The review of the pertinent literature in chapter II indicates that epinephrine (E) plays an important role in learning in the normal state. The present investigation was conducted to determine whether this hormone would facilitate learning in the anaesthetic state.
The investigator utilized a multigroup design, with four groups, to test the hypotheses formulated in chapter III. The experiment was conducted in two phases. In the first phase, two groups were trained and tested to determine whether peripheral administration of E would enable learning to occur under deep anaesthesia. During the pilot work the investigator found that intraperitoneal (ip) administration of 50 mg/kg of sodium pentothal to albino rats resulted in complete anesthesia within 10 minutes which lasted for 60 minutes. Also, the depth of anaesthesia was not reduced when 10 electric shocks (5 mA for .5 seconds) were delivered to the hind limbs through needle electrodes inserted in both the thighies of the rat.
Keeping the duration of anaesthesia in view
the following paradigm was used for classical conditioning
of the E and saline groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anaesthetic treatment</th>
<th>After 10 minutes of anaesthetic treatment</th>
<th>Training after 7 minutes of anaesthetic treatment</th>
<th>Testing after 48 minutes of hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Sodium pentothal to be administered intraperitoneally in a dose of 50 mg/kg.</td>
<td>Saline to be administered intraperitoneally (.25 ml of .9% NaCl/animal.)</td>
<td>A pair of CS-UCS to be presented for 10 times with testing by using an ITI of 1 minute. supression task.</td>
<td>The extent of Pavlovian fear conditioning to be tested by using a drink suppression task.</td>
</tr>
<tr>
<td>II</td>
<td>Sodium pentothal to be administered intraperitoneally in a dose of 50 mg/kg.</td>
<td>Epinephrine to be administered intraperitoneally (250μg/kg)</td>
<td>A pair of CS-UCS to be presented for 10 times with testing by using an ITI of 1 minute. supression task.</td>
<td>The extent of Pavlovian fear conditioning to be tested by using a drink suppression task.</td>
</tr>
</tbody>
</table>

On the basis of the results of the above two groups
(learning did occur in the state of anaesthesia under the
influence of E) two more groups were experimented upon
to investigate whether the facilitative effect of
Epinephrine on learning under anaesthesia is mediated via the peripheral/central adrenergic receptors and/or increase in plasma glucose levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Anaesthetic treatment</th>
<th>Training after 7 minutes of anaesthetic treatment</th>
<th>Testing after 48 minutes of hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>Sodium pentothal to be administered intraperitoneally in a dose of 50 mg/kg.</td>
<td>Epinephrine A pair of (250 μg/rat) CS-UCS to plus insulin be presented for 10 times with an ITI of 1 minute.</td>
<td>The extent of pavlovian fear conditioning to be tested by using a drink suppression task.</td>
</tr>
<tr>
<td></td>
<td>The depth of anaesthesia to be determined by tail pinch reflexes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Sodium pentothal to be administered intraperitoneally in a dose of 50 mg/kg.</td>
<td>Epinephrine A pair of (250 μg/rat) CS-UCS to plus propranolol (.05 mg/kg)</td>
<td>The extent of pavlovian fear conditioning to be tested by using an ITI of a drink suppression task.</td>
</tr>
<tr>
<td></td>
<td>The depth of anaesthesia to be determined by tail pinch reflexes.</td>
<td>plus propranolol (.05 mg/kg) to be administered an ITI of a drink</td>
<td></td>
</tr>
</tbody>
</table>
Sample:
Initially a sample of 16 albino rats weighing 230 ± 10 gms. were selected randomly from the rat population of the animal house of the Department of Psychology for the first phase of the experiment. When it was confirmed that learning did occur under anaesthesia, 16 more albino rats weighing 230 ± 10 gms. were selected randomly for the second phase. The animals were marked on their tails for identification and kept in separate plastic cages in small groups (2 animals/cage).

Instrumentation:

Classical conditioning apparatus:
It consisted of a shock generator, which was used to deliver the UCS and a tape recorder with a cassette of white noise—which provided the CS. The shock generator provided an alternating current stimulus of 50 cycles per sec. The electric timing circuit contained in the apparatus automatically passed stimulus current for a pre-set period which could be varied from 0.1 second to 1 second in steps of 0.1 second. The current could be varied from .25 to 360 mA. Two electrodes were used to give the shock. One point of the electrodes was connected to the external sockets of the shock generator and the other ends were brought in
contact with needle electrodes which were inserted in the hind thighies of the animal.

White noise:

White noise was used as a conditioned stimulus in the experiment. When sounds of different frequencies are mixed up in such a way that they have the same amount of energy output, the resultant hiss like sound is called white noise. According to Chapnis (1949) white noise resembles the noise obtained from the exhaust of an air-jet. A Philips (AM 124/10S) cassette recorder was used to play the taped white noise.

Epinephrine:

Epinephrine is a hormone which is released endogenously from the adrenal medulla. It increases the cardiac output although it is not a central stimulant in the usual sense. It also has an agonistic effect on alpha and beta adrenergic receptors, and increases the blood sugar level by inhibiting the release of insulin by its influence on the alpha adrenergic receptors.

It occurs as a white or light brownish micro-crystalline, odourless powder which is very slightly soluble
Figure 2. Showing the Chemical Structure of Human Insulin.
in water but dissolves readily in water acidified with HCl acid. In the market, it is available under the tradename of Adrenaline Tartrate in an injectable water base form (dilution 1:1000 parts).

Insulin:

Insulin is a protein composed of 51 amino-acids arranged in two chains (Figure II) linked by two disulfide bridges. The precursor of insulin in the beta cell consists of insulin connected by large peptide. The pancreas secret insulin constantly at a rate which varies with the blood glucose level.

