Glucogenesis from Glycine and Serine in the Rat*

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Although it is assumed that amino acids constitute the main source of glucose production in animals, relatively little modern work has been done on the magnitude of glucogenesis from amino acids or on the factors which might influence this process. Studies conducted in early years were based principally on the experimental techniques of measuring glucose excretion in phlorizinized or depancreatized animals (1) or of measuring increases in the source of glucose production in animals, relatively little modern work has been done on the magnitude of gluconeogenesis from amino acids or on the factors which might influence this process.

In previous studies from this laboratory (6), a method was devised, based on isotopic dilution of C14-labeled glucose, to measure the rate of output, presumably hepatic, of newly synthesized glucose. It was recognized that the same procedure could be used to measure the formation of blood glucose from labeled precursors. If the assumption is correct that in the fasting animal essentially all of the newly synthesized glucose is released from the liver into the bloodstream, then the measurement of the uptake of C14 in the blood glucose should provide a reliable indication of the rate of glucogenesis from a C14-labeled amino acid. Experiments testing this assumption, together with data on gluconeogenesis from glycine and serine, are presented in this report.

EXPERIMENTAL

Labeled Amino Acids—Glycine-2-C14 and D,L-serine-3-C14 were purchased from the Volk Radiochemical Company. D- and L-serine-3-C14 were prepared in our laboratory by resolution of D,L-serine, with a small-scale modification of the Greenstein procedure, starting with the chloroacetetyl derivative (7). A typical preparation is described below.

One gram of D,L-serine was dissolved in 9.5 ml of 1 N NaOH, and the solution was partially frozen by immersion in an ice-salt mixture. Then 4.0 ml of 1 N NaOH and 3 ml of redistilled acetyl chloride were added in portions and the reaction mixture was shaken vigorously for a few minutes between additions. The solution was acidified to pH 1 with 2 ml of 2 N H2SO4, then saturated with MgSO4 and subjected to continuous ether extraction for 48 hours. Ether was evaporated from the extract, and the residue was washed with petroleum ether to remove chloroacetic acid formed during the reaction, and dried in a vacuum. Crude crystals (1.4 g) of chloroacetyl-DL-serine thus obtained were used for resolution.

One gram of chloroacetyl-DL-serine was dissolved in 500 ml of water, the pH was adjusted to 7.6 with 1 N LiOH, and 10 ml of an aqueous solution containing 20 mg of acylase-I (280 units per g) were added. This enzyme was a commercial product obtained from the Pentex Company. The mixture was allowed to stand at 37-38° for 3 hours. The pH, which had dropped to 6.3, was again adjusted to 7.6 with LiOH, an additional 5 ml of the acylase I solution was added, and the mixture was placed in an incubator at 37-38° for 12 hours. The digested solution (pH 7.4) was brought to pH 5.0 with glacial acetic acid, and was treated with small portions of activated carbon (Norit) for 1 hour and filtered. The treatment with carbon was repeated and the filtrate was evaporated to a small volume under reduced pressure at 40°. A large amount of 95% ethanol was added and the mixture was stored in a cold room (0-2°) overnight. The crystals of L-serine which appeared were washed free of LiOH with ethanol and were dried over sulfuric acid in a desiccator; they weighed, yielding 0.23 g (87% of the theoretical yield). MDP = 0.03° in water: MDP recorded = 7.9° (7).

The mother liquor was concentrated under reduced pressure at 30°, acidified to pH 1.0 with 50% H2SO4, saturated with MgSO4, and subjected to continuous extraction with ether for 48 hours. Ether was evaporated under reduced pressure at 30°, the residue was refluxed for 2 hours with 5 ml of 2 N HCl, and the solution was evaporated to dryness under reduced pressure and dissolved in water. The pH was brought to 5 with 1 N LiOH, and the solution was treated with active carbon as before and then filtered. The filtrate was evaporated to a small volume, a large quantity of 95% ethanol was added, and the mixture was stored in a cold room overnight. The crystals which separated were washed with ethanol and dried over sulfuric acid in a desiccator; they weighed 0.19 g (72.5% of the theoretical yield). MD + 8.07° in water.

