[CONTRIBUTION FROM THE DIVISION OF PLANT BIOLOGY, CARNEGIE INSTITUTION OF WASHINGTON]

Studies on Atmospheric Oxidation. III. The Catalytic Oxidation of Trioses and Related Compounds

BY H. A. SPOEHR AND HAROLD W. MILNER

In the complex series of biocatalytic reactions comprising the catabolism of hexoses, the trioses and closely related compounds have been shown to play a most important role.[1] The behavior of some of these compounds has been studied in the model of biocatalytic oxidation described by Spoehr,[2] in which sodium ferropyrophosphate acts as an oxygen carrier under what may be termed biological conditions, *i. e.*, a hydrogen-ion concentration close to neutrality, body temperatures and by the use of the oxygen of the air.

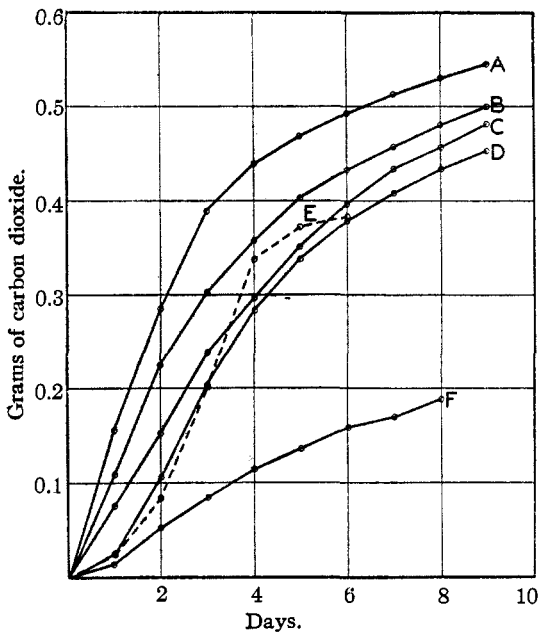

Fig. 1.—Carbon dioxide formed in the catalytic oxidation of 1.5 g. of: A, glyceraldehyde; B, dihydroxyacetone; C, acetol; D, glucose; E, glycerol; F, methylglyoxal.

It should perhaps again be emphasized that no claim is made that this system actually represents the one obtaining in any living organism, but rather that it is to our knowledge the only model in which reactions, simulating those occurring in living cells, can be carried out and that it is, therefore, not without interest in possibly throwing some light on the chemistry of the compounds involved in these complex reactions. Wind[3] has already demonstrated the decidedly stimulating effect which additions of iron and of sodium pyrophosphate, in sufficient quantity, exert on the oxidations of the trioses with air on the basis of oxygen absorbed.

The results here briefly described show a striking difference in this oxidation system between glyceraldehyde and dihydroxyacetone on the one hand and methylglyoxal on the other. The ease with which the trioses and methylglyoxal undergo condensation in solution with disodium phosphate or neutral phosphate[4] mixture must, of course, also be considered in this system. Glyceric acid is not oxidized to carbon dioxide in this system.[2]

Experimental

Unless stated otherwise the reaction mixtures were prepared by dissolving 6.7 g. of sodium pyrophosphate decahydrate in 100 cc. of water and adding thereto a solution of 1.0 g. of ferrous sulfate heptahydrate in 10 cc. of water. The precipitate of ferrous pyrophosphate dissolves by gently shaking the solution. To this was added 17 g. of disodium phosphate dodecahydrate, the organic compound to be oxidized, and finally 50 cc. of water. In some cases, where noted, 5 cc. of 0.5 *M* phosphoric acid was added to the mixture. These solutions are perfectly clear and remain so throughout the course of the experiments. The oxidations were carried out in 300-cc. Kjeldahl flasks with rubber stoppers provided for aeration with an entrance tube with constricted opening reaching to the bottom of the flask and an exit tube projecting just below the stopper. The flasks were in a water thermostat kept at 37.9°. In all the experiments the oxidations were carried out with air which was drawn over soda-lime and through several plugs of cotton. From the reaction flasks the air stream passed through a trap to collect condensed vapors and then through 0.2 *N* barium hydroxide. The carbon dioxide was determined by titration with 0.1 *N* hydrochloric acid.

