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GLOMERULAR FILTRATION AFTER OBSTRUCTION OF THE URETER

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Since its introduction by Malvin, Wilde & Sullivan in 1958 'stop-flow' analysis has been widely used to localize reabsorptive and secretory processes and the site of action of diuretics in the nephron. The concentration patterns revealed by this method are not always easy to interpret, one of several reasons being that it is uncertain to what extent they are affected by the continued passage of glomerular filtrate into the renal tubules during the period of occlusion. Hungarian workers in particular have long maintained that after ligation of the ureter glomerular filtration continues at one quarter to two thirds the normal rate (Jancsó, 1955), and stop-flow analysis has been condemned as 'useless' on these grounds (Foeldi, Papp & Koltay, 1958).

We have attempted to estimate, by a modification of the original stop-flow procedure (Malvin, Wilde & Sullivan, 1958), how long and at what rate glomerular filtration will continue in the dog's kidney if its ureteric outflow is suddenly obstructed during intense osmotic diuresis. Substances believed to enter the renal tubules only by glomerular filtration were injected intravenously at various times after blocking the ureter. The subsequent changes in plasma concentration were determined, as well as the total amount of these substances appearing when flow recommenced ahead of urine derived from filtrate formed after release of the obstruction. From the data the rate of entry of glomerular filtrate during the period of occlusion was calculated. The distribution of the reference material in the early (or 'distal') and late (or 'proximal') samples was also considered. In addition, the pressure changes in the ureteric catheter above the obstruction were recorded. A few experiments were performed in a somewhat different manner in order to study the effect on glomerular filtration of obstructing the outflow from the kidney at lower rates of urine flow.

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METHODS

Bitches weighing from 10 to 20 kg were used. They were anaesthetized with morphine tartrate 2 mg/kg subcutaneously, followed 40 min later by intravenous injection of a mixture of 50 mg/kg of chloralose (Roche) and 500 mg/kg of urethane dissolved in normal saline. The solution is strongly hypertonic and caused mild osmotic diuresis, with resting urine flows of 0·2–1·0 ml./min.

Through a flank incision the left ureter was cannulated retro-peritoneally with fine plastic tubing, which was advanced until the upper orifice lay 2 or 3 mm below the hilum of the kidney. In some experiments the right ureter was also cannulated, but in the majority the right kidney drained through a self-retaining catheter inserted into the bladder.

All injections were made into the left external jugular vein and slow sustaining infusions entered by the same route. Hypertonic solutions given at fast rates were infused through polythene tubing introduced into the abdominal vena cava via the right femoral vein. Arterial blood samples were taken from the right carotid, and blood pressure was recorded from the right femoral artery. Mercury or capacitance manometers were used to record arterial and ureteric pressures. Heparin (Liquemin, Roche, 100 u./kg approx.) was used as anticoagulant.

The routine followed in all experiments was based on the method of 'stop-flow analysis' described by Malvin, Wilde & Sullivan (1958) but differed from their procedure in various ways according to the type of experiment.

(1) *Experiments to demonstrate filtration during ureteric obstruction.* Diuresis was produced by infusion of 20% mannitol in 0·85% NaCl solution at a constant rate of 8–12 ml./min, depending on the size of the animal. No other chemicals were added to the infusion, except para-aminohippurate (PAH), 0·045 g/100 ml. on three occasions only, after 0·15 g PAH had been given as a priming injection. When the urine flow from the left kidney had reached a steady level exceeding 7 ml./min the ureteric catheter was clamped for 8 min. At the moment of occlusion (zero time in Fig. 1) 1·0 g creatinine in 10 ml. saline was rapidly injected into the jugular vein; 0·7 or 0·8 g inulin in 10 ml. saline was rapidly injected 2 or 4 min after the start of occlusion; and a slow steady injection of 1·0 g sodium thiosulphate in 50 or 100 ml. saline was begun 6 min 45 sec after zero time and completed 15 sec before release of the occlusion. Immediately following this, thirty serial urine fractions of 0·4–1·1 ml. each were collected in 1½–2½ min. From the moment of occlusion onward thirty-eight to forty serial blood samples of *ca.* 2 ml. were taken, every 5 sec during the first half minute following each injection of creatinine or inulin, and at gradually increasing intervals thereafter. The last sample was taken at the end of the collection of urine fractions. Before and after the period of occlusion several timed 'free-flow' urine specimens and corresponding blood samples were collected for clearance determinations.

(2) *Experiments to estimate the rate of residual filtration.* These were similar to those described above except for the following modifications. Diuresis was induced by infusion of 23% mannitol, or alternatively, of 10% mannitol and 15% glucose in normal saline, without addition of other chemicals. If glucose was used a priming injection of 10·0 g dextrose in 25 ml. saline was given before starting the infusion. The ureteric outflow was obstructed for 11 min and the pressure in the ureteric catheter was registered throughout this period by a mercury manometer with minimal fluid displacement. One gram creatinine in 10 ml. saline was rapidly injected exactly 6 min after the start of occlusion and serial blood sampling was begun at the same moment, intervals being staggered as described above. A slow injection of 0·7 or 0·8 g inulin in 50 ml. saline was begun 9 min 45 sec after the start of occlusion and completed 30 sec before release of the obstruction. Thirty fractions were collected in 2 min and the last of twenty-four blood samples was taken at the end of this period.

(3) *Control experiments.* In order to study the stop-flow pattern for creatinine which

developed when plasma creatinine concentration was not changing during the period of ureter obstruction, control experiments were performed. In these a steady raised plasma level was maintained by continuous infusion of creatinine, but no injections were made at the start or during the occlusion period apart from the 'new filtrate marker' (sodium thiosulphate, or inulin) which was given just before the release of the block, as usual. Blood was sampled only during the clearance periods before and following occlusion. For comparison with the first type of experiment another five dogs were given an intravenous priming injection of 0·7 g creatinine in 10 ml. saline, and 0·2 g creatinine/100 ml. was added to the 20% mannitol solution infused subsequently to raise the urine flow. The ureter was clamped for 8 min. Control runs for the second series of experiments described above were performed after completion of the main experimental run on the same animals. The creatinine injected during the experiment proper served as 'priming' injection for the control run. After collection of all experimental samples the infusion fluid was changed to one containing 0·2 g creatinine/100 ml. in addition to mannitol, or mannitol and dextrose, in the same concentration as before. Approximately 15–20 min after the end of the first run the ureter was again clamped for 11 min. Finally, a 'new filtrate' marker was injected and a second set of urine fractions was obtained in the usual manner.

(4) *Experiments to estimate filtration following ureteric obstruction at low rates of urine flow.* Both ureters were cannulated and the simultaneous output of the two kidneys was compared. A priming dose of 1 g creatinine in 25 ml. saline was injected intravenously and a constant plasma level was maintained by a sustaining infusion of a solution of 5% creatinine in saline, given at the rate of 0·35–0·50 ml./min. The left ureter was blocked 30–40 min after the priming injection. The duration of occlusion varied. The right kidney drained freely throughout. Inulin, and on some occasions sodium thiosulphate, was injected slowly starting 75 sec before the end of the occlusion period, to mark filtrate formed after the release of the obstruction. Fifteen to thirty fractional urine samples were collected, the time necessary to obtain adequate volumes varying with the rate of flow. After a brief interval a second experiment was performed in which the duration of occlusion was a third of that in the first run. Blood samples were taken at the mid points of preliminary and final 'free-flow' periods, and at regular intervals of 6 or 10 min during the rest of the time.

Chemical analyses. The volume of urine fractions was determined by weighing. Creatinine in plasma and urine was determined colorimetrically, as the alkaline picrate. Inulin in plasma and urine was estimated by the resorcinol method of Schreiner (1950). If glucose is present this method gives high plasma and urine blanks. By using yeasted samples, and by control occlusions without injection of inulin it was ascertained that despite the high blank the method was adequate for urine analysis; the arrival of the first trace of inulin in the urine samples also was recognizable without difficulty. The method was found not to be sufficiently accurate for determination of inulin in plasma in the presence of glucose and was not used for this purpose. Sodium was determined with an EEL flame photometer. Thiosulphate in urine was determined according to the method described by Gilman, Philips & Koelle (1946). This substance was used infrequently. As only 90 sec or less elapsed between injection of the material and release of the ureter any effective 'shortening' of the occluded fluid column which might have been caused by its being secreted into the tubules was probably negligible.

Calculations

The occluded volume, or renal 'dead space'. The serial urine samples emerging after the release of the ureteric block were of three kinds: samples derived entirely from glomerular filtrate formed *before* or *during* the period of ureteric block; samples derived entirely from 'new' filtrate which had passed into the nephrons after the injection of the final filtrate marker (inulin or thiosulphate); and samples which were mixtures of urine derived from both types of filtrate. The first kind contained none of the final filtrate marker (0–13·8 ml. in Fig. 2); the second kind contained it in the same (or a slightly higher) concentration as the

'free-flow' specimens collected after the fractions (18·4–20·6 ml. in Fig. 2); the third kind contained the marker at lower concentrations (13·4–18·4 ml. in Fig. 2). The part v_n of a mixed fraction of volume v_m which consisted of 'new' filtrate was calculated as

$$v_n = v_m \frac{c_m}{c_M},$$

where c_m = concentration of 'new filtrate' marker in the mixed sample, and
c_M = concentration of 'new filtrate' marker in urine derived wholly from 'new' filtrate.

