Chapter 10

Effect of Binding to Plasma Proteins on the Distribution, Activity and Elimination of Drugs

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With 9 Figures

The binding of a drug to plasma proteins is of importance in so far as it modifies the pharmacological activity of that drug. Protein-binding may affect drug activity in one of two ways: either by changing the effective concentration of the drug at its site of action or by changing the rate at which the drug is eliminated, thus affecting the length of time for which effective concentrations are maintained. In this chapter Section A deals with the effects of protein-binding on drug distribution and hence on activity; Section B discusses the ways in which protein-binding can affect drug elimination and Section C attempts a quantitative analysis of the ways in which these various factors interact in drug kinetics.

A. Effect on Distribution and Activity

1. The Drug-Protein Complex

The great majority of the drugs which combine with plasma proteins do so by means of a freely reversible bond with plasma albumin (GOLDSTEIN, 1949; MEYER and GUTTMAN, 1968). The drug-albumin interaction may be described by the Mass-Action Law thus:

$$D_F + \text{Free Sites} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} D_B \tag{1}$$

where D_F and D_B are the free and bound drug respectively and k_1 and k_2 are the rate constants for association and dissociation. Hence

$$K = \frac{k_1}{k_2} = \frac{[D_B]}{[D_F]\,[\text{Free sites}]} = \frac{[D_B]}{[D_F]\,(nP - [D_B])} \tag{2}$$

where K is the association constant for the drug-albumin bond, n is the number of binding sites per mole of protein and P is the molar concentration of albumin and $[D_B]$ and $[D_F]$ are in molar concentrations (GOLDSTEIN, 1949). The assumptions inherent in this equation are that the drug may be bound at n discrete sites on the albumin molecule, that all these sites have the same affinity for the drug and that all sites are mutually independent.

Most measurements of drug binding are made under equilibrium conditions when the extent of binding is governed by K (Eqn. 2). However, it is also important to know how rapidly a drug can be released from binding, i. e. the value of k_2. The bond between an azo dye and albumin dissociates with a half-life of 20 msecs (FROESE et al., 1962); thus it appears likely that in most cases the rate of dissociation of the drug-albumin bond does not limit the rate at which the drug passes out of the plasma.

B. B. Brodie et al. (eds.), *Concepts in Biochemical Pharmacology*

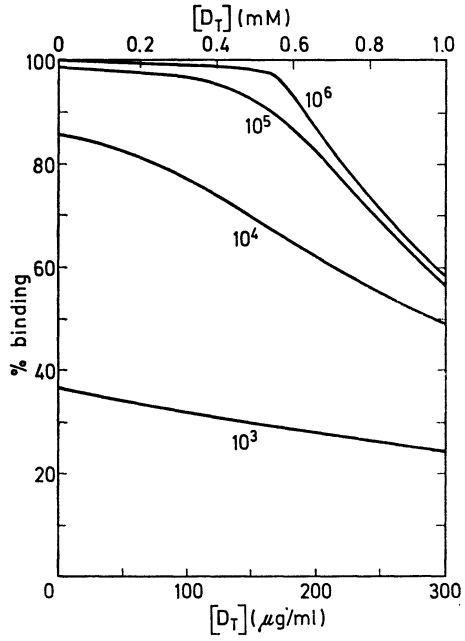

Fig. 1. Effect of drug concentration, $[D_T]$, on per cent binding of a drug in plasma. Each curve represents a different value of K. Calculated from Eqn. (6) assuming n = 1 and P = 5.8×10^{-4}M. Values for $[D_T]$ in μg/ml apply to a drug with mol. wt. = 300

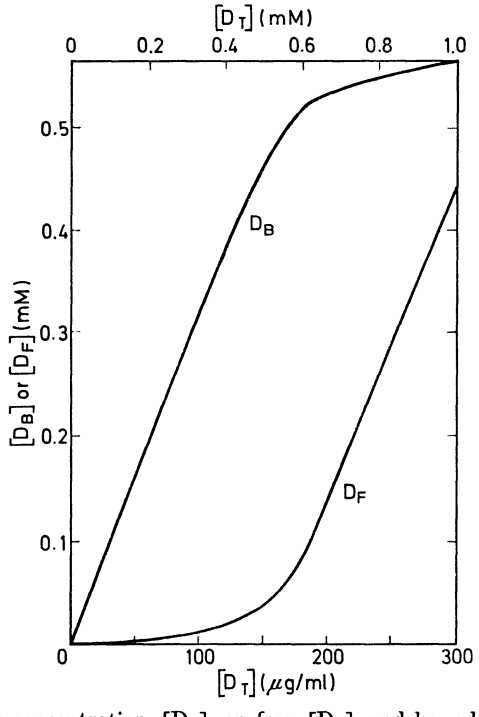

Fig. 2. Effect of drug concentration, $[D_T]$, on free, $[D_F]$, and bound, $[D_B]$, drug in plasma. Calculated from Eqn. (6) assuming n = 1, P = 5.8×10^{-4}M and K = 10^5. Values for $[D_T]$ in μg/ml apply to a drug with mol. wt. = 300

Protein-binding data are frequently expressed in terms of per cent bound. This is a very useful measure; however its limitations should be recognised. GOLDSTEIN (1949) pointed out that as drug concentration is lowered per cent binding increases as shown in Fig. 1. It will be seen that if a drug has a high affinity for albumin per cent binding falls sharply when the total drug concentration, $[D_T]$, exceeds a certain value. BRODIE and HOGBEN (1957) gave data for binding of phenylbutazone in plasma which showed that $[D_F]$ increases dramatically as $[D_T]$ is raised above the point at which the plasma proteins become saturated. At this point the value of $[D_B]$ is equal to the molar concentration of albumin in plasma, suggesting that phenylbutazone is bound to only one site on albumin. Figure 2 shows the theoretical values of $[D_B]$ and $[D_F]$ for a drug with $K = 10^5$.

