The Adaptation of Kidney Tests to Small Laboratory Rodents

by

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Methods of estimating the excretory capacity of the kidney by the administration of various compounds and the determination of their concentration in the urine have long been in use. The specificity of these tests has increased to the point, where, today,we are able to determine not only the efficiency of the kidney as a whole,but of the various parts of that organ.

Some of the earliest tests were performed on man and among these were the observations of Todd (69) and Charcot (5), Roberts (56),Duckworth (13) and Chauvet (7) who published accounts of the excretion,in disease, of various drugs, among which were mercury,iodides,quinine and many others.In 1897 Achard and Castaigne (1) introduced the methylene blue test which enjoyed extensive popularity. They administered the drug intramuscularly and noted the time of the first appearance in the urine,the time of maximum intensity of excretion and the time required for its complete elimination.In kidney disease, the time of appearance was delayed, as was also the time of maximum excretion, while the duration of excretion was prolonged. The test was quite rough,however, and the intramuscular administration of the dye caused pain.

Indigocarmine was introduced into this field by Heidenhain (21). He also administered the dye intramuscularly and found that the elimination was prolonged and delayed in disease.This test was considered more accurate than the methylene blue test, because indigocarmine was excreted more rapidly.

Lepine(28),Dreyfus(12) and Pugnat and Revilliod(47)worked with rosaniline. Klemperer(25),in 1895,introduced an important drug into this work when he used phlorizin to prove the existence of renal diabetes.Carrying this further,Achard and Delamere(2) found a diminution or absence of phlorizin glycosuria in kidney disease.The value of these tests in giving an accurate picture of the kidney seemed somewhat dubious, however, when it was found that in acute or subacute nephritis, the glycosuria might be absent,while the methylene blue test showed a normal or even hyperfunctioning kidney.

In 1910,Geraghty and Rowntree(55)introduced phenolsulphonephthalein and suggested that it possessed advantages over the tests previously employed because of the completeness of its excretion by the kidney and because it is easily determined quantitatively.Their work seemed to show that the excretion of this dye paralleled the functional capacity of the kidney and was decreased in the presence of renal disease,and further,that it would bring to light impending uremia which might otherwise go unnoticed.But since this test was influenced by cardiac decompensation,slight impairment of renal function was difficult to judge with certainty.

Folin introduced the use of blood creatinine and this work was carried on by Myers,Fine and Lough(41,42,43,44,45,46).The retention of uric acid was also used as an index of renal insufficiency.

The excretion of urea as an estimate of the condition of the kidney was used from early times.The work followed several lines

of approach of which one was the estimation of blood urea and non-protein nitrogen by Folin,Marshall and Rowntree,and another the study of the rate of excretion of urea.The latter led to the formulation of Ambard's constant(5) which is a mathematical expression for the relationship between the concentration in blood and urine of urea and other substances.The rule states that the ration of the concentration of urea in the blood to the square root of the urea output in twenty four hours is constant.Another result of this work was the Van Slyke urea clearance test which was,and still is, of great clinical value.Van Slyke(40) defines "clearance" as the volume of blood cleared of urea per minute, corrected for urine volume when the latter is below 2 cc. per minute.It is determined by dividing the quantity of urea excreted per minute by the quantity contained in each cc. of blood,i.e.,$C(max.)=\frac{UV}{B}$,or,below 2 cc per minute,$C(stand)=\frac{U\sqrt{V}}{B}$, where U=concentration of urea in urine,V= cc. of urine formed per minute and B=concentration of urea in blood.

All of the above tests,while they were of some use, particularly at the time they were introduced,did not give an accurate or truly quantitative picture of renal activity,with the exception of the Van Slyke urea clearance and even that does not give strictly quantitative and repeatable results.As knowledge of kidney physiology has increased,it has become obvious that more exact methods of measuring excretory function are necessary.

After much experimentation and years of contradictory theories,kidney physiology has reached its present status,where the theory now accepted is that there are three distinct processes concerned in excretion by the kidney(29).These are glomerular filtration together with tubular reabsorption and tubular excretion.Glomerular filtration has been thoroughly investigated

by the micropipette technique of Richards(49,50,51,70),which has demonstrated beyond doubt that glomerular urine is an ultrafiltrate of plasma containing all the constituents of plasma in the same concentration as they appear there,except the plasma proteins.The proof of tubular reabsorption lies in this same work,since certain substances,such as glucose and chloride,which are present in the capsular fluid,may be nearly or wholly absent from the urine as finally excreted.Tubular excretion was first demonstrated in the kidney of the aglomerular fish(14,50,54).This fish kidney consists of tissue analogous to that of the tubular tissue of other animals,but none bearing any resemblance to the tissue of the glomeruli,and since it is capable of excreting all the more important constituents of normal fish urine,it must do so by tubular excretion alone.In the higher animals it has been shown that substances appear in the urine in much higher concentration than could possibly be accounted for by glomerular filtration alone.

Since these three processes are involved in renal excretion, and since each can be separately affected by the action of drugs and disease,it would obviously be of value to be able to measure the part played by each separate function in the final elaboration of the urine.It is along these lines that recent work has progressed.

As a result of the participation by the tubules in the process of excretion,it becomes difficult to estimate quantitatively what happens to any particular substance as it is excreted.For example,a substance may appear in the urine in twice as concentrated a form as it does in the plasma(and hence in the glomerular filtrate due to water reabsorption and not necessarily to tubular excretion. Because of this,early investigators in the field realized that as ~~a basis for any test to measure the separate~~

a basis for any test to measure the separate processes involved, it would be necessary to have some standard substance known to be filtered through the glomerulus and then to pass inertly past the tubules and into the urine.Any change in concentration which this substance undergoes in passing down the tubules will indicate the extent of water reabsorption,while if any other substance present in the urine at the same time is concentrated to a lesser or greater extent,it can only be due to tubular reabsorption or tubular excretion of this second substance.

This standard would have to be physiologically inert so that subjects as nearly normal as possible could be examined;it would have to be completely filterable from plasma;and it would have to be some substance which could be accurately determined in both plasma and urine.With these criteria in mind,the search was begun for a suitable substance.It had been shown(29,34,14)that the tubules of the aglomerular kidney can excrete magnesium,sulphate, creatinine,chloride etc.,therefore,probably the tubules of other animals would also be able to excrete these and perhaps similar substances.But,on the other hand,Marshall and Grafflin had shown (31,33,34)that while glucose may appear in the urine of the glomerular fish and higher animals,it is never present in the urine of the aglomerular fish.This seemed to indicate that kidney tissue never actually secretes glucose,because it is of value to the organism,and since no glucose appears in the urine normally,it must be reabsorbed by the tubules; while,when,under abnormal circumstances,glucose does appear in the urine,it is because it is not adequately reabsorbed from the glomerular filtrate.If this is so,it was suggested that other sugars might

also not be secreted by the kidney,and perhaps those which are not metabolic products might not be reabsorbed either. As a result of this reasoning,much work was carries out on xylose(10,11,17,20, 38,39)and sucrose(20,24,36,37).Creatinine was also studied,since Rehberg had advocated and used the creatinine clearance as a measure of glomerular filtration in man(48).

The dogfish,dog and man were thoroughly investigated.In brief, the results seemed to indicate that in the dogfish,creatinine is excreted by tubular secretion as well as glomerular filtration(8, 55,34)and that xylose and sucrose are excreted exclusively by filtration,without any part being taken by the reabsorptive process which is normally present for glucose.The results in the dog,which seems to resemble man more closely with respect to renal function than any other animal studied,again indicated that xylose and sucrose are excreted independently of the tubules,and that creatinine,whose clearance is higher than that of these two sugars, is removed from the blood by tubular secretion as well as by filtration(60).In man,as in the dog and dogfish,it appeared that xylose and sucrose are neither secreted nor reabsorbed,while Jolliffe and Chasis(23)showed that the creatinine clearance exceeds the xylose clearance by an average of 74%,indicating that creatinine is secreted.

Further work by the same investigators showed these conclusions to be false,because the reasons for believing that these sugars are not actively reabsorbed,as is glucose, were based on a comparison of their concentration ratios with the concentration ration of urea before and after the administration of phlorizin, and it was later found that urea is excreted in a variable manner, and is hence an unreliable standard of reference.Nevertheless, this work was very valuable because it was in the right direction and was the link to the next phase of the work,which was the

use of inulin.

Inulin was first introduced here by Shannon(56) and independently by Richards,Westfall and Bott(52).As in the case of xylose and sucrose,inulin was chosen because it is a non-metabolized sugar;and in addition,because the large size of the molecule would tend to minimize any possiole diffusion across the tubules. It is a starch-like polysaccharide composed of hexose molecules,mostly fructose, and because of the elongate nature of the polysaccharide molecule,its difussion coefficient is considerably less than would oe expected from its molecular size (4).Inulin is completely filterable from plasma through collodion, although it is about as large a molecule as could be expected to filter through the glomeruli.Physiologically inactive,it is rapidly and quantitatively excreted in the urine.

Since the inulin clearance is widely used and accepted to-day as a measure of the rate of glomerular fitration,it would be well to re-examine briefly the specifications which Smith(64)has formulated for a substance suitable for measuring this datum,with special reference to inulin.

1. To be completely filterable through the glomeruli,the substance must be complerely filterable from plasma through artificial membranes permeable to smaller molecules,but not to plasma proteins.

Inulin is completely filterable in the amphibia and is not bound by plasma proteins(22,56).

2. As presumptive evidence against tubular excretion,the substance should not be excreted by the aglomerular fish kidney.

