Short communications

Determination of the renal clearance of inulin in rats: lowered values at low urine flow rates

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The renal clearance of inulin (Cl_{IN}) was measured in anaesthetized male rats using a continuous intravenous infusion method.

Cl_{IN} was independent of urine flow rate provided this was greater than 0·03 ml/minute.

At flow rates lower than this, Cl_{IN} was reduced and appeared to correlate with the rate of flow.

In experiments where a constant kidney function is required. e.g., pharmacokinetic studies, it is advisable to maintain urine flow rates in the rat above 0·03 ml/minute.

We are currently studying the distribution kinetics of neostigmine bromide in the rat. The clearance of inulin (Cl_{IN}) is monitored routinely in this work to assess renal function. For inulin, the clearance should be independent of plasma concentration and urine flow rate; Harvey & Malvin (1965) reported that in rats there is a lack of correlation between inulin clearance and flow rate. However, in the present distribution studies it was noted that at low rates of urine flow Cl_{IN} was reduced and correlated with flow. These results are reported here.

Methods.—The method for the measurement of clearance was essentially that of Harvey & Malvin (1965). Male Wistar rats weighing 320–420 g were anæsthetized by an intraperitoneal injection of ethyl carbamate (1·4 g/kg).

The trachea, jugular vein, carotid artery and both ureters were cannulated with polyethylene tubing (Portex, PP50). Heparin (125 I.U./100 g body weight) and a priming dose of inulin (40 mg) were injected intravenously. A constant intravenous infusion of inulin (4 mg/ml) in 4% or 6% mannitol dissolved in isotonic saline was delivered by a constant infusion pump. ^{3}H-Inulin (Radiochemical Centre, Amersham; 300 mCi/mM) was used to 'trace' the non-radioactive inulin; 5 μCi were infused over a 90 min period. After 30 min equilibration, Cl_{IN} was measured over several 10 min periods. Urine flow rate was calculated by weighing, assuming unit density. Blood (0·1 ml) was collected from the carotid artery 2 min before the midpoint of the urine collection period to allow for dead space and delay time. The plasma was separated by centrifugation.

In some experiments after measuring three control clearance periods neostigmine bromide C-14 was administered intravenously (10 μg/100 g or 20 μg/100 g body weight). In these experiments and also those of control groups the infusion rate was either 0·038 or 0·075 ml/minute. In other experiments urine flow rate was altered by changing the infusion rate randomly in the range 0·009–0·075 ml/minute. In these experiments arterial blood pressure (1 mmHg≡1·333 mbar) was monitored from the carotid artery by means of a Condon manometer, arterial blood samples being obtained for assay from the femoral artery.

The radioactive inulin was measured in plasma and urine samples using a Nuclear Chicago Unilux II liquid scintillation spectrometer. Samples (0·05 ml) were assayed in a Triton X-100 scintillant (666·6 ml toluene, 333·3 ml Triton X-100 and 6 g butyl P.B.D. [2(4'-t-butyl phenyl)-5(4" biphenylyl)-1,3,4, oxadiazole]. All samples were corrected for quenching by the external standard ratios method.

Results.—In control experiments, Cl_{IN} was measured at a constant infusion rate, mean urine flow was about 0·03 ml/min, Cl_{IN} was fairly constant and apparently independent of urine flow.

In the first experiments in which neostigmine was administered the infusion rate of inulin was 0·038 ml/minute. The Cl_{IN} fluctuated about the mean for any particular experiment, notably after the rapid intravenous injection of neostigmine. After neostigmine, the mean urine flow rate was only about 0·015–0·020 ml/min and the Cl_{IN} appeared to correlate with the flow rate. In later experiments infusion rate was raised to 0·075 ml/min to produce flow rates comparable to the controls. The Cl_{IN} was then fairly constant, except that a fall in Cl_{IN} was observed in the first collection period following the intravenous in-

jection of neostigmine and at the same time the flow rates were low, but by the next period Cl_{IN} was again normal and independent of urine flow rate. This latter averaged about 0·045 ml/min in these experiments.

When urine flow rate was deliberately altered by the random adjustment of the infusion rate, Cl_{IN} was again noted to correlate with urine flow rates particularly when these were low. The blood pressure did not correlate with any change in infusion rate.

