THE EXCRETION OF URINE IN THE DOG

III. THE USE OF NON-METABOLIZED SUGARS IN THE MEASUREMENT OF THE GLOMERULAR FILTRATE

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No substance is known to be present normally in the plasma and urine of vertebrates which can be safely used to evaluate the quantity of glomerular filtrate under physiological conditions. Creatinine of exogenous origin has been recommended for this purpose by Rehberg, (1926) but when it is recognized that this substance is secreted by the renal tubules of the lower vertebrates (Marshall and Grafflin, 1932; Clarke and Smith, 1932) and that it is not present normally in significant quantities in the blood of mammals (Behre and Benedict, 1922; Gaebler and Keltch, 1928; Gaebler, 1930) its use is open to question.

We have sought for a foreign substance, practically and theoretically suitable for this purpose, among several groups of organic and inorganic compounds and have concluded that the non-metabolized sugars are satisfactory.

The properties which we believe such a substance should possess are as follows:

1. It must be determinable in plasma and urine with quantitative accuracy.
2. It must be non-toxic and it must exert no local stimulating or depressing action upon the kidney.
3. It must be completely filtrable from plasma.
4. It must not be secreted by the renal tubules.
5. It must not be reabsorbed by the renal tubules.

The choice of sugars for this purpose was suggested by the recent observations of Marshall (1930) that glucose is excreted by glomerular, but not by aglomerular fishes. On broad principles we relate this circumstance to the fact that, being a food and not a waste product, glucose has been invariably conserved by the vertebrates throughout their evolution and at no time continuously rejected from the body. The renal tubules have never been called upon, therefore, to secrete this substance, and are incapable of doing so. There is good evidence that this is equally true of
the mammals. The appearance of glucose in the urine of glomerular animals during hyperglycemia or after phlorizin is in this view an incidental phenomenon referable to incomplete tubular reabsorption of glucose from the glomerular filtrate. But because it is normally reabsorbed by the tubules, glucose itself is not suitable for the evaluation of the glomerular filtrate. Although this reabsorption can be paralyzed by phlorizin there is reason to believe that other physiological activities of the kidney are also influenced by this drug, so that its use is of dubious physiological value.

It seemed that if glucose is not secreted by the tubules, other sugars would not be secreted by them, and that among the metabolically inert sugars one or more might be found which was not reabsorbed. Our attention has been directed toward three which appear to be most suitable: the pentose, xylose; the disaccharide, sucrose; and the trisaccharide, raffinose.

1. Quantitative determination. The introduction of the rapid absorption of glucose by washed yeast cells as a method of determining the true glucose content of plasma and urine has made it possible to distinguish this sugar from other reducing sugars, such as xylose. When the concentration of xylose is maintained at a level of 70 mgm. per cent or higher in the plasma, the non-glucose reducing substances of dog plasma and urine can be neglected. (These average about 5 and 150 mgm. per cent, respectively, when using Somogyi's copper precipitation method.) Methods have been devised for the determination of either sucrose or raffinose in the presence of glucose by means of sucrase, for a full description of which the reader is referred to the section of this paper dealing with methods.

2. Toxicity. Xylose, sucrose and raffinose can be administered in large amounts without evidence of toxic action. The possibility of a local action upon the kidney will be referred to subsequently in the paper.

3. The filterable character of the sugars. There is no evidence that a combination exists in the plasma between glucose and protein, and although it is frequently suggested that the glucose in normal blood exists in some complex condition, there is ample reason to believe that in the phlorizinized animal the glucose is entirely filterable at the glomerulus—a conclusion which is supported by the experiments reported in this paper.

There is no reason to suppose that a physiologically inert sugar such as xylose can combine with protein or otherwise form non-filterable complexes, but to test this point we have filtered dog plasma containing 150 mgm. per cent of xylose through collodion membranes under a pressure of 100 to 150 cm. of Hg; the filtrate contained no protein, as shown by heating and the addition of trichloracetic acid, but the residue and the filtrate contained xylose and glucose in the same concentrations, within the limits of experimental error in the sugar analysis (± 2 per cent).

