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Clinical Norm for Pseudocholinesterase with distribution of atypical enzymes in Ceylonese subjects; with 2 families exhibiting abnormal enzyme.

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Abstract : The ChE, DN and FN of 135 Ceylonese subjects were investigated and a clinical norm established. The ChE lies between 40.9 and 154.5 with a mean of 106.6 and a standard deviation (SD) of 14.1. The DN lies between 21.0 and 100 with a mean of 73.6 and a SD of 14.4. The FN lies between 10.3 and 88.8 with a mean of 48.0 and a SD of 30.7.

The families of 2 individuals sensitive to succinyl choline were investigated for ChE and DN with a view to tracing other members of their families who had low ChE and DN values and observing their inheritance patterns.

1. Introduction

Suxamethinium was first used as a muscle relaxant in 1951 and soon after Bourne et al[1] and Evans and co-workers,[3,4] reported prolonged activity. They attributed this unusual sensitivity to suxamethonium to low values of pseudocholinesterase (acylcholine-acyl hydrolase, ChE) in the patient's blood. Lehmann and co-workers,[16,17] postulated an inherited enzyme defect. Kalow and Genest[10] and Kalow and Staron[12] made it possible by using an enzyme inhibitor, to show the presence of an 'atypical, enzyme. By means of dibucaine (nupercaine) inhibition of the ChE, it was possible to differentiate three inherited serum cholinesterase phenotypes : those homozygous for the usual enzyme, those homozygous for the 'atypical' enzyme, and those individuals who are heterozygous or intermediate. Harris and Whittaker[6] showed that fluoride could also be used in a similar way ; and in a study of families sensitive to suxamethonium, they demonstrated that fluoride inhibition revealed three further phenotypes. These were due to another gene which they termed fluoride resistant. In 1962 Liddell, Lehmann and Silk[18] demonstrated the presence of a 'silent' gene.

Not all prolonged apnoeas following suxamethonium are due to genetic causes. Non-anaesthetic causes may be due to a grossly reduced total quantity of ChE in the blood. In normal individuals the ChE activity varies in certain diseases but unless the activity falls below 25% of the original value for that individual, no adverse.

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reaction is seen to suxamethonium.[23] In those persons who have the atypical enzyme, apnoea occurs even though the ChE is somewhat higher, implying that there is a qualitative difference in the genetically abnormal enzyme.

The present investigation was carried out to (a) establish clinical norms for (1) ChE activity (2) dibucaine and fluoride numbers of Ceylonese subjects, (b) detect the mode of inheritance of atypical forms of ChE in the families of two patients who were sensitive to suxamethonium. These parameters are useful in the treatment of cases of suxamethonium and in tracing their families. It is also an index for the screening of persons before employment in places where they may come in contact with organophosphates or carbamates, and for the later follow up of these persons.[20]

2. Materials

ChE activity, dibucaine and fluoride numbers (DN and FN) were determined in 135 subjects, comprising 100 consecutive donors from the blood bank before screening for Wasserman reaction, and 35 healthy volunteers. No patients were included in this series. The families of two patients who suffered from prolonged apnoea following the administration of suxamethonium were investigated for ChE and DN.

3. Method

ChE activity was measured by the method of Kalow and Lindsay[11] using a Unicam Spectrophotometer Model S.P. 500. The substrate used was benzoyl choline 5×10^{-3} M concentation in M/15 phosphate buffer, at pH 7.4. The measurements were made at 240 mμ at 26° C or at 37° C. All solutions before mixing were pre-incubated at the desired temperature and during the readings, water at that temperature was circulated through the cell compartment of the spectrophotometer.

For the determination of the DN, the inhibitor dibucaine was added to the mixture so that the final concentration of dibucaine was 10^{-5} M,[10] the other parameters being the same. For the estimation of the FN the concentration of the inhibitor sodium fluoride in the final mixture was 5×10^{-5} M.[6] A temperature of 26° C was chosen for the reaction because it was observed by King that the effect of sodium fluoride was more marked at or near 25° C than at a higher temperature. Hence all the FN were done at 26° C and the results corrected to 37° C.[14]

4. Results

The distribution of the ChE activity is shown in figure I. The range of activity is between 40.9 and 154.5 units with a mean value of 106.6 and standard deviation of 14.1 (A unit is equal to the number of micromoles of benzoyl choline hydrolysed in one hour by one ml. of serum at 37° C). This range is equivalent to 380—1430 I.U. units with a mean of 986.6 and S.D. of 130.5 as shown in Figure I. The DN lies between 21.0 and 100 with a mean of 73.6 and a S.D. of 14.4. The FN lies between 88.8 and 10.3 with a mean of 48.0 and a S.D. of 30.7 (Figure II & III). The correlation between DN and FN is provided in Figure IV.

