The use of kanamycin in equilibrium dialysis at 37°

Measurement of plasma concentrations of drugs is a useful means of monitoring drug therapy but since only the free (unbound) drug is pharmacologically active, estimates of the degree of plasma binding of a drug are necessary for the meaningful interpretation of total plasma drug concentrations. Plasma binding has most often been measured by equilibrium dialysis at temperatures varying from 4 to 37° (Meyer & Guttman, 1968). However, the temperature at which dialysis is carried out may influence the extent of drug binding in the plasma (Ballard, 1974) and therefore such estimates of plasma drug binding should be made at 37° to enable calculation of the free drug concentration which will actually occur at body temperature. Dialysis at 37° is often complicated by the occurrence of bacterial growth and this paper reports an investigation of the use of the antibiotic kanamycin to inhibit bacterial growth during equilibrium dialysis at body temperature.

Blood was obtained by venepuncture from adult mongrel dogs of either sex (10–20 kg) or from volunteers, and lithium heparin (2 units ml^{-1}) was added before separation of plasma from red cells by centrifugation at 1200 *g* for 15 min.

Binding of drugs to plasma proteins was determined by equilibrium dialysis at either 4 or 37°. Cellulose dialysis tubing (A.H. Thomas & Co., Cat. No. 3787–D22) was soaked in distilled water initially at 99° and later in cold distilled water for 4 h before use. A 3 ml sample of plasma containing one of 4 different drugs was placed in a dialysis bag, sealed in an apparatus similar to that described by Anton (1960) and dialysed to equilibrium against 10 ml of an isotonic phosphate buffer (K_2HPO_4, 14·11; KH_2PO_4, 2·59; NaCl, 1·99 g $litre^{-1}$; pH 7·4) containing kanamycin sulphate when appropriate.

After dialysis for 18 h, the concentration of drug in appropriate aliquots of both plasma and buffer was analysed. [^{3}H]Haloperidol was measured by liquid scintillation counting, propranolol and quinidine by spectrophotofluorimetry (Shand, Nuckolls & Oates, 1970; Cramér & Isaksson, 1963) and salicylate was determined by the method of Trinder (1954).

The drugs used were: [^{3}H]haloperidol (specific activity = 133 μCi mg^{-1}; G. D. Searle & Co., U.K.), kanamycin sulphate (Kantrex, Bristol Labs., Aust.), lithium heparin (Calbiochem), (±)-propranolol hydrochloride (ICI, Aust.), quinidine sulphate B.P. and sodium salicylate B.P.

Table 1. *Plasma protein binding of quinidine in dogs.* Showing the effect of kanamycin (3·3–100 μg ml^{-1}) on the % protein binding of quinidine sulphate (3 μg ml^{-1}) to dog plasma at 4 or 37°. All results are mean ± s.e. (number of observations in parentheses). Each of the 4 experiments was performed on plasma from a different dog. *, statistically significant difference from control ($P < 0.05$).

Temperature (°C)	Concentration of kanamycin in buffer (μg ml^{-1})	Plasma binding %	
		Control—No kanamycin	With kanamycin
4	3·3	85·77 ± 0·49 (6)	85·32 ± 0·44 (6)
4	10·0	95·21 ± 0·09 (7)	95·15 ± 0·11 (7)
4	100·0	91·98 ± 0·17 (9)	90·50 ± 0·17 (8)
37	10·0	83·48 ± 1·95 (5)	90·08 ± 1·68 (5)*

Table 2. *Drug binding to human plasma.* Showing the % plasma protein binding of various drugs to human plasma at 4 and 37° in the presence or absence of kanamycin (10 μg ml^{-1}). All results are expressed as mean ± s.e. (number of observations in parentheses). *, statistically significant difference from control at same temperature ($P < 0.02$). †, statistically significant difference from group with kanamycin at 4° ($P < 0.01$).

Drug (concentration in plasma)	% Binding at 4°C		% Binding at 37°C	
	Control	With kanamycin in buffer (10 μg ml^{-1})	Control	With kanamycin in buffer (10 μg ml^{-1})
Haloperidol (100 ng ml^{-1})	91·73 ± 0·11 (7)	91·95 ± 0·26 (8)	90·28 ± 0·38 (9)	90·15 ± 0·26† (10)
Propranolol hydrochloride (200 ng ml^{-1})	94·46 ± 0·47 (7)	91·93 ± 1·13 (6)	82·34 ± 1·56 (8)	82·33 ± 2·02† (8)
Quinidine sulphate (3 μg ml^{-1})	93·12 ± 0·21 (8)	93·30 ± 0·35 (8)	86·14 ± 0·35 (8)	86·98 ± 0·72† (8)
Salicylate (0·25 mg ml^{-1})	93·90 ± 0·35 (5)	93·48 ± 0·34 (5)	84·41 ± 0·98 (5)	87·89 ± 0·28†* (5)

All results are expressed as mean ± standard error with the number of observations in parentheses. Student's *t*-test was used to determine statistical significance.

