THE INFLUENCE OF INSULIN ON THE RATE OF GLUCOSE OXIDATION BY PASTEURELLA PESTIS*

BY H. B. LEVINE

(From the Office of Naval Research, Task V, Department of Bacteriology, and United States Naval Medical Research Unit No. 1,‡ University of California, Berkeley)

(Received for publication, July 13, 1950)

The possible role of insulin as a biocatalyst related to one or more of the enzymatic steps involved in intermediary glucose metabolism has been studied by numerous workers (1–6). Data have been presented (1, 5, 6) which indicate that the hormone may be intimately associated with the phosphorylation of glucose to glucose-6-phosphate via the hexokinase reaction. Soskin and Levine (7) have stated that the "fundamental action of insulin may be considered as being the increased rate of entry of glucose from the blood and extracellular fluids into the tissue cells of the body." Levine et al. (8) have demonstrated that galactose, which is not utilized by the dog, diffuses more rapidly into certain tissues from the blood and body fluids of eviscerated nephrectomized animals in the presence of insulin. These workers have postulated that insulin may have acted upon certain cellular membranes in such a manner that the transfer of the hexose from the extracellular fluid into the cell was facilitated.

The data which form the basis of this paper demonstrate that glucose is oxidized more rapidly by resting cells of Pasteurella pestis in the presence of insulin. The hormone itself is not oxidized by the organism and exerts its stimulatory effect most notedly on freshly harvested intact cells.

Experimental Methods

The rate of glucose oxidation was measured by direct Warburg methods described by Umbreit, Burris, and Stauffer (9). A typical, smooth appearing colony of Pasteurella pestis strain A-1122 (avirulent) was used to inoculate several heart-infusion agar slants which were incubated for 24 hours at 25–27°C. and then placed in the refrigerator to serve as the parent stocks.

* This work was supported by a contract between the University of California and the Office of Naval Research and sponsored in part by the Biological Department, Chemical Corps, Camp Detrick, Maryland.

‡ The opinions and assertions contained in this report are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. Article 1252, United States Navy Regulations 1948.
Cell suspensions were prepared by growing the organisms for 20 to 22 hours at approximately 26°C in tryptose broth (Difco) which was aerated by continuous shaking. The cells were then harvested by centrifugation at 2600 R.P.M. for 30 minutes in the International No. 1 centrifuge and the pellet was resuspended in 0.066 M KH₂PO₄-K₂HPO₄ buffer at pH 6.8 prepared in physiological saline or in 0.033 M KH₂PO₄-K₂HPO₄ buffer at pH 6.8 prepared in distilled water. The organisms were washed twice by centrifugation in the respective buffer (see tables and figures) and diluted to a turbidity of approximately 300 on the Klett-Summerson photoelectric colorimeter (660 mm filter, 15 mm. I.D. cuvette, water blank). Aliquots were removed for dry weight determinations and the resting cells were stored in the refrigerator when not in use. Since the respiratory rate of the suspensions increased with time of storage under these conditions, the age reported is the number of hours the suspensions were kept in the refrigerator after harvest.

Cell-free enzyme preparations were obtained by disrupting the cells mechanically. The washed 20 to 22 hour old harvest from 1 liter of tryptose broth (approximately 0.5 gm. dried cells) was concentrated by centrifugation and the cell paste was mixed with 0.5 gm. of powdered pyrex glass. The mixture was ground for 10 minutes at approximately 0°C. in a modified Potter-Elvehjem homogenizer affixed to a rotary motor. After adding 15 ml. of 0.066 M phosphate saline buffer, grinding was continued for approximately 2 minutes. The homogenate was then centrifuged at 2000 R.P.M. for 1 hour and the clear supernatant fluid was used as the source of enzyme.

Glucose was used in a final concentration of 0.0033 M or 0.00083 M in the Warburg vessels and insulin (Eli Lilly Iletin) in a final concentration of 3.3 units per ml. The hormone was mixed with the substrate and introduced into the side arms of the vessels. Appropriate endogenous control vessels with and without insulin were included in each determination.

It was found convenient to use insulin preparations in an initial concentration of 40 units per ml. This was mixed with an equal volume of substrate of twice the desired molarity prior to introducing 0.5 ml. into the side arm of the vessel. After tipping, the hormone and the substrate were diluted sixfold by the enzyme or cell suspension. When 80 unit insulin was diluted with either water or buffer, a precipitate formed upon the subsequent addition of glucose solution. Such preparations consistently failed to accelerate the organism's "normal" rate of oxidation of glucose.

**Experimental Results**

The effect of insulin on the rate of oxidation of glucose by freshly harvested cells of *P. pestis* strain A-1122 is shown in Fig. 1. The exogenous rate was accelerated by approximately 37 per cent when the hormone was present in the reacting vessel. In 6 determinations made within 24 hours after cell suspensions were harvested, the increased rate of glucose oxidation in the presence of insulin was always noted and ranged between 20 and 41 per cent. The data in Fig. 1 illustrate further that insulin alone did not influence the endogenous rate of the organism. Substrate controls failed to reveal any gas uptake resulting from the interaction of glucose, buffer, and insulin in the absence of cells.
FIG. 1. The rate of oxidation* of glucose in the presence and absence of insulin by Pasteurella pestis strain A-1122.

* Measurements begun 2 hours after harvest. Vessels contained 2.5 ml. cell suspension in 0.066 M phosphate saline buffer in flask body, 0.5 ml. 0.02 M glucose or 0.02 M glucose + 10 units insulin in side arms, and 0.2 ml. 20 per cent KOH in center cup. Temperature 35°C., pH 6.8.

