Introducing a New Drug into Clinical Practice

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A NEW DRUG usually begins at the bench of an organic chemist. The chemist may be attempting to prepare derivatives of substances with known pharmacologic activity or may be exploring new areas of medicinal chemistry. The first task is to confirm or identify the structure. This is done by the inventor in collaboration with physical chemists.

Chemistry and Pharmacology

The large numbers of new chemical compounds and the increasing complexity of their structures have led to the development of highly sophisticated techniques for the elucidation of structure. The methods employed include gas chromatography, infrared, ultraviolet, and nuclear magnetic resonance spectroscopy, and computerized mass spectroscopy. Microanalytic methods are available for the determination of molecular weight, water, and the elemental composition of the material to be tested. When the structural formula is established, the chemist then prepares sufficient quantities of the chemical for initial biological screening. The extent of this early screening process depends on the nature of the drug. If it is one of a series, it may be tested only for its relative potency in a specific activity. If it is novel, it may be screened for a variety of activities. The limiting factor is often the quantity of chemical available at ths stage. Table 1 is a representation list of pharmacologic screening tests commonly employed. If the substance shows favorable activity in one or more of these tests, a series of analogs is usually synthesized to find the most promising candidate for more extensive evaluation. The chemist must then prepare a larger supply of the candidate selected for more elaborate and definitive pharmacologic studies.

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The degree to which pharmacologic studies predict the actions of drugs in man depends on physiologic and pharmacologic species differences relative to the particular test and the particular drug. For instance, antimicrobial agents tend to be effective in both animals and man because infectious disease processes are similar. On the other hand, there are no good animal models for human mental disorders, so that in dealing with possible psychotherapeutic agents, pharmacologists employ model systems based on the pharmacologic actions of drugs known to be effective in man. Prominent among such studies are various types of conditioning procedures. Their predictability, however, appears to be related more to structural similarities between the proven and unknown agents than to specific pharmacologic actions.

Hypnotics, sedatives, and analgesics are qualitatively similar in man and laboratory animals, but there are substantial species differences relative to safety, potency, duration of action, development of tolerance or physical dependence, and drug interactions.

Cardiovascular studies in animals are of particular interest to anesthesiologists because information is provided relative to safety as well as possible utility. Primary cardiovascular screening, conducted in anesthetized dogs, measures the effects of test drugs on blood pressure, respiration and the responses to a variety of vasoactive substances such as epinephrine, norepinephrine, angiotensin, serotonin and histamine. Secondary cardiovascular studies also employ anesthetized dogs, which are prepared for more elaborate hemodynamic measurements such as cardiac output, myocardial contractility, end-diastolic pressure and peripheral resistance. Blood pressure studies in unanesthetized rats are also part of secondary screening. Such studies are conducted on most new drugs even if their intended use is not related to the cardio-

vascular system. When direct effects are suspected, isolated perfused hearts and tissues or individual vascular beds may be used to evaluate effects on rhythmicity, basic contractile mechanism, or vascular tone.

Biochemical investigations are becoming increasingly important in the pharmacologic program. Such studies are largely concerned with the effects of test drugs on specific enzyme systems *in vitro* and *in vivo*. Other types of experiments might involve drug effects on tissue constituents such as biogenic amines. Such effects are known to be associated with important clinical activity. For example, the action of catecholamines can be influenced by drugs which selectively inhibit or stimulate specific enzymes all along the biosynthetic and metabolic chains, including tyrosine hydroxylase, dopa decarboxylase, amine oxidase and phosphodiesterase. Tissue amines can be depleted by such sympatholytic drugs as reserpine and guanethidine, or replaced, as is the case with levodopa treatment in Parkinson's disease.[1,2]

If the data accrued during the pharmacologic and biochemical studies are sufficiently promising, the decision to proceed toward clinical trials may be made. Such a decision turns on several new interrelated activities: manufacturing, quality control, biopharmaceutics, and drug metabolism studies.

Manufacturing chemists are responsible for producing sufficient drug for animal toxicity studies and, possibly, early clinical trials. The amounts needed are too large for the test tube methods of the organic chemist and too small for the mass production equipment used for marketed drugs. This interim production, therefore, is carried out in a special facility, frequently called a "Kilo Lab" or pilot plant. Since the material produced has clinical relevance, control procedures are necessary to measure its content and purity. This is done by quality-control chemists, who first must devise and standardize methods to identify and quantitate the test substance and potential impurities. These assays are then applied to all production batches. If the material does not meet the rigid criteria established by the quality-control laboratory, the batch is rejected and must then be reworked or discarded. The quality-control laboratory also checks the potency and purity of stored material before it is certified and released for use.

TABLE 1. Pharmacologic Screening Tests

Acute toxicity
Analgesic activity
Anticonvulsant activity
Antiemetic activity
Anti-inflammatory activity
Antimicrobial activity
Antiobesity activity
Antitumor activity
Antiviral activity
Cardiovascular activity
CNS activity
Diuretic activity
Endocrinologic activity
Effect on blood sugar and cholesterol

The past decade has witnessed an increasing appreciation of biopharmaceutics in drug development. This science deals with relationships between physical and chemical properties of drugs and the formulations in which they are administered and their biological availability and eventual disposition, *i.e.*, absorption, distribution, metabolism, and elimination. The actions of a drug depend not only upon its intrinsic pharmacologic properties but also on its ability to reach target sites in the body. The physical and chemical properties which influence the biological processes of a drug are its 1) aqueous solubility as a function of *p*H; 2) dissolution rate; 3) dissociation constant (pK_a); 4) partition coefficient; and 5) permeability characteristics across a biological membrane. The biopharmaceutical chemist measures these properties *"in vitro"* to determine the potential absorbability of the drug. The conclusions regarding the absorbability of a new drug drawn from these *"in vitro"* studies are tested *"in vivo"* by measuring the drug blood levels after intravenous and oral administration.[3] If the absorption characteristics of the drug are not satisfactory, it may be subject to improvement by altering the crystal form, reducing the particle size (micronization), changing the salt form, or even selecting another member of the chemical series with similar pharmacologic activity, but possessing physical and chemical properties more conducive to good absorbability. In many instances the dosage formulation itself

can cause this absorption problem, and this factor must be studied individually.

