525. α-1,4-*Glucosans. Part XII.*[1] *End-group Assay of Glycogens by Oxidation with Sodium Metaperiodate.*

By D. J. Manners and A. Wright.

Four methods of end-group assay of glycogen by oxidation with sodium periodate have been critically compared. In three methods, varying degrees of overoxidation occurred, resulting in the production of additional formic acid. Determination of the initial amount of formic acid produced by the oxidation of 0·2% glycogen solution with 0·1M-sodium metaperiodate at room temperature (18—20°) gave values for the chain length in good agreement with those deduced by other methods of assay.

Although the end-group assay of glycogen by oxidation with sodium metaperiodate has been widely applied, the experimental conditions used by various workers differ considerably. Some examples are given in Table 1. We have therefore determined the maximum production of formic acid under different conditions from two glycogen samples whose average chain length ($\overline{CL}$) had previously been assayed by oxidation with potassium

Table 1. *Experimental conditions for the periodate oxidation of glycogen.*

Workers	Glycogen concn. (mg./ml.)	Periodate concn. (M)	Temp.	Duration of oxidn. (hr.)
Abdel-Akher and Smith [a]	0·4—2·0	0·05	5—6°	80
			0	180
Perlin [b]	2·0	0·1	16·6	*ca.* 30
Polglase, Smith, and Tyler [c]	10·0	0·16	2	*ca.* 72
Manners and Archibald [d]	4·0	0·032	2	*ca.* 240

[a] *J. Amer. Chem. Soc.*, 1951, **73**, 994. [b] *Ibid.*, 1954, **76**, 4101. [c] *J. Biol. Chem.*, 1952, **199**, 97. [d] *J.*, 1957, 2205.

Table 2. *Chain lengths of glycogens determined by oxidation with sodium metaperiodate under different conditions.*

Method	Time (hr.)	Apparent chain length (glucose residues) 49	96	168	216	260	336	696
Oyster glycogen *								
Abdel-Akher and Smith		—	—	—	10·1	9·3	8·6	7·8
Perlin		10·4	9·8	8·8	8·3	7·6	—	5·0
Polglase *et al.*		10·9	10·1	9·6	8·9	8·7	—	6·8
Manners and Archibald:	at 0°	14·9	12·8	11·9	11·3	10·5	—	10·0
	at *ca.* 17°	12·3	10·8	10·2	9·9	9·4	—	—
Rabbit liver glycogen †								
Abdel-Akher and Smith		—	—	—	12·9	11·6	10·3	8·5
Perlin		14·7	13·2	12·1	11·3	10·6	—	6·1
Polglase *et al.*		16·3	14·7	13·3	12·1	11·4	—	8·1
Manners and Archibald:	at 0°	21·6	18·2	16·9	16·4	16·0	—	14·0
	at 17°	18·4	16·7	15·6	15·0	14·7	—	12·5

* $\overline{CL}$ 10·4 by oxidation with potassium periodate.
† $\overline{CL}$ 13·9 by oxidation with potassium periodate.

metaperiodate.[2] This is a reliable and accurate method for the analysis of glycogens.[3] The results (Table 2) show that overoxidation (defined here as the production of formic acid in excess of that required by a Malaprade reaction) occurred in three of the experimental conditions, and that it was related to the temperature of the oxidation and the relative excess and concentration of oxidant. In all cases, an initial rapid production of formic acid was followed by a slower secondary reaction. The results in Table 2,

[1] Part XI, Archibald, Fleming, Liddle, Manners, Mercer, and Wright, *J.*, 1961, 1183.
[2] Bell and Manners, *J.*, 1952, 3641.
[3] Manners and Archibald, *J.*, 1957, 2205.

and those obtained previously,[3] show that the rate of oxidation of different glycogens may differ; $\overline{CL}$ values cannot, therefore, be calculated with certainty from the production of formic acid after a particular period of oxidation.

