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ON THE MECHANISM OF SUGAR ELIMINATION IN PHLORRHIZIN GLYCOSURIA. A CONTRIBUTION TO THE FILTRATION-REABSORPTION THEORY ON KIDNEY FUNCTION.

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It was formerly assumed that the sugar in the blood was bound and non-diffusible (Lépine, Pavy). Its appearance in the urine then implied a specific secretion of glucose by the kidney cells. More recent investigations, however, have led to the conclusion that the blood sugar is freely diffusible. This was on general physiological considerations to be expected. The greatest theoretical difficulties were involved in the supposition that this most important food material did not freely permeate the capillary walls.

The fact that glucose normally does not appear in the urine could be attributed to a specific impermeability of the glomeruli to the glucose molecule, an impermeability that is known to exist in the case of the red blood corpuscles in the most widely different species of animals, and such a theory, founded on perfusion experiments with the frog's kidney, was a decennium ago presented by Hamburger and Brinkman(8). Hamburger perfused the frog's kidney from the aorta under a pressure of 60 cm. of water, and assumed that the fluid issuing from the ureters represented the pure glomerular filtrate. The results of his highly interesting experiments were that the percentage of glucose entering the urine was dependent on the interrelation between the amounts of cations in the Ringer solution employed, especially the ratio between the Ca and K ions; the correct proportion was assumed to tighten the glomeruli membranes, and excess of glucose in the perfusion fluid so to alter the membrane that the sugar leaked out. Against the interpretation that Hamburger offers for his experimental findings it may be objected that his assumption that the urine in his perfusion experiments was pure glomerular fluid is in no way justifiable; and it seems impossible to reconcile his theory with the modern view on kidney function. He

attributes to the glomerular membrane a specific impermeability for glucose, which can hardly be maintained in view of the general permeability of this membrane and the relatively high osmotic pressure of the blood sugar. This last Hamburger himself recognizes in his hypothesis that the fluid elimination in the glomeruli is due to a transudation in Cohnheim's sense, *i.e.* a combination of filtration, diffusion and osmotic action. Even the last assumption is not tenable according to the latest developments of our conception of the kidney function.

That Hamburger and Brinkman's explanation of their results is really not the correct one has been demonstrated by recent investigators in this field. With a very fine capillary tube Wearn(20) was able to puncture the capsular space and procure some fluid from a glomerulus in the frog's kidney. The fluid contained glucose while, at the same time, the urine in the bladder was sugar free. In the same year Clark(4) made some very valuable experiments on the frog's kidney. Under appropriate pressures he perfused the kidney from the renal artery and renal portal vein, and clearly demonstrated that the appearance of glucose from the glomerular filtrate in the elaborated urine was dependent on the concentration of sugar in contact with the tubule cells, which he was able to modify by changing the amounts of glucose added to the perfusion fluid sent through the renal portal vein.

The important results of Wearn's and Clark's work are that the glomerular fluid probably always contains glucose, and the non-appearance of this substance in the urine must be due to a quantitative reabsorption of sugar by the renal tubule cells.

Until evidence to the contrary has been obtained we are justified in assuming that the excretion of sugar by the mammalian kidney is, in the main, brought about in the same way as that found in experiments on frogs.

A peculiar and interesting form of glycosuria was observed by von Mering(15) in 1888. The glucoside phlorrhizin causes the urine of dogs into which it is injected to contain abundant amounts of sugar without the animals coincidently showing hyperglycæmia. Von Mering himself attributed the glycosuria to an increased permeability of the kidney for sugar. Some years later another explanation of the phenomenon was afforded by Levene(11), who assumed that the kidney during phlorrhizin poisoning actually produced glucose. Levene's theory has not been supported by modern work. The increase in the sugar concentration in the renal vein under phlorrhizin, the observation on which his assumption was based, Zuntz afterwards(23) attributed to experi-

mental errors, and has further not been verified by Nash jr. (16) in a recent series of experiments. Nash was unable to observe such an increase, and in some of his experiments he even found a decrease in the sugar content of the renal vein during the action of phlorrhizin. It may be computed from the amount of sugar excreted and the large blood flow through the kidney that this difference lay within the limits of experimental error. Strong evidence against renal sugar production is further brought forward by experiments on the isolated heart-lung-kidney preparation (de Boër and Verney(2)). Under these conditions the quantity of sugar found in the urine exactly corresponds to the extra amount removed from the blood after addition of phlorrhizin.

