in controls (Table 4.7.5.1.), despite the presence of unsaturated FA's in the p-oils. It was also possible that incubation with p-OO, p-SSO or p-EPO decreased the rate of PGL turnover and therefore the size of the FFA pool. The converse, however, could explain the highest cellular lipoperoxide concentrations induced with 40 or 60mg/l p-LO or p-FO supplementation, which probably related to the amount of ALA and EPA in these p-oils, respectively (Table 2.3.3.2.), as well as their susceptibility to oxidation.

The absence of significant lipoperoxide amounts in the spent media derived from dosed cultures (Table 4.7.5.1.) supported cellular uptake of supplemented p-oil FA's (Tables 4.7.3.1. and 4.7.3.2.). This was also consistent with the lack of cytotoxicity found with 20, 40 or 60mg/l p-oil dosage, and therefore retension of cellular FA's, lipoperoxides and peroxidising enzymes.

Supplementation of cat skin fibroblasts with 20, 40 or 60mg/l p-oil stimulated and/or limited, but never

inhibited, cell viability (Figs. 4.7.1.2-4.7.1.8.), thus no correlation between lipid peroxide formation and cytotoxicity could be established. These compounds should, however, be considered as a possible mechanism involved in the modulation of cell death with higher p-oil concentrations. On the other hand, comparison of MDA amounts with the growth limitation induced with 20, 40 and/or 60mg/l p-oil dosage indicated that lipid peroxides were not solely involved in the modulation of growth l itation. It was likely, therefore, that other mechanisms, such alterations in membrane fluidity, were also involved.

The finding that total eicosanoid formation accounted for 20% of the lipoperoxides quantified in control cat skin fibroblasts (Tables 4.7.5.1. and 4.7.6.1.) reflected the poor capability of such cells to form eicosanoid precursors via desaturation (Table 4.7.3.1). It seemed likely therefore that skin was reliant on an external supply of eicosanoid precursors. The fact that the eicosanoids studied comprised a fraction of the molar amount quantified indicated that these cells were largely involved in the production of other eicosanoids. Prostanoid detection nevertheless supported functional cyclooxygenase expression in cat skin fibroblasts under the experimental conditions employed, although it was apparent that PGH_1-PGE_1 and PGH2-PGD2 isomerases were suppressed/inhibited since no significant PGE_1 or PGD2

formation was shown, even with p-oil supplementation. Alternatively, it was possible that these PG's were degraded at a faster rate than other prostanoids by cytoplasmic enzymes such as 15-hydroxy PG dehydrogenase. On the other hand, the finding that the rate of TXB_2 and PGF_{α} biosynthesis were considerably greater than that of PGE_2 and PGI_2 could have been the result of increases in the activity/expression of enzymes forming TXB_2 and PGF_{α} and/or relative decreases in PGH_2-PGE_2 isomerase and prostacyclin synthetase.

The eicosanoid spectra obtained from dosed cells (Table 4.7.6.1.) were consistent with the ability of the p-oils to modulate prostanoid production. This, however, did not correlate in any way with the degree of p-oil unsaturation or the ability of the p-oils to provide direct eicosanoid precursors (Table 2.3.3.2.). Cells enriched with p-EPO elongated incorporated 18:3ω6 to the 1-series eicosanoid precursor, 20:3ω6 (Table 4.7.3.1.), p-MO contained 20:4ω6, the direct 2-series precursor, whereas 2-series prostanoid inhibitors were present in p-FO and p-LO (20:5ω3 and 18:3ω3, respectively). The prostanoid changes these p-oils induced, however, were not significantly different, even when compared to those mediated with identical concentrations of other p-oils. These findings implied that exogenous FA's <u>per se</u> induced little effect on the production of the eicosanoids studied, and suggested that any enhancement

of prostanoid production related to utilisation of endogenous membrane 20:3ω6 and/or 20:4ω6, which were detected in cat skin fibroblasts (Table 4.7.3.1.). The fact that supplementation with 40mg/l p-oil had little effect on, or suppressed, prostanoid formation, whereas 20 and 60mg/l p-oil stimulated eicosanoid production nearly 2 fold primarily by enhancing TXB_2 and PGF_α synthesis (Table 4.7.6.1.), nevertheless implied p-oil concentration involvement in the modulation of prostanoid synthesis. This could have influenced the release of 1- and 2-series eicosanoid precursor FA's from membrane lipids, or the expression of enzymes involved in the formation of the prostanoids studied from PGH. However, the fact that no correlation could be found between the eicosanoid profiles (Table 4.7.6.1.) and cell viability changes (Figs. 4.7.1.2-4.7.1.8.) induced with p-oil dosed was <u>in vitro</u> evidence against the concept that the endogenously generated prostanoids studied played a direct role in the modulation of cell proliferation. It was more likely that such related to alterations in membrane physical properties and possibly the generation of FA oxidation products.

4.8 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM CAT ADIPOSE TISSUE.

4.8.1 Effects of pseudo-Oils on Cell Viability.

Microscopic examination before, during and after each experiment showed no changes in the morphology of control or p-oil dosed cells at any time, although cytoplasmic droplets were observed with p-oil incubation at high concentrations in some instances.

Investigation of the effects of albumin with final medium concentrations ranging from 0 to 250mg/l showed no significant effect on cell viability (Fig. 4.8.1.1.). The effects induced with the dosed p-oils were therefore a result of the FA's in the p-oil mixture and not the albumin used as FA carrier.

The number of control cells, seeded at $10x10^4$/ml, at the end of the 24 hour post-trypsinisation recovery period, was $10.1x10^4$/ml. This equated to about 92% of the final control cell number at the end of the 48 hour incubation period, and represented the cytostatic number. Only p-oil concentrations limiting cell viability to significantly below 92% were thus considered cytotoxic.

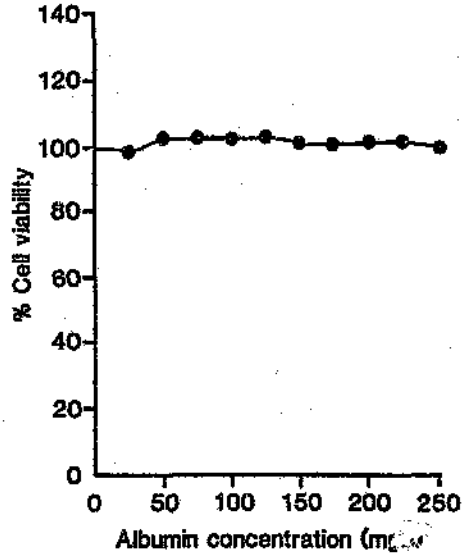
Figs. 4.8.1.2-4.8.1.8. depict the cell viability changes induced with p-oil incubation. Cell growth was enhanced with low concentrations of p-OO and p-FO, but was both

Legend to Figs. 4.8.1.1-4.8.1.9.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where "n" is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 4.8.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.8.1.2-4.8.1.8. depcit the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 4.8.1.9. shows the mean percent cell viability versus the albumin concentration (mg/l) for cells dosed with the amount of pseudo-oil which limits cell viability to 50% of the controls.

Fig. 4.8.1.1.

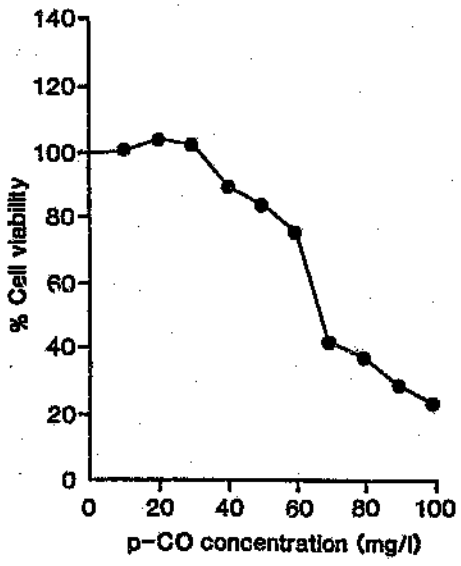
The percentage viability of cells derived from cat adipose tissue incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.7	12
25	98.5	4.0	12
50	102.6	3.9	12
75	102.4	3.8	12
100	102.4	2.5	12
125	103.1	3.6	12
150	100.4	2.2	12
175	100.9	2.5	12
200	101.7	3.1	12
225	101.3	2.5	12
250	99.6	3.6	12

Fig. 4.8.1.2.

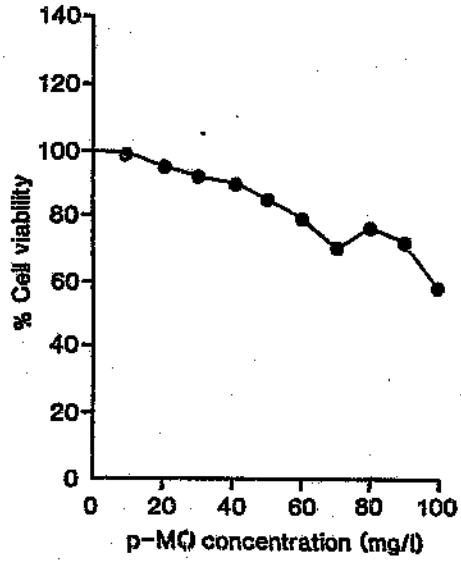
The percentage viability of cells derived from cat adipose tissue incubated with p-CO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.9	6
10	100.5	3.3	6
20	103.3	3.1	6
30	101.6	3.8	6
40	89.7	2.8	6
50	83.7	4.1	6
60	75.5	3.9	6
70	42.4	3.5	6
80	31.5	3.2	6
90	28.3	1.8	6
100	22.8	3.5	6

Fig. 4.8.1.3.

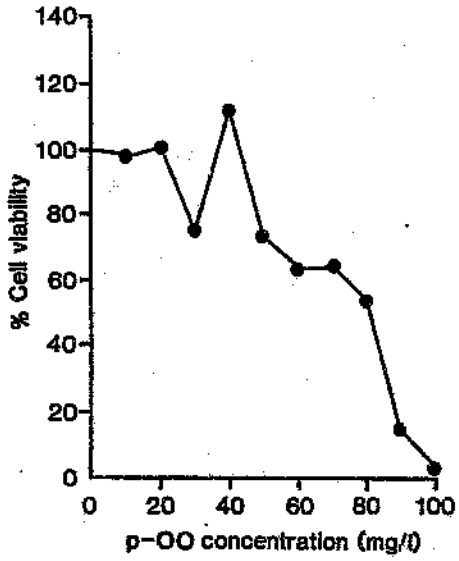
The percentage viability of cells derived from cat adipose tissue incubated with p-MO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.7	6
10	98.8	7.1	6
20	94.8	4.8	6
30	92.4	2.7	6
40	90.4	1.8	6
50	84.5	2.0	6
60	79.6	2.2	6
70	69.7	2.0	6
80	76.1	2.1	6
90	71.7	2.1	6
100	58.4	2.1	6

Fig. 4.8(\.4.

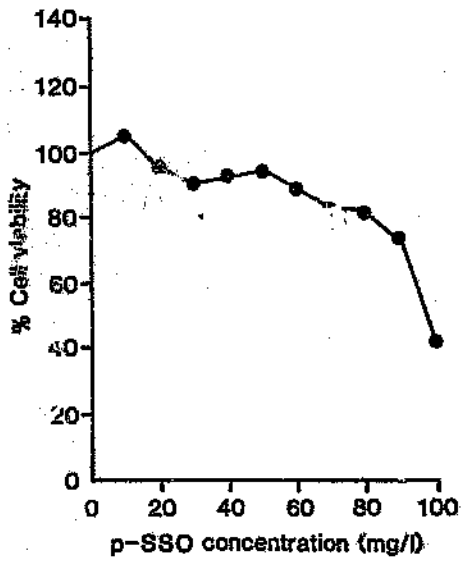
The percentage viability of cells derived from cat adipose ssue incubated with p-OO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
1[illegible]	98.0	4.7	6
20	100.2	5.3	6
30	75.1	1.9	6
40	111.3	2.1	6
50	72.7	2.1	6
60	63.8	2.5	6
70	64.4	3.3	6
80	53.7	1.5	6
90	14.7	2.3	6
100	2.5	0.8	6

Fig. 4.8.1.5.

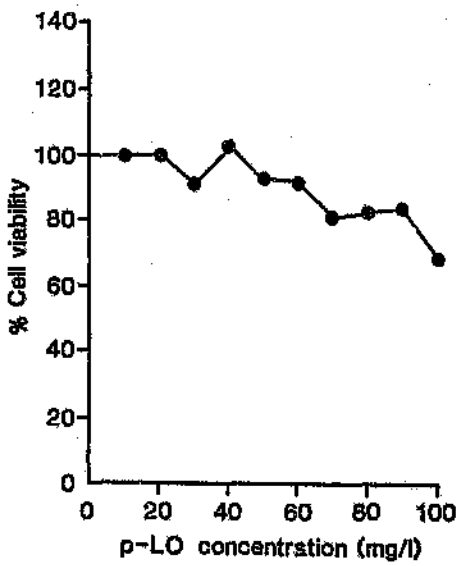
The percentage viability of cells derived from cat adipose tissue incubated with p-SSO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	105.0	3.1	6
20	95.2	4.7	6
30	90.3	1.7	6
40	91.9	2.1	6
50	94.4	3.8	6
60	88.7	2.1	6
70	81.8	5.7	6
80	81.4	2.7	6
90	72.4	1.9	6
100	41.8	3.2	6

Fig. 4.8.1.6.

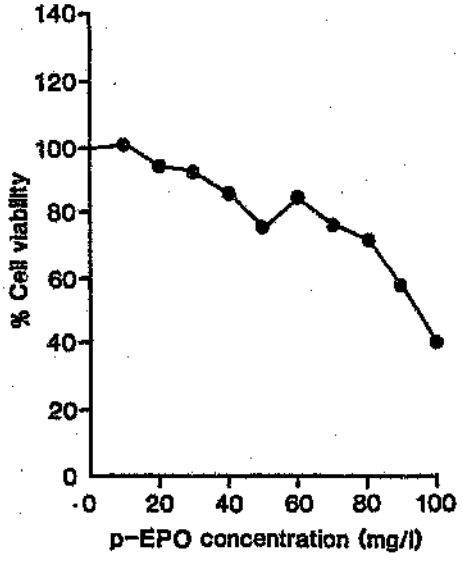
The percentage viability of cells derived from cat adipose tissue incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.4	6
10	99.6	3.7	6
20	100.4	3.5	6
30	91.0	4.5	6
40	102.3	3.0	6
50	92.5	2.1	6
60	92.0	4.4	6
70	80.8	3.5	6
80	82.7	3.4	6
90	83.1	2.6	6
100	68.1	2.4	6

Fig. 4.8.1.7.

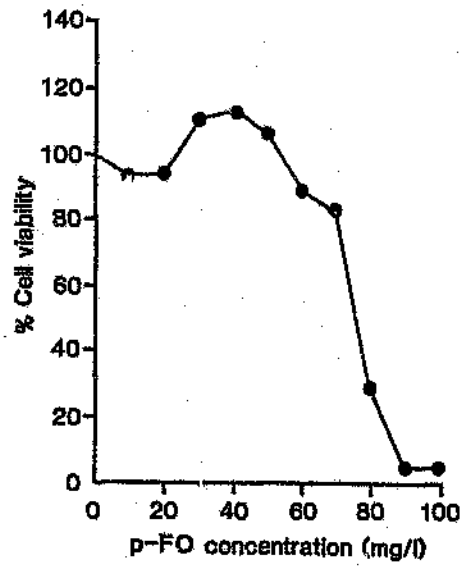
The percentage viability of cells derived from cat adipose tissue incubated with p-EPO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.9	6
10	101.3	3.4	6
20	93.7	2.5	6
30	92.4	2.6	6
40	86.1	2.6	6
50	76.0	2.9	6
60	84.0	2.2	6
70	76.8	3.0	6
80	71.7	2.2	6
90	57.8	2.2	6
100	40.1	1.8	6

Fig. 4.8.1.8.

