THE METABOLISM OF GLUCONIC ACID

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(Received for publication, June 16, 1950)

In a previous study the appearance of deuterioglucose in the urine of the phlorhizinized rat was shown to follow the injection of meso-inositol which had been tagged with deuterium in the carbon-bound positions (1). This result was taken as proof of the glucogenic nature of inositol in the rat and has led the authors to the investigation of another possible glucose precursor, gluconic acid, by a similar approach.

Early studies of the fate of gluconic acid in the intact animal have related chiefly to its oxidation. Thus it has been demonstrated that a large portion of gluconic acid administered is utilized even by the diabetic organism (2-5) and it has been claimed that saccharic acid is one of the oxidation products (3), a claim which has been refuted (6). Several investigators have found that the administration of gluconic acid is followed by the appearance of some unaltered gluconic acid in the urine (5, 6).

Apart from the extensive literature relating to 6-phosphogluconic acid, its formation from glucose-6-phosphate, and its further oxidation and decarboxylation, there are numerous reports of enzyme systems derived from various biological sources capable of oxidizing glucose to gluconic acid, without the demonstrated intervention of a phosphorylated intermediate (7-9). Of particular interest to the present discussion is the glucose dehydrogenase of Harrison (10) isolated from mammalian liver, capable of performing the specific oxidation of glucose to gluconic acid in vitro in good yield aerobically in the presence of diphosphopyridine nucleotide (DPN) and methylene blue. The presence of such an enzyme leaves open the possibility that free gluconic acid is formed in the intact liver as a normal oxidative fate of glucose.

In the present experiments doubly labeled gluconic acid has been prepared by the mixing of two samples of sodium gluconate, the one labeled with C¹⁴, the other with deuterium. The former was prepared from the starch of bean leaves which had been exposed to C¹⁴O₂, the latter from the urinary glucose of a diabetic rat receiving D₂O. Such doubly labeled material has been injected into normal and phlorhizinized rats and the distribution of isotopes in expired CO₂, liver glycogen, urinary gluconate, glucose, and other body constituents has been studied.
Preparation of Sodium Gluconate Labeled with $^{14}C$ and Deuterium—Starch labeled with $^{14}C$ was prepared essentially according to the method of Putman et al. (11) with modifications suggested by Gibbs et al. (12). Three trifolium were cut from a bush bean plant which had been kept in the dark for 24 hours. These leaves with their stems submerged in water were exposed, in a large desiccator, at slightly less than 1 atmosphere pressure to the CO$_2$ derived from 500 mg. of BaCO$_3$ containing 1 mc. of $^{14}C$. Two 100 watt incandescent bulbs illuminated the desiccator, which was maintained at 25° for 24 hours. At the end of this period there was no detectable CO$_2$ in the gas phase. Starch was isolated over the iodine complex; yield, 74 mg. Of this radioactive starch 36 mg. were treated with 2 ml. of 3 HCl at 100° for 3 hours, the resulting solution neutralized with methanolic NaOH, and the glucose oxidized to gluconate by further treatment with I$_2$ and methanolic NaOH (13).

An adult alloxan-diabetic rat, excreting 4 to 9 gm. of glucose daily, was given 20.9 ml. of an isotonic NaCl solution in 99.8 per cent D$_2$O in two intraperitoneal injections. The drinking water was replaced by 16 per cent D$_2$O and during the succeeding 8 days 40 gm. of glucose were excreted in the urine. Half of this urine was concentrated in vacuo to a syrup which was then oxidized with I$_2$ by the procedure of Moore and Link (13) with the sole modification that NaOH replaced KOH. The sodium gluconate was recrystallized from aqueous methanol and it was noted that recrystallization of this compound was more troublesome than that of the potassium salt. 19.3 gm. of recrystallized sodium gluconate were obtained.

5 gm. of deuteriosodium gluconate were added to an aqueous solution of the $^{14}C$-sodium gluconate which had been obtained from 36 mg. of $^{14}C$-starch, and, after clarification, cautious addition of methanol yielded 3.9 gm. of crystals. One further recrystallization from aqueous methanol gave 3.15 gm. of doubly labeled sodium gluconate; deuterium, 3.49 atom per cent excess; $^{14}C$, 160,000 c.p.m. per milliatom of C.

