SECRETION BY THE RENAL TUBULES IN THE FROG

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(Received April 27th, 1906)

INTRODUCTION

In spite of the large amount of experimental work which has been performed in order to elucidate the function of the renal tubules, it cannot be said that any experiment has yet shown, beyond all question, either that they can secrete or that they can absorb, although it is clear that they must do one or both of these. Nussbaum's experiments still seem to offer the only crucial method of testing the truth of the Bowman-Heidenhain view.

Halsey has fully confirmed and extended Nussbaum's results. He found in ligatured frogs that urea and indigo-carmin were secreted, whereas dextrose, egg-albumin, peptone, and carmine were not. He confirmed the statements of Mosberg and Marcuse that under the influence of phloridzin dextrose is secreted, and showed that the same was true when diuretin was injected simultaneously with dextrose. He found sodium chloride, sodium phosphate, and sodium sulphate regularly present in the urine after they had been injected. These experiments do not prove conclusively that the tubules can secrete. For a microscopical examination of the kidneys showed in every case, without exception, a greater or less number of glomeruli still in circulation.

Owing to the importance of the subject it seemed necessary to perform further experiments of the same kind, with the object of deciding whether or not results similar to those of Nussbaum and Halsey could be obtained when all the glomeruli were out of circulation.

One of us (A.P.B.) has previously confirmed Nussbaum's statement that complete ligation of the arteries supplying the kidneys cut all the glomeruli permanently out of the circulation. Nussbaum's statement that injections of urea into a fully ligated frog cause a secretion of urine was not confirmed, at any rate when the injections were begun as late as two days after the ligation. It was also found that cutting off the arterial blood supply of the kidneys caused the epithelium of the tubules to degenerate rapidly, presumably owing to a lack of a sufficient supply of oxygen. The failure of urea to cause a secretion of urine might have been due either to the absence of glomeruli in circulation, or to the degeneration of the epithelium.

The present experiments deal firstly with the injection of urea beginning directly after the ligation; the result of this was negative. Secondly, they deal with the questions whether by keeping ligated frogs in oxygen the degeneration of the epithelium can be prevented,¹ and a secretion of urine then obtained; both of these points are answered in the affirmative.

METHOD OF EXPERIMENT

The method of operation was essentially the same as that used previously. The frogs were always anaesthetised with the A.C.E. mixture throughout the operation. Before the operation was begun, the bladder was emptied with a catheter, and the bladder was seen to be empty during the operation. The kidneys were reached through a ventral incision. All the branches given off from the aorta between its bifurcation and the origin of the coeliaco-mesenteric artery were divided by means of the cautery; the coeliaco-mesenteric artery was exposed in its course from the aorta to the spleen, and all vessels given off from it tailwards were divided. The whole operation lasted about half an hour. Male specimens of *Rana temporaria* alone were used, and in all cases the testes and fat bodies were removed.

1. Miss Cullis, *Journ. of Physiol.* (1906), xxxiv, p. 250, states that Brodie suggested the use of oxygen in November, 1905, and that we have carried his suggestion into effect. We wish to point out that our experiments were begun early in October, 1905. Further, in Starling's *Elements of Human Physiology*, 5th edition (1902), p. 451, at the end of the account of Beddard's results this statement is made:—'It is evident that some means must be devised of repeating these experiments while ensuring an adequate supply of oxygen to the tubular epithelium.'

At the end of the operation, the anus was tied and a solution of urea, etc., was injected. All injections were made either into the dorsal lymph sac, or under the skin of the thigh. The frogs were placed with water under a large bell-jar filled either with air or with nearly pure oxygen under atmospheric pressure.

The experiments were carried out during the winter months. The frogs were not fed, and were kept in a very cool place. Throughout the course of an experiment the anus remained ligatured, except when the bladder was being catheterised. At the end of the experiment the frog was pithed and the vascular system was well washed out from the aorta with normal salt solution, and then thoroughly injected with a saturated solution of soluble Prussian Blue. Serial sections of each pair of kidneys were cut out of paraffin.

RESULTS

Three frogs, after the operation, were injected with urea and kept in air (Experiments 1-3). Each received one or two subsequent injections of urea ; they secreted no urine, and at the end of three days were killed. In their kidneys no glomeruli were injected, and the epithelium of the tubules had undergone degeneration. It would be surprising if epithelium, undergoing such a profound change within three days, were capable of secreting at any period after the ligature. If however, as in Halsey's experiments, the ligature was incomplete, urine could be produced not only by the glomeruli in circulation, but also by some of the tubules. For it has been shown previously that the epithelial degeneration does not take place in those portions of a kidney still supplied with arterial blood.

We conclude, therefore, that the positive results obtained by Nussbaum and Halsey were possible only because their ligature was never complete. Whilst Halsey admits that the ligature in his experiments was incomplete, he adds that the number of glomeruli in circulation was too small to be of physiological importance. Nussbaum did not inject the vascular system of the frogs at the end of his experiments, and there is, consequently, no evidence that his ligature was ever complete when he obtained a secretion of urine.

