& Layne (1970). Demonstration of bilirubin glucoside in dog bile (Heirwegh, Van Hees, Compennolle & Fevery, 1970) stimulated attempts to synthesize *in vitro* xylose and glucose conjugates of bilirubin.

By incubating microsomal preparations from rat liver with bilirubin and UDP-glucuronic acid, UDP-xylose or UDP-glucose in the presence of Mg^{2+} transfer of the sugar moieties to bilirubin was obtained. Reaction rates were measured quantitatively by treatment of the conjugated pigments with diazotized ethyl anthranilate (Van Roy & Heirwegh, 1968). Analysis of the derived azopigments (Heirwegh, Van Hees, Leroy, Van Roy & Jansen, 1970) allowed structural elucidation of the synthesized bilirubin conjugates. The same types of conjugates were formed by using either digitonin-activated or untreated enzyme preparations.

In the presence of either UDP-xylose or UDP-glucose conjugate formation was optimum at pH 7.1. Digitonin treatment increased reaction rates by the factor 2.1. Conservation of the sugar moieties was indicated by t.l.c. with known preparations of azobilirubin β -D-xylopyranoside and of azobilirubin β -D-glucopyranoside. Enzymic hydrolysis of the conjugates with emulsin and Mylase-P together with inhibition studies with glucono-(1 $\rightarrow$ 5)-lactone demonstrated that the conjugates derived from both UDP-sugars are in the β -D-configuration.

With UDP-glucose bilirubin β -D-monoglucoside was formed under a variety of reaction conditions. With UDP-xylose mixtures of β -D-monoxylsides and β -D-dixylsides were obtained. Formation of the dixylsides was favoured by, e.g., lowering the pH of the incubation mixtures.

Previous studies of bilirubin UDP-glucuronyl-transferase with digitonin-activated (Heirwegh & Meuwissen, 1968) and untreated preparations (Van Roy & Heirwegh, 1968) were completed. Enzyme activities were increased 5–15-fold by digitonin treatment and were optimum at pH 7.9. Under all conditions bilirubin β -D-monoglucuronide was formed, as indicated by t.l.c. of derived azopigments, with azobilirubin and azobilirubin β -D-monoglucuronide as reference substances (Compennolle, Jansen & Heirwegh, 1970). The β -D-configuration of the synthesized conjugate was indicated by complete hydrolysis of the derived conjugated azopigment with β -glucuronidase and complete inhibition of the process by saccharo-(1 $\rightarrow$ 4)-lactone.

Relative values of the transferase activities found with UDP-glucuronic acid, UDP-xylose and UDP-glucose were 1:2.1:1.4 for untreated microsomal preparations and 1:0.38:0.25 after activation with digitonin.

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The Incorporation of Glycylglycine into Humic Acid

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A large proportion of the nitrogen present in soil humic acid can be released as amino acids by acid hydrolysis (Bremner, 1955). Of the remainder, it is probable that some exists as α -amino nitrogen. The association between α -amino nitrogen and aromatic units within humic acid has remained a problem, although Haider, Frederick & Flaig (1965) and Ladd & Butler (1966) have presented possible solutions.

We have investigated the reaction between humic acid and specifically radioactively labelled glycylglycine. The humic acid was extracted from the A_0 horizon of a Hiraethog podzol (Ball, 1960) with 0.1 M-sodium hydroxide. [$1^{14}C$]Glycylglycine and glycyl[$1^{14}C$]glycine were each allowed to react with humic acid in sodium borate-HCl buffer, pH 8.5. The humic acid products of each reaction were radioassayed and subjected to hydrolysis with 6M-hydrochloric acid for 24h at 110°C. The amino acids released on hydrolysis were separated by ion-exchange chromatography from the non-hydrolysable residue and non-cationic acid-soluble material.

The specific radioactivities of the humic acid products of each reaction before hydrolysis differed by 9%. The non-hydrolysable fraction of humic acid that had been allowed to react with [$1^{14}C$]glycylglycine contained 11.3% of the total specific radioactivity incorporated. The radioactivity within this non-hydrolysable fraction was eight times that in the same fraction of humic acid that had been allowed to react with glycyl[$1^{14}C$]glycine.

