THE CONVERSION OF PROTEIN TO GLUCOSE IN DE-PANCREATIZED AND PHLORHIZINIZED DOGS

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The phlorhizinized dog has been used extensively for the quantitative estimation of glucose formation from protein, amino acids, other organic acids, and products of metabolism. The depancreatized dog has not been similarly used; i.e., fasted and then fed a pure protein. This work was undertaken to determine whether the depancreatized dog converts protein to glucose to the same extent as the phlorhizinized dog when the experimental conditions are as analogous as possible. For this purpose the glucose yield of casein was ascertained by similar procedures in both depancreatized and phlorhizinized dogs. And, since the yields in the depancreatized animals might be influenced by the unavoidable removal of pancreatic acinous function, the glucose yield of casein was also determined in phlorhizinized dogs after ligation of the pancreatic ducts.

Methods

Phlorhizinized Dogs. Intact—On the 3rd day of fasting and on each day thereafter normal dogs were injected subcutaneously with 1 gm. of phlorhizin (Merck) suspended in 10 cc. of olive oil, according to the method of Coelen (1895). It was found necessary to recrystallize the phlorhizin by the method of Deuel and Chambers (1925). The glucose to nitrogen ratio had usually reached a constant level by the 4th day on phlorhizin, at which time from 20 to 30 gm. of casein (Merck's according to Hammarsten) were given by stomach tube. One series of these animals was fed 100 gm. quantities of a special bread (soy bean) with a high protein content. The animals were used for a daily deter-
mination only for as long as they remained in good condition and their urine remained free of albumin.

Ligated Pancreatic Ducts—The same procedure was employed, except that the dogs were used 2 or 3 weeks after the pancreatic ducts had been ligated and the pancreas separated from the duodenum. They were all in excellent condition at the time the experiment was started. At the conclusion of the experiment autopsy examinations of the animals were made in order to establish that the pancreatic ducts had not regenerated. These animals did not receive raw pancreas in their diet. A series of these animals was also fed the special bread.

Depancreatized Dogs—Normal dogs were depancreatized, then standardized for several weeks on insulin and a diet including raw pancreas. At the beginning of an experiment they were deprived of food and insulin. On each day of fasting they were given 500 cc. of 0.9 per cent sodium chloride under the skin in order to combat the dehydration to which they are subject. Preliminary experiments had convinced us that this precaution is essential in order to maintain the animals in good condition and to insure a good yield of "extra sugar." Usually by the 3rd day of fasting without insulin the glucose to nitrogen ratio had reached a constant level, and the animals were fed casein or the special bread. Ordinarily these dogs were used for determinations on only 2 or 3 successive days, since they fail to recover from longer experimental periods.

Urine was collected under toluene from metabolism cages over 24 hour periods, each period being marked by catheterization. Urinary sugar was determined by the Benedict method (1911). Urinary nitrogen was determined by the Koch and McMeekin (1924) micromethod, the nitrogen content of the casein and bread by the macro-Kjeldahl method. The extra glucose was calculated by the Lusk method.

Results

Glucose to Nitrogen Ratio—It has been suggested that no difference exists between the glucose to nitrogen ratio of the fasting depancreatized and the fasting phlorhizinized dog (Dann, Chambers, and Lusk, 1931–32; Dann, 1933; Cori and Cori, 1935). Since the interpretation of our results depends on whether such a
difference exists, it seemed necessary to investigate this matter, although the suggestion was based on the results obtained in only two thyroidectomized animals.

It was found that the average glucose to nitrogen ratio of forty-two fasting dogs on phlorhizin for 4 days was $3.55 \pm 0.06$ (maximum 4.38, minimum 2.26). The average glucose to nitrogen ratio of thirty-two fasting depancreatized dogs without insulin for 3 days was $2.91 \pm 0.09$ (maximum 4.37, minimum 1.31). The average glucose to nitrogen ratio of nine fasting phlorhizinized dogs with ligated ducts was $3.60 \pm 0.08$ (maximum 4.07, minimum 2.95). In order to avoid the necessity of making a subjective selection of the “typical” fasting ratio for each animal, the fasting ratios of only the 4th day for the phlorhizinized dogs and of only the 3rd day for the depancreatized dogs were considered in arriving at these average values. As a result the range of variation is perhaps wider than it might otherwise be. However, similar variations are to be found in the literature.

