RESEARCH COMMUNICATION
5-Octadecenoic acid: evidence for a novel type of fatty acid modification in schistosomes

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The lipid metabolism of schistosomes is characterized by several intriguing adaptations to a parasitic way of living. The surface of the parasite consists of two closely apposed phospholipid bilayers, a structure unique to blood flukes. Schistosomes do not synthesize fatty acids de novo, but are able to modify fatty acids, which they obtain from the host, by chain elongation. Here we present evidence that schistosomes are capable of another type of fatty acid modification, resulting in the formation of 5-octadecenoic acid [$C_{18:1(5)}$]. This highly unusual fatty acid, which is absent in the blood of the host, was shown to be almost exclusively located in the outer membrane complex of the schistosome. Within these membranes, it was almost exclusively present in one molecular phospholipid species, 1-palmitoyl-2,5-octadecenoyl phosphatidylcholine [$C_{16:0-18:1(5)}$PtdCho]. Apart from dipalmitoyl phosphatidylcholine, this was the most abundant phosphatidylcholine species in the outer membrane complex. The specific synthesis by the schistosome of $C_{18:1(5)}$ and the highly specific localization of this fatty acid to the tegumental membranes suggest an important tegument-mediated role for this lipid.

INTRODUCTION

The parasitic blood fluke *Schistosoma mansoni* is a long-term inhabitant of the mesenteric veins of its final host. The presence of a membrane complex that surrounds the schistosome is a very characteristic adaptation to the continuous contact between the parasite and the immune system of the host. This membrane complex, which consists of two closely apposed lipid bilayers, is synthesized from discoid and multilamellar bodies that are made in cell bodies just below the tegument [1,2]. Maintenance of the integrity of these outer tegumental membranes is essential for the survival of the parasite. Phospholipids, and, in particular, phosphatidylcholine (PC; referring collectively to all diradyl choline glycerophospholipid species), are the main constituents of these membranes [3].

Many important physical and biological properties of membranes are determined by their phospholipid composition. The degree of unsaturation of the fatty acyl constituents of the phospholipids determines the fluidity of the membranes, and therefore plays an important role in events such as endocytosis and exocytosis, sorting of lipids and membrane fusion. Furthermore, after hydrolysis of phospholipids by the appropriate phospholipases, diacylglycerol and fatty acids can serve as important mediators (or precursors thereof) in events such as signal transduction, immune modulation (e.g. via the synthesis of prostaglandins and leukotrienes) and lipid metabolism. Therefore a rigorous characterization of the fatty acids present in the tegumental membranes is essential in order to assess lipid-related functions of the tegument.

Here we demonstrate the presence in the schistosome of the unusual fatty acid, 5-octadecenoic acid [$C_{18:1(5)}$], which is very abundant in the outer tegumental membrane complex, but absent in the blood of the host and nearly absent in the worm body. This fatty acid, which does not observe the general rule that the first double bond in a fatty acid is introduced in the 9 position [4], was present in one PC molecular species only. Considering the absence of $C_{18:1(5)}$ in the host and its highly specific location in the tegument, we postulate that $C_{18:1(5)}$ is synthesized by the schistosome, thereby representing a novel type of fatty acid modification employed by the parasite. The highly specific localization of this fatty acid in the tegumental PC fraction suggests a tegument-related role for $C_{18:1(5)}$.
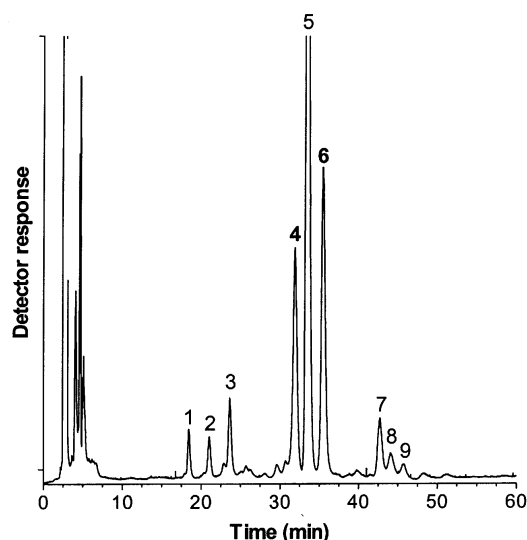
MATERIALS AND METHODS

Chemicals

All solvents were of HPLC grade and obtained from Labscan (Dublin, Ireland). Hepes was purchased from Gibco (Paisley, Renfrewshire, Scotland, U.K.) All other chemicals, including standards of molecular species and fatty acids, were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) or Baker (Deventer, The Netherlands) and were of the highest purity available. [1-^{14}C]stearic acid was obtained from Amersham (Amersham, Buckinghamshire, UK).

