THE SECRETION OF URINE. By JOSEPH BARCROFT and HERMANN STRAUB.

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The researches of Schlayer[1], his co-workers and others have made it desirable that some further investigation of the metabolism of the kidney during diuresis, should be made. Schlayer recognises two kinds of diuresis, the mechanism of the one is vascular and the other tubular. The action of the vascular diuretics is maintained after the cells of the tubules have been poisoned with salts of the heavy metals. The original intention of our research was to investigate the vascular diuretics for the purpose of finding out whether, or no, their action was associated with increased metabolism of the kidney, and if so whether such had its seat in the glomerular epithelium.

Heretofore the only diuretics whose action on the mammalian kidney[2] has been studied in this way are urea, sodium sulphate and phloridzin. There is a considerable amount of evidence culled from various organs in the body—striped[3], and cardiac muscle[4], submaxillary[5] and parotid glands[6], pancreas[7], kidney with certain diuretics[8], intestine[9]—which leads us to suppose that activity of the organ is associated with an immediate increase in the oxygen taken up by it, assuming that the oxygen is available, and we have regarded it as a fair assumption that if urine appears without increased oxidation in the kidney itself, the mechanism which accounts for its appearance is a purely physical one, whereas if there is a well marked increase in the metabolism of the kidney a secretory process is called into play.

General method of experiment. Our experiments have been performed on cats and rabbits; dogs proved unsuitable, because the large doses of diuretic necessary were retained in the tissues to such an extent that it was found impossible to free the animal from them. The dissections do not differ materially from those described by

Barcroft and Brodie[2]. The small amount of blood which comes from the cat's kidney (and the still smaller amount from the rabbit's) makes it possible to use the method of collection and of measurement of rate of flow described by one of us for the investigation of the salivary glands[10]. When possible we used but one kidney and in some cases put our venous cannula into the ovarian vein—a very convenient arrangement when this vein is large enough.

The oxygen analyses have been made by the differential method[11]. Blood samples of 1 c.c. or 2 c.c. each have been analysed. The blood has been kept from clotting by the use of a trace of hirudin[12] in the collecting tubes.

The anæsthetic, unless otherwise stated, has been urethane.

Diuresis evoked by Ringer's solution.

In our first experiments the diuretic used was a solution of the following composition, NaCl ·9%, KCl ·042%, $CaCl_2$ ·024%, Na_2CO_3 ·03%. The diuresis evoked by this solution will be called throughout "normal saline diuresis." Isotonic sodium chloride solution has been used by several observers, *e.g.* Magnus[13] and Frey[14]. Injected into rabbits in large quantities, up to the total body weight of the animal, it has been found to produce an immediate and considerable diuresis. This phenomenon is, of course, distinct from that observed by Thompson (This *Journal*, XXV. p. 487). We have obtained a good flow of urine with much smaller injections than they describe. This may be due directly or indirectly to the fact that our animals were eviscerated, for they had a good blood-pressure and small blood volume and there was no opportunity of their water being excreted into the intestinal tract.

Whilst injection of Ringer's solution, into the jugular vein of a cat in good condition, very shortly produces a flow of urine, it produces no increase, and what is equally important no decrease in the oxidation of the kidney. Before we can accept this statement as a valid proof of a mechanical production of urine it is necessary to consider several questions.

(1) Is the "normal saline" diuresis due to injury of the kidney? In order to settle this question we have controlled the diuresis as produced by Ringer's solution with a sodium sulphate diuresis before and after. Sodium sulphate is known to cause increased oxidation[2] in the kidney. The general course of such an experiment (Exp. 1) may be seen in Fig. 1 in which the oxygen used by the kidney and the volume

of urine are plotted vertically and the time horizontally. The points on which we would insist are:

(*a*) During the sodium sulphate diuresis, at the beginning and at the end of the experiment there was a great rise of the oxygen taken.

(*b*) During the "normal saline diuresis," which was greater and more prolonged than that produced by sodium sulphate, there was no corresponding rise.

(*c*) The kidney, in the matter of oxidation, responded as well to the sodium sulphate at the end as at the beginning of the experiment.

(*d*) The urine did not contain either albumen or hæmoglobin at any stage of the experiment.

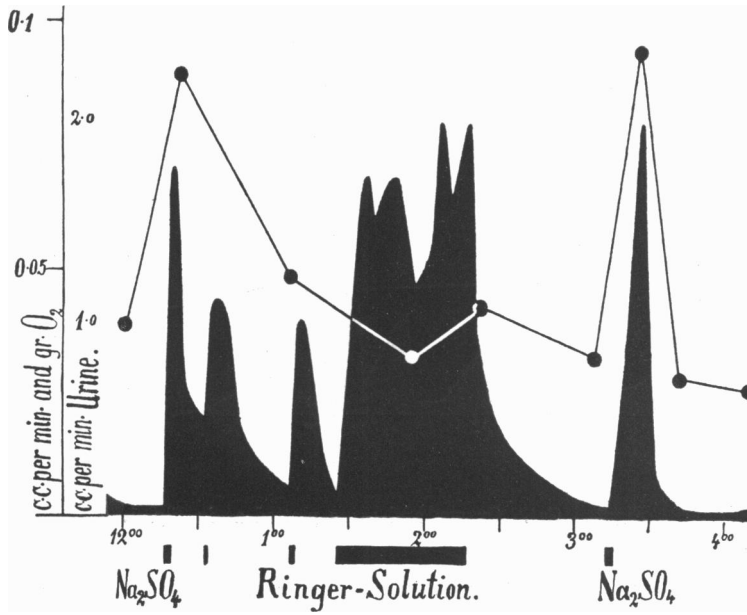

Fig. 1. Cat. The black line represents the oxygen taken up by the kidney in c.c. per gram of kidney per minute, the black areas represent the volume of urine secreted; urine per minute being plotted vertically and the time horizontally.