1 gm. of this material was used in the experiment with the phlorhizinized rat, described below. 2 gm. were further mixed in solution with 1.2 gm. of deuteriosodium gluconate, and this was the solution which was subsequently injected into a normal rat. The sodium gluconate in this solution exhibited 102,000 c.p.m. per milliatom of C.

To establish the freedom of this material from radioactive glucose, 20 mg. of the doubly labeled sodium gluconate were dissolved together with 200 mg. of non-isotopic anhydrous glucose in 75 ml. of H$_2$O. Treatment at 100° for 2 hours with 1.5 ml. of phenylhydrazine and 2 ml. of acetic acid gave a crystalline osazone which, after exhaustive washing with hot water and recrystallization from 60 per cent ethanol, was devoid
of radioactivity. This finding demonstrated that no radioactive glucose contaminated the sodium gluconate preparation. It further showed that no detectable amount of gluconic acid is reduced to glucose under the conditions of osazone formation and that the isolation procedure used is effective in separating glucosazone from all traces of contaminating gluconic phenylhydrazide.

Administration of Isotopic Sodium Gluconate to Normal Rat—The rats employed in the present study were kept in a large vacuum desiccator modified to permit the collection of expired CO₂. The desiccator was equipped with a coarse wire screen flooring and beneath this a fine wire gauze to separate feces from urine, an inlet and an outlet tube for air, a food jar, and a container for drinking water. Room air was drawn in succession through a flowmeter, a soda-lime tower, the desiccator, and finally through two gas absorption bottles with sintered glass distributing plates. Into the first of these were placed 300 ml., into the second 150 ml. of 7 N NaOH. A few crystals of thymol were placed in the bottom of the desiccator for preservation of the urine. During each experiment a flow of 500 to 600 ml. of air per minute was continuously maintained. The diet, which was offered ad libitum, contained 15 per cent casein, 68 per cent corn starch, 5 per cent yeast powder, 4 per cent salt mixture (14), 2 per cent cod liver oil, and 6 per cent refined cotton seed oil. Each rat was acclimated to the desiccator for 1 day prior to the isotope experiment.

An adult male white rat, weighing 227 gm., was given on each of 3 successive days by intraperitoneal injection 3 ml. of a solution containing 1 gm. of doubly labeled sodium gluconate. Prior to injection the pH was adjusted to 7 by addition of HCl. It was estimated that 95 per cent of the material was actually introduced into the animal. The rat appeared normal throughout the experiment except for marked polydipsia and transient evidence of peritoneal irritation manifested by splinting of the hind legs following each injection. On the 3 experimental days the rat consumed approximately 8, 4, and 10 gm. of diet. The desiccator was opened only to permit the daily injection and at these times the 24 hour accumulations of urine and expired CO₂ were collected. After 3 days the rat was killed by a blow on the head; final weight, 212 gm.

The liver, which weighed 9.1 gm., was promptly removed and ground with trichloroacetic acid. Glycogen was precipitated from the trichloroacetic acid solution (15) by the addition of ethanol and was purified by reprecipitation from KOH solution and finally from H₂O (16); yield, 434 mg. The residue from the trichloroacetic extract was defatted with boiling ethanol and ether. The nucleic acids were extracted with hot 10 per cent NaCl solution and their sodium salts precipitated with ethanol.
(17). The residue from the NaCl extraction was the material analyzed as "liver protein."

Each urine sample was carefully collected, filtered, and made up to a volume of 100 ml. Aliquots were taken for total C and radioactivity determinations (cf. "Analytical methods" below). A solution containing 1 gm. of neutral lead acetate was added to each remaining solution. After 24 hours the mixture was centrifuged and the precipitate washed once with water. Lead was removed from the combined supernatant fluids by treatment with H₂S and the volume reduced in vacuo to about 25 ml. 0.5 ml. of phenylhydrazine, 0.3 ml. of acetic acid, and a few crystals of NaHSO₃ were added and the mixture heated at 100° under a reflux for 3 hours. The solution was cooled and extracted with ether to remove excess phenylhydrazine. The small amount of precipitate that formed on refrigeration was removed by filtration. The volume of the filtrate was reduced to about 5 ml., and after refrigeration overnight the precipitated yellow crystals were collected by filtration. These were recrystallized from water and colorless needles of gluconic phenylhydrazide were obtained.

