detected with 20mg/l p-FO (2.0% vs 0.4% in controls), but no increases in 20:4ω6 or 22:4ω6 were found. Dosed lymphocytes incorporated 20:5ω3 in p-FO (1.6%, 1.7% and 2.1%, respectively vs 1.0% in controls), but only trace amounts (≤0.5%) of 22:5ω3 and 22:6ω3 were detected.

3.2.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

Results obtained from the measurement of lipid peroxides both in rat lymphocytes and their respective spent media are shown in Table 3.2.4.1. The values are reported as nmoles MDA/10^6 cells, but in the case of spent growth media, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained.

Lipoperoxide concentrations for dosed lymphocytes ranged from a low of 1.2nmoles MDA/10^6 cells (with 20mg/l p-MO or p-EPO) to a high of 102.6nmoles MDA/10^6 cells (with 60mg/l p-LO), compared to 0.4nmoles MDA/10^6 control lymphocytes. Lipid peroxide amounts increased in every instance with increments in p-oil concentration dosed, and the greatest increase was detected with ω3 PUFA rich-p-oils (5.0, 26.1 and 102.6nmoles MDA/10^6 cells with p-LO, and 7.3, 12.7 and 65.2nmoles MDA/10^6 cells with p-FO, respectively). On the other hand, lymphocytes supplemented with 20, 40 or 60mg/l p-MO or p-EPO induced the smallest concentration dependent increase in lipid peroxides, although the amounts generated were similar (1.2, 3.0 and 8.3nmoles MDA/10^6 cells, and 1.2, 5.1 and 8.4nmoles MDA/10^6 cells, respectively). Lipid peroxide production with p-CO, p-SSO or p-OO dosage was intermediate between the two extremes, ranging from 2.9 to 18.2nmoles MDA/10^6 cells.

Table 3.2.4.1.

Lipid peroxide formation by rat lymphocyte 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		2.9	11.1	15.3
MO		1.2	3.0	8.3
OO		2.8	11.2	18.2
SSO		4.4	9.9	12.4
LO		5.0	26.1	102.6
EPO		1.2	5.1	8.4
FO		7.3	12.7	65 2
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM 0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		-	-	-
LO		-	31.5	102.7
EPO		-	-	-
FO		-	-	10.4

Only spent medium derived from cultures incubated with ω3 PUFA-rich p-oils contained lipid peroxides. Medium derived from cultures incubated with p-FO contained fewer lipoperoxides than the lymphocytes themselves, with significant amounts measured in spent medium only with 60mg/l p-FO (10.4nmoles MDA/10^6 cells). As with the cells, the greatest amounts of medium lipoperoxides were found with 40 or 60mg/l p-LO dosage (31.5 and 102.7 nmoles MDA/10^6 cells, respectively), which correlated with cellular levels.

3.2.5 Discussion.

No reference has been found in the literature with regard to the influence of exogenous FA's on normal rat lymphocyte viability in culture. However, investigation of the effects of exogenous FA's on mitogen-induced DNA synthesis and cell activation in the modulation of mouse T and B lymphocyte proliferation showed that saturated FA's alone were more potent inhibitors of these processes than unsaturated FA's, and that the simultaneous addition of equimolar amounts of a saturated and an unsaturated FA abrogated the inhibitory effect of the former (Tsang et al 1977 and Buttke 1984). The finding that mitogen-activated B cells were much less susceptible to inhibition by FFA's than T cells (Buttke 1984) implied differential sensitivity of lymphocytes to the effects of FFA's. This was clear from comparison with our data. p-Oil supplementation affected rat lymphocyte viability (Figs. 3.2.1.2-3.2.1.8.), but it was unclear whether PUFA-rich p-oils exhibited greater cytotoxic potential than monoenoic or saturated FA-rich p-oils, since the effects induced were p-oil-specific rather than dependent on the degree of p-oil unsaturation. This implied that the modulation of rat lymphocyte viability related both to the nature (type) and proportion of saturated, monoenoic and/or polyenoic FA's dosed (Table 2.3.3.2.). p-EPO was most, and p-SSO least, cytotoxic, despite similar FA compositions,

implying involvement of both major and minor p-oil FA components, and possibly also p-oil FA synergism and/or antagonism, in the effects induced. Comparison of cell viability with p-EPO, p-SSO or p-LO dosage with the respective p-oil FA profiles indicated that GLA was a more effective cytotoxic agent than ALA, and both GLA and ALA exhibited greater cytotoxic potential than LA. This illustrated that both FA chain length and the number and position of double bonds present were involved in the modulation of rat lymphocyte viability.

Lymphocytes do not normally divide in culture without appropriate mitogenic stimulation, thus increased cell viability only with supplementation of 10mg/l p-MO, or 20 or 40mg/l p-OO, indicated FA involvement in the stimulation of rat lymphocyte proliferation at low concentrations. This, together with the finding of Dubin et al (1965) that an optimum concentration of only 0.07mg/l LA effectively promoted macrophage growth in culture, suggests that p-oil concentrations below 10mg/l may also effectively stimulate rat lymphocyte proliferation.

The data in Table 3.2.3.1. confirmed the ability of rat lymphocytes to incorporate exogenous FA's (Tsang et al 1977, Stubbs et al 1980 and Conroy et al 1986). A mechanism by which p-oils affected lymphocyte viability may therefore relate to differential uptake of p-oil

FA's and alterations in membrane fluidity. Studies have indeed shown that modification of rat diets either with olive oil or fish oil altered lymphocyte membrane fluidity, with the fish oil diet more effective in increasing the unsaturation index of the membrane than olive oil (Conroy et al 1986). p-Oil dosed lymphocyte FA compositions were modified compared to controls, thus it was likely that overall membrane fluidity was altered and this involved in the viability changes reported in Figs. 3.2.1.2-3.2.1.8. Supplementation with optimal proportions of saturated and unsaturated p-oil FA's at low concentrations may have allowed the cells to maintain a degree of membrane fluidity compatable with viability, whereas addition of large amounts of highly saturated or unsaturated p-oils may have led to pronounced changes in membrane fluidity such that lymphocyte viability could not continue. However, it was unlikely that eicosanoids were directly involved in the modulation of lymphocyte viability with p-oil dosage since this mechanism could not explain the effects of p-oils rich in saturated or monoenoic FA's, which can not be metabolised by this route. Even EFA-rich p-oils could not act as sources of eicosanoid precursors since the data in Table 3.2.3.1. indicated that rat lymphocytes exhibited no significant capability for PUFA desaturation (Δ6D, Δ5D and Δ4D) or elongation. Others have similarly shown that neither [^{14}C]-18:2ω6 nor [^{3}H]-20:4ω6 were further metabolised by these enzymic

pathways in rat leukocytes, despite considerable and rapid uptake into lipid pools, and suggested that the desaturase cascade was a minor route for FA metabolism in these cells (Brenner et al 1966, Mead et al 1976, Kanau et al 1977 and Cunnane et al 1984).

Detection of small, but significant, increases in 20:3ω6 levels with p-OO or p-FO dosage, which contained small amounts of 20:2ω6 (Table 2.3.3.2.), implied possible Δ8D capability. This may reflect an attempt by the cells to maintain membrane PUFA levels by activating Δ8D when Δ6D expression is suppressed/inhibited. However, it appeared that this mechanism was unable to sufficiently maintain lymphocyte PUFA levels since Δ8D substrate availability was limited by the inability to elongate 18:2ω6 incorporated with p-oil supplementation.

Zevallos et al (1989) showed that when rat lymphocytes were incubated with either [^{14}C]-18:0 or [^{14}C]-18:2ω6, in the presence of either diheptadecanoylphosphatidyl-choline or dioleylphosphatidylcholine, the fluidity of all membranes, including those of the microsomes, increased. Such membrane fluidisation was associated with depressed conversion of 18:0 to 18:1ω9, and 18:2ω6 to 18:3ω6 via Δ9D and Δ6D, respectively. The data in Table 3.2.3.1. showed that p-oil incorporation altered rat lymphocyte FA profiles. This may have altered the physical state (fluidity) of microsomal membranes and

depressed desaturase activity. Such may have contributed to absence of significant desaturase/elongase activity reported, and in particular, may explain the lack of PDFA formation from 18:2ω6 or 18:3ω3 incorporated with p-SSO, p-EPO or p-LO incubation (Table 3.2.3.1.). 18:1ω9 levels were significantly increased with p-oil dosage, but such probably reflects decreased PDFA levels and the fact that all p-oils contained 18:1ω9 in amounts ranging from about 5% to 70% (Table 2.3.3.2.), rather than 18:1ω9 formation from 16:0, 16:1ω9 and/or 18:0. It was unlikely, however, that the desaturase and elongase activity reported related to p-oil induced cytotoxicity since no significant evidence for such enzyme capability was found even when p-oils induced little or no toxicity.

Rode et al (1982) found between 4.5 and 6.0μg total protein/10^6 control lymphocytes, and this was in close agreement with the data reported in Table 3.2.2.1. The results presented also indicated that rat lymphocyte protein synthesis was significantly stimulated in a concentration dependent manner with increased p-oil concentration dose (Table 3.2.2.1.), despite the inverse correlation with cell viability (Figs. 3.2.1.2-3.2.1.8.). p-Oils were thus potent agents for enhancing rat lymphocyte protein synthesis, and the extent to which such was stimulated was relatable to p-oil FA composition (Table 2.3.3.2.). The finding that saturated

FA-rich p-oils were gene y most, and PUFA-rich p-oils least, effective in enhancing protein synthesis implied that the ability to modulate rat lymphocyte protein production related partly to p-oil unsaturation. This, did not explain why p-MO, for example, induced more protein than p-CO, although it was possible that such differences related to the mediation of synergistic or antagonistic effects between p-oil FA's.

Increased lymphocyte protein synthesis may reflect promotion of lymphocyte activation, enhanced protein synthesis in different cell components, as was shown by Rode et al (1982) in concanavalin A stimulated lymphocytes, increased enzyme synthesis to metabolise incorporated p-oil FA's, and/or enhanced biosynthesis of proteins required to maintain the integrity of cell membranes damaged directly with p-oil dosage, or indirectly by lipoperoxide formation (Table 3.2.4.1.). However, it was unlikely that increased protein synthesis related to enhanced desaturase or elongase expression in light of the data reported in Table 3.2.3.1.

The data pr ented in Table 3.2.4.1. was consistent with intact cellular enzymic and/or non-enzymic mechanisms for the production of lipoperoxides. Large amounts of PUFA's were detected in control lymphocytes (Table 3.2.3.1.), thus the small amounts of lipoperoxides measured may reflect cellular anti-oxidant and enzymic

mechanisms of protection against free radical attack. It was also possible that cellular PUFA's were present as components of complex lipids, eg. membrane PGL's, which would therefore not be so readily accessible to oxidation. On the other hand, saturation of the cellular anti-oxidant mechanisms and/or increased cellular FA availability may have occurred with incorporation of supplemented p-oils, and the lipoperoxides measured thus reflected the p-oil concentration dosed, p-oil FA composition (Table 2.3.3.2.) and FA susceptibility to oxidation. Measurement of the largest amounts of cellular and medium lipoperoxides with p-LO or p-FO dosage correlated with the significant ω3 PUFA levels in these p-oils. The significantly greater amount of ALA in p-LO relative to EPA in p-FO (approximately 63% and 18%, respectively) was consistent with the greatest lipid peroxide levels measured, both in the cells and spent medium, despite the greater susceptibility of EPA to oxidation. Rat lymphocytes were shown to incorporate p-oil FA's (Table 3.2.3.1.), thus the medium lipid peroxides measured with p-LO or p-FO dosage probably reflect cellular lipoperoxides released upon cell lysis, and/or released cellular FA's oxidised extracellularly by released cellular peroxidising enzymes.

Mosconi et al (1988) reported that superoxide production by white blood cells increased significantly when rat diets were supplemented with fish oil compared to olive

oil, and this correlated with the findings in Table 3.2.4.1. The significantly lower amounts of cellular lipoperoxides measured with p-MO, p-OO, p-SSO or p-EPO, compared to p-LO or p-FO, suggested that more substrates for peroxidation were lipid bound and stable with incubation of the former group, despite all these p-oils containing significant amounts of unsaturated FA's. This finding was consistent with the absence of lipoperoxides in the corresponding spent medium with p-MO, p-OO, p-SSO or p-EPO dosage, despite the mediation of cell lysis. The cellular lipid peroxides formed with p-SSO or p-EPO incubation possibly related to oxidation of incorporated LA or other cellular FA's. Both p-oils exhibited similar FA compositions, thus the greater lipoperoxide amounts induced with p-SSO suggested enhanced PGL cycling, which would have increased the rate of FA incorporation, release and pool of FFA's available for peroxidation. Alterations in PGL turnover could also have been consistent with the greater amounts of lipid peroxides measured with dosage of p-CO or p-OO than p-MO or p-EPO.

Current evidence suggests that lipid peroxides affect normal cell metabolism and cause membrane damage (Chio et al 1969, Mead 1976, Tappel 1980, Gavino et al 1981c, Morisaki et al 1982b and Frankel 1984), thus it was possible that such compounds were involved in the modulation of cytotoxicity induced with p-oil dosage. This may explain the inverse relationship between

lipid peroxide levels (Table 3.2.4.1.) and lymphocyte viability (Figs. 3.2.1.2-3.2.1.8.) with increased p-oil dosage. However, the finding that the greatest lipid peroxide levels did not correlate with the greatest cytotoxicity implied that these compounds were only partly involved in the modulation of rat lymphocyte viability. The data presented rather suggested that both lipoperoxides and alterations in membrane fluidity were involved in the effects induced.

3.3 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM RAT AORTIC ENDOTHELIUM.

3.3.1 Effects of pseudo-Oils on Cell Viability.

No morphological changes were observed between control cells before, during or after each experiment, and p-oil dosed cells were morphologically similar to controls, although cytoplasmic droplets were observed with p-oil incubation at high concentrations in some instances.

Investigation of the effects of 0 to 250mg albumin/l growth medium showed no significant changes in rat aortic endothelial cell viability (Fig. 3.3.1.1.), nor were any synergistic effects evident. The effects induced with p-oil dosage were thus a result of the mixture of 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 $10.2x10^4$/ml. This equated to approximately 89% 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 89% were considered cytotoxic.

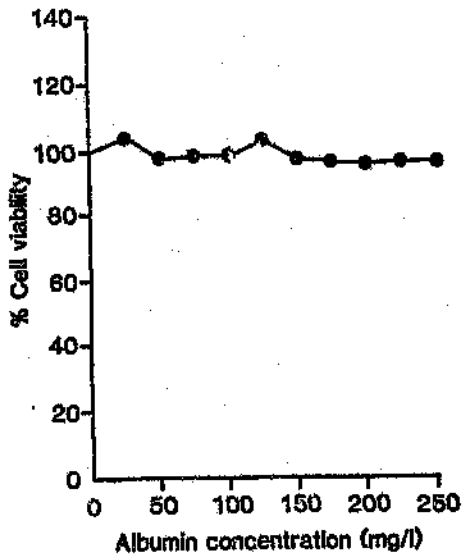
Figs. 3.3.1.2-3.3.1.8. show the results obtained from the incubation of rat endothelial cells with p-oils.

Legend to Figs. 3.3.1.1-3.3.1.9.

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. 3.3.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 3.3.1.2-3.3.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 3.3.1.9. 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. 3.3.1.1.

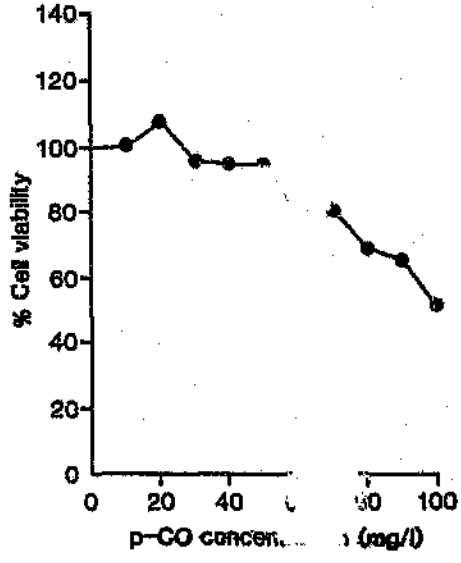
The percentage viability of cells derived from rat aortic endothelium incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.3	12
25	103.4	5.5	12
50	96.5	3.0	12
75	98.0	3.4	12
100	97.7	2.3	12
125	102.8	5.1	12
150	97.1	3.7	12
175	95.9	3.3	12
200	94.9	2.6	12
225	95.5	2.8	12
250	96.0	4.4	12

Fig. 3.3.1.2.

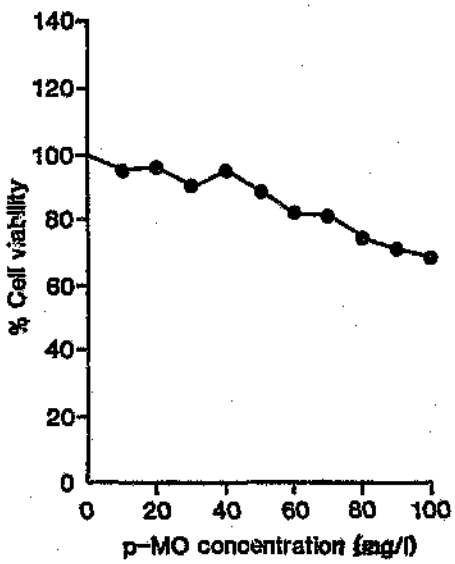
The percentage viability of cells derived from rat aortic endothelium incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.4	6
10	100.5	2.1	6
20	108.4	3.6	6
30	95.6	2.7	6
40	95.3	4.1	6
50	95.0	3.5	6
60	80.3	4.4	6
70	81.2	4.6	6
80	69.0	1.8	6
90	65.6	2.7	6
100	51.2	4.3	6

Fig. 3.3.1.3.

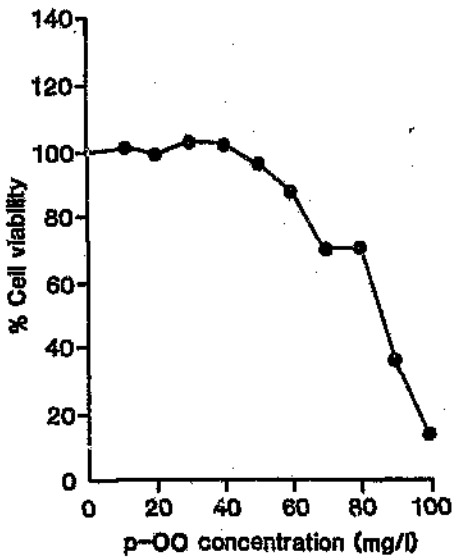
The percentage viability of cells derived from rat aortic endothelium incubated with p-MO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.3	6
10	96.0	2.0	6
20	97.0	2.3	6
30	90.9	2.6	6
40	96.3	1.0	6
50	89.9	2.4	6
60	82.8	2.3	6
70	82.5	2.8	6
80	75.4	2.3	6
90	72.1	2.1	6
100	68.7	1.5	6

Fig. 3.3.1.4.

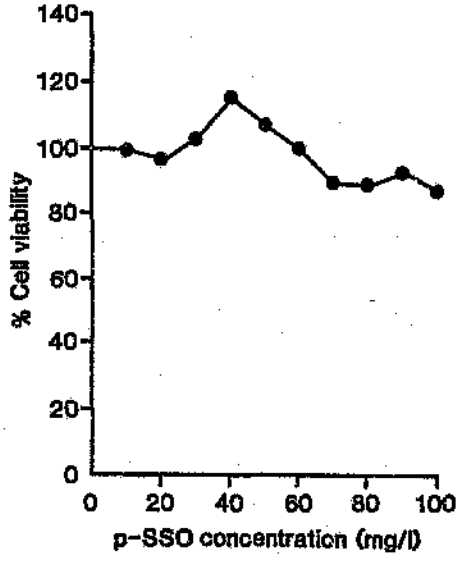
The percentage viability of cells derived from rat aortic endothelium incubated with p-OO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.3	6
10	101.2	3.5	6
20	99.6	2.9	6
30	103.2	3.2	6
40	102.0	2.7	6
50	97.2	2.9	6
60	88.5	2.4	6
70	70.8	2.2	6
80	72.3	2.8	6
90	36.8	3.2	6
100	13.4	2.0	6

Fig. 3.3.1.5.

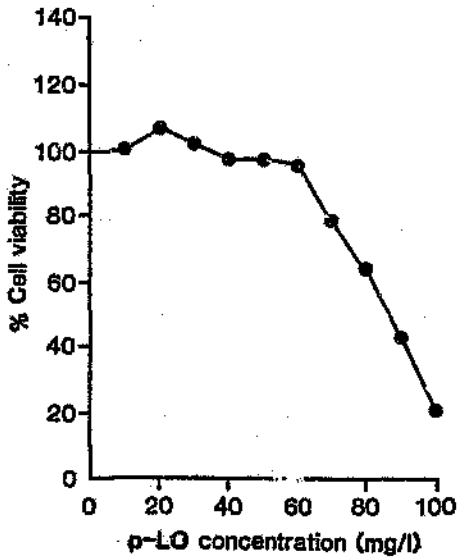
The percentage viability of cells derived from rat aortic endothelium incubated with p-SSO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.6	6
10	99.2	3.7	6
20	96.1	6.8	6
30	102.0	5.7	6
40	115.4	2.3	6
50	106.7	4.5	6
60	99.8	4.4	6
70	89.5	3.6	6
80	88.3	3.3	6
90	92.6	6.3	6
100	86.4	4.0	6

Fig. 3.3.1.6.

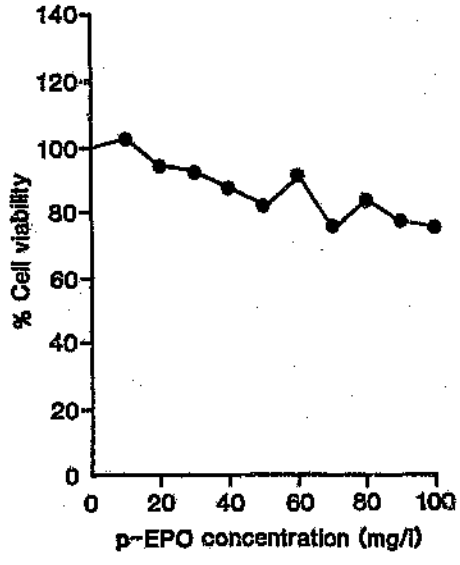
The percentage viability of cells derived from rat aortic endothelium incubated with p-LO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	101.0	3.5	6
20	107.5	3.8	6
30	102.0	3.3	6
40	98.0	3.5	6
50	97.5	4.2	6
60	96.0	4.0	6
70	79.1	3.5	6
80	64.7	2.6	6
90	42.8	3.2	6
100	20.9	3.2	6

Fig. 3.3.1.7.

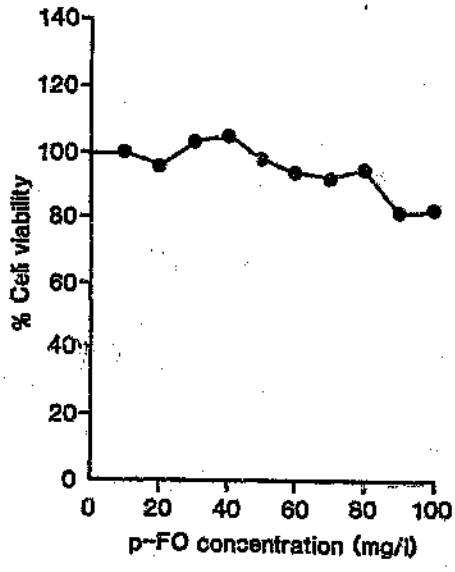
The percentage viability of cells derived from rat aortic endothelium incubated with p-EPO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.5	6
10	102.8	3.5	6
20	93.9	4.0	6
30	92.1	3.7	6
40	87.7	4.2	6
50	82.1	1.8	6
60	91.5	4.6	6
70	75.9	3.3	6
80	83.5	5.1	6
90	77.5	4.0	6
100	75.6	5.1	6

Fig. 3.3.1.8.

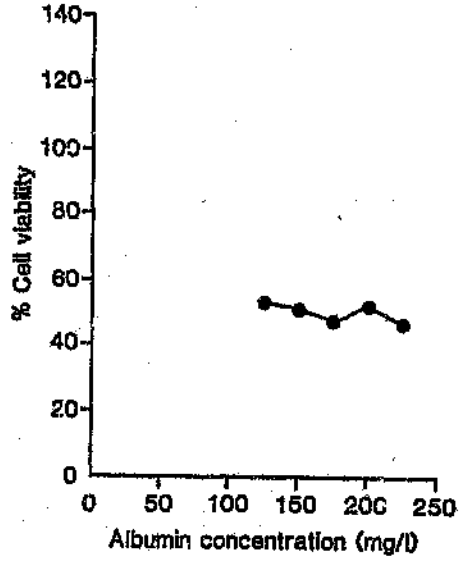
The percentage viability of cells derived from rat aortic endothelium incubated with p-FO.



pseuso-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.1	6
10	100.9	4.2	6
20	96.4	3.0	6
30	103.3	3.6	6
40	105.4	3.1	6
50	97.9	2.5	6
60	94.3	5.1	6
70	92.5	4.2	6
80	94.9	4.0	6
90	82.2	3.4	6
100	83.4	3.3	6

Fig. 3.3.1.9.

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



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.1	6
125	53.4	2.5	12
150	51.7	5.1	12
175	47.7	4.5	12
200	52.2	3.1	12
225	46.4	2.2	12

Certain concentrations of some p-oils stimulated cell proliferation, and all p-oils limited cell viability to varying extents, but only p-CO, p-OO and p-LO induced significant cytotoxicity.

Dosage with 20mg/l p-CO stimulated cell proliferation to 108.4%, whereas p-MO did not cause any significant rise in cell viability with any concentration supplemented (Figs. 3.3.1.2. and 3.3.1.3., respectively). Both p-oils induced cytostasis in the range 50 to 60mg/l and cytotoxicity with greater concentrations, although p-CO was slightly more effective than p-MO. Cell viability was reduced to 51.2% and 68.7% with 100mg/l p-CO and p-MO, respectively, thus ID_{50} values could not be calculated.

No significant change in cell viability occurred with dosage of up to 50mg/l p-OO or 60mg/l p-LO, although 20mg/l p-LO stimulated cell proliferation to a maximum of 107.5% (Figs. 3.3.1.4. and 3.3.1.6., respectively). These p-oils induced the greatest cytotoxicity with concentrations greater than 60mg/l, and reduced cell viability almost linearly to 13.4% and 20.9% with 100mg/l p-oil, respectively. ID_{50} values for p-OO and p-LO were alike (about 86mg/l and 87mg/l, respectively), and this supported the similar effects these p-oils induced.

p-SSO dosage induced the greatest growth stimulatory and least limiting effects (Fig. 3.3.1.5.). Incubation with

10 or 20mg/l p-SSO had little effect on cell viability, but such was stimulated in the range 20 to 60mg/l p-SSO to a maximum of 115.4% with 40mg/l, and limited to approximate the cytostatic number (about 89%) with concentrations greater than 60mg/l p-SSO.

10mg/l p-EPO had little effect on cell viability, but concentrations of 20 to 60mg/l p-EPO were slightly growth limiting or cytostatic (Fig. 3.3.1.7.). Cell viability was not significantly altered with p-FO dosage up to 80mg/l, but was marginally stimulated to 105.4% with 40mg/l p-FO (Fig. 3.3.1.8.). Both p-oils, however, induced little cytotoxicity as relative cell viability was only slightly lower than the cytostatic number with 100mg/l p-oil (75.6% and 83.4%, respectively).

Cells were incubated with p-OO to correspond with its ID_{50} (about 86mg/l) and dosed with varying amounts of albumin to exclude the possibility that the effects induced with p-oil dosage 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 concentration 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. 3.3.1.9., and indicated that the concentration of p-OO supplemented reduced cell viability to about 50% of controls, and that such was not significantly influenced

by different albumin concentrations. 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.

Rat 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 performed. Cell viabilities were assessed upon harvesting, and found not t. be statistically different from those in Figs. 3.3.1.1-3.3.1.8. All further biochemical assays were performed on these samples.

3.3.2 Effects of pseudo-Oils on Total Protein.

The results obtained from the determination of total protein in control and dosed cells are shown in Table 3.3.2.1. as µg total protein/10^6 cells seeded.

181.1µg protein was measured per 10^6 control cells seeded, while cells incubated with 60mg/l p-CO exhibited the lowest protein levels (174.2µg/10^6 cells seeded). All other dosed cells contained more protein than controls, and the highest amount measured was 254.7µg protein/10^6 cells seeded (with 40mg/l p-SSO).

p-Oil dosage induced concentration dependent changes in cellular protein. Total protein concentrations increased in direct correlation with the amount of p-OO dosed, but were generally inversely related to the p-CO, p-MO, p-LO or p-EPO concentration supplemented. With p-SSO or p-FO incubation, however, the highest protein concentrations measured were with a concentration of 40mg/l.

Table 3.3.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	181.1			
CO		212.3	188.1	174.2
MO		195.0	195.5	188.5
OO		198.4	215.7	226.1
SSO		201.5	254.7	201.9
LO		227.9	195.0	187.5
EPO		201.9	195.4	194.2
FO		184.6	227.9	219.3

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

Table 3.3.3.1. shows the FA spectra of rat aortic endothelial cells incubated with 0, 20, 40 or 60mg/l of each p-oil.

Control cells exhibited a FA spectrum in which no one FA was present in amounts greater than 20.5% (for 16:0). Other FA's contributing ≥5.0% to the total control spectrum included 18:0 (19.4%), 16:1ω9 (6.1%), 18:1ω9 (15.0%) and 22:4ω6 (13.5%). The remaining 25.5% was made up of small amounts (<5.0%) of 18:2ω6, 18:3ω6, 18:3ω3, 18:4ω3, 20:3ω6, 20:4ω6, 20:4ω3, 20:5ω3, 22:5ω6, 22:5ω3 and 22:6ω3. The above FA's were detected in varying amounts in the dosed cells.

In general, 16:0 levels were variable, and 18:1ω9 levels similar, between control and dosed cells, while 16:1ω9 and 18:0 percentages were higher in control cells. Dosed cells contained at least twice as much 18:2ω6 than controls, ω6 PDFA levels were variable depending on the p-oil and concentration supplemented, while small but significant increases in 22:5ω3 and 22:6ω3 were detected in dosed cells.

16:0 levels were increased with dosage of 20mg/l p-CO (27.5%), but parallel to controls (20.5%) with 40mg/l

Legend to Table 3.3.3.1.

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 3.3.3.1.

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

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	20.5±0.20	27.5	19.9	16.7	24.6	20.2	26.1	23.4	17.8	21.6	12.9	17.8	24.3	27.3	18.3	6.5	27.7	16.3	24.1	29.4	20.4	23.7
	18:0	19.4±0.70	14.3	14.2	26.1	13.7	15.1	16.3	11.5	10.7	10.3	14.3	12.8	13.9	13.9	15.8	28.0	16.6	15.9	13.5	14.4	14.3	11.9
	20:0	-	-	0.2	2.5	-	-	-	-	-	-	-	0.2	-	-	0.1	1.2	-	-	-	-	-	-
	22:0	-	0.2	0.2	-	0.1	0.2	0.1	-	-	-	-	-	-	0.1	0.2	-	-	-	-	-	-	-
	24:0	-	-	0.5	-	-	0.3	-	-	0.3	-	-	0.5	-	-	0.2	-	-	0.2	-	-	-	-
ω9 MONOS.	16:1	6.1±0.20	6.1	1.5	1.4	4.7	4.6	4.1	6.0	5.6	3.8	0.3	1.5	5.0	6.9	1.0	0.7	6.2	1.4	5.2	8.9	7.8	9.8
	18:1	15.0±0.15	15.9	15.6	24.2	14.7	15.9	18.9	21.5	29.5	35.1	15.9	14.8	16.0	15.6	15.8	22.5	14.9	13.3	13.7	15.1	16.1	16.3
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	2.8±0.05	6.9	4.7	11.6	8.2	9.2	10.8	8.2	8.6	10.2	19.4	21.6	19.0	10.5	10.9	15.6	13.4	20.1	19.4	7.6	5.6	8.2
	18:3	0.8±0.05	0.9	-	-	0.9	0.2	0.7	0.6	0.1	0.6	1.7	-	0.7	1.2	-	-	1.1	0.3	0.9	1.2	0.2	0.8
	20:2	-	-	-	-	-	0.2	-	0.4	0.6	1.2	0.5	1.5	0.8	-	-	-	0.3	1.0	0.4	-	0.3	0.2
	20:3	4.7±0.35	1.3	2.2	0.8	1.1	1.7	1.2	1.0	1.5	1.0	1.2	1.6	1.1	0.9	1.5	2.1	2.1	5.0	3.6	0.7	1.5	1.0
	20:4	4.1±0.05	3.0	5.0	2.4	3.7	6.0	3.5	2.3	3.7	2.2	2.4	2.7	2.0	2.8	5.1	2.3	1.8	2.8	1.7	2.0	3.7	2.2
	22:4	13.5±0.30	15.1	19.7	6.4	18.9	16.3	11.1	18.0	11.0	7.2	20.4	15.0	10.7	10.7	12.1	0.7	9.4	11.6	11.3	10.4	12.8	10.2
	22:5	0.9±0.00	-	2.3	0.3	0.1	0.9	-	0.2	1.3	0.1	0.4	1.5	0.2	0.2	1.4	0.2	0.2	2.7	0.2	0.3	1.6	-
ω3 POLYS.	18:3	0.2±0.01	0.8	0.6	2.2	1.1	0.7	0.7	1.0	1.2	1.0	1.5	0.5	0.6	3.8	8.4	11.7	0.9	0.3	0.5	1.1	0.5	0.9
	18:4	3.9±0.30	0.4	1.2	0.5	0.4	1.0	0.2	0.3	1.3	0.2	0.7	0.6	0.3	0.3	2.0	7.3	0.4	1.7	0.3	0.5	0.1	0.3
	20:4	2.0±0.10	1.5	0.9	1.3	2.4	0.8	0.9	1.5	0.2	0.7	3.5	0.4	1.2	1.8	0.5	0.1	1.7	0.7	1.6	2.4	0.7	2.1
	20:5	3.3±0.10	0.9	2.5	0.5	0.5	0.7	0.5	0.3	1.2	0.7	0.2	1.6	0.2	0.5	0.9	0.1	0.2	1.7	0.1	1.6	3.8	3.7
	22:5	1.2±0.10	2.1	3.3	1.4	2.0	2.6	2.0	1.6	2.4	1.6	2.0	2.3	1.8	1.6	2.5	0.4	1.4	2.2	1.5	3.1	8.0	6.6
	22:6	1.7±0.30	3.1	5.3	1.9	2.9	3.4	3.0	2.4	3.4	2.4	2.8	3.3	2.5	2.3	3.5	0.5	1.9	2.9	2.1	1.4	2.8	2.3

p-CO (19.9%), and decreased with 60mg/l p-CO (16.7%). 16:1ω9 levels decreased considerably with 40 and 60mg/l (1.5% and 1.4%, respectively vs 6.1% in controls), while notable increases in 18:0, 20:0 and 18:1ω9 occurred with 60mg/l (26.1%, 2.5% and 24.2% vs 19.4%, 0% and 15.0% in controls, respectively). Dosed cells contained 4.7% to 11.6% 18:2ω6 and 0.6% to 2.2% 18:3ω3 (2.8% and 0.2% in controls, respectively), and the greatest increasr in 22:4ω6, 22:5ω6, 22:5ω3 and 22:6ω3 occurred with 40mg/l p-CO (19.7%, 2.3%, 3.3% and 5.3% vs 13.5%, 0.9%, 1.2% and 1.7% in controls, respectively).

p-MO dosage induced an overall increase in 16:0 levels (20.2% to 26.1% vs 20.5% in controls), a decrease in 16:1ω9 and 18:0 (4.1% to 4.7% and 13.7% to 16.3% vs 6.1% and 19.4% in controls, respectively), but little change in 18:1ω9 (14.7% to 18.9% vs 15.0% in controls). 18:2ω6 incorporation increased significantly from 8.2% to 9.2% and 10.8% with 20, 40 and 60mg/l p-MO, respectively (2.8% in controls). 20:4ω6 was only increased with 40mg/l (6.0% vs 4.1% in controls), although 18.9% and 16.3% 22:4ω6 was found with 20 and 40mg/l, respectively (13.5% in controls). Small but significant increases in 22:5ω3 (2.0% to 2.6%) and 22:6ω3 (2.9% to 3.4%) were also found (1.2% and 1.7% in controls, respectively).

Supplementation with 20, 40 or 60mg/l p-OO resulted in concentration dependent 18:1ω9 incorporation (21.5%, 29.5% and 35.1%, respectively vs 15.0% in controls),

followed by a concommitant decrease in both 16:1ω9 (6.0%, 5.6% and 3.8%, respectively vs 6.1% in controls) and 18:0 (11.5% 10.7% and 10.3%, respectively vs 19.4% in controls), but little overall change in 16:0 levels (23.4%, 17.8% and 21.6%, respectively vs 20.5% in controls). Neither 18:3ω6, 20:3ω6 nor 20:4ω6 levels were increased, despite 18:2ω6 and 20:2ω6 incorporation, although 18.0% 22:4ω6 and 1.3% 22:5ω6 were detected with 20 and 40mg/l p-OO (13.5% and 0.9% in controls, respectively). Dosed cells also contained slightly elevated proportions of 22:5ω3 and 22:6ω3, especially with 40mg/l (2.4% and 3.4% vs 1.2% and 1.7% in controls, respectively).

p-SSO supplementation had little effect on 18:1ω9, but decreased overall 16:0, 16:1ω9 and 18:0, levels (15.0%, 20.5%, 6.1% and 19.4% in controls, respectively). 2.8%, 19.4%, 21.6% and 19.0% 18:2ω6 were found with dosage of 0, 20, 40 or 60mg/l p-SSO, with small amounts of 20:2ω6 detected over the entire range dosed (0.5% to 1.5% vs 0% in controls). 18:3ω6 was increased only with dosage of 20mg/l p-SSO (1.7% vs 0.8% in controls), and 20:3ω6 and 20:4ω6 were decreased over the entire range dosed. However, 20.4% and 15.0% 22:4ω6 were found with 20 and 40mg/l, respectively, and 1.5% 22:5ω6 with 40mg/l (13.5% and 0.9% in controls, respectively). Increases in 22:5ω3 and 22:6ω3 were also detected, especially with 40mg/l (2.3% and 3.3% vs 1.2% and 1.7% in controls,

respectively).

The results obtained for cells dosed with 20, 40 or 60mg/l p-LO indicated concentration dependent 18:3ω3 incorporation (3.8%, 8.4% and 11.4%, respectively vs 0.2% in controls). 18:4ω3 increased significantly only with 60mg/l (7.3% vs 3.9% in controls), although 22:5ω3 and 22:6ω3 were enhanced slightly with 20mg/l, and approximately two fold with 40mg/l, compared to controls (1.2% and 1.7%, respectively). 16:0 levels dropped from 27.3% to 18.3% and 6.5%, and 18:0 increased from 13.9% to 15.8% and 28.0% with 20, 40 and 60mg/l p-LO (20.5% and 19.4% in controls, respectively). 6.9% 16:1ω9 was found with 20mg/l, but this decreased to 1.0% and 0.7% with 40 and 60mg/l, respectively (6.1% in controls), and 18:1ω9 levels increased significantly only when 60mg/l p-LO was dosed (22.5% vs 15.0% in controls). 18:2ω6 percentages ranged from 10.5% to 15.6% (2.8% in controls), with 1.2% 18:3ω6 detected with 20mg/l (0.8% in controls), and 5.1% 20:4ω6 with 40mg/l (controls 4.1%). There was, however, a concentration dependent decrease in 22:4ω6 from 13.5% in controls to 0.7% with 60mg/l, although 1.4% 22:5ω6 was detected with 40mg/l (0.9% in controls).

16:0 amounts ranged from 16.3% to 27.7% with p-EPO incubation (20.5% in controls), while overall 16:1ω9, 18:0 and 18:1ω9 levels were slightly decreased (6.1%,

19.4% and 15.0% in controls, respectively). 18:2ω6 incorporation was marked (13.4%, 20.1% and 19.4% with 20, 40 and 60mg/l p-EPO, respectively vs 2.8% in controls), with up to 1.0% 20:2ω6 detected (0% in controls). With the exception of 2.7% 22:5ω6 with 40mg/l p-EPO (0.9% in controls), ω6 PDFA levels were parallel to controls, or decreased, although up to 2.2% 22:5ω3 and 2.9% 22:6ω3 were detected (1.2% and 1.7% in controls, respectively).

Incubation with 20mg/l p-FO resulted in significant 16:0 incorporation (29.4%), while 18:0 percentages were slightly decreased over the entire range dosed, in relation to controls (20.5% and 19.4%, respectively). 18:1ω9 levels were parallel to controls (15.0%), with 7.8% to 9.8% 16:1ω9 and 7.6% to 8.2% 18:2ω6 detected (6.1% and 2.8% in controls, respectively). With the exception of 1.6% 22:5ω6 with 40mg/l p-EPO (0.9% in controls), ω6 series PDFA levels were parallel to controls, or decreased. 1.6%, 3.8% and 3.7% 20:5ω3 were detected with 20, 40 and 60mg/l p-FO, respectively (3.3% in controls), although 22:5ω3 levels were significantly, and 22:6ω3 slightly, increased compared to controls (1.2% and 1.7%, respectively).

3.3.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 3.3.4.1. shows the lipoperoxides measured in the cultured cells and their respective growth media. The values are shown as nmoles MDA/10^6 cells, but in the case of the spent incubation media, this reflects the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained.

Lipid peroxide formation in dosed cells ranged from 0.7 to 2.7nmoles MDA/10^6 cells (with 20mg/l p-FO and 60mg/l p-LO, respectively) compared to 0.7nmoles MDA/10^6 controls cells. Lipid peroxide formation increased in an overall concentration dependent manner with increased amount of p-oil dosed. Cells incubated with 20mg/l p-oil generally induced <1.0nmole MDA/10^6 cells, while amounts ranged from 1.1 to 2.3 and 1.1 to 2.7nmoles MDA/10^6 cells with supplementation of 40 or 60mg/l p-oil, respectively. No clear relationship between lipid peroxidation and degree of p-oil unsaturation was evident, although cells incubated with 40 or 60mg/l p-LO generated the largest lipoperoxide amounts at these concentrations (2.3 and 2.7nmoles MDA/10^6 cells, respectively). Lipid peroxides were, however, absent from all spent media.