The quantities of carbon dioxide formed in the catalytic oxidation of the trioses and some related compounds are shown in Fig. 1.

dl-**Glyceraldehyde.**—This triose, prepared[4] according to the method of Wohl,[5] m. p. 141°, was found to be very readily oxidized. The striking fact is that there is no indication of an induction period; the largest amount of

(1) Neuberg and Kobel, *Naturw.*, **18**, 427 (1930): Meyerhof, *Nature*, **132**, 337, 373 (1933): Barrenscheen and Beneschovsky, *Biochem. Z.*, **265**, 159 (1933).

(2) Spoehr, THIS JOURNAL, **46**, 1494 (1924): Spoehr and Smith, *ibid.*, **48**, 236 (1926): Smith and Spoehr, *ibid.*, **48**, 107 (1926).

(3) Wind, *Biochem. Z.*, **159**, 58 (1925).

(4) Spoehr and Strain, *J. Biol. Chem.*, **89**, 503 (1930).

(5) Wohl, *Ber.*, **31**, 2394 (1898).

carbon dioxide was produced during the first twenty-four-hour period and this mole per mole was higher than any other compound thus far studied. The amount of carbon dioxide formed varies with the concentration of glyceraldehyde used. The rate of oxidation of different concentrations of glyceraldehyde in the catalyst–phosphate mixture, containing 5 cc. of 0.5 *M* phosphoric acid, is shown in Table I.

TABLE I

RATE OF OXIDATION OF GLYCERALDEHYDE

Orig. concn. of glyceraldehyde, g. per 160 cc.	Moles of carbon dioxide per mole of glyceraldehyde formed in 1 day	3 days	9 days
0.10	0.6244	0.8743	1.0155
.25	.5125	.7654	0.8849
.50	.4200	.7617	.8915
1.0	.3782	.7228	.8636
2.0	.2354	.5496	.7536
3.0	.1732	.3963	.6609

After the first twenty-four-hour period the rate of carbon dioxide formation shows a gradual decrease. The rate of this decrease is larger with smaller amounts of glyceraldehyde originally present. This decrease is in general parallel to the decrease in reducing power found by Spoehr and Strain[4] for solutions of glyceraldehyde in disodium phosphate. It is probable that the decrease in the rate of oxidation may in part at least be due to the rearrangement or condensation of the triose in phosphate solution. That is, a portion of the glyceraldehyde undergoes these changes before it can be oxidized. This fact is brought out in comparative experiments in which the glyceraldehyde is added in small portions each day. Under these conditions both the daily rate and the total amount of carbon dioxide is considerably higher than when the same total amount of the triose is added at the beginning of the experiment. The percentage of glyceraldehyde oxidized is also considerably higher when this triose is added in small amounts. With the single addition 32% and in the case of the additions of small portions 54% of the theoretical quantity of carbon dioxide was formed in fifteen days. In Fig. 2 are given the results of two oxidation experiments: (A) in which 0.5 g. of glyceraldehyde was added at the beginning of the experiment and (B) in which the same total amount of the triose was added in 0.1-g. portions each third day. In both experiments the rate of oxidation was determined for fifteen days. (A) produced 0.2345 g. of carbon dioxide, or 0.9600 mole per mole of glyceraldehyde, while (B) produced 0.3943 g. of carbon dioxide, or 1.6140 moles.

In all of the experiments carried out with small portions, of which (B) above is an example, the greatest amount of carbon dioxide was formed in the first twenty-four hour period after the addition of the triose; the second day the amount decreased decidedly and on the third day fell to almost zero. Following another addition of the triose the rate of carbon dioxide formation rose again very appreciably and attained a higher value than after the previous addition. The total amount increased with each addition. Obviously, therefore, the decrease in the rate of oxidation after the first and second days is not due to an inactivation of the catalyst, but rather to the conversion of the glyceraldehyde into some product which is oxidized with greater difficulty. Examination of the reaction mixture after this experiment showed that there was very little glyceraldehyde remaining and no pyruvic acid present.

An experiment was carried out with the aim of determining whether the monomolecular form is more easily oxidized than the dimolecular.[5] One-tenth gram of glyceraldehyde was dissolved in 5 cc. of water, heated gently until the solid was dissolved and allowed to stand at room temperature for twenty-two hours. It was then added to a catalyst–phosphate mixture and the rate of oxidation determined for three days; total carbon dioxide formed was 0.8782 mole per mole of triose. A control in which the crystalline glyceraldehyde was added directly to the catalyst–phosphate mixture formed 0.9497 mole of carbon dioxide.

