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Intratubular Fluid Movement in Dog Kidney during Stop Flow. (24951)

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The stop flow technic introduced by Malvin, Wilde and Sullivan(1,2,3) provides a convenient method for localizing reabsorptive and secretory activities in the mammalian kidney tubule. Briefly, the technic consists of clamping the ureter for a short period (*e.g.*, 8 minutes) during the steady state that arises from constant infusion of an osmotic diuretic. The intratubular fluid at the end of this period of ureteral occlusion differs from intratubular fluid during free flow because the former has had longer contact with tubular epithelium and, accordingly, its composition reflects reabsorptive and secretory activities to a greater extent. Immediately following the period of stop flow, serial samples of urine are collected and analyzed. The first sample is considered to be representative of fluid which is located most distal to the glomerulus. Each succeeding sample approximates fluid lying in more and more proximal positions. The arrival of fluid that entered the tubules after release of the occlusion is signaled by the presence of an indicator (*e.g.*, inulin) which was injected intravenously during the occlusion period. In interpreting the data so obtained, it has been assumed that the column of tubular fluid is stationary during the stop flow period. The present investigation explored the possibility that the column of tubular fluid does, indeed, move during the period of stop flow as a necessary consequence of water reabsorption. Thus, tubular fluid would be replaced in part by new filtrate throughout the stop flow period. This possibility has been mentioned before(2,4) but the magnitude of the effect may necessitate more attention than has previously been given. To demonstrate movement of tubular fluid during stop flow, we have repeated the technic described by Malvin, Wilde and Sullivan(2) with the modification that 2 or 3 indicators of

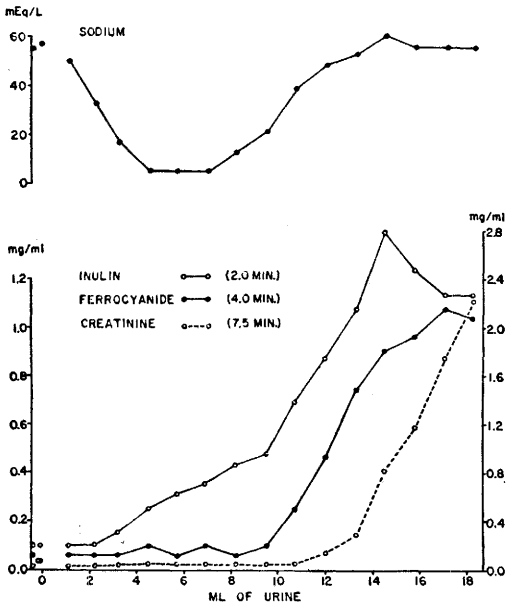

FIG. 1. Appearance of filtration indicators in stop flow samples. Figures in parentheses refer to time of inj. following ureteral occlusion. Left hand scale refers to ferrocyanide and creatinine; right hand scale refers to inulin.

glomerular filtration were injected at different times during the stop flow period. For example, in one experiment creatinine was injected 2 minutes after the ureter was clamped and sodium ferrocyanide was injected a few minutes later before the ureter was reopened. If movement of fluid along the tubule is negligible, creatinine and ferrocyanide should begin to appear in the same urine sample. On the other hand if there is appreciable fluid movement, creatinine should appear earlier than ferrocyanide. Further, the extent of the difference between the appearance of creatinine and ferrocyanide would be an indication of the extent of fluid movement during the period between injections.

Results. Fig. 1 illustrates the type of data we have obtained. In this experiment, 3 indicators were injected at different times during the 8 minute occlusion period. The first indicator, inulin, was injected 2 minutes after occlusion, sodium ferrocyanide was injected 4 minutes after occlusion and, finally, 7½ minutes after occlusion (*i.e.* ½ minute before reopening the ureter) creatinine was injected. Plasma samples taken from the femoral artery established that plasma concentration rose to its maximum at or before 15 seconds following each injection. Concentrations of inulin(5), creatinine(6), and ferrocyanide(7) were determined by conventional methods.

