BIOCHEMICAL STIJDIES ON THE PREVELENCE OF A TIPICAL PSEUDOCHOLINESTERASE

IN NOMAL SUBJECTS AND PATIENTS WITH SICKLE-CELL-DISEASE IN ZARIA

BY

UKWENI NWANKWO NWOKE

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*Ahm*adu

Department of Biochemistry,
Faculty of Science,
Bello University,
Zaria.

D E D I C A T I O N

To my grandmother, Ma Aina Ada Mong, whose advice and encouragement geared me into the Master of Science Programme. May her beloved soul rest in perfect peace.

Amen.

D E C L A R A T I O N

I declare that this work is wholly the author's and has not been part of any presentation for any other qualification.

Signed:_____
Ukweni N. Nwoke (author)

Date:_____

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A B S T R A C T

Succinylcholine, a short-acting muscle relaxant, has been reported as the cause of prolonged apnea following administration to some surgical patients. A relationship between increased sensitivity to succinylcholine and decreased levels of serum cholinesterase (pseudocholinesterase) EC.3.1.1.8 has been established. The cases of atypical or deficient pseudocholinesterase activity have been traced to variant forms of the enzyme at the gene E_1 locus.

The atypical variant of pseudocholinesterase has been looked for in 202 control (apparently normal) subjects and 28 patients with sickle-cell disease, all living in Zaria, Nigeria. The type was determined by inhibition tests with dibucaine and sodium fluoride; percent inhibition achieved under specified conditions were termed dibucaine number (DN) and fluoride number (F.N).

The Frequency of the carriers of the heterozygous I phenotype amounts to 1.98 percent in the control group. The frequency of the corresponding E_1^a gene is 0.009. Neither I nor A phenotype has been detected in the sickle-cell patients. The frequency of the carriers of the heterozygous UF phenotype amounts to 1.5 percent in the control group, and the frequency of the corresponding E_1^f gene is 0.0074. Neither UF nor F phenotype occurred in the sickle-cell patients as compared with apparently normal control subjects. In the total population the frequencies of the atypical gene and fluoride-insensitive gene were calculated to be 0.9 percent and 0.7 percent respectively. Thus, roughly one out of 60 persons has an intermediate DN but only one out of 12,500 is expected to have an atypical DN, and one out of 80 persons has the 'UF' FN but only one out of 20,500 is expected to have the 'F' FN.

Data on esterase levels have been obtained as by-product of the determination of DN and FN. The mean pseudocholinesterase activity for sickle-cell subjects is lower than (0.80 ± 0.18 I.U./L), but not significantly different from the control mean (0.85 ± 0.33 I.U./L).

No statistically significant correlation was observed between pseudocholinesterase activity (also DN and FN) and blood group, genotype, age and ethnic origin.

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ABBREVIATIONS

1. ACh = Acetylcholine
2. AChE = Acetylcholinesterase
3. BeCh = Benzoylcholine
4. BuCh = Butyrylcholine
5. ChE = Cholinesterase
6. DN = Dibucaine number
7. EDTA = Ethylene diamine tetracetic acid
8. FN = Fluoride number
9. Hb = Haemoglobin
10. I.U. = International unit (of enzyme activity)
11. Km = Michaelis constant
12. PrCh = Propionylcholine
13. SCh = Succinylcholine
14. SGOT = Serum glutamate oxaloacetate transaminase (Aspartate aminotransferase)
15. Vmax = Substrate concentration at which enzyme activity is maximal.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION.

There are two recognised types of cholinesterases in the animal tissues:

(a) acetylcholinesterase ("true cholinesterase"), found in erythrocytes and at the neuromuscular junction, specific for the hydrolysis of acetylcholine, and

(b) serum cholinesterase ("pseudocholinesterase"), present in the liver, heart and intestine, with no clearly defined physiologic function.

While the physiologic role of pseudocholinesterase is unclarified and highly controversial, its pharmacologic significance is universally accepted. It hydrolyses many choline and non-choline esters and from the point of view of the anaesthesiologist, the hydrolysis of the ester type neuromuscular and local anaesthetic agents has the greatest importance. Whereas the activity of the microsomal enzymes of the liver, responsible for the metabolic transformation of most drugs, is greater in most mammalian species than in man, the ability of pseudocholinesterase to hydrolyse ester type neuromuscular and local anaesthetic agents is much greater in man than in other mammals (Foldes and Rhodes, 1953). The high pseudocholinesterase activity of human plasma has important implications: It will decrease the relative potency and even more so, the duration of action of hydrolysable relaxants and decrease the systemic toxicity of ester type local anaesthetic agents. Hence, any qualitative or quantitative alteration of plasma pseudocholinesterase activity may significantly influence the intensity and

duration of action and toxicity of ester type neuromuscular blocking and local anaesthetic agents.

Evans et al. (1952) observed low pseudocholinesterase activity in patients who had received succinylcholine and who had subsequently remained in apnea much longer than the expected time. (Apnea is a transient cessation of respiration caused by certain drugs). Lehmann and Ryan (1956) pointed out that this low enzyme activity was in some patients not the result of disease but of hereditary factor, and studies by Kalow (1956) showed that the activity was not only low but qualitatively changed owing to a hereditary alteration of the enzyme molecule itself. The adjective "atypical" was then introduced to designate this altered enzyme. After the administration of conventional doses, the intensity and duration of action of hydrolysable relaxants (e.g. succinylcholine) is markedly, but not excessively, increased when pseudocholinesterase activity is low, but the enzyme is qualitatively normal, and decreased when the enzyme activity is abnormally high. In the presence of the atypical pseudocholinesterase variants, the duration of action may be excessively increased after the administration of conventional doses of succinylcholine (Kalow and Gunn, 1957; Foldes et al. 1963), and can result in respiratory failure (Bush, 1964) and death (Beecher and Todd, 1954).

Kalow and Genest (1957) have shown that the atypical variant can be reliably distinguished from the usual serum cholinesterase by the dibucaine number. The dibucaine number (D.N.) is the percentage inhibition of the serum enzyme by 10^{-5}M dibucaine using benzoylcholine as substrate. The D.N. clearly differentiates three

types of individuals: the normal homozygote with a dibucaine number of 70 - 85; the atypical homozygote with a value below 30; and the heterozygote with a value between 40 and 70. The three phenotypes are usual (U), atypical (A) and Intermediate (I); the corresponding genotypes are $E_1^u E_1^u$, $E_1^a E_1^a$ and $E_1^u E_1^a$ respectively.

Harris and Whittaker (1961) found that sodium fluoride was a differential inhibitor of the pseudocholinesterase variants in a manner similar to dibucaine. They defined the percentage inhibition produced by 5×10^{-5} M Sodium fluoride as the fluoride number (F.N.) and suggested that the corresponding gene E_1^f was allelic to the E_1^u and E_1^a genes.

A wide variety of inhibitors have been used to differentiate the pseudocholinesterase variants (Kalow and Davies, 1958; Harris and Whittaker, 1963; Whittaker, 1969; Hanel and Viby - Moginsen, 1977) and comparable differentiation has been obtained.

Beside the above mentioned variants detected by kinetic methods, other molecular variants of pseudocholinesterase have been detected by electrophoresis, because of a difference in the net electric charge of their molecules (Harris et al, 1962; Van Ros and Druet, 1966).

What could be done to minimize the incidence and severity of untoward reactions to drugs metabolized by pseudocholinesterase? The most important single factor is awareness of the possibility of such reactions. Ideally, it would be desirable to determine routine serum pseudocholinesterase activity of all patients in whom the use of such drugs are contemplated. While the knowledge of serum cholinesterase activity is very useful it should be noted that the normal level of this enzyme does not necessarily exclude prolonged

succinylcholine induced apnea or systemic reaction to hydrolyzable local anaesthetic agents (Foldes et al, 1963). The technique of differential inhibition, particularly with dibucaine, unlike the mere estimation of cholinesterase activity, clearly identifies the heterozygote. It has therefore become a standard technique in the investigation of families and in population surveys. In some cases it is a combination of two differential inhibitors that give an unambiguous phenotype which can be confirmed by family studies. Whittaker (1977) has recommended the use of dibucaine and sodium Fluoride as differential inhibitors with benzoylcholine as substrate in phosphate buffer. When atypical phenotype or low level of pseudocholinesterase value is reported, the technique for anaesthesia is modified, either by omiting succinylcholine or by using it with extreme caution, and warning cards should be issued to all affected individuals and relatives on the basis of such biochemical findings (Oropollo, 1978; Whittaker, 1980).

The incidence of sickle-cell anaemia has been reported to be high in the negro race, and the negroes of Africa have a higher incidence (14.32 percent) than those of North America (9.0 percent) (Shapiro and Poe, 1955). Low pseudocholinesterase levels have been implicated in various pathologic conditions (i.e. rheumatoid arthritis, chronic haemolytic anaemia, pneumonia, tuberculosis, enlarged liver, heart and spleen, jaundice and fever) which are characteristic symptoms of sickle-cell disease. Hilkowitz and Jacobson (1961) found marked reduction of pseudocholinesterase activity, associated with hypoalbuminemia in patients with sickle-cell disease. It is not known whether the enzyme present was atypical variant. There have been no studies to determine the presence

of an atypical variant of pseudocholinesterase in sickle-cell patients and even in apparently normal Nigerians.

The purpose of this work is to investigate the levels of pseudocholinesterase and the nature of the enzyme as indicated by the dibucaine and fluoride numbers in apparently normal individuals and in sickle-cell patients living in Zaria, Nigeria. Such information will be of clinical importance especially to the anaesthetist, and will also elucidate further any genetic coincidence between abnormal pseudocholinesterase and sickle-cell disease.

1.2 HISTORY OF CHOLINESTERASES.

Dale (1914) predicted the existence of cholinesterases based on his observation of the effect produced when acetylcholine was injected into an animal. He wrote, "In the blood at body temperature it seems not improbable that an esterase contributes to the removal of the active ester from the circulation and the restoration of the original condition of sensitiveness". Stedman et al (1932) prepared the first crude extract of cholinesterases from horse serum. In 1937, Marnay and Nachmansohn showed that almost all the cholinesterase present in the tissue was localized at nerve endings, and that the concentration was quite adequate to hydrolyse acetylcholine with the rapidity demanded by the theory of cholinergic transmision (Marnay and Nachmansohn, 1937; 1938). It thus became established that acetylcholine acted as a neurotransmitter.

During the second world war 1939 - 1945, cholinesterases acquired a special significance in the context of chemical warfare. Very many of the agents developed as potential war gases were powerful inhibitors of these enzymes./ (Silver, 1974) Soon after the war, the study

of cholinesterases received a new stimulus with the introduction of a histochemical method for their detection. This enabled correlations to be made between biochemical, physiological and morphological findings. In the last decade a variety of modern techniques have been used to examine the enzymes and are providing the answers to some questions.

1.3 DEFINITION OF CHOLINESTERASES.

Since their discovery, cholinesterases have been described in a number of ways (Mounter and Whittaker, 1950; Augustinsson, 1963, 1971). A composite definition which incorporates their main characteristics is that cholinesterases are hydrolases which, under optimal conditions, catalyse the hydrolysis of choline esters at a higher rate than that of other esters and which are inhibited by low concentrations (10^{-5} molar or less) of physostigmine (eserine) and organophosphorous compounds. Some cholinesterases are not totally inhibited by eserine even at a concentration of 10^{-4}M, and this lack of conformity underlines an important point: cholinesterases are not identical in all species. They constitute a family of enzymes which fall broadly into two types, those which preferentially hydrolyse acetyl esters such as acetylcholine and those with a preference for other types of choline esters such as butyrylcholine.

1.4 NOMENCLATURE OF CHOLINESTERASES.

Mendel and Rudney (1943a,b) showed that the serum contained two enzymes which were capable of hydrolysing acetylcholine. The greater part of the hydrolytic activity of serum was due to a non-specific esterase which could split both choline and non-choline esters. The remainder of the activity was attributable to a second enzyme,

thought to act exclusively on esters of choline. They suggested that this latter enzyme should be called true cholinesterase while the other, the non-specific enzyme should provisionally be called pseudocholinesterase.

Over the years other names have been introduced, true cholinesterase has been variously termed ChEI, specific, erythrocyte-, E-, aceto- or acetylcholinesterase. In 1964 the enzyme commission proposed acetylcholine acetylhydrolase (EC.3.1.1.7.) as the systematic name with acetylcholinesterase as the trivial name. Names which have been used for pseudocholinesterase include ChEII, non-specific, serum- (or plasma-), S-, butyro- or butyrylcholinesterase. The Enzyme Commission (1964) recommended acylcholine acylhydrolase (EC. 3.1.1.8) as the systematic name and simply 'cholinesterase' as the trivial name.

1.5 DISTRIBUTION OF PSEUDOCHOLINESTERASE.

Pseudocholinesterase is less widely distributed among various animal species than acetylcholinesterase. It has not been detected in any invertebrate species studied. In man and other mammals the highest activity is present in the plasma (Alles and Hawes, 1940) and in the liver (Ecobichon and Kalow, 1961). Moderate pseudocholinesterase activity has been demonstrated in the brain (Foldes et al, 1962), retina and milk (Svensmark, 1965), cerebrospinal fluid (Foldes and Aven, 1952), muscle and heart (Smith et al, 1963), intestines (Koelle, 1951), pancreas (Augustinsson, 1948), kidney and skin (Liddell et al., 1963).

1.6 BIOSYNTHESIS.

It is widely accepted that in most animal species plasma pseudocholinesterase is synthesized in the liver (Sawyer and Everett, 1947) and this accounts for the profound effect of liver disease on

blood levels (Gurtner et al., 1963). Pseudocholinesterase may also be synthesized in other tissues. Variations in the properties of this esterase obtained from different tissues of the same animal supports the assumption (Bergmann and Segal, 1955; Gerebtzoff, 1959).

1.7 ISOLATION AND PURIFICATION

Pseudocholinesterase, in contrast to acetylcholinesterase which is membrane bound, is a solubilized enzyme present in solution in plasma, cerebrospinal fluid and milk, and in the muscle, most pseudocholinesterase activity is recovered only after differential centrifugation of muscle homogenates from the supernatant.
A variety of methods have been employed for the purification of pseudocholinesterase. Steadman and Steadman (1935) achieved a 50- to 100-fold purification of horse serum pseudocholinesterase with ammonium sulphate precipitation. Surgenor and Ellis (1954) attained a 3,400-fold purification of human serum pseudocholinesterase by an ethanol fractionation procedure. Das and Liddell (1970) used a three-stage procedure consisting of chromatography on DEAE - cellulose, electrofocussing and gel filtration on sephadex G-200. The percentage yield was 54 and the purification factor 13,000. A 5,000-(Strelitz, 1944) to 14,000- (Svensmark, 1965) fold purification of horse plasma pseudocholinesterase has been obtained. Purified pseudocholinesterase is very stable and when stored at room temperature retains most of its activity for several years (Cole and Leadbeater, 1968).

1.8 PROPERTIES OF PSEUDOCHOLINESTERASE.

1.8(i) Physical and Chemical Properties.

The molecular weight of human pseudocholinesterase was estimated to be between 300,000 and 365,000 (Surgenor and Ellis, 1954; Das and

Liddell, 1970). The enzyme consists of two or more protomers. Each protomer contains two polypeptide chains each of molecular weight about 80,000 (Leuzinger et al 1969; Whittaker, 1980). The concentration of pseudocholinesterase in human plasma is about 2×10^{-8}M (Goedde and Altland, 1967) and the isoelectric point is 3.0 (Svensmark and Kristensen, 1963) Pseudocholinesterase appears to be a glycoprotein. As yet, the carbohydrate moiety has not been precisely defined but in both man and horse the enzyme is associated with sialic acid (N-acetylneuraminidic acid). If this is split off with neuraminidase, the electrophoretic mobility is decreased and the enzyme becomes less stable, but its hydrolytic activity is unchanged (Silver, 1974).

1.8(ii) The active site and Kinetics.

The active centre of pseudocholinesterase, similar to acetylcholinesterase, has an anionic and an esteratic site (Wilson, 1954). Jansz et al. (1959) and Oosterbaan (1967) have suggested the amino acid sequence of the active site of pseudocholinesterase to be: Phe-Gly-Glu-Ser-Ala-Gly- (Ala, Ala, Ser).

It seems probable that serine (Cohen et al., 1955) and histidine (Wilson and Bergmann, 1950; Mounter et al., 1957) are the basic groups at the esteratic site. The strong negative charge at the anionic site is probably carried by an w-carboxyl group of a dicarboxylic amino acid (e.g. glutamic acid) or phosphate groups which have the necessary negative charge at physiologic pH. The high reactivity of the esteratic site is supposed to be the result of the interaction of hydroxyl group of serine with the imidazole group of histidine (Silver, 1974; Foldes, 1978). It seems likely that although it is the serine hydroxyl group which functions as the ultimate acyl acceptor, the histidine imidazole group plays an important part in

the catalytic action. Cunningham (1957) has suggested that imidazole acts as a general base catalyst and enhances the nucleophilicity of the serine oxygen by forming a hydrogen bond with the hydroxyl group (Fig. 1.1).

It is generally held that the differences in the behaviour of acetylcholinesterase and pseudocholinesterase are mainly attributable to differences in or around their anionic sites, although minor differences between the esteratic sites may contribute. Some workers have suggested that the differences between the two cholinesterases could be explained if it were assumed that pseudocholinesterase had only one and acetylcholinesterase two anionic sites (Bergmann and Segal, 1954) Bergmann, 1955). In addition to the anionic site, Augustinsson (1966) postulated the presence of another non esteratic site in pseudocholinesterase but not in acetylcholinesterase. The dominant forces at this site were assumed to be Van der Waal's forces. Kabachnik et al.(1970) have proposed that both the anionic and esteratic sites may be virtually identical in the two types but

structures surrounding or adjacent to these active sites are different and these differences determine the characteristic behaviour of each enzyme. According to this scheme a hydrophobic area surrounds the anionic site in both enzymes but it exerts a greater effect on pseudocholinesterase than in acetylcholinesterase. In addition there is a second hydrophobic group near the anionic site which is thought to extend further in acetylcholinesterase than in pseudocholinesterase. There are also hydrophobic regions in the vicinity of the esteratic site, acetylcholinesterase having one and pseudocholinesterase, two.

1.8(iii) Mechanism of action.

At the simplest level, the events occuring in hydrolysis may be described (Silver, 1974; Foldes, 1978) as follows:

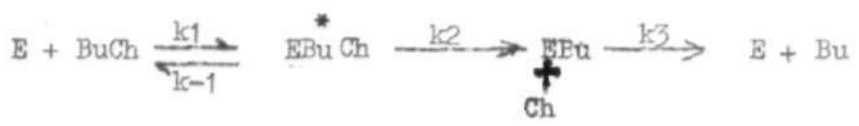
(1) the quaternary nitrogen of butyrylcholine is bound by van der Waals and coulombic forces to the negatively charged anionic site and so oriented that the carbonyl group is presented to the positively charged esteratic site, nucleophilic group consisting of histidine and serine and a high-energy unstable complex is formed which can dissociate into enzyme and unchanged substrate; or

(2) electron shifts occur at the esteratic site with the result that choline is liberated leaving an acylenzyme;

(3) The acylated enzyme is hydrolyzed very rapidly yielding

butyric acid and resulting in the restoration of the esteratic site to its inactive state.

Step I Step II Step III



In this equation E = free enzyme; BuCh = substrate; EBuCh = enzyme substrate complex; Ch = Choline; Bu = butyrate; k_1 and k_{-1} are the forward and backward reaction rate constants of step I, k_2 and k_3 are the reaction rate constants of steps II and III, EBu = Acylenzyme.

The turnover number of human plasma pseudocholinesterase is considerably lower than that of acetylcholinesterase. It is estimated to be about 50,000 with acetylcholine substrate, while acetylcholinesterase is of the order of 2,000,000 to 3,000,000.

.8(iv) Substrate Specificity

Although pseudocholinesterase hydrolyses some of the substrates of acetylcholinesterase (e.g. acetyl-β-methylcholine) at only a negligible rate it is capable of splitting much greater variety of substrates than AChE. Thus in addition to ACh, pseudocholinesterase hydrolyses a number of fatty acid and ω-amino fatty acid esters of choline (e.g. PrCh, BuCh, Valeryl-choline, 7-aminoheptanoylcholine), dicarboxylic acid esters of choline (e.g. succinylcholine), aromatic esters of choline such as benzoylcholine, ester-type local anaesthetic agents (e.g. procaine, tetracaine, isobucaine), their halogenated analogues (e.g. 2-chloroprocaine) and many other esters. The thio-analogues of ACh and BuCh are hydrolyzed at a faster rate than their oxy-analogues, both by human and horse plasma pseudocholine-

sterase. There is wide variation in the absolute and relative hydrolysis rates of various substrates by pseudocholinesterase, Thus, for example, although horse plasma pseudocholinesterase hydrolyses ACh at about the same or faster rates than human plasma cholinesterase, it hydrolyses procaine or succinylcholine at significantly lower rates than human pseudocholinesterase. Of all mammalian plasmas investigated, human plasma cholinesterase hydrolysed succinylcholine most rapidly. It is of interest that the plasma pseudocholinesterase of the cat, an animal widely used for the pharmacologic testing of neuromuscular blocking agents, does not hydrolyse succinyl choline (Foldes and Foldes, 1965).

In contrast to AChE, excess substrate does not inhibit pseudocholinesterase. The substrate concentrations at which pseudocholinesterase activity is maximal (Vmax) is about 2×10^{-2} molar for aliphatic (foldes et al, 1962) and 5×10^{-5} molar for aromatic compounds, e.g. benzoylcholine, procaine (Foldes et al., 1955; 1963). In mammalian plasmas the order of the relative hydrolysis rates of various substrates is usually

BuCh > PrCh > ACh > BeCh > SCh > Procain.

The optimal temperature for pseudocholinesterase activity is between 37°C and 40°C. Changes in temperature affect the activity of pseudocholinesterase much more than AChE. Decreasing the temperature by 10°C causes a 50 percent decrease of the reaction rate of pseudo ChE and only about 15 percent decrease of that of red cell AChE. The pH optimum of human pseudocholinesterase for most substrates is between 8 and 9 (Foldes, 1978).

1.9 MULTIPLE FORMS OF PSEUDOCHOLINESTERASE.

1.9(i) Isoenzymes.

Pseudocholinesterase has been found to exists in several isoenzymic forms.

Harris et al. (1962) used two-dimensional filter paper-starch gel electrophoresis to separate human plasma pseudocholinesterase into four bands C_1, C_2, C_3, and C_4, which were discernible in the region between the α_2 and β-globulins. Of these isoenzymes, C_4 contained about 90 percent of the total activity (Goeddo et al., 1965). The number of forms present in an individual appears to be genetically determined and the occurrence of 'extra' isoenzymes (e.g. C_5) in some population has been reported in the vicinity of C_4. Sera containing C_5 usually have relatively high pseudocholinesterase activity. Some evidence suggests that the physiological state may also influence the form of pseudocholinesterase which is detectable at a particular moment. Augustinsson (1968) using starch gel found two bands of pseudocholinesterase activity in plasma from rat during its pregnancy but only one band after parturition. Recently as many as 12 isoenzymes of pseudocholinesterase were identified in human plasma (Juul, 1968).

1.9(ii) Atypical Variants.

Apart from the molecular forms differentiated by their electrophoretic characteristics and their behaviour on columns, other variants of pseudocholinesterase exist which do differ in their activity towards substrates and inhibitors and an 'abnormal' form and the normal form can occur together in one individual.
The socalled 'atypical' or 'Dibucaine-insensitive' variant is

unable to hydrolyze a number of substrates, including succinylcholine, at the normal rate and is usually resistant to cholinesterase inhibitors. Kalow and Genest (1957) expressed this resistance in terms of the Dibucaine number which is the percentage inhibition of the enzyme produced by 10μm Dibucaine. Harris and Whittaker (1961) used 50μm sodium fluoride as an inhibitor in a similar way and showed that the Dibucaine-resistant variant had a low fluoride number. In addition they distinguished another variant which had a low fluoride number but a Dibucaine number which was only slightly lower than normal; this they termed the 'fluoride-resistant' variant. Its hydrolytic activity towards succinylcholine was found greater than that of the Dibucaine-resistant type but was still below that of the normal enzyme. Liddell et al. (1962) reported a patient whose serum was apparently devoid of pseudocholinesterases and dubbed the gene responsible for the condition the 'silent gene'. In summary, the four behaviourally different pseudocholinesterases which have been recognised are, (1) normal or usual, (2) atypical or Dibucaine-resistant, (3) fluoride resistant, (4) silent gene type.