In Exp. 2 (Fig. 2) the above statements are confirmed in every respect save that the initial oxidation with sodium sulphate was greater than the final one, a fact which is reflected in the composition of the urine secreted. In the first diuresis the chlorides (reckoned as NaCl) were ·17 % and the sulphates (reckoned as Na_2SO) were 2·3 %, the urea ·5 %;

in the second sulphate diuresis the chlorides were ·42 and the sulphates 1·25 and the urea ·25 %; clearly in the second case the urine approximated more to the plasma than in the first.

(2) Is "normal saline" diuresis due to inhibition? Such for instance as might be supposed to take place if the reabsorptive function of cells of the tubules were abolished? The answer to this question is negative, since the metabolism of the kidney is not reduced.

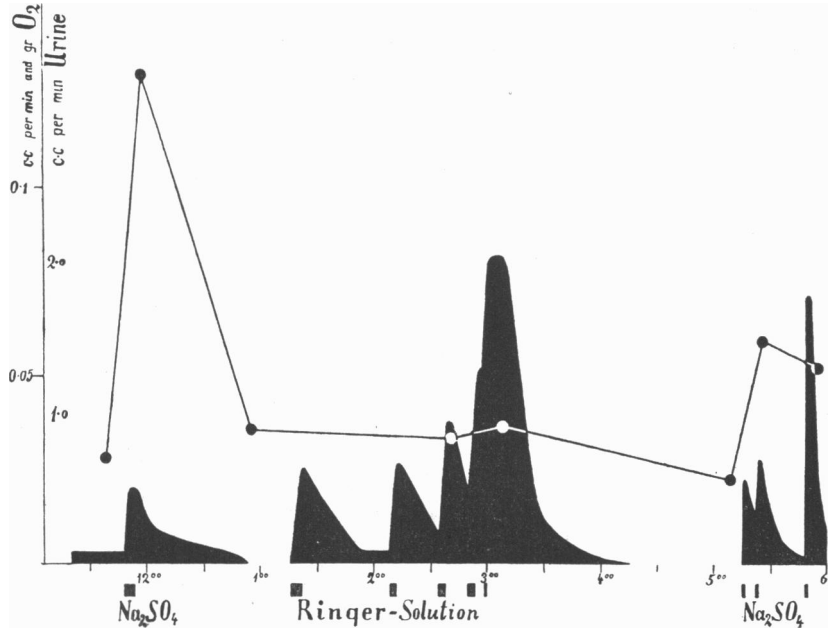

Fig. 2. Cat. Oxygen and urine plotted as in Fig. 1.

The view that "normal saline" diuresis is the result of a physical process which does not entail any increased output of energy on the part of the tubules, is in harmony with the nature of the urine itself. The sulphate diuresis in each case was marked by an amount of sulphate in the urine which was much greater, and an amount of chloride which was much less, than that of the plasma, whilst in the "normal saline" diuresis the salts in the urine are very like those of the plasma in composition. The following table will illustrate this point—for purposes of comparison we give also the salts in the urine which was collected from the bladder prior to the operation.

Salts in cat's urine. Exp. 1.

	Bladder prior to operation	1st sulphate diuresis	Ringer diuresis	2nd sulphate diuresis
Chlorides as NaCl	·42 %	·22 %	·86 %	·39 %
Sulphates as Na_2SO_4	·26 %	1·25 %*	·38 %	2·0 %
Urea	4·4 %	·28 %	·15 %	·25 %
Oxygen used up per grm.	0·4 c.c.	0·9 c.c.	0·3 c.c. 0·4 c.c.	0·9 c.c.

* At close of diuresis.

In confirmation of the observation that there is no increase in the oxygen used up by the kidney during "normal saline" diuresis we give the following data from other experiments.

Oxygen used up per gram of kidney per minute, and urine secreted per minute.

		Before injection	During Ringer diuresis			After
Exp. 3.	Oxygen	0·03	0·033	0·030	0·026	—
	Urine	0·1	0·4	0·5	0·4	—
Exp. 4.	Oxygen	0·10	0·11	0·11	—	0·09
	Urine	0·06	2·3	0·6	—	0·1
Exp. 5.	Oxygen	0·11	0·10	—	—	—
	Urine	·02	0·9	—	—	—

Sodium chloride. The transition from Ringer's solution to hypertonic and hypotonic solutions of sodium chloride was the natural extension of the above experiments. The case of hypotonic sodium chloride is quite clear, namely it consists in a physical production of urine without the aid of work done by the kidney. This is illustrated in Exp. 6 (Fig. 4) in which there was a considerable diuresis amounting to 1 c.c. of urine per minute, the oxygen used before the diuresis was ·73, during the diuresis ·71 and afterwards ·68 c.c. per gram per minute. As confirmatory of this result is one which was obtained at the end of Exp. 7 (Fig. 3), the oxygen taken in during the water diuresis was ·94 c.c. per gram per minute; this was preceded by an estimation of ·93 when the urine secreted was not more than a twentieth part of what it was during the diuresis: there was however a slight but appreciable fall after the diuresis to ·66 c.c. of oxygen.

In the case of hypertonic salt solutions diuresis does not seem to be the result of an active secretion. The most copious diuresis which we have obtained in the cat has resulted from the injection of 5 c.c. of 5 %

sodium chloride. In Fig. 3, it has been necessary to shorten the height of the record by cutting out a portion. The quantity of urine reached 5 c.c. per minute whilst before the diuresis the kidney was secreting only 0·04 c.c., nevertheless the oxygen used up in the normal period was 0·81 c.c. per gram per min. and during the diuretic period 0·83. About half an hour subsequently, the diuresis having by that time passed off, the metabolism seemed to have risen slightly, viz. to 0·93 c.c. Whether this rise was due to some stimulating effect of the chloride, or whether it was adventitious, we were not in a position to say.

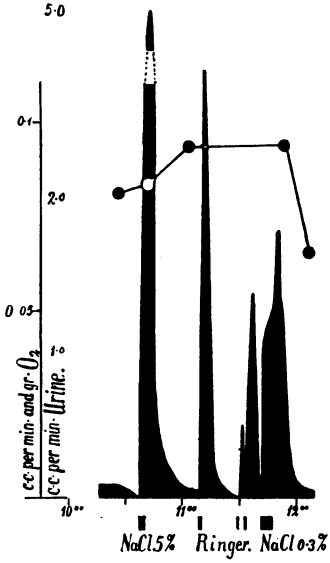

Fig. 3. Cat.

