PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **315th Meeting** *of the Biochemical Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* 1, *on Saturday,* 21 *February* 1953, *at* 11.0 *a.m. and took the form of a Symposium on:*

"BIOLOGICAL TRANSFORMATIONS OF STARCH AND CELLULOSE"

COMMUNICATIONS

The Biological Synthesis of Starch. BY E. J. BOURNE. (*Chemistry Department, University of Birmingham*)

A review will be given of the methods by which unbranched 1:4-α-glucosans of the amylose class have been synthesized *in vitro*. These include the following syntheses: (*a*) by phosphorylase from dipotassium glucose-1-phosphate, (*b*) by amylomaltase from maltose, (*c*) by Schardinger dextrinogenase from *cyclo*amyloses, and (*d*) by amylosucrase from sucrose. The formation of amylopectin and glycogen from amylose, by means of *Q*-enzyme and Cori's 'branching factor', will be discussed. It will be shown that all of these conversions involve transglycosylation, and thus fall into line with polysaccharide syntheses in general. Such reactions entail retention of the initial ring size and of the anomeric linkage. Attention will be drawn to the specificity of the enzymes and to the reversibility of the reactions. Some new results with the *Q*-enzyme of *Polytomella coeca* will be presented.

The Enzymic Breakdown of Starch. By W. J. WHELAN. (*Department of Chemistry, University College of North Wales, Bangor*)

The enzymes to be discussed under this heading are those which initiate reaction between starch molecules and water, resulting in the scission of glycosidic linkages and an increase in the number of free reducing groups. These comprise enzymes hydrolysing (i) α-1:4-linkages, (ii) α-1:6-linkages, (iii) α-1:4 and 1:6-linkages, and (iv) the β-linkages present in amylose. In their actions the enzymes fall into two groups, (*a*) enzymes able to penetrate the molecule and, subject to certain limitations, hydrolyse any appropriate linkage, e.g. α-amylase, *R*-enzyme, and (*b*) enzymes which can only hydrolyse linkages exposed at terminal non-reducing chain ends, e.g. β-amylase, amylo-1:6-glucosidase, glucamylase.

Recent unpublished work defining the action patterns and specificities of α-amylase, β-amylase and *R*-enzyme will be described, together with an account of the use of these enzymes in exploring the fine structures of ramified starch-type polysaccharides and in determining the proportions of branch linkages. A new type of enzyme action will be reported in which the presence of two active enzyme preparations (α-amylase and *R*-enzyme) is necessary before hydrolysis of the substrate takes place.

The possible *in vivo* functions of some of these enzymes will be discussed.

Starch Synthesis and Degradation *in vivo*. By HELEN PORTER. (*Research Institute of Plant Physiology, Imperial College of Science and Technology, London, S.W.* 7)

Starch synthesis occurs in plants when supply of primary assimilate exceeds capacity for utilization in growth; thus under conditions of nitrogen deficiency starch levels are high.

Starch formation may be induced in the dark, from sugars and related compounds, in most leaves, whether or not they normally contain starch. Etiolation does not prevent synthesis, and oxygen is necessary for both synthesis and degradation (Nurmia, 1935).

α-Amylase and phosphorylase are widely distributed, but these enzymes do not appear to attack the native starch grain, nor do the products of their action upon starch *in vitro* accumulate *in vivo*. Sucrose is the principal intermediate formed *in vivo*, and recently production of phosphate esters of glucose and fructose has been reported. The mode of synthesis of sucrose and its quantitative relation to starch formation is an outstanding problem of carbohydrate metabolism (Hassid & Putman, 1950;

Albaum, 1952). The presence of a thermolabile inhibitor, probably α-amylase, prevents synthesis of polysaccharide from glucose-1-phosphate (G-1-P) by many leaf extracts; similarly, β-amylase inhibits phosphorylase, and such interactions may be one way in which synthesis is regulated (Porter, 1952). Leaf disks will effect polysaccharide synthesis from G-1-P at the cut edges, and throughout the disk if entry is facilitated by pretreatment with toluene. The product is probably amylose (Stumpf, 1952; van Fleet, 1952). Toluene treatment, or addition of phosphate, at pH 7·0, inhibit synthesis in leaf disks, from simple sugars. Concentration of phosphate determines the degree of inhibition. Preliminary experiments using ^{14}C-labelled sugars indicate that fructose and glucose supplied together are equally available for starch synthesis, and that sucrose concurrently synthesized is labelled equally in both moieties, whichever hexose is initially labelled.

