COMPARATIVE HISTOPHYSIOLOGY OF THE VERTEBRATE NEPHRON

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The Vertebrate kidney is formed of a conglomerate of small tubular units named nephrons (Braus), the function of which is to form the urine. With their distal end, they open into the collecting ducts, which convey the urine towards the urinary bladder.

Two different kinds of nephrons are found among the Vertebrates.

(1) The glomerular nephrons. They are the most common, and usually the only ones the physiologist is dealing with. Their proximal end is dilated

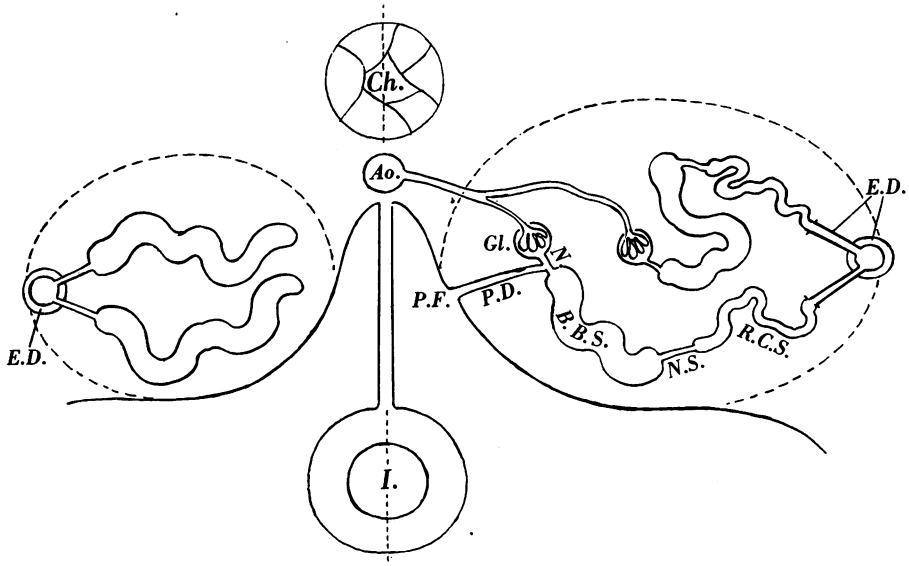

Fig. 1. Schema of the different kinds of nephrons. On the left, the aglomerular nephrons. On the right, the two varieties of glomerular nephrons: below, the open; above, the closed ones. *Ao.* = aorta; *B.B.S.* = brush border segment; *Ch.* = chorda; *E.D.* = excretory ducts; *Gl.* = glomerulus; *I.* = intestine; *N.* = neck; *N.S.* = narrow segment; *P.D.* = peritoneal duct; *P.F.* = peritoneal funnel; *R.C.S.* = rodded cell segment.

in a small ampulla, the glomerular chamber, in which a capillary tuft, the Malpighian glomerulus, hangs by a vascular pedicle; the blood enters the tuft by an artery, and leaves it by another artery which soon opens into an intertubular net of capillaries from which the veins emerge.

(2) The aglomerular nephrons are those the proximal end of which does not present a glomerulus. They are far less common than the glomerular ones; till now, they have been described in some marine Teleosts only (fig. 1).

Amongst the glomerular nephrons we can distinguish two varieties: the open and the closed ones. The former are characterised by the fact that the glomerular chamber, or the neck which connects it with the proximal end of the tube, presents a diverticulum which opens into the splanchnocoele by a wide opening or by a ciliated duct, the latter expanding to form a funnel-shaped orifice as it opens into the peritoneal cavity. Through the peritoneal canal, the nephron and the collecting duct, the body cavity communicates with the external world. On the contrary, the closed nephrons do not possess a peritoneal canal. Usually the glomerular nephron (closed or open) is divided into the following portions: the glomerulus, the neck, the proximal convoluted tube, the narrow segment (which is not constant) and the distal convoluted tube. These names, taken from mammalian anatomy, are somewhat inadequate. The proximal convoluted tube, for example, is sometimes straight and not convoluted. Therefore it seems advisable to rename these portions according to their histological structure. The suggested names would be as follows: glomerulus, neck, brush border (or striated border) segment, narrow segment, and rodded cell segment (fig. 1). As for the aglomerular nephron, it has, in its whole length, the structure of a brush border segment.

The histological structure of these segments is too well known to be described in detail. Nevertheless, it may be mentioned that the cells of the brush border segment contain, regularly in the Poikilotherms and rarely in the Homoiotherms, yellowish granules of varied size, spoken of as secretory granules. As the neck and the narrow segment may be absent, the glomerular nephron comprises three main segments: the glomerulus, the brush border segment, and the rodded cell segment.

Glomerular and aglomerular nephrons are homologous, and originate in the same embryological "Anlage". As the latter are derived from the former, and are strictly limited to certain marine Teleosts, it is logical to take up first the study of glomerular nephrons.

The histophysiology of the nephrons will be first considered, and then subsequently two processes which are intimately connected in the renal function, viz. the glomerular permeability and the storage phenomena in the brush border cells, known under the name of athrocytosis, will be discussed.

I. GENERAL VIEW OF THE HISTOPHYSIOLOGY OF THE NEPHRON

There are two theories of urinary secretion.

The Bowman-Heidenhain theory asserts that the water and the salts are filtered along the glomerulus, while "the tube" secretes into its lumen the characteristic substances of the urine (uric acid, urea, creatinine, etc.).

The Ludwig-Cushny theory admits that through the glomerulus are filtered not only all the components of the normal urine, but some others also, which are dissolved in the blood plasma. This glomerular urine is in a state of high

dilution, and when it passes through the tube it is concentrated by resorption of water and its composition changed through resorption of some of the components of the glomerular urine.

These two theories have one point in common, viz. the filtration of water through the glomerulus. They differ from each other, however, on the composition of the glomerular filtrate, and on the tubular function.

A. Function of the glomerulus

In the year 1878 Nussbaum (33, 34) succeeded in dissociating in the frog the glomerular and the tubular functions by ligation of the renal arteries. In this animal, as in the other Batrachians, the kidney is irrigated by two systems of afferent vessels. The renal arteries form the first one; they give off exclusively the afferent arteries to the glomeruli from which the efferent vessel branches soon into an intertubular capillary net; the second system is represented by the portal vein of the kidney, which runs along its external side, giving off numerous transverse branches which soon pass into the intertubular capillary net: so that in this net runs a mixture of arterial and venous bloods, which finally collects in the renal veins. Taking advantage of this anatomical position, Nussbaum tied the renal arteries. The circulation ceases in the glomeruli, and these are put out of function. The tubular system keeps its own blood supply through the portal vein. Immediately after the operation, the urinary flow stops nearly completely. One conclusion only may be drawn from this experiment: most of the urinary water comes from the glomerulus.

Since then, Wearn and Richards (39, 40), White and Schmidt (41) have succeeded in micropipetting the glomerular space, and collecting the glomerular fluid. In *Necturus*, the glomerular flow amounts to 0·1 mm.^{3} in the space of 10–15 min.

It is thus proved that much water passes through the glomerulus.

Wearn and Richards demonstrated also that the glomerular urine is an ultrafiltrate: it does not contain any proteids; but numerous normal urinary components can be detected in it, as chlorides, urea, uric acid, phosphates; the concentration of the two latter substances is equal to that in the blood plasma (Bordley and Richards (3); Walker, Ellinwood and Reisinger (38)).

When certain dyes are injected into the blood, they are partly excreted with the urine; but it is impossible to ascertain by direct exploration through which portion of the nephron (glomerulus or tube) they are passed. With the micropipette method it has been possible to ascertain that the following dyes are filtered through the glomerulus: methylene blue, phenol red, indigo carmine, which are not colloidal (Wearn and Richards); also ammonium carminate, trypan blue, which are colloidal dyes (Hayman and Richards (24)). Their extreme dilution in the glomerular urine, the narrowness of the glomerular space, and the slight opacity of the interposed membranes prevented the recognition of their presence in the glomerular urine by direct investigation.