Table 3.3.4.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.7			
CO		1.1	1.8	1.7
MO		0.9	1.1	1.4
OO		1.3	1.3	1.9
SSO		0.9	1.3	1.1
LO		0.9	2.3	2.7
EPO		0.9	1.1	1.7
FO		0.7	1.1	1.8

pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		-	-	-
LO		-	-	-
EPO		-	-	-
FO		-	-	-

3.3.5 Discussion.

No reference has been found in the literature in which the effects of FA's on the growth of rat aortic endothelial cells has been found. This study showed that p-oil supplementation affected rat aortic endothelial cell viability (Figs. 3.3.1.2-3.3.1.8.). However, it was unclear whether PUFA-rich p-oils generally exhibited greater growth stimulatory or cytotoxic potential than monoenoic or saturated FA-rich p-oils as the effects induced were not consistent with the degree of p-oil unsaturation. This may relate to differences in p-oil FA composition (Table 2.3.3.2.) and to FA's in the p-oils acting synergistically or antagonistically with each other to modulate the effects induced. Such may explain why p-oils of similar FA composition (p-EPO and p-SSO) induced different effects. The large amount of LA in p-EPO and p-SSO (about 70%) probably accounted for the effects these p-oils induced, although the greater capability for growth limitation and inability to stimulate cell proliferation significantly with p-EPO dosage may have related to the presence of about 9% GLA in p-EPO only. These findings implied LA involvement in the stimulation of cell proliferation and that GLA was a more potent growth inhibitory agent than LA. The large proportion of ALA in p-LO and OA in p-OO (approximately 63% and 71%, respectively) probably accounted largely for the extensive toxicity these p-oils induced with

high concentrations dosed, unlike both p-EPO and p-SSO. These moieties therefore appeared considerably more cytotoxic than LA, and such could reflect differences in the number and position of double bonds in these C18 FA's.

Rat aortic endothelial cells incorporated the exogenous FA's dosed (Table 3.3.3.1.), thus the p-oils may have induced their effects by alterations in membrane fluidity as there is substancial evidence to suggest that dietary supplementation with FA's induces changes both in membrane physical properties and membrane-associated cell functions and (King et al 1971, Ginsberg et al 1981, Simon et al 1982 and Stubbs et al 1984). Incubation with large amounts of p-OO or p-LO may have increased the membrane unsaturation index to such an extent that cell viability could not continue. In contrast, the large complement of saturated FA's (approximately 95%) and absence of PUFA's in p-CO could have inhibited cell viability with high concentrations by shifting membrane fluidity to a less stable state by increasing its rigidification. Such may explain the greater inhibition of cell viability with high concentrations of p-CO than p-MO, p-SSO, p-EPO or p-FO. On the other hand, the finding that p-FO had little overall effect on cell viability may relate to its more even balance of saturated, monoenoic and polyenoic FA's.

The cellular protein concentrations reported with p-oil dosage (Table 3.3.2.1.) generally paralleled the changes observed in cell viability (Figs. 3.3.1.2-3.3.1.8.). The highest protein concentration was for cells dosed with 40mg/l p-SSO, which corresponded to the greatest cell viability increase. This may have represented increased cell number, but with constant or increased protein per cell. The latter possibility seemed more likely as enhanced protein levels were measured even when cell proliferation was parallel to controls, or limited, with p-oil supplementation. The finding that dosed cells contained more protein than controls when cell viability was not significantly impaired indicated that p-oil FA's stimulated protein synthesis, possibly when the requirement for lipid metabolising enzymes increased. On the other hand, decreased protein only with 60mg/l p-CO dosage could have represented decreased cell numbers either with constant or decreased protein per cell.

Endothelial cells play a central role in eicosanoid, and especially prostacyclin, production, and therefore in vascular homeostasis (Moncada et al 1976a). Thus, the ability to desaturate FA's to generate eicosanoid precursors is of prime importance. The data in Table 3.3.3.1. indicated that cultured rat endothelium has the capability to desaturate and elongate PUFA's. Evidence for Δ6D, Δ5D, Δ4D and elongase expression were found with both w3 and w6 series PUFA's with p-oil dosage. The

finding that Δ6D and Δ5D product levels were generally lower, and that Δ4D substrates and products were higher, in dosed cells than in controls implied that the initial reaction steps in the desaturase cascade occurred rapidly. Δ4D product formation was more limited with ω6 than ω3 PUFA substrates, and this confirmed the preference for ω3 desaturase substrates (eg. Brenner 1974 and Kanau et al 1977). The formation of 22:5ω3 and 22:6ω3 even with p-oils which did not contain ω3 PUFA's implied that such p-oils enhanced desaturation and elongation of cellular ω3 PUFA's, and such may reflect a mechanism to restore membrane fluidity altered with exogenous FA incorporation.

Hornstra et al (1981) showed the endothelium of the rat to be very specific in the way in which it metabolises long chain polyenoic FA's. These workers found that rat aorta endothelium PGL 20:4ω6 levels were low when fed diets rich in 18:2ω6, implying inability of these cells to express the rate limiting enzyme, Δ6D. Low 20:4ω6 levels were also found with dosage of p-oils rich in 18:2ω6 (p-EPO or p-SSO) in our study (Table 3.3.3.1.). However, increased 22:4ω6 detected with 20mg/l p-SSO, and enhanced 22:5ω6 levels with 40mg/l p-SSO and p-EPO were indeed consistent with at least some expression of desaturase cascade enzymes. Hornstra et al (1981) did not comment on the levels of these moieties, and decreased 20:4ω6 may merely have reflected further

metabolism to 22:4ω6 and/or 22:5ω6. Hornstra et al (1981) also found low 20:4ω6 levels when fish oil was fed, but 20:5ω3 and 22:6ω3 increased up to 4.0% in the vessel walls. Their findings supported the data in Table 3.3.3.1. with p-FO dosage, although the increments we showed in 20:5ω3 and 22:6ω3 were not as great. On the other hand, 22:5ω3 levels increased by up to 6.8% with p-FO dosage, and 22:5ω6 increased almost 2 fold with 40mg/l p-FO. The data from both studies were nonetheless consistent with elongase and Δ4D expression.

Decreased 16:0 and increased 18:0 levels when rat aortic endothelial cells were incubated with 60mg/l p-CO or p-LO suggested that 16:0 was elongated, and the concommitant increase in 18:1ω9 indicated the potential for Δ9D expression (Table 3.3.3.1.). 16:1ω9 elongation could possibly be excluded as contributing to the enhancement of 18:1ω9 since the incorporation of 16:1ω9 with p-FO supplementation did not produce a concommitant increase in 18:1ω9. Both 18:3ω3 and 18:2ω6 competitively inhibit desaturation of 18:1ω9 (Brenner et al 1966, 1969, Brenner 1977, 1982 and Holman 1986b). Relative EFA percentages were enhanced with p-oil incubation (Table 3.3.3.1.), and such probably explains the lack of 20:3ω9 formation despite significant 18:1ω9 incorporation with p-OO supplementation, significant 18:1ω9 formation with 60mg/l p-CO or p-LO dosage, and the capability of these cells to express Δ6D and Δ5D.

Supplementation with 20, 40 or 60mg/l p-oil induced no significant growth limitation or cytotoxicity (Figs. 3.3.1.2-3.3.1.8.), thus it was unlikely that such limited the extent of desaturase cascade enzyme activity reported (Table 3.3.3.1.). It was more likely that the relative amounts of cellular FA's, both of the same and different series, modulated desaturase expression by competitive interactions which have been well documented (eg. Garcia et al 1956, Brenner et al 1966, Brenner 1974, Ullman et al 1971, de Schr- -r et al 1982 and Nassar 1986). ω3 PUFA's were desaturated and elongated to a greater extent than ω6 PUFA's (Table 3.3.3.1.), thus it was possible that the ω3 PDFA's formed suppressed, or inhibited, ω6 PUFA metabolism, depending on the overall ω3/ω6 PUFA balance in the cell. On the other hand, incubation of cells with saturated FA-rich p-oils (p-CO or p-MO) could have induced a relative EFA/PDFA deficiency, and such may have enhanced desaturase cascade enzyme expression in an attempt to restore membrane PUFA levels.

The results presented in Table 3.3.4.1. indicated that rat aortic endothelial cells exhibit intact enzymic and/or non-enzymic mechanisms for the production of lipoperoxides. Control cell lipid peroxides reflected oxidation of endogenous unsaturated FA's, whereas increased lipid peroxide production in dosed cells reflected oxidation of incorporated exogenous (p-oil)

unsaturated FA's. The finding that lipid peroxide generation increased overall as greater amounts of p-oil were supplemented was consistent with saturation of the protective mechanisms against free radical production, eg. glutathione peroxidase, and therefore increased FA availability for oxidation. However, the similar lipid peroxide amounts formed with incubation of p-CO or p-MO compared to p-OO, p-SSO, p-EPO or p-FO, despite differences in p-oil FA composition and unsaturation (Table 2.3.3.2.), suggested that the substrates for lipoperoxidation in the latter group were not so readily accessible to oxidation, possibly because they were present as components of complex membrane lipids. The absence of lipoperoxides in the spent medium of all dosed cultures indeed supported p-oil FA incorporation. It was also possible that incubation with p-CO or p-MO increased, or that p-OO, p-SSO, p-EPO or p-FO decreased, the rate of PGL turnover and therefore the size of the FFA pool. The above mechanisms could also explain the greatest cellular lipoperoxide amounts measured with 40 or 60mg/l p-LO supplementation, which probably related to the large amount of ALA in p-LO (about 63%) and its susceptibility to oxidation.

Measurement of relatively low lipoperoxide levels in dosed cells and their absence in all spent media (Table 3.3.4.1.) correlated with the lack of significant cytotoxicity induced with dosage of up to 60mg/l p-oil

(Figs. 3.3.1.2-3.3.1.8.). This suggested lipoperoxide involvement in the modulation of cell proliferation, although no correlation with cytotoxicity could be established. Lipoperoxide amounts greater than those reported with dosage of 60mg/l p-oil should, however, not be overlooked as a possible mechanism involved in the modulation of cytotoxicity. Overall, it seems likely that the modulation of rat aortic endothelial cell viability relates both to lipoperoxides and alterations in membrane fluidity.

3.4 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM RAT SKELETAL MUSCLE.

3.4.1 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 induced no significant effect on cell viability (Fig. 3.4.1.1.). The effects induced with the p-oils dosed were therefore 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 $10.6x10^4$/ml. This equated to about 76% 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 76% were considered cytotoxic.

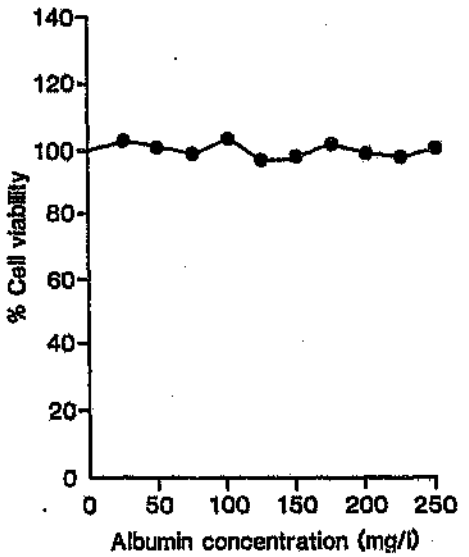
The results of the incubation of cells with p-oils are depicted in Figs. 3.4.1.2-3.4.1.8. Cell proliferation was enhanced only with certain concentrations of p-SSO, p-EPO or p-LO, all p-oils exhibited growth limiting

Legend to Figs. 3.4.1.1-3.4.1.9.

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. 3.4.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 3.4.1.2-3.4.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 3.4.1.9. 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. 3.4.1.1.

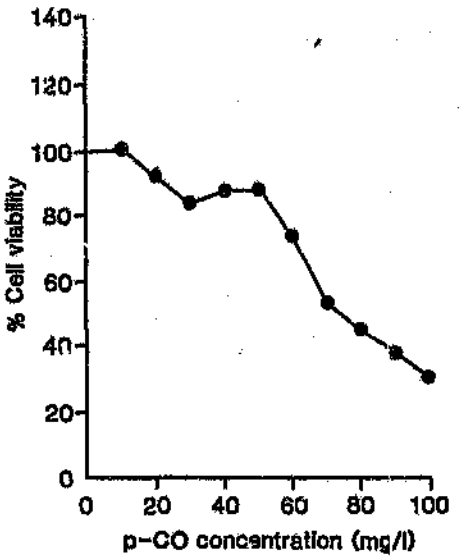
The percentage viability of cells derived from rat skeletal muscle incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.4	12
25	102.3	4.2	12
50	100.7	4.2	12
75	98.9	4.2	12
100	103.6	4.6	12
125	96.7	5.1	12
150	97.9	6.2	12
175	101.4	4.4	12
200	99.1	2.3	12
225	98.1	4.5	12
250	100.8	3.7	12

Fig. 3.4.1.2.

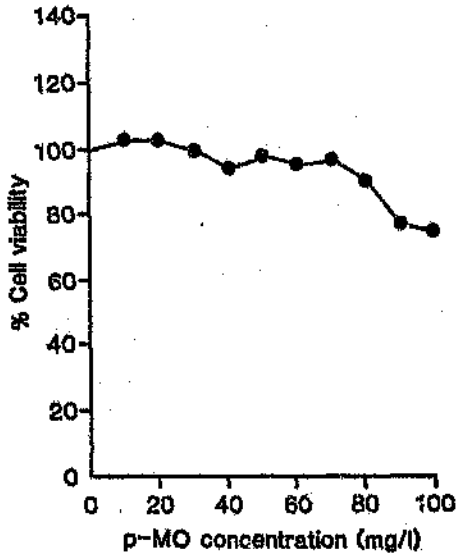
The percentage viability of cells derived from rat skeletal muscle incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	100.2	5.7	6
20	92.2	2.4	6
30	83.6	3.0	6
40	87.6	4.7	6
50	88.4	4.4	6
60	73.7	4.0	6
70	53.5	3.3	6
80	45.4	2.3	6
90	38.0	3.2	6
100	30.4	1.8	6

Fig. 3.4.1.3.

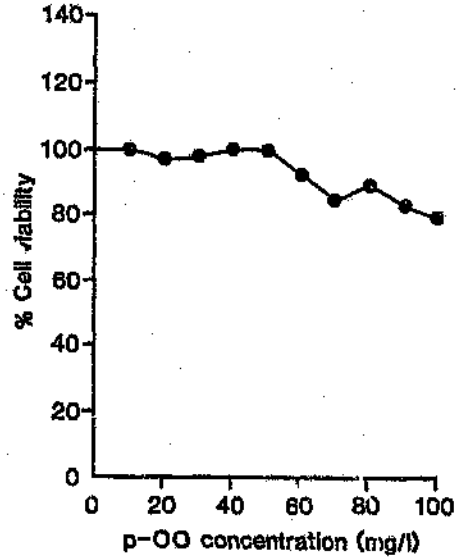
The percentage viability of cells derived from rat skeletal muscle incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.9	6
10	102.7	2.2	6
20	102.1	2.1	6
30	100.0	2.8	6
40	94.2	2.5	6
50	97.6	2.4	6
60	95.4	2.7	6
70	97.3	2.9	6
80	90.6	2.7	6
90	77.2	1.5	6
100	74.8	2.7	6

Fig. 3.4.1.4.

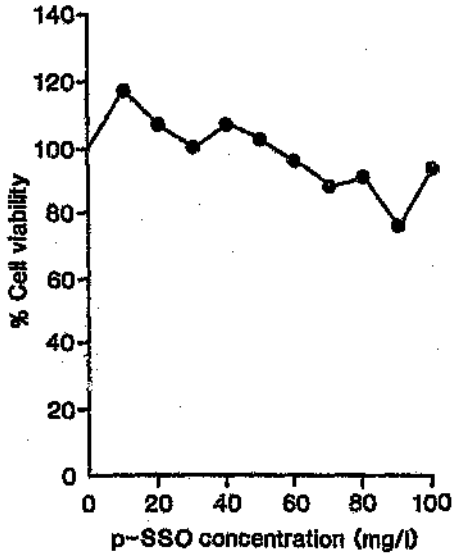
The percentage viability of cells derived from rat skeletal muscle incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.1	6
10	99.8	2.7	6
20	96.8	1.4	6
30	98.0	1.8	6
40	99.8	1.7	6
50	99.8	1.2	6
60	92.6	1.5	6
70	84.7	2.0	6
80	89.6	3.1	6
90	83.5	2.3	6
100	79.0	3.0	6

Fig. 3.4.1.5.

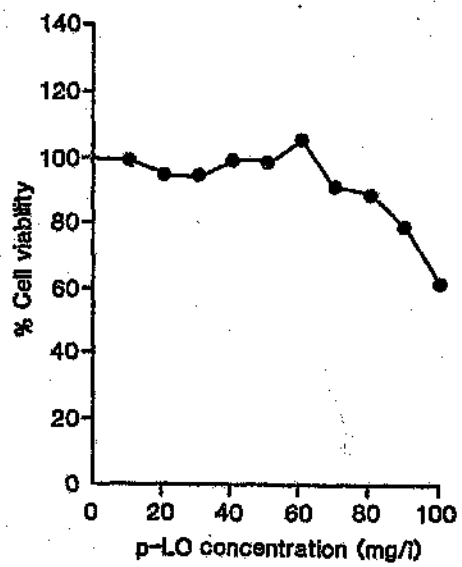
The percentage viability of cells derived from rat skeletal muscle incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.1	6
10	116.9	3.9	6
20	106.7	3.7	6
30	99.8	2.6	6
40	107.2	4.3	6
50	102.8	5.3	6
60	96.4	5.2	6
70	88.3	4.2	6
80	91.0	6.2	6
90	75.1	2.8	6
100	94.3	4.8	6

Fig. 3.4.1.6.

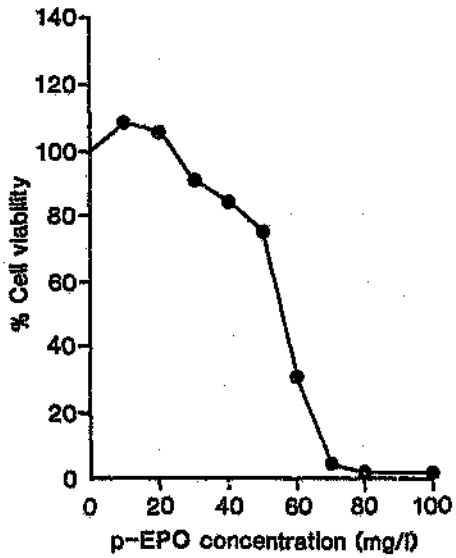
The percentage viability of cells derived from rat skeletal muscle incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	99.6	2.7	6
20	95.0	3.4	6
30	94.6	5.2	6
40	99.3	4.2	6
50	98.4	4.0	6
60	106.1	1.6	6
70	90.9	1.2	6
80	89.6	1.7	6
90	79.7	2.6	6
100	61.4	3.0	6

Fig. 3.4.1.7.

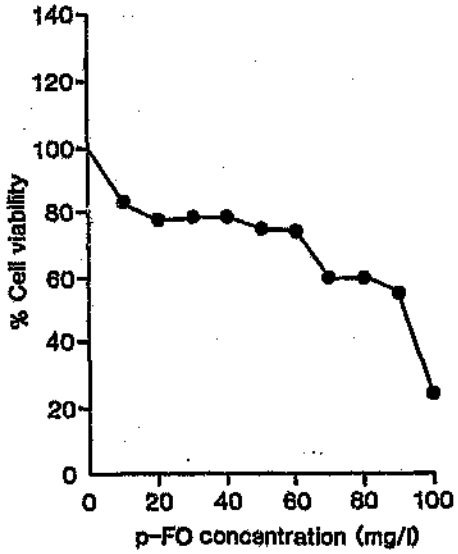
The percentage viability of cells derived from rat skeletal muscle incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.7	6
10	107.9	2.3	6
20	105.1	3.8	6
30	90.7	3.2	6
40	83.6	3.3	6
50	74.5	3.4	6
60	29.9	2.5	6
70	3.0	1.1	6
80	0.2	0.2	6
90	0.0	0.0	6
100	0.0	0.0	6

Fig. 3.4.1.8.

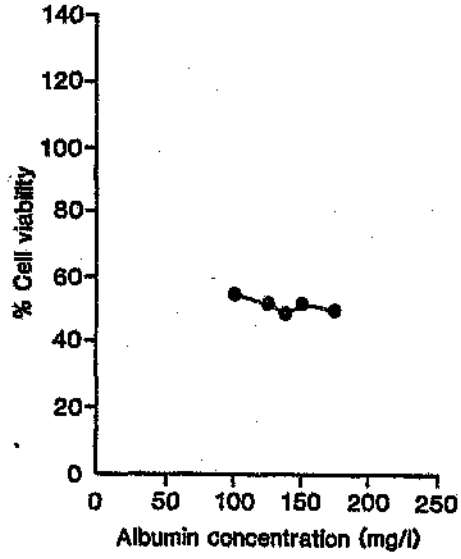
The percentage viability of cells derived from rat skeletal muscle incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.2	6
10	84.0	3.1	6
20	77.9	3.3	6
30	79.3	4.3	6
40	79.5	3.5	6
50	75.9	2.6	6
60	74.2	3.2	6
70	60.8	3.1	6
80	60.8	3.4	6
90	56.2	2.5	6
100	25.0	2.3	6

Fig. 3.4.1.9.

The percentage viability of cells derived from rat skeletal muscle incubated with albumin at the ID_{50} of r-EPO.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.9	6
100	55.5	5.6	6
125	52.1	4.4	6
137.5	50.0	3.7	6
150	52.3	3.6	6
175	50.5	4.4	6

potential dependent on the concentration dosed, while significant cytotoxicity was induced only with p-CO, p-FO or p-EPO incubation.

10mg/l p-CO had little effect on cell proliferation, but greater amounts dosed induced an overall concentration dependent reduction in cell viability (Fig. 3.4.1.2.). Cytostasis was induced with 60mg/l p-CO, while 50% of cells remained viable with about 75mg/l p-CO (ID_{50}), and 30.4% with 100mg/l p-CO.

Cell proliferation approximated to controls with dosage of up to 80mg/l p-MO or 60mg/l p-OO (Figs. 3.4.1.3. and 3.4.1.4., respectively). Greater amounts incubated were growth limiting, and 74.8% and 79.0% of cells remained viable with 100mg/l p-MO or p-OO, respectively, which approximated to the cytostatic number (about 76%). Cell viability was stimulated to 116.9% with 10mg/l p-SSO, ranged from 99.8% to 106.7% with concentrations between 10 and 60mg/l p-SSO, and was limited with greater amounts dosed to a minimum of 75.1% with 90mg/l p-SSO (Fig. 3.4.1.5.). No cytotoxicity was induced with p-MO, p-OO or p-SSO dosage, thus ID_{50} values could not be calculated.

Cell viability ranged from 94.6% to 106.1% with dosage of up to 60mg/l p-LO, but was reduced in a concentration dependent manner to 79.7% with 90mg/l p-LO (Fig. 3.4.1.6.). Despite cytotoxicity with 100mg/l p-LO, no

ID_{50} value could be calculated as cell viability was 61.4%.

The cell viability changes induced with p-EPO incubation followed a sigmoidal relationship (Fig. 3.4.1.7.). Cell proliferation increased to 107.9% and 105.1% with 10 and 20mg/l p-EPO, respectively, was progressively reduced to equate the cytostatic number (about 76%) with 50mg/l p-EPO, and was markedly inhibited with greater amounts dosed. p-EPO was the most cytotoxic p-oil dosed since a concentration of about 55mg/l reduced cell viability to 50% of controls (ID_{50}), and practically all cells took up Trypan blue with concentrations of 70mg/l or greater.

Cell viability was effectively limited to 84.0% with 10mg/l p-FO (Fig. 3.4.1.8.), and ranged from 74.2% to 79.5% when amounts of 20 to 60mg/l p-FO were incubated (cytostasis). Supplementation with higher concentrations induced cytotoxicity. Cell viability was reduced by half with about 92mg/l p-FO (ID_{50}), and by three quarters with 100mg/l p-FO.

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 (55mg/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. two points above,

two 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. 3.4.1.9. 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. Exclusion of the synergistic involvement of albumin indicated that the effects induced with the p-oils could be attributed solely to the exogenous FA's.

Rat skeletal muscle cells were subsequently 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. Upon harvesting, cell viabilities relative to controls were compared and found not to be statistically different from those obtained in Figs. 3.4.1.1-3.4.1.8., thus all further biochemical assays were performed on these samples.

3.4.2 Effects of pseudo-Oils on Total Protein.

The protein concentrations for cells supplemented with 0, 20, 40 or 60mg/l p-oil are shown in Table 3.4.2.1. as µg total protein/10^6 cells seeded.

Cells dosed with 60mg/l p-EPO contained only 362.4µg protein/10^6 cells seeded compared to 401.7µg protein/10^6 control cells seeded. All other dosed cells, however, contained significantly more protein than controls, with amounts ranging from 451.4 to a maximum of 607.4µg protein/10^6 cells seeded (with 60mg/l p-FO and 20mg/l p-EPO, respectively). The changes reported in cellular protein were p-oil concentration dependent. Total protein levels increased in direct correlation with the amount of p-LO incubated, but were generally inversely related to the p-MO, p-SSO, p-EPO or p-FO concentration supplemented. With p-CO or p-OO dosage, however, the highest protein amounts measured were with a concentration of 40mg/l.

Table 3.4.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	401.7			
CO		524.2	537.7	498.0
MO		551.1	537.9	537.9
OO		499.7	597.7	504.4
SSO		602.5	569.8	481.4
LO		516.8	548.7	600.1
EPO		607.4	501.5	362.4
FO		563.5	499.7	451.4

3.4.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Rat Skeletal Muscle.

Table 3.4.3.1. shows the FA spectra of rat skeletal muscle cells incubated with 0, 20, 40 or 60mg/l of each of the p-oils. The greatest amount of any single FA in control cells was 22.7% 22:4ω6, but significant levels of 16:0 (17.8%), 18:0 (13.7%), 18:1ω9 (13.7%) and 20:4ω6 (9.5%) were also found. Varying amounts of these FA's were found in the dosed cells.

16:0 and 16:1ω9 percentages in dosed cells generally approximated to controls, or were decreased, while the converse was true for 18:0, 18:1ω9 and 18:2ω6. ω6 PDFA levels were raised only with certain concentrations of some p-oils, although no increments in 22:5ω6 were found. Dosed cells generally contained 18:4ω3 amounts parallel to, or greater than, controls, but 20:4ω3, 20:5ω3, 22:5ω3 and 22:6ω3 were again increased only with certain concentrations of some p-oils.

15.1%, 16.8% and 24.0% 16:0 was found when cells were dosed with 20, 40 or 60mg/l p-CO_2 respectively (17.8% in controls), although 16:1ω9 was decreased (0.6% to 2.3% vs 3.9% in controls). 18:0 and 18:1ω9 levels, in contrast, were significantly increased with 20mg/l p-CO (32.4% and 22.4%, respectively vs 13.7% in each control), but approximated to controls with 40 or

Legend to Table 3.4.3.1.

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 3.4.3.1.

The fatty acid spectrum of cells derived from rat 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	17.8±0.05	15.1	16.8	24.0	13.3	15.6	25.4	16.0	19.4	12.3	10.4	18.7	7.9	16.6	20.5	22.4	3.6	18.6	4.1	10.6	17.7	4.8
	18:0	13.7±0.10	32.4	13.9	16.8	12.0	16.1	16.2	12.8	11.9	24.1	13.6	12.2	25.8	20.7	13.6	10.6	13.3	13.1	12.8	13.0	13.8	29.9
	20:0	0.3±0.00	-	0.3	-	-	0.4	0.3	-	0.4	-	-	0.1	-	-	0.2	-	-	0.2	-	-	0.1	-
	22:0	0.2±0.05	-	0.2	0.1	0.1	0.1	0.1	-	0.1	-	-	0.1	-	-	0.1	-	-	-	-	-	0.2	-
	24:0	0.3±0.00	-	0.6	-	-	0.2	-	-	0.1	-	-	-	-	-	-	-	-	0.1	-	-	0.1	-
ω9 MONOS.	16:1	3.9±0.25	0.6	1.6	2.3	1.2	0.8	5.9	1.0	1.4	1.1	0.4	1.1	0.8	0.7	1.9	2.8	0.3	2.4	0.2	2.1	4.1	0.9
	18:1	13.7±0.20	22.4	12.3	14.8	13.0	13.3	15.5	23.1	27.5	28.3	30.4	15.5	24.7	18.3	15.4	18.5	31.7	14.1	30.0	26.1	12.6	23.8
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	3.8±0.10	3.6	3.2	3.6	5.3	4.9	5.1	5.5	6.0	18.2	24.7	9.0	26.8	5.4	4.0	7.2	32.1	6.9	32.7	17.9	3.6	24.2
	18:3	-	-	-	-	-	-	-	0.2	-	-	-	-	-	0.1	-	-	-	-	-	-	0.1	-
	20:2	-	-	-	-	-	-	-	0.4	1.0	2.0	-	0.2	-	-	-	-	-	0.1	-	0.2	0.1	-
	20:3	1.6±0.10	0.9	1.7	2.1	1.2	1.7	1.4	1.4	1.4	1.9	2.5	1.7	0.2	1.5	1.4	-	2.2	1.5	0.2	3.3	2.4	1.9
	20:4	9.5±1.00	7.1	11.7	12.2	10.4	11.7	9.5	9.3	8.9	2.8	3.2	7.6	1.7	8.2	7.1	7.8	2.9	5.6	2.3	4.3	10.7	2.4
	22:4	22.7±1.40	10.9	26.4	10.3	32.7	21.9	13.8	18.8	9.6	2.3	4.9	22.0	3.1	16.1	24.5	17.2	3.5	26.0	2.2	3.2	15.9	1.9
	22:5	2.8±0.00	0.4	1.8	0.3	0.5	2.5	-	0.8	2.5	0.4	0.4	2.7	0.2	1.9	1.4	1.4	0.3	2.2	0.3	0.4	2.3	0.1
ω3 POLYS.	18:3	0.5±0.05	-	0.4	0.5	0.4	0.4	0.4	0.6	0.8	3.0	-	0.2	-	1.4	1.5	6.0	-	0.5	-	0.7	0.4	-
	18:4	1.1±0.30	1.1	1.9	1.0	0.7	3.2	0.5	0.7	1.6	2.2	6.3	1.6	7.3	0.8	2.4	2.5	7.7	2.3	12.7	12.5	2.3	7.5
	20:4	3.3±0.00	2.1	1.5	2.5	4.9	3.2	1.8	3.3	2.3	0.2	1.9	3.5	0.8	3.0	3.0	1.6	1.8	3.6	2.3	1.4	2.3	0.6
	20:5	0.4±0.10	0.2	1.7	3.0	0.2	0.2	0.3	0.2	0.2	0.1	-	0.2	-	0.2	0.1	-	-	0.4	-	1.0	2.3	0.7
	22:5	2.1±0.10	1.7	3.2	3.2	2.2	2.8	2.3	3.2	2.6	0.5	0.7	2.0	0.3	3.3	1.6	1.2	0.4	1.4	0.1	2.8	6.9	1.1
	22:6	2.1±0.10	1.6	0.9	3.3	1.9	0.9	1.9	2.7	2.3	0.6	0.6	1.7	0.2	1.9	1.2	0.8	0.3	1.0	0.1	0.6	2.1	0.3

60mg/l p-CO. No significant changes in 18:2ω6 or 18:3ω3 percentages were observed, but small increases in some PDFA's were found with incubation of 40 or 60mg/l p-CO. The most significant increases were for 22:4ω6 (26.4% with 40mg/l vs 22.7% in controls) and 20:5ω3 (1.7% and 3.0% with 40 and 60mg/l, respectively vs 0.4% in controls), with smaller increments shown for 20:3ω6, 20:4ω6, 18:4ω3, 22:5ω3 and 22:6ω3.

Both 16:0 and 16:1ω9 percentages were decreased with dosage of 20 or 40mg/l p-MO, but increased with 60mg/l p-MO (13.3%, 15.6% and 25.4%, and 1.2%, 0.8% and 5.9% vs 17.8% and 3.9% in controls, respectively). 18:0 and 18:1ω9 levels ranged from lows of 12.0% and 13.0% with 20mg/l p-MO, to highs of 16.2% and 15.5% with 60mg/l p-MO, respectively (13.7% in each control), while 4.9% to 5.3% 18:2ω6 was found (3.8% in controls). Only 10.4%, 11.7% and 9.5% 20:4ω6 were detected with 20, 40 and 60mg/l p-MO, respectively (9.5% in controls), although the elongation product, 22:4ω6, was significantly raised with 20mg/l (32.7% vs 22.7% in controls). However, no increase in 22:5ω6 was found in dosed cells (2.8% in controls). ω3 PUFA's were absent in p-MO, yet 18:4ω3 and 22:5ω3 were increased in cells dosed with 40mg/l p-MO (3.2% and 2.8% vs 1.1% and 2.1% in controls, respectively), while 20:4ω3 was increased with 20mg/l p-MO (4.9% vs 3.3% in controls).

Concentration dependent 18:1ω9 incorporation occurred with 20, 40 or 60mg/l p-OO incubation (23.1%, 27.5% and 28.3%, respectively vs 13.7% in controls). 16:0, 16:1ω9 and 18:0 levels were generally lower than controls (17.8%, 3.9% and 13.7%, respectively), although 18:0 was significantly increased with 60mg/l p-OO (24.1%). Concentration dependent increases in 18:2ω6 and 20:2ω6 were observed (5.5% to 18.2% and 0.4% to 2.0% vs 3.8% and 0% in controls, respectively), but ω6 PDFA levels were parallel to controls, or significantly decreased. On the other hand, small increases in 18:3ω3, 18:4ω3, 22:5ω3 and 22:6ω3 were found with p-OO incubation.

p-SSO dosage decreased overall 16:0 and 16:1ω9 levels, significantly increased 18:0 only with 60mg/l (25.8% vs 13.7% in controls), and raised 18:1ω9 levels with all concentrations dosed (15.5% to 30.4% vs 13.7% controls). 24.7% and 26.8% 18:2ω6 were detected with 20 and 60mg/l p-SSO, respectively, but only 9.0% with 40mg/l (3.8% in controls). 18:3ω6 was absent both in control and p-SSO dosed cells, 2.5% 20:3ω6 was found with 20mg/l p-SSO (1.6% in controls), but no increases in 20:4ω6, 22:4ω6 or 22:5ω6 were detected. Surprisingly, 18:4ω3 amounts were increased with p-SSO incubation (1.6% to 7.3% vs 1.1% in controls), but this did not induce significant increments in 20:4ω3, 22:5ω3 or 22:6ω6.

16:0 levels increased to 16.6%, 20.5% and 22.4% with

20, 40 or 60mg/l p-LO incubation, respectively (17.8% in controls), while 18:0 decreased from 20.7% to 13.6% and 10.6%, respectively (13.7% in controls). 16:1ω9 amounts were decreased (0.7% to 2.8% vs 3.9% in controls), while 18:1ω9 percentages increased slightly (15.4% to 18.5% vs 13.7% in controls). 4.0% to 7.2% 18:2ω6 was found (3.8% in controls), but ω6 PDFA levels were not significantly increased. Cellular 18:3ω3 amounts increased to 1.4%, 1.5% and 6.0% with addition of 20, 40 or 60mg/l p-LO; respectively (0.5% in controls). Furthermore, increased levels of 18:4ω3 were detected with 40 and 60mg/l p-LO (2.4% and 2.5%, respectively vs 1.1% in controls), and 22:5ω3 with 20mg/l p-LO (3.3% vs 2.1% in controls), but no increment in 22:6ω3 was found.

Supplementation with 20 or 60mg/l p-EPO caused a marked decrease in 16:0 (3.6% and 4.1%, respectively vs 17.8% in controls), but a significant increase in 18:1ω9 (31.7% and 30.0%, respectively vs 13.7% in controls). p-EPO had little effect on 18:0 levels, induced a reduction in 16:1ω9, and increased 18:2ω6 levels nearly 2 fold with 40mg/l and 10 fold with 20 and 60mg/l (6.9%, 32.1% and 32.7%, respectively vs 3.8% in controls). 18:3ω6 was absent in control and dosed cells, but 2.2% 20:3ω6 was found with 20mg/l p-EPO (1.6% in controls) and 26.0% 22:4ω6 with 40mg/l p-EPO (22.7% in controls). 18:4ω3 amounts were significantly increased over the range dosed (2.3% to 12.7% vs 1.1% in controls), yet the

levels of its products were not s'gnificantly raised.

16:0 and 16:1ω9 levels were parallel to controls (17.8% and 3.9%, respectively), or significantly decreased, with p-FO incubation, whereas marked increases in 18:0 were found with 60mg/l p-FO (29.9% vs 13.7% in controls) and 18:1ω9 with 20 and 60mg/l p-FO (26.1% and 23.8% vs 13.7% in controls). Significant increments in 18:2ω6 and 20:3ω6 were detected with p-FO dosage, yet overall 20:4ω6, 22:4ω6 and 22:5ω6 percentages were decreased. 22:6ω3 levels were not increased, despite raised amounts of 22:5ω3 found with 20 and 40mg/l p-FO (2.8% and 6.9%, respectively vs 2.1% in controls), and 18:4ω3 and 20:5ω3 detected with all p-FO concentrations supplemented (2.3% to 12.5% and 0.7% to 2.3% vs 1.1% and 0.4% in controls, respectively).

3.4.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

The results obtained from the quantitation of lipid peroxides in rat skeletal muscle cultures are shown in Table 3.4.4.1., expressed as nmoles MDA/10^6 cells. In the case of spent incubation media, however, this represents the nmoles of MDA in the volume of medium from which 1x10^6 cells were derived.

Lipid peroxide concentrations in dosed cells ranged from 0.1 to 8.0nmoles MDA/10^6 cells (with 20mg/l p-CO and 60mg/l p-EPO, respectively), compared to 0.1nmoles MDA/10^6 control cells. The lipoperoxide amounts measured both in the cells and spent medium varied with the p-oil supplemented, and generally increased in a concentration dependent manner. Cultures dosed with p-CO, p-MO or p-OO induced 0.1 to 0.8nmoles MDA/10^6 cells, and a maximum of 0.6nmoles MDA/10^6 cells was measured in the spent medium with p-MO (none in control medium). Surprisingly, cells supplemented with 20, 40 or 60mg/l p-LO contained only 0.2nmoles MDA/10^6 cells, although corresponding spent media lipid peroxides were the largest amounts measured (0.7, 2.1 and 3.9nmoles MDA/10^6 cells, respectively). Similarly, p-FO induced only 0.2 to 0.6nmoles MDA/10^6 cells, but 0.1, 0.5 and 1.4nmoles MDA/10^6 cells in the corresponding spent media, respectively. Only 0.3 and 0.4nmoles MDA/10^6 cells were measured with incubation of

Table 3.4.4.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.1			
CO		0.1	0.2	0.8
MO		0.2	0.3	0.4
OO		0.5	0.7	0.6
SSO		0.3	0.4	7.7
LO		0.2	0.2	0.2
EPO		1.8	1.9	8.0
FO		0.2	0.2	0.6
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		0.6	0.4	-
OO		-	-	-
SSO		-	-	-
LO		0.7	2.1	3.9
EPO		-	-	-
FO		0.1	0.5	1.4

20 or 40mg/l p-SSO, respectively, compared to 7.7nmoles MDA/10^6 cells with 60mg/l p-SSO, whereas p-EPO induced the largest cellular lipid peroxide amounts measured at any concentration (1.8, 1.9 and 8.0nmoles MDA/10^6 cells with 20, 40 or 60mg/l, respectively). However, neither p-SSO nor p-EPO induced any lipid peroxides in the corresponding spent media.

3.4.5 Discussio

The effects of FA's on the growth of rat skeletal muscle have not been found in the literature, and this is the first report describing the modulation of such cell growth in culture with supplementation of exogenous FA mixtures mimicking the composition of dietary oils. The data reported in Figs. 3.4.1.2-3.4.1.8. showed that rat skeletal muscle cell viability was affected with p-oil supplementation. The effects induced, however, were not consistent with the degree of p-oil unsaturation, and it was unclear whether the potential to modulate cell proliferation was greater with p-oils rich in polyunsaturated, monounsaturated or saturated FA's. The p-oil-specific effects induced rather appeared to relate to the unique FA composition of each p-oil (Table 2.3.3.2.), although the possibility also exists that certain effects could have been enhanced or deminished by the mediation of synergistic or antagonistic effects between FA's within any p-oil. The FA spectra of p-OO, p-SSO and p-LO suggested that the effects induced with dosage of each p-oil related to the large amount of OA, LA or ALA present, respectively, and that these C18 FA's exhibited little, or no, growth inhibitory capability at the concentrations dosed. The FA composition of p-EPO was similar to that of p-SSO, although the mediation of significantly more growth limitation and cytotoxicity

with p-EPO dosage was consistent with the presence of approximately 9% GLA in p-EPO only. It was apparent from the above, therefore, that GLA exhibited greater growth limiting and cytotoxic ability than LA, ALA and OA. This may relate to the presence of a double bond at the ω12-position in GLA, and the fact that this moiety is a PDFA. Such may also explain the marked cytotoxicity induced with high concentrations of p-FO, which contained 18% EPA. Indeed, the finding that pronounced growth inhibition was induced with supplementation of p-EPO or p-FO, but not with p-MO, p-OO, p-SSO or p-LO which contained insignificant PDFA amounts, implicated PDFA involvement in the modulation of cytotoxicity. On the other hand, the finding that cell viability was stimulated with certain concentrations of p-SSO, p-EPO or p-LO only, rich either in LA or ALA, implied EFA involvement in the enhancement of cell proliferation.

Evidence to support exogenous FA incorporation was found with p-oil supplementation (Table 3.4.3.1.), hence the changes reported in cell viability could relate to differential uptake of p-oil FA's, alterations in membrane unsaturation and fluidity. It was possible that the abundance of saturated FA's (about 95%) in p-CO inhibited cell viability at high concentrations by increasing membrane rigidification, whereas addition of large amounts of p-EPO or p-FO elevated the unsaturation index of the membrane sufficiently to induce cell death.

Modifications of the membrane PGL FA composition may desatabilise the membrane and induce changes in the activity of membrane-bound enzymes (Stubbs et al 1984 and Spector et al 1985). A possible mechanism whereby these p-oils induced cell death may therefore relate to uncoupling of calcium ATPase activity and altered calcium transport. At this stage, however, the effects of FA's on sarcoplasmic reticulum calcium ATPase are unclear (Hidalgo et al 1982, East et al 1984, Almeida et al 1986, Hidalgo 1987, Infante 1987 and Stubbs et al 1990). On the other hand, maintenance of basal membrane fluidity may explain why p-MO, p-OO, p-SSO and p-LO were not significantly cytotoxic. During periods demanding extra energy, skeletal muscle TAG stores are mobilised, releasing FFA's which this tissue can utilise as an energy source (Lehninger 1982). Skeletal muscle may therefore be well equipped to tolerate high FFA concentrations, and this could also partly explain the findings that many p-oils had little effect on cell viability, stimulated cell proliferation, or were merely growth limiting.

