5-16-1938

Some Aspects of the Physiology of the Nephron

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SOME ASPECTS OF THE PHYSIOLOGY OF THE NEPHRON

A thesis submitted in partial fulfillment of the requirement for department honors in Biology

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May 16, 1938

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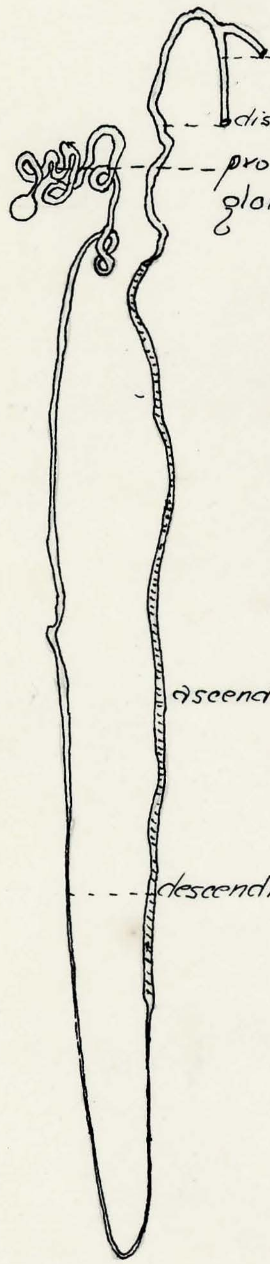
The nephron is the unit of function of the kidney. Through its activity the urine, or kidney excretion, is elaborated. Consisting of a Bowman's capsule, neck, proximal convoluted tubule, descending limb of Henle's loop, ascending limb of Henle's loop, and a distal convoluted tubule, the nephron performs a service without which the animal could not exist. The above named parts do not all appear in some of the lower vertebrate forms; the above being an enumeration of the parts found in the mammalian type. More will be said of phylogenetic variation of nephronic expression later in this paper.

The phrases "kidney function" and "nephronic function" are synonymous and will be used interchangeably herein. Kidney function consists of two aspects: the collection and subsequent discharge of certain wastes, and the maintenance of the osmotic pressure of the blood. Failure to perform either of these would most certainly result in death for the organism. It will be the purpose of this paper to discuss the various theories of kidney function, and to present the latest information which the research workers in the field have made available. Before presenting the main theme of the paper, I will briefly describe the histological aspects of the structures to be considered.

The capsule of Bowman (Fig.1) is the first functional portion of the nephron. It arises, as do the other nephronic elements, from the metanephroic blastema. Its visceral wall lies in contact with the endothelium of the glomerulus. This latter is a rete mirabile of capillaries which receive their vasculation from an afferent arteriole and are collected into an efferent arteriole. The visceral epithelium of Bowman's capsule consists of very thin cells which contain a few mitochondria and a Golgi net. The parietal layer is composed of cells of the ordinary squamous epithelial type. Leading

Fig. I



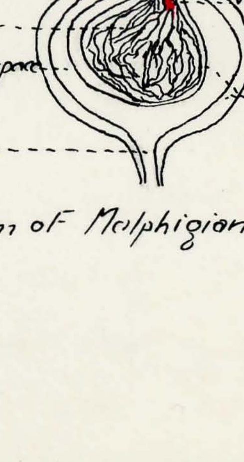


diagram of Malphigian corpuscle

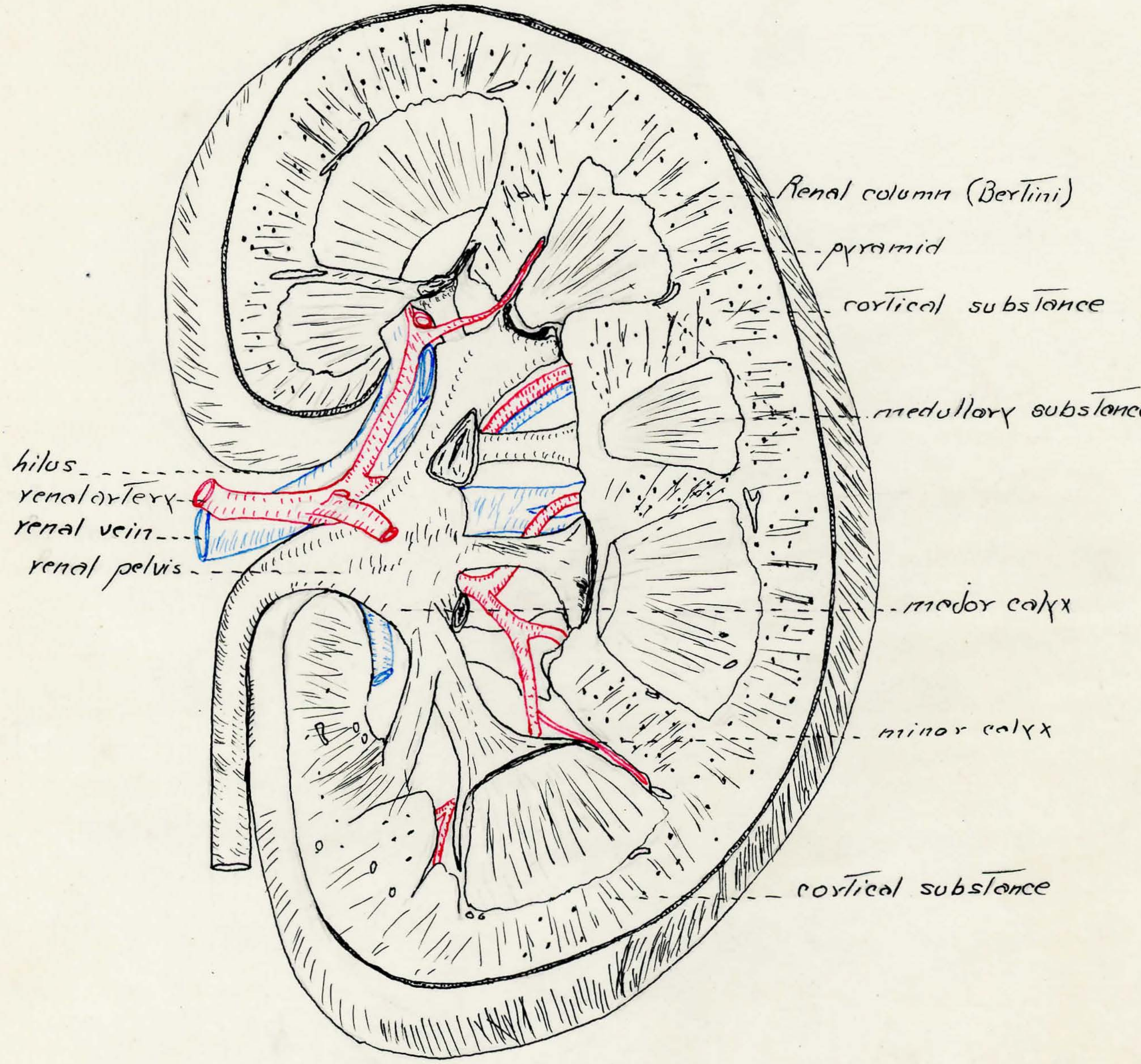
Metanephroic Renal Tubule (mammal)

