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Linoleic acid and arachidonic acid metabolism in human peripheral blood leucocytes: comparison with the rat

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1. Peripheral blood leucocytes from human male volunteers and from male rats were incubated in vitro in the presence of ^{14}C-labelled linoleic acid ([^{14}C]LA) or ^{3}H-labelled arachidonic acid ([^{3}H]AA). The time-course of [^{14}C]LA and [^{3}H]AA incorporation into human leucocyte total lipids was maximal at 80–90% of the initial dose within 20–30 min of dosing the cells.

2. Compared with mixed leucocytes, isolated polymorphonuclear leucocytes were only marginally different in the differential incorporation of [^{14}C]LA and [^{3}H]AA into total lipids.

3. In human leucocytes, [^{14}C]LA was incorporated initially into triglycerides but predominantly into phosphatidylcholine thereafter. In the rat, [^{14}C]LA remained as the free acid (63%), with lesser amounts entering the phospholipids (9%), monoglycerides–diglycerides (12%) and triglycerides (< 1%).

4. Utilization of [^{14}C]LA by the Δ6-desaturase was only a minor route of its metabolism in both human and rat leucocytes.

5. ^{3}H-labelled prostaglandins E_2 and $F_{2\alpha}$ accounted for up to 30% of the radioactivity released into the incubation medium from human leucocytes incubated with [^{3}H]AA for 60 min.

6. Stimulation of phagocytosis in the human leucocytes with latex beads or with unopsonized zymosan did not alter the differential incorporation of [^{14}C]LA or [^{3}H]AA into the leucocyte lipid fractions.

The essential fatty acids (EFA) have been recognized as being essential to humans for 35 years (Hansen *et al.* 1947) but the EFA status of humans is still only assessed on the basis of plasma and erythrocyte fatty acid composition. Static measurement of fatty acid composition can identify proportional imbalances of fatty acids, but it would be valuable if a metabolically active cell population were available in which EFA metabolism could be measured by in vitro techniques. Both platelets and peripheral blood leucocytes might be appropriate for this purpose. The platelet has been widely used to determine prostaglandin (PG) synthesis from arachidonic acid (Bills *et al.* 1977) and the leucocyte has been similarly used to study leukotriene synthesis from arachidonic acid (Borgeat *et al.* 1976). In neither case has the metabolism of other EFA been documented in any detail.

In the present study we have assessed the uptake of ^{14}C-labelled linoleic acid ([^{14}C]LA) and ^{3}H-labelled arachidonic acid ([^{3}H]AA) into mixed human peripheral blood leucocytes. We present evidence that both fatty acids are readily taken up into the leucocyte lipid pools but, in comparison with the rat, the pattern of incorporation is markedly different. In both man and the rat, Δ6-desaturation was a minor route of [^{14}C]LA metabolism, but did appear to be lower in man than in the rat. Basal PG synthesis, although readily monitored in the medium surrounding the leucocytes following incubation with [^{3}H]AA, was also a minor metabolic pathway of [^{3}H]AA.

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MATERIALS AND METHODS

Leucocyte preparation

Isolation of peripheral blood leucocytes from healthy male volunteers (aged 30–45 years) and from male Wistar rats (250–300 g) was performed as previously described (Keeling *et al.* 1981). Heparinized blood (50 ml) was mixed with 10 ml dextran in saline (9 g sodium chloride/l, Dextraven 150; Fisons) and the erythrocytes and platelets allowed to sediment for 20–30 min. The leucocyte-rich supernatant fraction was decanted into 15 ml test-tubes and centrifuged at 250 ***g*** for 5 min. The supernatant plasma fraction was discarded and the leucocytes resuspended in 3 ml deionized water for 15 s to lyse contaminating erythrocytes. Quadruple-strength Hartmann's solution (1·0 ml) was added to restore osmolarity and the leucocytes were then centrifuged for 3 min at 250 ***g*** and resuspended in the incubation medium.

Leucocyte incubation

The incubation medium was TC 199 minimal essential medium (Wellcome) with 20 mM-HEPES (Sigma) added as a buffer (final pH 7·4). The leucocytes were resuspended in varying amounts of medium (5–20 ml) according to the experiment. Leucocyte counts and viability tests were performed before and after incubations. Viability was found to be $> 95\%$, as found in previous studies under similar conditions (Keeling *et al.* 1981).

