REVIEWS

In Vitro-in Vivo Correlation: An Unrealistic Problem

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Abstract: This review demonstrates that any true *in vivo – in vitro* correlation cannot exist. A thorough analysis of the available results shows that any attempt to qualitatively or quantitatively correlate *in vitro* results with biopharmaceutical or pharmacokinetic data in humans is a chimerical problem.

The endeavor to obtain objective and generally applicable data on drug bioavailability and bioequivalence is a current problem in formulation studies of controlled drug delivery. There is an increasing tendency to project performance in biological systems on the basis of rational *in vitro* measurements. Numerous research groups concern themselves with perfecting methods and equipment to demonstrate the existence of relevant *in vivo-in vitro* relationships. However, all these studies are based on the assumption that an *in vitro-in vivo* correlation is obtainable. Judging by the number of publications, this area is very topical and ranks in first place in biopharmaceutics.

In opposition to a solution of the *in vitro-in vivo* correlation stands our increasing knowledge of human pharmacology and pharmacokinetics, with its non-homogeneity, complexity, variability and adaptability of the human system. This complexity casts doubt on the ability to correlate living processes with simple laboratory tests on the basis of physico-chemical principles with a concept of "man as machine" (La Mettrie: L'homme machine, 1747). The facts show that the correlation is an unrealistic or *pseudo*-problem, using the definition of Max Planck, "no solution to the problem is possible, since no methods free from objection for its investigation are available"(1).

In this study, arguments are gathered to refute systematic *in vivo* predictions from *in vitro* results and to define the value of mechanistic experimental models. It is not intended to discuss the philosophy of mechanistic biology ("biologic mechanism") as opposed to vital forces ("vitalism") (2, 3) or the ability or inability to simulate living processes, but to point out the sense and non-sense of basic drug delivery and biopharmaceutical problems. Also, it is not proposed to discuss the data in their entirety, but to substantiate the different aspects with characteristic examples.

Endogenous Factors

Inter-Subject Factors

Genetically-Induced Individual Variability

The differing predisposition of humans may greatly influence the sensitivity and response to drugs. In addition to biologic variations that are genetically induced, there are patients who show above average variation because of genetically conditioned fluctuations. The hereditary of this variability implies that family members may demonstrate a similar abnormal under- or over-response. Pharmacogenetics concerns itself with the elucidation of these relationships.

A typical and often cited example is the differing activity of *dicumarol:* the elimination half life, calculated from the plasma level-time curve, may vary between 7 and 100 hours, i. e. by a factor of 15 (after i. v. injection of 5 mg/kg). (Fig. 1) (4–6).

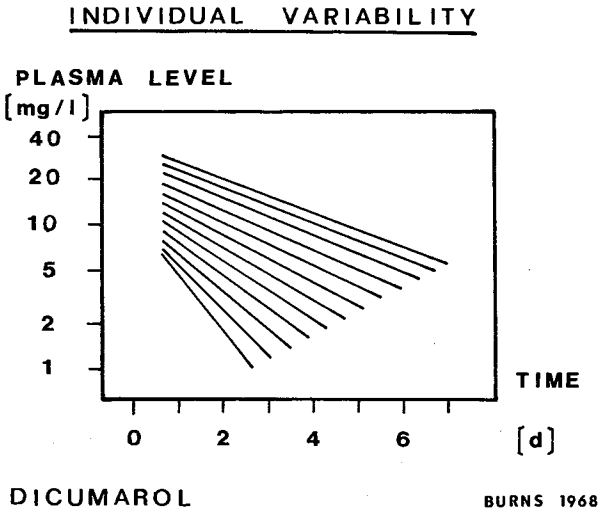

Fig. 1

The molecular cause for such variation is thought to arise from genetic differences in the level and nature of enzyme expression. The pattern of human enzymes can show great differences that must be clarified during the initiation of a dosage regime for some drugs. The hereditary frequency of enzymopathies in relation to drug activity has been reported (7).

Noteworthy is the individual variability with succinylcholine chloride, which is hydrolyzed to choline and succinic acid under the catalysis of serum cholinesterases. With reduced cholinesterase activity against the drug (atypical cholinesterase), an increased myorelaxation and apnoe occurs. However, in a few individuals the drug's efficacy is only 10 to 20 % of the average response because of a higher rate of drug hydrolysis (8). Therefore, the activity may vary by a factor of 10 to 100 (Fig. 2).

Ascorbic acid represents another example with a bioavailability that can vary up to threefold between individuals (9). The sum of all anatomical, physiological, and biochemical abnormalities is reflected in the dramatic non-uniformity of drug action. Moreover, all factors of drug absorption must be taken

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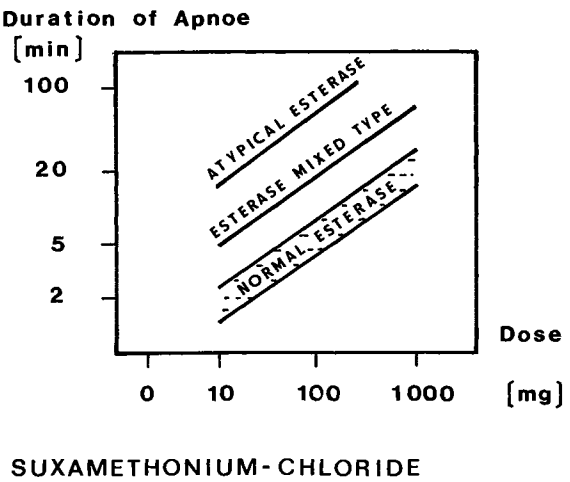

Fig. 2

into account. For example, the anatomy and the metabolic activity of the gastro-intestinal system, its motility and the metabolic activity of the intestinal mucosa and the liver contribute to individual variability after peroral application (10). Similarly, the skin displays interindividual differences for transdermal drug absorption (11).

Several comprehensive reviews of pharmacogenetics are available (11–15).

Genotype

A special form of genetically-induced differences is demonstrated by racial differences. Ethnographic studies show that variations arise between population groups in the same geographical area and therefore cannot be attributed to climatic conditions.

A different mydriatic action of ephedrine and cocaine is demonstrable between Caucasians, Chinese and Africans (6, 16). The strongest mydriasis is observed with Caucasians and the weakest with Africans; the effect ratios are 9:2.2:1 for ephedrine and 8.2:2.8:1 for cocaine, a variation of almost one order of magnitude.

The differing predispositions of the various races can also be seen in anatomical differences. For instance, the skin shows qualities that imply significant differences in drug absorption because of structural differences (Table I) (17).

