

SECTION II

CONTINUED

DR. CARNEVALE: We're going to continue this afternoon with a wrap-up of today's sessions. We'll have two speakers on the agenda this afternoon with a discussion period following which will include questions and answers for the speakers you heard a few minutes ago and the two speakers coming up now.

Our first speaker on the agenda needs little introduction. He comes to us from The Ohio State School of Veterinary Medicine where he's Professor and Chairman of the Pharmacology and Physiology Department. Dr. Tom Powers is also the immediate past president of the AAVPT. He's also, of course, the Chairman of the Planning Committee for this Symposium as he was with the meeting we had at Ohio State. I think what Tom's going to try to do today is tie in everything you've heard during today's session and kind of wrap it up in a package. This morning he told me that if you didn't hear anything today, you ought to listen to him because he's going to give you all the answers to the questions that were posed. Without further ado...Tom Powers.

DR. T. POWERS: Thank you, Dick.

Some Scientific Bases for Extrapolation

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INTRODUCTION

The Webster's New International dictionary defines extrapolation as "To project by inference into an unexplored situation from observations in an explored field, on the assumption of continuity or correspondence."

In most of the investigations which employ animal species, there is a tacit assumption that the data from one species may somehow be extrapolated to other species. Unfortunately, such methodologies to extrapolate quantitative drug metabolism from one species to another have been elusive so far. Some factors pertaining to interspecies variation in drug metabolism and excretion are reported in the literature.¹⁻⁵

Since we are trying to actually go from a basis or foundation of data, facts and/or premises to a conclusion or an inference, it is probably better termed "The use of scientific reasoning and logic in the determination of individual dosage schedules by extrapolation." These bases could be used to extrapolate the dose within the same species or from one species to another. Dose titration or dose bracketing studies as they are presently done may involve groups of from 6 to any larger number of individual animals in at least three treatment groups. An extrapolation from this small data base alone will probably lead to the wrong conclusion in most cases. One would expect a better extrapolation to occur when one uses all the scientific knowledge available at the time, such as, pharmacokinetic data, pharmacodynamic data, variability of the disease condition of the animal, etc. That is to say that the proper dose must be tailored for the individual animal by using scientific reasoning and logic and employing the total data base available.

This paper will discuss the important factors needed to make up this larger data base, how they are related to host and infectious organisms and their effect on the outcome of treatment with an antimicrobial agent.

HOST-PARASITE RELATIONSHIP

In case of infectious diseases, there exists a complex relationship among the host, the microorganism and the therapeutic agent which can be depicted as the chemotherapeutic triangle (Fig. 1).

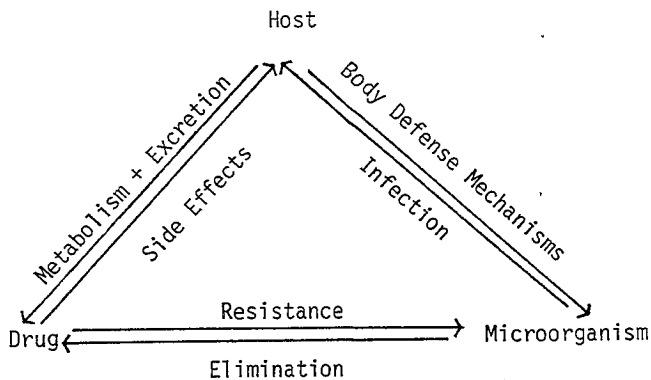


Fig. 1--The chemotherapeutic triangle

When one gives an antimicrobial drug to the host, it may produce an action (inhibitory, bacteriostatic, bactericidal) against the micro-organism and may also effect the host--directly or indirectly--to produce an untoward effect. Direct untoward (or side) effects include nephrotoxicity or hepatotoxicity, whereas indirect side effects include alteration of intestinal or cutaneous flora. Either of these effects may result in a more dire outcome than would have been produced by the pathogen for which the animal is being treated.

The host eliminates the drug through metabolism and/or by excretion. At the same time, the host is responding to the invasion of the microorganisms and the various body defense mechanisms are attempting to rid the animal of infection. It is our desire that the antibacterial agent produce its desired effect without interfering with the host defense mechanisms.

The therapeutic outcome or drug response depends not only upon microorganisms involved, host defense mechanisms and proper administration of effective drugs but also upon proper supportive therapy and maintenance of an environment not conducive to reinfection.

