REVIEW ARTICLE

How is the level of free arachidonic acid controlled in mammalian cells?

Robin F. IRVINE

Department of Biochemistry, A.R.C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, U.K.

Introduction

Over the last 25 years a considerable research effort has concentrated on the eicosanoids, and great progress has been made in the elucidation of the chemical structure of these compounds, of their physiological roles, and of the ways in which their synthesis is controlled. It is on the last of these that this Review will concentrate, for it is now established that in most tissues the synthesis of eicosanoids is limited by the availability of their common precursor, free arachidonic acid, which must be liberated from esterified stores in complex lipids (van Dorp *et al.*, 1964; Bergström *et al.*, 1964; Vogt *et al.*, 1966; Lands & Samuelsson, 1968; Vonkeman & van Dorp, 1968). Control of the subsequent metabolism of the free acid will not be discussed here, nor will the original derivation of arachidonate from dietary sources (reviewed by Willis, 1981). The purposes of this Review are twofold: firstly, it is useful to summarize briefly the present state of knowledge in order to help newcomers in the field; secondly, and more importantly, in order to move forward both conceptually and experimentally it is necessary to take a critical look at the complexities and problems associated with studying arachidonate liberation.

Liberation of arachidonate, as defined here, must involve hydrolysis of an ester bond i.e. lipase activity. The three essential questions concerning the control of arachidonate availability are therefore:

- (1) Which lipases are involved?
- (2) How are they controlled?
- (3) Which substrates are hydrolysed?

Definitions: Arachidonic acid is 5,8,11,14-icosatetraenoic acid. Eicosanoid is the term introduced by Corey *et al.* (1980) to cover all products of arachidonate metabolism, whether by the cyclo-oxygenase pathway (prostaglandins, endoperoxides, thromboxanes, etc.) or the lipoxygenase pathway (hydroxyicosatetraenoic acids, leukotrienes, etc.). The term 'liberation' of arachidonate will be employed to mean solely the hydrolytic cleavage of the free acid from its esterified form. Increases in the level of the free acid in the extracellular space will be called 'release', though the choice of this word implies no assumption as to whether the passage of arachidonate across the plasma membrane occurs by active transport or passive diffusion.

Neutral lipids

Generally, triacylglycerol (the principal neutral storage lipid) is less rich in arachidonoyl moieties than are phospholipids. Only in tissues where the mass of neutral lipids matches or exceeds that of phospholipids, such as adrenal cortex or adipose tissue, might they be expected to make a significant contribution to the free arachidonate pool (Christ & Nugteren, 1970; Vahouny *et al.*, 1980). In other tissues, the contribution of neutral lipids can be difficult to assess accurately. For example, in rabbit polymorphonuclear leucocytes, Kaplan-Harris & Elsbach (1980) could not quantify the extent to which neutral lipids were contributing to arachidonate release because even a small (unquantifiable) phospholipid breakdown would have exceeded the arachidonate loss from neutral lipids.

However, even in tissues where storage lipids are comparatively scarce, such as brain, there may be a high turnover of their arachidonoyl moieties, at least as judged by isotopic methods (McDonald *et al.*, 1975), so neutral lipid lipases may yet play an important role, undefined as yet, in arachidonate liberation. The enzymic regulation of triacylglycerol metabolism has been adequately documented elsewhere; the control of a hormone-sensitive lipase by cyclic AMP (Hales *et al.*, 1978) may be supplemented by calcium regulatory mechanisms (Wise & Jungas, 1978) perhaps through the action of α -adrenergic receptors (Jones & Michell, 1978). In tissues which lack this hormone-sensitive lipase, however, it is not yet clear whether the lysosomal triacylglycerol lipase is (Debeer *et al.*, 1979; Groener & Knauer, 1981) or is not (Hülsmann *et al.*, 1981), depending perhaps on the tissue, the only intracellular enzyme hydrolysing these lipids.

Phospholipids

The bulk of the arachidonate in mammalian cells is esterified in the fatty acyl chains of glycerophospholipids, almost exclusively in the 2-acyl position (Fig. 1). This has led to suggestions that phospholipases are responsible for controlling free arachidonate levels (Vogt *et al.*, 1966; Lands & Samuelsson, 1968; Vonkeman & van Dorp, 1968; Van den Bosch, 1980) and this concept is now

Fig. 1. Deacylation and reacylation of glycerophospholipids

The phospholipid is diagrammatically represented; thus P is a phosphate group, X is a base (choline in phosphatidylcholine, ethanolamine in phosphatidylethanolamine, etc.) and R_1 and R_2 are the fatty acyl chains. More detailed structures of phospholipids and their routes of biosynthesis can be found in standard biochemical texts. R_2 is where most of the arachidonate in mammalian cells is esterified.

generally accepted. However, the free fatty acid level in a tissue actually represents a balance between the liberation of the acid by hydrolysis and its re-esterification into complex lipids by acyl transferases.

Acyltransferases and their role in controlling free arachidonate levels

Arachidonate is unusual amongst the major fatty acids in that the level of the free acid in the cytoplasm (Bills *et al.*, 1977) and plasma (Hagenfeldt, 1975) is low, disproportionately so compared with its esterified levels. Following the pioneering work of Lands and his colleagues (Lands, 1979) it has become clear that arachidonate is not incorporated during synthesis *de novo* of glycerophospholipids (i.e. by acylation of glycerol-3-phosphate or lysophosphatidic acid to yield phosphatidic acid), but it enters at a later stage, by acylation of a glycerolysophospholipid such as lysophosphatidylcholine (Fig. 1; see Van den Bosch *et al.*, 1972, for a review). This reaction is catalysed by distinct acyl transferase(s) with different specificities from those which synthesize phosphatidic acid (Hill & Lands, 1968; Yamashita *et al.*, 1973, 1975). For long chain fatty acids there is probably only one acyl-CoA synthetase and this enzyme has no pronounced specificity for any fatty acid (Normann *et al.*, 1981; see Lands, 1979 for a review). The low level of arachidonic acid in the cytosol is therefore likely to

be a reflection of the activity of the enzyme(s) that transfer the arachidonate from arachidonoyl-CoA to phospholipids.

The degree to which acyl-CoA:1-acylglycerol-3-phosphoglyceride *O*-acyl transferase (EC 2.3.1.23) favours arachidonoyl-CoA varies markedly depending on the tissue employed, and the conditions under which the enzyme is assayed (compare Hill & Lands, 1968; Baker & Thompson, 1973; Ferber & Resch, 1973; Yamashita *et al.*, 1973, 1975; Colard *et al.*, 1980). For example, using a saturating concentration of lysophosphatidylcholine, Ferber & Resch (1973) found the apparent K_m (the term 'apparent' K_m is used because exact concentrations of lipid substrates are of questionable significance above the critical micellar concentration) of the lymphocyte enzyme to be $0.5 \mu\text{M}$ for arachidonoyl-CoA and $9 \mu\text{M}$ for oleoyl-CoA. Hasegawa-Sakai & Ohno (1980) confirmed this specificity, but found that the preference for arachidonate was less pronounced when endogenous lysophospholipids were used as substrates.

Despite these quantitative discrepancies *in vitro*, it seems likely that the low level of free arachidonate in the cytosol and plasma can be accounted for by a high affinity for arachidonoyl-CoA of the lysophosphoglyceride acyltransferase(s) *in vivo*. This preference might be partly the result of the existence of separate acyl transferase(s) for arachidonate, though that is not clearly established (Jezyk & Lands, 1968; Hasegawa-Sakai & Ohno, 1980). The specificity for arachidonoyl-CoA is more pronounced if lysophosphatidylinositol is an acceptor (Baker & Thompson, 1973; Holub, 1976), and has been used to explain the very high arachidonate content of phosphoinositides (see White, 1973); this may also be due to a separate enzyme. The high arachidonate level in phosphatidylethanolamine and its plasmalogen equivalent (Marcus *et al.*, 1969; White, 1973) suggests that acyl transfer to ethanolamine lysophospholipids might also favour arachidonoyl-CoA.

The data of Lands & Merkel (1963) serve to summarize the likely mechanism by which free arachidonate levels are kept low in the cell. These authors described a balance for a given acyl-CoA between two reactions, the release of the free fatty acid by acyl-CoA hydrolase, and its esterification into phospholipids by acyl-CoA transferase. This balance depended on which acyl-CoA was used, and increasingly favoured esterification with more unsaturated fatty acids. Though arachidonoyl-CoA was not studied, it probably represents an extreme case of this phenomenon, whereby acyltransferase is very much favoured over acylhydrolase and also predominates over phospholipase activity. This in turn prompts the question of whether changes in the rate of this acylation reaction (rather than the rate of

hydrolysis of phospholipids) are what control arachidonate availability in stimulated tissues.

In the elegant experiments of Isakson *et al.* (1977) on the ureter-obstructed kidney, free arachidonate release could only be detected in the perfusate if an albumin 'trap' was added to bind arachidonate and thus to reduce its re-esterification. Others have used a similar technique, for example Hong & Levine (1976b). Isakson *et al.* (1977) employed radiolabelled arachidonate; it should be noted here that Schwartzman & Raz (1979) conducted very similar experiments with the same tissue, but did not use isotopes, and obtained opposite results (in the presence of albumin the recovery of all fatty acids except arachidonate was enhanced). The reason for this discrepancy is not clear. Isakson *et al.* (1977) argued that the stimulation by bradykinin of arachidonate release in their experiments must be due to stimulated hydrolysis (rather than to a decrease in esterification), because they could not detect the stimulated release in the absence of albumin. However, an albumin 'trap' is only a tool for quantifying extracellular arachidonate release, and it cannot provide information about the mechanism of the intracellular liberation. It is possible that bradykinin inhibited re-esterification sufficiently for arachidonate to be available to cyclo-oxygenase, or to escape from the cell to be trapped by albumin, but not sufficiently for it to escape from the vascular bed of the kidney if no albumin is present.

