THE INFLUENCE OF PHLORIDZIN ON THE BILE AND LYMPH.

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In my article on Phloridzin Glycosuria, published in 1894,* I had in view the demonstration of two propositions: (1) That there existed no sufficient grounds for v. Noorden's assumption that phloridzin glycosuria does not at all resemble any forms of spontaneous diabetes mellitus, and that, therefore, the mechanism of this experimental glycosuria cannot be applied to the explanation of any clinical forms of diabetes; and (2) that the appearance of sugar in the urine in this experimental glycosuria could not be satisfactorily explained by mere elimination from the blood.

Several facts had led me to doubt the "elimination theory." In the first place v. Mering himself, who examined the blood for sugar only after the injection of phloridzin, found that the amount of sugar did not differ much from that which occurs very often in normal dogs, and therefore could not be called subnormal. Again, the work of Minkowski proved that after the extirpation of the kidneys and subsequent phloridzin injection there was in fact some slight increase of sugar in the blood. Lastly, Cornevin found an increase of sugar in the milk after phloridzin injection. To these facts I was able to add from my own experiments that the venous blood of the kidneys never contained less sugar than the arterial, and that the amount of sugar in the whole kidney after the phloridzin injection was higher than normal.

Very recently Zuntz, again recurring to the study of "phloridzin diabetes," has shown in a very ingenious way that the appearance of

* References to the literature are at the end of this article.

sugar in the urine after phloridzin injection may be due exclusively to the activity of the kidneys. This investigator injected very small quantities of phloridzin directly into the renal artery and afterward collected the urine of each ureter separately. He found that the urine of the kidney into which phloridzin had been injected contained sugar almost immediately, whereas in the other it was demonstrable only after some time, and then in much smaller quantities. Zuntz attempted further to limit the action of phloridzin to one kidney only, and therefore ligated the renal veins of the side upon which the glucoside was injected. But immediately after ligation the secretion of urine of the corresponding kidney was suppressed and sugar at once appeared in the urine of the other kidney, where without ligation it was usually demonstrable only after some time.

In these later experiments Zuntz sees a new corroboration of the elimination theory of v. Mering and a new disproof of the supposition expressed in my previous article.

Zuntz says:

"Verfasser hat die Nierenvene behufs Einführung einer Canüle abgeklemmt. Darnach aber hört die Urinsecretion für längere Zeit auf, es kann also auch keine Abnahme des Zuckergehaltes in der Vene erwartet werden. Noch weniger berechtigt ist Levene aus dem erhöhten Zuckergehalt der Niere selbst auf eine Beziehung derselben zur Zuckerbildung zu schliessen. Unvermeidlich findet sich in dem Canalsystem der Niere eine gewisse Menge Urin; da dieser Urin nach Phloridzinzufuhr 10 pro cent und mehr Zucker enthält, muss die Zuckerbestimmung in der gesammten Niere einen höheren Gehalt ergeben, als bei normalen Thieren, ohne dass man daraus folgern darf, der Zucker sei im Nierenparenchym gebildet worden."

But, if the suppression of the secretion of the urine immediately after stagnation actually takes place, this fact by itself does not by any means show that all the functions of the renal epithelium are suppressed at the same time, and there is no reason why the epithelium, stimulated or influenced in some way by the glucoside, could not for a time at any rate continue to absorb sugar from the blood.

Beyond doubt Zuntz's experiment affords a very striking argument

in favor of the supposition that the appearance of sugar in the urine after administration of phloridzin can be produced by the kidneys alone. Nevertheless by itself it proves nothing more, and fails utterly to explain the way in which the kidneys produce this effect. The latter might just as well be due to mere elimination as to local overproduction of sugar in the kidney. Important, therefore, as is Zuntz's discovery in showing the possibility of the occurrence of renal diabetes, it does not add much to the previous facts at our command for the explanation of the mechanism of phloridzin glycosuria.

The fact discovered by Zuntz, that the time which elapses between the injection of phloridzin directly into the arteries of the kidneys and the appearance of sugar in the urine is so short, induced me to attempt to apply the same method to another secretory organ, in order to see whether or not it would respond to the stimulus in the same way as the kidneys. As I have stated before, there is some mention in the literature that the mammary glands resemble the kidneys in this respect.

