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

(Communicated in the meeting of Sept. 29, 1917).

III. THE $NaHCO_3$ PERCENTAGE IN THE TRANSMISSION-FLUID [1]).

In a former paper [2]) on this subject we discussed the reason which induced us to enter upon a systematical investigation of the effect which the composition of the RINGER-fluid had upon the retention-power of the frog's kidney with respect to glucose. This investigation

TABLE I.

Effect of the Ca-concentration in the RINGER-fluid on the retention of glucose.

% NaCl	% $NaHCO_3$	% KCl	% $CaCl_2$ 6 aq.	Reduction of transmission-fluid.	Reduction of urine		Retention of glucose.	
					Left	Right		
0.6	0.020	0.010	0.000	0.098	0.095	0 096	0	
0.6	0.020	0.010	0.001	0.10	0.095	0.094	0	v. porta renalis ligatured
0.6	0.020	0.010	0.002	0.090	0.092	0.088	0	
0.6	0.020	0.010	0.004	0.090	0.090	0.090	0	
0.6	0.020	0.010	0.006	0.098	0 10	0.096	0	
In like manner rising with 0.002 % $CaCl_2$ 6 aq. to 0.012 % no retention of sugar.								
0.6	0.020	0.010	**0 012**	0.098	0.080	0.082	0.017	
0.6	0.020	0.010	**0 014**	0.098	0.076	0.075	0.022	
0.6	0 020	0.010	**0.015**	0.09	0.060	0.061	**0.030**	
0.6	0.020	0.010	**0.016**	0.096	0.066	0.068	**0.030**	v. porta renalis ligatured [3])
0.6	0.020	0.010	0.018	0.102	0.102	0.096	0	
0.6	0.020	0.010	0.020	0.098	0.10	0.10	0	

[1]) A more detailed account will be given in the Biochemische Zeitschrift.

[2]) HAMBURGER and BRINKMAN: Verslagen van de Koninklijke Akademie v. Wetenschappen van Jan 27, 1917, p. 944.

[3]) Obviously the results relate to the glomerulusproduct. Cf the above-mentioned paper p. 946.

brought to light that, apart from other factors, the permeability of the glomerulus membrane is, to a high degree, dependent on the $CaCl_2$ percentage of the transmission-fluid and further that this permeability is also affected by the KCl and NaCl concentrations.

We subjoin a series of experiments which demonstrates the effect of *calcium* and which was not published in our first paper. (See Table I, preceding page).

This series of experiments was carried out in February—March 1917. [1])

Evidently glucose-retention took place only when the $CaCl_2$-concentration varied between 0.012% and 0.016%, that is to say the Ca-concentration has its strict limits, and admits of but little variation. Of the ± 0.1% of glucose in the transmission fluid at most 0.03% of glucose was retained.

To determine the effect of *Potassium* in the transmission-fluid the KCl concentration was modified while the NaCl, $NaHCO_3$ and $CaCl_2$ remained the same. Increasing quantities of KCl were therefore added to the fluid composed of NaCl 0.6%, $NaHCO_3$ 0.02%, $CaCl_2$. 6 aq.

TABLE II.

Effect of the KCl concentration in the RINGER fluid. Experiments of March 1917.

% NaCl	% $NaHCO_3$	% K Cl	% $CaCl_2$ 6 aq.	Reduction of transmission fluid.	Reduction urine		glucose retention
					Left	Right	
0 6	0.020	0.000	0.015	0.095	0.070	0.072	0 025 %
0.6	0.020	0.000	0.015	0.090	0.068	0.069	0.021
0.6	0.020	0 002	0.015	0.095	0.070	0.070	0.025
0.6	0.020	0.004	0.015	0.115	0.092	0 088	0.025
0.6	0.020	0.006	0.015	0.10	0.098	0.10	0
0.6	0 020	0.006	0.015	0.085	0.080	0.084	0
0.6	0.020	**0.008**	0.015	0.10	0.070	0.070	**0.03**
0.6	0.020	**0.010**	0.015	0.098	0.070	0.070	**0.03**
0.6	0.020	**0.014**	0.015	0.10	0.065	0 070	**0 03**
0.6	0 020	**0.016**	0 015	0.10	0.070	0.075	**0.028**
0.6	0.020	0.018	0.015	0.092	0.094	0.092	0
0.6	0.020	0.020	0.015	0.080	0.070		0.010
0 6	0.020	0.022	0.015	0.098	0.095	0.098	0

