J. Pharm. Pharmac., 1972, **24**, 532–537 *Received November* 15, 1971

The pharmacokinetics of inulin and urea: a comparison of the dose eliminated from a compartmental model and that eliminated in urine

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The distribution kinetics of inulin and urea were studied in the male rat to test the validity of a proposed compartmental model. The plasma curves could be adequately described by a biexponential function. For inulin, analysis of the results gave valid parameters in terms of an open two compartment model. These parameters did not necessarily predict the amount eliminated from the central compartment but did so under constant clearance conditions after allowance for a time lag between filtration and appearance of the drug in the collecting vessel. A triexponential fit interpreted in terms of a three compartmental model described the urea data.

The rate constants for distribution and elimination, and the fractions of the dose in the various compartments of compartmental models proposed to describe the kinetics of drug distribution, are determined from the changing plasma concentrations of the drug after rapid intravenous injection (e.g., Riegelmann, Loo & Rowland, 1968). Since conclusions from plasma data are hypothetical it has been recommended that plasma studies should be supplemented by measuring the elimination of the drug in urine (Riggs, 1963; Wagner, 1968). This we have done for inulin and urea which we chose as test compounds because they penetrate biological barriers by diffusion and are eliminated unchanged by the kidney.* This enables a direct measurement of the fraction eliminated and thus a check of the proposed pharmacokinetic model from plasma data. Also these compounds have no pharmacological effect to alter the system studied.

METHODS

Male Wistar rats, 200–350 g, were anaesthetized by an intraperitoneal injection of urethane (ethyl carbamate, 1·4 g/kg). The trachea, jugular vein, carotid artery and both ureters were cannulated; heparin was injected intravenously (125 I.U./100 g); a 6% solution of mannitol in 0·9% saline was infused into the jugular vein at 0·075 ml/min. [^{3}H]Inulin or [^{14}C]urea (1 to 4 μCi/animal) was administered either alone or with non-labelled material (10 or 20 mg/100 g) by rapid intravenous injection. Blood samples (0·1 ml) were taken from the carotid artery at 2, 5, 8, 12, 15, 20, 25, 35, 45, 55 min after the injection and urine was collected from both ureters at 2 min intervals from 0 to 10 min then for five 10 min intervals. Additional blood samples for urea were taken at 1 and 3 min.

Samples of plasma and urine (0·02 or 0·05 ml) were assayed for inulin or urea by liquid scintillation counting (Barber & Bourne, 1971a). The fraction of the administered dose eliminated in the urine and also the renal clearance (see: Smith,

* Elimination in bile under the conditions of the experiments accounted for only 0·5% of the dose; this minor route of elimination was therefore ignored.

