A Study of the Mechanism of Conversion of Galactose to Glucose in the Intact Rat

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In 1950 Caputto et al. (1) found that the biological interconversion of D-galactose and D-glucose, which differ only in the configuration of the hydroxyl group at carbon 4, occurred not with the free sugars or phosphate esters, but while the carbohydrates were bound as uridine diphosphate derivatives. Since that time the mechanism of the conversion has been the subject of numerous investigations (2-12). A variety of possible mechanisms has been suggested and examined, each of which could account for the formation of glucose from galactose or galactose from glucose. These include (a) cleavage to 3 carbon or other fragments followed by recondensation of the fragments, (b) removal of water between carbon atoms 3 and 4 or between carbon atoms 4 and 5 followed by rehydration, (c) ring closure to an inositol followed by ring opening at a different position, (d) nucleophilic attack by OH of the medium to bring about inversion of configuration, (e) oxidation of the secondary alcohol group at carbon 4 to a keto group, followed by reduction, and (f) dehydrogenation between carbon atoms 3 and 4, or 4 and 5, to give an ene-diol intermediate, followed by rehydrogenation.

Attempts to elucidate the mechanism of interconversion have relied heavily on the use of isotopes. Studies carried out have utilized galactose-1-C14 (2), galactose-2-C14 (9, 10), D2O (6), T2O (6), T2O18 (7), H2O18 (4), and NAD+ (8, 11). The conclusions drawn from the results of these experiments appear to eliminate all of the postulated mechanisms except oxidation-reduction. This mechanism is further supported by the finding by Maxwell (5-7) that NAD+ was required for the action of uridine diphosphate galactose 4-epimerase from calf liver. Other studies revealed a similar co-factor requirement of a yeast enzyme (11, 12) and a bacterial enzyme (7). Analogous conclusions have been drawn concerning the mechanism of other epimerizations (13). An oxidation-reduction mechanism appears to be the operative mechanism, however, not because of direct evidence but because all other postulations have apparently been eliminated by (a) the failure of the systems to incorporate deuterium, tritium, or O18 from labeled water to a significant extent, (b) the location of C14 in glucose formed from labeled galactose, and (c) the demonstrated requirement for NAD+. Attempts to obtain direct evidence for the participation of NAD+ were unsuccessful when the reaction was studied in the presence of nicotinamide adenine dinucleotide, in the oxidized and reduced forms and labeled with tritium in position 4 of the nicotinamide ring (8, 11). It was assumed that the failure to observe incorporation of isotope resulted from the fact that the NAD+ involved in the reaction was firmly bound to the enzyme, rendering added NAD+ ineffectual. Good evidence for such binding has been obtained (11, 12).

The present investigation was undertaken for the purpose of further clarifying the mechanism of interconversion. In view of the fact that isotope from labeled NAD+ was not incorporated into the carbohydrates involved, an opposite approach was used. The studies were carried out with galactose doubly labeled with C14 and tritium to determine whether tritium is lost in the process of its conversion to glucose, as would be expected if an oxidation-reduction reaction took place involving unbound elements of the medium. The results show no such loss, and can be interpreted as evidence lending further support to the suggestion that bound NAD+ participates in both phases of the process or to indicate that an unknown mechanism is involved.

**Experimental Procedure**

Preparation of Isotopic Galactose—D-Galactose-U-C14,4-T1 was prepared through the reduction of 2,3,5,6-di-O-isopropylidene-4-keto-D-glucose-U-C14 dimethyl acetal with lithium aluminum hydride-H2 as described (14). The final product melted at 166–168, had an equilibrium rotation of [c]20° + 79.4° (c = 1, 1)

1 U denotes uniform or random labeling.

2 Obtained from New England Nuclear Corporation.

3 Melting points were obtained on the Kofler hot stage micro melting point apparatus.
in the conversion of galactose to glucose, showing anticipated loss of tritium from carbon 4.

Past work (5–8, 11–13) indicated that NAD+ was involved in metabolism cage and killed by a blow on the head after 4 hours. The animals were placed in a metabolism cage and killed by a blow on the head after 4 hours. The animals were placed in a metabolism cage and killed by a blow on the head after 4 hours. The animals were placed in a metabolism cage and killed by a blow on the head after 4 hours. The animals were placed in a metabolism cage and killed by a blow on the head after 4 hours.

