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University of Louisville

RENAL DYSFUNCTION IN PROSTATISM

A Dissertation

Submitted to the Faculty

of the

Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Pathology

By

Robert Lich, Jr.

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Robert Lich, Jr.

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RENAL DYSFUNCTION IN PROSTATISM

I. INTRODUCTION

Individuals suffering from prostatism often have renal function which is definitely though not irreparably damaged. That is to say, after varying periods of uninterrupted vesical drainage renal function materially improves. What then is the portion of the kidney that is not irreversibly impaired by the urethral obstruction? Is the primary effect on the tubule, the glomerulus or both? An attempt to localize the pathology in partially reversible renal dysfunction constitutes the essence of this thesis.

In order to ascertain the site of pathologic physiology in the kidney, it was considered imperative that some means must be employed to quantitatively estimate separately the function of the glomerulus and the tubule. Two such tests have been described, inulin clearance for the determination of glomerular function and phenol red clearance as an index of tubule activity. However, before approaching the experimental aspects of this problem it will be advisable to review briefly the essentials of renal microscopic anatomy and physiology.

A. Basic Renal Structure

The mammalian kidney, though apparently at first sight a very complicated organ, is a relatively simple anatomic structure composed of almost innumerable similar functional units. The nephron, the essential functional unit of the kidney, is composed of two distinct

parts, the glomerulus and the tubule. The glomerulus and tubule are primarily concerned with urine formation while the joining collecting tubule constitutes the first of a series of conduits; the collecting tubules, the kidney pelvis, the ureter, the bladder and the urethra.

Embryologically the glomerulus and tubule are derived from different anlagen and develop independently. Eventually the 'S' shaped tubule which is an entirely closed structure, expands on one end and invaginates. By invagination it develops a concavity on one side into which a tuft of blood vessels grows and becomes engulfed. The other end of the tubule during this process connects with a collecting tubule and thus the basic element of the kidney, the nephron, is created.

The glomerulus consists of an almost spherical tuft of capillaries supplied with blood through a short, wide afferent arteriole which in turn is a branch of the interlobular artery. The capillary tuft is formed by the abrupt division of the afferent arteriole into 2 to 4 primary branches which in turn subdivide again, at times into as many as 50 capillary loops, each loop having a length of 2 to 3 times the diameter of the whole tuft. The capillaries do not anastomose with each other, but coalesce into an efferent arteriole which breaks up further into a secondary capillary system around the tubules. The efferent arteriole of a particular glomerulus does not necessarily supply the adjoining

tubule. In fact, the medullary portions of the longer tubules are often supplied by the efferent arteriole of the nearest glomerulus.

The glomerulus or capillary tuft is thrust so far into the expanded but closed end of the tubule that the tuft is completely enveloped by a double layer of tubule epithelium; the inner or visceral layer embracing the individual capillary loops and the outer or parietal layer forming a smooth spherical capsule (Bowman's capsule) about the tuft as a whole. The space within the lumen of the tubule, the arrangement being such that any fluid passing through the capillaries drains from the capsular space down the tubule. Between the visceral layer of the capsule and the capillary endothelium there is a thin glomerular basement membrane, which is anatomically continuous with that of the remaining portion of the tubule. These three layers, the capillary endothelium, the basement membrane and the capsular epithelium are normally permeable though their exact cytological morphology is disputed.

The tubule is divided structurally into three distinct portions: (1) the proximal convoluted tubule, (2) the thin segment incorporating the loop of Henle and (3) the distal convoluted tubule. These regions of the tubule are products of developmental elongation. The thin segment is produced by the elongation of the unattached mid-portion of the primitive 'S' shaped tubule.

Usually the central part of the thin segment bends directly back on itself forming the so called "loop of Henle" which is located near the pelvis of the kidney. The portions of the tube known as the proximal and distal convoluted segments lie in the proximity of the glomerulus of origin. These two tortuous portions of the tubules are formed by the local elongation of the primitive tube at its attached extremities.

Cytologically the various divisions of the renal tubules are distinct. The proximal segment has the greatest diameter of any portion of the tubule. Its brush border cells are large and cuboidal with abundant moderately granular cytoplasm and large spherical nuclei. Along the basement membrane there are perpendicular mitochondrial striations. The thin segment, though variable in length and position, is characterized by its smaller diameter and flattened epithelium. The cells are composed of a perfectly clear cytoplasm containing slightly compressed nuclei. The thin segment may include, as it often does, the loop of Henle though on occasion this segment may occur before the "hair pin" bend occurs. Also, on occasion the thin segment may be entirely absent. However, it must still be considered as a purposeful functional unit of the nephron because of its persistent mammalian presence and less distinct development in the more primitive forms.

In the ascending loop of the tubule the cells gradually

become cuboidal and finally columnar. The cytoplasm is more finely granular than in the proximal part of the tubule and has no brush border. The mitochondrial striations are less distinct.