TABLE I
The Rate of Oxidation of Glucose by Pasteurella pestis Strain A-1122 in the Presence and Absence of Insulin at Varying Intervals after Harvest

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Time after harvest, hrs.</th>
<th>2</th>
<th>8</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose + insulin</td>
<td>Q_{O_2}*</td>
<td>36.0  (2.5)‡</td>
<td>35.8  (3.4)</td>
<td>38.3  (2.5)</td>
<td>37.1  (4.0)</td>
<td>35.8  (5.5)</td>
</tr>
<tr>
<td>Glucose</td>
<td>Q_{O_2}</td>
<td>25.5  (2.5)</td>
<td>29.9  (3.4)</td>
<td>29.5  (2.5)</td>
<td>32.8  (4.0)</td>
<td>34.3  (5.5)</td>
</tr>
</tbody>
</table>

* Q_{O_2} microliters O_2 uptake per mg. dry cell weight per hour.
‡ Endogenous values in parentheses. Vessels contained 2.5 ml. cell suspension in 0.066 M phosphate saline buffer in flask body, 0.5 ml. 0.02 M glucose or 0.02 M glucose + 10 units insulin in side arms, and 0.2 ml. 20 per cent KOH in center cup. Temperature 35°C., pH 6.8.
FIG. 2. The oxidation* of glucose in the presence and absence of insulin by *Pasteur-
ella* pestis strain A-1122.

*Measurements begun 8 hours after harvest. Vessels contained 2.5 ml. cell sus-
pension in 0.033 M phosphate buffer in flask body, 0.5 ml. 0.005 M glucose or 0.005 M
glucose + 10 units insulin in side arms, and 0.2 ml. 20 per cent KOH in center cup.
Temperature 35°C., pH 6.8.

**TABLE II**

The Rate of Oxidation* of Glucose in the Presence and Absence of Insulin by a Crude Enzyme
Preparation Obtained from *Pasteurella* pestis Strain A-1122

<table>
<thead>
<tr>
<th>Substrate</th>
<th>O&lt;sub&gt;2&lt;/sub&gt; uptake after 130 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose + insulin</td>
<td>67.5 (41.4)‡</td>
</tr>
<tr>
<td>Glucose</td>
<td>68.3 (40.8)</td>
</tr>
</tbody>
</table>

*Measurements begun 6 hours after harvest.
‡ Endogenous values in parentheses. Vessels contained 2.5 ml. enzyme preparation in
0.066 M phosphate saline buffer in flask body, 0.5 ml. 0.02 M glucose or 0.02 M glucose +
10 units insulin in side arms, and 0.2 ml. 20 per cent KOH in center cup. Temperature 35°C.,
pH 6.8.
After storage of cells in the refrigerator for varying periods of time up to 3 days, the oxidative rate of strain A-1122 on glucose substrate in the presence of insulin remained relatively constant. However, during this time the respiratory rate on glucose in the absence of insulin increased until it approximated that rate at which the organism oxidized glucose when the hormone was added simultaneously. These data are shown in Table I. Since the viable count and turbidity of suspensions stored at 0-5°C. were known to decrease with time, it is possible that cell lysis was occurring. Hence the effect of insulin on the respiratory rate of cells which were disrupted deliberately was studied. Freshly prepared cell extracts were used in place of the intact organisms to oxidize glucose. The data presented in Table II indicate that the hormone had no apparent effect on the endogenous or exogenous rates.

The molar ratios of oxygen consumed to substrate added were determined by allowing the organism to oxidize known amounts of glucose in the presence and absence of insulin until the exogenous rates declined and paralleled the endogenous rate. The measured volumes of oxygen consumed (Fig. 2) were computed after correcting for the endogenous value (9). The data in Fig. 2 indicate that the oxidation of glucose was altered in the presence of insulin inasmuch as 36 μl. more oxygen was taken up per unit weight of substrate added. The computed molar ratios were: 3.7 moles oxygen consumed per mole of glucose oxidized in the presence of insulin; 3.1 moles oxygen taken up per mole of glucose oxidized in the absence of insulin.

DISCUSSION

The data reported above serve to illustrate that insulin stimulates the oxidative rate of P. pestis strain A-1122 on glucose. It is of interest that the hormone did not influence the organism’s endogenous respiration but did accelerate respiration in the presence of a carbohydrate. The nature of this action or its possible relationship to carbohydrate metabolism in the animal kingdom is not known at the present time.

The fact that only young intact cells responded to insulin stimulation may indicate that a cell membrane or diffusion phenomenon was involved. It was noted that deliberate rupture of the cell membrane obviated the effect of insulin. However, these data are not definitive since the grinding procedure used to disrupt the membrane may have altered the organized system of enzymes as it existed in the intact cell (9).

Since the total oxygen consumption per unit weight of glucose oxidized by whole cells was increased in the presence of insulin, the hormone may have influenced one or more of the enzymatic steps concerned in intermediary glucose metabolism. This would appear to be the case if it could be established that insulin did not interfere with direct assimilation of glucose or its intermediary products. As yet, detailed data on the stoichiometry of the reaction are lacking.
INFLUENCE OF INSULIN ON GLUCOSE OXIDATION RATE

SUMMARY
The rate of oxidation of glucose by freshly harvested resting cells of *P. pestis* strain A-1122 was accelerated by 20 to 41 per cent in the presence of insulin. The stimulatory action was not noted when cell-free enzyme preparations were employed and was less marked after storage of cells for 3 days. Although insulin was not oxidized by the organism, the amount of oxygen consumed during the dissimilation of a unit weight of glucose was increased in the presence of the hormone.

The writer wishes to thank Mr. Donovan Cartwright for technical assistance.

BIBLIOGRAPHY