DEMONSTRATION

The Automated Fluorescent Assay of 4-Methylumbelliferone

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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

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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

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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that mimics closely many of the histological features of rheumatoid arthritis in man (D. P. P. Thomas & M. J. James, unpublished work).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

unable to detect any lactate dehydrogenase activity in a number of media that contained high activities of 'extracellularly' released enzyme, although lactate dehydrogenase activity is considerable in the synovial cells.

Rabbits that received either one or two injections of Congo Red showed similar patterns of enzyme activity for both the tissue-extract *N*-acetyl- β -D-glucosaminidase activity and the 'extracellular' enzyme. Increased activities were always found in the Congo Red-injected knees. The elevated enzyme activities in the animals that had received one injection fell to about normal by 2 weeks after the injection, but the enzyme activities were still slightly elevated in the animals that had received two injections 3 weeks previously. Changes in the elevation were even more marked in animals that had received four injections. Elevated enzyme activities were found for at least 6 weeks after the last injection. The relationship of the experimental disease process and the enzyme changes will be discussed.

The existence of sialic acid in one of the forms of *N*-acetyl- β -glucosaminidase suggests that this also may arise from membrane elements.

Plasma membranes were prepared from a rat liver homogenate in 1mM-NaHCO₃. This was filtered through muslin and centrifuged at 3000g for 5 min. in an MSE 18 centrifuge. The resulting pellet was resuspended in 1mM-NaHCO₃ and fractionated by zonal centrifugation in the MSE 'A' rotor at 5000 rev./min. for 2 hr. at 5°. The sucrose density gradient used was first steep [8–40% (w/w) in 300 ml.] followed by a slow rise in density (40–60%, w/w) in a further 900 ml. Much of the *N*-acetyl- β -glucosaminidase remained in the steep portion of the gradient, but a small peak of activity was recovered at $d = 1.22$. This fraction had 5'-nucleotidase and glucose 6-phosphatase activity, but very low amounts of other lysosomal enzymes, and when the particulate matter had been recovered by centrifugation electron microscopy revealed extensive sheets of plasma membranes, some still in apposition, but no traces of mitochondria or intact lysosomes.

Similar results have been obtained with a membrane preparation from human spleen prepared by the method of Takenchi & Terayama (1965), and a preparation of erythrocyte 'ghosts' has also been shown to have *N*-acetyl- β -glucosaminidase activity.

It is possible that the A form of the enzyme may arise from an association with plasma-membrane elements whereas the B form is that initially sequestered in primary lysosomes.

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Activity of Aminotransferases in the Tissues of Developing Lambs

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Ballard & Oliver (1965) have shown that liver slices from 4-month-old foetal sheep can incorporate pyruvate into glucose and that the rate of incorporation increases markedly by 1 week after birth. Since alanine and aspartate may be substrates for

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Localization of *N*-Acetyl- β -Glucosaminidase on Rat Liver Membranes

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Two forms of *N*-acetyl- β -glucosaminidase, distinguishable by their electrophoretic mobility, have been demonstrated in a number of tissues (Robinson & Stirling, 1968). The rapid anodic mobility of glucosaminidase A is attributed to sialic acid residues and both A and B forms occur in preparations of rat liver lysosomes. In the 3.5×10^5 g-min. supernatant from differential centrifugation of homogenates in 0.25M-sucrose, the A form predominates. Conchie & Hay (1963) and Weismann, Rowin, Marshall & Frederici (1967) have observed that the enzyme is tightly bound to particulate matter after the lysosomal particles have been ruptured, and other glycosidases, e.g. β -glucosidase (Beck & Tappel, 1968) and β -glucuronidase (Fishman, Goldman & DeLellis, 1967), are thought to be associated with membranes.