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# Drug displacement from protein binding: source of the sulphadoxine liberated by phenylbutazone

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## Summary

1. A quantitative study has been made of the redistribution of sulphadoxine produced by phenylbutazone in live sheep.

2. From calculations based on plasma measurements, 73% of the sulphadoxine in the body can be accounted for as the sum of the plasma-bound, extracellular-free and intracellular-free drug in the whole animal. This sum is termed the accountable sulphadoxine.

3. After the injection of phenylbutazone there was a large shift of sulphadoxine from the plasma-bound form into the free form. The gain in free drug significantly exceeded the loss of bound sulphadoxine from the intravascular compartment, but the discrepancy could easily be explained by displacement of some bound sulphadoxine from extravascular plasma protein.

4. Phenylbutazone did not displace sulphadoxine from sheep erythrocytes *in vitro*, nor from homogenates of liver, kidney or skeletal muscle.

5. Although the existence of other susceptible binding sites cannot be entirely excluded, this evidence strongly suggests that all the sulphadoxine liberated by phenylbutazone comes from binding sites on plasma protein.

## Introduction

In the preceding paper (McQueen & Wardell, 1971), it was shown that when phenylbutazone was injected intravenously into sheep previously loaded with sulphadoxine, the concentrations of total and free sulphadoxine in plasma underwent large, rapid and reciprocal changes. It was shown furthermore that these changes were the result largely or solely of the redistribution of sulphadoxine, uncomplicated by effects of phenylbutazone on the absorption, metabolism or excretion of sulphadoxine.

The redistribution of sulphadoxine consists, at least in part, of its liberation from binding sites on plasma protein followed by diffusion of this unbound (free) sulphadoxine into extravascular compartments. Qualitatively, this process by itself could cause the observed fall in the concentration of total sulphadoxine and the rise in free sulphadoxine in plasma. It is not yet clear, however, whether plasma protein is the only source of bound sulphadoxine susceptible to displacement, nor has it been established whether this can account quantitatively for the observed

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changes. Are there, for example, extravascular binding sites (interstitial or intracellular) which contribute free sulphadoxine in this interaction? In this paper I have analysed the redistribution with the aim of identifying the origin and fate of all the bound sulphadoxine which is displaced by phenylbutazone.

The first part of the paper deals with quantitative aspects of the redistribution *in vivo*. Starting with the measured concentrations of bound and free sulphadoxine in plasma samples, a balance sheet has been constructed for each animal to compare the amount of bound sulphadoxine displaced by phenylbutazone from plasma protein with the observed amount of free sulphadoxine gained by the extracellular and intracellular fluids. One can then test whether plasma proteins supplied all the sulphadoxine liberated by phenylbutazone. If more free sulphadoxine were gained by the body than were liberated from plasma protein, then clearly some binding site other than plasma protein must have contributed to the liberation. Conversely, if the gain in free sulphadoxine were equal to, or less than, the observed loss from plasma binding, then plasma protein could be the sole source of bound sulphadoxine participating in the redistribution.

The second part of the paper deals with the converse question of whether phenylbutazone can displace sulphadoxine from tissues, as distinct from plasma proteins.

The results of both types of experiment were consistent with the hypothesis that plasma protein is the sole source of liberated sulphadoxine.

## Methods

The administration of sulphadoxine and phenylbutazone to live sheep, and the techniques for ultrafiltration and drug analyses were described by McQueen & Wardell (1971).

*In vitro* experiments were performed on four types of sheep tissue, by loading with sulphadoxine and subsequently adding phenylbutazone. If phenylbutazone displaced sulphadoxine from binding sites, this would cause a rise in the concentration of sulphadoxine in ultrafiltrates of fluid bathing the tissues. The four tissues were: intact erythrocytes and homogenates of liver, muscle and kidney.

