0031-6997/97/4904-0369$03.00/0
PHARMACOLOGICAL REVIEWS
Vol. 49, No. 4

Printed in U.S.A.

Pharmacogenetics in Biological Perspective

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I. Introduction

Pharmacogenetics is a field of growing interest in medicine and within the pharmaceutical industry. To physicians whose patients do not respond to drug therapy as expected, pharmacogenetics is increasingly a worry or a nuisance. Numerous drugs that tended to produce variable and sometimes serious responses had to be withdrawn from the market and thus became a loss to the industry. Today, many pharmaceutical companies attempt to minimize such losses by pretesting their products for metabolism, using genetically variable enzymes that may lead to response variations. These practical considerations are valuable but tend to produce a narrow view of pharmacogenetics.

This article provides a brief overview of pharmacogenetics, emphasizing those features that make it a part of population biology rather than merely an item that is from time to time of medical concern. Pharmacogenetic variation is truly important with respect to the adaptability and survival of populations. Pharmacogenetic diversities are a precondition for Darwinian selection.

To present pharmacogenetics from a biological point of view, we start by avoiding confusion with a linguist's look at the word "pharmacogenetics." This is followed by a bird's-eye view of examples of pharmacogenetic variations in humans, insects, and bacteria. Next is a comparison between responses to toxicants and to carriers of infections. We raise the question of the biological cost of pharmacogenetic variation. Section VI. includes a discussion of some quantitative aspects of pharmacogenetics, contrasting monogenic and gaussian variation and recommending a new way to estimate heritabilities. In the final discussion, we consider pharmacogenetic variation in the context of evolution.

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II. The Word "Pharmacogenetics"

The broad applicability of pharmacogenetic principles to all forms of life (e.g., bacteria, insects, mammals, and plants) is sometimes missed for linguistic reasons; to ancient Greeks, the word "pharmakon" meant magic charm, drug, or poison (*Webster's*). Hence, a meaningful translation of the word could be "xenobiotic," indicating a biologically active material formed outside the host's body (in contrast to an endobiotic, e.g., a hormone). In modern life, however, the meaning of the prefix "pharmaco-" is often equated in a narrow sense with medicine or drug. Hence, geneticists and other scientists sometimes referred to "ecogenetics" when concerned with variable response to environmental chemicals. Other terms used are "toxicogenetics" or "environmental genetics." Agriculturists concerned with insecticide resis-

tance or herbicide resistance, or microbiologists concerned with bacterial resistance to antibiotics, are indeed dealing with a pharmacogenetic phenomenon, but in their communications, they tend to refer to response variations without invoking pharmacogenetics.

Another restriction in customary use of the word "pharmacogenetics" lies in the fact that, at present, it almost always refers to monogenic variants. The old twin studies of Vesell and colleagues (1968, 1992) gave the best experimental indication of strong genetic components in drug elimination, although no specific components of variability were known; Vesell calculated heritabilities. Such heritability data are clearly part of pharmacogenetics. Thus, in the present context, pharmacogenetics refers to any kind of inborn variation in any group of creatures in response to xenobiotics. This definition excludes the induction of mutations by xenobiotics and the research by modern industry that uses DNA sequences of receptor proteins to develop new drugs.

III. The Nature of Examples

When humans apply chemicals to bacteria, insects, or other "pests" such as rats and mice, it is usually with the purpose of killing them. The usual purpose of applying chemicals to humans is to support life or well-being. There is no reason to assume that such differences in purpose will affect the principles that govern the interactions between biological systems and chemicals in the form of drugs or toxicants. Human data provide the most detailed measurements and records of pharmacogenetic differences between individuals and also between populations, but they are biological snapshots. Toxicological data in bacteria cover thousands of generations, in insects, dozens of generations. Therefore, they are better suited than human studies to reveal principles that relate to survival of a population or of a species, as distinct from survival of the individual.

A. Human Data

1. Pharmacokinetic compared with pharmacodynamic variation. Numerous physicians and investigators have devoted efforts to the study of therapy-related clinical pharmacogenetics. Genetic deviations from the common response to a given chemical may be due to alterations of the drug target [e.g., receptor systems (Propping and Nothen, 1995; Vernier et al., 1995)], or abnormalities of the drug's fate in the body [e.g., drug metabolism (Kalow, 1992b; Pacifici and Fracchia, 1995)]. In most cases so far, genetically controlled exceptional reactions to commonly used drugs were due to the variation of drug-metabolizing enzymes (Daly et al., 1993; Kalow, 1993; Price Evans, 1993; May, 1994). It is not clear whether this prominence is incidental to the development of analytical techniques; the chemical methods to determine drug metabolism are older than the molecular techniques used in the study of drug receptor variations. However, it is possible that receptor variations tend to be rarer because they are often associated with pathology, a factor that would reduce their frequencies in a population.

Mammalian drug-metabolizing enzymes consist of several classes that together are able to metabolize almost every chemical to which the body is exposed. The development of enzymes capable of metabolizing most chemicals may represent coevolution of plants and herbivores (Gonzalez and Nebert, 1990). In humans, approximately 3 dozen of the enzymes that metabolize foreign compounds have been shown to be genetically variable. Most variants show deficiency but some show excessive activity (Kalow, 1992b; Pacifici and Fracchia, 1995).

