Chapter - III

METHODOLOGY

In this chapter, the method adopted for the selection of subjects, selection of variables, collection of data, tester’s reliability, instrument reliability, training programme for yogasana, administration of tests and methods employed for statistical treatment of data have been explained.

Selection of Subjects

Prior to the test, the investigator visited many hospitals in Chidambaram and Annamalainagar and met diabetes patients (type - II) and explained to them about the purpose and nature of the study and requested the patients to volunteer for the study. Only women diabetes patients, those who were aged between 35 and 40 years were contacted and around fifty one subjects gave their voluntary consent to work as subject for the study. A qualified medical officer of the Rajah Muthiah Medical College & Hospitals, Annamalai University, Annamalainagar, examined 51 diabetes patients and declared 46 of them to be fit for this study, and from the 46 subjects, only 30 were selected. They were selected by lot method
and divided randomly into two equal groups as the yogic practice group and the control group. Their written voluntary consent was obtained after clearly explaining the nature of the study, the training programme and the variables under which they would be tested and they were assured that their data would not be used for any purpose other than the present study. They were also assured that the results would be kept strictly confidential. They were also informed that they were free to opt out of the study at any time if they feel any discomfort or difficulty in continuing with the training programme.

The selected thirty subjects were randomly divided into two groups of fifteen each, out of which group - I (n = 15) underwent yogic practice six days per week (Monday to Saturday) for twelve weeks and group - II (n = 15) remained as the control. All the subjects have revealed that they had no ailments of any sort and they were taking medicines for treatment after a general medical check up done on them. The physician confirmed this and the subjects were given clearance to take part in the yogic practices.
Selection of Variables

People in the 21st century seem to be facing several grave health related problems. Some of the grave health problems can be cured with the newly introduced medicines, machines and techniques. Some of them can be avoided, but may not be cured. Before proceeding with the study, the researcher went through the available literature in the area, and had, on the basis of her personal experience, discussed the results with her research supervisor. Consultation with the other experts was made for considering the factors of feasibility, availability of proper techniques and instruments; certain variables were selected during this study.

Heredity, age, gender, race are some of the factors which cannot be altered and over eating, physical inactivity and psychological stress are some of the factors which a person consciously adapts to, in spite of knowing the health hazards associated with them. No external help can be of any use to these people, unless they themselves control their eating and involve themselves in physical activity. After eliminating the risk factors, which are mentioned above, the researcher’s task was to identify,
those that may be altered as a consequence of yogic practices. Taking into consideration all these factors, a set of variables was selected to test on the selected subjects, to observe the variations in their levels due to the training effect. The variables selected and tested were

**I. Physical Fitness Variables:**

1. Flexibility
2. Muscular Endurance

**II. Physiological Variables:**

1. Blood pressure - Systolic and Diastolic
2. Resting pulse rate
3. Breath holding time

**II. Biochemical Variables:**

1. Total Cholesterol
2. Triglycerides
3. HDL–Cholesterol
4. Blood glucose
5. Uric acid

**Selection of Tests**

The present study was undertaken primarily to assess the effectiveness of yogic practices on selected physical fitness, physiological and biochemical variables. The investigator analysed
various literatures and also consulted with physical education professionals to use the most suitable tests for the purpose of the study and it was presented in Table - I.

**Table – I**

**TEST ITEMS FOR THE SELECTED VARIABLES**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Dependent Variables</th>
<th>Test Items</th>
<th>Measuring Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Flexibility</td>
<td>Sit and reach test</td>
<td>Inches</td>
</tr>
<tr>
<td>2.</td>
<td>Muscular Endurance</td>
<td>Sit-ups</td>
<td>Numbers</td>
</tr>
<tr>
<td>3.</td>
<td>Systolic and Diastolic Blood Pressure</td>
<td>Sphygmomanometer</td>
<td>mmHg</td>
</tr>
<tr>
<td>4.</td>
<td>Resting Pulse Rate</td>
<td>Pulse rate per minute</td>
<td>Numbers</td>
</tr>
<tr>
<td>5.</td>
<td>Breath Holding Time</td>
<td>Holding the breath for maximum duration</td>
<td>Seconds</td>
</tr>
<tr>
<td>6.</td>
<td>Total Cholesterol</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>7.</td>
<td>Triglycerides</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>8.</td>
<td>High Density Lipoproteins</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>9.</td>
<td>Blood Glucose</td>
<td>Boehringer Mannheim kit</td>
<td>mg/dl</td>
</tr>
<tr>
<td>10.</td>
<td>Uric Acid</td>
<td>Uricase Method</td>
<td>mg/dl</td>
</tr>
</tbody>
</table>
Orientation to the Subjects

