initiated by an acid protease as in cartilages (Ali *et al.*, 1967; Morrison, 1970; Dingle *et al.*, 1971), possibly cathepsin D. Extracts of autopsy specimens of the disc, from which almost all of the proteoglycans had been removed by precipitation with protamine sulphate (Dziewiatkowski *et al.*, 1968), contained significant activity against acid-denatured haemoglobin at pH 3.6. Supernatants obtained by using cetylpyridinium chloride or cetyltrimethylammonium bromide had much lower activities. At 37°C and pH 5 a protamine sulphate supernatant rapidly lowered the viscosity of the light fraction (PPL fraction) of purified disc proteoglycans, which contained a large proportion of keratan sulphate as well as chondroitin sulphate (unlike most cartilage proteoglycans). Incubation for 1.5 h resulted in complete disappearance of the excluded peak found for untreated PPL fraction in gel filtration on Sepharose 2B (Pharmacia Ltd.) and an increase in retarded polyanions, shown by monitoring turbidimetrically with rivanol (6,9-diamino-2-ethoxyacridine lactate) (Nemeth-Csoka, 1961). Preheating the protamine supernatant at 100°C for 15 min diminished but did not entirely eliminate its effects on the viscosity and gel-filtration pattern of PPL fraction.

Autolysis at pH 4–5 of slices of nucleus pulposus for 24 h at 37°C appeared to induce breakdown of the proteoglycans, as judged by gel filtration, but unequivocal evidence that the total quantity released from the tissue was increased was not obtained. Possibly partial autolysis had occurred before autopsy. A more definite mobilization of a proteoglycan fraction that resisted extraction with cold iso-osmotic saline from tissue slices before autolysis was demonstrated.

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Cation-Dependence of Phosphatase Activity in Bull Spermatozoa

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Active transport of alkali-metal cations in spermatozoa has been inferred both from studies on intracellular ionic concentrations (Quinn & White, 1967) and by the demonstration of a $\text{Na}^+ + \text{K}^+$ -stimulated ouabain-sensitive adenosine triphosphatase activity in whole sperm and in extracts (Uesugi & Yamazoe, 1966; Quinn & White, 1968). A metabolically dependent binding of ouabain to bull sperm has also been demonstrated (O'Donnell & Ellory, 1970). The present experiments investigated the ion-dependence of bull sperm nucleotide phosphatase activity in more detail.

Fresh washed bull spermatozoa suspended in an iso-osmotic sucrose medium buffered at pH 7.5 with 10 mM-tris showed an adenosine triphosphatase activity of $1.2 \pm 0.1 \mu\text{mol}$ of P_i/h per 10^8 cells at 37°C. Addition of MgCl_2 (1.5 mM) increased the activity to $3.2 \pm 0.3 \mu\text{mol}$ of P_i/h per 10^8 cells, and the addition of NaCl (165 mM) further stimulated the rate to $5.9 \pm 0.2 \mu\text{mol}$ of P_i/h per 10^8 cells. Rapid freezing and thawing of fresh washed cells gave a supernatant, after mild centrifugation, that had an activity of $3.2 \pm 0.2 \mu\text{mol}$ of P_i/h per mg of protein under the same conditions, and which was also stimulated by Mg^{2+} and further stimulated by Na^+ , or by $\text{Na}^+ + \text{K}^+$. Neither the whole cells nor the extract adenosine triphosphatase activity was inhibited by 0.1 mM-ouabain.

Both cells and extract hydrolysed ITP and CTP as well as ATP in the presence of Mg^{2+} , though further activation by alkali-metal cation was confined, in the extract, to the hydrolysis of ATP. Ca^{2+} could substitute for Mg^{2+} , and ADP but not AMP was also an effective substrate. *p*-Nitrophenyl phosphate was not hydrolysed. Whole sperm hydrolysed ATP more rapidly at alkaline pH, but the extract, in the presence of Mg^{2+} , Na^+ and K^+ , showed an optimum at pH 7.4. Whole cells and extract also differed in the efficacy with which other members of the alkali-metal cation series enhanced the Mg^{2+} -dependent adenosine triphosphatase; for the extract Na^+ was most effective and Cs^+ least so, whereas K^+ stimulated the cell activity most and Li^+ least (Li^+ was also least effective in maintaining the motility of intact cells when other alkali-metal cations were substituted for K^+ in an artificial incubation medium).

