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The Prevention of Long-Term Memory Formation by 2,6 Diaminopurine

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KOBILER, D. AND C. ALLWEIS. *The prevention of long-term memory formation by 2,6 diaminopurine.* PHARMAC. BIOCHEM. BEHAV. 2(1) 9–17, 1974. – 2,6 Diaminopurine, an inhibitor of RNA synthesis was injected intracisternally into rats which had been taught an avoidance response. The metabolic effect of the drug was followed by measuring the incorporation of intracisternally injected tritium-labelled uridine into macromolecular RNA, and the behavioural effect by testing the retention of memory at various times. The results are presented in a series of three dimensional graphs, depicting the time course of the behavioural and metabolic effects of the drug. If RNA synthesis is reduced by 60% over the period from 30–200 min after training the elaboration of long term memory is prevented. The above metabolic and behavioural effects are reversible by intracisternally injected adenosine.

2,6 Diaminopurine Long term memory RNA synthesis Adenosine

BY THE USE of inhibitors, it has been possible to obtain evidence that ribonucleic acid and protein synthesis are related to the consolidation of memory in laboratory animals. (For a recent review see Barondes [2]).

The RNA inhibitors used until now in these studies have certain disadvantages. 8-Azaguanine if administered into the subarachnoid space of rats before training impairs acquisition [8]. Since actinomycin-D [6] and 2,6 diaminopurine (discussed in this paper) both inhibit RNA synthesis without impairing acquisition it must be concluded that 8-azaguanine has other effects which are responsible for its action on learning. Actinomycin-D must be injected intracerebrally in large doses to produce the desired effect and this may result in illness and death within hours to days later [2].

Reinis discovered that the intracranial injection of 2,6 diaminopurine into mice before or one hour after acquisition of a passive avoidance task impairs subsequent performance [12].

We report here on the use of 2,6 diaminopurine (DAP) in experiments designed to throw more light on the probable role of RNA synthesis in the consolidation of memory. It appears in most respects to be preferable to the above mentioned agents and has the added advantage that its metabolic effects can be abolished by an injection of adenosine, and providing this is done within a certain period following training its behavioural effects are prevented from occurring.

MATERIALS AND METHODS

Animals

Local (Sabra) white rats 35–45 days old (120–150 g) were used. When 21 days old, the rats were separated and kept one to a cage. (No difference in learning ability was found between these rats and Hooded Listers in our test).

Avoidance Apparatus

A training apparatus similar to that described by Albert [1] was used. It consisted of a wooden box 36 x 10 x 18 in. deep, divided into two equal sections by a vertical sliding door. One half of the box and the corresponding side of the door were painted white; the other half of the box and door were painted black. Each side of the box contained a separate grid floor which could be electrified by a current limited to about 0.33 mA.

At the start of training, the rat was placed on the safe side with the door open. After a 1 min delay, a uniselector advanced automatically and opened the door. The rat was then lifted out of the box and transferred to the other side by hand.

If the rat ran back to the safe side within 7 sec, the trial was scored as a successful response. If the rat failed to run back within 7 sec, the 7-sec delay relay closed and the rat received a shock. Further brief shocks were then given at 7 sec intervals until the rat ran back to the safe side of the box. This trial was scored as a failure. A record of the

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experiment was made with the aid of a potentiometric recorder.

Training continued until the animal reached a criterion of 10 avoidances in 10 or 11 successive trials (10/10 criterion) or 5 out of 5 or 6 trials (5/5 criterion).

Animals (<2%) which in their first training reached criterion after a single trial or more than 20 trials (not counting the criterion trials), were discarded.

Intracisternal Injection

Intracisternal injections were done with an Agla micrometer syringe, connected to the needle by a polythylene tube to permit free handling of needle. The rats were very lightly anaesthetized with ether just prior to injection. The dispersion of intracisternally injected materials in brain was studied using fluorescein (40 mg/ml) which was injected into anaesthetized rats. At various times the heads of the narcotized rats were frozen in liquid air. The frozen brain was exposed by rough dissection and the distribution of the flourescein in the frozen skull was studied under ultraviolet light. It was considered that this method minimized artifacts introduced by dissection of the unfrozen skull.

Fifteen minutes after injection, fluorescence was seen over the entire brain surface.

DAP (2,6 diaminopurine)

The DAP (California Foundation for Biochemical Research, 3408 Fowler St., Los Angeles, 63, California) injection was in a solution of 7 mg DAP (base) per ml 0.15 M $NaHCO_3$ solution. Because of the low solubility of the DAP, the injecting apparatus containing the solution was maintained at 37°C. The controls were injected with the sodium bicarbonate solution alone under similar conditions.

Adenosine

Some experiments included an intracisternal injection of 10 μl of a solution of adenosine (15 mg/ml) in $NaHCO_3$ (0.15 M) into each rat.

Determination of the Rate of Incorporation of Uridine-5-^{3}H into the Brain RNA Fraction

Brain RNA was separated using the Schmidt-Tannhauser method [13] as modified by Fleck and Munroe [9]. At various times after intracisternal DAP injection, rats received an intracisternal injection of 10 μl per rat of uridine-5-^{3}H (20.1 Ci/mM, 100 μC/ml) and were sacrificed half an hour later. The brain was homogenized in 5 ml cold water and rehomogenized to give a 1:20 suspension in cold water. To 5 ml of this suspension (250 mg tissue) was added 2.5 ml $HC10_4$ (2.1N). After standing for 15 min in the cold, centrifuging and washing twice with 0.7 N $HC10_4$, we dispersed the precipitate in 1 ml water, using a vortex stirrer and added 1 ml of 0.6 N KOH. The suspension was then incubated at 37°C for 15 min to dissolve the suspended material. Soluene (Packard) was used in some experiments to dissolve the precipitate. No significant radioactivity was found in the DNA fraction. Radioactivity was determined by counting 0.5 ml of the KOH solution or the whole Soluene solution in a scintillation counter. The scintillator used contained toluene, naphthalene and methylcellosolve.

