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Drug distribution as a function of binding competition. Experiments with the distribution dialysis technique

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In the distribution dialysis technique each of the two dialysis chambers contains a binding system, and a drug is allowed to distribute between them. This technique was tested by using various intracellular and extracellular binder preparations over wide concentration ranges, and model drugs selected for their known binding properties. The drugs were then tested at therapeutic concentrations in standardized systems of liver homogenate (0·5 g ml^{-1}) and whole blood (0·02 ml ml^{-1}). The resulting intracellular/extracellular concentration ratios were characteristic for the binding properties of the various drugs. Thus, for imipramine, a drug with strong tissue and weaker plasma binding properties, the concentration ratios were 25 for the system homogenate/buffer, 0·8 for buffer/blood, and 15 for the competitive system homogenate/blood. In experiments with homogenates from various tissues (liver, lung, kidney, intestine, brain) and blood in the standard system, the following approximate ratios were obtained: 1 for antipyrine, 2 for phenylbutazone, 14 for imipramine (but only 8 with muscle, skin and adipose tissue). These results reflect both the individual binding to intracellular and extracellular components and the tissue/blood concentration ratios in vivo. It is suggested that distribution dialysis is an in vitro method for characterizing the distribution of drugs. It is also concluded that drug distribution is largely determined by a binding competition between tissue and blood sites.

Distribution of a drug in the body is commonly considered to be dependent on the drug's plasma binding, its permeation properties, and the blood flow rates in various tissues. Even though the potential of tissue components to bind drugs is larger than that of plasma proteins, it is only in recent years that tissue binding has found its way into pharmacokinetic considerations (Gillette 1973; Wilkinson 1975; Jusko & Gretch 1976; Gibaldi et al 1978; Benet 1978). In earlier studies (Bickel & Steele 1974) we have found that basic lipophilic drugs like imipramine or chlorpromazine show considerable tissue binding, whereas phenylbutazone shows little and salicylate none. Tissue binding of the above basic drugs is largely due to binding to the phospholipids of intracellular membranes (Di Francesco & Bickel 1977; Römer & Bickel 1979). Such drugs, however, are also bound to serum albumin and/or other blood components (Bickel 1975). Therefore, an intracellular/extracellular binding competition must be assumed to be operative, and this binding competition, rather than blood binding alone, is likely to be a major determinant of drug distribution.

We have set up a simple in vitro model to quantify distribution of a drug between two binding systems. The model has been used to test the hypothesis that distribution is a function of binding competition. In this model each of the two chambers of an equilibrium dialysis device contains a binder simulating intracellular and extracellular phases, respectively, and a drug is then allowed to distribute in this system. This technique, although it has been described many years ago under the name 'distribution dialysis' (Bischoff & Stauffer 1957; Kallee et al 1957; Kallee & Oppermann 1958), has been surprisingly neglected ever since.

This study demonstrates that distribution dialysis can be used as a method for characterizing the distribution of drugs. In addition, it supports the view that distribution of many drugs is largely controlled by intracellular/extracellular binding competition. These conclusions are based on the results of the following types of experiments:

Distribution dialysis was tested as a suitable method by using various binders over wide concentration ranges and by determining the distribution of drugs.

Tissue homogenates and whole blood were used in fixed proportions simulating intracellular and extracellular phases. Distribution of model drugs with known binding affinities and capacities was measured.

These values were compared with tissue/blood concentration ratios in vivo, measured after intravenous injection of the same drugs in rats.

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MATERIALS AND METHODS

Distribution dialysis technique

When equilibrium dialysis is used for the purpose of binding studies, a drug or other ligand is dialysed against plasma, protein, or other binders. Once diffusion-equilibrium is reached, the free and bound drug concentrations can be determined. By contrast, in the distribution dialysis technique both dialysis chambers contain a binder, and a drug is allowed to distribute between the two binding systems. Distribution then is expressed as drug concentration ratio of the two chambers. The same equipment can be used for the two techniques. In this study a Dianorm apparatus was used according to Weder & Bickel (1970) and Weder et al (1971). Its two 1 ml chambers were separated by a Visking cellulose membrane of 10000 to 20000 awu (unified) exclusion limit. Time to reach equilibrium was 2·5 h. All experiments were carried out at 37° C with drugs and binders dissolved in phosphate buffer pH 7·4, 0·01 M containing 0·9% NaCl.