For the Δ6-desaturase studies in intact leucocytes, 0·5 μCi [1-^{14}C]LA (10 nmol; Amersham International) was incubated with the leucocytes for 30 min at 37°. In the leucocyte and rat liver microsomal fractions, Δ6-desaturase assays were run using appropriate cofactors and the particle-free supernatant fractions added to a Tris-buffered medium as previously described (Cunnane & Wahle, 1981).

The time-course and lipid distribution studies were carried out by resuspending the leucocytes in TC 199 medium with 0·5 μCi each of [1-^{14}C]LA and [5,6,8,9,11,12,14,15-^{3}H]AA (Amersham International) simultaneously.

Stimulation of the leucocytes was carried out with either latex beads or unopsonized zymosan (Sigma). Latex beads were incubated with the leucocytes in the ratio 20:1, and zymosan in the ratio 2:1, or approximately 100–150 μg zymosan/ml incubation medium. The incubations with zymosan were done on a time-course basis: 1 h incubation before stimulation, followed by sampling at 1 h and 2 h after stimulation. Comparisons of lipid incorporation and distribution were made between stimulated and unstimulated cells.

Lipid extraction

To determine the lipid incorporation and distribution of [^{14}C]LA and [^{3}H]AA, the leucocyte lipids were extracted with chloroform–methanol (2:1, v/v) containing butylated hydroxytoluene (100 mg/l) as an antioxidant. Lipid extracts were spotted on Whatman LK5D pre-coated plates (Pierce and Warriner) which were developed with the following solvents: for neutral lipids (light petroleum (b.p. 40–60)–diethyl ether–acetic acid–methanol (85:15:2·5:1, by vol.); for phospholipids, chloroform–methanol–water (60:30:4, by vol.). After development, radioactive peaks on all plates were identified using a radiochromatogram scanner (Panax). Lipid bands on the plates were further identified by charring and comparison with authentic standards.

For the Δ6-desaturase assay, the incubation was stopped and the lipids saponified with ethanolic potassim hydroxide followed by acidification, extraction with diethyl ether, neutralization, and methylation with diazomethane (Cunnane & Wahle, 1981). Argentation thin-layer chromatography was used for fatty acid separation, according to the number of double bonds, with the solvent hexane–diethyl ether (40:60, v/v).

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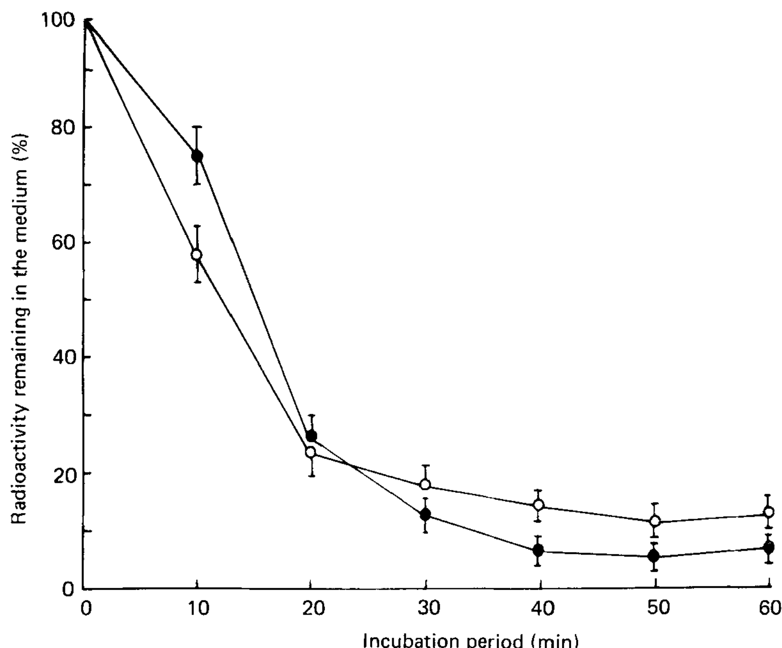

Fig. 1. Time-course of the uptake of [^{14}C]linoleic acid (○) and [^{3}H]arachidonic acid (●) into human leucocytes. Points are mean values with their standard errors, represented by vertical bars, for four experiments utilizing $1-2 \times 10^7$ cells/incubation. [^{14}C]linoleic acid was absorbed less rapidly than [^{3}H]arachidonic acid at 10 min ($P < 0.01$) but more rapidly from 40 to 60 min ($P < 0.05$).

