A METHOD FOR THE STUDY OF RENAL FILTRATION

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The most accurate quantitative methods for the study of renal clearance of various substances were developed by Smith and his co-workers in the 1930's [13, 14]; they have not been taken up in clinical practice, and certainly not in pediatrics, because of their complication and of difficulties for the patient.

Many investigators [2, 3, 5, 10, 12] have proposed simplifying Smith's method by replacing the prolonged intravenous infusion by a single injection of the substance to be studied. The basis for this proposal was that the fall in concentration over a certain interval of time of a substance introduced by a single injection is of an exponential type. In a study of glomerular filtration in terms of inulin clearance, repeated blood analyses, a graph to be drawn on a semi-logarithmic plot which represents a linear fall of the concentration of inulin with time; hence the concentration of inulin at any given time may be calculated and the renal clearance deduced from the classical Van Slyke formula: $C = U \cdot V/P$, where C is the clearance of the given substance; U is the concentration of inulin in the urine; V the diuresis (in ml/min), and P the concentration of inulin in the blood in the middle of the period during which the urine is collected.

Some authors [5, 10] used the method of a single injection and have found it possible to calculate the clearance without collecting and studying the urine. For example: Newman [10] showed that the distribution of the injected substance in the body fluids (O), the excretion constant (K), and the clearace of the given substance (C) are related by the equation: $C = O \cdot K$. The volume of the distribution is calculated from the formula: $O = M/P_0$, where M is the amount of substance (in g) injected intravenously, P_0 is the concentration in g/liter of this substance in the blood at time zero, as established graphically by extrapolation of the exponential curve to intersect the ordinate. The excretion constant is calculated from the formula: $K = \log P_0 - \log P_t/t$, where P_0 and P_t are the concentrations of the substance in the blood at the end of the injection and at the end of the time t respectively.

However, this method gives not the renal but the total clearance of the substance, because clearance through routes other than the kidney is not excluded. Because of the disagreement concerning the reliability of simplified methods of studying renal filtration with inulin we have made a comparative study of renal and total inulin clearances after a single injection, and of their relationship to the clearance of endogenous creatinine. The latter substance was used as a means of comparison, because it is known that in the dog creatinine is neither secreted nor reabsorbed in the tubules, and its clearance is equal to the glomerular filtration (some reduction in the value of the creatinine clearance may be caused by nonspecific chromogens of the blood).

Studies were made on the dogs Ace (14 kg) and Malyshka (11 kg) in which the ureters were brought to the outside by Orbeli's method. A single intravenous injection of 0.1 g/kg was made in isotonic sodium chloride. Before the injection 1-1.5 ml of blood were taken from a vein for determination of creatinine and for control photometry for determination of inulin. Precisely at the 40th, 60th, 80th, and 100th min after the termination of the injection 1 ml of blood was taken from a vein to determine the inulin content. Urine was collected at 10-min intervals starting at the 35th min after the end of the inulin injection, because from published reports and from our own observations the curve of the reduction of inulin concentration in the blood becomes exponential at the end of this time. The amount of creatinine in the blood and urine was determined by a modification of the method of Lib and Zakharl', and the amount of

Results of Measurements of Endogenous Creatinine Clearance and of Renal and Total Inulin Clearance Based on a Single Injection (Mean Values are given for 5 Periods, in ml/min)

Ace			Malyshka		
Creatinine	Inulin		Creatinine	Inulin	
	Renal	Total		Renal	Total
27,9	35,3	55,5	21,7	25,0	40,0
32,7	32,6	47,0	32,0	31,5	38,4
34,0	25,1	44,0	28,1	25,1	37,0
29,0	33,8	56,5	26,9	26,6	37,6
28,9	28,4	48,5	25,1	28,2	36,8
28,0	30,8	49,0	27,7	33,7	46,5
30,2	31,4	49,0	20,3	23,6	37,0
29,0	30,0	56,3	34,3	33,5	48,7
30,0	33,6	—	24,4	24,3	—
30,5	28,9	—	21,8	23,2	—
Mean 30,0	31,0	50,7	26,2	27,5	40,3

inulin by means of the antron reagent [8]. Antron was obtained from anthraquinone in a laboratory investigation made in the Department of Pediatrics of the Central Institute for Advanced Training. The following reagents were required: 1) sulfuric acid (72 ml of concentrated sulfuric acid are mixed with 28 ml of twice-distilled water); 2) the antron reagent (0.2 g of antron are dissolved in 100 ml of dilute sulfuric acid); 3) 4% trichloracetic acid.