Behavioural Results

The ability of the animal to learn in our experiments is quantitated as the number of trials required to reach an arbitrary criterion of success in avoiding shock. The rats were found to learn in 6.7 trials, S.D. ± 2.9 (N=192).

The learning ability of the animals was unaffected by intracisternal administration of DAP or any other procedure used and will therefore not be discussed in what follows.

The animal's ability to remember is operationally quantitated as the number of trials required in a subsequent TEST procedure to reach an arbitrary criterion of success in avoiding shock. Our behavioural findings are limited to results of this kind.

The test results expressed as the number of trials required to reach criterion (TTC) were the same when testing was carried out within the period of 4–5 hr after learning or up to 4 days after learning. We did not test at longer times.

Controls

All experiments were performed with controls, which received an intracisternal injection of 0.15M sodium bicarbonate solution instead of DAP when appropriate. A few experiments needed special controls and these are described in the text. (Some critical experiments were performed blind).

Presentation of Data

When this work was completed, we decided in the interests of clarity and brevity to present our main findings in graphic form. Since our tests for LTM within each group were run at various convenient times greater than 4 to 5 hr after training, we were unable to present individual time/TTC experimental points for each curve. However, as may be seen in Fig. 6 values for TTC do not change significantly with time later than 4–5 hr after learning under any of our experimental conditions. Hence the average TTC for the group as a whole was taken as the TTC value over the entire period.

Furthermore, to simplify the graphic presentation only, groups differing in their average TTC by less than 0.4 TTC from the average values 0.7 (remembers), 5.5 (does not remember) are assigned those nominal values respectively in the graphs. The exact numbers are always given in the text.

The biochemical effect of DAP is documented as the decreased incorporation (D.I.) of Uridine-5-^{3}H into the brain RNA measured over a half hour period and it is expressed as a percentage of the control value.

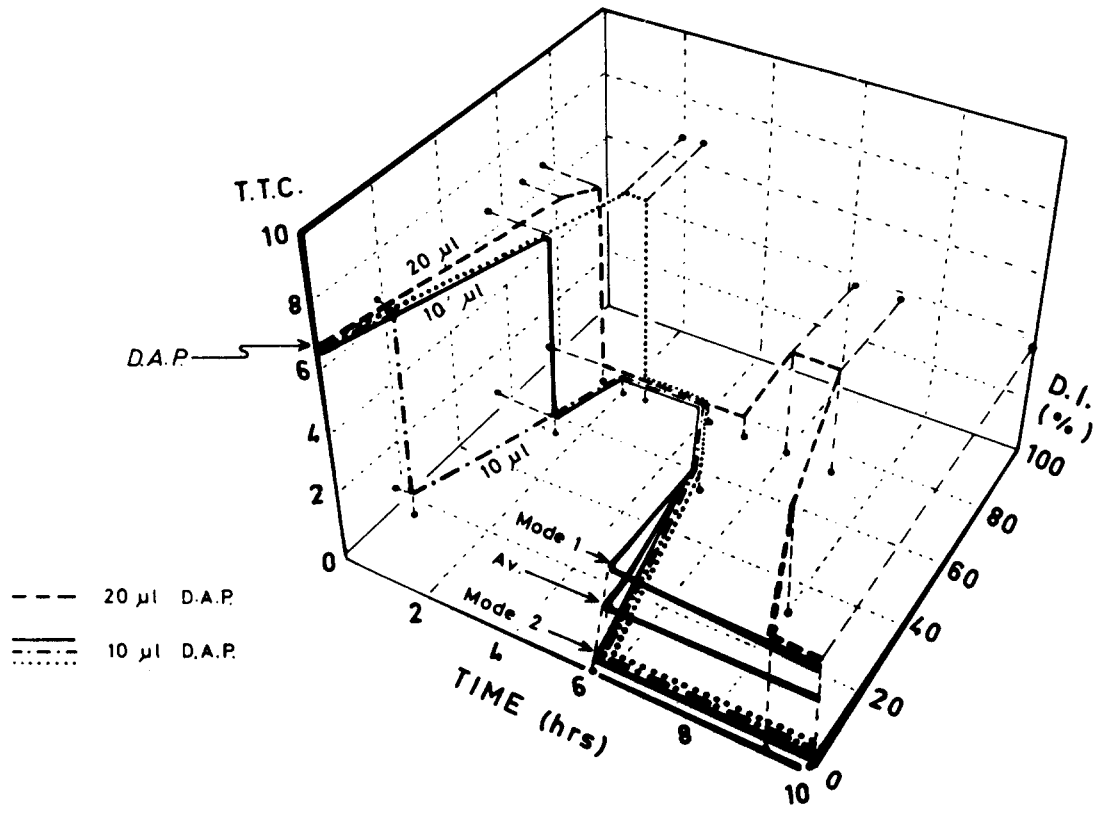
The interval between the administration of DAP and training will be referred to for brevity as Interval $_{\text{DAP}}$ - train.

Any line that can be traced out on a graph represents the behaviour of an animal which was subjected to the experimental treatment indicated by the graph and any additional annotation.