Inulin makes its appearance much earlier than ferrocyanide and ferrocyanide appears much earlier than creatinine. The Na curve (determined by flame photometry) has been included as a landmark. The region where Na concentration falls has been commonly interpreted as distal tubule. In these terms, our data indicates that new filtrate (*i.e.* plasma filtrate entering the tubules after the first 2 minutes of stop flow) has found its way into the distal regions of the tubules. Essentially similar results were obtained in 8 other experiments using various combinations of indicators, such as inulin and creatinine and inulin and ferrocyanide.

We interpret these movements of tubular fluid as being due primarily to water reabsorption by the tubules. It is true that a finite time is required for intratubular pressure to rise immediately after ureteral occlusion to the value existing in the glomerulus and during that time hydrodynamic flow would occur regardless of any water reabsorption. However, the experiments of Malvin, Wilde and Sullivan(2) indicate that ureteral pressure rises to a maximum within 1 to 1.5 minutes after occlusion. Since our first indicator was injected 2 minutes after urine flow was stopped, it would appear that this phenomenon plays an insignificant role in explaining our results.

Gross movement of tubular fluid during stop flow could be anticipated from U/P (urine concentration/plasma concentration) ratios of a continuously administered indicator. Our data as well as that of other investigators have shown that during a typical stop flow of 8 minutes duration, the intratubular U/P ratios vary from 2 to 6. Taking a mean U/P ratio of 4, for example, would indicate that total volume of water reabsorbate during the stop flow is roughly 3 times intratubular volume.

If new filtrate continuously enters the tubules during stop flow, then any change in plasma concentration occurring during or immediately following the stop flow period will

influence the composition of the collected samples. It follows that this factor must be accounted for if the method is to be used to calculate statistical distribution of nephron lengths and total volume of the nephron plus collecting system.

Finally, these results indicate that the actual localization of tubular activity by the stop flow method is perhaps less precise than realized. In particular, any quantitative conclusion that does not account for fluid movement and consequent introduction of new filtrate must be interpreted with caution.

Summary. The movement of intratubular fluid during stop flow has been investigated by injecting 2 or more filtration indicators at different intervals during period of ureteral occlusion. Appearance of these indicators in different samples collected upon release of the occlusion demonstrates that appreciable fluid movement takes place during stop flow.

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Differences between Antigenic Specificity of Nonsoluble Particles and Soluble Extracts Prepared from Brucellae by Disintegration.* (24952)

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Olitzki and Sulitzeanu(1,2,3) demonstrated that soluble antigens obtained by disintegration of Brucellae by sonic vibration or by a disintegrator reacted in agar gels with antisera and produced with them precipitation bands. On the other hand, Sulitzeanu(4) showed that nonsoluble particles resulting after disintegration of the majority of cells and after removing the rest of the intact bacteria by centrifugation, although unable to produce precipitation bands in agar gels, absorb agglutinins, while the soluble antigens do not possess this ability. Since in these experiments only *Brucella suis* was employed, the following experiments were carried out with *Br. suis, Br. abortus* and *Br. melitensis* to ascertain whether the antigens involved in these 2 different seroreactions are specific or not.

Methods. The antigens were prepared by the method of Olitzki and Sulitzeanu(3). From the 2 methods of immunization described by them the subcutaneous method with employment of an adjuvant was preferred. The bacteria were repeatedly disintegrated in a disintegrator (Mickle, Mill Works, Gomshall, Surrey) and remaining intact bacteria and cell walls were removed by centrifugation. For the agglutination test with intact bacteria acetone dried bacteria suspended in physiological saline were employed. 0.04 mg/ml of dried bacterial substance still gave a measurable opacity (O.D. 0.014) at 660 mμ and a visible agglutination. Cell wall antigens were prepared from disintegrated bacteria by centrifugation. At first the intact bacteria were removed by repeated centrifugation at 3400 rpm until no more sediment was produced. Then the supernatant was centrifuged 30 minutes at 9000 rpm. The absence of intact bacteria was verified by microscopical examination. The resuspended sediments gave opalescent suspensions, and

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