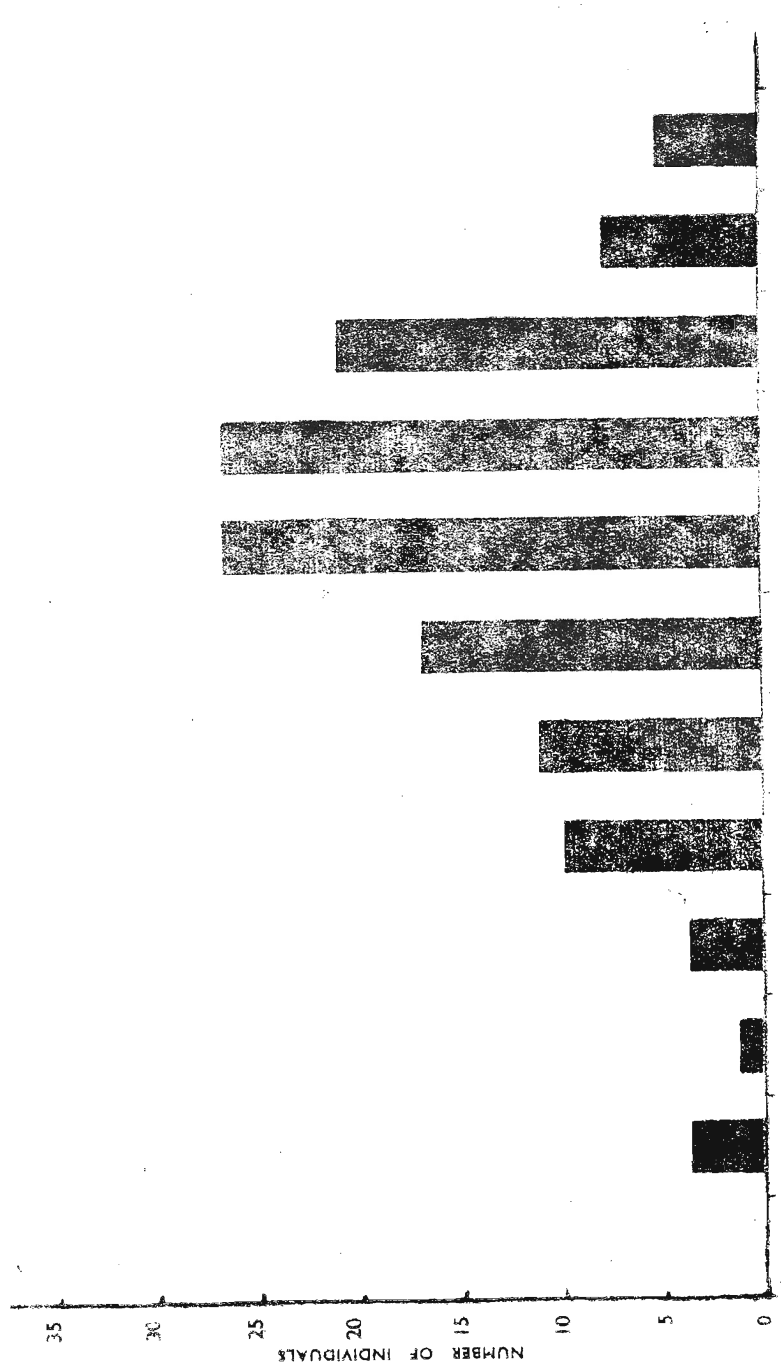

Fig. 1 Distribution of pseudocholinesterase activity among Ceylonese subjects
The activity was determined on serum as described under 3.1.

