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Inhibition of plant fatty acid synthesis by nitroimidazoles

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1. The effect of the addition of a number of nitroimidazoles was tested on fatty acid synthesis by germinating pea seeds, isolated lettuce chloroplasts and a soluble fraction from pea seeds. 2. All the compounds tested had a marked inhibition on stearate desaturation by lettuce chloroplasts and on the synthesis of very-long-chain fatty acids by pea seeds. 3. In contrast, the effect of the drugs on total fatty acid synthesis from [^{14}C]acetate in chloroplasts was related to the compound's electron reduction potentials. 4. Of the compounds used, only metronidazole had a marked inhibition on palmitate elongation in the systems tested. 5. The mechanism of inhibition of plant fatty acid synthesis by nitroimidazoles is discussed and the possible relevance of these findings to their neurotoxicity is suggested.

Nitroimidazole drugs are used in the treatment of protozoal infections and anaerobic bacteria (O'Brien & Morris, 1972). Part of the reason for the selective toxicity of the compounds may lie in the redox potential of the nitro group, which can only be reduced by anaerobes, having redox reactions that are more negative than those found in aerobes (Edwards *et al.*, 1973). Furthermore, compounds such as reduced misonidazole [1-(2-hydroxy-3-methoxypropyl)-2-nitroimidazole] have been shown to have a nuclease-like activity specific for thymidine in DNA. This then results in increased damage to organisms such as the protozoans *Trichomonas vaginalis* and *Entamoeba histolytica*, which have high adenine and thymine contents (Edwards *et al.*, 1980).

In addition, several nitroimidazole drugs are under current clinical investigation as radiosensitizers of hypoxic tumours (Wardman, 1977; Edwards *et al.*, 1980). Unfortunately, the neurotoxicity characteristic of such nitroaromatic compounds presently limits their usefulness in radiotherapy (Dische *et al.*, 1979).

Recently, while using metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole] in plant tissues we observed the inhibition of several enzymes involved in fatty acid synthesis (Harwood *et al.*, 1980). These included desaturases acting on stearate and linoleate as well as palmitate elongase. In view of the importance of the membrane in nerve-cell function, these observations on fatty acid synthesis may be of relevance to the neurotoxic effect of nitroimidazoles mentioned above. Accordingly, we have extended our experiments with metronidazole to include different plant systems and various alternative nitroimidazoles. In so doing, it was also hoped to see how specific were the previously observed effects of metronidazole on plant fatty acid synthesis (Harwood *et al.*, 1980).

Experimental

Materials

Imidazole drugs were gifts from the following sources: metronidazole from Dr. C. G. Curtis, University College, Cardiff, U.K.; misonidazole and compound Ro-05-9963 [1-(2,3-dihydroxypropyl)-2-nitroimidazole] from Roche Products, Welwyn Garden City, Herts., U.K.; nimorazole [1-(2-morpholinoethyl)-5-nitroimidazole] from Montedison Pharmaceuticals, Barnet, Herts., U.K.; compound RGW-611 [1-(2-morpholinoethyl)-4-nitroimidazole] from Dr. R. G. Wallace, Brunel University, Uxbridge, Middlesex, U.K., and compound AM-1 [1-(2-hydroxy-3-methoxypropyl)-2-methyl-4-nitroimidazole] from Dr. A. Michalowski, Instytut Onkologii, Warsaw, Poland.

[1-^{14}C]Acetate (sp. radioactivity 60 Ci/mol) and [2-^{14}C]malonyl-CoA (sp. radioactivity 53 Ci/mol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K., and ATP, NADPH, NADH and CoA were from Sigma (London) Chemical Co., Kingston upon Thames, Surrey

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KT2 7BH, U.K. Acyl-carrier protein was isolated from *Escherichia coli* (Sauer *et al.*, 1964). Other reagents were of best available grades as detailed by Bolton & Harwood (1977*a*).

Chloroplast isolation and incubation

Chloroplasts were isolated from locally-purchased lettuce (*Lactuca sativa*) by the method of Nakatani & Barber (1977) and were incubated under the conditions of Roughan *et al.* (1979). Incubations were terminated by the addition of KOH and fatty acids were extracted as detailed previously (Jones & Harwood, 1980). Chlorophyll was determined by the method of Bruinsma (1961).

