MAO activity proved to be linear for a minimum of 60 min at temperatures between 20 °C–37 °C. The Q_{10} (27 °C–37 °C) was found to be 1.9. At 0 °C no activity was found, which is interesting in view of the fact that snails can survive for months at this temperature. This, together with other data[13], showing an uptake mechanism for 5-HT to exist at 0 °C, supports the idea that MAO plays only a minor role in the inactivation of neurotransmitters in the snail.

MAO properties were studied in more detail in the suboesophageal ganglia. The K_m value for tryptamine was 2.1×10^{-5} M, similar to that found in the rabbit brain of 1.6×10^{-5} M[11]. Using 5-HT and DA as substrates, rather than tryptamine, it was found that 5-HT was deaminated at a rate of only 20% and DA only 50% of that of tryptamine. This contrasts with studies in the vertebrates where higher specific activities were found for 5-HT and DA substrates in contrast to tryptamine[11].

The effects of various drugs on MAO activity were also studied. Nialamide (10^{-4} M), a MAO inhibitor, produced a 50% decrease of MAO activity. Chlorpromazine (10^{-4} M), a DA receptor blocker, also inhibited MAO by 50%: Concentrations of less than 5×10^{-5} M of either drug had no measurable effect. Reserpine (5×10^{-5} M or greater) enhanced the MAO activity, with a 50% increase at 10^{-4} M. Incubation of ganglia with 5-HT (10^{-5} $M - 10^{-3}$ M) resulted in a non-linear decrease in measured activity, though this might be due to competition of the accumulated 5-HT with the ^{14}C-tryptamine.

The effects of pargyline (MAO inhibitor) were also examined. 5-HT deamination was only inhibited with pargyline concentrations greater than 5×10^{-4} M. DA and tryptamine deamination were both partially inhibited by pargyline between 5×10^{-4} M and 5×10^{-5} M. Little effect was seen at concentrations lower than 10^{-6} M. This is interesting in view of the fact that multiple forms of MAO have been proposed[14–16] to occur in the vertebrates, based on the differential degradation of various monoamines and the actual separation of 'isoenzymes' from purified preparations. The suggestion has been put forward that pargyline (MAO inhibitor) selectively inhibits the B type enzyme[17] (substrate tyramine) at low concentrations, while the A type enzyme (where 5-HT is a substrate) is only inhibited by higher concentrations of pargyline. DA und tryptamine (being) substrates for both types, are partially inhibited at low concentrations. Such is found to be the case in the snail. It is therefore possible that more than one form of MAO occurs in the snail, though the significance of this is not clear, especially in view of the apparant minor role MAO might have in snails.

The significance of the small but definite activity of MAO in the snail central nervous system is not clear, especially since other studies[13] have shown re-uptake of the released transmitter to be the probable mechanism of inactivation of DA and 5-HT in the snail. It may well be that MAO has an inactivating function in only a small proportion of nerve cells, but the 'background noise' of the other cells, caused when analyzing whole ganglia, blurs the discovery of this functional significance. However, it could be that MAO has an even distribution, as has been shown for other inactivating enzymes (acetylcholine-esterase[18] and catechol-*O*-methyl-transferase[19]). In this case, the enzyme could be functioning more as an extraneuronal regulating mechanism, guarding against non-specific stimulation by extraneous amines.

MAO activity in various tissues

Tissue	Tissue (nmole/g/min)	Protein (nmole/g/min)
Albumen Gland	ND	ND
Flagella	ND	ND
Radula retractor muscle	ND	ND
Liver	8.053 ± 0.390	62.11 ± 4.05
Kidney	2.506 ± 0.282	25.15 ± 2.81
Salivary gland	3.065 ± 0.268	39.09 ± 3.23
Spermatheca	0.465 ± 0.099	33.41 ± 0.52
Optic tentacle	0.105 ± 0.015	0.80 ± 0.15
Ventricle	0.067 ± 0.001	0.99 ± 0.08
Ganglia		
Buccal	3.253 ± 0.262	26.81 ± 4.30
Supraesophageal	1.502 ± 0.103	16.86 ± 1.07
Suboesophageal	0.904 ± 0.069	13.34 ± 0.81
Suboesophagel[a]	0.189 ± 0.069[b]	2.63 ± 0.96[a]
Suboesophagel[b]	0.415 ± 0.018[b]	5.77 ± 0.25[b]

Tissues analyzed as described in text with tryptamine as substrate except[a] with 5-HT as substrate and[b] with DA as substrate. ND indicates not detected. Values are ± SEM for n between 9-37.

Summary. The distribution and characterization of MAO in various tissues of the snail were analyzed. Only low amounts of the enzyme exist in the different tissues and data suggest that there is more than one type of MAO.

