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THE MECHANISMS OF DILUTION DIURESIS IN THE ISOLATED KIDNEY AND THE ANAESTHETIZED DOG

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The important contributions to the theory of renal secretion made by students of dilution diuresis began with Starling's [1899] consideration of the influence of the colloid osmotic pressure of plasma proteins on the rate of glomerular filtration. Despite Magnus's [1900] impression, based on extensive observations, that the diuresis was too great to be attributed solely to a change in glomerular activity, the view that tubular activity was not directly affected gained ground and was supported by Barcroft & Straub's [1910] finding that dilution diuresis was unaccompanied by increase in metabolism of the kidney and was therefore a "mechanical" diuresis. This view was reinforced by Knowlton's [1911] comparison of the effects of saline with gum-saline and gelatine-saline solutions, which identified the diuretic stimulus as a reduction in colloid osmotic pressure of the plasma. The purely "mechanical" view of dilution diuresis was strongly advocated by Cushny [1917] and has since been generally accepted as evidence in favour of the filtration theory of glomerular function in the mammal.

Cushny [1917] concedes that "the change in the amount of urine in Magnus's experiments is out of all proportion to the change in the concentration of the colloids", but he felt able to explain away the discrepancy on the grounds that the proportion of filtrate reabsorbed in the tubules would be lower during the diuresis, and that this would magnify the change in glomerular filtration rate which he apparently took to be proportional to the reduction in concentration of plasma colloids.

More recently, observations on perfused kidneys have directed attention to the disproportionately great diuresis produced by a small reduction in colloid osmotic pressure of the plasma in comparison with that

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produced by an equivalent rise in arterial pressure [Winton, 1937], with the implied suggestion that dilution diuresis is in part due to a direct action on tubular activity, inhibiting reabsorption of water. It is the main purpose of the experiments described below to measure this disproportion in the anaesthetized dog and in the isolated kidney, and to attempt to evaluate the glomerular and tubular factors co-operating in the production of dilution diuresis.

The decision as to whether a form of diuresis is to be attributed to change in glomerular or tubular activity encounters a difficulty which is simply explained by an illustration. Suppose we may measure the glomerular filtration rate in terms of the creatinine clearance, and that a twofold diuresis involves an increase of urine flow from 1 to 2% of the filtration rate. If the amount of water reabsorbed in the tubules remains unchanged, we should expect only 1% increase in creatinine clearance, a change too small to detect with confidence in view of the other hazards of biological experiment. It would seem difficult to discover, therefore, whether even a considerable diuresis is due to a small increase in glomerular filtration rate or to a small diminution of reabsorption in the tubules. Fortunately, the difficulty can be surmounted if the meanings attached to "change in glomerular activity" and "change in tubular activity" or more shortly "glomerular diuresis" and "tubular diuresis" are properly chosen and precisely defined. As far as we know, a change in glomerular filtration rate always involves characteristic and determinate changes in tubular activity, and glomerular diuresis should, therefore, be taken to imply the whole complex of glomerular and tubular changes consequent upon an increase in filtration rate. Similarly, tubular diuresis implies the changes occurring when diuresis is unaccompanied by increase in filtration rate.

To assign a particular type of diuresis to the glomerular or tubular class requires standards of reference. The standard here chosen for a glomerular diuresis is the change in a number of the constituents of urine produced by an increase in renal arterial pressure, and the standard chosen for a tubular diuresis is the corresponding change produced by increase in the urea content of the plasma. It is a further aim of this communication to give rather more formal precision to the ideas underlying this way of distinguishing between changes in glomerular and tubular activity, though the ideas themselves have been implicit in several earlier communications [e.g. Verney & Winton, 1930].

The mechanism of dilution diuresis is particularly concerned with the difference between the glomerular capillary pressure and the intracapsular pressure, and in suitable circumstances [Winton, 1931*a*] changes in the latter can be estimated if the pressure fall from the glomerular capsule to the ureter be kept approximately constant by preventing changes in urine flow by appropriate changes in ureter pressure. We have, therefore, compared dilution diuresis with the standard forms of diuresis not only when the increase in urine flow is allowed to become manifest, without intentional changes in pressure head, i.e. "isobaric diuresis", but also when the urine flow is kept constant by suitable adjustment to the pressure head, and the "isorrheic diuresis" is measured in terms of the change in arterial or ureter pressure.

METHODS

The technique employed in experiments with the double pump-lung-kidney preparation was the same as that described in our previous communication [Eggleton, Pappenheimer & Winton, 1940*a*]. The chemical methods were also as there described.

Observations on the kidney *in situ* were conducted on dogs anaesthetized with chloralose, 0·1 g./kg. Ureters were cannulated, and in some experiments the left kidney was denervated. The pressure in the renal artery was varied by adjusting a clamp placed on the thoracic aorta. This and certain details of procedure are described in more detail by Shannon & Winton [1940].

Dilution of the blood was achieved by adding a Ringer-Locke's solution containing NaCl 0·76%, KCl 0·042%, $CaCl_2$ 0·024%, $MgCl_2$ 0·0005%, $NaHCO_3$ 0·20%, dextrose 0·05%. The higher value of bicarbonate than is usual was chosen in pump-lung-kidney experiments so that an appropriate *p*H would result from ventilation of the lungs with 5% CO_2. The degree of dilution of the plasma was estimated by measuring the total solids.

Two or three consecutive samples of urine were collected under any given set of conditions after an appropriate time had been allowed for the composition to reach a steady value. It was observed that the usual procedure of allowing a definite volume of urine, say 3 c.c., to be formed in the hope that such a "wash-out sample" would be sufficient was unsatisfactory at high urine flows—the volume of the "wash-out sample" being greater the higher the urine flow. At high rates of flow we often discarded 10 c.c. or more before beginning the collection of test samples.

RESULTS

The comparison of dilution diuresis with glomerular and tubular diuresis under isobaric conditions

Isolated kidney. The disproportion between the degree of diuresis due to blood dilution and that due to an arterial pressure change which might be expected to produce about the same change in glomerular filtration pressure is shown in Table I. As a first approximation, the glomerular capillary pressure in the isolated kidney may be taken as 60% of the arterial pressure [Winton, 1931*b*], a reduction of 1% (e.g. from 8 to 7%) in the serum protein concentration would imply a reduction of colloid osmotic pressure of about 3 mm. Hg, and the equivalent rise in arterial pressure should, therefore, be about 5 mm. Hg. If this were so, the disproportion between pressure and dilution diuresis would be six- to sevenfold.

TABLE I. Diuretic action of the changes in arterial pressure and in plasma protein concentration which may produce comparable changes in glomerular filtration pressure

	Percentage increase in urine flow due to	
	Pressure diuresis (per 5 mm. Hg change in arterial pressure)	Ringer diuresis (per 3 mm. Hg change in colloid osmotic pressure)
Isolated kidney	21·1 ± 3·5 (9)	136 ± 20 (7)
Anaesthetized dog (denervated)	12·0 ± 1·5 (2)	145 ± 31 (10)
Anaesthetized dog (innervated)	12·3 ± 4·0 (8)	212 ± 30 (20)

More precisely, the relevant osmotic pressure is the properly calculated average of that in the serum entering the glomerular capillary, and that in the serum as it leaves the capillary deprived of the glomerular fluid. If this be taken into account, the reduction of colloid osmotic pressure effective in glomerular filtration corresponding with a change by 1% in the serum protein concentration may fairly be put at 4 mm. Hg and the equivalent increase in arterial pressure should, therefore, be about 6 mm. Hg. This consideration, however, only reduces the disproportion between dilution and pressure diuresis to one of fivefold.

