*LACK OF AN α-1,4-GLUCAN:α-1,4-GLUCAN 6-GLYCOSYL TRANSFERASE IN A CASE OF TYPE IV GLYCOGENOSIS**

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Although "amylopectinosis" was originally described in 1952 as a type of glycogen storage disease,[1] very few additional cases have been observed in the intervening years either because the disease is very rare or because it is not easily recognized clinically. The original designation of the disease was based on the accumulation in the liver of a polysaccharide whose structure resembled that of amylopectin rather than that of glycogen. Thus, the polysaccharide had long outer chains with relatively few branch points and was highly chromogenic with I_2–KI; the I_2 complex had an absorption peak at 530 mμ as compared to 460 mμ for glycogen. The cause of the accumulation of such an unusual polysaccharide was postulated to be a relative deficiency of the branching enzyme.[1, 2] No enzymic assays could be performed. A detailed clinical description of this case was published by Andersen,[3, 4] and the observations on polysaccharide structure were reported by Illingworth and Cori.[1] A second case of Type IV glycogenosis was described by Sidbury *et al.* in 1962,[5] and a third case by Holleman *et al.* in 1966.[6] We recently received tissues taken by biopsy from a 19-month-old child (M. G.) suspected of having Type IV glycogenosis. The purpose of this paper is to describe some new chemical and enzymatic findings which have been made on this material. A full clinical report of the case will be presented by Donnell.[7]

Methods and Results.—The liver had a polysaccharide content of 3.5 per cent, and the isolated material was shown to be highly chromogenic with I_2–KI (maximum absorption at 525 mμ), and to have long outer chains (degraded by phosphorylase to the extent of 47%) and a relatively low branch point percentage (6.0%). Muscle samples taken upon two different occasions had polysaccharide contents of 0.5 and 1.0 per cent. Unlike the polysaccharide from liver, that from muscle contained 7.4 per cent branch points and could be degraded by phosphorylase to the extent of 36 per cent, a value characteristic of glycogen. The absorption spectrum of the I_2 complex of this polysaccharide had a peak at 440 mμ, which is also that found for most glycogen samples. Thus, on the basis of the structure of the isolated polysaccharides, only that obtained from liver had a structure different from that of mammalian glycogen. When a deliberate attempt was made to fractionate the polysaccharide isolated from liver, a product was obtained (representing 50% of the total) which had an end group percentage of 6.6 per cent, was digested 41 per cent by phosphorylase alone as compared to 47 per cent for the original material, and gave an I_2 complex with an absorption maximum at 510 mμ. Due to the insoluble nature of the residual polysaccharide from this fractionation, no acceptable measurement of its end group percentage and outer chain length could be made. Dr. Ben Landing had observed variations in the staining characteristics of histological sections suggesting that both a glycogen-like and an amylopectin-like polysaccharide were present.[8]

Since a sensitive assay for "branching enzyme" (α-1,4-glucan:α-1,4-glucan 6-glycosyl transferase) had already been developed for following the purification of the enzyme from rabbit skeletal muscle,[9] this method was utilized to study the activity present in homogenates of liver and leucocytes from the patient suspected of having Type IV glycogenosis. The control series consisted of tissues obtained from other types of glycogenosis and also from patients whose livers showed fibrotic changes without evidence of Type IV glycogen storage disease. The assay for "branching enzyme" depends upon the increase in the rate of formation of polysaccharide from glucose-1-phosphate by phosphorylase in the presence of the enzyme and in the absence of added primer.[10] That the effect on the rate of polysaccharide synthesis in the present experiments was not due to the introduction of primer into the system by any one of the branching fractions prepared from human liver was shown by boiling it and then adding it to control reaction mixtures in an amount equal to 5–10 times the quantity added in the test assay. No acceleration of rate was observed in these control experiments.

