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GENETIC CONTROL OF DRUG METABOLISM AND DRUG ACTION IN MAN

Introduction : The study of genetic factors that modify the individual response to drugs, referred to as "pharmacogenetics" is a relatively new field, a discipline at the interface between genetics and clinical pharmacalogy. In the short period since Motulsky (1) emphasized the importance of genetics to pharcology and Vogel coined the term "pharmacogenetics," an impressive number of examples in man has accumulated in which inherited differences account for strikingly exaggerated responses to drugs, novel drug effects, or lack of effectiveness of drugs given in the usual dosage. More recently, we have come to realize that genetic factors are in large measure responsible for the individual variability in response to drugs. conferring on each patient a "pharmacologic individuality."

The objectives of pharmacogenetice include the identification of genetically controlled variations in response to drugs and the study of the molecular basis for these conditions, their clinical significance, and most important, the development of simple methods by which susceptible individuals can be recognized before the drug is administered.

Genetic control of drug action : The basic tenets of pharmacogenetics are simple. The genetic endowment of the individual, phenotypically expressed in protein structure, concentration, and configuration may alter drug action in multiple ways.

A drug entering the body comes into contact with numerous enzymes and other proteins. Nearly all drugs undergo enzymatically controlled transformations during their passage through the liver and other organs. Theoretically, genetic mutations that alter the quality of any of these proteins could lead to a recognizable disturbance in drug-cell interactions. For instance, variations in enzyme components of drug metabolism and conjugation may retard inactivation and consequent excretion of drugs, resulting in toxic concentrations after small doses. They may modify the site of enzymatic attack on the drug molecule, producing different metabolites with differing properties. Alternatively genetic mutations may increase the activity or inducibility of drug-metabolizing enzymes and prevent the drug from reaching effective concentrations in plasma or tissues. Structural differences in serum proteins could presumably change binding affinities and alter the ratios of bound to free drug. Similarly, aberrant gene products

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at the site of a drug's action in organs, tissues, or cells may confer increased or decreased responsiveness to usual therapeutic concentrations of a drug,

In some subjects, unusual reactions to drugs may be inherited without the hereditary defect being directly associated with the pharmacokinetic behaviour or with the usual response of organs, tissues, or cells to a particular drug. The prototype of this phenomenon is represented by the syndrome of drug-induced hemolytic anemia in subjects genetically deficient in glucose- 6 -phosphate dehydrogenase (G-6-PD) in their erythrocytes.

Pharmacogenetic conditions may exist due to the action of a single mutant gene. This leads to discontinuous variation in the measure of drug action or-metabolism, and the population can be divided into two or more groups (with continuous variation within each group) suggesting single. gene polymorphism. The variations most frequently observed have, however, a Gaussian distribution resulting from polygenic and/or environmental factors or influences.

Detection of pharmacogenetic conditions : An unexpected drug response or unusual metabolism is the starting point of the study detailed studies of the affected patients and their close relatives show whether the condition is present in other family members, permitting recognition of the transmission by classic mendelian inheritance, i.e., as an autosomal dominant or recessive, or X-linked trait.

A survey in a sample population can be conducted looking for either a distinct subpopulation or for individuals with. an unusual response. This method was successfully employed by evans and co-workers (2) In their classical studies on the inherited variability in the metabolism or inactivation of isoniazid. This method; is not favoured, because of ethical consideration and other difficulties.

Another approach is to conduct studies with a small series of identical and fraternal twins to determine whether an observed variation in drug response can be attributed primarily to genetic or environmental factors This is achieved by the calculation of the heredity co-efficient (H) from the formula :

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

The value of H ranges from 0 to 1, from "negligible hereditary contribution" to "virtually complete hereditary

REFERENCES :

1 Motulsky, A. G. Drug reaction's, enzymes and biochemical genetics. J.A.M.A. 165: 35-37, 1957.

2 Evans, D.A.P., Manley, K.A. & McKusick, V.C. Genetic control of isoniazid metabolism in men. Brit. Med. J. 2:485-91, 1960.

3 Osborne, R H. & De George, F V. : In Genetic basis of morphological vatiation. Vol. 17. p 60 Harvard University Press, Cambridge, Mass , 1959.

