

# A Discourse

ON

## PERMEABILITY IN PHYSIOLOGY AND PATHOLOGY.

*Delivered at the University of London, under a Scheme for Exchange of Lectures in Medicine between England and Holland,*

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### I. INTRODUCTION: SOME HISTORICAL REMARKS.

THE problem of permeability plays a fundamental rôle in physiology as well as in pathology and pharmacology. For permeability means a power of admitting certain substances into the cell and of excluding others, and thus in physiology and pathology we endeavour to substitute for the mysterious principle of "conscious selection" of cells towards some substances, and for the so-called "vital activity," simple physical and chemical factors, no matter how complicated the coöperation of these factors may ultimately prove to be. This means also in pharmacology the finding of those forms of drugs which by penetrating into the interior of the cell are able to be of use there.

As an example, let us consider the acinus of the salivary gland. From the outside the epithelial cells are supplied with nutritive materials from the blood, while from the side next the lumen the saliva is secreted. The neo-vitalist has a simple explanation for this. According to him, the cells of the salivary glands take up, actively and selectively, substances from the blood, and secrete saliva through the so-called physiological motive force. If he introduces a salt solution into the alimentary canal, it is again the physiological vital force, an attribute of the living cell alone, that brings about the absorption. For, if the epithelium be injured by a poison, the process of absorption is entirely altered; this, he says, is because the dead epithelium lacks the physiological motive force. The same line of argument is sufficient for him to explain secretion and absorption in any part of the body. The neo-vitalist, it is true, freely acknowledges that physical factors also play a part in secretion and absorption, but the fundamental principle of these processes is a specific attribute of the living cell alone.

But what does this term "physiological motive force," so often used by Heidenhain, and by many after him, imply? Only this: we do not know and have to abide by that. It appears to me, however, that it is the duty of the biologist to endeavour at least to explain the phenomena along physical and chemical lines. Is it not probable that in the formation of saliva the cells by virtue of their properties of permeability allow the permeation of certain food-stuffs from the blood? That they convert these by chemical action and allow the products—namely, the saliva—to pass out at the other end, aided probably during this process by electrical currents, the cataphoresis? Such lines of thought appeal more to the modern physiologist than the idea that the cell, according to its needs, takes up actively, by virtue of a vital power, the necessary elements from the blood and discharge these into the lumen of the acinus. Some think that this physico-chemical explanation is much too simple and that the delicateness of the processes of life are thereby ignored. Yet upon more thorough investigation this proves not to be so, and one becomes more and more impressed by the marvellous intricacy and efficiency of these physico-chemical processes, while the number of unsolved questions continually increases.

I could add to these two examples, where permeability must play an important part, a great many others; but a detailed discussion would practically

involve the whole of physiology. I shall be obliged, therefore, to limit my discussion to a few illustrative examples. In the choice of these I shall, while regardful of the *suum cuique*, also refer to my own work which, alas, has of late been too often overlooked. Who is there, however, of the senior among us who has no reason to complain of neglect? Moreover, these lectures are, I think, expected to have a personal flavour, and it is better so.

The study of the permeability of the animal cell may be said to have been introduced by researches which I carried out in 1889.<sup>1</sup> These investigations arose from my work in 1883 on the influence of salt solutions on the escape of colouring matter from the red blood corpuscles.<sup>2</sup> A year previously<sup>3</sup> (1882) the well-known botanist Hugo de Vries had conducted experiments upon the force with which plant cells attract water. He employed three biological methods, of which the plasmolytic gave the most reliable results. This method consisted in using a salt solution strong enough to bring about a slight separation of the contents of the plant cell from the cell membrane—in other words, to induce plasmolysis in the cell. This separation of the protoplast from the membrane (plasmolysis) was due to the fact that the water-attracting power of the surrounding fluid was something greater than that of the cell-contents. Hence de Vries concluded that solutions of any salts which caused the same degree of shrinkage when acting upon the same cell must have identical water-attracting powers. He called such solutions, having identical water-attracting powers, *isotonic* solutions; and the simple relations which appeared to exist between the concentrations of such solutions of different salts he named their isotonic coefficients. De Vries thus regarded the protoplast as semi-permeable—in other words, perfectly permeable to water but impermeable to substances dissolved in it. In the following year now (1883) I investigated the question whether the same phenomena which de Vries had observed in the plant cell applied also to the animal cell.<sup>2</sup>

I chose the red blood corpuscle as my material. The first step was to try to find a concentration capable of inducing plasmolysis in these cells. But I failed to find it. No plasmolysis could be observed in my experiments. Then I turned to the study of the escape of coloured matter from the red corpuscles. And so it was found that the red blood corpuscles were also subject to the law of isotonic coefficients. Between the concentration of salt solutions causing the escape of coloured matter from the same blood corpuscles the same numerical relation appeared to exist as between the concentration of salt solutions inducing plasmolysis in the same plant cell. These investigations on the blood corpuscles, as you no doubt recollect, marked the beginning of modern physico-chemical research in the medical sciences.