In all the frogs, twelve in number, which were kept in oxygen after the operation, and in which the ligature was found to be complete, the epithelium of the tubules was microscopically normal. It seems clear, therefore, that the degeneration observed in the kidneys of the frogs kept in air is due to a diminished supply of oxygen to the renal epithelium.

In six of these twelve frogs fluid was found in the bladder after injections of urea, alone or in combination with dextrose, phloridzin or Na_2HPO_4 (Experiments 4-9). In the other six no fluid, or at most a few drops was found in the bladder. (Experiments 10-15). In one of these normal salt solution alone had been injected, in another urea and Na_2HPO_4 , and in four urea and phloridzin.

Before concluding that the six positive results were due to a secretion of urine by the renal tubules, it is necessary to show that the fluid could have reached the bladder by no other route than the ureters. The other possibilities are the anus, the rectum, and secretion by the walls of the bladder itself. The first is excluded by the continuous ligature of the anus, and the other two would seem to be excluded by our six negative results. But in order to exclude definitely the possibility of secretion by the walls of the bladder, two other experiments were performed. (Experiments 16-17). In these two frogs both ureters, the anus and the rectum above the bladder were ligatured and injections of urea, or urea in combination with Na_2HPO_4 or phloridzin were given. In neither case was any fluid found in the bladder. The possibility that fluid in the bladder could have been derived from the rectum is excluded by the following considerations:—In no case, *post mortem*, was the rectum found distended with fluid; the drop or two of fluid in the rectum is brown and contains brown flakes, whilst that in the bladder is clear and almost colourless. Further, in two of the positive results (Experiments 8 and 9) the rectum had been tied above the bladder at the operation. This was done because it was found that injections of Na_2HPO_4 and phloridzin sometimes set up slight diarrhoea, so that in passing the catheter into the bladder the eye might become fouled with a few brown flakes which might interfere with determinations of the acidity of the urine.

SECRETION BY THE RENAL TUBULES IN THE FROG 259

We conclude, therefore, that the fluid obtained from the bladder was urine secreted by the renal tubules, and that secretion of urine can be obtained in fully ligatured frogs, provided they have been kept in oxygen.

No. of Experiment	Material injected			Quantity of urine in twenty-four hours	Acidity	Urine contained
4	Urea	...	...	$\begin{Bmatrix} a & 0.5 \text{ c.c.} \\ b & 1.0 \text{ c.c.} \end{Bmatrix}$	...	Urea and salts
5	"	...	...	1.0 c.c.	...	" "
6	"	...	...	0.5 c.c.	1.9	" "
7	Urea and dextrose			0.5 c.c.	...	Dextrose, urea and salts
8	"	phloridzin		0.5 c.c.	2.0	" " "
9	"	Na_2HPO_4		$\begin{Bmatrix} a & 0.5 \text{ c.c.} \\ b & 0.25 \text{ c.c.} \end{Bmatrix}$	$\begin{Bmatrix} a & 2.4 \\ b & 2.5 \end{Bmatrix}$	Urea and salts

The table shews the quantity and the composition of the urine secreted by the six fully ligatured frogs. None of them secreted during the first day after ligature, and this is not surprising considering the severity of the operation. Incompletely ligatured frogs, however, may secrete urine within twenty-four hours of the operation. (Experiments 21 and 22). The greatest quantity of urine secreted in a day by a fully ligatured frog was 1 c.c. Observations on normal frogs of the same size kept under the same conditions showed that they might produce as little as 0.25 c.c., and not more than about 1 c.c. in a day. When, however, the normal frogs had received injections of urea, etc., the quantity of urine was four or more times greater than that produced by ligatured frogs after the same injections. This difference may be amply accounted for by the absence of glomeruli in the one case. For whatever difference of opinion there may be as to how the glomerulus works, a consideration of its structure hardly leaves room for doubt that it is a mechanism which can pass out large quantities of water.

The urine secreted by the ligatured frogs contained, in all cases, urea. This was recognised by the production of urea nitrate crystals. It contained also chlorides and sulphates, which were demonstrated by the ordinary qualitative tests. No quantitative estimations of these constituents could be made in the small quantities of urine available.

Urates and phosphates never could be demonstrated even after the injection of Na_2HPO_4 , and the same was true of similar quantities of urine obtained from normal frogs. The urine, like that of normal frogs, gave a reddish colour with a saturated solution of picric acid and 10 per cent. NaOH similar to that given by kreatinin in solution. But no attempt was made to demonstrate conclusively the presence of kreatinin.

Five fully ligatured frogs had received injections of phloridzin and urea. In one only (Experiment 8) was a secretion of urine obtained. This urine gave a red colour with a solution of ferric chloride similar to that given by the original phloridzin solution, and reduced Fehling's solution readily. No attempt could be made to identify the reducing substance present, but it was considered to be dextrose for the following reasons:—It is recognised that injections of phloridzin produce glycosuria, and it was found that the urine of neither ligatured nor unligatured frogs ever contained a substance which reduced Fehling's solution unless phloridzin or dextrose had been injected.