Samples of liver (200–400 mg) were homogenized in 0.25 *M* sucrose–0.001 *M* EDTA, pH 7.1, using TenBroeck glass homogenizers (Kontes Glass Co.). The homogenates (1:6–1:10) were centrifuged briefly at 1000 × *g* and the supernatant fluid was then centrifuged at 100,000 × *g* for 45 min. To the supernatant fluid was added an equal volume of neutral, saturated ammonium sulfate (at 4°). After 20 min the precipitate was collected by centrifugation, dissolved in 1.0 ml of 0.005 *M* Tris–0.001 *M* EDTA–0.005 *M* 2-mercaptoethanol, pH 7.2, and dialyzed for 2 hr in the cold against a large volume of the buffer. After centrifugation to remove insoluble material, the solution was assayed for branching activity both by measuring the acceleration of the rate of formation of inorganic phosphate from glucose-1-phosphate as described above, and also by the decrease in the absorption of the I_2 complex of corn amylopectin at 520 mμ after incubation with this polysaccharide. The use of this substrate for the detection of branching enzyme activity in rat liver has been described by Larner[11] and by Krisman.[12] The results are given in Table 1 and represent the average of two or more experiments with different quantities of liver fraction in the assay system. The M. G. preparation is the only one which had

TABLE 1

ACTIVITY OF A BRANCHING ENZYME IN HUMAN LIVER

Glycogenosis type*	Case	μMoles P_{inorg}/min/mg protein†	$\Delta\epsilon_{520}$/min/mg protein‡
I	S. B.	8.7	−0.17
I	M. T.	8.1	−0.24
I	J. R.	11.0	−0.11
III	W. M.	9.9	−0.33
III	B. T.	6.3	−0.17
III	V. M.	4.3	
IV	M. G.	0.0	−0.008
§	S. S.	4.0	
§	F. R.	12.4	−0.34
§	J. D.	8.5	−0.45

* For the classification of glycogenoses see ref. 2.

† Reaction mixtures contained 20 μmoles G-1-P, pH 6.4, 0.5 μmoles 5′-AMP, 2 units phosphorylase *a*, 5–20 μg of protein of the liver fraction; vol, 0.5 ml. Incubated at 30°. Aliquots of 0.05 ml were analyzed for P_{inorg} at 15-min intervals.

‡ Reaction mixtures contained 0.1% corn amylopectin, 100 μmoles of sodium citrate, pH 6.0, 75–150 μg of protein of the liver fraction; vol, 0.5 ml. Aliquots of 0.10 ml were taken at 0, 10, and 20 min and mixed with 0.65 ml H_2O and 0.25 ml of 0.2% I_2–0.4% KI; the extinction of the I_2 complex was measured in a cuvette with a 1-cm light path.

§ Tissues from patients other than those with glycogen storage disease.

no branching activity. No change in activity of any fraction was found after storage of the enzyme preparation from liver for 4 days in the 4° cold room. Overnight incubation of the reaction mixtures containing added amylopectin resulted in the formation of products which gave little or no color with iodine, suggesting that some α-amylase was present. Since it was known that α-amylase can interfere in synthesis of polysaccharide without added primer,[13] the possibility had to be considered that the M. G. preparation appeared to have no branching enzyme activity because it contained excessive amounts of α-amylase rather than because a "branching enzyme" was in fact missing. Accordingly, aliquots of the M. G. preparation containing 100 μg of protein were added to aliquots of the J. R. (7 μg) and B. T. (15 μg) preparations, and the branching enzyme activities of the latter two tissues were then reassayed. No decrease in their branching activity was found. Hence, the failure to measure branching action in the M. G. preparation was not due to α-amylase contamination. Homogenates of the M. G. liver had normal levels of activity when assayed for glucose-6-phosphatase, phosphorylase, and amylo-1,6-glucosidase.

A similar type of assay has been utilized in studying the branching activity present in leucocyte homogenates. In Figure 1 the solid blocks represent the values obtained for two separate leucocyte homogenates from M. G. Leucocytes were isolated by a modification of the method of Huijing,[14] who has used these cells in assaying for many of the other enzymes implicated in the etiology of the various glycogenoses. Leucocytes separated from 10 ml of heparinized blood were washed well with 0.15 *M* NaF and then homogenized in 1 ml of 0.05 *M* NaF. Aliquots containing 150 μg of protein were incubated at 30° with 20 μmoles of glucose-1-phosphate, pH 6.4, and 2 units of added phosphorylase *a* in the presence of 5′-AMP. Samples were removed for determination of inorganic phosphate at 15-min intervals. Results are expressed in terms of μmoles polysaccharide glucose formed/min/mg protein. The M. G. leucocytes had virtually no activity in this assay, showing that the enzyme pattern of the leucocytes reflects that of the liver in this patient as well as in other individuals. When assayed for phosphorylase, the M. G. leucocyte preparations had activities of 33 and 39 mμmoles P_{inorg}/min/mg (milliunits/mg), as compared to a series of 25 other leucocyte preparations with activities between 17 and 52 milliunits/mg.

