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PHARMACOGENETICS

**Report of a
WHO Scientific Group**

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Geneva, 4-8 December 1972

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PHARMACOGENETICS

Report of a WHO Scientific Group

The WHO Scientific Group on Pharmacogenetics met in Geneva from 4 to 8 December 1972. The meeting was opened by Dr T. A. Lambo, Assistant Director-General, who welcomed the participants on behalf of the Director-General.

1. INTRODUCTION

Subsequent to a resolution adopted by the Seventeenth World Health Assembly in 1964¹ and later resolutions, the World Health Organization has convened several scientific groups to study the problems involved in the evaluation of safety and efficacy of drugs and several technical reports have been published.²

In 1970 the WHO Advisory Committee for Medical Research suggested that a scientific group should be convened to review the data concerning the role of genetic factors in responses to drugs and to discuss problems related to the methodology of pharmacogenetic studies, as well as the practical implications of their results. In view of the mass use of drugs all over the world it was suggested that attention also be given to the public health aspects of pharmacogenetics, including adverse reactions.

2. GENERAL CONSIDERATIONS

This report is concerned especially with the ways in which heredity influences those responses to drugs that are of particular clinical significance. It also considers the problems presented by differences in the distribution of genetic phenotypes sensitive to drugs and other toxic chemicals in different populations.

The fate of drugs in man is influenced by several processes including absorption, protein binding, distribution and transfer across cell membranes, interaction with cell receptors and organelles, biotransformation, and excretion. Both specific and nonspecific enzymes are involved in these processes. A high degree of genetic control of biochemical and enzymatic mechanisms has recently become apparent. Thus, in a significant proportion of human populations, about one-third of all enzymes exist as electrophoretically detectable variants (enzyme polymorphisms). Such normal

¹ Resolution WHA 17.39 (*Off. Rec. Wld Hlth Org.*, 1964, No. 135, p. 17).

² *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 341, No. 364; 1969, No. 426; and 1971, No. 482.

enzyme variants are frequently associated with differences in enzyme activity. The effects of the variants on the different processes involved in the fate of the drug may therefore result in differences between individuals in drug response. If the effect of such a difference at one reaction step is large, well-defined differences in response may occur in affected individuals and lead to the existence of distinct phenotypes. Much recent work has shown that the fate of most drugs is affected by multiple interacting genetic factors.

3. METHODS

3.1 General remarks

The methods used in pharmacogenetics are derived from those used in biochemistry, human genetics, and pharmacology. Pharmacology is the study of drugs and other foreign substances and genetics is the study of biological variation and the transmission of this variability. Pharmacogenetics is a new interdisciplinary field, that deals with the influences of heredity on responses to drugs.

Species differences in the metabolism and clearance of almost all drugs are largely genetic in origin. Since man's genome differs from that of other species there is a need for critical pharmacogenetic studies to be performed in human subjects. Occasionally, mutations similar to those found in man may be encountered in other species and this allows more detailed investigations to be undertaken. This important aspect requires further study with particular reference to the applicability to man of the results obtained with animals.

Many investigations in pharmacogenetics require the administration of a drug to normal persons. The reluctance on ethical grounds to give drugs to healthy individuals is a factor that has prevented more rapid growth in this field. In this regard the use of subtherapeutic quantities of the drug would be highly desirable wherever practical, but would require the development of sensitive microtechniques. The use of gas chromatography combined with mass-spectrometry is an example of one such approach.

Another possible way of avoiding the difficulties of drug administration to normal subjects would be the use of tissue culture cells, which could be manipulated by a variety of techniques. At the present time only fibroblasts can be used successfully (Krooth, 1971) since differentiated tissues, such as liver cells, do not maintain their characteristics in culture. Since all the cells in one organism, regardless of their differentiation, carry identical genes, which, however, are often not expressed, special techniques such as stimulation of lymphocytes by phytohaemagglutinin (Goldstein et al., 1972) may lead to the expression of some previously unexpressed genes. Gene expression may also be induced as a result of cell fusion.

3.2 Pharmacological methods

3.2.1 *Drug concentration measurements*

For many studies in pharmacogenetics, measurements of the drug concentration in biological fluids are required. Simple and reliable methods are essential.

3.2.2 *Pharmacokinetic constants*

Pharmacokinetics is the study of the fate of a drug throughout an organism in quantitative terms. The kinetic behaviour of the drug is a complex interaction of both physically and enzymatically mediated reactions.

Specific measurements include drug half-life, plasma clearance, steady-state plasma concentration, and urinary excretion. For many drugs a straight line is obtained when the log plasma concentration is plotted against time after drug administration, and this implies that the rate of elimination of the drug from plasma is proportional to its concentration. The plasma half-life of the drug is a commonly used indicator for the kinetic behaviour of a drug and is equal to 0.3010 divided by the slope of this line: another important pharmacokinetic parameter, the elimination rate constant (k_{el}), equals 0.693 divided by the half-life.

A third important parameter is the apparent volume of distribution (V_D) of the drug in the body. This volume is the total amount of drug absorbed by the body (for well-absorbed drugs it is almost equal to the dose administered) divided by the calculated concentration of the drug in plasma at zero time (derived from extrapolation of the curve previously described). The plasma clearance of the drug equals $V_D \times k_{el}$ (Dost, 1968; Wagner, 1969).

The steady-state plasma concentration of a drug is an important parameter because it represents: (1) the concentration maintained during prolonged therapy, and (2) an equilibrium between input and elimination. The time taken to attain 99% of this concentration is often about seven times the plasma half-life.

It is probable that individuals vary in each of these pharmacokinetic parameters and since the above kinetic measurements involve the products of genes at several loci it is hardly surprising that genetic studies employing them have revealed polygenic patterns of inheritance.

3.2.3 *Drug metabolites*

Searches for drug metabolites in serum and, particularly, urine have been made more informative by the use of sensitive methods such as gas chromatography. New pharmacogenetic phenomena may be found by detecting previously unknown metabolites.

3.2.4 *Protein binding*

Amino acid substitutions of transport proteins may cause differences in protein binding. Such variants may not be electrophoretically detectable and may be uncovered only by quantitative drug binding.

3.2.5 *Drug receptors*

The study of drug receptor variability is in its infancy, but among the different approaches, work with membranes is likely to produce significant pharmacogenetic data.

3.3 **Biochemical genetics**

The elucidation of differences in drug metabolism between individuals requires the use of a wide range of biochemical methods (Harris, 1969). The most meaningful data have been obtained using specific enzyme techniques. The discovery of enzyme variants has been facilitated by the use of electrophoresis and chromatographic methods are often useful for the isolation of proteins of pharmacogenetic interest. Additional characterization for genetic purposes can be obtained by the careful determination of such parameters of enzyme kinetics as substrate affinity, inhibition constant, substrate analogue utilization, thermal stability, pH range, and others.¹

Often it is necessary to study several members of an affected family by these techniques to ensure that a *bona fide* genetic variant has been detected. Ultimately, the specific amino acid substitution of the enzyme protein will define exactly the genetic defect. Microtechniques for these tests are under development.

Often a mutation may result in an enzymatically inactive protein that can be detected by immunological techniques; proteins of this type are referred to as cross-reacting material (CRM).

3.4 **Human genetics methods**

3.4.1 *Population surveys*

When a drug is tested in a general population sample, or is used therapeutically in patients, considerable variability in effectiveness and in side effects is commonly noted. Even when drug potency and mode of administration have been carefully standardized, the following sources of variation must still be expected. First, some individuals will need a larger or a smaller dose than the average in order to obtain the same effect or to attain the same plasma concentration of the drug; some individuals may fail to show the

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 366.

desired effect at any reasonable dose. Second, some patients may fail to respond because the diagnosis is incorrect or because the diagnostic category comprises two or more distinct subpopulations, only one of which is responsive to the drug therapy provided.

If a significant proportion of the population carries a Mendelian trait that makes the level of the parameter under study, such as plasma concentration of the drug, distinctly different from that of the remainder of the population, the frequency distribution curve may show more than one mode. Such distributions can only be detected when allele frequencies are fairly high. If the trait is rare, the values at the extremes of the distribution fail to produce discernible multimodal patterns. The finding that the frequency distribution differed for different ethnic or racial groups would be another indication that genetic factors might be involved, particularly if the groups lived in the same environment.

3.4.2 *Family studies*

The establishment of genetic patterns requires family studies. For many purposes the study of first-degree relatives such as parents and children may be sufficient but to differentiate monogenic from polygenic inheritance it is particularly useful to study large kindreds. In contrast to population studies, studies of the families of affected individuals are most revealing for rare monogenic traits, since the proportion of affected relatives is expected to be high (i.e., 50% for autosomal dominant inheritance, and 25% for autosomal recessive). Family studies also have the advantage that an identical mechanism is responsible for the unusual drug response in all affected members of the family; this would probably not be so with affected members of a population. If the distribution of variation is more nearly continuous, a search for indications of the genetic pattern may be especially fruitful when individuals at the extremes of the distribution curves are selected for family study. Family studies may reveal a bimodal distribution for specific genetic mechanisms that are of too low frequency in the general population to produce a discernible "hump" in the distribution curve.

