RELATIONSHIP BETWEEN DRUG DISTRIBUTION AND THERAPEUTIC EFFECTS IN MAN

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INTRODUCTION

The intensity and duration of therapeutic effects derived from drugs other than those exerting an immediate, irreversible action depend theoretically on maintaining adequate concentrations of the active form of the drug at receptor sites. For the purposes of this review receptor sites may be considered to be molecules located anywhere in the body that combine with a drug or its metabolite to produce a pharmacological effect. It follows that the distribution of drugs must be carefully considered in selecting an appropriate dose, dosage form, dosage interval, and route of administration; judicious modification of these parameters permits attainment of effective concentrations of the active form of drugs at receptor sites. Quantitative measurement of the pharmacological response is often difficult or impossible. Furthermore, in man and most experimental animals, concentrations of the free active form of drugs at many receptor sites cannot be conveniently determined. Instead, drug concentrations in more accessible locations, such as plasma and urine, have had to suffice. These technical problems in quantitating precisely the pharmacological responses and receptor site concentrations of many drugs have seriously impaired efforts to define the relationship between drug distribution and therapeutic effects in man. Unfortunately, most assays of drug concentrations in plasma do not distinguish between the free active and the protein-bound inactive forms of a drug. Some assays fail to separate the parent drug from its metabolites. This flaw is serious because if only the parent drug or its metabolites possess pharmacological activity, an assay combining both cannot satisfactorily serve as the basis for gaining a true picture of the relationship between drug concentrations and pharmacological effect.

Distribution of a drug mainly depends upon such physical properties as its lipid solubility and degree of ionization. The degree of ionization is determined by the pKα of a drug and the pH of the fluid in which it is dissolved. For example, the

nonionized form of a drug is much more lipid soluble than the ionized form and hence much more capable of penetrating lipid cell membranes such as line the gastrointestinal tract.

Distribution refers to the various body fluid compartments a drug enters after its administration. Conceptually, body water exists as three functionally distinct compartments: the vascular, the extracellular (interstitial), and the intracellular fluids. The apparent volume of distribution (*Vd*) of a drug is the fluid volume in which the drug seems to be dissolved. Gillette (1) defines the kinetic *Vd* as the total amount of drug in the body at any given time after the distribution phase is completed divided by the plasma concentration of drug at that time. The distribution of some drugs, like dicumarol, phenylbutazone, and diphenylhydantoin, is largely limited to the space occupied by plasma protein, because these compounds bind avidly to albumin (the circulating plasma volume is approximately 3 liters in normal 70 kg subjects). Other drugs, such as bromide salts, thiocyanate, iodide, sucrose, and inulin, do not readily penetrate cell membranes and are therefore distributed mainly in the extracellular fluid compartment (12 liters in normal 70 kg subjects). Antipyrine passes through cell membranes without difficulty and thus distributes in the total body water (41 liters in normal 70 kg subjects), which antipyrine has been employed to quantitate (2). As a result of macromolecular binding, fat solubility, or active transport, some compounds such as phenoxybenzamine, thiopental, and quinacrine accumulate in certain tissues of the body that may or may not contain the receptor sites through which the drugs produce their therapeutic effects. In a two-compartment model, the *Vd* is frequently approximated by extrapolation to the *y*-intercept of the straight terminal portion (β-phase) of the curve relating the log of the plasma concentration of drug to time after its administration and dividing the total amount of drug in the body at time 0 by this *y*-intercept value. Certain apparent anomalies can arise. For example, if a drug such as thiopental or cyclopropane is sequestered by tissue binding to extravascular sites, so much of the drug can be withdrawn from the circulation that the apparent *Vd* of the drug may greatly exceed the entire fluid volume of the body.

The distribution of a drug is generally rapid as evidenced by the steep initial component of the curve relating the log of drug concentration in plasma to time. For a drug that is rapidly metabolized or localized in tissues, only this portion of the curve may be observed after a single dose; continuous infusion of drug can be used to test whether this single phase is due mainly to distribution or elimination. Appropriate treatment of the infusion data can yield a biphasic curve, indicating that distribution is followed by metabolism and elimination (1). Initial distribution of a drug is influenced by such factors as blood flow (bone and adipose tissue, being poorly supplied with blood, require a much longer time for drugs to attain equilibrium concentrations), the availability of and the drug's avidity for binding sites on albumin and tissue proteins, and finally the degree of ionization and lipid solubility of the drug. During or after this initial distribution of the drug in the body, processes of drug metabolism and excretion ensue.

A sharp break appears in the curve relating the log of plasma drug concentration to time after most of the drug has been distributed and drug metabolism and

elimination have begun. This terminal portion of the curve is called the β-phase. Because metabolism and elimination can proceed prior to the β-phase, extrapolation of the β-phase portion of the curve to the y-intercept may constitute an underestimate of the total amount of drug in the body at the time of drug administration. Other approaches can be employed; in small laboratory animals the *Vd* can be directly measured by determining, simultaneously in tissue and plasma, drug concentrations at various times. In humans, mathematical models have been developed to avoid the underestimation of the y-intercept if metabolism is rapid (1, 3, 4).

During the β-phase certain tissues may undergo drug redistribution, through which process drug concentrations in tissues may shift dramatically. Redistribution may generate additional inflections or phases in the curves relating drug concentrations in plasma to time and is often encountered in tissues such as bone or fat where affinity for the drug may be great; however, blood flow is insufficient to allow for rapid accumulation. The classical example of the key role played by drug redistribution in modifying the therapeutic effects of a drug is that of thiopental (5). A small intravenous dose of thiopental produces anesthesia of rapid onset but short duration. Because of its high lipid to water partition coefficient, thiopental gains extremely quick access to the brain, but blood concentrations of thiopental decline rapidly, mainly due to its distribution into other tissues (not its metabolism). Consequently the drug moves rapidly out of the brain to remain in equilibrium with blood concentrations, and the subject awakens. Much later, thiopental becomes highly localized in fat depots. Elegant pharmacokinetic analyses of the relationship between drug distribution and pharmacologic effects have been published by Levy (6–9).

