Genetic polymorphism and interethnic variability of plasma paroxonase activity

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Summary. A method for determining plasma paroxonase activity using an auto-analyser is described. Frequency distributions for British and Indian subjects show bimodality. A study of 40 British families confirms the presence of a genetic polymorphism with regard to plasma paroxonase activity. Two phenotypes can be defined, controlled by two alleles at one autosomal locus. The frequency of the low activity phenotype is less in the Indian population than in the British population. Malay, Chinese, and African subjects fail to show obvious bimodality.

Paroxon (O, O-diethyl-O P-nitro phenyl phosphate) is an organophosphorus anticholinesterase compound. It has been used in man as a local preparation in the treatment of glaucoma (Fagerlind, Holmstedt, and Wallen, 1952). It is produced in mammals by microsomal oxidation of the widely used insecticide parathion (Fukuto and Metcalf, 1969). Parathion is biologically inert until transformed to paroxon.

Paroxonase is an arylesterase (EC 3.1.1.2) which is capable of hydrolysing paroxon to produce p-nitrophenol (Fig. 1). It has no known natural substrate. The enzyme is widely represented in mammals and has been isolated and purified in sheep (Main, 1960). It was described in human plasma by Erdos and Boggs (1961) and has been characterized by Krisch (1968).

Geldmacher v. Mallinckrodt *et al* (1973) suggested that the activity of human plasma paroxonase exhibited a genetic polymorphism.

This paper describes: (i) an autoanalyser method for determining plasma paroxonase activity, (ii) the existence of a genetic polymorphism, shown by the study of 40 families, and (iii) large interethnic variability in paroxonase activity distributions.

Materials and methods

- (1) British Caucasian subjects were unrelated blood transfusion volunteers from Merseyside.
- (2) British Caucasian subjects in 2 generation family units ascertained with the help of local doctors.
- (3) Other ethnic groups.
 - (a) 74 Chinese, 70 Indian, and 71 Malay subjects were blood transfusion volunteers in Kuala Lumpur, Malaya. The Indians were mainly Northern Indian in derivation.
 - (b) A further 175 Chinese subjects came from Hong Kong.
 - (c) Kenyan Africans were healthy staff from the Medical Research Council Project, Kisumu, and were taking weekly pyrimethamine.
 - (d) Nigerian Africans were 11 healthy medical

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FIG. 1. Biotransformation of parathion and paroxon. Parathion is converted to paroxon by means of microsomal oxidation and paroxon is hydrolysed to yield p-nitrophenol. The reaction is speeded by the plasma enzyme, paroxonase.

student and doctor volunteers, and 22 patients with miscellaneous disorders in Benin; 145 were blood transfusion volunteers in Ibadan.

Blood specimens

These were collected in heparinized containers. Plasma was separated and stored at -20°C . Specimens were transported in a frozen state with the assay being performed within three months of collection. No deterioration was shown within this period and no change in activity was seen on repeated freezing and thawing.

Reagents

(1) Glycine pH 10.5 buffer: 50 ml 1.0M Na OH adjusted to pH 10.5 with 2M glycine and diluted to 1L with deionized water.

(2) TRIS/HCl buffer pH 7.7: 250 ml 0.2M TRIS adjusted to pH 7.7 with 0.1M HCl and diluted to 1L with deionized water.

(3) A.M.P. buffer pH 10.25 0.625M (Technicon formula T01-0312-38).

(4) Paroxon in TRIS/HCl buffer pH 7.7. Paroxon is stable in aqueous solution below pH 7.0. Paroxon was obtained from Koch-Light Laboratories, Colnbrook, Bucks. 1 ml was diluted to 500 ml with distilled water whose pH was approximately 6.5. This solution was stable. For each day's estimation procedure a fresh working substrate solution was prepared by diluting the stock solution with TRIS/HCl pH 7.7 so that when 2.4 ml was treated with 0.6 ml of 4N NaOH and assayed, the released p-nitrophenol was equal to $0.52\text{ }\mu\text{mol/ml}$. The stock solution was kept at room temperature.

(5) p-nitrophenol standards. A stock solution of $10\text{ }\mu\text{mol/ml}$ of p-nitrophenol (Sigma Chemical Co., London) was diluted with Technicon T01-0312-38 AMP buffer, to produce working standards comprising 0.1, 0.25, 0.5, 1.0, 1.5, and $2.0\text{ }\mu\text{mol/ml}$ concentrations of p-nitrophenol. All standard solutions were kept at -4°C in the dark, when not in use.

(6) Ethylene diaminetetra-acetic acid (EDTA). 84.51 mg EDTA were dissolved in one litre of glycine buffer

pH 10.5. When diluted in the auto-analyser this gave a final concentration of $1 \times 10^{-4}\text{ M}$.

(7) p-Chloromercuribenzoate (PCMB). 81.834 mg PCMB were dissolved in one litre of glycine buffer pH 10.5. When diluted in the auto-analyser this gave a final concentration of $1 \times 10^{-4}\text{ M}$.

