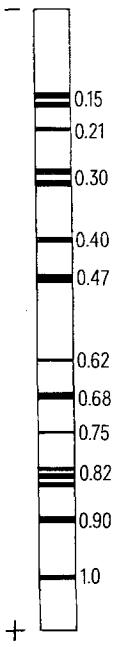

Fig. 2. A composite drawing of the neural retina and brain tissue bands in Figure 1 with mobilities indicated. Band 1.0 runs with the front and albumin has a mobility of 0.68 in this system.

Résumé. En examinant par électrophorèse en gel de polyacrylamide les protéines solubles de la rétine neurale et du cerveau du poulet au cours de sa croissance, nous avons comparé 15 bandes nettement visibles dans la série de ces deux tissus; 8 furent très semblables et 7 notablement différentes.

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[1] A. Lajtha, *Protein Metabolism of the Nervous System* (Plenum Press, New York 1970).
[2] E. M. Shooter and E. R. Einstein, A. Rev. Biochem. *40*, 635 (1971).
[3] B. J. Davis, Ann. N.Y. Acad. Sci. *121*, 404 (1964).
[4] L. Ornstein, Ann. N.Y. Acad. Sci. *121*, 321 (1964).
[5] B. S. McEwen and H. Hyden, J. Neurochem. *13*, 823 (1966).
[6] J. Alcala and A. LaVelle, Abstr. Am. Soc. Cell Biol. *11*, 6 (1971).
[7] This work was supported by a General Support Grant No. RR-5384-12 to Wayne State University School of Medicine from the National Institutes of Health.

Sulphite Oxidase from Pacific Hake (*Merluccius productus*)

Sulphite oxidase (sulphite:oxygen oxidoreductase E.C. 1.8.3.1) catalyzes the oxidation of sulphite to sulphate in mammals[1–5], plants[6] and bacteria[7]. With ferricytochrome c as the electron acceptor, the enzymic reaction may be depicted as: 2 ferricytochrome c^{3+} + SO_3^{2-} + H_2O = SO_4^{2-} + 2 ferrocytochrome c^{2+} + $2H^+$. The enzyme system can also transfer electrons from sulphite to a variety of other acceptors, e. g., molecular oxygen, methylene blue, ferricyanide, and 2,6-dichloroindophenol[1]. A clinical case of deficiency of the enzyme has been observed in an infant who excreted abnormally large quantities of sulphite and thiosulphate in the urine. The patient at 9 months showed severe neurological signs, mental retardation, and dislocated lenses[2].

Sulphite waste liquors are associated with activities of the paper and pulp industry. Because of possible effects of these spent liquors on fish, we decided to assay for sulphite oxidase in the Pacific hake (*Merluccius productus*), a commercially important fish of local waters. Buffer extracts (0.05 *M* K phosphate, pH 8.00) of acetone powder (75 mg/ml) prepared from hake liver were assayed spectrophotometrically by observing the oxidation of sulphite by ferricyanide through decrease in absorption of the latter at 420 nm. The reaction mixture

[1] R. M. MacLeod, W. Farkas, I. Fridovich and P. Handler, J. biol. Chem. *236*, 1841 (1961).
[2] L. Laster, F. Irreverre, S. H. Mudd and W. D. Heizer, J. clin. Invest. *46*, 1082 (1967). – S. H. Mudd, F. Irreverre and L. Laster, Science *156*, 1599 (1967).
[3] H. J. Cohen, I. Fridovich and K. V. Rajagopalan, J. biol. Chem. *246*, 374 (1971).
[4] H. J. Cohen and I. Fridovich, J. biol. Chem. *246*, 359, 367 (1971).
[5] D. L. Kessler, J. L. Johnson, H. J. Cohen and K. V. Rajagopalan, Biochim. biophys. Acta *334*, 86 (1974).
[6] P. Fromageot, R. Vaillant and H. Perez-Millan, Biochim. biophys. Acta *44*, 77 (1960).
[7] R. M. Lyric and I. Suzuki, Can. J. Biochem. *48*, 334 (1970).

Characteristics of sulphite oxidase from various sources

Characteristics	Sulphite oxidase		
	Fish (hake liver)	Mammal[4] (ox liver)	Bacterium[7] (*Thiobacillus thioparus*)
Type of protein	haemoprotein	haemoprotein	non-haemoprotein
Molecular weight	—	115,000	54,000
Absorption maximum (nm)	286, 410	280, 413	270
Optimum pH	8.7	8.6	8.0
Substrate	sulphite	sulphite	sulphite
Electron acceptor used	cytochrome c	cytochrome c	cytochrome c
Km for sulphite (*M*)	1.5×10^{-4}	1.4×10^{-4}	8.8×10^{-5}

contained 0.4 m*M* potassium ferricyanide, 0.4 m*M* sodium sulphite, 0.1 m*M* ethylenediamine tetracetate (EDTA) buffered at pH 8.7 with 0.1 *M Tris*-HCl, and 0.4 ml of acetone powder extract (final volume 5.0 ml). The assay was carried out for 10 min at 25 °C. Sulphite oxidase was demonstrated at a level of 3.3 μmoles/min/g acetone powder. For the measurement of Km of the partially purified enzyme, ferricytochrome c (horse heart, Type XII, Sigma Chemical Co.) was also used. In this case 10 μmole ferricytochrome c was applied instead of ferricyanide and the increase in absorbance at 550 nm followed as described above.

