The possibility was considered that *within* the cell benzaldehyde might be formed, whilst at the same time it was proved that *outside* the cell important quantities of amygdalin by no means should be converted into glucose, benzaldehyde and hydrogen cyanide.

This result was confirmed in another way by a new series of experiments, from which it is to be concluded with certainty that *amygdalin without any preceding conversion* into glucose benzaldehyde and hydrogen cyanide, is *absorbed* by the cells.

The referential experiments are united in the table.

From these experiments it follows that the addition of amygdalin diminishes the noxious influence of benzaldehyde. Compare $E_1, F_1$ and $G_1$ on one side with $E_2, F_2, G_2$ and $E_3, F_3, G_3$ on the other side.

If a conversion into glucose, benzaldehyde and HCN should precede the absorption of amygdalin just the contrary should be stated.

**Physiology**. — "*Experimental researches on the permeability of the kidneys to glucose*" [1]). By Prof. H. J. HAMBURGER and R. BRINKMAN.

I. THE PROPORTION BETWEEN K AND Ca IN THE CIRCULATING FLUID.

(Communicated in the meeting of January 27, 1917).

## 1. *Introduction.*

No solution has been offered to the question of importance to physiologists as well as to clinicists, viz. why the urine of a normal person is entirely or all but entirely free from sugar as long as the sugar-percentage of the blood serum does not rise above a certain concentration, and why as a rule glucosuria only sets in when accompanied by hyperglycaemia.

Two explanations suggest themselves:

It may be supposed that the normal glomerulus epithelium is proof against ± 0.1 % of glucose without becoming permeable to it, but cannot keep back all the glucose of a higher concentration. Not much can be said in favour of this view, for it is not very likely that cells which are permanently exposed to a 0.1 % solution of a physiological non-electrolyte such as glucose should be changed by a 0.2 % solution [2]).

[1]) A more detailed account will be published elsewhere.

[2]) We shall not discuss here the hypothesis of a glomerulus-epithelium absolutely permeable to glucose, with back-resorption of it through the kidney-ducts, nor the oxidation of the glucose in the kidney.

The second explanation, which has found many advocates, is that the blood sugar in the serum is not present in a free state, but is in normal circumstances only met with as a colloidal compound (LÉPINE's sucre virtuel), which cannot pass the glomerulus membrane. If the serum does not contain a quantity of this substance sufficient to bind the glucose, then part of the glucose remains circulating in a free state and can pass the glomerulus-epithelium, in other words glucosuria sets in. Several colloidal glucose compounds have been suggested already (jecorin, lecithin glucose, globulin-glucose).

Objections have been raised, however, against this retention of sugar by a substance, present in the serum. Serum has been made to dialyze against glucose-solutions (ASHER, RONA and MICHAELIS) and it was found that the percentage of glucose became equal on both sides of the membrane. A retention of sugar in a colloid form was, therefore, manifestly impossible. This statement has produced a considerable impression, and the result seems to be that matters have come to a dead stop.

We have asked ourselves, however, if the results obtained in experiments with parchment membranes might be applied to glomerulus epithelium. Obviously there is a possibility of compounds of glucose with some serumsubstance diffusing through a parchment membrane, but not through a membrane of glomerulus epithelium. BECHHOLD's experiments have amply demonstrated that certain colloidal particles diffuse through one membrane, but not through another with smaller pores.

We experimented, therefore, with celloidin membranes of various celloidin-percentages and ultrafiltrated under a pressure of 4 or 5 atmospheres serum through it to which known quantities of glucose had been added, but the reduction-power of the ultrafiltrates did not warrant us to conclude that a colloidal glucose-compound had been kept back by the ultrafilter.

Pursuing the same line of thought it seemed advisable now to investigate systematically whether in spite of the results of these diffusion- and ultrafiltration-experiments the second view was not the right one after all.