The atypical variant occurs in combination with the normal type in about 4 percent of European and North American populations. It is more sensitive than the normal form to inhibition by n-butanol (Whittaker, 1968) and by formaldehyde (Whittaker, 1969).

Important differences were found between the properties of normal and atypical plasma pseudocholinesterase. For instance, plasmas containing the atypical enzyme hydrolysed ACh and various other substrates 2 - 10 times more slowly than the normal enzyme (Davies et al., 1960; Foldes et al, 1963). The decreased rate of

hydrolysis was attributed to the much lower affinity of the various substrates to the atypical than to the normal enzyme.

Besides the lower affinity of the various substrates, there are other differences between the normal and atypical pseudocholinesterase. Thus, for example, increasing the ionic strength of the assay medium from 0.7mM to 125mM increased the activity of normal pseudocholinesterase two- to three fold, but had no influence on the atypical enzyme (swift and LaDu, 1966; Clark et al., 1968). Various cationic activators (e.g. Ca^{2+}, Mg^{2+}, tetramethylammonium, choline) also had less effect on the atypical than the normal enzyme. It was suggested that the different kinetics of the normal and atypical pseudocholinesterase are due to differences in the structure of the anionic site, which in turn affect the hydrolytic process at the esteratic site (Kalow and Genest, 1957; Clark et al., 1968).

Beckett et al. (1968) are of the opinion that there is no difference in the amino acid composition of the active site of the normal and atypical enzyme, but the configuration of this site is distorted by structural changes at secondary sites in a way that interferes with the binding of the substrate.

iii) Genetic Studies.

The discovery of the genetically determined variants of pseudocholinesterase started with the observations (Bourne et al., 1952; Evans et al., 1952) that after the administration of the usual doses of succinylcholine, prolonged apnea was occasionally encountered in apparently healthy individuals. Further investigations showed that not only the patients involved, but also some of their family members had abnormally low plasma pseudocholinesterase (Kalow

and Lindsay, 1956).

Based on the trimodal distribution of the Dibucain number in large population samples, Kalow and Staron (1957) postulated that the type of pseudocholinesterase is determined by non-dominant, allelic, autosomal genes. Other investigators suggested that the gene expressivity of the atypical variant is inbetween dominant and recessive (Allot and Thompson, 1956; Lehmann et al, 1958). Kalow and Staron (1957) designated the normal gene by "N" and the atypical dibucaine resistant gene by "D". Those individuals who have two N genes (NN) are normal homozygotes, with qualitatively and quantitatively normal pseudocholinesterase; those with two atypical genes (DD) are atypical homozygotes; and those with one N and one D gene (ND) are heterozygotes.

It was originally thought that the polymorphism of pseudocholinesterase, as indicated by the usual and atypical variants, could be accounted for on the basis of only two allelic genes. Based on the demonstrated existence of normal, atypical, fluoride resistant, and silent cholinesterase variants, Lehmann et al. (1963) proposed that the type of pseudocholinesterase present in any individual is controlled by four allelic autosomal genes acting at the same site. The four genes were originally designated as: N (normal), D (dibucaine resistant), F (fluoride resistant), and S (silent) genes. When both genes are identical the individual ('Propositus') is a homozygote; when the two genes are different the propositus is a heterozygote. There are at present four known types of homozygotes (NN, DD, FF, and SS) and six possible combinations of heterozygotes (ND, NF, NS, DF, DS, and FS).

Subsequently two other nomenclatures (Table 1.1.) were proposed, one by Motulsky (1964) and the other by Goedde and Baitsch (1964). These systems of classification also distinguish between genotype, which is based on the presence of one or two of the genetically determined variants (genes) and the phenotype which is the manifestation of the genes in the individual.

Clear-cut differentiation of the various homozygotes from one another is relatively simple. The discrimination between the various heterozygotes and between heterozygotes and homozygotes is much more difficult and requires detailed genetic studies of the family members of the propositus. Thus, because of the wide variation of pseudocholinesterase activity among normal subjects this parameter may be very similar in normal homozygotes and in normal - atypical (ND) or normal-silent (NS) heterozygotes. The dibucaine number of these heterozygotes may be so close to that of normal homozygotes that differentiation between them is not possible. Determinining the ratio of the hydrolysis rates of procaine and tetracaine by pseudocholinesterase, and multiplying the ratio of the two rates by 100 gives a number termed the P/T ratio (Smith and Foldes, 1968, 1972). In one study, the P/T ratio was found to be 364 ± 7.7 for normal homozygotes, 203 ± 5.9 for normal-atypical (ND) heterozygotes and 43.2 ± 2.8 for atypical homozygotes. This wide divergence of the P/T ratios allows unequivocal differentiation between the most frequent NN or ND and also between the ND and DD variants, but not between the NN and NS or the DD and DS variants. For similar reasons, differentiation between the FN, FF, FD and FS variants may be very difficult without detailed genetic studies of the family members.

Table 1.1. Nomenclature of Pseudocholinesterase Polymorphism (Goeddo and Baitsch, 1964) (Gene locus 1: Ch_1; Alleles: Ch_1^U, Ch_1^D, Ch_1^F, Ch_1^S,)

Author	Lehmann and Liddell (1964)		Motulsky (1964)		Goeddo and Baitsch(1964)	
	Gene locus 1		Gene locus 1		Gene locus 1	
	Genotype	Old designation	Genotype	Phenotype	Genotype	Phenotype
Homozygote	1. N-N	Usual	$E_1^U E_1^U$	U	$Ch_1^U Ch_1^U$	Ch_1(UU)
	2. D-D	Atypical	$E_1^a E_1^a$	A	$Ch_1^D Ch_1^D$	Ch_1(DD)
	3. F-F	-	$E_1^F E_1^F$	F	$Ch_1^F Ch_1^F$	Ch_1 (FF)
	4. S-S	Silent	$E_1^S E_1^S$	S	$Ch_1^S Ch_1^S$	Ch_1(SS)
Heterozygote	N-D	Intermediate	$E_1^U E_1^a$	UA	$Ch_1^U Ch_1^D$	Ch_1 (UD)
	N-F	U_1	$E_1^U E_1^F$	UF	$Ch_1^U Ch_1^F$	Ch_1 (UF)
	N-S	Usual	$E_1^U E_1^S$	US	$Ch_1^U Ch_1^S$	Ch_1 (US)
	D-F	I_1	$E_1^a E_1^F$	AF	$Ch_1^D Ch_1^F$	Ch_1(DF)
	D-S	Atypical	$E_1^a E_1^S$	AS	$Ch_1^D Ch_1^S$	Ch_1 (DS)
	F-S	-	$E_1^F E_1^S$	FS	$Ch_1^F Ch_1^S$	Ch_1 (FS)

*(i) E and Ch refer to cholinesterase enzyme.

(ii) Subscripts (1) refer to gene locus one.

(iii) Superscripts refer to the variant forms.

1.10 ACTIVATORS OF CHOLINESTERASES.

Various chemical compounds may accelerate cholinesterase activity.

Divalent cations increase the activity of acetylcholinesterase in the order Ba^{2+}, Mg^{2+}, Ca^{2+} and Mn^{2+}. Relatively high concentrations of monovalent ions (e.g. Na^{+}, K^{+}, Li^{+}) are required for acceleration of enzymatic activity.—Their effect is usually non-specific and may be attributed to the increase of the ionic strength of the medium. Compounds like tetramethyl- or tetraethyl ammonium activate acetylcholinesterase by inducing conformational changes in the enzyme molecule that facilitate the access of the substrate to the osteratic site.

Various amines, such as trimethyl- or triethylamine, tryptamine, benzylamine, histamine (Erdos et al., 1957, 1958), various narcotic analgesics (Erdos et al., 1959), amphetamine and quaternary ammonium compounds (Erdos et al., 1958; Feldes et al., 1960) are capable of significantly accelerating the hydrolysis of aromatic substrates (e.g. BeCh or procaine) by plasma cholinesterase.

The accelerating effect of substances containing positively charged nitrogen aton on the hydrolysis of aromatic substrates by pseudocholinesterase has been explained (Feldes, 1978) by the following hypothesis: It is generally accepted that the N^{+} of aromatic substrates is attracted by coulombic forces to the anionic and their ester group by coulombic and other forces to the osteratic site of cholinesterases. Pseudocholinesterase also contains a second non-osteratic site where Van der Waal's forces supply the dominant reactive force. This site may play an important role in the binding

of aromatic substrates such as benzoylcholine or procaine containing a relatively large flat benzene ring. It is conceivable that if the N^+ of an aromatic substrate interacts with the anionic site and its benzene ring with the above non-specific site by Van der Waal's forces, the ester group of the substrate will not be in a position favourable for its interaction with the esteratic site and the rate of hydrolysis will be relatively slow. When the anionic site is occupied by another group containing N^+, it is conceivable that the aromatic substrate will be adsorbed by van der Waal's forces to a non-specific site of pseudocholinesterase molecule in a way that will favour the interaction of its ester group with the esteratic site resulting in increased rate of hydrolysis observed in the presence of such N^+ compounds.

1.11. INHIBITORS.

The normal reaction between a cholinesterase molecule and its substrate involves the attachment of the substrate to the enzyme with the production of an acylated form of enzyme which undergoes rapid hydrolysis to yield re-usable enzyme. Anticholinesterases interfere with this orderly sequence, the point of attack depending on the nature of the inhibitor. They may block (i) the anionic site, (ii) the anionic and esteratic sites, or (iii) the esteratic site, but in almost all the cases the reaction is to some extent similar to that between substrate and enzyme.

The inhibitors of cholinesterases (ChE) may be reversible or irreversible. The reversible inhibitors dissociate readily from the enzyme and can be easily removed (e.g. by dialysis) with full restoration of enzyme activity Or, in other words, there is a reversible equilibrum between enzyme and inhibitor:

$$E + I \rightleftharpoons [EI]$$

In contrast, with irreversible inhibitors there is no such equilibri
and the reaction between enzyme and inhibitor is unidirectional:

$$E + I \longrightarrow [EI]$$

The binding of enzymes and irreversible inhibitors can only be broke
up by the use of 'reactivators' (Wilson, 1951, 1952), causing the
return of enzyme activity.

The reversible inhibitors may be competitive, noncompetitive,
and uncompetitive. Competitive inhibitors compete with the substrate
for the same site of the enzyme and are usually structurally similar
to the substrate. Their presence increases the Km of the substrate
but has no effect on Vmax. Noncompetitive inhibitors combine with
the enzyme at another site different from the active site meant for
the substrate. They do not influence the Km, but decrease Vmax.
Uncompetitive inhibitors combine with the enzyme-substrate complex.

The effect of competitive inhibitors on cholinesterase depends
on numerous factors. These include:

(a) the type of cholinesterase. Some inhibitors are almost
equally active against acetylcholinesterase and pseudocholinesterase,
but most have relatively greater inhibitory effect on pseudocholine-
sterase than on acetylcholinesterase. As already mentioned, the
inherited abnormal variants of pseudocholinesterase are, as a rule,
significantly less sensitive to the inhibitory effects of various
compounds;

(b) the choice of the substrate. The inhibitory effect of
various inhibitors on the hydrolysis of substrates with a low K_m
(high affinity to the enzyme) is less than on those which have a
high Km;

(c) the time of equilibration between enzyme and inhibitor before adding the substrate. The optimal incubation time varies with the type of enzyme and the inhibitor. With pseudocholinesterase it is 10 minutes for dibucaine and about 2 hours for neostigmine;

(d) the relative concentrations of the inhibitor and substrate.. With some compounds, complete inhibition of one enzyme is achieved only by concentrations which cause, in certain species at least, significant ihibition of the other enzyme as well. With other compounds there may be little or no overlap in effective concentrations and it is these compounds which are termed 'selective';

(e) Whether the inhibition is observed in situ or in a homogenate or solution. If the inhibitor is susceptible to detoxication by systems in the liver or other organs, it may prove less toxic than its performance in vitro would suggest. On the other hand, some compounds are metabolized in a way that a relatively weak inhibitor is rendered more potent. The metabolism of Malathion to Malaoxon and Parathion to Paraoxon exemplify just such an increase in toxicity;

(f) the presence of certain reactive compounds or groups in the assay system to which the inhibitor or the substrate may be bound reversibly or irreversibly, thereby leaving only a relatively small fraction available in free form for interaction with the enzyme;

(g) the methods used for the purification of the enzyme. The specific interaction forces in the enzyme protein that are responsible for its conformation can be broken up by placing the enzyme into certain environments. The transition from the active conformation to a 'disordered' random conformation is referred to as denaturation. Denaturation changes the orientation and location of the amino acid

residues and thereby disrupts the steric requirements for catalytic activity; and (h) the pH, ionic stength, and temperature of the assay system. These factors may influence the state of ionisation of the active groups of the enzyme, of the substrate, of the enzyme-substrate complex, and of the inhibitor. They may cause conformational changes of the cholinesterase molecule.

The interaction of cholinesterase and inhibitors depends on the chemical structure of the inhibitor, Inorganic ions, such as F^-, are uncompatitive inhibitors (Cimasoni, 1966; Krupka, 1966). They become attached to sites different from the active centre of cholinesterase and can get adsorbed, not only to the enzyme, but also to the enzyme-substrate complex and interfere with both the acylation and deacylation.

Inhibitors like simple tetraalkylammonium compounds, which contain a quaternary ammonium group but no ester group, are adsorbed only to the anionic site of ChE. The quaternary nitrogen attaches itself to the anionic site so rendering it unavailable to the substrate. This inhibitor-enzyme reaction differs from the substrate-enzyme reaction in that there is no direct reaction with the esteratic site. The orientation and dimensions of the compounds may, however, be such that when the molecule is attached to the anionic site it extends far enough to mask the esteratic site as well. The inhibitors which interact primarily with the anionic site of cholinesterases have a greater inhibitory effect on acetylcholinesterase than on pseudocholinesterase. These findings are in agreement with the observation that the negative charge of the anionic centre of acetylcholinesterase is greater than that of pseudocholinesterase and the quaternary ammonium group is relatively more important for the binding of inhibitors (and also substrates) to acetylcholinesterase

than to pseudocholinesterase (Wilson, 1954).

Neostigmine and other carbamates containing both an ester and a quaternary ammonium group are adsorbed to both the esteratic and anionic sites of cholinesterases (Myers, 1951; Foldes et al. 1958, 1960). The reaction between the enzyme and compounds of this type is qualitatively analogous to the reaction between enzyme and substrate. The inhibitory action arises because of the relative slowness with which the enzyme is regenerated by hydrolysis of the intermediate complex. Some organophosphorus compounds (e.g. echothiophate iodide) also have a quaternary ammonium group and interact with both the esteratic and anionic site. They yield a phosphorylated enzyme as intermediate complex.

The majority of organophosphorus compounds, selenophosphorus compounds, and organosulphonates react with cholinesterases only at the esteratic site. The phosphorylated enzyme intermediate is extremely resistant to hydrolysis. Interference, by steric hindrance, with the adsorption of the ester group of an inhibitor to cholinesterases causes a greater loss of pseudocholinesterase than of acetylcholinesterase activity (Foldes et al., 1958) indicating that binding at the esteratic site is relatively more important for the inhibition of pseudocholinesterase than for that of acetylcholinesterase.

1.12 MUSCLE RELAXANTS.

The history of muscle relaxants dates back to the 16 century when some English travellers reported the use of poisoned arrow-heads by natives of South America. The poison was made from the leaves and roots of a local plant 'curare'. Back in England, the pharmacological effects of curare was investigated and it was found that the poison interfered with nerve function as a neuromuscular

blocker, and the victims had died of asphyxia from paralysis of their respiratory muscles. The refined drug was later employed as a relaxant in inducing insensitivity to pain, but due to its side effects other drugs were developed. The various drugs used as anaesthetic agents differ in their mode of action, but essentially all anaesthetic drugs are tissue poisons.

1.12(i) Nerve - Function

Nerve fibres consist of a cytoplasm encased by a lipoprotein membrane. In the resting state neurons have a negative charge of -50 to -70 mV on the inside of their cell membrane. This charge - called the resting membrance potential - is primarily due to the difference in concentrations of K^+ across the cell membrane, the intracellular concentration being 30 times higher than that of the extracellular fluid. On the other hand while the extracellular concentration of Na^+ is ten times higher than the intracellular concentration, it contributes little to the resting membrance potential; however, its entry into the cell after neuron is excited represents the prime event in the process of depolarization.

Under normal circumstances while the neuron is at rest, both K^+ and Na^+ find the cell membrane relatively impermeable though the latter ion has greater difficulty in penetrating the cell because the calcium ions which are adherent to the cell membrane obstruct the normal pathway of Na^+ through the pores into the cell. When the neuron is excited, the permeability characteristics are altered, allowing the passive but brisk influx of Na^+ into the cell. The rapid accumulation of those positively charged ions inside the cell produces a reversal of membrane polarity. Immediately following the influx

of Na^+, K^+ leave the cell. This rapid alteration of membrane polarity is termed'depolarisation' and is electrically seen as the 'action potential'.

On the arrival of a nerve impulse at the myoneural junction, a motor nerve liberates acetylcholine from the dense projections in the nerve terminal. Acetylcholine crosses the junctional cleft and becomes fixed at lipoprotein receptors on the junctional folds of the end-plate membrane. The motor end-plate is specifically sensitive to acetylcholine and becomes depolariced. This current of depolarization passes along the membrane of the muscle-fibre and is the final stimulus forcausing the contraction of the contractile part of the muscle-fibre. The released acetylcholine is meanwhile hydrolyzed by acetylcholinesterase in the region of the motor end-plate, so that when the excited muscle-fibre has come out of its refractory state it will not become excited again by a depolarized end-plate unless a new nerve impulse has arrived and released a new supply of acetylcholine.

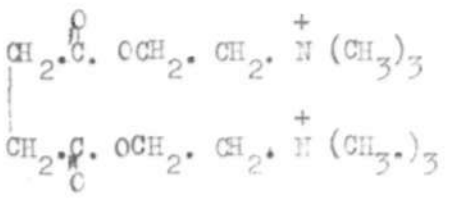
1.12(ii) Neuromuscular Block.

Neuromuscular blocking agents interfere with the transmission of impulses from motor nerves to muscles at the skeletal neuromuscular junction. They may be classified into non-depolarizers and depolarizers.

The non-depolarizers of the myoneural junction (competitive inhibitors of acetylcholine) prevent access of acetylcholine to the receptor protein so that no depolarization, no change in resting potential of the motor end-plate, and no muscular contraction - with consequent paralysis - take place.

Depolarizers cause depolarization of the end plate like that caused by acetylcholine which in this case persists. They do not become bound to receptor sites. The depolarization spreads to the muscle fibres making them electrically unresponsive to subsequent stimuli, even after repolarization is complete. A short-acting depolarizer clinically used is suxamethonium (Bourne et al. 1952).

Suxamethonium is the dicholine ester of succinic acid.



Suxamethonium.

The compound inhibits acetylcholinesterase in addition to its depolarizing effect at the neuromuscular junction (Evans et al. 1952), and the removal of the drug by pseudocholinesterase normally curtails its effect. The enzymatic hydrolysis takes place rapidly in two stages:



A few individuals having low or abnormal pseudocholinesterase activity are unable to hydrolyse the drug quickly and in consequence a prolonged apnea occurs after administration of suxamethonium.

(iii) Local anaesthetics.

Local anaesthetics, render neurons inexcitable; they prevent a stimulus from altering the resting membrane potential sufficiently to cause depolarisation. They are believed to do this by displacing the calcium ions from their binding sites on the cell membrane, thereby obliterating the pores through which influx of sodium occurs

(Oyama and Cohen, 1978). Local anaesthestics further inhibit the release of acetylcholine at the nerve endings and also inhibit the cholinesterases. A typical example is dibucaine (Nupercaine). Figure 1.2.

While dibucaine is not hydrolyzed by pseudocholinesterase, the enzyme has considerable affinity for the anaesthetic. This mutual attraction is put to good use in laboratory tests for certain atypical forms of the esterase which fail to hydrolyze succinylcholine, so causing prolonged apnea. Dibucaine has proven useful for this purpose, as its affinity for typical plasma cholinesterase is some twenty times greater than for the atypical form (Kalow and Genest 1957).

1.13. VARIABILITY OF PSEUDOCHOLINESTERASE LEVELS.

1.13(i) Influence of Physiologic States.

Interesting changes in plasma pseudocholinesterase activity occur in infancy and childhood. At birth and within the first six months the activity is low (McCance et al. 1949; Bleisch and Shwachman, 1954; Brody, 1960) compared with 50 percent of non-pregnant adults (Zsigmond and Downs, 1971). Hutchinson and Widdowson (1951) reported the mean cholinesterase activity level between three and six years to be 30 percent above the adult level, but begins to decrease during the fifth year and continues to do so until the adult level is reached . Zsigmond (1971) observed no sex difference in pseudocholinesterase activity in the first six months. The pseudocholinesterase in young infants showed similar sensitivity to inhibition by dibucaine and succinylcholine as the adult enzyme but was extremely resistant to neostigmine.

Shanor et al. (1961) reported a decrease in plasma cholinesterase activity with age in adult males. There is no difference in activity

of pseudocholinesterase in young and aged females.

Conflicting results have been reported for the influence of age and sex on the pseudocholinesterase activity in normal healthy adults. Whereas no age or sex effect was found in 247 adults (Callaway et al. 1951) and in 120 adults (Vorhaus and Kark, 1953), others have found a conflicting correlation of enzyme activity with age negative for 1,200 Canadians (Kalow and Gunn, 1959) and positive for 1,154 Australians (Properi and Brackenridge, 1976). The mean adult pseudocholinesterase activity using a variety of analytical procedures has been summarized (Wetstone and LaMotta, 1965). Adult males appear to have a higher pseudocholinesterase activity than females (Shanor et al., 1961).

Plasma pseudocholinesterase activity decreases in pregnancy (Shnider, 1965; Hazel and Monier, 1971). This diminished activity probably represents a decreased rate of synthesis in the liver. The activity of the enzyme returns to normal levels within a few weeks after delivery.

1.13(ii) Influence of Pathologic Conditions.

Changes of plasma pseudocholinesterase activity have the greatest diagnostic and prognostic significance in various pathologic conditions of the liver. Thus, for example in acute hepatitis or obstructive jaundice the elevation of the SGOT activity and icterus precedes the fall of pseudocholinesterase activity. Similarly low pseudocholinesterase activity may be the first sign of decreased liver function in incipient cirrhosis of the liver. Low pseudo-cholinesterase activity is also implicated in acute myocardial infarction and decreased liver function caused by right-sided congestive heart failure.

Besides liver dysfunction various other conditions can also

reduce pseudocholinesterase level. These include carcinoma (Vaccarezza and Willson, 1965), eczema (Trotter and Fairburn, 1966), Cancer (Robins et al., 1969), Secondary anaemia (Milstoc and Wolfson, 1972), Malnutrition due to protein poor diet (Bourne et al., 1952) and sickle-cell anaemia (Hilkowitz and Jacobson, 1961). Plasma cholinesterase activity may decrease postoperatively following the transient impairment of liver function caused by anaesthesia, surgery, and impaired nutrition in the immediate postoperative period. Bush (1964) observed a decrease in pseudocholinesterase activity in burnt children. In these patients the reduced enzyme activity affects the rate of enzymatic breakdown of succinylcholine and the effect of this drug is intensified and prolonged at the neuromuscular junction and on the myocardium. There is significant increase in the amount of potassium ions (K^+) released by succinylcholine from the muscle (Schaner et al., 1969). The hyperkalemia combined with the vagotonic effect of the relatively high concentrations of succinylcholine reaching the myocardium may result in a high incidence of cardiac arrest. The incidence of this complication can be decreased by decreasing the dose of succinylcholine from 1.1 mg/kg body weight (Bourne et al., 1952) to 0.4 mg/kg (Gronert et al., 1969).