The cytology of the collecting tubules suggests their physiologic unimportance. The cells of this branched tubular system are essentially a single layer smooth cells of different height in various portions of the collecting system. This abrupt cytological transition from the nephron is indicative of their different embryologic origin.

B. Theories of Renal Function

Bowman⁷ writing in 1842 advanced his theory which he supported purely on morphological grounds. The glomerulus in his conception excreted only water and salts whereas the tubule of the kidney excreted such urinary constituents as uric acid, urea, etc.

Ludwig²⁶ in 1844 claimed the glomerulus acted only as a filter, permeable to everything in the plasma but protein. The elaboration of urine was consummated in the uriniferous tubules.

Heidenhain²¹, in 1874, suggested that glomerular activity was concerned chiefly with the secretion of water and salts and that further elaboration of urinary products was brought about by the specific activity of the tubule cells. Immediately there was introduced dissension in the ranks of renal physiologists,

Heidenhain and his students as opposed to Ludwig and his followers. The latter group accused the Heidenhain supporters of introducing 'vitalism' into renal physiology. This dispute continued for fifty years until Cushny's¹⁰ monumental work was published in 1917.

Cushny considered, as did Ludwig, that the energy of glomerular fluid was that of the plasma lacking the protein constituents. From this basic assumption, Cushny reasoned that substances in the plasma but absent from the urine must have of necessity been reabsorbed. In addition, substances whose concentration in the urine exceeded the concentration in the plasma must have been created by the reabsorption of water in the tubules. Cushny denied tubule excretion and maintained that the variation of salt and urinary solid concentration was determined exclusively by water reabsorption. Cushny's 'threshold substances' were such ingredients as were essential for the economy of the organism and reabsorption took place so long as the plasma level remained below a certain or 'critical level'. On the other hand, such substances as creatinine were supposedly rejected by the tubules regardless of the plasma level and were termed 'no-threshold substances'. Here again we have another form of 'vital activity' entering into renal physiology. The tubule cells of Cushny by innate ability were capable of reabsorbing certain materials, in other words anticipating plasma concentration. Cushny it is true attempted

to overcome this objection by introducing the complicated explanation that the reabsorbed fluid was of a constant predetermined composition, and 'optimal fluid'.

Modern views of renal physiology were modified by such investigators as Richards and Marshall. Richards^{40,41,42} through his microchemical methods demonstrated conclusively the existence of glomerular filtration and tubule reabsorption. Marshall^{27 to 35} demonstrated the presence of tubule excretion.

Starling⁶², in 1899, introduced perfusion experiments in an attempt to clarify mammalian kidney physiology, but it was soon found that this method proved to be of little physiologic significance. In other words, normal renal function has never been attained with either the perfused kidney or the heart-lung preparation. However, these experiments demonstrated the mean arterial pressure (glomerular pressure being approximately $2/3$ of the arterial pressure. (Winton⁶⁹)

That the glomerular membrane acts only as a filter is strengthened by the work of Bayliss, Keridge and Russel² since they found by injecting various substances intravenously into cats, that the glomerular membrane would pass substances with molecules smaller than that of serum albumin (molecular weight 72,000).

A more recent method of demonstrating glomerular filtration is the identical excretion of several substances relative to their respective plasma concentrations. This has been demonstrated

specifically in the dog for inulin, creatinine and ferrocyanide and after the administration of phlorizin to block the reabsorption of sugars, it has been likewise demonstrated for glucose, xylose and sucrose. Filtration rather than cellular activity must be the explanation since it is inconceivable that six different products can be handled identically by cellular activity.

Tubule activity has been studied though its physiology has not been entirely clarified. The tubules have in addition to the mechanical function of conduits the function of reabsorption and excretion.

The activity of reabsorption can be demonstrated experimentally in a number of ways. Although the bulk of this work has been done on Amphibia much of the evidence can be transposed to a degree to elucidate mammalian tubule physiology. Richards and his co-workers⁴¹, who catheterized the tubule, demonstrated that glucose was actively removed from the tubule in its proximal portion. Chlorides on the other hand were removed in the distal tubule where sodium bicarbonate was also returned to the body since it was found that in this portion of the tubule the urine suddenly became acid (Montgomery and Pierce³⁹). Furthermore, the distal segment was found largely responsible for the reabsorption of water. This can be seen by the fact that after giving phlorizin, the concentration ratio of glucose in the tubule urine increases rapidly in the distal tubule reaching finally a value of 2.5. Richards⁴³ estimated that

only one-fifth of the total fluid reabsorbed was removed by the proximal tubules. In the mammalian kidney Starling and Verney⁶³ demonstrated tubule reabsorption by the addition of cyanide to the blood pumped through the kidney which caused the urine to resemble closely deproteinized plasma with an increase of volume. Bickford and Winton⁴ by cooling the kidney demonstrated the same phenomenon. Though cooling reduced the blood flow the urinary secretion greatly increased, and the similarity of the chloride concentration in the urine and plasma suggested that the urine produced was merely a filtrate. This is further borne out by the fact that creatinine, which is normally concentrated by the kidney, likewise approaches the plasma level.