Unlike previous results, therefore, the present experiments did not detect any ouabain sensitivity in the Mg^{2+} -dependent adenosine triphosphatase activity of sperm that was stimulated by Na^+ or by $\text{Na}^+ + \text{K}^+$. The measured phosphatase activities were

not specific for Mg^{2+} or ATP, and are likely to be composites of several enzyme systems.

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An Electron-Microscopic Technique for Measuring Ribosomal Profiles from Cardiac Muscle

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Current methods of studying ribosomal profiles from cardiac tissue involve the use of sucrose density gradients and measurements of the absorbance. Such methods produce results that are difficult to quantify. An electron-microscopic technique has therefore been developed that attempts to describe ribosomal profiles in numerical terms.

Ribosomes and polyribosomes are separated from cardiac tissue by the method of Earl & Morgan (1968). One drop of a suspension of ribosomes is layered on to a carbon-coated Formvar-backed copper grid. The grids are positively stained with a 1% uranyl acetate solution in the manner described by Burghouts *et al.* (1907). They are then ready for electron microscopy.

Randomly selected fields are photographed with a magnification of 63000 and the subsequent print is enlarged twice. A differential count is performed on each photograph, the results being expressed as a percentage of the total number of ribosomes and polyribosomes per photograph.

Three studies were performed to determine the degree of variation in distribution of the ribosomal populations prepared in this way. In the first, ten micrographs were taken at regular intervals across an individual square of the grid. In the second, 30 micrographs were taken diametrically across an entire grid. In the third, five grids were spread with the same ribosomal preparation and four micrographs taken from each.

Differential counts were performed on each micrograph and the mean percentage of monoribosomes in each study was 42.1 (S.D. ± 4.8), 45.6 (S.D. ± 7.3) and 42.5 (S.D. ± 5.1) respectively. These results show that there is a random distribution of ribosomes between grids, within grids and within squares, and that the results are reproducible.

To examine the sensitivity of the method, it has been used to study the effect of actinomycin D on cardiac and hepatic ribosome profiles. Treatment with this drug produces an increase in the percentage of monoribosomes whereas the percentage of

polyribosomes containing six or more ribosomes decreases. This effect is more noticeable in the liver.

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Giant Mitochondria in Iron- or Copper-Deficient *Candida utilis*

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Dallman & Goodman (1970) demonstrated that rats fed on an iron- or copper-deficient diet developed enlarged mitochondria in their livers and hearts. These effects were more marked with copper deficiency. It was decided to investigate the possible generality of these phenomena, and we have therefore made an electron-microscopic study of *Candida utilis* cells grown in continuous culture under conditions of glycerol, iron or copper limitation (Light & Garland, 1971; Light, 1972; Downie & Garland, 1972). Sections of glycerol-limited cells were of typical appearance for aerobically grown yeast, showing up to 10–15 oval mitochondrial profiles per cell. The larger dimension of these mitochondria was about 0.4–0.5 μm . Sections of copper-limited cells exhibited in many instances as few as one mitochondrial profile per cell, the mitochondria being elongated to a length of 3.0 μm or more. Similar but less marked effects were observed with iron-limited cells. These observations provide strong evidence that the morphological changes described by Dallman & Goodman (1970) are due to abnormal composition of the mitochondria rather than to any more generalized whole-animal effect. It is of significance that deficiency of riboflavin (in mice) also causes mitochondrial enlargement (Tandler *et al.*, 1969). The mechanisms and controls that effect mitochondrial division are unknown; nevertheless it seems possible that one signal for mitochondrial division could be the presence of an inner mitochondrial membrane containing appropriate concentrations of cytochrome oxidase, other iron proteins and flavoproteins. Deficiency of any one appears to inhibit mitochondrial division.

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