Gowans and Knight (*11*) have shown that, in the rat, lymphocytes recirculate from blood to thoracic duct lymph via the postcapillary venules of lymph nodes, spleen, and intestinal Peyer's patches. Since the peritoneal space encloses most of these viscera, the possibility that the lymphocytes of the peritoneal fluid of mice also participate in the recycling deserves to be tested.

Little is known about the origin and homeostatic control of the normal peritoneal macrophage population in mice. The bulk of the literature relates either to the mobilization of these cells in response to various stimuli or the repopulation of peritoneal fluid in irradiated animals (*4, 12, 13*). It is clear that peritoneal macrophages normally have a very low mitotic rate in vivo and in vitro (*14*), but a rapidly proliferating population can be elicited by injecting antigen intravenously (*15*), or foreign materials intraperitoneally (*13*). Volkman (*13*) provides evidence that these rapidly dividing macrophages are derived from the bone marrow but their relationship to the normal peritoneal macrophage population is not clear. Experiments by North (*16*) indicate that rapidly dividing macrophages are not necessarily mobilized from a distant site since a high percentage of mature resident peritoneal macrophages can be stimulated to divide by the intravenous injection of *Listeria monocytogenes*. What emerges from the present quantitative study is the fact that the peritoneal macrophage population is numerically stable in normal adult mice. The numerical stability of the peritoneal macrophage in comparison with the numerical fluctuations of the peritoneal lymphocyte suggests that there is no direct relationship between the body pools of these two types of cells, a conclusion similar to that reached by Volkman (*13*).

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Extinction in Goldfish: Facilitation by Intracranial Injection of RNA from Brains of Extinguished Donors

Abstract. *Extracts rich in RNA were prepared from the brains of goldfish that had acquired and then extinguished a light-signaled avoidance to shock in an aquatic shuttle-box. Recipient fish injected intracranially with such extracts extinguished the response significantly faster than those injected with extracts prepared from brains of naive donors.*

Investigators attempting to demonstrate effects of biochemical transfer from trained donors to naive recipients have used, almost exclusively, acquisition paradigms. A review of the literature on vertebrates revealed no transfers, nor attempted transfers, of experimental extinction. The injection of brain homogenates from donor rats given successive trials for acquisition, extinction, and reacquisition of a response facilitates indicant responses in naive recipient rats, as compared with controls (*1*). However, no attempt has been made to transfer extinction itself. The successful transfer of some extinction effect, besides extending the generality of the biochemical transfer phenomenon, would be invulnerable to any criticism that the effect was due to some general activating or energizing property of the extract employed. Furthermore, a successful transfer of extinction would yield information about the nature of the extinction process itself.

That my attempt might succeed was suggested by the positive results obtained by Ungar (*2*) in his chemical transfers of another decremental effect, the habituation of the startle response to auditory and air-puff stimuli. Habituation and experimental extinction appear to be quite similar processes, a major difference being that repeated presentation of stimuli leads to a decrease in an unconditional response in the former case, and in a conditional response in the latter. Goldfish were chosen as experimental subjects because they offer many advantages for biochemical research (*3*), and because Fjerdingstad (*4*) using a shuttle-box avoidance task had already demonstrated an acquisition transfer effect in the species.

The first experiment was a systematic replication of Fjerdingstad's acquisition study. The apparatus and procedure were identical to those used in the acquisition phase of the extinction experiment described below. Sixteen goldfish were given 8 days of training for acquisition of shock avoidance in an aquatic shuttle-box; a daily session consisted of 20 trials. The performances of these experimental donors, in mean percentage of avoidance responses, for the 8 days were, respectively: 18.75, 53.35, 75.31, 90.00, 93.75, 91.25, 93.44, and 92.50. These animals, along with 16 naive control donors, were killed 24 hours after the last trial; extracts of brain RNA were prepared according to the method described below. Such extracts might best be described as RNA-rich, since a Folin-Ciocalteu test reveals the presence of protein or polypeptide, in addition to RNA. Two groups of 14 naive recipient fish, chosen randomly, were injected intracranially with the equivalent of the extract of one brain from either a control or a trained fish in 40 μl of $10^{-3}M$ saline. All animals were tested in the shuttle-box without reinforcement 48, 72, 96, and 120 hours after injection. A daily test consisted of 20 trials with light only (without shock) in which avoidance responses were scored (Table 1). Performance of the fish injected with brain extract from trained fish was reliably greater than that of fish injected with extract from control fish at the first

