CHAPTER I

Review of literature

In his fascinating book, "Lectures on the Kidney", HOMER W. SMITH quotes a French chemist, Fourcroy, who defines the physiological significance of urinary investigations in the following words:

"The urine of man is one of the animal matters that have been most examined by chemists and of which the examination has at the same time furnished the most singular discoveries to chemistry, and the most useful applications to physiology as well as the art of treating. This liquid which commonly inspires men only with contempt and disgust, which is generally ranked amongst vile and repulsive matter, has become, in the hands of the chemists, a source of important discoveries and is an object in the history of which we find the most singular disparity between the ideas which are generally formed of it in the world, and the valuable notion which the study of it affords to the physiologist, the physician and the philosopher."

These words were written close upon 150 years ago, and still this source of important discoveries shows no signs of becoming exhausted. The reverse happens.

In the course of time, the conceptions held by physiologists of the renal function and urinary secretion, have been embodied in a long series of theories. BOWMAN (1842), LUDWIG (1844) and HEIDENHAIN (1874), have been followed by CUSHNY (1926) and VOLHARD (1931), AMBARD, REHBERG (1926) EKEHORN (1931) and SMITH (1937). The actual significance of all these theories is not that they elucidate the problem of renal function, but rather in the fact that they have stimulated further research. A great deal has gradually become illuminated, but renal physiology still offers a large field for research.
GLomerular filtration

Wearn's and Richards' investigations of fluid from the glomerular capsule simultaneously with bladder urine in the frog, have provided a solid basis for establishing some essential features of renal physiology. Glomerular urine is practically free from protein, as a result of a filtration process. The effective filtration pressure is the resultant of the blood pressure in the glomerular capillary and the colloid osmotic pressure in the blood plasma and the pressure in Bowman's capsule, the latter two acting in the opposite direction.

This work by Wearn and Richards and others, substantiates the hypothesis of glomerular filtration proposed by Ludwig. In amphibians, studies have shown that the pH, vapor pressure, conductivity and concentrations of urea, glucose, chloride, inorganic phosphorus, exogenous uric acid, exogenous creatinine, bicarbonate, etc., are practically identical in the glomerular fluid and the protein-free serum filtrate. These observations indicate that, at least in certain amphibians, the glomerulus acts merely as an ultrafilter.

In certain circumstances, such as, orthostatism, physical exertion, renal disease or increased plasmatic protein concentration, the plasma protein is, apparently, filtered through the glomeruli in larger quantities than normally, and protein will then be found in the bladder urine. Brandt and Gruhn, as well as Lippman, have, however, shown that the presence of protein in bladder urine may also be due to a reduced reabsorption in the tubules (tubular reabsorption). Albumin is often found in infants, aged from two to five days, and likewise, and even more constantly, in the urine of prematures (Smith C. A. 1946). Some albumin is, very likely, normally filtered through the glomeruli to be reabsorbed in the tubules in Man. Such renewed resorption is less marked, or non-existent, in prematures and newborns.

On the other hand, when the blood flow is reduced, the filtration diminishes with a retention of waste products, i.e. the following factor lower the effective filtration pressure: 1st, decreasing the glomerular blood pressure, 2nd, increasing the plasma oncotic pressure or: 3rd, increasing the capsular pressure.

Gérard applies the term athrocytosis to indicate the intracellular flocculation that follows the absorption of electronegative colloids and their storage in the brush border segment of the tubules. Evidently,
the reabsorption through the tubuli of colloidal particles differs from that of crystalloids.

**ACTIVE AND PASSIVE TUBULAR REABSORPTION**

The reabsorption of water, which in the human kidney produces a urine of a higher osmotic pressure than that of plasma, is supposed to be due, at least partly, to an *active* reabsorption. When a concentration ratio, *i.e.* urinary concentration: plasma concentration, of any solute is different from 1, either it, or the water, or both, may be undergoing an active reabsorption. This is presumed to be brought about through an expenditure of metabolic energy by the tubules (Wolf).

A solution that freely diffuses through the tubular walls is reabsorbed *passively*. The reabsorption of, *e.g.*, acetone is supposed to take place passively, probably also urea (Shannon).