This experiment shows that phloridzin can produce glycosuria by acting on the renal tubules. It confirms the conclusions reached by Mosberg, Marcuse, and Halsey as the result of similar experiments. It cannot, however, be held that any of these previous experiments have proved the point. For Halsey admits the presence of glomeruli in circulation. And Mosberg in only one of his experiments injected carmine in order to see that his ligature had been complete, and in that experiment he found glomeruli in circulation. He quotes two experiments with phloridzin performed by Marcuse in which also no injection of the vascular system was made.

Although it has been shown that phloridzin can produce glycosuria by an action on the renal tubules, it does not follow that in normal animals there is no action also upon the glomeruli. The fact that it was found difficult in fully ligatured frogs to obtain a secretion of urine after phloridzin, although secretion occurred readily in unligatured ones (Experiment 20), is open to two interpretations. It would be possible to believe that phloridzin acts upon the glomeruli as well

as on the tubules. It might equally well be that the tubule epithelium of ligatured frogs kept in oxygen, although normal in appearance, is not sufficiently normal in function to react to phloridzin or that the epithelium is not in condition to withstand the deleterious action of phloridzin. These experiments seem to favour the latter supposition. For it was found much more easy to obtain a secretion of urine when urea had been injected alone than when the same dose of urea had phloridzin added to it.

Dextrose together with urea was injected into two frogs. In one the ligature was slightly incomplete (Experiment 22), but in the other it was complete (Experiment 7). Both secreted urine which reduced Fehling's solution readily. The injection of 0.1 gram dextrose into a frog of this size leads to hyperglycaemia. For it was found that when this dose had been injected into a normal frog the urine of the next twenty-four hours contained 0.06 gram and for another day reduced Fehling's solution slightly (Experiment 19). We conclude that in a condition of hyperglycaemia the renal tubules can secrete dextrose.

This conclusion is opposed to the results obtained by Nussbaum, Mosberg, and Halsey. It is only in Halsey's experiments that there is any information about the completeness of the ligature. If, as he believes, dextrose is passed out by the glomeruli alone, and in all his experiments glomeruli were in circulation, it is difficult to understand why he never found dextrose in the urine, except when he had given diuretin at the same time. And the same is true of Nussbaum's and Mosberg's experiments. Although in these there is no microscopical examination of the kidneys to show that glomeruli were in circulation, we infer that such must have been the case, otherwise their frogs kept in air would not have secreted urine at all.

Some observations were made upon the acidity to phenolphthalein of the urine of ligatured and unligatured frogs. A method of estimation suitable to the small quantities of urine available had to be employed; and the following was found to give concordant results. Urine was sucked up to the mark 1 on a Thoma-Zeiss white blood corpuscle haemocytometer. The stem of this instrument below the

bulb is divided into tenths up to 1. This volume of urine was blown into a watch-glass and .05 of the same volume of a weak solution of phenolphthalein was added. With the same pipette measured quantities of $\frac{N}{100}$ NaOH were added, the mixture was stirred with a fine glass rod after each addition and held over white paper in a good light. The first tinge of pink in the fluid was taken at the end point. The urine of frogs is almost colourless; consequently the end point of the reaction is not obscured by the colour of the fluid. The acidity of the urine is expressed in vols. of $\frac{N}{100}$ NaOH necessary to make 1 volume of urine just alkaline to phenolphthalein.

The acidity of the urine of normal frogs was found to vary widely. Thus one frog (Experiment 18) passed 0.25 c.c. urine with an acidity of 2.3, whilst in another (Experiment 9) the acidity approximated to that of the blood to phenolphthalein, in that the slightest addition of alkali made the urine alkaline. Between these two extremes intermediate figures were obtained in other cases. The most frequent figures were about and generally less than 1. The acidity was influenced in the usual way by the injection of drugs. Thus in Experiment 18 urea injections reduced the acidity from 0.6 to 0.2, and the injection of Na_2HPO_4 raised it to 1.5.

Four estimations were made of the acidity of the urine of fully ligatured frogs. In Experiment 6 after injections of urea the acidity was 1.9, and in Experiment 8 after injections of phloridzin and urea it was 2.0. In Experiment 9 the urine before the operation was only just acid to phenolphthalein, but after ligature and the injection of Na_2HPO_4 and urea it had an acidity of 2.4 and 2.5 on successive days.

