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Use of the analogue computer to examine the quantitative relation between urinary pH and kidney reabsorption of drugs partially ionized at physiological pH

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A method, involving the use of an analogue computer, is described for determining a quantitative relation between measured urinary pH and the kidney tubular reabsorption of certain drugs under normal conditions of fluctuating urinary pH. The method is based on the use of drug excretion versus time profiles under normal conditions and of absorption, metabolism and excretion rate constants, determined under conditions of controlled urinary pH, e.g. constant acidic pH (<5) in the case of basic drugs. In support of the theoretical treatment, experimental results, using amphetamine as a model drug, are presented. The implications of the method are discussed with respect to the evaluation of drug formulations under normal urinary pH conditions.

THE dependence of the urinary excretion of acidic and basic drugs on urinary pH is now well established (Milne, Scribner & Crawford, 1958; Weiner & Mudge, 1964; and others), and although the effect may be explained by the change in pH reversing the direction of an active transport system (Baer, Paulson & others, 1956), it is more generally considered that passive or non-ionic diffusion is responsible (Orloff & Berliner, 1956; Torretti, Weiner & Mudge, 1962; Weiner & Mudge, 1964). Furthermore, evidence indicates that diffusion of drugs across the lipid membranes of the kidney tubules is predominantly in a reabsorptive direction, from the urine back into the blood (Torretti & others, 1962; Ullrich, Kramer & Boylan, 1961).

This hypothesis has recently been used to explain excretion versus time profiles of amphetamine (Beckett & Rowland, 1965a) and other related amines such as the "ephedrines" (Beckett & Wilkinson, 1965) under normal conditions in which the pH of urine fluctuates. When urinary pH was maintained at a constant acidic level (pH about 5), variations in the excretion rates of the amines, in response to normal rhythmic diurnal changes in urinary pH, were abolished, thus allowing an evaluation of the kinetics of drug absorption and elimination from the smooth excretion versus time profiles so obtained.

Maintenance of a constant acidic urinary pH is a valuable procedure for examining biological availability of basic drugs from various formulations in volunteers (Beckett & Tucker, 1966, 1968), but it is impractical and probably undesirable under clinical conditions. Thus, only general indications of how a drug, sensitive to urinary pH, will be eliminated under the conditions of its use, can be deduced from studies in which urinary pH is closely controlled at acidic values (or alkaline values for

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acidic drugs). Furthermore, although it is important to know how the drug in a particular formulation is being released from the preparation and absorbed from the alimentary tract, it is equally important to know how different formulations will affect the amount of drug in the body when the urinary pH is fluctuating.

The present paper attempts to show how a numerical relation between drug reabsorption and urinary pH can be determined, with the aid of an analogue computer. Such a relation is a necessary prerequisite for the evaluation of drug formulations by studying urinary excretion of drugs under normal conditions. In support of the theoretical treatment, experimental results, using amphetamine as a model drug, are also presented.

Theoretical

When the urinary pH is maintained at a constant acidic level, the absorption, metabolism and excretion of drugs such as amphetamine (see Beckett & Tucker, 1968) and the "ephedrines" (Wilkinson, 1966), administered in aqueous solution of "free" forms, can essentially be described by simple compartmental models of the type shown in Fig. 1, where k values represent apparent first-order rate constants.

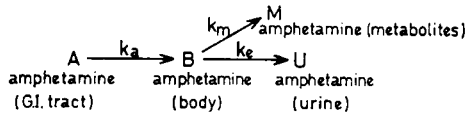

FIG. 1. Pharmacokinetic model for the absorption, metabolism and excretion of amphetamine in man at constant acidic urinary pH.

