CIHM
Microfiche
Series
(Monographs)

ICMH
Collection de
microfiches
(monographies)

Canadian Institute for Historical Microreproductions / Institut canadien de microreproductions historiques

Technical and Bibliographic Notes / Notes techniques et bibliographiques

The Institute has attempted to obtain the best original copy available for filming. Features of this copy which may be bibliographically unique, which may alter any of the images in the reproduction, or which may significantly change the usual method of filming, are checked below.

L'Institut a microfilmé le meilleur exemplaire qu'il lui a été possible de se procurer. Les détails de cet exemplaire qui sont peut-être uniques du point de vue bibliographique, qui peuvent modifier une image reproduite, ou qui peuvent exiger une modification dans la méthode normale de filmage sont indiqués ci-dessous.

- [x] Coloured covers/ Couverture de couleur
- [ ] Covers damaged/ Couverture endommagée
- [ ] Covers restored and/or laminated/ Couverture restaurée et/ou pelliculée
- [ ] Cover title missing/ Le titre de couverture manque
- [ ] Coloured maps/ Cartes géographiques en couleur
- [ ] Coloured ink (i.e. other than blue or black)/ Encre de couleur (i.e. autre que bleue ou noire)
- [ ] Coloured plates and/or illustrations/ Planches et/ou illustrations en couleur
- [ ] ·nd with other material/ avec d'autres documents
- [x] Tight binding may cause shadows or distortion along interior margin/ La reliure serrée peut causer de l'ombre ou de la distorsion le long de la marge intérieure
- [ ] Blank leaves added during restoration may appear within the text. Whenever possible, these have been omitted from filming/ Il se peut que certaines pages blanches ajoutées lors d'une restauration apparaissent dans le texte, mais, lorsque cela était possible, ces pages n'ont pas été filmées.

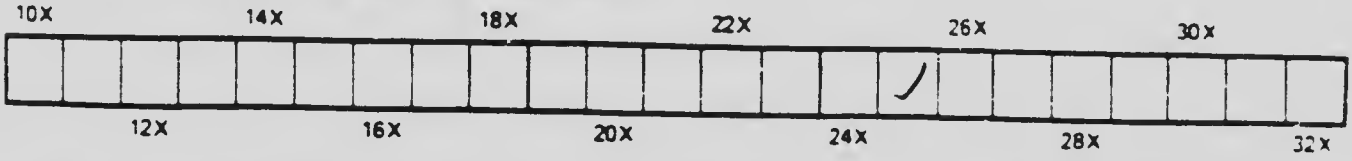
- [ ] Coloured pages/ Pages de couleur
- [ ] Pages damaged/ Pages endommagées
- [ ] Pages restored and/or laminated/ Pages restaurées et/ou pelliculées
- [x] Pages discoloured, stained or foxed/ Pages décolorées, tachetées ou piquées
- [ ] Pages detached/ Pages détachées
- [x] Showthrough/ Transparence
- [ ] Quality of print varies/ Qualité inégale de l'impression
- [ ] Continuous pagination/ Pagination continue
- [ ] Includes index(es)/ Comprend un (des) index

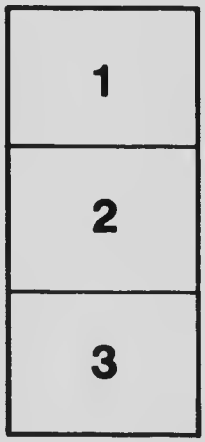
Title on header taken from:/ Le titre de l'en-tête provient:

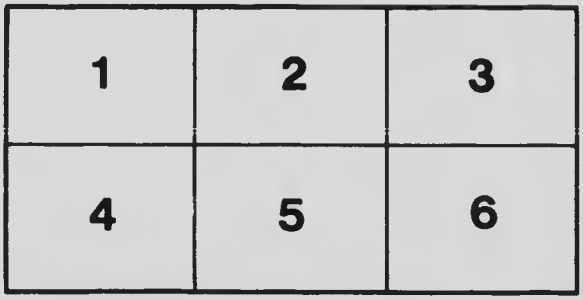
- [ ] Title page of issue/ Page de titre de la livraison
- [ ] Caption of issue/ Titre de départ de la livraison
- [ ] Masthead/ Générique (périodiques) de la livraison

- [x] Additional comments:/ Commentaires supplémentaires: Pagination is as follows : p.[387]-407, [8]

This item is filmed at the reduction ratio checked below/
Ce document est filmé au taux de réduction indiqué ci-dessous.







UNIVERSITY OF TORONTO
STUDIES

PHYSIOLOGICAL SERIES

No. 9: ON THE DISTRIBUTION OF POTASSIUM IN RENAL CELLS, BY C. P. BROWN

(REPRINTED FROM TRANSACTIONS OF THE CANADIAN INSTITUTE, VOL. IX.)

THE UNIVERSITY LIBRARY: PUBLISHED BY
THE LIBRARIAN, 1912

University of Toronto Studies

COMMITTEE OF MANAGEMENT

Chairman: Robert Alexander Falconer, M.A., Litt.D., LL.D., D.D.
President of the University

Professor W. J. Alexander, Ph.D.

Professor W. H. Ellis, M.A., M.B.

Professor A. Kirschmann, Ph.D.

Professor J. J. Mackenzie, B.A.

Professor R. Ramsay Wright, M.A., B.Sc., LL.D.

Professor George M. Wrong, M.A.

General Editor: H. H. Langton, M.A.
Librarian of the University

ON THE DISTRIBUTION OF POTASSIUM IN RENAL CELLS

BY

C. P. BROWN, M.A.

ON THE DISTRIBUTION OF POTASSIUM IN RENAL CELLS.

By C. P. Brown, M.A.,

Fellow in Biochemistry in the University of Toronto, 1910-11.

I. Introduction.

Although investigation of the problems of renal secretion has given us some information regarding the manner in which the kidney carries out its functions, it has hitherto thrown very little light on the character of the cellular processes which are fundamentally concerned in the elimination of salts and metabolites from the blood by the renal tubules. This is in large part due to the fact that although our cytological and histological methods are highly developed, yet the results which they give when applied to the kidney do not enable us to understand how a renal cell acts when performing the excretory function. We can by the application of histological and cytological methods distinguish stages in the activity of the cells in the pancreatic and peptic tubules, and we are able to recognize the presence in these cells of the antecedent substances, the zymogens, of the ferments which the glands produce. The changes which we distinguish in such gland cells do not give us a very profound view of the processes of cellular secretion involved, for those changes as we observe them are of a more or less superficial character, but in the renal cells the changes which precede, or are consequent on, rest and activity are much less in evidence. It has indeed been claimed that in the active renal cell there are "secretion vesicles" which are not present when the cell is at rest or is relatively inactive, and more or less marked vesiculation of the free border of the renal cells obtains under the influence of powerful diuretics, but the "secretion vesicles" are held by some to be artefacts, and the vesiculation of the free border is not necessarily an indication of the processes which the cell under ordinary conditions undergoes.