Thanks to a method devised by Ellinger and Hirt (12, 13), it is now possible to follow the elimination of an injected fluorescent dye in a normally irrigated kidney when illuminated with ultra-violet light. The slightest traces of the dye become fluorescent and can be easily detected. By this means Ellinger and Hirt succeeded in demonstrating that the fluorescine passes through the glomerulus in both Amphibians and in Mammals. In the frog, Singer obtained similar results with aesculin.

But the glomerular liquid contains substances which are not found in the vesical urine. Wearn and Richards have demonstrated with the micropipette method that glucose is a normal component of glomerular urine. According to Walker, Ellinwood and Reisinger, it amounts to 95 per cent. of the blood glucose. There is no doubt that most of the substances dissolved in the blood plasma are filtered—or, better, ultrafiltered.

These experiments, however, do not exclude the possibility of the same substances being at the same time secreted through the tubule. We are thus led to examine with close attention the tubular function, and particularly that of the brush border segment.

B. Functions of tubules

(*a*) *Secretion*

In favour of the secretory function of the brush border segment, the following arguments have been put forward.

(1) In the cells of the striated border segment of many Poikilotherms and of some Mammals, numerous granules can be seen; they are yellowish, variously sized, and from their similarity to the secretory granules of the glandular epitheliums are known as secretory granules. Some colloidal dyes also appear in these same cells in a granular form, and this has been interpreted as a secretion process. Now, these conclusions are based on wholly superficial resemblance. We shall see later that yellow granules and coloured granules are witnesses of a secretory function.

(2) During these last years, American physiologists (Edwards (8, 9), Marshall (30, 31), etc.) have studied in some detail the urinary function in aglomerular Fishes, the nephron of which is composed only of a brush border segment. In these Fishes, the quantity of urine seems to be slightly inferior to that of the glomerular ones: *Myoxocephalus* (glomerular) supplies 4 c.c. of urine per kg. per day; *Opsanus* (aglomerular) 2·5 c.c. per kg. per day (Grafflin (21, 22)). But in both the components are the same, and in about the same proportions; measurements made on creatine, creatinine, uric acid and sulphates (Marshall (30) and Grollman (23)). The aglomerular kidneys excrete fairly well indigo sulphate and phenol red, this latter in a higher concentration than in the blood. All these substances must necessarily pass through the tubule.

Now, are we allowed to extend to the glomerular nephrons the information gained from the aglomerular kidney, and to infer that their brush border

segment secretes the urinary substances? This would not be justifiable. With the micropipette method, the filtering function of the glomerulus has been demonstrated. The aglomerular structure is a secondary state, which sometimes develops late (Grafflin). It might be possible then that the atrophy—or the non-development—of the glomerulus awakes or increases the secretory capacities of the tubule. Therefore, the physiological features of the aglomerular nephrons must be considered as rather exceptional, and cannot be invoked in favour of the secretory power of the segment in the glomerular nephrons.

So far the evidence for the secretory power of the striated border segment is of little value. This problem cannot really be solved unless the glomerular can be experimentally dissociated from the tubular functions.

To Heidenhain and Neisser (26) must be ascribed the first experimental research in this direction. After sectioning the spinal cord in the cervical region in a rabbit (operation which is followed by a fall of the blood pressure and a nearly complete cessation of the urinary flow), indigo sulphate is injected into the veins, and some minutes later the kidney fixed with a fluid which precipitates the dye. In the sections, a coloured precipitate is observed in the lumen of the tube, and none in the glomerular space, while in the cells of the striated border segment appears a diffuse coloration. For a long time this experiment seemed convincing. Nowadays it is no longer so, for the following reasons: firstly, the circulation is not entirely suppressed in the glomerulus, and it might be supposed that a small quantity of the dye has been passed through it, and has been concentrated as soon as it reaches the tubule.

Secondly, as for the dye found in the cells, close examination shows that only the nuclei are tinged, while the cytoplasm is colourless. This fact proves that diffusion of the dye has taken place, leading to its adsorption on previously coagulated cellular organites.

This experiment was repeated by Nussbaum, in a far more satisfactory way, on frogs with suppressed renal arteries. In these conditions, the indigo sulphate is found as a precipitate in the lumen of the tubes, and as a diffuse coloration in the cells of the segment. This experiment is much more convincing than that of Heidenhain, and in these last years it has been confirmed by Bensley and Steen (2) in the living kidney. After having suppressed the arterial blood supply, these authors examined by direct observation the elimination of the indigo sulphate. Soon after the injection, the dye is detected in the lumen of the brush border segment, and a very slow flow of coloured to tubular urine can be observed.

The phenol red gives the same results, and, moreover, here the dye is more concentrated in the lumen than in the blood plasma.

Ellinger and Hirt were able with their method to follow the elimination of the fluorescine in the kidney of a rat after section of the cervical spinal medulla. The blood flow ceases in the glomeruli. Then fluorescine is injected into the blood, and no fluorescence can be detected in the glomerular space,

nor in the lumen of the tube, but the cells of the striated border segment become slightly fluorescent.

After a subsequent injection of urea, no change is observed in the glomerular space, but fluorescent liquid appears in the lumen of the striated segment, and coloured urine collects in the bladder.[1]

In the Amphibian as in the Mammal, the secretory power of the brush border segment is thus demonstrated.

(*b*) *Resorption*

In the front rank we find the experiments of Richards and his school. We have already seen that Wearn and Richards demonstrated in the Amphibian the presence of glucose and sodium chloride in the glomerular fluid. Now, the vesical urine is always devoid of glucose, and in winter of sodium chloride. These two substances must then have been reabsorbed during their passage through the tubule.

White and Schmidt showed, by micropipetting at more and more distal points of the brush border segment, that the glucose resorption takes place in the first third of this segment. They showed also that the glomerular fluid, which is nearly isotonic with blood serum, becomes highly hypotonic when it has run past this first third.

On the other hand, Hayman and Richards succeeded in introducing with the micropipette some dyes into the glomerular space: methylene blue, phenol red, trypan blue, and ammonium carmine. All these substances (which filter through the glomerulus) are absorbed by the cells of the striated segment where they can be detected *in vivo* or after fixation: but this is a "partial argument", for, as the authors point out, it ought to be proved that the same substances are not secreted through the segment.

Von Möllendorff (37) seems to have found convincing proofs of the resorption of certain dyes (colloidal acid) by the brush border segment.

When such a substance, trypan blue for instance, is injected it is eliminated in part through the kidney. At the same time, the cells of the brush border segment fill with coloured granules.

This storage of the dye presents two peculiarities: there is no concordance between the moment of the maximal dye concentration in the vesicular urine and the moment of the maximal storage in the cells. The dye concentration in the urine reaches its maximal height a few hours after injection; the maximum of storage is obtained after a few days, and will remain unchanged during a long time, while the urine contains but traces of the colour. If the dye were secreted by the tube, no such discrepancy between the curves of the elimination and of the storage would occur.

[1] These results are in contradiction with those of Ghiron; but this author could observe the tubes only and not the glomeruli, and admitted that these latter had recovered their filtering power after the urea injection.

Moreover, when the topographic distribution of the dye in the brush border segment is studied, it appears that the storage is the densest at the initial part of the segment; from this point it decreases gradually, and ceases about the middle part of the segment. This phenomenon may be easily explained in this way. The dye is filtered through the glomerulus in a diluted state (this we know by the micropipetting experiment). As it progresses through the striated border segment, the glomerular fluid is cleared up through the absorption of its coloured particles; and at a certain level, the concentration of the dye becomes so low that no storage phenomena follow its resorption phenomena. Von Möllendorff has thoroughly studied the way by which these storage granula are formed and has come to the conclusion that their formation is the same in the kidney as in the reticulo-endothelial system of Vertebrates. Before him this process had been described by Burian in the Invertebrates under the name of athrocytosis. We will make use of this convenient word to indicate the intracellular flocculation which follows the absorption of electro-negative colloidal particles.

Experiments undertaken by Dawson (6, 7) on *Necturus* afford supplementary proof of the resorption in the brush border segment. In this animal—as in all the Urodeles—the lumbar kidney is composed of a mixture of open and closed glomerular nephrons. After subcutaneous injections of trypan blue, an athrocytosis of the dye develops, with the same intensity, in the two kinds of nephrons.

