Trial 1 choice and then alternated. Strong-preference Ss were not likely to leave the arm chosen on Trial 1 if they reentered it on Trial 2. Hughes (1966), investigating the effect of stimulus change on SA, found it did affect frequency of partial body entries but not the arm which was finally selected. The present study indicates that position preference also may be reflected more strongly in variations in body entry of the nonselected side than in frequencies of final choice.

The primary hypothesis gains support from Iwahara's (1960) finding that shocked rats, with strong position preferences, showed an unusually sharp decline in SA as ITI was increased from 0 sec to 3 min. In further examination of the hypothesis, position preference differences could be obtained by appropriate selection of the strains used (see Douglas & Isaacson, 1965), with the confounding this would entail, or preferences could be artificially created by using arms of different brightness (see Eisenberg, Myers, Sanders, & Shanab, 1970; O'Connell, 1964; Pate & DeLoache, 1970). Preferences could also be established by variations in amount of reward training. SA data obtained under these conditions would invite comparisons with results of studies of proactive inhibition (e.g., Gleitman & Jung, 1963).

REFERENCES

- DEMBER, W. N. Alternation behavior. In D. W. Fiske and S. R. Maddi (Eds.), *Functions of varied experience*. Homewood, Ill: Dorsey Press, 1961. Pp. 227-252.
- DENNY, M. R., & LECKART, B. Alternation behavior: Learning and extinction one trial per day. *Journal of Comparative & Physiological Psychology*, 1965, 60, 229-232.

- DOUGLAS, R., & ISAACSON, R. Homogeneity of single trial response tendencies and spontaneous alternation in the T-maze. *Psychological Reports*, 1965, 16, 87-92.
- EISENBERGER, R., MYERS, A. K., SANDERS, R., & SHANAB, M. E. Stimulus control of spontaneous alternation. *Journal of Comparative & Physiological Psychology*, 1970, 70, 136-140.
- GLANZER, M. Stimulus satiation: An explanation of spontaneous alternation and related phenomena. *Psychological Review*, 1953, 60, 257-268.
- GLEITMAN, H., & JUNG, L. Retention in rats: The effect of proactive interference. *Science*, 1963, 142, 1683-1684.
- HUGHES, R. N. Stimulus-change effects of forcing in alternation behavior. *Psychological Reports*, 1966, 19, 515-518.
- IWAHARA, S. Effect of intertrial interval upon alternation behavior in the shocked rat. *Psychological Reports*, 1960, 6, 266.
- KLEIN, S. B., & SPEAR, N. E. Forgetting by the rat after intermediate intervals ("Kamin effect") as retrieval failure. *Journal of Comparative & Physiological Psychology*, 1970, 71, 165-170.
- O'CONNELL, R. H. Comparison of alternation and response to stimulus change. *Journal of Comparative & Physiological Psychology*, 1964, 57, 362-366.
- O'CONNELL, R. H. Trials with tedium and titillation. *Psychological Bulletin*, 1965a, 63, 170-179.
- O'CONNELL, R. H. Spontaneous alternation following long intertrial intervals. *Psychological Reports*, 1965b, 17, 131-134.
- PATE, J. L., & DELOACHE, J. Brightness and direction as cues for spontaneous alternation behavior. *Psychonomic Science*, 1970, 18, 27-28.
- SIEGEL, S. *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill, 1956.
- SQUIRE, L. R. Effects of pretrial and posttrial administration of cholinergic and anticholinergic drugs on spontaneous alternation. *Journal of Comparative & Physiological Psychology*, 1969, 69, 69-75.
- THOMPSON, R. Transient memory in albino rats. *Science*, 1959, 129, 842-843.

regenerated animals were tested for 3 consecutive days for retention of the conditioned response. Significant results were obtained when regenerated-head planaria, kept in RNase, were tested. This group behaved randomly as though it had not been conditioned. It was assumed that memory was impaired and that RNA was involved in retention of a conditioned response. All other regenerated Ss evidenced no deficit in performance of the response.

