A STUDY OF THE METABOLISM OF D-ARABINOSE IN THE RABBIT*

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Breusch (1) reported the existence in cat liver of a D-arabinose dehydrogenase; Wainio (2) found that D-arabinose is oxidized in the presence of diphosphopyridine nucleotide, triphosphopyridine nucleotide, methylene blue, and a preparation from lyophilized lamb liver. In view of these results, and because of the interest in the metabolic relationships of 5-carbon sugars in animal tissues, we have made studies on the fate of D-arabinose when administered to rabbits.

EXPERIMENTAL

The D-arabinose, obtained from the Pfannstiehl Chemical Company, was prepared from calcium gluconate by the method of Hackett and Hudson (3). Its specific rotation was $-104.5^\circ$. The dosage used in all experiments was 2 gm. per kilo of body weight.

Studies of Blood Levels—After collecting a control sample of blood, D-arabinose was administered orally to a fasting rabbit, and blood samples were collected at hourly intervals for 6 hours. Somogyi blood filtrates (4) were analyzed by the method of Benedict (5) for total sugar and non-fermentable reducing substances, and by the method of Roe and Rice (6) for pentose content. A typical result is shown in Fig. 1. The blood levels of total sugar and non-fermentable reducing substances reached a peak in about 1 hour and returned to the preingestion concentrations in about 6 hours; the curves representing these substances closely paralleled each other, indicating that there was not a shift of pentose into fermentable substance.

Recoveries after Oral Administration—D-Arabinose was given orally to rabbits fasted overnight. The animals were anesthetized with nembutal and sacrificed at different intervals after administration. After laparotomy the gastrointestinal tract and the urinary bladder were removed. The gastrointestinal tract was homogenized with water in a Waring blender and

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its pentose content was determined by analysis of a filtrate from an aliquot treated with Somogyi's reagents. The urine in the bladder was added to that collected in the metabolism cage, and the pentose content of the pooled sample was determined. Samples of blood, liver, kidney, brain, and muscle were taken, and, in some experiments, of the gallbladder and spleen, and samples of the hide were removed for analysis. The tissue samples were homogenized in a Waring blender and Somogyi filtrates were prepared. All analyses for pentose were made with the method of Roe and Rice (6).

The results of these experiments are shown in Table I. Quantitative recoveries were obtained from the gastrointestinal tract of control rabbits.
sacrificed 5 minutes after administration. A gradual decrease in the D-arabinose in the intestinal tract was observed, only 2 per cent remaining 9 hours after administration. Traces of free pentose were found in the tissues at the 1 and 2 hour intervals after administration, but the analyses performed 6 hours, or longer, after administering the sugar gave negative values. Pentose appeared in the urine early in the experiment, and the total excretion was less than 10 per cent of the amount administered in all cases. The urine analyses for rabbits allowed to remain in the metabolism cage for 48, 72, and 144 hours showed no greater excretion of pentose than was observed in the 24 hour experiments.

The results of Table I, and other studies made upon blood and urine, indicate that, at a dosage of 2 gm. per kilo of body weight, 90 per cent of orally administered D-arabinose undergoes a metabolic transformation in the rabbit, as a result of which it no longer gives a colorimetric pentose reaction based on furfural formation or reduces alkaline copper solution.

An extension of these studies was made to ascertain whether D-arabinose might become loosely bound in the tissues of the rabbit in a form that would not be detected by the colorimetric method. D-Arabinose was given to young rabbits weighing 200 to 300 gm. With nembutal anesthesia, the animals were sacrificed at different intervals after administration. The hide and paws were removed and discarded. A laparotomy was performed, and the gastrointestinal tract and urinary bladder were removed. The bladder contents were added to the urine voided in the metabolism cage after arabinose administration. Somogyi filtrates of the gastrointestinal tract and the urine were prepared. The remainder of the animal was homogenized with 0.6 N HCl. An aliquot of this mixture was neutralized with solid Na₂CO₃, and a Somogyi filtrate was made and analyzed for free pentose. Another portion of the carcass homogenate was refluxed for 3 hours, cooled, and neutralized with solid Na₂CO₃. A Somogyi filtrate was made and analyzed for pentose and free furfural. The pentose was determined by the usual method, and the furfural was estimated by the same procedure without warming to 70°. The results of these experiments are shown in Table II. The recoveries of free pentose were similar to those shown in Table I. These experiments, in which an analysis of the whole carcass for pentose content was performed, confirmed the recovery studies made by analysis of separate tissues of adult rabbits. The data also showed that there was no additional pentose or furfural released by hydrolysis for 3 hours with 0.6 N HCl. This is evidence that the arabinose was not stored in the animal's tissues in a loosely bound form.