Ligation of the pancreatic ducts did not alter the glucose to nitrogen ratio of phlorhizinized dogs. It is clear, however, that an experimental difference between the glucose to nitrogen ratio of the fasting phlorhizinized and depancreatized dogs does exist. In accordance with many previous observers, we occasionally obtained ratios of approximately 2.80 in phlorhizinized animals; but unselected data recorded in the literature show that of 100 phlorhizinized dogs only 15 per cent had ratios below 3.0.

During the course of these experiments it was found incidentally in a series of seven dogs which were sacrificed after the daily administration of phlorhizin for from 16 to 26 days that the liver was devoid of gross and microscopic fat (Sudan III). On a shorter period of phlorhizin administration we found, as is well known, that the liver was fatty.

Glucose Yield of Casein—The results are shown in Tables I to III. In Table I, the results on the duct-ligated animals are given in some detail, since data on such animals are not available in the literature. In Table II the averaged results of the glucose yield of casein in the three types of animals are given; the analyses have been averaged and presented in the form of a single determination. In Table III the glucose yields of casein are compared with those of the special high protein bread.
Conversion of Protein to Glucose

The intact phlorhizinized dog yielded an average of 48 per cent glucose from casein, which confirms Janney's (1915) value of 48 per cent. The depancreatized dog yielded an average of 43.5

**TABLE I**

Response of Duct-Ligated Phlorhizinized Dogs to Casein

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Glucose</th>
<th>Nitrogen</th>
<th>Glucose to nitrogen ratio</th>
<th>Extra glucose</th>
<th>N fed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Casein*</td>
<td>Fasting</td>
<td>Casein*</td>
<td>Fasting</td>
</tr>
<tr>
<td>1</td>
<td>15.90</td>
<td>20.77</td>
<td>4.57</td>
<td>6.32</td>
<td>3.49</td>
</tr>
<tr>
<td>2</td>
<td>30.05</td>
<td>24.65</td>
<td>8.45</td>
<td>8.30</td>
<td>3.57</td>
</tr>
<tr>
<td>3</td>
<td>21.00</td>
<td>19.62</td>
<td>6.12</td>
<td>7.22</td>
<td>3.44</td>
</tr>
<tr>
<td>4</td>
<td>12.55</td>
<td>15.40</td>
<td>3.98</td>
<td>5.68</td>
<td>3.15</td>
</tr>
<tr>
<td>5</td>
<td>20.09</td>
<td>17.72</td>
<td>7.12</td>
<td>6.91</td>
<td>2.95</td>
</tr>
<tr>
<td>Average</td>
<td>19.92</td>
<td>19.63</td>
<td>6.05</td>
<td>6.88</td>
<td>3.32</td>
</tr>
</tbody>
</table>

* Average of two tests. The amount of pure casein fed was calculated from the nitrogen content, the factor 6.41 (Janney, 1915) being used.