The residue, $v_o = v_m - v_n = v_m(1 - c_m/c_M)$ was derived from filtrate formed *before* or *during* the period of occlusion. For example, in Fig. 2 sample 24, which brought the accumulated volume to 16 ml., contained 22 m-mole $Na_2S_2O_3$/l. In later samples the concentration rose to a steady value of 50 m-mole/l. Thus the fraction of new filtrate in sample 24 was 22/50 = 0·44. This calculation is valid only if (*a*) the plasma concentration of the final marker remained constant during the relevant period, and (*b*) if all urine fractions containing it were concentrated to the same extent during their passage through the tubules.

During the 2–2½ min spent in collecting thirty urine fractions there was usually a just detectable fall in the plasma concentration of the final filtrate marker. Usually only the last twelve to sixteen urine fractions contained the marker. Because of delay time these must have come from filtrate formed during the first 1–1½ min after release of the block. The decline of plasma concentration during this short interval was negligible. We have no direct evidence on whether or not all fractions were concentrated to the same degree. However, it was improbable that large differences arose within the 60–100 sec between the appearance of the first and last of the fractions containing the marker, since these late fractions, in contrast to the first three to five samples, all emerged at the same rate of flow.

The total 'occluded' volume V_o was the sum of all fractions of urine derived from filtrate formed *before* or *during* ureteric obstruction.

The amount of creatinine and/or inulin excreted in the occluded volume. The total creatinine or inulin content of all fractions consisting wholly or partly of 'occluded' urine was determined, and the amount due to admixture of 'new' (i.e. formed after flow recommenced) filtrate urine subtracted (see Fig. 2). The residue must have entered the tubules before the 'new filtrate' marker was injected.

The admixture of inulin or creatinine due to 'new filtrate' was calculated as $\Sigma v_n . U$, where
v_n = urine derived from 'new' filtrate in the mixed fractions, and
U = concentration of creatinine (or inulin) in the first fraction which was derived wholly from 'new' filtrate.

This was based on the following reasoning: 'New' filtrate contained inulin or creatinine at the concentration existing in plasma at the time of its formation. From analysis of timed blood samples (Fig. 1) it was known that the plasma concentration of the material remained virtually constant during the brief interval in which the 'new' filtrate for all mixed and the early unmixed fractions must have entered the nephrons. As before, it was assumed that all were concentrated to the same degree during their passage through the nephrons. Hence, if the portion v_n of a mixed sample had emerged from the ureter uncontaminated by occluded urine it would have contained creatinine or inulin in the same concentration as the first fraction which was wholly derived from 'new' filtrate.

The total inulin or creatinine present in the total occluded volume was obtained by summation of the amounts present in the 'occluded' fractions, and designated Q.

In the case of creatinine, Q included an unknown quantity q of endogenous creatinine which was already present in the nephrons at the moment of occlusion of the ureter. This could not be measured directly. An estimate of its magnitude was made according to the formula

$$q = 1{\cdot}2 \times V_o \times \frac{U_I}{100},$$

where V_0 = total occluded volume, and

U_1 = concentration of endogenous creatinine in 'free-flow' urine collected immediately before the start of occlusion.

The values found indicated that endogenous creatinine accounted for from 1·5 to 9·6% of the total recovered in the 'occluded' urine, according to the type of experiment. This is likely to be an over-estimate rather than the converse, for the following reasons:

(1) The recovered occluded volume V_0 was probably larger than the volume of fluid present in the nephrons at the moment of occlusion;

(2) Study of stop-flow patterns for creatinine in control experiments on the same animals during which the plasma creatinine level was maintained at a *steady* raised level showed that the *average* creatinine concentration for all fractions of 'occluded' urine was always less than 1·2 times the concentration in the free-flow specimen collected just before occlusion. The factor 1·2 in the formula above thus represents the upper limit of change. In the same control studies it was also established that no single fractional sample contained creatinine at a concentration as high or higher than twice the free-flow concentration. Hence in those experiments in which creatinine was injected during occlusion, samples were regarded as containing exogenous material if the concentration exceeded 2·5 times the free-flow concentration.

Mean plasma concentration of injected creatinine and/or inulin. The concentrations of the test materials in the serial plasma samples were plotted against time in seconds (Fig. 1). The area below the curve between time 0 (instant of injection of test material) to time t (instant of release of ureteric block) was equal to $\bar{c}t$, $\bar{c}$ being the mean plasma concentration.

Rate of glomerular filtration during occlusion of the ureter. For the purpose of estimating the volume of filtrate which entered the 'occluded' kidney certain assumptions were made which are discussed in the text. If the test materials (e.g. inulin in Figs. 1 and 2) were injected after an interval of 2 min or more had elapsed since the start of ureteric obstruction, the rate of residual filtration, RFR was calculated as follows:

$$\text{RFR} = \frac{Q-q}{\bar{c}t} \times 60 \text{ ml./min} \quad \text{(symbols having the same meaning as before).}$$

This tended to underestimate the true RFR, first because the values of q used may have been too large, as discussed above, and secondly because t was the time in seconds between injection of the test material and release of ureteric block, and not between injection of test material and injection of final filtration marker. An error, for which no correction could be readily applied, must have resulted from the fact that an unknown quantity of the final filtration marker very probably passed through the glomeruli into the tubules during the 75 sec between its first appearance in the blood stream and the release of the ureteric clamp.

In five experiments an estimate was sought of the total volume of filtrate which entered the kidney in the course of 8 min of occlusion. In these experiments, with the time (t) measured in minutes from the beginning of occlusion, creatinine was injected at $t = 0$, and inulin at $t = 2$.

In the first attempts on this problem it was assumed that the filtration rate $F(t)$, declined exponentially to zero from its initial value (F_1) determined during the control clearance period before occlusion. It appeared, however, that on this basis $F(t)$ would be down to about 1% of its initial value at the end of 2 min and the observed excretion of inulin in the subsequent period (see Fig. 2) could not be accounted for. The assumption was therefore made that the filtration did not decline to zero, but to a constant value (F_0), which it attained, for practical purposes, by $t = 2$, i.e.

$$F(t) = F_0 + F_1 e^{-\beta t},$$

which is the general equation of the heavy line in the lower part of Fig. 1. The line thus represents the assumed relation between filtration rate and time. The total filtration during the period of occlusion is given by the area beneath this curve, i.e. by

$$\int_0^8 (F_0 + F_1 e^{-\beta t})\, dt \sim 8F_0 + \frac{F_1}{\beta}.$$

The quantities of F_0, F_1 and β were deduced from the amounts of inulin and creatinine excreted. At any moment, the rate of entry into the occluded volume of either of these substances is given by the product of the filtration rate at that moment and their concentration in the plasma at the same moment; the total quantity in the occluded volume will be the integral of the momentary rates of excretion.

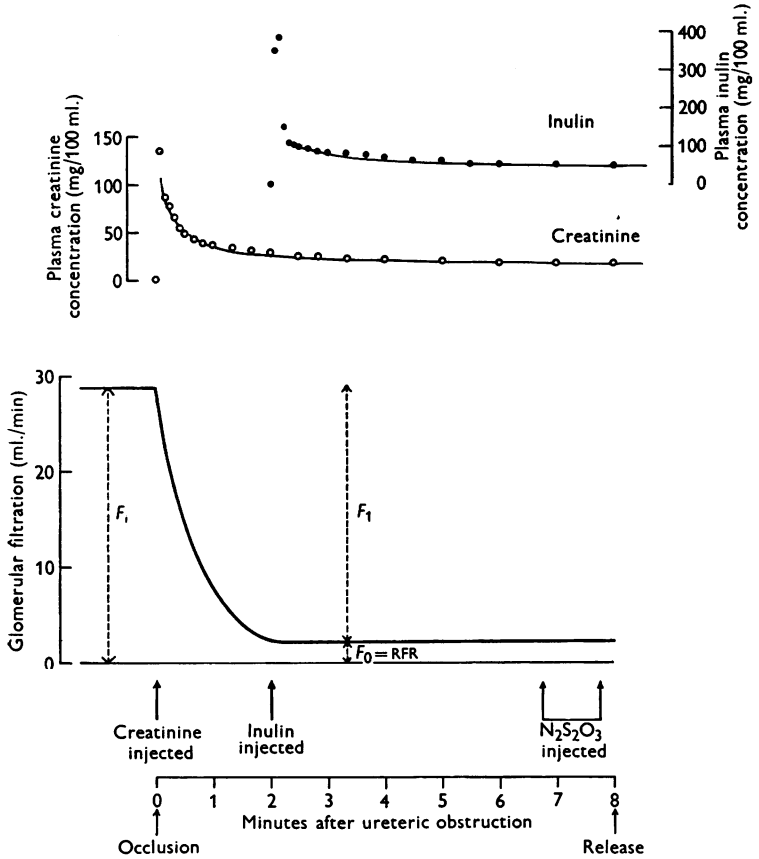

Fig. 1. The period during which the ureter was obstructed in the same experiment as that of Fig. 2. Abscissa, time (t) after ureteric obstruction. The upper curves show the observed concentrations of creatinine and inulin following their intravenous injection at 0 and 2 min respectively.