Because the topic of the influence of drug distribution on therapeutic effects is broad, many different aspects could be reviewed; for example, in this series last year, autoradiographic methods for investigating tissue distribution of drugs were discussed (10). Additional aspects in drug distribution include physiocochemical mechanisms of passage into or through tissues, membranes, and subcellular particles; physiocochemical mechanisms of drug binding to proteins (now being illuminated by extremely sensitive electron spin resonance and nuclear magnetic resonance techniques); analysis of dose response curves; pharmacokinetic and pharmacogenetic influences; effects of acidosis, alkalosis, fever, starvation, blood flow, and of cardiovascular, hepatic, renal or hormonal status. Exploration of these topics would provide interesting interrelationships between drug distribution and therapeutic effects, but could not all be summarized satisfactorily in a single article. Instead, attention here is focused on an area of current controversy in pharmacology: the relationship of drug blood levels to therapeutic effects. Much discussion of this topic has taken place; a recent conference was held to help define the underlying pharmacological significance of drug blood levels and to place them into better perspective (11). In describing the relationship of drug blood levels to the therapeutic effects of drugs, this review dwells on those pharmacologic principles that offer insight into the rational use of blood concentrations and that make some drugs more suitable than others for its application.

HISTORICAL BACKGROUND

The relationships between drug distribution and pharmacological effects were ill defined until sensitive, accurate methods were developed for measuring drug concentrations in the blood, urine, and tissues of an organism. Such techniques became available in the early 1950s, mainly as a result of work by Brodie and associates (12–16), who based their methods on the differential polarity and hence lipid solubility of drugs and their metabolites. It was recognized previously that for most drugs a poor correlation exists between dose and pharmacological effects. Introduction of spectrophometric, fluorimetric, and, more recently, gas-liquid chromotographic, mass spectrographic, and radioimmunologic techniques for assay of drugs and their metabolites permitted investigations of the correlation between drug concentrations in various body compartments and tissues and pharmacological effects.

Studies by Brodie and associates revealed a close relationship between the blood concentrations of certain drugs and their effects. Even among different species, similar pharmacological effects were observed when similar blood concentrations of a drug were achieved. For example, Quinn et al (17) showed that the same hypnotic dose of hexobarbital (100 mg/kg for mouse, rabbit, and rat) produced markedly different durations of action in these species; an inverse relationship existed between the duration of action of the drug and the enzyme activity in liver microsomes responsible for metabolizing hexobarbital (Table 1) (17). Thus species variations in the duration of action of hexobarbital could be traced to species differences in enzymatic capacity to metabolize the drug. However, once the same blood drug level was attained in different species, pharmacological response was similar as indicated by the observation that all species awakened at similar blood concentrations of hexobarbital. From these and other studies, several concepts emerged. First, pharmacologic effects relate more closely to blood concentrations of certain agents than to the dose of drug administered. Second, as a corollary, large interindividual variations exist in the dose of drug required to achieve the same blood concentration in various subjects. Third, normal experimental animals of the same species or even of different species exhibit great similarity in certain drug receptor sites.

CURRENT VIEWS ON DRUG DISTRIBUTION AND PHARMACOLOGICAL EFFECTS

Concentrations of a drug and its metabolites in biological fluids now constitute fundamental facts from which such critical properties of a drug as its absorption, distribution, biotransformation and excretion can be partially deduced. Therefore, it is understandable that data on blood levels are gathered during phase one studies and that assessment of dosage forms, dose intervals, and routes of administration rests largely on comparisons of blood levels. Blood levels of drugs have also become widely used in checking on patient compliance, since several studies revealed that a large segment of patients fail to take medicines as directed or, unknown to their physician, may consume other agents that interfere with the pharmacological actions of prescribed drugs (18).

Table 1 Species difference in duration of action and in metabolism of hexobarbitone[a] (Dose of barbiturate: 100 mg/kg for mouse, rabbit, and rat, and 50 mg/kg for dog)

Species	Duration of action	Biologic half-life	Plasma level of hexobarbitone on awakening	Relative enzyme activity
	min	min	μg/ml	μg/g/hr
Mouse (12)[c]	12 ± 8	19 ± 7	89 ± 31[b]	598 ± 184
Rabbit (9)	49 ± 12	60 ± 11	57 ± 12	196 ± 28
Rat (10)	90 ± 15	140 ± 54	64 ± 8	134 ± 51
Dog (8)	315 ± 105	260 ± 20	19 ± 4	36 ± 30
Man[d]	–	360	20	–

[a]Reproduced by permission from Quinn et al 1958 (17).

[b]Micrograms per gram of tissue. Tissue levels are about 50% higher than plasma levels.

[c]Figures in brackets refer to number of animals.

[d]Unpublished data (J.J. Burns and E.M. Papper).

A major difficulty in relating drug blood levels to pharmacological effects has been the tendency to generalize too broadly. With few exceptions (19–21) most treatments of the subject have failed to identify which drugs are amenable to this technique and to define those situations in which drug blood levels are particularly useful. There are several distinguishing characteristics that render a drug suitable for blood level analysis as a basis for monitoring pharmacological effects.

For drug blood levels to correlate with pharmacological effects, the free concentration of the drug in blood must be in equilibrium with the concentration of drug bound to the receptor site through which it produces its actions. Furthermore, the pharmacological responses being investigated must bear a direct relationship to the drug's concentration at receptor sites. Certain drugs, such as monoamine oxidase and cholinesterase inhibitors, must accumulate at receptor sites until a certain level is attained before pharmacological effects will ensue. During this initial period of drug accumulation at receptor sites, no direct relationship between the drug blood level and pharmacological effects is discerned. Similarly, although appreciable blood levels of various coumadin anticoagulants may be obtained shortly after their administration, the pharmacological effect, prolongation of prothrombin time, may be delayed by many hours due to persistence in the blood of previously synthesized clotting factors. Brodie has referred to a group of drugs that continue to produce their pharmacological actions on receptor sites long after they disappear from plasma as "hit-and-run" drugs (17); alkylating agents that form irreversible covalent bonds exemplify this group of drugs whose blood levels bear no direct relationship to their pharmacologic effects.

If a drug exerts easily quantifiable therapeutic effects, such as changes in blood pressure, prothrombin time, or heart rate, the appropriate dose of drug may be

determined by titration against changes in these parameters. In the absence of easily measured endpoints, other methods must be used to relate the dose of drug to therapeutic effect; in such situations drug blood levels may be useful as a guide to selecting appropriate drug dosage.