**TABLE II**

Averaged* Results with Casein

<table>
<thead>
<tr>
<th>Preparation of dog</th>
<th>No. of dogs used</th>
<th>No. of tests</th>
<th>Casein fed</th>
<th>N fed</th>
<th>Urine sugar</th>
<th>Urine N</th>
<th>Glucose to nitrogen ratio</th>
<th>Glucose yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact, phlorhizinized</td>
<td>6</td>
<td>11</td>
<td>Fasting 24.35</td>
<td>7.13</td>
<td>3.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30.12</td>
<td>4.72</td>
<td>28.07</td>
<td>8.63</td>
<td>3.24</td>
<td>48.6</td>
</tr>
<tr>
<td>Duct-ligated, phlorhizinized</td>
<td>5</td>
<td>10</td>
<td>Fasting 20.09</td>
<td>6.05</td>
<td>3.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22.21</td>
<td>3.53</td>
<td>19.63</td>
<td>6.88</td>
<td>2.84</td>
<td>37.1</td>
</tr>
<tr>
<td>Depancreatized</td>
<td>4</td>
<td>6</td>
<td>Fasting 11.24</td>
<td>3.90</td>
<td>2.88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31.4</td>
<td>4.92</td>
<td>16.79</td>
<td>5.98</td>
<td>2.81</td>
<td>43.5</td>
</tr>
</tbody>
</table>

* The results have been averaged and presented in the form of a single determination for each type of animal.

per cent glucose from casein. This determination has not been made before in the depancreatized dog by the method of urinary analysis. The results of Ralli, Canzanelli, and Rapport (1931), who determined the r.o. of the depancreatized dog fed casein,
implied a yield of approximately 50 per cent. However, their animals received 50 gm. of raw pancreas with the casein. The duct-ligated phlorhizinized dog yielded only 37 per cent glucose from casein. It is to be noted in Table II that on feeding casein the glucose to nitrogen ratio definitely decreased in the duct-ligated dogs, but not in the depancreatized dogs. The glucose yields when the more complex food, a special high protein bread, was fed confirm the differences noted for casein in the different canine preparations, except that the yield in the duct-ligated dogs is not significantly different from that in the depancreatized animals.

**TABLE III**

*Glucose Yields of Dogs Fed Casein and Bread*

The values are given in per cent.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Intact, phlorhizinized</th>
<th>Duct-ligated, phlorhizinized</th>
<th>Depancreatized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>Bread</td>
<td>Casein</td>
</tr>
<tr>
<td>1</td>
<td>44.7</td>
<td>28.0</td>
<td>48.5</td>
</tr>
<tr>
<td>2</td>
<td>51.3</td>
<td>26.8</td>
<td>33.9</td>
</tr>
<tr>
<td>3</td>
<td>31.9</td>
<td>29.1</td>
<td>30.9</td>
</tr>
<tr>
<td>4</td>
<td>48.5</td>
<td>29.4</td>
<td>38.2</td>
</tr>
<tr>
<td>5</td>
<td>52.9</td>
<td>30.5</td>
<td>34.3</td>
</tr>
<tr>
<td>6</td>
<td>55.9</td>
<td>28.7</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>47.5</td>
<td>28.7</td>
<td>37.1</td>
</tr>
<tr>
<td>11†</td>
<td>21†</td>
<td>10†</td>
<td>30†</td>
</tr>
</tbody>
</table>

* This special bread contained 22.8 per cent protein, 20.5 per cent of digestible carbohydrate by chemical analysis, and 3.65 per cent nitrogen.
† These numbers refer to the total number of individual tests.

*Recovery of Ingested Glucose*—As an aid in the interpretation of the observed results, it is desirable to know how much ingested glucose may be recovered in the urine of the phlorhizinized and depancreatized dogs. Barker, Chambers, and Dann (1937) have recently obtained an average recovery of 95 per cent in fasting depancreatized dogs in twenty-one experiments. We have fed 10 and 15 gm. of glucose to one fasting depancreatized dog and recovered 93 per cent in one instance and 87 per cent in the other. These values are within the range reported by the investigators just mentioned and also by Minkowski (1893), Allard (1908),
and Moorhouse and collaborators (1915), who fed glucose in addition to a meat diet.