Experimental Procedures—Male rats obtained from Carworth Farms and weighing about 200 g were fasted for 24 hours, then given a "trace" dose of the C14-labeled amino acid (about 1 to 20 mg containing 1 to 2 μc of C14) by intraperitoneal injection, with or without simultaneous administration of glucose (5 mmoles/3 ml of water) by stomach tube. In experiments with postabsorptive rats, described in Table III, animals which had been fasted for 24 hours previously were given 5 mmoles of glucose by stomach tube 4 hours before injection of labeled amino...
acid and were immediately placed in a Modes metabolism chamber (8). The respiratory CO₂ was collected in hourly portions by absorption in NaOH and precipitation as BaCO₃. After stated intervals the animals were decapitated and the blood was collected in small beakers containing heparin. The liver was quickly removed, weighed, and placed in hot 30% KOH for isolation of liver glycogen by the method of Boxer and Stetten (9). Skeletal muscles were treated similarly for isolation of muscle glycogen.

Representative specimens of liver glycogen obtained from D-serine-3-C¹⁴ and L-serine-3-C¹⁴ were degraded to determine the distribution of radioactivity in each carbon atom of glucose. For this purpose a fermentation procedure which used Leuconostoc mesenteroides was carried out as described earlier (10).

In the estimation of incorporation of labeled amino acid carbon into blood glucose, advantage was taken of a method previously used for following blood glucose turnover (8). This consists in oxidation of blood glucose to formic acid by means of periodate, followed by oxidation of formate to CO₂ by mercuric ions. Treatment of the blood filtrate with Amberlite MB-3 ion exchange resin was found to remove amino acids quantitatively, and control tests run with radioactive glycine and serine added to blood filtrates demonstrated that the blood glucose carbon can be recovered quantitatively from an Amberlite MB-3 eluate without interference from radioactive amino acids in the blood.

Two milliliters of blood were deproteinized by the method of Somogyi (11), and 5 ml of the resulting filtrate were passed through an Amberlite MB-3 column, 0.6 × 20 cm. The eluate and washings were made to 25 ml, from which 1 or 2 ml were used for blood sugar determination by the anthrone method (12) and 20 ml taken for duplicate determinations of blood glucose radioactivity (6).

The glycogen samples obtained from liver and muscle were purified by repeated solution in 10% trichloroacetic acid and precipitation with 1.2 volumes of ethanol. Finally the glycogen was dissolved in water, reprecipitated with ethanol, and dried in a vacuum desiccator and weighed. For radioactivity assay, samples were oxidized quantitatively to CO₂ by the persulfate method (13).

**RESULTS**

Gluconeogenesis from Serine-3-C¹⁴—Data on the quantities and isotope distribution in four products of serine metabolism, viz. liver, muscle, blood glucose, and respiratory CO₂ are recorded in Table I. As anticipated, the quantity of liver glycogen was high in animals given glucose in addition to the labeled serine, averaging about 2%, and was negligible in the fasted rats. Similarly, the incorporation of radioactivity in liver glycogen was negligible in the fasted rats and quite high (5.30 and 8.05% on the average for L- and D-isomers, respectively) in the glucose-fed rats.

<table>
<thead>
<tr>
<th>Amount</th>
<th>Activity</th>
<th>Amount</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver glycogen</td>
<td>0.088 (0.018-0.085)</td>
<td>2.13 (1.44-2.62)</td>
<td>5.30 (4.03-7.40)</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td>0.22 (0.20-0.24)</td>
<td>0.44 (0.42-0.53)</td>
<td>1.77 (0.79-3.32)</td>
</tr>
<tr>
<td>Blood sugar</td>
<td>76 (73-79)</td>
<td>0.08 (0.06-1.00)</td>
<td>0.30 (0.25-0.36)</td>
</tr>
<tr>
<td>Respiratory CO₂</td>
<td>34.7 (34.0-36.4)</td>
<td>32.3 (32.1-32.5)</td>
<td>40.1 (39.8-40.3)</td>
</tr>
</tbody>
</table>

**Table I**

Distribution of isotope from D- and L-serine-3-C¹⁴ in tissue glycogens, blood sugar, and respiratory CO₂, in fasted and glucose-fed rats

Experimental details are given in the text. Numbers in parentheses at the head of each column give the total number of experiments. Observed ranges are given in parentheses under each value. Amounts of liver and muscle glycogen are given in per cent of fresh weight of tissue; blood sugar is in mg per 100 ml of blood; and respiratory CO₂ in mmoles excreted per rat over the designated experimental period. Duration of experiment, 4 hours.

