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# Pharmacogenetics

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The term Pharmacogenetics is defined as the study of genetically determined variations in animal species which are revealed by the effects of drugs.

I am going to deal with examples of these variations which have been found in Man. I must apologise for a certain amount of repetition of some facts which have already been aired in this conference, but it is my intention to give you a bird's eye view of the subject.

## Acatalasia

The first example that I would like to draw your attention to is the phenomenon of acatalasia. Professor Takahara has described this polymorphic system in detail earlier in this conference.

This condition of acatalasia was found by Dr. Takahara and almost all the cases so far described have occurred in Japan. The way this phenomenon came to light is a model of clinical observation. Dr. Takahara was cleaning out the maxillary sinus of a patient with hydrogen peroxide when he noticed that no frothing occurred on the raw surface and that the free blood turned black.

He speculated that this might be due to absence of catalase in the blood, and such was proved to be the case.

Figure 1 shows the blood catalase values in the acatalasemic subjects, the intermediate values of heterozygotes and values for " normal " homozygous individuals.

Reviewing the families clinically in the light of the biochemical findings it has become apparent that there is a characteristic clinical syndrome in half the subjects with acatalasia.

Below 12 years of age there is a predisposition to dental sepsis, to ulceration of the gums, to granulating tumors of the nasal sinuses and destructive lesions in the nasopharynx. Improvement occurs after the teeth have been lost.

The point that I want to make is this. Here we have a situation where observing different responses in patients has led to the finding of a beautiful biochemical polymorphism; and subsequently reapplying this knowledge back in the clinic it has led to the recognition of a new disease entity.

## Primaquine Sensitivity

As soon as primaquine was introduced into treatment on a wide scale it was realized that some subjects — mainly Negroes, developed haemolytic anaemia, whilst others did not.

By means of haematological studies the reason was found to be inside the sensitive subject's red cells.

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Sensitive subjects, red cells were found to lose their reduced glutathione with abnormal readiness in the presence of primaquine.

This is shown in Fig. 2. In the top 2 lines we see cells from a non-sensitive subject. The presence of primaquine does not make any difference to the glutathione. The bottom 2 lines are cells from a sensitive subject. When primaquine is present the reduced glutathione content

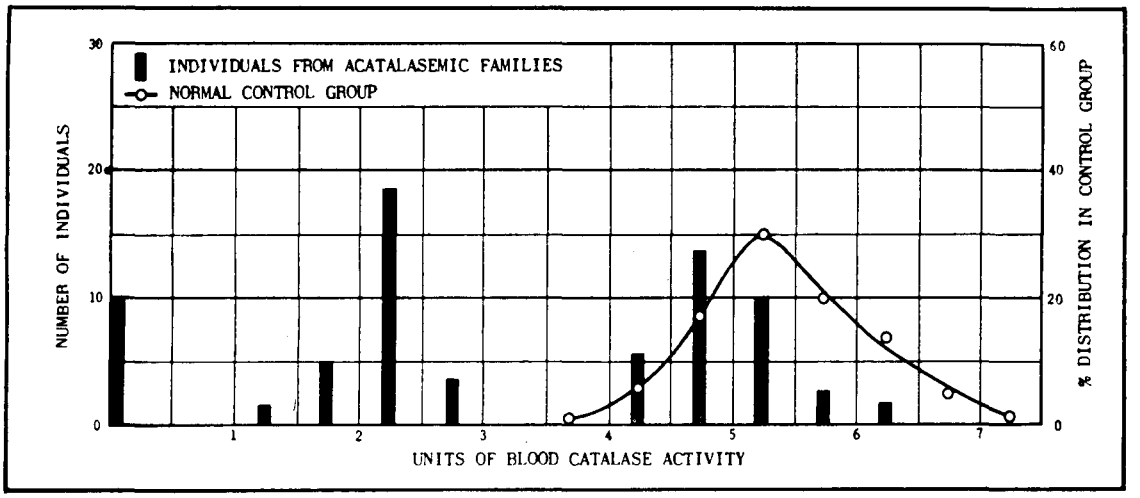


Fig. 1. Distribution of catalase values for members of the five acatalasemia families and comparison with a percentage distribution curve of values from a normal control group (From Nishimura et al., 1959)

falls sharply — even though plenty of glucose is available. This suggests that these sensitive cells have something missing, so that they cannot use the glucose to reduce glutathione.

Fig. 3 shows three things:

(1) There is a bimodal distribution of glutathione values in a Negro population.

(2) The lower mode does not represent absence of activity, but rather a low level.

(3) The bimodality is much more marked in males (top histogram) than in females (bottom histogram).

To go on with the biochemistry, it is known that the glucose pathway links up with glutathione at one point shown in Fig. 4.

Glucose 6 phosphate dehydrogenase (G6PD) was found to be present at a very low level in primaquine sensitive subjects. Hence they are unable to reduce glutathione properly.