\[C_{12}H_{18}O_{4}N_{4}\]. Theory, N 9.8; found, N 10.0

An attempt to isolate saccharic acid as its phenylhydrazide from the insoluble lead salt fraction of the urine was unsuccessful. A carrier method of isolation was therefore employed. The three urine samples from which gluconic phenylhydrazide had been isolated were combined and 100 mg. of non-isotopic saccharic phenylhydrazide were added. From a solution of the mixture saccharic phenylhydrazide was isolated and three times recrystallized from large volumes of aqueous ethanol. To insure the absence of contaminating radioactive gluconic phenylhydrazide in this material, 50 mg. of it were dissolved together with 50 mg. of non-isotopic gluconic phenylhydrazide and the saccharic acid derivative reisolated and recrystallized. The specific activity of saccharic phenylhydrazide was as follows: twice recrystallized, 47 c.p.m. per milliatom of C; three times recrystallized, 51 c.p.m. per milliatom of C; and "washed out" with gluconic phenylhydrazide, 48 c.p.m. per milliatom of C.

The freedom of the injected sodium gluconate from contaminating radioactive saccharic acid was established by a similar "washing out" procedure in which non-isotopic saccharic acid was added to and reisolated from a solution of the radioactive gluconic acid.

A preliminary exploration of the fate of gluconic acid in the rat was carried out by the same methods. The material injected was sodium gluconate exhibiting 12,800 c.p.m. per milliatom of C, and the study of
the animal was somewhat less complete than in the experiment described above. The pertinent results from this study are included in the present report.

**Administration of Isotopic Sodium Gluconate to Phlorhizinized Rat**—An adult male white rat, weighing 232 gm., was injected subcutaneously with 0.25 ml. of a 10 per cent suspension of phlorhizin in sesame oil. A similar preliminary injection had resulted in an excretion of 0.75 gm. of glucose in 17 hours. No food was given for 3 hours previous to the phlorhizin injection nor during the course of the experiment. 1 gm. of sodium gluconate (160,000 c.p.m. per milliatom of C), dissolved in 6 ml. of H2O and adjusted to pH 7 with HCl, was injected intraperitoneally 2 hours after administration of the phlorhizin. During the next 24 hours the rat was kept in the desiccator equipped as previously described and expired CO2 and excreted urine were collected. The rat was stuporous for several hours after the injection, had marked dyspnea, and slept for most of the remaining time. At the end of the 24 hour period the rat was killed by a blow on the head and the liver quickly removed and dissolved in hot 30 per cent aqueous KOH. No glycogen could be isolated. C and C14 analyses were carried out on aliquots of the NaOH solution in which the expired CO2 had been collected. 0.326 mole of CO2 exhibiting 1400 c.p.m. per milliatom of C was expired in 24 hours.

The small amount of voided urine and that remaining in the bladder were combined and glucose isolated as its osazone. The osazone was twice extracted with 50 ml. of boiling water and filtered from the hot solution. This procedure had previously been shown to remove all traces of gluconic phenylhydrazide from glucosazone. The resulting 201 mg. of osazone were recrystallized first from 60 per cent ethanol and then from pyridine-benzene without change in radioactivity. The yield of twice recrystallized glucosazone was 125 mg.

**Degradation and Allocation Studies**—Glucosazone was oxidized with periodate by a combination of methods described by others (18–20). 36 mg. of recrystallized glucosazone from the urine of the phlorhizinized rat were dissolved in 15 ml. of hot 66 per cent ethanol and the solution was cooled to 30° and treated with 1 ml. each of 1 N NaOH and 0.3 M periodic acid. After 15 minutes the precipitated 1,2-bisphenylhydrazone of mesoxaldehyde was removed by centrifugation and recrystallized from 66 per cent ethanol. The supernatant was promptly acidified with HCl and freed of excess periodate by addition of 1 ml. of 1.2 N Na2HAsO4. The solution was made alkaline and formaldehyde distilled in a stream of N2 into a chilled receiver. Formaldehyde was precipitated as its dimedon derivative (18). The formic acid in the undistilled residue was
oxidized by treatment with HgO in acid solution and the CO₂ collected in Ba(OH)₂ solution. From the analyses of these derivatives the radioactivity in C-(1 + 2 + 3), in C-(4 + 5), and in C-6 was calculated.