Inulin is not excreted by the tubules of several species of aglomerular fish(52,56).

3. The rate of excretion of the substance(UV)should increase over wide limits in simple,direct proportion to the plasma concentration(P),i.e.,the clearance $\frac{UV}{P}$ should be independent of the plasma concentration.This condition in large measure excludes the possibility of tubular excretion and tubular reabsorption.

Inulin has been shown to follow this condition in the dog (57) and man(61).

4. Assuming that adequate doses of phlorizin completely block the tubular reabsorption of glucose(which is known to be excreted by filtration plus reabsorption without any tubular excretion), then,in the phlorizinized animal,the clearance of the substance should be equal to the glucose clearance.

Inulin shows this over a wide range of species(56,57,61).

5. Where the simultaneous clearances of two or more substances are identical under a wide variety of conditions,this may be taken as evidence that both substances are excreted by the glomeruli without interference from the variable tubular factors.

The inulin and creatinine clearances are identical over a wide range where creatinine is known to be only filtered(53,57,59, 63) and,after phlorizin,over a wide range where creatinine is known to be excreted by filtration plus tubular excretion(65).

6. Where a completely filterable substance is excreted in part by tubular activity,its clearance,when depressed by elevating the plasma level,should approach the clearance of the test substance as the limiting asymptote.

The creatinine clearance in the dogfish(56), bird(65) and man(58)approaches the inulin clearance as the limiting asymptote, as the plasma level of creatinine is raised.

On the basis of all this evidence,it seems reasonable to assume as a starting point for clearance work that inulin is filtered through the glomeruli in the same concentration per unit of water as it is present in the plasma,and then passes inertly down the tubules remaining in the same quantity,being neither excreted further from the blood coming to the tubules,nor being reabsorbed.Since this occurs,the degree of water reabsorption can be determined by the ratio of the concentration of inulin in urine to the concentration of inulin in plasma, $\frac{U}{P}$. In addition, the rate of glomerular filtration is given by the quantity of inulin excreted per minute,divided by the concentration of inulin in plasma,$\frac{UV}{P}$.Using inulin to measure the filtration rate,it has been found that in the dog and man,it is fairly steady,regardless of the rate of urine formation except at great extremes,indicating that regulation of the rate of water excretion is effected primarily by regulation of the fraction of water reabsorbed,not by the filtration rate.Since the reabsorption of water varies,inulin and other substances appear in the urine in varying concentrations,but this does not necessarily mean any variation in the filtration rate itself.(6).

Inulin thus becomes an extremely valuable standard,as now, anything which has a clearance less than that of inulin,such as glucose or Vitamin C,may be assumed to be reabsorbed by the tubules;while anything which has a clearance greater than that of inulin,such as diodrast or phenol red,may be assumed to be excreted by the tubules,as well as filtered through the glomeruli. For example,it has been shown that the creatinine clearance is greater than the inulin clearance in man(58),the apes(66),the

chicken(65),the teleost fishes(65)and the dogfish(56),indicating that in these animals,creatinine must be excreted in partby the tubules.On the other hand,the evidence is that there is no tubular excretion of creatinine in the dog(52a,57,59,69a),the rabbit(23a),the sheep or seal(63).Hence,the creatinine clearance cannot be used to measure the filtration rate in man as was so long believed,but it can be used for this purpose in the dog.(this clears up the false evidence which was accumulated when the creatinine clearance was tested against the xylose and sucrose clearances as a standard of filtration.)

Changing plasma concentrations of inulin have no effect on the inulin clearance,for a high plasma concentration will mean a high urine concentration,and the ratio $\frac{UV}{P}$ will remain the same.This is contrary to the effect of changing the plasma concentration of substances which are excreted or reabsorbed by the tubules,as will be seen in the case of diodrast.

In all this work,the term "clearance" is used with a meaning slightly different to that originally proposed by Van Slyke for urea.Clearance is here defined as the virtual volume of blood which is cleared of a particular substance in one minute's time,and is given by the formula $C = \frac{UV}{B}$ where these terms have the same meaning as defined above for urea(U=concentration of the substance in the urine,V= cc.of urine formed per minute and B=concentration of the substance in the blood.) The important difference is that here, the volume of urine makes no difference to the calculation and clearance can be determined by the same formula at any urine flow. In most cases now,plasma is used rather than whole blood,since it is only the plasma which is filtered through the glomeruli,and

much error is saved by not introducing the haematocrit.Thus,the formula becomes $C = \frac{UV}{P}$, and "plasma" is substituted for "blood" in the definition.

As an example,if the amount of a particular substance which appears in the urine is 1000 mg. a minute and the plasma concentration is 10 mg./cc.,then the amount of plasma cleared of this substance per minute is 100cc.In the case of inulin,this figure would give not only the volume of plasma cleared of inulin, but also the glomerular filtration rate,since here these two are identical.In the case of a substance excreted in part by the tubules, the value would be greater by an amount proportional to the part played by the tubules.If there were some substance for which the tubules had such a highly developed excretory capacity that they completely removed it from the blood coming to them,then the clearance of this substance would be a "complete clearance",since in one circulation through the kidneys,part of it would be removed by filtration and all the rest by tubular excretion,with the result that each cc. of blood would be wholly cleared. Obviously,the volume of plasma cleared of that substance per minute would be equal to the amount of plasma circulating through the kidneys,provided the substance were neither synthesized nor destroyed by the kidneys,and provided it were concurrently transferred to the urine.That is,this value would give the renal plasma flow,and once the renal plasma flow is determined,it is a simple matter,knowing the percentage of plasma in whole blood,to estimate the renal blood flow.The renal blood flow constitutes the highest possible figure which the clearance of any substance not synthesized by the kidney can have.Since it is often of great value to know the volume of blood flowing through the kidneys,such a

substance would be very useful.

Phenol red has a much higher clearance than inulin wherever it has been studied and is known to be excreted by the tubules. Marshall and Crane(32),studying the excretion of phenol red in the dog,noticed that whereas raising the plasma level of inulin caused the clearance to increase proportionally,raising the plasma level of phenol red above certain values caused a progressive decrease in the clearance.This observation has since been extended and shown to apply in all clearances which involve either tubular excretion or tubular reabsorption.It has been interpreted to mean that a given mass of renal tubular tissue can transport from blood to urine - or from the urine to blood in the case of active reabsorption - only a fixed,maximal quantity of a substance per unit time(62).Thus,as the plasma concentration of phenol red is raised,the phenol red clearance is depressed,approaching the inulin clearance as a limiting factor,because above the maximum amount which the tubules can handle,the amount which they excrete stays the same,and although the amount filtered through the glomeruli rises parallel to the plasma concentration of free,filterable phenol red,in the ratio $\frac{UV}{P}$,U does not increase as rapidly with regard to P as it did at lower plasma levels,and the net result is a lowered ratio,and hence a lowered clearance value.This is referred to as "self-depression of the clearance" and was first demonstrated in man for phenol red by Goldring,Clarke and Smith (19),and in the dog for diodrast and hippuran by Elsom,Bott and Shiels(15).

Observations on the clearances of other substances soon showed that the clearance of phenol red is not the highest known

and thus not complete; as a result it cannot be used to measure renal blood flow.Elsom,Bott and Shiels (15) and Landis,Elsom,Bott and Shiels (26) showed that the organic iodine compounds,diodrast and hippuran,are copiously excreted by the tubules in dog,man and rabbit (16),are completely filterable from plasma, and that both the hippuran and diodrast clearances approach more nearly a complete clearance than does phenol red. Actually, it appears that for all practical purposes, the diodrast clearance approaches closely enough a complete clearance to be considered identical with the effective renal plasma flow. The term "effective" is used because this value does not measure the total amount of blood flowing through the kidney,but that part which flows to active excretory tissue. Studying these clearances in man with relation to the simultaneous inulin and phenol red clearances,Smith (67) found that increasing the plasma concentration of these iodine compounds not only depresses their own clearances, but it also depresses the simultaneous clearance of phenol red,indicating that the iodine compounds and phenol red are excreted by a common tubular mechanism.

Diodrast, or neo-skiodan,is 2,5 diiodo-4-pyridon-N acetic acid, dissolved with the aid of diethanolamine.It contains 49.8% iodine, is clear and almost colourless.Commercial diodrast (Winthrop) is 35% weight by volume.

Because of the phenomenon of self-depression, the plasma level of diodrast must be kept below the range where self-depression occurs,if diodrast is to be used as a measure of effective renal plasma flow.In this range,the kidney actually excretes all the

diodrast which is brought to it in the plasma,and the clearance is "complete" and hence equal to the effective renal plasma flow. In man,self-depression does not begin until a level of about 5 mg% iodine (67) and here the renal blood flow as measured by the diodrast clearance constitutes about 1/3 of the total cardiac output.It is not probable that the actual total renal blood flow can be much in excess of this.

Many of the above facts now known about the mechanism of tubular excretion might lead to error in the interpretation of renal blood flow using the clearance method,therefore Smith has again propounded certain rules which must be followed(67). Diodrast,if properly controlled will follow all these rules, and is,therefore,widely used for this purpose.In brief, the rules are:

1. The substance should have the highest possible clearance, since the highest clearance will approach most nearly the true blood flow (synthesis by renal tissue is excluded in the case of diodrast on the grounds that it is not likely that a substance foreign to the body should be synthesised).

In this respect,diodrast approaches more nearly a complete clearance than phenol red,as has been seen, and has the advantage that it is to a lesser extent bound by plasma proteins,a fact which is important in the calculation of Tm (v.infra) and in addition does not penetrate the red blood cells (19).Thus, as already stated the diodrast clearance approaches closely enough a complete clearance to be considered identical with renal plasma flow (63).

