ADVANCES IN

Pharmacology and Chemotherapy

VOLUME 7

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FOREWORD

We would like to congratulate Dr. Robert J. Schnitzer on his seventy-fifth birthday.

Dr. Schnitzer was born in Berlin on May 16, 1894. He studied at the University in Berlin, and received his M.D. degree in 1919. From 1919 to 1928 he worked as a bacteriologist at the Institut Robert Koch in Berlin, and soon became involved in chemotherapy studies which have remained his major life interest. From 1928 to 1938 he was head of the Chemotherapy Laboratory, Farbwerke Hoechst, Hoechst/Main; from 1939 to 1941 he worked at the Connaught Laboratories, University of Toronto, and from 1941 to 1960 he was Director of the Chemotherapy Department of Hoffman La Roche, Inc., Nutley, New Jersey.

Dr. Schnitzer was the first to institute extensive routine screening tests of new compounds on many different infections—metazoal, protozoal, fungal, bacterial, viral—and on experimental neoplasms. This led to numerous discoveries: the action of quinoline compounds on *Trypanosoma congolense* and *T. vivax* (a step toward the discovery of Antrycide); the production of sulfisoxazole (Gantrisin) and sulfadimethoxine (Madribon); and, perhaps the greatest of all, the contribution to the discovery of isoniazid, the most active and widely used compound for the treatment of tuberculosis. He has also made many other important contributions to the practical and theoretical aspects of chemotherapy, including the relationship of immunological phenomena and drug resistance.

Since 1960 Dr. Schnitzer has served as a Consulting Editor to Academic Press, and has been instrumental in initiating and organizing the treatise "Experimental Chemotherapy," and other works, particularly the *Advances in Chemotherapy*, of which the present volume is an amalgamation and continuation.

Dr. Schnitzer, who is still as active as ever, is now Professorial Lecturer at the Mount Sinai School of Medicine, New York. We congratulate him on his achievements to date and wish him many more fruitful years.

SILVIO GARATTINI
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PREFACE

An observer of the developments in the fields of pharmacology and chemotherapy during the last decade could not fail to notice that the artificial separation of the two areas of experimental research in drug action cannot be maintained any longer. As the study of mechanism of drug action gained in depth, the correlation of active compounds with the biochemically and genetically defined properties of the target cells moved into the foreground of quantitative evaluation. Interpretations beyond the cellular level by the specific functions of subcellular structures and their enzymes seem to offer general concepts which may include organ, neoplastic, and microbial cells. Moreover, interactions of the response of host cells with pathological and pathogenic cells are characteristic for many therapeutic and toxic effects regardless of the etiological factors involved.

These considerations among others induced the editors and publisher of *Advances in Pharmacology* and *Advances in Chemotherapy* to combine these serial publications in the hope that the wider scope of the new work will offer to a larger audience a more complete insight into the interdigitations of chemical and biological action and open the way to new experimental approaches.

This volume, entitled *Advances in Pharmacology and Chemotherapy*, contains articles on pharmacological topics in a strict sense, namely, the contributions by Vesell, Hammar *et al.*, Kizer and Bressler, and Collier, whereas the articles by Hartwell and Abbott, Browne, Jawetz, Perkins, and McFadzean are devoted to topics of chemotherapy.

We cannot conclude the Preface without announcing with deep regret that Dr. Parkhurst A. Shore has completed his term of editorship of the Advances. Unfortunately, the burden of his academic commitments makes it impossible for him to continue his editorial work. The success of the first six volumes of *Advances in Pharmacology* was due to a great extent to his dedication to the difficult task as editor, his foresight, and his understanding of the essential events in pharmacology. We thank him for a job well done.

August, 1969

SILVIO GARATTINI
ABRAHAM GOLDIN
FRANK HAWKINS
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Recent Progress in Pharmacogenetics

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I. History

In 1957 A. Motulsky and A. Vogel independently coined the term pharmacogenetics and laid the conceptual foundations for this new field (Motulsky, 1957; Vogel, 1959; Kalow, 1962). These two geneticists used the word to draw attention to several hereditary conditions then recently discovered through unusual responses elicited by the administration of various drugs. The examples available in 1957 were acatalasia, suxamethonium sensitivity, slow inactivation of isoniazid, inability to taste phenylthiourea, to smell hydrocyanic acid, and a self-limited hemolytic anemia occurring after ingestion of various drugs

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caused by glucose-6-phosphate dehydrogenase deficiency. Increasing exposure of large numbers of individuals to various therapeutic agents and development of sensitive techniques for measuring activities of drug-metabolizing enzymes enabled the elucidation of these entities. Much recent work reviewed below has been devoted to these genetically transmitted conditions but few new examples have been described.