The failure of cytological methods to reveal fully the cellular processes of renal excretion has made it necessary to employ other lines of investigation on this subject. In the microchemistry of the cell from its inorganic side there are methods some of which at least are already available for developing to a certain extent a knowledge of these processes, and it is probable that a full achievement of that knowledge will

occur when more than one of those methods, specially adapted, are skilfully applied to investigate the distribution of salts in the cells of the renal tubules in the various stages of activity. Of the methods now available, that for the microchemical demonstration of potassium, first employed by Professor A. B. Macallum,[3] has been already used by him in studying the excretion of the salts of that element in the renal cells of frogs kept in the laboratory tanks through the winter. The results thus obtained were such as to indicate that the method might profitably be applied in a more extended investigation along the same line, and the author, acting on the suggestion of Professor Macallum, undertook the research of which the present contribution is the outcome.

II. METHODS OF INVESTIGATION

The method used is that fully described by Professor Macallum for the microchemical demonstration of potassium in animal and vegetable cells. It will, therefore, be necessary only to review here very briefly the reaction and preparation of the reagent.

The reagent is prepared by dissolving 20 grams of cobalt nitrite and 35 grams of sodium nitrite in 75 c.c. of dilute acetic acid (i.e., 10 c.c. glacial acetic diluted to 75 c.c.). A vigorous evolution of nitrogen peroxide results. It is allowed to stand for some hours, when, if any trace of potassium has been present in the sodium nitrite used, a precipitate forms which may be removed by filtration. The filtrate is diluted with water to 100 c.c., and is then ready for use. The precipitate produced by the reagent, when added to a solution of potassium salt, is a triple compound, the hexanitrite of cobalt, potassium and sodium. Its composition is given by Gilbert[4] as $Co(NO_2)_3$ $3(K/Na)$ NO_2, nH_2O, the value of n being 1 1/2, 2, or 2 1/2. Its composition varies, however, with the potassium salt content of the original solution. It consists of chrome yellow crystals of dodecahedra of varying microscopic size.

The tissue examined was removed from the body as soon as possible after the animal was killed, so as to insure a perfectly fresh, normal condition. It was at once placed on the plate of a CO_2-freezing microtome, where it was immediately frozen and sections made from it, and placed immediately in the reagent. Here lay perhaps the crucial point of the technique. First, the tissue must be frozen quickly and while perfectly fresh. In the second place, it is absolutely essential that the knife and the atmosphere in which the sectioning is done should be thoroughly chilled to a distinctly freezing temperature in order that the section should not thaw before the entrance into its reagent. If this

object is attained the diffusion of the salts in the sections is reduced to a minimum. The slightest thawing permits diffusion of the salts, in which case the distribution of the potassium compounds, as observed, would be other than that obtaining in the living tissue. Even with the greatest care the knife will warm a little and cause surface diffusion in cutting. It is thus necessary to prepare a large quantity of material and examine each section carefully under the microscope after it has been appropriately treated. Nearly all the drawings of the plates of this contribution have been made from sections in the preparation of which these precautions were observed, and in which surface diffusion, consequently, had been almost wholly prevented. The sections were cut at 10 to 20μ, the thickness depending on the firmness of the tissue.

The sections were allowed to remain in the reagent from three-quarters of an hour to an hour and a half, according to convenience. The longer time was preferable to attain better fixation of the tissue. They were then washed gently from half an hour to an hour in ice-cold water which was frequently changed. This dissolved the uncombined reagent and the precipitates which the reagent forms with creatin or ammonium salts. Immediately after washing, the sections were mounted on a glass slide in a mixture of equal parts of 50 per cent. glycerine and concentrated ammonium sulphide solution.

After this treatment the distribution of potassium in the sections was revealed by the localization of the black precipitate due to the formation of cobaltous sulphide. The staining of the preparation by the reagent itself varies in colour from a deep brownish yellow to a light gray. In animal tissues I have found the nuclei, if apparent, to be so from their lighter colour as compared with the rest of the section. In nerve cells the nuclei are stained much deeper, and Professor Macallum states that in vegetable cells they are stained still deeper and of a reddish shade. This is probably due to the nitrous acid reaction affecting the cytoplasm and the nuclear material differently.

That the uncombined reagent is readily removed from the sections by washing with ice-cold water has been many times shown in preparations of which Fig. 1 is representative. This was drawn from a section which, immediately after it was cut, was put in distilled water to remove all the potassium salts present, then placed in the reagent and treated further as described. The absence of a black reaction or of a dark shading in the preparations is evidence that all the cobalt reagent has been extracted.

Frogs were largely used, other animals as occasion offered. The frogs had been kept in a cool tank until January, and so were considered to have ceased to secrete actively. Hence they are referred to

as being in a condition of inanition. This condition was varied by keeping them in a warm room for some days and also by subcutaneous injections, into the dorsal lymph sac, of various diuretics. In some instances the spinal cord had been previously cut. Details on these points are given in the index to the Figures. The various animals used were *Necturus*, frog, dog, cat, rabbit, and pigeon.

III. RESULTS.

In the kidney from the inanition frog there were heavy condensations of potassium about the periphery of the tubules and at the margins of their lumina (Fig. 2). Practically no potassium was found in the cytoplasm except in the region bordering on the lumen, although the cellular outline was not sufficiently evident to state definitely in the majority of instances whether it was within or without the cell. By comparison, however, of a large number of preparations obtained under different conditions the peripheral deposit was judged to be on the lymph-tubule interface outside the cells, the central deposit being in the cytoplasm adjacent to the lumen.

The active kidney did not differ quantitatively from the inactive one, but the distribution of potassium was more uniform about the periphery of the tubules and frequently about the lumina as well. This was shown best in the frog under a mild dextrose diuresis (Fig. 3), also in the frog under moderate sodium sulphate diuresis (Fig. 4). In the latter the spinal cord had been cut previous to injection, to lower the blood pressure so as to prevent a flow of urine and, consequently, the actual localization of the potassium in the active kidney was more evident. The peripheral distribution can be clearly recognized as being external to the tubule and the central as being in the cytoplasm bordering on the lumen.