It may be observed in passing that it has repeatedly been stated that Van't Hoff's theory of osmotic pressure laid the foundation for these investigations on the blood corpuscles, but this is decidedly a mistake. The real basis for my work was given in de Vries's researches in plant physiology of 1882. These investigations and my own hæmatological researches furnished important data for an experimental proof of Van't Hoff's theory, which was based principally on thermodynamic considerations and on Pfeiffer's researches. Besides, Van't Hoff's theory was published for the first time two years later, in 1885,<sup>4</sup> and absolutely needed Arrhenius's theory of electrolytic dissociation (1887)<sup>5</sup> as a supplementary one to be accepted.

As already mentioned, I found that for every salt a concentration could be obtained in which the least resistant blood corpuscles lost their colouring matter, and in these concentrations the salts had the same power of attracting water. How could this phenomenon be explained? Could it be possible that these salt solutions, which had the same power of attracting water, brought about a slight shrinkage as in the case of the plasmolysis of plant cells? This

could hardly be accepted. It was more plausible that the salt solutions used caused a swelling which a number of the blood corpuscles could not withstand without losing their colouring matter. If this assumption were true, then it would follow that the serum of blood could also be diluted with water without the occurrence of hæmolytic. This, indeed, proved to be the case. The serum of animals could be diluted with percentages of water up to multiples of ten, that of man with 50 per cent. and more before colouring matter was discharged, and the conclusion was that a diluted serum which just caused hæmolytic to set in was isotonic with a salt solution which could do the same. A simple calculation now showed that the blood serum of the majority of warm-blooded animals, including man, was isotonic with a NaCl solution of 0.9 per cent., and not of 0.6 per cent., as was generally thought at the time, and which had always been called the physiological NaCl solution. In connexion with this I therefore introduced the terms *hyperisotonic* and *hypoisotonic*, according as the solutions had respectively a stronger or weaker power of attracting water than normal serum. All this was proved by determination first of the corpuscular volume<sup>6</sup> and afterwards of the freezing-point. I, and after me Hedin<sup>7</sup> and others, found that the blood corpuscles shrink in hypertonic and swell in hypotonic solutions. I considered, and so did they, that the blood corpuscles, like the plant cells of de Vries, were semi-permeable, and thus allowed only water to pass through.

## II. PERMEABILITY OF RED CORPUSCLES TO ELECTROLYTES (ANIONS AND CATIONS).

Experiments by quantitative chemical methods in 1889<sup>1</sup> convinced me quite unexpectedly, however, that this was not the case—in other words, the blood corpuscles were not semi-permeable. For when they were mixed with a salt solution which was isotonic with the serum their volume, it is true, remained unchanged, but they lost chlorine, and, as I was able to demonstrate afterwards, an interchange took place between the contents of the red corpuscles and their environment. The elimination of chlorine was especially studied. Through these researches the question of permeability was introduced for the first time, and since then permeability has been a prominent item on the programme of large numbers of physiological inquiries. When we come to think of it, it is somewhat remarkable that it had not been earlier realised that permeability to dissolved substances is necessary for the life of cells, whether for the assimilation of nutritive substances or for the elimination of waste products. Yet it was only with difficulty that we succeeded even in recent times in getting the idea of permeability, to inorganic substances for instance, universally accepted.\*

After the permeability of blood corpuscles to chlorine was established in 1889 I was able in 1892<sup>9</sup> to offer additional proofs. I inquired whether, seeing that the hæmolytic of the red corpuscles is so sensible to slight modifications in the concentration of the surrounding fluid, the erythrocytes of the arterial and venous blood of the same animal would show any difference in respect to the salt concentration at which colouring matter was discharged. Such a difference proved to exist; the carotid blood of a horse showed commencement of hæmolytic in a NaCl solution of 0.61 per cent., the jugular blood, on the other hand, had already hæmolytic in a NaCl solution of 0.62 per cent. This fact was the starting-point of a new series of investigations on permeability. On the assumption that this phenomenon was in some way connected with the carbon