Besides structural variation of the enzymes that metabolize xenobiotics, there may be variation in the control of enzyme levels. A special case, originally observed in mice (Conney, 1967; Nebert, 1979) and still under study in humans, is variability in response to enzyme inducers via the Ah receptor (Okey, 1992). Because the Ah receptor controls several enzymes, it is interesting to think that a single variant could control several products.

Ziegler (1991), in his B. B. Brody Award Lecture, emphasized the general principle that the "enzymes of detoxication" are more variable than the "enzymes of biosynthesis". This is a shared impression (Kalow and Grant, 1995), but objective counts of variability are not yet available.

Genetically determined variations in drug-metabolizing enzymes are of obvious importance in the presence of drugs that are substrates for these enzymes. It is not always evident, however, whether or not such variation has important biological consequences in the absence of these drugs (see Section VI.). This is true of some of the best-studied pharmacogenetic variations, such as the example of CYP2D6[b] (debrisoquine hydroxylase) given below.

2. The example of debrisoquine hydroxylase deficiency. An illustrative example from human pharmacogenetics is the deficiency of the cytochrome P450 CYP2D6 (Balant et al., 1991; Meyer et al., 1992; Eichelbaum and Gross, 1992). This example is chosen because of the extent and the intensity of investigations devoted to it. At least six different mutations lead to a sufficiently faulty gene so that the enzyme is not formed and thus is absent (Kalow and Grant, 1995). CYP2D6 participates in the metabolism of over 40 drugs. The consequences of deficiency depend on the metabolized chemical; the deficiency may cause an exaggerated, even fatal, response because of failure of drug elimination (e.g., perhexiline, sparteine), or it may cause a lack of response because of a failure of pro-drug activation (e.g., codeine not con-

[b] Abbreviations: CYP2D6, debrisoquine hydroxylase; DDT, dichlorodiphenyltrichloroethane.

verted to morphine), or the effect may be negligible because there are alternative processes that compensate for the genetically altered ones. Recessive enzyme deficiency is like the tip of an iceberg (Kalow and Bertilsson, 1994): The heterozygotes average half of the wild-type enzyme activity.

Functional CYP2D6 is absent in 7% of Caucasians and in approximately 1% of East Asians, a typical case of ethnic differences. A distribution of functional capacity of CYP2D6 in four human populations is shown in figure 1 (Kalow, 1991). There are differences not only in enzyme deficiency. Besides variation within each population, differences of enzyme activity between populations are evident. The average enzyme activities of both Africans and Chinese are lower than in both European populations, but the enzyme variants are not identical (Kalow and Bertilsson, 1994; Johansson et al., 1991; Masimirembwa et al., 1993; Masimirembwa, 1995). The main CYP2D6 difference between Spaniards and Swedes is a more frequent gene duplication in the former (Dahl et al., 1995), leading to more cases with very high enzyme activity; this almost certainly represents North African admixture (Aklillu et al., 1996).

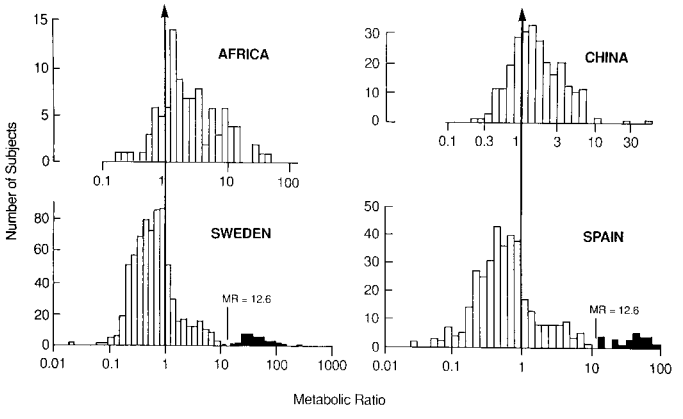

FIG. 1. Frequency distributions of the activities of CYP2D6 (debrisoquin hydroxylase) in four human populations. The abscissa indicates, on a logarithmic scale, the ratio of debrisoquine/4-OH-debrisoquine in urine after administration of a test dose of debrisoquine; these are conventional plots in which the increasing ratios reflect decreasing metabolic rate. Each of the four inserts represents an adaptation of a published illustration. Their abscissas are comparable, and the graphs have been aligned for metabolic ratios of unity. The insert marked "China" represents a study of 269 Han (Lou et al., 1987), "Africa," a study of 92 Venda (Sommers et al., 1989), "Sweden," a study of 752 Swedes (Steiner et al., 1988), and "Spain," a study of 377 Spaniards (Benitez et al., 1988). Comparability of the measurements has been assured: for the Venda data from Africa, only recently, and indirectly by investigations of the Shona in Zimbabwe (Masimirembwa et al., 1993). In the meantime, the phenotypic population differences shown here have been defined at the DNA level (Masimirembwa 1995). High enzyme activity due to gene duplication is more common in Spain than in Sweden. [In the publications from Sweden and Spain, subjects with a metabolic ratio >12.6 ("poor metabolizers") were indicated by black bars. MR, metabolic ratio. Parts of figure reprinted with permission from the following: Kalow, W.: Interethnic variation of drug metabolism, Trends Pharmacol. Sci. 12: 102–107, 1991; "AFRICA": Sommers, D. K., Moncrieff, J., and Avenant, J.: Non-correlations between debrisoquine and metoprolol polymorphism in the Venda. Hum. Toxicol. 8: 365–368, 1989; "CHINA": Lou, Y. C., Ying, L., Bertilsson, L., and Sjoqvist, F.: Low frequency of slow debrisoquine hydroxylation in a native Chinese population. Lancet 2: 852–853, © by the Lancet Ltd., 1987; "SWEDEN": Steiner, E., Bertilsson, L., Sawe, J., Bertling, I., and Sjoqvist, F.: Polymorphic debrisoquine hydroxylation in 757 Swedish subjects. Clin. Pharmacol. Ther. 44: 431–435, 1988; and "SPAIN": Benitez, J., Llerena, A., and Cobaleda, J.: Debrisoquine oxidation polymorphism in a Spanish population. Clin. Pharmacol. Ther. 44: 74–77, 1988.

