contrary, would work only to see another monkey.

There is no ready explanation for the difference between these two types of monkeys in the case of "mirror" display. The manner in which they are obtained commercially makes their territorial origin uncertain. Answers to inquiry suggest that the Gothic type is Colombian or Peruvian in origin, whereas the Roman is a native of Brazil. The Gothic type appears to correspond to Hill's description of *Saimiri sciurea petrina,* which is characterized by a pierrot-like face and is indigenous to northeast Peru (*1*). It will be of interest to learn whether or not there has existed any environmental difference between the Gothic and Roman type monkeys in regard to ancestral exposure to reflecting pools and streams from overhanging boughs.

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Puromycin Effect on Memory Fixation in the Goldfish

Abstract. *Puromycin injected intracranially into the goldfish produces impairment of memory for a shock-avoidance response. Intracranial injection of puromycin aminonucleoside, or of saline has no effect. Puromycin does not affect performance in naive or overtrained goldfish.*

There have been several recent reports that antimetabolites may affect learning or recall. Dingman and Sporn (*1*) reported that intracisternal injection of 8-azaguanine in the rat does not affect performance of a previously learned maze, but blocks acquisition of a new one. Chamberlain *et al.* (*2*) found that intraperitoneal injection of the same drug prolongs critical fixation time for the persistance of an asymmetry after section of the spinal cord. Flexner *et al.* (*3*) have shown that subcutaneous injections of puromycin into mice inhibit synthesis of brain protein but have no effect on learning and retention of simple or discrimination avoidance responses. More recently, Flexner *et al.* (*4*) found that injection of puromycin by a different route (intracerebral) into mice one or more days after a training session causes loss of memory of avoidance discrimination learning.

Table 1. Effect of puromycin on the shuttlebox response. Group A, fish injected with saline after trial 20 or not injected; group B, injected with puromycin after trial 20; group C, injected with puromycin aminonucleoside after trial 20; group D, injected with puromycin 72 hours before trial 1; group E was not injected. The results are expressed as the mean number of correct responses in 10 trials ± the standard errors.

Group	*N*	Trials		
		Day 1		Day 4
		1–10	11–20	21–30
A	36	1.31 ± .28	2.89 ± .43	4.56 ± .41
B	36	1.44 ± .30	2.86 ± .41	2.89 ± .34
C	23	2.04 ± .39	3.22 ± .57	5.13 ± .65
D	50	1.29 ± .25	2.48 ± .40	
E	31	1.26 ± .21	2.78 ± .36	

We have used a simple training apparatus in testing the effect of antimetabolites and physical agents on a relatively primitive vertebrate. In the experiments reported here, a simplified semi-automated version of the shuttle box for goldfish developed in Bitterman's laboratory was used (*5*).

Goldfish, 7.6 to 10 cm long, were obtained from Ozark Fisheries, Stoutland, Missouri. They were stored in 55-liter aquariums. The day before an experiment, they were transferred to individual clear plastic tanks measuring 13.3 by 18.7 by 9.5 cm (*6*). The fish were kept in continuous light and fed daily at noon.

The training apparatus consisted of six shuttle boxes made from clear plastic boxes measuring 12.4 by 30.1 by 7.6 cm deep (*6*). Each had a 12.4-cm paraffin-impregnated solid wooden barrier centered across the bottom, 3.2 cm high, 3.2 cm wide at the bottom, and 1.9 cm wide at the top. Two stainless steel mesh electrodes, 11.4 by 6.4 cm, were affixed to the sides near each end of the box. Two stimulus lights (Sylvania 120PSB) were mounted outside the box 1.3 cm from each end. The boxes were filled to a depth of 5 cm with aged tap water, leaving 1.9 cm of water over the barrier. The observer and apparatus were in a quiet, darkened room.

Trial cycles consisting of 20 seconds of light, 20 seconds of light coupled with shock, and 20 seconds of darkness. were controlled by a set of microswitches activated by a 1-rev/min cam. The shocks, 3 v a-c and 0.2 seconds long, were delivered at a rate of 40 per minute. A ratchet relay alternated the stimulus lights and shock after each trial. Thus the fish was trained to avoid shock by swimming over the barrier to the dark side of the box.