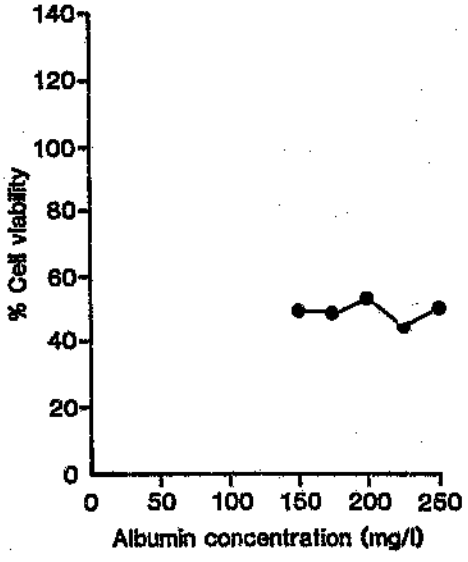
The percentage viability of cells derived from cat adipose tissue incubated with p-FO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.1	6
10	93.3	3.7	6
20	95.5	5.2	6
30	110.9	3.8	6
40	117.8	2.6	6
50	107.2	3.6	6
60	89.9	3.7	6
70	81.8	1.9	6
80	27.9	3.1	6
90	4.6	2.4	6
100	4.4	1.6	6

Fig. 4.8.1.9.

The percentage viability of cells derived from cat adipose tissue incubated with albumin at the Iso of p-00.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.1	6
150	49.9	2.0	6
175	49.1	1.8	6
200	53.1	2.0	6
225	45.0	1.8	6
250	50.1	1.9	6

limited and inhibited with all p-oils, although the magnitude thereof varied greatly dependent on the p-oil and concentration dosed. The overall pattern of growth inhibition was least with p-LO or p-MO, and greatest with p-CO, p-OO or p-FO, dosage.

Dosage with up to 30mg/l p-CO had little effect on cell growth (Fig. 4.8.1.2.), but cytostasis was induced with 40mg/l p-CO as cell viability (89.7%) approximated to the cytostatic number. Concentrations greater than 40mg/l p-CO were cytotoxic, and progressively reduced cell viability to 50% of controls with about 68mg/l p-CO (ID_{50}), and to 22.8% with 100mg/l p-CO.

Cell proliferation was not significantly different from controls with 10 or 20mg/l p-MO dosage, cytostasis was induced with 30mg/l p-MO, and cytotoxicity was prominent with concentrations greater than 40mg/l (Fig. 4.8.1.3.). No ID_{50} value was found, however, as 58.4% of cells were viable with 100mg/l p-MO.

Cell growth mirrored that of controls with up to 20mg/l p-OO incubation (Fig. 4.8.1.4.). Cytotoxicity increased progressively with higher concentrations, reducing cell viability to 75.1% and 72.7% with 30 and 50mg/l p-OO, respectively, although cell proliferation was enhanced with 40mg/l p-OO to 111.3%. Concentrations greater than 70mg/l p-OO induced marked cell death as cell viability was 50% with 81mg/l p-OO (ID_{50}), and only 2.5% with

100mg/l p-OO.

10mg/l p-SSO enhanced cell proliferation to 105.0%, but 20 to 60mg/l p-SSO induced cytostasis (Fig. 4.8.1.5.). Cytotoxicity reduced cell viability to 81.4% with 80mg/l p-SSO, but more markedly to 41.8% with 100mg/l p-SSO. 10 and 20mg/l p-EPO had little effect on cell growth, while 30mg/l p-EPO induced cytostasis (Fig. 4.8.1.7.). Higher concentrations were progressively cytotoxic, and resulted in 40.1% viable cells with 100mg/l p-EPO. The cell viability changes p-SSO and p-EPO induced were thus similar, and this was supported by the ID_{50} values (approximately 98mg/l and 95mg/l, respectively).

Dosage with up to 40mg/l p-LO generally had little effect on cell growth, while cytostasis was induced with 30, 50 or 60mg/l p-LO (Fig. 4.8.1.6.). Concentrations greater than 60mg/l p-LO were only slightly cytotoxic, and cell viability ranged from 80.8% to 83.1% with 70 to 90mg/l p-LO. No ID_{50} value could be calculated as 68.1% viable cells were found with 100mg/l p-LO.

No marked cell viability changes were induced with 10 or 20mg/l p-FO, while cell proliferation was enhanced to 110.9%, 117.2% and 107.2% with 30, 40 and 50mg/l p-FO (Fig. 4.8.1.8.). 60mg/l p-FO induced cytostasis, while cytotoxicity reduced cell viability to 81.8% with 70mg/l p-FO and to 50% with 76mg/l p-FO (ID_{50}). Cytotoxicity was considerably greater with higher concentrations, and

Trypan blue uptake was about 95% with 90 and 100mg/l p-FO.

Cells were incubated with p-OO to correspond with its ID_{50} (about 81mg/l) and dosed with varying amounts of albumin to exclude the possibility that any cell viability changes induced with p-oil incubation were influenced by the amount of albumin bound to the FA's. Five points were chosen around which to vary the albumin concentration, keeping the p-oil level fixed, viz. two points above, two points below, and the ID_{50} of p-OO. The appropriate amount of albumin was added to these cultures to give the desired concentrations. The results are shown in Fig. 4.8.1.9., and indicated that the p-OO concentration supplemented reduced cell viability to approximately 50% of controls, and that such was not significantly influenced by different concentrations of albumin. Exclusion of the synergistic involvement of albumin therefore implied that the effects induced with the p-oils were attributed solely to the exogenous FA's.

Subsequent to these studies, cells derived from cat adipose tissue were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient amounts for all quantitative and qualitative analyses to be carried out. Upon harvesting, cell viabilities were compared and found not to be statistically different from those in Figs. 4.8.1.1-4.8.1.8., hence all further biochemical assays were performed on these samples.

4.8.2 Effects of pseudo-Oils on Total Protein.

The total cellular protein content determined at each of the 3 p-oil concentrations dosed are shown in Table 4.8.2.1. in relation to controls, expressed as µg total protein/10^6 cells seeded.

475.3µg protein/10^6 control cells seeded was measured, while concentrations were considerbly greater in cells supplemented with 20, 40 or 60mg/l p-oil, ranging from 533.0 to 774.2µg/10^6 cells seeded. The protein increment was greater with supplementation of 20 than 40 or 60mg/l p-CO (681.3, 668.0 and 664.9µg, respectively) or p-EPO (638.9, 575.4 and 533.7µg, respectively), but did not vary significantly with p-SSO dosage (668.5 to 673.6µg). On the other hand, the protein increment was noticeably greater with supplementation of 40 than 20 or 60mg/l p-OO (695.3, 609.8 and 533.0µg, respectively) or p-FO (774.2, 694.0 and 681.3µg, respectively), while the converse was found with p-MO (668.5, 708.9 and 694.0µg, respectively).

Table 4.8.2.1.

The protein content of cells derived from cat adipose tissue, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	475.3			
CO		681.[illegible]	668.[illegible]	664.9
MO		708.9	668.5	694.0
OO		609.8	695.3	533.0
SSO		672.2	673.6	668.5
LO		740.2	767.0	763.3
EPO		638.9	575.4	533.7
FO		694.0	774.2	681.3

4.8.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Cat Adipose Tissue.

The FA spectra for cells incubated with 20, 40 or 60mg/l p-oil are shown in Table 4.8.3.1. in relation to controls.

Control cells exhibited a FA spectrum in which 18:1ω9 comprised 21.6% of all FA's detected, compared to 18.1% 16:0, 12.7% 18:0, 9.0% 20:4ω6, 15.5% 22:4ω6 and smaller percentages of other FA's.

In general, 16:0, 18:0, 16:1ω9 and 18:1ω9 percentages approximated to that of controls, or were decreased, in dosed cells. Similarly, ω6 PDFA levels were generally decreased in dosed cells, despite increments in 18:2ω6. ω3 PUFA levels in dosed cells were generally parallel to controls, or decreased, although small increments in some higher ω3 PDFA's were detected.

Supplementation with 20, 40 or 60mg/l p-CO resulted in increased percentages of 16:0 (22.7%, 25.4% and 25.6%, respectively vs 18.1% in controls), 18:1ω9 (23.8%, 25.4% and 26.8% vs 21.6% in controls), and 16:1ω9 (3.5%, 5.7% and 10.1%, respectively vs 4.7% in controls), while 18:0 proportions approximated to controls (12.7%). Slightly increased levels of 20:5ω3 (1.1% to 1.7% vs 0.8% in controls) and 22:6ω3 (2.6% to 2.8% vs 1.9% in controls)

Legend to Table 4.8.3.1.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where 'n' is the number of experiments. '[p-Oil]' refers to the pseudo-oil concentration used.

Table 4.8.3.1.

The fatty acid spectrum of cells derived from cat adipose tissue.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	18.1±0.20	22.7	25.4	25.6	20.9	20.5	23.7	16.6	14.9	15.5	17.0	15.2	14.5	16.5	14.9	14.1	16.0	15.6	15.3	18.1	16.1	17.1
	18:0	12.7±0.15	13.3	12.4	10.5	12.6	12.1	12.0	8.9	6.3	5.7	11.1	9.5	8.0	17.1	9.7	8.1	13.7	9.9	9.0	11.3	8.1	7.5
	20:0	0.1±0.00	-	0.1	-	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
	22:0	0.1±0.00	0.1	0.2	0.1	0.1	0.3	0.1	0.1	0.1	-	-	0.2	-	-	0.1	-	-	0.1	-	2.3	0.3	0.1
	24:0	0.1±0.00	0.2	0.4	-	-	0.4	0.2	-	0.1	-	0.2	0.3	-	-	0.3	0.1	0.1	0.2	0.4	-	-	-
ω9 MONO.	16:1	4.7±0.05	3.5	5.7	10.1	2.3	2.5	2.9	3.2	2.6	3.1	2.5	2.7	2.5	1.7	2.2	2.2	1.7	2.8	2.7	9.7	13.8	20.4
	18:1	21.6±0.20	23.8	25.4	26.8	23.0	23.1	26.7	38.2	45.3	50.4	21.7	21.5	21.8	22.1	18.4	17.3	18.7	16.6	15.5	22.0	18.5	20.2
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	4.3±0.05	3.9	3.8	3.4	6.3	7.4	9.2	7.3	9.0	9.6	19.9	26.6	35.2	7.9	10.0	11.4	20.0	29.1	34.5	6.0	6.0	7.5
	18:3	-	-	0.1	0.1	-	0.1	0.2	0.1	0.1	0.1	1.2	0.1	0.3	-	-	0.1	0.3	1.0	1.3	-	-	0.1
	20:2	0.1±0.00	0.1	0.1	-	0.2	0.4	0.3	1.1	1.5	1.7	1.1	1.3	1.4	3	0.3	0.2	0.7	1.1	1.1	0.3	0.5	0.6
	20:3	2.9±0.05	2.5	2.4	2.0	2.8	3.1	2.7	2.4	2.0	1.5	3.1	2.8	2.3	1.8	1.8	1.2	5.1	5.4	5.0	-	1.8	1.4
	20:4	9.0±0.25	9.6	8.1	6.8	10.6	10.0	9.9	7.0	5.0	3.6	7.3	5.8	4.2	7.9	7.1	5.6	7.2	5.6	4.1	7.1	4.1	3.7
	22:4	15.5±0.45	10.9	6.8	6.8	11.4	10.3	5.9	8.0	6.8	4.4	8.4	7.6	5.5	7.4	7.1	4.8	9.7	7.1	6.4	8.3	15.3	4.0
	22:5	1.2±0.00	-	0.4	0.1	-	0.4	0.3	0.1	0.2	-	0.1	0.3	-	0.2	0.3	-	0.1	0.3	0.1	0.1	0.5	-
ω3 POLYS.	18:3	0.7±0.00	0.6	0.7	0.4	1.0	0.7	0.3	0.9	0.8	0.7	0.4	0.5	0.4	10.5	20.9	29.8	0.3	0.4	0.3	0.7	0.6	0.7
	18:4	1.2±0.05	0.5	0.7	0.5	0.3	0.8	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.5	0.6	0.3	0.4	0.4	0.2	0.4	0.9	0.3
	20:4	2.4±0.05	1.1	0.7	0.8	0.8	1.3	0.3	0.7	0.6	0.5	0.9	0.8	0.6	1.3	1.8	1.5	1.4	0.4	0.8	1.8	2.7	0.9
	20:5	0.8±0.05	1.7	1.3	1.1	1.9	1.3	0.8	0.8	1.0	0.5	1.1	1.0	0.5	0.8	1.0	0.7	0.5	0.7	0.9	4.9	7.1	9.4
	22:5	2.8±0.00	3.1	2.6	2.4	3.3	2.6	2.3	2.5	1.7	1.2	2.3	1.9	1.4	2.4	1.9	1.4	2.4	1.8	1.3	5.3	3.8	4.7
	22:6	1.9±0.05	2.6	2.8	2.6	2.4	2.5	2.4	2.2	1.8	1.3	1.6	1.7	1.3	1.6	1.6	1.2	1.8	1.6	1.3	1.8	0.1	1.6

were found, but all other PUFA proportions were parallel to controls, or significantly decreased.

The levels of 16:0 and 18:1ω9 increased slightly with p-MO dosage (20.5% to 23.7% and 23.0% to 26.7% vs 18.1% and 21.6% in controls, respectively), 18:0 mirrored controls (12.7%), while 16:1ω9 decreased about 2 fold compared to controls (4.7%). No significant increments in ω6 PDFA's were detected, despite slightly increased 18:2ω6 levels (6.3% to 9.2% vs 4.3% in controls). 20:5ω3 increased approximately 2 fold with 20mg/l p-MO dosage (1.9% vs 0.8% in controls), while 22:6ω3 was slightly increased with all concentrations dosed (2.4% to 2.5% vs 1.9% in controls).

20, 40 or 60mg/l p-OO supplemenation increased cellular 18:1ω9 levels (38.2%, 45.3% and 50.4%, respectively vs 21.6% in controls), but decreased 16:0, 18:0 and 16:1ω9 percentages. p-OO dosage induced significant increments in 18:2ω6 (7.3% to 9.6% vs 4.3% in controls) and 20:2ω6 (1.1% to 1.7% vs 0.1% in controls), but not in other ω6 or ω3 PDFA's, which levels either mirrored, or were lower than, their respective controls.

Supplementation of cells with p-SSO caused 16:0, 18:0 and 16:1ω9 levels to decrease, but had no effect on 18:1ω9. A concentration dependent increased in 18:2ω6 was induced with 20, 40 and 60mg/l p-SSO (19.9%, 26.6% and 35.2%, respectively vs 4.3% in controls), and 20:2ω6

levels increased over the same range (1.1%, 1.3% and 1.4%, respectively vs 0.1% in controls). 18:3ω6 was only slightly increased with 20mg/l p-SSO dosage (1.2% vs 0% in controls), but other ω6 and ω3 PDFA's were found in proportions similar to, or lower than, controls.

An overall decrease in 16:0, 16:1ω9, 18:0 and 18:1ω9 was detected when p-LO was dosed, although 18:0 was slightly increased with 20mg/l p-LO (17.1% vs 12.7% in controls). ω6 and ω3 PDFA percentages were parallel to controls, or significantly decreased, despite increases in 18:2ω6 (7.9%, 10.0% and 11.4% with 20, 40 and 60mg/l p-LO respectively vs 4.3% in controls) and 18:3ω3 (10.5%, 20.9% and 29.8% with 20, 40 or 60mg/l p-LO, respectively vs 0.7% in controls).

Feeding cells p-EPO had little effect on, or decreased, 16:0, 18:0, 16:1ω9 and 18:1ω9 proportions in relation to controls. Dosage with 20, 40 or 60mg/l p-EPO resulted in increased detection of 18:2ω6 (20.0%, 29.1% and 34.5%, respectively vs 4.3% in controls), but small increments in 18:3ω6 (0.3%, 1.0% and 1.3%, respectively) and 20:2ω6 (0.7% to 1.1% vs 0.1% in controls). 20:3ω6 percentages nearly doubled over the same range (5.1%, 5.4% and 5.0%, respectively vs 2.9% in controls), but higher ω6 PDFA amounts were significantly decreased in relation to controls. Furthermore, ω3 PUFA levels were parallel to controls, or decreased.

Supplementation of cells with 20, 40 or 60mg/l p-FO had no significant effect on 16:0 and 18:1ω9 levels, but caused a decrease in 18:0, and significant increases in 16:1ω9 (9.7%, 13.8% and 20.4%, respectively vs 4.7% in controls) and 22:0 with 20mg/l p-FO (2.3% vs 0.1% in controls). No significant increments in ω6 PDFA levels were found, despite increased 18:2ω6 detection (6.0% to 7.5% vs 4.3% in controls). Dosage with 20, 40 or 60mg/l p-FO raised the levels of cellular 20:5ω3 (4.9%, 7.1% and 9.4%, respectively vs 0.8% in controls) and 22:5ω3 (5.3%, 3.8% and 4.7%, respectively vs 2.8% in controls), but not 22:6ω3 (1.8%, 0.1% and 1.6%, respectively vs 1.9% in controls).