But if intraperitoneal injections be made, the athrocytosis is much more developed in the open nephrons than in the closed ones (fig. 2, left).

This difference must be ascribed to the fact that the striated border segment of the closed nephrons receives only the dye which has filtered through the glomerulus, while it in the open ones in addition to the same glomerular fluid receives the much more concentrated solution entering through the peritoneal canals. No wonder then that the athrocytosis is by far more conspicuous in the open nephrons. But the athrocytic power of this segment is not restricted to particles which can pass through the glomerular barrier: it is much more extensive, as Lambert (27, 28), Gérard and Cordier (17, 19) have shown, in the Urodeles and the Discoglossidae tadpoles. When solutions of low dispersion (as soluble Prussian blue, or colloidal thorium oxide, or Indian ink Pelikan), the particles of which are unable to pass through the glomerulus, are injected into the peritoneal cavity, athrocytosis takes place in the open nephrons only (fig. 2, right). But, if previously the peritoneal funnels have been experimentally occluded, no athrocytosis at all occurs.

Therefore, this phenomenon depends on the fact that the particles come in close contact with the apical surface of the brush border cells.

It was then interesting to inquire if particles larger than those of Indian ink would also be absorbed by the cells of the segment, and to determine the limit at which this phenomenon ceases.

This we have done by injecting in the same Amphibians a suspension of

minute crystals of cinnabar, or of sepia ink (which is a suspension of very small melanin granules). In both cases these visible granules were absorbed and stored in the cells of the brush border segment, not in its initial portion, but in its distal one. This process can no more be called athrocytosis but phagocytosis. This phagocytosis is restricted to particles smaller than 1 μ, for carbon dust of this size is left unabsorbed.

Thus we may consider the cells of the brush border segment not only as athrocytic, but also as phagocytic cells: they are athrophagocytes in the sense used by Cuenot, with the restriction that they are polarised athrophagocytes, which can exert this power through their apical surface only.

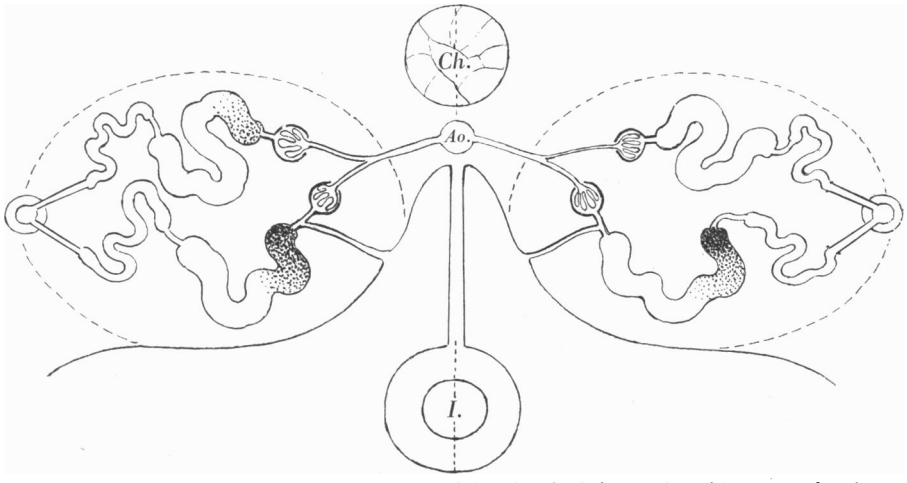

Fig. 2. Schema of the lumbar kidney in an Urodele. On the left, results of Dawson after intraperitoneal injection of trypan blue—athrocytosis much more developed in the open nephrons than in the closed ones. (When the dye is injected subcutaneously, the amount of athrocytosis is equal in both nephrons.) Note the proximally located athrocytosis. On the right, results of Gérard and Cordier, and Lambert, after intraperitoneal injection of Prussian blue, or Indian ink. Athrocytosis takes place in the open nephrons only, and is distally shifted. The same result is obtained after injection of a suspension of melanine granules, or of cinnabar crystals (phagocytosis).

The arguments in favour of the resorbing activity of the brush border segment are thus as impressive as those in favour of its secretion power.

It would then seem quite logical to ascribe to it a double function of secretion and of resorption. But it seems that this idea is regarded as nonsense by most of the experimentalists.[1]

This must be ascribed to the fact that most of the researches have been undertaken with the view of confirming or upsetting one of the two theories.

Gérard and Cordier (15) examined the problem again, restricting the experiments to one species, in which it was possible to dissociate the glomerular

[1] Ellinger and Hirt appear to be the only ones who, after experimentation, support the double function of the brush border segment.

from the tubular function. In this manner they could get results comparable with one another.

After preliminary trials they chose the toad (*Bufo vulgaris*, *B. calamita*) to experiment on. This Anuran is much better suited to experimental work than the frog, because the suppression of the arterial blood supply in its kidney does not provoke the desquamation of the tubular epithelium. They sectioned first, with the thermocautery, the renal arteries of one-half of the organ (there are four to six renal arteries). As there are no anastomoses between the different arterial territories, the arterial blood supply is abolished in the operated part; the other half of the kidney, which keeps its normal circulation, serves as control.

First of all they had to ascertain the behaviour of the operated portion. This was done by histological examination of the kidney at longer and longer intervals after operation.

This preliminary work enabled them to affirm that, in the region with suppressed arteries, no recuperation of the circulation takes place during three weeks following the operation. The tubes in that region remain quite normal, and can be distinguished from those of the control region by their shrunken lumen. As soon as the circulation is restored in the glomeruli, the lumen of the corresponding tubes widens immediately. The soundness of the tubular epithelium in the operated portion may last much longer than three weeks. In many cases some glomeruli are still without a circulation two months after the operation. They are only recognisable by their narrow lumen.

A. Experiments proving the secretory function of the striated border segment

(1) 1–2 c.c. of a 1 per cent. methylene blue solution is given subcutaneously to an operated toad. When the elimination of the dye is in full progress, a fixing fluid, which acts also as a precipitant towards the colour, is injected through the aorta. By this means the fixing fluid reaches the cells immediately, the shifting of the dye is avoided, and it is precipitated in the vacuoles in which it has segregated. In the sections the dye can be demonstrated in the striated border cells of the operated as well as in those of the normal part of the kidney; it is even more abundant in the former. This fact, which had been observed previously *in vivo* by Ellinger, can be explained by a delayed transit of the dye through the cells of the operated region (fig. 3).

(2) Under the same experimental conditions, uric acid previously dissolved by addition of piperazine (2 injections of 0·05 gm. of uric acid at 20 min. interval) is injected, and, half an hour after the last injection the kidney is fixed by an intra-aortic injection of the van Gehuchten fluid, in which urate of piperazine is insoluble. Thanks to its low diffusibility this salt is precipitated without shifting, and its localisation can be traced with the polarisation microscope.

In the normal region of the kidney crystals are detected in the cells of the brush border segment and more abundantly in its lumen. Occasionally a crystal may be discovered in the glomerular space. In the operated region most of the crystals are included in the cells of the same segment, and few in its lumen. On the contrary in both regions of the kidney the cells of the rodded segment or those of the collecting tubules appear quite free from crystals (fig. 3).

Thus the brush border segment is able, alone, to eliminate methylene blue and uric acid: it behaves as a secretory organ.