2. The plasma concentration of the substance must be kept below the level where the clearance is significantly self-depressed.

There are available methods of measuring diodrast at

sufficiently low plasma levels.

3. It must be ascertained whether other solutes in the plasma and particularly substances which are themselves excreted by the tubules, can interfere with the tubular excretion of the substance in question.

While phenol red and hippuran,if present,depress the diodrast clearance (67) no other substances are known which specifically affect these clearances and it is probable that there is nothing normally present in plasma in concentrations sufficient to have an effect of this kind.

4. It must be shown that the substance is concurrently transferred to the urine and not stored in the kidney.

Goldring,Clarke and Smith have shown in man (19) that the clearance of phenol red, diodrast and hippuran, has the same value on rising and falling concentration curves which would indicate that they are not stored but are transferred immediately to the tubular urine.

Diodrast,following these specifications,can hence be used to measure effective renal blood flow.

Dividing the filtration rate (inulin clearance) by the renal plasma flow (diodrast clearance) it is possible to calculate the fraction of the plasma which is filtered through the glomeruli, i.e.,the Filtration Fraction or F.F.Thus,if the afferent arterioles are constricted,the glomerular pressure is reduced and F.F. tends to decrease;while,on the other hand,if the efferent arterioles are constricted, the glomerular pressure increases and hence the F.F. tends to increase.Actually,the construction of the glomeruli is such that when the renal blood flow is altered as a result of changes in arteriolar tone, a simultaneous opposite change in glomerular

pressure tends to maintain the filtration rate at a constant level.

When diodrast excretion is at its highest value,the rate of tubular excretion,T,is of course,maximal also.This maximum value of tubular excretion has been termed Tm_D.This datum is independent of glomerular activity and renal blood flow and is proportional to the number or mass of normal,active excretory tubules.The greater the number of functional tubules,the more diodrast will be excreted, and the higher Tm_D will be,i.e.,Tm_D measures the tubular excretory mass.Diodrast Tm,unlike renal blood flow,is independent of the plasma concentration of diodrast so long as that concentration is adequate at the existing blood flow to supply a sufficient quantity of diodrast per unit time to maintain a maximum rate of excretion; it is similarly independent of renal blood flow so long as that blood flow is adequate at the existing plasma concentration to maintain the maximum rate of excretion.Thus,Tm_D can actually be measured at levels where the clearance is depressed,because the tubules are still excreting to their full capacity.Diodrast Tm as an index of the residual quantity of functional tubular tissue is an extremely important value,especially when it is assumed, as is usually done,that tubular functions related to excretion vary in a manner closely paralleling Tm_D .It is possible to speak of "glucose Tm" also, referring here to tubular reabsorptive mass.Recent work has shown that Tm determined by reabsorption,or glucose,and Tm determined by excretion, or diodrast, do not necessarily parallel one another quantitatively (18).

T,the rate of tubular excretion,is determined (67) by subtracting from the total excretion of diodrast per minute,the quantity excreted by filtration alone:

$$T = UV - PI \cdot F$$

$$\left(\frac{X}{I} - WF\right) PI$$

where U = concentration of the solute per cc. of urine,

V = the rate of urine formation in cc. per minute,

P = the quantity of solute in each cc. of plasma,

I = the concurrent rate of glomerular filtration as measured by the simultaneous plasma inulin clearance,

X = the plasma clearance of diodrast,

W = the fraction of water in the plasma,

F = the fraction of solute which is free in the plasma and therefore available for filtration.

WF comes into the calculation here, because diodrast, like phenol red, and unlike inulin, is bound by plasma proteins; hence, not all the diodrast in the plasma is free for filtration and excretion. The amount of diodrast which is excreted through the glomeruli is determined by the extent to which diodrast is bound by plasma proteins, and hence this latter datum is necessary in the calculation of Tm. The fraction of free solute in the plasma, or F, can be determined by ultrafiltration through collodion membranes at 37°C., and 40 mm. pCO_2. Since it is usual to express the observed composition of the ultrafiltrate in concentrations per unit of water, allowance must be made for the water content of the plasma in calculating the quantity of solute filtered through the glomeruli, W, since the inulin clearance is conventionally expressed as cc. of plasma and not as cc. of water.

In all cases where the diodrast and inulin clearances of one individual are being compared,they must be determined simultaneously.By the simultaneous use of inulin and diodrast, it is possible to learn many things about the kidney,including the rate of glomerular filtration,the fraction of substance which is being filtered,the effective renal blood flow,and the amount of functional tubular tissue.These tests have been widely applied in the clinic and it has been found that the average renal blood flow in an adult male of 1.73 sq. m. is about 1300 cc.per minute or about one-third of the average cardiac output;this is reduced by adrenalin and similar acting drugs, through constriction of the efferent arteriole,while certain disturbances of the autonomic nervous system tend to increase the renal blood flow through relaxation of the efferent arteriole.

Because of the many advantages possessed by the small rodent as an experimental animal,it seemed advantageous to attempt an adaptation of the methods which have been devised for larger animals,to the rat.With this end in view,the work to be described was undertaken.

The general plan described by Smith(67) was used as a basis, although because of the obvious limitations of the rat, considerable modification of the method had to be introduced. For example,in man, inulin and diodrast are administered by constant intravenous infusion,and numerous urine samples are collected by an inlying catheter,while blood samples are taken at the beginning,end,and several times during the infusion period,and the mid point value determined by interpolating semi-logarithmically.In the rat,by contrast,infusions cannot be given to an unanaesthetized animal,urine cannot be

collected over too short a period of time,because not enough is formed,and only relatively small amounts of blood can be drawn if the animal is to be considered physiological.Many different modifications to circumvent these difficulties were tried and discarded,until.a satisfactory method was evolved.It was hoped that once the method was established,it would serve as a basis of comparison of the functional efficiency of the kidneys in rats treated in different ways,since it was felt that a picture of how the kidneys are functioning is preferable to a gross or even histological examination.

Experimental.

The following is a detailed account of the steps taken in evolving a method suitable for estimating renal function in the rat by the simultaneous use of inulin and diodrast.

As it is obviously impossible to collect any urine from the rat in one minute,urine was collected over a longer period, and the urine concentration was divided by the number of minutes of collection time in the calculation of clearance.Immediately after it was drawn the blood was centrifuged at high speed for one half hour,the plasma drawn off and the proteins precipitated. Inulin was determined according to the method of Corcoran and Page (9) using 0.2 cc. of yeast treated filtrate.Diodrast was determined according to the method of White and Rolfe (72) using 0.5 cc. of filtrate and 0.0005 N sodium thiosulphate in the determination of plasma diodrast and,in most cases,1 cc. aliquots of urine which had been made up to 50 cc. with water immediately after collection and 0.0025 N thiosulphate in the determination of urine diodrast.All determinations were carried out at least in duplicate.

Work was begun on diodrast alone,then inulin alone was investigated,then both together.Essentially the same procedure was followed for each substance individually: a) determination of known quantity in aqueous solution, b) determination of known quantity added to plasma, c) determination of the actual clearance of the substance in the rat.

Diodrast. a) Determination of known quantity in aqueous solution.

Diodrast is obtained in solution 35% weight/volume,containing 49.8% iodine.From this, solutions of different strength were made on the basis of 174.3 mg. of iodine per cc. of the original solution.

Solution	Content	Recovery	% Recovery
A	1.743 mg%I	1.625 mg%I	93.2
B	12.201	12.190	99.8
C	17.430	17.013	97.3
D	24.402	24.221	99.2
E	34.860	33.019	94.7

The above is on the basis of numerous determination (at least twelve in each case) and was considered a sufficiently good recovery to warrant proceeding to work on plasma.

b) Determination of known quantity added to plasma.

Solution	Content	Recovery	% Recovery
A	17.43 mg%I	15.992 mg%I	91.8
B	24.90	24.337	98.0

c) Determination of diodrast clearance.

The adaptation of a method which had been originated for the dog and man to a such a small animal as the rat presented certain problems. Many modifications were made in the handling of the animals as work progressed,since only by continued trial was it possible to determine how much and how often to draw blood,how to collect urine,how long to wait before collecting urine,how best to administer the diuretic,what strength sodium thiosulphate to use and so on. Many animals were lost and many were carried through with no result before the method was finally standardised.These latter are included,as they give an indication of how the final method was arrived at.

Male albino rats in apparently good condition were used in all cases.In general,the routine used at this stage was: 1)fluids and diodrast administered, 2) 1.2 cc. of blood drawn from femoral artery and concentration of diodrast in plasma determined,3) urine

collected,diluted, and the amount of diodrast present determined,

4) 1.2 cc. of blood drawn from carotid artery and the concentration of diodrast in plasma determined.

Animal #D1

Zero time - 10 cc. of 2% sodium sulphate administered by stomach tube with 1 cc. of diodrast (Winthrop) in 5 cc. of water subcutaneously (fluids were here administered as approximately 7% of body weight.)

60 min. - 1.2 cc. of blood drawn from femoral artery under ether anaesthesia. Five cc. sodium sulphate administered by stomach tube. Animal placed over metabolism funnel for urine collection during next hour.

120 min. - Blood drawn from carotid artery and jugular vein (it was here attempted to determine the arterio-venous difference but this was much too small to be estimated). 0.7 cc. urine collected and diluted to 1.4 cc. with water.

The blood and urine were both titrated with 0.0005 N thiosulphate. This was found to be much too weak for the urine.