The majority of conditions which affect pseudocholinesterase cause a fall in activity but kidney diseases tend to cause abnormally high serum levels. Some workers have suggested that the increased level might be due to a general increase of protein synthesis by the liver in response to protein loss resulting from renal failure (Faber, 1943; Levine and Hoyt, 1949, 1950). Raab (1969) postulated that in addition to an increase in synthesis, enzyme might escape from the damaged kidney cells and so add to the level in the

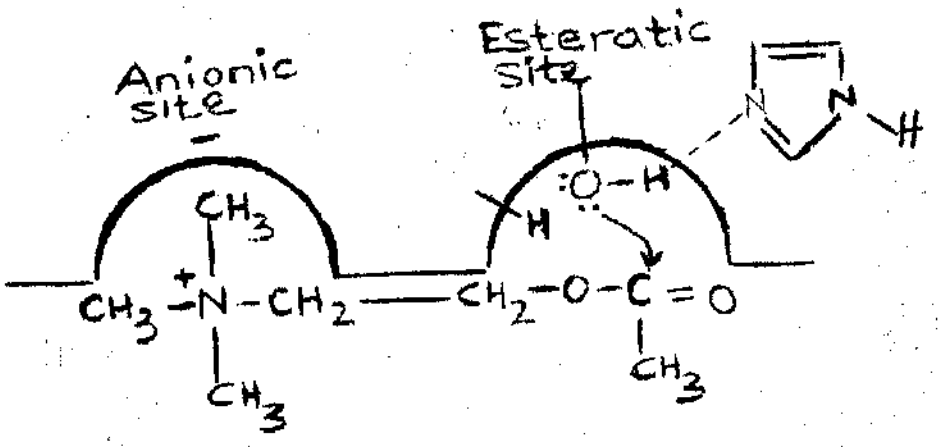
serum.

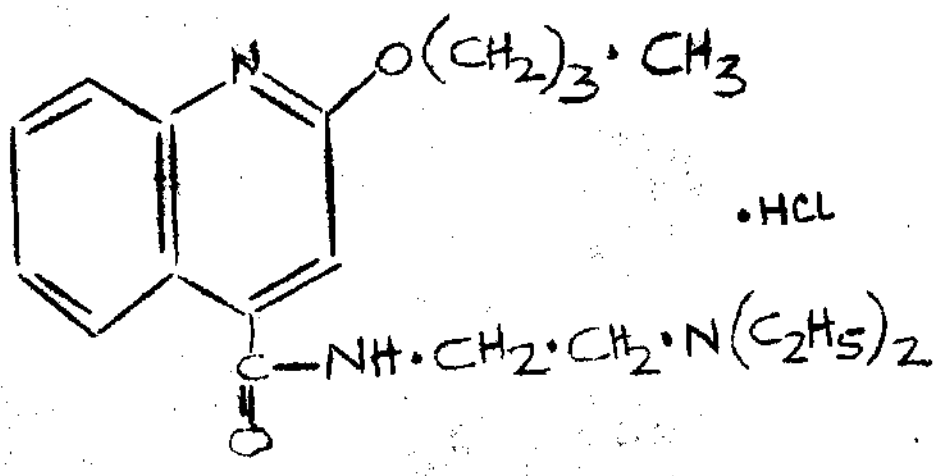
Pseudocholinesterase activity is increased in hyperthyroidism and decreased in hypothyroidism (Thompson and Whittaker, 1965). Mckerracher et al. (1966) reported raised levels of pseudocholinesterase in patients with mental abnormalities.

1.4. PHYSIOLOGICAL FUNCTIONS OF PSEUDOCHOLINESTERASE.

A number of possible roles for pseudocholinesterase have been mooted and for each there is some evidence but for none is this convincing and unequivocal. It was suggested that its primary function is the hydrolysis of compounds that would otherwise inhibit the physiologically important acetylcholinesterase (Lehmann et al., 1961). Pseudocholinesterase achieves this by its ready availability in the liver, through which ingested, and in the plasma, through which both ingested and parenterally administered inhibitors pass in the course of their distribution to various parts of the body. The fact that the affinity of eserine and most other inhibitors to pseudocholinesterase is greater than to acetylcholinesterase increases its ability to protect the functionally important acetylcholinesterase. Thus, for example, in patients treated for glaucoma (Pantuck, 1966) with ecothiopate, plasma pseudocholinesterase may be completely inhibited without any sign or sympton of anti-cholinesterase intoxication.

Other functions proposed for the enzyme include its involvement in assimilation of food (Gorebtzoff, 1959), in the destruction of butyryl choline formed by butyryl-CoA in the course of fatty acid metabolism in the liver (Clitherow et al., 1963), in the maintenance of the optimal Ch: ACh balance in plasma (Funnell and Oliver 1965), in lipid metabolism in the liver (Clitherow et al., 1963; Ballantyne, 1968) and in the regulation of tissue growth (Ballantyne and Burwell, 1965).


Anionic site
Esteratic site
CH3
CH3 -N- CH2
CH3
CH2-O-C=O
CH3
:O-H
H
N
N
H


N
O(CH2)3·CH3
·HCl
C-NH·CH2·CH2·N(C2H5)2
O

CHAPTER TWO

MATERIALS AND METHODS

2.1 THE SUBJECTS STUDIED.

All subjects have been described in the appendix, and have been grouped into four, (table 2.1) based on related ethnic background. The group other Northerners include Fulanis, Kanuri, Nupes, Tivs, Idomas, Igalas, and Southern Zarians. The Westerners include the Yorubas and Binis. The Easterners include Ibos, Eket and Ijaws. Unlike the Hausas, these other ethnic groups were not sufficient for statistical analysis.

The population consisted of 202 blood donors, apparently normal people as examined by the Department of Haematology before blood was taken from them (such people have been used by researchers as controls), and 28 patients with sickle-cell disease.

After studying the two groups of subjects, sicklers and controls, independently, all their data were pooled together for the purpose of studying the prevalence of the atypical and other genotypes in Zaria.

Approval for this work was obtained from the Medical Ethical Committee of the Ahmadu Bello University, Zaria.

2.2. CHEMICALS AND REAGENTS.

The following chemicals used for analysis were obtained from Sigma Chemical Company, St. Louis, MO. U.S.A; benzoylcholine; and ibucaine free base (2-butoxy-N-[2-diethyl aminoethyl] cinchoninamide).

The following chemicals were obtained from Hopkins and Williams, Chadwell Heath, Essex England: di sodium hydrogen orthophospheric acid (Na_2HPO_4); and sodium dihydrogen orthophosphoric acid ($NaH_2PO_4.\ H_2O$).

Sodium fluoride was obtained from Carlo Erba, Division Chemica, Milano, Italy. Other standard laboratory reagents used were obtained either from M and B, Dagenham England, or from B.D.H., Poole, England, and were of analytical grade.

2.3 REAGENTS PREPARATION

All weighings were done with Mettler P.C. 440 balance (Made in Switzerland), and the pH meter used was obtained from Pye Unican Ltd., England.

Reagents were prepared as follows:-

(a) 0.05M sodium phosphate buffer pH 7.4, 6.07g disodium hydrogen orthophosphoric acid and 2.00g sodium dihydrogen orthophosphoric acid were dissolved in some distilled water in one litre flask. The volume was made up to the litre mark with more of the distilled water.

(b) 1mM substrate benzoylcholine chloride, 24.4mg was dissolved in 100ml of distilled water.

(c) 0.2mM dibucaine inhibitor stock solution, 68.7mg dibucaine (free base) was dissolved in 10ml of distilled water and one ml taken from this stock solution was diluted to 100ml with distilled water.

(d) 1mM sodium fluoride inhibitor stock solution, 42mg sodium fluoride was dissolved in 10ml of distilled water and one ml taken from this stock solution was diluted to 100ml with distilled water.

2.4. SAMPLE PREPARATION

Blood samples were obtained from donors at the blood transfusion centre and from patients at the sickle-cell clinic, Ahmadu Bello University Teaching Hospital Zaria, without selection.

About 5ml of blood was taken from the cubital vein of each subject and shared into two sample tubes, one without any anticoagulant and the other with anticoagulant E.D.T.A. (sodium salt). The blood samples in tubes containing E.D.T.A. were used for ABO-Rh blood typing and Hb electrophoresis (Dacie and Levis, 1975; Fleming, 1983).

The blood in tubes without anticoagulant were allowed to clot for 3 hours at room temperature and later centrifuged at 3,000 rpm for 30 minutes at 10°C, using a clinical centrifuge. The supernatant sera were collected and analysed within 48 hours after storage at - 17°C. Preliminary investigation revealed that the samples are stable for 7 days.

2.5. ENZYME ASSAY, DETERMINATION OF DIBUCAINE AND FLUORIDE NUMBERS.

The samples obtained as described above were assayed for pseudocholinesterase activity by the method of Kalow and Lindsay (1955) as modified by Foldes (1978).

ASSAY PRINCIPLE

Certain aromatic esters absorb maximally at longer wavelengths in the ultraviolet region and have high absorbance compared with the hydrolysis products which absorb maximally at shorter wavelengths in this region. Thus, benzoylcholine has a greater absorbance in the ultraviolet than its split products.

Benzoylcholine $\xrightarrow{ChE}$ Choline + Benzoic acid.

Benzoylcholine is hydrolysed at a great velocity by ChE, and the disappearance of the absorption of this ester at 246 nm was followed, which gave rate curves with each assay.

Prior to use, the sera samples were diluted 10-fold in the phosphate buffer. Table 2.2 summarises the composition of the assay mixtures. All measurements were performed manually in a Beckmann Spectrophotometer (model DB-GT) at 246nm using silica cuvettes of 10mm light path, with 3ml minimum volume. The assay mixtures were incubated at 37°C in a

waterbath with $\pm$ 0.1° error.

A substrate blank was used to zero the instrument. Thereafter the substrate was added to the experimental tube at zero time. The mixture was poured once back and forth in the test tube, while the mouth was covered with cellophane paper, then filled into an absorption cell, and the measurement started 30 seconds after the mixing. The change of optical density (extinction coefficient) was monitored for 5 minutes at 30 seconds interval using a stop watch.

Similar procedure was used to determine the activity in the presence of inhibitors. In this case the enzyme and inhibitor were allowed to equilibrate for 10 minutes before the substrate was added. The inhibitor disturbed the substrate blank, so an inhibited blank was used to zero the instrument.

2.6 EVALUATION AND UNITS.

The data obtained for each serum sample were plotted on graph paper as decrease of absorbance per five minutes. The best fitting straight line was drawn in the linear part of the graph and the slope of the line gave the enzyme activity. The micromoles of benzoylcholine hydrolysed per minute is one I.U. Results are expressed as I.U. per litre of serum.

$$\text{ie., I.U/litre serum} = \frac{\Delta E}{720} \times \frac{1}{t} \times 50 \times 1000$$

In the above equation, ΔE is the observed change of extinction coefficient at 246nm; t is the time in minutes during which the measured change of the extinction coefficient occured; 50 is the factor that converts the results from the 0.02ml serum present in the reaction mixture to one ml; 1000 is the factor that converts the results from one ml serum to one litre; and 720 is the change in the extinction coefficient caused by the hydrolysis of one micromole of benzoylcholine.

The dibucaine numbers (DN) and Fluoride numbers (FN.) were calculated as

$$\frac{\text{uninhibited rate - inhibited rate}}{\text{uninhibited rate}} \times 100$$

The method of Richterich as cited by Van Ros and Vervoort (1973) was followed for classifying dibucaine numbers. With this method the U phenotype gives dibucaine numbers above 60, the I phenotype between 30 and 60 and the A phenotype below 30, all determined at 37°C. Fluoride inhibition studies were also done at 37°C. On the basis of parallelism with dibucaine numbers, it was considered justifiable to classify U phenotype for fluoride numbers above 40, the UF phenotype between 20 and 40, and the F phenotype below 20. The procedure of Whittaker (1980) for phenotyping was followed, employing a combination of dibucaine and sodium fluoride as differential inhibitors.

Table 2.1. PERCENTAGE COMPOSITION OF THE ETHNIC GROUPS IN THE POPULATION STUDIED.

Description		Percentage subjects	
		Apparently normal (control)	Sickle-cell patients
1. Hausa	Males	74.26	46.43
	Females	-	14.29
2. Other Northern			
	Males	14.36	17.86
	Females	-	7.14
3. Western	Males	8.42	7.14
	Females	-	3.57
4. Eastern	Males	2.97	3.57
	Females	-	-

Table 2.2. COMPOSITION OF PSEUDOCHOLINESTERASE ASSAY MIXTURE

Reagent	Substrate blank (ml)	Normal reaction (ml)	Inhibited substrate blank (ml)	Inhibited reaction (ml)	Final concentration in assay mixture
Phosphate buffer (0.05M)	2.8	2.8	2.8	2.8	35mM
Diluted serum	0.2	0.2	0.2	0.2	0.02mL
Distilled Water	1.0	0.8	0.8	0.6	
Inhibitor: Bibucaine (0.2ml)	-	-	0.2	0.2	10µm
Inhibitor: NaF(1mM)	-	-	0.2	0.2	50µm
Substrate BeCh (1mM)	-	0.2	-	0.2	50µm

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 REPRESENTATIVE RESULT.

Table 3.1 shows some spectrophotometric readings for benzoylcholine hydrolysis obtained during assay of a serum sample in the absence and presence of inhibitor. Figure 3.1. shows plots of the data to determine pseudocholinesterase activity in the representative serum before and after inhibition.
The dibucaine number, 75.83 and fluoride number, 51.66 calculated as described in chapter two show the enzyme to be the typical type of ChE. Detailed result obtained for the population is presented in appendix one. The data agree with findings by other workers that the DN and FN did not depend on esterase level (Kalow and Genest, 1957; Kalow and Staron, 1957; Lehmann and Liddell, 1969; and Whittaker, 1980).

3.2 DISTRIBUTION OF ACTIVITY IN A POPULATION

The frequency distribution of ChE activity in the population studied is shown in table 3.2. The distribution for the control (apparently normal) subjects and sickle-cell patients are represented in Figures 3.2(a) and (b) respectively. The distribution pattern is similar for both groups of subjects. The control subjects had a mean activity value of 0.85 $\pm$ 0.33 I.U./L, while the sickle-cell patients had a mean value of 0.80 $\pm$ 0.18 I.U./L. The 5.88 percent difference was not statistically significant ($t = 0.77$, $tp0.95 = 1.645$). However, it should be noted that sickle-cell patients had a lower activity mean than the control subjects. Such finding had been

Table 3.1. REPRESENTATIVE SPECTROPHOTOMETRIC READINGS OBTAINED DURING ASSAY OF SERUM SAMPLE FOR ChE ACTIVITY AT 246nm.

Time (Secs.)	Control	+ Dibucaine	+ NaF
30	0.190	0.210	0.193
60	0.180	0.208	0.191
90	0.170	0.206	0.188
120	0.161	0.204	0.185
150	0.153	0.202	0.180
180	0.145	0.200	0.174
210	0.140	0.198	0.170
240	0.130	0.196	0.165
270	0.122	0.194	0.163
300	0.115	0.192	0.160
ChE Activity (I.U./L.)	1.15	0.26	0.56
D.N.	-	75.83	-
F.N.	-	-	51.66

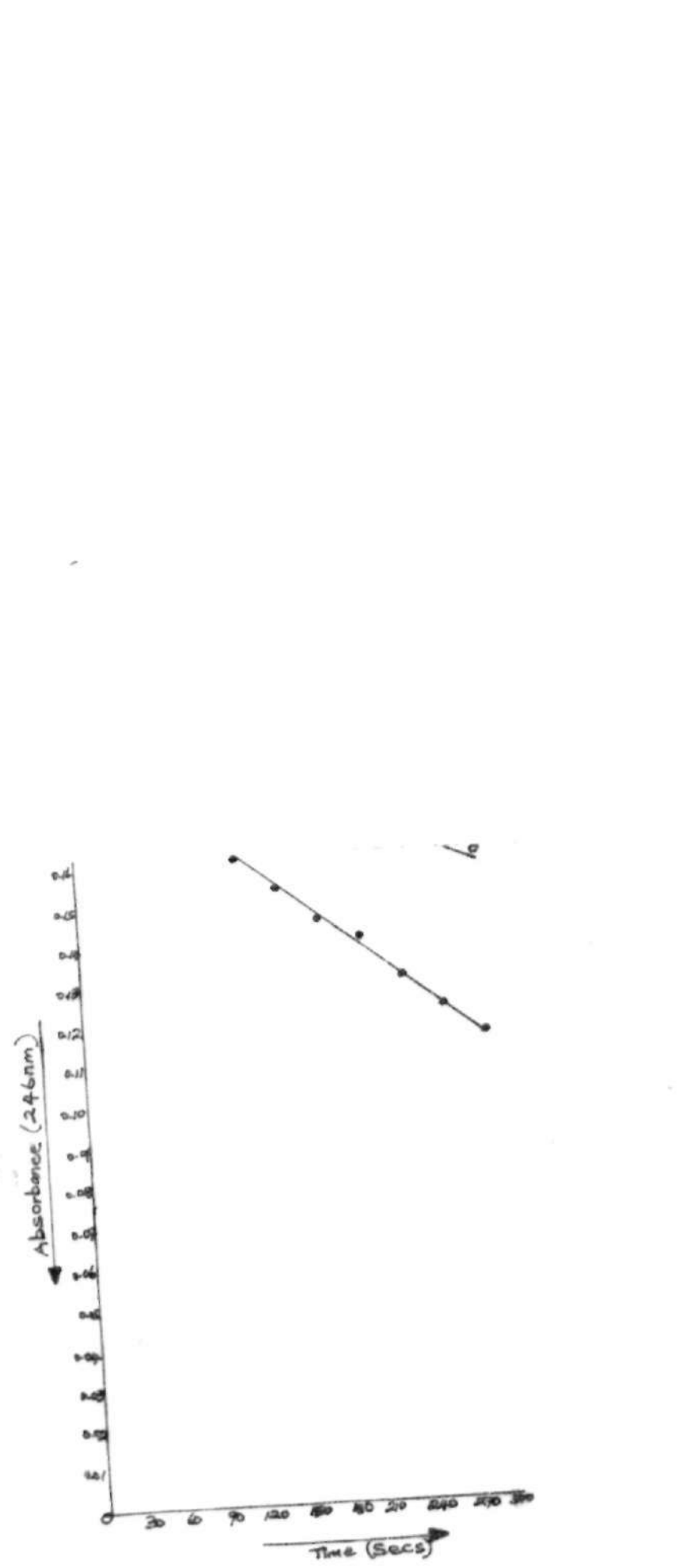

Absorbance (246nm)
Time (Secs)
KEY :
Dibucaine - inhibited enzyme.
Fluoride - inhibited enzyme.

Table 3.2. FREQUENCY DISTRIBUTION OF UNINHIBITED PSEUDOCHOLINESTERASE ACTIVITY.

ChE activity (I.U./L.)	Frequency Percentage		
	Control Subjects	Sickle-Cell Patients	Total Population
0.17	0	0	0
0.17 - 0.33	1(2)	0	0.9 (2)
0.34 - 0.50	6.4(13)	7.4 (2)	6.5(15)
0.51 - 0.67	12.9(26)	10.7 (3)	12.6(29)
0.68 - 0.83	30.2(61)	46.4(13)	32.2(74)
0.84 - 1.0	24.3(49)	21.4(6)	23.9(55)
1.01 - 1.17	11.9(24)	10.7(3)	11.7(27)
1.18 - 1.33	7.4(15)	3.6(1)	7.0(16)
1.34 - 1.50	4.5(9)	0	3.9(9)
1.51 - 1.67	1 (2)	0	0.9(2)
1.68 - 1.83	0.5(1)	0	0.4(1)
Total	(202)	(28)	(230)
Percentage of grand total	87.8	12.2	100
Activity range	1.53	0.72	1.53
Mean ChE Activity	0.85 ± 0.33	0.80 ± 0.18	0.85 ± 0.27

* Figures in parenthesis indicate the actual number of subjects.

Control Vs. Sicklers: t = 0.77

tpo.95 = 1.645.

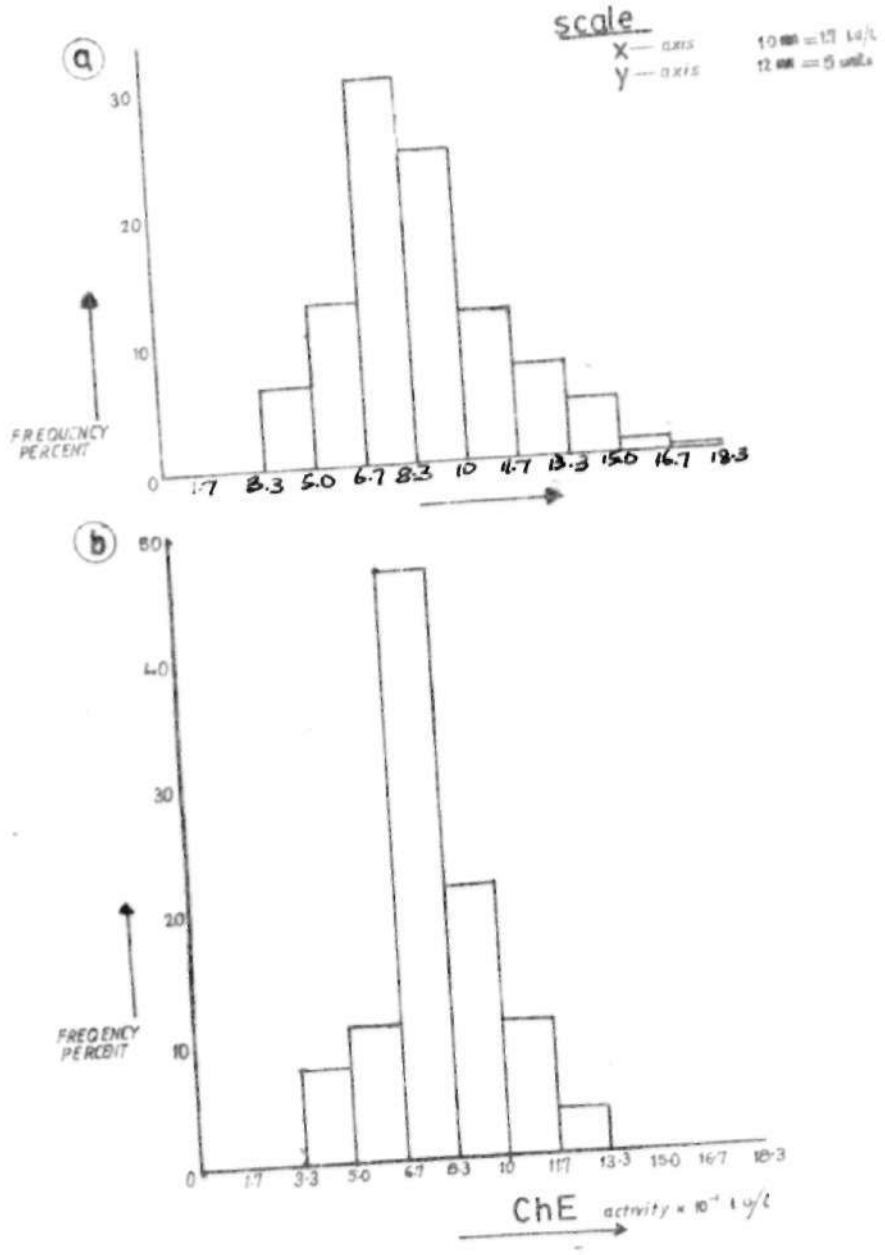

scale
x — axis
y — axis
a
30
20
10
0
FREQUENCY PERCENT
1·7 3·3 5·0 6·7 8·3 10 11·7 13·3 15·0 16·7 18·3
b
40
30
20
10
0
FREQENCY PERCENT
1·7 3·3 5·0 6·7 8·3 10 11·7 13·3 15·0 16·7 18·3
ChE activity

Table 3.3. FREQUENCY DISTRIBUTION OF PSEUDOCHOLINESTERASE ACTIVITY IN RELATION TO BLOOD GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

