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Displacement from protein binding of sulphormethoxine by phenylbutazone using *in vivo* dialysis in rats

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1. Displacement of the ultra-long-acting sulphonamide sulphormethoxine from binding to serum proteins by phenylbutazone has been demonstrated by *in vivo* dialysis in rats. The technique used involved the introduction of dialysis sacs into the peritoneal cavity. The regression line relating free to total sulphormethoxine was significantly displaced after addition of phenylbutazone to the regime although the mean level of free drug was not significantly raised. No significant effect was observed on the proportion of phenylbutazone in the free form. The range of concentrations employed corresponded to that encountered clinically.

2. *In vitro* experiments with comparable concentrations of both drugs confirmed displacement of sulphormethoxine by phenylbutazone. Insignificant displacement of phenylbutazone from binding occurred. These experiments indicated the relationships between the relative affinities of sulphormethoxine and phenylbutazone for serum proteins and the extent to which mutual displacement from binding occurred.

Displacement of protein-bound drugs by others which compete for the non-specific binding sites is well known, particularly in the case of weak organic acids. *In vitro* studies have demonstrated the extent of this displacement for many such substances (Anton, 1961 ; Rolinson & Sutherland, 1965 ; Brodie, 1965 ; Kunin, 1964, 1966a ; Keen, 1966 ; Engel & Melville, 1966 ; Solomon, Schrogie & Williams, 1968 ; see also Meyer & Guttman, 1968). There are relatively few studies of this form of drug interaction *in vivo*. Anton (1961) presented evidence of displacement of a highly bound sulphonamide by sulphinpyrazole, but this was based on indirect evidence only. Kunin (1966b) demonstrated the displacement of protein bound penicillins by sulphonamides and aspirin in humans. There are also instances of untoward reactions occurring clinically in which displacement seems likely to have played a significant part. Thus the anticoagulant activity of warfarin is increased dangerously by the concurrent use of phenylbutazone (Eisen, 1964 ; Aggeler, O'Reilly, Leong & Kowitz, 1967). Sulphonamides enhance the hypoglycaemic action of tolbutamide (Christensen, Hansen & Kristensen, 1963 ; Büttner & Portwich, 1967).

In the present experiments the technique of *in vivo* dialysis already described (McQueen, 1968) has been used to study the interaction of sulphormethoxine

('Fanasil,' Roche) and phenylbutazone. This technique involves implantation of dialysis tubing sacs containing dextran into the abdominal cavities of rats. Sulphormethoxine is a sulphonamide with a long half-life (about 24 hr in the rat, McQueen, unpublished), and rats receiving the drug in their diet show reasonably stable blood and tissue levels over the equilibration period. Phenylbutazone is highly bound to serum proteins and hence it has a high displacement potential. Corresponding *in vitro* experiments using the same concentrations as those obtained in the *in vivo* experiments are also described.

Methods

The animals used were male white Wistar rats aged 4–6 months and weighing 350–400 g. In the experiments investigating the displacement of sulphormethoxine by phenylbutazone, the rats were fed mash containing sulphormethoxine either 2 or 4 g/kg to give a range of serum levels and this was continued for 4 days before the implantation of the dialysis sacs. The dextran-containing dialysis membrane sacs were then implanted intraperitoneally as previously described (McQueen, 1968). Phenylbutazone ('Butazolidin' injectable, Geigy) was administered by injection in a dose of 75 mg into the back muscles at the same time as the sacs were implanted. After being left overnight the animals were anaesthetized and exsanguinated, the sacs were removed and serum and sac concentrations of sulphormethoxine were estimated. Phenylbutazone concentrations at exsanguination were also measured in those rats which received this drug.

In the experiments investigating the possible displacement of phenylbutazone by sulphormethoxine, phenylbutazone was given in the same dose of 75 mg. The group designated to receive sulphormethoxine as well were given it as above in the 4 g/kg concentration.