These several experiments are indicative of tubule reabsorption. Specifically then, to what extent does tubule excretion influence the composition of urine?

Nussbaum, making use of the anatomical arrangement of the vascular supply of the frog's kidney, ligated the renal artery which supplies the glomeruli alone and found urea and phenol red were excreted; hence, a product of tubule activity. Though for some time Nussbaum's work was disputed, it was eventually confirmed by Bensley and Steen³. In the mammal the excretory function of the tubule was demonstrated by Marshall and Vickers³⁵. They found that the amount of phenol red brought to the kidney was entirely inadequate to account for its rate of excretion, assuming even a

maximal renal blood flow and complete plasma filtration by the glomerulus. Sheehan⁵⁷ and Sheehan and Southworth⁵⁸ confirmed these experiments in the dog and rabbit.

We have seen now that the glomerular activity is limited primarily to filtration and tubule activity is a complex physiologic mechanism embracing both excretion and reabsorption. How then are we to measure renal function as a whole, as well as the specific functional ability of the two components of the nephron; namely, the glomerulus and the tubule?

C. Plasma Clearance as a Measure of Renal Function

The term 'clearance' was first employed in connection with the excretion of urea by Möller, McIntosh and Van Slyke³⁸ in 1928, who defined it as the "volume of blood which one minute's excretion of urine suffices to clear of urea". It is obtained by dividing the quantity of urea excreted per minute by the quantity contained in each cubic centimeter of blood; ie., if U = concentration of urea in the urine, V = cc. of urine formed per minute and B = concentration of urea in the blood, the (urea) clearance is given by the expression UV/B . Möller, McIntosh and Van Slyke developed this expression primarily for the purpose of describing the capacity of the diseased kidney to excrete urea. Since 1932 clearance tests have been applied to substances other than urea and it is now possible to investigate not only renal function per se, but indeed the physiologic

activity of the various anatomical components.

It was originally asumed that if a foreign substance was to be investigated from a standpoint of renal clearance, its plasma concentration must of necessity be kept constant, at least within certain limits. Technically the maintainance of definite plasma concentrations is very difficult. However, Miller, Alving and Rubin³⁶ demonstrated that inulin in plasma concentrations of 5.0 mgms. per 100 cc. produced clearances that closely approximated the clearances of much higher concentrations. This led to Alving and Miller's¹ work which demonstrated that mass intravenous injections of inulin produced constant clearances. That is to say, concentrations of inulin plotted against time on semilogarithmic paper decreased at first in a curvilinear manner and later in a linear relation. Presumably the curvilinear relation marks the period of equilibrium of the sugar between the blood plasma and the extracellular fluid and the straight line indicates the rate at which inulin is cleared from the plasma by the kidneys.

D. Evidence that Inulin is a Measure of Glomerular Filtration Alone

In order to ascertain the value of any substance for the estimation of glomerular function, certain specific criteria must be fulfilled.

1. The substance must be completely filterable through artificial membranes that are impermeable to plasma proteins, but permeable to smaller molecules.

2. As presumptive evidence against tubule excretion, the substance should not be excreted by the aglomerular fish kidney.
3. The clearance of the substance should be independent of a wide range of plasma concentration.
4. Whenever the simultaneous clearances of two or more substances are identical under a wide variety of conditions it may be assumed that the activity is glomerular since the variable factors of tubule function are not evident.
5. If one assumes that phlorizin completely blocks the tubules, then the substance in question should have the same clearance as simultaneously administered glucose.

In the following paragraphs inulin will be examined on the basis of these criteria.

Inulin is not bound by plasma proteins and is completely filterable in the Amphibia^{22,51}. The molecular weight of inulin is 5200 and it passes readily through collodion membranes which are impermeable to plasma proteins.

Inulin is excreted freely by the glomerular kidney, but is not excreted by the aglomerular kidney of the toadfish^{47,51}, batfish⁶⁰ or goosefish⁵¹.

The clearance of inulin is constant regardless of the plasma concentration, which in itself excludes the possibility of tubule reabsorption or excretion^{52,56}.

The simultaneous inulin and creatinine clearances are identical in the rabbit²⁵, and dog^{48,52,54,67}, in which there is no evidence to support the idea that there is any creatinine excreted by the tubules. In the case of man and chicken, where there is evidence of

creatinine excretion by the tubules, the clearance ratio is unaltered by the administration of phlorizin. The simultaneous clearance of inulin and ferrocyanide are identical in the dog⁶⁷ though not in man³⁷, since in the latter there is evidence that tubule reabsorption of ferrocyanide occurs.

