Reports of Scientific Meetings

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Conference on Drug Metabolism

Studies of drug metabolism have become an essential part of many pharmacologic and toxicologic investigations. The number of investigators with familiarity and experience in this area is limited, and, at the suggestion of the Drug Research Board, workshops on principles and methodology in drug metabolism have been organized. The third such workshop was held June 24–28, 1968, at the University of California, San Francisco Medical Center.

In the introductory session E. Jorgensen noted that the distribution, metabolism, and biological effects of drugs are mediated by a variety of physicochemical factors, those related to stereochemistry and to solubility or partition behavior being among the most important. The Newman projection, in which a molecule is seen end-on along the C–C axis, is helpful in visualizing torsion around a single C–C bond and the effect on stereochemical positioning of critical groups in drug–receptor or drug–enzyme interactions. Cori-Pauling-Koltun space-filling molecular models were recommended. The partition behavior of molecules can be predicted using the approach of Corwin Hansch (J. Med. Chem. 11: 430, 1968), according to which the partition characteristic of a new molecule is the sum of the partition characteristics of the substituent groups.

C. Davison discussed factors affecting the distribution of drugs and methods of studying protein binding. The latter include equilibrium dialysis between a protein and buffer solution in a dialysis bag and the surrounding medium, ultrafiltration, crossing electrophoresis, and gel filtration. D. P. Rall showed how the foregoing applied to the absorption and elimination of drugs by the central nervous system. The pressure-sensitive one-way flap valves in the villi of the arachnoid granulations allow bulk movement of cerebrospinal fluid into the sinus while preventing the reflux of red blood cells. Perfusion studies, in which drugs are introduced into the lateral ventricle and a core of brain tissue is subsequently removed for measurement of drug content and distribution, indicate that the extracellular space available for diffusion in the dog brain varies from 12 to 19 per cent. Failure to visualize the space in electron micrographs is best explained as a fixation artifact. The tight cell junctions of the capillary endothelium demonstrated by Reese and Karnovsky (J. Cell. Biol. 34: 207, 1967) account for the pharmacologic blood–brain barrier. Analogous junctions in the placenta (Enders: Amer. J. Anat. 116: 29, 1967) explain the maternal–fetal barrier. Brain entry is favored by small size of molecules, high lipid solubility, low ionization at body *p*H and limited binding with proteins.

E. J. Cafruny outlined the role of the kidney in drug metabolism. Measurements using slices of renal cortex in a beaker of oxygenated medium are useful, but active transport in the tubules can be studies *in vivo* by the retrograde stopped-flow technique in which a catheter is tied into a ureter and a drug solution is introduced retrogradely into the renal tubular system under a pressure of 180 torr maintained for a few minutes. Recovery of the residual solution in fractions allows inferences concerning the extent and site of active transport and the tubular segment in which this occurred.

G. L. Plaa dealt with the biliary excretion of drugs. At least three transport systems can be demonstrated, one for organic acids, one for organic bases, and one for nonionic substances. Saturation of one of these does not affect the others. In the rat, biliary secretion declines 5–10 per cent with every 1 C drop in body temperature. Substances with molecular weights exceeding 300 tend to be excreted in the bile, those below 300 in the

urine. Nonpolar compounds have to be conjugated or metabolized to more polar, and hence more water-soluble, substances. H. C. Mandel summarized the major pathways of drug metabolism, including oxidations by liver microsomal enzymes (requiring O_2 and NADPH), reductions, hydrolysis and conjugation. The effect of metabolism is not always one of protection or deactivation. For example, heroin conversion to morphine enhances potency and toxicity; imipramine converts to the more active desmethylimipramine. The neonate has very little capacity to form glucuronides due to the low levels of microsomal transferase and uridine diphosphate. In consequence, drug levels safe for women near term may be dangerous to their babies; kernicterus can result. It remains difficult to predict the metabolic fate of a new drug.

G. J. Mannering's subject was oxidation and reductive reactions. He described recent work on the microsomal system responsible for many of the known biotransformations of drugs. Oxidation requires reduced NADP, magnesium and cytochrome P450, and extramitochondrial enzyme that cannot be solubilized and is therefore measured as the soluble carbon monoxide derivative. This enzyme is a terminal oxidase reacting directly with molecular oxygen. Several forms probably occur. Induction of the microsomal system accelerates drug metabolism and also raises the cell content of P450. Preparation SKF 525A prolongs the action of drugs such a barbiturates and meperidine because it is itself subject to metabolism and hence spares the metabolism of the other drugs.

A. H. Conney discussed induction of drug-metabolizing enzymes. More than 200 drugs that stimulate microsomal metabolism are known, but there is no obvious molecular feature to explain the similarity of their effects. The rate of metabolism of zoxazolamine has become a favorite method of measuring the intensity of stimulation of microsomal metabolism by other drugs. The rate-limiting step in hydroxylation of this drug is not yet known, but there is an associated increase in microsomal protein. In man barbiturates enhance the urinary excretion of 6b-hydroxycortisol and the metabolic inactivation of coumarin anticoagulants; a regime balanced while both drugs are administered will become unbalanced when pentobarbital is withheld. Enzyme induction is responsible for the growing insensitivity of insect populations to many insecticides. The possibility of a similar effect on the metabolism of oral contraceptives is being studied.

