EXPERIMENTAL NEPHRITIS IN THE FROG

II. Perfusion Methods of Testing the Kidney by Dissociation of Its Functions*

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The hypothesis that correlation of function and anatomical change may be more accurately examined by the study of experimental nephritis in the kidney of the frog than in the complex mammalian kidney is the basis of the present work. A preliminary investigation (1) has shown that the morphological changes observed in mammals after the kidney is damaged by renal toxic agents are reproduced in all their essential characteristics in these relatively simple kidneys when such substances are administered *in vivo* to the frog. Moreover it has been found that in the frog the consequent lesions are less complex and are therefore more readily open to interpretation. With this encouragement the next step has been an examination of function.

As we have pointed out in another place (2), the chief factor that makes it difficult to achieve any successful result with the mammalian kidney in such a correlation, is the impossibility of distinguishing in the total function of the kidney the function of its component parts. For example, judging from present physiological experience, damage to tubules may compensate for disturbances due to damage in the glomeruli and the consequent presence of two or perhaps more variables leaves the observer confused. In studying the problem in the frog our attention must therefore be directed towards a dissociation of

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these complex mechanisms and the examination of them under conditions of isolation and control.

The method of perfusion of the frog's kidney approaches at least the accomplishment of this ideal. Its arterial-glomerular and venous-tubular circulation, together with the possibility of administering dyes to it which are reciprocally excreted in significant amount by only one of the renal mechanisms, is the basis of its value. Controversy concerning these concepts indeed exists, but as will be shown later, the use of the method with the pathological kidney adds further evidence of their verity, in the same way that the use of toxic damage by Bainbridge, Collins, and Menzies (3) and Höber (4) has illuminated obscure aspects of normal kidney function.

Technique

The method of perfusion has been previously described (5). It is based on Höber's (4) modification of the Barkan, Broemser, and Hahn (6) technique by which isotonic Locke's solution containing 0.025 per cent sugar and a small amount of glycocol maintained at a pH of 7.3 is led from separate containers to the arterial and the portal-renal venous systems. The urine is collected from each kidney in cannulae. The urine formed by the procedure when successful is sugar-free, its electrolyte content is less than one-half that of the perfusion fluid, and the rate of its excretion is comparable to that of the formation of urine by the living frog. If urea is added to the perfusion fluid it is concentrated in the urine. Failure in method is therefore easily detected by the presence of sugar in the urine, by a rise in salt content towards the level existing in the perfusion fluid, or by abnormal rates, either high or low, of water excretion. The methods of determination of the constituents of the urine were as follows: Benedict's method for sugar, determination of electrolyte content by the Christiansen ionometer, and dye content by the usual colorimetric method.

The Manner of Excretion of Phenol Red and Neutral Red

We have previously stated our reasons for believing that phenol red is excreted by the perfused frog's kidney chiefly through the glomerulus and that neutral red is excreted principally through the tubular epithelium. Misunderstanding may perhaps be avoided if we emphasize that our interest in the present study is not how these dyes are eliminated by the kidney of the living frog but how they are excreted by the isolated kidney under our experimental procedure, for these procedures as used here are not designed to examine the intricacies of

renal physiology, but to test the reaction of the two components of the renal mechanism under abnormal conditions.

Dissociation of Glomerular and Tubular Function

In the study of the function of the normal kidney a frequent procedure is to damage or narcotize one or the other of the kidney elements on the assumption that this depresses its activity and hence removes its influence from the total function of the kidney. This method has been applied especially to the examination of tubular activity. An excessive excretion of water or the appearance of sugar in the urine after such treatment of the tubules has therefore been interpreted as the result of an abolition of a normal absorptive function inherent in them. Although there is a considerable amount of indirect evidence to support this idea, the fact remains that there are other possible interpretations. The concept of tubular absorption depends largely on the assumption that the bulk of the water in the urine is derived from the glomeruli. Moreover it is entirely possible that damage to the tubular epithelium might allow both water and sugar to leak through its wall from the surrounding capillaries and thus explain the experimental findings. Further evidence on the question is therefore needed and the following experiment, an example from a series of twelve essentially similar findings, is given.

The isolated kidneys of *R. catesbiana* were perfused through both the arteries and the renal-portal vein in the usual way with modified Locke's solution containing 0.05 per cent phenol red and 0.025 per cent sugar (Chart 1). As soon as a normal output of urine was established the flow through the renal-portal venous system was gradually restricted until in the fourth, fifth, and sixth periods of the experiment it was only a fraction of its original figure. The tubules were thus deprived of a part of their supply of fluid. It will be seen that there resulted a gradually increasing output in the amount of water until in the seventh period a rate of 20.8 cc. per hour was reached, more than twice the original volume. Accompanying this diuresis there went an increase in the rate of salt and phenol red excretion and sugar appeared in the urine.

In the seventh period the restriction of the venous-tubular supply was removed. The supply of fluid to the tubules was thus increased and in the next (eighth) period the flow of fluid through the arterial system was reduced. A sharp fall in water output immediately resulted and with this fall a decrease in the output of salt and phenol red occurred so that the original rates of excretion of these two substances were again reached. As the supply of arterial fluid was decreased still

further these values reached ultimately an amount approximately one-tenth of their original normal values, the urine was highly concentrated, and sugar disappeared from it.