The changes in cellular protein concentrations reported with p-oil supplementation (Table 3.4.2.1.) reflected the changes induced in cell viability (Figs. 3.4.1.2-3.4.1.8.). Enhanced cellular protein amounts with 20 and 40mg/l p-SSO, 20mg/l p-EPO and 60mg/l p-LO correlated with increased cell viabilities at these concentrations,

but it was apparent that increments in cell number alone could not justify the marked protein increases. This implied FA involvement in the stimulation of rat skeletal muscle protein synthesis, particularly since protein concentrations were significantly increased even when p-oils induced growth limitation and cytostasis. The extent to which protein synthesis was stimulated varied with dosage of identical amounts of different p-oils, even when such yielded similar cell numbers. This suggested that the capability to enhance protein synthesis varied with FA, and was probably relatable to FA structure and concentration in any p-oil. The enhancement of protein synthesis with p-oil dosage may, nevertheless, reflect the extremely high metabolic activity of skeletal muscle in vivo (Lehninger 1982) and a mechanism whereby the cells enhanced the production of lipid metabolising enzymes in an attempt to metabolise the exogenous FA's incorporated with p-oil incubation. On the other hand, the reduction of cellular protein concentations only with 60mg/l p-EPO correlated with the marked cytotoxicity this p-oil induced, and reflected decreased cell numbers either with constant or decreased protein per cell.

Comparison of control cell FA spectra with those derived from p-oil-dosed cells indicated the potential of cultured cells derived from rat skeletal muscle to express desaturase and elongase enzymes (Table 3.4.3.1.). The

capability for 16:0 desaturation and elongation, and 18:0 desaturation to 18:1ω9 via Δ9D, were suggested. However, it was apparent that delta-6-desaturation of 18:1ω9 was competitively inhibited by 18:2ω6 and/or 18:3ω3 since evidence for Δ6D and subsequent elongase activities were found with both ω3 and ω6 series PUFA's as substrates. PDFA product formation was clearly more limited with ω9 than ω6, and ω6 than ω3, series FA's, and this confirmed the established substrate preference (Brenner 1974, Mead et al 1976 and Kanau et al 1977). Increased 18:4ω3 levels in cells supplemented with p-oils deficient in 18:3ω3 was consistent with delta-6-desaturation of endogenous 18:3ω3 and/or extramicrosomal retroconversion of 20:4ω3. The significant increase in 20:5ω3 amounts with p-CO dosage implied Δ5D capability, and the finding that Δ5D expression was more restricted with supplementation of PUFA-rich p-oils supported the formation of 18:4ω3 by retroconversion of 20:4ω3. Gavino et al (1981b) similarly showed limited conversion of 20:3ω6 to 20:4ω6 via Δ5D in rodent smooth muscle cell cultures, which elongated 20:4ω6, but did not desaturate 22:4ω6. The present study demonstrated elongation both of 20:4ω6 and 20:5ω3, but the formation of small amounts of 22:6ω3 only with p-CO or p-OO incubation reflected limited/suppressed Δ4D activity. This explained the accumulation of the Δ4D substrates, 22:4ω6 and 22:5ω3, particularly with dosage of p-MO or p-FO, respectively.

Desaturation is regulated by competitive interactions between FA's of the same and different families (eg. Garcia et al 1965, Brenner et al 1966, Ullman et al 1971, de Schriver et al 1982 and Nassar et al 1986), and this partly determined the pattern of desaturation and elongation reported in Table 3.4.3.1. with p-oil dosage. Studies have also shown 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). Detection of increased PDFA levels with p-CO incubation, which contained about 95% saturated FA's and no PUFA's, implied enhanced desaturase cascade enzyme expression and subsequent desaturation and elongation of endogenous PUFA's. This reflected an attempt to re-establish membrane fluidity altered with uptake of exogenous saturated FA's. On the other hand, PDFA formation with dosage of PUFA-rich p-oils was consistent with the availability of desaturase cascade enzyme substrates. However, once optimal PUFA levels were established to maintain normal membrane fluidity and cellular functions, it was likely that desaturase cascade enzyme expression was suppressed by negative feedback reactions which have been well documented (Garcia et al 1965, Brenner et al 1966, Brenner 1974, Ullman et al 1971, de Schriver et al 1982 and Nassar 1986). Such probably

explains why long chain polyenoic FA levels were not markedly raised with dosage of PUFA-rich p-oils, despite incorporation of such moieties. The potential of these cells to desaturate and elongate FA's nevertheless reflects a mechanism to maintain its own FA levels.

Detection of large proportions of unsaturated FA's in control cells (Table 3.4.3.1.), but only small amounts of lipid peroxides (Table 3.4.4.1.) implied chemical and enzymic mechanisms of protection against free radical attack, eg. vitamin E and/or superoxide dismutase. 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 therefore not be so readily accessible to oxidation. Increased lipid peroxide measurement in dosed cells, however, could have reflected saturation of the cellular anti-oxidant mechanisms induced with exogenous unsaturated FA uptake and/or increased cellular unsaturated FA availability. The lipid peroxides detected in dosed cells were nevertheless consistent with intact cellular enzymic and/or non-enzymic mechanisms for their generation, and reflected the p-oil concentration incubated, p-oil FA composition (Table 2.3.3.2.), and FA availability and susceptibility to oxidation.

The small increment in cellular lipoperoxides detected with p-CO or p-MO dosage (Table 3.4.4.1.) correlated with the large concentration of saturated FA's these

p-oils contained (Table 2.3.3.2.). However, quantitation of small cellular lipoperoxide amounts also with p-OO, p-LO or p-FO incubation, despite these p-oils containing significant levels of unsaturated FA's, implied that the substrates for peroxidation were lipid bound and stable. Furthermore, the finding that overall lipid peroxide levels were lowest in the cells and greatest in the spent medium with p-LO supplementation in the absence of cytotoxicity (Fig. 3.4.1.6.) suggested that the cellular lipid peroxides formed with incorporation of this p-oil were transfered into the incubation medium through the intact plasma membrane. This correlated with studies which have implicated lipid peroxides in increasing membrane permeability (Chio et al 1969, Mead 1976, Tappel 1980, Gavino et al 1981c, Morisaki et al 1982b and Frankel 1984). This may also explain why cellular lipid peroxide levels were greater in the spent medium than in the cells with p-FO supplementation. It was also possible that these medium lipoperoxides reflected released cellular FFA's which were oxidised extracellularly by released cellular peroxidising enzymes. Nevertheless, measurement of the largest medium lipid peroxide amounts with p-LO or p-FO dosage correlated with the significant ω3 PUFA levels in these p-oils. The significantly greater concentration of ALA in p-LO than EPA in p-FO (approximately 63% and 18%, respectively) was consistent with the greatest medium lipoperoxide

levels measured, despite the greater susceptibility of EPA to oxidation.

The cellular lipid peroxides formed with p-SSO or p-EPO supplementation (Table 3.4.4.1.) probably related mainly to oxidation of incorporated free LA. Both p-oils exhibited similar FA compositions (Table 2.3.3.2.), but the presence of about .9% GLA in p-EPO only probably explains the greater lipid peroxides amounts induced overall with this p-oil. Morisaki et al (1984) have indeed shown that GLA generates large quantities of lipoperoxides in rodent smooth muscle cell cultures, and that such could induce cytotoxicity. The greatest cellular lipoperoxide levels quantitated with dosage of 60mg/l p-EPO correlated with the most extensive growth inhibition induced at this p-oil concentration (Fig. 3.4.1.7.), and suggested lipid peroxide involvement in the mediation of cytotoxicity. This supported current evidence which indicates that lipoperoxides affect normal cell metabolism and cause membrane damage (Chio et al 1969, Mead 1976, Tappel 1980, Gavino et al 1981c, Morisaki et al 1982b and Frankel 1984). Demonstration of cell lysis and the absence of lipoperoxides in the spent medium with p-EPO supplementation implied that the cytotoxicity induced arose partly as a result of oxidation of membrane FA's. Lipoperoxides were also absent in the spent medium with p-SSO incubation, and similar amounts of cellular lipid peroxides were

quantitated with 60mg/l p-EPO or p-SSO supplementation, yet cell viability was not significantly limited with this amount of p-SSO (Figs. 3.4.1.5. and 3.4.1.7.). These findings suggested that a critical lipid peroxide concentration may have to be attained before lipid peroxides become membrane damaging and detrimental to cell survival, dependant on the overall state of cellular equilibrium. On the other hand, it may indicate that lipoperoxides are less involved in FA mediated cytotoxicity than postulated, and that changes in membrane fluidity are equally or more important. The absence of significant amounts of unsaturated FA's in p-CO would indeed imply that the reduction of cell viability this p-oil induced related to alterations in membrane fluidity rather than lipoperoxide production. However, the finding that the low concentrations of cellular lipoperoxides measured with p-MO, p-OO, p-LO or p-FO dosage (Table 3.4.4.1.) correlated with the absence of cytotoxicity (Figs. 3.4.1.3., 3.4.1.4., 3.4.1.6. and 3.4.1.7., respectively), indicated the possiblity that lipoperoxides were partly involved in the modulation of cell viability.

3.5 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM RAT BRAIN.

3.5.1 Effects of pseudo-Oils on Cell Viability.

Cells isolated from rat cerebral cortex were largely astrocytic, and microscopic examination of control and p-oil dosed cells before, during and after each experiment showed no morphological changes at any time, 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 had no significant effect on cell viability (Fig. 3.5.1.1.), nor were any synergistic effects evident. Thus, the cell viability changes induced with the p-oils 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 $12.8x10^4$/ml. This equated to approximately 62% 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 62% were thus considered cytotoxic.

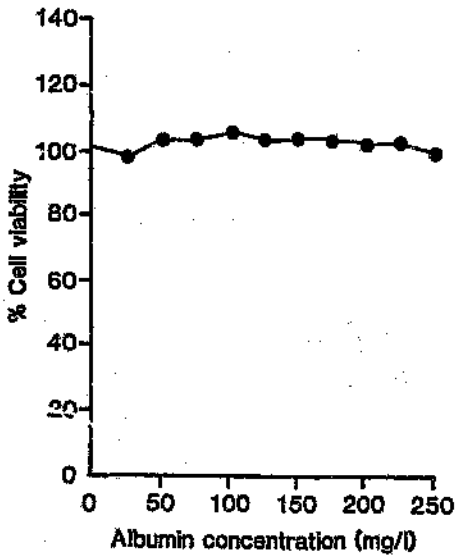
Figs. 3.5.1.2-3.5.1.8. depict the data derived following incubation of rat brain cells with p-oils. Certain

Legend to Figs. 3.5.1.1-3.5.1.9.

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. 3.5.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 3.5.1.2-3.5.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 3.5.1.9. 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. 3.5.1.1.

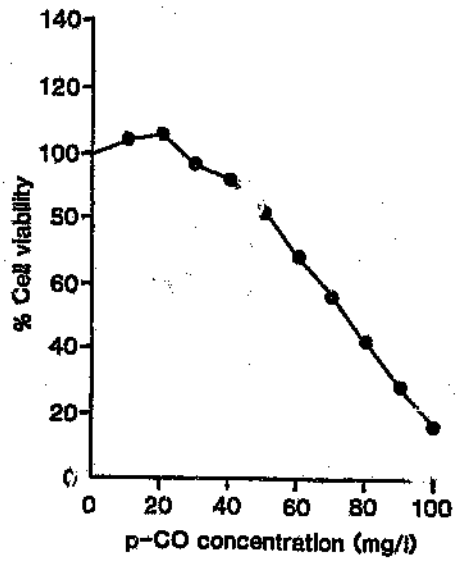
The percentage viability of cells derived from rat brain incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	12
25	96.8	5.4	12
50	102.5	3.3	12
75	102.9	4.3	12
100	104.9	2.7	12
125	102.9	3.2	12
150	103.5	5.2	12
175	102.8	4.4	12
200	101.5	5.0	12
225	102.9	3.1	12
250	99.0	2.9	12

Fig. 3.5.1.2.

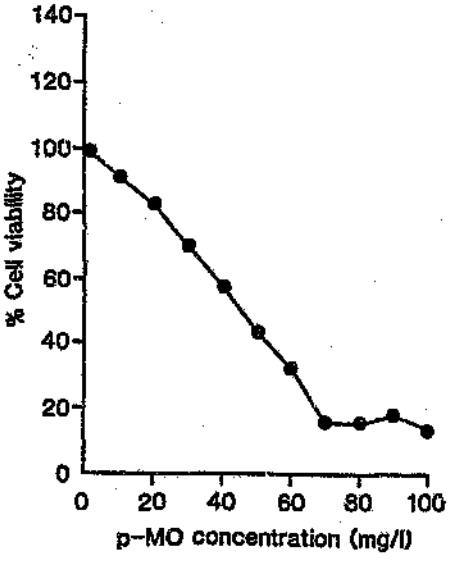
The percentage viability of cells derived from rat brain incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.2	6
10	104.4	2.6	6
20	105.8	1.6	6
30	97.1	1.9	6
40	91.8	3.0	6
50	82.0	1.8	
60	67.9	1.3	6
70	55.8	1.6	6
80	42.4	1.9	6
90	28.4	1.5	6
100	15.8	1.0	6

Fig. 3.5.1.3.

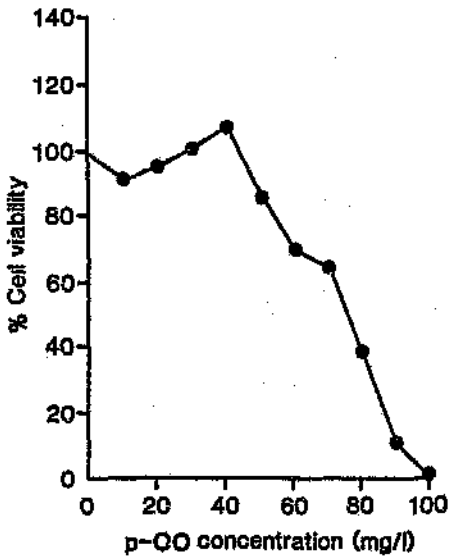
The percentage viability of cells derived from rat brain incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.7	6
10	91.3	3.5	6
20	83.1	2.6	6
30	69.6	6.3	6
40	56.8	5.1	6
50	43.5	2.5	6
60	32.3	2.4	6
70	15.3	2.4	6
80	15.4	4.6	6
90	18.0	2.8	6
100	12.5	1.8	6

Fig. 3.5.1.4.

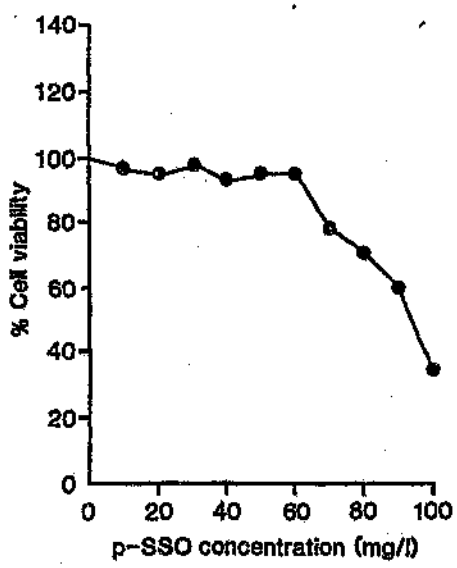
The percentage viability of cells derived from rat brain incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.9	6
10	90.9	4.8	6
20	94.8	4.9	6
30	100.4	5.8	6
40	107.3	4.5	6
50	86.2	5.7	6
60	70.4	3.7	6
70	64.9	3.0	6
80	38.2	4.1	6
90	10.8	2.4	6
100	0.0	0.0	6

Fig. 3.5.1.5.

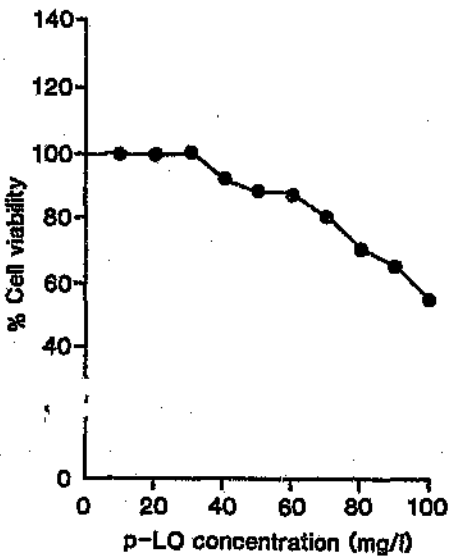
The percentage viability of cells derived from rat brain incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.4	6
10	96.7	2.3	6
20	95.0	2.0	6
30	98.0	1.9	6
40	93.5	3.1	6
50	95.2	2.8	6
60	95.2	2.4	6
70	78.3	5.3	6
80	70.8	3.0	6
90	60.1	2.4	6
100	34.5	1.3	6

Fig. 3.5.1.6.

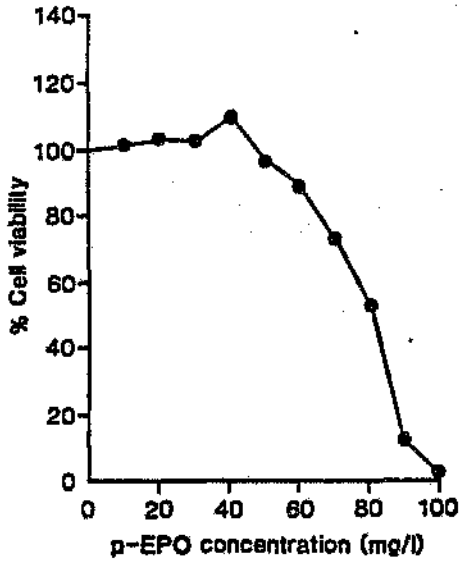
The percentage viability of cells derived from rat brain incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.8	6
10	99.2	2.8	6
20	99.1	2.6	6
30	99.5	2.3	6
40	90.9	2.4	6
50	87.5	2.7	6
60	86.8	2.3	6
70	80.6	3.4	6
80	70.3	1.4	6
90	64.5	2.2	6
100	54.3	1.4	6

Fig. 3.5.1.7.

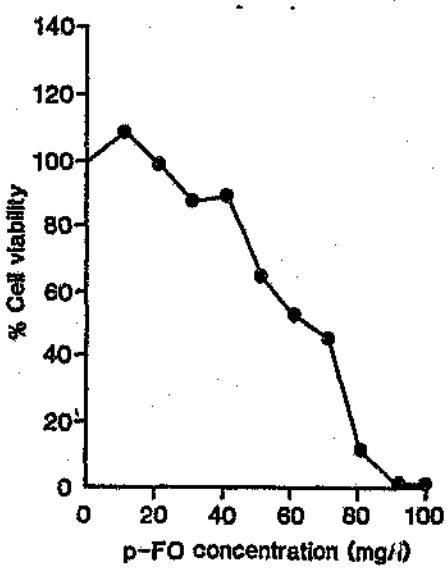
The percentage viability of cells derived from rat brain incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	100.2	2.9	6
20	102.1	2.7	6
30	101.3	3.0	6
40	109.2	2.5	6
50	96.3	2.3	6
60	89.0	2.1	6
70	73.1	2.7	6
80	52.1	2.9	6
90	11.9	0.9	6
100	1.0	0.3	6

Fig. 3.5.1.8.

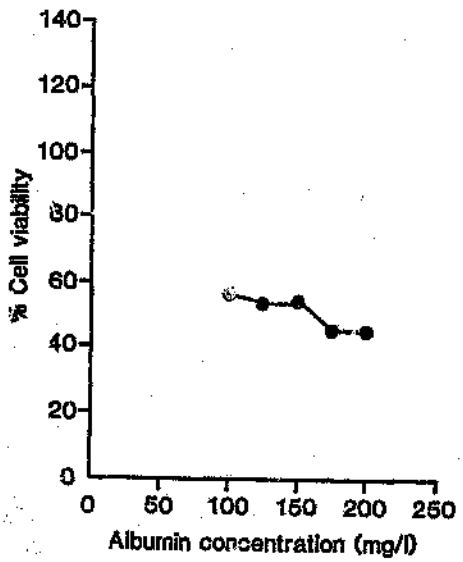
The percentage viability of cells derived from rat brain incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.8	6
10	109.1	3.4	6
20	99.0	3.1	6
30	88.0	2.5	6
40	89.8	2.7	6
50	64.8	2.3	6
60	52.6	5.5	6
70	45.1	2.1	6
80	10.5	3.3	6
90	0.0	0.0	6
100	0.0	0.0	6

Fig. 3.5.1.9.

The percentage viability of cells derived from rat brain incubated with albumin at the ID_{50} of p-00.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.5	6
100	56.6	3.1	6
125	54.6	5.7	6
150	55.3	3.6	6
175	46.1	4.8	6
200	45.8	4.0	6

concentrations of p-CO, p-OO, p-EPO and p-FO stimulated cell proliferation slightly, whereas all p-oils limited and inhibited cell growth. The magnitude of the effects induced, however, varied with the nature of the p-oil and concentration dosed.

Cell proliferation was stimulated to a maximum of 105.8% with 20mg/l p-CO and to 107.3% with 40mg/l p-OO (Figs. 3.5.1.2. and 3.5.1.4., respectively). Incubation with greater amounts of p-CO or p-OO limited cell viability in a concentration dependent manner, while amounts greater than 70mg/l p-CO or p-OO induced cytotoxicity and reduced the viable cell yield, almost linearly, to 15.8% and 0% with 100mg/l p-CO and p-OO, respectively. The ID_{50} for both p-oils was approximately 75mg/l, which reflected the similar effects these p-oils induced.

Dosage with up to 70mg/l p-MO reduced cell viability in an approximately linear concentration dependent manner (Fig. 3.5.1.3.). p-MO was the most effective p-oil to impair cell proliferation as cytotoxicity was induced with amounts greater than 30mg/l p-MO, and 50% of cells were killed with only 45mg/l p-MO (ID_{50}). The cytotoxic effect of p-MO plateaued with concentrations of 70mg/l to 100mg/l as cell viability ranged from 12.5% to 18.0%.

Cell proliferation was not significantly influenced with supplementation of up to 60mg/l p-SSO or 30mg/l p-LO (Figs. 3.5.1.5. and 3.5.1.6., respectively). Higher

concentrations limited cell growth almost linearly, but cytotoxicity was induced only with amounts greater than 80mg/l p-SSO and 90mg/l p-LO. Half the cells were viable with about 94mg/l p-SSO (ID_{50}), compared to 34.5% with 100mg/l p-SSO. More than half the cells, however, were still viable with 100mg/l p-LO (54.3%), thus no ID_{50} could be calculated. This was the least cytotoxic p-oil.

Supplementation with up to 30mg/l p-EPO had little effect on cell viability and increased cell numbers were found with 40mg/l p-EPO (109.2%), whereas proliferative effects were induced only with 10mg/l p-FO (109.1%) (Figs. 3.5.1.7. and 3.5.1.8., respectively). These were the greatest increments in cell proliferation found. Concentrations greater than 50mg/l p-EPO and 20mg/l p-FO induced growth limitation, but amounts greater than 70mg/l p-EPO and 50mg/l p-FO were cytotoxic. Calculation of ID_{50} values showed that p-EPO was less cytotoxic than p-FO (approximately 81mg/l and 65mg/l, respectively), and practically all cells took up the Typan blue with 100mg/l p-EPO and 90mg/l p-FO.

To exclude the possibility that any of the effects induced 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-OO corresponding to its ID_{50} (about 75mg/l). Five points were selected around which to vary the albumin concentration, viz. two points above, two points below, and the ID_{50} of p-OO, and the appropriate

amount of albumin added to these cultures to give the desired concentrations. The results are depicted in Fig. 3.5.1.9., and indicated that the amount of p-OO dosed reduced cell viability to approximately 50% of controls, and that such was not influenced by the concentration of albumin present as FA carrier. Hence, synergistic effects of albumin could be excluded, and the effects induced with the p-oils could be attributed solely to the exogenous FA's.

Subsequent to these studies, cells derived from rat brain were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient amounts to permit all the quantitative and qualitative analyses to be performed. Upon harvesting, cell viabilities relative to controls were compared and found to be statistically similar to those in Figs. 3.5.1.1-3.5.1.8. All further biochemical assays were thus performed on these samples.

3.5.2 Effects of pseudo-Oils on Total Protein.

Table 3.5.2.1. shows the total protein concentrations of cells incubated with 0, 20, 40 or 60mg/l p-oil, expressed as µg total protein/10^6 cells seeded.

455.5µg protein/10^6 control cells seeded was detected, whereas dosed cells contained protein amounts ranging from 198.7 to 500.5µg/10^6 cells seeded (with 60mg/l p-MO and 20mg/l p-EPO, respectively). Cells supplemented with 20mg/l p-oil (except p-OO) exhibited the highest protein concentrations quantitated over the range dosed (422.1 to 500.5µg/10^6 cells seeded), which approximated to control amounts. Cellular protein levels were related inversely to the p-oil concentration dosed, although slightly more protein was found in cells incubated with 40mg/l than with 20mg/l p-OO (478.2 vs 461.5µg/10^6 cells seeded). Cells dosed with 40mg/l p-OO or p-EPO exhibited enhanced protein levels, whereas significantly reduced protein concentrations were found in all cells incubated with 60mg/l p-oil, compared to controls.

Table 3.5.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	455.5			
CO		471.9	351.2	297.9
MO		451.5	254.9	198.7
OO		461.5	478.2	309.7
SSO		450.5	428.1	397.7
LO		474.9	409.9	387.1
EPO		500.5	496.5	409.9
FO		422.1	400.8	223.2

3.5.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Rat Brain.

Table 3.5.3.1. lists the FA profiles of cells incubated with 0, 20, 40 or 60mg/l p-oil. The greatest percentage of any single FA detected in control cells was 18:1ω9 (25.9%). FA's comprising >5.0% of the total spectrum in control cells included 16:0 (20.5%), 18:0 (18.6%), 18:2ω6 (5.4%) and 22:4ω6 (6.4%).

p-Oil dosed cells contained 16:0 and 18:1ω9 percentages parallel to, or greater than, those in control cells, whereas 16:1ω9 proportions were generally, and 18:0 levels were always, lower in dosed cells. 18:2ω6, 18:3ω6, 20:4ω6, 18:3ω3 and 20:5ω3 percentages were generally only comparable to, or higher than, control amounts if the p-oils dosed contained these moieties, and the levels of most other polyenoic FA's were parallel to, or lower than, control levels.

Incubation with 20, 40 or 60mg/l p-CO increased 16:0 levels to 27.9%, 29.0% and 41.8%, respectively (20.5% in controls), while 16:1ω9 and 18:1ω9 amounts ranged from 1.0 to 5.5% and 23.8% to 29.6%, relative to 3.8% and 25.9% in controls, respectively. 18:0 and 18:2ω6 levels were both decreased (12.9% to 13.2% and 2.8% to 4.6% vs 18.6% and 5.4% in controls, respectively), although up to 4.7% 20:4ω6 and 8.6% 22:4ω6 were found (3.5% and 6.4% in controls, respectively).

Legend to Table 3.5.3.1.

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 3.5.3.1.

The fatty acid spectrum of cells derived from rat brain.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	[p-CO] (mg/l) 40	[p-CO] (mg/l) 60	[p-MO] (mg/l) 20	[p-MO] (mg/l) 40	[p-MO] (mg/l) 60	[p-OO] (mg/l) 20	[p-OO] (mg/l) 40	[p-OO] (mg/l) 60	[p-SSO] (mg/l) 20	[p-SSO] (mg/l) 40	[p-SSO] (mg/l) 60	[p-LO] (mg/l) 20	[p-LO] (mg/l) 40	[p-LO] (mg/l) 60	[p-EPO] (mg/l) 20	[p-EPO] (mg/l) 40	[p-EPO] (mg/l) 60	[p-FO] (mg/l) 20	[p-FO] (mg/l) 40	[p-FO] (mg/l) 60
SATURATES.	16:0	20.5±0.50	27.9	29.0	41.8	22.8	23.1	30.7	17.7	19.3	17.9	23.1	25.8	17.8	19.0	32.3	22.9	16.8	26.9	22.1	19.5	41.1	33.5
	18:0	18.6±0.30	13.2	12.9	13.0	11.9	12.1	18.0	7.3	6.2	3.4	11.3	13.5	16.8	10.6	15.3	7.9	9.5	14.6	11.8	9.1	10.2	6.1
	20:0	1.2±0.05	-	0.2	0.1	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1
	22:0	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24:0	0.3±0.05	-	0.3	-	-	-	0.1	-	-	-	-	0.2	-	-	0.1	-	-	0.2	0.2	-	0.1	-
ω9 MONOS.	16:1	3.8±0.10	5.5	3.1	1.0	1.7	1.9	3.5	1.8	0.7	1.1	4.4	4.0	1.1	1.5	1.7	2.7	1.5	1.2	2.7	12.4	13.3	20.5
	18:1	25.9±0.55	29.6	28.1	23.8	32.3	31.4	26.7	48.5	58.6	66.3	26.8	28.8	31.0	28.0	24.4	30.1	21.1	21.6	22.9	26.0	20.7	23.6
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	5.4±0.00	4.6	3.6	2.8	10.4	13.0	7.8	9.2	6.4	6.0	21.2	18.2	27.1	10.0	5.5	11..	31.7	23.2	32.4	9.1	4.3	6.6
	18:3	-	0.1	-	0.2	0.1	-	-	0.1	-	-	0.1	-	-	0.1	0.1	0.2	0.5	0.7	2.2	0.1	-	-
	20:2	1.9±0.05	0.2	0.3	-	0.3	0.3	0.3	1.0	2.5	1.5	0.9	0.2	0.3	0.3	0.3	0.1	1.1	0.2	-	0.4	0.6	0.5
	20:3	1.8±0.05	1.3	1.2	0.9	1.3	1.0	0.5	0.9	0.3	0.2	1.0	0.6	0.3	0.9	0.7	0.5	3.9	0.8	0.4	1.0	0.5	0.4
	20:4	3.5±0.10	4.7	3.9	2.7	5.5	5.6	2.8	2.7	0.6	0.6	2.5	1.4	0.8	4.6	1.7	1.4	3.2	1.4	1.1	3.4	0.9	1.0
	22:4	6.4±0.05	5.0	7.6	8.6	5.8	5.4	6.5	5.5	3.1	1.7	4.2	3.6	2.6	6.8	5.8	3.1	5.6	4.2	1.5	6.1	3.6	3.5
	22:5	1.2±0.10	0.1	1.0	0.1	-	0.3	-	-	0.5	-	0.1	0.6	0.1	-	0.6	-	-	0.7	0.1	0.1	0.4	0.1
ω3 POLYS.	18:3	0.9±0.00	0.9	0.7	0.6	1.1	0.8	0.6	0.9	0.3	0.2	0.6	0.3	0.2	12.7	7.2	18.0	0.4	0.4	0.8	0.9	0.6	0.5
	18:4	0.9±0.05	0.2	0.9	0.2	0.2	0.4	0.2	0.1	0.3	0.1	0.2	0.6	0.2	0.1	0.5	0.1	0.1	0.6	0.2	0.2	0.4	0.2
	20:4	1.8±0.10	0.4	1.6	0.9	0.2	0.3	0.7	0.4	0.3	0.1	0.1	0.7	0.7	0.6	0.7	0.3	0.3	0.9	0.1	0.8	0.6	0.6
	20:5	0.4±0.00	0.5	0.9	0.4	1.0	0.6	0.2	0.2	0.1	-	0.1	0.1	-	0.8	0.2	0.4	0.3	0.3	0.1	3.8	1.1	1.6
	22:5	3.0±0.30	2.9	2.5	1.7	2.8	1.9	0.9	1.9	0.5	0.5	1.9	1.1	0.6	2.2	1.4	0.9	2.0	1.2	0.8	4.7	1.0	0.7
	22:6	2.5±0.30	2.9	2.4	1.4	2.8	2.1	0.7	1.8	0.4	0.3	1.7	0.6	0.4	1.9	1.0	0.7	2.0	0.9	0.6	2.5	0.6	0.6

Cells dosed with 20, 40 or 60mg/l p-MO contained 22.8%, 23.1% and 30.7% 16:0, respectively (controls 20.5%), but decreased amounts of 16:1ω9 (1.7% to 3.5% vs 3.8% in controls). 18:0 was decreased with 20 and 40mg/l p-MO (11.9% and 12.1%, respectively) and parallel to controls with 60mg/l p-MO (18.0%), while 18:1ω9 levels were greatest with 20 and 40mg/l p-MO (32.3% and 31.4%, respectively), and comparable to controls with 60mg/l p-MO (26.7%). 18:2ω6 uptake increased significantly over the range dosed (7.8% to 13.0% vs 5.4% in controls), and 5.5%, 5.6% and 2.8% 20:4ω6 was detected with 20, 40 and 60mg/l p-MO, respectively (3.5% in controls), yet other PDFA levels were not significantly increased.

A marked increase in 18:1ω9 proportions was found with 20, 40 or 60mg/l p-OO supplementation (48.5%, 58.6% and 66.3%, respectively vs 25.9% in controls). 16:0 levels were slightly decreased, but 16:1ω9 and 18:0 amounts were considerably reduced, compared to controls (20.5%, 3.8% and 18.6%, respectively). 6.0% to 9.2% 18:2ω6 was found (5.4% in control), with 2.5% 20:2ω6 detected with 40mg/l p-OO (1.9% in controls), but other PUFA levels were not significantly raised.

16:0 percentages were 23.1% and 25.8% with 20 and 40mg/l p-SSO, respectively, but only 17.8% with 60mg/l p-SSO (20.5% in controls). 16:1ω9 levels were slightly raised when incubated with 20mg/l p-SSO (4.4%), decreased with

60mg/l p-SSO (1.1%), and parallel to controls (3.8%) with 40mg/l p-SSO (4.0%). Decreased amounts of 18:0 were found (11.3% to 16.8% vs 18.6% in controls), but 18:1ω9 levels were increased (26.8% to 31.0% vs 25.9% in controls). Despite marked 18:2ω6 incorporation (5.4%, 21.2%, 18.2% and 27.1% with 0, 20, 40 and 60mg/l p-SSO, respectively), no significant increases in PDFA's were detected, and all ω3 PUFA levels were decreased.

16:0, 16:1ω9, 18:0 and 18:1ω9 percentages were parallel to controls or decreased with p-LO dosage, although increased 16:0 and 18:1ω9 were found with 40 and 60mg/l p-LO, respectively (32.3% and 30.1% vs 20.5% and 25.9% in controls, respectively). 18:2ω6 levels increased only with 20 and 60mg/l p-LO (10.0% and 11.1%, respectively vs 5.4% in controls), and 20:4ω6 was slightly increased with 20mg/l p-LO (4.6% vs 3.5% in controls). Incubation with p-LO raised cellular 18:3ω3 percentages markedly (7.2% to 18.0% vs 0.9% in controls), yet ω3 PDFA amounts were not significant increased.

The levels of the saturated and monoenoic FA's reported in Table 3.5.3.1. were parallel to controls or decreased with p-EPO supplementation, although increased 16:0 was detected with 40mg/l p-EPO (26.9% vs 20.5% in controls). A marked elevation in cellular 18:2ω6 occurred with all p-EPO concentrations dosed (23.2% to 32.4% vs 5.4% in controls), and 0.5%, 0.7% and 2.2% 18:3ω6 were detected with 20, 40 and 60mg/l p-EPO, respectively (no 18:3ω6 in

controls). 20:3ω6 levels increased approximately 2 fold with 20mg/l p-EPO (3.9% vs 1.8% in controls), but all other ω6 and ω3 PUFA percentages were decreased relative to controls.

Incubation with 20mg/l p-FO induced no significant change in 16:0 levels (19.5% vs 20.5% in controls), but amounts were dramatically raised with 40 and 60mg/l p-FO (41.1% and 33.5%, respectively). 16:1ω9 levels increased from 5.8% in controls to 12.4%, 13.3% and 20.5% with 20, 40 or 60mg/l p-FO, respectively, while 18:0 amounts were decreased with all concentrations dosed (6.1% to 10.2% vs 18.6% in controls), and 18:1ω9 percentages were generally parallel to control levels (25.9%). 18:2ω6 proportions ranged from 4.3% to 9.1% (5.4% in controls), and no significant increases were found for any other ω6 PUFA's. Significantly increased 20:5ω3 amounts were detected with 20, 40 and 60mg/l p-FO (3.8%, 1.1% and 1.6%, respectively vs 0.4% in controls), but with the exception of 4.7% 22:5ω3 detected with 20mg/l p-FO (3.0% in controls), no increases in other ω3 PUFA's were observed.

3.5.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 3.5.4.1. shows the lipoperoxides measured in rat brain cells and their respective growth media following incubation with 0, 20, 40 or 60mg/l p-oil. 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 10^6 cells were obtained.

0.4nmoles MDA/10^6 control cells was measured, while the amounts detected for dosed cells ranged from a low of 0.5nmoles MDA/10^6 cells (with 20mg/l p-EPO) to a high of 2.7nmoles MDA/10^6 cells (with 60mg/l p-MO). The cellular lipoperoxide amounts measured varied with the p-oil dosed and generally increased in a p-oil concentration dependent manner, but showed no correlation overall with the degree of p-oil unsaturation. Cells dosed with p-EPO contained only 0.5 to 0.8nmoles MDA/10^6 cells, whereas 0.9, 1.6 and 1.0nmole MDA/10^6 cells were detected with 20, 40 and 60mg/l p-SSO, and 0.7, 0.7 and 1.3nmoles MDA per 10^6 cells quantitated with p-LO, respectively. In comparison, cells incubated with 20, 40 or 60mg/l p-CO or p-OO contained 0.7, 1.1 and 1.8 or 0.8, 1.1 and 2.0 nmoles MDA/1x10^6 cells, respectively, and p-MO induced 0.6, 0.9 and 2.7nmoles MDA/1x10^6 cells. However, p-FO generated the largest cellular lipid peroxide amounts overall across the range dosed (1.4, 1.4 and 2.4nmoles

Table 3.5.4.1.

Lipoperoxide formation by rat brain 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		0.7	1.1	1.8
MO		0.6	0.9	2.7
OO		0.8	1.1	2.0
SSO		0.9	1.6	1.0
LO		0.7	0.7	1.3
EPO		0.5	0.6	0.8
FO		1.4	1.4	2.4
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	2.7
MO		-	-	4.8
OO		-	-	3.4
SSO		-	-	3.3
LO		-	1.0	4.9
EPO		-	1.8	7.8
FO		-	2.9	12.3

MDA/10^6 cells, respectively).

Lipoperoxides were absent both in spent control medium, and in the spent media derived from cultures dosed with 20mg/l p-oil. Further, only p-LO, p-EPO and p-FO induced medium lipoperoxides when 40mg/l p-oil was incubated (1.0, 1.8 and 2.9nmoles MDA/1x10^6 cells, respectively). On the other hand, lipoperoxides were measured in all spent media derived from cultures supplemented with 60mg/l p-oil (2.7 to 12.3nmoles MDA/10^6 cells). Unlike the cells, however, the lowest concentrations of lipid peroxides in the spent media occurred with p-CO dosage, whereas larger MDA concentrations were quantitated with p-OO or p-SSO, followed by p-MO, p-LO, p-EPO or p-FO incubation, respectively.

3.5.5 Discussion.

This study showed that supplementation of primary cultures of normal rat brain with p-oils affected the viability of these cells (Figs. 3.5.1.2-3.5.1.8.), and that the p-oil-specific cell viability changes induced reflected the unique FA profile each p-oil exhibit (Table 2.3.3.2.). The FA compositions of p-OO, p-SSO and p-LO suggested that the effects induced with incubation of each p-oil were largely attributable to the high concentrations of 18:1ω9 (71%), 18:2ω6 (70%) or 18:3ω3 (63%) present, respectively. Since the capability to kill cultured rat brain cells increased markedly in the order p-LO, p-SSO and p-OO, it seemed possible that OA was the most, and ALA the least, cytotoxic of these moieties, and that such related inversely to the number of double bonds in these C18 FA's. This, however, was in direct contrast with a previous study in our laboratory which showed LD_{50} values of about 55, 50 and 35mg/l for rat brain primary cultures incubated with 18:1ω9, 18:2ω6 or 18:3ω3, respectively (Girao 1988). 18:3ω3 and 18:3ω6 were equally cytotoxic, whereas comparison of the FA profiles of p-EPO, p-LO, p-SSO and p-OO with the cell viability changes these p-oils induced suggested that 18:3ω6 exhibited greater cytotoxic capability than 18:3ω3, 18:2ω6 and 18:1ω9. Girao (1988) found that 18:0 induced cytotoxicity in the range 60 to 100mg/l and cytostasis overall in the range 0 to 60mg/l, although

amounts of 1, 25, 35 and 45mg/l 18:0 increased growth by 6% to 12%. Low concentrations of p-CO, p-OO, p-EPO and p-FO enhanced cell proliferation similarly by between 5% and 10% (Figs. 3.5.1.2., 3.5.1.4., 3.5.1.7., 3.5.1.8., respectively), but this was not found when individual C18 unsaturated FA's (18:1ω9, 18:2ω6, 18:3ω6 or 18:3ω3) were supplemented (Girao 1988). Others found that addition of 18:2ω6, 18:3ω3, 20:4ω6 or 22:6ω3 at seeding time to cultured fetal mouse brain cells increased [^{3}H]-thymidine incorporation into DNA, stimulated cell proliferation with optimal concentrations of 1.0mg/l FA, and were toxic with higher amounts (Bourre et al 1983). The finding that optimum cell growth was achieved with p-oil concentrations of 20mg/l p-CO, 40mg/l p-OO, 40mg/l p-EPO or 10mg/l p-FO, and cytotoxicity was induced only with significantly higher amounts of these compounds, clearly demonstrated that p-oils were less growth inhibitory than individual FA's.

The quantitative and qualitative differences described above with supplementation of individual FA's or p-oils were not surprising, but in fact supported the mediation of synergistic or antagonistic effects between FA's within a p-oil which may have enhanced or diminished the effects caused by individual FA's. This may indeed explain the different effects which p-SSO and p-EPO induced, despite the similar FA compositions these p-oils exhibited. Furthermore, this phenomenon more than

likely occurs *in vivo* when organisms consume FA mixtures as dietary oils, thus the modulation of cell viability reported with p-oil dosage probably reflects closer to the "real world" than studies using individual FA's.