4 Vesell, E.S. & Page, J.G. Genetic control of drug levels in man: antipyrine. Science 161: 72-73, 1968.

5 Vesell, E.S & Page, J. G, Genetic control of dicumarol levels in man. J. Clin. Invest. 47: 2657-63, 1968.

6 Vesell, E S. & Page, J.G. Genetic control of drug levels in man: phenylbutazone. Science 159:1479-80, 1968.

7 Alexanderson, B., Price - Evans, D A. & Sjoqvist, F. Steady-state plasma levels of nortryptyline in twins : influence of genette factors and drug therapy. Brit. Med J. 4:784-68, 1969.

8 Raghupati Sarma, G., Kailasam, S., Nair, N.G.K , Narayana, A.S.L. & Tripathy, S.P. Effect of prednisolone and rifampin on isoniazid metabolism in slow and rapid inactivators of isonazid. Antimicrob. Agents Chemother. 18: 661-666, 1980.

9 La Du, B.N. Isoniazid and pseudocholinesterase polymorphisms. Fed. Proc. 31: 1276-85, 1972

10 Gangadharam. P. R. J , Bhatia. A. L., Radhakrishna, S. & Selkon J B. Rate of inactivation of isoniazid in South Indian, patientswith pulmonary tuberculosis. Bull. WHO 25 : 765-77, 1961

influence" (3). A number of drugs such as isoniazid antipyrine, dicumarol and phenylbutazone (4-6) and nortryptiline (9) have been investigated using this approach. These studies however, do not permit a distinction between modes of mendelian or polygenic inheritance.

Specific pharmacogenetic disorders

Slow and rapid acetylation of isoniazid : Isoniazid is an antituberculosis drug and the primary step in its metabolism is acetylation to acetylisoniazid (Fig.1). The metabolizing enzyme is a hepatic N-acetyltransferase, which displays genetic polymorphism. Analysis of the inheritance of the two phenotypes, slow and rapid acetylators of the drug, indicate that slow metabolism is due to an autosomal recessive gene, and that slow acetylators are homozygous for the recessive gene; rapid acetylators could either be heterozygous or homozygous for the dominant gene. The difference in the two phenotypes is due to the difference in the quantity rather than the quality of the enzyme, the rapid acetylators having nearly 4-5 times the quantity of the enzyme as the slow inactivators (10-12). The incidence of slow acetylators in different populations varies remarkably, ranging from about 5% in Canadian Eskimoes to about 83% in Egyptians (13). Human N-acetyltransferase catalyzes the acetylation of a number of other drugs such as sulphadimidine, hydralazine, dapsone and phenelazine, and the acetylation of these drugs parallels that of isoniazid.

A number of methods involving determination of concentrations of isoniazid in serum (13, 14, 15) or the ratio of acetylisoniazid to isoniazid in urine collections (16-18) following dosage with isoniazid have been developed for classifying subjects as slow or rapid acetylators. Methods based on the estimation of free and acetylsulphadimidine in blood and urine following oral administration of sulphadimidine have also been employed for this purpose (19-21). A simple qualitative test based on the excretion of free sulphadimidine in urine has also been developed at our Centre (22)

A number of controlled clinical trials of the treatment of pulmonary tuberculosis (23, 27) have shown that the rate of acetylation of isoniazid is of no prognostic significance when patients are treated with either daily or twice-weekly regimens containing this drug; however, with once-weekly regimens of isoniazid, but without rifampicin (15, 25, 28), the response to treatment in rapid-acetylators was considerably inferior to that in slow acetylators (Table 1). Detailed pharmacological investigations undertaken at our Centre (15, 29, 30) have established that the failure of once-weekly regimens in rapid acetylators was predominantly due to inadequate exposure (area under the time-concentration curve) and coverage (hours for which a bacteriostatic concentration of isoniazid 0.2 pg/ml is maintained).

REFERENCES

11 Venkataraman, P., Menon, N.K., Nair, N.G.K. et al Classification of subjects as slow or rapid inactivators of isoniazid. Indian, J. Med. Res. 60 : 68 -93, 1972

12 Raghupati Sarma, G., Chandra Immanuel Kailasam, S., et all. A modified method for the estimation of acetylisoniazid in man. Indian J. Med. Res. 62:945-52, 1974.

13 Raghupati Sarma, G., Kannapiran, M., Narayana, A.S.L., et al Determination of acetylator phenotype Indian J. Med. 64:1-8, 1979

14 Rao, K,V.N, Mitchison, D.A., Nair, N.G.K, Ethyl Sulphadimidine acetylation test for classification of patients. Brit. Med. J. 3 : 405-67, 1970.

