CONFERENCE LECTURE

PHARMACOGENETICS—A STUDY OF INHERITED VARIABILITY IN THE RESPONSE TO DRUGS

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Multifactorial Inheritance

You know a lot about drugs and I know a little about genetics and I thought it might be interesting to marry up our two interests for a short while today. However, before we discuss this so-called pharmacogenetics I must remind you of the two principal ways in which characters are inherited. On the one hand they may be controlled by many genes (multifactorial inheritance) and then, according to whether you have a few, a medium number or the complete set so will you manifest more or less of the trait in question. The usual example given is that of human height where you can be anything from very short to very tall but with a likelihood of being somewhere around 5 ft. 8 in., assuming the nutritional side of the matter to be adequate throughout.

Single Gene Inheritance

On the other hand, some characters are all-or-none, that is, you either have them or you do not and these are controlled by single genes which you either do or do not possess. A good example of single gene inheritance in normal people is afforded by the blood groups. Thus you are either group A *or* group O *or* group B *or* group AB and there is no grading.

Polymorphism

The next concept that I want to introduce to you is that of genetic polymorphism. The best way to explain it is by taking an example. Let us again consider the blood groups. Now the various components of the system I have mentioned exist in certain frequencies. Thus here in Liverpool about 50 per cent of people are group O, 40 per cent A and 10 per cent B or AB. Since geneticists think that genes are never entirely neutral as regards survival value there must be balancing forces which keep the members of any polymorphic system, such as the blood groups, in equilibrium, otherwise one of them would increase at the expense of the others and in due course the most advantageous would be the only one left. On reflection it is clear that susceptibility to diseases of one sort or another might well be an important factor in stabilising this equilibrium, and in fact it has been found that people who are group A are slightly more prone to gastric cancer than the other groups and that duodenal ulcer patients are particularly likely to be group O. It must be remembered, however, that factors responsible for maintaining genetic equilibrium must operate during the reproductive period for the effects of selection to be passed on. If you die of duodenal ulcer aged 60 it will

not have affected the frequency of group O in the next generation because you will have had your children by then. If you die of the disease aged 20 then there *will* be a drain on group O. It only needs a moment of reflection to see that the effects of duodenal ulcer and gastric cancer on maintaining the equilibrium of groups O and A are very small.

There are fortunately much more easily understood examples of factors that stabilise polymorphisms and if we turn to some of our humbler relatives in the animal kingdom we find that they are most informative. The British peppered moth exists in two forms, "typical" and *carbonaria.* The two patterns are inherited and a single gene converts "typical" to *carbonaria* and there are no intermediates. A hundred years ago the whole population in England was "typical"; then in Manchester about 1850, there arose the *carbonaria* and it has spread so rapidly that at the present time in certain areas 95 per cent are *carbonaria*; however, nowhere has the "typical" form been completely eliminated. *Carbonaria* and "typical" represent a polymorphism just like the blood groups and one of the reasons why the change has taken place is the better camouflage of the black form in areas affected by industrial pollution. In the moth, therefore, a change in environment has altered a genetic situation favouring those insects of a particular genetic constitution which previously had enjoyed no advantage. We shall see later that there is a parallel in Man.

Salicylic Acid and Isoniazid

Now when we come to consider the response of the body to drugs the two types of inheritance that I have mentioned hold good. For example, if we consider the metabolism of salicylic acid we find that on administration of a standardised dose to 100 healthy individuals we obtain a normal distribution for the serum salicylic acid 3 hr. after the

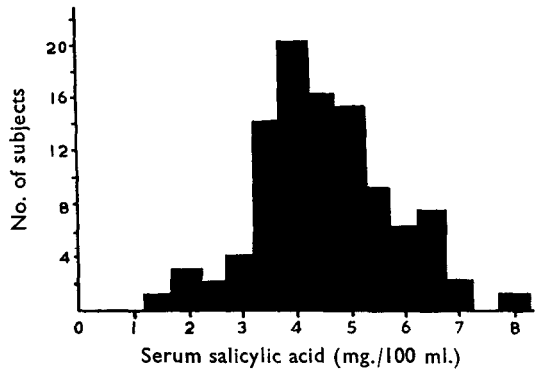

FIG. 1. Serum concentrations of salicylic acid 3 hr. after ingestion of 50 mg. of sodium salicylate per kg. of metabolically active mass (= wt.$^{0.71}$).

