CXVII. ON THE MECHANISM OF SUGAR EXCRETION.

I. GLUCOSE.

BY TSANG-G. NI AND POUL BRANDT REHBERG.

From the Laboratory of Zoophysiology, University of Copenhagen.

(Received July 2nd, 1930.)

WHEN the blood-sugar is raised and sugar is excreted in the urine, this excretion is, according to the secretion theory, due to a stimulation of the tubule cells by the increased sugar percentage. In answer to this stimulation the tubule cells secrete sugar into the urine. The filtration-reabsorption theory, on the other hand, holds that sugar normally filters out through the glomeruli, but that all that is filtered is, under normal conditions, reabsorbed. When the percentage of sugar in the blood is raised, the tubule cells fail to reabsorb all; some of the sugar is left in the urine and is excreted.

It was part of our plan to use the experiments reported in this paper to contribute to an answer as to which of the two theories is right. Our viewpoint was that it would perhaps be possible to demonstrate that the kidney during prolonged sugar-excretion became fatigued. If so, the result of a fatigued secretion should be different from that of a fatigued reabsorption. If the mechanism of sugar-excretion is a secretion by the tubule cells and if the cells responsible for the secretion could become fatigued, one might expect that the excretion of sugar after repeated injections of sugar would diminish. If, on the other hand, sugar is excreted as postulated by the filtration-reabsorption theory the cells which could become fatigued would be those responsible for the reabsorption. Fatigue should show itself in failure of reabsorption, that is, increased excretion after the second injection.

While our experiments cannot contribute to this part of the problem because—as will be shown later—no definite difference could be demonstrated in the sugar-excretion after repeated sugar injections, experiments have been made by another author which answer the question.

Poulsøn [1930] has made a series of experiments in which he studies phloridzin-glycosuria by means of the creatinine method [Rehberg, 1926]. The result of the experiments is, briefly, that the amount of sugar excreted in a completely phloridzinised dog corresponds closely to the amount which should be excreted according to the calculations of filtrate made by the creatinine method.

The amount of blood cleared of sugar is for each minute nearly the same as the amount cleared of creatinine.

While this fact is in complete accordance with the filtration-reabsorption theory and only needs the assumption that phloridzin abolishes the sugar reabsorption, it is hard to explain on the basis of the secretion theory. It makes it necessary to assume that under phloridzin poisoning the tubules begin to secrete sugar and moreover do so at exactly the same rate as that at which they secrete creatinine—an assumption which is possible but certainly not likely.

We think that the experiments of Poulsson together with what is further known about the excretion of sugar—especially the presence of sugar in the glomerular filtrate and its absence from the urine of the frog [Richards, 1929]—practically proves that sugar is actually filtered and not secreted, a fact which once accepted leads of necessity to the conclusion that the whole theory is in the main right.

The other part of our programme was to study how the glycosuria arising out of a too high sugar level in the blood should be looked upon according to the filtration-reabsorption theory, if this was once accepted. Our experiments may be regarded as a supplement to those of Poulsson, which were concerned with the mechanism of phloridzin glycosuria, while ours are concerned with the mechanism of normal glycosuria.

The explanation offered for glycosuria in the normal state is that any excess above the normal percentage present in the glomerular filtrate fails to be taken up by the tubule cells and passes into the urine, but how we are to look upon this failure is not clear.

Different possibilities can be conceived. (1) A regulation might exist, whereby the reabsorption of sugar was either stopped completely or decreased, when the blood-sugar was increased above a certain level. (2) The reabsorption could go on at unchanged rate, thus leaving the excess of sugar to be excreted. (3) The reabsorption could increase with the blood-sugar, but fail to follow the increase completely and in this way leave some sugar to be excreted.

To ascertain which of these possibilities was responsible for the excretion of sugar was the further aim of our experiments.

The plan of our experiments was to use the creatinine excretion as an index for the filtration rate [Rehberg, 1926].

That creatinine really is an indicator of the filtration rate is made highly probable by the following facts.

(1) Creatinine is the substance which is concentrated most by the kidney. In man the concentration ratio $\left(\frac{\text{percentage in urine}}{\text{percentage in blood}} \right)$ can reach 400. More blood is cleared of creatinine than of any other substance.