In cases of excessive diuresis the conditions were less uniform, the potassium being found on the intercellular membranes, in the intracellular spaces, on the surface of the nuclei, and, if the diuresis had been induced by an injection of potassium salts, frequently diffused throughout the cytoplasm as well. This distribution was not associated with any apparent increase in amount of potassium in the preparations from the frog after excessive dextrose diuresis (Fig. 5), and also after excessive sodium sulphate diuresis (Fig. 6). No potassium was found throughout the cytoplasm of the cells in these. The preparations from one of these kidneys under excessive sodium sulphate diuresis showed curious protrusions which by comparison with stained preparations were recog-

nized as migrating leucocytes (Fig. 7*a*), protruding red blood cells (Fig. 7*b*) and epithelial cells (Fig. 7*c*). These were found in restricted regions of the kidney where large numbers of leucocytes had gathered, the excess of diuretic apparently having acted as an irritant producing a necrosis similar to the condition found in preparations from the kidney of a dog which had been treated with cantharidin. Whether the striking aggregation of potassium salts on the surface of these structures is due to precipitation or condensation it is difficult to say. It appears to be analogous to that found by Professor Macallum in the root hairs of *Equisetum arvense* and in the outgrowing processes of conjugating *Spirogyra*.[b]

Where the excessive diuresis was occasioned by injection of potassium salts, *e.g.*, potassium phosphate, a similar distribution was observed (Fig. 8), accompanied, as one would expect, by an increase in the amount of potassium present. Not only were the intercellular membranes and the surfaces of the nuclei heavily charged, but the potassium was frequently found throughout the cytoplasm as well, either in a sort of network, or else distributed fairly uniformly. The rapidity with which an iodide penetrates tissues was demonstrated in one animal injected with potassium iodide and killed five minutes later. The heavily loaded tubules showed different states of activity. In the inactive tubules, as illustrated in Fig. 9, the potassium iodide was more or less irregularly distributed, but in the very active tubule the salt was chiefly condensed on the external surfaces of the tubules and in the cell at the lumen border (Fig. 10).

The preparations from the kidney of a dog which had been under A.C.E. anæsthesia for two hours, and in which the kidney would thus be in a fair state of activity, illustrated the peripheral and central condensations most strikingly (Fig. 11). The peripheral condensation was more frequent than the central, the latter being oftener seen when the urine flow was checked by pithing. Where the tubules had been thrown into activity by phloridzin the same distribution was found, differing only in that the condensations of potassium were distributed more uniformly about the tubules and a larger proportion of the tubules manifested the condensation (Fig. 12). It is difficult to illustrate this, as one only comes to this conclusion after examining a considerable number of preparations, but I have endeavoured to select tubules representative of each condition. In these preparations, in all of which the different regions of the kidney could be recognized, the localization was found in the convoluted tubules and in the loops of Henle, but in no case was a distinct localization evident in the region of the collecting tubules. Owing to the unstained nature of the preparations it was impossible to

refer the localization of potassium more definitely to the individual portions of the tubule.

Similar condensations were found in the rabbit. But, comparing the sections of the kidney from the pithed animal (Fig. 13, *a*, *b*), which had been excised before the injection of the dextrose, with those from the remaining kidney (Fig. 14, *a*, *b*) after it had been acted on by the diuretic, a distinct difference would be seen between the two. In the inner portion of the boundary zone from the former kidney there were only about half as many tubules which exhibited the condensation about the lumen as compared with those from the same region in the diuretic kidney, a larger number of these latter also showing the potassium in the lumen as well. These two kidneys gave the distinction between active and inactive most clearly, the localization in the active kidney being much more definite and uniform.

The cat (Fig. 15) and pigeon (Fig. 16) also evidenced the peripheral and central condensations. In some preparations from the kidney of a fœtal cat (Fig. 17) a few isolated convoluted tubules showed a certain amount of peripheral condensation, but no central condensation was observed. Sections from the kidney of *Necturus* were also prepared, and in them was found a similar distribution, except that the cells seem to retain their individuality to a greater degree than in the kidneys from the higher forms. This occasioned a distribution which tended to be cellular rather than tubular in character. The same peculiarity was observed in preparations of the pancreas from *Necturus*.

The glomeruli in all the forms showed a characteristic pattern which would be traced as lines composed of black dots of cobaltous sulphide (Fig. 19). By comparison with stained and other preparations this was judged to be largely in the walls of the blood vessels, with less marked precipitations in the interepithelial walls and in the connective tissue. In one preparation in which a surface view of Bowman's capsule (Fig. 20) was seen, the localization was confined to the cement substance of the cells and to the connective tissue strands. Some of the potassium had apparently slightly diffused before the reagent completely penetrated. In the intertubular spaces the potassium was found concentrated on the strands of connective tissue (Figs. 9 and 10). In a few preparations from the dog's kidney the basement membrane became slightly separated during preparation and was observed to be impregnated with the potassium salt. In no instance was any potassium demonstrated in a cell nucleus.

That these concentrations of potassium were the result of actual localization of the potassium salts in the kidney, and not artefacts, was apparent from inspection of the sections. They could not have been so

distributed by the knife in cutting, for, if so, the heavy precipitates would appear in more or less parallel lines at right angles to the direction of cutting and in much coarser aggregations, as was the case in some preparations in which diffusion had occurred before precipitation. Nor could they have been due to a penetration of the reagent into the lumina and intertubular spaces first, then into the cells. The character of the section excludes this possibility. The reagent comes in direct contact with a cross section of a tubule from 10μ to 20μ thick. It will penetrate directly at all points and not first to the interspaces, then to the basement membrane and so diffuse throughout a tubule of 18μ radius.

It might be urged that the reagent, penetrating the tissue at some points quicker than at others, precipitates the potassium with which it first comes in contact at these spots. Then the rest of the potassium in the cytoplasm, from its great diffusibility, would immediately gather and precipitate at the centres thus formed. This might be a real danger in organs limited by a cuticle or membrane, but in these preparations it could not obtain. The conditions are all against such a result, for a frozen cross-section dropped into the reagent while still frozen thaws equally and uniformly throughout and permits of the penetration of the reagent at all points of the surface at the same time. If this tendency toward diffusion to the point of initial precipitation operated, one would expect to find a heavy surface precipitate over the membranes, for these, being firmer, stand out as it were and first meet the reagent. Here would be the first precipitation, and to this would diffuse, not only the potassium in the cytoplasm, but that from the deeper portions of the membranes themselves. This would be apparent immediately on adjusting the microscope so as to focus the deeper portions of the section with little or no precipitate in contrast with the heavy peripheral and central surface precipitates thus supposed to occur. If only the surface layers were affected by this first diffusion, then, as the section thaws, the reagent would penetrate the cytoplasm more quickly than the membrane, and a heavy deposit in the deeper portion of the cytoplasm would result.

But none of these conditions obtains, actually the opposite being the case. Where diffusion has taken place on the surface before contact with the reagent, it is found that the tendency is for a uniform distribution over the entire surface of the section. The same applies to the deeper portions as well, the tendency being to a uniform distribution throughout. In Fig. 21*a* is shown a tubule in which a large measure of surface diffusion has taken place, this being a drawing of the surface view only, while Fig. 21*b* is of the same tubule observed at a deeper level of the section. What, however, may be considered the strongest argument

for the normal character of the result is that in the same section the regions of the convoluted tubules and Henle's loops show the localization while the regions of the collecting tubules do not. If it were due to an artefact occasioned in the freezing or subsequent treatment the whole section would be uniform. Again, there is the fact that in many tubules where a cut and an uncut surface can be distinguished, the uncut surface will show the potassium deposit while the cut section will be entirely free. Further, those tubules that evidence the localization are as a rule more heavily stained by the reagent than those that do not exhibit it, thus showing that the protoplasm in the two instances must differ in some respect.

