Physicochemical Studies on Starches. Part II. The Oxidation of Starches by Potassium Metaperiodate.*

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[Reprint Order No. 5721.]

The oxidation of 18 different starches by potassium metaperiodate has been studied. At constant temperature, the time taken for the theoretical uptake of periodate ion varies from starch to starch. Only traces of glucose were found in the hydrolysates from oxidised starches when oxidation was continued until over-consumption of periodate had occurred. Potentiometric-titration studies showed that, in the presence of all reaction products, formic acid is quantitatively determined by titration to pH 6·25. Oxidised starches are stable below this pH, but decompose rapidly in dilute alkali, yielding acidic products in quantity. Oxidised starches bind about 2% of the formic acid released during oxidation; hence complete estimation of this acid can only be made by titrating the *heterogeneous* reaction mixture to pH 6·25. The effect of the presence of protein on the periodate oxidation of oat-starch samples has been investigated. Values for the ratio of non-terminal to terminal groups of the starches studied are presented, the accuracy claimed being ±0·5 glucose unit. The average length of unit-chain of the corresponding amylopectin components has been calculated from these values and the results of determinations of the percentages of amylose.

In recent years there has been an increasing tendency for the methylation method of determining the ratio of non-terminal to terminal groups [*i.e.*, $(\bar{R})$] in starches to be superseded by the method involving the estimation of formic acid released from the non-reducing end-groups during periodate oxidation. The periodate method has the advantages of simplicity and speed and requires about ten times less material. Theoretically, it can also provide additional evidence for the presence or absence of inter-chain linkages involving $C_{(2)}$ or $C_{(3)}$ (Halsall, Hirst, Jones, and Roudier, *Nature*, 1947, **160**, 899) and, in conjunction with methylation data, of glucose residues linked solely through $C_{(1)}$ and $C_{(6)}$ (Brown, Halsall, Hirst, and Jones, *J.*, 1948, 27). However, the accuracy of periodate determinations of the value of $(\bar{R})$ for starches has been quoted as ±10% (*idem*, *loc. cit.*). There has also been considerable disagreement in the values obtained for the average lengths of unit-chain of amylopectins by different workers (see Table 1). The studies reported here were undertaken in an effort to reduce the experimental errors

Table 1. *Values quoted from periodate oxidation results for the average length of unit chain of amylopectins.*

Source of starch	Reference A	B	C	D
Maize	20	—	25—26	25·2
Potato	24—26	—	27	—
Tapioca	20	22	23	26·6
Wheat	21	—	23	—

A, Brown, Halsall, Hirst and Jones, *loc. cit.*; B, Meyer and Settele, *Helv. Chim. Acta*, 1953, **36**, 197; C, Potter and Hassid, *J. Amer. Chem. Soc.*, 1948, **70**, 3488; D, *idem*, *ibid.*, 1951, **73**, 997.

involved in periodate oxidations, and so obtain accurate values of $(\bar{R})$ for use in conjunction with the results from differential potentiometric-titration studies of the iodine uptake of starches and their components.

Oxidations were carried out with potassium metaperiodate (Halsall, Hirst, and Jones, *J.*, 1947, 1399) at 15—16° and at 20—21°. At both temperatures, the time required for the theoretical uptake of 1·03—1·05 moles of periodate per anhydroglucose unit varied for

* Part I, *J.*, 1954, 3769.

different starches. Some typical results are shown in Table 2. In no case was the theoretical uptake reached in less than 240 hr. at 15—16°, and even after 350 hr. at this

