Chapter III

METHODOLOGY

In this chapter the subjects, selection of haematological and body composition variables, criterion measures, design of the study, procedure of establishing instrument reliability and tester reliability, procedure of administration of tests, administration of exercise programme and analysis of data are described.

Selection of Subjects

From the list of 200 students studying in eighth, ninth and tenth grades of Rabbani School, Gwalior, 60 male subjects were selected randomly by using the table of random numbers. It was ensured from the health examination records of the subjects maintained as a part of regular school procedure that all the subjects were medically fit for going through the experimental requirements of the project. According to the school records, the average age was fifteen years, ranging from fourteen to seventeen years.

The requirements of the project were explained to all the subjects in the presence of their doctor and all of them agreed voluntarily to undergo testing and training programme. The procedure of testing blood sample and the harmless nature of taking such samples under strict conditions of hygiene and sterility was explained to them so that the subjects would not have any reservation in this matter.
The subjects were randomly assigned to three experimental groups and one control group, each consisting of fifteen subjects. The Experimental groups were aerobic, anaerobic and combination of aerobic and anaerobic.

It was heartening to note that subjects took this as a challenge and were very enthusiastic to take part in the study to prove their mettle.

**Selection of Haematological and Body Composition Variables**

A feasibility analysis as to which of the variables could be taken up for investigation, keeping in mind the availability of equipments, instruments, acceptability to the subjects and the legitimate time that could be devoted for the tests was done in consultation with the experts. With the above criteria in mind, the following haematological and body composition variables were chosen for the study.

I- Haematological Variables :

1. Formed Elements
   a) Red Blood Corpuscles (total count)
   b) White Blood Corpuscles (total count)
   c) Haemoglobin.

2. Plasma Constituents.
   a) Serum Cholesterol
   b) Serum Protein.

II- Body Composition Variables :

a) Total Body Weight
b) Body Fat
c) Lean Body Weight.
Criterion Measures

The criterion measures chosen for testing the hypotheses were:

1. Red Blood Corpuscles count in millions per cubic millimetre of blood.

2. White Blood Corpuscles count in thousands per cubic millimetre of blood.

3. Haemoglobin content recorded in gram/dl.

4. Amount of Serum Cholesterol in the Blood recorded in mg/dl.

5. Amount of Serum Protein (Total) in the blood recorded in gram/dl.

6. Body Weight recorded in kgs.

7. Body Fat recorded in Kgs.

8. Lean Body Weight recorded in Kgs.

Experimental Design

Random group design was used for the experimental study because it was considered the most appropriate. The subjects numbering 60 were equally divided into three experimental groups and one control group. Each group consisted of 15 subjects. The experimental treatment to each of the three groups was assigned at random by drawing lots. The experimental groups were given aerobic, anaerobic and combination of aerobic and anaerobic training programme for a period of ten weeks excluding the period utilized for pre-test and post-tests. The control group did not participate in any activity during the experimental period. The training for the
three experimental groups were given thrice a week i.e. on Mondays, Wednesdays and Fridays.

**Instrument and Tester Reliability**

To ensure collection of accurate and precise data and also to ascertain investigator's ability to administer tests on selected haematological and body composition variables, the reliability of the instruments and the tester was established.

**Instrument Reliability**

The Colorimeter (Model CL 20-A - See Figure 1, Page 55) was available in the Pathology Laboratory of the College. This instrument was supplied to the college by Elico Pvt. Ltd., Hyderabad, INDIA one of the reputed firms in the country.

The other instruments such as Automatic Pipette (Eppendori Geratebau Netheler + Hinz ComBh, Hanburg, Germany - See Fig. 2, Page 55), Haemocytometer (Pein - Optic, Jena, GDR - See Fig. 3, Page 56) and Lange Skin Fold Caliper (See Fig. 4, Page 56) were used to assess the haematological and body composition variables.

**Tester Reliability**

For administering tests in selected haematological variables, the procedure as described in the Text Book of Practical Physiology\(^1\) and

Fig. 1. Calorimeter.

Fig. 2. Automatic Pipette
Fig. 3. Haemocytometer.

Fig. 4. Lange Skin Fold Caliper.
and Practical Haematology\textsuperscript{2} was strictly followed. Reagent kits supplied by the Span Firm only were used in blood analysis to ensure accurate results.

The investigator had a number of practice sessions under the guidance of Mr. A.K. Tomar\textsuperscript{3} to ensure accuracy of measurements. Finally, tests in all the selected blood variables were conducted on the five subjects selected at random. The coefficient of correlation computed between the results obtained by the investigator and expert are presented in Table 1.