[1]) It is desirable to know whether summer- or winter frogs are used for the experiments. Cf. Verslagen van Jan. 27, 1917 p. 949.

0.15% and glucose 0.1% Some of the results are given in Table II.

It becomes evident that if from the RINGER-fluid *containing the right $CaCl_2$. 6 aq. concentration* viz. 0.015% (see Table I) all the potassium is omitted, glucose is still retained viz. ± 0.02%; if the KCl is increased to 0.005—0.006% all the glucose passes through; at a further increase to KCl 0.008—0.017% the maximum amount of glucose is retained (0.03%); at higher KCl concentrations the retention decreases again.

Hence we see that the potassium is not absolutely necessary [1]); the chief function of the K in the transmission-fluid is probably to balance an excess of Ca.

It appeared from out last paper that the NaCl concentration also affects the results.

The composition of the transmission-fluid thus found could, however, be hardly looked upon as the optimum one *since from a transmission-fluid with* 0,1% *of glucose at most only* 0,03% *was retained.* And this value decreased even when the glucose-concentration in the transmission-fluid was lowered. The reason why also experiments with glucose-concentrations below 0.1% were made was due to the fact that the normal glucose-concentration of frog's blood varies between 0,03 and 0,06%. If we used a glucose-concentration of 0,03—0,04% not 0,03% was retained but at most 0,015%. The glucose retention was, consequently, dependent on the glucose-concentration of the transmission-fluid; a decrease in the glucose-concentration causes a corresponding decrease in the retention. In spite of a great number of experiments, we did not succeed in obtaining a glomerulus-filtrate which contained no glucose. But even if the transmission-fluid contained 0.1% of glucose and moreover the abovementioned favourable Ca- and K-concentrations were used, it not unfrequently occurred, more especially in summer when the frog's have less vitality, that little or no glucose was retained. Probably the most effective composition of the Ringer-fluid had, therefore, not been arrived at.

Increase of the usual $NaHCO_3$-concentration.

We, therefore, attempted to improve upon our transmission-fluid.

[1]) The fact that it is necessary in the transmission-fluid for the heart need not surprise us, because the heart uses potassium in its muscular labour; things are different for the kidney, which is mainly a passive though complicated and sensitive living filter. Besides, the same arterial blood must supply all organs and provide every one with what it needs. Thus it may be understood that the most effective artificial transmission fluid need not have the same composition for every separate organ.

The effect of $CaCl_2$, KCl and NaCl had already been determined; *it only remained to examine the effect of the $NaHCO_3$ concentration.*

Since RINGER it has been generally assumed that in artificial transmission fluids $NaHCO_3$ is indispensable. The present researches have likewise shown that it cannot be dispensed with in the transmission-fluid. One of the functions of $NaHCO_3$ consists as we know in maintaining a very slight actual alkalinity of the body-fluids which would otherwise, owing to the continual formation of acids, pass into an acid reaction. Like serum protein it acts as a buffer; hence we also speak of a tampon or moderator. Besides a specific HCO'_3-action may have to be assumed (E. LAQUEUR).

It appears already theoretically that a concentration of $NaHCO_3$ 0.01 % is too low to act as a sufficient buffer. We shall revert to this later on, in connection with other more theoretical considerations.

RINGER himself added 5 cc. of a 1 % $NaHCO_3$-sol. to 100 cc. of fluid. TYRODE even used 0.1 % of $NaHCO_3$. But 0,02 % of $NaHCO_3$ is the rule in RINGER's fluid. [1]) That the usual concentration of 0.02% of $NaHCO_3$ is too slight for frogs' kidneys could be determined experimentally in the following manner.