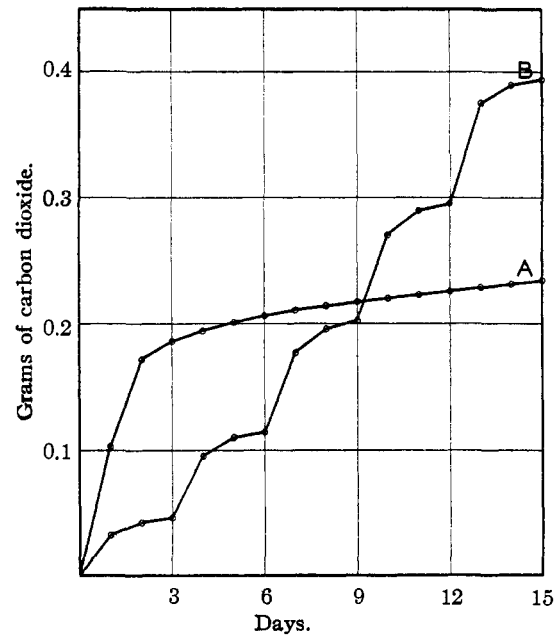

Fig. 2.—Carbon dioxide formed from 0.5 g. of glyceraldehyde: A, added at beginning of experiment; B, added in 0.1 g. portions each third day.

Wind[3] has studied the effect of phosphates and of iron on the oxidation of the trioses on the basis of oxygen absorbed and has demonstrated the stimulating effect on the rate of oxidation of complex formation between the iron and pyrophosphate. The results of a comparative experiment, on the basis of carbon dioxide formed, are shown in Fig. 3 in which 0.1 g. of glyceraldehyde was oxidized with air in identical phosphate mixtures as given above, but with the addition of 5 cc. of 0.5 *M* phosphoric acid, to each, (C) containing 1.0 g. of ferrous sulfate heptahydrate and (D) containing no added iron. From this the much higher initial rate of oxidation in the presence of iron is evident.

Dihydroxyacetone, m. p. 80–81°, prepared as previously described,[4] was easily oxidized to carbon dioxide with no induction period in the rate of oxidation when 1.5 g. of the triose was used. The rate of oxidation was slightly less than that of glyceraldehyde, Fig. 1; 0.6821 mole of carbon dioxide was formed from dihydroxyacetone and in a corre-

sponding experiment 0.7434 mole from glyceraldehyde in nine days. In this experiment the amount of carbon dioxide formed from dihydroxyacetone was also slightly less on the first day than on the second day. It has previously been found[4] that the reducing power of dihydroxyacetone decreases more rapidly than that of glyceraldehyde when these trioses are in solution with sodium phosphate.

It is a well-known fact that dihydroxyacetone easily undergoes autocondensation,[4,6] resulting in the formation of compounds of higher molecular weight and higher melting point. All attempts to break down these compounds into the monomolecular form in water solution (*e.g.*, through heating the solutions) and thus possibly to obtain a higher rate of oxidation to carbon dioxide were fruitless.

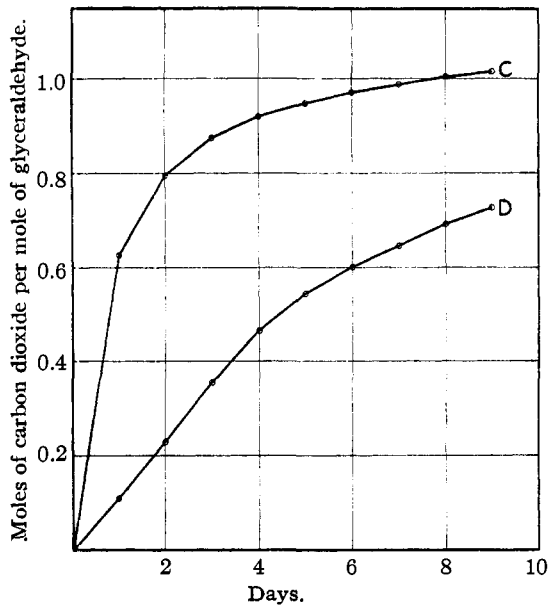

Fig. 3.—Rate of oxidation of 0.1 g. of glyceraldehyde: C, with iron; D, without iron.