For drugs whose blood levels do correlate with their pharmacological actions, several additional properties of the drug make blood level measurements of clinical value in selecting appropriate dosage to insure therapeutic effects while avoiding toxicity. The ideal drug for this approach is one that possesses a low therapeutic index but has clearly separable ineffective, therapeutic, and toxic regions of drug concentration in the blood. The agent should be potent and exhibit large interindividual variations in rates of elimination from the body, so that the same dose could conceivably yield ineffective, therapeutic, or toxic blood concentrations in different subjects. Only a few therapeutic agents have been described in which certain clearly defined blood concentrations have the desired therapeutic effects, above which toxicity may occur, and below which little therapeutic benefit is obtained. Koch-Weser (21) cites the following ten drugs with their usual therapeutic ranges of serum concentrations: digitoxin (14–30 μg/liter), digoxin (0.9–2 μg/liter,) diphenylhydantoin (10–20 mg/liter), lidocaine (1.5–4 mg/liter), lithium (0.5–1.3 meq/liter), nortriptyline (50–140 μg/liter), procainamide (4–8 mg/liter), propranolol (20–50 μg/liter), quinidine (2–5 mg/liter), and salicylates (150–300 mg/liter).

It should be stressed that, like all other clinical chemical determinations, drug concentrations are maximally useful when placed in the broad context of a particular patient's problem; taken out of this context, such measurements may prove misleading. Presently, the availability of drug level measurements constitutes a major advance in avoiding therapeutic accidents with an important group of commonly used potent drugs with low therapeutic indices. However, even for a small group of carefully selected drugs this approach carries significant drawbacks; and an occasional patient may experience toxic reactions at therapeutic drug blood levels. One possible explanation for these anomalies is that in several disease states receptor sites on which drugs act may be aberrant. The clinical utility of drug blood levels is predicated on the usually safe assumption that the responsiveness of drug receptor sites does not exhibit large interindividual variations. Numerous environmental perturbations as well as supervention of several diseases may render this assumption invalid. A second cause of apparent dissociation between drug blood levels and drug effects involves the drug assay. For example, radioimmunoassays of digoxin are so specific that they fail to detect digitoxin. Patients with digitalis toxicity may take both drugs. Thus a normal digoxin blood level may be reported in patients with digitalis toxicity; unless blood digitoxin levels are also determined, anomalous dissociations of drug blood levels and effects may occur.

Drugs such as salicylates and certain antibiotics have high therapeutic indices that permit wide latitude in dosage; large amounts of these agents can be administered with low risk of severe toxicity. Normally, no major advantage may be obtained from precise quantitative data on the concentrations of these drugs in biological fluids. However, when continuously high blood levels of salicylates are desirable, as in the therapy of rheumatoid arthritis or the carditis of rheumatic fever, blood level

measurements of salicylates become useful, because they serve as checks on the bioavailability of different commercial preparations, on patient compliance, and on the normal function of the processes of drug absorption, distribution, metabolism, and excretion.

BIOLOGICAL BASIS FOR THE NEED TO MEASURE DRUG BLOOD LEVELS

The need to measure drug blood levels in clinical medicine and in pharmacological studies on outbred species arises from the large interindividual variations that occur after administration of the same dose of an agent to different subjects of the same species. For example, Figure 1 shows threefold variations in the plasma half-lives of phenylbutazone after a single intramuscular dose of 800 mg in six unrelated cirrhotic subjects (22). Figure 2 shows that tenfold variations in the plasma decay of the anticoagulant ethyl biscoumacetate occurred in eight unrelated normal individuals after a single intravenous dose of this drug (23). Tenfold differences in plasma half-life are impressive compared with the much smaller range of variation for other biochemical parameters in normal subjects. Moreover, if blood concentrations of the anticoagulant 3 hr after its administration are compared, the range of interindividual variation becomes twentyfold. If chronic administration of such an agent were contemplated for individuals at the extremes, the magnitude of this variation expands considerably beyond twentyfold.

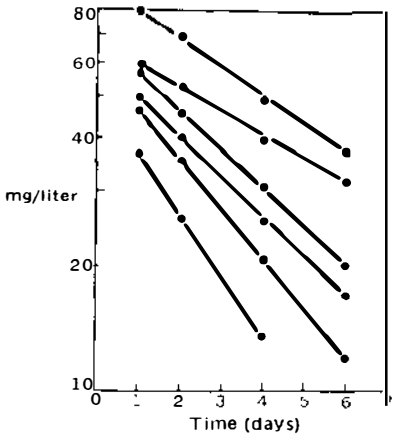

Figure 1 Decay of phenylbutazone in the plasma of six cirrhotic subjects after a single intramuscular dose of 800 mg. Reproduced by permission from Burns et al (22).

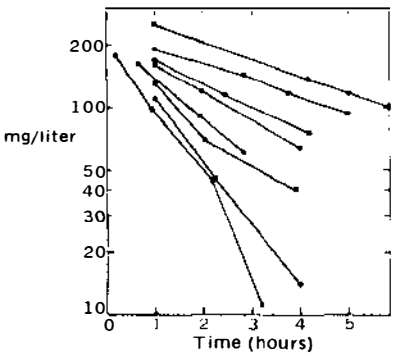

Figure 2 Decay of ethyl biscoumacetate in the plasma of eight normal volunteers after a single intravenous dose of 20 mg/kg. Reproduced by permission from Brodie et al (23).

With respect to switching from single to multiple doses, the pharmacokinetic relationship between the plasma half-life ($t½$) of a drug after a single dose and the steady-state blood concentration (C) of that drug is given by the equation of van Rossum & Tomey (24):

$$C = 1.44\ (Q \cdot t½ / Vd \cdot \Delta t)$$

where Q is the maintenance dose, Vd is the apparent volume of distribution, and Δt is the dosage interval. For completely absorbed drugs and for situations where the maintenance dose and dosage interval do not change, this formula indicates that alterations in the steady-state plasma concentrations arise from differences in $t½$, Vd, or both.

Studies in animals revealed that numerous factors can alter drug blood levels under certain conditions (25–29). Such factors include exposure to agents that stimulate or depress rates of drug metabolism; agents that alter the binding of a drug to plasma or tissue proteins; agents that alter rates of drug excretion in urine, saliva, sweat, bile or milk; and changes in hepatic blood flow, gastrointestinal absorption, and hormonal or nutritional status. In mice, responsiveness to a drug such as hexobarbital depends upon age, strain, sex, litter, painful stimuli, ambient temperature, degree of crowding, time of day of drug administration, type of bedding, and even the frequency with which this bedding is changed (28). Numerous compounds (30), including antipyrine (31), will on repeated administration enhance their own metabolism by induction of hepatic microsomal enzyme systems; thus, shifting from a single dose to chronic administration of some agents can substantially decrease the blood levels. Other drugs, such as diphenylhydantoin, produce metabolites that inhibit biotransformation of the parent drug, thereby tending to augment drug levels of the parent compound after chronic administration (32, 33).