I chose for this purpose the liver. The experiments were conducted in the following way: The animals were narcotized by means of ether. After the gall bladder had been emptied of its contents a cannula was inserted into it and a few cubic centimetres of a 0.5 per cent solution of phloridzin in alkaline water were injected directly into the portal vein. The specimens of bile collected before and at different times after the injection were separately treated with 10 volumes of 95 per cent alcohol for 24 hours and filtered. The filtrate was decolorized by means of bone-black, evaporated to dryness, dissolved in water, filtered, and the resulting filtrate was tested with Fehling's solution. The experiment was repeated on five dogs. In two of them the blood-vessels of the kidneys were ligated previous to the injection of phloridzin. In all cases a very distinct reduction of the copper appeared with bile collected after the injection. In some cases a slight reduction took place also in the normal bile; this was probably due to the effects of the anæsthetic, and was never so great as in the other cases. As the amount of sugar in the bile was not quite sufficient for a quantitative examination, I decided to observe the influence of

phloridzin injection on animals with biliary fistulæ, where I could collect pure bile without inconvenience to the animal and hence without the use of narcotics. I performed this operation on two dogs with the following results:

Experiment, February 17, 1896.—Dog; weight, 20 kilos; operation for biliary fistula performed under anæsthesia. The cannula was kept open till the following morning, when it was closed. February 18, 11 A. M.: the normal bile was collected and a solution of 2.5 grammes of phloridzin in alkaline water was injected hypodermically. At 3 P. M. 25 cubic centimetres of bile were collected. At 6 P. M. 19 cubic centimetres of bile were collected. The cannula was left open till the following morning, when it was again closed for a few hours and the bile collected. These operations were repeated until February 21, when the dog was killed, as a slight suppuration had taken place around the wound. All the specimens of bile were treated in the above-mentioned way. Reduction of the copper solution took place in all after the injection of phloridzin, but did not appear in the normal bile. On February 21 the urine still possessed high reducing power.

Experiment, March 24, 1896.—Dog; weight, 9 kilos; operation for biliary fistula performed under anæsthesia; the fistula was left open until the following morning. March 25, 10 cubic centimetres of normal bile were obtained and a solution containing 2 grammes of phloridzin was given to the dog by the mouth; a portion was vomited. At 3 P. M. 7 cubic centimetres of bile were collected. As the quantity of bile secreted thereafter was very small, the dog was killed. The bile obtained before the injection of phloridzin possessed no reducing power, that after the injection showed a distinct reduction.

Though it is worthy of note that the reducing power of the bile was never so high as that of the urine, it must be remembered that the question for me was whether phloridzin acts exclusively on the kidneys or not. The foregoing experiments, as well as those of Cornevin, tend to answer the question in the negative. On the other hand these facts alone do not exclude the elimination theory.

In order to obtain more data relating to the cause of the appearance of sugar in the urine and other secretions, I decided to examine the lymph of the thoracic duct. I thought that over-production of sugar might coexist with a normal percentage of sugar in the blood

by virtue of the glycolytic power of the blood, which in this case might be increased. In such case one would expect to find the amount of sugar in the lymph increased after the injection of phloridzin. The experiment was conducted in the following way: The animals were allowed to fast for at least 24 hours previous to the operation. They were narcotized by means of morphine and ether. In all the operations artificial respiration was employed. In order to prevent coagulation the lymph was collected in a saturated solution of Na_2SO_4, which was then boiled with a few drops of acetic acid. The precipitate was once more boiled with a saturated solution of Na_2SO_4, again filtered, and the precipitate again washed with a hot Na_2SO_4 solution. All the filtrates and wash waters were combined, and the sugars determined by means of the following modification of Ahlin's method, which I have used for the last two years.