drug ingestion (Evans and Clarke, 1961), and this is consistent, as far as genetics is concerned, with the multifactorial inheritance that I mentioned earlier in which most of the individuals fall into the middle grades (see Fig. 1). All a curve such as this suggests is that the individual

variation is continuous and it is not a promising situation to investigate either from a genetic or an environmental point of view.

The normal curve probably holds for the majority of compounds but occasionally we meet with the single-gene, all-or-none situation where there is a clear-cut difference in the way a drug is metabolised. The best example is isoniazid. Here we find that, in this country, the drug is inactivated by acetylation rapidly by 50 per cent of the population and slowly by the other 50 per cent and that there are very few intermediates. Furthermore, family studies have shown that the situation is controlled by a single pair of genes, "rapid" being dominant to "slow" (Evans, Manley and McKusick, 1960) (see Fig. 2).

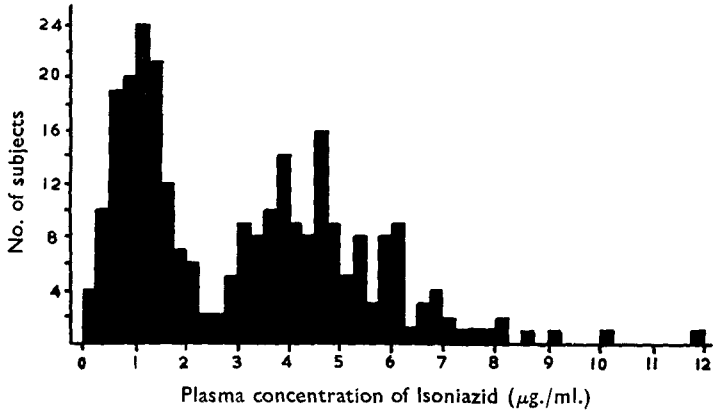

FIG. 2. Plasma concentration of isoniazid 6 hr. after oral administration of 9·7 mg./kg. body weight. There were 53 families, 267 family members.

Several things follow from this knowledge. First, you can use isoniazid as a genetic marker, just like the blood groups, but this is not pharmacologically very interesting. Second, and much more stimulating, is to consider whether the disease for which the drug is used—in this case tuberculosis—is modified by what we may call the "inactivator status" of the individual. *A priori* we might think that the "slows" would benefit more from the compound than the "rapids" and there is a suspicion that this may be so. However, the effect is not a big one and is not likely to be of much importance from the practical point of view of the management of the disease. Nevertheless, when we come to look at the toxic effects there is much more to it—the "slows" are heavily loaded compared with the "rapids" in their liability to develop polyneuritis (Devadatta, Gangadharam, Andrews, Fox, Ramakrishmam, Selkon, and Velu, 1960) (see Table I). It would also be interesting to look at the response to the drug of lupus vulgaris, which is often treated by isoniazid alone, and it would be easy to measure the size of the lesion and assess its rate of disappearance in relation to the "inactivator status" of the patient.

Thirdly, we can look at the matter from the much broader biological point of view to try and find out the balancing advantages and disadvantages of being "rapid" or "slow." We have actually no idea what

factors are involved but they must be different in different parts of the world, because in Japan and in the Eskimos (Harris, Knight and Selin, 1958) 90 per cent of the population are "rapid" and 10 per cent "slows" whereas in Africa there are more "slows" than "rapids." Therefore, it seems likely that it is more advantageous in northern latitudes to be "rapid"—though not overwhelmingly so because 10 per cent of people there still remain "slow." It is clear that the metabolism of isoniazid *per se* is unlikely to have been a stabilising factor in earlier times since the drug has only been recently discovered, though it might become important in the future. It is, however, possible that in the past we ate naturally occurring, chemically related compounds possessing anti-tuberculosis activity and that these may have been metabolised in the same way as isoniazid. If this were so it would cause the "slows" to be preserved in populations which were particularly exposed to the disease.