We conclude from these results that the tubules can secrete a fluid which is more acid to phenolphthalein than the blood or than the urine turned out by the glomeruli during a glomerular diuresis. Our results confirm those of Dreser. He showed that by injecting acid fuchsin into normal frogs the glomeruli remained uncoloured, and that the fluid and cells of the tubules were red and therefore acid to this indicator. It must be remembered that the uncoloured condition of the glomeruli might be due, when the secretion of urine was small, just as much to the absence of secretion by the glomeruli as to

the secretion by them of an alkaline fluid. When acid fuchsin was injected into ligatured frogs the red colour was still seen in the tubules. From this Dreser concluded that the difference in reaction between blood and urine was due to the secretion of acid radicles by the tubules and not to the absorption of bases from a glomerular filtrate. The only source of fallacy in Dreser's experiments is the possibility that his ligature was not complete. He did not inject the vascular system at the end of an experiment, and as he used a dorsal method of ligature similar to that used by Adami it is certain—as has been shewn by one of us—that his ligature was incomplete. His control experiments in which he tested his method of ligature by injecting vermilion into the anterior abdominal vein, never showed injected glomeruli. This must mean that his method of injection was unable to show with certainty when glomeruli were in circulation.

Our results, like those of Dreser, are not compatible with the view put forward by Cushny, namely, that the normal difference between the reaction of blood and urine is brought about, not by the addition of HPO_4 anions to a glomerular filtrate, but solely by the absorption from that filtrate of Na^+ kations in combination with OH^- or HCO_3^- . These experiments on ligatured frogs do not prove that absorption may not play a part, but only that it is not the sole mechanism at work.

It seems at first sight a little surprising, if secretion is a normal function of the renal tubules, that this epithelium after ligature should not secrete spontaneously but should need a powerful stimulus in the shape of large quantities of urea. Nussbaum, Adami, and Beddard have all found that frogs kept in air after ligature do not secrete spontaneously; and this is true even when the ligature is moderately incomplete. But in these frogs much of the epithelium has visibly degenerated and probably none of it is strictly normal. The case seems different, however, with frogs kept in oxygen. In Experiment 15, normal salt solution alone was injected in the hope of obtaining a spontaneous secretion. The frog apparently had bled more at the operation than was thought at the time. The result was negative. The attempt to obtain a spontaneous secretion was not persisted in as our primary object was to obtain positive results if possible. But even

in oxygenated frogs the epithelium can hardly be functionally normal, although it appears so microscopically. For in Experiment 14, in which no adverse circumstance such as haemorrhage was present, injections of urea and Na_2HPO_4 failed to produce any secretion of urine. Nor can it be a matter of indifference to the kidney that it should be cut off from its high pressure blood supply.

The frogs in the other four experiments with negative result had received injections of phloridzin as well as of urea. As in two of these (Experiments 10, 11) not more than the very slight unavoidable haemorrhage had taken place at the operation and there had been no post-operative oozing of blood, we believe that the main adverse factor at work was damage done by phloridzin to an already depressed epithelium.

CONCLUSIONS

The experiments which we have carried out seem to prove two facts, namely, (1) that the renal tubules of the frog can secrete ; and (2) that they secrete urea, chlorides and sulphates, and dextrose during hyperglycaemia and after injections of phloridzin. It is possible that they secrete kreatinin and, judged by the reaction of the urine, phosphates—in fact most of the normal urinary constituents. This fully confirms the view of the function of the tubules put forward originally by Bowman and then by Heidenhain.

According to the view of Ludwig, elaborated by Starling, the fluid turned out by the glomeruli is a mechanical filtrate and must be isotonic and identical with the blood plasma except for the absence of proteids. This view entails the further supposition that the main function of the tubules is to absorb water, dextrose, bases, etc., and so to concentrate the glomerular filtrate. In order to explain the production, during some diureses, of urine with Δ very much less than that of the blood plasma, Starling has suggested that the tubules may then secrete an extremely hypotonic fluid. We have found, however, that, when the tubules do secrete in response to urea and water, they turn out a urine which contains besides other crystalloids certainly as much urea as that of normal frogs. Although this observation does not disprove the view that the glomerulus is a mechanical filter, it

renders filtration extremely unlikely, and, therefore, necessitates a belief that the glomerular epithelium secretes. And again, if both the glomeruli and the tubules can secrete, there is no longer any necessity to believe that the tubules also absorb. But, whilst there certainly is no experiment which proves that the tubules can absorb, there is equally none which shows that they are unable to do so. Absorption by the tubules must be left an open question to be proved or disproved by future experiments. It is clear, however, that no view of the function of the renal tubule can be considered adequate and final which does not explain the function of its different parts corresponding to their variations in structure.