2. Tissue Distribution

The capillary wall, through which the blood is in contact with the tissue fluids, contains pores through which drugs of molecular weight less than 1,000 can pass with ease (PAPPENHEIMER, 1953). Plasma albumin, however, is largely prevented from passing through these pores and so retains in the blood stream any drug bound to it. Thus the dye Evans Blue, because it is almost completely bound to albumin, is effectively restricted to the blood stream and so may be used to mea sure plasma volume. In the case of a drug which is not completely bound it might be expected that, as free drug diffused from the capillaries, bound drug would dissociate from the protein until the drug in the tissue fluid came into equilibrium with the free drug in the plasma. To test this hypothesis a means of sampling tissue fluid is required. VERWEY and WILLIAMS (1963a) have developed an elegant technique for the cannulation of peripheral lymphatics in the dog, thus obtaining lymph which has been recently derived from tissue fluid. VERWEY and WILLIAMS (1963b) gave intravenous infusions of a series of penicillins whose binding in plasma ranged from 21%—81% and used the lymphatic cannulation technique to study distribution between plasma and lymph at equilibrium. Lymph-plasma ratios ranged from 0.96—0.59 and were inversely related to the extent of protein binding. However, the protein content of lymph was 1.9% (as compared with 6% in plasma) so that, in the case of the most highly-bound penicillin, half of that present in the lymph was in the bound form and the concentration of free penicillin in the lymph was only 0.3 of the total plasma concentration. It appeared that in each case the free penicillin in lymph had come into equilibrium with that free in plasma, although the former was consistently higher by a factor of 1.2. These authors noted that binding in lymph was less than binding in plasma which had been diluted to the same protein concentration. This could be so because, in diluting the plasma, they had diluted substances which compete with penicillin for its binding sites. Of great practical importance was the subsequent finding (VERWEY et al., 1966) that, following a single intramuscular injection, the rise and fall of the concentration of penicillin in tissue fluid followed the plasma concentration very closely, with a latent period of only a few minutes. This latent period was similar for each of the penicillins tested, regardless of extent of protein-binding, showing that, as discussed in Section A.1., the rate-limiting factor for entry of penicillin into tissue fluid is diffusion of the free penicillin and not the rate of dissociation of the penicillin-albumin complex. Thus it appears that, for penicillin at least, the best available indication of the level of free drug in tissue fluid is the free level in plasma.

BROWN (1964) studied the distribution of penicillins between blood and lymph following intramuscular injection in rats. Lymph was collected by cannulation of

the thoracic duct and penicillin levels in lymph followed plasma levels with a latent period of about an hour. The peak lymph level of ampicillin (which is only 5% bound to plasma proteins in the rat) was twice the peak plasma level whereas for a group of highly-bound penicillins lymph levels exceeded plasma levels by a factor of 1.3. To explain the discrepancy between these results and their own VERWEY et al. (1966) suggested that in BROWN's experiments the thoracic duct lymph contained an artificially high concentration of penicillin because it included lymph which had originated at the site of injection or in the kidneys. The composition of lymph does not necessarily reflect that of tissue fluid and it has been suggested that lymph becomes progressively concentrated with respect to protein (but not with respect to chloride or urea) as it flows towards the thoracic duct (BELL et al., 1968). The thoracic duct lymph in BROWN's experiments contained 3.2% protein (as compared with 1.9% in peripheral lymph) which might indicate that it had been concentrated two-fold since leaving the tissues. If it had been similarly concentrated with respect to penicillin the results of BROWN (1964) and of VERWEY and WILLIAMS (1963a, b) would be very comparable. The same concentrating process, occurring to a much lesser extent, would also explain the discrepancy between the free penicillin levels in lymph and plasma in the latter authors' experiments.

MCQUEEN (1968) used a technique of '*in vivo* dialysis' in which a cellulose dialysis sac (filled with a dextran solution to maintain osmotic pressure) was placed in the peritoneal cavity of a rat which was receiving sulphormethoxine (SOM) in the diet. The bag was allowed to equilibrate for 24 hours during which time a similar bag was dialysed against rat serum containing SOM. Not surprisingly perhaps, for a given serum concentration the final concentrations of SOM in the two dialysis sacs were similar. On administration of phenylbutazone, which displaces SOM from plasma proteins, the SOM levels in the peritoneal sac increased (MCQUEEN, 1969). Whilst this technique is an alternative to ultrafiltration as a means of measuring the extent of protein-binding *in vivo* the fluid in the sac does not represent tissue fluid because the effective diameter of the pores in cellulose tubing is very much less than that of the capillary pores (CRAIG et al., 1957; PAPPENHEIMER, 1953).

ANTON (1961) studied the distribution of sulphaethylthiadiazole (SET) between plasma and tissues of the rat. When sulphinpyrazone (SNPZ) was administered it displaced SET from binding to plasma proteins; plasma levels of SET fell and tissue levels rose indicating that protein-binding maintains plasma levels at the expense of tissue levels. Thus, when SNPZ was given, the concentration of SET in muscle rose considerably relative to the total concentration of drug in plasma. However, the ratio between the concentration of SET in muscle and the concentration of free SET in plasma remained the same (albeit considerably less than unity), suggesting that tissue levels are a function of free concentration rather than total concentration of drug. KUNIN (1965) came to a similar conclusion when he studied the distribution of penicillins in heart, muscle and lung of rabbits given intravenous infusions of a series of penicillins whose binding in plasma ranged from 46—73%. Due to these differences in protein-binding the ratio of organ concentration to total drug concentration in plasma varied both between organs and between penicillins. However, when $[D_F]$ was calculated, it was found that the ratio of organ concentration to $[D_F]$ in plasma still varied between organs but for any one organ was similar for each of the penicillins. DAYTON et al. (1967) studied the effect of protein-binding on the distribution of thiopentone in patients who had been given an injection of the drug some hours previously so that, it was assumed, the drug had come into equilibrium between plasma and fat. At high plasma levels

the percent of the drug bound to protein was lower and fat-plasma ratios were higher than at lower levels. When fat-plasma ratios were calculated in terms of free drug this difference disappeared.

In the case of drugs which are highly bound to tissues, drug levels per unit weight of tissue may be well in excess of the concentration of free drug in plasma. Thus BURNS et al. (1953) found that in a patient who had been given phenylbutazone over a period of time, $[D_F]$ was only 0.02 of $[D_T]$ in plasma, whereas the levels in lung, heart and muscle were 0.68, 0.48 and 0.39 respectively of the $[D_T]$ in plasma.

LÜLLMAN and VAN ZWIETEN (1969) studied the effect of blood on the binding of a series of cardiac glycosides to isolated atria. The results, which are reproduced in Table 1, demonstrate very clearly that tissue levels are governed by the concentration of free glycoside in the blood and that those glycosides which are highly bound to plasma proteins are also highly bound to tissue. These authors further demonstrated that tissue-binding did not slow washout when atria were transferred to a glycoside-free Tyrode solution i. e. that the k_2 for binding to tissue, like that for binding to plasma proteins, is higher than the rate constant for diffusion. Extent of tissue-binding was not directly proportional to biological activity indicating that plasma-binding and tissue-binding are similar processes each sequestering drug and so lowering free drug concentration. Thus, tissue-binding to non-active sites cannot increase the concentration of a drug at its site of action. Indeed the concentration of free drug in tissue fluid and intracellular water does not normally exceed the concentration of free drug in plasma unless there is an active transport process (IVERSEN, 1963) or a pH gradient (WADDELL and BUTLER, 1959) across the cell membrane.