The subjects were allowed to familiarize themselves with the techniques involved to execute the yogic practices. It helped them to perform well in yogic practices and they were informed of the benefits of yogic practices. The control group had no specific exercises and was advised not to involve themselves in any sort of exercise related to yogasana. Further, they were informed of the seriousness of this project, which needed good orientation and co-operation on the part of the subjects.

Collection of Data

Data on the selected physical fitness physiological, and biochemical variables were collected as per the method prescribed in test administration one day prior to the commencement of training and one day after the completion of training. The data on physical fitness were collected after administering the sit and reach test with a yard stick scale and sit-ups test. The subjects were asked to sit relax and the pulse rate was taken during the early morning after waking up from the bed. The breath holding time was measured by asking
the subjects to hold their breath for a maximum duration after a long inhalation.

For the purpose of collection of data on selected biochemical variables, the subjects were asked to report early morning, one day prior to the commencement of training and one day after the training, in fasting condition. It was ensured that the subjects had not consumed any food or beverages in the past 9 to 10 hours. The subjects were made to relax and then their blood pressure levels were measured with a sphygmomanometer and stethoscope. Later, 5 ml of blood was collected from each subject by venous puncture method and the blood, thus collected, was stored in small bottles for the pre-test. The post-test data was collected 24 hours after the completion of the 12 weeks of the yogic practice period.

**Tester Reliability**

Prior to the commencement of the study, the investigator had undergone training in various techniques and testing procedures under experts working the fields of physical education, physiology, biochemistry and general medicine. Collection of data on flexibility, muscular endurance, blood pressure, resting pulse rate and breath
holding time was done under the supervision of experts in physical education.

Estimation of lipoproteins, blood glucose and uric acid was done with the help of experts in a professional laboratory and all the instruments such as centrifuge and auto analyzer were of high quality manufactured by companies of repute and showed excellent accuracy in giving results. Blood pressure was measured using standard equipment and with the help of a physician. The testing procedure was started only after ensuring that the investigator was competent enough to do so.

Instrument Reliability

All the instruments and equipment, used for the study were standard ones and of high quality. None had any functional defect and were being used for the same purposes. Each instrument was tested several times and then being satisfied with the performance of the instrument used on subjects. The sphygmomanometer and stethoscope used for measuring blood pressure were acquired from a physician who had been using it for diagnostic purposes on his patients for quite some time. Estimation of lipids and lipoproteins
was done with the help of experts in a professional laboratory and all
the instruments, such as, centrifuge and auto analyzer were of high
quality manufactured by companies of repute and they showed
excellent accuracy in giving results. The testing procedure was
stored only after establishing reliability.

Reliability of the Data

Reliability of the data was established by test and re-test
process where consistency of scores was statistically tested by
computing univariate co-efficient for ten subjects on the entire
criterion variables. All the variables revealed high correlation when
tested and re-tested, thus ensuring their reliability. The results are
presented in Table – I.
Table – I

Univariate Correlation on Selected Criterion Variables

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Variables</th>
<th>r.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Muscular endurance</td>
<td>0.87*</td>
</tr>
<tr>
<td>2.</td>
<td>Flexibility</td>
<td>0.82*</td>
</tr>
<tr>
<td>3.</td>
<td>Systolic blood pressure</td>
<td>0.99*</td>
</tr>
<tr>
<td>4.</td>
<td>Diastolic blood pressure</td>
<td>0.99*</td>
</tr>
<tr>
<td>5.</td>
<td>Resting pulse rate</td>
<td>0.98*</td>
</tr>
<tr>
<td>6.</td>
<td>Total Cholesterol</td>
<td>0.99*</td>
</tr>
<tr>
<td>7.</td>
<td>Triglycerides</td>
<td>0.99*</td>
</tr>
<tr>
<td>8.</td>
<td>High density lipoproteins</td>
<td>0.99*</td>
</tr>
<tr>
<td>9.</td>
<td>Blood glucose</td>
<td>0.99*</td>
</tr>
<tr>
<td>10.</td>
<td>Uric acid</td>
<td>0.99*</td>
</tr>
</tbody>
</table>