Kröner *et al.* (1981) could observe arachidonate liberation in bone marrow macrophages (with or without albumin present) only if acylation was inhibited by various agents. This led them to suggest that in those cells acylation is the factor that controls free arachidonate levels. These cells are unusual in that no arachidonate liberation was detectable unless their metabolism was altered by inhibitory compounds; furthermore no stimulation of liberation by a physiological effector was studied. Nevertheless, the thesis that a decrease in the reacylation rate of arachidonate could cause a rapid rise in the free levels of this fatty acid bears serious consideration, at least in some tissues. Morita & Murota (1980) observed that ATP levels could markedly influence the fate of arachidonate in liver homogenates, suggesting that cellular ATP levels could influence the liberation/reacylation balance. In mitochondria incubated *in vitro* this is clearly so (Parce *et al.*, 1978) and again, re-esterification is probably the rate-limiting factor in fatty acid liberation.

However, although in resting tissues acylation may control the overall levels of arachidonate in the cell (in equilibrium with acyl-CoA hydrolase and phospholipases), an inhibition of this process cannot account for stimulated arachidonate liberation in all tissues. For example, a stimulation of acyl transfer accompanies increased arachidonate liberation in

blood platelets aggregating in response to thrombin (Rittenhouse-Simmons *et al.*, 1976) and in mitogen-stimulated lymphocytes (Ferber & Resch, 1973). In many, and probably most, tissues, stimulation of arachidonate liberation is most likely to be controlled by changes in phospholipase activity (Vogt *et al.*, 1966; Lands & Samuelsson, 1968; Vonkeman & van Dorp, 1968).

Phospholipases

With the exception of phosphatidylinositol (discussed subsequently) the major route of phospholipid degradation in mammals is by deacylation catalysed by the action of phospholipase A₁ or A₂ activities (Fig. 1). The remaining fatty acid is removed from the lysophospholipid so formed by a lysophospholipase (phospholipase B). To answer questions (1) and (2) posed at the beginning of this Review, a survey of intracellular phospholipase A activities and their control mechanisms is necessary. Fortunately, this field has recently been excellently reviewed by Van den Bosch (1980), and only some updating and extension of those areas relevant to arachidonate liberation are necessary here. The first of these areas concerns the specificity of the phospholipases liberating arachidonate.

Do arachidonoyl-specific lipases exist?

The original evidence for this idea came from the pioneering work of Bills *et al.* (1977), studying fatty acid liberation by platelets aggregating in response to thrombin, and of Hsueh *et al.* (1977) measuring fatty acid release from the perfused heart or kidney stimulated by bradykinin; both these groups discovered that if the liberation or release of radioactive arachidonate was compared with that of other 2-acyl fatty acids, a pronounced specificity for arachidonate was observed. These observations have been confirmed by others in other tissues, for example 3T3 cells, (Lindgren *et al.*, 1978; Hong & Deykin, 1979; Shier, 1980), lymphocytes (Parker *et al.*, 1979) and mouse peritoneal macrophages (Bonney *et al.*, 1978; Scott *et al.*, 1980).

There are, however, several problems in interpreting these data in terms of specific liberation of arachidonate. For example, a puzzling aspect of the experiments on platelets (Bills *et al.*, 1977) is that the liberation of free arachidonic acid showed a 15-fold specificity over 8,11,14-icosatrienoic acid, whereas in other tissues the trienoic acid, along with arachidonate, showed specificity of release over other fatty acids (Lindgren *et al.*, 1978; Hong & Deykin, 1979). One reason for this apparent anomaly may be that, whereas the last two named groups were measuring release of radiolabel from the cell (i.e. into the incubation medium), Bills *et al.* (1977) were measuring loss of label from phospholipids (liberation as defined in this Review). The low

concentration of cytosolic free arachidonate (see above) could mean that liberation of a small mass of arachidonate (or icosatrienoic acid, generally a minor fatty acid and therefore also low in tissues) may be easily detectable above the low background, whereas with other fatty acids liberation of a similar mass may not represent a significant proportion of the total. Additionally, we do not yet know the pattern and mechanism of incorporation of radio-labelled icosatrienoic acid (as compared with arachidonate) into phospholipids, so the relative specificity of the liberation of radiolabel in experiments using these two fatty acids is not easy to assess. This difficulty applies also to linoleate and oleate, the other two acids employed by Bills *et al.* (1977): the fact that radioactive arachidonate may be incorporated by a different acyltransferase (see above), quite possibly in a different part of the cell (see below), makes the greater percentage loss of arachidonate label from phospholipids difficult to interpret.

Further evidence for specificity of arachidonate release in the perfused kidney came from the data of Hsueh *et al.* (1977) who observed an apparent dichotomy between 'specific' release of arachidonate stimulated by bradykinin, and 'non-specific' release of all fatty acids caused by ischaemia, a dichotomy that has been observed in other tissues (Hong & Deykin, 1979; Kröner *et al.*, 1981). This 'non-specific' release could, however, be explained either by a greater contribution of fatty acids from triacylglycerol to the 'non-specific' release (confirmed in bone marrow macrophages by Kröner *et al.*, 1981), or by hydrolysis of phospholipid pools not preferentially labelled by radioactive arachidonate; when Parker *et al.* (1979) measured the release of fatty acids from lymphocytes by absolute mass measurements, the specificity of arachidonate release was considerably reduced from that observed with radiolabelling techniques, which supports the possibility that such preferentially labelled pools could exist (discussed further below). In summary, although data from experiments with radiolabelled fatty acids appear to provide evidence for a strong preference for the liberation of arachidonate, they are open to other interpretations which cannot be eliminated as yet.

Broekman *et al.* (1981) measured the absolute mass of fatty acids liberated in the human platelet in response to thrombin, instead of employing radiolabel techniques. They showed that the liberation of fatty acids occurred in roughly the proportion expected if phosphatidylethanolamine were the main substrate (Broekman *et al.*, 1980), as it is when isolated platelet membranes are incubated *in vitro* (Jesse & Cohen, 1976). However, the determination of whether arachidonate is liberated faster than would be expected from its esterified levels is

complicated because a substantial proportion is further metabolized to eicosanoids. For example, we have found that in platelets 10 s after stimulation only 20–25% of [^3H]arachidonate liberated was still the free acid, the rest having been converted to products of cyclo-oxygenase and lipoxygenase (R. F. Irvine, A. J. Letcher & R. M. C. Dawson, unpublished work). If the amounts of free arachidonate measured by Broekman *et al.* (1981) must be multiplied by four or five, some evidence for preferential liberation of that fatty acid may still exist.

Schwartzman & Raz (1979) measured non-radioactive fatty acid release from the perfused ureter-blocked kidney, and observed a relatively small release of arachidonate compared with the other common fatty acids. This suggests, incidentally, that there is probably no preferential transfer of arachidonate out of the cell, though this possibility has not been investigated fully. As with the isotopic experiments of Hsueh *et al.* (1977), bradykinin caused a large percentage increase in the release of only arachidonate. However, because of the much higher overall levels of the other fatty acids, a similar mass increase in these would probably have been undetectable above experimental error.

In conclusion, data from experiments measuring the mass liberation (or release) of fatty acids, like those using radiolabelled acids, have yet to produce unequivocal evidence in favour of a specific liberation of arachidonate. However, as the verdict is still open, some consideration must be given to possible mechanisms of phospholipase specificity.

Specificity of phospholipases

As yet no phospholipase A has exhibited any pronounced specificity for arachidonoyl groups *in vitro*, though Ohkazaki *et al.* (1978) did find some preferential arachidonoylphosphatidylethanolamine hydrolysis in foetal membranes. The possible means by which a phospholipase not specific *in vitro* could be so *in vivo* have been discussed by Van den Bosch (1980) [see also Dawson (1973) for a general discussion on the pitfalls of assessing phospholipase specificity]; only one point needs expansion here. Perret *et al.* (1979) digested the outer half of the bilayer of the plasma membrane of intact platelets with a snake venom phospholipase A₂, and found that much less than the expected arachidonate (expressed as a percentage of the total in the cell) was liberated. From these data they concluded that 90% of the plasma membrane's arachidonoyl residues were on the inner layer and suggested this localization as a reason for the specificity of arachidonate liberation. However, they did not produce control data (% of the cell's total esterified level liberated) for any other fatty acid. If arachidonate liberated is expressed as a percentage

of the total free fatty acids liberated (Perret *et al.*, 1979, Table 1), it is present in only slightly less than the expected amount assuming no asymmetric distribution. The same is true for oleate, the other major 2-acyl fatty acid. Probably the only unusual thing about the outer half of the membrane is its high proportion of disaturated phospholipids, which Perret *et al.* (1979) noted. There is no clear evidence for a gross distortion of the ratio of arachidonate to other unsaturated fatty acids in the inner bilayer. Arachidonate esterified in phosphatidylethanolamine is symmetrically distributed across this membrane (Schick *et al.*, 1981) and this phospholipid is likely to be the major early source of fatty acids when platelets are aggregated by thrombin (Blackwell *et al.*, 1977; Broekman *et al.*, 1980, 1981). If there is any pronounced specificity of arachidonate liberation over other fatty acids, its origin is as yet unexplained.