A first approach to the problem whether the disproportionate magnitude of dilution diuresis is due to an unexpectedly large increase in glomerular filtration or to a direct influence on the tubules reducing their reabsorptive activity may be made by comparing the data represented in Figs. 1–3. The curves illustrate the changes in urine flow, and in the chloride, creatinine and urea clearances characteristically associated with diuresis due to (1) dilution of serum, (2) increase in arterial pressure, and (3) increase in the urea content of the serum. The figures represent observations on different kidneys. The experiment on pressure diuresis (Fig. 2) was performed on the single pump-lung-kidney preparation. In the other experiments (Figs. 1, 3), each kidney was perfused alternately from two pump-lung circuits, the control circuit containing defibrinated blood the composition of which was kept as far as possible constant throughout the experiment, the second circuit being used for the addition of the diuretic

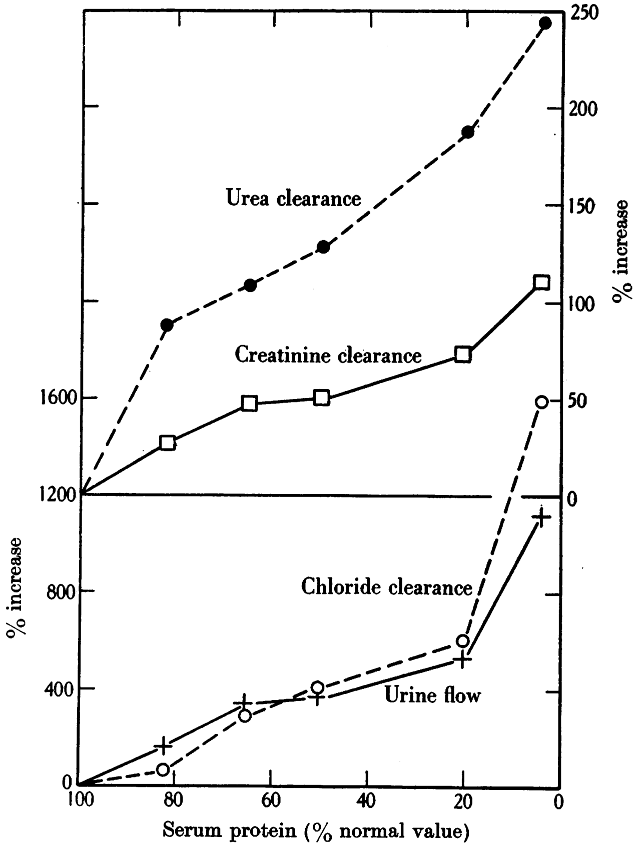

Fig. 1. Dilution diuresis in the isolated kidney. The percentage increase in urine flow, and in creatinine, urea, and chloride clearances with increasing dilution of serum protein by Ringer's fluid. Double pump-lung single kidney preparation. Initial values: kidney weight 15·5 g., urine flow 0·18 c.c./min., creatinine clearance 8·7 c.c./min., urea clearance 2·44 c.c./min., chloride clearance 0·029 c.c./min.

agent in several stages. The blood in a double pump-lung single kidney preparation was well mixed, the two circuits only being separated shortly before the transfer of the kidney from the control circuit to that in which either Ringer's solution or extra urea had been added to the defibrinated blood.

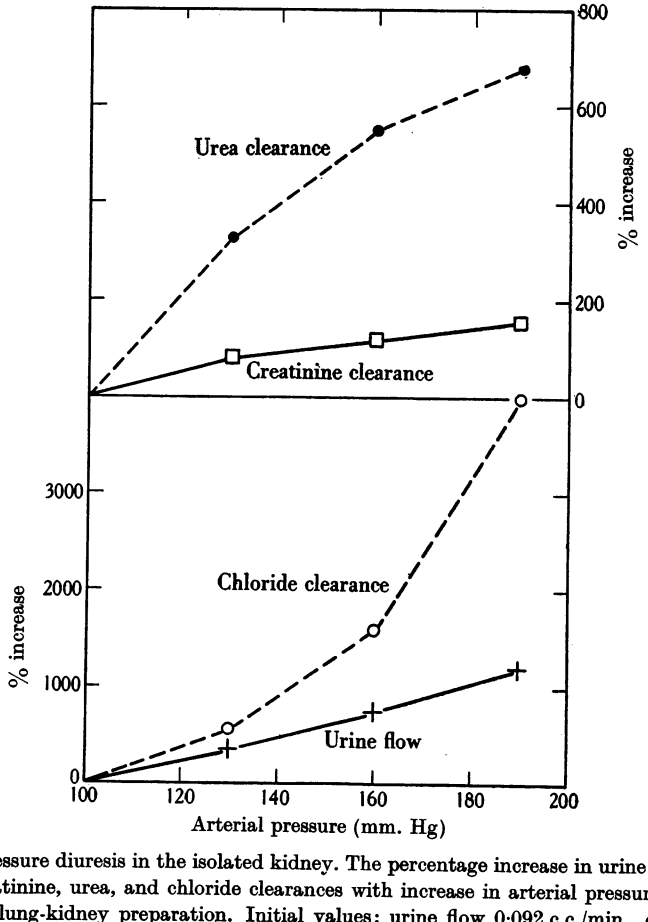

Fig. 2. Pressure diuresis in the isolated kidney. The percentage increase in urine flow, and in creatinine, urea, and chloride clearances with increase in arterial pressure. Single pump-lung-kidney preparation. Initial values: urine flow 0·092 c.c./min., creatinine clearance 5·4 c.c./min., urea clearance 0·59 c.c./min., chloride clearance 0·0019 c.c./min.

Any point on the curves represents the increase in value when the kidney was on the diuretic circuit over the average of the two values obtained just before and after, when the kidney was on the control circuit. Inspection of Figs. 1–3 immediately shows that dilution diuresis is much more closely related to pressure diuresis than it is to urea diuresis.

In both dilution and pressure diuresis the creatinine clearance increases, and the urea clearance increases relatively more. The increase in chloride clearance is less marked in dilution diuresis than in pressure diuresis, but in both there is a systematic increase which contrasts with the more complicated relation shown in urea diuresis.

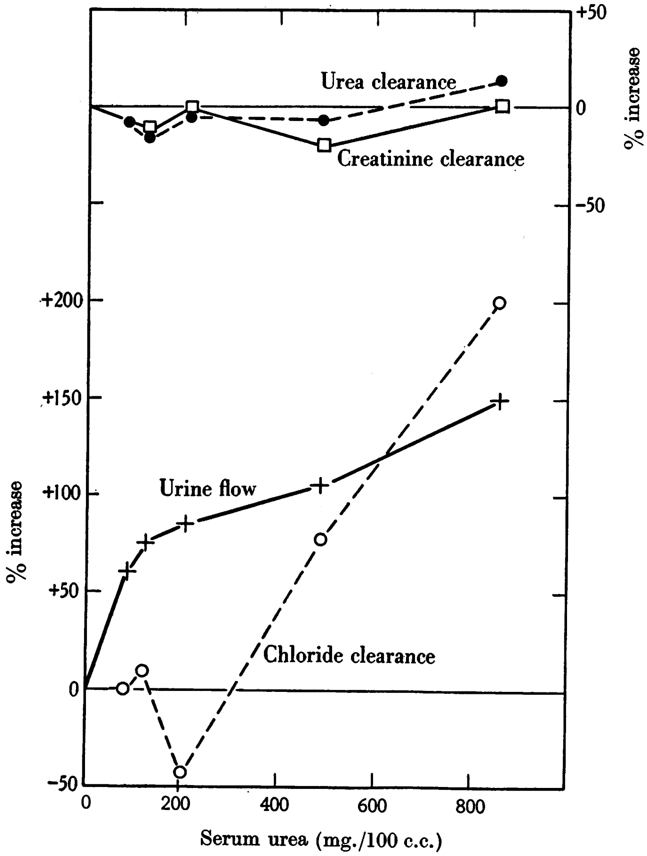

Fig. 3. Urea diuresis in the isolated kidney. The percentage changes in urine flow, and in creatinine, urea, and chloride clearances with increasing serum urea concentration. Double pump-lung single kidney preparation. Initial values: kidney weight 23 g., urine flow 0·307 c.c./min., creatinine clearance 12·5 c.c./min., urea clearance 4·2 c.c./min., chloride clearance 0·0006 c.c./min.

