Dr P. Johns (GGRA) dealt with the use of gelatine for the preparation of lymphocytes from whole blood. Gelatine has certain advantages over the other methods; it could be the basis of a one-step process, particularly important for rapid tissue typing. When gelatine solution is added to whole blood, the red cells sediment more rapidly, leaving behind the slower sedimenting white cells in an upper layer, which by simple means can be converted to a lymphocyte-rich suspension. Johns described experiments which showed the optimum conditions for the production of lymphocyte suspensions and the most suitable types and amounts of gelatine required.

ENZYME DEFECTS

Two Functions Missing

from our Medical Biochemistry Correspondent

THE absence of an enzyme with two different hydrolytic activities has been identified as the cause of glycogen II storage disease. This is an inherited metabolic disorder which is normally fatal within the first year of life, although in some cases it runs a more benign course. It is characterized by the presence of large quantities of glycogen in the liver and muscles in vacuoles which are probably enlarged lysosomes. Unlike the situation with other inherited abnormalities in glycogen metabolism, patients with this disease can use glycogen normally, and death seems to be due to destruction of muscle tissue by the disrupted lysosomes.

These patients have been shown to lack an a-1,4glycosidase which is present in normal human tissues. This enzyme, active at acid pH, was assumed to be in the lysosomes and could not be detected in the liver, heart or skeletal muscle of children with the fatal form of the disease. This lysosomal glucosidase has been purified recently from rat liver lysosomes and its kinetic properties have been studied (Jeffrey *et al.*, *Biochemistry*, **9**, 1403, 1416; 1970). It seems to have a-1,6-glucosidase activity as well as a-1,4-glucosidase activity. The two activities stayed together during purification and behaved in a similar manner, suggesting that a single enzyme protein in the lysosomes can completely break down glycogen in rat liver.

The same group has looked at normal human tissues to see whether the enzyme missing from patients with glycogen II storage disease had the same properties as those in rat liver. Tissue samples from ten patients and normal controls were obtained by biopsy or at autopsy within six hours of death and frozen quickly (Brown *et al.*, *Biochemistry*, **9**, 1423; 1970). Homogenates were assayed at pH 4.0 for *a*-1,4-glucosidase activity using both maltose and glycogen as substrates and for *a*-1,6-glucosidase activity using isomaltose as substrate.

No activity of either type could be detected in skeletal muscle, heart, leucocytes or fibroblasts from patients with glycogen II storage disease although there were very slight activities in some kidney homogenates. The individual variation in normal subjects was very large, but all samples contained considerable activity, much greater than the barely detectable traces in the kidney from the patients. Several patients with other abnormalities of carbohydrate metabolism were also examined, but their enzyme activities were all within the normal limits. It seems unlikely that all ten patients with glycogen II storage disease would have lost two distinct enzyme proteins, and so this work confirms the studies of rat liver lysosomal enzyme, and suggests that one enzyme protein has the two different hydrolytic activities.

The differences between this enzyme and a-amylase would be of great interest to molecular enzymologists, and the importance of this enzyme in normal human tissues is also an intriguing question. Attempts to treat glycogen II storage disease with fungal glycosidases have been disappointing, but another source of enzyme with the same properties as that lacked by patients might now be sought.

REPRODUCTION

Egg and Embryos

from a Correspondent

SPERM, eggs and embryos were the chief topics for discussion when the Society for the Study of Fertility held its annual conference at the University of Liverpool from July 14-18. Speaking in a symposium on spermatogenesis and sperm maturation, Dr D. W. Fawcett (Harvard) stressed the multiple function of the seminiferous epithelium in the production of both germ cells and exocrine secretions. With the aid of superb electron micrographs he also discussed the site of the so-called blood-testis barrier and its role in creating and maintaining the environment of the differentiating cells. Using a technique of micropuncture of seminiferous tubules, Drs B. P. Setchell and G. M. H. Waites (Cambridge) have been able to sample the exocrine secretions for chemical analysis. It is not yet clear, however, whether this fluid fulfils any function other than transport of spermatozoa. There was further evidence that cell biologists are turning their attention to the study of mammalian embryonic systems. Dr V. Monesi (Rome) emphasized the relative activity of the XY complex and the absence of ribosomal synthesis in spermatocytes. He thought it possible that in this situation histones act as gene repressors.

A symposium on eggs and embryos underlined the significance of the development of in vitro culture systems for the study of cellular events during early mammalian differentiation. Mouse embryos can now be cultured in chemically defined media from the single cell to the blastocyst stage, when they would normally implant in the uterus. Drs A. McLaren and T. Menke (Edinburgh) used carbon dioxide output as a measure of the metabolic state of mouse blastocysts. and suggested that the stimulus for implantation and for subsequent trophoblast outgrowth depends on a certain threshold of metabolic activity. Both Dr A. K. Tarkowski (Warsaw), who described how to induce parthenogenesis in the mouse by electrical stimulation of the oviduct (Nature, 226, 162; 1970), and Dr C. F. Graham (Oxford), who discussed the possibility of introducing alien genetic material into mammalian eggs by fusion with somatic cells using Sendai virus, emphasized that their principal aim is to understand normal development and not to produce parthenogenetic adult mammals.