The first blood gave a plasma value of 43.167 mg%I.

The second blood (arterial) gave a plasma value of 18.868 mg%I.

The second blood(venous) gave a plasma value of 20.988 mg%I.

The urine was not known accurately but was estimated to contain about 5 mg. I.

No calculation of clearance was attempted.

Animal #D2 - 255 gm.

Procedure same as for #D1, but no venous blood taken. 0.1 cc. of urine collected and diluted to 25 cc.

Blood 1.- 30.19 mg%I in plasma.Blood 2.- 14.92 mg%I.

The urine was still too concentrated for the strength of thiosulphate used.The amount of iodine present was estimated as

roughly 21.2 mg.

No calculation of clearance was attempted.

Animal#D3 - 245 gm., #D4 - 310 gm.

These two animals were done on the same day using the same method for each.

Zero time - 7cc. of 2% sodium sulphate by stomach tube with 1 cc. diodrast in 5cc.sodium sulphate subcutaneously.

15 min. - 7cc. 2% sodium sulphate by stomach tube .

30 min. - 6cc. 2% sodium sulphate by stomach tube.

60 min. - Blood drawn from femoral artery and animal placed over funnel.

120 min. - Urine colleted(0.5 cc. from each animal,made up to 10 cc. with water.) Blood drawn from carotid artery.

	#3	#4
Urine diodrast	1.99 mg.	1,246 mg.
Plasma I	66.14 mg.%	50.456 mg.%
Plasma II	23.76 mg.%	45.15 mg.%
Midpoint	39.0 mg.%	47.7 mg.%
Clearance	0.085 cc./min.	0.043 ccc/min.,

Clearance is determined by the formula,

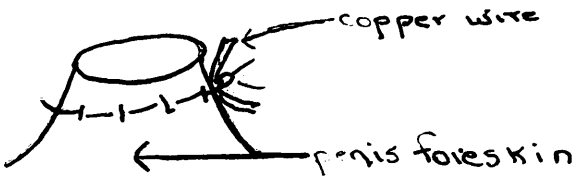
$$C = \frac{UV}{60 \times P}$$

where, UV = total amount of diodrast excreted in 60 min.

P = plasma concentration of diodrast in mg./cc. at the mid point.The mid point is determined by semi-logarithmic interpolation from the first and second plasma values,as this is considered to be the most accurate method(64).

It was felt at this point that the method of urine collection was not sufficiently accurate,and this is very important,since the loss of even one drop can make a large difference in the clearance value.The following operation was hence adopted and is used in

all animals hereafter,with slight modifications as to timing. The bladder is expressed,then the urethra is ligated at the beginning of the urine collection period by a purse-string suture which is passed through the penis foreskin with a fine needle and tightly tied.To facilitate removal of the ligature, a small L-shaped piece of copper wire is included in the ligature as sketched;originally,anaesthetic was administered for this operation,but was later found to be unnecessary.



At the end of the collection period,the animal is held over a large funnel leading into a graduated centrifuge tube,and,while in this position,the ligature is removed and the bladder expressed by gentle pressure.In this way,no urine is lost.Collection time is reckoned from the tying of the ligature to the end of urine collection.The urine as collected was made up to 50cc. with water and determinations done on 1cc.aliquots of this solution using 0.0025 N.thiosulphate in all cases from this point on.

Animals #D5,D6,D7,D8,D9,D10.

Using the above method of urine collection and with blood collection and fluid administration the same as already described for animals D4 and D5,the following results were obtained:

No.	Wt.	Time.	Urine vol.	Urine dio.	Diodrast PlasmaI	Diodrast PlasmaII	C learance cc/min	C learance cc/gm/min
D5	250	62min.	1.0cc	18.76mg	42.55mg	56.99mg	0.76	0.0030
D6	250	60	0.5	10.80	29.25	12.08	0.83	0.0033
D7	270	60	0.5	14.7	20.14	22.05	1.10	0.0040
D8	315	60	0.4	13.4	27.34	13.14	1.05	0.0033

No.	Wt.	Time	Urine vol.	Urine dio.	Diodrast PlasmaI	Diodrast PlasmaII	Clearanc e cc/min	Clearanc e cc/gm/min
D9	250	60	0.36	15.1	53.00	19.08	0.78	0.0031
D10	320	60	0.5	20.6	46.21	16.74	1.18	0.0036

This was considered sufficient work on the diodrast clearance of normal animals for that time.

Animal #D11

As a means of checking the relation of clearance to kidney mass,one kidney was removed in this animal one hour before the above procedure was started.The rest of the procedure was the same.

No.	Wt.	Time	Urine vol.	Urine dio.	Diodrast PlasmaI	Diodrast PlasmaII	Clearance cc/min	Clearance cc/gm/min
D11	250gm	65	0.35	12.45	58.93	58.30	0.33	0.0013

The diodrast clearance expressed per gram of body weight is here slightly less than $\frac{1}{2}$ that obtained in the normal animals.

With these facts about the diodrast clearance,it was decided to begin introductory work on inulin.

Inulin.

Inulin contains a pyrogen which causes undesirable physiological changes and which,therefore,must be removed before administration.This was done at this time by suction filtering a hot solution of inulin through a Seitz filter using an EK asbestos pad. It was later found that this is insufficient treatment and certain discrepancies in the following work may be due to the presence of pyrogen in the inulin.

Since the quantitative determination of inulin involves the use of the photoelectric colorimeter,percent recovery was not calculated,but curves were drawn up and the results of all determinations calibrated against these.Numerous determinations

were made at each concentration level, and curves were plotted for a) aqueous solutions of inulin, b) inulin added to urine, c) inulin added to plasma.

The determination of inulin clearance proved to be somewhat more difficult than that of diodrast and involved several further changes in method as will be seen.

Plasma determinations were done on 0.2 cc. of yeast treated filtrate. Urine, collected as already described, was made up to 50 cc. with water and determinations were done on 0.2 cc. aliquots of this solution after treatment with yeast.

Inulin is known to be poorly absorbed from subcutaneous injection (67). For this reason, it was decided to give inulin intravenously by injection into the jugular vein. At this point in the work, several animals were lost while the preliminary fluids were being administered by stomach tube, due to rupture of the oesophagus, consequently, fluids were administered subcutaneously henceforth.

<u>Animals #In1, In2</u>

Zero time,- 0.7 cc. 2% sodium sulphate subcuataneously.

15 minutes,- repeated.

30 min.- repeated

60 min.- Bladder expressed and urethra ligated, then 2 cc. of 2% inulin injected into the jugular vein under ether anasethesia. Blood taken from femoral artery. The whole operation took about twenty minutes with 5 minutes between the injection of inulin and the drawing of blood.

120 min. (60 mins. after ligation) -Ligature removed. Urine collected and made up to 50 cc. Immediately after all the urine was collected blood was taken from the carotid artery.

No.	Wt.	Time.	Urine vol.	Urine Inulin	Inulin PlasmaI	Inulin PlasmaII	Clearance cc/min	Clearance cc/gm/min
In1	280	70	1.3	10.0	125.0	17.5	0.32	0.0011
In2	290	70	1.7	27.5	100.0	20.0	0.92	0.0031

The first plasma values here are too low considering that 40 mg. of inulin had just been administered.The difference between the first and second plasma value was very great which shows that the time interval between the first and second blood could be shortened. In addition, 20 minutes under anaesthesia is too long if the animal is to be considered normal.On the assumption that inulin falls sharply from a peak immediately after intravenous administration and that the curve then declines more slowly, it was decided to let 15 to 20 minutes elapse between the time of administration and the drawing of the first blood.If the assumption were correct then the plasma values would represent a period of slowly declining concentration and the mid-point interpolation would represent more nearly a true mean. For the above reasons the procedure was modified as follows:

Animals #In3,In4,In5,In6,In7.

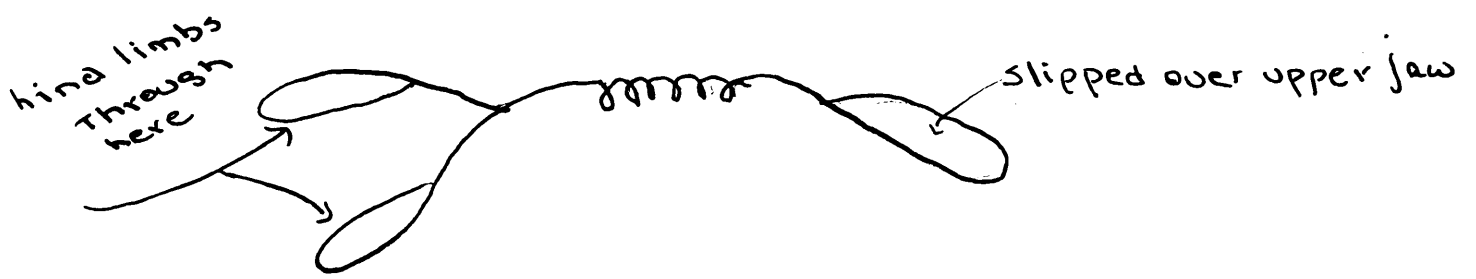
Zero time.- 7 cc. 2% sodium sulphate subcutaneously.

15 mins.- repeated.
30
30 mins.- repeated.

60 mins.- Animal was placed in a special holder as illustrated, and 2 cc. of 2% inulin in physiological saline injected into the jugular vein without anaesthesia.

75 mins.- Bladder expressed,anaesthesia given,the urethra ligated and blood drawn from the femoral artery.The whole time under the anaesthetic was less than 10 minutes.

