MATERIAL AND METHODS
This study was conducted on patients admitted to the M.L.B. Medical College and Hospital, Jhansi, from August, 1981 to February, 1982. The cases were chosen from patients undergoing various surgical procedures under General Anaesthesia.

**SELECTION OF CASES:**

Apart from the normal healthy subjects chosen as control group, the selection of other cases was done on the basis of some factors known to influence serum cholinesterase level. A total No. of 93 cases belonging to both sexes and various age groups from (0-10) years to (61-70) years were divided into :-

1. **Control Group:** This constituted of 26 normal healthy individuals with no apparent factor likely to have any bearing on the serum cholinesterase level.

2. **Anaemia and Malnutrition:** This included 21 individuals with haemoglobin levels less than 10 gm. % or with a history of marked weight loss and evident by their asthenic to cachectic look. These patients often complained of listlessness, easy fatiguability and were prey to frequent infections of various types. Borderline cases of malnutrition were not included in this study.

3. **Pregnancy:** This comprised of 15 women who underwent Caesarean section or medical termination of pregnancy.
Fig. 4 - SHOWING THE DISTRIBUTION OF POPULATION INTO DIFFERENT STUDY GROUPS.
4. Post-Partum: Six patients in early puerperium who underwent abdominal ligations composed this group.

5. Dehydration: This group contained a total of nine patients with acute abdominal conditions having clinically evident dehydration.

6. Malignancy: This group comprised of four patients with malignancies proved on histopathological examination.

7. Liver and Biliary diseases: A group of three patients with liver dysfunction proved by albumin levels, less than 3 gm. % (normal 3.5 to 5.5 gm. %) and abnormal liver function tests.

8. Mental disorder: Making up this group were three patients of diagnosed mental disorder who underwent electroconvulsive therapy or some other necessary operation.

9. Thyroid disorder: Assembled in this group were four patients of thyroid conditions such as thyroid adenoma, goitre and mild thyrotoxicosis.

10. Obesity: This group had two patients both grossly overweight in respect to their frame and height.

11. Children: The group consisted of six normal healthy children between 10 and 14 years of age.

Due to the presence of multiple factors in some (7) patients, they were placed separately under more than one group.

The patients were classified into high, high middle, low middle and low socioeconomic groups on the basis of a criteria prepared for the purpose (Appendix II).
The patients were also grouped according to their place of residence (Fig. 5).

**PREOPERATIVE ASSESSMENT** :-

The patients included in the study were clinically examined preoperatively before any premedication was administered. Details were recorded on a proforma prepared for the purpose (Appendix I).

**SAMPLE COLLECTION** :-

5 ml. blood was withdrawn before operation into a clean dry test tube. As soon as clot retraction and serum separation had occurred, the serum was withdrawn and kept in a plain vial which was maintained in a deep freezer at -20°C till needed for estimations.

**PREMEDICATION** :-

Patients were premedicated with 0.01 mg./kg. of body weight of Atropine. Narcotic analgesics were not used for premedication to avoid any influence on the duration of apnoea.

**ANAESTHESIA** :

The patients were induced with sleep dose of 2.5% intravenous (I.V.) thiopentone, followed immediately by a 1 mg./kg. I.V. dose of suxamethonium. The stopwatch was switched on to record the start of suxamethonium apnoea as heralded by disappearance of reservoir bag movement.

Patients were oxygenated with mask and intermittent positive pressure ventilation (I.P.P.V.) followed by direct vision intubation and connection to the Boyle's apparatus.
Fig. 5: PIE CHART SHOWING RESIDENTIAL DISTRIBUTION OF THE STUDY POPULATION.

- Rural
- Urban
The chest was auscultated. The patient was subsequently maintained with oxygen and nitrous oxide at the rate of 12-16 min. The I.P.P.V. was withheld every 1/2 minute to catch the first return of respiratory effort as noted by reservoir bag movement. The stopwatch was then switched off.