Examples of the effects of physical properties of drugs on clinical activity are readily available. Many drugs exist in two or more crystal forms which are chemically identical but differ in intrinsic dissolution rates. Obviously the form which goes into solution most rapidly is most likely to produce rapid and reproducible clinical responses. The crystalline forms of novobiocin and chloramphenicol are not absorbed from the gastrointestinal tract and are therefore therapeutically inactive, while the amorphous forms are readily absorbed and provide effective blood levels.[4,5] The role of dissolution rate and the effect of particle size on biological availability is exemplified by griseofulvin. This antifungal drug is so poorly soluble in water that its absorption is limited by the dissolution rate. This process, and hence absorbability, is more than doubled when the mean particle size of the administered material is decreased (specific surface area increased) four- to fivefold.[6]

Most drugs are organic acids or bases, that is, they are capable of dissociating into ionized ad unionized portions. Since it is essentially the undissociated portion which is capable of penetrating through biological membranes, the dissociation constant, pK_a, is an important parameter for absorption. The pK_a and pH levels determine the degree of undissociation. Acidic drugs such as aspirin and the barbiturates are less dissociated, and therefore better absorbed on the acid side of the pK_a, and the reverse is true for bases.[7] Barbiturate derivatives exemplify the importance of the partition coefficient in absorption. Barbituric acid derivatives having similar pK_a values have exhibited different absorbabilities. This has been shown to be directly related to differences between the partition coefficients of the undissociated forms of the individual derivatives.[8] Under experimental conditions, secobarbital, with a partition coefficient of 50.7, was twice as well absorbed as phenobarbital, with a partition coefficient of 4.8.[9] The inhalation anesthetics do not undergo ionization and are metabolized only slightly in the body. Thus, their biological availabilities to the brain, and therefore their relative potencies, are directly related to their lipid solubility coefficients.[10]

The role of formulation is exemplified by aspirin, the absorption of which is enhanced if it is mixed with antacids and properly granulated with starch.[11] On the other hand, antacids containing divalent and trivalent cations impair the absorption of tetracycline.[12] When a drug is administered in a tablet, the ability of the tablet to break up must be demonstrated. This is done by a standard disintegration time test described in the U. S. Pharmacopeia. However, disintegration is not tantamount to availability, because the active substance itself must have the physical properties needed for absorption.[13] These factors relate to the differences in biologic availabilities sometimes observed among drugs considered generically equivalent.[14,15] Since formulation can clearly affect the absorption, and therefore the activity, of the active substance, every effort is made to develop the final formulation as early as possible so that clinical trials can be conducted with a uniform product.

Drug metabolism also plays an important role in biological availability and pharmacologic activity. If a drug is rapidly and extensively metabolized, it may be available to carry out its desired action only briefly. Such is the case with levodopa.[16] The pharmacologic and toxicologic effects of a drug which is subject to biotransformation are, in fact, the effects of the drug and its active metabolites. For example, the activities of chlordiazepoxide and diazepam are associated with at least two and three metabolites, respectively.[17,18] Furthermore, there is considerable species variation in drug metabolism. As a new drug approaches the animal toxicology stage of development, it is clearly important to be able to measure the drug in biologic fluids and tissues and to identify its principal metabolites. Assay procedures used in studies of drug metabolism must have high degrees of specificity, sensitivity and selectivity.[19] This is due to the fact that the drug and its metabolites are often present in very small amounts (usually 10^{-9} to 10^{-12} g) as a result of low doses, high distribution volumes, or extensive metabolism and rapid elimination. Therefore, selective extraction and extensive sample clean-up are usu-

ally necessary to separate the drug and its metabolites from interfering substances in biological specimens. The extraction and separation steps are based on the same physical properties discussed in the section on biopharmaceutics, especially pK_a, solubility in water and various organic solvents, and the partition coefficients between different solvent systems. Thin-layer and paper chromatography and adsorption on certain stable adsorbents such as alumina or ion-exchange resins may also be used during the separation and clean-up process. Detection and quantitation of the drug residues so isolated are usually accomplished by absorptiometric, fluorescence, phosphorescence, gas chromatographic, or radioisotope techniques.[19] Once the test drug and its metabolites have been isolated and their quantitation is confirmed by recovery experiments with authentic standards, the assay procedures are applied to determining the fate of the test drug in animals, and later in man. The resulting pharmacokinetic profiles allow for the correlation of species differences with pharmacologic, toxicologic and clinical information; provide an endpoint for the design and evaluation of dosage formulations; and aid in the design of dosing regimens and clinical protocols for further evaluation studies.[20-22] Many aspects of this program may be so intricate that it continues long after the drug is marketed.

Animal Toxicology

Animal toxicity experiments are a critical part of the preclinical activities. Properly done, these experiments are based upon, and integrated with, the pharmacologic, biochemical, and biopharmaceutical programs. Pharmacologic data provide an estimate of the clinical dose range, and therefore the dose levels, needed in the toxicity studies to justify human exposure. Biochemical tests may identify potential toxicity as well as potential activity. For example, *in-vitro* effects on important enzyme systems may occur *in vivo* if the drug reaches the sites of the enzyme systems in adequate concentration. Biopharmaceutical studies guarantee that the drug administered is biologically available to the tissues during the toxicity experiments. The physical and chemical properties responsible for the presence of a drug in a tissue where a desirable effect takes place also govern the distribution of the drug to other tissues where undesirable effects may occur.

Acute toxicity experiments comprise LD_{50} determinations in three to four species and pyramiding single-dose studies in dogs.[23] These procedures are conducted as part of primary pharmacologic screening because they provide information about the general potency of a compound and the dose range likely to be tolerated during pharmacologic and subacute toxicity studies. A comparison of the LD_{50} values after oral and parenteral administration may give an early estimate of gastrointestinal absorption. The LD_{50} is a statistical estimate of the dose which is lethal to 50 per cent of treated animals. In each species four groups of six to ten animals are given the test drug in graded doses by several routes of administration. The drug is generally given orally, intravenously, intraperitoneally, and subcutaneously and the animals observed for 72 hours or longer. If the toxicity of the drug is too low to define a lethal dose, the highest dose administered is recorded.