IV. Discussion.

Special attention was drawn by Professor Macallum[8] two years ago to the various suggestions that surface tension might play a part in different processes of the living organism. He has discussed these suggestions and expanded them to apply to cellular activity in general. In any attempt to relate surface tension to renal function one must bear in mind that protoplasm is of more or less colloidal nature, and also that it is living material. What factors modifying the ordinary forces of surface tension this introduces have not been fully investigated, but, as surface tension appears to be involved in renal secretion, a discussion of its action in relation thereto may be permitted.

The kidney cells, whether considered individually or collectively in the tubule, form peripherally a system with lymph-cytoplasm interfaces, a condition somewhat analogous to a liquid-liquid interface, the two liquids forming the latter differing greatly in density. Centrally, if the lumen be filled with fluid, a cytoplasm-fluid interface would result, though if the tubule were collapsed this would not obtain. Now in a drop of liquid surrounded by air, organic substances lower the surface tension, while most inorganic solutes raise it. Hence in accordance with the law that energy tends ever to a minimum in a system, the organic solute is found concentrated at the surface of the drop, while the inorganic solute is more dilute at the surface as compared with the interior of the drop. This is called the Gibbs-Thomson principle, and had been expressed mathematically in a formula deduced by Gibbs for the concentration of one of the phases at an interface separating a two-phase system. When, however, the drop is in contact with another fluid with which it does not mix or is in contact with a solid (*e.g.*, glass), the surface tension of the drop on the contact surface is greatly diminished and, in consequence, there the solutes condense whether they ordinarily tend to

raise or to lower surface tension at the liquid-air surface. As far as has been investigated the tendency is for all substances in solution under these conditions to concentrate at the interface where the tension is lowered.

The degree of concentration has been investigated experimentally by Lewis[7] in the case of a number of solutes. For this purpose he allowed a known volume of a hydrocarbon oil to ascend through aqueous solutions in a tall cylinder. As the diameter and, consequently, the number of the droplets were ascertained the total surface area they presented was also known. The oil was collected at the top of the cylinder in a suitable apparatus. Each aqueous solution contained a substance soluble in water but insoluble in oil, viz., sodium glycocholate, methyl orange or Congo red. These lowered the surface tension in proportion to their concentration in solution. The degree of adsorption of each of these solutes on the surface of the oil droplets was from twenty to eighty times the theoretical value calculated according to the Gibbs formula. Lewis further investigated the effect of electrolytes[8] and found they manifested a similar but less marked condensation, the adsorption of potassium in potassium chloride solution being thirty times that calculated. Indeed, in all cases the adsorption was such that a process of gelatinisation at the surface was suggested as an explanation. It would further appear that all substances in solution are adsorbed at a fluid-fluid or fluid-solid interface.

Mayer[9] has shown that the surface tension of blood plasma is lower than that of an isotonic salt solution, and this would favour adsorption on cell-lymph interfaces. We may, therefore, perhaps be justified in concluding from this that the concentrations of potassium on the renal tubules, which we have described, are the result of surface tension differences on the surface of the tubules. In the production of these differences metabolism may play a part. We know that the oxygen intake in the kidney is very high, indicating a considerable liberation of energy, which the cells may transform to surface energy, using it either to obtain material for metabolic purposes or in the functional activities of excretion. The excess absorption of oxygen during the period of diuresis over the period of rest in the experiments of Barcroft and Brodie[10] is equal to 0.0401 c.c. O_2 per gram per minute. The secreting area for dog's kidney has not been calculated, but for comparison the area of a human kidney of similar weight may be given. According to Pütter[11] the secreting area of a kidney of 45 gr. is 2.2 m^2. Transforming the excess of oxygen consumption into dynes per cm^2., after allowing 1/20 for concentration of urine, we find an expenditure of 271.3 dynes per cm^2.

When we consider that only a portion of the kidney is active at one time we see the energy expenditure, if expressed as surface units, would appear very ample for excretory purposes.

As found in the preparations referred to, the potassium is localized on the periphery of the excreting portion of each tubule and in the cytoplasm of the cells forming it. As indicated above, the peripheral deposit can be explained only as condensation, due to surface tension, from the lymph bathing the surface of the tubule. This condensation effect would involve also the sodium, magnesium and calcium salts and the metabolites, such as urea and uric acid, and if there were a microchemical reaction for each of these as sensitive as the one employed for potassium the application of it would probably show such a condensation layer, although perhaps in every case not to the degree illustrated by the potassium preparations. The results of Lewis' estimations of the concentration of the condensation layers of different compounds from their solutions on the surface of droplets of paraffin oil rising, or of mercury falling, through such solutions seem to indicate very distinctly that the concentrations may greatly exceed the proportions postulated by the concentrations in the solutions, and, as already pointed out, this is specially the case with potassium salts. We might, therefore, expect the respective concentrations of the salts and metabolites on the external surface of each excreting tubule to exceed very greatly their concentrations in the lymph.

It is not unreasonable to suppose that the basal surface of each excreting renal cell, on which the condensation has occurred, is permeable to the constituents of the deposit. The entrance of potassium and other salts into the cell would bring them into a new system in which their distribution would be affected by the surface tension of the cell as a whole and of its individual parts. In consequence, condensations would occur, and particularly on interfaces and surfaces where the surface tension is very low. In fluids this condensation occurs quickly, but it is possible that in a colloidal system, such as the renal cell illustrates, the condensation might not be effected so quickly, and yet it would occur with such rapidity as to keep down the concentration in the cytoplasm generally.

The occurrence of a condensation of potassium salt in the cytoplasm of the renal cell immediately adjacent to the lumen of the tubule would seem to indicate that this border of the cell has a low surface tension as compared with the other surfaces of the cell, and especially with the basal one, and, consequently, other salts than those of potassium would be condensed there also. This difference in surface tension could only be maintained by a constant expenditure of energy in the cell, and

perhaps this would account for the very large amount of energy liberated in the kidney during diuresis, as calculated from the oxygen consumption of the organ by Barcroft and Brodie.

The maintenance of this difference in the tensions of the free and basal surfaces of the cell would not, however, be of much service in the way of explaining the mode of action by the cell if the condensation of the free (lumen) surface of the cell were not affected by another force. In renal activity the glomeruli, it is believed, separate from the blood plasma a fluid which is more or less free from salts and metabolites, and this thin watery fluid passing down the lumina of the convoluted tubules, sweeps over the free surfaces of the excreting renal cells in which the surface condensation of potassium and other salts obtains. The membrane of each cell at this point is extraordinarily thin and presumably permeable to water, which, however, owing to the low tension in the cell at the lumen surface, would not convey the salts there condensed into the interior of the cell, and they, therefore, would diffuse only into the lumen. The fluid in the latter would thus, as it passed down the tubule, become more and more concentrated, and in this way the higher concentration of the urine in solutes, as compared with the blood plasma and lymph, would be accounted for. This removal of the potassium and other compounds in the condensation layer would not cause the latter to disappear, for so long as a difference exists in the tension on the basal and free surfaces of the cell, and so long as the latter is permeable to salts on its basal border, the concentration of the adsorption layer in the cell at its free border would be maintained. The condensation process would thus parallel the extraction process so long as the cell is in activity.

