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The Estimation of Small Amounts of Formaldehyde Liberated during the Oxidation of Carbohydrates and other Substances with Periodate

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In the course of investigations into the chemistry of the blood-group mucoids and closely related polysaccharides, it was necessary to measure the formaldehyde produced on oxidation of these materials with periodate. The gravimetric estimation of the aldehyde as its dimedon derivative was used by Aminoff & Morgan (1951) in their studies on blood-group A substance, but the method has two disadvantages. First, relatively large amounts (30–50 mg.) of the material to be oxidized are required in order to yield a suitable amount of the dimedon derivative, and secondly, other aldehydes such as acetaldehyde will also give a dimedon derivative and the quantitative separation of these non-specific materials, without loss of the formaldehyde derivative, may be difficult. Furthermore, the reagent blank is appreciable (Courtois, 1951).

A method for the determination of small amounts of formaldehyde was therefore sought which was specific for formaldehyde, and which was applicable to small amounts of sugars, polysaccharides, mucoids, or other materials yielding formaldehyde on oxidation with periodate. Eegriwe (1937) observed that formaldehyde reacted with chromotropic acid (1:8-dihydroxynaphthalene-3:6-disulphonic acid) on heating in strong sulphuric acid to yield a highly coloured compound, and that the reaction appeared to be specific for formaldehyde.

This reaction has already been adapted for quantitative use by several authors (Boyd & Logan, 1942; MacFadyen, 1945; Bricker & Johnson, 1945), but where the estimation was preceded by oxidation with periodate, the formaldehyde was in most instances recovered from the reaction mixture by distillation. Thus methods have been proposed for the analysis of serine in protein hydrolysates (Boyd & Logan, 1942) and steroids containing the α-ketol group (Daughaday, Jaffe & Williams, 1948; Corcoran, Page & Dustan, 1950) which depend on periodate oxidation and subsequent distillation of the oxidation mixture, the formaldehyde in the distillate being estimated colorimetrically after condensation with chromotropic acid. Quantitative distillation of small amounts of formaldehyde, however, is difficult and is seldom attained; moreover, the products of oxidation of some carbohydrate materials are unstable and on heating during distillation decompose to give additional formaldehyde or materials which interfere with its estimation. It is therefore desirable to estimate the formaldehyde *in situ*. Chromotropic acid, however, is rapidly oxidized by periodate, and the complete removal of the excess periodate together with the inorganic reaction products is essential.

Fleury, Courtois & Perles (1951) state that, after removal of the interfering ions as insoluble salts, they were able to estimate with chromotropic acid the glyoxylic acid formed on oxidation of some organic acids with periodate. The direct application of their technique using barium hydroxide and silver acetate as precipitants was found impracticable at the pH (7·5–8) which is optimal for formaldehyde production. A method devised by Rabinovitch, Decombe & Freedman (1951) for the estimation of α-ketol steroids, which involved the use of sulphite as reducing agent and the subsequent removal of the iodide so formed as the silver salt, was likewise inapplicable owing to the large amounts of silver sulphate required. When silver salts are used as precipitants, the photochemical formation of colloidal silver interferes with the spectrophotometric estimation.

The success of a method which employs chromotropic acid for the micro-estimation of formaldehyde after periodate oxidation thus depends on the discovery of an agent which precipitates quantitatively periodate and iodate. As mentioned above, barium and silver salts are unsatisfactory, as are salts of mercury, zinc, magnesium, aluminium, beryllium, manganese, titanium, bismuth, cadmium and calcium. Compounds (e.g. sodium bisulphite or meta arsenite) which reduce the periodate to iodate or iodide which are subsequently removed, are likewise unsuccessful. The most encouraging results were obtained using lead salts, as lead periodate and lead iodate are insufficiently soluble to cause any interference in the reaction. The choice of a soluble lead salt was, however, limited. Lead nitrate interferes with the chromotropic acid reaction; lead acetate is moderately successful, but gives a yellow colour with the chromotropic acid solution. Lead dithionate is, however, very suitable, principally because of its high solubility and also since the dithionate ion decomposes in acid into sulphate and sulphur dioxide. The lead sulphate is conveniently removed by centrifugation and the sulphur dioxide does not interfere with the formaldehyde reaction (see also Bricker & Vail, 1950), and, in fact, stabilizes the chromotropic acid against oxidation by air and light.