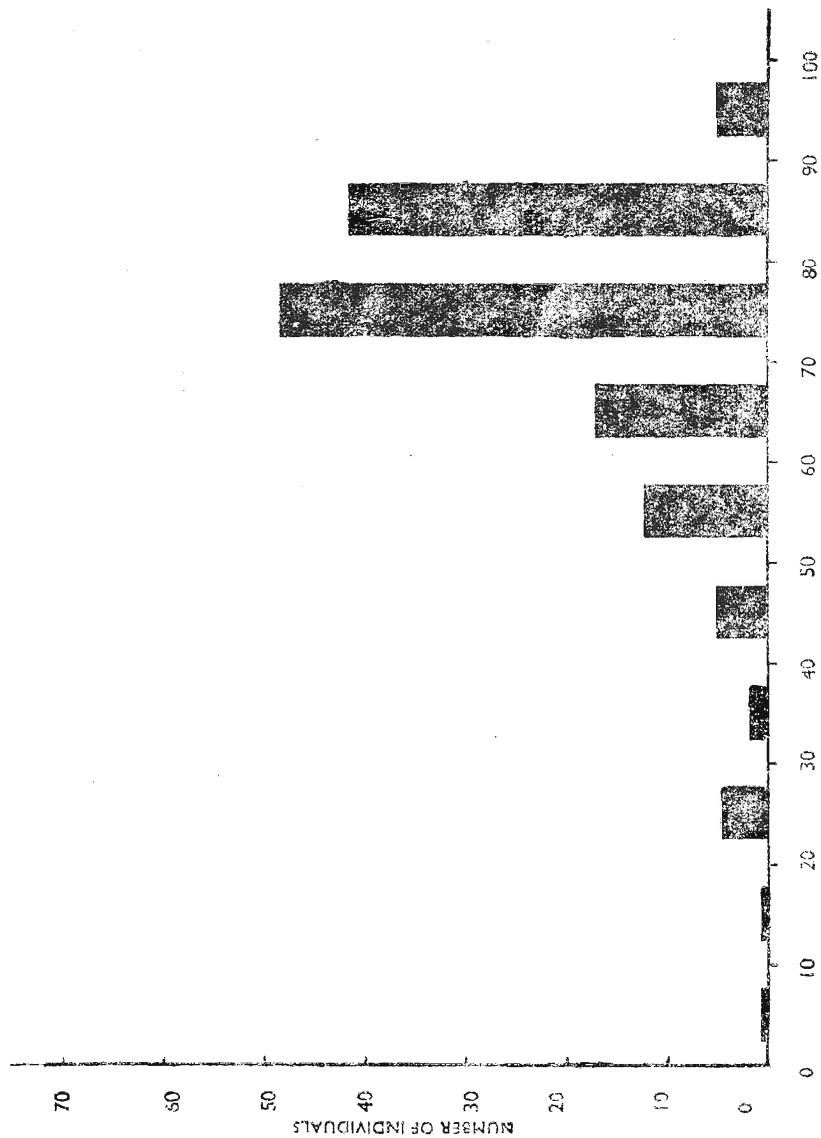

Fig. 11 Distribution of dibucaine numbers on Ceylonese subjects. The pseudocholinesterase activities were determined under 3.2 with and without the addition of dibucaine to the system. From the results, the percentage inhibition calculated (DN).

5. Discussion

There is considerable variation amongst different workers in the mode of expression of their results,[7] and this makes comparison of values for ChE difficult. The variation in the units used is due to the differing techniques and different substrates that are used. Since an I.U.[8] is defined as the number of micromoles of benzoyl choline hydrolysed per minute by one millilitre of serum at 25°C, we could, by expressing our results in mU/ml compare them with the results of Kalow and Lindsay,[11] as converted by King.[12] The normal range of values obtained by Kalow and Lindsay[11] is 620 to 1370 mU/ml at 25°C. Our results range from 388 to 1468 mU/ml at 25°C, with a mean of 1008 ± 134. It is not possible to compare our results with those of other workers as they have used substrates other than benzoyl choline.[2,9,11]

The activity of both acetyl and acyl hydrolase is reduced in the blood of patients with various types of anaemia and returns to normal after treatment.[13] Malnutrition and debilitating diseases are accompanied by low serum cholinesterase activity.[22] It has been suggested that not only are serum albumin and serum cholinesterase both formed in the liver but that their synthesis is carried on in parallel. The low levels of albumin and ChE in malnutrition and cirrhosis are then a consequence of diminished production due to the depletion of raw material and synthesising mechanisms.[13] These factors may explain why some low values are lower than the lowest values reported by Kalow and Lindsay.[11] Of the 135 subjects studied only 6 (4.5%) had values below 620 mU/ml, which is the lowest value obtained by Kalow and Lindsay.[11] Blood donors are screened for haemoglobin percentage and it is unlikely that these six persons would have been from that group. These six persons may have been from the volunteers in whom the haemoglobin percentage was not done. 40% of the population in 1970 were from the lower socio-economic group.[21] It is therefore possible that these six persons were within that group. This aspect was not investigated further.