after Huber - From Maximow

from the capsule to the proximal convoluted tube is a short neck which is not always seen. The proximal tubule is the most important segment of the nephron, and is composed of a truncated pyramidal epithelium whose free edge bears a brush border. The appearance of this epithelium varies with the functional state of the kidney. In diuresis it is low and flattened with the brush border protruding. While resting, its cells are taller and the border less evident. After pursuing its course, the proximal tubule passes abruptly into the descending limb of Henle's loop. Here the epithelium is a very thin squamous type and in appearance closely resembles the capillary endothelium. Continuing from the descending to the ascending limb of Henle's loop, the nephronic epithelium becomes again cuboidal, and mitochondria are once more evident. There is no brush border. The ascending limb joins the distal convoluted tubule which constitutes the terminal portion of the nephron. The latter presents a cuboidal epithelium which is lower than that of the proximal segment and a lumen which is larger. A brush border is absent, and its cells are more numerous. From this terminal portion the nephron is joined to the collecting tubule system, the latter arising embryologically from the Wolffian duct.

As regards the orientation of the various nephronic elements within the kidney, the following facts are known: the capsules, with their enclosed glomeruli, all lie within the cortical material of the kidney. The proximal tubule after leaving the capsule proceeds toward the periphery; turns, and entering a medullary ray runs toward the medulla. In the outer zone of the renal pyramid, it enters the descending limb of Henle's loop. The latter proceeds deep into the medulla; while the ascending limb, which for a part of its course runs parallel to its fellow, returns through the medulla to the cortex and attaches itself to the capsule near the efferent arteriole. At this point the

Fig. II



Cortex, medulla, and contents of the sinus of human kidney - dorsal view - part of organ removed

After Braus: From Maximow

distal tubule arises and passes to a medullary ray where it is connected to a collecting tubule. This account of the nephronic histology is far from complete, but will serve the purpose intended here.

Physiologists attacking the problem of renal function possess one great advantage. They know exactly what the kidney does. It elaborates a substance we call urine which possesses a chemical content which has been quantitatively analyzed with the most delicate techniquies as yet devised by the bio-chemist. What then is the problem? It is to determine just how the kidney performs its work, and according to the latest accounts (1937) the problem is far from being solved. The gross end point of kidney function is evident, and the problem remaining consists of assigning specific functions to the v_rious portions of the nephron. There are two principle courses open to the physiologist, either of which he may pursue in solving the problems concerned with nephronic activity.

The first of these is a study of the comparative physiology of the kidney. The nephrons of certain lower forms are glomerular and contain only a proximal segment. By studying these types (the toadfish is one) a certain amount of light may be thrown upon the function of the particular nephronic expressed. Another aspect for study is the noting of the functions added to the kidney as the various parts of the nephron are added in the phylogenic ascent. This work will be more fully discussed later and a criticism of it given at that time. The second method of attacking the renal function problem is direct study of the kidney with an attempt to devise ways for determining just what the various parts of the nephron are doing. THis method has received far more attention, and from applications of it most of our knowledge of the kidney physiology has come. Most of this type work has been done upon the frog and lower mammals (rabbit and cat). Many of the theories and statements applicable to these types have never been directly proven when applied to the human. However, the experimental difficulty involved is evident, and the better work (as far as it goes) probably presents a true picture of the function of the human nephron.

The Bowman's capsule, with its included glomerulus, provides a perfect condition for filtration to occur from the blood fluids in the capillary plexus into the intra-capsular space and the lumen of the proximal tubule. It was this histological set-up which convinced Ludwig that the urine is formed by a simple process of filtration and diffusion. He believed that in this portion of the nephron a complete but dilute urine is formed, and that concentration occurred by exosmosing of water from the luma of the tubules into the more concentrated plasma which bathed their outer surfaces. This is the mechanical or filtration theory,and while it has been attacked in the not distant past, is to-day generally accepted with certain reservations and modifications. Bowman, after Ludwig's work, introduced a theory of urinary secretion which states that in the Malphigian body, water and inorganic salts are produced in the capsule; while urea and related bodies are actively secreted by the epithelial cells in the convoluted tubules. Since the latter theories receive very little, if any, direct support from modern investigators, it will be briefly discussed at this time.