Parasites

S. mansoni worms were isolated from diethyl ether-anaesthetized

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Figure 1   HPLC chromatogram, as monitored by light-scattering detection, showing the separation of molecular species of PC isolated from the outer tegumental membranes of *S. mansoni*

Isolated peaks were identified with on-line ESI-MS. Note the occurrence of two $(C_{16:0-18:1})$PtdCho species (labelled in bold). Major species were identified as: 1, $(C_{16:0-22:6})$PtdCho; 2, $(C_{16:0-20:4})$PtdCho; 3, $(C_{16:0-18:2})$PtdCho; 4, $(C_{16:0-18:1})$PtdCho; 5, $(C_{16:0-16:0})$PtdCho; 6, $(C_{16:0-18:1})$PtdCho; 7, $(C_{16:0-16:0})$alkylacyl PC; 8, $(C_{20:1-16:0})$PtdCho; and 9, $(C_{18:0-18:1})$PtdCho.

hamsters 45–48 days after infection by perfusion of the heart at 37 °C, as described previously [5]. After isolation, schistosomes were plunged into liquid nitrogen in Hanks' balanced salts solution. After thawing, the outer tegumental membrane complex was removed by the method of Roberts et al. [6].

Isolation of lipid fractions

Lipids were extracted by the method of Bligh and Dyer [7]. Approx. 80–100 worm pairs were used per HPLC analysis of tegumental phospholipids. Phospholipid classes were separated on HPLC using a 125 mm × 4 mm Lichrospher 4 μm normal-phase column (Merck, Darmstadt, Germany). Elution was performed with a gradient of hexane:propan-2-ol: dichloromethane (20:27:3, by vol.) to hexane:propan-2-ol:dichloromethane:water (20:23:3:4, by vol.) during 10 min, and an additional 10 min elution with the latter solvent. Detection was performed with a Varex MKIII light-scattering detector (Alltech, Deerfield, IL, U.S.A.) operated at a gas flow rate of 2 l·min^{-1}, and the drift tube temperature set to 100 °C. Phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), sphingomyelin and PC were isolated with the aid of a flow splitter inserted between column and detector.

Molecular species analysis

After isolation of the lipid fractions, PC molecular species were separated as described previously [8]. Briefly, PC was eluted isocratically from two Lichrospher RP18 end-capped columns in series using a mobile phase of acetonitrile:methanol:triethylamine (8:11:1, by vol.). The light-scattering detector was set to 1.8 l·min^{-1} and 100 °C. The positions of the fatty acyls along the glycerol backbone were determined by phospholipase A$_2$ digestion of individual components collected at the outlet of the column. Free fatty acids were converted into their phenacyl ester derivatives, eluted with acetonitrile from the reverse-phase columns described above, and detected at 242 nm [5,9]. Identification of molecular species eluting from the column was performed with on-line electrospray ionization–MS (ESI–MS), as described below.
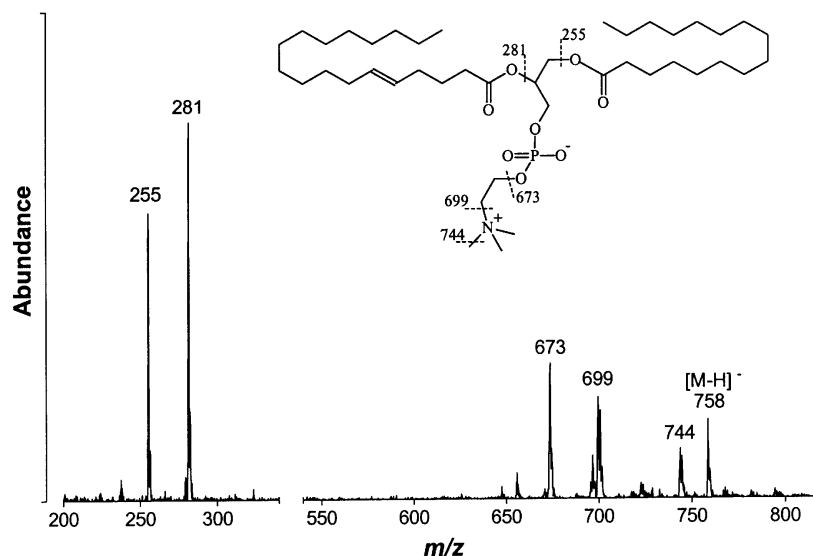
Figure 2   Negative-ion-mode electrospray mass spectrum of component 6 from Figure 1, identifying $(C_{16:0-18:1})$PtdCho

