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Drug displacement from protein binding: isolation of a redistributional drug interaction *in vivo*

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Summary

1. The pharmacokinetic interaction between phenylbutazone and sulphadoxine has been studied in live sheep.

2. Intravenous injection of phenylbutazone causes a rapid fall in the concentration of total sulphadoxine together with a simultaneous rise in the concentration of free sulphadoxine in plasma. Both processes are complete within 3 min of the injection of phenylbutazone.

3. These reciprocal changes in the concentrations of total and free sulphadoxine in plasma do not depend on changes in the absorption, hepatic metabolism or renal excretion of sulphadoxine. By exclusion therefore, it is concluded that the effect of phenylbutazone in this interaction is solely to cause redistribution of the sulphadoxine.

Introduction

Competition between coadministered drugs for binding sites in the body may lead to the displacement of one drug by another. This is one type of pharmacokinetic drug interaction. It involves the redistribution of a drug, as distinct from interactions at other pharmacokinetic levels involving drug absorption, metabolism or excretion.

When one drug displaces another, the unbound or free concentration of the latter increases. This has been well demonstrated *in vitro* (Meyer & Guttman, 1969) and is thought to explain the observed potentiation of the effect of one drug by another in certain clinical situations (Brodie, 1965; Prescott, 1969).

It should be noted, however, that most of the evidence for drug redistribution as a cause of potentiation *in vivo* comes from experiments performed *in vitro*, in particular on plasma proteins. Interactions between coadministered drugs *in vivo* are more difficult to interpret than those performed *in vitro*, because more than one type of interaction may be occurring simultaneously. There have been few studies of the redistribution phenomenon in intact animals or man, and in none of these have the contributions of all interactions at the levels of absorption, metabolism and excretion been measured or excluded. Consequently, there is no firm information available to define the quantitative significance of a purely redistributional drug interaction *in vivo*. Such information is necessary before one can assess the part that redistribution plays in causing drug potentiation in man.

The experiments described here concern the interaction *in vivo* of two drugs (phenylbutazone and the sulphonamide, sulphadoxine) which compete for binding sites on plasma protein. The properties of this type of interaction *in vivo* were first studied by Anton (1961) using live rats. Anton showed the characteristic fall in the concentration of total sulphonamide in plasma, resulting at least in part from the diffusional loss of displaced sulphonamide from the vascular compartment, which occurred after the injection of the displacing agent sulphinpyrazone. The interpretation of Anton's experiments was complicated by the fact that the effect of sulphinpyrazone on the renal excretion of the sulphonamide was not measured in the rat, and the effect on metabolism was not examined. Furthermore, the free drug concentrations were not measured directly in the plasma.

These studies were extended by McQueen (1969) who demonstrated, using *in vivo* dialysis in rats, the concomitant rise in free sulphonamide in the extracellular fluid when a displacing agent (phenylbutazone) was injected. Because of the small size of the rat, only one observation could be made on each animal after the injection of the displacing drug, and so the time course of the interaction could not be followed in a single animal.

In this study we have used sheep, which are large enough to supply many blood samples in the course of each experiment. Thus it has been possible to follow in detail the time course of the interaction between phenylbutazone and the sulphonamide sulphadoxine in individual animals, and to obtain values for the concentrations of both total and free sulphonamide in each plasma sample. By combining this with experiments designed to assess the roles of hepatic metabolism and renal excretion, we have been able to identify the redistribution component of the interaction and study it in isolation.

This paper presents the evidence for identification of the redistribution component of the interaction between phenylbutazone and sulphadoxine in live sheep. Wardell (1971) has analysed the redistribution process in detail.

Preliminary accounts of some of these findings have been published (Wardell & McQueen, 1969, 1970).

Methods

Thirty experiments were performed on ten crossbred sheep weighing 45–55 kg. With a few stated exceptions the animals were not anaesthetized and were standing in a cage for the greater part of each experiment. Each animal was used in rotation for several experiments some weeks apart.