Activities are total activities expressed as percentage of injected radioactivity; those for liver glycogen are on the basis of the weight of the freshly excised liver; those for muscle glycogen are estimated on the basis of a total weight of muscle equivalent to 50% of the body weight; total activities of blood sugar are based on the assumption that the glucose space is essentially that of the extracellular fluid, taken to be 30% of the body weight (20).
of either D- or L-serine was not greatly affected by the dietary condition; the quantities of CO₂ excreted in fed and fasted rats, and also the incorporation of radioactivity therein, were of similar magnitude.

Glucogenesis from Glycine-2-Cl⁴—In Table II there are recorded data on the metabolism of glycine-2-Cl⁴. In this experiment, fasted rats were given “trace” doses of the labeled glycine, with or without a simultaneous intragastric injection of 5 mmoles of glucose in 3 ml of water, and were sacrificed 1, 2, or 4 hours later. The experiment provided additional data on the time course of glucogenesis from glycine. As was already reported for serine, the glucose-fed rats displayed a remarkably different pattern of glucogenesis from that of the fasted animals. With glucose feeding, relatively large amounts of radioactivity were found in the liver glycogen, and the incorporation increased from 2.26% in 1 hour to 5.02% in 4 hours. In contrast, in the fasted, the small initial quantity of liver glycogen was further depleted during the 4-hour period and no appreciable incorporation of glycine occurred in liver glycogen.

Although the levels of muscle glycogen did not change during the 4-hour experimental period, far more radioactivity was incorporated therein in the fed than in the fasted rats. In the former, the incorporation increased from 0.4% at 1 hour to 0.8% in 4 hours; in the latter, the already low average incorporation of 0.07% at 1 hour declined to 0.02% at 4 hours.

At each time interval studied, the blood sugar level was somewhat higher in the glucose-fed rats, but the differences declined with time, as expected. However, marked differences were found in the incorporation of activity therein. In 1 hour 3.7% of the injected dose appeared in the blood sugar of the fasted rats; the activity subsequently declined to 1.7% in 2 hours and to 0.85% in 4 hours. In contrast, the incorporation of radioactivity in blood sugar of the glucose-fed rats was less than one-fourth that of the fasted in 1 hour, about one-fifth after 2 hours, and about one-seventh after 4 hours.

As was observed previously (10), glycine was oxidized to CO₂ but not nearly so rapidly as serine; in 4 hours only about 8% of the injected dose was recovered in the respiratory CO₂, whereas after the same interval over 30% of D- or L-serine was thus converted. A somewhat surprising finding was the increased oxidation of glycine in the glucose-fed rats. Despite the presence of large amounts of glucose, the fed animals oxidized glycine from 1.4 to 1.8 times as rapidly as did the fasted rats.

Postabsorptive Rats—The low levels of liver glycogen precluded an accurate estimation of liver glucogenesis in the fasting rat. In order to obtain better data on neogenesis of liver glycogen without the complication of glucose feeding, a series of similar experiments was performed with postabsorptive rats. Cori (14) observed that 4 hours after ingesting 5 mmoles of glucose, rats have a normal blood sugar, but a reproducibly high level of liver glycogen. Trace doses of glycine were given to such treated rats. As shown in Table III, essentially the same pattern of glycine metabolism appeared in the treated as in the fasted rats. Blood sugar radioactivity was high, reaching its

| Table II |

Distribution of Cl⁴ of glycine-2-Cl⁴ in tissue glycogens, blood sugar, and respiratory CO₂, in fasted and glucose-fed rats

Values are as given in Table I.

<table>
<thead>
<tr>
<th></th>
<th>Amount</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr.</td>
<td>2 hr.</td>
</tr>
<tr>
<td></td>
<td>(2)</td>
<td>(2)</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Fasted</td>
<td></td>
<td>0.24 (0.09, 0.40)</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td></td>
<td>0.26 (0.26, 0.26)</td>
</tr>
<tr>
<td>Blood sugar</td>
<td></td>
<td>86 (86, 87)</td>
</tr>
<tr>
<td>Respiratory CO₂</td>
<td></td>
<td>8.8 (8.2, 9.5)</td>
</tr>
<tr>
<td>Glucose-fed</td>
<td></td>
<td>(3)</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td></td>
<td>0.98 (0.72-1.25)</td>
</tr>
<tr>
<td>Muscle glycogen</td>
<td></td>
<td>0.28 (0.21-0.30)</td>
</tr>
<tr>
<td>Blood sugar</td>
<td></td>
<td>132 (129-140)</td>
</tr>
<tr>
<td>Respiratory CO₂</td>
<td></td>
<td>10.1 (10.1-10.2)</td>
</tr>
</tbody>
</table>