The frequency of distribution of dibucaine and fluoride numbers in the population is shown in figures II and III.

Figure II indicates that 78% of the subjects have DN between 21.0 to 100 with a mean of 73.6 and a S. D. of 14.4. It is the usual practice to describe DN data using 3 groups viz.

1. High, usual or typical
2. Intermediate,
3. Low, rare or atypical

the dividing line between the groups being chosen at 70.0 and 20.0.[12] In our results we have observed that 97 subjects had values over 70.0 and therefore were usual, 38 subjects were between 20.00 and 69.0 and were intermediate, while there were no

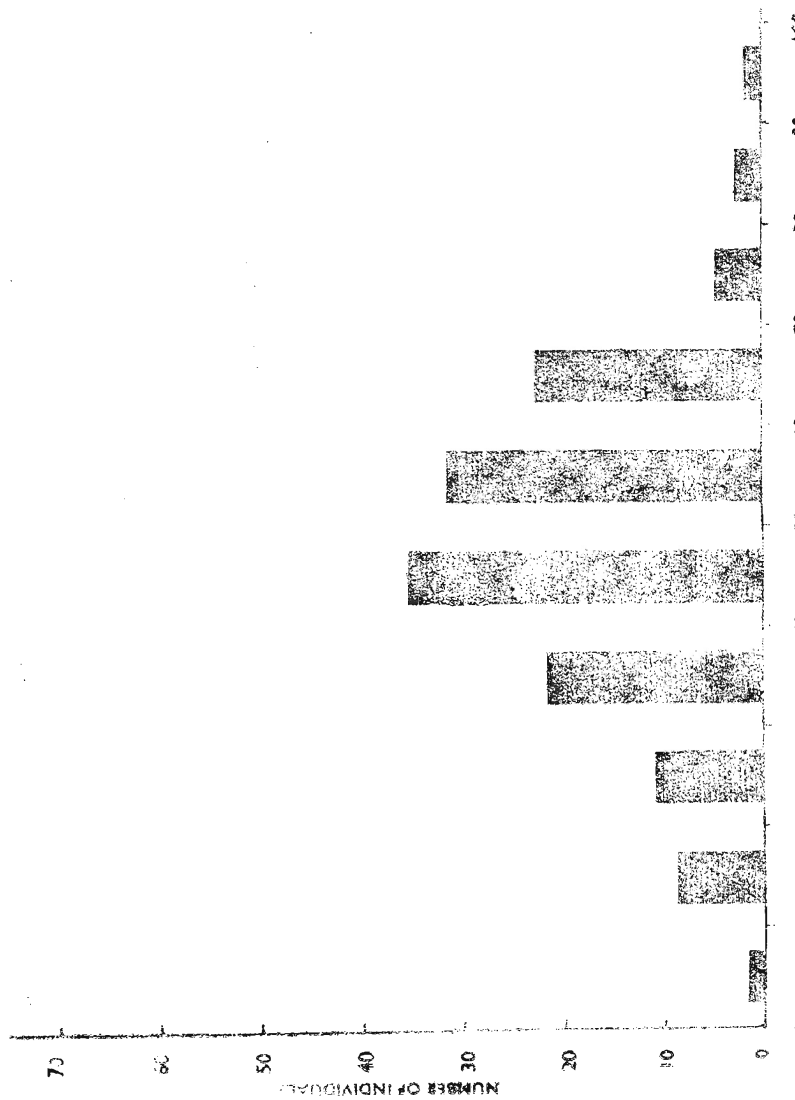

Fig. 1[illegible] Distribution of fluoride numbers among Ceylonese subjects. The pseudocholinesterase activities were determined as described under 3.2 with and without added sodium fluoride to the system. From the results, the percentage inhibition calculated (FN).

