THE MEASUREMENT OF GLUCOSE Tm IN THE NORMAL DOG

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A cellular limitation in the renal tubular reabsorption of glucose is manifested by the inability to transfer more than a certain maximal quantity per unit time. For convenience we term this quantity glucose Tm and express it in milligrams per minute. In the normal, well hydrated dog, glucose reabsorption remains essentially complete with successive increments in plasma concentration until the filtered load1 approximates glucose Tm. Further elevation of plasma glucose results in abrupt glycuresis and the quantitative excretion of the excess glucose. Under these circumstances glucose excretion is equal to the difference between the filtered load and glucose Tm (1). The usefulness of a Tm measurement in physiological investigations depends to a large extent upon its stability and reproducibility. This report presents an examination of the glucose Tm with specific reference to these qualities.

Experimental Procedure. Seven healthy, well-trained female dogs were used. In general the experimental procedure was the same as previously described (1). The plasma level was quite constant during any series of observations except in those which specifically examined the effect of a changing plasma level. As a routine the absolute plasma concentration was sufficiently high that the filtration load was at least 1.5 times glucose Tm. The hydration of the animal was assured by the preliminary administration of 50 ml. of water per kilogram. This was an adequate fluid reserve in those experiments where the urine output exceeded the infusion rate. The experiments which simply evaluated glucose Tm followed the routine of the first three periods of the experiment shown in table 1.

Glomerular filtration rate was assumed to be equal to the plasma creatinine clearance. Tubular reabsorption of glucose was calculated as the difference between the glucose load and its concurrent rate of excretion. A correction was made for renal dead space if there was a rapid change in plasma concentration. This was measured in our animals by determining

1 In milligrams per minute this is equal to the product of the rate of glomerular filtration (ml. per min.) and the plasma glucose concentration (mg. per ml.).
the amount of clear urine excreted following the intravenous injection of 10 ml. of concentrated cyanol solution. The dead space was roughly proportional to urine flow over a considerable range and was quite close to the amount of urine formed in two minutes (mean = 2.23 min.).

**Experimental results.** The influence of changing plasma glucose concentration on its renal tubular reabsorption. In a series of 13 experiments glucose Tm was measured in three periods at a constant elevated plasma glucose concentration; the glucose in the infusion fluid was then removed or decreased in concentration and additional observations made as the

**Table 1**

An experiment which examines the effect of falling plasma glucose concentration on the renal tubular reabsorption of glucose in the normal dog.

6/14/37; dog G:

<table>
<thead>
<tr>
<th>Period Number</th>
<th>Concurrent Time</th>
<th>Urine Flow</th>
<th>Plasma Level</th>
<th>Creatinine Clearance</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>ml. per min.</td>
<td>Creatinine</td>
<td>Glucose</td>
<td>Filtered</td>
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<tr>
<td>1</td>
<td>85–94</td>
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<td>35.2</td>
<td>666</td>
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</tr>
<tr>
<td>2</td>
<td>–104.5</td>
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<td>33.8</td>
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<td>77.6</td>
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<td>467</td>
<td>82.2</td>
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<td>31.6</td>
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<td>–165</td>
<td>1.3</td>
<td>31.6</td>
<td>175</td>
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</table>

*Less than 0.5 mgm. per minute.*

plasma concentration was falling (table 1, fig. 1). Glucose Tm was taken as the mean of the three initial observations. Reabsorption in the subsequent periods was calculated as the ratio of the observed rate to glucose Tm (i.e., T/Tm). In figure 1 these ratios are plotted against the filtration load for the period, also expressed in terms of Tm (i.e., load/Tm). In other experiments, observations on a rising plasma glucose were contrasted to subsequent periods when the plasma concentration was falling. In still others, observations on a rising curve were compared to subsequent periods at the high plasma concentration.
The experiments of the first group (fig. 1) are the only ones which suggest that glucose reabsorption may be conditioned by the direction and rate of change in the plasma glucose. Even in this group the effect is neither constant nor extensive. The ratio $T/Tm$ on a falling plasma glucose is significantly below 1.0 in a few experiments but the mean of the entire group is 0.941 ($\sigma = \pm 0.070$, 37 observations) and there is extensive overlapping between these and the control observations ($1.00 \pm 0.53$, 36 observations). This is an uncertain demonstration that a rapidly falling blood glucose level influences reabsorption. It is our belief that the mechanical factor of renal dead space is quantitatively more important than this. The latter makes it difficult to obtain a plasma concentration which is representative of the period. However, the necessity for a constant plasma glucose in the measurement of $Tm$ is derived from both factors.

