The present investigation was conducted to study the effect of E on Pavlovian fear conditioning under anaesthesia. Some researchers have reported that E can enable learning even under the state of general anaesthesia (Weinberger, Gold and Sternberg, 1984; Gold, Weinberger and Sternberg, 1985). In the present study, the experiment was conducted into two phases.
The first phase was designed to determine whether 
E would enable learning to take place under a state of general 
aesthesia and whether this learning could be detectable, 
behaviourally, later. The aim of the second phase was to 
determine the mechanism through which the memory modulatory 
effects of E are mediated. The methodology used has been 
already discussed in detail in the preceding chapter. In 
brief, animals treated with different treatments were trained 
on pavlovian fear conditioning (noise paired with shock) 
while under sodium pentothal induced general anaesthesia. 
Retention was tested 2 days later by using a conditioned 
drink suppression task. The scores (in terms of cumulative 
number of seconds spent on drinking) of the animals of each 
group, during the first and second minutes of the test 
session, were calculated.

Table I  Showing the mean drinking scores of the four groups 
during the first and second minute of the conditioned 
water drink suppression test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean drinking scores (in seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First Minute</td>
</tr>
<tr>
<td>I</td>
<td>Saline</td>
<td>43.75</td>
</tr>
<tr>
<td>II</td>
<td>E</td>
<td>41.00</td>
</tr>
<tr>
<td>III</td>
<td>E + Insulin</td>
<td>48.00</td>
</tr>
<tr>
<td>IV</td>
<td>E + Propranolol</td>
<td>47.00</td>
</tr>
</tbody>
</table>
FIGURE 3 SHOWING THE MEAN DRINKING TIME SCORES OF GROUP I, II, III AND IV DURING FIRST AND SECOND MINUTE OF THE CONDITIONED WATER DRINK SUPPRESSION TEST.
From Table I, it appears that animals of the four groups spent almost an equal amount of time in drinking during the first minute of testing, or in other words, they continued drinking almost throughout the first minute. During the second minute, when the white noise (CS) was turned on, animals of Group I, III and IV kept drinking for nearly the entire minute. However, the drinking time of Group II (i.e. E injected group) animals was reduced drastically (Figure III). The individual drinking time of each animal was converted into suppression scores (number of seconds spent drinking during minute second divided by number of seconds spent drinking during the minute first).

Table II showing the mean suppression scores of the four groups on a conditioned water drink suppression test given 48 hours after training.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean suppression scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline</td>
<td>1.04</td>
</tr>
<tr>
<td>II</td>
<td>E</td>
<td>.541</td>
</tr>
<tr>
<td>III</td>
<td>E + I</td>
<td>1.04625</td>
</tr>
<tr>
<td>IV</td>
<td>E + P</td>
<td>1.1325</td>
</tr>
</tbody>
</table>

From Table II, it can be seen that the mean suppression scores of Group I, III and IV were more than one while that of Group II (E group) was less than one.
Figure IV. Showing the mean suppression score of group I, II, III, IV on a conditioned water drink suppression test.
(Figure IV). In order to determine whether those differences in the suppression scores were significant or the variation was due to chance factor, Kruskal-Wallis, the non-parametric counterpart of Analysis of variance (for independent group) were applied. The investigator of the present study deviated from the usual practice of applying a parametric test, to test the significance of difference between the means, because application of Hartley's F-Max test of Homogenity (Appendix-B) revealed that the variance among the suppression scores of the groups (F-max,10.10 < .01) was not homogenous.

The Kruskal-Wallis analysis (Appendix C) indicated a significant difference between the suppression scores of the four groups (H=264.75 < .01).

The differences between the test scores of the individual groups were further analysed by using Mann-Whitney-U Test to determine where actually the difference lay.