Contrary to the reabsorption, *tubular secretion* involves a transport of substance from the plasma to the tubular fluid. The fact that a secretion actually occurs, has been well demonstrated by Marshall and Vickers with phenol red, though Cusnhy has tried to refute this. Pitts et al. have suggested a pseudosecretory mechanism by means of which sodium ions are taken up by the tubular cells from the tubular fluid, in exchange for hydrogen ions given up to the tubular fluid. The phosphate ion is likewise reabsorbed.

Through the tubular function, the glomerular filtrate is concentrated 98—99 per cent. Ludwig regarded this process as a diffusion, but later investigators have shown it to be a more complicated process. Wearn demonstrated that the glomerular urine of the frog, after an injection of glucose, contains a reducing substance which is not present in the bladder urine. Phlorhizin renders the tubules non functional and prevents reabsorption. In the opinion of Lundsgaard, the reabsorption depends on a phosphoric regulation process in the tubules which is inhibited by the phlorhizin, and since this substance is more concentrated in the kidney than in any other organ, its effect is strongest there.

Thus, if the function of the renal glomeruli may be regarded as that of ultrafiltration (protein-free filtrate of the bloodplasma), the function of the tubular epithelium may be regarded as, chiefly, a “selective” reabsorption. However, there is evidence that certain of the urinary constituents pass from the blood into the urine at some point in the
tubules beyond the glomeruli (creatine, ammonia, diodrast, phenol red and hippuran). Inasmuch as the average rate of glomerular filtration is 120—130 cc per minute, it follows that more than 170 litres are filtered through the glomeruli from the plasma daily, the tubules subsequently reabsorbing about 168.5 litres of water, 1000 gm of NaCl, 360 gm of NaHCO₃, 170 gm of glucose and smaller amounts of phosphate, sulfate, amino acids, urea, etc., in order to excrete about 60 gm of NaCl, urea and other waste products in about 1500 cc of urine (Homer W. Smith).

Rehberg divides the glomerular filtrates into 3 groups, according to their behaviour from the point of view of the reabsorption process, as follows:

1. Substances that are being actively reabsorbed, i.e., apart from phosphates and sulfates, the forementioned high threshold substances. These substances occur in lower concentrations in the urine, and in higher concentrations in the reabsorbed fluid, than in plasma.

2. Substances that pass back through the tubular epithelium by means of a simple diffusion process, once their concentration in the tubular fluid exceeds the plasma concentration. They correspond to the low threshold substances. They are reabsorbed passively. These substances are never to be found in lower concentrations in the urine, or in higher concentrations in the reabsorbed fluid, than in plasma.

3. No-threshold substances that suffer neither active reabsorption nor any back diffusion. They are, therefore, absent from the reabsorbed fluid.

At a low plasma concentration, inorganic phosphate is almost completely reabsorbed, but even a slight increase in the plasma concentration will make it appear in the urine.

Tubular secretion has always formed a burning subject. Without entering into the various hypotheses, the known data may be summarized as follows: although dyes, creatinine, diodrast, etc., are excreted through the proximal tubules of the mammalian kidney (except creatinine which is not excreted through the kidney in dogs) this process probably plays but an insignificant part under normal conditions, being, possibly, only a functional relic from a more primitive stage. The urea filtration is reduced in acute nephritis where the glomeruli are affected by inflammation, but it remains intact in lipid rephrosis where the glomeruli are unimpaired or inconsiderably damaged. On the other
The primary excretory function of the tubules may be altered in chronic nephritis with glomerular damages, and function quite differently than the healthy kidneys.

PRESENT OPINION CONCERNING THE KIDNEY FUNCTION

After the investigations of recent years, chiefly American (by Shannon 1936, Elsom, Bott and Shiel's 1936, Smith 1937, and others), we are able to summarize the renal function as a result of the following fundamental processes (Hogeman 1948):

1. Ultrafiltration in the glomeruli of a protein-free solution containing all the substances freely dissolved in the plasma, in the same concentration as in the plasma, with the exception of the small difference due to the Donnan-effect.

2. Active reabsorption in the tubules of the larger part of this filtrate, i.e. water and other substances necessary for metabolism or for the maintenance of the electrolyte balance in the body.

3. Passive rediffusion of certain substances through the tubules, this diffusion being entirely dependent on the degree of concentration of the respective substances in the tubular urine and on its diffusibility.

