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"APARTHEID" PARADIGM IN THE XENOBIOTIC BIOTRANSFORMATION

By P. A. F. Dixon

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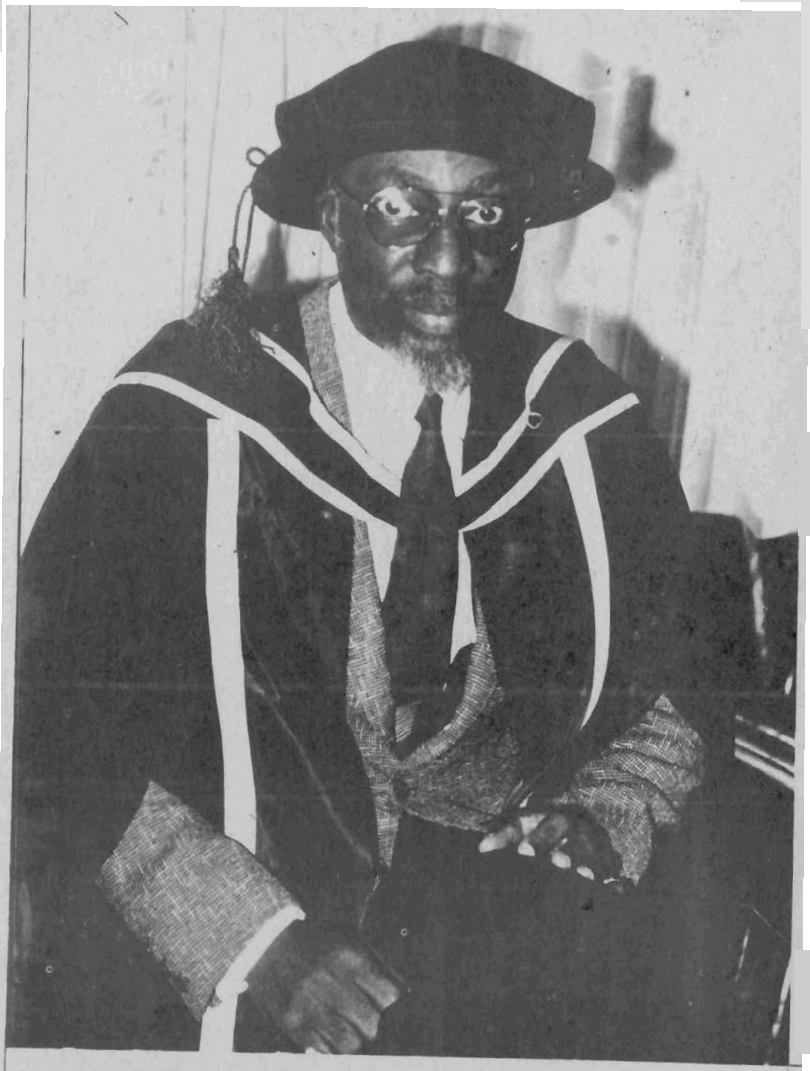

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This is the 4th Inaugural Lecture emanating from the Faculty of Pharmacy of this University and the 2nd from the Department of Pharmacology. It is the First from the University in Biochemical Pharmacology.

INTRODUCTION

In Genesis, we read about the origin of the World and the human race. God created man on the 6th biblical day after creating creatures of the sea, air and land. We also read about the tower of Babel and how the Lord confused the human language such that they could not understand one another and then scattered humans all over the World. This must have been, in my opinion, when the seed of tribal/ethnic or racial basis of human affairs was sown.

Scientists, however, hold that man evolved from lower animals and have anatomical, physiological, biochemical and anthropological evidence to substantiate this position. Logically, therefore, one might expect to see in man some conserved characteristics of the lower animals either structural or functional. Man is still evolving and there are already geographical races with distinct characteristics.

Races do differ in both their physiology and biochemistry. Some of these differences are manifested in the blood, endocrine hormones, nervous system and to certain extent behaviour. All these are adaptive features for the effective survival of the races.

Blood group B is very common in Eastern Asia, India and Africa, and is of minor occurrence in Europe, but is not found among isolated American Indians and Australian Aborigins.

There are some diseases that are known to be restricted to certain races. Sickle-cell anaemia and thalassemia are restricted to people of African and Mediterraen ancestry respectively. Glucose-6-phosphate dehydrogenase and lactase deficiencies are common in Africans and Orientals.