105 mins.-The ligature was removed,urine collected and timed to the last drop.Anesthetic given and second blood taken from the carotid artery.



No.	Wt.	Time	Urine vol.	Urine Inulin	Inulin PlasmaI	Inulin PlasmaII	Clearance cc/min	Clearance cc/gm/min
In3	235	30	0.9	2.5	30.0	18.5	0.33	0.0014
In4	236	30	0.4	2.75	32.5	27.5	0.305	0.0013
In5	280	30	0.3	5.25	55.0	32.5	0.42	0.0015
In6	252	30	0.2	2.25	25.0	17.5	0.35	0.0014
In7	250	30	0.2	3.75	41.0	26.0	0.38	0.0015

Work was now commenced on the simultaneous inulin and diodrast clearances.

Diodrast and Inulin Clearances.

As a compromise,it was necessary to arrange the timing so that the urine collection period began not more than 60 minutes after the giving of diodrast,and also so that the second blood was collected not more than 60 minutes after the intravenous injection of inulin.This necessitated slight changes in the timing of the administration of fluids.Modifications are noted as they were introduced.

Animals #1,2.

Zero time.- 6 cc. 2% sodium sulphate with 1 cc. diodrast subcutaneously and 4 cc. sodium sulphate intraperitoneally.

20 mins.- 4 cc. sodium sulphate subcutaneously and 4 cc. intraperitoneally.

40 mins.- 1½ cc. 2% inulin in saline injected intravenously using holder.Operation lasted about 5 minutes.

50 mins. - Animal anaesthetised,urethra ligated,and blood drawn from femoral artery.Operation took about 10 minutes, hence the blood was taken about 20 minutes after the injection of inulin.

95 mins.(45 minutes after ligation)- urine collected and timed to the last drop.Animal then anaesthetised and carotid blood taken.

No.	Wt.	Time	Urine vol.	Urine Inulin	Urine Diodr.	Inulin PlasmaI	Inulin PlasmaII	Diodrast Pl.1	Diodrast Pl.2
1	273	45	0.4	1.5	3.97	56.0	35.0	18.86	17.8
2	265	60	0.7	7.25	5.56	102.5	26.0	39.44	63.4

Inulin clearance cc/min	Inulin clearance cc/gm/min	Diodrast clearance cc/min	Diodrast clearance cc/gm/min
0.08	0.0003	0.47	0.0017
0.23	0.0009	0.17	0.0007

In the case of animal #1,urine collection was probably incomplete,because although the plasma levels were falling, little inulin or diodrast was found in the urine.Moreover the urine volume was small and was obtained with difficulty.Animal #2 had frank blood in the urine and the diodrast clearance was less than the inulin clearance indicationg kidney damage.Neither of these animals can, therefore,be considered standard normals.

Animals #3,4,5,6,7,8,9.

It was decided to return to a 60 minute collection period to ensure adequate urine collection.To do this,and still keep close to a 60 minute interval between the intravenous injection of inulin and the drawing of the second blood, it was necessary to perform the injection of inulin,urethra ligation and first blood collection as close together as possible.

Zero time.- 1 cc. diodrast with 4 cc. 2% sodium sulphate subcutaneously,4 cc. sodium sulphate intraperitoneally, and an additional 3 cc. subcutaneously.

20 mins.- 4 cc. sodium sulphate subcutaneously and 4 cc. intraperitoneally.

55 mins.- Intravenous injection of 1.5 cc. of 2% inulin in saline using holder (finished 1 hour from the giving of diodrast). Urethra ligated immediately after intravenous injection,animal anaesthetised and first blood taken from femoral artery.In all these animals,the taking of the first blood was completed in from 6 to 9 minutes after the injection of inulin.

115 mins.(60 minutes after ligation)- urine collected,timed to the last drop and second blood drawn from carotid artery.

The results are summarised in Chart I,page 31.

Diodrast Tm was here determined for the first time because both clearances were done simultaneously.These animals (#3 - 9) are not at all in good agreement,considering the values previously obtained for inulin and diodrast alone.An alteration in method was hence introduced and the following points were considered,a) the level of the first plasma inulin in the above animals is not very high considering the amount injected,therefore it was felt that a smaller injection might give a truer first plasma value, b)the urine volume is very large,therefore it was thought advisable to cut down on the amount of fluids and thus obtain a truer urine flow, rather than such a great diuresis.

<u>Animals #10 - 18.</u>

Zero time.- 1 cc. diodrast in 4 cc. 2% sodium sulphate subcutaneously with 3 cc. sodium sulphate intraperitoneally and an additional 3 cc. subcutaneously.

No.	Wt.	Time	Urine vol. cc.	Urine inul. mg.	Urine diod. mg.	Inulin pl.1 mg%	Inulin pl.2 mg%	Diodrast pl.1 mg%I	Diodrast pl.2 mg%I	Inulin clearance cc/ min	Inulin clearance cc/ gm/ min	Inulin clearance cc/ gm.ky/ min	Diodrast clearance cc/ min	Diodrast clearance cc/ gm/ min	Diodrast clearance cc/ gm.ky/ min	Diodrast Tm mg/ min	Diodrast Tm mg/ gm/ min	Diodrast Tm mg/ gm.ky/ min
3	240	60		17.7	21.12	82.5	25.0	50.03	36.88	0.6	0.0025	0.30	0.81	0.0034	0.41	0.10	0.0004	0.05
4*	238	60		3.0	8.49	126.0	26.0	42.4	11.23	0.09			0.64					
5	280	60		16.25	18.8	205.0	15.0	31.95	8.48	0.49	0.0014	0.21	1.9	0.0067	0.82	0.24	0.0009	0.10
6	223	60	1.2	7.8	15.1	90.0	17.5	43.03	55.22	0.32	0.0014	0.17	0.51	0.0023	0.28	0.091	0.0004	0.05
7**	220	60	1.8	21.25	39.5	56.5	10.0	49.4	38.16	1.4	0.0063		1.5	0.0067				
8	225	60		18.7	35.7	72.5	37.5	35.61	24.38	0.59	0.0026	0.30	2.0	0.0088	1.0	0.41	0.0018	0.21
9	235	60	1.2	10.25	28.5	50.0	7.5	50.03	40.7	0.65	0.0028	0.34	1.05	0.0044	0.55	0.183	0.0008	0.09

* Right kidney found to be hydronephrotic

**Figures indicate no tubular excretion

CHART - I

20 mins.- 2 cc. sodium sulphate subcutaneously, 2 cc. intraperitoneally (this step was omitted in animals #13 - 18)

55 mins.- Intravenous injection of 0.8 cc. of 2% inulin in saline.

Remainder of procedure as described above for animals #3 - 9. Results are summarised in Chart II, page 33.

The clearance ranges of inulin and diodrast are too large here to be useful for comparison. The diodrast clearances would be expected to vary over a wide range due to differences in self-depression at different plasma levels, but the inulin clearance should be more constant. Even diodrast Tm, which should give a steady value over a series of normal animals, varies more than is desirable.

The range in the unilaterally nephrectomised animals was determined.

Animals #19 - 24.

One hour before the procedure was started, the left kidney was removed through a left lumbar incision. The remainder of the procedure was as already described for animals #13 - 18. The results are summarised in Chart III, page 34.

Diodrast Tm here is in all cases lower than in the normal animals and falls in a fairly definite range. The results in Charts II and III indicated that the method was not sufficiently accurate in giving a picture of the functioning rat kidney. In addition, two drawbacks were a) because of the total amount of blood which had to be drawn, it was necessary to use very large rats which were often beginning to show senility changes, b) the procedure was rather long and cumbersome and, as a result, not more than 2 animals could easily be assessed on one day.

No.	Wt.	Time	Urine vol. cc.	~~Urine~~ Urine inul. mg.	~~Urine~~ Urine diod. mg.	Inulin pl.1 mg%	Inulin pl.2 mg%	Diodrast pl.1 mg%I	Diodrast pl.2 mg%I	Inulin clearance cc/ min	Inulin clearance cc/ gm/ min	Inulin clearance cc/ gm.ky/ min	Diodrast clearance cc/ min	Diodrast clearance cc/ gm/ min	Diodrast clearance cc/ gm.ky/ min	Diodrast Tm mg/ min	Diodrast Tm mg/ gm/ min	Diodrast Tm mg/ gm.ky/ min
10.	233	60	0.8	6.75	16.50	65.0	17.5	26.07	16.11	0.33	0.0014	0.19	1.01	0.0043	0.58	0.21	0.0009	0.12
11.	235	64		12.5	36.0	37.5	5.0	50.88	29.04	0.81	0.0034		1.5	0.0063		0.25	0.0010	
12.	225	66		4.25	18.55	57.5	7.5	68.01	51.09	0.29	0.0013	0.15	0.52	0.0023	0.27	0.14	0.0006	0.08
13	228	60	0.4	0.5	13.20	62.5	17.5	84.8	53.0	0.02	0.0001		0.40	0.0017		0.22	0.0009	0.12
14	228	60	0.4	5.25	19.21	62.5	17.5	55.12	27.56	0.26	0.0012	0.14	0.82	0.0036	0.45	0.22	0.0009	0.12
15	220	60	1.0	10.25	29.1	82.5	22.5	62.96	51.72	0.38	0.0017	0.20	0.84	0.0038	0.44	0.26	0.0011	0.13
16	237	60		9.5	35.6	60.0	15.0	49.8	26.28	0.52	0.0021	0.96	1.60	0.0066	0.89	0.39	0.0016	0.20
17	220	65	1.2	15.0	26.2	60.0	15.0	47.27	29.46	0.76	0.0034	0.45	1.07	0.0048	0.63	0.12	0.0006	0.07
18	237	65		8.75	33.3	50.0	0.0	33.49	17.69	0.59	0.0024	0.35	2.08	0.0087	1.2	0.36	0.0015	0.121