Subcellular location of arachidonate liberation

The subcellular distributions of the various phospholipases A are reviewed by Van den Bosch (1980), who remarks that we have almost certainly not found them all yet, and the subcellular location of those known is sometimes contentious. Furthermore, it is not always certain that it is a phospholipase A₂ we are seeking. In a foregoing section, the difficulties in assessing whether arachidonate is preferentially (compared with other fatty acids) liberated from phospholipids were discussed. This argument can be extended to the saturated fatty acids (stearate and palmitate) which are primarily esterified in the 1-acyl position. That is, if no detectable saturated fatty acids are liberated or released in a tissue where arachidonate release or liberation is demonstrable, we cannot be certain that a phospholipase A₂ is involved. A phospholipase A₁ followed by lysophospholipase (Fig. 1), an active and soluble enzyme, at least in liver (Moonen *et al.*, 1977), could effectively liberate arachidonate. In some tissues, however, arachidonate liberation parallels lysophospholipid accumulation, for example, the platelet (Broekman *et al.*, 1980; McKean *et al.*, 1981) or fibroblasts (Hong & Deykin, 1981), and here the involvement of a phospholipase A₂ is more likely.

The subcellular site of the cyclo-oxygenase or the lipoxygenase that metabolize the free arachidonate is also unclear. The latter enzyme is probably soluble (Narumiya *et al.*, 1981). Although cyclo-oxygenase has been assumed to be in the endoplasmic reticulum, the membrane fractions involved were not always well characterized (Kataoka *et al.*, 1967; Bohman & Larsson, 1975); for example, depending on the tissue, the absence of plasma membrane 'sheets' does not prove an absence of this membrane in a microsomal preparation (Wisher & Evans, 1975). Erman & Raz (1981) recently presented

more convincing evidence that in the kidney both plasma membrane and mitochondrial fractions contain cyclo-oxygenase which cannot be accounted for by contamination with endoplasmic reticulum. In platelets cyclo-oxygenase seems to be predominantly associated with the dense tubular system (Gerrard *et al.*, 1976), which makes the recent description of a phospholipase A₂ (Lagarde *et al.*, 1981) possibly in the same location, of interest (see also Erman & Raz, 1981).

The subcellular location of the acyltransferase(s) (reviewed by Van den Bosch *et al.*, 1972) is also not certain, but is of some significance when examining data from experiments involving radiolabelled arachidonate (see above). Kaulen *et al.* (1970) maintained that, in liver, arachidonoyl-CoA transferase is absent from the plasma membrane, though the rat liver plasma membrane is so heterogeneous (Wisher & Evans, 1975) that this warrants further investigation. Oleoyl-CoA transferase, which may be a different enzyme (see above) is also in the endoplasmic reticulum in liver and brain (Eibl *et al.*, 1969; Fisher & Rowe, 1980) but its presence in the plasma membrane may depend on the tissue. For example, it is in that membrane in lymphocytes (Ferber & Resch, 1973) and possibly in liver (Stein *et al.*, 1968; Yousef *et al.*, 1977) but problems of heterogeneity in subcellular enzyme markers (evident from the data of Stein *et al.*, 1968; Wisher & Evans, 1975; Fisher & Rowe, 1980) make proof of its absence or presence in plasma membranes difficult. Similar problems will no doubt occur with acyl-CoA ligase; this enzyme is found in the endoplasmic reticulum and mitochondria (Lands, 1979; Normann *et al.*, 1981), but its distribution elsewhere in the cell has not been fully explored.

The subcellular location of arachidonate liberation and of its subsequent metabolism are important when considering that the two processes are often coupled. Several workers, using fibroblasts, kidneys and macrophages (Hong & Levine, 1976b; Schwartzman & Raz, 1979; Needleman *et al.*, 1979; Schremmer *et al.*, 1979; Schwartzman *et al.*, 1981; Hsueh *et al.*, 1981), have shown that if eicosanoid release is stimulated over basal levels, or it is increased by two different agonists, the coupling efficiency (arachidonate/eicosanoid ratio) and/or the proportion of different eicosanoids formed is altered. In the examples employing a homogeneous population of cultured cells, the existence of different subcellular locations for stimulated arachidonate liberation is a likely explanation for these data. This in turn suggests the interesting possibility of several phospholipases liberating arachidonate, which are independently controlled (Hong & Levine, 1976b; Hsueh *et al.*, 1981).

In the kidney, the presence of several cell types within the organ complicates the data. Differences in

eicosanoid ratios synthesized between cortex and medulla (Whorton *et al.*, 1978; Needleman *et al.*, 1979; Schwartzman & Raz, 1981) or the predominance of the hormone-sensitive component of arachidonate release in the vascular tissue of the cortex (Hsueh & Needleman, 1978) are illustrations of this problem. Tissue heterogeneity is also the most likely explanation for the differential sensitivity of arachidonate release to various inhibitors, as documented by Blackwell *et al.* (1978) and Robinson & Hoult (1980).

Schwartzman *et al.* (1981) have distinguished a number of arachidonate pools in the kidney, and shown that radiolabelled arachidonate enters a pool from which it can be released by bradykinin only if turnover of that pool is stimulated by the agonist (i.e. radiolabelled arachidonate does not directly enter the hormone-sensitive pool). A distinct hormone-sensitive pool is further implied by tachyphylaxis in the kidney's response (Isakson *et al.*, 1977), and Jose & Seale (1979) showed similar pool compartmentalization phenomena in the perfused lung. Finally, unlabelled arachidonate perfused into the kidney prelabelled with radioactive arachidonate is not converted directly to prostaglandins, but enters an esterified pool first and displaces from there radiolabelled arachidonate which serves as a substrate for cyclo-oxygenase (Schwartzman *et al.*, 1981). A tight coupling between arachidonate liberation and cyclo-oxygenase is further suggested by the data of Schwartzman & Raz (1981).

However, the phenomenon of displacement of radiolabelled by unlabelled arachidonate is also suggested by the experiments on lymphocytes conducted by Parker *et al.* (1979), and indeed, most of the evidence for heterogeneity of arachidonate pools described in the previous paragraph can be found in cell cultures (Hong & Levine, 1976b; Schremmer *et al.*, 1979; Parker *et al.*, 1979). Here intracellular heterogeneity is the more likely explanation. These various arachidonate pools can be stable over several hours and they make interpretation of many experiments employing radiolabelled arachidonate difficult (as discussed above and below). However, they may also provide us with a tool for dissecting intracellular heterogeneity, and for exploring the possibly independent control mechanisms of the different phospholipases involved.

Control of phospholipase activity

This topic needs only a little addition to the extensive review by Van den Bosch (1980). There the regulation of phospholipases by conversion of zymogen forms, by non-enzymic proteins, by calcium, by cyclic AMP, by hormones, and by membrane structure are fully discussed. Three particular control mechanisms which have been

implicated in arachidonate liberation will be briefly expanded here.

The phospholipases about whose regulation we know least are those in the lysosome. Although the enzymes are well characterized (Waite *et al.*, 1976) and a role for lysosomes in eicosanoid release in some tissues is quite likely (Anderson *et al.*, 1971; Åkesson & Gustavii, 1975; Dawson & Irvine, 1978; Hsueh *et al.*, 1981), the mechanisms by which lysosomal activity is controlled are unknown at present.

Another difficult area is the role of calcium in controlling the activity of the phospholipases outside the lysosome. Most of these enzymes are calcium-dependent, but that does not necessarily mean they are calcium-controlled. The bulk of the evidence for the latter comes from the application of the calcium ionophore A23187 as a cell stimulant (Van den Bosch, 1980). This compound was first discovered as a toxin (Reed & Lardy, 1972) and it may be that it is continuing to act as one when it is used experimentally. It can liberate intracellular calcium stores (Pickett *et al.*, 1977) as well as stimulating the influx of extracellular calcium, but many workers have had problems in interpreting the results obtained because of secondary effects of the ionophore on cellular metabolism (as documented, for example, by Tamarit-Rodriguez *et al.*, 1978). Furthermore, an increase in cell calcium is likely to have some effects on phospholipid hydrolysis whether the increase results from application of an ionophore, from a breakdown of the plasma membrane's barrier properties induced by cell damage (for example, Chien *et al.*, 1978), or from increasing calcium concentrations outside the cell (Erman & Raz, 1979), but the relationship of these changes to those induced by physiological agonists is debatable. In the human platelet the radical difference between thrombin- and A23187-induced lipid changes have been well documented by Rittenhouse-Simmons (1981) and it seems likely that similar differences may become apparent when other systems are studied in more detail. In summary, although it is frequently assumed that a major (or the major) controlling factor in arachidonate liberation is the concentration of intracellular calcium, as yet there is no clear evidence that this is so *in vivo* (see also Van den Bosch, 1980).