### *Erythrocytes*

Plasma from oxalated or heparinized sheep blood was separated and the cells were washed 3 times in 10 times their own volume of Hanks solution (Paul, 1970) and resuspended in Hanks solution at a packed cell volume of 50%. Sulphadoxine from a 10-times concentrated standard solution was then added to the erythrocyte suspension to give a desired concentration (see below), and the suspension incubated at 37° C for 1 hour. The sulphadoxine loaded erythrocyte suspension was then divided into two halves. To one half, a concentrated standard solution of phenylbutazone was added to give final drug concentrations of 300 $\mu$g/ml of phenylbutazone and 200 $\mu$g/ml of sulphadoxine. To the other half, an equivalent volume of isotonic phosphate buffer solution (which had no displacing ability) was added to act as a control. The control suspension thus had the same concentration of total sulphadoxine as the test suspension, but no phenylbutazone. The suspensions were again incubated at 37° C for 1 hour. At the end of incubation, the cells were spun down, the supernatant was ultrafiltered, and the ultrafiltrate was analysed for sulphadoxine. The free sulphadoxine thus determined in

the phenylbutazone loaded tube was then compared with that of the control. Duplicate results were averaged.

### *Tissue homogenates*

A sheep was killed by exsanguination and the liver, a kidney, and a hindquarter were perfused arterially with Hanks solution at a pressure of 180 mmHg for 90 min, in order to wash out the blood (1 mmHg≡1·333 mbar). The liver was perfused via the portal vein in addition to the hepatic artery. A 50 g sample of liver was then chopped finely, added to 150 ml of Hanks solution at 4° C, and homogenized in a Waring Blendor using two 1 min runs with cooling in between. Phase contrast microscopy of the homogenate confirmed that there were essentially no intact cells or tissue particles. Homogenate (180 ml) was incubated at 37° C with sulphadoxine (222 μg/ml) for 30 minutes. To an aliquot, phenylbutazone in phosphate buffer solution (pH 7·4) was then added to give final drug concentrations of 300 μg/ml phenylbutazone and 200 μg/ml sulphadoxine. The homogenate was incubated at 37° C for a further 30 minutes. Part of the solid matter was removed by centrifugation and duplicate samples of the supernatant were ultrafiltered at 37° C, a 5-ml sample of supernatant producing 2 ml of ultrafiltrate in 3 hours. Two control runs were performed at the same time, identical in all respects except that equivalent volumes of phosphate buffer or Hanks solution, respectively, were added to the homogenate instead of phenylbutazone. A blank run was also performed, adding equivalent volumes of buffer solution, instead of the two drug solutions, to the original homogenate. Sulphadoxine was then analysed in the ultrafiltrates.

The above procedure was repeated in every detail with another 50 g sample of liver, except that 0·25 M sucrose was used as the suspending medium instead of Hanks solution. Samples of skeletal muscle and kidney were then treated in the same way. Thus, six experiments were performed, each of the three tissues being tested in both Hanks solution and sucrose, with duplicate ultrafiltration and analysis in each case.

The dry weight of all homogenates amounted to 7% (±0·5%) of the wet weight.

## Results

### *Accountable sulphadoxine in the sheep*

The amount of drug present in the body in any given form can be obtained by multiplying the concentration of that form by the volume in which it is distributed. From measurements of the bound and free concentrations of drug in a plasma sample, one can derive the concentrations of three forms of that drug present in the body: (i) the concentration of plasma bound drug is obtained directly; (ii) the concentration of free drug in extracellular water is equal to that in an ultrafiltrate of plasma; (iii) the concentration of free drug in intracellular water is obtained by multiplying the concentration in extracellular water by a factor which corrects for pH partitioning of the drug between intracellular and extracellular fluids. These arguments assume that bound drug is completely mixed throughout the plasma volume and that free drug has equilibrated throughout total body water. The validity of these assumptions is discussed later.

The following figures for body fluid volumes in the sheep (ml/kg body weight) were obtained from the literature: plasma volume=44·6 (Hodgetts, 1961; Evans blue method); extracellular fluid volume=198·0 (McDonald, Coats & Munro, 1954; inulin space); total body water=558·0 (Hansard & Lyke, 1956; antipyrine space); intracellular fluid volume by subtraction=360·0.

The pH partitioning of free sulphadoxine between intracellular and extracellular water was calculated from the following relationship (Jacobs, 1940):*

$$\frac{\text{Intracellular concentration}}{\text{Extracellular concentration}} = \frac{1 + 10^{(pH_{ic} - pK_a)}}{1 + 10^{(pH_{ec} - pK_a)}}$$

The extracellular pH has been taken as 7·4 (Borrie, Lichter & Miller, 1967). The whole body intracellular pH has been taken as 6·9 (Waddell & Bates, 1969) and in addition the calculations have been repeated for several alternative assumed intracellular pH values ranging from 6·5 to 7·2. Sulphadoxine is a weak acid with a pKa of 6·1 (Struller, 1969). Thus, when the intracellular pH was 6·9, the extracellular sulphadoxine concentration was multiplied by 0·349 to obtain the intracellular concentration.