Experiments like that of Corning and John, purporting to demonstrate conditioning in planaria and other species, have been criticized on many grounds. Sensitization rather than conditioning of the planaria seems to have been indicated in these experiments. In addition, the data on whether or not planaria are even capable of learning are extremely equivocal (Jensen, 1965). However, the above study is illustrative of those favoring the implication of RNA in learning and memory. The role of DNA has been less extensively studied, perhaps because of its lesser lability than RNA. A nucleoprotein, highly resistant to alternation, could not transcribe the changes necessary for learning and retention. However, it would seem impossible for a learned response to be maintained unless DNA were involved, for after the initial altered RNA is depleted, from where would come the instructions to keep encoding this newly formed structure? This suggests that some structural modification must occur to DNA so that it could keep transcribing the altered RNA which would, by some complex and uncertain process, enable the response to be retained over a period of time.

The purpose of the present experiment was to determine if there was a time-dependent involvement of DNA and RNA in the maintenance of an acquired response pattern. Planaria were taught to crawl to a particular position in a glass bowl to escape an aversive light stimulus. By transecting the animals at different times after training and placing them in solutions containing RNase or DNase to regenerate, it could be ascertained whether RNA or DNA were involved in retention and if there was a temporal relationship. Testing for retention of responses after regeneration enables testing of the hypothesis that altered RNA must migrate back to the nucleus of the cell and bring about structural changes in DNA. The implication is that if the migration is interrupted along the way, for example by electroshock or more probably by enzymatic activity, memory consolidation would be

Temporal effects of RNase and DNase in disrupting acquired escape behavior in regenerated planaria

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Trained planaria, transected immediately after the last training trial and regenerated in RNase, exhibited impairment of an acquired escape pattern of responses. Regeneration in DNase resulted in no response decrement. Ss transected 24 h after completion of the last training sessions and regenerated in either RNase or DNase, displayed significant response decrement. Results suggest a time-dependent involvement of RNA and DNA in maintenance of the acquired response pattern.

The possible roles of RNA and DNA in learning and memory have received much attention by investigators in the past decade.¹ Corning & John (1961) trained planaria to make a contraction

to a light stimulus in order to avoid an electric shock. Ss were then transected and allowed to regenerate, half in pond water, the other half in pond water containing RNase. The

Table 1
Mean Times Necessary for Task Completion on the Last Four Training Trials and for Groups After Regeneration. *t* tests were performed to compare the mean times of each regenerated group to the mean time of the last four training trials.

	Last Four Training Trials	Immediate Transection Group			Delayed Transection Group		
		A(Control)	B(RNase)	C(DNase)	D(RNase)	E(DNase)	F(Control)
Time (Sec)	116.25	115.75	132.75	116.25	135.75	134.50	117.25
<i>P</i> *		.05 < <i>P</i> < .10	.01	.10	.01	.01	.05

* *t* test

prevented. As Corning & John (1961) have demonstrated that significant effects of RNase occur only in the regenerated-head animals, only tail sections were placed in the bowls to regenerate.

METHOD

Thirty-six planaria (*D. dorotocephala*) were obtained from Wallace Lake in Berea, Ohio, and kept in a salt solution of 10^{-3} M CaCl_2 , 10^{-6} M KCl, 10^{-4} M NaCl, and 10^{-6} M ethylenediaminetetraacetate (EDTA). (This salt solution also constituted the regenerating medium in which the transected Ss were kept throughout the regeneration period—see below.) The Ss were trained to crawl to a particular position in a small glass bowl, filled with lake water, in order to escape a high-intensity light stimulus. Each animal was placed in the bowl near the light, and, since planaria are negatively phototactic, they would crawl away

from the light along the sides and bottom of the bowl. When an S reached the position in the bowl farthest from the light, the light was turned off and the animal placed back into its experimental environment. Each animal was given eight training sessions, one per day. After every planarian had completed a daily session and the time necessary for completion of the task had been recorded for each S, the mean time for all the Ss was computed. *No shock was used, nor was it necessary to decide arbitrarily if the Ss had been conditioned.* If the animals were "learning" to make a beeline for the light-off position in the bowl, the mean time per day should become progressively shorter (see Fig. 1). This would indicate elimination of random side-to-side movement.