Bowman (1842) first completely described the histology of the capsule which bears his name and the relationship it bears to the capillary tuft or glomerulus. In the statement of his theory of renal function, he believed that the glomeruli and capsules behaved as a sort of automatic sluice. The fluid admitted to the intra-capsular space served the purpose of washing away the secreted products of the tubules. This work was substantiated by the later efforts of Mussboum (1878) and Brodie and Mackenzie (1914). The most successful attack upon the mechanical filtration theory was initiated by Heidenhain. He based his contention upon the fact that certain dyes are secreted by the renal tubules; proved by the fact that dye granules were seen, after alcohol fixation, to be contained in the cytoplasm of the tubule cells. Another piece of work by Heidenhain in which he proved that ligation of the renal vein does not cause increased urine flow proved to be a powerful argument against the filtration theory for many years. The significance of this experiment is this: if the glomerulus and capsule act as a mechanical filter, their function must conform to the physical law of filtration. The law stated in equation form is $F = P - p$ where F is the rate of filtration, P the pressure in the glomerular capillaries, and p the pressure in the intra-capsular space and tubule luma. Heidenhain believed that blocking of the renal vein should cause increased glomerular pressure with consequent diuresis. When, upon the carrying out of the experiment, increased urine flow did not appear, he concluded that the activity of the capsule and glomerulus was of a secretory or "vital" nature and not a purely mechanical process; his contention being that the anuria was produced by a localized asphixia of the capsular and glomerular cells due to the accumulation of their own wastes and CO_2 arising to the stagnation of the blood in the rete. Heidenhain's theory then states that the capsule extracts a fluid from the blood, and the tubules contribute to the urine by secretion. He further opposed Ludwig by stating that no reabsorption occurs in the tubules.

The filtration theory was revived by Starling, and has been supported by the work of Richards and many other contemporary investigators. It had been stated in early expositions of the filtration theory that all constituents of the blood except the plasma colloids passed through the capsular wall. These colloids are not found in appreciable amounts in normal or non-pathologic urine. Starling studied the relationship between the osmotic pressure of these plasma colloids and the pressure within the glomerular capillaries; realizing that if filtration were the process occurring, the capillary pressure must exceed that of the colloids. His conclusions were that the excess of the former over the latter was sufficient to permit an assumption that filtration did occur, not necessarily secretion. Richards in one of his earlier experiments stumbled upon a proof for the filtration theory quite by accident. He had devised a method of entering the intracapsular space and extracting the fluid contained in it before it could reach the neck or tubule. During one of these experiments, a piece of mercury which had become lodged in the pipette dropped into the opening of the proximal tubule where it leaves the capsule. After the introduction of the obstruction, fluid continued to rise in the pipette; proving that the Malphigian corpuscle does eliminate a fluid and does not act merely as a pressure valve as certain adherents of this secretion theory believed.

Working further with this technique, Wearn and Richards found the capsular filtrate to be protein free and containing such metabolic substances as sugar and chlorides. Since these compounds are not found in bladder urine, the fact that they are reabsorbed in other parts of the nephron becomes evident. In regard to the nature of glomerular and capsular function, the appearance of Cl and sugar in the filtrate, in as great a concentration as it appears in plasma, proves that the passage of the liquid across the corpuscle membranes is non selective except that colloids are retained in the blood. Essential nutrients and other necessary constituents of the blood are passed through as well as the waste materials, which are ultimately voided in the urine. If the former were not reabsorbed and returned to the blood, the functioning of the upper part of the nephron would prove immediately fatal to the organism. Richards concludes that if the glomerular and capsular functions were secretory and therefore "vital", there would be an immediate selectivity exhibited with a retention in the capillary blood of the essential constituents. In other words, a "vital" process would work in such a manner as to be favorably correlated with the best interests of the organism. On the other hand, a purely mechanical process would not differentiate between the essential and non-essential and would pass through any substances within the permeability limits of the membrane. Since the capsular epithelium exhibits no selectivity as regards the utility of the substance it diffuses, the process may logically be concluded to be a filtration. We have stated that if glomerular and capsular functions are expressions of a simple, mechanical filtration a variation in the blood pressure of the rete mirable of the glomerulus should initiate a directly proportional change in the urine flow. Conversely, if the function is a secretory one a change in the velocity of blood flow through the rete would produce a similar change. This would be due to the fact that changes in flow velocity would result in lower or more rapid changes of the vascular fluid bathing the capsular epithelium and thus decrease or increase its secretory activity. Therefore, if the filtration process is to be proven it will benecessary to devise an experiment wherein the factor of blood flow will be kept constant and the pressure varied by one means or another. This problem was solved by A. N. Richards and O. H. Plant in 1922 while they were working in the physiology laboratories of the University of Pennsylvania.

The essential technique of the experiments was as follows: A rabbit was eviscerated after the ligation of the coeliac axis, superior and inferior mesenteric arteries and the portal vein. The vessels of the right kidney were tied off. Ligatures were placed on the inferior vena cava and abdominal aorta at points proximal to the entrance of the renal vessels. Cannulae were then inserted pointing toward the heart into the main trunks. These cannulae were connected with each other and with the pump. Cannulae were also inserted into the carotids one of which was connected to the inlet of the pump, the other to a mercury manometer. After the set-up was completed hirudinized rabbit's blood was introduced into the perfusion system, the ligature on the vena cava was loosened, and the pump started. The result accomplished was this: a mechanical pump of sufficient power so that no possible variation in the size of the blood vessels in the system could produce an increase of resistance sufficient to change the action rate, was used to drive blood through a mammalian kidney. A constant rate of flow was assured, and by various means the pressure could be changed. Some of the procedures used to accomplish this latter effect were: medullary stimulation (ischemia) splanchnic nerve stimulation, and addition of such vaso constrictors as adrenalin, nitro-glycerine, etc. to the perfusate. These procedures all result in the constriction of the renal blood vessels. The pressure was also increased by the compression of the renal vein in order to investigate the validity of the contention of Heidenhain that this procedure would produce anuria.