4.8.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Cat Adipose Tissue.

Table 4.8.4.1. shows the radioactivity recovered from cultures incubated with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3.

The total counts recovered in the spent incubation media ranged from 2.7 to 3.2x10^6cpm, while only 0.5x10^6cpm was recovered from the pooled buffers after washing the cells, which accounted for non-specific binding. For both [^{14}C]-18:1ω9 and [^{14}C]-18:3ω3, the equivalent of 4.3x10^6cpm were dosed to the incubation medium, and total recovery of radiolabel from these cultures was approximately 91%. For [^{14}C]-18:2ω6, the equivalent of 4.8x10^6cpm were dosed to the incubation medium, and the total recovery of this radioisotope from the cultures was similarly about 92%. However, the total amount of each of these radioisotopes incorporated into the cells equated to 16% for [^{14}C]-18:1ω9, 14% for [^{14}C]-18:2ω6 and 9% for [^{14}C]-18:3ω3.

Table 4.8.4.2. shows the percentage conversion of each of the incorporated radiolabelled C18 FA's above to ω9, ω6 or ω3 series products after 48 hours incubation with cells derived from cat adipose tissue.

32.1% of the total [^{14}C]-18:1ω9 counts derived upon GLC

Table 4.8.4.1.

The radioactivity (cpm) recovered from cat adipose cultures incubated with radiolabelled C18 fatty acids, expressed as mean (±s.e.m.), n=3.

SPENT MEDIA (M)	POOLED WASHINGS (W)	TOTAL (M+W)	CELLS	TOTAL $\times 10^6$
Total 18:1 counts dosed to incubation medium = 4.3×10^6				
2730200 (±16020)	509275 (±7735)	3239475	665235 (±1740)	3.9
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
3195893 (±8035)	524588 (±5039)	3720481	646281 (±894)	4.4
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
3101520 (±5900)	450320 (±6435)	3551840	368823 (±1113)	3.9

Table 4.8.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from cat adipose tissue, expressed as a percentage of the total counts recovered upon GLC analysis.

ω9 MONOS.	[^{14}C]-18:1ω9	ω6 POLYS.	[^{14}C]-18:2ω6	ω3 POLYS.	[^{14}C]-18:3ω3
18:1	32.1	18:2	27.5	18:3	19.5
20:1	34.3	18:3	8.4	18:4	9.2
22:1	30.8	20:2	19.1	20:3	30.0
24:1	2.8	20:3	23.9	20:4	19.6
		20:4	10.2	20:5	8.0
		22:4	7.6	22:5	9.2
		22:5	3.3	22:6	4.7
TOTAL CPM	1300	TOTAL CPM	2823	TOTAL CPM	905

analysis (1300cpm) remained as 18:1ω9, with similar amounts shown for the subsequent elongation products, 20:1ω9 (34.3%) and 22:1ω9 (30.8%). However, only 2.8% 24:1ω9 was detected.

27.5% of the total [^{14}C]-18:2ω6-derived counts detected (2823cpm) remained unchanged, while 8.4% was detected for 18:3ω6, compared to 19.1% 20:2ω6 and 23.9% 20:3ω6. Approximately 20% of the total counts detected were converted to post-Δ5D PUFA's, comprising 10.2% 20:4ω6 and 7.6% 22:4ω6, but only 3.3% 22:5ω6.

The total counts derived following GLC analysis of cells incubated with [^{14}C]-18:3ω3 were 905cpm, of which 19.5% remained as 18:3ω3. Only 9.2% 18:4ω3 was detected, compared to 30.0% 20:3ω3 and 19.6% 20:4ω3, while 8.0% of the recovered radiolabel was shown as 20:5ω3 and 9.2% as 22:5ω3, but only 4.7% as 22:6ω3.

As Δ6D represents the first and rate limiting enzyme in the desaturase cascade, total activity of this enzyme is reflected by the sum of all subsequent desaturase and elongase products.

4.8.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides measured in dosed cat adipose cells and their respective growth media are shown in Table 4.8.5.1. in relation to controls. The data is expressed as nmoles MDA/10^6 cells, but in the case of the spent media, this represents the nmoles of MDA in the volume of medium from which 1x10^6 cells were obtained.

0.4nmoles MDA/10^6 control cells were quantitated, while concentrations in dosed cells varied with the p-oil supplemented, ranged from 0.5 to 4.5nmoles MDA/10^6 cells and increased in an overall concentration dependent manner. Cells incubated with p-CO, p-MO or p-OO induced similar lipoperoxide amounts (0.5 to 0.9nmoles MDA/10^6 cells), while larger concentrations were measured with dosage of p-oils more abundant in PUFA's. Comparable lipoperoxide amounts were induced with 20, 40 or 60mg/l p-EPO or p-SSO supplementation (0.9, 1.0 and 1.2 vs 0.9, 1.2 and 1.3nmoles MDA/10^6 cells, respectively), while p-FO generated significantly greater amounts only with a concentration of 60mg/l (2.2nmoles MDA/10^6 cells). p-LO, however, induced the greatest amounts of cellular lipid peroxides at the p-oil concentrations studied (1.1, 1.9 and 4.5nmoles MDA/10^6 cells with 20, 40 and 60mg/l, respectively).

Lipoperoxides were absent in spent control media, and in

Table 4.8.5.1.

Lipoperoxide formation by cat adipose cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.4			
CO		0.5	0.7	0.8
MO		0.8	0.9	0.9
OO		0.7	0.8	0.9
SSO		0.9	1.2	1.3
LO		1.1	1.9	4.5
EPO		0.9	1.0	1.2
FO		0.8	0.8	2.2
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	0.6
OO		-	-	2.2
SSO		-	-	1.7
LO		0.8	2.1	2.5
EPO		-	-	2.1
FO		-	-	1.9

that derived from cultures incubated with p-CO. On the other hand, small lipoperoxide amounts were measured in spent medium with 60mg/l p-MO dosage (0.6nmoles MDA/10^6 cells). Higher concentrations were found with 60mg/l p-OO, p-SSO, p-EPO or p-FO incubation (2.2, 1.7, 2.1 and 1.9nmoles MDA/10^6 cells, respectively), whereas spent medium lipid peroxide levels were greatest with p-LO dosage (0.8, 2.1 and 2.5nmoles MDA/10^6 cells with 20, 40 and 60mg/l, respectively).

4.8.6 The Eicosanoid Profile of Cells Derived from Cat Adipose Tissue.

Table 4.8.6.1. shows the eicosanoids positively detected in cells supplemented with 0, 20, 40 or 60mg/l p-oil. All data is presented as a percentage of the total area quantitated, but an indication of the actual eicosanoid concentration found is given for controls in "( )" as pmoles total eicosanoids/10^6 cells.

Total eicosanoid production by control cells amounted to 208.0pmoles/10^6 cells, of which 19.3% correlated with the retention times of the prostanoid standards used. This fraction was largely composed of PGF_{α} (14.4%), with smaller amounts of PGI_2 (1.2%), TXB2 (3.6%) and PGE2 (0.1%), but no PGE_1 or PGD2 were found in control cells.

The percentage of total prostanoids detected in dosed cells varied significantly with p-oil concentration, as such was decreased in relation to controls (19.3%) with dosage of 40mg/l p-oil (11.7% to 15.8%), comparable to controls or slightly increased with 60mg/l p-oil (17.0% to 25.4%), but significant increased with 20mg/l p-oil (28.3% to 32.9%). Such changes related to alterations in the production of individual prostanoids in these cells. PGI_2 production varied with supplementation of 20mg/l p-oil (0.4% to 1.7%), but increased slightly with 60mg/l p-oil (2.0% to 3.3%), and considerably with 40mg/l p-oil (3.8% to 5.2%) in relation to controls (1.2%). Every

Legend to Table 4.8.6.1.

Values are tabulated as relative percent of the total area detected, and as pmoles total eicosanoids/10^6 control cells.

Table 4.8.6.1.

The profile of the eicosanoids detected in extracts of cells derived from cat adipose tissue.

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	PGF_{α} (1+2)	PGE_2	PGE_1	PGD_2
CONTROL (208.0)		19.3	1.2	3.6	14.4	0.1	-	-
CO	20	30.4	1.7	18.3	9.2	1.2	-	-
	40	12.8	4.7	4.2	3.8	0.1	-	-
	60	21.0	2.8	10.4	6.2	0.6	0.8	0.2
MO	20	29.3	0.9	17.9	9.9	0.6	-	-
	40	15.1	3.8	6.2	5.0	0.1	-	-
	60	23.5	2.8	11.3	6.7	1.2	1.3	0.2
OO	20	29.5	0.7	17.4	10.5	0.9	-	-
	40	11.7	5.2	3.8	2.6	0.1	-	-
	60	19.6	2.0	9.1	6.1	1.2	1.0	0.2
SSO	20	32.9	0.8	18.6	12.7	0.8	-	-
	40	12.5	4.5	4.6	3.1	0.3	-	-
	60	17.0	2.0	8.9	5.4	0.5	0.1	0.1
LO	20	28.3	0.4	16.8	10.3	0.8	-	-
	40	16.8	4.4	7.6	4.7	0.1	-	-
	60	19.4	2.6	8.9	5.6	0.9	1.3	0.1
EPO	20	29.5	0.5	16.2	12.1	0.1	-	-
	40	11.8	3.9	3.7	4.0	0.2	-	-
	60	20.7	3.2	10.3	5.8	1.2	0.1	0.1
FO	20	29.2	1.6	17.4	9.3	0.9	-	-
	40	14.7	4.1	6.4	3.9	0.3	-	-
	60	25.4	3.3	13.4	7.6	0.6	0.3	0.2

p-oil concentration dosed enhanced TXB_2 biosynthesis, but this was greatest with a concentration of 20mg/l (16.2% to 18.6% vs 3.6% in controls) and least with 40mg/l (3.7% to 7.6%). In contrast, p-oil incubation decreased PGF_α production (14.4% in controls), with concentrations of 20mg/l being least (9.2% to 12.7%), and 40mg/l most (2.6% to 5.0%), effective. Dosage with 40mg/l p-oil had little effect on cellular PGE_2 levels (0.1% in controls), but such was increased with 20 and 60mg/l p-oil (0.5% to 1.2%). PGE_1 and PGD_2 were absent in control cells, but trace amounts were detected only with 60mg/l p-oil supplemenation.

4.8.7 Discussion.

The effect of FA's on cat adipocyte growth has not been described in the literature. We demonstrated that the viability of cultured cells derived from cat adipose tissue was affected when subjected to supplementation with p-oils for 48 hours (Figs. 4.8.1.2-4.8.1.8.), and it was apparent that the modulation of cell viability related to p-oil FA composition and concentration dosed, rather than the overall degree of p-oil unsaturation (Table 2.3.3.2.). Thus, the similar extent of growth limitation and cytotoxicity induced with p-EPO or p-SSO dosage was not surprising as the FA compositions of both p-oils were similar. The presence of 9% GLA in p-EPO, but not in p-SSO, seemed to have had little additional influence on cell proliferation, and the effects induced seemed more likely to relate to the abundance of LA in these p-oils (approximately 70%). Comparison with the cell viability changes p-LO induced, however, indicated that LA exhibited greater growth limiting and cytotoxic potential than ALA, the ω3 series EFA abundant in p-LO. On the other hand, the marked cytotoxicity p-OO induced with high concentrations in the range dosed implied greater OA involvement in the modulation of cytotoxicity than LA or ALA, although the increased cell viability 40mg/l p-OO induced also supported OA involvement in the stimulation of cell proliferation. The different effects these FA's appeared to induce probably related to

variations in FA structure between such moieties, and this indicated that FA's of different structures could modulate cell proliferation in different ways. The cell viability changes which p-FO induced, however, could not be related to any individual FA as this p-oil contained a more even balance of saturated, monounsaturated and polyenoic FA's rather than a single FA which contributed to the bulk of the spectrum. Although the modulation of cell growth could be related in some cases to particular p-oil FA's, every FA in a p-oil had the potential to induce an effect on cell viability to a greater or lesser extent. The possibility exists, therefore, that p-oil FA's operated either in concert with each other to enhance, or antagonistically to suppress, the effects induced by individual FA's. This could explain the greater stimulation of cell growth found with 40mg/l p-OO or p-FO dosage than with other concentrations of these p-oils, as well as the dramatic reduction in cell viability observed over small increments of certain p-oils at high concentrations dosed.

Supplementation of the culture medium for 48 hours with p-oils resulted in exogenous FA incorporation into the cells, which generally increased with the amount of p-oil dosed and altered the FA composition of these cells compared to controls (Table 4.8.3.1.). Thus it was possible that membrane fluidity was altered to a greater or leser extent, and such was involved in the modulation

of growth limitation and cytotoxicity, particularly when large amounts of p-oil were dosed. Whereas an increased complement of membrane unsaturated FA's could explain the marked cytotoxicity induced with p-OO or p-FO supplementation, the modulation of growth inhibition with p-CO dosage, in contrast, may well relate to rigidification of cell membranes.

p-Oil supplementation enhanced the total protein content of cat adipose cells (Table 4.8.2.1.). However, it was apparent from comparison with the cell viability changes induced (Figs. 4.8.1.2-4.8.1.8.) that increased cellular protein did not reflect increments in cell number alone, particularly since the amount of protein/cell increased significantly even when growth limitation and/or cytotoxicity were induced. These findings supported p-oil FA involvement in the stimulation of protein synthesis. As the extent of protein stimulation varied quantitatively when dosage with identical amounts of different p-oils yielded similar cell numbers, it was suggested that FA's vary in their capability to modulate protein synthesis, and that this related to variations in FA structure. The enhancement of protein synthesis could nevertheless represent increased production of lipid metabolising enzymes such as lipases or enzymes related to protection against lipid peroxidation, particularly in the light of p-oil FA's incorporated (Table 4.8.3.1.) and involvement of adipocytes in lipid metabolism (Lehninger 1982).

It was unlikely that absolute increases in cellular protein related to increased expression of desaturase cascade enzymes as the FA spectra of dosed cat adipose cells indicated that such exhibited relatively little capability for FA desaturation or elongation (Table 4.8.3.1.). Saturated and monoenoic FA's were neither desaturated nor elongated, although the potential for 16:0 and 18:0 desaturation (suggested with p-CO or p-MO dosage) could not be excluded. The finding that cellular 18:3ω6 and 18:4ω3 levels were not significantly raised, even with p-EPO, p-SSO or p-LO incubation, supported the lack or suppression of Δ6D activity in these cells. Even direct 18:2ω6 elongation to 20:2ω6 was limited, despite substrate availability with p-SSO or p-EPO incubation in particular. These findings indicated that the formation of 20:3ω6 only with p-EPC dosage more than likely represented elongation of incorporated 18:3ω6, but the inability to induce increased post-Δ5D FA levels implied that 20:3ω6 was not desaturated. It was also apparent that the small 20:4ω6 amounts incorporated with p-MO supplementation could not be elongated. This elongation step, however, was shown when 20:5ω3 was incorporated with p-FO dosage, but no evidence was found to suggest that this moiety was converted to 22:6ω3 via Δ4D. On the other hand, the slightly increased 20:5ω3 and 22:6ω3 levels found in cells dosed with p-CO or p-MO suggested limited Δ5D and Δ4D potential. These p-oils did not

contain ω3 PUFA's (Table 2.3.3.2.), thus the increases in post-Δ5D FA's reported must represent desaturation and elongation of cellular (membrane) ω3 PUFA's; the significantly decreased 18:4ω3 and 20:4ω3 levels found suggested utilisation of these moieties as substrates. It was theoretically possible that incorporation of saturated FA's present in these p-oils, as well as prolonged EFA/PDFA deprivation, enhanced Δ5D, subsequent elongase and Δ4D expression in an attempt to maintain membrane fluidity. In contrast, PUFA incorporation from more unsaturated p-oils may have suppressed expression of such enzymes to preserve membrane fluidity. Previous studies from our laboratory showed that cats fed a diet deficient in ω3 and ω6 PDFA's contained no post-Δ5D FA products in adipose tissue. However, PUFA levels in these cells were maintained by incorporation of greater amounts of 18:2ω6 compared to cats fed diets sufficient in ω3, ω6, or all PDFA's (Davidson et al 1989, 1990a). Such also reflected an attempt to maintain membrane fluidity. The finding that ω3 PDFA's were desaturated and elongated to a greater extent than those of the ω6 series (Table 4.8.3.1.) nevertheless confirmed the substrate preference established by others (Brenner et al 1966, Brenner 1974, 1982, Mead et al 1976 and Kanau et al 1977).