Sulphadoxine (Fanasil, Roche) and phenylbutazone (Butazolidin, Geigy or phenylbutazone, B.P.) were administered by one jugular vein as the sodium salt in aqueous solution at a concentration of 200 mg base/ml, at pH 8–9. Appropriate control experiments were performed as described. Blood samples were obtained from the opposite jugular vein, anticoagulated with potassium oxalate 1·5 mg/ml (Rieder, 1963) and the plasma deep frozen promptly on separation.

Sulphonamide analysis

Unconjugated sulphadoxine in plasma and in ultrafiltrates was measured by the method of Morris (1941), which gave a recovery of 95% from plasma and a

standard deviation of 2·1% (n=29). Unconjugated urinary sulphadoxine was measured by the method of Bratton & Marshall (1939).

Phenylbutazone had no effect on the measurement of unconjugated sulphadoxine by either method, but it interfered when a solution of sulphadoxine was heated, so that neither method was suitable for the measurement by hydrolysis of conjugated sulphadoxine after the displacement.

Phenylbutazone analysis

This was performed by the method of Burns, Rose, Chenkin, Goldman, Schulert & Brodie (1953). Sulphadoxine did not interfere with this estimation.

Ultrafiltration

Five millilitre samples of plasma were adjusted to pH 7·4 by gassing with 5% CO_2 in oxygen, and then ultrafiltered through washed Visking dialysis tubing at 37° C using the same gas mixture at a pressure of 1080 mbar. Although the partial pressure of CO_2 was thus excessive, the pH did not fall but actually rose slightly during ultrafiltration to a maximum of 7·6. Control experiments showed that this small rise produced negligible change in the binding of sulphadoxine. Two millilitres of ultrafiltrate were produced in approximately 1 hour. The presence of protein was looked for in all ultrafiltrates when paratoluenesulphonic acid was added during the sulphonamide estimation. Very occasionally turbidity was observed, in which case the sample was discarded.

In control experiments with large volumes of plasma, no progressive change of the sulphonamide concentration in the ultrafiltrate could be detected as ultrafiltration progressed, even if as much as 60% of the initial plasma volume was removed as ultrafiltrate. In other tests using plasma samples loaded with sulphadoxine *in vitro*, ultrafiltration gave results very close to those obtained by dialysis of the same plasma samples.

Calculation of free sulphonamide in plasma

The concentration of free sulphonamide in each plasma sample was obtained by applying the following corrections to the value measured in the ultrafiltrates (Klotz, 1953 ; Keen, 1965 ; Rieder, 1963).

Volume occupied by the plasma proteins. The total protein content of mixed sheep plasma was 7·0 g/100 ml (microbiuret method). Taking the specific volume of plasma proteins as 0·75, concentrations in ultrafiltrate were therefore multiplied by 0·948 to convert to the corresponding concentration in whole plasma.

Donnan effect. A correction for this is required since sulphadoxine (pK=6·1 ; Struller, 1968) is more than 95% dissociated at pH 7·4. The magnitude of this effect was determined from the distribution of sodium (measured by flame photometry) between mixed sheep plasma and its ultrafiltrate. The ratio [Na] ultra-

filtrate: [Na] plasma water was 0·932 (mean of three experiments). Thus (inverting for anions) the final expression incorporating both corrections becomes

$$[\text{Free sulphadoxine}]_{\text{plasma}} = [\text{Sulphadoxine}]_{\text{uf}} \times 0{\cdot}932 \times 9{\cdot}948$$
$$= [\text{Sulphadoxine}]_{\text{uf}} \times 0{\cdot}884$$

Membrane binding. There was no measurable binding of sulphadoxine to the Visking membrane, and so no filter-loss correction was required.

Results

In vitro *displacement of sulphadoxine from sheep plasma protein by phenylbutazone*

To confirm that phenylbutazone would displace sulphadoxine from sheep plasma, the effect of adding 300 μg/ml of phenylbutazone (a concentration typical of that attained in the *in vivo* experiments) was tested on a sample of mixed sheep plasma *in vitro*, containing 100 μg/ml of sulphadoxine. The results of duplicate analyses are shown in Fig. 1, where the concentration of free sulphadoxine rose from 36·7 μg/ml to 75·2 μg/ml on addition of the phenylbutazone. These values also represent the percentage of free sulphadoxine, since the total sulphadoxine was 100 μg/ml. Conversely the fraction of sulphadoxine bound (determined by subtracting the free from the total) fell from 63·3% to 24·8%.