Methylglyoxal, prepared by the method of Denis,[4] and by the method of Neuberg, Farber, Levite and Schwenk,[7] and assayed by means of *m*-nitrobenzohydrazine was used in these experiments. Prepared in either way the rate of carbon dioxide formation was decidedly lower than that formed from any of the trioses, Fig. 1. The rate of carbon dioxide formation from the oxidation of methylglyoxal is also more regular over a period of ten days as compared with the high initial rates observed with the trioses. The rates of carbon dioxide formation for different concentrations of methylglyoxal are shown in Table II. It was observed that the barium hydroxide solutions, used for the absorption of the carbon dioxide formed in the oxidation, were slightly yellow, indicating the presence of a volatile aldehyde. In fact, separate experiments showed that an appreciable amount of methylglyoxal was carried into the barium hydroxide solutions. Although it was not possible to calculate exactly from these the error in the quantities of carbon dioxide reported due to this cause, it is evident that these values are too high due to the reaction of the methylglyoxal with the barium hydroxide.

TABLE II

RATE OF OXIDATION OF METHYLGLYOXAL

Orig. concn. of methylglyoxal, g. per 160 cc.	Moles CO_2 per mole of methylglyoxal in: 1 day	3 days	10 days
0.15	0.0893	0.2384	0.5363
.76	.0513	.1507	.3646
1.52	.0375	.1138	.2791

Neuberg and Kobel[8] have shown that methylglyoxal–sodium bisulfite in the presence of phosphates is oxidized to pyruvic acid with molecular oxygen at room temperatures. The reaction mixtures from the oxidation experiments of methylglyoxal with sodium ferropyrophosphate were examined, according to the method of Neuberg and Kobel with 2,4-dinitrophenylhydrazine, for the presence of pyruvic acid. None of these showed the presence of this compound. Moreover, separate oxidation experiments with pyruvic acid yielded only exceedingly small quantities of carbon dioxide, so that it is highly improbable that, if methylglyoxal had been oxidized to pyruvic acid, this would have been removed from the reaction mixture through further oxidation. Consequently it may be concluded that methylglyoxal is not oxidized to pyruvic acid in this system.

Lactic acid formed only exceedingly small quantities of carbon dioxide when aerated in solution with the iron pyrophosphate complex. It is interesting, however, that under certain conditions lactic acid is easily oxidized to pyruvic acid. With iron pyrophosphate in the ferrous condition large yields of pyruvic acid were obtained, while with the ferric complex no oxidation occurred. In 350 cc. of water were dissolved 23.25 g. of sodium pyrophosphate decahydrate and 20 g. of disodium phosphate dodecahydrate. This was contained in a three-necked flask which was arranged so that the flask could be evacuated and all air replaced by oxygen-free nitrogen. To this solution was added 3.5 g. of ferrous sulfate heptahydrate in 15 cc. of water and the mixture stirred with a stream of nitrogen until all the ferrous pyrophosphate had dissolved. To this was added 0.5 g. of lactic acid neutralized with 0.5 *N* sodium hydroxide. Air was then drawn through the solution, kept at 40°, for fifteen hours. After acidifying the solution with sulfuric acid and adding 1.1 g. of 2,4-dinitrophenylhydrazine dissolved in 6 *N* sulfuric acid, it was heated for one hour on the boiling water-bath. The crude dried hydrazone, 0.99 g., treated according to Neuberg and Kobel,[8] yielded 0.9 g. of pyruvic 2,4-dinitrophenylhydrazone, m. p. 206°, corresponding to a yield of 68.7% of pyruvic acid.

Anal. Calcd. for $C_9H_8O_6N_4$: N, 20.9. Found: N, 20.56, 20.49.

A similar experiment in which, however, the sodium ferropyrophosphate mixture was aerated *before* adding the lactic acid, in order to convert the ferrous into the ferric complex, yielded on further aeration only 0.06 g. of pyruvic 2,4-dinitrophenylhydrazone, m. p. 213°.

The oxidation of the lactic acid is probably due to an in-

(6) P. A. Levene and A. Walti, *J. Biol. Chem.*, **78**, 23 (1928).

(7) Neuberg, Farber, Levite and Schwenk, *Biochem. Z.*, **83**, 244 (1917).

(8) Neuberg and Kobel, *Biochem. Z.*, **252**, 215 (1932).

duced reaction associated with the formation of the ferric from the ferrous complex. Lactic acid does not reduce the ferric complex to the ferrous, therefore the reaction cannot proceed catalytically. With a substance such as glucose, on the other hand, some of the iron is always reduced to the ferrous condition, so that under these conditions the oxidation of the glucose can proceed catalytically.

