MATERIAL AND METHODS

The case material for the present study consisted of healthy junior doctors, patients attending the O.P.D. and wards of M.L.B. Medical College, Hospital, Jhansi.

Informed consent was taken from every case. In each case a detailed history was taken, meticulous clinical examinations done and investigations carried out. The cases were divided into following categories -

GROUP A

It consisted of 10 healthy doctors in age group of 20-30 years.

GROUP B

It consisted of 10 patients of hypertension in age group of 25-60 years.

GROUP C

It comprised of 10 patients suffering from IHD diagnosed on the basis of clinical history and 12 lead ECG (report of WHO task force on standardization of clinical nomenclature, 1979) in the age group of 20-65 years.

GROUP D

It consisted of 8 patients of NIDDM (maturity onset and 3 patients of IDDM (juvenile onset).

A detailed history was elicited to assess the amount of fat consumed daily and weekly by these subjects.
in their usual routine diet. Specific consideration was given to record the weekly amount of ghee and its type (saturated/unsaturated) oil and its type, milk and milk products, eggs and food additives. All the healthy subjects (Group A) were hostlers eating a common type of food in hostel messes. Thus per head consumption of fat was calculated by giving consideration to the total amount of oil, ghee purchased monthly and number of members eating in the same mess.

Any recent change in diet, oral or parenteral modification before and during the study were noted. Hospitalised patients were given diet from hospital for one week prior to the test.

DESIGN OF TEST

All the subjects were asked to have their dinner at around 6 PM on the previous day and then after they were instructed to have nothing in the night except water till the next morning when the test is over. Fasting blood samples were collected about 8 AM next morning in the recumbent posture without producing venous stasis (Koerserman et al, 1961). After this they were given the test meal consisting of three boiled eggs with 250 ml sweetened milk, which supplies approximately 750-800 mg of egg yolk cholesterol. Thereafter three postprandial blood samples were taken at an hourly intervals for three consecutive hours. During this whole procedure the subjects were not allowed
to take any thing orally except water and full instructions were given before hand to each subject to be completely relaxed and make no considerable movements during the test. Smoking was strictly prohibited during the test procedure.

Plasma was separated from each sample and the following tests were performed.

1. **SERUM TOTAL CHOLESTEROL (STC)**

   The estimation was done by one step method utilizing the kit provided by Ortho Diagnostic system, Ethnor Division.

   **Procedure**

   Three test tubes are taken and labelled as Test (T), Standard (S) and Blank (B) and then:

<table>
<thead>
<tr>
<th>Test (T)</th>
<th>Standard (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ortho cholesterol reagent</td>
<td>4 ml</td>
<td>4 ml</td>
</tr>
<tr>
<td>Serum</td>
<td>20 ul</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol standard (250 mg%)</td>
<td>-</td>
<td>20 ul</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

   Mix contents of each test tube simultaneously for 10 seconds and immediately place them to a boiling water bath for exactly 45 seconds followed by cooling with running tap water or cold water for 5 minutes. Dry the exterior of tube, mix their contents.
Measure optical density (OD) of each solution at 560 nm (range 560 to 600 nm). Set blank at calorimetric zero and calculation is done as

\[
\text{Cholesterol concentration (mg\%)} = \frac{\text{OD (T)}}{\text{OD (S)}} \times 250
\]

(Cholesterol mg/dl or mg\%/38.7 = mmol/l).

(Range of normal expected values = 150-250 mg\%).

2. **SERUM TRIGLYCERIDES (STG)**

It was estimated by using GPO-PAP method for quantitative determination (Enzymatic method) of STG.

**Procedure**

The reagents in kit are:

- 4 vial of reagent I (Lyophilised enzymes)
- 1 vial reagent II (phenol solution)
- 1 vial of triglyceride standard (300 mg\%)

Reconstitute reagent I in 2.5 ml of distilled water, while reagent II and triglyceride standard are supplied ready to use.

<table>
<thead>
<tr>
<th></th>
<th>Test (T)</th>
<th>Standard (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent I</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Reagent II</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
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</table>

Mix well and add

<table>
<thead>
<tr>
<th></th>
<th>20 ul</th>
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<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglyceride standard (300 mg/dl)</td>
<td>-</td>
<td>20 ul</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>20 ul</td>
</tr>
</tbody>
</table>
Mix and incubate in water bath at 37°C for 10 minutes.