Phenylbutazone, then, is an effective agent for displacing sulphadoxine from sheep plasma.

Speed of displacement versus time required for ultrafiltration

A separate experiment was performed on plasma *in vitro* to determine whether the speed of the displacement was slow enough to be detectable by ultrafiltration.

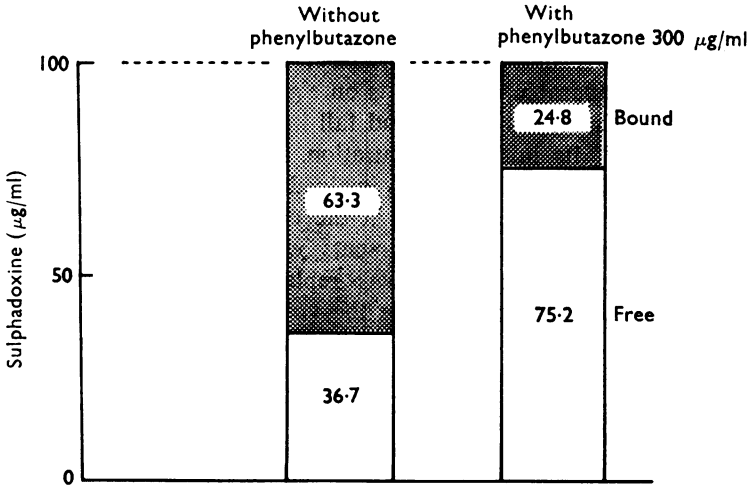

FIG. 1. Effect of phenylbutazone (300 μg/ml) on the binding of sulphadoxine (100 μg/ml total concentration) to mixed sheep plasma *in vitro*. The values given for the concentrations of bound and free sulphadoxine are in μg/mil and also represent percentages of the total sulphadoxine.

Sheep plasma was loaded with sulphadoxine (100 μg/ml) and then phenylbutazone was added (200 μg/ml). Part of the plasma was ultrafiltered immediately; large volumes of plasma were used so that a usable volume of ultrafiltrate was obtained 12 min after adding the phenylbutazone. The remainder was ultrafiltered 4 days later.

The results were as follows (mean of duplicate experiments):

Concentration of free sulphadoxine:

(a) Immediately after adding phenylbutazone 47·5 μg/ml.

(b) Four days after adding phenylbutazone 45·0 μg/ml.

Thus, full displacement of sulphadoxine had occurred within the 12 min needed to produce a rapid ultrafiltrate. This finding has a bearing on the validity of free drug levels *in vivo* at the time of sampling, and is considered later.

In vivo *displacement: Time course of the changes in the concentration of sulphadoxine in plasma after intravenous injection of phenylbutazone*

In these experiments the animals were first given an intravenous dose of 1·0 g of sulphadoxine. Two hours or more were then allowed for the drug to distribute throughout the body (a process with a half life of 12 min when measured in one sheep), and to enable its subsequent plasma half life to be established. The postdistributional plasma half life varied widely among different sheep. In six animals the half life ranged from 4–22 h with a mean of 10·7 h (S.D.=4·3). Phenylbutazone (2·0 g) was then given by rapid intravenous injection and 20-ml blood samples were taken at appropriate intervals thereafter to follow the time course of the change in drug concentrations in the plasma. Part of each plasma sample was ultrafiltered, so that for each point the concentrations of total and free sulphadoxine were obtained, together with total phenylbutazone.