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Distribution of Cl⁴ of glycine-2-Cl⁴ in tissue glycogens, blood sugar, and respiratory CO₂, in fasted and glucose-fed rats

Values are as given in Table I.
peak in 1 hour and declining thereafter. Muscle glycogen content remained steady at 0.46% over the 4-hour period, with essentially no incorporation of radioactive carbon. At all time periods, liver glycogen was high, so that there was no difficulty in the isolation and assay of radioactivity. Nevertheless, the incorporated radioactivity was very low and declined with time. It is certain, therefore, that under postabsorptive conditions there is no rapid replacement or turnover of liver glycogen derived from glycine.

**Glycogenesis from D- and L-Serine**—It was reported earlier (10) that glycogenesis from D,L-serine-3-$\text{Cl}^4$ occurs, in part at least, by a pathway more direct than would be suggested by its transformation to pyruvic acid. The basis for this conclusion was the observation that in glycogen produced from pyruvate-3-$\text{Cl}^4$, pyruvate carbon 3 was more highly randomized among the 6 glucose carbons than was serine carbon 3 in glycogen produced from serine-3-$\text{Cl}^4$. It was pointed out earlier (15-17) that pyruvate yields glucose apparently only by way of 4-carbon acids, since the glycogen synthesized in rats receiving pyruvate-3-$\text{Cl}^4$ has almost as much activity in glucose carbon atoms 2 and 5 as in carbons 1 and 6. Thus, a lower degree of randomization than that exhibited by pyruvate is indicative of a by-pass in metabolism allowing entry to glycogenic pathways above pyruvate. That such a path exists for D-serine, but not for L-serine, is demonstrated in Table IV, in which are given the results of degradation of liver glycogen. When L-serine was the glycogen precursor, the $(\text{C}_1 + \text{C}_4) - (\text{C}_2 + \text{C}_6)$ values were 17 and 12% respectively, indicating a randomization consonant with the intermediary participation of pyruvate in its conversion to glucose. On the other hand, D-serine-3-$\text{Cl}^4$ yielded glycogens with $(\text{C}_1 + \text{C}_4) - (\text{C}_2 + \text{C}_6)$ values of 64 and 62% respectively, indicating a low degree of randomization and hence the elimination of pyruvate as a major intermediate in its conversion to glucose.

**DISCUSSION**

The conversion of substantial quantities of glycine and serine carbon to blood glucose and tissue glycogen confirms previous balance studies on the glycogenic properties of these amino acids (1-9). The appearance of large amounts in the blood glucose of fasting and postabsorptive rats, coupled with the almost complete absence in liver glycogen, indicates that in these conditions essentially all of the glucose synthesized by liver passes into the blood without appreciable equilibration with liver glycogen. However, when glucose was fed simultaneously with the injection of the labeled amino acid, incorporation in blood glucose was depressed while incorporation in liver glycogen was correspondingly enhanced. These results are in keeping with present views (18), in which it is assumed that the net flow of glucose is normally outward from the liver but is reversed during hyperglycemia. Although the mechanism of the regulation of hepatic glucose transport is uncertain, there is evidence that the suppression of hepatic output is mediated by means of insulin (6, 19). Although the site of this regulation is likewise uncertain, it is reasonable to assume that an interference with the normal process of glucose release results in the accumulation of glucose 6-phosphate, which then follows the alternate pathway to glycogen. But this is probably a gross oversimplification of an exceedingly complex control mechanism.

Glucose entering the blood of fasting animals undergoes constant replacement at a rate approximating a half-time of 60 minutes (20). Hence one can assume that a major proportion of "new" glucose derived from a trace quantity of labeled precursor would enter the blood quickly and thereafter would be removed at the same rate as the blood glucose. This process is...
Glucose

\[ \uparrow \downarrow \]

Triose phosphate

\[ \downarrow \]

Pyruvate

\[ \leftarrow \]

Hydroxy pyruvate

\[ \rightarrow \]

L-serine

\[ \rightarrow \]

Glucose was also considerably higher (possibly 4 to 8 times) than is indicated in Table I.