PGE_2 and $PGF_{2\alpha}$ synthesis from [^{3}H]AA was determined by acidification of the incubation mixture to pH 3·2, extraction of the prostaglandins with diethyl ether–ethyl acetate (4:1, v/v) and thin-layer chromatographic separation of PGE_2 and $PGF_{2\alpha}$ using the organic phase of a mixture of ethyl acetate–iso-octane–acetic acid–water (10:5:2:11, by vol.) as solvent.

For all radioactivity studies, bands of silica containing radioactivity were scraped into scintillation vials, 10 ml toluene-based scintillation fluid was added and the radioactivity determined using a liquid-scintillation counter (Intertechnique).

Statistical analyses were performed using Student's paired *t*-test.

RESULTS

[^{14}C]LA and [^{3}H]AA uptake

Fig. 1 shows the time-course of [^{14}C]LA and [^{3}H]AA uptake from the incubation medium by the leucocytes. For both fatty acids, at least 50% of the label had been incorporated into the leucocytes within 15 min, and within 30 min incorporation was maximal. The amount of [^{14}C]LA remaining in the incubation medium was significantly less than that of [^{3}H]AA after 40 min.

Incorporation of [^{14}C]LA and [^{3}H]AA into mixed leucocytes v. *separated polymorphonuclear leucoctyes*

The incorporation of [^{14}C]LA and [^{3}H]AA into the lipid fractions of populations of mixed leucocytes (polymorphonuclear and mononuclear leucocytes) and separated polymorphonuclear leucocytes were compared (Table 1). There were only minor differences in the

Table 1. *Comparison of percentage ^{14}C-labelled linoleic acid ([^{14}C]LA) and ^{3}H-labelled arachidonic acid ([^{3}H]AA) incorporation into mixed leucocytes and that of separated polymorphonuclear (PMN) leucocytes utilizing 1–2 × 10^7 cells/incubation*

(Each value is the mean of two determinations)

	[^{14}C]LA		[^{3}H]AA	
	Mixed	PMN	Mixed	PMN
PGE_2	—	—	0·7	0·4
$PGF_{2\alpha}$	—	—	1·6	0·9
TG	73·1	66·8	61·1	58·3
CE	< 0·1	< 0·1	< 0·1	< 0·1
MG–DG	2·1	2·5	2·6	3·1
FFA	10·3	11·6	11·1	8·6
PI–PS	0·1	0·1	0·1	0·1
PC	12·5	14·0	21·7	24·3
PE	1·6	2·0	1·9	2·6

PG, prostaglandin; TG, triglyceride; CE, cholesteryl ester; MG, monoglyceride; DG, diglyceride; FFA, free fatty acid; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

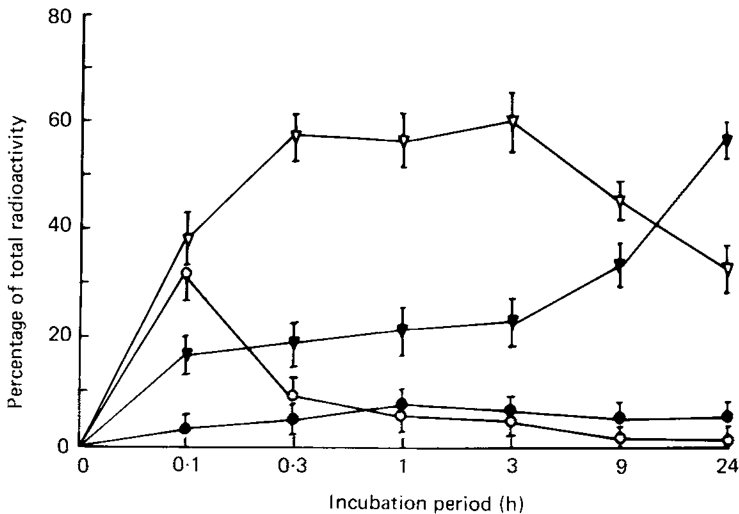

Fig. 2. Time-course of the distribution of [^{14}C]linoleic acid in human leucocyte lipids over 24 h: triglycerides (▽), free fatty acids (○), phosphatidylcholine (▼), phosphatidylethanolamine (●). Points are mean values with their standard errors, represented by vertical bars, for four experiments utilizing 1–2 × 10^7 cells/incubation. There was less [^{14}C]linoleic acid in phosphatidylcholine than in triglyceride at all time-points except 24 h ($P < 0·01$), and less [^{14}C]linoleic acid in the free fatty acid fraction than in phosphatidylcholine at all time-points except 0·1 h ($P < 0·01$).