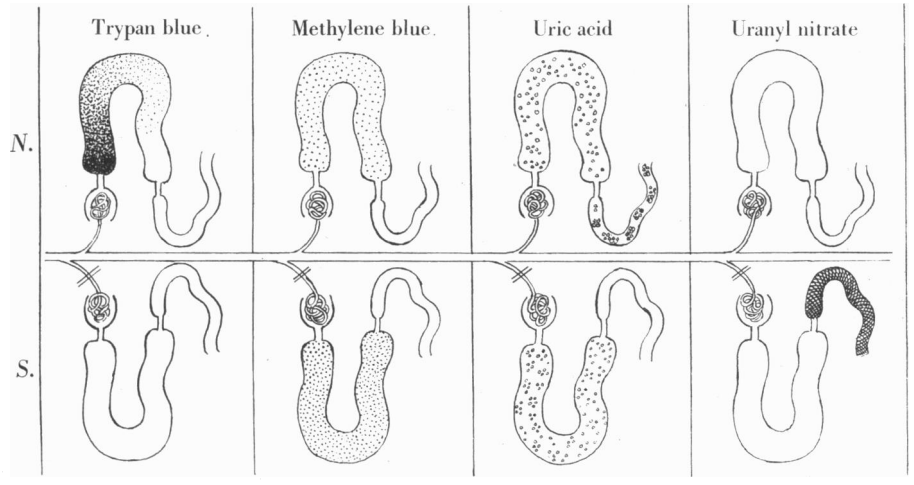

Fig. 3. Schema of the experiments of Gérard and Cordier on the toad. The upper part represents the normal nephron (*N.*), the lower one, the nephron with the glomerular blood supply suppressed (*S.*) after injection of various substances.

B. Experiments proving the resorbing function of the striated border segment

Quite different are the results obtained after injections of a colloidal electronegative dye, as trypan blue or ammonium carminate. In these instances, the dye is only found in the normal portion of the kidney, where it has been stored with a maximal athrocytosis at the beginning of the brush border segment. On the contrary, in the operated part, all the cells of the segment are empty.

Thus, the brush border segment behaves quite differently towards a non-colloidal basic and a colloidal acid dye. It can absorb the first one through its basal surface, which proves quite impermeable for the second one; on the contrary, the same acid dye is absorbed and stored when it comes in contact with its apical surface (fig. 3). Moreover, there is a parallelism between the intensity of the glomerular circulation and the extent of the athrocytosis. This is rendered evident when injecting toads more than three weeks after

operation. At that time, there exists in the operated region of the kidney a mixture of glomeruli which have recovered their normal circulation, of others which remain streamless, and lastly of others in which the blood supply is still small (this is detected by the slight quantity of Indian ink sticking to the capillar endothelium). The brush border tubules related to these three species of glomeruli exhibit a quite different degree of athrocytosis: in the first tubules it is as developed as in the normal region; in the second there is none; in the third it is but poorly apparent.

The same results are obtained with other electro-negative colloids: solganal (aurothio-glucose), ammonium iron citrate, etc. Ovalbumin behaves also as an electro-negative colloid: it is stored in the brush border cells of the non-operated region, and is quite absent from the operated one.

These proofs of the resorbing function of the brush border tubule are also strengthened by another experiment (Gérard (14)).

The Teleost *Lepadogaster Gouanii* possesses a very peculiar kidney. In the front part persists the pronephros constituted by a single glomerular nephron; posteriorly, a set of mesonephric lobules are found, each of which is formed by some aglomerular nephrons. If trypan blue be injected into this fish, the dye is found stored in the striated border segment of the pronephros, the athrocytosis being there at its height in the initial part. On the contrary, no dye can be found in the mesonephros. Now, the two species of nephrons differ from each other by the glomerulus only. It must then be concluded that the athrocytosis in the pronephric nephron originates in the resorption of the dye which has been passed through the glomerulus (fig. 4).

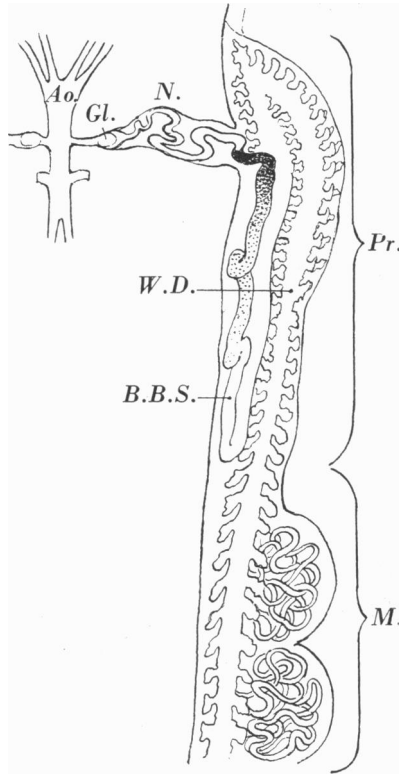

Fig. 4. Schema of the anterior part of the kidney of *Lepadogaster Gouanii* after an injection of trypan blue. Athrocytosis takes place in the brush border segment of the glomerular nephron only. *Ao.* = aorta; *B.B.S.* = brush border segment, showing initial athrocytosis; *W.D.* = Wolffian duct; *Gl.* = glomerulus; *M.* = mesonephros; *N.* = neck; *Pr.* = pronephros.

Summing up all the experimental data, these two conclusions may be drawn: through its basal membrane the cell of the striated border segment acts as a glandular cell: it is secretory. Through its apical membrane it resorbs some of the plasmatic substances which have filtered through the glomerulus—crystalloids and highly dispersed colloids—the resorption of these latter being

followed by their athrocytosis. In the case of open nephrons, resorption and athrocytosis of much bigger particles takes place.

C. Function of the rodded cells segment

All the histophysiologists agree to ascribe to this segment a water-resorbing function. When a living kidney is examined while excreting a dye, it can be noticed at first sight that the coloration of the liquid flowing in the lumens is much more marked in the rodded cell segment than in the striated border tubule. Never has there been a secretory process demonstrated in it. On the contrary, we have obtained numerous proofs of water resorption at its level.

(1) Bensley and Steen observe *in vivo* the elimination of indigo sulphate through functionally isolated tubules. The urinary flow, of course, had become very slow. When the fluid reaches the rodded cell segment, crystalline deposits, formed by minute needles of the dye, appear in the lumen.

(2) During the experiments on the elimination of uric acid, it is always observed, in the normal portion of the kidney, that the lumen of the brush border segment contains a very fine crystalline dust, while in that of the rodded cells segment there appear very large birefringent deposits. As no secretion is conspicuous in this latter segment, this picture shows evidently that the uric acid has been concentrated in the rodded cell segments.

(3) If uranyl nitrate be injected into a toad, this salt is eliminated through the kidney. It occasions a necrosis in the rodded cell segment where it can be easily detected.[1] It is quite impossible, with this sole experiment, to discover the mechanism of the lesions. But if we repeat it after partial suppression of the renal arteries, no injury at all can be discovered in the operated portion of the kidney. The suppression of the glomerular function has protected the kidney against the nocuous action of the uranyl salt. Thus, this substance is eliminated through the glomerulus and passes through the brush border segment without causing any injury because of its insufficient concentration; but as soon as it reaches the rodded cell segment, its concentration increases to such a degree, through resorption of water, that necrosis follows (fig. 3). Thus, to each segment of the glomerular nephron may be ascribed a different function. The glomerulus acts as an ultrafilter; the glomerular urine which it passes undergoes quantitative and qualitative changes during its course through the brush border segment, by a double process of secretion and resorption. Finally, as it reaches the rodded cells segment, it concentrates through water resorption.

II. The athrophagocytic function of the nephron and its significance

One of the most astounding features of the brush border segment is the storage process of colloidal substances known as athrocytosis. From this standpoint, a difference of behaviour exists between the closed and the open

[1] In the Mammals, the lesions are located in the striated border segment.

nephrons. In the former indeed are only stored the micellae which can pass through the glomerular barrier; the second are capable of exerting their athrocytic function on much larger particles. This difference must be ascribed to the presence, in the open nephrons, of the peritoneal duct, and not to a difference in the glomerular permeability.

Now if we examine the localisation of the athrocytosis all along the brush border segment, we are struck by the fact that it is not the same for the small or for the large micellae. Von Möllendorff had already noticed that the athrocytosis of the trypan blue is localised at the proximal end of the brush border segment, while that of the less diffusible pyrrol blue is shifted somewhat distally. From this observation he had drawn the conclusion that the distal portions of the brush border segment are more permeable than the proximal ones, since they can reabsorb bigger particles. But exact data concerning the dispersity of colloidal solutions were not available then, and without these it was impossible to attempt accurate investigations.

