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.

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In vitro protein synthesizing activity of monkey lung subcellular fractions 4 months after intratracheal administration of silica

Subcellular particulate fraction	Soluble fraction	Incorporation of (^{14}C)-amino acids into protein	
		cpm/mg particle RNA	Control (%)[a]
Ribosomes	Cell sap		
	Silica treated	2880	45
	Control	3103	49
	pH 5 enzyme		
	Silica treated	22,055	66
	Control	24,561	73
Rough membranes	Cell sap		
	Silica treated	1770	48
	Control	1992	54
	pH 5 enzyme		
	Silica treated	9279	65
	Control	8180	57

[a] Control is the incorporation obtained with control particulate and soluble fractions.

The reduced overall protein synthesis, at a time when fibrogenesis is actively taking place, implies effects which are not discernible by histological examination. It remains to be established what effect the diminished protein synthesis has on the functional integrity of the lung[8].

[8] I wish to thank Mrs. G. KUNZ for technical assistance and Dr. I. WEBSTER for providing the monkeys used in this study.

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Zusammenfassung. Die Proteinsynthese der Lungenribosomen (frei oder membrangebunden) vom Affen nach Injektion von Silika wurde untersucht. Die Abnahme der Proteinsynthese nach 4 oder 6 Monaten ist nicht von löslichen Faktoren abhängig.

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Lathyrogenic Activity of 2 (2-Cyanoethyl)-3-isoxazolin-5-one from *Lathyrus odoratus* Seedlings

Several isoxazolin-5-one derivatives have recently been isolated from *Lathyrus odoratus* (sweet pea) seedlings[1,2]. One of these, 2 (2-cyanoethyl)-3-isoxazolin-5-one, component VIII, can be regarded as a new derivative of β-aminopropionitrile (BAPN), the well-known active principle[3–5] of the substance γ-GluBAPN in sweet pea seeds that produces changes in collagen in a variety of species including chicks, turkeys, baboons, rats and tadpoles. In the rat, effects of BAPN may include skeletal deformities, hernia and aortic rupture. In this report the isoxazolin-5-one derivative VIII has been examined in the weanling rat for signs of lathyrogenic activity.

Materials and methods. Component VIII was isolated from 10-day-old *L. odoratus* seedlings by extraction with ethanol and ion exchange chromatography as described[2]. Weanling white male Sherman rats, 33–38 g body wt., were used. The basal diet was Teklad 4% Mouse/Rat diet (Animal Feeds, Inc., Bronx, N.Y .) containing 24% crude protein, 4% fat, 6% crude fibre, supplemented with vitamin A, palmitate, irradiated dried yeast, niacin, calcium pantothenate, riboflavin, menadione, vitamin B_{12}, 1% calcium carbonate, 0.5% dicalcium phosphate, 1% salt, and traces of manganese oxide, copper oxide, cobalt carbonate, iron carbonate, zinc oxide, and calcium iodate. Comp VIII was incorporated as 0.4% of the basal diet. A control group received the equivalent (0.3%) of BAPN·HCl (Calbiochem. Calif.) and another control group received the unsupplemented diet. All diets were consumed ad libitum. After 18 days, rats were x-rayed under ether anesthesia.

After 10 days, urine was collected in metabolic cages over a 24-h period in the presence of thymol. Excreted BAPN was detected by electrophoresis of untreated urine on Whatman No. 3 paper in 0.1 *M* pyridine acetate buffer, pH 5.6, for 3 h at 10 V/cm. The sample contained material that travelled with the mobility of the authentic basic BAPN and gave its characteristic green color when treated with ninhydrin[6]. Excreted BAPN was determined quantitatively by chromatography on a Beckman Spinco analyzer[7] on the 15-cm column with Type 15A resin and 0.35 *N* sodium citrate buffer, pH 6.5, at 30 °C. The

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