For all collection periods Cl_{IN} per 100 cm^2 body surface (Friedman, Polley & Friedman, 1947) was determined. Five groups of clearance values were obtained for the following flow intervals: 0·008–0·02, 0·02–0·03, 0·03–0·04, 0·04–0·05 and 0·05–0.075 ml/minute. The mean clearance value and mean flow rate in each group are shown in Fig. 1. It is seen that Cl_{IN} falls at low rates of flow, this reduction being highly significant using a non-parametric method for the statistical test (Quenouille, 1959).

Discussion.—The results indicate that with the present method an average constant Cl_{IN} of (0·28 ml/min)/100 cm^2 is obtained at urine flow rates in excess of about 0·03 ml/minute. Harvey & Malvin (1965) also obtained Cl_{IN} which were independent of flow rate. Their flow rates were higher than those attained in the present experiments, as were their clearance values. It is possible that the faster infusion rates used by them caused an increase in the extracellular fluid space leading to an actual increase in the Cl_{IN}. Friedman, Polley & Friedman (1947) using a constant injection method in unanaesthetized rats obtained a value of (0·36 ml/min)/100 cm^2 for Cl_{IN} which corresponds more favourably with the present work. Dicker & Heller (1945) again using unanaesthetized rats obtained values for Cl_{IN} similar to those presented here, but they found that Cl_{IN} was independent of urine flow.

The arterial blood pressure of the rat falls acutely after a rapid intravenous injection of neostigmine but returns to control values within a few minutes. It is not unexpected therefore that the Cl_{IN} and the urine flow rate fall after administration of neostigmine. However, with this important exception it is difficult to explain why low values of the Cl_{IN} were obtained and the correlation with flow at low flow rate. Sellwood & Verney (1955), using dogs, found that a high water load increased glomerular filtration (GFR) to a maximum which was reached about 35–40 min after a single dose of water; when diuresis was produced by a smaller water load, GFR remained steady. If Cl_{IN} monitors glomerular filtration, then the clearance should be independent of flow rate. It would appear likely that in this and the cited work the GFR is artificially increased by the experimental technique possibly due to an expansion of extracellular space, thus

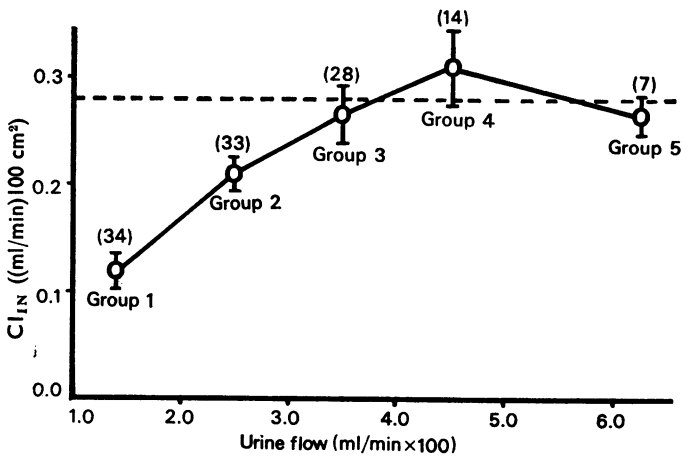

FIG. 1. Relationship of clearance of inulin to urine flow rate. The interrupted line represents the mean clearance ((0·28 ml/min)/100 cm^2) independent of flow rate. Results are mean ± S.E., with the numbers of determinations in parentheses. *p between groups 1–2 = <0·01 ; p between groups 2–3 = $\ll$0·05 ; p between groups 3–4 = $\gg$ 0·05 ; p between groups 3–5 = $\gg$0·05.
* Non parametric test of Quenouille (1959).

allowing reversible lower values of Cl_{IN} to be observed. Alternatively, a correlation of clearance with low flow rates may indicate a reabsorptive component in the handling of a filtrate by the renal tubular cells (Pitts, 1963). The possibility of inulin reabsorption has previously been considered (Ferguson, Olbrich, Robson & Stewart, 1950) although their results have been interpreted as being due to the heterogeneity of the inulin polymer (Barnard, Bassir & Hough, 1955).

Certainly the clearance value is one measure of kidney function and it is recommended that in experiments where a constant kidney function is required, for example, pharmacokinetic studies, it is advisable to maintain urine flow rates in the rat above 0·03 ml/minute.

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