

https://dspace.org/

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

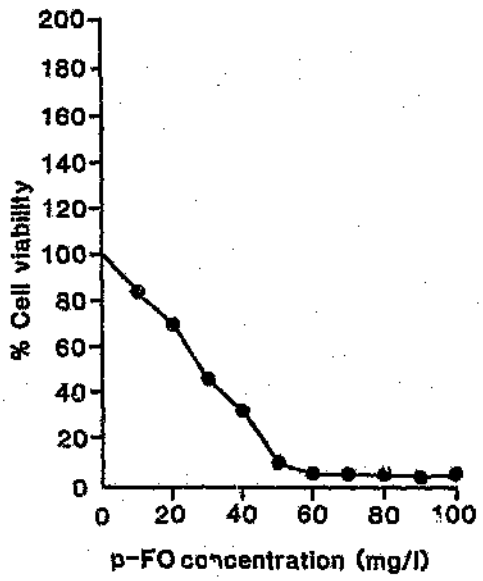
Giangregorio, Alfredo

1991
https://hdl.handle.net/10539/25153

Downloaded from a DSpace repository. https://dspace.org/

Fig. 5.2.1.8.

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



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.8	6
10	82.8	5.5	6
20	69.5	4.2	6
30	45.2	3.6	6
40	33.0	1.2	6
50	9.6	0.2	6
60	4.4	0.6	6
70	4.0	1.0	6
80	4.6	1.0	6
90	3.7	0.6	6
100	4.0	1.4	6

saturated FA's. This correlated with the respective ID_{50} values, which were highest for p-MO and p-CO (about 95mg/l and 85mg/l, respectively), intermediate for p-OO, p-SSO, p-LO and p-EPO (approximately 50mg/l, 47.5mg/l, 47.5mg/l and 49mg/l, respectively), and lowest for p-FO (about 28mg/l).

Lymphocyte viability approximated to that of controls with dosage of up to 20mg/l p-CO, but was reduced almost linearly as the concentration dosed was progressively increased to yield 28.5% viable cells with 100mg/l p-CO (Fig. 5.2.1.2.). Supplementation with up to 40mg/l p-MO had no significant effect on lymphocyte viability, but cytotoxicity also increased progressively with higher concentrations dosed, although 41.9% viable lymphocytes were found with 100mg/l p-MO (Fig. 5.2.1.3.).

Lymphocyte viability was not significantly altered with 0 to 30mg/l p-OO dosage, but was progressively inhibited to 23.6% with 60mg/l p-OO and to practically 100% with 100mg/l p-OO (Fig. 5.2.1.4.).

Lymphocyte viability was reduced by approximately 20% with dosage of 10 or 20mg/l p-SSO, but not significantly with p-LO (Figs. 5.2.1.5. and 5.2.1.6., respectively). However, the cytotoxicity these p-oils induced at higher concentrations were similar as lymphocyte viability was progressively reduced to about 70% with a concentration of 40mg/l, 20% with 60mg/l, and further to only 6% with

100mg/l p-SSO or p-LO.

Incubation with increasing amounts of p-EPO resulted in an overall linear increment in lymphocyte killing, with viable cell yields ranging from 86.0% with 10mg/l p-EPO, 47.5% with 50mg/l p-EPO, and 1.4% with 100mg/l p-EPO (Fig. 5.2.1.7.).

Lymphocyte killing increased linearly with incubation of up to 50mg/l p-FO, at which concentration 9.6% viable lymphocytes were found, while Trypan blue uptake was about 95% with 60 to 100mg/l p-FO (Fig. 5.2.1.8.).

Human lymphocytes 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 performed. Upon harvesting, relative cell viabilities were compared and found to be statistically similar to those in Figs. 5.2.1.1-5.2.1.8. Hence, all further biochemical assays were performed on these samples.

5.2.2 Effects of pseudo-Oils on Total Protein.

Table 5.2.2.1. shows the total protein concentrations of human lymphocytes dosed with 20, 40 or 60mg/l p-oil in relation to controls.

14.8µg protein/10^6 control cells seeded was measured. Dosed lymphocyte protein concentrations approximated to, or were greater than, controls levels, and ranged from from 14.1 to 66.6µg/10^6 cells seeded, although amounts were decreased with 20mg/l p-LO dosage (9.0µg/10^6 cells seeded). Increments induced in lymphocyte protein were smallest with dosage of 20mg/l p-oil (9.0 to 25.3µg/10^6 cells seeded), and greatest with 60mg/l p-oil (18.1 to 66.6µg/10^6 cells seeded), while p-CO and p-MO were most, and p-LO least, effective in mediating these changes.

Table 5.2.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	14.8			
CO		18.1	44.7	66.6
MO		25.3	30.4	40.6
OO		21.9	28.0	34.6
SSO		21.6	29.6	33.7
LO		9.0	15.1	18.1
EPO		14.9	32.7	33.5
FO		14.1	20.0	32.1

5.2.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Human Lymphocytes.

The FA spectra of human lymphocytes dosed with 0, 20, 40 or 60mg/l of each of the p-oils are shown in Table 5.2.3.1.

Control lymphocytes exhibited a FA spectrum in which 22:4ω6 was found to be present in amounts of 41.3%, compared to 14.2% 16:0, 9.8% 18:0, 9.3% 18:1ω9, 5.4% 18:4ω3, 7.6% 20:4ω3, and smaller amounts of other FA's.

Dosed lymphocytes generally contained less 18:0 and 16:1ω9 than controls, while 16:0 and 18:1ω9 amounts approximated to controls or were considerably increased. 18:2ω6 levels were generally significantly increased in dosed lymphocytes, but other ω6 and ω3 PUFA percentages were parallel to controls or decreased.

16:0 percentages increased significantly with 20, 40 or 60mg/l p-CO dosage (21.8%, 52.8% and 59.3%, respectively vs 14.2% in controls), 18:1ω9 increased approximately 2 fold over the same range (18.1%, 19.6% and 15.4%, respectively vs 9.3% in controls), while 18:0 levels approximated to control amounts overall (9.8%). However, all PUFA percentages were parallel to controls or significantly decreased with p-CO dosage, particularly that of 22:4ω6 with 40 and 60mg/l (11.5% each vs 41.3% in controls).

Legend to Table 5.2.3.1.

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

Table 5.2.3.1.

The fatty acid spectrum of human lymphocytes.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	14.2±0.75	21.8	52.8	59.3	23.8	29.5	33.2	18.3	15.3	14.6	14.4	18.3	19.7	20.0	22.1	23.9	13.7	24.8	21.2	34.6	30.0	24.9
	18:0	9.8±0.25	6.2	9.5	9.2	8.6	14.7	15.1	3.4	1.1	0.7	6.5	8.5	6.2	6.1	5.1	4.5	9.8	11.5	7.4	7.1	4.8	2.4
	20:0	0.4±0.00	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:0	0.3±0.00	-	-	0.1	-	-	0.1	-	-	-	-	0.1	-	-	-	-	0.1	-	-	-	-	-
	24:0	-	-	-	-	0.1	-	-	0.1	-	-	-	-	0.4	-	1.5	0.5	0.3	-	0.5	-	-	-
ω9 MONOS.	16:1	2.9±0.20	0.6	-	-	0.1	-	-	0.3	-	-	0.7	0.1	0.1	-	0.1	-	2.5	1.0	0.1	11.7	27.3	32.3
	18:1	9.3±0.10	18.1	19.6	15.4	34.6	26.7	22.6	54.4	71.6	73.4	17.7	36.1	34.4	45.6	34.8	30.3	9.4	19.4	17.2	13.2	22.2	23.2
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	2.5±0.05	2.7	1.4	1.1	8.8	6.3	5.5	6.4	6.7	7.1	18.6	28.6	34.9	5.9	10.5	11.7	4.4	31.2	42.6	3.5	7.4	8.8
	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	0.8	1.1	-	-	-
	20:2	-	-	-	-	0.1	-	-	1.4	1.9	1.9	-	-	-	0.5	0.3	0.1	-	-	-	0.4	0.9	0.8
	20:3	0.1±0.00	0.3	0.3	0.5	0.1	-	0.1	-	-	0.1	0.1	-	-	-	-	-	-	0.1	-	-	-	0.1
	20:4	1.6±0.05	1.0	0.6	0.8	1.0	0.5	0.6	0.2	0.1	0.1	0.7	0.2	-	-	0.1	-	-	0.3	-	-	0.1	0.1
	22:4	41.3±2.05	42.9	11.5	11.5	15.4	14.0	19.8	13.7	2.4	1.8	36.9	5.0	3.5	18.2	7.7	4.7	52.7	6.4	8.9	25.6	3.0	3.0
	22:5	4.3±0.05	0.5	0.8	-	-	1.1	0.2	0.3	0.1	-	0.4	0.5	0.1	0.1	0.4	0.1	0.8	0.7	0.2	0.8	0.5	0.1
ω3 POLYS.	18:3	0.2±0.00	0.9	0.6	0.4	5.7	2.6	1.0	0.1	0.1	-	-	0.1	0.1	1.6	15.6	23.7	-	1.3	0.1	0.3	0.4	0.5
	18:4	5.4±0.30	0.4	1.2	0.1	0.2	2.1	0.2	0.3	0.3	-	0.4	1.3	0.2	-	0.8	0.2	0.8	-	-	0.4	0.7	0.1
	20:4	7.6±0.25	4.5	1.5	0.7	1.7	2.5	1.5	1.2	0.3	0.1	2.3	0.9	0.2	2.0	1.1	0.3	5.4	1.4	0.4	2.0	0.6	0.3
	20:5	-	-	-	0.7	-	-	-	-	-	0.2	1.2	0.1	-	-	-	-	-	0.5	-	0.5	2.1	3.3
	22:5	0.2±0.00	-	0.1	0.2	-	-	-	-	0.1	0.1	-	-	-	-	-	-	-	-	-	-	-	-
	22:6	0.2±0.00	-	0.1	0.2	-	-	-	-	0.1	-	-	0.2	0.3	-	0.1	0.1	-	0.6	0.2	-	0.1	0.1

Lymphocyte incubation with 20, 40 or 60mg/l p-MO induced extensive increments in the levels of 16:0 (23.8%, 29.5% and 33.2%, respectively vs 14.2% in controls) and 18:1ω9 (34.6%, 26.7% and 22.6%, respectively vs 9.3% in undosed cells), compared to smaller changes in 18:0 (8.6% to 15.1% vs 9.8% in controls). 18:2ω6 and 18:3ω3 increased over the entire range dosed (5.5%, 6.3% and 8.8%, and 5.7%, 2.6% and 1.0% vs 2.5% and 0.2% in controls, respectively), but no significant increments were detected for PDFA's, including 20:4ω6 which levels were decreased (1.0%, 0.5% and 0.6%, respectively vs 1.6% in controls).

Supplementation of the incubation medium with 20, 40 or 60mg/l p-OO induced marked increments in cellular 18:1ω9 levels (54.4%, 71.6% and 73.4%, respectively vs 9.3% in controls). 16:0 levels were largely unaltered (14.6% to 18.3% vs 14.2% in controls), but 18:0 and 16:1ω9 were significantly decreased in relation to controls (9.8% and 2.9%, respectively). Small increments were detected for 18:2ω6 (6.4% to 7.1% vs 2.5% in controls) and 20:2ω6 (1.4% to 1.9% vs 0% in controls), but PDFA percentages were parallel to controls or significantly decreased with p-OO dosage, particularly that of 22:4ω6 (41.3%, 13.7%, 2.4% and 1.8% with 0, 20, 40 and 60mg/l p-OO, respectively).

16:0 levels were marginally increased with p-SSO dosage

(14.4% to 19.7% vs 14.2% in controls), but the converse was true for 18:0 (6.2% to 8.5% vs 9.8% in controls). On the other hand, more marked changes were induced in the levels of 16:1ω9 (0.1% to 0.7% vs 2.9% in controls) and 18:1ω9 (17.7% to 36.1% vs 9.8% in controls). A concentration dependent increased in 18:2ω6 was detected with 20, 40 or 60mg/l p-SSO incubation (18.6%, 28.6% and 34.9%, respectively vs 2.5% in controls), yet all PDFA levels were decreased. No significant increments in ω3 PUFA levels were detected.

Significant increases in 16:0 and 18:1ω9 were induced with p-LO dosage (20.0% to 23.9% vs 14.2% in controls, and 30.3% to 45.6% vs 9.3% in controls, respectively), but the converse was true for 18:0 (6.1% to 4.5% vs 9.8% in controls) and 16:1ω9 (<0.1% vs 2.9% in controls). Dosage with 20, 40 or 60mg/l p-LO induced concentration dependent increases in 18:2ω6 (5.9%, 10.5% and 11.7%, respectively vs 2.5% in controls), and 18:3ω3 (1.6%, 15.6% and 23.7%, respectively vs 0.2% in controls), yet PDFA levels approximated to controls or were decreased.

The levels of 16:0 and 18:1ω9 increased overall, 16:1ω9 was decreased, and 18:0 not significantly altered, with p-EPO supplementation. A marked increase in 18:2ω6 was induced when cells were incubated with 20, 40 or 60mg/l p-EPO (4.4%, 31.2% and 42.6%, respectively vs 2.5% in controls), and small amounts of 18:3ω6 were detected with 40 and 60mg/l p-EPO (0.8% and 1.1%, respectively vs

0% in controls). 22:4ω6 was increased only with 20mg/l p-EPO (52.7% vs 41.3% in controls), but no significant increments in other PUFA's were detected.

Lymphocyte incubation with 20, 40 or 60mg/l p-FO caused a fall in 18:0 (7.1%, 4.8% and 2.4%, respectively vs 9.8% in controls), but increments in 16:0 (34.6%, 30.0% and 24.9%, respectively vs 14.2% in controls), 16:1ω9 (11.7%, 27.3% and 32.3%, respectively vs 2.9% in undosed cells), and 18:1ω9 (13.2%, 22.2% and 23.2%, respectively vs 9.3% in controls). Small increments were detected for 18:2ω6 (3.5% to 8.8% vs 2.5% in controls) and 20:5ω3 (0.5%, 2.1% and 3.3% with 20, 40 and 60mg/l p-FO, respectively), but neither ω6 PDFA, nor 22:5ω3 and 22:6ω3, percentages were increased.

5.2.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 5.2.4.1. shows the lipoperoxides quantitated in lymphocytes and their respective spent culture media following incubation with 0, 20, 40 or 60mg/l p-oil for 48 hours. The values are as nmoles MDA/10^6 cells, but in the case of the spent incubation media, this represents the nmoles of MDA in the volume of medium from which 1x10^6 cells were obtained.

Lipoperoxides was absent from control lymphocytes, while concentrations in dosed lymphocytes ranged from 0 to 38.8nmoles MDA/10^6 cells. Cellular lipoperoxide levels approximated to controls with 20mg/l p-oil dosage (0 to 0.3nmoles MDA/10^6 cells), but generally increased with higher p-oil concentrations dosed. Incubation with p-MO did not result in significant lipoperoxide formation (0.1nmoles MDA/10^6 cells), p-OO, p-SSO and p-EPO induced small amounts (0.1 to 0.7, 0.2 to 1.2, and 0.1 to 1.3 nmoles MDA/10^6 cells, respectively), while p-CO induced significant levels with 40 and 60mg/l (0.8 and 3.9nmoles MDA/10^6 cells, respectively). The highest lipoperoxide levels were measured with p-LO or p-FO dosage, although the amounts induced were greater with 40 or 60mg/l p-FO than p-LO (7.0 and 38.8 vs 3.0 and 9.6nmoles MDA/10^6 cells, respectively).

Spent medium lipoperoxide levels were greatest with

Table 5.2.4.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	-			
CO		-	0.8	3.9
MO		0.1	0.1	0.1
OO		0.1	0.3	0.7
SSO		0.3	0.2	1.2
LO		0.1	3.0	9.6
EPO		0.1	0.6	1.3
FO		0.1	7.0	38.8

pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	-			
CO		-	-	0.2
MO		-	-	-
OO		-	-	-
SSO		3.6	3.2	-
LO		-	-	-
EPO		3.3	-	19.2
FO		-	-	19.5

60mg/l p-EPO or p-FO dosage (19.2 and 19.5nmoles MDA/10^6 cells, respectively), while smaller amounts were found with 20mg/l p-EPO or p-SSO, 40mg/l p-SSO, or 60mg/l p-CO incubation (3.3, 3.6, 3.2 and 0.2nmoles MDA/10^6 cells, respectively). Lipoperoxides were absent from all other spent media, however, including that from which control lymphocytes were derived.

5.2.5 Discussion.

Supplementation of resting human lymphocytes with p-oils induced cytotoxicity in every instance (Figs. 5.2.1.2-5.2.1.8.). Modulation of significantly more cytotoxicity with dosage of p-OO, p-SSO, p-LO, p-EPO or p-FO than with p-CO or p-MO was consistent with large amounts of unsaturated FA's in the former p-oils (Table 2.3.3.2.). This implied that unsaturated FA's were more effective cytotoxic agents than saturated FA's. The greater potential for induction of membrane rigidification with p-CO than p-MO dosage, however, could explain why p-CO, containing only 5% unsaturated FA's, induced more cell death than p-MO, containing about 20% unsaturated FA's. It was nevertheless apparent that the modulation of cell viability related not only to the total amount of unsaturated FA's present in any p-oil, but also the degree of FA unsaturation. Indeed, the presence of EPA in p-FO could account for the fact that this p-oil induced the greatest degree of cytotoxicity with its dosage, despite containing intermediate amounts of unsaturated FA's compared to other p-oils. The similar ID_{50} values found with dosage of p-OO, p-SSO, p-LO or p-EPO were consistent with the similar amounts of unsaturated FA's present in these p-oils (about 90%), while the small differences observed in lymphocyte viability were relatable to variations in the FA compositions between these p-oils. The abundance of DA

in p-OO, LA in p-SSO and p-EPO, and ALA in p-LO probably accounted for the cell viability changes these p-oils induced overall. Thus, the more effective reduction of lymphocyte viability reported with dosage of low concentrations of p-EPO or p-SSO than with p-OO or p-LO suggested that LA was slightly more effective than OA and ALA in mediating cytotoxicity. The converse was true, however, with incubation of these p-oils at high concentrations. The similar cell viability changes p-EPO and p-SSO induced with supplementation at low concentrations were not surprising in the light of their similar FA compositions; however, modulation of slightly less cytotoxicity with p-EPO than p-SSO supplementation at high concentrations more than likely related to the presence of about 9% GLA in p-EPO only, which may have limited the cytotoxicity of LA. While the data suggested that specific p-oil FA's were largely responsible for certain cell viability changes, it was also possible that synergistic and antagonistic interactions between p-oil FA's enhanced or diminished particular effects of individual FA's, and contributed to the overall changes reported.

Ruck et al (1986) incubated resting (unstimulated) human monocytes derived from normal periferal blood with single C18 FA's under similar experimental conditions to the present study. No growth enhancement was induced with any FA over the range dosed (0-100mg/l), and this

correlated with the findings reported in Figs. 5.2.1.2-5.2.1.8. Stearic acid dosage did not influence lymphocyte viability, whereas concentration dependant cytotoxicity was induced with unsaturated C18 FA supplementation. GLA and LA induced cytotoxicity at much lower concentrations than OA and ALA (ID_{50} = 4.4, 5.0, 12.0 and 13.0mg/l, respectively), and a similar pattern of effects was apparent only with supplementation of p-OO (rich in OA), p-LO (rich in ALA), p-EPO or p-SSO (rich in LA) at low concentrations. Furthermore, higher p-oil concentrations were required to induce the same degree of cytotoxicity in relation to that shown by Ruck et al (1986) when individual FA's were dosed. This probably related to the different human cell types used and/or the mediation of synergistic/antagonistic effects between p-oil FA's. The studies of Weyman et al (1977) indeed indicate that individual FA's mediate different effects to FA combinations. Addition of albumin-bound saturated or unsaturated FA's to human lymphocyte cultures inhibited phytohaemaglutanin-stimulated uptake of radioactive thymidine or uridine into DNA or RNA. However, when a saturated and an unsaturated FA were present together in the medium, the inhibition was much less pronounced, or even abolished. Furthermore, when 2 or more different FA's of the same degree of unsaturation were dosed, the inhibition was the same, or greater, than for single FA's (Weyman et al 1977). Their data therefore supported

the lower degree of cytotoxicity induced with p-oils in our study (Figs. 5.2.1.2-5.2.1.8.) compared with the more pronounced effects of individual FA's in the study of Ruck et al (1986).

Weyman et al (1975, 1977) demonstrated that both stimulated and unstimulated human lymphocytes were able to take up exogenous albumin-bound saturated and unsaturated FA's in vitro to varying extents and incorporate them into membrane PGL's. The present study similarly showed evidence of p-oil FA incorporation into cultured lymphocytes (Table 5.2.3.1.). A mechanism by which p-oil dosage affected lymphocyte viability may therefore relate to differential uptake of p-oil FA's, and alterations in membrane fluidity. Supplementation of p-oils abundant in unsaturated FA's probably increased membrane fluidisation, while membrane rigidification was likely with dosage of saturated FA-rich p-oils. Marked alterations in membrane fluidity would impair normal cell functions, and thus account for the cell death induced particularly with high p-oil concentrations in the range dosed.

p-Oil supplementation to the culture medium stimulated human lymphocyte protein synthesis in a concentration dependent manner with increasing amount of p-oil dosed (Table 5.2.2.1.), despite the inverse correlation with cell viability (Figs. 5.2.1.2-5.2.1.8.). FA's were thus potent agents for the enhancement of lymphocyte protein

synthesis, and the extent to which such was stimulated was relatable to p-oil FA composition (Table 2.3.3.2.). Measurement of more protein with dosage of p-CO or p-MO, compared to other p-oils, however, suggested that saturated FA's were more powerful agents than unsaturated FA's in stimulating human lymphocyte protein synthesis. These findings implied that FA's of different structures exhibit varying abilities to modulate protein synthesis. The med' cion of synergistic/antagonistic effects between FA's, however, probably also contributed to the protein changes the p-oils induced. Stimulation of lymphocyte protein synthesis with p-oil dosage could well reflect increased enzyme synthesis for metabolising incorporated p-oil FA's. It may also reflect increased synthesis of membrane proteins to maintain the integrity thereof damaged directly by dosed p-oil FA's. Reports that lipoperoxides damage membrane proteins (Chio et al 1969, Tappel 1975, 1980 and Frankel 1984) would indeed support their replacement to maintain normal cell functions. Stimulation of protein synthesis may also reflect promotion of lymphocyte activation, a process which has been shown to require FA's (Cuthbert et al 1986). Increased immunoglobin production was unlikely, however, since exogenous FA's induce immunosuppressive responses in lymphocytes <u>in vitro</u> (Weyman et al 1975, 1977).

The possibility that increased protein synthesis related

to enhanced expression of desaturase cascade enzymes was excluded as human lymphocytes exhibited no significant capability for FA desaturation or elongation (Table 5.2.3.1.). No evidence to indicate significant 16:0 desaturation or elongation was found, and despite marked 16:1ω9 incorporation with p-FO dosage, 18:1ω9 levels were not consistent with 16:1ω9 elongation. The levels of 18:0 were decreased and 18:1ω9 increased with p-oil supplementation, yet Δ9D capability seemed unlikely as all p-oils contained sufficient 18:1ω9 (Table 2.3.3.2.) to explain the data. Poor Δ6D, Δ5D, Δ4D and elongase capability was also shown in p-oil dosed cells. This supported the work of Cunanne et al (1984) who showed little further metabolism of [^{14}C]-18:2ω6 to longer chain more unsaturated FA's in such cells, despite pronounced and rapid uptake of this radioisotope into lipid pools. They postulated that 18:2ω6 utilisation by the desaturase cascade was only a minor route of its metabolism, while the present study suggested that such was probably true for all FA's. There is also evidence that the eicosanoid pathway is of minor significance in leukocytes and particularly lymphocytes (Dy et al 1980, Kennedy et al 1980, Bankhurst et al 1981, Cunnane et al 1984, Ferreri et al 1986, Poubelle et al 1987 and Yi et al 1988), but this appears unrelated to the poor desaturation capability of this cell (Goldyne et al 1982, 1984, 1989).

Our data suggested that desaturase and elongase enzyme activity was suppressed rather than absent since the low level of 18:2ω6 and concommitant formation of 22:4ω6 only with dosage of 20mg/l p-EPO was consistent with Δ6D, Δ5D and elongase capability. Alternatively, 18:2ω6 was elongated and converted to 20:3ω6 via an active Δ8D, prior to further desaturation. It was unlikely, however, that enzyme suppression related to cytotoxicity induced with p-oil dosage since no significant evidence for desaturase or elongase capability was found even when p-oils had little effect on lymphocyte viability. Zevallos et al (1989) showed that alteration of the physical state of lymphocyte membranes suppressed desaturase enzyme activity, thus it was possible that a similar effect in microsomal membranes was responsible for suppression of desaturase and elongase activity with p-oil dosage.

Lymphocyte lipoperoxide formation with p-oil dosage (Table 5.2.4.1.) indicated intact cellular enzymic and/or non-enzymic mechanisms for the production of such compounds. The lack of lipoperoxide formation in control lymphocytes (Table 5.2.4.1.), despite an unsaturated FA complement of 75% (Table 5.2.3.1.), however, reflected sufficient cellular vitamin E to prevent FFA oxidation. Alternatively, the cells possess enzymes such as superoxide dismutase, catalase and peroxidase which remove different species of activated oxygen that promote lipo-

peroxidation. Saturation of these protective mechanisms against oxidation probably occurred with p-oil dosage, and the lipoperoxides measured thus reflected the amount and FA composition of the p-oils dosed (Table 2.3.3.2.). The finding that p-FO induced the greatest lipoperoxide amounts when dosed, both in the cells and spent medium, probably related both to the availability of EPA and the susceptibility of this moiety to oxidation. However, formation of significantly more lipoperoxides with p-FO than p-LO dosage, despite more ALA in p-LO than EPA in p-FO (about 63% vs 18%, respectively), was consistent with fewer double bonds in ALA. The similar cellular lipoperoxide amounts induced overall with p-EPO or p-SSO dosage reflected the similar FA compositions of these p-oils, and probably related to oxidation of the large amount of LA present in each (about 70%). Oxidation of GLA in p-EPO only, however, could account for the greater lipoperoxide amounts found with supplementation of 40 or 60mg/l p-EPO than p-SSO. However, such may also relate to alterations in PGL cycling and FA release from PGL's, which would have influenced the availability of FA's for oxidation. This mechanism could also account for the greater lipoperoxide amounts measured with p-CO than p-MO or p-OO dosage, despite the presence of more unsaturated FA's in the latter p-oils. On the other hand, a very slow rate of PGL turnover could explain the absence of lipoperoxides in control lymphocytes, despite the presence of large amounts of cellular PUFA's.

Human lymphocytes were shown to incorporate p-oil FA's (Table 5.2.3.1.), thus the lipoperoxides measured in the spent medium (Table 5.2.4.1.) may well have originated intracellularly, and were transferred to the medium upon cell lysis or if plasma membrane permeability of viable cells was increased by the formation of cellular lipid peroxides. However, it seemed unlikely that cellular FFA's released in this process would be susceptible to extracellular oxidation, even in the presence of released cellular peroxidising enzymes, since incubation media contained albumin as p-oil FA carrier.

Lipoperoxides have been associated with protein and membrane damage (eg. Chio et al 1969, Mead 1976, Tappel 1975, 1980 and Frankel 1984), thus it was possible that such compounds were involved in the modulation of p-oil-induced cytotoxicity. This could well explain the inverse correlation found between lymphocyte viability (Figs. 5.2.1.2-5.2.1.8.) and lipoperoxide concentrations (Table 5.2.4.1.) as greater amounts of p-oil were dosed. The finding that the induction both of cytotoxicity and MDA formation were greatest with p-FO, and least with p-MO, dosage, indeed supported lipoperoxide involvement in the modulation of cell viability. The fact that 60mg/l p-SSO and p-OO also induced marked cytotoxicity, but only small MDA amounts, however, implied that lipid peroxides were not solely involved in this process. In the light of the parameters investigated in the present

study, it was proposed that the modulation of human lymphocyte viability related to the involvement of lipoperoxides as well as alterations in membrane fluidity.

5.3 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM HUMAN SKELETAL MUSCLE.

5.3.1 Effects of pseudo-Oils on Cell Viability.

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

The effects of albumin, with final medium concentrations ranging from 0 to 250mg/l, were investigated and shown to have no significant effect on cell viability (Fig. 5.3.1.1.). Hence, the cell viability changes induced with the dosed p-oils were a result of the exogenous FA's and not the albumin used as FA carrier.

The control cell number, seeded at $10x10^4$/ml, at the end of the 24 hour post-trypsinisation recovery period, was $10.1x10^4$/ml. This equated to 99% 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 99% were thus considered cytotoxic.

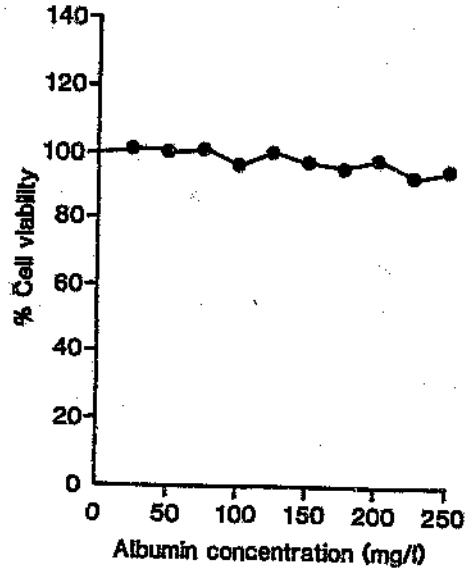
The results of the incubation of cells with p-oils are depicted in Figs. 5.3.1.2-5.3.1.8. Low concentrations

Legend to Figs. 5.3.1.1-5.3.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where 'n' is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 5.3.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 5.3.1.2-5.3.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 5.3.1.1.

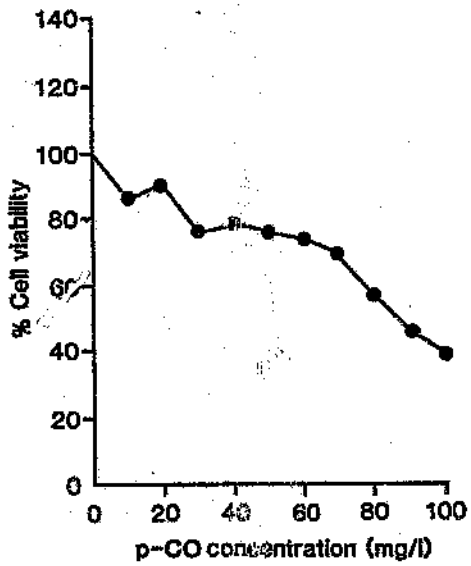
The percentage viability of cells derived from human skeletal muscle incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.2	12
25	101.4	3.4	12
50	100.5	2.6	12
75	101.3	2.5	12
100	96.5	3.7	12
125	100.8	4.8	12
150	98.1	3.7	12
175	96.1	4.2	12
200	98.9	2.9	12
225	93.4	4.1	12
250	96.1	4.7	12

Fig. 5.3.1.2.

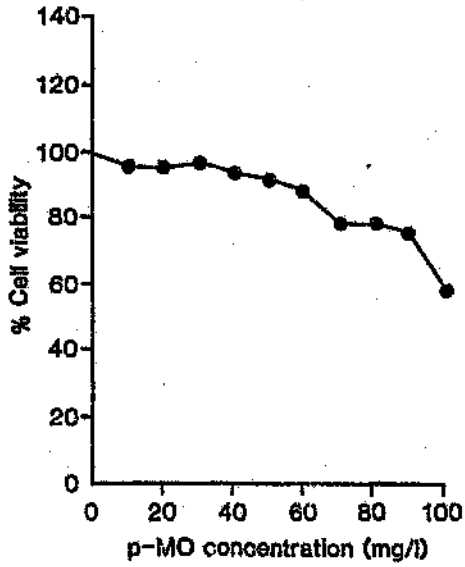
The percentage viability of cells derived from human skeletal muscle incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.4	6
10	86.1	4.5	6
20	90.6	4.6	6
30	75.7	3.9	6
40	78.3	5.8	6
50	75.7	3.9	6
60	74.0	4.6	6
70	68.1	3.0	6
80	56.3	2.3	6
90	45.1	2.5	6
100	37.5	3.6	6

Fig. 5.3.1.3.

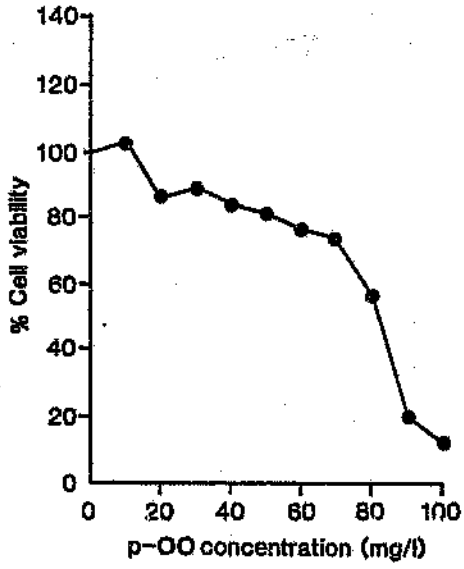
The percentage viability of cells derived from human skeletal muscle incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.4	6
10	94.8	4.4	6
20	94.9	4.5	6
30	96.8	4.0	6
40	93.6	3.5	6
50	91.1	4.5	6
60	87.3	3.8	6
70	77.3	2.9	6
80	79.6	3.6	6
90	75.8	3.9	6
100	58.1	4.4	6

Fig. 5.3.1.4.

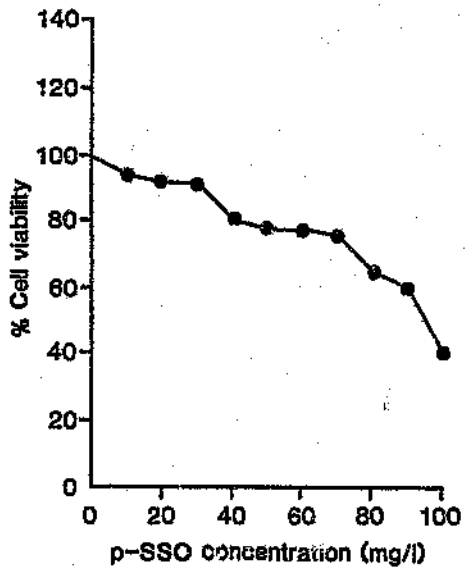
The percentage viability of cells derived from human skeletal muscle incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	102.0	4.8	6
20	85.9	5.4	6
30	88.6	4.3	6
40	82.6	4.5	6
50	80.5	3.9	6
60	75.2	4.6	6
70	73.2	2.8	6
80	56.4	6.0	6
90	20.1	3.9	6
100	11.4	3.2	6

Fig. 5.3.1.5.

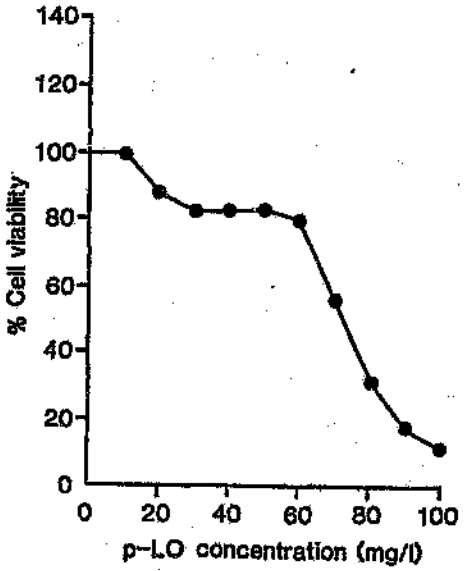
The percentage viability of cells derived from human skeletal muscle incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.8	6
10	93.3	3.1	6
20	91.5	3.9	6
30	91.5	4.1	6
40	80.6	2.6	6
50	77.0	2.9	6
60	77.6	3.2	6
70	75.2	4.5	6
80	64.2	2.6	6
90	60.0	4.1	6
100	40.6	3.2	6

Fig. 5.3.1.6.

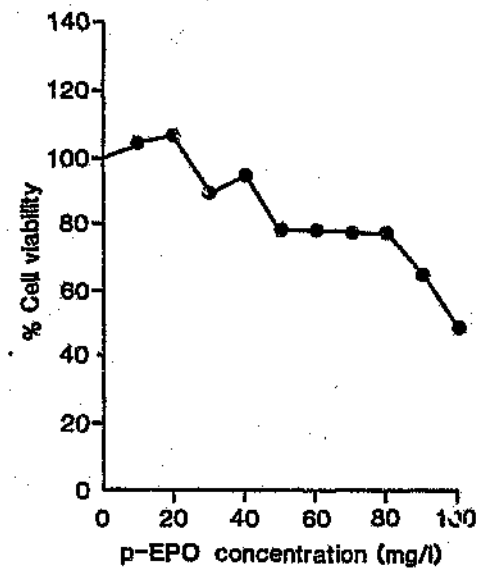
The percentage viability of cells derived from human skeletal muscle incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.6	6
10	100.2	3.7	6
20	88.0	3.5	6
30	79.8	3.8	6
40	83.3	2.9	6
50	83.3	2.1	6
60	79.3	2.3	6
70	56.5	4.9	6
80	30.9	5.1	6
90	16.9	3.1	6
100	10.5	3.1	6

Fig. 5.3.1.7.

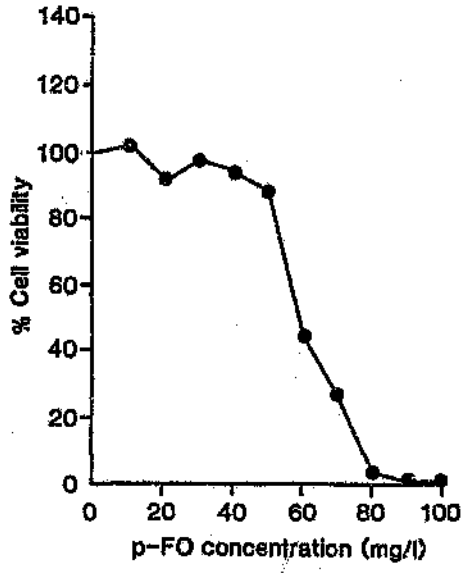
The percentage viability of cells derived from human skeletal muscle incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.1	6
10	104.1	4.0	6
20	107.2	3.5	6
30	89.6	3.9	6
40	95.1	2.9	6
50	78.8	3.4	6
60	77.9	3.1	6
70	77.8	2.3	6
80	77.0	4.4	6
90	64.9	4.4	6
100	48.7	2.9	6

Fig. 5.3.1.8.

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



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.4	6
10	101.8	2.6	6
20	90.5	4.2	6
30	97.1	3.2	6
40	93.2	3.2	6
50	86.6	2.5	6
60	43.6	4.4	6
70	27.1	4.2	6
80	3.3	1.7	6
90	0.7	0.7	6
100	0.0	0.0	6

of p-EPO enhanced cell proliferation, but all p-oils were growth inhibitory, the extent of which varied with the p-oil and concentration dosed. p-MO modulated the least, and p-FO the most, cytotoxicity, while the pattern of growth inhibition induced with p-OO and p-LO were generally alike, as were those of p-CO, p-SSO and p-EPO.

Concentration dependant cytotoxicity was induced with increments in the amount of p-CO dosed (Fig. 5.3.1.2.). The number of viabile cells decreased to 75.7% with 30mg/l p-CO, and further to 68.1% with 70mg/l p-CO. Cell viability was reduced to 50% of controls with 85mg/l p-CO (ID_{50}), and the yield of viable cells was 37.5% with 100mg/l p-CO.

Supplementation with up to 30mg/l p-MO had little effect on cell viability, while higher concentrations induced progressively more cytotoxicity, to yield 75.8% viable cells with 90mg/l p-MO and 58.1% with 100mg/l p-MO (Fig. 5.3.1.3).

10mg/l p-OO had little effect on cell viability, but such was decreased to 85.9% with 20mg/l p-OO and 73.2% with 70mg/l p-OO (Fig. 5.3.1.4.). Higher concentrations induced marked cytotoxicity, reducing the number of viable cells to 50% of controls with 82mg/l p-OO (ID_{50}), 20.1% with 90mg/l p-OO, and 11.4% with 100mg/l p-OO.

Cell proliferation was inhibited in a concentration

dependant manner with increasing amounts of p-SSO dosed (Fig. 5.3.1.5.). More than 90% of cells were viable with dosage of up to 30mg/l p-SSO, but the yield was reduced to 80.6% with 40mg/l p-SSO and 75.2% with 70mg/l p-SSO. Further concentration increases reduced cell viability to 50% of controls with 95mg/l p-SSO (ID_{50}) compared to 40.6% with 100mg/l p-SSO.

Little change in cell proliferation was shown with 10mg/l p-LO dosage, but this was progressively reduced to 79.8% with 30mg/l p-LO (Fig. 5.3.1.6.). p-LO induced no further cytotoxicity up to a concentration of 60mg/l, as cell viability ranged from 79.3% to 83.3%. Higher concentrations, however, induced marked cytotoxicity, reducing the yield of viable cells almost linearly to 50% of controls with 73mg/l p-LO (ID_{50}) and to 10.5% with 100mg/l p-LO.

Dosage with 10mg/l p-EPO had little effect, but 20mg/l p-EPO enhanced cell viability to 107.2% (Fig. 5.3.1.7.). 40mg/l p-EPO induced cytostasis, while cell viability ranged from 78.8% to 77.0% with 50 to 80mg/l p-EPO. Cytotoxicity was increased, however, with 90mg/l p-EPO, and approximately 50% of cells were stained with Trypan blue with 100mg/l p-EPO.

Cell growth approximated to that of controls with dosage of up to 40mg/l p-FO, while higher concentrations were cytotoxic (Fig. 5.3.1.8.). Cell viability was reduced to

50% of controls with 59mg/l p-FO (ID_{50}), and further to 3.3% with 80mg/l p-FO, while Trypan blue uptake was 100% with 100mg/l p-FO.

Cell 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. Cell viabilities were compared upon harvesting to those in Figs. 5.3.1.1-5.3.1.8., and were found not to be statistically different. All further biochemical assays were therefore performed on these samples.

5.3.2 Effects of pseudo-Oils on Total Protein.

The total protein concentrations determined in cells dosed with 20, 40 or 60mg/l p-oil for 48 hours are shown in Table 5.3.2.1. compared to controls, and expressed as µg total protein/10^6 cells seeded.

Cells dosed with 60mg/l p-FO contained 424.2µg total protein/10^6 cells seeded compared to 593.1µg protein/10^6 controls cells seeded. All other dosed cells, however, contained significantly more protein than controls, and ranged from 697.3 to 974.4µg/10^6 cells seeded (obtained with 60mg/l p-LO and 20mg/l p-EPO dosage). A slightly greater increment in cellular protein was induced with 40 than 20mg/l p-FO dosage (874.2 vs 854.1µg/10^6 cells seeded, respectively). In contrast, cellular protein levels changed inversely with increments in the amount of p-CO (828.3 to 773.8µg), p-MO (872.2 to 765.2µg), p-OO (743.9 to 703.5µg), p-SSO (828.2 to 764.8µg), p-LO (800.6 to 697.3µg) or p-EPO (974.4 to 713.6µg) dosed.

Table 5.3.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	593.1			
CO		828.3	796.7	773.8
MO		872.2	839.7	765.2
OO		743.9	716.1	703.5
SSO		828.2	791.8	764.8
LO		800.6	751.5	697.3
EPO		974.4	859.0	713.6
FO		854.1	874.2	424.2

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

Table 5.3.3.1. shows the FA spectra of cells incubated with 20, 40 or 60mg/l of each of the p-oils compared to controls.

18:1ω9 contributed 24.7% to the total FA spectrum in control cells, compared to 21.3% 16:0, 12.8% 18:0, 6.8% 16:1ω9, 5.5% 20:4ω6, 14.5% 22:4ω6 and smaller proportions (≤5%) of other FA's.

In general, cells dosed with p-oils contained similar amounts of 16:0, 18:0 and 18:1ω9, but less 16:1ω9, than controls. More 18:2ω6 was generally found in dosed than control cells, whereas ω6 PDFA and ω3 PUFA proportions either approximated to controls, or were decreased.

Cells incubated with 20, 40 or 60mg/l p-CO exhibited consistently higher 16:0 levels (24.1%, 26.1% and 30.5%, respectively) than controls (21.3%), but only half as much 16:1ω9 (3.2%, 3.6% and 3.5%, respectively vs 6.8% in controls). 18:0, 18:1ω9, 18:2ω6 and 18:3ω3 levels were largely unchanged, while ω6 and ω3 PDFA percentages approximated to controls, or were decreased; 20:4ω3 was slightly increased, however, with 40mg/l p-CO dosage (2.2% vs 1.6% in controls).

Cells incubated with 20, 40 or 60mg/l p-MO exhibited

Legend to Table 5.3.3.1.

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 5.3.3.1.

The fatty acid spectrum of cells derived from human 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	21.3±0.20	24.1	26.1	30.5	22.7	23.0	24.2	18.4	17.3	17.2	19.9	17.8	20.9	20.4	20.3	24.8	20.2	19.4	22.7	20.-	20.3	20.4
	18:0	12.8±0.20	14.4	11.3	10.4	12.5	12.6	12.2	9.6	7.1	6.0	12.7	9.6	10.2	12.7	11.0	13.8	12.1	10.2	12.8	10.6	10.7	6.1
	20:0	0.1±0.00	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	0.3	-
	22:0	-	-	-	0.1	-	-	-	-	0.2	-	-	-	-	-	-	0.1	-	-	0.2	-	-	-
	24:0	0.4±0.00	-	0.3	-	-	-	-	0.2	0.3	-	0.4	0.1	0.2	-	0.3	0.3	-	0.2	-	-	-	-
ω9 MONOS.	16:1	6.8±0.07	3.2	3.6	3.5	2.4	1.9	2.0	3.4	3.7	4.1	3.5	3.7	4.1	3.9	4.9	2.6	3.5	3.8	2.6	10.9	13.4	20.5
	18:1	24.7±0.21	25.6	25.2	28.9	26.8	26.5	29.3	42.9	45.3	47.8	26.1	24.2	23.9	25.8	22.5	23.8	23.4	19.7	21.7	25.3	23.1	23.1
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	3.7±0.05	3.2	3.2	3.6	5.2	6.6	9.5	6.5	7.9	8.9	17.0	23.3	26.0	8.4	8.3	6.0	20.7	23.4	17.5	5.1	6.6	8.7
	18:3	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	0.2	0.3	0.2	-	-	-
	20:2	0.2±0.00	0.1	-	0.1	0.1	0.1	0.2	0.6	1.1	1.3	0.5	1.1	0.9	0.2	0.2	0.1	0.7	0.7	0.3	0.2	0.7	0.5
	20:3	0.9±0.00	0.9	1.0	1.0	0.9	0.8	1.0	0.7	0.9	0.6	0.9	1.1	0.8	0.9	1.2	0.8	2.2	2.7	1.7	0.8	0.8	0.6
	20:4	5.5±0.13	6.0	5.3	4.8	7.3	6.1	6.0	3.9	3.2	2.4	4.7	3.6	3.0	6.2	5.3	3.4	5.0	4.1	3.4	4.4	3.6	2.5
	22:4	14.5±0.55	14.8	14.6	10.7	15.6	14.1	10.4	8.8	7.5	7.5	9.5	10.0	6.2	7.6	7.9	13.2	7.1	9.8	13.3	13.1	7.9	7.2
	22:5	1.1±0.05	0.3	0.8	0.1	-	0.6	0.1	0.2	0.3	0.1	0.2	0.5	0.2	0.2	1.8	0.3	0.2	0.7	0.2	0.1	1.0	0.1
ω3 POLYS.	18:3	0.5±0.00	0.4	0.4	0.5	0.5	0.3	0.4	0.7	0.8	0.8	0.5	0.4	0.4	8.7	11.1	7.1	0.4	0.4	0.3	0.5	0.5	0.6
	18:4	0.8±0.05	0.6	0.4	0.2	0.3	0.7	0.2	0.3	0.4	0.1	0.2	0.4	0.2	0.3	0.4	0.4	0.3	0.6	0.3	0.3	1.5	0.2
	20:4	1.6±0.05	1.6	2.2	0.7	0.7	2.2	0.1	-	0.7	0.3	-	0.7	0.2	0.2	0.9	0.9	0.1	0.9	0.3	0.9	2.5	0.8
	20:5	1.0±0.00	0.8	1.2	1.0	0.9	1.1	1.0	0.8	0.8	0.9	1.0	0.6	0.8	1.0	0.9	0.3	0.6	0.6	0.6	2.6	2.8	5.5
	22:5	2.1±0.00	2.2	2.1	2.0	2.3	1.8	1.8	1.6	1.4	1.1	1.6	1.4	1.3	1.9	1.6	1.3	1.9	1.4	1.1	3.3	3.4	2.7
	22:6	2.0±0.00	2.0	2.2	2.0	1.9	1.6	1.7	1.4	1.2	1.0	1.4	1.3	0.9	1.6	1.3	0.9	1.5	1.1	0.8	1.3	1.0	0.7

16:0 and 18:0 percentages which mirrored controls (21.3% and 12.8%), decreased 16:1ω9 (2.4%, 1.9% and 2.0%, respectively vs 6.8% in controls), and sightly increased 18:1ω9 (26.8%, 26.5% and 29.3%, respectively vs 24.7% in controls) levels. Significant increases were induced in 18:2ω6 with 20, 40 and 60mg/l p-MO (5.2%, 6.6% and 9.5%, respectively vs 3.7% in controls) and 20:4ω3 with 20mg/l p-MO (2.2% vs 1.6% in controls), unlike 20:4ω6 (7.3%, 6.1% and 6.0%, respectively vs 5.5% in controls); all other PDFA levels levels approximated to controls, or were decreased.