* Significant at .05 level of confidence. (The table value required for significance at .05 level of confidence with df 9 was 0.767)

Training Programme

The subjects were divided into two groups, namely, yogic practice group and control group. The control group was not given any training. The experimental group practiced yoga, weekly for six days i.e., Monday to Saturday, between 6.00 a.m. to 8.00 a.m., for a period of twelve weeks.
**Training Schedule**

The experimental group (yogic practice group) underwent the training programme six days per week (Monday to Saturday) for twelve weeks. Control group was instructed not to participate in any exercise programme and were requested to do regular work throughout of the study. The experimental group, who participated in it, was informed to report at the early hours of the day around 5.30 a.m. at the training place.

**Description of Asanas**

The experimental factor selected is yogasana and since it is innumerable, the scholar consulted with experts in the field of yogasana, then selected certain yogasanas which presented in Appendix – I.

**TEST ADMINISTRATION**

**Sit and Reach Test**

**Purpose:**

To measure the horizontal forward mobility of the hip region and elasticity of the hamstring, gluteus and gastrocnemious group muscle.
Equipments:

Flex measure scale with yard stick scale.

Procedure:

The investigator directed the subjects to take a long sitting position. Hands were kept by the side of the body and heels were placed 10 cm apart. The equipment was placed between the legs that the 40 cm mark of the scale with a line on the floor.

The subjects were asked to sit erect then slowly raise both the hands till they come to a vertical position and palms facing each other, they were asked to reach forward to the yard stick (scale) and the maximum possible measurement was taken one quarter of the centimeter.

Scoring:

The best among the three trials were his test score.

Bent Knee Sit-ups

Purpose:

To assess strength endurance.

Equipments:

Mat, floor, or dry turf.
**Procedure:**

The subjects lies on their backs with their knees bent, feet on the floor, and heels not more than 12 inches from the buttocks. The angle at the knees should be less than 90 degrees. The subjects put their hands at the back of their necks with fingers clasped and place their elbows squarely on the mat, floor or turf. Their feet are held by their partner to keep them in touch with the surface. The subject tightens their abdominal muscles and brings their head and elbows forward as they curls up, finally touching elbows to knees. This action constitutes one sit-up. The subject returns to their starting position with their elbows on the surface before they sits up again. The timer gives the signal “ready-go” and the sit-up performance is started on the word “go”. Performance is stopped on the word “stop”. The number of correctly executed sit-ups performed in 60 seconds is the score.

**Rules:**

1. Only one trial shall be allowed unless the teacher believes the subject has not had a fair opportunity to perform.

2. No resting is permitted between sit-ups.
3. No sit-ups shall be counted in which the subject does not (a) keep the fingers clasped behind the neck; (b) bring both elbows forward in starting to sit-up, without pushing off the floor with an elbow; or (c) return to starting position, with elbows flat on the surface, before sitting up again.

**Scoring:**

Record the number of correctly executed sit-ups the subject is able to do in 60 seconds. A foul nullifies the count for that sit-up. The watch is started on the word “go” and stopped on the word “stop”.

**Measurement of Blood Pressure**

Blood pressure was measured by the indirect method using sphygmomanometer and stethoscope, as recommended by Cromwell *et al.*

For measuring blood pressure, the subjects were asked to report early in the morning and were allowed to relax for half an hour by lying down on the mattress. After ensuring that the subjects were relaxed mentally and physically, they were asked to sit in a

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chair and the cuff of the sphygmomanometer was placed on the right upper arm of the subject. The stethoscope was placed over the brachial artery down stream from the cuff.