Table I. Human skin structure of different genotype

	Number of glands per cm^2 on fingertips	
European USA	559	100 %
Black USA	597	107 %
Philippinos	654	117 %
Pygmeans	709	127 %
Hindu (India)	738	132 %

On the basis of the density of sweat ducts as a measure for general structural variation, one may assume the absorption function of the skin to vary by approximately 30 %, although the pores themselves are not much involved in this process.

Age

It is customary to differentiate between perinatal, neonatal, childhood, adolescent, adult and senescent periods in life. Medical care is then adapted to these phases. The aging process, however, does not progress step-wise, but rather is continuous, so that the exact planning and evaluation of pharmacokinetic experiments requires a more detailed classification of the life stages.

The well-known decline in physical function after 25 years of age can be approximated by a zero-order reaction (Table II) (18).

Table II. Loss of body functions due to the aging process

Function	approximate loss past the age of 25 (%/year)
General metabolism	0.38
heart-activity	0.75–1.01
cell weight (mass)	male: 0.20 – female: 0.16
total body fluid	male: 0.20 – female: 0.13
intracellular liquid	0.38
glomerula filtration velocity	0.66
tubular secretion	0.62
maximum respiration capacity	1.18
vital capacity	0.81
blood circulation:	
– brain	0.35 – 1.50
– heart	0.5
– liver	0.30 – 1.50
– kidney	1.10 – 1.90
– tissue	1.3

Some parameters change by approximately 1 % per year, which in the course of several decades results in a drastic alteration.

Age-induced changes are multi-faceted. The plasma level-time profile is influenced by numerous factors (6, 18–20) such as cell permeability, circulatory function, endocrine activity, and by the function of the immune system and the nervous system. While in the most cases absorption is not affected (18), distribution, plasma protein binding, biotransformation and elimination alter considerably. For example, the proportion of total body water decreases from 82 to 55 % during the course of life and the proportion of extracellular fluid decreases from 36 to 18 % (5). Body fluid changes alter the distribution compartments and the dosage proportions when adjusted to body weight. The age-dependent changes of the volume of distribution can be approximated by a linear function (8). In the case of plasma protein binding, differences of approximately 20 % are observed between neonates and adult; in some cases, e. g. ampillicin and digoxin, the binding doubles with old age (21).

Moreover, increasing the biotransformation rate decreases the plasma level-time curve. In general the half-life of drug elimination decreases from the new-born to the adult: with phenobarbital, diazepam and phenytoin the ratio is 3:1, and with amidopyrine the ratio is 10:1 (21). Essential experimental errors are not to be expected, as much of this change occurs during the first year of life, and babies are included with *in vitro-in vivo* comparisons only in exceptional circumstances.

The kidney function is a particularly important determinant of drug elimination rates. With increasing age glomerular filtration and tubular secretion diminish, with a corresponding increase of the biological half-life of the active substance, if it is cleared mainly by the kidneys. The age-dependence of renal clearance is calculable (18). For example the elimination rate of theophylline varies by a factor of approximately 2 between the 20th and 80th year (Fig. 3) (22), which results from changes of the renal and, more importantly, metabolic clearance.

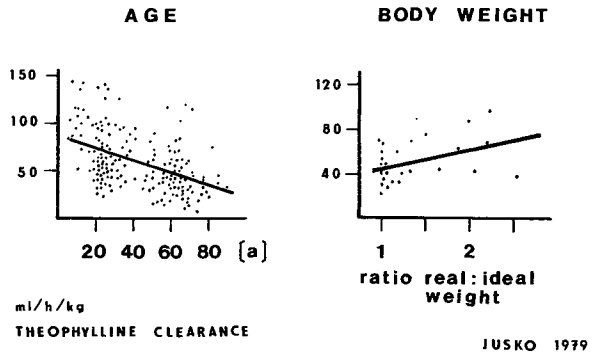

Fig. 3

Similar results were obtained for the renal excretion of penicillin (5, 23). When a group of human subjects with an average age of 30.6 years was compared to a group with an average of 80 years, the half-lives were 23 and 52 minutes, respectively. Neglect of the age-factor and a simplistic differentiation between children and adults may lead to variations of ± 50 %, and in some cases even more.

Various authors have considered the diminished capacity of organs in old age to be comparable to certain disease states affecting those organs.

State of Health

Among the various bodily functions that can be diminished by illness, those of pharmacokinetic interest are not always evident and are therefore often overlooked. Rather typical are the consequences of an altered kidney function. For patients with an elevated serum creatinine level the half-life of penicillin may increase from 52 to 118 minutes (23). Amobarbital, phenytoin and propranolol, however, show a reduction in half-life with kidney disease because of decreased protein binding. Moreover, the half-life of practolol doubles when the creatinine clearance increases from 10 to 60 ml/min, while renal insufficiency extends the half-life from 9.5 hours to 58 hours, which means a 600 % prolongation (26).

Pregnancy also affects drug pharmacokinetics. As with aging, a range of factors are changed with many activities being decreased. Of relevance to drug absorption is the fact that the gastro-intestinal activity is only 50 to 70 % of control, while the volume of gastric secretions is reduced to 60 %, causing a reduction in stomach peptidases. This diminution of enzyme activity is a general phenomenon influencing the rate of metabolism (5). Absorption variability of a factor of 2 is quite common and is rarely accounted for in pharmacotherapy.

Body Weight and Size

These factors are often neglected. For dose corrections the body surface area can be calculated as follows:
Body surface area = weight 0.425 x height 0.725 x 71.84 (25). The average area is 1.73 m^2. With increasing age as well as with increasing weight, absorption and transport requirements change, because of changes in the composition and proportion of distribution compartments (27). Therefore, lipid-soluble drugs require higher dosages for adipose patients than for athropic patients.

The elimination rate constant is also weight dependent. If the ratio of real weight to ideal weight is increased, the clearance values relative to body weight increase for theophylline. Correspondingly, biological half-life decreases and therewith the bioavailability. Doubling of body weight causes an increase in clearance of approximately 40 % (Fig. 3) (22).

Sex

Numerous studies document the pharmacokinetic importance of gender. The difference in kinetics is mainly due to variations in enzyme activity. In part, different metabolites are formed in men and women.

A comparison of acetylsalicylic acid esterase activities shows that the average plasma enzyme activity is 33 % higher in men than in women (5, 28). On the other hand, the half-life of antipyrine is increased by 30 % in men as compared to women because of a higher metabolic activity in women (29). Again using the example of theophylline clearance, men on an average show a 50 % higher value than women (Fig. 4). The importance of sexual gender can also be shown with animal experiments (5, 30).