Preliminary experiments indicated that there were no significant differences in total recovery of drug from dialyses carried out at 4° and 37°. Recoveries were 89·7 ± 1·2% (12) for haloperidol, 92·7 ± 0·5% (20) for quinidine, 95·4 ± 1·9% (16) for propranolol and 97·1 ± 1·9% (15) for salicylate. Equilibrium was attained for all drugs within the 18 h dialysis period both at 4 and 37°.

At 4° the binding of quinidine (3 μg ml^{-1}) to dog plasma proteins was unaffected by the addition of kanamycin (3·3–100 μg ml^{-1}) to the dialysis buffer and no bacterial growth occurred in any of the samples. At 37°, however, bacterial growth occurred in the control dialyses, but not in those with kanamycin incorporated into the buffer. The bacterial growth in the controls was associated with a significant decrease ($t = 2{\cdot}57$, $P < 0{\cdot}05$) in the plasma binding of quinidine (Table 1).

To test whether the use of kanamycin (10 μg ml^{-1}) could be extended to other drugs the binding of haloperidol, propranolol, quinidine and salicylate in human plasma were investigated at both 4 and 37°. These results are summarized in Table 2.

The addition of kanamycin (10 μg ml^{-1}) to the dialysis buffer did not significantly alter binding of any of the four drugs tested when determined at 4°. Binding at 37° was consistently and significantly lower than at 4° for haloperidol ($t = 4{\cdot}9$, $P < 0{\cdot}001$) propranolol ($t = 4{\cdot}2$, $P < 0{\cdot}01$) quinidine ($t = 7{\cdot}9$, $P < 0{\cdot}001$) and salicylate ($t = 12{\cdot}7$, $P < 0{\cdot}001$). No bacterial growth occurred in dialyses containing kanamycin in the buffer or in those conducted at 4° without the antibiotic. Dialyses conducted at 37° in the absence of kanamycin exhibited slight but variable bacterial growth and in the case of salicylate this was sufficient to cause a significant ($t = 3{\cdot}1$, $P < 0{\cdot}02$) reduction in binding.

The experiments with quinidine in dog plasma and salicylate in human plasma indicate that bacterial growth during dialysis can reduce the percent protein binding. To overcome this problem we have incorporated the antibiotic kanamycin into the dialysis buffer. Kanamycin has a broad spectrum of activity and is reputed not to bind to plasma proteins (Tolhurst, Buckle & Williams, 1972). It has a molecular weight of 484 and can therefore diffuse from the buffer, through the dialysis bag

(molecular weight exclusion = 12 000) and into the plasma. For routine equilibrium dialysis we chose to use a concentration of 10 μg ml^{-1} of kanamycin in the dialysis buffer, since at equilibrium this yields a final concentration of kanamycin which is some 10 times the minimal inhibitory concentration for a heterogenous population of bacteria from this laboratory (unpublished results).

At 4°, where bacterial growth did not occur, the addition of kanamycin to the dialysis buffer did not alter drug-plasma protein binding. Thus, in the concentration range investigated, kanamycin itself does not affect plasma protein binding and the decreased binding observed at 37° in the absence of kanamycin is most likely due to bacterial growth.

Previous studies have shown that increase in the temperature of dialysis decreases the extent of protein binding for penicillins (Scholtan & Schmid, 1962; Klotz, Urquhart & Weber, 1950), sulphonamides (Scholtan, 1962, 1964; Clausen, 1966), diazepam (van der Kleijn, 1969), diphenylhydantoin (Lunde, Rane & others, 1970), uric acid (Sheik & Møller, 1968) and warfarin (O'Reilly, 1967, 1973). The present study shows that this temperature dependence of binding extends to propranolol, quinidine and salicylate and to a lesser extent to haloperidol. Plasma binding of drugs should, therefore, always be determined at body temperature so that correct concentrations of free drug in plasma may be calculated. Moreover, kanamycin can be added to the equilibrium dialysis system to prevent problems associated with bacterial growth at 37° without interfering with the protein binding of the drugs tested.

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