In addition to the assumptions implicit in the use of these models (see Beckett & Tucker, 1968), the following assumptions are made for the purposes of the present theoretical treatment: (i) The value of the excretion rate constant, ke, determined when urinary pH is controlled, is a direct measure of the rate at which drug is presented to the kidney for excretion, and reabsorption is negligible under such conditions. This is reasonable if essentially smooth exponential curves can be drawn through experimental rate of excretion versus time data when urinary pH is controlled at an acidic value (about 4·7 for amphetamine), and minor variations in pH at such levels have little effect on excretion rate. (ii) Intra-subject variation in the values of the rate constants determined under controlled acidic urine conditions is small. (iii) The values of these rate constants are the same under conditions of controlled and uncontrolled urinary pH. (iv) Of the factors determining the extent of tubular reabsorption, i.e. the amount of drug in the glomerular filtrate plus that actively excreted, the permeability of the tubules at the reabsorption site(s) to unionized and ionized drug species, respectively, the pH of the peritubular fluid, and the pH of tubular fluid, only the latter is significantly altered by the induction and maintenance of an acidic urine.

Even if the first assumption is not completely valid, but providing that

the second assumption is verified experimentally and any changes making the final assumptions invalid are progressive and consistent, it should still be possible to establish a relation between measured urinary pH and the urinary excretion of appropriate drugs. In the following treatment, excretion figures obtained under closely controlled conditions are merely used as a reference to calculate perturbing effects on urinary excretion due to normal changes in urinary pH.

Equations 1 and 2, derived from the model shown in Fig. 1, describe the rate of change of body level of drug and of its excretion, respectively, under acidic urine conditions.

$$\frac{dB}{dt} = ka.A - km.B - ke.B \quad .. \quad .. \quad .. \quad 1$$

$$\frac{dU}{dt} = ke.B \quad .. \quad .. \quad .. \quad .. \quad .. \quad 2$$

Thus, the term ke.B represents the rate of presentation of drug to the kidney at any time. At relatively alkaline pH, some of the drug which has passed into the kidney tubule is reabsorbed back into the body compartment, thus effectively acting as an additional dose of the drug. For drug initially appearing in the tubules, the value of the constant ke is assumed to remain the same as under controlled acidic urine conditions, but the amount of drug in the body will differ for any particular time depending on the amount which has previously been reabsorbed. This new body level of the drug, which depends on the amount of reabsorbed drug, is designated B* where:

$$B^* = B + R \quad .. \quad .. \quad .. \quad .. \quad 3$$

(R = amount of drug which has been reabsorbed).

Reabsorbed drug, as well as drug already in the body, will be susceptible to metabolism in the body and filtration in the kidney and thus equation 1 must be re-written (see eqn 4) by substituting B* for B.

$$\frac{dB^*}{dt} = ka.A - km.B^* - ke.B^* \quad .. \quad .. \quad 4$$

When integrated, equation 4 gives the amount of drug present in the body at any time (B*) if the amount of drug previously reabsorbed in in the kidney tubules (R) can be determined. The value of R, at any time during a drug excretion study in which urinary pH is not controlled, may be calculated using the analogue computer. Using the appropriate values of ka, km and ke for a particular subject, a continuous calculation of U (see eqn 2) as a function of time can be made on the computer. At the same time, the actual rate of excretion of drug when the subject's urinary pH is fluctuating, (dU*/dt), can be programmed on the computer as a function of time using a variable diode function generator. By integrating the simulated function dU*/dt, the value of U* (the amount of drug excreted when urinary pH is fluctuating), can be continuously calculated on the same time scale as that used to calculate U. The value of R can be determined up to any time by continuously subtracting

U* from U. Knowing the value of R at any time, equation 3 can then be solved on the computer by continuously adding R into a new body compartment, multiplying by the constants km and ke, and integrating. The results, B*, will be calculated as a function of time, taking into account the metabolism of reabsorbed drug. The analogue computer program required to carry out these calculations is represented diagrammatically in Fig. 2.

The term ke.B* in equation 4 represents, at any time, the rate of presentation of the drug to the kidney for excretion when urinary pH is fluctuating. The difference between rate of presentation of drug to the kidney and rate of appearance of drug in the urine is a measure of the rate of reabsorption of drug from the kidney tubules. Therefore, by subtracting the actual rate of excretion (dU*/dt) from the value of ke.B* at the same time, the rate of reabsorption (dR/dt) at that time can be calculated. Since dU*/dt and ke.B* are produced continuously on the computer, with the same time scale, their difference (dR/dt) can be plotted as a continuous function (see Fig. 2). Percentage reabsorption can be calculated by taking the ratio of dR/dt to ke.B* at any time, and likewise the ratio of dU*/dt to ke.B* gives the percentage excretion at any time.