TABLE I

THE DEVELOPMENT OF POLYNEURITIS ON ISONIAZID THERAPY

Inactivator phenotype	Without polyneuritis	Polyneuritis	Totals
Rapid	58	2	60
Slow	66	17	83
Totals ..	124	19	143

$\chi^2 = 8{\cdot}87.$ $p < 0{\cdot}01$

Dr. D. A. Price Evans (1962 and in preparation), of the Department of Medicine of the University of Liverpool, has now advanced the understanding of the difference between the rapid and slow inactivators of isoniazid by *in vitro* experiments making use of the enzymes of the liver which carry out the process of acetylation. This they do by enabling acetyl coenzyme A to give up its acetyl radical to an acceptor molecule, for example, a drug. Biopsy specimens of liver were ground up, suitable additions made and the homogenate then mixed with isoniazid. After a given period the amount of free drug was estimated and from this the amount acetylated was calculated. The result showed clearly that sometimes the isoniazid was acetylated slowly and sometimes quickly, and this tallied with the "inactivator status" of the patient from whom the liver specimens were obtained.

The great advantage of being able to carry out such an investigation *in vitro* is that it becomes possible to find out exactly which enzymes are responsible for particular metabolic steps.

In a second experiment, the amount of acetylation of sulphadimidine by volunteer medical students given a standard dose of the drug was estimated in urine collected for 8 hr. after the drug was taken. Dr. Price Evans found that there was a clear bimodality in the acetylation of the drug, some of the students carrying it out quickly and others slowly, and that the rapid acetylators of sulphadimidine were also the rapid inactivators of isoniazid and the same was true of the "slows", so it looks as if the same gene is controlling the metabolic step in each case. Now what follows from this?

Dr. Price Evans feels that the enzyme basis for these acetylation polymorphisms lies either in the rate of transfer of acetyl groups from acetyl coenzyme A to the substrate (drug) molecule; or alternatively in the rate of production of acetyl coenzyme A itself. Only further experiment will be able to decide which is in fact the case, but the matter generally seems of great interest.

Primaquine

We next turn to another drug, namely primaquine, which has been found to uncover a most interesting genetical situation. Let us begin at the beginning. Haemolytic anaemia was recognised as an occasional complication of the now out-of-date pamaquine when it was introduced in 1925 as an effective agent against the sexual forms of the malarial parasite, *Plasmodium vivax*. At first the anaemia was thought to be

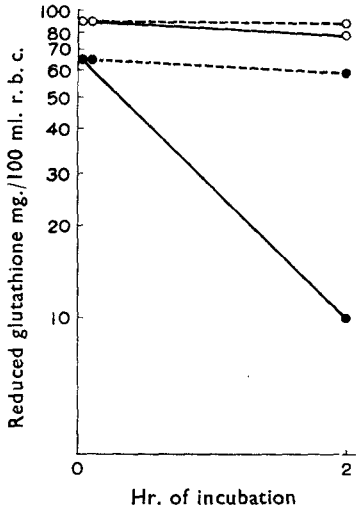

FIG. 3. The effect on reduced glutathione of incubating sensitive and non-sensitive red cells in the presence of glucose (Beutler, 1960, by kind permission of McGraw Hill Book Co.).