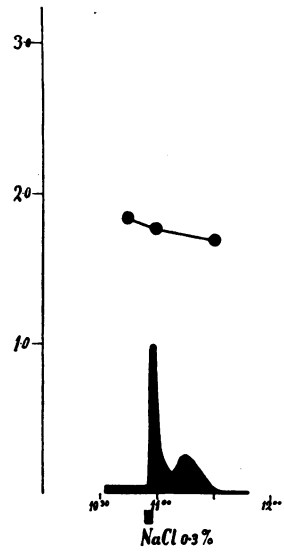

Fig. 4. Cat.

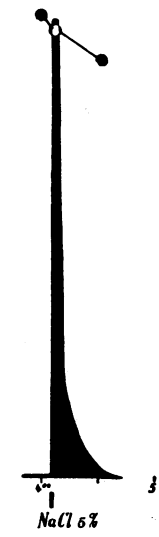

Fig. 5. Rabbit.

In Fig. 3 the column representing the first diuresis has been shortened, the apex being at 5 c.c. The apex of Fig. 5 is 3 c.c.

We therefore performed Exp. 8 shown in Fig. 5 which gave no rise either during or after the diuresis.

Caffeine and urea. The effects produced by caffeine and urea are somewhat similar to one another, but different from those already studied. Both these substances appear to cause a rise in the metabolism during the period of diuresis, followed by a fall of the metabolism to an abnormally low level. This depressing or poisoning action has been figured by Barcroft and Brodie in the case of urea, though its significance was not appreciated by them (*Journ. of Physiol.* XXXIII.

p. 61, Fig. 2, Exp. 6). In the single experiment with urea, a record of which appears in the present paper (Exp. 9, Fig. 6), the metabolism rose during the extended period of diuresis, then fell to about $\frac{2}{3}$ of its original level and subsequently recovered completely from the depression caused by the first dose of urea (10 c.c. of 5 %). A second and very large dose, 5 c.c. of 80 % urea, produced a still further depression from which the recovery was but very slight.

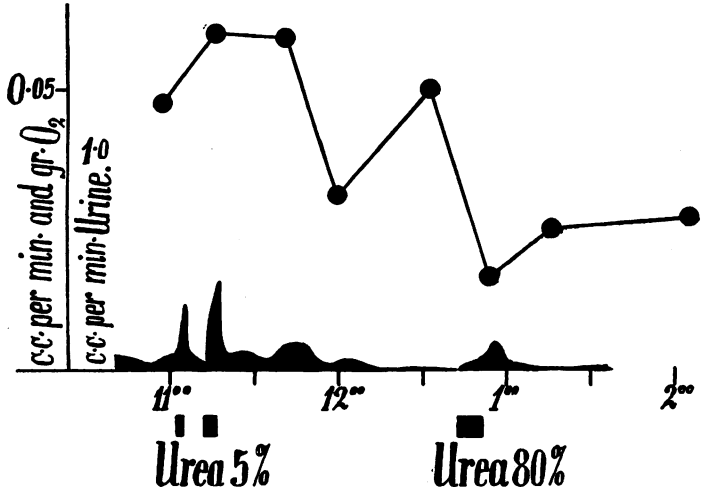

Fig. 6. Cat. Oxygen and urine plotted as in Fig. 1.

Two experiments (10 and 11, Figs. 7 and 8) have been performed with caffeine, one in the cat and the other in the rabbit. In both the same general features may be seen—namely (1) the stimulating effect of the drug during the diuretic period, (2) the subsequent depressing effect. In the case of the second dose there was a still further depressant effect in both animals, though with this difference: in the cat the second dose caused no diuresis, the depression set in at once; in the rabbit, which is notoriously tolerant to caffeine, the second dose caused a small diuresis, and with it the metabolism was augmented temporarily. The doses of caffeine used in these experiments were as follows. In the rabbit (weight 2 kilos) the first dose was 2 c.c. of 5 % caffeine sodium benzoate solution in 10 c.c. of defibrinated blood, the second injection was the same. In the cat (weight 2·6 kilos) the first dose was 1·5 c.c. caffeine solution in 7·5 c.c. of blood, the second dose was 2·2 c.c. of caffeine in 9 c.c. of blood. We have found it advantageous

to dilute the caffeine solution with defibrinated blood rather than with saline solution.

At this point something may be said about the theory of caffeine diuresis. Up to the present time two theories have been put forward to explain it, (1) that the caffeine acts as a specific stimulant to the kidney cells [15] and (2) that it acts by causing vaso-dilatation [16], accompanied to some extent with a paralysis of the hypothetical reabsorptive mechanism of the tubules [17].

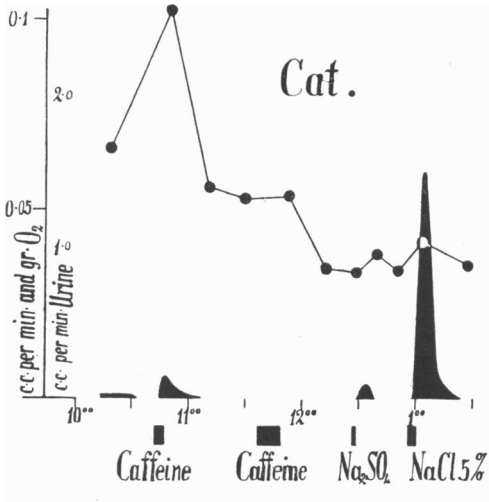

Fig. 7.

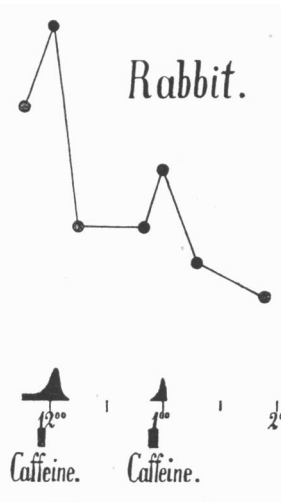

Fig. 8.