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Phospholipids of *Mycobacterium phlei*[1]

Amongst the phospholipids of mycobacteria, cardiolipin has been reported to be the major component in *M. phlei* (penso)[2], in an unclassified mycobacteria P_6[3] and also in $H_{37}Rv$, $H_{37}Ra$ and *M. avium* (all NCTC) strains[4]. Recently CHANDRAMOULI and VENKITASUBRAMANIAN[5] have reported from this laboratory that the cardiolipin is the major component in *M. smegmatis* and

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Table I. Individual phospholipid components of *M. smegmatis* and *M. phlei* strains[a]

Strain	Total lipids (mg/100 mg dry wt.)	Total phospholipid phosphorus (μmoles/100 mg dry wt.)	Phospholipid components (% of total phospholipid phosphorus)			Phospholipid components (μmoles per 100 mg dry wt. of bacteria)		
			CL[b]	PE[c]	PMx[d]	CL	PE	PMx
M. smegmatis CDC	10.00	4.50	45.0	22	27.0	2.02	0.99	1.22
M. phlei NCTC	6.32	3.60	32.0	25	40.0	1.15	0.90	1.44
M. phlei CDC	10.80	4.40	38.0	23	38.5	1.67	1.01	1.69
M. phlei NADL	10.00	5.00	37.5	21	38.5	1.88	1.04	1.93
M. phlei ATCC 354	11.50	4.10	29.0	14	46.5	1.19	0.57	1.91

[a]Average of 3 separate experiments. [b]CL, cardiolipin. [c]PE, phosphatidylethanolamine. [d]phosphatidylinositolmannosides.

$H_{37}Rv$ of NCTC; *M.* 607 (strain No. K 155 ATCC) and *M. avium* (TMC 701). In the course of study on the effect of environment on different constituents of mycobacteria, it was found that one of the strains of *M. phlei* showed a pattern different from other strains. This prompted us to investigate the levels of individual phospholipids in other strains of *M. phlei* also. To the best of our knowledge, this is the first report where phosphatidylinositolmannosides, monomannoside in particular, are the major component of phospholipids of mycobacteria.

Materials and methods. The cells were grown as steady cultures at 37 °C on YOUMANS and KARLSONS[6] medium, except that magnesium citrate was replaced by citric acid and magnesium carbonate. The cells were harvested during exponential phase of growth. Thus *Mycobacterium phlei* (ATCC 354), *M. phlei* (CDC, USA) and *M. smegmatis* (CDC, USA) were harvested on the 5th day, whereas *M. phlei* (NCTC, London) and *M. phlei* (NADL, USA) were harvested on the 10th day. Lipids were extracted by the method of Folch et al.[7]. Total lipids were determined gravimetrically. Phospholipids were separated by thin-layer chromatography using as solvent system chloroform: methanol: ammonia (115:45:7.5, v/v/v)[8]. Phosphorus was estimated by the method of BARTLETT as modified by MARINETTI[9]. Mannose was estimated by the method of DIETRICH and ANDERSON[10] and molar ratio of phosphorous to mannose was used as criterion for mono, di- and pentamannosides.

Results and discussion. Comparison of individual phospholipids as shown in Tables I and II reveals cardiolipin as major component in *M. smegmatis* CDC and phosphatidylinositolmannosides as major component in *M. phlei* ATCC 354 of total phospholipids. Among the individual phosphatidylinositolmannosides, monomannophosphoinositide is the major component in all the strains studied, in agreement with other reports[4,6,11].

M. phlei ATCC 354 seems to be a unique strain in that monomannophosphoinositide itself is more than the concentration of cardiolipin as against the opposite observation reported in other mycobacteria. Since as many as 12 strains of mycobacteria (5 in the present investigation and 7 reported elsewhere[4,5]) have been screened for their phospholipid components in this laboratory, the unique finding in case of *M. phlei* ATCC 354 could not be due to variations in extraction procedure and laboratory conditions, but is solely due to the characteristics of the strain itself.

Simultaneously, *M. phlei* ATCC 354 exhibits decreased levels of phosphatidylethanolamine. However, still lower values have been reported in some strains of *M. phlei*[12].

The presence of antibodies to phospholipids of mycobacteria has been demonstrated in tuberculous patients[13–15]. Phosphatides capable of reacting with corresponding antibodies in tuberculous sera has also been observed in *M. phlei* and *M.* 607. (both NCTC strains) and the antigen responsible for this serological reaction has been purified and identified as phosphatidylinositolmannosides[16]. The specificity of the phosphatide-antiphosphatide reaction suggests that phosphatidylinositolmannosides are of considerable value in the serodiagnosis of tuberculosis. Therefore, it is possible that *M. phlei* ATCC 354 has a high antigenic activity by virtue of its high content of phosphatidylinositolmannosides.

Table II. Individual phosphatidylinositolmannosides of *M. smegmatis* and *M. phlei* strains[a]

Strain	Total phospholipid phosphorous (%)			μmoles per 100 mg dry wt. of bacteria		
	PM_1[b]	PM_2[c]	PM_5[d]	PM_1	PM_2	PM_5
M. smegmatis CDC	14	11	2.0	0.63	0.50	0.09
M. phlei NCTC	27	10	3.0	0.97	0.36	0.11
M. phlei CDC	25	11	2.5	1.10	0.48	0.11
M. phlei NADL	26	10	2.5	1.30	0.50	0.12
M. phlei ATCC 354	32	11	3.5	1.31	0.45	0.14

[a]Average of 3 separate experiments. [b]PM_1, monomannophosphoinositide. [c]PM_2, dimannophosphoinositide. [d]PM_5, pentamannophosphoinositide.