A portion of the liver glycogen from the normal rat was converted to glucosazone, which was degraded and analyzed in the same way, giving the distribution of radioactivity in C-(1 + 2 + 3) in C-(4 + 5), and in C-6. Another portion of liver glycogen was hydrolyzed and oxidized directly to give values for C-(1 + 2 + 3 + 4 + 5) and C-6.

Analytical Methods—Aliquots of alkaline solutions which contained radioactive CO₂ were transferred to the Van Slyke manometric apparatus, where the CO₂ was liberated and measured. All organic compounds to be analyzed for C¹⁴ were oxidized by the wet ash procedure of Van Slyke and Folch (21) and the CO₂ collected in the pipette of the manometric apparatus. Aliquots of materials such as urine were evaporated to dryness in combustion tubes of the type described by the above authors and the residues were oxidized by the wet ash procedure. The CO₂, which at the termination of this manometric analysis was in the gas phase, was then transferred to a 15 ml centrifuge tube containing 5 ml of a solution of approximately 0.25 N Ba(OH)₂ in 2 per cent of BaCl₂ (22). The transfer was effected through a short piece of capillary tubing connected to the side arm of the Van Slyke apparatus and fitted with a 1-hole rubber stopper which accommodated the centrifuge tube. The precipitated BaCO₃ was twice washed with CO₂-free water by centrifugation with protection against contaminating atmospheric CO₂ and finally suspended in water.

The BaCO₃ was plated for counting by filtration on a disk of scinttered alundum of medium porosity. Disks nominally 1 × 1/8 inches¹ were ground to flatness and uniform thickness by rubbing their wet surfaces against each other. These were mounted under glass chimneys constructed of precision bore tubing of 0.750 inch internal diameter with ground flat ends. The BaCO₃ suspension in ample water was poured into the chimney and gentle suction applied. The precipitate was sucked dry, the chimney carefully removed, and the disk placed upon clean aluminum foil on an electric hot plate which was equipped with a thermometer. The temperature was slowly allowed to rise to 160° (23).

Planchets were uniformly mounted beneath a thin mica window Geiger-Müller tube. A standard sample of BaC¹⁴O₃, similarly mounted, was frequently counted and all analyses were normalized with reference to this sample. In addition to the conventional corrections for background, self-absorption, and coincidence error, an additional correction for the

¹ E. Machlett and Son, No. 34-230-B.
contaminating non-radioactive carbon, based on the value of the blank in the manometric analysis, was applied.

The weight of the sample of BaCO$_3$ was determined at the end of the counting operation by weighing the alundum disk before and after dissolving the precipitate in dilute HCl. No significant residual radioactivity could be detected at the end of an analysis in the combustion tube, in the Van Slyke pipette, or in the cleaned planchet. With the procedures and equipment described, “infinite thickness” of BaCO$_3$ was found to have been reached when the precipitate weighed 34 mg. Whenever possible, “infinitely thick” samples were counted.

All analytical values are expressed as specific activity which is defined for the purpose of the present communication as counts per minute per milliatom of C.

**DISCUSSION**

The sodium gluconate administered in the present study was a mixture of the products derived from the oxidation of two biosynthetic sugars, deuterioglucose excreted by a diabetic rat and C$^{14}$-starch photosynthesized by bean leaves. Glucose excreted by a diabetic animal receiving D$_2$O has previously been shown to be approximately uniformly labeled in all its stable positions (24) and starch accumulating in a leaf after prolonged exposure to C$^{14}$O$_2$ has likewise been reported to be uniformly labeled (25), a finding confirmed in this laboratory. The sodium gluconate administered was therefore approximately uniformly labeled with respect to both isotopes.