The thick line shows the assumed course of glomerular filtration during the occlusion period. To calculate total filtration which is the area below this line, it is necessary to know F_0, and how the line falls from F_t (i.e. the exponential β in the text).

The course of the creatinine and inulin concentrations in the plasma subsequent to injection (Fig. 1) was expressed in the forms

$$C(t) = C_0 + C_1 e^{-\alpha t}$$

and

$$D(t) = D_0 + D_1 e^{-\gamma t}, \text{ respectively.}$$

Thus, for inulin

$$Q_I = F_0 \int_2^8 (D_0 + D_1 e^{-\gamma t})\, dt,$$

where Q_I was the amount of inulin excreted, and the terms D_0, D_1 and γ had been determined from the inulin plasma concentration curve (Fig. 1). F_0 was thus obtained, and F_1 was found by subtracting F_0 from the initial filtration rate F_1.

The exponent β was then found, using the data on creatinine, by evaluating the expression:

$$Q_C = \int_0^8 (F_0 + F_1 e^{-\beta t})\,(C_0 + C_1 e^{-\alpha t}) dt,$$

when Q_c was the amount of creatinine excreted, C_0, C_1 and α were known from the creatinine plasma concentration curve (Fig. 1), and F_0 and F_1 had been determined as described above.

Integration of this expression gave

$$Q_C = F_0\left[8C_0 + \frac{C_1}{\alpha}\right] + F_1\left[\frac{C_0}{\beta} + \frac{C_1}{\alpha+\beta}\right],$$

where terms in $e^{-8\beta}$, $e^{-8(\alpha+\beta)}$ and $e^{-8\alpha}$ have been neglected, being small with respect to unity.

One thus obtained a quadratic equation for β

$$\beta^2 - \beta\left[\alpha - \frac{B+G}{A}\right] - \frac{\alpha B}{A} = 0,$$

where $A = [Q_C - F_0(8C_0 + C_1/\alpha)]$, $B = F_1 C_0$ and $G = F_1 C_1$

and the positive root was the quantity sought. The values obtained for β, by this method, were such that the filtration rate did in fact appear to approach a constant value for $t > 2$, thus confirming the reasonableness of the initial assumption. It should be noted that there was no restriction set to the value of β.

The total filtration during the period of occlusion was finally obtained from

$$\int_0^8 (F_0 + F_1 e^{-\beta t})\, dt \sim 8F_0 + \frac{F_1}{\beta}.$$

It is obvious that apart from the assumption of a simple form for $F(t)$, the major simplification involved in this procedure was the neglecting of the short rising limb of the plasma concentration curve; with the sampling frequency employed, this could not, in any case, be accurately defined, although the subsequent course of the concentration could be followed with confidence. In the form assumed, the concentration was taken to be maximal at the instant of injection; the effect of this would have been to make the estimates of filtration slightly lower than the true value.

In some cases the plasma concentration curves were not adequately fitted by the forms given above, and an additional linear trend was incorporated; the method of solution, though somewhat lengthier than that outlined here, was in principle the same.

RESULTS

Persistence of glomerular filtration after obstruction of the ureter

Creatinine injected intravenously immediately after occlusion of the ureter was found after release of the obstruction 8 min later in all serial urine samples, with the occasional exception of the first. Thus 96·3–100 % of the 'occluded' volume was contaminated with material that had crossed the glomerular membrane after the ureter was clamped. One of six

experiments, all of which gave similar results, is illustrated in Fig. 2. The simultaneous concentration patterns for sodium and para-aminohippurate (PAH) are also shown.

When inulin was injected 2 min after clamping the ureter, and allowed to circulate for 6 min before the obstruction was removed, the first 10 %

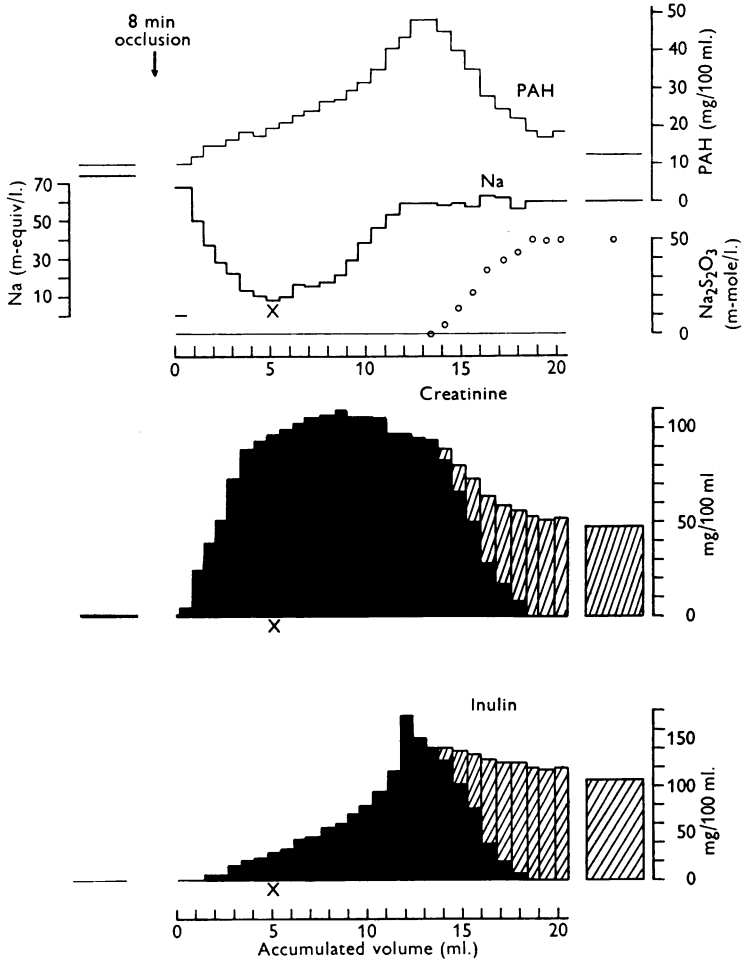

Fig. 2. The creatinine and inulin content of urine collected after 8 min obstruction of the ureter. Infusion of 20 % mannitol in saline containing PAH, at 10 ml/min. Left ureter occluded when urine flow on this side was 9·9 ml./min. 1 g creatinine injected I.V. at the time of occlusion; 1 g inulin injected I.V. 2 min after occlusion; 0·9 g sodium thiosulphate injected I.V., starting 6 min 30 sec after occlusion. Top: Stop-flow concentration patterns for sodium, para-amino hippurate and thiosulphate. Centre and bottom: Creatinine and inulin present in the fractions. Black: filtered during ureteric occlusion. Hatched: filtered after release of obstruction.

of the occluded column of fluid to emerge was inulin-free in four experiments; in the remaining two, inulin was recovered from all fractions. In two further experiments, in which inulin was injected at the mid point of an occlusion lasting 8 min, the distal 10 and 27 % respectively of the occluded fluid were inulin-free.

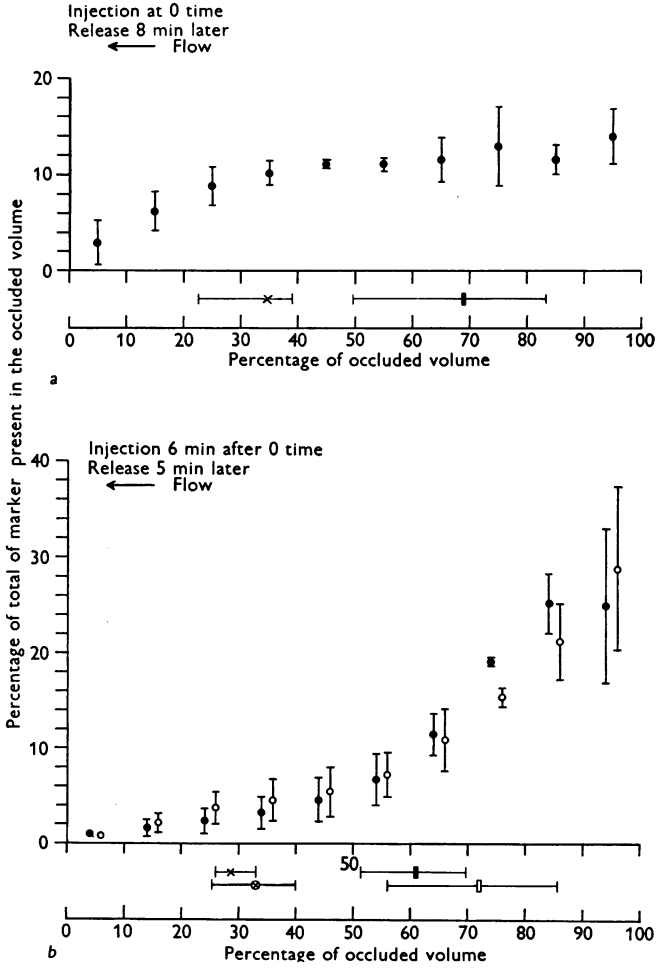

Fig. 3. Distribution in the occluded volume of creatinine injected after occlusion of the ureter. Mean values and standard deviations for groups of six experiments.