18:1ω9 percentages increased almost 2 fold with 20, 40 or 60mg/l p-OO supplementation (42.9%, 45.3% and 47.8%, respectively vs 24.7% in controls), but 16:0, 18:0 and 16:1ω9 levels decreased in relation to controls (21.3%, 12.8% and 6.8%, respectively). Concentration dependant increases were detected for 18:2ω6 and 20:2ω6 (6.5%, 7.9% and 8.9%, and 0.6%, 1.1% and 1.3% vs 3.7% and 0.2% in controls, respectively), but all other PUFA amounts were either parallel to controls, or significantly decreased.

p-SSO dosage had little overall effect on cellular 16:0, 18:0 and 18:1ω9 percentages, but reduced the amount of 16:1ω9 (3.5% to 4.1% vs 6.8% in controls). A marked increase in 18:2ω6 was detected (17.0%, 23.3% and 26.0% with 20, 40 and 60mg/l p-SSO, respectively vs 3.7% in

controls), with a small increment induced in 20:2ω6 over the same range (0.5%, 1.1% and 0.9%, respectively vs 0.2% in controls), yet all other ω6 and ω3 PUFA percentages mirrored controls, or were decreased.

16:0, 18:0 and 18:1ω9 proportions were not significantly changed in relation to controls when 20, 40 or 60mg/l p-LO was dosed, but 16:1ω9 was decreased (3.9%, 4.9% and 2.6%, respectively vs 6.8% in controls). Increments 18:2ω6 and 18:3ω3 were induced over the same range dosed (8.4%, 8.3% and 6.0%, and 8.7%, 11.1% and 7.1% vs 3.7% and 0.5% in controls, respectively), yet PDFA amounts were not significantly increased.

p-EPO supplementation had little overall effect on cellular 16:0, 18:0 and 18:1ω0 levels; however, 16:1ω9 was significantly decreased (2.6% to 3.5% vs 6.8% in controls). Marked 18:2ω6 changes were induced (20.7%, 23.4% and 17.5% with 20, 40 and 60mg/l, respectively vs 3.7% in controls). Further, 20:3ω6 was a significantly increased over the same range (2.2%, 2.7% and 1.7%, respectively vs 0.9% in controls), but the levels of other ω6, as well as all ω3, PDFA's detected were decreased.

No significant changes were induced in cellular 16:0 and 18:1ω9 levels with addition of 20, 40 or 60mg/l p-FO to the incubation medium, while 18:0 amounts were decreased (10.6%, 10.7% and 6.1%, respectively vs 12.8% in undosed

cells) and 16:1ω9 significantly increased (10.9%, 13.4% and 20.5%, respectively vs 6.8% in controls). Despite increments in 18:2ω6 (5.1% to 8.7% vs 3.7% in controls), no significant increases in ω6 PDFA's were induced. 18:4ω3 and 20:4ω3 amounts were slightly increased with 40mg/l p-FO dosage (1.5% and 2.5% vs 0.8% and 1.6% in controls, respectively). 22:6ω3 levels, however, were decreased with 20, 40 or 60mg/l p-FO incubation (1.3%, 1.0% and 0.7%, respectively vs 2.0% in controls, despite increments in 20:5ω3 (2.6%, 2.8% and 5.5%, respectively vs 1.0% in controls) and 22:5ω3 (3.3%, 3.4% and 2.7%, respectively vs 2.1% in controls).

5.3.4 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides quantified in the cells and spent media of cultures dosed with 20, 40 or 60mg/l p-oil are shown in Table 5.3.4.1. in relation to controls. The data is expressed as nmoles MDA/10^6cells, but in the case of the spent incubation media, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained. Lipoperoxide levels varied with the p-oil supplemented, but increased overall as greater p-oil amounts were dosed.

0.2nmoles MDA/10^6 control cells were measured, while amounts in dosed cells were greater in every instance, ranging from 0.3 to 2.5nmoles MDA/10^6 cells. Saturated FA-rich p-oils induced only small lipoperoxide amounts, although levels were slightly higher with dosage of p-CO (0.4 to 0.5nmoles MDA/10^6 cells) than p-MO (0.3nmoles MDA/10^6 cells). However, slightly larger, but consistent lipoperoxide levels were induced with 20, 40 or 60mg/l p-OO, p-SSO, p-LO or p-EPO incubation (0.4 to 1.0nmoles MDA/10^6 cells), while p-FO induced significantly greater amounts only with a concentration of 60mg/l (2.5nmoles MDA/10^6 cells).

Greater lipoperoxide amounts were measured in the spent incubation media of control and dosed cultures than in the cells themselves. Spent incubation medium derived

Table 5.3.4.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	0.2			
CO		0.4	0.5	0.5
MO		0.3	0.3	0.3
OO		0.4	0.9	1.0
SSO		0.4	0.7	1.0
LO		0.9	0.9	1.0
EPO		0.7	0.8	1.0
FO		0.6	0.8	2.5
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	0.5			
CO		2.8	3.6	4.1
MO		1.8	2.0	2.2
OO		1.6	1.7	2.1
SSO		1.7	4.3	6.4
LO		3.7	6.5	11.0
EPO		1.9	4.6	6.6
FO		4.1	4.2	10.2

from control cultures contained 0.5nmoles MDA/10^6 cells, while levels were considerably higher in dosed cultures, ranging from 1.6 to 11.0nmoles MDA/10^6 cells. Spent media derived from cultures incubated with 20, 40 or 60mg/l p-MO or p-OO contained similar lipid peroxide amounts (1.8, 2.0 and 2.2, and 1.6, 1.7 and 2.1nmoles MDA/10^6 cells, respectively), while slightly higher levels were measured in the presence of p-CO (2.8, 3.6 and 4.1nmoles MDA/10^6 cells). Spent media lipoperoxide concentrations, however, were generally lower with p-CO, p-MO or p-OO than p-SSO, p-LO, p-EPO or p-FO incubation. Comparable spent media lipoperoxide amounts were found with dosage of p-SSO (1.7, 4.3 and 6.4nmoles MDA/10^6 cells) or p-EPO (1.9, 4.6 and 6.6nmoles MDA/10^6 cells), but concentrations were greatest in the presence of p-FO (4.1, 4.2 and 10.2nmoles MDA/10^6 cells) and p-LO (3.7, 6.5 and 11.0nmoles MDA/10^6 cells).

5.3.5 Discussion.

No reference has been found in the literature relating to the effects of FA's on the growth of human skeletal muscle. The present study showed that human skeletal muscle cells were largely susceptible to growth inhibition when supplemented with p-oils in culture (Figs. 5.3.1.1-5.3.1.8.), and this appeared to relate partly to the long population doubling times of these cells (Table 2.3.1.1.). The cell viability changes induced could nevertheless be related to the unique FA compositions of the p-oils dosed (Table 2.3.3.2.). The similar degree of cytotoxicity induced with p-EPO or p-SSO supplementation was consistent with the similar FA compositions of these p-oils, and was probably relatable to the large amount of LA present therein (about 70%). The presence of 9% GLA in p-EPO only, however, may explain the growth enhancement this p-oil induced with dosage at low concentrations. This PDFA may have stimulated cell proliferation directly, or limited the cytotoxic capability of LA, or other FA's, in p-EPO. The similar proportions of LA in p-EPO and p-SSO, OA in p-OO and ALA in p-LO, and the more pronounced cytotoxicity induced with p-LO or p-OO, than p-EPO or p-SSO, dosage implied that ALA and OA exhibited greater cytotoxic potential than LA, while the similar degree of cytotoxicity p-LO and p-OO induced suggested that ALA was as effective as OA in mediating growth inhibition. On the

other hand, the finding that p-FO exhibited the greatest capability to induce cytotoxicity with dosage at high concentrations could relate to the significant amounts of 20:5ω3 and 16:1ω9 (about 18% and 31%, respectively) present in this p-oil only. The different cell viability changes which p-oil FA's appeared to induce probably related to variations in their structure, although it was also likely that the effects induced by single FA's were diminished or enhanced by antagonistic and synergistic interations between p-oil FA's. Evidence that these cells incorporated exogenous FA's with p-oil dosage (Table 5.3.3.1.) nevertheless suggested that the mediation of cytotoxicity related to alterations in membrane fluidity. Incubation with unsaturated FA-rich p-oils could have increased the membrane unsaturation index to such an extent that cell viability could not continue, whereas membrane rigidification probably accounted for growth inhibition p-CO induced. On the other hand, the amount and structure of unsaturated FA's present in p-MO (about 11% OA, 8% LA and 2% AA) may have conteracted any membrane rigidification the saturated FA's incorporated from this p-oil induced, and explain why p-MO was least growth inhibitory p-oil.

The large total protein amounts measured in cultured human skeletal muscle cells reflected the high metabolic activity of this tissue <u>in vivo</u>, which accounts for over 50% of total metabolism (Lehninger 1982). Dosage of

such cells with p-oils resulted in cellular protein changes (Table 4.3.2.1.) which generally reflected the cell viability changes induced (Figs. 5.3.1.2-5.3.1.8.). However, while p-oil FA's were generally not effective in enhancing cell proliferation, it was clear that these moieties were very effective in stimulating protein synthesis, even when p-oils induced 20% to 30% growth inhibition. The finding that the extent of protein stimulation varied with dosage of different p-oils at identical concentrations, even when such yielded similar cell numbers, suggested that the capability to enhance cellular protein synthesis varied with FA, and was probably relatable to FA structure. It was also likely that synergistic/antagonistic interactions between p-oil FA's played a role in this process. Enhancement of cellular protein synthesis may, nevertheless, reflect increased enzyme production to metabolise exogenous FA's incorporated with p-oil dosage.

Stimulation of protein synthesis did not appear to relate to increased expression of desaturase cascade enzymes since the overall capability of these cells to desaturate or elongate FA's was very limited (Table 5.3.3.1.). Indeed, studies have shown that skeletal muscle is less capable of synthesising FA's than tissues such as mammary gland, adrenal cortex, liver and adipose (Lehninger 1982). 16:0 and 16:1ω9 taken up with p-oil dosage were not significantly desaturated or elongated,

while 18:0 and 18:1ω9 levels parallel to controls supported impaired Δ9D capability. 18:3ω3 and 18:2ω6 incorporated with p-LO and p-SSO dosage, respectively were not further metabolised to PDFA's, and such was consistent with impaired Δ6D capability. Thus 20:3ω6 formation with p-EPO supplementation probably related to elongation of incorporated 18:3ω6. Lack of further 20:3ω6 metabolism, however, was consistent with impaired Δ5D capability. On the other hand, formation of small 22:4ω6 amounts with 20mg/l p-MO, and significant 22:5ω3 amounts with p-FO, dosage supported the capability for 20:4ω6 and 20:5ω3 elongation, respectively. The fact that no significant increase in 22:5ω6 or 22:6ω3 levels were detected, however, implied impaired Δ4D capability. It was clear from the above that elongation was more frequent than desaturation, and this confirmed the rate limiting nature of the desaturases (Marcel et al 1968, Brenner 1971, Bernert et al 1975 and Hassam et al 1975).

The formation of small amounts of 18:4ω3 and 20:4ω3 with 40mg/l p-FO, 20:4ω6 with 20mg/l p-LO, and 22:5ω6 with 40mg/l p-LO, for example, suggested the potential for limited desaturase expression in these cells. Thus it was possible that the impaired Δ6D, Δ5D and Δ4D capabilities reported above related to enzyme suppression rather than their absence in these cells. It was unlikely that such related to growth inhibition induced with p-oil dosage, however, since desaturase

activity was impaired even when p-oils had little effect on cell viability. Studies have shown that dietary FA's modulate the lipid composition of microsomal membranes (eg. Nervi et al 1968 and Garg et al 1988a, 1988b, 1988c), thus it has been suggested that the microsomal lipid composition is involved in the regulation of desaturase enzyme activity (Kurata et al 1980 and Garda et al 1984, 1985). This phenomenon may therefore explain the overall suppression of desaturase activity reported with p-oil dosage, although such may be a characteristic of this particular cell type <u>in vitro</u> under the culture conditions employed. Even if desaturase capability is poor <u>in vivo</u>, skeletal muscle has a generous vascular supply, and may obtain PDFA's from other tissues.

MDA measurement in control cells (Table 5.3.4.1.) was not surprising as such contained unsaturated FA's which were potential candidates to act as substrates for the formation of cellular lipoperoxides (Table 5.3.3.1.). This could occur enzymatically and/or via auto-oxidation of unsaturated FA's released during the continuous cycling of cellular PGL's when the mechanisms of protection against oxidation were saturated. The FA composition of new incubation medium was primarily composed of saturated FA's (Table 2.3.3.3.), thus su[illegible] was unlikely to have contributed significantly to any medium lipoperoxides reported (Table 5.3.4.1.). Cellular lipoperoxides, however, are able to increase membrane

permeability (eg. Chio et al 1969, Tappel 1975, 1980 and Frankel 1984). Thus it was possible that the greater lipoperoxide amounts measured in spent control medium than in control cells themselves reflected the formation of cellular lipoperoxides, and their subsequent release into the medium through the plasma membrane of viable cells. Evidence to support p-oil FA incorporation into the cells from the culture medium was shown (Table 5.3.3.1.), thus it was unlikely that the greater lipid peroxide amounts measured in the spent medium than in the cells of dosed cultures (Table 5.3.4.1.) were primarily the result of auto-oxidation of unincorporated p-oil FA's, particularly since these moieties were albumin-bound. It rather appeared that the lipoperoxides measured in the spent medium of dosed cultures also originated intracellularly, and were released into the incubation medium as a result or increased plasma membrane permeability or cell lysis. The considerably greater lipoperoxide amounts shown in the spent medium of dosed cultures than in the cells themselves was indeed consistent with the cytostasis and cytotoxicity the p-oils induced (Figs. 5.3.1.2-5.3.1.8.).

The greater lipoperoxide concentrations measured in dosed than in control cultures, both in the spent medium and cells themselves (Table 5.3.4.1.), reflected increased unsaturated FA availability for oxidation. This correlated with the increased lipid peroxide

concentrations found overall with the amount of p-oil dosed, while the quantitative variations in MDA levels reported reflected differences in p-oil FA composition (Table 2.3.3.2.). This related not only to the amount of unsaturated FA's present in any p-oil, but also to their susceptibility to oxidation, dependent on the number and position of double bonds in a FA. The rate of PGL turnover probably also played a role in cellular lipid peroxide formation as such influences the rate of exogenous FA incorporation and FA release from membrane lipids. This would have determined the size of the cellular FFA pool, and thus the availability of FFA's for intracellular oxidation. An increased rate of PGL turnover with p-CO dosage could well account for the greater lipoperoxide amounts this p-oil induced compared to p-MO and p-OO, which contained a greater complement of unsaturated FA's. Alternatively, more unsaturated FFA's may have been components of complex membrane lipids and therefore less susceptible to peroxidation with p-MO or p-OO than p-CO incubation. The greater lipoperoxide amounts measured overall with p-SSO, p-LO, p-EPO or p-FO than with p-CO, p-MO or p-OO, dosage were nevertheless consistent with significantly greater PUFA amounts in the former p-oils. The similar lipoperoxide amounts measured with p-EPO or p-SSO supplementation, both in the cells and spent media, were not surprising as these p-oils exhibited similar FA compositions and

induced similar degrees of cytotoxicity. Such probably related primarily to oxidation of the large amount of LA present in both p-oils (about 70%), while formation of the greatest lipoperoxide amounts overall with p-LO or p-FO dosage could well have related both to the greater availability and susceptibility of ALA and EPA to oxidation, respectively. p-LO contained approximately 63% ALA compared to 18% EPA in p-FO, but the greater susceptiblity of the latter to oxidation probably explains the similar lipoperoxide amounts these p-oils induced overall.

Lipoperoxide formation by cultures supplemented with p-oils generally correlated directly with the amount of p-oil dosed (Table 5.3.4.1.), and inversely with cell viability (Figs. 5.3.1.2-5.3.1.8.). Furthermore, p-FO induced the greatest cellular lipoperoxide amounts and cytotoxicity, whereas such was least with p-MO dosage. These findings supported lipoperoxide involvement in the modulation of cell viability. The lipoperoxide amounts found, however, did not always correlate quantitatively with the degree of cytotoxicity the p-oils induced, and this suggested that these compounds were only partly involved in the modulation of human skeletal muscle growth. The data presented rather suggested that both lipoperoxides and membrane fluidity changes were involved in this process.

5.4 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM HUMAN LUNG.

5.4.1 Effects of pseudo-Oils on Cell Viability.

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

Investigation of the effects of albumin with final medium concentrations ranging from 0 to 250mg/l showed no significant effect on cell viability (Fig. 5.4.1.1.). The effects induced with the 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 $12.8x10^4$/ml. This equated to approximately 61% 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 61% were hence considered cytotoxic.

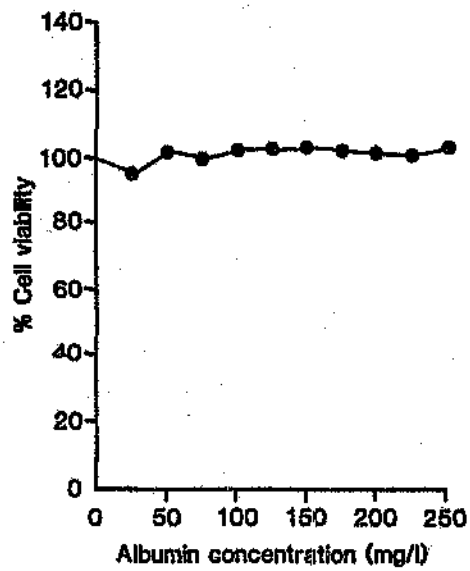
Figs. 5.4.1.2-5.4.1.8. depict the results obtained from

Legend to Figs. 5.4.1.1-5.4.1.8.

The results are expressed as mean percent (%) cell viability±standard error of the mean (s.e.m.), where 'n' is the number of experiments. The concentrations given are as mg albumin or pseudo-oil per litre of growth medium. Fig. 5.4.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 5.4.1.2-5.4.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l).

Fig. 5.4.1.1.

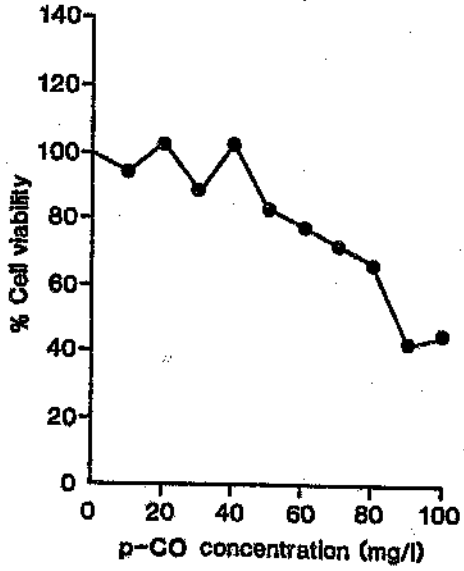
The percentage viability of cells derived from human lung incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.3	12
25	95.4	3.4	12
50	101.8	2.9	12
75	99.3	3.0	12
100	102.8	4.3	12
125	102.8	4.7	12
150	103.0	1.1	12
175	102.2	2.7	12
200	101.8	3.7	12
225	100.7	4.5	12
250	103.6	4.9	12

Fig. 5.4.1.2.

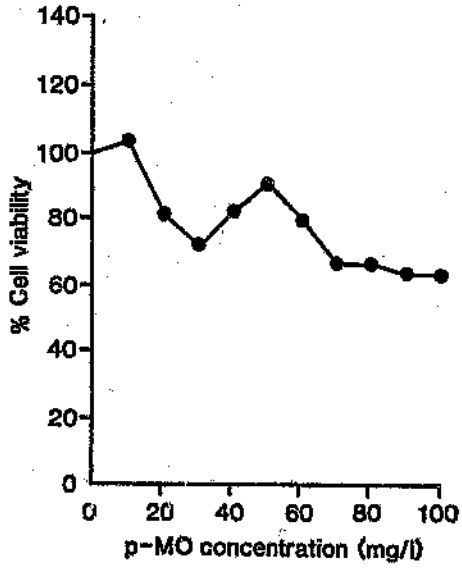
The percentage viability of cells derived from human lung incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.6	6
10	93.9	4.7	6
20	102.5	5.5	6
30	88.3	3.5	6
40	102.7	3.6	6
50	82.7	5.3	6
60	77.2	3.2	6
70	72.0	3.6	6
80	66.7	3.2	6
90	42.3	5.1	6
100	45.0	4.3	6

Fig. 5.4.1.3.

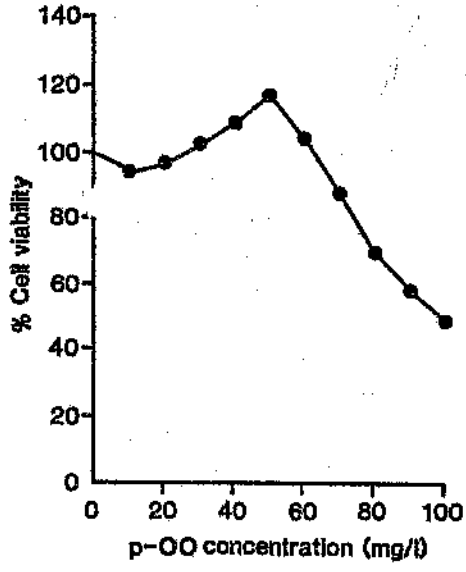
The percentage viability of cells derived from human lung incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.6	6
10	103.3	4.3	6
20	80.9	2.5	6
30	71.7	2.7	6
40	82.3	4.2	6
50	90.2	4.2	6
60	79.3	3.3	6
70	66.6	4.8	6
80	65.9	3.0	6
90	63.1	4.5	6
100	62.3	3.9	6

Fig. 5.4.1.4.

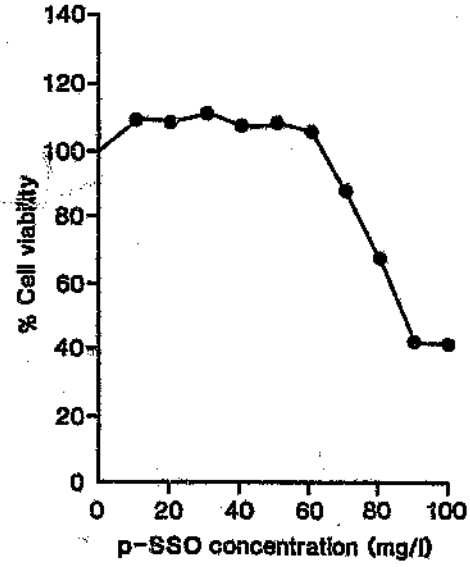
The percentage viability of cells derived from human lung incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.9	6
10	93.5	4.0	6
20	96.1	3.6	6
30	101.8	4.6	6
40	108.2	4.6	6
50	116.2	4.3	6
60	103.0	4.6	6
70	86.5	3.7	6
80	69.2	4.7	6
90	57.3	4.0	6
100	48.2	4.7	6

Fig. 5.4.1.5.

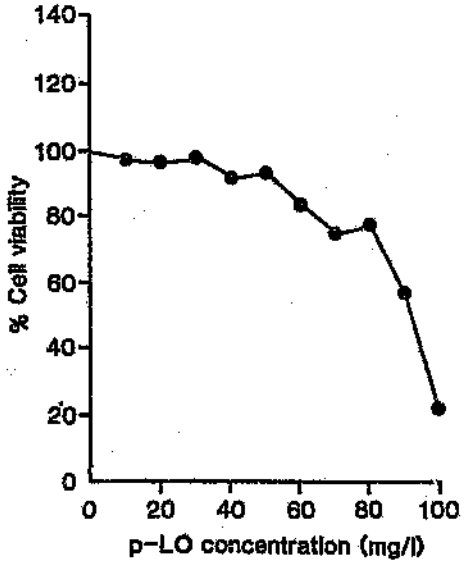
The percentage viability of cells derived from human lung incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.5	6
10	109.0	2.6	6
20	107.9	2.7	6
30	110.9	4.5	6
40	107.4	5.7	6
50	108.4	5.3	6
60	105.3	5.2	6
70	87.4	5.0	6
80	67.3	1.7	6
90	42.5	2.0	6
100	41.7	1.1	6

Fig. 5.4.1.6.

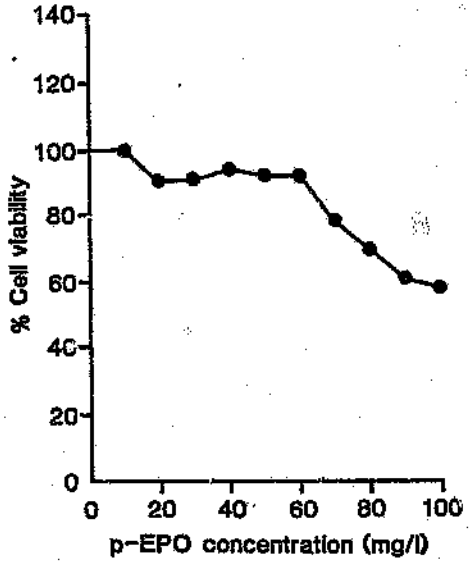
The percentage viability of cells derived from human lung incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.5	6
10	97.0	4.9	6
20	95.7	5.0	6
30	98.0	4.0	6
40	90.9	3.4	6
50	92.9	4.8	6
60	83.4	2.3	6
70	74.6	3.2	6
80	77.5	4.1	6
90	57.0	4.4	6
100	21.7	2.3	6

Fig. 5.4.1.7.

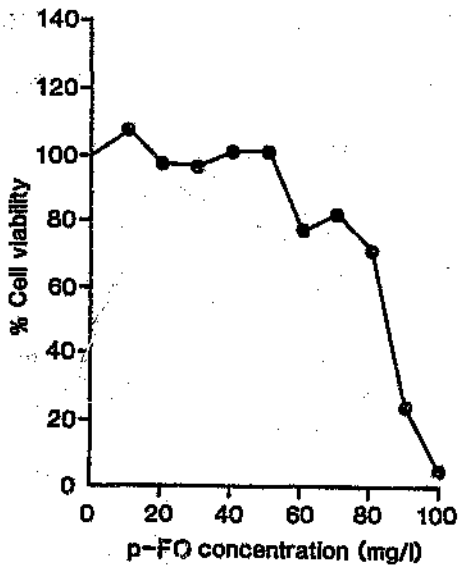
The percentage viability of cells derived from human lung incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.1	6
10	99.7	3.1	6
20	90.1	4.4	6
30	91.3	3.5	6
40	93.9	4.4	6
50	92.2	1.7	6
60	92.3	5.7	6
70	78.8	2.8	6
80	69.7	3.7	6
90	61.0	1.6	6
100	57.5	5.3	6

Fig. 5.4.1.8.

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



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	4.5	6
10	107.5	5.4	6
20	96.8	5.3	6
30	96.3	5.4	6
40	100.5	4.4	6
50	100.3	3.6	6
60	76.4	1.9	6
70	82.4	5.0	6
80	70.6	4.2	6
90	23.3	4.2	6
100	4.3	1.6	6

was enhanced with certain concentrations of p-OO, p-SSO and p-FO, all p-oils were growth limiting, while only p-MO and p-EPO induced no cytotoxicity. The magnitude of the cell viability changes, however, varied with p-oil and concentration incubated.

Dosage with up to 80mg/l p-CO induced growth limitation, although cell viability was parallel to controls with 20 and 40mg/l p-CO (Fig. 5.4.1.2.). Higher concentrations induced cytotoxicity, and approximately 87mg/l p-CO reduced the number of viable cells to 50% of controls (ID_{50}), compared to 42.3% and 45.0% viability with 90 and 100mg/l p-CO, respectively.

10mg/l p-MO had no significant effect on cell viability, but 20 and 30mg/l p-MO were increasingly limiting, yielding 80.9% and 71.7% viable cells, respectively (Fig. 5.4.1.3.). Growth limitation, however, was not increased with 40, 50 and 60mg/l p-MO as cell viability ranged from 79.3% to 82.3%, while this decreased from 66.6% to 62.3% with 70 to 100mg/l p-MO, and approximated to the cytostatic number (61%). No cytotoxicity was induced, thus no ID_{50} was found.

Dosage with up to 30mg/l p-OO induced no marked cell viability changes, while 40 and 50mg/l p-OO enhanced cell proliferation to 108.2% and 116.2%, respectively (Fig. 5.4.1.4.). Higher concentrations, however, reduced

p-OO to 69.2% with 80mg/l p-OO, while this was further reduced by cytotoxicity to 57.3% and 48.2% with 90 and 100mg/l p-OO, respectively. An ID_{50} of 98mg/l p-OO was calculated.

Cell proliferation was enhanced with dosage of up to 60mg/l p-SSO, and ranged from 105.3% to 110.9% (Fig. 5.4.1.5.). Cell growth, however, was linearly reduced to 67.3% with concentration increases to 80mg/l p-SSO, and Trypan blue uptake was 50% with approximately 87mg/l p-SSO (ID_{50}). Cytotoxicity reduced cell viability to 42.5% and 41.7% with 90 and 100mg/l p-SSO, respectively.

No significant cell viability changes were induced with up to 30mg/l p-LO dosage, but higher concentrations were increasingly growth limiting to yield 74.6% and 77.5% viable cells with 70 and 80mg/l p-LO, respectively (Fig. 5.4.1.6.). Cytotoxicity reduced cell viability to 50% of controls with approximately 92mg/l p-LO (ID_{50}), and to 21.7% with 100mg/l p-LO.

No marked cell viability changes occurred with dosage of up to 60mg/l p-EPO, but further concentration increases progressively limited cell growth to 61.0% with 90mg/l p-EPO, which equated to cytostasis (Fig. 5.4.1.7.). No ID_{50} value was found, however, as cell viability was 57.5% with 100mg/l p-EPO.

Cell proliferation increased to 107.5% with 10mg/l p-FO,

cell viability (Fig. 5.4.1.8.). Further concentration increases limited cell growth to 70.6% with 80mg/l p-FO, while cytotoxicity yielded 50.0%, 23.3% and 4.3% viable cells with 84, 90 and 100mg/l p-FO, respectively. This was the most cytotoxic p-oil.

Subsequent to these studies, cells were plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient quantities for all the quantitative and qualitative analyses to be carried out. Upon harvesting, cell viabilities relative to controls were compared, and found not to be statistically different from those in Figs. 5.4.1.1-5.4.1.8. Hence, all further biochemical assays were performed on these samples.

5.4.2 Effects of pseudo-Oils on Total Protein.

Table 5.4.2.1. shows the total protein concentrations for cells incubated with 0, 20, 40 or 60mg/l p-oil, expressed as µg total protein/10^6 cells seeded.

168.5µg total protein/10^6 control cells seeded was measured, while concentrations in dosed cells were higher, and ranged from 195.7 to 258.4µg/10^6 cells seeded (with 60mg/l p-FO and 60mg/l p-MO, respectively). Total cellular protein concentrations decreased with increments in the amount of p-CO or p-SSO dosed (230.3 to 202.1 and 254.0 to 223.6µg, respectively), but the converse was found with p-MO (204.8 to 258.4µg). On the other hand, greater protein amounts were induced when cells were supplemented with 40 than 20 or 60mg/l p-OO (249.7, 202.7 and 248.1µg, respectively), p-LO (220.7, 211.2 and 204.3µg, respectively), p-EPO (235.0, 223.3 and 224.2µg, respectively) or p-FO (248.7, 204.2 and 195.7µg, respectively).

Table 5.4.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	160.5			
CO		230.3	224.3	202.1
MO		204.8	221.9	258.4
OO		202.7	249.7	248.1
SSO		254.0	250.4	223.6
LO		211.2	220.7	204.3
EPO		223.3	235.0	224.2
FO		204.2	248.7	195.7

5.4.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Human Lung.

Table 5.4.3.1. shows the FA spectra of cells incubated with 0, 20, 40 or 60mg/l p-oil.

Control cells exhibited a FA spectra comprising 21.8% 18:1ω9, 17.6% 16:0, 11.8% 18:0, 6.0% 20:4ω6, 19.8% 22:4ω6, and smaller amounts (≤5%) of other FA's. In comparison, dosed cell 16:0, 18:0, 16:1ω9, ω6 and ω3 PDFA levels were generally parallel to controls or decreased, while the converse was found for 18:1ω9, 18:2ω6 and 18:3ω3.

Increased 16:0 and 18:1ω9 levels were found when cells were dosed with 20, 40 or 60mg/l p-CO (21.7%, 24.6% and 27.8%, and 25.4%, 26.3% and 28.8% vs 17.6% and 21.8% in controls, respectively), but 18:0 and 16:1ω9 levels were not significantly altered compared to controls (11.8% and 4.1%, respectively). With the exception of small increments in 20:4ω6 and 20:5ω3 with 20mg/l p-CO (6.9% and 2.4% vs in 6.0% and 1.7% in controls, respectively), PUFA percentages approximated to controls, or were significantly decreased.

Dosage with 20, 40 or 60mg/l p-MO had little overall effect on 16:0 and 18:0 levels, whereas 18:1ω9 was slightly increased (23.3% to 27.6% vs 21.8% in controls)

Legend to Table 5.4.3.1

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 5.4.3.1.

The fatty acid spectrum of cells derived from human lung.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	17.6±0.10	21.7	24.6	27.8	18.4	18.9	21.8	16.2	15.4	15.6	16.0	14.4	13.1	16.5	14.9	13.1	15.9	14.7	13.1	17.8	15.2	17.5
	18:0	11.8±0.20	11.4	14.0	10.0	12.4	11.7	12.2	8.6	8.7	5.5	10.8	8.6	7.7	11.2	9.6	7.8	11.0	8.9	7.7	10.4	14.4	6.7
	20:0	0.1±0.00	-	0.1	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	-
	22:0	0.1±0.00	0.1	-	0.1	0.1	0.1	0.1	-	0.1	-	0.1	-	0.1	0.1	0.1	0.1	-	-	-	0.1	-	0.1
	24:0	-	-	-	-	-	-	-	-	-	-	0.1	-	0.3	-	-	-	-	0.3	0.2	-	-	-
ω9 MONOS.	16:1	4.1±0.05	3.9	3.9	3.7	2.7	2.3	2.0	3.4	3.1	3.2	3.6	2.4	1.8	3.7	2.4	2.1	3.5	2.6	2.0	9.8	10.6	16.7
	18:1	21.8±0.28	25.4	26.3	28.8	25.4	23.3	27.6	37.6	44.7	50.7	23.9	22.0	21.8	23.5	19.4	18.6	20.4	17.1	15.9	23.1	24.6	20.9
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	3.7±0.06	3.7	3.3	3.3	5.4	6.9	9.5	6.9	8.7	9.9	19.1	29.4	37.5	7.4	9.3	10.8	21.7	29.8	38.9	5.8	6.5	8.7
	18:3	-	0.1	-	0.2	0.2	0.1	0.3	0.2	0.2	0.3	0.4	0.3	0.2	0.1	0.1	0.1	1.0	1.4	2.2	0.2	0.2	0.1
	20:2	-	-	-	-	0.1	-	0.2	0.4	1.0	1.1	0.3	0.5	0.8	-	0.1	0.1	0.4	0.2	0.5	0.2	0.5	0.5
	20:3	1.1±0.05	1.1	0.8	0.9	1.2	1.1	1.2	0.9	0.7	0.8	1.4	1.3	0.9	0.9	0.8	0.7	2.7	2.8	3.0	1.1	0.8	0.8
	20:4	6.0±0.15	6.9	5.9	6.0	7.1	6.5	7.7	4.4	3.7	3.8	5.8	4.5	3.5	6.1	5.2	4.7	5.8	4.7	3.9	5.9	3.8	3.9
	22:4	19.8±0.50	15.4	10.2	10.8	16.7	17.9	9.8	13.9	6.6	4.6	10.6	8.8	7.4	11.3	8.3	8.5	10.6	8.7	7.5	12.4	6.7	6.3
	22:5	1.7±0.15	0.1	1.0	0.1	0.2	0.8	0.2	-	0.4	0.1	0.2	0.4	0.2	0.2	1.7	0.1	0.2	1.1	0.1	0.1	1.2	0.1
ω3 POLYS.	18:3	0.4±0.00	0.5	0.4	0.5	1.0	0.6	0.5	0.4	0.5	0.5	0.4	0.3	0.3	10.3	19.3	27.2	0.3	0.4	0.2	0.5	0.4	0.7
	18:4	1.9±0.10	0.4	1.3	0.3	0.5	1.6	0.3	0.3	0.7	0.2	0.3	0.9	0.2	0.3	1.5	0.4	0.3	0.8	0.2	0.4	1.8	0.4
	20:4	3.1±0.0	1.6	2.5	1.0	1.1	2.5	0.5	1.3	1.7	0.7	0.6	1.8	0.5	1.5	2.1	0.9	1.1	1.8	0.6	1.5	2.1	0.6
	20:5	1.7±0.10	2.4	1.8	1.9	2.4	1.4	1.7	1.7	1.0	0.6	1.9	0.8	1.1	2.1	1.7	1.6	1.1	1.1	1.2	5.1	6.2	10.7
	22:5	1.6±0.05	1.7	1.2	1.3	1.7	1.4	1.5	1.2	0.9	0.9	1.6	1.2	1.0	1.7	1.3	1.2	1.4	1.0	0.9	3.2	2.7	3.1
	22:6	3.7±0.00	3.5	2.8	3.1	3.4	3.0	3.1	2.4	1.9	1.7	3.1	2.4	1.8	3.0	2.4	2.1	2.8	2.8	1.9	3.3	2.2	2.3

18:2ω6 increased significantly (5.4%, 6.9% and 9.5%, respectively vs 3.7% in controls), and 20:4ω6 slightly (7.1%, 6.5% and 7.7%, respectively vs 6.0% in controls), but no increments in 22:4ω6 or 22:5ω6 levels were found. With the exception of a small increase in 20:5ω3 with 20mg/l p-MO (2.4% vs 1.7% in controls), no significant increases were noted for ω3 series PUFA's.

A marked increased in 18:1ω9 was induced when cells were incubated with 20, 40 or 60mg/l p-OO (37.6%, 44.7% and 50.7%, respectively vs 21.8% in controls), while 16:0, 16:1ω9 and especially 18:0 percentages were decreased. A concentration dependant increment in 18:2ω6 was detected over the range dosed (6.9% to 9.9% vs 3.7% in controls), and 20:2ω6 increased slightly (0.4, 1.0 and 1.1% with 20, 40 and 60mg/l p-OO, respectively vs 0% in controls). All other ω6 and ω3 PUFA amounts, however, approximated to controls, or were significantly decreased.

16:0, 18:0 and 16:1ω9 percentages were decreased with p-SSO supplementation, while little changed occurred in 18:1ω9 proportions in relation to controls (17.6%, 11.8%, 4.1% and 21.8%, respectively). On the other hand, a concentration dependant increased in 18:2ω6 was induced with 20, 40 or 60mg/l p-SSO dosage (19.1%, 29.4% and 37.5%, respectively vs 3.7% in controls), yet ω6 PDFA levels were not significantly increased, as were ω3 PUFA percentages.

A decrease in 16:0, 16:1ω9, 18:0 and 18:1ω9 was induced with p-LO supplementation, especially with increments in concentration dosed. PDFA levels were also decreased, or not significantly altered, despite increments in 18:2ω6 and 18:3ω3 found with 20, 40 and 60mg/l p-LO (7.4%, 9.3% and 10.8%, and 10.3%, 19.3% and 27.2% vs 3.7% and 0.4% in controls, respectively).

16:0, 18:0, 16:1ω9 and 18:1ω9 percentages decreased progressively with increments in the amount of p-EPO dosed, while the converse was shown for 18:2ω6 (21.7% with 20mg/l, 29.8% with 40mg/l, and 38.9% with 60mg/l p-EPO (3.7% in controls). 18:3ω6 increased significantly over the same range (1.0%, 1.4% and 2.2%, respectively vs 0% in controls), while 20:3ω6 increased 2 to 3 fold (2.7%, 2.8% and 3.0%, respectively vs 1.1%), although decreased product amounts were found.

An increase in 16:1ω9 was induced with 20, 40 or 60mg/l p-FO incubation (9.8%, 10.6% and 16.7%, respectively vs 4.1% in controls), but little change occurred overall in 16:0, 18:0 or 18:1ω9. Despite an increment in 18:2ω6 (5.8% to 8.7% vs 3.7% in controls), ω6 PDFA proportions approximated controls, or were decreased. Similarly, decreased 22:6ω3 levels were detected with 20, 40 or 60mg/l p-FO dosage (3.3%, 2.2% and 2.3%, respectively vs 3.7% in controls), even though increments were induced in 20:5ω3 (5.1%, 6.2% and 10.7%, respectively vs 1.7% in

controls) and 22:5ω3 (3.2%, 2.7% and 3.1%, respectively vs 1.6% in controls).

5.4.4 Incorporation of Radiolabelled C18 Fatty Acids into Cells Derived from Human Lung.

Table 5.4.4.1. shows the radioactivity recovered from cultures incubated with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3.

For both [^{14}C]-18:1ω9 and [^{14}C]-18:3ω3, the equivalent of $4.3x10^6$cpm were dosed to the incubation medium, and the total recovery approximated to 91% in each case. For [^{14}C]-18:2ω6, the equivalent of $1.8x10^6$cpm were dosed to the culture medium, and the total recovery of this radioisotope after 48 hours incubation amounted to 98%. The total counts recovered from the spent growth media ranged from 1.3 to $2.6x10^6$cpm, with 0.7 to $1.1x10^5$cpm recovered from the pooled buffers after washing the cell, which accounted for for non-specific binding. The amount of each of these radioisotopes incorporated into the cells themselves equated to 34% for [^{14}C]-18:1ω9, 29% for [^{14}C]-18:2ω6 and 27% for [^{14}C]-18:3ω3.

Table 5.4.4.2. shows the percentage conversion of incorporated [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 to ω9, ω6 or ω3 series products, respectively after 48 hours incubation with human lung fibroblasts.

26.6% of the total [^{14}C]-18:1ω9 counts derived upon GLC analysis (2202cpm) remained unchanged, while 39.6% was found for 20:1ω9, and 24.0% for 22:1ω9, but only 9.8%

Table 5.4.4.1.

The radioactivity (cpm) recovered from human 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 x 10^6				
1278280 (±2240)	1140230 (±6435)	2418510	1477332 (±1653)	3.9
Total 18:2 counts dosed to incubation medium = 4.8 x 10^6				
2599077 (±4886)	710943 (±4171)	3310020	1397004 (±2118)	4.7
Total 18:3 counts dosed to incubation medium = 4.3				
2005060 (±8920)	745550 (±7085)	2750610	1147896 (±870)	3.9

Table 5.4.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from human 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	26.6	18:2	25.9	18:3	18.3
20:1	39.6	18:3	10.0	18:4	23.5
22:1	24.0	20:2	28.5	20:3	22.3
24:1	9.8	20:3	17.1	20:4	7.8
		20:4	10.0	20:5	14.2
		22:4	3.9	22:5	10.3
		22:5	4.6	22:6	3.6
TOTAL CPM	2202	TOTAL CPM	3848	TOTAL CPM	3226

for 24:1ω9.

25.9% of the total [^{14}C]-18:2ω6-derived counts detected following GLC analysis (3848cpm) remained as 18:2ω6, while 28.5% was detected as 20:2ω6, compared to 10.0% 18:3ω6. 20:3ω6 and 20:4ω6 accounted for 17.1% and 10.0% of total counts, respectively, but only 3.9% 22:4ω6 and 4.6% 22:5ω6 were detected.

3226cpm were derived upon GLC analysis of cells dosed with [^{14}C]-18:3ω3, of which 18.3% remained unchanged, compared to 23.5% 18:4ω3 and 23.5% 20:3ω3. Only 7.8% 20:4ω3 was detected, but 20:5ω3 levels were twice as high (14.2%). 10.3% 22:5ω3 was found, but only 3.6% 22:6ω3.

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.

5.4.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

Table 5.4.5.1. shows the lipoperoxides measured in human lung cultures incubated with 0, 20, 40 or 60mg/l p-oil for 48 hours. The results are expressed 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.

1.1 to 3.0nmoles MDA/10^6 dosed cells were quantitated, compared to 1.1nmole MDA/10^6 control cells. Dosage with 20, 40 or 60mg/l p-MO resulted in the formation of slightly greater cellular lipoperoxide amounts than p-CO (1.7, 1.6 and 2.1 vs 1.5, 1.6 and 1.4nmoles MDA per 10^6 cells, respectively). The lipoperoxide amounts generated by cells dosed with p-OO or p-SSO, however, approximated to controls with all concentrations. On the other hand, cells supplemented with p-LO, p-EPO or p-FO induced the largest lipoperoxide amounts overall. Levels were comparable with 20, 40 or 60mg/l p-LO or p-EPO dosage (1.3, 2.0 and 2.8 vs 1.3, 2.4 and 3.0nmoles MDA/10^6 cells), while the amounts p-FO induced were larger with a concentration of 20mg/l, comparable with 40mg/l and lower with 60mg/l (2.0, 2.0 and 2.2nmoles MDA/10^6 cells, respectively).

The concentration of lipoperoxides measured in the spent medium of dosed cultures ranged from 0.3 to 7.1nmoles

Table 5.4.5.1.

Lipoperoxide formation by human 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		1.5	1.6	1.4
MO		1.7	1.6	2.1
OO		1.2	1.1	1.0
SSO		1.1	1.1	1.3
LO		1.3	2.0	2.8
EPO		1.3	2.4	3.0
FO		2.0	2.0	2.2
pseudo-Oil (mg/l)	SPENT INCUBATION MEDIUM			
	0	20	40	60
Control	0.5			
CO		1.3	1.5	1.6
MO		1.0	0.9	1.4
OO		0.7	0.9	1.0
SSO		0.5	0.8	2.1
LO		1.1	2.9	6.6
EPO		0.3	0.7	2.1
FO		2.1	3.1	7.1

MDA/10^6 cells, compared to 0.5nmoles MDA/10^6 cells in control media. Unlike the cells, media dosed with 20, 40 or 60mg/l p-CO contained greater lipoperoxide amounts (1.3, 1.5 and 1.6nmoles MDA/10^6 cells, respectively) than that derived from incubation with p-MO or p-OO (1.0, 0.9 and 1.4, and 0.7, 0.9 and 1.0nmoles MDA/10^6 cells, respectively). Spent media lipoperoxide levels were similar with 20, 40 or 60mg/l p-SSO or p-EPO dosage (0.5, 0.8 and 2.1 vs 0.3, 0.7 and 2.1nmoles MDA/10^6 cells, respectively), while amounts were larger in the presence of p-LO (1.1, 2.9 and 6.6nmoles MDA/10^6 cells, respectively). The greatest spent media lipoperoxide levels were nevertheless shown with 20, 40 or 60mg/l p-FO incubation (2.1, 3.1 and 7.1nmoles MDA/10^6 cells, respectively).

5.4.6 The Eicosanoid Profile of Cells Derived from Human Lung.

Table 5.4.6.1. shows the eicosanoids positively detected in control and p-oil dosed human lung fibroblasts as a percentage of the total area detected. An indication of the total eicosanoid amount quantitated, however, is given for controls in '( )', expressed as pmoles/10^6 cells.

Total eicosanoid production amounted to 332.6pmoles/10^6 control cells, of which 19.9% could be positively identified with the prostanoid standards available. Approximately half this proportion was composed of TXB_2 (8.8%), while the remainder was made up of smaller proportions PGI_2 (2.5%), PGF_α (5.8%), PGE2 (0.2%) and PGE_1 (2.6%). However, no PGD2 was detected in control or dosed cells.

Total percentages for the prostanoids detected were greater in dosed than control cells. The increments were least with dosage of 40mg/l p-oil (21.2% to 30.9%), but significantly greater both with 20mg/l (36.0% to 40.9%) or 60mg/l p-oil (30.9% to 48.9%). These differences were reflected by appropriate changes in the amount of individual prostanoids. Dosed cells generally produced more PGI_2 than controls (2.5%). The largest PGI2 percentages were detected with 40mg/l p-oil (2.8% to 10.9%), compared to lesser amounts with 20 and 60mg/l

Legend to Table 5.4.6.1.

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

Table 5.4.6.1.