Thanks to a better instrumental equipment, Gérard and Cordier (17,18,19) were able to undertake the study of this problem in the Anurans and some other Vertebrates, while Lambert (28) did the same for the Urodeles. The starting-point of our researches consists in an observation made after an intramuscular injection of tellurium in the toad. It had been shown by Levaditi that if a suspension of tellurium be injected intramuscularly, it is resorbed and eliminated partially through the kidney, in the brush border segments of which the metalloid appears as a deposit of black granules. This experiment was repeated in the toad with the same results, and we started to inquire through what portion—glomerular or brush border tube—tellurium was eliminated. Having injected the metalloid into toads whose renal arteries had been partially suppressed, we found the telluric granules only in the normal portion of the kidney; in consequence they must have been filtered through the glomerulus, reabsorbed and stored into the cells of the striated border segment. Moreover, the athrocytosis of the tellurium does not begin immediately after the neck, as it does for trypan blue; but it has shifted distally, and its maximum density occupies the region of the yellow granules. This can be better demonstrated on operated toads which have been injected successively with tellurium and ammonium carminate: the initial portion of the brush border segment presents thus a very dense athrocytosis of the red dye, which decreases gradually; then comes an intermediate portion where scarce granules of carmine and of tellurium are found together; more distally, telluric granules only are conspicuous (fig. 5). This experience demonstrated that the striated border tube behaves, in its storage of substances filtered through the glomerulus, as if it were composed of portions performing each a different function; but it was then impossible to give a satisfactory explanation of this phenomenon.

When Lambert's researches had shown that the Indian ink was stored by the striated border segment in open nephrons (which was confirmed afterwards

by Dawson), we decided to make use of the open pro- and mesonephric nephrons in the tadpoles of *Rana*, *Discoglossus* and *Alytes* in order to investigate the athrocytic localisation of colloids presenting a different degree of dispersion. If we inject into the splanchnocoele of tadpoles a solution of trypan blue or of ammonium carminate, which is able to be filtered through the glomerulus, an athrocytosis of this substance develops in the proximal end of the striated border tube, and presents a maximum of density just at its beginning.

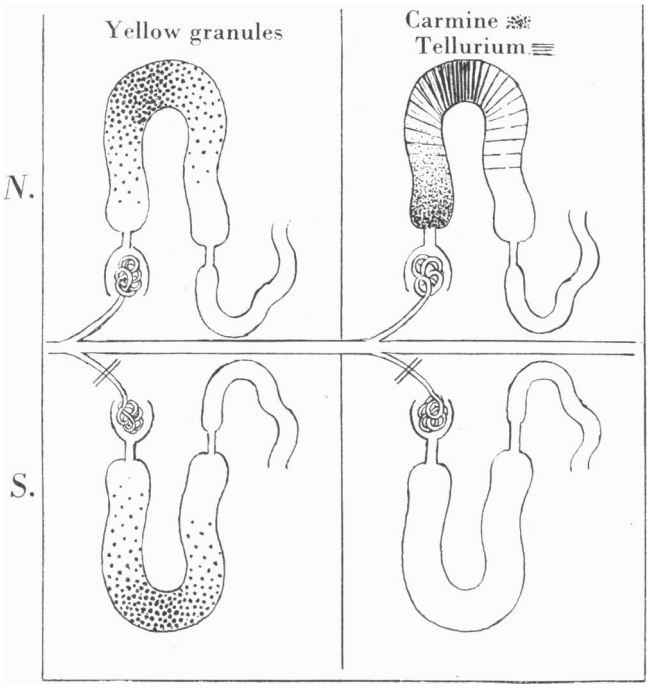

Fig. 5. Schema of the localisation (*a*) of the yellow (so-called secretory) granules, (*b*) of the athrocytosis of carmine and tellurium, in the toad. Above, normal nephron (*N*); below, nephron with suppressed arterial glomerular supply (*S*). The level of the tellurium athrocytosis corresponds to that of the yellow granules. The suppression of the glomerular blood supply does not affect the amount of yellow granules, which form very slowly, and, when stored, remain in the cell.

On the contrary, after an injection of Indian ink, no athrocytosis can be detected in the initial portion of the tube; it appears, but very sparse, near the middle part of the tube, and from this point increases distally till it reaches a maximum density at the end of the segment. Moreover, if ammonium carminate (or trypan blue) and Indian ink are injected on successive days into the same tadpole, the first substance is found stored in the initial, the second one in the distal portion of the striated border tube. And the same result may be obtained if the order of injections be reversed. Here we

find, but with an extremely developed shift, the figures we had obtained in the toad after the double injection of carmine and tellurium. The difference in dispersion between trypan blue (or ammonium carminate) and Indian ink is easy to control by simple methods. Biologically, it can be easily demonstrated by the fact that the first two dyes are filtered through the glomerulus, while the ink is retained by it: but these data are but approximate, and it would have been advisable to get precise measures of their respective dispersions. It would have been interesting also to make use of substances the particles of which are of intermediate size between these two extremes.

The ultrafiltration method giving poor results, we used for this purpose Nistler's apparatus. It consists of a specially devised comparison microscope which enables one to measure the diffusion coefficient in water[1] of the substances employed. From this data the particle dimensions can be deduced with the help of the Einstein formula.[2] Using this method, we have calculated the following data for the substances which give rise to athrocytosis in the kidney:

Substances	Diffusion coefficient (D)	Radius of particles Å.	Substances	Diffusion coefficient (D)	Radius of particles Å.
Trypan blue	3·03	6·5*	Colloidal thorium oxide (Thorotrast)	—	80 (from the von Heyden firm)
Ammonium carminate	1·94	10·2*			
Ink R. A. L.	1·83	10·8	Soluble Prussian blue	0·17	115*
Chlorophyll	0·83	23·3	Indian ink (Pelikan 541)	—	>1000
Iron saccharate	0·76	24·6			

* See footnote 2 below.

With this series of colloids, whose micellar dimensions vary between 6·5 and 1000 Å., we determined the site of their athrocytosis in the open nephrons of tadpoles.

We know already that trypan blue, ammonium carminate, the micellar radius of which lies between 6·5 and 10·8 Å., are stored in the brush border segment, with an initial maximum of athrocytosis. On the other hand, the Indian ink for bacteriology R. A. L. gives also maximal initially located athrocytosis: its micellar radius is 10·8 Å. The athrocytosis of these four substances is equally intense in the closed and in the open nephrons, when injected subcutaneously. When the injections have been given into the splanchnocoele, it appears of course more intense in the open nephrons, which receive, through the peritoneal canal, a supplementary dose of the dye.

After an injection of chlorophyll (23·3 Å.) or of iron saccharate (24·7 Å.) athrocytosis always takes place in the open nephrons: its maximum is no more initial; it has shifted somewhat distally, and is situated near the end of the first quarter of the striated border tube. In the closed nephrons,

[1] Hanut and Fautrez(25) have shown that the diffusion coefficient of the dyes used *in these experiments* is not affected by addition of serum, nor of salts in the same proportion as in the blood.