Preparation and incubation of the soluble fraction from pea

Pea (*Pisum sativum* cv. Feltham First) seeds were purchased from Asmer Seeds, Leicester, U.K., surface-sterilized with NaOCl and germinated for 24h at 15°C. A particle-free supernatant was prepared from the seeds and incubated with [^{14}C]malonyl-CoA as detailed by Bolton & Harwood (1977*b*). Protein was estimated by the Lowry method (Lowry *et al.*, 1951) with bovine serum albumin as standard.

Experiments with pea seeds

Pea seeds were surface-sterilized and germinated in 0.5ml of water or drug solution containing 1μCi of [^{14}C]acetate/seed for 6h at 15°C. After this period a further 0.5ml of solution without radioisotope was added and germination was continued for a further 18h. After this period, the lipids were extracted as described by Harwood & Stumpf (1970).

Fatty acid analysis

Fatty acid methyl esters were prepared from the lipid extracts by the use of 2.5% (w/v) H_2SO_4/methanol. The methyl esters were separated by g.l.c. in 15% (w/v) ethylene glycol succinate silicone (EGSS-X) on Chromosorb W AW (80–100 mesh; Supelco, Bellefont, PA, U.S.A.) at 185°C. Glass columns (2m × 5mm int. diam.) were used and analysis was with either a Pye GCD or a Pye 104 gas chromatograph connected to a Panax radioactivity detector. Fatty acids were identified as described previously (Jones & Harwood, 1980). Total fatty acid radioactivity was estimated using PCS™ (Amersham–Searle)/xylene (2:1, v/v) scintillation cocktail and an Intertechnique PG 4000 counter. Quenching was estimated by the channels-ratio method.

Results

Since previous results with metronidazole had indicated that one of the most sensitive fatty-acid-synthesizing enzymes was the stearoyl–acyl-carrier-protein desaturase (Harwood *et al.*, 1980), we first tested the nitroimidazoles on a system that could form oleate. Chloroplasts from higher-plant leaves are known to carry out the desaturation of stearate to oleate with ferredoxin as the preferred electron-donor (Jacobsen *et al.*, 1974) and, since metronidazole was capable of being reduced by ferredoxin (Schmidt *et al.*, 1977), a rationale was available for its inhibitory action. The action of a series of nitroimidazoles on the incorporation of radioactivity from [^{14}C]acetate into fatty acids by isolated lettuce chloroplasts is shown in Table 1. Drugs with a redox

Table 1. *Effect of nitroimidazoles on fatty acid synthesis by isolated lettuce chloroplasts*

Fatty acid synthesis in the control samples was in the range 30–110nmol of [^{14}C]acetate incorporated/h per mg of chlorophyll. Results are means ± S.E.M. for independent experiments carried out in triplicate. Electron reduction potentials (referring to the reduction of the nitro radical anions) were obtained by pulse radiolysis (Wardman & Clarke, 1976). For details of the incubations and of the individual structures see the Experimental section.

Addition	Final concn. (mM)	E'_7 (mV)	Number of experiments	Fatty acid synthesis (% of control)
Compound Ro-05-9963	5	−389	2	38 ± 5
(desmethylmisonidazole)	20		2	29 ± 4
Misonidazole	5	−389	5	51 ± 4
	20		5	38 ± 6
Nimorazole	5	−457	2	87 ± 13
	20		2	51 ± 7
Metronidazole	5	−486	6	81 ± 5
	20		6	63 ± 6
Compound RGW-611	5	−554	3	151 ± 17
	20		3	108 ± 8
Compound AM-1	5	−564	2	235 ± 3
	20		2	207 ± 7

potential of −486 E'_7(mV) or less result in an inhibition of total fatty acid synthesis from [^{14}C]-acetate. The amount of inhibition was dependent on the concentration of the drug and on its redox potential, with more inhibition occurring for drugs with the less negative potentials. There was some evidence that the ability of the drug to partition into hydrophobic systems might also have some influence on its inhibitory effect. For example, misonidazole and its demethylated metabolite, compound Ro-05-9963, both have the same redox potential. However, misonidazole has a higher partition coefficient (octanol/water) of 0.43 in contrast with compound Ro-05-9963, where it is 0.11. At equivalent added concentrations, compound Ro-05-9963 was somewhat more inhibitory (Table 1). The two nitroimidazoles with the most negative redox potentials, compounds AM-1 or RGW-611, were not only non-inhibitory with regard to total fatty acid biosynthesis but, instead, caused a significant increase in the incorporation of radioactivity from [^{14}C]acetate.