Diabetes (a genetic disease) for example may diminish as a medical problem among populations that eat little carbohydrate and take most of their calories from protein foods, such as meat, beans and fish. In essence you may be tempted to conclude that many genetically determined differences between races are "diseases" only under specific circumstances. This assertion will become evident in the later part of this lecture.

Many is constantly exposed to xenobiotics in the form of drugs, chemical constituents of foods and environment. These xenobiotics

of necessity must be changed into such forms for easy excretion from the body. This biotransformation process is made possible by metabolising enzymes. Enzymes are proteins and are products of gene expression, and provide the most tangible source of diversity in drug plasma levels and response.

During the course of evolution once a metabolic task is achieved, this is repeated and modified to suit any new metabolic challenge. This phenomenon is now known to have occurred for numerous multigene families by means of duplication of the ancestral gene and subsequent modification of extra genes for evolving functions (1).

In a given species there exist families of proteins with strikingly similar structure. Insulin - like growth factor, relaxin and nerve growth factor evolved from common ancestral gene (2). Among many different species, homologous enzymes and other proteins are structurally similar, for example, the growth hormone mRNA in cow, rat and man have been shown to be related. (3, 4).

Xenobiotic biotransformation is a necessary process for the eventual elimination of the xenobiotics. This involves a functionalisation (phase 1) and synthetic or conjugation (phase 11) reactions. The pattern and degree of this biotransformation is influenced by genetic factors, species and race among many other factors, such as, age, sex, diet, stress, disease state etc. This discriminating pattern is the meat of this Inaugural Lecture "*Apartheid paradigm in the xenobiotic biotransformation.*" Apartheid in this context has no derogatory undertone like the prevailing situation in South Africa. However, pharmacogenetic information could degenerate to such base use as the prevailing dehumanising situation in the apartheid enclave. It is this later position that forced on me the choice of the word "*Apartheid*".

SPECIES DIFFERENCES IN XENOBIOTIC BIOTRANSFORMATION

Differences in xenobiotic biotransformation between species generally arise from variation in the types and quantities of various drug metabolising enzymes, or relative extents of various reactions which a compound may undergo. This may be seen with my earlier research work on arylacids, which are metabolised by conjugation with either glucuronic acid or amino acids. The relative extent of these conjugation options varies greatly between species.

While man, the old World monkeys (except the marmoset) and bushbaby conjugate 1-naphthylacetic acid mainly with amino acid

and to a small extent with glucuronic acid, the cat extensively with amino acid; the rat and rabbit principally do with glucuronic acid, and the fruit bat entirely with glucuronic acid (5). Diphenylacetic acid on the other hand is conjugated with glucuronic acid irrespective of the specieis (6) but hyratropic acid is conjugated mainly with glucuronic acid in man, rhesus monkey, rat and rabbit, and with both amino acid and glucuronic acid in the cat (7). There is a shift from amino acid to glucuronic acid conjugation with increase in complexity of the chemical structure of arylacetic acids in most species (8).

Using chlorpromazine (9), metyrapone (10) cimetidine and metiamide (11) as probes, we have adduced evidence for an evolutionary trend in the metabolic oxidation reactions, and also to the effect that susceptibilities to inducers and inhibitors are substrate dependent. With respect to chlorpromazine biotransformation, the lizard and tortoise (reptiles) did not form sulphoxides, and tortoise was unable to N-demethylate the drug. The pigeon (bird) which has a close phylogenetic relationship to reptiles showed limited capacity as the toad (amphibian) and the cat for these two biotransformation pathways.

Birds form mainly ornithine, while amphibians form glycine conjugates of arylacids and I have shown (12) that the retention of the glycine-conjugating mechanical alongside the main ornithine detoxication in lizard and tortoise seems to be a relic of the amphibian ancestry. It also shows the close phylogenetic relationship of reptiles to bird. Sulphoxidation and N- demethylation were well marked in the rat and rabbit respectively and each showed a limited capacity for the other biotransformation routes.

The defect in suphoxidation in the cat adds to the other known defects in the biotransformation of xenobiotics associated with carnivores namely glucuronidation in the cat and N-acetylation in the dog (13).

Man, rhesus monkey (Old World Monkey) and capuchin monkey (New World Monkey) showed a similar capacity for all the biotransformation options of chlorpromazine. These show some of the conserved characteristics in the lower animals that are still retained in man but of comparative degrees depending on the species of reference in relation to man.