Table 1. *The binding of cardiac glycosides to guinea-pig atria in whole blood and in Tyrode.* From LÜLLMANN and VAN ZWIETEN (1969)

	per cent bound in blood	Tissue/Medium Ratio		
		$\frac{[D_T]\text{tissue}}{[D_T]\text{blood}}$	$\frac{[D_T]\text{tissue}}{[D_F]\text{blood}}$	$\frac{[D_T]\text{tissue}}{[D]\text{Tyrode}}$
Ouabain	0	0.52	0.52	0.6
Digoxin	30	1.25	1.79	2.8
Digitoxin	88	1.12	9.32	9.3
Digitoxigenin . . .	92	0.61	7.63	8.0

3. Pharmacodynamic Activity

It has been shown that protein-binding inhibits the activity of digitoxin (FAWAZ and FARAH, 1944; FARAH, 1945), tubocurarine (ALADJEMOFF et al., 1958) and salicylates (REYNOLDS and CLUFF, 1960) and that recovery from thiopentone anaesthesia is directly related to free concentrations in blood (TAYLOR et al., 1954). The action of cortisol is reduced in the presence of serum and it has been suggested that the greater potency of the synthetic analogues of cortisol may be in part attributable to their being less highly protein-bound (BLECHER, 1966). The finding that the activity of drugs is increased when they are displaced from protein-binding (FOX, 1964) also shows that protein-binding inhibits activity. In one isolated case, that of noradrenaline acting upon the aortic strip, the presence of plasma proteins has been reported to *increase* activity (WURZEL et al., 1964) but this is probably because binding to serum albumin prevents autoxidation of noradrenaline.

4. Antibacterial Activity

With pharmacodynamic agents the dose required to produce a given effect is determined empirically and can rarely be compared with the activity of the drug *in vitro*. With antibacterial agents, on the other hand, the sensitivity of bacteria *in vitro* is known and a dosage regimen can be planned so as to maintain levels in excess of the minimum inhibitory concentration (MIC) at the site of infection. Thus it is very important to be able to predict from the plasma level what is likely to be the effective concentration of the antibacterial agent in the tissues. ROLINSON (1967) has reviewed this problem.

A number of experiments have been designed to test whether plasma proteins inhibit the antibacterial activity of drugs. The chief difficulty in testing antibacterial activity in serum is that any dilution of the serum causes dissociation of bound drug and so, to simulate conditions *in vivo*, the test should be carried out with undiluted serum. Only certain organisms will grow in serum (WOLFE and MCGUIRE, 1961); of these many grow less well in serum than in broth (QUINN, 1964) and this may give rise to spurious results when comparing MIC_{broth} with MIC_{serum} (ROBERTS et al., 1961).

a) Sulphonamides

NEWBOULD and KILPATRICK (1960) overcame this difficulty by bringing a solution of inorganic salts into equilibrium with plasma across a dialysis membrane. They then separated the fluids, which were thus identical in composition except for the non-diffusible plasma components, and compared the MIC of a series of sulphonamides for staphylococci in the two fluids. They also measured the protein-binding of the sulphonamides in the plasma phase and established that in each case only free sulphonamide was exerting antibacterial activity. ANTON (1960) used a dialysis system in which a solution of serum albumin was dialysed against a culture medium which contained the sulphonamide under test. Irrespective of whether the test organism was added to the inner (albumin-containing) solution or to the outer compartment only free sulphonamide was active. This was thus a crude model for both intra- and extra-vascular infections. Phenylbutazone displaced sulphonamide from binding and anti-bacterial activity was correspondingly increased.

Notwithstanding the findings of NEWBOULD and KILPATRICK (1960) and ANTON (1960) cited above the impression persists that the antibacterial activity of the highly-bound sulphonamides *in vivo* cannot be explained by the free plasma concentration. For example, MADSEN et al., (1963) reported that protein-binding did *not* inhibit the antibacterial activity of sulphonamides, but this can probably be discounted on the grounds that their determination of antibacterial activity involved dilution of serum with a non protein-containing medium, so that most of the bound sulphonamide would have been released from the protein. Further work on this problem would be most welcome.

b) Antibiotics

QUINN et al. (1963), ROLINSON and SUTHERLAND (1965) and KUNIN (1966) have between them determined MIC_{broth} and MIC_{serum} for eleven penicillins and have correlated the extent to which activity is diminished in serum with the extent of binding as determined by dialysis or ultrafiltration. In each case they have concluded that only free penicillin is able to exert any antibacterial activity. This is corroborated by the finding that the activity of penicillins in serum is enhanced by drugs which are known to displace them from protein-binding

(KUNIN, 1964). Taking these findings in conjunction with those of VERWEY and WILLIAMS (1963a, b), showing that free drug is in equilibrium between plasma and lymph, it seems that free plasma levels are the best available indication of effective antibiotic levels in the tissues.

Does this relationship still hold for inflamed tissues? It is known that during inflammation total antibiotic levels in tissues rise, largely due to efflux of plasma proteins from the capillaries into the tissue space. Thus BROWN (1964) found that the protein content of a peritoneal exudate was similar to that of serum and that consequently highly-bound penicillins were equally distributed between the two fluids. Since it has been established that protein-bound antibiotic is inactive, the *effective* antibiotic concentration would still be represented by the free tissue fluid level which in turn would be in equilibrium with the free plasma level. However, BROWN (1964) found also that the poorly-bound antibiotic ampicillin reached a concentration in inflammatory fluid 2.5 times that in plasma and UNGAR (1950) found that total levels of benzylpenicillin in an inflammatory exudate exceeded total plasma levels and persisted after the antibiotic had disappeared from the blood-stream. It would be interesting to know what proportion of the penicillin in the inflammatory exudate was in the bound form.

Two attempts have been made to assess the effect of protein-binding on the effectiveness of antibiotics against infections *in vivo*. GOUREVITCH et al. (1960) tested the ability of a series of penicillins to protect mice against an experimental staphylococcal infection. They also measured the activity of each penicillin against the same organism *in vitro*, both in serum and in broth. They found that effectiveness *in vivo* correlated more closely with MIC_{serum} than with MIC_{broth}. ROLINSON (1967) collected data for a wide range of antibiotics, all clinically effective in the treatment of staphylococcal infections in man but differing widely with respect to the peak serum levels typically achieved (1.5—35 μg/ml), concentrations at which they show antistaphylococcal activity *in vitro* (0.02—3.0 μg/ml), and extent of serum binding (30 %—99 %). The data are reproduced graphically in Fig. 3. Despite the differences between these antibiotics, peak *free* serum levels are in each case 2—5 times the MIC_{broth}. Exceptions are novobiocin and benzylpenicillin. It is to be expected that activity *in vivo* will depend not only on peak blood levels but also on the length of time for which these are maintained and this may explain why novobiocin (the most slowly-excreted) and benzylpenicillin (the most rapidly-excreted) are at either end of the scale. The high dose of benzylpenicillin administered may also reflect the fact that it is cheap and, in most patients, non-toxic.