S. Riegelman presented an interesting new "mamillary" model of the kinetics of drug distribution (J. Pharmacol., June 1968). The model represents drug distribution and elimination as made up of a central compartment interchanging with one peripheral compartment. The central compartment includes the plasma volume but is larger than this because an injected solute penetrates rapidly into a much larger volume including the highly perfused tissues; these can be conceived mathematically to be part of the central compartment and are included in the first term. Metabolism and excretion are conceived to take place from this compartment. All the other interchanging pools can be represented mathematically by one additional term. As in other pharmacokinetic models, the volume of distribution is assumed to be constant; this volume can be calculated only if a steady state of equilibrium exists between compartments. The model provides a convenient means of using blood concentration data to calculate absorption, metabolism and elimination rate constants, important in evaluating drug toxicity, efficacy and dosage.

R. M. Featherstone described how x-ray diffraction techniques have been used to study the details of drug receptor interaction and illustrated this by remarkable evidence showing the precise location of the site at which a xenon atom locates in a myoglobin molecule. Ehrlich's dictum that "drugs are molecules and react only with other molecules" should be modified to "with *parts* of other molecules."

R. T. Williams discussed at length a topic that had been briefly alluded to by many speakers: the species differences that characterize the metabolism of many drugs. In the body a drug is usually metabolized in two phases: Phase I includes oxidations, reductions and hydrolyses. The products of phase I may proceed to the second phase, the reactions of which are syntheses. The reactions are catalyzed by enzymes, and qualitative and quanti-

tative variations in these enzymes explain many of the species differences. In some cases differences in the gut flora, causing differences in the elaboration of cofactors, are responsible. Metabolic reactions frequently do not go to completion, so that several products of the original compound may be excreted.

The influence of genetic factors was expounded by B. N. LaDu. Two ways of evaluating these factors were available: population studies and the analysis of individual differences. Both have to be done in man. For example, population studies reveal a bimodal distribution in the disappearance rate of isoniazid from plasma. Comparisons of identical twins with fraternal twins have shown that the basis of the difference is genetic; pedigree studies therefore were made; these revealed that two alleles at one locus are involved, the slow phenotype occurring in subjects homozygous for the recessive allele. Another example cited is the exceptionally low plasma cholinesterase activity that results from the presence of an atypical enzyme, a homozygous recessive trait present in 1 in 2,800 of the Canadian population, although many more are heterozygous carriers of the trait. In the homozygous the dibucaine number (per cent inhibition of hydrolysis of acetylcholine by 10^{-5} M dibucaine) averages about 14, in the heteroxygous about 60, as compared with a normal value of about 75. Several variants of the enzyme have been discovered.

J. J. Burns classified the application of drug metabolism in research as (1) study of species and individual or genetic differences, (2) formation of active and toxic metabolites, (3) impaired metabolism in the newborn or young, and (4) enzyme induction and inhibition. A drug may stimulate its own metabolism on chronic administration, as in the case of the antiarthritic agent phenylbutazone in the dog. This has important bearing on the design of chronic toxicity studies since the greatest toxicity is likely to be met after the early doses. He proposed that the study of a new drug should proceed in four steps: (1) acute and chronic toxicity tests in at least two animal species; (2) cautious initial clinical pharmacologic investigations in man, beginning with very low doses with measurements of plasma level, rate of metabolism, absorption, type of metabolite; (3) long-term chronic toxicity tests and detailed pharmacologic studies in animals; and (4) extensive clinical investigation. Burns argued that because of species differences, too much should not be required at stage 1 before a verdict of potential usefulness in man at stage 2 was returned.

B. B. Brodie's concluding seminar was a discussion of the biochemistry of drug effects. The systems on which drugs act can be thought of as transducers, for example, a sympathetic nerve ending transduces the nerve impulse into release of norepinephrine. Adverse effects are of two types: (a) an exaggeration of the desired action on the transducer, and (b) structural damage elsewhere. A classic example of the latter is thalidomide. The quantity of drug bound at a receptor cannot be measured, but in the absence of damage the binding is reversible. The quantity bound reversibly is related to the plasma level; where irreversible, covalent binding occurs, and plasma levels are no indication of the amount of binding. Tissue levels are not a guide to dosage unless the extent of reversible binding in tissue is known. At the same molar plasma concentration, a drug only 90 per cent bound to plasma proteins has five times as many therapeutically-active molecules as an analogue that is 98 per cent bound, and so may appear five times more potent.

The workshop was designed to provide a working knowledge of basic principles and methodology and to attract investigators into an important field where manpower is critically short. The organization of the workshop was flawless and bespoke an enormous investment of time by those responsible. As far as the metabolism of anesthetic drugs is concerned, knowledge is still rudimentary. A vast amount of work waiting to be done beckons investigators into this field.

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