If a given drug measurement shows different ranges in males and females, or varies with age or weight, appropriate corrections need to be applied. Such correction usually requires the determination of the parameter under study in a significant number of normal individuals.

3.4.3 *Twin studies*

Comparison of the rates of concordance of a trait or of intra-pair similarity of a quantifiable measure, such as rate of elimination of a drug in monozygotic (identical) and dizygotic (fraternal) twins, gives an estimate of the extent to which variation is a result of inherited factors. As with a

population study, the twin method may provide a clue to the mode of inheritance (i.e., monogenic or polygenic). The contribution of environmental variables can also be assessed by changing the environment, as in the following examples: chronic administration of a drug as compared with acute administration, retesting the same twin pairs, or studying monozygotic twin pairs reared apart and reared together. In the last case, the extent to which the environments differ for the monozygotic twins reared apart must be estimated independently.

4. PHARMACOGENETIC VARIANTS WITH MENDELIAN INHERITANCE

Simple genetic differences, due to the action of a single gene, that clearly affect disposition and response to a drug have been demonstrated in relatively few instances (Table 1).

A. Common Pharmacogenetic Traits

4.1 G6PD deficiency

The types of G6PD deficiency of public health importance are those that are frequent (Table 2) and carry the risk of haemolysis on administration of certain drugs (Table 3). The African (A⁻) and the Mediterranean varieties have been characterized most thoroughly.

Different types of G6PD¹ deficiency may often coexist in the same population, as in Greece, the Philippines, and Thailand. Detailed studies on large populations are required to distinguish between the different types of G6PD deficiency that occur in regions other than Africa and the Mediterranean. G6PD-deficiency is an X-linked trait; males with G6PD deficiency have a single enzyme-deficient red cell population and homozygous females also have a single red cell population. Heterozygous females are more frequently encountered in a population than deficient males and they have two red cell populations, one normal and one deficient. The ratio of the two populations varies widely, with a mode of 50:50; in rare cases the proportion of abnormal cells may be as low as 1% or as high as 99%. Only abnormal cells are drug-sensitive, and in most heterozygous females drug-induced haemolysis is mild, since in the typical heterozygous female only half of the cells are enzyme deficient. Only about one-third of all heterozygous females have a high enough proportion of abnormal cells to predispose them to clinically significant haemolysis.

¹ Classified as enzyme EC 1.1.1.49 in: International Union of Biochemistry (1965) *Enzyme nomenclature*, Amsterdam, Elsevier Publishing Co.

TABLE 1
PHARMACOGENETIC TRAITS WITH PROVEN MENDELIAN INHERITANCE

Trait	Frequency	Population distribution	Drugs involved	Adverse drug effect	Mode of transmission	No. of variant alleles
Common traits						
G6PD-deficiency polymorphism	see table 2	tropical and subtropical populations	see table 3	haemolysis	X-linked	~90 known, not all of clinical significance
acetyltransferase polymorphism slow acetylator	~60% in Caucasians and Negroes	5-20% in Orientals	isoniazid, phenelzine, hydralazine, dapson, sulfadimidine	more frequent side effects unique to each drug	autosomal recessive	1
intraocular pressure response to glucocorticoids polymorphism	66% with low response 29% heterozygotes (intermediate) 5% homozygotes (high response)	unknown	topical application of glucocorticoids to the eye	increased intraocular pressure	autosomal dominant	1 (polygenic ?)
methaemoglobin reductase deficiency	~1% heterozygous	unknown	dapsone, chloroquine, primaquine	cyanosis	presumably heterozygous expression of rare type of autosomal recessive methaemoglobinemia	several
Rare traits						
suxamethonium sensitivity (cholinesterase variants)	see table 4	rare in Negroes, practically absent in Orientals	suxamethonium	prolonged apnoea	autosomal recessive	3
malignant hyperthermia	~1 : 20 000 anaesthetized children	observed in Caucasians, Negroes and Orientals	general anaesthetics	hyperthermia; death in two-thirds of cases	autosomal dominant	1 recognized, probably heterogenous
warfarin resistance	exceedingly rare	not applicable	coumarin, oral anti-coagulants	affected patients require 25x the conventional dose	autosomal dominant	1 recognized
some unstable haemoglobins	exceedingly rare	not applicable	oxidant drugs	haemolysis	autosomal dominant	3-4 recognized
pseudocholinesterase cynthiana	exceedingly rare	not applicable	suxamethonium	suxamethonium resistance	autosomal dominant	1
acetophenetidine-induced methaemoglobinemia	exceedingly rare	not applicable	phenacetin	cyanosis	autosomal recessive ?	1
phenytoin hydroxylation deficiency	exceedingly rare	not applicable	phenytoin	ataxia, nystagmus	autosomal dominant; polygenic ?	1

TABLE 2
POPULATIONS WITH G6PD DEFICIENCY OF MORE
THAN 1% IN MALES

Africans
All populations with African ancestry, i.e., American Negroes, Puerto Ricans
Arabs (Egyptians, Kuwaiti, Lebanese)
Filipinos
Greeks
Indians from the Indian subcontinent
Indonesians
Jews (primarily Oriental and Sephardic)
Kurds
Malaysians
New Guineans
Pakistanis
Persians
Romanians
Sardinians
Sicilians
Southern Chinese
Thais

4.1.1 Types of G6PD deficiency

African type (A⁻). The A⁻ type of G6PD deficiency is characterized by mild enzyme deficiency (mean enzyme activity 8–20% of normal) and high electrophoretic mobility. The youngest red cells have normal or almost normal enzyme levels, and red cells younger than 50 days have sufficient enzyme activity to be protected against damage by haemolytic drugs. Thus, only the older cells are susceptible to destruction and, even if the offending drug continues to be administered, haemolysis will be self-limited. The risk of potentially fatal haemolysis is therefore less than with the Mediterranean type of enzyme deficiency and fewer drugs are potentially toxic (Table 3).

Mediterranean type. The Mediterranean type of G6PD deficiency is characterized by severe enzyme deficiency (0–4% enzyme activity). Identification requires further enzyme characterization. Enzyme deficiency affects even the younger red cells, and haemolytic episodes are therefore not self-limited. Haemolysis is thus more severe and more often life-threatening, and more drugs are potentially harmful (Table 3).

Other common types of severe G6PD deficiency. The variants of G6PD deficiency that are common in East and South-East Asia (e.g., the variants Canton and Union) differ from the Mediterranean and A⁻ types. The changes in enzyme activity and the clinical implications with regard to haemolysis have not yet been determined with these variants. It is likely that some at least may be as severe as the Mediterranean types of deficiency.

TABLE 3
DRUGS REPORTED TO INDUCE HAEMOLYSIS IN SUBJECTS WITH G6PD DEFICIENCY

Drug	Haemolysis		References ^b
	Negro ^a subjects	Caucasian subjects	
A. Drugs producing clinically significant haemolysis			
acetanilide	+ + +		1, 8
dapsone	+ +	+ + +	1, 8
furazolidone	+ +		1, 8
furaltadone	+ +		1, 8
nitrofurantoin	+ + + +		1, 8
nitrofurantoin	+ +	+ +	1, 2, 8
sulfanilamide	+ + +		1, 8
sulfapyridine	+ + +	+ + +	1, 6, 8
sulfacetamide	+ +		1, 8
salazosulfapyridine	+ + +		1, 8
sulfamethoxy-pyridazine	+ +		1, 8
thiazosulfone	+ +		1, 8
quinidine		+ +	8
primaquine	+ + +	+ + +	1, 3, 5, 8
pamaquine	+ + + +		1, 8
pentaquine	+ + +		1, 8
quinocide ^c	+ + +	+ +	1, 8
naphthalene	+ + +	+ + +	1, 8
neocarsphenamine	+ +		1, 8
phenylhydrazine	+ + +		1, 8
foluidine blue	+ + + +		1
trinitrotoluene		+ + +	8
B. Drugs reported as haemolytic agents in some cases, but usually not producing clinically significant haemolysis under normal conditions (e.g., in the absence of infection)			
phenacetin	+		1, 8
acetylsalicylic acid	±	+	1, 7, 8
sulfadiazine		+ +	6, 8
sulfafurasole	+ ±		1, 8
sulfoxone	+		1, 8
chloramphenicol	0, +	+ +	1, 4, 8
nitrite	+	+ + +	8
methylene blue	+		1, 8
ascorbic acid	+		1, 8
dimercaprol	+		1, 8
chloroquine	±		1, 8
mepacrine	±		8

^a There have been many more studies in Negroes.