CHART - II -

No.	Wt.	Time	Urine vol. cc.	Urine inul. mg.	Urine diod. mg.	Inulin pl.1 mg%	Inulin pl.2 mg%	Diodrast pl.1 mg%I	Diodrast pl.2 mg%I	Inulin clearance cc/min	Inulin clearance cc/gm/min	Inulin clearance cc/gm.ky/min	Diodrast clearance cc/min	Diodrast clearance cc/gm/min	Diodrast clearance cc/gm.ky/min	Diodrast Tm mg/min	Diodrast Tm mg/gm/min	Diodrast Tm mg/gm.ky/min
19	215	60	0.3	1.5	8.9	65.0	17.5	87.76	53.84	0.07	0.0003	0.07	0.22	0.0010	0.23	0.09	0.0004	0.09
20	210	60	0.8	10.0	21.8	75.0	25.0	77.53	58.51	0.38	0.0013	0.47	0.53	0.0025	0.65	0.107	0.0005	0.13
21*	225	60	0.3	5.0	6.3	56.0	32.5	61.48	59.26	0.19	0.0008		0.19	0.0008				
22	235	60	0.5	2.5	10.6	85.0	25.0	48.54	44.09	0.09	0.0004		0.39	0.0016		0.134	0.0005	
23	250	60	0.8	10.0	21.5	100.0	22.5	52.15	65.12	0.35	0.0014		0.61	0.0024		0.157	0.0006	
24	250	60	0.5	7.0	19.74	87.5	25.0	48.76	53.84	0.25	0.0010		0.65	0.0026		0.205	0.0008	

* Figures indicate no tubular excretion.

CHART - III -

A radical change in procedure was hence introduced at this point.The resulting method was shorter,could be used in smaller rats,and yielded more consistent results.One blood only,at the mid-point,was drawn.

Animals #25 - 35.

Zero time.- 0.6 cc. diodrast in 3.4 cc. 2% sodium sulphate subcutaneously.

20 mins.- 2 cc. 2% inulin in saline subcutaneously and 1.5 cc. intraperitoneally (although inulin is slowly absorbed from these routes,enough was absorbed in the required time to give a colorimetric reading on a 0.2 cc. sample of filtrate.This change may account partially for the more consistent results obtained because it approaches more nearly the infusion method.)

60 mins.- Bladder expressed (this must be thoroughly done,so that no urine is left to dilute the urine formed in the next 60 minutes).Urethra ligated without anaesthesia.This is usually done in holder,which itself causes voluntary micturition.

85 mins.- Blood taken from carotid artery under anaesthesia.This is completed 30 minutes from the time of ligation.

110 mins.(50 minutes from ligation) - ligature removed and urine collected.Total collection time was estimated to the end of urine collection and the time was usually not greater than 60 minutes.

The results are summarised in Chart IV,page 36. These results show more consistent diodrast Tm values. As a check,the clearances of a series of unilaterally nephrectomised animals were determined.

Animals #36 - 40.

One hour before the start of the experiment,the left kidney was removed.The rest of the procedure was the same as that described for animals #25 - 35. The results are summarised in Chart V,page 37.

No.	Wt.	Time	Urine vol. cc.	Urine inul. mg.	Urine diod. mg.	Plasma inul. mg%	Plasma diod. mg%I	Inulin clearance cc/ min	Inulin clearance cc/ gm/ min	Diodrast clearance cc/ min	Diodrast clearance cc/ gm/ min	Diodrast Tm mg/ min	Diodrast Tm mg/ gm/ min	Diodrast Tm mg/ gm.ky/ min
25	160	60	0.4	16.75	21.5	50.0	30.74	0.56	0.0035	1.10	0.0070	0.179	0.0011	0.139
26	195	57	1.0	27.5	42.1	45.0	46.44	1.0	0.0050	1.50	0.0080	0.265	0.0013	0.177
27	150	70	0.4	15.0	23.85	42.5	33.92	0.504	0.0033	1.04	0.0069	0.170	0.0011	0.126
28	165	65	0.6	8.0	26.75	20.0	25.44	0.61	0.0036	1.6	0.0096	0.225	0.0013	0.125
29	150	60	0.3	12.5	17.49	45.0	28.77	0.45	0.0030	1.01	0.0067	0.161	0.0010	0.122
30	180	55	0.8	18.75	33.39	37.5	36.04	0.90	0.0050	1.68	0.0092	0.282	0.0015	0.170
31	148	60	0.7	18.5	30.7	47.5	57.24	0.64	0.0043	0.89	0.0060	0.150	0.0010	0.103
32	165	59	0.7	8 .0	22.5	42.5	61.48	0.31	0.0018	0.62	0.0037	0.185	0.0011	0.145
33	150	56	0.7	16.25	23.8	32.5	27.56	0.89	0.0059	1.5	0.0100	0.175	0.0011	0.128
34	175	60	0.5	11.75	21.73	35.0	18.86	0.56	0.0032	1.92	0.0109	0.255	0.0014	0.180
35	176	51	0.6	10.5	22.52	36.0	17.808	0.478	0.0027	2.07	0.0117	0.274	0.0015	0.186

CHART - IV -

No. //	Wt.	Time	Urine vol. cc.	Urine inul. mg.	Urine diod. mg.	Plasma inul. mg%	Plasma diod. mg%I	Inulin clearance cc/ min	Inulin clearance cc/ gm/ min	Inulin clearance cc/ gm.ky/ min	Diod. Clearance cc/ min/	Diod. Clearance cc/ gm/ min	Diodrast Tm mg/ min	Diodrast Tm mg/ gm/ min	Diodrast Tm mg/ gm.ky1/ min	Diodrast Tm mg/ gm.ky2 min
36	175	61	1.5	12.75	23.58	37.5	56.81	0.557	0.0031	0.78	0.68	0.0038	0.070	0.0004	0.098	0.074
37	158	59	1.1	17.25	29.41	45.0	59.78	0.649	0.0041	1.0	0.834	0.0052	0.111	0.0006	0.222	0.154
38	180	58	0.6	13.25	25.86	35.0	58.088	0.652	0.0034	1.0	0.767	0.0043	0.083	0.0004	0.137	0.087
39	170	57	0.7	10.37	17.49	42.5	61.586	0.428	0.0025	0.71	0.498	0.0029	0.044	0.0002	0.073	0.052
40	168	54	0.8	9.5	14.04	50.0	67.628	0.351	0.0021	0.58	0.380	0.0022	0.030	0.0002	0.049	0.037
Mean										0.81				0.0003	0.116	0.081

ky.1. is extirpated kidney.
ky.2. is surviving hyperaemic kidney

CHART - V -

These diodrast Tm's all fell in a range well below that of the normal animals and showed consistency.

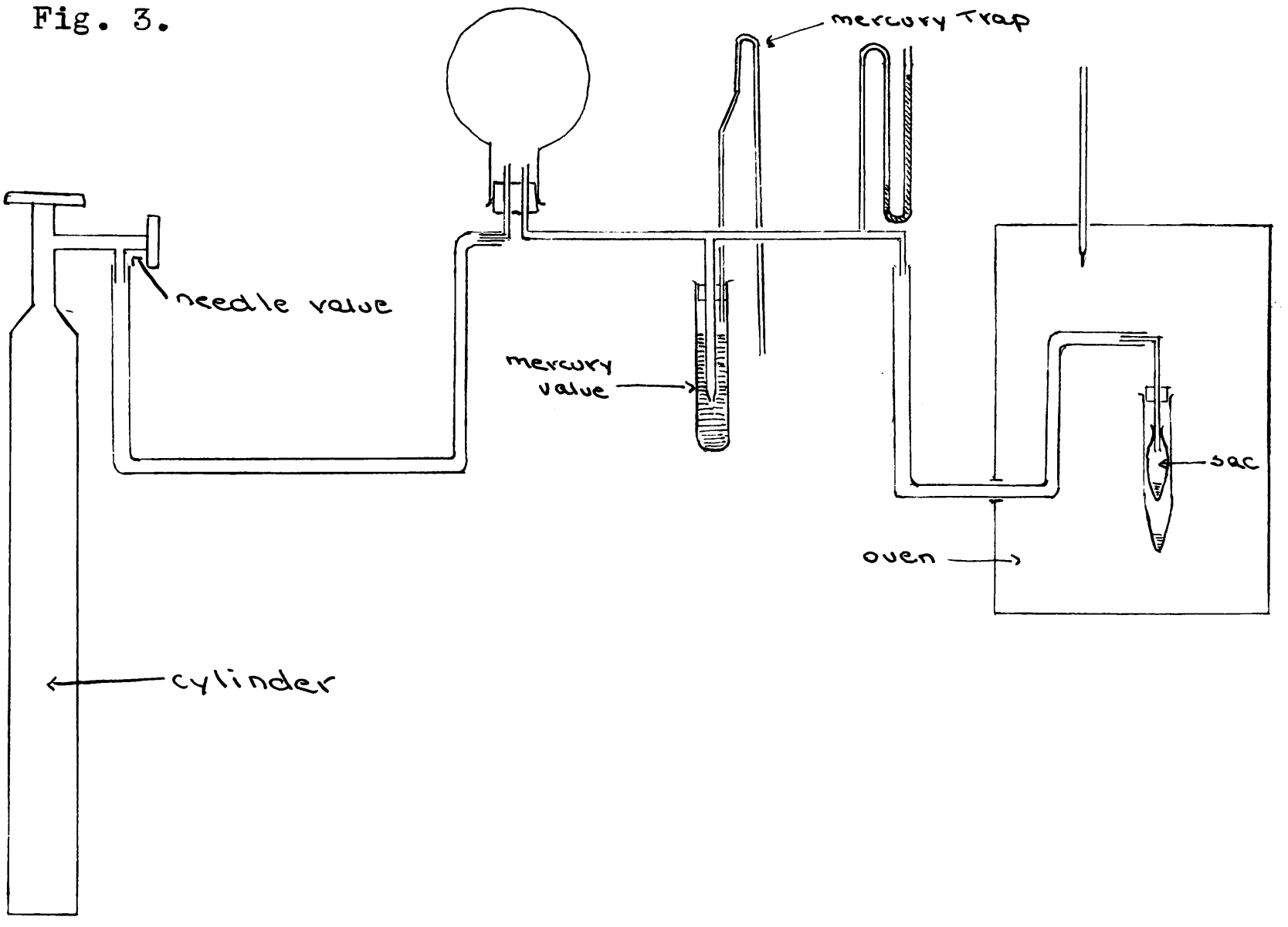
In both these groups(Charts IV and V) inulin clearance still varies considerably and seems high.This may be due to a hyperaemia caused by the inulin since it was learned at this time that Seitz filtration alone is not enough to remove the pyrogen completely; the inulin must also be treated with charcoal.