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The Chemistry and Chemical Degradation of Cellulose. By G. O. Aspinall. (*Chemistry Department, King's Buildings, Edinburgh*)

Cellulose, the chief constituent of the cell walls of the majority of plants, consists of long chains of 1:4-linked β-D-glucopyranose units. That over 99 % of the linkages are of this type is indicated by the high yield of 2:3:6-trimethyl D-glucose on hydrolysis of methylated cellulose and confirmed by studies of the kinetics of this reaction. Chemical measurements of chain length by the end-group method indicate a minimum value of 1700 glucose residues, and physical measurements of molecular size give values of 3000 or greater for the degree of polymerization of native cellulose.

X-ray studies show that the length of the unit cell along the axis of the cellulose fibre corresponds to that of two glucose residues linked as in cellobiose and X-ray studies together with chemical studies show that the fibre contains crystalline and amorphous regions. Hydrogen bonding occurs between the hydroxyl group of adjacent cellulose chains in the crystalline region, and the high tensile strength of cellulose fibres can be explained on the assumption that cellulose chains run through two or more crystalline regions.

Chemical degradation of cellulose occurs with acids and with oxidizing agents. The mechanical strength of the fibres disappears on treatment of cellulose with dilute acids with the formation of hydrocellulose (D.P. *c.* 200). The products of oxidative breakdown are either acidic in character due to oxidation of the —CH_2OH groupings or aldehydic in the case of glycol-splitting reagents. Preferential reaction of certain residues in the cellulose chain has been explained chemically on the assumption of periodic modified linkages and physically on the basis of the greater accessibility of the amorphous regions.

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Cellulases. By M. V. Tracey. (*Rothamsted Experimental Station, Harpenden, Herts*)

Until about 20 years ago the snail was the source of material for work on cellulase. In the recent war textile deterioration in the tropics assumed great economic importance; consequently knowledge of micro-organisms producing powerful cellulases was rapidly expanded. Recently, research on rumen digestion has added further to our knowledge of microbiological cellulase producers. It is only in these two fields that our knowledge of the distribution of cellulase is at all thorough. The known distribution of cellulase among other organisms is extremely patchy. Differentiation between cellulase

production by an organism with a gut and production of cellulase by organisms living in that gut is often difficult in the smaller invertebrates. It seems likely, however, that cellulases are produced by some molluscs and arthropods.

The first report of the preparation of a cellulase in an apparently pure state appeared a year ago (Whitaker, 1951). For the first time some evidence can be produced about the course of enzymic cellulose breakdown. It had been, and still is, assumed that this is formally similar to the breakdown of amylose by β-amylase and maltase. More recent work has led to the supposition that an additional 'disaggregating' enzyme is involved. Whitaker's results using purified *Myrothecium* cellulase suggest that this enzyme is capable alone of the complete hydrolysis of cellulose to glucose and that interpretation of results of the action of crude extracts may be made uncertain by the absorption phenomena inevitable in the action of an enzyme on an insoluble polymeric substrate.