The pressure cuff on the upper arm was inflated by pressing the rubber bulb and the cuff was inflated till no sounds were heard in the stethoscope, as the brachial artery has been collapsed by the pressure of the cuff.

The pressure in the cuff was then gradually reduced by deflating the cuff through the valve. As the cuff started deflating gradually a small sound called “korotkoff” sounds were heard through the stethoscope, at this stage, the mercury level in the manometer was recorded and this recording was taken as systolic blood pressure.

The pressure of the cuff that was indicated on the manometer when the first “korotkoff” sound was heard, was recorded as the systolic blood pressure. As the deflation continued and the pressure started falling, at one stage the “korotkoff” sounds disappeared as the pressure was no longer sufficient to occlude the vessel.
The cuff pressure shown in the manometer was recorded as soon as the “korotkoff” sounds disappeared and this reading was considered as diastolic pressure.

The measured values were expressed in terms of mmHg.

**Pulse Rate**

**Purpose:**

The purpose of the test was to find out the pulse rate at rest of the individuals.

**Instruments:**

A stopwatch, pencil and score sheet was used to assess the pulse rate at rest.

**Procedure:**

The pulse rate of the subject was recorded in the sitting position. Before taking the normal pulse rate, the subject was asked to relax in a sitting position for 30 minutes. The pulse rate was taken at the radial artery at the wrist in such a manner that palpitation was clearly felt by the fingertips.

**Scoring:**

The measurement of palpitation was counted for one minute.
Breath Holding Time

**Purpose:**

The objective was to measure the ability of the subjects to hold the breath for longer time.

**Equipments:**

A stopwatch with calibration of 1/10 seconds, score sheet and a pencil were used to administer the test.

**Test Description:**

The subject stood at ease and inhaled deeply after which he held his breath for a length of time possible to him. The index finger of the respondent served as the indicator for the investigator to know the start and end of the recording time. The thumb and center finger were used to hold the nose to avoid letting the air through the nostrils. The subjects were requested not to let the air out by opening the mouth while recording the breath holding time.

**Scoring:**

The time of holding the breath till the subject let the air out was closed by using the stop to the nearest one tenth of a second as breath holding time.
Estimation of Total Plasma Cholesterol

For the purpose of the study, all the thirty subjects were tested for an amount of total cholesterol in 150 ml. of blood.

Procedure for cholesterol estimation:

CHOD-POP method, recommended by Katterman, Jaworek and Möller\textsuperscript{85} was used for this purpose. The kit used for this purpose was Boehringer Mannheim, West Germany with Photometer – 4010 (auto analyzer). Boehringer Mannheim kit consisted of one bottle.

Bottle 1: Cholesterol reagent (MPR1).

Preparation: MPR1 dissolve contents of one bottle 1 by adding 32 ml redistilled water. The reagent solution is ready to use after 10 minutes.

Procedure:

Wave length: Hg 546 nm (470 – 560 nm)

Spectrophotometer: 500nm

Cuvette: 1 cm light path

Incubation temperature: 20 – 25°C or 37°C

Measure against reagent blank (RB)

One reagent blank is sufficient for each assay series.

Sample material pipette into test tubes will 0.02 ml and reagent solution pipette into test tubes 2.00 ml of reagent blank (RB) and 2.00 ml of sample. Mix and incubate RB and sample for 10 minutes at 20 – 25°C or 5 minutes at 37°C. Read absorbance of sample against RB within 1 hour = A sample.

Dilution threshold: 1000 mg/dl.
At higher cholesterol concentrations, dilute 0.1 ml of sample material with 0.2 ml of 0.9% of NaCl solution and repeat assay (result x 3).

Calculation: Cholesterol concentration (C) in the samples was calculated by the following formula:

\[ \text{Hg 546 nm} - c = 853 \times A \text{ sample} \]
\[ 500 \text{ nm} - c = 575 \times A \text{ sample} \]

**Estimation of Triglycerides**

Triglycerides were estimated by using GOP – PAP method recommended by Searcy\(^86\) contains two sample bottles

- Bottle 1: Buffer
- Bottle 1a: 6 Reagent strips

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**Preparation and stability of solution:**

Do not touch the reagent patches or the surrounding area. Immerse one reagent strip in one bottle of buffer solution and use to stir the bottle contents for Ca – 10 seconds. Leave to stand in buffer solution for 5 minutes stir once again for Ca – 10 seconds and then discard reagent strip with 20°C.