It is only fair to point out that this does not tell us exactly why these sensitive subject's cells lyse with primaquine.

What of the genetics? As I pointed out earlier, the sensitive subjects are much more common in men that in women. This suggests at once that the character is controlled by the sex chromosomes, rather than by the autosomes. This has been substantiated by family studies. Primaquine sensitivity is a sex-linked intermediate dominant which is present on the x chromosome and linked with colour blindness.

Knowledge of this biochemical defect has shed light on certain clinical phenomena:

1. Haemolytic anaemia is seen as a response to small doses of certain drugs, and it is the possession of this enzymic defect which is often responsible. Amongst these drugs are phenacetin, acetanilide, probenecid, PAS and ordinary aspirin.

2. Favism — a severe haemolytic anaemia is due to the sensitivity of certain subjects to beans, and whilst there may be something additional in the set-up, this G6PD deficiency is an essential prerequisite.

3. It has been shown that severe neonatal jaundice may be due to G6PD deficiency. Possibly it may be the administration of drugs to the mother before delivery that make the baby jaundiced.

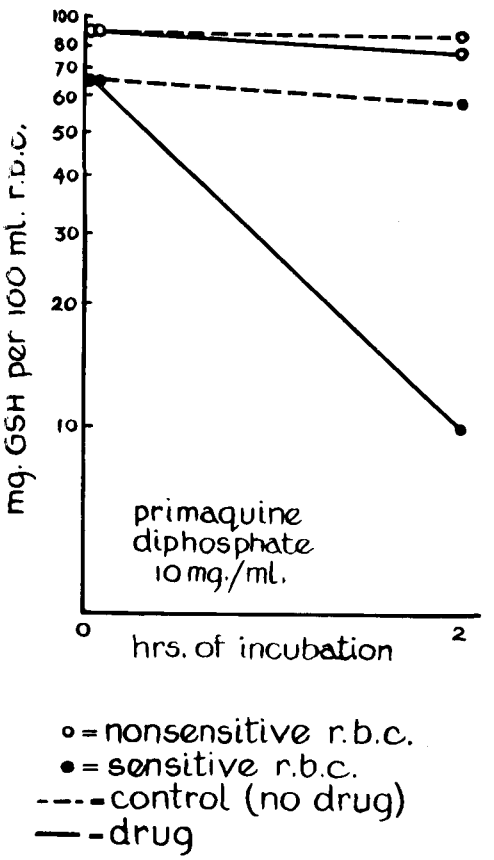


Fig. 2. The effect on reduced glutathione of incubating sensitive and non-sensitive red cells in the presence of glucose (Adapted from Beutler, 1960)

Here again we have this biochemical defect, once it has been elucidated, being applied in the analysis of clinical syndromes and shedding light on what were previously mysteries.

Lastly, geographic distribution suggests that G6PD deficiency like sickling gives protection against Plasmodium Falciparum malaria. The protection by both genetic mechanisms would seem to be of the same order.

## Suxamethonium Sensitivity

When suxamethonium was introduced into anaesthesia as a relaxant it was found that some people developed prolonged apnoea as a result of the administration of this drug.

It was found that these people lacked the capability to break down suxamethonium du to their possessing an abnormal esterase.

These people occur about 1 in 2,000 in the population.

Dr. Lehmann will be giving a fuller account of these biochemical variants later.

It has been found that the simplest way to show the differences between these pseudocholinesterases is to measure the inhibition of the enzymes caused by the local anaesthetic dibucaine. The result is the Dibucaine Number.

Fig. 5 shows the dibucaine numbers from two families which contained affected individuals. They fall again like the acatalasemics into three modes. The people who get the profound apnoea are on the left, the heterozygotes in an intermediate mode, and homozygous normals on the right.

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The people who get the profound apnoea are autosomal recessives.

Kalow has shown that here it is not an absence of enzyme or a reduced amount of enzyme that is responsible for the abnormal phenotype but rather an enzyme with quite different biochemical characteristics.

There has been a further extension of this subject as shown in the Fig. 6. The pseudocholinesterases can be inhibited by fluoride as well as by dibucaine. When the two results