EXPERIMENTAL

Materials

Chromotropic acid. Technical chromotropic acid gives rise to very high blank readings which can be greatly reduced by purifying the reagent via the lead salt (Boyd & Logan 1942).

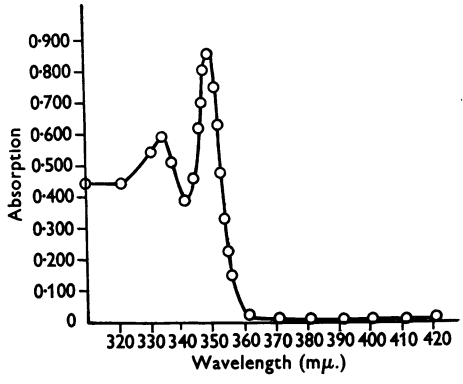

Fig. 1. The absorption spectrum of sodium salt of chromotropic acid (0·003%) in 0·3 N-H_2SO_4.

The most satisfactory material, however, is obtained by using the method of purification described by Ovenston, Parker & Hatchard (1952), and their criteria of purity. The impure sodium salt is recrystallized from twice its weight of hot water and the crystals obtained after standing overnight are dried over H_2SO_4. The whole operation is carried out in complete darkness except when manipulations are taking place, and even then the light is reduced to the absolute minimum necessary. The purity of the product is assessed by spectrophotometric examination of its solution in 0·3 N-H_2SO_4, at a concentration of 3 mg./100 ml. Values for extinction are obtained at 340, 348 and 420 mμ. The pure product, usually obtained after one cryst allization, should satisfy the following specifications: E_{420}/E_{348} to be not greater than 0·015 and $E_{348}/E_{340}/c$ to be not less than 0·130 where c is concentration in mg./100 ml. The pure reagent should be stored in a dark bottle away from light. The absorption spectrum of a satisfactory sample is shown in Fig. 1. (Hopkin and Williams Ltd. now supply a grade of chromotropic acid (sodium salt), which satisfies these

criteria and has been used successfully without further purification.) In aqueous solution, chromotropic acid and the sodium salt are unstable. The decomposition encountered is of two types, one photochemical and the other independent of light. Photochemical decomposition of aqueous solutions is accompanied by variation in extinction readings at 340, 348 and 420 mμ. and also by the appearance of a clearly visible orange colour which gives rise to a diffuse absorption between 390 and 480 mμ. The decomposition which occurs in the absence of light likewise affects the values of extinction at 340, 348 and 420 mμ., but causes only very slight absorption in the 390—480 mμ. range. However, in this instance the decomposition is accompanied by the appearance of a secondary absorption peak at 362 mμ. Both types of decomposition are minimized by the addition of $SnCl_2$ and storage away from light, and the stability of the acid is further increased by keeping it in strongly acid solution.

Chromotropic acid reagent. The pure dry sodium salt of chromotropic acid (1 g.) is dissolved in hot water (100 ml.) and the solution filtered through glass wool to remove any insoluble sulphones. $SnCl_2$ (0·1 g.) is added and the turbid solution so obtained is diluted to 500 ml. with H_2SO_4 (66%, v/v). All operations are carried out in the dark and the final solution stored in a stoppered, brown glass container. The solution should be prepared fresh every 2 or 3 days.

Periodate solution. Sodium metaperiodate (0·015M) in H_2SO_4 (0·045N) is diluted immediately before use with an equal volume of N-$NaHCO_3$. This reagent is suitable for the oxidation of the simple substances described in this paper. However, since it is intended to use this technique to investigate the rate and extent of formaldehyde production during the oxidation with periodate of polysaccharides and mucoids, substances which consume very much larger amounts of periodate than simple sugars to yield a given amount of formaldehyde, a more concentrated reagent has been used for much of this work. The reagent is prepared by adding a solution of $NaIO_4$ (0·06M) in H_2SO_4 (0·18N) to an equal volume of a suspension of $NaHCO_3$ (2N). This reagent deposits a crystalline precipitate, presumably Na_5IO_6, on standing.