The impairment of Δ6D, and potential for Δ5D, expression we demonstrated above correlated with similar findings

which others have reported in this species (eg. Rivers et al 1975a, Frankel et al 1978, Sinclair 1979, Sinclair et al 1981 and Davidson et al 1989, 1990a). The present study also indicated the potential for Δ4D expression in cat adipocytes, while <u>in vivo</u> studies have been unable to demonstrate either its presence or absence in this tissue (Davidson et al 1989, 1990a). Our data indicated that the inability of cat adipose cells to efficiently express desaturase cascade enzymes (Table 4.8.3.1.) did not relate to limitation or inhibition of cell viability induced with p-oil supplementation, as such enzyme capability did not improve even when p-oils enhanced cell proliferation (Figs. 4.8.1.2-4.8.1.8.). Impaired desaturase cascade enzyme expression may rather be a characteristic of this tissue, implying a requirement of preformed PDFA's from the diet or other cat tissues to maintain normal physiology.

Comparison of p-oil desaturation (Table 4.8.3.1.) with that of individual FA's (Table 4.8.4.2.) was assessed to establish whether the pattern of p-oil desaturation reported above was modulated by the presence of FA mixtures. The pattern of radioactivity derived from cells supplemented with [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 was consistent with desaturation and elongation of these moieties (Table 4.8.4.2.). However, conversion of incorporated [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6 to PDFA's via Δ6D was unlikely as the cat lacks significant Δ6D

capability (eg. Rivers et al 1975a, Frankel et al 1978, Sinclair 1979, Sinclair et al 1981 and Davidson et al 1989, 1990a). Instead, the data suggested that [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6 were directly elongated to 20:3ω3 and 20:2ω6, and such converted to 20:4ω3 and 20:3ω6, respectively via an active Δ8D. As elongase reactions are reversable, it was possible that 20:4ω3 and 20:3ω6 retroconversion accounted for the small amounts of 18:4ω3 and 18:3ω6 detected, respectively, although such could also theoretically relate to Δ6D expression with EFA incorporation and/or prolonged PDFA deprivation. Nevertheless, conversion of approximately 20% [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6 each to post-Δ5D FA's was evidence for Δ5D activity. However, the apparent accumulation of 20:4ω3 and 20:3ω6 may reflect that the rate of their formation was greater than the rate of their conversion to post-Δ5D products and/or that Δ5D activity was not fully expressed. Indeed, this would support 20:4ω3 and 20:3ω6 retroconversion. Although 20:5ω3 and 20:4ω6 were elongated, the formation of less than 5% 22:6ω3 and 22:5ω6 indicated that Δ4D was not significantly expressed. The lack of desaturation found with [^{14}C]-18:1ω9 supplementation supported impaired Δ6D capability, whereas 22:1ω9 formation was consistent with expression of elongases.

The above findings indicated that capability for desaturation and elongation not only varied numerically

with dosage of individual C18 FA's or p-oils (Tables 4.8.3.1. and 4.8.4.2.), but such was also more limited with p-oils. Competitive interactions between FA's for desaturase cascade enzymes are well known (eg. Brenner et al 1966, 1967, 1969 and Brenner 1974, 1982), and similar reactions may have occurred between p-oil FA's which could explain the data. However, as p-oil FA composition mirrored that of similar dietary oils, the desaturase capability shown with p-oils probably related closer to the situation in cat adipocytes <u>in vivo</u> than that suggested with single FA's.

MDA measurement in control and p-oil dosed cat adipose cells (Table 4.8.5.1.) supported the capability of those cells to generate lipoperoxides by auto-oxidation or enzymatically. This could have occurred when the availability of unsaturated FA's outweighed the amount of vitamin E which serves as anti-oxidant. Both the p-oils and cells themselves contained FA's which were potential candidates to act as substrates for cellular lipoperoxide formation (Tables 2.3.3.2. and 4.8.3.1.). However, the presence of albumin as FA carrier and abundance of saturated FA's in new culture medium (Table 2.3.3.3.), as well as the ability of these cells to incorporate p-oil FA's (Table 4.8.3.1.), supported the likelyhood that most lipoperoxides found in the spent media of dosed cultures originated intracellularly. Reports that cellular lipoperoxides disrupt biological

membranes and increase their permeability (Chio et al 1969, Mead 1976, Tappel 1975, 1980, Gavino et al 1981c, Morisaki et al 1982b and Frankel 1984) supported the transfer of these compounds into the incubation medium through the plasma membrane, eg. with p-LO, 60mg/l p-SSO or 60mg/l p-FO supplementation, or as a direct result of p-oil-induced cytotoxicity, eg. with 60mg/l p-MO, p-OO or p-EPO dosage. Cellular peroxidising enzymes and FFA's could also have been released into the culture medium in this process, thus the possiblity that further FA oxidation occurred extracellularly, and contributed to the spent medium lipoperoxides measured, could not be ignored. Such may well account for the greater MDA amounts found in the spent medium of cultures enriched with 60mg/l p-OO, p-SSO or p-EPO, or 40mg/l p-LO than in the cells themselves.

The quantitative variations in MDA levels found between cultures incubated with identical p-oil concentrations reflected the different p-oil FA compositions (Table 2.3.3.2.). This related not only to the amount of unsaturated FA's present in any p-oil, but also to their availability and susceptibility to oxidation. The small lipoperoxide amounts induced with p-CO or p-MO dosage reflected the abundance of saturated FA's in these p-oils, and probably related to oxidation of their small amounts of unsaturated FA's and/or cellular FA's released during PGL turnover. The finding that p-OO also

induced small cellular lipoperoxide amounts when dosed, despite significant OA uptake (Table 4.8.3.1.), and the capability of OA to undergo oxidation (Frankel 1984), implied that such was largely lipid-bound and thus not readily accessible to oxidation, although lipoperoxide release into the medium with 60mg/l p-OO supplementation could explain the low cellular levels. The greater lipoperoxide amounts induced overall with dosage of p-SSO, p-EPO, p-FO or p-LO than with p-CO, p-MO or p-OO, was consistent with the presence of larger amounts polyenoic FA's in the former. The similar lipoperoxide amounts p-EPO and p-SSO induced, which probably related to oxidation of free LA, reflected their similar FA compositions and effect on cell viability. The similar EFA levels present in p-EPO, p-SSO and p-LO (about 73% LA, 70% LA and 63% ALA, respectively) and greater lipoperoxide amounts induced with dosage of p-LO than p-EPO or p-SSO, however, indicated that ALA was more susceptible to oxidation than LA, probably relatable to the greater number of double bonds in ALA. The presence of 5 double bonds in EPA compared to 3 in ALA implied greater susceptiblity of the former to oxidation. However, the considerably lower amount of EPA in p-FO (about 18%) than ALA in p-LO was consistent with lower lipoperoxide levels induced with p-FO dosage. Such could also reflect a faster rate of PGL cycling and therefore FFA release into the cellular pool from membrane lipids with p-LO incubation.

Total lipoperoxide formation by cultures supplemented with p-oils was directly related to the amount of p-oil dosed (Table 4.8.5.1.), and generally inversely related to changes in cell viability (Figs. 4.8.1.2-4.8.1.8.). Thus, it was possible that lipoperoxides were involved in the modulation of cell proliferation. The finding that the greatest cytotoxicity 60mg/l p-OO induced did correlate with the highest lipoperoxide amount found (with 60mg/l p-LO dosage), however, indicated that other mechanisms were also involved in the modulation of cell growth. It was possible that the lipoperoxide levels reported in dosed cultures induced cell damage/death only once membrane fluidity had been significantly altered. Thus, the modulation of cell proliferation with p-oil supplementation may relate both to alterations in membrane stability and lipoperoxidation.

Control cat adipose cells produced a significant amount of total eicosanoids, which accounted for approximately 50% of all lipoperoxides quantitated (Tables 4.8.5.1. and 4.8.6.1.). This was rather surprising in the light of the poor capability for p-oil desaturation reported (Table 4.8.3.1.), but could well reflect a requirement for eicosanoids in cat adipocytes, which may, for example, relate to the control of lipid metabolism or mobilisation of storred FA's (Mayes 1981). Control cells indeed contained PUFA's which were potential candidates to act as substrates for the formation of eicosanoids

(Table 4 .1.), but the finding that the prostanoids studied comprised only a fraction (19.3%) of the molar amount quantitated indicated that these cells were primarily involved in the making of other eicosanoids. Prostanoid detection nevertheless supported cyclo-oxygenase expression, and it was apparent that the enzymic pathways for PGF_{α} biosynthesis were greatly favoured over those involved in TXB_2, PGI2 or PGE2 production. Furthermore, the small PGE_1 and PGD2 amounts produced by dosed cells, but not in controls, suggested that PGH_1-PGE1 and PGH2-PGD2 isomerases were suppressed or inhibited rather than absent.

Supplementation of cat adipose cultures with p-oils for 48 hours influenced the rate of prostanoid production in these cells (Table 4.8.6.1.), but such was independent upon the degree of p-oil unsaturation or ability of the p-oils to provide eicosanoid precursor PUFA's (Table 2.3.3.2.). Indeed, incorporation of 20:4ω6, the direct 2-series eicosanoid precursor, with p-MO incubation, and formation of the 1-series precursor, 20:3ω6, from 18:3ω6 incorporated with p-LPO dosage (Table 4.8.3.1.), had no significant effect on prostanoid production compared to other p-oils supplemented at the same concentrations. This phenomneon was also found with p-LO or p-FO dosage, which contained significant amounts of 18:3ω3 and 20:5ω3 that normally inhibit 2-series prostanoid production by preferentially occupying the active site on cyclo-

oxygenase (Lands et al 1973, 1977, Hamazaki et al 1982, Corey et al 1983, Fischer et al 1983, 1984 and Nassar et al 1987). The inability to induce significant changes in TXB_2, PGF_α, PGI2, PGE2, PGE1 or PGD2 levels with dosage of different p-oils at the same concentration implied that exogenous FA's per se had little effect on prostanoid production, and detection of 20:4ω6 and smaller 20:3ω6 levels in control cells (Table 4.8.3.1.) rather supported endogenous PUFA utilisation for prostanoid formation. On the other hand, the finding that total prostanoid production was suppressed with 40mg/l p-oil, unaltered overall with 60mg/l p-oil, and stimulated with 20mg/l p-oil, supplementation primarily due to changes in PGF_α, TXB2 and/or PGI2 levels, implied p-oil concentration involvement in the modulation of prostanoid biosynthesis. This could have influenced eicosanoid precursor availability or the expression of enzymes involved in prostanoid formation from PGH. The prostanoid changes induced with p-oil supplementation of cells were nevertheless found not to correlate with the changes reported in cell viability (Figs. 4.8.1.2-4.8.1.8.), total protein (Table 4.8.2.1.), lipoperoxides (Table 4.8.5.1.), or desaturation capability (Table 4.8.3.1.). This implied that while p-oil supplementation influenced eicosanoid production, or the balance between individual prostanoid groups, the endogenously generated prostanoids studied were probably not directly involved

in the modulation of effects demonstrated. Indeed, this mechanism could not explain the growth limitation and cytotoxicity induced with p-oils rich in saturated or monounsaturated FA's, which could not be metabolised to eicosanoids. Alterations in membrane physical properties and the generation lipoperoxides were proposed as more likely mechanisms involved in the modulation of cell proliferation.

4.9 General Discussion.

The effect of p-oil supplementation on the viability of different types of cultured normal cat cells was presented in this chapter. The capability of each cell type to incorporate, desaturate and elongate FA's, the formation of lipid peroxides and eicosanoids and the possible roles these compounds played in the modulation of cell viability, were also examined.

The cat is a model for many human diseases (O'Brien 1986), yet the literature indicates that relatively little work has been conducted in this species, particularly with regard to lipid metabolism. The lack of commercially available cat cell lines (Flow 1984 and Gibco 1984) possibly explains why tissue culture has not been used as a tool to study aspects of lipid metabolism in the cat *in vitro*. To the best of our knowledge, this study was the first to use a range of normal cat cells in tissue culture to investigate the above parameters. This therefore permitted valid comparisons to be made between different cat cell types under standard experimental conditions.

p-Oils had the capability to modulate cat cell viability when dosed for 48 hours, but the effects induced and magnitude thereof varied to a greater or lesser extent between different cat cell types. All p-oils were cytotoxic to cat erythrocytes and lymphocytes,

except p-CO which induced no effect on the erythocytes. In general, suspension cultures of erythrocytes and lymphocytes were killed with very low (sections 4.1.1 and 4.2.1, respectively), adipose and brain cells with intermediate (sections 4.8.1 and 4.5.1, respectively), and endothelium, skeletal muscle, lung and skin cells only with high (sections 4.3.1, 4.4.1, 4.6.1 and 4.7.1, respectively), p-oil concentrations in the range dosed. Adherent cells were thus more resistent to cytotoxicity than suspension cultures, and such may relate to the fact that the adherent cells used divided in culture without prior stimulation. It was also clear that the threshold for cytotoxicity varied with different adherent cells. This was found to be relatable to cell population doubling times (Table 2.3.1.1.) as relatively slow dividing cells, eg. brain and adipose, required lower p-oil concentrations to induce growth limition and/or cytotoxicity than cells with shorter population doubling times, eg. lung and skin. The lower cytostatic number established with faster, than with slower, replicating cells, was consistent with the broader growth limiting range the former induced. This range is nevertheless important and should always be defined as it distinguishes the p-oil concentration(s) which induce mere growth limitation or cytostasis from those promoting cell proliferation or cytotoxicity. In this regard, the data presented therefore has practical

applications for those wishing to modulate cell growth with p-oils, single FA's or other agents to achieve a specific effect, and also allows for valid comparisons to be made between cells of different growth rates.

Previous work from our laboratory showed that both ω6 and ω3 PDFA's were required for normal cat growth. Feeding with diets deficient in either ω3 or ω6 PDFA's limited the rate of growth significantly, while the poorest growth rate was observed when fed a diet deficient both in ω3 and ω6 PDFA's (Davidson et al 1989, 1990a). No consistent correlation between cell viability and p-oil PDFA levels were found in the present study. Dosage with low to intermediate concentrations of p-oils containing EFA's and/or PDFA's, however, induced less growth limitation than p-CO, which was deficient in all PUFA's. Furthermore, EFA and/or PDFA containing p-oils enhanced cell proliferation significantly in numerous instances, whereas such was never mediated with p-CO feeding. These findings therefore suggested a PUFA requirement for normal proliferation of cat cells and tissues.

The data presented supported the involvement of dietary FA's in the modulation of cat cell growth, and the different cell viability changes found between the cat cell types studied to dosage with identical p-oils may well relate to the different physiology of these tissues in vivo. Careful consideration should therefore be

given to the selection of cat tissues for experimental purposes, and extrapolation of cell viability results from one cat cell type to another should therefore be avoided to ensure valid interpretation of data.

The present chapter provided substancial evidence to support the incorporation of exogenous FA's by cat cells in vitro. The resulting FA modifications induced in the cells led to the proposal that differential uptake of exogenous FA's caused alterations in membrane physical properties such as fluidity, and this was a mechanism whereby p-oils reduced cell viability, particularly with high concentrations in the range dosed.