If namely to a transmission-fluid composed: NaCl 0.6 %, $NaHCO_3$ 0.02 % $CaCl_2$ 6 aq 0.015 % some neutral red [2]) is added, the colour of the indicator is orange yellow (slightly alkaline), which corresponds with $[H^{\cdot}] = 1.10^{-8}$. It is necessary to use boiled out aq. dest. and to prevent the absorption of CO_2. Now we need only shake this fluid for a moment with air or lead it through an india rubber tube and the colour turns to pink, which points to an acid reaction of $[H^{\cdot}] > 1.10^{-7}$. If, however, one is careful in preparing this fluid then one succeeds in keeping it slightly alkaline. Now if this fluid is transmitted through the kidney, the latter becomes evidently acid, which is manifested by the red colour it assumes and also the metabolism products which have passed into the urine, colour the indicator red after some time. We have made the oxygenation in the experiment as intense as we could to eliminate metabolism products of greater acidity as much as possible, without succeeding, however, in keeping the reaction of the urine neutral.

What is the reaction of the normal urine of the frog?

It is not difficult to obtain it by squeezing out the bladder of

[1]) Cf. e.g. BAYLISS: Principles of General Physiology, 1916, p 211.

ZWAARDEMAKER and his collaborators also use this concentration of 0 02 %. (See e g. Proceedings 1916, April 28, May 27, Sept. 30).

[2]) The reader will be aware that the colour of this vital indicator is at $[H^{\cdot}] = 1.10^{-7}$ pink, at $[H^{\cdot}] = 1.10^{-8}$ orange yellow and at $[H^{\cdot}] = 1.10^{-9}$ yellow.

the animal. It appears then that the liquid is slightly alkaline.

The same can be demonstrated in the following way. If 1 cc of a saturated watery neutralred solution is injected into the back lymphsac, an investigation, half an hour after, brings to light the following facts: skin, muscles, brain and spinal cord are pink, intestine yellow and pink, depending on place and degree of peristalsis, but the urine is yellow, and is, therefore, though only slightly, alkaline. When we followed the practice hitherto adopted and transmitted a RINGER fluid containing 0.02% of $NaHCO_3$, the quantity generally used for the heart, then the urine after some time became permanently pink, that is to say, acid. *Hence we see that the protective value of $NaHCO_3$ 0.02 % is not great enough.*

At the same time it appeared that the acidity of the urine and the diminution or loss of the kidney's retention-power to glucose went hand in hand. As an example we add the following experiment.

Transmission from the aorta with a sol. of NaCl 0,6 %, $NaHCO_3$ 0,02 % KCl 0,01 %, $CaCl_2$.6 aq. 0,016 %, and glucose 0,098 %, saturated with O_2; no india rubber tube was used; the colour of the solution is orange owing to neutralred. The first urine is yellow and has a reduction of 0,06 %; the latter red, its reduction being 0,090 %, in other words: *now that the urine has become acid, the kidney is found to have lost the power of retaining glucose.*

The obvious course was now to increase gradually the $NaHCO_3$-conc. of the transmission-fluid. It was raised to **0,090** %. Now we had therefore a transmission-fluid of the following composition: NaCl 0.6 %, $NaHCO_3$ 0.90 %, KCl 0 010 %, glucose ± 0.1 % and had to discover the suitable $CaCl_2$ 6 aq. concentration. Table III contains the results of these experiments.

In the first place it is observed that a much greater quantity of glucose is retained than before. It amounts to no less than 0.079%. But this requires a concentration of $CaCl_2$ 6 aq. of 0.024—0.030 %.