There is evidence that in man⁵⁶ and dog⁵² about 25 per cent of xylose is reabsorbed; however, the constancy of the clearance ratio of xylose and inulin would indicate non-tubular influence on the latter.

In the normal animal glucose is absent from the urine; however, after phlorizin the glucose/inulin ratio reaches 1.0 in the dogfish⁵¹, dog⁵² and chicken, indicating that phlorizin completely blocks the reabsorption of glucose in these animals. In man⁵⁶ the ratio has never been raised above 0.89 and this is very likely due to inadequate phlorizin dosage.

Hence, from the observations related in the preceding few paragraphs, it is practically certain that the renal elimination of inulin is primarily by filtration, at least any reabsorption by the tubules is negligible.

E. Evidence that Phenol Red is a Measure of Tubule Activity

Phenol red, like inulin, is normally a foreign substance to the body, though this does not influence its use as a measure of renal activity. Phenol red was first introduced into renal

physiology by Rowntree and Geraghty^{48,49} who demonstrated its clinical worth as a measure of renal dysfunction. DeHaan¹¹ in 1922 demonstrated that phenol red combined with plasma protein and assigned the glomerulus as the functional unit of excretion. Marshall and his collaborators^{30,33,35} showed that the uriniferous tubule removed the dye from the reversible combination with the plasma proteins and excreted it. The evidence for this contention of Marshall's is listed below:

1. Considering the glomerular filtrate to be protein free and renal blood flow within reasonable limits, there is an insufficient quantity of unbound dye in the plasma to account for its urinary concentration by filtration.
2. Phenol red excretion is not dependent on the plasma level, as would be expected for a substance excreted by filtration, but decreases as the plasma level is raised.
3. Low plasma levels of phenol red produce clearances of this dye which greatly exceed creatinine in the dog and there is good evidence that creatinine is excreted only by filtration in this animal.

Further in support of tubule excretion of phenol red we have the observation that this dye is excreted by the aglomerular fish kidney^{12,13,17,18,28,34}. Chambers and Kempton⁸ demonstrated in cultured tubules of the chick mesonephros which originally are sealed completely and finally form elongated cysts, that when phenol red is added to the culture medium the dye is taken up by the tubule cells and excreted in high concentration within the cyst. None of the dye is stored in the tubule cells. When the dye is injected directly into the lumen of the cysts none of the dye is taken up by the cells.

This cellular activity is demonstrated only by the cells of the proximal tubule and the distal cells of the tubules show no capacity to excrete phenol red.

The reversible combination of phenol red with serum albumin is closely correlated with its value as a renal function test (Grollman^{19,20}). When, for example, the plasma concentration in man is 1.0 mgm. per cent, about 20 per cent of the dye is free and 80 per cent bound to the plasma proteins (Smith⁶⁰). In order to upset this equilibrium it would be necessary to have the capsular dye concentration built up to a level that would further tend to draw free dye from the plasma. However, since the glomerular membrane acts merely as a passive filter, such a condition cannot exist. Richards and Walker⁴⁵ demonstrated in the frog that the dye concentration in the capsular fluid is identical to the free dye of the plasma; this bit of experimental evidence not only supports the idea of glomerular filtration but supports the contention that dye equilibrium in the plasma can not be disturbed by filtration. However, in the case of tubule excretion the problem takes on a different aspect. The tubules actively remove the free dye and thus cause a dissociation of the plasma bound dye so that the entire amount of dye within the plasma is available for tubule excretion. Grollman^{19,20} has pointed out that the combination between dye and protein in man can be demonstrated by the usual adsorption isotherm - $x/m = Kc^{1/n}$. For man the factor for K remains 0.85 irrespective of renal dysfunction

and the value for $1/n$ is less than 1.0 which precludes the idea that the free dye in plasma increases as the total concentration of phenol red increases.

There is one phenomenon, in so far as tubule excretion of dye is concerned, that must be considered at this point, although its mechanism is not entirely clear. At high concentrations of phenol red in the plasma the excretion of dye is markedly depressed. This phenomenon was first reported by Marshall and Crane³³ in the dog and has since been substantiated by Marshall and Grafflin³⁴ and Bieter⁵ in the glomerular kidney of the toadfish.

High concentrations of phenol red in the plasma have two effects: (1) it elevates the amount of free dye in the plasma according to the dictates of the adsorption isotherm equation and (2) it depresses the tubular excretion of the dye. No adequate explanation of this phenomenon has been offered though it might be based partially on an endothermic reaction of the tubule cells. That is, these cells are capable of doing only a specific maximum of work and if the limit of this is exceeded, the tubule ceases to function as actively. Since the depression of the phenol red phenomenon is reversible it is indicative of two facts: (1) that the dye is not toxic to the cellular structure of the tubule and (2) that no dye stuff is stored with the tubule cells.⁵⁹ This depressed excretion phenomenon is typical of renal tubule activity and demonstrated by all substances excreted by the tubules, i.e., creatinine^{51,53},

phenol red, hippuran^{14,16} and urea^{33,68}.