Branching points lead to different behaviours caused usually by different experimental procedures and in graphs 2 and 3 only, by different statistical treatments.

These lines were drawn on the basis of results obtained with different animals used in experiments which were terminated at different times. In reading the graph, it is helpful to remember that DAP was given at zero time, and that the abrupt vertical fall in the trajectory represents the initial training session.

By projecting a given trajectory vertically downwards onto the Time /DI plane, a graph of the time course of the



GRAPH NO. 1. The effect of 60 and 120 µgm intracisternal doses of DAP given at zero time on memory retention in rats trained to a criterion of 10/10. In this and all other graphs D.I. refers to the decrease in incorporation of tracer. Training is indicated by an abrupt decrease of TTC. Only the Interval DAP → train 90′ group (full lines) showed impairment of memory. The average result for the group and the subdivision of the results into mode 1 and mode 2 is explained in the text. The impairment was greater when twice the amount of DAP was given.

biochemical activity of a administered substance can be obtained alone. By projecting a given trajectory horizontally forwards onto the TTC/Time plane a graph of the behavioural changes alone can be obtained.

RESULTS

Graph No. 1: The Effect of 60 and 120 µg Doses of DAP on Rats Trained to a Criterion of 10/10

1. The time course of the decreased incorporation of Uridine-5-^3H into brain RNA. As may be seen from the graph, in all groups there was a progessive decrease in the incorporation of the labelled precursor into RNA following the intracisternal injection of 60 µg DAP in a volume of 10 µl. The effect reached a maximum value of a 75% decrease after 2 hr, and remained at that value for about two hours before diminishing. By the end of the 6th hr the effect had disappeared.

When double this amount of DAP was administered (broken line) its effect was seen to become maximal earlier and was prolonged up to seven hours at its maximal value before diminishing. However, the larger dose did not cause any further decrease in incorporation.

2. The short term effect of DAP on behaviour. With the 10 µl dose, the Intervals DAP → train were 30 min (dot-dash line) 90 min (full line) and 150 min (dotted line). At these times groups of rats were trained to a criterion of 10/10. When these groups were tested at various times after training we found no impairment in performance up to four hours after training.

3. The long term effect of DAP. Animals from the above groups were tested for retention more than 5 hours after learning.

(a) The groups Interval DAP → train 30′ and 150′ did not show any impairment of performance (TEST - 0.75 ± 0.66 TTC; N = 12)

(b) The Interval DAP → train 90′ group showed a somewhat higher average TTC value (TEST - 2.05 ± 1.51 TTC; N = 18). On closer inspection these results seemed to have a bimodal distribution. The results were divided into two groups in accordance with this distribution.

DAP Mode I	TEST–0.8 ± 0.6 TTC; N=10
DAP Mode II	TEST–3.62 ± 0.48 TTC; N=8

The results of Mode II are the same as controls. This suggests the presence of an effect of DAP on some of the

animals in the Interval DAP → train -90′ group and its absence in others.

(c) The dotted line represents the results obtained in a similar experiment using 20 μl DAP (120 μg). The impairment of performance (TEST -3.5 ± 0.68 TTC; N = 8) is of the same degree as the impairment seen in the animals of Mode II of the Interval DAP → train 90′ group with a single dose.

These results show that intracisternally injected DAP is capable of interfering with long term memory, but that the outcome in a particular experiment is greatly dependent on the interval which elaspsed between the injection of the DAP and training. The impression gained was that if DAP slows RNA synthesis below about 60% of its normal value during a critical period subsequent to training, then the establishment of LTM_2 is prevented. The critical period in question appears to extend approximately from 30 min to 200 min after training.

Graph No. 2: The Effect of DAP on Rats Trained to a Criterion of 5/5

In the hope of rendering the animals more sensitive to the effect of DAP by limiting their training, we carried out a similar experiment using a criterion of 5/5 in both training and testing instead of 10/10. We also tested the effect of twice the amount of DAP under these experimental conditions.

(1) As in the previous experiment no short term effects on performance were found up to 4 hours after training.

(2) The long term effect of DAP. (a) The 10 μl (60 μg) DAP group, Interval DAP → train 90′ (full line) needed an average of 4.21 ± 2.61 TTC; N = 19. This is a higher value than that found for the parallel group in the previous experiment. As in the previous experiment, the results showed a bi-model distribution and resolution into two sub-groups gave the following results.