Lead dithionate. One mol. prop. of H_2SO_4 is added to a solution of BaS_2O_6 (20%, w/v), the $BaSO_4$ precipitate removed by centrifugation, and a slight excess of $PbCO_3$ added to the acid solution. After the reaction is complete the excess $PbCO_3$ is removed by centrifugation and the supernatant fluid added to ethanol (2–3 vol.). After standing at 0° overnight, the crystals obtained (PbS_2O_6, $4H_2O$), are washed in ethanol and dried over $CaCl_2$. The reagent consists of a 10%, w/v, solution in water. A 20%, w/v, solution is employed when the more concentrated oxidant is used. Solutions of the salt kept frozen at −10° are stable for long periods.

Formaldehyde solution. Commercial formalin solution, assayed by oxidation with H_2O_2 in alkaline solution and back titration with acid, was used as a standard in the early part of this work. Such a solution may contain variable amounts of finely divided insoluble polymer in suspension, and it was preferable to use a formaldehyde solution prepared by acid hydrolysis of hexamethylenetetramine (MacFadyen, 1945). The formaldehyde so obtained was steam-distilled according to the method described by Boyd & Logan (1942), and preserved by addition of 2–3 ml. N-H_2SO_4/l.

Method

Freshly prepared periodate-bicarbonate solution (1 vol.) is added to water (1 vol.) containing the material to be oxidized. The concentration of this material should be of the order of 0·01% for a monosaccharide, and appropriately reduced if serine or ethylene glycol are used. The initial pH of the solution is about 7·5, and the oxidation is allowed to proceed in the dark, at or below room temperature.

At appropriate intervals, 1 ml. portions are withdrawn and pipetted into 1 ml. of PbS_2O_6 solution in a conical centrifuge tube. After mixing and centrifuging, a 1 ml. portion of the supernatant is withdrawn (care being taken to avoid inclusion of any of the precipitate), placed in a second centrifuge tube, and chromotropic acid reagent (9 ml.) is added. (This and subsequent operations should be carried out away from direct light.) The mixed reagents are allowed to stand for 30 min.; the $PbSO_4$ is removed by centrifugation and the supernatant fluid transferred to a glass-stoppered tube and heated on a boiling-water bath for at least 30 min. The absorption at 570 mμ. is read on a Hilger spectrophotometer, or a colorimeter using a filter giving maximum transmission at 570 mμ. can be used.

Blank determinations. Blanks using distilled water, and suitable standards have been included in all determinations. A variation in the quality of any of the reagents was usually detected as a changed value for the blank. For example, solutions of periodate which had stood for a long time gave a brown colour to the blank, which normally was colourless. Exposure to light either of the mixture during the oxidation or of the final chromotropic acid solution likewise gave a colour and some absorption in the blank. As a means of comparing day-to-day variations in the blank and also of preventing increase in reading during exposure in the spectrophotometer, all readings have been taken against 66% (v/v) H_2SO_4 and appropriate correction made. Slightly increased values of the absorption of blanks, standards and unknown materials, dependent on the age of the chromotropic acid reagent, have been observed. Such increase was accompanied by a slight discoloration in the blank. The use of freshly prepared reagent eliminated this effect, but in practice such a procedure was unnecessary in that, despite discoloration, the corrected absorption value was unaltered.

Formaldehyde standard. It has been established by MacFadyen (1945) that solutions containing from 0 to 17 μg./ml. of formaldehyde when heated in sulphuric-chromotropic acid solutions under the conditions described here for the development of the colour give rise to a chromotropic acid complex with a maximum absorption at 570 mμ. The extinctions at this wavelength are linear with concentration over this range. This has also been found to be true for standard formaldehyde solutions when these are made up in periodate-bicarbonate and precipitated with PbS_2O_6. Since standard solutions of ethylene glycol or glucose may be conveniently prepared by direct weighing, it has been found preferable to use these substances as standards in place of formaldehyde solutions.