In Table II we have brought together for quantitative comparison a series of values from a considerable number of experiments of the same kind. We have calculated all values for a doubling of urine flow assuming proportionality between the increase in the value and the increase in the

TABLE II. Isolated kidney. Isobaric diuresis. Showing the percentage changes in clearances, etc., calculated for a doubling of urine flow by different diuretic agents

Percentage increase in	Arterial pressure	Ringer's fluid	Urea
Creatinine clearance	31 ± 8	24 ± 7	- 5 ± 4
Urea clearance	60 ± 10	45 ± 5·7	- 1 ± 4
Chloride clearance	220 ± 50	90 ± 13	63 ± 16
Total osmotic work	38 ± 3	41 ± 4	63 ± 7
Number of observations	9	7	8
Mean percentage increase in urine flow	183 ± 24	396 ± 130	132 ± 51

urine flow. It will be seen from the curves given that such an assumption is only an approximation. Each value is expressed in terms of the mean, and the standard error of the mean. The increases in urine flow actually observed are included in the table, and the fact that the Ringer diuresis was considerably greater than the two other forms of diuresis should not be entirely ignored in a comparison of the other values shown.

The salient features of the results expressed in Table II are (1) the increase in creatinine clearance in dilution diuresis and in pressure diuresis is considerable and not very different in amount, whereas, by contrast, there is no such increase in urea diuresis. Considerable urea diuresis usually involves a small reduction in creatinine clearance due to increased intrarenal pressure [Winton, 1933], and comparable with the reduction of 18% found [Bickford & Winton, 1937] when water reabsorption is blocked by cooling the kidney. (2) In view of the greater increase in urine flow in the dilution diuresis, the increase in urea clearance is probably rather greater in pressure diuresis than in dilution diuresis, there being no increase in urea diuresis. The lessening in reabsorption of urea with diuresis is, therefore, on the average less marked with dilution than with pressure diuresis. (3) Increases in chloride clearance occur in all three forms of diuresis, the increase being larger in pressure diuresis. Dilution diuresis differs from pressure diuresis in promoting the reabsorption of chloride.

The evidence of Table II as to whether dilution diuresis is to be regarded as "glomerular" or "tubular" is, therefore, conflicting. It appears to be mainly glomerular with reference to the creatinine and urea clearances, but mainly tubular with reference to chloride clearance. As far as the elimination of water is concerned, however, and the problem of the disproportionately great dilution diuresis is primarily concerned with this constituent of the urine, the creatinine clearance is the surest guide to the mechanism involved, and this would suggest that in the isolated kidney dilution diuresis is mainly a glomerular diuresis. This suggestion is supported by our previous finding [Eggleton *et al.* 1940*a*] that the increase in total osmotic work done in pressure and dilution diuresis is not significantly different, whereas that in urea diuresis is usually somewhat higher. This involves the conclusion that a given change in colloid osmotic pressure of the serum produces a much greater change in glomerular filtration rate than does a comparable change in hydrostatic pressure across the glomerular membrane, i.e. that either the permeability or the surface area of the glomerular membrane has undergone substantial increase.

Anaesthetized dog. The disproportion between dilution diuresis and the pressure diuresis due to a change in hydrostatic pressure comparable with the change in colloid osmotic pressure is even greater in the anaesthetized dog than in the pump-lung-kidney preparation. If a change in arterial pressure of 5 mm. Hg be regarded as equivalent to a change by 1 % in the concentration of serum protein, as above, dilution produces about fifteenfold the diuresis produced by an equivalent change in hydrostatic pressure (Table I). We have separated our results on innervated and denervated kidneys in Table I to indicate that they are sufficiently alike to justify considering them together in Table III. If, for reasons given in the previous section, the arterial pressure equivalent of a change by 1 % in serum protein be taken as 6 mm. Hg, the disproportion in diuresis is reduced to one of about thirteenfold, which still implies a major discrepancy in the theory of dilution diuresis currently adopted.

The plan of the experiments on anaesthetized dogs from which the data in Table III were obtained was less elaborate than that adopted in connexion with Table II; it is illustrated in Fig. 4 and involves following the progressive changes due to successive doses of the diuretic agent without the intermediate returns to control conditions rendered possible by the double perfusion arrangement. Such a procedure has greater justification in the anaesthetized dog, for under our experimental conditions the urinary output and composition remain fairly constant if not

TABLE III. Anaesthetized dog. Isobaric diuresis. Showing the percentage changes in clearances, calculated for a doubling of urine flow by different diuretic agents

Percentage increase in	Arterial pressure	Ringer's fluid	Urea
Creatinine clearance	14 ±3	4 ±3	-10 ±2
Urea clearance	35 ±2	20 ±5	1 ±4
Chloride clearance	475 ±70	400 ±70	52 ±30
Number of observations	10	30	4
Mean percentage increase in urine flow	167 ±44	439 ±87	249 ±30

