PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 520th Meeting of the Society was held jointly with the Association of Clinical Biochemists at The Middlesex Hospital Medical School, London W1P 7PR, on Friday, 12 November 1971, when the following papers were presented:

COLLOQUIUM ON 'SPECIFIC BINDING PROTEINS FOR BIOLOGICALLY ACTIVE SUBSTANCES'

Cyclic Nucleotides and Thyroid Hormones: Radioimmunoassay or Protein-Binding Assay?

By R. P. EKINS and B. L. BROWN (*Institute of Nuclear Medicine, The Middlesex Hospital Medical School, Nassau Street, London W1N 7RL, U.K.*)

Traditionally, saturation assay techniques have relied upon specific antisera in the case of proteins and polypeptide hormones (radioimmunoassays), and on naturally occurring serum or cellular binding proteins in the case of the thyroid hormones, the steroid hormones and other similar classes of compound (protein-binding assays). Recent developments have run counter to this general rule, and radioimmunoassay techniques have now been developed for a number of non-protein compounds of relatively low molecular weight. Conversely, receptor-site protein-binding assays have been developed for the polypeptide hormones adrenocorticotrophin and glucagon, and there is little doubt that this tendency will continue as methods of identification and isolation of specific cellular receptors are evolved and exploited.

These developments raise some interesting theoretical points relating to the sensitivity and specificity of saturation assays that are relevant to the choice of the best experimental technique to adopt in particular situations. Some of these points are illustrated by assay techniques for the cyclic nucleotides [particularly cyclic AMP (adenosine 3':5'-cyclic monophosphate)] and the thyroid hormones (particularly tri-iodothyronine).

Both radioimmunoassay and protein-binding saturation assay techniques have been developed for cyclic AMP and cyclic GMP. Difficulty has been experienced in the raising of antisera against these compounds, and published observations suggest that the equilibrium constants displayed by the antisera used in reported methods is lower than that characterizing specific binding proteins (for cyclic AMP) found in muscle, adrenal and other tissues. On the other hand, specific antisera permit the use of iodine-labelled tyrosine-nucleotide conjugates of very high specific radioactivity as competitor reagents, which, in certain circumstances, can yield higher

assay sensitivity than is obtainable with cellular binding proteins by using relatively low-specific-radioactivity ^3H -labelled nucleotide. (A similar situation can arise in the radioimmunoassay of certain steroid hormones.) The circumstances under which each approach is the more advantageous will be discussed.

The thyroid hormones can likewise be assayed by using either specific antibodies or naturally occurring serum binding proteins. In neither situation is complete specificity achieved; in practice, however, the antibodies against the thyroid hormones are more specific against the individual compounds than are the binding proteins, and on both sensitivity and specificity grounds the antibody methods are clearly superior. The cross-reaction displayed by thyroxine and tri-iodothyronine with respect to thyroxine binding globulin nevertheless illustrates some basic theoretical conclusions relating to the relative potency of competing compounds in a saturation assay system. It is demonstrable that, in certain circumstances, a very weakly reacting competitor can display a potency very close to that of a much more reactive compound. These theoretical observations are clearly relevant to the interpretation of studies on the relative affinities with which a particular protein binds different, cross-reacting, ligands. They also emphasize that the specificity of a saturation assay system (with given reagents) is variable, and can be maximized by a suitable choice of reagent concentrations.

Protein Binding of Steroid Glucuronosides

By A. E. KELLIE (*Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London W1P 5PR, U.K.*)

A high proportion of steroid assays requested as a routine clinical service are carried out on urine and are designed to measure conjugates of steroid hormones and their metabolites, e.g. 17-oxo steroids, 17-hydroxy corticosteroids, pregnanediol, pregnanetriol, oestrogens etc.; most of these conjugates