A new approach has shown the gene to be more variable than previously thought. Marez et al. (1997), using polymerase chain reaction-single–strand conformation polymorphism analysis, screened 672 subjects of European descent and identified 48 point mutations, 29 of which were novel. Because of doubling or tripling of some point mutations, there were 53 enzyme variants. The functional significance of any of the new variants has been tested by phenotyping the carriers with either debrisoquine, sparteine, or dextromethorphan. Sixteen genotypes were found to be poor metabolizers, and three cases displayed genotype-phenotype discrepancies. These results illustrate the power of the new technique; we have to count on prominent variation of many enzymes and other proteins, and genotyping and phenotyping of individual subjects may never completely agree. Today, it is technically easier to identify a new point mutation than to investigate its function. Among functional tests, absence of enzyme activity is much more readily demonstrated than K_m variation, which may alter only the enzyme specificity.

Nothing is known about any particular advantage or disadvantage of any CYP2D6 variant. Because CYP2D6 occurs not only in liver but also in brain (Tyndale et al., 1991), it might affect some personality traits (Bertilsson et al., 1989; Llerena et al., 1993), and this might conceivably affect fitness and, thereby, frequency of the variants. Most pharmacogenetic differences currently known have not yet been shown to have any nonpharmacological effects that could affect their prevalence and their biological importance. To gain some insight into this question, it is necessary to look at other examples for which the biological consequences have been more clearly defined.

3. Epidemiological implications of kinetic variants. The consequences of drug metabolism as terminator of the action of medicines is a matter of daily observation by patients and physicians. The need for drug-metabolizing enzymes with functions that are unrelated to drug therapy may be less obvious. However, the need is clear in the case of the exposure to natural toxins, which can be catastrophic. Exposures of populations to some mycotoxins (fungus-produced toxins; World Health Organization, 1979; Jelinek et al., 1989; Bhatnagar et al., 1992; Kwon-Chung and Bennett, 1992) have been incisive medical events of epidemiological proportions in human history. Examples are the different epidemics of ergot intoxication (Bettmann, 1956); one was characterized by

gangrene of hands and feet and known as "St. Anthony's Fire" in the Middle Ages in Europe, the other by high frequency of abortions. It is not known which human drug-metabolizing enzymes would be able to attack the ergot alkaloids, but the variability of such enzymes must have affected survival in these epidemics.

A high level of activity of a drug-metabolizing enzyme is not necessarily a safeguard. A modern case is the identification of aflatoxin and the discoveries of aflatoxin-caused liver cancers (Lillehoj, 1992). Drug-metabolizing enzymes play a role in both the bioactivation and destruction of aflatoxins (Guengerich and Kim, 1990). Depending on the balance between these two processes, increased drug-metabolizing activity may actually increase the risk of aflatoxin-induced cancer.

The mycologist Stormer (1992) cited various periods of high mortality and low fertility in England since 1541 that could be ascribed to mold poisoning. He concluded that the sudden increase in the size of the English population after 1830 cannot be explained by an improvement of sanitation or medical care. There was a dramatic increase in potato consumption in the diet of the poor. The increase has been traditionally ascribed to improved nutrition by the introduction of potatoes. The modern mycological explanation is that the potatoes did not provide more calories than did the customary grains, but that potatoes contain fewer fungi than untreated grains always do. (In modern times, fungal growth is prevented by predrying the grains before storage.) The implication is that the toxins produced by fungi present in the cereal grains in England during the last 400 years sometimes killed enough people to significantly diminish population size and thereby, in the long run, impaired population expansion.

We do not know specifically the toxic chemicals nor the drug-metabolizing enzymes that must have been involved; however, there is no doubt that human biochemical defense systems must have been called into action. If so, it is an example to show that drug-metabolizing enzymes are not merely serving one drug-exposed patient, but that they serve a population. One may speculate that some people had better defense systems than others, but there is nothing to indicate any development of tolerance to the fungal toxicants in the population; perhaps the toxic impacts were not strong enough for such an effect to become visible.

B. Toxicant Resistance in Arthropods and Bacteria

In summarizing the principal aspects of resistance by bacteria to antibiotics and by arthropods to pesticides, Graham-Bryce (1987) wrote, "Untreated populations of organisms can be assumed to be so large that they will contain, as a result of mutation, individuals capable of withstanding toxicants applied at a rate which will kill the great majority (i.e., the resistance is preadaptive)".