[2] The radius calculated by this method is but approximate for salts (and dyes behave as salts); it is quite reliable for such substances as chlorophyll, haemoglobin and other proteids.

athrocytosis appears irregularly: present in some animals it is lacking in others. In this case, individual factors have been set in action to prevent or to allow the glomerular filtration of particles of 23–24 Å. Thus this dimension of the particles marks the limits of the glomerular permeability in the Anurans. Above these numbers, the athrocytosis takes place solely in the open nephrons. Likewise thorium oxide, collargol, soluble Prussian blue, the particulate radius of which ranges between 80 and 115 Å., are only stored in the open nephrons,

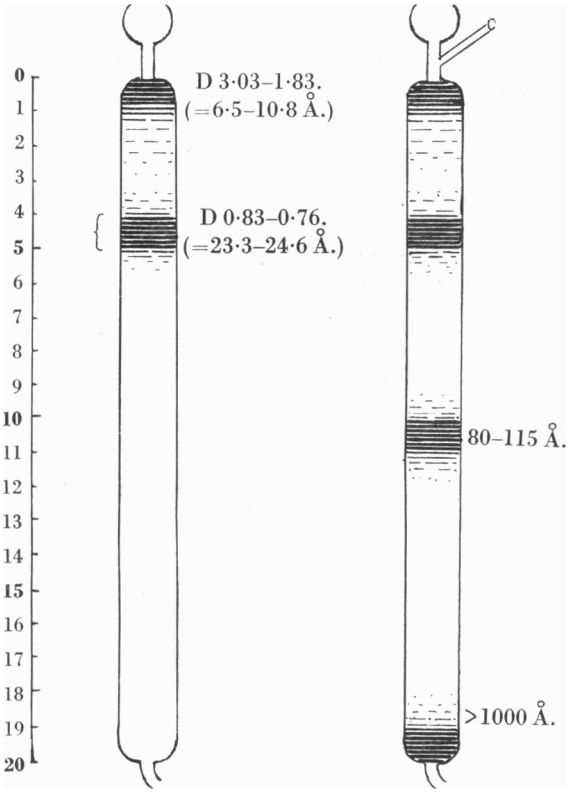

Fig. 6. Schema showing the levels of the maximum of athrocytosis for different substances in the brush border segment of Discoglossidae tadpoles. On the left, closed; on the right, open nephrons. This localisation is the more distally shifted as the diffusion coefficient is low (or as the particle dimension is great).

their maximal athrocytosis being located near the middle portion of the striated border tube (fig. 6).

Finally, at the end of our series, Indian ink Pelikan 541, whose particulate radius measures more than 1000 Å., is stored in the distal portion of the segment, with a terminal maximum of athrocytosis (fig. 6). Moreover, if the same animal be injected successively with solutions differing widely from one another in dispersion, as trypan blue, thorium oxide, Indian ink Pelikan 541, these three substances are received in the brush border segment, each

with a localisation of its own: the trypan blue in the proximal, the thorotrast in the middle, and the ink in the distal portion. In each of these portions athrocytosis reaches its maximum at a very characteristic level: initial in the first one, median in the second, and distal in the third. These portions are separated from each other by transitions in which are found side by side scanty granules of the two adjacent areas.

This athrocytic picture remains unchanged, whatever be the order in which the injections have been given. From these experiments the following conclusions may be drawn: all along the brush border segment the cells do not enjoy the same apical permeability; the cells of the initial portions are pervious to the smallest colloidal particles only, those which, indeed, are filtered through the glomerulus.

As the distance from the glomerulus increases the permeability becomes greater and greater, till it reaches its maximum at the end of the segment. At this level, phagocytic processes also take place. Thus a gradient of apical permeability may be postulated in the striated border segment, which increases from its initial to its distal portion.

In the open nephrons of adult *Salamandra* the general outline of the athrocytosis is the same, except that the maximal athrocytosis of the substances with a particulate radius from 80 to 115 Å. is much more distally shifted, and is distinguishable with difficulty from that of the Pelikan Indian ink. But the gradient is here as easily observed as in the Anurans. Further, the limit of the glomerular permeability is more distinct: so, the chlorophyll (23·3 Å.) is filtered through the glomeruli and is stored in all the nephrons, while the iron saccharate is to be found in the open nephrons only. The limit of the glomerular permeability, in the *Salamandra*, may be fixed at 24 Å. In the closed nephrons, the gradient of permeability exists also, but it is less conspicuous, the resorption and the athrocytosis being only exerted on the particles filtered through the glomerulus: it is thus limited by the glomerular permeability.

But this gradient is not only apparent in the case of electro-negative colloidal dyes; it is as clear for the proteids, as has been demonstrated by Lambert in *Salamandra* (28).

Unhappily, our information about the micellar dimensions of the different proteids is still very scanty and, as they are colourless, it becomes impossible to follow their diffusion speed with the Nistler microscope. But we can draw up a list in which they are arranged according to the increasing order of their molecular weight, and we get the following table:

Substances	Molecular weights	Observations
Ovalbumin	34,000 (after Bayliss, Kerridge and Russell)	—
Haemoglobin	68,000 ,,	—
Serum albumin	68,000 ,,	69,000 (after Roche, Dorier and Marquet)
Serum globulin	103,000 ,,	150,000 (after Roche and Braco)
Casein	788,000 ,,	—

We already know that in the Anuran kidney injections of ovalbumin, as of coloured electro-negative colloids, are followed by athrocytosis.

How do the Salamander nephrons behave towards these different-sized proteids? After injecting them into the splanchnocoele, all of them are found stored in the striated bordered segment of the open nephrons, but the maximum of athrocytosis has, for each one, a different localisation. In order to locate it accurately, reconstructions must be made from serial sections of the

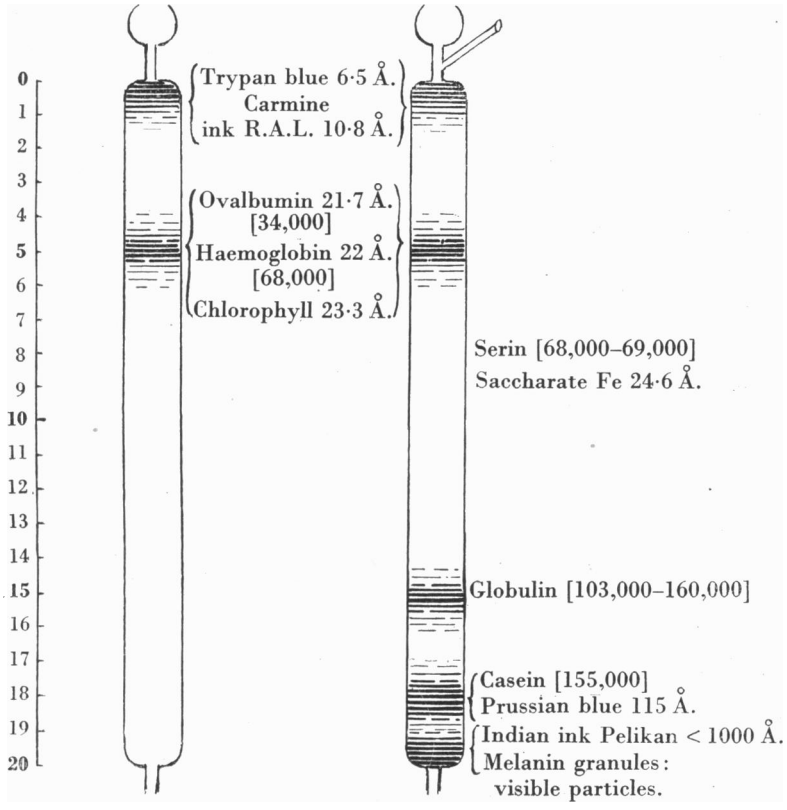

Fig. 7. Schema showing the levels of the maximum of athrocytosis for different substances in the brush border segment of *Salamandra*. On the left, closed; on the right, open nephrons. The localisation of athrocytosis follows the same law as in the Anuran nephrons.

tube. For the sake of an easy study, let us take as reference an uncoiled brush border segment, divided into twentieths, on which these maxima are plotted.

The maximum of athrocytosis, for ovalbumin, is not initial; but it has shifted in the region of the $\frac{4}{20}$th–$\frac{5}{20}$th. Its limits are not sharply marked distally, the ovalbumin producing in that region vacuolisations in the cytoplasm of the segment (fig. 7).

It is impossible to give precise details about the location of the haemoglobin athrocytosis; the histochemical detection of this substance is only practicable

on frozen sections, from which reconstructions of the tube cannot be made; nevertheless it may be suggested that this maximum is located in the first half of the segment.

As for the serum albumin, its athrocytosis is maximal at the level of the $\frac{9}{20}$th; for the serum globulin, between the $\frac{16}{20}$th and $\frac{17}{20}$th; for the casein, in the last twentieths of the brush border tube (fig. 7).

If we compare now these results with the data given in the table, we may infer that there exists a relation between the localisation of the maximum of athrocytosis and the molecular weight: the former is the more distal as the latter increases.