The sum of the plasma bound, extracellular-free and intracellular-free sulphadoxine calculated in the above manner represents the total amount of sulphadoxine in the body that can be accounted for by measurements on plasma samples. I shall call this total the 'accountable' sulphadoxine.

In order to determine how much of the body content of sulphadoxine was accountable in sheep, four animals were each given a 1·0 g dose intravenously. The concentrations of total and free sulphadoxine in plasma were measured 1 h after injection to allow time for distribution (the half-time of the distribution phase measured by rapid sampling in one sheep was 12 min).

The results are shown in Table 1, which gives the individual and mean values for the four sheep at the 1 h point, assuming a value of 6·9 for the intracellular pH. It can be seen that a mean of 13% of the original 1·0 g dose of sulphadoxine was bound to the plasma protein; a further 34% was free in the extracellular fluid; and 21% was free in the intracellular fluid. Thus, 68% of the original dose was accountable. Allowing for drug elimination during the 1 h equilibration time (assuming a mean half life of 10·7 h; McQueen & Wardell, 1971) increases the

TABLE 1. *Accountable sulphadoxine in four normal sheep 1 h ± 10 min after intravenous injection of 1,000 mg sulphadoxine*

| | | Sulphadoxine per animal (mg) (intracellular pH=6·9) | | | |
|---|---|---|---|---|---|
| Sheep no. | Wt. (kg) | Plasma bound | Extracellular-free | Intracellular-free | Accountable |
| 321 | 49·5 | 145·1 | 316·0 | 200·5 | 661·6 |
| 322 | 49·5 | 143·5 | 343·7 | 218·1 | 705·3 |
| 324 | 50·3 | 130·3 | 325·6 | 206·6 | 662·5 |
| 330 | 43·5 | 112·7 | 359·5 | 228·1 | 700·3 |
| | Means: | 132·9 | 336·2 | 213·3 | 682·4 |

If intracellular pH=6·5, mean accountable sulphadoxine becomes 571·8 mg.
If intracellular pH=7·2, mean accountable sulphadoxine becomes 865·3 mg.

* Jacobs' (1940) equation 6, p. 31, has the numerator and denominator transposed. The correct form shown here is derived from Jacobs' equation 5, p. 31, and is the same as that given by Schanker (1962) p. 505.

accountable fraction to 73% of the sulphadoxine present at that time in the body. If the intracellular pH were as low as 6·5 or as high as 7·2, the accountable sulphadoxine would be, respectively, 57% or 87% of the original dose.

These figures should not be confused with the conventional plasma protein-binding of the drug. At 100 μg/ml in the plasma, sulphadoxine is 63% protein-bound (McQueen & Wardell, 1971), but the above figures show that this plasma bound fraction forms only 20% of the accountable sulphadoxine, or 14% of the sulphadoxine actually present at that time in the body.

*Change in accountable sulphadoxine after administration of phenylbutazone*

Ten experiments were performed on six sheep which had been given intravenous loading doses of 1·0 g sulphadoxine 2 h or more previously. In eight experiments the phenylbutazone was given as an intravenous dose of 2·0 g. These experiments were similar to, and included, those illustrated in Fig. 2 of the paper by McQueen & Wardell (1971). In the remaining two experiments the phenylbutazone was given as an oral dose of 5·0 g in aqueous suspension.

Accountable sulphadoxine was calculated from the plasma concentrations measured immediately before, and at intervals after, the administration of the phenylbutazone. The main interest lies in comparing the accountable sulphadoxine 1 h after the administration of phenylbutazone with that immediately before it. These calculations are summarized in Tables 2 and 3, which give results for nine of the ten experiments. (The tenth experiment, which belongs in Table 2, was not included because the 60 min point was lacking; nevertheless, the result at the 30 min point in this experiment agreed closely with the seven experiments in the table.) There was little overall change in accountable sulphadoxine: taking