The very large variation in diodrast clearance values is,of course,due to differences of self-depression because of the wide range of plasma levels.As a result,these diodrast clearance values are,per se,meaningless.Although Tm_D can be determined above the level of maximum clearance,the true value of this datum here is rendered questionable,particularly without the calculation of FW. Above plasma levels where the tubules are saturated,any futther increment in excretion is due solely to filtration,hence at the plasma levels used above a large fraction of the total excretion is probably by way of filtration.It thus becomes very important to know what per cent of diodrast is not bound to plasma proteins,but is freely filterable. Any attempt to estimate T acurately at such widely different plasma levels is of little use,without FW.

The next step in the work should have been to sacrifice a large number of rats and determine,by ultrafiltration,the adsorption isotherm for diodrast in rat blood in relation to albumin content, and then to correct the above Tm figures for FW. For various reasons,it was impossible to undertake a systematic investigation of FW at this time.As a substitute, a large series of animals was observed at lower plasma diodrast levels,and with a smaller range of plasma diodrast.This has given an indication of the concentration of diodrast in plasma at which the clearance is greatest - and hence of renal blood flow.In addition,F was determined roughly on separate

animals by an application of the ultrafiltration method described by Smith and Smith (68),and a mean value was applied in the calculation of Tm_D although at these lower plasma levels,F does not contribute such a large factor.

The apparatus used for ultrafiltration is that shown in Fig. 3.



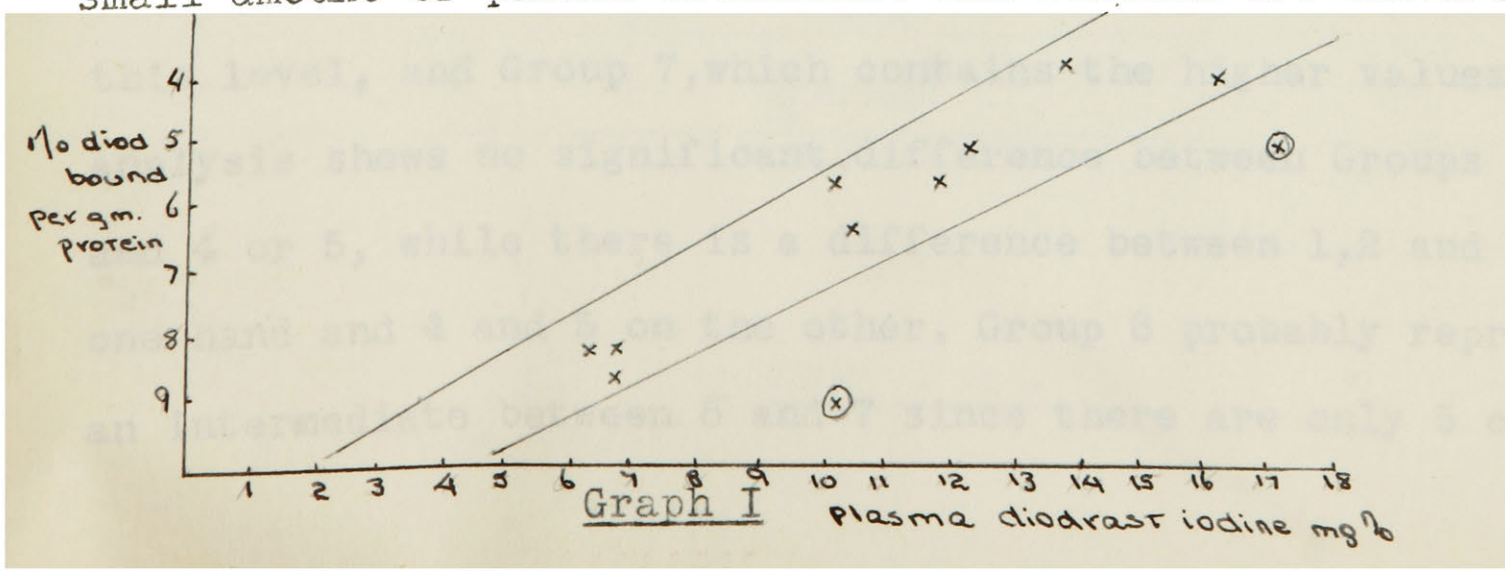
A pressure tank containing CO_2 25 vols.%, and O_2 75 vols.%, with an outflow needle valve,is connected through an inverted litre flask to a mercury manometer and a mercury pressure valve. The outflow of gas is directed into a small collodion sac made in a centrifuge tube and the ultrafiltrate is collected in a stoppered centrifuge tube which is placed in an oven at a constant temperature of 37° Centigrade.The collodion is prepared according to the method of Marshall and Vickers (35).Gas from the tank is slowly bubbled through the apparatus until the pressure in the manometer reaches 160 mm Hg., which,with the gas mixture used,gives pCO_2 40 mm Hg.

Constant pressure at this level is maintained by adjusting the mercury valve so that excess pressure is released in the form of bubbles.Filtration is allowed to proceed for about 3 hours.

The blood for ultrafiltration is taken immediately at the end of the clearance period and hence represents a plasma level somewhat lower than the mid-point.The blood is centrifuged in a capped tube to prevent aeration and after one-half hour,the plasma is carefully transferred to a collodion sac, 0.3 cc. being reserved for diodrast analysis. At the end of filtration, the amount of diodrast in the filtrate is determined, and F is calculated from the formula $\frac{\text{Concentration diodrast in the ultrafiltrate}}{\text{Concentration diodrast in plasma}}$.

This merely gives a rough idea of F and is by no means accurate since the removal of a second blood sample 30 minutes after the first probably means that the blood is hydraemic.In addition, diodrast is at a lower concentration in the plasma than at the time the clearance is determined and further,with such small amounts of plasma, changes in pH can probably not be avoided. At the low plasma levels used here,however, a variation in F does not contribute a great factor to the calculation of Tm.

In an effort to obtain a rough idea of the correlation between plasma protein concentration and the amount of diodrast bound, protein was determined by the falling drop method (71). This method, although not accurate,was the only one which could be used on the small amount of plasma available. The results are shown in Graph I.



Graph I

There is some indication of a linear correlation between the percent of diodrast bound per gram of protein and the plasma concentration of diodrast ; in general more diodrast is bound at lower plasma levels.

Charts VI,VII and VIII summarise the final results obtained in this work. Chart VI gives a summary of the complete findings on a series of 31 animals (3 animals previously shown are included in this table because their range of plasma level is within the desired limits).Chart VII is a statistical analysis of the diodrast clearance with relation to plasma level,dividing the clearance values into groups. Chart VIII gives the values for T_D in groups treated statistically.

Chart VI (p.42).

Inulin clearance shows a mean value of 0.0027 cc/gm/min with a deviation from the mean of ± 0.0006.

A certain difficulty was encountered in 3 animals in which it was found at autopsy that the bladder had not been completely emptied.These animals are not included.This can be avoided in further work by drawing the urine directly out of the bladder under anaesthesia at the end of the collection period.

Chart VII (p.43).