Finally, phospholipid methylation has been proposed as another regulator of phospholipase A₂ activity, by Axelrod and co-workers (see Hirata & Axelrod, 1980, for a review). Arachidonic acid is liberated from a pool of phosphatidylcholine that labels to a higher specific activity with [³H]methionine than with [³H]choline in leucocytes and platelets (Hirata *et al.*, 1979; Kannagi *et al.*, 1980). This demonstrates a pool compartmentalization of the sort described above for radiolabelled ara-

chidonate, but its physiological function is unknown. It should be remembered in this context that phosphatidylcholine is probably not the major source of arachidonate during the early stages of platelet aggregation (Broekman *et al.*, 1980, 1981).

We do not yet know the role of lipid methylation in cell activation. This is not a topic to be discussed here; the considerable evidence for this hypothesis has been summarized by Hirata & Axelrod (1980), and some recent criticisms can be found in Vance & De Kruijff (1980) and Randon *et al.* (1981). As far as arachidonate liberation is concerned, it is important to emphasize that if phospholipid methylation does play a role in cell activation, a coincidence of methylation and arachidonate liberation is likely in tissues which do liberate arachidonate, and any effects of methylation inhibitors on this liberation (Crews *et al.*, 1980; Hirata *et al.*, 1980a) may be indirect. The same reservation applies to consideration of the possible role of calcium in regulating intracellular phospholipases, which was discussed briefly above; i.e. phospholipase stimulation may be a secondary consequence of a primary cell activation, rather than an integral part of that activation mechanism. Distinguishing between these two possibilities is not easy, and the principal means of doing so has been to apply phospholipase inhibitors, and to monitor the effect of these compounds on arachidonate liberation and on other cell functions. A brief, but critical, look at these inhibitors is therefore necessary.

Phospholipase inhibitors and their effect on arachidonate liberation

Compounds which inhibit phospholipases (usually the extracellular phospholipases of venoms or pancreatic secretions) can also inhibit arachidonate liberation in some tissues. To what extent soluble extracellular phospholipases are homologous with intracellular enzymes is at present an unknown quantity that requires further exploration. More importantly, it is central to the interpretation of the results of experiments employing these compounds that they are merely inhibiting phospholipases and nothing more; this may not always be so. For example, mepacrine (Blackwell *et al.*, 1977), dibutyl cyclic AMP (Minkes *et al.*, 1977), bromophenacyl bromide (Vargaftig, 1977), serine proteinase inhibitors (Feinstein *et al.*, 1977) and propanol and chlorpromazine (Vanderhoek & Feinstein, 1979) have each been shown to decrease liberation of free arachidonate in the platelet.

However, it is now well established that if platelets are aggregated by high doses of thrombin, although arachidonate liberation is stimulated, the aggregation is not dependent on the free arachidonate, nor on its metabolites (see Lapetina *et al.*, 1978, for references). Yet bromophenacyl bromide, serine

proteinase inhibitors and dibutyl cyclic AMP block thrombin-induced platelet aggregation; mepacrine or serine proteinase inhibitors, and propanolol or chlorpromazine, can block platelet aggregation in response to arachidonate and thromboxane A_2 respectively; and bromophenacyl bromide can prevent the conversion of arachidonate to prostaglandins in uterine homogenates (Blackwell *et al.*, 1977; Minkes *et al.*, 1977; Mitchell *et al.*, 1977; Vargaftig, 1977; Vanderhoek & Feinstein, 1979; Kosaki *et al.*, 1980). In short, all these compounds are doing more than just inhibiting arachidonate liberation.

Indomethacin, which was originally discovered as a cyclo-oxygenase inhibitor, can inhibit phospholipase A_2 in the test tube depending on the calcium concentration (Franson *et al.*, 1980). It can also inhibit the liberation of arachidonate in some tissues, but this may be caused partly by the re-esterification of free arachidonate (which indomethacin has prevented from being converted to other eicosanoid products) rather than by an inhibition of liberation (Hong & Levine, 1976b). The inhibition by this drug of diacylglycerol lipase has been correlated with the accumulation of diacylglycerol in the platelet (Rittenhouse-Simmons, 1980), but as in the same cell it also inhibits the release of arachidonate from phosphatidylcholine (Rittenhouse-Simmons, 1981) and its transfer to plasmalogen phosphatidylethanolamine (Rittenhouse-Simmons & Deykin, 1981), it is clearly pluripotent in its action (see also Northover, 1977; Kantor & Hampton, 1978). In summary, it could be that in these examples the platelet is providing us with a cautionary tale: that it is difficult to draw conclusions from inhibitors which are non-specific and acting on more than one site, about the role of an enzyme that they can inhibit in the test tube. Whether or not the range of phospholipase inhibitors recently described by Wallach & Brown (1981) also have other sites of action has yet to be established. If they do not (as the available data suggest), then they could prove to be valuable tools in investigating the phospholipases responsible for arachidonate liberation.

Effect of corticosteroids on arachidonate release

The pioneering work of Gryglewski *et al.* (1975) and Hong & Levine (1976a) first demonstrated that in responsive tissues the main effect of glucocorticoids on eicosanoid metabolism was to lower the availability of free arachidonate; this observation has been confirmed in many laboratories and is now generally accepted (Blackwell *et al.*, 1980). [For a review of other effects of corticosteroids on eicosanoid metabolism, see Skidmore (1982)]. Danon & Assouline (1978) first demonstrated that this effect required RNA and protein synthesis and thus that the steroid was probably acting through its

cytosolic receptor; again this has been confirmed by many others.

The elegant demonstration of a corticosteroid-induced protein in lung and macrophages that can inhibit phospholipase activity and arachidonate release (Flower & Blackwell, 1979; Carnuccio *et al.*, 1980) has been confirmed in neutrophils (Hirata *et al.*, 1980b). Macrophages react very rapidly to corticosteroids, and they may be unusual in that this protein (macroscortin) is not induced by corticosteroids (except as a later, secondary event) but rather that the steroids induce a protein-synthesis-dependent release of the stored protein (Blackwell *et al.*, 1980; Carnuccio *et al.*, 1981). Once the macroscortin is released, the macrophages are no longer responsive to dexamethasone until more protein is made; they are, however, responsive to added macroscortin (Carnuccio *et al.*, 1981). In other words, the macrophage cannot respond to its own macroscortin (which must therefore be sequestered in a cell compartment remote from its site of action), but is responsive to exogenous macroscortin. This suggests that, in the macrophage at least, the site of action is either at the plasma membrane or inside the lysosomal system. The site of the phospholipase activity assayed by Blackwell *et al.* (1978, 1980) is not clear. In their experiments [^{14}C]oleoyl-labelled phosphatidylcholine is perfused into the system (the lung) and the release of oleic acid is monitored; it is not known whether (or how) the phospholipid enters the cells, and so the site of hydrolysis is unknown.

Lipomodulin, the equivalent protein to macroscortin in neutrophils, (Hirata *et al.*, 1980b) has an M_r of 40 000 as against macroscortin's approx. 15 000, though recent work from Flower's laboratory (R. J. Flower, personal communication) suggests that the true molecular weight of macroscortin is about 40 000 but that smaller fragments of it retain some biological activity. Thus lipomodulin and macroscortin could well be the same protein. Hirata and co-workers (Hirata *et al.*, 1981; Hirata, 1981) have correlated the appearance of an antibody to lipomodulin with the incidence of rheumatic diseases, and postulated a phosphorylation-dephosphorylation cycle to control its activity.

Dexamethasone can also affect arachidonate availability in the long term by its effect on the enzymes making arachidonate from linoleate (Dumm *et al.*, 1979; Holloway & Margoulis, 1980). Possible effects on phospholipid methyltransferases and acyltransferases are also suggested by dexamethasone's effect on sphingomyelin and lysophosphatidylcholine levels in HeLa cells (Johnston *et al.*, 1980), and an increase in arachidonate esterification induced by this steroid has been suggested by Araki *et al.* (1981).

Finally, if a tissue exhibits a dichotomy of 'specific' versus 'non-specific' arachidonate

liberation (above) then it is generally the specific liberation that is blocked by corticosteroids (for example, MacMillan *et al.*, 1980). [There are some exceptions, such as the inhibition by dexamethasone of the release of fatty acids induced in BALB/3T3 cells by removing them from their substratum (Hong & Levine, 1976b)]. Triacylglycerol catabolism is probably not inhibited by dexamethasone; in fact, in adipose tissue corticosteroids have a potentiating or permissive effect on lipolysis (Goodman, 1970). It is therefore possible that, as mentioned above, in some tissues 'non-specific' fatty acid liberation involves a greater contribution from neutral lipids.

Relative contribution of the various phosphatidate esters to arachidonate release

A number of attempts have been made to examine this by preincubating a tissue with radioactive arachidonate, stimulating the liberation and seeing which phospholipid loses most label, e.g. in platelets (Bills *et al.*, 1977; Blackwell *et al.*, 1977; Rittenhouse-Simmons, 1979), macrophages (Hsueh *et al.*, 1979; Kröner *et al.*, 1981), lymphocytes (Parker *et al.*, 1979), BALB/3T3 cells (Hong & Deykin, 1979), DSDM $_1$ C $_1$ cells (Schremmer *et al.*, 1979), post-mortem brain (Marion & Wolfe, 1979) and exocrine pancreas (Marshall *et al.*, 1981). The conclusions from such experiments are subject to several reservations which must be borne in mind when comparing the various tissues and agonists.