TABLE 2. *Effect of phenylbutazone (2·0 g intravenous) on accountable sulphadoxine in seven experiments on five sheep (intracellular pH assumed to be 6·9)*

| | Mean amount of sulphadoxine (mg per animal) in these locations: | | | |
|---|---|---|---|---|
| Time | Plasma-bound | Extracellular-free | Intracellular-free | Accountable |
| 'Initial' (immediately before phenylbutazone) | 107·0 | 206·7 | 131·1 | 444·8 |
| 'Final' (1 h after phenylbutazone) | 34·3 | 261·1 | 165·6 | 461·1 |

Mean final accountable sulphadoxine equals 104% of initial value. Individual final values as % initial: 106·0%, 98·6%, 97·6%, 117·3%, 92·5%, 109·2%, 110·0%. Range=92·5% to 117·3%.
Effect of other values for intracellular pH: If intracellular pH=6·5, mean final value=100·0% of initial value. If intracellular pH=7·2, mean final value=109·7% of initial value.

TABLE 3. *Effect of phenylbutazone (5·0 g orally) on accountable sulphadoxine in two sheep*

| | Sulphadoxine (mg per animal) in these locations: | | | |
|---|---|---|---|---|
| Time | Plasma-bound | Extracellular-free | Intracellular-free | Accountable |
| Before phenylbutazone | 111·5 | 217·4 | 138·0 | 466·9 |
| 15 min after phenylbutazone | 46·7 | 261·4 | 165·9 | 474·0 |
| 60 min after phenylbutazone | 29·6 | 279·4 | 177·3 | 486·3 |
| Final accountable sulphadoxine=104·2% of initial value | | | | |
| Before phenylbutazone | 168·6 | 273·8 | 173·7 | 616·1 |
| 15 min after phenylbutazone | 64·0 | 370·2 | 234·9 | 669·1 |
| 60 min after phenylbutazone | 45·0 | 378·0 | 239·9 | 662·9 |
| Final accountable sulphadoxine=107·6% of initial value | | | | |

all nine experiments at the 1 h point, there was a mean rise of 4%, which was not significant ($P>0{\cdot}5$, paired $t$ test). It was more informative, however, to examine in detail the large shift of sulphadoxine between the bound and free compartments. At the 1 h point, the plasma-bound sulphadoxine showed a mean fall of 79·1 mg/animal, while the free sulphadoxine per animal had risen by a mean of 99·1 mg. Thus the gain in free sulphadoxine per animal exceeded the fall in plasma-bound sulphadoxine by 20·0 mg, that is, 26%. The difference was not quite significant ($0{\cdot}05<P<0{\cdot}1$), but this calculation does not make any allowance for elimination of sulphadoxine during the 1 h equilibration period. Allowing for a 6% loss of accountable sulphadoxine by elimination during the 1 h period would widen the difference and make it significant: in that case the mean rise in free sulphadoxine would exceed the fall in plasma bound sulphadoxine by 48·0 mg, that is, 61% ($P<0{\cdot}005$).

*Failure of phenylbutazone to displace sulphadoxine from tissues* in vitro

In these experiments, sulphadoxine was first added to a suspension of red cells or to homogenates of liver, kidney or skeletal muscle. Subsequently, the phenylbutazone was added. The concentration of free sulphadoxine in the phenylbutazone treated suspension was then compared with that in controls to which no phenylbutazone had been added.

The results are shown in Table 4, where the main information appears in columns 4 and 5.

It can be seen that phenylbutazone caused no appreciable change in the concentration of free sulphadoxine in any of the tissues tested.

TABLE 4. *Effect of phenylbutazone on the concentration of sulphadoxine in ultrafiltrates of tissue suspensions*

| Column no. 1<br>Tissue and suspending medium | 2<br>Nature of experiment (phenylbutazone or control) | 3<br>Total concentration of sulphadoxine added to suspension ($\mu$g/ml) | 4<br>Sulphadoxine concentration measured in duplicate ultrafiltrates ($\mu$g/ml) | 5<br>% change of ultrafilterable sulphadoxine (with respect to mean control) due to phenylbutazone |
|---|---|---|---|---|
| Erythrocytes in Hanks solution | Buffer control | 200 | 155 | |
| | Phenylbutazone | 200 | 152 | −2% |
| Liver homogenate in Hanks solution | Buffer control | 200 | 197 | |
| | Hanks control | 200 | 189 | |
| | Phenylbutazone | 200 | 195 | +1% |
| Liver homogenate in sucrose | Buffer control | 200 | 193 | |
| | Sucrose control | 200 | 196 | |
| | Phenylbutazone | 200 | 194 | −0·25% |
| Muscle homogenate in Hanks solution | Buffer control | 200 | 216 | |
| | Phenylbutazone | 200 | 207 | −4% |
| Muscle homogenate in sucrose | Buffer control | 200 | 213 | |
| | Sucrose control | 200 | 206 | |
| | Phenylbutazone | 200 | 196 | −6% |
| Kidney homogenate in Hanks solution | Buffer control | 200 | 175 | |
| | Hanks control | 200 | 164 | |
| | Phenylbutazone | 200 | 171 | +1% |
| Kidney homogenate in sucrose | Buffer control | 200 | 170 | |
| | Sucrose control | 200 | 182 | |
| | Phenylbutazone | 200 | 178 | +1% |