^b (1) Beutler, E. (1971) *Seminars hemat.*, 8, 311
 (2) Jeannet, M., Perrier, C. V. & Tonz, O. (1964) *Schweiz. med. Wschr.*, 94, 939
 (3) Larizza, P. et al. (1958) *Minerva med.*, 49, 3769
 (4) McCaffery, R. P. et al. (1971) *Ann. intern. med.*, 74, 722
 (5) Panizon, F. & Vullo, C. (1962) *Haematologica*, 47, 205
 (6) Szeinberg, A. et al. (1959) *Israel med. J.*, 18, 176
 (7) Szeinberg, A. et al. (1960) *Acta haemat.*, 23, 58
 (8) *Wld Hlth Org. techn. Rep. Ser.*, 1967, No. 366

^c 8-(4-aminopentylamino)-6-methoxyquinoline.

Much more work is required to characterize these G6PD types, and to assess the susceptibility to haemolysis of red cells containing them before their public health importance can be fully evaluated.

Until more information becomes available, variants that have not yet been defined should be considered a potential risk if they are associated with an enzyme activity of less than 10%. It should be stressed, however, that there is no complete correlation between enzyme activity, as estimated by standardized *in vitro* techniques, and clinical severity.

4.1.2 *Clinical manifestations of haemolysis*

The most typical clinical manifestation of G6PD deficiency is a haemolytic crisis precipitated by drugs (or by food or infections). Mild episodes may be recognized only by the incidental discovery of haemolytic anaemia in a patient receiving treatment for an unrelated condition. Neonatal hyperbilirubinaemia leading to kernicterus, occurs in G6PD-deficient infants in Africa, China, and the Mediterranean region after exposure of the child or mother to certain drugs or to naphthalene, and at times even in the absence of such exposure.

The haemolytic drugs do not appear to have common chemical structural characteristics. The exact mechanisms of drug haemolysis are not fully understood but they are related to defective metabolism of glutathione. Kidney and liver damage may increase blood levels of potentially toxic drugs and thereby cause a relatively safe drug to be a highly haemolytic substance. Similarly, diabetic acidosis and other electrolyte disturbances may alter the haemolytic sensitivity of the red cell. The extent of haemolysis usually appears to be dose-related.

Haemolytic episodes may be precipitated by cutaneous contact with an offending agent or by inhalation of its vapour (e.g., naphthalene) without actual ingestion.

It is often difficult to distinguish the effect of infection from that of drugs. It has been demonstrated, however, that infectious hepatitis, infectious mononucleosis, and probably other infections can provoke haemolysis in both the A⁻ and the Mediterranean type of deficiency in the absence of drugs. In patients with hepatic damage and haemolysis, high levels of bilirubin in the blood cause further damage to the liver.

4.1.3 *Prevention of drug haemolysis*

Awareness of the frequency of G6PD deficiency in many populations is an important prerequisite for the prevention of haemolysis. More data are required on the haemolytic potential of different drugs in different types of G6PD deficiency. The existing list of drugs (Table 3) serves as a guide to the

avoidance of certain medicaments. The difference in the severity of haemolysis between the African and Mediterranean variants is clinically important. The decision to use or cease administration of a potentially haemolytic drug depends upon (a) the type of G6PD deficiency, (b) the sex of the patient, (c) the severity of the disease, (d) the need for the drug, and (e) the availability of alternative drugs. Thus, chloramphenicol appears to be safe in the treatment of typhoid fever in G6PD Canton carriers in Asia, but is contraindicated in male patients with the Mediterranean type of G6PD, in whom severe haemolysis occurs and in whom synergism between G6PD deficiency and the infection has been described. Ampicillin might be substituted for chloramphenicol in such patients.

It is sometimes possible to haemolyse the oldest and most sensitive red cells by employing small doses of a drug without significant clinical effects, after which the dose can be increased gradually. While such regimens have not been tested in a controlled manner, they may be applicable even in the Mediterranean type of deficiency. It should be recognized that offending drugs have different degrees of haemolytic potential.

Haemolysis is likely to recur with the same drug on subsequent administration, and patients who have had haemolytic episodes should be informed of this danger. It is valuable to provide patients with a list of the common drugs that may produce haemolysis, so that they can inform medical personnel about possible problems.

It is important to stress the synergism between G6PD deficiency and infection (or chronic illness such as liver failure, renal failure, or diabetic acidosis) in the pathogenesis of haemolysis. Clinically benign haemolysis with a given drug may become severe in the presence of additional infection or disease. There is much need for careful prospective studies on the frequency of clinically important haemolytic episodes in patients with all types of G6PD deficiency, including the A⁻ and Mediterranean variants. Many drugs that are manufactured in countries where G6PD deficiency is rare may be used extensively in countries with high prevalence of enzyme deficient subjects. There is an urgent need therefore to establish adequate cooperation between drug manufacturers, the health authorities, and practising clinicians in the countries where the drugs are sold, to safeguard against unnecessary haemolytic hazards of inadequately tested drugs.

It is recommended that all hospital patients from populations at risk should be screened for G6PD deficiency when they are hospitalized for any cause, since the largest number of haemolytic episodes occurs in this group.

Several G6PD screening methods have been described.¹ These techniques are excellent for the detection of G6PD males, but miss many heterozygous

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1966, No. 338 ; 1967, No. 366 ; 1972, No. 509.

females. However, most heterozygous females at haemolytic risk are likely to be recognized by means of simple detection techniques.

4.2 Acetyltransferase polymorphism

When a standard dose of the antituberculosis drug isoniazid (INH) was administered to a group of individuals, the histograms of blood levels of INH 6 hours later and of the percentage of the administered INH excreted free and unacetylated in the urine showed a bimodal distribution (Bönicke & Reif, 1953; Evans et al., 1963). Twin studies showed great similarity between monozygotic twins and considerable difference between dizygotic twins (Bönicke & Lisboa, 1957). Family studies showed that slow acetylators of isoniazid develop higher blood levels, excrete a higher proportion of free drug in the urine, and have a trait recessive to rapid acetylation (Evans et al., 1963). The responsible enzyme is an *N*-acetyltransferase (EC 2.3.1.5) in the liver that acetylates INH and probably phenelzine and partly acetylates hydralazine, and dapsone (Evans et al., 1965). Rapid acetylators do not show impairment of the antituberculosis effect with a standard dose of INH. However, with slow acetylators there is a greatly enhanced risk of undesirable side effects, in particular peripheral neuropathy (Devadattor et al., 1960), which can be avoided by concomitant administration of the B-vitamin pyridoxine. When patients with both epilepsy and tuberculosis are given INH and phenytoin an important drug-drug interaction may occur. Among the slow acetylators, INH concentrations reach levels that inhibit metabolism of phenytoin by hepatic microsomal oxidases, thus leading to the accumulation of toxic concentrations of phenytoin, with ataxia, nystagmus, and drowsiness. Other drugs that are hydroxylated might be similarly inhibited by isoniazid. Similarly, severe toxic side effects of the antidepressant phenelzine occurred only in subjects with the slow acetylator phenotype (Evans et al., 1965). It should be noted that about 60% of Caucasians, 60% of Negroes, and only 10–15% of Orientals are slow acetylators (La Du, 1972) (Table 1). Certain other drugs, such as *p*-aminosalicylic acid, *p*-aminobenzoic acid and sulfanilamide are acetylated in a unimodal manner by another acetylating enzyme (Motulsky & Steinmann, 1962). The role of the acetylating enzyme in the acetylation of sulfa drugs such as sulfamethoxy-pyridazine, sulfafurazole and sulfadiazine is uncertain (Peters & Levy, 1971). With other compounds that can be acetylated, including serotonin, it is not yet clear whether the polymorphic acetylating system is responsible (White et al., 1969; Schloot et al., 1969; Goedde et al., 1970).

There is no single *in vitro* screening technique for detecting *N*-acetyltransferase deficiency. Acetylator phenotyping requires the administration of sulfamethazine or isoniazid, followed by measurement of serum or urine drug levels. The simplest procedure is considered to be sulfamethazine administration followed by urine assay 3–6 hours after drug administration.

4.3 Genetic variation of the intraocular pressure response to glucocorticoids

Repeated topical application of glucocorticoids to the eye is followed by an increase in intraocular pressure (Armaly, 1968; Schwartz et al., 1972). The change in pressure obtained under standard conditions seems to be inversely related to the ease of aqueous outflow and shows an apparent trimodal distribution in a randomly selected population, with relative frequencies of 66%, 29%, and 5% for groups characterized by low, intermediate, and high pressure change in response to the drug. These effects were larger in subjects aged over 40 years, and were much more pronounced in eyes with both low-tension and high-tension open-angle glaucoma. Data from family studies selected by means of propositi of the 3 different phenotypes revealed that a two-allele model is sufficient to explain the segregation of genotypes $P^L P^L$, $P^L P^H$ and $P^H P^H$, where the allele P^L is responsible for low pressure and the allele P^H for high pressure, but a polygenic inheritance cannot be excluded. The rise of intraocular pressure appears to be continuous in individuals of genotype $P^L P^H$, so that the eye may become damaged. In most cases this response is totally reversible by withdrawal of the drug.