I have described the technique used in these experiments, because I believe them to be among the most important ever carried out in the investigation of kidney function. They create a situation wherein the filtration theory can be put to direct empirical test. The results verified most conclusively this theory. In all experiments there was a definite tendency for urine flow to vary directly with blood pressure. Thus the filtration formula, $F = P - p$ is directly applicable to the glomerular phenomenon. Richards in his paper (1922) acknowledges some possible sources of error which may appear in his work. While Huber, Gerard, and Gross say there are no arteriolae rectae verae in the rabbit's kidney which supply the tubules directly, they may exist and their presence would make a constancy of glomerular supply uncertain.

On the other hand, there may be a varying number of glomeruli acting at any one time, and indeed this is so in the normal mammalian kidney as will be stated later. This would tend to cast uncertainty as to the significance of the experiments. The kidney of the eviscerated rabbit is not an entirely normal kidney. The factor of operative shock is an important one and must not be overlooked. Finally, the substance hirudin which was used in the perfusate to prevent clotting is known to be a diuretic and could introduce a source of error.

Considering all of these facts, however, there are certain things which cannot be disputed. In this technique, an increase in renal blood pressure would result in distension of the glomerular capillaries which would obviously result in a decrease in rate of flow. Therefore, if the contention of Heidenhain, that the rate of flow is the important factor in the glomerular vasculation, was correct an increase in pressure would produce an anuria. This was certainly not the case, but the contrary result was reported. The constriction of the renal vein produces, for Richards, an increased urine flow if not carried to a complete blocking of the vein. If the latter occurs, urine flow ceases due to a distension of the kidney which causes a complete stoppage of blood flow through the organ. Thus the experiment of Heidenhain upon which he placed so much significance is repeated and interpreted differently. Instead of saying that urine stops flowing because the rate of blood flow stops, Richards states that upon complete ligation of the renal vein no blood at all reaches the glomeruli and therefore no filtration can occur.

Richards, in acknowledging certain sources of error which may occur in his perfusion experiments upon the evicerated rabbit, has stated that varying number of glomeruli may be functioning at any given time. A paper by Hayman and Starr (1925) throws much light upon this problem. These investigators accomplished the intravital staining of the glomeruli by injecting Janus Green B in a .9% Na Cl solution (warm) into the abdominal aorta. After allowing the dye to flow into the blood stream for a very short time, the kidneys were removed, sectioned, and a count of the number of stained glomeruli taken. This count gives an accurate picture of the number of elements functioning at the time the dye was introduced. Nelson's method was used to count the total number of glomeruli, and from this figure and estimation of the per cent of functional structures can be made. It was found that the number of functional elements varies widely, and changes in it can be produced experimentally. Vaso constrictors, such as adrenalin and carbon dioxide, reduce the number to an extent that only ten per cent of the total glomeruli may be functioning when these substances are present in the blood stream in appreciable amounts. Caffeine and sodium chloride, which act as vao dilators, cause an increase in the number of open glomeruli up to 100%. It was found that as a rule the number of functional glomeruli, the kidney volume, the renal blood flow, and the rate of urine elimination, vary in the same direction but exceptions may occur. A decrease in kidney volume may be accompanied by one of the following: (a) The number of open glomeruli decrease, but the average blood flow per glomeruli continues high; (b) The number of open glomeruli continues high, constriction here simply diminishing the blood flow through them; and (c) a reduction in the number of open glomeruli may be accompanied by reduced blood flow through those remaining open.

Richards and his co-workers have performed experiments upon the frog's kidney to determine the effect of certain drugs upon the number of glomeruli functioning and the effects upon the afferent and efferent arteriales. Because its effect is more profound upon small or peripheral vessels than upon larger vessels, adrenalin is an ideal substance for use in this type of work. The calibre of the efferent glomerular vessel is about one half that of the afferent, due to the fact that a difference in size is necessary for the maintenance of blood pressure after the extraction of the urine constituents has been accomplished in the capillary plexus lying between the two vessels. Using .3 c.c. injections of a one to one million dilution of adrenalin, it was found that expansion of the kidney (detected by an oncometer) diminution in the blood flow, and diureses occurred simultaneously. By direct observation it was possible to note the constriction of the efferent and dilation of the afferent vessels. This was accomplished by noting the volume of erythrocytes passing through the vessels. Pituitrin was found to produce the same results. Large doses of adrenalin and pituitrin cause kidney shrinkage and anuria, due to the fact that in sufficient concentration their constrictor action is extended to the afferent vessels producing a stoppage of blood flow through the rete. These two drugs in high concentration are used to check the excessive urine flow which occurs in cases of diabetes insipidus.

