in semen from vasectomized rams were extremely low: acid phosphatase activity was <1% of the total activity of normal semen. Thus the bulk of 'soluble' enzymes recovered in the plasma presumably diffused from organelles (e.g. droplets and acrosomes).

We conclude that the acrosome is a specialized lysosome that has evolved to facilitate fertilization in multi-cellular organisms.

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Distribution and Behaviour of some Glycolytic Enzymes in Spermatozoa and Cytoplasmic Droplets from Mammalian Semen

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Semen from the bull, boar and ram normally contains, in addition to spermatozoa, small spherical particles 2–3 μ in diameter known as cytoplasmic droplets (Dott & Dingle, 1968). It is difficult to obtain a preparation of droplets essentially free of spermatozoa or a preparation of spermatozoa essentially free of droplets. One method for preparing droplets has been published (Dott & Dingle, 1968).

Another method has been developed whereby a single sample of semen can be separated easily into populations of droplets and spermatozoa, each preparation containing less than 5% contamination of the other particle. This method involves slow cooling and storage at 4° in a milk diluent, followed by slow differential centrifugation and washing in a sucrose medium.

Analysis of hexokinase, glucose phosphate isomerase and lactate dehydrogenase activities in our preparations revealed certain intrinsic differences in enzyme distribution between the spermatozoa and droplets: in particular, although both spermatozoon and droplet contain very large amounts of glucose phosphate isomerase (the amount per particle is approximately equal), the droplet contains less than one-tenth the amount of lactate dehydrogenase activity that is found in

the spermatozoon. Hexokinase is partly bound in each type of particle, glucose phosphate isomerase is entirely soluble, but lactate dehydrogenase is partly bound in the spermatozoon and soluble in the droplet.

Because of the great practical importance of storage of deep-frozen semen for artificial insemination, much research has been done on the mortality of spermatozoa when deep-frozen or cold-shocked, and emphasis has been placed on the leakage of enzymes from the spermatozoon during these treatments (Murdoch & White, 1968). We therefore cold-shocked, deep-frozen, and shocked hypo-osmotically our preparations and studied the leakage of enzymes by using glucose phosphate isomerase and lactate dehydrogenase as differential markers. The results show that the droplet preparations are very susceptible to all of the treatments, particularly cold-shock and deep-freezing, and leakage of enzymes occurs readily under these conditions. In all cases, although leakage from spermatozoa occurred, the droplets were more fragile and a much greater percentage of their total enzyme content leaked out.

Thus it seems that when enzyme analyses are carried out on seminal constituents, care must be taken to differentiate between the contribution from the droplets and the contribution from the spermatozoa.

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An Electron - Microscope - Autoradiographic Investigation of the Localization of ²⁴¹Pu in Rat Liver

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The intracellular sites of storage in the liver of various colloidal metals have been investigated as a result of the interest in 'bone-seeking' elements (Langham, 1959; Taylor, 1964). Plutonium has been shown to remain in the liver for several months (Taylor, Sowby & Kember, 1961). The reports from biochemical investigations of cell fractions have been conflicting as regards the association of injected plutonium with lysosomes. Rahman & Lindenbaum (1964) demonstrated a close relationship between lysosomal enzymes and cytoplasmic vacuoles containing segregated polymeric ²³⁴Pu in rat liver. Taylor and associates partially confirmed these findings but proposed in addition that mitochondria may also be sites of

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

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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