Below this concentration and above it little is retained. The $CaCl_2$ 6 aq. conc. necessary for a maximum glucose retention has, therefore, risen from 0.015 % (Cf. tables I and II) to 0.024 %—0.030%. This need not surprise us for the concentration of ions of Ca is repressed by $NaHCO_3$ and the ions of Ca are an important factor. It may, therefore, be said that *an increased $NaHCO_3$ conc. in a transmission-fluid with* ± 0.10 % *of glucose raises the maximum retention from* 0.03 % *to an average of* 0.06 %.

Further increase of the $NaHCO_3$ concentration.

We did not stop short, however, at this increase of the $NaHCO_3$.

concentration. *We have namely made the titration-alkalinity of our transmission fluid equal to that of frog's serum.*

TABLE III.

Effect of an increased $NaHCO_3$-concentration.

Transmission from the aorta of NaCl 0,6 %, $NaHCO_3$ **0,090** %, KCl 0,010 % and $CaCl_2 . 6$ aq. 0,020 %—0,050 %; colour of transmission-fluid orange-yellow caused by neutral red.
(Experiments of June—July 1917).

$CaCl_2 . 6$ aq.	Reduction transmission fluid.	Reduction urine.	Retention glucose	Colour urine [1])
0.020	0.100	0.098	0.020 %	colourless
0.020	0.105	0 080	0.025	colourless
0.022	0.105	0.080	0.025	colourless
0.024	0.115	0.062	**0.053**	light-yellow
0 025	0.100	0.040	**0.060**	light-yellow
0.025	0.10	0.041	**0.059**	yellow
0.026	0.115	0.058	**0.057**	yellow
0.028	0.115	0.064	**0.051**	greenish yellow
0.028	0.111	0.052	**0.059**	yellow
0.030	0.105	0.042	**0.063**	light yellow
0.030	0.105	0.026	**0.079**	light yellow
0.030	0.105	0.031	**0.074**	light yellow
0.031	0.115	0 102	0.013	colourless
0.032	0.115	0.10	0.005	very light yellow
0.032	0.115	0.091	0.024	colourless
0.035	0.10	0.089	0.011	colourless
0.040	0.102	0.090	0.022	first light yellow afterwards colourless
0.045	0.098	0.075	0.023	colourless
0.050	0.098	0 080	0.018	colourless

For this purpose frog's serum was titrated with $^1/_{25}$ normal tartaric acid with neutralred paper as an indicator, according to the method of SNAPPER [2]). 1 cc. of defibrinated only slightly haemolytic

[1]) For the meaning of this column see p. 677.

[2]) J. SNAPPER: Biochemische Zeitschrift **51**, (1913), 88.

frog's serum required 0.85 cc. of $^1/_{25}$ normal tartaric acid. The titration alkalinity of frog's serum is, therefore equal to that of a 0.034-normal or a 0.285 % $NaHCO_3$-sol. We have, therefore, given a $NaHCO_3$ conc. of 0.285 % to our transmission-fluid; to prevent a resulting increase of osmotic pressure the NaCl conc. was lowered to 0.5 %. Now again it was obvious that the suitable $CaCl_2$ 6 aq. conc. would have to be raised again, as the conc. of the free ions of Ca would again be repressed.

The result will be found in Table IV.

The maximum quantity of glucose begins to be retained at $CaCl_2$ 6 aq. 0.030 %; so this concentration is still somewhat higher than if $NaHCO_3$ 0.9 % is used (then the conc. of $CaCl_2$ 6 aq. was, as appears from Table III 0.024 %).

Table III shows that if the $CaCl_2$ 6 aq. rose to above 0.030 %, the retention of glucose began to decrease. In Table IV, however, when a higher conc. of $NaHCO_3$ was used, this was not the case; even if the $CaCl_2$ 6 aq. conc. rises to 0.080 %, the glucose-retention remains pretty well invariably high viz. an average of 0.07 %. One will be inclined to assume that this is due to the fact that in the latter case the most favourable conc. of ions of Ca is brought about automatically.