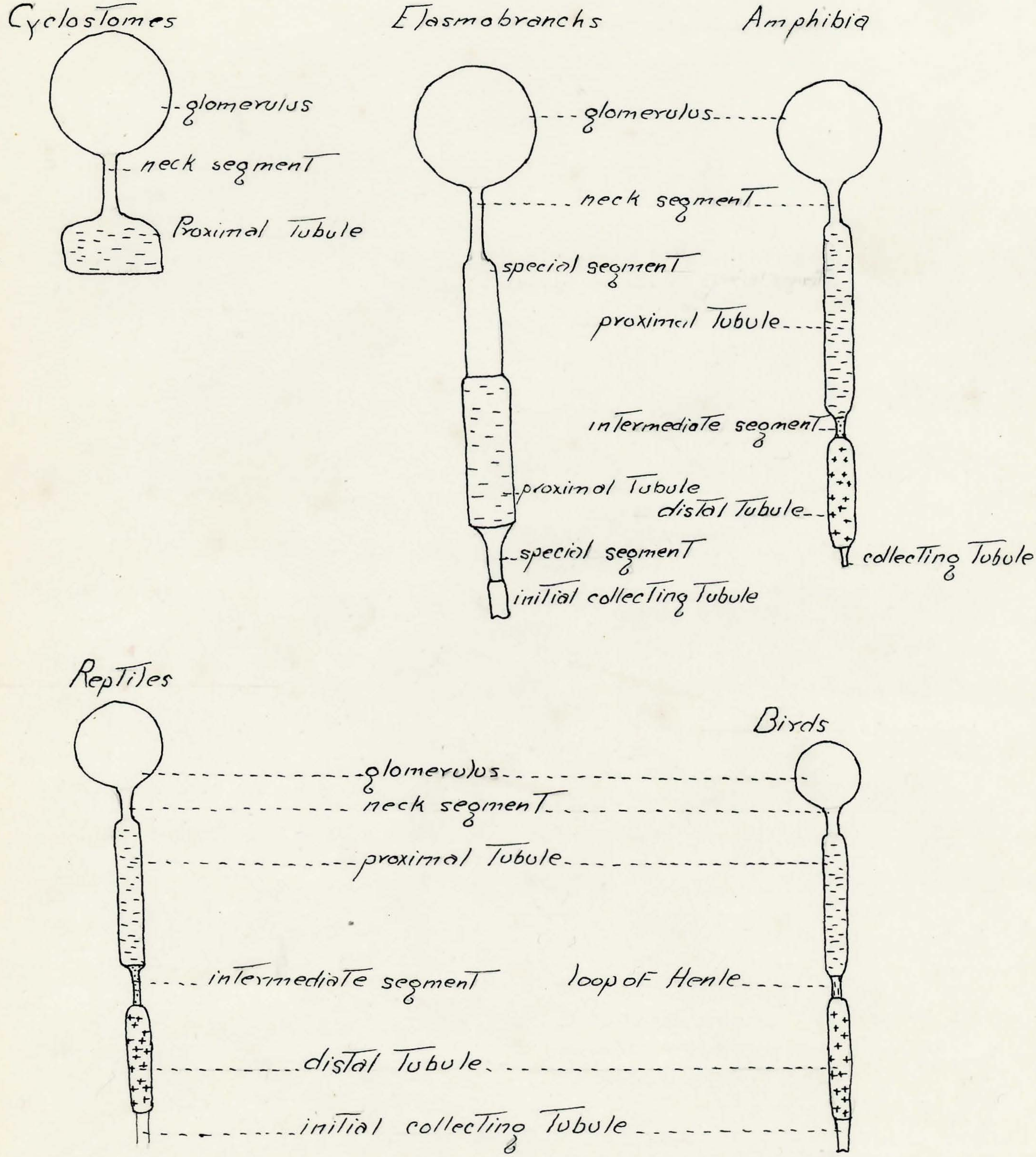
The significance of these experiments is great since they add another point in the mass of evidence in support of the filtration theory of glomerular-capsular function and also cast a great light upon the functioning of the kidney in the normal body. In great dilution certain vaso-constrictors (adrenalin and pituitrin) cause diuresis, because they increase glomerular pressure. This effect is accomplished by a constriction of the efferent and a dilation of the afferent vessels; the difference in action being due to the difference in their sizes. When administered in greater concentration the vaso-constrictors produce anuria by cutting down both glomerular flow and pressure.

These constrictor substances occur naturally in the human body and are secreted normally according to the needs and demands of the body. Through their action a nicety of renal control is obtained that is truly remarkable when we consider the minuteness of the amount of these substances which will produce marked changes in renal function.

That these changes are occurring at every instant is proven by Richards through his experiments of direct observation of the functioning of glomeruli. He has seen the constriction and dilation of the glomerular anterioles and has observed the functional cycle of the Malphigian bodies. It is apparent to him that each unit repeatedly goes into and out of an active period. There appears to be no rythmaticity concerned with the process. The theory to explain this states that the afferent arterioles constrict, due to nervous impulses causing a diminution in the blood flowing through them. The lessened blood flow results in a partial asphixia of the muscular elements of the blood vessel and under these conditions the vessels dilate readmitting a full supply of blood. Thus a cycle results which causes alternate flooding and emptying of the capillary plexus of the glomerulus. This process is being repeated at all times and may be altered by the presence in the blood stream of certain physiological substances such as adrenalin and pituitrin.

Other diuretics which have been used for experimental purposes are Na Cl, H_2O, $NaHCO_3$, Na_2SO_4, sugar, and urea. It has been found that the effect of all of these is due to the afferent dilation, efferent constriction phenomenom first noticed during the administration of dilute amounts of adrenalin. It has been noted that the plasma colloids are not filtered through the capsular membrane. Therefore a high concentration of plasma colloids must occur in the efferent arterioles. That these substances produce a demulcent effect upon the vaso dilators has been proven and another body adaptation discovered. By the tempering effect of the plasma colloids, excessive diuresis is prevented when any of the above named substances is administered. Another check on excessive glomerular filtration is the back pressure developed in the intracapsular space. If this pressure is built up sufficiently, as occurs during diuresis, the filtration rate is lowered or the process may stop altogether.