The results obtained from the experiment in which isotopic sodium gluconate was injected intraperitoneally into a normal rat for three successive days are given in Table I. It will be seen that, of the C$^{14}$ administered as gluconate, 57 per cent was promptly excreted in the urine while 14 per cent appeared in the expired CO$_2$. Thus, of the 43 per cent which was not lost in the urine, approximately a third appeared as respiratory CO$_2$. This finding gives some idea of the extent to which gluconic acid is catabolized and indicates furthermore that the observed oxidation involves more than mere decarboxylation to pentose. If only C-1 were contributing to CO$_2$, no more than a sixth of the carbon of retained gluconate would have been recoverable in this fraction.

Several of the components of the liver were found to contain C$^{14}$. It is noteworthy that the nucleic acids were almost as rich in isotope as was the glycogen. Because of the shortage of material no attempt has been made to determine the specific activity of nucleic acid pentose, but the present finding is compatible with the suggestion that gluconic or phosphogluconic acid (26, 27) may serve as a precursor of the 5-carbon sugars.
From the urine excreted on each of the 3 days the phenylhydrazide of gluconic acid was isolated in the pure state and analyzed for both C\textsuperscript{14} and D. When these values are compared with the corresponding values obtained on the material administered, it is apparent that, within the limits of the analytical methods, neither isotope has undergone any dilution incident to the passage of gluconate through the body. Had the tissues of the rat contributed as little as 50 mg. of gluconic acid per day to the material which was presented to the kidney for excretion, a dilution of both isotopes in the urinary product would at once have been apparent and these results are therefore taken to mean that free gluconic acid is formed very little if at all in the tissues of the normal rat. This conclusion is not to be construed as in any way affecting the possibility of the formation of phosphogluconic acid from hexose phosphate (28), since, in the absence of an appropriate phosphatase, phosphogluconic acid would not mix with gluconic acid. It would appear, however, that the oxidation of glucose to free gluconic acid observed to be catalyzed \textit{in vitro} by glucose dehydrogenase prepared from normal liver does not, in the intact animal, make any detectable contribution to gluconic acid in the blood.

### Table I

**Distribution of Isotopes after Administration of Doubly Labeled Sodium Gluconate to Normal Rat for 3 Days**

<table>
<thead>
<tr>
<th>Sample analyzed</th>
<th>C\textsuperscript{14} specific activity ((\text{a}))</th>
<th>Total carbon ((\text{b}))</th>
<th>Total radioactivity, ((\text{a}) \times \text{(b)} = \text{(c)})</th>
<th>Per cent dose administered (100 \times \text{(d)})</th>
<th>Deuterium excess (\text{atom per cent})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na gluconate injected</td>
<td>102,000 (\text{c.p.m. per milliatomic C})</td>
<td>78.5</td>
<td>8.0 (\times 10^6)</td>
<td>100</td>
<td>3.40*</td>
</tr>
<tr>
<td>Urine C, Day 1</td>
<td>66,600</td>
<td>28.2</td>
<td>1.88 (\times 10^6)</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; 2</td>
<td>46,900</td>
<td>20.2</td>
<td>0.95 (\times 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; 3</td>
<td>61,200</td>
<td>27.6</td>
<td>1.69 (\times 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expired CO\textsubscript{2}, Day 1</td>
<td>757</td>
<td>252</td>
<td>0.221 (\times 10^6)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; 2</td>
<td>1,790</td>
<td>306</td>
<td>0.548 (\times 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; 3</td>
<td>1,100</td>
<td>292</td>
<td>0.322 (\times 10^6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver, glycogen</td>
<td>492</td>
<td>16.1</td>
<td>0.008 (\times 10^6)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>&quot; nucleic acids</td>
<td>468</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; proteins&quot;</td>
<td>305</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluconate from urine, Day 1</td>
<td>104,000(\dagger)</td>
<td></td>
<td></td>
<td></td>
<td>3.54*</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; 2</td>
<td>101,000(\dagger)</td>
<td></td>
<td></td>
<td></td>
<td>3.54*</td>
</tr>
<tr>
<td>&quot; &quot; &quot; &quot; 3</td>
<td>102,000(\dagger)</td>
<td></td>
<td></td>
<td></td>
<td>3.50*</td>
</tr>
</tbody>
</table>