15 Raghupati Sarma, G., Kannapiran, M , Narayanan, A.S.L. & Radhakrishna, S. A qualitative test for the determination of isoniazid acetylator phenotype. Indian J. Med. Res 68 : 335-40, 1978.

16 Tuberculosis Chemotherapy Centre, Madras. Isoniazid plus thioacetazone compared with two regimens of isoniazid plus PAS in the domiciliary treatment of pulmonary tuberculosis Bull. WHO 34 : 483-515, 1966.

17 Tuberculosis Chemotherapy Centre, Madras. Controlled comparison of oral twice-weekly and oral daily isoniazid plus PAT in newly diagnosed pulmonary tuberculosis Brit. Med. J. 2: 7-11, 1273.

18 Tripathy, S.P. A slow-release preparation of isoniazid : Therapeutic efficacy and adverse side-effects. Bull. Int. Union Tuberc. 51 : 133-41, 1976.

19 Raghupati Sarma, G. Kailasam, S., Mitchison, D.A., Etal. Studies of serial plasma isoniazid concentrations with different doses of a slow-release preparation of isoniazid. Tubercle 56 : 314-23, 1975.

20 Devadatta, S., Gangadharam, P.R.J., Andrews R.H., Etal, Peripheral neuritis due to isoniazid. Bull. WHO 23 : 587-98, 1960.

The main adverse reaction during isoniazid therapy is peripheral neuropathy (22, 23). In studies undertaken at our Centre (30), it was established that the incidence of this adverse reaction was related to the dose of isoniazid, and that it was substantially higher in slow than in rapid acetylators (Table II) This adverse reaction is caused by the more rapid elimination of pyridoxine as a hydrazone of isoniazid, and pyridoxine is an essential co-enzyme required for the formation of Y-aminobutyric acid from glutamic acid. Deficiency of Y-aminobutyric acid leads to epileptiform convulsions and peripheral neuropathy. This can be overcame with the administration of prophylactic doses of pyridoxine.

Another adverse reaction encountered during isoniazid treatment is hepatitis. Mitchell and co-workers (21) have suggested that isoniazid may be more hepatotoxic for rapid than for slow acetylators because rapid acetylators might be expected to form monoacetylhydrazine more rapidly than do slow acetylators (Fig. 1), and monoacetylhydrazine can be converted by p-450-dependent hepatic microsomal enzymes to a potent acylating agent capable of causing hepatic necrosis. This hypothesis has been refuted on the ground that rapid acetylators acetylate monoacetylhydrazine more rapidly than do slow acetylators to the non-toxic diacetylhydrazine and that exposure of rapid acetylators to monoacetylhydrazine is, in consequence, similar to that of slow acetylators (29,36) Indeed, a retrospective analysis of the incidence of hepatitis among 3000 patients with pulmonary tuberculosis during treatment with a variety of isoniazid-containing regimens without rifampicin has shown that the incidence was low (1.3%), and that rapid acetylators were no more prone to develop isoniazid-induced hepatitis then were slow acetylators (27). This is a remarkable example of an interaction between genetic and environmental factors in the pathogenesis of a drug-induced disease.

Slow metabolism of diphenylhydantoin : Diphenylhydantoin is used in the treatment of epilepsy. Over-dose toxicity was observed in several family members when the drug was given in the usual doses. Diphenylhydantoin is metabolized to a p-hydroxy compound (Fig. 2); it has been shown that the metabolism of this drug-is genetically controlled and that patients who developed over-dose toxicity are probably deficient in this microsomal hydroxylase (28). Kutt and co-workers (24) noted that some patients given both diphenylhydantoin and isoniazid in the usual-doses developed signs of diphenylhydantoin overdosage, and that patients most likely to show intolerance were slow acetylators of isoniazid. It has been established that this is due to the inhibition by isoniazid of the microsomal hydroxylase responsible for the metabolism of diphenylhydantoin.

REFERENCES :

21 Mitchell, J.R., Thorgeirssop U.P., Black, M., Etal, Increased incidence of isoniazid hepatitis in rabid acetylators : possible relation to hydrazid metabolites. Clin. Pharmacol. Ther. 18 : 70-79, 1975.