Firstly, and most importantly, the existence of several distinct pools of arachidonate (discussed above) leads to difficulties in quantifying arachidonate liberation from measurements of radioactivity alone. In many tissues phosphatidylethanolamine and its plasmalogen derivative are rich in esterified arachidonate, yet in the platelet (Rittenhouse-Simmons & Deykin, 1981), synaptosomes (Yau & Sun, 1974) and macrophages (Scott *et al.*, 1980) both incorporate radiolabelled arachidonate very slowly. Consequently, comparing absolute mass measurements and radioactive data, Blackwell *et al.* (1977) found remarkable discrepancies between the two methods in determining the source of arachidonate liberated in rabbit platelets. Similar discrepancies can be found between the two techniques in human platelets (compare Bills *et al.*, 1977 and Broekman *et al.*, 1980, 1981). A second problem is that reacylation of deacylated phospholipids may obscure the contribution of a given phospholipid: accumulation of lysophospholipids (Broekman *et al.*, 1980, 1981; McKean *et al.*, 1981) merely reflects the degree to which reacylation is not keeping up with deacylation. Thirdly, in some tissues a particular phospholipid may take up so much [^3H] or [^{14}C]arachidonate that, even if it made the major contribution to the radiolabel liberated, this loss

would not be detectable over experimental error (for example, Marshall *et al.*, 1981).

Completing a balance sheet of arachidonate is essential when assessing the loss from phosphatidylinositol, as esterified arachidonate can remain in products of the catabolism of this phospholipid (see below and Fig. 2) so that what appears to be a large loss of arachidonate from phosphatidylinositol is actually balanced by an equal rise in arachidonoyl-phosphatidic acid and diacylglycerol (Fig. 2). In platelets, such analyses have shown that, despite the large breakdown of phosphatidylinositol in response to thrombin, it makes little net contribution to the arachidonate liberated (Rittenhouse-Simmons, 1979; Broekman *et al.*, 1980, 1981). [This applies at high thrombin doses. At low doses the relative contribution of phosphatidylinositol may be greater (Guichardant & Lagarde, 1980; Rittenhouse-Simmons & Deykin, 1981). However, the various platelet responses induced by thrombin or platelet-activating factor show different agonist dose-response curves (Minkes *et al.*, 1977; Shaw *et al.*, 1981) and phosphatidylinositol turnover may serve alternative (or additional) functions to arachidonate liberation (see Irvine *et al.*, 1982).] In summary, although we have a lot of data, we do not yet have a clear picture of which phospholipid fraction(s) contribute most of the arachidonate liberated in any tissues other than the platelet.

Mechanism by which arachidonate is liberated from phosphatidylinositol

Generally, the hydrolysis of this arachidonate-rich phospholipid [phosphatidylinositol is not rich in arachidonate in all tissues (White, 1973)] proceeds in all organisms other than fungi (see Irvine *et al.*, 1982) by a route different from other phospholipids. Phosphatidylinositol is hydrolysed by a specific phosphodiesterase (phospholipase C) to diacylglycerol and inositol phosphate or cyclic phosphate (Fig. 2; Dawson, 1959; Kemp *et al.*, 1961; Dawson *et al.*, 1971). The diacylglycerol is then either deacylated to complete the degradation (Cabot & Gatt, 1976, 1977; Mauco *et al.*, 1978) or recycled to phosphatidate and thence to phosphatidylinositol (Hokin, 1968; see Fig. 2); this recycling is called the phosphatidylinositol turnover cycle and the mechanisms of its control and its function have been the subject of considerable study (see Hokin, 1968; Michell, 1975; Irvine *et al.*, 1982, for reviews).

It is therefore of considerable interest that several reports have recently appeared of stimulated deacylation of phosphatidylinositol in tissues during the liberation of arachidonate (Schrey & Rubin, 1979; Rubin *et al.*, 1981a,b; Walsh *et al.*, 1981; Hong & Deykin, 1981). In all these cases the considerable evidence for direct deacylation (Fig. 1) of phosphatidylinositol has been well argued by the authors

concerned, and limitations of space preclude a summary of this evidence. The purpose of this section can therefore only be to summarize briefly some possible artefacts that may lead to misinterpretation.

Firstly, lack of accumulation of any intermediates of the phosphatidylinositol cycle (see Fig. 2) may simply mean that the cycle is occurring, but re-synthesis is able to keep up with degradation. Secondly, a lack of stimulated turnover of the phosphoinositol group (when it has been sought), as judged by isotope incorporation or a pulse chase, cannot be taken as clear evidence that the cycle is not operating, as pool sizes, compartmentalization and other problems may confuse the issue (discussed further by Irvine *et al.*, 1982). Thirdly, a specific stimulation of radiolabelled arachidonate incorporation into phosphatidylinositol might be expected if this phospholipid is turning over more rapidly than other phospholipids; phosphatidylinositol is frequently the phospholipid that shows the highest specific activity with radiolabelled arachidonate (see also the section on acyltransferases above). [To incorporate arachidonate it could be argued that phosphatidylinositol must first be deacylated (Baker & Thompson, 1973; Holub, 1976). However, the removal of the 2-acyl fatty acid might occur by reversal of acyl transfer (see the next section) or by an acyl exchange of the sort demonstrated in plant tissues (Stymie & Glad, 1981). Alternatively, a phospholipase A₂ may indeed deacylate the phosphatidylinositol, but the point of debate here is whether deacylation constitutes a significant component of the arachidonate liberation.] Fourthly, if deacylation induced by zymosan has occurred in neutrophils (Walsh *et al.*, 1981), it may be caused by a contaminating phospholipase activity in the zymosan (Kröner *et al.*, 1981). Fifthly, several of these studies employ A23187, and reservations about this ionophore have been discussed above; in the platelet it does stimulate deacylation of phosphatidylinositol, but during physiological stimulation (by thrombin) hydrolysis occurs by the classical (Fig. 2) pathway (Rittenhouse-Simmons, 1981). Finally, a specific liberation of arachidonate over stearate or palmitate (which are mostly incorporated into the 1-position of phospholipids) has been used as evidence for phospholipase A₂ activity, but this is subject to all the reservations discussed above concerning the demonstration of specificity using radiolabelled fatty acids.

Unequivocal evidence for deacylation of phosphatidylinositol has been found in transformed 3T3 cells, where the accumulation of lysophosphatidylinositol has been well documented (Hong & Deykin, 1981). It is interesting that glycerophosphoinositol (the final product of phosphatidylinositol deacyl-

Fig. 2. *The phosphatidylinositol cycle*

The phosphatidylinositol is represented diagrammatically, thus $\textcircled{\text{P}}$ is a phosphate group, and $\textcircled{\text{I}}$ is inositol. For a more detailed structure of this molecule and the metabolites thereof, see Irvine *et al.* (1982) Notes: (1) The order in which the acyl chains of diacylglycerol are removed is discussed by Chau & Tai (1981), but see also Cabot & Gatt (1976). (2) The phosphatidic acid in this cycle, being derived from phosphatidylinositol, is rich in stearate (R_1) and arachidonate (R_2). It is therefore a different (and probably distinct) pool from the phosphatidate which is an intermediate in glycerolipid synthesis *de novo*. This latter pool is formed by acylation of glycerol-3-phosphate, and, as discussed in the text, the acyltransferases responsible do not favour arachidonate (Yamashita *et al.*, 1975).

ation) is found in abnormally high levels in another transformed cell culture (Koch & Diring, 1974) suggesting perhaps an unusual metabolism of phosphatidylinositol in such cells. In summary, phosphatidylinositol can be deacylated in some tissues; what is difficult to assess is the relative importance of this route for liberating arachidonate in comparison with the usually predominant phosphodiesterase–diacylglycerol lipase pathway.

Finally, the other mammalian inositides, di- and tri-phosphatidylinositol are also arachidonoyl-rich (Baker & Thompson, 1972) and catabolized by phosphodiesterase or phosphomonoesterase action (Dawson & Thompson, 1964), but their role (if any) in arachidonate release, and the extent to which they may be deacylated, are unknown.

Other routes of arachidonate release

Liberation of arachidonate from the probable main sources (phosphatidylethanolamine and phosphatidylcholine) may not take place by direct hydrolysis, but by sulfolysis, a CoA-mediated transfer of arachidonate followed by hydrolysis of another lipid. This is really no more than reversal of the acyltransferase reaction (Fig. 1), which *in vitro* shows some specificity for arachidonate (Irvine & Dawson, 1979; Trotter & Ferber, 1981). This specificity is enough to account for the transfer of arachidonate between phospholipids observed in

erythrocytes (Bérezat *et al.*, 1978) and it is the probable mechanism of arachidonate transfer to plasmalogen phosphatidylethanolamine in the aggregating platelet (Rittenhouse-Simmons *et al.*, 1976; Rittenhouse-Simmons & Deykin, 1981). If direct arachidonoyl transfer (involving no phospholipase A_2) does occur *in vivo*, its function (if any) in arachidonate liberation is not clear at present.