Several urine samples obtained after administration of D-arabinose were refluxed for 3 hours with 0.6 N HCl without showing an increase in the pentose content. These results indicated that pentose was not excreted in the urine in a bound form.
Liver Glycogen Formation—Since our preliminary studies showed that n-arabinose undergoes a metabolic transformation in the rabbit, it was of interest to investigate whether this sugar enters into pathways of normal hexose metabolism. A study was therefore made of the formation of liver glycogen following administration of n-arabinose.

In the experiments of Table III, Groups 1, 2, and 3, the method of Cohn and Roe (7) was used. Rabbits were fasted for exactly 24 hours. Nembutal in a dosage of 30 mg. per kilo of body weight was administered intravenously, and a laparotomy was performed. Samples of liver were removed from the outer margin of the right and left lobes of the liver and placed in 30 per cent KOH. Bleeding was controlled by the application of Hemopak and skin clamps. Saline or sugar solution was injected into the duodenum, and the animal’s abdomen was closed with sutures. 6 hours later nembutal was given, the abdomen was opened quickly, and samples of liver were excised from the outer margin of each liver lobe and placed in 30 per cent KOH. The glycogen content of the liver samples was determined by the method of Good, Kramer, and Somogyi (8).

This method of studying glycogenesis has the theoretical advantage of permitting one to obtain a control value for liver glycogen before administering the test substance. It requires careful control of anesthesia and bleeding, and it is desirable to work rapidly with the surgical procedure. As a check on this procedure for measuring glycogenesis, the experiments of Groups 4 and 5, Table III, were carried out. In these experiments the same technique was followed except that the initial laparotomy and removal of control samples of liver were omitted, and the test substances were given orally instead of intraduodenally.

The results of the experiments of Group 1, Table III, confirmed the studies of Cohn and Roe (7), in which it was found that there is a significant
increase in liver glycogen in rabbits fasted for 24 hours following saline administration, when nembutal and surgery are used. The only conclusions that can be drawn from the experiments of Groups 1, 2, and 3 must be based upon a comparison of the increases in liver glycogen 6 hours after administration of the test substances. The observed mean increase in liver glycogen in 6 hours was 0.22 per cent with saline, 0.71 per cent with arabinose, and 2.45 per cent with glucose. The mean increase in liver glycogen following d-arabinose administration is significantly greater than that obtained with saline ($t = 3.5, P < 0.01$). But d-arabinose must be considered a poor glycogen former in comparison with glucose, since the mean increase in liver glycogen after glucose administration is significantly greater than that obtained with d-arabinose ($t = 9.1, P < 0.01$). The

**Table III**

*Formation of Liver Glycogen*

The rabbits were fasted for 24 hours. In Groups 1, 2, and 3, nembutal was administered, a laparotomy was performed, and control liver samples were taken. Saline or sugar was injected into the duodenum, the abdomen was closed, and liver samples were taken 6 hours later. In Groups 4 and 5, nembutal was administered, saline or arabinose was given orally, and liver samples were taken 6 hours later. Dose of d-arabinose, 2 gm. per kilo of body weight.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Solution administered</th>
<th>No. of rabbits</th>
<th>Control (mean)</th>
<th>6 hrs. later (mean)</th>
<th>Increase (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline</td>
<td>6</td>
<td>0.31</td>
<td>0.53</td>
<td>0.22</td>
</tr>
<tr>
<td>2</td>
<td>d-Arabinose</td>
<td>5</td>
<td>0.46</td>
<td>1.18</td>
<td>0.71</td>
</tr>
<tr>
<td>3</td>
<td>d-Glucose</td>
<td>4</td>
<td>0.19</td>
<td>2.64</td>
<td>2.45</td>
</tr>
<tr>
<td>4</td>
<td>Saline</td>
<td>6</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>d-Arabinose</td>
<td>5</td>
<td>1.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The data of Groups 4 and 5, Table III, are confirmatory of the results in Groups 1, 2, and 3, though they are less significant statistically ($t = 2.71, P = 0.04$).