In this study extracellular (e) and intracellular (i) preparations were used as binders. The following parameters were varied in certain experiments:

L = ligand, $c_{L(o)}$ = initial ligand concentration
B = binder, B(e), B(i), $c_{B(e)}$, $c_{B(i)}$

After equilibration, the concentrations of the ligand (drug) $c_{L(i)}$ and $c_{L(e)}$ were determined in the two dialysis chambers. The concentration ratio $c_{L(i)}/c_{L(e)}$ was used as a measure for distribution or binding competition. Since the concentrations were determined in either chamber, adsorption of drug to the dialysis membrane was not a critical factor in these experiments.

Model drugs have been used at a concentration $c_{L(o)}$ which was of the order of therapeutic plasma concentrations in man. The intracellular and extracellular binder concentrations were chosen in an attempt to simulate "physiological" proportions. In standard experiments a $c_{B(i)}/c_{B(e)}$ ratio of 25 was chosen, based on physiological ratios of intracellular/extracellular protein of 15 in terms of concentration and 35 in terms of mass. Since homogenate concentrations above 50% are not feasible, standard experiments were carried out with tissue homogenate $c_{B(i)}$ = 0·5 g ml^{-1} against whole (citrated) blood $c_{B(e)}$ = 0·02 ml ml^{-1}. Other intracellular and extracellular preparations have also been used. Rat liver microsomes were prepared as described earlier (Bickel & Steele 1974). Identical results were obtained with human or rat blood, and with human or bovine serum albumin (BSA). In the first series of experiments one of the binders was either omitted, or its concentration was varied over a wide range.

Distribution in vivo

Male Sprague-Dawley rats, about 250 g, were given model drugs intravenously in therapeutic doses. Animals were decapitated 2, 10, and 60 min after injection and immediately dissected. Drug concentrations were determined in tissue samples and blood and expressed as tissue/blood concentration ratios.

Analytical procedures

Imipramine was used ^{14}C-labelled as purchased from The Radiochemical Centre, Amersham, U.K. Imipramine was extracted from biological materials at pH 13 with heptane. Phenylbutazone was determined by the method of Burns et al (1953) which was slightly modified. Phenazone (antipyrine) was used as the *N*-methyl[^{14}C] compound purchased at New England Nuclear and extracted from biological material according to Yoshimura et al (1968).

RESULTS

To obtain optimum conditions and to gain insight into the factors influencing the distribution of drugs in distribution dialysis, experiments shown in Figs 1–5 were carried out. In these experiments the distribution of model drugs between rat liver homogenates and whole blood at varying concentrations was determined. The ligand concentrations were kept constant. Fig. 1 shows the binding of imipramine at

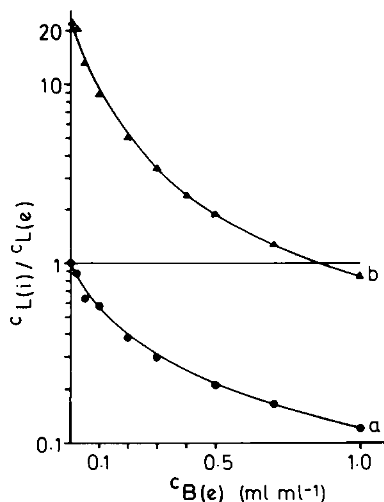

FIG. 1. Tissue/blood concentration ratios of imipramine (5 μM) in vitro. Distribution dialysis with whole blood as extracellular binder B(e). Intracellular binder B(i): none in curve a, rat liver homogenate (0·5 g ml^{-1}) in curve b. Mean values of 2–4 experiments.

increasing extracellular binder concentrations (whole blood) in the absence and in the presence of competitive intracellular binder (liver homogenate). In Fig. 2 distribution of the same drug is shown as a function of intracellular binder concentration up to the highest feasible liver homogenate concentration of 0·5 g ml^{-1}. With some tissues lower homogenate concentrations must be used. To extrapolate distribution values obtained under such conditions, the curve in

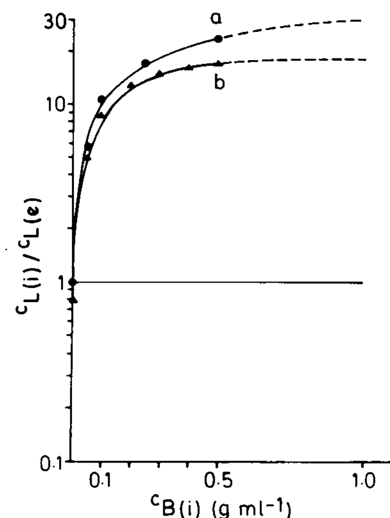

FIG. 2. Tissue/blood concentration ratios of imipramine (5 μM) in vitro. Distribution dialysis with rat liver homogenate as B(i). B(e): none in curve a, whole blood (0·02 ml ml^{-1}) in curve b. Mean values of 3 experiments.