At Period 12 the arterial supply was reestablished. The volume of urine thereupon increased to its original value, and was accompanied by increases in the rate of phenol red and salt excretion which also approached their normal figures. The final conditions, including the absence of sugar from the urine, therefore approximated the original conditions of the experiment.

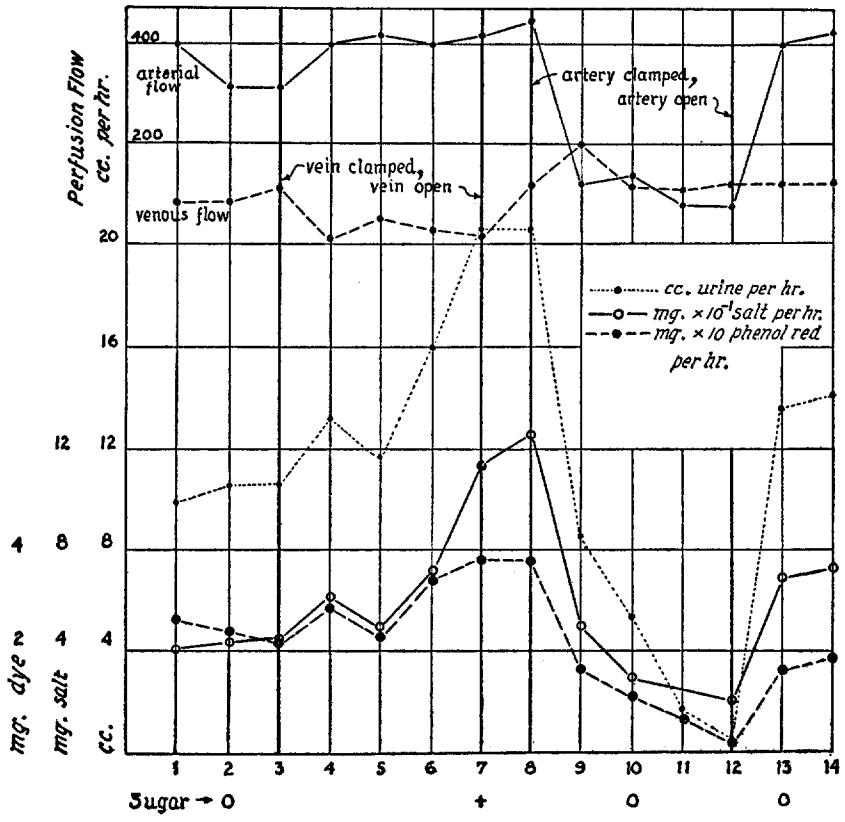

CHART 1

The experiment emphasizes certain points. First, that an increase in urine volume may result from a *restriction* of the supply of fluid to the tubules but that the ultimate source of water depends on adequate glomerular supply. Second, that a restriction of the supply of fluid to the tubules produces those changes which have formerly been re-

ported as a result of damage by toxic agents. The experiment therefore supports the theory that diuresis, increased salt output, and appearance of sugar in the urine are not the result of any increased passage of these substances through a damaged tubular epithelium but that they are due rather to a lack of absorptive activity of this portion of kidney mechanism. In other words, these phenomena represent an abolition of a normal absorptive function, a disturbance which in our experiment was due to a lack of an essential supply of materials to the tubule cells.

It would also appear that the source of the greater part of phenol red in the urine is the glomerular apparatus, since the proper excretion of this substance in our experiment is dependent on a proper supply of fluid and dye to this structure. This fact and its converse, that neutral red is excreted in greater part through the tubular epithelium, we shall not discuss at this time. Experiments similar to the one we have just described may be found in another place (7) where it has been shown that though the changes in water, salt, and sugar excretion are consistently similar no matter which dye is used in the perfusion fluid, the dyes themselves behave in an opposite manner to the experimental variations in fluid supply.

These experiments in which it is believed glomerular and tubular functions have been dissociated to such an extent that their individual characteristics may be recognized, form the basis for the interpretation of the results in the varied experiments that follow. Certain phenomena such as diuresis, increase in salt output, the appearance of sugar in the urine, or fall in rate of phenol red or of neutral red elimination, to name only a few, have been found to follow mechanical and relatively simple procedures, such as reducing the supply of fluid, or its pressure, to a certain part of the kidney unit. It is by the recognition of these same functional variations in experiments where toxic substances have damaged the kidney that the attempt to localize the seat of lesion will be made.

The Demonstration of Tubular Dysfunction by the Administration of Toxic Agents to the Kidneys

In devising methods for the detection of those disturbances of function in the kidney that follow the administration of toxic sub-

stances an attempt at simplification and control has been made in that we have not given the poison to the living animal where its activity may be modified by the infinite complexities that of necessity develop when a substance is introduced into the living organism, but to the organ itself isolated from these uncontrollable and in great part unknown factors. Furthermore, in the frog's kidney at least, such a method allows to a certain degree the administration of the toxic agent to specific structures in the organ, and the concentration of its effects to definite localities. The importance of this factor is apparent when one calls to mind the current theory which claims that renal toxins are by themselves able to produce specific lesions in one or the other part of the renal unit.