The difference in surface tension between the free and the basal borders and the surface tension in the lymph on the external surface of the excreting tubule would thus, of course, be subject to the metabolic activity of the renal cells and to the composition of the lymph. Anything that affects this metabolic activity, therefore, would affect surface tension in the renal cells. The difference of surface tension between the basal and free surfaces of the cells would thus be diminished or enhanced. In the former case the elimination of salts would be lessened, in the latter case it would be increased. On the other hand, a change in the composition of the lymph must influence to a greater or less degree the condensation on the surface of the tubules, and this would involve a lessened or increased diffusion of the condensed salts into the renal cells.

Such alterations in the surface tension of the lumen border of the cell and of its basal surface may be brought about by diuretics. This would explain the results obtained in the dog's kidney under the influence

of phloridzin and in the rabbit's kidney in dextrose diuresis. A substance that promotes very vigorous diuresis may, directly or indirectly, influence unequally the tension of the lymph and the tension on the basal and free surfaces of the excreting renal cells. This would explain many differences observed between the renal cells in very marked diuresis and the renal cells engaged in ordinary activity. In the renal cells in excessive diuresis, as already described, the condensations are irregular, occurring not rarely on the surface of the cell nuclei and the lateral walls of the cells, particularly so in the case in which injections of potassium salts, and especially the iodide, had been given. Similar but less striking condensations of potassium were found in kidneys in diuresis caused by injections of sodium sulphate.

Potassium salts were occasionally found in the lumina of the tubules in all preparations, but particularly in those from the rabbit that had been subjected to the action of dextrose after the animal was pithed. In these the amount of glomerular fluid formed was diminished, and, in consequence, the potassium diffused into the lumen of the tubule from the adjacent condensation layer was to a certain extent retained in the fluid of the lumen. Where, therefore, potassium salts were found in the lumina of some of the renal tubules of unpithed animals, it may be reasonably explained as due to diminished activity on the part of their glomeruli.

No evidence of the occurrence of potassium salts in the free space of the glomerular capsule was obtained.

Whether potassium in its salts or compounds serves in the kidney any special purpose in renal excretion is a question which presents itself. The potassium of the condensation layers, abundant though it is, cannot be regarded as the whole of the element which obtains in the kidney, for although the hexanitrite reagent is an exceedingly sensitive one for potassium, it cannot demonstrate what is below the limit of sensitivity. Indeed, the very fact that in the cells of the convoluted tubules which show condensation of potassium in the cytoplasm adjacent to the lumen borders, the remaining cytoplasm itself does not give a reaction for potassium is evidence of this, for the condensation itself predicates a certain concentration, although excessively dilute, in the fluid system constituted by the cell as a whole.

Support for the view that potassium serves a special function in the kidney is afforded by analyses which the author made and which show the amount of the sodium and potassium in the organ. For this purpose frogs and dogs were used. In the case of the frog a large number of kidneys removed from recently killed animals were collected in bulk to weigh 17–22 grm.; each mass thus made was carefully dried in a platinum

dish till a constant weight was obtained; it was then carefully incinerated and the soluble portion of the ash extracted with hot water acidulated with hydrochloric acid. From the combined extraction fluids the calcium and magnesium and the sulphuric and phosphoric acids were removed by precipitation and filtration, and the filtrate, treated with hydrochloric acid, was evaporated to dryness, then heated to expel any traces of ammonium chloride. The residue, which consisted of the chlorides of sodium and potassium, was carefully weighed, then appropriately treated by the platinum method to determine the amount of potassium present, which, calculated as the chloride and subtracted from the total chloride found, gave the amount of sodium chloride present. In the dogs' kidneys the same method of estimating the total sodium and potassium present was used, but only one kidney was used in each analysis.

The analyses gave the following:—

Frog

Analyses	Sodium	Potassium	Na(=100 : K
1	0.1995	0.2031	100 : 101.8
2	0.2319	0.1995	100 : 86.3
3	0.160	0.1897	100 : 114.8
Average	0.1974	0.1974	100 : 100.2

Dog.

Analyses	Sodium	Potassium	Na(=100) : K
1	0.1995	0.2227	100 : 111.6
2	0.1746	0.2235	100 : 128.0
3	0.1657	0.2471	100 : 150.5
4	0.1758	0.2394	100 : 136.3
5	0.1846	0.2452	100 : 132.8
Average	0.1798	0.2356	100 : 130.9

In the analyses are, of course, included the sodium and potassium of the blood and lymph held in the excised kidney, and this constitutes a source of error, for the more blood and lymph the dog's kidney contains the lower is the proportion of the potassium to the sodium. Abderhalden's[13] analyses of dog's blood gave 0.2721 per cent. of sodium and

0.0212 per cent. of potassium. The value* Na(=100) : K of the blood would, therefore, be 100 : 7.8. The quantitative composition of dog's lymph has not been determined, but it cannot be very different from blood plasma and serum, so far as the inorganic constituents are concerned, and in the serum according to Abderhalden's analyses the sodium and potassium are 0.3175 and 0.0217 per cent. respectively, or in the proportion of 100 : 6.8.

If, therefore, the dog's kidney could, before analysis, be freed of all its blood and lymph, it would show a very considerable excess of potassium over sodium. It would be much more than ten times richer in potassium than the blood or blood plasma, while it would contain much less sodium than the blood or blood plasma.

No analyses have been published giving the composition of the blood in the frog, and consequently one cannot determine whether the retention of blood or lymph in the frogs' kidneys analyzed influenced very materially the analyses, but, on comparison with the results furnished by the analyses of the dogs' kidneys, it would appear as if there were no important difference between the two series of analyses in regard to the potassium.

The kidney is not the only organ in which potassium is richer than sodium. Katz[14] found that in the striated muscle of the dog potassium is three and a half times as abundant as the sodium, and Stoklasa[15] determined that the dried pancreas of the pig contains 2.08 per cent. of potassium and 0.28 per cent. of sodium. It is also known from Geoghegan's[16] analyses that potassium in brain tissue exceeds the sodium in amount.

In muscle certainly, and probably in the pancreas, the metabolic processes are very active. In striated muscle fibre Macallum has shown that the potassium is confined to the doubly refractive portion of the sarcous elements in which, there is reason to believe, the chief metabolic processes of the fibres occur. It is not at all unlikely that potassium plays a part in these processes, and that in the pancreas a similar rôle is filled by the element. In the active kidney, as shown by its consumption of oxygen, the metabolism is very pronounced, and consequently it may be suggested that here, also, the potassium so abundantly present is in some manner associated with that metabolism.