Fig. 3 was used. This was obtained by diluting both B(i) and B(e) without altering their proportion. A plateau reached at homogenate concentrations above 0·25 g ml^{-1} was also obtained with phenylbutazone (400 μM). By analogy with Fig. 1, but with characteristic differences, Fig. 4 depicts the distribution values of phenylbutazone as a function of whole blood concentration.

In a second series of experiments the attempt was made to characterize model drugs in distribution dialysis carried out under standardized conditions (Tables 1–3). In addition to imipramine (Tables 1 and 2), phenylbutazone and antipyrine (phenazone) were used as model drugs (Table 3) in the following types of experiments listed in tabular form: liver homogenate, liver microsomes, or buffer against whole blood, plasma, serum albumin, or buffer (Tables 1 and 3) and various tissue homogenates against whole blood (Tables 2 and 3).

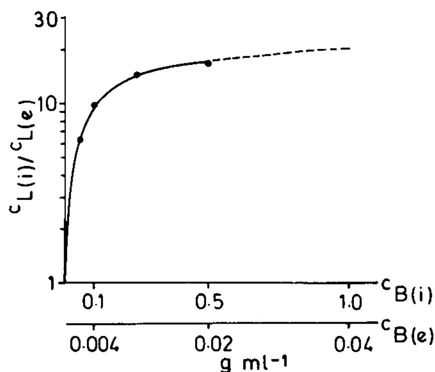

FIG. 3. Tissue/blood concentration ratios of imipramine (5 μM) in vitro. Distribution dialysis with rat liver homogenate as B(i) against whole blood as B(e) in constant proportion. Mean values of 2 experiments.

To compare the values obtained with distribution dialysis of tissue homogenates against blood (Tables 2 and 3) with distribution in vivo, the model drugs were injected into rats i.v. and the tissue/blood concentration ratios determined after 2, 10 and 60 min (Table 4).

DISCUSSION

Binding values of various drugs with biological materials have been determined earlier (Bickel & Steele 1974). According to these, both binding

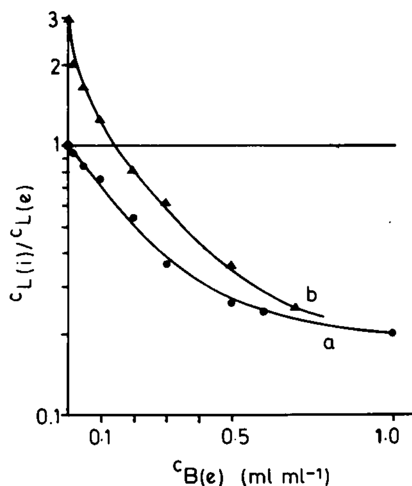

FIG. 4. Tissue/blood concentration ratios of phenylbutazone (400 μM) in vitro. Distribution dialysis with whole blood as B(e). B(i): none in curve a, rat liver homogenate (0·5 g ml^{-1}) in curve b. Mean values of 3 experiments.

Table 1. Tissue/blood concentration ratios of imipramine (5 μM) *in vitro*. Distribution dialysis with model binder preparations from rats.

Preparation* intracellular	extracell.	n	$c_{L(i)}/c_{L(e)}$	*P*
Liver homogenate	whole blood	14	15·2 s.d. 2·6	< 0·05
Liver homogenate	plasma	7	17·5 s.d. 1·8	> 0·05
Liver homogenate	BSA	6	20·1 s.d. 2·3	< 0·05
Liver homogenate	buffer	8	25·2 s.d. 4·7	
Buffer	whole blood	5	0·81 s.d. 0·02	< 0·025
Buffer	plasma	5	0·83 s.d. 0·01	< 0·050
Buffer	BSA	5	0·87 s.d. 0·03	< 0·001
Buffer	buffer	5	1·01 s.d. 0·04	
Liver microsomes	whole blood	5	15·1 s.d. 2·5	> 0·2
Liver microsomes	plasma	5	16·8 s.d. 1·9	> 0·2
Liver microsomes	BSA	5	18·1 s.d. 2·2	> 0·1
Liver microsomes	buffer	5	20·6 s.d. 2·3	

* Liver homogenate (0·5 g ml^{-1}) or microsomes contained therein (protein 20 mg ml^{-1}) in left dialysis chamber; whole blood (0·02 ml ml^{-1}) or equivalent amounts of plasma (0·01 ml ml^{-1}) or bovine serum albumin (0·4 mg ml^{-1}) plus ligand in right chamber. Phosphate buffer 0·01 M, pH 7·4, containing 0·9% NaCl. Dialysis time 2·5 h, 37 °C.
Mean values with s.d., n number of experiments.