Uptake of FA's by rat brain *in vivo* has been reported by Cook (1978a, 1978b, 1980). FA's must be in their activated state before being incorporated, and this requires plasma membrane acyl CoA synthetase, which has been demonstrated in rat brain synaptosomal plasma membranes in our laboratory (Davidson 1986). Thus, the potential exists within the brain to incorporate exogenous FA's, and there is no evidence that such is lost when cells are maintained in culture. Numerous studies have in fact shown rat brain cells in culture to be capable of exogenous FA incorporation. Yavin et al (1974) demonstrated that the rate of 12:0, 14:0, 16:0, 18:0, 18:1ω9, 18:2ω6 and 18:3ω3 uptake into cultured cells derived from rat cerebral tissue was greatest with 18:0 and decreased progressively with decreasing chain length, although no clear relationship was evident between uptake and degree of FA unsaturation. These cells have been reported to incorporate the above FA's into TAG's, PGL's and glycerolipids, although such moieties could also remain in the free form (Menkes 1972 and Yavin et al 1974). On the other hand, addition of 18:2ω6, 18:3ω3, 20:4ω6 or 22:6ω3 at seeding time to cultured fetal mouse brain cells resulted in these

FA's being incorporated predominantly into the PGL fraction (Bourre et al 1983). The site(s) of p-oil FA uptake were not investigated in the present study, but the data in Table 3.5.3.1. nevertheless supported the capability of cultured rat brain cells to incorporate exogenous FA's to varying extents. Hence, it was likely that membrane fluidity changes occurred. Brain membrane lipids have been reported to be highly unsaturated and conservative in nature (Crawford et al 1971), and any alteration in membrane stability induced with p-oil incubation may consequently have caused cell death. The cytotoxicity reported with p-CO and p-MO could well have related to a marked decrease in the unsaturation index of brain membrane lipids when these saturated FA-rich p-oils were dosed. This mechanism alone, however, could not fully explain why p-MO was more growth inhibitory than p-CO, despite the more saturated nature of the latter. The inclusion in p-MO of the PDFA and endoperoxide substrate, 20:4ω6, may be an explanation. Indeed, considerably more cytotoxicity was induced with incubation of p-OO, p-EPO or p-FO which also contained EFA desaturation and/or elongation products, than with p-SSO or p-LO which were EFA-rich and PDFA-deficient. In fact, the finding that p-FO was significantly more growth inhibitory than p-EPO correlated with the presence of almost 20% PDFA's in p-FO, compared to 10% in p-EPO. These moieties may have altered membrane

stability, either directly or indirectly, to such an extent that cell viability could not continue.

The cellular protein concentrations reported with p-oil dosage (Table 3.5.2.1.) generally paralleled the changes observed in cell viability (Figs. 3.5.1.2-3.5.1.8.). The slight increments in cellular protein levels found with 20mg/l p-CO, 40mg/l p-OO or 40mg/l p-EPO supplementation primarily reflected increased cell numbers with constant or slightly elevated protein per cell. Comparison of cellular protein concentrations with cell viability indicated that 20mg/l p-LO or p-EPO enhanced protein synthesis slightly, whereas such was suppressed with 20mg/l p-FO, 40 or 60mg/l p-SSO. On the other hand, the finding that dosed cells contained less protein than controls when cell viability was impaired correlated with decreased cell numbers with constant or decreased protein per cell; the latter may relate to the low turnover of proteins known to exist in the brain (Lehninger 1982). When Vignikin et al (1989) incubated primary cultures of newborn rat glial cells with single FA's at 30mg/l, they found that oleic acid induced a greater protein increment over a two week period than cells treated with linoleic, alpha-linolenic or especially arachidonic acid. This correlated with the mean protein concentrations reported in Table 3.5.2.1. for cells supplemented with 20 or 40mg/l p-OO, p-SSO, p-LO or p-MO, respectively, but the finding that such

largely reflected changes in cell viability extended the work of Vignikin et al (1989).

The results obtained from the analysis of FA's in control and p-oil dosed cells (Table 3.5.3.1.) were consistent with an overall lack or suppression both of desaturase and elongase expression in cultured rat brain. However, slightly increased levels of some PDFA's with dosage of certain p-oils supported the potential for limited desaturase cascade enzyme activity. 18:3ω3 incorporated with p-LO supplementation was not further metabolised, and such was consistent with impaired Δ6D expression. 18:2ω6 incorporated with p-SSO incubation was also not converted to PDFA's, thus 20:3ω6 formation with 20mg/l p-EPO incubation probably related only to elongation of incorporated 18:3ω6. The lack of further 20:3ω6 metabolism with p-EPO supplementation, however, was consistent with impaired Δ5D expression. Slightly increased 20:4ω6 and/or 20:5ω3 levels when certain concentrations of p-CO, p-MO or p-LO were supplemented reflected limited Δ5D expression or extramicrosomal retroconversion of cellular 22:4ω6 and 22:5ω3, respectively. No evidence for elongation of 20:4ω6 incorporated with p-MO dosage was found, although 22:5ω3 formation with 20mg/l p-FO supplementation supported elongation of incorporated 20:5ω3. However, the lack of significant increments in 22:5ω6 or 22:6ω3 with p-oil dosage indicated impaired Δ4D capability.

Literature reports on the capability of rat brain cells to desaturate and elongate FA's are contradictory. Menkes (1972) showed the capability of such cells in lipid-free culture medium to desaturate and elongate both [^{14}C]-16:0 and [^{14}C]-18:0 prior to incorporation into cellular lipids. The potential for 16:0 and 18:0 desaturation were also indicated in the present study, but neither these moieties nor their immediate desaturation products were further elongated (Table 3.5.3.1.). A possible explanation for the latter finding came out of the work of Yavin et al (1974) who found that incorporated FA's underwent elongation in cultured rat brain cells and that such was maximal for 12:0 and decreased with increased FA chain length. The same group demonstrated that these cells were also able to synthesise 20:4ω6 from 18:2ω6 and convert 18:3ω3 and 20:5ω3 to 22:6ω3 (Yavin et al 1974, 1975). Similar results were reported by Dhopeshwarkar et al (1971), Sinclair et al (1972) and Anding et al (1986) in neonatal and adult rat brain <u>in vivo</u>. Cook et al (1978a) found that developing rat brain had the capacity to desaturate both 18:0 and EFA's, but Δ6D activity clearly appeared a rate limiting step in suckling rats, and its activity was hardly above background in the adult. Hassam et al (1975) showed that when suckling rats were orally fed radiolabelled 18:2ω6 or 18:3ω6, most 18:2ω6 activity remained unchanged, while 18:3ω6 radioactivity

had been incorporated into 20:3ω6 and 20:4ω6, in brain lipids. Sinclair (1975b) also reported that desaturation of 18:2ω6 was very slow after birth in rat brain. The present study used newborn rat pups, hence this may partly account for the relatively low desaturase cascade enzyme activity reported overall (Table 3.5.3.1.). It was unlikely, however, that such activity was suppressed primarily as a result of the growth limitation or cytotoxicity induced with p-oil dosage, since the capability for PDFA formation was limited/impaired even when p-oils had little effect on, or stimulated, cell viability. Desaturase cascade enzyme suppression may rather be a characteristic of this cell type *in vitro* under the present culture conditions. Our results, nevertheless, supported the finding of Novelot et al (1986) that the liver is implicated in desaturating and elongating FA's *in vivo*, and packaging such moieties for transport to the brain.

MDA measurement in rat brain cultures both in the absence and presence of dosed p-oils (Table 3.5.4.1.) supported the ability of these cells to oxidise FFA's, as others have shown (Pitas et al 1987). This reflected intact cellular enzymic and/or non-enzymic mechanisms for lipoperoxide generation when the lines of protection against FA oxidation were saturated, impaired or absent. Control cell lipid peroxides implied oxidation of endogenous unsaturated FA's released during PGL

turnover, whereas the enhanced lipoperoxide amounts measured in dosed cultures were consistent with oxidation of unsaturated p-oil FFA's.

The abundance of saturated FA's in new incubation medium (Table 2.3.3.3.) and the ability of these cells to incorporate exogenous FA's (Table 3.5.3.1.) supported the likelyhood that most peroxides found in the spent media of dosed cultures originated intracellularly. Such were released as a result of cell death with 60mg/l p-MO or p-FO dosage, whereas the ability of cellular lipoperoxides to alter membrane permeability (Mead 1976, Tappel 1980 and Frankel 1984) implied their leakage through the plasma membrane of intact cells with incubation of p-oils which did not induce cytotoxicity at the concentrations studied. Cellular FFA's and peroxidising enzymes could also have been released into the culture medium in the above processes, thus the possiblity that enzymic and/or spontaneous FFA oxidation occurred extracellularly, and contributed to the lipid peroxides measured in the spent medium, can not be ignored. Such may explain the greater lipoperoxide amounts shown in the spent medium of cultures dosed with 40mg/l p-LO, p-EPO, p-FO, or 60mg/l p-oil, than in the cells themselves.

The finding that lipoperoxide generation increased overall with the amount of p-oil supplemented (Table 3.5.4.1.) reflected increased FFA availability for

oxidation. However, the numerical differences in the lipoperoxide amounts reported with p-oil supplementation at a particular concentration 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 susceptibility to oxidation, dependent on the number and position of double bonds in a FA. The susceptibility of 20:5ω3 to oxidation may indeed explain why cultures dosed with p-FO exhibited the largest lipid peroxide amounts measured overall. The greater cellular lipoperoxide concentrations found with dosage of p-CO than with p-LO or p-EPO, for example, was surprising considering the significantly greater unsaturated FA content of the latter p-oils. This suggested that the substrates for lipoperoxidation in the p-LO and p-EPO were not so readily accessible to oxidation, possibly because they were present as components of complex membrane lipids. It may also reflect a slower rate of PGL turnover and therefore FA release into the cellular pool from membrane lipids with p-LO and p-EPO. Alternatively, low cellular lipoperoxide levels may reflect their release into the culture medium. This supported the higher lipid peroxide amounts found in the spent medium with p-EPO, compared to in the cells with p-SSO, dosage; these lipoperoxides probably related mainly to oxidation of free 18:2ω6. p-EPO and p-SSO both exhibited similar FA compositions, thus the greater total lipoperoxide amounts induced with the

former may reflect the presence of about 9% 18:3ω6 in p-EPO only. This PDFA merely requires chain elongation to be usable as a substrate for enzymatic peroxidation, whereas 18:2ω6 requires desaturation first. The lipid peroxides formed with p-MO or p-FO supplementation could similarly have related partly to the utilisation of exogenous 20:4ω6 and 20:5ω3, respectively as direct endoperoxide substrates.

The observation that the cell viability changes reported in Figs. 3.5.1.2-3.5.1.8. with p-oil incubation were generally inversely related to the MDA amounts produced (Table 3.5.4.1.) suggested lipoperoxide involvement in the modulation of cell proliferation. Support for this was the fact that 60mg/l p-MO induced the greatest increase in cellular lipoperoxide amounts and the most pronounced cytotoxicity. However, the finding that the greatest lipoperoxide levels in the spent incubation media did not correlate with the greatest cytotoxicity indicated that the modulation of cell viability also related to other mechanisms, such as alterations in membrane fluidity. Indeed, the lack of significant ability to desaturate or elongate FA's overall implied that these cells were largely unable to redress membrane lipid FA composition and fluidity altered with exogenous FA incorporation, and that such was involved in the modulation of growth limitation and inhibition reported in Figs. 3.5.1.2-3.5.1.8.

3.6 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM RAT LUNG.

3.6.1 Effects of pseudo-Oils on Cell Viability.

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

The results depicted in Fig. 3.6.1.1. showed that final medium albumin concentrations ranging from 0 to 250mg/l had no significant effect on rat lung cell viability. The effects induced with the supplemented p-oils were therefore 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 $13.1x10^4$/ml. This equated to about 60% of the final control cell number at the end of the 48 hour incubation period, and represented the cytostatic number. Only p-oil amounts reducing cell viability to significantly below 60% were therefore considered growth inhibitory.

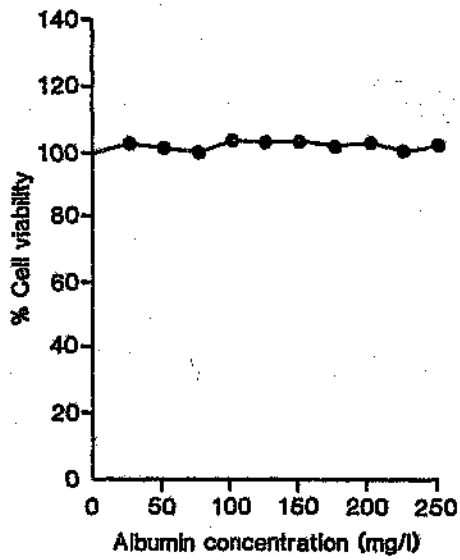
The results of the incubation of cells with p-oils are shown in Figs. 3 6.1.2-3.6.1.8. Overall, cell viability

Legend to Figs. 3.6.1.1-3.6.1.9.

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. 3.6.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 3.6.1.2-3.6.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 3.6.1.9. 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. 3.6.1.1.

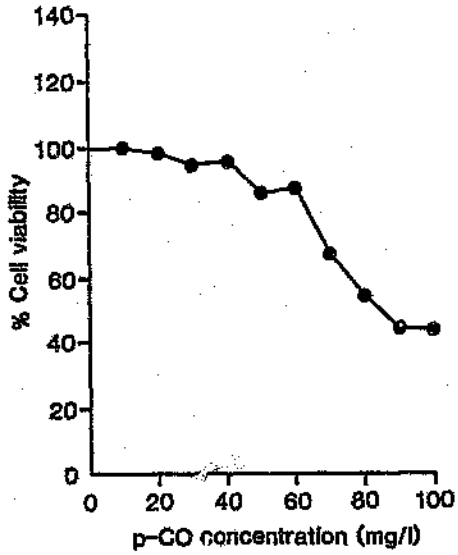
The percentage viability of cells derived from rat lung incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.6	12
25	103.2	4.0	12
50	101.1	5.3	12
75	100.0	4.9	12
100	104.7	5.5	12
125	103.3	3.8	12
150	104.5	3.9	12
175	102.1	3.1	12
200	104.7	4.2	12
225	100.9	2.3	12
250	103.7	5.1	12

Fig. 3.6.1.2.

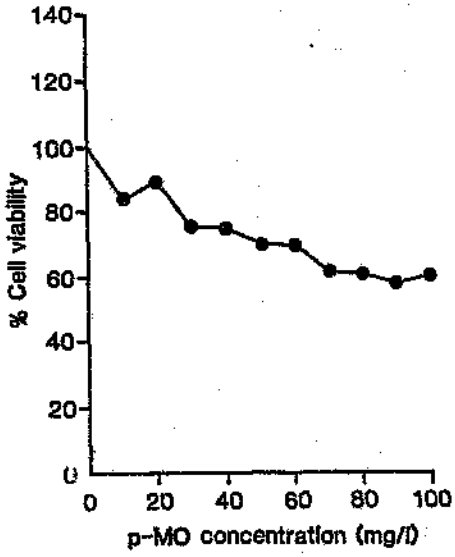
The percentage viability of cells derived from rat lung incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.7	6
10	100.0	2.5	6
20	97.8	2.3	6
30	94.2	2.9	6
40	95.6	2.9	6
50	85.4	2.5	6
60	87.3	2.7	6
70	67.2	2.2	6
80	54.3	1.2	6
90	44.3	1.6	6
100	43.3	1.5	6

Fig. 3.6.1.3.

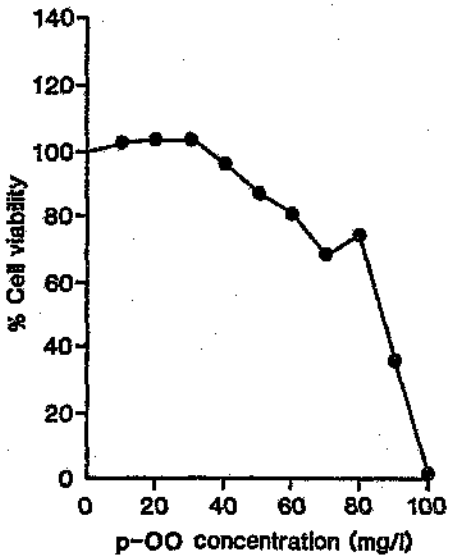
The percentage viability of cells derived from rat lung incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.3	6
10	84.0	4.1	6
20	89.3	2.5	6
30	75.7	1.4	6
40	74.7	4.7	6
50	69.6	4.0	6
60	70.4	2.9	6
70	61.4	4.7	6
80	61.1	2.9	6
90	58.3	1.1	6
100	60.6	1.4	6

Fig. 3.6.1.4.

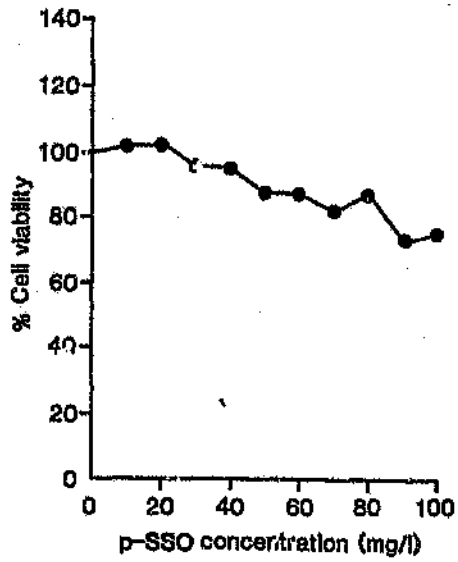
The percentage viability of cells derived from rat lung incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	102.2	3.9	6
20	103.8	1.8	6
30	103.4	5.6	6
40	96.3	2.4	6
50	86.8	7.4	6
60	81.1	4.7	6
70	68.5	2.4	6
80	74.6	5.6	6
90	35.9	2.9	6
100	0.8	0.5	6

Fig. 3.6.1.5.

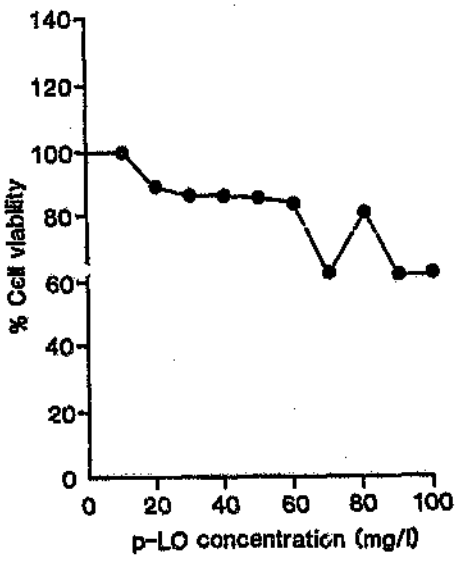
The percentage viability of cells derived from rat lung incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	101.8	4.4	6
20	102.4	2.8	6
30	94.6	4.1	6
40	93.7	3.4	6
50	87.1	4.2	6
60	86.5	4.0	6
70	80.7	4.7	6
80	86.7	5.9	6
90	72.9	4.7	6
100	74.5	2.4	6

Fig. 3.6.1.6.

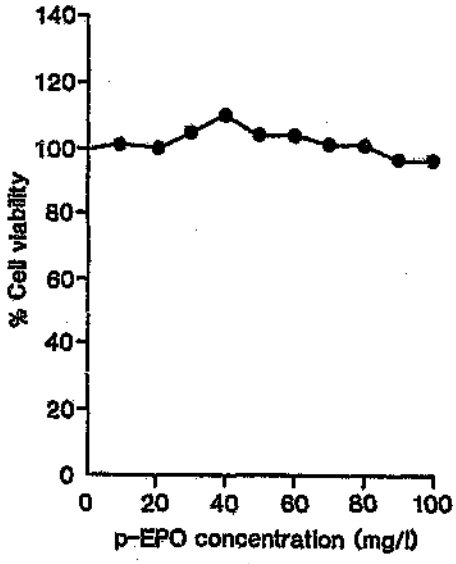
The percentage viability of cells derived from rat lung incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.5	6
10	99.8	1.5	6
20	89.4	2.2	6
30	86.3	2.1	6
40	87.1	1.3	6
50	86.1	2.0	6
60	83.5	2.2	6
70	63.0	1.9	6
80	80.8	2.9	6
90	62.1	3.0	6
100	62.6	2.5	6

Fig. 3.6.1.7.

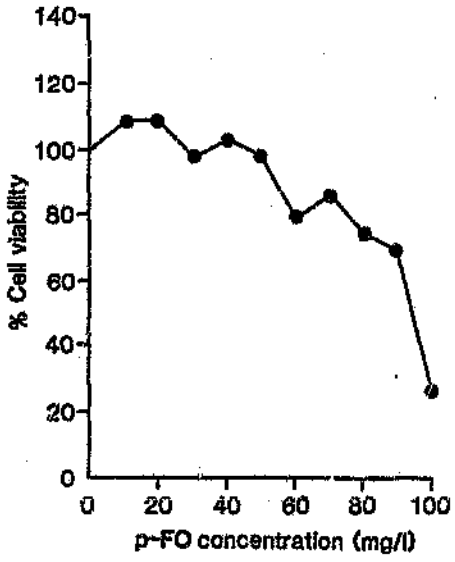
The percentage viability of cells derived from rat lung incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.9	6
10	101.4	1.5	6
20	99.5	1.8	6
30	105.1	2.8	6
40	110.4	2.7	6
50	104.2	1.8	6
60	105.0	1.9	6
70	101.1	2.2	6
80	101.2	1.3	6
90	96.6	1.6	6
100	97.7	1.7	6

Fig. 3.6.1.8.

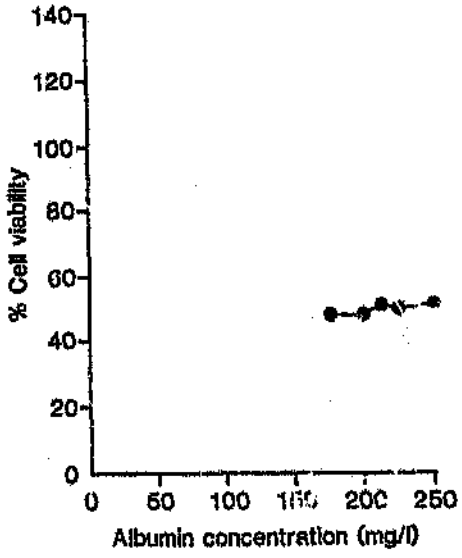
The percentage viability of cells derived from rat lung incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.1	6
10	111.0	4.7	6
20	109.6	4.2	6
30	98.3	4.3	6
40	104.4	5.8	6
50	98.5	3.9	6
60	80.4	3.6	6
70	87.9	5.8	6
80	75.4	4.1	6
90	69.8	4.3	6
100	26.2	4.6	6

Fig. 3.6.1.9.

The percentage viability of cells derived from rat lung incubated with albumin at the ID_{50} of p-QD.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.6	6
175	49.1	2.8	6
200	48.4	2.1	6
212	51.6	3.2	6
225	50.5	4.6	6
250	52.6	2.7	6

was stimulated only with low amounts of p-EPO and p-FO, all p-oils besides p-EPO limited cell proliferation to a greater or lesser extent, and cytotoxicity was induced only with high concentrations of p-CO, p-FO and p-OO.

p-CO concentrations up to 40mg/l had little effect on cell viability, whereas 50 to 70mg/l p-CO limited cell proliferation, and greater amounts were cytotoxic (Fig. 3.6.1.2.). Half the cells were viable with 85mg/l p-CO (ID_{50}) and 43.3% with 100mg/l p-CO. On the other hand, p-MO induced concentration dependent growth limitation with amounts up to 60mg/l, and cytostasis with 70 to 100mg/l p-MO (Fig. 3.6.1.3.), therefore no ID_{50} could be calculated.

Concentrations up to 40mg/l p-OO induced no significant change in cell viability, but 50 to 80mg/l p-OO limited cell proliferation to a minimum of 68.5% with 70mg/l p-OO (Fig. 3.6.1.4.). 50% of cells were killed with about 86mg/l p-OO (ID_{50}), but higher amounts induced the greatest cytotoxicity observed and practically all cells took up Trypan blue with 100mg/l p-OO.

Dosage with up to 30mg/l p-SSO had little effect on cell proliferation, but greater amounts progressively limited the viable cell yield in an overall concentration dependent manner to 72.9% and 74.5% with 90 and 100mg/l p-SSO, respectively (Fig. 3.6.1.5.). Cell proliferation with 10mg/l p-LO mirrored controls, but ranged from

83.5% to 89.4% with 20 to 60mg/l p-LO (Fig. 3.6.1.6.). Higher p-LO concentrations induced cytostasis and were therefore slightly more growth limiting than p-SSO, although 80.8% viability occurred with 80mg/l p-LO.

30 to 60mg/l p-EPO stimulated cell proliferation to a maximum of 110.4% with 40mg/l, but other concentrations had little effect on cell viability, and no growth limitation was observed (Fig. 3.6.1.7.).

10 and 20mg/l p-FO enhanced cell proliferation to 111.0% and 109.6%, respectively, 30 to 50mg/l p-FO had little effect on cell viability, and higher amounts limited cell growth to a minimum of 69.8% with 90mg/l p-FO (Fig. 3.6.1.8.). Half the cells were viable with 95mg/l p-FO (ID_{50}), but only 26.2% with 100mg/l p-FO.

Rat lung cells were incubated with an amount of p-OO corresponding to its ID_{50} (approximately 86mg/l), and dosed with varying albumin concentrations to exclude the possibility that any effects induced with p-oil dosage were influenced by the amount of a bumin bound to the FA's. Five points were chosen around which to vary the albumin concentration, keeping the p-oil concentration fixed, viz. two points above, two points below, and the ID_{50} of p-OO. The results are depicted in Fig. 3.6.1.9., and indicated that the p-OO concentration incubated reduced cell viability to approximately 50% of controls, and that such was not significantly influenced by

different albumin concentrations. Exclusion of the synergistic involvement of albumin thus implied that the effects reported in Figs. 3.6.1.2-3.6.1.8. were solely attributed to the exogenous FA's.

Cells were subsequently plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in amounts sufficient to permit all quantitative and qualitative analyses to be carried out. Cell viabilities relative to controls were compared upon harvesting and found to be statistically similar to those in Figs. 3.6.1.1-3.6.1.8. Hence, all further biochemical assays were performed on these samples.

3.6.2 Effects of pseudo-Oils on Total Protein.

The results derived from the quantitation of total protein in control and p-oil dosed cells are shown in Table 3.6.2.1.

176.5µg protein/10^6 control cells seeded was measured, while concentrations in dosed cells ranged from 158.5 to a maximum of 247.5µg protein/10^6 cells seeded (obtained with 60mg/l p-MO and 20mg/l p-OO, respectively). Cells contained protein amounts equal to, or greater than, controls when 20mg/l p-oil (except p-EPO) or 40mg/l p-oil were dosed (176.9 to 247.9µg/10^6 cells seeded), while concentrations were increased with 60mg/l p-EPO or p-OO (209.5 and 199.1µg/10^6 cells seeded, respectively), parallel to controls with 60mg/l p-SSO (176.8µg/10^6 cells seeded) and slightly decreased with 60mg/l p-CO, p-MO, p-LO or p-FO (158.5 to 165.6µg/10^6 cells seeded). The changes induced in total cellular protein were p-oil concentration dependent as protein levels increased in direct correlation with the amount of p-EPO dosed, but were inversely related to the p-CO, p-MO or p-LO concentration supplemented. With p-SSO or p-FO dosage, however, the highest protein levels measured were with a concentration of 40mg/l, but the reverse was true with p-OO supplementation.

Table 3.6.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	176.5			
CO		189.0	184.4	163.2
MO		187.1	182.0	158.5
OO		247.9	186.7	199.1
SSO		204.0	213.9	176.8
LO		186.7	176.9	160.0
EPO		167.9	203.5	209.5
FO		182.0	187.7	165.6

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

The FA spectra of cells incubated with 20, 40 or 60mg/l p-oil are shown in Table 3.6.3.1. in relation to undosed cells. The greatest proportion of any FA in controls was 16:0 (23.4%), but FA's contributing at least 5.0% to the total spectrum in controls included 18:0 (18.4%), 18:1ω9 (15.9%), 20:4ω6 (11.8%) and 22:4ω6 (9.0%). Significant proportions of these moieties were also detected in dosed cells.

In general, dosed cells contained 16:0 and 18:1ω9 proportions parallel to controls, but decreased 18:0 and more varied 16:1ω9 percentages. 18:2ω6, 20:2ω6, 18:3ω6, 22:4ω6, 18:3ω3 and 20:4ω3 levels were generally equal to, or greater than, controls, and 18:4ω3 amounts were consistently increased with 40mg/l p-oil. The relative percentages of 20:5ω3 and 22:5ω3 were more variable, but 22:5ω6 and 22:6ω3 levels were generally lower in dosed cells.

16:0 percentages were parallel to controls (23.4%) with p-CO incubation, 16:1ω9 was slightly increased with 20 and 40mg/l p-CO and decreased with 60mg/l p-CO (4.1%, 4.2% and 2.4%, respectively vs 3.5% in controls), while 18:0 levels were marginally reduced (15.8%, 15.2% and 13.8%, respectively vs 18.4% in controls). p-CO had no marked effect on 18:1ω9 and 18:2ω6 levels, but 20:4ω6

Legend to Table 3.6.3.1.

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 3.6.3.1.

The fatty acid spectrum of cells derived from rat lung.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MC] (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	23.4±0.15	23.8	20.9	25.1	23.1	20.3	27.1	18.3	20.3	22.2	23.4	18.7	12.5	24.6	19.6	26.5	22.4	18.9	26.8	26.8	20.4	23.2
	18:0	18.4±0.30	15.8	15.2	13.8	13.0	15.4	16.5	12.9	12.4	11.3	19.6	14.6	25.2	15.9	15.6	14.1	16.6	15.8	15.2	19.2	14.6	10.7
	20:0	0.1±0.00	-	0.1	-	-	-	-	-	-	-	-	0.2	3.2	-	-	-	-	-	-	-	0.1	-
	22:0	-	0.2	-	0.3	-	0.1	0.1	0.1	-	-	0.1	-	-	0.1	0.1	0.2	-	0.1	0.1	-	0.1	0.1
	24:0	0.2±0.00	-	0.7	-	-	-	-	-	-	-	-	0.2	-	-	0.6	-	-	0.8	-	-	0.7	-
ω9 MONOS.	16:1	3.5±0.05	4.1	4.2	2.4	11.2	2.9	-	0.9	1.0	3.8	4.6	3.0	1.1	6.6	1.4	4.6	4.9	1.4	4.8	6.8	5.9	8.3
	18:1	15.9±0.10	16.0	14.8	18.0	17.8	17.8	21.6	24.8	31.2	35.6	18.5	14.3	22.7	14.9	14.8	18.2	14.4	12.7	16.4	17.8	14.9	15.7
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	4.0±0.10	5.2	3.7	6.3	7.3	7.7	9.3	8.0	7.4	8.7	14.1	15.4	20.3	7.5	8.1	9.1	16.3	19.3	15.3	7.4	5.8	7.5
	18:3	-	0.7	-	0.7	0.5	-	0.8	0.6	-	0.6	1.8	0.1	-	0.7	-	1.0	-	-	1.1	0.6	-	0.4
	20:2	-	-	-	-	-	-	0.1	0.3	1.1	0.9	0.2	0.9	1.2	-	-	-	-	0.8	0.1	-	0.3	0.3
	20:3	2.0±0.00	1.7	2.0	1.7	1.3	1.4	1.0	1.5	1.3	1.0	1.0	1.8	0.7	1.3	2.1	1.2	2.4	5.2	1.7	0.9	1.5	1.1
	20:4	11.8±0.20	8.2	9.7	7.3	6.8	8.6	6.2	8.5	6.6	3.9	5.0	6.5	2.0	5.3	7.3	4.5	6.7	5.7	3.7	5.2	6.7	3.8
	22:4	9.0±0.65	14.7	12.3	13.0	10.9	13.6	9.4	13.4	9.8	4.9	4.3	12.0	2.7	12.9	12.8	9.3	8.2	6.1	9.5	7.8	10.2	10.5
	22:5	1.3±0.10	0.2	1.5	0.2	0.2	1.5	0.2	0.1	0.8	-	0.4	1.0	0.2	0.1	1.4	0.2	0.8	0.8	0.1	0.2	0.8	0.1
ω3 POLYS.	18:3	0.5±0.05	0.8	0.6	1.0	0.7	0.6	1.0	1.2	1.0	1.3	1.3	0.6	4.0	2.2	4.9	6.2	-	0.5	0.9	0.5	0.5	0.7
	18:4	0.5±0.00	0.3	1.9	0.5	0.3	2.2	0.3	0.3	1.3	0.2	0.3	1.0	1.2	0.3	1.9	0.3	-	1.7	0.2	0.3	1.4	0.4
	20:4	0.7±0.00	1.0	1.0	1.7	1.4	0.7	1.3	1.1	0.4	0.6	0.1	1.0	0.4	1.4	0.7	1.4	0.3	0.8	1.0	0.3	1.0	1.9
	20:5	1.2±0.05	1.3	2.7	1.0	0.9	1.4	0.5	1.6	0.2	0.8	0.5	1.3	0.2	0.9	1.9	0.4	0.1	1.1	0.3	1.2	4.5	5.3
	22:5	3.1±0.15	2.6	3.7	2.9	2.1	2.6	2.1	2.8	2.4	1.8	2.2	3.4	1.2	2.4	3.2	1.5	2.9	3.8	1.3	2.9	7.8	7.5
	22:6	4.3±0.20	3.5	5.1	4.2	2.5	3.3	2.5	3.8	3.0	2.4	2.6	4.3	1.4	3.1	3.9	1.5	4.0	4.7	1.4	1.9	3.0	2.7

percentages were at least 2.0% lower than controls (11.8%), whereas 22:4ω6 levels were 3.0% to 6.0% greater than controls (9.0%). However, no significant increase in 22:5ω6 was found. 18:4ω3, 20:5ω3, 22:5ω3 and 22:6ω3 amounts increased only with 40mg/l p-CO (1.9%, 2.7%, 3.7% and 5.1% vs 0.5%, 1.2%, 3.1% and 4.3% in controls, respectively), but 20:4ω3 was increased slightly with all concentrations (1.0% to 1.7% vs 0.7% in controls).

20.3% to 27.1% 16:0 was detected with p-MO dosage (23.4% in controls), whereas 16:1ω9 levels were significantly increased with 20mg/l p-MO, but decreased with 40 and 60mg/l p-MO (11.2%, 2.9% and 0%, respectively vs 3.5% in controls). p-MO induced a slight decrease in 18:0 (13.0% to 16.5% vs 18.4% in controls), but an increase in 18:1ω9 (17.8% to 21.6% vs 15.9% in controls) and 18:2ω6 (7.3% to 9.3% vs 4.0% in controls), percentages. 20:4ω6 levels were lower than controls (11.8%), and a greater proportion of its elongation product, 22:4ω6, was found (up to 13.6% vs 9.0% in controls), but 22:5ω6 levels were parallel to controls (1.3%) or decreased. The only ω3 PUFA's that were significantly increased were 18:4ω3 (2.2% with 40mg/l p-MO vs 0.5% in controls) and 20:4ω3 (1.4% and 1.3% with 20 and 60mg/l p-MO, respectively vs 0.7% in controls).

18:1ω9 levels increased significantly with dosage of 20, 40 or 60mg/l p-OO (24.8%, 31.2% and 35.6%, respectively

vs 15.9% in controls), whereas 16:0, 18:0 and 16:1ω9 proportions were parallel to controls (23.4%, 18.4% and 3.5%, respectively), or decreased. Raised percentages of 18:2ω6 (7.4% to 8.7% vs 4.0% in controls) and 20:2ω6 (0.3% to 1.1% vs 0% in controls) were found, but 20:4ω6 levels were lower than controls (11.8%). However, 22:4ω6 was significantly raised with 20mg/l p-OO (13.4% vs 9.0% in controls), yet 22:5ω6 levels were significantly lower than controls (1.3%). 18:3ω3 increased at least 2 fold with p-OO dosage (1.0% to 1.3% vs 0.5% in controls), and 40mg/l p-OO raised 18:4ω3 percentages (1.3% vs 0.5% in controls), but other ω3 FA levels were only marginally raised, or decreased.

16:0 and 16:1ω9 percentages decreased inversely with the amount of p-SSO dosed (23.4% to 12.5% and 4.6% to 1.1% vs 23.4% and 3.5% in controls, respectively), but 18:0, 20:0 and 18:1ω9 proportions increased significanty with 60mg/l p-SSO (25.2%, 3.2% and 22.7% vs 18.4%, 0.1% and 15.9% in controls, respectively). p-SSO supplementation raised cellular 18:2ω6 amounts (14.1% to 20.3% vs 4.0% in controls), and induced small increments in 18:3ω6, 22:4ω6 and 20:2ω6 with concentrations of 20, 40 and 60mg/l, respectively (1.8%, 12.0% and 1.2% vs 0%, 9.0% and 0% in controls, respectively). Up to 4.0% 18:3ω3 and 1.2% 18:4ω3 were detected (0.5% each in controls), but other ω3 PUFA levels were parallel to controls, or decreased.

Cells dosed with p-LO exhibited 16:0 and 18:1ω9 levels similar to controls (23.4% and 15.9%, respectively), marginally decreased 18:0 (14.1% to 15.9% vs 18.4% in controls), and varied 16:1ω9 amounts (1.4% to 6.6% vs 3.5% in controls). A concentration dependent increase in 18:2ω6 incorporation (7.5% to 9.1% vs 4.0% in controls), and up to 1.0% 18:3ω6 (none in controls), were detected, and 22:4ω6 was increased with 20 and 40mg/l p-LO (12.9% and 12.8%, respectively vs 9.0% in controls). 18:3ω3 incorporation increased from 0.5% in controls to 2.2%, 4.9% and 6.2% when 20, 40 or 60mg/l p-LO were added to cultures, respectively. Increased 18:4ω3 and 20:5ω3 were detected with 40mg/l p-LO (1.9% each vs 0.5% and 1.2% in controls, respectively) and 20:4ω3 with 20 and 60mg/l p-LO (1.4% vs 0.7% in controls), yet 22:5ω3 and 22:6ω3 levels were parallel to controls, or reduced.

p-EPO dosage had no pronounced effect on cellular 16:0 and 18:1ω9 levels, but decreased 18:0 marginally (15.2% to 16.6% vs 18.4% in controls), and had a more variable effect on 16:1ω9 (1.4% to 6.6% vs 3.5% in controls). 18:2ω6 increased from 4.0% in controls to 16.3%, 19.3% and 15.3% with 20, 40 and 60mg/l p-EPO, respectively, and 1.1% 18:3ω6 was detected with 60mg/l p-EPO, but none in controls, with 20 or 40mg/l p-EPO. 20:3ω6 increased significantly with dosage of 40mg/l p-EPO (5.2% vs 2.0% in controls), but no such changes were shown for other ω6 PUFA's. With the exception of slight increments in

18:4ω3, 22:5ω3 and 22:6ω3 detected with 40mg/l p-EPO, ω3 FA levels were either parallel to controls or decreased.

16:0 and 18:1ω9 levels were not markedly altered with p-FO incubation, whereas 18:0 was decreased overall, and 16:1ω9 significantly increased (5.9% to 8.3% vs 3.5% in controls). 5.8% to 7.5% 18:2ω6 was detected (4.0% in controls), and up to 10.5% 22:4ω6 (9.0% in controls), but 22:5ω6 levels were decreased. A maximum of 1.4% 18:4ω3 was detected with 40mg/l p-FO and 1.9% 20:4ω3 60mg/l p-FO (0.5% and 0.7% in controls, respectively), whereas 20:5ω3 was significantly increased both with 40 and 60mg/l p-FO (4.5% and 5.3%, respectively vs 1.2% in controls). 22:5ω3 increased by at least two fold with 40 and 60mg/l p-FO (7.8% and 7.5%, respectively vs 3.1% in controls), yet 22:6ω3 was decreased (1.9% to 3.0% vs 4.3% in controls).

3.6.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Rat Lung.

The radioactivity recovered from cultures incubated with 2μCi of each of the radiolabelled C18 FA's is shown in Table 3.6.4.1.

The total counts recovered in the spent incubation media ranged from 2.4 to 3.0×10^6cpm, with only 0.5 to 0.8×10^6 cpm recovered from the pooled buffers after washing the cells; this accounted for non-specific binding. For both [^{14}C]-18:1ω9 and [^{14}C]-18:3ω3, the equivalent of 4.3×10^6cpm were dosed to the incubation medium. Total recovery of radiolabel from cultures dosed with these FA's was approximately 84% and 91%, respectively. For [^{14}C]-18:2ω6 the equivalent of 4.8×10^6cpm were dosed to the incubation medium, and total recovery of this radioisotope from the cultures was about 96%. The total amount of each of these radioisotopes incorporated into the cells was approximately 13% for [^{14}C]-18:1ω9, 15% for [^{14}C]-18:2ω6, and 7% for [^{14}C]-18:3ω3.

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 rat lung fibroblasts is shown in Table 3.6.4.2. As Δ6D represents the first enzyme in the desaturase cascade, total activity of this enzyme is reflected by the sum of all subsequent desaturase and elongase products.

Table 3.6.4.1.

The radioactivity (cpm) recovered from rat 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				
2350600 (±26420)	664370 (±14630)	3014970	548552 (±1989)	3.6
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
3043260 (±18423)	784740 (±7191)	3828000	734574 (±1176)	4.6
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
3044360 (±36020)	521690 (±5265)	3566050	304473 (±1779)	3.9

Table 3.6.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from rat 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	12.7	18:2	4.8	18:3	3.4
20:1	34.5	18:3	1	18:4	22.8
22:1	37.9	20:2	24.2	20:3	14.7
24:1	15.0	20:3	25.5	20:4	18.7
		20:4	14.7	20:5	19.0
		22:4	7.2	22:5	11.1
		22:5	6.5	22:6	10.3
TOTAL CPM	1107	TOTAL CPM	1865	TOTAL CPM	804

12.7% of the total [^{14}C]-18:1ω9 counts derived upon GLA analysis (1107cpm) remained as 18:1ω9, whereas 34.5%, 37.9% and 15.0% were elongated to 20:1ω9, 22:1ω9 and 24:1ω9, respectively.

Only 4.8% of the total [^{14}C]-18:2ω6-derived counts measured (1865cpm) remained unchanged, whereas 17.2% was shown to be 18:3ω6. 24.2% and 25.5% of the total counts were found as 20:2ω6 and 20:3ω6, respectively, compared to 14.7% 20:4ω6. Furthermore, 7.2% and 6.5% 22:4ω6 and 22:5ω6 were detected, respectively.

Only 3.4% of total counts (804cpm) remained as 18:3ω3, and 14.7% was found as the immediate elongation product, 20:3ω3. 22.8% 18:4ω3 was detected, compared to 18.7% 20:4ω3 and 19.0% 20:5ω3. 11.1% of total [^{14}C]-18:3ω3-derived counts were shown to be 22:5ω3 and 10.3% 22:6ω3, and these percentages were larger than those found for corresponding products in the ω6 series.

3.6.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 3.6.5.1. shows the lipoperoxides quantitated in dosed rat lung fibroblasts and their respective spent incubation media, compared to controls. The results are 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.

Spent medium derived from cultures supplemented with 60mg/l p-SSO 60mg/l p-FO, 40mg/l p-LO, or p-60mg/l p-LO contained 0.3, 0.6, 0.1 and 3.7nmoles MDA/10^6 cells, respectively, but MDA was absent from all other spent media. However, control and all dosed cells formed MDA. 1.1nmoles MDA/10^6 control cells was measured, whereas concentrations in dosed cells ranged from a low of 1.0 nmole MDA/10^6 cells (with 20mg/l p-SSO and p-EPO) to a maximum of 10.8nmoles MDA/10^6 cells (with 60mg/l p-EPO). Cellular lipoperoxide amounts varied with the p-oil supplemented, but increased in an overall concentration dependent manner. p-OO and p-SSO produced the smallest cellular lipoperoxide amounts overall, ranging from 1.0 to 1.3nmoles MDA/10^6 cells, whereas p-CO and p-MO generated 1.4 to 2.1nmoles MDA/10^6 cells. Cells dosed with 20mg/l p-LO, p-EPO or p-FO contained no more MDA than controls, but p-LO and p-FO generated more MDA than p-EPO with a concentration of 40mg/l (1.9, 2.1 and

Table 3.6.5.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	1.1			
CO		1.4	1.4	1.5
MO		1.6	1.7	2.1
OO		1.2	1.2	1.3
SSO		1.0	1.1	1.3
LO		1.1	1.9	7.2
EPO		1.0	1.2	10.8
FO		1.1	2.1	2.6
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		-	-	0.3
LO		-	0.1	3.7
EPO		-	-	-
FO		-	-	0.6

1.2nmoles MDA/10^6 cells, respectively). p-EPO, however, induced the greatest cellular lipoperoxide amounts with a concentration of 60mg/l, followed by p-LO and p-FO (10.8, 7.2 and 2.6 nmoles MDA/10^6 cells, respectively).