The administration of pyramiding single oral doses of the compound to dogs is used to determine acute symptomatology and prepare for subacute and chronic experiments. Usually a dose of 1 to 5 mg/kg is given on the first day of observation, and this is increased in twofold increments until definite untoward reactions or death occur.

During the acute toxicity studies the animals are observed for certain symptoms and signs, such as increased motor activity, tremors, convulsions, ataxia, spasticity, sedation, Straub tail reaction, and respiratory depression. These observations, and identification of the apparent mechanism of death, provide early information about the toxic potential of the drug. The time factor is also important. Death in minutes or hours may simply reflect the potency of the test compound. Death in two to three days may be a better indicator of tissue damage.

The acute toxicity experiments will readily identify frank poisons such as organic solvents, other lipid-soluble substances which irreversibly combine with vital enzyme systems, and corrosive chemicals. Damage produced by

organic solvents such as ether, chloroform, and carbon tetrachloride is related to the concentration which comes in contact with the tissue and has direct clinical relevance. The acute toxicity experiments are not helpful in identifying the selective toxic actions more commonly encountered clinically. Selective toxic actions are those which occur at specific sites or tissues at concentrations which are not generally destructive. The potentiality for this kind of toxicity is investigated during the subacute and chronic toxicity experiments and clinical trials.

The design and duration of the subacute and chronic toxicity studies depend on the results of the acute toxicity, pharmacologic, and metabolic studies, and the intended use in man. The FDA guidelines for such studies (table 2) reflect usual practices and the recommendations of the World Health Organization study group.[24, 25] Rats and dogs are the animals most often used, but subhuman primates are also being used with increasing frequency as second or third species. Four groups of animals comprising three dose levels and a group of controls are studied in each species. The drug is usually given as a dietary admixture to rodents, with food consumption measured to document delivery. The drug is administered directly to dogs. Ingestion and absorption of the drug may also be monitored by determining blood levels of the drug at intervals during the toxicity trials. The three dose levels employed include a high dose, which is expected to produce toxic effects, a low dose, approximately two to five times higher than the highest anticipated human dose, and a dose intermediate between the two. The use of three dose levels permits correlation with dose of any toxic effect which may be encountered. During the studies the animals are observed daily for signs of drug activity or toxic manifestations, and hepatic, hematologic, and renal function tests are performed at regular intervals. Following completion of the treatment, the animals are sacrificed and macroscopic and microscopic examinations of the organs and tissues are conducted.

The acute and subacute toxicity trials must be completed in all respects before administration to humans can be considered. Longer-term toxicity trials are needed to support Phase III studies in man and to document claims of safety prior to marketing.

In evaluating changes observed in these experiments one must first exclude all environmental, dietary, disease, and other non-drug-related factors which can produce toxic effects. Most of these have been controlled by air conditioning, light control, sterilization of cages, vaccination, and diet supplementation. Non-drug-related factors would probably produce effects in the control as well as in the treated animals, but such an event might disqualify the entire experiment. Dietary factors are particularly important. Intestinal antiseptics are very toxic in rats deficient in vitamins E and K[26]; blindness may occur in dogs deficient in vitamins A and B given 1-hydroxyl-2(1H)-pyridinethione[27]; thiopental is markedly potentiated in mice deficient in niacin.[28] While, on one hand, obesity increases the cardiotoxic effects of isoproterenol,[29] pentobarbital and caffeine are less toxic in malnourished rats.[30, 31] In chronic toxicity experiments the animals may be maintained on drugs for very long periods; thus, they may develop degenerative organ changes or chronic infections which must be distinguished from drug effects. For example, slight accumulations of fat in the livers of untreated rats and inflammatory cells in the livers of dogs are common.

In considering drug-related factors, one must distinguish toxic manifestations which are highly specific from those which are more or less nonspecific consequences of the experimental procedure. For example, the drug may produce anorexia, leading to malnutrition and subsequent organ changes. The excessive doses often used may simply overload the animal and cause widespread chemical damage. Such effects may be quite nonspecific yet difficult to distinguish from specific organ damage.

Specific organotoxic effects uncovered during toxicity experiments are, of course, much more important for the evaluation of the drug's safety. These organ changes occur at a dose which may not initially impair the general well-being of the animal. The changes are observed in most treated animals, and a clear-cut dose–activity relationship is often

TABLE 2. Synopsis of Food and Drug Administration Guidelines for Animal Toxicity Studies*

Category	Duration of Human Administration	Phase	Subacute or Chronic Toxicity
Oral or parenteral	Several days	I, II, III, NDA	Two species; two weeks
	As long as two weeks	I	Two species; two weeks
		II	Two species; as long as four weeks
		III, NDA	Two species; as long as three months
	As long as three months	I, II	Two species; four weeks
		III	Two species; three months
		NDA	Two species; as long as six months
	Six months to unlimited	I, II	Two species; three months
		III	Two species; six months or longer
		NDA	Two species; 12 months (nonrodent) 18 months (rodent)
Inhalation (general anesthetics)		I, II, III, NDA	Four species; five days (three hours/day)
Dermal	Single application	I	One species; single 24-hour exposure followed by two-week observation
	Single or short-term application	II	One species; 20-day repeated exposure (intact and abraded skin)
	Short-term application	III	As above
	Unlimited application	NDA	As above, but intact skin study extended for as long as six months
Ophthalmic	Single application	I	Eye irritation tests
	Multiple application	I, ", III	One species; three weeks daily applications, as in clinical use
		NDA	One species; duration commensurate with period of drug administration
Vaginal or rectal	Single application	I	Local and systemic toxicity in two species
	Multiple application	I, II, III, NDA	Two species; duration and number of applications determined by proposed use
Drug combinations		I	LD_{50} determinations
		II, III, NDA	Two species; as long as three months

* Adapted with modifications from Goldenthal.[21]

evident. Although toxicologists are most eagerly on the lookout for such changes, the general experience is that they are not common. If, however, such a change is uncovered during the toxicity evaluation, one must decide whether the drug may be given to humans. In making this decision, various factors have to be considered. These include: 1) the therapeutic potential of the new compound; 2) the nature of the toxic effect; 3) the dose at which the toxic effect was observed relative to the therapeutic and tolerated doses; 4) the mechanism of action of the toxic effect; and finally, 5) the problem of species differences.