22 Ellard, G.A , Mitchison, D.A., Girling, D J , Nunn, A J. & Fox, W. The hepatic toxicity of isoniazid among slow and rapid acetylators of the drug. Am. Respir Dis 118, 628-9, 1978.

23 Prema Gurumurthy, Krishnamurthy, M.S., Nazareth, O., Etal. Lack of relationship between hepatic toxicity and acetylator phenotype. Am. Rev. Respir. Dis 129 : 58-61, 1984.

24 Kutt, H., wolk, M., Sherman, R. & McDowell, F. Insufficient parahydroxylation as a cause of diphenylhydantoin toxicity, Neurol. 14: 542-48, 1964.

25 Kclow, W. In pharmacogedetics : Herity and the response to drugs. W.B. Saunders Co., Philadelphia, 1962.

26 Krishnan, H.N. Glucose+phosphate dehydrogenase variants and drug-induced hemolysis. Ann. N.Y. Acad. Sci 151 : 753-94, 1968

27 Motlusky, A G. Hemolysis In glucose-6-phosphate dehydrogenase defloiencs. Fed Proc. 31 : 1286-92, 1972.

28 Urs. A. M. Role of genetic factors in the rational use of drugs. In Clinical pharmacology-Basic principles in therapeutics (Rd. Melmon, K. L. & Morrelli, HF.) 2ud ed., p. 913 McMillan Publishing Co. Inc., New York, 1978

Sensitivity to succinylcholine : Succinylcholine is used as adjunct to anaesthesia. It is a muscle relaxant and causes muscle paralysis for a short duration of time. Soon after its introduction, it was seen that a small number of patients had prolonged apnea following a single dose. A familial background was soon disclosed (7, 25). Succinylcholine, a dicholine ester of succinic acid, is hydrolyzed by pseudocholine esterase in serum. Normally the homozygous carrier genotypes being referred to as E^u E^u , this enzyme rapidly converts succinylcholine to succinylmonocholine, an inactive compound, allowing very little of the parent compound to reach myoneural receptor sites. In patients with unexpected sensitivity or resistance to the drug variant forms of the enzyme with altered qualitative Or quantitative properties have been detected (Table Ill).

The most common variant, so called "atypical" pseudocholinesterase has a lower affinity for choline-esterase substrates and less susceptible to inhibition by dibucaine. Using the percentage inhibition by dibucaine of esterase activity in samples of plasma, homozygote and heterozygote carriers of the gene defects have been identified (25). Sensitivity to succinyicholine is inherited as an autosomal recessive trait.

Drug-induced hemolytic anemia : A number of individuals treated with certain drugs (e.g., primaquine) developed hemolytic anaemia, it was shown that these individuals were deficient in glucose-6-phosphate dehydrogenase (G-6-PD) in their erythrocytes (26). A number of variants of G-6-PG (at least 80) have been identified; these may have an activity ranging from 0 - 100% of normal, and there are corresponding variations in the clinical consequences. Thus, one form which occurs not uncommonly in black Africans has about 15% of normal activity. Another variant which is most common in Mediterranean people has an activity of 0-7% of normal. and predisposes to favism (consumption of feva beans). Four other types of the enzyme, with activities ranging from 0-26% of normal appear to be responsible for hemolytic anaemia (27).

G-6-PD catalyses the formation of NADPH (through the pentose pathway of glucose metabolism), end NADPH is essential for maintaining glutathione in its reduced from (Fig. 3). Reduced glutathione is essential for the conversion of methemoglobin (oxidised form) to hemoglobin (reduced form). Lack or deficiency of G-6-PD, in the presence of several oxidant drugs, causes a more rapid conversion of reduced glutathione to the oxidised form; and results in the accumulation of methemoglobin, Heinz body formation, hemolysis and hemolytic anaemia. A number of drugs (28, 29) such as antimalarial% antibacterials, analgesics and antipyretics are known to cause methemeglobinemia and hemolysis (Table IV).

REFERENCES :

29 Shahidi, N.T. Acetophenetidin-induced methemoglobinema, Ann. N.Y. Acad. Sci. 151 : 822-32, 1968

30 Seegmiller. J.E., Rosebloom, F.M. & Kelley, W.N. Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. Science 155 : 1682-84, 1967.

31 O'Reilly, R.A., Aggeler, P.M., Hoag, M S., Leong, L.S. & Kropatkin, M.L. Hereditary transmission of exceptional resistance to coumarin anticoagulant drugs. The-first reported kindred. New Eng. J. Med. 271 : 809-15, 1964.