Add distilled water & mix 2 ml 2 ml 2 ml

Take reading by measuring optical density (OD) of each solution at 500 nm (500-530 nm). Set blank at calorimetric zero. Calculation of STG is done as:

\[
\text{STG in test sample (mg\%)} = \frac{\text{OD (T)}}{\text{OD (S)}} \times 300
\]

(for conversion \( \text{mmol/l} = \text{mg/dl} \times 0.0114 \))

(Normal expected values = 30-150 mg%).

This test assay has validity for values up till 600 mg% only. If higher values expected then dilute serum suitably with distilled water and multiply value by dilution factor.

3. SERUM HIGH DENSITY LIPOPROTEINS (HDL)

It was estimated by kit provided by Ortho Diagnostic System, Ethnor Division.

Procedure

Two phases of test are involved:

Phase I: Lipogent™ agent (prepared solution for ready use) is used with serum to precipitate LDL and VLDL so that only HDL fraction remains.

Phase II: The supernatant of the above centrifuged solution is proceeded on with cholzyme-M kit for estimating HDL.
**Phase I**

Mix 0.5 ml of lipogen™ with 0.5 ml of test serum. Keep at room temperature for 10 minutes and then centrifuge at 2000 rpm for 20 minutes to obtain a clear supernatant.

**Phase II**

Dilute one part of cholzyme M standard (200 mg%) with seven parts of distilled water = Cholzyme™ working reagent.

<table>
<thead>
<tr>
<th></th>
<th>Test (T)</th>
<th>Standard (S)</th>
<th>Blank (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholzyme™ working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Supernatant</td>
<td>100 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Working standard</td>
<td>-</td>
<td>100 ul</td>
<td>-</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>-</td>
<td>100 ul</td>
</tr>
</tbody>
</table>

Mix and incubate in water bath at 37°C for 15 minutes.

Add distilled water 4 ml 4 ml 4 ml

Mix and read optical density (OD) by 515 nm (Range 500 - 530 nm) within 60 minutes.

Calculation is done as:

\[
\text{HDL concentration in test serum (mg\%)} = \frac{\text{OD (T)}}{\text{OD (S)}} \times 50
\]

(Conversion into SI unit mmol/l = mg%/38.76)

(Range of normal expected values = 30-60 mg%).
4. **SERUM VERY LOW DENSITY LIPOPROTEINS (VLDL)**

It was calculated by using formula given by Friedwald et al (1972):

\[ VLDL \text{ (mg\%)} = \frac{STG}{5} \text{ (This formula is valid only up to STG value \( \leq 500 \text{ mg\%} \)).} \]

5. **SERUM LOW DENSITY LIPOPROTEINS (LDL)**

It was also calculated by the formula given by Fredrickson DS (1972).

\[ LDL \text{ (mg\%)} = STC - (STG/5 + HDL) = STC - (VLDL + HDL) \]

Charts were made for individual subjects and the pattern of change of lipid lipoprotein profile was noted. Remarks were specifically given for any marked change in any factors viz. marital status, occupation, physical activities, dietary habits, smoking, alcoholism, CAD in family history, fat consumption per day, history of any drug intake and finally conclusion was drawn regarding the change in lipid levels.

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<table>
<thead>
<tr>
<th>HCFB</th>
<th>Cholesterol (mg%)</th>
<th>Fat (gm%)</th>
<th>Saturated fat (gm%)</th>
<th>Polyunsaturated fat (gm%)</th>
<th>Monounsaturated fat (gm%)</th>
<th>P/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 egg (Hen)</td>
<td>250</td>
<td>6.0</td>
<td>2.2</td>
<td>0.85</td>
<td>3.00</td>
<td>0.4</td>
</tr>
<tr>
<td>Butter (25 gm)</td>
<td>70</td>
<td>21.5</td>
<td>12.9</td>
<td>0.64</td>
<td>7.95</td>
<td>0.05</td>
</tr>
<tr>
<td>250 ml milk (sweetened whole fat buffalo)</td>
<td>27</td>
<td>22.0</td>
<td>13.2</td>
<td>0.66</td>
<td>8.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Crystalline cholesterol</td>
<td>500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Slices (4)</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Fat, cholesterol and fatty acid contents were calculated on the basis of values given by Swaminathan MS: Essentials of Food Nutrients, (1974).