If the same test for intraocular pressure change is performed on a sample of individuals with open-angle hypertensive glaucoma, a trimodal distribution of phenotypes is also obtained, but the frequency of the $P^L P^L$ genotype is much lower than in a randomly selected population.

The pressure change resulting from the application of glucocorticoids in patients with hypertensive glaucoma is unaffected by the simultaneous application of hypertensive drugs such as pilocarpine. If the risk of developing glaucoma for $P^L P^L$ is unity then the risk for $P^L P^H$ is 18 and for $P^H P^H$ is 101.

4.4 Methaemoglobin reductase deficiency

Hereditary methaemoglobinaemia not requiring any exogenous agents can be caused by several mutations affecting the haemoglobin molecule or by the homozygous state for methaemoglobin reductase deficiency. Heterozygous carriers of this enzyme deficiency have approximately 50% of the normal enzyme activity: the frequency of such carriers in the population is about 1%. Heterozygous carriers are more likely than normal persons to develop methaemoglobinaemia and cyanosis when they are given methaemoglobin-forming drugs such as dapsone, primaquine, and chloroquine (Cohen et al., 1968).

4.4.1 *Phenacetin-induced methaemoglobinaemia*

Phenacetin-induced methaemoglobinaemia (Shahidi, 1968) has been observed in two sisters who metabolized the drug to hydroxy metabolites

of phenacetin. Since the 2-hydroxy compounds are highly potent oxidants of haemoglobin, methaemoglobinaemia occurs. Since both parents and two additional sibs of the affected patients metabolize the drug normally, autosomal recessive inheritance was suggested.

B. Rare Pharmacogenetic Traits

4.5 Cholinesterase variants

Suxamethonium is a drug commonly used by anaesthetists as a short-acting muscle relaxant during surgery. Normally the drug is inactivated rapidly by the serum enzyme cholinesterase (EC 3.1.1.8) (Kalow, 1962; Goedde et al., 1967). Suxamethonium sensitivity is occasionally manifested as prolonged apnoea. Genetic variants of this enzyme that fail to hydrolyse the drug have occasionally been detected (Kalow & Staron, 1957). The most common enzyme is specified by a gene known as E_1^a which is allelic to its normal counterpart, E_1^u (a = atypical, u = usual) (Kalow & Gunn, 1959). The atypical enzyme can be identified most readily by its resistance to inhibition by a variety of agents, the most common being cinchocaine.

Most suxamethonium-sensitive patients are homozygous for the atypical allele. Some suxamethonium-sensitive patients may be homozygous or heterozygous for two of the variant alleles detailed in Table 4. The fluoride-resistant allele E_1^f can be detected by inhibition with sodium fluoride (Harris & Whittaker, 1961). The so-called silent allele E_1^s is associated with complete absence of, or trace amounts of, activity (Liddel & Lehmann, 1962; Simpson & Kalow, 1964; Hodgkin et al., 1965). Homozygotes can be readily detected, but detection of the heterozygous state is more difficult. In some cases immunological cross-reacting material (CRM) has been found, but in most instances no CRM could be detected (Goedde & Altland, 1971).

About 70% of suxamethonium-sensitive patients have been found to have detectable variants of cholinesterase (Kalow, 1966; Thompson & Whittaker, 1966). The remaining patients may have had undetected cholinesterase variants or other mechanisms may be involved. A simple screening test is available (Morrow & Motulsky, 1968). Details of the pseudo-cholinesterase system are given in *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 401.

Before clinical use of suxamethonium, inquiries should be made concerning a personal or family history of sensitivity and equipment for sustained artificial respiration should be available. If use of the drug cannot be avoided altogether, the doses should be reduced as much as possible. In patients with prolonged apnoea caused by this genetic defect, enzyme replacement has been accomplished by injection of purified pseudocholinesterase (Goedde et al., 1967).

TABLE 4
PSEUDOCHOLINESTERASE TYPES AND SUXAMETHONIUM SENSITIVITY

Genotype of clinical significance	Activity	Cinchocaine number	Fluoride number	Phenotype frequency in "European" populations ^a	Suxamethonium sensitivity
E ₁ ^a E ₁ ^a	moderately decreased	22	27	1 : 3 200	+ + +
E ₁ ^e E ₁ ^e	absent	0	0	1 : 170 000	+ + + +
E ₁ ^f E ₁ ^f	slightly decreased	66	35	1 : 28 000	+ +
E ₁ ^a E ₁ ^e	decreased	22	27	1 : 11 000	+ + +
E ₁ ^a E ₁ ^f	slightly decreased	49	33	1 : 2 500	+ + +
E ₁ ^f E ₁ ^e	slightly decreased	67	43	1 : 33 000	+ +
E ₁ ^u E ₁ ^u	normal	80	59	95%	none
E ₁ ^u E ₁ ^a	slightly decreased	62	48	3%	(+)
E ₁ ^u E ₁ ^f	slightly decreased	74	50	1%	(+)
E ₁ ^u E ₁ ^e	slightly decreased	80	59	1 : 200	unknown

^a Based on the frequency of the heterozygous state of 3.5% for the atypical allele, 1.2% for the fluoride-resistant allele and 0.5% for the silent allele. The frequencies of the homozygous and the heterozygous states were calculated by an expansion of the Hardy-Weinberg theorem for multiple alleles.

4.5.1 Population distribution of the common atypical allele E₁^a

The frequency of heterozygotes in populations of European origin is 2-4% (Kalow, 1962; Goedde & Altland, 1963; Szeinberg et al., 1963). The highest frequencies observed so far have been in an Oriental Jewish population (9% Iraqi Jews; 11% Iranian Jews) (Szeinberg et al., 1972). The clinically significant homozygote frequencies corresponding to heterozygote frequencies of 3% and 10% are 1 in 2 500 and 1 in 400, respectively.

In populations of African origin the gene is extremely uncommon, and its somewhat higher frequency in American Negroes could be accounted for by admixture with Caucasians (Motulsky & Morrow, 1968). The gene is extremely rare in populations of Oriental origin such as Eskimos, Filipinos, Japanese, South American Indians, and Thais (Omoto & Goedde, 1965; Motulsky & Morrow, 1968). A high frequency of the silent allele, with 1.5% of the population being homozygous (suggesting a heterozygous frequency of 20%), has been observed in Alaskan Eskimos (Gutsche et al., 1967).

4.6 Malignant hyperthermia

A definite genetic entity exists that is characterized by severe hyperthermia often leading to death and that is precipitated by general anaesthetic agents (Kalow, 1972).

Potent anaesthetics for inhalation (such as halothane, methoxyfluorane, and ether) and muscle relaxants (such as suxamethonium) may trigger a rapid rise in body temperature and progressive muscular rigidity. Temperatures have reached 44.4° C, with tachycardia, tachypnoea, hypoxia, respiratory and metabolic acidosis, hyperpotassaemia, hypocalcaemia, and death from cardiac arrest in about two-thirds of reported cases. In all, 180 cases have been described and the population frequency of the condition is about 1 per 15 000 among juveniles undergoing surgery in Canada. The predisposition is inherited as an autosomal dominant trait, with variable expressivity and incomplete penetrance. Some form of muscular dysfunction such as ptosis is sometimes observed. An elevated serum creatine phosphokinase (EC 2.7.3.2) level in family members at risk can be shown in the majority of affected families. The basic defect remains unexplained, although intracellular calcium metabolism in skeletal muscle seems to be involved. There is no relationship between suxamethonium-induced malignant hyperthermia and the activity of plasma cholinesterase or its variants.

4.7 Coumarin resistance

Two large families have been described in which certain individuals required a much higher than usual dose of oral anticoagulants. Extensive studies showed that drug absorption and drug half-life were within normal limits, and that resistance was a dominant character. When these resistant patients were given anticoagulants they were unusually sensitive to the prothrombinogenic effect of vitamin K₃ and they were found to possess a qualitatively different receptor in the liver with increased affinity for vitamin K (O'Reilly & Aggeler, 1970).

4.8 Drug-sensitive haemoglobins

Among the unstable haemoglobins that are characterized by mutations affecting the stability of the molecule, several examples (Hb Zurich and Hb Torino) are associated with increased lability following the administration of certain oxidant drugs (that cause haemolysis): the harmful drugs are similar to those listed in relation to G6PD deficiency. The red cells of affected patients contain the denatured haemoglobin in the form of small inclusion bodies (Heinz bodies). Hb-H is an aggregate of normal β -chains in Hb-H disease and α -thalassaemia.¹

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1972, No. 509.