3.6.6 The Eicosanoid Profile of Cells Derived from Rat Lung.

Table 3.6.6.1. shows the eicosanoids positively detected in control and dosed rat lung 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 total eicosanoids/10^6 cells.

Total eicosanoid production by control cells amounted to 727.6pmoles/10^6 cells, of which 22.0% correlated with the retention times of the prostanoid standards used. This fraction was shown to be composed of 11.9% PGE_1, 1.6% PGI_2, 3.0% TXB2, 4.2% PGF_α and 1.3% PGE2. Varying proportions of these prostanoids were found in dosed cells, but neither control nor dosed cells formed PGD_2.

The percentage of total prostanoids detected in dosed cells ranged from 15.8% (with 40mg/l p-MO) to a maximum of 28.5% (with both 40mg/l p-MO and p-LO), compared to 22.0% in controls, but no consistent correlation was evident between total eicosanoid percentages and p-oil concentration. p-Oil supplementation, however, altered the production of individual prostanoids in these cells. PGI_2 levels were lower in dosed cells than in controls (0.3% to 1.0% vs 1.6%, respectively), and no particular p-oil concentration consistently induced greater PGI_2 percentages than any other concentration. Cellular TXB_2

Legend to Table 3.6.6.1.

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

Table 3.6.6.1.

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

pseudo-Oil (μg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	$PGF_{\alpha(1+2)}$	PGE_2	PGE_1	PGD_2
CONTROL (727.6)		22.0	1.6	3.0	4.2	1.3	11.9	-
CO	20	25.4	0.9	14.6	8.5	1.2	0.2	-
	40	23.6	0.8	4.3	6.6	-	11.9	-
	60	24.3	0.5	14.9	7.4	1.2	0.3	-
MO	20	24.9	0.7	14.9	7.9	1.2	0.2	-
	40	15.8	1.0	3.2	4.2	-	7.4	-
	60	28.5	0.3	17.4	8.7	1.8	0.3	-
OO	20	21.0	0.7	11.0	8.1	1.1	0.1	-
	40	26.1	1.0	4.3	4.4	-	16.4	-
	60	26.5	0.3	14.9	9.7	1.1	0.5	-
SSO	20	26.6	0.3	15.5	9.1	1.5	0.2	-
	40	24.8	0.9	4.3	3.4	-	16.2	-
	60	27.5	0.4	15.9	8.0	2.1	1.1	-
LO	20	22.2	0.7	11.8	8.0	1.5	0.2	-
	40	18.1	0.7	3.7	4.3	-	9.4	-
	60	28.5	0.5	16.1	9.2	2.1	0.6	-
EPO	20	24.1	0.5	13.1	8.8	0.8	0.9	-
	40	20.1	0.7	5.1	3.1	-	11.2	-
	60	26.9	0.4	15.0	7.9	1.4	2.2	-
FO	20	28.3	0.9	17.0	8.7	1.5	0.2	-
	40	19.1	0.8	4.0	1.7	-	12.6	-
	60	20.7	0.8	12.3	5.7	1.2	0.7	-

percentages were comparable to controls (3.0%) with 40mg/l p-oil (3.2% to 5.1%), but were increased between 3 and 6 fold with 20 and 60mg/l p-oil (11.3% to 17.4%). PGF_{α} production was also enhanced with dosage of 20mg/l (7.9% to 9.1%) or 60mg/l (5.7% to 9.7%) p-oil, but more variable with 40mg/l p-oil (1.7% to 6.6%). compared to controls (4.2%). Incubation with 20 or 60mg/l p-oil had no profound effect on PGE_2 levels (0.8% to 2.1% vs 1.3% incontrols), but such was inhibited with 40mg/l p-oil. PGE_1 percentages, in contrast, were comparable in cells dosed with 0 or 40mg/l p-oil (11.9% vs 7.4% to 16.2%), and markedly decreased both with 60mg/l (0.3% to 2.2%) and 20mg/l (0.1% and 0.9%) p-oil.

3.6.7 Discussion.

It was apparent from lack of literature that very little is known regarding the effects of exogenous FA's on normal rat lung fibroblast growth. The present study indicated that p-oil supplementation affected the viability of such cells (Figs. 3.6.1.2-3.6.1.8.), and that this was dependent both on the p-oil concentration dosed and p-oil FA composition (Table 2.3.3.2.). The mediation of cytotoxicity only with p-OO, p-FO or p-CO dosage at concentrations greater than 80mg/l may relate partly to the relatively low population doubling time these cells exhibited (Table 2.3.1.1.). Furthermore, the ability of cultured rat lung fibroblasts to withstand large amounts of PUFA-rich p-oils without being killed may relate to the finding that this tissue has been implicated as the primary site for eicosanoid synthesis (Mathe et al 1977, Hyman et al 1978 and Harper et al 1984), the precursors of which are PUFA's.

OA, LA or ALA comprised about 71%, 70% and 63% of all FA's in p-OO, p-SSO and p-LO, respectively, thus it was likely that the cell viability changes induced with incubation of either p-oil related largely to these C18 FA's. Such moieties were all Δ6D substrates, but the variation in the number and position of their double bonds could explain the mediation of cytotoxicity with high p-OO concentrations, but not with p-SSO or p-LO.

The similar extent of growth limitation induced overall with p-SSO or p-LO dosage, however, implied that ALA and LA modulated similar effects in these cells, which may relate to the fact that both moieties are EFA's and exhibit a double bond at the ω6 position, unlike OA. On the other hand, the similar FA compositions of p-SSO and p-EPO suggested that the inability to limit cell viability with dosage of up to 100mg/l p-EPO related to the presence of 9% GLA only in this p-oil. Such may be important for timal rat lung fibroblast proliferation, or may suppress the growth limiting capability of other FA constituents in p-EPO, particularly LA. The finding that the greatest enhancement of cell viability occurred with incubation of p-oils containing significant PDFA amounts (9% GLA in p-EPO and 18% EPA in p-FO) indeed implied involvement of these moieties in the modulation of rat lung fibroblast proliferation. Synergistic and antagonistic interations between FA's in any p-oil should, however, not be overlooked as mediators of the growth responses induced. This may explain why cell viability did not always relate inversely to the p-oil concentration dosed and why p-oils of similar FA composition induced different effects.

The data in Table 3.6.3.1. supported the ability of rat lung fibroblasts to incorporate exogenous FA's, which modified dosed cell FA compositions to a greater or lesser extent compared to controls. There is substancial

evidence to indicate that dietary FA supplementation induces changes in membrane physical properties (eg. King et al 1971, Ginsberg et al 1981, Simon et al 1982, Stubbs et al 1984 and Spector et al 1985). A possible mechanism whereby p-oils were involved in the reduction of cell viability reported may therefore relate to differential uptake of exogenous FA's and alterations in membrane fluidity, dependent on p-oil FA composition. Furthermore, incubation with low p-oil concentrations could have allowed the cells to maintain a similar degree of membrane fluidity to controls, whereas larger p-oil amounts could have led to more pronounced changes in the membrane unsaturation index such that cell viability was limited or inhibited.

The finding that the cellular protein concentrations reported with p-oil supplementation (Table 3.6.2.1.) did not always correlate with the cell viability changes induced (Figs. 3.6.1.2-3.6.1.8.) implied FA involvement in the modulation of protein synthesis. Increased protein levels when cell viability was parallel to controls or limited indeed indicated that protein synthesis was stimulated, whereas the finding that decreased cell numbers alone could not justify the lowest amount of protein measured with 60mg/l p-MO implied suppression of protein synthesis. Since the modulation of protein synthesis varied in magnitude with dosage of identical amounts of different p-oils, even

when such yielded similar cell numbers, it was suggested that the capability to enhance or suppress protein synthesis varied with FA, and was relatable to FA structure and concentration in any p-oil. Absolute changes in cellular protein with p-oil dosage may nevertheless relate to alterations in the production of structural membrane proteins and/or enzymes involved in FA metabolism.

The lung has been implicated as the primary site for eicosanoid synthesis (Mathe et al 1977, Hyman et al 1978 and Harper et al 1984), thus its ability to desaturate and elongate FA's to provide eicosanoid precursors is of major importance. Comparison of control cell FA spectra with those derived from p-oil dosed cells indicated the capability of cultured rat lung fibroblasts to express desaturase and elongase enzymes (Table 3.6.3.1.). The raised 16:1ω9 levels found in dosed cells implied 16:0 desaturation, and the possibility for 16:1ω9 elongation also existed. In addition, the significantly decreased 16:0 and increased 18:0, 20:0 and 18:1ω9 levels detected with 60mg/l p-SSO dosage supported the capability for elongase and Δ9D expression. Comparison of ω3 PUFA desaturase cascade substrates and products indicated Δ6D, subsequent elongase and Δ5D expression. 18:3ω6 and 20:3ω6 formation with 20mg/l p-SSO and 40mg/l p-EPO, respectively supported Δ6D and elongase capability. Further, raised 22:4ω6 levels in dosed cells suggested

rapid 20:3ω6 desaturation and 20:4ω6 elongation, and this could reflect a requirement for higher PDFA's to maintain membrane fluidity. 22:4ω6 accumulation may also reflect a lack of significant 22:5ω6 formation via Δ4D. Slightly increased 22:6ω3 levels with supplementation of 40mg/l p-CO suggested the capability to express Δ4D, although 22:6ω3 levels lower than controls with p-FO dosage implied suppression of Δ4D expression, despite substrate availability. It has been suggested that 22:5ω3 is the storage form of 20:5ω3 in rat lung (Swanson et al 1987). This could explain the preference to form 22:4ω6 and 22:5ω3 rather than 22:5ω6 and 22:6ω3, especially since the former moieties can undergo extra-microsomal retroconversion and further desaturation, thus giving the cells greater flexibility to utilise these PUFA's efficiently to form the products required, particularly for 2- and 3-series eicosanoid synthesis. Overall, the potential to express desaturase cascade enzymes implied that rat lung fibroblasts could control membrane fluidity, and this may explain why cytotoxicity was induced only with p-CO, p-OO and p-FO concentrations greater than 80mg/l.

Desaturation with individual radiolabelled C18 FA's was assessed to establish whether the pattern of p-oil desaturation reported in Table 3.6.3.1. was modulated with FA mixtures. 24:1ω9 detection when cultures were dosed with [^{14}C]-18:1ω9 was consistent with elongase

expression, although no evidence to suggest [^{14}C]-18:1ω9 desaturation was found (Table 3.6.4.2.). This probably reflects the inhibition of 18:1ω9 desaturation by EFA's, which has been well documented (Brenner et al 1966, 1969, Brenner 1977 and Holman 1986b). On the other hand, the pattern of radioactivity derived from cultures incubated with either [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 was consistent with significant Δ6D and subsequent elongase activity (Table 3.6.4.2.), and the significant amounts of [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6 directly elongated to 20:3ω3 and 20:2ω6, respectively could have served as EFA storage products for retroconversion. Conversion of about 40% [^{14}C]-18:3ω3 and 28% [^{14}C]-18:2ω6 to post-Δ5D PUFA's supported Δ5D expression. 20:5ω3 and 20:4ω6 elongation was shown, but Δ4D activity was more limited. The finding that desaturase capability was greatest with EFA substrates and progressively decreased as more unsaturated PDFA's were formed nevertheless comfirmed the rate limiting nature of Δ6D in the cascade (Castuma et al 1972, Brenner 1974 and de Gomez Dumm et al 1976). Furthermore, the greater proportion of [^{14}C]-18:3ω3 converted to PDFA's than [^{14}C]-18:2ω6 supported the established preference for ω3, rather than ω6, series PUFA substrates for desaturation (Brenner et al 1966, Mead et al 1976 and Kanau et al 1977). This was consistent with the direct elongation of more [^{14}C]-18:2ω6 to 20:2ω6 than [^{14}C]-18:3ω3 to 20:3ω3 (Table 3.6.4.2.).

Comparison of the data in Tables 3.6.3.1. and 3.6.4.2. clearly indicated that the capability for desaturation and elongation were more limited with p-oil than with individual C18 FA incubation. Competitive interactions between p-oil FA's could well have suppressed desaturase cascade enzyme expression, particularly since the competitive nature of FA's for such enzymes have been well documented both *in vivo* and *in vitro* (Brenner et al 1966, Mohrhauer et al 1963a, Ullman et al 1971, de Schriver et al 1982, Holman 1986a, 1986b, Nassar et al 1986 and Cook et al 1987). Cells *in vivo* are exposed to FA mixtures rather than to individual FA's, hence the desaturase cascade enzyme capability reported with individual FA dosage may not necessarily reflect the situation in the 'real world'. On the other hand, p-oil FA composition mimicked that of dietary oils, thus enzyme expression reported with p-oil dosage probably reflects the capability of rat lung fibroblasts to desaturate and elongate dietary oils *in vivo*.

The cellular lipoperoxides measured both in the presence and absence of p-oil supplementation (Table 3.6.5.1.) supported enzymic and/or non-enzymic mechanisms for their production in rat lung fiboblasts. Control cells contained unsaturated FA's that were potential oxidation substrates (Table 3.6.3.1.), hence the lipid peroxides measured in such were not surprising. Control cell lipid peroxides could reflect the lack of sufficient

alpha-tocopherol to limit FA oxidation, or inefficient mechanisms to remove different species of activated oxygen that promote lipoperoxidation, such as catalase, peroxidase and superoxide dismutase (Lands et al 1971, Smith et al 1972, Tappel 1972, 1980 and Pryor 1976). The greater lipid peroxide amounts reported in certain dosed cultures than in controls implied oxidation of unsaturated p-oil FA's, and the numerical differences in lipoperoxide concentrations reported between dosed cultures 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 susceptibility oxidation, which in turn related to the number and position of double bonds in a FA.

It was apparent that the small cellular lipoperoxide amounts formed with 60mg/l p-SSO incubation related largely to oxidation of free LA, but the greatest cellular lipoperoxide amounts induced with 60mg/l p-EPO incubation probably also reflected oxidation of GLA incorporated only with p-EPO dosage, as both p-oils otherwise exhibited similar FA compositions. It followed from comparison of the amount of C18 trienoic FA's present in p-EPO and p-LO (about 9% GLA and 63% ALA, respectively) with the lipid peroxides these p-oils induced that GLA was more susceptible to oxidation than ALA. This could relate to the easier production of endoperoxides from GLA than ALA because the former

merely requires chain elongation to be usable as an endoperoxide substrate, while ALA requires elongation and two desaturations first. On the other hand, the fact that incubation for 48 hours with 60mg/l p-EPO or p-LO induced markedly more lipoperoxides than p-FO, despite the presence of 18% EPA in the latter, may relate to FA availability for oxidation. The finding that lipoperoxide amounts approximated to controls with supplementation of certain unsaturated FA-rich p-oils (20mg/l p-LO, 20mg/l p-FO, 20 and 40mg/l p-EPO, and all concentrations of p-OO and p-SSO) indeed implied that the incorporated exogenous FA's were largely components of complex membrane lipids, and therefore not so readily accessible to oxidation. In fact, the ability of rat lung to peroxidise lipids <u>in vitro</u> has been shown to correlate with the presence of PUFA's in the TAG fraction (Kehrer et al 1978). It was also possible that dosage with different p-oils altered the rate of PGL turnover and thus the release of FA's from membrane lipids into the cellular pool. Such may explain the slightly greater cellular lipoperoxide amounts noted with supplementation of p-CO or p-MO than with certain more unsaturated FA-rich p-oils.

The significantly greater amounts of cellular than spent medium lipid peroxides (Table 3.6.5.1.) supported the ability of these cells to incorporate exogenous FA's (Table 3.6.3.1.). Hence it was unlikely that the lipid

peroxides measured in the spent medium of dosed cultures were formed primarily as a result of auto-oxidation of unincorporated p-oil FA's, particularly since these moieties were albumin-bound. The absence of cytotoxicity with dosage of 60mg/l p-oil (Figs. 3.6.1.2-3.6.1.8.) and the known ability of lipoperoxides to increase membrane permeability (Chio et al 1969 and Frankel 1984) rather suggested that the spent medium lipoperoxides originated intracellularly via passage through the plasma membrane. Cellular FFA's and lipoperoxidation enzymes could also have been released in this process, thus the possiblity that spontaneous and/or enzymic FFA oxidation occurred extracellularly can not be ignored as contributing to the spent medium lipid peroxides reported.

Growth inhibition was not induced with supplementation of 20, 40 or 60mg/l p-oil (Figs. 3.6.1.2-3.6.1.8.), thus no correlation between lipid peroxide formation (Table 3.6.5.1.) and cytotoxicity could be established. Lipid peroxides should, however, not be overlooked as a possible mechanism involved in the modulation of cytotoxicity with dosage of higher p-oil concentrations. It was nevertheless apparent that if lipid peroxides were directly involved in the inhibition of rat lung fibroblast proliferation, the concentrations required would have to be considerably greater than those reported. On the other hand, the inverse relationship generally found between rat lung cell viability and MDA

concentrations with p-oil incubation suggested lipid peroxide involvement in the modulation of growth limitation. However, the fact that the greatest cellular lipoperoxide concentrations induced with 60mg/l p-EPO correlated with the absence of growth limitation implied that the modulation of cell viability also related to other mechanisms, such as alterations in membrane fluidity. As the lung is continuously exposed to high oxygen levels in vivo, this tissue may be particularly resistant both to the formation and effects of lipid peroxides. The extent of lipoperoxidation in rat lung microsomes has indeed been shown to be considerably lower than in liver microsomes, and this was relatable to its markedly greater vitamin E content and affinity for the vitamin (Krishnamurthy et al 1963, Gallo-Torres et al 1971, Kelleher et al 1972, Kornbrust et al 1980 and Sevanian et al 1982).

Total eicosanoid production accounted for 66% of all cellular lipoperoxides measured in control cells (Tables 3.6.5.1. and 3.6.6.1.), and this supported studies which have shown the lung to be an important site for eicosanoid production (Mathe et al 1977, Hyman et al 1978 and Harper et al 1984). In the presence of exogenous AA, Pace-Asciak et al (1977) found that the primary eicosanoids formed by rat lung homogenates were 6-keto $PGF_{1\alpha}$, followed by TXB2, whereas such exhibited relatively little capacity to produce PGE_2, $PGF_{2\alpha}$ and

PGD_2. Others reported some PGE2, $PGF1_\alpha$ and $PGF2_\alpha$, but no PGD_2, formation in this tissue (Moore 1985). Under the conditions used in the present study, control rat lung fibroblasts similarly produced no PGD_2 and small amounts of PGE_2, PGI2, TXB2 and PGF_α, although marked PGE_1 formation was additionally shown (Table 3.6.6.1.). The preferential synthesis of PGE_1 may be the result of increased PGH_1-PGE_1 isomerase expression or relative decreases in the biosynthesis of other prostanoids. However, the finding that dosage with 20 or 60mg/l p-oil markedly enhanced TXB_2 and PGF_α, and suppressed PGE_1, production implied modulation of eicosanoid synthesis, probably at the level of the synthetase enzymes. Changes in the proportions PGI_2 and PGE2 were also found with p-oil dosage, although total eicosanoid production was not markedly altered compared to controls (Table 3.6.6.1.). Others showed that eicosanoid synthesis (6-keto $PGF_{1\alpha}$, TXB2 and PGE2) was not altered in this tissue when olive oil enriched diets were fed compared to controls (fed a lard enriched diet), despite displacement of AA with OA in membrane lipids (Lokesh et al 1988). Menhaden oil also displaced AA with ω3 PUFA's, although eicosanoid synthesis was decreased (Lokesh et al 1985). This implied that different FA components of these oils mediated eicosanoid production in a specific manner. It was apparent, however, that the modulation of eicosanoid production in our study did not relate primarily to incorporation of exogenous FA's per se,

since no clear differences in total or individual eicosanoid production was found between cells dosed with identical amounts of different p-oils, either containing or devoid of eicosanoid precursors. The production of the prostanoids reported was also not related to the degree of p-oil unsaturation (Table 2.3.3.2.) or the ability of these cells to form eicosanoid precursors with p-oil supplementation (Table 3.6.3.1.).

The inability to induce marked differences in the levels of the eicosanoids reported with dosage of the same concentration of different p-oils suggested utilisation of endogenous rather than exogenous PUFA precursors for eicosanoid production. The enhanced prostanoid levels reported in Table 3.6.6.1. could indeed relate to the presence of 20:3w6 and particularly 20:4w6 in undosed cells (Table 3.6.3.1.), which are usable by this tissue for prostanoid synthesis (Lokesh et al 1985). The eicosanoids detected nevertheless implied that the cyclooxygenase pathway was functional in these cells, although the exact mechanism by which prostanoid production was modulated with p-oil dosage was unclear. The possibility exists that incubation with different p-oil concentrations influenced eicosanoid precursor availability, or modulated various enzymic steps in the prostanoid biosynthetic pathway either by activating, suppressing or inhibiting their activity under the culture conditions used.

Eicosanoid profiles (Table 3.6.6.1.) did not correlate in any way with the changes reported in cell viability (Figs. 3.6.1.2-3.6.1.8.), total cellular protein (Table 3.6.2.1.), desaturation capability (Table 3.6.3.1.), or lipid peroxide production (Table 3.6.5.1.) with p-oil dosage. This implied that although p-oil supplementation influenced the overall production of the eicosanoids detected, or the balance between individual prostanoid groups, these endogenously synthesised eicosanoids were probably not directly involved in the modulation of the effects demonstrated with p-oil dosage. Indeed, this mechanism could not explain the effects of p-oils rich in saturated or monoenoic FA's, which can not be metabolised by this route. Membrane fluidity changes and lipoperoxides were proposed as alternative mechanisms.

3.7 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM RAT SKIN.

3.7.1 Effects of pseudo-Oils on Cell Viability.

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

Final medium albumin concentrations ranging from 0 to 250mg/l had no significant effect on the viability of these cells (Fig. 3.7.1.1.). The effects observed with p-oil dosage were therefore 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 $10.8x10^4$/ml. This equated to approximately 72% 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 the cytostatic number were hence considered cytotoxic.

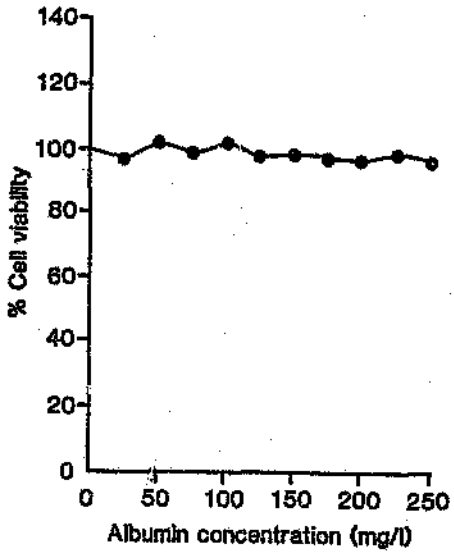
Figs. 3.7.1.2-3.7.1.8. show the data obtained from the incubation of cells with p-oils. Cell proliferation was significantly enhanced only with low concentrations of

Legend to Figs. 3.7.1.1-3.7.1.9.

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. 3.7.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 3.7.1.2-3.7.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 3.7.1.9. 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. 3.7.1.1.

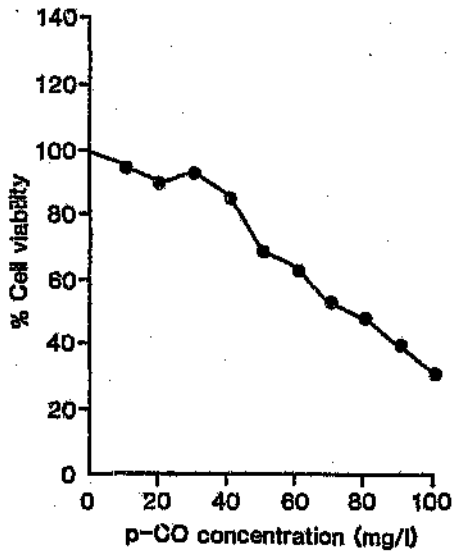
The percentage viability of cells derived from rat skin incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.8	12
25	96.2	3.2	12
50	101.1	3.2	12
75	97.9	1.1	12
100	101.4	3.5	12
125	96.6	2.4	12
150	97.5	2.3	12
175	97.2	3.1	12
200	96.4	3.0	12
225	98.4	3.2	12
250	96.3	5.4	12

Fig. 3.7.1.2.

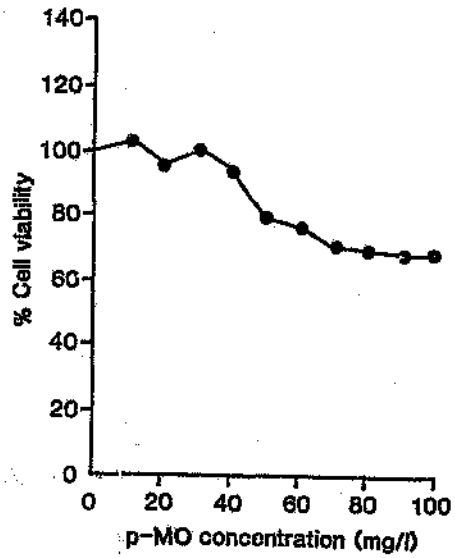
The percentage viability of cells derived fron rat skin incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.6	6
10	94.9	2.8	6
20	90.3	4.5	6
30	94.2	1.1	6
40	86.5	5.5	6
50	69.4	4.6	6
60	63.8	6.1	6
70	53.1	4.4	6
80	49.0	2.4	6
90	40.3	2.3	6
100	30.9	3.3	6

Fig. 3.7.1.3.

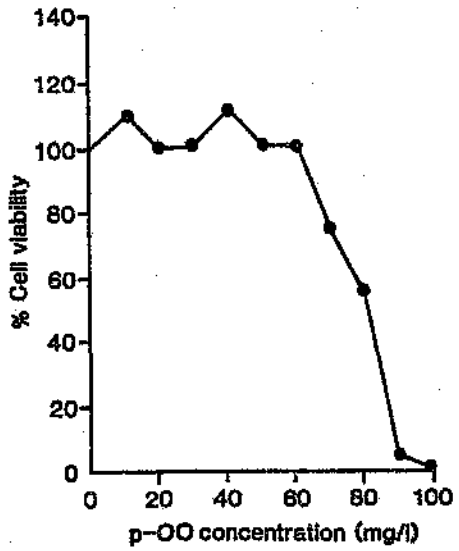
The percentage viability of cells derived from rat skin incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	103.3	2.7	6
20	96.2	1.8	6
30	100.3	1.9	6
40	93.8	2.4	6
50	80.0	1.7	6
60	77.8	2.0	6
70	70.5	2.0	6
80	69.7	2.1	6
90	68.8	2.0	6
100	69.7	1.8	6

Fig. 3.7.1.4.

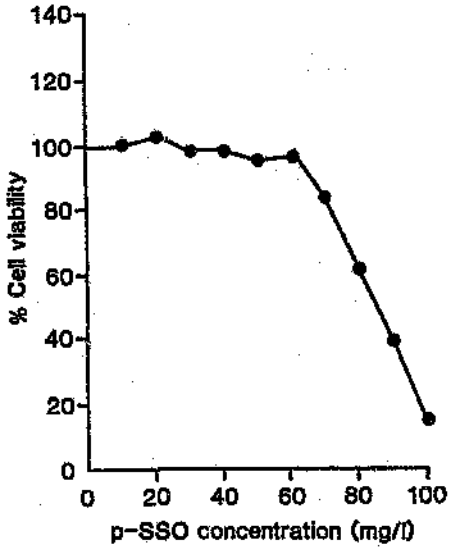
The percentage viability of cells derived from rat skin incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	110.6	2.9	6
20	100.4	2.0	6
30	101.8	4.7	6
40	112.4	2.7	6
50	101.9	3.1	6
60	101.4	5.0	6
70	76.9	3.0	6
80	56.9	3.4	6
90	4.8	0.8	6
100	0.0	0.0	6

Fig. 3.7.1.5.

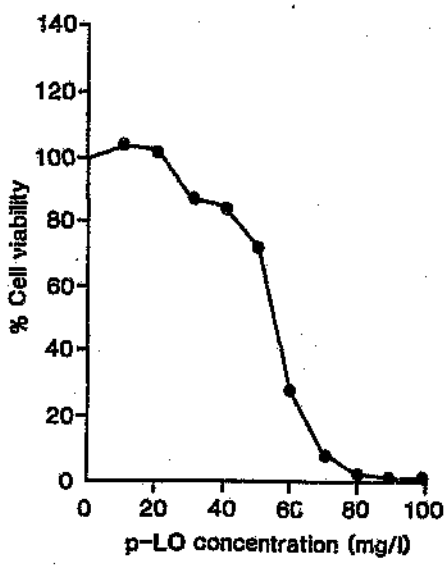
The percentage viability of cells derived from rat skin incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.1	6
10	100.7	2.0	6
20	103.4	2.3	6
30	98.8	2.2	6
40	99.0	1.8	6
50	95.8	1.8	6
60	97.6	2.2	6
70	83.9	2.4	6
80	61.6	3.1	6
90	39.1	1.9	6
100	14.9	1.2	6

Fig. 3.7.1.6.

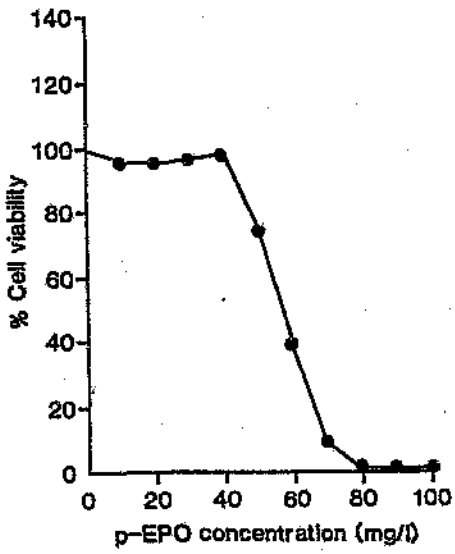
The percentage viability of cells derived from rat skin incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.4	6
10	103.9	4.9	6
20	102.7	3.9	6
30	88.2	4.4	6
40	86.0	2.9	6
50	74.0	4.7	6
60	29.4	5.0	6
70	9.2	1.9	6
80	2.1	1.0	6
90	0.5	0.3	6
100	0.3	0.3	6

Fig. 3.7.1.7.

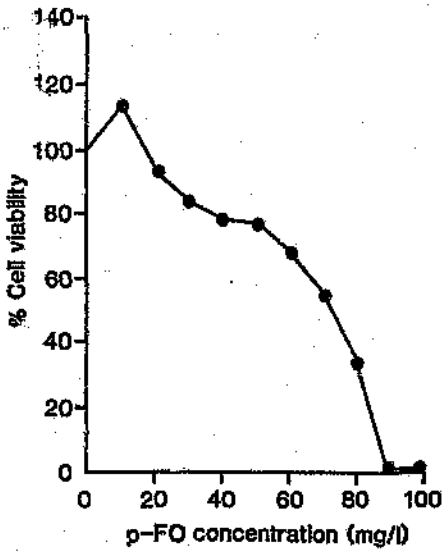
The percentage viability of cells derived from rat skin incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.7	6
10	95.9	2.1	6
20	96.3	6.0	6
30	97.6	2.8	6
40	98.9	2.7	6
50	74.3	4.6	6
60	38.9	4.3	6
70	8.9	1.1	6
80	0.9	0.6	6
90	0.0	0.0	6
100	0.0	0.0	6

Fig. 3.7.1.8.

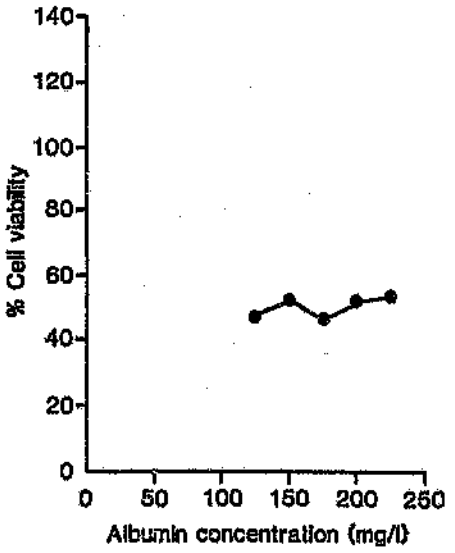
The percentage viability of cells derived from rat skin incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.3	6
10	114.8	2.5	6
20	94.1	2.5	6
30	84.5	2.9	6
40	79.4	3.4	6
50	78.7	4.0	6
60	68.9	3.4	6
70	56.0	2.3	6
80	35.5	2.5	6
90	0.0	0.0	6
100	0.0	0.0	6

Fig. 3.7.1.9.

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



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.7	6
125	47.9	2.5	6
150	53.5	1.4	6
175	47.1	3.5	6
200	52.8	1.1	6
225	54.0	3.7	6

p-OO or p-FO, whereas all p-oils limited, and with the exception of p-MO all p-oils inhibited cell viability to a greater or lesser extent, dependent on the p-oil and concentration supplemented.

Dosage with increasing amounts of p-CO reduced cell viability in an overall concentration dependent manner (Fig. 3.7.1.2.). Cytostasis was induced with 50mg/l p-CO, whereas half the cells were viable with 76mg/l p-CO (ID_{50}) and 30.9% with 100mg/l p-CO.

Enrichment of the culture medium with up to 30mg/l p-MO had little effect upon cell viability, but such was progressively reduced to 77.8% with 60mg/l p-MO (Fig. 3.7.1.3.). Cell viability plateaued and ranged from 68.8% to 70.5% with dosage of 70 to 100mg/l p-MO. This approximated to the cytostatic number, thus no ID_{50} could be calculated.

Cell viability was similar to controls with dosage of up to 60mg/l p-OO or p-SSO (Figs. 3.7.1.4. and 3.7.1.5., respectively), although proliferation was stimulated to 110.6% with 10mg/l p-OO and 112.4% with 40mg/l p-OO. Growth limitation occurred with 70mg/l p-OO or p-SSO, but greater amounts were increasingly cytotoxic. Both p-OO and p-SSO exhibited comparable ID_{50} values (about 82 and 85mg/l, respectively), but no viable cells were found when cultures were enriched with 100mg/l p-OO, compared to 14.9% with 100mg/l p-SSO.

Dosage with up to 20mg/l p-LO or 40mg/l p-EPO had little significant effect on cell viability, but growth was limited to a point of cytostasis with 50mg/l p-LO or p-EPO (Figs. 3.7.1.6. and 3.7.1.7., respectively). These were the most effective p-oils to inhibit rat skin fibroblast proliferation, and this correlated with the lowest ID_{50} values calculated (about 55mg/l for p-LO and 57mg/l p-EPO). More than 90% of the cells took up Trypan blue with 70mg/l p-LO or p-EPO, and this increased to practically 100% with concentrations of 90 or 100mg/l.

Cell proliferation was enhanced to a maximum of 114.8% with 10mg/l p-FO, and was progressively limited with concentrations of 20 to 50mg/l p-FO such that cytostasis occurred with 60mg/l p-FO and cytotoxicity with higher amounts (Fig. 3.7.1.8.). Half the cells were viable with approximately 73mg/l p-FO (ID_{50}) compared to 35.5% with 80mg/l p-FO, but 100% cell death was observed with 90mg/l and 100mg/l p-FO.

To eliminate the possibility that any of the effects induced with p-oil dosage were influenced by the amount of albumin bound to the FA's, these cells were incubated with a fixed amount of p-FO corresponding with its ID_{50} (approximately 73mg/l) and dosed with varying amounts of albumin. Five concentrations were chosen around which to vary the amount of albumin, viz. two points above, two points below and the ID_{50} of p-FO. The results shown in

Fig. 3.7.1.9. indicated that the amount of p-FO dosed reduced cell viability to approximately 50% of controls, and that such was not affected by the amount of albumin used as FA carrier. Synergistic effects of albumin could hence be excluded, and any effects induced by the p-oils could be attributed solely to the exogenous FA's.

Fig. 3.7.1.10. depicts the growth of cells obtained from rat skin in culture medium supplemented with 10% serum derived from its own species compared to commercially available fetal calf serum (FCS) in order to establish whether FCS modulated cell growth in any way. These cells grew from 10.0 to 13.1×10^4 over a 72 hour period when culture medium was supplemented with 10% FCS. Plating efficiency was low in the presence of 10% rat serum (RS), however, resulting in an initial decrease in the number of viable cells at 24 hours to 7.0×10^4. The cells grew in a linear manner over the next 48 hours, although only 8.2×10^4 viable cells were found at the end of the incubation period. Thus, the routine use of 10% FCS did not limit cell growth compared to 10% RS, and promotion of growth limitation and cytotoxicity were attributed solely to the exogenous FA's incorporated.

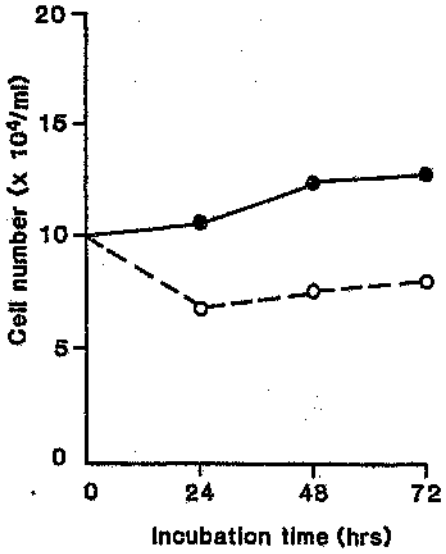
Rat skin fibroblasts were subsequently plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient amounts for all quantitative and qualitative analyses to be carried out. Upon harvesting, cell numbers were compared and found to be statistically

Legend to Fig. 3.7.1.10.

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. 3.7.1.10. shows mean cell numbers versus he incubation period (hours).

Fig. 3.7.1.10.

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



Incubation Time (hrs)	Mean Cell Number (x10^4/ml)	±s.e.m.	n
Foetal calf serum:			
24	10.8	0.3	9
48	12.7	0.2	9
72	13.1	0.4	9
Adult rat serum:			
24	7.0	0.2	9
48	7.7	0.3	9
72	8.2	0.3	9

similar to those obtained in Figs. 3.7.1.1-3.7.1.8. All biochemical assays were thus performed on these samples.

3.7.2 Effects of pseudo-Oils on Total Protein.

Table 3.7.2.1. shows total protein content of rat skin fibroblasts at each of the 3 p-oil concentrations dosed compared with controls, expressed as µg total protein per 10^6 cells seeded.

Control fibroblasts exhibited 374.7µg total protein/10^6 cells seeded, whereas p-oil dosed fibroblasts contained 269.4 to 579.6µg protein/10^6 cells seeded (obtained with 60mg/l p-LO and 40mg/l p-OO, respectively). Protein levels were higher than controls in every instance with incubation of 20mg/l p-oil (392.2 to 473.6µg protein/10^6 cells seeded). This was also true with dosage of 40mg/l p-oil (436.1 to 579.6µg protein per 10^6 cells seeded), although cells exhibited less protein than controls with addition of 40mg/l p-CO (327.4µgprotein/10^6 cells seeded). Enhanced protein levels were found with 60mg/l p-OO supplementation (445.4µg protein/10^6 cells seeded), but amounts were decreased with incubation of other p-oils at this concentration (269.4 to 366.8µg protein per 10^6 cells seeded). Cellular protein levels decreased inversely with the amount of p-CO, p-SSO, p-LO or p-FO supplemented, whereas the greatest and lowest amounts measured with p-MO, p-OO or p-EPO dosage occurred with a concentration of 40 and 60mg/l, respectively.

The spent incubation media derived from these cultures were also analysed for total protein. The values for all

Table 3.7.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	374.7			
CO		392.2	327.4	305.9
MO		422.5	449.3	366.8
OO		471.2	579.6	445.4
SSO		444.9	436.1	361.4
LO		473.6	466.3	269.4
EPO		436.1	468.2	348.4
FO		458.1	440.5	366.9

samples were summed, and a mean protein concentration of 46.8±0.35mg/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).

3.7.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Rat Skin.

Table 3.7.3.1. lists the FA spectra of cells incubated with 0, 20, 40 or 60mg/l p-oil.

Control cells exhibited a FA spectrum in which 18:0 comprised 19.1% of all FA's detected. 16:0, 18:1ω9, 20:4ω6 and 22:4ω6 contributed 18.3%, 16.5%, 13.3% and 9.9% to the total spectrum, respectively, while other FA's contributed less than 5% each to the total.

Dosed cells generally contained variable proportions of 16:0, less 18:0 and 16:1ω9, and more 18:1ω9 and 18:2ω6, than control cells. 20:4ω6 and 22:5ω6 percentages were decreased in dosed cells, although 22:4ω6 was markedly increased with supplementation of 20mg/l p-oil. Dosed cells generally contained less 18:4ω3 and 20:4ω3 than controls, while the relative amounts of 18:3ω3, 20:5ω3, 22:5ω3 and 22:6ω3 were more variable.

Dosage with 20mg/l p-CO had little effect on 16:0 levels (17.3% vs 18.3% in controls, respectively), but this was increased with 40 and 60mg/l p-CO (25.9% and 27.7%, respectively). Similar changes occurred in 18:1ω9 levels (16.5%, 15.1%, 19.3% and 23.3% with 0, 20, 40 and 60mg/l p-CO, respectively), while 18:0 and 16:1ω9 proportions were decreased compared to controls (19.1% and 4.2%, respectively). p-CO had little effect on 18:2ω6 levels

Legend to Tables 3.7.3.1. and 3.7.3.2.

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 3.7.3.1.