○ - - - - ○ Non-sensitive red cells incubated without primaquine.
○ ——— ○ Non-sensitive red cells incubated with primaquine.
● - - - - ● Sensitive red cells incubated without primaquine.
● ——— ● Sensitive red cells incubated with primaquine.

due to a hypersensitive or immune mechanism but no antibody was ever discovered and the problem remained unsolved until many more cases occurred during World War II when the very similar drug primaquine came extensively into use. We must next discuss the clinical features of this anaemia which are of some interest. A sensitive subject when given 30 mg. of primaquine daily does not develop the blood changes for 2 or 3 days. Thereafter his urine gradually turns dark, muscular pains occur and anaemia and possibly jaundice appear. Discontinuing the drug results in a return to normal over a few weeks. However, if

the symptoms are not severe and primaquine ingestion is continued he will, surprisingly, also improve. This is a most important observation and the reason for it was discovered by labelling red cells of different ages with radioactive iron (Dern, Weinstein, Leroy, Talmage and Alving, 1954). This has shown that red cells in a sensitive subject can be lysed by primaquine when 63 to 76 days old but not when 8 to 21 days old Therefore, it seems that it is the ageing erythrocyte which is destroyed by primaquine, and spontaneous clinical recovery, while continuing to take the drug, is due to the regeneration of a red cell population with a low mean age.

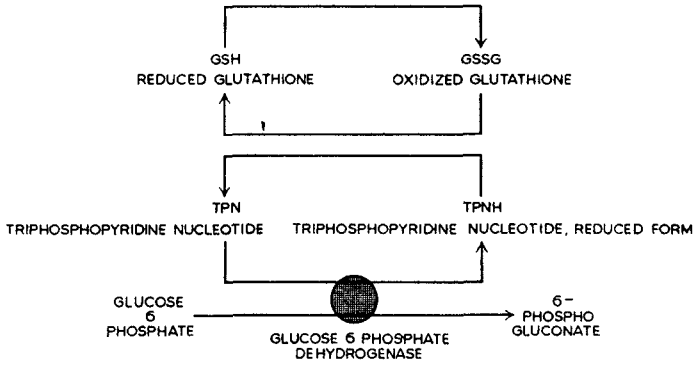

FIG. 4. The hydrogen atom removed from G6P by G6PD is taken up by TPN and TPNH is formed. This in turn reduces GSSG to GSH. If G6PD is deficient, the cycle is interrupted and no GSH is formed.

These are the facts regarding primaquine and we must turn to biochemistry for the explanation. The normal red cell possesses enzyme systems which are concerned with the metabolism of glucose. One of these is glucose-6-phosphate dehydrogenase and the important finding is that this is greatly diminished in primaquine-sensitive individuals. The first observation which led to this discovery concerns reduced glutathione (Childs and Zinkham, 1958). Thus when both primaquine-sensitive and non-sensitive red cells are incubated with the drug *in vitro*, with glucose added to the buffer, the content of reduced glutathione falls in the cells from sensitive individuals but not in those from normals. The continued fall in sensitive red cells is due to a fault in glucose metabolism, the result of a defect in glucose-6-phosphate oxidation brought about by a deficiency in the appropriate dehydrogenase (G6PD) and more marked, as stated above, in the older cells. The two diagrams (Figs. 3 and 4) give the details of the normal and faulty mechanisms.

Glucose-6-phosphate Dehydrogenase Deficiency

The next point to appreciate is that the lack of G6PD and hence the presence of primaquine-sensitive individuals is an inherited characteristic, and males are readily assignable either to the sensitive or non-sensitive group on both glutathione and direct G6PD studies. Women do not give

so clear-cut a division and intermediate values are observed with both methods of assessment. These sex differences together with pedigree analysis, led to the conclusion that the gene controlling the presence or absence of G6PD is sex-linked, that is, on the X chromosome, and that the trait is semi-dominant to its normal partner (Childs and Zinkham, 1959). In women with two Xs only one carries the abnormal gene and the other lessens its effect. This is not the case in men who will have either a normal or an abnormal X with a clear-cut result in either event.

The important point to appreciate is that primaquine has divided individuals into two groups, non-sensitive and sensitive and the latter can comprise as many as 10–15 per cent of some populations, for example, in certain Mediterranean countries, Persia, parts of India and Indonesia, though in England sensitivity is excessively rare. When we consider the world distribution of G6PD deficiency it is seen that a high frequency coincides with a high incidence of malaria and there is now additional evidence that G6PD deficient cells are less favourable to the proliferation of the malarial parasite than are normal ones (Allison and Clyde, 1961). This is an overriding advantage compared with the possibility of developing haemolytic anaemia from primaquine and incidentally from other substances as well. For example, the bean *Vicia fava* produces a similar syndrome in G6PD deficient individuals and so also may naphthalene mothballs—children sometimes eat them or they may be taken by adults in pregnancy as a perversion of appetite.