Starting from the observed fact that the tubule can secrete and from the suppositions that the glomerulus also secretes and is a mechanism whose most important function is to pass out any excess of fluid, and remembering that the only known difference between the urines secreted by the glomerulus and tubule, is that one is always and the other never acid to acid fuchsin, we would suggest the following working hypothesis of the secretion of urine :—Both the glomerular and tubule epithelia definitely secrete urine, and in all probability both can secrete much the same urinary constituents in solution. At times when the quantity of fluid to be secreted from the blood is comparatively small the urine represents for practical purposes the secretion of the tubules, because the epithelium covering the glomeruli is insignificant in extent as compared with that of the tubules. When, however, as during saline diureses, large quantities of water and salts have to be passed out, the mechanism which removes rapidly the bulk of this fluid is the glomeruli ; consequently at the height of such a diuresis the urine secreted by the glomeruli very greatly exceeds that being secreted at the same time by the tubules. During saline diuresis the urine approximates more and more to the composition of blood plasma minus proteid, whereby the maximal amount of material is excreted with minimal expenditure of energy by the kidney cells. This power of the glomeruli to get rid of fluid is a true secretion which is generally greatly assisted by increased blood flow through the kidney, but can take place independently of the vascular changes. In

other words the primary factor in such a diuresis is not the vascular change but the state of activity of the glomerular epithelium. If once it is admitted that vascular changes alone cannot set up diuresis by increased glomerular filtration, then it seems necessary to believe with Eckhard that the state of activity of the renal epithelium can be influenced directly by the central nervous system, and that the nerve endings described by Berkeley in the renal epithelium represents the terminations of this nervous supply. Otherwise it seems impossible to explain a diuresis caused by disturbance of the medulla oblongata; for there is no reason to believe that a change in the composition of the blood takes place in such a nervous disorder. It is further probable that all diureses are not solely and entirely of glomerular origin, but that some depend largely upon an increased activity of the tubular epithelium.

REFERENCES

- Adami, *Journ. of Physiol.* (1885), VI, p. 382.
 Beddard, *Journ. of Physiol.* (1902), XXVIII, p. 20.
 Cushny, *Journ. of Physiol.* (1904), XXXI, p. 188.
 Dreser, *Zeitschr. f. Biol.* (1885), XXI, p. 41.
 Halsey, *Amer. Journ. of Physiol.* (1902), VI, p. xvi.
 Mosberg, *Inaug-Dissert. Universit. Würzburg* (1898).
 Nussbaum, *Pflüger's Archiv.* (1878), XVII, p. 580; *Anat. Anzeig.* (1886).
 Starling, *Schafer's Text-Book of Physiol.* (1898), Vol. I, p. 639; *Journ. of Physiol.* (1899), XXIV, p. 317.

DETAILS OF THE EXPERIMENTS

SOLUTIONS USED FOR INJECTION.

- (a) Urea solution = 10 per cent. urea in normal salt solution.
- (b) Dextrose and urea solution = 5 per cent. dextrose and 10 per cent urea in water.
- (c) Phloridzin and urea solution = 5 per cent. phloridzin in the urea solution.
- (d) Na_2HPO_4 solution = 5 per cent. Na_2HPO_4 in water.
- (e) Dextrose solution = 10 per cent. dextrose in water.

EXPERIMENT I

1905

- Oct. 31. Usual operation. Emptied bladder. Injected 1 c.c. urea solution. Placed in air.
- Nov. 2. No urine. Injected 2 c.c. urea solution. Tied anus. Air.
- „ 3. No urine. Washed out and ligatured the vascular system. Microscopically. No injected glomeruli. Epithelium throughout much degenerated.

SECRETION BY THE RENAL TUBULES IN THE FROG 267

EXPERIMENT 2

1905

- Nov. 3. Usual operation. Very little haemorrhage at operation. Emptied bladder. Tied anus. Injected 2 c.c. urea solution. Placed in air.
- „ 4. No urine. Tied anus. Injected 2 c.c. urea solution. Air.
- „ 5. No urine. Tied anus. Injected 2 c.c. urea solution. Air.
- „ 6. 11 a.m. Frog lively. Injected 1 c.c. urea solution. 5 p.m. Pithed frog. *Post-mortem*. Viscera of normal colour and not pale. Two drops of blood-stained urine found in bladder. Washed out and injected the vascular system. Microscopically. No injected glomeruli. Epithelium moderately degenerated throughout.

EXPERIMENT 3

- Nov. 10. Usual operation. Some haemorrhage from one of the renal arteries during the operation. Emptied bladder and tied anus. Injected 1 c.c. urea solution. Placed in air.
- „ 11. Injected 1 c.c. urea solution. Air.
- „ 12. No urine. Tied anus. Injected 1 c.c. urea solution. Air.
- „ 13. No urine. Frog rather feeble. *Post-mortem*, bladder found empty. All viscera very pale. Much blood in the peritoneal cavity. Washed out and injected the vascular system. Microscopically. No injected glomeruli. Epithelium very degenerated throughout.

EXPERIMENT 4

- Nov. 14. Usual operation. Emptied bladder and tied anus. Injected 2 c.c. urea solution. Placed in oxygen.
- „ 15. No urine. Tied anus. Injected 2 c.c. urea solution. Oxygen.
- „ 16. 0.5 c.c. urine in bladder. Tied anus. Injected 2 c.c. urea solution. Oxygen. Urine contained urea, chlorides, and sulphates.
- „ 17. 1.0 c.c. urine, which contained urea, sulphates, and chlorides. Frog fairly lively, but generally oedematous. *Post-mortem*. Viscera not pale. Rectum empty. Washed out and injected vascular system. Microscopically; no injected glomeruli; epithelium normal in appearance.