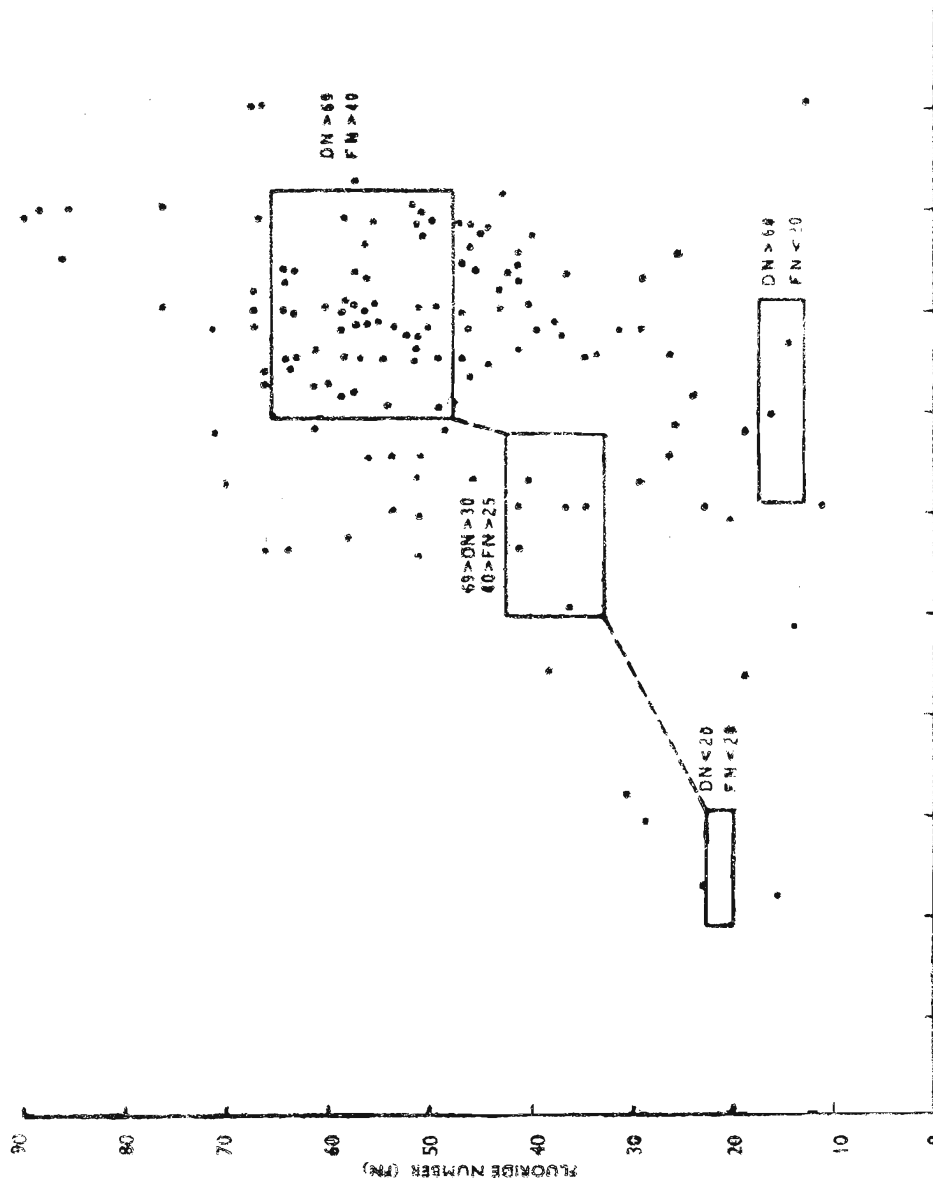

Fig. IV Plot of dibucaine numbers (DN) against fluoride numbers (FN). The DN and FN were estimated as described under 3.2.

persons below 20.0. Their survey however did not contain a single case between 20.0 and 43.0.[12] Our results are not in agreement with theirs ; we did not observe any cases between 31 and 43.