Fragment ions result from fragmentation at the positions indicated in the structural formula. The positions of palmitate (*m/z* 255) and oleate (*m/z* 281) along the glycerol backbone were determined by phospholipase A$_2$ digestion of the isolated molecular species. The spectrum shown is identical to the mass spectrum recorded during elution of component 4 in Figure 1.

MS

Identification of PC molecular species was performed with a Fisons VG Platform II single quadrupole mass spectrometer (Micromass, Manchester, U.K.), fitted with an electrospray ion source operated at atmospheric pressure and in the negative mode, which was connected to the outlet of the HPLC columns via a flow splitter. Nitrogen was used as nebulizer gas ($0.2 \, l \cdot min^{-1}$) and as curtain gas ($7 \, l \cdot min^{-1}$). The capillary voltage was set to 3.0 kV and the cone voltage to 200 V.

Determination of the position of the double bond within the fatty acid was performed with tandem MS on a Jeol JMS SX/SX 102A instrument, equipped with a fast-atom-bombardment (FAB) gun and source used for the generation of the negative precursor ion M^- (m/z 281) from individual PC molecular species that were purified by HPLC. Triethanolamine was used as a matrix. Collision-induced dissociation (CID) spectra of the precursor ion selected by MS-1 were acquired by MS-2. Air was used as the collision gas.

RESULTS

Identification of two isomers of octadecenoic acid ($C_{18:1}$) in the tegumental membranes

In Figure 1 the separation of molecular species of PC from the outer tegumental membranes is shown, as monitored by light-scattering detection. Species that eluted from the column were identified by on-line ESI–MS, resulting in a mass spectrum for each component that allowed the identification of the choline headgroup, the two fatty acyl chains and the molecular mass of the component. This resulted in the identification of the main molecular species, as shown in Figure 1. Most notable was the fact that two major molecular species (Figure 1; peaks 4 and 6) displayed identical mass spectra. In Figure 2, this mass spectrum is shown (collected during the elution of component 6 in Figure 1). From the mass of the molecular ion (m/z 758) it can be deduced that the acyl chains of the PC species under investigation have a total of 34 carbon atoms, contain one single double bond and are linked to the glycerol backbone via ester bonds [10]. In the m/z range from 250 to 350 the fatty acid carboxylate anions are present that are lost from the glycerol backbone during fragmentation. The two fatty acids present are $C_{16:0}$ (m/z 255) and $C_{18:1}$ (m/z 281), which are in agreement with the expected total mass of the acyl chains. The presence of the fragment ions m/z 744 $[M-15]^-$, m/z 699 $[M-60]^-$ and m/z 673 $[M-86]^-$ further confirms that the components under investigation are indeed PC species, since these fragment ions result from typical losses from the choline headgroup ([10]; see structural formulae and fragmentation sites in Figure 2). Peak 4 (Figure 1) co-eluted with an authentic standard of palmitoyl oleoyl PC [$C_{16:0-18:1(9)}$PtdCho].