From 1 to 30 min after the phenylbutazone

The effect of phenylbutazone was rapid and profound. Figure 2A and B illustrates two experiments in separate animals; similar results were obtained in twelve essentially comparable experiments on eight sheep. Within 30 s of injecting the phenylbutazone there was a profound fall in the total sulphadoxine in plasma to approximately half its initial concentration. The fall was complete within 3 min, after which the total sulphadoxine remained relatively constant for 30 minutes. Simultaneously there was a sharp rise in the concentration of free sulphadoxine in the same samples. Control experiments were performed in four sheep, in which the phenylbutazone injection was replaced by an injection of isotonic phosphate buffer solution having double the volume and a higher pH (10·2) than the phenylbutazone solution. These control injections resulted in negligible change in the concentration of total or free sulphadoxine in plasma: Figure 3 illustrates a control experiment in one animal: it is typical of the results obtained in four animals.

Immediate effect

In two animals, the time course of the interaction was examined at even shorter intervals. For these experiments, the animals were anaesthetized with halothane

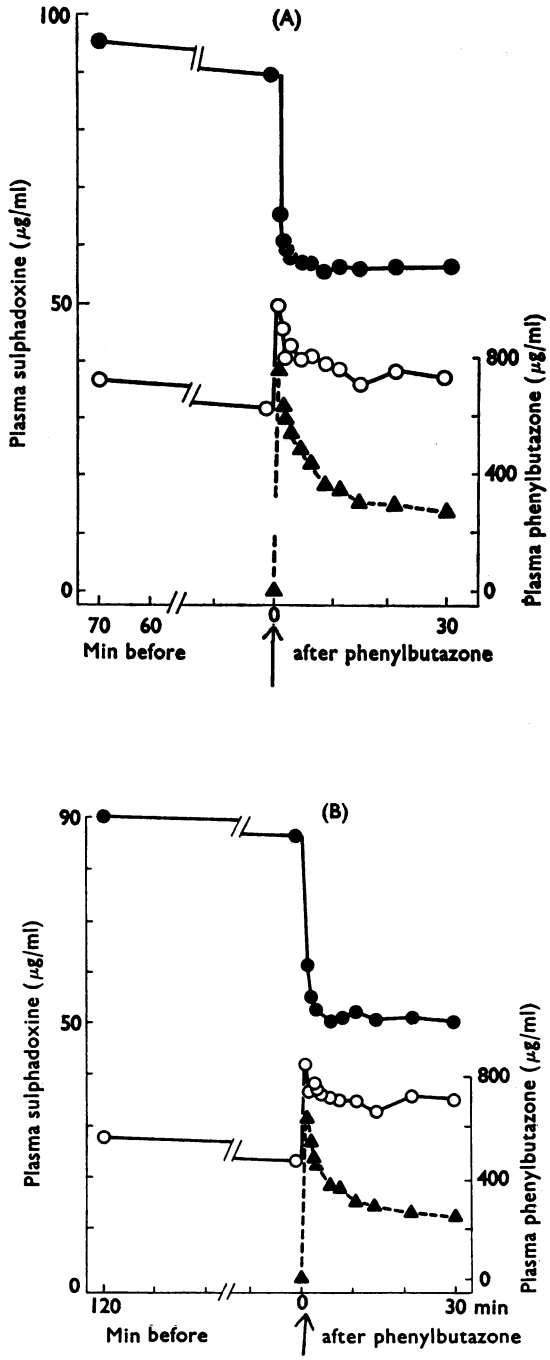

FIG. 2. (A), Effect of 2·0 g intravenous phenylbutazone at zero time (arrow) on the total and free concentrations of sulphadoxine in plasma in one sheep. The three traces from above down are the concentrations of total sulphadoxine, free sulphadoxine and total phenylbutazone. Abscissa: time in minutes before and after phenylbutazone. Left ordinate: sulphadoxine scale. Right ordinate: phenylbutazone scale. (B), Experiment in another sheep, other details as for (A).