incorporation of both [^{14}C]LA and [^{3}H]AA into triglycerides (TG) and phosphatidylcholine (PC), so all further experiments were conducted using the mixed population of leucocytes to reduce handling of the cells and maintain high viability.

[^{14}C]LA incorporation into leucocyte lipids

The 24 h time-course of [^{14}C]LA incorporation into leucocyte lipids is shown in Fig. 2. At 0·1 h, 60% of the [^{14}C]LA was already incorporated into tissue lipids, predominantly TG, which remained the main pool of [^{14}C]LA accumulation for at least 9 h. The amount of

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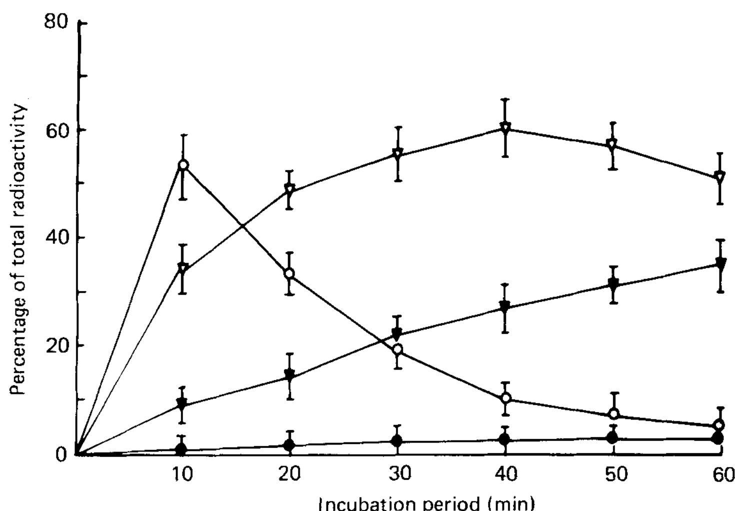

Fig. 3. Time-course of [^{3}H]arachidonic acid distribution in human leucocyte lipids over 60 min: triglyceride (▽), free fatty acid (○), phosphatidylcholine (▼), phosphatidylethanolamine (●). Points are mean values with their standard errors, represented by vertical bars, for four experiments utilizing 1–2 × 10^7 cells/incubation. There was less radioactivity incorporated into the phosphatidylcholine fraction than into triglyceride at all time-points to 60 min ($P < 0{\cdot}01$) and less radioactivity in the free fatty acid fraction than in triglyceride at all time-points except 10 min ($P < 0{\cdot}01$).

[^{14}C]LA in the TG pool peaked at 50–60% of the total [^{14}C]LA incorporated at between 0·3 and 3 h. This amount was variable between subjects. The amount of [^{14}C]LA incorporated into the PC fraction increased to a maximum at 24 h. Phosphatidylethanolamine (PE) took up about 5–7% of the [^{14}C]LA within 0·3 h and this did not change significantly within 24 h. The amount of [^{14}C]LA remaining as the free fatty acid (FFA) in the leucocyte lipids was maximal at about 30% and occurred at the 0·1 h time-point, after which it decreased to 5% at 24 h. The other lipid fractions measured, i.e. monoglycerides (MG), diglycerides (DG), cholesteryl esters (CE), phosphatidylinositol (PI), and phosphatidylserine (PS) accumulated no more than 1% of the total incorporated [^{14}C]LA at all time-points measured.