The fatty acid spectrum of cells derived from rat skin.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l)			[p-MO] (mg/l)			[p-OO] (mg/l)			[p-SSO] (mg/l)			[p-LO] (mg/l)			[p-EPO] (mg/l)			[p-FO] (mg/l)		
			20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
SATURATES.	16:0	18.3±0.00	17.3	25.9	27.7	17.0	20.8	24.3	14.6	15.9	12.0	19.2	16.5	9.5	14.2	16.7	21.2	20.9	16.5	14.7	15.4	19.0	15.0
	18:0	19.1±0.05	12.0	13.7	16.7	14.3	13.4	16.6	8.6	7.1	15.2	11.6	11.0	21.7	12.1	16.1	9.9	11.5	10.1	16.2	11.7	12.2	19.7
	20:0	0.4±0.00	-	0.2	-	-	0.2	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	0.1	-
	22:0	0.2±0.00	0.4	0.2	0.2	0.2	0.2	0.1	0.1	0.2	-	0.2	-	0.1	0.2	0.1	0.1	-	-	0.3	-	0.1	0.1
	24:0	0.3±0.00	0.7	0.8	0.2	-	0.8	-	0.3	0.1	-	0.1	0.1	-	-	-	-	0.2	1.0	-	-	0.3	-
ω9 MONOS.	16:1	4.2±0.10	2.3	2.7	3.9	0.8	1.2	2.0	1.1	1.4	1.6	4.0	1.1	1.1	1.1	0.8	3.5	4.3	1.7	2.2	5.4	11.1	11.0
	18:1	16.5±0.10	15.1	19.3	23.3	15.6	19.0	28.5	30.9	41.8	55.1	13.6	18.2	22.5	14.1	16.8	16.1	9.8	12.5	17.9	15.9	18.7	22.6
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	3.2±0.00	3.2	3.4	2.7	6.2	7.8	8.2	6.4	8.0	5.8	15.3	25.9	36.3	8.0	9.2	11.2	15.2	23.1	28.9	5.3	6.8	15.8
	18:3	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	3.1	-	-	-
	20:2	0.1±0.00	-	-	-	-	0.3	0.1	1.0	1.5	1.6	1.2	1.8	0.6	-	0.3	-	0.8	1.1	1.2	0.1	0.3	0.3
	20:3	1.6±0.05	1.4	1.4	1.1	1.5	1.6	0.9	1.1	1.1	0.4	1.2	1.4	0.5	1.1	1.1	0.7	3.5	3.8	2.4	1.1	1.0	0.4
	20:4	13.3±0.05	11.1	10.1	6.5	13.2	11.8	7.5	8.3	5.7	2.3	6.9	6.3	1.8	10.8	8.9	5.1	7.4	4.8	2.5	8.7	6.4	2.5
	22:4	9.9±0.35	26.6	11.3	9.1	21.1	14.3	6.3	20.3	10.0	3.0	20.4	10.7	3.4	26.0	11.5	8.0	20.0	17.2	4.2	23.6	8.0	2.8
	22:5	1.8±0.03	-	1.3	-	-	0.3	0.2	-	0.8	-	0.1	0.9	0.1	-	1.3	0.1	0.2	1.6	0.1	0.1	1.1	0.1
ω3 POLYS.	18:3	0.3±0.00	0.4	0.6	0.4	0.7	0.6	0.4	1.1	1.2	0.5	0.4	0.5	0.1	4.0	9.1	19.	0.2	0.3	2.4	0.4	0.5	0.3
	18:4	1.6±0.05	0.3	1.7	0.8	0.5	1.1	0.3	0.2	0.8	0.3	0.3	0.9	0.3	0.2	2.1	0.2	0.2	1.1	1.2	0.3	1.2	0.5
	20:4	4.3±0.05	1.7	2.0	2.6	2.3	2.3	1.4	1.2	1.8	1.2	1.6	1.6	1.2	1.5	2.9	1.5	1.9	3.1	1.2	2.1	1.4	1.5
	20:5	0.7±0.00	1.4	1.2	1.0	0.4	0.5	0.2	0.2	0.2	-	0.2	0.3	-	2.0	0.5	0.3	0.1	0.3	0.1	2.3	4.1	4.1
	22:5	3.1±0.03	2.9	2.7	1.7	3.2	2.5	1.4	2.4	1.8	0.5	2.0	2.1	0.5	2.5	2.0	1.1	2.0	1.4	0.6	6.1	6.-	2.7
	22:6	1.2±0.10	3.1	1.7	2.3	3.2	1.4	1.6	2.4	0.9	0.7	1.9	0.6	0.6	2.3	0.6	1.4	1.7	0.5	0.8	1.6	0.-	0.8

(2.7% to 3.2% vs 3.2% in controls), but decreased 20:4ω6 amounts from 11.1% to 10.1% and 6.5% with 20, 40 and 60mg/l, respectively (13.3% in controls). Significantly increased 22:4ω6 was found with 20mg/l p-CO (26.6% vs 9.9% in controls), but this did not raise 22:5ω6 levels. On the other hand, increased 20:5ω3 and 22:6ω3 reflected decreased 18:4ω3, 20:4ω3 and 22:4ω3 proportions.

Decreased 18:0 and 16:1ω9 levels were found when cells were incubated with p-MO, but 16:0 and 18:1ω9 increased significantly with 60mg/l p-MO (24.3% and 28.5% vs 18.3% and 16.5% in controls, respectively). 18:2ω6 increased from 6.2% to 7.8% and 8.2% with 20, 40 and 60mg/l p-MO, respectively (3.2% in controls), and 20:4ω6 decreased from 13.2% to 11.8% and 7.5%, respectively (13.3% in controls), whereas the elongation product, 22:4ω6, was markedly increased with 20mg/l p-MO (21.1% vs 9.9% in controls). ω3 PDFA levels were parallel to controls or decreased, although 22:6ω3 was significantly increased with 20mg/l p-MO (3.2% vs 1.2% in controls).

The percentages of 18:1ω9 detected in cells dosed with 0, 20, 40 or 60mg/l p-OO increased from 16.5% to 30.9%, 41.8% and 55.1%, respectively, whereas 16:0, 16:1ω9 and 18:0 levels were decreased. Significant increments in 18:2ω6 (5.8% to 8.0% vs 3.2% in controls) and 20:2ω6 (1.0% to 1.6% vs 0.1% in controls) were detected, but 20:3ω6 and 20:4ω6 were decreased. However, 22:4ω6 increased about two fold with 20mg/l p-OO (20.3% vs 9.9%

in controls). With the exception of a two fold increase in 22:6ω3 with 20mg/l p-OO (2.4%), ω3 PDFA levels were reduced with p-OO dosage.

16:0, 16:1ω9, 18:0 and 18:1ω9 percentages were either parallel to controls or decreased in cells dosed with p-SSO, although 18:1ω9 was increased with 60mg/l p-SSO (22.5% vs 16.5% in controls). The provision of p-SSO resulted in concentration dependent 18:2ω6 incorporation from 3.2% in controls to 15.3%, 25.9% and 36.3% with concentrations of 20, 40 or 60mg/l, respectively. 20:2ω6 levels were raised (0.6% to 1.8% vs 0.1% in controls), and 22:4ω6 increased approximately two fold with 20mg/l p-SSO (20.4% vs 9.9% in controls), but other ω6 PDFA levels were parallel to controls or decreased. With the exception of a slight increase in 22:6ω3 with 20mg/l p-SSO (1.9% vs 1.2% in controls), ω3 PDFA levels were also decreased.

p-LO generally had little effect on cellular 16:0 and 18:1ω9 levels, but lowered the amount of 16:1ω9 and 18:0. 18:2ω6 increased from 3.2% in controls to 8.0%, 9.2% and 11.2% with 20, 40 and 60mg/l p-LO, respectively and 26.0% 22:4ω6 was detected with 20mg/l p-LO (9.9% in controls). Other ω6 PDFA levels, however, approximated to controls or were decreased. The percentage of 18:3ω3 increased from 4.0% to 9.1% and 19.5% when 20, 40 or 60mg/l p-LO was incubated with the cells, respectively

(0.3% in controls). 18:4ω3 was enhanced with 40mg/l p-LO (2.1% vs 1.6% in controls), 20:5ω3 with 20mg/l p-LO (2.0% vs 0.7% in controls) and 22:6ω3 with 20mg/l p-LO (2.3% vs 1.2% in controls), while other concentrations of p-LO had little effect on, or lowered ω3 PUFA levels.

16:0, 16:1ω9, 18:0 and 18:1ω9 levels were parallel to controls or decreased in cells incubated with p-EPO, but 18:2ω6 increased markedly (15.2%, 23.1% and 28.9% with 20, 40 or 60mg/l, respectively vs 3.2% in controls). 18:3ω6 was absent from controls, but up to 3.1% was found in dosed cells. Significant increments in 20:2ω6 (0.8% to 1.2% vs 0.1% in controls) and 20:3ω6 (2.4% to 3.8% vs 1.6% in controls) were found, but 20:4ω6 levels decreased from 13.3% to 7.4%, 4.8% and 2.5% with 0, 20, 40 and 60mg/l p-EPO, respectively. 22:4ω6 percentages were significantly raised both with 20 and 40mg/l p-EPO (20.0% and 17.2%, respectively vs 9.9% in controls), but this did not induce an increase in 22:5ω6. Incubation of cells with 20mg/l p-EPO caused a slight increase in 22:6ω3 (1.7% vs 1.2% in controls), but other ω3 PUFA levels were parallel to controls or decreased.

Cells supplemented with p-FO exhibited 16:0 and 18:0 percentages either approximating to controls (18.3% and 19.1%, respectively) or decreased, raised 16:1ω9 levels (5.4% with 20mg/l, 11.1% with 40mg/l and 11.0% with 60mg/l, respectively vs 4.2% in controls), and increased 18:1ω9 with 40 and 60mg/l p-FO (18.7% and 22.6% vs 16.5%

in controls). 18:2ω6 increased significantly from 5.3% with 20mg/l p-FO to a maximum of 15.8% with 60mg/l p-FO (3.2% in controls), and 22:4ω6 increased more than two fold with 20mg/l p-FO (23.6% vs 9.9% in controls), but no increment in 22:5ω6 was detected. 20:5ω3 percentages increased with p-FO dosage (2.3% with 20mg/l and 4.1% each with 40 and 60mg/l vs 0.7% in controls), and its elongation product, 22:5ω3, increased approximately two fold with 20 and 40mg/l (6.1% and 6.8%, respectively vs 3.1% in controls). Yet, 22:6ω3 was only slightly raised with 20mg/l p-FO (1.6% vs 1.2% in controls).

The FA spectra of the spent growth medium removed from each of the above cultures is shown in Table 3.7.3.2. The medium in which control cells had been grown contained 96.6% 14:0, 2.0% 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 0.4% to 2.7% 17:0, but only trace amounts of the major FA components present in the p-oils remained in the medium following the 48 hour incubation period.

3.7.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Rat Skin.

Table 3.7.4.1. shows the radioactivity recovered from cultures incubated with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3.

Total counts recovered from the spent incubation media ranged from 2.0 to 2.8x10^{6}cpm. Only 0.3 to 0.5x10^{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 77% and 84%, respectively. For [^{14}C]-18:2ω6 the equivalent of 4.8x10^{6}cpm were dosed to the incubation medium, and the total recovery of this radioisotope amounted to about 88%. However, the total amount of each of these radioisotopes incorporated into the cells approximated to 22% for [^{14}C]-18:1ω9, 23% for [^{14}C]-18:2ω6 and 10% for [^{14}C]-18:3ω3.

Table 3.7.4.2. shows the percentage of each incorporated radiolabelled C18 FA converted to ω9, ω6 or ω3 series products after incubation with rat skin fibroblasts.

15.5% of the total [^{14}C]-18:1ω9 counts derived upon GLC analysis (1151cpm) remained unchanged, while the proportions of 20:1ω9, 22:1ω9 and 24:1ω9 detected were 35.3%, 39.1% and 10.2%, respectively.

Table 3.7.3.2.

The fatty acid spectrum of the spent culture medium of cells derived from rat 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	[n-FO] (mg/l) 20	40	60
SATURATES.	12:0	-	0.1	0.2	0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14:0	96.6±0.38	98.3	97.7	98.1	98.5	98.1	96.3	97.9	96.5	97.8	97.4	97.1	96.6	97.5	98.2	97.9	97.8	97.7	96.1	98.3	97.9	97.9
	16:0	0.4±0.09	0.2	0.2	0.2	0.2	0.2	0.7	0.2	0.2	0.2	0.2	0.4	0.7	0.2	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	17:0	2.0±0.06	0.6	0.9	0.5	0.4	1.1	1.4	0.8	2.2	1.0	1.2	1.3	1.5	1.5	0.6	0.7	0.9	0.9	2.7	0.4	0.7	0.6
	18:0	0.2±0.08	0.2	0.2	0.2	0.1	-	0.4	0.2	-	-	0.2	0.1	-	-	0.1	0.2	0.2	0.2	-	0.2	0.1	0.2
	20:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24:0	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MONO.	16:1	0.1±0.03	-	-	-	-	-	0.2	-	-	-	-	0.1	0.1	-	-	-	-	-	-	0.1	0.2	0.3
	18:1	0.4±0.05	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.6	0.6	0.3	0.4	0.3	0.3	0.2	0.2	0.2	0.3	0.4	0.3	0.3	0.3
ω6 POLYS.	18:2	-	-	-	-	-	-	0.1	0.1	0.1	0.1	0.1	0.2	0.2	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	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-
	22:4	0.8±0.03	0.2	0.4	0.3	0.4	0.2	0.4	0.4	0.3	0.2	0.4	0.3	0.4	0.3	0.2	0.4	0.4	0.3	0.2	0.3	0.4	0.3
	22:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω3 POLYS.	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2	0.3	-	-	-	-	-	-
	18:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:4	-	-	-	-	0.1	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	-	0.1	0.1	-	-	-	-	-
	20:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.7.4.1.

The radioactivity (cpm) recovered from rat 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				
1946880 (±15780)	421260 (±3240)	2368140	959907 (±5226)	3.3
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
2524200 (±22620)	528840 (±2520)	3053040	1121016 (±3027)	4.2
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
2831960 (±11900)	344100 (±5040)	3176060	407862 (±3507)	3.6

Table 3.7.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from rat 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	15.5	18:2	6.7	18:3	7.0
20:1	35.3	18:3	16.1	18:4	17.1
22:1	39.1	20:2	16.7	20:3	16.8
24:1	10.2	20:3	18.3	20:4	12.2
		20:4	23.2	20:5	23.2
		22:4	10.9	22:5	10.6
		22:5	8.2	22:6	13.1
TOTAL CPM	1151	TOTAL CPM	2689	TOTAL CPM	1011

On the other hand, only 6.7% of the total [^{14}C]-18:2ω6-derived counts measured (2689cpm) remained unchanged. 16.7% and 16.1% of total counts recovered were found as 20:2ω6 and 18:3ω6, respectively, compared to 18.3% 20:3ω6, 23.2% 20:4ω6, 10.9% 22:4ω6 and 8.2% 22:5ω6.

The total counts derived following GLC analysis of cells incubated with [^{14}C]-18:3ω3 were 1011cpm, of which only 7.0% was detected for 18:3ω3. The percentage for 18:4ω3 (17.1%) was comparable with that of 18:3ω6 (16.1%), as were those for the immediate elongation products of 18:3ω3 and 18:2ω6 (16.8% 20:3ω3 and 16.7% 20:2ω6). 12.2% 20:4ω3 was detected, while the levels of the subsequent products of desaturation and elongation (23.2% 20:5ω3 and 10.6% 22:5ω3) were almost identical to those found for corresponding products in the ω6 series. However, a larger proportion of total [^{14}C]-18:3ω3-derived counts were shown as 22:6ω3 (13.1%) than for the corresponding Δ4D product in the ω6 series (8.2% for 22:5ω6).

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.

3.7.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides quantified in dosed cells and their respective growth media are presented in Table 3.7.5.1. 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 derived.

Medium derived from cultures incubated with 60mg/l p-LO or p-FO for 48 hours contained 1.0 and 0.9nmoles MDA/10^6 cells, respectively, but MDA was absent from all other spent media. On the other hand, MDA was measured both in control and all dosed cells. The MDA amounts quantitated in dosed rat skin fibroblasts ranged from 0.1 to 2.6 nmoles MDA/10^6 cells (with 20mg/l p-MO or 60mg/l p-FO incubation, respectively), compared to 0.2nmoles MDA/10^6 control cells. Little lipoperoxide formation occurred when cells were dosed with 20, 40 or 60mg/l p-CO, p-MO, p-OO or even p-SSO since only 0.1 to 0.4nmoles MDA/10^6 cells were measured. Cells supplemented with 20mg/l p-LO, p-EPO or p-FO induced similar lipoperoxide amounts (0.4 to 0.5nmoles MDA/10^6 cells), but p-EPO formed more lipoperoxides than p-LO or p-FO at quantities of 40mg/l. (1.3, 0.4 and 0.5nmoles MDA/10^6 cells, respectively). However, p-FO induced the greatest quantity of cellular lipoperoxides with a concentration of 60mg/l, followed

Table 3.7.5.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.2			
CO		0.2	0.2	0.4
MO		0.1	0.4	0.2
OO		0.2	0.2	0.4
SSO		0.2	0.2	0.2
LO		0.4	0.4	0.7
EPO		0.5	1.3	2.1
FO		0.4	0.5	2.6
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	C	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		-	-	-
LO		-	-	1.0
EPO		-	-	-
FO		-	-	0.9

by p-EPO and p-LO (2.6, 2.1 and 0.7nmoles MDA/10^6 cells, respectively).

3.7.6 Eicosanoid Profile of Cells Derived from Rat Skin.

The eicosanoids positively detected in control and dosed rat skin fibroblasts are presented in Table 3.7.6.1. as a percentage of the total area found. However, an indication of the total eicosanoid amounts quantitated is given for controls in "( )", expressed as pmoles/10^6 cells.

Total eicosanoid formation amounted to 165.7pmoles/10^6 control cells, of which 15.4% could be positively identified with the prostanoid standards available. Approximately half of this was TXB_2 (7.3%) and one third PGF_{α} (5.2%), compared to 1.3% PGI2, 1.0% PGE2 and 0.6% PGE_1. Varying proportions of these prostanoids were also found in dosed cells, whereas PGD_2 was not produced by control, or dosed, rat skin fibroblasts.

Total percentages for the prostanoids detected in dosed cells varied significantly with p-oil concentration as such approximated to controls (15.4%) with 40mg/l p-oil (9.5% to 15.1%), but were significantly increased both with 20mg/l (22.9% to 29.4%) and 60mg/l (24.1% to 33.7%) p-oil. These differences were reflected by appropriate changes in the amount of individual prostanoids. PGI_2 levels generally approximated to controls (1.3%) with supplementation of 40mg/l p-oil, but none was detected with 20 or 60mg/l p-oil. Cellular TXB_2 percentages were

Legend to Table 3.7.6.1.

Values are tabulated as relative percent of the total area detected, and as pmoles total eicosanoids/10^5 control cells.

Table 3.7.6.1.

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

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	PGF_{α} (1+2)	PGE_2	PGE_1	PGD_2
CONTROL (165.7)		15.4	1.3	7.3	5.2	1.0	0.6	-
CO	20	29.0	-	17.5	9.3	1.9	0.3	-
	40	13.9	0.9	6.2	6.5	0.3	-	-
	60	33.7	-	18.2	15.2	0.3	-	-
MO	20	22.9	-	13.0	8.4	1.5	-	-
	40	15.1	0.6	5.5	8.8	0.2	-	-
	60	33.4	-	20.6	12.5	0.3	-	-
OO	20	27.5	-	16.6	8.8	1.7	0.4	-
	40	13.0	0.8	6.8	5.1	0.3	-	-
	60	29.2	-	18.0	11.1	0.1	-	-
SSO	20	23.7	-	13.3	8.9	1.2	0.3	-
	40	11.2	0.4	7.4	3.1	0.3	-	-
	60	24.1	-	13.4	10.0	0.1	0.6	-
LO	20	24.3	-	14.9	7.8	1.4	0.2	-
	40	9.7	1.9	4.5	3.1	0.2	-	-
	60	24.5	-	13.5	10.4	0.1	0.5	-
EPO	20	27.6	-	16.9	9.1	1.4	0.2	-
	40	14.8	1.0	8.8	4.4	0.6	-	-
	60	27.2	-	16.3	10.3	0.1	0.5	-
FO	20	29.4	-	17.7	9.9	1.5	0.3	-
	40	9.5	0.9	5.3	3.1	0.2	-	-
	60	33.2	-	20.3	12.3	0.6	-	-

comparable to controls (7.3%) with 40mg/l p-oil dosage (4.5% to 8.8%), but these increased 2 to 3 fold with incubation of 20 or 60mg/l p-oil (13.0% to 20.6%). Control PGF_{α} proportions (5.2%) were also parallel to those found in cells supplemented with 40mg/l p-oil (3.1% to 8.8%), but levels increased slightly with 20mg/l p-oil (7.8% to 9.9%) and 2 to 3 fold with 60mg/l p-oil (10.0% to 15.2%). Incubation with 20mg/l p-oil enhanced PGE_2 production only slightly (1.2% to 1.9% vs 1.0% in controls), but decreased PGE_2 levels were detected with 40 and 60mg/l p-oil (0.1% to 0.6%). p-Oil supplementation, however, had little effect on PGE_1 production as trace amounts (≤0.6%) of this compound were found both in control and dosed cells.

3.7.7 Discussion.

Supplementation of cultured rat skin fibroblasts with p-oils affected the viability of such cells. The finding that the p-oils used exhibited varying abilities to modulate cell proliferation reflected the different FA compositions of these compounds (Table 2.3.3.2.). The greater potential to inhibit cell viability with high concentrations of p-OO, p-SSO, p-LO, p-EPO and p-FO than with p-CO and p-MO (Figs. 3.7.1.2-3.7.1.8.) implied that monoenoic and polyenoic FA's were more effective growth inhibitory agents than saturated FA's. The greater cytotoxic potential of p-EPO compared to p-SSO could relate to the presence of approximately 9% GLA in the former only as both p-oils contained similar proportions of other FA's. It seemed possible therefore, that induction of the greatest degree of cytotoxicity with p-EPO or p-LO supplementation related to the GLA and ALA concentrations in these p-oils, respectively, especially since both p-oils contained similar complements of saturated and monounsaturated FA's. However, in the light of the similar effects these p-oils induced, and the fact that the proportion of GLA in p-EPO was much lower than ALA in p-LO (9% vs 63%), it was apparent that GLA exhibited greater growth inhibitory potential than ALA. This probably relates to the different double bond positions in these C18 trienoic FA's. It should be borne 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 phenomenon may explain the enhanced cell growth induced only when certain concentrations of p-OO or p-FO were dosed, and 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 3.7.3.2.) indicated the capability of these cells to incorporate exogenous FA's, even in the presence of high 14:0 levels derived from 90% DMEM+10% FCS itself (Table 2.3.3.3.). Support for p-oil FA uptake from the culture medium was also shown in Table 3.7.3.1. Modifications of the membrane PCL FA composition have been reported to induce changes in membrane physical properties (Stubbs et al 1984 and Spector et al 1985). A mechanism whereby p-oils affected cell viability may therefore relate to differential exogenous FA uptake and alterations in membrane stability, dependent on p-oil FA composition and concentration dosed. Pronounced shifts in membrane fluidity would indeed support the limitation and inhibition of cell proliferation shown when large quantities of saturated or unsaturated FA-rich p-oils were fed (Figs. 3.7.1.2-3.7.1.8.).

p-Oil supplementation induced concentration changes in total cellular protein (Table 3.7.2.1.) which did not always parallel the changes found in cell viability (Figs. 3.7.1.2-3.7.1.8.). In fact, cell numbers could not justify the marked protein increase found with 40mg/l p-OO dosage, nor the increased cellular protein measured when p-oil dosage induced little effect on, or limited, cell viability. This, however, indicated p-oil FA involvement in the stimulation of protein synthesis, which may reflect enhanced protein turnover or enzyme expression to metabolise the incorporated p-oil FA's. Numerical differences in the protein concentrations measured when supplementation with identical amounts of different p-oils yielded similar cell numbers suggested that the ability to modulate protein synthesis related to differences in FA structure and probably also to FA synergism and antagonism. On the other hand, the protein amounts quantified in the spent medium of dosed cells were not statistically different from controls (section 3.7.2), thus it was unlikely that medium protein itself contributed significantly to the cellular protein changes demonstrated (Table 3.7.2.1.).

Cultured rat skin fibroblasts had little capability to desaturate or elongate 16:0 when incubated with p-oils, although decreased 18:0 and enhanced 18:1ω9 levels in dosed cells suggested the potential for Δ9D expression (Table 3.7.3.1.). The fact that all p-oils contained

18:1ω9 (Table 2.3.3.2.), however, could have contributed to the enhancement of cellular 18:1ω9 levels, although 16:1ω9 elongation could possibly be excluded in this regard since 16:1ω9 incorporated with p-FO dosage did not produce a concommitant increase in 18:1ω9. The lack of 18:1ω9 desaturation or elongation nevertheless relate to competitive inhibition by PUrA's (Brenner et al 1966, 1969, Brenner 1977 and Holman 1986a, 1986b).

Cultured rat skin fibroblasts exhibited the potential to desaturate and chain elongate PUFA's in the presence of p-oil supplementation (Table 3.7.3.1.). significant 18:3ω6 production from 18:2ω6 was apparent with p-oil dosage, although elevated 20:3ω6 levels with incubation of p-EPO, providing 18:3ω6, suggested that delta-6-desaturation of 18:2ω6 was slow, probably due to the rate limiting nature of this enzyme (eg. Stoffel 1961, Holloway 1963, Marcel et al 1968, Brenner 1971, 1974, 1977, 1982 and de Gomez Dumm et al 1976). Detection of small amounts of 20:2ω6 supported the capability for limited 18:2ω6 elongation, while decreased 20:3ω6/20:4ω6 and increments in 22:4ω6 levels, particularly with 20mg/l p-oil dosage, suggested Δ5-desaturation of 20:3ω6 and rapid subsequent elongation. However, the inability to form 22:5ω6 via Δ4D was consistent with 22:4ω6 accumulation. The finding that the immediate products of desaturation were lower than those following elongation supported documentation implicating the desaturases as

the rate limiting steps in the desaturase enzyme cascade (eg. Stoffel 1961, Holloway 1963, Marcel et al 1968 and Brenner 1971, 1977, 1982). A similar pattern of slow Δ6- and Δ5-desaturation was found with ω3 PUFA substrates. Despite 22:5ω3 formation by elongation of 20:5ω3 incorporated with p-FO dosage, only a small increase in 22:6ω3 production occurred via Δ4D with 20mg/l p-FO, suggesting suppressed expression of this enzyme, possibly because membrane unsaturation index was increased. Detection of low levels of long chain more unsaturated PDFA's when PUFA-rich p-oils were dosed could indeed relate to the known inhibitory effect of such moieties on desaturase enzymes, particlarly when product levels are sufficient for maintaining cell functions and membrane fluidity (eg. Garcia et al 1965, Brenner et al 1966, 1967, Brenner 1971 and Sprecher 1981). On the other hand, the greatest increase in relative 22:6ω3 percentages found when saturated or monoenoic FA-rich p-oils were dosed implied an increased need for highly unsaturated PDFA's to re-establish membrane fluidity altered with exogenous FA uptake, for example. However, increased detection of higher PDFA levels with p-CO, p-MO or p-OO dosage suggested enhanced desaturation and/or elongation of endogenous PUFA's as these p-oils contained relatively little, or no, PUFA's. The degree of FA unsaturation attained in the cells with p-oil dosage was therefore a factor involved in control

of desaturase cascade enzyme expression, particularly since modifications of the membrane PGL FA composition have been shown to induce changes in the activity of membrane bound enzymes (eg. Brenner et al 1965a, 1965b, 1966, 1967, 1969, Stubbs et al 1984 and Spector et al 1985). This could explain why the capability for desaturation and elongation were generally greatest with addition of low p-oil concentrations, despite increased cellular uptake of major p-oil FA components with the amount dosed (Table 3.7.3.1.).

To assess whether the pattern of p-oil desaturation described above was modulated by the presence of FA mixtures, p-oil desaturation was compared with that of individual radiolabelled C18 FA's. Only the pathway for elongation was shown with [^{14}C]-18:1ω9 dosage, whereas the pattern of radioactivity derived from cells enriched with either [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 was consistent with both desaturation and elongation of these moieties (Table 3.7.4.2.). Conversion of approximately 76% of incorporated [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 to PDFA's supported Δ6D expression, and the formation of 20:3ω3 and 20:2ω6 by elongation could have served as storage substrates for retroconversion or further desaturation. Conversion of about 47% of incorporated [^{14}C]-18:3ω3 and 42% [^{14}C]-18:2ω6 to post-Δ5D FA's was evidence for Δ5D activity, but Δ4D expression was more limited. Chapkin et al (1984) demonstrated a lack of conversion of 18:2ω6

to 18:3ω6 and 20:3ω6 to 20:4ω6 in isolated microsomes of rat epidermis, even when different amounts of 18:2ω6 or 20:3ω6 were fed as Δ6D or Δ5D substrates, respectively. They suggested that 20:4ω6 in rat skin was synthesised elsewhere, presumably the liver, and transported to the skin. Others reported that Δ6D and Δ5D activities were suppressed in the skin of EFA-deficient rats only, and that significant Δ6D and Δ5D expression occurred in the skin when the diet of normal rats was supplemented with 18:2ω6 (Lowe et al 1978). The variations in desaturase activity described above may relate to different experimental designs, but could also reflect the ability of this tissue to selectively regulate PDFA production.

The finding that desaturase cascade enzyme expression was more limited with p-oil compared to individual FA supplementation (Tables 3.7.3.1. and 3.7.4.2.) 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 <u>in vivo</u> than that shown with single FA's.

MDA quantitation showed that rat skin fibroblasts exhibited the capability to produce lipoperoxides (Table 3.7.5.1.). This could occur either enzymatically or by auto-oxidation of free monoenoic and/or polyenoic FA's. Measurement of relatively low MDA concentrations in

control and most dosed fibroblasts, however, indicated efficient intracellular mechanisms of protection against free radical formation. Indeed, it is recognised that aerobic organs are protected from oxygen toxicity by an array of defence systems, including preventative anti-oxidants such as vitamin E, and anti-oxidant enzymes such as peroxidase, superoxide dismutase and catalase which remove different species of activated oxygen that promote lipoperoxidation (Lands et al 1971, Smith et al 1972, Tappel 1972, 1980 and Pryor 1976). Alternatively, FA oxidation could have been suppressed by limited substrate availability. If FA's were present within the membranes as components of complex lipids they would not have been so readily accessible to oxidation, although the rate of PGL turnover could have influenced the release of FA's from membrane lipids into the cellular pool. These factors probably accounted for the greater cellular lipoperoxide amounts p-EPO induced compared to p-SSO, despite the similar FA compositions of these p-oils, although such could also relate to the presence of 18:3ω3 in p-EPO only (Table 2.3.3.2.) and the greater capability to form 20:3ω6 with p-EPO incubation (Table 3.7.3.1.). This could also explain the greater amounts of total lipoperoxides generated with p-EPO than p-LO supplementation (despite 63% 18:3ω3 in p-LO and only 9% 18:3ω6 in p-EPO), which probably related to the easier production of endoperoxides from 18:3ω6 and 20:3ω6 than

from 18:3ω3. The suppression/inhibition of microsomal PG biosynthesis or oxygenase activity by ω3 series PUFA's (Morisaki et al 1982b) could probably be excluded in this regard since dosage with 60mg/l p-FO induced the greatest cellular lipid peroxide amounts measured, which was probably largely attributable to the susceptibility of 20:5ω3 to oxidation.

Lipoperoxides have been implicated in the disruption of biological membranes (Mead et al 1976, Tappel et al 1980 and Frankel 1984), thus the small MDA amounts found in the spent medium of cultures dosed with 60mg/l p-FO or p-LO may have arisen from the release of cellular lipid peroxides through the membrane of intact cells or as a result of cell lysis. This could also have resulted in the escape of cellular FFA's and enzymes responsible for peroxidation into the medium, thus extracellular auto-oxidation should not be overlooked as a contributor to these medium lipoperoxide levels. On the other hand, the absence of MDA from all other spent media supported cellular uptake of dosed p-oil FA's.

Low cellular lipoperoxide levels generally correlated with high cell yields, whereas the significant increases in cellular and/or medium lipoperoxides levels found only with p-LO, p-EPO or p-FO dosage (Table 3.7.5.1.) correlated inversely with cell viability (Figs. 3.7.1.6-3.7.1.8.). It was apparent, however, that lipoperoxides were only partly responsible for the modulation of cell

viability since the greatest cytotoxicity induced with 60mg/l p-LO or p-EPO did not correlate with the highest cellular lipoperoxide amounts found. Skin *in vivo* is continuously exposed to oxygen in air, thus this tissue may be particularly resistant both to the generation and effects of lipoperoxides, and the changes induced in cell viability with p-oil dosage probably involved other mechanisms, such as alterations in membrane stability.

The finding that total eicosanoid formation by control rat skin fibroblasts comprised 83% of all lipoperoxides quantified (Tables 3.7.5.1. and 3.7.6.1.) was not surprising considering endogenous 20:4ω6 levels and the capability of these cells to form 1-, 2- and 3-series eicosanoid precursors via desaturation and elongation (Tables 3.7.3.1. and 3.7.4.2.). 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 rat skin fibroblasts. Under the experimental conditions employed, the enzymic pathways for TXB_2 and PGF_{α} synthesis were greatly favoured over those involved in PGI_2, PGE_2 and PGE_1 production, and it was possible that PGH_2-PGD_2 isomerase was inhibited rather than absent as PGD_2 formation has been reported in rat skin *in vivo* (Ujihara et al 1988).

Lowe et al (1978) showed that 18:2ω6 supplementation of the diet of normal mice resulted in at least a two fold increase in cutaneous PGE and PGF levels compared to controls, but had little effect in EFA-deficiency. p-Oil supplementation also influenced the ability of rat skin fibroblasts to produce the prostanoids studied (Table 3.7.6.1.), but this was not related to the degree of p-oil unsaturation, the ability of the p-oils to provide direct eicosanoid precursors (Table 2.3.3.2.), or the capability of the cells to form eicosanoid precursors via the desaturase cascade (Table 3.7.3.1.). Indeed, the detection of increased levels of the 1-series eicosanoid precursor, 20:3ω6, only when cells were dosed with p-EPO induced no marked effect upon eicosanoid production compared to other p-oils 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 therefore 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, TXB2, PGF_α, PGE_2 and PGE_1 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 rat skin fibroblasts (Table 3.7.3.1.). However, the finding that 40mg/l p-oil had little effect on or suppressed prostanoid formation, whereas 20 and 60mg/l p-oil significantly stimulated eicosanoid production primarily by enhancing TXB_2 and PGF_{α} synthesis, 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.

Numerous studies have implicated PG involvement in the modulation of cell division in skin when such compounds were provided exogenously (Ziboh et al 1972, Bem et al 1974, Lupulescu 1976, Lowe et al 1977 and McCullough et al 1978). PGE compounds in particular, have been associated with increased epidermal proliferation in vivo (Lowe et al 1977), and similar results have been obtained in culture (Bem et al 1974). Others, however, found that the rate of epidermal proliferation increased 5 fold over controls in EFA-deficient rats with impaired PGE_2 synthesis, and that topical PGE2 application daily for one week restored the slower, normal rate of growth (Ziboh et al 1972 and McCullough et al 1978). The present study, in contrast, could not establish any correlation between the eicosanoid profiles (Table

3.7.6.1.) and cell viability changes (Figs. 3.7.1.2-3.7.1.8.) induced with p-oil supplementation, and this implied that the endogenously synthesised 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.

3.8 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM RAT ADIPOSE TISSUE.

3.8.1 Effects of pseudo-Oils on Cell Viability.

No morphological changes were found between control cells before, during and after each experiment, and dosed cells were morphologically similar to controls, except for cytoplasmic droplets observed with p-oil incubation at high concentrations in some instances.

The data depicted in Fig. 3.8.1.1. showed that final medium albumin concentrations ranging from 0 to 250mg/l induced no significant changes in cell viability. This excluded the possibility that albumin as FA carrier had any effect on cell viability, hence the effects induced with the p-oils were a result of the exogenous FA's.

11.4×10^4 control cells/ml culture medium were found at the end of the 24 hour post-trypsinisation recovery period. This equated to 70% of the final control cell number at the end of the 48 hour p-oil incubation period and represented the cytostatic number. Growth inhibition was therefore induced only with p-oil concentrations which reduced cell viability to significantly below 70%.

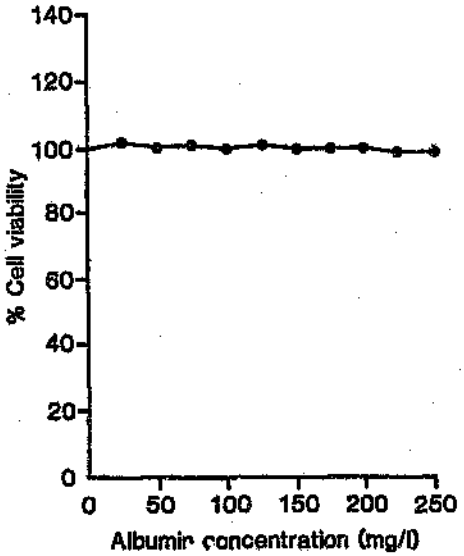
The cell viability changes induced following incubation with 0 to 100mg/l p-oil for 48 hours are shown in Figs. 3.8.1.2-3.8.1.8. All p-oils enhanced cell proliferation

Legend to Figs. 3.8.1.1-3.8.1.9.

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. 3.8.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 3.8.1.2-3.8.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 3.8.1.9. 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. 3.8.1.1.

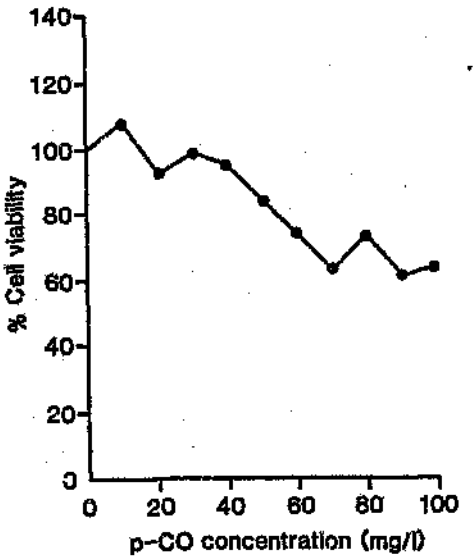
The percentage viability of cells derived from rat adipose tissue incubated with albumin.



Albumin Concentration (mg/l).	Mean	±s.e.m.	n
0	100.0	2.3	12
25	102.8	2.0	12
50	100.7	2.6	12
75	101.6	2.1	12
100	99.3	2.0	12
125	101.1	2.1	12
150	99.8	3.1	12
175	99.9	3.6	12
200	100.3	2.8	12
225	98.8	3.7	12
250	98.1	2.1	12

E' 1. 3.8.1.2.

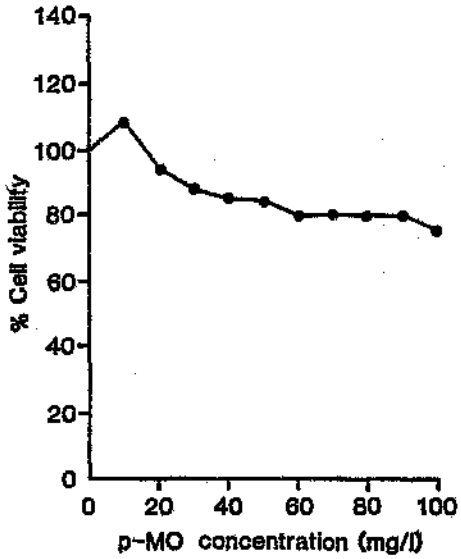
The percentage viability of cells derived from rat adipose tissue incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.6	6
10	108.8	5.6	6
20	93.4	4.5	6
30	99.5	5.5	6
40	96.0	3.9	6
50	84.8	3.6	6
60	74.4	2.6	6
70	63.0	1.8	6
80	73.3	5.1	6
90	61.5	3.4	6
100	64.4	3.3	6

Fig. 3.8.1.3.

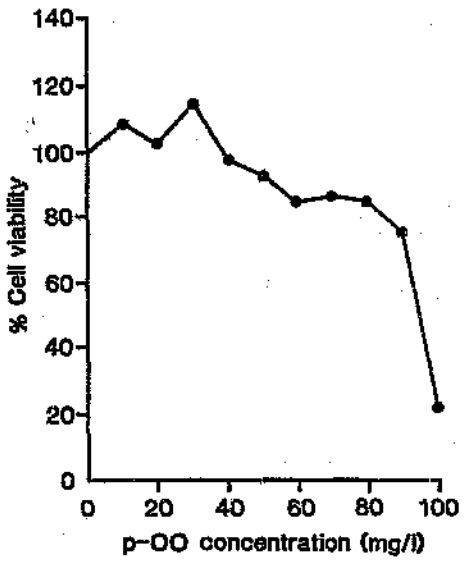
The percentage viability of cells derived from rat adipose tissue incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.1	6
10	108.6	4.2	6
20	95.1	5.7	6
30	88.3	4.9	6
40	86.0	5.9	6
50	84.8	2.1	6
60	80.5	5.0	6
70	80.9	3.8	6
80	80.3	5.3	6
90	82.3	2.0	6
100	76.9	2.7	6

Fig. 3.8.1.4.

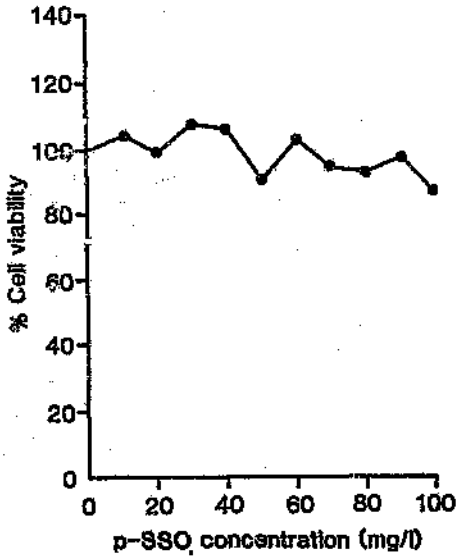
The percentage viability of cells derived from rat adipose tissue incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.7	6
10	110.1	5.2	6
20	102.2	6.4	6
30	114.1	6.1	6
40	97.8	1.9	6
50	93.4	3.2	6
60	85.1	4.3	6
70	87.0	2.7	6
80	85.1	7.0	6
90	75.9	3.4	6
100	21.9	2.1	6

Fig. 3.8.1.5.

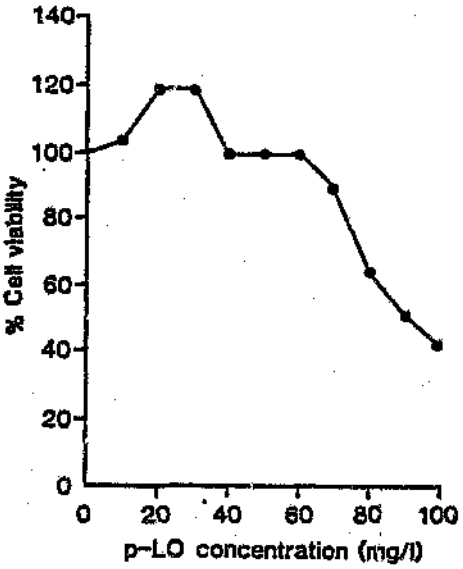
The percentage viability of cells derived from rat adipose tissue incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	1.6	6
10	104.7	4.7	6
20	98.9	4.3	6
30	107.0	3.6	6
40	105.4	2.2	6
50	90.4	2.2	6
60	102.8	3.7	6
70	93.7	2.5	6
80	92.2	4.7	6
90	97.0	3.6	6
100	87.0	3.6	6

Fig. 3.8.1.6.