In the first place it would have to be ascertained, which had not been done previously, that *free* glucose diffuses through the kidney. To investigate this, RINGER-fluid, to which sugar had been added, would have to be transmitted through the vascular system of the kidney. If it was found then that the fluid flowing from the ureters contained the same concentration of sugar as the transmission-fluid, and if further it became evident that a RINGER-fluid

containing sugar, to which serum had been added, produced an artificial urine *free* from sugar, then it was, as we thought, conclusively shown that the serum contains a substance retaining sugar in a form which cannot diffuse through the glomerulus epithelium, and then further researches might be made as to the nature of this substance.

Before entering upon the description of the experiments we wish te make a few observations of a technical nature.

*Some remarks of a technical kind.*

The experiments were exclusively carried out with frogs, viz. with large male specimens of the Rhine frog. The spinal marrow was destroyed with a needle, and all organs except kidneys, testicles and bladder were removed at once. Then a thin injection-needle was inserted into the aorta communis and a canula into each ureter. The fluid which circulates through the vascular system must be amply provided with oxygen. The pressure amounted to 60—80 centimetres of water. In this way from 300 to 800 cubic c.m. of fluid flows through the kidneys per hour. The amount of fluid passing through the ureter is 0.5 c.c. or less. This fluid must be looked upon as a glomerulus product, for if at the same pressure fluid is transmitted through the vena Jacobsonii, then no fluid is secreted in the ureters. At a higher pressure some fluid is formed but very slowly.

The glucose-percentage of the urine is not affected by the vena Jacobsonii being tied off. This makes it probable that the kidney ducts have little to do with the glucose-motion.

The glucose-determination of transcirculating-fluid and kidney-product was carried out by means of the excellent method of I. BANG [1]). It enables one to determine the glucose-percentage in 0.1 c.c. of fluid to within 0.006 %.

## 2. *The permeability of the frog's kidney to glucose which has been dissolved in* RINGER-*fluid.*

### 1st Series of Experiments.

As we said before the fundamental problem to be solved in the first place was whether at a transmission of RINGER-fluid containing

[1]) J. BANG. Methoden zur Mikrobestimmung einiger Blutbestandteile. Wiesbaden, J. F. BERGMANN. 1916.

glucose, the glucose-percentage of the urine would become equal to that of the circulating fluid. Repeated experiments showed that the glucose concentrations were exactly the same in both experiments. We shall give some of the values obtained. Each experiment was repeated at least three times and all gave the same results.

Jan. 20 to Jan. 26. 1916.

1. RINGER-solution containing 0,1 % of glucose transmitted from the aorta through the kidneys under a pressure of ± 50 c.m. Reduction of transmission-fluid 0,098 %. Reduction (expressed in glucoseconcentration) of the left kidney 0,095 %, right kidney 0,095 %.

2. Circulation of RINGER-solution containing exactly 0,05 % of glucose, from the aorta. Pressure 60 c c. of water. Reduction of urine to the left 0,05 %, to the right 0,045 %.

3. Circulation from the aorta with pure RINGER-solution. The urine shows no reduction.

These results formed, as it seemed, a reliable foundation for further researches. It was now expected that on serum being added to the RINGER-fluid containing glucose, the free glucose would be entirely or partially bound; in other words that the reduction capacity of the ureter-fluid would be smaller than that of the circulation-fluid.

3. *The permeability of the kidneys to glucose when it is dissolved in a mixture of serum and* RINGER-*fluid.*

2nd. Series of Experiments.

For these experiments horse's or neat's serum was diluted with a 2-, 3-, 4- and 5-fold quantity of RINGER-fluid to which mixtures in every instance a known quantity of glucose was added. The secretion of ureter-fluid took place very slowly, but could be promoted by the addition of urea.

We subjoin a few of the many experiments.

1. Frog's kidney through which flows a fluid consisting of 50 c.c. of horse's serum + 150 c.c. of RINGER + glucose + urea. Reduction of this mixture 0,17 %, reduction urine 0,086 %. Consequently 0,09 % of glucose has been kept back (= the quantity of glucose in normal horse's serum).