Oxidation of glycogens by cold dilute solutions of sodium metaperiodate has been used extensively by F. Smith and his co-workers.[4] However, in our experiments (Tables 2 and 3) a very slow production of formic acid was noted after 180 hr. at 0° (cf. Table 1), so that there was some uncertainty about the final end-point. This may cause an error of 1—3 glucose residues for a 12-unit glycogen. In these estimations, the frequency of sampling is important; at short intervals of time the increase in production of formic acid may not exceed the experimental error involved in the titration with standard alkali, but at 48 or 72 hr. intervals a small increase may be detected. These conclusions are in agreement with recent studies by Professor R. Montgomery.[5]

TABLE 3. *Chain lengths of glycogens determined by oxidation with sodium metaperiodate in the conditions of Abdel-Akher and Smith.*

Glycogen sample	$\overline{CL}$ (KIO_4)	Time of oxidation (hr.):	Apparent chain length 216	288	360
Ascaris lumbricoides	12·0		13·5	10·8	9·4
Oyster	10·4		10·4	10·1	9·1
Rabbit liver VII	13·9		13·9	12·3	9·8

The conditions used by Polglase and his co-workers [6] were considered by them to give comparative rather than absolute values of $\overline{CL}$. Nevertheless, Warren and Whittaker [7] concluded that under these conditions oxidation was complete after 72 hr.; however, inspection of their results does not support this conclusion, and our results in Table 2 show clearly that formic acid production continues for several hundred hours. After oxidation for 72 hr., the apparent $\overline{CL}$ values of the oyster and rabbit-liver glycogen are 10·5 and 15·5, respectively (cf. values of 10·4 and 13·9 by oxidation with potassium periodate). The $\overline{CL}$ values obtained by Warren and Whittaker may therefore be too high by an amount varying from 0 to 2 glucose residues. This would account for the small but significant difference noted by them between the arithmetic mean of the $\overline{CL}$ values of some twenty glycogens as determined by periodate oxidation (12·9) and by enzymic assay of the same samples (12·4).

Under the conditions developed previously,[3] with a very limited excess of oxidant, no appreciable overoxidation occurs at 2° although the rate of oxidation is reduced to that found with potassium metaperiodate. The method gives reliable results: *e.g.*, human liver glycogen [8] and horse muscle glycogen [9] had $\overline{CL}$ values of 14·4 and 16·8, respectively (cf. values of 14·6 and 16·6 by the potassium method); it also enables the reduction of periodate to be analysed. The temperature of the oxidation is, however, critical and at room temperature (18—20°) slight overoxidation is apparent.

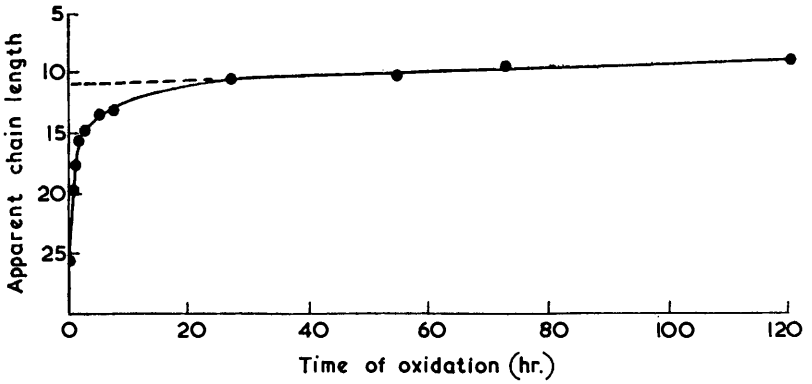
We conclude, in agreement with Peat, Turvey, and Evans,[10] that, provided experimental conditions are carefully controlled, oxidation by sodium metaperiodate provides an accurate method for the end-group assay of glycogens.

Alternative Method of Analysis.—When glycogen was oxidised under the conditions used by Perlin,[11] an approximately linear increase of production of formic acid with time occurred after 25 hr. (see Figure). In similar conditions, trehalose gave 2·2—2·3 mol.