The clearances are here grouped for comparison in units representing a spread of 2mg% in plasma level,except for Group 6 in which a spread of 3 mg% is used because of the fewer data at this level, and Group 7,which contains the higher values.The analysis shows no significant difference between Groups 1,2 or 3, and 4 or 5, while there is a difference between 1,2 and 3 on the one hand and 4 and 5 on the other. Group 6 probably represents an intermediate between 5 and 7 since there are only 5 chances in

No.	Wt.	Time	Urine vol. cc.	Plasma inul. mg%	Plasma diod. mg%I	Inulin clearance cc/gm/min	Diodrast clearance cc/gm/min	T mg/gm/min	F
41	160	60	0.3	37.5	4.4	0.0022	0.0101	0.00036	
42	157	58	0.1	31.0	5.0	0.0026	0.0080	0.00032	
43	154	58	0.1	36.0	5.7	0.0034	0.0095	0.00046	
44	180	60	0.2	45.0	5.9	0.0029	0.0140	0.00072	
45	178	60	0.2	31.0	6.3	0.0024	0.0096	0.00046	
46	126	60	0.1	37.5	7.4	0.0028	0.0080	0.00052	
47	162	60	0.3	47.5	7.6	0.0026	0.0110	0.00072	
48	133	60	0.1	50.0	7.8	0.0037	0.0109	0.00068	
49	152	58	0.2	12.5	8.0	0.0011	0.0119	0.00082	0.5
50	150	60	0.5	40.0	8.9	0.0028	0.0112	0.00082	
51	181	60	0.2	31.0	9.1	0.0044	0.0088	0.00068	
52	155	60	0.1	40.0	9.5	0.0016	0.0079	0.00068	
53	176	58	0.5	25.0	10.1	0.0027	0.0171	0.00158	
54	180	55	0.2	35.0	10.1	0.0017	0.0111	0.00110	0.76
55	135	58	0.2	52.5	10.8	0.0025	0.0120	0.00118	
56	185	60	0.5	30.0	10.8	0.0024	0.0142	0.00138	
57	195	60	0.6	42.5	10.9	0.0023	0.0120	0.00170	0.66
58	200	60	0.6	42.5	11.2	0.0031	0.0133	0.00150	0.5
59	200	60	0.7	37.5	11.5	0.0034	0.0160	0.00160	0.46
60	178	58	0.4	25.0	13.0	0.0034	0.0149	0.00160	0.69
61	205	55	0.4	26.0	13.3	0.0032	0.0114	0.00130	0.53
62	195	60	0.5	25.0	14.6	0.0024	0.0143	0.00180	0.64
63	185	63	0.5	31.0	16.1	0.0026	0.0104	0.00140	0.71
64	176	51	0.6	36.0	17.8	0.0027	0.0117	0.00170	
65	175	60	0.5	35.0	18.8	0.0032	0.0109	0.00160	
66	192	62	0.5	33.3	23.3	0.0022	0.0070	0.00120	0.77
67	200	60	0.5	30.0	25.3	0.0025	0.0063	0.00120	0.70
28	165	65	0.6	20.0	25.4	0.0036	0.0096		
25	160	60	0.4	50.0	30.7	0.0035	0.0070		
27	150	70	0.4	42.5	33.9	0.0033	0.0069		
68	180	58	0.4	25.0	35.6	0.0032	0.0061	0.00140	0.5
Mean						0.0027			0.62
σ						$\pm$ 0.0006			

CHART - VI -

Plasma diod.	Clearance cc/gm/min	Group	Mean	Probability between gps.	Group	Probability between gps.	Mean	σ	$\frac{\sigma}{\bar{X}} \times 100$	F.F.
4.4	0.0101									
5.0	0.0080	1	0.0104	1-2 $p>0.05$						
5.7	0.0095			1-3 $p>0.05$						
5.9	0.0140			1-4 $p<0.05$						
6.3	0.0096			2-3 $p>0.05$						
7.4	0.0080	2	0.0099	2-4 $p<0.05$	I		0.0101			
7.6	0.0110									
7.8	0.0109									
8.0	0.0119			3-4 $p<0.01$						
8.9	0.0112	3	0.0100							
9.1	0.0088									
9.5	0.0079									
10.1	0.0171			4-5 $p>0.05$						15.7
10.1	0.0111			4-6 $p<0.05$						15.3
10.8	0.0120	4	0.0137			II-I $p<0.01$				20.8
10.8	0.0142					II-III $p<0.01$				16.9
10.9	0.0120				II		0.0133 $\pm$0.0021		15.8	19.1
11.2	0.0133									23.3
11.5	0.0160									21.2
13.0	0.0149			5-6 $p<0.05$	II					22.8
13.3	0.0114	5	0.0135							28.0
14.6	0.0143									16.7
16.1	0.0104									
17.8	0.0117	6	0.0110	6-7 $p<0.01$						
18.8	0.0109									
23.3	0.0070									
23.3	0.0063									
25.4	0.0096	7	0.0071		III		0.0090			
30.7	0.0070									
33.9	0.0069									
35.6	0.0061									

CHART - VII -

a hundred that it is part of Group 5 and less than 1 chance that it is part of Group 7.There are really too few data here to come to a definite conclusion about this.

On regrouping into broader units,further analysis shows that there is significant difference beween groups I,II and III. According to these data,it is possible to plot only 3 points on the curve of diodrast clearance against plasma concentration; a) a low clearance range below 10 mg%I, b) a range of maximum clearance certainly between 10 and 14 mg%I and possibly between 10 and 18 mg%I, and c) a depressed clearance range beyond this level. The data are too few to attempt any further correlation.

From the data in the group with maximum clearance,it appears that the mean renal blood flow is 0.03 cc/gm/min,using an average haematocrit of 60% for cells. This is approximately 1/3 of the total circulating blood volume in the rat if 7% of body weight is used to determine blood volume.This roughly approximates the value in man which is 1/3 of the average cardiac output.

From the maximum diodrast clearance it is also possible to calculate Filtration Fraction, $\frac{\text{inulin clearance}}{\text{diodrast clearance}} \times 100$

This shows what proportion of the total excretion is due to glomerular filtration. The mean value here is 20.02% with a standard deviation of $\pm$ 3.99%. This may be compared with the data for man (18) where F.F. is 18.9% $\pm$ 2.4%, on a larger series.

Chart VIII (p.45).

The rate of tubular excretion,T, is the difference between the total excretion per minute and the quantity excreted by filtration, that is,

$$T = \frac{\text{diodrast excretion}}{\text{time}} - PIWF$$

Plasma diod.	T mg/gm/min	Group	Mean	Probability between gps.	Group	Probability between gps.	Mean	σ	$\frac{\sigma}{\bar{X}} \times 100$
4.4	0.00036			1-2 $p>0.05$					
5.0	0.00032	1	0.00046	1-3 $p<0.05$					
5.7	0.00046			1-4 $p<0.01$					
5.9	0.00072								
6.3	0.00046								
7.4	0.00052	2	0.00059	2-3 $p>0.05$					
7.6	0.00072			2-4 $p<0.01$	I	I-II $p<0.01$			
7.8	0.00068								
8.0	0.00082								
8.9	0.00082	3	0.00075	3-4 $p<0.01$					
9.1	0.00068								
9.5	0.00068								
10.1	0.00158			4-5 $p>0.05$					
10.1	0.00110			4-6 $p>0.05$					
10.8	0.00118			4-7 $p>0.05$					
10.8	0.00138	4	0.00143						
10.9	0.00170								
11.2	0.00150								
11.5	0.00160								
13.0	0.00160			5-6 $p>0.05$	II		0.00145	$\pm$0.00021	13.7
13.3	0.00130	5	0.00156	5-7 $p>0.05$					
14.6	0.00180								
16.1	0.00140			6-7 $p>0.05$					
17.8	0.00170	6	0.00156						
18.8	0.00160								
23.3									
23.3	0.00120								
23.3	0.00120								
35.4		7	0.00129						
30.7									
33.9									
35.6									

CHART - VIII -

where P is the quantity of solute in each cc. of plasma, I the concurrent rate of glomerular filtration as measured by the simultaneous inulin clearance, W the fraction of water in the plasma, and F the fraction of solute which is free in the plasma and therefore available for filtration. Free diodrast was roughly determined in some of the animals by the method already described, and in these cases, the result is tabulated in Chart I. For others a mean value of 0.62 was used and is incorporated into the value for T. The fraction 0.62 was considered valid only at the lower plasma levels where a difference of $\pm$ 0.2 in F would give a maximum difference of only $\pm$ 0.0001 mg. in the result for T expressed in terms of body weight.

W is determined by the formula $1 - \frac{\text{percent plasma protein}}{100}$ (18). Since the average plasma protein value was 5.6% with a deviation from the mean of $\pm$0.4 , this would give a value for W of 0.944 $\pm$ 0.004 which would influence the calculation of T maximally by $\pm$ 0.00002. Since a variation of this magnitude would have a negligible effect on the net result, W is considered as unity in the calculation.

The data for T are grouped in a similar manner to the clearance data in Chart VII. Again, there is no statistical difference between Groups 1,2 and 3 on the one hand, and 4,5,6 and 7 on the other. Above 10 mg%I the tubular excretion is significantly higher than below this level. Hence it appears that above this plasma level the calculation yields Tm_D . In these animals σ is $\pm$ 0.00021 or 13.7% which compares favourably with the variation found in man (18) of 18.2%.

Conclusions.

Thus, in a range of diodrast plasma level of 10 to 14 mg%I, maximum diodrast clearance can be calculated in the rat. From this, together with the simultaneous inulin clearance, it is possible to deduce effective renal blood flow,glomerular filtration rate, filtration fraction,urine concentration, and diodrast Tm, all of which give further information about the kidney. In view of the fact that these results show no greater variation than that encountered by other investigators in the dog and man it is felt that the clearance method may be applied satisfactorily to a study of the function of the rat kidney under various experimental conditions.

Summary.

1. A brief historical survey of the methods of investigating renal function by clearance tests is presented.

2. The essential features of the simultaneous inulin and diodrast clearance test as currently applied to larger animals is outlined.

3. Experimental work is presented to show a) the steps by which the inulin-diodrast method was adapted to the rat. b) The results obtained with the method as finally devised.

Addendum

It has recently been shown that p-amino-hippuric acid can be used as a substitute for diodrast in this work but the preparation has not as yet been made available. (Finklestein,N., Aliminosa,L.M. and Smith,H.W. 1941. Am. J. Physiol., 133, 276.)

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