affinity and capacity of imipramine are higher with tissue homogenates or microsomal fractions than they are with serum albumin. The opposite was observed with phenylbutazone. No binding with extracellular or intracellular components could be detected with antipyrine (unpublished results). If distribution is controlled by binding competition, then these model drugs should show different and characteristic distribution patterns. This is indeed the case as is demonstrated by tissue/blood concentration ratios obtained *in vivo* (Table 4). Thus, imipramine shows values far above unity, particularly in liver, lung, kidney, small intestine, and brain. In contrast, the values for phenylbutazone are below unity and those for antipyrine are around unity. This experiment does not differentiate between total drug and metabolites. However, since sampling times were chosen before appreciable metabolism could have taken place, and again after distribution equilibrium, the values obtained are likely to characterize the distribution of the unchanged drugs.

Table 2. Tissue/blood concentration ratios of imipramine (5 μM) *in vitro*. Distribution dialysis with rat tissue homogenates against whole blood as binders.

Tissue*	$c_{L(i)}/c_{L(e)}$
Liver	16·9 s.d. 1·5
Lung	12·6 s.d. 2·3
Kidney	12·5 s.d. 1·2
Small intestine	13·7 s.d. 3·2
Brain	15·6 s.d. 1·5
Skeletal muscle	6·4 s.d. 0·7
Skin	7·5 s.d. 3·2
Epididymal fat	9·9 s.d. 2·7

* Homogenate and whole blood concentrations either 0·1 and 0·004 or 0·25 and 0·01 g ml^{-1}, respectively, extrapolated to 0·5 and 0·02 g ml^{-1} according to Fig. 3. Other conditions as Table 1. Mean values of 7 experiments with s.d.

Table 3. Tissue/blood concentration ratios of phenylbutazone (400 μM) and antipyrine (300 μM) *in vitro*. Distribution dialysis with binder preparations from rats.

Preparation* Intracellular	extracell.	$c_{L(i)}/c_{L(e)}$ Phenylbutazone	Antipyrine
Liver homogenate	whole blood	2·3	1·02
Liver homogenate	plasma	2·6	1·01
Liver homogenate	BSA	2·7	1·1
Liver homogenate	buffer	3·0	1·1
Buffer	whole blood	0·88	0·99
Buffer	plasma	0·89	—
Buffer	BSA	0·92	—
Buffer	buffer	1·01	—
Liver microsomes	whole blood	1·3	—
Liver microsomes	plasma	1·3	—
Liver microsomes	BSA	1·4	—
Liver microsomes	buffer	1·5	—
Liver homogenate	whole blood	2·5	1·07
Lung. hom.	whole blood	1·9	1·05
Kidney hom.	whole blood	2·1	1·05
Small intest. hom.	whole blood	2·1	0·98
Brain hom.	whole blood	1·9	1·03
Sk. muscle hom.	whole blood	1·9	1·01
Skin hom.	whole blood	1·9	1·03
Epidid. fat hom.	whole blood	2·0	0·98

* Experimental conditions see Tables 1 and 2. Mean values of 2–3 experiments.

In this study the emphasis is on the simulation of drug distribution between binding systems by means of the distribution dialysis technique. For imipramine Fig. 1 shows the binding curve with blood and the 8- to 25-fold elevation of this curve in the presence of the intracellular binder. This drastic elevation of the curve demonstrates the existence of a binding competition, one which is strongly in favour of tissue binding. Fig. 2 specifically shows that extracellular binding has little influence on the distribution of imipramine in this system. In contrast, phenylbutazone distribution is much less influenced by tissue binding, the elevation of the curve being 1-3-fold only (Fig. 4). This is not only in agreement with the distribution of imipramine and phenylbutazone in

Table 4. Tissue/blood concentration ratio of imipramine (5 mg kg^{-1}), phenylbutazone (50 mg kg^{-1}), and antipyrine (50 mg kg^{-1}) in rats *in vivo* after i.v. administration.