The results indicate that the dipeptide became bound to the humic acid and that a proportion of the radioactivity in glycylglycine was not hydrolysable. The *N*-terminal glycine residue was considerably more resistant to hydrolysis than was

the C-terminal residue, indicating that the bond between humic acid and the N-terminal glycine residue is more stable to hydrolysis than is the peptide bond. These observations support the suggestions of workers using model systems involving the reaction of amino acids and phenols undergoing oxidation. It therefore could well be that at least a proportion of the stable amino nitrogen in humic acid is bound by N-phenyl linkages, as suggested by Flaig (1966).

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Effect of Gibberellic Acid on the Development of some Cereal Carbohydrases

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Although it is generally accepted that gibberellic acid stimulates the production of α -amylase by the aleurone layer of the barley endosperm (MacLeod, 1967), the effect of this hormone on the development of other carbohydrases is not so well established. For example, although MacLeod & Millar (1962) and MacLeod, Duffus & Johnston (1964) have suggested that the formation of endo- β -glucanase is substantially increased by gibberellic acid, a recent report by Bourne & Pierce (1970) does not support this view.

Extracts of malted barley contain at least two β -glucosidases, three endo- β -glucanases and a limit dextrinase (Manners & Marshall, 1969; Manners, Marshall & Yellowlees, 1970). Since all of these enzymes may be involved either directly or indirectly in the utilization of the carbohydrate reserves of barley, we now describe the effect of gibberellic acid on their development.

Barley (var. Proctor) was malted on a pilot scale, by using the following procedure. The grain was steeped in water for 8 h, followed by an air rest (24 h) and a further steep for 24 h in either gibberellic acid solution (0.5 p.p.m.) or water. The grain was allowed to germinate at 16°C for 5 days, samples being removed every day, and then air-dried

at 90.5°C (195°F). Samples of barley (500 grains) were taken, weighed and ground. Protein was then extracted from the flour as described by Manners & Marshall (1969).

The activity of the extracts towards laminaribiose and cellobiose varied during the 5 days of germination, but there was no significant difference between the gibberellic acid-treated and control samples. The endo- β -(1 $\rightarrow$ 4)-glucanase activity, assayed viscometrically against sodium carboxymethylcellulose, was also unaffected by gibberellic acid. However, there was a significant increase in the rate of development of both endo- β -glucanase activity towards barley β -glucan and endo- β -(1 $\rightarrow$ 3)-glucanase activity that was stimulated by gibberellic acid. When assayed viscometrically against barley β -glucan, the activity during a germination time of 4 days increased from 0.03 to 0.13 unit in the control, and from 0.03 to 0.27 units after gibberellic acid treatment. With sodium carboxymethylpachyman as substrate the activities increased from 0.13 to 0.17 unit and from 0.13 to 0.41 unit respectively.

Assays for limit dextrinase activity were also carried out with pullulan as the substrate (Manners & Yellowlees, 1970). There were 10-fold and 15-fold increases between the first and fourth days of germination in the control and in the gibberellic acid-treated barley. These results were also confirmed when samples of abraded barley (Palmer, 1969) were steeped in either water or gibberellic acid and then malted. Again there was a significant stimulation of the production of limit dextrinase by gibberellic acid.

The present results suggest that there is not a common mode for the production of cereal carbohydrase activity. It seems likely that some of these enzymes [α -amylase, limit dextrinase, endo- β -(1 $\rightarrow$ 3)-glucanase, endo- β -glucanase activity towards barley β -glucan] are produced in the aleurone cells by a mechanism that is sensitive to exogenous gibberellic acid. However, it is now clear that other carbohydrases [laminaribiase, cellobiase, endo- β -(1 $\rightarrow$ 4)-glucanase] are produced by a pathway that is not affected by exogenous gibberellic acid. The biochemical explanation for this difference remains for future investigations.

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