Chapter IV

DESIGN AND METHODOLOGY

Review of the pertinent literature in Chapter II indicates that blood glucose levels play an important role in memory modulation. The present investigation was conducted in order to determine the dose and age dependent effect of glucose. An attempt was also made to determine the effect of abnormal glucose metabolism (diabetics) and dietary proteins on memory.

Design

In order to test the hypotheses formulated in Chapter III, the investigator conducted three experiments and a correlational study.

Experiment I

A multi-group (three groups, n₁ = 12, n₂ = 23, n₃ = 8) repeated testing design was used in order to study the dose-dependent effect of glucose on memory. In this experiment subjects served as their own controls in the different dose groups. The basic design for testing and treatment is shown in Table 1.
Table 1

Showing the basic design used for treatment and testing of the subjects of Experiment I and II.

<table>
<thead>
<tr>
<th>Day</th>
<th>Blood glucose (B.G.) levels</th>
<th>Recall of glucose in 250 ml of water</th>
<th>Performance of VR (immediate recall) and learning of PA (Form I).</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>measured initially (in fasting), 15 minutes after treatment and after learning.</td>
<td>- + orange flavour.</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>- do - Recall of n drops of Similar to day one PA learnt Saccharine (to except that Form PA learnt on day 1 be varied in II to be used. such a away that the sweetness become equal to that in the glucose condition) in 250 ml of water + orange flavour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Initial testing only Recall of PA learnt on day 2</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
The amount of glucose to be given to each group to be varied (Gr I = 25 g, Gr II = 50 g and Gr III = 100 g) and the amount of saccharine also to be varied in such a manner that the beverage would be as sweet as that in the experimental condition. Treatment (glucose or saccharine) and learning tasks to be varied in a random and counterbalanced manner.

Experiment II

In order to study the age-related effect of glucose a two-group repeated testing design (Gr IV elderly, mean age = 50.50 years; Gr V young = 15, mean age = 22.40 years), in which subjects served as their own controls, was utilized. The basic design of this experiment was similar to that of Experiment I.
The treatment and learning tasks to be administered in a random and counter-balanced manner in the following way.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subjects</th>
<th>Treatment &amp; Learning tasks</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV</td>
<td>Elderly (n = 10)</td>
<td>The same procedure as in Experiment I to be used except that the dose of glucose found</td>
</tr>
<tr>
<td></td>
<td></td>
<td>to be facilitative in Experiment I would be used.</td>
</tr>
<tr>
<td>V</td>
<td>Young (n = 15)</td>
<td></td>
</tr>
</tbody>
</table>

**Experiment III**

A two-group design (Gr VI (diabetics, n = 9) and Gr VII (non diabetics, n = 9) was used in order to study the effect of abnormal blood glucose levels on memory. An attempt was made to make these two groups equal by matching their age and academic qualifications. The testing and learning of the two groups to be done in the following manner.
<table>
<thead>
<tr>
<th>Group</th>
<th>Subjects</th>
<th>Measurement Learning Tasks of B.G. levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>VI</td>
<td>Diabetics (n = 9)</td>
<td>Initial testing of Learning of PA blood glucose levels and VR sub-tests after fasting of at least 7 - 8 hours. Memory scale. (Form I)</td>
</tr>
<tr>
<td>VII</td>
<td>Non diabetics (n = 9)</td>
<td>Recall of PA to be taken after 24 hours.</td>
</tr>
</tbody>
</table>

**Experiment IV**

A correlational research, in which 17 post-graduate students (20 - 25 years, mean age = 23.33) was conducted. Initially a demographic profile of the subjects was to be obtained. A detailed diet chart of the subjects to be obtained over a period of seven days. On the eighth day learning tests (PA and VR) to be administered after measuring the blood protein levels clinically.

**Sample**

A sample of 104 subjects (normal = 60, mean age = 22.5; elderly = 35, mean age = 50.25 years and diabetics = 9, mean age = 55 - 77 years) were used. Additional 50 subjects were selected for pilot work. The subjects who
voluntarily agreed to participate in the study were taken on the basis of availability. The experimenter had to resort to this method of selection as, the experiment was rather cumbersome for the subjects (experiment was to be conducted in fasting state on three consecutive days and blood samples had to be taken at least seven times) and therefore, very few subjects were agreed to participate. However, the subjects of the various groups were equated with regard to educational qualifications (all were at least graduates). Further, all the subjects were in good physical health and were non-diabetic (except the 9 diabetics).

Instrumentation:
Glucometer: The apparatus used for measuring blood glucose levels, i.e. reflectance photometer – II, was manufactured by the Ames – division of Miles India Limited. It was a portable, battery operated instrument that was to be used with "Ames Dextrostix Reagent Strips for Glucometer – II" to measure the amount of glucose in the whole blood. The unit of measurement was milligrams per deciliter (mg/dl).
It was a rectangular plastic moulded box (Length = 13 cm; width = 6.5 cm; height = 2.25 cm; weight = 175 g), with a digital display window which had a display range of 0 - 399 mg/dl. It operated on an alkaline battery (9 V). A single unit was sufficient for 1000 readings.