Thus when comparing two antibiotics of the same type it seems that a good index of their likely efficacy *in vivo* is either

$$\frac{\text{Peak total plasma concentration}}{MIC_{serum}} \text{ or } \frac{\text{Peak free plasma concentration}}{MIC_{broth}} \qquad (3)$$

If only free antibiotic is active *in vitro* these indices have the same value. BOND et al., 1963 compared four orally-administered penicillins in this way. It has been suggested that mean plasma concentration may be a more useful parameter than peak concentration (WARREN, 1966).

The data of ROLINSON (1967) reproduced in Fig. 3 suggest that the indices in Eqn. (3) can also be used to predict the relative efficacy *in vivo* of antibiotics of *different* types. This can only be done, however, if the site of infection is freely accessible to all the antibiotics concerned. If the bacteria are either surrounded by an inflammatory reaction, in a transmembrane compartment or within a cell the efficacy of an antibiotic will depend not only on its antibacterial activity *in vitro*

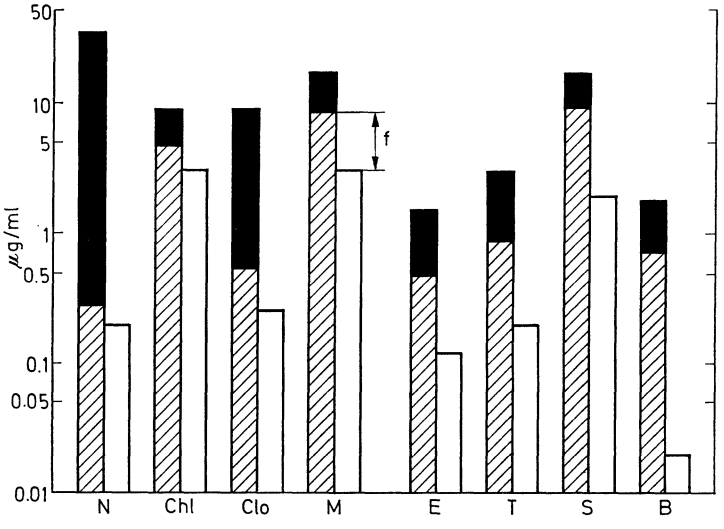

Fig. 3. Histogram showing peak serum level (left-hand column) and MIC for staph. aureus (right-hand column) for each of eight antibiotics. Peak serum level divided into bound (black) and free (hatched). Concentrations shown on log scale hence height off represents $\frac{\text{Peak free serum level}}{\text{MIC}}$. N = novobiocin; Chl = chloramphenicol; Clo = cloxacillin; M = methicillin; E = erythromycin; T = tetracycline; S = streptomycin; B = benzylpenicillin. Data from ROLINSON (1967)

and the free plasma level attained but also on its ability to penetrate to the site of infection (MCCUNE, 1960).

WARREN (1966) has criticised the use of the indices in Eqn. (3) on two grounds: firstly that the free plasma concentration may not accurately reflect the concentration in tissue fluid (this has been discussed above) and secondly that the MIC determined *in vitro* may not represent antibacterial activity *in vivo*. He points out that certain antibiotics may sensitise bacteria to body defences (WARREN and GRAY, 1965) so that the MIC may underestimate their activity *in vivo*. The distinction between bactericidal and bacteriostatic antibiotics could be important in this respect. On the other hand the density of the bacterial population in a natural infection may exceed that used to determine the MIC so that, especially in the case of penicillinase-producing organisms, the MIC might alternatively *over* estimate activity *in vivo*.

5. Entry into Transmembrane Compartments

A number of body compartments, including the eye, the mammary gland, the foetus and the greater part of the brain, are effectively separated from the circulation by membranes lacking the pores which render the capillary walls permeable to drugs. Free drug tends to come into equilibrium across these membranes but the rate of attainment of equilibrium is determined by the degree of ionisation of the drug and by its lipoid-solubility (SCHANKER, 1962). HOLDER and HAYES (1965) studied the effect of plasma-binding on the diffusion of sulphonamides across membranes using the red blood cell as a model system. They found that the rate of diffusion was determined by the concentration of *free* sulphonamide and that, although drug continuously dissociated from binding to replace that lost by diffusion, at any given instant only unbound drug was free to diffuse into the cell.

a) The Brain

The brain contains only a small proportion of extracellular fluid and this is largely protein-free. Hence a protein-bound drug attains only low levels in the brain and the high protein-binding of the vital dyes used in early studies effectively excluded them from the brain. That the apparent exclusion of a substance from the brain may be due to protein-binding alone and not to the 'blood-brain barrier' is shown by the classical finding that when bilirubin is displaced from plasma-binding by sulphonamides it enters the brain to cause kernicterus (SILVERMAN et al., 1956; ODELL, 1959). Equilibrium levels of sulphonamides (VAN OS et al., 1964) and thiopentone (DAYTON et al., 1961) in cerebrospinal fluid (CSF) are similar to free plasma levels and the rate at which drugs enter the brain is also governed by the free plasma level (MAYER et al., 1959; BRODIE et al., 1960). Drugs enter brain tissue both via the CSF and directly from the brain capillaries (BRADBURY and DAVSON, 1964; LITHANDER and LITHANDER, 1962). Evidently the latter process is also limited by protein-binding because displacement of sulphonamides from binding increases sulphonamide levels in brain tissue while the ratio between the concentrations in brain water and in plasma water remains the same (ANTON, 1961). In some instances, however, changes in drug distribution may be due to alterations in the permeability of the blood-brain barrier. For example, free penicillin levels in the CSF of rabbits with experimental meningitis are 10—20 times as high as those in controls (RUEDY, 1965) since inflammation increases the permeability of the brain capillaries.

b) The Eye

Protein-binding also limits distribution of drugs into intraocular fluids; thus free thiopentone comes into equilibrium between aqueous humor and plasma (DAYTON et al., 1961). However, GENAZZANI et al. (1968) found that the concentration of a series of sulphonamides in aqueous humor was 2—2.5 times the free plasma concentration and even exceeded the total plasma concentration. Aqueous humor-blood ratios may have been high because the sulphonamides were administered by single intravenous injection and samples were taken thirty minutes later at which time plasma levels would have been falling rapidly.

c) The Mammary Gland

Protein-binding limits the distribution of drugs into milk although this effect may be obscured by the unequal distribution which results from the difference between the pH of plasma and of milk (RASMUSSEN, 1958, 1959; see also chapter 20, this volume).

d) The Foetus

Transfer from maternal to foetal plasma differs from transfer into other transmembrane compartments because there are similar concentrations of protein on either side of the barrier. Hence, even with a highly bound sulphonamide such as sulphamethoxypyridazine, *total* levels in maternal and cord serum come into equilibrium (SPARR and PRITCHARD, 1958). However, as GOLDSTEIN et al. (1968) point out, protein-binding greatly decreases the rate of transfer across the placenta by reducing the concentration gradient of free drug. Free drug, once transferred, binds to foetal plasma proteins which thus act as a 'sink' delaying the attainment of equilibrium.