TABLE 2. *Periodate uptake, in moles per anhydroglucose unit.*

Source of starch	Temp.	Time of oxidation (hours) 72	120	160	200	240	280	336	432
Barley I [a]	15—16°	—	—	0·91	0·99	1·01	1·04	—	—
Oat [b]	,,	0·77	0·80	0·86	0·96	1·02	—	1·05	—
Potato I [c]	,,	0·81	0·86	0·89	0·95	0·98	1·00	1·02	—
Potato II [d]	,,	—	—	0·88	0·94	0·96	—	1·01	1·04
Potato III [e]	,,	—	—	0·88	0·94	0·97	—	1·03	1·03
Rice [c]	,,	—	—	0·72	0·84	0·93	1·01	1·03	—
Sweet potato [c] ...	,,	—	—	—	0·97	1·01	—	—	—
Waxy maize [c]	,,	—	—	0·91	0·92	0·96	1·00	1·03	1·05
Oat [b]	20—21	—	0·97	1·02	1·03	—	1·08	—	—
Potato II [d]	,,	—	0·97	1·03	1·04	1·09	1·13	—	—
Rice [c]	,,	—	0·95	1·01	1·03	1·06	1·09	—	—
Waxy maize [c]	,,	—	0·98	1·02	—	1·03	1·05	—	—

[a] McWilliam and Percival, *J.*, 1951, 2259; [b] Anderson and Greenwood, unpublished work; [c] Samples described in *J.*, 1948, 27; [d] *Var.* "Golden Wonder"; [e] *Var.* "Redskin" ([d,e] Greenwood, unpublished work).

temperature, very little over-consumption of periodate (and hence very little over-oxidation of starch) occurs. At 20—21°, the oxidation is about 30% faster, but there is a greater tendency for over-oxidation.

It follows that the time required for the periodate-uptake of a starch to reach the theoretical value *at constant temperature* must be determined. The amount of formic acid liberated in a shorter time cannot be quantitative. Although a longer time may even be necessary to allow for hydrolysis of intermediate complexes (Hughes and Nevell, *Trans. Faraday Soc.*, 1948, **44**, 941), it has been found that in the final stages of oxidation the release of formic acid follows consumption of periodate without apparent delay (see Fig. 1). The differing oxidation times required could be explained by differences in chain length and degree of multiple branching of the amylopectin fractions, by differences in natural granular structure, or by alteration in the physical characteristics of the granules arising from differing methods of preparation. As a result, it appears unlikely that the oxidation time for any simple saccharide can be taken as a standard for starch. In particular, it is difficult to justify the procedure of Morrison, Kuyper, and Orten (*J. Amer. Chem. Soc.*, 1953, **75**, 1502), who apply a fixed correction factor, based on the percentage of the theoretical formic acid released in the same time from sucrose; results agreeing with those of Halsall, Hirst, and Jones (*J.*, 1947, 1427) have been obtained here which show that release of formic acid from sucrose is abnormally slow and non-quantitative. There is no reason to suspect that starches differ from the model of methyl β-D-maltoside (Brown, Halsall, Hirst, and Jones, *loc. cit.*) which releases formic acid quantitatively in 150 hr. at 15°, in any respect other than having variably slower rates of oxidation.

After several starches had been oxidised for the time found necessary for the theoretical uptake of periodate, they were examined by the methods described by Hirst, Jones, and Roudier (*J.*, 1948, 1779) for the isolation and hydrolysis of the oxidation products, and for the determination of glucose in the hydrolysates. Quantities of glucose between 0·5 and 1% were found. When further samples of the same starches were oxidised for at least 100 hr. longer in each case, traces of glucose, too small for estimation, were detectable only by examination of the chromatograms under ultra-violet light. Recent papers have reported the presence of 1—2% of glucose in hydrolysates after oxidation at room temperature for 240 hr. (Hirst, Jones, and Roudier, *loc. cit.*) and for 150 hr. (McWilliam and Percival, *loc. cit.*), but these authors were undecided whether this was of structural significance or due to incomplete oxidation. The latter explanation is supported by the present work, which shows that about 1% of glucose residues in the starches examined are abnormally but not completely resistant to periodate attack. This suggests that 1 : 2- or 1 : 3-glucosidic linkages are not present, and is in agreement with the work of Gibbons

and Boissonnas (*Helv. Chim. Acta*, 1950, **33**, 1477). However, the polyaldehydic oxidation products are very easily hydrolysed, giving solutions containing a brown precipitate, and Jackson and Hudson (*J. Amer. Chem. Soc.*, 1938, **60**, 989) reported that during hydrolysis some destruction of material occurred and polymer degradation was incomplete. For this reason, Abdel-Akher, Hamilton, Montgomery, and Smith (*ibid.*, 1952, **74**, 4970) hydrolysed the corresponding polyalcohols and claimed that 1 : 3-linkages exist since 1% of glucose residues was found. However, the period of oxidation used may not have been sufficient for complete oxidation.