Species differences in the toxic and pharmacologic responses to drugs may result from many factors. Variations in the rate and pattern of drug metabolism appear to be the most important, but differences in renal handling, protein binding, tissue distribution and end-organ responses must also be considered.[22] The application of metabolic data to the evaluation of drugs was recently discussed in detail by the Committee on Problems of Drug Safety of the Drug Research Board, National Academy of Sciences—National Research Council.[23] In general, laboratory animals metabolize drugs faster than humans. This is one reason animals are given substan-

tially larger doses during subacute and chronic toxicity studies than are expected to be used in humans. Furthermore, because of the existence of species differences in drug metabolism, clinical pharmacologists and biochemists try to identify drug metabolic patterns in animals and man as early in the life of a new drug as possible, so that chronic toxicity studies can be conducted in the appropriate species and at the appropriate dose levels to give maximum information relative to safety in man.

As examples, phenylbutazone has a plasma half-life of 72 hours in man, but it is eliminated ten times more rapidly by several animal species, including the dog, monkey and horse.[32] The patterns are similar for oxyphenbutazone, antipyrine and meperidine.[32] On the other hand, man metabolizes ethylbiscoumacetate faster than the dog.[34] Cats metabolize aspirin and morphine poorly, and therefore analgesia is achieved at very low doses compared with dogs and man, while toxicity occurs if doses appropriate to the dog and man are given to the cat.[35, 36] Species differences in enzyme induction may influence the interpretation of toxicity studies. Phenylbutazone and tolbutamide accelerate their own metabolism in dogs, thus reducing their pharmacologic and toxicologic actions in this species.[37, 38] Such an effect has not been observed in man.[32] As examples of species differences at the end-organ level of interest to anesthesiologists, man is 15 times more sensitive to atropine than rabbits and 100 times more sensitive to decamethonium than rats.[25]

It has been suggested that a toxic effect which occurs in more than one species of laboratory animal is more likely to occur in man than an effect which appears in a single species.[39] In an evaluation involving 25 anticancer drugs, it appeared that predictability for man is greater when dogs and monkeys are used rather than dogs and a rodent species.[40] In the same investigation it was found that toxicity studies in these large animals predicted well for bone marrow, gastrointestinal, hepatic and renal toxicity, and poorly for neurologic and dermatologic side effects. However, a considerable number of false-positive predictions were also encountered; these were attributed to the toxic nature of the drugs, the large doses used, and the extensive laboratory and histologic tests carried out in the animals.

The potential for adverse effects due to drug interactions may also be evaluated during the preclinical animal toxicity experiments. One procedure determines whether the LD_{50} of the test drug is altered when it is administered to rats or mice together with a number of commonly used medicinal agents. The ability of the drug to induce or inhibit hepatic microsomal enzyme systems, and thus alter its own metabolism or that of other drugs, can be tested by measuring its effect on hexobarbital sleeping time, zoxazolamine paralysis time, phenylbutazone half-life or hydroxycortisone excretion.

Young individuals tend to have defective detoxification mechanisms; therefore, special studies in newborn animals must be carried out before a drug may be used in children. These include acute toxicity studies to compare the newborn rat with the adult.[24] Information is also supplied by Segment III of the reproductive studies.

The influence of drugs on reproduction has been of considerable interest since the thalidomide disaster in Europe. It is generally agreed that there is still no reliable and sensitive animal model for the human reproductive process. Teratogenic effects are easy to demonstrate, but among the agents possessing such action are insulin, penicillin, streptomycin, cortisone, salicylates, caffeine, nicotine and phenobarbital.[41] The FDA has issued guidelines for reproduction studies in animals to be conducted in three segments, each related to a specific area of the mammalian reproduction process.[42] The "General Study of Fertility and Reproductive Performance" (Segment I) involves administration of the drug to male rats for 60 to 80 days prior to mating to assess the effect on spermatogenesis. Female rats are treated for 14 days prior to mating, and half of these are sacrificed on the thirteenth day of pregnancy. This allows a determination of the number and state of embryos. The remaining females are allowed to litter and the pups are examined for litter size, viability and abnormalities. The "Teratologic Study" (Segment II) may employ mice, rats or rabbits. Pregnant females are treated from the period of organ formation to the end of

pregnancy. The fetuses are delivered by cesarean section one or two days prior to anticipated parturition, and are carefully examined for external and internal anomalies. Special techniques are used to examine the viscera and skeleton. In the "Perinatal and Postnatal Studies" (Segment III) the pregnant females are given the drug during the final third of gestation and throughout lactation to weaning. In addition to examining the pups, the processes of labor, delivery, nursing and lactation are carefully observed.

Segment II and the female portion of Segment I must be completed before a new drug can be given to women of childbearing potential. All aspects of the reproductive studies are completed before Phase III clinical trials are initiated.

Animal toxicity experiments have become an undertaking of awesome proportions. It has been estimated that a complete program for testing the toxicity of one drug by one route of administration may involve more than 1,000 animals, more than 1,200 hemograms, about 5,000 laboratory tests, more than 700 autopsies, and histologic examinations of more than 6,000 organs, and may cost close to $100,000.[43]

The completion of the preclinical animal toxicity experiments represents a critical point in the career of a new drug. At this point all available pharmacologic, toxicologic, biopharmaceutic, metabolic, and other information is reviewed and a decision made as to whether the drug should be submitted to clinical trial. If the decision is affirmative, the drug has its first brush with the law.