Isolated lettuce chloroplasts characteristically synthesize a mixture of saturated fatty acids (laurate, myristate, palmitate) and oleate from [^{14}C]acetate (Jones & Harwood, 1980). When incorporation was examined in the presence of nitroimidazoles, a notable increase in the proportion of radioactivity present in stearate was seen. Whereas the fatty acid only contained 3% of the total radioactivity in fatty acids of control chloroplasts, this percentage increased as high as 28% in individual experiments with nitroimidazoles. Presumably, this was due to an inhibition of the stearoyl–acyl-carrier-protein desaturase, which is not normally rate-limiting for chloroplastic fatty acid synthesis (Stumpf, 1977). Interestingly, compounds AM-1 and RGW-611, which stimulated overall fatty acid synthesis, both resulted in accumulation of ^{14}C-labelled stearate like the other drugs. However, because of their stimulation of [^{14}C]acetate incorporation, net synthesis of oleate was increased. In addition, metronidazole also caused an increase in the relative labelling of myristate (Table 2) and a decrease in the relative amount of the total C_{18} fatty acids. These alterations by metronidazole indicate that the compound not only inhibits oleate synthesis but also palmitate elongation, such as has been observed in other systems (Harwood *et al.*, 1980, and see below). The build-up of myristate may be due to an action on fatty acid synthetase, which was found, in addition, with a soluble fraction from pea (see below).

It had previously been noted that metronidazole apparently inhibited the synthesis of stearate in germinating pea seeds (Harwood *et al.*, 1980). At concentrations of up to 20mM (the maximum solubility of the drug), no statistically-significant inhibition of total fatty acid synthesis was seen. However, the incorporation of radioactivity into stearate and

Table 2. *Effect of nitroimidazoles on the distribution of radioactivity between fatty acids synthesized from* [^{14}C]*acetate by isolated lettuce chloroplasts*

For details of incubations and nitroimidazoles see the Experimental section. Results are expressed as means ± S.E.M. (where appropriate) for independent experiments carried out in triplicate. Significance was analysed by Student's *t* test for paired samples. Abbreviation used: tr., trace (<0.5%).

Addition (concentration)	Number of experiments	Fatty acid ...	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids) 12:0	14:0	16:0	18:0	18:1
None (control)	8		3 ± 1	15 ± 2	40 ± 2	3 ± 3	37 ± 4
Compound Ro-05-9963 (20mM)	1		4	17	39	18	22
Misonidazole (5mM)	4		2 ± tr.	14 ± 1	45 ± 3	14 ± 4 ($P<0.10$)	25 ± 2
Misonidazole (20mM)	4		3 ± 1	16 ± 1	43 ± 1	12 ± 3 ($P<0.05$)	27 ± 3
Nimorazole (20mM)	1		2	16	42	17	20
Metronidazole (5mM)	5		3 ± 1	21 ± 4 ($P<0.01$)	41 ± 7	16 ± 8 ($P<0.05$)	17 ± 6 ($P<0.025$)
Metronidazole (20mM)	5		7 ± 4	24 ± 3 ($P<0.005$)	39 ± 8	16 ± 8 ($P<0.10$)	12 ± 6 ($P<0.01$)
Compound RGW-611 (5mM)	3		3 ± 1	11 ± 2	32 ± 5	22 ± 2 ($P<0.001$)	27 ± 3
Compound RGW-611 (20mM)	3		2 ± 1	15 ± 2	36 ± 3	22 ± 2 ($P<0.001$)	24 ± 1 ($P<0.01$)
Compound AM-1 (5mM)	2		2 ± 1	13 ± 2	32 ± 1	21 ± 1 ($P<0.10$)	33 ± 1

the very-long-chain fatty acids (C_{20} and greater) was inhibited (Table 3). In contrast, none of the other drugs affected the relative labelling of stearate. However, misonidazole inhibited the relative labelling of the very-long-chain fatty acids (Table 3) as did all the other nitroimidazoles (A. V. M. Jones, J. L. Harwood, M. R. L. Stratford & P. K. Stumpf, unpublished work).