The influence of excess insulin on glucose reabsorption. This was observed in six experiments. In each of these, a series of three control periods was obtained at an elevated plasma glucose level, 50 units of insulin were administered intravenously, and after an interval varying from 0 to 50 minutes a second series of observations was made. A summary of these

Fig. 1. The effect of rapidly falling plasma concentrations of glucose on its renal tubular reabsorption.

The control periods which serve as the standard of reference in each experiment are not shown. Each dot represents the ratio observed in a single experimental period. Periods in which the ratio load/$Tm$ spans the value of 1.0 have been omitted.

Fig. 2. Showing the lack of correlation between glomerular filtration rate and glucose $Tm$.

Each point represents an experiment of three or more periods. The mean glomerular filtration rate and glucose $Tm$ has been calculated for each animal. The values for each experiment have been plotted as the fractions of these means. The symbols used to represent each of the animals are as follows: A, dots; H, circles; B, open triangles; G, filled triangles; CT, half filled squares, C, half filled circles; P, crossed circles.

* The rate of change of plasma glucose in most of these experiments was comparable to that shown in table 1.
GLUCOSE Tm

755

experiments is given in table 2. A depression of 10 per cent or more in the reabsorption of glucose was demonstrated in 4 of 6 experiments.

The influence of adrenaline on glucose reabsorption. We have not examined this in the dog, since there is an adequate series of observations on man (2). These experiments were similar in design to the insulin experiments. There was no change in glucose Tm which could be related to the influence of adrenaline on the transport system.

The reproducibility of glucose Tm. The measurements of glucose Tm in a series of normal dogs subjected to repeated examination are given in table 3. These are sufficiently numerous in 5 dogs to define the variability which may be expected over a period of months. No precautions were taken to control the diet, nutritional status or general activity of the

<table>
<thead>
<tr>
<th>DOG NUMBER</th>
<th>BEFORE INSULIN</th>
<th>AFTER 50 UNITS INSULIN INTRAVENOUSLY</th>
<th>RATIO, GLUCOSE REABSORPTION AFTER INSULIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine clearance</td>
<td>Plasma glucose</td>
<td>Glucose Tm</td>
</tr>
<tr>
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<td>Number of periods</td>
<td>ml. per min.</td>
<td>mgm. per cent</td>
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<td>3</td>
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<td>48.4</td>
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<td>H</td>
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<tr>
<td>B</td>
<td>3</td>
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animals except in the case of dog C. The protein content of the diet was varied considerably in this animal with no effect upon glucose Tm (general mixed diet 5/1/37 to 5/19/37; high protein diet 5/19/37 to 5/28/37; low protein diet 5/29/37 to 6/9/37; general mixed diet 6/10/37 to 10/26/37, see 3 for composition of diets). We did not observe the changes in glomerular filtration rate which usually result from such dietary changes (3, 4, 5). However the maintenance of a high filtration rate may be attributed to the generous use of intravenous fluids.

Dog P died two days after the last experimental observation. At the time of this experiment (6/11/37) he gave no evidence of distress. The lowering of glucose Tm in this experiment may be a true expression of variability in the normal animal; however, we believe that this experiment should be withheld from inclusion in the general consideration of the data.
TABLE 3

Demonstrating the reproducibility of glucose Tm in the normal dog when repeated examination is made over a period of months

<table>
<thead>
<tr>
<th>DOG</th>
<th>DATE</th>
<th>NUMBER OF PERIODS</th>
<th>NUMEROSIS FILTERATION RATE</th>
<th>GLUCOSE LOAD Tm</th>
<th>GLUCOSE Tm</th>
<th>GLUCOSE Tm OBSERVED Tm MEAN</th>
<th>GLUCOSE Tm FILTERATE PER MOM. Tm</th>
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<tr>
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<td>206</td>
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<td>0.374</td>
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<td>0.94</td>
<td>0.247</td>
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<tr>
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<td>1.99</td>
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<td>0.98</td>
<td>0.438</td>
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The reproducibility of glucose Tm in any one animal is quite striking. The variation is more than ±10 per cent of the mean for any animal in only 3 of 35 observations. This consistency is quite impressive since the measurement itself has an error in the order of magnitude of ±5.0 per cent. The mean glucose Tm derived from any three consecutive experiments would be quite adequate as a standard of reference for the study of subsequent change and a difference of 10 per cent or more would have significance if it were capable of consistent reproduction. The independence of glomerular filtration rate and glucose Tm previously noted in a limited series of observations (1) is clearly evident in the present data (fig. 2).

