sponse reached a peak within about 5 min and during prolonged exposure to dopamine the secretory rate fell usually to a maintained lower value after about 10 min. In the same preparation the amplitudes of the maximal responses to dopamine and nerve stimulation were identical (figure 2) and in the range 60–100 nl/min.

Glands were stimulated for 20–30 min periods with dopamine (10^{-7}–10^{-6} M) and the secreted fluid collected for either chloride (14 samples) or sodium and potassium analysis (9 samples). The results given in table indicate that the major cation in the saliva is sodium and that chloride is present at a concentration almost equal to the combined concentrations of sodium and potassium. The composition of certain other salivas is also shown in the table for comparison. It can be seen that cockroach saliva is different from both insect salivas and is somewhat similar to the mammalian ones. Other experiments have shown that salivary secretion evoked by dopamine in Nauphoeta glands is reversibly abolished in sodium-free medium. Coupled with the analytical results this suggests that fluid secretion in this tissue is driven by active sodium transport.

The effects of emetine hydrochloride on brain protein synthesis and on dark avoidance response in the goldfish

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Summary. Emetine hydrochloride at 100 μg injected intracranially blocked more than 80% of protein synthesis of the brain in the goldfish for at least 4 days, but was not lethal. Testing the effect of emetine hydrochloride on dark avoidance go-no go learning, the fish injected with emetine hydrochloride showed poorer performance than those injected distilled water throughout the experiment, except on the 1st day, suggesting potentialities of emetine as a new blocking agent of memory consolidation.

It is generally believed that memory consolidation is inhibited by the blockage of protein synthesis in animals[3]. The mechanisms of chemical actions of protein synthesis blockers, however, are neither simple nor yet completely clarified. Cycloheximide[4–7] and acetoxycycloheximide[8–11], for example, have been said to block directly memory consolidation through the interruption of protein synthesis, whereas the inhibitory actions of puromycin[12,13] are less clear than that of other antibiotics. Thus, the relation between protein synthesis and memory consolidation has been suggested by means of only a few specific drugs, but is not generalized up to now. So, in the present experiments, emetine hydrochloride (E-HCl), which is an ipecac alkaloid and is reported to block the protein synthesis for long duration[14], was used for the first successful attempt to clarify relations between its inhibitory effects on protein synthesis and blocking actions on memory consolidation.

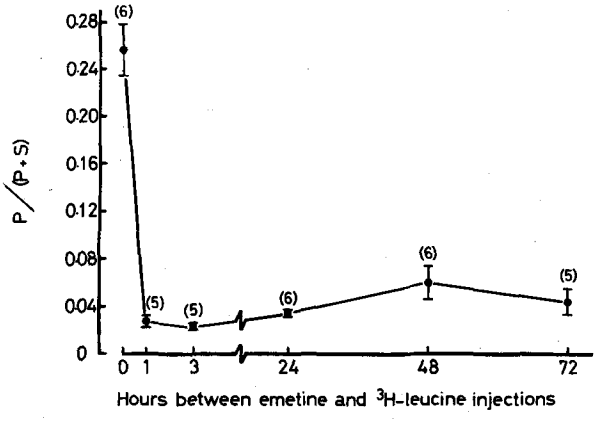

Fig. 1. The time course of protein inhibition in goldfish brain resulting from an intracranial injection of emetine hydrochloride (100 μg/10 μl). Abscissa 0 is the control injection of 5 μCi of ³H-leucine. Number of fish for each point is shown in parentheses. Dots and bars represent, respectively, means and standard errors of P/(P+S).

Materials and methods. The subjects used were goldfish, 9.5–11.0 cm long, in these experiments. Time course of inhibition of protein synthesis caused by 100 μg/10 μl of E-HCl was determined in goldfish brain. At 1, 3, 24, 48 or 72 h after the injection of E-HCl, 5 μCi/5 μl of L-leucine-4, 5-³H (specific activity 58 Ci/mmole, The Radiochemical Center, Amersham, England: ³H-leucine) was injected. Fish were decapitated 30 min later, the whole brain was quickly removed and protein was isolated as the trichloroacetic acid (TCA) precipitate fraction. The degree of protein synthesis was determined by the following formula:

$$P/(P+S) = \frac{\text{cpm in TCA precipitate (P)}}{\text{cpm in TCA precipitate (P)} + \text{cpm in TCA supernate (S)}}$$

When ³H-leucine was injected intracranially, the temperature of the water in the home tank was 18 °C.

Goldfish were given dark avoidance go-no go training for 3 days. For intracranial injections, a small hole was drilled in the centre of the skull between the 2 eyes, 3 days before the beginning of training trials[15]. Fish were not fed. On the previous day of training, fish were divided randomly into experimental and control groups, and the experimental group was injected with E-HCl 100 μg/10 μl in distilled water, while the control group was given 10 μl of distilled water.

For 3 days of training, a fish was given 2 sessions of 20 trials of dark avoidance go-no go training in each day which consisted of 10 go training tirals and 10 no-go training trials, randomly ordered. Intersession interval was 2 min.

The apparatus used was a gray plastic shuttle box with a trapezoidal barrier[16]. The temperature of water in both apparatus and home tanks was 16.0–18.0 °C. An electric light bulb (20 W/100 V a.c.) was placed over each compartment at 20 cm from surface of the water. These bulbs were ordinarily lighted and the CS was to put off either of the 2 bulbs for 10 sec. Which bulb was put off was determined in accordance with both the sequence of go and no-go trials and where the fish was, and it was con-

sidered to be a correct response if the fish avoided shock by leaving the dark compartment during the 10 sec of CS. If it failed to respond correctly, it was given an unconditioned stimulus of 4 V a.c. electric shock, for 5 sec at the longest.