[^{3}H]AA incorporation into leucocyte lipids

The time-course of [^{3}H]AA incorporation into human leucocyte lipids is shown in Fig. 3. Since the major short-term (< 6 h) changes in the lipid distribution of [^{14}C]LA occurred within 1 h of dosing the cells, a time-course within this time-period only was studied with [^{3}H]AA. Over 1 h, the incorporation of [^{3}H]AA was similar to that of [^{14}C]LA, but the lipid distrubution was different: significantly more [^{3}H]AA was incorporated into the PC fraction and less into the TG fraction. At 20 min, significantly more [^{3}H]AA remained as the FFA than did [^{14}C]LA. As with [^{14}C]LA, < 1% of the incorporated [^{3}H]AA was found in the MG, DG, CE, PI, or PS fractions up to 1 h after dosing. In addition, < 0·8% of the [^{3}H]AA remaining in the leucocytes was converted to [^{3}H]PGE_2 or [^{3}H]$PGF_{2\alpha}$.

[^{3}H]AA incorporation into lipids in the incubation medium

The distribution of [^{3}H]AA in the lipids present in the incubation medium showed a different pattern from that in the leucocyte intracellular lipids (Fig. 4). [^{3}H]PGE_2 and [^{3}H]$PGF_{2\alpha}$ were major products of [^{3}H]AA metabolism by the leucocytes and were detected in the incubation medium after 20 min. The amount of conversion of [^{3}H]AA to lipoxygenase products was not measured. The amount of [^{3}H]AA remaining as the FFA decreased rapidly to a

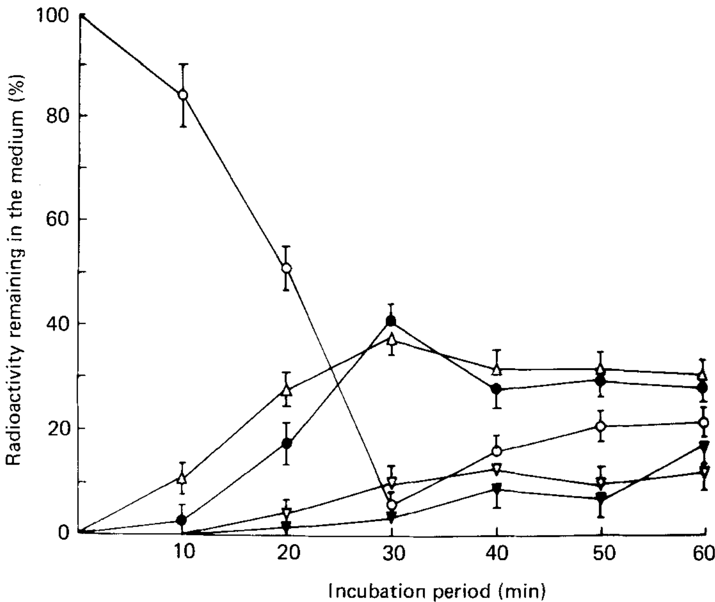

Fig. 4. Time-course of [^{3}H]arachidonic acid incorporation into the leucocyte incubation medium over 60 min: PGE_2 (▼), $PGF_{2\alpha}$ (▽), free fatty acid (○), neutral lipid (△), phospholipid (●). Points are mean values with their standard errors, represented by vertical bars, for four experiments utilizing $1–2 \times 10^7$ cells/incubation. There was less [^{3}H]arachidonic acid remaining as the free fatty acid after 30 min than was incorporated into the neutral lipids or phospholipids ($P < 0·01$), and less [^{3}H]arachidonic acid converted to $PGF_{2\alpha}$ after 20 min than was in the neutral lipid or phospholipid fractions ($P < 0·01$).

minimum at 30 min which coincided with the rate of its incorporation into the leucocyte lipids. In the incubation medium, the amount of [^{3}H]AA in the neutral lipids and phospholipids was similar: [^{3}H]AA in the phospholipids was equally distributed between PC and PE and in the neutral lipids was mainly in the MG and DG fractions (60–70%), with much smaller amounts in the TG fraction than in the intact cells. Very little (0·5%) [^{3}H]AA was detected in the PI, PS or CE fractions in the incubation medium.

Leucocyte stimulation

Stimulation of phagocytosis with latex beads in the presence of [^{14}C]LA and [^{3}H]AA did not significantly affect the intracellular lipid distribution pattern of these fatty acids. Zymosan particles (100–150 μg/ml) also did not significantly affect incorporation of [^{14}C]LA or [^{3}H]AA into TG, PC, PE or FFA fractions up to 24 h after stimulation.