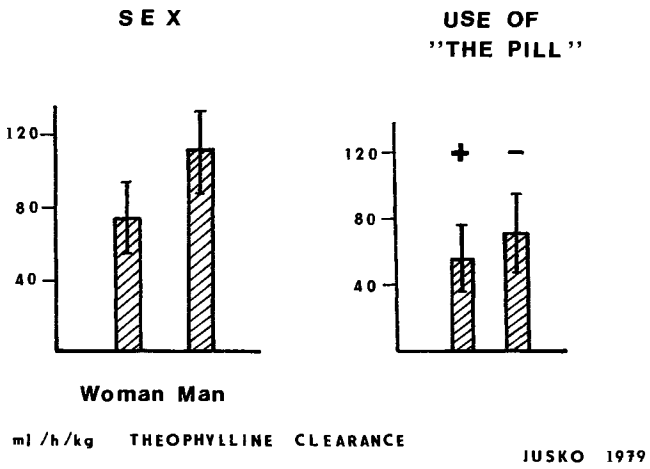

Fig. 4

Lifestyle

Regular habits can affect the pharmacokinetics both between and within individuals, either irreversibly or temporarily. Some habits are characteristic for certain racial or religious groups, for climatic regions or job groups, and as such they may vary between seasons, days, during therapy or due to a single event. Since factors affected by time are dominant, these aspects are dealt with in the section on intra-subject factors.

Intra-Subject Factors

Body Region

Especially for *topical* application the body region can play an essential role. For example the antiseptic parathion shows region-dependent percutaneous uptake which may vary in extreme cases by one order of magnitude (Fig. 5) (31). The values in Fig. 5 represent relative drug uptake.

The application of hydrocortisone (0.06 mg in 0.1 ml acetone) yields a relative absorption of only 0.14 on the sole of the foot, but 13 in the region behind the ear, and 42 on the scrotum (32); the uptake varies therefore by a ratio of 1:93:300.

These intra-subject factors have particular importance when transdermal therapeutic systems (TTS) are being developed. With scopolamine these conditions have been studied carefully (33, 34). TTS preparations should therefore

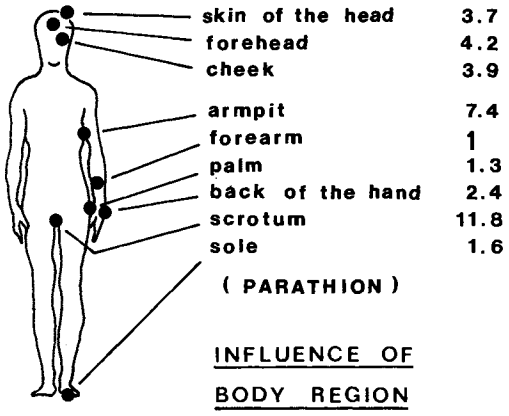

Fig. 5

always be applied to the same body region (35, 36). Application behind the ear results in a 3 to 4 times higher bioavailability as compared to application on the back (25).

With ointments this knowledge is often neglected. In addition, weather, job and age can modify skin structure and thereby increase differences in skin absorption, among body region so that systemic side-effects of topical preparations can often be attributed to the region of the body treated. There has been no lack of efforts to simulate these relationships *in vitro* and to correlate *in vitro-in vivo* studies. These generalized statements have to be severly circumscribed, and the ability to correlate is called into question (37).

Biorhythm

For more than two decades rhythmic variations in the behavior of organisms have been studied. Endogenous factors cause a time-dependent variation in sensitivity that is important for bioavailability and effect, but remains underestimated (38). New fields such as chronomedicine, chronotherapy, chronobiology, chronopharmacology, chronotoxicology and chronopharmacokinetics have developed very quickly. Biorhythm may influence the whole body, single organs, or particular cells. The fluctuation may be repeated in intervals of seconds, minutes, hours, days (circadian), months (circatrigintan) or yearly (circannual). Some examples of this "human inner clock" (39, 40, 41, 42) will be cited.

Differences in activity which are extremely time-dependent have been observed in animal experiments (43). When phenobarbital is administered i. p. to rats at 2 p. m., 100 % mortality occurs; at 1 a.m., however, the contrary result of 0 % mortality occurs (Fig. 6) (44).

The factors which determine the oscillation and underlie a biorhythm may be attributed in part to absorption, distribution, metabolism and excretion. Thus, at midnight Vitamin K 1 is absorbed in the jejunum of rats 2.6 times better than at 6 a.m. With humans the excretion of the griseofulvin metabolite, 6-demethylgriseofulvin, is doubled at noon, compared to 8 p.m. (43). In the case of sulfisoxazole the elimination rate increases 150 % between sleeping and waking, while the elimination of sulfadiazine is increased by 190 % (45). Variations of at least 100 % are quite common.

Important factors of the circadian rhythm are living habits such as sleeping-waking cycles, timing of food-intake, climatic factors and light and temperature variations. There are even morning and evening types with a biorhythm which is shifted time-wise (Fig. 7) (46).

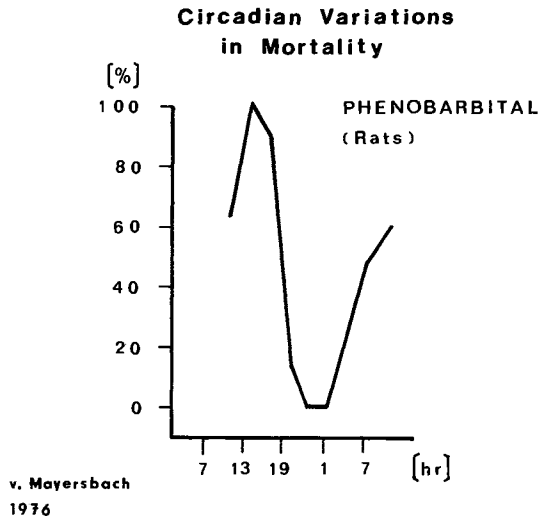

Fig. 6

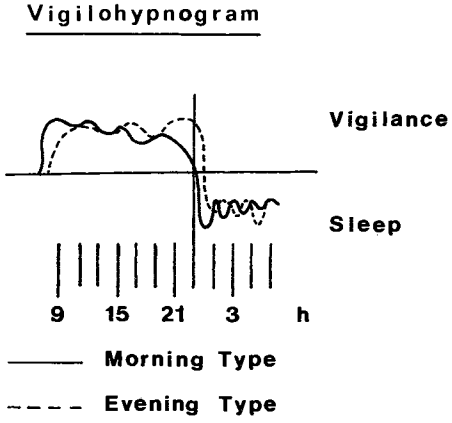

Fig. 7

Best known is the circadian variation in body temperature (47, 48). The strength and the time-course of the rhythm varies with the different body regions (Fig. 8). The biological morning (ca. 3 a.m. to 3 p.m.) evinces increases in oral and rectal temperature, whereas in the biological afternoon and evening loss of warmth occurs. Moreover, a difference can be demonstrated even between the sides of the feet (49).