Oxygen and urine plotted as in Fig. 1.

We may first clear the ground to some extent by considering the action of caffeine on the vessels. In experiments to be subsequently described we have injected caffeine directly into the kidney vessels for the purpose of depressing the renal epithelial cells; the incidental effect noticed on the kidney vessels has been constriction. The following table shows the rate of flow through the kidney before and after the injection together with mean arterial pressure.

Rate of flow seconds per c.c. of blood		Mean arterial pressure	
Before	After	Before	After
0·8	1·8	78 mm.	72 mm.
0·9	1·2	90 mm.	103 mm.
1·9	3·9	138 mm.	122 mm.

The mechanism of the constriction is not clear, but presumably it is to some extent of central origin. Certainly in the first and third instances there was evidence, from the B.-P. tracing, of the caffeine having reached the general circulation to some extent: it is not the mechanism but the fact with which we are concerned. We do not wish to push these data beyond their legitimate sphere. It might be argued, and with some truth, that if there was not dilatation there was also no diuresis. Our point just here is merely this, that there was no gross inhibition of the tone of the kidney vessels, such as would be produced by pilocarpine on the heart or if injected into the artery of the salivary gland.

According to the first of the two views given above we should expect increased oxygen consumption on the part of the kidney cells, on the second view we should get decreased oxidation, an approximation to plasma in the constituents of the urine and large vasodilatation.

In Exp. 10, in which the caffeine was not injected into the renal vessels but into the general circulation, there was diuresis accompanied by only a very trivial increase in the blood flow, before and after the diuresis the blood flow was ·9 c.c. per second and during the diuresis 1·1 c.c. Similarly in Exp. 11, the rate of flow was increased from ·3 c.c. per second to ·4 c.c. This slight increase seems to us quite insufficient to account for the diuresis.

Nor was there a cessation of the metabolism of the cells suggesting diminished reabsorption of water, on the other hand there was increased metabolism.

The simple explanation then of caffeine diuresis is that caffeine stimulates the cell, and like so many protoplasmic stimulants ultimately poisons it. This view at once follows from the changes observed in the metabolism, and explains the reason why the cell should eliminate the drug. Moreover it is suggestive that in the caffeine diuresis observed in the rabbit, the chlorides in the urine were low 0·3—0·4 %. They presented no approximation to those of the plasma. In the percentage of chlorides therefore the urine resulting from caffeine contrasts markedly from the mechanical flow produced by Ringer's solution.

The poisoning action of caffeine upon the tubules accounts for the fact noticed by Loewi and known clinically that a second injection of caffeine gives rise to a smaller effect than the first.

Discussion of mechanical factors in diuresis.

It is desirable to make some analysis of the possible factors which might combine to render a mechanical flow of urine possible. Of these the most obvious is increased arterial pressure.

I. *Arterial pressure.* In our experiments this factor has not been the subject of special investigation so that it is quite unnecessary to publish a complete record of the arterial pressures which we have observed. We have taken a tracing in each experiment and we are convinced that other things being equal an increased arterial pressure tends to augment the flow of urine, but we have observed very many cases in which a copious diuresis has taken place without any such rise; we need only note that of Exp. 7 in which the flow of urine was the largest that we have obtained namely 5 c.c. per minute, nevertheless there was scarcely any change in the arterial pressure as compared with the previous period of rest. In the pre-diuretic period it was 126 mm., in the diuretic period 122 mm.

Indeed nothing could be more striking than the low pressure at which considerable quantities of urine may be secreted. In one case, see Fig. 9, for instance with an arterial pressure of 13 mm. of mercury, there was a secretion of 0·8 c.c. of urine per minute, the same animal secreted urine with an arterial pressure of 6 mm., maintained by massaging the thorax, after the heart had ceased to beat. It was one in which the blood had largely been replaced by a suspension of red corpuscles in Ringer's solution in a manner subsequently to be described.

II. *Venous pressure.* We are in agreement with de Souza[18] in so far as we are unable to regard increased venous pressure as the cause of diuresis. In some cases, after the injection of considerable quantities of saline solution, we have clearly had a considerable venous pressure. On the whole, other things being equal, a high venous pressure seems to go hand in hand with a weak heart; in cases where we measured the venous pressure during diuresis in vigorous animals it was very low, for instance at the height of the "normal saline" diuresis shown in Fig. 2, the venous pressure was less than 1 cm. of blood.

III. *Rate of flow.* In the majority of cases in which we obtained urine as the result of filtration there was an increase in the rate of flow of blood through the kidney. Such a condition with a constant arterial pressure would indicate an increased capillary pressure. We

have not however obtained increased rate of flow in all our experiments. The following are examples of cases in which the diuresis was not accompanied by any considerable increase of rate of blood flow.

Exp.:—	12	13	19	14	6	15	16	5
Rate before diuresis (secs. per c.c.)	1·2	1·2	1·8	0·9	2·5	·7	2·9	4·9
„ during „ „ „	1·2	1·1	2·0	1·0	2·5	·8	4·3	4·5

IV. *Dilution of the blood* has been the one constant change in the vascular conditions which has been present in all our experiments which have yielded diuresis without increased oxidation. We must therefore consider whether this in itself would tend to promote a flow of urine. Starling[19] has drawn attention to the importance of the osmotic pressure of the proteins in relation to the glomerular function. He accounts for the fact that a considerable capillary pressure is necessary for the production of urine in the glomerulus by supposing that the filtration can only take place when the capillary pressure exceeds the osmotic pressure of the non-diffusible constituents of the plasma. On the basis of this hypothesis it is clear that the injection of Ringer's solution, or water, or hypertonic salt solutions (which would attract much water from the tissues) would dilute the proteins in the plasma and would render the filtration more easy.