**Procedure:**

Wavelength : Hg546 mm  
Spectrophotometer: 500 nm  
Cuvette : 1 cm light path  
Incubation temperature: 20 – 25°C or 37°C  
Measure against reagent solution : One time is sufficient for each series (increase in absorbance).

Serum or plasma pipette into test tubes with 0.02 ml and reagent solution with 2.00 ml.

Mix and incubate for 10 minutes at 20 – 25°C. Read absorbance of sample against reagent solution within

60 min = A_{Sample}  
Dilution threshold : 1000 mg/dl  
Calculation via factor : calculated the concentration (c) of triglycerides as follows : 20 – 25°C
Wavelength : Hg 546 nm - \( c = 1040 \times A_{\text{sample}} \)

\[ \text{500 nm} - c = 760 \times A_{\text{sample}} \]

calculation via standard : calculated the concentration (c) of triglycerides as follows

Incubation temperature: 20 – 25 °c or 37 °c

\[ C = 200 \times \frac{A_{\text{Sample}}}{A_{\text{Sample}}} \]

**Estimation of HDL-Cholesterol**

HDL – Cholesterol was estimated by using CHOD – PAP method recommended by Lopes – Virella *et al.*

Serum must be separated from the blood clot as rapidly as possible.

**Bottle 1: Cholesterol (reagent)**

Dissolve Contents of one bottle 1 by adding 32 ml redistilled water.

Sample preparation: Precipitation – sample pipette into centrifuge test tubes 200 ml and precipitant 500 ml.

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Mix and let it stand for 10 minutes at room temperature, then centrifuge for 10 minutes at 4000 rpm or more or for 2 minutes at 12000 rpm.

After centrifugation, separate the clear supernatant within two hours and determine the cholesterol content by the CHOD-PAP method.

Assay procedure:
Wave length : Hg 546 nm
Spectrophotometer : 500nm
Cuvette : 1 cm light path
Incubation temperature : 20 – 25 °C or 37 °C

One reagent blank is sufficient for each assay series. Redistilled water, pipette into test tube 100 ml of reagent blank supernatant pipette into test tubes with 100 ml of sample. Reagent solution pipette into test tubes with 1600 ml reagent blank and 1000 ml sample. Mix and incubate at 37 °C then measure absorbance of sample \( A_{\text{sample}} \) against blank within one hour.

Calculation:
Wavelength – Hg 546 nm = 325.1 \times A_{\text{sample}}
500 nm = 219.2 \times A_{\text{sample}}
3.14.8 Estimation of Glucose

GOD-PAP method, recommended by Trinder\textsuperscript{88} was used for this purpose. The kit was used for this purpose was Boehringer Mannheim, West Germany with Photometer – 4010 auto analyzer.

The kit consists of 2 bottles:

Bottle 1: Buffer/Enzymes/Chromogen (5 bottles for 5 x 20 ml)

Bottle 2: Phenol (5 bottles for 5 x 200 ml).

\textbf{Preparation and stability of Reagent solution:}

Dissolve the contents of one bottle by adding 200 ml distilled water. Then add the contents of one bottle la (Phenol) store in amber glass bottles.

\textbf{Sample Preparation:}

Note: Deproteinise immediately

Plasma should be separated from cellular constituents immediately if possible, run later than one hour after collecting the blood specimen.

URAC (deproteinizing solution) pipette into a centrifuge tube with 1.00 ml sample pipette into a centrifuge tube with 0.10 ml.

Flush the pipette with the mixture several times. Centrifuge the suspension and use 0.20 ml of the supernatant for the assay.

**Procedure:**

Wave length: Hg 546 nm (470-560 nm)

Spectrophotometer: 510 nm

Cuvette: 1 cm light path

Incubation temperature: 20 – 25° C.