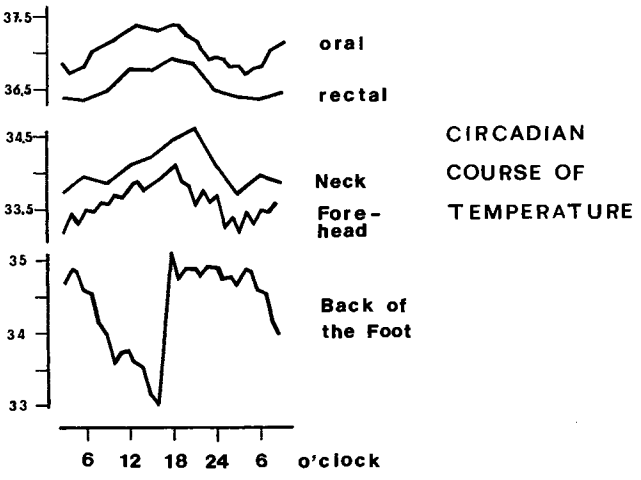

Fig. 8

Direct consequences for pharmacokinetics arise from this oscillation. Variable parameters include solubility, pK_a-value, partition coefficient, diffusion, disintegration of solid dosage-forms, stability, metabolism and protein binding. At the same time various physical functions are altered: increasing temperature diminishes the gastric acidity, the time of passage of food through the gut is shortened, but bile secretion is increased (50). Targeted hyperthermy and hypothermy have been used successfully in therapy (51).

Since melting and drug release of the suppository bases depend on body temperature, the biorhythm has a definite role during rectal application. A temperature difference of 2 K can already be significant, for example, when aging has caused changes in the suppositories (Fig. 9) (52).

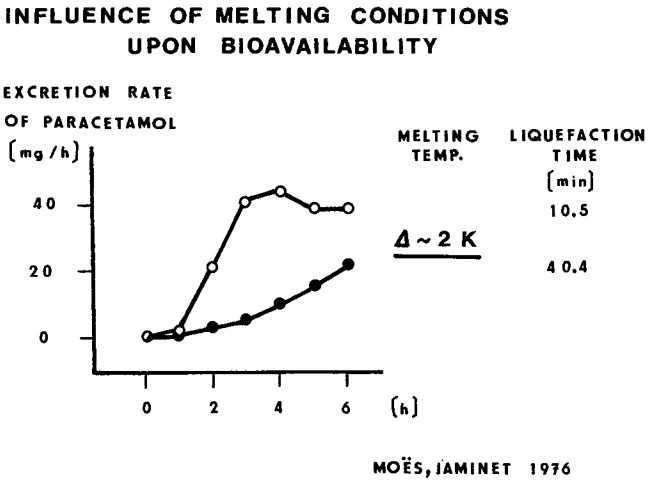

Fig. 9

Consequently, the alterations in external melting conditions as well as shifts in environmental temperature produce clear variations in bioavailability. The consideration of biorhythms in drug delivery has been named chronogalenics (53, 54). In the case of suppositories, reproducible and time-dependent bioavailability can be obtained using chronogalenic technology, adjusting the melting characteristics of the dosage form to the body temperature (Fig.10).

If the circadian rhythm of body temperature is not considered, as is usual, then the bioavailability can vary by a factor of 2.

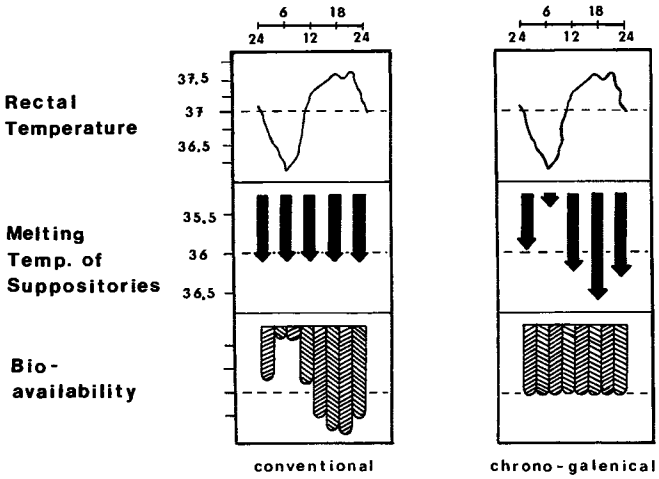

Fig. 10

Finally, a circadian biorhythm of cell division has been shown to exist (38, 55). If healthy cells are compared with neoplastic transformed cells, the stronger periodicity of cancer cells is clearly recognizable. The differential periodicity increases further with the increasing age of the cancer organ (Fig. 11) (56).

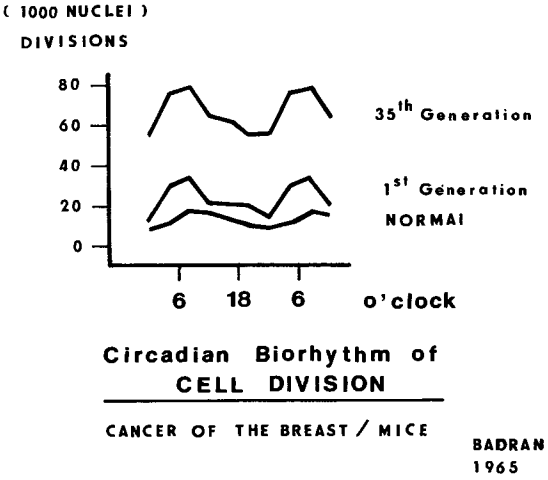

Fig. 11

Since dividing cells are exceptionally sensitive, the above observation is of importance to chemotherapy of cancer and for the "timing" of the medication. Variations of the *in-vivo* behavior in the organism, and hence, the efficacy of a pharmaceutical preparation could be caused by the rhythm of this division. Moreover the activity of cell membranes is directly connected to the endocytotic uptake of certain drug carrier systems, and therewith to the effect of lysosomotropic dosage forms. The efficacy of organ specific and target-orientated preparations such as liposomes, nanoparticles and DNS-complexes, would therefore be governed by the biorhythm of the cell.

Nutrition

In the area of nutrition qualitative and quantitative aspects are encountered. The range and frequency of food intake are in a sense personal habit and *inclination* but are also ethnologically determined. Considerations include the differences between main meals, as well as seasonal variations, and sporadic specialties. Not only the composition of the nutrition is important for bioavailability but also the time interval between consumption and drug administration. Extensive experimental data have given rise to numerous reviews that differentiate between drugs of which the absorption is reduced, delayed, increased or not influenced by food (57), or drugs that are to be taken with water on an empty stomach, with a meal, or where a particular component like alcohol should be avoided (58). By themselves the food-induced interactions merely cast doubt on an *in vitro-in vivo* correlation. Pharmacotherapy is linked in only a few cases to an international standard diet. There are no rational measures that would account for the extent of this variability.