The power of selective athrocytosis in the brush border segment can be yet better demonstrated in this way. Instead of injecting separately serum-albumin or serum globulin, let us inject diluted serum. Theoretically, we may predict that in the brush border segments two maxima of athrocytosis will appear. And these we do really find; the first one is conspicuous at the level of the $\frac{7}{20}$th; from there the athrocytosis decreases and then increases gradually till it reaches a new maximum in the $\frac{17}{20}$th of the brush border segment. The first region of athrocytosis is that of the serum albumin, the distal one, that of the serum globulin. Thus, the striate bordered tube acts as a sorting apparatus towards the mixture of the protein micellae; those with a low molecular weight have been resorbed in the proximal parts of the brush border tube; those with a high molecular weight in its distal parts.

Instead of expressing the relation between the maximum of athrocytosis and the molecular weight, let us try if it can be done in terms of the micellar dimensions of the different proteids. Till recently, we had but one measure, that of ovalbumin, the micellar radius of which is 21·7 Å. (Nichols). With Nistler's method, Lambert and Fautrez (29) have just found that the radius of the haemoglobin micella amounts to 22 Å.

Now, if we compare two substances of widely different chemical formula, but of near molecular weight as ovalbumin and chlorophyll, we see that the maximums of their athrocytosis are located nearly at the same point of the brush border segment. From the level of its athrocytosis, we may thus infer the approximate particulate radius of a proteid whose dispersion is unknown. By this means we may estimate the particulate radius of the serum albumin at 22–24 Å. (its maximum of athrocytosis agreeing with that of iron saccharate); the radius of the serum globulin particle must be situated between 40 and 50 Å., since its maximum of athrocytosis is localised before that of thorotrast (80 Å.); and the radius of the casein must be greater yet.

Through an indirect means it is possible to get a more accurate value for the micellar radius of the serum albumin. The researches of Bayliss, Kerridge and Russell (1) have shown that, in the dog, proteins with a molecular weight greater than 68,000 are not eliminated through the kidney, while those with an equal or inferior weight are excreted. But their experiments do not inform us about the site of elimination in the nephrons. We have already seen that

in *Salamandra* the ovalbumin and the haemoglobin are filtered through the glomerulus, and that they are stored in both open and closed nephrons. On the other hand, injections of serum globulin and of casein are followed by athrocytosis in the open nephrons and only after intraperitoneal injections.

As for the serum albumin, subcutaneous injections of this substance are followed by a slight athrocytosis in both nephrons, but only after numerous injections. Thus serum albumin is just at the limit of the glomerular permeability, which can be said to be a little higher in *Salamandra* than in the Mammals. We have found previously that this limit, when expressed in terms of the micellar radius, is 24 Å. Therefore we may fix at 24 Å. the value of the micellar radius of the serum albumin.

In the Vertebrates, other than Urodeles, whose kidney is formed by closed nephrons, athrocytosis is also found in the brush border segment; but it is restricted to those particles which can filter through the glomerulus.

In the Mammals, the glomerular filtration limit is lower than in the Salamander, as no athrocytosis can be obtained by injections of chlorophyll. Moreover, the permeability gradient is more developed in the Mammals, for the athrocytary maximum of trypan blue (6·5 Å. radius) is not localised at the very beginning of the tube, but is shifted a little distally. It may be inferred that in Mammals the apical permeability in the initial cells of the brush border segment is less than in the Poikilotherms, and that in this region are resorbed particles of very minute radius (less than 6·7 Å.), and which therefore do not give rise to athrocytic granules (fig. 8).

It was interesting to inquire about the existence of athrocytosis in the renal organs of Invertebrates. In most of them indeed the urinary organs or nephridia are homologous to the tubular part of the Vertebrate nephron; these nephridia are often closed at their proximal end, like the aglomerular nephrons, and consequently never will exhibit any athrocytosis phenomenon. But some nephridia are open, and, like the open Vertebrate nephron, connect the body cavity with the external world. The most propitious of these open nephridia for experimentation is that of the earthworm. It has a very complicated structure, which we will schematise in this way. It is made of three lobes, joined together at their base, the middle one being the longest. At its beginning, a ciliated nephrostome is differentiated, which is continuous with the nephridial tube; this much-coiled tube runs in a very complicated fashion through the three lobes, and opens at its end into a small urinary bladder. Cytologically, it is differentiated in several portions, one of which, the ciliated segment, presents a structure rather similar to the brush border segment. It runs straightway through the median lobe, and opens into a dilated ampulla. The coelomic liquid is forced through the nephrostome by the long cilia which cover it; after a long run it enters the ciliated segment at the base of the second lobe, and leaves it at its summit, where begins the ampulla; from that point it comes back to the first lobe, and finally passes into the third one, and from it into the bladder.

Cordier(4) has undertaken a series of experiments on this nephridium. After injections of electro-negative colloids into the coelom, athrocytosis, which can never be obtained after injections of non-colloids, occurs in the ciliated segment; here also it presents a maximum, the localisation of which is different according to the injected substances. For colloids, the radius of which ranges from 6·5 Å. to 115 Å., the maximum of athrocytosis is at the initial part of the ciliated segment; from that point it decreases gradually. But if particles of 1000–1200 Å. radius be injected (Indian ink Pelikan), the maximal athrocytosis is shifted distally and located in the middle portions of the ciliated segment[1] (fig. 8).

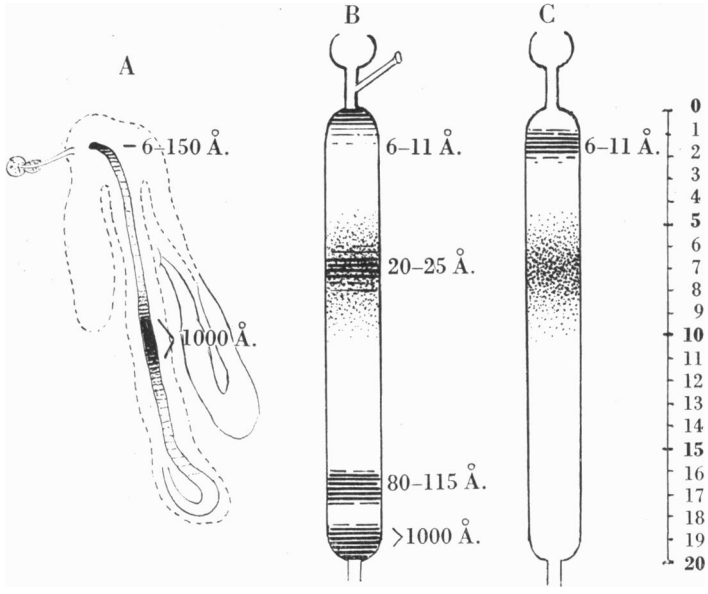

Fig. 8. Schema of the localisation of the maximal athrocytosis. A, in the Oligochaete nephridia; B, in the Urodele nephron; C, in the Mammalian nephron. The stippled areas correspond to the portion filled with yellow granules.

Thus it appears that there exists also an apical permeability gradient in the nephridia as in the Vertebrate nephron. But its value is not the same in the animal kingdom; it varies gradually from the Invertebrate to the Mammal, and is characterised by a progressive diminution of the apical permeability in the initial portion of the tubular system. In the earthworm this initial portion stores particles the radius of which ranges from 6·5 to 115 Å.; in the Amphibians it stores particles from 6·5 to 10·5 Å. radius; in the Mammals it does not allow these same particles to pass. The discriminating power of the athrocytic segment for particles of different size is only slightly indicated in the Oligochaete; it becomes very efficient in Amphibia; it is

[1] In this area also are phagocytosed the small melanin granules of *Sepia*. This portion exhibits an athrophagocytic function similar to that of the distal end in the Vertebrate open nephrons.

highly developed in Mammals. This physiological improvement conforms with the morphological evolution of the animal kingdom.

Thus in the brush border segment of the Vertebrate, as in the ciliated segment of the earthworm, the resorbed substances can be readily recognised by these two characteristics: they are found in a granular form; they are unequally distributed in all the cells of the segment. As granules they are stored in limited portions of the segment, the localisation of their athrocytosis varying with their dimensions. Consequently, whenever a localised region of athrocytosis is located in a nephron, it may be concluded that the stored substance is a colloid which has been reabsorbed by the cells of that part.