three times of testing; the greatest difference occurred 72 hours after injection.

In the second experiment, 64 common goldfish (*Carassius auratus*), 2½ to 3 inches long, obtained from Ozark Fisheries, Stoutland, Missouri, were maintained in pairs in 1½-gallon clear plastic cylindrical aquariums provided with constantly aerated and filtered water at 20°C. All fish were fed once daily with Shrimp-el-etts pellets. The training apparatus was a clear plastic aquatic shuttle-box 30 cm long, 10 cm wide, and 18 cm high. The box was filled with aquarium water to a depth of 10 cm. A wooden partition which cleared the bottom by 2 cm divided the tank into two equal compartments. Two photobeams positioned at either side of the space below the partition were programmed so as to track the position of the fish, record crossings, and determine which compartment would be illuminated by a General Electric pilot lamp (No. 313) centered at the top edge of the end wall. Shock was applied through two wire-mesh electrodes which completely covered the two sides of the tank.

On day 1, 16 subjects were given a single 20-minute session in the shuttle-box. This consisted of 20 presentations of light only, each 25 seconds long, with an intertrial interval (ITI) of 1 minute. The light was always presented in the compartment occupied by the fish. Any fish which escaped the light during its first 12½ seconds on more than 10 of these 20 trials with light only was not used in the experiment and was replaced by another fish. Of the 64 fish tested in these experiments, only two were replaced. On days 2 through 6, the 16 subjects were given daily 20-minute sessions for shock avoidance. Such a trial consisted of the following: a 12½-second presentation of light, a 12½-second simultaneous presentation of light and pulsed shock, and then a 35-second period of darkness. Twenty such trials were administered daily, with an ITI of 1 minute. Shock was 9 volts (d-c) and was given at a rate of 17 pulses per 12½ seconds; the duration of each pulse was 50 msec. The terminations of light and shock stimuli were contingent on response. If no response occurred within the intervals specified above, the various events were terminated automatically. On days 7 through 13, the 16 subjects were given daily 20-minute sessions for extinction. Each session consisted of 20 trials identical to the light-only trials described above. The 16 fish which supplied brain material for the control recipients were maintained in their home tanks throughout the experiment and were never exposed to the shuttle apparatus.

Table 1. Mean percentage of avoidance in experimental and control recipients for the various nonreinforced test sessions.

Value	Time after injection (hours)				Average
	48	72	96	120	
Experimental	27.15	49.30	50.35	41.80	42.15
Control	14.65	28.55	33.55	32.15	27.22
Difference	12.50	20.75	16.80	9.65	14.93
P*	$<.025$	$<.01$	$=.05$	$>.05$	$<.01$

* Mann-Whitney U test.

Twenty hours after the last extinction session, the subjects were killed by immersion in a mixture of solid carbon dioxide and acetone for 4 seconds. This treatment froze surface tissues, but not the brain itself. The cranium was sliced open with a scalpel; the entire brain was removed in less than 1 minute and frozen in powdered solid carbon dioxide. After all brains had been frozen solid, they were removed from the carbon dioxide and sealed in a glass vial in a freezer at −20°C. Ribonucleic acid was extracted from the brains by the method of Laskov, Margoliash, Littauer, and Eisenberg (*5*); the method was modified in that RNA was precipitated with isopropanol according to Haselkorn's (*6*) technique of obtaining RNA from infectious viruses.