Animals belonging to the same zoological order are likely to have similar tissue enzymes due to common habits, environment and

similar gene pools. Some support for this view comes from the study of blood groups in primates, which shows that there are similarities between the serum proteins of different species and man (14). One might therefore suggest that man and other primates have broadly similar drug-metabolising enzymes in their tissues and that the animals nearest to man on the evolutionary scale are more likely to biotransform xenobiotics as does man, than an animal zoologically far removed from man such as rat, cat, rabbit or the reptiles. I must also emphasise that other factors such as absorption, excretion, tissue distribution and the biotransformation by the gut flora may all influence the overall fate of a xenobiotic in the body.

INTERINDIVIDUAL DIFFERENCES IN DRUG METABOLISM

We have just seen the cases of species differences in the drug metabolism and also the case of metabolic deficiencies in the carnivores. Since man evolved from these lower animals, are some of these characteristics evident in man?

It is now possible to state that human interindividual differences in the quantitative metabolism of xenobiotics has its origins in the variation in the nature and activities of drug- metabolising enzymes as regulated and influenced by a multiplicity of genetic, environmental, physiological and pathological factors. Of dominant importance is genetic constitution but metabolism can be modulated by a variety of other factors as stated earlier.

The knowledge that humans vary in their responses to therapeutic agents and toxic substances has been known for hundreds of years and aptly epitomised in the aphorism attributed to Lucretius (95-55BC) that:

> "What is the food to one man may be fierce poison to others".

The usual saying "One man's meat is another man's poison". One of the earliest documented examples of food idiosyncracy pertains to the consumption of asparagus. Some individuals who have consumed asparagus pass a characteristic and pungent smelling urine (15). This is as a result of genetically determined differences in the metabolic handling of two sulphur containing acids present in asparagus, namely asparagusic and dihydroasparagusic acids.

A second example of dietary idiosyncracy known to represent a pharmacogenetic polymorphism is that of trimethylaminuria or better known colloquially as the fish-odour syndrome (16). In this condition, affected individuals develop an offensive personal body odour, particularly in the breath and sweat, attributable to the presence of trimethylamine. The condition must have been known to that acute observer of the human situation - William Shakespare who writes.

> "What have we here? a man or a fish? dead or alive?
>
> A fish, he smells like a fish . . . . .a very ancient and fish-like smell" (The Tempest Act 2 scene 2)

This inability to metabolise trimethylamine leads to a lot of psychosocial problems like, complications of social isolation and rejection, poor school performance, sexual and marital problems, depression and suicidal tendencies.

Chlorpromazine is used in the treatment of schizophrenia because of its dopamine receptor blocking activity but it has shown wide variability in plasma level with its attendant clinical responsiveness. Drug concentration in plasma seems to be only weakly correlated to the therapeutic effect and this low correlation resides in the interindividual differences in CPZ metabolism resulting in different level of active and inactive metabolites in different patients.

We resolved this problem of which pharmacokinetic parameters of CPZ would be best correlated to the therapeutic responsiveness. We showed that the area under the plasma time curve (AUC) to have an inverted U-shaped relationship with % clinical responsiveness and were able to identify three groups of patients, responders, L- and h-group non-responders. L- and h-groups respond on further treatment with haloperidol, and electroconvulsive shock respectively (17).

A genetic polymorphism can be considered as an organisational arrangement of nature whereby the population can be structured to give it a better chance of survival should changes occur in the environment. You might simply say that it is a kind of variation in which individuals with sharply distinct qualities coexist as normal members of a population. With respect to drug metabolism two phenotypes, extensive metabolisers (EM) and poor metabolisers (PM) have been identified. The PM have impaired or nearly absent capacity to metabolise these drugs. The variations in the incidence of PM have been demonstrated among different ethnic or racial groups.

There are now many polymorphisms identified (18) but the most

studied drugs for their oxidation polymorphism are debrisoquine, sparteine and several B-adrenoceptor blocking drugs.

The oral bioavailability of a drug given to PM may be 2-5 times that seen in individuals of EM phenotype. It is not surprising therefore that PM subjects display exaggerated responses to "normal' doses of some therapeutic agents.