The importance of nutrition to drug interactions is illustrated by several drug studies (59). The plasma concentration-time curve of isoniazid can be reduced to 50 % by food intake, while moderate alcohol consumption can reduce the bioavaila-

bility by 96%. In the case of propranolol, however, a 60% higher AUC-value results after breakfast than on an empty stomach. Hence, a variability factor of 2 is no exception.

An important aspect of gastro-intestinal drug absorption is the passage time. This is dependent on the type of nutrition: the half-life of gastric emptying is 15 minutes for water, 60 minutes for sugar solutions, and 100 to 120 minutes for solid food (59). The plasma levels correlate to transit time when drug is absorbed in the stomach, as is observed with paracetamol. Emptying is accelerated with an empty stomach, by warm or by fat-poor meals, by substances of low viscosity such as liquids, by lying on the right side, or by a high body activity. The opposite conditions of body and food cause the contrary results. Similar influences affect the intestinal passage time, and pathological changes such as diarrhoea and constipation may cause differences of a factor of 10.

Non-Medical Use of Drugs

The non-medical use of drugs forms a part of life style. Their consumption corresponds to the habits of individuals or to whole population groups. The most frequently misused agent is alcohol. As already mentioned a moderate alcohol intake can decrease the bioavailability of isoniazid to 4% of control. Numerous drugs show additive effects with alcohol, and excretion can be limited through alcohol consumption. With the already cited example of theophylline clearance, alcohol consumption (in this case in asocial quantities) reduces the elimination of the drug to half the level of moderate drinkers (Fig. 12) (22).

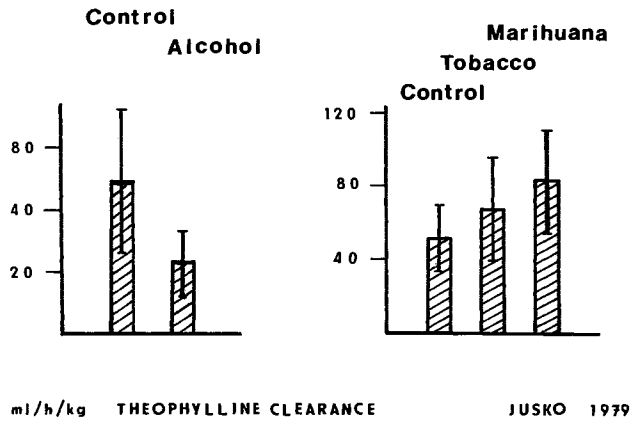

Fig. 12

The clearance values for abstainers is 65 ml/h/kg, for social drinkers 58.3 ml/h/kg and for heavy drinkers 22 ml/h/kg. In the extreme case a variability factor of 2.9 occurs.

In second place comes the consumption of *tobacco*. With smoking the theophylline clearance is increased (Fig. 12) (22). With non-smokers the clearance value is 57 ml/h/kg and for light smokers 61 ml/h/kg. An increase in clearance also occurs with caffeine, antipyrine, lidocaine, propranolol, imipramine, phenacetin and pentazocine. There is also a clear age-dependence; under the influence of smoking clearance decreases with increasing age. The clearance of propranolol in smokers, as compared to non-smokers, between the age of 21 and 37 varies by a factor of 2.7, while between the ages of 46 and 73 the variability factor is only 1.3 (63).

An increase in clearance also follows consumption of narcotics such as marihuana. Again citing studies on theophylline clearance, the ingestion of marihuana increases the clearance from 56 to 82 ml/h/kg (Fig.12) (22). Hence the influence of drug abuse on pharmacokinetics cannot be neglected.

Therapeutic Drug Use

Therapeutic drugs in most cases also are xenogenic substances that are regularly applied. It involves an inter-individual factor if a chronic illness such as diabetes or high blood pressure necessitates continuous medication, or if there is habitual use of laxatives, contraceptives or analgesics. The number of substances that have been reported to interact therapeutically or pharmacokinetically is rather large (64). Simplified charts are available to rapidly inform physicians and pharmacists on drug interactions in the prescribing of the most frequent combinations (65).

Rather typical for long-term therapy with the potential for drug-drug interaction is the peroral administration of contraceptive steroid hormones (Fig. 4). Theophylline clearance is decreased from 58 to 50 ml/h/kg so that the drug plasma levels increase by approximately 15% (22). In contrast, during pregnancy clearance increases by approximately 7% (22).

Especially noteworthy is the large number of interactions where the metabolism of one drug is induced or inhibited by a second drug (66). Presently, more than 200 substances are known that can activate the microsomal enzyme system. Less numerous are the drugs that inhibit the metabolism of other drugs after therapeutic dosages. As with enzymopathies this can be an important factor. For example testosterone increases the enzymatic degradation of hexobarbital in rats four-fold, thereby shortening the duration of drug induced sleep (67). For this reason hexobarbital should be more potent in the female organism.

Impairment of drug effects may be due to changes in absorption. Through stimulation of gut motility, changes in bile secretion, variation of pH and damage of the mucosa through toxins, variations can occur that often remain unnoticed and in general cannot be simulated *in vitro*. Drug-drug interactions may also involve distribution by competing for binding to transport proteins. Effects can also occur through competition at the receptor site, or the site of excretion, e. g. the renal transport systems.

All of these effects differ not only with the type of drug, the interfering substance, dosage, administration intervals and methods of administration, but they also vary with the immediate disposition of the patient. Therefore, it would appear impossible to find a reasonable "normal value" as a datum point drawn from an *"average situation"*. When another drug is simultaneously administered, the biosystem reacts, not infrequently shifting half-life and other pharmacokinetic parameters by 100% and more.

Physical Activity

It is well known that physical exercises promote enzymatic processes and thus reduces the lipid blood level (69). Physical activity also clearly increases the bioavailability of drugs. The AUC can double (Fig. 13) (70).

A correlation between physical exercise and pharmacokinetics has been demonstrated with different drugs. Hence pharmacokinetic studies may be affected by the occupation of the subject and the treatment on an in-patient or out-patient basis.