1. Arthropods. Arthropod resistance to pesticides poses serious problems in agriculture (Georghiou and Saito, 1983; Ford et al., 1988; Roush and Tabashnik, 1990). To retain susceptibility to pesticides in agricultural practice, the times and kinds of chemical exposure and measurements of resistance may follow sophisticated schedules (Forrester et al., 1993). The large variety of types and kinds of pesticides used against the large variety of living targets is associated with many different modes of resistance that range from alterations of toxicant metabolism to changes of toxicant target. Moderate degrees of resistance may represent enzyme induction, a reversible process without heritable consequences (Okey, 1992). However, the agriculturally important high resistance levels of whatever cause must be due to multiplication of initially uncommon, naturally insensitive individuals who survive insecticide exposure and who reproduce in spite of continuous exposure.

An old example is dichlorodiphenyltrichloroethane (DDT) resistance (Brown, 1958). On continued exposure of houseflies to DDT, some individuals who were able to rapidly metabolize DDT survived and became the parents of a resistant strain. In many cases, the resistance was due to the flies' rapid metabolic dechlorination of DDT to form dichlorodiphenyl-dichloroethylene. In 1971, Brown made the summarizing statement, "In laboratory strains of houseflies that are not yet pure for DDT-resistance, the susceptible minus-variants outgrow the resistant plus-variants because they complete their larval and pupal developments slightly faster". In other words, if DDT is absent, this retardation of development in the individuals carrying the gene for resistance causes the resistance to disappear from a fly population within a few generations. However, after 30 generations of laboratory breeding in the continuous presence of DDT, the DDT resistance persisted in spite of termination of the DDT exposure.

Roush and McKenzie (1987) indicated that some of these traditional data have to be viewed with reservation. In their review they state that resistance evolved in the laboratory tends to be multifactorial and is often seen to be clearly associated with decreased fitness, fitness being defined in terms of fertility by counting the offspring produced. After relaxation of the selection pressure, resistance tends to revert because of the fitness deficit. Resistance developing in field strains typically involves selection from much larger numbers than in the laboratory. This tends to produce a higher specificity of resistance that will often be monogenic. If termination of exposure is followed by loss of resistance, it is mostly by dilution, i.e., by immigration of unexposed and therefore nonresistant individuals, a factor that is difficult to control and quantify under field conditions.

A modern set of field data is shown in figure 2. It illustrates the development of resistance of an Australian moth (*Helicoverpa armigera*) to two different pesticides over a period of 12 years in three different regions (Forrester, 1996, personal communication). Pyrethroids are complex organic chemicals derived from a natural

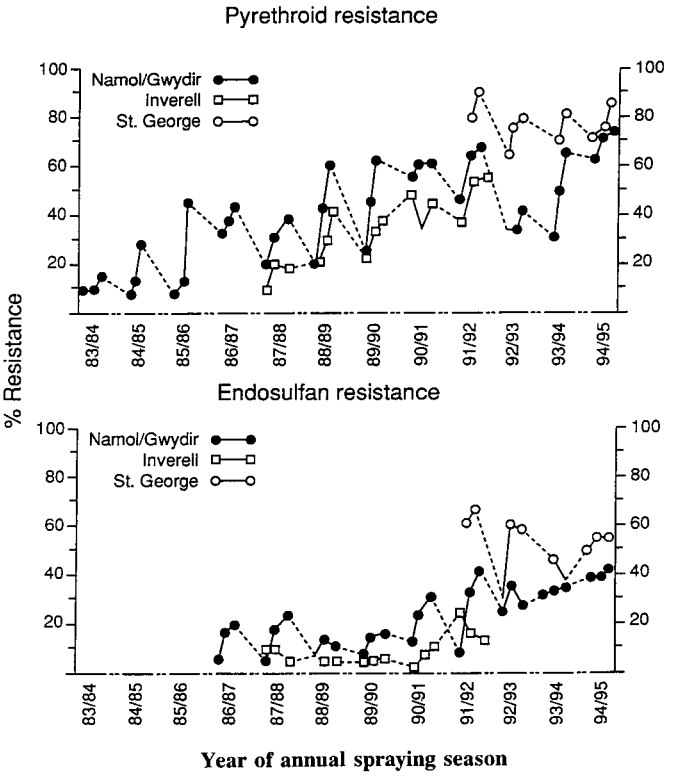

FIG. 2. Development of pesticide resistance of a lepidopteran insect (*Helicoverpa armigera*). The abscissas indicate 12 years of fully monitored, seasonal pesticide application, a pattern designed to slow the development of resistance. The ordinates indicate the pesticide resistance measured in percent of larvae surviving the discriminating dose of a synthetic insecticide; the larvae were raised and tested in the laboratory from moth eggs collected from sprayed plants. The dashed lines indicate the periods without spraying. The insecticides are pyrethroid (derivative of pyrethrum, a plant-produced insecticide) and endosulfan (a polychlorinated synthetic). Namoi/Gwydir, Inverell, and St. George are adjoining districts in Australia. See text for further information. This represents unpublished data by courtesy of Dr. Neil W. Forrester from New South Wales Agriculture, Australian Cotton Research Institute, Myall Vale via Narrabri, NSW, Australia (1990).

plant-produced insecticide; endosulfan contains a fully chlorinated ring structure. The data reflect closely controlled, seasonal applications of the insecticides and precise measurements of resistance by collecting moth eggs from the sprayed plants, rearing the larvae, and testing their responsiveness to a lethal dose in the laboratory; the tests are timed to always target a new generation of moths. The dashed-line portions of the graphs indicate the times of seasonal interruption of insecticide spraying; the loss of resistance during this period mostly reflects immigration of moths from unsprayed areas. This immigration tends to fade toward the end of the recording, indicating that the abundance of unaffected moths capable of migrating into the sprayed area is gradually diminishing.