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

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	$PGF_{2\alpha}$ (1+2)	PGE_2	PGE_1	PGD_2
CONTROL (332.6)		19.9	2.5	8.8	5.8	0.2	2.6	-
CO	20	40.9	5.5	23.3	12.1	-	-	-
	40	30.9	8.2	9.6	7.6	5.1	0.4	-
	60	41.6	4.7	19.5	14.4	2.4	0.6	-
MO	20	36.0	4.6	21.7	9.7	-	-	-
	40	28.2	4.6	11.9	7.7	3.2	0.8	-
	60	48.9	6.0	24.1	16.4	2.4	-	-
OO	20	36.3	1.2	21.2	13.9	-	-	-
	40	21.5	10.9	6.1	4.5	-	-	-
	60	37.2	1.0	22.3	13.9	-	-	-
SSO	20	36.3	4.8	20.3	10.9	0.3	-	-
	40	21.2	6.4	10.1	4.5	0.2	-	-
	60	37.9	3.8	20.8	12.8	0.5	-	-
LO	20	38.1	1.2	21.4	15.1	0.4	-	-
	40	25.6	2.8	15.1	7.7	-	-	-
	60	30.9	2.9	18.0	10.0	-	-	-
EPO	20	40.5	2.9	25.4	12.1	0.1	-	-
	40	24.5	4.5	12.3	7.7	-	-	-
	60	37.3	2.3	22.1	12.0	0.9	-	-
FO	20	36.5	3.5	20.2	12.4	0.4	-	-
	40	21.5	10.6	4.6	4.2	2.1	-	-
	60	38.4	5.1	17.7	13.3	2.3	-	-

p-oil (1.0% to 6.0%). Variable TXB_2 amounts were induced with 40mg/l p-oil (4.6% to 15.1% vs 8.8%), but this increased 2 to 3 fold with 20 and 60mg/l p-oil (17.7% to 25.4%). PGF_α levels approximated to that of controls (5.8%) with 40mg/l p-oil (4.2% to 7.7%); considerably greater amounts, however, were formed with 20 and 60mg/l p-oil (9.7% to 16.4%), which were comparable. PGE_2 production was more variable in dosed cells (0% to 5.1% vs 0.2% in controls), while only small PGE_1 amounts (≤0.8% vs 2.6% in controls) were formed with p-CO or p-MO dosage.

5.4.7 Discussion.

Supplemention of cells derived from human fetal lung with p-oils modulated the viability thereof (Figs. 5.4.1.1-5.4.1.8.), and the finding that cytotoxicity was induced only with greater than 80mg/l p-oil could have related to the relatively low population doubling time these cells exhibited (Tab'e 2.3.1.1.). The different cell viability changes induced nevertheless reflected the variations in p-oil FA composition (Table 2.3.3.2.). OA, LA or ALA comprised approximately 70% of all FA's in p-OO, p-SSO and p-LO, respectively, thus it was likely that the cell viability changes induced with dosage of either p-oil related primarily to these C18 FA's. Such moieties were all Δ6D substrates, but they varied in the number and position of their double bonds. This may explain the stimulation of cell proliferation with p-OO or p-SSO, but not with p-LO, supplementation, as well as the greater capability to reduce cell viability with incubation of p-LO, than with p-SSO or p-OO. The FA composition of p-EPO was similar to that of p-SSO, thus modulation only of growth limitation with p-EPO dosage suggested that such related to the presence of about 9% GLA in p-EPO only. This moiety may have limited the capability of LA to stimulate, or inhibit, cell proliferation with incubation of low, or high, p-EPO concentrations, respectively. Dosage with high p-LO or

cytotoxicity. ω3 PUFA's were present in these p-oils only, thus it was possible that the amount and structure of ALA in p-LO and EPA in p-FO enhanced the capability of these p-oils to inhibit cell proliferation. However, as the proportion of ALA in p-LO was greater than that of EPA in p-FO, the induction of significantly more cytotoxicity with dosage of high concentrations of the latter may relate partly to the greater degree of EPA unsaturation compared to ALA. Synergistic and/or antagonistic interations between p-oil FA's should also not be overlooked as mediators of the growth responses induced. This could explain why cell viability did not always relate inversely to the p-oil concentration dosed, while other workers showed that the growth of human fetal lung cultures correlated inversely with FFA concentration (Boone et al 1972). The ability of these cells to incorporate exogenous FA's (Table 5.4.3.1.) nevertheless suggested the involvement of membrane fluidity changes in the modulation of cell viability, particularly with high p-oil concentrations dosed.

The results obtained from the measurement of total protein in control and dosed cells indicated significant stimulation of protein synthesis with p-oil dosage, even when such induced significant growth limitation (Table 5.4.2.1.). The extent to which protein synthesis was stimulated varied with supplementation of identical

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 incorporation of exogenous FA's with p-oil dosage increased the content of cellular FA's, thus it was possible that increased protein synthesis related to enhanced expression of lipid metabolising enzymes.

It was unlikely that an increased amount of protein per cell related to enhanced expression of desaturase or elongase enzymes, since the evidence obtained from supplementing cultures with p-oils suggested that these fibroblasts exhibited limited capability to desaturate or elongate FA's (Table 5.4.3.1.). Neither 16:0 nor 16:1ω9 incorporated with p-oil dosage were significantly desaturated or elongated, while 18:0 and 18:1ω9 levels parallel to controls suggested impaired Δ9D capability. It was apparent that 18:2ω6 incorporated with p-SSO dosage was not significantly converted to PDFA's, thus 20:3ω6 formation with p-EPO supplementation probably related to elongation of incorporated 18:3ω6 rather than delta-6-desaturation and elongation of incorporated 18:2ω6. In addition, lack of further 20:3ω6 desaturation was consistent with impaired Δ5D expression. On the other hand, 18:3ω3 incorporation and the formation of small 20:5ω3 amounts with supplementation of 20mg/l p-LO suggested the capability for Δ6D, elongase and Δ5D

activity. Also, the formation of 22:5ω3 with p-FO dosage supported elongation of incorporated 20:5ω3. However, no increase in 22:5ω6 or 22:6ω3 was detected with dosage of any p-oil, and this indicated impaired Δ4D capability. Maeda et al (1978) showed that human embryonic lung fibroblasts (WI-38) dosed with 15mg/l albumin-bound 18:3ω6, 20:2ω6 or 20:5ω3 for 24 hours incorporated these moieties, elongated 18:3ω6 and 20:5ω3 to 20:3ω6 and 22:5ω3, respectively, but did not exhibit the capability to desaturate 20:2ω6, 20:3ω6 or 22:5ω3. This supported the findings we reported above. p-CO and p-MO contained no ω3 PUFA's, thus the formation of small amounts of 20:5ω3 with dosage of 20mg/l p-CO or p-MO may reflect an attempt to enhance desaturation and/or elongation of cellular ω3 PUFA's to maintain membrane fluidity altered with incorporation of these p-oils.

The formation of small PDFA amounts with supplementation of certain p-oils suggested that desaturase and elongase capability was suppressed rather than absent in these cells. It was unlikely, however, that such related to growth limitation induced with p-oil dosage, since the capability for PDFA formation was impaired even when p-oils had little effect on, or stimulated, cell viability. Suppression of desaturase cascade enzyme expression may rather relate to alterations in microsomal lipid composition induced with p-oil dosage,

as the modulation of microsomal lipid composition has been implicated in the regulation of desaturase enzyme activity by others (Nervi et al 1968, Kurata et al 1980, Garda et al 1984, 1985 and Garg et al 1988a, 1988b, 1988c).

Desaturation with individual radiolabelled C18 FA's was assessed to establish whether the pattern of p-oil desaturation reported (Table 5.4.3.1.) was modulated with FA mixtures. The detection of 24:1ω6 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 5.4.4.2.). On the other hand, the pattern of radioactivity derived from cultures supplemented with either [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 was consistent with Δ6D and subsequent elongase capability (Table 5.4.4.2.). Significant amounts of [^{14}C]-18:3ω3 and [^{14}C]-18:2ω6 were also directly elongated to 20:3ω3 and 20:2ω6, respectively, thus the possibility that such moieties were converted to 20:4ω3 and 20:3ω6, respectively via an active Δ8D can not be dismissed, particularly since humans have been shown to exhibit limited Δ6D capability (Dyerberg et al 1980 and Horrobin 1983). Nevertheless, conversion of about 30% [^{14}C]-18:3ω3 and 20% [^{14}C]-18:2ω6 to post-Δ5D FA's supported Δ5D expression. 20:5ω3 and 20:4ω6 elongation were shown, but the detection of less than 5% 22:6ω3 and 22:5ω6 each indicated that Δ4D was

not significantly expressed.

The data reported in Table 5.4.4.2. was supported by the work of Dunbar et al (1975), who demonstrated Δ6D, subsequent elongase and Δ5D capability when the culture medium of human embryonic lung fibroblasts (WI-38) was supplemented with 2μCi [^{14}C]-18:2ω6. These workers showed that approximately 45% of the incorporated [^{14}C]-18:2ω6 (34%) was converted to PDFA's, and such correlated numerically with the present study (Tables 5.4.4.1. and 5.4.4.2.). However, we showed 10 fold more [^{14}C]-18:2ω6 elongation to 20:2ω6 than these workers (28.5% and 2.8%, respectively), and this was consistent with the smaller proportion of incorporated [^{14}C]-18:2ω6 left unchanged in our study (25% and 50%, respectively). The conversion of [^{14}C]-18:2ω6 to 18:3ω6 and 20:4ω6 was also shown in WI-38 human embryonic lung cells by Maeda et al (1978). However, such capability was shown to be very limited since 85% of incorporated [^{14}C]-18:2ω6 (50%) remained unaltered, and only 4% 20:4ω6 was detected, compared to the formation of approximately 35% 20:4ω6 in Dunbar's study and 20% post-Δ5D FA's in our study (Table 5.4.4.2.). Dunbar et al (1975) used a FA incubation period of 4 days, while Maeda et al (1978) used 1 day, and this may explain the different extent to which 18:2ω6 was desaturated and elongated in these two studies, despite the use of the same cell line and similar experimental conditions. Numerical differences

between studies may also relate partly to subtle differences in lipid metabolism between human embryonic lung cultures, which Spector et al (1979) showed even under identical culture conditions. Unfortunately, the formation of 22:5ω6 from 20:4ω6 was not examined either by Dunbar et al (1975) or Maeda et al (1978). The latter group showed the capability of these cells form 22:5ω3 when [^{14}C]-18:3ω3 was dosed, and this supported our data (Table 5.4.4.2.). However, these workers showed that such capability was limited as only 30% of incorporated [^{14}C]-18:3ω3 was converted to longer chain more unsaturated ω3 FA's, compared to more than 80% in our study. Nevertheless, both our data and that of Maeda et al (1978) demonstrated that desaturation and elongation of incorporated [^{14}C]-18:3ω3 was greater than for [^{14}C]-18:2ω6, and this confirmed the established preference for ω3, rather than ω6, PUFA substrates for such reactions (Brenner et al 1966, Mead et al 1976 and Kanau et al 1977).

Spector et al (1979) reported that the formation of 20:2ω6, 20:3ω6 and 22:4ω6 when human embryonic lung fibroblasts (IMR-90) were incubated with 15mg/l 18:2ω6 was consistent with Δ6D, Δ5D and elongase, but no significant Δ4D, capability, as shown in our study (Table 5.4.4.2.). On the other hand, when these workers added 15mg/l 18:1ω9 to the culture medium, this moiety

probably reflected 18:1ω9 incorporation into neutral lipids, compared to 18:2ω6 incorporation primarily into PGL's. If the exogenous FA's dosed in our study were primarily incorporated into neutral lipids rather than into membrane PGL's, such would have limited the capability of these moieties to undergo desaturation or elongation. This may explain why the capability for desaturation and elongation was more limited with p-oil (Table 5.4.3.1.), than with individual C18 FA's (Table 5.4.4.2.), supplementation, although such may also relate to modulation of enzyme expression by p-oil FA's. Nevertheless, when relating <u>in vitro</u> experimental data to the 'real world', the capability for desaturation and elongation with p-oil, rather than individual FA, dosage may be a better indication of such capability <u>in vivo</u>, since p-oil FA composition reflects that of dietary oils. Although the ability to desaturate and elongate FA's clearly is an important source of PDFA's for eicosanoid production, impairment of PDFA formation may not necessarily limit the ability of human lung to synthesise eicosanoids <u>in vivo</u> provided PDFA precursors are obtained from other tissues or from the diet.

The results obtained from the measurement of cellular lipoperoxides showed evidence of such compounds both in the presence and absence of p-oil dosage (Table 5.4.5.1.), and this reflected cellular enzymic and/or non-enzymic mechanisms for their production. The

measurement of lipoperoxides in control cells was not surprising as the cells themselves contained FA's which were potential substrates for lipoperoxidation (Table 5.4.3.1.). It was also possible that lipoperoxide formation in control cells reflected inefficient cellular mechanisms to couteract and/or limit oxidation of cellular FA's. The greater amount of lipid peroxides measured with p-oil dosage than in controls, both in the spent medium and cells themselves, was consistent with oxidation of unsaturated p-oil FA's. We showed evidence which supported the ability of these cells to incorporate p-oil FA's (Table 5.4.3.1.), thus it was unlikely that the lipoperoxides measured in the spent medium of dosed cells were formed primarily as a result of auto-oxidation of unincorporated p-oil FA's, particularly since these moieties were bound to albumin. The FA composition of the incubation medium was primarily composed of saturated FA's (Table 2.3.3.3.), thus it was also unlikely that such contributed significantly to the medium lipoperoxide levels reported in control and dosed cells. The data therefore suggested that the lipoperoxides measured in the spent medium originated intracellularly, although it was apparent that these moieties were not released as a result of cell death, since cytotoxicity was absent even with dosage of 60mg/l p-oil (Figs. 5.4.1.1-5.4.1.8.). There is evidence, however, that lipid peroxides increase

membrane permeability (eg. Chio et al 1969 and Frankel 1984). It was therefore possible that the lipoperoxides measured in spent medium reflected the formation of cellular lipid peroxides which altered membrane permeability and permitted the release of these moieties into the medium. Cellular enzymes responsible for lipoperoxidation and FFA's may also have been released into the incubation medium in this process, thus the possiblity that enzymic and/or spontaneous FA oxidation occurred extracellularly, and contributed to the lipid peroxides measured in the spent medium, can not be ignored.

The numerical differences in the concentration of lipoperoxides reported with p-oil supplementation (Table 5.4.5.1.) reflected the different FA compositions of the p-oils (Table 2.3.3.2.). This relates not only to the amount of unsaturated FA's present in any p-oil, but also to the susceptibility of FFA's to oxidation, which in turn relates to the number and position of double bonds in a FFA. It was clear that cultures dosed with with p-LO or p-FO induced the greatest amount of lipoperoxides overall. The FA compositions of these p-oils suggest that such may relate to their complement of ω3 PUFA's. The ALA content of p-LO was greater than the amount of EPA in p-FO (about 63% and 18%, respectively), although the greater number of double bonds in EPA increased its susceptibility to oxidation

compared to ALA. This probably accounted for the similar lipoperoxide amounts induced overall with p-LO or p-FO dosage. The FA composition of p-SSO indicated that the lipoperoxides formed with supplementation of this p-oil related largely to oxidation of LA. Greater amounts of cellular lipoperoxides were induced with p-EPO, than with p-SSO, dosage, and this may well reflect oxidation of GLA present in p-EPO only, as both p-oils otherwise exhibited similar FA compositions. It may also relate to the utilisation of DGLA, formed only with p-EPO dosage (Table 5.4.3.1.), as an endoperoxide substrate. The measurement of more lipid peroxides with p-CO or p-MO, than with p-OO, dosage was surprising considering the significantly greater unsaturated FA content of p-OO. However, this may reflect a slower rate of PGL turnover and therefore FA release from membrane lipids into the cellular pool with p-OO, than with p-CO or p-MO, dosage.

Growth inhibition was not induced with supplementation of up to 60mg/l p-oil (Figs. 5.4.1.1-5.4.1.8.), thus no correlation between lipid peroxide formation (Table 5.4.5.1.) and cytotoxicity could be established. Nevertheless, lipoperoxides should not be overlooked as a possible mechanism involved in the modulation of cytotoxicity with dosage of higher p-oil concentrations. On the other hand, measurement of the smallest amount of lipoperoxides overall with p-OO or p-SSO dosage correlated with the greatest stimulation of cell

viability, whereas the greater amounts of lipoperoxides measured with supplementation of other p-oils correlated with growth limitation. Lipoperoxides may therefore be involved in the modulation of cell viabililty. However, the fact that growth limitation was not most pronounced with p-LO or p-FO dosage, despite measurement of the greatest lipoperoxide amounts with supplementation of these p-oils, indicated that the modulation of cell viability also related to other mechanisms, such as alterations in membrane fluidity.

PG's and related eiccsanoids are important mediators of normal lung function (Hyman et al 1978 and Newman et al 1984), thus it is not surprising that this tissue has been shown to be the primary site for eicosanoid production (Mathe et al 1977, Hyman et al 1978 and Harper et al 1984). The total eicosanoid concentration quantitated in control cells in the present study (Table 5.4.6.1.) supported the capability for human lung fibroblasts to biosynthesise significant amounts of eicosanoids, even though this only accounted for about 30% of all lipid peroxides (Table 5.4.5.1.). The lung is indeed rich in enzymes that synthesise PG's and TX's, as well as inactivating these compounds (Samuellson et al 1975, 1978). McLemore et al (1988) showed that normal human lung tissue biosynthesised small amounts of $PGF_{2\alpha}$ and PGE_2, larger amounts of PGD2 and 6-keto-$PGF1_{\alpha}$, while TXB_2 production was greatest. This confirmed and

extended previous reports (Hyman et al 1978, Newman et al 1984 and Hubbard et al 1986). Human lung cultures have also been shown to express the enzymes necessary for prostanoid synthesis and release, although leukotrienes appear to be released only upon stimulation (Chagnon et al 1985 and Robidoux et al 1988). The abundance of TXB_2 in the lung has also been reported in culture, and such has been routinely monitored to reflect TXA_2 synthesis (Robidoux et al 1988). Feinmark et al (1982) reported that monolayer cultures of human diploid embryonic lung fibroblasts (WI-38) metabolised exogenously supplied [^{14}C]-20:4ω6 to TXB_2, and showed that PGL was the source of the radioactive precursor. Their studies indicated that lung fibroblasts were a prime source of pulmonary thromboxane in vivo, although significant PGE_2 synthesis also occurred. Other studies, however, showed that cultured human lung cells produced considerably smaller amounts of PGE_2 than TXB_2 (Robidoux et al 1988). Our study not only confirmed that cultured human lung fibroblasts synthesise large amounts of TXB_2, but also showed that these cells produced significant amounts of PGF_{α}, PGE_1 and PGI_2, but not PGE_2 and PGD_2 (Table 5.4.6.1.).

Supplementation of cultures with any p-oil modulated the proportions of the individual eicosanoids detected in these cells, although overall prostanoid production was enhanced (Table 5.4.6.1.). Dosage with 60mg/l p-MO

induced the greatest enhancement of prostanoid synthesis, but such did not appear to relate primarily to incorporation of exogenous 20:4ω6, the 2-series eicosanoid precursor, since dosage with other p-MO concentrations did not induce appreciable increases in eicosanoid production relative to cells dosed with p-oils devoid of exogenous 20:4ω6. Cells supplemented with p-EPO contained higher levels of the 1-series eicosanoid precursor, 20:3ω6, than cells dosed with any other p-oil (Table 4.7.3.1.), yet no clear differences in eicosanoid production were found between cells dosed with identical p-oil concentrations. 18:3ω3 and 20:5ω3, abundant in p-LO and p-FO, respectively, are potent inhibitors of 2-series prostanoid production as they preferentially occupy the active site on cyclo-oxygenase (Lands et al 1971, 1973, 1977, Hamazaki et al 1982, Corey et al 1983, Fischer et al 1983, 1984 and Nassar et al 1987). Yet, dosage with these p-oils also yielded prostanoid profiles similar to that derived from cells supplemented with p-oils devoid of such moieties. It was apparent, therefore, that p-oil FA's per se induced little effect on the formation of the endogenous prostanoids reported as such was unrelated to the degree of p-oil unsaturation (Table 2.3.3.2.), the ability of the p-oils to supply direct eicosanoid precursors, or the capability of the cells to form eicosanoid precursors with p-oil supplementation (Tables 5.4.3.1.).

The lack of significant differences in the levels of the eicosanoids reported with dosage of different p-oils at the same concentration suggested utilisation of endogenous, rather than exogenous, PUFA precursors for eicosanoid production. The enhanced prostanoids levels reported may thus relate to utilisation of membrane 20:3ω6 and 20:4ω6. The detection of these moieties in control cells (Table 4.8.3.1.) indeed supported their utilisation for eicosanoid formation. The levels of the prostanoids detected were nevertheless modulated dependent on p-oil concentration. The enhancement of total eicosanoid production, particularly with dosage of 20 or 60mg/l p-oil, was manifested primarily by enhanced production of TXB_2, PGF_α and PGI2. However, p-oil supplementation suppressed or inhibited PGE_1 synthesis, and this may reflect the significantly lower amounts of the 1-series eicosanoid precursor (20:3ω6) detected in these cells (Table 5.4.3.1.) than the 2-series precursor (20:4ω6). While the data indicated that the cyclo-oxygenase pathway was functional in these fibroblasts, the exact mechanism by which prostanoid production was modulated with p-oil dosage was unclear. The possibility exists that dosage with different p-oil concentrations influenced eicosanoid precursor availability, or that p-oil FA's modulated various enzymic steps in the prostanoid biosynthetic pathway, dependent on p-oil concentration.

The eicosanoid profiles reported in this study (Table 5.4.6.1.) did not correlate in any way with the changes reported in cell viability (Figs. 5.4.1.2-5.4.1.8.), total protein (Table 5.4.2.1.), lipoperoxide production (Table 5.4.5.1.), or desaturation capability (Table 5.4.3.1.) with p-oil dosage. The data therefore suggests that although p-oil supplementation influenced the overall production of the eicosanoids studied, 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 supplementation.

5.5 THE EFFECTS OF PSEUDO-OILS ON CELLS DERIVED FROM HUMAN SKIN.

5.5.1 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 cells, or of p-oil dosed cells, although cytoplasmic droplets were observed with p-oil supplementation at high concentrations in some instances.

Investigation of the effects of 0 to 250mg albumin/l culture medium showed no significant effect on human skin fibroblast viability (Fig. 5.5.1.1.). The effects induced 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 $11.7x10^4$/ml. This equated to about 61% 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 61% were thus considered cytotoxic.

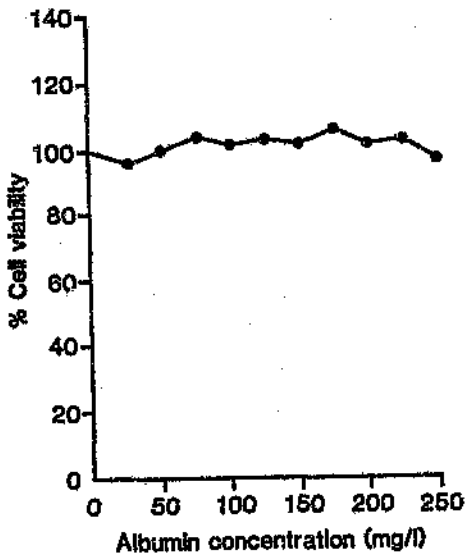
The results of incubation of cells with p-oils for 48 hours are depicted in Figs. 5.5.1.2-5.5.1.8. p-MO, p-OO, p-SSO and p-FO enhanced cell proliferation with low

Legend to Figs. 5.5.1.1-5.5.1.9.

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. 5.5.1.1. shows the mean percent cell viability versus the albumin concentration (mg/l), and Figs. 5.5.1.2-5.5.1.8. depict the mean percent cell viability versus the pseudo-oil concentration (mg/l). Fig. 5.5.1.9. 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. 5.5.1.1.

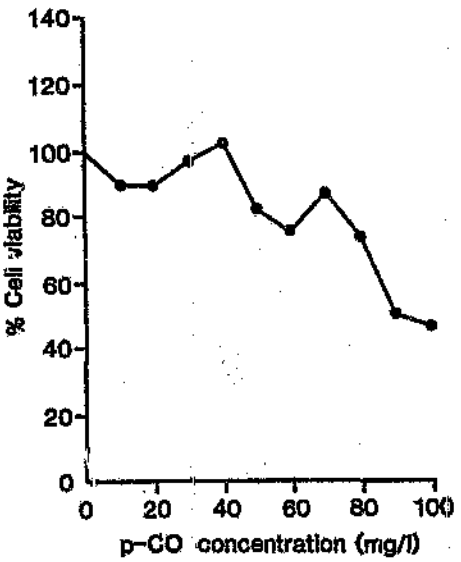
The percentage viability of cells derived from human skin incubated with albumin.



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	12
25	96.2	5.7	12
50	100.0	6.4	12
75	103.9	6.7	12
100	101.5	5.7	12
125	103.4	5.6	12
150	101.5	4.8	12
175	105.8	4.6	12
200	101.9	6.9	12
225	103.3	4.8	12
250	97.1	4.9	12

Fig. 5.5.1.2.

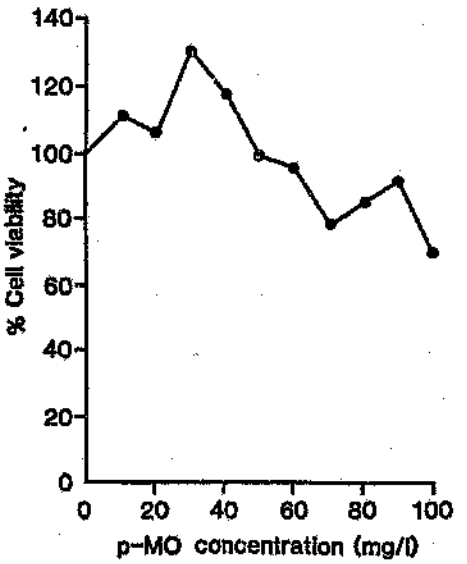
The percentage viability of cells derived from human skin incubated with p-CO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	5.7	6
10	89.1	5.6	6
20	89.1	3.1	6
30	96.6	6.8	6
40	102.5	4.5	6
50	82.4	2.5	6
60	74.8	6.6	6
70	86.6	5.7	6
80	74.0	5.6	6
90	49.6	4.6	6
100	46.2	6.1	6

Fig. 5.5.1.3.

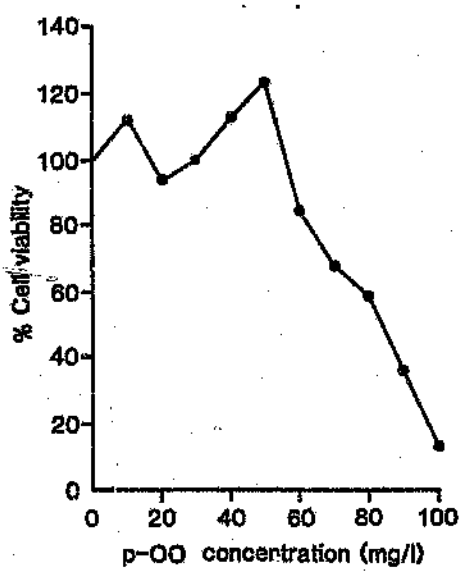
The percentage viability of cells derived from human skin incubated with p-MO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	8.5	6
10	111.0	5.6	6
20	105.5	6.4	6
30	130.6	3.6	6
40	117.1	8.5	6
50	98.6	4.6	6
60	95.3	2.3	6
70	78.8	6.6	6
80	84.4	4.8	6
90	92.7	3.6	6
100	69.5	1.5	6

Fig. 5.5.1.4.

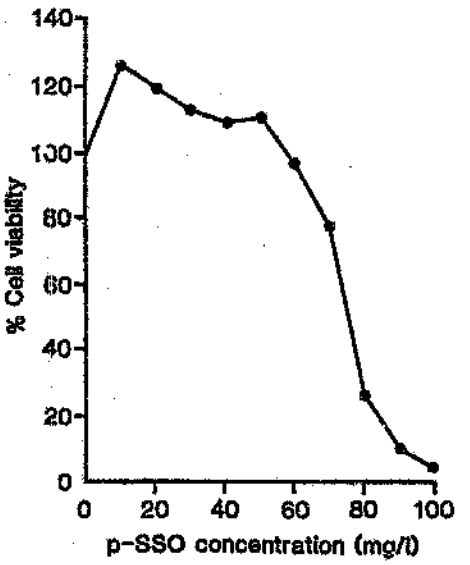
The percentage viability of cells derived from human skin incubated with p-OO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	6.4	6
10	113.5	7.0	6
20	93.9	4.1	6
30	100.2	2.6	6
40	113.7	7.8	6
50	124.7	4.8	6
60	84.7	4.4	6
70	68.5	5.9	6
80	58.8	3.5	6
90	35.9	3.8	6
100	12.9	2.4	6

Fig. 5.5.1.5.

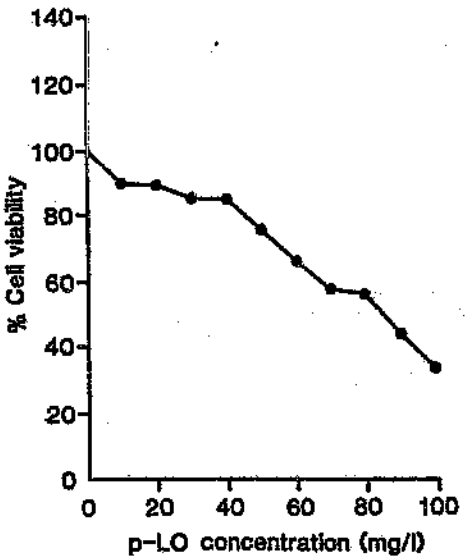
The percentage viability of cells derived from human skin incubated with p-SSO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.2	6
10	126.2	1.7	6
20	118.8	2.6	6
30	112.3	3.0	6
40	109.4	3.4	6
50	111.1	4.3	6
60	97.0	1.9	6
70	77.8	3.5	6
80	26.5	2.9	6
90	9.8	1.4	6
100	3.4	0.5	6

Fig. 5.5.1.6.

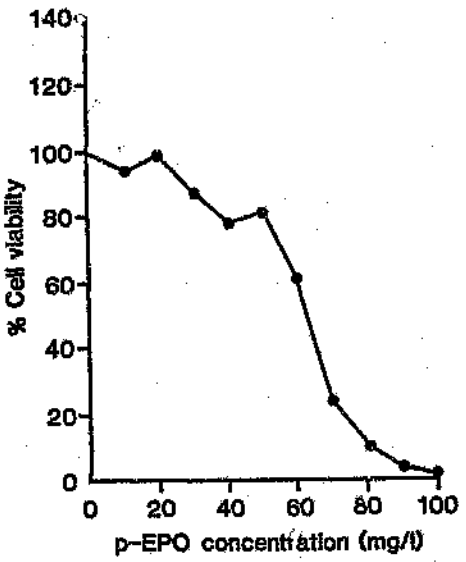
The percentage viability of cells derived from human skin incubated with p-LO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	2.5	6
10	89.5	3.9	6
20	88.7	4.0	6
30	85.2	3.7	6
40	84.7	4.3	6
50	75.6	5.3	6
60	65.0	5.3	6
70	56.8	5.0	6
80	55.0	5.5	6
90	43.2	2.9	6
100	33.2	4.5	6

Fig. 5.5.1.7.

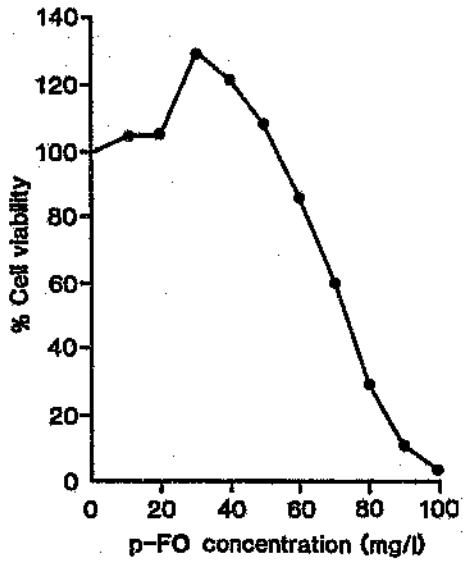
The percentage viability of cells derived from human skin incubated with p-EPO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.5	6
10	93.1	5.0	6
20	99.2	2.6	6
30	86.8	5.5	6
40	78.5	4.0	6
50	80.8	4.4	6
60	60.0	2.7	6
70	22.3	3.9	6
80	8.5	2.2	6
90	2.3	1.0	6
100	0	0	6

Fig. 5.5.1.8.

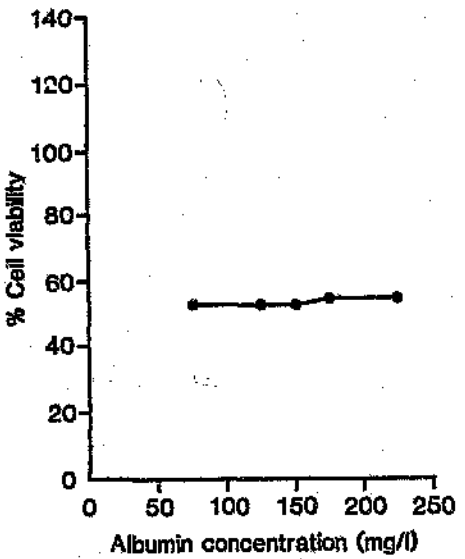
The percentage viability of cells derived from human skin incubated with p-FO.



pseudo-Oil Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.1	6
10	106.1	5.3	6
20	105.7	6.2	6
30	131.1	5.1	6
40	122.6	5.1	6
50	108.5	5.3	6
60	86.4	5.1	6
70	61.0	5.5	6
80	29.5	2.9	6
90	10.2	2.0	6
100	2.0	1.3	6

Fig. 5.5.1.9.

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



Albumin Concentration (mg/l)	Mean	±s.e.m.	n
0	100.0	3.3	6
75	53.0	0.8	6
125	53.4	1.8	6
150	52.5	2.3	6
175	55.2	1.2	6
225	54.7	1.9	6

concentrations, while all p-oils, without exception, were growth limiting; such potential, however, varied greatly between p-oils and concentrations supplemented. Saturated FA-rich p-oils were the least effective in mediating growth inhibition; an ID_{50} was found for p-CO (90mg/l), but not for p-MO. In contrast, p-oils abundant in unsaturated FA's were considerably more cytotoxic. ID_{50} values for p-OO and p-LO equated to approximately 85mg/l each, whereas that for p-SSO and p-FO was lower, at about 75mg/l. Cell dosed with p-EPO exhibited an ID_{50} of 63mg/l, thus p-EPO was the most cytotoxic p-oil.

Cell viability was limited to 89.1% with 10 and 20mg/l p-CO dosage, but approximated to controls with 30 and 40mg/l p-CO (Fig. 5.5.1.2.). Concentration increases to 80mg/l p-CO limited cell growth to a minimum of 74.0%, while cytotoxicity reduced cell viability to 49.6% and 46.2% with 90 and 100mg/l p-CO, respectively.

Cell proliferation was enhanced with incubation of up to 40mg/l p-MO, to a maximum of 130.6% with 30mg/l (Fig. 5.5.1.3.). 50 and 60mg/l p-MO had little effect on cell viability, and growth limitation was induced with higher concentrations, but no cytotoxicity was induced, even with 100mg/l p-MO, as relative cell viability was 69.5%.

Cell proliferation was enhanced to 113.5%, 113.7% and 124.7% with 10, 40 and 50mg/l p-OO, respectively, while

(Fig. 5.5.1.4.). Higher concentrations limited cell growth to 68.5% with 70mg/l p-OO, and cytostasis was induced with 80mg/l p-OO as cell viability (58.8%) approximated to the cytostatic number (61%). Further concentration increases induced cytotoxicity, and only 12.9% of cells were viable with 100mg/l p-OO.

10 and 20mg/l p-SSO enhanced cell proliferation to 126.2% and 118.8%, respectively, while cell viability ranged from 109.4% to 112.3% with 30 to 50mg/l p-SSO (Fig. 5.5.1.5.). Concentrations greater than 60mg/l were growth limiting and yielded 77.8% viable cells with 70mg/l p-SSO, while extensive cytotoxicity reduced cell viability to 26.5% with 80mg/l. The proportion of cells taking up Trypan blue increased progressively as greater amounts of p-SSO were dosed, such that only 3.4% viable cells were counted with 100mg/l p-SSO.

p-LO dosage reduced cell viability in an almost linear concentration dependant manner (Fig. 5.5.1.6.). 50 and 60mg/l p-LO induced cytostasis, while concentrations of 70mg/l or greater induced cytotoxicity such that cell viability was reduced to 33.2% with 100mg/l p-LO.

10 and 20mg/l p-EPO had little effect on cell growth, but this was progressively reduced with higher amounts dosed such that cytostasis was induced with 60mg/l p-EPO (Fig. 5.5.1.7.). Further concentration increases induced

70mg/l p-EPO, while Trypan blue uptake increased to 100% with 100mg/l p-EPO.

Cell proliferation was enhanced slightly with 10, 20 and 50mg/l p-FO, but significantly to 131.1% and 122.6% with 30 and 40mg/l p-FO, respectively (Fig. 5.5.1.8.). Growth limitation reduced cell viability to 86.4% with 60mg/l p-FO, 70mg/l p-FO induced cytostasis, while cytotoxicity progressively increased cell killing with dosage of higher concentrations such that Trypan blue uptake was 98% 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 (63mg/l p-EPO, ID_{50}) and dosed with varying amounts of albumin. Five points were chosen around which to vary the albumin concentration, keeping the p-oil concentration fixed, viz. 2 points above, 2 points below, and the ID_{50} of p-EPO. The appropriate amount of albumin was added to these cultures to give the desired concentrations. The results shown in Fig. 5.5.1.9. indicated that the concentration of p-EPO dosed reduced cell viability to approximately 50% of controls, and that such was not influenced by different albumin concentrations. Thus, the effects induced with the p-oils could be attributed solely to

could be excluded.

Fig. 5.5.1.10. depicts the growth of cells derived from human skin in culture medium enriched with 10% serum from its own species was compared to commercially available fetal calf serum (FCS) in order to determine whether FCS modulated cell growth in any way. Over a 72 hour period, human skin fibroblasts grew from 10.0 to $14.8x10^4$ when culture medium was enriched with 10% FCS. Cell growth was slower, however, with 10% human serum (HS) over the same time period, from 10 to $12.2x10^4$. Thus, the routine use of FCS did not limit cell growth compared to 10% HS, and promotion of growth limitation and inhibition could therefore be attributed solely to the exogenous FA's incorporated.

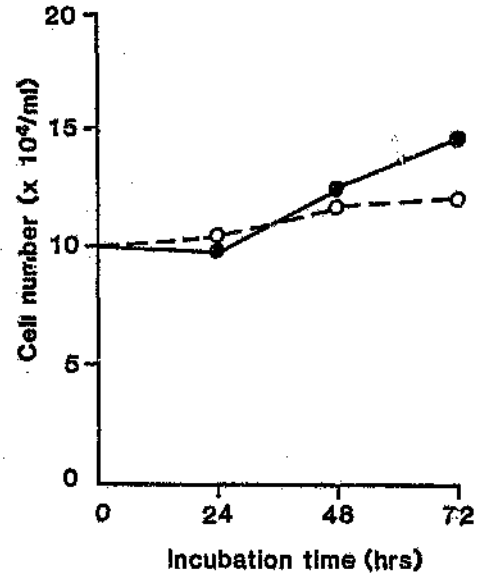
Human skin fibroblasts were subsequently plated and dosed appropriately with 0, 20, 40 or 60mg p-oil/l culture medium in sufficient amounts for all qualitative and quantitative analyses to be performed. Cell viabilities were compared upon harvesting and found to be statistically similar to those in Figs. 5.5.1.1-5.5.1.8., thus all further biochemical assays were performed on these samples.

Legend to Fig. 5.5.1.10.

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. 5.5.1.10. shows mean cell numbers versus the incubation period (hours).

Fig. 5.5.1.10.

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



Incubation Time (hrs)	Mean Cell Number (x10⁴/ml)	±s.e.m.	n
Foetal calf serum:			
24	10.0	0.1	9
48	12.6	0.2	9
72	14.8	0.3	9
Adult human serum:			
24	10.7	0.2	9
48	11.8	0.4	9
72	12.2	0.3	9

5.5.2 Effects of pseudo-Oils on Total Protein.

The total protein concentrations determined at each of the 3 p-oil concentrations incubated with human skin fibroblasts are shown in Table 5.5.2.1. in relation to controls. Results are expressed as µg total protein/10^6 cells seeded.

308.2µg protein/10^6 control cells seeded was found, while concentrations in dosed cells ranged from 238.2 to 423.0µg/10^6 cells seeded. In every instance, cells dosed with 20 or 40mg/l p-oil contained more total protein than controls, while most cells dosed with 60mg/l p-oil contained protein concentrations parallel to, or lower than, controls. Total cellular protein concentrations correlated inversely with the amount of p-LO or p-EPO supplemented (334.9, 311.0 and 307.0, and 349.9, 333.5 and 300.1µg, respectively), while protein levels were alike with dosage of 40 or 60mg/l p-SSO, but increased 20mg/l p-SSO (345.3, 348.5 and 372.2µg, respectively). On the other hand, cellular protein amounts were greater with incubation of 40 than 20 or 60mg/l p-CO (352.5, 336.5 and 238.2µg, respectively), p-MO (423.0, 342.6 and 349.7µg, respectively), p-OO (378.2, 366.3 and 247.1µg, respectively) or p-FO (369.5, 355.6 and 280.9µg, respectively).

The spent incubation media derived from these cultures were also assayed for total protein. The concentrations

Table 5.5.2.1.

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

pseudo-Oil (mg/l)	CELLS			
	0	20	40	60
Control	308.2			
CO		336.5	352.5	238.2
MO		342.6	423.0	349.7
OO		366.3	378.2	247.1
SSO		372.2	345.3	348.5
LO		334.9	311.0	307.0
EPO		349.9	333.5	300.1
FO		355.6	369.5	280.9

for all samples were summed, and a mean of 40.6±0.38mg protein/10^6 cells seeded obtained (n=22). Statistical comparison of individual samples, by analysis of variance, however, showed no significant differences in the spent media protein concentrations between control and dosed cultures (F=136.5, p=0.001).

5.5.3 Effects of pseudo-Oils on the Fatty Acid Spectrum of Cells Derived From Human Skin.

The FA spectra of cells incubated with 20, 40 or 60mg/l p-oil are shown in Table 5.5.3.1. in relation to controls.

The greatest FA proportion in control cells was detected for 18:1ω9 (27.5%). Other FA's contributing ≥5.0% to the total spectrum in control cells were 16:0 (21.3%), 18:0 (15.7%), 16:1ω9 (7.4%) and 22:4ω6 (10.7%).

Dosed cells contained variable amounts of 16:0 and 18:1ω9 compared to controls, while 18:0 proportions were lower in dosed than control cells in every instance. Decreased 16:1ω9 levels were generally found in dosed cells, while the converse was true for 18:2ω6. However, ω6 and ω3 PDFA levels were more variable.

16:0 levels increased when cells were dosed with 20, 40 or 60mg/l p-CO (27.5%, 28.9% and 29.7%, respectively vs 21.3% in controls), 18:1ω9 levels were not significantly altered (27.5% in controls), while decreased proportions of 18:0 (9.5% to 11.0% vs 15.5% in controls), 16:1ω9 (2.9% to 5.4% vs 7.4% in controls) and 18:2ω6 (2.6% to 2.9% vs 3.6% in controls) were found. 20:4ω6 and 22:4ω6 levels were slightly increased (4.5% to 5.7% and 12.3% to 13.0% vs 4.0% and 10.7% in controls, respectively), but 22:5ω6 was decreased. Increased 20:5ω3 proportions

Legend to Tables 5.5.3.1. and 5.5.3.2.

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 5.5.3.1.

The fatty acid spectrum of cells derived from human skin.

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	16:0	21.3±0.10	27.5	28.9	29.7	24.6	25.1	25.1	17.4	15.8	18.6	20.1	18.0	16.5	20.1	17.8	27.2	19.4	17.6	17.1	20.3	18.8	35.6
	18:0	15.7±0.70	11.0	10.2	9.5	11.0	12.2	9.9	6.4	4.3	4.9	9.8	8.9	7.1	10.4	9.7	13.9	10.1	11.9	7.6	9.0	10.7	9.1
	20:0	0.2±0.00	-	0.1	-	-	0.1	-	-	0.1	-	-	-	-	-	-	0.1	-	-	-	-	-	-
	22:0	-	0.1	0.1	0.3	0.1	0.1	0.1	0.1	0.1	-	0.1	-	-	-	-	0.1	-	-	-	-	-	-
	24:0	-	-	-	-	-	0.4	0.4	-	-	-	-	0.1	-	-	-	-	0.4	0.2	-	0.1	-	-
ω9 MONOS.	16:1	7.4±0.15	5.4	4.1	2.9	2.7	2.4	1.6	3.7	4.5	1.9	4.1	3.0	2.1	3.8	2.2	2.4	4.4	2.6	2.3	12.6	17.1	12.6
	18:1	27.5±0.25	28.9	27.2	28.4	27.6	28.4	29.6	46.0	47.8	50.8	28.4	26.2	25.0	25.5	23.0	21.1	22.5	20.9	18.9	24.9	24.3	18.9
	24:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω6 POLYS.	18:2	3.6±0.13	2.6	2.6	2.9	4.6	6.4	9.2	5.7	7.7	6.5	16.7	24.8	31.1	7.0	10.0	5.9	18.0	25.7	34.9	4.5	6.6	4.5
	18:3	-	-	-	-	-	-	0.1	0.1	0.1	-	0.2	0.1	-	-	-	-	0.4	0.4	0.6	-	-	-
	20:2	-	-	-	0.1	-	0.3	0.3	0.7	1.5	1.3	1.0	1.5	1.5	0.2	0.4	0.1	1.2	1.3	1.5	0.2	0.5	0.2
	20:3	0.7±0.00	0.9	0.9	1.1	1.2	1.3	1.3	1.0	1.2	0.5	2.0	2.0	1.1	0.9	0.8	0.7	3.6	3.8	3.7	0.9	0.7	0.4
	20:4	4.0±0.10	5.7	4.7	4.5	6.3	5.8	5.8	3.8	3.5	1.5	4.3	3.1	2.9	4.9	4.9	2.9	4.8	3.2	3.0	3.7	2.5	1.5
	22:4	10.7±0.60	12.3	13.0	12.8	16.2	9.8	10.4	10.1	8.5	10.5	9.2	7.0	8.6	14.4	7.2	13.7	10.5	7.0	6.6	15.7	7.2	11.1
	22:5	0.8±0.00	-	0.6	0.2	0.1	0.7	0.1	-	0.4	0.2	0.1	0.6	0.2	-	0.6	0.1	0.2	0.6	0.1	0.2	0.9	0.4
ω3 POLYS.	18:3	0.5±0.00	0.5	0.5	0.5	0.7	0.5	0.5	0.9	0.7	0.5	0.6	0.5	0.4	6.2	17.4	8.4	0.5	0.3	0.3	0.5	0.5	0.4
	18:4	2.4±0.00	0.4	1.2	0.4	0.2	1.2	0.3	0.3	0.6	0.2	0.2	0.8	0.2	0.4	1.1	0.2	0.2	1.1	0.1	0.3	1.6	0.3
	20:4	1.9±0.07	0.6	1.8	1.3	0.9	1.1	1.2	0.4	0.4	1.0	0.4	0.6	0.8	1.3	1.9	0.9	0.4	0.8	0.5	1.2	1.6	1.2
	20:5	0.7±0.00	0.3	0.7	1.7	0.2	0.7	1.3	0.9	0.5	0.5	0.2	0.4	0.3	1.6	0.6	0.7	0.7	0.4	0.4	1.8	3.9	2.3
	22:5	1.1±0.10	1.7	1.5	1.6	1.7	1.5	1.3	1.2	1.1	0.5	1.3	1.0	1.1	2.0	1.3	0.9	1.3	0.9	1.1	2.9	2.6	1.0
	22:6	1.5±0.05	2.3	2.2	2.2	1.9	2.0	1.7	1.4	1.5	0.6	1.5	1.3	1.3	1.4	1.3	1.0	1.5	1.3	1.3	1.2	0.8	0.5

were found with 60mg/l p-CO (1.7% vs 0.7% in controls), while 22:5ω3 and 22:6ω3 levels were slightly increased with all concentrations (1.5% to 1.7% and 2.2% to 2.3% vs 1.1% and 1.5% in controls, respectively in controls.

Dosage with p-MO had little effect on cellular 16:0 and 18:1ω9 levels, while 18:0 and 16:1ω9 were decreased. On the other hand, increased 18:2ω6 was detected (4.6% to 9.2% vs 3.6% in controls) and 20:3ω6 percentages (1.2% to 1.3%) were almost twice as great as controls (0.7%). 20:4ω6 levels were raised with all concentrations (6.3% with 20mg/l, and 5.8% each with 40 and 60mg/l vs 4.0% in controls), while 22:4ω6 was significantly increased with 20mg/l p-MO (16.2% vs 10.7% in controls), but no increment was shown for 22:5ω6. 20:5ω3 proportions increased approximately 2 fold with 60mg/l p-MO dosage (1.3% vs 0.7% in controls), while small increments in 22:5ω3 and 22:6ω3 were found with all concentrations dosed (1.3% to 1.7% and 1.7% to 2.0% vs 1.1% and 1.5% in controls, respectively).

Supplementation with 20, 40 or 60mg/l p-OO resulted in a marked elevation of cellular 18:1ω9 (46.0%, 47.8% and 50.8%, respectively vs 27.5% in controls), while 16:0, 16:1ω9 and especially 18:0 percentages decreased in relation to controls. Small increments were induced in 18:2ω6 (5.7% to 7.7% vs 3.6% in controls) and 20:2ω6 (0.7% to 1.5% vs 0% in controls), while all other PUFA levels approximated to, or were lower than, controls.

p-SSO dosage had little effect on, or decreased the proportions of the saturated and monounsaturated FA's detected, while 18:2ω6 increased in a concentration dependant fashion (16.7%, 24.8% and 31.1% with 20, 40 and 60mg/l, respectively vs 3.6% in controls). Small but significant increments in 20:2ω6 (1.0% with 20mg/l and 1.5% with 40 and 60mg/l vs 0% in controls) and 20:3ω6 (1.1% to 2.0% vs 0.7% in controls) were also found, but all other PDFA levels were parallel to controls or decreased.