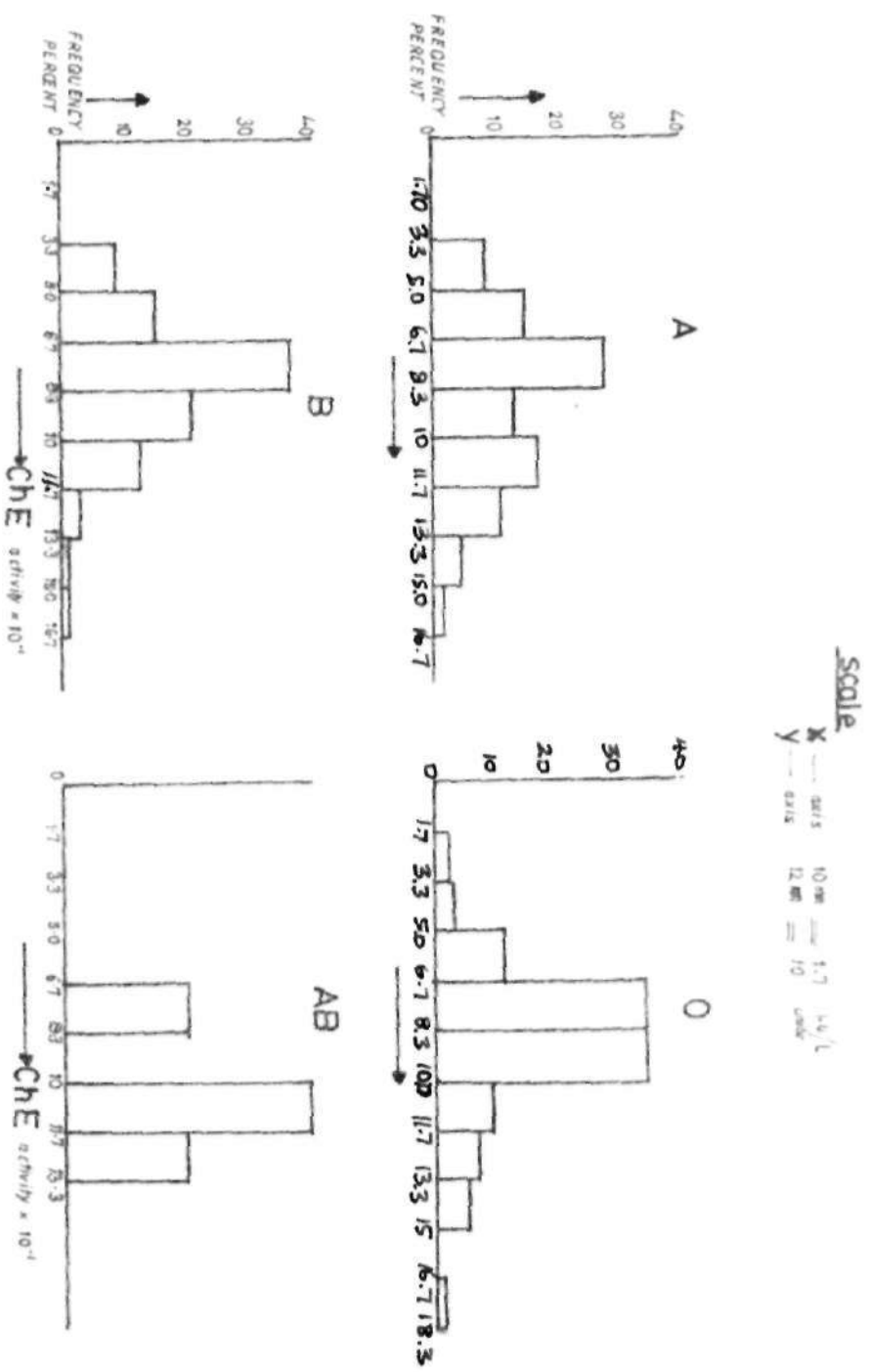
ChE activity (I.U./L.)	Frequency Percent				Total
	A	B	AB	O	
0.17	0	0	0	0	0
0.17-0.33	0	0	0	2.1(2)	0.9(2)
0.34-0.50	0.3(5)	9.5(7)	0	3.1(3)	6.5(15)
0.51-0.67	14.8(8)	14.9(11)	0	10.3(10)	12.6(29)
0.68-0.83	27.8(15)	36.5(27)	20(1)	32(31)	32.2(74)
0.84-1.0	13(7)	21.6(16)	20(1)	32(31)	23.9(55)
1.01-1.17	16.6(9)	12.2(9)	0	9.9(9)	11.7(27)
1.18-1.33	11.11(6)	2.7(2)	40(2)	62(6)	7.0(16)
1.34-1.50	5.6(3)	1.4(1)	20(1)	4.1(4)	3.9(9)
1.51-1.67	1.9(1)	1.4(1)	0	0	0.9(2)
1.68-1.83	0	0	0	1.0(1)	0.4(1)
Total	(54)	(74)	(5)	(97)	(230)
Percentage of grand total	23.5	32.2	2.2	42.2	100
Mean activity	0.87±0.28	0.80±0.21	1.13±0.27	0.86±0.25	0.85±0.27

* Figures in parenthesis indicate the actual number of subjects.

(i) Mean blood group O Vs. A: t = 0.23
tp0.95 = 1.71

(ii) Mean blood group O Vs. B: t = 1.65
tp0.95 = 1.645

(iii) Mean blood group O Vs. AB: t = 2.33
tp0.95 = 1.66



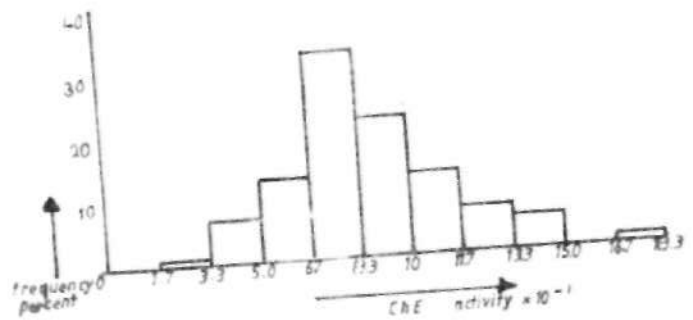
* Figures in parenthesis indicate the actual number of subjects.

(i) Genotype AA Vs. AS/AC: t = 0.24
tp0.95 = 1.645

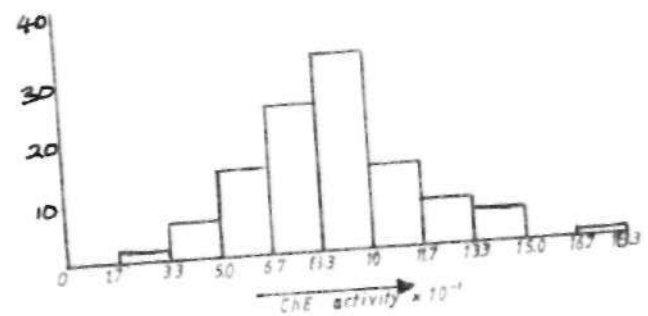
(ii) Genotype AA Vs. SS/SS+F: t = 1.2
tp0.95 = 1.645

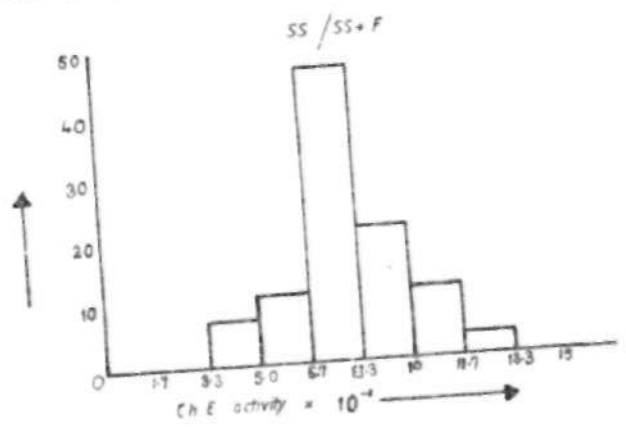
scale
x-axis 10 mm = 1.7 Iu/l
y-axis 12 mm = 10 units

AA



AS AC





t = 0.91

$t_{x0.95} = 1.66$

(iv) 21-30 Vs. 41-50:

Table 3.6. FREQUENCY DISTRIBUTION OF PSEUDOCHOLINESTERASE ACTIVITY IN RELATION TO ETHNIC GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

ChE activity (I.U./L)	Frequency percent Ethnic group				Total
	Hausa	Other North	West	East	
0.17	0	0	0	0	0
0.17-0.33	0	2.8(1)	5(1)	0	0.9(2)
0.34 - 0.50	7.2(12)	2.8(1)	5(1)	14.3(1)	6.5(15)
0.51 - 0.67	13.2(22)	11.11(4)	10(2)	14.3(1)	12.6(29)
0.68 - 0.83	33.5(56)	36.1(13)	15(3)	28.6(2)	32.2(74)
0.84 - 1.0	24(40)	19.44(7)	30(6)	28.6(2)	23.9(55)
1.01 - 1.17	10.2(17)	19.44(7)	15(3)	0	11.7(27)
1.18 - 1.33	7.8(13)	2.8(1)	10(2)	0	7.0(16)
1.34 - 1.50	2.4(4)	5.6(2)	10(2)	14.3(1)	3.9(9)
1.51 - 1.67	1.2(2)	0	0	0	0.9(2)
1.68 - 1.83	0.6(1)	0	0	0	0.4(1)
Total	(167)	(36)	(20)	(7)	(230)
Percentage of grand total	72.6	15.7	8.7	3.0	100
Mean activity	0.85±0.25	0.85±0.27	0.94±0.30	0.80±0.28	0.85±0.27

* Figures in parenthesis indicate the actual number of subjects.

Other Northerner Vs. Hausa: $t = 0$

$tp0.95 = 1.645$

Other Northerner Vs Westerner: $t = 1.12$

$tp0.95 = 1.67$

Other Northerner Vs Easterner: $t = 1.56$

$tp0.95 = 1.68$

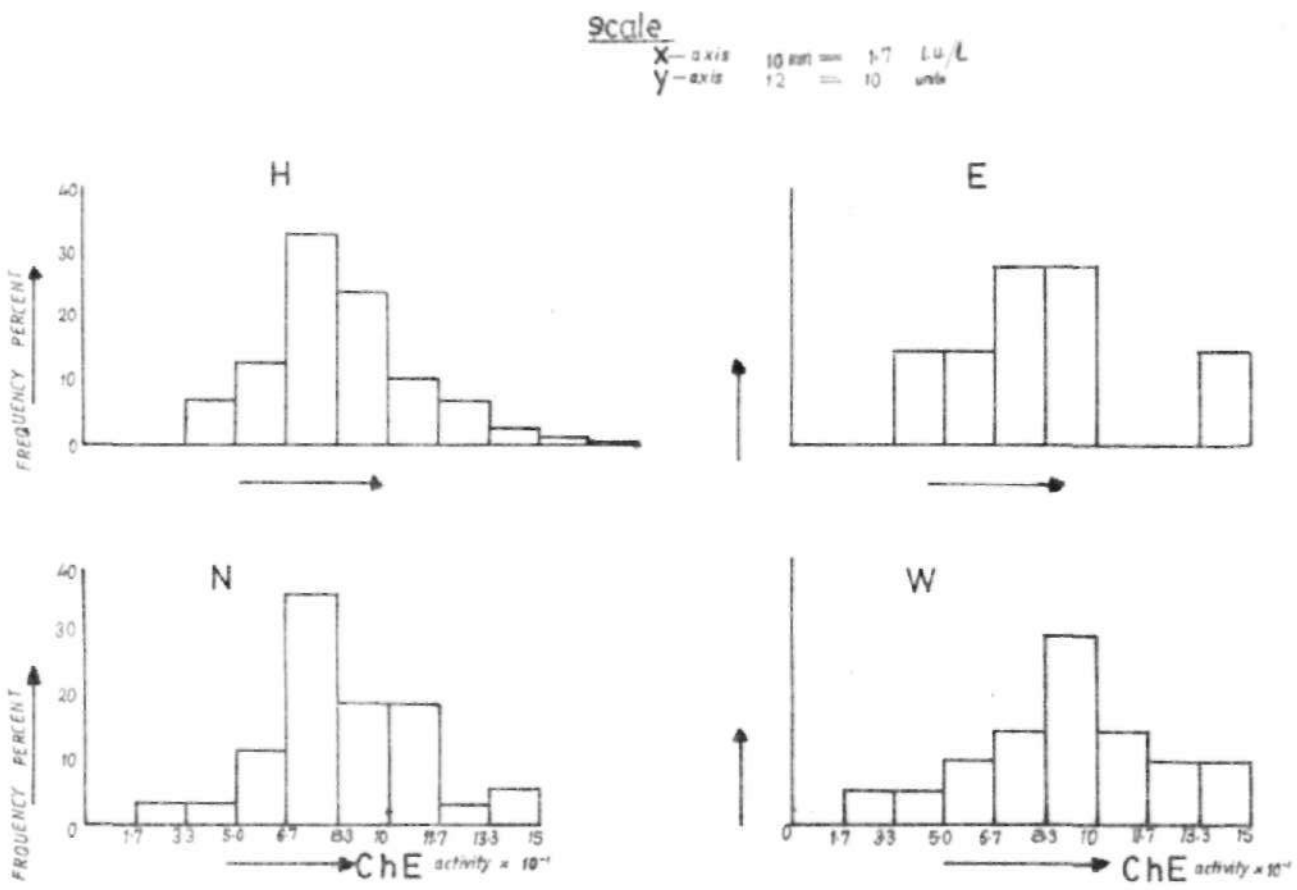
Table 3.6 Figure 3.6 FREQUENCY PERCENTAGE DISTRIBUTION OF PSEUDOCHOLINESTERASE ACTIVITY IN RELATION TO ETHNIC GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

H = Hausa

N = Other Northerner

W = Westerner

E = Easterner


Scale
x-axis 10 mm = 1·7 Lu/L
y-axis 12 = 10 units
H
E
N
W
FREQUENCY PERCENT
0
10
20
30
40
1·7
3·3
5·0
6·7
8·3
10
11·7
13·3
15
ChE activity x 10⁻¹

in these age groups (54.4 percent). The higher activity observed for the age group 41 - 50 (0.93 ± 0.27) is due to small number of subjects involved (5.2 percent of the population). The data of table 3.5. does not reveal any significant difference between the various age groups (t=0.24 to 1.62, tp0.95 1.66) inspite of the variations in mean activity.

Table 3.6 and Figure 3.6 show the distribution of ChE activity in relation to Ethnic group. The 0.80 ± 0.20 mean activity found for Easterners is lower than, and the 0.94 ± 0.3 for Westerners higher than, but not significantly different from (t = 1.2, tp0.95 1.67 for Easterners, and t = 1.56, tp0.95 1.68 for Westerners) the 0.85 ± 0.25 for Kausas and 0.85 ± 0.27 for other Northerners.

3.4 DISTRIBUTION OF DIBUCAINE NUMBERS.

The distribution of dibucaine numbers is shown in table 3.7 and represented in figure 3.7(a) for control subjects and (b) for sickle-cell patients. No difference was observed in the mean values for the control subjects (75.44 ± 7.42) and Sicklers (74.5 ± 5.85) (t = 0.6, tp0.95 = 1.645). Altogether, 226 sera (98.26 percent of the population) contained the 'usual' or 'typical' type of enzyme. Of these, 198 sera from control subjects (representing 98.02 percent) and 28 sera from sickle-cell patients (representing 100 percent) were involved. The 'usual' enzyme was described by a mean of 76.01 ± 6.41 for controls and 74.5 ± 5.58 for sicklers. The difference of 1.99 percent in the occurrence of 'usual' type of enzyme in the two groups of subjects was not sifnificant (t = 1.18, tp0.95 = 1.645). It should be noted that both male and female sickle-cell patients had the same mean D.N.

Table 3.7. FREQUENCY DISTRIBUTION OF DIBUCAINE NUMBER.

Dibucaine number	Frequency percentage		Total Population
	Control subjects	Sickle-cell patients	
0 - 9	0	0	0
10 - 19	0	0	0
20 - 29	0	0	0
30 - 39	0	0	0
40 - 49	0.5(1)	0	0.4(1)
50 - 59	1.5(3)	0	1.3(3)
60 - 69	13.4(27	17.9(5)	13.9(32)
70 - 79	56.9(115)	64.3(18)	57.8(133)
80 - 89	27.2(55)	17.9(5)	26.1(60)
90 - 99	0.5(1)	0	0.4(1)
Total	(202)	(28)	(230)
Percentage of grand total	87.8	12.2	100
Range of D.N.	44.84	24.51	44.84
Mean	75.44±7.42	74.5±5.85	75.33±7.27
Mean for 'usual' enzyme	76.01±6.41	74.5±5.85	75.82±6.14
Mean for 'I-type' enzyme	52.0±4.33	-	52.0±4.33

* Figures in parenthesis indicate the actual number of subjects.

Control Vs. Sickle-cell patients: $t = 0.6$

$tp_{0.95} = 1.645$

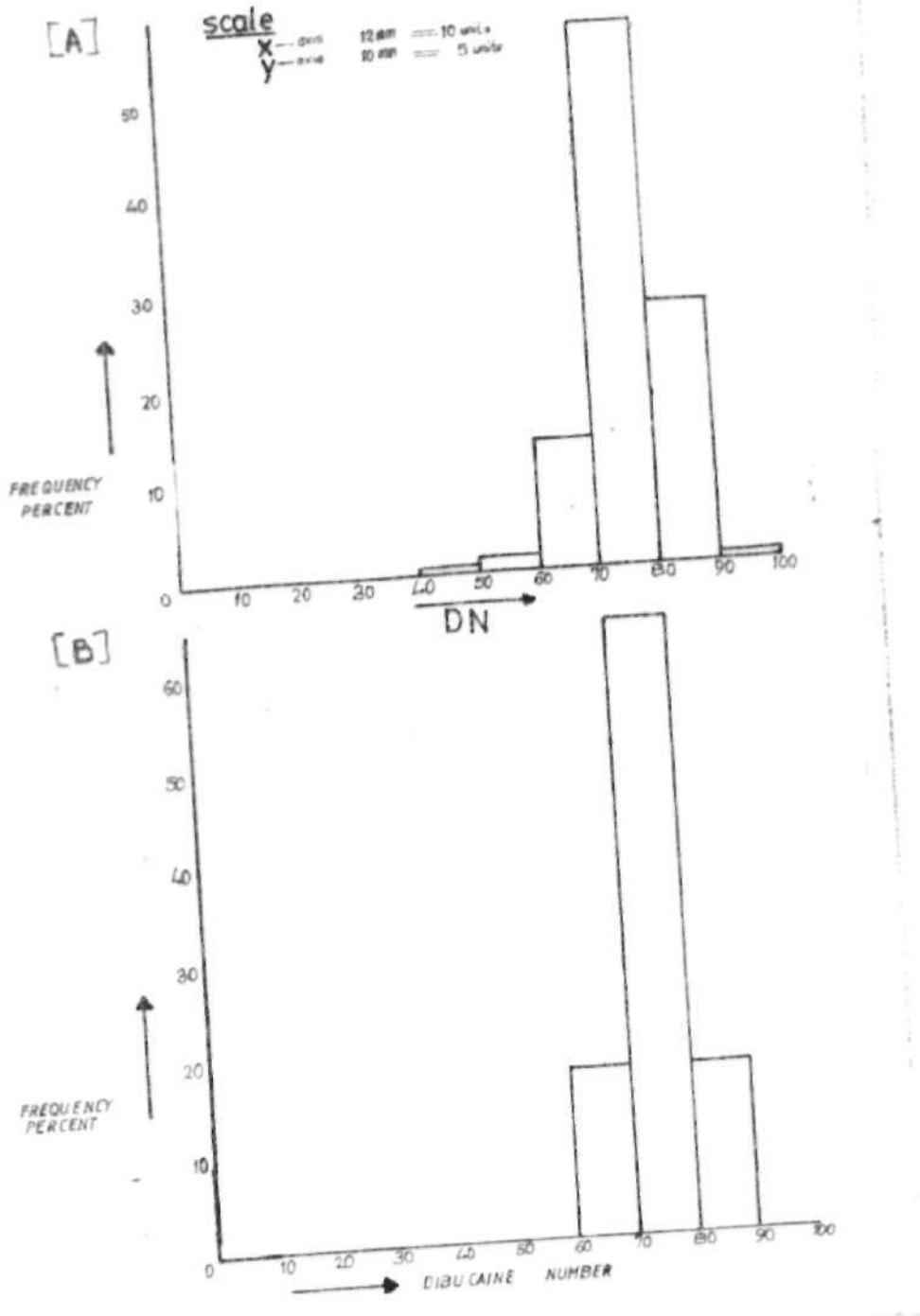

[A]
scale
x — axis 12 mm ==== 10 units
y — axis 10 mm ==== 5 units
50
40
30
20
10
0
FREQUENCY PERCENT
10 20 30 40 50 60 70 80 90 100
DN
[B]
60
50
40
30
20
10
0
FREQUENCY PERCENT
10 20 30 40 50 60 70 80 90 100
DIBUCAINE NUMBER

Table 3.8. FREQUENCY DISTRIBUTION OF DIBUCAINE NUMBER IN RELATION TO BLOOD GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

Dibucaine number	Frequency percentage Blood group				TOTAL
	A	B	AB	O	
10	0	0	0	0	0
10-19	0	0	0	0	0
20 - 29	0	0	0	0	0
30 - 39	0	0	0	0	0
40 - 49	0	0	0	1.0(1)	0.4(1)
50 - 59	1.9(1)	0	0	2.1(2)	1.3(3)
60 - 69	9.3(5)	16.2(12)	0	15.5(15)	13.9(32)
70 - 79	64.8(35)	58.1(43)	80(4)	52.6(51)	57.8(133)
80 - 89	22.2(12)	25.7(19)	20(1)	28.9(28)	26.1(60)
90	1.9(1)	0	0	0	0.4(1)
Total	(54)	(74)	(5)	(97)	(230)
Percentage of grand total	23.5	32.2	2.2	42.2	100
Mean D.N.	75.8±6.68	75.3±6.73	76.76±4.16	75.02±8.04	75.33±7.27

* Figures in parentheses represent the actual number of subjects.