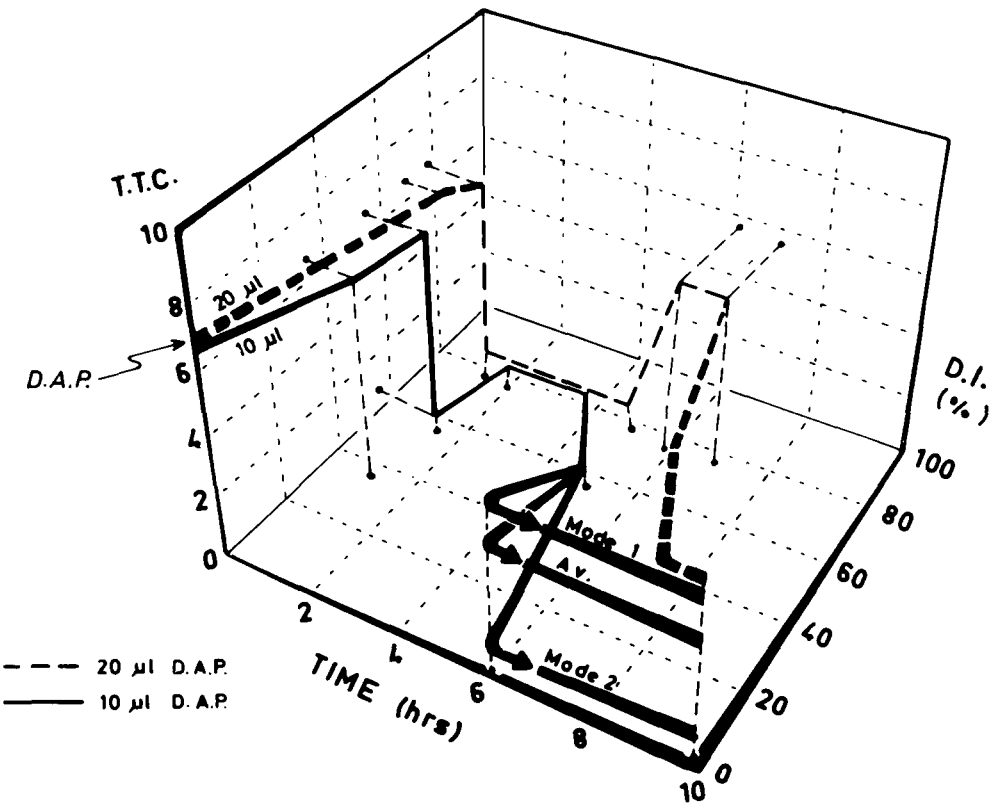
DAP Mode I	0.6 ± 0.49 TTC; N = 5
DAP Mode II	5.5 ± 1.68 TTC; N = 14

In the previous experiment in which the criterion was 10/10, half of the group behaved like the controls, whilst in this experiment only a quarter of the groups behaved like controls.

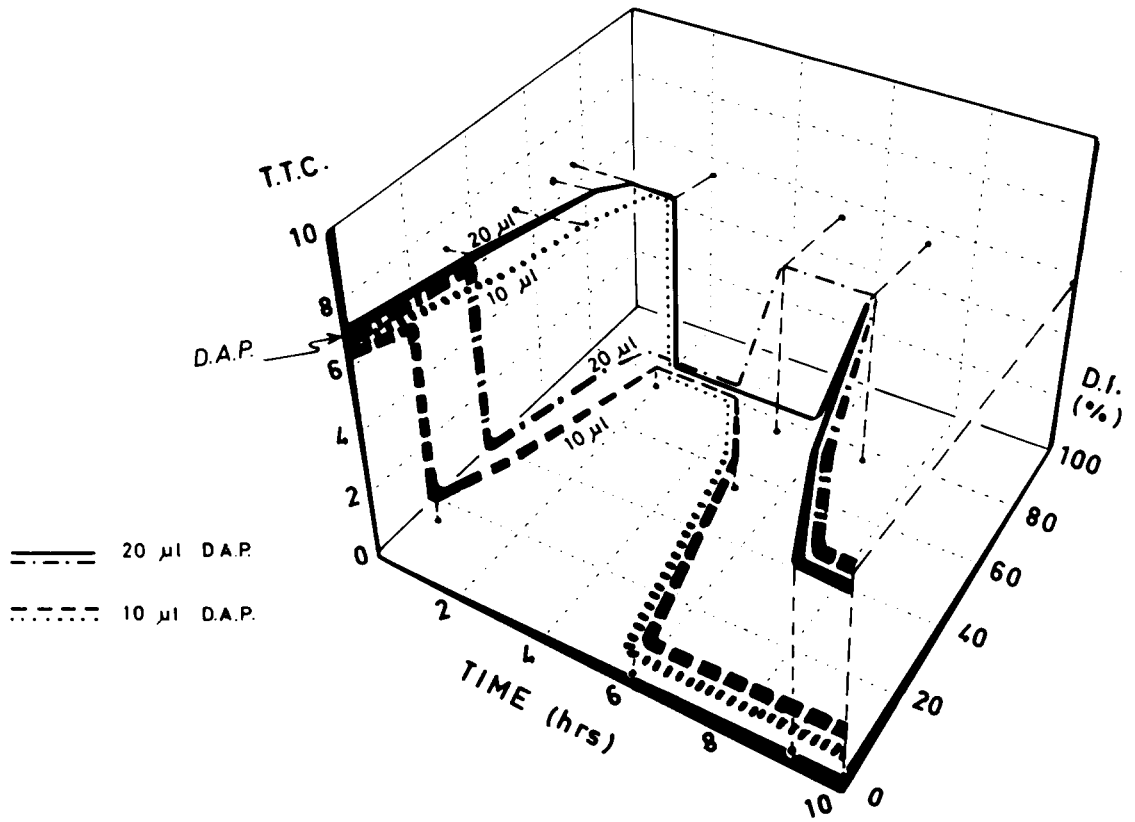
(b) The 120 μg (20 μl) DAP group (broken line) showed an impairment of performance: TEST 5.46 ± 1.14 TTC; N = 15.

These results suggest that in rats trained to a criterion of 5/5, the processes leading to consolidation of memory for the long term are more susceptible to interference by DAP than was the case with rats trained to a criterion of 10/10, so that the critical period might be shorter than was found in the previous experiment.

These results also indicate that an interaction between RNA synthesis and another mechanism responsible for temporary memory retention till such synthesis occurs is essential for the establishment of long term memory. From the data presented in Graph 2 it may be inferred that this interaction may occur either before the DAP becomes ef-



GRAPH NO. 2. The effect of DAP on rats trained to a criterion of 5/5.