Problems can arise with polymorphically handled drugs when they produce effects which are not overt or readily monitored or when they can accumulate to toxic proportions. The case of phenformin, where a dramatic lactic acidosis occurs in PM's which correlated with elevated serum levels of the drug is a good example (19). Perhaps the most serious complication and the most difficult to detect, is where drug accumulation occurs to toxic burden levels of metabolic impairment. Such accumulation may go on silently over a long period of therapy until the drug burden expresses itself in some form of major target organ toxicity. The best example of this is the antianginal drug perhexiline (20).

Metabolic switching is a phenomenon that may occur in PM's because of the reduced availability of a normal pathway of oxidation metabolism. One example of this appears to be phenacetin which is normally metabolised by O-dealkylation by microsomal P-450 enzymes and only to a minor extent by deacetylation leading to toxic phenetidines (21).

Therapeutic failure may also arise by inability to generate active drug by PM's, and encainide is a good example.

ANIMAL MODELS USED FOR PHARMACOGNENETIC INVESTIGATION

There are animal models which exhibit the precise genetic trait of drug metabolism as in man. All these are part of the evidence of some conserved traits during evolution. Female DA strain of rats are used as PM models for debrisoquine (22) while a particular strain of chicken *Gallus domesticus* is used as model for 'fish odour' syndrome (23). Rabbit is used for the study of acetylation polymorphism (24).

DIET

High protein diet increases hepatic drug metabolism but the reverse is induced by high carbohydrate diet. Think of the case of Ijaw people - fish and the Ekitis - pounded yam. In malnourished

adults with nutritional edema (may be as a result of "SAP") drug metabolism is impaired with significant increases in plasma half-life of the drug.

Severe nutrient deficiences decrease enzyme activity except in cases of iron, vitamin B_1 and general starvation which appear to increase the biotransformation of certain drugs. Folic acid and Vitamin C supplements increase phenytoin and phenazone biotransformation respectively while zinc deficiency in adults decrease drug biotransformation. Food culture and social status therefore do play a role in influencing xenobiotic biotransformation.

HEALTH STATUS

Diseases that affect the ultrastructure and function of the liver, for example, liver cancer, cirrhosis and hepatitis have been shown to affect drug biotransformation. In this environment, for example, malaria is known to decrease the metabolism of quinine. In recent work in my laboratory we have shown significant decrease of quinine biotransformation in model cirrhosis and necrosis of the liver. Cardiac disease that would limit blood flow to the liver may impair the disposition of drugs whose metabolism is flow limited.

ETHNIC OR RACIAL DIFFERENCES IN DRUG METABOLISM

The pronounced variation in the incidence of the PM phenotype for debrisoquine/sparteine have been demonstrated among different ethnic groups. PM incidence appears to decline in moving from Western to Middle Eastern African population Groups. In the caucasian subjects, the incidence is 8%, San Bushmen of South Africa 19%, Nigeria 15%, Middle East and Far-East population groups 1-2%, (Saudis & Egyptians), the Chinese orientals 30% Japanese 0%.

The incidence of PM of mephenytoin is less than 5% in the Caucasian population (25) but much higher (20%) in the Japanese population (26). There are marked differences between orientals and caucasians with respect to the metabolism of diphenylhydramine, desipramine, notriptyline, amobarbitone and caffeine.

Erythrocyte catechol-O-methyltrasferase activity is the same in Filipinos and Chinese but is lower in caucasians.. Liver alcohol dehydrogenase variants occur in identical proportion among Japanese and Chinese but are much more rare among caucasians.

The amount of alcohol to intoxicate an oriental is not likely to in-

toxicate a caucasian. So by virtue of their genetic apparatus, they are outright winners against the orientals in any alcohol drinking competition.

Slow acetylators of isoniazid differs slightly between Japanese and Chinese 10-15% and 22% respectively yet both of these frequencies contrast markedly, with that among caucasians, 60%.

Comparing the paracetamol metabolism in Chinese and Indians in Singapore, it was found that the Indians excreted significantly lower amounts of sulphate conjugate, whereas the excretion of glutathione-derived metabolites in both Chinese and Indians was found to be closer to that of the Ghanians than the Scots.

Mephobarbital, a drug whose metabolism consegregates with mephenytoin trait has an incidence of adverse effects of about 4% in white subjects living in Australia, but 20% in Japanese patients.