4. Xylose and sucrose are not secreted by the renal tubules. We have already referred to Marshall's (1930) observation that the agglomerular
MEASUREMENT OF GLOMERULAR FILTRATE BY SUGARS

Fish kidney is incapable of excreting glucose, even after large doses of phlorizin, indicating that glucose is excreted only by glomerular filtration. Jolliffe (1930) and Clarke and Smith (1932) have shown that xylose and sucrose, although freely excreted by glomerular fish, are not excreted by agglomerular fish from plasma levels of 300 to 400 mgm. per cent.

Though evidence derived from lower vertebrates should not be transferred directly to the higher animals, our rapidly growing knowledge of the comparative physiology of the kidney leads us to believe that sugars are never secreted by the renal tubules. Direct evidence, however, against the secretion of sugars by the renal tubules in the dog is now available in our observations, reported in this paper, that xylose and sucrose, and in the phlorizinized dog xylose and glucose, or raffinose and glucose, are excreted at the same rate relative to the plasma concentration; i.e., the respective simultaneous glomerular clearances for these substances are identical. This fact is consonant with filtration, but irreconcilable with secretion.

5. **Xylose is not reabsorbed.** It would appear probable that the normal tubular reabsorption of glucose, which prevails in all vertebrates that have been examined, is related to the metabolic importance of this substance and that the actual process of reabsorption possibly involves to some extent a process of metabolism, a suggestion which has been frequently made. In any case, one would not expect xylose, which is not metabolized to any appreciable extent by the rabbit or the dog (Corley, 1926, 1928; Greenwald, 1931) to be conserved by active tubular reabsorption in the manner of glucose. But if any active reabsorption occurred one would expect phlorizin, because it blocks the reabsorption of glucose, to block the reabsorption of xylose, also.

Xylose is excreted by all normal animals so far examined (glomerular fishes and the dog) with a relatively high U/P ratio when glucose is absent or present only in small quantities, in the urine. This fact in itself indicates that no extensive reabsorption of xylose prevails in the normal animal, but it is not sufficient to warrant the assumption that there is no reabsorption at all, because the quantitative relations between the plasma, the urine and the renal threshold might be different for the two sugars. So we have attempted to answer the question of whether xylose is reabsorbed by comparing the U/P ratios before and after phlorizin of glucose, xylose and a third and indifferent substance—indifferent, that is, so far as sugar metabolism is concerned. In the present experiments on the dog urea has been chosen for this third substance.

**DISCUSSION.** With the background furnished by the above facts we may turn to the experimental data. The following experiments are given in full:

Table 1. Experiments 87, 88 and 100, showing the excretion of xylose, glucose and urea before and after the administration of phlorizin.
Table 2. Experiments 92 and 96, showing excretion of raffinose, glucose and urea before and after the administration of phlorizin.

<table>
<thead>
<tr>
<th>Exp. Number</th>
<th>Date</th>
<th>Duration of Expt.</th>
<th>THERMOPHERIC AREA</th>
<th>CM. = UV ( \frac{P}{S.A.} )</th>
<th>CM. XYLENE</th>
<th>CM. GLUCOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Xylose</td>
<td>Xylose</td>
<td>Glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
<td>mgm. per cent</td>
</tr>
<tr>
<td>87</td>
<td>26</td>
<td>0.885</td>
<td>10.4 271.0</td>
<td>99.1 6,260</td>
<td>121.8 1,090</td>
<td>29.9 72.7</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0.906</td>
<td>10.8 225.0</td>
<td>113.5 5,520</td>
<td>125.2 830</td>
<td>24.5 67.6</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>1.291</td>
<td>11.2 134.5</td>
<td>112.8 9,370</td>
<td>115.8 4,007</td>
<td>20.1 59.0</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.39</td>
<td>11.9 119.0</td>
<td>102.6 3,760</td>
<td>109.0 4,320</td>
<td>18.1 66.2</td>
</tr>
<tr>
<td>88</td>
<td>31</td>
<td>0.906</td>
<td>11.0 6,780</td>
<td>85.1 Trace</td>
<td>102.6* 0.00</td>
<td>0.00</td>
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<td></td>
<td>30</td>
<td>1.006</td>
<td>114.0 5,940</td>
<td>82.0 Trace</td>
<td>102.9 0.00</td>
<td>0.00</td>
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<td></td>
<td>32</td>
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<td>89.0 3,940</td>
<td>75.7 3,280</td>
<td>107.6 104.60.97</td>
<td>97.6 98.0.93</td>
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<td>31</td>
<td>1.290</td>
<td>96.0 4,270</td>
<td>71.2 2,930</td>
<td>106.3 98.4 0.93</td>
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<tr>
<td>100</td>
<td>24</td>
<td>2.290</td>
<td>6.5 70.7 150.4 2,792</td>
<td>32.8 56.1 0.585</td>
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<td>26</td>
<td>1.537</td>
<td>6.5 98.9 152.7 4,040</td>
<td>30.8 53.6 0.575</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>29</td>
<td>1.620</td>
<td>6.5 92.5 169.9 4,185</td>
<td>79.1 1,895 0.581</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Average after phlorizin ............................................. 0.90