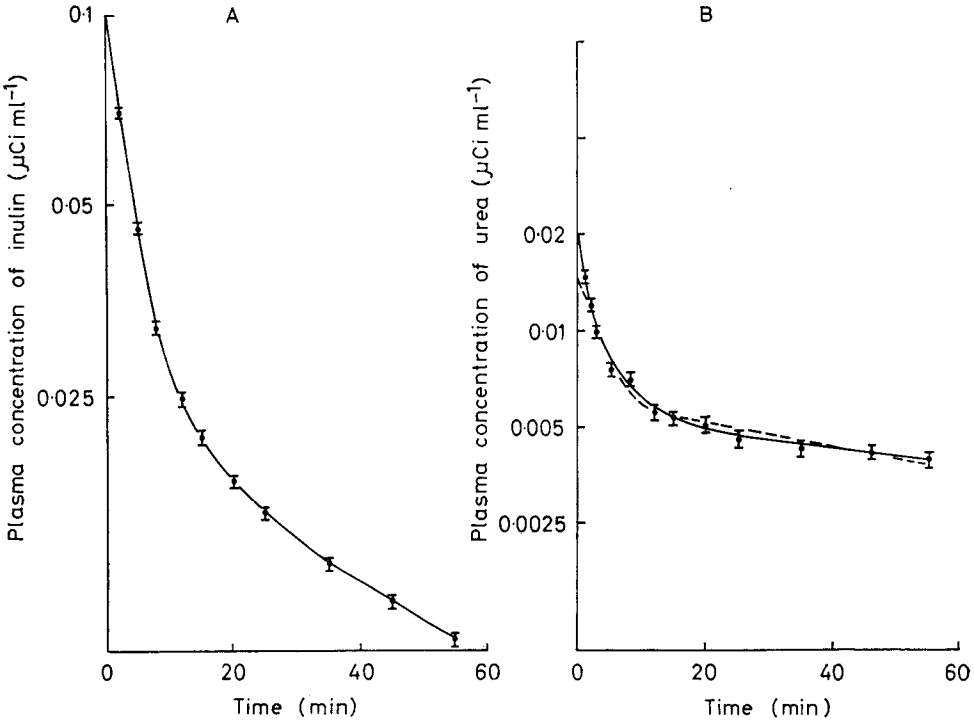

FIG. 1A. Inulin: Plasma concentration-time curve. Solid line: Mean computer prediction, a biexponential function. Results are mean ± s.e.
B. Urea: Plasma concentration-time curve. Solid line: Mean computer prediction, a triexponential function. Dotted line: Mean computer prediction, a biexponential function. Results are mean ± s.e.

1956) were calculated for known times after the intravenous injection of the compounds. An adequate urine flow was always maintained to facilitate a constant renal clearance (Barber & Bourne, 1971a).

The processing of the experimental data from the spectrometer, the analysis of multiexponential functions and the calculation of model parameters were determined with the aid of a digital computer using programs developed by the authors (Barber & Bourne, 1971b; Barber, Bourne & Buckley, 1971).

Table 1. *Parameters of an open two compartment model for inulin (mean results ± s.e.).*

	Experiments 1a–6a	Experiments 1b–6b
"Fast disposition" half life (min^{-1})	3·03 ± 0·18	3·03 ± 0·163
"Slow disposition" half life (min^{-1})	49·50 ± 4·56	44·70 ± 3·75
First order distribution rate constant		
(i) k_{ct} (min^{-1})	0·131 ± 0·013	0·126 ± 0·009
(ii) k_{tc} (min^{-1})	0·065 ± 0·003	0·070 ± 0·005
First order elimination rate constant k_{el} (min^{-1})	0·052 ± 0·004	0·054 ± 0·003
Volume of the central compartment V_c (ml)	17·20 ± 1·27	16·30 ± 1·22
Volume of distribution at the steady state V_{dss} (ml)	55·30 ± 5·42	49·20 ± 5·64
Clearance from the central compartment $k_{el}V_c$ (ml/min)	0·894 ± 0·095	0·880 ± 0·082

RESULTS

In all experiments the plasma concentration-time curve could be described by a multiexponential function. The inulin plasma curve was described by a least squares biexponential function and interpreted in terms of a two compartment open model (Riegelmann & others, 1968); the urea plasma curve was interpreted in terms of this model and also by analysis of a triexponential function according to the three compartment model described by Nagashima, Levy & O'Reilly (1968). The plasma concentration-time curves are shown in Fig. 1.

Inulin

The individual parameters of the two compartment model were obtained for each experiment and these results are shown in Table 1. The division of the results into two series is explained below, but it is apparent that the two sets have similar half lives. The predicted cumulative fraction of the total dose which is eliminated from the model was calculated from these parameters and compared to the experimentally determined fraction of the dose eliminated in the urine. This was either above (Experiments 1a–6a) or below (Experiments 1b–6b) the predicted fraction. When urine was collected in 2-min samples at the beginning of an experiment it was found that peak activity appeared in the third sample. Thus there is a delay time of up to 4 min from glomerular filtration to appearance of the drug in the collecting vessel. When allowance is made for this, computer predicted and experimentally determined fractions eliminated are in agreement. All these results are shown in Fig. 2.

The renal clearance of inulin was determined for 10 min periods. In the series 1a–6a the clearance fell throughout the experiments but the value was higher than the clearance value computed from the model, $k_{el}V_c$. In experiments 1b–6b the clearance was fairly constant and was similar to $k_{el}V_c$. These results are shown in Fig. 3.

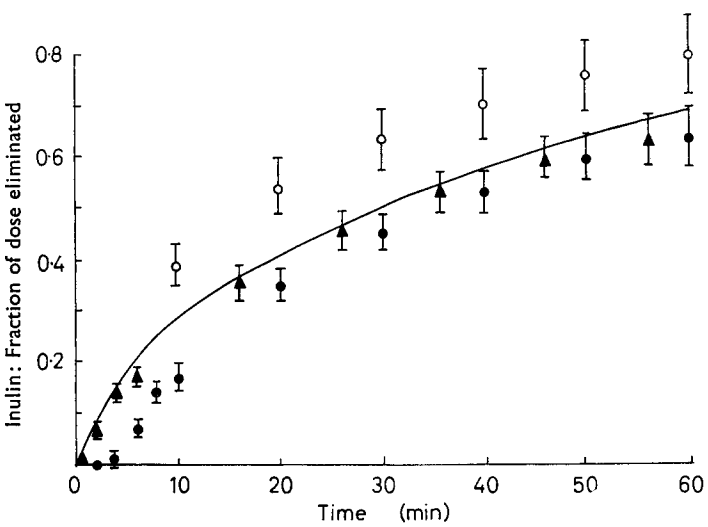

FIG. 2. Inulin: cumulative fraction eliminated. Mean computer prediction: solid line. Mean fraction eliminated (± s.e.). ○ experiments 1a–6a; ● experiments 1b–6b; ▲ experiments 1b–6b after allowing a 4 min time delay.