The dose of a drug can affect blood concentrations in several other ways. For example, when the dose is small, the drug can be so strongly bound to plasma or tissue proteins that only a small proportion is available for metabolism and elimination. At higher doses, the protein binding capacity may be exceeded so that more of the drug is present in the free form, the form available for metabolism. At still higher doses, the biotransforming capacity of the body may become saturated, causing sustained elevations in drug blood levels. The mathematical treatment of the influence of different degrees of drug binding on drug elimination has been ingeniously described by Gillette (29), who also developed a mathematical model for the influence of the rate of hepatic blood flow on the biological half-life of a drug.

The multiple factors affecting drug blood levels can be divided into genetic and environmental subgroups. Such a division has broad implications in understanding the mechanisms that underlie interindividual variations and in attempting to translate such observations into practical clinical measures to improve therapy. Earlier passages in this review might suggest that in humans an important reason for variations among individuals in drug blood levels would be environmental. As we shall see, this prediction is incorrect in healthy, otherwise nonmedicated, normal volunteers.

Study of human twins permits separation of the control of a trait into hereditary and environmental components. This method, introduced in 1875 by Francis Galton

(34), has the advantage of comparing age- and sex-matched individuals and depends on the fact that identical twins have identical genomes, whereas fraternal twins share, on the average, only half their genes. The major assumption implicit in all twin studies is that identical and fraternal twins live within similar environments; this assumption has been challenged on the grounds that adult identical twins living in different households tend to create more similar environments for themselves than do fraternal twins under the same circumstances.

The twin method has been employed to identify the relative contribution of genetic and environmental factors to large interindividual differences in rates of decay of commonly used drugs. Healthy, adult, nonmedicated twins were studied pharmacokinetically for rates of elimination of the following drugs after a single oral or intravenous dose: phenylbutazone (35), antipyrine (36), bishydroxycoumarin (37), ethanol (38), and halothane (39). Steady-state blood levels of nortriptyline were measured in twins after 8 days of nortriptyline administration (40). Large interindividual differences in half-life or steady-state blood level tended to vanish within a set of identical twins, but to be preserved within a set of fraternal twins. Table 2 shows data for three drugs investigated at different times in seven sets of identical and seven sets of fraternal twins.

Several methods exist for estimating the relative contributions of environmental and genetic factors to the control of a trait (41); most of these are too complex to consider here. A rough estimate of the hereditary component of variation can be made by determination of the mean variance within sets of identical and fraternal twins; these variances are then treated according to the following formula for heritability (42, 43):

$$\frac{\text{variance within pairs of fraternal twins} - \text{variance within pairs of identical twins}}{\text{variance within pairs of fraternal twins}}$$

This expression yields values from 0, indicating negligible hereditary and complete environmental control, to 1, indicating virtually complete hereditary influence. For phenylbutazone, antipyrine, bishydroxycoumarin, and ethanol, values for the contribution of heredity were 0.99, 0.98, 0.97, and 0.99, respectively. Our studies on twins yielded intraclass correlation coefficients close to theoretical expectation solely on the basis of genetic control, according to which fraternal twins, having in common approximately half of their total number of genes, should have a value of 0.5, whereas identical twins should have a value of 1. For rates of metabolism of phenylbutazone, antipyrine, bishydroxycoumarin, and ethanol the intraclass correlation coefficients of identical twins were 0.83, 0.85, 0.85, and 0.82, respectively; and for fraternal twins, 0.33, 0.47, 0.66, and 0.38, respectively. Evidently for these drugs and for these subjects, large interindividual differences in rates of drug elimination from plasma are surprisingly free of environmental influence. As shown by repeated plasma drug half-life determinations, normal subjects have remarkably reproducible plasma half-lives for these drugs. Because phenylbutazone (22) and bishydroxycoumarin (44) are 98% bound to plasma proteins, differences among individuals in plasma elimination rates might involve variability in the binding of the drug to albumin. However, antipyrine is not appreciably bound to plasma proteins (45). Therefore, it seems reasonable to conclude that for antipyrine, if not also for phenyl-

Table 2 Dicumarol, antipyrine, and phenylbutazone half-lives with smoking and coffee history in 28 twins[a]

Twin	Age, sex	Half-life			Smoking	Coffee
		Dicumarol (hr)	Antipyrine (hr)	Phenylbutazone (days)	(pack/day)	(cups/day)
Identical twins						
Ho. M.	48,M	25.0	11.3	1.9	0.5	2
Ho. M	48,M	25.0	11.3	2.1	1	3
D. T.	43,F	55.5	10.3	2.8	0	5-6
V. W.	43,F	55.5	9.6	2.9	2	8-10
J. G.	22,M	36.0	11.5	2.8	1	1-2
P. G.	22,M	34.0	11.5	2.8	1	1-2
Ja. T.	44,M	74.0	14.9	4.0	0	6
Ja. T.	44,M	72.0	14.9	4.0	0	2-3
C. J.	55,F	41.0	6.9	3.2	0	2
F. J.	55,F	42.5	7.1	2.9	0	2
Ge. L.	45,M	72.0	12.3	3.9	0	4
Gu. L.	45,M	69.0	12.8	4.1	0	4
D. H.	26,F	46.0	11.0	2.6	0	0-1
D. W.	26,F	44.0	11.0	2.6	0	3-4
Fraternal twins						
A. M.	21,F	45.0	15.1	7.3	1.5	2
S. M.	21,M	22.0	6.3	3.6	0	0
D. L.	36,F	46.5	7.2	2.3	0	2-3
D. S.	36,F	51.0	15.0	3.3	2	3-4
S. A.	33,F	34.5	5.1	2.1	1	2
P. M.	33,F	27.5	12.5	1.2	0.5	2
Ja. H.	24,F	7.0	12.0	2.6	0	10-15
Je. H.	24,F	19.0	6.0	2.3	1.5	10
F. D.	48,M	24.5	14.7	2.8	0	1
P. D.	48,M	38.0	9.3	3.5	1.5	8
L. D.	21,F	67.0	8.2	2.9	1	6
L. W.	21,F	72.0	6.9	3.0	1	2-3
E. K.	31,F	40.5	7.7	1.9	0	0
R. K.	31,M	35.0	7.3	2.1	1	0

[a]The difference between identical and fraternal twins in intrapair variance is significant: $P < 0.005$ ($F = 36.0$, $N_1 = N_2 = 7$). Reproduced by permission from Vessell and Page, 1968 (37).

butazone and bishydroxycoumarin, interindividual variability in plasma half-life arises from genetic differences in drug metabolism rather than in drug distribution. That appreciable variations do exist in rates of metabolism of these drugs is indicated by ranges for the plasma half-lives of ethanol, antipyrine, phenylbutazone, and bishydroxycoumarin of twofold, threefold, sixfold, and tenfold, respectively, among the 28 individuals in the study (Table 2).

