Experimental Pharmacogenetics

PHYSIOPATHOLOGY OF HEREDITY AND PHARMACOLOGIC RESPONSES

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Preface

This monograph is an outgrowth of a chapter "Potentialities for and Present Status of Pharmacologic Research in Genetically Controlled Mice" published in Volume 2 of Advances in Pharmacology. While some of this material, with modifications, is repeated, much information on the hereditary aspects of the response to drugs in mice and a variety of other animal species has been added. It reviews heritable factors in animals recognized by the use of drugs and hereditary defects altering drug responses. Hereditary disorders that by virtue of their similarity to those of man may serve as useful models for further intensive investigation are presented in great detail regarding their physiopathology and biochemistry; also a great many "new areas" for future pharmacologic research are suggested. Therefore it is hoped that this monograph, while primarily addressing itself to research workers in pharmacology and genetics, may be of interest to investigators of problems in physiology, pathology, and biochemistry as well. In fact, because of the complexity of the subject, it has been necessary to assume on the part of the reader considerable familiarity with all of these scientific disciplines. It is hoped, however, that this will not discourage those whose backgrounds in any one of these areas are minimal.

HANS MEIER

August, 1963

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1. Introduction

A certain interdependence of drug action and heredity has long been recognized through the experiences of workers studying the genetics of bacteria, of human geneticists classifying man according to pharmacologic assays (e.g., taste), and of cytologists observing drugs that influence heredity. However, it was only because research in the broad aspects of pharmacology and genetics has been rapidly expanding in recent years, and by focusing greater attention on biochemical aspects, that a link has been established between the two disciplines. As a result a new science, termed "pharmacogenetics," has evolved. Pharmacogenetics is defined as the study in animal species of genetically determined variations that are revealed by the effects of drugs (Motulsky, 1958; Vogel, 1959). Since the total variation in the response to drugs is caused by both hereditary and non-hereditary forces, pharmacogenetics deals only with part of the modifying factors of drug action.

While mechanisms of heredity cannot operate in a vacuum—a gene must attain its expression within the framework of a material environment (Snyder, 1955)—it is sometimes difficult to separate environmental components from genetic ones. There are many environmental events which can operate prior to and after the time a gene begins to take its effect. These environmental influences also include the effect on the genetic background (the other genes) in which a particular gene attempts to exert its own effect. Among inherited characteristics of which environmental variations do not affect the ultimate expression of the end product are the blood groups of man and animals; however, the residual genetic background may modify their expressivity and penetrance.

Although the hereditary determination of drug reactions may not always be clear, the existence of hereditary influences is without a doubt. Observations in animals are advantageous because they may be subject to special analysis by breeding procedures. Since mutations

TABLE I

ANALOGOUS HEREDITARY DISEASES OF MAN AND ANIMALS[a]

Organ system and disease or anomaly	Man	Rabbit	Guinea pig	Rat	Mouse	*Peromyscus*	Cat	Dog	Pig	Cattle	Horse
Dermal system											
Albinism (albino series)	X	X	X	X	X	X	X	X			
Elephantiasis	X								X		
Hydroa aestivale	X										X
Hypotrichosis, atrichia	X	X		X	X	X	X	X	X	X	
Hypotrichosis/anhidrosis/anodontia	X										X
Ichthyosis	X				X					X	X
Keratosis	X	X									
Nervous system											
Ataxia	X	X			X	X					
Dystrophia muscularis	X				X						
Epilepsy	X	X			X	X					
Hydrocephalus	X	X		X	X						
Spina bifida	X	X			X						
Syringomyelia	X	X									
Sense organs											
Anophthalmos/microphthalmus	X	X	X	X	X			X	X		
Cataract	X	X		X	X			X		X	X
Coloboma	X	X		X	X						
Deafness	X		X		X		X	X			
Hydrophthalmos	X	X									
Retinitis pigmentosa	X			X	X			X			
Skeletal system											
Brachydactylia	X	X			X						
Calve-Perthes' disease	X							X			
Chondrodystrophia	X	X						X		X	
Harelip, cleft palate	X				X			X	X		
Oligodactylia	X				X						
Osteopetrosis	X	X									
Polydactylia	X		X		X		X	X	X	X	X
Syndactylia	X				X				X		
Split-hand	X						X				
Tibial aplasia	X				X						

[a] Nachtsheim (1958).