Urea

The fractions eliminated as predicted by the two compartment model from the analysis of the biexponential function shown in Fig. 1B were a very poor fit to the experimental fractions eliminated both in the individual and mean results. The three compartment model derived from the triexponential function which described the plasma concentration-time curve was able to predict these experimental fractions better. These results are shown in Fig. 4. The individual experimental results and the triexponential analyses are listed in Table 2. Despite similar plasma concentrations in the three experiments and steady clearances, the predicted fraction eliminated in Experiment 3 is greater than the experimental fraction. This point will be discussed.

DISCUSSION

For both inulin and urea, when only plasma data are analysed, a biexponential function and hence a two compartment model gives a good description of the experimental data. However, when urine and plasma data are studied together, this model is an inadequate description of the urea results and is apparently also inadequate for the inulin results, the experimental fraction eliminated being above or below the levels predicted from plasma.

Compartmental theory requires that clearance ($k_{el}V_c$) from a system should be constant (Riggs, 1963). In experiments 1a–6a the experimental clearance of inulin (C_{IN}) was higher in the first collection periods (0–20 min) than later in the experiment and was also higher than $k_{el}V_c$. It is to be expected therefore that the experimental rate of excretion is greater initially than predicted from the model parameters.

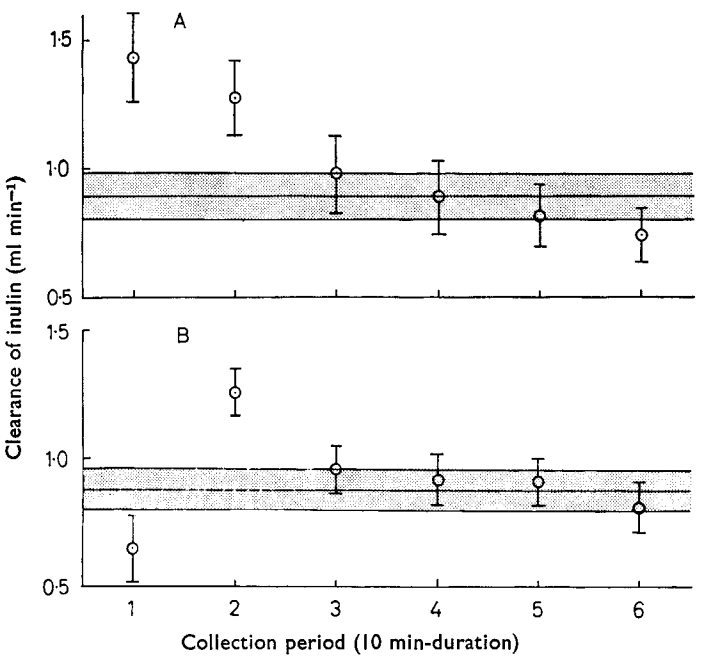

FIG. 3. Renal clearance of inulin. A. Experiments 1a–6a. B. Experiments 1b–6b. Circles: mean results ± s.e. Solid line: rate of clearance from the central compartment of an open two compartment model, $k_{el}V_c$.

Because the fraction eliminated is cumulative, the greater elimination initially is carried through to later periods and this experimental curve is consistently above the predicted curve. For experiments 1b–6b the C_{IN} approximates to $k_{el}V_c$ throughout the experiment; the observed deviation is therefore not due to inconsistencies in renal clearance. It is considered that the delay time referred to in the results section is responsible for this discrepancy. When allowance was made for the delay time,