4.9 Phenytoin hydroxylation

A single family with phenytoin toxicity has been reported, in which several members failed to hydroxylate the drug. Autosomal dominant inheritance is suggested although multigenic inheritance cannot be entirely ruled out.

5. MULTIFACTORIAL VARIABILITY AND POLYGENIC INHERITANCE

Multifactorial analysis is concerned with continuous unimodal distributions of measured characters. There are both genetic and environmental contributions to the variability of these measured characters or phenotypes. It is of great interest and practical importance to determine the relative contributions of both heredity and environment because the latter can be changed (particularly in this context by giving or withdrawing drugs), whereas the former is a more stable property of the population.

Wherever a trait shows a high variability among individuals in the population, accompanied by smaller variability within an individual on different occasions, and the variability is not caused by obvious environmental factors, genetic differences should be suspected.

It is a well-known rule of thumb that a bimodal distribution points to a simple Mendelian mode of inheritance, whereas a unimodal distribution is believed to be based on a more complicated, multifactorial genetic model. This rule, however, is not without exceptions, for a unimodal distribution may result even from a simple mode of inheritance, if the within-genotype variation of this variable is large in comparison with the between-genotype variation. On the other hand, a bimodal distribution can be produced by a multifactorial genetic model under certain circumstances.

5.1 The paucity of pharmacogenetic polymorphisms

A polymorphism is recognizable because the variability conferred by one factor, namely the existence of allelic genes at one locus, overshadows other contributions to the total variability. For example, in the case of the red cell acid phosphatase polymorphism (Spencer et al., 1964) there are electrophoretic enzyme variants controlled by only three allelic genes, and the 6 genotypes have different enzyme activities; yet the frequency distribution of enzyme activity for the population is approximately a normal curve. In this case, the polymorphism was identified because the gene products were resolved by electrophoresis. In another case a polymorphism has been demonstrated by means of cinchocaine inhibition of plasma cholinesterase (Kalow & Genest, 1957). The unimodal distribution curve of plasma

pseudocholinesterase activity gave way to a trimodal distribution curve of "cinchocaine numbers" each mode representing a genotype and the whole, a polymorphism, being governed by the (then known) two alleles (Kalow & Staron, 1957).

Most of the phenomena observed in relation to drug metabolism or pharmacological responses are unimodally distributed in the population. This is why many pharmacological procedures aim to produce a sigmoid dose-response curve that is merely the cumulative form of the normal distribution. (The concepts of probit analysis and LD_{50} estimates are closely allied.)

Uncritical acceptance of these standard pharmacological techniques could result in discontinuities in frequency distributions (which might indicate polymorphisms) being overlooked (Kalow, 1965). Such techniques are employed more in animal than in human studies and thus this criticism does not explain the relative rarity of polymorphisms in human pharmacogenetics.

Strong indications exist (Harris, 1966, 1969) that polymorphisms are common at the protein structure level. Electrophoresis of blood cell homogenates followed by specific enzyme staining has revealed common variants with different enzyme activities in a high proportion of cases. It, therefore, seems very likely that the enzymes involved in drug metabolism and the proteins constituting the "receptors" in tissues upon which drugs act will be polymorphic at the molecular level.

There are several reasons why polymorphisms have not been revealed more readily by studying pharmacogenetic phenomena in whole mammals (Motulsky, 1971). Many steps are involved in the action of drugs in mammals—absorption, distribution, metabolism, penetration to site of action, and excretion—and all these contribute to the total variability. Consequently, a discontinuous variation that results from polymorphism at one site may be obscured. The experimental techniques required to determine the genetic control of each of these processes are not sufficiently refined and thus the contributions of individual genes are obscured. It should be stressed that polygenic inheritance may be mimicked by a polyallelic system as in the case of red cell acid phosphatase. In other cases, only two or three genes may be involved in a polygenic system. Thus, with new techniques it might be possible to recognize the individual loci concerned in the control of these processes.

Some further specific difficulties should be explained:

(1) Human tissue receptors have not yet been isolated (Rang, 1971) and so are not available to the close scrutiny that might well reveal polymorphism.

(2) Most drugs are principally metabolized by means of mechanisms located in the smooth endoplasmic reticulum of the liver. The process of

oxidation (the most frequent is hydroxylation) is an important and typical example (Parke, 1968). An electron transport chain, analogous to that involved in respiration, is involved in the process. In mammals the individual components of this electron transport chain cannot be examined individually, and therefore the existence of genetically controlled variation involving one member of the chain would be almost impossible to detect.

(3) A large number of compounds (especially drugs) when given *in vivo* result in "induction", i.e., enhancement of microsomal metabolic activity (Remmer & Merker, 1965; Conney, 1967; Mannering, 1968). A typical example of an inducer is phenobarbital. It is impossible to know to what extent induction by unknown environmental agents occurs in individuals not exposed to drugs.

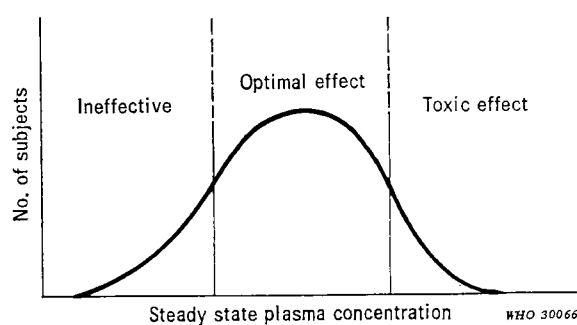
(4) Individuals may differ in the extent to which they respond to inducing agents.

5.2 Practical relevance of considerations of variability

Much drug therapy is only partially effective. Many adverse reactions result from a hypersensitivity or an immunological mechanism, and do not fall within the framework of this section. Even when adverse reactions of this type are excluded, too high a proportion of patients either do not show a satisfactory response to properly administered medication, or exhibit a toxic effect, which may be a direct outcome of the existence of biological variability. Consequently, it is hoped that an improved understanding of such variability might lead to safer and more successful therapeutic practice. Fig. 1 illustrates the distribution of plasma concentrations among a population of individuals who received the same dose of a drug; three types of drug effect can be distinguished: ineffective, optimal, and toxic.

FIG. 1

FREQUENCY DISTRIBUTION OF STEADY STATE PLASMA CONCENTRATIONS RESULTING FROM THE ADMINISTRATION OF A STANDARD DOSAGE REGIME



5.3 Genetic methods of investigating multifactorial variability

The two main methods of defining the relative contributions of heredity and environment to observed unimodal distributions are twin studies and family studies.

5.3.1 *Twin studies and polygenic inheritance*

Twin studies may be very useful as the first approach to genetic investigations, and may sometimes be easier to perform than family studies. The variability between pairs of monozygotic twins must be environmental and is contributed to by the error of the assay method employed. If the response of identical twins to a drug were just as variable as that of unrelated persons then the genetic contribution to the variability would be extremely small.

In fact, the kinetics of all drugs examined so far by twin studies—ethanol, dicoumarol, phenylbutazone, phenazone (Vesell et al., 1971), halothane (Cascorbi et al., 1971), nortriptyline (Alexanderson et al., 1969), isoniazid (Bonike & Lisboa, 1957)—have shown greater similarity between identical than fraternal twins.

Twin studies alone do not give definite information on the mode of inheritance, although distribution curves of the variable being studied can provide useful hints. Family studies are always required to investigate further the role of genetic factors in drug metabolism.

The important question of the genetic control of inducibility was investigated by Vesell & Page (1969) and it was concluded that the response of a subject's metabolic capacity to phenobarbital administration for two weeks was largely under genetic control.

As regards tissue responses to drugs, the influence of phenylephrine eye-drops upon pupillary diameter in twins was examined (Butler & Smith, 1971). The initial pupillary size and the response to phenylephrine showed a much higher intraclass correlation for monozygous than for dizygous twins, thus suggesting a considerable measure of genetic control of both the physiological and pharmacological variable.

5.3.2. *Family studies and polygenic inheritance*

There have not been any family studies of polygenic systems controlling responses of tissues to a fixed concentration of drug. However, pharmacokinetic variations have been studied in families.

Dicoumarol (unpublished observations quoted by Motulsky, 1964) was given to 20 families and there was considerable variation in the half-life of the drug. A significant sib-sib correlation was shown. There was no significant correlation between husbands and wives in regard to the half-life of the drug. Had such a correlation between present, it would have indicated a large environmental contribution to variability.

The plasma half-life of phenylbutazone was found to follow a normal logarithmic distribution in subjects who had been given phenobarbital for the preceding 3 days (Whittaker & Evans, 1970). A significant regression of the mean values for the offspring upon the mean values for the parents was found in 24 families. Under the circumstances of this experiment about two-thirds of the variability observed in the phenylbutazone half-life (an indicator of the metabolism of the drug) was due to the additive effects of genes.

