ON THE ACTION OF PHOSPHATE UPON HEXOSES. II.

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Is Potassium Phosphate the Unique Catalyst by whose Action Acetol is formed from Glucose under the Experimental Conditions? In the previous work,(1) we observed that when a mixture of glucose and a slightly acidic (*p*H 7–5) concentrated solution of potassium phosphate was distilled, its volume being kept constant by adding water drop by drop, acetol was found in an appreciable quantity (ca. 5% of glucose used) in the distillate, accompanied by traces of methylglyoxal and diacetyl. We can, however, anticipate that such special catalytic action will not be limited solely to potassium phosphate.

To solve this question, similar distillation experiments at *p*H 6.2–7.0 were tried with certain other alkali salts. And it was found that a concentrated solution of sodium phosphate, potassium arsenate, or sodium sulphite, behaves like that of potassium phosphate, actually giving acetol in the distillate from glucose. On the contrary, concentrated solutions of alkali chlorides or sulphates gave no distillate showing the iodoform reaction, and the distilling mixtures turned only faintly yellow in colour even after a prolonged distillation (10 hours).

We may so far infer that there are two classes of alkali salts, one giving acetol when its neutral or slightly acidic concentrated solution is distilled with glucose, and the other not. Since the boiling temperatures all lay between 104° and 109°, this striking difference seems to be attributable simply not to the difference in the reaction temperatures but rather to that in their catalytic activities.

By the same iodometric method as described in the previous article, some determinations were made on yields of the iodine-consuming substances (estimated as acetol) from glucose in the distillation experiments with about 40% solutions of some of these catalytically active salts. It was found that the yield in the experiment with potassium arsenate was about one-fourth, and that with sodium sulphite was less than three-fifth, of that with potassium phosphate. In the previous work, it was also established that the iodine-consuming substances in the experiment with potassium phosphate are mainly (more than 60%) composed of

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(1) This Bulletin, **10** (1935), 122.

acetol. Taking these facts into consideration, it is probable that among these salts potassium phosphate is the most favourable catalyst for the production of acetol.

Influence of Iron and Copper Ions, etc. In the literature, there is abundant evidence that certain metals have some characteristic actions in the process of oxidative decomposition of carbohydrates under various experimental conditions. According to Meyerhof and Matsuoka,(2) for example, fructose is oxidized to carbon dioxide by aerial oxygen even in an acidic solution of sodium phosphate when copper or iron salt is present. Further, Bernhauer, and Tschinkel(3) have found that various sugars split into methylglyoxal when they are distilled with dilute sulphuric acid and hydrogen peroxide in the presence of ferrous sulphate. The nature of the catalytic action of these metals is not yet well known. It is, nevertheless, not impossible that these catalysts activate the sugar molecules as well as those of the oxidizing agents. There is, therefore, a possibility that iron or some other metals might play some important rôles in the characteristic non-oxidative decomposition of glucose by potassium phosphate, since our experimental materials always contained some metals as impurity, e.g., calcium, magnesium, etc., and especially iron.

To examine this possibility, the rates of appearance of the iodine-consuming substances (estimated as acetol) in the distillates from a mixture of glucose and potassium phosphate solution, either alone or along with iron or other metallic salts, were compared. The potassium phosphate used for this purpose was purified and proved to be free from any heavy metal and alkali earth. The purified glucose was, however, not quite free from a trace of ash—calcium—but it was free from iron. All of the distillations, of course, were carried out under conditions as similar as possible, and in each distillation experiment a rate-curve(4) was prepared. Fig. 1 shows the rate-curves from the experiments with potassium phosphate alone. As seen in the figure, the two curves practically coincide.

Fig. 2 contains the rate-curves for the experiments with phosphates of calcium, magnesium, zinc, and copper, respectively (in such a quantity as to make 1/100–1/300 molar solution) in addition to potassium phosphate. Fig. 2 indicates that the addition of calcium or magnesium

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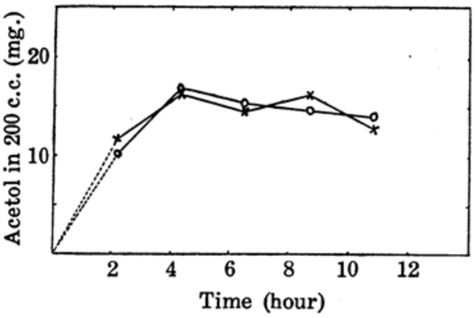
(2) O. Meyerhof and K. Matsuoka, *Biochem. Z.*, **150** (1924), 1.