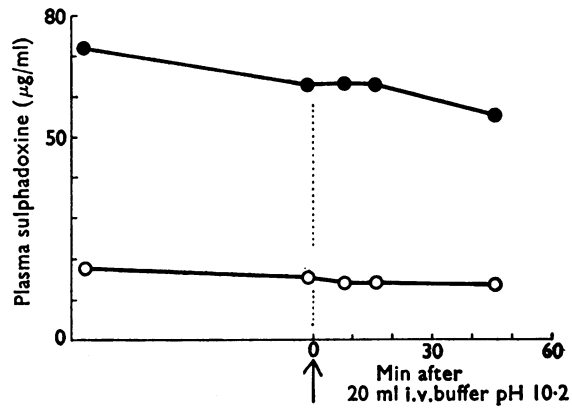

FIG. 3. Control experiment in one animal injecting buffer solution instead of phenylbutazone. Upper trace, concentration of total sulphadoxine; lower trace, free sulphadoxine. Other details as for Fig. 2(A).

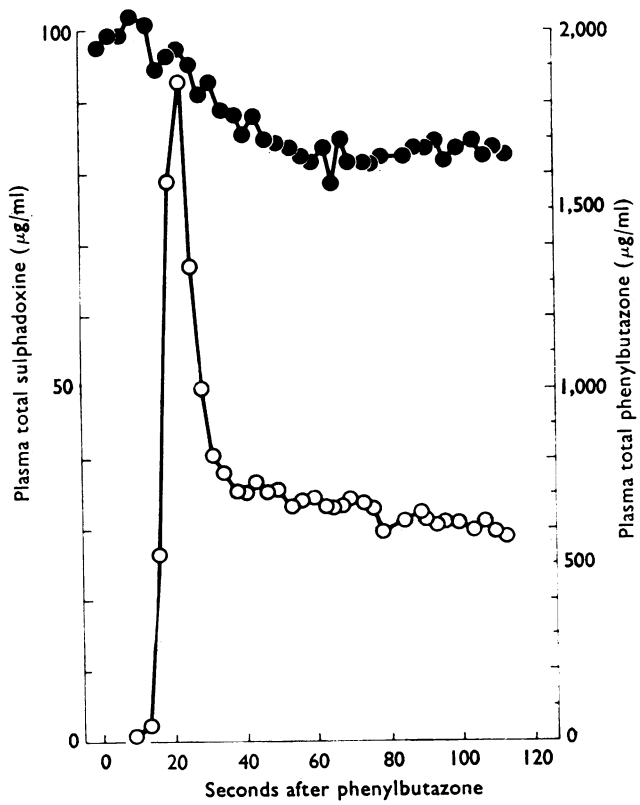

FIG. 4. Earliest stage of the interaction. Concentration of total sulphadoxine in plasma (top trace) falls during the first circulation of the bolus of phenylbutazone (lower trace), which has an appearance time of 16 seconds.

and heparinized, and blood samples were collected in rapid sequence from a freely running jugular cannula from the moment phenylbutazone was injected. The result of one of the experiments is illustrated in Fig. 4. The bottom trace shows the arrival of the bolus of phenylbutazone in the contralateral jugular vein, with an appearance time of 16 s following one complete circulation. At the moment the phenlbutazone appeared, the sulphonamide total in the same plasma samples (top trace) had begun to fall, and this fall neared completion during the first passage of the bolus of phenylbutazone. The magnitude of the fall was smaller than in the previous experiments where unanaesthetized, unheparinized animals were used.

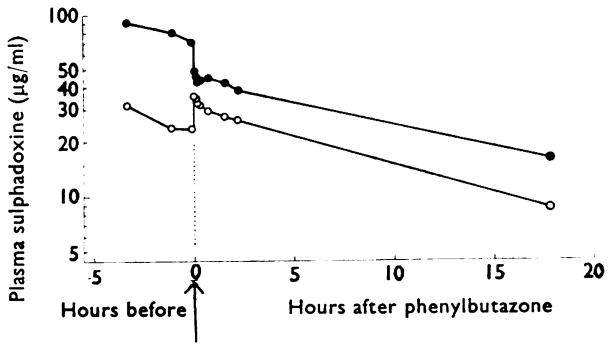

FIG. 5. Result of a displacement experiment on a longer time scale, using a semilogarithmic plot. Upper trace, concentration of total sulphadoxine ; lower trace, free sulphadoxine. Other details as for Fig. 2(A).