(i) Blood group B Vs. AB: t = 0.47
tp0.95 = 1.66

(ii) Blood group B. Vs. A: t = 0.41
tp0.95 = 1.645

(iii) Blood group B Vs. O: t = 0.025
tp0.95 = 1.645

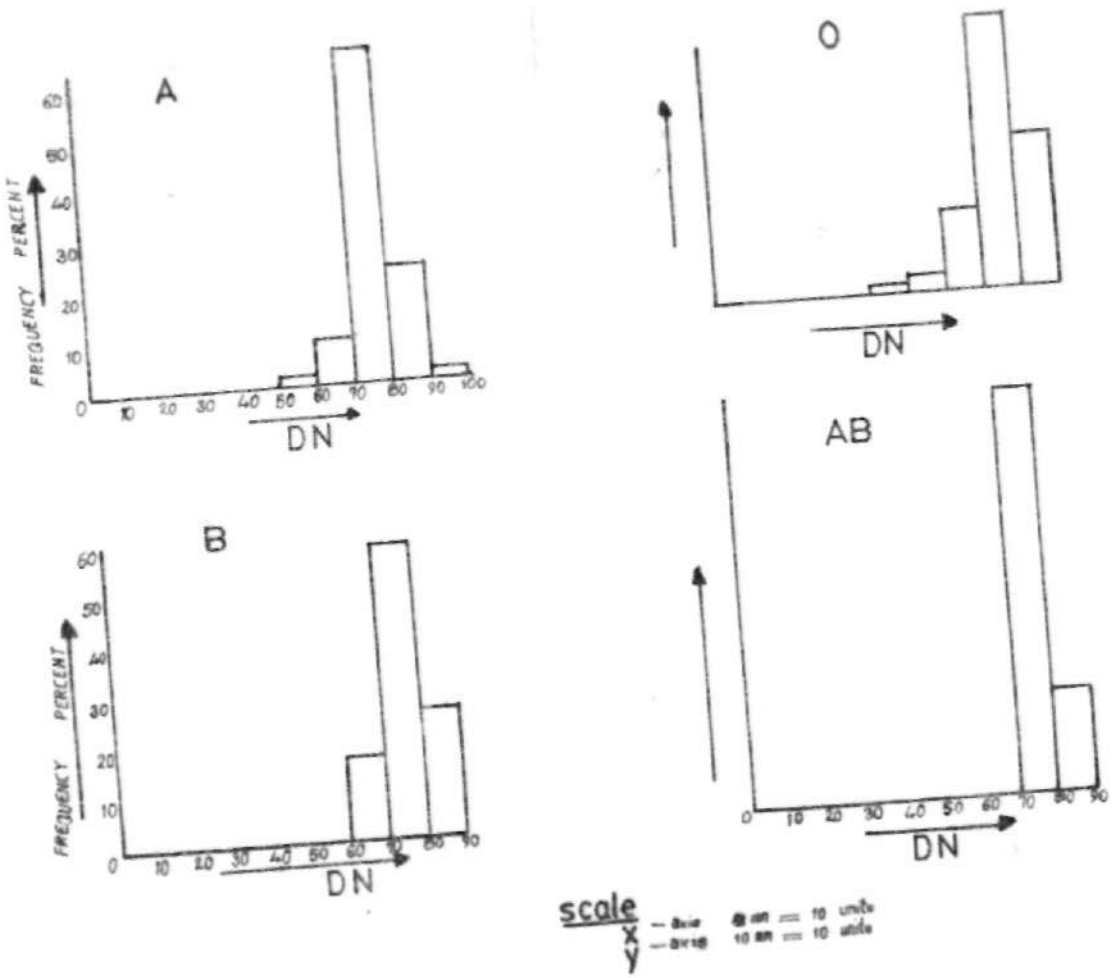

A
FREQUENCY PERCENT
0
10
20
30
40
50
60
10 20 30 40 50 60 70 80 90 100
DN
O
DN
B
FREQUENCY PERCENT
0
10
20
30
40
50
60
10 20 30 40 50 60 70 80 90
DN
AB
0 10 20 30 40 50 60 70 80 90
DN
scale
x
y

Table 3.9. FREQUENCY DISTRIBUTION OF DIBUCAINE NUMBER IN RELATION TO GENOTYPE OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

Dibucaine number	Frequency percentage genotype			Total
	AA	AS/AC	SS/SS+F	
10	0	0	0	0
10 - 19	0	0	0	0
20 - 29	0	0	0	0
30 - 39	0	0	0	0
40 - 49	0	2(1)	0	0.4(1)
50 - 59	2(3)	0	0	1.3(3)
60 - 69	13.2(20)	13.7(7)	17.9(5)	13.9(32)
70 - 79	57.6(87)	54.9(28)	64.3(18)	57.8(133)
80 - 89	27.2(41)	27.5(14)	17.9(5)	26.1(60)
90 - 99	0	2(1)	0	0.4(1)
Total	(151)	(51)	(28)	(230)
Percentage of grand total	67.7	22.2	12.2	100
Mean D.N.	75.36±7.18	75.68±8.08	74.5±5.98	75.33±7.27

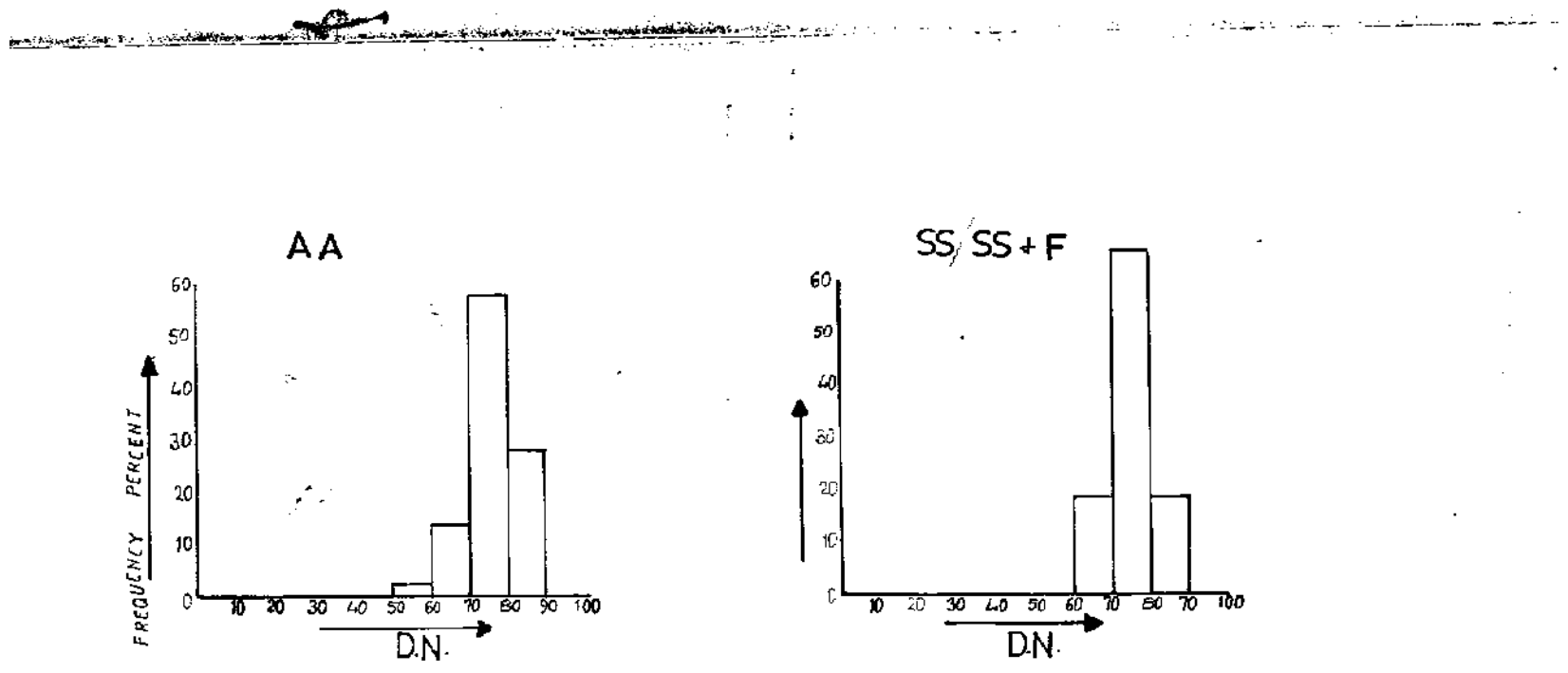
* Figures in parenthesis indicate actual number of subjects.

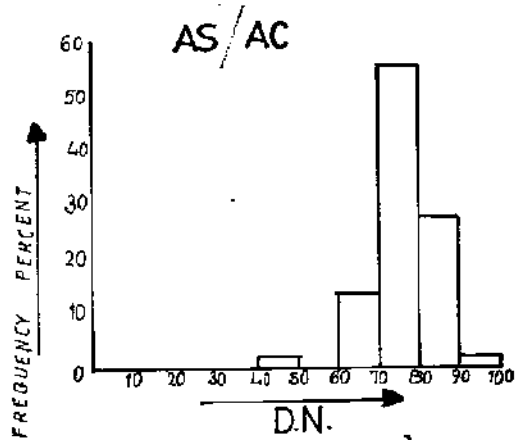
Genotype AA Vs. AS/AC: t = 0.26

tp0.95 = 1.645

Genotype AA Vs. SS/SS+F: t = 0.82

tp0.95 = 1.645





scale

x — axis 8mm = 10 units

y — axis 10 mm = 10 units

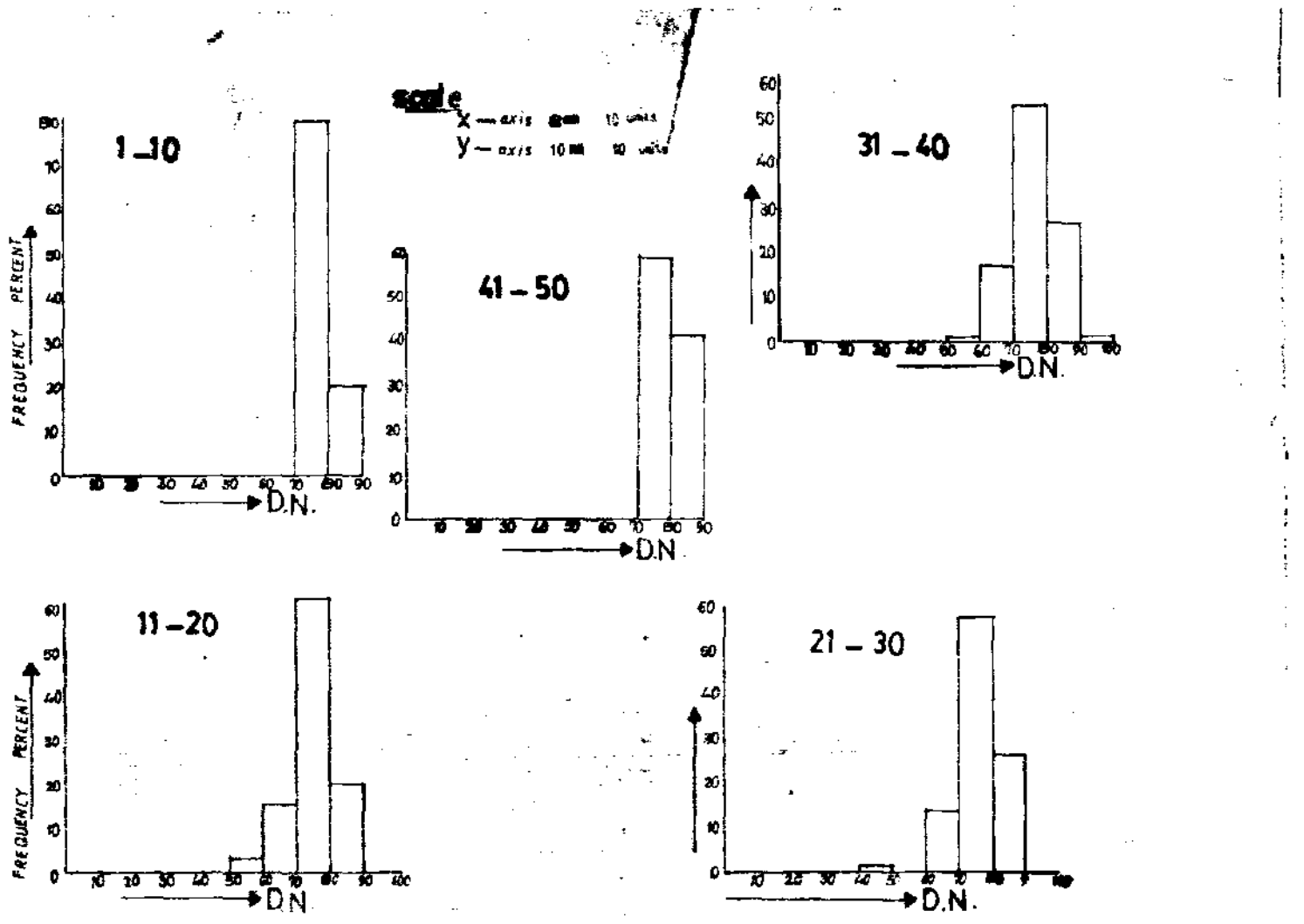

scale
x — axis
y — axis
1 – 10
11 – 20
21 – 30
31 – 40
41 – 50
FREQUENCY PERCENT
D.N.

Table 3.11; FREQUENCY DISTRIBUTION OF DIBUCAINE NUMBER IN RELATION TO ETHNIC GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

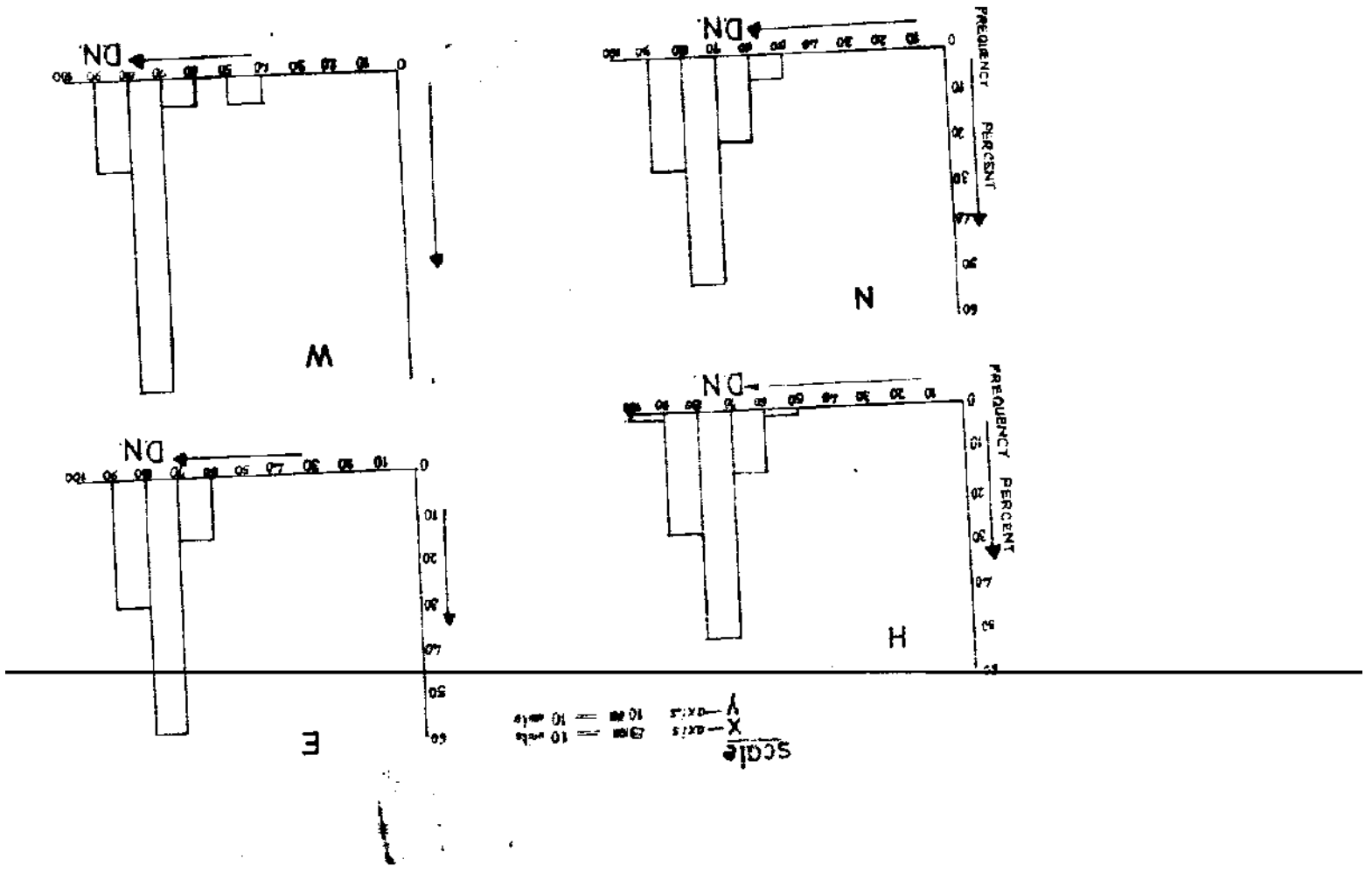
Dibucaine number	Frequency percent				Total
	Hausa	Other North	West	East	
10	0	0	0	0	0
10 - 19	0	0	0	0	0
20 - 29	0	0	0	0	0
30 - 39	0	0	0	0	0
40 - 49	0	0	5.0(1)	0	0.4(1)
50 - 59	0.6(1)	5.6(2)	0	0	1.3(3)
60 - 69	13.8(23)	19.4(7)	5(1)	14.3(1)	13.9(32)
70 - 79	50.1(97)	50(18)	70(14)	57.14(4)	57.8(133)
80 - 89	26.9(45)	25(9)	20(4)	28.6(2)	26.1(60)
90 - 100	0.6(1)	0	0	0	0.4(1)
Total	(167)	(36)	(20)	(7)	(230)
Percentage of grand total	72.6	15.7	8.7	3.0	100
Mean D.N.	75.69±6.90	73.94±8.15	74.5±0.37	75.93±6.39	75.33±7.27

* Figures in parenthesis indicate the actual number of subjects.

Hausa Vs. Other Northerner: $t = 1.33$
$tp0.95 = 1.645$

Hausa Vs. Westerner: $t = 0.71$
$tp0.95 = 1.645$

Hausa Vs. Easterner $= 0.09$
$tp0.95 = 1.645$


DN
W
N
E
H
FREQUENCY PERCENT
SCALE

Only four sera, representing 1.96 percent of control subjects (and 1.74 of the total population) had intermediate D.N. The mean value for the intermediate-type enzyme was 52.0 ± 4.33. No intermediate D.N. was recorded among the sickle-cell patients studied, and there was no case of low or atypical D.N. encountered in the population studied.

The relationship of D.N. to blood group is shown in table 3.8 and figure 3.8. The higher mean DN for blood group AB (76.76 ± 4.16) is due to the small number of subjects involved (2.17 percent of the population). Analysis of variance shows the difference to be insignificant ($t = 0.47$ $tp0.95 = 1.66$) In other words, there is no correlation between D.N and blood group. Similar analysis did not reveal any correlation between D.N. and genotype ($t = 0.26$ to 0.82, $tp0.95 = 1.645$) as shown in table 3.9 and figure 3.9; between DN and age ($t = 0.25$ to 1.43, $tp0.95 = 1.66$) shown in table 3.10 and figure 3.10, the higher mean DN (78.67 ± 4.93) recorded for 41 - 50 years is due to the small number of subjects (5.22 percent of the population); and between DN and ethnic group ($t = 0.09$ to 1.33, $tp0.95 = 1.645$) as shown in table 3.11 and figure 3.11.

.5 DISTRIBUTION OF FLUORIDE NUMBER

The frequency distribution of Fluoride numbers is presented in table 3.12 and represented in figure 3.12(a) for controls and (b) for sickle-cell patients. The mean values calculated for the two groups of subjects were 54.95 ± 7.66 (controls) and 55.21 ± 5.93 (sicklers). The difference was not significant ($t = 0.17$, $tp0.95 = 1.645$). In other words, fluoride had similar

Table 3.12 FREQUENCY DISTRIBUTION OF FLUORIDE NUMBER.

Fluoride number	Frequency percentage		Total Population
	Control Subjects	Sickle-cell patients	
0 - 9	0	0	0
10 - 19	0	0	0
20 - 29	2(4)	0	1.7(4)
30 - 39	0.5(1)	0	0.4(1)
40 - 49	8.9(18)	14.3(4)	9.6(22)
50 - 59	67.8(137)	64.3(18)	67.4(155)
60 - 69	19.3(39)	21.4(6)	19.6(45)
70 - 79	1,5(3)	0	1.3(3)
Total	(202)	(28)	(230)
Percentage of grand total	87.8	12.2	100
Range of F.N.	54.84	19.69	54.84
Mean F.N.	54.95 ± 7.66	55.21 ± 5.93	54.98 ± 7.47
Mean for 'UF-type' enzyme	26.5 ± 4.0	-	26.5 ± 4.0

* Figures in parenthesis indicate the actual number of subjects.

Control Vs Sickle-cell patients: t = 0.17

tp0.95 = 1.645

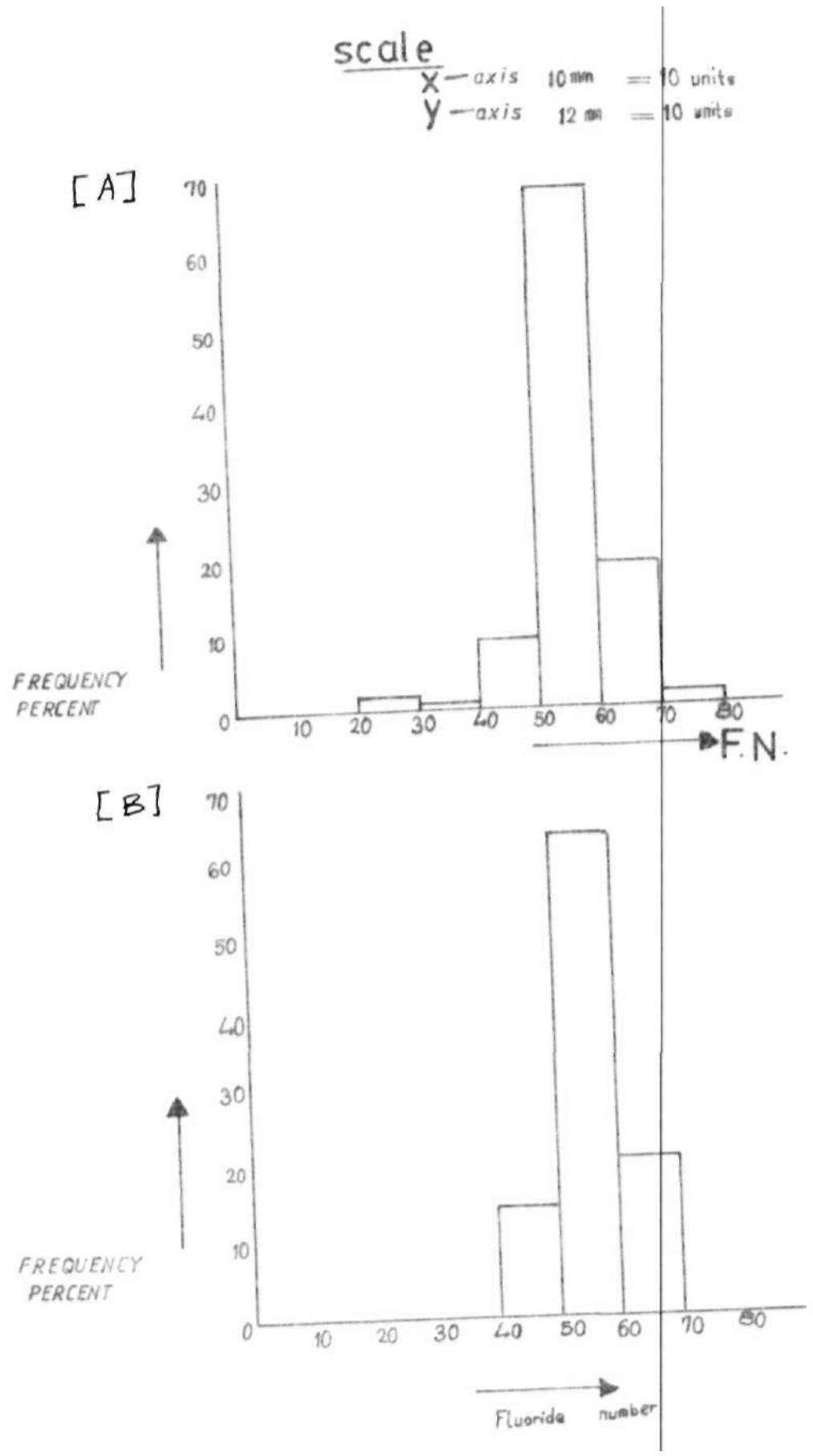

scale
x — axis 10 mm = 10 units
y — axis 12 mm = 10 units
[A]
70
60
50
40
30
20
10
0
10 20 30 40 50 60 70 80
F.N.
FREQUENCY PERCENT
[B]
70
60
50
40
30
20
10
0
10 20 30 40 50 60 70 80
Fluoride number
FREQUENCY PERCENT

Table 3.13. FREQUENCY DISTRIBUTION OF FLUORIDE NUMBER IN RELATION TO BLOOD GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