4. Active secretion through the tubular cells of other substances, either normally present in the plasma or artificially administered.

5. A synthesis of certain substances in the kidney.

By means of these processes, the kidneys perform their various tasks, i.e. to remove from the body waste matter and other unwanted substances, and to regulate the water balance and the osmotic equilibrium between the blood plasma and the tissue fluids, as well as the acid-base equilibrium.

Such quantitative renal function tests as are in practical clinical use belong to one or the other of the following four groups: retention tests, concentration-dilution tests, excretion tests and clearance tests (Wolf). These tests supplement one another in so far as a clinician can hardly do without any of them, and tests pertaining to any single group will fail to produce a clear picture of the renal function. The literature on renal physiology is enormous. Above, I have tried to give a short résumé without any pretension to completeness. Detailed accounts of
the quantitative renal function tests will be found in Homer W. Smith’s Monographs (1937, 1943), Addis’ book on Glomerular nephritis (1948), A. V. Wolf’s “The Urinary Function of the Kidney” (1950), Högeman’s excellent survey of the clearance tests (1948), Ekehorn (1945, 1946), Laake (1945), Hilden (1946), Josephson (1947), Raaschou (1948) and Hood (1949).

THE RENAL EXCRETION OF PHOSPHATE

The phosphate excretion plays an important part in the regulation of the phosphate concentration in the body fluids.

Walker & Hudson have shown in frogs, that the plasma phosphate is filtered through the glomerular membranes. They also noted that, in certain circumstances, an active reabsorption takes place in the proximal parts of the tubules and that the urine in the distal parts and in the bladder may be almost free from phosphate. There was no suggestion that phosphate is excreted through the tubular wall. In experiments on dogs, Pitts has demonstrated that the phosphate clearance increases with the plasma phosphate. With a normal phosphate level in the blood, the urine is almost free from phosphate and only a small part of the phosphate that is filtered through the glomerulus is found in the urine.

The process of the reabsorption of glucose has been elucidated by Shannon and Fisher, Govaerts and Müller and Shannon, Farber and Troast. Apparently, a reabsorption of phosphate takes place also in mammals during the passage of the glomerular filtrate. Harrison and Harrison have studied the way in which the excretion of phosphate is controlled and influenced by vitamin D and the parathyroid hormone. They used dogs as test animals, finding that, under standard conditions, there is a limiting maximal rate of reabsorption of phosphate by the renal tubules, which does not vary when the plasma is elevated by the administration of phosphate salts. The phosphate in the glomerular filtrate, over the maximal quantity that can be reabsorbed by the tubules, is excreted with the urine. After an administration of vitamin D to young dogs that had been kept on a rachitogenic diet, a conspicuous rise in the maximal rate of reabsorption of phosphate by the renal tubules was noted, thus increasing the concentration of inorganic phosphate in the plasma, at equilibrium. This effect remains noticeable for 24 hours.
after an administration of vitamin D. In the opinion of the forementioned authors, it constitutes an important factor in the antirachitic activity of the organism. The parathyroid hormone has an entirely contrary affect. In a work of 1947, LAMBERT, VAN KESSEL and LEPLAT have found a complete conformity between the excretion mechanism for inorganic phosphate and glucose, respectively, in Man. Both for glucose and phosphate a urinary threshold is to be found. The concept of a renal threshold is attributed to BERNARD (1877) and GOTTLIEB and MAGNUS (1900). They assumed that glucose was excreted in the urine when the plasma level of a certain substance exceeded a critical value which they called the threshold concentration. CUSHNY defines the renal threshold of excretion as the plasma value above which the substance appears in the urine, and below which it does not appear in the urine in any perceptible quantities. The threshold for phosphate in dogs is 1.1—1.5 mm/l acc. (PITTS). The term threshold of retention indicates the value at which the concentration of urine and plasma are identical (WOLF). The threshold of retention is not invariably the same as a normal plasma concentration. Urea and acetone exemplify no-threshold substances. Their concentration ratio constantly equals or >1, while threshold substances may possess a concentration ratio above or below 1.