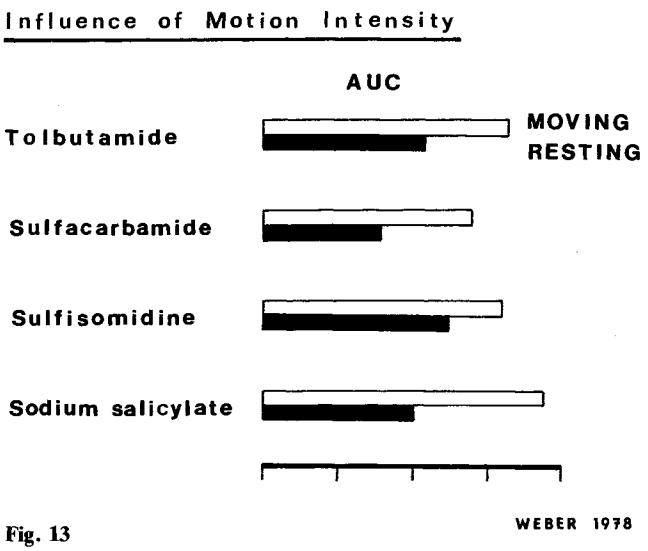

Fig. 13

Personal Hygiene

More clearly demonstrable as a factor of life style is personal hygiene which is determined by personal upbringing, personality traits, religion and public awareness. Further factors are the level of civilization and technological development, climatic conditions and social milieu. Primarily affected are the actions and reactions of the skin with consequences for topical preparations. The condition of the skin alters with washing, the cleaning method plays an essential role, and the original condition is regenerated gradually (Fig. 14) (71).

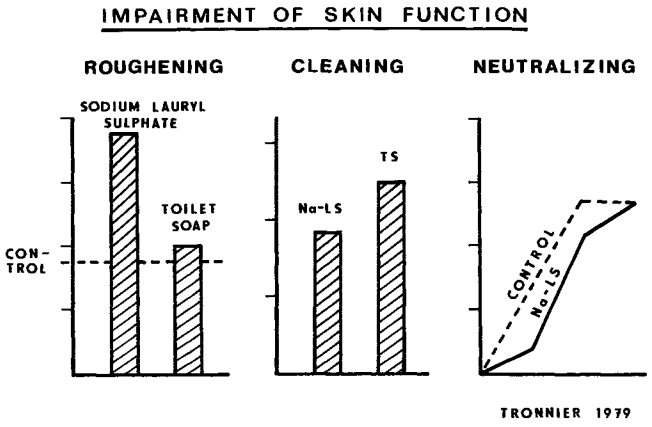

Fig. 14

The influence of the choice of the cleaning agent can serve as an example. While toilet soap cleans better than a 2% surfactant solution (sodium lauryl sulfate), the rawness of the skin is increased more by the surfactant. This deleterious effect can be likened to the effect of aging of the skin ("artificial aging"). In old age the skin possesses a decreased ability to regenerate, and the lipid mantle decreases and deteriorates which is similar to the effect of washing.

The influence of surfactants on percutaneous absorption (72), and altered permeability of transdermal therapeutic systems (73) can be demonstrated with cleaning agents. The surfactant-treated skin shows increased permeability (Fig. 15) and, thus, drug absorption.

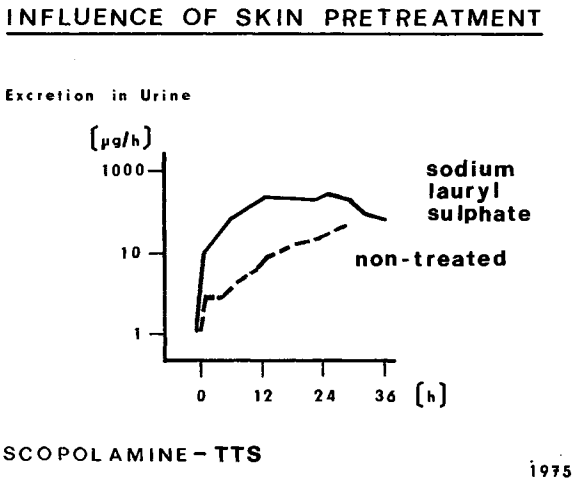

Fig. 15

The frequency and kind of personal hygiene and the preferred cosmetics represent contributory factors of dermal and transdermal drug application.

Exogenous Factors

Periodic Factors

Climatic factors can be regarded as exogenous factors that determine the bioclimate of humans (74, 75). Sensitivity to changes in the weather has long been recognized, i.e. a reaction to short or seasonally-induced alterations in the environment. Day-length and air temperature vary throughout the course of a year (Fig. 16) (75) (Middle European latitude).

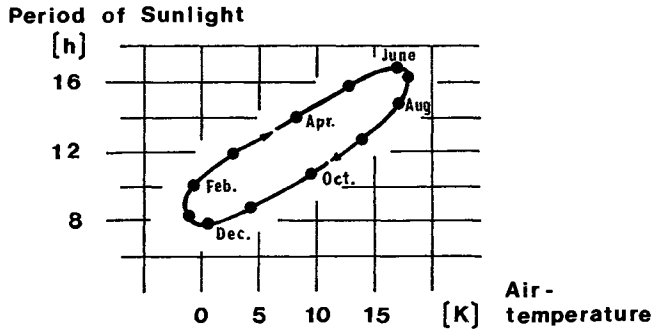

Fig. 16

A relationship between time of the year and frequency of certain diseases is demonstrable. The disease profile can worsen with changes in atmospheric conditions. For example the course of a fatal illness is dependent on the time of the year (Fig. 17) (75).

In the case of influenza, mortality occurs frequently with the sudden change of weather in April, while during the months from May to September a worsening of the weather exercises no influence on the death rate. The influence of the weather leads to a variation of ± 50%. The greater suspectibility to weather changes does not persist generally but occurs only in certain months. With heart and circulatory diseases the mortality with bad weather in May and October is especially high and lies 30–40% above the average. In contrast, good weather

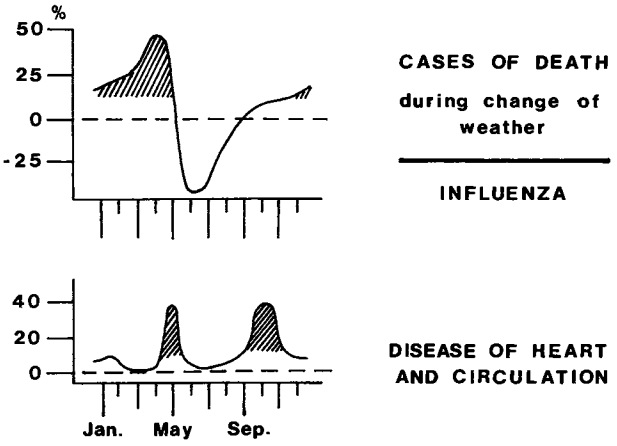

Fig. 17

decreases the mortality rate below the average value. This implies that sensitivity to drugs could also show annual periodicity.