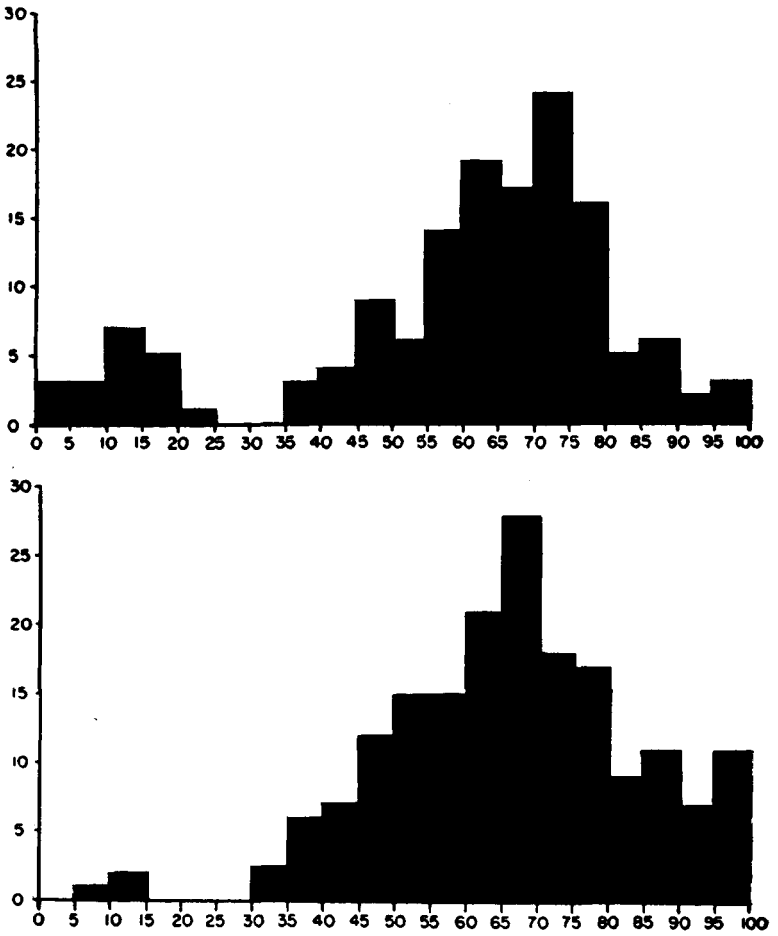


Fig. 3. Distribution of post-incubation glutathione values in a survey of Negro outpatients (From Childs B. and Zinkham W., 1958)

are combined then a small number of subjects are away from the three main modes. The reality of these sub-groups has been confirmed by family studies.

These findings suggest that there may be a third gene which can influence the character of the pseudo-cholinesterase synthesized. Possibly this new gene determines yet a further variant of the enzyme which is qualitatively distinct.

## Isoniazid Metabolism

Since the discovery of isoniazid (1 isonicotinyl-acid hydrazide) (Fig. 7) in 1952 it has become apparent that there are considerable variations in the manner in which different individuals metabolize the drug.

Two distinct classes were shown in the population by Biehl and the way was then open to perform family studies.

Fig. 8 shows the distribution of plasma isoniazid concentrations 6 hours following an oral dose of 9.7 mg. per kg. Isoniazid concentrations are shown along the abcissa and the number

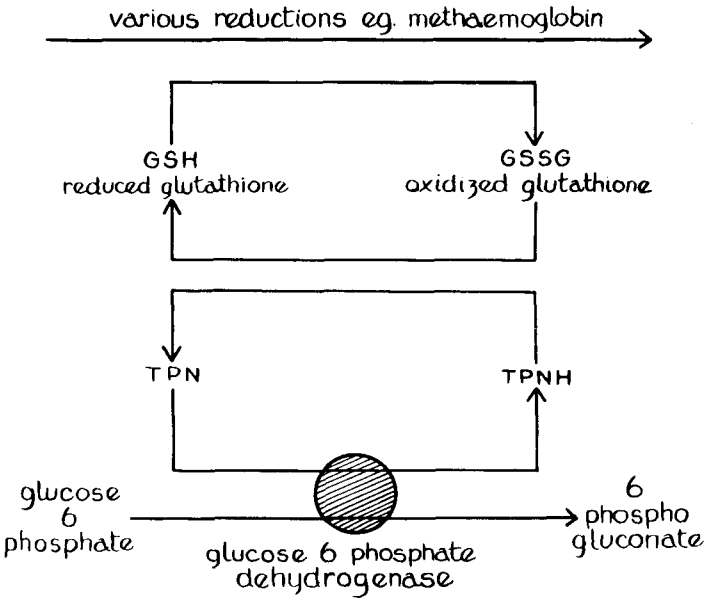


Fig. 4. The site of the metabolic defect in primaquine sensitivity is indicated by the shaded area

of subjects along the ordinate. There is a clear antimode at 2.5 $\mu$g/ml concentration. Subjects with values less than this are rapid inactivators and those with higher values are slow inactivators of the drug. There are 267 members of 53 Caucasian (white) 2 generation family units.

The mating data are shown in Table I. The expected numbers of children are derived from the hypothesis that slow inactivators are autosomal recessives.

From the first mating type (slow $\times$ slow) there are 4 rapid inactivator offspring. This is due to the overlap of the two phenotypes at the antimode in the previous figure. In the other two matings (rapid $\times$ slow) and (rapid $\times$ rapid) there is good agreement between the observed and expected numbers of offspring.

This shows that slow inactivators of isoniazid are autosomal recessives.