According to modern views of kidney function the probable explanation of phlorrhizin glycosuria is that the glucoside completely or partially prevents the tubules from reabsorbing glucose.

The correctness of this idea may be experimentally decided. Rehberg(18) recently made it highly probable that creatinine is typically what in renal physiology is termed a "no-threshold" body, *i.e.* a substance that appears in the urine in the amount in which it is present in the glomerular filtrate. This conception was already formulated in an important paper by Mayrs(13), and the same author has further shown what has been since confirmed by Poulsson(17) that inorganic sulphate in the kidney behaves in much the same way. In regard to threshold bodies, *i.e.* substances that disappear from the urine during its passage through the tubules, Rehberg has introduced a distinction which admirably supplements the filtration-reabsorption theory advanced by Ludwig and Cushny. When a substance passes back to the blood through the tubule cells this may be due either to active reabsorption by the tubule cells or merely to passive diffusion resulting from differences in concentration of the substance on the two sides of the thin walls of the tubules. In the case of the threshold body chlorine both these factors are conspicuous, whereas the fact that urea is concentrated by the kidney to a less degree than inorganic sulphate or creatinine, must be attributed to mere diffusion.

If the sugar during phlorrhizin poisoning is filtered through the glomeruli in the same concentration as obtains in the plasma, and neither is reabsorbed nor diffuses back during the passage through the tubules, we should find the same concentration ratios for glucose and for creatinine. If phlorrhizin eliminates only active reabsorption of sugar while the glucose still diffuses back in some degree, it consequently will be concentrated to a less extent according to the degree of diuresis,

but the concentration index of sugar will vary in accordance with that of creatinine, as demonstrated by Rehberg in the case of urea.

Two investigations bearing upon this question are reported in the literature. Mayrs (14) observed that after intravenous infusion of sulphate into rabbits treated under urethane with phlorrhizin, 0·2 g. per kg., the sulphate ion was concentrated by the kidneys 0·98–1·91 times more than the sugar, and offers the explanation that phlorrhizin only partially prevents the reabsorption of glucose by the renal tubule cells. White (22), who advocates the secretion theory, in a dog under morphia and ether and the same dose of phlorrhizin, observed a close connection between the figures for plasma sugar and the absolute amounts of glucose excreted in the urine. His findings clearly point to filtration. The result of another experiment indicates that sugar is concentrated by the kidney somewhat more (1·17–1·80) but closely parallel to the inorganic sulphate. Probably his method for the estimation of sulphate in plasma yields figures that are a little too high.

Experimental.

The aim of my own researches was to study the quantitative relation between plasma and urinary sugar in alimentary glycosuria produced in normal individuals, and in phlorrhizin diabetes. The first part of the investigation had to be abandoned. Like Folin and Berglund (6) I found it impossible to produce glycosuria even after excessive amounts of glucose by mouth. Two experiments on myself were done. In the first on an empty stomach 100 g. of glucose dissolved in 500 c.c. of tap water was drunk at 8.45 in the morning, the same dose was repeated at 10.35 and at 12.10. No glycosuria occurred. In the next experiment 100 c.c. of a 25 p.c. glucose solution was taken every fifteen minutes for 3 hours beginning at 9.15. In the urine samples from the first 2 hours doubtful traces of sugar seemed to be present (Benedict's reaction), but it did not seem worth while to continue the experiments as the glycosuria later on disappeared and there was not sufficient diuresis to get frequent samples of urine.

The second part of my investigation concerning phlorrhizin glycosuria is reported here. Two female dogs were used. On the day preceding the experiment food was withheld from 15.00 and only water ad libitum allowed. I used phlorrhizin (Merck) in doses from 3·8 to 5 g., *i.e.* somewhat more than 0·2 g. per kg. The glucoside was given in about 20–25 c.c. of water to which was added just enough 10 p.c. sodium carbonate solution to give a clear solution in the cold. Injections were administered subcutaneously 2 hours before the collection of urine and blood began.

Creatinine (B.D.H.) 4 g. was given in about 300 c.c. of water by stomach tube, and in order to get urine of widely different concentration water was supplied in the same way during the experiments. The urine was obtained by catheterization and careful expression of the bladder every hour, blood was obtained from the veins on the hind and fore legs exactly midway between the two catheterizations. The experiments lasted for 5 hours.