18:1ω9, 16:1ω9 and 18:0 levels were decreased relative to controls (27.5%, 7.4% and 15.7%, respectively) with p-LO incubation, and increased 16:0 was found only with 60mg/l p-LO (27.8% vs 21.3% in controls). Raised 18:2ω6 levels were found with all concentrations (5.9% to 10.0% vs 3.6% in controls), but 22:5ω6 was decreased, despite small increments in 20:4ω6 with 20 and 40mg/l p-LO (4.9% each vs 4.0% in controls), and 22:4ω6 with 20 and 60mg/l p-LO (14.4% and 13.7%, respectively vs 10.7% in undosed cells). An increase in 18:3ω3 was induced with 20, 40 or 60mg/l p-LO dosage (6.2%, 17.4% and 8.4%, respectively vs 0.5% in controls). No significant increments in 18:4ω3 or 20:4ω3 were induced, but 20:5ω3 and 22:5ω3 increased approximately 2 fold with 20mg/l p-LO (1.6% and 2.0% vs 0.7% and 1.1% in controls, respectively).

p-EPO supplementation caused a reduction in cellular

16:0, 18:0, 16:1ω9 and 18:1ω9, but a marked increase in 18:2ω6 (18.0% with 20mg/l, 25.7% with 40mg/l and 34.9% with 60mg/l vs 3.6% in controls). No significant increment in 18:3ω6 was induced (0.4% to 0.6% vs 0% in controls), unlike 20:2ω6 and 20:3ω6 (1.2% to 1.5% and 3.6% to 3.7% vs 0% and 0.7% in controls, respectively). However, all other PDFA percentages were parallel to controls, or decreased.

Supplementation with 20, 40 or 60mg/l p-FO induced a marked increase in 16:1ω9 (12.6%, 17.1% and 12.6%, respectively vs 7.4% in controls), 16:0 was increased only with 60mg/l (35.6% vs 21.3% in controls), while 18:0 and 18:1ω9 proportions were decreased. 18:2ω6 levels were slightly increased with all concentrations (4.5% to 6.6% vs 3.6% in controls), and 22:4ω6 with 20mg/l p-FO only (15.7% vs 10.7% in controls), but all other ω6 PDFA amounts were parallel to controls or decreased. 20:5ω3 levels were significantly increased with 20, 40 or 60mg/l p-FO dosage (1.8%, 3.9% and 2.3%, respectively vs 0.7% in controls). Furthermore, 22:5ω3 was increased with 20 and 40mg/l p-FO (2.9% and 2.6%, respectively vs 1.1% in controls), although 22:6ω3 percentages decreased (1.2, 0.8% and 0.5%, respectively vs 1.5% in controls).

The FA spectra of the spent growth medium removed from each of the above cultures as shown in Table 5.5.3.2. The medium in which control cells had been grown

Table 5.5.3.2.

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

FATTY ACID SPECTRUM (%)		CONTROLS (n=3)	[p-CO] (mg/l) 20	40	60	[p-MO] (mg/l) 20	40	60	[p-OO] (mg/l) 20	40	60	[p-SSO] (mg/l) 20	40	60	[p-LO] (mg/l) 20	40	60	[p-EPO] (mg/l) 20	40	60	[p-FO] (mg/l) 20	40	60
SATURATES.	12:0	-	0.4	0.8	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14:0	97.7±0.46	96.7	96.5	96.9	97.7	97.5	97.3	96.9	96.6	95.2	97.3	96.9	95.6	97.2	96.5	95.5	96.8	96.9	96.6	96.4	95.9	96.0
	16:0	0.2±0.02	0.5	0.3	0.3	0.4	0.4	0.4	0.4	0.5	0.5	0.3	0.4	0.5	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.5	0.4
	17:0	1.6±0.09	1.4	1.4	1.0	0.8	1.1	0.9	1.3	1.4	1.6	0.9	0.9	1.4	1.0	1.6	1.3	1.6	1.4	0.9	1.9	1.6	1.1
	18:0	-	-	-	-	0.2	-	0.3	-	-	-	0.3	0.4	0.4	0.3	-	-	-	-	0.2	-	-	-
	20:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	22:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	24:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MONO.	16:1	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	-	0.1	0.2	0.6	0.9
	18:1	0.3±0.00	0.5	0.5	0.4	0.4	0.5	0.6	0.8	0.9	1.9	0.5	0.6	0.9	0.5	0.6	0.8	0.5	0.6	0.6	0.6	0.7	0.7
ω6 POLYS.	18:2	-	0.1	-	-	0.1	0.1	0.1	0.1	0.1	0.3	0.2	0.3	0.7	0.1	0.1	0.4	0.2	0.4	0.7	0.1	0.2	0.3
	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:4	-	0.1	0.1	-	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	22:4	0.2±0.04	0.3	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.1	0.2	0.2	0.3	0.2
	22:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ω3 POLYS.	18:3	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.4	1.0	-	-	-	-	-	-
	18:4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	20:4	-	-	-	-	-	-	-	-	0.1	0.1	-	-	-	-	-	-	0.1	0.1	0.1	-	0.1	-
	20:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.2
	22:5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-
	22:6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	0.1	0.1

contained 97.7% 14:0, 1.6% 17:0 and less than 0.5% each of 16:0, 18:1w9 and 22:4w6. Spent media obtained from cultures dosed with p-oils similarly contained at least 95.0% 14:0 and 0.8% to 1.9% 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.

5.5.4 Incorporation of Radiolabelled Fatty Acids into Cells Derived from Human Skin.

The radioactivity recovered from cultures incubated with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 is shown in Table 5.5.4.1.

For both [^{14}C]-18:1ω9 and [^{14}C]-18:3ω3, the equivalent of $4.3x10^6$cpm were dosed to the incubation medium, and total recovery of radiolabel from these cultures was approximately 86% and 91%, respectively. Similarly, the equivalent of $4.8x10^6$cpm [^{14}C]-18:2ω6 were dosed to the incubation medium, and total recovery of this radiolabel from cultures was about 98%. The total counts recovered from the spent incubation media ranged between 1.6 to $2.6x10^6$cpm, with only 0.6 to $0.8x10^6$cpm recovered from the pooled buffers after washing the cells; this accounted for non-specific binding. The total amount of each of these radioisotopes incorporated into the cells themselves, however, equated to 34% for [^{14}C]-18:1ω9, 32% for [^{14}C]-18:2ω6 and 19% 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 human skin fibroblasts is shown in Table 5.5.4.2. As *Δ6D* represents the first and rate limiting enzyme in the desaturase cascade, total activity thereof is reflected by the sum of all subsequent desaturase and elongase products.

Table 5.5.4.1.

The radioactivity (cpm) recovered from human 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				
1583080 (±5520)	640380 (±1900)	2223460	1470507 (±2817)	3.7
Total 18:2 counts dosed to incubation medium = 4.8×10^6				
2410120 (±12780)	785340 (±7380)	3195460	1541535 (±2340)	4.7
Total 18:3 counts dosed to incubation medium = 4.3×10^6				
2561820 (±5340)	567000 (±3660)	3128820	799068 (±540)	3.9

Table 5.5.4.2.

The counts detected for the radiolabelled fatty acids dosed and their resulting metabolites formed after incubation with cells derived from human 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	30.7	18:2	17.8	18:3	11.4
20:1	21.4	18:3	10.6	18:4	19.9
22:1	36.4	20:2	25.4	20:3	18.2
24:1	11.5	20:3	19.1	20:4	17.5
		20:4	10.9	20:5	14.1
		22:4	11.4	22:5	8.5
		22:5	4.7	22:6	10.4
TOTAL CPM	2130	TOTAL CPM	3026	TOTAL CPM	1711

30.7% of the total [^{14}C]-18:1ω9 counts derived upon GLC analysis (2130cpm) remained unchanged, while 21.4% was found for 20:1ω9, 36.4% for 22:1ω9, and only 11.5% elongated to 24:1ω9.

17.8% of the total [^{14}C]-18:2ω6-derived counts measured upon GLC analysis (3026cpm) remained as 18:2ω6, whereas 25.4% 20:2ω6 was detected. 10.6% of total counts were found as 18:3ω6 and 19.1% as 20:3ω6, compared to 10.9% 20:4ω6 and 11.4% 22:4ω6. However, only 4.7% 22:5ω6 was detected.

11.4% of total [^{14}C]-18:3ω3-derived counts (1711cpm) remained unchanged, and 18.2% was found for its immediate elongation product, 20:3ω3. Significantly more 18:4ω3 was detected than 18:3ω6 following the same Δ6D step (19.9% and 10.6%, respectively), but the converse was true with regard to 20:3ω3 and 20:2ω6 levels (18.2% and 25.4%, respectively). 17.5% 20:4ω3 was detected, while 14.1% and 8.5% of total counts were present as 20:5ω3 and 22:5ω3, respectively. Desaturation to 22:6ω3 accounted for 10.4% of total counts.

5.5.5 Effects of pseudo-Oils on Lipid Peroxide Formation.

The lipoperoxides quantitated in cells dosed with p-oils and their respective growth media are shown in Table 5.5.5.1. in relation to controls. The data is expressed as nmoles MDA/10^6 cells, but in the case of the spent media, this represents the nmoles of MDA in the volume of medium from which $1x10^6$ cells were obtained.

1.1nmoles MDA/10^6 control cells was measured, while amounts ranged from 1.0 to 2.3nmoles MDA/10^6 dosed cells, dependant on the p-oil and concentration used. Lipoperoxide production by cells dosed with p-MO or p-OO approximated to that of controls, and p-SSO only induced greater amounts with a concentration of 60mg/l (2.3nmoles MDA/10^6 cells). Cells supplemented with 20, 40 or 60mg/l p-CO or p-FO, however, induced identical lipoperoxide concentrations (1.3, 1.4 and 1.6nmoles MDA/10^6 cells, respectively), which were comparable to those induced with p-LO or p-EPO (1.4, 1.6 and 1.6, and 1.0, 1.3 and 1.5nmoles MDA/10^6 cells, respectively).

Spent medium from control cells contained 0.2nmoles MDA/10^6 cells, while amounts measured in spent media of dosed cultures ranged from 0.1 to 4.3nmoles MDA/10^6 cells. Spent media from cultures incubated with p-CO, p-MO or p-OO generally contained slightly lower lipid peroxide amounts (0.1 to 0.8nmoles MDA/10^6 cells) than

Table 5.5.5.1.

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

pseudo-Oil	CELLS			
(mg/l)	0	20	40	60
Control	1.1			
CO		1.3	1.4	1.6
MO		1.0	1.0	1.0
OO		1.2	1.0	1.0
SSO		1.2	1.0	2.3
LO		1.4	1.6	1.6
EPO		1.0	1.3	1.5
FO		1.3	1.4	1.6
pseudo-Oil	SPENT INCUBATION MEDIUM			
(mg/l)	0	20	40	60
Control	0.2			
CO		0.3	0.3	0.8
MO		0.5	0.2	0.3
OO		0.4	0.1	0.6
SSO		0.4	0.2	1.3
LO		1.3	1.5	4.3
EPO		0.2	0.6	1.0
FO		1.5	2.4	3.4

that derived from p-SSO or p-EPO incubation (0.2 to 1.3 nmoles MDA/10^6 cells. On the other hand, the greatest spent media lipid peroxide levels were found with incubation of 20, 40 or 60mg/l p-FO (1.5, 2.4 and 3.4 nmoles MDA/10^6 cells, respectively) or p-LO (1.3, 1.5 and 4.3nmoles MDA/10^6 cells, respectively).

5.5.6 The Eicosanoid Profile of Cells Derived from Human Skin.

The eicosanoids positively detected in control and dosed human skin fibroblasts are presented in Table 5.5.6.1. as a percentage of the total area found. An indication of the total eicosanoid amount quantified, however, is given for controls in "( )", expressed as pmoles/10^6 cells.

Total eicosanoid production by control cells amounted to 232.5pmoles/10^6 cells, of which 31.2% correlated with the retention times of the prostanoid standards used. This was composed of 12.4% TXB_2, 9.4% PGF_α, 4.7% PGI_2, 4.1% PGE_2 and 0.6% PGE_1. Varying proportions of these prostanoids were also found in dosed cells, but neither control or dosed cells formed PGD_2.

Total prostanoid percentages detected in dosed cells varied significantly with p-oil concentration, as such was increased in relation to controls (31.2%) with supplementation of 20mg/l p-oil (39.3% and 45.3%), decreased with 40mg/l p-oil (14.9% to 18.4%), but was more variable with 60mg/l p-oil (19.5% to 37.9%). Such changes related to alterations in the percentages of individual prostanoids in these cells. PGI_2 percentages were lower in dosed (1.5% to 4.1%) than in control (4.7%) cells. On the other hand, TXB_2 levels were comparable to controls (12.4%) with 60mg/l p-oil (8.4%

Legend to Table 5.5.6.1.

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

Table 5.5.6.1.

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

pseudo-Oil (mg/l)		EICOSANOIDS (%)						
		TOTAL	PGI_2	TXB_2	PGF_{α} (1+2)	PGE_2	PGE_1	PGD_2
CONTROL (232.5)		31.2	4.7	12.4	9.4	4.1	0.6	-
CO	20	39.3	2.9	20.2	13.1	2.4	0.7	-
	40	18.0	3.4	6.0	5.8	2.0	0.8	-
	60	28.9	3.6	11.9	10.2	3.1	0.1	-
MO	20	44.8	3.2	21.5	15.2	4.4	0.5	-
	40	17.4	2.3	6.0	7.2	1.6	0.3	-
	60	37.9	4.1	15.9	10.8	6.8	0.3	-
OO	20	42.0	3.8	18.5	14.1	5.1	0.5	-
	40	15.0	2.9	5.6	4.5	1.5	0.5	-
	60	23.4	2.8	10.3	8.8	1.1	0.4	-
SSO	20	41.4	3.7	21.7	11.9	3.1	1.0	-
	40	18.0	3.3	6.4	4.9	1.3	2.1	-
	60	19.5	3.0	9.0	5.9	1.2	0.4	-
LO	20	45.3	2.5	24.8	16.0	1.4	0.6	-
	40	16.2	2.9	6.0	5.8	1.2	0.3	-
	60	23.0	3.6	8.4	8.7	2.2	0.1	-
EPO	20	43.6	2.4	23.2	14.5	2.8	0.7	-
	40	14.9	2.1	6.6	4.0	1.3	0.9	-
	60	24.1	1.9	11.4	9.1	1.5	0.2	-
FO	20	40.7	3.4	20.0	12.5	4.0	0.8	-
	40	18.4	3.9	6.5	5.8	1.2	1.0	-
	60	28.0	1.5	14.8	10.3	1.3	0.1	-

to 15.9%), but approximately 2 fold lower with 40mg/l p-oil (5.6% and 6.6%) and up to 2 fold greater with 20mg/l p-oil (18.5% to 24.8%). PGF_{α} production was lower than controls (9.4%) with dosage of 40mg/l p-oil (4.0% to 7.2%), enhanced with 20mg/l p-oil (11.9% to 16.0%), and more variable with 60mg/l p-oil (5.9% to 10.8%). Dosed cells generally produced less PGE_2 than controls (4.1%), although such was slightly increased with 60mg/l p-MO and 20mg/l p-OO (6.8% and 5.1%, respectively). Comparable PGE_1 levels were induced in control (0.6%) and dosed cells with all p-oil concentrations, although production increased 3 to 4 fold with 40mg/l p-SSO (2.1%).

5.5.7 Discussion.

The data presented showed that the p-oils dosed to human skin cultures exhibited varying abilities to modulate cell viability (Figs. 5.5.1.1-5.5.1.8.), dependent on p-oil FA composition (Table 2.3.3.2.). The significantly greater ability to inhibit cell viability with dosage of p-OO, p-SSO, p-LO, p-EPO or p-FO than with p-CO or p-MO was consistent with large amounts of unsaturated FA's in the former p-oils. This indicated that unsaturated FA's were more effective cytotoxic agents than saturated FA's. The FA spectra of p-OO, p-SSO and p-LO suggested that the effects each p-oil induced with dosage related largely to the abundance of OA, LA or ALA present in such, respectively. The ability to stimulate cell proliferation with dosage of low concentrations of p-OO or p-SSO, but not with p-LO, as well as the greater ability to reduce cell viability with p-OO or p-SSO, than with p-LO, dosage at high concentrations, appeared to relate to the absence of a double bond at the ω3 position in OA and LA, but not in ALA. The FA composition of p-EPO was similar to that of p-SSO, although the presence of about 9% GLA in p-EPO only probably accounted for the inability of this p-oil to stimulate cell viability and the mediation of significantly more growth limitation and cytotoxicity than with p-SSO dosage. It was apparent therefore, that GLA exhibited greater cytotoxic ability than LA.

Comparison of the amount of GLA in p-EPO and ALA in p-LO (approximately 9% and 63%, respectively) with the growth profiles induced with dosage of each of these p-oils suggested that GLA was also a more effective cytotoxic agent than ALA. This could reflect the presence of a double bond at the ω12-position in GLA. The presence of GLA in p-EPO and ALA in p-LO may explain why dosage with these p-oils induced only growth limitation and cytotoxicity, whereas dosage with other p-oils devoid of these moieties also exhibited the ability to enhance cell growth. Although the changes induced with p-oil dosage may relate to specific p-oil FA's, the mediation of synergistic and/or antagonistic effects between p-oil FA's may have enhanced or suppressed the ability of a FA to induce a particular effect. This may explain, for example, the greater ability to stimulate cell growth with dosage of 30mg/l p-MO or p-FO, or the ability to effectively suppress growth limitation with dosage of 30 or 40mg/l p-CO, than with other concentrations of these p-oils.

This is the first report describing the modulation of human skin fibroblast growth following dosage with exogenous FA mixtures mimicking the FA composition of dietary oils; prior to this study, only the effects of individual FA's have been reported. Huttner et al (1978) showed that the growth responses induced following incubation of human foreskin fibroblasts with up to

100μM 18:1ω9, 20:3ω6 or 20:4ω6 for 7 to 9 days were both FA and concentration specific. 18:1ω9 or 20:4ω6 enhanced cell proliferation with concentrations between 0 and 60μM, to a maximum of 150% and 120%, respectively in the range 10-20μM; higher concentrations were growth inhibitory, but 20:3ω6 inhibited cell proliferation with concentrations of 1.6μM, or greater. On the other hand, Gavino et al (1981a) reported that the proliferation of primary neonatal foreskin cultures was inhibited with dosage of 16μM 20:3ω6 or 22:4ω6, and with 40μM 20:4ω6, or greater; none of these FA's, however, enhanced cell proliferation. Human skin fibroblast growth was not significantly influenced when Spector et al (1979) enriched the culture medium containing 10% fetal bovine serum with up to 100μM 18:1ω9 or 18:2ω6 for 7 days. This was also found with 16:0, 18:3ω3 or 20:4ω6 dosage in the range 10-20μM, although these moieties reduced cell growth by up to 50% with amounts of 50μM, or greater. Rosenthal (1981) also observed no morphological evidence of cytotoxicity when human skin fibroblasts were cultured with 15-20μM 16:0 or 18:0 for 24 hours, although dosage with 70μM 16:0 resulted in cytotoxicity. On the other hand, cells supplemented with 140μM 18:1ω9 contained numerous lipid droplets, but were otherwise normal. It was suggested that the ability to induce more cytotoxicity with saturated, than unsaturated, FA dosage related to solid depositions of saturated fat found with

16:0 dosage, rather than to the discrete osmiophilic droplets found with 18:1ω9 dosage. This, however, was not consistent with a previous study in our laboratory which showed that the inhibition of human skin fibroblast growth with C18 FA supplementation increased in the order 18:0, 18:1ω9, 18:2ω6, 18:4ω3, 18:3ω3 and 18:3ω6 (Girao 1988). No FA concentration dosed (3-350μM) enhanced cell proliferation, although Vignikin et al (1989) reported that human infant skin fibroblasts exhibited a faster growth rate over a 7 day period in the presence of 250μM 18:1ω9, compared to controls, and thus implicated 18:1ω9 involvement in the stimulation of cell proliferation. The rate of cell growth, however, was unaltered with supplementation of 250μM 18:2ω6 or 20:4ω6, despite ultrastructural modifications (lipid droplet formation). Their data nevertheless showed that dosage with 250μM 18:1ω9, 18:2ω6 or 20:4ω6 did not pose a cytotoxic threat to these cells.

It was clear from comparison of the literature reports discussed above that there is both aggreement and dissagreement between workers regarding the effect of exogenous FA supplementation on cultured human skin fibroblast growth. Much of the discrepency reported, however, may reflect variations found in culture conditions between workers. The data reported both in the literature and the present study nevertheless clearly indicate that FA's have the ability to modulate

human skin fibroblast proliferation and that the effects induced are both FA and concentration specific. It was apparent from comparison of the literature reports with the results obtained in Figs. 5.5.1.1-5.5.1.8. that the effects induced with p-oil dosage reflected some of the effects reported with supplementation of single FA's, although the growth response to p-oil dosage may be quite different to that induced with supplementation of individual FA's. This supported the mediation of synergistic and/or antagonistic effects between p-oil FA's. The modulation of cell viability reported with individual FA dosage may thus not necessarily reflect the situation in the 'real world' as cells in vivo are exposed to mixtures, rather than individual, FA's. p-Oil FA composition mimicked that of dietary oils, thus the data reported in Figs. 5.5.1.1-5.5.1.8. may reflect the effect of dietary oil ingestion on cell growth in vivo.

Cultured human skin fibroblasts readily take up exogenous FFA's from the surrounding medium and incorporate them into cellular lipids, thus altering the FA composition of the cells (Spector et al 1979, 1981a, Rosenthal 1978, 1980, 1981, Gavino et al 1981b and Banerjee et al 1985). These workers report up to 40% FA incorporation, and this correlated with the data in Table 5.5.4.1., following [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 dosage. The FA profiles of human skin fibroblasts and the corresponding spent medium (Tables

5.5.3.1. and 5.5.3.2.) supported p-oil FA incorporation. Thus it was possible that membrane fluidity was altered, and that such was involved in the mediation of growth limitation and cytotoxicity reported in Figs. 5.5.1.1-5.5.1.8. Such a mechanism was possible since greater exogenous FA incorporation has been shown into membrane PGL's than into TAG's when [^{14}C]-16:0, [^{14}C]-18:0, [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 were added to human skin fibroblast cultures (Rosenthal et al 1978 and Rosenthal 1981). However, when Spector et al (1979) added 16:0, 18:1ω9, 18:2ω6, 20:3ω6 or 20:4ω6 to the culture medium of such cells, the greatest changes were induced in the neutral lipids, despite significant changes in the PGL fraction. Cellular TAG accumulation results in cytoplasmic lipid droplet formation (eg. Rosenthal et al 1981 and Spector et al 1981a), and such may also be involved in the modulation of cytotoxicity through cell lysis when excess TAG accumulates.

Fujimoto et al (1977) and Pentland et al (1986) quantitated cellular protein in human skin fibroblast cultures to monitor cell growth, and measured similar amounts of protein at the end of the incubation period (2-3 days) to that reported for control cells in our study (308μg protein/10^6 cells; Table 5.5.2.1.). A previous study in our laboratory, utilising similar culture conditions to those described in section 2.2.1, showed about 8μg protein/10^6 cells in the plasma

membrane fraction obtained from control human skin fibroblast cultures (Girao 1988). It was thus apparent from comparison of the above studies that <5% of total cellular protein is plasma membrane related.

Total protein quantitation of p-oil supplemented human skin fibroblasts showed changes (Table 5.5.2.1.) in such which generally reflected the cell viability changes induced (Figs. 5.5.1.2-5.5.1.8.). However, measurement of increased cellular protein when p-oil dosage induced little effect on, or limited, cell viability implied p-oil FA involvement in the stimulation of protein synthesis. Quantitative variations in the amount of cellular protein measured when dosage with identical concentrations 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. However, it was unlikely that medium protein itself contributed significantly to the cellular protein changes found since the protein amounts measured in the spent medium of dosed cells were not statistically different from controls (section 5.5.2). Supplementation of human skin fibroblast cultures with individual C18 FA's has been shown to have little effect on the plasma membrane protein content per viable cell, irrespective of changes in cell viability (Girao 1988). Thus it was likely that the changes reported in total protein per cell with

p-oil dosage reflected alterations in intracellular, rather than plasma membrane, protein. Stimulation of protein biosynthesis with p-oil dosage may therefore reflect enhanced expression of enzymes to metabolise incorporated p-oil FA's.

Comparison of FA profiles for p-oil dosed cells and controls indicated that human skin fibroblasts exhibit the capability to desaturate and elongate PUFA's, although enzyme expression varied with p-oil dosed (Table 5.5.3.1.). 18:3ω3 incorporation and the formation of small amounts of 20:5ω3 and 22:5ω3 with 20mg/l p-LO supplementation suggested limited Δ6D, Δ5D and elongase capability. 22:5ω3 formation with p-FO dosage also indicated expression of the elongase enzyme responsible for addition of 2 carbon atoms to the C20 backbone of incorporated 20:5ω3. However, 22:6ω3 levels lower than controls with p-LO or p-FO supplementation indicated suppressed Δ4D activity. On the other hand, formation of small amounts of 20:5ω3, 22:5ω3 and 22:6ω3 with p-CO or p-MO dosage reflected desaturation and elongation of cellular ω3 PUFA's as these p-oils were deficient in such moieties. This reflected an attempt to enhance PDFA production possibly to maintain membrane fluidity altered with supplementation of these saturated FA-rich p-oils. Such may also explain the formation of 20:4ω6 and 22:4ω6 with p-CO dosage, while the inability to form 22:5ω6 via Δ4D with p-CO or p-MO dosage may relate to

the greater preference for ω3 rather than ω6 substrates for desaturation established by others (Mohrhauer et al 1963a, Brenner et al 1966 and Brenner 1974). The formation of small 20:3ω6 amounts when p-MO or p-SSO were dosed reflected limited delta-6-desaturation and elongation of incorporated 18:2ω6, and 20:3ω6 formation with p-EPO dosage was thus consistent with desaturation and/or elongation of incorporated 18:2ω6 and 18:3ω6. On the other hand, the small proportion of 20:3ω6 formed with p-OO supplementation suggested limited capability for Δ8-desaturation of incorporated 20:2ω6. Small but significant amounts of 20:2ω6 were formed via direct elongation of 18:2ω6 incorporated with p-SSO or p-EPO dosage, thus it was possible that delta-8-desaturation of 20:2ω6 also contributed to the amount 20:3ω6 detected. It was apparent, however, that the inability to form 22:4ω6 or 22:5ω6 with p-OO, p-SSO or p-EPO dosage related to 20:4ω6 deficiency due to insufficient Δ5D expression. The formation of small amounts of 22:4ω6 with p-FO or p-LO incubation nevertheless reflected limited capability for desaturation (Δ6D and Δ5D) and elongation of incorporated 18:2ω6. While it is well known that 20:5ω3 and 18:3ω3 inhibit 18:2ω6 desaturation (Mohrhauer et al 1963b, Garcia et al 1965, Brenner et al 1966, 1967, Brenner 1974 and de Schriver et al 1982), detection of relatively small 20:5ω3 and 18:3ω3 amounts with p-FO or p-LO supplementation, respectively may have permitted limited desaturation of incorporated 18:2ω6.

The pattern of radioactivity derived from human skin fibroblasts dosed with [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 supported Δ6D and subsequent elongase expression (Table 5.5.4.2.). 20:3ω6 and 20:4ω3 formation may also relate partly to direct EFA elongation and subsequent delta-8-desaturation, particularly if Δ6D activity was limiting. Conversion of about 30% [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 each to post-Δ5D FA's supported Δ5D expression, but significant Δ4D expression was found only with [^{14}C]-18:3ω3 dosage.

Numerous studies support the capabililty of cultured human skin fibroblasts to actively desaturate and elongate incorporated exogenous FA's. Using similar culture conditions to our study, Dunbar et al (1975) demonstrated Δ6D, subsequent elongase and Δ5D capability when the culture medium of human skin fibroblasts containing 10% FCS was supplemented with 2μCi [^{14}C]-18:2ω6. The proportion of incorporated [^{14}C]-18:2ω6 (34%) converted to post-Δ6D and post-Δ5D FA's correlated with the present study (Tables 5.5.4.1. and 5.5.4.2.), but the greater proportion of [^{14}C]-18:2ω6 elongated to 20:2ω6 in our study (about 25% and 3%, respectively) was consistent with the smaller proportion of incorporated [^{14}C]-18:2ω6 left unchanged compared to Dunbar's group (approximately 18% and 55%, respectively). Aeberhard et al (1978) not only confirmed Δ6D, Δ5D and elongase expression in human skin fibroblasts, but also showed

traces of Δ4D, but not Δ9D, activity. Rosenthal et al (1982) found that human skin fibroblasts grown in medium with 10% fetal bovine serum converted only 15-20% of incorporated [^{14}C]-18:2ω6 to PDFA's. However, Δ6D and Δ5D activities were enhanced by prior growth of the cells without serum lipids, and only 20% of incorporated [^{14}C]-18:2ω6 remained unchanged at the end of the 24 hour incubation period (Rosenthal et al 1983a). There are also reports that EFA deficiency enhances desaturase expression (eg. Brenner 1982). The large proportion of incorporated [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 metabolised in the present study (80-90%; Table 5.5.4.2.) may thus relate partly to enhancement of desaturase expression due to EFA deficiency in the incubation medium prior to [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 dosage. Using similar culture conditions to the present study, Girao (1988) reported evidence for Δ6D, Δ5D and Δ4D expression in skin fibroblasts cultured with 18:3ω3, although activity for the latter enzyme was not shown with 18:2ω6 dosage. This correlated with the data in Table 5.5.4.2. when [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 was supplemented. The inability to significantly desaturate or elongate 16:0, 18:0, 16:1ω9 or 18:1ω9 incorporated with p-oil dosage (Table 5.5.3.1.) supported and extended the work of Girao (1988) which indicated that these cells were unable to desaturate or elongate 18:0 or 18:1ω9. However, 24:1ω9 detection when we dosed cultures with [^{14}C]-18:1ω9 was consistent with elongase expression,

although [^{14}C]-18:1ω9 desaturation was not found (Table 5.5.4.2.). [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3 dosage nevertheless confirmed the pattern of substrate preference for desaturation (18:1ω9 < 18:2ω6 < 18:3ω3) established by Rosenthal et al (1982) in cultured human skin fibroblasts.

There are some reports which indicate that cultured human skin fibroblasts are unable to efficiently convert 18:2ω6 to 18:3ω6, 20:3ω6 to 20:4ω6, or 22:4ω6 to 22:5ω6, via Δ6D, Δ5D, and Δ4D, respectively, despite the capability to incorporate and elongate FA's (Spector et al 1979, 1980a, 1981a, Gavino et al 1981c, Chapkin et al 1984, 1986a, 1986b and Ziboh et al 1988). These studies implied suppression of desaturase activity and suggested that the skin may be reliant on other tissues for PDFA's. On the other hand, this could also reflect the ability of human skin fibroblasts to selectively control PDFA formation. There is indeed evidence to suggest 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). Elongase expression appears to be influenced to a lesser extent since cultured human skin fibroblasts elongate saturated and unsaturated FA's, even when exogenous FA concentrations are increased (Dunbar et al 1975, Spector

et al 1979, 1981a, Gavino et al 1981a, 1981b, Tsuji et al 1984 and banerjee et al 1986). The greater capability for elongation than desaturation in the present study supports the fact that desaturase steps are rate limiting (Bernert et al 1975, Hassam et al 1975 and Brenner 1982). Banerjee et al (1986) showed that elongase substrates may act as positive modulators of their own elongation, and this may also explain elongase product formation in our study (Tables 5.5.3.1. and 5.5.4.2.). There is also some evidence that cultured human skin fibroblasts exhibit the capability for extramicrosomal retroconversion of elongated PUFA's (Gavino et al 1981a), thus the possibility that such may have occurred in the present study can not be dismissed.

We showed that the overall capability for desaturation and elongation was more limited with p-oil (Table 5.5.3.1.), than with individual C18 FA (Table 5.5.4.2.), dosage. This may relate to competitive interactions between p-oil FA's for the enzymes. Enzyme expression reported with individual FA dosage may nevertheless not necessarily reflect the situation in the "real world" as cells in vivo are exposed to FA mixtures rather than to single FA's. As p-oil FA composition mimicked that of dietary oils, enzyme expression reported with p-oil supplementation probably reflects the capability of human skin to desaturate and elongate dietary oils in vivo.

The results obtained from the measurement of cellular lipoperoxides showed evidence of such compounds both in the presence and absence of p-oil dosage (Table 5.5.5.1.), and this reflected cellular enzymic and/or non-enzymic mechanisms for their production. Lipid peroxide measurement in control cells was not surprising as these cells contained FA's which were potential substrates for oxidation (Table 5.5.3.1.). The greater lipoperoxide amounts measured with p-oil dosage than in controls, both in the spent medium and cells themselves, was consistent with oxidation of unsaturated p-oil FA's. However, the ability of these cells to incorporate p-oil FA's (Table 5.5.3.1.) indicated that it was unlikely that the lipoperoxides measured in the spent medium of dosed cells were formed primarily due to auto-oxidation of unincorporated p-oil FA's, particularly since these moieties were albumin-bound. The FA composition of the incubation medium was primarily composed of saturated FA's (Table 2.3.3.3.), thus it was also unlikely that such contributed significantly to the spent medium lipoperoxide levels reported for control and dosed cultures. The data therefore suggested that the lipoperoxides measured in the spent medium originated intracellularly, although it was apparent that these products were not released as a result of cell lysis since cytotoxicity was absent even with 60mg/l p-oil incubation (Figs. 5.5.1.1-5.5.1.8.). However, lipid peroxides can increase plasma membrane

permeablity (Chio et al 1969, Tappel 1975, Mead 1976 and Frankel 1984), thus spent medium lipoperoxides probably reflect intracellular lipoperoxide leakage through the plasma membrane. Cellular FFA's and enzymes responsible for lipoperoxidation may also have been released into the medium, 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.

Gavino et al (1981c) found that lipid peroxide formation increased in the order 20:3ω6, 20:4ω6 and 22:4ω6 when 120μM of each moiety was incubated with human neonatal foreskin fibroblasts for 52 hours. Girao (1988) showed that lipid peroxidation in human skin fibroblast cultures dosed with individual C18 FA's related both to the number and position of double bonds in the FA chain. No more lipoperoxides were induced with saturated or monoenoic FA dosage than were produced by controls, while lipoperoxide production increased in the order 18:4ω3, 18:2ω6, 18:3ω3 and 18:3ω6 when these PUFA's were dosed. Control cell lipoperoxide levels correlated with the present study (Table 5.5.5.1.), reflecting similarities in the culture conditions employed, while the lower increment in lipoperoxide formation when these cultures were dosed with p-oils than individual C18 FA's is probably a better indication of <u>in vivo</u> production when dietary oils are fed.

Numerical differences in the lipoperoxide concentrations reported with p-oil dosage (Table 5.5.5.1.) reflected the different p-oil FA compositions (Table 2.3.3.2.). This relates to the amount of unsaturated FA's in any p-oil and their susceptibility to oxidation, dependent on the number and position of double bonds in a FFA. The total PUFA concentration of p-EPO and p-SSO was similar to that of p-LO, and significantly greater than that of p-FO, yet total lipoperoxide production was greatest with p-LO or p-FO dosage. p-Oil FA composition suggested that such related to the greater susceptibility of the ω3 PUFA's in p-LO and p-FO to oxidation, compared to the ω6 PUFA's in p-EPO and p-SSO. The ALA content of p-LO was greater than the amount of EPA in p-FO (about 63% and 18%, respectively), although the greater number of double bonds in EPA increased its susceptibility to oxidation compared to ALA. This probably explains the similar lipid peroxide amounts induced overall with p-LO or p-FO dosage. The lipoperoxides detected may also relate to the rate of PGL turnover. Increased PGL cycling increases the rate of FA release from membrane lipids, and this may explain, for example, the significantly greater amount of lipoperoxides generated by cells dosed with 60mg/l p-SSO than with p-EPO, despite both p-oils exhibiting similar FA compositions. On the other hand, decreased PGL cycling may explain why fewer lipid peroxides were measured with supplementation

of p-MO or p-OO, than with p-CO, despite the significantly greater unsaturated FA content of the former p-oils. It was also possible that lipo-peroxidation related to the utilisation of free DGLA, AA and/or EPA formed with p-oil dosage (Table 5.5.3.1.) as endoperoxide substrates.

The concentration dependent increase in lipoperoxide formation shown when cultures were incubated with 20, 40 or 60mg/l p-LO . p-EPO correlated inversely with the changes reported in cell viability (Figs. 5.5.1.6. and 5.5.1.7., respectively). This suggested lipid peroxide involvement in the modulation of cell proliferation, particularly since lipid oxidation products have been implicated in cellular protein and tissue damage (Tappel 1975, 1980). As the epidermis is exposed to oxygen <u>in vivo</u>, the skin may be partly resistant to the effects of lipoperoxides. This may explain the stimulation and limitation, but not inhibition, of cell proliferation shown with dosage of 20, 40 or 60mg/l p-oil, even when significant amounts of cellular and/or medium lipid peroxides were measured. However, lipoperoxides should not be dismissed as a possible mechanism involved in the modulation of growth inhibition with supplementation of higher p-oil concentrations. Growth limitation was not most pronounced with p-LO or p-FO dosage, despite the greatest amount of lipoperoxides measured overall with these p-oils. The modulation of cell viability with

p-oil supplementation thus probably also involves other mechanisms, such as alterations in membrane fluidity.

The finding that total eicosanoid production accounted for approximately 20% of all lipoperoxides measured in control human skin fibroblasts (Tables 5.5.5.1. and 5.5.6.1.) suggested that the eicosanoid requirement of these cells was low, that the cells themselves exhibited limited capability to synthesise eicosanoids and/or that production was limited by substrate availability (Table 5.5.3.1.) or enzyme expression. There is evidence that PG synthesis relates inversely to cell density (Rice et el 1984 and Pentland et al 1986), thus it was also possible that overall eicosanoid production was suppressed in our study as the degree of cell confluence increased over the incubation period as a function of the cell population doubling time (Table 2.3.1.1.). Prostanoid quantitation nevertheless indicated the capability of cultured human skin fibroblasts to express enzymes for TXB_2, PGF_α, PGI2, PGE2 and PGE1 synthesis (Table 5.5.6.1.), and the amount of each moiety shown reflected quantitative differences in enzyme expression involved in their biosynthesis.

The eicosanoid profiles obtained from dosed cells (Table 5.5.6.1.) were consistent with the ability of the p-oils to modulate prostanoid synthesis, but such was unrelated to the degree of p-oil unsaturation, the ability of the p-oil to supply direct eicosanoid precursors (Table

2.3.3.2.), or the capability of the cells to form such from the exogenous PUFA's dosed (Table 5.5.3.1.). Even enrichment of these cultures with p-FO or p-LO yielded prostanoid profiles similar to that derived from cells dosed with other p-oils at the same concentration, despite the presence of 2-series prostanoid inhibitors in p-FO and p-LO (20:5ω3 and 18:3ω3, respectively; Lands et al 1971, 1973, 1977, Hamazaki et al 1982, Corey et al 1983, Fischer et al 1983, 1984 and Nasqar et al 1987). It was therefore apparent that p-oil FA's <u>per se</u> induced little effect on PGI_2, TXB2, PGF_α, PGE2, PGE_1 or PGD2 production. The absence of significant changes in the production of these prostanoids with dosage of different p-oils at the same concentration suggested utilisation of endogenous membrane, rather than exogenous, PUFA's as precursors for eicosanoid production. This was indeed possible as control cells contained 20:3ω6 and 20:4ω6, the precursors for the 1- and 2-series eicosanoids, respectively (Table 5.5.3.1.). The percentage of 20:3ω6 was significantly lower than 20:4ω6, and this may explain the significantly lower proportion of PGE_1 detected compared to 2-series prostanoids. Overall eicosanoid production, or the balance between the individual prostanoid groups studied, was nevertheless modulated dependent on p-oil concentration dosed. The mechanism responsible for the changes in prostanoid biosynthesis reported was unclear, although the p-oil

concentrations dosed may have influenced eicosanoid precursor availability or modulated the expression of enzymes involved in formation of these prostanoids.

Studies have shown that intradermal PGE_1 or PGE2 injection enhanced human epidermal proliferation and DNA synthesis (Eaglestein et al 1975 and Bentley-Phillips et al 1977), and similar changes have been shown in culture after dosage with PGE_1 (Bem et al 1974). There is also conflicting evidence which shows that addition of exogenous PGE_1, PGE2, $PGF_{1\alpha}$ or $PGF_{2\alpha}$ to the culture medium of human epidermal explants inhibits cell proliferation (Harper 1976). Others showed that PGD_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ synthesised endogenously following [^{14}C]-20:4ω6 incubation with human keratinocyte cultures had no effect on cell proliferation, although PGE_2 was growth promoting in non-confluent cultures (Pentland et al 1986). Dosed cell eicosanoid profiles (Table 5.5.6.1.), however, did not correlate in any way with changes in cell viability (Figs. 5.5.1.1-5.5.1.8.), total protein (Table 5.5.2.1.), lipoperoxide production (Table 5.5.5.1.), or desaturation capability (Table 5.5.3.1.). The endogenously synthesised eicosanoids studied were therefore probably not directly involved in the modulation of the effects demonstrated with p-oil dosage, and alterations in membrane stability and lipid peroxides were proposed as more likely mechanisms involved in the modulation of cell proliferation.

5.6 General Discussion.

This chapter reported on the effect of p-oil supplementation on the viability of cultured normal human cells. The capability to incorporate, desaturate and elongate FA's was assessed, as were the formation of lipoperoxides and eicosanoids and the roles these compounds played in the modulation of cell viability. The findings presented thus allowed for valid comparison with regard to the parameters examined between human cell types.

Comparison of the cell viability changes reported between the human cell types studied clearly showed that the effects induced with p-oil supplementation were tissue-specific. p-Oil dosage mediated cytostasis and cytotoxicity to erythrocytes, lymphocytes and skeletal muscle (sections 5.1.1, 5.2.1 and 5.3.1, respectively), whereas some p-oils had the additional capability to enhance human lung and particularly skin fibroblast proliferation (sections 5.4.1 and 5.5.1, respectively) with low and/or intermediate p-oil concentrations in the range dosed. This implied FA involvement in the stimulation of cell proliferation, dependent on human cell type, p-oil FA composition and concentration dosed. Suspension cultures of erythrocytes and lymphocytes were most susceptible to p-oil-induced cytotoxicity, followed by skeletal muscle, skin and lung, thus it was clear

that the threshold for cytotoxicity varied with cell type. Such appeared to relate to cell population doubling times (Table 2.3.1.1.), since cells which did not divide, or divided slowly, viz. erythrocytes, lymphocytes and skeletal muscle, were killed with low, whereas the faster growing skin and lung fibroblasts were only killed with high, p-oil concentrations in the range dosed. Cells which divided relatively fast exhibited a lower cytostatic number and thus a broader growth limiting range than slower growing cells. This range determined the p-oil concentration(s) which induced mere growth limitation or cytostasis from those promoting cell proliferation or cytotoxicity, and such should always be defined to ensure valid expression of cell viability results.

Begin et al (1986b) showed that dosage of normal human fibroblasts (CCD-41SK) with 20mg/l LA, GLA, or ALA induced little effect on cell viability, whereas 20mg/l DGLA, AA, EPA or DHA decreased the rate of cell division but did not kill these cells. In comparison, we showed that p-oils induced little or no cytopathic effects with low concentrations in the range dosed, although p-oils rich in unsaturated FA's were generally more growth limiting and/or cytotoxic than p-oils rich in saturated FA's, particularly at high concentrations. p-CO was more effective in reducing cell viability than p-MO, but no consistent pattern was established with supplementation

of p-oils rich in unsaturated FA's, indicating that the effects induced were dependent upon cell type. The different cell viability changes shown with p-oil dosage between the human cell types could well relate to the different physiology of these tissues in vivo. Thus, extrapolation of cell viability results from one human cell type to another should be avoided.

Evidence to support p-oil FA uptake by human cells was presented in this chapter. FA incorporation has also been demonstrated by numerous workers when single FA's were dosed to a variety of normal human cell types, including erythrocytes, lymphocytes and fibroblasts (eg. Bailey et al 1972, Weyman et al 1977, Rosenthal 1978, 1980, Spector et al 1979, 1980a and Stubbs et al 1984). Human embryonic fibroblasts grown in medium containing 10% FCS not only incorporate saturated and unsaturated FA's, but also utilise these moieties for synthesis of lipids (Bailey et al 1972 and Waite et al 1977). Lipid biosynthesis was reported both in growing and stationary phases of cell growth, although the process was more rapid in the former. Spector et al (1979) modified the FA composition of cultured human fibroblasts by addition of single FA's to the culture medium, and showed that the degree of modification attained was dependent on the FA incorporated and whether such was further metabolised via the desaturase cascade. We similarly showed that p-oil incorporation

modified the overall FA composition of the cells, thus alterations in plasma membrane fluidity were likely. Such may have been one of several mechanisms contributing to loss of cell viability, particularly when large p-oil amounts were supplemented.

Total protein concentrations varied significantly between the human cell types studied (Tables 5.2.2.1., 5.1.2.1., 5.4.2.1., 5.5.2.1. and 5.3.2.1.). Amounts were lowest in control lymphocytes (14.8µg), and increased in the order erythrocytes (26.4µg), lung (168.5µg), skin (308.2µg) and skeletal muscle (593.1µg). The ability of adherent cells to divide in culture without prior stimulation could once again explain their considerably higher protein amounts than control erythrocytes or lymphocytes. Control cell protein levels, however, showed no clear correlation with the proliferation rates of these cells (Table 2.3.1.1.), and probably reflected differences in size and physiology of each cell type in vivo. The significantly greater amount of protein reported for human skeletal muscle compared to the other human cell types studied may also relate to the high metabolic activity of this tissue, which accounts for over 50% of total metabolism in vivo (Lehninger 1982). Total protein quantitation served as a measure of cell viability in dosed cells, although it was apparent that p-oil FA's had the capability to stimulate protein synthesis in nucleated cells. This was proposed to

reflect enhanced expression of lipid metabolising enzymes or increased membrane protein biosynthesis. Variations therein between the dosed cells studied nevertheless supported specificity between human tissues.

Current evidence suggests that the human has a limited capability to desaturate PUFA's (de Gomez Dumm et al 1975b, Anon 1979, Stone et al 1979, Dyerberg et al 1980, Blond et al 1981, Horrobin 1983, Mest et al 1983 and El-Boustani et al 1986), although much of this has been based on studies pertaining to desaturation in human liver. Results obtained from cell culture studies, however, are more conflicting. There is evidence that human adult liver cells express Δ5D, but lack Δ6D and Δ4D capability (Maeda et al 1978). Others showed that human diploid lung (WI-38) and skin (Ric Mil and Lala) fibroblasts all exhibit Δ6D, Δ5D and elongase capability since these cells converted approximately 50% of dosed [^{14}C]-18:2ω6 to a mixture of 20:2ω6, 20:3ω6 and 20:4ω6 (Dunbar et al 1975). The expression of such enzymes were subsequently confirmed (Aeberhard et al 1978 and Maeda et al 1978), while traces of Δ4D were also reported in human skin fibroblasts (Aeberhard et al 1978). Other investigators, however, suggest that desaturation is considerably more limited, or absent, in these human fibroblasts (Spector et al 1979, 1980a). Primary monolayer cultures of human endothelium were also

reported not to readily form 20:4ω6 when exposed to high concentrations of 18:2ω6 (Spector et al 1981c), while others showed that human umbilical vein endothelial cells exhibited significant Δ6D, Δ5D and elongase capability (Rosenthal et al 1983b). It was therefore apparent that there may be a wide range of desaturase enzyme activities within human cell types, and this could well relate to variations in experimental conditions between workers.

In vitro evidence to support the presence or absence of desaturase cascade enzyme capability in a range of normal human cells exposed to p-oil FA's under identical culture conditions was presented in this chapter. We showed that human lymphocytes and erythrocytes exhibited no significant potential to desaturate or elongate FA's (Tables 5.2.3.1. and 5.1.3.1., respectively). In contrast, all adherent cells exhibited a degree of desaturation and/or elongation capability, but such was greatest with skin, followed by lung and skeletal muscle (Tables 5.5.3.1., 5.4.3.1. and 5.3.3.1., respectively). Human lung and skeletal muscle cells exhibited poor desaturation capability, and FA metabolism was limited primarily to PUFA elongation. Human skin fibroblasts, in contrast, exhibited limited potential to metabolise both ω3 and ω6 PUFA substrates via Δ6D, Δ5D and elongases, while Δ4D activity was limited to ω3 PUFA substrates only. Δ6D, Δ5D and elongase expression were

also indicated when human lung and skin fibroblasts were incubated with [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6, while limited Δ4D was expressed only when skin human fibroblasts were dosed with [^{14}C]-18:3ω3 (Tables 5.4.4.2. and 5.5.4.2.). The data also proposed conversion of EFA's to PDFA's via direct elongation and Δ8-desaturation. This route has been demonstrated in human testes (Albert et al 1979). Others similarly detected 20:3ω6 and 20:4ω6 when microsomes isolated from human ovarian, colonic or urinary bladder were incubated with [^{14}C]-20:2ω6 (Nakazawa et al 1976), but there are no reports for or against this possibility in other normal human tissues. Numerous workers have, however, indicated limited Δ6D capability in the human (de Gomez Dumm et al 1975b, Spector et al 1979, 1980a, 1981c, Dyerberg et al 1980, Blond et al 1981, Horrobin 1983 and Mest et al 1983), and Δ8D expression may therefore serve as an alternative pathway for PDFA formation. Desaturation with [^{14}C]-18:3ω3 or [^{14}C]-18:2ω6 dosage nevertheless confirmed the greater capability of human skin than lung fibroblasts to form PDFA's shown with p-oil supplementation. This was in contrast to the work of Dunbar et al (1975), who demonstrated that both human fetal lung (WI-38) and skin (Lala and Ric Mil) fibroblasts exhibit similar capabilities to desaturate [^{14}C]-18:2ω6. However, as these tissues differ in physiology <u>in vivo</u>, it may be reasonable to expect some difference in desaturation capability, as we have demonstrated.