Non-periodic factors

Air Temperature

Temperature influences bioavailability and actions in many respects. It alters the rate as well as the vigor of transport and reactions. Certainly, each process is differently sensitive to temperature. For chemical reactions a temperature difference of 10°C can increase or decrease the rate by a factor of 2 to 3 (van't Hoff's rule), while the physico-chemical processes involved in absorption, such as diffusion and osmosis, vary by factors of only 1.1 to 1.5. These variations can be used therapeutically with hypo- or hyper-thermia. Therapeutic local cooling retards the dissipation of a local anaesthetic or limits the absorption of a toxin. Moreover, it stops the diffusion into the local blood circulation and from there into the tissues and into individual cells. A similar effect can also result from a drastic change in environmental temperature. In experiments with rats and mice, methadone distribution after subcutaneous injection at 18°C shows a 1.4 fold delay in comparison with administration at 29°C (76). Differences in absorption of this magnitude (40 %) are also possible with humans. With the temperature differentials of the seasons much greater temperature differences from −10° to +30°C are readily realized.

The influence of temperature on the intensity of action can cause altered toxicity. However, the temperature dependence of drug action is surprisingly varied. Cortisone, ephedrine, methadone and reserpine possess increased toxicity with increased temperature, while procaine, caffeine, and pentetrazol have an induction period that prevents temperature to affect the pharmacokinetics; with chlorpromazine, atropine and digitalis glycosides a temperature of minimal toxicity exists, while either warming or cooling cause increased toxicity (77).

Some specific measurements of the half-lives of drug elimination document the temperature dependence. In perfusion experiments in rabbits, the following half-life values occur (50):

	24°C	37°C
Morpine	94 min	3.7 min
Thiopental	185–530 min	46 min

With a 13 K variation in temperature the pharmacokinetic data vary 4- to 25-fold, probably as a result of accelerated metabolism. Because of *homoio*thermic control this variability is much smaller in humans, but fever or an increase in temperature because of strenuous activity elicit a marked effect. The air temperature is also involved in this chain of events.

Finally, there is an apparently paradoxical situation in the case of excretion. With differently acclimatized rats the glucuronide excretion is temperature dependent. Under cold conditions more glucuronide is excreted than normal; apparently, the kinetics of the conjugation mechanisms are promoted by cooling (Fig. 18) (78). Therefore, predictions on the effects of temperature must be treated with restraint.

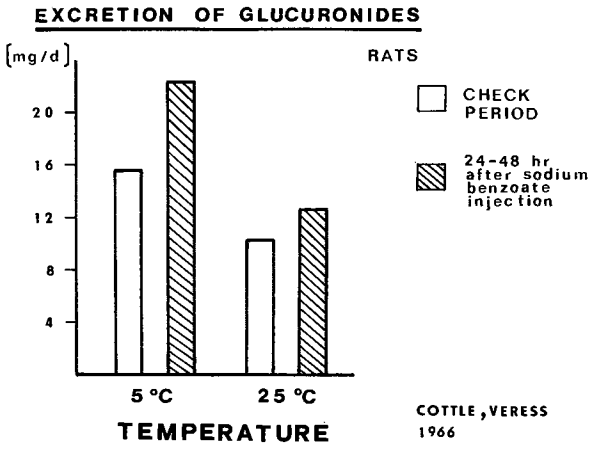

Fig. 18

Changes in temperature shift numerous physiological functions, that affect drug blood levels. For instance, a decrease in stomach passage time occurs with higher and lower temperatures (i.e. hot and cold meals) (50). The variation involves 45 minutes (59), which can constitute 25 % of the emptying time and drug absorption.

For the maintenance of body temperature the skin plays a fundamental role, with variations in air temperature directly affecting skin function. The circulation in the skin is regulated according to air temperature, with ratios in the total body of 1:7, in the hand of 1:30 and in the fingers of 1:600 (79). Simultaneously, the oxygen concentration in blood varies along with changes of the volume (Fig. 19) (80, 81).

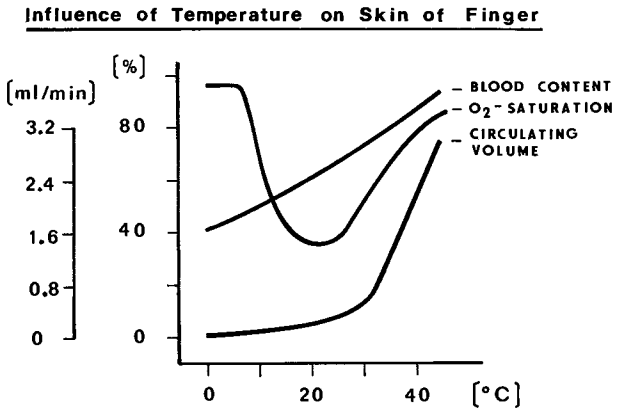

Fig. 19

The dynamics of the blood vessels are pharmaceutically relevant for percutaneous absorption. In ape studies, all the animals could endure a certain amount of sarin intoxication at 25°C, but at 38°C only 28 % of the apes survived (5).

Humidity

Among the relevant bioclimatic factors, air humidity affects drug activity, which is one of the reasons for requiring air-conditioned surroundings for reproducible animal experiments (GLP). For the daily environment of humans such requirements are not realizable.

High humidity tends to congest body temperature and diminishes endurance. For example, the LD_{50} of nicotine bitartrate in rats is 83 mg/kg above 80 % relative humidity, and 94 mg/kg under 60 % r.h. (82). Humid conditions induce stronger drug action.

Atmospheric Pressure

Pressure differences occur according to the altitude of the normal residence, or to exceptional stress such as mountain climbing or air travel. The oxygen partial pressure changes along with overall pressure change.

A clear pressure effect was demonstrated (Fig. 20) (83) in an experiment with methionine sulfoxime, in which the increase of spasms in mice was chosen as the parameter. In this case the same level of effect occurred at relative dosages of 4.2:1.4:1 at 760, 364 and 280 mm Hg, respectively. Hence, the influence on drug effects can be considerable. Probably, weather-related pressure changes suffice to elicit changes in the biosystem. One assumes that through pressure the circulation, enzyme activity, membrane permeability and other parameters may be influenced.

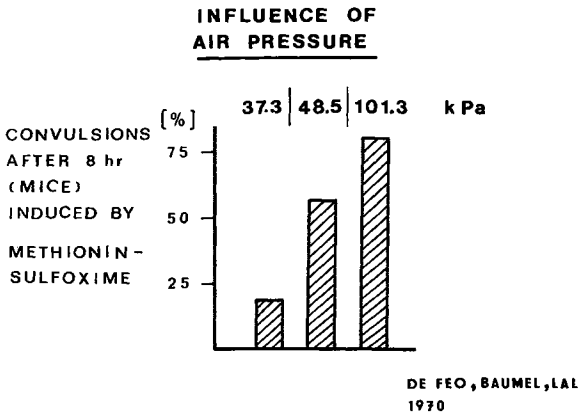

Fig. 20

Conclusions

Considering the relatively large number and the importance that endogenous and exogenous factors possess for absorption, transport, transformation, action and elimination of drugs, it is surprising to notice the almost "careless" interpretation of pharmacokinetic data, its generalization, and the strenuous attempts at *in vitro* simulation. Synchronization of results from different laboratories, research groups and various individuals does not prove feasible. In view of the many sources of variability and their possible summation, the occurrence of an *in vitro* and *in vivo* correlation can be seen as an unrealistic problem.