(*a*) Distribution of creatinine injected at time of occlusion. Infusion fluid, 20 % mannitol in saline. (*b*) Distribution of creatinine injected 6 min after start of occlusion. Filled circles, 23 % mannitol infused; open circles, 10% mannitol + 15 % glucose infused.

Also shown are: the mean position and range of the point of lowest sodium concentration (crosses) and of the first trace of material derived from filtrate formed after release of occlusion (vertical bars).

If injected 6 min after ureteric occlusion and left to circulate for 5 min before releasing the obstruction, exogenous creatinine likewise appeared in most of the serial urine samples, except the most distal fractions which comprised from 3 to 28 % of the occluded volume. These results showed that substances believed to enter the nephrons only by glomerular filtration continued to do so when the outflow from the kidney was blocked, not only in the initial stages during which ureter pressure rose steeply, but also after it had attained very high levels, as indicated by the records which will be discussed later.

Distribution of the marker in the occluded fluid

Contrary to the usual conventions of stop-flow analysis the urinary concentration patterns of creatinine (or inulin) in this type of experiment could obviously not be taken to reflect the extent of water reabsorption at different points of the nephron. Nor did the quantity of the reference substance found in any particular fraction indicate in how much filtrate it was originally contained, because after injection the plasma creatinine or inulin concentration first rose to a peak and then fell, and the precise moment during the period of obstruction when the material entered the nephron was not known.

If creatinine was injected at the moment of clamping the ureter the first 30 % of the occluded volume to emerge contained, on average, 18 % of the total exogenous creatinine recovered. The remainder was fairly evenly distributed through the rest of the 'dead space', with 25 % of the total coming out in the last 20 % of the occluded fluid (Fig. 3*a*). By contrast, if creatinine was injected 6 min after the start of occlusion not more than 5–7 % of the total found in the occluded volume was in the first 30 %, and almost half was in the last 20 % of the column of occluded fluid as it emerged from the ureter (Fig. 3*b*). Both distribution patterns may give a misleading picture in that they appear to exaggerate the contamination of the distal fractions by filtrate entering during the obstruction compared with samples coming from proximal sites. It is perhaps not unreasonable to assume that the molecules of the marker which had travelled farthest down the nephron were the first to pass across the glomerular membrane after injection, at the time when the plasma concentration was at its peak. If this was so the small quantities of marker which reached the distal sites probably represented very small volumes of filtrate. It follows that of a non-reabsorbable substance present in *constant* concentration in filtrate formed after ureteric obstruction only a small quantity would reach the distal segment, whereas much greater amounts would continue to pass into the proximal third of the occluded tubules.

In one experiment out of six the distribution in the 'dead space' of

inulin injected 2 min after clamping the ureter resembled that of creatinine injected at the moment of occlusion (Fig. 3*a*); in three experiments it resembled that of creatinine injected 6 min after blocking the outflow (Fig. 3*b*); and in the remaining two it was intermediate between these

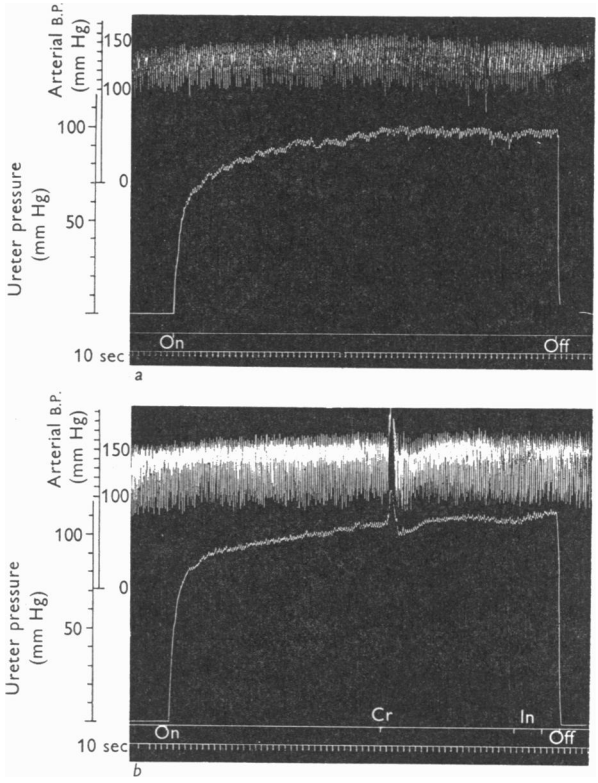

Fig. 4. Typical pressure records obtained from the obstructed ureter (two dogs). In each record, upper trace: femoral arterial pressure, lower trace: ureter pressure. In both experiments 23% mannitol was continuously infused at 10 ml./min, and the ureter was occluded for 11 min.

(*a*) Initial urine flow from left kidney 12·2 ml./min. No injections were made during occlusion.

(*b*) Initial urine flow from left kidney 11·8 ml./min. 6 min after start of occlusion rapid I.V. injection of 1 g creatinine (Cr). On this occasion this caused a transient rise of arterial B.P., which was reflected in the ureter pressure. Final slow injection of 0·7 g inulin (In) caused no disturbance of blood pressure, but a slight rise of ureter pressure.

types. This suggested that the initial phase during which filtrate apparently could still enter rapidly was almost over at the end of 2 min as is shown in the curve in Fig. 1. A change of conditions at about this time was also suggested by the records of ureter pressure.

The pressure in the obstructed ureter

Typical records of ureter pressure are reproduced in Fig. 4. In these tracings four characteristic stages could usually be distinguished. In the first 10–40 sec the pressure above the obstruction rose rapidly to levels ranging from 55 to 95 mm Hg. During the next 30–90 sec the pressure curve became inflected. This was followed by a slow steady pressure rise, with the slope of the curve remaining constant for several minutes. In the

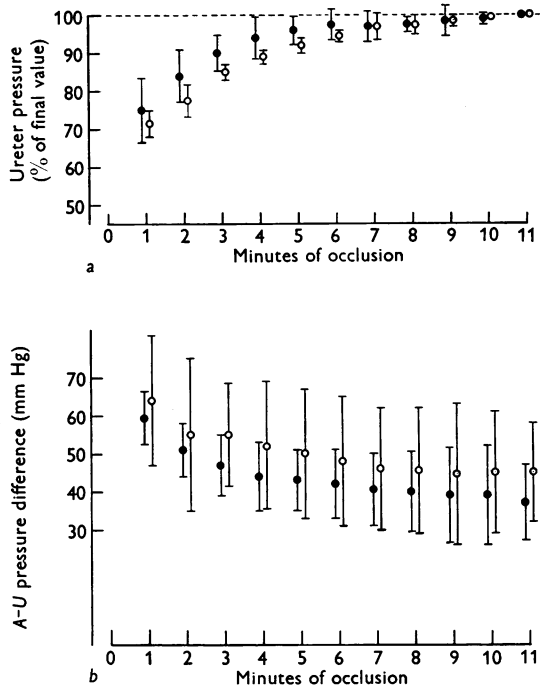

Fig. 5. (*a*) Ureter pressure after occlusion expressed as a percentage of the value attained immediately before release of the obstruction. Mean values and standard deviations. Filled circles, experiments without glucose; open circles, experiments with glucose in infusion fluid. (*b*) *A–U* pressure differences (femoral artery–ureter). Mean values and standard deviations. Same experiments as in (*a*).

final phase the rise of pressure virtually ceased and the curve flattened out. This rarely occurred before the end of the fifth minute of occlusion, and in some experiments this last stage was not reached at all. Any alteration of the mean arterial pressure, whether sudden or gradual, was reflected in the ureter pressure by a change in the same direction but of lesser magnitude. A small upward step in pressure frequently followed upon the injection of each filtration marker.