[^{14}C]LA desaturation

The rate of conversion of 10 nmol (0·5 μCi) [^{14}C]LA to [^{14}C]-γ-linolenic acid and [^{14}C]dihomo-γ-linolenic acid (Δ6-desaturase and elongase products respectively) and then to [^{14}C]AA (Δ5-desaturase product) during 30 min incubation is shown in Table 2. The results are expressed both as a percentage of the total radioactivity recovered as products of [^{14}C]LA and as pmol [^{14}C]LA converted/10^7 cells. The total Δ6- and Δ5-desaturase products of [^{14}C]LA were greater in the leucocyte microsomal fraction than in the intact cells when expressed on the basis of percentage total radioactivity recovered (1·77 *v.* 2·71, $P < 0·01$).

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Table 2. *Percentage incorporation and distribution of ^{14}C-labelled linoleic acid ([^{14}C]LA) into rat and human leucocyte lipids after 1 h incubation using 2–3 × 10^7 cells (human) or 4–5 × 10^6 cells (rat)*

(Mean values with their standard errors for five determinations)

	Human		Rat	
	Mean	SE	Mean	SE
Percentage [^{14}C]LA in supernatant fraction	5·2	2·0	18·2**	5·1
Free fatty acid	9·4	1·4	73·0**	5·1
Monoglyceride–diglyceride	0·3	0·1	12·3**	5·2
Total phospholipid	23·1	1·8	9·9**	0·8
Triglyceride	63·6	3·3	0·6**	0·3

** $P < 0·01$.

Table 3. *Products of Δ5- and Δ6-desaturation of ^{14}C-labelled linoleic acid in human leucocytes, rat leucocytes and rat liver microsomal fractions*

(Mean values with their standard errors; results based on a 1 h incubation of intact leucocytes or a 30 min incubation of the microsomal fractions)

	[^{14}C]γLA and [^{14}C]DγLA		[^{14}C]AA		Total products	
	Mean	SE	Mean	SE	Mean	SE
Human						
Intact leucocyte (*n* 6):						
Percentage radioactivity	0·72	0·10	1·05	0·11	1·77	0·12
pmol/h per 10^7 cells	9·05	1·25	13·5	1·84	22·6	1·71
Leucocyte microsomal fraction (*n* 3):						
Percentage radioactivity	1·01	0·16	1·70	0·15	2·71	0·24
pmol/h per 10^7 cells	9·16	1·31	15·7	1·64	24·9	1·57
Rat						
Intact leucocyte (*n* 6):						
Percentage radioactivity	1·13*	0·23	1·51	0·40	2·64**	0·31
pmol/h per 10^7 cells	79·4**	4·62	107·9**	8·19	186·3**	9·28
Liver microsomal fraction (*n* 2):						
Percentage radioactivity	1·62	0·31	10·2‡	0·87	11·8‡	0·98

[^{14}C]γLA, [^{14}C]-γ-linolenic acid; [^{14}C]DγLA, [^{14}C]dihomo-γ-linolenic acid; [^{14}C]AA, [^{14}C]arachidonic acid.
Mean values were significantly different from those of human leucocytes: * $P < 0·05$, ** $P < 0·01$.
Mean values were significantly different from those of intact human or rat leucocytes: † $P < 0·05$, ‡ $P < 0·01$.

Comparison with rat leucocytes

Table 2 illustrates the differences in the distribution of [^{14}C]LA into the leucocyte lipids in rats and humans. After 1 h incubation, 73% of the [^{14}C]LA still remained as the FFA in the rat leucocytes, compared with only 9% in the human leucocytes. Differences in the incorporation of [^{14}C]LA into phospholipids (9 *v.* 26% in humans) and the MG–DG fraction (12 *v.* 1% in humans) also occurred, but the major difference was in the TG fraction:

< 1% of the [^{14}C]LA was incorporated into TG in the rat leucocytes whereas in the human leucocytes it was 60%.

Table 3 illustrates the differences of desaturation of [^{14}C]LA by leucocytes from man and the rat. The intact rat leucocytes had an eightfold greater activity of Δ5- and Δ6-desaturases combined than did human leucocytes (measured on the basis of pmol [^{14}C]LA converted/10^7 cells). Both rat and human leucocytes were less capable of desaturating [^{14}C]LA than was rat liver ($P < 0{\cdot}001$).