The frozen brains were weighed and then homogenized with four volumes of chilled 88 percent phenol and four volumes of a $10^{-4}M$ ethylenediaminetetraacetate (EDTA) in $0.02M$ phosphate buffer at pH 7.6 in a glass homogenizing tube with a Teflon pestle for 2 minutes. The mixture was stirred magnetically for 20 minutes at 4°C, then centrifuged at 1000g for 30 minutes. The aqueous phase was carefully removed, the interphase and phenol phase were again extracted as before, and the two aqueous phases were combined and centrifuged at 25,000g for 30 minutes. The supernatant was acidified with one drop of $1.0M$ acetate buffer (pH 5.5) per milliliter and slightly more than one volume of isopropanol, mixed thoroughly, and allowed to stand at 4°C for 20 minutes. The precipitate formed was centrifuged down at 1000g for 30 minutes at 4°C, then dissolved in 5 ml of 10^{-4} EDTA. The resulting solution was lyophilized, and the dry powder was stored at −20°C. Immediately before injection, enough $10^{-3}M$ NaCl solution was added to adjust the volume of the material so that 40 μl was equivalent to the material from one donor brain.

On day 1, all recipient subjects were first tested with light only as described above. On days 2 and 3, the subjects were given daily sessions for acquisition of shock avoidance. On the basis of percentage of avoidance responses

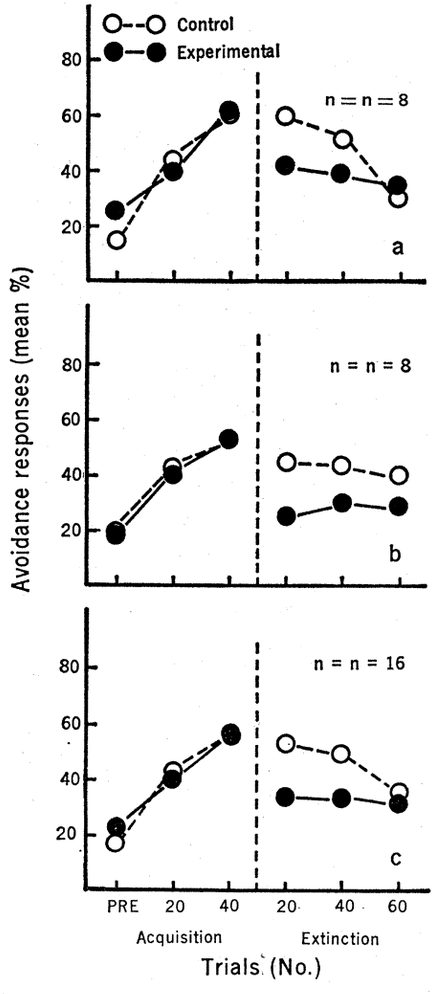

Fig. 1. Mean acquisition and extinction performance of recipients of extracts of brains from experimental (extinguished) and control (naive) goldfish. Panels a, b, and c represent, respectively, the first, second, and combined replications. Vertical dashed line indicates injection.

on day 3, the 16 subjects were rank ordered and divided into two matched groups which would receive the respective extracts. Two hours after the last recipient fish had been tested on day 3, 40 μl of solution was injected intracranially into each of the 16 recipient fish. Eight fish received material from donors trained and then extinguished, and eight fish received material from naive donors. Injections were done according to the method of Agranoff and Klinger (*7*). The 16 fish were injected within approximately 20 minutes.

The 16 recipient animals were given single 20-minute sessions for extinction on days 4, 5, and 6, approximately 19, 43, and 67 hours, respectively, after injection. The testing of the recipient fish was done by an experimenter who was unaware of the group membership of the animals. Two weeks later, the experimental phases from initial testing with light only through final testing of recipients were repeated with 16 additional recipient fish.