TABLE I (*Continued*)

Organ system and disease or anomaly	Man	Rabbit	Guinea pig	Rat	Mouse	*Peromyscus*	Cat	Dog	Pig	Cattle	Horse
Circulatory system											
Hemolytic jaundice	X			X							
Hemophilia	X							X			
Hydrops fetalis	X	X			X						
Spherocytosis	X					X					
Pelger's anomaly	X	X									
Urogenital system											
Kidney anomalies	X			X	X						
Digestive system											
Absence of second incisors	X	X									
Metabolism and endocrine organs											
Adiposity, obesity	X				X						
Diabetes	X				X						
Dwarfism	X	X	X	X	X				X	X	X
Porphyria	X								X	X	

identical to man have occurred in animals (Table I), it has been possible to establish colonies that simulate human groups in their response to drugs. One of the alternate purposes of this monograph is to demonstrate the feasibility of this approach. Animals in which the exact mode of inheritance of an hereditary disorder is established and the time of onset of the disease predictable lend themselves uniquely for studies on both therapy and prophylaxis.

While differences in response to drugs between individuals of a given species as well as between species have been considered impediments to the assessment of drug action, they are actually of value in several ways: in the general understanding of the metabolic fate of drugs, in the knowledge of the function of enzymes, and in the elucidation of possible and probable modes of action. Alterations of pharmacologic responses may result from multigenic differences between species, races, groups, breeds, and strains, but they may also be due to single genes or pairs of genes (e.g., affecting enzyme activities).

In the latter instance, the presence of (a) specific gene(s) would explain the special susceptibility of (man or) animals to certain drug effects. Since gene frequencies vary greatly between species and populations the incidence of different drug responses also varies (polymorphism).

II. Factors Influencing Drug Metabolism

From a recent analysis it becomes evident that a great many factors may markedly influence drug metabolism (Conney and Burns, 1961). Some of these include the effects of drug pretreatments on the metabolism of other drugs and the influence of drug action by either stimulating or depressing the activity of drug-metabolism enzymes in liver microsomes. The interaction of drugs and (hepatic) microsomes (endoplasmic reticulum) has been admirably reviewed by Fouts (1962); he points out the types of studies which might be most valuable and calls for the cooperation of biochemists, cytologists and cytochemists, and pharmacologists. One should add the geneticists to this list since there appears to be some correlation between endoplasmic reticulum structure and the levels of certain enzymes in the microsomes; this correlation is especially good for the drug-metabolizing enzymes. A decrease in smooth reticulum has been associated with a decrease in drug enzyme activity; conversely, preliminary results indicate that drugs such as phenobarbital which stimulate microsomal drug-metabolizing enzymes also have a marked effect on smooth reticulum as seen in electron micrographs. The effects of CCl_4 on certain microsomal enzymes involved in lipid metabolism have also been associated with change in endoplasmic reticulum structure. Such studies could be extended to other enzyme systems involved in steroid metabolism (C-20 keto reduction, etc.) and glycogen storage (epinephrine); also to drugs affecting hepatic microsomal drug metabolism, both drug-enzyme inhibitors, e.g., diethylaminoethyl diphenylpropyl acetate (SKF 525A) and stimulators, e.g., phenobarbital. In addition, triparanol (MER 29) (which influences cholesterol synthesis) and 3′-methyldimethylaminobenzene (a hepatic carcinogen) may affect the endoplasmic reticulum. Other factors influencing the drug metabolism are the physicochemical properties of the drugs themselves, e.g., pH and solubilities that alter absorption and excretion, structural molecular changes that influence penetrance into microsomes, localization of drugs in certain tissues,

displacement and strong or weak binding of drugs. None of these will be dealt with; however, certain physiologic factors (age and sex) will be considered in the following section.