Initially let us compare the mean suppression score of Group I (Saline) and Group II (E) to determine whether E facilitates learning under anaesthetic state.
FIGURE V SHOWING THE MEAN DRINKING TIME SCORES OF GROUP I AND II DURING FIRST AND SECOND MINUTE OF THE CONDITIONED WATER DRINK SUPPRESSION TEST.
Table III  

Showing the significance of difference between the mean suppression scores of Group I (Saline) and Group II (E) during conditioned water drink suppression test, by application of Mann-Whitney-U test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Mean Suppression Score</th>
<th>Z-Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline</td>
<td>1.04</td>
<td>3.150*</td>
</tr>
<tr>
<td>II</td>
<td>E</td>
<td>.541</td>
<td></td>
</tr>
</tbody>
</table>

* P < .01

The statistical analysis revealed that mean suppression scores of Group II injected with E prior to training under anaesthesia, was significantly less than that of the saline treated group (Group I). In fact the mean suppression scores of the saline group was slightly more than I (1.04) indicating that an almost equal time was spent in drinking by these animals during minute second as compared to duration of time spent in drinking during minute first (Figure V). On the other hand, the mean suppression score of Group II was .541 showing that in case of the E treated animals, duration of time spent in drinking during second minute was suppressed to nearly half of
that spent in drinking during minute one. Since white noise (CS) was paired with shock (UCS) during training it served as a predictor of the aversive stimulus (shock) and obviously, acquired the aversive property of the UCS, itself. Thus, if animals, presented with CS-UCS pairing had acquired the association between noise and shock then it should be expressed later, through behavioural suppression, in the presence of CS. The same has been observed in E treated but not in saline treated animals. Thus the animals that received E at the time of training under anaesthesia demonstrated very good retention of the Pavlovian fear conditioning as evidenced by the conditioned suppression of water drinking when tested two days later. However, no evidence of retention was observed in saline treated animals as they continued drinking throughout the CS presentation.

The present results are in congruence with those obtained by Weinberger, et al. (1984), and Gold et al. (1985) who also observed a facilitative effect of E (1 mg/kg) in rats, trained for the pavlovian aversive conditioning, while under the state of general anaesthesia.

However, it might be argued that E enables learning by reducing the depth of anaesthesia. Earlier Lico et al. (1968) reported conditioned responses of blood pressure in
in rabbits only if anaesthesia was light enough to permit reflexes to be elicited. This possibility was also checked in the present investigation by measuring reflexes before and at the end of training. A single reflex, the tail reflex, was measured by strong pinch of the tail by a needle. However, the reflex was not observed in both the groups either during training or at the end of training. Therefore, the differential behaviour during the test for conditioned suppression can not be attributed to an anti-anaesthetic action of E. Earlier Weinberger et al. (1984) had also checked this possibility in detail. They measured heart rate and reflexes during and at the end of training. Results indicated that E does not lighten the state of anaesthesia. Further, the group receiving the lowest dose of E had the highest retention score, thus further indicating that E does not enable learning merely by lightening the level of anaesthesia. Additionally, the animals treated with E but never presented with paired CS-UCS showed no retention, thus indicating that retention in the experimental group was not due to some non-associative effect of E-such as increased sensitivity to the conditioned stimulus, but the effect was due to an influence on the processes underlying memory.
Thus our first hypothesis which predicted that peripheral administration of Epinephrine would have a facilitative effect on learning under an anesthetic state has been proved.

Since, the CNS processes are depressed during the anaesthetic state it is possible that E enables learning under the anaesthetic state either by directly initiating the CNS and/or peripheral processes underlying memory.

The second phase of the present experiment was conducted to test this possibility. Two additional groups i.e. E+insulin (Group III) and E+Propranolol (Group IV) were run and the performance of these animals were compared with Group one (saline) and Group II (E) to test the second and third hypothesis formulated in Chapter III.

In order to test the second hypothesis which predicted that peripheral administration of insulin would antagonise the facilitative effect of E on learning under an anaesthetic state, the mean suppression scores of Group I,II and III were compared.
FIGURE VI SHOWING THE MEAN DRINKING SCORES OF THE GROUPS I, II, III ON A CONDITION WATER DRINK SUPPRESSION TEST.
Table IV  Showing the significance of difference between the mean suppression scores of Group I (Saline) II (E) and III (E+I) during conditioned water drink suppression test by application of Mann-Whitney-U test.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean suppression scores</th>
<th>Z-scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Saline) and Group III (E+I)</td>
<td>1.04 and 1.046</td>
<td>.315</td>
</tr>
<tr>
<td>Group II (E) and Group III (E+I)</td>
<td>.541 and 1.046</td>
<td>3.360*</td>
</tr>
</tbody>
</table>

* p < .01

From Table IV, it can be seen that no significant difference was observed between the mean suppression scores of Group I (Saline) and Group III (E+I) indicating an absence of learning under anaesthesia in these animals. In fact, the mean suppression scores of Group III (E+I) was found to be significantly higher as compared to the mean suppression scores of Group II (E), (Figure VI). As discussed earlier a high suppression score is indicative of poor retention. Thus, insulin (a glucose inhibitor) at .05 units/kg antagonized the facilitative effect of E on learning under anaesthesia.