## Discussion

### *Do plasma concentrations of bound and free drug represent concentrations in body compartments?*

In using a plasma concentration to derive the concentration of drug in a body compartment, the first requirement is that complete mixing has occurred. In these experiments, we need to have mixing of bound sulphadoxine throughout the plasma volume and equilibration of free sulphadoxine throughout the total body water. Since most of the plasma mixing occurs within 1 min (Lawson, 1962), it is the time needed for the equilibration in body water that is the limiting factor.

The exact rate at which sulphadoxine penetrates total body water is unknown, but I have assumed that equilibration is complete within 1 h on the following evidence: (i) I have found a half life of 12 min for the distribution phase of sulphadoxine in a whole animal. (ii) The rapid changes in free sulphadoxine were complete within 5 min of the injection of phenylbutazone. Thereafter, the free concentration remained relatively constant for at least 30 min (McQueen & Wardell, 1971). (iii) Where rates are known, the equilibration of other drugs in body water is complete within 1 h: antipyrine in the sheep (Hansard & Lyke, 1956) and dimethyloxazolidinedione (DMO) in the dog (Waddell & Butler, 1959).

I have therefore assumed that 1 h after the injection of phenylbutazone, the concentration of sulphadoxine in ultrafiltrates of plasma samples is representative of that in total body water. Somewhat less than 6% of the sulphadoxine in the body will be eliminated during this 1 h period (McQueen & Wardell, 1971): a half life of 10·7 h is equivalent to the overall elimination of 6%/h, and phenylbutazone reduced the rate of renal excretion). It is therefore feasible to compare the accountable sulphadoxine 1 h after the phenylbutazone injection with the value existing immediately before it, making an allowance of up to 6% for elimination.

The other requirement is that the details of the distribution of free drug throughout total body water be accurately known. Two aspects of this which require comment are the partition of drug between intravascular and interstitial water, and the partition between extracellular and intracellular water.

In assuming that ultrafiltrates of plasma are representative of all extracellular water, I have ignored the effect of the Donnan distribution of drug across the capillary wall. At worst, this would mean that the concentration of free drug in plasma water had been overestimated by 7·5% (see Paper I (McQueen & Wardell, 1971) **Methods**). Since plasma water forms only 21% of the extracellular water, the maximum error in my estimate of extracellular free drug would be +1·5%. Moreover, interstitial fluid also contains plasma protein, the concentration of which may range up to half that of the intravascular concentration (Landis & Pappenheimer, 1963). This would tend to reduce the magnitude of the overestimate due to the Donnan effect, so that any such error in these experiments is small enough to be ignored.

In calculating the pH-partitioning of free sulphadoxine between extracellular and intracellular fluids, I have assumed that the aqueous phases are homogeneous; that the cell membranes are permeable to the unionized form of the drug but not to the ionized form; and that the concentration of the unionized form is equal in extracellular and intracellular water. These are the same as the assumptions

made by Waddell & Butler (1959) and Waddell & Bates (1969) for deriving intracellular pH from the distribution of DMO between intracellular and extracellular fluids. The present argument is essentially the inverse of Waddell & Butler's in that a value for intracellular pH has been assumed in order to infer the intracellular/extracellular partitioning of free sulphadoxine. It should be noted that the present calculations are not affected by the question (Waddell & Bates, 1969) of whether the DMO method or certain microelectrode methods give the best indication of the 'true' intracellular pH; nor are these calculations affected by the complexities of the concept of intracellular pH. We are taking the value for intracellular pH simply as a parameter which describes the partitioning of an unbound weak acid (DMO), and using it to calculate the partitioning of the unbound fraction of another weak acid (sulphadoxine).