Indeed when through the RINGER fluid containing 0.285 % of $NaHCO_3$ and 0.080 % of $CaCl_2$ 6 aq., oxygen is led for some time, a precipitate is formed of $CaCO_3$ [1]). The following physico-chemical exposition will make matters clearer.

$$\frac{[Ca^{\cdot\cdot}]\,[HCO_3']^2}{H_2CO_3} = K, \text{ or } \frac{[Ca^{\cdot\cdot}]\,[HCO_3']}{[H^{\cdot}]} = K_1.$$

The latter formula teaches that the concentration of the free ions of Ca is only dependent on the conc. of the ions of H and those of HCO_3 or also that the amount of Ca salt makes no difference, when $[H^{\cdot}]$ and $[HCO_3^{\cdot}]$ are present in a certain suitable proportion. Hence we see that there must be a buffer-system for ions of Ca in this fluid.

To sum up: in order to maintain a proper concentration of ions of Ca it appears that not only the conc. of ions of HCO'_3, but also that of ions of $H^{\cdot}$ is of importance. A satisfactory regulation of the conc. of ions of H is not so easy to arrive at in our circumstances, where, if the kidney is to function well, the fluid must be saturated

[1]) We have invariably observed at the ultrafiltration of bloodserum that the clear filtrate becomes troubled when shaken with air, owing to the formation of $CaCO_3$, which was kept in solution by CO_2.

with O_2; this regulation will have to be further investigated.

An experimental confirmation of our view was obtained by determinations of the electric conductivity of the system $NaHCO_3$ and $CaCl_2$. This will be discussed in a subsequent paper.

TABLE IV.

Effect of a still greater increase of the $NaHCO_3$-Concentration.

Transmission of NaCl 0,5 %, KCl 0,010 %, $NaHCO_3$ 0,285 %, $CaCl_2.6$ aq. 0,028—0,080 %, glucose ± 0,1 %.

All solutions have been made again in boiled out water and saturated with oxygen.

(Experiments of July 1917.)

% $CaCl_2.6$ aq.	Reduction transmission fluid.	Reduction urine.	Difference (Retention of glucose).
0.028	0.145	0.130	0.015
0.028	0.091	0 076	0.015
0.030	0.091	0.038	0.055
0.030	0.092	0.027	0.065
0 032	0.088	0.066	0.022
0.032	0.091	0.056	0.035
0.034	0.098	0 042	0.056
0.034	0.091	0.040	0 051
0.036	0 098	0.052	0.046
0.036	0.125	0.040	0 085
0.038	0.125	0.035	0.090
0.040	0.106	0.031	0.075
0.042	0 105	0.029	0.076
0.044	0.105	0.045	0.060
0 048	0.105	0.035	0.070
0.050	0.105	0.031	0.074
0 052	0.105	0.053	0.052
0.056	0.105	0.050	0.055
0.060	0.115	0.062	0.053
0.064	0.115	0.058	0.057
0.080	0.115	0.041	0.074

But the state of things in the RINGER fluid is still more complicated than in the system $CaCl_2$ and $NaHCO_3$, especially because the fluid contains a rather considerable quantity of NaCl. This renders the determination of the conc. of free ions of Ca rather difficult.

It seems that the equilibrium of the system $CaCl_2$, $NaHCO_3$ and NaCl, so important to life, has hitherto not been studied. We intend to revert to this subject later on. *At any rate we have now obtained a transmission fluid of which, of the* 0.1 % *of glucose, on an average* 0.07 % *is retained, and in which automatically that conc. of ions of* $Ca^{\cdot\cdot}$ *sets in which causes* $\pm$ 0.07 % *of the* 0.1 % *of glucose to be retained.*

It is this transmission-fluid which has enabled us to obtain a *urine free from sugar*, which had hitherto been found impossible. According to BANG[1]) frogs' blood gives a reduction which corresponds to 0.03—0.05 % of glucose. We accordingly found in September a reduction value of 0.04—0.06 %. Now the question was: will the kidney be able to keep back all the sugar from a RINGER-fluid of the above composition and containing 0.05 % of glucose. The unanimous result of our experiments proved that this was indeed the case,

All glucose was likewise retained, even when the RINGER-fluid contained 0.06 % of glucose.