* Xylose clearance abnormally high, possibly because room was cold and dog shivered throughout experiment.

Expt. 87. Dog 18, wgt. 20 kgm., S.A. 0.77 sq. m. Mixed diet. Twenty-four grams xylose in 60 cc. water injected subcutaneously at 6:00 a.m. and 6 grams in 20 cc. water at 6:45 a.m. Water ad lib. Period 1 began at 7:03 a.m.

Two hundred milligrams phlorizin per kgm. in 50 cc. 1.25 per cent NaHCO\(_3\) solution injected subcutaneously at end of period 2, or 8:02 a.m. Six grams xylose in 20 cc. water injected subcutaneously at 8:45 a.m. Period 3 began at 9 a.m. All blood samples drawn at middle of urine collection periods.

Expt. 88. Dog 31, wgt. = 12 kgm., S.A. = 0.54 sq. m. Cracker meal diet. Fourteen and five-tenths grams xylose in 40 cc. water injected subcutaneously at 6:45 a.m. and 3.6 grams in 15 cc. water at 7:30 a.m. Three hundred cubic centimeters water by stomach at 6:50 a.m. and 300 cc. water at 7:30 a.m. Period 1 began at 7:45 a.m.

One hundred milligrams phlorizin per kgm. in 2.5 per cent NaHCO\(_3\) injected intravenously and same quantity subcutaneously at end of period 2, or 8:45 a.m. Six grams xylose in 15 cc. water injected subcutaneously at 9:30 a.m. Period 3 began at 9:45 a.m. Blood samples drawn at middle of urine collection periods.

Expt. 100. Dog 20, wgt. 19.0 kgm., S.A. 0.76 sq. m. Cracker meal diet 3 days. Fasted 2 days; 46 grams xylose in 100 cc. water injected subcutaneously at 9:50 a.m. and 11 grams xylose at 10:35 a.m. Period 1 started at 10:56 a.m.

One hundred milligrams phlorizin per kgm. in NaHCO\(_3\) intravenously and same quantity subcutaneously at end of period 2, or 11:55 a.m. Period 3 started at 12:40 a.m. Blood samples drawn at irregular intervals and interpolated.
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Table 3. Experiments 84, 85 and 86, showing the excretion of xylose, sucrose and urea in the normal animal.

The facts to be noted in these experiments are as follows:
1. Xylose, sucrose and raffinose are normally excreted by the dog with a relative high U/P ratio, a fact which qualitatively indicates that these sugars are not actively reabsorbed by the tubules.