Because each measurement of larval survival is applied to a new generation of moths, and in view of the short generation times of the moths in comparison with the slow, steady increase in resistance over a period of approximately 10 years, the resistance development can only be genetic. The resistance to endosulfan developed more slowly than that to the pyrethroid. The growth of the initial resistance may involve the gradual elimination of susceptible individuals and effects of breeding to create homozygotes from the initially heterozygous resistance genes, and there might be independent adaptive processes. In any case, the proportion of resistance-conveying genes could have been initially small with all these mechanisms.

2. Bacteria. The development of resistance of bacteria to antibiotics over the past 30 years has been a process of "stunning effectiveness" (Bennett, 1995). The resistance has reached proportions that put in question the future utility of antibiotics for treating infections. Resistance has become a problem that calls for new pharmaceutical solutions (Service, 1995). For many years already, there has been bacterial resistance to all clinically used antibiotics (Levy and Novick, 1986), although some resistant strains fortunately are still isolates (Levin, 1995).

Whichever the mechanism of resistance (Bryan, 1995), there is a fundamental distinction between resistance by chromosomal mutation (changes in the base sequence of DNA) and resistance carried by plasmids (extrachromosomal genetic elements that replicate independently of the chromosome; Levin, 1995). Chromosomal mutation may or may not be associated with a decrease of general fitness; it varies from case to case. LeClerc et al. (1996) found hypermutation in several strains of nonlaboratory *Escheria coli* and salmonellas collected in the wild; hypermutation provides special opportunities for developing chromosomal antibacterial resistance and for adapting to new hosts. Bennett (1995) raised the interesting point that most antibiotics are native or modified products of bacteria or fungi that must have been naturally resistant to their own products. DNA of some of these resistant genes was found in preparations of antibiotics. If this resulted in a DNA transfer to bacterial targets of therapy, it could enrich a plethora of resistance mechanisms.

Plasmid-determined antibiotic resistance is the most common, although it comes regularly with a modest biological cost, as reflected by lower rates of multiplication of the plasmid carriers. A bacterium may carry several plasmids (Bennett, 1995). Plasmids can be transferred even between different bacterial species so that clinically important resistance tends to persist as long as there are some carriers of appropriate plasmids (Russell and Chopra, 1990).

The current bane of therapy are the R-plasmids conveying multiple antibiotic resistance. Levin (1995) writes "it seems almost certain that the ancestors of contemporary R-plasmids and the transposable elements responsible for the movements of resistance genes to and from plasmids existed before the human use of

antibiotics". It seems that mechanisms of antibiotic resistance in bacteria are often more complex and of a greater variety than is pharmacogenetic variation in the more complex forms of life.

IV. Comparing Intoxication and Infectious Disease

"[I]t is an advantage to a species to be biochemically diverse∞For the biochemically diverse species will contain at least some members capable of resisting any particular pestilence" (Haldane, 1949). If we replace the word "pestilence" with "toxicant," we have a case description of what has been called pharmacogenetics, that is, the lore of inborn dissimilarities in response to xenobiotics. Let us compare infectious diseases and intoxications.

Different life spans of bacterial pathogens and mammalian hosts result in a complex pathogen-host coevolution (Brunham et al., 1993). Bacterial pathogens are able to modify their virulence and will change defensively with time to accommodate different host antigens. Thus, the timing of the most visible antipathogen defenses of the host is geared to the life span of the pathogen; the immune system has to adapt rapidly to the entry of an infectious agent (Langman, 1989; Schultz and Lerner, 1995).

In addition to the anti-infection defenses controlled by the immune system, there may be genetic variations in the host that have consequences that are relevant to the host's life span. In many aspects, this latter type of variation is comparable to pharmacogenetic variation of the host. Four examples can be offered.

The best known example is the protective effect of genetic deficiency of glucose-6-phosphate dehydrogenase against malaria (Beutler, 1993; Luzatto and Mehta, 1995). This particular deficiency happens to be relevant also to pharmacogenetics; it was discovered in the attempt to explain ethnicity-related primaquine hemolysis. Approximately 400 genetic variants of that enzyme are known. Also, a particular human lymphocyte antigen determined at locus A is associated with survival of infection with malaria (Hill et al., 1991).

Against tuberculosis, there is host protection by a genetic variant affecting monocytes in different mammals, which is effective before the immune system comes into play (Vidal et al., 1993). It is not yet clear to what extent this protection determines different rates of tuberculosis in different populations.