The finding that desaturase enzyme capability was considerably more limited with p-oil than single FA dosage both in human lung and skin fibroblasts (Tables 5.4.3.1., 5.4.4.2., 5.5.3.1. and 5.5.4.2.) supported competitive interactions between p-oil FA's for such enzymes. Similar reactions more than likely occur in vivo when humans ingest dietary oils, although changes in fatty acyl unsaturation, the cellular requirement for PDFA's, and the balance between cellular FA's of the same and different series, modulate desaturase cascade enzyme expression (Holman et al 1964, Brenner et al 1966, 1967, Brenner 1974, 1982, Holloway et al 1977, Pugh et al 1979, Sprecher 1981 and Holman 1986a, 1986b), the maximum potential which is genetically determined.

The findings of this chapter clearly indicated that desaturase cascade enzyme capability varies between human tissues. This probably relates to the different physiology of tissues in vivo, and implied dependence of some human tissues on others for a supply of PDFA's. In this regard, one should not extrapolate experimental findings to reflect the desaturase capability of the species as a whole, or even other human tissues.

Cells have the capability to produce lipid peroxides from unsaturated FA's via enzymic and/or non-enzymic mechanisms (Pryor et al 1976, Tappel 1980 and Frankel 1984). Erythrocytes lack microsomes, but are never-

theless capable of producing such compounds via auto-oxidation (Lehninger 1982) and thus serve as non-enzymic controls for other human tissues. Comparison of the results obtained from MDA quantitation in control and dosed cells indicated that different human tissues vary in their capability to produce lipoperoxides (Tables 5.1.4.1., 5.2.4.1., 5.3.4.1., 5.4.5.1. and 5.5.5.1.). Lipoperoxides were not formed by control erythrocytes and lymphocytes, but were measured in all adherent cells, although such was least with control skeletal muscle (0.2nmoles MDA/10^6 cells), and greatest with lung and skin fibroblasts (1.1nmole MDA/10^6 cells each). This pattern of lipid peroxide formation correlated with the inability of erythrocytes and lymphocytes to divide in culture without prior stimulation, the very limited replicative ability shown for skeletal muscle, and the significant ability for cell division reported with cultured lung and skin fibroblasts (Table 2.3.1.1.). Thus, the ability to produce lipid peroxides from endogenous unsaturated FA's appeared to relate partly to the rate of cell division, which in turn could have influenced the rate of PGL turnover and therefore FA availability for cellular oxidation. There is indeed evidence that dividing cells synthesise PGL more rapidly than in the stationary phase (Waite et al 1977 and Spector et al 1979), and this may explain the variation in lipid peroxide concentrations reported between the human cell types studied, despite the greater total PUFA

proportions detected in control erythrocytes and lymphocytes (Tables 5.1.3.1. and 5.2.3.1.) than in adherent cells (Tables 5.3.3.1., 5.4.3.1. and 5.5.3.1.). It was also possible that the mechanisms to prevent FFA oxidation, or to scavenge lipid radicals formed, were more efficient in erythrocytes and lymphocytes than in the adherent cells. Variations in the rate of FFA oxidation between human tissues could also explain the findings.

p-Oil supplementation affected lipoperoxide production to a greater or lesser extent, but quantitative differences were once again evident between the human tissues studied. Lipid peroxide concentrations less than 5nmoles MDA/10^6 cells were found in dosed human skin fibroblasts or their corresponding spent medium (Table 5.5.5.1), while the range was slightly wider for lung (0-7.1nmoles MDA/10^6 cells) and skeletal muscle (0-11.0nmoles MDA/10^6 cells) adherent cultures (Tables 5.3.5.1. and 5.4.5.1.), but significantly wider for lymphocyte (0-38.8nmoles MDA/10^6 cells) and erythrocyte (0-77.1nmoles MDA/10^6 cells) suspension cultures (Tables 5.2.4.1. and 5.1.4.1., respectively). These variations in susceptibility to p-oil-induced oxidation between the human cultures probably related to alterations in the rate of cellular FFA incorporation and release induced with p-oil dosage. It was also evident that the amount and structure of unsaturated FA's present in a p-oil

influenced lipoperoxide formation. Despite evidence that incorporated FA's can be released back into the culture medium subsequent to uptake (Rosenthal et al 1978 and Figard et al 1986), the presence of albumin as p-oil FA carrier in the present study's incubation medium suggested that most spent media lipoperoxides originated intracellularly and were released as a direct result of cell lysis or increased plasma membrane permeability induced with p-oil dosage. The finding that lipoperoxide formation often correlated inversely with cell viability when the human cultures were enriched with p-oils nevertheless led to the proposition that these compounds are involved, to a greater or lesser extent, in the modulation of cell proliferation. This supported the limitation of normal human fibroblast growth by intracellularly generated lipoperoxides when others dosed single PUFA's (Gavino et al 1981c).

Detection of 20:3ω6, 20:4ω6 and 20:5ω3 in control lung and skin fibroblasts, and the possibility for 22:4ω6 and 22:5ω3 retroconversion (Tables 5.4.3.1. and 5.5.3.1., respectively) supported the ability of these cells to synthesise eicosanoids (Tables 5.4.6.1. and 5.5.6.1., respectively). Total molar eicosanoid production, however, was greater with control lung than skin fibroblasts (332.6 vs 232.5pmoles/10^6 cells, respectively), despite the greater desaturation capability of skin fibroblasts (Tables 5.4.3.1., 5.4.4.2, 5.5.3.1. and

5.5.4.2.). These findings correlated with the greater availability of 1-, 2- and 3-series eicosanoid precursors in lung than skin fibroblasts (Tables 5.4.3.1. and 5.5.3.1., respectively) as well as reports that the lung is the primary site both of eicosanoid production and inactivation (Samuellson et al 1975, 1978, Mathe et al 1977, Hyman et al 1978 and Harper et al 1984). Furthermore, they reflected variations in the eicosanoid requirements and activities of enzymes involved in eicosanoid metabolism between such cells rather than the ability to provide eicosanoid precursors via desaturation. The different amounts of TXB_2, PGF_α, PGI_2, PGE_2 and PGE_1 these cells synthesised indeed supported differences in the expression and activity of enzymes involved in prostanoid synthesis between human tissues and/or modulation by other eicosanoids. There is evidence 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). Low PGE_1 production induces low cAMP levels, and AA mobilisation is subsequently increased. The 2-series prostanoids produced by control lung and skin fibroblasts therefore probably relate to the low PGE_1 levels reported (Tables 5.4.6.1. and 5.5.6.1.). In the wake of this phenomenon, it was also possible that a greater proportion of the PGF_α fraction detected was composed of $PGF2_\alpha$ rather than $PGF_{1\alpha}$. PGE_1 production significantly lower in skin

than in lung fibroblasts may also explain the greater formation of PGI_2, TXB2, PGF_α and PGE2 detected in the former. The finding that prostanoid production differed quantitatively between lung and skin fibroblasts nevertheless implied that eicosanoid synthesis probably varies between other human tissues as well.

Evidence was presented which supported exogenous FA involvement in the stimulation of human lung fibroblast prostanoid synthesis with all p-oil concentrations fed, unlike skin fibroblasts (Tables 5.4.6.1. and 5.5.6.1.). This was in spite of the greater desaturation capability of human skin than lung fibroblasts, which once again supported the importance of the lung in eicosanoid biosynthesis (eg. Hyman et al 1978). The finding that the modulation of human lung and skin fibroblast prostanoid synthesis was dependent only upon p-oil concentration supplemented nevertheless implied sufficient endogenous PUFA's in the precursor pool and/or the unavailability of incorporated p-oil FA's for prostanoid production, as well as p-oil concentration involvement in prostanoid pathway enzyme expression. The possibility that dosage with different p-oils induced variations in other eicosanoid levels, however, could not be excluded. The fact that human lung and skin fibroblast prostanoid profiles showed no clear p-oil concentration dependent correlation with cell viability, desaturation capability or lipoperoxide production implied that the endogenously

synthesised prostanoids studied were probably not directly involved in, or responsible for, the effects induced with p-oil supplementation. Hori et al (1989) showed that both intracellularly produced and exogenously administered PGE_2, $PGF2_\alpha$ and PGD2 inhibit human fibroblast growth to a greater or lesser extent. Considerable evidence, however, indicates that while certain exogenous PG's mediate potent effects on cell multiplication, endogenous PG's have little effect on the proliferation of human diploid cells in vitro (Thomas et al 1974, Cornwell et al 1979 and Bettger et al 1981). This supported the findings we presented, particularly with regard to p-oil-induced cytotoxicity, and eicosanoids have rather been implicated in the regulation of cellular metabolism and multiplication (Bettger et al 1981).

Overall, this chapter presented comparative data on a range of cell parameters for different cultured human tissues. The control human cells studied exhibited different growth rates, FA and prostanoid profiles, contained different protein concentrations, and produced different molar lipoperoxide and eicosanoid amounts. When p-oils were supplemented, variations in cell viability, total protein and lipoperoxide formation, eicosanoid and FA profiles, and desaturation capability, were also demonstrated between different human cells. Variations in physiology and FA metabolism between these

human tissues were important in the modulation of the effects shown, thus careful consideration should be given to the selection of human tissues for experimental purposes, and extrapolation of experimental findings from one human tissue to another should be avoided to ensure valid interpretation of data.

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION.

6.1 GENERAL DISCUSSION.

6.1.1 The Influence of p-Oil Supplementation on Cell Viability and Total Protein.

Most workers dosing FA's to growing cells in culture report the number of viable cells at the end of the experimental incubation period relative to controls either at the time of seeding or at the end of the experiment. Since most adherent cells are dosed subsequent to a post-trypsinisation recovery period, this method of data representation is not ideal if comparison to the 'real world' is intended. A more valid comparison is to the control cell number at the time of dosing. We have termed this the 'Cytostatic Number', which is that concentration of agent which causes zero nett proliferation compared to controls at the time of dosing. If the dosed cell number at the end of the experimental period is greater than the cytostatic number, but less than that for undosed cells, then mere growth limitation has occurred; if the viable cell count is less than the cytostatic number, then the compound dosed is truly cytotoxic. On the other hand, if cell numbers are greater than the cytostatic number and the control cell number at the end of the incubation period, then cell proliferation has occurred. As a corollary to this, it is not necessary to kill cells to be an effective adjunct - mere prevention of nett cell

proliferation, which equates to cytostasis, may be sufficient. Failure to recognise the cytostatic number concept will result in workers not using the correct concentrations of agents, eg. FA's, to induce a desired effect. Indeed, this would influence the effectiveness of a therapeutic agent *in vivo*. The cytostatic number is of particular importance if it is required to limit cancer, but not normal, cell growth.

This thesis showed that p-oils influence the viability of cultured normal rat, cat and human tissues (chapters 3, 4 and 5). The effects induced and magnitude thereof, however, varied with the p-oil and concentration dosed between different tissues within any species, and it was proposed that such related to variatic s in the *in vivo* localisation and/or physiology of different tissues. Endothelial cells *in vivo*, for example, are continuously exposed to FA's circulating in the serum, whereas tissues such as skin are not so directly exposed. This was reflected in the growth profiles of these cells *in vitro* as endothelial cells, irrespective of species, were less susceptible to p-oil-induced growth inhibition than skin fibroblasts. The finding that suspension cultures of erythrocytes and lymphocytes were most susceptible to p-oil-induced cytotoxicity correlated with the suspension culture work of others using single monoenoic and polyenoic C18 FA's dosed to unstimulated

myeloma cells (Girao 1988) under similar culture conditions to those in this thesis (section 2.2.1). The fact that less than half of the available surface of adherent cells were exposed to the dosed moieties, compared to 100% of the area in suspension cultures, could account for the greater susceptibility of suspension cultures to single FA's (eg. Ruck et al 1986) or p-oils (chapters 3, 4 and 5). The susceptibility of erythrocytes and lymphocytes to low p-oil concentrations may nevertheless explain why circulating FFA levels are maintained at relatively low levels in vivo (Fredrickson et al 1958 and Geigy 1984). p-Oil supplementation to identical rat, cat and human tissues also induced significantly different cell viability changes overall between these species, particularly with certain p-oils. Some of these are highlighted in the following paragraphs to illustrate this concept.

Overall, rat erythrocytes were more susceptible to p-oil-induced hemolysis than cat or human erythrocytes (sections 3.1.1, 4.1.1 and 5.1.1, respectively). Low to intermediate concentrations of p-CO, however, stabilised erythrocyte viability in the rat, had no effect in the cat, but was hemolytic in the human. Cat and human erythrocytes responded similarly to equal concentrations of p-SSO and p-MO, p-EPO was more hemolytic to rat and cat than human erythrocytes, but p-OO and p-FO induced

converse was found with p-LO dosage. Similarly, cat and human skin fibroblast growth were generally shown to be less susceptible to inhibition by p-oils than that of the rat (sections 4.7.1, 5.5.1 and 3.7.1, respectively). Furthermore, low concentrations of p-MO, p-SSO and p-FO were more effective in promoting cell growth in human than in cat or rat skin fibroblasts. p-OO enhanced and inhibited cell proliferation in all 3 species, but less growth enhancement and slightly more cytotoxicity was induced in the rat. p-Oils were generally also more able to restrict rat than cat endothelial cell proliferation (sections 3.3.1 and 4.3.1, respectively). p-SSO was more effective in the promotion of cat than rat endothelial cell proliferation, while p-EPO and p-FO largely induced similar growth limiting and cytostatic effects in both species. On the other hand, rat endothelial cells were more susceptible to p-OO-induced cytotoxicity than cat endothelial cells, but the converse occurred with p-LO dosage.

Considerably more growth inhibition was shown with p-oil supplementation to human than rat or cat skeletal muscle (sections 5.3.1, 3.4.1 and 4.4.1, respectively). p-OO was merely growth limiting to rat, cytotoxic only at high concentrations to cat, but growth inhibitory with practically all concentrations to human, skeletal muscle. p-SSO promoted and limited cell proliferation in

cytotoxic in the human. Low p-EPO concentrations were more effective in stimulating cat than rat or human skeletal muscle growth, while cat was least and human most susceptible to cytotoxicity with higher amounts dosed. The growth limitation and inhibition p-LO induced was similar in rat and cat, but again markedly more pronounced in human skeletal muscle cells.

Cat brain growth was more susceptible to impairment with p-oil dosage than rat brain (sections 4.5.1 and 3.5.1, respectively), although the converse was true with p-MO dosage. Furthermore, p-SSO and p-OO exhibited greater ability to promote cat than rat brain cell growth at low concentrations, in contrast to p-EPO and p-FO. Cells derived from rat adipose tissue were also significantly more resistant to growth inhibition than those from the cat (sections 3.8.1 and 4.8.1, respectively), except with dosage of high p-EPO concentrations. Low p-oil concentrations were more effective in stimulating rat than cat adipose cell growth, although the converse was shown with p-FO dosage.

p-Oil-induced changes in lung fibroblast viability varied between the 3 species (sections 3.6.1, 4.6.1 and 5.4.1). The growth limitation p-CO and p-MO induced was greater in rat and human than cat lung. Cat and human lung fibroblast proliferation was stimulated with low and intermediate p-OO concentrations in the range dosed,

respectively, while the susceptibility to cytotoxicity at higher concentrations increased in the order human, rat and cat. p-SSO promoted growth limitation in rat, growth inhibition in cat, and both proliferation and cytotoxicity in human, lung fibroblasts. On the other hand, p-EPO had little effect on or enhanced rat lung fibroblast growth, but limited and inhibited that of the human and cat, respectively. Differences in lymphocyte viability relating to p-oil supplementation between the three species were also found (sections 3.2.1, 4.2.1 and 5.2.1). Lymphocyte killing increased in the order human, cat and rat with p-CO or p-MO dosage, while p-OO increased lymphocyte viability only in the rat with low concentrations and was more cytotoxic to cat and human than rat lymphocytes with high concentrations. p-FO was more cytotoxic to cat and human than rat lymphocytes, but the converse was found with p-EPO dosage.

The findings presented indicated that exogenous FA's induce cell viability changes which vary between normal tissues from different mammalian species. Variations in cell proliferation are also documented for different transformed cells incubated with identical FA's and similar culture conditions. Girao et al '1986) and Begin et al (1987), for example, obtained different results when mouse myeloma (SP-210) and human breast carcinoma (ZR-75-1) cells, respectively, were incubated with

suggests that such effects are universal for different cell types.

Cell viability was often increased with low to intermediate concentrations of certain p-oils in the range dosed, while higher amounts were generally growth inhibitory (chapters 3, 4 and 5). The cell viability changes induced with p-oil dosage supported exogenous FA involvement in the modulation of normal mammalian cell viability, which others have shown with single FA's. Dubin et al (1965), for example, found that an optimum concentration of 0.25μM 18:2ω6 promoted cultured chick-embryo macrophage growth. On the other hand, Ruck et al (1986) showed that resting human monocytes were killed with low unsaturated C18 FA concentrations (15-50μM) and were unaffected by 18:0, while Rice et al (1981) reported that 18:1ω9 increased, and 18:3ω3 decreased, cytotoxic activity in human lymphocytes. Csordas et al (1984) showed that addition of 18:1ω9, 18:2ω6 or 18:3ω3 to isolated chicken or sheep erythrocytes <u>in vitro</u> induced some hemolysis with concentrations of 0.1-10μM and 100% lysis of the erythrocytes with greater amounts, although 10-30μM 18:1ω9 stabilised erythrocyte membranes and no lysis was observed. FA's have also been shown to influence the proliferation of numerous adherent normal cultured cells. Wicha et al (1979) found that rat mammary epithelial cell growth was stimulated when

inhibited with saturated, FA's, whereas higher concentrations were all inhibitory, although the extent thereof varied with FA. Bourre et al (1983) achieved optimal fetal mouse brain cell growth with 3.5μM 18:2ω6, 18:3ω3, 20:4ω6 or 22:6ω3, but inhibition thereof with higher FA concentrations. Huttner et al (1978) similarly demonstrated that low unsaturated FA concentrations (1-80μM) stimulated, and higher amounts inhibited, human skin fibroblast and guinea pig aorta smooth muscle cell growth, whereas saturated FA's had little effect or were inhibitory. The proliferative response, however, was a specific effect of the FA used, its concentration dosed and the cell line involved, as shown in the present study (chapters 3, 4 and 5). 18:1ω9, for example, was more effective in stimulating skin than muscle cell proliferation, but was also more cytotoxic to skin at high concentrations (Huttner et al 1978). There is also evidence that 20:3ω6, 20:4ω6 and 22:4ω6 are more effective in decreasing human skin fibroblast and guinea pig aorta smooth muscle cell proliferation than 18:1ω9 or 18:2ω6 (Huttner et al 1977, 1978, Cornwell et al 1979, Miller et al 1980 and Gavino et al 1981a, 1981b), although the extent thereof varied with PDFA and cell type. Spector et al (1979) similarly showed that human skin fibroblast proliferation was not influenced with 10 to 100μM 18:1ω9 or 18:2ω6, but was reduced by up to 50% when 16:0, 18:3ω3 or 20:4ω6 were added at concentrations

were non-toxic. Morisaki et al (1982b) stimulated guinea pig aorta smooth muscle cell proliferation with all 18:1ω9 concentrations tested (30, 60 and 90μM), whereas other ω9 FA's inhibited such in a concentration dependant manner, increasing in the order 18:2ω9, 20:2ω9 and 20:3ω9. Both 18:2ω6 and 18:3ω3 were least cytotoxic for their series and induced growth inhibition only with concentrations greater than 90μM, but cytotoxicity was increased when desaturation and elongation products thereof were dosed such that the most potent inhibitors of cell proliferation both in the ω6 and ω3 series were the eicosatrienoic acid. However, 20:3ω9 was even more growth inhibitory than 20:3ω6, followed by 20:3ω3 (Morisaki et al 1982b). More recently, Vignikin et al (1989) demonstrated that cultured rat brain glial, human skin and human liver cell growth responded differently to supplementation with various single FA's, supporting the work of this thesis with p-oil supplementation (chapters 3, 4 and 5).

The findings of the literature reports and this thesis indicated that both single FA's and p-oils influence mammalian cell viability, and supported earlier evidence implicating FA's as mediators of <u>in vitro</u> cell proliferation (Boone et al 1972 and Huttner et al 1977). FA's may thus be important in proliferation-linked phenomena <u>in vivo</u>. The present study indicated, however,

and species, probably due to inherent genetic differences. This emphasised the importance of selecting the same tissue and species for control and experimental studies, and avoiding generalisation/extrapolation of results from one cell type to another.

While some literature reports discussed above show correlation between different workers, or reflected the effects of certain p-oil FA's, reports were more conflicting in other instances. It is important to bear in mind, however, that experimental conditions vary with design between laboratories. Furthermore, culture variables including the source and concentration of serum, FA concentration, incubation period and cell type have been shown to influence the inhibition of normal and tumor cell proliferation (Begin et al 1986b). Similarly, the findings of this thesis supported the involvement of factors such as the p-oil FA composition and concentration dosed, as well as variations in cell type, proliferation rate and density in the modulation of mammalian cell viability. Certain factors may be characteristic of a particular cell type, the species from which it was derived, or whether the cells were adherent or not. However, this would appear not only to be true for normal cells, but also for cancer cells, since a similar proposal was put foreward by Begin et al (1988).

Evidence was presented which indicated that the albumin used as FA carrier did not influence cell viability in any significant way (chapters 3, 4 and 5), as was also shown previously by others (McGee 1981 and Bourre et al 1983). In addition, supplementation of the growth medium with 10% FCS had little effect on cell viability since the number of dead cells were minimal in control cultures. FCS concentrations less than 10%, however, have been shown to accelerate the rate of FA-induced cell killing (Begin et al 1986b). When rat, cat or human skin fibroblasts were grown in medium supplemented with 10% serum derived from their own species (section 2.2.3.3.), plating efficiency and cell growth were not as good as when supplemented with 10% FCS (Figs. 3.7.1.10., 4.7.1.10. and 5.5.1.10., respectively). Others have shown similar effects when adult human serum replaced fetal bovine serum in cultures of human endothelium or skin (Cooper et al 1977 and Lagarde et al 1984). This could well relate to a higher concentration of growth promoting factors in fetal than adult serum as the FA composition, total protein and lipid contents of FCS were similar to that of adult rat, cat and human sera (Tables 2.3.3.1. and 2.3.3.3.). These findings nevertheless excluded the possibility that routine supplementation of culture medium with 10% FCS limited cell viability.

with p-oil supplementation were transient, at least over relatively short incubation periods, since previous work in our laboratory demonstrated similar changes in growth whether cells were incubated with single FA's for 24, 48 or 72 hours (Girao et al 1987 and Girao 1988). The effects induced were, however, related to the growth rates of the cells (Girao 1988). Indeed, cultured cells which did not divide, viz. erythrocytes and lymphocytes, or which were relatively slow in dividing, eg. cells derived from rat aortic endothelium, cat brain and adipose tissue, or human skeletal muscle (Table 2.3.1.1.), required lower p-oil concentrations to induce growth limitation, cytostasis or cytotoxicity (chapters 3, 4 and 5) than cells with shorter population doubling times, eg. cells derived from rat, cat and human lung or skin. This supported the broader growth limiting range found with faster than slower growing cells. The present study showed that rapidly dividing normal cells are generally less susceptible to p-oil-induced cytotoxicity than slower growing normal cells, while others showed the converse for transformed cells exposed to single FA's (Girao 1988). These findings would, therefore, support the use of FFA's, and FFA mixtures, as a means of limiting the growth of cancer <u>in vivo</u>. Clinically, this would permit selective limitation of neoplastic, but not non-tumorigenic, tissue.

showed that PUFA's (LA, GLA, DGLA, AA, ALA and EPA) inhibited human breast, lung and prostate cancer cell growth at concentrations (20mg/l) which decreased the rate of unrelated normal human or animal cell division, but did not kill them. Subsequent work using genetically related normal and transformed cells showed that a tumorigenic phenotype renders cells more sensitive to PUFA-induced growth inhibition (Begin et al 1989 and Sircar et al 1990). Our laboratory similarly found that normal rat brain primary cultures were generally less susceptible to C18 FA-induced growth inhibition than a transformed rat neuroblastoma x glioma cell line (Davidson et al 1988a). It is thus reasonable to suggest that FA mixtures could induce a similar effect, or be more effective than single FA's in limiting cancer cell growth. This may have significant clinical implications.

Girao (1988) demonstrated that when single C18 FA's were dosed to cells in culture, the number and not the position of the double bonds in the FA chain generally determined the extent of cytotoxicity. Thus, the greater the degree of unsaturation, the greater the cytotoxic effect generally induced. The cell viability changes obtained with p-oil supplementation, however, could not be fully predicted by the results obtained utilising single FA's. While the modulation of cell viability with p-oil incubation could be related to certain exogenous

p-oil FA's could well have enhanced or diminished the effects of single FA's. This implied that the efficacy of an individual FA depends on the presence or absence of other FA's in a mixture. We have indeed shown significant differences between nett cell viability results from summing individual FA effects and actually using FA mixtures mimicking dietary oils (Davidson et al 1988b, 1990b and Giangregorio et al 1988b, 1990). Figure 6.1.1. illustrates the cell viability changes observed when human skin fibroblasts were dosed with each of the p-oils used in this thesis compared to that when individual C18 or C20 FA's were incubated and such effects summed to equate with the C18 and C20 FA's detected in the p-oils (Table 2.3.3.2.). The final concentrations were identical whether p-oils or single FA's were dosed. In general, there was little difference in cell viability with saturated FA's whether effects were summed, or whether FA mixtures (p-CO) were used (Fig. 6.1.1.1.). However, when unsaturated FA's were dosed individually, or as mixtures representing various p-oils, dramatic differences were demonstrated (Figs. 6.1.1.2-6.1.1.7.). Single FA's were more growth limiting than p-oils with low to intermediate concentrations in the range dosed, while the converse occurred with higher concentrations. These findings reinforce the observation that single FA's, while useful from a basic biochemical

Legend to Fig. 6.1.1.

The graphs in Fig. 6.1.1.1-6.1.1.7. show mean percent cell viability versus the concentration (mg/l) of C18 and C20 FA's, or p-oil, dosed.

Fig. 6.1.1.

The effect of p-oils versus the summed effect of single C18 and C20 FA's on human skin fibroblast viability.

Fig. 6.1.1.1.

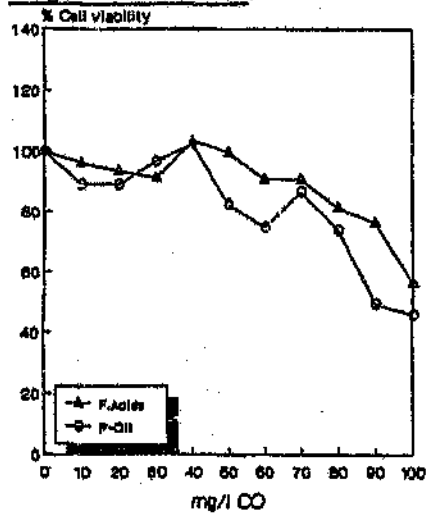


Fig. 6.1.1.2.

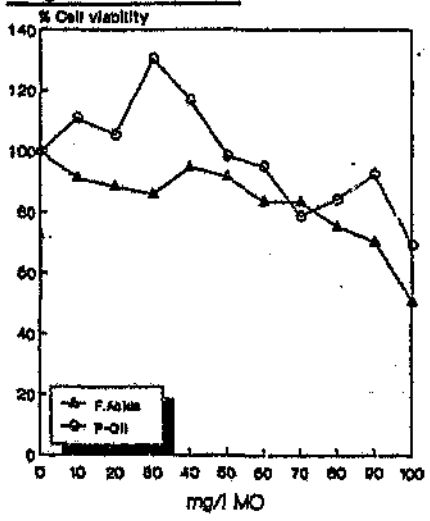


Fig. 6.1.1.3.

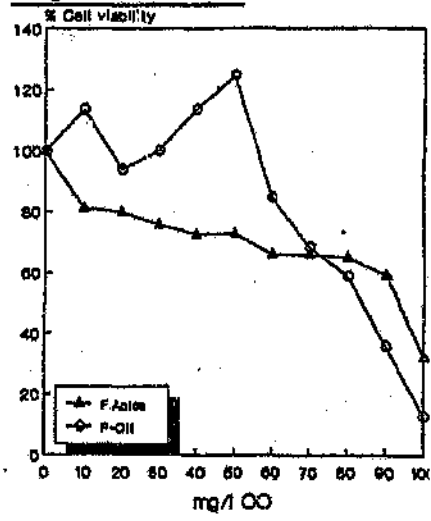


Fig. 6.1.1.4.

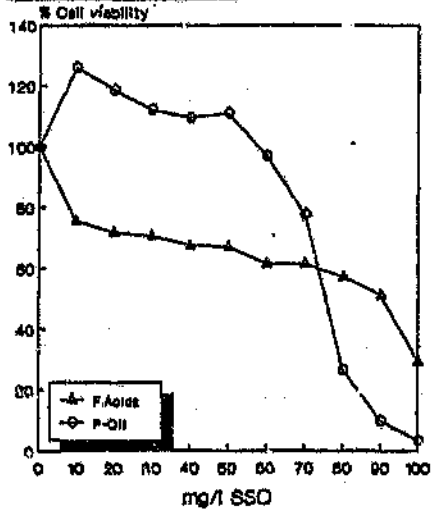


Fig. 6.1.1.5.

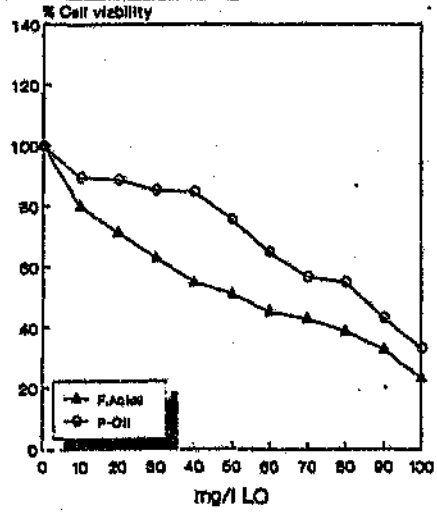


Fig. 6.1.1.6.

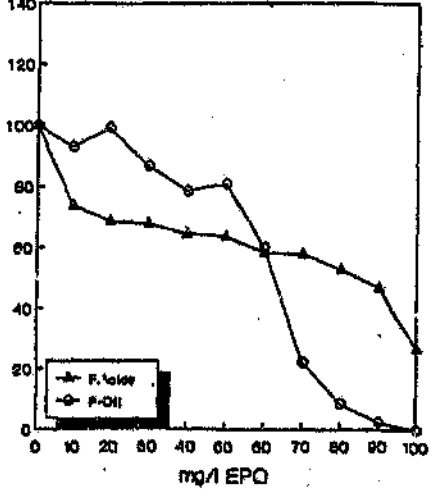
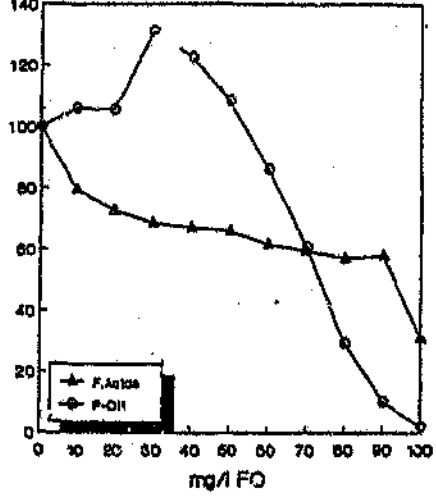


Fig. 6.1.1.7.



situation where FA mixtures are both present in the body and fed as dietary oils, and where the phenomenon of FA synergism/antagonism occurs. This should therefore be considered when workers using single FA's extrapolate results to the 'real world'.

The extact mechanism(s) whereby FA's influence cell proliferation is unclear, but likely mechanisms have been proposed (see below). The potential for PUFA's to be converted to 20:3ω6, 20:4ω6, 20:5ω3 and subsequently eicosanoids increases the number of possible mechanisms whereby these moieties exert their effects. However, it also raises the question as to why those cells which are unable to efficiently form eicosanoid precursors via desaturation and elongation are killed. Furthermore, such transformations to eicosanoids are not possible with saturated or monounsaturated FA's, suggesting that the native FA may somehow be inhibitory.

Hoover et al (1977) found that low PUFA concentrations (10-40mg/l ALA, AA and particularly LA) decreased the cell-to-cell and cell-to-substrate adhesive properties of cultured baby hamster kidney (BHK) cells in a concentration dependent manner, while long chain non-polyenoic FA's (OA, PA and SA) were less effective or marginally enhanced adhesion. Similar effects were observed when these authors investigated the effect of FA's on cell adhesion with chinese hamster ovary (CHO)

cell lines. This suggested that such effects may be a common feature of many cell lines.

Hoover et al (1977) also reported that as the LA concentration dosed increased from 10 to 20mg/l and particularly to 40mg/l, the growth rate of the cells decreased compared to controls. Their findings suggested that this effect was not due to FA toxicity, although the morphological alterations found implied that exogenous FA supplementation induced structural changes in cells.

Considerable evidence supports the fact that addition of exogenous FA's to culture medium induces differential FA uptake and appreciable changes in the FA composition of animal cells, which is associated with membrane structural changes, eg. fluidity (Williams et al 1974, Wisnieski et al 1974, Ferguson et al 1975, Weyman et al 1977, King et al 1978, Burns et al 1979, Jeffcoat 1979, Spector et al 1979, 1985, Simon et al 1982 and Stubbs et al 1984). The perturbation of membrane structure, function and fluidity by alteration of the FA profile of membrane PGL's may thus be another mechanism whereby p-oils affected cell viability, since uptake of exogenous FA's by rat, cat and human cells was shown in this thesis (chapters 3, 4 and 5). Indeed, the degree of membrane fluidity has been shown to be important for cell proliferation and mitogenesis (Farber et al 1975,

Cheng et al 1979 and Spector et al 1985). It has been reported that supplementation of mouse LM cells with saturated FA's resulted in an imbalance of membrane PGL FA's which inhibited growth, probably by decreasing membrane fluidity (Doi et al 1978). Incorporation of dosed p-CO could similarly have increased the proportion of saturated FA's in cells and induced membrane rigidification, thus increasing the susceptibility of those cells to lysis. In contrast, membrane 'over-fluidity' and a subsequent lack of stability could well have inhibited cell viability with dosage of PUFA-rich p-oils, particularly since an increased content of membrane PDFA's has been associated with an increased degree of membrane disorder (Salem et al 1980). There is also evidence that changes in membrane lipid composition can affect a number of cellular functions including carrier-mediated transport, endocytosis, exocytosis and membrane-bound enzyme activity (eg. Horwitz et al 1974, Engelhard et al 1976 and Spector et al 1985). The fact that many functional responses are directly related to membrane structural changes emphasised the importance thereof in the modulation of cell viability.

The formation of cytoplasmic droplets in adherent cells with p-oil incubation at high concentrations in some instances (chapters 3, 4 and 5) has also been reported by other workers in cultured cells dosed with single

1981, Rosenthal 1981, Spector et al 1981a and Begin et al 1986b). These droplets have been shown to accumulate in the cytoplasm when cells are exposed to excess lipid in the culture medium, and are composed primarily of TAG's (Mackensie et al 1967, Schneeberger et al 1971, Spector et al 1979, 1981a, Miller et al 1980, Gavino et al 1981b and McGee 1981). Excessive TAG accumulation, however, can damage cells and interfere with normal metabolism (Rosenthal et al 1978), and could therefore have contributed to the reduction of cell viability with p-oil dosage. The ability to induce more cytotoxicity with saturated than unsaturated FA dosage, however, has been reported to relate to solid depositions of saturated fat rather than discrete osmiophilic droplets induced with unsaturated FA's (Rosenthal 1981).

The mechanisms discussed above provide a means whereby FA's affect cell proliferation. Each mechanism could individually explain the cell viability changes induced with p-oil dosage, although involvement of a combination of mechanisms seems more realistic.

This thesis showed that cultured cells vary in the amount of total protein they contain both within and between species (chapters 3, 4 and 5). Concentrations were lowest in control lymphocytes (6.4-14.8µg/10^6 cells seeded) and erythrocytes (12.3-26.4µg/10^6 cells seeded), but the considerably higher protein amounts found in

correlated overall with the range reported by Patterson (1979) for some cultured cells. No clear trends were obvious when comparing control cell protein levels with their proliferation rates (Table 2.3.1.1.), thus the diversity of results could well reflect differences in cellular morphology and physiology. There is also evidence that the rate of protein biosynthesis varies with cell type (Vignikin et al 1989). While the protein concentrations and variations therein may be an inherent property of the cells themselves, such could also be a result of the culture conditions employed. There is indeed evidence that culture conditions, length of the incubation period and passage number influence cellular protein levels (Patterson 1979 and Vignikin et al 1989).

Total protein quantitation in dosed cells served as a measure of viability and also supported the ability of n-oil FA's to modulate absolute protein synthesis in nucleated mammalian cells, primarily by stimulating such (chapters 3, 4 and 5). This supported the work of Jacobson et al (1988) who observed a stimulatory effect of PUFA's on animal cell protein levels and growth rate. Vignikin et al (1989) similarly demonstrated that the rate of protein synthesis in rat brain and liver cells was influenced by exogenous FA's. Protein synthesis was stimulated in both cell types with OA, LA or AA incubation; however, the increase was greater with OA

cells (Vignikin et al 1989). This supported the present study's findings which indicated that the modulation of protein synthesis within a tissue related to variations in FA structure. We additionally proposed that synergistic and antagonistic effects between FA's can modulate protein synthesis when FA mixtures are dosed. The finding that spent medium protein levels of skin cultures dosed with p-oils were statistically similar to controls, however, indicated that medium protein probably did not contribute significantly to the changes demonstrated in cellular protein.

Differences in protein concentration in dosed cells could represent differences in cell number, protein synthesis, degredation, exchange and retention, or enzyme production to protect against lipid peroxidation, for example. Increased protein synthesis in p-oil dosed cells could well have reflected increased production of lipid metabolising enzymes. There are reports that lipid changes in membranes can affect the activity of lipid-dependent enzymes and that FA's activate membrane enzymes such as adenylate-cyclase (Carreau et al 1971, Counis 1973 and Weyman et al 1977). Our laboratory found that supplementation of cultured cells with single C18 FA's under similar experimental conditions to those employed in the present study (2.2.1) induced little effect upon the plasma membrane protein content per viable cell, irrespective of changes in cell viability

(Girao 1988). Thus it was likely that the changes reported in total protein per cell with p-oil dosage primarily reflected alterations in intracellular rather than plasma membrane protein. There is indeed evidence that FA's can activate cytoplasmic lipolytic and lipogenic enzymes (Loriette et al 1971, 1972 and Carreau et al 1972) and regulate FA biosynthesis in cultured animal cells by influencing the activity of enzymes involved in lipid metabolism (Spector et al 1981a and Vignikin et al (1989). Numerous studies have indicated that dietary FA's can modulate cellular DNA synthesis both in vivo and in vitro to a greater or lesser extent dependent on the tissue and degree of FA unsaturation (Launay et al 1968, 1969, 1981, Desnoyers et al 1971, Goureau-Counis et al 1974, Holley et al 1974, Manzoli et al 1974 and Andreis et al 1981a, 1981b). Thus it was also possible that the lipid environment in p-oil doped cells affected genetic expression and therefore total protein levels.

6.1.2 Fatty Acid Incorporation, Desaturation and Elongation in Mammalian Cells.

Comparison of the control rat, cat and human cell types studied in this thesis showed variations in cellular FA composition both between tissues and species (chapters 3, 4 and 5). This supported and extented the work of others who demonstrated species differences in the FA composition of certain mammalian tissues (Crawford et al 1970, 1971, Horrobin et al 1984c and Arakawa et al 1986). These findings probably reflect variations in physiology and lipid metabolism between tissues from different species, and should therefore be taken into consideration when selecting tissues for experimental purposes.

The FA spectra of the cultured rat, cat and human cells dosed with single radiolabelled C18 FA's or p-oils, as well as the spent incubation media of skin cultures supplemented with p-oils (chapters 3, 4 and 5) supported exogenous FA incorporation, which has been demonstrated in a variety of cultured cells (eg. Spector et al 1979, Christensen et al 1968, Lokesh et al 1988 and Weithmann et al 1989). There is evidence, however, that FA uptake varies between cell types (eg. Gavino et al 1981a, 1981b), and this was indeed confirmed with the different amounts of [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3

in the present study, summarised in Table 6.1.2.1. Work in our laboratory indicated that incorporation of polyenoic C18 FA's was greater in transformed (3T6D) than benign (3T6, 3T3), and least in normal (human skin) fibroblasts (Girao et al 1988, 1989). Similarly, the greater [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 and [^{14}C]-18:3ω3 amounts incorporated in rat than cat adipose cells in the present study (Table 6.1.2.1.) correlated with the faster rate of rat than cat adipose cell proliferation (Table 2.3.1.1.), and a direct relationship between FA incorporation and population doubling time was also found with skin fibroblasts between the 3 species. The finding that FA incorporation and cell proliferation rates were not always directly related with rat, cat and human lung fibroblasts for example, however, could well reflect selective regulation of FA incorporation. FA uptake by cells has been shown to involve a first step transfer to an exchangeable plasma membrane surface pool, followed by entry into a second 'deeper' non-exchangable membrane pool prior to utilisation (eg. Howard et al 1974, 1976, Yavin et al 1974, Shohet 1968, 1976, Weyman et al 1977 and Morand et al 1982a, 1985). Howard et al (1974) reported that the rate of FA uptake increased with increasing FA chain length and decreased with increasing degree of FA unsaturation, while this thesis showed that [^{14}C]-18:1ω9 and [^{14}C]-18:2ω6 incorporation were generally similar, but greater than

Table 6.1.2.1.

The incorporation of C18 FA's, expressed as the radioactivity (cpm) recovered from cells dosed with 2μCi [^{14}C]-18:1ω9, [^{14}C]-18:2ω6 or [^{14}C]-18:3ω3, and as a [percentage of the total amount dosed].

CELL TYPE	18:1ω9	18:2ω6	18:3ω3
LUNG:			
Rat	548552 [13%]	734574 [15%]	304473 [7%]
Human	1477332 [34%]	1397004 [29%]	1147896 [27%]
Cat	769494 [18%]	862419 [18%]	644634 [15%]
SKIN:			
Rat	959907 [22%]	1121016 [23%]	407862 [10%]
Human	1470507 [34%]	1541535 [32%]	799068 [19%]
Cat	1343979 [31%]	1463025 [31%]	969108 [23%]
ADIPOSE:			
Rat	938754 [22%]	1368009 [29%]	613707 [14%]
Cat	665235 [16%]	646281 [14%]	368823 [7%]

mechanism against alteration of the conservative and highly unsaturated PUFA composition of brain lipids, the proportions of which are essential for normal brain function (eg. Svennerholm 1968, Crawford et al 1971 and Davidson et al 1988c, 1988d). Δ6D, Δ5D, little Δ4D, and elongase, expression was shown in cultured rat skeletal muscle (Table 3.4.3.1.). In comparison, PDFA production was primarily limited to elongase activity both in cat and human skeletal muscle (Tables 4.4.3.1. and 5.3.3.1.), but the overall capability to form PDFA's was poorer in the cat than human. Aortic endothelial cells were shown to exhibit Δ6D, Δ5D, Δ4D and elongase activity in the rat, compared to Δ8D, Δ5D, Δ4D and elongase capability in the cat (Tables 3.3.3.1. and 4.3.3.1.); however, such enzyme potential was once again greater in the rat. The finding that endothelial cells from both species could form PDFA's may nevertheless reflect their involvement in eicosanoid, particularly prostacyclin, production (Moncada et al 1976a, 1976b and Vane et al 1987). Only elongation and retroconversion occurred in cat lung fibroblasts with p-oil incubation (Table 4.6.3.1.), while human lung fibroblasts exhibited elongase and some Δ5D, but no Δ6D or Δ4D, capability (Table 5.4.3.1.). On the other hand, Δ6D, Δ5D, some Δ4D, and elongase expression was shown in cultured rat lung with p-oil dosage (Table 3.6.3.1.). Rat skin fibroblasts incubated with p-oil similarly expressed Δ6D, Δ5D, Δ4D

limited in cultured human skin (Table 5.5.3.1.), while cat skin fibroblasts had elongase but no significant desaturase capability (Table 4.7.3.1.). Cells derived from cat adipose tissue exhibited elongase, very limited Δ5D and Δ4D, but no significant Δ6D, activity (Table 4.8.3.1.). Cultured rat adipose cells, in contrast, not only expressed Δ6D, Δ5D, Δ4D and elongases (Table 3.8.3.1.), but exhibited the greatest desaturase cascade capability of all the cells studied. Incubation of rat, cat or human lung, skin or adipose cultures with single C18 FA's induced more desaturase cascade enzyme expression than with p-oils, but PDFA formation was still greater in rat than human, and least in cat, cells (chapters 3, 4 and 5).

It was evident comparing desaturase cascade enzyme capability in the 3 species that such was greatest overall in growing rat, lower in human and least in cat, tissues. This supported current evidence with regard to the activity/expression of Δ6D in these species in vivo (eg. Brenner 1971, 1974, Rivers et al 1975a, Frankel et al 1978, Dyerberg et al 1980 and Horrobin 1983). The finding that the cat and human tissues studied exhibited a closer degree of similarity than the rat tissues with regard to desaturation capability, however, could relate to the high degree of genetic homology reported by O'Brien et al (1986) between cat and Man. The data from

mammalian species exhibit different capabilities to desaturate and elongate FA's and that these enzymes are not distributed evenly between tissues (eg. Gellhorn et al 1964, Brenner 1971, Rivers et al 1975a, Maeda et al 1978, Sinclair 1979, Sinclair et al 1981, Dyerberg et al 1980 and Chapkin et al 1984, 1986a, 1987). Desaturase cascade enzyme capability (chapters 3, 4 and 5) was unrelated to the proliferation rates of the cell types studied (Table 2.3.1.1.), but was rather characteristic of those tissues and species from which the cells were derived. These findings implied dependence of certain tissues on others in vivo for a supply of PDFA's.

The greater capability reported for FA elongation than desaturation (chapters 3, 4 and 5) confirmed that the rates of elongation were faster than desaturation, and that desaturases are rate limiting (Marcel et al 1968, Brenner 1971, 1974, 1977, Bernert et al 1975, Haasam et al 1975, Sprecher 1977 and Naughton 1981). Furthermore, this thesis confirmed the established order of substrate preference ($\omega3 > \omega6 > \omega9$) for desaturation (Brenner et al 1966, Mohrhauer et al 1967, Christiansen et al 1968, Ullman et al 1971a, Mead et al 1976 and Kanau et al 1977), and showed retroconversion capability in some cultured rat, cat and human tissues studied, supporting the retroconversion demonstrated by others in rat liver, bovine endothelial, guinea pig arota smooth muscle, and human skin, cells (Gavino et al 1981b, Hagve et al 1986

and Mann et al 1986). The magnitude of retroconversion reactions, however, were probably determined by the overall FA balance in the cultured cells.