Even under these controlled conditions and with the relatively simple kidney of the frog, as the experiments will show the reactions are exceedingly complex. We can only describe, therefore, the simpler and more typical of the "syndromes" which are observed. No attempt has been made to determine the action of all the known renal poisons as we are interested at this time only in methods which allow us to recognize where the damage to the kidney has been done. A study of the action of the poison itself will be considered at a later time when the kidney of the nephritic frog is examined.

Evidences of Absorptive Failure

If a renal toxic agent is introduced into the renal-portal circulation of the perfused kidney, the arterial circulation remaining normal, a damage to the tubular elements occurs whose functional aspects correspond exactly with those phenomena which our basic experiment has shown to be the result of a depression of the absorptive function of the tubule. Sugar appears in the urine, there is an increased output of water, and an increased excretion of salts. The rate of excretion of phenol red is not significantly decreased and may in fact be increased. Experiments illustrating these effects as a result of several toxic agents are given in Table I.

In these experiments it will be noted that no significant alteration in the flow of the perfusion fluid followed the administration of the toxic substance. Attention should be drawn to the fact that the lack of function of the tubular epithelium following urethane was not

simple anesthesia for there was no recovery of tubular activity when the urethane was discontinued, and finally, that we are warranted in assuming that actual tissue damage occurred after the introduction of all these substances, will be apparent in a later description of the anatomical changes that occurred in the kidneys of these and similar experiments.

TABLE I

Evidences of Absorptive Failure

	Arterial flow	Venous flow	Urine flow	Phenol red	Salt	Sugar
	Corrosive sublimate					
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*	
11:40–11:50	540	500	7.8	1.01	42.9	0
11:50–11:53		10 cc. 1/10,000 sublimate to tubules				
12:00–12:10	600	180	13.2	1.25	72.6	+
12:10–12:20	540	200	12.0	0.78	72.0	++
	Uranium nitrate					
11:30–11:45	240	280	2.4	1.09	—	0
11:45–11:50		15 cc. 1/5000 uranium nitrate to tubules				
12:00–12:15	120	280	2.4	—	—	0
12:15–12:30	280	400	6.4	0.740	—	Tr.
12:30–12:45	120	400	5.2	0.806	—	+
	Urethane					
11:20–11:30	540	300	12.0	1.2	66.0	0
11:30	2 per cent urethane to tubules continuously through following periods					
11:30–11:45	540	540	17.4	1.4	104.0	0
11:45–12:00	600	480	20.0	1.0	110.0	Tr.
12:00–12:15	600	520	25.4	1.0	152.0	+

*Evidences of Secretory Failure**

When the perfused kidney is treated as described above it can be shown by testing with neutral red that the failure of absorptive ac-

* The term secretion is used here in only a general sense to denote the passage of a substance from the blood stream through the epithelial cell into the lumen of the tubule. The term excretion is applied to the activity of the kidney as a whole. As will be seen the *excretory* activity of the kidney may be increased by changes which at the same time abolish the *secretory* activity of the tubular epithelium.

tivity may be accompanied by a decrease or even complete failure of the secretory activity of the tubule cells. In the experiments of Table II neutral red was added in concentrations varying from 0.01 to 0.02 per cent to the perfusion fluid which was passing by way of the renal-portal vein to the tubules. In the first two experiments the toxic agent was introduced into the venous circulation after the establishment of normal urine formation and when a normal excretion

TABLE II

Evidences of Secretory Failure

	Arterial flow	Venous flow	Urine flow	Neutral red	Salt	Sugar
			Urethane			
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*	
11:30–11:40	540	600	7.8	0.63	42.9	0
11:40	2 per cent urethane to tubules throughout following periods					
11:40–11:50	600	540	18.0	0.36	99.0	+
11:50–12:00	600	600	21.0	0.36	115.0	++
12:00–12:10	540	660	21.6	0.26	147.0	++
			Potassium bichromate			
10:45–11:00	480	660	3.6	2.01	—	0
11:02–11:05		10 cc. 1/2000 potassium bichromate to tubules				
11:15–11:30	320	320	4.0	1.12	—	+
11:30–11:45	300	520	4.6	1.09	—	++
			Corrosive sublimate			
11:45–12:00	360	440	4.8	—	24.0	0
12:00–12:03		10 cc. of 1/2500 sublimate to tubules Neutral red to venous fluid supply				
12:15–12:30	200	480	8.0	Ftst. tr.	48.0	+
1:30– 1:45	160	480	1.2	Ftst. tr.	9.0	++

of the dye was in process. Along with the evidences of absorptive failure, diuresis, high salt output, and glycosuria, there went a definite decrease in the rate of excretion of neutral red. In the third experiment, after normal urine formation was established by the perfusion with clear Locke's solution, the tubule cells were damaged by the toxic agent. After a proper interval, which was characterized by the same absorptive abnormalities described above, neutral red

was then supplied to the tubules. It will be seen that there was an almost complete lack of elimination of the dye in spite of the relatively large output of water, salt, and sugar, conditions which, as other experiments have shown, are not incompatible with the excretion of considerable amounts of phenol red.