GRAPH NO. 3. Dose dependence of DAP effects. The larger dose of DAP prevents the fixation of memory over a greater Interval $_{DAP \rightarrow train}$ than does the smaller dose.

fective following the 60 µg dose or after its effects was worn off.

Graph No. 3: Dose Dependance of DAP Effects

Judging from the difference in the shape of the incorporation curves obtained with the 10 µl or 20 µl DAP injections, we expected to find prevention of LTM formation over a greater range of Interval $_{DAP \rightarrow train}$ using the larger dose.

The results of the groups Interval $_{DAP \rightarrow train}$ 30′ and 150′ when the dose was 60 µg (0.75 ± 0.72 TTC; N = 12) showed no impairment of performance. After 120 µg DAP, marked impairment of performance was found over the same range of Interval $_{DAP \rightarrow train}$ as expected (TEST 5.5 ± 1.21 TTC; N = 16).

This parallel extension in time of the biochemical and behavioural effects of DAP is further evidence that RNA synthesis is an essential step in the fixation of memory.

The biochemical effect of the larger dose of DAP as determined by our method was of longer duration but not more intense than the smaller dose. However the possibility exists that local differences of the intensity of the effect which were not detected by our methods were responsible for the difference in the results. To clarify this point we performed the next experiment.

Graph No. 4: The Effect of Two Injections of DAP

The experiments depicted in this graph were carried out in an attempt to decide if the duration of DAP-induced decrease in the incorporation of uridine into RNA was the critical factor in preventing the establishment of LTM, rather than the intensity of its effect. The technique was to extend the period of DAP action without increasing its intensity by injecting a second dose of the drug before the effect of the first dose wore off.

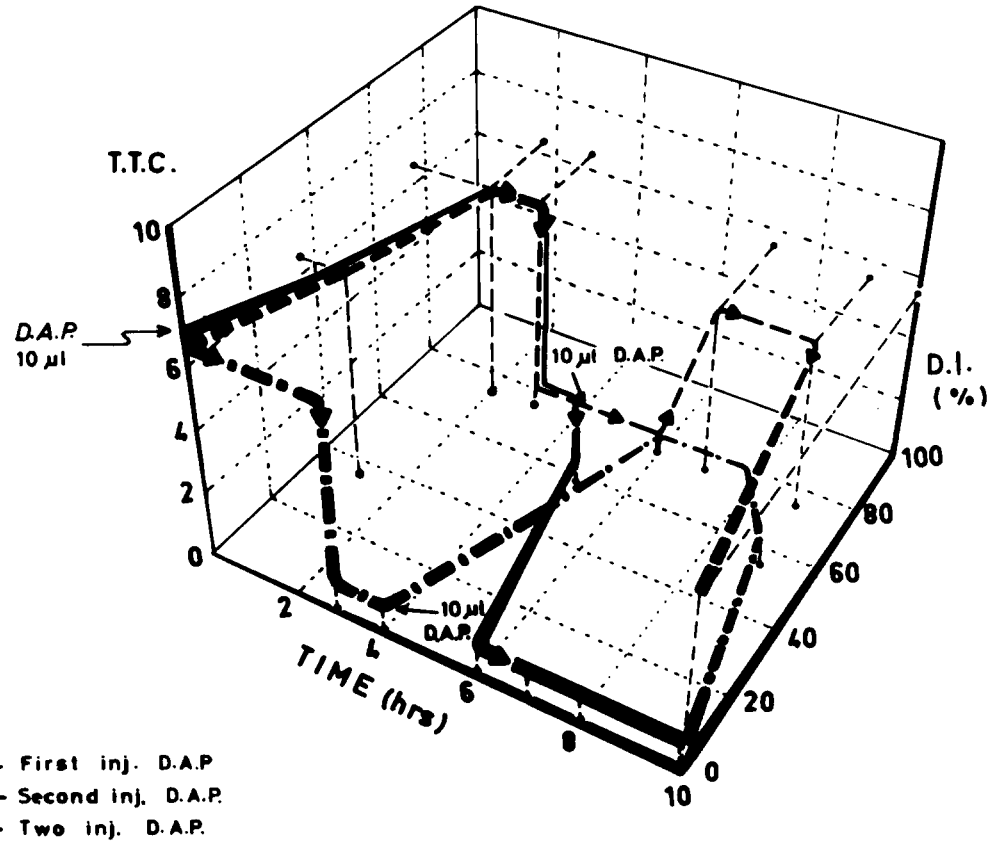
(1) The time course of the decrease in the incorporation of Uridine-^3H into brain RNA fraction using repeated doses of DAP. The two control groups each received a single 60 µg dose of DAP in 10 µl of solution. The first group (Control I full line) received 60 µg DAP in the first injection and 10 µl of $NaHCO_3$ solution in the second. The second group (Control II, — · — ·) received the same injections in the opposite order. In the experimental group (broken line) the first dose of 60 µg DAP produced the expected metabolic effect, and the second injection at the 4th hour maintained the effect at its full intensity up to the 9th hour.

(2) The effect on performance. The TTC of DAP-treated rats is seen in this graph to be the same as the TTC of naive untreated rats. In this and in many other experiments we found no evidence that DAP impairs learning ability.

The performance of the two control groups was unaffected by the DAP (Control I TEST 0.3 ± 0.49 TTC; N = 9. Control II TEST 0.7 ± 0.46 TTC; N = 10). The experimental group showed a marked impairment of performance (TEST 5.55 ± 1.26; N = 9).

Comments on Graphs 2–4.