In regard to the recovery of glucose in the fasting phlorhizinized dog, the values reported in the literature vary from 46 to 108 per cent. The average of all the values (56) in the literature, including two of our own (82 per cent), is 87 per cent (Ringer, 1912; Ringer and Frankel, 1914; Csonka, 1915; Sansum and Woodyatt, 1916; Olmsted, 1920; Ringer, 1923-24; Nash and Benedict, 1923; Nash, 1925; Deuel and Chambers, 1925; Wierzuchowski, 1927; Deuel, Wilson, and Milhorat, 1927; Boothby, Wilhelmj, and Wilson, 1929; Loewi, 1902; Reilly, Nolan, and Lusk, 1898). It is apparent from these data that more ingested glucose is recoverable in the urine in the depancreatized dog than in the phlorhizinized dog.

DISCUSSION

Low glucose yields from casein were obtained in the depancreatized dogs and in the duct-ligated phlorhizinized dogs. These were the animals in which there was a disturbance of the acinous pancreatic function. These low yields may be due to (a) impaired digestion, (b) utilization of a greater proportion of the sugar formed, or (c) incomplete conversion of the protein to glucose. In regard to the first possibility we believe that protein in 25 to 30 gm. quantities should be completely digested and absorbed (feces were rarely passed by these animals). Further, if one assumes that these animals convert casein to glucose to the same extent as intact phlorhizinized dogs, namely 49 per cent, and inserts such a yield in the Lusk equation which can be solved backwards for nitrogen intake, one obtains impossible answers. If the low yields were due to incomplete absorption of nitrogen, this procedure would reveal the true absorption. The fact that impossible answers are obtained proves that deficient digestion cannot be responsible for the low yields. These results further suggest that the depancreatized and duct-ligated animals do not convert casein to glucose to the extent of 49 per cent. That this is probably not the result of utilizing a greater proportion of the sugar formed is demonstrated by the fact that recovery of ingested glucose is greatest in the depancreatized dog. The only possibility left is that the low yields indicate impairment of
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Glucogenesis, so that less sugar is formed from a given amount of protein. It is well known that the depancreatized dog receiving neither insulin nor raw pancreas very rapidly develops a fatty liver. Berg and Zucker (1931), Ralli, Rubin, and Present (1938), and Beazell and Schmidt find similar changes follow complete ligation of the pancreatic ducts. Thus it is reasonable to consider that in the absence of acinous function of the pancreas, sufficient impairment of liver function occurs to depress glucogenesis.

Although impaired glucogenesis may explain the low yields on casein, it can hardly explain the low glucose to nitrogen ratio of the depancreatized dog after a 3 day fast, for the obvious reason that the duct-ligated dogs showed a high ratio in spite of their liver damage. However, it should be remembered that in contrast to the phlorhizinized dog, the long fasted depancreatized dog shows a progressively falling glucose to nitrogen ratio, and it is wholly possible that this phenomenon is a reflection of progressive liver damage, with consequent inability completely to convert protein to sugar. If it is true that the factor of liver damage is not operating in dogs fasted 3 days, why should it operate in the fed animals? Apparently the necessity of metabolizing a sudden influx of exogenous protein throws a strain on the liver which it cannot successfully withstand.

The low glucose to nitrogen ratio during fasting of the depancreatized dog may be due to oxidation of a greater proportion of sugar formed by glucogenesis, or to an inability to form sugar from unknown non-nitrogenous sources. We know of no satisfactory evidence which permits a choice between these alternatives.

**Summary**

1. A comparative study has been made of the glucose to nitrogen ratios during fasting, the recovery of ingested glucose, and the conversion of protein to sugar in intact phlorhizinized dogs, in phlorhizinized dogs with ligated pancreatic ducts, and in depancreatized dogs.

2. There is a significant difference between the glucose to nitrogen ratios during fasting of depancreatized and phlorhizinized dogs.

Conversion of Protein to Glucose

3. A greater proportion of ingested sugar is recoverable in the urine of depancreatized dogs than in that of phlorhizinized dogs.

4. Depancreatized dogs and duct-ligated phlorhizinized dogs give low glucose yields from ingested protein. Since impaired digestion cannot account for this finding, it is suggested that impaired gluconeogenesis in the liver may be responsible.

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