Widespread use of drugs constitutes a notable change in our environment. The possibility that this widespread dissemination of drugs might reveal additional hereditary disorders characterized by abnormal responses to potent commonly used therapeutic agents was anticipated in 1957. However, these expectations have not been realized over the past 12 years. Only rare and isolated examples have been reported: Sporadic cases of sensitivity to dicoumarol (Solomon, 1968) and Dilantin (Kutt *et al.*, 1964a) and of resistance to warfarin (O'Reilly *et al.*, 1964, 1968) have been published.

Clearly, adverse reactions to drugs are common; few new therapeutic agents lack side effects, either on the skin, gastrointestinal tract, blood, central nervous system, cardiovascular system, endocrine organs or genitourinary tract. However, the hereditary nature of these untoward responses remains to be established. Perhaps in the future some cases of blood dyscrasias after chloramphenicol, antibody formation to penicillin and other drugs, extrapyramidal symptoms after prochlorperazine, or a syndrome resembling disseminated lupus erythematosus appearing after hydralazine administration will be shown to be familial and their mode of inheritance elucidated.

II. Definitions

The term pharmacogenetics was employed originally to refer to hereditary disorders revealed solely by the use of drugs. Use of pharmacogenetics in this sense appears to be too restrictive. It excludes from consideration several hereditary diseases previously well described but only relatively recently discovered to be exacerbated by the administration of drugs: (1) diabetes mellitus precipitated by adrenocortical steroids, (2) acute gouty attacks after thiazide diuretics, and (3) porphyria worsened by barbiturates.

At the other extreme, pharmacogenetics has been defined to include conditions in which drug responses are modified by hereditary factors. This definition lacks specificity.

Experience during the 12 years since the introduction of the term pharmacogenetics has led to some disagreement over what limits should be imposed on the term and over how significant pharmacogenetic conditions and concepts will prove to be in the future. In this review the term pharmacogenetics will be applied to clinically significant consequences of hereditary variations in the handling of drugs. This article deals mainly with an increasing body of data

on the defects in man enumerated above and only tangentially with the very large literature concerning experimental animals reviewed extensively and well earlier (Kalow, 1962; Meier, 1963).

III. Types of Response to Drugs

Search for hereditary variations affecting the way the body handles drugs has until recently turned up almost exclusively traits inherited as single factors; that is, traits produced by point mutations at a single genetic locus and transmitted either as Mendelian dominants or recessives.

Investigation of the responsiveness of the general population to a drug in terms of the amount of a drug required to produce a given effect may take the form of a continuous unimodal distribution curve or of a discontinuous polymodal curve (Fig. 1). Until recently, studies of drug responses that yield a

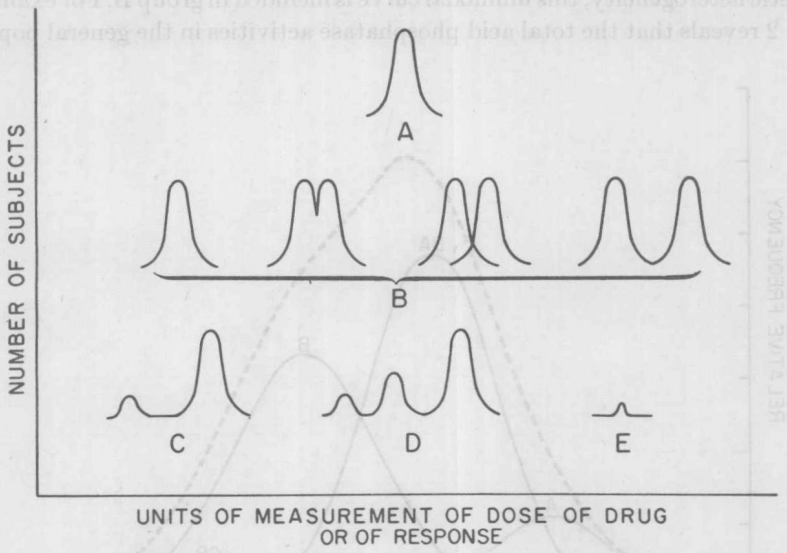

FIG. 1. Types of frequency distribution for the responsiveness of individuals to various drugs. Modified from Kalow (1962).