Some patients being treated with the customary clinical doses of nortriptyline and desipramine showed very high steady-state plasma levels of these drugs (Sjoqvist et al., 1968). The families of 3 propositi with high plasma levels were also investigated (Asberg et al., 1971). There was no tendency towards bimodality in the distribution of levels in either the relatives or random subjects. The values for the full sibs of the propositi and for the offspring of the full sibs of the propositi (but not spouses) lay towards the upper end of the distribution curve. It was concluded that the manner of inheritance of nortriptyline kinetics was likely to be polygenic. In these healthy subjects, there was a significant association between the occurrence of side-effects and a plasma concentration above the population median.

5.4 Correlation between the rates of elimination of different drugs in the same individual

It would be genetically interesting and clinically useful to be able to predict how an individual would metabolize several different drugs from the result of a "typing" test performed with one compound. Several attempts in this direction have been made by different groups but no clear conclusions have emerged (Vesell & Page 1968; Sjoqvist et al., 1971; Davies & Thorgeirsson, 1971).

5.5 The influence of miscellaneous factors on the variability of drug metabolism and pharmacological response

Disease generally increases the environmental component of variability. For example, it was shown that in non-pretreated subjects with liver disease, the variance of the plasma half-life of phenylbutazone was 3.7 times that in non-pretreated normal subjects (Levi et al., 1968).

The necessity for careful drug prescription and for graded dosages among neonates and young children is well known. It is also known that the incidence of adverse drug reactions is higher in elderly people (Hurwitz, 1969). Age is probably a factor contributing to variability within the population, as shown by O'Malley et al. (1971).

5.6 Practical applications

It is clear from the twin and family studies that plasma concentrations of drugs are in many cases determined to a considerable extent by genetic factors. It is also known that in some cases there is a direct correlation between pharmacological effect and plasma concentration; and, of course, there are drugs for which this is not so. The model outlined in Fig. 1 seems to apply for digitoxin, digoxin, phenytoin, lidocaine, lithium, nortriptyline, phenylbutazone, procainamide, propranolol, quinidine, and salicylates. Toxic plasma concentrations of digitoxin, digoxin, and nortriptyline, for example, are also accompanied by therapeutic ineffectiveness, so there can be a double disadvantage in being at the upper end of the distribution curve.

5.7 A polymorphism involved in a polygenic system

If it was known how genes at individual loci influence the disposition of and the response to drugs it would not be necessary to describe these phenomena in terms of polygenic inheritance. This goal is not in sight, however, and meanwhile empirical attempts to associate common genes with unknown physiological functions to polygenically determined phenomena may sometimes be successful. A recent example is the finding of a significant association of blood group A with thrombosis in women taking steroid contraceptives. In Britain the risk of hospital admission for thromboembolism is estimated to be 1 in 2 000 for women using oral contraceptives, compared with 1 in 20 000 for women not using them (Westerholm, 1970).

Women of blood group A are 3 times more likely to develop thrombosis when taking oral contraceptives than are women with other blood groups of the ABO system (Mourant et al., 1971). In genetic terms, blood group A represents one of an undefined number of genes predisposing to thrombosis. The mechanism is not known but it is interesting that blood group A is associated with increased antihemophilic globulin levels (Preston & Barr, 1964). This is one of the strongest associations known between the ABO blood groups and disease (Mourant et al., 1971). In general, most attempts to relate a given polymorphism to a particular disease in the absence of any pathophysiological relationship will be fruitless, but with computers such studies are relatively easy to carry out and occasionally interesting results such as the above may be obtained (for example see, Boston Collaborative Drug Surveillance Program; 1972).

5.8 Interethnic differences in polygenic systems of drug metabolism

In the polymorphisms of glucose-6-phosphate dehydrogenase, plasma pseudocholinesterase and *N*-acetyltransferase, there are wide interethnic

differences in allele frequencies. Other pharmacokinetic properties and pharmacological effects, which at present appear to be polygenic characters, may also show interethnic differences, but information is lacking on this topic. Consequently it would be of value to examine pharmacokinetic properties and drug responses in different ethnic groups under standardized environmental conditions.

A preliminary observation in this area is concerned with the disposition of ethanol *in vivo*. It has been shown in studies of twins that there is an important genetic influence on the disposition of ethanol in Europeans (Vesell et al., 1971). The decline of blood ethanol concentrations was studied in Europeans, Eskimos, and North American Indians (Fenna et al., 1971), and was found to be slower in the Eskimos and Indians than in the Europeans.

Ethnic differences in vascular reactions to alcohol ingestion have been found in man (Wolff, 1972). Adults and infants from several countries in East Asia responded with marked facial flushing, mild symptoms of intoxication, and increased pulse pressure to doses of alcohol that had little or no effect on European adults and infants. Presumably, these differences in the reactions of the autonomic nervous system are genetically determined.

Since most drugs are at present initially tested on limited populations, interethnic variability could be of practical importance as well as academic interest when the drugs are later used on a world-wide scale.

6. GENETIC DISORDERS WITH ALTERED DRUG SENSITIVITY

In this section attention is drawn to established abnormalities of drug response that may be detrimental to the well-being of patients with genetic disease, or that may interfere with therapy. It seems to be a general principle that a pharmacological effect that is not harmful in most subjects can be disastrous in persons with certain genetic disorders. No attempt has been made to cover here the numerous alterations of drug response (1) that may be biochemically interesting but seem to be clinically trivial; (2) that serve as a well-known basis for diagnostic procedures; or (3) that are too rare to be of general interest. Neither have genetic differences in the response to, or in the need for, vitamins or other nutrients been covered systematically.

The examples are grouped by disorders of metabolic pathways, or by organs involved, rather than by mode of genetic transmission, because diseases of the same system can have a different genetic basis.

6.1 Gout and HGPRT¹ deficiency

Most forms of primary gout are a result of hereditary disorders of purine metabolism that lead to an overproduction of uric acid. Secondary gout may arise as a consequence of chronic renal diseases or haematological disorders. The factors capable of producing secondary gout in all persons may do so more easily in genetically predisposed subjects.

From the pharmacogenetic point of view at least 3 drug effects should be taken into consideration.

(1) The induction of gout by alcohol may be partly explained by the reduction of nicotinamide adenine dinucleotide that occurs during ethanol metabolism, this favouring lactate formation from pyruvate in sufficient amounts to impair renal elimination of uric acid (Lieber et al., 1962).

(2) Diuretic agents, such as chlorothiazide or furosemide always cause hyperuricaemia because of a reduction in renal excretion of uric acid (Wyngaarden, 1970). Pre-existing hyperuricaemia is enhanced by these drugs, and the risk of diuretic-induced gout is increased in genetically predisposed subjects (Burns et al., 1957).

(3) Allopurinol reduces uric acid production by two different mechanisms. It reduces the conversion of hypoxanthine and xanthine into uric acid and it also inhibits the synthesis of purines. The second effect is absent in 0.5% of patients with gout in whom the "purine salvage enzyme" HGPRT is partially deficient (Stanbury et al., 1972; Fox & O'Sullivan, 1971). In these patients, allopurinol ribonucleotide is not formed and thus purine synthesis is not inhibited and xanthine oxidase (EC 1.2.3.2) is subjected to a higher concentration of allopurinol. As a result, these patients are liable to formation of xanthine stones.

Some other drugs are also transformed by the same phosphoribosyl transferase into ribonucleotides. The antineoplastic agent mercaptopurine must be converted to its ribonucleotide by HGPRT in order to be active *in vivo*. The immunosuppressive agent azathioprine is first converted to mercaptopurine and then activated in the same manner. It is therefore likely that HGPRT-deficient patients with gout are resistant to this form of therapy.

6.2 Hepatic porphyrias

The hepatic porphyrias are a group of disorders involving the metabolic pathway of porphyrins and haem biosynthesis in the liver. They may be classified according to clinical manifestations and to the patterns of urinary

¹ Hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8).

and faecal excretion of porphyrins and their precursors. The prevalence of the porphyrias varies greatly in different communities. Three genetically transmitted, autosomal dominant types are clearly established:¹

- (1) intermittent acute porphyria (Swedish type porphyria, pyrroloporphyria);
- (2) porphyria variegata (South African type porphyria, protocoproporphyria); and
- (3) hereditary coproporphyria.

The characteristic feature of the hepatic porphyrias is an overproduction of the rate-limiting enzyme, δ -aminolaevulinic acid (ALA) synthetase (EC 4.2.1.24) in the liver. Several common drugs induce ALA synthetase production in the liver and may precipitate clinical exacerbations when given in therapeutic doses to patients in remission or in latent stages of hereditary hepatic porphyrias. These include barbiturates, sulfonamides, griseofulvin, estrogens including those used in contraception, some anticonvulsants and tranquillizers, and possibly general anaesthetics, ethanol, and chloroquine. This effect is not invariable, however, and patients with latent porphyria have been known to receive barbiturates or other drugs without developing clinical symptoms (Tschudy, 1970; Goldswain & Eales, 1971; Marver & Schmid, 1972). Further investigations are required to determine which drugs are potentially harmful in different forms of hepatic porphyria and to estimate the relative frequencies of such reactions. A recent study in South Africa suggests that the following drugs should be avoided by patients with porphyria variegata (Eales, 1971): barbiturates (all varieties); nonbarbiturate hypnotics (e.g., glutethimide, meprobamate); pyrazolone compounds (e.g., aminophenazone, phenazone); anticonvulsants (e.g., hydantoin); sulfa drugs; griseofulvin; synthetic estrogens, and progestogens; ergot preparations.