Total protein concentrations varied considerably between the different cat cell types studied (sections 4.1.2, 4.2.2, 4.3.2, 4.4.2, 4.5.2, 4.6.2, 4.7.2 and 4.8.2). Protein amounts in control adherent cells were greatest in adipose and aortic endothelium, intermediate in skin and skeletal muscle, and lowest in lung and brain (475.3, 424.4, 304.5, 300.1, 230.4 and 175.1µg/10^6 cells seeded, respectively). The finding that control adherent cells exhibited markedly more protein than suspension cultures of erythrocytes or lymphocytes (12.3 and 6.4µg per 10^6 cells seeded, respectively) could have related to the ability of the former to replicate in culture without prior stimulation. Control cell protein levels,

however, showed no clear correlation with the proliferation rate of these cells (Table 2.3.1.1.), and probably reflected variations in morphology, physiology and rate of protein turnover (Lehninger 1982) with cell type in vivo. Total protein measurement in dosed cells reflected the relative changes induced in cell number to a greater or lesser extent, but such also provided evidence implicating exogenous FA involvement in the stimulation of protein synthesis in nucleated cells. This varied in magnitude with cat cell type, p-oil FA composition and concentration supplemented. It was suggested that absolute increases in cellular protein induced with p-oil dosage related to increased synthesis of membrane proteins, particularly if the integrity of such was threatened by growth limiting and/or cytotoxic p-oil concentrations. Increased expression of lipid metabolising enzymes were also proposed, although it was apparent that such probably did not represent increased desaturase and elongase enzyme production.

The FA spectra derived from the incubation of cat cells with p-oils provided a means of assessing the capability of those cells to desaturate and elongate FA's. Studies investigating the capability of the carnivorous cat to desaturate FA's have primarily been limited to liver and monitoring serum FA profiles subsequent to dietary oil or FA supplementation (Rivers et al 1975a, 1976, Frankel et al 1978, Sinclair 1979, MacDonald et al 1983a and

Davidson et al 1990a). The present study, in contrast, examined the <u>in vitro</u> desaturase capability of a variety of cat tissues not previously examined by other workers. Neither cat erythrocytes nor lymphocytes exhibited any significant capability for FA desaturation or elongation (Tables 4.1.3.1. and 4.2.3.1., respectively). Similarly, no significant desaturase enzyme expression occurred overall with p-oil dosage to cat skeletal muscle, lung, skin or adipose cells, and PUFA metabolism was primarily limited to elongation of 18:2ω6, 18:3ω6 and 20:5ω3 (Tables 4.4.3.1., 4.6.3.1., 4.7.3.1. and 4.8.3.1., respecitvely). Cat brain cells exhibited elongation, retroconversion, Δ8- and Δ5-desaturation, but not Δ6- or Δ4-desaturation, potential (Table 4.5.3.1.), whereas Δ8D, Δ5D, Δ4D and elongase, but not Δ6D, activity was suggested in cat endothelial cells (Table 4.3.3.1.). The above findings clearly demonstrated that desaturase cascade enzyme capability varies between different cat tissues. It also supported current evidence indicating that the domestic cat is Δ6D-deficient (eg. Rivers et al 1975a, 1976, Frankel et al 1978 and MacDonald et al 1983a). The results obtained by Sinclair (1979) after supplementing cat diets with 18:2ω6 similarly showed that cat tissues do not exhibit significant Δ6D capability, even in liver. When cats were fed diets either sufficient in all PDFA's, or deficient in ω6, ω3 or all PDFA's, Davidson et al (1989, 1990a) failed to detect in different tissues 20:3ω9, the unusual FA

considered indicative of PUFA deficiency in other mammals (Holman 1977 and Sprecher 1977); this also confirmed the lack or suppression of Δ6D activity in the cat. In contrast, Δ5D expression was demonstrated by both Sinclair (1979) and Davidson et al (1989, 1990a), although the presence or absence of Δ4D could not be proven. There is evidence, however, that this species probably has the capability to express both Δ5D and Δ4D (Kane et al 1981 and MacDonald et al 1983a), although enzyme activity appears to vary with experimental conditions.

The pattern of radioactivity shown with [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 dosage to cat lung, skin or adipose cells (Tables 4.6.4.2., 4.7.4.2. and 4.8.4.2., respectively) was consistent with impaired Δ6D capability, but supported the expression of elongases, Δ8D and Δ5D for which adipose cells exhibited slightly greater potential than skin or lung fibroblasts. When Sinclair et al (1981) fed cats EFA-deficient diets, they detected 20:3ω9 in various tissues and also proposed that such was formed by an active Δ8D and Δ5D. MacDonald et al (1983a) similarly demonstrated the capability of cat liver to elongate 18:2ω6 to 20:2ω6 and desaturate such to 20:3ω6 via Δ8D when SSO was fed in the absence of 18:2ω6 desaturation. Cat testes were also shown to use the Δ8D, rather than the Δ6D, route to convert 18:2ω6 to 22:5ω6 (MacDonald et al 1984). This could well be a

mechanism whereby the cat is able to form PDFA's even when EFA desaturation is impaired, and thus provide 20:3ω6, 20:4ω6 and 20:5ω3 as substrates for eicosanoid production.

The more limited desaturation capability we found with p-oil than single FA dosage in all cat cell types studied indicated differences in the metabolism of single and mixtures of FA's, which probably related to the mediation of competitive interactions between p-oil FA's for such enzymes. Similar reactions probably occur in vivo when cats ingest dietary oils, although FA's of the same and different series/positions in the desaturase cascade, as well as the cellular demand for PDFA's, will modulate desaturase/elongase expression, the maximum potential which is genetically determined. The fact that the cat cell types studied exhibited different capabilities to desaturate and elongate PDFA's implied in vivo dependence of some cat tissues on others as a source of higher PDFA's, particularly under conditions of limiting dietary supply. Nevertheless, the variations in desaturation capability found between the cat cell types studied, whether dosed with single FA's or p-oils, may well relate to the different physiology of these tissues in vivo. Extrapolations in this regard to other cat cell types should therefore be avoided.

Comparison of the MDA amounts quantitated between the

cat cell types studied indicated varying capabilities to generate lipoperoxides. These compounds were absent from control erythrocytes (section 4.1.4), but were formed by all nucleated control cat cells, although relative amounts were low in brain (0.1nmoles MDA/10^6 cells), intermediate in lymphocytes, lung, skin and adipose (0.3 to 0.4nmoles MDA/10^6 cells), and greatest in skeletal muscle and endothelium (0.6 to 0.8nmoles MDA/10^6 cells) (sections 4.5.4, 4.2.4, 4.6.5, 4.7.5, 4.8.5, 4.4.4 and 4.3.4, respectively). Others found that FA oxidation occurred more rapidly in cat adipose than liver slices (Richard et al 1989). These findings supported variations in the rates of FA oxidation, the content of vitamin E as cellular anti-oxidant and/or the efficiency of enzymes such as catalase, peroxidase and superoxide dismutase in the removal of different species of activated oxygen that promote lipoperoxidation between different cat tissues. The fact that control cell lipid peroxide concentrations showed no clear correlation with the total unsaturated FA contents or population doubling times (Table 2.3.1.1.) of these cells, however, could well have related to the different physiology and rates of PGL turnover with cell type (Lehninger 1982).

Incubation of cat cultures with p-oils for 48 hours induced the formation of lipoperoxides from exogenous unsaturated FA's, although the magnitude thereof once again varied quantitatively between the different cell

types studied even when identical p-oils were dosed. Lipoperoxide concentrations greater than 5nmoles MDA/10^6 cells were rarely found in dosed adherent cells or the corresponding spent medium (Tables 4.3.4.1., 4.4.4.1., 4.5.4.1, 4.6.5.1, 4.7.5.1. and 4.8.5.1.), while the range was considerably wider (0 to 73nmoles MDA/10^6 cells) for dosed erythrocyte or lymphocyte cultures (Tables 4.1.4.1. and 4.2.4.1.). The overall increment in cellular lipoperoxide formation found when cat cultures were enriched with increasing amounts of p-oil supported exogenous FA incorporation from the growth medium. The variations found in lipoperoxide concentrations between the dosed cat cell types studied, however, could have related to alterations in the rate of cellular FA uptake and release induced with p-oil supplementation. p-Oil-induced lipoperoxidation was also shown to relate to the amount of unsaturated FA's present in the p-oils (Table 2.3.3.2.) and their susceptibility to oxidation, dependent on FA structure and availability of these moieties in the free form. These factors could indeed explain the greater lipoperoxide amounts induced in all cells with dosage of p-FO or p-LO than other p-oils. The evidence we presented in this chapter indicated that the p-oils induced the spent medium lipoperoxides found occasionally in dosed cultures, but that these compounds largely originated intracellularly and were released as a result of p-oil-induced cytotoxicity or increased plasma membrane permeability. Both intracellular and

extracellular lipoperoxides could nevertheless have induced cellular damage, and the involvement of these compounds in the modulation of cell viability was proposed.

Eicosanoid quantitation supported the ability of cat lung, skin and adipose cells to generate such compounds (Tables 4.6.6.1, 4.7.6.1 and 4.8.6.1, respectively). This correlated with the detection of 20:4ω6, 20:3ω6 and 20:5ω3 in control cells as eicosanoid precursors (Tables 4.6.3.1., 4.7.3.1. and 4.8.3..., respectively). It was also possible that 22:4ω6 and 22:5ω3 retroconversion provided 2- and 3-series eicosanoid substrates, respectively, particularly since PDFA formation was limited by impaired Δ6D expression. We showed that total molar eicosanoid production varied considerably between different cat cells, being greatest for control adipose, while skin and lung formed considerably smaller amounts (208.0, 80.5 and 32.9pmoles $/10^6$ cells, respectively). The finding that these cells produced similar amounts of lipid peroxides (0.3 to 0.4nmoles MDA/10^6 cells) thus implied greater potential to synthesise eicosanoids in cat adipose, than in skin or lung under the experimental conditions used. This probably related to the different physiology, eicosanoid requirements, and activities of enzymes involved in eicosanoid formation/inactivation between such cells. The lower molar eicosanoid amounts generated by lung than skin or adipose cells could also

have related to a greater incorporation of FA's into the TAG rather than the PGL pool.

Control cat lung, skin and adipose cells differed in the relative amounts of PGI_2, TXB_2, PGF_α and PGE_2 they formed (Tables 4.6.6.1., 4.7.6.1. and 4.8.6.1.), and it was apparent that enzymic pathways for the synthesis of TXB_2 in lung and skin fibroblasts, and PGF_α in adipose cells, were preferred to that of other prostanoids. This implied differences in the expression and activity of enzymes involved in prostanoid synthesis between cat tissues and/or modulation by other eicosanoids. PGE_1, for example, is partly responsible for the control of cAMP levels, a potent inhibitor of phospholipase and thus AA release (Feinstein et al 1977, Minkes et al 1977 and Horrobin 1980b). Low PGE_1, as a result of impaired LA desaturation for example, will cause low cAMP levels, and thus increased AA mobilisation. As control lung, skin and adipose cells did not produce PGE_1, such may explain the production of 2-series prostanoids (Tables 4.6.6.1., 4.7.6.1. and 4.8.6.1.). In the light of this phenomenon, it was possible that a greater proportion of the PGF_α fraction detected in these cells was composed of $PGF_{2\alpha}$ rather than $PGF_{1\alpha}$.

Cat lung, skin and adipose prostanoid synthesis was modulated with p-oil dosage (Tables 4.6.6.1., 4.7.6.1. and 4.8.6.1., respectively), and its relationship with

the p-oil amount incubated suggested a preference for certain p-oil concentrations to selectively modulate enzyme activity involved in the synthesis of these compounds. The finding that the modulation of prostanoid production did not correlate with p-oil FA composition, however, suggested sufficient endogenous PUFA's in the precursor pool, or an unavailability of incorporated p-oil FA's, for prostanoid production. The prostanoid profiles of dosed cat lung, skin and adipose cells also showed no p-oil concentration dependent correlation with cell viability, desaturation capability or lipoperoxide production, thus we proposed that the endogenously produced prostanoids studied were not directly involved in, or responsible for, the effects induced with p-oil supplementation; these compounds may rather be involved in the overall regulation of cellular metabolism.

To sum up, comparative data on a range of cell parameters for different cultured cat tissues were presented in this chapter. We showed that control cells from different cat tissues varied with respect to their rates of cell proliferation, FA and eicosanoid profiles, total protein concentrations, and molar lipoperoxide and eicosanoid production. In addition, variations in cell viability, total protein and lipoperoxide formation, FA and eicosanoid profiles, and desaturation capability were demonstrated between different cat cells dosed with p-oils, although the impairment of Δ6D expression was

common to all cat cells studied. The data implied that variations in FA metabolism between different cat tissues were important in the modulation of the effects reported. Care should therefore be taken when selecting cat tissues for experimental purposes, and extrapolation of findings from one cat cell type to another should be avoided to ensure valid interpretation of data.

THE INFLUENCE OF FATTY ACIDS *IN VITRO* ON MAMMALIAN CELLS FROM SPECIES DIFFERING IN THEIR FATTY ACYL DESATURASE CAPABILITIES.

VOLUME 3

ALFREDO GIANGREGORIO

CHAPTER 5: THE HUMAN: RESULTS AND DISCUSSION.

5.1 THE EFFECTS OF PSEUDO-OILS ON HUMAN ERYTHROCYTES.

5.1.1 Effects of pseudo-Oils on Cell Viability.

Microscopic examination showed the human erythrocyte cultures to be free of any white blood cell or platelet contamination. Most cultured erythrocytes retained their biconcave disc shape, although a small proportion lost this characteristic after 2 days culture. In addition, 10% to 20% of erythrocytes lost their distinct 'red' colour after 48 hours in culture and became difficult to visualise. Hence, only erythrocytes which retained their pigmentation were regarded as viable, normal and representative of the situation *in vivo*.

The results obtained from the investigation of the effects of 0 to 250mg albumin/l growth medium showed no significant effect on erythrocyte viability (Fig. 5.1.1.1.). The effects induced with the dosed p-oils were therefore a result of the exogenous FA's, and not the albumin used as FA carrier.

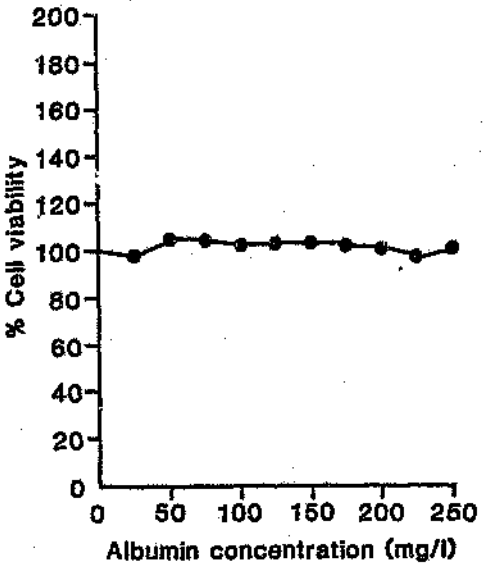
Erythrocytes do not divide nor require trypsinisation, thus no 24 hour post-trypsinisation recovery period was needed. Erythrocytes were dosed immediately subsequent to plating, hence the seeding concentration was the cytostatic number.

Legend to Figs. 5.1.1.1-5.1.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where "n" is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 5.1.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 5.1.1.2-5.1.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 5.1.1.1.

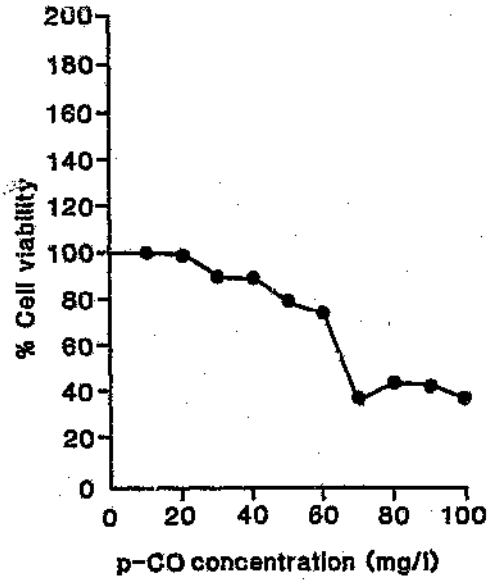
The percentage viability of human erythrocytes incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.7	12
25	97.5	4.1	12
50	103.5	3.7	12
75	104.4	5.2	12
100	101.6	3.1	12
125	103.3	5.4	12
150	102.2	5.0	12
175	100.7	4.6	12
200	100.4	3.9	12
225	96.1	2.9	12
250	99.8	4.4	12

Fig. 5.1.1.2.