Pyruvic acid is not oxidized, or only exceedingly slowly, in this system. Nor was it possible to attain a reduction of this compound to lactic acid with the ferrous complex in an atmosphere of nitrogen.

Acetol, prepared by the method of Nef,[9] was easily oxidized to carbon dioxide, Fig. 1, the rate reaching a maximum on the third day and forming 18% of the theoretical amount of carbon dioxide in nine days. The reaction mixture was examined for pyruvic acid by the method of Neuberg and Kobel[8] and 0.93 g. of crude pyruvic 2,4-dinitrophenylhydrazone was obtained, recrystallized from acetic acid, m. p. 216–217°. When mixed with an authentic preparation no lowering of the melting point was observed.

Glycerol was oxidized with surprising ease, although the initial rate was slow. With 1.5 g. of glycerol the rate of carbon dioxide formation, Fig. 1, rose rapidly after the first twenty-four-hour period until the fourth day. Thereafter the rate dropped suddenly, so that in six days, after which period of time the carbon dioxide formation almost ceased, 18.5% of the calcd. carbon dioxide had been formed, *i. e.*, 0.555 mole or a total of 0.3833 g.

Sodium β-Glycerophosphate.—From 4.1 g. only 0.0134 g. of carbon dioxide was formed during four days of aeration.

Isopropyl alcohol did not produce any carbon dioxide. However, with the sodium ferropyrophosphate it is easily oxidized to acetone with air. The ferrous complex was prepared as described under lactic acid, 31 g. of sodium pyrophosphate decahydrate, 20 g. of disodium hydrogen phosphate dodecahydrate, 4.6 g. of ferrous sulfate heptahydrate in 500 cc. of water and 0.5 g. of isopropyl alcohol were used. The mixture was aerated for two hours at 40°, acidified with sulfuric acid and 100 cc. of the solution distilled. The acetone in this distillate was determined as the *p*-nitrophenylhydrazone; 0.287 g., m. p. 145°, was formed equivalent to 17.8% of the theoretical quantity, recrystallized m. p. 148–148.5°.

Anal. Calcd. for $C_9H_{12}O_2N_3$: N, 21.76. Found: N, 21.76, 21.70.

With the ferric complex, formed by drawing air through the mixture before adding the isopropyl alcohol, barely perceptible traces of acetone *p*-nitrophenylhydrazone were formed.

Other C_3 compounds which were subjected to this oxidation system included propyl aldehyde, *n*-propyl alcohol, propylene glycol and trimethylene glycol. None of these yielded any carbon dioxide, but formed volatile aldehydic products which were carried into the barium hydroxide solution by the air stream.

Absence of Bacterial Action

Recently Theriault, Butterfield and McNamee[10] in criticizing results previously obtained[2] with this oxidation system stated that: "Discounting known thermal, bacterial or enzymatic and hydroxyl-ion effects, it appears doubtful whether the purely catalytic oxidation of such a fairly stable compound as dextrose has ever been accomplished *in vitro*." They suggest that microorganisms were responsible for the oxidations observed and, although their conclusions are based upon experiments the conditions of which in no case were a duplication of ours and in most cases differed very materially, they express doubt regarding the validity of the claims which have been made for the catalytic activity of iron pyrophosphate. These authors also cite results of the inhibiting action of pyrophosphate on oxidations induced by iron as being difficult to reconcile with our findings of the catalytic properties of iron pyrophosphate. They did not, however, include in this discussion the fact, established by Wind,[3] that this inhibition caused by pyrophosphate is not only entirely removed by the addition of iron but that such addition about quadruples the rate of oxidation, the possible biological significance of which was also discussed by Meyerhof and Lohman.[11]

Below are given the results of experiments designed to test whether the oxidation of glucose to carbon dioxide is due to the catalytic activity of sodium iron pyrophosphate or to the activity of living organisms. In these experiments the same conditions, *viz.*, concentration of reactants, temperature, method of determining rate of oxidation, etc., were followed as in our previous investigations, the validity of which has been questioned by Theriault, Butterfield and McNamee, and no attempt has been made to modify the procedure as was done by these workers. The results, therefore, apply to the systems and methods described, as quite obviously radical changes in these may affect the results materially.

