plutonium concentration (Boocock, Popplewell & Taylor, 1968; Taylor, 1969).

The isotope ^{241}Pu is suitable for an electron-microscopic-autoradiographic approach to this problem, since the β -radiation emitted is of low mean energy, comparable with that of tritium. The resolution afforded by this isotope, which also has a half-life of 14 years, allowed the storage sites in hepatic parenchymal cells to be observed. At periods up to 4 days after intraperitoneal injection there is an increase in peribiliary dense bodies and approximately 70% of the developed silver grains were found in the vicinity of these organelles.

There are many reports of the uptake and disposal of foreign materials such as dextran, polyvinylpyrrolidone, sucrose, iron compounds and carbon (see Hampton, 1958; de Duve & Wattiaux, 1966) by hepatic parenchymal cells, which indicate the involvement of lysosomes with heterophagy.

The possible involvement of lysosomes and other components of the 'vacuolar apparatus' with the heterophagy occurring with ^{241}Pu will be discussed.

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Inhibition of Protein Biosynthesis by Salicylate in Gastric-Mucosal Scrapings of Pig and Man *in vitro*

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Salicylate (0.1–20 mM) has been shown to inhibit protein biosynthesis in the rat diaphragm and in cell-free systems of rat liver (Dawkins, Gould & Smith, 1966). Kent & Allen (1968) found that salicylate (15 mM) inhibited the incorporation of L-[U- ^{14}C]threonine into the glycoprotein fraction of sheep colonic-mucosal scrapings. An inhibitory effect of the drug on protein biosynthesis in the gastric mucosa may be relevant to the problem of salicylate-induced gastrointestinal damage in man. We therefore studied the effects of this drug on the incorporation of ^{14}C -labelled amino acids into the gastric-mucosal scrapings of pig and man.

The tissue preparations were incubated in the

presence of salicylate (3–60 mM) in Krebs medium III at pH 7.3 with added L-[U- ^{14}C]threonine or U- ^{14}C -labelled *Chlorella* protein hydrolysate. The time of incubation ranged from 5 min. to 3 hr. with pig mucosal scrapings and from 15 min. to 1 hr. with the human material. With the pig preparations a significant inhibition of L-[U- ^{14}C]threonine uptake was observed after 5 min. incubation with 6–60 mM-salicylate. After incubation for 5 min. with the labelled *Chlorella* protein hydrolysate, inhibition was obtained only with 30–60 mM-salicylate. With the human material, significant inhibition of the incorporation of the radioactive threonine occurred after 15 min. incubation with 30 mM-salicylate and after 30 min. incubation with 3 mM-salicylate. In all the experiments the inhibition usually increased with both time of incubation and concentration of salicylate.

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Bacterial Oxidation of Dimethylamine, a New Mono-oxygenase Reaction

By R. R. EADY and P. J. LARGE. (*Department of Biochemistry, University of Hull*)

Washed cell suspensions of *Pseudomonas aminovorans* (den Dooren de Jong, 1927), grown on dimethylamine hydrochloride as sole carbon source, oxidized dimethylamine, methylamine and formate (Q_0 values, endogenous subtracted, respectively 92, 52 and 32 $\mu\text{l./hr./mg. dry weight}$), but not methanol or formaldehyde. Semicarbazide hydrochloride (75 μM) produced 90% inhibition of methylamine oxidation while leaving dimethylamine oxidation unaffected (J. M. Cook, K. M. Dawson & P. J. Large, unpublished work). Crude extracts of dimethylamine-grown cells catalysed the NADPH-dependent formation of formaldehyde from dimethylamine. Methylamine could not replace dimethylamine. No methylamine-oxidizing activity has so far been detected in cell-free extracts of this organism (cf. *Pseudomonas* AM1; Eady & Large, 1968).

By using as assay the measurement of NADPH oxidation spectrophotometrically at 340 m μ , the dimethylamine oxidation system has been partially purified about sixfold. The preparation is rather labile but can be stabilized by the presence of 5 mM-dimethylamine hydrochloride. The reaction has a broad pH optimum over the range pH 5.8–7.3. The partially purified system catalysed the

stoichiometric oxygen- and NADPH-dependent conversion of dimethylamine into methylamine and formaldehyde (Eqn. 1).

The identity of methylamine as product was shown by comparison of the spectrum of its *N*-2,4-dinitrophenyl derivative (Dubin, 1960) with that of authentic methylamine, and by its activity with the purified primary amine dehydrogenase of *Pseudomonas* AM1 (Eady & Large, 1968). The enzyme had K_m 59 μM for dimethylamine and was inhibited by dimethylamine concentrations above 1 mM. NADH could replace NADPH as electron donor and was slightly (1.2-fold) more active. Extracts of succinate-grown cells had no detectable activity. The partially purified enzyme showed no significant activity with primary, tertiary and quaternary amines, nor with sarcosine. Diethylamine was about 28% as active as dimethylamine.

These results suggest that during growth of *P. aminovorans* on dimethylamine, the first step is the reduced nicotinamide nucleotide-dependent oxidative *N*-demethylation of dimethylamine by a mono-oxygenase reaction. We have not yet determined whether the enzyme is a single flavo-protein (cf. imidazoleacetate hydroxylase; Hayaishi, 1966) or a multicomponent system like the fatty acid ω -hydroxylase of *Pseudomonas oleovorans* (Peterson, Basu & Coon, 1966) or the camphor methylene hydroxylase of *Pseudomonas putida* (Katagiri, Ganguli & Gunsalus, 1968).

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Some Problems Associated with the Amino Acid-Sequence Analysis of Proteins by Mass Spectrometry

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In the mass-spectrometric sequence determination of oligopeptides one of the more difficult problems encountered has been the low volatility

of the free peptides. Of the procedures suggested to overcome this difficulty (see Biemann, Cone, Webster & Arsenault, 1966) we have used *N*-acetylation of the peptide methyl ester. In certain cases it has been necessary to enhance further the volatility by using the permethylation technique described by Das, Gero & Lederer (1967).

We have studied derivatives of various synthetic analogues of protein fragments in addition to several readily available small peptides. In every case it has been possible to determine the sequence by using only data obtained at low resolution together with a knowledge of the amino acid composition, though high-resolution techniques have proved valuable for confirmation. The results were sufficiently encouraging for an attempt to be made at the sequence determination of peptides isolated from a protein.

The additional problem was encountered of contamination both from the ion-exchange resin used to fractionate the enzymic digest, and from small amounts of peptide impurity travelling in the main peptide peaks. These small peptide impurities give rise to low-intensity peaks, which do not interfere with the interpretation of the main spectrum. In an extreme case we have been able to show that the sequence of two very similar peptides in an approximately equimolar mixture can be easily identified on the same spectrum. The contamination from the column seems to be a mixture of polymers dissolved out from the resin; these impurities can completely obscure the peptide sequence-determining peaks.

Although further separative procedures might be desirable we have been able to show that a satisfactory spectrum can be obtained without prior removal of the impurities. The sequence of an octapeptide isolated from a chymotryptic digest of silk fibroin by means of ion-exchange chromatography has been unequivocally determined by mass-spectrometric analysis of the permethylated derivative (Geddes *et al.* 1969).

The spectra were obtained on a GEC-AEI MS902 mass spectrometer, and we are grateful to Dr M. Barber and Dr W. A. Wolstenholme of the Consultants Laboratory for their co-operation. We also thank Dr F. Lucas of the Shirley Institute, Manchester, for his help, and the Muscular Dystrophy Association of America Inc. for financial support.

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