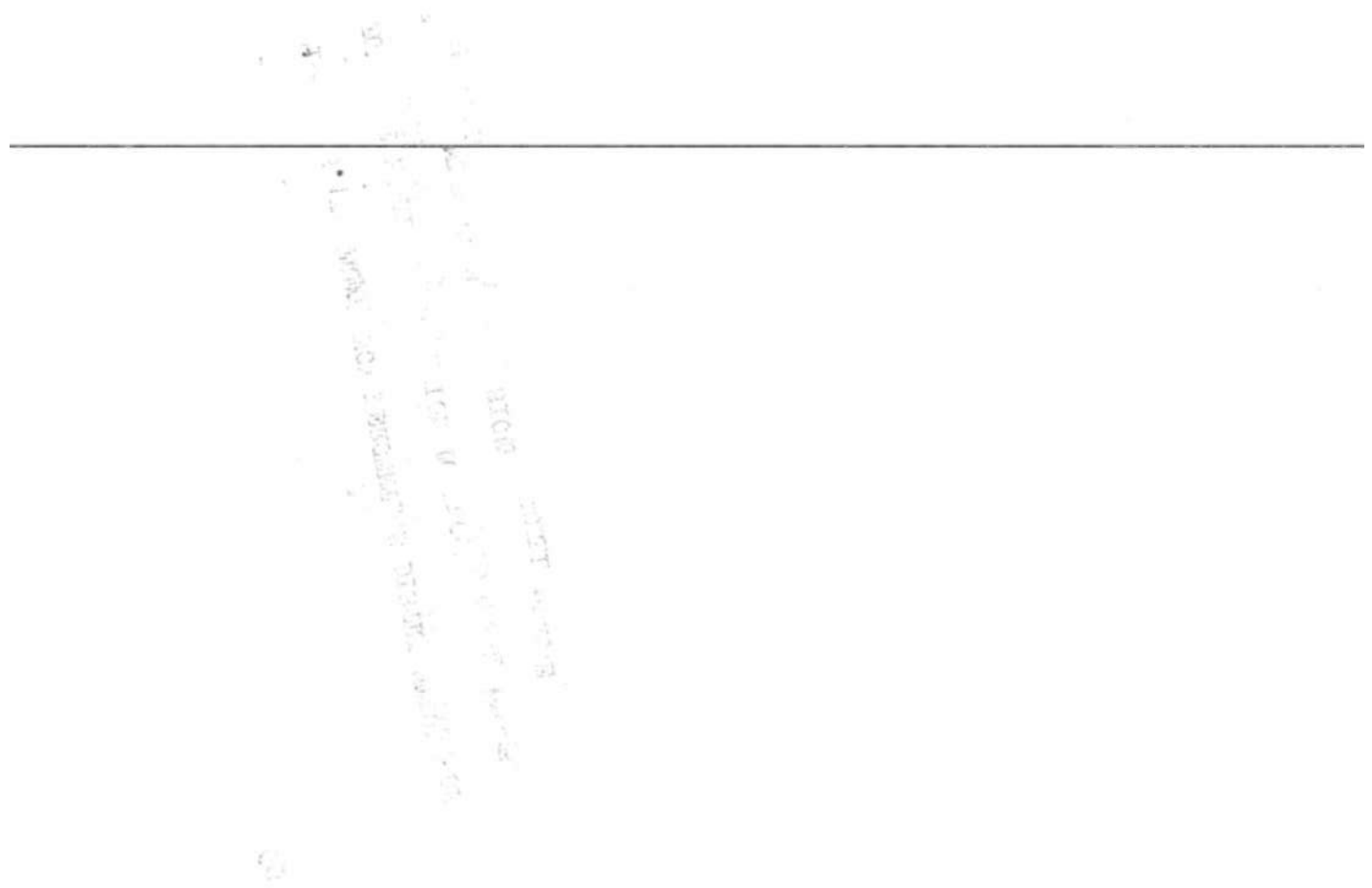
Fluoride number	Frequency percentage				Total
	A	B	AB	O	
10	0	0	0	0	0
10 - 19	0	0	0	0	0
20 - 29	3.7(2)	1.4(1)	0	1.0(1)	1.7(4)
30 - 39	0	0	0	1.0(1)	0.4(1)
40 - 49	3.7(2)	12.2(9)	0	11.3(11)	9.6(22)
50 - 59	75.9(41)	68.9(51)	40(2)	62.0(61)	67.4(155)
60 - 69	16.6(9)	17.6(3)	40(2)	21.6(21)	19.6(45)
70 - 79	0	0	20(1)	2.1(2)	1.3(3)
Total	(54)	(74)	(5)	(97)	(230)
Percentage of grand total	23.5	32.2	2.2	42.2	100
Mean F.N.	54.5±7.70	54.36±5.54	62.5±7.40	55.35±7.40	54.90±7.47

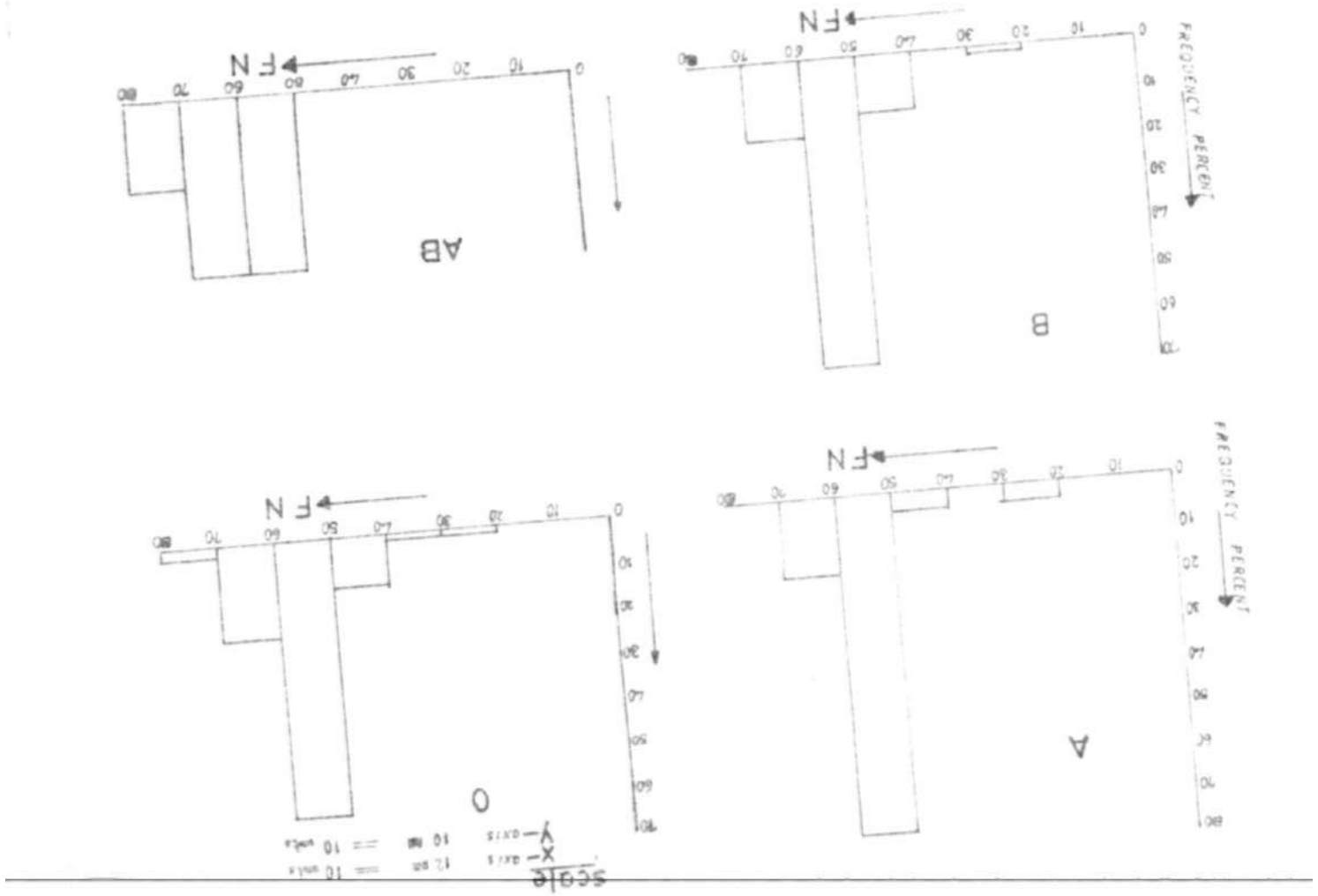
* Figures in parenthesis indicate the actual number of subjects.

(i) Blood group A Vs. B: t = 0.12
tp0.95 = 1.645

(ii) Blood group A Vs. AB: t = 2.12
tp0.95 = 1.67

(iii) Blood group A Vs. O: t = 0.62
tp0.95 = 1.645




FREQUENCY PERIOD
FREQUENCY PERIOD
FREQUENCY PERIOD
FREQUENCY PERIOD
FN
FN
FN
FN
A
B
AB
O
SCALE
X— axis 12 mm = 10 units
Y— axis 10 mm = 10 units

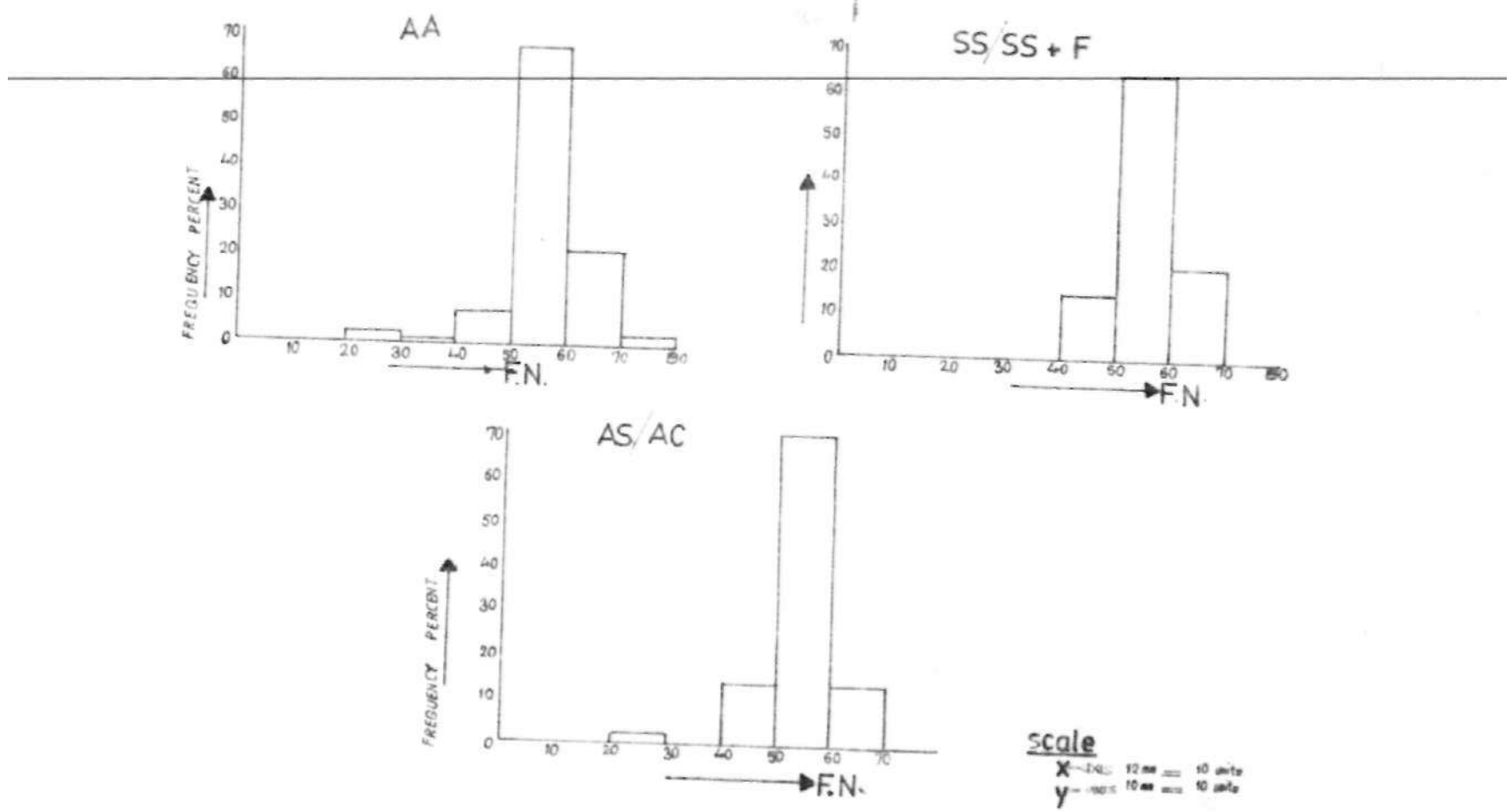

AA
SS SS + F
AS AC
FREQUENCY PERCENT
F.N.
scale

Table 3.15. FREQUENCY DISTRIBUTION OF FLUORIDE NUMBER IN RELATION TO AGE OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

Fluoride number	Frequency percentage of age (Years)					Total
	1 - 10	11 - 20	21 - 30	31 - 40	41 - 50	
10	0	0	0	0	0	0
10 - 19	0	0	0	0	0	0
20 - 29	0	2.5(1)	1.8(2)	1.6(1)	0	1.7(4)
30 - 39	0	0	0	1.6(1)	0	0.4(1)
40 - 49	0	15(6)	11(12)	6.3(4)	0	9.6(22)
50 - 59	100(5)	60(24)	66.1(72)	71.9(46)	66.7(8)	67.4(155)
60 - 69	0	22.5(9)	19.3(21)	18.8(12)	25(3)	19.6(45)
70 - 79	0	0	1.8(2)	0	8.3(1)	1.3(3)
Total	(5)	(40)	(109)	(64)	(12)	(230)
Percentage of grand total	2.2	17.4	47.4	27.8	5.2	100
Mean F.N.	52.71±1.2	54.25±8.21	54.27±7.77	54.90±6.72	58.67±6.4	54.98±7.47

* Figures in parenthesis indicate the actual number of subjects.

(i) 31 - 40 Vs. Under 10: $t = 0.74$, $tp0.95 = 1.66$

(ii) 31 - 40 Vs. 11 - 20: $t = 0.48$, $tp0.95 = 1.66$

(iii) 31 - 40 Vs. 21 - 30: $t = 0.08$, $tp0.95 = 1.645$

(iv) 31 - 40 Vs. 41 - 50: $t = 1.74$, $tp0.95 = 1.66$

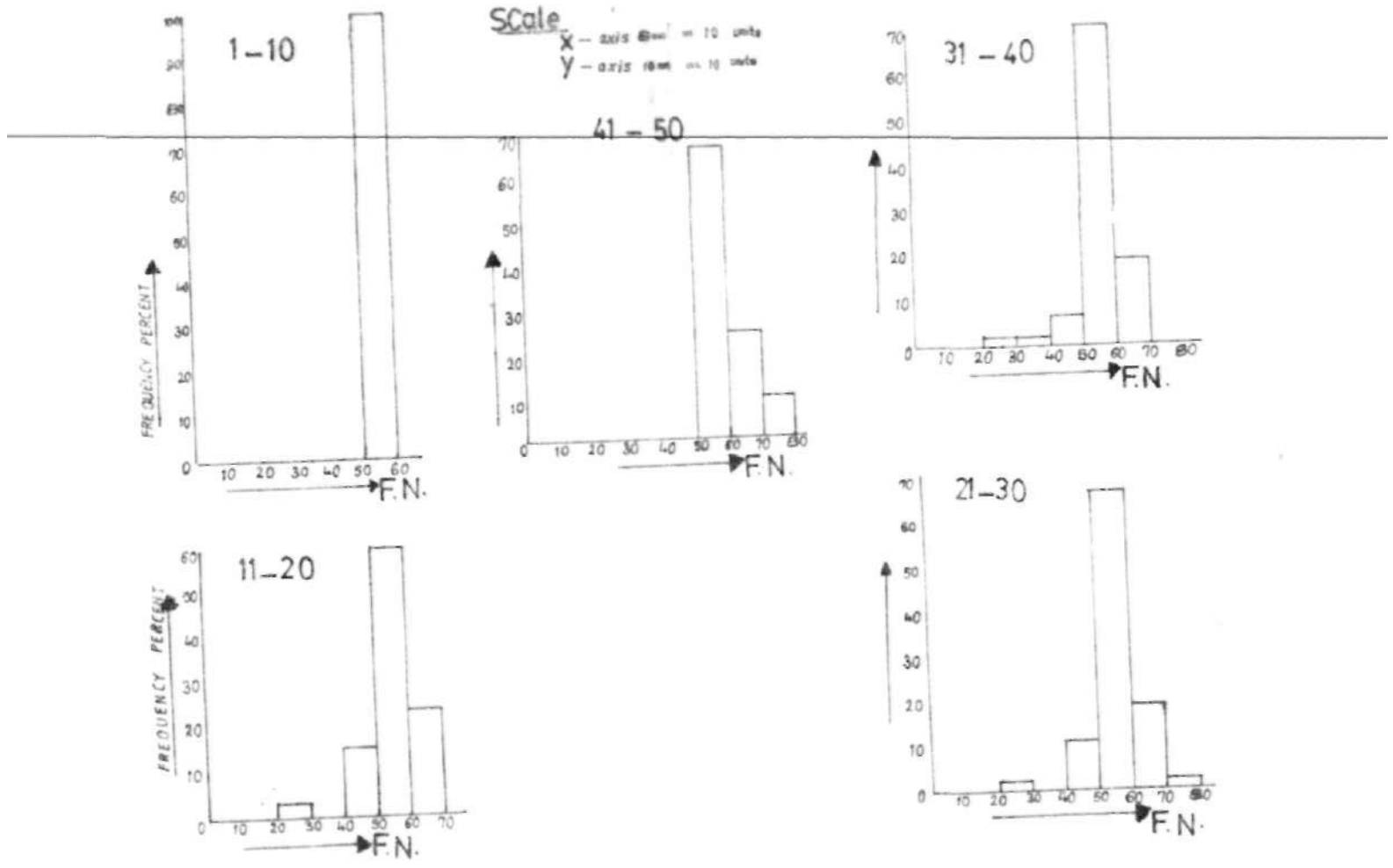

Scale
x – axis
y – axis
1–10
11–20
21–30
31 – 40
41 – 50
FREQUENCY PERCENT
F.N.

Table 3.16. FREQUENCY DISTRIBUTION OF FLUORIDE NUMBER IN RELATION TO ETHNIC GROUP OF 230 SUBJECTS WITH OR WITHOUT SICKLE-CELL DISEASE.

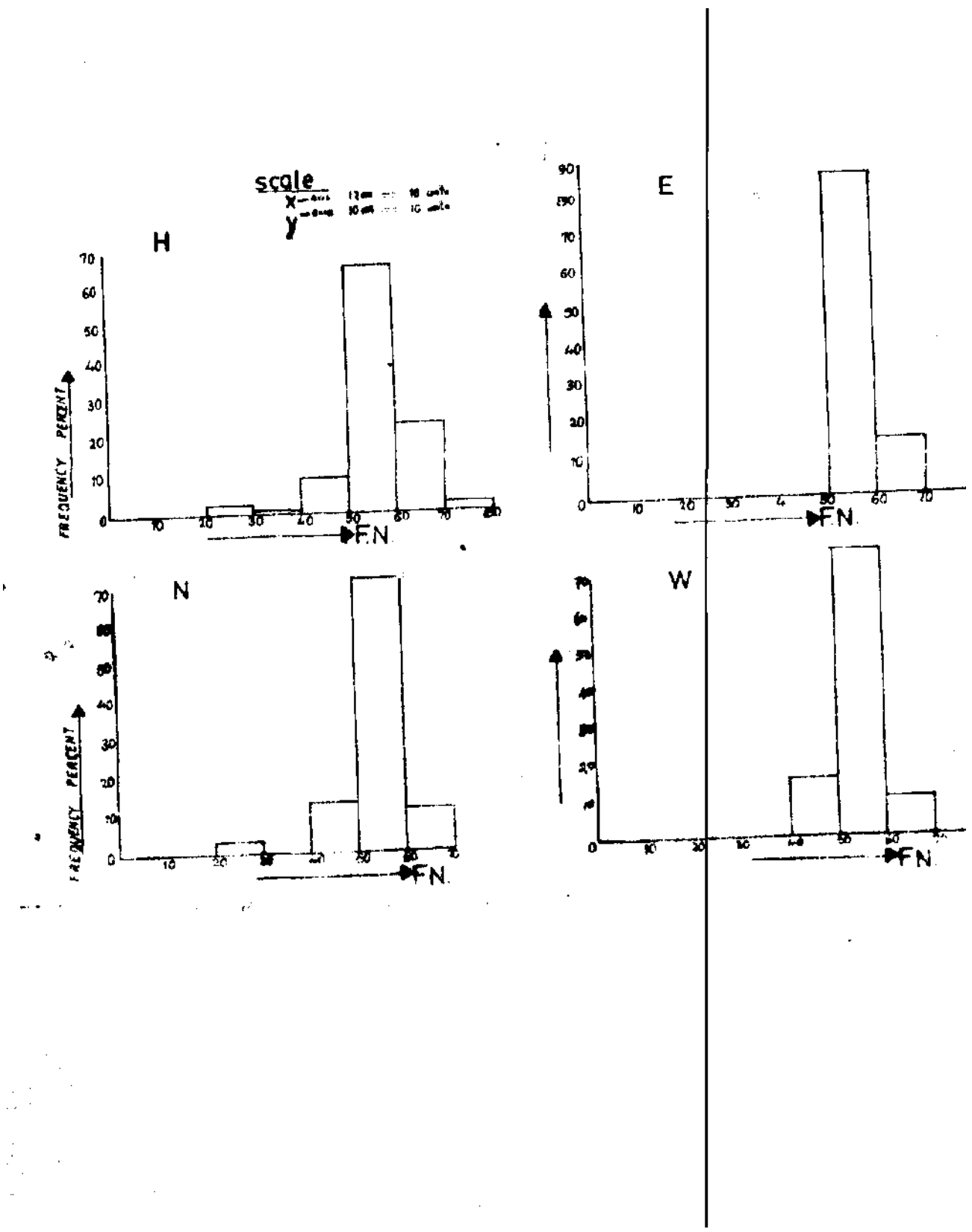
Fluoride number	Frequency percent Ethnic group				Total
	Hausa	Other North	West	East	
10	0	0	0	0	0
10 - 19	0	0	0	0	0
20 - 29	1.8(3)	2.8(1)	0	0	1.7(4)
30 - 39	0.6(1)	0	0	0	0.4(1)
40 - 49	8.4(14)	13.9(5)	15(3)	0	9.6(22)
50 - 59	64.7(108)	72.2(26)	75(15)	85.7(6)	67.4(155)
60 - 69	22.8(38)	11.11(4)	10(2)	14.28(1)	19.6(45)
70 - 79	1.8(3)	0	0	0	1.3(3)
Total	(167)	(36)	(20)	(7)	(230)
Percentage of grand total	72.6	15.7	8.7	3.0	100
Mean F.N.	55.46±7.76	53.11±7.5	54.0±1.90	55.93±3.5	53.98±7.47

* Figures in parenthesis indicate the actual number of subjects.

West Vs. Hausa: t = 0.82
tp0.95 = 1.645

West Vs. Other Northerner: t = 0.47
tp0.05 = 1.67

West Vs. Easterner: t = 0.92
tp0.95 = 1.70


scale
H
E
N
W
FREQUENCY PERCENT
F.N.

inhibitory effect on ChE from control (apparently normal) subjects and sickle-cell patients. Altogether 225 sera (97.83 percent of the population) had F.N. corresponding to the 'usual' type of enzyme. Of these, 197 sera from control subjects (representing 97.52 percent) and 28 sera from sickle-cell patients (representing 100 percent) were involved. The mean FNs. calculated for the 'usual' enzyme were 55.87 ± 5.76 for control subjects and 55.21 ± 5.93 for sicklers. The difference in the occurrence of 'usual' type of enzyme in the two groups of subjects is not significant ($t = 0.56$ $tp0.95 = 1.645$).

There were five cases of the 'UF' fluoride number, representing 2.47 percent of the control subjects (and 2.17 percent of the population). The 'UF' group had a mean FN. of 26.5 ± 4.0. There was no 'UF' FN recorded among the sickle-cell patients studied. Also no case of the 'Fluoride-insensitive' enzyme was encountered in the population.

Table 3.13 shows the distribution of FN in relation to blood groups. The distribution is represented in figure 3.13. The higher mean FN (62.5 ± 7.48) for blood group AB is due to the small number of subjects ($t = 2.12$, $tp0.95 = 1.67$). No other blood group showed correlation with F.N. ($t = 0.12$ to 0.62, $tp0.95 = 1.645$). Table 3.14 shows the distribution of FN in relation to genotype. The distribution is represented in figure 3.14. Analysis of variance did not reveal any significant difference between the genotypes ($t = 0.05$ to 1.11, $tp0.95 = 1.645$). Similar analysis showed no correlation between FN and age ($t=0.00$ to 0.74, $tp0.95 = 1.66$) as shown in table 3.15 and figure 3.15,

the high value obtained for 41 - 50 years ($t = 1.74$, $tp0.95 = 1.66$) is due to the small number of samples involved, and between FN and Ethnic group ($t = 0.47$ to 0.92, $tp0.95 = 1.645$ to 1.7) as shown in table 3.16. The distribution of FN in relation to ethnic group is represented in figure 3.16.

3.6 PSEUDOCHOLINESTERASE VARIANTS OBSERVED.

The carriers of the U and I phenotypes in the two groups of subjects studied are given in table 3.17(a), as well as the frequency of the E_1^a allele; no I phenotype was found.

Table 3.17(b) shows the carriers of the U and UF phenotypes and the frequency of the E_1^f allele; no F phenotype was found.

Table 3.17(c) shows the variants found in the two groups of subjects and in the total population using a combination of DN and FN as recommended by Whittaker (1980). The distribution of the E_1^u, E_1^a and E_1^f genes are also given.

3.7 DISCUSSION

Pseudocholinesterase is an enzyme produced in the liver under genetic control. The enzyme is polymorphic, involving many alleles and isoenzymes. Though the enzyme has no clearly defined biological function, pharmacologically it is necessary for the degradation of succinylcholine. Atypical variants are of interest to the clinician because of their association with prolonged respiratory cessation after succinylcholine administration.