Table 1

TESTER COMPETENCY FOR TESTS IN SELECTED HAEMATOLOGICAL VARIABLES

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient of Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Corpuscles</td>
<td>0.93</td>
</tr>
<tr>
<td>White Blood Corpuscles</td>
<td>0.95</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>0.96</td>
</tr>
<tr>
<td>Serum Cholesterol</td>
<td>0.92</td>
</tr>
<tr>
<td>Serum Protein</td>
<td>0.92</td>
</tr>
</tbody>
</table>


\textsuperscript{3}A.K. Tomar, I/C Pathology Laboratory, Lakshmibai National College of Physical Education, Gwalior.
Procedure of Administration of Tests

The tests for haematological and body composition variables were administered to the subjects belonging to all the four groups in the health centre of Rabbani School. The blood samples were collected there and tested in the Pathology Laboratory of the Lakshmibai National College of Physical Education, Gwalior.

Collection of Blood Samples

Sterilization

All syringes were sterilized before drawing the blood samples, at 210° C, in the hot air oven. All vials were cleaned proper in the chromic acid and rinsed with distilled water.

Collection of Blood

First of all, the student's arm was cleaned with diluted savlon and again cleaned with rectified spirit. 5 ml. of blood was drawn with minimum stress from each student. Out of 5 ml. of blood 1 ml. of blood was delivered in EDTA (Ethylenediamine Tetra-acetic Acid) vial and shaken properly for anticoagulation. Remaining 4 ml. of blood was delivered in plain vial and kept in the incubator for clotting and retraction.

The following procedures were employed for testing the subjects:

Red Blood Corpuscles (Total Count)

Apparatus

Haemocytometer, Micro Pipette, Cotton, 75x12 mm. tube (Borosil).
Sample

Anti-coagulated Blood (E.D.T.A.)

Reagent

R.B.C. fluid.

Procedure

The blood was sucked into the R.B.C. Pipette exactly upto .05 mark on the pipette and any excess of blood was wiped out from the tip of the pipette with a blotter. The tip of the pipette was immediately plunged into the diluting fluid (R.B.C. Fluid) placed in a watch glass. The fluid was sucked upto the mark"101" into the same pipette and during the process of sucking the pipette was gently rotated. When the fluid was sucked in, the blood moved into the bulb of the pipette being pushed by the diluting fluid so that in the end there was blood and fluid in the bulb and only fluid in the pipette upto the mark "1". The fluid in the pipette upto the mark "1" did not take part in dilution of the blood. The blood upto the mark "0.5" in the pipette was actually diluted by the volume of fluid present in the bulb i.e. a dilution 200 times of its own volume.

The cover slip was placed on the side pillars of the haemocytometer slide so that it lay centrally. The fluid in the pipette was thoroughly mixed by rotating it repeatedly and the fluid in the steam of the pipette was discarded. A drop of the diluted blood fluid from the bulb was brought to the tip of the pipette. The pipette was held at an angle of approximately 45° to the haemocytometer slide and the drop was made to touch the junction
between the cover slip and the central platform and thus the fluid got
drawn into the space between the platform and the slip by capillary attra-
tion. Care was taken to prevent over flow as well as entry of air bubbles
under the cover slip. Allowing five minutes for the corpuscles to settle
down, the fluid in the central R.B.C. chambers was examined with the lower
power lens of the microscope for even distribution of the corpuscles. When
such distribution was observed, counting of the R.B.C. in the five small
squares of the R.B.C. counting area was done by observing under the high
power lens of the microscope. Of the corpuscles lying on the borders, only
those which touched the upper and left borders were counted as being inside
the squares.

The score of red blood corpuscles count was computed from the
obtained number by applying the formula suggested by Shrivastava, Das
and Sahay⁴ and recorded in units of millions of R.B.C. per cubic millimeter
of blood.

White Blood Corpuscles

**Apparatus**

Haemocytometer, Micro-pipette, Cotton, 75x12 mm. Tube (Borosil).

**Sample**

Anti-coagulated Blood.