**METHOD OF SERUM CHOLINESTERASE ESTIMATION:**

The serum cholinesterase was estimated by the Steinitz et al. modification (1963) of Rappaport et al. method (1959). This method is based on the decolourisation of meta-nitrophenol by the acetic acid freed during the action of cholinesterase on acetyl choline substrate. This decrease in colour is a measure of the acetic acid formed and hence of the enzyme activity.

For "atypical" and "fluoride-resistant" cholinesterases additional substrates containing dibucaine for the former and sodium fluoride for the latter were used.

**REAGENTS:**

1. Buffer - (a) 6.65 gm. anhydrous disodium phosphate (Na₂HPO₄) and 0.43 gm. of monopotassium phosphate (KH₂PO₄) were dissolved in about 200 ml. of distilled water.
2. 0.30 gm. of m-nitrophenol was dissolved in about 200 ml. of distilled water while heating it slightly. After mixing (a) and (b) and adjusting pH to 7.8 by the addition of 0.1 N NaOH, the volume was made upto 1000 ml. with distilled water.

The colorimetric reading of this solution was about 300 in the Klett Summerson colorimeter. If it was higher, dilution with the same buffer (6.65 gm. Na₂HPO₄ and 0.43 gm. KH₂PO₄ made upto 1 L. and adjusted to pH 7.8) was done.
(2) Acetyl choline chloride or bromide (15%) (kept in refrigerator) - 0.1 ml. in 3.5 ml. final volume resulted in approximately $2 \times 10^{-2}$ M solution.

(3) 0.9% NaCl.

(4) 0.1 N Acetic Acid.

(5) (a) Dibucaine (mol. wt. 380). Stock solution of 44.3 mg. in 100 ml. distilled water.

(b) Stock solution was diluted 10 times before use (0.3 ml. in 3.5 ml. final volume resulting in $10^{-3}$ M concentration).

(6) Sodium Fluoride - 210 mg./L. This resulted in a $5 \times 10^{-3}$ M solution.

(7) Neostigmine - (ampoules of 0.5 mg./ml.).

**PROCEDURE:**

Three test tube designated T (test), D (dibucaine) and F (fluoride) were used for each determination. Two additional test tubes, S (standard) and B (blank) were used for each series of determination. For all above determinations haemolysed or turbid serum was not used, specially so in case of the Standard and Blank tests where in addition to above the serum was not icteric also.

Contd. .........
To the test tubes T, D, F, S and B were added reagents in the order and amounts shown in the table below:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>T (ml.)</th>
<th>D (ml.)</th>
<th>F (ml.)</th>
<th>Standard (ml.)</th>
<th>Blank (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Serum</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>* Buffer</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>* 0.9 % Nacl</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>* Neostigmine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>* 0.1 N Acetic acid (0.1 ml. corresponds to 100 units)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>* Dibucaine (diluted)</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Sodium Fluoride</td>
<td>-</td>
<td>-</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>* Acetyl choline</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>WATER</td>
<td>-</td>
<td>BATH</td>
<td>-</td>
<td>25°C, 30 min.</td>
<td>-</td>
</tr>
<tr>
<td>* Neostigmine</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results were read on the Klett-Summerson colorimeter. The absorbance was read at 420 millimicrons (filter 42), setting to zero absorbance with water.

For values of cholinesterase above 96 units/ml., the serum was diluted with an equal volume of the sodium chloride solution and the test was repeated taking 0.1 ml. of diluted serum and multiplying the result by 2.

**CALCULATIONS:**

1. Cholinesterase units = \( \frac{\text{Standard units}}{B - St} \) x (B - T)
2. Activity after Dibucaine = \( \frac{\text{Standard units}}{B - St} \) x (B - D)
3. Dibucaine Number = 100 - \( \frac{100 \times \text{Dibucaine Activity}}{\text{Cholinesterase units}} \)
4. Activity after Fluoride = \( \frac{\text{Standard units}}{B - St} \) x (B - F)
5. Fluoride Number = 100 - \( \frac{100 \times \text{Fluoride Activity}}{\text{Cholinesterase units}} \)