As in Ahlin's method, an excess of Fehling's solution was taken. After the reduction of the copper, the excess of the Fehling's solution was decanted on an asbestos filter, and the main part of the residue was left in the evaporating dish. The residue was then washed and the wash water decanted again. The precipitate of Cu_2O on the asbestos filter was also washed; and the Cu_2O in the dish and that on the filter were dissolved by means of nitric acid, and mixed together. After it had been neutralized by means of sodium carbonate, a few drops of ammonia were added to the solution, which was then titrated by means of a standard solution of KCy. This method has also been in use for the last year in the Analytical Laboratory of the School of Mines of the Columbia University of New York. The results of these experiments may be summarized as follows:

Experiment 1, *April* 17.—Dog; weight, 7 kilos; no food for 48 hours previous to the operation; 0.05 gramme of morphine two hours before the operation; ether; artificial respiration. A cannula was inserted into the thoracic duct. During the first thirty minutes 24.1 grammes of lymph were collected. Then 20 cubic centimetres of a 2 per cent solution of phloridzin in alkaline water were gradually injected into the right jugular vein, and in the next twenty minutes 36.2 grammes of lymph were collected. The urine obtained from the ureter contained sugar.

The amount of Cu reduced by the lymph before injection of phloridzin = 0.0313 gramme, corresponding to 0.078 per cent of sugar.

The amount of Cu reduced by the lymph after injection of phloridzin = 0.0686 gramme, corresponding to 0.0967 per cent of sugar.

In this case the first lymph was not collected in Na_2SO_4 at once, but was only later transferred to this solution, the greater part being then coagulated.

Experiment 2, *May* 27.—Dog; weight, 20 kilos; fasting about 24 hours previous to the operation; 0.15 gramme of morphine administered about two hours before the operation. During the first thirty-five minutes 24 grammes of lymph were collected; 28 cubic centimetres of a 2 per cent solution of phloridzin in alkaline water were then gradually injected into the right jugular vein. The lymph collected in the next twenty minutes was lost, but a third portion was collected during forty minutes equal to 43.6 grammes.

The amount of Cu reduced by the first lymph = 0.098 gramme = 0.279 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.0634 gramme = 0.074 per cent of glucose.

Experiment 3, *June* 1.—Dog; weight, 10 kilos; fasting 48 hours previous to the operation; 0.1 gramme morphine injected two hours before the operation. During the first thirty minutes after the operation 10 grammes of lymph were collected. 30 cubic centimetres of a 1 per cent solution of phloridzin were then injected into the right jugular vein. In the next forty-five minutes 22.5 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.049 gramme = 0.259 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.078 gramme = 0.177 per cent of glucose.

Experiment 4, *June* 2.—Dog; weight, 18 kilos; fasting 24 hours previous to the operation; 0.15 gramme of morphine injected one hour before the operation. During the first twenty-five minutes after the operation 29.3 grammes of lymph were collected. 0.4 gramme of phloridzin in 30 cubic centimetres of very dilute Na_2CO_3 solution was injected into the jugular vein. In the next 15 minutes 27.4 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.0625 gramme = 0.110 per cent of glucose. (Part of the Cu solution prepared for titration was lost during neutralization.)

The amount of Cu reduced by the second lymph = 0.0666 gramme = 0.125 per cent of glucose.

Experiment 5, *June* 4.—Dog; weight, 12 kilos; fasting 24 hours before the operation; 0.15 gramme of morphine injected two hours before the operation. During the first twenty minutes after the operation 24.6 grammes of lymph were collected. 0.4 gramme of phloridzin in 30 cubic centimetres of H_2O containing some Na_2CO_3 was injected into the right jugular vein.

The amount of Cu reduced by the first lymph = 0.095 gramme = 0.196 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.078 gramme = 0.175 per cent of glucose.

Experiment 6, *June* 8.—Dog; weight, 20 kilos; fasting twenty-four hours before the operation; 0.15 gramme of morphine injected two hours before the operation. During the first thirty-five minutes 3 grammes of lymph were collected. 0.3 gramme of phloridzin dissolved in 30 cubic centimetres of H_2O containing some Na_2CO_3 was then injected. During the next 45 minutes 10 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.0161 gramme = 0.300 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.0397 gramme = 0.130 per cent of glucose.

The results are here grouped in tabular form.

No.	Percentage of Glucose in the lymph before injection of phloridzin.	Percentage of Glucose in the lymph after injection of phloridzin.	Difference expressed in percentages.
1	0.078	0.096	+ 23
2	0.279	0.074	— 73
3	0.259	0.177	— 32
4	(0.110) part lost	0.125	— ?
5	0.196	0.175	— 11
6	0.300	0.130	— 57

All these experiments except No. 1 indicate a considerable decrease of sugar in the lymph after the injection of phloridzin.