The Dissociation of the Tubular Functions

The question arises if it is possible to produce one of the phases of tubular functional damage without the appearance of the other. Both depression of absorptive activity with intact secretory function and the converse condition of depressed secretory function and normal absorption by the tubule cells have been observed. The former case, however, has been less frequently encountered than the latter. Table III gives examples of such experiments.

The Demonstration of Glomerular Dysfunction

(*a*) *Evidences of Increased Filtration*

Our experiments have given no direct evidence as to whether the mechanism of passage of substances through the glomerular membrane is one of filtration or active secretion. Under certain pathological conditions at least the process closely resembles filtration and we have therefore used this term descriptively with reservations as to the exact nature of the process.

The simplest and most objective demonstration of an increase in the permeability of the glomerular membrane is obtained by testing it with substances which do not pass through it and appear in the urine under normal conditions. One thinks immediately of the proteins of the blood plasma which are thus held back by the normal kidney and in analogy to them the hydrophilic colloid gum arabic was used. If this substance is added in a concentration of 1.0 per cent to the perfusion fluid and passed through both the arterial and venous circulations of the frog's kidney, it does not appear in the urine so long as the urine is normal. If the tubules are damaged by a toxic substance and the typical results of such damage as described above appear, the urine may still remain free of gum when tested with a concentrated solution of picric acid. If, however, the toxic sub-

stance is introduced into the arteries and thus reaches the glomeruli in high concentration, a trace of precipitate increasing to a light or

TABLE III

Dissociation of the Tubular Functions

	Arterial flow	Venous flow	Urine flow	Neutral red	Salt	Sugar
			Potassium bichromate			
			Secretion of neutral red and absorption of water, normal: absorption of sugar, abnormal			
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*	
10:30–10:45	360	630	4.0	0.52	—	0
10:45–10:48		15 cc. 1/3000 potassium bichromate to tubules				
10:50–11:05	440	100	4.0	0.60	—	Tr.
11:05–11:20	400	560	4.4	0.62	—	+
			Potassium bichromate			
			Secretion of neutral red, normal: absorption of water and sugar impaired			
10:30–10:45	600	600	4.4	7.7	—	0
10:45–10:48		15 cc. 1/4000 potassium bichromate to tubules				
11:00–11:45	600	640	7.2	7.9	—	0
11:15–11:30	520	500	6.0	6.0	—	+
			Urethane			
			Permanent failure of neutral red secretion: transient failure of water and sugar absorption with recovery			
10:45–11:00	320	600	3.2	2.7	—	0
11:00		0.5 per cent urethane to tubules through following periods				
11:00–11:15	320	280	2.4	0.40	—	+
11:15–11:30	320	440	8.0	1.7	—	+
11:30			Urethane to tubules stopped			
11:20–11:45	320	380	6.4	1.6	—	+
12:00–12:15	240	240	4.0	0.8	—	Tr.
12:30–12:45	280	240	3.2	0.5	—	Ft. tr.
			Phloridzin			
			Failure of neutral red secretion: failure of sugar absorption, maintenance of water and salt absorption			
11:00–11:15	360	440	4.4	8.8	15.4	0
11:15–11:18		15 cc. 1/15,000 phloridzin to tubule				
11:30–11:45	360	280	3.2	1.2	11.2	+
11:45–12:00	280	360	4.4	1.1	17.6	+

heavy cloud promptly develops. Table IV shows the results of an experiment where paraphenylendiamine was administered first to the

tubules and then to the glomeruli by way of the arteries after the perfused kidney had established an output of normal urine.

As we have shown in another place the excretion of such colloidal dyes as brilliant red and trypan blue is also altered by damage to the glomerular membrane (8). The former does not pass through the normal membrane, but appears in the urine if this structure is damaged, while the latter alters the color of the urine, in which it normally appears in small amount, not only quantitatively but also qualitatively as the damage and consequent permeability of the membrane increases.

TABLE IV

Evidences of Increased Filtration

	Arterial flow	Venous flow	Urine volume	Phenol red	Salt	Sugar	Gum
	Paraphenylendiamine						
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*		
11:15–11:30	300	480	4.2	0.31	21.0	0	0
11:30–11:35	10 cc. 0.8 per cent paraphenylendiamine to tubules						
11:45–12:00	360	300	4.5	0.27	27.0	0	0
12:00–12:15	360	300	6.3	0.36	37.0	0	0
12:15–12:18	10 cc. 0.8 per cent paraphenylendiamine to glomeruli						
12:30–12:45	150	240	3.6	0.29	21.6	0	Tr.
12:15–1:00	220	480	4.2	0.22	25.2	0	++

It would seem likely that if the glomerular membrane becomes more permeable to substances in the perfusion fluid it should under these conditions allow an increased passage of water. A direct determination of this point is however difficult, for a similar end result, that is an increase in the volume of the urine, is obtained when the tubular absorptive activity is depressed. As we shall show later a combined damage to both parts of the renal unit sometimes follows the introduction of the toxic agent into either one of the renal circulations, and special means not always possible in an experiment designed for some specific purpose may be necessary to discover that more than one element of the kidney is affected. The experiment we shall describe is therefore not as direct a demonstration of increased permeability to water following membrane damage

as those which demonstrated its increased permeability for other constituents of the urine.