Hydrogen Peroxide and Acatalasia

We next come to a very ordinary drug, so mundane indeed that I doubt whether an audience such as this would deign to call it a drug at all—hydrogen peroxide. Now the polymorphism which this has uncovered is of particular interest to me because it shows that the art of clinical observation is neither dead nor useless. In 1946 a Japanese oto-rhino-laryngologist noticed that the operative field in a patient from whom he was removing a maxillary antral tumour turned black and did not froth when he dropped hydrogen peroxide on to it; normally, of course, the raw surface of a wound does froth and the blood does not alter in colour. It was found that the blood of this particular patient lacked an enzyme called catalase which normally degrades hydrogen peroxide and prevents the oxidation of haemoglobin by peroxide. Relatives of the patient were next investigated and it was found that some of them also lacked catalase. These people, usually children, lose their teeth and develop necrosis of the jaw. This is because haemolytic streptococci and certain pneumococci themselves produce hydrogen peroxide, and in a patient lacking catalase the haemoglobin of the blood reaching a lesion is oxidised so that necrosis occurs since the infected area is deprived of oxygen. In this situation the bacteria multiply, the hydrogen peroxide production increases and a vicious circle is established. Several points of interest about the syndrome are now known. Only about half of those who lack the enzyme actually show symptoms, and once all the teeth have been removed the lesions heal and many patients

remain permanently free of trouble. A similar condition has also been found in certain breeds of dog and guinea-pig (Wyngaarden and Howell, 1960; Allison, Rees and Burn, 1957).

It seemed not unlikely that when the condition was better known cases would be found in countries other than Japan, and recently it has been described in Switzerland. Acatalasia has been found, by pedigree studies, to have a recessive method of inheritance.

Suxamethonium Sensitivity

We now turn to anaesthetics. It has been known for some years that patients given suxamethonium to ensure muscle relaxation during anaesthesia have occasionally been unduly sensitive to the drug. Prolonged apnoea occurs and although the breathing can almost always be restored yet the situation is temporarily alarming. The reason for this is as follows. Normally suxamethonium is broken down by the serum cholinesterase but in sensitive individuals the value for the activity of this enzyme is low, and the same is true for some of the relatives of those known to be affected. On further investigation of those who tolerated the drug badly it was found that the enzyme present was different from that in normal individuals—it was not just a question of producing normal cholinesterase in smaller amounts than usual. Now the activity of the usual type of serum cholinesterase can be assessed quantitatively. The local anaesthetic drug cinchocaine (dibucaine) is a convenient inhibitor of the hydrolysis of benzoylcholine by the enzyme. The percentage inhibition so produced is called the "dibucaine number", and in normal people it is about 80 whereas in those affected it is around 16. A third group where the value is about 62 represents carriers of the condition (Lehmann and Ryan, 1956; Davies, Marton and Kalow, 1956). The condition has a recessive method of inheritance.

Presumably both in suxamethonium sensitivity and in acatalasia there is some advantage in having the gene in single dose, otherwise the polymorphism would never have developed.

Phenylthiocarbamide (PTC)

You might think from what I have said that biochemical explanations of genetic polymorphisms are readily forthcoming, but in fact the examples I have given you are highly selected and in many cases we are in difficulties. Take for instance what I will call the PTC story. Phenylthiocarbamide (PTC) was discovered in 1932 and it was soon found that 75 per cent of people in this country could taste it in solution whereas the other 25 per cent could not—in other words, here again we have an inherited polymorphism and "tasting" is a mendelian dominant to "non-tasting" (see Fig. 5). What are the advantages and disadvantages of belonging to one of the two classes? We still do not know the answer to this but because PTC is chemically allied to some of the anti-thyroid drugs, thioracil in particular, it was thought worth while investigating the "taster" status of patients with thyroid disease and sure enough it was found that certain types of goitre had a considerably higher incidence of non-tasters than

