of its conversion into fibrous hydroglucan (Houwink & Kreger, 1953).

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The Specificity of Barley Limit Dextrinase

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Extracts of germinated barley contain a mixture of carbohydrases including two α -1,6-glucosidases (MacWilliam & Harris, 1959). One of these, R-enzyme, can hydrolyse the outermost α -1,6-glucosidic interchain linkages in amylopectin, but has no action on α -1,6-glucosidic linkages in glycogen or in branched oligosaccharides. The second enzyme, limit dextrinase, hydrolyses the α -1,6-glucosidic linkages in certain branched oligosaccharides, but has no action on amylopectin. These 'debranching' enzymes can be separated by column chromatography (MacWilliam & Harris, 1959) or by continuous electrophoresis (Manners & Sparra, 1966), although these methods do not entirely separate limit dextrinase from α -amylase. We now report studies on the properties of purified limit dextrinase.

Dehusked barley grain was allowed to germinate at 25° for 36 hr. and then homogenized in citrate buffer, pH 5.9. Protein was precipitated from the extract by the addition of solid ammonium sulphate (70%, w/v), the precipitate was dissolved in water, and, after dialysis, the protein was isolated by freeze-drying in citrate buffer, pH 5.5. This preparation showed maltase, isomaltase, panase, α -amylase, limit dextrinase and laminarinase activity.

The preparation (0.336 g. of protein) was dialysed and fractionated by continuous electrophoresis (Manners & Sparra, 1966). Those protein fractions showing limit dextrinase activity were combined and then subjected to gel filtration at 2° on a column of Sephadex G-200 with 0.02 M-citrate buffer,

pH 5.3, as eluent. Appropriate protein fractions showing limit dextrinase activity were combined and freeze-dried to give 0.135 g. of protein.

This preparation had no effect on the iodine-staining power and β -amylolysis limit of amylopectin, and was therefore free of R-enzyme and α -amylase. It had no action on the following substrates: maltose, isomaltose, maltotetraose, isomaltotetraose, panose, glucosyl-Schardinger dextrin, 6³- α -glucosylmaltotriose, 6³- α -glucosylmaltotetraose, or on 'stuffed' glucan (Stark, 1967). Enzyme action on higher branched oligosaccharides gave a mixture of maltose, maltotriose and other maltosaccharides. Maltosyl-Schardinger dextrin was also hydrolysed. The enzyme is therefore unable to remove single glucose side-chains from substrates and requires at least one α -1,4-linked glucose residue on either side of the susceptible α -1,6-glucosidic linkage. In this respect, the specificity is similar to that of pullulanase from *Aerobacter aerogenes* (Abdullah *et al.* 1966).

The limit dextrinase also differs from barley malt α -glucosidase, which can hydrolyse maltose, isomaltose and panose (Jorgensen & Jorgensen, 1963; Jorgensen, 1963).

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The Determination of Glycosaminoglycans present in Various Mammalian Brains

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Chemical studies from several Laboratories have confirmed the earlier histochemical findings that glycosaminoglycans occur in brain tissue. There is, however, a certain amount of controversy as to the exact nature and relative amounts of the glycosaminoglycans present in brain tissue obtained from different animals. The present study is concerned with the isolation and identification of glycosaminoglycans from brain tissue of ox, dog, horse, monkey, rabbit, rat and sheep.

Brains were removed from animals as soon after death as possible and carefully freed from adhering

meninges and blood vessels. After homogenization of the tissue in acetone, lipids were removed by treatment with chloroform-methanol (2:1, w/v). The residue was dispersed in 0.2M-tris-HCl buffer, pH 8.0, containing Ca^{2+} ions, and incubated at 60° for 24 hr. in the presence of Pronase. Polyanions were precipitated from the digest by the addition of cetylpyridinium chloride. After careful removal of nucleic acid, the glycosaminoglycans were again precipitated by the addition of cetylpyridinium chloride and separated into three fractions that differed in their solubility in aqueous potassium chloride (Scott, 1963).

The nature and relative amounts of the glycosaminoglycans present in each of the three fractions were determined by chemical analysis, electrophoresis on cellulose acetate (Stefanovich & Gore, 1967) and chromatography on cellulose columns (Svejar & Robertson, 1967). The hydrolysis of the susceptible glycosaminoglycans by testicular hyaluronidase was investigated, the extent of hydrolysis being estimated by chemical analysis, separation of the products by electrophoresis on cellulose acetate and chromatography on cellulose columns.

The results indicate that the following glycosaminoglycans are present in all the animal brain tissues examined; hyaluronic acid, heparan sulphate, chondroitin sulphate and dermatan sulphate.

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Phosphoprotein-bound Iron in the Plasma of the Laying Hen

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Plasma iron in the laying hen and the oestrogenized non-laying bird is 200–500% higher than in the non-laying bird (Ramsay & Campbell, 1954, 1956). About half the total appears to be transferrin-bound. Rodriguez & Planas (1964) suggested that the remainder is bound to conalbumin, the iron-binding protein characteristically found in egg-white, but their evidence overlooks the similarity in properties between conalbumin and transferrin. The phosphoprotein phosvitin has iron-binding properties (Taborsky, 1963), and Greengard, Mendelsohn & Gordon (1965) suggested without direct evidence that the non-transferrin iron in the

plasma of oestrogenized cockerels might be bound to phosphoprotein.

Magnesium carbonate fails to remove transferrin-bound iron from plasma (Ramsay, 1957), but it has now been found to remove all the phosphoprotein phosphorus and about half the total iron from the plasma of laying hens and oestrogenized cockerels. Gel filtration on Sephadex G-200 in 0.15M NaCl–5mM-tris-HCl buffer, pH 7.4, and ion-exchange fractionation on DEAE-cellulose in 5mM-tris-HCl buffer, pH 8.0, with an NaCl gradient (0.1–0.5M) both separate the iron into two fractions, of which one migrates with transferrin and the other with phosphoprotein. Combinations of these techniques lend further support to the view that the non-transferrin iron is associated with phosphoprotein and not with conalbumin.

When ^{59}Fe -labelled plasma from a normal cockerel is mixed with plasma from a laying hen, the non-transferrin iron in the latter does not become labelled in 4 hr. at 37°, nor is there any fall in the specific radioactivity of the transferrin iron. There is thus no exchange between the fractions *in vitro*. On the other hand, specific radioactivity measurements made at intervals for several hours after the intravenous injection of [^{59}Fe]-transferrin (normal cockerel plasma labelled *in vitro*) show that in the laying hen and the oestrogenized cockerel transferrin iron serves as a precursor of phosphoprotein iron (Zilversmit, Entenman & Fishler, 1943), presumably by some process other than simple transfer in the plasma.

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Disulphide Bonds of Ovalbumins

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Hen and duck ovalbumins differ in disulphide content (Gilmore & Fothergill, 1967) and show large differences in 'fingerprint' pattern (Fothergill & Perrie, 1966), although their immunological cross-reactivity suggests marked structural similarity. The present study was undertaken to examine the location of the disulphide bond of hen ovalbumin