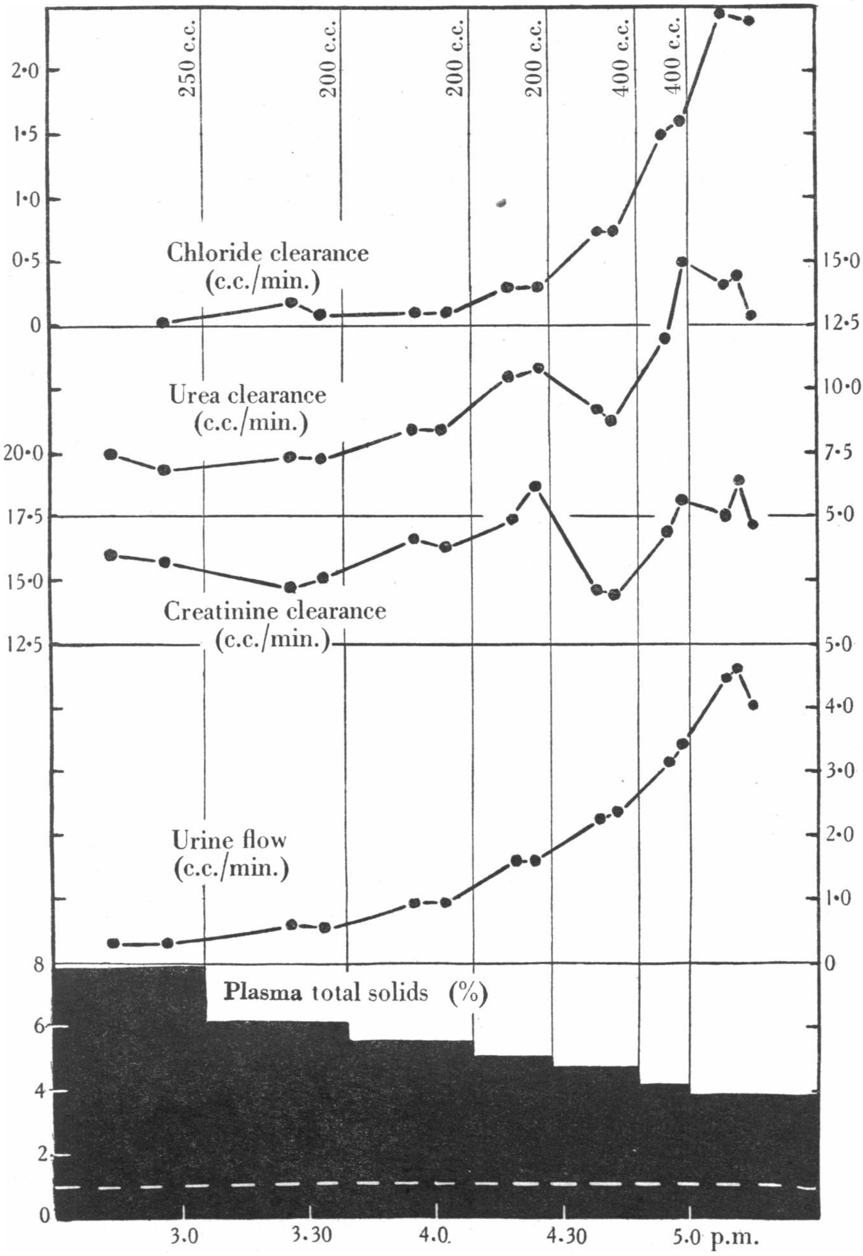

Fig. 4. Dilution diuresis in the chloralosed dog. The increase in urine flow, and changes in the creatinine, urea, and chloride clearances with decreasing concentration of serum protein due to addition of Ringer's fluid. A broken line is inserted at 1 % total solids, so that the values above this indicate the approximate concentration of plasma protein. Dog 10·5 kg. Kidney denervated. Arterial pressure increased from 95 mm. Hg to 110 mm. before 3.30 p.m. and was kept approximately at that value thereafter by appropriate removal of blood.

intentionally changed as indicated in Fig. 7, whereas the output of the isolated kidney systematically declines as the experiment progresses as indicated in Fig. 6. The characteristic effects of dilution diuresis are seen in Fig. 4 and in more quantitative form in Table III. In contrast with the isolated kidney, the kidney *in situ* responds to dilution of the plasma with a change in creatinine clearance which is definitely smaller than that associated with the same increase in urine flow produced by raising the arterial pressure. In our few observations on the action of urea in the anaesthetized dog secreting under isobaric conditions the creatinine clearance decreased, and it might be fair in respect of this variable to place dilution diuresis about half-way between pressure diuresis and urea diuresis, although dilution diuresis produced no significant increase in creatinine clearance. The fall in creatinine clearance during urea diuresis may well have been due to a rise in intrarenal pressure, and a similar rise may have prevented a greater increase in clearance during dilution diuresis. It may be recalled that pressure diuresis differs from urea diuresis in being unaccompanied by a change in intrarenal pressure [Winton, 1936].

The urea clearance increases in dilution diuresis by something of the order of one-half the increase in pressure diuresis, there being no change in clearance during urea diuresis.

The increase in chloride clearance due to dilution is about the same as that in pressure diuresis in the anaesthetized dog, whereas in the isolated perfused organ dilution diuresis resembles urea diuresis more closely in this respect.

The evidence with regard to the mechanism of dilution diuresis in the anaesthetized dog is, therefore, more definite than it is in the isolated kidney. A twofold increase in urine flow accompanied by little increase in creatinine clearance, which is substantially less than the increase produced in pressure diuresis of the same extent, cannot be attributed solely to an increased rate of glomerular filtration. It is clear that in this preparation dilution of the plasma produces a specific change in tubular activity inhibiting reabsorption of water, and that dilution diuresis is in large part due to this action on the tubules, and only in a smaller degree to an increase in glomerular filtration.

The comparison of dilution diuresis with glomerular and tubular diuresis under isorrheic conditions

Isolated kidney. A more exact analysis of the effects of dilution of the serum can be based on observations in which the increase in urine flow is just prevented by suitable reduction of the pressure head governing the formation of urine, and measuring both these changes in pressure and the changes in the composition of the urine. Such an experiment can be conducted in two ways, each having its advantages. The urine flow may be kept constant by variations in arterial pressure (Fig. 5); although the tubular activity can then be said to remain unaffected by the pressure change, for a purely "glomerular diuresis" has been defined so as to include the characteristically associated changes in tubule function (Fig. 2), the relation between the changes in arterial and glomerular capillary pressure are not certainly known. The urine flow may be kept

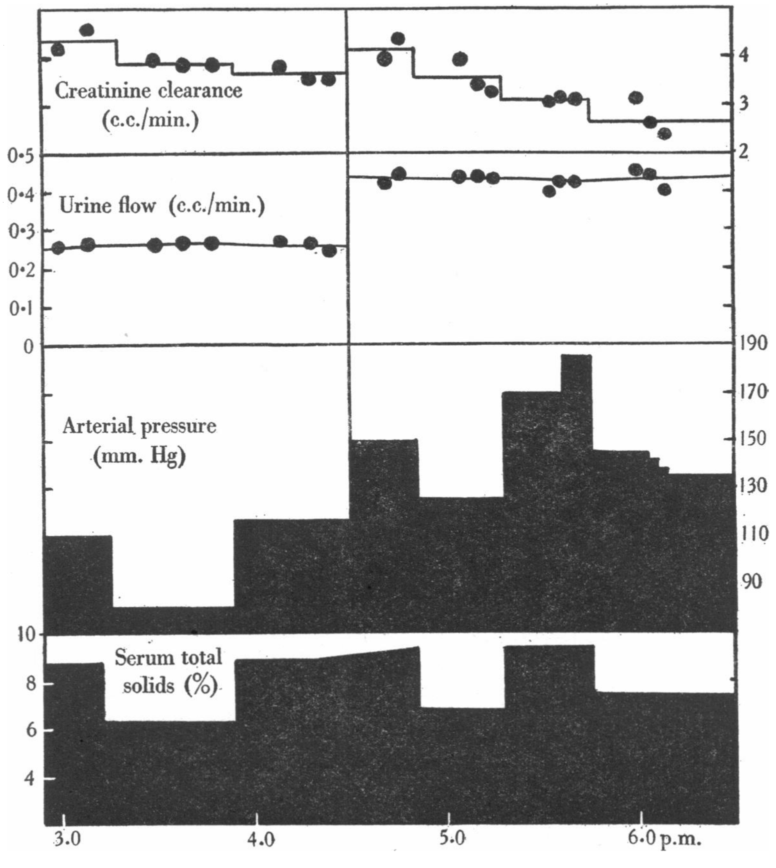

Fig. 5. Isorrheic dilution diuresis in the isolated kidney. Showing absence of effect of dilution on creatinine clearance, and the relation between arterial pressure and degree of dilution when urine flow is kept constant. Double pump-lung single kidney preparation. Kidney weight 22 g. Initial blood flow 105 c.c./min.

constant by variations in ureter pressure (Fig. 6); this provides for a direct comparison of the forms of diuresis due to arterial pressure, dilution and urea acting under isorrheic conditions, and probably enables the variations in pressure in the glomerular capsule to be measured fairly accurately [Winton, 1931*a*]; nevertheless we have recently discovered a small effect of ureter pressure on tubular activity [Eggleton, Pappenheimer & Winton, 1940*b*] and this complicates the interpretation of the changes in composition of the urine.