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⁴Shrivastava, Das and Sahey, *Text Book of Practical Physiology*,
pp. 67-68.
Procedure

The slide preparation for the W.B.C. counting was made similar to that of R.B.C. counting with the following differences:

A W.B.C. Micro-pipette was used, the blood was sucked up to the mark 0.5 and the diluting fluid (W.B.C. fluid) was taken up to the mark "11", the dilution resulting to 20 times the volume of blood taken was made. The counting of W.B.C. was done in four big W.B.C. counting areas under the low power microscope. The light was cut off to the minimum with the help of diaphragm attached to the condensor so that W.B.C. were clearly visible.

The score of the white blood corpuscles was computed from the obtained number by applying the formula suggested by Shrivastava, Das and Sahay and recorded in units of thousands of W.B.C. per cubic millimeter of blood.

Haemoglobin

Apparatus

Photoelectric Colorimetre, Automatic Pipette and Cuvette with stopper.

Sample

Anti-coagulated Blood.

Reagents

Drabkin's SaIn, Haemoglobin Standard and Distilled Water.

\(^5\)Ibid., pp. 74-75.
Procedure

Cyanmethaemoglobin Method was used to determine the amount of haemoglobin in the blood.

The glassware and the automatic pipette were thoroughly cleaned and dried. The colorimeter was connected to the main and power was switched-on. Add 0.02 ml. of blood to 4 ml. of diluent. Stopper was applied on the tube containing the solution and inverted several times. After being allowed to stand at room temperature for a sufficient period of time to ensure the completing of the reaction (10 minutes). The solution of cyanmethaemoglobin was ready to be compared with the standard in a photoelectric colorimetre, at 540 nm. against a reagent blank. Open a ampoule of cyanmethaemoglobin standard, (brought to room temperature) and measure the absorbance of the solution in the same photoelectric colorimeter against the blank.

Calculation:

\[ Hb \ g/l = \frac{A^{540 \text{ of test sample}}}{A^{540 \text{ of standard}}} \times \text{Concentration of haemoglobin standard.} \]

(Normal range 13.5 gm/dl - 18.0 gm/dl.)

Serum Cholesterol

Apparatus

Photoelectric Colorimeter, Automatic pipette, Cuvettes and conical Tubes.

Sample

Serum.
Reagent

1. Glacial Acetic Acid
2. Ferric Chloride
3. Sulphuric Acid
4. Standard: Cholesterol 200 mg/dl (prepared in glacial acetic acid).

Procedure

Determination of total cholesterol, by using reaction with Ferric Chloride and Sulphuric Acid. Zlatkis, Zak and Boyle (1958) used the red colour which cholesterol in acetic acid solution gives with Ferric Chloride and Sulphuric Acid.

Add 0.1 ml of serum of 10 ml. of ferric chloride - acetic acid reagent in a glass stoppered conical tube. Mix well and stand for fifteen minutes for the proteins to flacculate. Shake and centrifuge, then transfer 5 ml. of the clear supernatant fluid to a glass - stoppered tube. For the standard mix 0.1 ml. of cholesterol standard (200 mg/dl) in to 9.9 ml. of FAAR mix, and transfer 5 ml. to a second tube. As blank take 5 ml. of the ferric chloride acetic acid reagent in a third tube. Add 3 ml. of sulphuric acid from a burette to all three tubes, apply stopper tightly and mixed by repeated inversion. Loosen the stopper carefully and stand for thirty minutes. Read test and standard against the blank using yellow filter or at 570 nm.

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Calculation:

\[
\text{Cholesterol/100 ml.} = \frac{\text{Reading of Test}}{\text{Reading Standard}} \times \frac{\text{Concentration of Cholesterol Standard}}{}
\]

Normal Range (150 - 250 mg/dl.)

Serum Protein

**Apparatus**

Photoelectric Colorimetre, automatic pipette and cuvettes.

**Sample**

Serum.

**Reagent**

1. Biuret Reagent
2. Protein standard 7.3 gm/dl.

**Procedure**

Biuret Method was employed to determine the amount of total protein in the blood.

The cleaned and dry glassware, were used. The test tube were properly marked as Test 'T', Standard 'S' and Blank 'B'. The 0.1 ml. of distilled water. Sample and standard were drawn with the help of automatic pipette and poured into respective tubes. The 5.00 ml. of Biuret reagent was added to each tube and shaked gently two to three times. The tubes were placed at room temperature for 5 minutes for developing colour. Then read the optical density by using yellow-green filter e.g. 540 nm against blank 'B'.


Calculation:

\[
\text{Serum Total Protein} = \frac{\text{O.D. of Test}}{\text{O.D. of Standard}} \times \text{Concentration of Protein Standard.}
\]

Normal Value = Total Protein 6 to 8 gms./100 ml.