Clinical Studies

F.D.A. Regulations

The clinical investigation and marketing of new drugs in 1971 is governed by the 1938 Federal Food, Drug and Cosmetic Act expanded by the Kefauver-Harris Amendments of 1962. These statutes have been implemented by a series of regulations published by the Food and Drug Administration in the Federal Register. In essence, these laws and regulations define the conditions under which new drugs may be shipped interstate for clinical investigation and require that prior to marketing, substantial proof of safety and efficacy for the claimed indications be provided. A new drug is defined as any drug not generally recognized by qualified experts as safe and effective for use under the conditions prescribed, recommended, or suggested in the labeling.[44] A new drug, by this definition, may be a new chemical substance, a well known and accepted drug proposed for a new indication or a new combination of old and accepted drugs. Even a new dosage form, such as a sustained-release formulation, requires proof of safety and efficacy prior to marketing. It is to be noted that a practicing physician may use an approved drug for a nonapproved indication without legal consequence relative to the F.D.A. The lack of F.D.A. approval for such use, however, may be a burden in the event of malpractice proceedings. One example of the widespread use of an approved drug for a nonapproved indication was the use of lidocaine for cardiac arrhythmias. As mentioned, the government is concerned not only with the approval or disapproval of new drugs for marketing, but also with the regulation of clinical trials. The intent of this concern is to minimize the hazards inherent in new drug development by review of the chemical, pharmacologic and toxicity data prior to human administration and by surveillance of the clinical experience as trials proceed. When a sponsor, usually a drug company, wishes to initiate clinical trials, a Notice of Claimed Investigational Exemption for a New Drug (Form FD 1571), also known as an IND, is submitted to the FDA. This is a 14-schedule document, the principal features of which are outlined in table 3. Several of these schedules require further comment.

Schedule 1 calls for a description of dosage form. Although capsules and tablets may be included in one IND, investigation of an oral suspension, a sustained-release formulation, parenteral administration, or topical administration requires a separate IND for each formulation. However, common data may be included by reference. The schedules describing the synthesis, formulation, specifications and directions for testing of the new drug (2 through 5) have importance in the clinical evaluation. Any evidence that the test drug

proposed for trial differs significantly from that actually tested could result in the disqualification of the clinical data. Such differences could result from changes in manufacturing procedures or formulation, chemical instability or introduction of impurities. Thus, the quality-control procedures described in the preclinical section are essential not only for the potency and safety of the drug, but to insure the validity of the clinical trial.

Schedule 6, which describes the pharmacologic, toxicologic and similar data, is an extensive section, but the information contained in this schedule has been discussed under preclinical activities. Schedules 9 and 10 are of most direct interest to investigators. Schedule 9 identifies the monitors and investigators, including a summary of their training, experience and facilities. The sponsor affirms he has on file Statement of Investigator forms (FD 1572 for Phase I and II studies and FD 1573 for Phase III) from each investigator. These forms include the education, training and experience of the investigator, the location of the research facility, and the specific protocol for the trial to be conducted. Form FD 1572 additionally describes the expert committees responsible for approving the proposed project and gives an estimate of the numbers of subjects to be involved and the duration of the study. On these forms the investigator agrees: 1) to maintain adequate records of the study; 2) to submit a complete report to the sponsor when the study is completed; 3) to account for all supplies of the investigational drug in his possession; 4) to maintain his records, including case reports, for at least two years after studies with the drug are discontinued; 5) to administer the test drug only to subjects under his supervision; 6) to obtain the informed consent of the subjects involved in the study unless there are certain extenuating circumstances.

Schedule 10 provides an outline of the clinical investigations to be performed and must specify the phase involved. The first two phases are described as clinical pharmacology.[45] *Phase I* studies represent the first administration of the new drug to human beings. Only animal pharmacologic and toxicologic and *in-vitro* data are available. The purpose is to determine human tolerance, absorption, metabolism, elimination, pharmacodynamics, and dose range. Generally, healthy volunteers are employed. In *Phase II* the drug is first used in the prevention or treatment of the disease for which it is intended. The goals are the identification of efficacy and further proof of safety. Physicians involved in Phase I and II trials should be skilled in human pharmacology, toxicology, and pharmacokinetics and familiar with the disease states to be treated.

If the results of the Phase I and II studies provide reasonable evidence of safety and efficacy, extension of the investigation into *Phase III* may be considered. As noted in the preclinical section, additional animal toxicologic studies may be required before Phase III can begin. This phase is the broad clinical trial necessary to determine if the drug is a candidate for marketing. The studies involved may range from complex investigations of specific pharmacodynamic actions to controlled clinical trials by practicing physicians. In all cases careful monitoring and proper record-keeping is essential.

The research protocol is not entirely the concern of the investigator and sponsor alone. The 1962 Kefauver-Harris Amendments to the Federal Food, Drug and Cosmetic Act provided that, in addition to safety, "substantial proof of efficacy" is necessary prior to marketing.[46] Furthermore, the proof of efficacy must be established on the basis of "adequate and well-controlled clinical investigations." The F.D.A. interpretation of this phrase was published in the Federal Register,[47] and summarized in F.D.A. Papers.[48] For a clinical investigation to qualify as "adequate and well controlled": 1) The objectives of the study must be clearly stated. 2) The subjects must be selected by methods which insure that they are properly diagnosed and suitable for the purpose of the study. 3) The subjects must be assigned to test groups in a manner which minimizes bias and assures comparability of test and control groups. 4) The methods of observation and recording must be fully explained and these, too, designed to minimize bias. 5) The effects of the drug must be compared with a control in as quantitative and unbiased a manner as possible. Methods of "blinding," if used, must be documented.

TABLE 3. Composition of an IND

Schedule 1: Name, chemical structure, dosage form and route(s) of administration of the new drug.

Schedule 2: List of all components of the drug entity, including reasonable alternates for inactive components.

Schedule 3: Quantitative composition of the drug entity, including reasonable variations that may be expected during the investigational stage.

Schedule 4: Source and preparation of new drug substances used as components. This includes manufacturing processes for new drug substance(s) and dosage form.

Schedule 5: Methods, facilities and controls used for the manufacturing, processing, and packing of the new drug. The establishment and maintenance of appropriate standards of identity, strength, quality and purity.

Schedule 6: Preclinical pharmacology, toxicology, and drug metabolism data. Available clinical data if drug used previously (*e.g.*, in another country) or is a combination of previously investigated or marketed drugs.

Schedule 7: Informational material to be provided to investigators. This includes a copy of the labels to be on the drug containers identifying the drug as investigational and a clinical monograph describing the drug, possible utility, prior investigations and known hazards, contraindications, side-effects and precautions.

Schedule 8: A statement of the training and experience required of investigators.

Schedule 9: The names and credentials of the monitors and investigators. This schedule also describes the investigators' responsibilities regarding record-keeping, informed consent, and supervision of subjects.

Schedule 10: Outline of the clinical investigation, including specification of phase involved—Phases I and II, Clinical Pharmacology, or Phase III, Broad Clinical Trial.

