

hydrolysis¹⁰ of the proposed intermediate II. An analogous intermediate (II, R = Cl) may also be formed in the detoxification of ethylene dichloride which, together with ethylene bromohydrin, is known to produce the same metabolites as EDB⁴.

Fertility studies in rats with S-(β -hydroxyethyl)-cysteine (III) given orally (5×1 g/kg) have shown that it has no anti-spermatogenic activity. Ethylene dimethanesulphonate at either 1×100 mg/kg intraperitoneally or 10×25 mg/kg orally produces infertility in male rats by direct action on the intermediate range of spermatogenic cells (spermatids and spermatocytes), whereas ethylene dibromide shows no comparable activity. This suggests that the anti-spermatogenic and the anti-tumour activity of EDS is not due to any of its isolated metabolites but involves a mechanism of specific cellular alkylation².

Zusammenfassung. Äthylenglykol-dimethansulfonat (EDS) wird von der Ratte zu N-Acetyl-S-(β -hydroxyäthyl)-cystein (V), N-Acetyl-S-(β -hydroxyäthyl)-cystein-S-oxyd (VI), und Methansulfonsäure abgebaut. Äthylen-

dibromid (EDB) gibt ausser V und VI, S-(β -Hydroxyäthyl)-cystein (III) und wird weiter zu Kohlendioxyd und anorganischem Sulphat via S-(β -Hydroxyäthyl)-cystein-S-oxyd (IV) abgebaut. Keine der erwähnten Stoffwechselprodukte sind gegen Tumoren oder gegen Spermatogenese wirksam.

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¹⁰ T. A. CONNORS and W. C. J. ROSS, *Biochem. Pharmac.* 7, 93 (1958).

¹¹ E. A. BARNESLEY, A. E. R. THOMSON and L. YOUNG, *Biochem. J.* 90, 588 (1964).

¹² S. M. BIRNBAUM and J. P. GREENSTEIN, *Archs Biochem. Biophys.* 39, 108 (1952).

Xanthine Oxidase Activity in the Brain

Brain has been reported to be devoid of xanthine oxidase (XO) activity (xanthine oxidoreductase 1, 2, 3, 2 I.U.B.)¹ and in the recent treatises of McILWAIN² and of FRIEDE³ there is no reference to the presence of this enzyme in the brain. However, a low activity was found in the brain of the dog and the sheep⁴. The enzymatic activity of xanthine dehydrogenase (XD) in the brain of the mouse was found to increase up to 10 times in the animals infected with yellow fever and other virus⁵, and it was reported recently that XO decreases sharply in the brain of the rat during narcosis produced by sodium barbital (Medinal)⁶. These contradictory results led us to reinvestigate the XO activity of the brain of some commonly-used animals for experimental research of the nervous system.

Wistar rats, white mice (average 20 g), adult guinea-pigs and male rabbits were decapitated and the brains rapidly taken out washed with ice-cold distilled water and blotted with Whatman No. 50 paper to remove blood clots. Brain was carefully separated from the cerebellum, weighed and maintained in the freezer until homogenized with phosphate buffer 0.025 M, pH 7.0 in a Potter-Elvehjem all glass homogenizer using a teflon pestle. The volumes of the homogenates were adjusted to 1:10 with the buffer and centrifuged for 15 min at 10,000 g and the supernatant pipetted off. Cow brains were obtained from the slaughterhouse and kept frozen until used. XO was measured spectrophotometrically as explained in the Table.

XD was estimated using Thunberg evacuated tubes containing in the side arm 0.4 ml of 0.1% of 2, 3, 5-triphenyl tetrazolium chloride (TPTC) as hydrogen acceptor and 0.052 μ moles xanthine. The formazan produced after 30 min incubation at 37°C was extracted with petroleum ether and measured colorimetrically at 495 nm. In these conditions low values were obtained but when pre-incubated 10 min with 0.28 μ moles of nicotinamide adenine dinucleotide (NAD)/ml of the supernatant, as hydrogen acceptor, the reaction increased markedly. The values expressed in μ g formazan produced per mg protein were 15, 19, 17.5, 10 and 9 respectively for the rat, the mouse, the

guinea-pig, the rabbit and the cow. Protein was estimated spectrophotometrically using the formula of KALCKAR⁷.

The results are summarized in the Table. The rat and the guinea-pig showed the highest values in the brain, but

Xanthine oxidase in brain and cerebellum of some animals

Animals	No.	XO in μ moles/mg protein	
		Brain range	Cerebellum average
Mouse	25	0.012–0.022	0.003
Rat	35	0.023–0.068	0.018
Guinea-pig	5	0.018–0.042	0.020
Rabbit	2	0.008–0.010	0.010
Cow	2	0.004–0.008	0.008

XO measured in 0.5 ml of the supernatant incubated with 0.05 μ moles of hypoxanthine and Tris buffer pH 7.4 for 2 h at 37°C and the reaction stopped by the addition of 2 ml 12% (v/v) perchloric acid. The filtrate adjusted to 4 ml with the acid. Absorbancy read at 290 nm and the results calculated in uric acid and expressed in μ moles produced/mg protein of the supernatant. 2 blanks run for each determination: one with all the reagents plus brain but not incubated and the other with all reagents except the substrate and incubated for 2 h.

¹ W. W. WESTERFELD and D. A. RICHTER, *Proc. Soc. exp. Biol. Med.* 71, 181 (1949).

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³ R. L. FRIEDE, *Topographic Brain Chemistry* (Academic Press, New York 1962).

⁴ U. A. AL-KHALIL and T. H. CHAGLIASSIAN, *Biochem. J.* 97, 318 (1965).