As was stated before, a portion of phenol red in the plasma is combined and a portion is free. Hence, it must of necessity be true that the free form is subject to filtration and so long as the glomerular membrane does not pass albumin the combined dye must be excreted in some other way, tubule activity. How much then of the dye is subject to glomerular filtration? The phenol red clearance at plasma levels of dye below 1.0 mgm. per cent averages 400 cc. per minute in normal man, in contrast to inulin clearance of 120 cc. Since only about 20 per cent of the phenol red in the plasma at this concentration is free and filterable the volume of blood which can be cleared by filtration amounts to only about 20 per cent of 120 cc. or 24 cc. per minute, leaving 376 cc. of the dye clearance, or 94 per cent of the total dye excreted, to be accounted for by tubule excretion. On the other hand, hippuran and diodrast which are handled similarly by the kidney, demonstrate a plasma clearance of 600 cc. Therefore, since the renal blood flow cannot be less than this value (600 cc.), it may be assumed that the phenol red clearance is then but 66 per cent.

From the above discussion it may be assumed that phenol red is, within certain limitations, a satisfactory dye for estimating tubule function. Also, previously in this paper it was shown that inulin is a satisfactory substance for determining glomerular activity. The problem now arises as to whether or not these substances in

themselves are true indices of glomerular and tubule activity as whole figures or whether their ratio may not be the most satisfactory method of expressing renal function both in the diseased and in the normal state.

F. Phenol Red/Inulin Clearance Ratio

Comparison of the absolute values of clearance tests done at separate intervals is of little value.¹⁶ Variation under these circumstances must be expected not only in different individuals but in the same individual. In the former instance of comparing two individuals' clearance tests, it is obvious that the variation in kidney size, individual variation and possible anomalous conditions constitute factors for variation. Hence, simultaneous clearance tests with two or more substances are essential for an accurate estimate of total renal function at any given period of time.

The index of glomerular activity or inulin clearance exhibits greater constancy, since it involves the simpler physiologic mechanism; namely, simple filtration. For this reason, glomerular clearance should and does constitute a satisfactory standard reference point for the interpretation of simultaneous renal clearance tests.

In man, when the phenol red plasma concentration is kept below 1.0 mgm. per cent (the critical concentration), the phenol red/inulin clearance ratio has a value of 3.3. As the plasma concentration of the dye is increased, the ratio is necessarily depressed until it

may not exceed 0.89^{16} . The value of the phenol red/inulin clearance ratio will be discussed more fully in a later section of this paper.

II. EXPERIMENTAL METHOD

A. Selection and Preparation of Patients for Study

The patients used in this experiment were selected at random from admissions to the Urological Service of the Louisville City Hospital. The studies were undertaken on patients whose ages varied from 52 to 78 years, and who had symptoms of prostatism for more than two years, and whose clinical and laboratory picture did not indicate the presence of any nephritic lesion. Cystoscopic studies were done to determine the amount of prostatic obstruction in each case. Originally it was hoped that it would be possible to do these simultaneous clearance tests on individuals who had marked renal damage at the time of admission, but it so happened that during this experimental period no such cases were admitted to the hospital.

The patients were adequately hydrated before the beginning of the experiments so that their urinary output was more than 1.0 cc. per minute. Intravenous fluids were given if this urinary output could not be accomplished by liquids per orum. A total of six patients was studied and simultaneous clearance tests (inulin and phenol red) were done on three occasions in all but one individual. The clearance tests were done during the pre-operative drainage period, which varied

from six to sixteen days. The intervals at which these tests were done after the institution of vesical drainage were determined primarily by the patient's general clinical appearance and improvement. Each patient was finally subjected to prostatic surgery and every one enjoyed an uneventful post-operative course.

B. Measurement of Glomerular Filtration Rate (Inulin Clearance)

1. Preparation of Inulin Solution for Intravenous Use

The inulin used (Pfanstiehl, C.P.) was dissolved in water by heat and passed hot through a Seitz filter. The inulin was then thrice reprecipitated from the water solution with alcohol and dried in a desiccator over calcium chloride. The inulin was administered in 10 gm. doses dissolved in 100 cc. of normal saline heated to body temperature. The injection was carried out at the rate of approximately 10 cc. per minute.

2. Inulin Determination in Plasma

The method of measuring quantitatively the amount of inulin in plasma and urine was that described by Corcoran and Page⁹. In principle this method makes use of the fact that a blue compound results from the combination of diphenylamine and levulose in the presence of hot concentrated hydrochloric acid. This method was first described by Jolles and modified by van Creveld⁶⁶ and simplified by Herbert²³. The method described is essentially Herbert's applied to inulin and

the final determination is made in the single cell, compensating photoelectric colorimeter of Evelyn¹⁵. The glucose and levulose present in the material tested are removed by fermentation and the inulin hydrolyzed by the strong acid in which the reaction occurs.