B. Effect on Elimination

1. Renal Excretion

Three processes contribute to renal excretion: glomerular filtration, tubular secretion and tubular reabsorption (WEINER, 1967). KRÜGER-THIEMER (1968) has developed a mathematical model to predict renal clearance on this basis.

a) Glomerular Filtration

The glomerular capillaries contain pores which, like those in most other capillaries, permit the free passage of drugs but withold the plasma proteins. Hence the glomerular filtrate is a true ultrafiltrate of plasma and only free drug is filtered. If a drug is not subsequently secreted or reabsorbed by the tubules and is not protein-bound (e. g. inulin, creatinine) its total clearance indicates the glomerular filtration rate (GFR); if, on the other hand, it is protein-bound, its clearance in terms of *free* drug approximates to the GFR. Chlortetracycline (CTC) is 70% bound in plasma and the renal clearance of *free* CTC is similar to the inulin clearance and independent of urine flow (SIROTA and SALTZMAN, 1950), suggesting that CTC is filtered at the glomerulus and neither secreted nor reabsorbed. Hence the rate of renal excretion of tetracyclines is inversely related to their extent of binding (KUNIN et al., 1959).

b) Tubular Secretion

Tubular secretion, unlike glomerular filtration, is not limited by protein-binding as shown by the fact that phenol red and penicillins, despite protein-binding, can be largely cleared from the blood during one circulation through the kidney (MARSHALL, 1931; EAGLE and NEWMAN, 1947). Protein-bound drug probably becomes available for secretion because this is an open-ended system in which free drug is continuously removed by secretion so that the equilibrium shown in Eqn. (1) is moved sharply to the left and k_2 is sufficiently high for all the drug in the plasma to be removed during one passage through the kidney. Hence it is probable that protein-binding accelerates the rate of elimination of a drug which is secreted by the tubules because it increases $[D_T]$ in plasma (see Fig. 6) and so makes more drug available to the secretory process. Penicillins are secreted by the renal tubules and consequently their renal clearance is unrelated to protein-binding (ROLINSON, 1967). PITTS (1963) has developed an expression for the clearance of a drug which undergoes both glomerular filtration and tubular secretion; he relates these processes to $[D_F]$ and $[D_T]$ in plasma respectively. The model for renal clearance developed by KRÜGER-THIEMER (1968) assumes that tubular secretion is a function of $[D_F]$ in plasma but this would appear not to be so.

c) Tubular Reabsorption

Passive reabsorption of drugs from tubular urine takes place across an intact tubule membrane so that the extent to which a drug is reabsorbed will depend on its lipoid solubility and degree of ionisation. The rate of renal excretion of sulphonamides is not related to the extent of protein-binding (NEWBOULD and KILPATRICK, 1960) since, although protein-binding determines the amount of each which is filtered at the glomerulus, different sulphonamides are reabsorbed by the tubules at different rates, thus masking the effects of protein-binding (BERTAZZOLI et al., 1962). In this situation the effect of protein-binding is to place an upper limit on the clearance of a drug. Renal excretion of sulphonamides is further complicated by the fact some sulphonamides are secreted by the tubules (ANTON, 1961; PORTWICH et al., 1963).

2. Excretion into Bile

Protein-bound drug is available for active secretion into bile as it is for active secretion into tubular urine and presumably for the same reasons. For instance 90% of bromsulphthalein, which is very highly protein-bound, may nonetheless be cleared from plasma during a single circulation through the liver (BRADLEY et al., 1952). It has been suggested that, far from impeding biliary excretion, protein-binding may assist this process. LASSER et al. (1962), studying the excretion of a homologous series of radio-opaque agents, noticed that whereas poorly-bound compounds are excreted in the urine the highly-bound homologues are excreted in the bile. These authors further showed that, when the protein-binding of the latter was reduced by changing plasma pH, biliary excretion was concomitantly reduced. However, they were unable to demonstrate the converse: increasing the protein-binding of poorly-bound agents did not increase their excretion into bile. The initial process in biliary excretion, and hence the process which would be affected by protein-binding, is accumulation of drug by the liver cells (COMBES et al., 1956). Binding to plasma proteins appears to impede this process (BRAUER and PESSOTTI, 1959; PRIESTLY and O'REILLY, 1966). Possibly highly-bound drugs are excreted in the bile because, being highly-bound, they are poorly excreted by glomerular filtration and so are retained in the body to be excreted by the relatively slow biliary system (KNOEFEL, 1965 see also chapter 19, this volume).

3. Salivary Excretion

Drugs appear to enter saliva by a process of passive diffusion which excludes the protein-bound fraction. When the pH of the saliva differs from that of plasma, however, free drug may be unequally distributed between the two fluids (RASMUSSEN, 1964).

4. Drug Metabolism

ANTON and BOYLE (1964) found that binding to albumin decreased the rate at which sulphamethoxypyridazine (SMP) was acetylated by a liver extract. However, acetylation was reduced by only 15% in the presence of albumin although free drug concentration had been reduced by 60%. NEWBOULD and KILPATRICK (1960) studied the rate of acetylation of sulphonamides by the perfused liver. They found, using rather lower sulphonamide concentrations than ANTON and BOYLE, that plasma proteins reduced acetylation of SMP by 46% and that the rate of acetylation of the more highly-bound sulphaphenylpyrazole was reduced by 85%, whilst that of sulphadimidine was barely affected. These results suggest that only free sulphonamide is available to the drug-metabolising enzymes. However, protein-binding may not reduce acetylation to the same extent as it reduces free drug concentration, $[D_F]$, because the rate of acetylation will be directly proportional to $[D_F]$ (i. e. first-order) only if $[D_F]$ is much lower than K_m for the enzyme.

These results may apply only to drugs such as the sulphonamides which are metabolised in the soluble phase of the liver cell. For drugs metabolised by the microsomes, for instance, metabolism might be preceded by an uptake process which could be affected in a different way by protein-binding. It has been suggested without direct evidence (MARK et al., 1968) that the rapid metabolism of thiohexital in man is attributable to its high protein-binding which prevents accumulation in fat (DAYTON et al., 1967) but allows metabolism by the liver.