When the eighth training trial had been completed, 18 planaria were transected and placed randomly in three glass bowls. Group A (control) contained six Ss regenerating in 32 ml of the salt solution. Group B contained six animals regenerating in 40 ml of the salt solution plus 4.0 mg of RNase.² Into Group C were placed six planaria in 34 ml of the salt solution plus 3.4 mg of DNase.² A day later the remaining 18 animals were transected. Six were placed in a bowl containing 40 ml of the salt solution plus 4.0 mg of RNase (Group D); another six (Group E) were placed in a bowl containing 34 ml of the salt solution plus 3.4 mg of DNase. The final six Ss (Group F, control) regenerated in 34 ml of the salt solution.

At the end of the regeneration period (19 days, although some Ss completed the regeneration process before others), the animals were tested for retention of the task by the same procedure as outlined above. The regenerated Ss were tested for a period of 4 days, one trial per day. As before, the mean time for each group, per day, was recorded. Learning curves were plotted for mean times before transection and for mean trials for each group after regeneration was complete (Fig. 1).

RESULTS AND DISCUSSION

The *t* test was used to compare statistically the means of each regenerated group to the mean of the

last four conditioning trials. Results (Table 1) showed a significant increase in the time necessary to complete the task for those Ss in Groups B (immediate transection, RNase), D (delayed transection, RNase), and E (delayed transection, DNase).

An unequivocal explanation for the differences displayed between Groups C and E is not obvious—many possibilities may exist. Perhaps the involvement of DNA in memory consolidation and in performance of the acquired response pattern is different. That is, the biochemical events required for actual performance (cf. Group C) of an acquired response pattern may differ from those required for mere retention of the pattern sequence (cf. Group E). DNA dysfunction under these different conditions might yield differential effects. Other possibilities include RNA capability of self-replication (supported, for example, by studies done on the tobacco mosaic virus) if DNA is incapable of alteration by RNA (Group C).³ If DNA has been altered by RNA (Group E), the RNA may no longer self-replicate and must rely upon DNA to initiate the necessary biochemical events of task performance.

These are only two of the possible explanations of the existence of a profound difference between Groups C and E. At present, adoption of a particular hypothesis as being more valid than another would be purely speculative. Finally, performance of Groups B, D, and E during the 4 days of testing did not improve comparably to that during even the first 4 days of initial training. Perhaps learning as well as retention deficits are operative.

REFERENCES

- CORNING, W. C., & JOHN, E. R. Effects of Ribonuclease on retention of conditioned response in regenerated planarians. *Science*, 1961, 134, 1363-1365.
- GAITO, J. DNA and RNA as memory molecules. *Psychological Review*, 1963, 70, 471-480.
- GLASSMAN, E. The biochemistry of learning: An evaluation of the role of RNA and protein. *Annual Review of Biochemistry*, 1969, 605-632.
- JENSEN, D. D. Learning and associated phenomena in invertebrates: *Animal Behaviour Supplement* 1, 1965, 9-20.

NOTES

- For a recent review see Glassman (1969).
- The RNase used (5X crystalline) is active at room temperature with an optimal pH of 4.2. The DNase used (amorphous; 70,000 Dornase units/mg) is readily denatured by heat and at room temperature loses approximately half of its potency every 96 h. It has an isoelectric point in the region of pH 4.5-5.0. RNase and DNase obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.
- Some means by which the DNA template may be altered are offered by Gaito (1963).

Fig. 1. Mean times required for task completion during eight training trials and four testing trials after regeneration. Each regenerated group contained six Ss. Groups A, B, and C were transected immediately after the eighth training trial. Groups D, E, and F were transected 24 h after completion of the eighth training trial. The regenerating medium for Groups A and F (control) consisted of a salt solution. Groups B and D regenerated in the salt solution plus RNase. Groups C and E regenerated in the salt solution plus DNase.