RESULTS

Glucose and ethylene glycol. Glucose gives the theoretical yield of formaldehyde after oxidation for 30–40 min. with periodate at room temperature. In the range investigated (50–200 μg./ml.) the

Table 1. *Oxidation of glucose and ethylene glycol at* 16–18°

Concentration before addition of oxidant (μg./ml.)	Time of contact with oxidant (hr.)	Extinctions at 570 mμ. (2 cm. cell)	Formaldehyde found (μg.)	Formaldehyde found (% of theory)
		Glucose		
100	1	0·490	16·7	100·0
50	2	0·245	8·4	100·1
100	2	0·491	16·7	100·0
100	3	0·491	16·7	100·0
100	4	0·491	16·7	100·0
		Ethylene glycol		
22·66	1	0·640	21·8	99·5
22·66	2	0·632	21·6	98·2
22·66	3	0·630	21·5	98·1
22·66	4	0·630	21·5	98·1
15·5	24	0·430	14·9	99·2

theoretical yield is obtained with both the higher and lower concentrations of oxidant. Ethylene glycol is likewise oxidized rapidly and quantitatively to formaldehyde. Table 1 shows some results using these substances. In studying the rate and extent of formaldehyde formation by other sugars, polyhydric alcohols, and serine, known amounts of glucose or ethylene glycol have been oxidized at the same time and the formaldehyde produced by these substances used as reference standard. The extinctions shown in Table 1 have been regularly obtained. Glucose standards (Merck) were diluted as required from a 1 % solution which was kept frozen at −10°. Ethylene glycol was distilled *in vacuo* from sodium hydroxide, dispensed in ampoules and weighed out as required.

Serine. Using the dimedon reagent, Nicolet & Shinn (1941), and Martin, Synge & Bell (1941) assayed serine by estimating the formaldehyde produced by oxidation with periodate. Boyd & Logan (1942) similarly oxidized serine, distilled the formaldehyde, and estimated the latter by chromotropic acid. Desnuelle, Antonin & Daudet (1944) determined the formaldehyde produced by the Schryver reaction (Schryver, 1909). Recoveries in all cases have been from 90 to 99 %. Using the dithionate method, serine (0·005 %, w/v) has been estimated and 97·2 % of the theoretical yield of formaldehyde was obtained after oxidation at room temperature for 0·5 hr. (see Fig. 2). This figure did not alter appreciably after oxidation for 24 hr. At 2° the formaldehyde obtained was equivalent to 98·6 % of theoretical after oxidation for 3 and 10 hr.

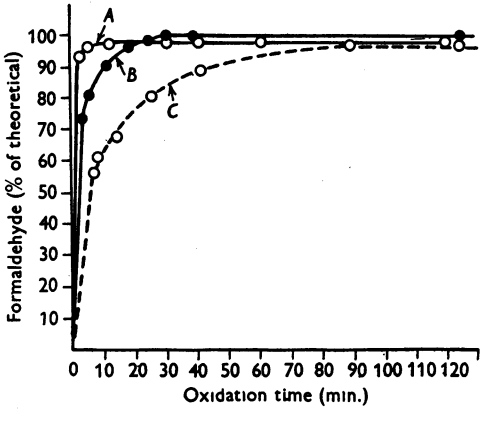

Fig. 2. Oxidation of serine, glucose and galactose at room temperature using 0·06 M-$NaIO_4$ in 0·18 N-H_2SO_4. *A*, serine (0·005 %, w/v); *B*, glucose (0·01 %, w/v); *C*, galactose (0·01 %, w/v).

Monosaccharides. The oxidation of glucose, galactose and mannose has been studied by many workers (see Jackson, 1944; Courtois, 1948, 1951, for review of literature). Using solutions of these monosaccharides (0·01 %, w/v) formaldehyde recoveries obtained are shown in Table 2. The course of the oxidation of galactose and glucose is shown in Fig. 2.