In addition the data have been analysed for a dosage effect. All 70 heterozygotes have been picked out of the 53 pedigrees. All the other rapid inactivators have been taken (55 of them)

**Tab. I.** Expected Numbers of Children of Each Phenotype Compared With Those Observed (From Evans et al. 1960)

| Phenotypic matings | N. of matings | N. of children | N. of children of each phenotype | | | | $\chi^2$ | D.F. |
|---|---|---|---|---|---|---|---|---|
| | | | R | | S | | | |
| | | | Expected | Observed | Expected | Observed | | |
| S × S | 17 | 54 | nil | 4 | 54 | 50 | — | — |
| R × S | 23 | 67 | 38.88 | 40 | 28.10 | 27 | 0.075 | 1 |
| R × R | 13 | 38 | 31.30 | 31 | 6.68 | 7 | 0.018 | 1 |
| | 53 | 159 | | 75 | | 84 | 0.093 | 2 |

$P > 0.95$

— and this group must contain about 27 homozygous dominants. When the mean plasma isoniazid concentrations are compared a significant difference is shown (Table II). This indicates that homozygous dominants have lower values than the heterozygotes — in other words a dosage effect is shown for the character.

Further work on this phenomenon has resulted in the recognition of the genotype.

Fig. 9 is taken from Dr. Sunahara's paper. The plasma isoniazid concentrations have been estimated by a very delicate microbiological technique, 4 hours and 6 hours following oral

**Tab. II.** Data for Family Members who are Recognisable as Heterozygotes, and Other Rapid-inactivator Family Members (Adapted from Evans et al. 1960)

| | Plasma Isoniazid Concentration ($\mu$g/ml) | | |
|---|---|---|---|
| | N. of Observations | Mean | Standard Error of Mean |
| All heterozygotes | 70 | 1.2661 | 0.0633 |
| All other rapid inactivators | 55 | 0.9080 | 0.0597 |

dosage of the drug. Family studies have shown that these three modes represent the three genotypes.

What of this character in the treatment of tuberculosis?

It has been proved to have a very definite influence on the production of polyneuritis on isoniazid therapy.

This is shown in Table III. In the 3rd column " Developed Polyneuritis " only 2 rapid inactivators were present in the 19 people who developed the complication whilst being treated with the drug. Hence slow inactivation predisposes to polyneuritis.

The influence of the inactivator character on the progress of tuberculous lesions under isoniazid treatment still remains to be properly worked out.

A word now about the anthropology of the isoniazid inactivator phenotype.

It seems clear from the literature that Eskimos are almost all rapid inactivators, Japanese have about 10% slow inactivators, whilst Europeans are 52% slow inactivators.

Fig. 10 shows some new studies. All these subjects have been studied by the same technique. In the top histogram are shown the results from 117 unrelated whites. There is a big spread

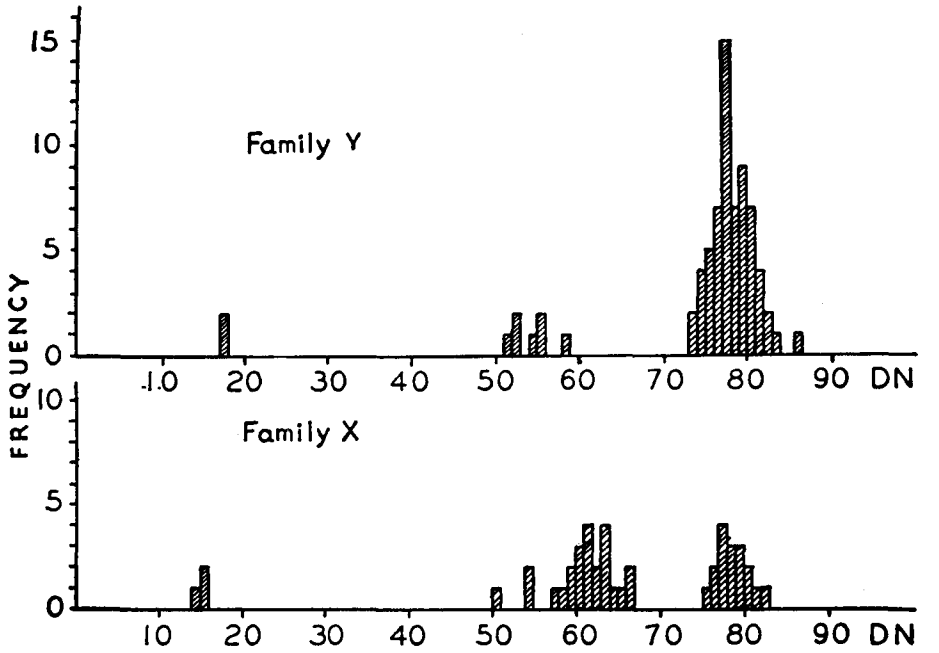


Fig. 5. The frequency distributions of dibucaine numbers in two families (From Kalow & Staron, 1957)

**Tab. III.** Development of Polyneuritis on Isoniazid Therapy from Devadatta S. et al. (1960)

| Inactivator Phenotype | Free of Polyneuritis | Developed Polyneuritis | Totals |
|---|---|---|---|
| Rapid | 58 | 2 | 60 |
| Slow | 66 | 17 | 83 |
| Totals | 124 | 19 | 143 |

$\chi^2 = 8.87$ $p < 0.01$

especially in the slow inactivator group — values up to 11 $\mu$g/ml. The slows are 52% of the population.