6.3 Hyperbilirubinaemia

Gilbert's syndrome represents a common form of chronic, mild, non-incapacitating hyperbilirubinaemia. The excess bilirubin is entirely in the unconjugated form and there is no serious liver dysfunction. Gilbert's syndrome is often accompanied by a moderate reduction in activity of hepatic glucuronyl transferase (EC 2.4.1.17) (Black & Billing, 1969), but defects of bilirubin uptake have also been implicated suggesting heterogeneity of causation. Alcohol, cholecystographic agents, and to some extent the estrogens in oral contraceptives (Fleischner & Arias, 1970) may cause an increase in plasma levels of bilirubin.

The Crigler-Najjar syndrome is now known to consist of two different disease entities (Arias et al., 1969). Both are rare and caused by a deficiency

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1968, No. 401.

of glucuronyl transferase. The more severe form leads relatively often to kernicterus and is life threatening. It is inherited as an autosomal recessive character, and is unaffected by administration of phenobarbital. The less severe form is usually without kernicterus, is inherited as an autosomal dominant character, and the hyperbilirubinaemia can be dramatically reduced by chronic administration of phenobarbital. The effect appears to be due to the induction of glucuronyl transferase by phenobarbital.

Jaundice with elevated levels of conjugated bilirubin occurs in the Dubin-Johnson syndrome and rotor disease. Oral contraceptive agents, particularly those containing estrogens may convert a mild chemical hyperbilirubinaemia of the Dubin-Johnson syndrome into overt clinical jaundice. The jaundice tends to revert quickly following the cessation of this drug administration (Fleischner & Arias, 1970).

6.4 Bleeding disorders

Many genetic disorders can give rise to haemorrhages.¹ They may be due to the deficiency of one or other clotting factor in plasma (e.g., haemophilia A, deficiency of Factor VIII), to vascular abnormality (telangiectasia), or to essentially unknown causes (e.g., von Willebrand's disease, characterized by prolonged bleeding time and variable Factor VIII deficiency). In all of these disorders, and probably some others as well, acetylsalicylic acid may promote bleeding as an exaggerated response (Goldstein et al., 1969; Quick, 1970; Weiss, 1970).

Haemophilia patients tend to take acetylsalicylic acid for the relief of pain in their bleeding joints. This in turn increases the bleeding and the subsequent pain so that a vicious cycle is established. Bleeding induced by this drug may of course occur in other locations, e.g., in the gastrointestinal tract or in the brain.

The prolongation of bleeding time after a single dose of acetylsalicylic acid lasts for several days and exceeds the presence of measurable levels of the drug in plasma (Weiss et al., 1968). Sodium salicylate does not cause bleeding. Experimental administration of acetylsalicylic acid does not prolong bleeding time in all patients with haemophilia A, but apparently does so in all subjects with von Willebrand's disease (Quick, 1970).

Bleeding induced by acetylsalicylic acid may be the result of a direct effect on the vascular wall.

6.5 Osteogenesis imperfecta

In patients with this disease, general anaesthesia conducted with halothane and suxamethonium may lead to a substantial elevation of body

¹ *Wld Hlth Org. techn. Rep. Ser.*, 1971, No. 504.

temperature (Solomons & Myers, 1972). This elevation is benign and more easily controlled than that occurring in patients with malignant hyperthermia.

6.6 Periodic paralysis

Periodic paralysis is characterized by episodes of muscular weakness or paralysis. There are 3 genetic types of the disorder, commonly distinguished by the behaviour of the plasma potassium level during paralysis. The potassium level may rise, fall, or remain unchanged. An apparently nongenetic, hypokalaemic, periodic paralysis is a well-recognized complication of thyrotoxicosis, a complication that seems to occur particularly in the Japanese, and therefore suggests the operation of yet undetected genetic factors.

Hypokalaemic paralysis has been induced by a variety of agents,—for instance, insulin, mineralocorticoids (except aldosterone), epinephrine, or ethanol. Particularly puzzling is the induction of attacks by ammonium glycyrrhizinate (liquorice). Hyperkalaemic paralysis can be initiated by administration of potassium chloride, and severe paralysis has also been precipitated by anaesthesia (Egan & Klein, 1959; Gross et al., 1966; Layzer et al., 1967; Pearson & Kalyanaraman, 1972). Abnormal effects of drugs, particularly of suxamethonium, in other muscle diseases have been reviewed recently (Kalow, 1972).

6.7 Familial dysautonomia (Riley-Day syndrome)

This disorder is inherited as an autosomal recessive character and causes protean manifestations of neurogenic origin. Most often affected are Jewish families originating from circumscribed areas in Eastern Europe (Brunt & McKusick, 1970).

The response of dysautonomic children to the parasympathomimetic drug methacholine was tested (Dancis, 1968) and was found to be exaggerated. The clinical difficulties presented by these patients arise from their labile blood pressure during anaesthesia, their lack of cough reflex, and their intolerance to halothane and methoxyflurane (Meridy & Creighton, 1971). Pretreatment with atropine and propranolol before anaesthesia is necessary to dampen their abnormal autonomic responses.

6.8 Huntington's chorea

The clinical manifestations of this autosomal dominant disorder, progressive deterioration of the personality and dementia, are delayed until the age of 30–40. Levodopa is known to induce involuntary, choreiform movements in patients with Parkinsonism and it was speculated that carriers of the gene for Huntington's chorea might manifest such movements at a

lower dose of levodopa than do normal people or patients with Parkinsonism. The challenge with levodopa of a group of persons at 50% risk of developing Huntington's chorea, induced mild transient chorea in the expected proportion. This test cannot be recommended, however, since it has not been validated or widely tested, and it could even hasten the onset of the disease.

7. STATES IN WHICH GENETIC FACTORS MAY ALTER DRUG RESPONSE

7.1 Toxic effects produced by environmental agents

In general, susceptibility to intoxication by pollutants and poisons varies as much between subjects as does the susceptibility to therapeutic drug effects. It is necessary to clarify the role of genetics in the predisposition of individuals to show deleterious effects from such poisons.

7.1.1 α_1 -antitrypsin deficiency

The rare homozygous state for the Z type of the serum protein antitrypsin is associated with severe deficiency of this substance and predisposes to chronic emphysema and pulmonary insufficiency at a relatively young age in both men and women. Heterozygotes for variants of this protein are more common and may also be predisposed to chronic pulmonary disease. These conditions are of interest in the context of pharmacogenetics, since pulmonary irritants and smoking are thought to worsen the pulmonary disease and hasten its onset. Abstention from smoking and avoidance of industrial irritants are therefore particularly indicated.

7.1.2 *Minamata disease*

This condition is characterized by different neurological and psychiatric signs and symptoms and has been found to be due to poisoning by organic mercury compounds. The two large-scale outbreaks of this disease that have occurred in Japan have shown that there is considerable variability between individuals in the level of exposure at which they may develop symptoms. Other examples, such as itai-itai disease, which may be due to cadmium poisoning, may be similar in this respect.

7.2 Pharmacogenetic effects of toxic substances

7.2.1 *Hyperoxaluria*

Primary hyperoxaluria is a rare disorder that can be caused by two different genetic defects and is characterized by the formation of oxalate stones in the kidneys and by nephrocalcinosis, frequently leading to renal

failure. This disease resembles poisoning by ethylene glycol fluoride, such as might occur within the body after breakdown of the anaesthetic agent, methoxyflurane. Renal damage and hyperoxaluria seem to be prominent in only some subjects receiving methoxyflurane. It is possible that such individuals may have a hereditary predisposition to respond in this particular way. Some degrees of hyperoxaluria have also been noted in patients with liver cirrhosis, renal tubular acidosis, experimental pyridoxine deficiency and Klinefelter's syndrome.

7.2.2 Heavy metal and other poisons

The renal defects associated with the Fanconi syndrome can be mimicked by poisoning with a degradation product of tetracycline and with heavy metals, such as lead, cadmium, mercury, and uranium. Transient features of the syndrome appeared during the recovery phase in a case of lysol poisoning.

A clinical syndrome similar to that of renal tubular acidosis can arise as a consequence of vitamin B intoxication, paraldehyde intoxication, cadmium poisoning, possibly chronic mercury intoxication, and in various other pathological conditions, such as hyperthyroidism or Wilson's disease.