Fig. III



Diagramatic Representation of Comparative Series of Nephrons after Marshall

The problem of the function of the nephronic parts distad to the Bowman's capsule is one which has not yet been successfully met. Some knowledge is available; none of which is above question. The comparative physiologist seeks to assign functions to the tubules by noting the changes in kidney action in the ascent of the phylogenetic scale from the lower to the higher animal forms. As figure 3 shows, parts are added in the higher forms, the mammal attaining the highest expression. These complications of the nephron are seen to be concurrent with an increase in the osmotic pressure of the blood. The cyclostomes secrete a large amount of dilute urine, this phenomenon being correlated with its very small proximal tubule. All of the aquatic forms from cyclostomes to amphibia have rather poorly developed tubules, but a gradual increase in their complexity is seen. The types in the above named order each have a higher vascular salt concentration and secrete a smaller, more concentrated volume of urine. When the reptilian, avian and mammalian kidney is examined, there is a much greater nephronic increase in complexity noted. In the first two, however, the glomerulus has degenerated; the capillary plexus being replaced by a synctial mass of cells. This is correlated with the highly concentrated urine and the inhibited excretion of certain substances known to be passed by the glomerulus only. In certain birds and in mammals the loop of Henle appears, being placed between the proximal and distal tubules. This structure has been correlated with the great degree of water reabsorption occurring in these kidneys, and in mammals is known to be under the control of the hypophysis by means of a hormone termed antidiurinace. This latter substance causes the loop to become active, resulting in a high rate of water reabsorption.

The result of this study has been the drawing of the conclusion that as the nephron evolves to the highest expression, an increase in the reabsorption of certain substances occurs. Since it is now held by most physiologists that the glomerulus and capsule behave as a non-selective filter, it must follow that all constituents of the blood plasma, except its colloids, appear in the glomerular filtrate. This has been proved by the efforts of many workers in the field. Since many of these substances do not appear in the bladder urine, it is logical to suppose that the tubules are actively concerned with the function of reabsorbing these substances, It is known that certain substances, such as chlorides and glucose, normally do not appear in the urine; but will be excreted if their concentration in the blood is made to exceed a certain value. The latter is called the sugar threshold or chloride threshold, etc. By comparing the threshold values of certain substances in the several classes of vertebrates, it is possible to correlate the degree of reabsorption with the degree of tubule expression. More will be said of reabsorption later in connection with a discussion of further experiments.

Most of the controversy concerning renal function today hinges about the question as to whether or not the nephronic tubule has a secretory function. Marshall and Grafflin (1932) have studied the function of certain kidneys which are aglomerular. It is obvious that in these animals any urine constituent must be secreted by the tubule since no glomerulus is present. Among the substances found to be actively secreted by the toad-fish kidney (aglomerular) were Mg, SO_4, creatinine, and phenol red. The secretory function of such a nephron is an obvious fact, but when the glomerular element is considered, and when we consider that every constituent of urine is filtered through the capsule to a certain extent, the complications of the problem become obvious. In certain fishes an injection of a large dose of phlorizin will render a glomerular kidney aglomerular in function. In these cases as much as 95% of the constituents of the urine appear to be secreted by the tubule.

If indigo carmine, sodium carminate, methylene blue, toluidin blue, trypan blue, ferric ammonium citrate, or urea be injected into the intracapsular space of a nephron, they may be detected microscopically in the tubules. However, each of these may also be detected in the glomerular filtrate after intra venous injection. Richards and Hayman conclude from this that substances introduced into the blood stream cannot be proven to be secreted by the tubule unless it can be also proven that they are not filtrable by the glomerulus. At present no substance is known which behaves in the above manner. In studying the kidney of Necturus and the frog, Walker and Hudson have reached the conclusion that in the first animal glomerular filtration and tubule reabsorption of water are sufficient to account for the urine concentrations of urea when it is compared with the plasma concentration. In the frog, however, the high urea concentration of the urine cannot be entirely accounted for by water reabsorption and glomerular filtration. They conclude, therefore, that some what less than 40% of the urea excreted by the frog kidney is secreted by the tubular portions of the nephron. Marshall and Vickers injected phenol red into a dog and found that the amount of dye excreted by the urine was larger than the calculated amount passing through the glomeruli. This dye was believed to be at first concentrated in the proximal tubule cells and then secreted into the lumen. Richards believes that the estimates of the rate of flow made by these investigators is low and their conclusions invalid.

I repeated an experiment which was introduced by Richards to investigate the function of the proximal tubule in respect to its secretory function. A modification was introduced in order to determine the effect of ethyl alcohol upon tubule function. Frogs' kidneys were excised and placed in .03% solutions of phenol red in Ringer's solution. The first kidney was placed in the pure dye - Ringer's solution, while other kidneys were placed in portions of original solution containing .1%, .25%, .5%, and 1% ethyl alcohol. After remaining in the solutions for three hours, the kidneys were examined microscopically from the ventral surface. In the non-alcoholic solution the dye was seen to be concentrated in the luma of the proximal tubules to a greater extent than in the external solution. In the .1% alcoholic solution the concentration had proceeded to a greater extent than in the control. The .25%, .5%, and 1% solutions showed a progressive decrease in the amount of dye held within the tubule. In the last case there was little or no evidence of tubule activity which was understandable since the dehydrating effect of the alcohol was evident from the puckering of the surface of the kidney.