One part of plasma is added to 8 parts of zinc sulphate solution (1.25 per cent $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ in 0.3125 N H_2SO_4), mixed, and the protein precipitated by the addition of 1 part of 0.75 N NaOH. The tube is then centrifuged and the supernatant fluid filtered and placed in a centrifuge tube containing an 1/8 volume of thoroughly washed packed yeast cells. The yeast and plasma filtrate are thoroughly mixed and after standing for 30 minutes the tubes are centrifuged for the same length of time used to pack the yeast cells (20 minutes at 2800 R.P.M.) and the supernatant fluid filtered. A 1.0 cc. portion of this filtrate is then placed in a test tube etched at 25 cc. and the sides of the tube are washed down with 5 cc. of acid alcohol diphenylamine reagent and the tubes are placed at once into a boiling water bath for exactly 15 minutes. At the end of this time the tubes are removed simultaneously from the boiling bath and plunged at once into a bath of ice water and allowed to remain there for at least 5 minutes. The tubes are then filled with absolute alcohol to the 25 cc. mark on the tubes and the color comparison made in the Evelyn colorimeter, using a filter transmitting wave lengths from 595 to 660 m μ (filter number 620). A compensating blank (plasma taken before the administration of inulin) to eliminate the error

introduced by the chromogenic factor of plasma, is treated in the same manner.

The inulin content of the plasma in question is determined by reference to the calibration chart (Graph I) which was prepared previously from samples of pure levulose in precipitating media treated as described for the final sugar free and protein free filtrate (treated as described above following the exhibition of yeast).

3. Inulin Determination in Urine

Urine inulin is determined by the method described above, provided the concentration of the polysaccharide is at least 2.5 per cent. The urine must be diluted 1:1000, to obviate false color reactions due to urinary nitrates and nitrites. The compensating blank in this instance is prepared from distilled water in which the urine was diluted.

C. Measurement of Tubule Excretion Rate (Phenol Red Clearance)

1. Preparation of Phenol Red Solution for Intravenous Use

The sodium salt of phenol red was dissolved in slightly alkalized distilled water and the solution filtered through a Seitz filter. The stock solution was made to contain 1.0 gm. of the dye per 100 cc. and was used in 50 cc. increments so that each intravenous injection contained 0.5 gms.

Graph I

2. Phenol Red Determination in Plasma

A calibration curve was first prepared using known dye dilutions (Graph I). The dilutions were prepared in 10 per cent sodium carbonate solution and diluted to 20 cc. with distilled water. The amount of sodium carbonate solution in each tube was limited to 1.0 cc.

In order to obtain a true value for tubule activity, it is necessary to keep the plasma concentration of the dye below 1.0 mgm. per cent⁶¹. Hence, the amount of dye in 2 cc. of plasma is so small that it can not be detected accurately. This difficulty was solved by adding a known amount of dye (0.003 gms.) to each tube so that the plasma concentration came within range of the standard calibration chart.

In making plasma dilutions it must be remembered that the alkali added in order to give the maximum of color should be added after the plasma has been diluted with water, otherwise the plasma will suffer precipitation and the colorimetric determination will be inaccurate. A plasma sample must always be taken before the beginning of the experiment so that the inherent chromogenic properties of the particular plasma can be compensated for in the colorimeter.

Each sample of plasma for phenol red determination¹ must be tested for hemolysis and the method used was that of Bing and Baker⁶. Two cubic centimeters of a 2 per cent benzidine solution in 20 per cent glacial acetic acid are added to 0.4 cc. of plasma plus 0.6 cc. of

water, the mixture agitated and 1.0 cc. of 1.5 per cent hydrogen peroxide added. A blank using 1.0 cc. of water is prepared in the same manner. The solutions are grossly compared in 30 to 60 minutes and if hemolysis is present the phenol red determination must be discarded.

3. Phenol Red Determination in Urine

The urine was diluted until its color fell within the range of the standard calibration curve. The chromogenic constituents of urine need not be considered since the dilution factor is so great that no appreciable error is introduced.

As in the case of plasma phenol red determinations the concentration of dye is measured in the Evelyn colorimeter using a #520 filter.

D. Intravenous Administration of the Inulin and Dye Solutions and Collection of Blood and Urine Samples

Inulin (10 gms.) dissolved in 100 cc. of normal saline at 98.6°F. was injected intravenously at the rate of 10 cc. per minute. Thirty minutes later 0.5 gms. of phenol red dissolved in 50 cc. of weak sodium bicarbonate solution was injected intravenously at the same rate as the inulin.