EVALUATION OF DRUG EFFICACY STUDIES

The major factors, which confound the evaluation of drug efficacy studies are given in Table 1.

TABLE 1--Major Factors Which Confound the Evaluation of Drug Efficacy Studies

- a. Underlying disease
 - b. Dose
 - c. Achievement of drug levels at infection site
 - d. Mode of administration
 - e. Host resistance factors
 - f. Use of combinations in severe or mixed infections (synergism or additive)
 - g. Drug resistance
 - h. Adjunctive therapy
 - i. Site of infection
 - J. Toxicity of drug
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The underlying disease may have several rates of progression and many degrees of severity. In addition, it may be acute or chronic. The etiological agent may have different susceptibility patterns in vivo than in vitro. For example, in our model studies we found Streptococcus zooepidemicus to be extremely sensitive to gentamicin in vitro but the drug was essentially ineffective in vivo.⁶ Another way to look at this problem is that gentamicin was more active in the aerobic environment (in vitro) than in the microaerophilic environment (in vivo). On the other hand, a drug may prove effective in vivo whereas in vitro it produces little or no response.

The dose chosen should be effective in alleviating the signs of disease. The values of sensitivity testing for the organism cannot be over-emphasized, although as mentioned earlier it has some limitations. One should determine the actual minimum inhibitory concentration in vitro. When the organism shows only moderate resistance, we usually can elevate the dose and reach levels adequate for successful therapy. By elevating the dose we are hoping to attain a higher level at the site of infection. As one increases the concentration of the antibiotic, one also broadens its spectrum of activity at the site of the infection.

The achievement of minimum activity concentration of drug at the site of infection is very important and depends not only on dose, but also on many

other factors such as route of administration (intravenous vs oral route), protein binding of the drug, etc.

The mode of administration could be continuous, intermittent or pulse dosing. By pulse dosing we are referring to intermittent therapy in which we have a peak and trough levels and the trough falls below the minimum inhibitory concentration (MIC) for some period of time. In pulse dosing we hope to take advantage of post antibiotic effect (PAE)⁷ as well as post antibiotic leucocyte enhancement (PALE)⁸ when the levels fall below the MIC of the organisms.

Inhibitors of protein synthesis (tetracyclines, chloramphenicol, aminoglycosides) have been shown to produce the longest suppression of growth (PAE) after the drug had been removed from the growth medium. This was of comparable duration for both gram-positive and gram-negative bacteria.⁷ Beta lactam antibiotics showed PAE mainly with gram positive organisms, unless extremely high levels were used. Chloramphenicol showed the PAE against a wider range of organisms. It produced a 3 hour PAE against E. coli following exposure to eight times the MIC. These in vitro studies proved that duration of the post antibiotic effect was related linearly to concentrations of the drug and exposure up to a point of maximal response. The PAE, however, was different for different organisms exposed to the same antibiotic.

McDonald et al, 1981⁸ found that exposure of Escherichia coli and Staphylococcus aureus to high levels of certain antibiotics for short periods increased the susceptibility of the organisms to the antimicrobial action of normal human leucocytes. This susceptibility varied with the antibiotic used and was most pronounced when E.coli was exposed to chloramphenicol. Bacteria exposed for one minute to chloramphenicol were sensitized to the post antibiotic leucocyte enhancement effect (PALE). The authors suggested that PALE operates through increased susceptibility of antibiotic damaged bacteria to intracellular killing mechanisms of leucocytes (probably due to perturbations of the cell wall). In addition, there is a marked reduction in the growth phase that lasts for hours. Both antibody and complement were shown to be necessary for maximum PALE.

The host defense mechanisms can play a dominant role in the outcome of the infection. A bactericidal drug given by continuous infusion rather than pulse dosing, may be more useful in an immunocompromised patient than a bacteriostatic drug. The use of combination therapy for severe or mixed infections is sometimes recommended to treat the multiple bacteria present. The synergistic combination of trimethoprim and sulfonamide has been a welcome combination in recent years.