Concerning this point, it is interesting to underline that ip administration of insulin alone had no effect on retention of a single trial passive avoidance task at
this dose (as evidenced by preliminary pilot work). The insulin induced antagonistic effect on the facilitative effect of E indicates that the memory modulatory effects of peripherally administered E are mediated via its influence on the peripheral glucose levels.

Since peripheral E releases glucose from the liver (Gorbman et al., 1983) and glucose, itself, also modulates memory processes (Gold, 1986; Gold, Vogt and Hall, 1986), it is possible that memory modulatory actions of E may be mediated by subsequent increases in circulating glucose levels. Glucose under normal circumstances is a major fuel for metabolic activity in the CNS (Ingvar and Lassen, 1975; Lowry, 1975; Sokoloff, 1980). Unlike E, glucose is readily and activity transported into the brain (Oldendorf, 1971; Pardridge and Oldendorf, 1975; Lund-Anderson, 1979). Earlier studies suggest that pyruvate dehydrogenase is activated after avoidance training (Morgan and Routtenberg, 1981) and this substance is found to be critical in the production of cellular energy. Since glucose is the primary precursor of the substrate for pyruvate dehydrogenase in the brain, it appears that circulating glucose levels may regulate the efficacy of the neural processes underlying central processing of information through activation of this or other enzymes indicating that the
mechanisms of memory storage may be highly dependent on energy production.

Glucose is also a key precursor of acetyl COA in the CNS, which is necessary for Acetylcholine (ACh) synthesis and ACh synthesis is sensitive to relatively small changes in plasma glucose levels (Gibson and Blass, 1966). Treatments which effect central cholinergic systems also affect memory processing (Gold and Zornetzer, 1983). Thus, an alternative to the energy regulation explanation is that peripheral E via glucose release, may exert control over central ACh functions in memory storage.

Thus, the obtained results support the hypothesis which predicted that peripheral administration of insulin would antagonise the facilitative effect of E on learning under anaesthetic state.

The effect of a centrally/peripherally acting adrenergic antagonist, propranolol on the memory modulatory action of E was also investigated by the present investigator.
FIGURE VII SHOWING THE MEAN DRINKING SCORES OF THE GROUP I, II, III AND IV ON A CONDITION WATER DRINK SUPPRESSION TEST.
Table V  Showing the significance of difference between the mean suppression scores of Group I (Saline) II (E), III (E+I) and IV (E+P) during conditioned water drink suppression test by application of Mann-whitney-U-test.

<table>
<thead>
<tr>
<th>Comparison between</th>
<th>Mean suppression scores</th>
<th>Z-scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Saline)</td>
<td>1.04</td>
<td>.420</td>
</tr>
<tr>
<td>and Group IV (E+P)</td>
<td>1.1325</td>
<td></td>
</tr>
<tr>
<td>Group II (E)</td>
<td>.541</td>
<td>3.048*</td>
</tr>
<tr>
<td>and Group IV (E+P)</td>
<td>1.1325</td>
<td></td>
</tr>
<tr>
<td>Group III (E+I)</td>
<td>1.046</td>
<td>.525</td>
</tr>
<tr>
<td>and Group IV (E+P)</td>
<td>1.1325</td>
<td></td>
</tr>
</tbody>
</table>

* p < .01

From Table V, it is evident that the animals treated with E+P (Group IV) had significantly higher mean suppression scores as compared to E treated animals. However, the mean suppression scores of Group IV did not differ significantly from the mean suppression score of Group I treated with saline. Additionally, no significant difference was observed between the mean suppression scores of Group III (E+I) and Group IV (E+P) indicating that their performance on the retention test was comparable. (Figure VII). Since E treated animals (Group II) had significantly smaller
suppression scores as compared to saline (Group I) or E+P treated (Group IV) animals, it appears that E facilitates learning under anaesthetic state and this facilitative effect is attenuated with an ineffective dose of propranolol (.05mg/kg). In the preliminary pilot work it had been observed that ip administration of propranolol at this dose immediately after training did not affect retention of a single trial passive avoidance task. The propranolol induced antagonism of the facilitative effect of E indicates that memory modulatory effect of peripherally administered E are mediated via the noradrenergic receptors.