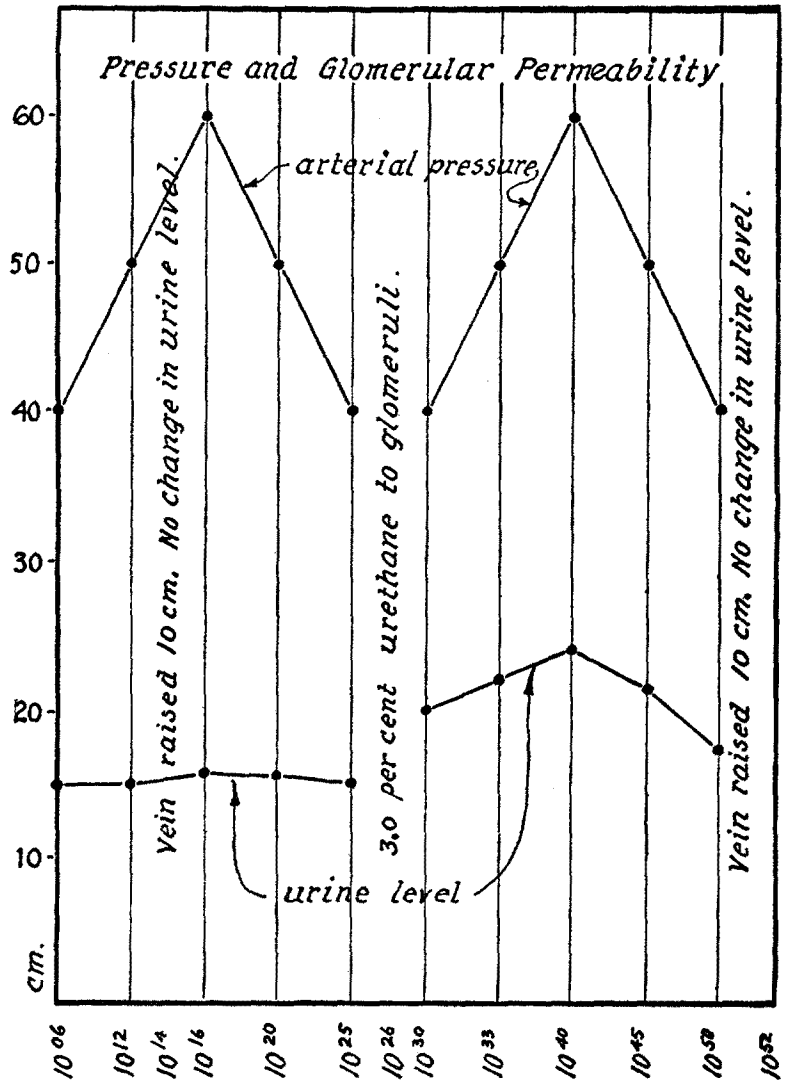

CHART 2

If a tube is placed in the ureter of one kidney and so fixed that it stands perpendicular the urine as it is formed by the perfusion will

rise to a certain height and then remain stationary. That this height depends in part at least on the height of the pressure head of the arterial perfusion fluid is evident from the fact that if the level of the arterial perfusion column is further raised, there is an accompanying rise in the level of the urine in the tube. A point is reached, however, when further elevations of the perfusion pressure, if moderate, produce little effect on the height of the urine. If the pressure is raised beyond this point to an extreme degree rupture of the vessels results and perfusion fluid as such flows through the kidney tubules into the upright tube. The following experiment shows the use of these points in our problem.

After normal urine formation had been established by the perfusion a glass tube was placed as described above in the ureter of the right kidney. The height of the arterial perfusion bottle was 40 cm. The urine rose slowly in the tube until it reached a height of 15 cm. and then remained stationary. The arterial bottle was then raised 10 cm. to a height of 50 cm. No change occurred in the level of the urine. Raising the level of the venous supply 10 cm. also produced no change. The arterial pressure was now raised to 60 cm. and a rise of 7 mm. in the urine was noted. It was then lowered to 50 cm., the urine falling 2 mm., and finally it was replaced at its original height of 40 cm., the urine level being now 15.2 cm.

3 per cent urethane was now added to the perfusion fluid which was passing through the arterial circulation. The catheter tube was emptied and allowed to fill again. The urine, with the arterial pressure level at 40 cm. now rose to 20 cm. when the pressure was increased to 50 cm. the urine rose 20 mm., and when raised to 60 cm. the urine level was increased 40 mm. over its original figure. As the arterial bottle was lowered to 50 and then to 40 cm., the urine level fell 25 and 60 mm. from its maximum height. At this point the venous pressure was increased 10 cm. with no effect on the level of the urine.

These results showing the reaction of a damaged glomerular membrane to the passage of fluid as affected by pressure are illustrated in Chart 2.

Such experiments are interpreted to mean that a damaged glomerular membrane allows the passage of fluid more readily when the pressure upon it is changed than does a normal one which offers a certain resistance to moderate variations in pressure. The fact that an increase in the venous pressure produces no change in the amount of urine secreted also adds evidence to the theory that water under abnormal conditions does not enter the lumen of the tubules in significant amount through the damaged tubular wall.