To allow for possible errors in the result for intracellular pH given by the DMO method itself, all the computations were repeated for a range of intracellular pH values from 6·5 to 7·2 in addition to the accepted value of 6·9. These are shown in Tables 1 and 2. Neither extreme value alters the conclusions of this paper, although the size of the accountable fraction of sulphadoxine changes somewhat with intracellular pH.

### *Source of the liberated sulphadoxine*

The experiments described here provide a substantial test of the hypothesis, proposed at the outset, that plasma proteins supply all the sulphadoxine liberated in the interaction with phenylbutazone.

#### *Experiments in intact animals*

The main evidence came from the experiments in which accountable sulphadoxine was calculated. We have seen that the rise in accountable sulphadoxine indicates a significant discrepancy between the amounts of sulphadoxine lost from plasma binding and gained by body water. The extent to which the gain in free sulphadoxine exceeded the loss in bound sulphadoxine could be as high as 61%, and there is a need to examine whether a difference of this size invalidates the hypothesis that all the liberated sulphadoxine came from plasma protein. The hypothesis appears to demand that there be no change in accountable sulphadoxine, that is, that the loss of plasma bound sulphadoxine be exactly balanced by the gain of free sulphadoxine. However, this demand only applies if all the plasma protein in the body is in the intravascular compartment. There is, moreover, strong evidence that a considerable amount of plasma protein is present in extravascular sites (Sellers, Katz, Bonorris & Okuyama, 1966). There is no reason to assume that this protein cannot bind sulphadoxine, and liberate it, in much the same manner as does intravascular protein. Drug bound to extravascular albumin is unaccountable, but displacement into the free form would render it accountable, thus raising the accountable pool by that amount and producing a discrepancy of the sort we have observed. The question should therefore be, does the extra gain in free sulphadoxine exceed the amount which could be displaced from extravascular albumin?

We have seen that the size of the extra gain in free drug was 61% of the amount displaced from intravascular plasma protein. Since the mean size of the extravascular albumin pool is 3 times as great as the intravascular pool (Sellers

*et al.*, 1966), only a small contribution from the extravascular pool would be needed to account for the extra 61%. The conclusion from these results is therefore that plasma protein supplies all the sulphadoxine liberated by phenylbutazone, provided that a modest contribution is allowed from extravascular plasma protein.

At this stage it is worth digressing to examine the concept of accountable sulphadoxine in the light of the foregoing discussion of extravascular plasma protein. It has been shown that 73% of the drug present in the body is accountable, before invoking any extravascular binding. If allowance were made for extravascular binding, the size of the accountable fraction of drug would rise accordingly. From Table 1 it can be calculated that if the amount of drug bound extravascularly were 1·94 times the amount bound intravascularly, then 100% of the drug in the body would be accountable. It is not even necessary for all this binding to be attributed to interstitial albumin since presumably some of the unaccountable sulphadoxine in the body will be sequestered in different sites—for example, bound to other interstitial or intracellular components or dissolved in fat depots. The accountable fraction rose by up to 10% following the injection of phenylbutazone, so that in that situation 80% of the body load of the drug was accountable.

*Experiments on tissues* in vitro

These showed unequivocally that in the four tissues tested (intact red cells, and homogenates of liver, kidney and muscle) there was no sulphadoxine susceptible to displacement by phenylbutazone. These results support those obtained in the intact animals, although one cannot exclude the possibility that binding sites which might exist in the whole organs could be destroyed by incubation of homogenates. This qualification does not apply to the red cell experiments.

In summary then, the results obtained in these experiments show that there is sufficient sulphadoxine displaced from plasma protein to account for all the free drug liberated ; and that conversely no sulphadoxine could be displaced from any of the four tissues tested *in vitro*. Although the possible participation of other sources of bound sulphadoxine has not been excluded, all these results are entirely consistent with the hypothesis that the redistributed sulphadoxine came entirely from binding sites on plasma protein.

## *Implications for redistributional drug interactions in man*

If a redistributional drug interaction consisted solely of the displacement of bound drug from plasma protein into body water, then one could predict from *in vitro* measurements the extent of a redistribution *in vivo*. Sellers & Koch-Weser (1970) have proposed such *in vitro* screening tests to predict the significance of possible displacement interactions clinically.