In mice, the gene for cystic fibrosis has been shown to convey resistance to cholera (Gabriel et al., 1994); it is possible that the high frequency of cystic fibrosis in human populations is a consequence of resistance of the heterozygous carriers of the gene to intestinal infections that have often been fatal in childhood.

Approximately 1% of Caucasians are immune to infection with the HIV virus and thereby are protected against acquired immunodeficiency syndrome (AIDS; Huang et al., 1996; Dean et al., 1996). The cause of immunity is homozygosity for a 32-nucleotide deletion within the chemokine receptor 5 gene. Infected heterozygotes show a delay in the development of AIDS. There is also human immunodeficiency virus resistance in African populations but by an obviously different (yet unidentified) mechanism.

It seems that one can think in terms of a parallelism between pharmacogenetic variation and genetic host variation against infectious diseases. However, although the immune system may have to cope with bacterial adaptations, this is not necessary when the host is exposed to a xenobiotic that is a clearly defined chemical. Hence, pharmacogenetic variability is principally simple when compared with the events that may follow the entrance of a pathogen into a host's body.

V. The Biological Cost of Variation

Does pharmacogenetic variation come with a biological cost? Cost in biology is measured in terms of reductions of fitness, whereby fitness is assessed by the number of offspring. It is instructive to raise this question, because many elements of pharmacogenetics have to be considered when looking for an answer.

The answer requires, in the first place, a specification of what is meant by pharmacogenetic variation. The question can apply usefully only to monogenic variation. There it is appopriate to make a distinction between rare and polymorphic variants. The distinction means, in principle, a dealing with two sources of variants. Rare variants may be the result of fresh mutations. Polymorphic variants occur by definition more frequently than could be expected from fresh mutations; their frequency is arbitrarily defined as $>1\%$. In other words, there must be some factor that helps to maintain polymorphic variants in a population.

Balanced polymorphism (Ford, 1940; Cavalli-Sforza and Bodmer, 1971) is a concept that was introduced to explain the high frequency of some genetic variants in a population. It means that a variant gene may be detrimental in homozygous double dose, whereas it may increase fitness in heterozygotes. The gene frequency in a population is determined by the balance between heterozygote advantage and homozygote disadvantage. The classical example is sickle cell hemoglobin; the homozygotes tend to die young from sickle cell disease, and the heterozygotes survive malaria better than do nonaffected persons. The sickle cell gene stays in a population only in areas with malaria.

The general principle displayed by this example means that the benefit or disadvantage of a given allelic gene does not represent an unequivocal or single value. In heterozygous form, the variant may be beneficial while disadvantageous in homozygous form (an independent experience resulting from this situation is hybrid vigor). When considering this principle, one might also think of the Hardy-Weinberg law that the proportion of

heterozygotes in a population will always be larger than that of homozygotes. This means that, in any population, the number of alleles in heterozygous form will always be larger than those in homozygous form (except when the gene frequency is 50%).

Pharmacogenetic variants are preadaptive in the sense that they occur before there has been any exposure to the drug. It is a different matter that each variant is noticed only after exposure to a xenobiotic; most exposures are unpredictable events. Because there are innumerable xenobiotics, pharmacogenetic variability must represent a multiplicity of variants. The majority of these variants must be present in heterozygous form and thus may tend to increase rather than decrease the fitness of a population.

Kimura (1968) introduced the now important concept of neutral mutations to indicate that not all polymorphic variants must represent "balanced polymorphism". If a new mutation is disadvantageous, it upsets an existing equilibrium and tends to be eliminated by the forces of selection. If a new mutation survives in an organism, it is likely more or less neutral, perhaps causing slightly decreased fitness (Cooper et al., 1995). The multiplicity of variants that represent pharmacogenetic variability in a population must be expected to be near neutral, besides the fact that they mostly occur in heterozygous form. Their presence should not mean much fitness reduction of the population.

In conclusion, pharmacogenetic variability will not be expected to mean any substantial biological cost to a population. This fits with the observations that most variants have no visible effect if the xenobiotic is absent and that many variants are polymorphic. Furthermore, any variant if rare and if somewhat disadvantageous would not strongly affect a population. However, this does not mean that pharmacogenetics cannot be costly under some circumstances.

Let me consider in this context a case of a clear-cut association between monogenic toxicant resistance and reduced fitness. After exposure to organophosphates, reduced fitness of insects has been associated with protective overactivity of carboxylesterases, which is often due to gene amplification (Roush and Daly, 1990). In clones of some aphids highly resistant to organophosphates, as much as 3% of body protein was devoted to esterases; the homozygous resistant genotypes appeared to be only half as fit as susceptible genotypes, producing only half the normal numbers of offspring. This is one clearly documented case in which maintenance of a resistance gene is biologically expensive for the population. However, in this case, the gene has become frequent in a population because it did provide protection against a toxicant. Therefore, the variant will be quite present in homozygous form and in duplicated form, in which it may be biologically costly.

A kind of fitness disadvantage that has been seen in insects represents "pleiotropy," that is, multiple phenotypic effects of a gene that is responsible for a toxicant resistance (Roush and Daly, 1990). In principle, pleiotropic effects can occur in any living subject, including humans. However, any disadvantage would depend on the specific effects of the gene. Sometimes, the only indication of a disadvantage in insects has been the loss of resistance after termination of exposure.