Data on Body Composition

Body Fat

The Lange Skinfold Caliper was used to assess the body fat. The instrument consisted of accurately calibrated dial which indicated in millimetres the thickness of the skinfold when the jaws were open, holding the Skinfold.\(^7\)

To eliminate the possible error, the reading was made between three to four seconds, when essentially all compressions had taken place. If this precaution was not taken then the skinfold would gradually have decreased, the tissue being squeezed out from the jaws of the calipers.

The right side of the body was used to determine the percentage of fat. The thickness of the skin and subcutaneous fat were grasped between the thumb and index finger and measurement was taken to the nearest millimetre from four different specific sites using the calipers.

The following were the sites used for taking skin fold measures:

1. Biceps
2. Triceps

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3. Subscapular Region

4. Supra-iliac Region

Biceps Skinfold

With the subject standing erect with arm hanging loosely, a fold of skin was picked up on the anterior of the mid part of the biceps and the skinfold thickness was measured. The position of the fold was vertical and the reading to the nearest half millimetre was recorded.

Triceps Skinfold

The skinfold thickness was taken over the triceps muscle at a point half way between the tip of the elbow (olecranon process). The point was located with forearm flexed to 90 degrees, and while taking the measurement the arm was kept hanging free. The fold of skin was lifted parallel to the long axis of the arm and the reading to the nearest half millimetre was recorded.

Subscapular Region Skinfold

The skinfold thickness was taken at the tip of the scapula (inferior angle) with the subject in a relaxed standing position. The fold was lifted in the diagonal plane at about 45 degree from vertical and horizontal planes and the reading to the nearest half millimetre was recorded.
Supra-iliac Region Skinfold

The skinfold thickness was taken three to five centimetres above the anterior superior-iliac spine on diagonal line going downward and inward and the reading to the nearest half millimetre was recorded.

The sum of the skinfold thickness of four sites of the body was converted into percentage body fat with the help of standard table suggested by Durnin and Rahaman. From each subject, body weight and the weight of the fat he possessed was calculated by using the following formula:

$$\text{Fat Weight} = \frac{\text{Body Weight} \times \text{Percentage of Value of Fat}}{100}$$

The four sites skinfold measurement are presented in Fig. 5, Fig. 6, Fig. 7 and Fig. 8 - Pages 68-69.

Lean Body Weight

The total body weight minus the weight of body's fat gave the lean body weight.

The weight of the fat was deducted from each subject's total body weight and recorded in kgs.

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Fig. 5. Measurement of Biceps Skinfold.

Fig. 6. Measurement of Triceps Skinfold.
Fig. 7. Measurement of Subscapular Region Skinfold.

Fig. 8. Measurement of Supra-iliac Region Skinfold.
Administration of Training Programme

The training schedule prepared by the investigator was imparted to three experimental groups and training was personally supervised by the investigator. The training was carried out thrice a week i.e. on Mondays, Wednesdays, and Fridays. The details of all the three training methods are:

Aerobic Training

The permitted time for aerobic training was fixed after conducting a pilot study with eight subjects, who were selected at random from among the subjects. They were asked to run certain distance for certain duration, to find out the time required to raise the pulse rate between 140-160 beats per minute. After several trials, it was found that 25 minutes of running at a moderate pace was sufficient to raise the pulse rate of the subjects between 140 to 160 beats per minute. A duration of two weeks was considered sufficient for adaptation of the body system to the work load. Therefore, load was increased after every two weeks in terms of duration of the running. The subject performed the work-out on the prescribed days during the morning sessions.
Table 2

BI-WEEKLY SCHEDULE OF STIMULUS INTENSITY FOR
AEROBIC TRAINING GROUP

<table>
<thead>
<tr>
<th>Week</th>
<th>Stimulus Intensity Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st and 2nd</td>
<td>25 minutes</td>
</tr>
<tr>
<td>3rd and 4th</td>
<td>30 &quot;</td>
</tr>
<tr>
<td>5th and 6th</td>
<td>35 &quot;</td>
</tr>
<tr>
<td>7th and 8th</td>
<td>40 &quot;</td>
</tr>
<tr>
<td>9th and 10th</td>
<td>45 &quot;</td>
</tr>
</tbody>
</table>

Anaerobic Training

The permitted distance and repetition for anaerobic training were fixed after conducting a pilot study with the eight subjects, who were selected at random from among the total subjects. After achieving the maximum speed, the sportsman is able to maintain this speed with minute fluctuations, depending upon this qualification, from 20-45 metres (Gundlach, 1963). Therefore, the distance can be from 50 to 80 metres. Better the sprinter, longer should be the distance. Harre (1979) suggested that the distance should be so long that the sportman can hold his maximum speed for 1-2 seconds. To improve the anaerobic capacity, the repetition should be between 5 to 10 times. More number of repetition leads to fatigue. After several trials it was found that the 60 M. distance with starting of 6 repetitions was sufficient to start the anaerobic training. The duration of two weeks was considered sufficient for adaptation of
the body system to the anaerobic load. The subjects performed the work-out on prescribed days during the morning session.