The procedure adopted in experiments designed to compare dilution and pressure diuresis under isorrheic conditions is illustrated in Fig. 5. When the kidney is transferred to the circuit containing diluted blood, the arterial pressure is also reduced to an extent necessitated by the condition that the urine flow is to remain unchanged. This adjustment is not easy and requires experience and constant attention throughout the experiment. The adjustments were made relatively quickly and precisely with the aid of the recording urine flow-meter described by one of us [Winton, 1939]. After a suitable delay during which a steady state was obtained, two or three consecutive samples of urine secreted from the diluted blood were collected. The kidney was then restored to the control circuit, the arterial pressure being raised just far enough to compensate for the more concentrated blood, and after suitable delay two or three successive samples of urine were collected. In this way the composition of the urine and the arterial pressure during isorrheic dilution diuresis could be fairly compared with the average value for the same variables obtained before and after on undiluted blood, in spite of the gradual decline in the creatinine clearance.

The main points demonstrated by Fig. 5 are the order of accuracy of our technique in keeping the urine flow constant, the fact that the steady decline in creatinine clearance is not substantially accelerated or retarded during the periods of dilution diuresis, and the relation between the degree of dilution of the serum and the change in arterial pressure needed to neutralize its effect on the urine flow. For simplicity, the variations in urea and chloride clearances have been omitted from Fig. 5, but included in the summary of data in Table IV.

A corresponding experiment in which an increase in ureter pressure was adjusted so as to neutralize the increase in urine flow due to serum dilution is illustrated in Fig. 6. It shows again the order of accuracy achieved in matching the urine flows, it shows the relation between the equivalent ureter pressures and serum dilution when the serum is progressively diluted, but it differs from Fig. 5 especially in showing a small but systematic reduction in creatinine clearance during dilution diuresis. We have found a similar decline in creatinine clearance when a rise in ureter pressure is neutralized by an increase in arterial pressure, the composition of the blood remaining unchanged [Eggleton *et al.* 1940*b*], and the decline shown in Fig. 6 cannot, therefore, be attributed to serum dilution.

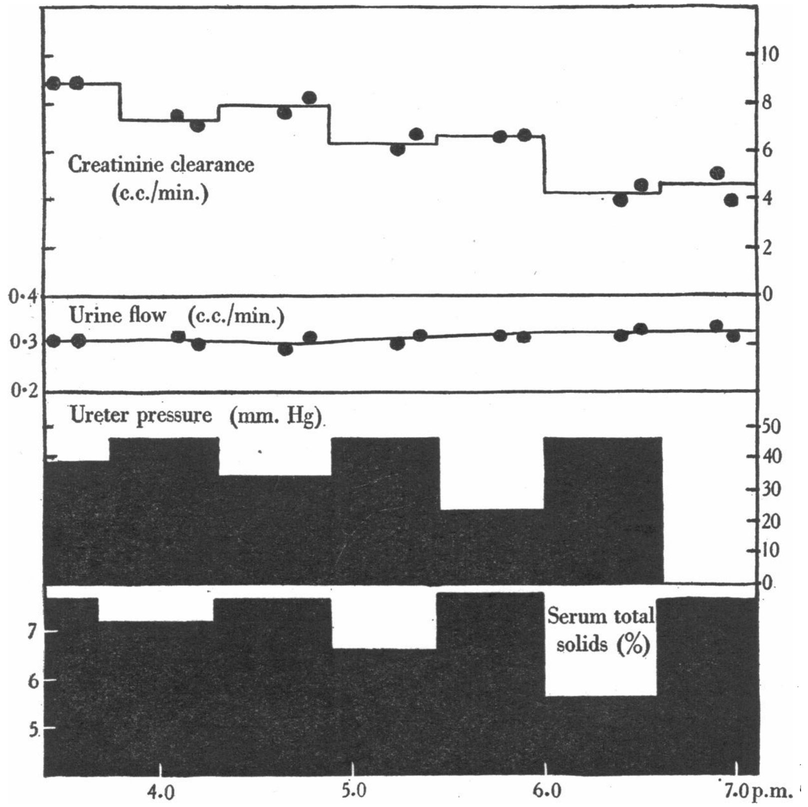

Fig. 6. Isorrheic dilution diuresis in the isolated kidney. Showing relation between ureter pressure and degree of dilution when urine flow is kept constant, and the small decrease in creatinine clearance due to rise in ureter pressure. Double pump-lung single kidney preparation. Kidney weight 20 g. Initial blood flow 135 c.c./min. Arterial pressure 130–132 mm. Hg.

Our observations on isorrheic diuresis in the isolated kidney are summarized in Table IV, all values being reduced to the percentage change per 100 mm. Hg, on the assumption that the change in value is proportional to the change in pressure. This assumption is made for simplicity in presentation, and is only approximately true. It should be

TABLE IV. Isolated kidney. Isorrheic diuresis. Showing the percentage changes in clearances when increase of urine flow due to various diuretic agents was prevented by decrease in arterial pressure or by increase in ureter pressure. Results expressed per 100 mm. Hg change in pressure

	Controlled by changes in arterial pressure	Controlled by changes in ureter pressure		
Diuretic agent ...	Ringer's fluid	Arterial pressure	Ringer's fluid	Urea
Percentage change in				
Creatinine clearance	-13·5 ±15	-47 ±12	-73 ±22	-148 ±15
Urea clearance	+6·6 ±8	-20 ±10	-17 ±20	-144 ±23
Chloride clearance	-80 ±39	-19 ±50	-127 ±53	-24 ±52
Number of observations	9	12	11	4
Mean increase in pressure (mm. Hg)	34	45	38	40

noted that whereas 100 mm. Hg change in ureter pressure probably represents nearly the same change in pressure in the glomerular capsule, a change of 100 mm. in arterial pressure probably represents only 60–65 mm. change in the glomerular capillaries. The salient features of these results are (1) with reference to the creatinine clearance, both arterial and ureter pressure experiments indicate an approach of dilution diuresis to pressure diuresis, but taken together both suggest that this approach may be incomplete and that a small inhibition of tubular reabsorption of water may contribute to the phenomena, (2) with reference to urea clearance, the approach of dilution to pressure diuresis seems complete in both types of experiment, urea diuresis giving widely different values, and (3) with reference to chloride clearance, there appears in both types of experiment to be a smaller output of chloride during dilution diuresis than in pressure diuresis, and the few observations on isorrheic urea diuresis suggest that dilution diuresis involves also a smaller output of chloride than does urea diuresis. The averages of the increases in pressure actually observed are given at the foot of the table, and show that the magnitude of isorrheic diuresis was of the same order in the different classes of experiment tabulated.