Schedule 11: Agreement to notify FDA if investigation is discontinued and why.

Schedule 12: Agreement to notify investigators if investigation is discontinued or an NDA is approved.

Schedule 13: Completed only if sponsor wishes to sell rather than distribute test drug free to investigators. The reason for the need to sell must be explained.

Schedule 14: Agreement not to ship drug or use in man until 30 days after receipt of IND by FDA.

Four types of comparisons are recognized: a) No treatment; objective measurements are available and placebo effect is negligible; thus, treated and untreated patients are compared. b) Placebo control. c) Active treatment control; a known active drug is employed when no treatment or a placebo may be harmful to the patient. d) Historical control; the effect of a drug may be measured against the untreated course in diseases with a predictable natural history, *e.g.*, acute leukemia of childhood and certain infections. 6) The report of the study must include the methods of analysis, including the statistical methods applied, and an evaluation of the significance of the data. 7) The test drug must be standardized as to identity, strength, quality, purity, and dosage form. Evidence of biologic availability is included in these considerations.

Although these criteria would appear to be rather rigid and formalized, the F.D.A. is willing to waive any one or all of them if evidence can be provided that these criteria are not appropriate for a specific clinical investigation and that the study will yield "substantial evidence of effectiveness" by the methods to be employed in place of these criteria. In addition to these general regulations, the F.D.A. is preparing a series of guidelines in specific clinical areas.

Informed consent is part of the larger consideration of the ethics of clinical investiga-

tion. The Declaration of Helsinki of the World Medical Association and the Ethical Guidelines for Clinical Investigation of the American Medical Association are guidelines for physicians engaged in research on man.[49-50] The principles set forth in these documents have been endorsed by the major societies concerned with clinical research. If human beings are to be subjected to any risks, the project should be worthy; that is, it should be a systematic study based on laboratory and animal experiments, or other scientifically established facts, and employ accepted standards of scientific research to produce valid and significant data. The importance of the objective of the study and the possible benefit to the subject should be in proportion to the inherent risks involved. The investigator should exercise the same concern for the welfare, safety and comfort of the persons involved as physicians providing medical care unrelated to clinical investigation.

Adequate informed consent, according to these guidelines, requires the physician to provide prospective subjects with a fair explanation of the nature, purpose, and duration of the study; possible inconveniences; risks involved; and the existence of alternative treatments. The subjects must be mentally, physically, legally and socially capable of exercising free choice. In the case of minors or mentally incompetent persons, consent in writing may be obtained from a legal guardian. Such subjects should be used in research only if mentally competent adults would not be suitable.

The F.D.A. requires that informed consent be obtained *in writing* from subjects involved in Phase I and II clinical investigations.[51] This regulation is consistent with the aforementioned ethical guidelines, since the basic purpose of these phases is the accumulation of scientific information, the toxicity of the drug is relatively unknown, and possible benefit to the patient is not established. During Phase III, investigators are again required to obtained informed consent, but if deemed in the best interests of the patient, the consent may be obtained orally rather than in writing. If this is done, the fact of consent must be recorded in the medical record of the subject. The requirement to obtain consent may be waived under very exceptional circumstances. These would include coma or other emergency situations in which the patient's representative cannot be reached and the need for the drug is urgent. Another, perhaps more common, situation would be when communicating the information necessary to obtain informed consent would be detrimental to the patient, such as in dealing with malignancies.[51]

Clinical Trials

Phase I studies are usually initiated with a single hospitalized or institutionalized individual. Informed consent is obtained and the subject's qualifications established by history and physical examination, appropriate x-rays, electrocardiography and a battery of hematologic, liver and renal function tests. The first dose is usually five to ten times smaller than that expected to show clinical activity, and many orders of magnitude smaller than that associated with adverse effects in animals. The dose is then gradually increased until desired or untoward effects are encountered. If the experience is satisfactory, new subjects are investigated in the same manner until the prescribed number of participants, usually 10 to 20, is under study. During and after the period of drug administration, the subjects are interviewed and examined regularly and the laboratory tests repeated once or twice weekly. The clinical and laboratory data are carefully recorded on special forms, usually provided by the sponsor. If an assay is available, blood and urine specimens are obtained at appropriate intervals and analyzed for the test drug and its metabolites. Thus, absorption, distribution and elimination data are developed and the drug blood levels can be correlated with any pharmacodynamic actions which may have occurred. The relationship, or its lack, between drug blood levels and a pharmacodynamic effect such as lowering of blood pressure or sedation may suggest whether the drug or a metabolite is responsible for the action. Time-course studies may also provide information about the accessibility of the drug in the blood to the target tissue of receptor. Furthermore, the metabolic data can be compared with that previously obtained in animals to provide guidelines for further animal studies, and to suggest the degree of relevance

the earlier animal pharmacologic studies may have for man.

In addition to ordinary clinical measurements such as blood pressure, pulse and electrocardiogram, simple physical observations often provide important information about drug effects. For example, the size of the pupil of the eye is sensitive to many types of drugs.[52] Other variables studied depend on the anticipated actions of the drug. For instance, many substances enter the brain and affect the electroencephalograph. Several specific patterns have now been described.[53] If the test drug affects sympathetic nerve tissue or receptors, pressor sensitivity tests with tyramine and norepinephrine may delineate the exact mechanism of action.[54]

Phase II studies continue and expand all activities initiated in Phase I. The prime purpose, however, is to establish the clinical activity of the new agent. In consultation with medical statisticians and investigators expert both in the disease or condition for which the drug is intended and in the conduct of clinical trials, protocols are devised which describe in detail the questions being asked, the controls and treatments to be used, and the measurements to be made. Forms are designed to record the clinical, pharmacodynamic and laboratory data to be obtained. These forms, hopefully, are constructed to facilitate both the recording and the subsequent analysis of the data. A discussion of the design and execution of clinical trials applicable to all drugs is beyond the scope of this paper. Some of the factors which must be considered are outlined in table 4. Side-effects encountered in Phase I and II studies are likely to be those related to the intended effects of the drugs, acting either in excess or on the wrong target organ.[23] An example of the former would be excessive hypotension from sympathetic blocking agents. Examples of the latter are dryness of the mouth from anticholinergic drugs and bone-marrow depression from anticancer agents. The exact protocol for a Phase I or II study depends on the nature of the test drug. Anesthetic agents represent a special situation which is considered elsewhere in this symposium. The completion of Phase II studies marks another major decision point in the life of a new drug. The sponsor must consider whether the evidences of efficacy and safety now available warrant the broad clinical trials, chronic animal toxicity studies, manufacturing commitment and the many other activities which must be done to make the drug a candidate for marketing. An affirmative decision launches Phase III.