Auto-analyser method

Plasma paroxonase activity was determined using a Technicon auto-analyser apparatus. The flow diagram is shown in Fig. 2. The method involved the enzymatic hydrolysis of paroxon at 37°C . Each plasma sample was automatically mixed in a single mixing coil with the substrate in TRIS/HCl buffer pH 7.7 and glycine buffer pH 10.5. The mixture was fed into a delay coil in a 37°C water bath. After 15 minutes of incubation, the p-nitrophenol enzymatically released from paroxon was dialysed into 2-amino 2-methyl 1-propanol (AMP) recipient buffer pH 10.25. p-Nitrophenol is yellow under alkaline conditions and the absorbance was measured at 412 nm in a 15 mm tubular flow cell. During the inhibition studies the solution of the inhibitor (see above) replaced the glycine buffer pH 10.5.

The p-nitrophenol liberated by plasma samples is measured by reference to a standard curve prepared at the start and end of every batch of determinations by running the p-nitrophenol (PNP) standard solutions (see above) through the sample line. It was shown that neither heparin nor the inhibitor solutions interfered with the determination of the PNP standard curve.

The enzyme activity of each sample was expressed in terms of the amount of PNP produced under the conditions specified.

The production of PNP with time in the presence of 'low' and 'high' activity plasmas defined below is shown in Fig. 3. The auto-analyser apparatus (Fig. 2) provided an incubation period of 15 minutes which was chosen arbitrarily, and the conditions in the auto-analyser circuit were constant for each assay.

The auto-analyser method was simple, reliable, and accurate. The intra-assay experimental error was estimated by running multiple samples of the same

FIG. 2. Diagram of technicon auto-analyser apparatus used to determine plasma paroxonase activity.

FIG. 3. The amount of pNP produced against time for: (1) a typical 'low activity' phenotype, (2) a typical 'high activity' phenotype. Samples from a typical 'high activity' phenotype and a typical 'low activity' phenotype (as determined by the autoanalyser method) were assayed using a SP 500 Unicam Spectrophotometer. The same reagents in the same proportions as used in the autoanalyser method were employed. The incubation was at 37° C and the assay was performed on duplicate specimens.

specimens during the same assay and was found to be $\pm 3\%$. The interassay experimental error was determined by running 10 specimens in several different assays and found to be $\pm 9\%$.

In 20 unrelated individuals 2 repeat specimens were obtained with an interval of between 5 days and 1 month. These 40 plasma samples were assayed in one single auto-analyser run. The phenotyping information on all individuals was the same from both samples. The inter-class correlation coefficient r was 0.988 for all 20 subjects. For 9 low activity phenotypes r was 0.627 and for 11 high activity phenotypes r was 0.945.

Results

Taking a random group of 190 British (Caucasian) blood donors the frequency distribution of paroxonase activities was bimodal (Fig. 4). Taking the antimodal point at 600 $\mu\text{mol}/\text{PNP}$ per l, two phenotypes, one 'low activity' and the other 'high activity' can be defined. A genetic polymorphism could

FIG. 4. Frequency distribution histograms of (i) British blood donors (ii) British families.

explain the bimodality if the low activity phenotype is a recessive character and the high activity is a dominant character. This hypothesis was tested by an analysis of paroxonase activity in 40 families.

The postulated alleles were designated N^H controlling 'high activity' and N^L controlling 'low activity'. If the proportion of N^L alleles is q and the proportion of N^H alleles is p then, according to the Hardy-Weinberg equilibrium the frequency of the homozygote recessive character in the population will then be q^2 . From the data in British (Caucasian) blood group donors (Fig. 4) the proportion of the low activity phenotype is $94/190 = 0.4947$ and therefore $q = 0.7034$ and $p = 0.2966$.

The allele frequency calculated from 80 unrelated parents in the families was not significantly different at $q = 0.6546$.

TABLE I
COMPARISON OF NUMBER OF OBSERVED WITH NUMBER OF EXPECTED PHENOTYPES
IN OFFSPRING OF 40 MATINGS

Matings Male $\times$ Female	Offspring				χ^2
	High Activity		Low Activity		
	Observed	Expected	Observed	Expected	
High activity $\times$ high activity	25	26.54	7	5.45	0.234
Low activity $\times$ low activity	0	0	11	11	
Total	25	26.54	18	16.46	
High activity $\times$ low activity	28	24.65	14	17.35	1.002
Low activity $\times$ high activity	11	12.90	11	9.10	0.677
					1.913

Degrees of freedom 2 $P = > 0.100$.

TABLE II
COMPARISON OF PHENOTYPE FREQUENCY IN MALES AND FEMALES

Source of Population Sample	Males			Females		
	No.	High Activity	Low Activity	No.	High Activity	Low Activity
British blood donors	154	78 51%	76 49%	36	18 50%	18 50%
Parents of British families	40	26 65%	14 35%	40	20 50%	20 50%

The gene frequencies obtained from the blood donor population were used to compute the expected numbers of phenotypic matings and offspring for comparison with data from the families. Table I shows that the observed numbers of offspring of each phenotype from matings of all the possible combinations of phenotypes were closely similar to those expected. By inference, the result substantiated the hypothesis that the low plasma paroxonase activity phenotype is an autosomal recessive Mendelian character.