In Fig. 5 the average minute-to-minute changes in ureter pressure are

expressed as percentage of the value reached at the end of 11 min of ureteric obstruction. The latter ranged from 75 to 148 mm Hg. The final difference between femoral arterial and ureter pressure ranged from 26 to 73 mm Hg. Figure 5 shows that 2 min after occlusion ureter pressure averaged about 81 % and 6 min after occlusion about 95 % of the final height attained. These percentages would have been slightly higher if the terminal injection of inulin or thiosulphate had not been made.

Estimation of the volume of filtrate formed by the obstructed kidney

The plasma clearance by the obstructed kidney in t sec between injection of the material and release of the ureteric obstruction was $C_t = (Q-q)/\bar{c}$ ml; and the apparent mean rate of clearance $C_{\bar{m}} = (Q-q) \times 60/\bar{c}t$ ml./min (each symbol having the meaning explained under Methods).

For creatinine injected at the moment of obstructing the ureter $C_{\bar{m}}$ averaged 4·7 ml./min (range: 3·0–6·2 ml./min). This was 18 % of the plasma clearance during free urine flow. However, in the obstructed kidney $C_{\bar{m}}$ is not a measure of filtration, because the rate of filtration obviously diminishes during the period of occlusion. As is illustrated in Fig. 1, it probably dropped most rapidly in the first minute in these experiments, at the same time as the plasma concentration of creatinine fell steeply from the maximum reached shortly after injection. In these circumstances, in order to calculate accurately the volume of filtrate formed during a given interval of time, an exact knowledge of the time course of the decline in filtration rate is required. In the absence of precise information on this point the assumption was made that the decline was exponential. A further assumption was that from the start of the slow steady rise in ureter pressure onward filtrate entered the nephrons at a much reduced, but approximately constant rate. Accordingly, when creatinine was not injected until 6 min after blocking the ureter the apparent mean plasma clearance $C_{\bar{m}}$ was taken to equal the 'residual' filtration rate (RFR). It must be stressed, however, that the values presented in what follows are to be regarded as estimates rather than precise measurements, and that they depend on premises whose validity has yet to be established.

Calculations based on the foregoing assumptions (see Methods) indicated that from the beginning of the occlusion to the moment of release 8 min later a total of 12–25 ml. of glomerular filtrate entered the obstructed kidney. This was 5·6–10·8 % of the total volume of filtrate that would have been formed in the same time in conditions of free urine flow (Table 1).

If these calculations are valid it follows that the volume of filtrate formed during ureteral obstruction was from 1·3 to 2·2 times larger than the occluded volume. The free-flow creatinine U/P ratios in these experiments ranged from 2·46 to 3·87. Control experiments carried out in comparable

TABLE 1. Mean plasma clearances and estimated total filtration during 8 min of ureteric obstruction. Creatinine injected at instant of blocking ureter; inulin injected 2 min later

		Free flow			Ureteric obstruction						
						Creatinine		Inulin		Estimated total volume filtered	
No.	Body weight (kg)	Urine flow (ml./min)	GFR (ml./min)	U/P creatinine	Occluded volume (ml.)	C_t (ml.) $\frac{Q-q}{\bar{c}}$	$C_{\bar{m}}$ (ml./min) $\frac{Q-q}{\bar{c}t} \times 60$	$C_{\bar{m}}$ = RFR (ml./min) $\frac{Q}{\bar{c}t} \times 60$	% of free-flow GFR	(ml./8 min)	% of vol. in 8 min of free flow
A	10·5	9·17	28·8	2·90	14·96	49·7	6·21	2·36	8·2	23·2	10·0
B	9·9	6·67	25·8	4·29	9·55	24·0	3·00	0·50	2·0	12·1	5·6
C	10·4	8·00	24·2	2·69	12·21	34·4	4·30	1·95	8·0	18·6	10·8
D	14·5	10·00	34·3	3·47	11·50	46·8	5·85	2·38	6·9	25·1	9·2
E	14·5	8·30	20·3	2·42	12·24	34·0	4·25	1·13	5·6	17·8	9·2
Mean	—	—	26·7	—	—	—	4·72	1·66	6·14 S.D. ± 2·54	—	—

conditions (see Methods), showed that with a constant plasma creatinine concentration the average U/P ratios for the total occluded volume were 1·05–1·20 times the free-flow ratios. In the five experiments under consideration in Table 1 this would correspond to maximal U/P ratios ranging from 2·95 to 4·65. It thus appeared that of the total volume of filtrate of which the occluded fluid was the residue between one quarter and three fifths entered the kidney after the ureter was clamped.

Residual filtration in the presence of high ureter pressures

Between the sixth and eleventh minutes of ureteric obstruction residual filtration (RFR) averaged 5·9% (S.D. ± 1·41) of the free-flow glomerular filtration rate (GFR) in six experiments in which diuresis was produced by infusing 23% mannitol. In another six experiments the infusion fluid contained 10% mannitol and 15% glucose, and in this series the residual filtration rate averaged 11·4% (S.D. ± 1·65) of the free-flow GFR. The difference of 5·5% between the mean RFR values in the two types of experiment was very highly significant statistically ($P < 0·001$).

The rate of urine flow, free-flow GFR and creatinine U/P ratio, occluded volume, and arterial blood pressure did not differ systematically in the two series (Table 2). The final ureter pressure averaged 118 mm Hg when mannitol alone was infused, and 95 mm Hg when the mannitol-glucose mixture was given, a significant difference ($P < 0·05$). The final $A–U$ gradient, i.e. the pressure drop between femoral arterial blood and the fluid in the blocked ureter, averaged 37·5 mm Hg in the former, and 45·0 mm Hg in the latter series. The difference between these two values was not statistically significant, and the difference in ureteric pressure was in part a reflexion of the higher average arterial blood pressure in the experiments in which mannitol only was used.

The results revealed a genuine difference between the two types of experiment which was independent of whether the assumptions made in calculating RFR were correct or not. This difference showed that filtration in the occluded kidney was greatly influenced by the composition of the fluid trapped in the nephron, even if this fluid contained much osmotically active solute. The presence of a substance which could be actively reabsorbed from the tubular lumen clearly favoured the continuing entry of new filtrate in comparison with an essentially non-reabsorbable compound. It was interesting that the additional exogenous creatinine that appeared in the occluded fluid if glucose was infused was not confined to the 'proximal' urine fractions, but seemed to diffuse, or flow, into the distal segment also. The distribution of the total recovered material in the 'dead space' was remarkably similar whether or not glucose was used. This is shown by the open and filled circles of Fig. 3*b*.

In phlorrhizinized animals (170–200 mg/kg) the difference between experiments with and without glucose disappeared. The results could not be compared directly with the findings in normal animals because of the profound depression of free-flow GFR by phlorrhizin.

TABLE 2. Residual filtration between the sixth and eleventh minute of ureteric obstruction

		Free flow			Ureteric obstruction					
						RFR		Final pressures (mm Hg)		
No.	Body weight (kg)	Urine (ml.)	GFR (ml.)	*U/P* creatinine	Occluded volume (ml.)	(ml./min)	% of free-flow GFR	A.B.P.	Ureter	*A–U*
				I. 23% mannitol infused						
*N*2	13·8	8·7	22·85	2·64	12·40	1·55	6·8	154	116	38
*N*3	11·8	9·0	26·80	2·98	16·05	1·59	5·9	142	109	33
*N*4	17·0	7·5	24·15	3·26	9·14	1·05	4·3	168	113	55
*N*6	18·5	16·2	45·50	2·80	20·33	1·98	4·3	160	124	36
*N*8	14·3	11·5	38·18	3·32	19·70	2·53	6·6	154	117	37
*N*19	16·2	11·8	34·10	2·89	18·18	2·67	7·8	156	130	26
Mean	15·3	10·78	31·93	2·98	15·97	1·89	5·95	156	118	37·5
S.D.	±2·4	±3·14	±8·91	±0·26	±4·41	±0·62	±1·41	±8·5	±7·6	±9·6
				II. 10% mannitol and 15% glucose infused						
*G*1	15·0	10·2	31·21	3·06	15·57	4·09	13·1	160	87	73
*G*2	11·8	8·0	28·80	3·60	18·00	2·88	10·0	122	75	47
*G*3	15·7	11·5	33·35	2·90	14·98	3·99	12·0	154	118	36
*G*4	13·5	12·0	33·36	2·78	15·42	3·85	11·5	144	102	42
*G*5	12·1	12·2	31·48	2·58	15·76	4·07	12·9	136	92	44
*G*7	19·2	13·0	46·30	3·56	25·08	4·12	8·9	144	116	28
Mean	14·55	11·15	34·08	3·08	17·47	3·83	11·40	143	98	45
S.D.	±2·75	±1·80	±6·22	±0·42	±3·89	±0·47	±1·65	±13·4	±16·9	±15·3
				III. 23% mannitol infused. Hydrochlorothiazide, I.V.						
*C*9	15·5	9·33	30·32	3·25	20·43	3·43	11·3	138	89	49
*C*10	17·8	7·00	20·58	2·94	17·02	1·81	8·8	114	84	30
*C*18	12·2	16·30	33·90	2·08	20·78	3·27	9·6	134	107	27
Mean	15·2	10·9	28·25	2·79	19·41	2·84	9·9	129	93	35
				IV. 23% mannitol infused. Mercaptomerin sodium, I.V.						
*M*11	10·1	10·50	22·36	2·13	11·00	1·03	4·6	126	105	21
*M*13	16·3	10·50	32·23	3·07	19·43	2·84	8·8	150	123	27
*M*16	13·8	11·00	25·66	2·33	15·03	2·04	7·9	150	112	38
Mean	13·4	10·70	26·75	2·51	15·15	1·97	7·1	142	113	29

In a further six experiments drugs known to diminish tubular reabsorption of sodium were used in order to determine whether residual filtration in the occluded kidney was thereby reduced or abolished. In all of them increased excretion of water and sodium and increased urinary sodium concentration in conditions of free urine flow showed the drugs to have been effective. Filtration during occlusion was not measured in the same animals before giving the drugs because a high background of creatinine

and inulin persisting in plasma and urine after the first run impaired the accuracy with which small changes of concentration could then be assessed in the second of two consecutive runs.