Polyhydric alcohols. These oxidations followed the course indicated by many workers and summarized by Courtois (1951). The oxidation of ethylene glycol has already been mentioned. Table 3 shows

Table 2. *Oxidation of glucose, galactose and mannose* (0·01 %) *at* 16–18° *and at* 2°

(The range of values obtained represents the limits of many determinations using either 0·015 M-$NaIO_4$ in 0·045 N-H_2SO_4 buffered with N-$NaHCO_3$ or 0·060 M-$NaIO_4$ in 0·18 N-H_2SO_4 buffered with 2 N-$NaHCO_3$.)

Temp.	Formaldehyde found (% of theoretical) Glucose	Galactose	Mannose
16–18°	99·3–100·4	96·0–96·8	96·0–98·5
2°	99·5–100·4	96·0–97·3	98·6–100·0

Table 3. *The formaldehyde liberated from erythritol* (0·004 %), *dulcitol* (0·005 %), *and mannitol* (0·005 %) *at* 16–18° *and at* 2°

(Oxidation mixture: 0·015 M-$NaIO_4$ in 0·045 N-H_2SO_4 buffered with N-$NaHCO_3$.)

Period of oxidation (hr.)	Formaldehyde found (% of theoretical)		
	Erythritol	Dulcitol	Mannitol
	Oxidation at 2°		
18	100·9	53·8	95·8
42	102·0	76·7	95·8
115	99·5	94·2	94·2
	Oxidation at 16–18°		
18	100·3	105·4	96·1
42	100·9	106·4	94·8

the course of oxidation of erythritol (0·004 %), dulcitol (0·005 %), and mannitol (0·005 %) at 16–18° and at 2°. Glucose (0·01 %) was used as standard throughout. The course of the oxidation was not followed in the early stages; more prolonged periods of oxidation were studied so that the stability of the formaldehyde, once formed, could be observed. The results confirmed those of Head & Hughes (1952), in that substantial oxidation of the formaldehyde by periodate did not occur. The rate of oxidation of the hexahydric alcohols was similar to that of the monosaccharides.

N-Acetylglucosamine. Jeanloz & Forchielli (1951) studied the oxidation of glucosamine and its derivatives with periodate and showed that the oxidation is complete and side reactions are eliminated if the oxidation is carried out in the dark and in a buffered medium at pH 4·5. The course of the oxidation was followed by measuring the consumption of periodate, but the formaldehyde produced was not estimated. The liberation of formic acid and ammonia, two other products of periodate oxidation of these compounds, was followed. The optimal pH for measurement of formaldehyde during the oxidation of sugars by periodate has been shown to be about neutral or slightly alkaline (Jeanloz, 1944; Bell, 1948). Aminoff & Morgan (1951), using buffered periodic acid at room temperature and following the procedure of Reeves (1941), oxidized *N*-acetylglucosamine and estimated the formaldehyde as the dimedon derivative. A yield of 93–95 % of the theoretical amount was obtained. Under similar conditions, but employing the chromotropic acid method, the course of oxidation of *N*-acetylglucosamine has been followed, (Fig. 3), and 90 % of the theoretical yield of formaldehyde was obtained. At 2° and under otherwise identical conditions, the yield of formaldehyde was increased to 97 %. The effect of varying the pH of

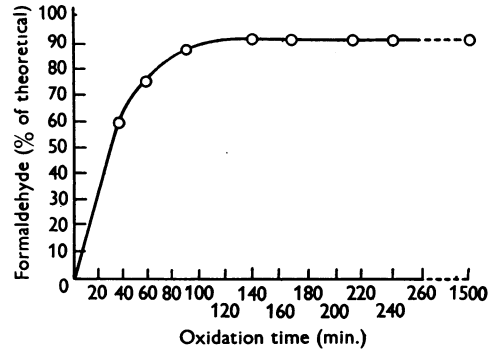

Fig. 3. Oxidation at room temperature of *N*-acetylglucosamine (0·01 %), using 0·015 M-$NaIO_4$ in 0·045 N-H_2SO_4 buffered with N-$NaHCO_3$.