TABLE 4. Considerations in the Design of Clinical Trials*

I. Patients
- Number required for statistical significance
- Criteria for inclusion or exclusion
- Informed consent
- Cross-over or single-treatment design
- Allocation to the treatments
- Frequency of interview and examination
- Standardization of environmental conditions

II. Drugs
- Dosage strength and route of administration
- Frequency and time of administration
- Fixed or flexible dosage
- Single or multiple courses
- Pharmaceutical form
- Use or exclusion of concomitant drugs
- Identification and matching of the drugs to be compared
- Mode of distribution to patients
- Control of ingestion and absorption
- Control of deviations from protocol
- Duration of treatment

III. Effects
- Values to be measured; subjective or objective
- Quantification of responses
- Frequency of observations
- Recording of observations
- Laboratory procedures
- Terms of comparison (pre- and post-drug, placebo, standard drug)
- Use of double-blind technique or not
- Experimental design
- Compilation of data
- Statistical methods to be used

* Adapted with modification from Joubert, L. and Abrams, W. B.: Drug testing and statistical methods. *In* Fundamentals of Clinical Pharmacology, Berlin, Urban and Schwarzenberg (in press).

Phase III trials are a massive effort to provide the "substantial proof of efficacy" based on "adequate and well controlled clinical investigations" required by the FDA before marketing can be considered. Hundreds or thousands of patients may be involved. The sponsor prepares a monograph describing the chemistry, pharmacology and toxicology of the new drug and the results of the Phase I and

II studies. Contraindications, precautions, and side-effects anticipated from the biologic or early clinical data are listed. Finally, the general areas of clinical utility for which the drug is proposed are described. This brochure is given to clinical investigators and specific protocols and report forms are prepared for individual studies. These protocols are designed to provide evidence for the claims the sponsor feels the drug will merit. It is often necessary to have a single protocol carried out in many centers in order to gather sufficient case material to achieve statistical significance. On the other hand, numerous single studies may be generated to investigate mechanisms of action, physiologic effects and drug interactions. It is now well known that a drug may influence the metabolism, and therefore the clinical effects, of other drugs.[55] Furthermore, the repeated administration of a drug may accelerate its own degradation. Such effects may be investigated by noting changes in the blood elimination rates of the drugs involved or by studying the test drug's influence on a readily measured and inducible agent such as coumadin.[55] Pharmacodynamic as well as metabolic interactions must be investigated. Blood-level measurements are also useful to monitor ingestion, absorption, and adequacy of dosage. An efficacy trial with oral propranolol in tetralogy of Fallot failed to produce the results obtained when the drug was given intravenously.[56] Recent blood-level studies suggest that the doses used in that trial were inadequate.[57]

The expanded clinical trials will tend to bring out more side-effects.[23] Some of these may be related to pre-existing pathology, such as the azotemia which may occur when blood pressure is lowered in the presence of renal disease, or the activation of peptic ulcer by corticosteroids, phenylbutazone and salicylates. Pharmacogenetics may play a role. Individuals deficient in glucose-6-phosphate dehydrogenase are subject to hemolytic reactions with such drugs as primaquine, pamaquine, sulfanilamide, and nitrofurantoin. Prolonged apnea has occurred in patients given succinylcholine when plasma cholinesterase was less efficient than normal.[58] As the clinical exposure increases, idiosyncratic and allergic reactions may begin to appear. Side-effects must be carefully recorded and their relationships to the test drug thoroughly documented. During Phase III such experiences are recorded on a special form (FD 1639). Unusual or serious side-effects must be reported immediately to the sponsor, who is obliged to notify the FDA within 15 days. In addition, the sponsor must send the FDA a status report on all clinical investigations at least once a year. Investigators who fail to supply reports may have to be deleted from the program.

Phase III can thus be characterized by protocol planning, solicitation of investigators, multipurpose and combined clinical trials, data collection and evaluation, chronic animal toxicity studies, and solidification of formulation, quality-control and manufacturing procedures. When all this is done, an encyclopedic document known as a New Drug Application is prepared and sent to the FDA to seek approval for marketing.

The New Drug Application (NDA) describes in detail all the chemical, pharmacologic, toxicologic, biopharmaceutic, manufacturing, and clinical information produced since the drug was synthesized. The document is organized into 13 sections (table 5). Section 1 is a table of contents. Section 2 provides a concise résumé of all the data being submitted in the NDA. This section is critical because it brings into focus the scientific rationale for marketing the test drug. In Section 3 the evidence for and against each recommended use is summarized. Side-effects are tabulated and qualified relative to significance and relationship to the drug. The statements made in Sections 2 and 3 include references to the detailed data provided elsewhere in the NDA. Labeling, Section 4, includes the package insert, which states the indications, contraindications, precautions, warnings, side-effects, and other information. Since the package insert sets the stage for the use, distribution and advertising of the new drug, this section is of considerable interest to both the sponsor and the FDA. Section 12, clinical studies, is of most interest to investigators. In addition to the results of the clinical trials, individually and collectively, complete case reports must be provided. These must include the age, sex, diagnoses, dosage, and results of clinical and laboratory studies

TABLE 5. Composition of a NDA

1. *Table of Contents.* Reference to volumes and pages in NDA where item is described in detail and summarized.

2. *Summary.* Summary demonstrating the application is well organized, adequately tabulated and coherent; includes chemistry, scientific rationale, references to IND and supporting documents, pharmacology, toxicology and clinical studies.

3. *Evaluation of Safety and Effectiveness.* Separate summary and tabulation of the favorable and unfavorable evidence for each claim, with reference by volume and page number to the complete data and reports.

4. *Labeling.* All material printed on container or in package insert: describes indications, mode of administration, contraindications, precautions and side-effects. Copies of proposed advertising material are included in this section.