dioxide concentration, we investigated the influence of CO<sub>2</sub> on the loss of colouring matter. This influence proved to be very great, and led to the study of the influence of carbon dioxide on the distribution of the constituents of the blood between corpuscles and serum. In the first place, it appeared that when blood was treated with a quantity of carbon dioxide, which was well within physiological limits, an interchange of constituents took place between blood corpuscles and blood serum. If the carbon dioxide was driven out the original partition was fully restored; the phenomenon was a reversible one, and the conclusion was drawn that it must be of importance to life. For while the blood flows through the capillaries of the tissues it is acted upon by carbon dioxide, and when it reaches the lungs the carbon dioxide is expelled again. Of the changes which carbon dioxide brings about in the distribution of the blood constituents between the blood corpuscles and serum the most important was a transit of chlorine into the corpuscles and an increase of alkalinity—that is, in the amount of diffusible alkali of the blood serum.<sup>†</sup> There was also a considerable swelling of the blood corpuscles. With every act of respiration, therefore, rhythmical changes take place; while the blood circulates through the tissues the blood serum becomes more alkaline, poorer in chlorine and phosphoric acid, and poorer in water. Owing to the latter the concentration of albumin and other organic substances in the plasma increases. When the blood reaches the lungs again the carbon dioxide is expelled and the partition of the constituents is as before. These results can be confirmed by comparative experiments on normal carotid and jugular blood. It would carry me too far if I were to enter upon the significance of these phenomena in metabolism. It is obvious that in venous congestion the interchange between blood corpuscles and plasma is much more pronounced than in the normal circulation.

These various facts about CO<sub>2</sub> were useful to British and American investigators during the war. It may be observed that they have also formed the basis for the well-known researches of Korányi on the diagnosis and treatment of heart diseases.<sup>11</sup> This investigator has shown how an uncompensated heart disease can be differentiated from renal insufficiency. In both cases the lowering of the freezing-point of the blood serum is increased, in the case of the heart disease through the accumulation of carbon dioxide, and in the case of renal insufficiency through the accumulation of waste products. In both cases a decrease in the amount of chlorine in the blood plasma is observed. To distinguish between these two diseases Korányi suggested that oxygen should be passed through the blood drawn from the patient. If it is a case of uncompensated heart disease, then there is an increase of chlorine, and also the freezing-point of the serum becomes normal again. If these phenomena are absent then the diagnosis is renal insufficiency. Korányi also worked on the same lines in the therapeutic direction. In cases of uncompensated heart disease he administers oxygen; the surplus of carbon dioxide disappears, and with it the cyanosis. The blood undergoes the same changes which are observed in vitro when oxygen is passed through; thus the blood corpuscles surrender water to the plasma, with the result that the percentage of chlorine increases. It is obvious that in consequence of this dilution of the plasma with water the viscosity of the blood will diminish and the circulation be improved. This improvement of the circulation serves a double purpose: firstly, the blood of the coronary system will feed the heart better than when the flow of blood is slow on account of the great viscosity of the plasma. The improvement in the circulation is shown by the kidney function. The diuresis increases, and this is further facilitated by the increased amount of chlorides in the plasma. The special value of the oxygen inhalation is seen in the fact that through the improved action of the

\* It may be remarked en passant that in the literature one repeatedly comes across the idea that the question of permeability originated in the lipid-theory of Meyer-Overton.<sup>8</sup> This theory, however, only appeared in 1899, ten years later, and its sole object was the study of the conditions under which organic substances could penetrate the wall of the cell. According to that theory, as you know, this penetration is considered to be subject to the solubility of the substance which is experimented with in the lipid cell-surface.

† An increase of the amount of alkali in the serum had been already seen by Zuntz<sup>10</sup> in saturating blood with CO<sub>2</sub>.



injection of foreign blood corpuscles, of complement-fixation, hæmolysis, agglutination, and so many other things? But also vice versa, and this is worth repeating, the study of the osmotic relationship of the erythrocytes was indispensable for the correct application of red corpuscles in the researches on immunity. It is owing to the above-mentioned advantages of the red corpuscles that the results obtained with them have repeatedly been the guides to important researches on cells and tissues which can only with difficulty be examined. We can also mention other cells which have the same advantages as the red corpuscles—namely, the white corpuscles; for these also are cells which can be easily examined in an isolated condition in their natural environment. But outside my laboratory they have, unfortunately, scarcely been examined as to their permeability. This is the more unfortunate because in the phagocytic power one has an excellent means of making accurate quantitative observations on life, or rather on one of the vital attributes of life, after the equilibrium has been disturbed by the addition of definite substances to the medium. I have outlined in a monograph (1912) some of the interesting results which can be obtained from a study of these cells.<sup>24</sup>

Of the isolated cells, which have been examined by me in a manner similar to that used for the red corpuscles on their osmotic behaviour towards salt solutions, can be mentioned spermatozoa, lymph gland and epithelial cells, the parenchymous cells of the liver, spleen and kidney, and also the nuclei of all these cells, but I cannot expatiate here on these.

I will now go over the question as to whether the permeability is constant for one and the same kind of cell.

#### V. THE PERMEABILITY CHANGES (VITAL PERMEABILITY).

It is now some 20 years since I pointed out for the first time that the permeability of one and the same kind of cell has no constant value, but is dependent on the physiological conditions of these cells.<sup>1</sup>

1. *Influence of CO<sub>2</sub>, Acids, and Alkalies on Red Corpuscles.*—As already mentioned, I stated in 1892 that the permeability of red and white corpuscles changes quantitatively in a rhythmic way with every act of respiration. Snapper also demonstrated (1912)<sup>25</sup> in my laboratory by purely chemical experiments that the permeability of red corpuscles is changed by the influence of traces of sulphuric acid, and also in diseases, accompanied by fever, especially in pneumonia.