Now it will be of importance to determine to what pitch hyperglycaemia can be raised before glycosuria sets in, in other words *how much sugar the kidney can bear*. This question will be treated in a subsequent paper.

CONCLUSIONS.

The fact that by modifying the composition of the RINGER-fluid the colloid state of the glomerulus-epithelium can be regulated in such a manner that it either admits or does not admit sugar, seems to us of great importance, for now it has become superfluous to assume as an explanation of physiological glucose-retention, that substances are found in the serum which keep back the glucose in colloid compounds and that the glucose cannot pass through the glomerulus-membrane in that form. That this supposition is no longer necessary will afford satisfaction, after MICHAELIS and RONA and also ABEL by dialysis-experiments and we by ultra-filtration have found that parchmentpaper and ultra-filters of celloidin allow all glucose in the

[1]) J BANG, Der Blutzucker 1913. J. F. BERGMANN, Wiesbaden.

serum to pass, which as we observed before [1]) is not a strict proof that sugar cannot be present in a composition with a serum-compound which can pass through these two membranes, but not through the glomerulus-epithelium. Now, however, it has been demonstrated that the glomerulus epithelium can keep back the glucose as such.

We have evidently to deal here with *a new form of permeability*: cells, here the glomerulus-epithelium, allow salts to pass, but not the likewise crystalloid sugar, which under the circumstances is highly useful; for thus a substance necessary for our nutrition is kept in circulation. As far as we can see we find ourselves confronted here by a phenomenon not observed before. The intestinal epithelium and likewise the pleura and the peritoneum are permeable to salts as well as to glucose, the red blood-corpuscles of most animals are *impermeable* to salts and to sugar both [2]).

Finally we wish to point out another fact. An examination of table III makes it evident that although the transmission-fluid contained neutral red, mostly a colourless urine was obtained in these cases, therefore, the neutral red had been kept back by the glomerulus-epithelium. That the urine was free from neutral red appeared from the fact that neither the addition of acid nor that of alkali to the urine caused colouring.

Hence we may assume that *if the $NaHCO_3$ concentration is high enough*, the glomerulus-membrane is impermeable to the colloid neutral red. If the $NaHCO_3$ conc. amounts to only 0.02 % then the glomerulus-filtrate becomes red, because the RINGER-fluid, on being transmitted, grows too acid. That this is really only a glomerulus-product appears when for instance the porta renalis is ligatured, for then the urine-secretion through the tubulus epithelium is prevented (cf. our first publication) [3]). According to table III, however, the urine in some of these experiments was yellow, but this colouring originated from neutral red, which is excreted by the tubulus epithelium; this is confirmed by the experiments of HÖBER and KONIGSBERG, to which we shall have to refer again presently.

Our experiments also throw a light on the contradiction between the results of the experiments of GERZOWITSCH [4]) and those of HÖBER [5]).

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[1]) Cf. our first paper in Verslagen Jan. 1917.

[2]) Only some blood corpuscles viz. those of man, of the monkey and of the dog seem, to a certain extent, permeable to sugar

[3]) Meeting of January 27, 1917.

[4]) GERZOWITSCH: Zeitschr f. Biologie, **66**, 391, (1916).

[5]) HÖBER und KÖNIGSBERG: Pflügers Archiv **108**, 324. (1905).

GERZOWITSCH namely dissolved neutral red in ordinary RINGER fluid, the composition of which is not stated, and obtained at *arterial* transmission a "*coloured*" glomerulus filtrate; he does not say whether the colour was red or yellow orange. HÖBER on the other hand injected neutral red into the back lymphsac, and on examining the capsule microscopically he saw a "*colourless*" glomerules filtrate.