The most active subcellular fraction for fatty acid synthesis that can be prepared from germinating peas is the soluble one (Bolton & Harwood, 1977*b*). Accordingly, this was tested directly with [^{14}C]-malonyl-CoA in the presence of nitroimidazoles. Fig. 1 shows that increasing amounts of metronidazole cause an increase in the relative proportion of [^{14}C]palmitate to [^{14}C]stearate. The increase in ratio occurs even before total fatty acid synthesis is affected. As expected, the subcellular system was more sensitive to metronidazole addition than the germinating seed, significant inhibition of total synthesis occurring at 5 mM or higher concentrations of metronidazole.

When other nitroimidazoles were compared with metronidazole in the soluble fraction from germinating pea a notable difference was seen (Table 4). Whereas metronidazole caused the inhibition of palmitate elongase, which resulted in increased radioactivity in myristate and palmitate, none of the other drugs caused any significant change in the pattern of ^{14}C-labelled fatty acids made. This agreed

Table 3. *Effect of nitroimidazoles on fatty acid synthesis from [^{14}C]acetate by germinating peas in vivo*

For details of incubation and analysis see the Experimental section. Total incorporation of radioactivity into fatty acids was in the range 10000–70000 c.p.m./seed. For statistical analysis see Table 2. Abbreviations used: n.s., not significant; VLCFA, very-long-chain ($C_{20:0}$, $C_{22:0}$) fatty acids.

Nitroimidazole (final concn.)	Number of experiments	Distribution of radioactivity (% of total ^{14}C-labelled fatty acids)		
		$C_{16:0}$	$C_{18:0}$	VLCFA
None	9	36±4	55±4	7±2
Metronidazole (20 mM)	6	57±2 ($P<0.05$)	38±3 ($P<0.001$)	2±1 ($P<0.05$)
Misonidazole (20 mM)	4	46±4 (n.s.)	53±2 (n.s.)	1±1 ($P<0.05$)

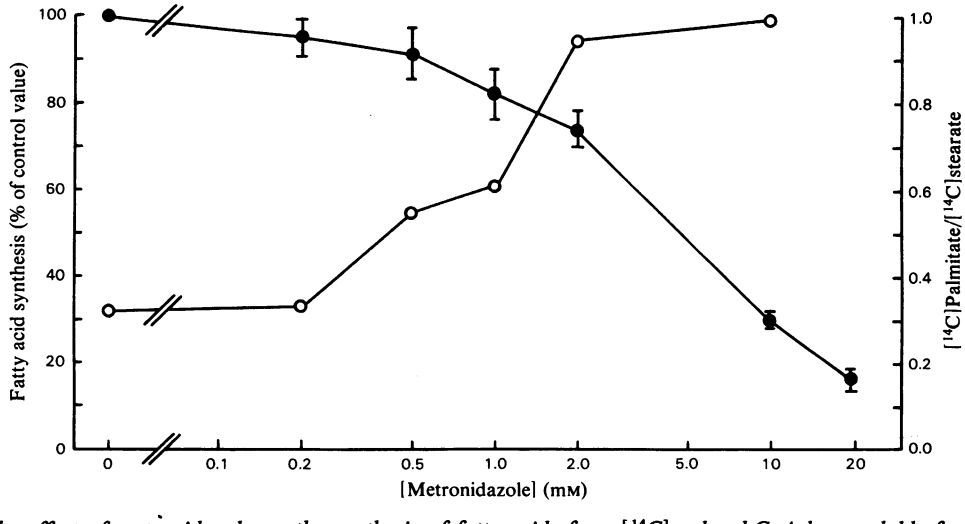

Fig. 1. *The effect of metronidazole on the synthesis of fatty acids from [^{14}C]malonyl-CoA by a soluble fraction from germinating pea*

●, Total fatty acid synthesis (% of control); ○, ratio of [^{14}C]palmitate/[^{14}C]stearate in the products.

Table 4. *Influence of nitroimidazoles on fatty acid synthesis from [^{14}C]malonyl-CoA by a soluble fraction from germinating pea*

Fatty acid synthesis was measured as described previously (Bolton & Harwood, 1977*b*). Means ± S.E.M. are given and statistical analysis was by Student's *t* test for paired samples.