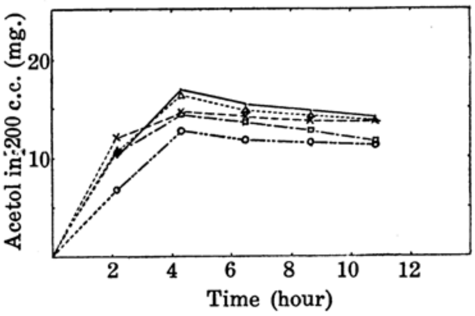
(3) K. Bernhauer and H. Tschinkel, *Biochem. Z.*, **230** (1931), 484.

(4) See the experimental part.



(I): 3 g. Glucose + 30 c.c. 40% K-phosphate solution, pH 6.2-6.4.

Fig. 1.



———— (I): 3 g. Glucose + 30 c.c. 40% K-phosphate solution, pH 6.2-6.4.

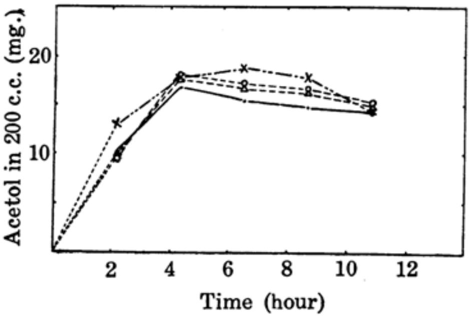
- - - △ - - - - - - △ - - - (II): (I) +1/200 molar Ca-phosphate.

- - - × - - - - - - × - - - (III): (I) +1/100 molar Mg-phosphate.

—·—□—·—·—□—·— (IV): (I) +1/300 molar Zn-phosphate.

—··—○—··—··—○—··— (V): (I) +1/100 molar Cu-phosphate.

Fig. 2.



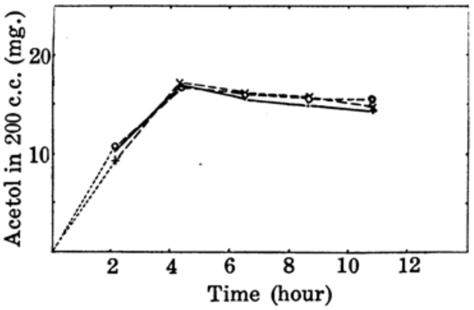
———— (I): 3 g. Glucose + 30 c.c. 40% K-phosphate Solution, pH 6.2-6.4.

—··×—··—×—· (VI): (I) +1/100 molar Ferrous sulphate.

- - - ○ - - - - - ○ - - - (VII): (I) +1/200 molar Ferrous phosphate.

- - - △ - - - - - - △ - - - (VIII): (I) +1/300 molar Ferrous phosphate.

Fig. 3.



———— (I): 3 g. Glucose + 30 c.c. 40% K-phosphate solution, pH 6.2-6.4.

- - - - - - - - - - - - - - - (IX): (I) +1/100 molar Ferric phosphate.

Fig. 4.

salt does not notably influence the reaction, whereas zinc and copper salts have a retarding action.

Fig. 3 shows the influence of addition of ferrous salts to the distilling mixture (in such a quantity as to make 1/100–1/300 molar solution). Thus ferrous sulphate or phosphate undeniably accelerates the rate of formation of the iodine-consuming substances. On the contrary, ferric phosphate showed almost no influence upon this rate (Fig. 4).

Thus, among the metal-ions studied, ferrous and cupric ions seem to have some particular effects upon the formation of iodine-consuming substances. In all of these experiments, the presence of acetol in the distillates was proved by preparing its semicarbazone. Since, however, it is not known to what extent the iodine consumption of these distillates is actually due to acetol, it is impossible to reach any absolute conclusion from these results, as to the catalytic effect of these metal-ions on the acetol formation. But it has become obvious that acetol is produced from pure glucose by the action of pure potassium phosphate under the experimental conditions.

Experimental Part.

The distillations, and the estimations (as acetol) of the iodine-consuming substances in the distillate were carried out in the same ways as described in the previous article, excepting that a distilling flask in Pyrex glass was used this time instead of an ordinary one.

Acetol Formation by Other Salts. The reagents used in these experiments were the commercial "chemically pure" products.

Sodium phosphate. Distilling mixture: 5 g. glucose, 50 c.c. ca. 40% sol. Na-phosphate (from $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot H_2O$). *p*H 6.2–6.5; boiling temperature 106–109°. From 1000 c.c. (0.14 g. as acetol) of the distillate, about 0.1 g. acetolsemicarbazone and a trace of diacetylbissemicarbazone were actually obtained.