The possibilities here presented are very great. Suppose for instance that under ordinary circumstances the capillary pressure were just higher than the osmotic pressure of the proteins, place the former at 27 mm. and the latter at 25, the available pressure for filtration would be 2 mm. Halve the osmotic pressure of the proteins, *i.e.* make it 12·5, the available filtering pressure becomes 14·5 mm., a sevenfold increase. In Fig. 9 it appears urine could just be filtered under the conditions of the experiment at 6 mm. If this figure represented the osmotic pressure of the proteins there would have been on the above assumption a tenfold increase of nett filtering pressure.

It occurred to us that if the dilution of the blood were really an important factor, it should be possible by bleeding the animal to obtain a copious diuresis with much smaller injections of Ringer's solution than those used by Magnus, Frey etc., which were measured in litres. Before the injection of Ringer's solution the animal was therefore bled to the maximum extent consistent with life, oxygen being administered when necessary.

The following is the record of one such experiment.

Cat 1·75 kilos.

12.11. 10 c.c. Ringer's solution injected.
12.14. 17 c.c. of blood withdrawn and 50 c.c. of Ringer injected.
12.30. 25 c.c. of Ringer's solution injected.
12.34. 20 c.c. of blood withdrawn and 50 c.c. Ringer injected.
12.49. 50 c.c. of Ringer's solution injected.
12.56. 25 ,, ,, ,,
1. 1. 12 ,, ,, ,,
1. 7. 25 c.c. blood withdrawn and 50 c.c. Ringer injected.

During this time 272 c.c. of Ringer's solution were injected whilst 62 c.c. of blood were withdrawn, leaving a balance of about 200 c.c. or $\frac{1}{8}$ of the animal's weight if this 26 c.c. were secreted.

In such circumstances we were much struck with the low arterial pressure at which the urine might be secreted. We here give the portion of the tracing which represents the termination of this experiment.

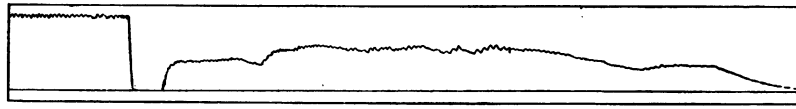

Fig. 9. $\times\frac{3}{4}$. Coincident with the first portion of the tracing 0·8 c.c. of urine per min. was flowing. In the second part the B.-P. was kept up mechanically and urine was secreted, none being secreted when there was no B.-P.

The urine and the plasma were analysed in this experiment. The analysis showed:

	Chlorides as NaCl	Urea	Albumen
Plasma	0·88 %	0·04 %	Absent
Urine	0·88 %	0·05 %	Absent

Everything in the above experiment, the comparatively small amount of the injection, the low blood-pressure, and the composition of the urine, pointed to our original assumption being justified. This experiment is only one of a number. That the urine did not appear as the result of gross mechanical injury is sufficiently attested by the fact that it contained no albumen (as shown by Heller's test) at any stage of the experiment, and also by the fact that in a similar experiment we replaced the original blood (which had been prevented from clotting by hirudin) in the animal; the diuresis after a time passed off and the urine secreted contained only 0·4 % of chloride.

We have endeavoured to eliminate factors other than dilution to a much greater extent than they were eliminated in the preceding experiment. With the object of maintaining the vascular conditions, *i.e.* the blood-pressure and the rate of flow, as constant as possible whilst at the same time diluting the plasma-proteins we conceived it possible to replace a considerable portion of the blood with a suspension of red corpuscles in Ringer's solution. For this purpose an animal was bled, the corpuscles centrifugalised out and washed, the process repeated and the corpuscles finally made up to about the original bulk

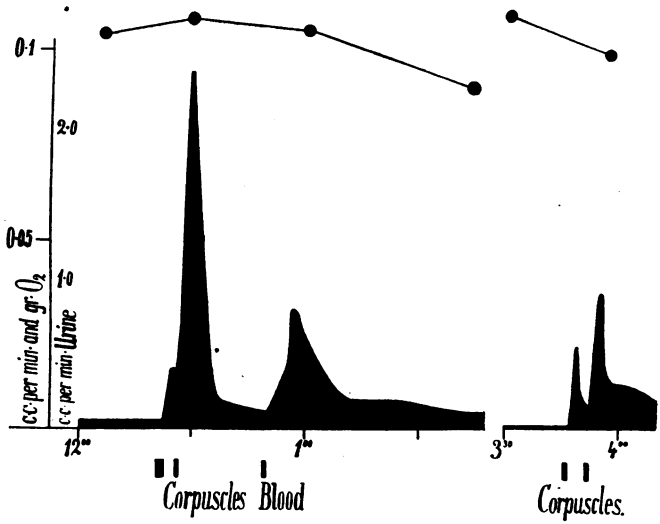

Fig. 10. Rabbit. Fig. 11. Rabbit.

Oxygen and urine plotted as in Fig. 1.

of the blood. The record of such an experiment is given in Fig. 10. The animal which was the subject of the experiment was secreting 0·05 c.c. of urine per minute, its blood-pressure was 95 mm. of mercury; 22 cubic centimetres of blood was taken out and 25 c.c. of Ringer's solution was injected, the secretion at once rose to 0·4 c.c. per minute (see the notch on the record of the diuresis at 12.23), the arterial pressure was then but 52 mm. The suspension of corpuscles (25 c.c.) was put into the jugular vein, the arterial pressure at once rose to 84 mm., and the diuresis reached the very large figure (for a rabbit) of 2·35 c.c. per minute. The rate of flow of blood through the kidneys was slightly slower in the diuretic period than before it, being 1 c.c. in 4·3 seconds

as opposed to 1 c.c. 2·9 secs. The urine attained a value of 0·95 % of chlorides during the diuresis, the oxygen taken in presented scarcely any variation.

	Before diuresis	During		After
Oxygen taken in per grm. of kidney per minute	0·104 c.c.	0·108	0·105	0·09

Similar results were obtained in a second experiment of the same nature (Fig. 11). In it the oxygen taken in before the diuresis was 0·11 c.c. per gram per minute, during the flow 0·10.

