was observed. These results strengthen the view that nicotinic acid inhibits the cyclization of squalene.

The general mechanism of sterol biosynthesis in yeasts is similar to that of cholesterol biosynthesis in animals[12]. From rat liver slice experiments, Gamble and Wright concluded[13] that nicotinic acid inhibits cholesterol biosynthesis prior to MVA. However, these experiments have been criticized[4]. Since divergence of pathways in sterol biosynthesis in yeast as compared to animal tissues occurs after the formation of lanosterol[12], it is possible that nicotinic acid acts at the squalene cyclization stage rather than prior to MVA in animals. This possibility is under continuing investigation.

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DAVID M. BOWEN
ROBERT E. OLSON

Department of Biochemistry and Nutrition,
Graduate School of Public Health,
University of Pittsburgh,
Pittsburgh, Pennsylvania.

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Metabolism of Phenoxyacetic Acid by *Aspergillus niger* van Tiegh

A RECENT communication[1] reported the isolation of *o*-hydroxyphenoxyacetic acid as the main acidic product from the metabolism of phenoxyacetic acid by *A. niger*, using a replacement culture technique. Since these findings contrasted with our earlier work[2] in which both *o*- and *p*-hydroxyphenoxyacetic acids had been isolated, it was thought advisable to re-investigate the problem.

It has now been shown using both paper and thin-layer chromatography that while ortho-hydroxylation is predominant, all three hydroxyphenoxyacetic acids are in fact produced. While the *m*- and *p*-isomers have very similar R_F values in the three solvents used, their presence is clearly indicated by the characteristic colours of the diazo-coupled spots (*m*-isomer, bright yellow; *p*-isomer, salmon-pink). Exposure of the chromatogram to ammonia vapour changed the colour of the *m*-spot to magenta and that of the *p*-spot to bright blue. Furthermore, the relative positions of the spots are reversed in the two solvent systems used in the paper chromatographic examination.

Cultures of *A. niger* (Mulder strain, C.M.I. 31283) were grown in penicillin flasks and after 3 days the medium was replaced by a solution of phenoxyacetic acid (10^{-3} M) in aqueous disodium hydrogen phosphate (10^{-2} M). After 24 h at 26°, the substrate was poured off and concentrated in a cyclone evaporator, and the acidified concentrate continuously extracted with ether.

In a typical experiment, from 31 l. of fungal substrate initially containing 4·7 g phenoxyacetic acid, 3 g of sodium bicarbonate-soluble material was isolated. Separation was achieved by partition chromatography using silica gel as the supporting medium for the stationary aqueous phase which was buffered to *p*H 8, the mobile phase being *n*-butanol–chloroform (successively 60 : 40, 70 : 30, 80 : 20). After elution of unidentified material (0·9 g) and unchanged phenoxyacetic acid (1·42 g), the monohydroxyphenoxyacetic acids followed in the order *o*-(0·4 g), *m*-(0·02 g) and *p*-(0·1 g), details of their identification being given in Table 1.

Table 1. HYDROXYPHENOXYACETIC ACIDS

Substituent	R_F solvent *A*	R_F solvent *B*	Diazo coupling colour	λ_{max} *p*H 2	λ_{max} *p*H 11	Identification
Ortho-	0·53	0·60	Purple (orange centre)	275	290	Mixed m.p. U.V. and I.R.
Meta-	0·31	0·45	Bright yellow	273 278	286	Mixed m.p. U.V. and I.R.
Para-	0·27	0·51	Salmon pink	286	307·5	Mixed m.p. U.V. and I.R.

Solvent *A*: butanol/ethanol/3 N ammonia (4 : 1 : 5).
Solvent *B*: propanol/0·880 ammonia (7 : 3).

The difference between these findings and our earlier report[2] may reflect an improved separation technique or it may be due to a strain difference in *A. niger*. The culture used in the earlier work was Mulder strain, which had been in use at Long Ashton for some years previously; current practice is to renew the inoculum at frequent intervals from the Commonwealth Mycological Institute. The general pattern of hydroxylation appears to be relatively non-specific—resembling the metabolism of 2- and 4-chlorophenoxyacetic acids[3], but contrasting with results obtained in the metabolism of 2-naphthyloxyacetic acid[4] and with recent results obtained with 2,4-dichlorophenoxyacetic acid[5].

The metabolism of phenoxyacetic acid by a wild-strain of *A. niger* (kindly supplied by Dr. S. M. Bocks) has also been investigated. Thin-layer (propanol/0·880 ammonia (7·5 : 2·5) using 'Silica Gel G') and paper chromatographic examinations of acidic material obtained from four separate experiments using the replacement culture technique clearly reveal the presence of all three hydroxyphenoxyacetic acids, though the *m*- and *p*-isomers are present in a lower proportion than in the case of the Mulder strain.

D. R. CLIFFORD
D. WOODCOCK

Research Station
(University of Bristol),
Long Ashton,
Bristol.

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PHYSIOLOGY

Effect of Intraventricularly Injected Anti-cerebral Antibodies on the Histamine-like Substance and Potassium Content of Various Regions of the Brain of the Cat

IN an attempt to investigate the possible differential reactivity of various cerebral structures when exposed to the action of different brain region anti-sera, it has been shown that the injection of heterologous anti-caudate nucleus antibody into the lateral cerebral ventricle of the cat was followed by pronounced electrographic abnormalities, confined mainly to the caudate nucleus. Since the electrical activity of other, simultaneously investigated brain regions was not essentially modified, the alterations

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