The frequencies of the atypical gene (E_1^a allele) have been determined by many authors in various populations and vary widely; the highest are generally found in European and Jewish population - 1.4 to 5.1 percent (Van Ros and Vervoort, 1973), 2 to 8.5 percent (Garcia and Diaz, 1972); they are lower in Negroes and

Table 3.17(a) FREQUENCY PERCENTAGE OF THE PSEUDOCHOLINESTERASE VARIANTS DETERMINED ACCORDING TO THE PERCENTAGE OF INHIBITION DUE TO DIBUCAINE (DIBUCAINE NUMBER).

Subjects	Number Studied	Pseudocholinesterase Phenotypes			Gene Frequency	
		U	I	A	E_1^u	E_1^a
Sickle-cell patients	(28)	100(28	0	0	100	0
Control Subjects	(202)	98.0(198)	2(4)	0	99.0	1.0
Total Population	(230)	98.3(226)	1.7(4)	0	99.1	0.9

(b) FREQUENCY PERCENTAGE OF THE PSEUDOCHOLINESTERASE VARIANTS DETERMINED ACCORDING TO THE PERCENTAGE OF INHIBITION DUE TO SODIUM FLUORIDE (FLUORIDE NUMBER).

Subjects	Number Studied	Pseudocholinesterase Phenotype			Gene Frequency	
		U	UF	F	E_1^u	E_1^f
Sickle-cell patients	(28)	100(28)	0	0	100	0
Control	(202)	97.5(197)	2.5(5)	0	98.8	1.2
Total Population	(230)	97.8(225)	2.2(5)	0	98.9	1.1

(c) DISTRIBUTION OF PSEUDOCHOLINESTERASE VARIANTS AS DETERMINED BY THE COMBINED PERCENTAGE OF INHIBITION DUE TO DIBUCAINE (DIBUCAINE NUMBER) AND SODIUM FLUORIDE (FLUORIDE NUMBER).

Subjects	Number Studied	Pseudocholinesterase Phenotypes			Gene Frequency		
		$E_1^u E_1^u$	$E_1^u E_1^a$	$E_1^u E_1^f$	E_1^u	E_1^a	E_1^f
Sickle-cell patients	(28)	100(28)	0	0	100	0	0
Control Subjects	(202)	96.5(195)	2(4)	1.5(3)	98.3	1.0	0.7
Total Population	(230)	97(223)	1.7(4)	1.3(3)	98.5	0.9	0.7

* Figures in parenthesis indicate the actual number of subjects.

Asiatic groups - 0 to 0.9 percent(Van Ros and Vervoort, 1973). Kalow (1964) found a gene frequency of 3.8 percent in 2,017 Caucasian Canadians, while Motulsky and Morrow (1968) reported a lower frequency among Seattle Negroes (1.05 percent heterozygotes) and Congolese Negroes (0.22 percent heterozygotes). The 0.9 percent reported for this study is much lower than the 3.8 percent reported by Kalow (1964) for Caucasian Canadians, and agrees with the values reported by Motulsky and Morrow (1968) for Seattle Negroes. It lies within the non-Caucasian range of 0 to 0.9 percent (Van Ros and Vervoort, 1973).

The frequency of 0.7 percent calculated for the fluoride-insensitive gene is higher than, but not significantly different from, the result 0.34 percent obtained by Whittaker (1980) for British students and 0.56 percent for random British population. The experiments were conducted at 26°C. Cimasoni (1966), Whittaker (1980) and Whittaker and Britten (1981) have reported that fluoride inhibition is strongly temperature-dependent, and it is impossible to recognise the fluoride-resistant variants when the reaction temperature is above 26°C. The inhibition of ChE by fluoride ion is immediate and its degree remains constant for long periods of time, up to 48 hours at room-temperature or for 30 minutes at 37°C. The higher value obtained here could be due to the high temperature, 37°C of the assay medium.

The findings of this study suggest that prolonged apnea after succinylcholine administration may be expected to occur less often among the Hausas and other Nigerians living in Zaria than other people of European stock. Based on the data provided the estimated frequency of the horozygous $E_1^{a}E_1^{a}$ is $\frac{1}{12,500}$ and for the horozygous $E_1^{f}E_1^{f}$ $\frac{1}{20,500}$.

In the present study no significant correlation was found between pseudocholinesterase activity, DN or FN and blood group, genotype, age or Ethnic group. Although the number of sickle-cell patients involved was small for statistical evaluation, it should be noted that there was no incidence of the atypical enzyme or even the intermediate type. When compared with the control subjects, with about ten times larger size, the difference in the distribution of pseudocholinesterase variants is not significant. The result shows that a locus regulating pseudocholinesterase inheritance and activity is not genetically linked to the locus regulating the inheritance of sickle-cell disease. In deed, whereas atypical variants of pseudocholinesterase are prevalent in Caucasians (Garcia and Diaz, 1972; Van Ros and Vervoort, 1973), sickle-cell disease is prevalent in non-Caucasians, particularly Africans (Shapiro and Poe, 1955).

Further, the works of Gilbertson (1955), Holzmann et al. (1969), Oduntan and Isaac (1971) and Searle (1973) reveal that patients with sickle-cell disease are at no greater risk during anaesthesia and surgery than patients with normal haemoglobin, provided that there is adequate oxygenation and ventilation that cardiac output and circulating blood volume are satisfactorily maintained, and acidosis avoided before, during and after surgery. These workers agree that choice of the anaesthetic agent is of less importance in patients with abnormal haemoglobin syndrome; what may be more important is the quality of anaesthetic management.

This is a pilot study, further studies involving larger population are needed to confirm the results presented here.

REFERENCES

1. Alles, G.A. and Hawes, R.C. (1940)
   Cholinesterases in the blood of man. Journal of Biological Chemistry 133, 375.

2. Allot, E.N. and Thompson, J.D. (1956)
   The familial incidence of low pseudocholinesterase level. Lancet 2, 157

3. Augustinsson, K.B. (1948)
   Cholinesterases: a study in comparative enzymology. Acta physiologica Scandinavica 15 suppl. 52,1.

4. Augustinsson, K.B. (1963)
   Classification and comparative enzymology of the cholinesterase and methods for their determination, in G.B. Koelle (1963) Cholinesterase and Anticholinesterase Agents. Springer, Berlin, pp 89.

5. Augustinsson, K.B. (1966)
   The nture of an 'anionic' site in butyryl-cholinesterase compared with that of a similar site in acetylcholinesterase. Biochemica et Biophysica Acta 128, 351.

6. Augustinsson, K.B. (1968)
   The evolution of esterases in vertebrates, in N. Van Thoai and J. Roche (1968) Homologous enzymes and biochemical evolution. Gordon and Breach, New York, pp 229.

7. Augustinsson, K.B. (1971)
   Determination of the activity of cholinesterases, in D. Glick (1971) Methods of Biochemical Analysis, Supplemental Volume, Analysis of Biogenic Amines and their related enzymes. Interscience publishers, New York, pp 217.

8. Ballantyne, B. (1960)
Histochemical and Biochemical aspects of cholinesterase activity of adipose tissue. Archives of International Pharmacodynamics and Therapy 173, 343. Cited by Foldes (1970).

9. Ballantyne, B. and Burwell, R.G. (1965)
Distribution of cholinesterase in normal lymphnodes and its possible relation to the regulation of tissue size.
Nature 206, 1123.

10. Beckett, A.H., Vaughan, C.L. and Mitchard, M.L. (1960)
Inhibition of the pseudocholinesterase in horse serum by some choline analogues. Biochemical Pharmacology 17, 1575.

11. Beecher, H.K. and Todd, D.I. (1954)
A study of deaths associated with anaesthesia in surgery
Annals of surgery 140, 2.

12. Bergmann, F. (1955)
Fine structure of the active surface of cholinesterase and the mechanism of enzymatic ester hydrolysis. Discussions of the Faraday Society 20, 126.

13. Bergmann, F. and Segal, R. (1954)
The relationship of quaternary ammonium salts to the anionic sites of true and pseudocholinesterases. Biochemical Journal 58, 692.

14. Bergmann, F. and Segal, R. (1955)
The characterization of tissue cholinesterases, Biochimica et Biophysica Acta 16, 513.

15. Bleisch, V.R. and Shwachman, H. (1954)
Serum cholinesterase values in childhood in health and disease.
Pediatrics 13, 426.

16. Bourne, J.G., Collier, H.O.J. and Somers, G.F. (1952)
Succinylcholine, muscle relaxant of short duration. Lancet 1, 1225.

17. Brody, S. (1960)
Plasma Cholinesterase activity in the new-born infant.
Acta obstetricia et Gynaecologica Scandinavica 39, 1.

18. Bush, G.H. (1964)
The use of muscle relaxant in burnt children. Anaesthesia
19, 231.

19. Calloway, S., Davies, D.R. and Rutland J.P. (1951)
Blood Cholinesterase levels and range of personal variation
in healthy adult population. British Medical Journal 2, 812.

20. Cimasoni, G. (1966)
Inhibition of Cholinesterases by Fluoride in vitro.
Biochemical Journal 99, 133.

21. Clark, S.W., Glaubiger, G.A. and LaDu, B.N. (1963)
Properties of plasma cholinesterase Variants. Annals of the
New York Academy of Science, 151, 710.

22. Clitherow, J. W., Mitchard, M. and Harper, N.J. (1963)
The possible biological Function of pseudocholinesterase
Nature (London) 199, 1000.

23. Cohen, J.A., Oosterbaan, R.A. and Warringa, M.G.P.T. (1955)
The turnover number of aliesterase, pseudo- and true
Cholinesterase and the Combination of these enzymes with
diisopropylfluorophosphate. Biochimica et Biophysica Acta
16, 220.

24. Cole, E.R. and Leadbeater, L. (1968)
Estimation of the stability of dry horse serum Cholinesterase
by means of an accelerated storage test. Journal of Pharmacy
and Pharmacology 20, 40.

25. Cunningham, L.W. (1957)

Proposed mechanism of action of hydrolytic enzymes

Science 125, 1145.

26. Dacie, J.V. and Levis, S.M. (1975)

Cellulose acetate electrophoresis, in J.V. Dacie and S. M. Levis (1975) Practical Haematology. E.L.B.S. and Churchhill Livingstone, Britain.

27. Dale, H.H. (1914)

The action of certain esters and ethers of choline and their relation to muscarine. Journal of Pharmacology and Experimental Therapeutics 6, 147. Cited by Silver (1974).

28. Das, P.K. and Liddell, J. (1970)

Purification and properties of human serum Cholinesterase. Biochemical Journal 116, 875.

29. Davies, R.O., Marton, A.V. and Kalow, W. (1960).

Action of normal and atypical Cholinesterase of human serum upon series of esters of choline. Canadian Journal of Biochemistry 38, 545.

30. Ecobichon, D.J. and Kalow, W. (1961)

Some properties of the soluble esterases of liver. Canadian Journal of physiology and pharmacology 39, 1329.

31. Enzyme commission (1965)

Enzyme Nomenclature. Recommendations (1964) of the international Union of Biochemistry on the nomenclature and classification of enzymes together with their units and the symbols of enzyme kinetics. Elsevier, Amsterdam. Cited by Silver (1974).

32. Erdos, E.G., Foldes, F.F., Daart, N. and Shamor, S.F. (1957).
Activating effect of tryptamine, benzylamine and histamine on plasma cholinesterase. Federation Proceedings, Federation of the American Society for Experimental Biology 16, 294.

33. Erdos, E.G., Foldes, F.F., Daart, N., Zsigmond, E.K. and Zwartz, J.A. (1958).
Acceleration of plasma Cholinesterase activity by quaternary ammonium salts. **Science** 128, 92

34. Erdos, E.G., Foldes, F.F., Daart, N., Zsigmond, E.K. and Zwartz, J.A. (1959).
The accelerating effect of narcotic analgesics on the hydrolysis of aromatic substrates by human plasma Cholinesterase.
Biochemical Pharmacology 2, 97.

35. Evans, F.T., Gray, T.W.S., Lehmann, H. and Silk, E. (1952).
Sensitivity to succinylcholine in relation to serum Cholinesterase.
Lancet 1, 1229.

36. Faber, M. (1943)
Relationship between serum choline esterase and serum albumin.
Acta medica scandinavica 114, 72.

37. **Fleming, A.F.** (1983)
Blood transfusion handbook, blood and rhesus grouping. Ahmadu Bello University Press, Zaria p 7.

38. Foldes, F.F. (1970)
Enzymes of acetylcholine metabolism, in Francis F. Foldes (1970) Enzymes in Anaesthesiology. Springer-Verlag, New York, Heidelberg, Berlin, p. 91.

39. Foldes, F.F. and Aven, M.H. (1952)
The hydrolysis of procaine and 2-chloroprocaine in spinal fluid.
Journal of Pharmacology and Experimental Therapeutics 105, 259.

40. Foldes, F.F. and Foldes, V.M. (1965)
a-amino acid esters of choline: interaction with ChE and neuromuscular activity in man. Journal of Pharmacology and Experimental Therapeutics 150, 220

41. Foldes, F.F. and Rhodes, D.H. (Jnr) (1953)
The role of plasma cholinesterase in anaesthesiology. Anaesthesia and analgesia (Cleveland) 32, 305

42. Foldes, F.F., Davis, D.L., Shanor, S.P. and Van Hees, G.R. (1955)
Hydrolysis of ester-type local anaesthetics and their halogenated analogues by purified plasma cholinesterase. Journal of the American Chemical Society 77, 5149.

43. Foldes, F.F., Davis, D.L., Shanor, S.P. and Van Hees, G.R. (1958)
The structure-action relationship of urethane type cholinesterase inhibitors. Journal of Pharmacology and Experimental Therapeutics 122, 457.

44. Foldes, F.F., Erdos, E.G., Zsigmond, E.K. and Zwartz, J.A.(1960)
Reactivation of neostigmine inhibited human plasma cholinesterase. Journal of Pharmacology and Experimental Therapeutics 129, 394.

45. Foldes, F.F., Zsigmond, E.K., Foldes, V.M. and Erdos, E.G.(1962)
The distribution of acetylcholinesterase and butyrylcholinesterase in the human brain. Journal of Neurochemistry 9, 559.

46. Foldes, F.F., Foldes, V.M., Smith, J.C. and Zsigmond, E.K.(1963)
The relation between plasma cholinesterase and prolonged apnea caused by succinylcholine. Anaesthesiology 24, 208.

47. Funnell, H. and Oliver, W.T. (1965)
Proposed Physiological function for plasma cholinesterase. Nature (London) 208, 689.

48. Garcia, C.H. and Diaz, P.M. (1972)
Atypical cholinesterase: Frequency in a Puerto Rican Population. Anaesthesiology 36, 81.

49. Gerebtzoff, M.A. (1959)
Cholinesterase: A histochemical Contribution to the Solution of some functional problems. Pergamon, Oxford London.

50. Gilbertson, A.A. (1965)
Anaesthesia in West African patients with sickle-cell Anaemia, Hb Hc disease and sickle-cell trait. British Journal of Anaesthesia 37, 614.

51. Goedde, H.W. and Altland, K. (1967) Biochemic and Genetik der pseudocholinesterasen, in H.W. Geedde, A. Doenicke and K. Altland (1967). Cholinesterasen: Pharmakogenetik, Biochemic Klinik. Springer-Verlag, Berlin, Heidelberg and New York.

52. Goedde, H.W. and Baitsch, H. (1964)
Nomenclature of pseudocholinesterase polymorphism. British Medical Journal 2, 310.

53. Goedde, H.W., Gehring, D. and Hoffman, R.A. (1965)
On the problem of a silent gene in Pseudocholinesterase Polymorphism. Biochimica et Biophysica Acta 107, 391.

54. Gronert, G.A., Botin, L.N., Ritchey, C.R. and Mason A.D. (Jnr) (1969)
Succinylcholine-induced hyperkalemia in burnt patients. II. Anesthesia and Analgesia (Cleveland) 48, 950.

55. Gurtner, J., Kreutzberg, G. and Doenicke, A. (1963).
Comparative studies on cholinesterase activity in serum and liver cells. Acta Anaesthesiologica Scandinavica 7, 69.

56. Hanel, H.K. and Viby-Mogensen, J. (1977)
The inhibition of serum cholinesterase by Urea. British Journal of Anaesthesia 49, 1251.

57. Harris, H. and Whittaker, M. (1961)
Differential inhibition of human serum cholinesterase with fluoride. Recognition of two new phenotypes. Nature (London) 191, 496.

58. Harris, H. and Whittaker, M. (1963)
Differential inhibition of 'usual' and 'atypical' serum cholinesterase by NaCl and NaF. Annals of Human Genetics 27, 53.

59. Harris, H., Hopkinson, D.A. and Robson, E.B. (1962)
Two dimensional electrophoresis of Pseudocholinesterase components in normal human serum. Nature 196, 1296.

60. Hazel, B. and Monier, D. (1971)
Human serum cholinesterase: Variations during pregnancy and Postpartum. Canadian Anaesthetists' Society Journal 18, 272.

61. Hilkowitz, G. and Jacobson, A. (1961)
Hepatic dysfunction and abnormalities of serum proteins and serum enzymes in sickle-cell anaemia. Journal of Laboratory and Clinical Medicine 57, 856.

62. Holzmann, L,, Finn, H., Lichtman, H.C. and Harmel, M.H. (1969)
Anaesthesia in patients with sickle-cell disease: A review of 112 cases. Anaesthesia and Analgesia 48, 566.

63. Hutchinson, A.O. and Widdowson, E.M. (1951)
Cholinesterase activities in the serum of healthy British Children. Nature 169, 204.

64. Jansz, H.S., Brons, D. and Warringa, M.G.P.J. (1959)

Chemical nature of the DFP-binding site of Pseudo cholinesterase. Biochimica et Biophysica Acta. 34, 573.

65. Juul , P. (1960).

Human plasma cholinesterase isoenzymes. Clinica Chimica Acta 19, 205.

66. Knbachnic, M.I., Brestkin, A.P., Godovikov, N.N., Michelson, M.J., Rosengart, E.V. and Rosengart, V.I. (1970).

Hydrophobic areas on the surface of cholinesterase. Pharmacological Review 22, 355.

67. Kalow, W. (1964).

Pharmacogenetics and anaesthesia. Anaesthesiology 25, 337.

68. Kalow, W. (1956)

Familial incidence of low pseudocholinesterase level. Lancet 2, 576.

69. Kalow, W. and Davies, R.O. (1958)

The activity of various esterase inhibitors towards atypical human serum cholinesterase. Biochemical Pharmacology 1, 183.

70. Kalow, W. and Genest, K. (1957)

Method for detection of atypical forms of human serum cholinesterase: Determination of Dibucaine numbers. Canadian Journal of Biochemistry and Physiology 35, 339.

71. Kalow, W. and Gurn, D.R. (1957)

The relationship between dose of succinylcholine and duration of apnea in man. Journal of Pharmacology and Experimental Therapeutics. 120, 203.

72. Kalow, W. and Gurn, D.R. (1959)

Some statistical data on atypical Cholinesterase of human serum. Annals of Human Genetics 13, 239.

73. Kalow, W. and Lindsay, H. (1955)
Comparison of optical and manometric methods for the assay of human serum cholinesterase. Canadian Journal of Biochemistry and Physiology. 33, 568.

74. Kalow, W. and Lindsay, H. (1956)
Abnormal Behaviour of human serum cholinesterase. Journal of Pharmacology and Experimental Therapeutics. 116, 34.

75. Kalow, W. and Staron, N. (1957)
On Distribution and Inheritance of Atypical forms of Human serum Cholinesterase, as indicated by Dibucaine numbers. Canadian Journal of Biochemistry and Physiology 35, 1305.

76. Koelle, G.B. (1951)
The elimination of enzymatic diffusion artifacts in the histochemical localization of Cholinesterases and a survey of the distributions. Journal of Pharmacology and Experimental Therapeutics 103, 153. Cited by Foldes (1970)

77. Krupka, R.M. (1966)
Fluoride inhibition of acetylcholinesterase. Molecular Pharmacology 2, 550.

78. Lehmann, H. and Liddell, J. (1964)
Genetic variants of human serum pseudocholinesterase. Progress in Medical Genetics 3, 75.

79. Lehmann, H. and Liddell, J. (1969)
Human cholinesterase (pseudocholinesterase): Genetic variants and their recognition. British Journal of Anaesthesia 41, 235.

80. Lehmann, H. and Ryan, E. (1956)
The familial incidence of low pseudocholinesterase level. Lancet 2, 576

81. Lehmann, H., Patson, V. and Ryan, E. (1956)
The inheritance of an idiopathic low plasma pseudocholinesterase level. Journal of Clinical Pathology 11, 554.

82. Lehmann, H., Silk, E. and Liddell, J. (1961)
Pseudocholinesterase. British Medical Bulletin 17, 230.

83. Lehmann, H., Liddell, J., Blackwell, B., O'Connor, D.C. and Daws, A.V. (1963).
Two further serum pseudocholinesterase variants as causes of suxamethonium apnea. British Medical Journal 1., 1116.

84. Leuzinger, W., Goldberg, M. and Cauvin, E. (1969)
Molecular properties of acetylcholinesterase. Journal of Molecular Biology 40, 217.

85. Levine, M.G. and Hoyt, R.E. (1949).
Serum Cholinesterase in some pathological Conditions Proceedings of the Society for Experimental Biology and Medicine 70, 50.
Cited by Foldes (1970).

86. Levine, M.G. and Hoyt, R.E. (1950)
The relationship between human serum cholinesterase and serum albumin. Science 111, 266.

87. Liddell, J., Lehmann, H. and Silk, E. (1962).
A silent pseudocholinesterase gene. Nature (London) 193, 561.

88. Liddell, J., Newman, G.E. and Brown, D.F. (1963)
A Pseudocholinesterase variant in human tissues
Nature (London) 198, 1090.

89. Marnay, A. and Nachmansohn, D. (1937)
Cholinesterase in voluntary Frog's muscle. Journal of physiology (London). Cited by Silver (1974). 92, 359.

90. Marnay, A. and Nachmansohn, D. (1938).
Cholinesterase in voluntary muscle. Journal of Physiology (London)
Cited by Silver (1974) 92, 37

91. McCance, R.A., Hutchinson, A.O., Doan, R.F.L. and Jones, P.E.H. (1949). The Cholinesterase activity of the serum of new-born animals and of colostrum. Biochemical Journal 45, 493.

92. McKerracher, D. W., McGuire, W.A., Aronson, A. and Scott, J. (1966) Pseudocholinesterase and the prediction of stability in subnormal and psychopathic offenders. British Journal of psychiatry 112, 717.

93. Mendel, B. and Rudney, H. (1943a). Studies on Cholinesterase. 1. Cholinesterase and pseudocholinesterase. Biochemical Journal 37, 59.

94. Mendel, B. and Rudney. H. (1943b) On the type of cholinesterase present in brain tissues. Science 98, 201.

95. Milstoc, M. and Wolfson, A.H. (1972) The role of cholinesterase in secondary anaemia: Experimental Phase. American Journal of Clinical Pathology 57, 494.

96. Mounter, L.A. and Whittaker, V.P. (1950) The esterase of horse blood. 2. The specificity of horse erythrocyte cholinesterase. Biochemical Journal 47, 525.

97. Mounter, L.A., Alexander, H.C. and Tuck, K.E. (1957). The pH dependence and dissociation constants of esterases and proteases treated with diisopropyl fluorophosphate. Journal of Biological Chemistry 226, 867.

98. Motulsky, A.G. (1964) Pharmacogenetics. Progress in Medical Genetics 3, 49.

99. Motulsky, A.G. and Morrow, A. (1968) Atypical Cholinesterase gene E_1^a: Rarity in Negroes and most Orientals. Science 159, 202.

100. Myers, D.K. (1951)
Differentiation of three types of competitive Cholinesterase inhibitors. Archives of Biochemistry 31, 29. Cited by Foldes (1978).

101. Oduntan, S.A. and Isaac, W.A. (1971)
Anaesthesia in patients with Abnormal Hb. Syndromes: A preliminary report. British Journal of Anaesthesia 43, 1159.

102. Oosterbaan, R.A. (1967)
Consititution of DFP enzymes, in E. Heilbronn (1967) Proceedings of the conference on structure and Reactions of DFP Sensitive Enzymes. p. 25 Swedish Research Institute of National Defence, Stockholm. Cited by Foldes (1974).

103. Oropollo, A.T. (1978)
Abnormal pesudocholinesterase levels in a surgical population. Anesthesiology 48(4), 284.

104. Oyama, T. and Cohen, M.S. (1970)
Local Anaesthetic Agents, in Lichtiger, M. and Moyn, F. (1970). Introduction to the practice of anaesthesia. Harper and Row, New York, San Francisco.