By phospholipase A_2 digestion of the isolated molecular species, it could be demonstrated that the $C_{16:0}$ chain of both components 4 and 6 was esterified at the *sn*-1 position and that, in both components, the *sn*-2 position contained the $C_{18:1}$ fatty acid. The longer retention time of component 6 compared with component 4 was a result of a difference in the $C_{18:1}$ fatty acid: after hydrolysis of the molecular species and conversion of the $C_{18:1}$ fatty acids to their phenacyl derivatives, the $C_{18:1}$ from component 4 co-eluted with an authentic standard of oleic acid [$C_{18:1(9)}$], whereas the $_{18:1}$ from component 6 had a longer retention time on the reverse-phase HPLC column (data not shown).
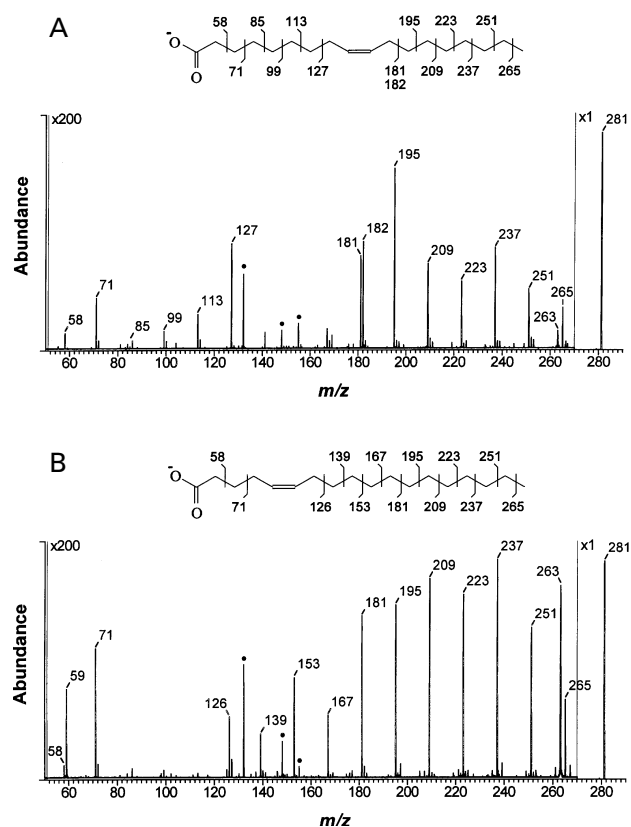
Figure 3   CID spectrum of *m/z* 281

Tandem MS was performed on components 4 and 6 (see Figure 1). After FAB ionization, MS-1 was used to select m/z 281 as precursor ion for MS-2. After collision activation of m/z 281, the charge-remote fragmentation of the precursor ion was recorded with MS-2. (**A**) shows the charge-remote fragmentation of m/z 281, derived from component 4 (Figure 1), identifying $C_{18:1(9)}$ (oleic acid). (**B**) shows the charge-remote fragmentation of m/z 281, derived from component 6 (Figure 1), identifying $C_{18:1(5)}$. Fragmentation sites and the resulting products are indicated in the structure formulae. The ion at m/z 263 results from the loss of water from the precursor ion. Fragment ions indicated by ● are matrix-derived, and are unrelated to the m/z 281 ion.

Determination of the position of the double bond in the fatty acid

The position of the double bond within the fatty acyl chain was determined by FAB–CID tandem MS. The fatty acid carboxylate anion selected with MS-1 was collisionally activated and the subsequent dissociation of the ion, mainly occurring via charge-remote fragmentation, was recorded by MS-2. The position of the double bond is identified by a gap in the regularly spaced series of fragment ions (Figure 3). This gap results from the fact that no fragmentation occurs at either the double bond or at the two adjacent vinylic bonds [11]. MS-2 spectra are presented of the m/z 281 ion of components 4 (Figure 3A) and 6 (Figure 3B). On the basis of the fragmentation pattern in Figure 3(A), the m/z 281 ion from component 4 was identified as $C_{18:1(9)}$ (oleic acid), which was confirmed by a standard of oleic acid having the same fragmentation pattern (results not shown). All commercially available positional isomers of $C_{18:1}$ [at $C_{(12)}$, $C_{(11)}$, $C_{(9)}$, $C_{(7)}$ or $C_{(6)}$] gave the typical regularly spaced series of fragment ions, in which a gap identifies the position of the double bond. Therefore the m/z 281 ion from component 6 was identified as $C_{18:1(5)}$, although an authentic standard of this fatty acid is not available (Figure 3B).