Family studies using pharmacokinetic measurements tend to support the results of the twin experiments; the family studies disclosed predominantly genetic control over large interindividual variations in plasma half-lives of bishydroxycoumarin (46), phenylbutazone (47), and nortriptyline (48). Furthermore, the results of all three studies suggest polygenic control.

There also exist in man at least a dozen monogenically controlled conditions that produce unusual responses to drugs (41, 49). These include acatalasia, atypical plasma pseudocholinesterase, slow acetylation of isoniazid, deficient parahydroxylation of diphenylhydantoin, sensitivity to bishydroxycoumarin, glucose-6-phosphate dehydrogenase deficiency, resistance to warfarin, and drug-sensitive abnormal hemoglobins. The first five conditions are hereditary abnormalities that affect enzymes directly involved in drug metabolism. These conditions may result in drug toxicity attributable to elevated blood levels from drug accumulation after continuous administration of even a low dose. By contrast, the last three conditions represent genetically transmitted aberrations, not of drug metabolism, but of sites of drug action. In these disorders toxicity or failure to elicit a therapeutic response can occur at what are generally considered therapeutic drug blood levels. Some structural alteration in a protein with which the drug or drug metabolite can interact causes these blood levels, safe in most subjects, to be either toxic or ineffective. For example, O'Reilly (50, 51) described two extensive pedigrees of warfarin resistance, an autosomal dominant trait; the normal mean daily dose of warfarin (6.8 ± 2.8 mg) was completely ineffective in anticoagulating these subjects although their warfarin blood levels were within normal range. A daily 20 mg dose of warfarin was required in affected individuals; resistance was attributed to a structurally altered hepatic receptor site with greater affinity than normal for Vitamin K (50, 51).

ROLE OF ENVIRONMENTAL FACTORS

Despite the abundant evidence described in the preceding section indicating predominantly genetic control over large interindividual differences in rates of drug metabolism for normal, nonmedicated subjects living in relatively uninduced environments, nongenetic factors can also significantly alter both drug distribution and therapeutic response. Many drugs, or environmental agents such as insecticides (26, 52, 53), can accelerate rates of drug metabolism and elimination from the body; other drugs and environmental agents can retard these rates (54). However, recognition of the numerous factors that accelerate or retard drug metabolism and elimination is insufficient to permit adequate adjustment of drug dosage, because individuals vary greatly in their quantitative response to inducing (31, 55, 56) or inhibiting (54, 57–62) agents. A twin study revealed that phenobarbital administered for two weeks

(2 mg/kg qd po) produced a wide range of responses, as estimated by the degree of shortening of plasma antipyrine half-lives (55). The extremes were total failure to alter antipyrine half-life and a shortening of antipyrine half-life from 18 to 6 hr. These variations in response to phenobarbital were shown to be predominantly under genetic control (55). Furthermore, the extent to which phenobarbital shortened antipyrine half-lives was related to the control value for plasma antipyrine half-life; subjects with relatively long antipyrine half-lives before phenobarbital administration reduced their half-lives much more than did subjects with initially short values (Figure 3) (55). In harmony with these observations, genetic control of the induction of aryl hydrocarbon hydroxylase activity in cultured human lymphocytes by 3-methylcholanthrene was demonstrated (56); this study of cells from 353 healthy subjects from 67 families revealed hereditary control by a single genetic locus with gene frequencies of the alleles for low and high inducibility being 0.717 and 0.283, respectively (56).

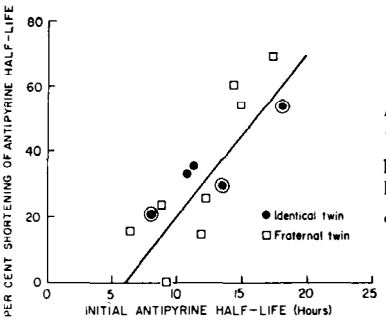

Figure 3 Positive correlation (0.84) between the initial antipyrine half-life in plasma and the phenobarbital-induced shortening of antipyrine half-life. Reproduced by permission from Vesell & Page (55).

As mentioned previously, large interindividual variations also exist in response to compounds that inhibit drug metabolism (57–62), but these differences appear from twin studies (63) to be more under environmental than genetic control. Inhibition of drug metabolism presumably arises from binding of compounds to hepatic microsomal proteins (64, 65) and does not involve protein synthesis, whereas induction requires protein synthesis that is under direct genetic control. For these reasons, predominantly environmental control of interindividual differences in response to inhibiting drugs, and underlying genetic control of large interindividual differences in drug metabolism, drug blood levels, and induction of drug metabolizing enzymes could have been predicted. On the other hand, the numerous, previously described environmental factors capable of altering drug blood levels by affecting processes of drug absorption, distribution, metabolism, interaction at receptor sites, and/or excretion might have been expected to obscure the fundamental genetic control. In this connection, several disease states should be mentioned as examples of altered environments capable of changing both drug blood levels and receptor site sensitivity. In hyperthyroidism, antipyrine metabolism is accelerated, whereas it is retarded in myxedema (54). However, in myxedema, decreased sensitivity of warfarin receptor sites has also been described (66). In myasthenia gravis, decreased sensitivity of the acetycholine receptors in the postjunctional membrane may be the

principal mechanism for skeletal muscle weakness, although it may develop only after prolonged deficiency of acetylcholine (67). Thus, for myasthenia gravis, the interrelationship between acetylcholine distribution and therapeutic response is complex and as yet not completely resolved. After injury or section of peripheral nerve to skeletal muscle, supersensitivity of the acetylcholine receptor sites on the muscle develops as a compensatory mechanism for decreased availability of acetylcholine. Here an inverse relationship exists between acetylcholine distribution and receptor site response.