Studies in male Hawaiians of different origins have noted the incidence of bladder cancer in white is over twice that in Japanese and this is probably in part a genetic predisposition linked to the inter-ethnic difference in xenobiotic metabolism characterised by the debrisoquine trait (27).

GHANAIAN AND GERMAN CONTROVERSY

Eichelbaum (a German) and Woolhouse (a Briton) from their work on the comparative sparteine and debrisoquine oxidation among Ghanaians and Germans concluded that poor metabolisers of debrisoquine/phenformin are metabolisers of sparteine in Ghanaians. Because of this, people started to propose a new gene for West Africa. In short that there is a dissociation of the genetic control of debrisoquine and sparteine metabolism among Ghanaians which might be due to a third allele at the debrisoquine/sparteine gene locus in Ghanaians (28).

The Pharmacogenetic fraternity, among whom were Professor R.L. Smith (Professor of Biochemical Pharmacology), Dr. J. Idle (now a Professor of Pharmacogenetics) and Professor Price Evans (Professor of Medical genetics) requested in 1982 that I should investigate this dissociation concept in the Nigerian population as a means of confirming or otherwise Woolhouse's findings. I believe that ideas must compete in the market place unadorned and naked like chickens.

Since my work did not confirm Woolhouse's findings but reasserted the concordance of the two metabolic oxidation in the

Nigerian population, the dissociation phenomenon and the new gene concept for West Africa were abandoned by the pharmacogenetic fraternity.

PHENOTYPE AND DISEASE DIASTHESIS

There is a present hypothesis that many idiopathic diseases arise from the interplay of genetic and environmental factors. The highly polymorphic nature of the genes regulating the enzymes of xenobiotic biotransformation and in particular those concerned with the oxidation metabolic pathways, is well recognised. These enzymes are known to be of crucial importance in the maintenance of safe homeostasis with the myriads of organic xenobiotic substances encountered in chemical environment. Allelic modifications of these genes can be predisposing factors for morbidity and mortality in individuals carrying them and when exposed to drugs and environmental chemicals.

Of great interest is the range of spontaneous disease which include diabetes, epilepsy, rheumatic fever, psoriasis, schizophrenia, manic-depression, psychosis and multiple sclerosis which encompass varying degree of heritability indicative of additive multifactoral inheritance.

Slow acetylator is a predisposing factor for bladder cancer and systemic lupus erythematosis whereas the fast acetylator phenotype is a predisposing factor for diabetes and breast cancer.

PM to debrisoquine 4-hydroxylation is a predisposing factor to systemic lupus whereas EM is for hepatoma (Nigeria), lung cancer associated with smoking, bladder cancer and Balkan nephropathy.

Poor suphoxidisers of s-carboxymethyl L-cysteine are very susceptible to primary biliary, cirrhosis, myasthenia gravis and allergic phenomena (18).

One might ask whether some spontaneous diseases represent chronic toxicity disorders arising from the persistent failure to metabolise and clear certain environmental chemicals? Drug- metabolising phenotypes because of linkage and association may simply represent markers for other genes more proximally involved in the expression and pathology process.

PM-DEBRISOQUINE AND PERSONALITY

Aim et al. (29) have shown that PM personality is characterised by high vitality, alertness, efficiency and ease of decision- making. They suggested that the debrisoquine hydroxylase is involved in the formation or catabolism of endogenous substance of importance for central nervous system function.

Will your phenotype for debrisoquine in future determine whether or not you get a particular appointment? I hope not, since these traits are characteristics expected of Chief Executives of organizations. Well in Nigeria at least the traits is found in 15% of the population.

A PENNY FOR YOUR THOUGHT

We now know that the major biochemical defense system, the mixed function oxidize system (MFO) which is involved in the oxidation biotransformation process is susceptible to induction or inhibition by numerous nutritional, pharmacological and toxicological factors. There are differences in the natural occurring toxic components of food or other environmental products in different ecological regions. Apart from these, there are areas where there are specific endemic diseases. All these factors could invariably modulate the genetic expression with respect to drugs metabolism and individual responsiveness to drugs or chemicals. It is therefore important to know the extent the ethnic or racial differences in metabolic oxidation capacity are temporary adaptations, and to what extent they are stabilized adaptations, due to ecologic imposed stress, expressed as genetic differences.