2. *Influence of MgSO<sub>4</sub> on Intestinal Epithelium.*—Further, in 1898,<sup>26</sup> I was able to show that intestinal epithelium with great difficulty allowed of the passage of common salt after it had been in contact with a solution of magnesium sulphate.

3. *Experiments on Permeability of Glomerular Membrane to Glucose.*—These experiments demonstrated the variable permeability to inorganic substances. Of late years, however, experiments have been performed in my laboratory with organic substances, more especially with glucose and its isomers and stereo-isomers. These experiments chiefly dealt with the permeability of the glomerular epithelium to these substances and the profound influence which is exercised on it by very minute physiological changes in the liquid flowing through the glomerular capillaries. I shall try to deal with the subject in a chronological order.<sup>27</sup> Since the time of Claude Bernard it has been known that though sugar is never absent from the blood it rarely appears in the urine of normal individuals. What is the cause of this, and why does sugar not appear in the urine until its amount in the blood exceeds a certain limit? Several possibilities have been considered. I shall not dwell on these; I only wish to point out that discussions on these possibilities have not reckoned with the fundamental question whether the kidney allows free glucose to pass through or not. Experiments were made with perfused kidneys; to ensure that the perfusing liquid contained sugar

exclusively free and not as a colloidal compound a Ringer's solution containing sugar was taken. If the concentration of sugar in the artificial urine formed was the same as that of the original perfusing liquid the conclusion could be drawn that the kidney is permeable to glucose. After this it could be determined whether addition of serum causes a retention of sugar. Should this be the case, it would be practically certain that there is present in the serum a substance which binds the sugar in a form that the glomerular epithelium does not allow to pass through, and eventually it could be investigated what the substance in the serum is. With these investigations Dr. R. Brinkman and I have kept ourselves busy for more than two years; through them we have come to the most unexpected results, which now and then led us into side-tracks which were important in themselves.

Frogs were exclusively used for the experiments; the kidneys were perfused through the aorta at a pressure up to 60 cm. with Ringer's solution (NaCl 0.6 per cent., KCl 0.01 per cent., NaHCO<sub>3</sub> 0.02 per cent., and CaCl<sub>2</sub> 0.02 per cent.) containing 0.05 per cent. of glucose. Approximately 150 c.cm. passed through the kidneys per hour.

The urine is formed in the glomeruli. To grasp this a knowledge of the circulation in the frog is needed; in contrast to what we find in warm-blooded animals, the renal arteries supply the glomeruli, and to a very small extent the tubules, which get their blood-supply almost wholly from the renal portal vein which carries off the greater part of the blood from the hindquarters. It now appears that if a liquid is made to flow through this latter vessel under a pressure of 60 cm. not a drop of urine is excreted. Excretion takes place only under a much higher pressure, and even then very slowly. It is therefore clear that in our experiments, where the liquid flows through the renal arteries, the urine is derived from the glomeruli.

The fact that it is possible thus functionally to separate the contents of the glomeruli and the tubes so well from each other makes the frog an admirable object for the study of the formation of the urine, and British authors are very well acquainted with this fact. In our earlier experiments the percentage of glucose in the urine and the perfusion liquid was always the same. These results were also obtained after ligature of the renal portal vein. It was therefore obvious that the urine obtained was a purely glomerular filtrate so far as glucose was concerned, and that the glomerular membrane was permeable to glucose.

But further experiments made it doubtful whether the usual Ringer's fluid was the physiological one for the perfusion of the kidneys. Systematic changes in the fluid taught us that for the permeability of the glomerular membrane the concentration of the free calcium-ions and of NaHCO<sub>3</sub> are factors of preponderant importance, and that in using the right concentrations the urine was free from sugar; in other words, the glomerular membrane now showed itself impermeable to physiological quantities of glucose. As regards the concentration of the NaHCO<sub>3</sub>, its proportion in the usual Ringer's solution is too low. No glucose was retained in the absence of NaHCO<sub>3</sub>. It is known that one function of NaHCO<sub>3</sub> is to preserve a certain alkalinity in the body fluids. The sodium phosphate and sodium protein also take part in this. In this connexion these substances are consequently called tampons, buffers, regulators, or moderators. Most investigators in their perfusion liquids use 0.02 per cent. NaHCO<sub>3</sub>, but it appears to us that this concentration is too weak for perfusion through the frog's kidney, for if to the solution made up of NaCl 0.6 per cent., NaHCO<sub>3</sub> 0.02 per cent., KCl 0.01 per cent., and CaCl<sub>2</sub> 0.0075 per cent. a little neutral red is added, then although the perfusion liquid is alkaline the urine becomes acid. The normal urine of the frog shows a weak alkaline reaction with neutral red. The buffer concentration of 0.02 per cent. was thus too weak to keep the urine reaction a weak alkaline one. But what is still more important, the retentive powers of the kidneys for sugar grow considerably weaker and finally disappear altogether. Clinicians will immediately think of the relation existing between acidosis and glycosuria.