\* Calculated from analyses on phenylhydrazides by multiplication by 18/11.
\dagger Calculated from analyses on phenylhydrazides by multiplication by 12/6.
The results of a preliminary experiment carried out in similar fashion, except that a much smaller quantity of C\textsuperscript{14} was employed, are given in Table II. The values obtained in this experiment were essentially in agreement with those just discussed and again it will be noted that the specific activity of the urinary gluconate was the same as that of the sodium gluconate injected.

As in the normal rat, so in the phlorhizinized rat, when isotopic sodium gluconate was injected, the expired CO\textsubscript{2} was found to have about 1 per cent of the specific activity of the material injected (Table III) and represented about 10 per cent of the total dose of C\textsuperscript{14}. Of particular interest in this case was the urinary glucose, the carbon of which proved to be about twice as radioactive as was the carbon of the CO\textsubscript{2}. Whereas this finding in itself made it unlikely that the radioactivity of the glucose had arisen exclusively from tissue CO\textsubscript{2}, it was deemed advisable to study the distribution of C\textsuperscript{14} among the 6 carbon atoms of this sample of glucose, the fact having been well established that CO\textsubscript{2} contributes exclusively to C-3 and C-4 of glucose in the body of the rat (29). The fragments analyzed represented carbon atoms 1, 2, and 3, atoms 4 and 5, and atom 6. As will be seen in Table III, the specific activities of each of these three fragments were of the same order of magnitude and indicated an approximately uniform distribution of C\textsuperscript{14} among the 6 carbon atoms of the excreted glucose. A similar degradation of the sample of liver glycogen obtained from the normal rat receiving isotopic sodium gluconate also revealed similar uniformity of specific activity of the frag-

<table>
<thead>
<tr>
<th>Sample analyzed</th>
<th>C\textsuperscript{14} specific activity</th>
<th>Total carbon</th>
<th>Total radioactivity, (a) x (b) = (c)</th>
<th>Per cent dose administered, 100(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) c.p.m. per milliatom C</td>
<td>(b) milliatoms</td>
<td>(c) c.p.m.</td>
<td>(d) per cent</td>
</tr>
<tr>
<td>Na gluconate injected</td>
<td>12,800</td>
<td>78.5</td>
<td>10.0 x 10\textsuperscript{6}</td>
<td>53</td>
</tr>
<tr>
<td>Urine C</td>
<td>6,870</td>
<td>77.0</td>
<td>5.28 x 10\textsuperscript{5}</td>
<td></td>
</tr>
<tr>
<td>Expired CO\textsubscript{2}, Day 1</td>
<td>152</td>
<td>248</td>
<td>0.377 x 10\textsuperscript{5}</td>
<td>53</td>
</tr>
<tr>
<td>&quot; &quot; &quot; 2</td>
<td>137</td>
<td>334</td>
<td>0.458 x 10\textsuperscript{5}</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot; &quot; 3</td>
<td>238</td>
<td>280</td>
<td>0.666 x 10\textsuperscript{5}</td>
<td>15</td>
</tr>
<tr>
<td>Liver, glycogen</td>
<td>51</td>
<td>17.2</td>
<td>0.009 x 10\textsuperscript{6}</td>
<td>0.1</td>
</tr>
<tr>
<td>&quot; nucleic acids</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gluconate from urine</td>
<td>12,500</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ments (Table IV). These findings, considered together with the fact that the gluconate administered was also uniformly labeled with C\textsuperscript{14}, demonstrate clearly the occurrence of a transformation of gluconic acid into glucose \textit{in vivo} which proceeds in some fairly direct fashion.