C. Effect on Pharmacokinetics

1. A Model

Figure 4 shows a model system which has been used by MARTIN (1965) and KRÜGER-THIEMER et al. (1966) to simulate the effects of protein-binding on drug distribution and kinetics. This model is valid on the assumption i) that the drug is given intravascularly; ii) that the drug in the body is distributed into two aqueous compartments: plasma water (volume V_1) and a second compartment (volume V_2) which represents that part of the residual body water which is accessible to the drug; iii) that there is protein-binding according to the Mass-Action Law only within the plasma water compartment; iv) that unbound drug equilibrates instantaneously between the two compartments. The studies of

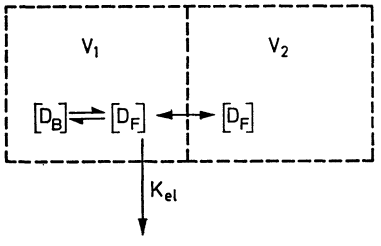

Fig. 4. Model to describe compartmentation of drug between plasma and extravascular fluid

VERWEY et al. (1966) cited above suggest that this may be a reasonable assumption; v) that drug metabolism and excretion are first order processes. As discussed in Section B.4. drug metabolism is likely to be a first-order process only when $[D_F] << K_m$ of the drug-metabolising enzyme; vi) that drug metabolism and excretion are a function of the concentration of unbound drug, $[D_F]$. The evidence for this has been reviewed in Section B. It will be apparent that the model cannot be used to predict the kinetic behaviour of drugs which are actively secreted by the renal tubules or by the liver, processes which are a function of $[D_T]$ in plasma. It can, however, be used to predict the distribution of such drugs at any particular time; vii) that the rate constants for metabolism and excretion, k_m and k_e can be combined to give k_{el}, the rate constant for elimination, as follows:

$$k_{el} = k_m + k_e \tag{4}$$

As WAGNER (1968) points out, this model is an oversimplification in that it does not take into account extravascular binding or nonuniform equilibration between compartments.

On the assumption that drug is bound only in the plasma compartment, the body content (which at time zero equals the dose administered) is given by

$$\text{Body content} = V_1 D_T + V_2 D_F \tag{5}$$

2. Distribution

Figure 5 shows drug concentration in tissue fluid $[D_F]$ following a standard dose of drug as a function of the percent binding. These curves have been calculated for two types of drug: Drug A is a lipoid-insoluble drug which is unable to cross cell membranes and is thus restricted to extracellular fluid so that V_2 is 9 litres; Drug B passes into cell water so that V_2 in this case is 39 litres. The effect of binding on $[D_F]$ in plasma is buffered by the extravascular volume so that for a given degree of binding $[D_F]$ in plasma is not lowered to the extent that one might

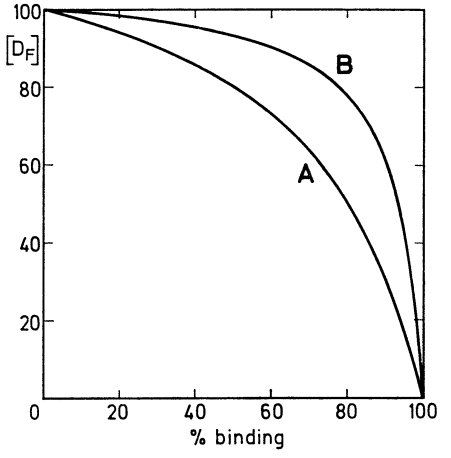

Fig. 5. A plot illustrating the potential effect of plasma-binding on the concentration of unbound drug, $[D_F]$, in tissue fluid. Calculated from Eqn. (5) for a drug distributed according to Fig. 4 assuming $V_1 = 3$ litres. Drug A, $V_2 = 9$ litres; Drug B, $V_2 = 39$ litres

expect. For instance 90% binding in plasma may lower $[D_F]$ in the body by only 40% (Drug B, Fig. 5). This is an important point as it is often erroneously implied that if a drug is 90% bound it should have only 10% of the activity of *the same dose* of an equiactive but unbound drug.

Now per cent binding may vary with $[D_T]$ (Fig. 1) and so the data of Fig. 5 can be applied only to drugs, such as penicillin, which are active at low doses so that binding does not change over the range of plasma concentrations usually achieved. In the case of drugs which are given at higher doses, Fig. 5 may be used to predict tissue fluid concentrations corresponding to a particular plasma concentration at which per cent binding is known.

For drugs whose binding varies with plasma concentration MARTIN (1965) derived the following equation from the mass-action relationship developed by GOLDSTEIN (1949) and shown in Eqn. (2)

$$[D_T] = [D_F]\left[(1 + \frac{nP}{K^{-1} + [D_F]})\right] \quad (6)$$

KRÜGER-THIEMER et al. (1966) use an expression which differs from this only in including a correction for the space occupied by the plasma proteins. The question arises as to what values of n and K are to be utilised in Eqn. (6). A number of detailed studies have been undertaken to determine values of n and K for particular drugs. Most of these studies agree in finding one main binding site with high affinity for the drug and a variable number of subsidiary sites having a much lesser affinity (CONN and LUCHI, 1961; KEEN, 1966; LUKAS and MARTINO, 1969; O'REILLY, 1967; THORP, 1964). Hence when predicting the effect of protein-binding on the distribution of a drug for which n and K have not been accurately determined, it is justifiable to assume that the drug forms a 1:1 complex with albumin. The association constant K for drug-binding to the main site varies from 10^3—10^6 (see refs. cited above) and Fig. 6 shows the theoretical relationship between $[D_T]$, $[D_F]$ and dose for a drug with $K = 10^5$. Tissue concentrations are low following low doses of drug but rise steeply as dose is increased beyond the point at which the binding sites become saturated.

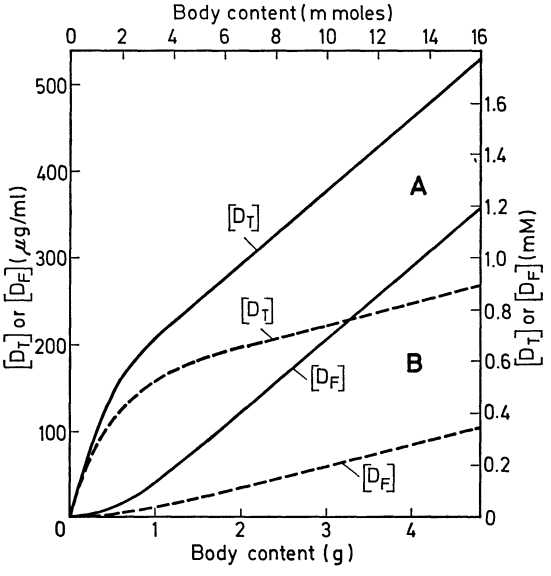

Fig. 6. Relationship between total amount of drug in the body, concentration of drug $[D_T]$ in plasma, and concentration of unbound drug, $[D_F]$, in tissue fluid. Calculated from Eqns. (5) and (6) for a drug distributed according to Fig. 4 assuming $K = 10^5$, $n = 1$, $P = 5.8 \times 10^{-4}$M and $V_1 = 3$ litres. Drug A, $V_2 = 9$ litres; Drug B, $V_2 = 39$ litres