The present work has shown that aqueous suspensions of the polyaldehydes obtained are stable in the range pH 3—6·25, but decompose readily in 0·01—0·001N-alkali, releasing acidic products even in a nitrogen atmosphere, with decomposition ceasing when the pH has dropped to 6·25. For example, immediately after potentiometric titration of a reaction mixture (Fig. 2, curve *abd*, of which part *bd* is time-dependent), the excess of alkali was back-titrated with standard formic acid (curve *dea*); 0·16 ml. of acid was found

FIG. 1. FIG. 2.

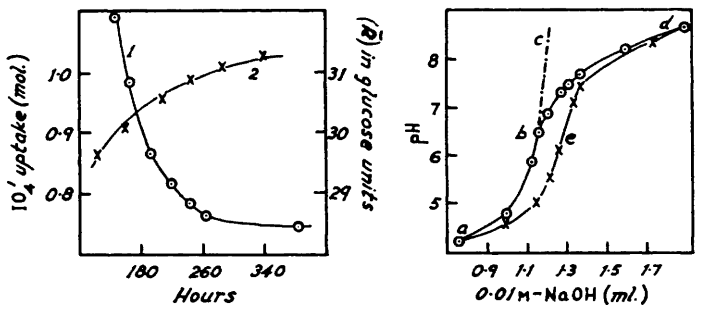

FIG. 1. *Variation of periodate-uptake and apparent value of R with oxidation time at* 15—16° *for oat starch.*

Curve 1. $(\bar{R})$ (*glucose units*).
Curve 2. IO_4'-*uptake* (*moles/anhydroglucose unit*).

FIG. 2. *Titration curves for a reaction mixture.*

Curve abc. Control H·CO_2H–NaOH.
Curve abd. Reaction mixture–NaOH.
Curve dea. Back-titration curve for mixture–H·CO_2H.

to have been liberated in the time taken (about 15 min.) to titrate from *b via d* to *e*. Liberation of acid in this manner may explain some anomalous results which have been reported involving over-production of acid during periodate oxidations in alkaline-buffered systems. Similarly, when a calculated excess of potassium hydroxide was added to a series of periodate oxidations of waxy maize starch at 15—16°, it was found that, although the rate of periodate uptake was normal (0·55 mole/162 g. of starch after 40 hr.), the acid liberated reached the theoretical value in only 38 hr. and continued to increase. The alkali-sensitivity of some aldehydes obtained by periodate oxidation has been investigated by Head (*J. Text. Inst.*, 1947, **38**, T 389), and it is considered that the acid formation reported here is due to alkaline hydrolysis of the acetal linkages accompanied by continued conversion of –CHO into –CO_2H by Cannizzaro-type reactions and *not* to over-oxidation by periodate, as the uptake did not exceed 1·1 moles/anhydroglucose unit (cf. Barry, *J.*, 1942, 578).

There was the possibility that esterification of $C_{(6)}$ in the starch may occur with some of the liberated formic acid (cf. Gottlieb, Caldwell, and Hixon, *J. Amer. Chem. Soc.*, 1940, **62**, 3342; Tarkov and Stamm, *J. Phys. Chem.*, 1952, **56**, 262). This would account for the release of some acid when the oxidation product is treated with alkali, and would imply that the estimation of formic acid by titration of aliquot portions centrifuged free from

oxidised starch granules would not be quantitative. As esterification might have already occurred during preparation of the polyaldehydes, quantities were shaken for twelve days at 15—16° with concentrations of formic acid ten times greater than that normally released during oxidation, so that further esterification could occur. The formic acid was recovered quantitatively: in a similar experiment with pure starches, evidence of 0·2% removal of acid was obtained. Hence no significant quantity of formic acid becomes chemically bound as ester during periodate oxidations lasting 12 days at 15—16°.

