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Estimation of the degree of binding of tetracyclines in human plasma

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The binding of tetracyclines in human plasma has been studied extensively since bound antibiotic can be considered to be temporarily inactive (Kunin, Craig & others, 1973). Table 1 lists the available data for the four tetracyclines of greatest clinical importance. Although these data show plasma binding to be significant the variation in the values quoted for each drug is such as to make a reliable estimate impossible. The values for percentage binding of tetracycline, for example, range from 24 to 64% (Table 1) whereas the percentage of free (and therefore active) doxycycline could be 9 or 27% of the total drug in the plasma (Table 1). Furthermore, some of the concentrations used (Table 1) have been markedly higher than the therapeutic concentration of 0·8 to 5·0 μg ml^{-1} (Fabre, Milek & Kaliopoulos, 1971). Because of this confusion we have re-examined the binding of these four tetracyclines in human plasma at concentrations covering the therapeutic range. Samples (as hydrochlorides) of tetracycline and doxycycline were gifts from Pfizer (Lot Nos. 203–71716 and 305–58708 respectively), oxytetracycline was purchased from Sigma (Lot No. 41C-0800) and minocycline from Cyanamid (Batch 205).

The two methods most suitable for evaluating drug binding in plasma are ultrafiltration and equilibrium dialysis, the latter method was used in this work since we found the tetracyclines bind extensively and variably to ultrafiltration membranes which may account for some of the variation in previously reported values (Table 1). In contrast, only relatively low binding (6%) of tetracyclines to the equilibrium dialysis apparatus occurred and this was corrected for by sampling from both sides of the membrane. The same basic procedure was used for each tetracycline: a solution of the drug in pooled citrated human plasma (5 ml) was dialysed against sterile Sørensen's phosphate buffer (pH 7·4 5 ml) using a Perspex dialysis cell and sterile, washed Visking membrane. The pH of the buffer is critical since the degree of binding is known to vary with pH (Williamson, 1969), this may also account for some of the variation in previously determined values (Table 1), consequently the buffer used was at physiological pH since calcium citrated-plasma was used, Ca^{2+} variations were not considered likely to affect the results. Preliminary studies showed that, with shaking, equilibrium was achieved between 48 and 72 h and that no dilution of the plasma occured and that the pH at equilibrium was 7·4. The dialysis cells were thus shaken for 72 h and at 4° in the dark to prevent decomposition. Eight samples were used for each tetracycline giving a range of concentrations between 0·1 and 10 μg ml^{-1} in the plasma at equilibrium and the procedure was repeated to give duplicate values. In previous studies bioassay methods have generally been used (Table 1),

Table 1. *Percentage binding of tetracyclines in human plasma (literature values).*

Tetracycline concn (μg ml^{-1})	Method	Assay	% Bound	Ref.
Tetracycline				
5–20	Dialysis	bioassay	24	1
1–10	Dialysis	radiochem.	34	2
1·5–4·1	Ultrafiltr.	bioassay	54	3
N.S.	Ultrafiltr.	bioassay	58	4
a	Dialysis	bioassay	36	5
30	Ultrafiltr.	bioassay & uv	55	6
30	Dialysis	bioassay & uv	56	6
1–10	Ultrafiltr.	bioassay	64	7
1	Dialysis	radiochem.	53	8
Oxytetracycline				
5–20	Dialysis	bioassay	20	1
0·69	Dialysis	bioassay	27	9
30	Dialysis	bioassay & uv	35	6
30	Ultrafiltr.	bioassay & uv	34	6
1–10	Ultrafiltr.	bioassay	35	7
Doxycycline				
N.S.	Ultrafiltr.	bioassay	73	10
1–30	Ultrafiltr.	bioassay	91	11
Minocycline				
N.S.	N.S.	N.S.	59	12
N.S.	Ultrafiltr.	bioassay	76	13

N.S. = not specified.
(*a*) Binding determined in human plasma 2 h after i.v. injection of 500 mg tetracycline.
1. Kunin, Dornbush & Finland (1959). 2. Wozniak (1960). 3. Remington & Finland (1962). 4. Kirby, Roberts & Burdick (1961). 5. Kunin (1962). 6. Schach von Wittenau & Yeary (1963). 7. Bennet, Mickelwait & others (1965). 8, Powis (1974). 9. Kaplan, Yugeoglu & Strauss (1960). 10. Rosenblatt, Barret & others (1966). 11. Williamson (1969). 12. Miura, Mizumoto & Shibaki (1969). 13. Macdonald, Kelly & others (1973).

* Correspondence.

Table 2. *Plasma binding and lipophilic nature of tetracyclines.*

Tetracycline	Concn ($\mu g\ ml^{-1}$)	% bound s.e.m. $P'=0{\cdot}05$, n=14)	App. part. coeff. (octanol—buffer pH 7·5)	Part. coeff. (octanol—buffer pH 5·5–8·0) relative to tetracycline
Tetracycline	0·1–10	35·50±3·18	0·036[a]	1[c]
Oxytetracycline	0·1–10	24·31±1·96	0·025[a]	0·15[c]
Doxycycline	0·1–10	59·99±2·04	0·60[a]	6[c]
Minocycline	0·1–10	54·98±1·54	1·10[b]	38–180[d]

a From Colaizzi & Klink (1969).
b Estimated from Fig. 5 in Colaizzi & Klink (1969).
c From Schach von Wittenau & Yeary (1963).
d From Macdonald, Kelly & others (1973).

however greater accuracy can be obtained by using radiochemical and spectrofluorimetric techniques. Consequently in this study tetracycline -7-^{3}H (Radiochemical Centre) was used and assayed according to Allison, Monro & Offerman (1972); the other three tetracyclines were assayed spectrofluorimetrically by the method of Lever (1972).

It was possible to calculate a mean value for the percentage binding of each tetracycline at therapeutic concentrations of antibiotic since the degree of binding was found to be independent of concentration over the range used: the mean values are listed in Table 2. The values for oxytetracycline, tetracycline and doxycycline (24, 36 and 60% bound respectively) follow the expected pattern, the percentage of drug bound increasing with increasing lipophilic nature of the molecule (Table 2). The binding of minocycline (55%) is lower than doxycycline despite its increased lipophilicity. This indicates there is an optimum lipophilicity for the binding of tetracyclines to some plasma constituent or that the steric effect of introducing a dimethylamino-group onto the planar region of the tetracycline nucleus gives a decrease in the affinity for some specific binding site on a plasma constituent.

The results clearly show that protein binding of tetracyclines is significant and the degree of binding varies within the group. For equal plasma concentrations not only will protein binding determine the relative concentrations of free (and therefore active) tetracycline in the plasma but, since only free antibiotic will equilibrate with extravascular fluids, protein binding will have a marked effect on the relative concentrations of these drugs in body compartments other than plasma. The methods used in this work have been selected critically to give a reliable determination of the degree of binding and so the values for % bound can be used as a guide to estimate the percentage of antibiotic free in the plasma in therapeutic situations.

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