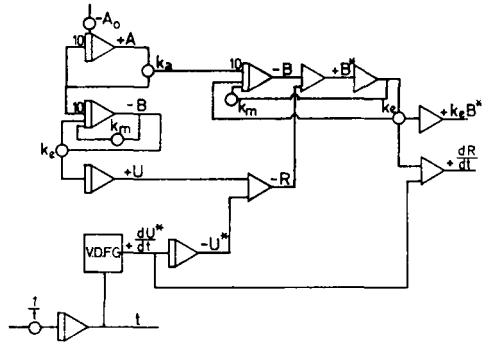

FIG. 2. Analogue computer program† for the absorption, metabolism and excretion of amphetamine in man at constant acid urinary pH.

When urine is collected at known time intervals and the pH accurately measured, the percentage drug reabsorption and percentage excretion derived as described above can be plotted against the average pH for each particular time interval.

† An alternative treatment of the problem is possible using a program based on the following equations:

$$\frac{dA}{dt} = -ka.A$$

$$\frac{dB}{dt} = ka.A - km.B - ke.B + \frac{dR}{dt}$$

$$\frac{dU}{dt} = ke.B - \frac{dR}{dt}$$

However, the introduction of the two different body level terms, B and B*, in the present treatment, was chosen to emphasize the fact that results are obtained by examination of two separate sets of experimental data, i.e. controlled and uncontrolled data.

The computer program given in the next paper of the series, for the prediction of urinary excretion under uncontrolled conditions (Beckett, Boyes & Tucker, 1968) is essentially derived from the above equations.

Experimental

Apparatus. An analogue computer (Electronic Associates Limited TR-20R), a digital computer (Elliot Automation 803B), a Perkin Elmer F11 gas chromatograph and a Pye Dynacap pH meter with a screened glass/saturated calomel electrode system.

CALCULATION OF REABSORPTION

Rate constants previously obtained for subjects given (+)-amphetamine under acidic urine conditions were used (Beckett & Tucker, 1968).

One subject was given two separate 15 mg doses and another subject was given one 15 mg dose of (+)-amphetamine sulphate in aqueous solution, on separate occasions, and the urinary pH was not controlled. Urine samples were collected at ½ hrly intervals for 16 hr. The pH of each sample was determined immediately after collection and after cooling to room temperature; the amphetamine content was measured by the gas chromatographic assay (Beckett & Rowland, 1965b). Further uncontrolled urine pH amphetamine excretion figures were also used to calculate reabsorption values (Rowland, 1965).

The analogue computer was programmed as shown in Fig. 2 and the experimentally determined rate of excretion during each trial, corrected for "lag time", was simulated using a variable diode function generator. The values of ka, km and ke, previously determined for each subject,

TABLE 1. URINARY EXCRETION OF AMPHETAMINE AFTER ORAL ADMINISTRATION OF 15 MG (+)-AMPHETAMINE SULPHATE TO SUBJECT 1 (UNCONTROLLED URINE pH)