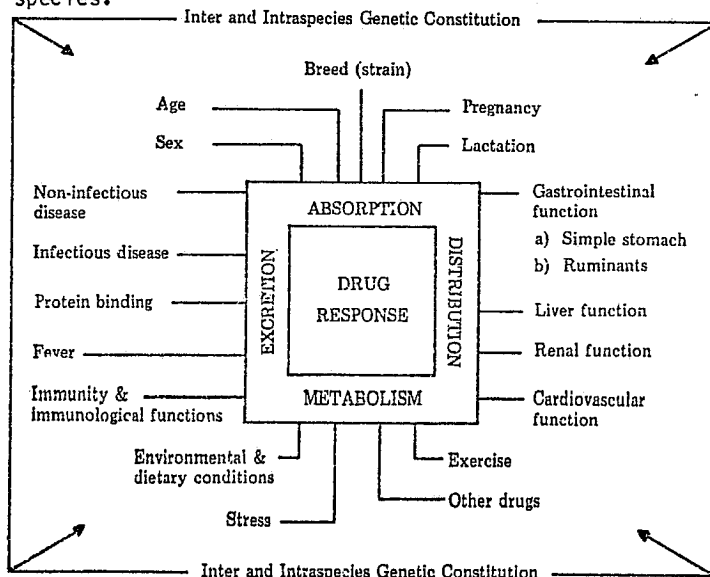
The development of resistance by a microorganism can and does occur sometimes during therapy. One must continually reevaluate the case for this possibility. There appears to be an inverse relationship between the MIC and overall clinical response. That is the higher the MIC, the poorer the response and the lower the MIC, generally the better the response.

The adjunctive therapy of fluids, etc. should not be overlooked. The use of some adjunctive therapy may assist in the drugs efficacy; whereas, others may hinder the drug's effect. The site of the infection can markedly influence the dosage required. Since many antimicrobial agents are concentrated in the urine against a particular pathogen, one may be able to use a lower and less frequent dose than if the pathogen were in the blood or in a "deep seated" tissue. Often, however, the type of pathogen involved in urinary tract infection requires large and frequent dosages due to their sensitivity pattern.

As doses are increased, the potential for a toxic reaction must be considered. For drugs like penicillins, which have a wide margin of safety, the dose can usually be increased greatly without causing complications, whereas, for aminoglycosides, which are potentially nephrotoxic, the range of safe dose levels is rather narrow.

FACTORS INFLUENCING DRUG RESPONSE AND DOSAGE

The complexities of factors that influence drug response and dosage are emphasized in the following diagram (modified from Vesell, 1982⁹). The response one obtains is first dependent upon the biological phenomena of absorption, distribution, metabolism, and excretion. These biological phenomena are known to be under the influence of a variety of factors which are depicted by the lines leading out from the second square. Then controlling these factors is the genetic constitution which varies from individual to individual, breed to breed, strain to strain and from species to species.



FACTORS INFLUENCING DRUG RESPONSE AND DOSAGE
(Modified from Vesell, 1982)

The drug response often varies between the neonate, adult and the geriatric patient thus causing a need to adjust the dose for optimum effects without toxicity, e.g., the biological half-life ($t_{1/2}$) of sulfadimidine in calves was found to 13.5-17.5 hr at 1 day of age, falling rapidly to 4-6 hr within 3 weeks, and did not change markedly thereafter (Nouws, et al, 1983).¹⁰ Similarly, the $t_{1/2}$ of chloramphenicol in 1 day old calves was 3-fold longer as compared to 10-12 week old animals (Reiche et al, 1980).¹¹ These responses can be related to the fact that renal and hepatic function are not mature at birth but do reach adult levels very rapidly. However, for chloramphenicol, the pharmacokinetic parameters in the neonate and adult horse were quite similar (Table 2).

TABLE 2--Chloramphenicol Pharmacokinetics in Horse

	Kinetic parameter			References
	$t_{1/2}$ min	$V_{d_{area}}$ L/kg	Cl_B ml/kg/min	
Neonate (1-10 days)	31-114	0.86-2.63	11.74-22.99	12
Adult	21-81	0.92-2.26	11.81-32.21	13

There is wide intraspecies variation and by the first week the hepatic, metabolic and kidney functions may have reached near adult levels, thus explaining the constancy of the pharmacokinetic parameters. For oxytetracycline the total body clearance (Cl_B) and volume of distribution ($V_{d_{area}}$) in 3 week old calves have been reported to be 2 to 3-fold greater than corresponding values in cows.¹⁴ This can be correlated with a decrease in extracellular and total body water as well as a progressive increase in gastrointestinal tract volume and weight (especially the rumen) with age in cattle. Gender of the animals can also influence drug response.