In order to ascertain the relation of this decrease of glucose in the lymph to the activity of the kidneys, I thought it advisable to ligate the whole hylus renalis previous to the collection of the lymph and the injection of the phloridzin; and, in order to determine to what extent this decrease might be due to the action of phloridzin, I examined the lymph collected in the same intervals as in the other experiments, but without the injection of the glucoside. Unfortu-

nately I did not have time to perform each of these control operations more than once.

Experiment, June 12, 1896.—Dog; weight, 18 kilos; fasting twenty-four hours before the operation; 0.1 gramme morphine injected two hours before the operation. The dog was narcotized, artificial respiration was employed; both hyli renales were ligated. The operation was then directed to the thoracic duct. During the first 30 minutes 13 grammes of lymph were collected. 0.5 gramme of phloridzin in 40 cubic centimetres of H_2O containing some Na_2CO_3 was injected into the jugular vein, and in the next thirty minutes 19.5 grammes of lymph were collected.

The amount of Cu reduced by the first lymph = 0.063 gramme = 0.240 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.568 gramme = 0.150 per cent of glucose.

Control Experiment, June 18, 1896.—Dog; weight, 18 kilos; no phloridzin injected. The operation was performed in the usual way with anæsthetics and artificial respiration. During the first 35 minutes 16.5 grammes of lymph were collected, during the following 35 minutes 15 grammes.

The amount of Cu reduced by the first lymph = 0.0725 gramme = 0.224 per cent of glucose.

The amount of Cu reduced by the second lymph = 0.0706 gramme = 0.240 per cent of glucose.

The foregoing experiments tend to show that the kidneys are not the only organs affected by phloridzin. This conclusion is based not only upon the result of phloridzin injection on the bile and the milk, but also upon the fact that the amount of sugar in the lymph decreases after the injection of phloridzin, even if the hyli renales are ligated previous to the injection of the glucoside.

The examination of the lymph shows a decrease of sugar after the administration of the phloridzin. If this is to be explained by the fact that the blood, being more than normally deprived of sugar by one organ, yields less to the other tissues, and, therefore, the lymph flowing from those tissues is poorer in sugar than under normal conditions, we have a strong argument in support of the elimination theory. But, on the other hand, it has been found that the urine

can eliminate during one hour after the administration of phloridzin more sugar than can be obtained from the daily amount of lymph. This fact, especially when taken in conjunction with the changes taking place in the different tissues and in the metabolism of the whole organism, offers at present some room for doubt in regard to the elimination theory. The same opinion has been expressed by Kraus.* At the same time the occurrence of spontaneous diabetes without hyperglycæmia, which has been proved by Seegen, does not justify v. Noorden in his assertion that the mechanism of phloridzin glycosuria does not resemble any form of spontaneous diabetes. Only recently Klemperer, at the meeting of the Verein für innere Medicin in Berlin (May 18, 1896), has called attention to a form of renal diabetes resembling the phloridzin glycosuria.

As to Zuntz's supposition of the existence of a certain mechanism regulating the amount of sugar in the blood through increased or decreased combustion of the sugar, I may remark that the study of phloridzin glycosuria has made virtually the same impression on myself, and I expressed in my previous paper the following opinion: "The absence of hyperglycaemia may be explained by the supposition that the organism has not lost its ability to rid itself of the superabundance of sugar on the one hand by its elimination through the kidneys, and on the other by its increased decomposition."

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REFERENCES TO LITERATURE.

Cornevin.—*Compt. rend.*, cxvi, 6, p. 263.
Klemperer.—*Centralblatt für innere Medicin*, 1896, no. 24.
Levene, P. A.—*Journal of Physiology*, vol. xvii, nos. 3 and 4, 1894.
von Mering.—*Centralblatt f. d. med. Wissensch*, 1885, p. 531.
von Noorden.—Lehrb. d. Path. des Stoffwechsels, Berlin, 1893.
Seegen, I.—Der Diabetes Mellitus, Berlin, 1893.
Zuntz, N.—*Verhandlungen der physiologischen Gesellschaft zu Berlin*, Jahrgang 1894–1895, nos. 16, 17, 18, p. 51.

* Lubarsch und Ostertag, *Ergebnisse der allg. Pathologie u. path. Anat.*, Abth. ii, p. 607.