It was suprising to find an almost exact coincidence in



GRAPH NO. 4. The effect of two injections of DAP. The first DAP injection extends the duration of the effect of DAP on RNA synthesis thereby affecting memory.

mean values obtained for the degree of impairment of memory in different experiments although the standard deviations were up to 25%. The distribution curves were similar. The difference in the degree of impairment of memory found between Graphs 1 and 2 seemed to depend on the training–testing criterion. Barondes and Cohen [3] and Cohen and Barondes [7], found that the impairment of memory by acetoxycycloheximide could be obscured by overtraining and suggested that with marked overtraining, the residual degree of cerebral protein synthesis was sufficient to lead to complete or partial long term memory storage.

Graph No. 5: The Prevention of DAP Effects on Memory by Administration of Adenosine

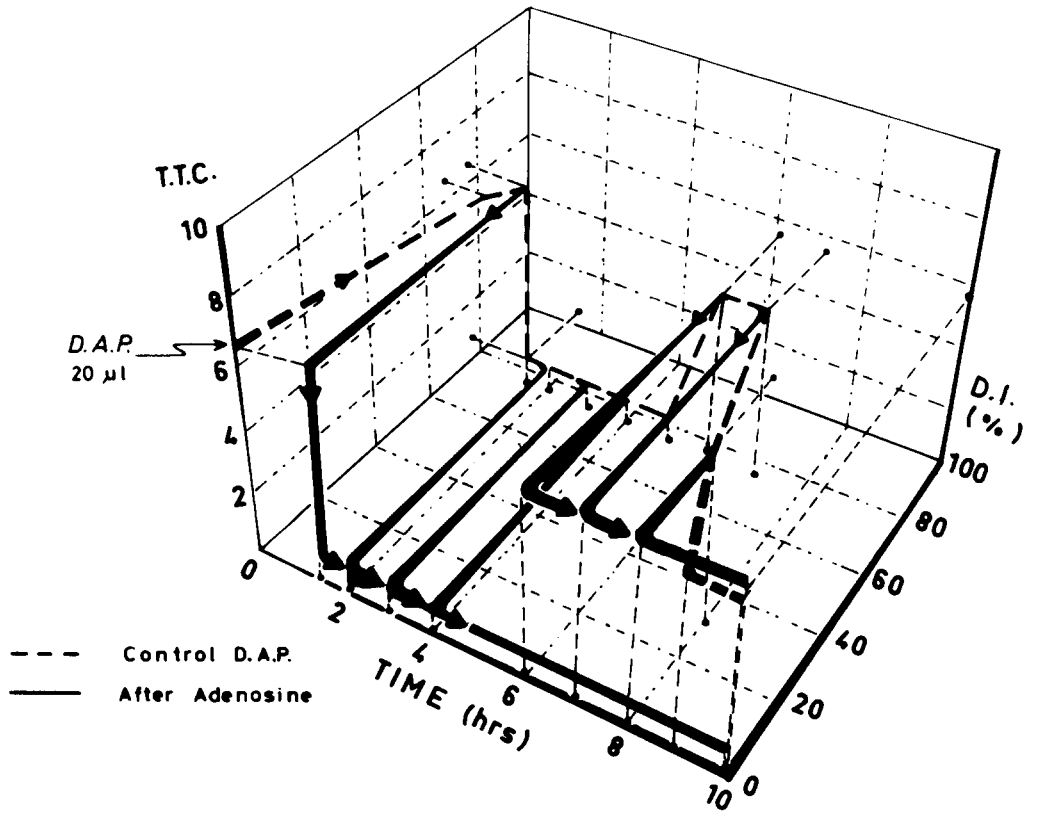
Knowing from the literature that the biochemical effect of DAP on RNA synthesis could be reversed by adenine derivatives, we tried to obtain a parallel reversal of its behavioural effect.

(1) Effect of adenosine on the time course of incorporation of uridine. The broken line represents the known effects of an injection of 20 µl DAP (120 µg) on the incorporation of precursor into brain RNA and on behaviour. The injection of 150 µg adenosine (10 µl) per rat at various times after the injection of DAP restored the rate of incorporation of precursor to values which were 95–100% of normal within 15 min.

(2) The effect of adenosine on DAP impairment of long term retention. If the adenosine was injected before or within the first 3 hr after training there was no impairment of performance (before adenosine TEST 0.7 ± 0.46 TTC; N = 9, after adenosine TEST 0.53 ± 0.48 TTC; N = 15).

The importance of this result lies in the fact that it indicates the absence of any secondary effect of DAP on the brain which might not be reversed by adenosine and yet be responsible for the effects of DAP on behaviour.

Our results indicate that even in the presence of DAP the establishment of long term memory may occur as late as 3 hr after learning when RNA synthesis is reinstated. This suggests that RNA synthesis is essential and that it is not some other action of DAP that is responsible for the effects we observed. Furthermore, LTM developed spontaneously following adenosine administration or when the biochemical effect of DAP injected before training wore off early enough (Graphs 1, 2 and 3) without the necessity for any additional activating factor. These results are in contrast to the work of Barondes and Cohen [4], who found that "arousal-producing manipulations" such as "foot shock", amphetamine or corticosteroids were required in addition to the persistence of the cognitive information acquired from training and an intact cerebral protein synthesis capacity in order for LTM to be produced. This difference could be due to a secondary effect of cycloheximide, similar to that demonstrated by Reilly *et al.* [11].



GRAPH NO. 5. Reversal of the DAP effect on RNA synthesis and the prevention of the DAP effect on memory by the administration of adenosine. The adenosine was effective if it was injected either before or within about 3 hours after learning, but not later.