There are, however, important limitations to the predictive value of *in vitro* tests. These hinge on the relative sizes of plasma bound and accountable drug, and on how much drug in the body is actually accountable. If 100% of the drug in the body is accountable (that is if it is either bound to plasma protein or free in body water, with none in other forms), then predictions from *in vitro* experiments should apply exactly *in vivo*, provided appropriate calculations are made for the volumes of the plasma, interstitial and intracellular compartments and the

partitioning of free drug between them. But if, for example, only 1% of the drug in the body is accountable, then no matter how much binding and displacement can be demonstrated on plasma protein *in vitro*, the effect of this redistribution on the overall distribution of drug in the body would be negligible. There could be a dramatic fall in the total drug in plasma, with only a trivial rise in free drug and its pharmacological effect.

One would expect that the clinical significance of redistributional drug interactions involving plasma binding, and the predictive value of experiments on plasma *in vitro*, would depend more on the size of the accountable fraction of the drug in the body than on its plasma binding alone. Although the size of the accountable fraction has not been calculated for many drugs in man, there is often sufficient data in the literature to enable the calculations to be made. In the case of drugs that are largely unaccountable in the body, binding and displacement experiments on tissue samples might have greater predictive value than experiments on plasma.

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## REFERENCES

BORRIE, J., LICHTER, I. & MILLER, W. N. (1967). Experimental heart surgery in sheep. *J. surg. Res.*, **7**, 560–568.

HANSARD, S. L. & LYKE, W. A. (1956). Measurement of total body water in sheep using $I^{131}$ labelled 4-iodo-antipyrine. *Proc. Soc. exp. Biol. Med.*, **93**, 263–266.

HODGETTS, V. E. (1961). The dynamic red cell storage function of the spleen in sheep. III. Relationship to determination of blood volume, total red cell volume, and plasma volume. *Aust. J. exp. Biol. med. Sci.*, **39**, 187–196.

JACOBS, M. H. (1940). Some aspects of cell permeability to weak electrolytes. *Cold Spring Harb. Symp. quant. Biol.*, **8**, 30–39.

LANDIS, E. M. & PAPPENHEIMER, J. R. (1963). Exchange of substances through the capillary walls. In: *Handbook of Physiology*, Vol. **2**, *Circulation*, pp. 961–1034. American Physiological Society. Baltimore: Williams & Wilkins.

LAWSON, H. C. (1962). The volume of blood—a critical examination of methods for its measurement. In: *Handbook of Physiology*, Vol. **1**, Section 2, *Circulation*, pp. 23–49. American Physiological Society. Baltimore: Williams & Wilkins.

MCDONALD, I. R., COATS, D. A. & MUNRO, J. (1954). Body water and electrolyte changes in bilaterally nephrectomised sheep. *Aust. J. exp. Biol. med. Sci.*, **32**, 275–284.

MCQUEEN, E. G. & WARDELL, W. M. (1971). Drug displacement from protein binding. Isolation of a redistributional drug interaction *in vivo*. *Br. J. Pharmac.*, **43**, 312–324.

PAUL, J. (1970). *Cell and Tissue Culture*, 4th ed. Edinburgh and London: E. & S. Livingstone.

SCHANKER, L. S. (1962). Passage of drugs across body membranes. *Pharmac. Rev.*, **14**, 501–530.

SELLERS, A. L., KATZ, J. K., BONORRIS, G. & OKUYAMA, S. (1966). Determination of extravascular albumin in the rat. *J. Lab. clin. Med.*, **68**, 177–185.

SELLERS, E. M. & KOCH-WESER, J. (1970). Displacement of warfarin from human albumin by dizaoxide and ethacrynic, mefenamic and nalidixic acids. *Clin. Pharmac. Ther.*, **11**, 524–529.

STRULLER, TH. (1969). Long-acting and short-acting sulphonamides. Recent developments. *Antibiot. Chemother, Basel*, **14**, 179.

WADDELL, W. J. & BATES, R. G. (1969). Intracellular pH. *Physiol. Rev.*, **49**, 285–329.

WADDELL, W. J. & BUTLER, T. C. (1959). Calculation of intracellular pH from the distribution of 5,5-dimethyl 2,4-oxazolidinedione (DMO). Application to skeletal muscle of the dog. *J. clin. Invest.*, **38**, 720–729.

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