(*b*) *Evidence of Decreased Filtration*

Decreased filtration may be the result of at least two mechanisms. The amount of fluid available for filtration may be decreased and, by what amounts to the same thing, there may be a decrease in the number of filters in action. A decrease in the pressure head of the fluid may in a similar way lead to the same result. Or the membrane of the filters may become more dense and even with pressure or amount of fluid remaining constant there will result a decrease in the amount of filtrate.

The first mechanism is definitely demonstrated in our basic experiment as a result of restriction of arterial supply. Doubtless both fall of pressure and decrease in fluid were factors in its production, and the same mechanism, a result of arterial spasm, may be observed when toxic agents are introduced into the arterial circulation. Table V illustrates such an experiment. The perfused kidneys were functioning normally and flow through the arterial and venous circulations was moderate yet adequate and constant from period to period of the experiment. Phenol red was excreted in moderate amount. 15 cc. of 1/7500 sublimate were slowly introduced through the arterial circulation to the glomeruli. There resulted a gradual drop in the flow through the arterial circulation, from 440 cc. per hour to 140 cc. per hour. Concomitantly there occurred a marked fall in the volume of urine and a consequent decrease in the rate of excretion of phenol red and salts. This decrease in urine continued until in the fifth period anuria was approached.

Though it has no bearing on the question of glomerular dysfunction we mention in passing the effects of spasm in the venous circulation of the perfused kidney. Its result may be predicted from the findings of our basic experiment in which the restriction of the venous circulation was a simple mechanical one. The contrast of the effects of spasm in the venous system that may follow the introduction of a drug into its circulation with the results of the analogous condition in the arterial system is shown in the second experiment of Table V. The perfused kidney was excreting a normal amount of normal urine. 10 cc. of 1/4000 potassium bichromate was introduced into the vein. There resulted a marked reduction in flow through the venous sys-

tem, but the volume of urine increased and sugar appeared in the urine. It is of course, impossible in such an experiment to estimate how much of the failure of absorption of water and sugar was due to vascular constriction and how much was the result of damage to the tubule cells from the direct toxic action of the drug.

As previous experiments have shown, glomeruli which have been subjected to the action of such a substance as sublimate become more permeable to the passage of fluid and when examined histologically are found to be definitely damaged. The significance of the coexistence of an increased permeability and decreased volume of urine from vascular spasm will be considered in the discussion.

TABLE V

Evidences of Decreased Filtration

	Arterial flow	Venous flow	Volume urine	Phenol red	Salt	Sugar
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*		
1:00–1:15	440	120	12.0	0.36	72.0	—
1:15–1:18		15 cc. 1/7500 sublimate to glomeruli				
1:30–1:45	300	120	7.6	0.23	56.0	—
1:45–2:00	300	120	4.8	0.19	33.6	—
2:15–2:30	200	120	0.2	—	1.4	—
2:30–2:45	140	120	0.1	0.003	0.7	—
			Effect of venous spasm			
10:40–10:55	400	400	4.8	—	—	0
10:55–11:00		10 cc. 1/4000 potassium bichromate to tubules				
11:00–11:15	240	40	4.8	—	—	Tr.
11:15–11:30	280	80	7.2	—	—	+

The other possible cause of decreased filtration, that is an increase in density of the glomerular membrane which may lessen the filtrate, can be only indirectly and perhaps not definitely demonstrated. In a certain number of cases after the toxic substance has passed to the glomeruli a decrease in the volume of urine occurred sometimes even to the establishment of an anuria without any lessening of flow through the glomerular circulation. Table VI gives an example of such an experiment.

It will be observed that although 340 cc. per hour of perfusion fluid

flowed through the arterial circulation only 0.5 cc. per hour of urine was formed. Since by far the greater amount of this perfusion fluid must have passed through the glomerular capillaries the conclusion is possible that fluid did not filter through the glomerular membrane. Another possible explanation will however be mentioned in the discussion.

The Demonstration of Combined Tubular and Glomerular Damage

We have already mentioned the possibility of damage to both elements of the kidney unit even when the toxic agent is introduced with care into only one of the circulations. This is possible not only by communicating channels that exist between the two circulatory

TABLE VI

Decreased Filtration with Adequate Arterial Supply

	Arterial flow	Venous flow	Urine volume	Phenol red	Salt	Sugar
	Corrosive sublimate					
	cc. per hr.	*cc. per hr.*	*cc. per hr.*	*mg. per hr.*	*mg. per hr.*	
1:45–2:00	360	200	6.0	0.12	42.0	0
2:00–2:05		15 cc.1/15,000 sublimate to glomeruli				
2:15–2:30	280	200	4.0	0.08	24.0	+
3:00–3:15	240	280	1.6	0.03	11.0	+
3:45–4:00	340	200	0.5	0.01	3.0	—

beds and by diffusion directly through the tissues but since in most cases the toxic substance is a diffusible one it may pass through the glomerular membrane along with the other constituents of the urine into the tubular lumen and thus come in contact with the tubular cells. As a result we have found it very difficult to damage the glomerular membrane without producing some lesion in the tubular epithelium.