In vitro equilibrium dialysis was performed as described previously (McQueen, 1968) using fresh rat serum with added sulphormethoxine to give a range of concentrations after dialysis approximating to that covered by the *in vivo* experiments. Phenylbutazone 0·25 mg/ml. (0·812 mM) was added in half the experiments. After dialysis this produced a concentration of approximately 0·21 mg/ml. (0·682 mMI. The dextran-containing sacs were then added and gently agitated at 37° C overnight. Sulphormethoxine was estimated by the Bratton & Marshall (1939) technique in the *in vivo* experiments and by the Morris (1941) method, as suggested by Keen (1966) in the *in vitro* series. Only unconjugated sulphormethoxine was measured, previous experiments having shown that conjugated sulphonamide could not be detected. Phenylbutazone was measured by the method of Burns, Rose, Chenkin, Golman, Schubert & Brodie (1953).

Results

In vivo *experiments*

Sulphormethoxine

The relationships between serum (total) and sac (free) sulphormethoxine are shown in Fig. 1. When plotted over the full range of attainable concentrations, the line relating free and total drug concentrations is a portion of a hyperbolic curve (McQueen, 1968). If linear regression is used to express the relationships over the

relatively limited range of concentrations involved in these experiments, however, the regression equations for the two sets of values are

For rats on sulphormethoxine alone: $y = -0{\cdot}231 + 0{\cdot}831\ x$

Sulphormethoxine plus phenylbutazone: $y = -0{\cdot}162 + 0{\cdot}910\ x$

The difference between these two estimated regression lines is significant at the probability level $0{\cdot}05 > P > 0{\cdot}01$.

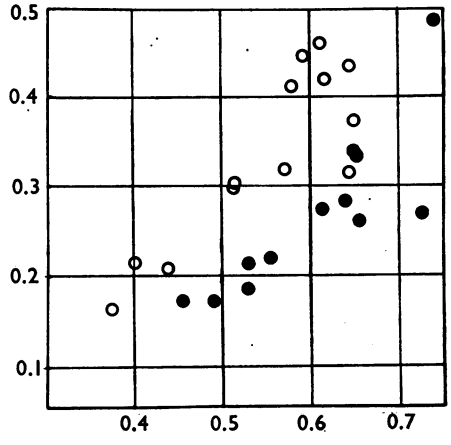

FIG. 1. *In vivo* dialysis in rats. Abscissa, serum (total) sulphormethoxine (mM); ordinate, sac (free) sulphormethoxine (mM). ●, Values for rats on sulphormethoxine alone; ○, sulphormethoxine plus phenylbutazone.

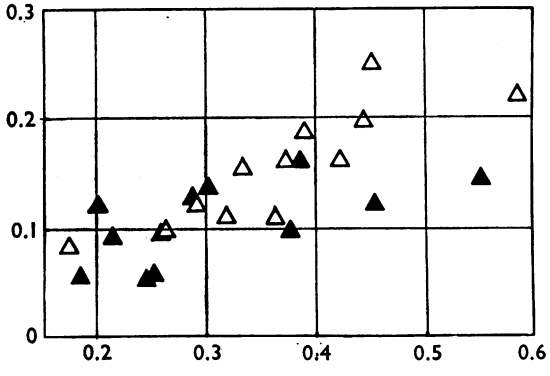

FIG. 2. *In vivo* dialysis in rats. Abscissa, serum (total) phenylbutazone (mM); ordinate, sac (free) phenylbutazone (mM). ▲, Rats receiving phenylbutazone alone; △, phenylbutazone plus sulphormethoxine.

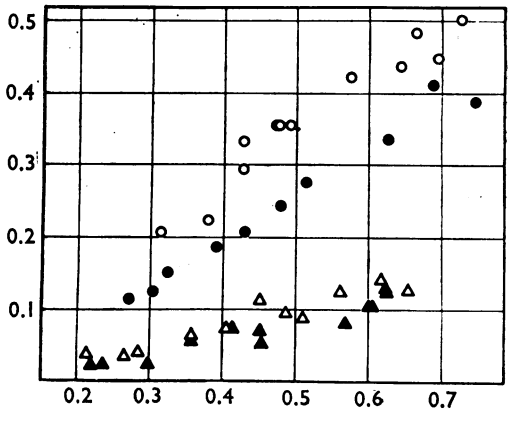

FIG. 3. *In vitro* experiments. Abscissa, serum (total) drug (mM); ordinate, sac (free) drug (mM). ●, Sulphormethoxine alone; ○, sulphormethoxine plus phenylbutazone; ▲, phenylbutazone alone; △, phenylbutazone plus sulphormethoxine.