One must not, however, exclude the possibility that potassium is so abundant in tissues simply because of a special tendency to condense on surfaces of systems affected by low surface tension. From Lewis'

*Calculated from Abderhalden's values which were given for Na_2O and K_2O respectively.

observations already referred to, it would appear that some solutes in such systems undergo condensations differing in concentration for each solute, and potassium from its chloride condenses more than silver does from its nitrate. The relation of potassium to sodium in this respect was not investigated, but one may suspect there is a difference, perhaps an important one. This might account for the contrast between the sodium, which is 0.3175 per cent. in the serum and 0.1783 per cent. in the kidney, on the one hand, and on the other, the potassium, which is 0.0217 per cent. in the serum and 0.2356 per cent. in the kidney. The sodium is thus decreased 44 per cent., while the potassium is increased over 1000 per cent. Such a difference, as suggested, in the degree of condensation would furnish an explanation for the difference in the quantities of sodium and potassium in the plasma or serum which are in the proportion approximately of 15 to 1.

V. SUMMARY.

1. The sodium cobalt hexanitrite reagent ($CoNa_3(NO_2)_6+nH_2O$), as prepared by Professor Macallum, is a suitable reagent for the localization of potassium in kidney tissue.

2. It is essential that the tissue be frozen while perfectly fresh, and that the sections prepared from it be kept frozen until they come in contact with the reagent.

3. There is a definite localization of potassium on the external surface of the convoluted tubules and frequently about their lumina as well.

4. The uniformity of this localization about the tubules tends to increase in direct proportion to the state of activity of the kidney.

5. In the resting condition or during ordinary activity the only potassium demonstrable in the cytoplasm of the cells of the convoluted tubules is condensed in a layer immediately adjacent to the lumen border in each cell.

6. This localization appears to be in accordance with surface tension phenomena.

7. The presence of potassium in the lumina of the tubules and absence of potassium in the glomerular cavity is evidence that the inorganic salts are excreted by the tubule cells.

8. No evidence bearing on the manner in which the glomeruli perform their function was obtained.

9. In no instance was any potassium found in a cell nucleus.

10. The amount of potassium in the kidney of the dog and even of the frog exceeds that of the sodium, and in the dog it exceeds greatly the amount of potassium in the blood or plasma.

I desire to acknowledge the kind criticism and advice which I received during the progress of this work from Professor Macallum, at whose suggestion and under whose guidance it was carried out.

VI. Literature.

1. A. B. Macallum, Ergebnisse der Physiologie, herausgegeben von L. Asher und K. Spiro, VII Jahrgang, p. 552.
2. A. B. Macallum, Jour. of Physiol., Vol. XXXII, p. 95, 1905.
3. A. B. Macallum, Surface Tension in Relation to Cellular Protoplasm. Presidential Address, Physiological Section, British Association, 1910. Rep. British Association, Sheffield.
4. K. Gilbert, Die Bestimmung des Kaliums nach quantitativer Abscheidung desselben als Kaliumnatriumkobaltnitrite. Inaugural Dissertation, Tübingen, 1898.
5. Whatmough, Zeitschr. physik. Chem., Vol. XXXIX, p. 129, 1907.
6. J. Willard Gibbs, Scientific Papers, English edition, Vol. I, p. 219.
7. Lewis, Phil. Mag., April, 1908, p. 499.
8. Lewis, Phil. Mag., April, 1909, p. 466.
9. Mayer, Arch. di Fisiol. IV, 2, p. 165.
10. Barcroft and Brodie, Jour. of Physiol., Vol. XXXIII,
11. Pütter, Zeitschr. für allg. Physiol., Vol. XII, p. 133, 1911.
12. Moore, Biochem. Jour., Vol. V, p. 32, 1910.
13. Abderhalden, Zeitschr. für physiol. Chem., Vol. XXV, p. 106, 1898.
14. Katz, Arch. für die ges. Physiol., Vol. LXIII, p. 1.
15. J. Stoklasa, Zeitschr. für physiol. Chem., Vol. LXII, p. 47, 1909.
16. E. G. Geoghegan, Zeitschr. für physiol. Chem., Vol. I, p. 330, 1877-8.

VII. EXPLANATION OF PLATE.

The figures were drawn with the camera lucida. The distribution of potassium salts is indicated by the black shading which represents the cobaltous sulphide reaction given by the triple salt of potassium sodium and cobalt hexanitrite when ammonium sulphide is applied to it.

Fig. 1. Kidney tubule, frog, diuresis with K_2HPO_4; the potassium salts were washed out of the freshly cut section, which was then treated with the cobalt and sodium hexanitrite reagent in the usual way. × 820.

Fig. 2. Kidney tubule, frog in inanition. × 820.

Fig. 3. Kidney tubule, frog, in the dorsal lymph sacs of which ½ c.c. of 30 per cent. dextrose solution had been injected 4 hours before it was killed. The frog had previously been kept several days in a room of about 20°C. temperature. × 820.

Fig. 4. Kidney tubule of frog which had been kept for several days in warm room, then pithed and injected with 1 c.c. of half saturated Na_2SO_4 solution, 17½ hours after which it was killed. × 820.

Fig. 5. Kidney tubule, frog, taken from cold room (temperature about 10°C.), into the dorsal lymph sacs of which 4 c.c. of 30 per cent. dextrose had been injected four hours before it was killed. × 820.

Fig. 6. Kidney tubule of frog which had been kept for several days in warm room and into which 4 c.c. of a half-saturated solution of Na_2SO_4 had been injected two hours before it was killed. × 820.

Fig. 7. Cells from kidney of frog which had been subjected to excessive Na_2SO_4 diuresis. *a*, leucocyte; *b*, red blood cell; *c*, epithelial cell. × 820.

Fig. 8. Kidney tubules of frog into which had been injected 4 c.c. $\frac{N}{10}$ K_2HPO_4 and which was killed two hours later. × 820.

Fig. 9. Portions of two kidney tubules of frog in which 4 c.c. $\frac{N}{10}$ KI had been injected. Animal killed five minutes later. × 820.

Fig. 10. Portions of three kidney tubules from same preparation as Fig. 9. × 820.

Fig. 11. Kidney tubules, dog, A.C.E. anæsthesia, otherwise normal. × 820.

Fig. 12. Kidney tubules, dog, A.C.E. anæsthesia; 0.5 grm. phloridzin in 10 c.c. of a 1 per cent. NaCl solution was injected into external jugular; kidney excised as soon as urine showed glycosuric condition. × 820.

Fig. 13. *a* and *b*. Tubule of Henle's loop and convoluted tubule respectively, rabbit pithed, and kidney immediately excised during slight A.C.E. anæsthesia. × 820.