It is unlikely that much of the arachidonoyl-CoA so formed is hydrolysed directly to liberate arachidonate (Kröner *et al.*, 1981) because this equilibrium seems to favour reacylation rather than liberation (as discussed above), so transfer to another lipid followed by hydrolysis is probably necessary if arachidonate is to be mobilized in this way. Transfer to phosphatidylinositol (Irvine & Dawson, 1979) seems unlikely, in the platelet at least, as there is no evidence for resynthesis in which an arachidonoyl-poor phosphatidylinositol accepts arachidonate (Broekman *et al.*, 1981, but see also Prescott & Majerus, 1981). Trotter & Ferber (1981) could not detect arachidonoyl transfer to phosphatidylinositol in mouse thymocytes; they could only find transfer from phosphatidylcholine to phosphatidylethanolamine (and not vice versa). These authors used endogenous arachidonoyl-labelled phospholipids, which are more likely to reflect the reaction *in vivo* than the experiments of Irvine & Dawson (1979) who employed exogenous

substrates. The data on arachidonate metabolism in the platelet are consistent with transfer from phosphatidylcholine to phosphatidylethanolamine (Broekman *et al.*, 1980; Rittenhouse-Simmons & Deykin, 1981), but, if this transfer does occur, its function (other than to replenish phosphatidylethanolamine with arachidonate) remains obscure.

Finally, Cuatrecasas and co-workers (Lapetina *et al.*, 1980, 1981; Billah *et al.*, 1981) have suggested a role for phosphatidic acid (derived initially from phosphatidylinositol, Fig. 2) as a receiver of transferred arachidonate, the arachidonate being released by a phosphatidate-specific phospholipase A_2 . These workers have presented some strong evidence for their hypothesis, though there are a few aspects that offer alternative interpretations. The rapid pulse of [^{14}C]arachidonate through phosphatidate (Lapetina *et al.*, 1980) is not consistent with the specificities of the known acyltransferases (see above) and it may alternatively reflect some reversal of the phosphatidylinositol synthesis pathway (Hokin-Neaverson *et al.*, 1977) from a pool of arachidonoyl-phosphatidylinositol that is turning over very quickly; Holub & Kuksis (1971) presented data consistent with the idea that arachidonate enters phosphatidylinositol in a pool where the phosphoinositol moiety is turning over rapidly.

Interpretation of the data derived from experiments employing mepacrine (Lapetina *et al.*, 1981) are complicated by the pluripotent effect of this inhibitor (see above) [phosphatidylinositol phosphodiesterase, for example, is inhibited by mepacrine (R. F. Irvine, A. J. Letcher & R. M. C. Dawson, unpublished), though not if deoxycholate is included in the assay (as it was by Billah *et al.*, 1981); surface-active inhibitors require a planar surface to exert their effect (Dawson *et al.*, 1980), and deoxycholate destroys this surface], and the apparent specificity of the phospholipase A_2 which hydrolyses phosphatidate (Billah *et al.*, 1981) is also open to discussion. Assessment of phospholipase specificity is fraught with many difficulties (Dawson, 1973), and in this particular case the problems are compounded by the fact that the enzyme in question is membrane-bound, and so hydrolyses phospholipids in a membrane. Unless details are known of the intramembranous concentrations which result from the external addition of different phospholipid substrates, it is difficult to be sure whether such an enzyme really favours one phospholipid over another. The high calcium-binding efficiency of phosphatidate could account for the low calcium requirement of the phospholipase A_2 with this particular substrate, and the apparently high rate of hydrolysis of phosphatidate in horse platelets compared with the human platelet phospholipase A_2 may be simply a species difference; rat platelets (Etienne *et al.*, 1979) have a (non-specific) phospholipase A_2

of similar activity to that found by Billah *et al.* (1981) in horse platelets.

Despite these difficulties in interpretation, the hypothesis that phosphatidate serves as a pivot in arachidonate release has considerable experimental evidence in its favour. If it can be extended to other tissues, then the effect of corticosteroids on the different stages of this pathway will be an interesting area for exploration.

Conclusions and speculations

The study of phospholipases proceeds apace; although many gaps in our knowledge remain, we are now beginning to build up a picture of what enzymes there are and how they might be controlled (Van den Bosch, 1980). Phospholipases have more functions than just liberating arachidonate, however, and which particular enzymes and control mechanisms are most intimately linked with arachidonate liberation is not well understood. The existence of considerable inter- and intracellular heterogeneity in the pools into which radiolabelled arachidonate can be incorporated may be a two-edged weapon; it provides us with a possible means of answering these questions (which enzymes and which control mechanisms), but it also makes interpretation of the results of many other experiments extremely difficult, as the above discussions have indicated.

With regard to control mechanisms, the most intriguing possibilities lie in the effect of membrane structure on the activity of phospholipases (Vogt, 1978; Van den Bosch, 1980). Recently, some understanding has emerged of the effect of membrane structure on the phosphatidylinositol-specific phosphodiesterase (phospholipase C: Irvine *et al.*, 1979; Dawson *et al.*, 1980; Hirasawa *et al.*, 1981) which is an enzyme depending on, but probably not controlled by, calcium; the major control of its activity is likely to be the physical form and composition of the lipids in the membrane that it hydrolyses. The possibility that intracellular phospholipases A may be controlled on similar principles is one that bears serious consideration.

Another interesting topic from the pharmacological point of view is the way in which acyltransferases could be manipulated therapeutically. Esterification of arachidonate plays an important role in maintaining intracellular levels, and acyl transfer may also be involved in the mobilization of arachidonate reserves, as discussed in the preceding section. Compounds which can modify these enzymes [which include pharmacologically active agents such as Δ^9 -tetrahydrocannabinol (Greenberg & Mellors, 1978)] may have potent effects on eicosanoid release.

Whether or not arachidonate liberation can be specific for this fatty acid is contentious at present; if

it is, then the mechanism of this specificity is one of the principal enigmas of this field. Another enigma is the role of phosphoinositides and plasmalogens. Both these lipid classes are very rich in arachidonate, but the control and mechanism of their catabolism is not clearly defined; plasmalogen catabolism in particular is a neglected area compared with most aspects of lipid research. There has been particularly exciting progress recently in the study of how anti-inflammatory steroids can influence arachidonate liberation. This is only a beginning, and it may be that further progress in this particular topic will help to clarify other areas of debate.

In the 16 years since it was first suggested that the availability of free arachidonate is the limiting factor in eicosanoid synthesis (Vogt *et al.*, 1966), great progress has been made in our understanding of how this availability is controlled. As in all areas of science, the questions that have been answered have in turn prompted yet more questions. If this Review has helped to clarify what those new questions are, and which of the old questions remain unanswered, then its main purpose will have been fulfilled.

While the responsibility for omissions and misinterpretations remains mine alone, it is a pleasure to acknowledge the contribution made to this review by Drs. R. M. C. Dawson, J. L. Gordon and J. D. Pearson.

References

- Åkesson, B. & Gustavii, B. (1975) *Prostaglandins* **9**, 667–673
- Anderson, A. J., Brocklehurst, W. E. & Willis, A. C. (1971) *Pharmacol. Res. Commun.* **3**, 3–19
- Araki, H., Peck, R. C., Lefer, A. M. & Smith, J. B. (1981) *Prostaglandins* **21**, 387–399
- Baker, R. R. & Thompson, W. (1972) *Biochim. Biophys. Acta* **270**, 489–503
- Baker, R. R. & Thompson, W. (1973) *J. Biol. Chem.* **248**, 7060–7065
- Béréziat, G., Chambez, J., Trugnan, G., Pepin, D. & Polonovski, J. (1978) *J. Lipid Res.* **19**, 495–500
- Bergström, S., Danielsson, M. & Samuelsson, B. (1964) *Biochim. Biophys. Acta* **90**, 207–210
- Billah, M. M., Lapetina, E. G. & Cuatrecasas, P. (1981) *J. Biol. Chem.* **256**, 5399–5403
- Bills, T. K., Smith, J. B. & Silver, M. J. (1977) *J. Clin. Invest.* **60**, 1–6
- Blackwell, G. J., Duncombe, W. G., Flower, R. J., Parsons, M. F. & Vane, J. R. (1977) *Br. J. Pharmacol.* **59**, 353–366
- Blackwell, G. J., Flower, R. J., Nijkamp, F. P. & Vane, J. R. (1978) *Br. J. Pharmacol.* **62**, 79–89
- Blackwell, G. J., Carnuccio, R., Di Rosa, M., Flower, R. J., Parente, L. & Persica, P. (1980) *Nature (London)* **287**, 147–149
- Bohman, S. & Larsson, C. (1975) *Acta Physiol. Scand.* **94**, 244–258
- Bonney, R. J., Wightman, P. D., Davies, P., Sadowski, S. J., Kuehl, F. A. & Humes, J. L. (1978) *Biochem. J.* **176**, 433–442
- Broekman, M. J., Ward, J. W. & Marcus, A. J. (1980) *J. Clin. Invest.* **66**, 275–283
- Broekman, M. J., Ward, J. W. & Marcus, A. J. (1981) *J. Biol. Chem.* **256**, 8271–8274
- Cabot, M. C. & Gatt, S. (1976) *Biochim. Biophys. Acta* **431**, 105–115
- Cabot, M. C. & Gatt, S. (1977) *Biochemistry* **16**, 2330–2334
- Carnuccio, R., Di Rosa, M. & Persico, P. (1980) *Br. J. Pharmacol.* **68**, 14–16
- Carnuccio, R., Di Rosa, M., Flower, R. J. & Pinto, A. (1981) *Br. J. Pharmacol.* **74**, 322–324
- Chau, L.-Y. & Tai, H.-H. (1981) *Biochem. Biophys. Res. Commun.* **100**, 1688–1695
- Chien, K. R., Abrams, J., Serroni, A., Martin, J. T. & Farber, J. L. (1978) *J. Biol. Chem.* **253**, 4809–4817
- Christ, E. J. & Nugteren, D. H. (1970) *Biochim. Biophys. Acta* **218**, 296–307
- Colard, O., Bard, D., Béréziat, G. & Polonovski, J. (1980) *Biochim. Biophys. Acta* **618**, 88–97
- Corey, E. J., Niwa, H., Flack, J. R., Miokowski, C., Arai, Y. & Marfat, A. (1980) *Adv. Prostaglandin Thromboxane Res.* **6**, 19–23
- Crews, F., Morita, Y., Hirata, F., Axelrod, J. & Siraganian, R. P. (1980) *Biochem. Biophys. Res. Commun.* **93**, 42–49
- Danon, A. & Assouline, G. (1978) *Nature (London)* **273**, 552–554
- Dawson, R. M. C. (1959) *Biochim. Biophys. Acta* **33**, 68–77
- Dawson, R. M. C. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C., eds.), pp. 97–116, Elsevier, Amsterdam and New York
- Dawson, R. M. C. & Irvine, R. F. (1978) *Adv. Prostaglandin Thromboxane Res.* **3**, 47–54
- Dawson, R. M. C. & Thompson, W. (1964) *Biochem. J.* **91**, 244–250
- Dawson, R. M. C., Freinkel, N., Jungawala, F. B. & Clarke, N. (1971) *Biochem. J.* **122**, 605–607
- Dawson, R. M. C., Hemington, N. & Irvine, R. F. (1980) *Eur. J. Biochem.* **112**, 33–38
- Debeer, L. J., Thomas, J., De Schepper, P. J. & Mannaerts, G. P. (1979) *J. Biol. Chem.* **254**, 8841–8846
- Dumm, I. N. T. de G., de Alaniz, M. J. T. & Brenner, R. R. (1979) *J. Lipid Res.* **20**, 834–839
- Eibl, H., Hill, E. E. & Lands, W. E. M. (1969) *Eur. J. Biochem.* **9**, 250–258
- Erman, A. & Raz, A. (1979) *Biochem. J.* **182**, 821–825
- Erman, A. & Raz, A. (1981) *Biochem. J.* **194**, 957–961
- Etienne, J., Gruber, A. & Polonovski, J. (1979) *Biochimie* **61**, 433–435
- Feinstein, M. B., Becker, E. L. & Fraser, C. (1977) *Prostaglandins* **14**, 1075–1093
- Ferber, E. & Resch, K. (1973) *Biochim. Biophys. Acta* **296**, 335–349
- Fisher, S. K. & Rowe, C. E. (1980) *Biochim. Biophys. Acta* **618**, 231–241
- Flower, R. J. & Blackwell, G. J. (1979) *Nature (London)* **278**, 456–459
- Franson, R. C., Eisen, D., Jesse, R. & Lanni, C. (1980) *Biochem. J.* **186**, 633–636
- Gerrard, J. M., White, J. G., Rao, G. H. R. & Townsend, D. (1976) *Am. J. Pathol.* **83**, 283–298
- Goodman, H. M. (1970) *Endocrinology* **86**, 1064–1070