Nitroimidazole addition (final concentration)	Number of experiments	Distribution of radioactivity (% of ^{14}C-labelled saturated fatty acids)		
		$C_{14:0}$	$C_{16:0}$	$C_{18:0}$
None	7	1 ± 1	58 ± 4	41 ± 5
Metronidazole (2 mM)	6	9 ± 3 ($P < 0.005$)	75 ± 4 ($P < 0.001$)	16 ± 8 ($P < 0.001$)
Misonidazole (5 mM)	1	tr.	53	47
Compound Ro-05-9963 (5 mM)	1	n.d.	41	59
Compound RGW-611 (5 mM)	1	2	48	50

with the data for intact seeds shown in Table 3, where synthesis of stearate was unaffected by imidazoles apart from metronidazole.

Discussion

Since metronidazole is believed to be active in organisms that are capable of reducing the drug (Edwards *et al.*, 1973) and because reduced ferredoxin is a suitable reductant (Schmidt *et al.*, 1977), an inhibition of stearoyl–acyl-carrier-protein desaturase was expected. This enzyme uses ferredoxin as the preferred electron carrier (Jaworski & Stumpf, 1974) and was severely inhibited by metronidazole (Harwood *et al.*, 1980). Other nitroimidazoles with less negative redox potentials than metronidazole would also be expected to act similarly in accepting electrons from ferredoxin and so preventing NADPH formation or desaturation. Indeed, as Table 1 shows, metronidazole and other drugs of lower redox potential inhibited fatty acid synthesis from [^{14}C]acetate by isolated chloroplasts. Such synthesis is dependent on photosynthesis (Stumpf & James, 1963) and the lowered synthesis of fatty acids is probably due to the smaller amounts of available cofactors such as NADPH. Compounds RGW-611 and AM-1, which are unlikely to be reduced by ferredoxin (redox potential approx. −420 mV), actually caused an increase in total fatty acid synthesis.

It was expected that oleate formation by isolated chloroplasts would be particularly impaired by those nitroimidazoles that also inhibited total fatty acid sythesis. This result is shown in Table 2. Because compounds RGW-611 and AM-1 cause a stimulation of total fatty acid synthesis, oleate formation, although reduced as a percentage of total ^{14}C-labelled fatty acids, is not significantly affected. However, stearate accumulation was seen with compounds RGW-611 and AM-1 to a similar extent to that with other imidazoles. This may be due to a stimulation of fatty acid synthetase and palmitate elongase such that stearate desaturation (which normally is very fast; Stumpf, 1977) becomes rate-limiting. Alternatively, they may inhibit stearoyl–acyl-carrier-protein desaturase directly. Since the redox potential of compounds AM-1 and RGW-611 is too negative for them to be reduced by ferredoxin, these nitroimidazoles would have to inhibit the desaturase in another way. In this connection, it should be mentioned that the imidazole misonidazole has been shown to inhibit cytochrome *c* oxidase and cytochrome *c* peroxidase while increasing catalase activity in *Candida albicans* (de Nollin *et al.*, 1977).

Likewise, the inhibition of palmitate elongase in a number of systems (Harwood *et al.*, 1980), including germinating peas (Tables 3 and 4), by metronidazole cannot be explained purely on the basis of redox potentials. The other imidazoles tested failed to alter the synthesis of stearate (Tables 3 and 4). Metronidazole therefore appears to be relatively specific in this regard and the inhibition may be compared with the varying actions of different imidazoles on mitochondrial respiration and oxidative phosphorylation (cf. Kerridge, 1980). However, as might be expected for hydrophobic molecules, which would partition into membranes to various extents, all the nitroimidazoles tested inhibited the synthesis of very-long-chain fatty acids (Table 3; see also the Results section). The elongation of stearate has been shown to be particularly sensitive to disturbances of the membrane environment (Bolton & Harwood, 1977*a*). There are a number of reports in the literature implicating imidazoles in the inhibition of membrane functions (cf. Kerridge, 1980). For example, membrane damage (Swamy *et al.*, 1974), changes in membrane transport (van den Bossche, 1974) and inhibition of membrane formation (van den Bossche *et al.*, 1978) have all been found.

In conclusion, we have found that nitroimidazoles have a number of effects on fatty acid synthesis in plants. Whereas all of the drugs tested affected

the desaturation and elongation of stearate, metronidazole also inhibited palmitate elongase. It appeared that their inhibitions were not simply due to the redox reaction of the imidazoles but also related to other actions such as the disturbance of membrane functions.