We then determined the alkalinity of the frog's serum by titration with neutral red paper as indicator (Snapper). This corresponded to a NaHCO<sub>3</sub> solution of 0.285 per cent., and consequently a Ringer's solution was made with the following composition: NaCl 0.5, KCl 0.01, NaHCO<sub>3</sub> 0.285, CaCl<sub>2</sub> 0.015 per cent. The urine now was perfectly free from sugar and was no longer acid. These results have been fully confirmed by Bahlmann (1920).<sup>28</sup> Hence the glomerular membrane has the power of retaining free glucose, and this power is governed by the chemical composition of the perfusion liquid, which cannot be that of



the usual Ringer's fluid, but must be NaCl 0.5 per cent.,  $\text{CaCl}_2$  0.02-0.04 per cent., KCl 0.01 per cent.,  $\text{NaHCO}_3$  0.285 per cent.

As to the amount of calcium chloride, it may be emphasised that the percentage of this salt may be much greater than 0.02 per cent., provided the concentration of the Ca-ions be the right one. And this concentration is fixed by the following formula (Rona and Takahashi<sup>29</sup>):

$$\sqrt{[\text{Ca}^{++}]} = K \cdot \frac{[\text{H}^+]}{[\text{HCO}_3']}, \text{ where } K = 3.50.$$

The  $[\text{H}^+]$  can be kept constant in the artificial perfusing liquid by the addition of a small quantity of  $\text{CO}_2$  and a little neutral red. The colour to be obtained in this way must be the same as in a definite mixture of primary and secondary phosphates, as was pointed out by Sørensen.<sup>30</sup> The concentration of  $[\text{HCO}_3']$  depends on the amount of  $\text{NaHCO}_3$ , and this amount can be determined by titration of the normal serum. It is obvious that in this way an increase of the concentration of the calcium salt does not affect the concentration of the free  $[\text{Ca}^{++}]$ , and, as already stated, the latter is of predominant importance.

The significance of the concentration of free Ca-ions for the permeability of cells other than the epithelium cells of the glomeruli is also evident from experiments conducted in our laboratory on the red corpuscles of man and other warm-blooded animals (Brinkman and Van Dam<sup>31</sup>). When red corpuscles are washed in 0.9 per cent. solution of NaCl they lose some of their colouring matter after again adding a 0.9 per cent. solution of NaCl. But this can be prevented if a definite amount of calcium-ions are present in the solution of NaCl. The amount of  $\text{CaCl}_2$  needed for this purpose may be between 0.015 and 0.020 per cent. On increasing or diminishing the calcium hæmolysis occurs. It is not necessary in this case to use a buffer, for no acids are formed here as in the perfusion of the kidneys.

Experiments similar to those on the kidney were performed on the automatic movements of the rectum (Brinkman and Van Creveld<sup>32</sup>) and stomach,<sup>33</sup> and even on the secretion of the gastric mucous membrane when the vagus is stimulated (Brinkman and Van Dam<sup>33</sup>). The concentration of the Ca-ions must always be confined within very narrow limits, and this is effected by using the modified Ringer's solution as well as in the normal serum.

The fact that in normal serum the concentration of the Ca-ions is kept fairly constant may be the reason why the therapeutic application of Ca-salts is so often of no value. When acidosis exists, however, the concentration of Ca-ions is altered.

#### SOME REMARKS.

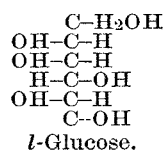
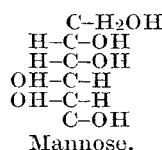
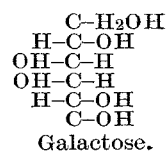
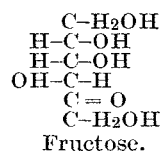
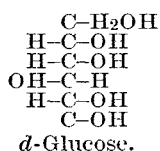
(a) *Tolerance*.—There are four other points in connexion with the permeability of the glomerular epithelium to glucose. In the first place; when the amount of glucose added to a suitable Ringer's solution is greater than that which is normally present in the blood plasma of the frog all the glucose is not held back. The amount of glucose let through increases with the amount added. It is even possible to add so much glucose that it all passes through the glomerular membrane. Is this phenomenon to be ascribed to damage done to the glomerular membrane by the increased amount of glucose? In any case the glomerular membrane becomes more and more permeable; in other words, the tolerance of the glomerular membrane decreases and even becomes nil. I shall not discuss this question here, only I wish to remark that the same phenomenon is observed with red corpuscles, which are also made more permeable by and to glucose.