In three experiments 10–20 mg hydrochlorothiazide (Esidrex, Ciba) was injected intravenously after full diuresis had developed during infusion of 23 % mannitol; the ureter was blocked 10–20 min later. Contrary to expectation RFR exceeded in every instance the highest single value found in the six experiments without the drug. It averaged 9·9 % of the free-flow filtration rate compared with 5·95 %, but none of the remaining measured variables showed any clear differences (Table 2/III).

The chlorothiazides are known to exhibit antidiuretic properties in certain conditions (Crawford & Kennedy, 1959). As this might conceivably have accounted for enhanced reabsorption of the static fluid in the occluded nephrons, and might thus have promoted the entry of new filtrate, a further three experiments were carried out in which sodium reabsorption was depressed by mercaptomerin sodium (Thiomerin, Wyeth): 2 mg Hg/kg was administered by slow intravenous infusion in the course of 45 min, followed by the usual rapid infusion of 23 % mannitol. The ureter was blocked 40–50 min after stopping the drug infusion. Again RFR was found to be somewhat greater than when no drug was given, averaging 7·1 % of free-flow GFR, but in this case there was some overlap between the groups (Table 2/IV). In no instance did mercaptomerin depress the residual filtration to a value below those found when sodium reabsorption was not inhibited. Neither drug enhanced RFR as much as did glucose.

Occlusion of the ureter during minimal osmotic diuresis

On a number of occasions the ureter was blocked while urine was excreted at much lower rates than in the experiments described so far. The osmotic activity of the anaesthetic by itself was responsible for unilateral flow rates between 0·1 and 0·35 ml./min; they could be increased to 1·0–2·5 ml./min by cautious infusion of 5 % mannitol. In this type of experiment ureteric obstruction was maintained for periods ranging from 18 to 120 min. During this time changes in filtration rate of the contralateral kidney sometimes occurred spontaneously, and sometimes as a result of the experimental procedure. It was therefore necessary to compare the performance of the obstructed and unobstructed kidneys while plasma creatinine concentration was maintained at a *constant* raised level. The mean plasma creatinine clearance of each kidney (as distinct from 'filtration' rate), while one of them was occluded, was expressed as a percentage of the average creatinine clearance in all free-flow control periods. The course of one such experiment is illustrated in Fig. 6. In this

and in most other low-flow experiments the ureter was obstructed twice; on the first occasion occlusion lasted three times as long as the interval which elapsed before ureter pressure reached a constant level; on the second for the length of this interval only.

Sections from a typical ureter pressure tracing are shown in Fig. 7. After a rapid rise to 20–30 mm Hg immediately after clamping, the

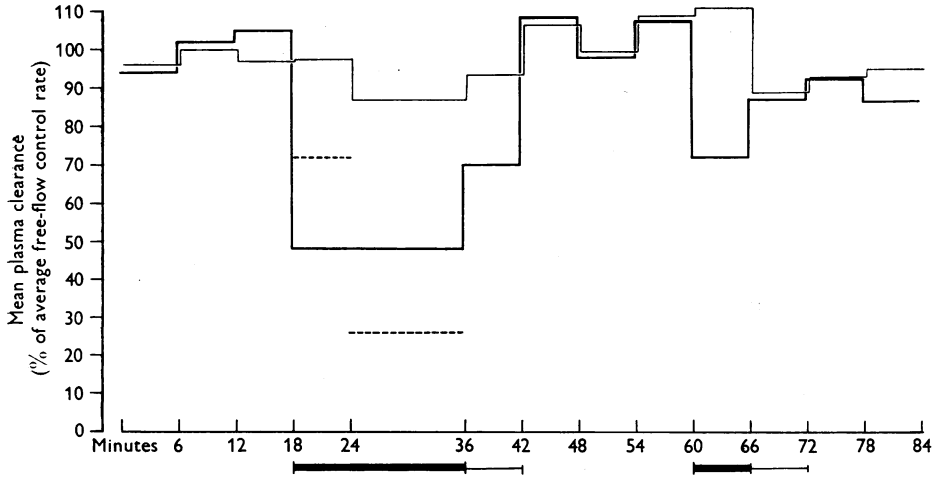

Fig. 6. Plasma creatinine clearance during slight osmotic diuresis in the freely draining and in the obstructed kidney. Thin line: right kidney, draining freely in all periods. Thick line: left kidney, whose ureter was blocked during two periods of unequal length, indicated by the heavy horizontal bars. Thin horizontal bars show periods of collection of fractional urine samples from left kidney. The mean plasma clearance for each period is calculated as a percentage of the average clearance during all free-flow periods for the same kidney. Plasma creatinine kept constant at a raised level by slow continuous infusion. Initial urine flows: left kidney, 2·17 ml./min; right kidney, 2·03 ml./min. Ureter pressure in the left kidney reached a constant level after 6 min obstruction. If on the first occasion mean plasma clearance during the initial 6 min was the same as on the second occasion (72 %), the calculated mean plasma clearance during the remaining 12 min of constant ureter pressure drops to 26 % of the control rate, as indicated by the broken lines.

pressure remained at or near this level for several minutes. It then climbed slowly and steadily and eventually reached a plateau. The time necessary for the level to become constant was greater the slower the rate of urine flow before occlusion. After this the only detectable further changes paralleled changes in blood pressure, i.e. the $A-U$ pressure difference appeared to remain constant indefinitely. It was greater than when the kidney was occluded during maximal osmotic diuresis, varying from 115 to 80 mm Hg. If, after it had remained apparently unaltered for 30–90 min, a fast infusion of 20 % mannitol was begun, ureter pressure

would immediately start to rise again steeply, and the *A–U* gradient was diminished. This phenomenon was first briefly described by Winton (1935) and was seen during micropuncture studies on the unobstructed diuretic kidney by Gottschalk & Mylle (1957). It invariably occurred in the

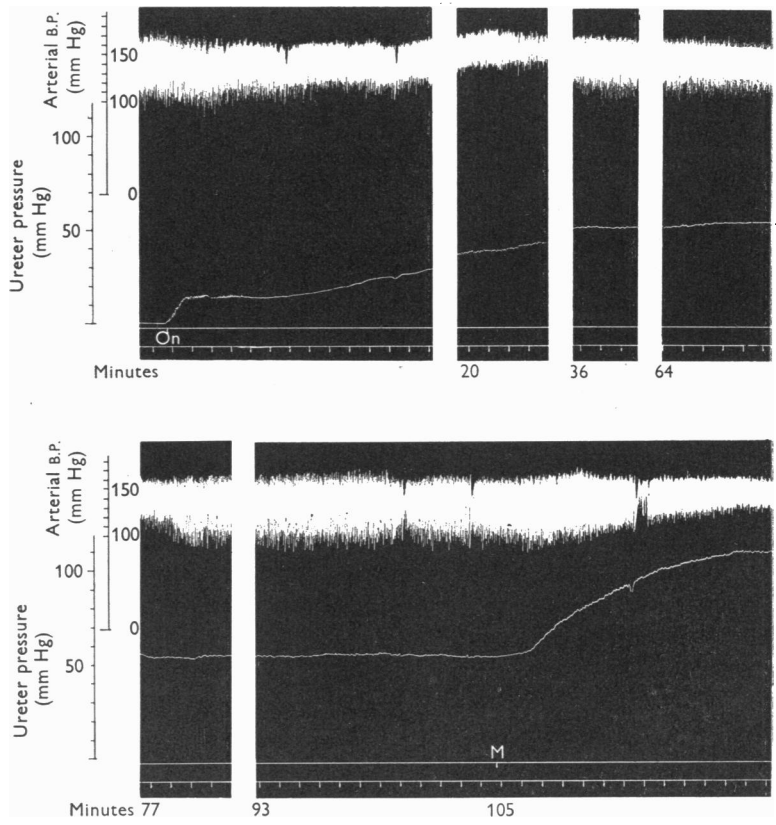

Fig. 7. Obstruction of the ureter during minimal osmotic diuresis. Sections from a continuous pressure record. Upper trace, blood pressure in femoral artery; lower trace, pressure in the blocked left ureter. Urine flow from left kidney before occlusion, 0·18 ml./min. No osmotic diuretic administered except chloralose-urethane mixture given as anaesthetic. 105 min after start of occlusion an infusion of 20% mannitol at the rate of 10 ml./min was begun (M). Ureter pressure began to rise almost at once and eventually levelled out again at a higher value.

present low-flow experiments after injection of the final filtration marker which was begun 90 sec and completed 30 sec before release of the obstruction; as already mentioned, it was seen also in vestigial form if the ureter was blocked during maximal osmotic diuresis.