⁵ D. J. BAUER and P. L. BRADLEY, *Br. J. exp. Path.* 37, 447 (1966).

⁶ G. G. VILLELA, O. R. AFFONSO and E. MITIDIERI, *Biochem. Pharmac.* 15, 1894 (1966).

⁷ H. KALCKAR, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955), vol. 3, p. 451.

no XO was detected in the cerebellum of the mouse. In spite of the high XO of blood serum of the cow⁸, the brain presented comparatively low values. The failure of previous workers to find XD activity in the brain tissues could be explained by the low sensitivity of the methylene blue method used and the necessity to increase the dehydrogenation reaction by the previous incubation with NAD. The function of XO in the brain is not yet known; however, the variations in virus infection and the decrease during narcosis by barbiturates suggest a possible role of this enzyme in the metabolism of the central nervous tissues^{9,10}.

Résumé. La xanthine oxydase a été déterminée dans la partie surnageante des homogenisats centrifugés de cerveau et cervelle de divers animaux (souris, rat, cobaye, lapin, vache) par spectrophotométrie de l'acide urique produit après incubation de 2 h avec l'hypoxanthine. Le

cerveau du rat et du cobaye sont les plus riches en cette enzyme, mais la cervelle de la souris est dépourvue d'activité. La xanthine déshydrogénase s'est montrée plus active après incubation avec le NAD.

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⁸ G. G. VILLELA, O. R. AFFONSO and E. MITIDIERI, *Experientia* 12, 477 (1956).

⁹ The analytic reagents used were: xanthine (British Drug House), hypoxanthine chromatographic pure (California Corporation for Biochemical Research), NAD (SIGMA) and TPTC (Eastman Organic Chemicals).

¹⁰ The author is grateful to Conselho Nacional de Pesquisas (Brazilian Research Council) for a fellowship.

Fatty Acid Composition of Mycelium of *Penicillium chrysogenum* Grown in Different Carbohydrates as a Sole Source of Carbon

Carbohydrate constituents of mycelia of *Penicillium chrysogenum* grown under different carbon sources have been studied by IRANI and GANAPATHI¹. The various carbon sources used were pentoses, hexoses and sugar acids. The amounts of various components of carbohydrates changed with the change in the carbon source.

On similar lines we are presenting some of our observations on the gross fatty acid composition of the mycelium of *P. chrysogenum* grown on different carbohydrates as sole carbon source. The different carbohydrates used include: 3 pentoses, namely D-arabinose, D-xylose and D-ribose; 2 hexoses, glucose and fructose and a disaccharide, sucrose. The synthetic medium of GITTERMAN and NIGHT² was used to cultivate the mycelia and consisted of 5 g/l ammonium nitrate, 5 g/l potassium dihydrogen phosphate, 0.5 g/l magnesium sulphate (7H₂O), 0.1 g/l ferrous sulphate, 0.01 g/l zinc sulphate, 0.01 g/l manganese chloride and 0.01 g/l calcium chloride; pH was adjusted to 6.5 with NaOH. The desired sugar was aseptically added to the medium at the concentration of 2%. 100 ml medium in 500 ml Erlenmeyer flask was inoculated with 10 ml of the seed culture of *P. chrysogenum* (HA-6 strain) and grown on the rotary shaker giving 250 rpm for 48 h. Mycelia were harvested by centrifugation, washed with phosphate buffer and dried under vacuum.

The dried mycelia were finely powdered, and pre-weighed amounts of this powder were used to extract the lipid by FOLCH's method³ using 2:1 chloroform and methanol mixture. After determining the amount of lipid, this fraction was hydrolysed and methylated by the usual standard procedures. The methyl esters of fatty acids were separated and estimated on gas chromatogram with flame ionization detector on a 6-foot column of DEGS (20%), coated on chromosorb P at 195°C.

The results of the various analyses are given in the Table. It appears from the Table, that in the presence of pentoses as the sole carbon source, total lipids of the mycelia are kept low, while it is 2–3 times more when pentoses are replaced by hexoses or disaccharide as a sole carbon source. Though there is vast difference in the lipid content of the mycelia grown on pentoses and hexoses, the gross fatty acid composition of all the mycelia appears to be more or less similar. Fatty acids ranged from C-12 to C-20, although the lower acids C-12 to C-15 were observed to be present in traces. C-17 was almost absent and only

glucose grown mycelia showed traces of this acid. C-18:2 was maximal in all the mycelia amounting to about 40–50% of the total fatty acids. A good portion of C-16 was also noted while the rest were below 10% or in trace amounts.

Fatty acid composition of mycelium of *P. chrysogenum* grown on different carbon sources

	Carbon source used					
	D-arab- inose	D-xylose	D-ribose	D-glucose	D-fructose	Sucrose
Total lipids (g %)	4.67	6.67	4.33	12.7	18.7	19.0
Fatty acids	Percentage distribution					
C-12	Trace	Trace	Trace	Trace	Trace	Trace
C-14	0.31	Trace	Trace	Trace	Trace	Trace
C-15	0.63	Trace	Trace	1.8	1.25	1.2
C-16	36.37	25.95	27.5	23.8	24.25	17.3
C-16:1	0.47	0.25	0.4	3.0	0.7	1.15
C-17	—	—	—	Trace	—	Trace
C-18	3.55	8.60	4.2	9.0	12.4	8.85
C-18:1	4.31	18.70	5.4	4.7	9.6	11.25
C-18:2	54.32	40.4	53.8	48.0	36.0	53.0
C-20	—	2.1	3.0	4.5	5.8	6.3
C-20:4	—	3.75	5.7	4.2	9.8	0.65

Zusammenfassung. Der bisher wenig bekannte Fettsäuregehalt in Mycelien wird untersucht.

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