TABLE 2
The effect of phlorizin on the excretion of raffinose and glucose in the dog

<table>
<thead>
<tr>
<th>EXPT. NUMBER</th>
<th>DURATION OF PERIOD</th>
<th>URINE VOLUME</th>
<th>Plasma</th>
<th>Raffinose</th>
<th>Glucose</th>
<th>CM = UP/S.A.</th>
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</thead>
<tbody>
<tr>
<td>92</td>
<td>32</td>
<td>1.940</td>
<td>10.0</td>
<td>197.0</td>
<td>120.63</td>
<td>553</td>
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<tr>
<td></td>
<td>29</td>
<td>1.380</td>
<td>9.7</td>
<td>262.0</td>
<td>107.44</td>
<td>900</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.933</td>
<td>8.7</td>
<td>303.5</td>
<td>82.04</td>
<td>750</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>0.967</td>
<td>9.1</td>
<td>276.0</td>
<td>70.94</td>
<td>324</td>
</tr>
<tr>
<td>96</td>
<td>30</td>
<td>2.233</td>
<td>6.5</td>
<td>86.4</td>
<td>90.22</td>
<td>3551</td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>1.545</td>
<td>6.3</td>
<td>112.3</td>
<td>96.13</td>
<td>942</td>
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<td>34</td>
<td>1.088</td>
<td>6.7</td>
<td>219.0</td>
<td>87.55</td>
<td>327</td>
</tr>
</tbody>
</table>

Average after phlorizin .............................................. 1.04

Expt. 92. Dog 20, wgt. 19 kgm., S. A. 0.76 sq. m. Cracker meal diet six weeks. Twenty-four grams raffinose in 100 cc. water injected subcutaneously and 300 cc. water by stomach at 6:10 a.m. Six grams raffinose in 30 cc. water subcutaneously at 7:00 a.m. Period 1 began at 7:14 a.m.

One hundred milligrams phlorizin per kgm. in NaHCO₃ solution subcutaneously and same quantity intravenously at end of period 2, or 8:10 a.m. Period 3 began at 9:17 a.m. All blood samples drawn at middle of collection periods.

Expt. 96. Dog 29, wgt. 13.5 kgm., S. A. 0.75 sq. m. Cracker meal diet four weeks. Twenty-four grams raffinose in 100 cc. water injected subcutaneously and 500 cc. water by stomach at 9:00 a.m. Six grams raffinose in 30 cc. water subcutaneously at 9:45 a.m. Period 1 began at 8:00 a.m.

One hundred milligrams phlorizin per kgm. in NaHCO₃ solution intravenously and 200 mgm. subcutaneously at end of period 2, or 9:10 a.m. Fourteen grams raffinose in 60 cc. water subcutaneously and 500 cc. water by stomach at 11:40 a.m. Period 3 began at 12:10 p.m. All blood samples drawn at middle of collection periods.

2. Phlorizin does not cause a rise in the absolute value of the U/P ratio for these sugars, as it does in the case of glucose, nor does it cause a rise in clearance (i.e., the quantity of sugar excreted per unit time from a constant blood level, or UV/P) as would be expected if active reabsorption normally occurred and if this reabsorption were blocked by phlorizin.
3. The ratio of the xylose or raffinose clearance to the urea clearance is not significantly affected by phlorizin; there is no reason to believe, even if this drug blocked the reabsorption of these sugars, that it would block the reabsorption of urea to exactly the same extent, if at all; therefore, since the sugar clearance is not increased relative to the urea clearance after phlorizin, it may be inferred that phlorizin is without effect upon the former.

4. Under the influence of phlorizin the glucose clearance rises to the xylose clearance or the raffinose clearance, as the case may be, but never significantly exceeds the latter. This indicates that there is no active reabsorption of xylose or raffinose which is not affected by phlorizin; for if