(b) *Hyperglycoplasma*.—The second point is connected in a natural way with the first. We all know that one is accustomed to speak about hyperglycæmia when the total amount of blood-sugar is greater than normal; but when investigating the relation between hyperglycæmia and glycosuria the important point is not the total amount of sugar in the blood as a whole but the amount of sugar present in the plasma; one must know the degree of hyperglycoplasma. Of late years a number of investigators have studied the partition of sugar between corpuscles and plasma; most of them agree that there may be a considerable amount of sugar in the corpuscles, but only in the case of man and the dog. The experiments of van Creveld and Brinkman recently conducted in my laboratory have established in a direct manner that

in circulating blood glucose is exclusively present in the plasma, and that the transit of sugar from plasma to corpuscles takes place when the blood is defibrinated;<sup>35</sup> the migration occurs as soon as coagulation commences, and for this reason is to be observed even in hirudinised blood.<sup>36</sup> If, however, the blood is collected in a tube coated with paraffin and at once centrifuged it is found that all the sugar is present in the plasma. This is of great importance for the quantitative determination of hyperglycoplasma, which is the only matter of real importance in the clinical determination of blood-sugar. From a theoretical point of view it is interesting to add that the change which takes place even in the beginning of coagulation of the plasma surrounding the superficial layer of red corpuscles is sufficient to alter the permeability of the red corpuscles to sugar.

(c) *Phloridzin*.—A third direction in which our work on the permeability of the glomerular membrane to glucose was extended was in connexion with the influence of traces of phloridzin on the passage of glucose into the urine. Brinkman<sup>37</sup> found that when only 0.0004 per cent. of phloridzin is added to the perfusion liquid no trace of sugar is held back by the glomerular membrane; the renal portal vein was ligatured, so that the renal tubules could not have exerted any influence. Thus we may conclude that the glomerular membrane has been rendered wholly permeable to glucose through the phloridzin added. This permeability is perfectly reversible, so that if a Ringer's solution free from phloridzin and sugar is passed through, and then a Ringer's solution containing the physiological quantity of sugar, all the sugar is once more retained. We have therefore before us an additional example of the alteration of the permeability of the glomerular membrane to glucose, and in this case it is caused not by an excess of glucose itself, but by the foreign substance phloridzin.

(d) *The Reason why the Glomerular Epithelium is Normally Impermeable to Glucose; Size of the Molecule of Glucose; its Configuration; Isomeric and Stereoisomeric Sugars*.—The fourth remark has reference to the question why the physiological amount of glucose is normally held back by the glomerular membrane. It is, indeed, remarkable that glucose is retained by the glomerular membrane, whereas other crystalline bodies, like NaCl, sulphates, and phosphates, pass through. We might imagine that the molecule of the monosaccharide glucose ( $\text{C}_6\text{H}_{12}\text{O}_6$ ) is so large that its passage through the glomerular membrane is hindered. We thought that if this notion were correct disaccharides, like sucrose (saccharose), maltose, and lactose, which have a still larger molecule ( $\text{C}_{12}\text{H}_{22}\text{O}_{11}$ ), would certainly be retained as well. Experiments proved, however, that the glomerular epithelium is highly permeable to these three disaccharides. It is even perfectly permeable to raffinose, which has a still larger molecule ( $\text{C}_{18}\text{H}_{36}\text{O}_{18}$ ). Thus the size of the molecule alone cannot be the determining factor.

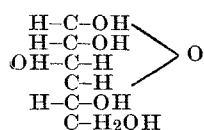


If the retention of glucose could not be ascribed to the size of its molecule there was the probability that a characteristic structure or configuration of the glucose molecule had something to do with it. For this reason some sugars which were either isomeric or stereo-isomeric with glucose were experimented with. For the sake of convenience I give above the formulæ of a few of these sugars.

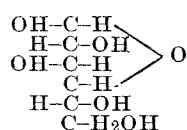
And it was found that the fructose passed through completely, likewise the mannose, while only part of the galactose was retained. You will notice how slight is the difference between the configurations of these five sugars, especially between d-glucose and its stereo-isomers galactose and mannose. Glucose, therefore, occupies a very peculiar place amongst the isomeric monosaccharides with regard to the glomerular membrane—that is to say, the glomerular epithelium distinguishes glucose from other sugars in a way which suggests the relation of sugars to ferments, like a lock and key. If we apply this to our case we would say that the key (glucose) does not fit the lock (glomerular membrane), and that the other sugars experimented with do fit and pass through. The power of the glomerular membrane to distinguish d-glucose from other sugars goes so far—and this is not without importance from a clinical point of view—that when d-glucose (dextrose) and lævulose are dissolved in the perfusion liquid the dextrose is still retained by the glomerular membrane, but the lævulose passes through. From this it is evident that the power of retention for dextrose is not quantitatively altered. The two sugars are separated from each other as by a filter. What is true for a mixture of dextrose and lævulose also applies to a mixture of glucose and lactose. The lactose passes completely into the urine and the glucose is retained just as when there is no lactose present.