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Résumé. La cardiolipine est le principal phospholipide dans *M. smegmatis* CDC. Il constitue le 45% du phospholipide total. Elle est suivie d'une quantité de phosphatidylinositolmannosides. Dans *M. phlei* ATCC 354 au contraire, ces derniers représentent le 46% du phospholipide total. Le monomannophosphoinositide y est présent en plus forte concentration que la cardiolipine.

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Aryl and Aniline Hydroxylases in Rat Nuclear Membranes after Pretreatment with Pregnenolone 16α-carbonitrile, Phenobarbital and Methylcholanthrene

The ability of pregnenolone 16α-carbonitrile, a potent catatoxic steroid, to protect rats against many toxicants[1], may be attributed to its capacity to induce mixed-function microsomal oxidases such as aryl hydrocarbon hydroxylase. The dual role of this enzyme system in either detoxification or in the formation of active intermediates in polycyclic hydrocarbon carcinogenesis has been established[2]. Pretreatment of rats with pregnenolone 16α-carbonitrile increases liver weight, stimulates smooth-surfaced endoplasmic reticulum proliferation in liver cells, and enhances NADPH cytochrome c reductase activity as occurs after phenobarbital treatment[3,4].

The inductive effect of pregnenolone 16α-carbonitrile in microsomes has been shown to be greater than that of phenobarbital but still less than the induction of the enzymes by methylcholanthrene[5]. However, the induction in microsomes of aniline hydroxylase, another inducible enzyme system, was the same for all 3 substances. KHANDWALA and KASPER[6] have recently demonstrated high levels of aryl hydrocarbon hydroxylase in the liver nuclei and nuclear membranes of methylcholanthrene-treated rats, but no differences were noted in the nuclei or nuclear membranes from phenobarbital-treated or control rats. Here we report that pregnenolone 16α-carbonitrile, which induced high quantities of aryl hydrocarbon hydroxylase and anilin hydroxylase in microsomes, as did methylcholanthrene and phenobarbital, was unable to induce the enzyme in purified nuclei and nuclear membranes.

For enzyme induction, male rats (WAG strain, C.E.S.A.L., Orleans, France) weighing 130–150 g were treated with phenobarbital, 3-methylcholanthrene, and pregnenolone 16α-carbonitrile in the following manner: 0.1% sodium phenobarbital was placed in the drinking water of 1 group of animals for 2 weeks, methylcholanthrene was injected into another group (20 mg/kg in 0.5 ml corn oil) once a day for 2 days, and a micronized suspension of pregnenolone 16α-carbonitrile in 2 ml water with a trace of Tween 80 was given per os to another group (50 mg/kg). The compound was administered at 8-h intervals (twice daily for 2 days and once on the 3rd day). Controls of injected rats received 0.5 ml corn oil only. The animals had free access to water and a standard diet. They were killed by decapitation 24 h after the last injection or last oral administration. The livers were excised quickly, chilled and weighed. The nuclei from rat liver were isolated (according to KASPER[4]) and the nuclear membranes were prepared by action of heparin on the nuclei as described by BORNENS[7]. The purity and integrity of both preparations were controlled by electron microscope studies.

Table I shows that, in the microsomes, methylcholanthrene and pregnenolone 16α-carbonitrile induce greater quantities of aryl hydrocharbon hydroxylase than phenobarbital. In the nuclei and nuclear membranes, however, neither pregnenolone 16α-carbonitrile nor phenobarbital induce the enzyme. On the other hand, methylcholanthrene, which induces 6 times more aryl hydrocarbon hydroxylase in the microsomes, induces 10 times more enzyme in the nuclei and 15 times more en-

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Table I. Specific activity of aryl hydrocarbon hydroxylase in the nuclei, the nuclear membranes and microsomes after induction by phenobarbital (PB), methylcholanthrene (MC) and pregnenolone 16α-carbonitrile (PCN)

Fraction	Treatment C	PB	MC	PCN
	(pMoles 3-hydroxy benzo(a)pyrene/30 min/mg protein)			
Nuclei	222 ± 48	202 ± 52 (−)[a]	2402 ± 342 (×10)	186 ± 42 (−)
Nuclear membranes	400 ± 120	416 ± 104 (−)	6956 ± 820 (×15)	422 ± 120 (−)
Microsomes	4200 ± 380	7450 ± 602 (×2)	26420 ± 2250 (×6.5)	12604 ± 620 (×3)

[a] The figures in parentheses show the degree of activation as compared to controls. Aryl hydrocarbon hydroxylase was determined by the method described by KINOSHITA and GELBOIN[2].