Methods.

As anti-coagulant heparine was employed; this preparation has for the last few years been almost exclusively used in this laboratory. It was ascertained that heparine has no influence on the reduction values in blood (three samples of blood from myself, with oxalate, fluoride and heparine respectively, gave 89 mg. p.c. glucose). To avoid a possible shifting of blood constituents between the red corpuscles and plasma, a rapid centrifuge (5000–6000 revolutions per minute) was used and only 3 to 5 minutes elapsed between the venous puncture and pipetting the plasma for analysis. In this creatinine was determined according to Rehberg's modification(18) of Folin's original method, glucose by the method of Hagedorn and Norman Jensen(7). To get the real content of sugar in plasma during phlorrhizin poisoning in some cases, the residual reduction was actually determined after fermentation of the glucose according to Hemmingsen(9). Hagedorn(7) has found that creatinine has a reduction value for ferricyanide of about 50 p.c. of glucose, corrections for plasma creatinine were accordingly introduced.

The determination of creatinine was performed in the appropriate dilutions of urine in 100 c.c. flasks and 1·5 mg. standards (Folin); the presence of glucose (I tried 5 p.c.) does not modify the colour. Estimation of sugar in urine was made according to Benedict(1).

A preliminary experiment showed the efficiency of different methods

Table I.

Urine treated with	Creatinine removed (p.c.)	Glucose found (p.c.)	Colour of filtrate
(1) Carbo medicinalis (Merck) after addition of hydrochloric acid to 10 p.c.	31·7	1·52	Water clear
(2) Kaolin	5·7	1·72	Somewhat more yellow than (5)
(3) Fuller's earth	23·5	1·75	"
(4) Mercuric nitrate according to Patein and Dufau. Hg in filtrate removed with Zn and HCl	60·0	1·57	Water clear
(5) Lead acetate	2·4	1·65	Faintly yellow
(6) Siliceous earth	4·5	(1·91)	Only slightly decolorized

for the removal of colouring matter and creatinine before the sugar titrations in urine. Glucose (Merck's *pro infusione*) 1·56 p.c. and some creatinine were added to a very concentrated specimen of human urine. Determination of creatinine—0·822 p.c.

The table indicates, as especially emphasized by Ivar Bang, that urochrome interferes to a certain degree with the sugar titration. In my own investigations I adopted the Pateïn and Dufau procedure, and removed the mercury in the filtrates by shaking them vigorously for 10 to 15 minutes with zinc dust after the addition of five drops of concentrated hydrochloric acid.

Extracts of my experiments are given below.

Exp. 1. Stella. Weight 17·2 kg. 8.15, 4 g. phlorrhizin subcutaneously: 9.00, 4 g. creatinine in 300 c.c. of tap water: 10.00, bladder emptied and urine samples taken every hour till 15.00: venous puncture 10.30 and every hour till 14.30: 10.58, 300 c.c. and 13.02, 150 c.c. of tap water by stomach tube. From 9 till 10 o'clock the dog had some diarrhœa and was apparently unwell.

Table II gives the results of sugar and creatinine determinations. Residual reduction assumed to be 8 mg. p.c., this amount and 50 p.c. of the plasma creatinine in mg. is subtracted from the original reduction values observed.

TABLE II.

	Urine vol. in c.c.	Sugar: In plasma mg. p.c.	Sugar: In urine mg. p.c.	Sugar: Concentration	Creatinine: In plasma mg. p.c.	Creatinine: In urine mg. p.c.	Creatinine: Concentration	$\frac{\text{C. sugar}}{\text{C. creat.}}$ p.c.
1.	23	112	8080	67·4	14·64	1249	85·4	79
2.	62	94	2870	30·5	16·05	571	35·5	86
3.	269	89	833	9·34	15·72	162	10·3	91
4.	192	94	1083	11·52	14·64	192	13·15	88
5.	83	85	2250	26·5	13·00	385	29·6	90

If on the basis of the creatinine analysis, according to Rehberg's formulæ

$$F \times \frac{\text{C. creat. plasma p.c.}}{100} = U \times \frac{\text{C. creat. urine p.c.}}{100}, \text{ and } R = F \div U,$$

we compute the amounts of fluid and glucose filtered and absorbed per minute, and the concentration of sugar in the reabsorbed fluid, we get the following data:

	Filtered fluid c.c. per min.	Reabsorbed fluid c.c. per min.	Filtered glucose mg. per min.	Reabsorbed glucose mg. per min.	Glucose mg. p.c. in reabsorbed fluid
1.	32·7	32·3	36·6	5·5	17·0
2.	36·7	35·7	34·5	5·8	16·2
3.	46·2	41·7	41·2	3·9	9·4
4.	42·1	38·9	39·6	5·5	14·1
5.	42·0	40·6	35·8	4·6	11·3

Exp. 2. Lisa. Weight 18 kg. 8.05, 3·9 g. phlorrhizin: 9.00, 4 g. creatinine in 300 c.c. water: 11.05, 500 c.c. of tap water by stomach tube: 10.00, bladder emptied, urine and blood samples as in Exp. 1. The dog seems to be quite well. No albuminuria.

Residual reduction in blood sample 1 = 17 mg. p.c., *i.e.* 17 mg. p.c. ÷ 0·5 × 16 mg. p.c. = 9 mg. p.c. without creatinine.

TABLE III.

	Urine vol. in c.c.	Sugar: In plasma mg. p.c.	Sugar: In urine mg. p.c.	Sugar: Concentration	Creatinine: In plasma mg. p.c.	Creatinine: In urine mg. p.c.	Creatinine: Concentration	$\frac{\text{C. sugar}}{\text{C. creat.}}$ p.c.
1.	160	104	1960	18·7	16·35	320	19·6	95·5
2.	166	108	1345	12·5	14·06	210	15·0	83·4
3.	272	99	840	8·5	12·33	127	10·3	82·5
4.	93	101	2270	23·0	11·05	327	29·6	77·8
5.	49	85	4025	47·3	9·03	544	60·2	76·4

Calculation according to Rehberg shows:

	Filtered fluid c.c. per min.	Reabsorbed fluid c.c. per min.	Filtered glucose mg. per min.	Reabsorbed glucose mg. per min.	Glucose mg. p.c. in reabsorbed fluid
1.	52·2	49·5	54·6	2·3	4·6
2.	41·5	38·7	44·8	7·6	20·2
3.	46·7	42·2	46·2	8·1	19·2
4.	45·9	44·3	46·4	11·2	25·3
5.	49·4	48·6	42·0	9·1	18·7

As will be seen from Exps. 1 and 2 a close parallelism exists between the concentration ratios of glucose and creatinine, the concentration of sugar by the kidney however is somewhat less efficient. The possible explanation may be that the phlorrhizination in these experiments was incomplete or perhaps that the blood samples from the leg vein do not give the correct expression regarding the glucose content in the renal artery. Probably the glucose-drainage through the kidneys under phlorrhizin leads to an emission of sugar not only from the liver but in part from the tissues. My tables show that the concentration of glucose in plasma is only slightly if at all reduced after injection of the glucoside.

Exp. 3. Lisa. Weight 18 kg. No food restriction during the day and night before the experiment. 8.15, 5 g. phlorrhizin: 8.35, 4 g. creatinine in 200 c.c. of water. The experiment was performed as 1 and 2, with the exception that the blood samples were taken from the ear vein after the shaved ear had been vigorously rubbed with toluene in order to paralyse the vessels, and to prevent coagulation a little vaseline was applied before the incision in the marginal vein. The blood was bright arterial in colour. Duplicate analyses of the residual reduction figures were made after Hemmingsen; the samples were incubated at 34° C. for 54 hours.

Values found: (1) 23 mg. p.c., (2) 15 mg. p.c., (3) 10 mg. p.c., (4) 12 mg. p.c., (5) 8 mg. p.c. During the drawing of the blood samples 3 and 4 the dog was slightly excited. At 12.02 the animal received 500 c.c. of water by stomach tube.

The result of this experiment is summarized in Table IV.

TABLE IV.