The level of radioactivity in blood glucose was, at all time periods studied, considerably higher in the fasted than in the fed rats. This difference can doubtless be attributed to two principal causes: (1) the lack of suppressive mechanisms for hepatic glucose release in the fasted animals, which makes more glucose available to the blood at the expense of liver glycogen synthesis; and (2) a more rapid turnover of blood glucose in the fed animals, which tends to remove the radioactivity from the blood, and thus accounts also for the higher incorporation of radioactivity in muscle glycogen. Since hardly any radioactivity is incorporated in the liver and muscle glycogen in fasting, it is clear that a study of the time course of blood glucose labeling after the injection of labeled precursors should provide a reliable measure of gluconeogenesis under steady state conditions. More detailed time studies are now under way.

The greater utilization of D-serine over L-serine for gluconeogenesis, as shown in Table I, is consonant with a more direct path for the incorporation of L-serine into blood glucose. Since hardly any radioactivity is incorporated in muscle glycogen in fasting, it is clear that a study of the time course of blood glucose labeling after the injection of labeled precursors should provide a reliable measure of gluconeogenesis under steady state conditions. More detailed time studies are now under way.

The situation with L-serine is considerably more complicated. There is good evidence for linking hydroxy pyruvate with the synthesis of L-serine from glucose (27-31), but the scanty evidence hitherto available did not clearly establish whether hydroxy pyruvate or pyruvate itself is on the main pathway of the reverse process. From the report of Chargaff and Sprinro (32) that pyruvate is formed in rat liver in the presence of L-serine, and the occurrence in many microorganisms of a serine deaminase which converts serine directly to pyruvate, it has been assumed that this is the mechanism whereby L-serine is metabolized in the rat, but substantiating evidence for the existence of this enzyme in animals has appeared only since completion of this study (33). The present results clearly establish that a high degree of randomization occurs during gluconeogenesis from L-serine-3-C\textsuperscript{14} and thus implicate pyruvate as a direct major intermediate. While this work was in progress, similar conclusions were announced by Minthorn et al. (34).

At first glance, the formation of pyruvate from serine is at variance with previous indications that pyruvate is not an intermediate of serine metabolism, based on its nonconversion to acetoacetate in rat liver slices (35). It is probable, however, that the result in vivo do not necessarily portray accurately the metabolism in the intact animal. The results here reported are also somewhat at variance with studies in vivo of Elwyn et al. (36) who found that D-serine was not oxidized at all and that L-serine was as efficient a precursor of glucose as was D-serine in rat liver slices.

The results of the present study, taken in the light of these previous findings, confirm the suggestion by Ichihara and Greenberg (37) that L-serine follows a cyclic path in its biosynthesis and its conversion to glucose, as shown in Fig. 1.

**SUMMARY**

The incorporation of radioactivity into the blood glucose, liver and muscle glycogen, and respiratory CO\textsubscript{2} was determined after the injection of D- and L-serine-3-C\textsuperscript{14} and glycine-2-C\textsuperscript{14}. In fasting rate, incorporation was rapid in blood glucose, but negligible in liver and muscle glycogen. In rats fed glucose simultaneously, incorporation was markedly reduced in blood glucose, greatly increased in liver glycogen, and moderately increased in muscle glycogen, but oxidation to CO\textsubscript{2} was not greatly affected. D-Serine was more readily incorporated in blood glucose and in liver and muscle glycogen than L-serine, but was oxidized to CO\textsubscript{2} at about the same rate. Distribution of C\textsuperscript{14} among the carbon atoms of glucose derived from liver glycogen, determined by a fermentative procedure, revealed a far greater randomization of carbon 3 of L-serine among glucose carbon atoms 1, 2, 5, and 6. These data confirm previous suggestions that D-serine is metabolized by means of hydroxy pyruvate, whereas L-serine yields pyruvate.

**Addendum**—After submitting this paper for publication, we became aware of the study by Dickens and Williamson (38), which showed (a) that B-hydroxy pyruvate was readily converted to glucose in the intact rat and in rat liver slices; and (b) that randomization of carbon 3 of B-hydroxy pyruvate-3-C\textsuperscript{14} was not very different from that observed here with D-serine-3-C\textsuperscript{14} and was similar to that previously reported by us (10) for D-, L-serine-3-C\textsuperscript{14}. They also showed that L-serine-3-C\textsuperscript{14} was converted to glucose slowly in rat liver slices, but did not degrade this. These investigators considered the various pathways of gluconeogenesis from serine, and their conclusions are in general consonant with those here presented. However, they envisioned the possibility of a direct conversion of L-serine to hydroxy pyruvate by means of transamination (31). This possibility seems to be ruled out as an important process since our degradation data show extensive randomization of L-serine carbon 3 in liver glycogen.

**REFERENCES**

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