Table 3

BI-WEEKLY SCHEDULE OF STIMULUS INTENSITY FOR ANAEROBIC TRAINING GROUP

<table>
<thead>
<tr>
<th>Week</th>
<th>Distance</th>
<th>Repetitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st and 2nd</td>
<td>60 M.</td>
<td>6 Repetitions</td>
</tr>
<tr>
<td>3rd and 4th</td>
<td>60 M.</td>
<td>7 '</td>
</tr>
<tr>
<td>5th and 6th</td>
<td>60 M.</td>
<td>8 '</td>
</tr>
<tr>
<td>7th and 8th</td>
<td>60 M.</td>
<td>9 '</td>
</tr>
<tr>
<td>9th and 10th</td>
<td>60 M.</td>
<td>10 '</td>
</tr>
</tbody>
</table>

Combination of Aerobic and Anaerobic Training

Combination group under-went the training with alternate workout of aerobics and anaerobics. First day of training, they started with aerobic group and the second day of training, along with anaerobic group. The training was carried out for ten weeks with alternation of aerobic and anaerobic work-out. The training was carried out thrice a week. Ten weeks of training work-out are given below for combined group. The stimulus intensity were increased after every two weeks as in the case of aerobic and anaerobic training schedule.
<table>
<thead>
<tr>
<th>Weeks</th>
<th>Days</th>
<th>Stimulus Intensity</th>
<th>Stimulus Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anaerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distance</td>
<td>Rept.</td>
</tr>
<tr>
<td><strong>1st Week</strong></td>
<td>Monday</td>
<td>60 M.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2nd Week</strong></td>
<td>Monday</td>
<td>60 M.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>3rd Week</strong></td>
<td>Monday</td>
<td>60 M.</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>4th Week</strong></td>
<td>Monday</td>
<td>60 M.</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>5th Week</strong></td>
<td>Monday</td>
<td>60 M.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>6th Week</strong></td>
<td>Monday</td>
<td>60 M.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Friday</td>
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</table>
Table 4 (continued)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Days</th>
<th>Stimulus Intensity</th>
<th>Stimulus Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Anaerobic Distance</td>
<td>Aerobic Distance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rept.</td>
<td>Duration</td>
</tr>
<tr>
<td>7th Week</td>
<td>Monday</td>
<td>60 M.</td>
<td>40 Minutes</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td>60 M.</td>
<td>40 Minutes</td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td>60 M.</td>
<td></td>
</tr>
<tr>
<td>8th Week</td>
<td>Monday</td>
<td>60 M.</td>
<td>40 Minutes</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td>60 M.</td>
<td>40 Minutes</td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td>60 M.</td>
<td></td>
</tr>
<tr>
<td>9th Week</td>
<td>Monday</td>
<td>60 M.</td>
<td>45 Minutes</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td>60 M.</td>
<td>45 Minutes</td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td>60 M.</td>
<td></td>
</tr>
<tr>
<td>10th Week</td>
<td>Monday</td>
<td>60 M.</td>
<td>45 Minutes</td>
</tr>
<tr>
<td></td>
<td>Wednesday</td>
<td>60 M.</td>
<td>45 Minutes</td>
</tr>
<tr>
<td></td>
<td>Friday</td>
<td>60 M.</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of Data

The difference between the pre-test and the post-test means of each group in the chosen variables was tested by applying 't' test. It was done inorder to find-out difference, if any, on each of the chosen variables before and after the experimental treatment. The mean difference method was applied for this purpose. In order to find-out the differential effects of the three experimental methods of training,
analysis of variance and co-variance (F-test) were carried out for the four groups with respect to mean gains in each chosen variables. A post-hoc 't' test was applied in cases where 'F' ratios were found significant, to find out which of the differences of the paired means were significant. For testing the hypothesis, the level of confidence was set at .05.