Graph No. 6: The Effect of DAP on Long Term Memory Formation

This graph summarizes data obtained on about 90 rats. It shows that when an adequate dose of DAP is given at the appropriate time, loss of memory becomes evident about 4 hr after training. No further changes in TTC were noted up to 96 hr after training.

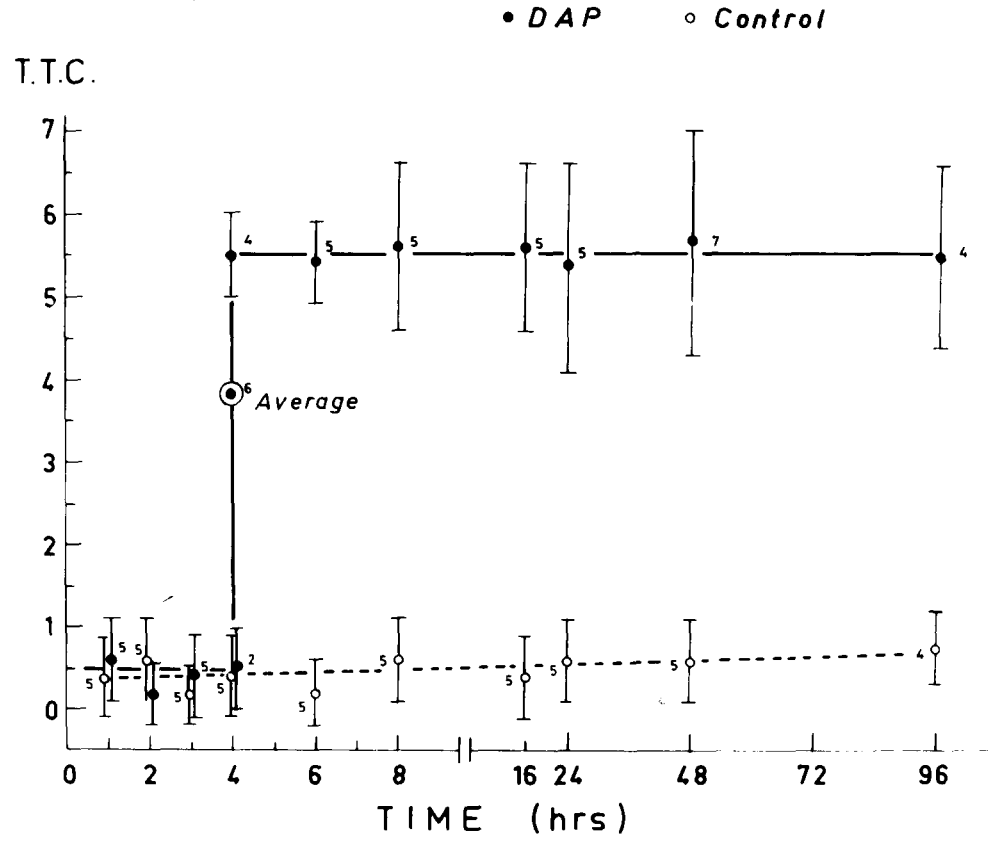
DISCUSSION

DAP is known to inhibit RNA synthesis and cell growth but the mechanism by which it does so is not clear [5,10]. Our experiments show that intracisternally-admininstered DAP decreases the incorporation of labelled uridine into brain RNA, and simultaneously under certain experimental conditions it also prevents the establishment of long term memory.

It was possible to decrease the incorporation of uridine into macromolecular RNA only 75% by intracisternal injection of DAP. Since the same route was used for both inhibitor and tracer, the same brain surfaces must have been exposed to both substances. The persistence of 25% of the normal incorporation rate does not appear to be due to limited concentration-dependent competition, since doubling the dose of DAP did not diminish the residual 25% incorporation rate, although its effect lasts longer. Neither does the persistence of 25% of the normal incorporation appear to be due to a time concentration-gradient limited diffusion of DAP within the brain as compared to uridine, since if that were the case the higher dose of DAP would have been expected to produce a further decrease in incorporation as well as increasing its duration. It therefore appears that the limit to the biochemical inhibition is chemically determined or due to the existence of a permeability barrier to DAP in some part of the brain.

A DAP-induced 60% decrease in incorporation, provided it was maintained over the period of from about 30 to 200 min after training, severely impaired the establishment of long-term memory in about half the animals. On changing from a criterion of 10/10 to one of 5/5 the fraction of animals in a group which showed impaired memory increased from about 1/2 to 3/4. This finding demonstrated that there was marginal competition between the extent of training and these time-dosage conditions. We did not determine the exact limits of the critical period with the 5/5 criterion and it is possible that it might differ appreciably from that found in the 10/10 criterion.

The biochemical and behavioural effects of DAP can both be extended in time by using a larger dose. The larger dose takes effect more rapidly and the duration of the effect is extended. This fact was used to demonstrate a parallel extension in time of the behavioural effects of DAP. (Graph 4) Whilst with the lower dose the behavioural effect was limited to Interval DAP → train 90 min, with the higher dose it could be extended backwards to Interval DAP → train 30 min and forwards to Interval DAP → train 150 min.



GRAPH NO. 6. The influence of time on test results in about 90 animals, which were all trained at zero time. The effect of DAP on memory became evident 4 hours after training. No further changes were seen up to 96 hours after training. Note break in abscissa.