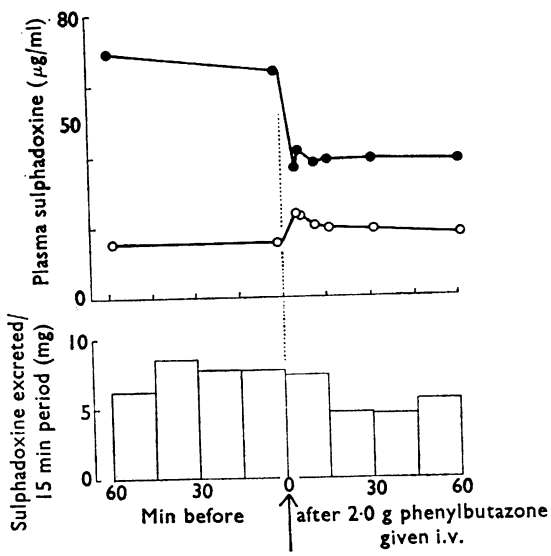

FIG. 6. Result of a displacement experiment in which the urinary sulphadoxine was measured at 15 min intervals (bars at bottom of figure represent sulphadoxine excreted in mg/15-min period). Upper trace, concentration of total sulphadoxine ; lower trace, free sulphadoxine. Other details as for Fig. 2(A).

Later effects

Thirty minutes or more after the rapid change produced by the phenylbutazone, the concentrations of both total and free sulphadoxine resumed their decline. An example is seen in Fig. 5, where the interaction is followed for 18 h and a semi-logarithmic scale is used.

It was possible to examine whether the injection of phenylbutazone caused a change in the plasma half life of sulphadoxine. In four sheep, the half life of sulphadoxine for 2 h before the phenylbutazone was compared with its half life from 30 min to 2 h after the phenylbutazone. The results were as follows:

Mean half life of total sulphadoxine before phenylbutazone=12·4 h (S.D.=4·16).
Mean half life of total sulphadoxine after phenylbutazone=12·9 h (S.D.=3·96).
Difference not significant ($P>0·9$; paired t test).

Thus, beyond the acute changes occurring in the first 30 min, phenylbutazone caused no significant change in the plasma half life of total sulphadoxine.

Assessment of the contribution of metabolism and excretion

It is conceivable that the fall in the concentration of total sulphadoxine in plasma (but not the rise in its free concentration) could be due in part to enhancement of its metabolic destruction or of its renal excretion, caused by the injection of phenylbutazone.

Although the very rapidity of the interaction renders these possibilities unlikely, it was possible to test the point directly. This was done in two ways: first, a displacement experiment was carried out while urine was simultaneously collected to determine whether a change in sulphonamide excretion occurred; second, a displacement experiment was performed in animals in which the kidneys had been removed and the liver excluded from the circulation.

Renal excretion

Four female sheep were catheterized with balloon catheters. Water was administered by oropharyngeal tube to maintain a diuresis. Urine was collected for 1 h before and 1 h after injection of phenylbutazone. Other details, including drug doses, were as for the previous experiments. In three sheep the excretion rate fell and in the fourth did not change significantly. Mean sulphadoxine excretion during the hour before the phenylbutazone was 23·7 mg and after phenylbutazone was 15·6 mg. There was thus no suggestion that a sudden marked increment in sulphadoxine excretion resulting from the increased free fraction could have contributed to the fall in the total sulphadoxine in plasma. One experiment is illustrated in Fig. 6.

Renal and hepatic exclusion

Two sheep were given loading doses of sulphadoxine. One of these sheep was unusually large (63·5 kg) so the doses of sulphadoxine and phenylbutazone were scaled up to 1·4 and 2·8 g, respectively, for this animal. They were then anaesthetized with halothane and methoxyflurane. The kidneys were removed, and all mesenteric arteries and the hepatic artery ligated. Finally, the portal vein was

ligated to exclude the liver from its afferent blood supply. The phenylbutazone was then injected and blood samples collected as before.