Large interindividual differences in drug blood levels after exposure to inducing or inhibiting compounds render hazardous predictions of the extent to which any particular therapeutic agent will affect the blood level of another drug in any given patient. The most direct solution to this problem lies in measuring drug blood levels in each subject to whom such inducing or inhibiting agents have been administered. Drug blood level determinations will reveal directly the degree to which each subject responds to an inducing or inhibiting agent. Nevertheless, problems arise if too much faith is placed in drug blood levels. Specific information is required on each drug whose blood level is being measured to insure a direct correlation between drug blood level and therapeutic effect. For example, a recent experimental study in dogs contrasted serum and interstitial levels of various antibiotics administered chronically or acutely (68). The results revealed that after a single dose the concentrations of certain antibiotics, including ampicillin, were much lower in the tissue fluid, where they normally act, than in serum (68). Furthermore, the shape of the curve relating antibiotic levels to time differed in serum and tissue fluid. For several antibiotics the peaks of the curves did not occur simultaneously. On chronic administration, higher tissue concentrations of antibiotics could be produced. These data suggest that reliance on blood levels as indicators of antibiotic concentrations in interstitial fluid may be invalid for several antibiotics after a single dose and that for certain rapidly excreted antibiotics frequent administration of high doses is required to achieve effective tissue concentrations.

Another possible misinterpretation that could arise from too literal a reading of drug blood levels is exemplified by the patient who fails to take his medication for several weeks or even months but decides to resume the prescribed drug several days prior to visiting his physician. High drug blood levels at the time of the visit without much therapeutic effect could mislead the physician into believing that either this particular patient was resistant to the drug or that poor correlation exists between blood levels of the drug and therapeutic effects.

Three diverse drug interactions are pertinent to this discussion. Although the literature on drug interactions is vast, few studies have succeeded in defining systematically whether a drug interaction occurs during drug absorption, distribution, biotransformation, binding at receptor sites, excretion or a combination of these. Too often a drug interaction is described without elucidation of its mechanism, or else a mechanism is proposed with insufficient supporting evidence. To illustrate how a drug interaction having profound therapeutic effects can occur without changing the drug blood level, the interaction between warfarin and Vitamin K is cited. In a patient taking warfarin, administration of Vitamin K does not alter the

blood level or plasma half-life of warfarin. However, through competition with warfarin at sites in the liver where certain clotting factors are synthesized, Vitamin K restores prothrombin times toward normal. Frequently, after a drug interaction has been documented, its precise clinical significance is unclear. The following three examples illustrate aspects of these and related problems in drug interactions.

One study with cyclophosphamide revealed that patients before and after exposure to microsomal enzyme-inducing drugs exhibited marked changes in their plasma cyclophosphamide half-lives and in the peak plasma alkylating concentrations (69). However, no significant alteration in clinical response occurred; this lack of interference was attributed by the authors to the relative constancy of the product of the total drug concentration and time (69).

The second example concerns displacement of one drug from albumin by administration of another drug that binds to albumin more avidly. Because only the unbound portion of a drug is considered available to produce therapeutic effects, this type of drug interaction has the potential for changing therapeutic effects by changing the ratio of bound, pharmacologically inactive drug to unbound, pharmacologically active drug. However, few reports contain quantitative data on the degree to which such drug displacements actually intensify therapeutic effects; as a result much confusion on this point has been generated. Gillette (29) has clarified the situation by developing a formula to describe the relationship between the biological half-life of a drug and its binding to albumin and muscle under specified conditions. According to these formulas, 50% binding of a drug to albumin increases the drug half-life by only 11% when the unbound drug is distributed in the total body water; if 75% of the drug is bound to albumin, the drug half-life is prolonged by only 33% (29). Reduction in drug binding to albumin from 95 to 90% would decrease the biologic half-life by only 50%, but reduction from 99 to 98% binding would diminish it by 86%. Greater effects would occur for drugs whose unbound portions are distributed in the extracellular, rather than the total body, fluids; for example, if 75% of such a drug were bound to albumin, its biological half-life would be prolonged by 111% (29). Furthermore, Gillette demonstrated that variations in binding of a drug to albumin would produce little effect if the drug were also highly bound to skeletal muscle. In instances of changes in the extent of binding of a drug to skeletal muscle, marked alterations in the biologic half-life of a drug could result because the volume of skeletal muscle greatly exceeds the plasma albumin volume. Thus more attention should be devoted to drug binding to tissues with large volumes such as skeletal muscle (29).

The third example concerns oversimplification in classification of drugs that alter rates of hepatic microsomal drug metabolism. It has become popular to distribute lists separating those drugs that increase from those that retard or produce no change in the rates of biotransformation of other drugs. This approach stems from the trend in studies on drug interactions to investigate the effect on drug metabolism only at a single time after chronic administration of a compound. While technical problems prevent the clinical pharmacologist from investigating effects on drug metabolism at multiple time points in his patients, restriction of such studies to a single time point can yield erroneous conclusions if results are generalized too broadly. Many so-called inducing drugs exhibit biphasic effects; 2 to 10 hr after their

administration certain inducing agents can inhibit drug metabolism by binding to hepatic microsomal proteins (30). For example, a recent study in rats on the effects of an experimental anti-inflammatory agent revealed that 2 hr after its oral administration hepatic microsomal aniline hydroxylase and ethylmorphine N-demethylase activities were inhibited, but 24 hr after oral administration of the same dose analine hydroxylase activity and cytochrome P-450 content were enhanced (70). After 4 daily doses of this experimental anti-inflammatory drug no significant change occurred in either enzyme activity, but cytochrome P-450 content remained slightly elevated (70). These studies suggest that interactions affecting rates of drug metabolism may be more complex than previously recognized and that the same agent can produce polyphasic alterations depending on the experimental conditions. From the point of view of this review, such investigations illustrate that the same dose of a drug can affect drug blood level and therapeutic response in markedly different, even opposite, ways depending on the time selected to examine the effects. In this connection changes in the dose of a drug or its route of administration (71, 72) can alter drug blood levels, metabolism and therapeutic effects.