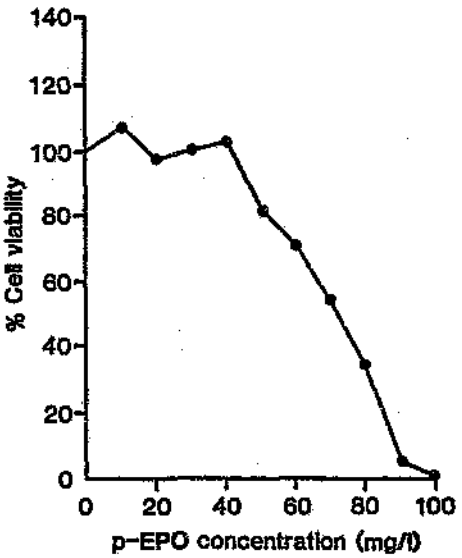
The percentage viability of cells derived from rat adipose tissue incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.0	6
10	104.0	5.2	6
20	120.5	3.9	6
30	120.3	4.0	6
40	99.9	4.0	6
50	100.9	2.4	6
60	99.8	5.1	6
70	89.1	5.8	6
80	64.4	5.4	6
90	51.8	4.4	6
100	42.0	3.6	6

Fig. 3.8.1.7.

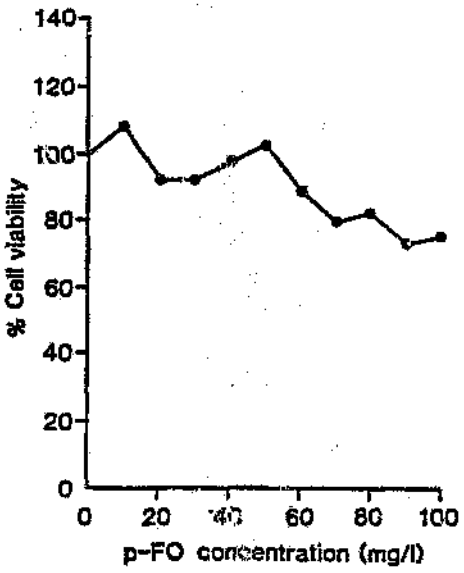
The percentage viability of cells derived from rat adipose tissue incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.6	6
10	108.1	2.6	6
20	97.8	4.3	6
30	100.5	4.8	6
40	103.3	4.6	6
50	80.9	5.4	6
60	71.2	5.4	6
70	53.9	1.9	6
80	35.2	1.9	6
90	4.7	1.2	6
100	0.3	0.3	6

Fig. 3.8.1.8.

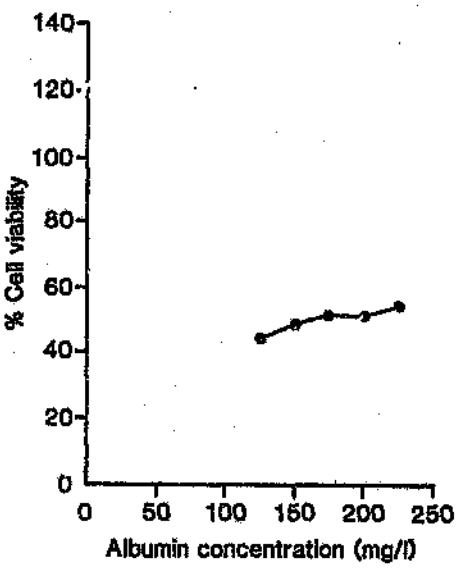
The percentage viability of cells derived from rat adipose tissue incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	107.8	7.1	6
20	91.7	4.3	6
30	92.2	3.7	6
40	97.5	4.5	6
50	102.4	4.4	6
60	88.5	4.8	6
70	79.1	3.5	6
80	81.9	5.6	6
90	72.6	4.4	6
100	75.2	5.0	6

Fig. 3.8.1.9.

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



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
125	45.0	4.5	6
150	49.3	2.4	6
175	52.0	4.1	6
200	51.0	3.1	6
225	54.1	3.1	6

slightly with low concentrations and limited growth to a greater or lesser extent with higher amounts dosed, although p-EPO, p-LO and p-OO were cytotoxic at high concentrations.

p-CO enhanced cell viability to 108.8% with 10mg/l, had little effect with 20 to 40mg/l, and limited growth to 84.8% with 50mg/l (Fig. 3.8.1.2.). Cell viability ranged from 61.5% to 74.4% with 60 to 100mg/l p-CO, relative to the cytostatic number of 70%, thus cytotoxicity was not marked and no ID_{50} was found. Cell proliferation was also stimulated with 10mg/l p-MO to 108.6%, and growth limitation increased progressively with higher amounts dosed to a minimum of 76.9% with 100mg/l p-MO (Fig. 3.8.1.3.), thus no ID_{50} could be calculated either.

Cell proliferation increased to 110.1% and 114.1% with 10 and 30mg/l p-OO, respectively, but approximated to controls with 20 and 40mg/l p-OO (Fig. 3.8.1.4.). Higher concentrations progressively limited cell growth to 85.1% with 60mg/l and 75.9% with 90mg/l p-OO, whereas 50% of cells were viable with 95mg/l p-OO (ID_{50}), but only 21.9% with 100mg/l p-OO.

Incubation with 10 to 90mg/l p-SSO had little effect on cell proliferation, although such was increased to 107.0% and 105.4% with 30 and 40mg/l p-SSO, respectively (Fig. 3.8.1.5.). This was the least effective p-oil to impair cell growth as 87.0* viability occurred even with

100mg/l p-SSO.

p-LO stimulated cell proliferation by 20% with 20 and 30mg/l and had little effect with 40 to 60mg/l, but limited growth to 89.1% with 70mg/l (Fig. 3.8.1.6.). p-LO induced cytotoxicity with concentrations of 80mg/l, or more, and half the cells were viable with 92mg/l p-LO (ID_{50}), compared to 42% with 100mg/l p-LO.

Cell viability was 108.1% with 10mg/l p-EPO, but in line with controls following 20 to 40mg/l p-EPO dosage (Fig. 3.8.1.7.). Cell proliferation was limited to 80.9% with 50mg/l p-EPO and parallel to the cytostatic number with 60mg/l p-EPO, but higher amounts induced concentration dependent cytotoxicity such that half the cells were killed with 73mg/l p-EPO (ID_{50}) and practically all with 90 and 100mg/l p-EPO. This was the most effective p-oil to impair the proliferation of these cells.

Cell viability remained within 10% of controls with dosage of up to 50mg/l p-FO, and was merely limited with higher concentrations to a minimum of 72.6% with 90mg/l p-FO (Fig. 3.8.1.8.). p-FO thus induced similar effects to p-MO (Fig. 3.8.1.3.) and p-SSO (Fig. 3.8.1.5.).

Cells were incubated with a fixed amount of p-EPO to correspond with its ID_{50} (70mg/l) and supplemented with varying amounts of albumin to eliminate the possibility that any effects induced with p-oil dosage were related to the amount of albumin used as FA carrier. The albumin

concentration was varied at 2 points above and below the ID_{50} of p-EPO, and the results obtained following the 48 hour incubation period are depicted in Fig. 3.8.1.9. This indicated that the concentration of p-EPO dosed reduced cell viability to about 50% of controls, and that such was not significantly influenced by different albumin concentrations. 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 rat adipose tissue were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient amounts to permitt all quantitative and qualitative analyses to be carried out. Upon harvesting, cell yields were compared and found to be statistically similar to those in Figs. 3.8.1.1-3.8.1.8. All further biochemical assays were thus performed on these samples.

3.8.2 Effects of pseudo-Oils on Total Protein.

The total protein concentrations determined for cells supplemented with 0, 20, 40 or 60mg/l p-oil are shown in Table 3.8.2.1. as µg total protein/10^6 cells seeded.

134.7µg total protein per 10^6 control cells seeded was quantitated, whereas concentrations in dosed cells were increased and ranged from 142.8 to 205.5µg protein/10^6 cells seeded (found with 60mg/l p-EPO and 20mg/l p-LO, respectively). Cellular protein concentrations decreased over the range dosed in the presence of p-CO, p-SSO or p-LO, but increased with the amount of p-MO added. On the other hand, more cellular protein was measured with 40mg/l than with 20 or 60mg/l p-EPO and p-FO, but the converse was found with p-OO.

Table 3.8.2.1.

The protein content of cells derived from rat adipose tissue, expressed as µg total protein/10^5 cells seeded.

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	134.7			
CO		162.4	161.1	157.5
MO		158.3	165.7	178.8
OO		167.4	149.4	155.3
SSO		164.5	165.6	143.9
LO		205.5	175.5	156.2
EPO		164.6	172.8	142.8
FO		172.4	196.9	167.4

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

The FA spectra for cells incubated with 20, 40 or 60mg/l p-oil are shown in Table 3.8.3.1. in relation to undosed cells. The greatest proportion of any single FA in the controls was 18:1ω9 (23.4%), but similar percentages were found for 16:0 (22.3%) and 18:0 (21.8%). Other FA's contributing >5.0% to the total spectrum in control cells included 18:2ω6 (9.0%) and 22:4ω6 (5.8%). Varying amounts of these moieties were present in dosed cells.

In general, dosed cells contained less 18:0 and 18:1ω9, variable proportions of 16:0 and 16:1ω9, and more 18:2ω6 and 18:3ω6, compared to controls. ω6 PDFA levels were generally parallel to controls or increased in dosed cells, although 22:5ω6 was decreased. 18:3ω3 and 20:4ω3 levels were decreased, but 20:5ω3, 22:5ω3 and 22:6ω3 amounts were generally increased in dosed cells.

16:0 levels increased from 22.3% and 21.4% to 28.1% and 31.6% when cells were supplemented with 0, 20, 40 and 60mg/l p-CO, respectively. On the other hand, 16:1ω9 was significantly raised only with 40mg/l p-CO (4.0% vs 1.5% in controls), while 18:0 and 18:1ω9 levels were parallel to controls or decreased. Dosed cells contained less 18:2ω6 than controls (9.0%), but 20:4ω6 was increased at least 2 fold (3.6% to 5.3% vs 1.6% in controls). 22:4ω6

Legend to Table 3.8.3.1.

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 3.8.3.1.

The fatty acid spectrum of cells derived from rat 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	22.3±0.55	21.4	28.1	31.6	19.3	21.9	23.2	17.7	16.0	19.1	17.5	17.6	19.8	26.1	14.3	23.6	11.0	24.4	23.5	18.5	20.6	21.2
	18:0	21.8±0.00	15.7	15.7	18.7	15.6	18.1	15.1	10.8	13.6	6.7	14.6	17.2	10.8	15.4	13.9	11.6	21.6	20.9	11.7	13.8	11.2	8.1
	20:0	0.8±0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.1	-	1.0	-	-	-	-
	22:0	0.2±0.00	0.2	0.6	0.1	0.1	1.0	0.1	-	-	-	-	-	-	0.1	1.8	-	-	0.1	0.1	-	0.1	0.1
	24:0	0.3±0.03	-	0.5	0.1	0.1	0.6	0.4	-	-	-	-	0.2	0.3	-	0.2	-	-	0.3	0.4	-	-	-
ω9 MONOS.	16:1	1.5±0.05	0.9	4.0	1.8	0.5	0.4	2.2	0.7	0.4	2.3	0.4	0.7	2.7	4.1	3.1	3.8	0.4	0.7	0.9	4.6	11.7	15.9
	18:1	23.4±0.75	20.7	.1	25.3	20.0	19.1	23.4	31.6	42.2	48.8	19.4	19.1	19.7	15.8	17.1	18.3	22.3	21.3	18.8	19.6	17.4	18.1
	24:1	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	9.0±0.85	8.4	3.4	5.6	13.0	10.3	14.3	11.1	11.8	11.6	24.7	26.7	31.6	11.1	11.9	11.7	31.1	20.7	33.7	11.5	7.8	9.2
	18:3	-	0.9	0.6	0.3	0.9	0.2	0.9	0.6	-	0.4	1.8	-	1.1	0.7	1.4	0.9	4.6	0.9	1.6	1.4	0.2	0.3
	20:2	1.4±0.05	-	3.9	-	-	3.7	0.3	0.9	3.7	1.7	0.9	2.0	1.3	-	1.7	-	0.5	0.5	-	-	0.9	0.5
	20:3	1.0±0.05	1.9	2.3	1.3	1.6	2.7	1.4	1.4	0.6	0.7	1.2	1.4	1.0	1.0	1.9	0.9	4.1	1.0	0.4	1.2	1.4	0.9
	20:4	1.6±0.05	5.3	4.0	3.6	5.0	4.6	5.6	3.5	1.5	1.7	2.7	2.3	1.9	3.7	3.2	2.7	1.6	1.5	0.6	3.5	2.7	2.3
	22:4	5.8±0.90	10.5	7.7	4.1	±0.8	7.2	5.5	11.4	4.0	2.1	8.0	4.3	5.0	8.4	6.6	7.4	1.0	4.4	6.5	8.5	5.4	4.9
	22:5	2.0±0.05	0.2	1.9	0.2	0.2	1.5	0.2	0.1	0.6	0.1	0.3	1.1	0.1	0.1	1.7	0.1	0.1	0.7	-	0.2	1.0	0.1
ω3 POLYS.	18:3	0.2±0.00	1.0	0.4	0.6	1.1	0.3	0.9	1.3	0.6	1.2	1.8	0.5	1.1	5.1	10.7	15.7	-	0.2	0.4	1.4	0.5	0.7
	18:4	2.9±0.20	0.4	-	0.6	0.7	0.2	0.2	0.3	1.0	0.1	0.4	1.5	0.2	0.3	1.5	0.2	0.1	0.9	0.2	0.3	0.2	0.3
	20:4	3.0±0.15	2.2	2.2	0.5	1.9	1.9	0.7	1.0	1.5	0.1	1.1	1.5	0.4	1.8	2.6	0.6	-	0.5	0.3	0.7	1.7	1.2
	20:5	0.4±0.00	1.3	1.4	0.7	1.5	1.2	0.6	1.4	0.2	0.4	0.3	0.4	0.4	1.6	0.6	0.3	0.2	-	-	4.3	5.8	9.5
	22:5	1.1±0.00	3.9	2.3	2.2	3.6	2.4	2.3	2.9	1.0	1.4	2.5	1.8	1.4	2.3	4.3	1.1	0.8	0.3	0.5	7.7	9.1	4.9
	22:6	1.3±0.10	4.6	3.0	2.8	4.1	2.9	2.7	3.2	1.3	1.6	2.5	1.9	1.4	2.6	1.5	1.0	0.6	0.2	0.4	3.0	2.3	2.1

percentages were raised with 20 and 40mg/l p-CO (10.5% and 7.7%, respectively vs 5.8% in controls), yet 22:5ω6 levels were parallel to controls (2.0%) or decreased. p-CO induced a marked reduction in 18:4ω3 (0% to 0.6% vs 2.9% in controls), but 20:5ω3, 22:5ω3 and 22:6ω3 levels increased 2 fold or more in relation to controls (0.4%, 1.1% and 1.3%, respectively).

p-MO dosage had little effect on 16:0 and 18:1ω9 levels, but reduced 18:0 and 16:1ω9 amounts, although the latter was slightly raised with 60mg/l p-MO (2.2% vs 1.5% in controls). Up to 14.3% 18:2ω6 and 2.7% 20:3ω6 were found (9.0% and 1.0% in controls, respectively), 20:4ω6 levels ranged from 4.6% to 5.6% (1.6% in controls), and 22:4ω6 was increased almost 2 fold with 20mg/l (10.8% vs 5.8% in controls), yet 22:5ω6 amounts were decreased. p-MO induced a marked reduction in 18:4ω3 and 20:4ω3, but up to 1.5% 20:5ω3, 3.6% 22:5ω3 and 4.1% 22:6ω3 were found (0.4%, 1.1% and 1.3% in controls, respectively).

A concentration dependent increase in cellular 18:1ω9 occurred with dosage of 0, 20, 40 or 60mg/l p-OO (23.4% to 31.6%, 42.2% and 48.8%, respectively), but 16:0 and 18:0 levels were decreased and increased 16:1ω9 was found only with 60mg/l p-OO (2.3% vs 1.5% in controls). p-OO had little effect on 18:2ω6 levels, but 20:2ω6 was increased with 40mg/l (3.7% vs 1.4% in controls). 20:4ω6 and 22:4ω6 increased about 2 fold with 20mg/l p-OO (3.5%

and 11.4% vs 1.6% and 5.8% in controls, respectively), but no increase in 22:5ω6 was found. 18:4ω3 and 20:4ω3 amounts were decreased with all concentrations, but 20:5ω3, 22:5ω3 and 22:6ω3 were significantly increased only with 20mg/l p-OO (1.4%, 2.9% and 3.2% vs 0.4%, 1.1% and 1.3% in controls, respectively).

p-SSO supplementation decreased cellular 16:0, 18:0 and 18:1ω9 amounts, and 16:1ω9 was significantly increased only with 60mg/l p-SSO (2.7% vs 1.5% in controls). 18:2ω6 percentages increased from 9.0% to 24.7%, 26.7% and 31.6% with 0, 20, 40 and 60mg/l p-SSO, respectively, and up to 1.8% 18:3ω6 was found (none in controls). 20:3ω6 levels were parallel to controls (1.0%), but 20:4ω6 amounts were increased (1.9% to 2.7% vs 1.6% in controls). 22:4ω6 levels were increased only with 20mg/l p-SSO (8.0% vs 5.8% in controls), and decreased 22:5ω6 was found. p-SSO decreased cellular 18:4ω3 and 20:4ω3 amounts, but 22:5ω3 and 22:6ω3 were increased about two fold with 20mg/l p-SSO (2.5% each vs 1.1% and 1.3% in controls, respectively).

16:0 levels ranged from 14.3% to 26.1% with p-LO dosage (22.3% in controls), 22:0 increased significantly with 40mg/l p-LO (1.8% vs 0.2% in controls), 16:1ω9 increased at least two fold (1.5% in controls), whereas 18:1ω9 and 18:0 percentages were decreased. p-LO had little effect on 18:2ω6 levels, 20:4ω6 and 22:4ω6 were increased (2.7% to 3.7% and 6.6% to 8.4% vs 1.6% and 5.8% in controls,

respectively), although 22:5ω3 was decreased. 18:3ω3 percentages increased from 0.2% to 5.1%, 10.7% and 15.7% with 0, 20, 40 or 60mg/l p-LO dosage, respectively, yet decreased 18:4ω3 and 4ω3 were found. Increments were, however, detected for 20:5ω3 with 20mg/l p-LO (1.6% vs 0.4% in controls), 22:5ω3 with 20 and 40mg/l p-LO (2.3% and 4.3%, respectively vs 1.1% in controls) and 22:6ω3 with 20mg/l p-LO (2.6% vs 1.3% in controls).

16:0, 16:1ω9, 18:0 and 18:1ω9 percentages approximated to controls or were decreased with p-EPO incubation, but 18:2ω6 proportions were greatly increased (31.1%, 20.7% and 33.7% with 20, 40 and 60mg/l, respectively vs 9.0% in controls). Cells contained 0%, 4.6%, 0.9% and 1.6% 18:3ω6 with 0, 20, 40 and 60mg/l p-EPO, respectively, and 4.1% 20:3ω6 was detected with 20mg/l p-EPO (1.0% in controls). 20:4ω6 levels, however, were not increased, and the percentage of 22:4ω6 was raised only with 60mg/l p-EPO (6.5% vs 5.8% in controls). On the other hand, ω3 PDFA levels were parallel to controls or decreased.

p-FO incubation increased cellular 16:1ω9 levels to 4.6% with 20mg/l, 11.7% with 40mg/l and 15.9% with 60mg/l (1.5% in controls), reduced 18:0 percentages from 21.8% in controls to 13.8%, 11.2% and 8.1%, respectively, but had no marked effect on 16:0, 18:1ω9 and 18:2ω6. Raised amounts of 20:4ω6 were found with 20, 40 and 60mg/l p-FO (3.5%, 2.7% and 2.3%, respectively vs 1.6% in controls),

and 22:4ω6 with 20mg/l p-FO (8.5% vs 5.8% in controls), but 22:5ω6 percentages were decreased. Dosage with 20, 40 or 60mg/l p-FO greatly increased the levels of 20:5ω3 (4.3%, 5.8% and 9.5%, respectively vs 0.4% in controls), and 22:5ω3 (7.7%, 9.1% and 4.9%, respectively vs 1.1% in controls), but the increment in 22:6ω3 was smaller (3.0%, 2.3% and 2.1%, respectively vs 1.3% in controls).

3.8.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Rat Adipose Tissue.

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 3.8.4.1.

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 77% and 91%, 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 radioisotope after 48 hours incubation amounted to 85%. The counts recovered from the spent incubation media ranged from 1.8 to $2.8x10^6$cpm, whereas only 0.4 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 22% for [^{14}C]-18:1ω9, 29% for [^{14}C]-18:2ω6 and 14% 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 cells derived from rat adipose tissue are shown in Table 3.8.4.2.

29.9% of the total [^{14}C]-18:1ω9 counts derived upon GLC

Table 3.8.4.1.

The radioactivity (cpm) recovered from rat 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				
1838100 (±10480)	528000 (±7440)	2366100	938754 (±771)	3.3
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
2235400 (±14720)	508380 (±4020)	2743780	1368009 (±5787)	4.1
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
2841400 (±11940)	419400 (±3360)	3260800	613707 (±2496)	3.9

Table 3.8.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from rat 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	29.9	18:2	2.8	18:3	6.7
20:1	28.1	18:3	18.3	18:4	26.9
22:1	30.4	20:2	17.6	20:3	11.4
24:1	11.7	20:3	16.3	20:4	11.4
		20:4	20.3	20:5	21.1
		22:4	12.8	22:5	8.2
		22:5	11.9	22:6	14.2
TOTAL CPM	1237	TOTAL CPM	2745	TOTAL CPM	1485

analysis (1237cpm) were unchanged. Similar percentages were detected for 20:1ω9 (28.1%) and 22:1ω9 (30.4%), but only 11.7% was found as 24:1ω9.

Only 2.8% of the total [^{14}C]-18:2ω6-derived counts detected following GLC (2745cpm) remained unaltered, whereas 18.3% was found as 18:3ω6 and 17.6% as 20:2ω6. 20:3ω6 and 20:4ω6 accounted for 16.3% and 20.3% of the total, respectively, compared to 12.8% 22:4ω6 and 11.9% 22:5ω6.

1485cpm were derived upon GLC analysis of cells dosed with [^{14}C]-18:3ω3, of which only 6.7% remained as 18:3ω3 and 11.4% found for its immediate elongation product, 20:3ω3. The percentage for 18:4ω3 (26.9%) was almost 10% higher than that of the corresponding ω6 series product, 18:3ω6 (18.3%), while 11.4% 20:4ω3 was found. 21.1% of the total counts were shown to be 20:5ω3 compared to 8.2% 22:5ω3, and the level of the subsequent product of delta-4-desaturation was similar to that found for the corresponding product in the ω6 series (14.2% 22:6ω3 vs 11.9% 22:5ω6).

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

3.8.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 3.8.5.1. shows the MDA concentrations measured in cells dosed with 0, 20, 40 or 60mg/l p-oil, as well as their respective growth media. 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.

0.5nmoles MDA/10^6 control cells were quantified, while concentrations in dosed cells ranged from 0.6 to 4.2 nmoles MDA/10^6 cells. MDA generation varied with p-oil, but generally increased with the concentration of p-oil supplemented. Cells incubated with 20, 40 or 60mg/l p-OO induced the lowest MDA amounts overall (0.6, 0.6 and 0.9 nmoles MDA/10^6 cells, respectively), concentrations were similar with p-CO, p-MO or p-FO supplementation (0.8 to 1.5nmoles MDA/10^6 cells), while PUFA-rich p-oils induced the largest MDA amounts. Cellular levels increased from 0.6 to 0.8 and 4.2nmoles MDA/10^6 cells in the presence of 20, 40 or 60mg/l p-LO compared to 1.1, 1.1, 2.1nmoles MDA/10^6 cells with p-SSO and 1.6, 1.6, 2.2nmoles MDA/10^6 cells with p-EPO, respectively. Medium derived from cultures incubated with 60mg/l p-EPO contained 2.1nmoles MDA/10^6 cells, but none was found in other spent media.

Table 3.8.5.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.5			
CO		0.9	1.3	1.3
MO		1.0	1.2	1.5
OO		0.6	0.6	0.9
SSO		1.1	1.1	2.1
LO		0.6	0.8	4.2
EPO		1.6	1.6	2.2
FO		0.8	1.2	1.5
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	-
MO		-	-	-
OO		-	-	-
SSO		-	-	-
LO		-	-	-
EPO		-	-	2.1
FO		-	-	-

3.8.6 The Eicosanoid Profile of Cells Derived from Rat Adipose Tissue.

Table 3.8.6.1. shows the eicosanoids detected positively in control and dosed cells. All data is presented as a percentage of the total area found, but an indication of the total eicosanoid amounts quantified is given for controls in "( )", expressed as pmoles total eicosanoids per 10^6 cells.

Total eicosanoid production by control cells amounted to 215.9pmoles/10^6 cells, of which 35.9% correlated with the retention times of the prostanoid standards used. This was composed of 16.6% PGF_{α}, 8.2% TXB2, 7.7% PGE2 and 3.4% PGI_2, and no PGD2 was shown in control cells. Varying proportions of these prostanoids were found in dosed cells, but neither control nor dosed cells formed PGE_1.

The percentage of total prostanoids detected in dosed cells varied significantly with p-oil concentration, as such was decreased with supplementation of 60mg/l p-oil (20.9% to 26.7%) and particularly 40mg/l p-oil (11.8% to 19.7%), but was more variable with 20mg/l p-oil (22.8% to 41.4%). Such changes related to alterations in the production of individual prostanoids in these cells. PGI_2 levels were increased with dosage of 40mg/l p-oil (3.8% to 5.8% vs 3.4% in controls), generally decreased with 60mg/l p-oil (1.9% to 3.4%), and more variable with

Legend to Table 3.8.6.1.

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

Table 3.8.6.1.

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

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	PGF_{α} (1+2)	PGE_2	PGE_1	PGD_2
CONTROL (215.9)		35.9	3.4	8.2	16.6	7.7	-	-
CO	20	22.8	4.8	11.0	7.0	-	-	-
	40	11.8	5.3	3.3	2.2	0.4	-	0.6
	60	20.9	2.5	10.3	6.1	2.0	-	-
MO	20	27.8	3.9	13.1	8.2	2.6	-	-
	40	17.2	5.3	5.4	4.0	1.9	-	0.6
	60	22.7	2.9	11.2	7.0	1.6	-	-
OO	20	36.3	2.5	15.3	18.5	-	-	-
	40	17.8	3.9	6.1	4.6	2.3	-	0.9
	60	26.7	2.0	14.6	7.7	2.4	-	-
SSO	20	41.4	3.1	23.0	9.3	6.0	-	-
	40	19.6	3.8	6.3	4.5	3.1	-	1.9
	60	24.3	2.1	12.5	7.8	1.9	-	-
LO	20	29.2	2.6	18.1	7.5	1.0	-	-
	40	15.5	5.8	4.4	3.6	1.5	-	0.2
	60	23.5	1.9	12.8	7.0	1.8	-	-
EPO	20	36.4	4.7	17.8	13.0	0.9	-	-
	40	19.7	5.5	7.1	4.5	1.8	-	0.8
	60	21.2	3.4	9.1	5.7	3.0	-	-
FO	20	30.1	4.1	13.5	8.5	4.0	-	-
	40	14.5	5.5	3.1	2.0	3.3	-	0.6
	60	22.2	2.3	12.2	6.8	0.9	-	-

20mg/l p-oil (2.5% to 4.8%). TXB_2 amounts were increased in relation to controls (8.2%) with 20mg/l (11.0% to 23.0%) and 60mg/l (9.1% and 14.6%) p-oil, but decreased with 40mg/l p-oil (3.1% to 7.1%). p-Oil supplementation suppressed the production of PGF_{α}, particularly with a concentration of 40mg/l (2.0% to 4.6%), although amounts were comparable to controls (16.6%) in the presence of 20mg/l p-OO (18.5%). Dosed cells also formed less PGE_2 than controls (0% to 6.0% vs 7.7%), and small amounts of PGD_2 were detected only with incubation of 40mg/l p-oil (0.6% to 1.9% vs 0% in controls).

3.8.7 Discussion.

p-Oil supplementation affected the viability of cultured rat adipose cells (Figs. 3.8.1.2-3.8.1.8.). The effects induced, however, were not consistent with the degree of p-oil unsaturation, hence it could not be established whether PUFA-rich p-oils exhibited greater potential to modulate cell proliferation than p-oils abundant in monounsaturated or saturated FA's. The p-oil-specific effects induced could rather be explained in terms of p-oil FA composition (Table 2.3.3.2.) and concentration dosed, although the possibility also existed that effects were enhanced or deminished by p-oil FA's operating either in concert with each other, or antagonistically. The data supported FA involvement in the enhancement of cell proliferation with incubation of low p-oil concentrations. However, the finding that cell proliferation was stimulated over a wider concentration range with incubation of p-OO, p-SSO or p-LO than with other p-oils implied greater involvement of 18:1ω9, 18:2ω6 and 18:3ω3, respectively in this process than other FA's. This may relate to the chain length of these moieties and the fact that 18:1ω9, 18:2ω6 and 18:3ω3 are Δ6D substrates, whereas the different number and position of double bonds in these C18 FA's may explain why 18:3ω3 was a more effective growth promoting agent than 18:2ω6 and 18:1ω9. This indicated that FA structure was an important determinant of cell viability. p-SSO

and p-EPO exhibited similar FA compositions with a rich supply of 18:2ω6 (about 70%), thus the cytotoxicity induced with p-EPO, but not p-SSO, dosage probably related to the presence of 9% 18:3ω6 in p-EPO only. This implied, therefore, that 18:3ω6 was a more potent growth inhibitory agent than 18:2ω6. The cell death p-LO and p-OO induced at high concentrations also indicated that 18:3ω3 and 18:1ω9 exhibited greater cytotoxic potential than 18:2ω6, but it was apparent that these C18 FA's were less effective than 18:3ω6. On the other hand, it seemed that 18:3ω3 was more effective than 18:3ω6 in stimulating cell proliferation. Such effects could have related to the presence of a double bond at the ω12-position in 18:3ω6.

No reference has been found in the literature in which the effects of FA's on rat adipose cell growth has been investigated, although the influence of exogenous FA's has been studied with rat mammary cells in culture (Wicha et al 1979). Mammary tissue is largely modified adipose tissue and is thus another cell type specialised for handling large amounts of FA's. Like adipose tissue, mammary tissue is actively involved in FA biosynthesis and is affected by dietary lipids (Lehninger 1982). In the presence of de-lipidised FCS, insulin, progesterone, estrogen and prolactin, Wicha et al (1979) showed that normal mammary epithelial cell proliferation in culture was inhibited with all concentrations of palmitic and

stearic acids tested (0.1-10mg/l), whereas unsaturated FFA's induced both stimulatory and inhibitory effects. Linoleic and alpha-linolenic acids were most stimulatory and resulted in a doubling of the growth rate with concentrations of 1.0 and 0.1mg/l, respectively, whereas both oleic and arachidonic acids were vastly less stimulatory. Linoleic acid could not induce cytotoxicity with any concentration dosed (0.1-10mg/l), but this was induced with greater thar 0.5mg/l arachidonic acid and 5mg/l oleic and α-linolenic acids (Wicha et al 1979). Comparison with our data clearly indicated that the effects individual FA's induced were more dramatic than with p-oil dosage, and this supported the mediation of antagonistic effects between FA's, which probably occurs in vivo when FA mixtures are consumed as dietary oils. On the other hand, it was possible tha: the individual FA effects Wicha et al (1979) reported on cell growth were mediated by the EFA-deficient culture conditions used, or modifications of membrane-bound hormone receptors as a result of hormonal supplementation. Wicha et al (1979) solubilised the FA's used in ethanol such that the final ethanol concentration in the culture medium was 1.0%. Our studies, however, have shown this concentration to be toxic both to normal and transformed cells (Davidson et al 1987a, 1987b, 1988a, 1988b, Giangregorio et al 1988b and Girao 1988), thus their data should be interpreted with caution. This could also explain discrepancies with our data (Figs. 3.8.1.2-

3.8.1.8.), whereas similar trends between the two studies may reflect similarities in these lipid storing tissues. Both studies nevertheless established that FA's played a role in cell growth, and it was possible that the response of the cells to the FA's reflected their physiological requirements in vivo.

We presented evidence which supported the ability of rat adipose cells to incorporate the exogenous FA's dosed (Table 3.8.3.1.). It was mechanistically possible, therefore, that the p-oil effects on cell growth were the result of changes in cell membrane fluidity and/or modifications of membrane-bound enzymes, as evidence suggests that dietary supplementation with FA's induces changes in membrane physical properties and membrane-associated cell functions (King et al 1971, Ginsberg et al 1981, Simon et al 1982, Spector et al 1985 and Stubbs et al 1984). On the other hand, the finding that growth inhibition occurred only with p-OO, p-LO or p-EPO dosage at high concentrations could reflect the ability of these cells to tolerate relatively large changes in FA concentration as adipose tissue is primarily involved in TAG storage and release (Lehninger 1982).

p-Oil dosage caused changes in the content of total cellular protein (Table 3.8.2.1.), but this did not always parallel the cell viability changes induced (Figs. 3.8.1.2-3.8.1.8.). The greatest increase in total protein correlated with the greatest stimulation of cell

viability with 20mg/l p-LO dosage. However, as protein increased approximately 50% and cell viability increased about 20%, compared to controls, increased protein did not appear to relate to increased cell numbers alone. In fact, increased cellular protein was found even when p-oil dosage induced growth limitation. It was apparent, therefore, that while total protein quantitation could reflect cell growth changes in terms of actual cell numbers, p-oil FA's were involved in the stimulation of protein synthesis. The finding that the extent of protein stimulation varied with dosage of identical concentrations of different p-oils, even when such yielded similar cell numbers, suggested that FA's of different chain lengths and degrees of unsaturation vary in their capability to modulate protein synthesis. Whether this related to specific FA's in any p-oil, or to synergistic effects induced between p-oil FA's, was unclear, although a combination of such effects seemed most likely. The enhancement of protein biosynthesis with p-oil supplementation may, nevertheless, reflect the extremely active metabolism of adipose tissue <u>in vivo</u> (Lehninger 1982). The involvement of this tissue in processes such as FA synthesis, TAG storage and release, as well as its ability to rapidly respond to metabolic changes/stimuli (Allman et al 1965, Fain et al 1966 and Lehninger 1982) suggested that the increased production of cellular proteins related to the enhanced expression

of lipid metabolising enzymes, such as lipases and those involved in FA synthesis and oxidation.

Comparison of control cell FA spectra with those derived from p-oil supplemented cells indicated the ability of cultured rat adipose cells to desaturate and elongate PUFA's (Table 3.8.3.1.). This correlated with the active involvement of adipose tissue in FA biosynthesis in vivo (Allman et al 1965, Fain et al 1966 and Lehninger 1982). Formation of small 18:3ω6 amounts and no increment in 18:4ω3 in dosed cells, even when significant EFA levels were present as substrate, supported the rate limiting nature of Δ6D (Stoffel 1961, Holloway 1963, Marcel et al 1968 and Brenner 1971, 1977, 1982). Slightly increased 20:2ω6 levels indicated the capability for limited 18:2ω6 elongation, whereas increments in 20:3ω6 and 20:4ω6 implied rapid 18:3ω6 elongation and subsequent Δ5-desaturation. On the other hand, the decreased 18:4ω3/20:4ω3 and increased 20:5ω3 levels found even with incubation of p-oils deficient in ω3 PUFA's implied elongation and Δ5-desaturation of endogenous 18:4ω3. 22:5ω3 and 22:6ω3 formation with p-FO dosage supported elongation of incorporated 20:5ω3 and subsequent Δ4-desaturation, while production of these moieties also with incubation of p-oils deficient in ω3 PDFA's implied elongation and desaturation of endogenous ω3 PDFA's. However, despite 22:4ω6 formation via elongation, this moiety was not further desaturated, hence explaining the

accumulation thereof. Such implied greater Δ4D activity with 22:5ω3 than 22:4ω6, and this supported the greater activity of desaturase cascade enzymes with ω3 than ω6 PUFA's (Brenner 1966, Mead et al 1976 and Kanau et al 1977) and the potential of ω3 PDFA's to suppress/inhibit ω6 PDFA desaturation (eg. Garcia et al 1965 and Sprecher 1981).

The raised 16:1ω9 levels found in p-oil dosed cells (Table 3.8.3.1.) implied 16:0 desaturation, but it was apparent that 16:1ω9 elongation was inhibited/suppressed since no increment in 18:1ω9 was found, despite 16:1ω9 uptake with p-FO incubation. 18:0 desaturation to 18:1ω9 via Δ9D was not demonstrated with p-oil supplementation (Table 3.8.3.1.), although Gellhorn et al (1964) found that rat adipose tissue was able to desaturate 18:0. Lack of Δ9D expression in our study, however, could have related to PUFA inhibition of the enzyme, which has been well documented in the rat (Ullman et al 1971, Mahfouz et al 1984, Pugh et al 1984 and Garg et al 1988b). This could also explain the lack of 18:1ω9 desaturation and elongation found, even when large amounts of 18:1ω9 were incorporated with p-OO dosage (Table 3.8.3.1.).

Dietary-induced alterations in microsomal FA composition and competitive interactions between cellular FA's of the same and different families modulate desaturase cascade enzyme expression (Garcia et al 1965, Brenner et al 1966, Nervi et al 1968, Ullman et al 1971, Kurata et

al 1980, de Schriver et al 1982, Garda et al 1984, 1985, Nassar et al 1986 and Garg et al 1988a, 1988b, 1988c). Such factors therefore contributed to the pattern of desaturation and elongation reported in Table 3.8.3.1. with p-oil dosage. In vitro studies using microsomes isolated from rat liver, known to exhibit desaturase activity, indicated that saturated FA's inhibited PUFA desaturation (Brenner et al 1966). This, however, did, not appear to be the case in cultured adipose cells, as detection of increased PDFA levels in cells dosed with saturated FA-rich p-oils (p-MO and p-CO in particular) implied that a relative EFA/PDFA deficiency enhanced desaturase cascade enzyme expression, probably in an attempt to restore membrane fluidity. PDFA formation with dosage of p-oils abundant in PUFA's was consistent with the availability of desaturase cascade enzyme substrates, although such moieties could suppress enzyme expression by negative feedback reactions once optimal PUFA levels were established to maintain normal membrane fluidity and cellular functions. This could explain the slightly greater cellular 22:6ω3 amounts found with supplementation of p-CO or p-MO than with other p-oils. The amount of FA synthesised in adipocytes has, however, been shown to decline with age in the rat (Henderson et al 1979 and Gandemer et al 1985), suggesting that PDFA availability and lipid deposition in adipose tissue of older rats is more dependent on exogenous lipid than on

de novo biosynthesis (Etherton et al 1980 and Jamdar et al 1986). The ability of these cells to desaturate and elongate FA's nevertheless reflected a mechanism to control their FA composition, and this could partly account for the lack of growth inhibition found with most p-oils and concentrations dosed (Figs. 3.8.1.2-3.8.1.8.).

The capability of these cells to desaturate and elongate individual C18 FA's was assessed to establish whether the pattern of p-oil desaturation found (Table 3.8.3.1.) was modulated in any way by the presence of FA mixtures. The pattern of radioactivity derived from cells enriched with [^{14}C]-18:1ω9 was consistent only with elongation of this moiety, but the finding that these cells converted 80% of incorporated [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 to PDFA's indicated Δ6D expression (Table 3.8.4.2.). Δ6D activity was also shown in rat mammary tissue microsomes by Cunnane et al (1981), although Brenner (1971) found that microsomes isolated from epididymal fat of young rats exhibited no potential to convert 18:2ω6 to 18:3ω6. This discrepancy may relate to the fact that Δ6D is extremely sensitive and easily inhibited by slight changes in the metabolic and physical environments (Cook 1979 and Brenner 1982); and could have been damaged upon microsomal isolation or inhibited by the experimental incubation conditions used. Enzyme activity expressed in whole cells is nevertheless more realistic and

relevant than microsomal assays since the former more closely parallels the situation in the intact organism. [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 elongation to 20:2ω6 and 20:3ω3, respectively (Table 3.8.4.2.) supported the capability for extramicrosomal retroconversion (Stearns et al 1967) and/or Δ8-desaturation. The presence or absence of Δ8D has not been reported in adipocytes, but such has been found in rat testes (Albert et al 1977). The capability for Δ8D expression in the present study could therefore not be ruled out as a second pathway for 20:3ω6 and 20:4ω3 synthesis. The formation of about 45% post-Δ5D FA's from incorporated [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 supported Δ5D expression, whereas the detection of 22:5ω3 and 22:6ω3 was consistent with Δ4D activity (Table 3.8.4.2.).

It was evident from comparison of the data in Tables 3.8.3.1. and 3.8.4.2. that desaturase cascade enzyme expression was more limited with p-oil than with [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 incubation. This implied suppressed enzyme expression as a result of competitive interations between p-oil FA's, particularly in the light of the competitive nature of FA's for such enzymes (Brenner et al 1966, 1967, Mohrhauer et al 1963a, Ullman et al 1971, de Schriver et al 1982, Holman 1986a, 1986b, Nassar et al 1986 and Cook et al 1987). Desaturase cascade enzyme expression reported with p-oil supplementation may nevertheless be a better indication

of such capability in rat adipose tissue in vivo than that reflected with individual FA's as cells are exposed to mixtures rather than single FA's in the "real world".

The presence of MDA in cultured rat adipose cells (Table 3.8.5.1.) supported the capability for enzymatic and/or spontaneous FFA oxidation when the protective mechanisms against free radical formation were saturated. Control cells contained unsaturated FA's which could have served as potential oxidation substrates (Table 3.8.3.1.), and the greater MDA amounts found in dosed cells reflected oxidation induced by unsaturated p-oil FFA's. Adipocytes are largely concerned with TAG storage (Lehninger 1982), and it is well known that the FA composition and level of dietary fat influence the FA composition of rat adipose tissue TAG's (Stein et al 1962, Knittle et al 1965, Awad 1981, Becker et al 1986 and Lhuillery et al 1988). Thus, the cellular lipoperoxides formed may well reflect oxidation of highly unsaturated FFA's in the TAG's.