32 O' Reilly, R.A., Pool, J G. & Aggelar, P.M. Hereditary resistance to coumarin anticoagulant drugs in man and rat Ann. N.Y. Acad. Sci. 151 : 913-31, 1968.

33 O'Reilly, R.A. The second reported kindred with hereditary resistance to oral anticoagulant drugs. New Eng. J. Med. 282 : 1448-51, 1970.

34 O'Reilley, R.A. & Aggeler, P.M. Coumarin anticoagulant drugs : hereditary resistance in man Fed. Proc. 24 : 1266-73, 1965.

35 Motulsky, A.G. & Stamatoyannopoulos, G. Drugs, anesthesia and abnormal hemoglobins. Ann. N.Y. Acad. Sci. 151: 808-21, 1968

36 Brit, B.A. & Kalow, W. Hyperrigidity and hyperthermia associated with anesthesia. Arm. N.Y. Acad. Sci. 151 : 947-57, 1968.

37 Kalow, W, B.A., Terreau, M.E. & Haist, C. Metabolic error. of muscle metabolism after recovery from malignant hyperthermia. Lancet 11 : 895-98, 1970.

G-6-PD deficiency is determined by a sex-linked gene carried on the X-chromosome. The X-chromosomes of males can be normal or defective and two male genotypes “reactor” or “normal” are expected; females can be divided into three groups, “reactor”, “intermediate” or “normal” depending on the presence of two defective X-chromosomes, one normal and one defective, and two normal X-chromosomes, respectively.

Acetophenetidin and methemoglobinemia : Acetophenetidin (phenacetin) is usually metabolized in the body to N-acetylphenetidin (paracentamol), the active ingredient (Fig. 4) The O-dealkylation is genetically controlled and in individuals deficient in this enzyme, acetophenetidin is metabolized through an allernate pathway resulting in the formation 2-hydroxyphenetidin. This compound is known to be toxic as it causes methemoglobinemia and hemolysis in G-6-PD deficient individuals (29).

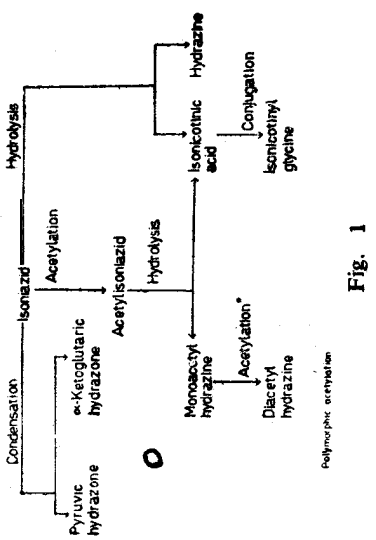

Fig. 1

HGPRT deficiency : Allopurinol is used in the treatment of gout. This drug inhibits the production of uric acid, by two different mechanisms; it decreases the conversion of hypoxanthine and xanthine to uric acid, and it inhibits the synthesis of purines (30). This latter effect is absent in children with Lesch-Nyhan syndrome and in some adult. patients with gout, in whom there is a deficiency of hypoxanthine-guanine phosphoribosyl transferase (HGPRT). This enzyme is required to allopurinol to its corresponding nucleotide, a prerequisite for the drug’s effect on purine systhesis. These patients are also unresponsive to this drugs such as 6-mercaptopurine and azathioprine that require transformation into ribonucleotides by HmPRT to be active.

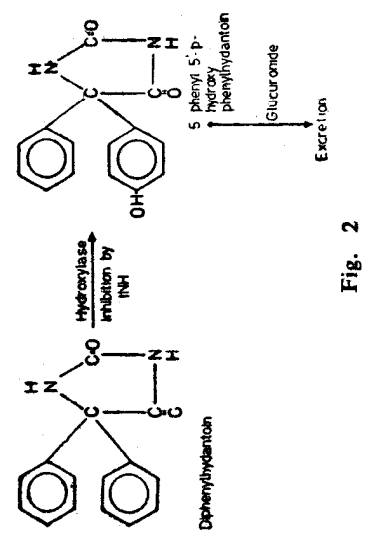

Fig. 2

Resistance to coumarin anticoagulants : A very unusual type of pharmacogenetic condition is the hereditary resistance to anticoumarin drugs such as warfarin. These patients require a much higher dose of the drug for adequate therapeutic response, monitored by the determination of prothrombin activity (31, 32 33) There were no unusual differences in the rate of metabolism, binding to plasma proteins or in the physiological distribution of warfarin in these patients. The unusual hereditary resistance is believed to be inherited as an autosomal mendelian dominant trait (34). Resistant individuals were also found to have unusual sensitivity to the antidotal effects of vitamin K. Thesefindings were interpreted to mean that an enzyme or a recpector site is involved in the synthesis of the clotting. factors II, VII, IX and X had been modified by genetic

mutation in some way to alter its affinity for both coumarin anticoagulants and vitamin k.