105. Pantuck, E.J. (1966)
Ecothiopate eye drops and Prolonged response to suxamethonium. British Journal of Anaesthesia. 38, 406

106. Properi, D.N. and Brackenridge, C.J. (1976)
The relation of sex, age, smoking status, birth rank and parental ages to pseudocholinesterase activity and Phenotypes in a sample of Australian Caucasian adults.
Human Genetics 32, 181. Cited by Whittaker (1980)

107. Raab, W. (1969)
Cholinesterase activity of rat serum following renal damage. Clinica Chimica Acta 24, 135.

108. Robins, G.S., Zsigmond, E.K. and Shanor, S.P. (1969).
Plasma cholinesterase activity and safe use of succinylcholine in Cancer patients. 6th Annual meeting of the American Society of Clinical Pharmacology and Chemotherapy, Atlantic City, May 1 - 3, 1969. Cited by Foldes (1970).

109. Sawyer, C.H. and Everett, J.W. (1947)
Cholinesterase in rat tissues and the site of serum non-specific cholinesterase production. American Journal of Physiology 148, 675.

110. Schanor, P.J., Brown, R.L., Kirksey, T.D., Gunther, R.C, Ritchey, C.R. and Grenert, G.A. (1969).
Succinylcholine induced hyperkalemia in burned patients.1. Anesthesia and Analgesia (Cleveland) 48, 764.

111. Searle, J.F. (1973)
Anaesthesia in Sickle-cell states, Anaesthesia 28, 48.

112. Shapiro, N.D. and Poe, M.F. (1955)
Sickle-cell disease: an anesthesiological problem. Anesthesiology 16, 771.

113. Shanor, S.P., Van Hees, G.R., Baart, N., Erdos, E.G. and Foldes, F.F. (1961).
The influence of age and sex on human plasma and red cell Cholinesterase. American Journal of Science 242, 357.

114. Shnider, S.M. (1965)
Serum cholinesterase activity during pregancy labor and puerperium. Anesthesiology 26, 335.

115. Silver, Ann. (1974)

The biology of Cholinesterase. North Holland publishing Company-Amsterdam, Oxford.

116. Smith, J.C. and Foldes, F.F. (1968).

An improved method for the recognition of atypical plasma Cholinesterase. Anaesthesiology 29, 211.

117. Smith, J.C. and Foldes, F.F. (1972)

The recognition of atypical pseudocholinesterase by relative substrate hydrolysis rate. Biochimica et Biophysica acta 289, 352.

118. Smith, J.C., Foldes, V.M. and Foldes F.F. (1963)

Distribution of Cholinesterase in normal human muscle. Canadian Journal of Biochemistry and Physiology 41, 1713.

119. Spiegel, M.R. (1961)

Schaum's Outline of Theory and Problems of statistics in S.I. units. McGraw-Hill, London. p. 190.

120. Stedman, E. and Stedman, E. (1935)

The purification of Choline-esterase. Biochemical Journal 29, 2563.

121. Stedman, E., Stedman, E. and Easson, L.H. (1932).

Choline-esterase: An enzyme present in the blood serum of the horse. Biochemical Journal 26, 2056.

122. Strelitz, F. (1944)

Studies on cholinesterase. IV. Purification of pseudocholinesterase from horse serum. Biochemical Journal 38, 86.

123. Surgenor, D.M. and Ellis, D. (1954).

Preparation and properties of serum and plasma proteins, plasma cholinesterase. Journal of the American Chemical Society 76, 6049

124. Svensmark, O. and Kristensen, P. (1963)
Isoelectric point of native and Sialidase-treated human serum cholinesterase. Biochimica et Biophysica Acta 67, 441.

125. Svensmark, O. (1965)
Molecular properties of cholinesterases. Acta physiologica Scandinavica 64, Suppl. 245.

126. Swift, M.R. and LaDu, B.N. (1966)
A rapid Screening test for atypical serum Cholinesterase. Lancet I, 513.

127. Thompson, J.C. and Whittaker, M. (1965)
Pseudocholinesterase activity in thyroid disease. Journal of Clinical Pathology. 18, 811.

128. Trotter, M.D. and Fairburn, E.A. (1966)
Serum cholinesterase in eczema. British Journal of Dermatology 78, 469. Cited by Silver (1974).

129. Vaccarezza, J.R. and Willson, J.A. (1965)
The relationship between blood cholinesterase activity and neoplastic disease. Experientia 21, 405. Cited by Silver (1974).

130. Van Ros, G. and Druet, R. (1966)
Uncommon electrophoretic patterns of serum Cholinesterase (Pseudocholinesterase). Nature 212, 543.

131. Van Ros, G. and Vervoort, T. (1973).
Frequencies of the "atypical" and C5 Variants of Serum Cholinesterase in Zairians and Belgians. Detection of the C5 variant by Agar Gel electrophoresis with an Acid Buffer. Annales de la societe Belge de Medicine Tropicale 53, (6), 633.

132. Vorhaus, L.J. and Kark, R.M. (1953)
Serum Cholinesterase in health and disease. American Journal of Medicine 14, 707.

133. Wetstone, H.J. and LaMotta, R.V. (1965)
The Clinical stability of serum cholinesterase activity. Clinical Chemistry 11, 653.

134. Whittaker, M. (1968)
Differential inhibition of human serum Cholinesterase with n-butyl alcohol: recognition of new Phenotypes. Acta Genetica et statistica Medica 18, 335. Cited by Whittaker (1980)

135. Whittaker, M. (1969)
The serum cholinesterase Variants: Differentiation by means of formaldehyde. Clinica Chimica Acta 26, 141. Cited by Whittaker, (1980).

136. Whittaker, M. (1977)
Estimation of plasma cholinesterase activity and the use of inhibitors for the determination of phenotypes. Association of clinical pathologists. Broadsheet 87, 1. Cited by Whittaker (1980).

137. Whittaker, M. (1980)
Plasma Cholinesterase variants and the anaesthetist. Anaesthesia 35, 174.

138. Whittaker, M. and Britten, J.J. (1981).
A comparison of some methods of phenotyping the plasma Cholinesterase Variants using benzoylcholine as substrate. Annals of Clinical Biochemistry 18, 1

139. Wilson, I.B. (1951)
Acetylcholinesterase. XI. Reversibility of tetraethyl pyrophosphate inhibition. Journal of Biological Chemistry 190, 111

140. Wilson, I.B. (1952)
Acetylcholinesterase. XIII. Reactivation of alkyl Phosphate inhibited enzyme. Journal of Biological Chemistry 199, 113.

141. Wilson, I.B. (1954)
The active surface of serum esterase. Journal of Biological Chemistry 208, 123.

142. Wilson, I.B. and Bergman, F. (1950)
Acetylcholinesterase. VIII. Dissociation constants of the active group. Journal of Biological Chemistry 186, 683.

143. Zsigmond, E.K. and Downs, J.R. (1971)
Plasma Cholinesterase activity in newborns and infants. Canadian Anaesthetists' Society Journal 18, 278.

Appendix 1. ChE activity of individual subjects studied for this presentation.

(M = Male, F = Female, H = Hausa, N = Other Northernor W = Westerner, and E = Easterner)

S/NO.	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
1.	M - 40 - H	AS(A)	1.59	91.29	52.98
2.	M - 35 - H	AA(B)	0.70	76.54	50.71
3.	M - 25 - H	AA(B)	1.13	87.40	68.39
4.	M - 25 - H	AA(O)	0.87	84.16	18.13
5.	M - 32 - H	AA(A)	0.40	65.13	49.83
6.	M - 30 - H	AA(B)	0.96	79.72	58.75
7.	M - 25 - H	AS(A)	0.99	78.02	49.66
8.	M - 20 - H	AA(B)	0.50	72.33	44.11
9.	M - 18 - H	AA(O)	0.47	70.77	41.14
10.	M - 19 - H	AS(O)	0.76	74.21	55.51
11.	M - 25 - H	AA(B)	0.52	61.61	46.13
12.	M - 24 - N	AA(O)	1.13	77.99	64.84
13.	M - 41 - H	AA(AB)	1.39	83.33	74.94
14.	M - 30 - H	AA(B)	0.70	80.1	59.95
15.	M - 35 - W	AC(B)	0.48	70.98	41.61
16.	M - 36 - H	AA(A)	0.70	80.1	59.95
17.	M - 24 - H	AS(A)	0.44	68.32	22.52
18.	M - 42 - N	AA(B)	1.03	86.55	67.90
19.	M - 37 - N	AA(O)	0.64	78.23	59.32
20.	M - 30 - H	AA(O)	1.2	79.22	72.25
21.	M - 38 - H	AS(O)	0.74	31.18	62.13
22.	M - 23 - H	AS(O)	0.68	80.1	59.95

S/NO.	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
23.	M - 33 - H	AA(B)	0.70	80.1	59.95
24.	M - 45 - W	AS(O)	0.80	82.60	56.39
25.	M - 25 - H	AS(O)	0.89	84.51	55.6
26.	M - 25 - H	AA(B)	0.89	84.51	55.6
27.	M - 40 - H	AA(O)	0.70	80.1	59.95
28.	M - 40 - H	AA(B)	0.76	81.68	52.76
29.	M - 36 - H	AA(O)	1.36	85.27	50.48
30.	M - 23 - N	AS(O)	1.16	85.10	54.53
31.	M - 19 - N	AA(O)	0.93	85.07	50.90
32.	M - 28 - H	AA(A)	0.93	85.07	50.90
33.	M - 20 - H	AS(B)	0.70	80.1	49.88
34.	M - 19 - N	AA(B)	0.87	84.07	54.32
35.	M - 40 - N	AA(O)	0.28	50.30	50.30
36.	M - 35 - H	AA(A)	0.80	64.99	50.94
37.	M - 30 - H	AS(O)	1.03	86.1	67.90
38.	M - 40 - H	AA(B)	0.80	70.86	62.05
39.	M - 35 - H	AS(B)	0.64	78.49	56.74
40.	M - 40 - H	AS(O)	1.05	77.34	52.13
41.	M - 35 - H	AA(A)	0.74	81.35	53.70
42.	M - 20 - H	AA(A)	1.16	73.96	51.94
43.	M - 45 - H	AA(B)	0.62	77.51	54.74
44.	M - 36 - E	AS(A)	0.95	70.84	56.29
45.	M - 26 - N	AA(A)	1.39	84.29	57.67
46.	M - 26 - H	AS(O)	0.70	65.71	51.32
47.	M - 35 - H	AA(B)	0.70	80.1	51.32

S/NO.	Sex-Age-Tribe	Genetype (Blood Group)	ChE(I.U/L.)	D.N.	F.N.
48.	M - 29 - H	AA(O)	0.97	85.79	69.35
49.	M - 35 - H	AA(B)	1.39	81.41	60.79
50.	M - 35 - H	AA(B)	0.80	70.02	56.84
51.	M - 32 - E	AA(O)	0.70	80.1	49.66
52.	M - 22 - H	AA(O)	1.79	78.92	44.40
53.	M - 35 - W	AA(A)	1.09	74.43	50.63
54.	M - 30 - H	AA(A)	1.11	74.96	54.13
55.	M - 45 - H	AA(A)	1.19	80.00	60.00
56.	M - 25 - H	AA(A)	0.95	70.80	53.14
57.	M - 35 - H	AA(O)	0.80	67.92	57.44
58.	M - 25 - H	AA(B)	0.70	74.34	50.12
59.	M - 35 - H	AA(O)	0.40	65.13	29.83
60.	M - 28 - H	AA(A)	0.70	71.46	50.88
61.	M - 25 - W	AA(O)	0.99	84.4	61.74
62.	M - 20 - H	AA(O)	0.54	59.34	26.09
63.	M - 22 - H	AS(O)	0.66	78.88	49.80
64.	M - 27 - H	AS(B)	0.87	70.23	55.34
65.	M - 29 - H	AA(B)	0.80	82.60	56.39
66.	M - 25 - N	AA(A)	0.33	58.5	22.00
67.	M - 28 - N	AA(A)	0.70	77.22	51.79
68.	M - 27 - N	AA(B)	0.52	65.48	46.13
69.	M - 30 - N	AA(B)	0.89	74.07	57.84
70.	M - 33 - N	AA(O)	0.58	69.14	52.01
71.	M - 35 - H	AA(A)	0.70	80.1	50.60

S/NO.	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
72.	M - 20 - H	AA(B)	0.42	66.8	57.2
73.	M - 28 - H	AA(B)	0.70	71.46	49.68
74.	M - 21 - H	AA(O)	0.72	80.65	61,07
75.	M - 20 - H	AA(A)	0.52	77.42	65.43
76.	M - 25 - H	AA(O)	0.70	80.1	59.95
77.	M - 32 - E	AA(B)	0.50	68.54	60.26
78.	M - 35 - H	AS(O)	0.58	76.01	51.44
79.	M - 27 - H	AA(A)	0.42	72.00	59.20
80.	M - 45 - N	AA(O)	0.72	69.82	64.39
81.	M - 22 - N	AA(B)	0.80	75.05	56.18
82.	M - 32 - N	AA(B)	0.50	72.15	50.95
83.	M - 19 - H	AS(O)	0.52	61.61	46.13
84.	M - 40 - H	AA(B)	0.35	60.29	20.1
85.	M - 38 - H	AA(B)	0.50	72.15	50.96
86.	M - 29 - H	AA(B)	0.80	62.47	50.10
87.	M - 24 - E	AS(A)	0.48	70.98	52.79
88.	M - 20 - E	AA(O)	0.70	71.46	50.12
89.	M - 21 - N	AA(O)	0.70	86.57	58.99
90.	M - 20 - N	AA(B)	0.68	79.75	66.10
91.	M - 27 - H	AA(B)	0.70	71.46	50.13
92.	M - 23 - H	AS(A)	0.50	73.57	62.42
93.	M - 25 - H	AA(B)	0.78	70.11	66.67
94.	M - 20 - H	AA(O)	0.70	74.34	59.95
95.	M - 30 - H	AA(O)	0.68	73.58	52.84
96.	M - 19 - H	AA(B)	0.72	72.26	51.52

S/NO.	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
97.	M - 25 - H	AA(A)	0.54	74.22	55.00
98.	M - 20 - H	AS(O)	0.91	76.09	59.48
99.	M - 37 - N	AS(A)	1.07	75.89	52.41
100.	M - 30 - W	AA(O)	0.81	75.61	53.69
101.	M - 22 - W	AS(O)	0.26	46.45	40.00
102.	M - 38 - H	AA(A)	0.80	82.60	57.44
103.	M - 26 - N	AA(O)	0.70	73.36	57.58
104.	M - 31 - N	AA(O)	1.27	79.69	56.88
105.	M - 35 - H	AA(B)	1.02	72.71	53.26
106.	M - 26 - H	AA(A)	0.70	80.10	60
107.	M - 28 - H	AA(A)	0.58	69.90	54.04
108.	M - 23 - H	AA(B)	0.46	70.14	55.04
109.	M - 50 - H	AA(A)	0.62	77.75	51.74
110.	M - 21 - H	AS(O)	0.61	77.26	50.68
111.	M - 35 - W	AS(O)	1.49	85.47	53.40
112.	M - 30 - W	AA(B)	0.64	78.22	51.96
113.	M - 50 - H	AS(A)	0.75	82.6	55.23
114.	M - 40 - H	AS(A)	1.27	78.11	58.45
115.	M - 30 - H	AA(O)	0.83	83.4	58.4
116.	M - 22 - H	AS(O)	0.80	70.86	51.78
117.	M - 21 - H	AS(A)	0.97	75.51	54.11
118.	M - 37 - H	AS(B)	1.56	73.35	49.68
119.	M - 30 - H	AA(B)	1.01	77.02	53.06
120.	M - 24 - H	AA(O)	0.93	80.32	62.43
121.	M - 22 - W	AA(O)	0.93	85.07	56.29

S/NO.	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
122.	M - 20 - W	AA(A)	0.64	78.39	53.31
123.	M - 28 - H	AA(O)	0.95	75.49	50.44
124.	M - 25 - H	AA(B)	0.80	82.71	56.46
125.	M - 22 - H	AA(O)	0.83	70.8	58.2
126.	M - 31 - H	AA(B)	1.31	78.7	60.84
127.	M - 40 - H	AA(AB)	1.33	77.23	62.14
128.	M - 18 - H	AA(B)	0.87	71.20	53.55
129.	M - 29 - H	AS(B)	0.99	85.79	50.84
130.	M - 39 - N	AA(O)	0.95	75.00	50.87
131.	M - 23 - N	AA(B)	1.45	75.40	53.33
132.	M - 29 - N	AA(B)	1.14	70.63	53.45
133.	M - 34 - H	AA(A)	1.39	76.62	57.33
134.	M - 19 - N	AA(O)	1.09	72.12	51.60
135.	M - 32 - H	AA(O)	0.97	69.52	54.50
136.	M - 30 - H	AA(B)	0.56	67.96	40.12
137.	M - 22 - H	AA(O)	0.78	82.15	56.98
138.	M - 30 - H	AS(O)	0.85	69.60	52.60
139.	M - 35 - H	AS(O)	0.64	75.26	54.69
140.	M - 38 - H	AA(B)	0.70	80.10	52.04
141.	M - 30 - H	AA(B)	1.01	86.28	50.08
142.	M - 35 - H	AA(B)	0.87	65.64	43.95
143.	M - 26 - H	AS(O)	1.31	84.10	60.94
144.	M - 26 - W	AS(B)	0.99	77.68	57.05
145.	M - 30 - W	AA(O)	1.25	72.97	61.92
146.	M - 30 - N	AS(O)	0.70	59.95	40.05

S/NO	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
147.	M - 27 - N	AA(A)	0.74	62.13	39.68
148.	M - 25 - H	AA(O)	0.98	76.06	69.60
149.	M - 22 - H	AS(O)	0.70	63.31	45.80
150.	M - 30 - H	AA(O)	0.99	71.98	54.70
151.	M - 36 - H	AS(O)	1.03	77.30	51.97
152.	M - 38 - H	AA(O)	0.89	75.56	54.78
153.	M - 39 - N	AA(B)	0.99	71.98	49.66
154.	M - 46 - H	AA(B)	0.95	70.10	56.29
155.	M - 19 - H	AA(O)	0.80	70.02	53.46
156.	M - 23 - H	AA(O)	1.30	74.71	60.33
157.	M - 28 - N	AS(O)	0.78	64.09	43.87
158.	M - 25 - H	AA(A)	0.78	70.11	56.56
159.	M - 30 - H	AA(A)	0.75	72.22	53.78
160.	M - 30 - H	AA(A)	0.61	77.38	54.5
161.	M - 35 - H	AA(O)	0.61	77.38	52.59
162.	M - 22 - H	AA(O)	0.62	60.16	50.95
163.	M - 32 - H	AA(A)	1.20	76.70	61.22
164.	M - 33 - H	AS(A)	0.97	75.51	59.93
165.	M - 40 - H	AA(O)	0.89	68.84	53.36
166.	M - 30 - H	AA(O)	1.14	75.58	62.23
167.	M - 27 - H	AS(O)	0.93	70.18	56.78
168.	M - 25 - H	AS(A)	1.01	81.74	56.60
169.	M - 30 - H	AA(A)	0.95	78.15	58.39
170.	M - 32 - H	AA(O)	0.80	70.02	50.94
171.	M - 27 - H	AS(B)	1.00	72.07	50.00

S/NO.	Sex-Age-Tribe	Genetypo (Blood group)	ChE(I.U/L.)	D.N.	F.N.
172.	M - 33 - H	AA(O)	0.89	68.84	44.40
173.	M - 27 - H	AA(B)	1.10	74.70	60.30
174.	M - 20 - H	AA(B)	0.93	72.12	55.57
175.	M - 35 - H	AA(A)	0.70	80.10	56.59
176.	M - 27 - N	AA(O)	0.93	72.12	57.91
177.	M - 24 - W	AS(O)	0.99	71.98	56.37
178.	M - 30 - E	AA(O)	1.35	77.90	56.00
179.	M - 42 - H	AS(B)	0.95	70.80	56.99
180.	M - 25 - H	AS(O)	0.70	80.10	59.95
181.	M - 30 - H	AS(O)	0.89	68.84	46.64
182.	M - 31 - H	AA(A)	1.19	71.19	53.29
183.	M - 25 - H	AA(A)	0.70	59.95	45.80
184..	M - 42 - H	AA(O)	0.93	69.96	55.76
185.	M - 38 - H	AA(O)	1.09	74.50	56.64
186.	M - 37 - H	AA(B)	0.89	68.84	46.64
187.	M - 26 - H	AA(B)	0.80	77.57	64.99
188.	M - 25 - H	AA(A)	1.01	72.53	56.6
189.	M - 35 - H	AA(A)	1.11	74.96	56.6
190.	M - 28 - H	AA(A)	0.60	70.03	53.22
191.	M - 22 - H	AS(O)	0.85	71.29	51.17
192.	M - 42 - W	AS(AB)	1.27	78.11	55.30
193.	M - 23 - W	AA(O)	1.36	76.56	56.00
194.	M - 25 - W	AA(A)	1.05	73.53	52.46
195.	M - 31 - W	AA(O)	0.99	71.98	59.39
196.	M - 30 - H	AA(B)	0.99	71.98	52.35

S/NC.	Sex-Age-Tribe	Genotype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
197.	M - 24 - H	AA(B)	0.74	62.13	51.47
198.	M - 24 - H	AA(B)	1.19	74.97	55.68
199.	M - 28 - H	AS(A)	1.33	76.07	54.01
200.	M - 23 - H	AA(A)	1.38	76.66	60.42
201.	M - 25 - H	AA(B)	1.03	73.06	56.13
202.	M - 35 - H	AA(B)	0.70	80.10	55.88
203.	F - 9 - H	SS+F(B)	1.15	75.83	51.66
204.	M - 12 - H	SS+F(O)	1.17	76.24	64.44
205.	M - 12 - H	SS+F(O)	1.70	80.10	59.95
206.	M - 14 - H	SS+F(B)	0.76	81.68	63.13
207.	M - 15 - N	SS+F(O)	0.70	71.46	59.23
208.	M - 15 - W	SS(O)	0.89	72.57	55.6
209.	M - 27 - W	SS(O)	1.09	74.50	53.59
210.	M - 21 - H	SS+F(B)	0.76	63.13	44.81
211.	M - 12 - H	SS(O)	0.70	59.95	49.16
212.	M - 9 - E	SS+F(O)	0.89	84.51	55.60
213.	M - 13 - H	SS+F(O)	0.95	75.00	60.48
214.	F - 19 - H	SS+F(A)	0.70	80.10	59.95
215.	M - 9 - H	SS(B)	0.60	76.75	53.22
216.	F - 30 - H	SS+F(B)	0.50	72.13	50.67
217.	F - 18 - H	SS+F(O)	0.89	84.51	55.60
218.	F - 12 - N	SS+F(O)	0.99	71.98	50.00
219.	F - 16 - N	SS(B)	0.80	64.99	47.59
220.	M - 16 - N	SS+F(A)	1.01	72.53	58.88
221.	M - 11 - H	SS(AB)	0.95	70.80	56.64

S/NO.	Sex-Age-Tribe	Genetype (Blood group)	ChE(I.U/L.)	D.N.	F.N.
222.	M - 9 - H	SS+F(B)	0.58	76.01	51.73
223.	F - 16 - W	SS(O)	0.70	59.95	47.48
224.	M - 12 - H	SS(B)	0.66	75.83	57.51
225.	M - 19 - H	SS+F(B)	0.70	74.34	54.2
226.	M - 15 - H	SS+F(AB)	0.70	74.34	59.95
227.	M - 7 - N	SS+F(B)	0.70	74.34	51.32
228.	M - 17 - N	SS+F(O)	0.74	67.57	56.68
229.	M - 22 - H	SS+F(O)	0.45	70.48	51.67
230.	M - 11 - N	SS(O)	0.70	71.46	54.20

APPENDIX 2

The mean values and standard deviations reported in this work were calculated using Casio fx-550 Scientific calculator (made in Japan).

Comparison of two mean values to determine significance of difference was done using the "student's" t distribution as outlined in Schaum's series (Spiegel, 1961)

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sigma\sqrt{1/N_1 + 1/N_2}}$$

where

$$\sigma = \sqrt{\frac{N_1 S_1 + N_2 S_2}{N_1 + N_2 - 2}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are mean values of two samples

S_1 and S_2 are the standard devations

N_1 and N_2 are sample sizes

$V = N_1 + N_2 - 2$ degrees of freedom.

and σ is population standard deviation.

The calculated t value was compared to a table of percentile values (tp) with V degrees freedom. Values of t less than or equal to tp meant the difference between the two mean values was insignificant (negligible).