Water, chloride, and sugar are quite obviously reabsorbed from the filtrate during the passage along the tubules. The term high threshold substances has been applied (CUSHNY 1926) to such substances, as glucose, amino acids, sodium, potassium and calcium, which are, to a comparatively large extent, absorbed with water and, consequently, absent from the urine, or present only in low concentrations. They appear in the urine in greater amounts when their concentrations in the plasma rise beyond a certain optimal threshold. Glycemia in diabetes is an instance.

The phosphates belong to the low threshold substances that are highly concentrated in the urine, since they are reabsorbed in relatively small amounts or not at all. Other low threshold substances are urea, uric acid, and sulfates, which are reabsorbed in varying amounts.

Inorganic phosphorus is reabsorbed in the proximal tubule like sugar, sodium, chloride and sulfur. In its transfer across the tubular membrane, phosphorylation of the glucose molecule appears to be a necessary preliminary step. The change is brought about by a specific enzyme, a kidney phosphorylase. Dephosphorylation of the hexose phosphate
thus formed, is effected by a second enzyme, “alkaline” phosphatase, present in the cells of the proximal convoluted tubules.

Despite intense researches of recent years, the mechanism of phosphorus excretion cannot, as yet, be said to be elucidated. Brull and his collaborators showed, as early as in 1928, that the excretion threshold of plasma phosphorus is not fixed to any definite value but that, with various factors, it can be raised or lowered. Brull later (1936) demonstrated that injected phosphorus is partly transformed into colloidal phosphorus (colloidal calcium phosphate) which the kidney is unable to excrete. In his important investigations, Govaerts, by applying an isotope technique, showed that the formation of colloidal phosphorus does not constitute the only transformation that phosphorus is subject to in plasma. He used the increased urine isotope ratio as evidence of a non-filtrable plasma fraction. His results, in experiments with radioactive calcium, deny the role of a non-filtrable calcium phosphate. In his opinion, the non-filtrable phosphate of plasma consists of some other, labile, phosphate complex which is very slowly synthesized under an enzymatic influence. He concludes that endogenous and exogenous phosphorus are treated differently by the kidneys, possibly because of the different physicochemical nature of the two different phosphorus fractions. Govaerts’ results were substantiated by the observations of Östling, who gave P32 intravenously to patients with renal diseases. Separate urine samples were taken from both kidneys by simultaneous ureteral catheterization. He obtained a different P32/P31 ratio from the diseased and healthy kidney in the same patient.

Lambert, Van Kessel and Leplat (1947) have raised certain objections to Govaerts’ conception. Govaerts’ determinations from plasma and urine were made simultaneously but during a period when the plasma phosphate was not constant, but was decreasing rapidly, in a relatively unphysiologic condition. It is uncertain whether an artificial raising of the blood phosphate level beyond a certain value is followed by the appearance in the blood of a colloidal, non-filtrable phospho-calcium complex. As yet, sufficient light has not been shed on the mechanism of excretion and reabsorption of organic phosphate under the condition of a constant blood phosphate level. These authors studied the urinary excretion in man in groups with different blood phosphate levels, viz. less than 65 mg per litre, 100 mg per litre and values above 100, respectively. In the first group the phosphate resorption was found to increase
with rising amounts of blood phosphate. In the second group, they noted a threshold for maximal phosphate resorption, where the amount of phosphate reabsorbed from the glomerular filtrate remained constant. In the third group, they actually noticed a non-filtrable phosphate fraction which increased with the rising blood phosphorus and corresponded to the colloidal calcium phosphate. Within the limits imposed by this third finding, the urinary excretion of phosphorus appears to conform to laws analogous to those for glucose.

In order to study the renal excretion from one side at a time Swedin has employed the technique with radioactive phosphorus. He made the observation that the kidneys seem to alternate in their function, with other words not always functioning with equal intensity at a given time.

Quite recently, Friedländer and Wilde (1951) applied isotope methods to studies of the urine isotope ratio in inorganic phosphate. They determined the ratio of the isotopes collected in the urine during a definite period, comparing it with the ratio of the isotopes in the plasma, as averaged for the same period. They also fixed the acid-soluble phosphate fraction in the urine and the blood, finding that the urine receives 2—5 times the amount of labelled phosphorus predicted from the plasma ratio; a result identical with Govaerts'. Various explanations were suggested without offering any definite solution of the question.