The glucometer measured the colour change which took place when the reagent area (pad) of the dextrostix strip reacted with glucose in a drop of blood. When the test chamber lamp’s light fell on the reacted reagent pad, it was reflected. This reflected light was measured by a micro computer chip and the amount of glucose in the whole blood was displayed. The glucometer had fixed programmes ranging from 0 - 9. Thus, the programme number in the glucometer window was matched with the programme number on the dextrostix. It also had a storage capacity of 25 results.

The glucometer was kept in a leather case along with the following accessories - a bottle of dextrostix reagent strips; low, high and normal calibrating solutions, calibrating chip, puncture device, extra lancets (disposable), paper
towel, a bottle of antiseptic liquid and cotton.

Learning Material

On the basis of review of past researches (Hall et al., 1989; Manning et al., 1990), the Wechsler's standardized memory scale, which has two equated forms (I & II), was chosen as the learning task. This scale consisted of seven sub - tests i.e. personal and current information, immediate orientation, mental control, logical memory, memory span, visual - reproduction and paired associate learning.

The investigator used only two of the seven sub - tests. Visual - reproduction (VR) was used as a measure of short term memory and paired - associate learning (PA) as a measure of long term memory.

Paired - Associate Learning: This sub - test consists of ten pairs of word (a stimulus word and response word in each pair). The subject was shown the pairs on three trials (study - test method).
Visual Reproduction: This sub-test consists of the three simple geometric figures. The subjects were required to draw each figure from memory after observing it for 5 seconds.

Glucose

Glucose, which is a major source of energy for each and every action of the body, is a simple sugar. It is a member of the general class of hexoses sugars ($C_6H_{12}O_6$). It is also stored in the body through the formation of complex carbohydrates and fats as reserves supplies, which can be utilized at the time of deprivation. Although most of the body parts can function adequately for fairly long intervals at low blood sugar levels, the brain is totally dependent on a fresh and continuous supply of glucose through the blood.

Glucose is available in powder form under various trade names and is easily soluble in water. In the present investigation Glucose - D, manufactured by Glaxo - Laboratories, was used.

Saccharine

It is a colourless liquid with a remarkable sweetening property yet it is totally calorie free. It is
commonly employed in the form of a sodium salt, which is comparatively free from the unpleasant after-taste of saccharine.

Continuous use of saccharine in small amount has been shown to be harmless. It is available in the market under various trade names and forms such as, tablets, powder, liquid and cube. The investigator used the liquid form, which is available under the tradename of Sweetex, manufactured by Boots Company (India) Limited.

Orange Flavour
In order to make the colour and odour (if any) of the treatment blind, orange flavour was used. It was in a liquid form and addition of just 2 or 3 drops produced a natural flavour and colour of orange juice. The flavour was manufactured by Bush Company (Madras) and available in a pack of 10 ml.

Methodology
In the present study a sample of 104 subjects was utilized. Additional, 50 subjects were used for pilot work. The subjects were assigned to various experiments according to the requirement.
All the experiments (except Experiment IV) were conducted in the morning hours from 6.30 AM to 7.30 AM in order to minimize the effect of variation in blood glucose levels due to time interval from waking and experimentation.

The general procedure for Experiment I & II was same. Both the experiments were conducted over a period of three consecutive days on each subject. Subjects were instructed not to eat or drink anything (except plain water) after midnight and prior to experimentation on each of the three consecutive mornings.

Measurement of blood glucose levels: Before measuring the blood glucose levels, the experimenter ran a control test (by applying the control solution on the pad instead of a blood drop) to ensure that the instrument was calibrated properly. The programme number of the glucometer was matched to that on the dextrostix bottle. The puncture site on the subject's finger and the puncture device (with disposable lancet) was cleaned with an alcohol swab. Then an Ames dextrostix strip was removed from the bottle and the cap was
replaced immediately. The finger was pricked and a drop of blood was squeezed out. The time button of the glucometer was pressed and at the sound of the first buzzer a large drop of blood was applied on the reagent pad of the dextrostix. The reaction was allowed to continue till the next buzzer (after 30 seconds) and immediately after the pad was blotted gently on a paper towel (lint free). The lid of the chamber was lifted and the reagent strip was gently placed in it with the reagent pad facing the chamber and the reading, which appeared on the display window, was recorded. The used strip was disposed off.