*Keto and Lactic Acid Formation after d-Arabinose Administration*—Since it has been shown that a plethora of utilizable carbohydrate in the tissues gives rise to increased blood lactate (9, 10) and pyruvate (11), it was decided to determine the blood levels of these intermediates after d-arabinose administration.

A non-fasted rabbit was placed in a well ventilated animal box which was covered with a removable wire screen. A 3 hour rest period was allowed to permit the animal to come to a basal condition. Two samples of blood were collected from the marginal ear vein at hourly intervals before and after injection of the test substances, fluoride and oxalate being used as
preservative and anticoagulant. Somogyi filtrates of these samples were analyzed for lactate by the method of Barker and Summerson (12). Blood keto acids were determined by the direct method of Friedemann and Haugen (13) for total hydrazones, with trichloroacetic acid filtrates of iodoacetate-treated, oxalated bloods. Care was taken to keep the movements of the animal at a minimum throughout the experimental period; this is imperative, since otherwise erratic levels of blood lactate and keto acids are obtained.

The results of these experiments are shown in Table IV. Two samples of blood collected 1 hour apart were taken before injecting the test sub-

<table>
<thead>
<tr>
<th>Solution administered</th>
<th>Keto acids</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg. per 100 cc. blood</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-1 hr.</td>
<td>0 hr.</td>
</tr>
<tr>
<td>Saline</td>
<td>3.09</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>2.23</td>
<td>1.61</td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>1.54</td>
<td>1.63</td>
</tr>
<tr>
<td></td>
<td>2.93</td>
<td>1.74</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.70</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>2.86</td>
<td>2.99</td>
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<td></td>
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<td></td>
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<td>3.34</td>
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<tr>
<td></td>
<td>2.62</td>
<td>1.36</td>
</tr>
</tbody>
</table>

stance to determine whether a basal condition had been obtained. The values at minus 1 hour are included in Table IV to show the essentially basal character of the zero hour levels. Statistical analysis is based on the zero hour values only as control levels. Analysis of variance showed non-significant variations in the hourly values for blood keto acids and lactate when saline was injected. After glucose injection, there was a highly significant variation ($P < 0.01$) in the tests for blood lactate and a moderately significant variation ($P < 0.05$) in the values for blood keto acid. When D-arabinose was injected, the tests were moderately significant for blood lactate ($P < 0.05$) and non-significant for blood keto acids. These
data showed that there is a significant increase in blood lactate, but not in blood keto acids, after D-arabinose injection.

DISCUSSION

Our data upon blood levels and urinary excretion after D-arabinose administration are essentially in agreement with the results reported by Corley (14), based on copper reduction techniques.

The observation that glucose administration gives rise to only moderately significant changes in the blood keto acids indicates that this is not a sensitive method for measuring carbohydrate degradation. The negative findings with blood keto acids after D-arabinose administration might, therefore, reflect the use of a method of low sensitivity.

It appears that these studies present unequivocal evidence to show that D-arabinose undergoes metabolic transformation in the rabbit and suggestive, but not rigorous, evidence that this sugar gives rise to intermediates that enter known pathways of carbohydrate metabolism.

SUMMARY

1. D-Arabinose, when administered orally to rabbits, undergoes a metabolic transformation, after which it is no longer capable of giving characteristic reactions of a free pentose.

2. Evidence was obtained suggesting that in the rabbit D-arabinose gives rise to intermediates that enter known pathways of carbohydrate metabolism.

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