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Digestion of Cellulose by Ruminants. By A. T. PHILLIPSON. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

The ability of the ruminant to digest large quantities of cellulose is the result of the activity of the bacterial population that inhabits the alimentary tract and, in particular, the rumen. Crude fibre as analysed by the current agricultural procedures is a fair measure of cellulose, and the two terms will be considered as synonymous. In spite of the importance of cellulose as a foodstuff to ruminants, very little is known of the stages of its digestion in the rumen. The end-products of fermentation found by the *in vitro* incubation of filter-paper by rumen bacteria appear to be largely acetic and propionic acids and carbon dioxide (Elsden, 1946; Marston, 1948); the intermediate stages that occur in the rumen are unknown, except that succinic acid is known to be a precursor of at least some of the propionic acid (Sijpesteijn & Elsden, 1952). It is noteworthy that acetylated or methylated celluloses are indigestible.

When natural fodders are fed potentially digestible fibre is excreted in the faeces, and although some 45–60 % of the total fibre disappears as it passes through the alimentary tract more could do so if it remained in the rumen long enough. Further digestion occurs in the caecum and colon, but this is slight compared to digestion in the rumen and does not remove all the potentially digestible fibre. Finely divided particles of food pass through the rumen more rapidly than large particles when both are fed together, but if the whole ration is ground it remains there for an abnormally long period and, strangely enough, fibre is less completely digested (Balch, 1950). The reason for this is not known.

Digestion of fibre is lowered by several other factors. Feeding starch or other soluble carbohydrates in quantity depresses the digestion of fibre, although this can to some extent be counteracted by increasing the protein of the feed. Increasing protein alone, however, also depresses fibre digestion, even though the fodder by itself is low in nitrogen. Cellulose-fermenting bacteria apparently receive adequate nitrogen from the fodder itself. Only one dietary factor increases digestion of fibre: adding the ash of lucerne at the rate of 3·7 % to a ration containing coarse fodder and a total fibre content of 17·8 % increases digestion of fibre by some 10 %; methane excretion is also increased (Swift, Cowan, Barron, Maddy & Grose, 1951). It is not known which of the mineral constituents of this ash is responsible for this effect. Rumen fluid normally contains nearly as much potassium as sodium, and only small concentrations of soluble calcium and magnesium. In contrast lucerne ash is rich in calcium and magnesium; sufficient data is not available to make a direct comparison of all inorganic ions and little is known of the trace element composition.

It seems probable from these facts that cellulose-fermenting bacteria are influenced by the inorganic composition of the media in which they live and, consequently, laboratory media for the cultivation of cellulose-fermenting bacteria should conform to the conditions in the rumen; this has been generally recognized as far as phosphorus is concerned but not as regards cations. The results of adding lucerne ash to the feed suggest that the only practical way of increasing cellulose fermentation in the rumen—apart from chemical treatment of the natural fodder—appears to be to produce optimal conditions by the judicious use of mineral supplements once the optimal conditions are known.

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The Occurrence and Prevention of the Biological Attack of Cellulose Textile Fibres. By R. G. FARGHER. (*Shirley Institute, Didsbury, Manchester* 20)

Since 1939, the pioneer work on the occurrence and prevention of the biological attack of cellulosic fibres, most of it undertaken in this country, has been extended considerably, particularly in the United States. Nevertheless, the biological techniques have been so variable and the failure adequately to characterize the substrates so frequent that the available data permit only a qualitative interpretation.

To the textile technologist, biological attack has two connotations. The first, and most common, is disfigurement without appreciable change in strength. The second, loss of strength ('tendering') of the fibres, is encountered less frequently, principally because of the high moisture requirement of the organisms which attack cellulose.

Apart from temperature and moisture content, the attack of cellulosic fibres by micro-organisms is controlled by the purity and crystallinity of the cellulose. It is very localized and occurs most readily in the least crystalline portions. As these are the most chemically reactive, resistance to attack can be enhanced by partial esterification—or by association with certain hydrous metallic oxides.

Resistance may also be increased by the 'barrier' protection claimed for the lignin component of the bast fibres or by the incorporation or organic antiseptics. The efficacy of insoluble copper compounds in 'burial' tests may afford a good example of barrier protection. The organic antiseptics present two interesting problems, namely, the very limited relationships between chemical constitution and toxicity and the comparative ease with which some of them are decomposed by micro-organisms.