Owing to the slow rates of urine flow a long time was required to collect, after occlusion, the volume of occluded fluid which was uncontaminated

by the final filtration marker. The latter tended to appear suddenly in relatively high but variable concentration, and in only three out of seven experiments was it possible to differentiate with some degree of confidence between 'occluded' volume and urine derived from filtrate formed after the release of the block. In these three experiments the mean plasma clearance during the long occlusion was approximately one half, and during the short occlusion approximately three quarters, of the average control value for the same kidney (Table 3).

TABLE 3. Mean plasma clearances in minimal osmotic diuresis. $C_{\overline{m}}$ expressed as percentage of average value for all free-flow control periods. Left ureter obstructed

No.	Free urine flow before occlusion (left kidney: ml./min)	Minutes of occlusion	$C_{\overline{m}}$ Left	$C_{\overline{m}}$ Right
*L*3	2·17	18	47·7	92·4
	2·58	6	72·0	111·2
*L*6	0·28	60	50·1	92·0
	0·58	20	69·4	96·8
*L*7	0·20	45	63·1	97·2
	0·18	17	95·6	92·1

These results demonstrated that in a kidney whose outflow is blocked while the urine flow is low filtration may continue at a considerable rate, even at a time when there is no further obvious change in ureter pressure. This conclusion was implicit also in the renewed rise of ureter pressure following injection into the blood stream of non-reabsorbable material. The simultaneous diminution of the $A-U$ gradient indicated that there was now osmotic retention in the nephron of fluid that could be continuously reabsorbed during the preceding phase, in which ureter pressure had remained steady at a lower level. But to exert this effect non-reabsorbable material which is not secreted must clearly be able to pass into the nephron through the glomerular membrane.

DISCUSSION

Ureter pressure and filtration in the occluded kidney

During osmotic diuresis the intratubular pressure in the distended nephrons is greatly elevated compared with the non-diuretic state, and is almost as high in the distal as in the proximal segments. At the same time the pressure in the renal pelvis is not increased, so that the pressure drop across the collecting duct system becomes much steeper. This was shown by Gottschalk & Mylle (1957) in direct micropuncture studies on the freely draining kidney of the rat. If the state of the dog's kidney in osmotic

diuresis resembles that of the rat the very rapid rise of ureter pressure seen immediately after occlusion probably represents the instantaneous equilibration of the low pelvic pressure with the existing high pressure in the proximal and distal tubules, and not, as is usually supposed, the filling up of the system with fluid that cannot escape. When creatinine was injected at the moment of occlusion the amount of exogenous material that found its way into the occluded nephrons was much greater than after later injection, but this does not necessarily imply that the volume contained in the system increased during the first minute or two. If before most of the normal reabsorptive processes came to a standstill the tubules continued for a brief time after ureteric obstruction to reabsorb, as during free flow, 50–60 % of the glomerular filtrate, a large quantity of fresh filtrate charged with injected creatinine could replace the fluid reabsorbed without any change in the total volume of fluid in the nephrons.

The subsequent gradual rise of ureter pressure, on the other hand, is almost certainly due to a slow increase of intratubular volume. It suggests steady accumulation of the non-reabsorbable residue of glomerular filtrate formed at this stage. Without knowledge of the actual glomerular capillary pressure and of the capacity of the system of nephrons it is not possible to tell from the ureter pressure curve and $A-U$ difference alone whether at this time filtration proceeds at a steadily diminishing or at a constant rate. If the rising intratubular pressure reduced the net filtration pressure across the glomerular membrane, or if it depressed the remaining reabsorptive processes which make room for new filtrate, filtration rate would be falling. But filtration could continue at a constant rate if fluid leaked out of the tubules (as discussed below), or if the pressure rise within the tubules was transmitted to surrounding blood vessels, and thence backward to the glomerular capillaries, so that the effective gradient across the membrane remained unaltered. Our findings are in favour of a constant filtration rate at this stage, since the mean plasma clearances of inulin and creatinine were of the same order of magnitude whether injections were made 2 min or 6 min after blocking the ureter.

Ureter pressure and $A-U$ difference eventually attain constant values; how soon, and at what absolute level of pressure depends on the osmotic activity of the filtrate before and during ureteric obstruction. The plateau was usually, but not invariably, reached at about the sixth minute of occlusion during maximal osmotic diuresis. However, the flattening of the pressure curve is not a valid sign that filtration has ceased. For not only do glomerular substances still find access to the tubules at this stage, but the pressure in the ureter can be made to rise further by suddenly raising their concentration in the circulating plasma. This is difficult to explain unless the osmotic activity of glomerular substances on entering

the tubules retains in the nephrons fluid which also entered, but was not so retained during the preceding 'steady' state.

The higher residual filtration rates encountered if glucose was substituted for part of the mannitol in the filtrate were clear evidence that continuing active reabsorption is an important factor determining filtration rate in these conditions. Part of the removal of fluid from the nephrons which permits continued filtration in the occluded kidney appeared however to be due to a physical mechanism, such as the difference in protein concentration between lumen and post-glomerular blood, or to 'distal' water reabsorption. The experiments which showed that inhibitors of sodium reabsorption did not reduce the rate of residual, i.e. 'replacement' filtration, though not conclusive, strongly suggested this.

In all conditions explored some fraction of the newly entering filtrate would normally be left behind in the tubules as non-reabsorbable residue. Thus if ureter pressure can become constant while filtration continues, a state of equilibrium must have been reached, but in what manner is far from clear.

If the tubules increased their capacity at the expense of the blood content of adjoining capillaries and venules the process would be rapidly self-limiting. It may be argued that the rate of deposition of non-reabsorbable material from the filtrate has become so slow that the associated rise of ureter pressure escapes detection in short periods of observation. But it was not possible to detect it even when the occlusion was continued for 50–60 min after an apparently constant pressure was reached during mannitol infusion, and for 120 min when no infusion was given. This failure was not conclusive proof against the hypothesis, since the rise may not have manifested itself because the animals' condition may have deteriorated during these long experiments. In an intact animal in the course of hours or days the pressure might well rise further.

Another possibility is that ureter pressure became constant because the 'non-reabsorbable residue' becomes reabsorbable after obstruction of the ureter. This was suggested and discussed by Winton (1935), who thought that there might be a leak from the distal segment. The distribution of the filtration marker after late injection in our experiments makes it more probable that leakage, if it occurs, is from the proximal tubule. Nor can it be a simple escape of fluid through holes in the tubular wall. The cells of the proximal tubule become thin and flattened when there is a high hydrostatic pressure in the lumen (Brodie, 1914; Gottschalk & Mylle, 1957). This, or stagnant anoxia in the congested kidney, might conceivably destroy their power to prevent selectively the outward passage of certain molecules, so that all the filtrate formed could then be returned to the blood stream by active transport or protein osmotic pressure. However, certain difficulties arise in this context. The postulated loss of selectivity

has to be reconciled with the fact that the tubular wall appears to remain an effective semipermeable membrane with respect to the osmotic activity of the fluid entering the nephrons, and of the protein in the post-glomerular blood. Also, as Winton has pointed out, if all substances become reabsorbable, including inulin and creatinine, filtration rate will be underestimated. The validity of the inulin and creatinine clearance as a measure of filtration at low urine flows is still a matter for debate (Bálint & Forgács, 1960). The depression of these clearances during osmotic diuresis in the freely draining kidney (Mudge, Foulks & Gilman, 1949; Dempster, Eggleton & Shuster, 1956), though open to several interpretations, also indicates that the possible reabsorption of inulin and creatinine in the distended kidney must be seriously considered. However, there is as yet no definite proof that it occurs.