Apparent proofs of the existence of such connections are fortuitous and are mostly induced through the limitations of the experiment. Furthermore, the apparatus or the calculations are often adapted to specified groups of people and in a specified milieu. Even the scatter of the correlation coefficient with constant *in vitro* methodology is hardly investigated, and much less the variation of factors between and within individuals. On the other hand, the statistical investigation of large populations possesses little meaning for individual subjects whose drug reactivity can vary by orders of magnitude from the avarage value.

The ability to correlate *in vitro* and *in vivo* data assumes the ability to determine both the individual characteristics and their invariability, an assumption that is at best equivocal.

For biopharmacy, or more correctly (84), for "biopharmaceutics" (85) or "biogalenics" (86), the necessity arises to separately consider and evaluate *in vitro* and *in vivo* investigations. Both are indispensable for drug research, and importantly, one cannot be substituted for the other. *In vitro* methods are relevant in the development of pharmaceutical procedures and optimization of dosage forms, while the *in vivo* model is irrenouncable to obtain qualitative estimates on the behavior of a medication in a living organism. On the one hand, it is therefore rather unimportant which apparatus with what limits is favored for *in vitro* measurements; the reproducibility of the results must have only limited latitude and possibly conform to some internationally determined standard. On the other hand, *in vivo* results must be separately obtained in each case, and in essence only those assertions are acceptable which include a comprehensive description of factors such as age, weight, height, time of day, nutrition, mobility, medical treatment and current climate.

These problems have long been recognized and are addressed in early articles, e.g. "Meaning and Limits of *in vivo* Experiments" (87). The inter- and intra-subject variations in drug absorption kinetics were shown to be considerable (88). Recent results on intra-subject variation with digoxin absorption demonstrate that these variations are not only important, but depend on the nature of the dosage form (89). Fig. 21 shows a comparison of results from two series of experiments with twelve subjects. The tablet produces a much wider scatter of bioavailability data than the soft gelatine capsule. The inter-individual variation supports our initial contention that *in vitro-in vivo* results cannot be correlated in meaningful fashion.

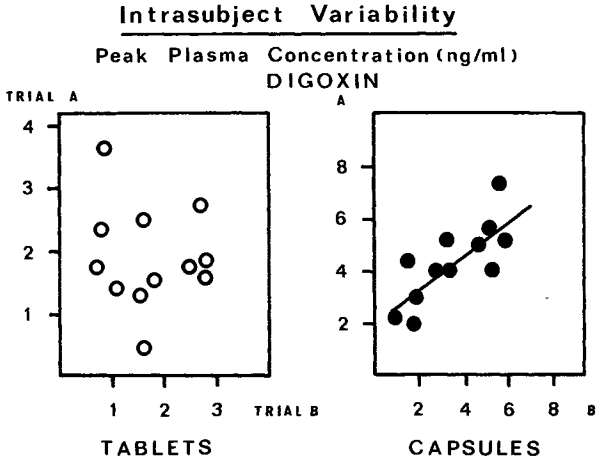

Fig. 21

On the basis of this "unsatisfactory" situation, proponents of the *in vitro-in vivo* connection have recently sought to maintain their concept through correction factors for the calculations or in circumscription of the statements. Notwithstanding, the discussion and the doubt remain (90). Levy correctly asserts that *in vitro* tests for the dissolution rate "render possible deliberations on absorption rate" (91). Much more than deliberations they are not, readily leading to erroneous conclusions.

The most consequential conclusion is to renounce generalized pharmacokinetic data like half-life and to orient drug therapy to individualized pharmacokinetics. With this procedure the dosage of highly active pharmacological agents such as cytotoxics can be determined by the concurrently determined individual pharmacokinetic values (92).

With the discussion of the different factors at work, a more wide-ranging complexity must be considered. Pharmacodynamic effects are determined not only by the various factors already discussed but also by the quality of the drug formulation product that is the responsibility of the manufacturer, by the judgement of the physician, and by the reaction of the patient. The therapeutic outcome is the product of these three participants (Table III).

Table III. Spheres of Influence and Responsibility in Pharmacotherapeutics

Manufacturer (Pharmacist)	Physician	Patient
Applicability Tolerance Accuracy of dosage Bioavailability Stability	Nature of drug Dose Way of application Timing of application Interactions	Disposition Conditions of life Environment Habits Compliance

Pharmacist

Communication, Information, Distribution, Cooperation, Control, Marketing

The manufacturer fulfills on the average 80 ± 10 % of his task, the physician achieves approximately 75 % of optimal diagnosis and prescribing (93, 94), and the patient realizes only 50 % of his potential benefit from drug therapy usually because of insufficient compliance (in the highly favorable case of digitalis glycosides this loss in benefit may be only 7 %). Therefore, pharmacotherapy on average realizes the following fraction of its full potential:

$$0.8 \times 0.75 \times 0.50 = 0.3,$$

and it is this remaining 30 % that affects the patients' condition.

New factors are constantly discovered. If for example the peroral dose of prednisolone is increased from 0.13 to 0.27 mg/kg, the bioavailability (measured as AUC) decreases from 1295 to 1008 mg/ml × h and the clearance increases from 0.10 to 0.13 l/kg × h (95). Similarly the blood profile of intramuscular injections varies with injection volume as well as with concentration. One cannot speak of a characteristic biological half-life.

Enormous uncertainty stems from patient compliance. It would lead too far at this point to expand on this very extensive area and the motivations of this behavior (96, 97), although in real life it represents a decisive factor. Compliance problems occur very early in therapy. A statistical study revealed that 20 % of the patients still had not redeemed their prescriptions after one month (98). A sarcastic interpretation would be that non-compliance is a reasonable and defensible stance of the patient against some prescriptions (99). Non-compliance possesses not only therapeutic but also economic consequences. It is estimated that annually in this fashion a drug volume with the value of US $ 30 billion is wasted worldwide (96).

It is conceivable that there is a single fictional standard person with exactly average characteristics and predispositions with a normalized life and environmental conditions, for whom a simulation methodology could be justified. But in reality this type of idealized situation is not encountered. The deviation from the accepted norm is normal, with the norm condition remaining a postulate. There is such a wide spectrum of variation that the *in vitro* model cannot with a "clear conscience" predict a specific therapeutic outcome. In science, the notion of "hope and belief" for any *in vitro-in vivo* correlation must sooner or later be abandoned.

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