Administration of Learning tasks: On the first day of the experiment the subject was instructed in the following manner:

"I am conducting this experiment to study the role of nutrition in memory processes. I will present some words, in pair form, before you. The first word would be the stimulus word and the second would be the response word. Read each pair aloud as it appears in the cardboard window. Later, you will be shown only the stimulus words and you will have to recall the corresponding response words. You would be
shown the entire list three times. Next, I will show you a geometric figure (drawn on a card) for five seconds. After that you will have to draw that figure from your memory. In this manner three figures will be shown to you.

Pilot work: Before conducting the actual experiment a pilot study was conducted in order to select the doses of glucose to be used in Experiment I. In an earlier study by Hall et al. (1989) a dose of 50 g of glucose had been found to have a facilitative effect on memory. Hence, the present investigator decided to select one dose above and one below it to study the full dose response curve.

For selecting the lower dose, five groups (n = 5) of subjects were taken. After measuring their basal blood glucose levels (in fasting state) they were given either 5, 10, 15, 20 or 25 g of glucose in 250 ml. of water+orange flavour, respectively. After 15 minutes their blood glucose levels were measured again. It was found that intake of only 25 g of glucose resulted in a perceivable change in the blood glucose levels.
For selecting the higher dose the same procedure was used except that now 65, 80, 100, 150 or 200 g of glucose in 250 ml of water + orange flavour was given, after measuring the basal blood glucose levels in fasting state. Though intake of 65 and 80 g of glucose increased the blood glucose levels but they were yet within the normal range. The two higher doses, i.e. 150 and 200 g resulted in physical discomfort (subject found it very difficult to drink the entire beverage) and vomiting. However, the intake of 100 g of glucose increased the blood sugar levels above the normal range but did not result in any kind of physical discomfort.

Thus, the three doses which were selected for Experiment I were 25 g, 50 g and 100 g of glucose in 250 ml of water.

Experiment I

After ensuring that the subject had understood the instructions, the experiment was started. The basal blood glucose level was tested (as per the method described earlier) and recorded. The subject was then given 25 g of glucose in 250 ml
of water flavoured with orange flavour. After 15 minutes of ingestion of the drink the blood glucose level was measured again.

Then, the two sub-tests of the Wechsler's memory scale (Form I) were administered. Initially, the ten paired associates were shown to the subjects, with the help of a cardboard strip and a metronome (fixed at the speed of 60 oscillations/per minute and bell after two oscillation). Each pair was shown for 2 seconds. As each pair appeared, the subject read the response word aloud. After presenting the entire list, only stimulus words were shown one by one and the responses of the subjects were recorded. This procedure was repeated until the subject achieved a criterion of 100% correct responses, subjected to a maximum of three trials.

Then, the visual reproduction sub-test was administered. The subject was shown Figure 1 for 5 seconds. After five seconds the figure was removed and the subject was asked to draw it. The same procedure was repeated for Figure 2 and 3.
On the second day, the subject's basal blood glucose level was measured and recorded. Then, recall of paired-associates learned on day 1 was taken. The subject was given saccharine (of equal sweetness as that in glucose condition) in 250 ml. of water-orange flavour and blood glucose levels were measured after 15 minutes. The PA and VR sub-tests of Form II were administered.

On the third day, the recall of the paired-associates (learnt on day 2) was taken after measuring the basal blood glucose level.

The same procedure was repeated for the rest of the subjects of Gr I except that the treatment and learning tests were given in a random and counter balanced manner.

Similarly, the remaining groups, i.e. Gr II (50 g glucose) and Gr III (100 g glucose) were tested.

Experiment II:

Analysis of the results of Experiment I, indicated that a dose of 50 g of glucose enhanced memory on both the sub-tests of Wechsler's Memory
Scale. Thus, in the second experiment a dose of 50 g of glucose was used. The blood glucose levels of two groups of subjects [(Gr IV, n = 15 (young); Gr V, n = 10 (elderly)] were measured and the PA and VR tests were also administered exactly in the same manner as in Experiment I.

Experiment III

This experiment was conducted in order to study the difference in the memory of normal and age-matched diabetics. The experiment was conducted over a period of two days. On day 1, the basal blood glucose level was measured and the two sub-tests of Form I were administered (exactly in the same manner as in Experiment I & II). Responses of the visual reproduction sub-test were taken immediately. On day 2, after measuring the basal blood glucose level, recall of the paired-associate, learnt on day 1, was taken.

Experiment IV

It was a correlational research. Seventeen post-graduate students were given performance mae (Appendix A-4) seeking demographic information and quantitative and qualitative description of
their daily diet. After seven days the diet charts were taken back and were analysed by a dietician for total caloric intake during the whole day. Further, protein intake of breakfast, was also calculated separately. On the eighth day all the subjects (in fasting state) were clinically tested for protein levels in the blood at the University Health Centre and learning tasks (PA & VR) of Form I were administered. Recall of VR was taken immediately and recall of PA was taken after 24 hours.

The obtained data were statistically analysed. These results are presented and discussed in Chapter V.