Table 1   Ratios of $C_{18:1(5)}$ to $C_{18:1(9)}$ in lipid fractions of the host and the parasite

The phospholipid compositions of the outer tegumental membranes (in mol%) are given in parentheses. *, the total amount of $C_{18:1(9)}$ in the injected equivalent of 0.2 ml of hamster blood was 67 nmol, whereas the total amount of $C_{18:1(5)}$ was below the threshold level of 50 pmol.

Sample	$C_{18:1(5)} : C_{18:1(9)}$
Hamster blood, total lipids	< 0.001*
S. mansoni, total lipid	0.007 ± 0.002
S. mansoni, tegumental phospholipids	0.41 ± 0.07
S. mansoni, tegumental PS + PI (6%)	< 0.005
S. mansoni, tegumental sphingomyelin (14%)	< 0.010
S. mansoni, tegumental PE (29%)	0.16 ± 0.09
S. mansoni, tegumental PC (50%)	1.4 ± 0.3

Specific localization of $C_{18:1(5)}$ to the tegumental PC fraction

The amount of $C_{18:1(5)}$ in the blood of the host, on which schistosomes feed, was too low to be detected. Within the schistosome, $C_{18:1(5)}$ was not distributed homogeneously, as it was almost exclusively present in the outer tegumental membranes (Table 1). The distribution of $C_{18:1(5)}$ among the phospholipid classes of the outer tegumental membranes was also found not to be homogeneous. The phospholipid composition of the outer tegumental membranes, given in Table 1, is in good aggrement with previous data on this membrane complex [3]. Of the major phospholipids in these membranes, sphingomyelin and PE contained only very small amounts of $C_{18:1(5)}$, whereas the amount of $C_{18:1(5)}$ exceeded the amount of $C_{18:1(9)}$ in the PC fraction (Table 1). Desaturation of [1-^{14}C]stearic acid to [1-^{14}C]$C_{18:1(5)}$ could not be demonstrated during *in vitro* incubations of intact adult schistosomal worm pairs (results not shown).

DISCUSSION

Identification of $C_{18:1(5)}$ in the outer tegumental membranes

$C_{18:1(5)}$ is rarely present in Nature. However, using the combination of a chromatographic procedure that is capable of isolating a single molecular species of PC and subsequent tandem MS, we were able to unequivocally demonstrate the presence of this fatty acid in schistosomes. The spectra of Figure 3 revealed the elimination of C_nH_{2n+2} units from the m/z 281 precursor ion, which is characteristic of charge-remote fragmentation of fatty acids [12]. Other types of fragmentation that occur include the loss of water from the carboxy group (resulting in m/z 263) and the loss of C_nH_{2n+1} units when fragmentation occurs in the vicinity of a double bond [m/z 58 and m/z 182 in Figure 3(A) and m/z 58 and m/z 126 in Figure 3(B)]. The observed fragmentations are in good agreement with CID spectra of monounsaturated fatty acids published previously [11].

Implications of the $C_{(5)}$ double bond

The double bond located at the $C_{(5)}$ position may be expected to result in considerable metabolic differences between (the common) oleic acid [$C_{18:1(9)}$] and the isomer identified here [$C_{18:1(5)}$]. Only fatty acids containing a double bond at $C_{(5)}$ were released following thrombin or histamine stimulation of vascular endothelial cells [13,14]. A comparable selectivity was seen in several lipases, e.g. the lipase from oilseed rape discriminates against $C_{(4)}$- and $C_{(6)}$-unsaturated fatty acids, but readily esterifies $C_{(5)}$- or $C_{(9)}$-unsaturated fatty acids [15]. The activity of pancreatic lipase is less specific, but it is inhibited by a double bond at any position up to $C_{(7)}$ [16]. A strong indication that, also in schistosomes, there is a fatty acyl specificity mediated by the position of the double bond in the fatty acid lies in the different ratios of $C_{18:1(5)} : C_{18:1(9)}$ found in the phospholipids of the outer tegumental membrane complex (Table 1). PC was particularly enriched in $C_{18:1(5)}$; lesser amounts were present in PE, and only trace amounts of $C_{18:1(5)}$ were present in sphingomyelin, PS and PI. It is not yet known, however, whether this heterogeneous distribution of $C_{18:1(5)}$ is already achieved during the *de novo* synthesis of the phospholipids, or whether it is introduced by the deacylating/reacylating system, which is very active in schistosomes [5]. It is of interest that Wolff [17] reported on the structural importance of the double bond at $C_{(5)}$ in channelling fatty acids to the *sn*-2 position of phospholipids (PI in particular), but it could not be shown whether this was a result of *de novo* synthesis or of deacylation/reacylation.