V. *Other factors.* Whilst we are of opinion that the urine in these cases finds its way through the glomerulus by a process of filtration, we are conscious that we have by no means exhausted the physical factors which may regulate the degree of filtration. In the case of chloride-diuresis for instance, the change in saline concentration in the plasma may alter the degree of aggregation of the proteins and so alter their osmotic pressure; moreover a similar effect may, in fact must, take place in the cell membranes through which the fluid passes and so must alter their permeability, but whether such factors are considerable or inconsiderable we do not know; such as they are however they affect a much less restricted field in physiology than the secretion of urine.

Isotonic urine.

In the cases which we have just quoted, the urine secreted differs from the serum only in so far as the cells of the tubule have been able to modify it as it hurries down these passages. If we are correct in viewing it as being a modified glomerular filtrate, it is clear that if we can suspend the activity of the tubule cells, we should get a fluid in which the salts were jointly and severally isotonic with those of the plasma. In this we have appeared successful so far as the matter can be tested. The difficulty which such testing presents is that of ascertaining the extent to which some of the saline constituents are attached to the proteins of the plasma. This point is forming the subject of special investigation.

Here we may put down three methods for suspending the activity of the kidney cells, with each of which we have met with some degree of success: (1) poisoning with corrosive sublimate, (2) anæmia, (3) poisoning with caffeine. In the case of the first and third method, the problem is to poison the cells sufficiently for the purpose in hand, and at the same time to preserve the vascular conditions in a satisfactory state.

Our method of poisoning (suggested to us by Prof. Langley) has been to put a cannula in the superior mesenteric artery, to clamp the aorta in the vicinity of the cœliac axis, the vena cava is also clamped above the renal veins. The drug is injected through the arterial cannula, is kept in the kidney for the time required and is washed out with Ringer's solution, finding its exit through the cannula in the distal end of the vena cava. In this way a minimal quantity of the drug gets into the general circulation.

Before using such a method some enquiry must be made into the effect upon the kidney of cutting it off from the circulation. From the statements which have been made on the subject we were inclined to think that clamping would in itself probably kill the cells. We have made no experiments in which the kidney vessels have been clamped when they contained blood and therefore our observations are not quite

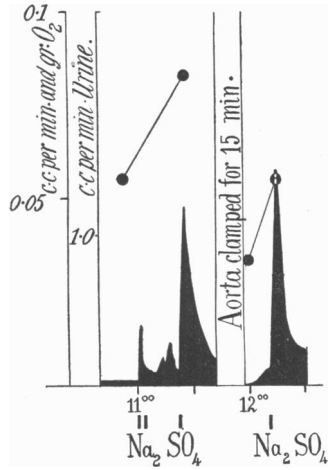

Fig. 12. Cat. Oxygen and urine plotted as in Fig. 1.

comparable with those of previous observers, but it is clear that a kidney survives the operation of clamping the vessels when the blood has been thoroughly washed out with Ringer's solution. This point is illustrated in Fig. 12. Sodium sulphate was used before and after the clamping (for 15 minutes) as a stimulus to the cells. The cells responded, as is shown both by the diuresis and the increased oxidation. Also the urine in each case was a typical secretion, at height of each diuresis it contained less than 0·05% NaCl. The general metabolism

of the kidney was not so great as before clamping, but its power to secrete remained.

1. *Sublimate poisoning.* The first poison which we employed was corrosive sublimate. It is known to constrict the vessels, indeed in the dose which we first used (2 c.c. of ·25 %), it did this with such effect that the blood flow was reduced from over 1 c.c. per second to 1 c.c. in 74 seconds. This experiment (Exp. 20) is worth putting on record partly on account of the fact that the venous blood which emerged even at this slow rate was bright red, the metabolism of the kidney having been for the time almost abolished; the gland which had been using 0·12 c.c. per gram, used only ·0011 c.c. after the sublimate. This experiment seems to dispose of the contention of those who hold that corrosive sublimate poisoning is due to anæmia produced by the vaso-constriction. Had this theory been correct the venous blood would

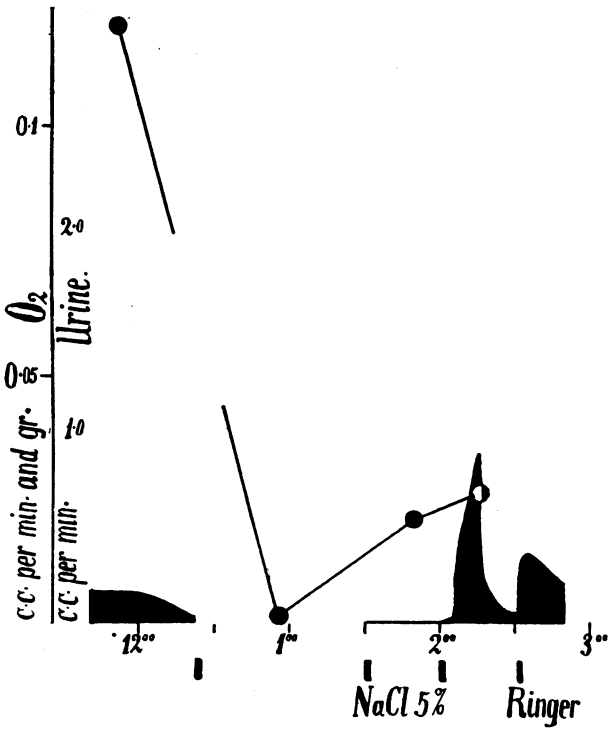

Fig. 13. Cat. Oxygen and urine plotted as in Fig. 1. The break in the O_2 line corresponds to the time during which the vessels were clamped, the signal underneath it to the injection of sublimate.

have been completely reduced. Clearly the sublimate acts on the kidney cell.

Usually the kidney in the cat or rabbit at the beginning of an experiment is using from 0·07 to 0·1 c.c. of oxygen per minute. Experience has shown us that this must be reduced to something like 0·02—0·03 c.c. before we can claim to have abolished the active influence of the tubule cells. At this point we may give details of Exp. 21 in which a rapid diuresis unaccompanied by increased oxidation was produced by injection of sodium chloride; the kidney at the same time had been poisoned with sublimate till its metabolism had fallen to 0·013 c.c. of oxygen per gram per min.