Now, in most of the Poikilotherms, a portion of the brush border segment—approximately the second quarter—contains regularly the yellow granules we mentioned before. In the proximal part of the segment these granules are small and scanty; distally they become bigger and more numerous, till a maximum of density is reached, localised roughly near the middle of the segment; from this point their volume and number decrease gradually, and soon the rest of the segment appears quite empty (figs. 5 and 8).

These granules are considered as secretory granules from their likeness to the granules found in the glandular cells. This resemblance is most striking if oblique sections of an Amphibian kidney be examined: here are found side by side sections of the segment in which cells are filled with large granules, while in others the cytoplasm contains only a filamentous chondriom; between these two extremes other sections exhibit all transitional stages. A mere seriation of these pictures would give all the stages of a secretory process as has been done frequently—if longitudinal sections, or teasings of the segments, were not examined: then, the localisation of the granules becomes conspicuous with their maximum of density in its middle part. This is rendered more striking after an injection of trypan blue or ammonium carminate: the athrocytosis of the dye—with its initial maximum—fills the first portion of the segment and then decreases; afterwards comes a region in which coloured and yellow granules are found in the same cells, till a region is reached in which yellow granules only are conspicuous.

Moreover, if the formation of these granules was the result of a cyclic process of secretion it would be logical to expect also the presence of large granules at the beginning or at the end of the supposed secretory region of the tube, and small ones in its centre. Now, this never happens, and the seriation of the granules occurs always in the same fashion. The yellow granules, with their characteristic localisation, have been found in the following families: Cyclostomata, glomerular Teleosts, Ganoids, Dipnoi, Anurans, Chelonia, Ophidians, and Lacertians. Let us point out here the absence of these granules in the aglomerular nephrons, which are purely secretory.

In the homoiothermic Vertebrates, the absence of granules in the brush border segment cells is an established idea. Still, two exceptions have been

pointed out, both concerning hibernating Mammals. Ferrata, in the marmot, and Suzuki, in the hedgehog, have found that during winter the brush border segment cells are filled with yellow granules, without noticing yet any special localisation. In teased preparations from the winter hedgehog kidney, it has been possible to demonstrate that the yellow granules are only present, with a median maximum of density, in the second quarter of the brush border segment. And this can be better illustrated by an injection of trypan blue into the animal: an initial coloured athrocytosis arises, behind which appears the yellow granule region: this figure is similar to that presented by the Poikilotherms.

For all these reasons we may conclude that the yellow granules are not secretory; they are the result of an athrocytosis exerted on colloidal particles which have filtered through the glomerulus, and the size of which is slightly under the filtration limit. When a great number of these granules has been heaped up in the cell, these undergo desquamation, readily followed by necrosis. Thus, athrocytosis is not merely an experimental phenomenon: it is a normal function performed by the kidney in many Vertebrates.

As we have seen, most of the homoiothermic kidneys do not exhibit any athrocytosis. It might then be questioned if their brush border segment has lost this property or if it does not show it for lack of opportunity.

Examples taken from pathology give full answer to this question. We will choose them from the human pathology for two reasons: first, because it is the best known pathology of all homoiotherms; secondly, because in the normal state the human kidney never exhibits athrocytosis.

These pathological conditions are characterised by an increased glomerular permeability: serum albumin which filters through the glomerulus is expelled with the urine, inducing in a limited portion of the brush border segment cytoplasmic changes known as albuminoid degeneration ("Trübe Schwellung" or "albuminöse tropfige Degeneration" of the German pathologists). In that state, the cytoplasm becomes filled with rounded, colourless, refringent granules; they give positive Derrien-Turchini reaction just as the protein granules produced in the Salamander nephron after serum albumin injections.

In another pathological state, the lipoid nephrosis, an increased glomerular permeability develops in a subject affected at the same time with hypercholesterolaemia. Serum albumin and sphero crystals of cholesterol are passed with the urine of these patients, in which they are easily detected.

Now, in the blood plasma, cholesterol is in a colloidal state. In this same state it is filtered through the glomerulus. During its course through the kidney its physical state must have been changed, since it is found crystallised in the urine. Now, if kidney sections coming from a case of lipoid nephrosis be examined, two different kinds of granular formations are discovered in the brush border segments, each one presenting its own and limited localisation. At one level of the tubule, protein granules fill the cells; in another, birefringent crystals or spherical crystals whose cholesterolic constitution may

be ascertained with the Lieberman reaction. Thus two regions of athrocytosis are detected in the same brush border segment (Gérard and Cordier (16)).[1]

If we recollect the experimental results we have obtained, we may explain in this way the successive phenomena which developed in the kidney. Owing to the increased glomerular permeability, superfluous blood cholesterol and serum albumin have been both filtered in a colloidal state through the glomerulus. Their dispersion being different they have been reabsorbed and stored in two different regions of the brush border segments; at the same time cholesterol has crystallised in the cells which subsequently have undergone desquamation when they became overloaded: this explains the occurrence of spherical crystals in the bladder urine.

It is easy to produce the same pictures in the Salamander. After two or three intraperitoneal injections of hypercholesterolised serum, cholesterol and serum proteins are reabsorbed and stored, causing a double athrocytosis in the brush border segment of the open nephrons only.

The same figures can be obtained in the closed nephrons after having increased artificially the glomerular permeability. This can be readily done with injections of ovalbumin: it is known that if ovalbumin is injected into a Mammal, it is filtered through the glomerulus; but, while passing through it, it increases the glomerular permeability to such an extent that serum albumin is also eliminated. Starting from that point, let us inject ovalbumin and hypercholesterolised serum into the body cavity of a toad, the renal arteries of which have been partially ligated. After numerous injections, an athrocytosis of proteins and cholesterol can be observed in the brush border segment of the normal part of the kidney, each one with a special localisation; in the ligated part, on the contrary, no athrocytosis at all can be detected.

Thus the closed nephrons of homoiothermic Vertebrates have preserved an athrocytic power similar to that existing in the open ones. Under normal conditions (beside the hibernating Mammals) they have no opportunity to exert it, either because their metabolism does not produce the colloidal mother substance of the yellow granules, or the amount of glomerular filtration of colloids is too low to give athrocytosis. But, as soon as the glomerular filter opens, the athrocytic properties, till then latent, awake and develop to the full.

From all these researches the following conclusions may be drawn. Athrocytosis is a characteristic property of the striated border segment of the Vertebrate nephron: it arises after the resorption of electro-negative colloids through the apical surface of its cells. This apical permeability is not equal all along the segment, but presents an increasing gradient of permeability from its proximal to its distal end. The degree of athrocytosis, or even its starting, depends on anatomical conditions, which do or do not allow the colloids to reach the apical surface of the cells of the brush border segment. So, athrocytosis can develop to the full in the open nephrons; in the closed

[1] See also Randerath, E., *Ziegler's Beitr.* vol. XCV (1935).

glomerular nephrons it is restricted to the particles which have been filtered through the glomerulus; it is absent in the aglomerular nephrons.

The athrocytic power of the brush border segment is incomprehensible when closed nephrons alone are considered. But if we remember the ontogenetic as well as the comparative anatomical data, we must consider the closed nephron as being the result of a long evolution which we may schematise in this way. Primitively, the nephron, as the nephridium, opened into the coelom through a wide aperture, which later turned into a peritoneal duct. Through this duct large quantities of coelomic fluid were forced into the nephron and conducted towards the outside: now, in this fluid are dissolved proteins and many other substances whose incessant loss would be detrimental to the organism. The brush border segment acted on them as an organ of recovery, whose function is witnessed by athrocytosis phenomena. When the nephron, having lost its communication with the body cavity, became closed, it received only highly dispersed colloids filtered through the glomerulus: since then its athrocytic function seems to have become imperceptible or even abolished. But it is only dormant; in pathological conditions it awakes and appears as developed as in the open nephrons.

Therefore the athrocytic power in the closed glomerular nephrons must be regarded as an ancestral character, transmitted through heredity, which has been kept intact, although under normal conditions it has no opportunity to reveal its existence.

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