5. *Limitation to Prescription.* Statement as to whether drug is, or is not, limited in its labeling to use by prescription only.

6. *Components.* Full list of the substances used in the synthesis and preparation of the new drug and its final dosage form.

7. *Composition.* Name and amount of each ingredient contained in the final dosage form and in a representative manufacturing batch formula.

8. *Manufacture, Processing and Packaging.* A full description of the methods, facilities and controls used in these activities. Includes physical facilities, qualifications of personnel, methods used in each step, analytical and other control procedures, record keeping, precautions to insure proper labeling and other information.

9. *Samples.* Representative samples of finished dosage forms and production batches provided so the FDA can confirm the control procedures and specifications described in previous sections.

10. *Preclinical Investigations.* Detailed reports of all studies made on laboratory animals, including facilities employed, qualifications of investigators, and reference to other preclinical safety and efficacy data known to the sponsor.

11. *List of Investigators.* Names and addresses of all investigators supplied with the drug, with reference to the location in the NDA where their work is reported.

12. *Clinical Investigations.* Reports of all clinical trials, including adequate information on each subject treated or used as a control. Clinical and laboratory data presented in detail. Beneficial or adverse effects identified as drug-related or not. Methods used to establish the identity and quality of drug substance used in each trial are described. Clinical safety and efficacy data from outside sources are included.

13. *Supplemental Applications.* Any change from an approved NDA, such as new claims, labeling revisions, or manufacturing changes, requires a new submission.

for each subject treated or used as a control. Both beneficial and adverse effects must be identified as drug-related or not. A Drug Experience Report (FD 1639) is submitted for each adverse effect, including such effects from comparison drugs. If efficacy and safety information from other sources, such as outside the United States or in the scientific literature, is known to the sponsor, it must be summarized in this section.

It is not unusual for a NDA to be hundreds or thousands of pages long, and for the three copies sent to the FDA to weigh hundreds of pounds. The FDA has 180 days to respond after a NDA is received. The response may be in the form of acceptance (unusual), "incomplete" relative to one or more sections (most common), or total rejection (also unusual). When a NDA is incomplete, a dialogue begins between the sponsor and the FDA in an attempt to resolve the differences. New data may be submitted in the form of amendments, supplements and reports. When the FDA is satisfied, the NDA is approved;

otherwise, the NDA stands as incomplete and subject to withdrawal by the sponsor or rejection by the FDA.

Approval of a NDA is not the end of research and record-keeping. Indeed, information about a drug is collected throughout its lifetime, and absolute certainty relative to safety and efficacy is rarely attained. One need only recall the controversies relative to anticoagulants in myocardial infarction and the preoperative prophylactic use of digitalis to appreciate this fact. It required a review of 850,000 cases to determine that the incidence of hepatic damage associated with the use of halothane is low,[59] but its exact incidence is still not clear.[60] During the first year a new drug is marketed, the sponsor must submit quarterly reports summarizing all new efficacy and safety information. This is done twice a year the second year and annually thereafter. Addition of new claims for use, changes in formulation, or revisions of manufacturing or control procedures requires supplemental applications. The extensive and stringent regulations which must be met during the development and marketing of therapeutic agents has produced a new kind of drug company expert, the manager of drug regulatory affairs. Finally, a word about cost and productivity. At a recent seminar, it was estimated that the total cost of developing a new therapeutic agent ranges from $2½ to $4½ million.[61] When the cost of unsuccessful compounds was included, the estimate increased to $10½ million. By way of contrast, the average research and development cost for a new drug during the 1950's was $1.5 million.[62] Furthermore, the number of new single chemical entities introduced in the United States declined progressively from 44 in 1958 to 11 in 1968.[62] It would appear that introducing a new drug into clinical practice under the prevailing circumstances is an expensive and low-yield procedure. Since many diseases are still with us, we have no choice but to continue and to make every effort to improve the procedure and the circumstances.

Summary

Preclinical activities with new drugs include confirmation of structure, pharmacologic evaluation, biopharmaceutics, drug metabolism studies, and acute and subacute animal toxicity trials. Quality control and production also require attention. Clinical investigation and marketing in the United States are regulated by the Food and Drug Administration. Prior to testing in humans, all available information must be sent to the FDA in a document known as an IND. The clinical phases of new drug development can be characterized by protocol planning, multiphasic clinical trials, data collection, and evaluation of results. Chronic animal toxicity studies, further drug metabolism investigations, and soldification of manufacturing and quality-control procedures are also carried out. Approval from the FDA for marketing is sought by means of a New Drug Application, which includes all available information in detail. The process is complicated, long and expensive, but must be continued in order to meet the therapeutic challenges still with us.

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Drugs

DRUG COMA The increasing use of diuresis and dialysis to treat patients with drug coma has created a need for rapid and detailed toxicologic information about the unconscious patient. A gas chromatographic method was used to screen plasma from 41 patients with suspected drug coma. In each of 37 a barbiturate, meprobamate, glutethimide, or a combination of these sedatives was found in sufficient concentration to explain the coma. In more than half of these cases, the histories available at the time of admission proved unreliable as guides for identifying the drugs causing the comas. Gas–liquid chromatography is uniquely suited for the rapid diagnosis of drug intoxication, since it permits simultaneous identification and measurement of a variety of sedative agents. (*Bloomer, A. A., and others: Rapid Diagnosis of Sedative Intoxication by Gas Chromatography, Ann. Intern. Med. 72: 223 (Feb.) 1970.*)

PRENYLAMINE LACTATE Prenylamine lactate (N-[3,3-diphenylpropyl]-methylphenethylamine) has been shown by double-blind techniques to have reduced the anginal attack rate from 6.1 to 4.2 per week in 12 subjects ($P < 0.01$). Prenylamine inhibits the uptake of norepinephrine by storage granules in sympathetic nerve endings. The 120–240-mg daily dose of prenylamine resulted in lower mean resting pulse rates than in control subjects (74 vs. 81), but did not affect blood pressure. No evidence of congestive heart failure or bronchospasm appeared in patients taking the drug for as long as two years. (*Cardoe, N.: A 2-Year Study of the Efficacy and Tolerability of Prenylamine in the Treatment of Angina Pectoris, Postgrad. Med. J. 46: 708–712, 1970.*)