[4] Abdel-Akher and Smith, *J. Amer. Chem. Soc.*, 1951, **73**, 994.
[5] Montgomery, personal communication.
[6] Polglase, Smith, and Tyler, *J. Biol. Chem.*, 1952, **199**, 97.
[7] Warren and Whittaker, *Biochem. J.*, 1959, **72**, 288.
[8] Calderbank, Kent, Lorber, Manners, and Wright, *Biochem. J.*, 1960, **74**, 223.
[9] Lawrie, Manners, and Wright, *Biochem. J.*, 1959, **73**, 485.
[10] Peat, Turvey, and Evans, *J.*, 1959, 3341.
[11] Perlin, *J. Amer. Chem. Soc.*, 1954, **76**, 4101.

of formic acid (theor. 2·0 mol.). It was concluded that production of formic acid from the non-reducing end-group was complete within about 25 hr. and that the remaining acid arose from further oxidation of dialdehyde residues. It follows that extrapolation of the linear portion of the formic acid production *v.* time curve to zero time should provide

The rate of oxidation of oyster glycogen by sodium metaperiodate at 20°. *The production of formic acid is shown as a decrease in apparent chain length.*



an accurate measure of the formic acid arising solely from the non-reducing end-groups. This procedure has been applied to various polysaccharides, and the results (Table 4)

TABLE 4. *Chain lengths of polysaccharides determined by the extrapolation method.*

Sample	$\overline{CL}$ by extrapoln.	$\overline{CL}$ by KIO_4 oxidn.	Sample	$\overline{CL}$ by extrapoln.	$\overline{CL}$ by KIO_4 oxidn.
Human liver glycogen (A. K.)	14	15	Maize amylopectin	19	18 *
Human liver glycogen (S. K.)	6	6	Malt amylopectin	18 *	18 †
Oyster glycogen	11	10	Potato amylopectin	24	23
Rabbit liver VII glycogen ...	15	14	Potato amylopectin β-dextrin	10	9
Rabbit liver VIII glycogen ...	14	13			

* Determined by Mr. G. A. Mercer. † Kindly supplied by Dr. G. O. Aspinall (cf. Aspinall, Hirst, and McArthur, *J.*, 1955, 3075).

are in good agreement with those from assays of the same samples by oxidation with potassium metaperiodate. Within certain limits, the extrapolated $\overline{CL}$ value is independent of the relative concentration of oxidant and of temperature, although the rate of over-oxidation is temperature-dependent. The method requires an oxidation period only one-half that of the other procedures and, with suitable modification, should be applicable to the microanalysis of the small quantities of glycogen which are available in most clinical and metabolic studies.

EXPERIMENTAL

Analytical Methods.—The methods described previously [3] were used, solutions of polysaccharide samples being adjusted to pH 5·8 before oxidation.

Comparison of Oxidation Conditions.—Glycogen samples (100 or 250 mg.) were oxidised under the conditions described in Table 1, the final volumes of the mixtures being increased proportionally. The results are shown in Table 2. Further samples of glycogen (100 mg.) were oxidised by the method of Abdel-Akher and Smith; [4] see Table 3 for results.

Overoxidation of Glycogen.—Oyster glycogen (200 mg.) was oxidised by Perlin's method [11] at 20°, the total volume being 100 ml. Samples (5 or 10 ml.) were removed at intervals; the results are shown in the Figure.

Human liver glycogen [8] (100 mg.) was then oxidised under three different conditions: (*a*) by Perlin's method [11] at 20°; (*b*) as (*a*) but with twice the concentration of periodate; (*c*)

Time of oxidn. (hr.)	Apparent $\overline{CL}$ values (*a*)	(*b*)	(*c*)	Time of oxidn. (hr.)	Apparent $\overline{CL}$ values (*a*)	(*b*)	(*c*)
24	—	—	11·1	95	12·0	11·8	4·1
49	12·9	13·0	8·4	140	11·0	11·0	—
75	—	—	5·5	180	10·7	10·6	—

as (*a*) but at 37°. In each experiment, a graph of formic acid production against time was prepared, and by extrapolation to zero time, a $\overline{CL}$ value obtained, namely, (*a*) 13·9, (*b*) 13·9, (*c*) 13·8 glucose residues.

Several samples of glycogen (70—100 mg.) and amylopectin (*ca.* 200 mg.) were oxidised by Perlin's method at 20° and $\overline{CL}$ values obtained by extrapolation as above. The results are given in Table 4.

Trehalose (10—20 mg.) was also oxidised under these conditions: after 26 hr., 2·1 mol. of formic acid were released, and after 94 hr., 2·2—2·3 mol.

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