did controls (Harris and others, 1941; Kitchin and others, 1959). Since goitrogens similar to PTC are present in certain foodstuffs, particularly vegetables such as cabbage, it was thought that dietetic and gustatory habits might have something to do with the maintenance of the polymorphism and it was clear that the next step was to investigate various substances with the chemical linkage $S = C\langle^{N=}$ to see if there were any clear-cut differences in their metabolism as between tasters and non-tasters. The short answer to this is that there are not. My colleague Dr. D. A. Price Evans has investigated the matter using the output of methyl thiouracil in the urine and also the uptake of thiopentone from the blood and in both he found no significant difference between tasters and non-tasters (Evans, Kitchin and Riding, 1962). It may be therefore, that the answer lies more superficially and that there are differences in the salivary enzymes between the two classes and this in fact is being investigated at the present time.

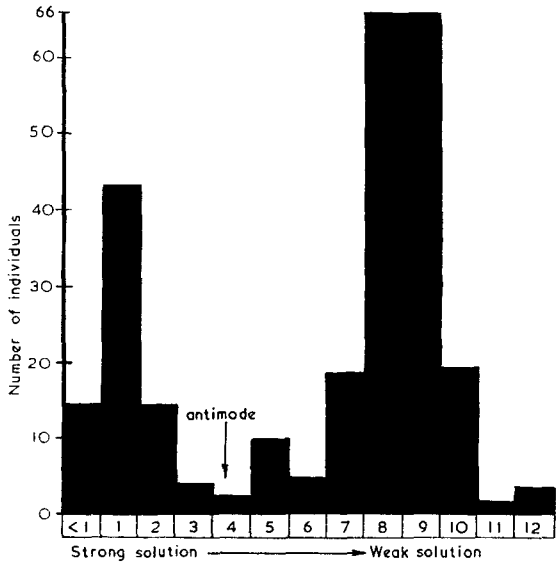

FIG. 5. Distribution of PTC taste response in 265 normal individuals (Kitchin and others, 1959).

Miscellaneous Information

I want to conclude by bringing to your notice very briefly some miscellaneous information.

Porphyria. Many forms of this disease are inherited and there are large numbers of afflicted individuals in Port Elizabeth in South Africa. Many of them used not to have much disability but since the introduction of barbiturates the matter has become much more serious since pentobarbitone in particular is extremely dangerous and because of this it is now the practice in Port Elizabeth always to test the urine for porphyrins before

giving this anaesthetic (Dean and Barnes, 1955). The porphyria story is a striking example of how a commonly used group of drugs can unfavourably influence an inherited disease.

Atropinesterase in rabbits. In some rabbits there is present in the serum an enzyme called atropinesterase. The ability to form this is genetically controlled and some strains possess it whereas others do not (Sawin and Glick, 1943). It would be interesting to know whether the enzyme is of any use to the rabbit by enabling it to inactivate solanaceous alkaloids, such as those contained in deadly nightshade and other plants. Is there anything comparable in Man? Confusional states occasionally occur after the administration of atropine eye-drops and it may be that different people metabolise the drug in different ways but whether there is an actual polymorphic system we do not know. Another interesting fact is that the mongoloid idiots are particularly sensitive to the effect of atropine.

Variations in pupillary responses. Blue-eyed Europeans are a little more sensitive than brown-eyed to the action of hydroxyamphetamine and other sympathomimetic drugs (Wells, 1958). The deep brown iris of the Negro is said to dilate only very little under the influence of these drugs. Here again therefore is a situation which would be worth investigating, particularly as nowadays many of the hypotensive drugs have ocular manifestations as one of their side effects, and it may be that patients with a particular eye colour are unduly sensitive.

I think you can be left in no doubt that pharmacogenetics is capable of shedding considerable light on normal biochemical processes. Moreover, when it comes to the question of drug trials, more attention should be paid to the possibility that patients may deal with a drug in two or more distinctly different ways rather than manifest minor individual variations.

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