A. Physiologic Factors: Influence of Age and Sex

Rats, guinea pigs, and rabbits are born without the ability to metabolize drugs (Williams, 1962); this specifically relates to the oxidation of drugs by microsomes and the formation of glucuronides and sulfates. In contrast, chick embryos already possess these synthesizing enzymes. This "arrangement" may have survival value since the chicken feeds freely from birth on while the newborn mammal drinks milk. It is of interest that the series of enzymes which metabolize aromatic amino acids appear in parallel with the times of reaching immunologic maturity (Knox, 1962).

In view of the fact that the guinea pig is born with a more advanced behavioral pattern compared with that of the rabbit and rat, it is noteworthy that the newborn guinea pig has the same concentration of brain amines as the adult, whereas the brain of the newborn rat has very low amounts of serotonin and catecholamines (Karki *et al.*, 1960). Regarding the questions as to whether the newborn lacks enzyme-forming systems or whether the enzymes are present in an inactivated form, the following observations are relevant: some rats show no sex difference in drug metabolism until they are 5 weeks old; it may be that sex hormones play a part in the production of enzymes. Also tyrosine transaminase which appears abruptly after birth in the rat and 12 hours later attains very high levels can be prevented from appearing by adrenalectomy at birth. This finding is interesting since adrenal cortical stimulation in the adult will also increase this enzyme very markedly (Sereni *et al.*, 1959). An example of an enzyme which is present in young animals in an inactive form relates to *p*-hydroxyphenylpyruvate oxidase (an enzyme in tyrosine metabolism); the active form of this enzyme is very low in the newborn rat but its inactive form is present in full amount and can be activated *in vitro* (Goswami and Knox, 1961). The presence of the inactive enzyme *in vivo* is proven because—if it is not fully active—a dose of tyrosine will result in the excretion of *p*-hydroxyphenylpyruvic acid. The fact that ethionine

does not block the conversion means tha. the enzyme is not synthesized *de novo* (from amino acids) but may be derived from a large precursor which is enzymatically inactive *in vitro* and *in vivo* until treated in a particular way (Goswami and Knox, 1961). Another possibility, namely the presence of inhibitors of drug metabolism, was suggested by the studies of Fouts and Adamson (1959) on drug metabolism in livers of baby rabbits; these included oxidation of hexobarbital, *N*-alkylation of aminopyrine, deamination of amphetamine, hydroxylation of acetanilide, etc.

While the most striking age-dependent changes in enzyme concentrations occur during the early part of life, certain senescent changes have also been observed. However, investigations of the mechanisms of the regulation of enzyme concentrations as a function of age must be supported by data on changes in the total number and proportion of different cell types within a tissue, and also changes in the enzymatic activity of particulates and in the number of particulates per cell. For example, in a study on senescent Sprague-Dawley rats, the concentration of succinoxidase in livers decreased similarly as the number of cells assessed by the concentration of deoxyribonucleic acid (DNA). Yet, since the concentration of DNA only estimates the total number of cells per unit weight, this measurement cannot properly indicate whether or not actual changes in enzymes occur (Barrows and Roeder, 1962). A decrease of succinoxidase in the renal cortex resulting from a loss of mitochondria during aging may be considered due to changes in enzyme activity of a given particulate within the cell. There seems to be, however, at least one change, increased catheptic activity, that occurs with age in rats. Increased catheptic activity, since it was observed without a comparable change in acid phosphatase activity, suggests that the number of lysosomes per cell did not increase with age, but rather that there is an increase in the catheptic activity of lysosomes.

It is clear that there are many facets to the biology of aging (see Shock, 1962); changes in body composition, electrolyte imbalance, and alterations in cellular structure and cell growth are but a few parameters that particularly relate to metabolism. Alterations in metabolism also lead to deviations in pharmacologic responses; virtually none have thus far been investigated thoroughly.