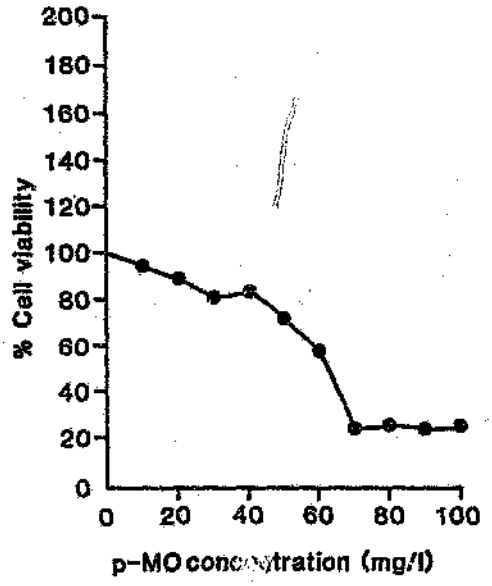
The percentage viability of human erythrocytes incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.9	6
10	99.9	3.2	6
20	98.2	4.0	6
30	88.8	3.8	6
40	89.6	3.0	6
50	78.4	2.5	6
60	74.1	2.1	6
70	37.5	1.7	6
80	44.7	1.7	6
90	42.7	1.0	6
100	38.4	1.5	6

Fig. 5.1.1.3.

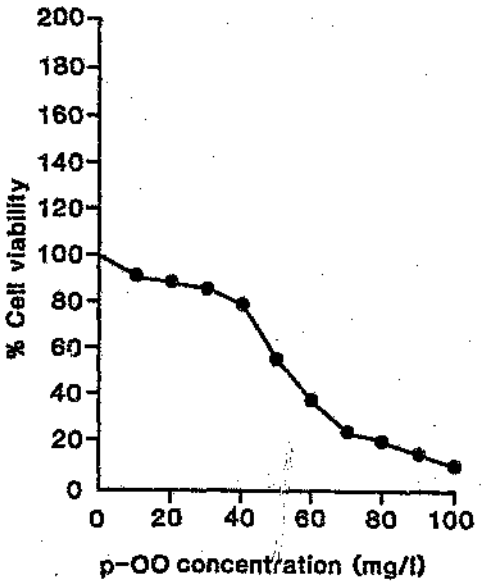
The percentage viability of human erythrocytes incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.1	6
10	93.8	5.1	6
20	88.6	4.7	6
30	80.7	2.8	6
40	82.5	2.7	6
50	70.2	4.0	6
60	57.8	3.3	6
70	25.1	5.5	6
80	25.9	5.3	6
90	23.9	5.2	6
100	25.5	5.3	6

Fig. 5.1.1.4.

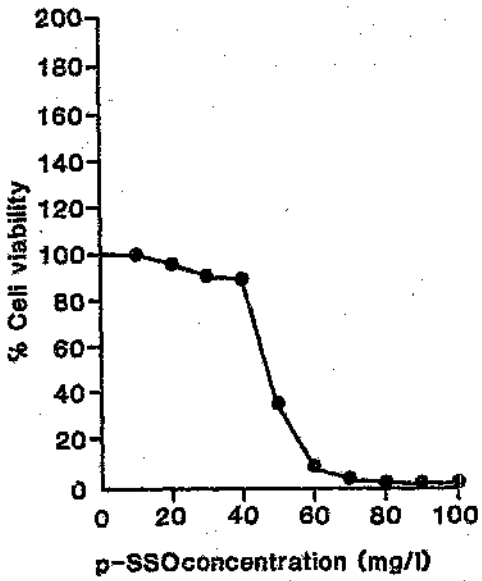
The percentage viability of human erythrocytes incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.6	6
10	90.8	4.5	6
20	87.6	3.4	6
30	85.0	1.5	6
40	78.9	5.1	6
50	55.2	4.8	6
60	37.4	3.7	6
70	24.2	1.9	6
80	20.8	3.4	6
90	14.6	1.6	6
100	9.6	1.7	6

Fig. 5.1.1.5.

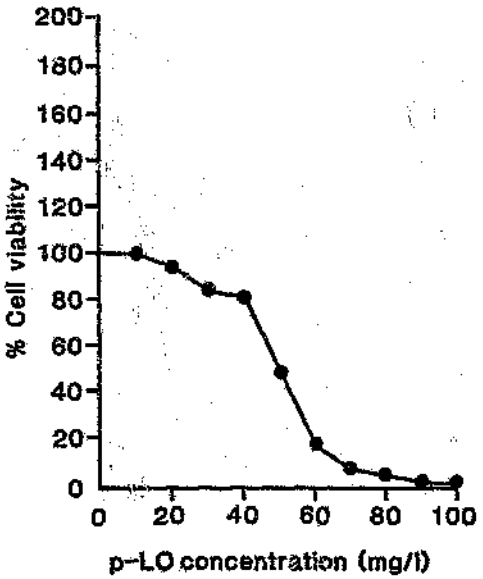
The percentage viability of human erythrocytes incubated with p-SSO.



pseudo-Cell Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.5	6
10	99.6	3.1	6
20	95.8	2.9	6
30	91.1	3.2	6
40	90.0	4.2	6
50	35.6	3.3	6
60	9.2	0.7	6
70	2.9	0.3	6
80	0.9	0.1	6
90	0.7	0.1	6
100	0.3	0.1	6

Fig. 5.1.1.6.

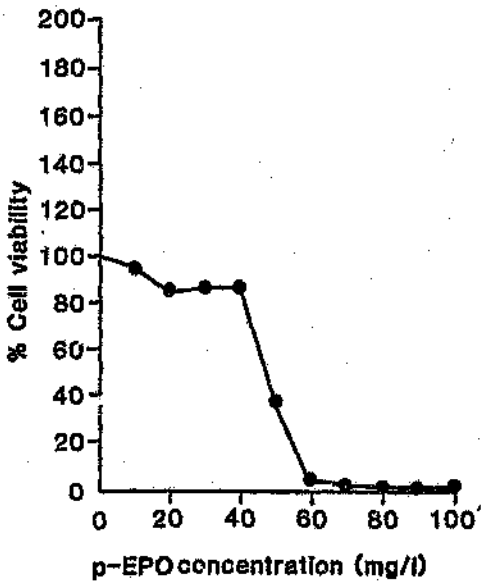
The percentage viability of human erythrocytes incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	99.1	3.8	6
20	93.7	3.2	6
30	84.4	1.4	6
40	79.7	2.9	6
50	48.5	3.7	6
60	17.6	1.9	6
70	7.4	0.6	6
80	4.0	0.5	6
90	1.0	0.3	6
100	0.1	0.1	6

Fig. 5.1.1.7.

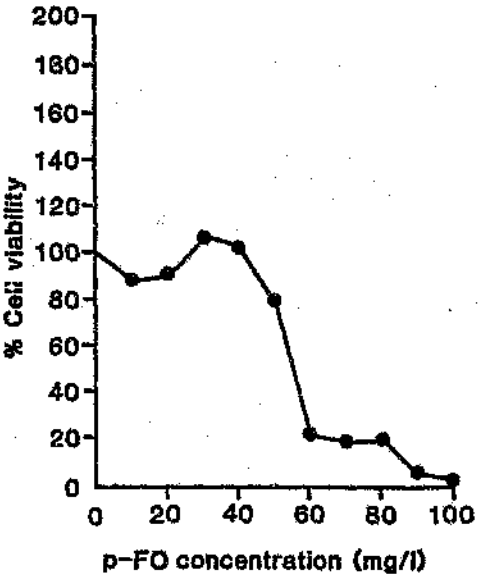
The percentage viability of human erythrocytes incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.9	6
10	95.2	2.3	6
20	86.0	3.4	6
30	86 3	3.6	6
40	86.6	4.5	6
50	38.1	3.1	6
60	4.8	0.7	6
70	2.1	0.4	6
80	0.8	0.1	6
90	0.7	0.1	6
100	0.2	0.1	6

Fig. 5.1.1.8.

The percentage viability of human erythrocytes incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.4	6
10	88.4	5.3	6
20	90.8	1.0	6
30	106.9	5.4	6
40	103.2	5.1	6
50	79.0	4.5	6
60	21.9	1.7	6
70	19.0	1.9	6
80	19.9	3.2	6
90	6.0	1.7	6
100	0.5	0.3	6

The results obtained from the incubation of erythrocytes with p-oils are shown in (Figs. 5.1.1.2-5.1.1.8.). All p-oils induced hemolysis, although the effects were more pronounced overall with unsaturated than saturated FA-rich p-oils, especially at high concentrations in the range dosed. ID_{50} values ranged from 45 to 55mg/l with p-OO, p-SSO, p-LO, p-EPO or p-FO dosage, while those for p-CO and p-MO were higher (67 and 63mg/l, respectively).

Dosage with up to 20mg/l p-CO had little effect on human erythrocyte viability, but higher concentrations induced hemolysis such that viability was 74.1% with 60mg/l p-CO and 37.5% with 70mg/l p-CO (Fig. 5.1.1.2.). Similarly, p-MO dosage progressively reduced erythrocyte viability to 25.1% with a concentration of 70mg/l (Fig. 5.1.1.3.). However, both p-CO and p-MO induced no further hemolysis with concentrations greater than 70mg/l as erythrocyte viability ranged from 38.4% to 44.7% with p-CO, and 23.9% to 25.9% with p-MO, dosage. These p-oils induced similar effects, although p-CO was less cytotoxic than p-MO with all concentrations tested. On the other hand, p-OO dosage progressively reduced the number of viable erythrocytes to 78.9% with 40mg/l, 24.2% with 70mg/l and 9.6% with 100mg/l (Fig. 5.1.1.4.).

p-SSO, p-LO and p-EPO had little effect on erythrocyte viability with a concentration of 10mg/l, whereas slight hemolysis was induced with amounts of 20 to 40mg/l since

80% to 90% of erythrocytes were viabile. However, this was dramatically reduced with higher concentrations to yield 17.6%, 9.2% and 4.8% viable erythrocytes with 60mg/l p-LO, p-SSO or p-EPO dosage, respectively, and Trypan blue uptake was practically 100% with addition of 100mg/l p-oil. The ID_{50} values of p-SSO, p-LO and p-EPO (approximately 48mg/l, 50mg/l and 48mg/l, respectively) correlated with the similar effects these p-oils induced (Figs. 5.1.1.5-5.1.1.7.).

Dosage with up to 40mg/l p-FO had no marked effect on erythrocyte viability, but 50 and 60mg/l p-FO induced extensive hemolysis and reduced cell viability from 79.0% to 21.9%, respectively (Fig. 5.1.1.8.). Hemolysis was increased with higher p-FO amounts dosed so that practically all erythrocytes were hemolysed with 100mg/l p-FO.

Subsequent to these studies, human erythrocytes were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient quantities for all the quantitative and qualitative analyses to be carried out. Cell viabilities were compared upon harvesting and found not to be statistically different from those in Figs. 5.1.1.1-5.1.1.8. All further biochemical assays were thus performed on these samples.

5.1.2 Effects of pseudo-Oils on Total Protein.

The total protein concentrations of human erythrocytes dosed with 0, 20, 40 or 60mg/l p-oil are shown in Table 5.1.2.1. as µg total protein/10^3 cells seeded.

26.4µg total protein/10^6 control erythrocytes seeded was measured, whereas amounts in dosed erythrocytes were similar to, or lower than, control levels and ranged from 2.9 to 28.4µg/10^6 cells seeded (induced with 60mg/l p-EPO and 40mg/l p-FO dosage, respectively). Erythrocyte protein concentrations were parallel to, or slightly below, control levels with 20mg/l p-oil supplementation, but decreased overall with increasing amounts of p-oil dosed. The smallest change in erythrocyte protein was induced with 20, 40 or 60mg/l p-CO dosage (27.2, 24.8 and 20.9µg/10^6 cells seeded), while the reduction in erythrocyte protein was more extensive with dosage of unsaturated FA-rich p-oils at a concentration of 60mg/l (2.9 to 8.9µg/10^6 cells seeded).

Table 5.1.2.1.

The protein content of human erythrocytes, expressed as µg total protein/10^6 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	26.4			
CO		27.2	24.8	20.9
MO		23.6	23.4	16.6
OO		20.6	19.0	8.9
SSO		27.3	24.9	3.6
LO		23.9	21.7	5.6
EPO		21.8	21.4	2.9
FO		21.5	28.4	6.2

5.1.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Human Erythrocytes.

Table 5.1.3.1. shows the FA spectra of human erythrocytes dosed with 0, 20, 40 or 60mg/l p-oil. Erythrocyte FA's contributing at least 5% to the profile in control cells included 16:0 (8.9%), 18:0 (5.2%), 18:1ω9 (6.0%), 22:4ω6 (55.0%), 18:4ω3 (5.6%) and 20:4ω3 (10.5%). These FA's were detected in varying amounts in dosed erythrocytes.

16:0 and 18:1ω9 levels were significantly greater in all dosed erythrocytes than in controls, and this generally applied for 18:0, while the converse was generally true for 16:1ω9. 18:2ω6 proportions were generally increased in dosed erythrocytes, but ω6 PDFA levels were parallel to, or lower than controls. 22:4ω6 percentages were particularly decreased with increments in the amount of p-oil dosed. ω3 PUFA levels in dosed erythrocytes generally approximated to controls or were significantly decreased.

Erythrocytes dosed with 20, 40 or 60mg/l p-CO showed a marked increased in 16:0 (42.0%, 45.2% and 55.6%, respectively vs 8.9% in controls). 18:0 levels increased about 2 fold (9.3% to 10.0% vs 5.2% in controls), and 18:1ω9 increased 2 to 3 fold (15.8% to 17.3% vs 6.0% in controls), but 16:1ω9 and all PUFA levels were parallel to controls, or significantly decreased.

Legend to Table 5.1.3.1.

All values are tabulated as relative percent total area. Control values are reported as mean±s.e.m., where 'n' is the number of experiments. '[p-Oil]' refers to the pseudo-oil concentration used.

Table 5.1.3.1.

The fatty acid spectrum of human erythrocytes.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-QO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	9.9±0.00	42.0	45.2	55.6	32.5	32.6	32.9	15.8	16.4	14.3	12.9	5.4	27.8	25.0	23.8	27.6	17.3	23.8	36.4	40.0	31.4	31.9
	18:0	5.2±0.15	9.3	10.0	9.4	16.5	19.1	16.1	6.9	3.5	3.8	7.1	3.1	10.4	10.3	7.8	6.2	12.0	16.2	16.3	8.9	4.0	3.2
	20:0	0.5±0.00	-	0.2	-	-	-	-	-	0.2	-	-	0.3	-	-	-	-	-	0.4	-	2.1	-	-
	22:0	0.1±0.00	0.1	-	0.1	-	0.2	-	-	0.1	-	0.2	-	0.1	-	-	-	-	0.2	-	-	-	-
	24:0	-	-	0.2	0.2	0.1	-	0.1	0.6	-	-	-	-	-	-	0.2	0.7	-	-	1.5	-	-	-
ω9 MONOS.	16:1	1.0±0.00	-	0.2	0.1	0.1	-	-	1.1	0.6	0.8	2.3	0.5	0.5	0.8	0.3	0.1	2.5	1.3	0.4	10.8	27.3	28.6
	18:1	6.0±0.05	16.9	15.8	17.3	21.4	25.1	30.6	28.7	38.4	38.2	14.5	13.7	29.4	19.2	26.0	25.0	13.4	11.6	18.8	15.1	21.2	22.0
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	1.5±0.00	2.0	1.6	1.2	6.1	6.2	9.6	2.6	1.5	1.4	3.1	1.8	6.6	3.4	9.6	11.6	1.7	3.3	4.5	3.8	5.0	6.4
	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.2	-	-	-
	20:2	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.1	0.6	0.6
	20:3	0.1±0.00	-	0.2	0.3	-	-	0.1	-	-	-	-	-	-	-	0.2	0.1	-	-	-	0.7	-	0.1
	20:4	1.0±0.05	0.5	0.4	0.4	0.7	0.6	1.1	-	-	-	-	-	-	-	0.3	0.1	0.8	-	-	-	-	-
	22:4	55.0±4.65	26.1	18.3	13.2	18.8	11.2	8.1	37.2	28.7	37.1	54.0	31.0	20.4	35.5	12.6	5.8	47.6	29.0	19.7	14.9	5.9	4.5
	22:5	4.4±0.30	0.4	2.0	0.2	0.3	0.6	0.1	0.8	2.4	0.7	0.8	4.5	0.7	0.4	0.8	0.1	2.3	2.1	0.6	0.6	1.3	0.4
ω3 POLYS.	18:3	0.2±0.00	0.2	0.2	0.2	1.3	0.4	0.2	-	0.1	-	0.3	-	-	1.8	15.3	22.0	0.5	0.2	-	0.1	0.3	0.4
	18:4	5.6±0.30	0.5	2.1	0.2	0.4	1.5	0.2	0.8	2.8	0.7	0.7	5.6	0.6	0.5	1.3	0.2	1.0	4.4	0.8	1.0	0.8	0.2
	20:4	10.5±1.80	2.0	3.6	1.5	1.9	2.3	0.6	5.5	5.0	3.1	4.1	9.2	3.5	3.3	1.9	0.5	1.0	7.4	0.6	1.8	1.2	0.5
	20:5	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.2	0.9	1.2
	22:5	-	-	-	0.1	-	-	0.1	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
	22:6	0.2±0.25	-	-	0.1	-	0.1	0.1	-	-	-	-	-	-	-	0.1	0.1	-	0.2	0.3	-	0.1	-

Table 5.1.3.1.