In the middle histogram 102 primitive Africans (Sudanese Dinkas and Dongalawis) 65% are slow inactivators (the highest value yet recorded) and there is a much smaller spread of values and more overlap at the antimode.

In the bottom histogram 60 Chinese show 85% rapid inactivators and a small spread.

There must be some factor apart from just variations in gene frequency to account for the shapes of these curves. It may be environmental, but it is just a possibility that it is the genetic background against which the isoniazid gene is set in the different races which is responsible for the differences.

What is the biochemical nature of this isoniazid polymorphism? There is some evidence,

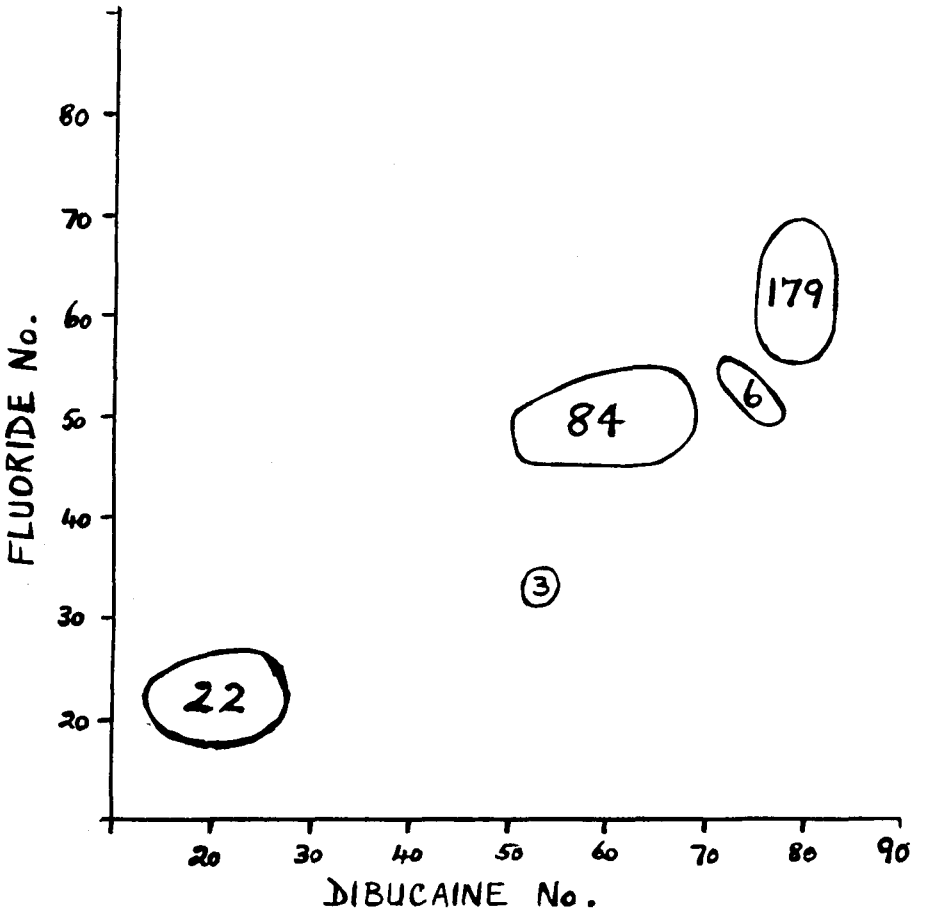


Fig. 6. Within the ringed areas are indicated the numbers of persons studied Adapted from Harris & Whittaker, 1961

but not conclusive proof, that it is differences in the acetylation of the free hydrazine moiety of the molecule which is responsible.

One can speculate, and I think Dr. Kallman that this is important in psychiatry, that there may be a similar polymorphism in the metabolism of other mono-substituted hydrazides such as " Nardil ", " Cavodil " etc. which are known to have such a variable effect in the treatment of depressive states.

