262. *The Structure of Glycogen. Ratio of Non-terminal to Terminal Glucose Residues.*

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An assay of the proportion of terminal groups present in various glycogens has been made by measuring the amount of formic acid liberated on oxidation of the polysaccharide by potassium periodate. All the glycogens examined gave results indicating that approximately 12 glucose residues were present per end group, except that certain samples of rabbit-liver glycogen contained approximately 18 residues per end group. The results are in agreement with determinations of the percentage of end groups arrived at by estimation of the tetramethyl glucose produced on hydrolysis of the methylated glycogen.

The proportion of terminal groups present in samples of glycogen isolated from various sources has been determined previously by methylation of the polysaccharide followed by the quantitative determination of the amount of tetramethyl methyl-*d*-glucoside in the products of methanolysis of the methylated polysaccharide, and in this way it was found that for most samples the ratio of terminal to non-terminal glucose residues was approximately 1 to 12. Nevertheless, in certain samples of rabbit-liver glycogen the ratio was nearer 1 to 18. No completely satisfactory explanation of this variation has yet been put forward, and further investigation was clearly needed. The examination of a large number of samples by the classical methylation technique is a lengthy operation, but starches and glycogens respond readily to the periodic acid oxidation process in which, under carefully standardised conditions, formic acid is liberated quantitatively from the terminal residues of each side chain, and it was decided, therefore, to examine various samples of glycogen by this method (cf. Brown, Dunstan, Halsall, Hirst, and Jones, *Nature*, 1945, **156**, 785; *idem*, *J.*, in the press). In some instances comparative experiments were made by the methylation method, using newly developed semi-micro-methods for the determination of the tetramethyl methylglucoside which arises from each end group (Bell, *J.*, 1944, 473; Jones, *J.*, 1944, 333; Brown and Jones, in the press).

Through the kindness of Dr. D. J. Bell of Cambridge University, we were able to investigate numerous samples of glycogens which he had isolated from various sources. These included glycogen from *Ascaris lumbricoides*, *Mytilus edulis*, human muscle, rabbit liver, rabbit muscle (fasted), and horse muscle. Some of these had already been assayed by him using the methylation procedure. A check was thus provided of the results derived by determination of the formic acid produced after oxidation of the glycogen with potassium periodate. Figures in excellent agreement with those obtained by the use of the methylation procedure were observed. Dr. F. Smith very kindly placed at our disposal samples of guinea-pig-liver glycogen and rabbit-liver glycogen which had been assayed by the methylation technique at Birmingham University.

The proportion of end groups in rabbit-liver glycogen was first determined by Haworth and Percival (*J.*, 1932, 2277) who reported the figure of 1 for every 12 glucose residues. Examination of another sample of rabbit-liver glycogen by Haworth, Hirst, and Isherwood (*J.*, 1937, 577) showed, however, that this glycogen contained approximately 18 glucose residues per end group. This result was confirmed by Bell (*Biochem. J.*, 1936, **30**, 1612) and by Bacon, Baldwin, and Bell (*ibid.*, 1944, **38**, 198) who demonstrated that the ratio of terminal to non-terminal residues in rabbit-liver glycogen may be either 12 to 1 or 18 to 1 approximately, the value observed being apparently dependent upon the carbohydrate diet of the rabbit. We have now confirmed by the periodate oxidation method the higher figure for the sample of rabbit-liver glycogen examined by Haworth, Hirst, and Isherwood, and have obtained also a ratio of 18 to 1 approximately, by both methods of assay, for another sample of rabbit-liver glycogen prepared

by Messrs. Hopkin & Williams Ltd. On the other hand, the sample of rabbit-liver glycogen which had been found by Bell to give the value 12 gave a low value by the periodate method also. Further investigation is clearly required to ascertain the precise reasons for these unpredictable variations which appear to occur only in rabbit-liver glycogen. In all other cases, including those cited in the table and the numerous samples of fish-liver and fish-muscle glycogen examined by Smith (*J.*, 1939, 1915), the value obtained has been 12 approximately.