(2) Creatinine excretion is independent of urine volume, which proves that nothing diffuses back or is reabsorbed in the tubules.

(3) Creatinine excretion seems to be dependent on the osmotic pressure of the blood colloids in such a way that the excretion decreases with increasing osmotic pressure of the blood colloids [Ni and Rehberg, 1930]. This is easily

understood if creatinine is excreted by a filtration process but hard to explain from the secretion standpoint.

We therefore think it justifiable to postulate that the amount of filtrate formed in the glomeruli per minute must be the same as the amount of blood cleared of creatinine per minute, and from this view-point our experiments have been planned.

The experiments were made on a dog in which ureter-fistulae had been made after the method of Orbeli¹. The operation was made in September and the dog recovered well from it and was in good health during the experiments. It was well trained and accustomed to the experimental conditions.

The experiments were made according to the following plan. The dog was fed in the afternoon and received no food from the evening before an experiment, but was allowed plenty of water for the night. At the beginning of an experiment an intravenous injection of a varying amount of glucose was given together with usually 1 g. of creatinine. The glucose used was dissolved the day before, made alkaline and kept for about 18 hours at 20°. Before injection it was neutralised. The amount of water in which the sugar was dissolved was varied. About 5 minutes after the injection the collection of urine was started—the urine from both ureters being collected together through a common funnel fastened over the ureter openings. Urine was collected in periods, the lengths of which were varied according to the magnitude of the diuresis.

At intervals during the experiment blood was drawn from the saphenous vein. The blood was kept from clotting by a small amount of lithium oxalate. Both urine and blood samples were cooled down immediately after taking and the blood was kept on ice until centrifuging took place.

Plasma was used for the determinations of sugar and creatinine.

For the calculation of the rate of the glomerular filtration and the estimation of creatinine in the samples of plasma and of urine, the method described in another paper [Rehberg, 1929] was followed. Control experiments showed that glucose added to urine up to 1200 mg. per 100 cc. did not change appreciably the creatinine readings taken between 5 to 100 minutes after the addition of sodium hydroxide. The urine was therefore diluted so that the sugar concentration was less than 1 % and the readings made as soon as possible.

Urinary glucose was estimated by polarisation. The polariscope² was a Schmidt and Haensch instrument which could be read to 0.01°. The urine was acidified with acetic acid and cleared by grinding with lead acetate and filtering.

The error caused by this treatment was found to be within the sensitivity of the polariscope. Tubes of 2 dm. length were used. Control experiments showed that fasting urine under various degrees of diuresis did not show any

¹ The operation was kindly performed by Prof. Orbeli himself during a visit to our laboratory and we hereby beg him to accept our best thanks for his assistance.

² We take pleasure here to express our thanks to Prof. Ege of the Biochemical Institute for the privilege of using this fine polariscope.

rotation. As a precaution a zero reading with distilled water was taken each time.

Plasma-glucose was determined by the technique of Hagedorn and Jensen [Hagedorn, 1921]¹. When the concentration was high, the same amount of undiluted plasma was always used. After precipitation of the protein, the filtrate was divided into three parts and the sugar determined in each part separately, so that there was no danger of being out of range of the reagents. This procedure was found to be more accurate than diluting the plasma.

As an example of an experiment the following may serve.

The first injection consisting of 50 g. glucose and 0.8 g. creatinine in 500 cc. of water was ended at 10 h. 13. Blood was drawn at 10 h. 20, 10 h. 28½ and 10 h. 37. Urine samples were collected for the following periods: (1) 10 h. 17–10 h. 22, (2) 10 h. 22–10 h. 27, and (3) 10 h. 27–10 h. 39.

A second injection consisting of 50 g. glucose in 1000 cc. of water was ended at 11 h. 11.

Blood samples were drawn at 11 h. 22, 11 h. 35 and 11 h. 42. Urine samples were collected at (4) 11 h. 16–11 h. 20, (5) 11 h. 20–11 h. 25, (6) 11 h. 25–11 h. 30, (7) 11 h. 30–11 h. 35, and (8) 11 h. 37–11 h. 45.