Results and discussion. The biochemical results of E-HCl are shown in figure 1. In comparison with the control value at time '0', the degree of inhibition of protein synthesis was about 90% at 1, 3 and 24 h, and about 80% at 48 h (3rd day) and 72 h (4th day) from the time of injection. The results indicated that 100 μg of this agent injected intracranially caused a rapid and long-lasting blockage of protein synthesis in the brain of goldfish. But, E-HCl at 100 μg was not lethal for at least a month. From these results, it appeared that the intracranial injection of 100 μg of E-HCl inhibits more than 80% of brain protein synthesis in the goldfish for at least 4 days, but it may not be noxious enough to be lethal.

Our previous reports on actinomycin D[15,16] suggested that if a drug shows a long-lasting effect, the actions of the drug on memory might appear more clearly after several days of training. Therefore, in the present experiment, the subjects were trained for 3 days from the day after injection of E-HCl.

The behavioral results are shown in figure 2. Mean numbers of correct responses are plotted against sessions and days. The analysis of variance was applied to these data. In the go performance (C), the correct responses of both groups were increased day by day (experimental group: $F = 28.36$, $df = 2/30$, $p < 0.01$; control group: $F = 109.30$, $df = 2/30$, $p < 0.01$). Whereas, in the no-go performance (B), the responses were kept at high level throughout the experiment. On the 1st day, fish did not go to the other compartment of both procedures, indicating that fish do usually not cross the barrier of this apparatus. As the experiment proceeded, the go correct responses increased day by day but no-go correct responses did not decrease. These results showed that fish had acquired the discrimination learning of the go-no go avoidance.

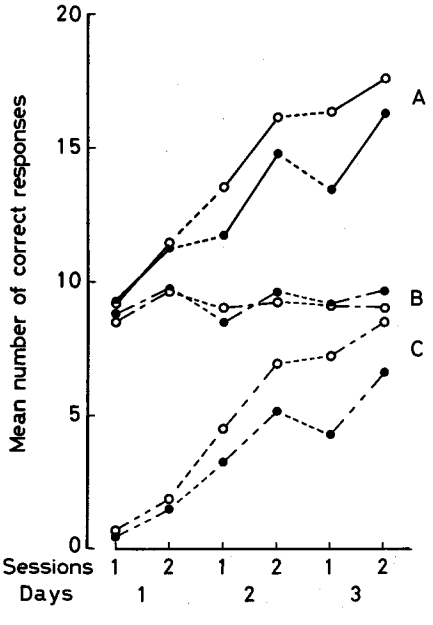

Fig. 2. Mean number of correct responses in each session of 20 trials in dark avoidance training as a function of sessions and days. Solid circles, experimental group (n = 16); open circles, control group (n = 16). Curves in A, B and C represent total, no-go and go correct responses, respectively.

From curve A in figure 2, however, the experimental group showed lower scores ($F = 4.30$, $df = 1/30$, $p < 0.05$) and lower increments of scores day by day (group X day interaction: $F = 3.45$, $df = 2/60$, $p < 0.05$) than the control group. But the scores of both groups increased in the same degree session by session within day. On the other hand, our prepared data appeared that injection of E-HCl after having learned this task does not inhibit the performance of the established learning in tests for 3 days from the day after injection. Thus, the above results in present experiment indicated that E-HCl blocked the formation of memory between days but did not inhibit the acquisition of learning within the day.

The actions of E-HCl in central nervous system, however, have hardly been clarified with the exception of its effect on protein synthesis. As it has appeared that some inhibitors of protein synthesis also decrease temporarily the synthetic rates of catecholamine[17], further research is necessary to clarify the interaction between the actions of E-HCl and the metabolism of catecholamine. However, the fact that the time course of inhibition of protein synthesis and the blockage of the formation of memory between days by E-HCl are parallel phenomena might suggest potentialities of E-HCl as a new blocking agent of memory consolidation.

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3 B. W. Agranoff, in: The Neuroscience: A Study Program, p. 756. Ed. G. C. Quarton, T. Melnechuk and F. O. Schmitt. The Rockefeller University Press, New York 1967.
4 E. E. Quinton, Psychon. Sci. *25*, 295 (1971).
5 J. F. Flood, E. L. Bennett, M. R. Rosenzweig and A. E. Orme, Physiol. Behav. *9*, 589 (1972).
6 L. R. Squire and S. H. Barondes, Proc. nat. Acad. Sci., USA *69*, 1416 (1972).
7 L. R. Squire, G. A. Smith and S. H. Barondes, Nature, Lond. *242*, 201 (1973).
8 L. B. Flexner and J. B. Flexner, Proc. nat. Acad. Sci., USA *55*, 369 (1966).
9 B. W. Agranoff, R. E. Davis, L. Casola and R. Lim, Science *158*, 1600 (1967).
10 S. H. Barondes and H. D. Cohen, Science *160*, 556 (1968).
11 R. G. Serota, R. B. Roberts and L. B. Flexner, Proc. nat. Acad. Sci., USA *69*, 340 (1972).
12 H. D. Cohen and S. H. Barondes, Science *157*, 333 (1967).
13 L. B. Flexner, P. Gambetti, J. B. Flexner and R. B. Roberts, Proc. nat. Acad. Sci., USA *68*, 26 (1971).
14 E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond and M. J. Waring, in: The Molecular Basis of Antibiotic Action. John Wiley & Sons, London 1972.
15 S. Ohi, Jap. J. Psychol. *46*, 191 (1975).
16 S. Ohi, Ann. Anim. Psychol. *22*, 61 (1972).
17 L. B. Flexner and R. H. Goodman, Proc. nat. Acad. Sci., USA *72*, 4660 (1975).