The responses of individual fish were recorded by direct observation. A correct response was scored when the fish crossed from the light to the dark end of the box before the onset of shock. Occasionally, a fish was out of position (on the dark side) at the start of a trial as he either failed to cross the barrier during the shock period on the previous trial, or crossed back between trials. This was scored as an incorrect response.

A 100-μl Hamilton syringe with a 1.3-cm 30-gauge needle was used to inject puromycin solutions or 0.154*N* NaCl into the cranial cavity just over the brain. The cranium was penetrated at the medial suture and in line with the posterior margin of the orbits. The needle was inserted 2 mm at an angle of about 45 degrees to the surface and directed posteriorly. This placed the tip in the midline over the tecta.

Two groups (A and B) of 36 fish each were given 20 trials, with 5 minutes of rest in darkness after every 5 trials. In group A, 18 fish were immediately injected with 10 μl of saline and the other 18 fish were not injected; all were placed in individual tanks (*7*). The other 36 fish (group B) were immediately injected with 90 μg of puro-

mycin hydrochloride in 10 μl of saline and placed in individual tanks. After 72 hours all fish were given 10 trials, again with 5 minutes of rest after 5 trials.

There was no significant difference between groups A and B in the 20 trials before the injection was given. However, the fish injected with puromycin showed no significant improvement and scored significantly fewer correct responses than the control group (A) in the 10 trials given 72 hours later ($t = 3.139$, $p < .005$).

When puromycin aminonucleoside was substituted for puromycin, no significant effect was seen (group C). To determine whether puromycin injected 72 hours before training affects learning, 50 naive fish (group D) were injected with 90 μg of puromycin in 10 μl of saline and placed in individual tanks. Thirty-one uninjected fish (group E) were placed in individual tanks. Both groups were given 20 trials 72 hours later. The results in Table 1 show that there was no significant difference in performance between groups D and E.

To establish whether puromycin has an effect on performance of trained goldfish, 30 goldfish were trained over a period of several days until they improved no further. They were then responding correctly 80 percent of the time. One-half of these fish were injected with puromycin and both groups were tested 72 hours later. There was no significant change in performance in either group, indicating that puromycin has no effect on performance or memory in overtrained fish.

A similar effect to that of puromycin was observed when electroconvulsive shock (ECS) was administered immediately following the 20th trial on the first day.

By giving intracranial injections of puromycin and tritium-labeled leucine, we measured the effect of puromycin on the incorporation of leucine into the trichloroacetic acid–insoluble fraction of fish brain (*8*). Under these conditions puromycin causes a decrease in the incorporation of leucine, the maximum inhibition, 80 percent, occurring 3 hours after the puromycin injection.

The known effect of puromycin on protein synthesis (*9*) may or may not relate directly to the behavioral effect reported here. Current concepts on the molecular site of action of puromycin favor a reversible block on the ribosomal surface with the premature release of partially formed proteins (*10, 11*). If it is assumed that a protein synthetic block is the mode of action here, it is not surprising that recall is also blocked. A number of possible models of behavior—including alteration in synaptic permeability, specific memory proteins, growth of new neurons, glia, or synaptic processes—all require protein synthesis.

The results are consistent with the hypothesis that a special metabolism participates in the fixation of experience, presumably with the formation of covalent changes. Temporary block of this special metabolism can apparently be accomplished without observable changes characteristic of general block of brain metabolism and subsequent degradation. The similar effect of electroconvulsive shock supports the concept of a brief, reversible action, during which information is lost.

These experiments with goldfish confirm the important observation of Flexner *et al.*, who found in the mouse that puromycin can block memory without having any apparent effect on learning. Our experiments differed from those of Flexner *et al.* in several particulars. The goldfish were given injections intracranially, but not directly into the brain substance. Saline-injected controls showed no loss of memory. The goldfish were injected immediately after the training session, while in the mouse (*4*), injections were made 1 or more days after training. The intracranial injection technique in goldfish has not, to our knowledge, previously been used. It should be noted that the fish brain is relatively accessible to these injections since the small size of the brain permits rapid diffusion from the surrounding cranial fluid. Goldfish appear to be ideally suited for studies on biochemical correlates of behavior. They are easily housed, learn quickly, and retain what they have learned for a long period of time. We found significant retention of learning for at least 1 month after the initial 20 trials. The availability of this simple technique should facilitate further studies on the biochemical elucidation of the fixation of experience.

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