Conclusion : The examples cited above have been those whose effects are striking and grossly unusual. There are bound to be a number of other drugs, whose response may not be so striking, but nevertheless important. The medical profession should be aware of such differences, and the dose of drugs adjusted accordingly. It may even be necessary to withhold certain drugs fear of serious adverse reactions. Some drugs are capable of producing chromosomal damage leading to mutagenesis, teratogenesis or carcinogenesis. Drugs should therefore, be released only after extensive pharmacological investigations.

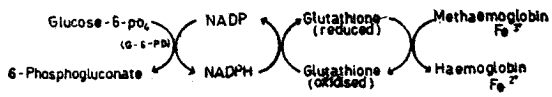

Fig. 3

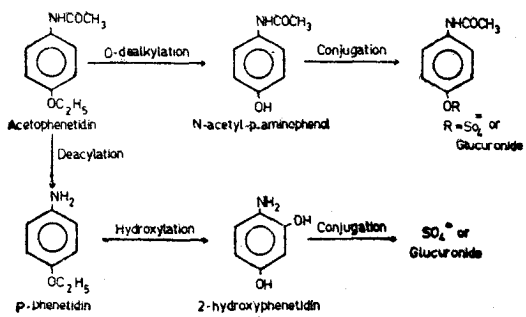

Fig. 4

TABLE I

Acetylator phenotype and response to treatment with regimens containing isoniazid in patients with pulmonary tuberculosis.

Rhythm of drug -administration	Favourable response at 1 year	
	Slow acetylators	Rapid acetylators
Daily	79-88%	84-85%
Twice-weekly	92-94%	82-91%
Once-weekly	82-95%	53-76%

Dosage of INH : Daily : 400 mg ;
Intermittent : 15 mg/kg.

TABLE II

Peripheral neuropathy during daily isoniazid treatment

Daily dose of INH (mg)	Slow acetylators			Rapid acetylators		
	Total patients	Palients with P.N.* No.	%	Total patients	Patients with P N.* No.	%
100 x 2	51	0	0	36	0	0
200 x 2	44	6	14	28	0	0
400 x 1	39	11	28	32	3	6
600 x 1	30	15	50	30	2	10

*P.N : Peripheral neuropathy

TABLE III

Heriditary variants of plasma cholinesterase and succinylcholice sensitivity.

Type of enzyme	Genotype	Prevalence	Activity
Homozygotes			
Usual	E_1^u E_1^u	94%	100%
Atypical (dibucaine-resistant)	E_1^a E_1^a	0.04%	50%
Fluoride resistant	E_1^f E_1^f	Rare	55%
Silent	E_1^s E_1^s	Rare	0.5%
Heterozygotes			
Usual and atypical	E_1^u E_1^a	4%	75%
Usual and fluoride-resisiant	E_1^u E_1^f	Rare	85%
Usual and silent	E_1^u E_1^s	0.5%	70%
Atypical and fluoride-resistant	E_1^a E_1^f	Very rare	60%
Atypical and silent	E_1^a E_1^s	Rare	25%
E^+		10%	130%
E^2 cynthiana		Very rare	200-300%

TABLE IV

Some drugs and other agents capable of inducing hemolysis in G-6-PD deficient individuals

Primaquine	Nitrofurazone*
Pamaquine	Nitrofurantion
Pentaquine	Furazolidone
Quinidine*	Sulphacetamide
Acetylsalicylic acid	Sulphacetamide
Acetanilide	Sulphapyridine
Phenacetin	Depsone
Antipyrine	Chloramphenicol*
Fava beans*	Viral respiratory infections
Bacterial pneumonias	Vitral hepatitis
Diabetic kectoacidosis	Uremia

*Hemolysis observed principally in the Caucasians