As Halsall, Hirst, Jones, and Sansome (*Biochem. J.*, 1948, **43**, 70) have obtained evidence that different samples of the same starch, derived from plants differing in botanical variety and growth-conditions, contain the same proportions of end-group, and

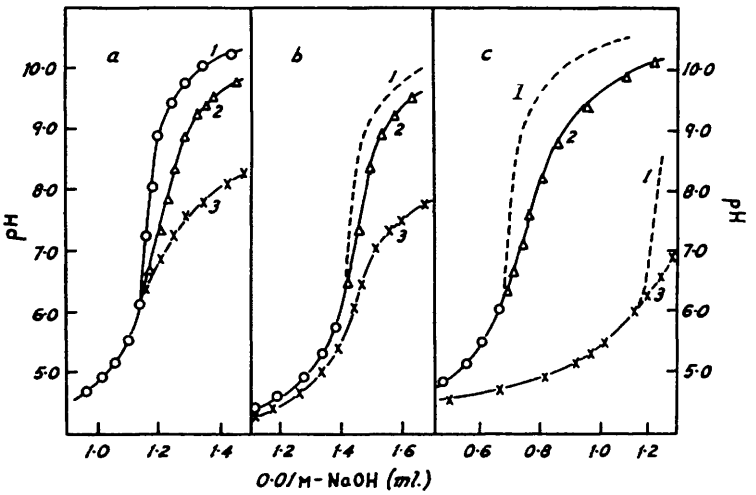

FIG. 3 *a*. *Titration curves for control solutions.*

Curve 1. $H{\cdot}CO_2H$–NaOH.
Curve 2. ($H{\cdot}CO_2H$ + KCl + $NaIO_4$ + *ethylene glycol*)–NaOH.
Curve 3. [*As* (2) + *oxidised starch*]–NaOH.

FIG. 3 *b*. *Titration curves for reaction mixtures.*

Curve 1. *Control* $H{\cdot}CO_2H$–NaOH.
Curve 2. *Centrifuged reaction mixture*–NaOH.
Curve 3. *Uncentrifuged reaction mixture*–NaOH.

FIG. 3 *c*. *Titration curves for a proteinaceous reaction mixture.*

Curve 1. *Control* $H{\cdot}CO_2H$–NaOH.
Curve 2. *Centrifuged reaction mixture*–NaOH.
Curve 3. *Uncentrifuged reaction mixture*–NaOH.

as this result has been substantiated (see Table 3), the deviating periodate values in Table 1 could possibly be explained by experimental errors arising through incomplete oxidation and the use of differing procedures for the determination of formic acid. Abdel-Akher and F. Smith (*J. Amer. Chem. Soc.*, 1951, **73**, 994) prefer the iodometric method; Kerr and Cleveland (*ibid.*, 1952, **74**, 4036) titrate to pH 7·1, and papers in which titration to pH 5·5, 6·0, 8·0, and 8·2 was used have been referred to by Morrison, Kuyper, and Orten (*loc. cit.*), who showed that choice of end-point in the range pH 5·5—8·0 should have little influence on quantitative titration of *pure* formic acid. This is correct, but potentiometric titration studies carried out here, supported by iodometric determinations, have shown that quantitative titration of formic acid in the presence of all normal oxidation products is complete at pH 6·25, and that over-titration to pH 8·0 would reduce the value obtained for a true ($\bar{R}$) value of 25—30 by about 20%. In Fig. 3 *a*, which shows typical potentiometric titration results for control solutions, the curve for pure formic acid (curve 1) differs