Childs (1995), writing about DNA, sums up the situation; it is likely that some of the mutants that are functionally neutral or nearly neutral when formed may assume a defensive function when called upon. What is neutral in one setting may be adaptive or maladaptive in another. It may constitute a reserve of variation against unforeseen need.

VI. Quantitative Aspects of Pharmacogenetic Variation

There are two kinds of variation in drug response: gaussian variation and all-or-none (monogenic) variation. Concurrent contributions of multiple genetic and environmental factors lead to random (gaussian) variation. Monogenic (often polymorphic) variation is based on a controlling effect of a single gene product that may lead to all-or-none responsiveness; pharmacogenetics is often understood to deal only with monogenic variation. There are always transitions or overlaps between these kinds of variation, because random variations are pervasive.

Because of frequent social and economic costs of differences in response to drugs, toxicants, and carcinogens, pharmacology uses some otherwise uncommon graphic representations designed to detect deviations from gaussian variation by monogenic influences (Probit plots, Normal-Test-Variable plots) (Endrenyi and Patel, 1991).

A. Monogenic Variation

In genetic terms, product deficiency of any autosomal gene will divide the population into three groups: one homozygous for the wild-type gene, one homozygous for the variant, which may mean complete absence of the gene product, and the third group being the heterozygotes. If the variant gene product means the absence of the only drug-metabolizing enzyme, the drug-metabolizing capacity of the heterozygotes will average half that of the wild-type homozygotes. Because there will be gaussian variation in each subgroup, the reduction of the drug-metabolizing capacity in the heterozygotes may be overlooked under clinical conditions.

Under these conditions, the genetically controlled absence of a particular protein may allow a population to be divided into two groups, one group whose members show a particular response to a given chemical and the other group whose members do not. Examples are the "Poor and Extensive" metabolizers of debrisoquine and other drugs, or the "slow and fast acetylators," referring to N-acetyltransferase type 2. If genetic variation of an

enzyme does not merely determine its presence or absence but its function (e.g., a K_m deviation), the effects tend to vary with the substrate.

B. Gaussian Variation

Gaussian variation is often viewed as environmentally determined, rather than as part of pharmacogenetics. Most variation in drug response is of the gaussian variety. Gaussian variation usually contains hereditary elements that can be defined in terms of heritability (Vesell, 1992). It is important to note that the edges of gaussian distribution curves are often of greater clinical interest than their means (Kalow, 1992a; Kalow and Bertilsson, 1994).

Gaussian variation is a mathematically defined variation, systematically used for the determination of the median effective or lethal dose of a drug (ED_{50} or LD_{50}; Trevan, 1927). In animal studies with 1000 or more individuals, there is often amazing precision with which the distribution curves adhere to the gaussian rules; an example among others is the dose-effect study by Morrell and Chapman (1933) who tested neoarsphenamine in 1331 rats and whose data were reanalyzed and reported by Clark (1937). If standard deviations are precisely measurable parameters, then small differences between them also become important and susceptible to biological analysis.

Clark (1937) called the size of the standard deviation of a drug response in different people or animals the "characteristic" of the drug. He thereby emphasized the behavior of different drugs in the same population, disemphasizing any differences in standard deviations of the same drug in different populations. In any case, all such differences become susceptible to genetic analysis with measurements of heritability. Questions arising are: What determines the magnitude of the standard deviations or, in more general terms, the coefficients of variation (standard deviation as percent of the mean). Is the number of factors contributing to gaussian variation decisive? To what extent do differences between these coefficients reflect different mutant frequencies in the different systems that might control drug effects? Do we see variation of the drug's target or of the forces determining its absorption or elimination? There have been very few attempts to come close to answering such questions.

I was excited as a young scientist (1949) by a paper by Lands et al. (1948) that related the slopes of logarithmic dose-effect curves of 12 catecholamines to their structures and their patterns of activity; the drugs were given to mice by intraperitoneal injection, and results were reported in terms of LD_{10}, LD_{50}, and LD_{90}. Compounds with the same N- or C-substitutions had the same slopes (i.e., the same standard deviations) and identical or similar patterns of activity; for instance, adrenaline and epinine had the same slope in spite of a 100-fold difference in LD_{50}. In this case, it must be variation of the drug target that determined variability of the drug response.

There is a recent observation that reveals a rule that affects the magnitude of variation. Hellriegel et al. (1996) conducted a meta-analysis of 143 suitable publications and concluded that "the lower a drug's bioavailability, the greater the intersubject variability in bioavailability". This is perhaps not surprising; low bioavailability could indicate, for example, excessive first-pass metabolism in gut or liver, or active countertransport by p-glycoprotein (Lown et al., 1994; Wacher et al., 1996). Nothing is known about the genetics of transport proteins.

Perhaps the rule of Hellriegel et al. (1996) is reflected by the following observation. Lindahl et al. (1996) compared the absorption of fluvastatin, antipyrine, metoprolol, and atenolol in nine subjects by testing plasma level kinetics after jejunal perfusion. The coefficients of variation were 35.1, 41.5, 47.3, and 78.1, respectively. Atenolol shows the largest variation and has a far lower octanol/water partition coefficient than the other drugs. Perhaps, the absorption of atenolol involves variability of active transport, whereas the other drugs depend mostly on diffusion.