Three different methods were followed to test the point in question: (1) the oxidation experiments were conducted in the usual manner, without any special precautions against microorganisms other than the use of strictly chemically clean glassware. In these reaction mixtures bacterial counts were made before aeration and when the rate of carbon dioxide formation had reached its maximum. (2) The rate of oxidation was determined in reaction mixtures which had been sterilized at fifteen pounds pressure for

(9) Nef, *Ann.*, **335**, 247 (1904).

(10) Theriault, Butterfield and McNamee, THIS JOURNAL, **55**, 2012 (1933).

(11) Meyerhof and Lohman, *Biochem. Z.*, **203**, 208 (1928).

thirty minutes. (3) The rates of oxidations were determined in mixtures containing mercuric chloride or mercuric cyanide. The results of these experiments have been confirmed repeatedly. They show that microörganisms cannot be responsible for the formation of significant amounts of carbon dioxide in the oxidation of glucose under the conditions described.

Bacteriological Examination

We are indebted to Dr. C. B. van Niel of Stanford University for the determination of the number of viable bacteria in the reaction mixtures. These determinations were made immediately after making up the solutions and at the time when the rate of carbon dioxide formation was close to maximal, *i. e.*, after six days at 37.9°. The oxidation was carried out with 3 g. of glucose and with the reaction mixture described in the first portion of this paper. During the six days of aeration there was formed 0.4081 g. of carbon dioxide, which represents an average yield. It was intended to determine, if possible, whether there might be a more or less normal bacterial flora at the start of the experiment which would gradually be replaced by a flora more especially adapted to the high salt concentration. Careful microscopical examination failed to reveal the presence of microörganisms either at the beginning or at the completion of the experiment. The number of viable bacteria was determined by the "plate count" method. Two types of solid media were used. The first one approached in composition that of the liquids used in the experiment, leaving out, however, the pyrophosphate and the iron. It consisted of yeast extract with the addition of 2% glucose, 10% disodium phosphate dodecahydrate and 2% agar. The second medium was made up in the same manner, but contained only 1% of the secondary phosphate. The yeast extract agar was sterilized in 90-cc. portions with the required amount of phosphate added; the glucose was sterilized separately in 10-cc. portions as a 20% solution. Before pouring the plates the previously melted agar was cooled to 40°, the glucose solution was then added, well mixed, and the complete medium poured into Petri dishes. As soon as the plates were hardened they were inoculated.

Before starting the aeration of the reaction mixture portions of 0.1 cc. of the mixture were transferred to plates containing the medium with the 10% phosphate and to the 1% phosphate mixture. Also portions of 1 cc. were added to sterile 9-cc. portions of 10% secondary phosphate in tap water and of 1% solutions of the same substance. Portions of 0.20 cc. of the well-shaken suspensions, corresponding to 0.02 cc. of the original solutions, were then plated out on the agar media with 10 and 1% phosphate, respectively. The second series of plate counts, made at the end of the aeration experiment, was prepared in a similar manner. The plates were examined for a period of six days. The number of colonies produced on the agar medium with 10% phosphate was as follows:

At beginning of experiment,	inoculated with 0.1 cc.:	none
	inoculated with 0.02 cc.:	none
At end of experiment,	inoculated with 0.5 cc.:	none
	inoculated with 0.05 cc.:	none

The number of colonies produced on the agar medium with 1% phosphate was as follows:

At beginning of experiment,	inoculated with 0.1 cc.:	two
	inoculated with 0.02 cc.:	none
At end of experiment,	inoculated with 0.5 cc.:	none
	inoculated with 0.05 cc.:	none

These figures show that neither at the beginning nor at the end of the experiment did the number of bacteria in the reaction mixture exceed 20 per cc. If it is borne in mind that this figure represents a maximum, because it is practically impossible to avoid contamination completely during the distribution of the inoculum over the agar surface, it may be justifiable to conclude that the number of living bacteria was actually smaller than one per cc. The fact that occasional colonies were found to develop on the plates shows clearly that the composition of the medium was not inhibitory to bacterial development.

Sterilization by Heat

Because of the effect of the phosphate mixture on glucose at higher temperatures it was necessary to sterilize the glucose separately. This was accomplished by placing 3 g. of glucose together with a number of silver pellets in a small bulb of thin glass; to this was added 3 cc. of water. The neck of the bulb was drawn out to a long capillary. The bulb was then placed in the reaction flask containing the sodium ferropyrophosphate-phosphate mixture with the capillary of the small bulb reaching almost to the mouth of the flask. The flask was sealed with a rubber stopper provided with entrance and exit tubes. The latter bore

cotton plugs 4 cm. long. These flasks containing solutions of the iron catalyst, the sugar bulbs and the aeration tubes were sterilized in an autoclave at 15–17 pounds pressure for thirty minutes. After the flasks had cooled in the autoclave they were given a few violent shakes which broke the glass bulbs containing the sugar solutions and the flasks were then attached to the aeration apparatus.