above pH 6·5 from that for the same quantity of acid in the equivalent of a centrifuged reaction mixture (curve 2). A still greater divergence occurs above pH 6·25 when 50 mg. of oxidised starch (carefully washed free from acid) are added, so giving the equivalent of an uncentrifuged reaction mixture (curve 3). Cori and Larner (*J. Biol. Chem.*, 1951, **188**, 17) noted " an apparent buffering action " during titration of periodate reaction mixtures : this is now explained by the alkaline degradation of oxidised starch. From the equivalence point of curve 1 at pH 7·1, it is seen that under these experimental conditions, titration of formic acid is complete by pH 6·5 for curve 2 and pH 6·25 for curve 3. The divergence of these curves above pH 6·25 is due to inclusion in the titration of substances other than formic acid.

Fig. 3 *b* shows the titration curves for a typical reaction mixture, curve 2 being for 10 ml. of centrifuged solution and curve 3 for 10 ml. of the heterogeneous mixture. Although both curves are superimposable with curve 1 for pure formic acid to pH 6·25, they are not themselves coincident over any range, and the end-points at pH 6·5 for (2) and pH 6·25 for 3 differ by 0·03 ml. Differences of this order (about 2% of the total titration) were consistently found. Investigation showed that the formic acid in the reaction mixture is not uniformly distributed, about 2% being loosely bound to the polyaldehydic

TABLE 3. *Average values of* $(\bar{R})$ *found for unfractionated starches, and calculated number of glucose residues per non-reducing end-group in the amylopectin fraction.*

Source of starch	Temp.	No. of determns.	Oxidation time (hr.) *	Av. value of $(\bar{R})$ †	Amylose ‡ content (%)	Calc. chain-length for amylopectin
Arrowroot [c]	15—16°	3	284—312	27·3	20·5	21·7
Banana [c]	15—16	2	244—336	26·3	16·8	21·9
Barley I [a]	15—16	4	260—282	29·5	22·0	23·0
Barley II [f]	15—16	2	260—282			
Iris germanica [g]	15—16	2	262—308	28·0	27·0	20·4
Maize [a]	15—16	2	300—384	26·5	24·0	20·1
Oat I and II [h]	15—16	5	240—318	27·4	26·0	20·3
	20—21	1	164—240			
Parsnip [h]	15—16	2	268—360	23·0	11·1	20·4
Pearl manioc [c]	15—16	2	244—312	24·1	15·7	20·3
Potato I [a]	15—16	2	291—383	28·3	20·4	22·5
Potato II [d]	15—16	2	336—455	28·3	21·0	22·4
	20—21	1	186			
Potato III [b]	15—16	2	335—455	28·3	21·0	22·4
Rice [c]	15—16	3	286—384	27·5	18·5	22·4
	20—21	1	164—212			
Sago [a]	15—16	2	244	25·0	26·0	18·5
Sweet potato [c]	15—16	2	266—310	28·2	17·8	23·2
Tapioca [c]	15—16	4	264—300	26·2	16·7	21·8
Waxy maize [c]	15—16	4	302—400	20·0	<1	20
	20—21	1	164—284			
Wheat [c]	15—16	4	260—306	26·2	25·0	19·6

* The time necessary (found by separate expt.) for periodate uptake to reach 1·03—1·05 moles/162 g. starch : the range quoted shows the period in which no over-oxidation occurred, the formic acid released being constant within the limits corresponding to $(\bar{R}) \pm 0{\cdot}5$ glucose unit.

† All values obtained were within $\pm 0{\cdot}5$ glucose unit from the average.

‡ Values obtained from potentiometric iodine titration curves (Anderson and Greenwood, unpublished work).

[a–d] See Table 1. [f] Aspinall, Hirst, and McArthur. [g] Aspinall and Johnstone. [h] Greenwood (all unpublished work).

oxidation product and removable by several washings with distilled water. Hence removal of the starch oxidation product before titration of formic acid gives values of $(\bar{R})$ which are about 0·5 glucose unit high. By careful titration of the heterogeneous reaction mixture to pH 6·25, any bound acid is included in the titre and there is no risk of including any acid arising from alkaline degradation of the polyaldehyde.