To precipitate the proteins 0.2 ml of serum were added to 1.8 ml of a 4% solution of trichloracetic acid. After 10 min, the mixture was centrifuged. Into a thick-walled test tube 5 ml of antron reagent were introduced, and upon it were placed 0.5 ml of protein-free centrifugate of serum. The mixture was shaken and placed for 10 min on a water bath at 55°. After cooling photometric measurements were made through a red filter (630 μm) with a FEK-M apparatus.

For comparison we took a mixture of 5 ml of antron reagent and 0.5 ml of trichloracetic acid. The urine was first diluted with distilled water 10-50 times or more according to the anticipated concentration coefficient. Then 1 ml of diluted urine was mixed with 9 ml of trichloracetic acid; when the protein precipitate was formed the mixture was centrifuged. The subsequent course of the determination was the same as for serum studies.

The inulin content of the blood (in mg%) was recorded on a semi-logarithmic graph, so that by interpolation a straight line of the reduction of concentration of blood inulin with time was obtained. From our observations the fall of inulin concentration is exponential between the 35th and 80-90th min after its injection. The graph made it possible to determine the blood inulin concentration precisely in the middle of each period of collection of urine; in addition, by extrapolation we could determine the volume in which the inulin was distributed in the body (from the dilution principle) in order to calculate the total clearance by Newman's method.

The results given in the Table show that the total clearance, calculated by Newman's method, greatly exceeded the endogenous creatinine clearance (by 69% in one and by 53.8% in the other set of experiments).

The reason for such a difference is primarily that the change of inulin concentration in the blood after a single injection is not determined solely by the activity of the glomeruli, and that a very small amount of inulin can be excreted by extra-renal routes or destroyed within the body [15]. The shortcoming of the dilution method of determining the volume in which the inulin was distributed in the body is evidently a further reason for increasing the value of the total clearance. It is known that inulin does not penetrate into cells and is confined to the extracellular fluid. Its concentration at zero time, used for calculation of the volume of the extracellular fluid, is only estimated; in addition it is determined from the curve of the fall of the intravascular concentration, which is usually lower than the extravascular concentration on account of clearance of inulin from the blood through the kidneys [12]. The net effect is an increased total clearance. In studying renal clearance in terms of inulin it has been shown that there is a satisfactory correlation between it and endogenous creatinine clearance. The ratio:

$$\frac{\text{creatinine clearance}}{\text{inulin clearance}}$$

had average values of 0.967 and 0.952.

Many authors [1, 6, 7, 9, 11, 14] have shown that measurement of renal clearance from a single injection is not free from errors. One difficulty is the occurrence of an arterio-venous difference in the content of the substance as its concentration in the blood falls. For inulin this difference is 7.4% [4]. Consequently, if only venous blood is measured the result is inevitably in error. Therefore, under clinical conditions it is recommended that capillary blood be taken, because in composition it is close to arterial blood, and it is easily obtainable; however, until recently the absence of any biochemical micromethod was an obstacle. The antron micrometric determination of inulin may be made with a very small amount of blood obtained from a finger prick.

Another source of error was the so-called delay time; this is the time required for the passage of the glomerular filtrate along tubules and ureters into the bladder. It has been shown that for a diuresis of 2 ml/min the delay time is $2\frac{1}{2}$ min. Therefore, with such a diuresis, under conditions under which the concentration of inulin in the blood is falling, the true value P in the Van Slyke formula should correspond to the concentration not in the middle of the collection period but $2\frac{1}{2}$ min earlier. The slower the excretion of urine the greater is the time delay. In our experiments we have attempted to produce a high diuresis to reduce the time delay to a minimum. However, in the dog, it was almost impossible to obtain rapidly a sufficient amount of capillary blood. We have, therefore, examined only venous blood. Nevertheless, the mean values of the creatinine clearance and the renal inulin clearance in the two sets of experiments (covering 100 periods) were quite close.

Therefore, the method with a single intravenous injection of inulin is valid for the determination of glomerular filtration, but only for the study of renal clearance. Total inulin clearance cannot be taken as an indication of glomerular filtration because it is considerably higher than the endogenous creatinine clearance.

It should be noticed that the application of the antron micromethod enabling measurements to be made on capillary blood may considerably facilitate studies in pediatric practice.

SUMMARY

Renal and total inulin clearances were measured after a single injection and were compared with the endogenous creatinine clearance. The investigation was made on two dogs in which the ureters had been brought to the outside by Orbeli's method. Inulin clearance was measured in capillary blood by the antron micromethod. We concluded that a single intravenous injection of inulin may be used only for the measurement of renal inulin clearance. Total inulin clearance considerably exceeds the creatinine clearance and therefore cannot be used to measure glomerular filtration.

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