	Rate of blood flow	Do. of urine	Oxygen used up	Chloride in urine
Before sublimate	1 c.c. in 2·5″	—	0·06 c.c.	—
After ,,	1 c.c. in 33″	none	0·013 c.c.	—
During chloride diuresis	1 c.c. in 24″	rapid	0·014 c.c.	·8 %

In the case of Exp. 20, Fig. 13, it is of interest to observe that with the tubules poisoned, it was also possible to evoke a considerable diuresis with 5 % NaCl as well as with Ringer's fluid, the final urine contained 0·8 % NaCl and the O_2 consumed was but 0·026 c.c. per gram per minute.

2. *Anæmia.* The second series of experiments in which we have obtained what appeared to be isotonic urine was that already alluded to in which we administered considerable quantities of Ringer's solution after profuse bleeding. In some such cases the tubules appear to have been temporarily asphyxiated, as the blood reaching them contained an inadequate supply of hæmoglobin.

The following data were derived from two such experiments (Exps. 18 and 3).

Exp. 18.

Period	Urine	NaCl	Urea	Oxygen taken up
I.	After 50 c.c. Ringer	—	—	0·05 c.c.
II.	After bleeding and Ringer ...	0·67 %	0·22 %	0·06 c.c.
III.	After further bleeding and injection	0·83 %	0·04 %	0·017 c.c.
IV.	Further injections	0·88 %	0·05 %	0·018 c.c.
	Blood at end of exp.	0·88 %	0·04 %	...

It will be seen that the very low oxygen consumption of the last two periods was associated with urine which must have been a very close approximation to serum as regards crystalline bodies. Probably the blood, or rather the circulating fluid, containing as it did but a small fraction of the original hæmoglobin, was completely reduced.

In Exp. 3 (of the same character) the chloride in the urine rose from ·37 % before the bleedings to ·83 %, the oxygen taken in at the final stages of the experiment was ·033, ·030 and ·026 c.c. per gram of kidney per minute. In the last analysis it corresponded to about $\frac{1}{5}$ of what the arterial blood at the commencement of the operation might have yielded if the kidney had taken all its oxygen, and as it is scarcely possible that the blood at the end can have been more than $\frac{1}{5}$ of the concentration that it was at the beginning, it is probable that the cells were not able to get enough to give them the opportunity of doing work.

3. *Caffeine poisoning.* A single experiment in which the kidney was poisoned with caffeine, gave a similar result, viz. that when the metabolism of the kidney had fallen to something approaching $\frac{1}{3}$—$\frac{1}{4}$ of its former value isotonic urine was secreted.

The following data may be given.

Exp. 15.	Urine per min.	NaCl %	Urea %	Albumen	Oxygen used
Normal kidney ...	·2 c.c.	0·35	1·03	none	0·13
Diuresis after caffeine poisoning ...	1 c.c.	0·95	·07	trace	0·04
Blood corresponding to diuresis ...	—	0·9	·065	—	—

In this case 10 c.c. of a 5 % solution of caffeine sodium benzoate had been injected into the superior mesenteric artery as above described and the kidney then clamped for five minutes. The immediate cause of the diuresis was the injection of 125 c.c. Ringer's fluid. That the kidney was not asphyxiated in this case is shown by the fact that the venous blood emerging from it was about 50 % saturated with oxygen.

The question of reabsorption.

In the foregoing section we have spoken of the glomerular filtrate being modified in its passage down the tubule. Whilst the major theme of the present paper is a proof that filtration can exist, it is scarcely possible to leave the subject without some enquiry into the much vexed question of whether the modification of the urine by the tubule is a process of secretion or reabsorption. So far as we can contribute anything to this controversy it is the data which are available in the case of kidneys whose tubules have been poisoned. Of these we would say:

That the poisoning of the tubule seems to restrict the amount of urine which can be obtained as the result of sodium sulphate diuresis; and to deprive it of its typical character. Such urine under normal

circumstances is very poor in chlorides (they may even be absent) and very rich in sulphates. On the reabsorption theory it is arrived at by a process of complete or almost complete reabsorption of chlorides and reabsorption in a very great measure of water. Were these reabsorptive functions done away with, a urine would result which was enormously greater in volume than a normal sulphate diuresis and containing a large quantity of chlorides, upwards of 0·8 %, or perhaps more.

In the matter of bulk then the reabsorption theory demands precisely the opposite of what we have obtained. We quote two experiments in which the kidneys have been poisoned and have yielded but a scanty secretion with sodium sulphate. That there was nothing in the nature of the case to prevent a copious filtration is shown by the control experiments which were performed in each case with sodium chloride.

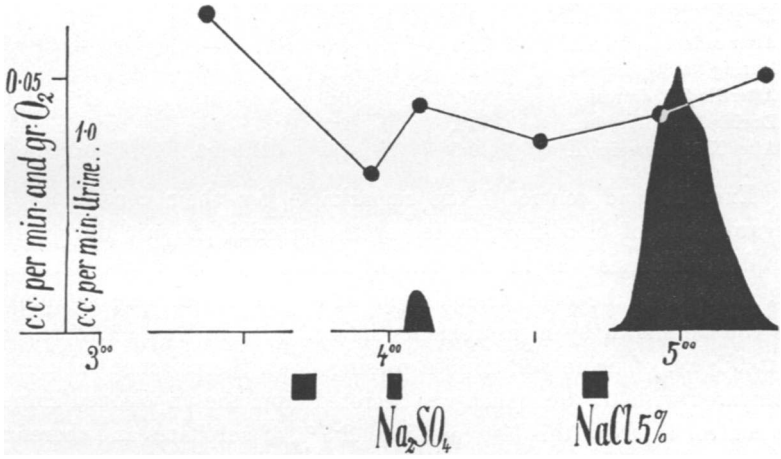

Fig. 14. Cat. Oxygen and urine plotted as in Fig. 1. The first signal represents the poisoning with caffeine.

These experiments (10 and 22) are figured in Figs. 7 and 14; the leading data are as follows.