It was found that with the iron catalyst–phosphate mixture usually some precipitate was formed in the sterilization. Separate experiments demonstrated that this could be obviated largely by the addition of a certain amount of phosphoric acid. The mixture used was the same as that described in the first part of this paper with the addition of 5 cc. of 0.5 *M* phosphoric acid. This mixture had a P_H of about 7.5 and produced slightly more carbon dioxide in the oxidation of glucose than the one without the phosphoric acid.

Several experiments were carried out in which the rate of carbon dioxide formation from sterilized and unsterilized solutions was determined under identical conditions. The results were always the same; an example is given in Table III. A control with the same mixture, not sterilized and containing no iron, yielded only very small amounts of carbon dioxide. The slightly lower values for the sterilized solutions may be ascribed to the fact that the catalyst in these did suffer some decomposition in the sterilization as was evidenced by their cloudy appearance.

Antiseptics

Mercuric Chloride.—The catalyst was prepared from ferrous sulfate in the usual manner, disodium phosphate and 5 cc. of 0.5 *M* phosphoric acid added and the solution shaken in contact with air for five hours in order to convert the ferrous complex into ferric, as the former rapidly reduces mercuric chloride. To this mixture were added 3 g. of glucose and 0.1 g. of mercuric chloride. A control experiment with exactly the same amounts but without mercuric chloride was carried out simultaneously. The rates of carbon dioxide formation are shown in Table III. The oxidation in the presence of mercuric chloride showed an induction period which is greater than that of the control. This in all probability was due to the fact that the ferrous complex (formed through the reducing action of the glucose) reacts with the mercuric chloride and thus reduces the amount of ferrous-moloxide formed and consequently decreased the rate of oxidation. The differences in rate of oxidation between the two experiments were not great; these differences decreased with time, finally the mixture with the mercuric chloride produced slightly more carbon dioxide than the control. At the end of the experiment there was a fine suspension in the mixture containing mercuric chloride. On centrifuging and filtering this suspension coalesced to a globule of metallic mercury.

Mercuric Cyanide.—In these experiments the catalyst was prepared from ferric sulfate: to 6.7 g. of sodium pyrophosphate decahydrate in 100 cc. of water were added 0.6 g. of ferric sulfate in 10 cc. of water, then 17 g. of disodium phosphate dodecahydrate, 3 g. of glucose in 50 cc. of water and finally 0.2 g. of mercuric cyanide. A control contained the same constituents but no mercuric cyanide. The solutions were aerated for eleven days and carbon dioxide determinations made for each twenty-four-hour period as usual. At the end of the period the solution containing the mercuric cyanide was slightly turbid, which proved to be due to finely suspended metallic mercury. The results of the carbon dioxide determinations are shown in Table III and, as in the case in which mercuric chloride was used, demonstrate that the total amount and course of oxidation of glucose with the catalyst is not materially different in the presence of these antiseptics.[12]

TABLE III

EFFECTS OF STERILIZATION, ANTISEPTICS AND ABSENCE OF IRON ON THE RATE OF OXIDATION OF GLUCOSE

Solution	Duration, days	Moles of CO_2 per mole of glucose
Sterilized	9	0.8694
Control	9	.9830
Control without iron	9	.0068
With $HgCl_2$	12	1.0926
Control	12	1.0769
With $Hg(CN)_2$	11	0.9154
Control	11	.8330

Summary

Glyceraldehyde, dihydroxyacetone, acetol and glycerol are readily oxidized, with the formation of carbon dioxide, by air in the presence of sodium ferropyrophosphate-phosphate mixtures. Methyl-

(12) Theriault, Butterfield and McNamee (Ref. 10) have questioned the values reported for the volumes of oxygen absorbed by 1 g. of $FeSO_4 \cdot 7H_2O$, which were reported in a previous article [Smith and Spoehr, THIS JOURNAL, **48**, 111 (1926)]. The values reported were obtained at low barometric pressures (Colorado Springs, Colo.) at 607 ± 4 mm. as stated in the previous article and the theory was calculated for such pressures.

glyoxal is oxidized more slowly than the trioses. Pyruvic acid is not oxidized. With the ferrous complex lactic acid is oxidized to pyruvic acid, and isopropyl alcohol to acetone; this does not occur with the ferric complex. There is no indication that, under the experimental conditions described, microörganisms are responsible for the formation of significant amounts of carbon dioxide in the oxidation of glucose.