Potassium arsenate. Distilling mixture: 3 g. glucose, 30 c.c. ca. 40% sol. K-arsenate (prepared by boiling 40 g. KH_2AsO_4+5.2 g. $KHCO_3$+62 g. H_2O). *p*H 6.2–6.4; boiling temperature 104–105°. The presence of acetol in the distillate from this mixture was proved by means of preparation of its semicarbazone. In another experiment, the distillation was continued until the distillate gave almost no more iodoform reaction and the acetol content of the total distillate was determined, which amounted to about 1.2% of glucose used.

Sodium sulphite. About 40% solution (*p*H 6.3–6.4; boil. temp. 104–106°) of sodium sulphite gave also acetol and its yield was about 3% of glucose used. In this case, however, the acetol yield should be less than the above value, since it was determined by iodometry and the distillate evidently implied some sulphur dioxide. It is noteworthy in this experiment that the distilling mixture underwent almost no caramelisation even after a prolonged distilling process.

Twenty three per cent. solution of potassium chloride (boil. temp. 105–106°), 28% solution of sodium sulphate (boil. temp. 104°), and 23% solution of potassium sulphate (boil. temp. 103–104°) gave no distillate showing iodoform reaction. A concentrated solution of sodium chloride behaved similarly. It is, however, notable in these experiments that when a distilling flask in ordinary glass instead of Pyrex was used, some iodoform giving substance occurred in a minute quantity in the distillates.

Influence of Metal Ions. A sample of commercial potassium biphosphate (implying Cu, As, Fe, Ca, Mg, etc. as impurities) and glucose (Merck, containing Fe, Ca, etc. as impurities) were purified as follows. To a solution of the potassium biphosphate made alkaline with potassium carbonate, hydrogen sulphide was passed and the precipitated sulphides were filtered off. The filtrate was made about *p*H 6.5 with phosphoric acid (containing a trace of iron) and then evaporated under a reduced pressure. The turbid concentrated solution was filtered through a pulp mat and further evaporated to crystallization. The crystals (KH_2PO_4) thus obtained were recrystallized twice from the solution of *p*H 6.5 (adjusted with purified potassium carbonate) and twice from their own solution. The product was free from all of the impurities mentioned above.

Glucose was twice recrystallized from glacial acetic acid, washed with alcohol containing a few drops of nitric acid, and dried at 60° under a reduced pressure. The sample thus obtained had still a trace of ash, mainly calcium, but no iron.

Using these materials, the following distillation experiments were performed in the same Pyrex flask and at the same tempo—200 c.c. distillate in ca. 130 minutes. The acetol contents (mg.) in each successive 200 c.c. distillate were plotted against time (hour) and the curves thus obtained are designated as "rate-curves". About 40% stock solution(5) of potassium phosphate (*p*H 6.2–6.4) was prepared by boiling a mixture of 340 g. KH_2PO_4, 68 g. $KHCO_3$ (purified), and 536 g. H_2O.

Distilling mixture (I): 3 g. glucose, 30 c.c. the stock solution.

The rate-curves are plotted in Fig. 1.

Distilling mixture (II): (I) + 0.046 g. $Ca_3(PO_4)_2$ (1/200 molar);
,, ,, (III): ,, + 0.074 g. $MgHPO_4 \cdot 7H_2O$ (1/100 molar);
,, ,, (IV): ,, + 0.046 g. $Zn_3(PO_3)_2 \cdot 4H_2O$ (1/300 molar);
,, ,, (V): ,, + 0.13 g. $Cu_3(PO_4)_2 \cdot 3H_2O$ (1/100 molar).

Each of these experiments was repeated twice and the mean rate-curves are plotted in Fig. 2.

Distilling mixture (VI): (I) + 0.083 g. $FeSO_4 \cdot 7H_2O$ (1/100 molar);
,, ,, (VII): ,, + 0.075 g. $Fe_3(PO_4)_2 \cdot 8H_2O$ (1/200 molar);
,, ,, (VIII): ,, + 0.05 g. ,, (1/300 molar).

The rate-curves for these experiments are plotted in Fig. 3.

Distilling mixture (IX): (I) + 0.067 g. $FePO_4 \cdot 4H_2O$ (1/100 molar).

The results are summarized in Fig. 4. In all of these experiments (I–IX) acetol-semicarbazone was obtained from the distillate and identified with an authentic specimen.

In conclusion the writers wish to thank Prof. S. Komatsu for his valuable advice and at the same time the Department of Education for a research grant.

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(5) No iron nor any other heavy metals were identified in the solution which had been boiled for 10 hours in the Pyrex flask.