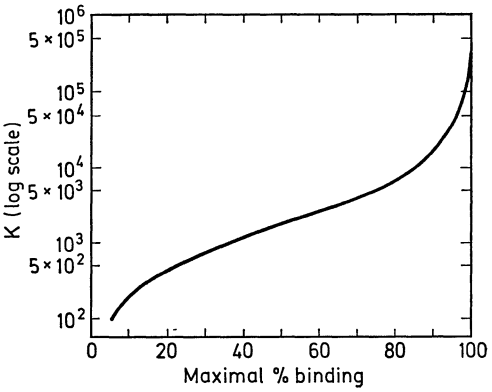

Fig. 7. Plot of K against maximal binding calculated from Eqn. (7) assuming $n = 1$ and $P = 5.8 \times 10^{-4}$M

In order to apply Eqn. (6) to a drug whose K is not accurately known there are two ways in which an estimate of K can be made from data of binding in plasma: i) if per cent binding in plasma is known for a particular $[D_T]$ and n is assumed to be unity, K can be calculated from Eqn. (2). Before a value of K is calculated in this way the data for binding in plasma should be corrected for Donnan effects and for space occupied by the proteins (KEEN, 1965). ii) MEYER and GUTTMAN (1968) point out that when fractional binding (β) is maximal at low values of $[D_T]$

$$nKP = \frac{\beta_{max}}{1 - \beta_{max}} \tag{7}$$

Fig. 7 shows a plot of K against maximal binding assuming n = 1. If the degree of binding at low drug concentrations is known, a *minimal* value can be allotted to K. Obviously if the drug concentration is not low enough for binding to be maximal this will underestimate K.

3. Kinetics

In drawing up the model shown in Fig. 4 it has been suggested that elimination, whether by metabolism or excretion, is a first-order process depending on $[D_F]$ and having a rate constant k_{el}. Hence $[D_F]$ will fall exponentially with time (t) according to

$$[D_F] = [D_F]^\circ e^{-k_{el}t} \tag{8}$$

where $[D_F]^\circ$ is the value of $[D_F]$ at the time of administration assuming instantaneous equilibration. Since elimination is a first-order process the concept of *clearance* can be introduced — i. e. elimination may be expressed as that volume of plasma water which is effectively cleared of drug in unit time.

The second factor governing rate of elimination will be the relative concentration of drug in plasma water as indicated by V_A, the apparent volume of distribution. V_A is calculated from

$$V_A = \frac{\text{Dose}}{[D_F]^\circ} = \frac{\text{Total drug in body at any time}}{[D_F]} \tag{9}$$

and may be defined as that volume of body fluids which would hold the drug in solution at the same concentration as in the plasma water. WAGNER and NORTHAM (1967) have discussed methods of determining V_A in practice. BUTLER (1958), in an admirably lucid article pointed out that

$$k_{el}\,(\text{min}^{-1}) = \frac{\text{Clearance (ml/min)}}{V_A\,(\text{ml})} \tag{10}$$

The analogy with a tank of water is obvious. The volume of the tank (V_A) can be calculated by dividing the amount of drug added (Dose) by the initial concentration of drug in the water, $[D_F]^\circ$. If the tank is being drained by a tap (and replenished with water at the same rate from another tap) the rate of drug elimination will be governed by the rate of outflow from the tap (the clearance) and by the concentration of drug in the water, $[D_F]^\circ$, which for any given dose will in turn depend on the volume of the tank (V_A). $[D_F]$ will approach zero asymptotically and so at no time can the drug be considered to have been completely excreted. Thus the rate of elimination of a drug is usually expressed as its half-life ($t_{1/2}$) i. e. the time taken for the amount of drug in the body to fall to half its initial value. It can be calculated from Eqn. (8) that

$$t_{1/2} = \frac{\ln 2}{k_{el}} \tag{11}$$

hence combining Eqns. (10) and (11):

$$t_{1/2} = \ln 2 \frac{V_A}{\text{Clearance}} \tag{12}$$

The parameters used in the foregoing equations have all been referred to plasma water and so binding to plasma proteins, by reducing $[D_F]$, will increase V_A (see Eqn. (9)). Now we have seen that the rate constant for dissociation of the drug-protein complex (k_2) is very rapid, hence although the drug is in two 'compartments' — bound and free — it will be eliminated as though it was all free in

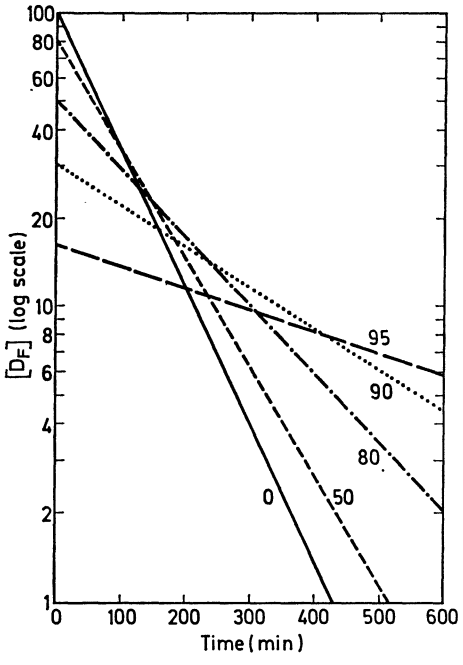

Fig. 8. Potential effect of plasma-binding on the change of unbound drug concentration, $[D_F]$, in tissue fluid with time. Each line represents a different value of per cent binding which is assumed not to change with time. Intercept on ordinate $[D_F]^\circ$ calculated from Eqn. (5). Slope of line calculated from Eqns. (9) and (12). Dose = 10^{-3} moles; V_1 = 3 litres; V_2 = 9 litres; clearance = 0.13 litres/min

one large compartment of volume V_A. Figure 8 shows the effect of various degrees of protein-binding on the plasma decay curve following a standard dose of drug. It will be seen that protein-binding, by increasing V_A has had two effects: i) it has reduced the initial concentration of free drug, $[D_F]^\circ$ (see Eqn. (9)), ii) it has increased $t_{1/2}$ so that $[D_F]$ falls less rapidly (see Eqn. (12)). Whether protein-binding confers a therapeutic advantage will depend on the level of $[D_F]$ above which the drug becomes toxic and the level below which it becomes ineffective. Thus if the toxic level in Fig. 8 is 50, binding of 80% or more will be beneficial because it keeps $[D_F]^\circ$ below the toxic level. On the other hand if the minimal effective level is 6 and if the drug is not eliminated mainly by active transport in the kidney, binding to plasma will confer an advantage by prolonging effective blood levels; for instance 95% binding will double the length of time for which the drug is effective (Fig. 8).