Table 4. *Oxidation of glucose and* N-*acetylglucosamine* (0·01 %), *at* 2° *at various* pH *values*

(Oxidation mixture 0·015 M-$NaIO_4$ in 0·045 N-H_2SO_4 with various buffers.)

pH and buffer	Formaldehyde found (% of theoretical) after			
	21 hr.	45 hr.	69 hr.	141 hr.
	Glucose			
7·5 (N-$NaHCO_3$)	99·5	99·5	99·5	99·5
5·0 (unbuffered)	9·8	14·9	20·4	27·4
9·5 (borate)	99·5	99·5	99·5	99·5
	N-Acetylglucosamine			
7·5 (N-$NaHCO_3$)	97·0	97·0	97·0	97·0
5·0 (unbuffered)	32·2	44·5	54·3	66·1
9·5 (borate)	42·8	51·4	52·9	60·5

the oxidation and the ions present is shown in Table 4.

Miscellaneous observations. MacFadyen (1945) has recorded that a large number of aldehydes and also such biological materials as suspensions of *Shigella sonnei* and allantoic fluid do not interfere with the estimation of formaldehyde with chromotropic acid. Oxidation of fucose and threonine, together with ethylene glycol or glucose, has shown that acetaldehyde does not substantially affect the estimation of formaldehyde unless it is present in very much higher concentration than formaldehyde, as stated by MacFadyen (1945). Serine in the presence of threonine likewise gave results similar to those for serine alone. Glyoxal bisulphite gave no colour on heating with the chromotropic acid reagent. The absence of abnormality in the oxidation of serine indicated that glyoxylic acid was not causing interference. This was confirmed by oxidizing tartaric acid whence no colour was produced with the reagent. Finally, the indiffusible material obtained after thorough dialysis of a specimen of

blood-group substance which had been oxidized with periodate (Aminoff & Morgan, 1951) also gave no colour with the chromotropic acid reagent.

DISCUSSION

The oxidation of sugars with periodate under different conditions confirms that those described by Jeanloz (1944), in which the oxidation is carried out in the presence of bicarbonate buffer at about pH 7·5, are the most satisfactory. Sodium metaperiodate instead of the sparingly soluble potassium salt has been used so that the solution can be kept at room temperature without crystallization occurring. Oxidations carried out in phosphate buffer at the same pH were not as successful as those in bicarbonate buffer, presumably due to the formation of complex ions (Bell, Palmer & Johns, 1949), although Greville & Northcote (1952) consider the nature of the buffer to be without influence on the oxidation of some methylated hexoses. In view of the findings of Price & Kroll (1938) and Sarkar (1951), a large excess of periodate has been used in the oxidation stage. An excess of periodate was also essential in our experiments owing to the high dilution of the material oxidized. Furthermore, the oxidations have invariably been carried out in the dark to obviate the occurrence of both photochemical side reactions and reagent decomposition described by Head & Hughes (1952).

The pH of the oxidant (7·5) is the initial pH only; the pH increases with time. Blank solutions as well as solutions containing oxidizable substances show a shift of pH to a more alkaline value; the changed values cannot, therefore, be entirely accounted for by iodate formation as assumed by Meyer & Rathgeb (1949). Using the conditions of Jeanloz (1944) and Reeves (1941), this shift was also apparent. While probably not affecting the work reported here, the pH change observed is likely to be of importance in the interpretation of the course of periodate oxidation of polysaccharides. It is suggested that the drift of pH may be the result of the slow attainment of an equilibrium between complex ionic species. Salts of the composition $Na_5[I(MoO_4)_6]$, $13H_2O$ and $K_5[I(Mo_6O_{24})]$ are known, i.e. HIO_4 (and its hydrates) will form complex heteropolyanions in suitable circumstances. So far as is known, analogous sulphato-periodates or carbonato-periodates have not been described, but it is not improbable that some such complex anions exist, at any rate in solution. If so, the drift of pH observed becomes explicable.

The conditions used by MacFadyen (1945) have been adapted with little modification in the procedure used to determine free formaldehyde in solution. It was necessary to use chromotropic acid of high purity and to carry out the reaction in the dark and in all-glass, stoppered reaction vessels. Amounts of formaldehyde of the order of 3–5 μg. are readily determined with an accuracy of 1–2 %.

