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.

Beck, C. & Tappel, A. L. (1968). *Biochim. biophys. Acta*, **151**, 159.

Conchie, J. & Hay, A. J. (1963). *Biochem. J.* **87**, 354.

Fishman, W. H., Goldman, S. S. & DeLellis, R. (1967). *Nature, Lond.*, **213**, 457.

Robinson, D. & Stirling, J. L. (1968). *Biochem. J.* **107**, 321.

Takenchi, M. & Terayama, H. (1965). *Exp. Cell Res.* **40**, 32.
Weismann, B., Rowin, G., Marshall, J. & Frederici, D. (1967). *Biochemistry*, **6**, 207.

Activity of Aminotransferases in the Tissues of Developing Lambs

By ELIZABETH M. SMITH, D. A. L. SHEPHERD and MARJORIE K. JEACOCK. (*Department of Physiology and Biochemistry, University of Reading, RG1 5AQ*)

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

Dingle, J. T. (1962). *Proc. R. Soc. Med.* **55**, 109.

Dingle, J. T. (1966). In *Modern Trends in Rheumatology*, p. 110. Ed. by Hill, A. G. S. London: Butterworths.

Hayashi, M. (1965). *J. Histochem. Cytochem.* **13**, 355.

Hendry, N. G. C. & Carr, A. J. (1963). *Nature, Lond.*, **199**, 392.

Sellinger, O. Z., Beaufay, H., Jacques, P., Doyen, A. & de Duve, C. (1960). *Biochem. J.* **74**, 450.

Thomas, D. P. P. (1967). In *Rheumatology*, vol. 1, p. 29. Ed. by Rotstein, J. Basle: S. Karger.

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

By D. ROBINSON [*Department of Biochemistry, Queen Elizabeth College (University of London), London W.8*] and J. L. STIRLING (*Makerere College, Kampala, Uganda*)

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.