RESULTS AND DISCUSSION

In view of the conclusion from past investigations, as discussed earlier, that the conversion of galactose to glucose involved an oxidation-reduction mechanism, the present experiment was carried out with the hope of obtaining direct evidence about the process. An oxidation would necessarily entail the loss of hydrogen from carbon 4, as shown in Fig. 1.

Past work (5–8, 11–13) indicated that NAD+ was involved in the process. If the reduction involved NAD+ from the metabolic pool rather than, or in addition to, the NAD+ utilized in the oxidation, then tritium would either be absent from, or decreased in amount in, the product obtained. The greater the amount of pool NADH utilized for the reduction, the smaller the amount of tritium that would be present. It would therefore be expected that the ratio of tritium to C14 in glucose formed from galactose would be decreased. From the last column in Table I it can be seen that the ratio of tritium to C14 in glucose was unchanged from that in the galactose from which it was formed. It must therefore be concluded that, if NAD+ is assumed to be a co-factor in an oxidation-reduction mechanism, the NAD+ must be bound to the enzyme and utilized in both the oxidative and reductive steps. This would be in accord with a theory proposed by de Robichon-Szuhajster (17).

It would be of interest to utilize the doubly labeled galactose employed in these experiments as the substrate in a system in vitro in an attempt to find formation of NAD+. This would afford direct evidence for the participation of NAD+ in the process and add considerably to our knowledge of the mechanism of the conversion. Without such direct proof it is conceivable, although not likely, that NAD+ participates in a manner different from that presently assumed.

Previously proposed mechanisms which have apparently been ruled out by studies of other investigators with labeled water might also be re-examined on the basis of an extension of the findings with NAD+. If, as appears to be the case, the same

Table I

<table>
<thead>
<tr>
<th>Animal</th>
<th>Liver glycogen</th>
<th>Glucose from liver glycogen</th>
<th>Total isotope administered</th>
<th>Isotope recovered in liver glycogen</th>
<th>Specific activity of glucose</th>
<th>$H_2^3C^{14}$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>mg</td>
<td>d.p.m.</td>
<td>$H_2^{14}$</td>
<td>$C^{14}$</td>
<td>%</td>
</tr>
<tr>
<td>Administered galactose</td>
<td>215</td>
<td>152</td>
<td>$2.19 \times 10^7$</td>
<td>$2.08 \times 10^6$</td>
<td>$9.80 \times 10^5$</td>
<td>$9.92 \times 10^4$</td>
</tr>
<tr>
<td>Rat I</td>
<td>215</td>
<td>134</td>
<td>$2.19 \times 10^7$</td>
<td>$2.08 \times 10^6$</td>
<td>$1.08 \times 10^7$</td>
<td>$1.04 \times 10^6$</td>
</tr>
<tr>
<td>Rat II</td>
<td>258</td>
<td>134</td>
<td>$2.19 \times 10^7$</td>
<td>$2.08 \times 10^6$</td>
<td>$1.08 \times 10^7$</td>
<td>$1.04 \times 10^6$</td>
</tr>
</tbody>
</table>

* Assays were carried out on glucose obtained from liver glycogen, and the activity in glycogen was calculated by using a correction for the water taken up during hydrolysis (16).
NAD$^+$ molecules are utilized for both the oxidation and reduction by virtue of binding to the enzyme, then it could be argued that the process might occur by a dehydration-hydration mechanism, with the same water molecules being involved in both processes by virtue of binding to the enzyme. Similarly, an aldol type of cleavage might occur but the fragments remain bound to the enzyme and recombine. Horecker, in a personal communication to Leloir (17), suggested the possibility of continuous binding of a 3 carbon fragment. In consideration of such arguments it appears that the only mechanisms thus far proposed which have been eliminated as possibilities are ring closure to an inositol and a Walden inversion involving attack by OH$^-$ from the medium.

**SUMMARY**

The conversion of galactose to glucose was studied by administering galactose labeled uniformly with $^{14}C$ and at position 4 with tritium to rats and examining glucose obtained from liver glycogen. The ratio of tritium to $^{14}C$ in the isolated glucose was unchanged from that in the administered galactose, indicating that if the mechanism of conversion involved the participation of NAD$^+$ in an oxidation-reduction process, the NAD$^+$ must have been bound to the enzyme and brought about both the oxidation and the reduction. Other possible mechanisms of conversion are discussed from the viewpoint of binding by the enzyme of reactants in the conversion.

**REFERENCES**

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