7.3 Mutagenic effects of environmental contaminants

The environment contains many mutagens, both naturally occurring (e.g., aflatoxin) and synthetic (e.g., pesticides, insecticides, and herbicides). Two agents can act in a potentiating or synergistic fashion, and it sometimes happens that a mutagenic agent can arise from a metabolic or degradation product of an agent not mutagenic in its original form. Thus genetic differences in metabolism may result in differences in the induction of mutations.

The genetic consequences of exposure of human populations to mutagenic agents are not clear, and it is unknown to what extent individuals differ in their susceptibilities.

7.4 Drugs of abuse and addiction syndromes

The commonest drug of abuse is ethanol. Family studies reveal a high prevalence of alcoholism among close relatives of alcoholics, but the roles of genetic and environmental influences have not been quantified. Such a distinction could be obtained by comparing the blood relatives and adoptive relatives of children adopted early in life, a method that has been used extensively in studies of schizophrenia. The areas of genetic influences in alcoholism could affect: (1) susceptibility to acute intoxicating effects;

(2) metabolism of ethanol; (3) central nervous system cellular adaptation to chronic intake—"addictability"; (4) predisposing factors, and (5) susceptibility to complications (e.g., cirrhosis).

7.5 Various neuropsychiatric conditions

7.5.1 *Schizophrenia*

There is genetic predisposition to schizophrenia, and it might be interesting to try and determine whether individuals who have schizophrenia-like reactions to drugs such as amphetamines have a relatively high risk of developing the disease spontaneously.

7.5.2 *Depression*

Depressive syndromes (especially manic-depressive psychosis) have an important genetic element in their etiology. Studies of the responses of patients to drugs indicate that they could be used to delineate particular syndromes within the heterogeneous group of depressions. This arises from the observation that affected relatives of depressed patients tend to respond to the same group of drugs (either monamine oxidase inhibitors or tricyclics) as the probands.

A proportion of patients treated with reserpine become depressed. It seems possible that persons who respond in this way may have a predisposition to depression.

7.5.3 "*Minimal brain dysfunction*" and the problem of hyperkinetic children

There is a loosely defined clinical syndrome in schoolchildren, consisting of motor hyperactivity, distractibility, and poor learning performance. Boys are more frequently affected than girls. While many of these children have behavioural problems, some may be suffering from an organic disorder (minimal brain damage). A twin study suggests that genetic factors may be of importance in the production of the syndrome in the latter group of children. A pharmacogenetic approach to this disorder may be fruitful in that it has been observed paradoxically that amphetamine quietens whereas phenobarbital excites hyperkinetic children. Studies of the metabolism of, and responses to drugs in, family members may serve to clarify the role of genetic factors.

7.6 Phenothiazine drugs

Phenothiazines induce extrapyramidal effects in a proportion of patients and there is evidence that spontaneous Parkinsonism occurs more frequently in their relatives than in the population at large.

8. PUBLIC HEALTH ASPECTS OF PHARMACOGENETICS

The extent of the impact of pharmacogenetics on public health needs better definition. Common pharmacogenetic conditions under monogenic control that occur in relatively high frequency (see above) include: G6PD deficiency, atypical pseudocholinesterase, acetyltransferase deficiency, and the heterozygous state of methaemoglobin reductase deficiency. No epidemiological studies have been performed to assess the frequency of drug reactions caused by these traits and this information is urgently needed. Undetected polymorphisms and rare single gene variants may also play a part in causing hitherto poorly understood adverse reactions.

The World Health Organization has launched a drug monitoring programme and receives reports from 15 countries. In 4 years, details of more than 80 000 adverse reactions have been gathered. The genetic contribution to these drug reactions needs to be defined; the genetic component of drug hypersensitivity reactions remains largely unknown. The details of these cases are on record at WHO and might constitute a source of probands for genetic studies. All cases of increased or decreased drug efficacy should be recorded.

Large variations in the elimination of drugs from blood may contribute significantly to adverse drug reactions. It has become clear that different individuals require different doses of a drug for effective therapy and that such variability is largely genetic in origin. From the studies that have been performed to define the relative magnitude of the genetic and environmental components of variations in plasma half-life of drugs it has been found that the genetic component exceeded the environmental component in each case. On the basis of numerous studies that indicate large variations between individuals in the plasma half-life of drugs it may be estimated that a small proportion of the population may be at risk for adverse drug reactions because they have a much slower capacity to clear drugs than others. For instance, it is estimated that drug plasma half-lives in approximately 2.5% of the population are about three times as long as the median for the population. These calculations are based on the administration of a single dose of drug to normal volunteers. With repeated administration relatively small differences in drug half-life can lead to very large differences in plasma drug concentrations. The risk of toxicity with drugs whose plasma half-lives are dose-dependent increases appreciably with long-term therapy. There is also an increased risk for persons who metabolize drugs slowly and who receive, over a long period, any therapeutic agent with a long biological half-life.

Another important application of pharmacogenetics in public health is to search for carriers and other affected individuals at high risk in families where a proband has been detected. Once a proband is identified a complete

family study should be performed. In searching for affected family members, extensive family investigation is justified only in disorders transmitted as dominant characters, whereas in the rare disorders that are autosomal recessive only the sibs would tend to be affected. However, it should be recognized that psychological harm may result from such screening programmes, and thus the counselling of individuals with genetic disease should be approached with caution.

Mass population screening for some pharmacogenetic traits, such as G6PD deficiency and pseudocholinesterase variants, is not recommended at this time, since these traits do not carry any significant general health hazards. An abnormal finding might be misconstrued by the carriers and might do more harm than good, particularly psychologically.

In well defined medical situations, pseudocholinesterase testing in surgical patients, prior to administering suxamethonium, or G6PD testing in patients receiving potent haemolytic agents would be of great value. In view of the ethnic distribution of these traits, logistic difficulties might arise in some countries. Thus G6PD deficiency almost always occurs in persons whose ancestors originated in tropical or subtropical countries, while cholinesterase abnormalities are more common in persons of European ancestry and are practically absent in Orientals and Africans.

9. RECOMMENDATIONS

Pharmacogenetics is an interdisciplinary field that requires close cooperation between clinicians, pharmacologists, geneticists, and others in allied disciplines. The development of pharmacogenetics has not been as rapid in the last decade as its public health implications would warrant, a problem common to most interdisciplinary fields. The Scientific Group believes that more rapid progress should now be possible. It was agreed that research effort would most usefully be concentrated on the following problems:

1. The improvement of pharmacogenetic methods:
 - (a) the application of more refined genetic analysis to polygenic systems;
 - (b) the development of methods to identify the discrete components of polygenic systems by laboratory techniques;
 - (c) the further development of chemical analytical methods and kinetic techniques for the recognition of phenotypes; and
 - (d) the use of *in vitro* techniques (including tissue culture) to predict the response of individuals to given drugs.
2. The study of population genetics with the object of:
 - (a) elucidating ethnic variability in the metabolism of, and in responses to, drugs; and

(b) identifying particular populations at high risk because of their possession of certain known alleles in high frequency.

3. The investigation of the genetic component in hypersensitivity reactions to drugs, since these reactions constitute a major public health problem and in most cases the underlying mechanism is poorly understood.

4. The elucidation of the pathophysiological mechanisms underlying proven associations between polymorphisms and disease, especially those produced or enhanced by drugs.

5. The study of the mental changes produced as adverse reactions to certain drugs. For example, it is known that mental changes are produced in a proportion of patients treated with corticosteroids, reserpine, levodopa, barbiturates, amphetamines, pentazocine, and methyl dopa. Studies of such adverse reactions might shed light on the processes that lead to the spontaneous occurrence of mental disorders.

6. The search for genetic factors when attempts are made to unravel the mechanisms of clinically significant drug interactions.

7. The application of the techniques and approaches to pharmacogenetics to the study of the effects of other environmental chemicals, including industrial wastes.

10. CONCLUSIONS

1. Sufficient examples exist to establish pharmacogenetics as a separate field of scientific inquiry. Pharmacogenetics is not simply of theoretical interest but of practical importance in the delivery of health care. For example, the carriers of certain genetic traits are at high risk of developing adverse reactions when they receive certain drugs.

2. In spite of the practical importance of pharmacogenetics, the field has not progressed as far as might have been anticipated in the discovery of new monogenic conditions. It has become apparent, however, that genetic factors play a significant role in the mechanisms affecting the fate of most drugs.

3. Research workers involved in evaluating drugs in man should receive training in pharmacogenetic principles to permit them to recognize the possible role of genetic factors in determining drug efficacy and in causing adverse drug reactions. Interdisciplinary educational efforts by geneticists, pharmacologists, and physicians should be fostered. The World Health Organization and other agencies should be encouraged to develop appropriate training programmes.

4. The requirements for progress in pharmacogenetic research have been identified and are described above.

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