The behaviour of some protein-contaminated oat starches [obtained as intermediates in the purification of the pure starch (Anderson and Greenwood, unpublished work)] on

periodate oxidation has been studied. For protein contents of less than 3%, uptake of periodate is normal, and the correct value of $(\bar{R})$ is given when the sample weight is corrected for the percentage of protein present. The differences in titration curves of centrifuged and non-centrifuged samples increase with increasing protein-content. Fig. 3*c* shows the curves obtained for a product containing 23% of protein. The end-point for the centrifuged solution (pH 6·5; curve 2) gives a value of $(\bar{R})$ of 40, whilst that for the non-centrifuged solution (pH 6·25; curve 3) gives the correct value of $(\bar{R})$ of 28. Thus the presence of protein causes further complex-formation with formic acid, and the *heterogeneous* reaction mixture must be titrated. For samples containing more than 23% of protein, periodate uptake exceeds the theoretical value and no reliable estimate of $(\bar{R})$ can be obtained from the potentiometric-titration curves.

The potentiometric-titration method has been found to be the simplest and most reproducible for determining the formic acid released on periodate oxidation. Whilst the iodometric method, which determines total acidity, gives good agreement in determinations on pure starches, the potentiometric method has the advantage of showing from the shape of the titration curve whether acids other than formic are being titrated, so indicating the presence of impurity. The steam-distillation method has been found to give less consistent results for the small amounts of formic acid normally released, and it is slower.

The average values of $(\bar{R})$ found for the starches studied are presented in Table 3, the experimental error being within $\pm 0{\cdot}5$ glucose unit. The values deduced for the average length of unit-chain in the corresponding amylopectin components are also shown.

Under the experimental conditions described, periodate oxidation is a reliable and easy routine method for determining values of $(\bar{R})$ for starches, having an accuracy and reproducibility better than that of the methylation technique.

Experimental

All starch samples were dried *in vacuo* at 80° for several hours. Reagents were of analytical grade, or were purified as described by Halsall, Hirst, and Jones (*loc. cit.*). Nitrogen and sodium hydroxide used during potentiometric titrations were free from carbon dioxide.

Periodate Oxidations.—Starch (250—400 mg.) was suspended in 0·56M-potassium chloride (60 ml.) to which was added 0·2M-sodium metaperiodate (20 ml.). Within these limits the rate of oxidation was independent of the weight of starch. Reaction flasks were shaken continuously in the dark in a constant-temperature room.

Potentiometric Titrations.—Samples (10 ml.) were withdrawn by pipette at the required times. Ethylene glycol (1 ml.) was added and the mixture shaken in the dark for at least 10 min., the time found necessary for complete reaction between the glycol and the suspension of potassium periodate. (All excess of periodate *must be destroyed* before the start of a potentiometric titration.) Nitrogen was bubbled through the mixture for 10 min. before titration with 0·01M-sodium hydroxide (semimicro-burette). The passage of nitrogen was continued throughout the titration, which was followed by means of a glass electrode and Pye mains-operated pH-meter. Blank determinations showed that generally no correction was required for the acidity of the starch samples or of other reagents.

Withdrawal of samples by pipette whilst the reaction flask was *shaken gently* introduced no error. Each of seven 10-ml. portions withdrawn consecutively from a reaction mixture gave the same titre with sodium hydroxide, and, further, the value of $(\bar{R})$ deduced was the same as that obtained from titrations of the entire contents of each of three individual reaction mixtures (10 ml. each) after the same oxidation time. This procedure did not, however, give consistent results for determinations of excess of periodate as the more rapid sedimentation of potassium metaperiodate made impossible the withdrawal of samples homogeneous with respect to this component.

It was shown that no loss of acid occurred when nitrogen was passed through 0·0015M-formic acid for 1½ hr.

Oxidation of Formic Acid by Potassium Metaperiodate.—No loss of formic acid or consumption of periodate occurred when 0·0015M-formic acid was shaken with a saturated solution of potassium metaperiodate for 28 days in the dark at 15—16°. At 20—21°, however,

the concentrations of formic acid and of periodate decreased by 3% after 15 days, and by 6% after 21 days.

