Gregory & Cocking (1965) are washed two or three times with an appropriate osmoliticum (diluting the pectinase about 100-fold or more) and kept for a few days in a suitable culture medium.

New cell-wall formation was most marked in a medium containing high levels of Ca^{2+} and K^{+} ions. The medium contained 9·8g. of sucrose, 0·13g. of $CaCl_2$, 0·13g. of KCl and 4·0g. of mannitol in 100ml. of a modified White's medium as described by Lamport (1964) but without 2,4-dichlorophenoxyacetic acid or coconut water.

New cell-wall formation was discernable in the light microscope and was particularly evident following plasmolysis or following freezing and thawing. Cell-wall formation was also evident, but to a lesser extent, in a medium containing Ficoll and coconut milk with a lower level of Ca^{2+} solely provided by the White's medium. The medium contained 9·0g. of sucrose, 1·25g. of Ficoll and 5·0g. mannitol in 100ml. of the modified White's medium containing 10% (v/v) coconut milk; and in this medium bud-like protrusions from some cells were evident (cf. Mitra, Mapes & Steward, 1960). Further evidence for the presence of a new cell-wall was provided by the greater resistance of regenerating protoplasts to osmotic shock as cell-wall regeneration proceeded (cf. Yoshida, 1961), and from electron microscopic observations.

The regeneration observed is of some interest since it raises the question of the mechanism of deposition and the nature of the composition of the new cell-wall. The failure of Ruesink & Thimann (1966) to observe cell-wall regeneration in isolated higher plant protoplasts cultured in somewhat comparable media may be due to their use of cellulase to release protoplasts.

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Effects of Cortisol and of Actinomycin D *in vitro* on the Hormone-Sensitive Lipase of Adipose tissue

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The activity of the lipase was determined (Vaughan, Berger & Steinberg, 1964) in rat epididymal fat-pads incubated for 4hr. either in bicarbonate buffer containing bovine albumin, or human (or rat) serum diluted (1:1) with the buffer. The enzyme activity was found to be higher, using both media, as the glucose concentration was increased. Serum yielded greater activity than buffer of the same glucose concentration and was effective even after dialysis provided glucose or pyruvate was added. Addition of norepinephrine for the final 5min. of the incubation raised the lipase activity to the same level whether the medium was buffer, or diluted serum.

An increment in lipase activity was observed if cortisol (30μg./ml.) was added to either medium. This effect was still observed when norepinephrine was added. Actinomycin D (11μg./ml.) also produced a higher lipase activity when present during a 4hr. incubation in diluted serum, but not when added only for the final 15min.

As puromycin in amount to suppress protein synthesis did not alter the lipase activity, it is difficult to apply to this lipase the hypothesis made for lipoprotein lipase that actinomycin D is inhibiting the synthesis of a protein involved in destruction or inactivation of the enzyme (Eagle & Robinson, 1964). It is suggested that the observation of increased lipase activity, susceptible to activation by norepinephrine, in tissue incubated with cortisol confirms the opinion (Fain, Kovacek & Scow, 1965) that the glucocorticoid hormones promote the formation of more of the lipolytic enzyme during incubation. The property of actinomycin D and of a non-dialysable component of serum of 'stabilizing' the enzyme will be discussed.

Part of the work was done when the author was a visiting scientist of the U.S.P.H.S. at the laboratory of Dr Daniel Steinberg, National Heart Institute, N.I.H. The D actinomycin was a gift from Merck, Sharp and Dohme, Rahway, N.J.

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Combined Biochemical and Morphological Ultrastructure Studies on Mast-Cell Granules

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Reports by Lindahl, Cifonelli, Lindahl & Rodén (1965) and Lindahl & Rodén (1965) have indicated

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that extensively purified commercial samples of heparin obtained from both lung and mucosal tissue are still firmly associated with residual peptide materials. The aims of the present work included, firstly, the development of methods for preparing ^{35}S-labelled heparin, together with any covalently-bound protein, from mast-cell granules and, secondly, an assessment of the relationship of this macromolecular component to the morphological ultrastructure of the sub-cellular organelles.

Suspensions of peritoneal cells were prepared from specific-pathogen-free Wistar rats by the method of Bloom & Haegermark (1965), 48hr. after the administration of $Na_2{}^{35}SO_4$ (4μc/g. body wt.) to each animal via the tail vein. Mast cells were separated by density-layer centrifugation as described by Chakravarty (1965) and mast granules then isolated by the method of Lagunoff, Phillips, Iseri & Benditt (1964). Electron microscopy revealed that mast granules prepared in this way were of reasonably preserved morphology but devoid of perigranular membranes (cf. Bloom & Haegermark, 1965).

Mast-granule preparations were treated sequentially at 4° for 30min. with aqueous KCl solutions in the concentration range 0·05M to 2·0M and residual material subsequently isolated by centrifugation at 100000*g* for 45min. Portions of the residual material in each case were then examined by electron microscopy and the corresponding supernatant solutions analysed for total ^{35}S-sulphur, uronic acid (Balazs, Berntsen, Karossa & Swann, 1965) and heparin (Lagunoff & Warren, 1962).

Electron microscopic examination of residual material obtained after treatment with 0·05M-KCl showed that the granules had coalesced into a continuous mass which, however, still exhibited the beaded reticular network of the original structures. The reticular appearance was retained in the declining amounts of residue obtained after extraction with 0·5M-KCl and 0·7M-KCl. Treatment with 1·0M-KCl produced significant changes in the characteristic fine structure, the latter disappearing completely after extraction with 2·0M-KCl.

Analyses of supernatant solutions obtained by these serial extractions revealed only a limited appearance of radioactivity, uronic acid and heparin after treatment with 0·05M-KCl, the values increasing slightly after suspension in 0·5M and 0·7M-KCl. Approximately 25% of granule heparin was recovered after treatment with 1·0M-KCl and an additional 57% after suspension of the residue in 2·0M-KCl.

The 1·0M and 2·0M-KCl solutions were examined by polyacrylamide gel electrophoresis (Davis, 1964). The 1·0M-KCl extract was resolved into two radioactive, metachromatic bands, a minor one having a mobility identical with that of commercial heparin and a second major immobile component. The 2·0M-KCl extract contained only the immobile metachromatic component, which was also radioactive. When both extracts were pretreated with pronase at 40° or dilute alkali at 4° for 8hr., only components with mobility equivalent to commercial heparin were observed on polyacrylamide gel electrophoresis. No defined metachromatic bands were observed on the gels after the electrophoresis of 2·0M-KCl extracts pretreated with an enzyme preparation obtained from *Flavobacterium heparinum* previously grown in media containing commercial heparin.

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Evidence for the Covalent Association of Heparin and Protein in Mast-Cell Granules

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The natural occurrence of the sulphated polysaccharide heparin as a component of the granular cytoplasmic inclusions of mammalian mast cells is a matter of long standing record (see review by Bloom, 1965). It is noteworthy, therefore, that the behaviour on polyacrylamide gel electrophoresis of the heparin contribution obtained by the extraction of rat mast-cell granules with 2·0M-KCl is consistent with the existence of the polysaccharide in a form having macromolecular proportions considerably in excess of that of commercial heparin (cf. Barret, 1966).

In an attempt to test the validity of this view mast-granule preparations were made from groups

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