These investigations form a new illustration for the law of stereo-isomerism, but here it is not of a chemical but of a physiological nature as they occur in the domain of permeability. We may repeat, therefore, that it is the configuration of the glucose molecule that imparts to this substance the peculiarity that causes it to be retained by the glomerular epithelium.

In order to know to what group of atoms the retention of glucose was due, we have examined other stereo-isomeric sugars, amongst which were pentoses, such as xylose, arabinose, &c., and some hexoses like galactose. Of these some passed through the glomerular membrane, others could only partially do so. Of those showing partial retention, we have examined especially d-galactose and l-xylose; the former has special importance as it is a constituent of the central nervous system. The partial retention, which was always of about 50 per cent. of the d-galactose and 25 per cent. of l-xylose, is very remarkable, because it appears to disagree with the idea of permeability. A mean between the two extremes of permeability and impermeability can hardly be conceived when we have to do with only a single substance. The reason for it lies in the fact that there are two modifications of d-galactose when dissolved in water—namely, the  $\alpha$  and the  $\beta$  variety, which only differ in the attachment of the H and OH groups to the asymmetrical carbon atom, as shown by the graphic formula given below. The  $\alpha$  variety is retained and the  $\beta$  variety allowed to pass through. The same is the case with l-xylose.



$\alpha$ -Galactose held back.



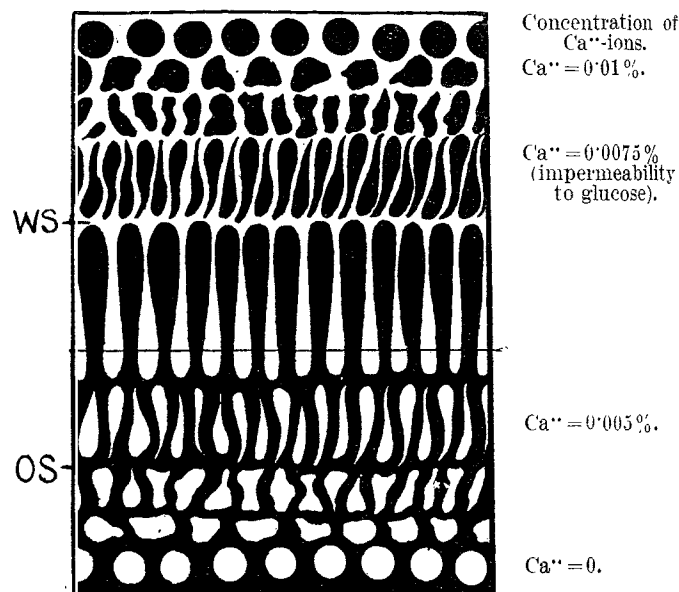
$\beta$ -Galactose not held back.

To summarise, we may say that the cells, with hardly any exceptions, are permeable to ions of electrolytes and often to organic substances; further, that the physiological, or more generally the vital condition of the cell, or better the composition of the surrounding fluid, exerts a great influence on the permeability.

#### VI. MECHANISM OF PERMEABILITY.

There are two theories which can be used to explain permeability; the one is based on the polarised condition of the surface of the cell, and this may be

called the electrical theory; the other is the sieve theory. The first is embodied to a certain extent in Girard's scheme, which we have previously mentioned in connexion with the influence of acidity or alkalinity on the movement of ions. The second (sieve theory) is embodied in the views of Clowes.<sup>38</sup> It would take me too far to consider both these in detail, but I would like to make some remarks on Clowes's theory. This originated in an observation by Bancroft (1913),<sup>39</sup> that a mixture of oil and water can be emulsified permanently in two ways; the one emulsion consists of oil drops suspended in a continuous watery phase as in cream; the other consists of drops of water suspended in a continuous phase of oil as in butter. Clowes was able to convert an emulsion of the first kind into one of the second kind by simply shaking an emulsion of oil in sodium soap with a solution of calcium chloride. The reverse took place when shaking with sodium hydroxide. If this is applied to the protoplasm of cells one will obtain two different states of permeability on the addition of Na or of Ca; Ca causes an oily canal system and Na a watery canal system. From this it follows that Ca will create an impermeability to watery substances. In other words, as regards their effects on permeability Na and Ca are antagonistic to each other. The following somewhat modified scheme of Clowes illustrates the experiment very well.



Transformation of emulsion of water in oil to emulsion of oil in water by means of a slight increase of the concentration of  $\text{Ca}^{++}$ -ions. Water = white. Oil = black. WS = watery canal system. OS = oily canal system.