Hepatic exclusion was technically difficult in the sheep. The major problem was to maintain the animal's fluid balance, and the results are probably complicated by the saline infusions which were needed to replace fluid oozing from the intestine after ligation of the portal vein. Furthermore, the phenylbutazone appeared to antagonize the anaesthetic, and changes in the depth of anaesthesia were required following the phenylbutazone. Despite these technical problems, clear-cut and consistent results were obtained in both experiments, and are illustrated in Fig. 7A and B; as in the intact animals, phenylbutazone caused a rapid fall in the concentration of total sulphadoxine in plasma together with a simultaneous rise in free sulphadoxine.

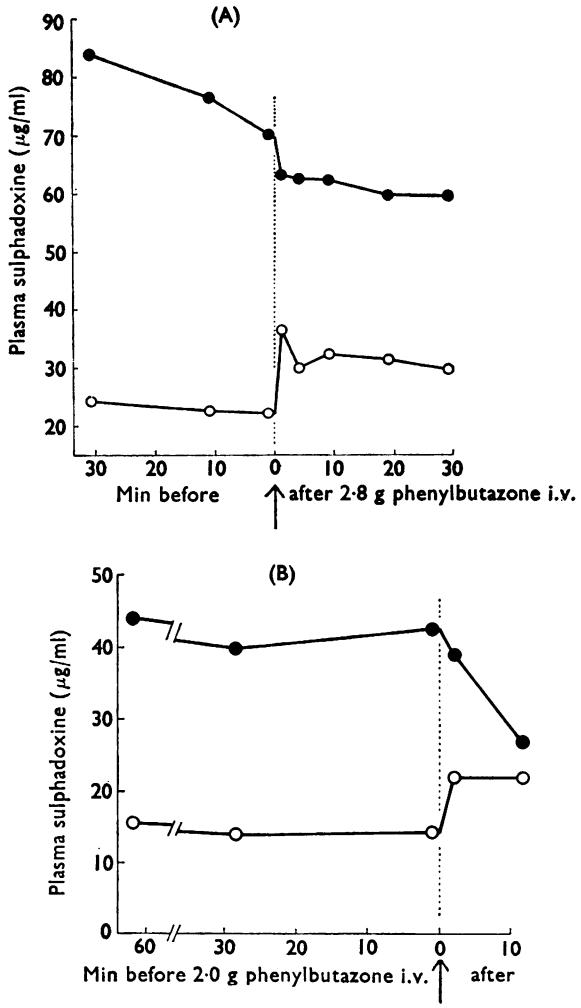

FIG. 7. Results of two displacement experiments performed in separate animals following bilateral nephrectomy and hepatic exclusion. Upper trace, concentration of total sulphadoxine; lower trace, free sulphadoxine. Other details as in Fig. 2(A), except for the drug doses shown in Fig. 7(A).

Discussion

Mechanism of the fall in the concentration of total sulphadoxine in plasma after the injection of phenylbutazone

Of the possible pharmacokinetic modes of drug interaction (absorption, distribution, metabolism and excretion), absorption can be disregarded since both drugs were administered intravenously.

Enhancement of its metabolism or excretion can lower the plasma concentration of a drug (Burns & Conney, 1965; Prescott, 1969). From the following evidence, however, we conclude that neither metabolism nor excretion plays an important part in the rapid fall of total sulphadoxine in plasma after the injection of phenylbutazone in these experiments.

The rapidity of the effect of phenylbutazone is difficult to explain on a metabolic or an excretory basis. It is clear that neither process could have contributed to the marked fall in total sulphadoxine which occurred in the first circulation of the bolus of phenylbutazone, since this blood had not by then been presented to any organ except the lungs. It is conceivable that the remainder of the fall could arise from enhanced metabolism or excretion; but in order to exert such a profound effect and then to cease, all within the space of 3 min, such processes would need to have extraordinary kinetics. Moreover there was a fall, rather than a rise in the urinary excretion of sulphadoxine. (This change could have been due to an effect of the phenylbutazone either on the glomerular filtration of sulphadoxine, for example via haemodynamic changes—or on its renal tubular handling, but since our interest lay mainly in the direction of the change we did not elucidate its mechanism.) Furthermore, the sharp fall in the concentration of total sulphadoxine in plasma still occurred in the two animals after renal and hepatic ablation. This is unequivocal evidence that the major part of the effect was independent of hepatic metabolism and renal excretion.