### Table 3

The excretion of urea, xylose and sucrose in the normal dog

<table>
<thead>
<tr>
<th>Exp. Number</th>
<th>Duration of Period</th>
<th>Urea</th>
<th>Xylose</th>
<th>Sucrose</th>
<th>CM = UV/S.A.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>cc.</td>
<td>Plasma</td>
<td>urine</td>
<td>Plasma</td>
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<tr>
<td>84</td>
<td>32</td>
<td>5.72</td>
<td>15.5</td>
<td>110.8</td>
<td>57.1</td>
</tr>
<tr>
<td>85</td>
<td>32</td>
<td>1.08</td>
<td>12.5</td>
<td>165.3</td>
<td>98.1</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>1.09</td>
<td>12.9</td>
<td>146.5</td>
<td>106.4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1.03</td>
<td>13.5</td>
<td>175.0</td>
<td>116.4</td>
</tr>
<tr>
<td>86</td>
<td>31</td>
<td>1.03</td>
<td>10.5</td>
<td>237.0</td>
<td>95.8</td>
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<td>1.17</td>
<td>11.0</td>
<td>215.0</td>
<td>105.2</td>
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<tr>
<td></td>
<td>32</td>
<td>1.22</td>
<td>9.5</td>
<td>203.0</td>
<td>101.3</td>
</tr>
</tbody>
</table>

**Average**.......................... 1.01

**Expt. 84.** Dog 20, wgt. 19.4 kgm., S.A. 0.76 sq. m. Cracker meal diet 11 days. Fifteen grams each xylose and sucrose in 60 cc. water injected subcutaneously at 6:00 a.m., and again at 8:00 a.m. Seven hundred fifty cubic centimeters water by stomach at 5:55 a.m. and 500 cc. at 7:55 a.m. Period 1 began at 8:08 a.m. All blood samples drawn at middle of urine collection periods.

**Expt. 85.** Dog 31, wgt. 12 kgm., S.A. 0.54 sq. m. Cracker meal diet 22 days. Nine grams each xylose and sucrose in 36 cc. water injected subcutaneously at 6:15 a.m. and again at 7:00 a.m. Four hundred cubic centimeters water by stomach at 6:20 a.m. and 200 cc. at 7:05 a.m. Period 1 began at 7:15 a.m. All blood samples drawn at middle of urine collection periods.

**Expt. 86.** Dog 20, wgt. 19.0 kgm., S.A. 0.76 sq. m. Cracker meal diet 31 days. Thirty-two grams xylose and 24 grams in 100 cc. water injected subcutaneously at 6:05 a.m. and 8 gms. xylose and 6 grams sucrose in 20 cc. water at 6:50 a.m. No water given. Period 1 began at 7:06 a.m. All blood samples drawn at middle of urine collection periods.
such reabsorption existed then the glucose clearance under phlorizin should rise above the xylose or raffinose clearance. This conclusion would be invalid if the action of phlorizin in blocking the reabsorption of glucose were an incomplete one, but all the evidence available on this subject indicates that in sufficient doses the contrary is the case; in fact, it has been observed in this laboratory that as little as 25 mgm. of phlorizin per kilogram given intravenously suffices to bring the glucose clearance up to that of xylose for short periods of time (1 to 2 hours), and larger doses do not raise it any higher. In experiments 88, 92, 96 and 100, we have given four times this quantity intravenously and an equal quantity subcutaneously, so we believe that the reabsorption of glucose is completely blocked.

We conclude from the above facts that there is no active reabsorption of xylose or raffinose in the kidney of the normal dog. It remains possible, however, that a small quantity of sugar might be reabsorbed into the blood stream by passive diffusion from the lumen of the tubule, since during the reabsorption of water a large concentration gradient is established between the urine and the plasma. That such a passive diffusion must be slight is indicated by the fact that urea can be concentrated 250 times, and urea is more diffusible than any of the sugars; but since it is possible that urea is itself extensively reabsorbed, this evidence is hardly conclusive. Therefore, to examine this question further we have compared the excretion of xylose with sucrose in the normal dog. Sucrose is not excreted by the tubules of Lophius, as shown by Clarke and Smith (1932), in any detectable quantity; certainly less than one per cent diffuses across the renal tubules from the plasma into the urine. (The excretion of raffinose by the aglomerular kidney has not been examined.) The molecular weights of unhydrated xylose, sucrose and raffinose are 150, 342 and 504, respectively, and it is to be expected that their rates of diffusion would vary inversely as the molecular size.