Anaesthetized dog. The observations on the kidney *in situ* secreting under isorrheic conditions were planned less elaborately than those on the isolated kidney for reasons described at the beginning of the section on isobaric diuresis in the whole animal. Many of the experiments summarized in Table V were simpler and more direct in design than that depicted in Fig. 7, which was chosen for illustration because it showed in one experiment many of the important features of both dilution and urea diuresis as manifested in both its isobaric and isorrheic forms. Isobaric dilution diuresis is shown to produce only a trivial increase in creatinine clearance, but a more considerable increase in the urea and chloride clearances. A rather severe isobaric urea diuresis, on the other hand, is accompanied by a fall in creatinine clearance, no great change in urea clearance and a rise in chloride clearance.

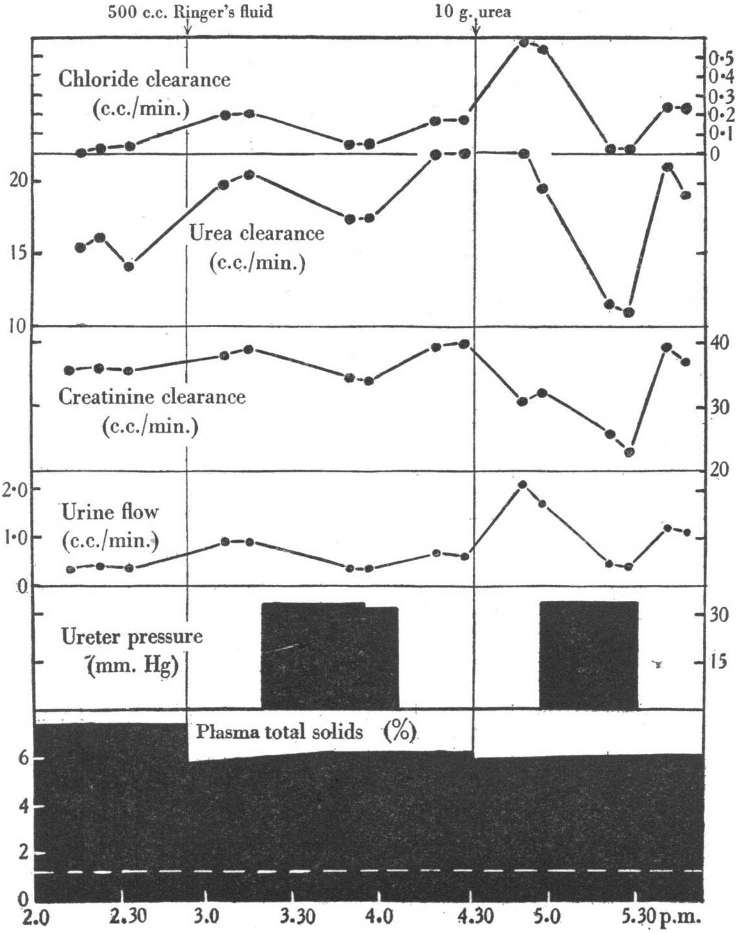

Fig. 7. Isorrheic diuresis in the chloralosed dog. Changes in creatinine, urea, and chloride clearances when the increased urine flow due to (*a*) Ringer's fluid, (*b*) urea, is reduced to the pre-diuretic level by rise in ureter pressure. A broken line is inserted at 1 % total solids, so that the values above this indicate the approximate concentration of plasma protein. Dog 18 kg. Kidney denervated.

The influence of isorrheic dilution diuresis on the composition of the urine is shown in the summary of our observations in Table V. The chief reason for the differences in the decline of creatinine and urea clearances shown under the headings "arterial pressure" and "ureter pressure" is that 100 mm. change in arterial pressure represents only about two-thirds of the change in glomerular pressure which is produced by 100 mm. change in ureter pressure. It is not known whether ureter pressure modifies tubular activity in the whole animal in any way comparable with the small effect of this kind it produces in the isolated kidney. It is clear that if dilution of the plasma is accompanied by a change in pressure, such that there is no change in urine flow, there is reduction in creatinine clearance, and probably also in urea clearance. The changes in chloride clearance are variable.

TABLE V. Anaesthetized dog. Isorrheic diuresis. Showing the percentage changes in clearances in dilution diuresis when urine flow is prevented from rising by a decrease in arterial pressure or by an increase in ureter pressure. Results expressed per 100 mm. Hg change in pressure

	Controlled by changes in arterial pressure	Controlled by changes in ureter pressure
Creatinine clearance	-57 ±17	-75 ±14
Urea clearance	-32 ±21	-75 ±20
Chloride clearance	-44 ±82	+465 ±245
Number of observations	5	8
Mean increase in pressure (mm. Hg)	56	32

The hydrostatic pressure equivalent to a change in the colloid osmotic pressure of the serum

The technique of measuring pressure changes during isorrheic dilution diuresis is sufficiently described in connexion with the experiments charted in Figs. 5–7. These measurements are summarized in Table VI, and for the sake of comparison are expressed as the change in pressure calculated for a change by 1% in plasma protein. The hydrostatic pressures given in the table are thus equivalent in their diuretic effect to a change in colloid osmotic pressure of the plasma of about 3 mm. Hg.

The disproportion between these pressure changes and the change in colloid osmotic pressure is immediately apparent, and corresponds with the disproportion in the extent of the isobaric diuresis due to dilution described above (Table I). The disproportion in the pressure equivalents in comparisons made on the anaesthetized dog might be attributed to the change in tubular activity accompanying dilution diuresis which seemed

TABLE VI. Pressure changes (mm. Hg) required to neutralize the diuretic action of a 1% reduction (e.g. from 8% to 7%) of plasma proteins due to dilution with Ringer's fluid

	Ureter pressure	Anaesthetized dog	
	Isolated kidney	Denervated	Innervated
Pressure increase	21 ±2·1	21 ±4	36 ±12
Number of observations	11	3	5
Average reduction in % concentration of plasma proteins	1·7	1·59	1·34
	Arterial pressure	Anaesthetized dog	
	Isolated kidney	Denervated	Innervated
Pressure decrease	15 ±1·9	18 ±0·3	50 ±3
Number of observations	15	2	3
Average reduction in % concentration of plasma proteins	2·06	2·23	1·5

to be indicated by observations summarized in Tables III and V. In the isolated kidney, however, the observations summarized in Tables II and IV have emphasized that an increase in glomerular filtration appears to be the major factor in dilution diuresis.

To appreciate the significance of the values of the pressure equivalents given in Table VI it is essential to take account of the presence of intrarenal pressure and the fact that it appears to increase during dilution diuresis but not during pressure diuresis [Winton, 1936]. Take, for example, the arterial pressure equivalent in the isolated kidney; the 15 mm. change in the artery would correspond with about 10 mm. change in the glomerular capillaries, but to this reduction in pressure head should be added a value, probably about 10 mm., corresponding to the increase in intrarenal pressure due to dilution of the serum, this 10 mm. acting like a back pressure comparable with that of ureter pressure. If there had been no change in intrarenal pressure, the arterial pressure equivalent might well have been 30 mm. instead of 15 mm. In our observations on the ureter pressure equivalent on the isolated kidney we contrived to prevent such masking of the true pressure equivalents by raising the ureter pressure at the beginning of the experiment above any likely intrarenal pressure, and measuring the further increase in ureter pressure equivalent to serum dilution from this base line. This value of 21 mm. may be a few per cent too high for reasons given elsewhere [Eggleton *et al.* 1940*b*], and our fairest estimate of the change in hydrostatic pressure head across the glomerular membrane equivalent to a change by 1% in serum protein would be 20 mm. Hg in the isolated kidney. The observations on the ureter pressure equivalent in the anaesthetized dog were unfortunately conducted without the precaution of an initial rise in ureter pressure. Since a pre-existing intrarenal pressure would act like a small ureter pressure, whereas when the ureter pressure was raised during isorrheic dilution diuresis this would mask all effects of intrarenal pressure, the values for ureter pressure equivalents may be too high by an amount equal to the intrarenal pressure, if any, in the anaesthetized dog before administration of the diuretic.