These experiments upon the excised frogs' kidney showed conclusively that there was secretion of phenol red by the renal tubule. That the phenomenon was attributable to the proximal tubule is justified when we consider that no glomerular circulation existed at the time of the experiment. The effect of alcohol seems to be to increase secretion up to a certain point in concentration and then inhibit it. Richards and Marshall have concluded that diffusion of water and phenol red occurs through the tubule wall into the lumen, and then reabsorption of water takes place resulting in a concentration of the dye remaining within the tubule. Many investigators claim to have proven that the kidney tubule secretes such substances as createnine, urea, and uric acid which are normal constituents of the urine. The effect of HCN and $Hg\ Cl_2$ upon the kidney seems to be to prevent the concentration of these substances within the tubule lumen. In other words, the selective permeability of the proximal tubule cells is destroyed and diffusion occurs in both directions; that is, both toward and away from the lumen. It seems unquestionable that secretion does occur in the proximal tubule of the amphibian kidney. This structure is closely akin to the analogous mammalian structure and may be used as a standard of reference. This secretion, as definitely proved by empirical investigation, has been concerned with substances foreign to the blood plasm. Whether or not the normal constituents of urine are secreted by the tubule still remains open for debate. Richards, whom I believe to be the outstanding authority on the subject of kidney physiology, contends that the amounts of all normal urine constituents may be entirely accounted for by a calculation of the rate of glomerular filtration. This calculation is rather simply made. A sbustance is injected introverously into the experimental animal. The rate of filtration of a single glomerulus is determined by inserting a cannula into the intracapsular space and recording the amount of filtrate per unit of time. This value is then multiplied by the total number of glomeruli which gives the total glomerular filtration value. The amount of urine voided per unit of time is noted and the concentration of the introduced substance is determined by analysis. By comparison of these values an estimate as to the amount of tubular secretion is possible. If it is found that the urine concentration of the substance is abnormally high, the logical conclusion must be that the excess of the substance has been secreted by the tubules. To reiterate; Richards has frequently stated that the excretion of all normal urine constituents can be accounted for by the rate of glomerular filtration; foreign substances such as dyes, certain drugs, and poisons such as As, Pb, etc. are secreted by the tubule if their concentration is not such as to injure the epithelium.

That reabsorption occurs in the renal tubule is a positively proven fact. The reabsorption of glucose and chlorides under experimental conditions has provided most of the knowledge we possess concerning the phenomenon. These substances occur in the glomerular filtrate in the same concentration as found in the blood plasma, although they are practically non-existent in the bladder urine. Walker and Hudson (1937) have examined the reducing power of the glomerular filtrate as it flows through the proximal tubule in an effort to determine just where reabsorption of glucose occurs. Richards states that glucose reabsorption in the proximal tubules of Necturi and frogs occurs in the following way: 1/8 of the distance down the tubule the glucose concentration is 20% less than in the plasma; at 1/4 the distance it is 50% less; and at 1/2 the distance there is no glucose detectable. Walker and Hudson believe that more of the tubule is involved than this. They have further noted that an increase in the rate of flow through the tubule decreases the absorptive phenomenon as does a high concentration of the substance being absorbed. This latter is illustrative of the threshold principle as it applies to sugars. The same investigators claim that the tubule may effectively reabsorb when the asmatic pressure of the tubule fluid is doubled, thus showing that the process is a "vital" one and can proceed against a greater than normal force.

By introducing phlorizin into the blood stream of a frog, the reabsorptive process may be stopped. When the reducing power of the tubule fluid is examined under these conditions, an increase in the value is seen. At the distal end of the proximal tubule of the frog, it is 40% higher than the plasma value, while at the distal end of the distal tubule it is 300% of the plasma value. This increase in the concentration of reducing substances (sugars) is accounted for by the reabsorption of water which is evidently not affected by phlorizin. We may then conclude that about 1/3 of the water reabsorbed is taken up in the proximal convoluted tubule and about 2/3 of it is reabsorbed by the distal convoluted tubule. The loop of Henle which achieves full expression only in mammals is the chief seat of water reabsorption in the higher types.

Montgomery and Pierce (1937) have examined the function of the Necturus and frog kidneys in an effort to determine the process by which acidification of the urine occurs. They have found it to occur in the distal convoluted tubule and specifically in the cells of about 1/5 the extent of the tubule. The cells participating in this action are nearer the distal than the proximal end of the tubule. They have noted that in many cases a widening of the lumen which these cells enclose is evident. The acidifying elements are capable of changing the pH of .37 $Na_3 PO_4$ from 7.5 and 6.8 in one minute. This phosphate solution has a buffer action 100 times greater than that of the glomerular filtrate. The acidifying action is retained after doses of $Na H CO_3$ sufficient to change the blood pH have been administered. There is no indication that other cells may assume this function if the pH value of the tubular fluid is made excessively low. Concerning the manner in which the pH of the urine is lowered, the authors have noted since the concentration of $Na H CO_3$ approaches zero in the urine, the reabsorption of this substance may result in the acidity. However, they are unable to deny that the cells secrete acid. Marshall suggests that the absorption of OH ions produces the acidity. He observed the progressively acid reaction of phenol red as it proceeded into the distal convoluted tubule.

This acidifying action of the distal tubule, as explained by the reabsorption of $Na H CO_3$, would constitute an important factor in the maintenance of the blood pH, since the latter depends to a great extent upon the buffer action of the bicarbonate ion. If this substance were actively excreted in the urine, a deficiency in the blood stream would soon arise resulting in a fatal acidosis. Although these experiments have not been developed for the mammalian kidney, the similarity existing between its proximal and distal convoluted tubules and those of the Amphibian type is so close as to make a conjecture that the process in the higher form is similar to that in the lower.