In view of the importance of the membrane in normal functioning of nerve axons, it is possible that these results could be relevant to the neurotoxicity of nitroimidazoles seen in the clinic. In this regard, metronidazole is less toxic than misonidazole and causes less inhibition of total fatty acid synthesis, whereas compound Ro-05-9963, the demethylated metabolite of misonidazole, which was predicted on pharmacokinetic grounds to be less toxic than misonidazole (Brown & Workman, 1980) owing to lower lipophilicity, has been recently shown to be unexpectedly equally toxic in man (Dische *et al.*, 1981), and in our study this drug in fact appeared to cause greater inhibition of fatty acid synthesis than misonidazole. Experiments with mammalian systems are required to see if the effects could indeed be relevant to the human situation.

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References

Bolton, P. & Harwood, J. L. (1977*a*) *Biochem. J.* **168**, 261–269

Bolton, P. & Harwood, J. L. (1977*b*) *Biochim. Biophys. Acta* **489**, 15–24

Brown, J. M. & Workman, P. (1980) *Radiat. Res.* **82**, 171–190

Bruinsma, J. (1961) *Biochim. Biophys. Acta* **52**, 576–578

de Nollin, S., van Belle, H., Goossens, F., Thone, F. & Borgers, M. (1977) *Antimicrob. Agents Chemother.* **11**, 500–513

Dische, S., Saunders, M. I., Flockhart, I. R., Lee, I. R. & Anderson, P. (1979) *Int. J. Radiat. Oncol. Biol. Phys.* **5**, 851–860

Dische, S., Saunders, M. I. & Stratford, M. R. L. (1981) *Br. J. Radiol.* **54**, 156–157

Edwards, D. I., Dye, M. & Carne, H. (1973) *J. Gen. Microbiol* **76**, 135–145

Edwards, D. I., Rowley, D. A., Knox, R. J., Skolimowski, I. H. & Knight, R. C. (1980) *Current Chemotherapy and Infectious Disease; Proc. ICAAC Am. Soc. Microbiol. 19th*, 561–563

Harwood, J. L. & Stumpf, P. K. (1970) *Plant Physiol.* **46**, 500–508

Harwood, J. L., Roberto, F., Murphy, D. J., McKeon, T. M. & Stumpf, P. K. (1980) *Biochem. Soc. Trans.* **8**, 543–544

Jacobsen, B. S., Jaworski, J. G. & Stumpf, P. K. (1974) *Plant Physiol.* **54**, 484–486

Jaworski, J. G. & Stumpf, P. K. (1974) *Arch. Biochem. Biophys.* **162**, 158–165

Jones, A. V. M. & Harwood, J. L. (1980) *Biochem. J.* **190**, 851–854

Kerridge, D. (1980) in *The Eukaryotic Microbial Cell* (Gooday, G. W., Lloyd, D., Trinci, A. P. J., eds.), pp. 103–127, Cambridge University Press, Cambridge

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randle, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Nakatani, H. Y. & Barber, J. (1977) *Biochim. Biophys. Acta* **461**, 510–512

O'Brien, R. W. & Morris, J. G. (1972) *Arch. Mikrobiol.* **84**, 225–233

Roughan, P. G., Holland, R. & Slack, C. R. (1979) *Biochem. J.* **184**, 193–202

Sauer, F., Pugh, E. L., Wakil, S. J., Delaney, R. & Hill, R. L. (1964) *Proc. Natl. Acad. Sci U.S.A.* **52**, 1360–1366

Schmidt, G. W., Matlin, K. S. & Chua, N-H. (1977) *Proc. Natl. Acad, Sci. U.S.A.* **74**, 610–614

Stumpf, P. K. (1977) *MTP Int. Rev. Sci.: Biochem. Lipids II* **14**, 215–237

Stumpf, P. K. & James, A. T. (1963) *Biochim. Biophys. Acta* **70**, 20–32

Swamy, K. H. S., Sirsi, M. & Rao, G. R. (1974) *Antimicrob. Agents Chemother.* **5**, 420–425

van den Bossche, H. (1974) *Biochem. Pharmacol.* **23**, 887–899

van den Bossche, H., Willemsens, G., Cools, W., Lauwers, W. F. J. & le Jeune, L. (1978) *Chem.-Biol. Interact.* **21**, 59–78

Wardman, P. (1977) *Curr. Top. Radiat. Res. Q.* **11**, 347–398

Wardman, P. & Clarke, E. D. (1976) *J. Chem. Soc. Faraday Trans. 1* **72**, 1377–1390