Discussion.—Since the assays used clearly show that the liver of the Type IV

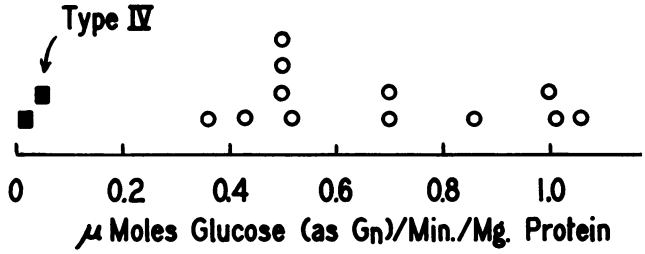

FIG. 1.—Activity of a branching enzyme in human leucocytes. See text for preparation of leucocytes and conditions of assay. ■, Leucocyte preparations made from blood samples from M. G. taken upon two occasions 1 week apart. O, Individual values obtained with leucocyte preparations from a variety of types of glycogenosis with some overlap of case material presented in Table 1.

glycogenosis patient (M. G.) is unique among those liver samples studied in being devoid of "branching enzyme" activity, the presence in it of an amylopectin-like polysaccharide containing α-1,6-linked glucose units as branch points requires explanation. The possibility might be considered that there are two different proteins with "branching enzyme" activity in liver and that the activity of only one of these is measured by the assay procedures used. When purification of the branching activity in skeletal muscle is followed by the assay involving synthesis of polysaccharide from glucose-1-phosphate by phosphorylase, the enzyme so obtained also acts well in a combined system with UDP glucose-α-glucan glucosyltransferase to increase the rate of glucosyl transfer in the presence of a limiting amount of a suitable polysaccharide acceptor.[15] The direct action of this "branching enzyme" on polysaccharides enlarged from UDP glucose has also been shown.[16] Thus, at least one enzyme responsible for branch point formation during glycogen synthesis *in vivo* is missing in Type IV glycogenosis, and this fact could account for the absence of glycogen of normal structure. How this enzyme might differ in its specificity from a second one whose presence in Type IV glycogenosis liver tissue is suggested above is not certain.

The analytical data presented on the polysaccharide from M. G. as well as those published previously on the original case,[1] show that the most prominent abnormality in the structure of these two liver polysaccharides is the length of their outer chains. The branching activity responsible for the synthesis of these amylopectin-like molecules may differ from that involved in glycogen synthesis in its specificity with regard to the length of the donor chain from which an oligosaccharyl fragment is removed prior to transfer and attachment elsewhere in α-1,6-glucosidic linkage. The probable natural substrate for the action of branching enzyme is a branched polysaccharide whose outer main chains have been substantially and selectively elongated from UDP glucose by the action of UDP glucose-α-glucan glucosyltransferase.[17] No direct information is available about the critical length to which such a chain must be extended before it becomes an effective donor in the branching reaction. Larner had shown in 1953 that glycogen enlarged from glucose-1-phosphate to average outer chain lengths of 11–21 glucose units could be acted upon by a "branching enzyme" prepared from liver.[18]

Verhue and Hers[19] have studied "branching enzyme" partially purified from rat liver according to the procedure of Krisman;[12] the enzyme was assayed by its ability to decrease the chromogenicity of the I_2 complex of amylopectin. It was found that when this enzyme acted on glycogen enlarged by phosphorylase from glucose-1-phosphate, the chain segment moved during branching was 6 or more glucose units in length, with some preference indicated for 7-unit oligosaccharyl transfer. We have studied the specificity of a highly purified "branching enzyme" from rabbit muscle[9] acting directly on glycogen enlarged from UDP glucose.[16, 20] Here, too, a preference was found for transfer of an oligosaccharide 7 glucosyl units long, and the specificity for this chain length appears to be even sharper than in the case of the liver enzyme.

The absence of an α-1,4-glucan:α-1,4-glucan 6-glycosyl transferase in Type IV glycogenosis has been clearly shown, but the special properties of the residual "branching enzyme" activity which could account for the presence of a branched polysaccharide in the liver of patients with this disease require further investigation.

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