normal or continuous distribution curve have been almost entirely ignored in pharmacogenetic investigations. To construct unimodal, Gaussian distribution curves large populations are required. Furthermore, genotypes are hard to deduce from such curves. In contrast, discontinuous, bimodal, or trimodal curves of response obtained from disorders transmitted as Mendelian dominants or recessives are more easily analyzed because each discrete curve generally corresponds to a different genotype. In other words, the mutant

genes and their corresponding phenotypes segregate both in pedigrees and in the distribution curves. Figure 1 compares the normal or Gaussian distribution curve obtained for graded metrical characters, typically under polygenic control (curve A), with the discontinuous multimodal curves for traits transmitted as Mendelian recessives and dominants. Curve E, though unimodal and continuous, actually indicates a response by individuals possessing a genetically transmitted polymorphic trait, a response which normal individuals do not exhibit. Such examples include hemolysis in some individuals with glucose-6-phosphate dehydrogenase deficiency after administration of antimalarials, or acute arthritic attacks in certain individuals with the gene for gout after receiving thiazide diuretics, or abnormal blood glucose tolerance curves in individuals with diabetes mellitus after receiving steroids.

Figure 1 includes under group B a unimodal curve, although all other examples under category B are multimodal. Because it may actually conceal genetic heterogeneity, this unimodal curve is included in group B. For example, Fig. 2 reveals that the total acid phosphatase activities in the general popula-

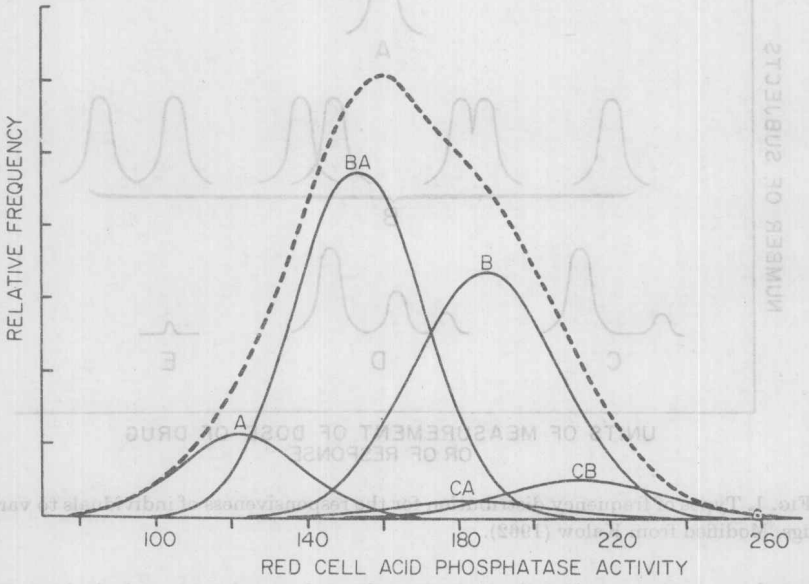

FIG. 2. Distribution of erythrocyte acid phosphatases in the general population (broken line) and in the separate phenotypes. The curves are reconstructed from data of Hopkinson *et al.* (1964) and are reproduced from Harris (1968).

tion superficially resemble a continuous unimodal curve, but in actuality this single curve is composed of five discrete curves representing five distinct

phenotypes and genotypes (Hopkinson *et al.*, 1964). The frequency of each of these five curves was determined in an English population by separating the total acid phosphatase activity of erythrocytes into isozymes by starch gel electrophoresis; relatively different electrophoretic mobilities and different distributions of total activity among the isozymes permit classification of individuals into one of the five phenotypes (Hopkinson *et al.*, 1964).