2. The fluid consists of 75 c.c. of neat's serum + 225 c.c. of RINGER + glucose + urea. Reduction of the transmission fluid 0,21 %, reduction of the urine only 0,12 %, to the right, 0,105 %. to the left.

3. The fluid consists of 60 c c. of horse's serum + 240 c.c. of RINGER + glucose + urea. Reduction of the transmission fluid 0,14 %. Reduction of the urine to the right 0,03 %, to the left 0,028 %. Hence at a 5-fold serum dilution 0,11 % of glucose in still kept back.

In the same way 0.07 % of glucose was kept back at a 6-fold, 0.06 % at a 7-fold dilution but at an 8-fold dilution *next to nothing.*

Evidently a considerable quantity of sugar is retained as long as the dilution of the serum is not an 8-fold one (0.17—0.086, 0.21—0.11, 0.21—0.105, 0.14—0.03, 0.14—0.028).

In stronger dilutions the retention of sugar grows less, and in an 8-fold dilution it is 0.

It was now attempted to trace the cause of this rather abrupt turning point, but in the midst of this somewhat elaborate investigation, which we shall not discuss here, the stock of RINGER-fluid gave out, and a fresh quantity had to be prepared. It soon became evident now that the retention power of the kidney for glucose in the serum-RINGER-mixtures was entirely different from what it had been in the previous experiments.

The possibility had to be taken into account that the RINGER-fluid was not identical with the one formerly used. Was the Ca-percentage different perhaps? We often read of a $CaCl_2$-solution of a given concentration without there being added if it has been made of anhydrous $CaCl_2$ or of $CaCl_2$ 6 aq. It was indeed found that an addition of some $CaCl_2$ strongly affected the glucose-excretion, for now the concentration of the ureter fluid was equal to that of the transmission-fluid. This observation, confirmed by parallel-experiments, induced us to determine whether the circulation of the new serumless RINGER-solution would cause all glucose to be diffused, as had been the case with the original RINGER-fluid.

To our surprise we discovered that when the new RINGER-fluid was transmitted, glucose was retained by the kidneys.

*Under these circumstances it became necessary to institute a systematic investigation of the way in which a change in the composition of the* RINGER*-fluid affects the permeability of the kidney.* The present paper confines itself to this investigation. We shall afterwards revert to the effect of serum being added.

### 4. *Change in the proportion of the quantities of K and Ca in the* RINGER*-fluid.*

#### 3rd Series of Experiments.

It appears from the following table that only the amount of $CaCl_2$ was modified, the KCl remaining the same.

Each of the four experiments was repeated 3 times with exactly the same results.

Evidently no glucose is retained if a solution of $CaCl_2$ 0,005 % is used; when however, the solution contains 0,0075 %, 0.095—0.065=

| | % of NaCl | % of $NaHCO_3$ | % of KCl | % of $CaCl_2$ (without crystallisation water) | % of Reduction | |
|---|---|---|---|---|---|---|
| | | | | | Circul. fl. | Urine |
| 1) | 0.7 | 0.02 | 0.01 | 0.005 | 0.09 | 0.09 |
| 2) | 0.7 | 0.02 | 0.01 | 0.0075 | **0.095** | **0.065** |
| 3) | 0.7 | 0.02 | 0.01 | 0.010 | 0.09 | 0.08 |
| 4) | 0.7 | 0.02 | 0.01 | 0.015 | 0.09 | 0.09 |

0,03 % of glucose is retained. If the Ca-perc. is raised to 0,010 % only 0,01 % is retained, at 0,015 % nothing again.