The frequency of distribution of the FN is shown in figure III. The values are considerably lower than those obtained by other workers in spite of the fact that the FN was done at 25°C for reasons referred to earlier. The FN lies between 10.3 and 88.8%, with a mean of 48.0 and a S. D. of 30.7. The plot of DN against FN is provided in Figure IV. It is seen from the Figure IV that the scatter of the FN was very wide and we were not able to group the FN into three distinct groups with small sub-groups in the manner of Harris and Whittaker.[6] The workers explain their sub-groups by the hypothesis that matings may have occurred between the I (intermediate) and U (usual) groups, (Liddell, Lehman and Davies as quoted by Lehmann and Liddell[16]). Our sample probably is more heterogenous, being an insular race, thus giving the wider scatter (Figure IV).

During the six month period of this study, we received many samples of blood for ChE estimation of which 5 were from patients who had true suxamethonium apnoea ; they alone had low ChE. Of these only two families could be traced to any degree. The data of the two investigated families is shown in detail in figures V and VI, and are compiled in geneological trees. The probands of both these families were patients who underwent caesarian section and are indicated by arrows.

In Figure V, the proband Mrs. W had a ChE of 22.5 with a DN of 15.0. Her baby's ChE was 12.5% estimated by the rapid field test method.[20] We were compelled to use this method as the mother refused permission to draw more blood from the baby. In view of this, we were not able to carry out the DN estimation.

The proband's brother also had a low ChE activity of 27.3 with a DN of 66.7, classifying him in the intermediate group, while the proband herself falls in the atypical group. In the previous generation (II) the proband's father appeared normal with ChE 104.0 units and DN 82.0 while her maternal uncle was found to be in the intermediate group with a ChE of 104.5 units and a DN of 69.0. The proband's mother was deceased and was probably either atypical or intermediate since the proband's father was normal and the maternal uncle showed the same defect ; since the proband was atypical it may be concluded that her mother was atypical. Generation I could not be studied.

In Figure VI, Mrs. A the proband, had a ChE of 15.3 with a DN of 33.3. Her baby's ChE was 25% and also estimated by the rapid field test method for the same reason as in the previous case. Mrs. A falls into the intermediate group. Mrs. A's brother and one sister could not be investigated. Her other sister had a ChE of 52.7 with a DN of 55.0 and thus was also in the intermediate group. Of the latter's children,

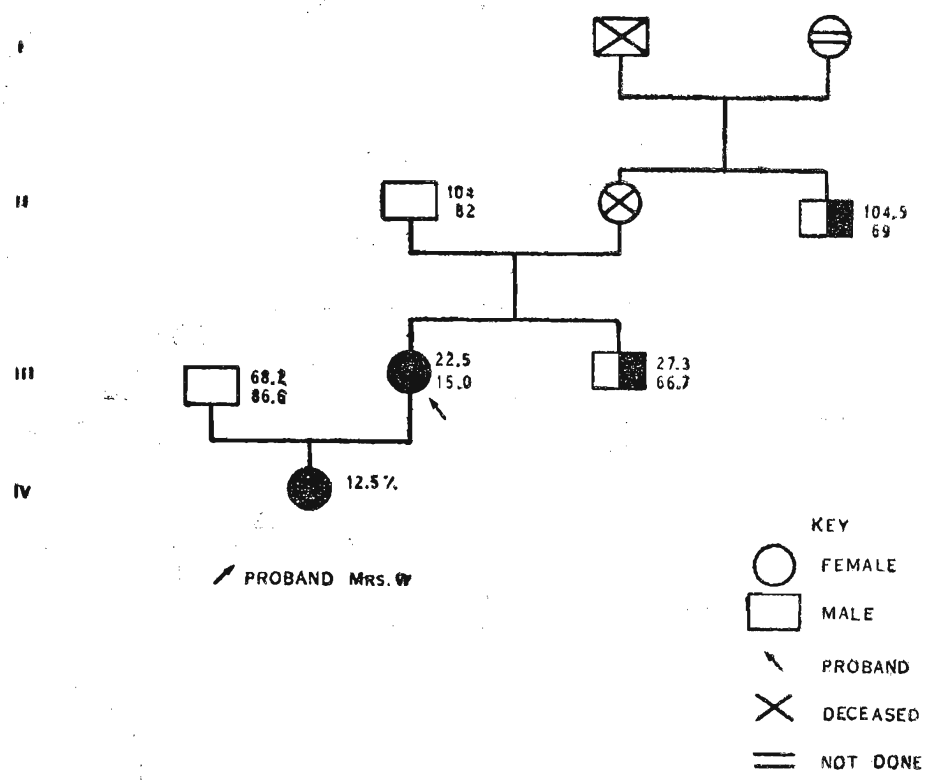

Fig. V Geneological Tree of Mrs. W who had suxamethonium apnoea during a caeserian section. ↗ — proband ; ○ — female ; □ — male ; unshaded — normal ; half shaded — intermediate ; fully shaded — abnormal ; crossed ones — deceased; = — not done, (either dead or could not be traced). Top values ChE; bottom values, DN.