Origin of the $C_{(5)}$ double bond

Schistosomes are not capable of either *de novo* synthesis or β-oxidation of fatty acids and, to date, fatty acid desaturation has not been demonstrated [18,19]. However, the fact that the uncommon $C_{18:1(5)}$ fatty acid is absent in the blood of the host on which schistosomes feed implies that $C_{18:1(5)}$ is synthesized by the parasite itself. This idea is further supported by the very specific localization of this fatty acid in the tegumental membranes. The phospholipids of these membranes are synthesized by highly specialized cells lying directly underneath the tegument, and the enzymes required for the synthesis of $C_{18:1(5)}$ might be expressed in these cells only. The small quantity of $C_{18:1(5)}$ present in schistosomes is likely to explain why no measurable conversion of [1-^{14}C]stearic acid into [1-^{14}C]$C_{18:1(5)}$ occurred. An alternative explanation for the presence of this specific fatty acid in the tegumental membranes, i.e. uptake (and subsequent transport) of undetectable trace amounts of $C_{18:1(5)}$ specifically to the PC fraction of the tegumental phospholipids, seems highly improbable.

In conclusion, we have reported the presence of a fatty acid [$C_{18:1(5)}$] in schistosomes that is almost exclusively present in the outer tegumental membranes of the parasite. On the basis of the absence of the fatty acid in the host and the highly specific localization within the schistosome, we believe that this fatty acid is synthesized by the parasite, thereby representing a type of fatty acid modification that was previously not known in schistosomes. The function of the fatty acid in the tegumental membranes remains to be resolved.

REFERENCES

1   Zhou, Y. and Podesta, R. B. (1989) J. Parasitol. **75**, 333–343
2   McLaren, D. J. and Hockley, D. J. (1977) Nature (London) **269**, 147–149
3   Allan, D., Payares, G. and Evans, W. H. (1987) Mol. Biochem. Parasitol. **23**, 123–128
4   Cook, H. W. (1996) in Biochemistry of Lipids, Lipoproteins and Membranes (Vance, D. E. and Vance, J. E., eds.), pp. 129–152, Elsevier Science, Amsterdam
5   Brouwers, J. F. H. M., Smeenk, I. M. B., van Golde, L. M. G. and Tielens, A. G. M. (1997) Mol. Biochem. Parasitol. **88**, 175–185

6   Roberts, S. M., MacGregor, A. N., Vojvodic, M., Wells, E., Crabtree, J. E. and Wilson, R. A. (1983) Mol. Biochem. Parasitol. **9**, 105–127

7   Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917

8   Brouwers, J. F. H. M., Gadella, B. M., van Golde, L. M. G. and Tielens, A. G. M. (1998) J. Lipid. Res. **39**, 344–353

9   Wood, R. and Lee, T. (1983) J. Chromatogr. **254**, 237–246

10   Murphy, R. C. (1993) in Handbook of Lipid Research, Part 7 (Snyder, F., ed.), Plenum Press, New York

11   Cordero, M. M. and Wesdemiotis, C. (1994) Anal. Chem. **66**, 861–866

12   Jensen, N. J., Tomer, K. B. and Gross, M. L. (1987) Lipids **22**, 480–489

13   Rosenthal, M. D., Garcia, M. C. and Sprecher, H. (1989) Arch. Biochem. Biophys. **274**, 590–600

14   Rosenthal, M. D. (1987) Biochim. Biophys. Acta **917**, 279–289

15   Jefferts, E., Morales, R. W. and Litchfield, C. (1974) Lipids **9**, 244–247

16   Heimermann, W. H., Holman, R. T., Gordon, D. T., Kowalyshyn, D. E. and Jensen, R. G. (1973) Lipids **8**, 45–47

17   Wolff, R. L. (1995) Lipids **30**, 893–898

18   Meyer, F., Meyer, H. and Bueding, E. (1970) Biochim. Biophys. Acta **210**, 257–266

19   Barrett, J. (1981) Biochemistry of Parasitic Helminths, MacMillan, London