	Exp. 10	Exp. 22	Control (1) unpoisoned	Control (2) unpoisoned
O_2 metabolism before poisoning with caffeine in c.c. per grm.	0·066	0·063	0·03	0·040
Do. after caffeine	0·034	0·031	—	—
Do. during Na_2SO_4 diuresis ...	**0·038**	**0·045**	**0·134**	**0·089**
Do. after Na_2SO_4 diuresis ...	**0·034**	**0·037**	**0·036**	**0·048**
Do. during NaCl diuresis ...	0·041	0·043	0·034 0·036	0·033 0·043
Do. after NaCl diuresis ...	0·035	0·050	0·023	0·033

The above-mentioned figures show:

(1) That the caffeine virtually abolished the specific effect of the sodium sulphate. The oxidation was much the same in Exps. 10 and 22 during the Na_2SO_4 diuresis as immediately before and immediately after it. In this control the oxidation was much increased during the Na_2SO_4 diuresis.

(2) Therefore such urine as came with the sulphate might be expected to be isotonic.

(3) This on the secretory theory should be small in amount, on the reabsorption theory great, unless the vascular conditions were modified in such a way as to interfere with the result, these therefore must be considered in the following table:

No. of c.c. of blood going through 1 *gram. of kidney per sec.*

	Exp. 10	Exp. 22	Control 1	Control 2
Normal	1·9	1·1	1·7	2·5
After caffeine ...	1·0	0·6	—	—
During Na_2SO_4 diuresis	2·1	1·6	2·0	3·6
After Na_2SO_4 diuresis	0·9	0·5	1·2	2·5
During NaCl diuresis...	3·1	2·7	2·5	1·6
After NaCl diuresis ...	0·9	1·3	1·6	0·9

Exp. 10 and control 1 are remarkable for their consistency as regards rate of blood-flow, moreover the arterial pressure during the sulphate diuresis was in the case of Exp. 10 83 mm. and control 1 82 mm.; thus the vascular conditions in the two cases were as similar as can be shown by measurements, nevertheless in Exp. 10 there was but 0·1 c.c. of urine per minute passing down the poisoned tubules as compared with 0·5 c.c. which was secreted from the unpoisoned kidney in control 1. Now this last contained 2·3 % of sulphates and therefore on the reabsorption theory as it is unlikely that the plasma contained more than 1 % this would represent a glomerular filtrate of at least $0{\cdot}5 \times 2{\cdot}3 = 1{\cdot}15$ c.c. per minute. This then we might have expected to get under the same vascular conditions in Exp. 10, instead we get less than $\frac{1}{10}$ of that amount. It is however fair to say that the kidneys in the case of Exp. 10 were only about half the size of what they were in control 1; to make the comparison fair we should consider that we got 0·2 c.c. where we looked for 1·15.

These facts are again controlled by the fact, that in the subsequent diuresis which was brought about mechanically (NaCl in one case, Ringer in the other) there was a rough equality in each case, both in the vascular conditions, the oxygen used up and the urine secreted.

Mechanical diuresis.

(All measurements in c.c. per grm. of kidney per minute.)

	Oxygen	Blood	Urine
Exp. 10	0·041	3·1	·056
Control 1	0·036	2·5	·044

From the above it will be seen that the two experiments ran a very consistent course, the slight balance of urine being on the side of Exp. 10.

The following are the details of the urine:

	Exp. 10	Exp. 22		Control 1		
Period	Quantity of urine, c.c.	Quantity, c.c.	NaCl %	Quantity, c.c.	NaCl %	Na_2SO_4 %
Normal	0·03	0+	0·6	0·08	0·78	0·7
After caffeine ...	none	none	—	—	—	—
During Na_2SO_4 diuresis	**0·1**	**0·2**	**0·6**	**0·5**	**0·17**	**2·27**
After Na_2SO_4 diuresis	none	none	—	none	—	—
During NaCl diuresis	1·5	1·3	0·6	2·1	0·78	0·37
After NaCl diuresis ...	none	0·05	—	none	—	—

The reader should note:

(1) That from the poisoned kidney the percentage of chlorides during this sulphate diuresis is equal to that secreted during the chloride diuresis.

(2) In this control the chloride during this sulphate diuresis is meagre as compared with the above and with what is secreted before and after.

Therefore the caffeine has abolished this characteristic paucity of chlorides during sulphate diuresis.

Whilst the possibility of some slight degree of absorption cannot be categorically denied we consider that two factors only are established, filtration in the glomeruli and secretion in the tubules, and that it is possible with their aid to account for all the facts of which we have a knowledge.

Conclusions.

1. The diuretics which we have studied fall into two groups (1) those which produce urine without alteration in the gaseous exchange of the kidney (Ringer's solution and NaCl in hyper- and hypotonic solutions); (2) those which cause increased gaseous exchange (urea, caffeine, sodium sulphate), to these may be added phloridzin

(Barcroft and Brodie). In the case of urea and caffeine there is a definite poisoning action as shown by subsequent depression of the gaseous exchange.

2. The distinctive features of the urine produced by the second class are attained by a process of secretion on the part of the tubules, rather than by a process of reabsorption.

We have failed to find any degree of inhibition during diuresis, suggesting that there is no suspension of a reabsorptive function. In the case of kidneys with the tubules poisoned the evidence points rather to their having lost the power of secretion than to their having lost that of reabsorption.

3. After poisoning the cells of the tubules it is possible to get a flow of urine with the first class (and a restricted flow with sodium sulphate due to the merely mechanical properties of the salt) which appears to be isotonic with the serum.

4. Clamping the kidney for 15 minutes after washing out the vessels with Ringer's solution, does not abolish its power of secretion.

5. Amongst the mechanical factors which play a part in the production of urine as the result of injection of Ringer's solution, is the lowering of the osmotic pressure of the blood proteins due to dilution, hence after repeated bleedings and injections of Ringer's solution, urine can be filtered at a very low pressure.

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We do not of course profess to have given a complete bibliography of the subject; such will be found in the text-books of Nagel or of Oppenheimer.