Support for this view comes from an earlier study conducted by Gold and van Buskirk (1978). They reported that peripherally administered E alters brain NE concentrations in a dose-dependent manner and these transient changes in NE levels were found to be correlated with later retention performance, thus indicating that E modulates memory processes, probably, by acting on the brain NE synapses. At the same time, neither the post-training (de Almedia et al, 1983) nor the pre-test effects (de Almeida and Izquierdo, 1983) of E on memory were obtained with icv injections suggesting the peripheral E might effect brain activity from the peripherally rather than the direct entry into the brain.

In the present investigation E induced memory,
facilitation is blocked with centrally acting adrenergic antagonist (Propranolol). Thus, the obtained results disprove our third hypothesis which predicted that peripheral administration of propranolol would not influence the facilitative effect of learning under the state of anaesthesia.

This negative finding does not imply that memory modulatory effects of E are mediated directly via the central NE synapses. There is still a possibility that memory modulatory effect of E are mediated primarily by the peripheral glucose release. The attenuating effect of propranolol observed in the present study may occur due to some other reason than the fact that it directly blocks the memory modulatory effect of E. Glucose release is under control of not only E derived from the adrenal medulla but may also be controlled by noradrenergic processes in the hypothalamus which appear to be independent of the adrenal medulla (Smythe et al., 1984). Further E induced hyperglycemia (glucose release) is mediated by a mixed adrenergic pharmacology (Ellis, 1956) in which both the alpha and beta adrenergic receptor antagonists can attenuate the glucose release (Ahlersova and Ahlers, 1976). Thus it is possible that peripheral administration of an adrenergic antagonist in the present investigation blocked the release of glucose either by acting at the hypothalamus and/or sympathetic nerve endings and thus changing the plasma glucose level necessary to obtain memory
enhancement. Since exogenously administered E as well as glucose has been found to interact with endogenously released E or glucose to produce memory modulatory effect, it might be possible that learning did occur in the animals treated with E+P (GroupIV) but it was certainly below behaviourally detectable levels as glucose released in response to E injection may not reach upto the optimum level due to the blockade of its release from the noradrenergic synapses. Finally, it appears that memory modulatory effect of peripheral E are mediated via glucose release.

We may now sum up the findings of the present investigation. A multi-group design experiment, with four groups was conducted in two phases to study the effect of E on Pavlovian fear conditioning under the anaesthetic state and implicate the mechanism through which the memory modulatory effects of E are mediated. It was hypothesized that peripheral administration of E prior to training would enable learning under anaesthesia and peripheral administration of insulin (a glucose inhibitor) but not propranolol (an adrenergic receptor blocker) would antagonize the facilitative effect of E on learning. A facilitative effect on learning under anaesthesia has been observed with pre-training E administration. However, the antagonistic effect has been observed with both the
antagonists. It appears that the memory modulatory effects of E are mediated via glucose.

Implication

Since E facilitates learning under anaesthesia, it appears that E can serve as an important memory modulatory agent and can facilitate memory in several conditions in which memory storage is deficient, such as persons suffering from Alzheimer's type senile dementia. Also the amnesia observed in persons suffering from acute diabetes could be attributed to the frequent fluctuations in their blood glucose levels.

Suggestion for further research

1. Further experimentation can be done to determine whether other adrenal or pituitary hormones which modulate memory in the normal state can facilitate learning under anaesthesia.

2. Since peripheral E releases brain beta-endorphin (Carraffco et al., 1982) experimentation can be done to determine whether nalaxone (opioid antagonist) can attenuate the memory modulatory effect of E under anaesthetic state.

3. Since E induced memory facilitative effects can be attenuated by both insulin (Glucose inhibitor) and propranolol (adrenergic blocker) there is the possibility that
the attenuating effects of propranolol may reflect decreases in plasma glucose levels. This possibility can be tested by assessing the glucose responses to training in normal and propranolol treated animals.