The importance of these facts becomes evident in such an experiment as follows. Sublimate is introduced in low concentration into the arterial system, no arterial spasm results, and there follows a diuresis with increased phenol red and salt excretion. Sugar is present in small amount, perhaps only in traces. The question now arises if all these phenomena may be the result of a marked increase in the

filtrate through the damaged and therefore more permeable membrane, the amount of the filtrate being too great for the absorptive ability of an intact tubular epithelium, or whether the excess water, salt, phenol red, and sugar are in part if not wholly due to an associated tubular damage. Since our ultimate problem will be to test the function of kidneys damaged *in vivo* by agents which have had access to all parts of the renal unit it is obvious that some method must be found which will disclose evidence of tubular damage when it is masked by the results of glomerular lesions.

This may be accomplished if the amount of filtrate is reduced by mechanically restricting the flow through the arterial circulation and observing if tubular absorption is then able with a small amount of filtrate, to concentrate the urine. Chart 3 shows an experiment which illustrates the method.

Normal urine formation was established by the perfused kidney. After the two 15 minute periods the volume was normal, 7.2 cc. per hour, phenol red was excreted at a normal rate and well concentrated and no sugar was present. At the end of the second period the arterial flow was reduced to about one-third its original amount. The volume of urine decreased about one-half and the concentration of phenol red rose, though the rate of elimination was definitely decreased. There was therefore evidence of efficient tubular activity. The reestablishment of the arterial supply increased the rate of phenol red excretion and produced the original conditions of normal concentration and volume. At the end of the fourth period the tubules were damaged by the passage of 12 cc. of 1/10,000 sublimate dissolved in Locke's solution through the venous circulation. During the next two periods there developed typical functional evidence of tubular damage, a diuresis with the appearance of sugar in the urine and though the concentration of phenol red fell sharply, the rate of its elimination remained fairly constant. The arterial supply was now restricted as before, and the volume of urine decreased to one-fourth its former figure. The concentration of phenol red did not rise however as previously noted but actually decreased somewhat.

An interpretation of these results to coincide with the theory stated above is possible. Intact tubules are able to concentrate phenol red more efficiently when the volume of glomerular fluid is lessened, but damaged tubules are unable to raise its concentration even under these optimal conditions.

Beside such combinations of glomerular and tubular damage many other combinations of lesions may be found in a series of experiments

of the sort that we have been describing. Very commonly vascular spasm complicates the action of either glomerulus or tubule, as for example, when after the introduction of sublimate through the ar-

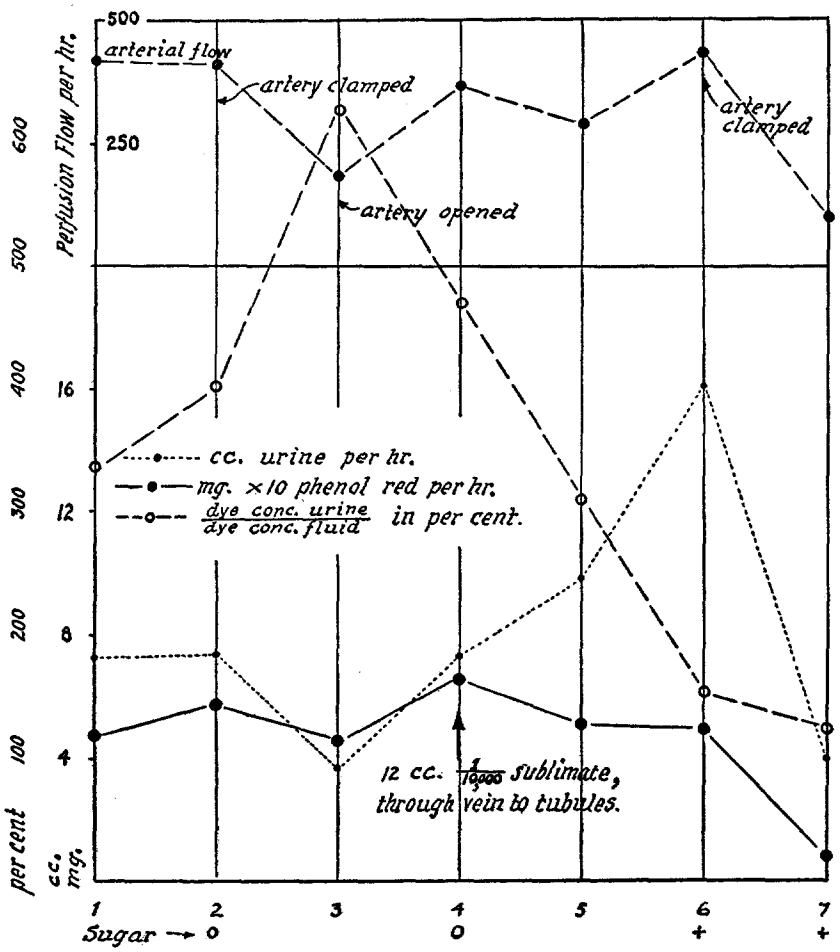

CHART 3

teries, decreased filtration, a result of arterial spasm, prevents a diuresis from the concomitant tubular lesion. Economy of presentation prevents a description of all the reactions we have observed and since in most cases they constitute variations of the simpler ones we

have described and may be recognized as such, a brief consideration of them is reserved for the discussion of our experimental findings.