5. The data given in table 3 show that xylose and sucrose are excreted by the normal kidney in an identical manner within the limits of the ex-

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1 It seems hardly necessary to discuss the view that phlorizin induces the secretion of glucose by the renal tubules. The extensive studies which have been made on the modus operandi of this drug have demonstrated to the satisfaction of most investigators that it acts by paralyzing the tubular reabsorption of glucose from the glomerular filtrate (Nash, 1927). There is conclusive evidence in Marshall's (1930) work on the fishes that the renal tubules are unable to secrete glucose and this fact, even if there were no other evidence, would lead us to believe that the tubular secretion of glucose in the mammals is a fiction. But we now have the additional evidence in our subjoined tables to the effect that the physiologically inert sugars, xylose, sucrose and raffinose, when excreted simultaneously are excreted in an identical manner (i.e., with an identical clearance) in the normal animal; a principle which is extended to glucose in the phlorizinized animal. These observations leave no doubt whatsoever that the excretion of all these sugars is effected exclusively by glomerular filtration.
experimental error in the determination of the sugars. The data given in tables 1 and 2 show that under phlorizin, glucose and xylose, on the one hand, and glucose and raffinose, on the other, are likewise excreted in an identical manner; so that except for the use of phlorizin to block the reabsorption of glucose, we may say that all these sugars are handled simultaneously by the kidney in exactly the same way. It would seem, therefore, that they must be reabsorbed to exactly the same extent, or else not reabsorbed at all. Since we are comparing substances of different molecular weights and diffusibility, the first alternative is very unlikely. This conclusion is reinforced by the observations of Clarke and Smith (1932) that the tubules of _Lophius_ do not permit xylose and sucrose to diffuse across them, whereas urea is nearly uniformly distributed between the blood and urine (Marshall and Grafflin, 1928). In the dog, on the other hand, where urea may be concentrated 250-fold it is inconceivable that the tubules would permit the passive diffusion of appreciable quantities of substances like raffinose, sucrose, xylose and glucose.

In résumé, the facts that the xylose clearance relative to the urea clearance is unaffected by phlorizin, that the glucose clearance under phlorizin does not exceed the xylose clearance or the raffinose clearance and that the xylose and the sucrose clearances in the normal animal and the glucose and xylose or glucose and raffinose clearances in the phlorizinized animal are identical (in simultaneous experiments), are interpreted to indicate that neither xylose, sucrose nor raffinose is reabsorbed by the renal tubules, and therefore, in view of the arguments set forth above, any of these substances may be used to measure the glomerular filtrate with a theoretical accuracy lying within the limits of the experimental error in the sugar analysis.

Of the three sugars examined xylose and sucrose are the most suitable for practical purposes. The accurate determination of xylose is perhaps not so easy as is the determination of sucrose, but the former possesses the advantages that it can be administered _per os_ and its chemical determination is not dependent upon the use of an enzyme.

The question of whether the administration of these sugars influences the excretory activity of the kidney with respect to other substances cannot be answered in this paper. It would seem that an inert sugar would exert as little action as any substance which might be used for the measurement of the glomerular clearance. But it is recognized that many factors, only a few of which are known at present, modify the activity of the kidney, and although we believe that these sugars have no specific stimulating or depressing action, either in respect to the excretion of urea or any other substance, the examination of this point must be postponed until certain conditions bearing on the maintenance of uniform renal activity can be discussed.
SUMMARY

It is shown that xylose is excreted by the normal dog kidney, at moderate to large urine flows, with a relatively high U/P ratio; under the action of phlorizin the glucose clearance rises to but never exceeds the xylose clearance, while the xylose clearance itself, or the xylose clearance relative to the urea clearance, remains unaffected. These facts indicate that there is no active reabsorption of xylose by the renal tubules.

The pentose, xylose, the disaccharide, sucrose, and the trisaccharide, raffinose, all of which are physiologically inert, are excreted (in simultaneous experiments) in an identical quantitative manner relative to each other or to glucose under phlorizin, indicating that there is no significant passive reabsorption (by diffusion) from the concentrated urine in the tubules.

From these and other considerations described in the paper we believe that these sugars can be used to evaluate the quantity of glomerular filtrate with an error that does not exceed a few per cent.