Motulsky (1964) reported genetic investigations of variations in the half-life of dicoumarol in human plasma. An approximately continuous distribution was observed (Fig. 3). Analysis of family data by the method of Fisher (1954)

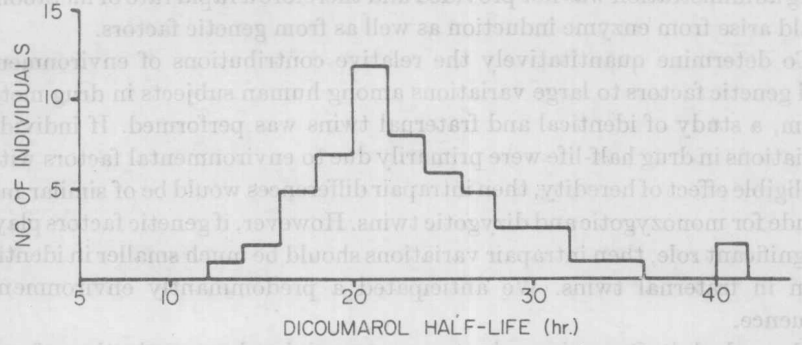

FIG. 3. Dicoumarol half-life in plasma in random subjects after a single oral dose of 2 mg/kg. (Reproduced from Motulsky, 1964.)

showed sib-sib correlations in the absence of sib-parent correlations. Attempts to fit the data to a single gene mechanism failed. To Motulsky these results suggested the operation of multiple recessive genes in controlling values for dicoumarol half-life in plasma. Such an interpretation is subject to the objection that young sibs may share a more similar environment than do parent and child. Thus sib-sib correlations in the absence of sib-parent correlations could result from the influence of environmental as well as genetic factors.

IV. Genetic Control of Drug Elimination from Human Plasma

Large differences in rates of elimination of phenylbutazone (Burns *et al.*, 1953), antipyrine (Brodie and Axelrod, 1950), and dicoumarol (Weiner *et al.*, 1950) from human plasma have been reported, but the basis for these differences was not established. Numerous environmental factors, such as exposure to inducing agents, degree of health or illness, and hormonal and nutritional status are known to alter the rates at which humans metabolize certain drugs. Several drugs such as phenylbutazone enhance their own metabolism (Conney,

1967). In mice, responsiveness to a drug such as hexobarbital differs according to age, sex, litter, painful stimuli, ambient temperature, degree of crowding, time of day of drug administration, and type of bedding (Vesell, 1968). Such experiments would imply that a large component in the causation of individual variations in human drug metabolism would be environmental.

Resistance to warfarin has been described in patients who metabolize the drug rapidly, with half-lives in plasma of 5.5 to 6.9 hours compared to normal values of 44 ± 10 hours (Lewis *et al.*, 1967). Abnormal plasma binding, anomalous apparent volume of distribution, and excretion of unchanged drug in the urine were eliminated as possible explanations; however, a history of prior drug administration was not provided and therefore a rapid rate of metabolism could arise from enzyme induction as well as from genetic factors.

To determine quantitatively the relative contributions of environmental and genetic factors to large variations among human subjects in drug metabolism, a study of identical and fraternal twins was performed. If individual variations in drug half-life were primarily due to environmental factors with a negligible effect of heredity, then intrapair differences would be of similar magnitude for monozygotic and dizygotic twins. However, if genetic factors played a significant role, then intrapair variations should be much smaller in identical than in fraternal twins. We anticipated a predominantly environmental influence.

Normal adult, Caucasian volunteers not receiving drugs at the time of, or for several weeks preceding, the study were typed for 30 blood groups to document the nature of their twinship. Each twin received a single oral dose of phenylbutazone (6 mg/kg); 2 months later a single oral dose of antipyrine (18 mg/kg) was given; 2 months later a single oral dose of dicoumarol (4 mg/kg) was administered. The half-lives of these three drugs determined in each individual are given in Table I (Vesell and Page, 1968a,b,c). Blood specimens were drawn at regular intervals after drug ingestion and the values for the concentration of drug in plasma plotted as shown in Figs. 4, 5, and 6. These curves illustrate for each of the three drugs tested typical examples of rates of elimination from plasma of identical twins and of fraternal twins. Half-lives of the three drugs, determined from these curves, appear in Table I.

The results clearly indicate that the major mechanisms for individual differences in rates of elimination of phenylbutazone, antipyrine, and dicoumarol are genetic rather than environmental. The contribution of heredity to the plasma half-life of these three drugs was estimated from the formula (Osborne and DeGeorge, 1959):

$$\frac{\text{Variance within pairs of fraternal twins} - \text{variance within pairs of identical twins}}{\text{variance within pairs of fraternal twins}}$$

Theoretically values derived from this formula could range from 0, indicating negligible contribution of heredity, to 1, indicating strong hereditary influence.