ON THE ESTIMATION OF GLUCOSE IN THE PRESENCE OF PHOSPHATE BUFFERS.

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During the course of some experiments upon the enzymatic hydrolysis of glycogen it was found that the copper equivalent of glucose in different buffer mixtures varied considerably. Since buffered solutions are used in most enzyme studies it is important to call the attention of investigators to the error that may arise from the failure to take the fact mentioned here into account.

A series of experiments was performed in which estimations were made of the amount of cupric salt reduced by a standard amount of glucose in the presence of different proportions of diacid and dibasic phosphates. The amount of copper reduced was estimated by one of the iodometric methods of Shaffer and Hartmann. Their macro method for cuprous copper estimation by thiosulfate titration of the excess iodine used to reoxidize the copper, was used. This gives the thiosulfate equivalent of glucose under the conditions studied.

In each case 10 cc. of 0.1 per cent glucose and 10 cc. of the Shaffer-Hartmann copper solution were used. To this solution was added a total of 10 cc. of 0.2 molar phosphate mixture containing varying proportions of the diacid and dibasic phosphates as indicated in Table I, where the results of the estimations are shown.

It is found that the presence of the larger quantities of potassium diacid phosphate interferes with the reduction of copper by glucose. There is a 20 per cent difference between the reducing power of glucose in the presence of the smallest and the largest amount of acid phosphate used in these experiments. The Shaf-

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Estimation of Glucose

fer-Hartmann method is accurate to within 1 per cent with pure solutions of glucose, therefore an error that may amount to 20 per cent of the total value is of importance to recognize.

**TABLE 1.**

*Influence of Different Proportions of Potassium Dihydrogen and Disodium Hydrogen Phosphates upon the Reduction of Copper by Glucose.*

10 mg. glucose used in each case. Heated 30 min. in a boiling water bath.

<table>
<thead>
<tr>
<th></th>
<th>0.2 M acid phosphate added, cc.</th>
<th>0.2 basic “ “ “ “ “</th>
<th>Thiosulfate equivalent of 10 mg. glucose, cc.</th>
<th>Thiosulfate equivalent of 1 mg. glucose, cc.</th>
<th>Glucose equivalent of 1 cc. of thiosulfate, mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.75 8.0 6.0 4.0 2.0 0.5</td>
<td>0.25 2.0 4.0 6.0 8.0 9.5</td>
<td>8.18 9.50 9.70 10.10 10.10 10.30</td>
<td>0.82 0.95 0.97 1.01 1.01 1.03</td>
<td>1.20 1.05 1.03 0.99 0.99 0.97</td>
</tr>
</tbody>
</table>

**SUMMARY.**

It is pointed out that the presence of potassium acid phosphate in glucose solutions lowers the reducing power of the glucose for copper. This fact must be taken into account whenever the sugar content of buffered solutions is measured.
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