A number of compounds have been oxidized and assayed for formaldehyde by the method described, the results in general confirming those of other workers. Galactose is oxidized more slowly than glucose, and a difference in rate of reaction is also apparent on oxidation of dulcitol and mannitol. The slight departures from theoretical yields of formaldehyde may be due, in some cases, to small amounts of impurities in the materials oxidized, since these were ordinary laboratory samples, but we have no direct evidence of this (see Tables 2 and 3).

The oxidation of *N*-acetylglucosamine has provided the only unexpected result. The oxidation of this compound is apparently much more sensitive to temperature change than is that of glucose. Moreover, it is not clear why oxidation of this acetylamino sugar at high pH and in borate buffer is incomplete and much slower than for glucose, and why the unbuffered oxidation at pH 5 is more rapid for *N*-acetylglucosamine than for glucose. It is possible that at low pH *N*-acetylglucosamine, unlike simple hexoses, tends to assume the furanose rather than the pyranose form (see Courtois, 1951).

Preliminary observations on the oxidation of disaccharides have shown that 1:4 linked sugars (lactose and maltose) yield more than the theoretical one molecule of formaldehyde per molecule of sugar, the yield in most instances being almost double this amount (Jeanloz, 1944). Small quantities of formaldehyde were detected in the oxidation of trehalose and sucrose, and the 1:6 linked melibiose yielded traces of formaldehyde on prolonged oxidation (3 days). The oxidation of disaccharides, polysaccharides and mucoids, as measured by the formaldehyde liberated, is being studied and will be reported elsewhere.

SUMMARY

1. A micro-method is described for the estimation, *in situ*, of the formaldehyde which arises from the oxidation of carbohydrates and other substances with sodium periodate.

2. Lead dithionate is used to remove the excess periodate and inorganic reaction products.

3. The method has been applied to the products of oxidation of some monosaccharides, polyhydric alcohols, serine and *N*-acetylglucosamine.

4. The conditions under which oxidations of carbohydrates with periodate are most suitably performed are discussed.

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The Respiratory and Adenosinetriphosphatase Activities of Skeletal-Muscle Mitochondria

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The study of the functions and enzymic nature of isolated mitochondria has been carried out mainly on particles obtained from liver. Kidney preparations have received some attention, but only recently have attempts been made to study mitochondria isolated from other tissues, such as, for example, from brain (Brody & Bain, 1952) and from mammary gland (Moore & Nelson, 1952).

A number of the oxidative systems, now usually associated with intact mitochondria, have been studied in muscle for several decades. Keilin & Hartree (1938, 1939) and Stern (1939) carried out early work on the nature of preparations from heart muscle which showed succinic oxidase activity and which were particulate in nature, but it is only very latterly that detailed studies have been made on heart-muscle particles isolated to correspond as closely as possible to their natural state in the cell (Harman & Fiegelson, 1952*a*; Slater & Cleland, 1953). Skeletal muscle has received even less attention, although recently communications have appeared on the enzymic properties of insect flight-muscle mitochondria (Watanabe & Williams, 1951; Sacktor, 1953), and the adenosine triphosphatase (ATPase) activity of the particulate components of rabbit skeletal muscle has been investigated by Perry (1952*a*).

Skeletal muscle is quantitatively by far the most important tissue for the oxidation of many metabolites, and for this reason alone its mitochondrial system merits study. This apparent neglect is no doubt due to the fact that the resting respiration rate of skeletal muscle from many animals is very low and that, in contrast to liver, the cells contain fewer granules, myofibrils being the dominant feature of the muscle cell. Paul & Sterling (1952) have prepared cyclophorase preparations from the skeletal muscle of various species, and report that many are poor in oxidative activity and that in general a correlation exists between granule count and oxidative activity. Cyclophorase preparations from muscle may, however, be very poor in mitochondria because this preparation contains, as main contaminant, the myofibrillar fraction which may account for 90–60 % of the total nitrogen, depending on the type of muscle.

We have investigated the oxidative activities of granules from back and leg muscle of the rabbit,