The avoidance behavior of the recipient fish was so consistent that matching on the basis of day-3 data also resulted in excellent matching on days 1 and 2 (Fig. 1). A Mann-Whitney U statistic was used to test the differences between the groups on each testing day. The dependent variable analyzed was a difference score, computed for each subject, which consisted of the subject's extinction score (percentage of avoidance) on the day in question minus that subject's acquisition score on day 3. The mean differences were −2.5 and −20.0, respectively, for the control and experimental groups on the first extinction session of the first 16 fish; this was a significant difference ($U = 11$, $n_1 = n_2 = 8$, $P = .014$). For the second 16 fish, the corresponding differences were −8.12 and −26.88 ($U = 11$, $n_1 = n_2 = 8$, $P = .014$). If the two groups were combined, the respective differences would be −5.31 and −23.44, a highly significant difference ($U = 47$, $n_1 = n_2 = 16$, $P < .001$). On days 5 and 6, the difference between groups gradually declined and was no longer significant; this effect is attributable to the extinction of the control group with testing. The performance level of all subjects declined to approximately 35 percent. In this laboratory, performance of fish in various "sensitization" control groups (light only, shock only, unpaired light and shock) is typically around 35 percent after 2 to 13 days under such control conditions. Also, fish given as many as 7 days of extinction training do not extinguish below the 30 to 35 percent level. Thus, 35 percent avoidance responses appears to be a relatively accurate base line, given the particular parameters of this task.

The effect obtained in this study was quantitatively great, statistically significant, and reliable enough to be reproduced in this laboratory. It is not known whether the results are due to an actual transfer of extinction information or to a general facilitation of the extinction process. In any case, one cannot argue that the effect is due to some general activating, exciting, or energizing property of the experimental extract. Of course, one might argue that such an extract exerts a generalized depressing effect on behavior, but then one would have to explain why some extracts activate while others depress.

My results suggest that extinction is a learned reaction to the conditional stimulus which opposes the original reaction conditioned to the conditional stimulus, rather than a simple weakening of the originally acquired reaction. Were the latter the case, one would expect extinction to be characterized by a gradual decrease in the chemical substrate of the conditioned response; it is difficult to conceive of such a lack of substrate from a donor producing a similar lack in a recipient. An interpretation of the extinction process as counterconditioning was also reached recently by Deutsch and Wiener (*8*) in an analysis of extinction through the use of the amnesic properties of physostigmine.

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Physiological Concentrations of Lactate Dehydrogenases and Substrate Inhibition

Abstract. *Lactate dehydrogenases at physiological concentrations are inhibited by high concentrations of pyruvate when the enzyme and the pyruvate are incubated in the presence of oxidized nicotinamide-adenine dinucleotide before assay. The inhibition is much more pronounced with the H-type than with the M-type lactate dehydrogenase. These results suggest that substrate inhibition may be operative in vivo.*

Substrate inhibition occurs with a number of pyridine nucleotide–linked dehydrogenases. In the case of lactate dehydrogenase (LDH), excess pyruvate significantly inhibits the H-type enzyme under conditions in which the kinetics of the M type are only slightly changed. This difference in substrate inhibition has been suggested as a basis for distinct physiological roles for the two main LDH's (*1*, *2*). The H type may function as a lactate dehydrogenase in heart, whereas the M enzyme may be geared to operate as a pyruvate reductase in voluntary muscle.

The mechanism by which excess pyruvate inhibits LDH may be related to the formation of an abortive ternary complex between pyruvate, nicotinamide-adenine dinucleotide (NAD^+), and the LDH (*2*, *3*). The dissociation constant of this complex is much lower for the H-type enzyme than for the M form. Wuntch *et al.* (*4*, *5*) reported that substrate inhibition may be an artifact of the assay system and hence may not be of any significance in vivo. These conclusions are based on the fact that the H type shows substrate inhibition when the enzyme is diluted, but at high concentrations of enzyme (equivalent to that present in tissues) there is no inhibition. The studies with high concentrations of the LDH were carried out with stopped-flow techniques. Wuntch *et al.* (*4*, *5*) suggest