DEMONSTRATION

The Automated Fluorescent Assay of 4-Methylumbelliferone

By D. P. PAGE THOMAS and M. J. JAMES. (*Medical Research Council Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks.*)

The commercial availability of a variety of 4-methylumbelliferone substrates has enabled very sensitive assays of group-specific hydrolases to be

developed. Thus fluorimetric assays of a number of glycosidases, esterases, lipases, phosphatases and sulphatases are now possible. The automated assay of 4-methylumbelliferone to be demonstrated is based on the Unicam AC.60 chemical processing unit. This unit has been adapted to automatically operate a Locarte fluorimeter flow cell, and the resulting fluorescence signal is fed into a Bryans recorder. Data on stability, replication and examples of practical assays will be shown.

COMMUNICATIONS

Secretory Activities of Cells in Culture: the Effect of Ouabain

By J. T. GALLAGHER and J. C. MARSDEN. (*Department of Biology, University of York.*)

Recently we described a secretory-cell culture system derived from rat submaxillary glands (Gallagher & Marsden, 1969). The extent of incorporation of [^{14}C]glucosamine into non-diffusible materials recoverable from the culture medium can be used as a quantitative index of secretory activity.

The negative charge on the secreted products as demonstrated by electrophoresis suggests that some form of counterion may be involved in the secretory process. It has been shown that secretion of amylase from rat parotid glands is accompanied by release of K^+ (Bdolah, Ben-Zvi & Schramm, 1964). The cardiac glycoside ouabain is known to inhibit linked Na^+ and K^+ movement through membranes (Glynn, 1957), and when added to the culture medium of secreting cells to a concentration of 1 mM it inhibits extracellular macromolecule production by 60% ($P < 0.01$). This result suggests that transport of alkali-metal ions may play an important role in the release of negatively charged materials from secretory cells and offers some experimental support for a theory recently advanced as to the cause of the genetic disease cystic fibrosis (Marsden, 1969).

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N-Acetyl- β -D-glucosaminidase Activity in Congo Red-Induced Arthritis

By D. P. PAGE THOMAS and M. J. JAMES. (*Medical Research Council Rheumatism Unit, Canadian Red Cross Memorial Hospital, Taplow, Maidenhead, Berks.*)

Intra-articular injections of certain bisazo-dyes into rabbit knee joints have been found to result in an arthritis that mimics closely many of the histological features of rheumatoid arthritis in man (D. P. P. Thomas & M. J. James, unpublished work). Congo Red and Trypan Blue were found to be among the most active dyes, and reliably produced the characteristic inflammatory arthritis after two to four injections at weekly intervals. We have followed changes in activity of N-acetyl β -D-glucosaminidase in rabbit synovia at various phases of the experimental arthritis. This enzyme is normally present in measurable quantities in normal rabbit synovium; it is present in greatly increased amounts in human rheumatoid synovia (Hendry & Carr, 1963) and has been demonstrated to be of lysosomal origin in liver (Sellinger, Beaufay, Jacques, Doyen & de Duve, 1960). It is probable that synovial N-acetyl- β -D-glucosaminidase is also of lysosomal origin in view of its histochemical distribution as shown by the method of Hayashi (1965) and the need for hypo-osmotic extractive media with added Triton X-100 to induce rapid solubilization of the enzyme from tissue homogenates. As the extracellular release of lysosomal enzymes is thought to be instrumental in the causation of some of the pathological changes in rheumatoid arthritis (Dingle, 1962, 1966; Thomas, 1967), an attempt has been made to study the enzyme release by the excised synovia into a suitable tissue culture medium under standard conditions. The enzyme released during this incubation period is not thought to be due to tissue damage incurred during excision, as we have been