EXPERIMENT 5

- Nov. 14. Usual operation. Very little haemorrhage at operation. Emptied bladder, and tied anus. Injected 1 c.c. urea solution. Placed in oxygen.
- „ 15. Two drops of blood-stained urine in bladder. Tied anus. Injected 2 c.c. urea solution. Placed in oxygen.
- „ 16. 1.0 c.c. clear urine, containing urea, sulphates, and chlorides. Frog lively. *Post-mortem*. Viscera not pale. Rectum empty. Washed out and injected the vascular system. Microscopically; no injected glomeruli; epithelium normal in appearance.

EXPERIMENT 6

1905

- Nov. 17. Unusual operation. Some haemorrhage at operation. Emptied bladder, and tied anus. Injected 2 c.c. urea solution. Placed in oxygen.
- „ 18. No urine. Tied anus. Injected 2 c.c. urea solution. Oxygen.
- „ 19. No urine. Tied anus. Injected 2 c.c. urea solution. Oxygen.
- „ 20. 0.5 c.c. clear urine. Frog fairly lively. *Post-mortem*. Viscera pale and much blood-stained fluid found in the peritoneal cavity. Rectum empty. Washed out and injected vascular system. Microscopically; no injected glomeruli; epithelium normal in appearance. Urine contained urea, chlorides, and sulphates. Gave a reddish colour with picric acid and caustic soda, similar to that given by solutions containing kreatinin. Phosphates and urates could not be demonstrated. Reaction was about neutral to neutral litmus paper. Acidity to phenolphthalein. 1 vol. urine + 1.9 vol. $\frac{N}{100}$ NaOH was just alkaline to phenolphthalein.

EXPERIMENT 7

- Nov. 28. Usual operation. Very little haemorrhage at operation. Emptied bladder and tied anus. Injected 2 c.c. urea and dextrose solution. Placed in oxygen.
- „ 29. Injected 2 c.c. dextrose and urea solution. Oxygen.
- „ 30. 0.5 c.c. clear urine. Frog appeared moribund. *Post-mortem*. Rectum empty. Viscera were of normal colour. Washed out and injected vascular system; microscopically; no glomeruli injected; epithelium normal in appearance. Urine reduced Fehling's solution readily. It contained urea, chlorides, and sulphates, and was neutral to neutral litmus paper.

EXPERIMENT 8

- Dec. 5. In addition to the usual operation the rectum was ligatured above the bladder. Very little haemorrhage at the operation. Emptied bladder and tied anus. Injected 2 c.c. phloridzin and urea solution. Placed in oxygen.
- „ 6. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- „ 7. 0.5 c.c. clear urine. Tied anus. Injected 2 c.c. phloridzin and urea solution. Frog lively but rather oedematous. Placed in oxygen. Urine reduced Fehling's solution readily, and gave a red colour, with a solution of ferric chloride. It contained urea, sulphates, and chlorides. It gave a red colour with picric acid and caustic soda. Phosphates and urates could not be demonstrated. Acidity to phenolphthalein; 1 vol. urine + 2.0 vols. $\frac{N}{100}$ NaOH was just alkaline.

SECRETION BY THE RENAL TUBULES IN THE FROG 269

1905

- Dec. 8. No urine. Frog moribund. *Post-mortem* viscera not pale. Rectum empty. Frog generally oedematous. Washed out and injected vascular system ; microscopically ; no glomeruli injected ; epithelium normal in appearance.

EXPERIMENT 9

- Dec. 5. In addition to the usual operation the rectum was ligatured above the bladder. Very little haemorrhage at the operation. Emptied bladder and tied anus. Injected 2 c.c. urea solution. Placed in oxygen. Urine drawn off at the time of the operation was alkaline to neutral litmus paper and became alkaline to phenolphthalein on the slightest addition of $\frac{N}{100}$ NaOH.
- „ 6. No urine. Tied anus. Injected 1 c.c. Na_2HPO_4 solution and 1 c.c. urea solution. Oxygen.
- „ 7. 0.5 clear urine. Tied anus. Injected 1 c.c. Na_2HPO_4 solution and 1 c.c. urea solution. Frog lively but rather oedematous. Placed in oxygen. Urine contained urea, chlorides, and sulphates. Gave a red colour with picric acid and caustic soda. It neither reduced Fehling's solution nor gave a reaction with ferric chloride. Phosphates and urates could not be demonstrated in it. Acidity to phenolphthalein ; 1 vol. urine + 2.4 vols. $\frac{N}{100}$ NaOH just alkaline.
- „ 8. 0.25 urine. Frog lively but oedematous. *Post-mortem*. Viscera not pale. Rectum empty. Washed out and injected vascular system. Microscopically ; no glomeruli injected ; epithelium normal. Urine. Acidity to phenolphthalein. 1 vol. urine + 2.5 vols. $\frac{N}{100}$ NaOH just alkaline.