Mean serum sulphormethoxine in the sulphormethoxine plus phenylbutazone group (0·548 mM) was lower than that in the group receiving sulphormethoxine alone (0·603 mM) but the difference was not statistically significant. The mean serum phenylbutazone level at exsanguination in the group on the combination was 0·315 mM.

Phenylbutazone

The relationships between free and total phenylbutazone for rats receiving phenylbutazone alone and phenylbutazone plus sulphormethoxine are shown in Fig. 2. There is no significant difference between the regression lines for the two sets of values.

The mean serum phenylbutazone in the phenylbutazone plus sulphormethoxine group (0·393 mM) was higher than that in the group receiving phenylbutazone alone, but the difference was not statistically significant. The mean serum level of sulphormethoxine in the group on the combination was 0.544 mM.

In vitro *experiments*

The results are shown in Fig. 3 for both drugs. Again there appeared to be significant ($P \simeq 0{\cdot}05$) displacement of the regression line for sulphormethoxine following the addition of phenylbutazone. The regression equations for these groups were as follows

Sulphormethoxine alone: $y = 0{\cdot}064 + 0{\cdot}648\ x$

Sulphormethoxine plus phenylbutazone: $y = 0{\cdot}009 + 0{\cdot}691\ x$

There was no significant difference between the regression lines for phenylbutazone with and phenylbutazone without sulphormethoxine. The regression equation for the phenylbutazone-alone group was $y = -0{\cdot}036 + 0{\cdot}242\ x$.

The affinity constants calculated from the *in vitro* experiments at total serum concentrations of 0·3 mM using the formula of Goldstein (1939) were $6{\cdot}3 \times 10^4$ for phenylbutazone and $6{\cdot}5 \times 10^3$ for sulphormethoxine. The calculations assume binding to a single site on the albumin molecule alone and utilize a figure of $3{\cdot}7 \times 10^{-4}$M for the concentration of albumin.

Discussion

In the *in vivo* experiments, the administration of phenylbutazone to rats under treatment with sulphormethoxine (concentrations of both drugs in the serum being of the same order as those encountered clinically) increased the proportion of the sulphonamide in the free state. This could be seen also to occur *in vitro*. The situation in the intact animal, however, is much more complicated than that *in vitro*. As has been pointed out by Gillette (1967), in the case of a drug bound to serum proteins only, the fraction displaced from binding would be freed to distribute itself and attain a fresh equilibrium in the whole volume of extracellular fluid rather than just in plasma. This would have the effect of minimizing the degree of increase in free drug. It would be expected also that the total level of drug in the plasma would be lowered by the addition of the displacing agent, and this was used by Anton (1961) as a measure of displacement.

In the present *in vivo* experiments the mean total sulphormethoxine level was indeed a little lower in the sulphormethoxine plus phenylbutazone group than in those receiving sulphormethoxine alone. The difference, however, was not significant.

Additionally, the displaced drug will be rendered more liable to excretion and biotransformation *in vivo*, both of which factors will help to diminish any increase in pharmacological activity resulting from displacement of the bound form. Variation in endogenous factors such as concentration of free fatty acids which have a strong displacement effect on acidic drugs (Solomon, Schrogie & Williams, 1968) must also be considered.

In fact the mean free (sac) level of sulphormethoxine was slightly greater in the group on the combination, although not significantly so. The relatively minor effect of displacement of a drug bound only to serum proteins predicted by Gillette (1967) is thus confirmed, even though the displacing drug, phenylbutazone, would appear to have a ten-fold greater affinity than sulphormethoxine for serum proteins.

Although there must have been some reciprocal displacement of phenylbutazone from binding, it was not really evident in the *in vitro* experiment. In the *in vivo* experiments, the phenylbutazone level would have been falling throughout and a meaningful result is scarcely to be expected from this technique. Furthermore, metabolic products of phenylbutazone such as oxyphenbutazone are not distinguished by the method used (Herrmann, 1959).

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