Consequently, referring to the values in Table VI, the arterial pressure equivalents are lower than and possibly only one-half the true equivalents, the ureter pressure equivalent on the anaesthetized dog is, if anything, too high, and only the ureter pressure equivalent in the isolated kidney should be accepted as fairly representing the pressure change across the glomerular membrane.

The question as to whether the hydrostatic pressure equivalent is proportional to serum dilution has not been investigated, but the average values of the dilutions actually used were sufficiently alike, as shown in Table VI, in the different classes of our experiments to yield fairly comparable results.

The change in blood flow through the kidney accompanying dilution diuresis

It has previously been observed [Whittaker & Winton, 1933] that when an isolated kidney perfused with defibrinated blood is suddenly transferred to perfusion with Ringer's solution at the same temperature, after a quite transient increase, the Ringer flow settles down to a lower value than the blood flow, and is often as low as one-third its original value. This was attributed [Winton, 1937] to the phenomenal increase in intrarenal pressure which obstructs the outflow of the Ringer's solution from the kidney, and so reduces the effective pressure head, thus masking the consequences of reducing the viscosity of the perfusion fluid from that of blood to that of Ringer's solution.

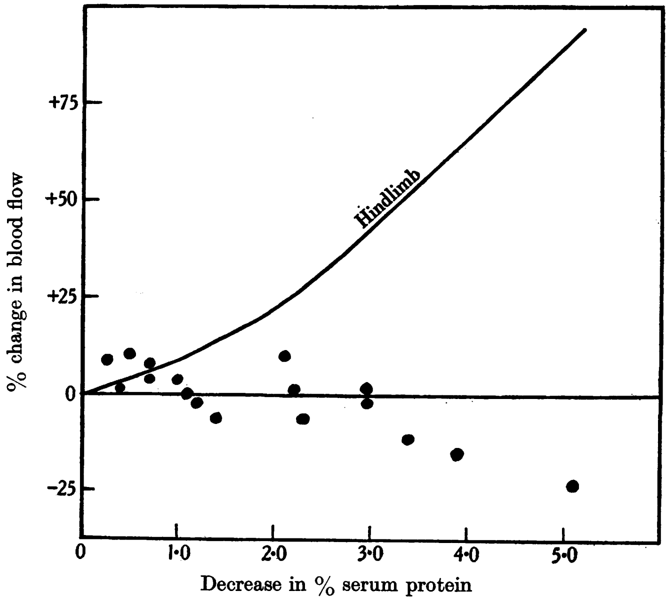

Fig. 8. The percentage change in blood flow through the isolated kidney during dilution diuresis, compared with changes in the hindlimb under similar perfusion conditions.

We have recorded the variations in blood flow in all our experiments on isolated kidneys, and though the effects of dilution of the serum are somewhat variable, it would be fair to say that we usually found very little change in blood flow with moderate dilution, and some reduction in flow with considerable dilution. The characteristic relation is well shown in Fig. 8, in which the change in blood flow through the isolated kidney is contrasted with that in the hindlimb of the dog perfused under similar conditions. The curve for the hindlimb is deduced from data given by Whittaker and Winton, appropriate allowance being made for the change in concentration of serum protein in the fashion indicated by Trevan [1918]. The interpretation of the relation in Fig. 8 would appear to be that a small dilution of the blood may produce a small increase in blood flow, appropriate to the reduction in the viscosity of the blood. If dilution proceeds beyond this, the development of intrarenal pressure obstructs the outflow of blood and thus produces a reduction of pressure head which about compensates for the further reduction in the viscosity of the blood. Beyond a dilution corresponding with a reduction of serum protein by about 3% (e.g. from 8 to 5%) this compensation breaks down, the intrarenal pressure rising more steeply and effectively reducing the blood flow.

DISCUSSION

The disproportionately great effect of serum dilution when the diuresis due to a given change in colloid osmotic pressure is compared with that due to about the same change in hydrostatic pressure across the glomerular membrane has been shown both in the isolated kidney and in the anaesthetized dog. In the isolated kidney the disproportion appears to average about sevenfold, there being good agreement between the measurements made under isobaric and isorrheic conditions. In the anaesthetized dog, while the disproportion appeared about the same or a little less in a few observations under isorrheic conditions, it was greater, amounting to fifteenfold in the more numerous observations on isobaric diuresis. The fact that the disproportion is greater in the organ *in situ* than after isolation is due to the smaller change in urine flow for a given change in arterial pressure; the increase in flow for a given degree of dilution of the plasma is about the same in the denervated kidney as it is in the isolated kidney (Table I). The interpretation of the disproportion appears to be rather different in the two preparations.

In the *isolated kidney*, comparing equal changes in serum colloid osmotic pressure and in hydrostatic pressure in the glomerulus, dilution produces not only a sevenfold increase in urine flow but about a sixfold increase in creatinine clearance. Moreover, if the increase in urine flow due to dilution be prevented by reducing the arterial pressure, the isorrheic dilution diuresis is accompanied by a hardly perceptible reduction in creatinine clearance. Now Shannon & Winton (1940) have shown that the creatinine clearance is not a safe guide to the glomerular filtration rate in the isolated kidney, particularly at high U/P ratios. In the experiments described in this communication, however, the U/P ratios were below 40, and, therefore, within the range over which creatinine and inulin U/P ratios are identical. It would seem fair, therefore, to adopt the creatinine clearance as at least a qualitative guide to the rate of glomerular filtration. If so, it is clear that the chief reason for the unexpectedly great diuretic action of serum dilution is an unexpectedly great increase in glomerular filtration, supplemented in quite a minor way by a change in tubular activity, involving a smaller reabsorption of water.

A possibility may be envisaged, that the excessive diuresis following dilution of the blood is due to an increase in glomerular capillary pressure, comparable with that produced by caffeine, without change in renal arterial pressure. This possibility can be excluded for two reasons: (1) diuresis due to considerable dilution could often only be explained by a rise of glomerular pressure well above the arterial pressure, and (2) isorrheic diuresis would be accompanied by a reduction in the viscosity of blood in the vasa afferentia and efferentia, which for reasons already indicated [Winton, 1937] would if anything lower the glomerular pressure slightly in relation to the arterial pressure rather than raise it.

The reason for this great increase in rate of glomerular filtration in dilution diuresis when the pressure head across the membrane is unchanged must be either that the surface area of the membrane is greatly enlarged or that the permeability of the membrane is correspondingly increased.

Reasons have been given against supposing that the proportion of active glomeruli varies in the mammalian kidney [White, 1939], and the grounds for regarding this proportion as constant in the isolated kidney under our conditions have also been given [Winton, 1937]. In any case, a considerable increase in the number of active glomeruli would give quite a different value of creatinine clearance from that observed in the isorrheic form of dilution diuresis. Increase in the surface area of individual capillaries in the glomerular tuft would, however, produce the required consequences, but such a change seems in conflict with the histological evidence [Brodie, 1914] that during diuresis it is the intracapsular space rather than the glomerular tuft which increases in size.