It is clear that gaussian variation may express variability of a drug target, drug metabolism, or drug transport. When considering variability, we usually do not know which of these factors are involved. Nor do we usually know how much of a given variation is due to heritability (Vesell, 1992) or environmental factors. However, this last question should be answerable more often than is currently the case.

In all the examples discussed so far, the kind of variation almost automatically considered is between-subject variation. Within-subject variation is formally considered only when conducting bioequivalence studies (Midha and Blume, 1993). However, a comparison of between- and within-subject variation can usually substitute to some extent for a twin study in sorting environmental and genetic factors. Twin studies are a necessity if one wants to determine the heritability of durable characteristics like body size or blood pressure. However, for studies of the heritability of drug effects, it seems that repeated applications of the same drug to the same person can often substitute for the use of twins.

The ratio of between- and within-subject variances of different caffeine metabolite ratios could clearly show the genetic control of N-acetyltransferase type 2 and the environmental control of xanthine oxidase; the activity of the P450 cytochrome CYP1A2 appeared to be under mostly environmental, but still mixed, control (Kalow, 1996). Such comparisons are less expensive than twin studies, and they should be reliable guides particularly in cases of low heritability.

VII. Pharmacogenetics and Evolution

Pharmacogenetic diversity provides a good illustration of Darwinian principles. Pharmacogenetic variation can be the saving grace for a population by creating survivors of most kinds of toxic impacts. If a population is exposed to a toxicant that destroys a majority of its members, survivors able to withstand the toxic effects become the fittest individuals under the new circumstances, and their propagation replaces the original population. However, diversity cannot give the kind of evolutionary direction that the Darwinian selection process provides by favoring the survival of the fittest individual. Diversity of a population and Darwinian selection are different milestones; the former makes the latter possible.

Diversity of a population is advantageous for its defense not only against chemical- or pathogen-produced adversities, but for its defense against all kinds of environmental dangers. With this realization, we come very close to thoughts expressed and critically discussed by Williams (1974). We have the advantage of being guided by the relatively simple and straightforward experiences from pharmacogenetics.

Here we remember that in humans, dealing with intoxication is not only a matter of biology and pharmacogenetics but of a brain that is capable of acquiring and using medical knowledge. Mental capacities directing behavior are vital in coping with environments. This means that useful variability in a population may be enhanced by individuals who are beyond procreational age.

VIII. Summary and Conclusions

What have we learned? Pharmacogenetics, heritable variation in response to xenobiotics, is present in all forms of life. Initially, human data perhaps have created the most excitement, and they provide much biochemical detail. However, if we look at pharmacogenetic variation of insects and bacteria, we see it as a characteristic of populations; individuals with inborn resistance to various toxicants can cause the survival of a population by the process of Darwinian selection. Diversity of a population and Darwinian selection are different milestones serving population survival.

Variation of drug response may represent variation of drug targets, drug metabolism, and probably drug transport. Metabolic variation appears to be the most prominent; at present, it is not clear whether this prominence has historical or biological causes.

It is an interesting exercise to compare pharmacogenetic resistance with intoxication and resistance to infection by invasion of disease-carrying bacteria or other pathogens. The big difference is that pathogens tend to show variabilities that drugs do not have. The immune system is made to deal with the genetic variabilities linked to the short life span of most pathogens. However, there are, besides the immune system, several cases of genetic host resistance associated with the long life span of mammalian hosts. Such genetic host resistances are factors equivalent to pharmacogenetic variation. Current data pertain to resistances against malaria, tuberculosis, cholera, and AIDS.

Most pharmacogenetic variants within a population are preadaptive, that is, they are established before xenobiotic exposure. Hence, one must postulate a multiplicity of variants in a population capable of resisting a multiplicity of drugs. The persistence of this multiplicity suggests that most variants are either present in heterozygous form and are thereby advantageous for their carriers, or they are selectively neutral mutants. It means that the biological cost of pharmacogenetic diversity, measured in terms of reduced fertility, should be low in a population.

The frequencies of variant genes are usually not the same in different populations. Also the nucleotide substitutions in a variable gene often differ between populations. In other words, pharmacogenetic differences between populations are typical events.

Pharmacogenetics is usually thought of as the study of a situation in which a single gene product exerts control over a given drug response so that a failure to respond, or an excessive response, may result. However, one should not forget that random variation is always present, probably reflecting the randomness of mutations plus variation of any environmental factors that might contribute. This underlying randomness of variation will always affect the picture of any all-or-none variation. Future pharmacogenetics must deal with both random and monogenic variation.

Acknowledgments. I owe special thanks to Dr. Harold Kalant in the Department of Pharmacology for his thoughtful critiques. I am grateful for stimulating discussions and criticism to Professor S. Pfeiffer of the University of Guelph and, in Toronto, to Dr. Richard Collins, Department of Genetics, to Dr. Spencer C.H. Barrett, Evolutionist in the Department of Botany, and in Pharmacology to my colleagues Dr. Laszlo Endrenyi and Dr. Rachel Tyndale. Thanks for special advice and for the permission to use unpublished data are due to Dr. Neil W. Forrester of the New South Wales Agricultural Research Station in Australia.

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