The question of the possible conversion of gluconic acid to saccharic acid and its subsequent excretion in the urine was explored by the addition of 108 mg. of non-isotopic carrier saccharic acid, as its phenylhydrazide, to the combined urine fractions from which gluconic

\begin{table}
\centering
\caption{Allocation of C\textsuperscript{14} in Urinary Glucose}
\begin{tabular}{l|l}
\hline
\textbf{Source} & \textbf{C\textsuperscript{14} specific activity} \\
\hline
Na gluconate injected & 160,000 \\
Expired CO\textsubscript{2} & 1,400 \\
Urinary glucose & 2,770 \\
C-(1 + 2 + 3) & 3,430 \\
C-(4 + 5) & 2,510 \\
C-6 & 2,380 \\
\hline
\end{tabular}
\label{table:urinaryGlucose}
\end{table}

\begin{table}
\centering
\caption{Allocation of C\textsuperscript{14} in Liver Glycogen from Normal Rat Given Isotopic Sodium for 3 Days}
\begin{tabular}{l|l}
\hline
\textbf{Source of carbon} & \textbf{C\textsuperscript{14} specific activity} \\
\hline
Liver glycogen & 492 \\
C-(1 + 2 + 3 + 4 + 5) & 420 \\
C-(1 + 2 + 3) & 455 \\
C-(4 + 5) & 383 \\
C-6 & 560 \\
\hline
\end{tabular}
\label{table:glycogen}
\end{table}

phenylhydrazide had been removed. This material was subsequently re-isolated, recrystallized to constant radioactivity, and then "washed out" to insure the absence of contaminating radioactive gluconic acid. Assuming any urinary saccharate to have the same specific activity as that of the gluconate, 102,000 c.p.m. per milliatom, and computing the activity of the finally isolated saccharate from that of its phenylhydrazide, \[ 48 \times (18/6) = 144 \text{ c.p.m. per milliatom}, \]
\[ (108 \times 144)/(102,000 - 144) = 0.15 \text{ mg. of saccharic acid is all that can be accounted for as having appeared in the urine}. \] This number, when compared with 3000 mg. of
sodium gluconate injected, reveals that only about 0.005 per cent of the injected gluconate, a negligible fraction, is recoverable as urinary saccharate.

The results, considered together, would make it appear that the reaction

\[ \text{Glucose} + \text{DPN}_\text{ox.} \xrightarrow{\text{glucose dehydrogenase}} \text{gluconic acid} + \text{DPN}_\text{red.} \]

certainly contributes very little if any gluconic acid to the fluids of the body, despite the demonstrated presence of the enzyme in the liver. It is clear that gluconic acid is oxidized in the animal body, but present evidence affords no support to the view that saccharic acid is an important intermediate. The transformation of gluconic acid to glucose which was found to occur has been shown not to proceed predominantly over CO₂. Alternative hypotheses to account for the data are the direct reduction of the acid to the aldehyde, or the degradation or fragmentation of gluconic acid to 3-carbon fragments and subsequent resynthesis of hexose. It seems unlikely that a reduction of gluconic acid by simple reversal of Reaction I can occur to any appreciable extent in view of the exergonic nature of the reaction as written.² Some reductant more potent than DPN_red. may, however, participate in the observed transformation.

The authors wish to acknowledge the valuable technical assistance of Mr. Steven Carson.

**SUMMARY**

Sodium gluconate uniformly labeled with C¹⁴ and D has been prepared and administered to normal and phlorhizinized rats. The occurrence of extensive biological oxidation of gluconate has been confirmed by the presence of C¹⁴O₂ in the expired air.

A considerable quantity of gluconic acid was excreted in the urine and was found to be isotopically identical with that injected. The failure of the tissues to contribute gluconic acid in vivo contrasts with the occurrence in vitro of a liver enzyme capable of converting glucose into free gluconic acid.

Only a negligible fraction of gluconate carbon could be recovered as urinary saccharate. Both nucleic acid and glycogen isolated from the liver of the normal rat exhibited significant radioactivity.

The urinary glucose from the phlorhizinized rat and the liver glycogen from the normal rat were found to be approximately uniformly labeled

² From published values of $E^o$'s (pH 7.0) for glucose to gluconate and for coenzyme I (30), the equilibrium constant for the reaction as written has been calculated as approximately $5 \times 10^6$ and $\Delta F$ as $-8000$ calories per mole.
with respect to C^{14}. This indicates a more or less direct conversion of
 gluconic acid into glucose.

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