One hour after the completion of the inulin injection all the urine was emptied from the previously catheterized bladder and discarded. At the same time 15 cc. of blood which was the second

specimen was taken from the antecubital vein; the first having been taken before the initiation of the experiment. Two other blood specimens were taken later, that is at 2 and 3 hours after the completion of the inulin injection.

Urine specimens were collected for analysis at 1 1/2, 2 1/2 and 3 1/2 hour intervals following the completion of the injection of inulin. The method of collecting the urine was carried out in the following manner: all the urine was allowed to drain from the bladder and then the bladder was washed out with three 30 cc. portions of saline and the bladder finally distended with air to insure complete emptying.

Blood samples were not taken during the first hour since during this period equilibrium is taking place between the plasma and tissues and the polysaccharide (Alving and Miller¹). The phenol red was injected 30 minutes after the beginning of the injection of inulin since the dye is more rapidly excreted. Oxalate was used throughout as an anticoagulant.

E. Calculation of Clearance Rates

The clearance rates for both substances were calculated at hourly intervals following the completion of the inulin injection which constituted the zero point of time. Since the samples of urine and blood actually were collected at alternating half hourly intervals, the clearance was ascertained by interpolation. That is, by plotting

the clearance on semi-logarithmic paper using the logarithmic scale for points of clearance rate and the linear scale for recording the time. A straight line is plotted and the clearance on the hour is read off the graph directly.

The clearance rate was determined by the formula $C = UV/B$ and no correction for surface area of the body was introduced, since the individuals used were of average size and for clinical work this correction has been shown to be superfluous (Alving and Miller¹). C = cc. of plasma cleared of inulin or phenol red per minute; U = urine inulin or phenol red concentration in mgm. per 100 cc.; B = mean plasma inulin or phenol red concentration per 100 cc.; and V = urine volume in cc. per minute.

Blood samples should be collected at the exact mid-point between urine samples; however, if this is not accomplished and the blood not taken within two minutes of this time a correction must be made. Regardless of when the blood sample is taken, the exact time from the zero time must be known and this information will allow for the correct value for inulin or phenol red concentration to be ascertained by the following procedure. On semilogarithm graph paper the two concentrations of blood inulin or phenol red are plotted on the logarithmic coordinate against time on the linear coordinate. A straight line is drawn between the two blood concentrations and the mid-point of the clearance period is then read. This is the mean plasma concentration for the period.

III. EXPERIMENTAL RESULTS

On the following two pages will be seen the tabulated results of the experimental work. Data such as the blood concentration of inulin and phenol red have been omitted purposely to maintain simplicity.

IV. INTERPRETATION OF THE EXPERIMENTAL RESULTS

The tabulated results demonstrate that continuous vesical drainage does improve total renal function in prostatism. However, there is no apparent relationship between the length of vesical drainage and the amount of renal functional improvement. That is, renal improvement is dependent upon the amount of acutal damage that has occurred before drainage is established and this no doubt, is dependent upon the length of time that the patient has had symptoms. This point is demonstrated in Case VI where there is little improvement though drainage was continuous over a 16 day period.

The Phenol Red/Inulin ratio was incorporated in the tables since it was felt that this ratio represented more accurately total renal function. The significance of this figure was first emphasized by Goldring, Clarke and Smith¹⁶ who showed that this ratio tended to eliminate experimental error since by setting the clearance values to a ratio it served to cancel out discrepancies. The normal Phenol Red/Inulin ratio is 3.3^{16} . That the ratio is more accurately

Case No.	Days of Catheter Drainage	Duration of Clearance Test (Hrs.)	Inulin Clearance	Phenol Red Clearance	Phenol Red : Inulin Ratio
I. #79746	0	1.0	112	260	2.3
		2.0	120	263	2.1
		3.0	106	244	2.2
	6	1.0	118	359	3.0
		2.0	127	370	2.9
		3.0	120	381	3.1
	10	1.0	101	302	2.9
		2.0	107	324	3.0
		3.0	93	289	3.1
II. #63707	0	1.0	122	270	2.2
		2.0	113	262	2.1
		3.0	119	276	2.3
	6	1.0	129	400	3.2
		2.0	134	381	2.9
		3.0	140	397	2.9
	10	1.0	123	376	3.0
		2.0	117	360	3.0
		3.0	127	370	2.9
III. #42772	0	1.0	96	168	1.7
		2.0	90	*	
		3.0	88	172	1.9
	6	1.0	89	224	2.5
		2.0	97	228	2.3
		3.0	101	239	2.3
	13	1.0	116	291	2.5
		2.0	121	302	2.5
		3.0	110	286	2.4