EXPERIMENT 10

- Nov. 18. Usual operation. Very little haemorrhage at the operation. Emptied bladder and tied anus. Injected 2 c.c. phloridzin and urea solution. Placed in oxygen.
- „ 19. No urine. Tied anus. Frog lively. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- „ 20. No urine. Tied anus. Frog lively. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- „ 21. One drop of clear urine drawn off from bladder. Frog lively and not oedematous. *Post-mortem*. Bladder and rectum empty. Viscera not pale. Washed out and injected vascular system. Microscopically ; no glomeruli injected. Epithelium normal in appearance.

EXPERIMENT 11

1905

- Dec. 1. Usual operation. A little arterial haemorrhage at operation. Emptied bladder and tied anus. Injected 2 c.c. phloridzin and urea solution. Placed in oxygen.
- „ 2. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- „ 3. Two drops of urine found in bladder. Frog fairly lively. Tied anus. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- „ 4. No urine. Frog appeared to be moribund and was slightly oedematous. *Post-mortem*. Bladder and rectum empty. Viscera not pale. Washed out and injected vascular system. Microscopically; no glomeruli injected; epithelium normal in appearance.

EXPERIMENT 12

- Nov. 23. Usual operation. Very little haemorrhage at the operation. Emptied bladder and tied anus. Injected 2 c.c. phloridzin and urea solution. Placed in oxygen.
- „ 24. No urine. Tied anus. Injected 2 c.c. urea solution. Oxygen.
- „ 25. Three drops of urine in bladder, which gave no reaction with ferric chloride. Tied anus. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- Nov. 26. One drop of urine in bladder. *Post-mortem*. Much blood-stained fluid in peritoneal cavity. Viscera pale. Bladder and rectum empty. Washed out and injected vascular system. Microscopically; no glomeruli injected; epithelium appeared normal.

EXPERIMENT 13.

- Nov. 24. Usual operation. Some arterial and venous haemorrhage at the operation. Emptied bladder and tied anus. Injected 2 c.c. phloridzin and urea solution. Placed in oxygen.
- „ 26. No urine. Tied anus. Frog lively and oedematous. Injected 2 c.c. phloridzin and urea solution. Oxygen.
- „ 27. No urine. Frog very oedematous. *Post-mortem*. Bladder empty. Viscera rather pale. Washed out and injected vascular system. Microscopically; no glomeruli injected; epithelium appeared normal.

EXPERIMENT 14

- Dec. 9. In addition to the usual operation the rectum was ligatured above the bladder. Very little haemorrhage at operation. Emptied bladder and tied anus. Injected 2 c.c. urea solution. Placed in oxygen.
- „ 10. Injected 2 c.c. urea solution. Frog fairly lively. Oxygen.
- „ 11. No urine. Tied anus. Frog lively. Injected 1 c.c. urea solution and 1 c.c. Na_2HPO_4 solution. Oxygen.

SECRETION BY THE RENAL TUBULES IN THE FROG 271

1905

- Dec. 12. No urine. Frog feeble and rather oedematous. *Post-mortem*. Viscera not pale. Bladder and rectum empty. Washed out and injected vascular system. Microscopically; no glomeruli injected; epithelium appeared normal.

EXPERIMENT 15.

- Dec. 1. Usual operation. Slight venous haemorrhage at operation. Emptied bladder and tied anus. Injected 2 c.c. normal salt solution. Placed in oxygen.
- „ 2. Injected 2 c.c. normal salt solution. Oxygen.
- „ 3. No urine. Tied anus. Frog lively and rather oedematous. Injected 2 c.c. normal salt solution. Oxygen.
- „ 4. Five drops of rather blood-stained urine in bladder, which contained urea. Frog very oedematous. *Post-mortem*. Much blood-stained fluid in peritoneal cavity. Viscera pale. Bladder and rectum empty. Microscopically; no glomeruli injected; epithelium normal in appearance.

EXPERIMENT 16

1906

- March 13. Tied both ureters and the rectum above the bladder and tied anus. Injected 2 c.c. urea solution. Placed in air.
- „ 14. Injected 2 c.c. urea solution.
- „ 15. No urine. *Post-mortem*. Bladder and rectum empty. Ureters and rectum properly ligatured.

EXPERIMENT 17

- March 13. Tied both ureters and the rectum above the bladder. Emptied bladder and tied anus. Injected 2 c.c. urea solution. Placed in air.
- March 14. Injected 2 c.c. urea solution.
- „ 15. Injected 1 c.c. urea solution and 1 c.c. Na_2HPO_4 solution.
- „ 16. Injected 2 c.c. phloridzin and urea solution.
- „ 17. No urine. Frog lively but rather oedematous. *Post-mortem*. Bladder empty. Rectum above colon contained a few drops of a brown fluid with numerous brown flakes in it. Ureters and rectum properly ligatured.