Hence the most favourable proportion between the concentrations of KCl and $CaCl_2$ is 4 : 3, which, expressed in the number of atoms, results in: K : Ca = 2 : 1. The following table demonstrates that the proportion between K and Ca and not the absolute amount of Ca is the important thing, because a slight increase of the K-perc. necessitates a corresponding increase of Ca.

| % NaCl | % $NaHCO_3$ | % KCl | % $CaCl_2$ | % Reduction | |
|---|---|---|---|---|---|
| | | | | Circul. fl. | Urine |
| 0.7 | 0.02 | 0.01 | 0.0075 | 0.095 | **0.065** |
| 0.7 | 0.02 | 0.015 | 0.0075 | 0.08 | 0.08 |
| 0.7 | 0.02 | 0.014 | 0.011 | 0.10 | **0.065** |

It should be noticed that the glucose perc. in the blood of the winter-frog (these were used for the experiments) amounts to 0.03 %. In the summer-frog it is 0.05 %.

*In accordance with this difference it was found that the kidneys of the summer-frog, when treated with* RINGER-*fluid containing glucose, retained* 0.05 % *glucose,* which is partly due to the temperature. We shall have occasion to mention the influence of the temperature again.

### 5. *The proportion of Na, K, Ca.*

#### 4th Series of Experiments.

In order to continue the experiments a new consignment of frogs had to be used. The RINGER-fluid, used for circulation, consisted again of NaCl 0.7 %, $NaHCO_3$ 0.02 %, KCl 0.01 %, $CaCl_2$ 0.075 %,

glucose 0.09 %. To our surprise little or no glucose was retained now. Then the possibility was debated whether perhaps the amount of Na might tell upon the results. The answer is supplied by the following table.

| % NaCl | % $NaHCO_3$ | % KCl | % $CaCl_2$ | % Reduction | |
|---|---|---|---|---|---|
| | | | | Circul. fl. | Urine |
| 0.7 | 0.02 | 0.01 | 0.0075 | 0.09 | 0.09 |
| 0.7 | 0.02 | 0.01 | 0.010 | 0.102 | 0.085 |
| 0.7 | 0.02 | 0.01 | 0.012 | 0.105 | 0.085 |
| 0.7 | 0.02 | 0.01 | 0.0075 | 0.085 | 0.085 |
| 0.6 | 0.02 | 0.01 | 0.0075 | 0.085 | 0.060 |
| 0.6 | 0.02 | 0.01 | 0.0075 | 0.010 | 0.070 |
| 0.6 | 0.02 | 0.01 | 0.005 | 0.09 | 0.070 |
| 0.6 | 0.02 | 0.01 | 0.0025 | 0.085 | 0.075 |
| 0.6 | 0.02 | 0.01 | 0.010 | 0.12 | 0.115 |

Obviously these results differ from those in the preceding table in which $KCl : CaCl_2 = 4 : 3$ and where 0.03 % of glucose was retained. Something indeed is retained when we use the new frogs, but not 0.03 %. 0.02 % is retained when we use $KCl: CaCl_2 = 4 : 4$ or $1 : 1$.

Why did the new consignment of frogs behave in a way different from the first? The temperature at which they were kept might be the cause. *It was ± 8° C.; formerly it had been higher.* That the difference is indeed due to the temperature is made manifest by the fact that, in order to obtain the same results some $CaCl_2$ must be added to the circulating fluid when the kidney is cooled down by ice. If we wish to maintain the proportion $KCl : CaCl_2 = 4 : 3$, then the NaCl must be reduced from 0.7 to 0.6 %.

*It follows that with every condition of the glomerulus epithelium must correspond, if it is to keep back a maximum amount of glucose, a certain proportion between Na, K and Ca.*

It is not improbable that the anions too play a part in the equilibrium, but at any rate one gets an impression that the proportion of cations preponderates.

The fact that a disturbance in the equilibrium of the cations

strongly affects the permeability of the kidney to sugar may explain two important observations, which have hitherto not been understood.

One relates to an experiment by UNDERHILL and CLOSSEN [1]), who injected into the ear-vena of a rabbit a solution of $CaCl_2$ and discovered that the hypoglycaemia is attended with glucosuria. The most obvious explanation is that we have to deal with a disturbance in the equilibrium between Na, K and Ca.