One cannot entirely exclude the possibility of some metabolic contribution to the fall in the concentration of total sulphadoxine in plasma at the longer time intervals after administration of phenylbutazone. Since hepatic exclusion involved anaesthesia together with massive cardiovascular and body fluid changes in these animals, one cannot obtain meaningful information by quantitatively comparing displacement experiments in the hepatectomized animals with those in the controls. Nor is the apparent constancy of plasma sulphadoxine half life of much help in detecting a metabolic contribution. In the absence of redistribution, the sulphadoxine half life after the injection of phenylbutazone should reflect the net result of any changes in both metabolism and excretion. After the injection of phenylbutazone, however, it is doubtful whether the concept of half life has much meaning: the concentrations of plasma bound and free sulphadoxine will change in a complex manner as elimination of the two drugs at different rates produces further mutual redistribution. It should be noted that none of the evidence obtained in this study points to any component involving enhanced metabolism, and for the reasons given it is likely to be insignificant if it exists at all. Therefore the fall in concentration of total sulphadoxine in plasma represents largely or solely a redistribution, namely the loss of part of the diffusible sulphonamide from the intravascular compartment following its displacement from binding sites on the plasma protein.

Rise in the concentration of plasma-free sulphadoxine after the injection of phenylbutazone

Validity of ultrafiltrates for measuring plasma free drug concentrations at the time of sampling

If the rate of the displacement interaction on the plasma protein were slow, it is possible that displacement might continue in the sampled blood after removal from the animal and before the specimen was ultrafiltered. If this happened it would cause the measured values of free sulphonamide to be falsely high, although the total sulphonamide would be unaffected.

In the few situations where exact measurement has been possible, the speed of interaction of small molecules with plasma proteins is very rapid. Froese, Sehon & Eigen (1962) using a temperature-jump method found that the half time for dissociation of albumin-dye complexes was of the order of tens or hundreds of milliseconds. No comparable experiments have been performed with drug molecules. However, when drug molecules have been studied by more conventional techniques such as ultrafiltration or dialysis (Rolinson & Sutherland, 1965 ; see also Meyer & Guttman, 1969) the interaction has occurred too rapidly to be measured. The fact that certain protein-bound molecules such as penicillin and phenol red can be almost entirely cleared from the renal blood in one passage through the kidney supports this idea of the rapidity at least of the dissociation (Goldstein, 1949).

In the case of the displacement of sulphadoxine by phenylbutazone, two of our own observations indicate that the results obtained in these experiments are not subject to serious error from this source. First, the interaction *in vitro* had taken place well within the 12 min required to produce an ultrafiltrate as rapidly as possible. Second, the fall in the concentration of total sulphadoxine in plasma *in vivo*, which involves the sequential processes of displacement of sulphadoxine from plasma protein followed by its loss from the intravascular compartment, was well advanced within a single circulation time (16 s).

From this evidence we conclude that the values given for free sulphadoxine in these experiments are valid measurements of the free drug at the moment of sampling, certainly for points taken 12 min or more after the displacement, and probably also for all earlier points.

Mechanism of the rise

As noted earlier, absorption can be disregarded since both drugs were administered intravenously.

Alteration in metabolism or excretion could not account for the rise in the concentration of free sulphadoxine because even if metabolism and excretion were suppressed entirely the concentration of sulphadoxine in plasma would, at best, only cease to fall. The direct experiments involving nephrectomy and hepatic exclusion confirmed that the rise was independent of renal excretion and hepatic metabolism. By exclusion therefore, the rise in plasma free sulphadoxine must be due to the redistribution of part of the body load of sulphadoxine so that more of it is transferred into the unbound form in the extracellular fluid.

Phenylbutazone caused rapid, reciprocal changes in the concentrations of total and free sulphadoxine in plasma. These changes were not due to effects of phenyl-

butazone on the absorption, metabolism, or excretion of sulphadoxine. They are therefore the result of a purely redistributional drug interaction.

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