This discussion of the role of environmental factors in altering drug blood levels will be concluded with two examples in which actual measurement of drug blood levels helped to identify the factors affecting drug blood levels and to develop safer, more effective ways of administering the drug in man. The first example concerns the use of procainamide in prophylaxis of antiarrhythmias after myocardial infarction (73). Until recently the usual dosage interval for procainamide was 6 hr. Koch-Weser et al (73) demonstrated that when the compound was administered in the usual dose every 6 hr, large fluctuations occurred in the serum concentrations of the drug (Figure 4) such that both the peak and lowest serum concentrations were out of the optimal therapeutic range (4–8 mg/liter). By changing the administration interval from 6 hr to 3 hr, they observed that the amplitude of these fluctuations was reduced and that patients could be maintained more consistently within therapeutic serum concentrations (Figure 4).

The second example involves biological availability of a dosage form. Lindenbaum et al (74) reported wide differences of serum digoxin levels in patients receiving various proprietary preparations of the drug. Each tablet contained equal

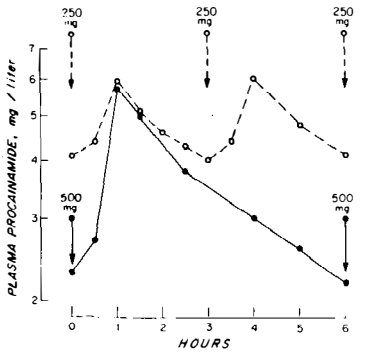

Figure 4 Fluctuation of plasma procainamide concentrations on two dosage schedules in the same patient. Reproduced by permission from Koch-Weser et al (73).

Table 3 Relationships between drug blood concentrations and therapeutic effects

Drug	Method (Ref.)	Drug Concentration in Serum	Reported Pharmacologic Effect
Acetaminophen	spectrophotometry (85)	10-20 μg/ml	analgesia
Acetohexamide	spectrophotometry (86)	20-55 μg/ml	hypoglycemia
Aminopterin	spectrophotofluorometry (87)	0.2-1 μg/ml	carcinostatic action
Amitriptyline	spectrophotometry (88)	0.3-0.9 μg/ml	antidepressant
Amphetamines	gas chromotography (89, 90)	1-2 μg/ml	analepsis
Antihistamines	gas chromatography (91)	0.008-0.016 μg/ml	antihistaminic
Barbiturates	spectrophotometry (92) & gas chromatography (93, 94)		sedation
Phenobarbital		20 μg/ml	
Amobarbital		5 μg/ml	
Pentobarbital		1 μg/ml	
Secobarbital		1 μg/ml	
Bishydroxycoumarin	spectrophotometry (95)	18-26 μg/ml	anticoagulant
Bretylium	gas chromatography (96)	0.5-1.3 μg/ml	antiarrhythmic
Bromide	spectrophotometry (97)	40-50 μg/ml	sedation
Brompheniramine	gas chromatography (98)	0.008-0.016 μg/ml	antihistaminic
Bromural	gas chromatography (99)	1-3 μg/ml	sedation - hypnosis
Butaperazine	spectrophotofluorometry (100)	4-6 μg/ml	antipsychotic
Captodiamine	spectrophotometry (101)	2-4 μg/ml	sedation, anti-spasmotic
Carbamazepine	gas chromatography (102)	2-10 μg/ml	anticonvulsive
Carbromal	gas chromatography (99)	1-3 μg/ml	sedation - hypnosis
Carisoprodal	gas chromatography (99, 103)	10-40 μg/ml	muscle relaxant
Chloral hydrate	gas chromatography (104)	5-10 μg/ml	hypnosis
Chlordiazepoxide	spectrophotofluorometry (105)	1-2 μg/ml	tranquilizer
Chlorpheniramine	gas chromatography (98)	0.008-0.016 μg/ml	antihistaminic
Chlorpromazine	gas chromatography (106)	0.5-0.7 μg/ml	tranquilizer, antiemitic
Chlorpropamide	spectrophotometry (86)	30-140 μg/ml	hypoglycemia
Chlorothiazide	spectrophotometry (107)	2-2.5 μg/ml	natriuretic action
Chlorprothixene	spectrophotofluorometry (105, 108, 109)	0.04 μg/ml	antipsychotic, antiemitic
Desipramine	spectrophotofluorometry (110)	0.6-1.4 μg/ml	antidepressant
Digoxin	radioimmunoassay (111)	0.0003-0.003 μg/ml	stabilize sinus rhythm & antiarrhythmic
Diphenylhydantoin	spectrophotometry (112)	6-17 μg/ml	anticonvulsive
	gas chromatography (113)	4-24 μg/ml	antiarrhythmic (Ventricular premature systoles)
		12-23 μg/ml	antiarrhythmic (Supraventricular tachycardia)
Ethchloroynol	gas chromatography (103)	4-6 μg/ml	sedation
Ethinamate	gas chromatography (99)	5-10 μg/ml	sedation
Ethyl ether	gas chromatography (97)	900-1000 μg/ml	anesthesia