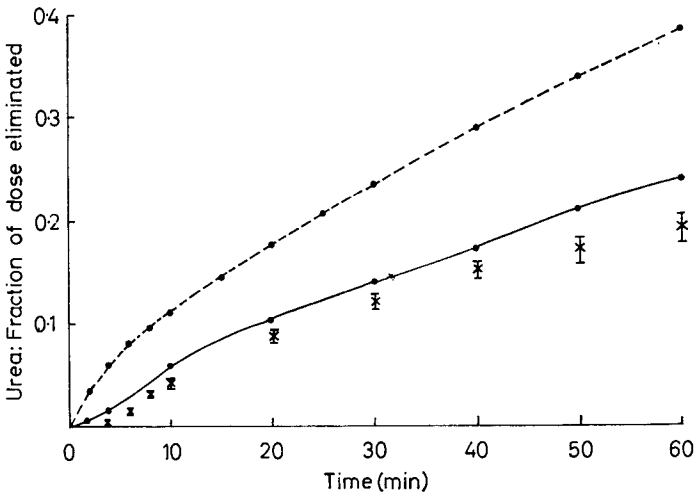

FIG. 4. Urea: cumulative fraction eliminated. Dotted line: mean computer prediction from the two compartment model. Solid line: mean computer prediction from the three compartment model. Results are mean ± s.e.

Table 2. *Urea: experimental results and predicted fraction eliminated from a two and three compartmental model.*

	Plasma concentration (nCi/ml) Experiment:					Experimental fraction eliminated in urine Experiment:			
Time (min)	1	2	3	Average	Time (min)	1	2	3	Average
1	15·7	13·6	16·3	15·2					
2	13·4	12·3	10·9	12·2	10	0·05	0·04	0·04	0·04
3	11·2	10·5	9·0	10·2					
5	7·8	8·3	7·0	7·7	20	0·10	0·08	0·08	0·09
8	8·9	6·8	6·0	7·2					
12	5·8	5·8	5·3	5·6	30	0·13	0·11	0·12	0·12
15	6·0	5·3	4·9	5·4					
20	5·6	5·1	4·7	5·1	40	0·16	0·13	0·15	0·15
25	4·7	4·8	4·5	4·7					
35	4·2	4·5	4·1	4·3	50	0·18	0·16	0·18	0·17
45	4·1	4·5	4·0	4·2					
55	4·2	4·2	3·8	4·1	60	0·19	0·17	0·21	0·19
	Predicted fraction eliminated two compartment model					Predicted fraction eliminated three compartment model			
10	0·12	0·08	0·12	0·11	10	0·05	0·04	0·08	0·06
20	0·19	0·12	0·20	0·17	20	0·09	0·07	0·14	0·10
30	0·24	0·17	0·26	0·22	30	0·13	0·10	0·19	0·14
40	0·30	0·21	0·33	0·28	40	0·16	0·13	0·23	0·17
50	0·35	0·24	0·38	0·32	50	0·19	0·16	0·27	0·21
60	0·39	0·28	0·43	0·37	60	0·22	0·18	0·31	0·24

computer predicted and experimentally determined fractions eliminated were in agreement. Therefore, for inulin under conditions of constant clearance, plasma and urine data are compatible with the two compartment model. Even when the clearances are changing, in Experiments 1a–6a, analysis of the plasma data alone gave model parameters which agreed with the other series.

The urea results emphasize that the fitting of multiexponential functions to results is arbitrary. A biexponential function can be described by a more complex triexponential function. The law of parsimony dictates that the simplest situation is tested first. However, despite a good fit of the plasma data by a biexponential function, the two compartment model could not describe both urine and plasma results together. The triexponential function fitted to the plasma data and analysed by means of the three compartment model of Nagashima gave a much better description of the experimental results. Gibaldi & Feldman (1969), using theoretical input data to pharmacokinetic models, have noted that the distinction between a two and three compartment model can only be made on the basis of experimental data obtained from blood during the first 2 or 3 min after injection. Thus from an experimental point of view it is reasonable to obtain an apparently biexponential curve after intravenous injection but be dealing with a three compartment model.

It is also instructive to examine the individual results shown in Table 2. Despite similar plasma concentrations in the three experiments described, the predicted fraction eliminated in Experiment 3 is greater than the experimental fraction. It is known (see: Westlake, 1971) that large differences can occur in the parameters of an exponential function with very little change in the error sum of squares of deviations about the experimental plasma concentration-time results. Thus numerous exponential functions exist which adequately describe plasma data but which will show considerable differences in their prediction of results in other compartments. The problem therefore of choosing the correct function to describe the overall experimental results is difficult. The functions presented here, for consistency, are least square fits, but this may not necessarily be the best fit to describe results, particularly for more complex exponentials. The statistical problems in fitting multiexponential functions to experimental data have been discussed more fully by Westlake (1971).

Acknowledgements

We should like to thank Professor A. Wilson for his advice in the writing of this manuscript and Miss S. Newby for technical assistance.

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