The mechanism of action of insulin is to facilitate the penetration of mono-saccharide amino acids through the cell membrane of insulin sensitive tissue. Glucose is the sugar primarily affected by insulin action.

Because of its protein nature, insulin is destroyed if administered orally; therefore, it is administered subcutaneously. Insulin, manufactured by Boots Company (India) under the trade name of Insulin I.P. (40 units per ml) was used in the present study.
Propranolol:

Propranolol is a beta adrenergic blocking drug. These types of drugs block the action of catecholamines mediated receptor stimulating actions of adrenaline. With the administration of propranolol, the automaticity is suppressed and the atrioventricular (AV) conduction is slowed. This also reduces the blood pressure. Highly lipid soluble propranolol readily crosses the blood-brain barrier (B.B.B.) and has an anticonvulsant effects in laboratory animals.

Propranolol Hydrochloride is available in the market under the trade name of Inderal in 10, 20 and 40 mg tablets soluble in water and 1 mg ampules.

Sodium Pentothal:

It belongs to the family of barbiturates. These barbiturates are the derivatives of barbituric acid which is prepared by condensation of urea. This is an ultra-short acting drug.

This drug has severe effects on CNS. Reticular activation system, which is necessary for wakefulness, is very sensitive to its depressant action. Sodium pentothal produces sleep which resembles normal physiological sleep.
In the peripheral nervous system the conduction is slowed. High doses reduce the cardiac contractility. This chemical is highly soluble in lipids.

Generally, it is used to perform any surgery, but is also used in hypnosis or as an anticonvulsant. It also produces excitement, restlessness, allergic reactions etc.

In the market it is available under the tradename of Sodium Pentothal. It is available in powder form. It is also available in the form of liquid of 2.5% concentration.
Photographic Plate I

Showing the shock generator and anaesthetized rat with the needle electrodes.
Methodology

Sixteen of the selected albino rats were randomly assigned to the two groups (saline and E, 8 animals per group). On the initial two days the animals were weighted daily and the average body weight of each rat was calculated. From the third day onwards the animals were placed on a partial water deprivation schedule, while they had free access to food. They were given access to water, once daily, for 3 minutes at a fixed time in a small plastic cage placed in a dark room where a small zero watt bulb was used to provide a very low level of illumination. The animals quickly learned to drink almost continuously during this time and maintained themselves at approximately 80-85% of the original body weight, during the experiment. The classical conditioning trials were given on the tenth day, i.e. on the 8th day of water deprivation.

On the day of training, after the drinking session the table, on which the trials were to be given, was swabbed with 50% spirit solution and dried with the help of tissue paper. The apparatus was arranged in the required manner (shown in the photographic plate). The electrodes were connected to the output sockets of the shock generator. The tape-recorder with the cassette of white noise (which was
to be used as the conditioned stimulus) was placed at a distance of 2 feet from the shock generator. The required concentration and amount of sodium pentothal was prepared half an hour before the experimentation. The concentration was made in such a way that .25 ml of the solution contained 11.75 mg of sodium pentothal (i.e. 50 mg/kg). The exact amount of solution to be injected to the animal was calculated on the basis of its body weight. For injecting the animals intraperitoneally it was held with its dorsal surface upwards and the abdomen stretched. The injection was given on either the right or left side of the midline. The hypodermic needle was inserted in such a manner that an angle of approximately 30° was maintained between the syringe and the surface of the abdomen. Before injecting the solution, the plunger was pulled back slightly to ensure that the needle had not pierced a blood vessel or the bladder.

The first rat of the saline group (Group I) was given an injection of sodium pentothal (50 mg/kg) intraperitoneally and the experimenter waited for complete anaesthesia which set in within approximately 10 minutes. The depth of anaesthesia was checked with the help of tail pinch reflexes. When the animal was under complete anaesthesia two sterilised needles were inserted into the thighs of the hind limbs. These were further connected to the electrodes. The animal was given an ip injection of saline
(.25 ml, .9% NaCl/rat), 1- minutes after the anaesthetic treatment. After 7 minutes of injecting saline the classical conditioning training was started. In this the animal was presented with a pair of white noise (of 120 db for 30 seconds) and a shock (of 5 mA for .5 seconds). In exactly the same way the remaining 9 trials were given with an inter-trial interval (ITI) of 1 minute. The rest of the 7 animals were trained in the same way. After training, the animals were shifted to their home cages. Two days after the training, testing was done. During the testing session a drink suppression task was used. The animal was placed in a plastic cage, in a dimly illuminated room where it was being given access to water daily. The tape-recorder with the white noise cassette was placed at a distance of 2 feet from the cage. A stop watch was started as soon as the animal started drinking and the drinking time for the first minute was noted. With the beginning of the second minute, the tape-recorder was started and the amount of time spent in drinking during the second minute was also noted. The remaining animals were tested in the same way.

The same training and testing procedure was utilised for the E group (Group II) except that they were injected with E (250 µg/rat) instead of saline before training.

The animals of the remaining two groups (Group III,
insulin group; and Group IV, propranolol group) were also trained and tested in the same way as the Saline group (Group I) except that they were given a second injection of insulin (.05 units/kg, Group III) or propranolol (.05 mg/kg, Group IV) immediately after the E injection.

The obtained results were tabulated and the suppression scores (obtained by dividing the time spent in drinking during the second minute of the test session by the amount of drinking time of the first minute) of each animal were calculated. The obtained ratio scores were further statistically analysed.

(We may now pass on to the fifth chapter dealing with the results and discussion of the present investigation.)