DISCUSSION

Our observtions suggest that the human peripheral blood leucocyte may be a useful cell in which to study EFA metabolism. Both [^{14}C]LA and [^{3}H]AA were rapidly incorporated into TG and PC lipid pools whereas, in the rat, the majority of these labelled fatty acids remained as the free acids. These results give a possible explanation for the apparent difference in desaturation of [^{14}C]LA between humans and the rat, which we have observed. If this difference (Table 3) is a real one, it may be linked to a difference in the rate of uptake of [^{14}C]LA into the leucocyte lipids and not just to the rate of conversion of LA to its desaturase products (Table 2). In rat leucocytes, > 60% of the [^{14}C]LA remained as the FFA for at least 1 h whereas, in human leucocytes, the intracellular pool of [^{14}C]LA only reached 30%, and after 1 h was < 10%. Hence the availability of free LA to the desaturase enzyme may be significantly less in the human leucocytes.

Our results also suggest that the TG pool is an important intermediate pool in the incorporation of EFA into human leucocyte cellular lipids. It was the major pool of [^{14}C]LA and [^{3}H]AA accumulation for as long as 9 h (Fig. 2). In addition, since the FFA pool of [^{14}C]LA and [^{3}H]AA was < 10% of the total after 30 min, the TG pool may be the short-term precursor pool of these fatty acids for both desaturases and PG synthesis respectively. These results contrast with those in platelets in which AA accumulates almost exclusively in phospholipids (Bills *et al.* 1977). This metabolic difference with the leucocytes suggests that the functional difference between these cells may be linked to a difference in their EFA metabolism.

Mouse periotoneal macrophages (Bonney *et al.* 1978, 1981; Scott *et al.* 1980) also esterify AA into phospholipids much more rapidly than do human peripheral blood leucocytes. However, rabbit peritoneal macrophages are similar to human peripheral blood leucocytes in their metabolism of LA (Elsbach, 1963, 1964; Cook *et al.* 1983). In fact, the 24 h time-course of [^{14}C]LA incorporation into rabbit peritoneal macrophages is virtually identical to that in human peripheral blood leucocytes. Since EFA metabolism in human tissues appears to be more like that in the rabbit and guinea-pig than that in the rat or mouse (Stone *et al.* 1979) EFA studies of relevance to man might therefore be more suitably conducted using the rabbit or guinea-pig.

We have confirmed previous reports that production of PGE_2 and $PGF_{2\alpha}$ by human peripheral blood leucocytes is low and is substantially lower than production by tissue macrophages (Morley, 1981). The low production of PG by peripheral blood leucocytes may be related to the high incorporation of AA into the TG rather than the phospholipid pool. This contrasts with tissue macrophages which readily produce PG but also incorporate AA into phospholipids. The rapid and long-term uptake of AA into TG by peripheral blood leucocytes may therefore be a protective mechanism preventing rapid release of AA for conversion to PG or leukotrienes. Our results also confirm that of Elsbach (1972) and suggest that peripheral blood leucocytes do not alter their uptake of EFA in response to phagocytic stimuli such as latex beads or zymosan.

Our time-course studies of [^{3}H]AA metabolism to PGE_2 and $PGF_{2\alpha}$ (Figs. 3 and 4) did not establish which pool was the source of free [^{3}H]AA for the cyclo-oxygenase enzyme

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under physiological conditions. This was because the amount of [^{3}H]PGE$_2$ and [^{3}H]PGF$_{2\alpha}$ secreted by the leucocytes in 1 h was only 2% of the total intracellular radioactivity, although it accounted for 25–30% of the radioactivity in the incubation medium. A 2% change in the amount of radioactivity associated with any of the lipid fractions was within the standard error of the results.

We conclude that the peripheral blood leucocyte may be valuable in the study of EFA metabolism in intact human cells. The possibility that the zinc status of the leucocytes may influence the lipid distribution profile of EFA in the leucocytes, as previously demonstrated in the rat (Cunnane & Huang, 1982), remains to be elucidated. The rapid uptake and distribution of EFA into four main leucocyte lipid fractions and the ease of sampling and assay should facilitate identification of defects in EFA metabolism in human diseases.

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