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[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF HEALTH, U. S. PUBLIC HEALTH SERVICE]

Studies of the Forms of Arabinose[1]

BY EDNA MONTGOMERY AND C. S. HUDSON

Although alpha and beta forms of arabinose are to be expected from analogy with such known forms of many other reducing sugars, only one modification has so far been crystallized; it is designated beta because for *l*-arabinose its initial specific rotation in water is more dextrorotatory than the final value. One can calculate the rotations to be expected for the two forms from the rotations of the alpha and beta methylarabinosides, which are known with fair precision. Thus in the *l*-arabinose series half the sum of the molecular rotations (sodium line) in water of the methyl *l*-arabinosides is 21,550,[2] the value *b* of the isorotation rules. Accepting the value $A_{OH} = 8500$ from the known rotations of alpha and beta *d*-glucose, the specific rotation of alpha *l*-arabinose (mol. wt. 150) at 20° and with sodium light is calculated to be $(b-A_{OH})/150$ or +87° and that of its beta-form, $(b + A_{OH})/150$ or +200°. Hudson and Yanovsky[3] found +175° for the beta modification, though they later raised this value to +186°. Riiber[4] has determined the rotation to be +192°, which is more nearly in agreement with the calculation. While the expected low rotating alpha form has never been obtained crystalline, Austin and Walsh[5] have recently described a crystalline double compound of it with calcium chloride, from the initial rotation of which they obtained a specific rotation of +75° for alpha *l*-arabinose, somewhat lower than the calculated value. We wish now to report the results of our reinvestigation of the rotation of beta arabinose carried out during the past several years and our recent study of Austin and Walsh's calcium chloride compound of alpha arabinose and of Dale's[6] calcium chloride compound of beta arabinose. We have found that the specific rotations of the two forms of the sugar are +202 and +89°, respectively, in excellent agreement with the calculations from the isorotation rules. These rotations are somewhat different from those obtained by Dale (+186°) and by Austin and Walsh (+75°), doubtless due to the fact that special precautions described below are essential to the obtaining of these substances in pure condition.

Experimental[7]

The specific rotations in every case were measured at 20.0 ± 0.5° with sodium light in a 2-dm. tube.

The Preparation of Pure Beta *l*-Arabinose.—The determination of the optical rotation of this substance involves the crystallization of the sugar and observation of its mutarotation, since the initial value can be obtained only by extrapolation. Since some commercial supplies of arabinose contain impurities very difficult to remove by recrystallization, the purity of the sugar should always be checked by observing its final specific rotation in water. This precaution, although necessary, is not sufficient for success in the preparation of the beta form in a state of optical purity. It has also been found essential to allow the crystallization to take place very slowly, preferably over a period of months.

Pure crystalline *l*-arabinose (50 g.) was left in an open dish at room temperature, protected from dust, in contact with distilled water (50 cc.) containing 10% of acetone to prevent bacterial action. More solvent was added from time to time so that all but 5 to 10% of the sugar remained in solution and after twelve weeks the crystals appeared to be all of one type and had ceased to change (increase) in initial specific rotation. The needles were filtered off, washed with 30% acetone followed by absolute alcohol and were dried to constant weight over calcium chloride under reduced pressure at room temperature. The yield was 3.8 g. Pure beta arabinose was also prepared by dis-

(1) Publication authorized by the Surgeon General, U. S. Public Health Service.

(2) Hudson, THIS JOURNAL, **47**, 268 (1925).

(3) Hudson and Yanovsky, *ibid.*, **39**, 1013 (1917); **52**, 1095 (1930).

(4) Riiber, *Forh. K. Norsk. Vidensk. Selsk.* Vol. III, 66 (1930).

(5) Austin and Walsh, THIS JOURNAL, **56**, 934 (1934).

(6) Dale, *ibid.*, **56**, 932 (1934).

(7) Both the *l*- and *d*-forms of pure arabinose were studied in the present research with agreeing numerical results except for the obvious differences in signs of the rotations.