DISCUSSION

Although perhaps no demonstration of the complexity of the reaction of the kidney to the introduction of a toxic agent is needed, the experiments we have described have at least the virtue of pointing out a few definite reasons for some of the complications. Any analysis, even if imperfect, is to be preferred to the uncertainty of our present knowledge as to why this organ is so peculiarly variable in its functional and anatomical responses to pathological conditions, for in just this feature lie the difficulties of the problems of experimental nephritis and Bright's disease in man.

With the perfused kidney of the frog the irritant may be introduced into one of two circulations and its action directed towards either glomerulus or tubule. But even under these controlled conditions the possibilities as to what may happen are enormous. There are anastomoses between the two systems the importance of which we have previously emphasized (5). The toxic substance may diffuse directly through the tissues or it may be excreted through the glomerulus and so reach the tubule. In this way its action may or may not be confined to the locality towards which it was directed. As a result any one or all of the following reactions may occur.

1. Arterial spasm.
2. Arterial dilatation.
3. Venous spasm.
4. Venous dilatation.
5. Increase in glomerular permeability.
6. Decrease in glomerular permeability.
7. Depression of tubular secretion.
8. Depression of tubular absorption.

And, based on the experiments of Richards (9) which will be mentioned later:

9. Increase in tubular absorption.

There are therefore 511 immediate possible combinations with which the functional response of the kidney may begin, and as the reaction proceeds so that permutations of the factors can occur a tremendous number of possibilities develop for the further course of

kidney's response. Actually, however, the situation does not prove as hopeless as our analysis would seem to indicate. In the majority of experiments response is determined by the experimental procedure. The toxic agent is confined in its action to the mechanism towards which it was directed and departure from this typical result can in many cases at least be detected, as we have shown, by proper methods of testing. However, our experiments are admittedly incomplete in many regards. For example, the experiment of Table VI, in which a repression of urine was found associated with a good flow through the glomerular capillaries, was interpreted to demonstrate the possibility of an increase in the density of the glomerular membrane as a cause of a lessened filtration of water. Richards (9) by direct visual observation of the kidney of the living frog after poisoning with sublimate has observed that the glomerular circulation may be active although no urine is being formed. The anuria he explains as due to an increased absorption of water by the damaged and therefore more permeable epithelium. Our experiments with a damaged tubular epithelium, and those of the following paper which show that this damage is a complete and structural one, have, in the absence of complicating vascular spasm, been accompanied by failure of rather than by increase of absorption, so that we have preferred the explanation we have given. This is only one of the many points that must be further examined, and it is therefore not so much the detailed findings of our experiments that we would emphasize as the importance of their method.

We believe it would be premature to draw any conclusions as yet in regard to the theory of disturbed kidney function even in the circumscribed field of experimental nephritis. This may be left until we have examined the function of kidneys from nephritic frogs. This has been done by the methods we have described in this study and will be reported later. Certain points deserve emphasis however and may be mentioned now, for they bear on general problems of interpretation of histological appearance in abnormal kidneys no matter what the cause of the abnormality may be. The literature of Bright's disease is full of assumptions as to the effect of damage to one part of the kidney or the other that have had hitherto neither support nor criticism except such as was logically convenient to the development of theory.

The functional variations in the glomerulus after toxic damage that we have demonstrated illustrate how useless it is to attempt any correlation from simple histological appearance. Such a glomerulus may be histologically plainly disrupted and so offer no barrier at all to the passage of fluid into the urine. Yet this increased permeability of its membrane may produce no increase in the volume of excreted water or other constituents of the urine, for vascular spasm, a concomitant response of the artery to the toxic agent, may reduce the supply of material available for excretion.

The mechanism by which tubular damage is intimately associated with the glomerular lesion is also illustrated. Any diffusible toxic substance that passes through the glomerular membrane into the tubular lumen comes in contact with the tubular epithelium and damage may result. We have found it therefore difficult to damage only the glomerulus with any of the substances we have used. Such a lesion of the tubule is however in no sense dependent or "secondary" to the glomerular lesion, but is part of the response of the kidney to the direct action of the toxin which has reached it by this circuitous route. There comes to mind the *nephrotische Einschlag* so common in "glomerular nephritis" and which on no very definite evidence, is considered by some to be a secondary and unimportant aspect of the kidney lesion in this form of Bright's disease.

Further evidence of the perils of deduction of function from morphology in our present state of knowledge is seen in our demonstration that the damaged tubule may absorb and yet fail to secrete or that the converse condition may exist. Until this paradox is solved, either by the localization of the two processes in different parts of the tubule or by some other explanation, a complete correlation of the histological and functional aspects of the kidney's response remains impossible.

SUMMARY AND CONCLUSIONS

1. A method of testing the frog's kidney by means of perfusion is described.

2. This is made possible by dissociating, as far as possible, from the total function of the organ the functions of its constituent parts.

3. The characteristics by which tubular, glomerular, and combined tubular-glomerular lesions may be recognized are described.

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