Phenotype frequencies were not found to be significantly different in the two sexes (Table II). For the British population, the high activity phenotype females had a mean value of 917 $\mu\text{mol/l}$ (SD 181) and males had a mean value of 894 $\mu\text{mol/l}$ (SD 193) which were not significantly different.

Increasing age up to about 60 years had no significant effect on plasma paroxonase activity. Fig. 5 shows the results in family members, viz. 80 parents, 2 grandparents, and 107 offspring. The range of ages of blood donors was more restricted.

Frequency distribution histograms of Indian, Kenyan, Nigerian, Malay, and Chinese populations are shown in Fig. 6. There is bimodality in the Indian population with an antimode at 540 $\mu\text{mol PNP/l}$, that is in about the same position as in the

British Caucasian population. The low activity phenotype in the Indians has a frequency of 15/70, giving an allele N^L frequency of $q = 0.4629$. In the African, Malay, and Chinese populations, separate phenotypes were impossible to define with certainty. It can be said that if the low activity allele N^L is present in these populations then it has a low frequency.

Studies on the inhibition of paroxonase activity with PCMB and EDTA were carried out at an early stage of the investigation and were not continued throughout the study. They were, therefore, not

Fig. 5. A scattergram of plasma paroxonase activity of British Caucasian family members of various ages.

Fig. 6. Frequency distribution histograms of various ethnic groups

FIG. 7. Inhibition of plasma paroxonase activity by parachloro-mercuribenzoate (PCMB) in various ethnic groups.

applied to all specimens but no selection was involved. The inhibition produced by PCMB did not help in phenotype definition, but there was a tendency for low activity phenotypes to be inhibited to a greater extent than high activity enzymes (Fig. 7). In the British population sample the low activity phenotype was inhibited to a greater extent than the high activity phenotype by EDTA (Fig. 8). This division was not so clearly apparent in other ethnic groups.

Discussion

The results indicate the existence of a genetic polymorphism for plasma paroxonase activity in the British population. Phenotypes are easily defined by the autoanalyser method, which was reproducible and accurate. The proposed model of a single autosomal locus with two alleles (N^H , N^L) is verified by the data presented. There is no significant difference of phenotype frequency or expression with sex. This indicates the probability of an autosomal locus. The allele frequencies of the blood donor group ($q=0.7034$; $p=0.2966$) are

FIG. 8. Inhibition of plasma paroxonase activity by ethylene diaminetetra-acetic acid (EDTA) in various ethnic groups.

arrived at independently from the allele frequencies in the family group ($q=0.6546$; $p=0.3454$) and are not significantly different. No significant difference can be shown between the expected and observed distributions, when allele frequencies from the blood donor group are applied to compute the expected distribution of phenotypes among offspring from 40 family matings. By inference, the single locus model appears to be correct.

The present findings can be compared with those of Geldmacher-v. Mallinckrodt *et al* (1973) who studied German subjects using a different technique. They claimed to be able to recognize three genotypes. Assuming that their low paroxonase activity genotype is equivalent to the low activity paroxonase phenotype in the present study, the allele frequencies derived ($q=0.7167$, $p=0.2833$) are closely similar.

The 11 families containing matings of high activity phenotypes can be analysed to yield information concerning polygenic inheritance. The correlation between parental values is not significant ($r=0.15$), which suggests no strong environmental influence within the home. Mean parental and mean offspring values have similar distributions.

The regression of mean offspring values on mean parental values gave a regression coefficient of 1.05, with an intercept of 3.06 units of y , $t=3.2$, and $P<0.02$. This regression coefficient is a direct estimate of 'heritability' (Falconer, 1960) and gives a value for $\frac{V_A}{V_P}$ where V_A is the variance caused by the additive effects of genes and V_P is the phenotypic variance. The estimated variance of heritability is 0.107. Most of the intramodal variability would, therefore, appear to be heritable.

There are obvious differences between the distributions of plasma paroxonase activities found in different ethnic groups. The Indian and British populations have a similar bimodality and it seems likely that they possess the same alleles though in different frequencies.

African, Chinese, and Malay populations have distributions differing from one another and which are not bimodal. It seems likely that the alleles (N^H and N^L) found in British and Indian populations are either replaced at the major locus or modified by the presence at other loci of different gene frequencies or new alleles.

The differential inhibition of the two phenotypes in the British population by EDTA suggests that the enzymatic polymorphism may have a structural basis but further work would be required to confirm this.

Paroxonase has no known natural substrate and its function *in vivo* is not clear. Many organo-phosphorus anticholinesterase compounds are known which are structurally similar to paroxon (O'Brien, 1967; Hartley and West, 1969; Satchell, 1973). It is possible that these are degraded by the same enzymes. Poisoning with anticholinesterase compounds is common in agricultural communities, and Taylor, Kalow, and Sellers (1965) have suggested that individuals with high enzyme activity are at an advantage when poisoning occurs.

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