The hypothesis of a variation in permeability of the glomerular membrane has fallen into disrepute because it has commonly been used as a cloak for ignorance of the mechanism of diuresis, and entirely without specific reference to the pressure-flow relation across the membrane. Dilution diuresis in the isolated kidney, however, has now been shown to involve a large increase in filtration rate in circumstances involving little change in filtration pressure (isobaric diuresis), whereas a large reduction in filtration pressure is required to prevent the filtration rate changing from its prediuretic value (isorrheic diuresis). This describes a change in pressure-flow relation which may fairly be taken to define an increase in permeability to water of the glomerular membrane.

When ultrafiltration is proceeding across the glomerular membrane at its usual high rate, the layer of serum in immediate contact with the membrane must contain protein in higher concentration than the average protein concentration of the serum in the capillary. Such a highly concentrated layer would presumably present considerable obstruction to ultrafiltration comparable with that encountered in ultrafiltration through artificial membranes. If so, dilution of the serum might produce a reduction in this obstruction and a fall in pressure head greatly in excess of the small theoretical change associated with the change in colloid osmotic pressure. It is not certain, therefore, that the increase in permeability due to dilution of the serum involves a change in the properties of the cells constituting the glomerular membrane.

The variations in the urea clearance during dilution diuresis will be discussed in a future communication devoted to an analysis of the urea/creatinine clearance ratios in various forms of diuresis. The chloride clearance is substantially lower in dilution diuresis, in both its isobaric and isorrheic forms, than in a corresponding pressure diuresis; this is so in spite of the increase in chloride content of the serum which accompanies dilution produced, as it has been in our experiments, by adding Ringer's solution to the blood. It would seem that such dilution has a direct effect on the tubule cells, promoting reabsorption of chloride, but whether the effective stimulus is the reduction of colloid osmotic pressure or some slight change in ionic balance in the serum cannot certainly be decided.

In the *anaesthetized dog* the justification for regarding the creatinine clearance as a measure of the rate of glomerular filtration is supported by the identity of the inulin and creatinine clearances at all U/P ratios [Shannon & Winton, 1940]. In isobaric dilution diuresis there may be no change in creatinine clearance, or there may be an increase which is, however, considerably smaller than that accompanying pressure diuresis of the same magnitude. The creatinine clearance in considerable isobaric urea diuresis falls, presumably owing to the development of intrarenal pressure, though this has not been measured in the anaesthetized animal. It is evident that dilution diuresis in the whole animal involves a much greater change in tubular activity than in the isolated kidney. The inhibition of tubular reabsorption of water is a factor in the diuresis at least as important as, and possibly more important than, the increase in the formation of glomerular filtrate.

This interpretation is confirmed by those observations on isorrheic diuresis in which dilution of the blood was accompanied by a suitable reduction in arterial pressure, there being a substantial fall in creatinine clearance although the urine flow is unchanged. The significance of the observations in which a rise in ureter pressure counteracted the diuretic influence of blood dilution is doubtful, because no information is yet available about the direct effect of ureter pressure on tubular activity in the anaesthetized animal.

It has become usual in recent years to regard the phenomena of water diuresis and of dilution diuresis as entirely distinct, the former being attributed to inhibition of the tubular reabsorption of water, and the latter to an increase in glomerular filtration. As we have now demonstrated that there is an inhibition of tubular reabsorption of water which is a major factor in dilution diuresis, this distinction is no longer so clear cut.

It should be emphasized that this tubular factor is detected by contrasting dilution with pressure diuresis, and the changes in tubule activity concomitant on increased glomerular filtration in pressure diuresis are not concerned in the evaluation of this factor. The question whether the effect of blood dilution on the tubules is due to a change in colloid osmotic pressure, and if so whether this effect is a direct one on the tubule cells or an indirect, involving possibly some hormonal mechanism in the animal, is under investigation by our colleague Dr L. Podhradszky.

The changes in chloride clearance during dilution diuresis are again rather different in the anaesthetized dog from those found in the isolated kidney. In the whole animal there is in isobaric diuresis an increase in chloride clearance nearly as great as that in pressure diuresis, but since this is accompanied by an increase in creatinine clearance which is much smaller than that in pressure diuresis, we may infer an inhibition of reabsorption of chloride as a consequence of blood dilution. A similar inference may be drawn from our observations on isorrheic dilution diuresis in the anaesthetized animal.

As disillusion has so often followed the most convincing advocacy of renal theories, it may be well, in conclusion, to summarize those of our results which can easily be expressed in a form independent of theoretical beliefs, even the belief that the creatinine clearance is related to the rate of glomerular filtration. We may set out (Table VII) the characteristic changes in the composition of the urine induced during dilution diuresis, compared with the standards of reference we have chosen, namely, pressure diuresis and urea diuresis. Each figure in the table represents the difference between the values for dilution and pressure diuresis in respect of one variable, expressed as a percentage of the corresponding difference between the values for pressure and urea diuresis. Those who believe that pressure diuresis is primarily due to increase in glomerular filtration, or who define "glomerular diuresis" in this way, and that urea diuresis is primarily due to change in activity of the tubules, or who define "tubular diuresis" in this way, would interpret the values in Table VII as giving a guide to the magnitude of the tubular factors operating in dilution diuresis.

TABLE VII. The difference between pressure and dilution diuresis expressed as a percentage of the difference between pressure and urea diuresis (from data in Tables II–V)

	Isolated kidney		Anaesthetized dog
	Isobaric diuresis	Isorrheic diuresis	Isobaric diuresis
Creatinine clearance	19	26	42
Urea clearance	24	0	42
Chloride clearance	83	—	17
Total osmotic work	12	—	—

Summary

1. The mechanism of dilution diuresis has been investigated in the anaesthetized dog and pump-lung-kidney preparation by comparison with pressure diuresis and urea diuresis, both under isobaric conditions, i.e. when the pressure head is not changed, and under isorrheic conditions, i.e. when the urine flow is kept constant by suitable adjustments of arterial or ureter pressure.

2. Dilution diuresis (isobaric) due to a given change in colloid osmotic pressure of the plasma is disproportionately greater than pressure diuresis due to an equivalent change in hydrostatic pressure across the glomerular membrane. The disproportion is about sixfold in the isolated kidney and fifteenfold in the anaesthetized dog (Table I).

3. The increase in urine flow due to a reduction in colloid osmotic pressure of the serum of 3 mm. Hg can just be prevented by a mean

increase in ureter pressure of 21 ± 2 (11) mm. Hg in experiments on the isolated kidney so designed as to prevent interference by changes in intrarenal pressure. Disproportionately high arterial and ureter pressure equivalents were also found in isorrheic diuresis in the anaesthetized dog (Table VI).

4. Comparisons of changes in creatinine and urea clearances in both the isobaric and isorrheic forms of diuresis due to dilution, pressure and urea show that dilution diuresis in the isolated kidney is mainly due to increase in the rate of glomerular filtration, whereas in the anaesthetized dog a change in tubular activity involving reduction in the reabsorption of water is a major factor (Tables II–V).

5. In spite of the increase in plasma chloride concentration after administration of Ringer's solution, there is an increased reabsorption of chloride in the isolated kidney. In the anaesthetized dog, on the other hand, dilution diuresis involves diminished reabsorption of chloride.

6. The disproportionately increased glomerular filtration rate during dilution diuresis in the isolated kidney is attributed to increased permeability to water of the glomerular membrane, possibly owing to lessening of an obstruction due to protein in the layer in immediate contact with the membrane, the protein being highly concentrated because left behind during rapid ultrafiltration.

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