The validity of quantitative estimates of filtration rate in the obstructed kidney

It will be evident from the foregoing discussion that the assumptions made in the calculation of actual filtration rates were no more than working hypotheses, based chiefly on the mean plasma clearances of the injected substances. For creatinine administered at the instant of occlusion $C_{\overline{m}}$ over 8 min averaged 18 % of the free-flow GFR; and 6·1 % over the remaining 6 min of the same experiments for inulin injected 2 min later. When the creatinine injection was made 6 min after the start of occlusion in another group of animals under comparable conditions, $C_{\overline{m}}$ over 5 min averaged 5·9 %. From this it was inferred that the rate of entry of new filtrate into the blocked kidney probably altered very little between the second and the eleventh minutes, and even if it did diminish somewhat toward the end of the period no great error would be introduced by regarding the rate as constant. In the first 2 min, taken by themselves, $C_{\overline{m}}$ must have been much greater than 18 %, but the mean plasma concentration of the marker was high at that time. Therefore the true filtration rate must have been decreasing rapidly at first, and an exponential decline was assumed as most probably correct for the initial phase. It was realized that this would represent the actual course of the change of filtration rate only approximately; but it was considered that any divergence there might be would not substantially affect the values for total entry of filtrate during occlusion which it was desired to estimate.

If in the obstructed kidney creatinine and inulin were not dealt with in the normal way this would invalidate our estimates in a much more unpredictable manner than errors arising from the assumptions on which the calculations were based. That filtration rate might have been *under*-estimated has already been discussed. It might also have been

over-estimated if creatinine were secreted or diffused into the occluded tubules. Despite the initially high plasma concentrations this probably did not occur, as it made no difference whether in the same type of experiment creatinine or inulin was employed; it is not likely that both were secreted. An erroneously high value for filtration would also have been obtained if, owing to congestion, the injected material had lain stagnant in the glomerular capillaries in high concentration, to be swept into the filtrate after release of the ureter at a time when the systemic arterial concentration had fallen to a much lower value. That this was not the true explanation for the higher concentration of the marker in the 'occluded' volume was shown by the fact that the amounts of creatinine which entered it differed greatly in experiments with or without glucose, which were alike in every other respect.

Implications for stop-flow analysis

For the practice of stop-flow analysis it is not without interest that glomerular filtration in the occluded kidney does not stop, even when ureter pressure reaches a steady level, an event which does not take place before the fifth or sixth minute of occlusion. Omachi & Macey (1959), in a brief report not seen by us before our work was completed, have described the presence in occluded fluid of various filtration markers injected successively during a stop-flow experiment. Their findings agree with ours, but in their deductions they do not appear to have taken into account that the *concentration* of the recovered marker is no direct guide to the volume of filtrate in which it was contained. It is apparent from our results that 'flow' during ureteric obstruction is restricted in the main to the upper portions of the nephrons. As during free urine flow, the volume of fluid reabsorbed from the distal segment and replaced from above must be very small in comparison with filtration. The fluid in the distal 30–40 % of the occluded fluid column, which usually includes the region of lowest sodium concentration, appears to be only very slightly contaminated by filtrate that has entered the proximal tubules after the start of occlusion; virtually no filtrate formed after the second minute reaches it. Thus in the distal segment reabsorption and secretion do indeed act on an almost stationary pool.

More proximal sites receive during occlusion progressively increasing amounts of new filtrate. Samples coming from these regions are therefore not in their entirety contemporaneous with the distal samples, nor within their own sequence. The arrival of new filtrate will make secretory processes appear exaggerated and will tend to conceal reabsorption. This can in part be amended by using the ratio $U/P_{(x)}: U/P_{(\text{creatinine})}$ when following the fate of substance x. But this correction does not eliminate inequalities between portions of the contents which are due to differences in time spent

in the tubules. That the interpretation of distal concentration patterns is more reliable and straightforward has, of course, been inferred from indirect evidence and other arguments by several users of the method.

From a study of the creatinine concentration of proximal samples Malvin, Wilde, Vander & Sullivan (1958) obtained the impression that replacement of occluded fluid by fresh filtrate was enhanced by substances like glucose which can be actively reabsorbed. Our findings have confirmed that this is in fact the case. It follows that if the composition of the solution infused is altered between two consecutive experiments performed on the same animal the two runs may not be strictly comparable, because of differences in the amount of filtrate formed during occlusion which will penetrate to a given distal or proximal site.

If the outflow is obstructed when no or only a minimal quantity of an osmotic diuretic is circulating filtration in the occluded kidney continues at such a high rate that stop-flow patterns obtained in these circumstances would appear to be meaningless. By imposing on the ureter from the start of occlusion a pressure close to or higher than arterial pressure, as was recently done by Jaenike & Berliner (1960), this difficulty can probably be overcome.

SUMMARY

1. By a modification of stop-flow analysis the amount and distribution of exogenous creatinine and inulin in the volume of fluid occluded in the dog's kidney were determined. These substances were injected into the circulation at various times after obstruction of the ureteric outflow during maximal osmotic diuresis. The changes in their plasma concentration were followed, and the pressure in the ureter above the obstruction was continuously recorded.

2. Ureter pressure was found to rise rapidly at first and then slowly and steadily. It did not attain a constant level until 5 min after obstruction, or later.

3. The levelling of the pressure curves did not coincide with cessation of glomerular filtration, which was found to continue throughout periods of occlusion lasting up to 11 min.

4. On the basis of specified assumptions the total volume of filtrate entering the obstructed kidney during 8 min of occlusion was estimated to average 9 % of the volume of filtrate that would have been formed in 8 min of free urine flow.

5. No systematic difference in the plasma clearance of filtration markers was detected whether they were injected 2 or 6 min after blocking the ureter. It was inferred that after the second minute of occlusion filtration continued at an approximately constant rate, averaging 5·9 % of the free-flow rate if 23 % mannitol was infused. The rate averaged 11·4 % of the

free-flow value if two thirds of the mannitol in the infusion was replaced by glucose.

6. From 96 to 100% of the occluded fluid was found to contain material that had crossed the glomerular membrane after the ureter was obstructed. The distal 30–40% of the occluded fluid column received a small fraction of the filtrate formed during the first 2 min, and practically none of that formed after the sixth minute. Considerable quantities of new filtrate continued to flow into the proximal third of the 'dead space'.

7. If the ureter was blocked for 18–120 min when no or little osmotic diuretic was circulating the occluded fluid contained up to 75% of the quantity of filtration marker excreted in the same time by the freely draining kidney. Ureter pressure became constant later, and at a lower absolute value than in maximal osmotic diuresis, but this again did not indicate cessation of filtration.

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REFERENCES

BÁLINT, P. & FORGÁCS, I. (1960). Zur Bewertung der Inulinclearance. *Pflüg. Arch. ges. Physiol.* **271**, 23–34.

BRODIE, T. G. (1914). A new conception of glomerular function. *Proc. Roy. Soc.* B, **87**, 571–592.

CRAWFORD, J. D. & KENNEDY, G. C. (1959). Chlorothiazid in diabetes insipidus. *Nature, Lond.*, **183**, 891–892.

DEMPSTER, W. J., EGGLETON, M. G. & SHUSTER, S. (1956). The effect of hypertonic infusions on glomerular filtration rate and glucose reabsorption in the kidney of the dog. *J. Physiol.* **132**, 213–224.

FOELDI, M., PAPP, N. & KOLTAY, E. (1958). Nierenfunction in der Hydronephrose. *Folia angiol.* **5**, 3–14.

GILMAN, A., PHILIPS, F. S. & KOELLE, E. S. (1946). The renal clearance of thiosulfate with observations on its volume distribution. *Amer. J. Physiol.* **146**, 348–357.

GOTTSCHALK, C. W. & MYLLE, M. (1957). Micropuncture study of pressures in proximal and distal tubules and peritubular capillaries of the rat kidney during osmotic diuresis. *Amer. J. Physiol.* **189**, 323–328.

JAENIKE, J. R. & BERLINER, R. W. (1960). A study of distal renal tubular functions by a modified stop-flow technique. *J. clin. Invest.* **39**, 481–490.

JANCSÓ, N. (1955). *Speicherung*, pp. 242–266. Budapest: Akademiai Kiado.

MALVIN, R. L., WILDE, W. S. & SULLIVAN, L. P. (1958). Localization of nephron transport by stop flow analysis. *Amer. J. Physiol.* **194**, 135–142.

MALVIN, R. L., WILDE, W. S., VANDER, A. J. & SULLIVAN, L. P. (1958). Localization and characterisation of sodium transport along the renal tubule. *Amer. J. Physiol.* **195**, 549–557.

MUDGE, G. H., FOULKS, J. & GILMAN, A. (1949). Effect of urea diuresis on renal excretion of electrolytes. *Amer. J. Physiol.* **158**, 218–230.

OMACHI, A. & MACEY, R. I. (1959). Intratubular fluid movement in dog kidney during stop-flow. *Proc. Soc. exp. Biol., N.Y.*, **101**, 386–388.

SCHREINER, G. E. (1950). Determination of inulin by means of resorcinol. *Proc. Soc. exp. Biol., N.Y.*, **74**, 117–120.

WINTON, F. R. (1935). The relation between diuresis and maximum ureter pressure, and its bearing on current methods of estimating the rate of glomerular filtration. *J. Physiol.* **83**, 38–39*P*.