Fig. 14. *a* and *b*. Tubule of Henle's loop and convoluted tubule respectively from rabbit from which preparation giving Fig. 13 was taken. Kidney excised 45 minutes after cord was cut; blood pressure 30 mm. Hg. 24 minutes before excision 4.5 grms. dextrose in warm water were injected into the internal jugular vein. × 820.

Fig. 15. Tubule of Henle's loop, cat, normal condition. × 820.

Fig. 16. Convoluted tubule, pigeon, normal condition. × 820.

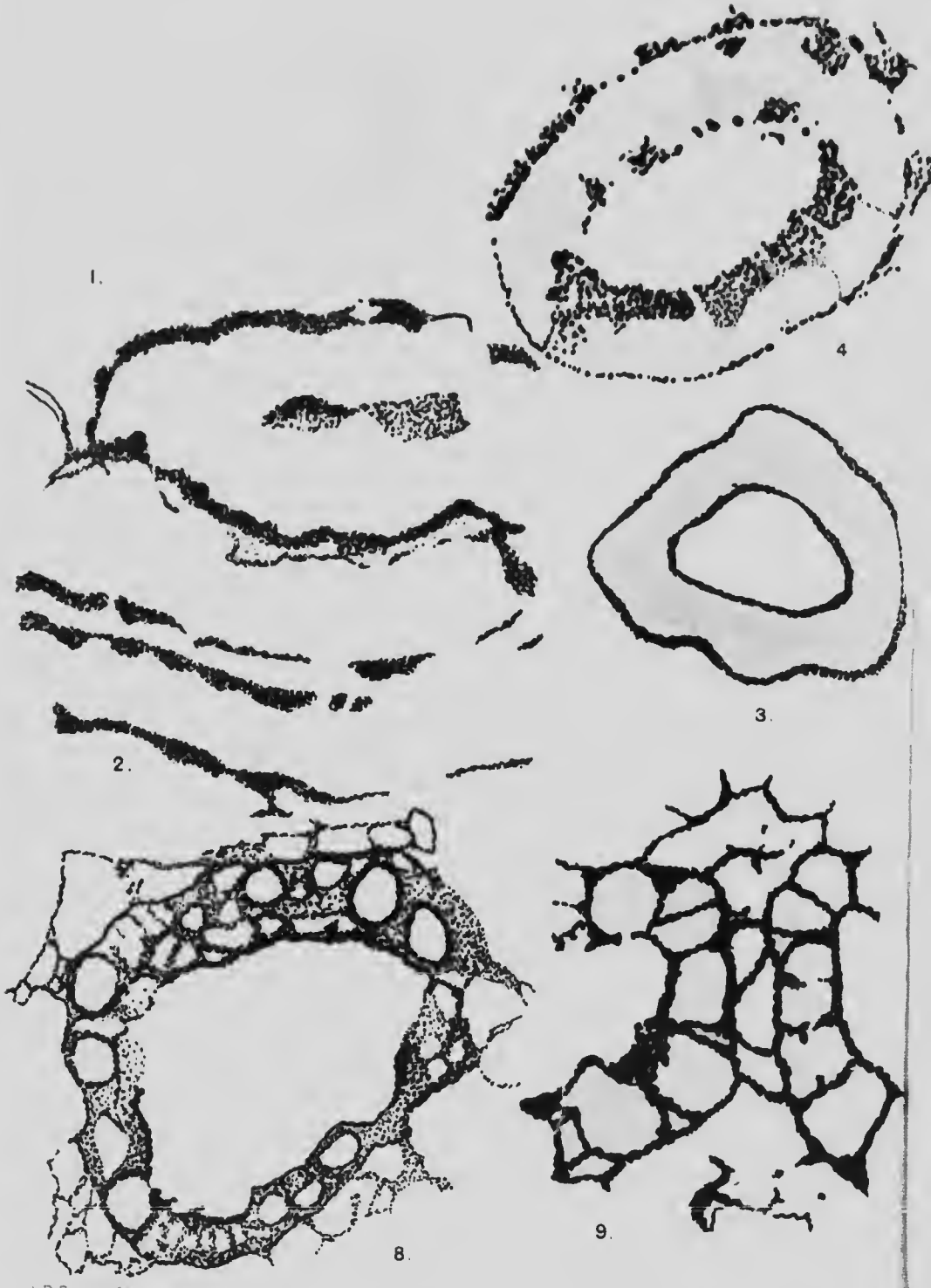
Fig. 17. Kidney tubule, fœtal kitten. × 820.

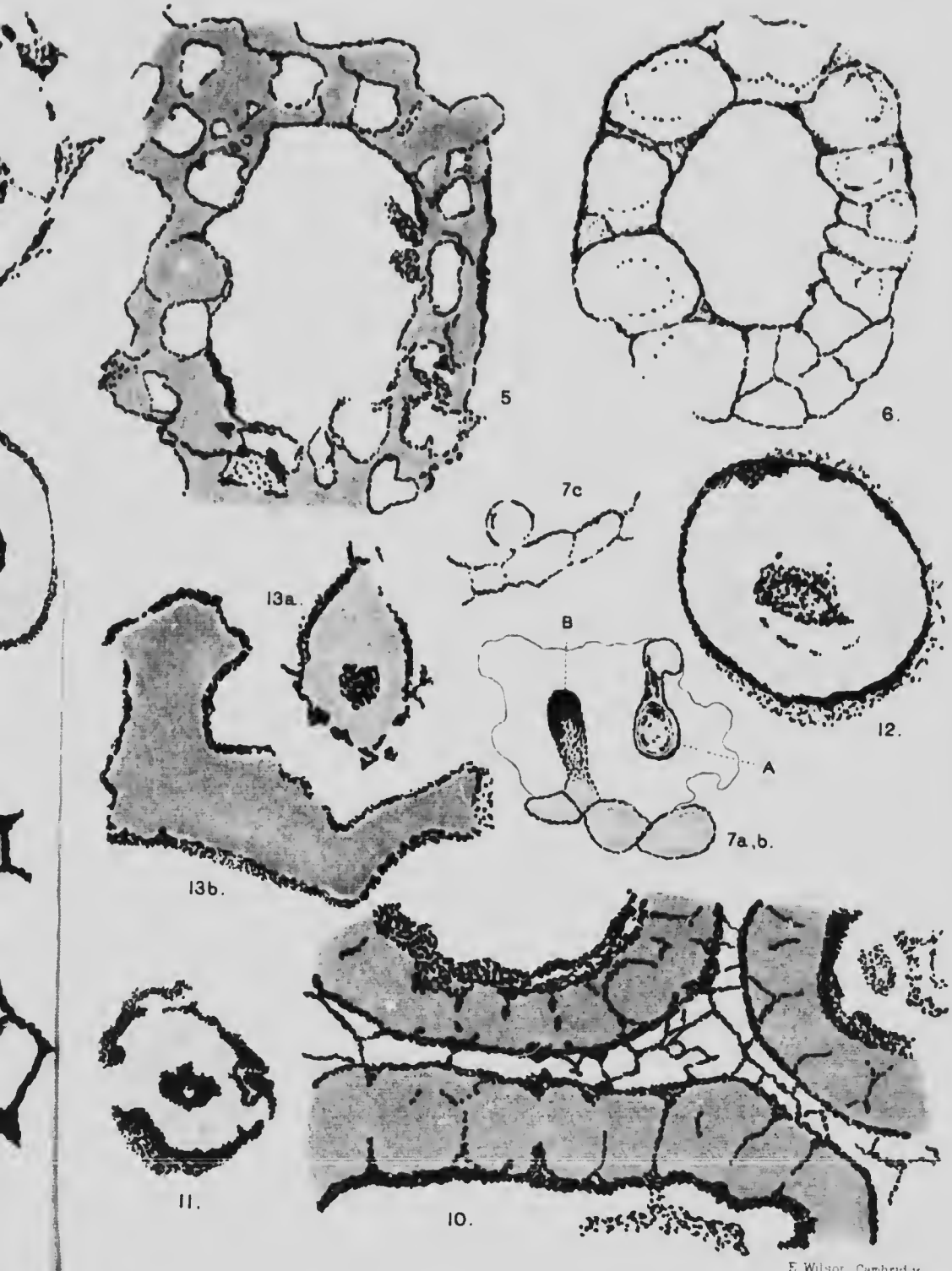
Fig. 18. Kidney tubule, *Necturus;* gastric digestion active, animal kept in cold aquarium. × 820.