Since this was our first demonstration of this enzyme in fish, we undertook purification following a modification of the method of COHEN et al.[4]. Details of the purification procedure will be reported later. Much effort by various investigators has gone into characterization of sulphite oxidase from different sources. Electron paramagnetic resonance revealed that the enzyme from rat liver was found principally in mitochondria, but with lesser activity occurring also in nuclei, microsomes, and soluble fraction[5]. Characteristics of the enzyme obtained from several sources show considerable variability (Table). The bacterial enzyme contains a non-haemoprotein and hence is different from that of bovine liver. Molecular weights, kinetics, pH optima and inhibition by various substances also differ.

Our preliminary studies with the partially purified hake liver enzyme indicates that it resembles that of bovine liver. The partially purified fish liver enzyme solution showed strong absorption at 286 nm and at 410 nm. These observations suggest that the fish enzyme is a haemoprotein. The pH optimum was 8.7 and a Lineweaver-Burke plot yielded a value for the Michaelis constant of 0.15 m*M* for sulphite when ferricytochrome c was used as the electron acceptor in our system. The hake liver sulphite oxidase was inhibited strongly by mercuric chloride (also in some pulp mill effluents) and by cadmium chloride. 50% inhibition for these 2 heavy metal ions took place at 0.35 m*M* with mercuric chloride and at 0.40 m*M* with cadmium chloride.

Our studies reveal that hake liver serves to detoxify sulphite through conversion to sulphate. We are currently undertaking further purification of the enzyme to make refined studies on its structural, kinetic, and inhibition properties.

Zusammenfassung. Sulfitoxidase, ein Hämoprotein, wurde aus der Leber des Fisches *Merluccius productus* isoliert. Das Enzym katalysiert die Oxydation von Sulfit zu Sulfat, wobei Ferricytochrom c oder Ferricyanid als Elektronenakzeptor dienen können. Das Enzym zeigt ein Aktivitätsmaximum bei pH 8,7; Km für Sulfitsubstrat beträgt 0,15 m*M*. 50% Aktivitätshemmung durch 0,35 m*M* Mercurichlorid, bzw. 0,40 m*M* Kadmiumchlorid.

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Protein Synthesis of Monkey Lung Subcellular Fractions after Exposure to Silica in vivo

In contrast to the well established histological changes in the lung of primates following exposure to fibrogenic dusts (see SCHEPERS[1]), very little is known about concomitant biochemical changes. The work presented here records changes in protein biosynthesis by subcellular components (rough membrane and ribosomes) of the monkey lung after administration of silica in vivo.

Twenty vervet monkeys (*Cercopithecus aethiops pygerythrus*) were each injected intratracheally with a suspension of 250 mg silica in 5 ml physiological saline (for details of silica dust see reference[2]), and sacrificed 4 and 6 months later. A control group of 10 animals was kept under the same conditions for similar periods. Histological examination of lungs injected 4 and 6 months previously showed an interstitial fibrosis with nodules showing a significant degree of collagenization (I. WEBSTER, personal communication). Macroscopic nodules (up to 1.5 cm diameter) corresponding to the sites of maximum silica deposition in the lung, were resected prior to homogenization and were not included in the material used for this study. Methods for the preparation of ribosomes, rough membranes, cell sap and pH 5 enzyme fractions from monkey lung, and for the determination of incorporation of (^{14}C)-amino acids (from yeast protein hydrolysate) into hot TCA precipitable material, have been described in detail elsewhere[3,4]. Similar amounts of RNA (rough membrane or ribosome) and protein (cell sap or pH 5 enzyme) at optimal concentration were used in each assay. RNA was determined by the method of SCOTT et al.[5] as modified by FLECK and MUNRO[6], and protein by the method of LOWRY et al.[7].

Protein synthesis of rough membranes and ribosomes from silica-treated lungs was assayed in the presence of cell sap and pH 5 enzyme from both silica treated and control lungs and compared with control rough membranes and ribosomes (Table). A marked decrease of protein synthesizing activity of both subcellular fractions tested was found after 4 months. The results also demonstrate that the differences in activity from control cannot be ascribed to differences of soluble factors in cell sap or pH 5 enzyme fractions as these show similar effects whether derived from control or silica-treated lung. The lower overall incorporation both by rough membranes and in the presence of cell sap is in accord with previous results[4]. The reduction in protein synthetic activity is expressed per unit RNA content of ribosome or rough membrane and is therefore not an artefact of general morphological changes in the lung. 6 months after injection, no significant changes from the above results were noted.

[1] G. W. H. SCHEPERS, Arch. envir. Health *5*, 278 (1962).
[2] W. FITSCHEN and I. WEBSTER, Medna Lav. *59*, 161 (1968).
[3] W. FITSCHEN, S. Afr. J. med. Sci. *32*, 112 (1967).
[4] W. FITSCHEN, S. Afr. J. med. Sci. *32*, 119 (1967).
[5] J. F. SCOTT, A. P. FRACCASTORO and E. B. TAFT, J. Histochem. Cytochem. *4*, 1 (1956).
[6] A. FLECK and H. N. MUNRO, Biochim. biophys. Acta *55*, 571 (1962).
[7] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).