Many workers have attempted to assess the desaturation and elongation capability of normal tissues, primarily in liver (eg. Brenner 1971, de Gomez Dumm et al 1975b, 1983, Rivers et al 1975a, Jeffcoat 1979 and Davidson et al 1987a), but also in other tissues including skin (eg. Dunbar et al 1975, Aeberhard et al 1978, Chapkin et al 1986b, 1987 and Ziboh et al 1988), endothelium (eg. Spector et al 1980b, 1981b, Weisiger et al 1981, Kaduce et al 1982 and Rosenthal et al 1983b), lung (eg. Brenner 1974 and Dunbar et al 1975), brain (eg. Cook 1978a, Sinclair 1972a, Naughton 1981 and Neuringer et al 1984) and smooth muscle (eg. Gavino et al 1981b). Comparison of literature reports with the findings of this thesis in previous discussions showed close correlations in some instances, but not in others. However, it must be pointed out that the origin of cell types and range of experimental designs employed between workers was often quite wide. There is indeed evidence that desaturase cascade enzyme expression varies not only between fetal, infant and adult tissues (Cook 1978a), but also between microsomes and cells. Microsomal preparations of rat, guinea pig and human epidermis, for example, lack the capacity to desaturate [^{14}C]-18:2ω6 and [^{14}C]-20:3ω6 (Chapkin et al 1984, 1986a, 1987 and Ziboh et al 1988).

while such was demonstrated in intact skin cells in this thesis (sections 3.7 and 5.5) and other studies (eg. Girao 1988). These findings, however, could well relate to the dependence of microsomal desaturation on cytosolic fractions present in intact cells (Catala et al 1975, 1977, Jeffcoat et al 1976, 1977a, 1978 and Leikin et al 1979, 1986, 1989). Enzyme expression is also influenced and regulated by the amount of serum and FA's in the culture medium (eg. Paulsrad et al 1970, Jacobs et al 1973a, 1973b, Brenner 1974, Jeffcoat et al 1977a, Spector et al 1981a, Rosenthal et al 1983b and Bar et al 1984), dietary FA's (eg. Paulsrad et al 1970, Castuma et al 1972, Holloway et al 1975 and Borgeson et al 1989), the FA composition and fluidity of microsomal membranes (eg. Nervi et al 1968, Kaduce et al 1977, Weyman et al 1977, Kurata et al 1980, Garda et al 1984, 1985, Stubbs et al 1984, Spector et al 1985 and Garg et al 1988a, 1988b, 1988c), and competition between FA's of the same and different series by complex mechanisms such as feedback inhibition, dependent on the relative substrate levels (Mohrhauer et al 1963a, 1967, Holman 1964, 1986a, 1986b, Garcia et al 1965, Christiansen et al 1968, Ullman et al 1971a, Brenner 1974, de Schriver et al 1982, Nassar et al 1986 and Cook et al 1987). Finally, it should be remembered that enzyme activity/expression is genetically determined for every tissue and species. Thus, the diversity of results with regard to the

literature, and between the tissues and species in this thesis, may relate to the factors discussed above.

A important feature of this thesis was that it assessed desaturase cascade enzyme capability in a variety of normal mammalian tissues, some of which have never been examined before, all under standard culture conditions. This permits valid comparison of results between different tissues and species. Furthermore, the present investigation clearly showed that desaturase cascade enzyme expression with p-oil dosage could not be fully predicted by the results obtained utilising single FA's due to synergism/antagonism between p-oil FA's (chapters 3, 4 and 5). While single FA's are useful to develop an understanding of the effects of specific FA's on desaturase cascade enzymes, such probably does not reflect the situation in the "real world", where FA's rarely, if ever, occur in isolation. On the other hand, the concept of using p-oils could well serve as an in vitro model to assess the desaturation capability of tissues in vitro when similar oils form part of the diet. The in vitro culture system using p-oils also has an advantage over the in vivo system which does not necessarily reflect the desaturation capability of the tissue(s) studied as PDFA's can readily be transported in vivo from tissues exhibiting desaturation capability to those having limited or no such potential. The present in vitro system, in contrast, is a more absolute

means of assessing tissue desaturase cascade enzyme expression/activity.

The fact that Δ6D is largely absent both in the cat (eg. Rivers et al 1975a) and diabetics (eg. Brenner et al 1968 and Horrobin 1988) implied that the desaturation and elongation induced with p-oil dosage in the cultured cat tissues in the present study may well serve as an indication of the response expected in human diabetics when similar dietary oils are ingested. Comparison of human and cat data could therefore reflect the situation between normal humans and diabetics, respectively.

6.1.3 Lipid Peroxide and Eicosanoid Production in Normal Cultured Mammalian Tissues.

The comparative data obtained from MDA quantitation indicated that the cultured rat, cat and human cells types studied varied in their capability to produce lipid peroxides (chapters 3, 4 and 5). All cells have the capability to produce these compounds via enzymic and/or non-enzymic mechanisms (Pryor et al 1976, Tappel 1980 and Frankel 1.84). However, lipid peroxides were not induced by control rat, cat and human erythrocytes and human lymphocytes (Tables 3.1.4.1., 4.1.4.1., 5.1.4.1. and 5.2.4.1., respectively), while control rat lung, human lung and human skin fibroblasts generated the highest concentrations (1.1nmoles MDA/10^6 cells) (Tables 3.6.5.1., 5.4.5.1. and 5.5.5.1., respectively). Similar lipoperoxide levels have been reported by other workers in cultured guinea pig aorta smooth muscle (Gavino et al 1981c), simian kidney (Begin et al 1986a) and human skin (Girao 1988) incubated in medium alone for a similar period to that used in the present study. The lipoperoxide levels and variations therein between the control cell types we studied may be an inherent property of the cells themselves, but could also be a result of the culture conditions employed. The finding of this thesis that lipoperoxidation varies both between mammalian tissues and species nevertheless supported and extended the work of Arakawa et al (1986) who reported

marked species differences in lung lipid peroxide formation (mouse > hamster > rat > guinea pig > rabbit). This implied that the rates of FA oxidation and efficacy of the mechanisms of protection against free radical formation varied between tissues from different species.

Studies have shown that there is a high degree of variation in the vitamin E content of different tissues (Taylor et al 1976). This implied differences amoung tissues with respect to protection against FA oxidation. Kornbrust et al (1980) indeed reported that the rates of in vitro lipoperoxidation varied widely amoung different species and tissues, and was inversely related to vitamin E levels. These workers found a 25-50 fold lower rate of lipoperoxidation in rat and rabbit lung and heart microsomes and homogenates than in liver, testes, [illegible] vitamin E content of the former. Higher rates of lipid peroxidation in mouse relative to human, rat and rabbit, lung microsomes similarly correlated with lower vitamin E contents in the former. The finding that rat and human lung microsomes contained similar amounts of vitamin E (Kornbrust et al 1980) could well explain the identical lipoperoxide levels measured in control rat and human lung fibroblasts in this thesis (Tables 3.6.5.1 and 5.4.5.1., respectively). These correlations implied that lipid peroxidation is partly determined by the ratio of

marked species differences in lung lipid peroxide formation (mouse > hamster > rat > guinea pig > rabbit). This implied that the rates of FA oxidation and efficacy of the mechanisms of protection against free radical formation varied between tissues from different species.

Studies have shown that there is a high degree of variation in the vitamin E content of different tissues (Taylor et al 1976). This implied differences amoung tissues with respect to protection against FA oxidation. Kornbrust et al (1980) indeed reported that the rates of in vitro lipoperoxidation varied widely amoung different species and tissues, and was inversely related to vitamin E levels. These worke s found a 25-50 fold lower rate of lipoperoxidation in rat and rabbit lung and heart microsomes and homogenates than in liver, testes, [illegible] vitamin E content of the former. Higher rates of lipid peroxidation in mouse relative to human, rat and rabbit, lung microsomes similarly correlated with lower vitamin E contents in the former. The finding that rat and human lung microsomes contained similar amounts of vitamin E (Kornbrust et al 1980) could well explain the identical lipoperoxide levels measured in control rat and human lung fibroblasts in this thesis (Tables 3.6.5.1 and 5.4.5.1., respectively). These correlations implied that lipid peroxidation is partly determined by the ratio of

vitamin E to peroxidisable FA's present in cells, a relationship found by Arakawa et al (1986) in lung.

Spent control media lipoperoxide concentrations were not only similar between the cultured rat, cat and human tissues studied (0 to 0.5nmoles MDA/10^6 cells), but also equal to, or lower than, cellular levels, except with human skeletal muscle cultures (chapter 3, 4 and 5). Gavino et al (1981c) similarly detected greater MDA amounts in guinea pig aorta smooth muscle cells than in the corresponding spent control medium, while the converse was reported by Begin et al (1986a) and Girao (1988) in normal and transformed cell lines. However, the fact that Begin et al (1986a) and Girao (1988) used ethanol as FA carrier implied that medium FA's were largely in free suspension and susceptible to auto-oxidation, while the use of albumin in the present study (section 2.2.2), and addition to culture medium of anti-oxidants by Gavino et al (1981c), probably protected medium FA's from significant auto-oxidation. These findings nevertheless indicated that discrepancies in results between workers could well relate to variations in experimental design, and should thus be considered in the interpretation of such data.

To a greater or lesser extent, lipoperoxide production increased overall when rat, cat and human cultures were incubated with p-oil for 48 hours, although quantitative

differences were once again evident between tissues from different species (hapters 3, 4 and 5). A wider range of lipid peroxides, however, was measured in dosed erythrocytes, lyr .cytes and their corresponding spent media than for adherent cultures (0 to 102.7 vs 0 to 14.5nmoles MDA/10^6 cells, respectively). Moderate levels of TBA reactive material (TBARM) have similarly been reported in normal adherent guinea pig aorta smooth muscle (Gavino et al 1981c), simian kidney (Begin et al 1986a and Horrobin 1990) and human skin (Girao 1988) cultures following addition of PUFA's. A possible explanation for the different lipid peroxide amounts induced with p-oil dosage between the cell types studied, however, could relate to alterations in the rate of PGL turnover, the concentration of FFA's and hence size of the peroxidisable pool.

Several workers reported higher lipoperoxide levels in cells than spent medium following dosage of single PUFA's to transformed and/or normal cell cultures (eg. Gavino et al 1981c, Morisaki et al 1984, Begin et al 1986a and Girao 1988). In our study, the distribution of cellular and medium lipoperoxides in dosed cultures was more varied (chapters 3, 4 and 5), particularly when growth limitation or inhibition was prominent with 60mg/l p-oil. This correlated with the findings of Begin et al (1986a) when a human breast tumor cell line was incubated for 7 days with single PUFA's solubilised in

ethanol. A possible explanation for our findings might relate to the proposal in previous discussions (chapters 3, 4 and 5) that most spent medium lipid peroxides originated intracellularly, and thus reflected the amount of lipoperoxides released either through the plasma membrane of viable cells or via cell lysis. Despite evidence that cellular FA's can be released back into the culture medium subsequent to uptake (eg. Cyong et al 1976, Rosenthal et al 1978 and Figard et al 1986), the presence of albumin as p-oil FA carrier in the present study's incubation medium (section 2.2.1) indeed supported the probability that extracellular auto-oxidation did not contribute significantly to spent medium lipoperoxide levels in the presence of cells when short incubation periods are used, particularly since the solubility of oxygen in water, and thus culture medium, is relatively low at 37°C (Merck 1983). Auto-oxidation, however, can contribute to medium lipid peroxide formation when dosed PUFA's are solubilised in ethanol and are thus in free suspension, as Begin et al (1986a) have shown.

Lipoperoxide generation varied with the p-oil and concentration dosed within a particular cell type or species, and between similar or different cell types in different species (chapters 3, 4 and 5). As a general rule, lipoperoxide production increased with the amount of p-oil dosed, and was greater with p-oils rich in

polyenoic than monoenoic or saturated FA's. Others similarly demonstrated an overall concentration dependent increase in lipid peroxide production with increasing amounts of FA dosed to normal cultured cells (eg. Morisaki et al 1982b and Girao 1988). Although lipoperoxide production is directly related to the number of double bonds when single PUFA's solubilised in non-toxic amounts of ethanol are dosed to culture medium alone (eg. Begin et al 1986a and Horrobin 1990), this is not necessarily true in all dosed cells. Little lipid peroxidation was measured in guinea pig aorta smooth muscle cultures incubated with 18:1ω9, more with 18:2ω6 or 20:2ω9, while FA's containing 3 or more double bonds induced greater amounts (Gavino et al 1981c and Morisaki et al 1982b). Other workers, however, report no obvious relationship between TBARM levels and the number of double bonds in the PUFA's added to cultured cells (eg. Horrobin 1990). It has been documented, for example, that the capability to induce lipoperoxides was in the order 18:3ω6 > 18:3ω3 > 18:2ω6 > 18:4ω3 > 18:1ω9 in human skin fibroblasts (Girao 1988), 20:5ω3 > 22:6ω3 > 18:3ω6 > 20:4ω6 in simian kidney cells, and 20:4ω6 > 18:3ω6 > 20:5ω3 > 22:6ω3 in human breast tumor cells (Begin et al 1986a). p-Oils abundant in unsaturated FA's similarly varied in ability to form TBARM between cell types in the present study (chapters 3, 4 and 5). These findings implied that the number and position of double

dependent on tissue and species. This could well relate to different effects which dosed FA's/p-oils had on the rate of PGL turnover, FA incorporation, FA release from PGL's and FFA availability for oxidation in the different cultured mammalian tissues, each of which exhibited unique FA compositions. The potential for p-oil FA synergism and antagonism probably also influenced lipid peroxide generation, and such could explain why lipoperoxide production with p-oil incubation could not be fully predicted by the results obtained by other workers (eg. Gavino et al 1981c, Morisaki et al 1982b and Girao 1988) utilising single FA's. Examination of findings both in the present study and literature discussed above nevertheless suggested an increased requirement for dietary anti-oxidants when unsaturated FA's/FA-rich oils are fed.

Some workers found that rapidly dividing normal cells, such as regenerating liver, generally contain lower lipoperoxide levels compared to tissues with a slow rate of division, eg. brain or non-regenerating liver (eg. Wolfson et al 1956 and Cornwell et al 1984). Lower lipid peroxide levels have similarly been reported in tumor than in normal cells <u>in vitro</u>, which was consistent with higher alpha-tocopherol levels in tumor cells (Cheeseman et al 1984), although such could also relate to a lower PUFA content in malignant than in normal cells. These findings nevertheless indicated that

a high rate of cell division generally correlated inversely with the rate of lipoperoxidation. Cheeseman et al (1986) demonstrated that the time of maximum DNA synthesis coincided with the maximum alpha-tocopherol content and minimum lipoperoxidation in dividing normal liver, and suggested that lipoperoxidation is decreased prior to cell division. This implied that lipoperoxide production may be related to the degree of confluency of cultured cells. Others showed that the mean lipoperoxide amounts generated by control human breast tumor and normal simian kidney cultures over a 7 day period were similar, whereas lipoperoxide production by tumor cultures dosed with PUFA's (20mg/l GLA, AA, EPA or DHA) approximated to, or was significantly greater than, amounts induced by dosed normal cultures (Begin et al 1986a). Similar findings have been reported by Das et al (1986) with regard to normal and tumor cells. Work in our laboratory on a normal (human skin), transformed (3T6D) and two benign (3T3, 3T6) cell lines showed that peroxidation of dosed C18 PUFA's increased with degree of cell transformation in the order HSF, 3T3, 3T6 and 3T6D cells (Girao et al 1988, 1989). We suggested that this may relate to impairment of desaturation capability in transformed cells (eg. Dunbar et al 1975, Bailey 1977 and Horrobin 1983), resulting in a build-up of peroxidation substances. Alternatively, the normal cells possessed efficient mechanisms to limit lipoperoxidation

while transformed cells could have lost the relevant mechanisms for controlled lipoperoxidation due to enzyme loss or anti-oxidant activity. Overall, the present study found no clear correlation between cell proliferation rates (Table 2.3.1.1.), or cellular unsaturated FA contents, and lipoperoxide production with regard to the cultured tissues studied (chapters 3, 4 and 5). This could well have related to variations between tissues from different species in the (i) amount of cellular vitamin E and activity enzymes of such as peroxidase, superoxide dismutase and catalase that remove different species of activated oxygen that promote lipid peroxidation; (ii) physiology and rates of PGL turnover; (iii) desaturation capability and thus peroxidation substrate availability; and/or (iv) size of the peroxidisable FFA pool (Resch et al 1975). The finding that no lipid peroxides were generated by control rat, cat and human erythrocytes (non-dividing) (Tables 3.1.4.1., 4.1.4.1. and 5.1.4.1.), and the direct correlation between control cell proliferation rates (Table 2.3.1.1) and lipid peroxide production in most cultured human tissues (chapter 5) nevertheless indicated that the rate of cell division may partly influence lipoperoxide generation.

Studies indicate that lipoperoxides are responsible to a greater or lesser extent for the inhibition of cell proliferation by PUFA's (eg. Cornwell et al 1979, Miller

et al 1980, Gavino et al 1981b, Morisaki et al 1982b, 1984, Horrobin et al 1984b and Begin et al 1985a, 1985b, 1986a, 1988). This thesis similarly supported p-oil-induced lipoperoxidation in the reduction of mammalian cell viability (chapters 3, 4 and 5), and thus the suggestion by other workers that controlled lipid peroxidation may have a physiological role in the regulation of cell division (eg. Cornwell et al 1984 and Begin et al 1986a). To date, research has been unable to unequivically clarify whether or not lipoperoxides are directly deleterious to cells, or merely an incidental outcome of induced damage (eg. Begin et al 1986b). The possibility exits, however, that p-oil/FA-induced growth limitation and cytotoxicity could well be the result of cumulative cellular damage arising from generated lipid peroxides, since these compounds are particularly destructive to cellular proteins, including DNA, enzymes and membranes (eg. Wills 1961, Desai et al 1963, Andrews et al 1965, Roubal et al 1966a, 1966b, Chio et al 1969, Mead 1976, Trotta et al 1982, Frankel 1984, de Groot et al 1985 and Gutteridge et al 1990). Another possibility is the stimulation of superoxide radicals. Unfortunately we could not determined this as the technology was unavailable. The present study nevertheless provided evidence which indicated that lipoperoxides are not solely involved in the modulation of cell viability, and proposed the involvement of other mechanisms, such as alterations in membrane fluidity (chapters 3, 4 and 5).

Indeed, this could explain the growth inhibition found when p-CO supplementation to human erythrocytes (section 5.1), for example, induced no lipoperoxides, and the finding by this and other studies (Morisaki et al 1982b) that lipoperoxidation does not always correlate with the degree of growth inhibition induced with dosage of FA's.

In the field of eicosanoid research, it has generally been accepted that "all nucleated cells can make PG's" (Moncada et al 1984) and that "cyclooxygenase is largely ubiqu[illegible]us for nucleated cells" (Janniger et al 1987). While eicosanoids were formed by all cultured tissues we studied (chapters 3, 4 and 5), eicosanoid production has been shown to be absent from human lymphocytes (Dy et al 1980, Bankhurst et al 1981 and Goldyne 1989), thus the above concept may be totally incorrect. Indeed, this thesis showed that cultured cells derived from different mammalian tissues and species produce different molar amounts of total eicosanoids (chapters 3, 4 and 5), which might well reflect the different physiology of tissues <u>in vivo</u>. The picogram eicosanoid concentrations detected, summarised in Table 6.1.3.1., supported the basal levels reported by Moore (1985) for tissues <u>in vivo</u>. Total molar eicosanoid concentrations, however, were always higher in the control rat and human than cat cells studied, and generally correlated with the desaturase capabilities of these species, which decreased in the order rat > human > cat (section 6.1.2).

Table. 6.1.3.1.

Comparison of Total Lipid Peroxide and Eicosanoid Concentrations in Control Cells.

Cell Type	nmoles total MDA/10^6 cells	pmoles total eicosanoids/ 10^6 cells	eicosanoids MDA (%)
Lung:			
rat	1.10	727.6	66.2
human	1.10	332.6	30.2
cat	0.30	32.9	11.0
Skin:			
rat	0.20	165.7	82.9
human	1.10	232.5	21.1
cat	0.40	80.5	20.1
Adipose:			
rat	0.50	215.9	43.2
cat	0.40	208.0	52.0

Molar eicosanoid production was greatest in control human, and particularly rat, lung compared to skin and adipose cells, confirming the lung as a major site of eicosanoid synthesis (eg. Mathe et al 1977, Hyman et al 1978 and Harper et al 1984). The finding that control cat lung fibroblasts produced the lowest total eicosanoid amounts detected may not necessarily reflect poor eicosanoid production, but possibly an increased capability to remove and inactivate eicosanoids, a function which the lung is very capable of performing (eg. Piper et al 1970 and Smith 1987). Eicosanoid production (Table 6.1.3.1.) and desaturation capability (section 6.1.2), however, were found not to correlate quantitatively between mammalian cell types. Both rat and human control skin fibroblasts, for example, exhibited relatively less capability to produce eicosanoids than lung, despite greater capability for desaturation in skin. Similarly, the potential to form eicosanoids was only marginally greater in rat than cat adipose cells, despite the greater capability of rat adipose cells to desaturate FA's. These findings may relate to variations between tissues from different species in the rate of eicosanoid synthesis and degredation, distribution of eicosanoid PDFA precursors between the TAG and PGL pools, and size of the eicosanoid PUFA precursor pool.

The greater molar lipoperoxide than eicosanoid levels

demonstrated in cultured rat, cat and human tissues (Table 6.1.3.1.) correlated with and extended the findings of Morisaki et al (1982b) in guinea pig aorta smooth muscle. Once again, we found that molar eicosanoid to lipoperoxide ratio's varied with cell type and species, ranging from 11.0% to 82.9%. A considerably greater proportion of total lipoperoxides found in control lung and skin fibroblasts were composed of eicosanoids in the rat than human, while ratios were almost identical in cat and human skin and lowest in cat lung fibroblasts. Thus, the enzymic pathways for eicosanoid production were greatest overall in rat, less in human, and least in cat, lung and skin fibroblasts. This pattern correlated with the greater capability of the rat to desaturate FA's, and therefore produce more eicosanoid precursors, than the human, and especially the cat (section 6.1.2). Furthermore, the greater correlation between total eicosanoid to lipid peroxide ratios in control cat and human than rat lung and skin fibroblasts (Table 6.1.3.1.) reflected the close degree of genetic homology demonstrated between the cat and human (O'Brien 1986). On the other hand, the finding that about half the total lipoperoxide amounts detected both in control rat and cat adipose cells were composed of eicosanoids implied that the pathways for eicosanoid generation were approximately equally favoured in both species. This may reflect the relative importance of

eicosanoids in adipose cells *in vivo*. Eicosanoids might well be associated with mobilisation of lipids into and/or out of this tissue by controlling the permeability of blood capillaries associated with adipose cells, thus allowing lipids to enter adipose tissue for storage or the circulation to supply other tissues with a metabolic energy source.

This thesis demonstrated considerable tissue and species specificity in the expression of enzymes involved in eicosanoid synthesis as control cells varied numerically in the amounts of PGI_2, TXB_2, PGF_{α}, PGE_2 and PGE_1 they produced (chapters 3, 4 and 5). Low concentrations of these prostanoids were detected in control cells, but PGF_{α} was the predominant prostanoid in adipose tissue, compared to TXB_2 in skin, while a more variable pattern was found in lung between the three species. This nevertheless indicated that the cyclooxygenase pathway was functional in the cultured mammalian tissues studied. These findings supported and extended the work of Pace-Asciak et al (1977) who demonstrated differences between rat lung, liver, spleen, stomach, kidney and heart homogenates to form PGE_2, $PGF_{2\alpha}$, PGD_2, TXB_2 and 6-keto $PGF_{1\alpha}$. These rat 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 formed specifically by stomach, although lung also generated significant amounts. TXB_2

was mostly directed to spleen, with lesser amounts in lung (Pace-Asciak et al 1977), while the converse was found in guinea pig lung and spleen (Hamberg 1976). This supported the ability of different mammalian species and tissues shown in the present study to selectively synthesise different eicosanoids and amounts thereof, probably to mediate specific functions. There is indeed evidence that the effects eicosanoids induce can vary both with tissue and species (Moore 1985 and Taylor et al 1986). For example, PGE_2 induces contraction of intestinal longitudinal muscle, but relaxation of lung smooth muscle (Taylor et al 1986). Similarly, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ decrease blood pressure and increase heart rates of rabbits and cats, while the converse occurs in the rat and dog (Moore 1985).

The eicosanoid levels and variations therein between the cell types we studied may be an inherent property of the cells themselves, but could also be a result of the culture conditions employed. Indeed, there are reports that serum concentration and cell age influence eicosanoid synthesis in culture (Dunn et al 1976, Hong et al 1976, 1979 and Hammarstrom 1977). There is also evidence that eicosanoid production is inversely related to cell density, being maximal during the first day in culture, and decreasing considerably after confluence (Hammarstrom 1977, Hong et al 1979 and Negrel et al 1981b). The present study, however, showed that cell

proliferation rates (Table 2.3.1.1.) and eicosanoid production (Table 6.1.3.1.) were not always directly related, thus the diversity of results could well reflect genetic variations between tissues from different species.

p-Oil supplementation modulated eicosanoid generation by enhancing, suppressing or inhibiting the production of invidivual prostanoids, dependant on cell type (chapters 3, 4 and 5). The modulation of prostanoid biosynthesis by single FA's has also been demonstrated by other workers. 20:3ω6 enhanced the release of PGE_1, and 20:4ω6 the formation of PGE_2 and TXA2, in cultured guinea pig aorta smooth muscle, human lung and skin (Huttner et al 1977 and Bryant et al 1978). Others found that cultured guinea pig aorta smooth muscle PGE_1, PGE2 and 6-keto-$PGF_{1\alpha}$ production was increased with 15-120μM 18:3ω6, 20:3ω6 and 20:4ω6, inhibited with 22:4ω6 and 22:6ω3, while 18:2ω9, 18:2ω6, 18:3ω3, 20:2ω9, 20:3ω3 and 20:5ω3 had no effect (Morisaki et al 1982b). On the other hand, ω6 and ω3 series PUFA's inhibited PGE_2 and $PGF_{2\alpha}$ synthesis in the order DHA+EPA > DHA > EPA > ALA > LA > DGLA > GLA in normal human gingival fibroblasts, and DGLA > EPA > DHA+EPA > DHA > ALA > LA in an oral human squamous carcinoma cell line, although normal cells were less susceptible to the inhibitory effects (El-Attar et al 1990). Others found that rats fed diets containing either safflower or coconut oils had similar prostanoid

generating capability (Codde et al 1985), and suggested that there was tight metabolic control of prostanoids and their precursors. This could well explain why the modulation of PGI_2, TXB_2, PGF_α, PGE_2, PGE_1 and PGD_2 production by exogenous FA's in this thesis was unrelated to the degree of p-oil unsaturation, the ability of p-oils to supply direct eicosanoid PUFA precursors (Table 2.3.3.2.), or the capability of the cells to form such via desaturation and elongation (section 6.2.1). The ability to influence eicosanoid precursor availability and enzyme expression in the prostanoid biosynthetic pathway with different p-oil concentrations was suggested, however (chapters 3, 4 and 5). Why eicosanoid production was enhanced in certain cultured tissues, for example cat lung, despite poor desaturation capability, and thus a lack of eicosanoid precursors, was unclear, although basal PUFA precursor levels may have been sufficient to maintain eicosanoid production. This could indeed explain the enhancement of eicosanoid production in certain instances to a similar extent with p-CO compared to p-oils containing PUFA's and eicosanoid precursors, although such may also relate to EFA-deficiency, which has been shown to increase PG-synthetase activity (Kaa 1976).

Literature reports indicate that exogenous PG's can have pronounced effects on the multiplication of a variety of cultured cell types (eg. Bem et al 1974, Thomas et al

1974, Eaglestein et al 1975, Armato et al 1977, Bentley-Phillips et al 1977 and Cornwell et al 1979), while reports are more conflicting for endogenously generated eicosanoids. The stimulation of DNA synthesis and mitotic activity in rat hepatocyte cultures following 20:4ω6 incubation was related to PGA_1, PGE_1 and PGE_2 production (Andreis et al 1981a), implying that PG's may be involved in the control of cell growth, probably by inducing mitogenic compounds, such as cAMP or cGMP (eg. Armato et al 1977). This supported the proposal that eicosanoid secretion may represent an autocrine mode of growth regulation for some fibroblasts (Taylor et al 1977 and Smith et al 1984). A larger school of other workers, in contrast, report that the inhibition of normal and cancer cell proliferation by incubation with single exogenous FA's does not relate to significant endogenous cyclooxygenase or lipoxygenase product involvement (eg. Cornwell et al 1979, Morisaki et al 1982b, 1984, Horrobin et al 1984b, Begin et al 1985a, 1985b, 1986c, 1988, Botha et al 1985, 1989, Metzger et al 1986 and Strange et al 1986). Even the enhancement of cell growth and DNA synthesis of an osteogenic cell line in the presence of 10% FBS and FA's (10^{-4}M 20:4ω6 or 18:3ω3) has been suggested recently to be independent of prostanoid involvement (Fujimori et al 1989). Others have gone as far as suggesting that endogenous PG biosynthesis is a consequence of, rather than a pre-requisite for, or important mode of control of, cell

proliferation in culture (Thomas et al 1974, Cornwell et al 1979, Negrel et al 1981a, 1981b and Durant et al 1989). The present study provided comprehensive *in vitro* evidence to support the fact that endogenously synthesised prostanoids were neither directly involved in the stimulation, limitation nor inhibition of mammalian cell viability induced with p-oil dosage under identical culture conditions (chapters 3, 4 and 5). Indeed, this mechanism could not explain the modulation of cell viability with incubation of saturated or monounsaturated FA-rich p-oils. The fact that p-oil FA composition mimicked that of dietary oils nevertheless implied with greater certainty than the studies discussed above using single FA's that this phenomenon could well occur *in vivo*

6.2 SUMMARY AND CONCLUSIONS.

This thesis demonstrated that p-oils modulate normal mammalian cell viability, and that the effects mediated vary considerably both between tissues and species. Furthermore, the modulation of cell viability, and p-oil-induced cytotoxicity in particular, was proposed to involve both the production of lipoperoxides and the perturbation of membrane structure, function and fluidity by alteration of the FA profile of membrane PGL's, but eicosanoid involvement was excluded. Direct FFA toxicity could not be completely ruled out as a mechanism involved in growth reduction, although it was likely that other mechanisms played a greater role. Studies have indeed shown that the changes in lipid composition induced in cells when exogenous FA's are incorporated can modify membrane structure, stability and permeability, mediate changes in membrane-associated receptors and enzyme activity, decrease cell adhesion and influence cell morphology and growth/viability (eg. Ginsburg et al 1973, Engelhard et al 1976, Hoover et al 1977, Doi et al 1978, Spector et al 1979, 1985 and Kidwell et al 1984). A correlation between TAG build up and cell damage has also been reported (eg. Rosenthal et al 1978). Thus, the accumulation of cytoplasmic lipid droplets in some instances in this thesis may indicate an inability of certain mammalian cells to metabolise particular p-oils or concentrations thereof, which may

subsequently have led to impaired cell function and death. Although we showed no distinct correlation between cell viability or FA incorporation, the desaturation capability of a cell type could well have affected its ability to respond to p-oil FA's. Indeed, desaturase cascade enzymes, and particularly Δ6D due to its rate limiting nature and position in the cascade, may be indirectly involved in the control of cell proliferation by influencing the supply of PUFA's to maintain membrane fluidity and to serve as substrates for lipoperoxidation. Although the cell viability changes the p-oils induced could be attributable to any one of the mechanisms discussed, a combination of the above would seem most likely, implying a multifcatorial mechanism.

Any investigation demonstrating an effect of FA's in a biological system must consider whether FA's functioned as anionic detergents. Significant detergent action was unlikely in this study, however, for a number of reasons. Firstly, the p-oil concentrations used were relatively low. Secondly, in addition to the albumin utilised as FA carrier, serum albumin itself has a number of strong FA binding sites (Ashbrook et al 1975 and Spector et al 1975), and there is evidence that 10% FCS contains sufficient albumin to completely block any detergent effect (Boone et al 1972), particularly at low p-oil concentrations dosed. Thirdly, despite the fact

that 18:1ω9 lowers surface tension to a greater extent than 18:0 (Patil et al 1977 and Huttner et al 1978), cell proliferation was stimulated more frequently and to a greater extent with dosage of certain concentrations of p-OO, rich in 18:1ω9, than with p-CO, rich in saturated FA's. Fourthly, the pattern of lipoperoxides detected would not have been observed if detergency played a significant role.

Cell cultures have provided a good system for studying lipid metabolism by offering more stable and controlled conditions with less biological variation than *in vivo* studies. Cultured cells derive most of their lipid from the incubation medium in general, and the serum in particular (eg. Geyer et al 1962, Mackenzie et al 1970, Bailey et al 1972 and Spector et al 1981a), thus the lipid content and FA composition of the incubation medium is an important, yet often overlooked, consideration in experimental studies. All tissue culture catalogues available (eg. Flow 1983 and Gibco 1984) extensively describe the protein, mineral, vitamin, glucose, etc. contents of various types of growth media and sera, but fail to consider the lipid, FA or even EFA contents, despite their well established importance *in vivo* (Burr et al 1930).

Examination of the literature clearly shows that the FA composition and PUFA distribution between different

kinds of commercially available sera used to supplement culture media differ widely (eg. Spector et al 1981a and Delplanque et al 1987). Our laboratory demonstrated that the FA composition of complete incubation medium, containing 10% FCS and 90% DMEM, was primarily composed of saturated FA's, with little PUFA's, and no significant EFA's, detectable (Table 2.3.3.3. and Girao 1988). Similar results were reported by Lagarde et al (1984) for medium containing 20% FBS, while medium supplemented with HS contained a greater molar amount of total FA's and a greater proportion of PUFA's. In the present study, however, only traces of EFA's and PDFA's were found in DMEM containing either 10% rat, cat or human sera (Table 2.3.3.3.). Taken together, such findings suggest that an EFA- and/or PDFA-deficiency state may exist in culture systems.

Both the quantity and quality of the incubation medium FA's can induce major differences in experimental results. For example, EFA deficiency has been implicated in enhancing endothelial cell Δ6D activity/expression (Spector et al 1981b and Rosenthal et al 1983b). Thus, EFA/PDFA deficiency in the incubation medium may explain the small amount of desaturation/elongation of cellular (endogenous) PUFA's found when particular cell types were dosed with p-oils containing primarily saturated FA's. Furthermore, incorporation of saturated FA's would decrease the unsaturation index in the cell membranes,

thus desaturation/elongation of cellular PUFA's could also reflect an attempt by the cells to counteract this effect to maintain membrane fluidity. Holloway et al (1977) indeed reported that EFA deficiency may enhance desaturase enzyme activity to serve this purpose.

The finding that p-oils containing unsaturated FA's stimulated the growth of many cell types with low to intermediate concentrations in the range dosed suggested that these moieties could well have growth promoting properties, and that this effect relates to a deficiency of EFA's and/or PDFA's in the growth medium. Such may also explain the enhanced cell growth reported in many other studies dosing low concentrations (0.1-120μM) of single PUFA's to a range of normal (eg. Ham 1963, Dubin et al 1965, Gerschenson et al 1967, Huttner et al 1978, Wicha et al 1979, Cornwell et al 1979 and Spector et al 1979) and even in some transformed (eg. Hillyard et al 1979, Wicha et al 1979 and McGee 1981) cell lines. While it has not conclusively been proven that 18:2ω6 or other PUFA's are required for cell multiplication in culture (Bailey et al 1973a, Spector et al 1981a and Bettger et al 1982), there is evidence that PUFA deficiency leads to alterations in morphological and biochemical properties (Monsen et al 1962, Walker 1966, 1967, Rao et al 1979 and Bourre et al 1983). These factors should therefore be considered in a situation of an EFA/PDFA deficiency in a cell, particularly if the tissue was

derived from a species already exhibiting limited desaturation capability.

As current conditions of tissue culture in general use may be sub-optimal, and the fact that the lipid and FA composition of different sera vary considerably, this thesis strongly advocates total lipid and FA analysis of all culture media prior to use, and appropriate supplementation thereof with polyenoic, or at least essential, FA's if required. Furthermore, as with all *in vitro* experimental systems, tissue culture conditions should, as far as possible, parallel conditions *in vivo*.

The variations found between the cultured rat, cat and human cell types with regard to the parameters investigated in this thesis indicated a unique pattern of lipid metabolism both between mammalian tissues and species, which probably related largely to variations in the genetic composition and physiology of those tissues *in vivo*. Thus, generalisation and extrapolation of experimental results between mammalian tissues and species is potentially highly misleading and invalid, and should be avoided if reliable interpretation of results is desired. This emphasised the importance of selecting the same tissue and species for control and experimental studies. Furthermore, with regards to interpretation of cell viability results, all effects must be related to the cytostatic number.

Many workers have used dosing of cultured transformed cells with single exogenous FA's as a means for limiti \ cell growth (eg. Begin et al 1985a, 1985b, 1986b, 1986c, Booyens et al 1984a, 1984b, 1984c, Davidson et al 1987b, 1987c, Girao et al 1986, 1987 and Giangregorio et al 1988b), while comparatively less work has been performed in this area on normal cells, and no work has been conducted using FA mixtures mimicking dietary oils (p-oils). It is nevertheless essential to understand the effects FA mixtures have on genetically entire normal cells before one is able to understand the situation in the "real world" and assess the significance of FA mixtures in the modulation of cancer cell growth.

The finding of this thesis clearly showed that the results obtained with p-oil dosage could not be fully predicted by those utilising single FA's. Clearly, the use of p-oils complicates interpretation of data to a greater extent than studies using single FA's. This is due to the greater complexity of the compound dosed, and the possibility for synergistic and antagonistic interactions between FA's. While individual FA's are useful to elucidate particular effects, the use of p-oils would nevertheless be a better reflection of the response expected in the "real world" where FA mixtures are ingested as dietary oils.

Since p-oil FA composition reflected that of dietary

oils, and the p-oil concentrations dosed were similar to the plasma FFA levels normally detected *in vivo* (Fredrickson et al 1958), the data presented in this thesis serves as an *in vitro* model and guide to how genetically entire normal mammalian cells *in vivo* may respond when similar oils form part of the dietary intake.

LITERATURE CITED.

Abdel-Halim M.S. and Anggard E., 1977.
Identification of prostaglandin D_2 as a major prostaglandin in homogenates of rat brain.
Prostaglandins, 14, 633-643.

Abdel-Halim M.S. and Anggard E., 1979.
Reigonal and species differences in endogenous prostaglandin biosynthesis by brai homogenates.
Prosaglandins, 67, 411-418.

Actis Dato, S.M. and Brenner, R.R., 1970.
Comparative effects of docosa-4,7,10,13,16-pentaenoic acid and docosa-4,7,10,13,16,19-hexaenoic acid on desaturation of linoleic and alpha-linolenic acid.
Lipids, 5, 1013-1015.

Actis Dato, S.M., Catala, A. and Brenner, R.R., 1972.
Circadian rhythm of fatty acid desaturation in mouse liver.
Lipids, 8, 1-6.

Adam, O., Wolfran, G. and Zollner, N., 1986.
Effect of α-linoleic acid in the human diet on linoleic acid metabolism and prostaglandin biosynthesis.
J. Lipid Res., 27, 421-426.

Adebonojo, F.O., 1975.
Monolayer cultures of disaggregated human adipocytes.
In Vitro, 11, 50-54.

Aeberhard, E.E., Corbo, L. and Menkes J.H., 1978.
Polyenoic acid metabolism in cultured human skin fibroblasts.
Lipids, 13, 758-767.

Aeberhard, E.E., Gan-Elepano, M. and Mead, J.M., 1981.
Metabolism of fatty acids in rat brain microsomal membranes.
Lipids, 16, 705-713.

Agradi, E., Tremoli, E., Petroni, H. and Galli, C., 1981.
Dietary linoleic acid, accumulation of arachidonic acid in tissue lipids and production of TXB_2 and PGI2 in the cardiovascular system.
Prog. Lipid Res., 20, 561-565.

de Alaniz, M.J.T. and Brenner, R.R., 1969.
Effect of insulin upon the lipids of subcellular proteins of rat liver.
Acta Physiol. Latinoam., 19, 1-15.

Albert, D.H. and Coniglio, J.G., 1977.
Metabolism of eicosa-11,14-dienoic acid in rat testes: evidence for delta-8-desaturase activity.
Biochim. Biophys. Acta, 489, 390-396.

Albert, D.H., Rhamy, R.K. and Conoglio J.G., 1979.
Desaturation of eicosa-11,14-dienoic acid in the human testes.
Lipids, 14, 498-500.

Alexander, R.W. and Gimbrone, M.A., 1976.
Stimulation of prostaglandin E synthesis in cultured human umbilical vein smooth muscle cells.
Proc. Natl. Acad. Sci. U.S.A., 73, 1617-1620.

Alfin-Slater, R.B. and Aftergood, L., 1968.
Essential fatty acids re-investigated.
Physiol. Rev., 48, 758-784.

Ali, A.E., Barrett, J.C. and Eling, T.E., 1980.
Prostaglandin and thromboxane production by fibroblasts and vascular endothelial cells.
Prostaglandins, 20, 667-688.

Allman, D.W., Hubbard, D.D. and Gibson, D.M., 1965.
Fatty acid synthesis during fat-free refeeding of starved rats.
J. Lipid Res., 6, 63-74.

Almeida, L.M., Vaz, W.L.C., Stumped, J. and Madiera, V.M.C., 1986.
Effect of short-chain primary alcohols on fluidity and activity of sarcoplasmic reticulum membranes.
Biochemistry, 25, 4832-4839.

Anding, R. and Hwang, D.H., 1986.
Effects of dietary linolenate on the fatty acid composition of brain lipids in rats.
Lipids, 21, 679-701.

Andreis, P.G., Whitfield, J.F. and Armato, U., 1981a.
Stimulation of DNA synthesis and mitosis of hepatocytes in primary cultures of neonatal rat liver by arachidonic acid and prostaglandins.
Exp. Cell Res., 134, 265-272.

Andreis, P.G. and Armato, U., 1981b.
Effects of epidermal growth factor and associated pancreatic hormones on mitotic cycle phases and proliferation kinetics of neonatal rat hepatocytes in primary culture.
Endocrinology, 108, 1954-1964.

Andrews, F., Bjorkstan, J., Trenk, F.B., Henick, A.S. and Koch, R.B., 1965.
The reaction of an autoxidised lipid with proteins.
J. Am. Oil. Chem. Soc., 42, 779-781.

Anon., 1968.
Phospholipid exchange between plasma and erythocytes in Man and the dog.
J. Clin. Invest., 47, 749.

Anon., 1973.
Are PUFA harmful?
Br. Med J., 5883, 1-2.

Anon., 1979.
Effects of feeding gamma-linolenic acid in Man.
Nutr. Rev., 37, 286-288.

Arakawa, K. and Sagai, M., 1986.
Species differences in lipid peroxides in lung tissue and investigation of their determining factors.
Lipids, 21, 769-775.

Arbogast, L.Y., Rothblat, G.H., Leslie, M.H. and Cooper, R.A., 1976.
Cellular cholesterol ester accumulation induced by free cholesterol-rich lipid dispersions.
Proc. Natl. Acad. Sci U.S.A., 73, 3680-3684.

Armato, U., Draghi, E. and Andreis, P.G., 1977.
Effects of purine cyclic nucleotides on the growth of rat hepatocytes in primary tissue culture.
Exp. Cell Res., 105, 337.

Ashbrook, J.D., Spector, A.A., Santo, S.E.C. and Fletcher, J.E., 1975.
Long-chain fatty acid binding to human plasma albumin.
J. Biol. Chem., 250, 2333-2338.

de Asua, L.J., Clingan, D. and Rudland, P.S., 1975.
Initiation of cell proliferation in cultured mouse fibroblasts by prostaglandin $F_{2\alpha}$.
Proc. Natl. Acad. Sci. U.S.A., 72, 2724-2728.

Awad, A.B., 1981.
Effect of dietary lipids on composition and glucose utilisation by rat adipose tissue.
J. Nutr., 111, 34-39.

Ayala, S., Gaspar, G., Brenner, R.R., Peluffo, R.O. and Kunau, W., 1973.
Fate of linoleic, arachidonic and docosa-7,10,13,16-tetraenoic acids in rat testicles.
J. Lipid Res., 14, 296-305.

Bailey, J.M., Howard, B.V., Dunbar, L.M. and Tillman, S.F., 1972.
Control of lipid metabolism in cultured cells.
Lipids, 7, 125-134.

Bailey, J.M. and Dunbar, L.M., 1973a.
Essential fatty acid requirements of cells in tissue culture: a review.
Exp. Mol. Path., 18, 142-161.

Bailey, J.M., Howard, B.V. and Tillman, S.F., 1973b
Lipid metabolism in cultured cells. XI. Utilisation of serum triglycerides.
J. Biol. Chem., 248, 1240-1247.

Bailey, J.M., 1977.
Lipid metabolism in cultured cells, in "Lipid Metabolism in Mammals" Vol. 2 (Snyder ed.), Plenum Press, New York, pg. 352.

Banerjee, N. and Rosenthal, M.D., 1985.
High-affinity incorporation of 20-carbon poly-unsaturated fatty acids by human skin fibroblasts.
Biochim. Biophys Acta, 835, 533-541.

Banerjee, N. and Rosenthal, M.D., 1986.
Elongation of C20 polyunsaturated fatty acids by human skin fibroblasts.
Biochim. Biophys. Acta, 878, 404-411.

Bankhurst, A.D., Hastain E., Goodwin J.S. and Peake G.T., 1981.
The nature of the prostaglandin producing mononuclear cell in human periferal blood.
J. Lab. Clin. Med., 97, 179-186.

Bar, R.S., Dolash, S., Spector, A.A., Kaduce, T.L. and Figard, P.H., 1984.
Effects of membrane lipid unsaturation on the interactions of insulin and multiplication stimulating activity with endothelial cells.
Biochim. Biophys. Acta, 804, 466-473.

Barker, M.O. and Brin, M., 1975.
Mechanisms of lipid peroxidation in erythrocytes of vitamin E-deficient rats and in phospholipid model systems.
Arch. Biochem. Biophys., 166, 32-40.

Barnard, G., Fosbrooke, A.S. and Lloyd, J.K., 1970.
Neutral lipids of plasma and adipose tissue in abetalipoproteinaemia.
Clin. Chim. Acta, 28, 417-422.

Barzanti, V., Biagi, P.L., Maranesi, M. and Turchetto, E., 1986.
Effect of lipids having different ω6 and ω3 fatty acid contents on some organs and subcellular structures of the rat.
Prog. Lipid Res., 25, 221-224.

Becker, W. and Bruce, A., 1986.
Retention of linoleic acid in carcass lipids of rats fed different levels of essential fatty acids.
Lipids, 21, 121-126.

Begin, M.E., Das, U.N., Ells, G. and Horrobin, D.F., 1985a.
Selective killing of human cancer cells by polyunsaturated fatty acids.
Prost. Leuk. Med., 19, 177-186.

Begin, M.E., Ells, G. and Das, U.N., 1985b.
Selected fatty acids as possible intermediates for selective cytotoxic activity of anticancer agents involving oxygen radicals.
Anticancer Res., 6, 291-296.

Begin, M.E. and Horrobin, D.F., 1986a.
Differential modulation of lipid peroxidation in normal and tumor cells by polyunsaturated fatty acids.
Personal communication.

Begin, M.E., Ells, G., Das, U.N. and Horrobin, D.F., 1986b.
Differential killing of human carcinoma cells supplemented with n-3 and n-6 polyunsaturated fatty acids.

Begin, M.E., Das, U.N. and Ells, G., 1986c.
Cytotoxic effects of essential fatty acids (EFA) in mixed cultures of normal and malignant human cells.
Prog. Lipid Res., 25, 573-576.

Begin, M.E. and Ells, G., 1987.
Effects of C18 fatty acids on breast carcinoma cells in culture.
Anticancer Res., 7, 215-218.

Begin, M.E., Ells, G. and Horrobin, D.F., 1988.
Polyunsaturated fatty acid-induced cytotoxicity against tumor cells and its relationship to lipid peroxidation.
J. Natl. Cancer Inst., 80, 188-194.

Begin, M.E., Sircar, S. and Weber, J.M., 1989.
Differential sensitivity of tumorigenic and genetically related non-tumorigenic cells to cytosol polyunsaturated fatty acids.
Anticancer Res., 9, 1049-1052.

Bem, J.L. and Greaves, M.W., 1974.
Prostaglandin E_1 effects on epidermal cell growth in vitro.
Arch. Dermatol. Forsch., 251, 34-41.

Bennett, A., 1984.
Prostanoids and cancer.
Ann. Clin. Res., 16, 314-317.

Bennett, A., 1986.
The production of prostanoids in human cancers, and their implications for tumor progression.
Prog. Lipid Res., 25, 539-542.

Bentley-Phillips, C.B., Paulli-Jorgensen, H. and Marks, R., 1977.
The effects of prostaglandins E_1 and $F2_\alpha$ on epidermal growth.
Arch. Dermatol. Res., 257, 233-237.

Beppu, M. and Kikugawa, K., 1987.
Periodate-induced lipid oxidation of erythrocyte membranes.
Lipids, 22, 312-316.

Bergstrom, S., Danielsson, H. and Samuelsson, B., 1964.
The enzymatic formation of prostaglandin E2 from arachidonic acid prostaglandins and related factors.

Bernert, J.T. and Sprecher, H., 1975.
Studies to determine the role rates of chain elongation and desaturation play in regulating the unsaturated fatty acid composition of rat liver lipids.
Biochim. Biophys. Acta, 398, 354-363.

Bernhard, K., von Bulow-Koster, J. and Wagner, H., 1959.
Die enzymatische dehydrierung der stearinsaure zu olsaure.
Helv. Chim. Acta, 42, 152-155.

Bettger, W.J. and Ham, R.G., 1981.
Effects of non-steroidal anti-inflammatory agents and anti-oxidants on the clonal growth of human diploid fibroblasts.
Prog. Lipid Res., 20, 265-268.

Bettger, W.J. and Ham, R.G., 1982.
The nutrient requirements of cultured mammalian cells.
Adv. Nutr. Res., 4, 249-286.

Bjerkvig, R., Steinsvag, S.K. and Laerum, O.D., 1986.
Re-aggregation of fetal rat brain cells in a stationary culture system. I: Methodology and cell identification.
In Vitro Cell. Dev. Biol., 22, 180-192

Blank, M.L., Lee, T.C., Piantadosi, C., Ishaq, K.S. and Snyder, F., 1976.
Membrane lipid modification and stearoyl-coenzyme A desaturase activity in L-M cells.
Arch. Biochem. Biophys., 177, 317-322.