The results of the analyses are given in the tables.

Urine samples	Amount (cc.)	cc. per min.	Creatinine (mg. per 100 cc.)	Glucose (mg. per 100 cc.)	Creatinine (mg. per min.)	Glucose (mg. per min.)
1	37.0	7.4	94.0	2500	6.96	186.0
2	10.0	2.0	236.0	3890	4.72	78.0
3	11.0	0.92	340.0	826	3.13	7.6
4	48.0	12.0	30.0	1420	3.60	170.0
5	38.0	7.6	35.8	1000	2.72	76.0
6	38.0	7.6	35.6	144	2.70	10.8
7	32.5	6.5	32.3	30	2.10	2.0
8	6.0	0.75	Lost	0	—	0

Blood samples	Creatinine (mg. per 100 cc.)	Glucose (mg. per 100 cc.)
I	5.0	592
II	3.6	324
III	2.7	225
IV	2.3	447
V	2.0	267
VI	Lost	156

The values of blood-creatinine and -glucose were then plotted in curves and from these curves the mean values for the various urine periods were read off. These values are used in the calculations, an example of which is given.

For the urine period (1) the excretion of creatinine was 6.96 mg. per min. For the same period the blood-creatinine averaged 5.08, so that in all 137 cc. of blood were cleared of creatinine per min. This amount is, according to the filtration theory, equal to the amount of filtrate formed in the glomeruli. For the same period the mean blood-sugar was 608 mg. per 100 cc., which means that 137 cc. filtrate contained 832 mg. glucose. 187 mg. were excreted

¹ We take pleasure to record our best thanks to Miss W. W. Smith for the determination of glucose in plasma.

and 645 mg. had thus been reabsorbed together with an amount of fluid which was $137 - 7.4$ (the urine voided per min.) = 129.6 cc. The content of glucose in the reabsorbed fluid was $\frac{645 \times 100}{129.6} = 498$ mg. per 100 cc.

Calculations like this lead to the following table:

For urine period	Average creatinine (mg. per 100 cc.)	Average glucose (mg. per 100 cc.)	Filtration (cc. per min.)	Filtered sugar (mg. per min.)	Re-absorbed sugar (mg. per min.)	Re-absorbed fluid (cc. per min.)	mg. per 100 cc. of glucose in re-absorbed fluid
1	5.08	608	137.0	832	646.0	129.6	498
2	4.26	450	111.0	498	420.0	109.0	385
3	3.12	172	100.0	172	164.4	98.2	168
4	2.39	502	151.0	757	587.0	139.0	422
5	2.29	440	119.0	523	447.0	111.4	322
6	2.17	371	124.5	461	450.0	116.9	386
7	2.06	302	102.0	308	306.0	101.3	302
8	1.86	172	—	—	—	—	—

$$\text{Filtration} = \frac{\text{mg. creatinine per min.}}{\text{mg. creatinine per 100 cc. blood}} \times 100.$$

$$\text{Filtered sugar} = \frac{\text{filtration} \times \text{blood-sugar}}{100}.$$

$$\text{Reabsorbed sugar} = \text{filtered sugar} - \text{urine-sugar}.$$

$$\text{Percentage of glucose in reabsorbed fluid} = \frac{\text{reabsorbed sugar}}{\text{reabsorbed fluid}} \times 100.$$

The results of a series of experiments of this kind are plotted in the figures and the results are the following.

(1) No difference can be shown between sugar excretion after one sugar injection and a second following shortly after the first. As seen in Fig. 1 the

Fig. 1. Sugar excretion at various blood-sugar levels.
 • = First injections. × = Second injections.

crosses representing sugar excretions observed after the second injection fall well in the middle of all the other points representing excretion after the first (or after a single) injection. Calculations of the amount of sugar which was reabsorbed at the various blood-sugar levels after the first and second injection

also failed to show any constant difference and we therefore must admit that the experiments do not show anything which can be explained as fatigue of either a secreting or a reabsorbing mechanism.

(2) The content of glucose in the reabsorbed fluid varies much. In the example given it varies between 168 mg. and 498 mg. per 100 cc., but even greater variations have been observed.