Methods for the analysis of mixtures of xylose, glucose and sucrose, or of glucose and raffinose in plasma and urine are described.

The xylose used in these experiments was kindly supplied by the Swann Chemical Company of Birmingham, Alabama. We are indebted to Miss Annie Breitweiser for her expert technical services in sugar analysis.

METHODS. A careful comparison of several sugar methods, tested by the recovery of added amounts of various sugars to plasma and urine, led us to choose the Folin (1929) method as yielding the best results in our hands. The Folin sugar reagent possesses the advantage that it is not reduced by phlorizin, which substance reduces ferricyanide; this fact, in our opinion, renders suspect all quantitative glucose observations on phlorizinized animals made with a ferricyanide method. The preparation of reagents and the sugar analysis were carried out precisely as described by Folin except that we have used copper filtrates as prepared by Somogyi (1931). Special blow-out pipettes were used in all measurements and great care was exercised in all details of technique.

Glucose + xylose. A portion of serum or oxalated plasma is added to 7 volumes of water in a 125 cc. Erlenmeyer flask; one volume of copper solution and one volume of sodium tungstate solution are added while spinning and the precipitated proteins are filtered out after 15 minutes using S. and S. 597 filter paper. Two cubic centimeters of this filtrate are taken for sugar analysis according to Folin (1929). Urine is diluted according to the expected sugar content, usually 1:25 or 1:50, with 0.06 per cent benzoic acid. A copper filtrate is prepared from this diluted urine as above. Glucose, xylose and sucrose will remain unchanged in the diluted urine over-night if kept in the ice-box.

Xylose. A weighed sample of yeast is made up to a 20 per cent suspension in water and washed by centrifuging until the supernatant fluid is clear (usually four times); 5 cc. of this suspension are centrifuged in a 15 cc. centrifuge tube for 15 minutes at high speed, decanted and drained for 30 minutes, when the sides of the tube are wiped dry up to the edge of the yeast. Five cubic centimeters of plasma or urine filtrate are added to the 1 cc. of dry yeast, stirred well and allowed to stand at room
temperature for 15 minutes with occasional agitation. The mixture is then centrifu-
ged for 15 minutes to throw down the yeast and 2 cc. of the supernatant fluid are
used for sugar analysis. (After Somogyi, 1928, and Van Slyke and Hawkins, 1929.)

We find that xylose is not actively absorbed by yeast, but that a small quantity
(depending upon the proportion of yeast and filtrate) disappears each time during
successive yeast treatments. This fraction is about 13 per cent when the propor-
tions of 1 cc. of dry yeast to 5 cc. of fluid are used. This absorption probably represents a
moiety which diffuses into the yeast. (Cf. also Van Slyke and Hawkins, 1929, who
found partial absorption of non-fermentable substances.) In this respect we do
not confirm Raymond and Blanco (1928) who obtained complete recovery of xylose
after yeast treatment and who make no mention of passive diffusion. The reducing
power of pure xylose by the Folin sugar method in our hands is 85 per cent of that of
glucose in contrast to 78 per cent obtained by Poe and Klemme (1930); consequently
after yeast treatment as above we recover 72 per cent of added xylose. This low
recovery is theoretically and actually constant and can be neglected in the calcula-
tion of U/P ratios when the plasma and urine filtrates are treated in exactly the same
manner. Consequently we do not make any correction in our data for reducing
power of xylose, but present only the observed glucose equivalent.

In calculating the true glucose, however, allowance must be made for the diffusion
of xylose into the yeast; therefore we calculate the glucose content of both plasma
and urine as glucose + apparent xylose—apparent xylose/0.87.

Sucrose. The total sugar content of plasma and urine filtrates prepared as above,
and containing glucose, xylose and sucrose was determined by inversion with sucrase.
We are indebted to Mr. Milton Levy, of the Department of Biochemistry, for the
sucrase used in these experiments. The enzyme was prepared from yeast by Hud-
son's method (cf. Morrow, 1927); we use a 0.26 per cent solution of a preparation 50
mgm. of which reduces the rotation of 20 cc. of 20 per cent sucrose solution to zero
degrees in twelve minutes.