The results so far discussed are taken to mean that RNA synthesis (which is slowed or may perhaps even be completely abolished by DAP in some key brain locations) must normally occur in the brain during the period from about 30 to 200 min after training in order for memory to be consolidated for a long term. Furthermore, it appears from the data that this essential RNA synthesis may occur to some extent either before the action of previously-injected DAP becomes intense or after it has worn off, suggesting that the DAP is without effect on the early memory retaining mechanism. The 30 minute period following training may be a period during which mechanisms which lead up to RNA synthesis are undergoing activation. Since all animals in a group are rendered amnesic, by prolonging DAP action by increasing the dose, it is assumed that the 30–150 min sensitive period referred to above is marginal. Implicit in this interpretation of our findings is the assumption of the existence of a qualitatively different memory retaining mechanism which is operative prior to consolidation.

The experiments with adenosine indicate that the early memory-retaining mechanism decays to an ineffective level in about 200 min after training.

In order to derive unequivocal conclusions from these experiments, it would be necessary to prove that the effects on behaviour found in our experiments were direct consequences of the effect of DAP on the rate of RNA synthesis and not due to side effects of DAP on brain function. The following facts support this viewpoint: (1) DAP did not interfere with the learning process itself (Graph 3), nor with performance over the next four hours (Graphs 2 and 3). (2) DAP did not interefere with the expression of behaviour which was learned prior to its administration (Graph 4). (3) No overt side effects were noted in animals which had received DAP. (4) When the known biochemical effect of DAP is reversed by adenosine in good time, loss of memory does not occur (Graph 5).

Whilst not conclusive, this evidence is in agreement with the hypothesis that the effects of DAP on memory are a direct consequence of its interference with RNA synthesis rather than due to a disruption of other aspects of normal brain function.

The use of different animal species and different training situations as well as different inhibitors makes it difficult to compare the results of different investigations in this field. Reiniš, working with mice, found that DAP produced impairment of memory of a passive avoidance task when it was injected 24 hr or 2 hr before training or 1 hr after training. However the impairment was evident only if the animals were tested 48 hr or more after training. Reiniš suggested that the effectiveness of DAP 24 hr after injection might be due to its deposition in nervous tissue at the lower pH of body fluids but did not present incorporation

studies to substantiate this hypothesis. Our incorporation studies show clearly the time course of activity of intracisternally injected DAP in relation to its effectiveness in preventing LTM formation.

Reiniš also found a delay of about 48 hr after training before impairment of memory became clearly evident in DAP - treated animals, and suggested that this was due to the persistance of "short-term memory mechanisms" up to 24 hr after training. Whether this is due to a species difference, or the existence of a MTM process whose temporal characteristics are dependent on the type of task learned remains to be seen.

The results presented here parallel in many respects results previously obtained by many other workers using the glutarimide derivatives cycloheximide and acetyoxycycloheximide to interfere with what may well be the next step in the consolidation of memory protein synthesis [2]. (These inhibitors avoid the complications which arise when puromycin is used). The main conclusion drawn from such experiments are that protein synthesis occurring between approximately 20′ and 180′ after training is necessary for the formation of long-term memory. Our results demonstrate that DAP can interfere with the consolidation process at an earlier stage, namely, RNA synthesis. DAP should therefore prove very useful in further investigations of the mechanism of memory consolidation.

REFERENCES

1. Albert, D. J. Memory in mammals. Evidence for a system involving nuclear RNA. *Neuropsychologia* **4**: 79–92, 1966.
2. Barondes, S. H. Cerebral protein synthesis inhibitors block long-term memory. *Int. Rev. Neurobiol.* **12**: 177–205, 1970.
3. Barondes, S. H. and H. D. Cohen. Delayed and sustained effect of acetoxycycloheximide on memory in mice. *Brain Res.* **58**: 157–164, 1967.
4. Barondes, S. H. and H. D. Cohen. Arousal and the conversion of 'short-term' to 'long-term' memory. *Proc. Nat. Acad. Sci. U.S.A.* **61**: 923–929, 1968.
5. Brockman, R. W. Mechanism of resistance to anticancer agents. *Adv. in Cancer Res.* **7**: 129–234, 1963.
6. Cohen, H. D. and S. H. Barondes. Further studies of learning and memory after intracerebral actinomycin-D. *J. Neurochem.* **13**: 207–211, 1966.
7. Cohen, H. D. and S. H. Barondes. Effect of acetoxycycloheximide on learning and memory of a light dark discrimination. *Nature* **218**: 271–273, 1968.
8. Dingman, W. and M. B. Sporn. The incorporation of 8-azaguanine into rat brain RNA and its effect on maze learning by the rat. *J. psychiat. Res.* **1**: 1–11, 1961.
9. Fleck, A. and H. N. Munroe. The precision of ultraviolet absorption measurements in Schmidt-Thannhauser procedure for nucleic acid estimation. *Biochimica et Biophysica Acta* **55**: 571–583, 1962.
10. Hitchings, G. H. and G. B. Elion. Purine analogues. In: *Metabolic Inhibitors* edited by R. M. Hochster and J. H. Quastel, New York: Academic Press pp. 215–237, 1963.
11. Reilly, C., G. F. Fuhrmann and A. Rothstein. The inhibition of K^+ and phosphate uptake in yeast by cycloheximide. *Biochimica et Biophysica Acta* **203**: 583–585, 1970.
12. Reiniš, S. Effect of 5-Iodouracil and 2,6 Diaminopurine on passive avoidance task. *Psychopharmacologia* **19**: 34–39, 1970.
13. Schmidt, G. and S. J. Thannhauser. A Method for the determination of DNA, RNA and phosphoproteins in animal tissues. *J. biol. Chem.* **161**: 83–89, 1945.