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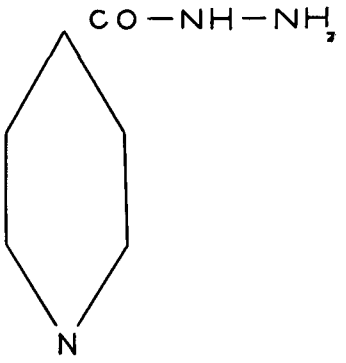


Fig. 7.
Isoniazid (1 isonicotinyl-hydrazine)

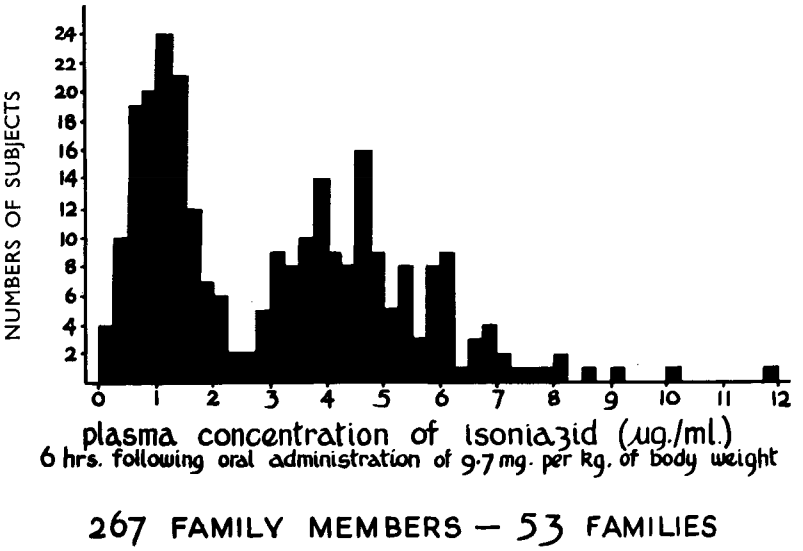


Fig. 8. From Evans et al., 1960

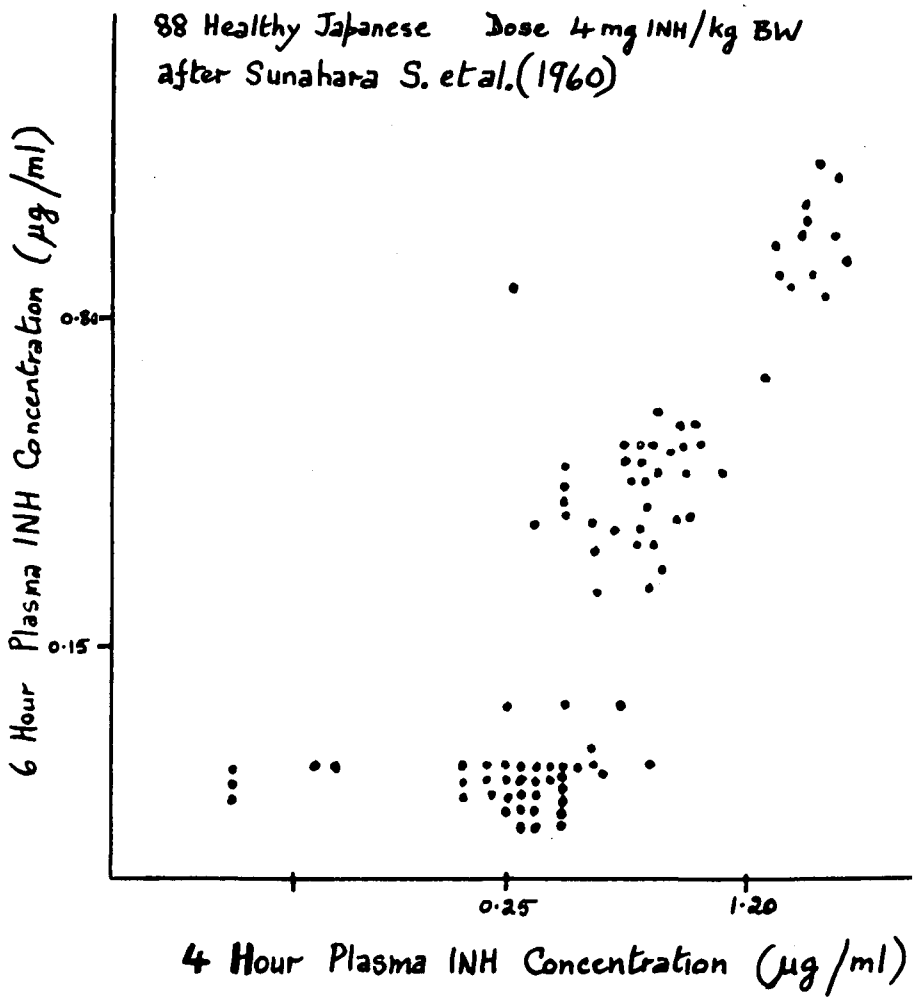


Fig. 9.