*Blood sample hemolyzed

Case No.	Days of Catheter Drainage	Duration of Clearance Test (Hrs.)	Inulin Clearance	Phenol Red Clearance	Phenol Red Inulin Ratio
IV.	0	1.0	110	220	2.0
		2.0	98	232	2.3
		3.0	104	223	2.1
	6	1.0	120	340	2.8
		2.0	117	358	2.8
		3.0	115	339	2.8
V.	0	1.0	114	260	2.2
		2.0	102*	234	2.2
		3.0		241	
	7	1.0	130	347	2.6
		2.0	141	332	2.3
		3.0	119	329	2.5
	14	1.0	96	250	2.6
		2.0	94	240	2.5
		3.0	104	261	2.5
VI.	0	1.0	101	180	1.9
		2.0	96	167	1.7
		3.0	90	150	1.6
	8	1.0	99	212	2.1
		2.0	111	193	1.7
		3.0	92	201	2.1
	16	1.0	103	217	2.1
		2.0	111	201	1.8
		3.0	110	213	1.9

*serum sample destroyed

the index of renal activity is demonstrated in Case I for the third hour clearance rate period on the 10th day of catheter drainage. Here it is seen that the clearance rate of inulin is 93 cc. per minute (normal 125 cc. per minute) and the phenol red clearance is 289 cc. per minute (normal 400 cc. per minute). These two figures would seem to indicate markedly impaired renal function though when the phenol red/inulin ratio is examined it is found to be 3.1 (normal 3.3). Hence, we can say that renal function is in this particular instance not impaired as seriously as one would first believe by examining the clearance rates separately. The advisability of simultaneous clearance rates has been definitely established in the literature and evidence here confirms this opinion.

Upon examination of the clearance rates before and after drainage it is seen that phenol red clearances seem to have improved more than the inulin clearances. This is depicted most clearly in the following tabulation:

Case Number	Mean Percentage Increase of Inulin Clearance following drainage	Mean Percentage Increase of Phenol Red Clearance following drainage
I	26.2	41.8
II	19.0	38.1
III	32.1	36.3
IV	20.0	41.7
V	5.5	27.1
VI	6.0	19.2
Average	<u>16.46</u>	<u>33.86</u>

From these figures it would seem that improvement in the tubule function, as compared to that of the glomerulus, occurs in a ratio of approximately 2:1. Obviously to establish this fact a larger series of cases would of necessity have to be investigated.

However, it can be said from even this small series that uriferous tubule function seems to be more seriously affected by partial urethral obstruction than the glomerular elements. Or that the tubule dysfunction is partially reversible especially in its early stages, though when the obstruction is long standing (Case VI) the renal dysfunction apparently becomes fixed and continuous vesical drainage serves little purpose in reestablishing a satisfactory renal status.

It can be said then, in the final analysis of the experimental results obtained in this study, that renal function is improved following prolonged periods of vesical drainage in prostatism and that the increase is apparently more pronounced in the tubule than the glomerular elements of the kidney.

The graph on the following page demonstrates that the improvement of total renal function continues to a certain point and then ceases. That is, apparently renal damage is reversible to a certain point and after that is irreversible. Just what determines the degree of irreversibility has not been determined. However, this factor of reversibility of renal functional damage has long been appreciated by urologists. In fact this stationary period has been

Graph. II

termed loosely by urologists as "renal stability" and constitutes one of the principle criteria indicative of the optimum time for prostatectomy. The plateau of renal stability is clearly shown in the foregoing graph.

V. DISCUSSION

The problem of renal dysfunction in prostatism has apparently received little or no experimental investigation. In fact, careful search of the literature reveals no single reference dealing with this problem. Reference was made on occasion to the observed fact that renal function improved in prostatism with the institution of bladder drainage, but further than advocating this observation as significant as an operative index nothing could be found.

Hence, it is impossible to compare this work directly with that of other authors. However, Strong⁶⁴ studied recently the nephron, by microdissection during the early process of hydronephrosis. This author found that early in hydronephrosis the proximal convoluted tubule became markedly reduced in volume and his findings were similar to those of Suzuki⁶⁵. Suzuki found that in vital staining with intravenous carmine the cells of the distal portion of the proximal convoluted tubule were stained very much less readily than the cells in the initial portion of the tubule. Hinman²⁴, working with rabbits and studying the problem of hydronephrosis, found that the

tubule suffers damage before the glomerulus. In these various experiments, the urinary back pressure, though created by different means, was still in its early stages and from that point of view one might at least imagine that the situation was somewhat comparable to the urinary obstruction of prostatism and it is of interest that the results of this work support his findings.

VI. SUMMARY AND CONCLUSIONS

Renal dysfunction was investigated by the use of simultaneous imulin and phenol red clearance tests and the following observations were made:

1. Renal dysfunction in prostatism is primarily tubule dysfunction.
2. The disturbed renal function in prostatism is definitely improved by continuous vesical drainage.
3. Renal damage due to prostatism is partially reversible.

Re-establishment of renal function is not complete, but reaches a peak beyond which no further functional improvement occurs.

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