Tissue	Imipramine 2	10	60 min	Phenylbutazone 2	10	60 min	Antipyrine 2	10	60 min
Liver	7	9	33	1·2	1·0	1·2	1·3	1·3	1·3
Lung	61	73	124	0·8	0·6	0·7	1·0	1·1	1·0
Kidney	57	34	43	0·8	0·6	0·8	1·2	1·0	1·1
S. intest.	13	14	24	0·5	0·3	0·4	0·7	0·8	0·6
Brain	25	22	32	0·3	0·2	0·3	1·0	0·8	0·8
Sk. muscle	2	8	10	0·4	0·2	0·4	0·4	0·7	0·9
Skin	2	3	12	0·6	0·4	0·6	0·4	0·7	0·9
Epidid. fat	1	1	4	0·3	0·2	0·4	0·2	0·5	0·3

Mean values of 2 experiments per time point.

vivo but also with their binding competition as demonstrated with liver perfusion experiments (Stegmann & Bickel 1977). The results of Figs 1–4 show that distribution dialysis is a suitable model to demonstrate the distribution of drugs according to the kind and concentrations of two binders competing for the drug.

Distribution dialysis experiments in the presence of intracellular, extracellular or both binders (Tables 1 and 3) clearly demonstrate that a binding competition exists and is decisive for the distribution of the drug. With imipramine (Table 1) the binding competition is strongly in favour of intracellular binding. This situation is typical for drugs with high volumes of distribution, e.g. basic lipophilic drugs. In addition, the first eight types of experiments in Table 1 confirm the finding that not only albumin, but also other blood constituents are binders for imipramine (Bickel 1975). That liver microsomes and homogenate yield comparable results, is in agreement with the finding that endoplasmic reticulum and other intracellular membranes are the major binder fractions for imipramine and similar drugs (Bickel & Steele 1974). Identical results were obtained with imipramine at 1, 5, and 10 μM and comparable results with 50 μM.

In Table 2, which shows the distribution of imipramine between blood and homogenates of various tissues in the distribution dialysis, it is easy to recognize two separate groups of tissues: one with tissue/blood ratios around 14 (liver, lung, kidney, intestine, brain) and the other with ratios around 8 (muscle, skin, adipose tissue). This again is in parallel with distribution in vivo as shown on Table 4, with very high tissue/blood ratios for the first group of organs and values as low as unity for the second group. The low but temporally increasing values for the second group of tissues is reminiscent of the pharmacokinetic concept of Bischoff & Dedrick (1968, 1970) which stresses the difference between rapidly perfused tissues (first group) and slowly perfused ones (second group) for the initial distribution of drugs. It is therefore remarkable that these differences in distribution can be seen in the in vitro model where not only flow limitations, but perfusion as such, are absent and the measurements are made after diffusion equilibrium has been reached. This finding strongly suggests that in addition to differences in perfusion rate, tissue binding is of major importance for the distribution of imipramine and similar drugs of high lipophilicity despite obviously low affinity for adipose tissue. Thus, the term "organ/blood partition coefficient" may be misleading and ought to be replaced by the concept of binding competition. Basic lipophilic drugs, in contrast to thiopentone or DDT-like compounds, do not accumulate in tissues rich in triglycerides but rather in phospholipid-rich organs. Indeed, phospholipids have been shown to be binding sites for imipramine-like drugs (Gillette 1973; di Francesco & Bickel 1977; Lee 1977; Elferink 1977; Schwendener & Weder 1978; Frenzel et al 1978; Römer & Bickel 1979; Minchin et al 1979).

The distribution dialysis results of the remaining model drugs are summarized on Table 3. For phenylbutazone the first eight experiments disclose a binding competition similar to imipramine but with values reflecting weaker tissue and stronger plasma binding. In addition, microsomes do not seem to be the major intracellular binding sites. Preliminary experiments with 50 μM phenylbutazone were comparable to those with 400 μM. The values for antipyrine are close to unity in all distribution dialysis experiments which was to be expected according to its lack of intracellular and extracellular binding. Tissue/blood values of antipyrine in vivo were also close to unity with most tissues (Table 4).

For phenylbutazone (weak intracellular and strong extracellular binding) tissue/blood ratios in the distribution dialysis $\leqslant 1$ respectively would be expected. The elevated values may result from the large tissue excess in the arbitrarily chosen standard system using 0·5 g ml^{-1} tissue homogeneate and 0·02 ml ml^{-1} whole blood. By altering the proportions and dilutions of the binders, the tissue/blood ratios can be lowered. With a system using 0·1 g ml^{-1} liver homogenate and 0·1 ml ml^{-1} whole blood the following ratios were obtained (preliminary results): imipramine 9, phenylbutazone 1·0, salicylate 0·9. These distribution values are close to those obtained in vivo (Table 4) and are in agreement with the intracellular and extracellular binding properties of the drugs. The latter system may therefore be better still for characterizing individual drugs or classes of drugs.

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