Distribution of Formic Acid in Reaction Mixtures.—After oxidation for 240 hr., a starch–potassium periodate reaction mixture (80 ml.) was divided into two. The first half was centrifuged, and portions (10 ml.) of the clear supernatant liquid were titrated (after destruction of excess of periodate) against 0·01024M-sodium hydroxide to pH 6·25. The average titre was 1·40 ml. From the second half, two *heterogeneous* 10-ml. portions were withdrawn, treated with glycol, and titrated to pH 6·25: the average titre was 1·43 ml. The remaining two 10-ml. portions were treated separately with glycol, then centrifuged, and the oxidised granules were washed three times with distilled water by centrifugation. The combined supernatant liquids and washings were then titrated to pH 6·25, the average titre being 1·43 ml.

Determination of Periodate Uptake.—Residual periodate concentration was determined by Fleury and Lange's indirect method (*J. Pharm. Chim.*, 1933, **17**, 107). This method gave satisfactory results in the presence of all reactants and products when the reaction mixture, to which had been added excess of bicarbonate, arsenite, and iodide, was *shaken in the dark for 15 min.* before back-titration with iodine. Periodate uptake was determined by analysis of a series of individual mixtures (10 ml., containing *ca.* 50 mg. of starch). The stoppers of the conical reaction flasks were lubricated with a little silicone grease.

Periodate Uptake and Formic Acid Release from Sucrose.—Results obtained (expressed in moles/mole of sucrose) were: (*a*) periodate uptake: 2·98 (262 hr.); 3·12 (300 hr.); (*b*) formic acid release: 0·87 (262 hr.); 0·88 (286 hr.); 0·89 (352 hr.); 0·91 (408 hr.); 0·92 (420 hr.); 0·93 (570 hr.).

Interaction of Formic Acid with Starches and their Oxidation Products.—The following mixtures were shaken for 240 hr. in the dark at 15—16°: (*a*) control solution of formic acid (10 ml.); (*b*) formic acid (10 ml.) and oat starch (64·35 mg.); (*c*) formic acid (10 ml.) and periodate-oxidised oat starch (65·18 mg.); (*d*) formic acid (10 ml.) and waxy maize starch (60·08 mg.); and (*e*) formic acid (10 ml.) and periodate-oxidised waxy maize starch (55·60 mg.). The contents of each reaction flask were then titrated to pH 6·25 against 0·00901M-sodium hydroxide, the titres obtained being (*a*) 16·15, (*b*) 16·05, (*c*) 16·15, (*d*) 15·85, and (*e*) 16·15 ml.

Examination of Polyaldehydic Oxidation Products.—No colour reaction occurred with (*a*) iodine–potassium iodide, (*b*) dilute sulphuric acid–potassium iodide, or (*c*) sulphuric acid–diphenylamine; Fehling's solution and Schiff's reagent were reduced. The release of acidic decomposition products from the oxidised starch in the presence of alkali was shown in the following experiments: (*a*) Oxidised oat starch (50 mg.) was added to water (5 ml.) which had been boiled, and then cooled, in the presence of nitrogen. 0·01024M-Sodium hydroxide (1 ml.) was added, and the mixture shaken for 15 hr. The pH was then 6·25, and did not decrease further during 24 hr. Further addition of 0·01024M-sodium hydroxide (1 ml.) gave an initial value of pH 10·5, which decreased overnight to pH 6·25 and did not decrease further. (*b*) Oxidised waxy maize starch (35·92 mg.) was shaken with 0·00901M-sodium hydroxide (20 ml.) for 17 hr. Titration of the excess of alkali to pH 6·25 required 1·70 ml. of 0·0147M-formic acid. Hence 232 g. of oxidised starch would liberate 1 l. of N-acid (duplicate experiment gave 273 g. as the apparent neutralisation equivalent).

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[*Received, September 13th*, 1954.]