Blond, J-P., Lemarchal, P. and Spielman, D., 1981.
Desaturation of linoleic and dihomogamma-linolenic acids by human liver in vitro.
C.R. Acad. Sci., 292, 911-914.

Bloomfield, D.K. and Bloch, K., 1960.
The formation of Δ^9-unsaturated fatty acids.
J. Biol. Chem., 235, 337-345.

Bonney R.J., Becker, J.E. and Walker, P.R., 1974.
Primary monolayer cultures of adult rat liver parenchymal cells suitable for study of the regulation of enzyme synthesis.
in Vitro, 9, 399-413.

Boone, C.W., Mantel, N., Caruso, T.D. and Stevinson, R.E., 1972.
Quality control studies of fetal bovine serum used in tissue cultures.

Booyens, J., Engelbrecht, P., le Roux, S., and Lowrens, C., 1984a.
Some effects of the essential fatty acids linoleic acid and α-linolenic acid and of their metabolites, γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and of prostaglandins A_1 and E_1 on the proliferation of human osteogenic sarcoma cells in culture.
Prost. Leuk. Med., 15, 15-33.

Booyens, J., Dippenaar, N., Fabbri, D., Engelbrecht, P. and Katzeff, I.E., 1984b.
The effect of gamma-linolenic acid on the growth of human osteogenic sarcoma and oesophageal carcinoma cells in culture.
S. Afr. Med. J., 65, 240-242.

Booyens, J., Dippenaar, N., Fabbri, D., Engelbrecht, P., Louwrens, C.C. and Katzeff, I.E., 1984c.
Some effects of linoleic acid and gamma-linolenic on the proliferation of human hepatoma cells in culture.
S. Afr. Med. J., 65, 607-612.

Bordoni, A., Biagi, O.L. and Torchetto, E., 1988.
Aging influence on delta-6-desaturase activity and fatty acid composition of rat liver microsomes.
Biochem. Int., 17, 1001-1009.

Borgeat, P., Hamberg, M. and Samuelsson, B., 1976.
Transformation of arachidonic acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes.
J. Biol. Chem., 251, 7816-7820.

Borgeat, P. and Samuelsson, B., 1979.
Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes.
J. Biol. Chem., 254, 2643-2646.

Borgeson, G.E., Pardini, L., Pardini, R.S. and Reitz, R.C., 1989.
Effects of dietary fish oil on human mammary carcinoma and on lipid metabo'ising enzymes.
Lipids, 24, 290-295.

Botha, J.H. and Robinson, K.M., 1985.
The response of human carcinoma cell lines to GLA with special reference to the effect of agents which influence prostaglandin and thromboxane synthesis.

Botha, J.H., Robinson, K.M., Ramchurren, N. and Norman, R.J., 1989.
The role of prostaglandins in the inhibition of cultured carcinoma cell growth produced by gamma-linolenic acid.
Prost. Leuk. EFA's., 35, 119-123.

Bourre, J.M., Faivre, A., Dumont, O., Nouvelot, A., Loudes, C., Puymirat, J., and Tixier-Vidal, A., 1983.
Effect of polyunsaturated fatty acids on fetal mouse brain cells in culture in a chemically defined medium.
J. Neurochem., 41, 1234-1242.

Boyum, A., 1968.
Isolation of mononuclear cells and granulocytes from human blood.
Scand. J. Clin. Lab. Invest., 21, 77-89.

Brandes, R. and Arad, R., 1983.
Liver cytosolic fatty acid binding proteins. Effect of diabetes and starvation.
Biochim. Biophys. Acta, 750, 334-339.

Brenneman, D.E. and Spector, A.A., 1974.
Utilisation of ascites plasma very low density lipoprotein triglycerides by Ehrlich cells.
J. Lipid Res., 15, 309-316.

Brenner, R.R. and Nervi, A.M., 1965a.
Kinetics of linoleic and arachadonic acid incorporation and eicosatrienoic depletion in the lipids of fat-deficient rats fed methyl linoleate and arachidonate.
J. Lipid Res., 6, 363-368.

Brenner, R.R. and Jose, P., 1965b.
Action of linoleic and docosahexaenoic acids upon eicosatrienoic acid level in rat lipids.
J. Nutr., 85, 196-204.

Brenner, R.R. and Peluffo, R.O., 1966.
Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic and linolenic acids.
J. Biol. Chem., 241, 5213-5219.

Brenner, R.R. and Peluffo, R.O., 1967.
Inhibitory effect of docosa-4,7,10,13,16,19-hexaenoic acid upon the oxidative desaturation of linoleic into γ-linolenic acid and of α-linolenic into octadeca-6,9,12,15-tetraenoic acid.
Biochim. Biophys. Acta, 137, 184-186.

Brenner, R.R., Peluffo, R.O., Mercuri, O. and Restelli, M.A., 1968a.
Effect of arachidonic acid in the alloxan-diabetic rat.
Am. J. Physiol., 215, 63-69.

Brenner, R.R., Peluffo, R.O., Nervi, A.M. and de Tomas, M.E., 1968b.
Competitive effect of alpha- and gamma-linolenyl-CoA in linoeyl-CoA desaturation to gamma-linolenyl-CoA.
Biochim. Biophys. Acta, 176, 420-422.

Brenner, R.R., and Peluffo, R.O., 1969.
Regulation of unsaturated fatty acid biosynthesis: effect of unsaturated fatty acids of 18 carbons on the microsomal desaturation of linoleic acid into γ-linolenic acid.
Biochim. Biophys Acta, 176, 471-479.

Brenner, R.R., 1971.
The desaturation step in the animal biosynthesis of polyunsaturated fatty acids.
Lipids, 6, 567-575.

Brenner, R.R., 1974.
The oxidative desaturation of unsaturated fatty acids in animals.
Mol. Cell Biochem., 3, 41-52.

Brenner, R.R., 1977.
Regulatory function of Δ6-desaturase enzyme of polyunsaturated fatty acid synthesis.
Adv. Exper. Med. Biol., 83, 85-97.

Brenner, R.R., 1982.
Nutritional and hormonal factors influencing desaturation of essential fatty acids.
Prog. Lipid Res., 20, 41-48.

Brett, D., Howling, D., Morris, L.J. and James, A.T., 1971.
Specificity of the fatty acid desaturases. The conversion of saturated to monoenoic acids.
Arch. Biochem. Biophys., 143, 535-547.

Bridges, R.B. and Coniglio, J.G., 1970.
The biosynthesis of delta-9,12,15,18-tetracosatetraenoic and of delta-6,9,12,15,18-tetracosapentaenoic acids by rat testes.
J. Biol. Chem., 245, 46-49.

Brush, M.G. 1982.
Efamol (Evening Primrose seed oil) in the treatment of premenstrual syndrome, in "Clinical Uses of Essential Fatty Acids" (Horrobin, D.F., ed.), Eden Press, Montreal, pg. 155-161.

Bryant, R.W., Feinmark, S.F., Makheja, A.N. and Bailey, M.J., 1978.
Lipid metabolism in cultured cells.
J. Biol. Chem., 253, 8134-8142.

Budowski, P., 1981.
Review: Nutritional effects of ω3-polyunsaturated fatty acids.
Isr. J. Med. Sci., 17, 223-231.

Bullaro, J.C. and Brookman, D.H., 1976.
Comparison of skeletal muscle monolayer cultures initiated with cells dissociated by the vortex and trypsin methods.
In Vitro, 12, 564-570.

Burns, C.P, Wei, S.P.L. and Spector, A.A., 1978.
Fatty acid metabolism in L1210 murine leukemia cells: differences in modification of fatty acids incorporated into various lipids.
Lipids, 13, 666-672.

Burns, C.P., Luttenegger, D.G., Dudley, D.T. Buettner, G.R. and Spector, A.A, 1979.
Effect of plasma membrane fatty acid composition on fluidity and methotrexate transport in L1210 murine leukemia cells.
Cancer Res., 39, 1726-1732.

Burr, G.O. and Burr, M.M., 1930.
On the nature and role of the fatty acids essential in nutrition.
J. Biol. Chem., 86, 587-621.

Buttke, T.M., 1984.
Inhibition of lymphocyte proliferation by free fatty acids. I. Differential effects on mouse B and T lymphocytes.
Immunology, 53, 235-242.

Campbell, W.B., Flack, J.R., Okita, J.R., Johnson, A.R. and Callahan, K.S., 1985.
Synthesis of dihomoprostaglandins from adrenic acid (7,10,13,16-docosatetraenoic acids) by human endothelial cells.
Biochim. Biophys. Acta, 837, 67-76.

Cantrill, R.C. and Davidson, B.C., 1984.
The role of essential fatty acids.
S. Afr. Med. J., 65:320.

Cantrill, R.C., Davidson, B.C., Katzeff, I. and Booyens, J., 1986
The effects of essential fatty acid supplementation on the fatty acid composition of cancer cells in culture.
Prog. Lipid Res., 25, 547-550.

Carpenter, M.P., 1981.
Antioxidants and prostaglandin synthesis: in vivo and in vitro effects.
Prog. Lipid Res., 20, 143-149.

Carreau, J.P., Counis, R. and Raulin, J., 1971.
Adenylcyclase foetale et neonatale du tissue adipeu. Relation avec la proportion d'acides gras essentiels de lipids exogenes.
J. Physiol., 63, 184A.

Carreau, J.P., Loriette, C., Counis, R. and Ketevi, P., 1972.
Demasquage des recepteurs de la noradrenaline par enrichissement en acide linoleique des phospholipides mambranaires de la cellule adipeuse.
Biochim. Biophys. Acta, 280, 440-443.

Carroll, K.K. and Hopkins, G.J., 1979.
Dietary polyunsaturated fat versus fat in relation to mammary carcinogenesis.
Lipids, 14, 155-158.

Carroll, K.K., 1984.
Role of lipids in tumorigenesis.
J. Am. Oil Chem. Soc., 61, 1888-1891.

Cartwright, I.J., Pockley, A.G., Galloway, J.H., Greaves, M. and Preston, F.E., 1985.
The effects of dietary ω3 polyunsaturated fatty acids on erythrocyte membrane phospholipids, erythrocyte deformability and blood viscosity in healthy volunteers.
Atherosclerosis, 55, 267-281.

Cash, R. and Berger, C.K. 1969.
Acerodermatitis enteropathica: defective metabolism of unsaturated fatty acids.
J. Pediatr., 74, 717-729.

Castuma, J.C., Catala, A. and Brenner, R.R., 1972.
Oxidative desaturation of eicosa-8,11-dienoic acid to eicosa-5,8,11-trienoic acid: comparison of different diets on oxidative desaturation at the 5,6 and 6,7 positions.
J. Lipid Res., 13, 783-789.

Castuma, J.C. and Brenner, R.R, 1983.
Effect of fatty acid deficiency on microsomal membrane fluidity and cooperativity of the UDP-glucuronyltransferase.
Biochim. Biophys. Acta., 729, 9-16.

Catala, A., Nervi, A.M. and Brenner, R.R., 1975.
Separation of a protein factor necessary for the oxidative desaturation of fatty acids in the rat.
J. Biol. Chem., 250, 7481-7484.

Catala, A., Leikin, A., Nervi, A.M. and Brenner, R.R., 1977.
Protein factor involved in fatty acid desaturation of linoleic acid.
Adv. Exp. Med. Biol., 83, 111-118.

Chagnon, M., Gentile, J., Gladu, M. and Sirois, P., 1985.
The mechanism of action of leukotrienes A_4, C_4 and D_4 on human lung parenchyma *in vitro*.
Lung, 163, 55-62.

Chang, H., Janke, J., Pusch, F. and Holman, R.T., 1973.
Effect of double and triple bonds in fatty acid inhibitors upon desaturation of stearic acid by rat liver microsomes.
Biochim. Biophys. Acta, 306, 21-25.

Chapkin, R.S. and Ziboh, V.A., 1984.
Inability of skin enzyme preparations to biosynthesise arachidonic acid from linoleic acid.
Biochem. Biophys. Res. Comm., 124, 784-792.

Chapkin, R.S., Ziboh, V.A., Marcelo, C.L. and Voorhees, J.J., 1986a.
Human epidermal preparations can elongate gamma-linolenic acid into gamma-homolinolenic acid.
J. Invest. Dermatol., 86, 468.

Chapkin, R.S., Ziboh, V.A., Marcelo, C.L. and Voorhees, J.J., 1986b.
Metabolism of essential fatty acids by human epidermal enzyme preparations: evidence of chain elongation.
J. Lipid Res., 27, 945-954.

Chapkin, R.S., Ziboh, V.A. and McCullough, J.L., 1987.
Dietary influences of evening primrose and fish oil on the skin of essential fatty acid-deficient guinea pigs.
J. Nutr., 117, 1360-1370.

Chase, H.P., Williams, R.L. and DuPont, J., 1979.
Increased prostaglandin synthesis in childhood diabetes melittis.
J. Pediatr., 94, 185-189.

Chase, H.P. and DuPont, J., 1980.
Abnormal levels of prostaglandins and fatty acids in blood of children with cystofibrosis.
Lancet, 687-709.

Cheeseman, K.H., Burton, G.W., Ingold, K.U. and Slater, T., 1984.
Lipid peroxidation and lipid anti-oxidants in normal and tumor cells.
Toxicol. Path., 12, 235-239.

Cheeseman, K.H., Collins, M., Maddix, S., Milia, A, Proudfoot, K., Slater, T., Burton, G.W., Webb, A. and Ingold, K.U., 1986.
Lipid peroxidation in regenerating liver.
Febs. Lett., 209, 191-196.

Cheng, S. and Levy, D., 1979.
The effects of cell proliferation on the lipid composition and fluidity of hepatocyte plasma membranes.
Arch. Biochem. Biophys., 196, 424-429.

Chiappe, L.E., de Tomas, M.E. and Mercuri, O., 1974.
In vitro activity of $\Delta 6$ and $\Delta 9$ desaturases in hepatomas of different growth rates.
Lipids, 9, 489-490.

Chio, K.S. and Tappel A.L., 1969.
Inactivation of ribonuclease and other enzymes by peroxidising lipids and by malondialdehyde.
Biochemistry, 8, 2827-2832.

Christ, E. J. and van Dorp, D.A., 1972.
Comparative aspects of prostaglandin biosynthesis in animal tissues.

Christ-Hazelhof, E., Nugteren, D.H. and van Dorp, D A., 1976.
Conversions of prostaglandin endoperoxides by glutathione-S-transferases and serum albumins.
Biochim. Biophys. Acta, 450, 450.

Christiansen, K., Marcel, Y. Gan, M.V., Mohrhauer, H. and Holman, R.T., 1968.
Chain elongation of α- and γ-linolenic acids and the effect of other fatty acids on their conversion in vitro.
J. Biol. Chem., 243, 2969-2974.

Christman, C.W., Wei, E.N., Kontos H.A., Povlishock, J.T. and Ellis, E.F., 1984.
Effects of 15-hydroperoxyeicosatetraenoic acid (15-HPETE) on cerebral arterioles of cats.
Am. J. Physiol., 247 (Heart Circ. Physiol.16); H631-H637.

Clejan, S., Castro-Magana, M., Collipp, P.J., Jonas, E. and Maddaiah, V.T., 1982.
Effects of zinc deficiency and castration on fatty acid composition and desaturation in rats.
Lipids, 17, 129-135.

Clement, J., 1980.
Intestinal absorbtion of triacylglycerols.
Reprod. Nutr. Dev., 20, 1285.

Codde, J.P., Beilin, L.J., Croft, K.D. and van Dongen, R., 1985.
Study of diet and drug interactions on prostanoid metabolism.
Prostaglandins, 29, 895-910.

Collins, F.D. and Connelly, J.F., 1965.
A fatty acid characteristic of a deficiency of linoleic acid in the case of hepatoma.
Lancet, 2, 883-885.

Collins, F.D., Sinclair, A.J., Royle, A.J., Coats, J.P., Maynard, D.A. and Leonard, R.F., 1971.
Plasma lipids in human linolenic acid deficiency.
Nutr. Metab., 13, 150-167.

Colquhoun, I. and Bunday, S., 1981.
A lack of essential fatty acids as a possible cause of hyperactivity in children.
Med. Hypotheses, 7, 681-686.

Conroy, D.M., Stul C.D., Belin, J., Pryor, C.L. and Smith, A.D., 1986.
The effects of dietary (n-3) fatty acid supplementation on lipid dynamics and composition in rat lymphocytes and liver microsomes.
Biochim. Biophys. Acta, 861, 457-462.

Cook, H.W. and Lands, W., 1976.
A mechanism for the suppression of cellular biosynthesis of prostaglandins.
Nature, 260, 630-632.

Cook, H.W., 1978a.
In vitro formation of polyunsaturated fatty acids by desaturation in rat brain. Some properties of the enzyme in developing brain and comparisons with liver.
J. Neurochem., 30, 1327-1334.

Cook, H.W., 1978b.
Incoporation, metabolism and positional distribution of trans-unsaturated fatty acids in developing and mature brain.
Biochim. Biophys. Acta, 531, 245-256.

Cook, H.W., 1979.
Differential alteration of Δ9 and Δ6 desaturation of fatty acids in rat brain preparations in vitro.
Lipids, 14, 763-767.

Cook, H.W., 1980.
Metabolism and positional distribution of isomers of 18-carbon dienoic fatty acids containing trans- and cis- double bonds by developing rat brain.
Can. J. Biochem., 58, 121-127.

Cook, H.W., 1981.
The influence of trans-acids on desaturation and elongation of fatty acids in developing brain.
Lipids, 16, 920-926.

Cook, H.W. and Spence, M.W., 1987.
Interaction of (n-3) and (n-6) fatty acids in desaturation and elongation of essential fatty acids in cultured glioma cells.
Lipids, 22,613-619.

Cooper, R.A., 1970.
Lipids of human red cell membrane: normal and comparative variability in disease.
in "Seminars in Hematology", Vol. 7 (no. 3),

Cooper, J.T. and Goldstein, S., 1977.
Comparative studies on human skin fibroblasts: life span and lipid metabolism in medium containing fetal bovine or human serum.
In Vitro, 13, 473-476.

Corey, E.J., Chuan, S. and Cashman, J.R., 1983.
Docosahexaenoic acid is a strong inhibitor of prostaglandin but not of leukotriene biosynthesis.
Proc. Natl. Acad. Sci. U.S.A., 80, 3581-3584.

Cornwell, D.G., Huttner, J.J., Milo, G.E., Sharma, H.M., Panganamala, R.V. and Geer, J.C., 1979.
Polyunsaturated fatty acids, vitamin E, and the proliferation of aortic smooth muscle cells.
Lipids, 14, 194-207.

Cornwell, D.G. and Morisaki, N., 1984.
Fatty acid paradoxes in the control of cell proliferation: prostaglandins, lipid peroxides and co-oxidation reactions.
in "Free Radicals in Biology" Vol. 6 (Pryor W.A. ed.), Academic Press Inc. New York, pg. 95-108.

Counis, R., 1973.
Demasquage presume des recepteurs de la nor-adrenaline par enrichissement en acide linoleique des phospholipides membranaires de la cellule adipeuse. II. Activites adenyl cyclase et phosphodiesterase.
Biochim. Biophys. Acta, 306, 391-395.

Crawford, M.A., Gale, M.M., Woodford, M.H. and Casperd, N.M., 1970.
Comparative studies on fatty acid composition of wild and domestic meats.
Int. J. Biochem., 1, 295-305.

Crawford, M.A. and Sinclair, A.J., 1971.
Nutritional influences in the evolution of mammalian brain.
in "Lipids, Malnutrition and the Developing Brain" (Elliot, K. and Knight, J. eds.), CIBA Foundation Symposium, Elsevier, Amsterdam, pg. 267-287.

Crawford, M.A. and Sinclair, A.J., 1972.
in "Lipids, Malnutrition and the Developing Brain" (Elliot, K. and Knight, J., eds.), Associated Scientific Publishers, London, pg.

Crawford, M.A., 1980.
The role of essential fatty acids and prostaglandins.
Post. Grad. Med. J., 56, 557-562.

Crawford, M.A., 1983.
Background to essential fatty acids and their prostanoid derivatives.
Br. Med. Bull., 39, 210-213.

Croft, K.D., Codde, J.P., Beilin L.J. and Vandongen, R., 1986.
Dietary modification of eicosanoid synthesis - a time course study.
Prog. Lipid Res., 25, 181-184.

Csordas, A. and Schauenstein, K., 1984.
Structure and configuration dependant effects of C18 unsaturated fatty acids on the chicken and sheep erethrocyte membrane.
Biochim. Biophys. Acta, 769:571-577.

Culp, B.R., Titus, B.G. and Lands, W.E.M., 1979.
Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid.
Prost. Med., 3, 269-278.

Cunnane, S.C. and Wahle, K.W.J., 1981.
Zinc deficiency increases the rate of delta-6-desaturation of linoleic acid in rat mammary tissue.
Lipids, 16, 771-774.

Cunnane, S.C., Keeling, P.W.N., Thompson, R.P.H. and Crawford, M.A., 1984.
Linoleic acid and arachdonic acid metabolism in human peripheral blood leucocytes: comparison with the rat.
Br. J. Nutr., 51, 209-217.

Cunningham, D.D., 1972.
Changes in phospholipid turnover following growth of 3T3 mouse cells to confluency.
J. Biol. Chem., 247, 2464-2470.

Cuthbert, J.A. and Lipsky, P.E., 1986.
Promotion of human T lymphocyte activation and proliferation by fatty acids in low density and high density lipoproteins.
J. Biochem., 261, 3620-3627.

Cyong, J. and Okada, H., 1976.
Histochemical studies on fatty acids in lymphocyte-mediated immune reaction.

Dalton, C. and Hope, W.C., 1974.
Cyclic AMP regulation of prostaglandin biosynthesis in fat cells.
Prostaglandins, 6, 227.

Dang, A.Q., Kemp, K., Faas, F.A. and Carter, W.J., 1989.
Effects of dietary fats on fatty acid composition and delta-5-desaturase in normal and diabetic rats.
Lipids, 24, 882-889.

Daniel, L.W., Kucera, L.S. and Whaite, M., 1980.
Metabolism of fatty acids by cultured tumor cells and their diploid precursor fibroblasts.
J. Biol. Chem., 255, 5697-5702.

Danon, A., Heimberg, M. and Oates, J.A., 1975.
Enrichment of rat tissue lipids with fatty acids that are prostaglandin precursors.
Biochim. Biophys. Acta, 388, 318-330.

Das, U.N., Huang, Y.S. and Begin, M.E., 1986.
Uptake and distribution of cis-unsaturated fatty acids and their effects on free radical generation in normal and tumor cells in vitro.
J. Free Radical Biol. Med., 2, 9-14.

Davidson, B.C. and Cantrill, R.C., 1985a.
Fatty acid nomenclature - a short review.
S. Afr. Med. J., 67, 633-634.

Davidson, B.C. and Cantrill, R.C., 1985b.
Erythrocyte membrane acyl:CoA synthetase activity.
Febs. Lett., 193, 69-74.

Davidson, B.C., 1986.
The activation of fatty acids in plasma membranes.
Ph.D. Thesis, University of the Witwatersrand.

Davidson, B.C. and Cantrill, R.C., 1986a.
Fatty acid activation by erythrocyte ghost plasma membranes.
Prog. Lipid Res., 25, 73-76.

Davidson, B.C. and Cantrill, R.C., 1986b.
Rat hepatocyte plasma membrane acyl:CoA synthetase activity.
Lipids, 21, 571-574.

Davidson, B.C., Cantrill, R.C. and Varaday, D., 1986c.
The reversal of essential fatty acid

Davidson, B.C., Morsbach, D. and Cantrill, R.C., 1986d.
The fatty acid composition of the liver and brain of Southern African cheetahs.
Prog. Lipid Res., 25, 97-99.

Davidson, B.C. and Traher, C., 1987a.
The importance of essential fatty acid evaluation and supplementation in feline diets.
J. S. Afr. Vet. Assoc., 58, 39-41.

Davidson, B.C. and Girao, L.A.F., 1987b.
A comparison of the effects of C18 fatty acids on the growth in culture of both malignant 3T6 and normal fibroblast cells.
J. Nutr. Growth Cancer, 4, 54-58.

Davidson, B.C. and Girao, L.A.F., 1987c.
The effects of C18 fatty acids on the growth in culture of 2 benign fibroblast derived cell lines - 3T3 and 3T6.
J. Nutr. Growth Cancer, 4, 59-64.

Davidson, B.C., Girao, L.A.F. and Giangregorio, A., 1988a.
Normal rat brain and neuroblastoma cells react differently to the presence of C18 fatty acids.
Anti Cancer Res., 8, 1057, 120A.

Davidson, B.C., Giangregorio, A. and Girao, L.A.F., 1988b.
C18 fatty acids influence the growth of normal and transformed fibroblasts in culture.
Anti Cancer Res., 8, 1085, 215A.

Davidson, B.C., Cantrill, R.C, Kursjens, N.P. and Patton, J., 1988c.
Polyenoic fatty acid deprevation of juvenile cats modulates [^{3}H]-dopamine release from presynaptic receptors in caudate slices.
In Vivo, 2, 295-298.

Davidson, B.C., Kursjens, N.P., Patton, J. and Cantrill, R.C., 1988d.
Essential fatty acid deprivation reduced the activity of apomorphine at presynaptic dopamine receptors modulating [^{3}H] dopamine release from cat caudate slices.
Europ. J. Pharmacol., 317.

Davidson, B.C., Giangregorio, A. and Girao, L.A.F., 1989.
The different polyenoic fatty acids provided by different purified diets affect domestic cat growth.
S. Afr. J. Food. Sci. Nutr., 1, 3-6.

Davidson, B.C., Giangregorio, A. and Girao, L.A.F., 1990a.
Essential fatty acids in cheetah and in domestic cats.
in "Omega-6-essential Fatty Acids. Pathophysiology and Roles in Clinical Medicine" (Horrobin, D.F., ed.), Alan R. Liss Inc., New York.

Davidson, B.C., Giangregorio, A., Girao, L.A.F., Mendelsohn, D. and Murphy, J., 1990b.
The effects of fatty acids, and fatty acid mixtures, on human skin fibroblasts.
Anti Cancer Res., 10, 1456, 333A.

Delplanque, B. and Jacotot, B., 1987.
Influence of enviromental medium on fatty acid composition of human cells: leukocytes and fibroblasts.
Lipids, 22, 241-249.

Denning, G.M., Figard, P.H., Kaduce, T.L., and Spector, A.A., 1983.
Role of triglycerides in endothelial cell arachidonic acid metabolism.
J. Lipid Res., 24, 993-1001.

Desai, I.D. and Tappel, A.L., 1963.
Damage to proteins by peroxidised lipids.
J. Lipid Res., 4, 204-207.

Desnoyers, F., Vodovar, N., Lapous, D. and Raulin, J., 1971.
Influence du degre d'insaturation des acides fras duregime sur la morphologie du depot adipeux mesenterigue et epididymarie.
C.R. Acad. Sci., 272, 2836-2840.

Dhopeshwarkar, G.A., Blomstrand, R. and Gustufsson, B.E., 1963.
Biohydrogenation in germ-free rats.
J. Atheroscler. Res., 3, 274-279.

Dhopeshwarkar, G.A., Subramanian, C. and Mead, J.F., 1971a.
Rapid uptake of (I-14C)acetate by the adult rat brain 15 seconds after carotid injection.
Biochim. Biophys. Acta, 239, 162-167.

Dhopeshwarkar, G.A., Subramanian, C. and Mead, J.F., 1971b.
Fatty acid uptake by the brain. IV. Incorporation of (I-14C)linoleic acid into the adult rat brain.
Biochim. Biophys. Acta, 231, 8-14.

Dhopeshwarkar, G.A. and Subramanian, C, 1976.
Intracranial conversion of linoleic to arachidonic acid: evidence for lack of delta-8-desaturase in the brain.
J. Neurochem., 26, 1175-1179.

Dippenaar, N., Booyens, J., Fabbri, D. and Katzeff, I., 1982a.
The reversability of cancer; evidence that malignancy in BL6 mouse melanoma cells is gamma-linolenic acid deficiency-dependant.
S. Afr. Med. J., 62, 505-509.

Dippenaar, N., Booyens, J., Fabbri, D. and Katzeff, I., 1982b.
The reversability of malignancy: evidence that malignancy in human hepatoma cells is gamma-linolenic acid deficiency-dependant.
S. Afr.Med. J., 62, 683-685.

Dise, CA., Goodman, D.B.P. and Rasmussen, H., 1980.
Definition of the pathway for membrane phospholipid turnover in human erythrocytes.
J. Lipid Res., 21, 292-300.

Do, O.H. and Sprecher, H., 1975.
Studies on the substrate specificity of the fatty acid desaturase using methyl branched isomers of eicosa-8,11,14-trienoic acid and the metabolism of these acids in rat liver.
Arch. Biochem. Biophys., 171, 597-603.

Documenta Geigy, 1962.
in "Documenta Geigy Scientific Tables" 6th Edition, (Diem, K., ed.), Ciba Geigy Publishers, Basle, Switzerland.

Doi, O., Doi, F., Schroeder, F., Alberts, A.W., and Vagelos, P.R., 1978.
Manipulation of fatty acid composition of membrane phospholipid and its effects on cell growth in mouse LM cells.
Biochim. Biophys. Acta, 509, 239-250.

Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H., 1964.
The biosynthesis of prostaglandins.
Biochim. Biophys. Acta, 90, 204-207.

Doyle, W., Hare, W.R. and Crawford, M.A., 1982.
Fatty acid metabolism.
Proc. Nutr. Soc., 42, 69A.

Draper, H.H., McGirr, L.G. and Hadley, M., 1986.
The metabolism of malondialdehyde.
Lipids, 21, 305-307.

Dubin, I.N., Bernard, B. and Herbst, [illegible], 1965.
Effects of albumin fraction and linoleic acid on growth of macrophages in tissue culture.
J. Natl. Cancer Inst., [illegible] 43-51.

Dugail, I., Quingnard-Boulange, A., Ardouin, B. and Brigant, L., 1986.
A method for separating cultured preadipocytes according to their density: application to stromal cells from overfed suckling rats.
In Vitro Cell. Develop. Biol., 22, 375-380.

Dumelin, E.E. and Tappel, A.L., 19[illegible]
Hydrocarbon gasses pr[illegible]d during in vitro peroxidation of polyun[illegible]ated fatty acids and decomposition of preformed hydroperoxides.
Lipids, 12, 894-900.

Dunbar, L.M. and Bailey, J.M., 1975.
Enzyme deletions and essential fatty acid metabolism in cultured cells.
J. Biol. Chem., 250, 1152-1153.

Dunham, E.W., Balasingam, M., Privett, O.S. and Nickell, E.C., 1978.
Effects of essential fatty acid deficiency on prostaglandin synthesis and fatty acid composition in rat renal medulla.
Lipids, 13, 892-897.

Dunn, M.J., Staley, R.S. and Harrison, M., 1976.
Characterisation of prostaglandin production in tissue culture of rat renal medullary cells.
Prostaglandins, 12, 37-49.

Durant, S., Duval, D. and Homo-Delarche, F., 1988.
Mouse embryo fibroblasts in c[illegible]ure: characteristics of arachidonic acid metabolism during early passages.
Prost. Leuk. EFA's., 32, 129-137.

Durant, S., Duval, D. and Homo-Delarche, F., 1989.
Effect of exogenous prostaglandins and non-steroidal antiinflammatory agents on prostaglandin secretion and proliferation of mouse embryo fibroblasts in culture.
Prost. Leuk. EFA's., 38, 1-8.

Dy, M., Astoin, M., Rigaud, M. and Hamburger, J., 1980.
Prostaglandin relese in the mixed lymphocyte culture: effect of presensitisation by a skin allograft; nature of the prostaglandin producing cell.
Eur. J. Immunol., 10, 121-126.

Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S. and Vane, J.R., 1978.
Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis.
Lancet, 2, 117-119.

Dyerberg, J., Bang, H.O. and Aagaard, O. 1980.
α-linolenic and eicosapentaenoic acid.
Lancet, 1, 199.

Dyerberg, J. and Jorgensen, K.A., 1982.
Marine oils and thrombogenesis.
Prog. Lipid Res., 21, 255-269.

Dyerberg, J., 1986.
Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis.
Nutr. Rev., 44, 125-134.

Eaglestein, W.H. and Weinstein, G.D., 1975.
Prostaglandins and DNA synthesis in human skin. F sible relationship to ultraviolet light effects.
J. Invest. Dermatol., 64, 386-389.

East, J.M., Jones, O.T., Simmonds, A.C. and Lee, A.G., 1984.
Membrane fluidity is not an important physiological regulator of the calcium-magnesium-dependent ATPase of sarcoplasmic reticulum.
J. Biol. Chem., 259, 8070-8071.

Ehrstrom, M., Harms-Rindahl, M. and Alling, C., 1981.
Osmotic fragility and fluidity of erythrocyte membranes from rats raised on an essential fatty acid deficient diet.
Biochim. Biophys. Acta, 644, 175-182.

El-Attar, T.M.A., Lin, H.S. and Platt, R.D., 1990.
Comparison of the inhibitory effect of polyunsaturated fatty acids on prostaglandin synthesis. II. Fibroblasts.
Prost. Leuk. EFA's., 39, 135-139.

El-Boustani, S.E., Descomps, B., Monnier, L. and Warnant, J., 1986.
In vivo conversion of dihomogamma-linoleic acid into arachidonic acid in Man.
Prog. Lipid Res., 25, 67-71.

Engelhard, V.H., Sko, J., Sturm, D. and Glaser, M., 1976.
Modification of adenylate cyclase activity in LM cells by manipulation of the membrane phospholipid composition in vivo.
Proc. Natl. Acad. Sci. U.S.A., 73, 4482-4486.

Erickson, B.N., Williams, H.H., Bernstein, I.A., Jones, R.L. and Macy, I.C., 1958.
The lipid distribution of posthemolytic residue on stroma of erythrocytes.
J. Biol. Chem., 122, 515.

Esterbauer, H., Cheeseman, K.H., Dranzani, M.U., Poli, G. and Slater, T.F., 1982.
Separation and characterisation of the aldehydic products of lipid peroxidation stimulated by ADP-Fe2+ in rat liver microsomes.
Biochem. J., 208, 129-140.

Etherton, T.D. and Allen, C.F., 1980.
Effects of age and adipocyte size on glucose and palmitate metabolism and oxidation in pigs.
J. Animal Sci., 50, 1073-1084.

Evans, V.J., Bryant, J.C., Kerr, H.S. and Schilling, E.L., 1965.
Chemically defined media for cultivation of long-term cell strains from four mammalian species.
Exp. Cell Res., 36, 439-474.

Faas, F.H. and Carter, W.J., 1980.
Altered fatty acid desaturation and microsomal facty acid composition in the streptozotocin diabetic rat.
Lipids, 15, 953-961.

Fain, J.N. and Scow, R.O., 1966.
Fatty acid synthesis *in vivo* in maternal and fetal tissues in the rat.
Am. J. Physiol., 210, 19-25.

Fain, J.N., 1975.
Isolation of free brown and white fat cells.
Methods Enzymol., 35, 555-561.

FAO/WHO, 1977.
Food and Nutrition Paper III.

Feinmark, S.J. and Bailey, J.M., 1982.
Lipid metabolism in cultured cells.
J. Biol. Chem., 257, 2816-2821.

Feinstein, M.B., Becker, E.L., Fraser, C. and Thromb, N., 1977.
Collagen and A23187 stimulated platelet arachidonate metabolism: differential inhibition by PGE_1, local anesthetics and a serine-protease inhibitor.
Prostaglandins, 14, 1075-1094.

Ferber, E., de Pasquale, G.G, and Resch, K., 1975.
Phospholipid metabolism of stimulated lymphocytes. Composition of phospholipid fatty acids.
Biochim. Biophys. Acta, 398, 364-375.

Ferguson, K.A., Glaser, M., Bayer, W.H., and Vagelos, P.R., 1975.
Alteration of fatty acid composition of LM cells by lipid supplementation and temperature.
Biochemistry, 14, 146-151.

Ferreira, S.H. and Vane, J.R., 1967.
Prostaglandins: Their dissapearance from and release into the circulation.
Nature, 216, 868-873.

Ferreri, N.R., Howland, W.C. and Spielberg, H.A.S., 1986.
Release of leukotrienes C_4 and B4 and prostaglandin E_2 from human monocytes stimulated with aggregated IgG, IgA and IgE.
J. Immunol., 136, 4188-4192.

Field, E.J. and Joyce, G., 1978.
Effect of prolonged ingestion of gamma-linolenate by multiple sclerosis patients.
Eur. J. Neurol., 17, 67-76.

Figard, P.H., Hejlik, D.P., Kaduce, T.L., Stoll, L.L., and Spector, A.A., 1986.
Free fatty acid release from endothelial cells.
J. Lipid Res., 27, 771-780.

Fischer, S. and Weber, P.C., 1983.
Thromboxane A_3 is formed in human platelets after dietary eicosapentaenoic acid.
Biochem. Biophys. Res. Comm., 116, 1091-1099.

Fischer, S. and Weber, P.C., 1984.
Prostaglandin I_3 is formed *in vivo* in Man after dietary eicosapentaenoic acid.
Nature, 307, 165-168.

Flow Laboratories, 1983.
in "Flow Laboratories Product Catalogue", Flow Laboratories, Scotland.

Flower, R.J., Cheung, H.S. and Cushman, D.W., 1973.
Quantitative determination of prostaglandins and malidonaldehyde formed by the arachidonate oxygenase system of bovine seminal vesicle.
Prostaglandins, 4, 325-341.

Folch, J., Lees, M. and Sloane-Stanley, G.H., 1957.
A simple method for the isolation and purification of total lipids from animal tissues.
J. Biol. Chem., 226, 497-509.

Forstermann, U. and Neutang, B., 1983.
Elimination from the circulation of cats of 6-keto-prostaglandin E_2 compared with prostaglandins E_2 and I_2.
J. Pharm. Pharmacol., 35, 724-728.

Fraga, C.G., Tappel, A.L., Leibovitz, B.E., Kuypers, F., Chiu, D., Iacono, J.M. and Kelley, D.S., 1990.
Lability of red blood cell membranes to lipid peroxidation: application to humans fed polyunsaturated lipids.
Lipids, 25, 111-114.

Frankel, T.L. and Rivers, J.P.W., 1978.
The nutritional and metabolic impact of γ-linolenic acid (18:3ω6) on cats deprived of animal lipid.
Br. J. Nutr., 39, 227-231.

Frankel, E.N., 1984.
Lipid oxidation: mechanisms, products and biological significance.
J. Am. Oil Chem. Soc., 61, 1908-1917.

Fredholm, B.B. and Hamberg, M., 1976.
Metabolism and effect of prostaglandin H_2 in adipose tissue.
Prostaglandins, 11, 507-518.

Fredrickson, D.S. and Gordon, R.S. Jr., 1958.
The metabolism of albumin-bound, ^{14}C-labelled unesterified fatty acids in normal human subjects.
J. Clin. Invest., 37, 1504-1515.

Freshney, I.R., 1983.
in 'Culture of Animal Cells, a Manual of Basic Technique', Alan R. Liss Inc., New York.

Freshney, I.R., 1986.
in 'Animal Cell Culture, a Practical Approach', IRL Press Limited, Washington DC.

Fujimori, A., Tsutsumi, M., Yamada, H., Fukase, M. and Fujita, T., 1989.
Arachidonic acid stimulates cell growth in an osteoblastic cell line, ML3T3-E_1, by non-eicosanoid mechanism.
Calcif. Tissue Int., 44, 186-191.

Fujimoto, W.Y., Teague, J. and Williams, R.H., 1977.
Fibroblast monolayer cultures in scintillation counting vials: metabolic and growth experiments using radioisotopes and a microfluorometric DNA assay.
In Vitro, 13, 237-244.

Fujiwara, F., Todo, S., and Imashuku, S., 1986.
Antitumor effect of gamma-linolenic acid on cultured human neuroblastoma cells.
Prost. Leuk. Med., 23, 311-320.

Fulco, A.J., 1974.
Metabolic alterations of fatty acids.
Annu. Rev. Biochem., 43, 215-241.

Galli, C., 1980.
Dietary influences on prostaglandin synthesis.
Adv. Nutr. Res., 3, 95-126.

Gallo-Torres, H.E. and Miller, O.N., 1971.
Tissue uptake and metabolism of d,1-3, 4-3 H2-a-tocopheryl nicotinate and d,1-a-tocopheryl-1',2'-3H2-acetate following intravenous administration.
Int. J. Vit. Nutr. Res., 41, 339-354.

Gandemer, G., Pascal, G. and Durand, G., 1985.
Comparative changes in the lipogenic enzyme activities and in the *in vivo* fatty acid synthesis in liver and adipose tissues during post-weaning growth of male rats.
Comp. Biochem. Physiol., 82B, 581-586.

Garcia, P.T. and Holman, R.T., 1965.
Competitive inhibitions in the metabolism of polyunsaturated fatty acids studied via the composition of the phospholipids, triglycerides and cholesterol esters of rat tissues.
J. Am. Oil Chem. Soc., 42, 1137-1141.

Garda, H.A. and Brenner, R.R., 1984.
Short-chain aliphatic alcohols increase rat liver microsomal membrane fluidity and affect the activities of some microsomal membrane bound enzymes.
Biochim. Biophys. Acta, 769, 160-170.

Garda, H.A. and Brenner, R.R., 1985.
In vitro modification of cholesterol content of rat liver microsomes. Effects upon membrane "fluidity" and activities of glucose-6-phosphate and fatty acid desaturation systems.
Biochim. Biophys. Acta, 819, 45-54.

Garg, M.L., Snoswell, A.M. and Sabine, J.R., 1986.
Influence of dietary cholesterol on desaturase enzymes of rat liver microsomes.
Prog. Lipid Res., 25, 639-644.

Garg, M.L., Sebokova, E., Thompson, A.B. and Clandinin, M.T., 1988a.
Δ^6 desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω3 fatty acids.
Biochem. J., 249, 351-356.

Garg, M.L., Wierzbicki, A.A., Thompson, A.B.R. and Clandinin, M.T., 1988b.
Dietary cholesterol and/or omega 3 fatty acids modulate delta-9-desaturase activity in rat liver microsomes.
Biochim. Biophys. Acta, 962, 330-336.

Garg, M.L., Thompson, A.B.R. and Clandinin, M.T., 1988c.
Effect of dietary cholesterol and/or omega 3 fatty acids on lipid composition and delta-5-desaturase activity of rat liver microsomes.
J. Nutr., 118, 661-668.

Garg, M.L., Wierzbicki, A.A., Thompson, A.B.R. and Clandinin, M.T., 1989.
Dietary saturated fat level alters the competition between α-linolenic and linoleic acid.
Lipids, 24, 334-339.

Garner, C.W., 1984.
Peroxidation of free and esterified fatty acids by horseradish peroxidase.
Lipids, 19, 863-868.

Gaspar, G., de Alaniz, M.J.T. and Brenner, R.R., 1975.
Uptake and metabolism of exogenous eicosa-8,11,14-trienoic acid in minimal deviation hepatoma 7788C cells.
Lipids, 10, 726-731.

Gavino, V.C., Miller, J.S., Dillman, J.M., Milo, G.E. and Cornwell, D.G., 1981a.
Effect of exogenous adrenic acid on the proliferation and lipid metabolism of cells in tissue culture.
Prog. Lipid Res., 20, 323-325.

Gavino, V.C., Miller, J.S., Dillman, J.M., Milo, G.E. and Cornwell, D.G., 1981b.
Polyunsaturated fatty acid accumulation in the lipids of cultured fibroblasts and smooth muscle cells.
J. Lipid Res., 22, 57-62.

Gavino, V.C., Miller, J.S., Ikharebha, S.O., Milo, G.E. and Cornwell, D.G., 1981c.
Effect of polyunsaturated fatty acids and antioxidants on lipid peroxidation in tissue cultures.
J. Lipid Res., 22, 763-769.

Geigy, 1984.
in "Geigy Scientific Tables", Vol. 3 (Lentner, C., ed.), 8th edition, Ciba-Geigy Publishers, Switzerland.

Gellhorn, A. and Benjamin, W., 1964.
The intracellular localisation of an enzymatic defect of lipid metabolism in diabetic rats.
Biochim. Biophys. Acta, 84,167-175.

German, J.B., Lokesh, B., Bruckner, G.G. and Kinsella, J.E., 1985.
The lipids of the rat erythrocyte membrane.
Nutr. Res., 5, 1393-1407.

Gerschenson, L.E., Mead, J.F., Harary, I. and Haggerty, D.F. Jr., 1967.
Studies on the effects of essential fatty acids on growth rate, fatty acid composition, oxidative phosphorylation and respiratory control of HeLa cells in culture.
Biochim. Biophys. Acta, 131, 42-49.

Geyer, R.P., Benett, A. and Rohr, A., 1962.
Fatty acids of the triglycerides and phospholipids of HeLa cells and strain L fibroblasts.
J. Lipid Res., 3, 80-83.

Giangregorio, A., Davidson, B.C. and Mendelsohn, D., 1988a.
in "Fats in Foods" (Giangregorio, A. and Davidson, B.C. eds.), 1st Edition, University of the Witwatersrand Press, South Africa.

Giangregorio, A., Murphy, J., Girao, L.A.F. and Davidson, B.C., 1988b.
C20 fatty acids influence the grown of normal and transformed fibroblasts in culture.
Anticancer Res., 8, 1085-1086, 217A.

Gibco Life Technologies Inc., 1984.
in "Gibco Product Catalogue", Gibco Limited, Scotland.

Gibson, R.A and Kneebone, G.M., 1981a.
Fatty acid composition of human colostrum and mature breast milk.
Am. J. Clin. Nutr., 34, 252-257.

Gibson, R.A. and Sinclair, A.J., 1981b.
Are Eskimo's obligate carnivores?
Lancet, 1, 1100.

de Gier, J. and van Deenen, L.L.M., 1961.
Some lipid characteristics of red cell membranes of various animal species.
Biochim. Biophys. Acta, 49, 286.

Gill, R. and Clark, W.R., 1980.
Membrane structure-function relationships in cell-mediated cytolysis.
J. Immunol., 125, 689-695.

Ginsburg, E., Salomon, D., Sreevalsan, T. and Freese, E., 1973.
Growth inhibition and morphological changes caused by lipophilic acids in mammalian cells.
Proc. Natl. Acad. Sci. U.S.A., 70, 2457-2461.

Ginsberg, B.H., Brovon, T.J., Simon, I. and Spector, A.A., 1981.
Effect of the membrane lipid environment on the properties of insulin receptors.
Diabetes, 30, 773-780.

Girao, L.A., Ruck, A.C., Cantrill, R.C. and Davidson, B.C., 1986.
The effect of C18 fatty acids on cancer cells in culture.
Anticancer Res., 6, 241-244.

Girao, L.A., Cantrill, R.C., Ruck, A.C. and Davidson, B.C., 1987.
The influence of C18 fatty acids on the growth of a 3T6 derived cell line.
Anticancer Res., 7, 133-138.

Girao, L.A.F., Davidson, B.C. and Giangregorio, A., 1988.
Incorporatior of exogenous fatty acids into, and lipoperoxide formation by, normal and transformed fibroblasts.
Anticancer Res., 8, 1054, 111A.

Girao, L.A.F., 1988.
To determine the in vitro effects of C18 fatty acids on normal, malignant and benign cell lines.
Ph.D Thesis, University of the Witwatersrand.

Girao, L.A.F., Davidson, B.C. and Giangregorio, A., 1989.
Incorporation and peroxidation of polyenoic fatty acids is increased in transformed compared to normal skin fibroblasts.
1st. International Conference on n-6 EFA's in Clinical Medicine, England, 1A.

Girotti, A.W., Bachowski, G.J. and Jordan, J.E., 1987.
Lipid peroxidation in erythrocyte membranes: cholesterol product analysis in photo-sensitised and xanthine oxidase-catalysed reactions.
Lipids, 22, 401-408.

Glaser, M., Ferguson, K.A. and Vagelos, P.R., 1974.
Manipulation of the phospholipid composition of tissue culture cells.
Proc. Natl. Acad. Sci. U.S.A., 71, 4072-4076.

Goetzl, E.J., 1980.
Mediators of immediate hypersensitivity derived from arachidonic acid.
New Engl. J. Med., 303, 822-825.

Goldyne, M.E. and Stobo, J.D., 1982.
Human monocytes synthesise eicosanoids from T-lymphocyte-derived arachidonate.
Prostaglandins, 24, 623-630.

Goldyne, M.E., Burrish, G.F., Poubelle, P. and Borgeat, P., 1984.
Arachidonic acid metabolism amoung human mononuclear leukocytes: lipoxygenase-related pathways.
J. Biol. Chem., 259, 8815-8819.