In the later stages of pregnancy the volume of distribution may increase due to increased fluid volumes thereby requiring a higher dose to maintain similar blood levels. In women, slower absorption of aspirin was noted as well as lower peak levels attained.

Male rats showed more severe nephrotoxic reaction to a given aminoglycoside than female rats. It has been shown that testosterone augments the number of lysosomal/vacuolar systems of the proximal tubules. It is now believed that the filtered aminoglycoside enters the proximal tubular cell by pinocytosis and subsequently becomes sequestered within these lysosomes. On the luminal membrane are polyphosphoinositides which act as binding sites. These lipids are also found in high content in brain, kidney and inner ear. None have been isolated from bacteria.

Various environmental and dietary conditions which can affect the drug response are given in Table 3.

TABLE 3--Environmental and Dietary Conditions

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- a. Circadian and seasonal variation
 - b. Starvation
 - c. Intensive farming methods
 - d. Sanitary conditions
 - e. Temperature, humidity, light, ventilation
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Stress and exercise are other factors which can influence drug response depending upon the type of drug used. An animal that is sedentary tends to eliminate more metabolites and less parent drug. Strenuous exercise may make urine less alkaline in horses and increase the ion trapping of base drugs which results in a higher elimination rate. In actuality the pH values of urine samples taken from horses at the race track have been shown to vary markedly.

The effect of multiple drug use is very important. It could lead to beneficial effects, i.e., synergism or even additive effects or on the other hand antagonistic effects. Some drugs may be antagonistic in vitro but may act synergistic in vivo, e.g., aminoglycosides inactivate certain penicillins in vitro; whereas, in vivo the penicillin acts on the bacterial cell wall and enhances the penetration of aminoglycosides.

Noninfectious diseases of kidney or liver can also affect the metabolism and excretion of drugs by the body, thereby altering the outcome of treatment. For example, an animal having chronic interstitial nephritis may show marked decrease in renal function, thus requiring lower dosages especially of such drugs as the aminoglycosides.

The drug response also depends upon the type of infectious disease as discussed earlier. Fever is another factor influencing the drug response. Varma et al 1983¹⁵ have related fever to the serum iron levels in horses infected with Streptococcus zooepidemicus. Soon after infection the serum iron levels dropped significantly with resulting rise in temperature. After starting treatment with Penicillin G the iron levels started to return to normal as did the temperatures. The horses getting the lower dose relapsed after cessation of therapy, thereby again showing a decrease in serum iron levels and an increase in temperature. The horses which relapsed showed a decrease in serum iron levels at least 6-12 hrs before the temperature showed a rise. The reduction in serum iron

deprives bacteria of a necessary substrate for multiplication and thus acts as one of the body's own defense mechanism.¹⁶

The immunity and immunological function can play an important part in determining drug response. Drugs like chloramphenicol may suppress the immune response in an animal and should be used with caution when vaccinating. As discussed earlier, in immunoincompetent patients, a bactericidal drug may be more beneficial than a bacteriostatic drug.

Protein binding has two important consequences from the chemotherapeutic point of view. First, protein bound drug is essentially without antimicrobial activity and second, the bound drug is nondiffusible. However, protein binding does not inactivate the antibiotic in an irreversible sense, the molecules are merely sequestered in a temporary inactive state.¹⁷ The extent of binding in serum will limit the amount of free drug available to the tissues but the total tissue level will be governed by the extent of binding which occurs in that particular tissue or tissue fluid. If the plasma levels are maintained for a considerable period of time, equilibrium will eventually be reached in the tissues despite a high level of serum binding and a diminished rate of diffusion from the vascular compartment. Hypoalbumenemia may lead to higher levels of free drug especially for those drugs that are normally highly bound. This could have beneficial and/or harmful effects. For drugs with low protein binding, this factor would not be as important.

Drugs may be absorbed differently in ruminants and simple stomach animals e.g., chloramphenicol should not be given to ruminants because of its degradation by the rumen microflora. The ruminants may also show a comparatively larger volume of distribution for certain drugs suggesting better tissue penetration. However, this is often due to the large volumes of the extracellular fluid in the gut which sequesters the drug and removes it from its site of action.