We have seen the racial differences in the metabolism of at least a few drugs. Could there be any military implications of this in the new age? A chemical by its nature may be innocuous but on interacting with ones genetic apparatus could precipitate a disastrous consequence. So only those with the particular genes are the target. Mr. Vice-Chancellor, Ladies and gentlemen, the effect on a nation therefore, depends on the frequency of such target genes in the population. We spend a lot of money on military defense in this country. How much do we spend on the possible understanding of our biochemical defense system ?

REFERENCES

1. Breathnach R. and Chambon A. (1981) *Rev. Biochem* 50, 349.
2. Marquardt H. Todaro, G.J. Henderson, L.E. and Oroszlan (1981). *J. Biol. Chem.* 256, 6859
3. Miller W. L. Martial, J.A. and Baxter, J.D. (1980). *J. Biol. Chem.* 255.7521
4. Nebert, D. W. and Negishi, M. (1982). *Biochem. Pharmacol.* 31(14), 2311.
5. Dixon, P.A.F. Caldwell, J. Smith, R.L. (1977), *Xenobiotica* 7, 717
6. Dixon, P.A.F. Caldwell, J., Smith, R.L. (1977), *Xenobiotical* 7,707
7. Dixon, P.A.F. Caldwell, J , Smith, R.L. (1977), *Xenobiotical* 7,727
8. Dixon, P.A.F. (1984), *J. Comp. Biochem. Physiol.* 77(1) 135.
9. Dixon, P.A.F., Okereke, S.E. and Enwelum, M.C.N. (1985), *J. Comp. Biochem. Physiol* 81C, 241.
10. Dixon, P.A.F; Okereke, N.O, and Ogundaunsi, O (1985). Biochem. Pharmacol 31, 2028.
11. Dixon, P.A.F. and Udeagha, A.U. (1986), *J. Comp. Biochem Physiol* 83C, 385
12. Dixon, P.A.F. (1980) *Eur. J. Drug. Metab. Pharmacokin,* 5,65
13. Caldwell, J. (1980). *In Enzymatic Basis of Detoxication* 1, 85-114
14. Goodman, M. (1963). *In Classification and Human Evolution* (Edited by Washburn S.L.), pp.204-231, Methuen, London.
15. Arbuthnot, J. (1735): *An Essay Concerning the Nature of Aliments* p. 64, and pp. 261-262 (3rd Edition), J. Tonson, London.
16. Humbert, J.R. Harmmond, K.B. Hathaway, W.E. Marcoux, J.G. and D.O. Brien (1970), *Lancet* 1:770
17. Dixon, P.A.F. Oforah, E. and Makanjuola R. (1982), *Br. J. Clin. Pharmac.* 14,273.
18. Ayesh, R. and Smith, R. *Recent Advances in Clin. Pharmacol. and Toxicol.* No.4, pp.137-157 (Edited by P. Turner and G.N. Volans), Church-hill Livingstone, 1989.
19. Oates N.S. Shah, R.R. Idle, J.R. and Smith, R.L. (1983), *Clin. Pharmacol. and Ther.* 34,827
20. Morgan, M.Y. Reshef. R. Shah R.R. Oates, N.S. Smith, R.L. Sherlock, S. (1984) Gut. 25, 1057.
21. Brodie, B.B. and Axelrod. J. (1949) J. Pharmacol. Expt! ther *9* , 58.

22. Kahn, G.C. Rubenfield, M. Davies, D.S. Murray, S. and Boobis A.R. (1985), *Drug Metab Disp.* 13,510
23. Pearson, A.W. and Butler, E.J. (1983). *Comp. Biochem. Phys.* 76C, 67
24. Weber, W.W. (1986), *Drug Metab. Disp.* 14,377
25. Kupfer, A. and Preisig, R. (1984) *Eur.J. Clin. Pharmacol*, 26,753
26. Jurima, M. and Inaba, T. Kadar, D. and Kalow, W. (1985(, *Br. J. Clin. Pharmacol.* 19,483
27. Young, J.L., Percy, C.L., Asire, A.J. (1981), *Natt Cancer Inst. Monogr.* 57,1-1081.
28. Echelbaum, M. and Woolhouse, N.M. (1985) *Eur. J. Clin. Pharmacol.* 28, 79.
29. Alm C., Bertilsson, L. de Las Carreras C., Edman, G. Schalling, D, and Widen J. (1989). *Eur. J. Clin. Pharmacol* 136, (Suppl.) pA201