	Urine vol. in c.c.	Sugar: In plasma mg. p.c.	Sugar: In urine mg. p.c.	Sugar: Concentration	Creatinine: In plasma mg. p.c.	Creatinine: In urine mg. p.c.	Creatinine: Concentration	C. sugar / C. creat. p.c.
1.	147	86	2035	23·5	18·25	393	21·5	110
2.	57	94	4750	50·6	15·38	856	55·6	91·2
3.	196	93	1450	15·6	11·72	207·5	17·7	88·1
4.	210	85	1335	15·7	9·68	152·7	15·8	98·8
5.	58	91	4490	49·4	7·60	453	59·6	83·0

	Filtered fluid c.c. per min.	Reabsorbed fluid c.c. per min.	Filtered glucose mg. per min.	Reabsorbed glucose mg. per min.	Glucose mg. p.c. in reabsorbed fluid
1.	52·8	50·3	45·4	[−4·4]	[−8·7]
2.	52·9	51·9	49·7	4·6	8·9
3.	57·9	54·6	53·9	6·7	12·3
4.	55·3	51·8	47·0	0·3	0·58
5.	57·7	56·7	52·5	7·7	13·3

In a previous investigation the author has gathered some evidence in favour of the opinion that pituitrin acts on the cells in the renal tubules. To determine if the phlorrhizin in some way interferes with the anti-diuretic action of pituitrin the following experiment was performed.

Exp. 4. Stella. Weight 17·1 kg. 8.00, 3·9 g. phlorrhizin. At 9.00, bladder emptied and urine sampling started every hour to 14.00. No blood samples.

9.00, 200 c.c. water by stomach tube
11.00, 300 c.c. „
12.00, 300 c.c. „
12.05, 5 units pituitrin injected subcutaneously.

TABLE V.

	Urine vol. c.c. per hour	Sugar in urine g. per hour
1.	41	0·71 (action of phlorrhizin evidently not yet complete)
2.	135	1·803
3.	200	2·150
4.	120	3·435
5.	70	2·740

The usual anti-diuretic action of pituitrin is obviously present. The increase in the sugar excretion is certainly due to the rise of blood sugar following injections of pituitrin. As demonstrated by Erlandsen(5) the phlorrhizin glycosuria is likewise augmented when hyperglycæmia is induced by bleedings or injections of adrenaline.

Discussion.

The result of my investigations is that a close agreement has been shown to exist in the behaviour of glucose in phlorrhizin poisoning and that of the typical no-threshold substance creatinine. This observation has a bearing upon two questions. In the first place, my findings lend some support to the filtration-reabsorption theory of kidney function. It is now known that three such fundamentally different substances as creatinine, inorganic sulphate and glucose in certain conditions behave in the kidney similarly. This seems to me to give a final proof of the filtration-reabsorption hypothesis now extensively adopted by leading physiologists.

The second point of some interest is that phlorrhizin apparently completely paralyses the reabsorption of glucose in the renal tubules. The data from my Exp. 3 indicate a very close correspondence between the concentration ratios of sugar and creatinine; in the first sample the glucose is even concentrated 10 p.c. more than creatinine.

The findings in Exp. 3 are put together in Fig. 1.

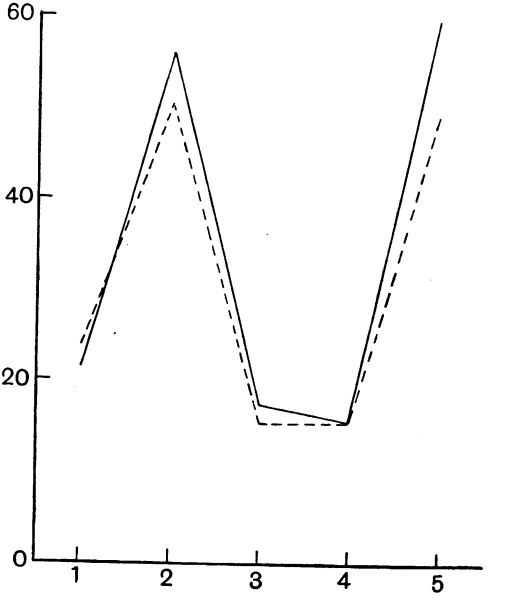

Fig. 1. The ordinate represents the concentration indices ——— of creatinine, and - - - - - - of sugar. The abscissa indicates time.

When we try to interpret the finding that glucose is in most cases

concentrated somewhat less than creatinine the following explanations offer themselves.

The sugar might in some degree during the concentration process diffuse back into the blood through the tubule walls. After what is already known about the rate of diffusion of glucose (von Brasol(3), Leathes(10)), this is not very probable. To throw some light on the question, Fig. 2 is constructed.