Probably the contradiction may be thus explained: GERZOWITSCH uses "eine für den Frosch physiologische RINGER-lösung". This must have been one, containing 0,02 % $NaHCO_3$ (see above p. 672) and this gives an acid i.e. a pink urine. HÖBER and KONIGSBERG, however, worked under physiological conditions, for normal blood flowed through the frogs; only some vital colouring-matter had been introduced into the back lymphsac. The glomerules filtrate was, just as with us, colourless, but in its passage through the ducts it took up neutral red, which was secreted by the epithelium of the tubuli. This would be in conformance with the yellow colour of the urine, which we obtained when under practically physiological conditions a suitable RINGER-fluid was used.

SUMMARY.

1. If, the usual RINGER-solution containing 0.02 % $NaHCO_3$, passes through the kidney, then it is found that of the 0.1 % glucose at most 0.03 % is retained (table II) in however favourable a manner we may vary the Ca and K percentage.

A considerable increase of the glucose retention may be attained if the $NaHCO_3$ conc. of the transmission fluid is raised from 0.02 % *to* 0.090 %.

2. *Experiments with neutralred teach that the cause of this phenomenon is connected with the reaction of the transmission-fluid.*

If the alkalinity of the latter (i. e. its protective value) is so slight that on being transmitted it is easily acidified, then the urine formed gives an acid reaction (neutralred becomes pink) and little or no glucose is retained.

If, however, the $NaHCO_3$ conc. is raised to 0.090 % then the artificial urine remains alkaline (neutral red remains yellow) *and of the* ± 0.1 % *of glucose about* 0.06 % *is retained.*

In order to obtain this favourale result, however, the Ca-concentration, the most effective conc. of which amounted hitherto to $CaCl_2$ 6 aq. 0.015 % (see table I) must be raised to 0.024—0.030% (table III), but not higher. That the $CaCl_2$ conc. should have to be raised if the $NaHCO_3$ conc. is increased need not surprise us, since an increase of the $NaHCO_3$ conc. impedes the dissociation of the $CaCl_2$ and a sufficient concentration of ions of Ca in the transmission fluid is of great importance.

3. *The kidney can retain even more than* 0.06 % *of glucose if*

the $NaHCO_3$ conc. is raised to 0.285 % *i.e. the conc. which corresponds to the titration-alkalinity of frogs' serum.* But then again more $CaCl_2$ must be added, *at least* 0.030% (table IV).

4. It is remarkable that otherwise than in the experiments in which $NaHCO_3$ 0.09 % was used (table III), now that the conc. is 0.285 %, an addition of more $CaCl_2$ 6 aq than 0.030 %, even of much more, does not impair the retention (table IV). There are reasons to assume that the most favourable conc. of ions of Ca brings itself about automatically, when more $CaCl_2$ is added. *The* RINGER-*sol. in the latter case, when of* ± 0.1 % *of glucose upon an average* 0.07% *was retained, was composed as follows*: *NaCl* 0.5 %, *$NaHCO_3$* 0.285 %, *KCl* 0.01 %, *$CaCl_2$ 6 aq.* 0.040 %.

5. *If the transmission-fluid contained* 0.05 % of glucose, the average concentration found in frogs' blood, *then a sugarless urine was obtained.* This was even the case when the RINGER-sol. contained 0.06 % of glucose.

6. This result seems important to us from a physiological-clinical and from a general biological point of view; from a physiological-clinical point of view, because the retention of sugar by the kidney has now been reduced to a question of permeability, so that the supposition that glucose is bound by one of the serum substances (sucre virtuel of LÉPINE) has become altogether unnecessary. Evidently the chemical composition of the transmission-fluid determines the state of the glomerulus epithelium, and consequently the permeability of the membrane to sugar. The results are important from a general biological point of view, because we have to deal here with a new form of permeability, one in which cells under physiological conditions, though easily permeable to salts, are impermeable to the likewise crystalloid glucose, *a form of permeability hitherto unknown and very useful under the circumstances.*

Groningen, September 1917. *Physiological Laboratory.*