The numerical variations in MDA concentrations between dosed cells (Table 3.8.5.1.) reflected the different FA compositions of the p-oils (Table 2.3.3.2.). This was a function of the amount of unsaturated FA's present in any p-oil and also their susceptibility oxidation, which related to the number and position of double bonds in a FA. It was also possible that supplementation with different p-oils altered the rate of PGL turnover in the

cells, which would have influenced FA availability for oxidation by affecting FA uptake and release. Indeed, FA's would not be so readily accessible to oxidation if they were components of the plasma membranes. This could explain the lowest cellular lipoperoxide amounts formed with p-OO dosage, despite significant OA incorporation (Table 3.8.3.1.) and a recognised mechanism for oleate auto-oxidation (Frankel 1984). The fact that p-CO and p-MO contained predominantly saturated FA's and induced larger cellular lipoperoxide amounts than p-OO, however, suggested the availability of endogenous polyenoic FA's for oxidation. Lipid peroxide formation with p-FO dosage reflected the more even balance of saturated, monoenoic and polyenoic FA's present in this p-oil, whereas the preference of polyenoic FA substrates for oxidation was reflected with p-SSO, p-LO or p-EPO dosage, particularly with a concentration of 60mg/l. Formation of more MDA with addition of 20 or 40mg/l p-EPO than p-SSO probably related to the availability of GLA present in p-EPO only, whereas the largest cellular lipoperoxide amounts formed with 60mg/l p-LO reflected the abundance of ALA in this p-oil.

The cytostasis induced with dosage of 60mg/l p-EPO and the known ability of lipoperoxides to increase membrane permeability (Chio et al 1969, Tappel 1975, Mead 1976 and Frankel 1984) suggested that the spent medium lipid peroxides originated intracellularly via passage through

the plasma membrane and/or as a result of extracellular oxidation of released cellular FFA's. On the other hand, the absence of MDA in all other spent growth media (Table 3.8.5.1.) supported p-oil FA incorporation and correlated with the lack of cytotoxicity 20, 40 or 60mg/l p-oil induced (Figs. 3.8.1.2-3.8.1.8.). As a result of the latter finding, it was unclear whether lipoperoxides were directly involved in the modulation of cytotoxicity, although this was a possible mechanism with dosage of higher p-oil concentrations. The inverse correlation generally found between cell viability and MDA concentrations with p-CO, p-MO, p-OO, p-EPO or p-FO dosage was suggestive of lipoperoxide involvement in the modulation of growth limitation. However, as 60mg/l p-LO induced the greatest cellular lipoperoxide amounts, but no growth limitation, it was proposed that the control of cell viability also related to other mechanisms, such as membrane fluidity changes.

The finding that total molar eicosanoid formation accounted for 43% of the lipid peroxides quantitated in control cells (Tables 3.8.5.1. and 3.8.6.1.) reflected the eicosanoid requirement of these cells and rate of biosynthesis under the experimental conditions employed. It was possible, however, that endogenous eicosanoid production was suppressed/limited as a result of the small 20:3ω6, 20:4ω6 and 20:5ω3 proportions found in control cells (Table 3.8.3.1.) as 1-, 2- and 3-series

eicosanoid precursors, respectively. Negrel et al (1981a 1981b) found that the rate of PGE_2 and 6-keto-$PGF_{1\alpha}$ biosynthesis in mouse epidermal adipocytes was inversely related to cell density, and decreased considerably after confluence. Hammarstrom (1977) also described this phenomenon, which could well explain the total molar eicosanoid amount found and the relative proportions to which the prostanoids studied contributed to the total molarity (Table 3.8.6.1.).

Prostanoid detection supported cyclooxygenase expression in these cells, but it was apparent that PGH_1-PGE_1 and PGH_2-PGD_2 isomerases were inhibited/suppressed since no significant PGE_1 and PGD_2 formation was shown in control or dosed cells (Table 3.8.6.1.), despite receptors for such compounds reported in rat adipocytes (Moore 1985). Alternatively, it was possible that these PG's were degraded at a faster rate than other eicosanoids by cytoplasmic enzymes such as 15-hydroxy PG dehydrogenase. On the other hand, the finding that the rate of PGF_α production was twice as great as that of TXB_2 and PGE_2, but considerably greater than PGI_2, synthesis in control cells may have been the result of increased enzyme expression for PGF_1 and/or PGF_2 formation, or relative decreases in the biosynthesis of other prostanoids. Lipinski et al (1978) similarly found that the <u>in vitro</u> production of $PGF_{2\alpha}$ was approximately two fold greater than PGE_2 in rat adipocytes. This supported the findings

of Lambert et al (1976), although earlier studies found very low $PGF_{2\alpha}$ levels in such cells (Dalton et al 1974). Others reported that isolated fat cells do not produce detectable amounts of TXB_2 (Fredhold et al 1976), unlike the present study (Table 3.8.6.1.). Possible reasons for such discrepancies were diet fed, incubation conditions and method of assay.

The eicosanoid profiles obtained from dosed cells (Table 3.8.6.1.) were consistent with the ability of the p-oils to modulate prostanoid synthesis. This, however, did not correlate in any way with degree of p-oil unsaturation, the ability of the p-oils to provide direct eicosanoid precursors (Table 2.3.3.2.), or the capability of the cells to form eicosanoid precursors via the desaturase cascade (Table 3.8.3.1.). Lipinski et al (1978) showed that changing the amount of dietary polyunsaturated fat altered PG production in rat adipocytes stimulated with norepinephrine <u>in vitro</u>. As the soyabean oil to beef tallow (P/S) ratio increased in the diet, so increased $PGF_{2\alpha}$ and PGE_2 formation. However, basal PGE_2 production was not altered in unstimulated adipocytes, even with increment in the P/S ratio, and $PGF_{2\alpha}$ increased only slightly. This implied hormonal involvement in the modulation of prostanoid biosynthesis, and the absence of hormonal supplementation in the present study could explain the findings we described. The modulation of prostanoid production could only be related to the p-oil

concentration dosed (Table 3.8.6.1.). This could have influenced intracellular calcium ion availability, which has been implicated as an essential factor regulating phosholipase activity (Moore 1985), and thus the release of FA's from membrane lipids for eicosanoid production. This phenomenon could explain why prostanoid synthesis was decreased overall with p-oil dosage. It was also possible that different p-oil concentrations influenced the expression of enzymes involved in the formation of the prostanoids studied from PGH to a greater or lesser extent and accounted for the alterations found. However, the fact that no correlation could be found between the eicosanoid profiles (Table 3.8.6.1.) and cell viability changes (Figs. 3.8.1.2-3.8.1.8.) induced with p-oil dosage was <u>in vitro</u> evidence against the concept that the endogenously synthesised prostanoids studied played a direct role in the modulation of cell proliferation. It was more likely such related to alterations in membrane physical properties and the generation of lipid peroxides.

3.9 General Discussion.

This chapter presented comprehensive data with regard to the effect of p-oil supplementation on the viability of cultured normal rat cell types, the capability of such cells to incorporate, desaturate and elongate FA's, the formation of lipoperoxides and eicosanoids and possible roles these compounds played in the modulation of cell viability. Furthermore, the data obtained from the parameters investigated permitted valid comparisons to be made between different rat cell types under standard experimental conditions.

p-Oil supplementation influenced rat cell viability, but the effects induced and magnitude thereof varied with rat cell type. The finding that cell viability was increased when particular p-oils and concentrations were dosed to nucleated rat cell types correlated with the work of Launay et al (1968, 1969, 1981), who showed that LA enrichment of rat diets increased cell numbers and the DNA content in liver and adipose tissue. The present study demonstrated that both saturated and unsaturated FA-rich p-oils exhibited the capability to promote cell viability, but such was usually induced with low and/or intermediate concentrations in the range dosed. This nevertheless implied FA involvement in the modulation of proliferation, dependent on rat cell type, p-oil FA composition and concentration dosed. Such factors also

determined the capability of the p-oils to reduce cell viability. All p-oils were cytotoxic to blood cells, but the fact that erythrocytes were more susceptible to the inhibitory effects of unsaturated FA-rich p-oils than lymphocytes (sections 3.1.1 and 3.2.1) may reflect the more limited metabolic capability of erythrocytes due to a lack of organelles. p-Oils were nevertheless far more effective in killing suspension cultures of blood cells than adherent cells, which could have related to the ability of the later to divide in culture without prior stimulation. Adherent cells exhibited different rates of proliferation (Table 2.3.1.1.), thus the growth limiting range established with p-oil incubation was broader with faster than with slower growing cells. This range should always be defined with dividing cells to ensure valid expression of the data as it clearly distinguishes between p-oil concentrations which induce mere growth limitation or cytostasis from those promoting cell proliferation or cytotoxicity. In this regard, rat endothelium, skeletal muscle, lung and adipose cell viabilities were generally limited with p-oil dosage and cytotoxicity induced only with high concentrations of certain p-oils (sections 3.3.1, 3.4.1, 3.6.1 and 3.8.1, respectively), whereas brain and skin cells were more susceptible to growth inhibition (sections 3.5.1 and 3.7.1, respectively). Hence, it was clear that the threshold for cytotoxicity varied with rat cell type, although no clear correlation could be established

between the rate of cell proliferation (Table 2.3.1.1.) and extent of growth inhibition induced. The different cell viability changes shown with p-oil dosage between these rat cell types may well relate to the different physiology and functions of such tissues in vivo. Extrapolation of cell viability results from one rat cell type to another should thus be avoided to ensure valid interpretion of data.

Evidence was presented in this chapter which supported the ability of rat cells to incorporate exogenous FA's to a greater or lesser extent. This modified the overall FA composition of the cells, suggesting that a mechanism whereby cell viability was reduced, particularly with supplementation of high p-oil concentrations, related to differential uptake of exogenous FA's and alterations in membrane fluidity.

Considerable variations were demonstrated with regard to the total protein concentrations of the different rat cell types studied (sections 3.1.2, 3.2.2, 3.3.2, 3.4.2, 3.5.2, 3.6.2, 3.7.2 and 3.8.2). Amounts were lowest in control lymphocytes and erythrocytes, intermediate in adipose, lung and endothelium, and greatest in skin, muscle and brain (6.5, 13.0, 134.7, 176.5, 181.1, 374.7, 401.7 and 455.5$\mu g/10^6$ cells seeded, respectively). The fact that control adherent cells contained considerably more total protein tha control erythrocytes or lymphocytes may once again have related to the ability

of the former to replicate under the culture conditions employed 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 the morphological and physiological differences of each cell type in vivo, as well as variations in the rate of total protein turnover with cell type (Lehninger 1982). The protein content of p-oil supplemented cells reflected the cell viability changes induced, although it was apparent that exogenous FA's had the capability to modulate protein synthesis in nucleated cells; the magnitude thereof varied with rat cell type, p-oil FA composition and concentration dosed. The enhancement or suppression of absolute cellular protein levels was suggested to relate to alterations in membrane protein synthesis and/or enzyme expression involved particularly in FA metabolism.

This chapter provided in vitro evidence to support the presence or absence of desaturase cascade enzyme capability in a range of normal rat cell types exposed to exogenous FA's. The lack of desaturase cascade enzyme activity was confirmed in erythrocytes (Table 3.1.3.1.) (Lehninger 1982), and these cells could thus be regarded as non-enzymatic controls within the species. Similarly, lymphocytes exhibited no significant Δ6D, Δ5D, Δ4D or elongase activity, but the potential to express Δ8D was suggested (Table 3.2.3.1.). Rat brain cells had limited

PUFA elongase, but no significant desaturase, capability (Table 3.5.3.1.), whereas skeletal muscle cells showed some Δ6D and Δ5D capability with PUFA substrates and Δ4D activity was implicated with ω3 PUFA's (Table 3.4.3.1.). On the other hand, Δ6D, Δ5D, Δ4D and elongase activities were found in rat endothelial cells (Table 3.3.3.1.). Rat lung and skin fibroblasts showed Δ6D, Δ5D and elongase activities both with ω3 and ω6 PUFA substrates, whereas Δ4D activity was found only with ω3 PUFA's and this was slightly greater in skin cells (Tables 3.6.3.1. and 3.7.3.1.). Δ6D and Δ5D were particularly active with both ω3 and ω6 PUFA substrates in rat adipose cells, although Δ4D activity was expressed only with ω3 PUFA's (Table 3.8.3.1.). These cells, however, exhibited the greatest capability for desaturation and elongation in relation to the rat cell types studied. Δ6D, Δ5D, Δ4D and elongase expression were also indicated when rat lung, skin and adipose cells were incubated with [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 (Tables 3.6.4.2., 3.7.4.2. and 3.8.4.2.). Such supported the slightly greater capability to form PDFA's with rat adipose, followed by skin and lung cells, as shown with p-oil dosage (Tables 3.6.3.1., 3.7.3.1. and 3.8.3.1), which was consistent with the greater incorporation of [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 into adipose than skin or lung cells (Tables 3.6.4.1., 3.7.4.1. and 3.8.4.1.).

The above findings clearly demonstrated that desaturase

enzyme capability varies between different rat tissues. A study in young rats has indeed shown that the percentage desaturation of LA to GLA via Δ6D was 20% adrenals, 14% in liver, 6% in testes and 2% each in heart, kidney and brain (Brenner 1971). No desaturation was detected in lung or epididymal fat (Brenner 1971), but this may have related to the fact that desaturation was assessed in isolated microsomes, which may not necessarily reflect the situation in a genetically entire cell. in vivo studies have indeed shown PDFA formation from LA in rat adipose tissue, heart, brain and liver (Mohrhauer et al 1963a, 1963b, 1963c, 1963d, 1963e). Little comparative data is available regarding the tissue distribution of other desaturases in the rat and the relative activities thereof. Δ5D, Δ4D and Δ9D activities have nevertheless been reported in several rat tissues, including adrenals, testes, liver, adipose, heart and brain (Mohrhauer et al 1963a, 1963b, 1963c, 1963d, 1963e, Gellhorn et al 1964, Brenner et al 1967, Bridges et al 1970, Takayasu et al 1970, Ayala et al 1973, Dannon et al 1975, Anon 1979, Stone et al 1979, Blond et al 1981 and Garg et al 1988b, 1988c). On the other hand, Δ8D activity has been found in rat testes (Albert et al 1977), but not in liver or brain (Ullman et al 1971b, Sprecher et al 1975 and Dhopeshwarkar et al 1976). There is evidence, however, that desaturase activity varies with tissue development as Cook (1978a)

detected limited Δ6D activity in fetal rat brain, which subsequently fell to very low levels 4 weeks after birth and was hardly detectable in the adult. In contrast, liver levels were very low after birth, but rose sharply after weaning (Cook 1978a). It was apparent, therefore, that desaturase activity relates both to the type and developmental state (age) of a tissue. Such factors warrant some consideration in the interpretation of the capability of different tissue types to desaturate FA's and in the comparison of the desaturase capabilities we reported with that of other workers.

The finding that desaturase enzyme capability was more limited with p-oil than individual FA dosage in all rat cell types studied supported competitive interations between p-oil FA's for such enzymes. Numerous studies have indeed indicated that desaturation can be modulated by FA's in dietary oils (eg. Brenner 1971, de Gomez Dumm et al 1983, Kirstein et l 1983, Barzanti et al 1986, Mosconi et l 1988, Garg et al 1989, Dang et al 1989). Δ9D, Δ6D and Δ5D expressions were enhanced in rat liver with corn oil, and suppressed with coconut oil, feeding (Pugh et al 1984), whereas others showed that hepatic Δ9D, Δ6D and Δ5D activities were not significantly altered by supplementing fat-free rat diets with 10% hydrogenated coconut oil, but were depressed with 10% safflower oil (de Schriver et al 1983). When rats were fed diets enriched with either hydrogenated beef tallow,

linseed or fish oils, Δ9D, Δ6D and Δ5D activities were inhibited by the latter two oils in liver microsomes, although the inhibition was greater with fish oil (Garg et al 1988a, 1988b, 1988c). Other investigations showed that hepatic Δ6D activity was greatest when rat diets were supplemented with linseed rather than safflower oil (Lee et al 1988), consistent with the fact that ALA is desaturated more efficiently than LA (Jeffcoat et al 1984). Linseed oil supplemenation of rat diets has also been shown to suppress/inhibit ω6 PUFA desaturation (Brazanti et al 1986), whereas fish oil administration decreased both Δ6D and Δ5D expression (Kirstein et al 1983). These studies indicated that the desaturase capabilities of the rat cell types we studied related to the overall balance of FA's both of the same and different series, as well as the cellular demand and/or requirement for longer chain more unsaturated FA's. Complex mechanisms control FA desaturation (eg. Holman 1964, Brenner et al 1966, 1967, 1969, Sprecher 1981 and de Schriver et al 1982), but maximum enzyme potential is genetically determined for every tissue type within a species.

The variations we reported in desaturation capability between the rat cell types studied may well relate to the different physiology of these tissues <u>in vivo</u>. However, the fact that desaturase capability was not distributed evenly between the rat tissues implied <u>in</u>

vivo dependence of some tissues on others for a supply of PDFA's. The fact that desaturase capability varied with rat cell type studied nevertheless indicated that extrapolations in this regard to other rat cell types should be avoided.

Comparative data obtained from the measurement of lipid peroxides indicated that the capability to produce these compounds varied with rat cell type. Lipoperoxides were absent from control erythrocytes (section 3.1.4), but were formed by all nucleated control cells, although relative concentrations were low in skeletal muscle and skin (0.1 to 0.2nmoles MDA/10^6 cells), intermediate in lymphocytes, brain, adipose and aortic endothelium (0.4 to 0.7nmoles MDA/10^6 cells), and greatest in lung (1.1nmoles MDA/10^6 cells) (sections 3.4.4, 3.7.5, 3.2.4, 3.5.4, 3.8.5, 3.3.4 and 3.6.5, respectively). This implied that the efficiency of the mechanisms of protection against free radical formation and rates of FFA oxidation varied between rat tissues. The finding that control cell lipid peroxide levels showed no clear correlation with the proliferation rates (Table 2.3.1.1.) or total unsaturated FA contents of these cells, however, probably reflected the physiological differences of each cell type in vivo, as well as variations in the rate of PGL turnover with cell type.

Supplementation of cultures with p-oils induced the formation of lipoperoxides from exogenous FA's, although

the concentrations varied numerically between rat cell types. Lipoperoxide concentrations greater than 10nmoles MDA/10^6 cells were rarely demonstrated in dosed adherent cells or their spent incubation media (Tables 3.3.4.1., 3.4.4.1., 3.5.4.1., 3.6.5.1., 3.7.5.1. and 3.8.5.1.), whereas a significantly wider range was found (0 to 103 nmoles MDA/10^6 cells) for erythrocyte and lymphocyte suspension cultures supplemented with p-oil (Tables 3.1.4.1. and 3.2.4.1.). Cellular lipoperoxide formation increased in an overall concentration dependent fashion when rat cultures were enriched with increasing amounts of p-oil, and this supported exogenous FA incorporation from the growth medium. Mouri et al (1984) similarly showed that TBARM levels increased in serum and liver when rats were fed increasing amounts of a marine oil diet containing a total of 15% ω3 PUFA's. Kobatake et al (1983) demonstrated that serum and liver lipoperoxide levels were more elevated in rats fed 5% marine oil (containing 75% ω3 PUFA's) compared to 5% oleate or linoleate supplementation, consistent with the greater susceptibility of ω3 PUFA's to oxidation. Others showed that lipid peroxidation decreased by 90% in rat liver microsomes when a 16% coconut oil diet was fed for 3 days, whereas normal lipid peroxidation was subsequently restored within 10 days of feeding a 16% safflower oil diet (Lokesh et al 1981). They suggested that diets rich in saturated fat reduced microsomal lipid peroxidation

by decreasing the availability of PUFA's in substrate PGL's. The findings we presented in this chapter indicated that p-oil-induced lipoperoxidation related to the amount of unsaturated FA's present in any p-oil (Table 2.3.3.2.) and their susceptibility to oxidation. This was dependent not only on the number and position of double bonds in a FA, but also the availability of these moieties. The possibility also existed that synergistic or antagonistic interactions between p-oil FA's influenced lipoperoxide formation to a greater or lesser extent.

The absence of lipoperoxides in spent control media from all rat cultures reflected the abundance of saturated FA's present in new incubation medium (Table 2.3.3.3.), and the retention of intracellular lipoperoxides. This implied that the p-oils induced the spent medium lipid peroxides found occasionally in dosed cultures. The ability of the rat cell types studied to incorporate exogenous FA's and the presence of albumin as p-oil FA carrier in the culture medium supported the likelyhood that most spent medium lipid peroxides originated intracellularly, and were released as a direct result of cell lysis or via leakage through the plasma membrane. 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.

The desaturation capability of rat lung, skin and adipose cells supported the production of DGLA, AA and EPA as precursors for 1-, 2- and 3-series eicosanoids, which correlated with the ability of these cells to form eicosanoids (Tables 3.6.6.1., 3.7.6.1. and 3.8.6.1.). Total molar eicosanoid production varied considerably between these different rat cell types, however, being greatest for control lung fibroblasts, whereas adipose and skin cells formed considerably smaller amounts (727.6, 215.9 and 165.7pmoles total eicosanoids per 10^6 cells, respectively). This correlated with reports implicating the lung as a primary site of eicosanoid biosynthesis (Mathe et al 1977, Hyman et al 1978 and Harper et al 1984). These variations in eicosanoid production may relate partly to the different eicosanoid requirements of such cells, rather than the ability to solely provide eicosanoid precursors via desaturation. Low eicosanoid production may also reflect greater FA incorporation into the TAG rather than the PGL pool. This could explain the lower molar eicosanoid levels generated by adipose than lung cells, despite the greater capability for desaturation with the former. The finding that the total eicosanoid:lipoperoxide molar ratio's for control skin (Tables 3.7.5.1. and 3.7.6.1.), lung (Tables 3.6.5.1. and 3.6.6.1.) and adipose (Tables 3.8.5.1. and 3.8.6.1.) cells ammounted to 83%, 60% and 43%, respectively, nevertheless implied a greater

preference to synthesise eicosanoids in skin than in lung or adipose cells under the experimental conditions employed. This probably related to the different physiology and requirements of these tissues *in vivo*.

The finding that control rat lung, skin and adipose cells differed in the relative amounts of PGI_2, TXB_2, PGF_{α}, PGE_2 and PGE_1 they produced signified that the expression and activity of enzymes involved in eicosanoid synthesis varied between rat tissues and that such was tissue-specific. This supported the work of Pace-Asciak et al (1977), who showed considerable tissue specificity in PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2 and 6-keto $PGF_{1\alpha}$ production in rat lung, liver, spleen, stomach, kidney and heart homogenates in the presence of AA. These tissues contained low PGE_2, $PGF_{2\alpha}$ and PGD_2 levels, although PGE_2 was detected in greater amounts than $PGF_{2\alpha}$ or PGD_2 in all tissues. 6-keto $PGF_{1\alpha}$ was specifically formed by stomach, although lung also formed significant amounts, whereas TXB_2 was mostly directed to spleen, with lesser amounts in lung. We showed that PGE_1 was the major prostanoid detected in control rat lung cells and this correlated with the considerably lower levels of other prostanoids reported (Table 3.6.6.1.). This could relate to the fact that PGE_1 is partly responsible for the control of cAMP levels, a potent inhibitor of AA release (Feinstein et al 1977 and Minkes et al 1977). High PGE_1 would have resulted in high cAMP levels, and

thus decreased AA mobilisation. In contrast, control skin and adipose cells produced little, or no PGE_1, and such could explain the synthesis of 2-series prostanoids (Tables 3.7.6.1. and 3.8.6.1.). In the light of this phenomenon, it was possible that a greater proportion of the PGF_{α} fraction detected was composed of $PGF2_{\alpha}$ rather than $PGF_{1\alpha}$.

p-Oil supplementation modulated the production of the prostanoids studied by influencing the balance between individual prostanoids to a greater or lesser extent (Tables 3.6.6.1., 3.7.6.1. and 3.8.6.1., respectively), although total prostanoid production was largely unaltered in lung fibroblasts, decreased in adipose cells and more varied in skin fibroblasts. These changes, however, could not be related to variations in p-oil FA composition. Lee et al (1988) similarly found no significant difference in TXB_2 and PGI2 production between rats fed safflower or palm oils, despite the large difference in P/S ratio. The reason for these findings were unclear, but could have related to sufficient endogenous PUFA's in the precursor pool to produce an adequate level of prostanoids, or the relative unavailability of incorporated exogenous FA's for the production of these prostanoids. The possibility that dosage with different p-oils induced variations in other eicosanoid levels, however, could not be excluded. The finding that the modulation of prostanoid production

related to the p-oil amount dosed suggested a preference for certain p-oil concentrations to selectively modulate enzyme activity involved in the synthesis of these compounds. However, the fact that rat lung, skin and adipose cell prostanoid profiles showed no p-oil concentration dependent correlation with cell viability, desaturation capability or lipid peroxide production implied that the endogenously synthesised prostanoids studied were not directly involved in, or responsible for, the effects induced with p-oil supplementation. Durant et al (1989) showed that exogenous prostanoids ($PGF_{2\alpha}$, 6-keto $PGF_{1\alpha}$ and PGE2) could alter endogenous PG synthesis/release and increase fibroblast proliferation, whereas endogenous PG's alone were not involved in the control of cell proliferation. This supported the findings we presented, particularly with regard to p-oil induced cytotoxicity. We extended the work of Durant et al (1989) by proposing lipoperoxides and alterations in membrane physical properties as alternative mechanisms involved in the modulation of cell proliferation. The role of eicosanoids in the regulation of cell functions, however, remains undisputed.

In summary, this chapter provided comparative data on a range of cell parameters for different cultured rat tissues. Control cells from different tissues exhibited different growth rates, FA and prostanoid profiles, contained different concentrations of total protein, and

produced different molar amounts of lipoperoxides and eicosanoids. Variations in cell viability, total protein and lipoperoxide formation, FA and eicosanoid profiles, and desaturation capability were also demonstrated between different rat cell types when p-oils were supplemented. These findings implied variations in FA metabolism between different rat tissues, and that these differences were important in the modulation of the effects reported. The data indicated that different rat tissues *in vivo* probably also respond differently when fed identical dietary oils due to inherent variations in cell physiology. Careful consideration should thus be given to the selection of rat tissues for experimental purposes, and extrapolation of experimental findings between rat cell types should be avoided to ensure valid interpretation of results.

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

VOLUME 2

ALFREDO GIANGREGORIO

CHAPTER 4: THE CAT: RESULTS AND DISCUSSION.

4.1 THE EFFECTS OF PSEUDO-OILS ON CAT ERYTHROCYTES.

4.1.1 Effects of pseudo-Oils on Cell Viability.

Cat erythrocyte cultures were found to be free of any white blood cell or platelet contamination upon microscopic examination. Most cultured erythrocytes retained their biconcave disc shape, although some lost this feature after 48 hours in culture. Further, 20% to 30% of erythrocytes lost their distinct 'red' colour after 2 days in culture and hence become difficult to visualise. Only erythrocytes which retained their pigmentation were therefore regarded as viable, normal and representative of the situation *in vivo*.

Final medium albumin concentrations ranging from 0 to 250mg/l were investigated on erythrocyte viability, and the results indicated that albumin had no significant effect, nor were any synergistic effects observed (Fig. 4.1.1.1.). The effects induced with p-oil dosage were thus a result of the exogenous FA's and not the albumin used as FA carrier.

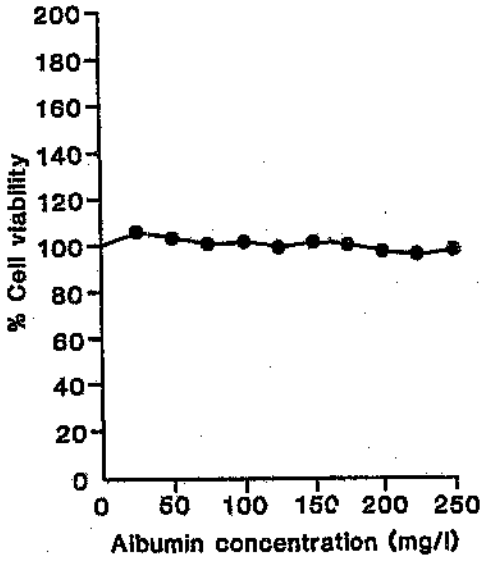
Erythrocytes do not divide nor require trypsinisation, thus no 24 hour post-trypsinisation recovery period was needed. Erythrocytes were dosed immediately subsequent to plating, thus the seeding concentration was the cytostatic number.

Legend to Figs. 4.1.1.1-4.1.1.8.

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. 4.1.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 4.1.1.2-4.1.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 4.1.1.1.

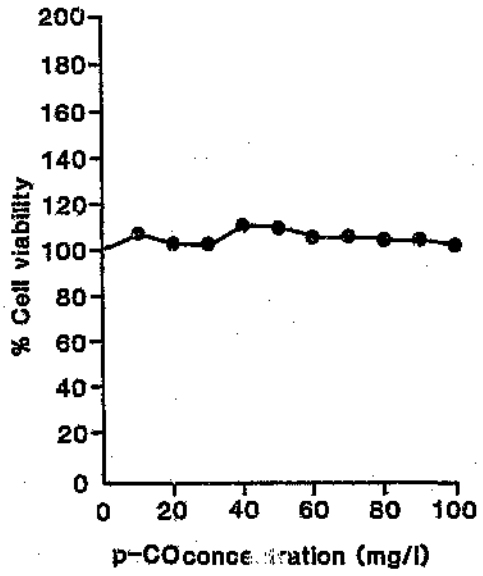
The percentage viability of cat erythrocytes incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.2	12
25	104.9	3.7	12
50	103.1	4.8	12
75	100.0	3.5	12
100	101.2	4.0	12
125	99.0	3.4	12
150	100.7	3.4	12
175	99.4	4.3	12
200	97.7	5.0	12
225	96.0	5.8	12
250	99.1	3.7	12

Fig. 4.1.1.2.

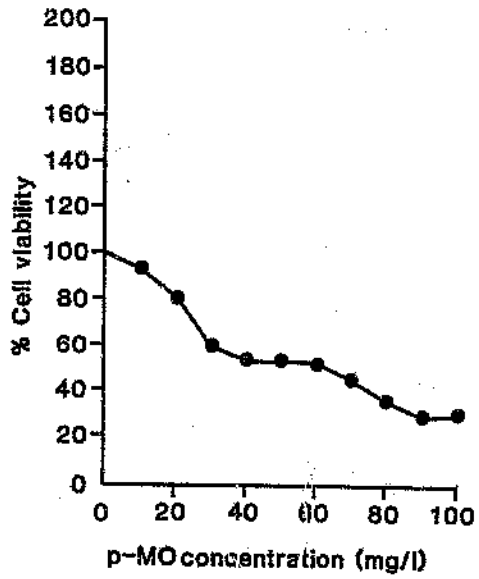
The percentage viability of cat erythrocytes incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.7	6
10	107.1	3.8	6
20	102.6	5.4	6
30	101.1	5.9	6
40	110.5	5.8	6
50	109.4	2.5	6
60	105.3	2.5	6
70	105.1	4.9	6
80	103.9	2.7	6
90	103.9	4.0	6
100	100.8	3.4	6

Fig. 4.1.1.3.

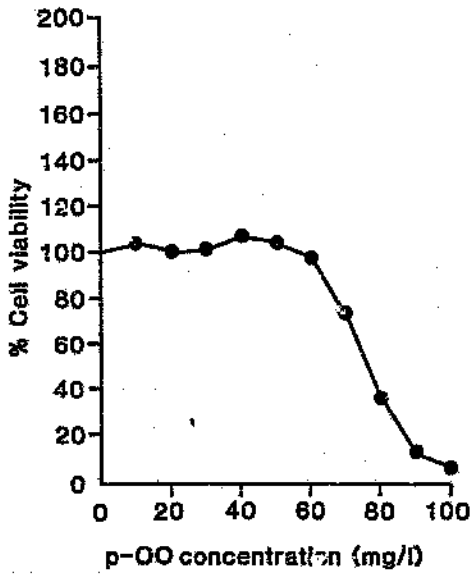
The percentage viability of cat erythrocytes incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.0	6
10	93.1	5.1	6
20	81.1	3.4	6
30	60.6	3.3	6
40	53.3	4.6	6
50	53.4	5.4	6
60	52.6	2.8	6
70	45.1	2.6	6
80	35.8	1.1	6
90	29.4	1.6	6
100	31.6	0.9	6

Fig. 4.1.1.4.

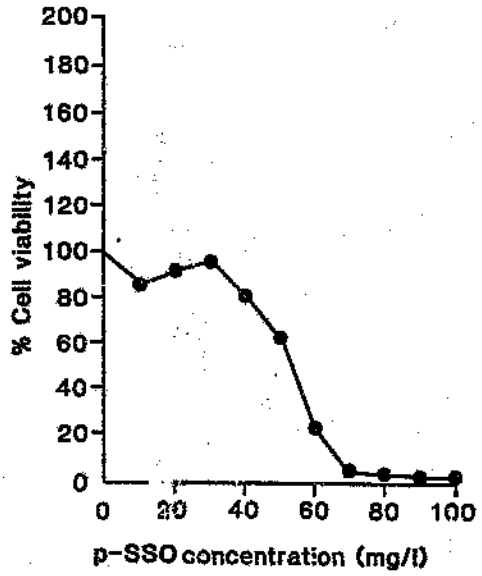
The percentage ibility of cat erythrocytes incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.8	6
10	104.5	4.0	6
20	99.5	1.5	6
30	101.4	3.8	6
40	107.0	4.3	6
50	103.4	2.8	6
60	97.5	3.4	6
70	72.9	2.2	6
80	35.8	2.4	6
90	12.7	1.5	6
100	6.0	1.0	6

Fig. 4.1.1.5.

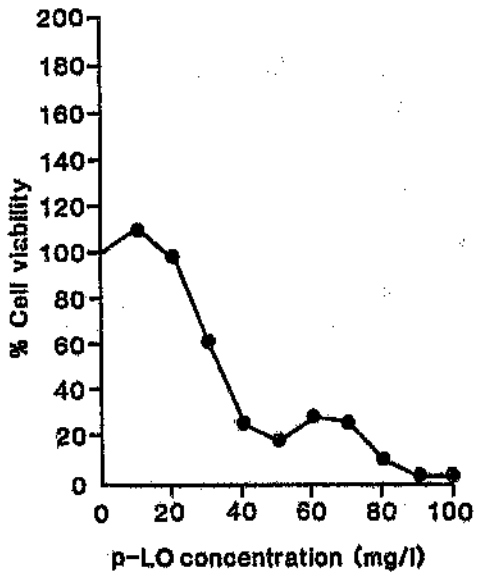
The percentage viability of cat erythrocytes incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.6	6
10	84.7	5.3	6
20	90.6	4.5	6
30	95.3	4.7	6
40	79.9	1.9	6
50	61.0	1.5	6
60	23.4	4.6	6
70	4.1	1.1	6
80	2.7	0.7	6
90	2.1	0.5	6
100	1.7	0.5	6

Fig. 4.1.1.6.

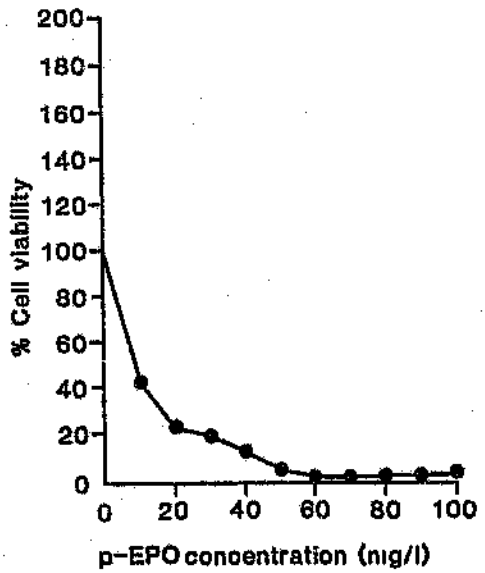
The percentage viability of cat erythrocytes incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.3	6
10	110.3	4.1	6
20	98.4	5.0	6
30	59.8	5.6	6
40	25.9	2.7	6
50	18.3	3.8	6
60	28.5	1.2	6
70	25.9	4.4	6
80	10.3	4.9	6
90	1.6	0.4	6
100	1.1	0.2	6

Fig. 4.1.1.7.

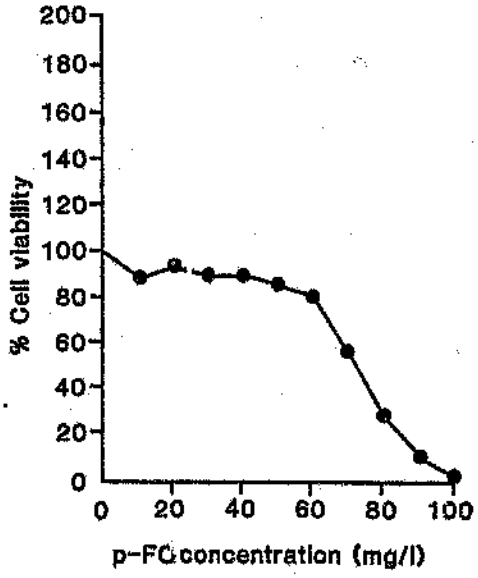
The percentage viability of cat erythrocytes incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.7	6
10	43.4	3.9	6
20	22.6	1.4	6
30	18.9	1.1	6
40	12.4	1.0	6
50	4.6	1.2	6
60	0.9	0.1	6
70	0.9	0.1	6
80	1.4	0.2	6
90	2.2	0.3	6
100	3.2	0.5	6

Fig. 4.1.1.8.

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



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.6	6
10	88.0	4.1	6
20	93.3	4.5	6
30	89.7	3.1	6
40	89.5	1.6	6
50	85.7	2.1	6
60	80.3	3.0	6
70	55.9	4.3	6
80	28.6	4.7	6
90	9.4	1.1	6
100	0.2	0.1	6

All p-oils, except p-CO, exhibited cytotoxic potential when amounts up to 100mg/l were added to erythrocytes, although the magnitude thereof varied with the p-oil and concentration dosed (Figs. 4.1.1.2-4.1.1.8.). EFA-rich p-oils (p-SSO, p-LO and p-EPO), however, induced the greatest hemolysis.

Incubation with up to 100mg/l p-CO induced no hemolysis (Fig. 4.1.1.2.), and both erythrocyte morphology and viability paralleled controls. 10, 40 and 50mg/l p-CO, however, appeared slightly more effective in stabilising erythrocyte viability in culture relative to controls. On the other hand, p-MO induced concentration dependent hemolysis such that approximately half the erythrocytes were viable with 40 to 60mg/l, and this was further reduced to 30% with 90 and 100mg/l (Fig. 4.1.1.3.).

p-OO maintained erythrocyte viability parallel to, or marginally better than, controls up to a concentration of 60mg/l, whereas extensive concentration dependent hemolysis was induced with greater amounts such that 50% of cells were killed with approximately 76mg/l p-MO (ID_{50}) and 94% with 100mg/l (Fig. 4.1.1.4.).

Erythrocyte viability decreased progressively from 95.3% with 30mg/l p-SSO to 23.4% with 60mg/l p-SSO, while 70 to 100mg/l p-SSO induced greater than 95% hemolysis (Fig. 4.1.1.5.). Erythrocytes were protected from lysis with 10 and 20mg/l p-LO, but marked hemolysis also

occured with greater concentrations, such that viability was 59.8% with 30mg/l p-LO and 18.3% to 28.5% with 40 to 70mg/l p-LO (Fig. 4.1.1.6.). Practically all the cells were killed with 90 and 100mg/l p-LO. On the other hand, erythrocyte viability was reduced to 43.4% with only 10mg/l p-EPO, and further to 22.6% with 20mg/l p-EPO, while at least 95% hemolysis occurred with 50 to 100mg/l p-EPO (Fig. 4.1.1.7.). p-EPO was the most toxic p-oil, followed by p-LO and p-SSO, and this correlated with the ID_{50} values (about 8, 33 and 53mg/l, respectively).

Concentrations up to 40mg/l p-FO induced approximately 10% hemolysis, which increased to 20% with 60mg/l p-FO (Fig. 4.1.1.7.). Hemolysis increased progressively with higher concentrations dosed, and practically all cells took up Trypan blue with 100mg/l p-FO. It was evident that the overall effects p-FO induced were parallel to those of p-OO (Fig. 4.1.1.4.), although p-FO was slightly more cytotoxic. This correlated with the ID_{50} values (about 73mg/l for p-FO and 76mg/l for p-OO).

Cat erythrocytes were subsequently 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 relative to controls were compared upon harvesting and found to be statistically similar to those in Figs. 4.1.1.1-4.1.1.8. All further biochemical assays were therefore performed on these samples.

4.1.2 Effects of pseudo-Oils on Total Protein.

Total protein was quantified for cat erythrocytes dosed with 0, 20, 40 or 60mg/l p-oil, and the results shown in Table 4.1.2.1. as µg total protein/10^6 cells seeded.

Control erythrocytes contained 12.3µg total protein/10^6 cells seeded, whereas amounts in dosed erythrocytes were similar to, or lower than, control levels and ranged from 1.6 to 12.3µg/10^6 cells seeded (induced with 60mg/l p-EPO and 20mg/l p-LO dosage, respectively). Erythrocyte protein concentrations approximated to controls with 20, 40 or 60mg/l p-CO or p-OO supplementation, but decreased overall with increasing amounts of other p-oils dosed. Cat erythrocytes incubated with 20, 40 or 60mg/l p-EPO, however, exhibited the lowest protein levels quantitated (4.2, 2.5 and 1.6µg/10^6 cells seeded, respectively).

Table 4.1.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	12.3			
CO		11.6	12.2	12.2
MO		10.0	7.6	6.7
OO		11.3	11.9	11.3
SSO		11.7	11.6	4.8
LO		12.3	3.8	3.3
EPO		4.2	2.5	1.6
FO		10.5	10.7	8.7

4.1.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cat Erythrocytes.

Table 4.1.3.1. shows the FA spectra of cat erythrocytes dosed with 0, 20, 40 or 60mg/l of each of the p-oils. FA's contributing >5.0% to the FA spectrum in control erythrocytes were 16:0 (11.2%), 18:0 (9.0%), 18:1ω9 (7.1%), 22:4ω6 (49.4%), 22:5ω6 (5.1%) and 20:4ω3 (9.3%). Overall, dosed erythrocytes contained greater 16:0, 18:1ω9 and 18:2ω6 amounts than controls, 18:0 and 16:1ω9 proportions were more varied, whereras ω6 PDFA and ω3 PUFA percentages were parallel to controls or decreased.

Erythrocytes dosed with p-CO showed a marked increment in 16:0 levels (34.8% with 20mg/l, 46.0% with 40mg/l and 55.9% 60mg/l p-CO, respectively vs 11.2% in controls), but 18:0 amounts were generally parallel to controls (9.0%). 24:0 and 16:1ω9 were increased significantly with 20mg/l and 40mg/l p-CO, respectively (1.8% and 7.9% vs 0% and 2.0% in controls, respectively), while 18:1ω9 levels increased approximately 2 fold with 20mg/l p-CO, and 3 fold with 40 and 60mg/l p-CO relative to controls (7.1%). On the other hand, p-CO incubation decreased erythrocyte PUFA levels.

Dosage with 20, 40 or 60mg/l p-MO induced increments in 16:0 (18.5%, 31.6% and 35.5%, respectively vs 11.2% in controls), 18:0 (25.4%, 17.2% and 19.7%, respectively vs 9.0% in controls) and 18:1ω9 (20.3%, 27.9% and 28.3%,