- Greenberg, J. H. & Mellors, A. (1978) *Biochem. Pharmacol.* **27**, 329–333
- Groener, J. E. M. & Knauer, T. E. (1981) *Biochim. Biophys. Acta* **665**, 306–316
- Gryglewski, R., Panczenko, B., Korbut, R., Grodzinska, L. & Ocetkiewicz, A. (1975) *Prostaglandins* **10**, 343–355
- Guichardant, M. & Lagarde, M. C. (1980) *Thrombosis Res.* **18**, 285–290
- Hagenfeldt, L. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 2246–2249
- Hales, C. N., Luzio, J. P. & Siddle, K. (1978) *Biochem. Soc. Symp.* **43**, 97–135
- Hasegawa-Sakai, H. & Ohno, K. (1980) *Biochim. Biophys. Acta* **617**, 205–217
- Hill, E. E. & Lands, W. E. M. (1968) *Biochim. Biophys. Acta* **152**, 645–648
- Hirasawa, K., Irvine, R. F. & Dawson, R. M. C. (1981) *Biochem. J.* **193**, 607–614
- Hirata, F. (1981) *J. Biol. Chem.* **256**, 7730–7733
- Hirata, F. & Axelrod, J. (1980) *Science* **209**, 1082–1090
- Hirata, F., Corcoran, B. A., Venkatasubramanian, K., Schiffman, E. & Axelrod, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2640–2643
- Hirata, F., Toyoshima, S., Axelrod, J. & Waxdal, M. J. (1980a) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 862–865
- Hirata, F., Schiffman, E., Venkatasubramanian, K., Salomon, D. & Axelrod, J. (1980b) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2533–2536
- Hirata, F., Del Carmine, R., Nelson, C. A., Axelrod, J., Schiffman, E., Warabi, A., De Blas, A. L., Nirenberg, M., Manganiello, V., Vaughan, M., Kumagi, S., Green, I., Decker, J. L. & Steinberg, A. D. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3190–3194
- Hokin, L. E. (1968) *Int. Rev. Cytol.* **23**, 87–208
- Hokin-Neaverson, M., Sadeghian, K., Harris, D. W. & Merrin, J. S. (1977) *Biochem. Biophys. Res. Commun.* **78**, 364–371
- Holloway, C. T. & Margoulis, R. N. (1980) *Lipids* **15**, 1037–1043
- Holub, B. J. (1976) *Lipids* **11**, 1–5
- Holub, B. T. & Kuksis, A. (1971) *J. Lipid Res.* **12**, 699–705
- Hong, S. & Deykin, D. (1979) *J. Biol. Chem.* **254**, 11463–11466
- Hong, S. L. & Deykin, D. (1981) *J. Biol. Chem.* **256**, 5215–5219
- Hong, S. L. & Levine, L. (1976a) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1730–1734
- Hong, S. L. & Levine, L. (1976b) *J. Biol. Chem.* **251**, 5814–5816
- Hsueh, W. & Needleman, P. (1978) *Prostaglandins* **16**, 661–681
- Hsueh, W., Isakson, P. C. & Needleman, P. (1977) *Prostaglandins* **13**, 1073–1091
- Hsueh, W., Kuhn, C. & Needleman, P. (1979) *Biochem. J.* **184**, 345–354
- Hsueh, W., Desai, V., Gonzalez-Crussi, F., Lamb, R. & Chu, A. (1981) *Nature (London)* **290**, 710–713
- Hülsman, W. C., Stam, H. & Breeman, W. A. P. (1981) *Biochem. Biophys. Res. Commun.* **102**, 440–448
- Irvine, R. F. & Dawson, R. M. C. (1979) *Biochem. Biophys. Res. Commun.* **91**, 1399–1405
- Irvine, R. F., Hemington, N. & Dawson, R. M. C. (1979) *Eur. J. Biochem.* **99**, 525–530
- Irvine, R. F., Dawson, R. M. C. & Freinkel, N. (1982) in *Contemporary Metabolism* (Freinkel, N., ed.), vol. 2, pp. 301–342, Plenum Press, New York
- Isakson, P. C., Raz, A., Denny, S. E., Wyche, A. & Needleman, P. (1977) *Prostaglandins* **14**, 853–871
- Jesse, R. L. & Cohen, P. (1976) *Biochem. J.* **158**, 283–287
- Jezyk, P. & Lands, W. E. M. (1968) *J. Lipid Res.* **9**, 525–531
- Johnston, D., Matthews, E. R. & Melnikovych, G. (1980) *Endocrinology* **107**, 1482–1488
- Jones, L. M. & Michell, R. H. (1978) *Biochem. Soc. Trans.* **6**, 673–688
- Jose, P. J. & Seale, J. P. (1979) *Br. J. Pharmacol.* **67**, 519–526
- Kannagi, R., Koizumi, K., Hata-Tanoue, S. & Masuda, T. (1980) *Biochem. Biophys. Res. Commun.* **96**, 711–718
- Kantor, H. S. & Hampton, M. (1978) *Nature (London)* **276**, 841–842
- Kaplan-Harris, L. & Elsbach, P. (1980) *Biochim. Biophys. Acta* **618**, 318–326
- Kataoka, K., Ramwell, P. W. & Jessup, S. (1967) *Science* **157**, 1187–1188
- Kaulen, M. D., Henning, R. & Stoffel, W. (1970) *Hoppe-Seyler's Z. Physiol. Chem.* **351**, 1555–1563
- Kemp, P., Hübscher, G. & Hawthorne, J. N. (1961) *Biochem. J.* **79**, 193–200
- Koch, M. A. & Diringer, H. (1974) *Biochem. Biophys. Res. Commun.* **58**, 361–367
- Kosaki, G., Nomura, T. & Kambayashi, T. (1980) *Thrombosis Res.* **20**, 587–598
- Kröner, E. E., Peskar, B. A., Fischer, H. & Ferber, E. (1981) *J. Biol. Chem.* **256**, 3690–3697
- Lagarde, M., Menashi, S. & Crawford, N. (1981) *Biochem. Soc. Trans.* **9**, 122–123
- Lands, W. E. M. (1979) in *Geometrical and Positional Fatty Acid Isomers* (Emken, E. A. & Dutton, H. J., eds.), pp. 181–212, American Oil Chemists Society, Champaign, Illinois
- Lands, W. E. M. & Merkel, I. (1963) *J. Biol. Chem.* **238**, 898–904
- Lands, W. E. M. & Samuelsson, B. (1968) *Biochim. Biophys. Acta* **164**, 426–429
- Lapetina, E. G., Chandrabose, K. A. & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 818–822
- Lapetina, E. G., Billah, M. & Cuatrecasas, P. (1980) *J. Biol. Chem.* **255**, 10966–10170
- Lapetina, E. G., Billah, M. M. & Cuatrecasas, P. (1981) *Nature (London)* **292**, 367–369
- Lindgren, J. A., Claesson, H.-E. & Hammarström, S. (1978) *Adv. Prostaglandin Thromboxane Res.* **3**, 167–174
- MacMillan, R. M., Brinckerhoff, C. E. & Harris, E. D. (1980) *Arthritis Rheum.* **23**, 717
- Marcus, A. J., Ullman, H. C. & Safer, L. R. (1969) *J. Lipid Res.* **10**, 108–114
- Marion, J. & Wolfe, L. S. (1979) *Biochim. Biophys. Acta* **574**, 25–32
- Marshall, P. J., Boatman, D. E. & Hokin, L. E. (1981) *J. Biol. Chem.* **256**, 844–847
- Mauco, G., Chap, H., Simon, M.-F. & Douste-Blazy, L. (1978) *Biochimie* **60**, 653–661
- McDonald, G., Baker, R. R. & Thompson, W. (1975) *J. Neurochem.* **24**, 655–661