Experimental

Potassium Periodate Oxidation of Glycogens. General Procedure.—The glycogen (*ca.* 500 mg.) was placed in a 500 ml. stoppered bottle which had been cleaned with chromic acid and steamed out. A known volume of water was added, followed by potassium chloride (5 g.) and a known excess of an aqueous solution of sodium metaperiodate. The oxidations were carried out at 15° with continuous shaking and in dim light (sunlight must be avoided). Portions of the solution (20 ml.) were withdrawn at intervals, excess of ethylene glycol was added, and the formic acid present was estimated by titration with 0·1N-barium hydroxide using a micro-burette and methyl-red as indicator. The resulting titres, after correction when necessary to allow for the slight acidity or alkalinity of the glycogen, were plotted against time to make sure that the normal type of reaction was proceeding, and the acid titre corresponding to 150 hours was used in the calculations, this being the time required for the liberation of 1 mol. of formic acid from β-methyl-*d*-maltoside under these conditions (Halsall, Hirst, and Jones, *J.*, *loc. cit.*). From this titre the size of the repeating unit was calculated. The results are given in the table.

Source of glycogen.	Wt. of glycogen (mg.).	Yield of formic acid (mg.).	Glucose residues per end group. (*a*) By periodate method.	(*b*) By methylation method.
1. *Ascaris lumbricoides*	567	13·5	12	13—14 (*a*)
2. *Mytilus edulis*	510	14·1	10	11 (*b*)
3 Human muscle	518·8	12·9	11	—
4. Rabbit (fasted) muscle	510·5	11·0	13	—
5. Horse muscle	400·4	8·1	14	12 (*c*)
6. Rabbit liver	365·5	7·2	14	12 (*d*)
7. Rabbit liver	509	9·0	16	18 (*e*)
8. Rabbit liver	871	13·8	18	18 (*f*)
9. Rabbit liver	216	4·2	14	12 (*g*)
10. Guinea-pig liver	174·5	3·9	13	12 (*g*)

Glycogens 1—6 were prepared by Dr. D. J. Bell.
Glycogen 7 was a sample of the material used by Haworth, Hirst, and Isherwood (*loc. cit.*).
Glycogen 8 was prepared by Messrs. Hopkin & Williams, Ltd.
Glycogens 9 and 10 were prepared by Dr. F. Smith.

(*a*) Bell, *J.*, 1944, 474.
(*b*) This figure was obtained for another sample examined by Meyer, *Naturwiss.*, 1941, **29**, 287.
(*c*) Bell, *Biochem. J.*, 1935, **29**, 2031.
(*d*) Bell, *Biochem. J.*, 1937, **31**, 1683.
(*e*) Haworth, Hirst, and Isherwood (*loc. cit.*).
(*f*) Present paper.
(*g*) F. Smith (private communication).

Size of Repeating Unit of Rabbit-liver Glycogen (*No.* 8 *in Table*), *by the Methylation Method.*—The glycogen was methylated with methyl sulphate and sodium hydroxide according to the method of Hirst and Young (*J.*, 1939, 1471). The methylated glycogen [759·5 mg.; $[\alpha]_D^{21°}$ + 206° in chloroform (*c*, 8·5); OMe, 45·1%] was hydrolysed with methyl-alcoholic hydrogen chloride (1·5%; 100 ml.) and the amount of 2 : 3 : 4 : 6-tetramethyl methylglucoside in the resulting mixture of glucosides was estimated by the partition method of Brown and Jones (this vol., p. 1344). Yield of 2 : 3 : 4 : 6-tetramethyl methylglucoside, 50·3 mg.; $n_D^{16°}$ 1·4445; $[\alpha]_D^{21°}$ + 66° in water (*c*, 2·3); corresponding to the presence of 18—19 glucose residues per end group.

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