Sample time (hr)	Mean time (hr)	Urine pH	Urine Vol (ml)	Total excretion (μg base)	μg base/min	% dose/hr	$\left(=\frac{dU^*}{dt}/ke.B^* \times 100\right)$ % excretion
0	0	5·56	—	—	—	—	—
0·5	0·25	7·10	66·0	35·7	1·19	0·67	8·5
1·0	0·75	6·66	53·0	73·8	2·46	1·39	17·3
1·5	1·25	6·32	61·0	26·3	4·21	2·38	22·9
2·0	1·75	5·42	114·0	165·0	5·50	3·11	32·4
2·5	2·25	5·50	238·0	197·4	6·58	3·72	40·1
3·0	2·75	5·19	110·5	229·8	7·66	4·33	75·5
3·5	3·25	5·48	176·0	215·4	7·18	4·06	43·0
4·0	3·75	5·60	161·0	186·3	6·21	3·51	36·0
4·5	4·25	5·76	126·0	155·1	5·17	2·92	32·9
5·0	4·75	6·00	110·0	121·5	4·05	2·29	26·9
5·5	5·25	6·17	59·0	101·4	3·38	1·91	24·1
6·0	5·75	6·28	63·0	78·6	2·62	1·48	17·4
6·5	6·25	6·68	53·5	63·6	2·12	1·20	14·7
7·0	6·75	6·59	41·0	39·9	1·33	0·75	11·5
7·5	7·25	6·35	41·0	52·5	1·75	0·99	18·1
8·0	7·75	5·96	48·0	75·3	2·51	1·42	29·7
8·5	8·25	5·95	60·5	111·9	3·73	2·11	36·5
9·0	8·75	5·34	120·0	132·6	4·42	2·50	46·1
9·5	9·25	5·43	140·0	138·6	4·62	2·61	48·2
10·0	9·75	5·30	140·0	139·5	4·65	2·63	53·0
10·5	10·25	5·29	63·5	138·0	4·60	2·60	55·1
11·0	10·75	5·26	42·0	134·4	4·48	2·53	56·4
11·5	11·25	5·19	40·0	130·5	4·35	2·46	58·1
12·0	11·75	5·49	28·5	106·8	3·56	2·01	55·3
12·5	12·25	5·40	31·0	97·8	3·26	1·84	51·4
13·0	12·75	5·70	41·5	84·9	2·83	1·60	48·5
13·5	13·25	5·58	26·0	74·4	2·48	1·40	42·4
14·0	13·75	5·90	25·0	50·4	1·68	0·95	30·5
14·5	14·25	6·15	18·0	34·5	1·15	0·65	23·3
15·0	14·75	8·00	21·0	11·7	0·39	0·22	0·076
15·5	15·25	7·85	24·0	6·3	0·21	0·12	3·7

were set using the appropriate potentiometers. The values of ke.B*, dR/dt and dU*/dt were plotted against time. The percentage reabsorption and excretion was then calculated for the times at which the urinary pH was known.

Results

Fig. 3 shows ke.B*, dR/dt and dU*/dt (for one subject) as calculated by the computer and plotted against time; Table 1 summarizes the data from which the curves were plotted. Similar results were obtained in all the trials. In Fig. 4 the logarithm of percentage excretion has been

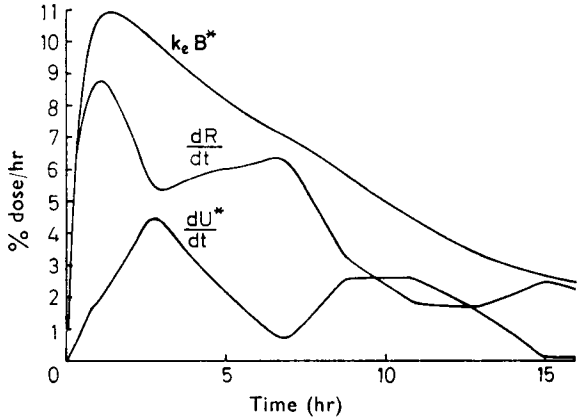

FIG. 3. Computer calculations of rate of presentation of amphetamine to the kidney (ke. B*); rate of kidney reabsorption of amphetamine $\left(\frac{dR}{dt}\right)$; and rate of urinary excretion of amphetamine as functions of time under controlled urinary pH conditions (Subject 1).