- McKean, M. L., Smith, J. B. & Silver, M. J. (1981) *J. Biol. Chem.* **256**, 1522–1524
- Mitchell, R. H. (1975) *Biochim. Biophys. Acta* **415**, 81–147
- Minkes, M., Standford, N., Chi, M. M.-Y., Roth, G. J., Raz, A., Needleman, P. & Majerus, P. W. (1977) *J. Clin. Invest.* **59**, 449–454
- Mitchell, S., Poyser, N. L. & Wilson, N. H. (1977) *Br. J. Pharmacol.* **59**, 107–113
- Moonen, H., Trienekens, P. & Van den Bosch, H. (1977) *Biochim. Biophys. Acta* **489**, 423–430
- Morita, I. & Murota, S.-I. (1980) *Biochim. Biophys. Acta* **619**, 428–431
- Narumiya, S., Salmon, J. A., Cottee, F. H., Weatherley, B. C. & Flower, R. J. (1981) *J. Biol. Chem.* **256**, 9583–9592
- Needleman, P., Wyche, A., Bronson, S. D., Holmberg, S. & Morrison, A. R. (1979) *J. Biol. Chem.* **254**, 9772–9777
- Normann, P. T., Thomassen, M. S., Christiansen, E. N. & Flatmark, T. (1981) *Biochim. Biophys. Acta* **664**, 416–427
- Northover, B. J. (1977) *Gen. Pharmacol.* **8**, 293–296
- Ohkazaki, T., Okita, J. R., McDonald, P. C. & Johnston, J. M. (1978) *Am. J. Obstetr. Gynecol.* **130**, 432–438
- Parce, J. W., Cunningham, C. C. & Waite, M. (1978) *Biochemistry* **17**, 1634–1639
- Parker, C. W., Kelly, J. C., Falkenstein, S. F. & Huber, M. A. (1979) *J. Exp. Med.* **149**, 1487–1503
- Perret, B., Chap, H. J. & Douste-Blazy, L. (1979) *Biochim. Biophys. Acta* **556**, 434–446
- Pickett, W. C., Jesse, R. L. & Cohen, P. (1977) *Biochim. Biophys. Acta* **486**, 209–213
- Prescott, S. M. & Majerus, P. W. (1981) *J. Biol. Chem.* **256**, 579–582
- Randon, J., Lecompte, T., Chignard, M., Siess, W., Marlas, G., Dray, F. & Vargaftig, B. B. (1981) *Nature (London)* **293**, 660–662
- Reed, P. W. & Lardy, H. A. (1972) *J. Biol. Chem.* **247**, 6970–6977
- Rittenhouse-Simmons, S. (1979) *J. Clin. Invest.* **63**, 580–587
- Rittenhouse-Simmons, S. (1980) *J. Biol. Chem.* **255**, 2259–2262
- Rittenhouse-Simmons, S. (1981) *J. Biol. Chem.* **256**, 4153–4155
- Rittenhouse-Simmons, S. & Deykin, D. (1981) in *Platelets in Biology and Pathology* (Gordon, J. L., ed.), vol. 2, pp. 349–372, Elsevier/North Holland, Amsterdam, New York and Oxford
- Rittenhouse-Simmons, S., Russell, F. A. & Deykin, D. (1976) *Biochem. Biophys. Res. Commun.* **70**, 295–301
- Robinson, C. & Houlst, J. R. S. (1980) *Eur. J. Pharmacol.* **64**, 333–339
- Rubin, R. P., Sink, L. E. & Freer, R. J. (1981a) *Biochem. J.* **194**, 497–505
- Rubin, R. P., Sink, L. E. & Freer, R. J. (1981b) *Mol. Pharmacol.* **19**, 31–37
- Schick, P. K., Schick, B. P., Brandeis, G. & Mills, D. C. B. (1981) *Biochim. Biophys. Acta* **643**, 659–662
- Schremmer, J. M., Blank, M. L. & Wykle, R. L. (1979) *Prostaglandins* **18**, 491–505
- Schrey, M. P. & Rubin, R. P. (1979) *J. Biol. Chem.* **254**, 11234–11241
- Schwartzman, M. & Raz, A. (1979) *Biochim. Biophys. Acta* **475**, 363–369
- Schwartzman, M. & Raz, A. (1981) *Biochim. Biophys. Acta* **664**, 469–474
- Schwartzman, M., Lieberman, E. & Raz, A. (1981) *J. Biol. Chem.* **256**, 2329–2333
- Scott, W. A., Zrike, J. M., Hamill, A. L., Kempe, J. & Cohn, Z. A. (1980) *J. Exp. Med.* **152**, 324–335
- Shaw, J. O., Kluvick, S. J. & Hanahan, D. J. (1981) *Biochim. Biophys. Acta* **663**, 222–229
- Shier, W. T. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 137–141
- Skidmore, I. F. (1982) *Mol. Aspects Med.*, in press
- Stein, Y., Widnell, C. & Stein, O. (1968) *J. Cell Biol.* **39**, 185–192
- Styme, S. & Glad, G. (1981) *Lipids* **16**, 298–305
- Tamarit-Rodriguez, J., Hellman, B. & Schlin, J. (1978) *Biochim. Biophys. Acta* **496**, 167–174
- Trotter, J. & Ferber, E. (1981) *FEBS Lett.* **128**, 237–241
- Vahouny, G. V., Chanberbhan, R., Hodges, V. A. & Treadwell, C. R. (1978) *Prostaglandins* **16**, 207–220
- Vance, D. E. & de Kruijff, B. (1980) *Nature (London)* **288**, 277–279
- Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604**, 191–246
- Van den Bosch, H., Van Golde, L. M. G. & van Deenen, L. L. M. (1972) *Rev. Physiol.* **66**, 14–145
- Vanderhoek, J. Y. & Feinstein, M. B. (1979) *Mol. Pharmacol.* **16**, 171–180
- van Dorp, D. A., Beerthuis, R. F., Nugteren, D. H. & Vonkeman, H. (1964) *Biochim. Biophys. Acta* **90**, 204–207
- Vargaftig, B. B. (1977) *J. Pharmacol.* **29**, 222–228
- Vogt, W. (1978) *Adv. Prostaglandin Thromboxane Res.* **3**, 89–95
- Vogt, W., Suzuki, T. & Babilli, S. (1966) *Mem. Soc. Endocrinol.* **14**, 137–142
- Vonkeman, H. & van Dorp, D. A. (1968) *Biochim. Biophys. Acta* **164**, 430–432
- Waite, M., Griffin, H. D. & Franson, R. (1976) in *Lysosomes in Biology and Pathology* (Dingle, J. T. & Dean, R. T., eds.), vol. 5, pp. 257–305, Elsevier/North Holland, Amsterdam and New York
- Wallach, D. P. & Brown, V. J. R. (1981) *Biochem. Pharmacol.* **30**, 1315–1324
- Walsh, C. E., Waite, B. M., Thomas, M. J. & DeChatelet, L. R. (1981) *J. Biol. Chem.* **256**, 7228–7234
- White, D. A. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C., eds.), pp. 441–482, Elsevier, Amsterdam and New York
- Whorton, A. R., Smigel, M., Oates, J. A. & Frölich, J. C. (1978) *Biochim. Biophys. Acta* **529**, 176–180
- Willis, A. L. (1981) *Nutr. Rev.* **39**, 289–301
- Wise, L. S. & Jungas, R. L. (1978) *J. Biol. Chem.* **253**, 2624–2627
- Wisher, M. H. & Evans, W. H. (1975) *Biochem. J.* **146**, 375–388
- Yamashita, S., Hosaka, K. & Numa, S. (1973) *Eur. J. Biochem.* **38**, 25–31
- Yamashita, S., Nakaya, N., Miki, Y. & Numa, S. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72**, 600–603
- Yau, T. M. & Sun, G. Y. (1974) *J. Neurochem.* **23**, 99–104
- Yousef, I. M., Fisher, M. M., Piekarski, J. & Holub, B. J. (1977) *Lipids* **12**, 140–144