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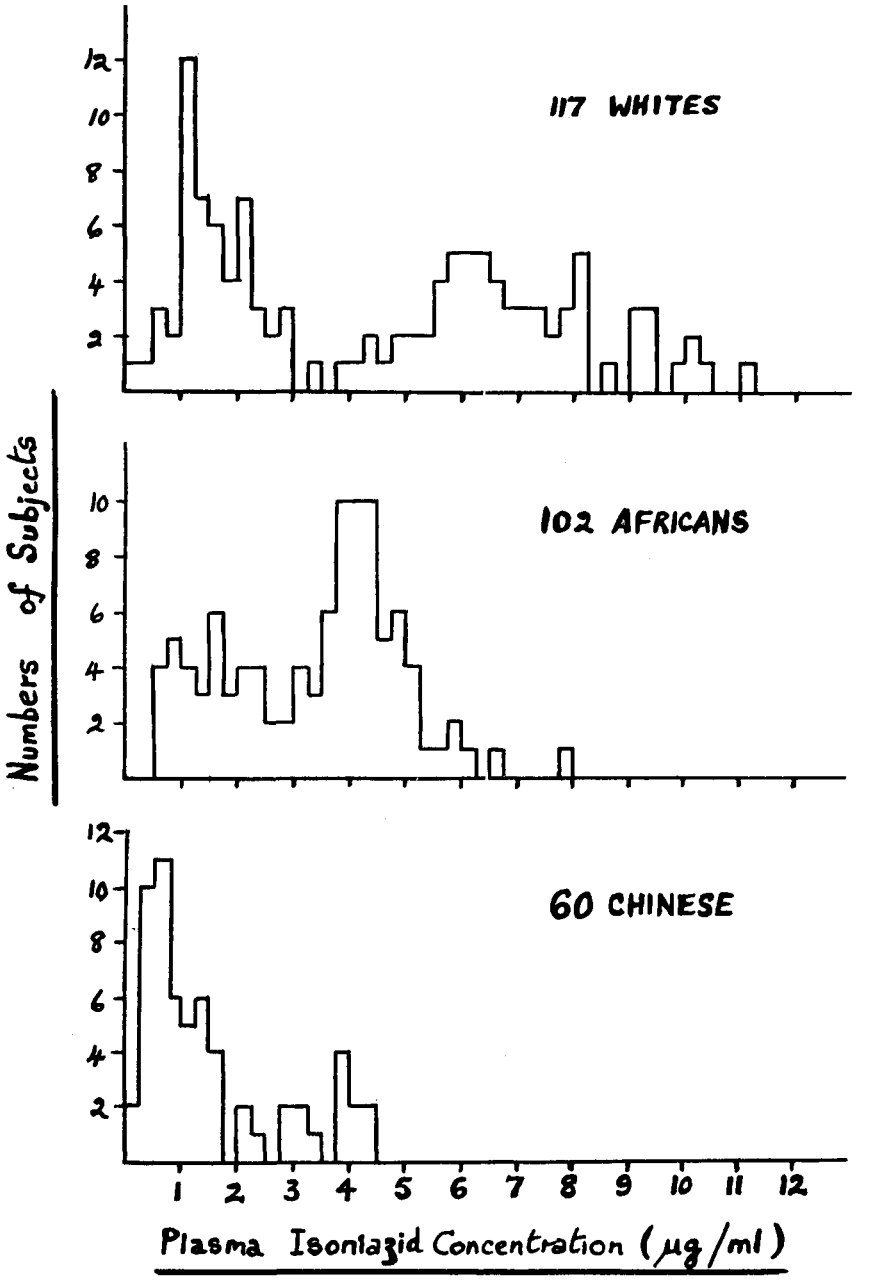


Fig. 10. Plasma isoniazid levels 6 hours following an oral dosage of 40 mg. isoniazid per kg. metabolically active mass (Evans 1961 Unpublished observations)

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## Extensions of the PTC Polymorphism

Fig. 11 shows the essential chemical linkage which gives rise to the PTC taste-testing polymorphism in Man.

About one-third of Europeans are unable to taste this compound unless they are given almost saturated solutions; they are called " non-tasters ". Two-thirds of the population can detect PTC on the back of the tongue in very low concentrations; they are called " tasters ".

It so happens that this chemical linkage is present in a number of drugs that we use in day-to-day medicine. Firstly, thiouracil (we actually use Methyl or Propyl Thiouracil) and secondly thiopentone, show a polymorphism, placed on the tongue.

The question that has been at the back of people's minds for a long time is this. Is the PTC polymorphism on the tongue an expression of a purely local phenomenon or does it represent a polymorphism for the metabolism of this linkage generalized throughout the body?

In favour of its being widespread is its association with thyroid diseases.

In an attempt to answer the question of whether this is a local or a general metabolic phenomenon we have done two studies.

Firstly, urine has been recovered for 4 hours after the ingestion of 15 mg. methyl thiouracil per kg. of metabolically active mass by fasting subjects and the amount of drug excreted in this time measured. The PTC taste-threshold of each subject has been independently determined.

Fig. 12 shows the result. There is no correlation between the percentage of the drug recovered and the PTC taste threshold.

The second study has been of plasma thiopentone concentration decay curves. Here also there is no bimodality after standardized doses of thiopentone have been given to fit subjects having minor operations.

These two snippets of information suggest that the PTC polymorphism is either a minor metabolic phenomenon or that it is a local effect possibly confined to the tongue and thyroid region. It is of interest to note that these two structures arise from embryologically adjacent sites.