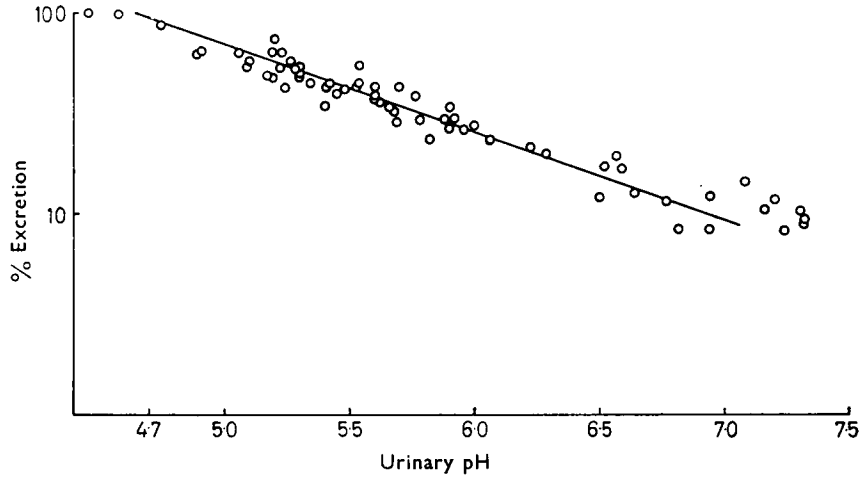

FIG. 4. Relation between log percentage urinary excretion of amphetamine and measured urinary pH (data from 2 trials in 2 subjects).

plotted against the corresponding urinary pH values and using a digital computer a straight line represented by equation 5 fitted by the method of least squares.

$$\log \% \text{ excretion} = -0{\cdot}4383 \text{ pH} + 4{\cdot}0384 \quad .. \quad .. \quad 5$$

The calculated correlation coefficient was −0·955.

Discussion

The method used to calculate the relation between kidney tubule drug reabsorption and measured urinary pH takes into account the higher body levels which result from reabsorption of the drug and the increased amount of metabolism which occurs compared with conditions under which reabsorption is assumed to be negligible. The high correlation coefficient obtained for the line resulting from a plot of log percentage excretion of amphetamine against pH (Fig. 4) supports the theory on which the calculations are based. Equation 5 gives a logarithmic relation between excretion (or reabsorption) of the drug and the measured urinary pH. Thus, there is a direct relation between the concentration of unionized drug in the kidney tubules and the extent of reabsorption. The present result apparently substantiates the theory that reabsorption is a passive process dependent on the concentration of unionized drug in the kidney tubules.

The rate of urine output varied between 0·5 and 5 ml/min in the present experiments. However, this does not explain the scatter of the points about the line in Fig. 4 since no direct relation between distance from the line and urine flow rate was apparent. In addition to departures from the assumptions listed in the theoretical section, possibly such factors as differences in measured urine pH and the actual pH at the site(s) of reabsorption, rate of change of urinary pH, and minor variations in kidney function, contribute to the relatively minor scatter of points.

The urinary excretion of most basic or acidic drugs will be influenced by urinary hydrogen ion concentration. If, using a basic drug, a smooth curve of rate of excretion against time can be obtained by rendering the urine acidic (about pH 5) (or alkaline for an acidic drug), the present method will reveal a relation between drug reabsorption (or excretion) and the pH of urine. For those drugs which are relatively slowly metabolized in man and are excreted in high percentage in the urine as unchanged drug, an estimate of the duration of action, under conditions of fluctuating urinary pH, should then be possible. Having established that there is little inter- or intra-subject variation in the rate constants describing absorption, metabolism and excretion of a drug, it should be possible to predict the excretion pattern for the drug in subjects whose urinary pH is not controlled, solely from a knowledge of the collection times and pH of urine samples. With amphetamine, inter- and intra-subject variation in the values of rate constants for absorption (ka) and excretion (ke) are relatively small (at least in healthy male subjects), although the inter-subject variation in the value of km, the metabolic constant, is somewhat larger (see Beckett & Tucker, 1968). However,

since the metabolism of amphetamine in man is a relatively slow process, differences in the value of km are very much less important in controlling the fate of the drug than major changes in the excretion rate of the drug, caused by fluctuations in urinary pH under normal conditions.

Once the necessary initial results have been obtained in volunteers under controlled conditions, it should, at least in theory, be possible to predict the performance of drug formulations solely on the basis of urine data from patients.

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