EXPERIMENT 18

- Feb. 9. Normal frog. Emptied bladder and tied anus. Placed in oxygen throughout the experiment.
- „ 10. 0.25 c.c. clear urine. Acidity to phenolphthalein; 1 vol. urine + 2.3 vols. $\frac{N}{100}$ NaOH just alkaline. Injected 1 c.c. Na_2PO_4 solution. Tied anus.

1906

- Feb. 11. 2 c.c. clear urine, which contained urea, chlorides, and sulphates. Phosphates and urates could not be demonstrated. It did not reduce Fehling's solution nor give any reaction with ferric chloride. Acidity to phenolphthalein; 1 vol. urine + 2.9 vols. $\frac{N}{100}$ NaOH just alkaline. No injection. Tied anus.
- „ 12. 1 c.c. clear urine. Acidity to phenolphthalein; 1 vol. urine + 0.6 vol. $\frac{N}{100}$ NaOH just alkaline.
- „ 14. 4.5 clear urine. This did not reduce Fehling's solution nor give a reaction with ferric chloride. Acidity to phenolphthalein; 1 vol. urine + 0.2 vol. $\frac{N}{100}$ NaOH just alkaline. No injection. Tied anus.
- „ 15. 2 c.c. urine. Acidity to phenolphthalein; 1 vol. urine + 0.3 vol. $\frac{N}{100}$ NaOH just alkaline. Injected 1 c.c. Na_2HPO_4 solution. Tied anus.
- „ 16. 1.5 urine, which did not reduce Fehling's solution. Acidity to phenolphthalein; 1 vol. urine + 1.5 vols. $\frac{N}{100}$ NaOH just alkaline. Tied anus.
- „ 18. 0.25 c.c. urine. Acidity to phenolphthalein; 1 vol. urine + 0.4 vol. $\frac{N}{100}$ NaOH just alkaline. Injected 1 c.c. urea solution. Tied anus.
- „ 19. 0.75 c.c. urine. Acidity to phenolphthalein; 1 vol. urine + 0.4 vol. $\frac{N}{100}$ NaOH just alkaline. Injected 1 c.c. Na_2HPO_4 solution. Tied anus.
- „ 20. 3.5 urine. Acidity to phenolphthalein; 1 vol. urine + 0.5 vol. $\frac{N}{100}$ NaOH just alkaline.

EXPERIMENT 19

- Feb. 21. Normal frog. Emptied bladder and tied anus. Placed in air throughout experiment.
- „ 22. 1 c.c. urine which did not reduce Fehling's solution. Injected 1 c.c. dextrose solution. (1 g.). Tied anus.
- „ 23. 2.5 urine, which gave no reaction with ferric chloride. The sugar was estimated in this by Pavy's method. 2.3 per cent. dextrose was found, which in 2.5 urine would amount to about 0.06 g. No injection. Tied anus.
- „ 24. 2 c.c. urine which gave a slight reduction with Fehling's solution.

EXPERIMENT 20

- Feb. 23. Normal frog. Emptied bladder and tied anus. Placed in air throughout experiment.

SECRETION BY THE RENAL TUBULES IN THE FROG 273

1906

Feb. 24. 1 c.c. urine, which neither reduced Fehling's solution nor gave a reaction with ferric chloride. Injected 2 c.c. phloridzin and Tied anus. urea solution.

„ 25. 2.5 c.c. urine which was acid to neutral litmus paper, and gave a red colour with ferric chloride. It contained 0.21 per cent. dextrose estimated by Pavy's method. The injections of phloridzin were continued for a week. The urine contained dextrose throughout. On the fifth day severe diarrhoea was set up.

EXPERIMENT 21

Feb. 7. Usual operation. Very little haemorrhage at operation. Emptied bladder and tied anus. Injected 1 c.c. urea solution. Placed in oxygen.

„ 8. 1 c.c. urine. Frog very sluggish, possibly due to cold weather, possibly to haemorrhage. *Post-mortem*. Much blood in the peritoneal cavity. Viscera pale. Washed out and injected the vascular system. Microscopically ; no glomeruli injected except at the extremities of both kidneys where numerous glomeruli contained the injection ; epithelium normal.

EXPERIMENT 22

Feb. 24. Usual operation. Very little haemorrhage at operation. Emptied bladder and tied anus. Injected 1 c.c. dextrose solution and 1 c.c. urea. Placed in oxygen.

„ 25. 0.5 c.c. urine which reduced Fehling's solution strongly. Tied anus. Injected 1 c.c. dextrose solution and 1 c.c. urea solution. Oxygen.

„ 26. Few drops of urine which reduced Fehling's solution. *Post-mortem*. Viscera not pale. Washed out and injected vascular system. Microscopically ; no glomeruli injected except at the anterior end of both kidneys ; epithelium normal.