Secondly it has been known for many years that uranium may also cause glucosuria [2]). Now ZWAARDEMAKER and FEENSTRA discovered [3]) that in the RINGER-fluid which sustains the beating of the frog's heart, the K may be replaced by the likewise radioactive uranium. It will appear from the ensuing communication that in the physiological circulation-fluid of the kidney the K may likewise be replaced by an equiradioactive quantity of uranium. Hence it is not assuming too much if we consider the uranium-glucosuria as being caused by an equilibrium-disturbance occasioned by a disturbance of the normal K-percentage.

It should be noticed that the glucosuria caused by $CaCl_2$- and by uranium-injections are the only two of which it may be stated with certainty that they are of a renal kind. Thus with warmblooded animals an equilibrium-disturbance in the relative cation-percentage of the circulating-fluid (here bloodplasm) might also be the cause of a modified permeability of the glomerulus epithelium to sugar.

## SUMMARY.

1. When a RINGER-fluid in which the atoms of K and Ca are as 2 to 1 and which contains glucose is transmitted through the frog's kidney at 7°—10° C, then a comparison of the glucose concentrations of circulating-fluid and ureter-fluid shows us that 0.03 % of glucose is retained by the kidneys.

2. If the proportion between K and Ca is somewhat modified, the glucose retention will decrease, further modification reducing it to 0; in other words the urine contains then as great a concentration of glucose as the circulating-fluid.

3. Evidently we have to deal here with a variable permeability of the glomerulus epithelium to glucose, not only depending on

[1]) UNDERHILL and CLOSSON. Americ. Journal of Physiol. **5**, p. 321, 1916. Quoted from BANG. Der Blutzucker 1913 p. 103.

[2]) POLLACK, Arch. für exp. Path. u. Pharmakol. **64** p. 415, 1911. See also BANG, Der Blutzucker.

[3]) ZWAARDEMAKER and FEENSTRA. These reports 1916, 28 April, 27 May, 30 September.

slight variations of the chemical composition of the circulation fluid, but also on slight differences of temperature. Thus this simple membrane forms a nice object for quantitative studies on permeability under various physiological conditions.

January 1917. *Physiological Laboratory, Groningen.*

**Physiology**. — *"Experimental researches on the permeability of the kidneys to glucose"*. By Prof. H. J. HAMBURGER and R. BRINKMAN.

## II. THE POTASSIUM REQUIRED IN THE CIRCULATING-FLUID IS REPLACED BY URANIUM AND RADIUM.

(Communicated in the meeting of January 27, 1917)

From our preceding paper it appeared that if a RINGER-fluid containing glucose and composed of NaCl 0.7 %, $NaHCO_3$ 0.02 %, KCl 0.01 %, $CaCl_2$ 0.0075 % was circulated through a frog's kidney, 0.03 % of glucose was retained. Now ZWAARDEMAKER and FEENSTRA availing themselves of the conclusions arrived at by N. R. CAMPBELL that potassium is the only radio-active element found in the body, have discovered that in the RINGER-fluid which maintains the beating of the heart, potassium may be replaced by uranium, radium and thorium and that in equivadioactive doses [1]. It seemed of importance to us to determine whether in the above-mentioned circulating-fluid this substitution may likewise be effected with regard to the kidney. Can here too uranium and radium take the place of potassium and if so in what proportion, *in a molecular or in a radioactive one?*

Hence the KCl in the RINGER-fluid which contained 100 milligrammes of KCl per litre was replaced by the equiradio-active quantity; viz. 15 milligrammes of $U(NO_3)_4$ per litre. And it was indeed found that here too the maximum quantity of glucose was retained. If, however, instead of 15 milligrammes of nitrate of uranium, 25 milligrammes are added, only very little glucose is retained. If the litre of RINGER-fluid without K, contains 35 mGr. of $U(NO_3)_4$, no glucose is retained at all.

Now 100 m.Gr. of KCl are chemically equivalent with 112 m.Gr.

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[1]) These Proceedings Vol. XIX p. 99, XX 341 and 633.
Compare also ZWAARDEMAKER, FEENSTRA and BENJAMINS, ibid. Nov. 10 1916.