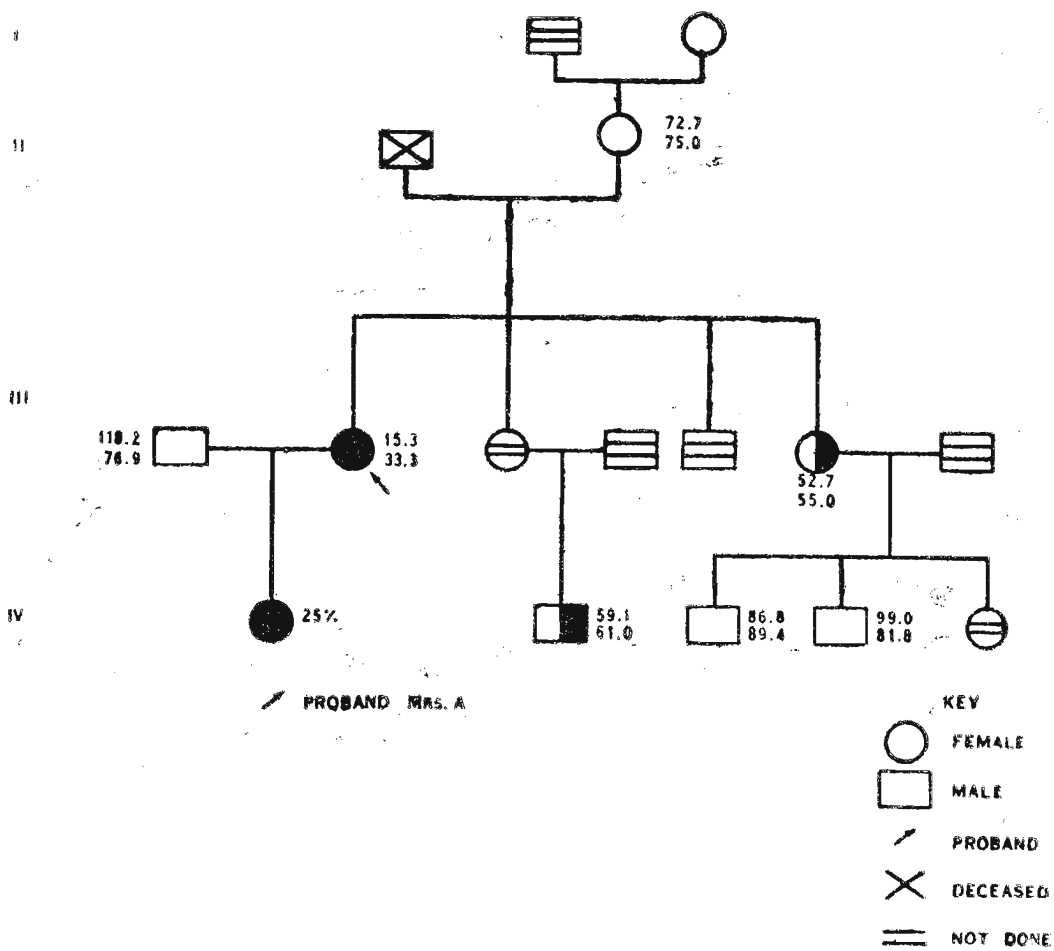

Fig. VI Geneological Tree of Mrs. A who had suxamethonium apnoea during a caeserian section. ↗ — proband ; ○ — female ; □ — male ; unshaded — not normal ; half shaded — intermediate ; fully shaded — abnormal ; crossed ones — deceased; = — not done, (either dead or could not be traced). Top values ChE; bottom values, DN.

two were typical while the third could not be investigated. The proband's other nephew was intermediate having ChE of 59.1 units with a DN of 61.0. Since the proband's mother was typical with ChE 72.7 units and DN 75.0 the defect was transmitted by the proband's father (deceased) who was either intermediate or atypical.

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