Measure against blank. One standard and one blank are sufficient for each assay series. Use the standard in the assay in the same way as the supernatant.

Distilled water pipette into test tubes .2 ml with blank, standard pipette into test tubes 0.2 ml with standard, supernatant pipette into test tubes 0.2 ml sample, reagent solution pipette into test tubes 2.0 ml with standard and 2.0 ml with sample.

Mix and incubate at 20 – 25° C. Avoid exposure to direct sunlight, after 35 – 60 minutes read the absorbance’s of the sample (A\text{sample}) and standard (A\text{sample}) against the blank.

**Concentration of Reagent Solution:**

GOD ≥ 18 U/ml; POD 1.1 U/ml; phenol 11 mol/l; 4 – aminophenazone: 0.77 mmol/l
Calculation the concentration (C) glucose in blood plasma.

\[ C = 100 \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \text{ (mg/dl)} \]

**Blood Uric Acid**

The decrease in absorption at 293 millimicrons resulting from the action of uricase (Felchtmeir and Wrenn)\(^{89}\) is measured in the spectrophotometer.

**Reagents:**
1. Glycine buffer, 2/3 M, pH 9.35. Dissolve 25 grams of glycine in about 200 ml of distilled water in a 500 ml volumetric flask add 110 ml of normal sodium hydroxide and make to the mark with water. Check the pH.

2. Uricase. Ampoules of uricase Lco are obtainable from light and Co. Dissolve 5 mg in 10 ml of the above glycine buffer diluted 1 to 10 with water.

3. Trichloracetic acid, 10 percent w/v in water.

4. Uric acid standard, 100 mg per 100 ml. Dissolve 150 mg of lithium carbonate in 30 ml of warm distilled water at 60°C and add

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to 200 mg of uric acid in a 200 volumetric flask. Shake until solution is complete, cool and make to the mark. Add a few drops of chloroform and keep at 4°C. Make freshly every fortnight. Dilute 1 to 5 with water to give a 20 mg per 100 ml of serum, 0.25 ml.

**Technique:** To each of two tubes add 0.25 ml of serum, 0.25 ml of the 2/3 M glycine buffer and 2 ml of distilled water. Add 0.5 ml of the uricase solution to one tube. Incubate for two hours at 37°C. Add 2 ml of 10 percent, trichloracetic acid to each tube and 0.5 ml of uricase solution to the control. Stand for twenty minutes, then centrifuge. Read the control against the test at 293 millimicrons in the ultraviolet spectrophotometer. At the same time put up 0.25 ml of standard solution in exactly the same way. The 20 mg per 100 ml standard gives an extinction of 0.55 when so treated.

It is advisable to check the activity of the uricase by putting up amounts of uricase solution from 0.1 to 0.5 ml as described for the test but using a uric acid solution containing 20 mg per 100 ml. Use a volume of uricase solution for actual determinations twice as great as that required just to remove the uric acid from the solution.
**Calculation:**

\[
\text{Mg. uric acid per 100 ml of serum} = \frac{\text{Reading of Unknown}}{\text{Reading of Standard}} \times 20
\]

**EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS**

The experimental design used for the study was similar to random group design involving thirty subjects, who were divided into two groups, such as, the yogic practice group and control group of fifteen subjects each.

This study consisted of only one independent variable such as yogic practice. The subjects in the experimental and control groups were tested prior (pre-test), and after twelve weeks (post test) on flexibility, muscular endurance, systolic and diastolic blood pressure, resting pulse rate, breath holding time, total cholesterol, triglycerides HDL-cholesterol, blood glucose and uric acid. The data collected from the two groups prior to the experimental treatment was the pre-test data and after twelve weeks of yogic practices on flexibility, muscular endurance, systolic and diastolic blood pressure, resting pulse rate, breath holding time, total cholesterol, triglycerides, HDL-cholesterol, blood glucose and uric acid were statistically examined
for significant difference, applying the analysis of covariance (ANCOVA). No attempt was made to equate the groups in any manner. In all the cases, .05 level of confidence was fixed to test the significance, which was considered as an appropriate.