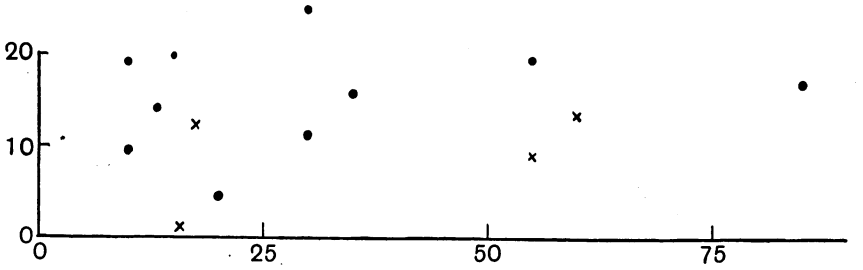

Fig. 2. Ordinate: mg. p.c. glucose in reabsorbed fluid. Abscissa: concentration of urine according to the creatinine determinations.
● Exps. 1 and 2. × Exp. 3.

The amount of retained glucose is evidently not dependent on the urine concentration. This is the case with substances as urea which, to a great extent, diffuses back to the blood; as we must expect, this diffusion is determined by the speed and the concentration with which the urine passes through the tubules (Rehberg(18)).

It is also not very probable that with the large doses used, the action of the phlorrhizin was incomplete. The interpretation which seems most likely to me is that the analytical methods employed have not been sufficiently accurate to demonstrate the complete parallelism between the concentration ratios of the two substances.

It is difficult, I think, to get the correct figures for the amount of glucose in the renal blood plasma, and it is particularly in regard to the figures for the residual reduction values that I have doubts. I incubated the blood filtrates for 24 to 54 hours; according to recent American investigators (see Somogyi, 1927, where literature is quoted) this is too long. As will be seen from my Exp. 3 sample 5 showed a residual reduction of 8 mg. p.c.; when the figures for creatinine are subtracted only 4 mg. p.c. remains, a value evidently too low. Finally, too, it is possible that a few mg. p.c. of the sugar in the plasma is non-diffusible. As already pointed out, however, I believe that the observed differences in concentration ratios between creatinine and glucose are not

real, and the expressions "mg. reabsorbed glucose" and "mg. p.c. glucose in reabsorbed fluid" in this connection consequently are misleading.

It has been a matter of some dispute (Loewi(12), Weber(21)) whether a superimposed diuresis increases the sugar elimination in phlorrhizin diabetes. This, of course, depends on whether the extra diuresis is accompanied by changes in the rate of filtration through the glomeruli or not. When the filtration is augmented, *e.g.* by raising the pressure in the renal artery in the heart-lung-kidney preparation (de Boër and Verney) the amount of sugar in the urine is also increased; if the rate of filtration remains very constant, as is the rule normally, the sugar elimination like the urinary content of inorganic sulphate and creatinine is independent of variations in the volume of water excreted. Fig. 3 (Exp. 3) illustrates this point.

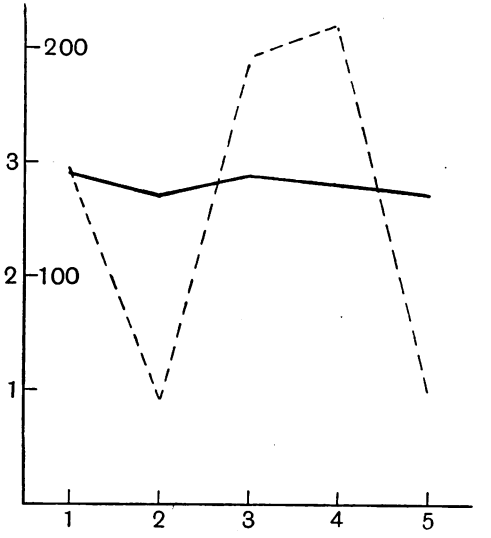

Fig. 3. Ordinate: ------ c.c. of urine per hour, ——— g. glucose excreted per hour. Abscissa: time in hours.

SUMMARY AND CONCLUSIONS.

During phlorrhizin poisoning in unanæsthetized dogs, the kidneys concentrate the glucose approximately in the same degree and in close parallelism with creatinine. This observation supports the Ludwig-Cushny view on kidney function, and furnishes evidence that phlorrhizin completely paralyses the renal tubules as to their power of reabsorbing sugar.

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