Most drugs are eliminated to a great extent by the kidney and liver. Therefore, as mentioned earlier, renal function is an important factor influencing the outcome of the treatment. Table 4 gives the renal functions in man and some domestic animals.

TABLE 4--Normal Renal Function in Man and Animals

Species	GFR ml/kg/min	ERPF ml/kg/min	Reference
Man	1.78	9.43	18
Dog	4.96 - 7.15	11.8 - 19.3	6
Cow	1.68 - 2.43	11.60 - 18.56	19
Buffalo	2.18 - 3.72	6.44 - 11.15	20
Horse	3.43 - 4.63	13.42 - 18.90	6

The values for renal function are very helpful in extrapolating data from one species to another for those drugs that tend to be eliminated predominantly by this route e.g., kidneys are predominant organs for elimination of aminoglycosides (primarily filtration). Aminoglycosides are not metabolized in the body and after single intravenous injection as much as 90% of the drug is excreted unchanged in urine in 24 hr.

Tables 5 and 6 show some data on aminoglycosides. Since they are physiochemically similar, are not metabolized and are excreted via glomerular filtration, we do not see marked differences in their pharmacokinetic patterns (Table 5). In such a case, the extrapolation of dose of one drug to another can be done within the same species.

TABLE 5--Pharmacokinetics of Aminoglycosides in Dog

Drug	Kinetic parameter			References
	t _{1/2} min	V _d area L/kg	Cl _B ml/kg/min	
Kanamycin	58	255	3.2	21
Gentamicin	74	308	2.9	6
Amikacin	51	250	3.4	22

Table 6 shows the pharmacokinetics of kanamycin in some domestic animals. As mentioned earlier, the aminoglycosides are excreted by glomerular filtration, thus knowing the renal status or function of a different species would help to adjust the dose of a given drug across species.

TABLE 6--Pharmacokinetics of Kanamycin in Domestic Animals

Species	Kinetic parameter			References
	$t_{1/2}$ min	$V_{d_{area}}$ L/kg	Cl_B ml/kg/min	
Horse	84.5	174	1.43	
Sheep	99.0	217	1.52	23
Dog	44.4	225	3.51	

For drugs like the tetracyclines, which are physiochemically different from one another, the extrapolation may not be easily achieved. Their pharmacokinetics vary from one member to another depending upon their lipophilicity and plasma protein binding variability. The only solution in such cases is to conduct the pharmacokinetic studies for each individual drug in each species.

The differences in functions of drug metabolizing enzyme systems may unravel some of the complexities of inter and intraspecies variations. Since the liver is considered to be the organ contributing most to the drug metabolic processes, its size and blood flow should be given major consideration. The liver blood flow, liver weight and hepatic clearance of drugs vary greatly due to species, strain, age, sex, weight, diet, nutritional state and exposure to environmental factors such as enzyme inducers.

CONCLUSIONS AND RECOMMENDATIONS

In summary, large biological variabilities exist which markedly influence the outcome of biological experiments, be they *in vitro* studies, studies in animal models, clinical trials and/or pharmacokinetic studies. The same variabilities are present when treating the individual clinical case. Knowing these facts to be truths, it is recommended that:

- (1) data be interpreted not only by statistical methods, but also by using scientific reasoning and logic based on the most current state of the science and art in clinical pharmacology and related biological fields,
- (2) if no biological significance can be established from the data, any statistical significance found may be meaningless,

- (3) each case or individual should be treated as a new clinical trial and the dose should be tailored to that case. We realize that this is much more difficult to do than selecting the drug of choice for the infectious disease but we must continue to extrapolate beyond any stated constant dosage regimens. Much more research is needed on pharmacokinetics, PAE and PALE to establish importance in predicting the proper dosage regimens.
- (4) the leeway we have for tailoring a dose is governed by the range between an efficacious and a toxic level. One must also consider the influence of dose alteration upon drug withdrawal times in food animals.
- (5) doses can be extrapolated across drugs within a single species and also across species for a single drug.
- (6) the package insert should contain information that will permit the veterinarian to practice better medicine rather than interfere with the practice of medicine. It is our recommendation that package inserts should be expanded, and for a prescription antibacterial agent it should include information such as
 - (a) bacterial spectrum based upon in vitro tests with specific organisms listed,
 - (b) the pharmacokinetic parameters such as volume of distribution, total body clearance and biological half-life. This information is required to enable the tailoring of dosage to the individual case.

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