Fig. 19. Glomerulus, dog, from same animal indicated in the case of Fig. 12. × 540.

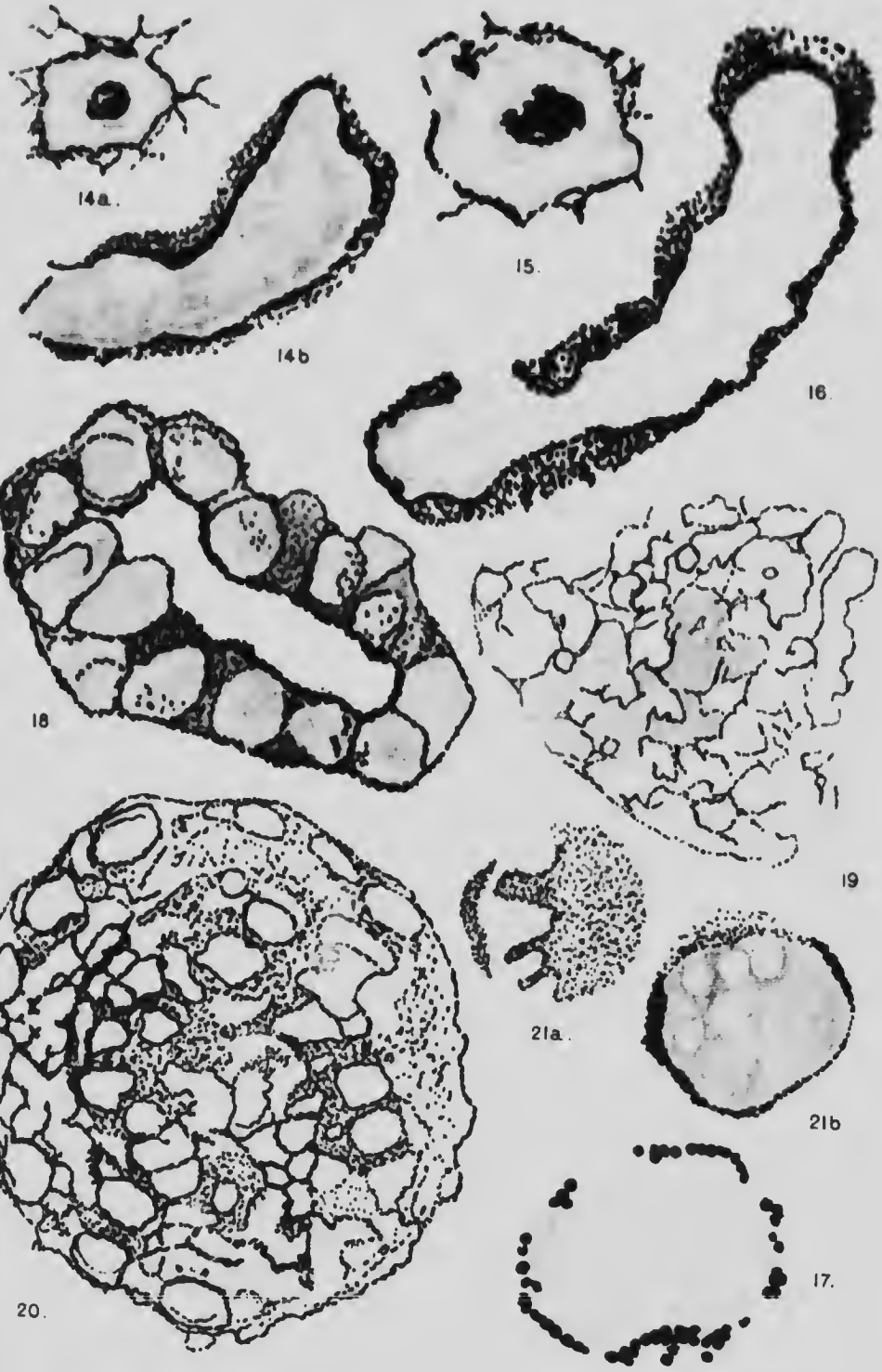
Fig. 20. Bowman's capsule, frog, from same specimen as in the case of Fig. 8. × 540.

Fig. 21. *a* and *b*. Kidney tubule, dog; *a*, surface view; *b*, deeper view of same. × 820.


1.
2.
3.
4
8.
9.
C P Brown, del.



E. Wilmot, Cambridge



C P Brown, del.

E. Wilson, Cambridge

UNIVERSITY OF TORONTO STUDIES

Physiological Series

No. 1. The structure, micro-chemistry and development of nerve-cells, with special reference to their nuclein compounds, by F. H. Scott $0.50

No. 2. On the cytology of non-nucleated organisms, by Professor A. B. Macallum 0.75

No. 3. Observations on blood pressure, by R. D. Rudolf 0.75

No. 4. The chemistry of wheat gluten, by G. G. Nasmith 0.50

No. 5. The palaeochemistry of the ocean, by Professor A. B. Macallum. 0.25

No. 6. The absorption of fat in the intestine, by G. E. Wilson 0.50

No. 7. The distribution of fat, chlorides, phosphates, potassium and iron in striated muscle, by Maud L. Menten 0.25

No. 8. Surface tension and vital phenomena, by Professor A. B. Macallum 1.00

No. 9. On the distribution of potassium in renal cells, by C. P. Brown 0.50

No. 10. On the probable nature of the substance promoting growth in young animals, by C. Funk and A. Bruce Macallum 0.25