In the diagram it is seen that the state is changed by adding more  $\text{Ca}$ -ions (part of the figure above the line), so that ultimately watery solutions might pass. But this is only the case with molecules of certain shapes. These shapes change by adding more  $\text{Ca}$ -ions, because the boundaries vary with the surface tension. If this is applied to our experiments it is clear why the glomerular membrane becomes impermeable to glucose with an efficient concentration of  $\text{Ca}$ -ions in the perfusion liquid. It may be stated here that something very similar is obtained in the hæmolysis of red corpuscles. Dr. Brinkman and Miss Van Dam have also observed a decided influence of the concentration of calcium-ions on gastric movements and gastric secretion when the vagus nerve is stimulated. We are not astonished at this when we come to think that, on stimulating a nerve, one has probably to do with the influence of the calcium-ions on the neuro-muscular junction, which is formed at the place where the nerve and the muscle meet.

Why the impermeability in the presence of a definite amount of  $\text{Ca}$ -ions in the fluid should be replaced by permeability again when there is a superabundance of  $\text{Ca}$  in the liquid we cannot now discuss. An analogue of such a contrast is seen in the recent

experiments of Neuschlosz,<sup>40</sup> who found that an emulsion of lecithin in water possesses a surface tension dependent on the amount of Ca present. Too much or too little Ca had the same effect. In the therapeutic application of lime salts one also often notices opposite effects according to the amount used.

To return for a moment to what Prof. Bayliss has called the "Clowes's effect," it would seem that the view of the American author is strongly supported by our experiments on the stereo-isomeric sugars. We have seen that pores are left between the oil-drops, and it is obvious that these pores, because they are subjected to the surface tension at the boundary, assume varying shapes. Now pores of a definite shape could allow sugars of a definite configuration—e.g., lævulose—to pass through, while holding back sugars—e.g., glucose—of another configuration. One might be inclined to explain this phenomenon by the aid of differences in viscosity or surface tension. But we have found with Miss S. C. Hamburger that these physical constants are the same for lævulose and for glucose. How can one explain this separation of glucose from lævulose, therefore, other than by supposing that the shape of the ultra-microscopic pores in the sieve plays a decisive part? Our experiments thus lend support to Clowes's theory, and vice versa the mechanism of specific permeability has become clearer.

Finally, it may be said that the conditions shown in our sketch by the portion below the line do not, as a rule, occur in physiological circumstances. In fact we know that, as a rule, all the cells of the body are permeable to water. But there are exceptions. When blood is diluted with a great deal of water most of the red corpuscles in absorbing water lose their colouring matter and the mixture has become transparent. But after centrifugalising I often saw a deposit which examined by the microscope proved to consist of red corpuscles. These cells must have been impermeable to water. Red corpuscles treated with cobra venom are also impermeable to water, and the same is true of the eggs of sea-urchins.<sup>41</sup> When put in distilled water the latter does not enter the eggs, as Ralph Lillie has pointed out, but on adding some Ca-ions their impermeability to water is lost.

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(Continued at foot of next column.)

## CRANIOTABES OF THE FŒTUS AND INFANT.

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In a previous paper<sup>1</sup> I recorded some results of a clinical inquiry into this subject. The account then given was composed under the combined disadvantages of military service and paper shortage; and this was unfortunate, because the contentious nature of certain of the findings called for their rather full presentment. I shall therefore make no apology for re-stating these findings in somewhat more adequate form.

Broadly, the position then reached was that the recognised "craniotabes" arising during the first few months of infancy is in many, and probably in most, cases only a fresh manifestation of a state of craniatrophie already existing in later foetal life. This foetal craniatrophie, having no physical differences from the later form, and occurring predominantly in the same individuals, was therefore held to deserve the same title. Verification of this finding would entail a re-scrutiny of the common belief that craniotabes is a sign of rickets. A simpler solution of the difficulty would be to infer the causality of syphilis. But the only definite contribution to the problem of causality I was able to make consisted of some fresh evidence tending to rule out syphilis as an acting or proximate cause. The evidence given was, I think, sufficient for that purpose; and it must be remembered that observation alone, though powerless to decide what is causal, can often determine what is not.

I will now go over the previous findings, incorporating such fresh material as has since been obtained. The total case-material is 154.

#### Immaturity of the Skull.

In connexion with yielding areas in the skull at birth, the problem was early raised in course of this inquiry as to what characters of this kind in the newborn skull can be legitimately ascribed to immaturity. To determine this point, a parallel study was made on some hundreds of living neo-natal crania at various stages of development, and of stillborn and non-viable crania, in situ and detached.\* Seeing that in all my cases of foetal craniotabes the parietal bones were attacked, and that all were born at or near term, interest here centres on the condition of the parietal at and near the end of the foetal period. The result of study makes it clear that the condition I call foetal craniotabes and the condition of the parietal due to immaturity are entirely distinct. In the first there is discontinuity of the bone-forming spicules—a true atrophy in some part of their course—

\* For the great majority (about 130) of these latter my thanks are due to Prof. J. M. Beattie, who kindly placed at my disposal the stillborn material brought to the Liverpool City Laboratory. This material has also furnished several morbid specimens.

(Continued from opposite column).

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