DISCUSSION. Relatively few precautions need be observed in measuring glucose Tm in the dog because of the stability which characterizes the system. The insulin experiments suggest that an extensive change in cellular metabolism may be a factor in conditioning the transport mechanism. However, the absence of any change when adrenaline is administered or when the dietary regime is extensively varied minimizes the practical significance of this. The constancy and the absolute level of plasma glucose are obviously important. Rapid changes in plasma concentration should be avoided if only to prevent an error due to renal dead space. An additional reason lies in the possibility that such changes may in themselves affect the activities of the system. Any absolute concentration which results in frank glycurcisis will be adequate to saturate all the nephrons in the normal, well hydrated dog since further elevation does not result in any increase in glucose reabsorption (1). When the measurement is applied to abnormal material higher plasma concentrations are advisable since this relationship between glomerular and tubular function may be altered (2).

4 This is an interesting finding since there is marked variability in the length of the proximal tubules in the dog (10). One may infer from this that there must be proportionate variation in glomerular development. If this were not so the precise balancing of tubular and glomerular function in the individual nephrons which contribute to total renal function could not occur.
The reproducibility of the glucose T_m is sufficient that it may be accepted as an excellent method for the characterization of the kidney. It is a quantitative functional measurement of the tubular tissue available for glucose reabsorption (presumably proximal) under the conditions of the experimental routine; hence the tissue with operating glomeruli (6, 7). It is to be stressed however, that the expansion of plasma volume which accompanies the maintenance of an elevated plasma glucose may bring into action glomeruli which otherwise would remain closed.

The relatively constant glucose T_m, despite variations in glomerular filtration rate, is in keeping with the histological evidence that essentially all the nephrons are continuously active in the normal dog (8). The absence of an increase in glucose reabsorption with progressive elevation of plasma glucose is also in favor of this interpretation. It seems likely that if there were a considerable number of inactive nephrons at moderate plasma levels some of these would be serviced by blood when the circulatory system is expanded by the high infusion rates necessary to produce severe hyperglycemia (1). In this view, variations in glomerular filtration rate such as we have observed are due to changes in the filtration pressure of the entire glomerular bed rather than to changes in the number of active nephrons. It may be possible that under certain circumstances nephrons can be withdrawn from activity as seems to be true in the avian kidney (9) and that some of the larger variations in our data may be the result of this. However, large variations in glucose T_m were too infrequent in our data to make this a safe conclusion at the present time.

SUMMARY AND CONCLUSIONS

1. The glucose reabsorptive system, as evaluated by glucose T_m, has considerable stability in the normal dog. Although we have observed

<table>
<thead>
<tr>
<th>DOG</th>
<th>GLUCOSE T_m</th>
<th>BODY WEIGHT</th>
<th>SURFACE AREA</th>
<th>KIDNEY WEIGHT</th>
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<tr>
<td></td>
<td>mgm, per min.</td>
<td>kgm.</td>
<td>sq.m.</td>
<td>grams</td>
</tr>
<tr>
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<td>100</td>
</tr>
<tr>
<td>H</td>
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<td>17.0</td>
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<tr>
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<td>1.03</td>
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<tr>
<td>P</td>
<td>340</td>
<td>26.0</td>
<td>1.04</td>
<td></td>
</tr>
</tbody>
</table>

* When first examined.
some depression when the plasma glucose falls precipitously, this is slight and inconstant. Adrenaline has no effect upon glucose transport and one must use excessive doses of insulin to produce a significant depression and then it is not a constant phenomenon.

2. For the valid measurement of glucose $Tm$ it is essential to work at a constant plasma level of adequate absolute concentration and to provide for the adequate hydration of the animal. It seems unlikely that close control must be maintained over other variables.

3. The reproducibility of glucose $Tm$ measured under these conditions is excellent. It is recommended as a dependable means for the quantitative characterization of tubular (proximal) reabsorptive function.

4. Contrary to the constancy of glucose $Tm$ the rate of glomerular filtration varied widely in these experiments. This feature of the data and other considerations indicate that under the conditions of our experiments essentially all the glomeruli are functioning and variations in filtration rate are the result of changes in the filtration pressure of all glomeruli rather than in the number of active nephrons.