We find that sucrose is quantitatively absorbed by yeast, either the small tin-foil
packages or baker's loaves, almost instantly. One cubic centimeter of yeast com-
pletely removes 40 mgm. from 5 cc. of solution (corresponding to 800 mgm. per cent
in our method) in less than 5 minutes. In this observation we do not confirm Ray-
mond and Blanco (1928) who observed only partial absorption, possibly because the
yeast which they used was not fresh. (Cf. also Ronzoni and Somogyi, 1929.)

One cubic centimeter of plasma or urine filtrate is placed in a Folin sugar tube with
one drop of 0.04 per cent bromocresol green, and 0.05 N acetic acid is added drop by
drop until the color indicates a pH between 4.0 and 4.6. One cubic centimeter of
sucrase is added and the mixture is agitated and allowed to stand in a water bath
at 40°C. for 30 minutes or more after which it is neutralized with 0.05 N NaOH.
One drop of phenolphthalein is added and the mixture is made alkaline with one
per cent Na₂CO₃ as in Folin's sugar method. The standards are treated by adding
the bromocresol green, acetic acid and NaOH. 2.0 cc. of Folin reagent are then
added and the routine sugar analysis completed. Blanks must be made on the
sucrase solution by adding it to known glucose solutions.

Sucrose is calculated as the difference between the glucose equivalents before and
after inversion. The glucose equivalent of pure sucrose solutions treated as above
is 105 per cent of glucose, and we obtain recoveries of added amounts (100 to 300 mgm.
per cent) from plasma and urine with an error of ± 2 per cent. In the Folin method
the reducing power of glucose is not impaired by the presence of sucrose in approxi-
mately equal concentrations, and since sucrose is completely removed by yeast (cf.
above) it offers no interference to the determination of xylose.

Raffinose. Raffinose (a non-reducing trisaccharide) is hydrolyzed by sucrase to
fructose and melibiose. Both sugars reduce Folin's Cu reagent, but the latter is
not absorbed on yeast. By the above method we find the reducing power of hydrolyzed raffinose at concentrations of 100 to 200 mgm. per cent to be 60 per cent of glucose. Since meiibiose is not absorbed by yeast, it is not possible to determine xylose in the presence of raffinose by sucrase hydrolysis, so we have compared the excretion of raffinose with glucose under phlorizin, the analyses being made in the same manner as in the case of sucrose. Our data express glucose equivalents of raffinose as calculated from the difference between the glucose equivalents of solutions before and after hydrolysis.

When present in approximately equal concentrations raffinose does not interfere with the quantitative determination of glucose.

All plasma and urine filtrates are prepared in duplicate and these duplicates are analyzed separately with separate standards. All analyses in which the unknown differs from the standard by more than 20 per cent are repeated, using a more appropriate standard. No standard corresponding to more than 200 mgm. per cent or less than 50 mgm. per cent was used.

Urea. Urea in both plasma and urine was analyzed by Van Slyke's (1927) plasma method, the plasma and urine analyses being alternated to prevent poisoning of the urease in the extraction chamber. One or 2 cc. samples of plasma and 1 cc. of diluted urine were used. All analyses were run in duplicate for 3 and 6 minutes, the variation in time serving to check the activity of the urease. The agreement between checks, even at concentrations of 6 mgm. per cent is usually better than 2 per cent.

General. Except as noted the general technique is similar to that used by Jolliffe and Smith (1931a, b). The dogs were maintained upon a cracker-meal and butter or lard diet. The greatest care was exercised in catheterizing to insure complete removal of urine from the bladder. In the present experiments the sugars were injected subcutaneously some time before the first urine collection period; injection is not necessary in the case of xylose which may be given by stomach, but sucrose and raffinose must obviously be administered parenterally. The essential details of each experiment are recorded in the tables.

Note on the filterable nature of plasma glucose (cf. p. 308): After submitting this paper we noted the work of Powers and Greene (1931) showing that all the plasma glucose is filterable by in vivo dialysis.

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