Goldyne, M.E., 1989.
Eicosanoid metabolism by lymphocytes: do all human nucleated cells generate eicosanoids?
Phar col. Res., 21, 241-245.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1970.
Effects of diet on linoleic acid desaturation and on some enzymes of carbohydrate metabolism.
J. Lipid Res., 11, 96-101.

de Gomez Dumm, I.N.T., Peluffo, R.O. and Brenner, R.R., 1972.
Comparative effect of a protein diet on the desaturation, elongation and simultaneous desaturation and elongation of linoleic acid.
Lipids, 7, 590-592.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1975a.
Effects of glucagon and dibutyryl adenosine 3′,5′-cyclic monophosphate on oxidative desaturation of fatty acids in the rat.
J. Lipid Res. 16, 264-267.

de Gomez Dumm, I.N.T. and Brenner, R.R., 1975b.
Oxidative desaturation of alpha-linolenic, linoleic and stearic acids by human liver microsomes.
Lipids, 10, 315-317.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1976.
Comparative effect of glucagon, dibutyryl cyclic AMP and epinephrine on the desaturation and elongation of linoleic acid by rat liver microsomes.
Lipids, 11, 833-836.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1979.
Effect of glucocorticoids on the oxidative desaturation of fatty acids by rat liver microsomes.
J. Lipid Res., 20, 834-839.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1980.
Effect of epinephrine and dibutyryl cyclic AMP on delta-5 desaturation activity of rat liver microsomes.
Lipids, 15, 1064-1066.

de Gomez Dumm, I.N.T., Peluffo, R.O., de Alaniz, M.J.T. and Brenner, R.R., 1982.
Effect of different carbon sources on desaturation activity of HTC cells.
Mol. Cell. Biochem., 48, 15-18.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1983.
Effect of dietary fatty acids on delta-5-desaturase activity and biosynthesis of arachidonic acid in rat liver microsomes.
Lipids, 18, 781-788.

de Gomez Dumm, I.N.T., de Alaniz, M.J.T. and Brenner, R.R., 1986.
Effect of malonyl-CoA on delta-6 desaturation activity of rat liver microsomes.
Lipids, 21, 721-723.

Goodman, D.S., 1958.
The interaction of human erythrocytes with sodium palmitate.
J. Clin. Invest., 37, 1729-1735.

Goodnight, S.H., Harris, W.S., Connor, W.E. and Illingworth, D.R., 1982.
Polyunsaturated fatty acids, hyperlipidemia and thrombosis.
Arteriosclerosis, 2, 87-113.

Goureau-Counis, M-F., Fichot, O., Raulin, J. and de Recondo, A-M., 1974.
Phospholipid configuration and template capacity of liver nuclei and hepatic DNA polymerase.
Physiol. Chem. Phys., 6, 379-392.

Gray, J.I., 1978.
Lipid and FA metabolism.
J. Am. Oil Chem. Soc., 55, 539A.

de Groot, H., Noll, T. and Tolle, T., 1985.
Loss of latent activity of liver microsomal membrane enzymes evoked by lipid peroxidation. Studies of nucleoside diphosphatase, glucose-6-phosphatase, and UDP-glucuronyltransferase.
Biochim. Biophys. Acta, 815, 91-96.

Gurr, I.M. and James, A.T., 1971.
in 'Lipid Biochemistry: An Introduction', Cornwell University Press, Ithaca, New York, pg. 52.

Gutteridge, J.M. and Halliwell, B., 1990.
The measurement and mechanism of lipid peroxidation in biological systems.
T.I.B.S., 15, 129-135.

Hagve, T. and Christophersen, B.O., 1984.
Effect of dietary fats on arachidonic acid and eicosapentaenoic acid biosynthesis and conversion to C22 fatty acids in isolated rat liver cells.
Biochim. Biophys. Acta, 796, 205-217.

Hagve, T. and Christophersen, B.O., 1986.
Evidence for peroxisomal retroconversion of adrenic acid and docosahexaenoic acid in isolated liver cells.
Biochim. Biophys. Acta, 875, 165-173.

Halliwell, B. and Gutteridge, J.M.C., 1985.
in 'Free Radicals in Biology and Medicine', Oxford University Press, New York.

Ham, R.G., 1963.
Albumin replacement by fatty acids in clonal growth of mammalian cells.
Science, 140, 802-803.

Hamazaki, T., Hirai, A. and Terano, T., 1982.
Effects of orally administered ethyl ester of eicosapentaenoic acid on PGI_2-like substance production by rat aorta.
Prostaglandins, 23, 557-567.

Hamberg, M., Svensson, J. and Samuelsson, B., 1975.
Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides.
Proc. Natl. Acad. Sci. U.S.A., 72, 2994-2998.

Hamberg, M., 1976.
On the formation of thromboxane B2 and 12l-hydroxy-5,8,10,14-eicosatetraenoic acid in tissues from the guinea pig.
Biochim. Biophys. Acta, 431, 651-654.

Hammerstrom, S., 1977.
Prostaglandin production by normal and transformed 3T3 fibroblasts in cell culture.
Eur. J. Biochem., 74, 7-12.

Hammerstrom, S., 1981.
Conversion of 5,8,11-eicosatrienoic acid to leukotrienes C3 and D3.
J. Biol. Chem., 256, 2275-2279.

Hanaoka, F. and Yamada,M., 1971
Localisation of the replication point of mammalian cell DNA at the membrane.
Biochem. Biophys. Res. Commun., 42, 647-653.

Hansen, A.E., 1933.
Serum lipid changes and therapeutic effects of various oils in infantile eczema.
Proc. Soc. Exp. Biol. Med., 31, 160-161.

Hansen, H.S., 1981.
Essential fatty acid supplemented diet increases renal excretion of PGE_2 and water in essential fatty acid deficient rats.
Lipids, 16, 849-854.

Harper, R.A., 1976.
Effect of prostaglandins on [^{3}H]-thymidine uptake into human epidermal cells *in vitro*.
Prostaglandins, 12, 1019-1025.

Harper, T.W., Nestott, T.Y., Voelkel, N. and Murphy, R.C., 1984.
Metabolism of leukotrienes B4 and C4 in the isolated perfused rat lung.
Biol. Chem., 259, 14437-14440.

Hassam, A.G., Sinclair, A.J. and Crawford, M.A., 1975.
The incorporation of orally fed linoleic acid and linolenic acid into the liver and brain lipids of suckling rats.
Lipids, 10, 417-420.

Hemler, M., Graff, G. and Lands, W., 1978.
Accelerative autoactivation of prostaglandin biosynthesis by PGG_2.
Biochem. Biophys. Res. Commun., 85, 1325-1331.

Hemler, M., Cook, H. and Lands. W., 1979.
Prostaglandin biosynthesis can be triggered by lipid peroxides.
Arch. Biochem. Biophys., 193, 340-345.

Henderson, R.J., Christie, W.W. and Moore, J.H., 1979.
Esterification of exogenous and endogenous fatty acids by rat adipocytes in vitro.
Biochim. Biophys. Acta, 573, 12-22.

Herold, P.M. and Kinsella, M.S. and J.E., 1986.
Fish oil consumption and decreased risk of cardiovascular disease: a comparison of findings from animal and human feeding trials.
Am. J. Clin. Nutr., 43, 566-599.

Herring, A.S., Raychaudhuri, R., Kelley, S.P. and Iype, P.T., 1983.
Repeated establishment of diploid epithelial cell cultures from normal and partially hepatectomised rats.
In Vitro, 19, 576-588.

Hidalgo, C., Petrucci, D.A. and Vergara, C., 1982.
Uncoupling of calcium transport in sarcoplasmic reticulum as a result of labeling lipid amino groups and inhibition of calcium-ATPase activity by modification of lysine residues of the calcium-ATPase polypeptide.
J. Biol. Chem., 257, 208-216.

Hidalgo, C., 1987.
Lipid-protein interactions and the function of the calcium-ATPase of sarcoplasmic reticulum.
C.R.C. Critical Reviews in Biochem., 21, 319-347.

Higgs, E.A., Moncada, S. and Vane, J.R., 1986.
Prostaglandins and thromboxanes from fatty acids.
Prog. Lipid Res., 25, 5-11.

Hilgers, J., Van der Sluis, P.J., van Blitterswijk, W.J. and Emmelot, P., 1978.
Membrane fluidity, capping of cell surface antigens and immune response in mouse leukemia cells.
Br. J. Cancer, 37, 329-336.

Hill, E.G., Johnson, S.B. and Holman, R.T., 1979.
Intensification of essential fatty acid deficiency in the rat by dietary trans fatty acids.
J. Nutr., 109, 1759-1766.

Hill, E.G., Johnson, S.B., Lawson, L.D., Mahfouz, M.M. and Holman, R.T., 1982.
Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil.
Proc. Natl. Acad. Sci. U.S.A., 79, 953-957.

Hill, M.J., 1987.
Dietary fa and human cancer (review).
Anticancer Res., 7, 281-292.

Hillyard, L.A and Abraham, S., 1979.
Effect of dietary polyunsaturated fatty acids on growth of mammary adenocarcinomas in mice and rats.
Cancer Res., 39, 4430-4437.

Hirata, Y., Uchihashi, M., Nakashima, H., Fujita, T. and Matsukura, S., 1985.
Stimulatory effects of epidermal growth factor on prostaglandin E_2 production in mouse fibrosarcoma cell line ($HSDM_1C_1$)
Horm. Met. Res., 9, 448-450.

Hoch, F.L., 1981.
Thydoid hormone control over rat liver membrane lipids.
Prog. Lipid Res., 20, 225-228.

Holley, R.W., Baldwin, J.H. and Kiernan, J.A., 1974.
Control of growth of a tumor cell by linoleic acid.
Proc. Natl. Acad. Sci. U.S.A., 71, 3976-3978.

Holloway, P.W., Peluffo, R. and Nakil, S.J., 1963.
On the biosynthesis of dienoic fatty acids by animal tissues.
Biochem. Biophys. Res. Commun., 12, 300-304.

Holloway, P.W., 1971.
A requirement for three protein components in microsomal stearoyl-Coenzyme A desaturation.
Biochem., 10, 1556-1560.

Holloway, C.T. and Holloway, P.W., 1975.
Stearyl coenzyme A desaturase activity in mouse liver microsomes of varying lipid composition.
Arch. Biochem. Biophys., 167, 496-504.

Holloway, P.W. and Holloway, C.T., 1977.
Desaturases in higher animals.
in "Polyunsaturated Fatty Acids" (Kanau, W-H. and Holman, R.T. eds.), Am. Oil Chem. Soc., Champaign, IL, pg. 37-50.

Holman, R.T., 1960.
The ratio of trienoic:tetraenoic acids in tissue lipids as a measure of essential fatty acid requirements.
J. Nutr., 70, 405-410.

Holman, R.T. and Mohrhauer, H., 1963a.
A hypothesis involving competitive inhibitions in the metabolism of polyunsaturated fatty acids.
Acta Chem. Scand., 17, S84-S90.

Holman, R.T. and Mohrhauer, H., 1963b.
The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver.
J. Lipid Res., 4, 151-159.

Holman, R.T., 1964.
Nutritional and metabolic interrelationships between fatty acids.
Fed. Proc., 23, 1062-1067.

Holman, R.T., 1968.
Essential fatty acid deficiency.
in "Progress in the Chemistry of Fats and Other Lipids", Vol. 9 (Holman, R.T. ed.), Pergamon Press, Oxford, pg. 275-348.

Holman, R.T., 1971.
Biological activities of and requirements for Polyunsaturated fatty acids.
in "Progress in the Chemistry of Fats and Other Lipids", Vol. 9 (Holman, R.T. ed.), Pergamon Press, Oxford.

Holman, R.T., 1977.
in "Polyunsaturated Fatty Acids" (Kunau H-W. and Holman, R.T. eds.), Am. Oil Chem. Soc., Champaign, IL, pg. 163-182.

Holman, R.T., Johnson, S.B., Mercuri, O.M., Harte, H.J., Rodrigo, M.A. and de Tomas, M.W., 1981.
Essential fatty acid deficiency in malnourished children.
Am. J. Clin. Nutr., 34, 1534-1539.

Holman, R.T. and Johnson, S.B., 1982.
Changes in essential fatty acid profiles of serum phospholipids in human diseases.
Prog. Lipid Res., 20, 67-74.

Holman, R.T., 1986a.
Control of polyunsaturated fatty acids in tissue lipids.
J. Am. Coll Nutr., 5, 183-211.

Holman, R.T., 1986b.
Nutritional and biochemical evidences of acyl interaction with respect to essential polyunsaturated fatty acids.
Prog. Lipid Res., 25, 29-39.

Hong, S.L., Polsky-Cynkin, R. and Levine, L., 1976.
Stimulation of prostaglandin biosynthesis by vasoactive substances in methylcholanthrene-transformed mouse BALB/3T3.
J. Biol. Chem., 251, 776-780.

Hong, S.L., Patton, G. and Deykin, D., 1979.
Arachidonic acid level in cellular lipids determines the amount of prostaglandins synthesised during cell growth in tissue culture.
Prostaglandins, 17, 53-59.

Hoover, R.L., Lynch, R.D. and Karnovsky, M.J., 1977.
Descrease in adhesion of cells cultured in polyunsaturated fatty acids.
Cell, 12, 295-300.

Hopkins, G.J. and West, C.E., 1976.
Possible roles of dietary fats in carcinogenesis.
Life Sci., 19, 1103-1116.

Hori, T., Kashiyama, S., Hayakawa, M., Shibamoto, S., Tsujimoto, M., Oku, M. and Ito, F., 1989.
Possible role of prostaglandins as negative regulators in growth stimulation by tumor necrosis factor and epidermal growth factor in human fibroblasts.
J. Cell Physiol., 141, 275-280.

Hornstra, G., Christ-Hazelhof, E., Haddeman, E., Ten Hoor, F. and Nugteren, D.H., 1981.
Fish oil feeding lowers thromboxane and prostacyclin production by rat platelet and aorta and does not result in the production of prostaglandin I_3.
Prostaglandins, 21, 727-738.

Horrobin, D.F., Ally, A.I. and Karmali, R.A., 1978.
Prostaglandins and schizophrenia: further discussion and evidence.
Psychol. Med., 8, 43-48.

Horrobin, D.F., 1980a.
The reversibility of cancer: the relevance of cyclic AMP, calcium, essential fatty acids and prostaglandin E_1.
Med. Hypotheses, 6, 469-486.

Horrobin, D.F., 1980b.
The regulation of prostaglandin biosynthesis: negative feedback mechanisms and the selective control of formation of 1 and 2 series prostaglandins: Relevance to inflammation and immunity.
Med. Hypotheses, 6, 687-709.

Horrobin, D.F., 1982.
Essential fatty acids: a review.
in "Clinical Uses of Essential Fatty Acids" (Horrobin, D.F. ed.), Eden Press, Montreal.

Horrobin, D.F., 1983.
The regulation of prostaglandin biosynthesis by the manipulation of essential fatty acid metabolism.
Rev. Pure App. Pharmacol. Sci., 4, 339-384.

Horrobin, D.F. and Manku, M.S., 1983.
How do polyunsaturated fatty acids lower plasma cholesterol levels?
Lipids, 18, 558-562.

Horrobin, D.F., Manku, M.S. and Huang, Y-S., 1984a.
Effects of essential fatty acids on prostaglandin biosynthesis.
Biomed. Biochys. Acta, 43, S114-S120.

Horrobin, D.F., Das, U.N. and Begin, M., 1984b.
Helicon Symposium in "Nutrition and Disease: Cancer" (Thomas, C.A. and Orgel, L., eds.), California, U.S.A.

Horrobin, D.F., Huang, Y-S, Cunnane, S.C. and Manku, M.S., 1984c.
Essential fatty acids in plasma, red blood cells and liver phospholipids in common laboratory animals as compared to humans.
Lipids, 19, 806-811.

Horrobin, D.F., 1988.
The roles of essential fatty acids in the development of diabetic neuropathy and other complications of diabetes mellitus.
Prost. Leuk. EFA's., 31, 181-197.

Horrobin, D.F., 1990.
Essential fatty acids, lipid peroxidation, and cancer.
in "Omega-6-Essential Fatty Acids. Pathophysiology and Roles in Clinical Medicine" (Horrobin D.F. ed.), Alan R. Liss Inc., New York, pg. 351-377.

Horrobin, D.F., Dobbin, S. and Reynolds, B., 1990.
Plasma fatty acids in dogs and their response to EPA supplementation.
in "Advances in Vetinary Dermatology", Vol. 1 (Von Tscharner, C. and Halliwell, R.E.W., eds.), Tindall, London.

Horwitz, A.F., Hatten, M.E. and Burger, M.M., 1974.
Membrane fatty acid replacements and their effect on growth and lectin-induced agglutinability.
Proc. Natl. Acad. Sci. U.S.A., 71, 3115-3119.

Hoshi, H. and McKeehan, W.L., 1986.
Isolation, growth requirements, cloning, prostaglandin production and life-span of human adult endothelial cells in low serum culture medium.
In Vitro Cell Dev. Biol., 22, 51-60.

Howard, B.V. and Howard,W.J., 1974.
Lipid metabolism in cultured cells.
Adv. Lipid Res., 12, 51-96.

Howard, B.V., Howard, W.J., de la Llera, M., and Kefalides, N.A., 1976.
Triglyceride accumulation in cultured human fibroblasts. The effects of hypertriglyceridemic serum.
Atherosclerosis, 23, 521-534.

Huang, Y-S., Cunanne, S.C, Horrobin, D.F. and Davignon, J., 1982.
Most biological effects of zinc deficiency corrected by gamma-linolenic acid but not linolenic acid.
Artherosclerosis, 41, 193-207.

Huang, Y-S, Horrobin, D.F., Manku, M.S., Mitchell, J. and Pyan, M.A., 1984.
Tissue phospholipid fatty acid composition in the diebetic rat.
Lipids, 19, 367-370.

Huang, Y-S, Mankin, M.S., Mitchell, J. and Horrobin, D.F., 1985.
Effect of high vitamin A intake on plasma and liver phospholipid amd the fatty acid composition in hamsters.
Nutr. Res., 5, 95-100.

Hubbard, V.S., 1983.
What is the association of essential fatty acid status with cystic fibrosis.
Eur. J. Pediatr., 141, 68-70.

Hubbard, W.C., Litterst, C.L., Liu, M.C., Bleecker, E.R., Eggleston, J., McLemore, T.L. and Boyd, M.R., 1986.
Profiling of prostaglandin biosynthesis in biopsy fragments of human lung carcinoma and normal human lung by capillary gas chromatography-negative ion chemical ionisation mass spectrophotometry.
Prostaglandins, 32, 889-906.

Huttner, J.J., Gwebu, E.T., Panganamala, R.V., Milo, G.E., Cornwell, D.G., Sharma, H.M. and Geer, J.C., 1977.
Fatty acids and their prostaglandin derivatives: inhibitors of proliferation in aortic smooth muscle cells
Science, 197, 289-291.

Huttner, J.J., Milo, G.E., Panganamala, R.V., and Cornwell, D.G., 1978.
Fatty acid and the selective alteration of *in vitro* proliferation in human fibroblast and guinea-pig smooth-muscle cells.
In Vitro, 14, 854-859.

Hwang, D.H., Mathias, M.M., DuPont, J. and Meyer, D.L., 1975.
Linoleate enrichment of diet and prostaglandin metabolism in rats.
J. Nutr., 105, 995-1002.

Hwang, D.H. and Kinsella, J.E., 1979.
The effects of trans,trans methyl linoleate on the concentration of prostaglandins and their precursors in the rat.
Prostaglandins, 17, 543-549.

Hwang, D.H. and Carroll, A.E., 1980.
Decreased formation of prostaglandins derived from arachidonic by dietary linolenate in rats.
J. Clin. Nutr., 33, 590-597.

Hyman, A.L., Spannhake, E.W. and Kadowitz, P.J., 1978.
State of the art: prostaglandins and the lung.
Am. Rev. Respir. Dis., 117, 111-136.

Hyman, B.T., Stoll, L.L. and Spector, A.A., 1981a.
Accumulation of (n-9)-eicosatrienoic acid in confluent 3T3-L1 and 3T3 cells.
J. Biol. Chem., 256, 8863-8866.

Hyman, B.T. and Spector, A.A., 1981b.
Accumulation of n-3 polyunsaturated fatty acids in cultured human Y79 retinoblastome cells.
J. Neurochem., 37, 60-69.

Ikai, K., Ujihara, M., Kashihara, M. and Fukushima, M., 1987.
Inhibition of the proliferation of transformed epidermal cells in culture by various prostaglandins.
J. Invest. Dermatol., 89, 69-72.

Infante, J.P., 1987.
Docosahexaenoate-containing phospholipids in sarcoplasmic reticulum and retinal photoreceptors. A proposal for a role in calcium-ATPase calcium transport.
Mol. Cell Biochem., 74, 111-116.

Inkpen, C.A., Harris, R.A. and Quackenbush, F.W., 1969.
Differential responses to fasting and subsequent feeding by microsomal systems of rat liver: 6- and 9-desaturation of fatty acids.
J. Lipid. Res., 10, 277-282.

Irvine, R.F., 1982.
How is the level of free arachidonic acid controlled in mammalian cells?
Biochem. J., 204, 3-16.

IUPAC, 1977.
IUPAC-IUB Commission on Biochemical Nomenclature Recomendations (1976): The Nomenclature of Lipids.
Lipids, 5, 455-468.

Jacobs, R.A., Sly, W.S. and Majerus, P.W., 1973a.
The regulation of fatty acid biosynthesis in human skin fibroblasts.
J. Biol. Chem., 248, 1268-1276.

Jacobs, R.A. and Mejerus, P.W., 1973b.
The regulation of fatty acid synthesis in human skin fibroblasts: Inhibition of fatty acid synthesis by free fatty acids.
J. Biol. Chem., 248, 8392-8401.

Jacobson, E.A., James, K.A., Frei, J.V. and Carroll, K.K., 1988.
Effects of dietary fat on long-term growth and mammary tumorigenesis in Sprague-Dawley rats given a low dose of DMBA.
Nutr. Cancer, 11, 221-228.

Jager, F.C., 1972.
Linoleic acid intake and vitamin E requirement in rats and ducklings.
Ann. N.Y. Acad. Sci., 203, 199-211.

Jamdar, S.C., Osborne, L.J. and Wells, G.N., 1986.
Glycerolipid biosynthesis in rat adipose tissue. Influence of age and cell size on substrate utilisation.
Lipids, 21, 460-464.

James, A.T., 1977.
The specificity of mammalian desaturases.
Adv. Exp. Med. Biol., 83, 51-74.

Janniger, C.K. and Racis, S.P., 1987.
The arachidonic acid cascade: an immunologically based review.
J. Med. Clin. Exp. Theor., 18, 69-87.

Jeffcoat, R., Brawn, P.R. and James, A.T., 1976.
The effect of soluble rat liver proteins on the activity of microsomal stearoyl-CoA and linoleoyl-CoA desaturase.
Biochim. Biophys. Acta, 431,33-44.

Jeffcoat, R., Brawn, P.R., Safford, R. and James, A.T., 1977a.
Properties of rat liver microsomal stearoyl-CoA desaturase.
Biochem. J., 161, 431-437.

Jeffcoat, R. and James, A.T., 1977b.
Interrelationship between the dietary regulation of fatty acid synthesis and the fatty acyl-CoA desaturases.
Lipids, 12, 469-474.

Jeffcoat, R. and Pollard, M.R., 1977c.
Studies on the inhibition of the desaturases by cyclopropenoid fatty acids.
Lipids, 12, 480-485.

Jeffcoat, R., Dunton, A.P. and James, A.T., 1978a.
Evidence for the different responses of Δ9-, Δ6- and Δ5-fatty acyl-CoA desaturases to cytoplasmic proteins.
Biochim. Biophys. Acta, 528, 28-35.

Jeffcoat, R. and James, A.T., 1978b.
The control of stearoyl CoA desaturase by dietary linoleic acid.
Febs. Lett., 85, 114-118.

Jeffcoat, R., 1979.
The biosynthesis of unsaturated fatty acids and its control in mammalian liver.
Essays Biochem., 15, 1-36.

Jeffcoat, R. and James, A.T., 1984.
The regulation of desaturation and elongation of fatty acids in mammals.
in "Fatty Acid Metabolism and its Regulation" (Numa, S., ed.), Elsevier Science Publishers, Amsterdam.

Jezyk, P.F. and Liberti, J.P., 1969.
Metabolic activities of mechanically and enzymatically prepared rat liver cells.
Arch. Biochem. Biophys., 134,442-449.

Johnson, M., Carey, F. and McMillan, R.M., 1983.
Alternative pathways of arachidonate metabolism: Prostaglandins, thromboxane and leukotrienes.
Essays Biochem., 19, 41-141.

Jones, D.B., Carter, R.D., Haitas, B. and Mann, J.I., 1983.
Low phospholipid arachidonic acid values in diabetic platelets.
Br. Med. J., 286, 173-175.

Jones, D.B., Carter, R.D. and Mann, J.I., 1986.
Indirect evidence of impairment of platelet desaturase enzymes in diabetes mellitus.
Horm. Metabol. Res., 18, 341-344.

Kaa, E., 1976.
In vitro biosynthesis of prostaglandin E_2 by kidney medulla of essential fatty acid deficeint rats.
Lipids, 11, 693-696.

Kaduce, T.L., Spector, A.A. and Bar, R.S., 1982.
Linoleic acid metabolism and prostaglandin production by cultured bovine pulmonary artery endothelial cells.
Arteriosclerosis, 2 380-389.

Kameda, K., Valicenti, A.J. and Holman, R.T., 1980.
Chain elongation of trans-octadecanoic acid isomers in rat liver microsomes.
Biochim. Biophys. Acta, 618, 13-17.

Kameda, T. and Otsuji, S., 1983.
Low levels of erythrcyte membrane fluidity in diabetic patients; a spin label study.
Diabetes, 32, 585-591.

Kanau, H-W. and Holman, R.T., 1977.
in "Polyunsaturated Fatty Acids" (Kanua, H-W and Homan, R.T eds.), Am. Oil Chem. Soc., Champaign, IL.

Kane, E., Morris, J.G., Rogers, Q.R., Ihrke, P.J., Cupps, P.T., 1981.
Zinc deficiency in the cat.
J. Nutr., 111, 488-495.

Kann, H.E., Mengel, C.E., Smith, W. and Horton, B., 1964.
Oxygen toxicity and vitamin E.
Aerospace Med., 35, 840-844.

Karmali, R.A., 1986.
Do tissue culture and animal model studies relate to human diet and cancer?
Prog. Lipid Res., 25, 533-538.

Kates, M., 1972.
in "Techniques of Lipidology: Isolation, Analysis and Identification of Lipids" (Work, T.S. and Work, E., eds.), North-Holland Publishing Company, Amsterdam.

Kehrer, J.P. and Autor, A.P., 1978.
Relationship between fatty acids and lipid peroxidation in lungs of neonates.
Biol. Neonate, 34, 61-67.

Kelleher, J., Davies, T., Smith, C.L., Walker, D.E. and Losowsky, M.S., 1972.
The absorbtion of tocopherol in the rat. I. The effect of different carriers and different dose levels.
Int. J. Vit. Nutr. Res., 42, 394-402.

Kennedy, M.S., Stobo, J.D. and Goldyne, M.E., 1980.
In vitro synthesis of prostaglandins and related lipids by populations of human periferal blood mononuclear cells.
Prostaglandins, 20, 135-145.

Kernoff, P.B.A., Willis, A.L. and Stone, K.J., 1977.
Antithrombotic potential of dihomogamma-linolenic acid in Man.
Br. Med. J., 2, 1441-1444.

Khan, O., Hensby, C.N. and Williams, G., 1982.
Prostacyclin in prostatic cancer: a better marker than bone scan or serum acid phosphatase?
Br. J. Urol., 54, 26-31.

Kidwell, W.R. and Shaffer, J., 1984.
[illegible]th stimulatory activity of unsaturated fatty acids for normal and neoplastic breast epithelium.
J. Am. Oil Chem. Soc., 61, 1900-1904.

King, M.E. and Spector, A.A., 1978.
Effect of specific fatty acyl enrichments on membrane physical properties detected with a spin label probe.
J. Biol. Chem., 253, 6493-6501.

Kinsella, J.E., Bruckner, G., Mai, J. and Shimp, J., 1981.
Metabolism of trans fatty acids with emphasis on the effects of trans,trans-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview.
Am. J. Clin. Nutr., 34, 2307-2318.

Kirstein, D., Hoy, C-E. and Holmer, G., 1983.
Effect of dietary fats on the delta-6 and delta-5-desaturation of fatty acids in the rat liver microsomes.
Br. J. Nutr., 50, 749-756.

Kitagawa, S., Endo, J. and Kametani, F., 1984.
Activation of bovine platelets induced by long-chain unsaturated fatty acids at just below their lytic concentrations, and its mechanism.
Biochim. Biophys. Acta, 802, 17-23.

Knittle, J.L. and Hirsch, J., 1965.
Effect of chain length on rates of uptake of free fatty acids during in vitro incubations of rat adipose tissue.
J. Lipid Res., 6, 565-571.

Ko, S.O., Page, R.C. and Narayanan, A.S., 1977.
Fibroblast heterogeneity and prostaglandin regulation of sub-populations.
Proc. Natl. Acad. Aci. U.S.A., 74, 3429-3432.

Kobatake, Y., Hirahara, F., Innami, S. and Nishide, E., 1983.
Dietary effect of omega-3 type polyunsaturated fatty acids on sera and liver lipid levels in rats.
J. Nutr. Sci. Vitaminol., 29, 11-21..

Kobayashi,S., Hirai, A., Terano, T., Hamazaki, T., Tamura, Y. and Kumagai, A., 1981.
Reduction in blood viscosity by eicosapenta-enoic acid.
Lancet, 2, 197-199.

Koningsberg, I.R., 1979.
Skeletal myoblasts in culture.
Methods Enzymol., 63, 511-527.

Kontos, H.A., Wei, E.P., Ellis, E.F., Jenkins, L.W., Povlishock, J.T., Rowe, G.T. and Hess, M.L., 1985.
Appearance of superoxide anion radical in cerebral extracellular space during increased prostglandin synthesis in cats.
Circ. Res., 57, 142-151.

Korn, J.H., Halushka, P.V. and Leroy, E.C., 1980.
Mononuclear cell modulation of connective tissue function: suppression of fibroblast growth by stimulation of endogenous prostaglandin production.
J. Clin. Invest., 65, 543-554.

Kornbrust, D.J. and Mavis, R.D., 1980.
Relative susceptibility of microsomes from lung, heart, liver, kidney, brain and testes to lipid peroxidation: Correlation with vitamin E content.
Lipids, 15, 315-322.

Kramer, J., 1980.
Comparative studies on composition of cardiac phospholipids in rats fed different vegetable oils.
Lipids, 15, 651-660.

Krishnamurthy, S. and Bieri, J.G., 1963.
The absorbtion, storage, and metabolism of alpha-tocopherol-C14 in the rat and chicken.
J. Lipid Res., 4, 330-336.

Kuehl, F.A., Egan, R.W.Jr. and Humes, J.L., 1981.
Prostaglandin cyclooxygenase.
Prog. Lipid Res., 20, 97-102.

Kulmacz, R.J. and Lands, W., 1983.
Requirements for hydroperoxide by the cyclooxygenase and peroxidase activities of prostaglandin H synthetase.
Prostaglandins, 25, 531-540.

Kumar, R., King, R.J., Martin, H.M. and Hanahan, D.J., 1987.
Metabolism of platelet-activating factor (alkylacetylphosphocholine) by type II epithelial cells and fibroblasts from rat lungs.
Biochim. Biophys. Acta, 917, 33-41.

Kurata, N. and Privett, O.S., 1980.
Effect of dietary fatty acid composition on the biosynthesis of unsaturated fatty acids in rat liver microsomes.
Lipids, 15, 512-518.

de Laat, S.W., van der Saag, P.T., Elson, E. and Schlessinger, J., 1977.
Microviscosity modulation during the cell cycle of neuroblastoma cells.
Proc. Natl. Acad. Sci. U.S.A., 74, 4458-4461.

Lagarde, M., Berciaud, P., Burtin, M. and Dechavanne, M., 1981.
Refractoriness of diabetic platelets to inhibitory prostaglandins.
Prostaglandin Med., 7, 341-348.

Lagarde, M., Sicard, B., Guichardant, M., Felisi, O. and Dechavanne, M., 1984.
Fatty acid composition in native and cultured human endothelial cells.
In vitro, 20, 33-37.

Lambert, S., Champion, S. and Jacquemin, C., 1976.
Relationship between prostaglandin biosynthesis and the effect of insulin on hormone stimulated lipolysis in rat adipose tissue.
Biochim. Biophys. Acta, 431, 132-134.

Lands, W.E.M. and Samuelsson, B., 1968.
Phospholipid presursors of prostaglandins.
Biochim. Biophys. Acta, 164, 426-429.

Lands, W.E.M., Lee, R. and Smith W., 1971.
Factors regulating the biosynthesis of various prostaglandins.
Ann. N.Y. Acad. Sci., 180, 107-122.

Lands, W.E.M., LeTellier, P.F., Rome, L.H. and Vanderhoek, J.Y., 1973.
Inhibition of prostaglandin biosynthesis.
Adv. Biosci., 9, 15-28.

Lands, W.E.M., Hemler, M.E. and Crawford, C.G., 1977.
Functions of polyunsaturated fatty acids: Biosynthesis of prostaglandins.
in "Polyunsaturated Fatty Acids" (Kanu, W.H. and Holman, R.T. eds.), Am. Oil. Chem. Soc., Champaign, IL, pg. 193-228.

Lands, W.E.M. and Byrnes, M.J., 1981.
The influence of ambient peroxides on the conversion of 5,8,11,14,17-eicosapentaenoic acid to prostaglandins.
Prog. Lipid Res., 20, 287-290.

Lands, W.E.M., 1982.
Biochemical observations on dietary long-chain FA's from fish oil and their effect on prostaglandin synthesis in animals and humans.
in "Nutritional Evaluation of Long Chain Fatty Acids in Fish Oil" (Barlow and Stansby eds.), Academic Press, New York, pg. 267-282.

Lands, W.E.M., 1986.
Renewed questions about polyunsaturated fatty acids.
Nutr. Rev., 44, 189-195.

Laposata, M., Kaiser, S.L. and Capriotti, A.M., 1988.
Eicosanoid production can be decreased without alterations in cellular arachidonate content or enzyme activities required for arachidonate release and eicosanoid synthesis.
J. Biol. Chem., 263, 3266-3273.

Launay, M., Vodovar, N. and Raulin, J., 1968.
Developpemant du tissu adipeux. Nombre et taille des cellules en function de la valeur energetique et de L'insaturation des lipides du regime.
Bull. Soc. Chim. Biol., 50, 439-450.

Launay, M., Dauvillier, P. and Raulin, J., 1969.
Developpement du tissu adipeux. Activite specifique des acides nucleiques et role des acides gras polyinsatures.
Bull. Soc. Chim. Biol., 51, 95-104.

Launay, M., Lapous, D. and Raulin, J., 1981.
Control of replication by dietary lipids and namely by linoleic acid in liver and adipose tissue in developing rats.
Prog. Lipid Res., 20, 331-338.

Leary, W.P., Robinson, K.M., Booyens, J. and Dippenaar, N., 1982.
Some effects of gamma-linolenic acid on cultivated human oesophageal carcinoma cells.
S. Afr. Med. J., 62, 681-683.

Lee, K.M. and Kim, K.H., 1977a.
Regulation of rat liver acetyl conenzyme A carboxylase.
J. Biol. Chem., 252, 1748-1751.

Lee, T.C., Baker, R.C., Stevens, N. and Snyder, F., 1977b.
Evidence for participation of cytochrome b_5 in microsomal Δ-6-desaturation of fatty acids.
Biochim. Biophys. Acta, 489, 25.

Lee, T.H., Hoover, R.L., Wiliams, J.D., Sperling, R.I., Ravalese, J., Spur, B.W., Robinson, D.R., Corey, E.J., Lewis, R.A. and Austen, K.F., 1985.
Effects of dietary eicosapentaenoic and docosahexaenoic acids on in vitro neutrophil and monocyte leukotrience generation and neutrophil function.
N. Engl. J. Med., 312, 1217-1224.

Lee, J.H., Sugano, M. and Ide, T., 1988.
Effects of various combinations of ω3 and ω6 polyunsaturated fats with saturated fat on serum lipid levels and eicosanoid production in rats.
J. Nutr. Sci. Vitaminol., 34, 117-129.

Lehninger, A.L., 1982.
in "Principles of Biochemistry" (Anderson, S. and Fox, J., eds.), Worth Publishers, Inc., New York.

Leikin, A.I., Nervi, A.M. and Brenner, R.R., 1979.
Lipid binding properties of a factor necessary for linoleic acid desaturation.
Lipids, 14, 1021-1027.

Leikin, A.I. and Brenner, R.R., 1986.
Regulation of linoleic acid delta-6-desaturation by a cytosolic lipoprotein-like fraction in isolated rat liver microsomes.
Biochim. Biophys. Acta, 876, 300-308.

Leikin, A.I. and Brenner, R.R., 1989.
Microsomal Δ5 desaturation of eicosa-8,11,14-trienoic acid is activated by a cytosolic fraction.
Lipids, 24, 101-104.

Lepran, I., Nemecz, G., Koltai, M. and Szekeres, L. 1981.
Effect of a linoleic acid rich diet on the acute phase coronary occlusion in concious rats: influence of indomethacin and asprin.
J. Cardiovasc. Pharmacol., 3, 847-853

Lhuillery, C., Mebarki, S., Lecourtier, M-J. and Demarne, Y., 1988.
Influence of different dietary fats on the incorporation of exogenous fatty acids into rat adipose glycerides.
J. Nutr., 118, 1447-1454.

Liepkalns, V.A., Icard-Liepkalns, C. and Cornwell, D.G., 1982.
Regulation of cell division in a human glioma cell clone by arachidonic acid and alpha-tocopherol-quinone.
Cancer Lett., 15, 173-178.

Lipinski, R.A. and Mathias, M.M., 1978.
Prostaglandin production and lipolysis in isolated rat adipocytes as affected by dietary fat.
Prostaglandins, 16, 957-963.

de la Llera, M., Rothblat, G. and Howard, B.V., 1979.
Cell triacylglycerol accumulation from very low density lipoproteins isolated from normal and hypertriglyceridemic human sera.
Biochim. Biophys. Acta, 574, 414-422.

Lloyd, D.H., 1990.
Essential fatty acids in dermatological disorders of dogs and cats.
in 'Omega-6-Essential Fatty Acids: Pathophysiology and Roles in Clinical Medicine' (Horrobin, D.F. ed.), Wiley-Liss.

Lodish, H.F. and Rothman, J.E., 1979.
The assembly of cell membranes.
Sci. Am., 240, 38-53.

Lokesh, B.R., Mathur, S.N. and Spector, A.A., 1981.
Effect of fatty acid saturation on NADPH-dependent lipid peroxidation in rat liver microsomes.
J. Lipid Res., 22, 905-915.

Lokesh, B.R., Bruckner, G. and Kinsella, J.E., 1984.
Reduction in thromboxane formation by n-3 fatty acids enriched lung microsomes from rat and guinea-pig following the ingestion of dietary menhaden oil.
Prost. Leuk. Med., 15, 337-348.

Lokesh, B.R. and Kinsella, J.E., 1985.
Lipid composition and prostaglandin synthesis in mouse lung microsomes following the ingestion of menhaden oil.
Lipids, 20, 842-849.

Lokesh, B.R., Hsieh, H.L and Kinsella, J.E., 1988.
Olive oil enriched diets decreases arachidonic acid without affecting prostaglandin synthesis in mouse lung and spleen.
Nutr. Res., 8, 499-507.

Loriette, C., Jomain-Baum, M., MaCaire, I. and Raulin, J., 1971.
Lipogenese de novo et insaturation des lipides exogenese dans le tissu adipeux du rat.
Eur. J. Clin. Biol. Res., 16, 366-379.

Loriette, C. and Raulin, 1972.
Commentaire sur l'effet regulateur de la lipogenese par les acides gras polinsatures. Activitis acetyl CoA carboxylase du foie et du tissu adipeux.
J. Biochimie, 54, 1467-1471.

Lowe, N.J., and Stoughton, R.B., 1977.
Effects of topical prostaglandin E_2 analog on normal hairless mouse epidermal DNA synthesis.
J. Invest. Dermatol., 68, 134-137.

Lowe, N.J. and DeQuoy, P.R., 1978.
Linoleic acid effects on epidermal DNA synthesis and cutaneous prostaglandin levels in essential fatty acid deficiency.
J. Invest. Dermatol., 70, 200-203.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J., 1951.
Protein measurement with the Folin phenol reagent.
J. Biol. Chem., 193, 265-275.

Lowry, R.R. and Tinsely, I.J., 1966.
Interactions in the metabolism of polyunsaturated fatty acids.
Biochim. Biophys. Acta, 116, 398-400.

Lubin, B. and Chiu, D., 1982.
Properties of vitamin E-deficient erythrocytes following peroxidative injury.
Pediat. Res., 16, 928-932.

Lupulescu, A.P., 1977.
Cytologic and metabolic effects of prostaglandins in rat skin.
J. Invest. Dermatol., 68, 138-145.

Lynch, R.E. and Fridovich, I., 1978a.
Effects of superoxide on the erythrocyte membrane.
J. Biol. Chem., 253, 1838-1845.

Lynch, R.E. and Fridovich, I., 1978b.
Permeation of the erythrocyte stroma by superoxide radical.
J. Biol. Chem., 253, 4697-4699.

MacDonald, M.L., Rogers, Q.R. and Morris, J.G., 1983a.
Role of linoleate as an essential fatty acid for the cat independant of arachidonate synthesis.
J. Nutr., 113, 1422-1433.

MacDonald, V.W. and Charach, S., 1983b.
Differences in the reaction sequences associated with drug-induced oxidation of hemoglobins E, S, A and F.
J. Lab. Clin. Med., 102, 762-772.

MacDonald, M.L., Rogers, Q.R., Morris, J.G. and Cupps, P.T., 1984.
Effect of linoleate and arachidonate deficiencies on reproduction and spermatogenesis in the cat.
J. Nutr., 114, 719-726.

Mackenzie, C.G., Mackenzie, J.B., Reiss, O.K. and Philpott, D.E., 1964.
The lipid content and structure of cells.
Fed. Proc., 23, 375 (Abst.).

Mackenzie, C.G., Mackenzie, J.B. and Reiss, O.K., 1967.
Increase in cell lipid and cytoplasmic particles in mammalian cells cultured at reduced pH.
J. Lipid Res., 8, 642-645.

Mackenzie, C.G., Mackenzie, J.B., Reiss, O.K. and Wineski, J.A., 1970.
Inclusion of albumin-bound fatty acids as the major factor in serum-induced accumulation by cultured cells.
J. Lipid Res., 11, 571-582.

Maeda, M., Doi, O. and Akamatsu, Y., 1978.
Metabolic conversion of polyunsaturated fatty acids in mammalian cultured cells.
Biochim. Biophys. Acta, 530, 153-164.

Mahfouz, M.M., Johnson, S. and Holman, R.T., 1980a.
The effect of isomeric trans-18:1 acids on the desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes.
Lipids, 15, 100-107.

Mahfouz, M. and Holman, R.T., 1980b.
Desaturation of isomeric cis-18:1 acids.
Lipids, 15, 63-65.

Mahfouz, M.M., Johnson, S. and Holman, R.T., 1981.
Inhibition of desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids in vitro by isomeric cis-octadecaenoic acids.
Biochim. Biophys. Acta, 663, 58-68.

Mahfouz, M.M., Smith, T.L. and Kummerow, F.A., 1984.
Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat-liver microsomes.
Lipids, 19, 214-222.

Mahfouz, M.M. and Kommerow, F.A., 1989.
Effect of magnesium deficiency on delta-6-desaturase activity and fatty acid composition of rat liver microsomes.
Lipids, 24, 727-732.

Manku, M.S., Horrobin, D.F., Morse, N., Kyte, V., Jenkins, K., Wright, S. and Burton, J.L. 1982a.
Reduced levels of prostaglandin precursors in the blood of atopic patients: defective delta-6-desaturase function as a biochemical basis for atopy.
Prost. Leuk. Med., 9, 615-623.

Manku, M.S., Horrobin, D.F., Wright, S. and Burton, J.L., 1982b.
Reduced levels of prostaglandin precursors in the blood of atopic patients: defective delta-6-desaturase function as a biochemical basis for atopy.
Prost. Leuk. Med., 9, 1-13.

Manku, M.S., Horrobin, D.F., Morse, N., Wright, S. and Burton, J.L., 1984.
Essential fatty acids in the plasma phospholipids of patients with atopic eczema.
Br. J. Dermatol., 110, 643-648.

Mann, C.R., Kaduce, T.L., Figard, P.H. and Spector, A.A., 1986.
Docosatetraenoic acid in endothelial cells: formation, retroconversion to arachidonic acid, and effect on prostacyclin production.
Arch. Biochem. Biophys., 244, 813-823.

Manzoli, F.A., Muchmore, J.H., Bonora, B., Capitani, S. and Bartoli, S., 1974.
Lipid-DNA interactions. II. Phospholipids, cholesterol, glycerophosphorylcholine, sphingosine and fatty acids.
Biochim. Biophys. Acta, 340, 1-15.

Marcel, Y.L., Christiansen, K. and Holman, R.T., 1968.
The preferred metabolic pathway from linoleic acid to arachidonic acid in vitro.
Biochim. Biophys. Acta, 104, 25-34.

Marcel, Y.L. and Suzue, G.J., 1972.
Kinetic studies on the specificity of long chain acyl coenzyme A synthetase from rat liver microsomes.
J. Biol. Chem., 247, 4433-4436.

Marcus, A.J., Weksler, B.B. and Jaffe, E.A., 1978.
Enzymatic conversion of prostaglandin endoperoxide H_2 and arachidonic acid to prostacyclin by cultured human endothelial cells.
J. Biol. Chem., 253, 7138-7141.

Marshall, L.A. and Johnson, P.V., 1982.
Modulation of tissue prostaglandin synthesising capacity by increased ratios of dietary alpha-linolenic acid to linoleic acid.
Lipids, 17, 905-913.

Mathe, A.A., Hedquist, P., Strandberg, K. and Leslie, C.A., 1977.
Aspects of prostaglandin function in the lung.
N. Engl. J. Med., 296, 850-855.

Mathias, M.M. and Dupont, J., 1979.
The relationship of dietary fats to prostaglandin biosynthesis.
Lipids, 14, 247-252.

Mayer, B., Rauter, L., Zenzmaier, E., Gleispach, H. and Esterbauer, H., 1984.
Characterisation of lipoxygenase metabolites of arachidonic acid in cultured human skin fibroblasts.
Biochim. Biophys. Acta, 795, 151-161.

Mayes, P.A., 1981.
in "Harper's Review of Biochemistry" (Martin, D.W., Mayes, P.A. and Rodwell, V.W., eds.), 18th ed., Lange Publishers, California.

Mayeux, P.R., Kadavitz, P.J. and McNamara, D.B., 1989.
Differential effects of ibuprofen, indomethacin and meclofenamate on prostaglandin endoperoxide H_2 metabolism.
Med. Cell Biochem., 87, 41-46.

McCance R.A. and Widdowson, E.M., 1978.
in "The Composition of Foods" (Paul, A.A. and Southgate, D.A.T., eds.), H.M.S.O., London.

McCullough, J.L., Schreiber, S.H. and Ziboh, V.A., 1978.
Cell proliferation kinetics of epidermis in the essential fatty acid deifcient rat.
J. Invest. Dermatol., 70, 318-320.

McDaniel, H.G., Papapietro, S.E., Rogers, W.J., Mantle, J.A., Smith, L.R., Russel, R.O. Jr. and Rackley, C.E., 1981.
Glucose-insulin-potassium induced alterations in individual plasma free fatty acids in patients with acute myocardial infarction.
Am. Heart J., 102, 10-15.

McGee, R.Jr., 1981.
Membrane fatty acid modification of the neuroblastoma x glioma hybrid, NG108-15.
Biochim. Biophys. Acta, 663, 314-328.

McGee, R. Jr. and Kenimer, J.G., 1982.
The effects of exposure to unsaturated fatty acids on opiate receptors, prostaglandin E_1 receptors and adenylate cyclase activity of neuroblastoma x glioma hybrid cells.
Mol. Pharm., 22, 360-368.

McGowan, J.A., Strain, A.J. and Bucher, N.L., 1981.
DNA synthesis in primary cultures of adult rat hepatocytes in a defines medium: effects of epidermal growth factor, insulin, glucagon and cyclic-AMP.
J. Cell Physiol., 108, 353-363.

McLemore, T.L., Hubbard, W., Litterst, C.L., Liu, M.C., Miler, S., McMahon, N.A., Eggleston, J.C. and Boyd, M.R., 1988.
Profiles of prostaglandin biosynthesis in normal lung and tumor tissues from cancer patients.
Cancer Res., 48, 3140-3147.

Mead, J.F. and Howtin, D.R., 1960
Metabolism of essential fatty acids. Conversion of 8,11,14-eicosatrienoic acid to arachidonic acid in the rat.
J. Biol. Chem., 235, 3385-3386.

Mead, J.F., 1968.
The metabolism of the polyunsaturated fatty acids.
Prog. Fats Other Lipids, 9, 159-192.

Mead, J.F., 1976.
Free radical mechanisms of lipid damage and concequences for cellular membranes.
in "Free Radicals in Biology", Vol. 1, Academic Press, New York, pg. 51-58.

Mead, J.F. and Fulco, A.J., 1976.
in "The Unsaturated and Polyunsaturated Fatty Acids in Health and Disease" (C.C. Thomas), Springfield.

Mengel, C.E., Kann, H.E., Heyman, A. and Metz, E., 1965.
Effects of in vivo hyperoxia on erythrocytes. II. Hemolysis in a human after exposure to oxygen under high pressure.
Blood, 25, 822-829.

Mengel, C.E. and Kann, H.E., 1966.
Effect of in vivo hyperoxia on erythrocytes. III. In vivo peroxidation of erythrocyte lipid.
J. Clin. Invest., 45, 1150-1158.