B. Genetic Factors: Species Dependence of Drug Metabolism

It has been known for quite some time in *microbial systems* that upon addition to a bacterial culture of an antibacterial agent, two changes occur. The first is adaptive and ensues very rapidly through "opening" of pre-existing minor metabolic pathways; the second, following selection of mutant strains possessing neutralizing enzymes, proceeds more slowly. Thus evolutionary forces can give rise to populations with a frequency of resistant enzyme systems very different from those of the ancestral type. A similar situation exists in higher animals and man, and the study of polymorphic systems provides the best approach. Evidently drugs are useful tools with which to investigate fundamentals of biochemical genetics and in particular enzymes that are controlled by allelic genes. In fact, genetically controlled drug reactions not only are of practical significance but may be considered pertinent models for demonstrating the interaction of heredity and environment in the pathogenesis of disease (Motulsky, 1958). Another facet of drug metabolism studies relates to the use of a drug as a substrate to uncover new enzyme systems (Kaplan *et al.*, 1960).

Polymorphic systems in *man* have developed for many drugs with differing proportions of phenotypes in various groups and races. The heritable factors recognized in man by the use of drugs have been summarized by Price Evans and Clarke (1961); in addition, a new book on pharmacogenetics also deals with human hereditary defects causing altered drug responses (Karlow, 1962).

The working hypothesis of making predictions from *animal* studies to man—assuming that many of the attributes of behavior found in man may also be observed in animals—has proved to be useful in drug screening and evaluative procedures despite the handicaps and limitations implicit in animal studies (Irwin, 1962). However, it is clear that in the case of drug metabolism, aside from dose effects, there is considerable species dependence (Fishman, 1961). For example, histamine may undergo acetylation, methylation, and oxidative deamination; the relative extent of these processes varies with both the animal species and the dose. While the amino acid, ornithine, is not used in metabolic conjugation by most species, it is employed almost exclusively by birds and some reptiles. The rabbit is a species which deacetylates

acetylated amino compounds only with great difficulty; evidence that acetylated compounds undergo deacetylation was reported for acetanilide in the dog, for a series of *N*-acetylsulfonilamides in the chicken, and for 2-acetamidofluorene in the rat. Perhaps physiopathologic significance relates to the enterohepatic circulation of phenols: thus, chloranystenicol in rats and morphine in dogs are excreted via the bile into the intestine as glucosiduronic acids from which they are freed by β-glucuronidase and may then be reabsorbed; this process may explain the origin of intestinal tumors of rats given 4-aminodiphenyl and its derivatives. The activity of β-glucuronidase is correlated with the action of certain hormones, e.g., estrogen-dependent fluctuations are recorded for both human mammary gland and vaginal fluid, rat preputial gland, seminal vesicle, estrus cycle, and mouse and rat liver. Other drug β-glucuronidase relationships pertain to the administration of menthol and borneol: an increase in the enzymes of liver, kidney, and spleen, but not of ovary, uterus, and pancreas, occur in the dog and mouse, respectively. Oral administration of *d*-glucosaccharo-1'4-lactose in mice (and also rat liver and kidney) strongly inhibits β-glucuronidase; other sugar lactones are only weakly inhibitory (Akamatsu *et al.,* 1961). Chlorpromazine metabolism is unquestionably complex; the total number of chlorpromazine metabolites is estimated to be close to 24. Both qualitative and quantitative differences are noted in the urinary metabolite pattern of man and dog (Goldenberg and Fishman, 1961); humans tend to favor the excretion of polar derivatives along with one or two major non-polar metabolites while dogs excrete less polar material, and the "blue" series is completely absent from dog urine. A unique feature of mouse liver has recently been recorded relative to the enzymatic degradation of azaserine; while absent from rat and pork liver, mouse liver contains an enzyme, serine-*O*-esterdeacidase which may be part of a detoxification mechanism and may give rise to toxic *O*-esters, possibly even azaserine (Jacquez and Sherman, 1962).

Recently, analogous to bacteria, mammalian somatic cells in *tissue culture* revealed differences in drug responses. Whether or not it will be possible to correlate an abnormal response *in vitro* with a similarly abnormal pharmacologic reaction *in vivo* remains to be elucidated.