The fatty acid spectrum of human erythrocytes.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	8.9±0.00	42.0	45.2	55.6	32.5	32.6	32.9	15.8	16.4	14.3	12.9	15.4	27.8	25.0	23.8	27.6	17.3	23.8	36.4	40.0	31.4	31.9
	18:0	5.2±0.15	9.3	10.0	9.4	16.5	19.1	16.1	6.9	3.5	3.8	7.1	3.1	10.4	10.3	7.8	6.2	12.0	16.2	16.3	8.9	4.0	3.2
	20:0	0.5±0.00	-	0.2	-	-	-	-	-	0.2	-	-	0.3	-	-	-	-	-	0.4	-	2.1	-	-
	22:0	0.1±0.00	0.1	-	0.1	-	0.2	-	-	0.1	-	0.2	-	0.1	-	-	-	-	0.2	-	-	-	-
	24:0	-	-	0.2	0.2	0.1	-	0.1	0.6	-	-	-	-	-	-	0.2	0.7	-	-	1.5	-	-	-
ω9 MONOS.	16:1	1.0±0.00	-	0.2	0.1	0.1	-	-	1.1	0.6	0.8	2.3	0.5	0.5	0.8	0.3	0.1	2.5	1.3	0.4	10.8	27.3	28.6
	18:1	6.0±0.05	16.9	15.8	17.3	21.4	25.1	30.6	28.7	38.4	38.2	14.5	13.7	29.4	19.2	26.0	25.0	13.4	11.6	18.8	15.1	21.2	22.0
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	1.5±0.00	2.0	1.6	1.2	6.1	6.2	9.6	2.6	1.5	1.4	3.1	1.8	6.6	3.4	9.6	11.6	1.7	3.3	4.5	3.8	5.0	6.4
	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.2	-	-	-
	20:2	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.1	0.6	0.6
	20:3	0.1±0.00	-	0.2	0.3	-	-	0.1	-	-	-	-	-	-	-	0.2	0.1	-	-	-	0.7	-	0.1
	20:4	1.0±0.05	0.5	0.4	0.4	0.7	0.6	1.1	-	-	-	-	-	-	-	0.3	0.1	0.8	-	-	-	-	-
	22:4	55.0±4.65	26.1	18.3	13.2	18.8	11.2	8.1	37.2	28.7	37.1	54.0	31.0	20.4	35.5	12.6	5.8	47.6	29.0	19.7	14.9	5.9	4.5
	22:5	4.4±0.30	0.4	2.0	0.2	0.3	0.6	0.1	0.8	2.4	0.7	0.8	4.5	0.7	0.4	0.8	0.1	2.3	2.1	0.6	0.6	1.3	0.4
ω3 POLYS.	18:3	0.2±0.00	0.2	0.2	0.2	1.3	0.4	0.2	-	0.1	-	0.3	-	-	1.8	15.3	22.0	0.5	0.2	-	0.1	0.3	0.4
	18:4	5.6±0.30	0.5	2.1	0.2	0.4	1.5	0.2	0.8	2.8	0.7	0.7	5.6	0.6	0.5	1.3	0.2	1.0	4.4	0.8	1.0	0.8	0.2
	20:4	10.5±1.80	2.0	3.6	1.5	1.9	2.3	0.6	5.5	5.0	3.1	4.1	9.2	3.5	3.3	1.9	0.5	1.0	7.4	0.6	1.8	1.2	0.5
	20:5	-	-	-	-	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.2	0.9	1.2
	22:5	-	-	-	0.1	-	-	0.1	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-
	22:6	0.2±0.25	-	-	0.1	-	0.1	0.1	-	-	-	-	-	-	-	0.1	0.1	-	0.2	0.3	-	0.1	-

FA analysis of erythrocytes dosed with 20, 40 or 60mg/l p-MO showed increased levels of 16:0 (32.5%, 32.6% and 32.9%, respecti y vs 8.9% in controls), 18:0 (16.5%, 19.1% and 16.1%, respectively vs 5.2% in controls), and 18:1ω9 (21.4%, 25.1% and 30.6%, respectively vs 6.0% in controls), but practically no 16:1ω9 was detected. With the exception of increased 18:2ω6 (6.1% to 9.6% vs 1.5% in controls) and 18:3ω3 with 20mg/l p-MO (1.3% vs 0.2% in controls), all PDFA amounts approximated to controls or were significantly decreased.

16:0 and 18:1ω9 incorporations were increased with 20, 40 or 60mg/l p-OO incubatio (15.8%, 16.4% and 14.3%, and 28.7%, 38.4% and 38.2% vs 8.9% and 6.0% in controls, respectively), while 18:0 and 16:1ω9 percentages were decreased overall compared to controls (5.2% and 1.0%, respectively). No significant increments in PUFA levels were detected, and these were largely considerably lower than in control cells over the entire range dosed.

p-SSO dosage induced increments in 16:0, 18:0 and 18:1ω9 (12.9% to 27.8%, 7.1% to 18.1% and 13.7% to 29.4% vs 8.9%, 5.2% and 6.0% in controls, respectively), but no marked changes in 16:1ω9. Surprisingly, 18:2ω6 levels were low (3.1%, 1.8% and 6.6% with 20, 40 and 60mg/l p-SSO, respectively vs 1.5% in controls), and no 18:3ω6 or 20:4ω6 were found. 22:4ω6 levels mirrored controls (55.0%) with supplementation of 20mg/l p-SSO (54.0%),

but decreased significantly with 40 and 60mg/l p-SSO (31.0% and 20.4%, respectively). The levels of all other PUFA's were parallel to, or lower than, control levels.

Dosage of erythrocytes with p-LO resulted in increased levels of 16:0, 18:0 and 18:1ω9 (23.8% to 27.6%, 6.2% to 10.3%, and 19.2% to 26.0% vs 8.9%, 5.2% and 6.0% in controls, respectively). No significant increments in PDFA's were found, however, despite 18:2ω6 and 18:3ω3 uptake (3.4%, 9.6% and 11.6%, and 1.8%, 15.3% and 22.0% with 20, 40 and 60mg/l vs 1.5% and 0.2% in controls, respectively).

Erythrocyte 18:2ω6 incorporation was low when dosed with 20, 40 or 60mg/l p-EPO (1.7%, 3.3%, 4.5%, respectively vs 1.5% in controls). No significant increase in 18:3ω6, 20:3ω6 or 20:4ω6 occurred, and there was a marked reduction of 22:4ω6 (47.6%, 29.0%, 19.7%, respectively vs 55.0% in controls). ω3 PUFA proportions were not significantly greater than in controls, but the converse was found for 16:0, 18:0 and 18:1ω9 (17.3% to 36.4%, 12.0% to 16.3%, and 11.6% to 18.8% vs 8.9%, 5.2% and 6.0% in controls, respectively).

Erythrocyte dosage with 20, 40 or 60mg/l p-FO induced marked increments in 16:0 (40.0%, 31.4% and 31.9%, respectively vs 8.9% in controls), 16:1ω9 (10.8%, 27.3% and 28.6%, respectively vs 1.0% in controls), and 18:1ω9 (15.1%, 21.2% and 22.0%, respectively vs 6.0% in undosed

cells), but not in 18:0 (5.2% in controls). Small 18:2ω6 and 20:5ω3 amounts were found (3.8% to 6.4% and 0.2% to 1.2% vs 1.5% and 0% in controls, respectively), but all other PUFA proportions were parallel to controls, or considerably decreased.

5.1.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides measured in human erythrocytes and their respective spent incubation media after 48 hours incubation with 0, 20, 40 or 60mg/l p-oil are shown in Table 5.1.4.1. The data is expressed as nmoles MDA/10^6 cells, but in the case of the spent media, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained.

No lipoperoxides were found in control erythrocytes, or in those dosed with p-CO or p-MO, while small amounts were induced with 40 and 60mg/l p-OO (0.2 and 0.7nmoles MDA/10^6 cells, respectively). However, lipoperoxidation increased when p-oils containing significant amounts of PUFA's were dosed. p-EPO and p-SSO induced identical lipoperoxide amounts with a concentration of 20mg/l, but levels were greater with 40 and 60mg/l p-EPO than p-SSO (0.1, 1.5 and 4.3 vs 0.1, 1.0 and 2.4nmoles MDA per 10^6 cells, respectively). Dosage with p-LO or p-FO, however, induced the largest overall amounts of lipid peroxides (0.7, 5.1 and 6.2, and 0, 3.6 and 8.6nmoles MDA per 10^6 cells with 20, 40 and 60mg/l, respectively).

No lipoperoxides were measured in spent control medium, or in that derived from cultures incubated with p-CO, p-MO or p-OO. As with the erythrocytes themselves, spent medium lipoperoxides were measured with p-SSO, p-LO,

Table 5.1.4.1.

Lipoperoxide formation by human erythrocyte cultures incubated with p-oils, expressed as nmoles MDA/10^6 cells.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	0.2	0.7
SSO		0.1	1.0	2.4
LO		-	3.6	8.6
EPO		0.1	1.5	4.3
FO		0.7	5.1	6.2

pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		0.1	-	3.2
LO		-	29.9	77.1
EPO		-	-	35.3
FO		-	6.4	6.8

p-EPO or p-FO incubation. The largest amounts of media lipoperoxides were quantitated with 40 and 60mg/l p-LO (29.9 and 77.1nmoles MDA/10^6 cells, respectively), and 60mg/l p-EPO (35.3nmoles MDA/10^6 cells), while markedly lower concentrations were found with 40 and 60mg/l p-FO (6.4 and 6.8nmoles MDA/10^6 cells respectively), and 60mg/l p-SSO (3.2nmoles MDA/10^6 cells).

5.1.5 Discussion.

p-Oil supplementation of human erythrocytes induced hemolysis and decreased the number of viable cells in every instance, although the extent thereof varied with p-oil (Figs. 5.1.1.2-5.1.1.8.). The more extensive hemolysis induced with incubation of p-oils rich in unsaturated, than saturated, FA's appeared to relate partly to p-oil PUFA levels (Table 2.3.3.2.). Hemolysis was least with p-CO (0% PUFA's), and greatest with p-EPO, p-SSO or p-LO (70% to 80% PUFA's), dosage, thus an increase in the percentage of total p-oil PUFA's was consistent with an overall increase in the extent of hemolysis induced. Indeed, the presence of approximately 10% PUFA's in p-MO was consistent with the slightly greater amount of hemolysis induced than with p-CO dosage, although the large amount of saturated FA's both in p-CO and p-MO possibly explains the similar pattern of hemolysis these p-oils induced. The slightly greater amount of hemolysis induced with incubation of p-EPO or p-SSO (rich in LA) than with p-LO (rich in ALA) did not necessarily imply that LA was more effective than ALA in mediating hemolysis, since slightly greater amounts of LA were present in p-EPO and p-SSO (about 70%) than ALA in p-LO (about 63%). On the other hand, the similar changes induced in erythrocyte viability with p-EPO or p-SSO dosage were not surprising as the FA compositions of both p-oils were similar. These changes probably

related to the large amount of LA present, whereas the slightly greater extent of hemolysis induced with p-EPO than p-SSO incubation was consistent with small amounts of GLA in p-EPO only (about 9%). Structural differences between FA's were important in explaining the different cell viability changes the p-oils induced, but the possibility that synergistic and antagonistic interactions occurred between p-oil FA's and contributed to the changes observed could not be ignored.

The exact mechanism(s) responsible for the erythrocyte viability changes reported are not fully understood, but studies have demonstrated an increase in the degree of unsaturation of human erythrocyte PGL's when moderate amounts of cod-liver oil were fed, which was indicative of increased plasma membrane fluidity (Popp-Snijers et al 1984). Subsequent studies by the same group examined erythrocyte membrane lipid composition, fluidity, and flexibility 8 weeks after the diets of human subjects had been supplemented with 3g of both EPA and DHA per day (Popp-Snijders et al 1986a). Suprisingly, no change in membrane fluidity or viscosity of erythrocyte suspensions were shown, despite an increased degree of unsaturation both in the phosphatidylcholine and phosphatidylethanolamine fractions. It was proposed that the changes induced were counteracted by alterations in PGL class distribution, thus resulting in overall maintenance of membrane fluidity. However, erythrocyte

deformability increased when subjects were fed 75-100g fatty fish/day for 4 weeks (Popp-Snijders et al 1986b). This supported earlier studies which similarly reported that altering the FA composition of human erythrocyte membranes induced changes in their deformability and viscosity (Kobayashi et al 1981 and Terano et al 1983). We showed that incorporation of dosed p-oil FA's altered the overall FA profile of human erythrocytes compared to controls (Table 5.1.3.1.). However, the surprising increase in total erythrocyte saturated and monounsaturated FA levels, even with dosage of p-oils rich in PUFA's, suggested selective incorporation of exogenous FA's. It was nevertheless possible that the modifications in erythrocyte FA composition reported altered membrane physical properties, particularly with supplementation of high p-oil concentrations, and such could well explain the hemolysis induced (Figs. 5.1.1.2-5.1.1.8.). In particular, the finding that saturated FA-rich p-oils were less effective in mediating hemolysis than p-oils abundant in unsaturated FA's could have related to the high polyenoic, and low saturated, FA content of control erythrocytes (Table 5.1.3.1.).

Erythrocyte membrane fluidity has been reported to be reduced in diabetics (Kameda et al 1983), who also show impaired desaturation capability (eg. Mercuri et al 1968, Brenner et al 1968a, Horrobin 1983 and Huang et al 1984a), thus the erythrocyte data from this study may

be clinically relevant in the diabetic situation. The finding that high concentrations of all p-oils induced considerable hemolysis (Figs. 5.1.1.2-5.1.1.8.) implied that human diabetics may be predisposed to greater hemolysis than non-diabetics when similar oils form part of the diet, although the extent of hemolysis would probably vary with oil. For example, high concentrations of p-SSO, p-EPO and p-LO induced almost complete hemolysis, thus such oils may predispose human diabetics to greater hemolysis than those inducing less hemolysis, eg. p-CO or p-MO. It may, therefore, be clinically relevant to monitor FA levels and erythrocyte membrane fluidity of diabetic patients.

The alterations reported in erythrocyte total protein concentrations (Table 5.1.2.1.) correlated with the cell viability changes induced (Figs. 5.1.1.2-5.1.1.8.) with p-oil dosage. Most erythrocyte protein is attributable to its hemoglobin (Geigy 1984), thus p-oil-induced hemolysis would be consistent with a reduction in erythrocyte protein, particularly with supplementation of high p-oil concentrations. Dosage with 60mg/l p-EPO induced 95% hemolysis (Table 5.1.1.7.), thus the amount of protein measured at this concentration (2.9µg/10^6 cells seeded) largely reflected erythrocyte membrane protein. Comparison of this amount of protein to that of controls confirmed that approximately 10% of total erythrocyte protein is membrane related, the remainder

being hemoglobin (Geigy 1984).

All erythrocyte lipids reside in the membrane (eg. Erickson et al 1958), where PGL turnover has been reported (Cooper 1970). Mature erythrocytes, however, are unable to synthesise FA's de novo (Pittman et al 1966), and the inability of these cells to desaturate or elongate PUFA's (Table 5.1.3.1.) was consistent with their lack of intracellular organelles and microsomes (Lehninger 1982). Erythrocytes therefore also lack the enzymes required for lipoperoxidation. These cells, however, contain large quantities of oxygen-carrying hemoglobin (Lehninger 1982), and studies have reported that oxygen, hemoglobin and iron are powerful catalysts of oxidative reactions in human erythrocytes, which augment oxidation of unsaturated FFA's (Tappel 1963, Beppu et al 1987 and Fraga et al 1990). It was likely, therefore, that the lipid peroxides measured in the erythrocyte cultures (Table 5.1.4.1.) were formed via auto-oxidation when incorporated unsaturated p-oil FA's, or FA's released from PGL's, were exposed to such compounds in intact cells.