Since so much of the physiology of the kidney is dependent upon the permeability of cell membranes, a brief discussion of this subject will be given. Richards has observed the fact that certain substances can diffuse across the cells of the proximal tubules in one direction but not the other. This results in the concentration of such substances as phenol red and urea in the tubule luma. Also he has noted that certain very diffusable substances, such as creatinine, will not diffuse through the renal tubule cells at all. This, he believes is due to the "vital" manifestations of the tubule which are destroyed by such toxic substances as H C N and Hg Cl_2. The existence of a cell membrane apart from the endoplasm is a proven fact. Chambers saw haemoglobin flowing from punctured erythrocytes and Fricke after studies of the same bodies has said that the membrane may be manomolecular with a calculated thickness of 33 Au or the length of a molecule of fatty acid. The action of membranes is today held to be that of a sieve. Wilbrant (1935) has attempted to compromise the sieve and lipoid solvent theories by explaining cell permeability upon a molecular basis. According to his idea the intermoleculer spaces are analogized to the pores of a sieve and the forces applied to them are analyzed. In his study of asymmetrical membranes, Wilbrant has hit upon a possible explanation of the one-way permeability of the cells of the proximal convoluted tubule. These membranes show different permeability capacities on either side, so that one side is anion permeable and cation imperineable while the other surface shows the reverse condition. Much of the work concerned with cell permeability has been done with artificial membranes made from collodion, cellulose acetate, silk-protein preparations, etc. Also the aquatic plant Velonia has figured to a great extent in the work. Whether the concepts drawn from this work are directly applicable to such complicated cell membrane phenomena as the tissue transport of HCl in the stomach and the secretion of phenol red by the proximal convoluted tubule of the kidney will perhaps be determined in the near future.

In concluding this discussion it may be said that the sieve theory is generally held at present to present the true picture of cell membrane behavior. The effects of certain substances upon permeability have been studied and these efforts may offer an explanation of the threshold phenomenon as expressed by the tubule. It is known that sucrose in hypotonic amounts will increase the permeability to water of the eggs of Arabacia punctulata, and it is likewise known that large amounts of glucose in the blood will present diuresis and decrease in sugar reabsorption by the kidney. These latter are certainly exhibitions of change in cell permeability. Concerning the nature of the sieve structure of membranes, it is probable that the pore size is variable and molecular size is an important factor in determining the permeability of a substance. The capsular epithelium and glomerular endothelium are evidently permeable to all blood constituents except the colloids and the erythrocytes, resulting in a mechanical, filter-like mechanism.

SUMMARY

(1) The nephron is the functional unit of the kidney. Its parts have been enumerated and a brief description of their histology given. Glomeruli and their capsules act as filters which can be proven by experiments that demonstrate the fact that their action conforms to the physical law of filtration, $F = P - p$. Recent attacks upon the filtration theory have been initiated by investigators who believe that the amuria attending ether anaesthesia is due to a disturbing of the "vital" activities of the glomerular cells. This contention is met by a statement of A. N. Richards to the effect that the anuria is a reault of the decrease of renal blood pressure.

(2) Diuretic action is produced due to the fact that the afferent arteriole is dilated by them to a greater extent than the efferent, thus producing an increase in glomerular pressure with concurrent increase in filtration. This dilation of the afferent vessels is attended by an increase in the size of the kidney detectable by oncometric measurements.

(3) Each Malphigian corpuscle is continually in a cycle which extends from functional activity to quiescence. No particular rhythmacity is evident and a theory to explain the phenomenon is given.

(4) The proximal tubules are known to reabsorb certain valuable constituents of the blood plasma which the non-selective Malphigian bodies have filtered. It is Richards' contention that no secretion of the normal urine constituents occurs in the proximal tubule, although many investigators claim the contrary. Certainly, foreign substances such as dyes and certain poisons are secreted by the proximal tubules and concentrated in their luma by a process of water reabsorption and retention of the substances in question. HCN and $HgCl_2$ prevent the one way diffusibility of such substances as phenol red and neutral red through the tubule and render the concentration of these substances in the lumen impossible.

(5) Water reabsorption and consequent concentration of the urine occurs chiefly in the distal convoluted tubule and the loop of Henle. The former is also concerned with the acidification of the excretion. Its functional capacity in this respect is at least 100 times greater than the maximum normal demand. This process of acidification may be attended by the reabsorption of $NaHCO_3$, the secretion of an acid by the cells of the distal tubule, or the absorption of OH ions. The question is undecided.

(6) The function of the kidney is to remove certain wastes and excess metabolic substances. In the latter instance we encounter the phenomenon of threshold substances such as sugar and the chlorides which, while appearing in the glomerular filtrate in a concentration equal to that of the blood plasma, will be reabsorbed by the proximal tubule as long as their concentrations, in the plasma, does not exceed a certain value. Beyond this point, called the threshold, the substances will appear in the urine. Phlorizin is a substance which will inhibit tubular absorption when administered intravenously. Another function of the kidney is the maintaining of the osmotic constant of the blood; this varying among the several vertebrate classes as does kidney expression. The lower forms possess simple kidneys whose reabsorptive function is less acute than those of the more completely evolved animals. This lack of reabsorptive powers is correlated with a small, poorly developed proximal tubule.

(7) Exquisite balance and control by innervation and endocrinal hormones is seen in case of the kidney. Among these substances adrenalin and pituitrin as well as the antidiuretic hormone, which is secreted by the posterior lobe of the hypophysis and controls the action of Henle's loop. The greatest amount of these controls is exerted upon the elements of the Malphigian corpuscles and specifically upon the afferent and efferent arterioles. By means of changes in their calibre through

vaso constriction and dilation, profound changes in the rate of urine formation and the urine composition are produced. It has even been suggested that these contain minute sphincter elements at their junction with the rete mirabile of the glomerulus. Variations in intra glomerular flow have been noted; various parts of the plexus receiving blood while others do not.

(8) In conclusion it may be said, that aside from its normal function the kidney continually acts as a safe guard against certain blood stream poisons and in general aids in the maintenance of proper body conditions through a delicately assigned and controlled functional apparatus, the nephron.

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