Since protein-binding lowers the $[D_F]^\circ$ which is attained following any given dose it allows a higher dose to be administered without producing untoward effects. This assumes (see Section A3) that the latter effects are a function of $[D_F]$ and not of $[D_T]$. Thus it may be helpful to examine the effect of protein-binding on the elimination of a series of drugs given at such a dosage that each attains the same $[D_F]^\circ$. Table 2 shows the factor by which protein-binding increases the length of time for which $[D_F]$ exceeds any given value under these conditions.

Differences in the length of action of different tetracyclines (KUNIN, 1962) and antihistamines (KUNTZMAN et al., 1967) have been attributed to differences in their apparent volumes of distribution caused by differences in protein-binding. BENNHOLD (1966) provided a direct demonstration of the effect of protein-binding

Table 2. *Potential effect of plasma-binding on drug half-life ($t^1/_2$) and apparent volume of distribution (V_A). F is the factor by which plasma-binding increases the length of time for which $[D_F]$ exceeds any given value assuming the initial free drug concentration, $[D_F]°$, is the same in each case. Calculated from Eqns. (5) and (12) assuming $V_1 = 3$ litres and clearance = 0.13 litres/min. Drug A, $V_2 = 9$ litres Drug B, $V_2 = 39$ litres*

per cent bound in plasma	Drug A			Drug B		
	V_A (litres)	$t^1/_2$ (minutes)	F	V_A (litres)	$t^1/_2$ (minutes)	F
0	12	64	—	42	224	—
50	15	80	1.25	45	240	1.07
80	24	128	2.0	54	288	1.29
90	39	208	3.25	69	368	1.64
95	69	368	5.75	99	528	2.36

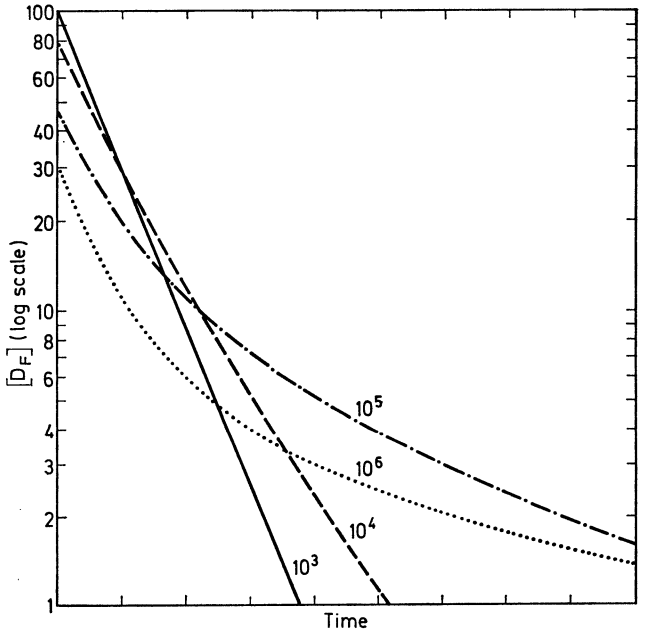

Fig. 9. Potential effect of plasma-binding on the change of unbound drug concentration, $[D_F]$, in tissue fluid with time. Each line represents a different value of K. Unlike the data shown in Fig. 8 these curves allow for changes in per cent binding with time. Reproduced from KRÜGER-THIEMER et al. (1966)

on drug elimination. He found that in a subject suffering from analbuminaemia the half-life of Congo red was 29 min; following the infusion of albumin the half-life of the dye was increased some seven-fold to 3 hours 42 min.

Binding to tissues increases V_A in the same way as binding to plasma proteins, hence the single parameter V_A, as determined for a particular drug, will embrace that drug's actual volume of distribution, its binding in plasma and its binding in tissue. For most drugs tissue-binding, and plasma-binding are similar processes each slowing elimination to the extent that it increases V_A. For drugs which are actively secreted into urine or bile, on the other hand, plasma-bound drug is available for excretion and so whilst tissue-binding slows elimination in accordance with Eqn. (12) plasma-binding does not.

The fall of $[D_F]$ with time will be linear as in Fig. 8 only if V_A has the same value at all drug concentrations. But, as shown in Fig. 1, the binding of some drugs changes with $[D_T]$. Hence for such drugs it would be predicted that at high values of $[D_T]$ per cent binding will decrease so that V_A will also decrease and as a result elimination will be more rapid (Eqn. (12)). Thus when a high dose of phenylbutazone is given to a patient who already has a 'plateau' blood level of the drug the rate of elimination is initially very rapid and then slows to its previous value (BURNS et al., 1953). Conversely, following a single high dose of a long-acting sulphonamide, as $[D_T]$ in plasma falls with time, per cent binding increases bringing about an increase in V_A which in turn slows the rate of elimination so that a semi-logarithmic plot of $[D_F]$ in plasma against time shows a diminishing steepness of slope with time (KRÜGER-THIEMER et al., 1966). KRÜGER-THIEMER (1968) has developed a rate equation for the change of $[D_F]$ with time using the model shown in Fig. 4 together with a binding expression analogous to Eqn. (6). This expression can be solved using a digital computer (KRÜGER-THIEMER, 1966) and the lines generated are reproduced in Fig. 9. This model accurately predicted the kinetic behaviour of a single oral dose of sulphaorthodimethoxine (KRÜGER-THIEMER, 1966).

In the examples shown in Fig. 8 plasma $[D_T]$ and $[D_F]$ and total body content all have the same $t_{1/2}$ because, V_A being constant, their relative proportions are constant. In the case shown in Fig. 9 however, KRÜGER-THIEMER (1968) has shown that the slope of the $[D_F]$ curve is initially steeper than the corresponding $[D_T]$ curve and changes more rapidly with time.

D. Conclusions

Binding to plasma proteins may have a marked effect on the distribution and activity of a drug and on the rate at which it is eliminated from the body. It should be borne in mind, however, that

i) the effect of binding in plasma is buffered by the extravascular volume so that the effect is less than would be predicted from the per cent binding in plasma (see Fig. 5). Concomitantly plasma-binding has less effect on a drug which is able to enter cells than on one which is restricted to extracellular fluid (see Figs. 5 and 6 and Table 2).

ii) plasma-binding has a marked effect on the properties of a drug only if K for the drug-protein bond is greater than 10^4, i. e. the maximal binding exceeds 85% (Figs. 5, 7 and 8 and Table 2).

iii) plasma-binding does not slow the elimination of drugs which are actively secreted into urine or bile.

Tissue-binding and plasma-binding are analogous processes which lower free drug concentration and (except in the case of actively-secreted drugs) slow drug elimination in a similar manner.

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