## Conclusions

1. It is obvious as a result of these studies that I have mentioned:
Acatalasia
Primaquine Sensitivity
Suxamethonium Sensitivity
Isoniazid inactivation polymorphism.
Ramifications of the PTC tasting polymorphism,
that the thing to do is to look for other bimodalities in the metabolism of drugs or in their actions in human populations. It would seem that the so-called " side effects " of drugs are worth looking at from this point of view.

2. It would seem that polymorphism of phenotypes of the orders of frequency of the isoniazid inactivators in whites (about 50%) or G6PD deficiency in Negroes (about 10%) are capable of interfering with the validity of the results of " double blind " trials of drugs espe-

cially where the tested groups are small. This is because they introduce a variability into the groups which may lead to false conclusions unless the possibility of polymorphism has been eliminated by preliminary studies.

3. The studies on primaquine and suxamethonium sensitivities are excellent exam-

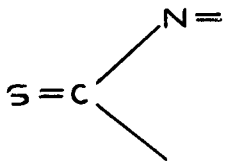


Fig. 11. The essential chemical linkage to detect the PTC polymorphism

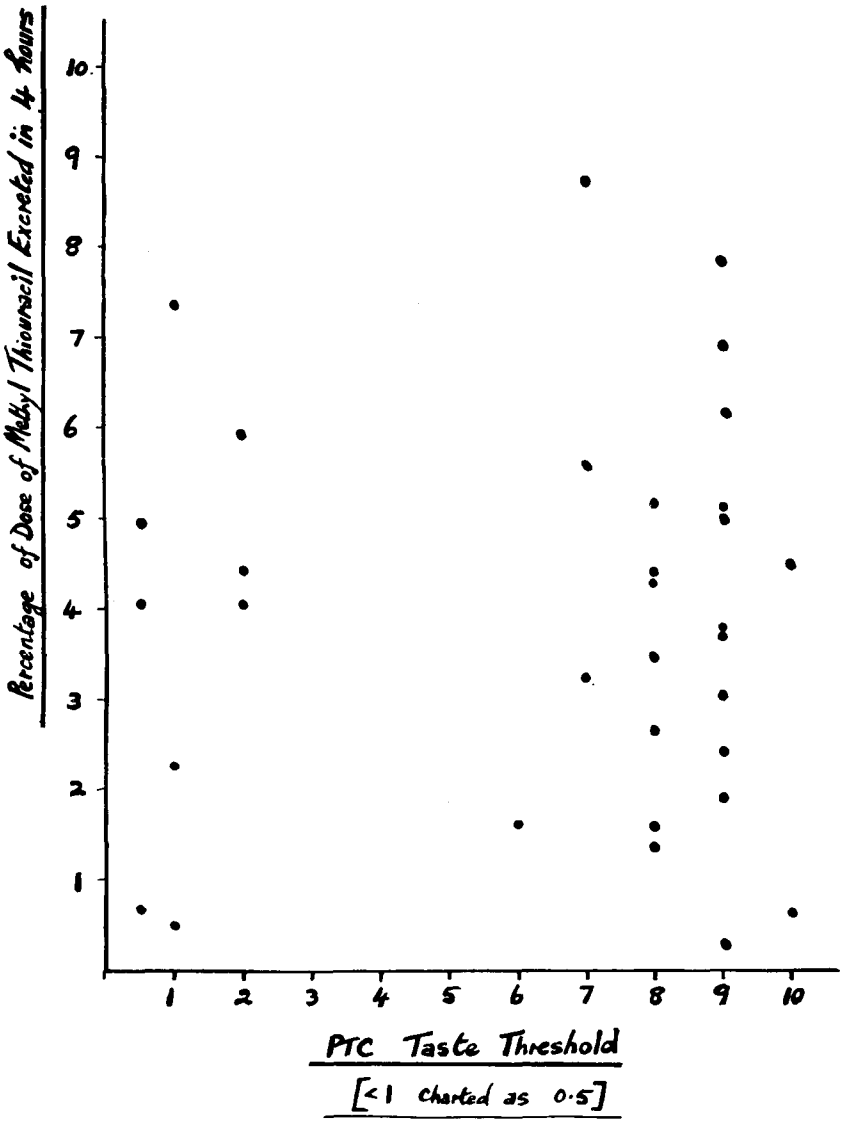


Fig. 12. The correlation between the percentage of a dose of Methyl Thiouracil excreted and the PTC taste threshold. Evans (1961) Unpublished observations

ples of the way in which observing diical respfferent clinonses to drugs ash led to important discoveries in the field of biochemical genetics.

4. Once the observations on the biochemistry of acatalasia and G6PD had been wor-

ked out, this being reapplied in the clinical field has given us a clearer understanding of hitherto rather obscure clinical syndromes.

5. These observations emphasize the interdependence of clinical practice and laboratory research in pharmacogenetics. It seems a fruitful field for further study.

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TIPOGRAFIA POLIGLOTTA VATICANA