APPENDIX. Chemical methods. The handling of the samples and the precautions taken in the analyses were the same as in our previous report (1). The chemical methods used in the experiments on dogs C, G and P were also the same. For the remaining dogs these were as follows: Plasma filtrates were prepared in 1:10 dilution using the ferric sulfate-barium carbonate method of Steiner, Urban and West (11). Excess barium in the filtrate was removed by the addition of minimal quantities of sulfuric acid, CO$_2$ was then shaken off and the pH adjusted to 7.0 with phenol red and sodium hydroxide. The urines if not contaminated by protein were not precipitated. Creatinine was determined by a modification (4) of Folin's alkaline picrate method. The optical density of each sample was determined exactly 10 minutes after the addition of the alkaline picrate. True glucose was determined as the difference between the reducing power of the samples before and after absorption on yeast (12) using the method outlined below. All colorimetric determinations were made with the Evelyn photoelectric colorimeter.

The Folin (13) sugar method proved to be unsatisfactory for adaptation to the photoelectric colorimeter. The reagent deteriorates rapidly, as evidenced by a progressive change in the slope of the standardization curve and there is a fairly rapid change in optical density after the addition of the acid phosphomolybdate and subsequent dilution. Furthermore the line relating optical density to glucose concentration does not extrapolate to 100 per cent transmission at zero glucose concentration.

Our modifications circumvent these difficulties to a large extent and introduce flexibility with respect to glucose concentration. In its present form, however, the method is not recommended for general application. Its most serious fault is its sensitivity to a change in the carbonate: bicarbonate ratio, a characteristic of copper reagents of this general type. For this reason it is essential that plasma proteins are precipitated by a method which yields a filtrate essentially neutral and with no significant buffer capacity. The method described above satisfies these requirements.
Solutions. The alkaline copper solution has the following composition:

Reagent I

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium carbonate (Na₂CO₃·H₂O)</td>
<td>15.0</td>
</tr>
<tr>
<td>Sodium tartrate</td>
<td>16.0</td>
</tr>
<tr>
<td>Copper sulfate (CuSO₄·5H₂O)</td>
<td>5.0 (50 ml. 10 per cent solution)</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>10.0</td>
</tr>
<tr>
<td>Water to 1.0 liter</td>
<td></td>
</tr>
</tbody>
</table>

This is prepared as directed by Folin except that the copper sulfate is added at the time the tartrate reagent is made. Some reduced copper usually precipitates in the first few days, but this may be disregarded if care is exercised when removing the supernatant fluid. The reagent is quite stable despite slow changes in the boiled blanks. Standardization curves are reproducible for a period of 5 or 6 months, which is as long as we have observed any one sample of reagent.

The acid molybdate solution is made up as directed by Folin except that the strength of sulfuric acid is increased by 30 per cent. This tends to stabilize the reduced phosphomolybdate color, although the degree of stability varies to some extent with different lots of reagent. The drift in optical density is generally less than two per cent in the interval of 20 and 30 minutes after the addition of the dilute phosphomolybdate reagent. We have routinely read our determinations from 20 minutes onwards.

The determinations are carried out as directed by Folin with the following exceptions. There is no preliminary adjustment of the pH of the test solution in the sugar tube prior to the addition of the copper reagent, the boiling time is taken as 10 minutes, and before reading the samples are permitted to stand for 20 minutes after mixing with the dilute acid phosphomolybdate. With each set of sugar determinations three boiled blanks (i.e., water and copper solution) are included and 100 per cent transmission taken from the tube most representative of the three. The variability encountered in the blanks enters as a serious difficulty only in the lower ranges of concentration (i.e., 2.5 mgm. per cent or lower).

Standardization curves are constructed as usual with aqueous solutions of glucose using the no. 635 filter for low concentrations (1.0–5.0 mgm. per cent) and the no. 540 filter for the higher ranges (2.5–15 mgm. per cent). The absorption curve of the reduced phosphomolybdate is such that no error results from the use of a wave length quite removed from the absorption maximum. Recoveries of glucose added in known amounts to plasma or plasma filtrates and submitted to our procedures were usually well within ±2.0 per cent of the theoretical. Reproducibility in practice was also within these limits. When proper care is taken in calculating dilutions, all samples can be read with the no. 540 filter.

REFERENCES

(1) SHANNON, J. A. AND S. FISHER. This Journal 132: 765, 1938.
GLUCOSE Tm

(8) White, H. L. This Journal 128: 159, 1939.