(3) The reabsorption of glucose goes on even when the blood-sugar is far above the normal. No regulation exists which stops the reabsorption.

(4) The reabsorption is not decreased with increasing blood-sugar.

(5) Sugar reabsorption thus goes on all the time, but the increase is less and less as the blood-sugar gets higher.

The higher the amount of filtered sugar, the smaller is the percentage of it which is reabsorbed. Up to an amount of 200 mg. per min. practically all

Fig. 2. The percentage of filtered sugar which is reabsorbed at various filtration rates.

is reabsorbed, but when about 500 mg. is filtered per min. only 75 % is reabsorbed and at a filtration of 1500 mg. per min. the reabsorbed amount is less than 50 % of the filtered.

We can next raise the question: what is the factor which limits the reabsorption?

Several factors perhaps work together. Possibly the amount of sugar which the tubule cells can transport per min. is limited, but it might also be possible that a maximum concentration difference between blood and urine could act as a limiting factor.

At a first glance it will perhaps seem contradictory to say so, as the concentration difference existing between urine and blood is such that the reabsorption ought to be favoured, because the concentration fall is from urine to blood. But if one tries to imagine how the reabsorption of sugar most likely is brought about, the possibility exists.

Experiments by White and Schmidt [1925] seem to indicate that the sugar is reabsorbed very soon after leaving the glomerular capsule. It is therefore conceivable and even probable that sugar is reabsorbed here until the concentration difference between blood and urine becomes too high. Some sugar

is left in the filtrate and this is then concentrated by reabsorption of water. The difficulty in trying to demonstrate this is that of course some water is reabsorbed together with the sugar. But as it is impossible to obtain any idea of how much this is, the only thing we can do is to calculate the percentage of sugar which would have been in the filtrate after reabsorption of the sugar if no water had been reabsorbed. This is done by the following formula :

$$\frac{\text{mg. urine-sugar per min.}}{\text{cc. filtrate per min.}} \times 100.$$

Example. In the experiment quoted the filtration is for the first period 137 cc. per min., and 832 mg. sugar are, according to the calculations, filtered and 645 mg. are reabsorbed, while 187 mg. are excreted. If we calculate the percentage of sugar in the filtrate after the reabsorption of sugar has taken place and assume that no water was reabsorbed we find $\frac{187}{137} \times 100 = 136$ mg. per 100 cc. In the same period the mean blood-sugar was 608 mg. per 100 cc.

Fig. 3. The difference (calculated) in sugar concentration between blood and urine after reabsorption of sugar.

The concentration difference between blood and filtrate on this assumption was therefore $608 - 136 = 472$ mg. per 100 cc.

The results of calculations like this are shown in Fig. 3, which may perhaps be explained in the following way. As the blood-sugar increases, the reabsorption increases at the same time, but the tubule cells are not able to reabsorb sugar against a concentration difference higher than 600–700 mg. sugar per 100 cc.

It thus seems possible that one of the factors determining the sugar reabsorption may be the concentration difference between blood and urine at the place of reabsorption.

SUMMARY.

1. The mechanism of glycosuria due to an increased blood-sugar level is discussed.
2. The results of a series of experiments bearing on this question are given graphically.
3. The experiments show that, according to the filtration-reabsorption

theory, the mechanism of glycosuria must be the failure of the reabsorption to increase as fast as the filtration of sugar increases with rising blood-sugar.

4. The percentage of sugar in the reabsorbed fluid varies within wide limits.

5. As a factor limiting the reabsorption of sugar we have probably the concentration difference between urine and blood at the site of reabsorption.

REFERENCES.

- Hagedorn (1921). *Dissertation* (Copenhagen).
Ni and Rehberg (1930). (To appear in *Amer. J. Physiol.*)
Poulsen (1930). *J. Physiol.* **69**, 411.
Rehberg (1926). *Biochem. J.* **20**, 447, 461.
— (1929). *Zentr. inn. Med.* **50**, 367.
Richards (1929). Methods and results of direct investigations of the function of the kidney (Baltimore).
White and Schmidt (1925). *Science*, **62**, 334.