The absence of lipid peroxides in control erythrocytes (Table 5.1.4.1.), despite the presence in these cells of oxygen-rich hemoglobin and a total PUFA complement of approximately 80% (Table 5.1.3.1.) suggested that these PUFA's were unavailable for oxidation, probably because these moieties were components of complex lipids, such

as membrane PGL's. There are also reports that a high hemoglobin concentration can protect the erythrocyte against oxidation because hemoglobin behaves as a lipid radical scavenger (Trotta et al 1981). Indeed, when erythrocytes were damaged as a result of exposure to increasing amounts of unsaturated FA-rich p-oils, loss of intra-cellular hemoglobin correlated with increased cellular lipoperoxide levels. It was also possible that the absence of lipoperoxides in control erythrocytes reflected cellular anti-oxidant activity, eg. vitamin E, preventing oxidation of FFA's released during PGL cycling. On the other hand, insufficient cellular anti-oxidant levels could explain the lipoperoxides formed with p-oil dosage. When the oxidant challenge was low, the physiological anti-oxidant defences were able to overcome the oxidative stress. When erythrocytes were exposed to greater p-oil concentrations, however, the anti-oxidant defences were probably overwhelmed, and lipid peroxidation became dependent on the exogenous FA substrate dosed. PGL oxidation could also be involved.

The lipid peroxides formed as a result of p-oil supplementation to erythrocyte cultures (Table 5.1.4.1.) related both to the amount and structure (number and position of double bonds) of unsaturated FA's present in the p-oils (Table 2.3.3.2.). The presence of only small amounts of unsaturated FA's both in p-CO and p-MO was consistent with the lack of lipoperoxide formation with

their dosage, while the detection of small quantities of cellular lipoperoxides with p-OO dosage probably related to the oxidation of free OA for which a mechanism has been reported (Frankel 1984). On the other hand, the larger lipid peroxide concentrations induced with p-SSO, p-LO, p-EPO or p-FO supplementation were consistent with the susceptibility of the PUFA's in these p-oils to oxidation. The lipoperoxides induced with p-SSO or p-EPO dosage probably related to oxidation of the large amount of LA present therein (about 70%), although the presence GLA in p-EPO only could explain the significantly greater lipoperoxide amounts this p-oil induced. The availability of free ALA probably accounted for the formation of the greatest lipoperoxide amounts when p-LO was supplemented. However, if one considers the amount of GLA present in p-EPO compared to ALA in p-LO (about 9% vs 63%, respectively) with the MDA concentrations these p-oils induced, then the data suggests that GLA is more susceptible to oxidation than ALA. This has also been reported by Morisaki et al (1982b). The reason for this phenomenon is unclear, but could relate to the fact that GLA is a PDFA. The greater lipoperoxide amounts p-EPO induced compared to p-FO, despite 18% EPA in the latter, however, implied that this ω3 PDFA was largely lipid-bound. The larger amount of ALA in p-LO than EPA in p-FO was neveretheless consistent with the greater lipoperoxide amounts p-LO induced, despite the greater susceptibility of EPA to oxidation.

Erythrocytes were shown to incorporate the albumin-bound p-oil FA's dosed (Table 5.1.3.1.), thus the lipid peroxides measured in the spent medium probably reflect released cellular lipid peroxides. Such may have been expelled into the medium upon cell lysis, implying that medium lipoperoxide levels were partly dependent on the degree of p-oil-induced hemolysis (Figs. 5.1.1.2-5.1.1.8.). The pronounced hemolysis induced with 60mg/l p-SSO, p-LO, p-EPO or p-FO dosage was thus consistent with greater lipoperoxide amounts in the spent medium than in the cells. The finding that superoxides readily cross the erythrocyte membrane in either direction (Lynch et al 1978a, 1978b) indicated that lipoperoxides generated intracellularly as a result of p-oil dosage could have been released into the medium through the plasma membrane of intact cells. This may explain the spent medium lipoperoxides measured in the absence of significant hemolysis, eg. with 40mg/l p-FO incubation. Evidence that erythrocyte FFA's exchange directly with extracellular FFA's (Tarlov et al 1967 and Anon 1968), and the potential for cellular FFA release into the culture medium upon hemolysis, could therefore not rule out the possibility that extracellular auto-oxidation contributed to the medium lipoperoxides detected.

There are numerous reports that lipid peroxides induce membrane damaging effects (Chio et al 1969, Mead 1976, Tappel 1980 and Frankel 1984). There is also evidence

that oxygen radicals induce cross-linking of erythrocyte aminolipids and alter plasma membrane permeability, and hemolysis has been associated with lipid peroxide production (Kann et al 1964, Mengel et al 1965, 1966, Oski et al 1967, Beppu et al 1987 and Girotti et al 1987). Recent studies have shown that increasing the degree of unsaturation of lipids in human erythrocyte membranes resulted in leakage from the cells of glucose, the prime energy source, the extent of which was relatable to the susceptibility of erythrocytes to peroxidation (Urano et al 1991). In the light of these findings and the inverse correlation found between lipoperoxide levels and cell viability induced with dosage of monoenoic or polyenoic FA-rich p-oils, it was likely that lipoperoxides played a role in the modulation of the hemolysis we reported. The fact that the lipoperoxide levels found did not always correlate quantitatively with the degree of cytotoxicity induced with p-oil incubation, however, indicated that these compounds were only partly involved in the modulation of human erythrocyte viability. Indeed these compounds could not explain the reduction of cell viability with p-CO or p-MO dosage which induced no lipoperoxides. Our data suggested that membrane physical changes were probably also involved in the modulation of erythrocyte viability.

5.2 THE EFFECTS OF PSEUDO-OILS ON HUMAN LYMPHOCYTES.

5.2.1 Effects of pseudo-Oils on Cell Viability.

Microscopic inspection showed human lymphocyte cultures to be free of erythrocyte or platelet contamination, and no morphological changes were evident between dosed or control lymphocytes either before, during or after each experiment.

Fig. 5.2.1.1. shows that human lymphocyte viability was not significantly altered with incubation of 0 to 250mg albumin/l culture medium, nor were any synergistic effects induced. Hence, the cell viability changes induced with p-oil dosage were a result of the exogenous FA's alone, and not the albumin used as FA carrier.

Lymphocytes do not normally divide in culture without appropriate mitogen stimulation nor do they require trypsinisation, thus no 24 hour post-trypsinisation recovery period was required. As lymphocytes were dosed immediately subsequent to plating, the seeding concentration was the cytostatic number.

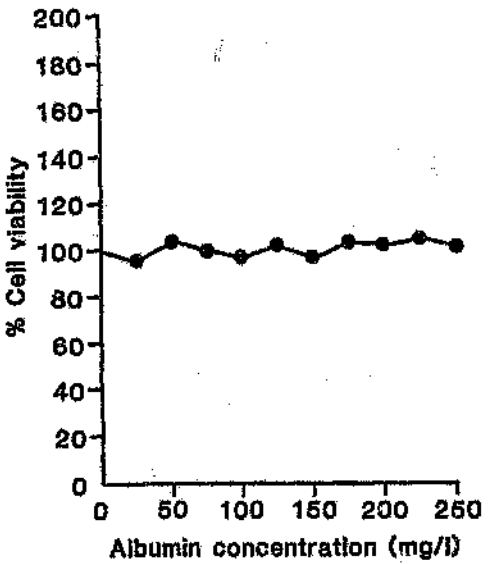
Figs. 5.2.1.2-5.2.1.8. depict the effects of p-oil dosage on human lymphocyte viability. All p-oils induced concentration dependant cytotoxicity, but unsaturated FA-rich p-oils more effective that those abundant in

Legend to Figs. 5.2.1.1-5.2.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where "n" is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 5.2.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 5.2.1.2-5.2.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 5.2.1.1.

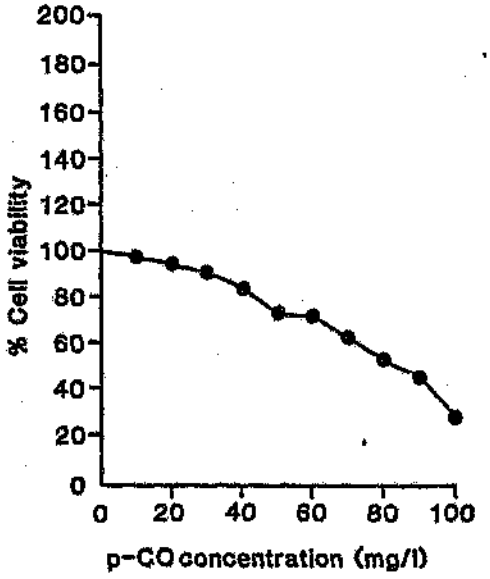
The percentage viability of human lymphocytes incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.3	12
25	95.0	5.0	12
50	102.2	3.6	12
75	98.7	5.2	12
100	95.4	4.0	12
125	101.1	2.9	12
150	95.6	4.7	12
175	102.9	4.8	12
200	100.9	5.3	12
225	104.2	2.4	12
250	100.3	3.5	12

Fig. 5.2.1.2.

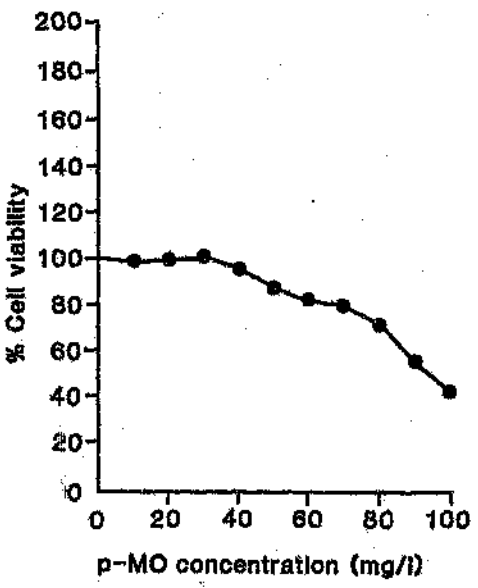
The percentage viability of human lymphocytes incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.9	6
10	96.7	1.6	6
20	95.2	1.8	6
30	90.5	2.2	6
40	83.6	3.2	6
50	73.3	1.7	6
60	72.7	1 7	6
70	62.5	1.1	6
80	53.3	1.3	6
90	45.1	1.2	6
100	28.5	1.3	6

Fig. 5.2.1.3.

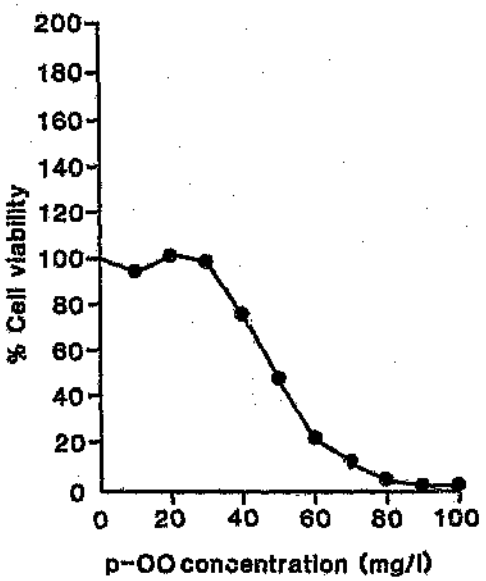
The percentage viability of human lymphocytes incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.4	6
10	97.2	3.9	6
20	98.5	3.1	6
30	99.6	3.9	6
40	95.3	3.0	6
50	87.3	2.9	6
60	80.9	3.6	6
70	78.8	3.3	6
80	70.6	2.9	6
90	54.5	2.3	6
100	41.9	2.1	6

Fig. 5.2.1.4.

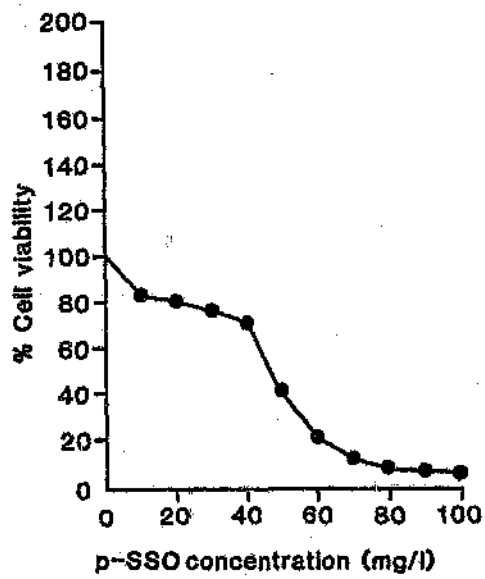
The percentage viability of human lymphocytes incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.0	6
10	94.5	2.7	6
20	101.4	3.9	6
30	98.1	3.5	6
40	75.5	2.7	6
50	47.8	2.6	6
60	23.6	1.8	6
70	12.7	1.7	6
80	4.8	0.7	6
90	1.6	0.5	6
100	0.3	0.2	6

Fig. 5.2.1.5.

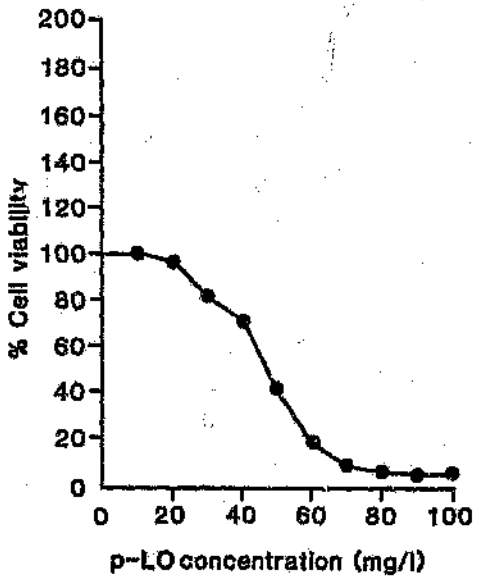
The percentage viability of human lymphocytes incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.4	6
10	82.3	2.0	6
20	80.7	1.6	6
30	76.3	3.6	6
40	70.3	2.2	6
50	41.8	2.8	6
60	22.2	1.3	6
70	13.1	1.0	6
80	8.3	1.6	6
90	7.1	1.2	6
100	6.2	1.1	6

Fig. 5.2.1.6.

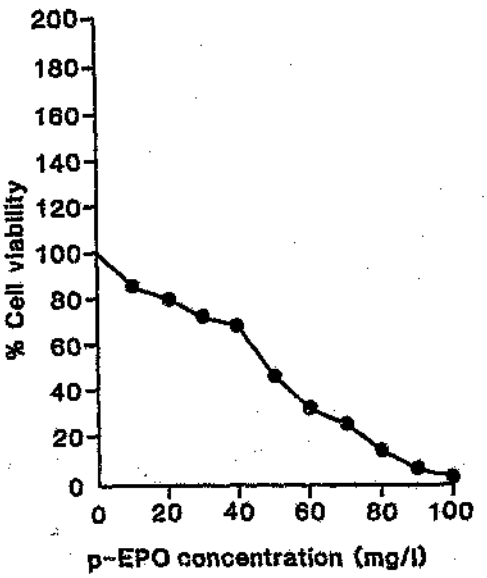
The percentage viability of human lymphocytes incubated with p-LO.



pseudo-Dil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.6	6
10	100.1	5.0	6
20	96.6	4.2	6
30	81.8	5.3	6
40	70.9	3.7	6
50	41.3	0.8	6
60	19.8	1.0	6
70	8.9	1.6	6
80	5.9	1.3	6
90	4.7	0.7	6
100	5.6	2.1	6

Fig. 5.2.1.7.

The percentage viability of human lymphocytes incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.6	6
10	86.0	2.4	6
20	80.4	2.3	6
30	72.7	3.2	6
40	68.0	1.8	6
50	47.5	3.5	6
60	32.2	3.5	6
70	26.2	1.7	6
80	14.5	1.9	6
90	5.8	1.4	6
100	1.4	0.7	6