Table 3 Continued

Drug	Method (Ref.)	Drug Concentration in Serum	Reported Pharmacologic Effect
Glutethimide	spectrophotometry (114, 115)	0.2-0.4 μg/ml	sedation
Griseofulvin	spectrophotofluorometry (87)	0.3-1.3 μg/ml	antifungal
Halofenate	gas chromatography (116)	150-250 μg/ml	hypolipidemic
Hydroxyphenamate	gas chromatography (99)	5-10 μg/ml	muscle relaxant, tranquilizer
Imipramine	spectrophotofluorometry (117)	2-6 μg/ml	antidepressant
Lidocaine	gas chromatography (118)	1.0-2.0 μg/ml	local anesthesia
Lithium	flame emission spectrometry (119)	1.0-2.0 mEq/liter	antidepressant
Meperidine	spectrophotometry (120)	0.6-0.75 μg/ml	sedation
Meprobamate	gas chromatography (103)	10-20 μg/ml	tranquilizer
Methaqualone	spectrophotometry (121)	2-5 μg/ml	sedation
Methohexitone	gas chromatography (122)	1-4 μg/ml	anesthesia
Methyprylon	gas chromatography (99)	10 μg/ml	sedation
Methapyrilene	spectrophotometry (123)	2-4 μg/ml	antihistaminic
Nitrofurantoin	spectrophotometry (124)	1-2 μg/ml	antibiotic
Nortriptyline	spectrophotometry (88) & gas chromatography	0.015-0.035 μg/ml	antidepressant
Oxazepam	gas chromatography (97)	1-2 μg/ml	antidepressant
Paracetamol	spectrophotometry (121)	4.5-25 μg/ml	analgesia
Paraldehyde	gas chromatography (103)	30-150 μg/ml	hypnosis
Pentazocine	spectrophotofluorometry (125)	0.14-0.16 μg/ml	analgesia
Phenylbutazone	spectrophotometry (22)	40-60 μg/ml	analgesia
Prilocaine	gas chromatography (118)	less than 2 μg/ml	local anesthesia
Probenecid	spectrophotometry (126)	100-200 μg/ml	uricosuric
Procainamide	spectrophotofluorometry (127)	4-8 μg/ml	antiarrhythmic
Propranolol	spectrophotofluorometry (128)	0.035-0.200 μg/ml	β-adrenergic blockade
Propoxyphene	gas chromatography (129)	0.1-0.2 μg/ml	analgesia
Quinacrine	spectrophotofluorometry (87)	0.005-0.05 μg/ml	antimalarial
Quinidine	spectrophotofluorometry (87)	3-6 μg/ml	antiarrhythmic
Quinine	spectrophotofluorometry (87)	2-5 μg/ml	antimalarial
Salicylate	spectrophotometry (130)	50-100 μg/ml	analgesia (therapeutic)
		350-400 μg/ml	antiarthritic
		> 250 μg/ml	rheumatic fever therapy
Sulfonamides	spectrophotofluorometry (105)	50-100 μg/ml	bacteriostatic
Tetracycline	spectrophotofluorometry (117)	1.2-1.9 μg/ml	antibiotic
Thiobarbiturate	spectrophotometry (131)		hypnosis
Thiopental		30 μg/ml	
Thiamylal		30 μg/ml	
Thioridazine	spectrophotofluorometry (109)	0.04-0.3 μg/ml	tranquilizer
Tolbutamide	spectrophotometry (86, 132)	50-95 μg/ml	hypoglycemia
Trimethobenzamide	spectrophotofluorometry (105)	1-2 μg/ml	antivertigo
Zoxazolamine	spectrophotometry (133)	3-12 μg/ml	muscle relaxant

amounts of digoxin as measured in vitro. Wide differences in serum digoxin levels, ranging from four- to sevenfold, occurred not only between preparations from different companies but even between different lots from the same company (74). Previously there had been reported similar differences in the bioavailability of other drugs, including diphenylhydantoin (75, 76), bishydroxycoumarin (77), phenylbutazone (78), prednisone (79), thyroid (80), tolbutamide (81), chloramphenicol (82), and oxytetracycline (83, 84). Thus differences in bioavailability of dosage forms can contribute to intraindividual and interindividual variations in drug blood levels and therapeutic response.

Gathered from the current literature, Table 3 summarizes the correlation between drug blood levels and therapeutic effects. It is presented with the recognition that certain of these drug levels represent approximations, that many change under various environmental circumstances and are not invariably associated with the therapeutic effects listed. The diverse causes for such limitations in the use of drug blood levels have been the topic of this review.

CONCLUSION

Certain pharmacological principles governing relationships between drug distribution and therapeutic effects have been described. A major obstacle to a clear understanding of the subject and a principal cause for the controversy surrounding it are tendencies to generalize too broadly rather than to recognize that the individual properties of a drug define the relationship between its distribution and therapeutic effects. Genetic and environmental factors represent important determinants of the relationship between the distribution and therapeutic effects of specific drugs. The close relationship that applies for certain drugs between blood levels and pharmacologic effects is exemplified by diphenylhydantoin. Administration of the usual doses of diphenylhydantoin produces serum concentrations from 2 to 50 mg/liter due to large interindividual differences in rates of hepatic diphenylhydantoin metabolism (134); however, the therapeutically effective range of serum concentration (10–20 mg/liter) is comparatively quite narrow. Progressive increments in diphenylhydantoin toxicity are associated with progressive elevations of blood concentrations above the therapeutic serum levels, as shown in Figure 5 (135).

In certain disease states or after administration of avidly bound compounds, the free, pharmacologically active fraction of a drug may be increased; thus, correlations between pharmacodynamic effects should be attempted with the unbound rather than with the total plasma drug concentration. For example, in renal failure with uremia the capacity of plasma albumin to bind drugs is decreased; and the apparent volume of distribution of such highly bound drugs as digitoxin and diphenylhydantoin is increased (136, 137). In addition to actual drug concentrations at the receptor sites for cardiac glycosides, many other environmental factors, such as potassium concentration, anoxia, and acidosis, influence the pharmacodynamic effects of the cardiac glycosides. Limitations exist in the precision and accuracy with which many pharmacodynamic effects can be measured. If correlations are to be sought, only plasma drug levels should be used that produce a pharmacodynamic response on

linear, rather than the plateau, portion of the dose-response curve. Although methodology for measuring drug blood levels has developed tremendously, many problems remain. One difficulty involves the distinction between the free and the bound forms of a drug. For drugs administered as racemic mixtures, such as warfarin (138), hexobarbital (139), methadone (140), propranolol (141), and amphetamine (142), problems exist in establishing the distinction between the pharmacologically active and inactive isomers and between potentially different rates of metabolism and hence different blood levels of the isomers.

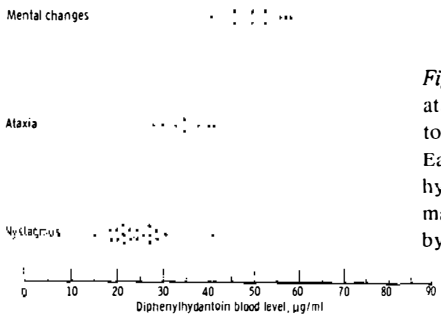

Figure 5 Progression from nystagmus to ataxia to mental changes in relationship to diphenylhydantoin blood concentrations. Each circle represents the blood diphenylhydantoin concentration and toxic clinical manifestation of a single patient. Reproduced by permission from Kutt et al (134).

ACKNOWLEDGMENTS

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Note added in proof: A high correlation between drug toxicity and plasma drug concentrations has actually been observed for relatively few therapeutic agents in clinical practise; Prescott et al (143) documents this conclusion for thiopental, α-methyldopa, methaqualone, glutethimide, salicylates, and paracetamol (143).

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