LD_{50} (lethal dose for 50 percent) values found with male mice are as follows (mg/kg): rotenone, 2.8; 8′-hydroxyrotenone, 2.6; 6′,7′-dihydro-6′,7′-dihydroxyrotenone, 10; rotenolone I, 4.1; rotenolone II, > 25.

The biological instability of rotenone in insects and mammals is attributable to its susceptibility to attack, by the microsome–$NADPH_2$ enzyme system, at the positions indicated in Fig. 1. In addition to the eight metabolites discussed here, at least two additional minor ether-extractable metabolites exist, but their structure is not yet known. These metabolites represent only the initial steps in rotenone metabolism because more polar products are subsequently formed. There is reason to believe that the more polar products comprise, in part, conjugates of the hydroxylated rotenone derivatives. It is interesting that 8′-hydroxyrotenone is a natural constituent in some plants in the form of a glycoside (amorphin) (*10*) and 11-hydroxyrotenone (sumatrol) also occurs in certain plants; however sumatrol is not one of the rotenone metabolites found in animals.

Our results suggest that variation in the ease of biodegradation of rotenone in different species may be one factor in its selective toxicity. Also, they suggest that certain of the metabolites are toxic and, therefore, need to be considered in further studies on the toxicology of rotenone and in biochemical studies where rotenone is used as a specific inhibitor of the electron-transport system.

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14. Supported by PHS grant ESGM 00049, and AEC contract AT(11-1)-34, project agreement No. 113. We thank J. L. Engel, E. C. Kimmel, L. Lykken, E. S. Oonnithan, and M. Tsukamoto of this laboratory for advice and assistance.

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Conditioning with Delayed Vitamin Injections

Abstract. *Rats deficient in thiamine were allowed to drink saccharin-flavored water. They were then given an injection of thiamine which caused their intake of the nonnutritive fluid to increase. Delay of the intramuscular injection up to 30 minutes had no effect upon the acquisition of this conditioning. Presumably, this delay reflects specialization in the central integrative mechanisms which serve these afferent modalities.*

Lorenz has proposed that an animal samples small amounts of novel foods and then forms an engram of its reaction to each. The fact that omnivorous animals eat only small amounts of novel foods supports this hypothesis (*1*). Animals tend to select beneficial diets. However, feeding behavior is a complex sequence in which many events (licking, chewing, and stomach distention) intervene between the discrimination of food by taste and smell and the correction of the homeostatic imbalance. Such intervening events can serve to mediate learning as they demonstrably reduce hunger (*2*).

Factors such as novelty and palatability of the food and dietary deficiencies also have a regulatory effect on food preference and intake; thus, the precise role of learning and memory is difficult to assess. For example, Rodgers and Rozin have demonstrated that rats deficient in thiamine will invariably select novel diets, and they will develop a stable preference for this novel diet if it contains the needed thiamine. Exclusive ingestion of the novel food for several days provides the opportunity for the development of a stable preference for the vitamin-rich diet, presumably by means of causal sequence of mediating events (*3*).

One way to circumvent some of these complexities which obscure the role of memory is to follow the ingestion of a discriminable nonnutritive substance with an intramuscular injection that introduces the dietary requirement directly into the blood stream. Patients with a thiamine deficiency often report that injections of thiamine make them "feel better" soon afterward. Therefore, we tested the effect of conditional associations of ingestion of a mixture of saccharin and water and a subsequent injection of thiamine in rats deficient in thiamine.

Forty-day-old male rats were placed on an unrestricted thiamine-deficient diet (Nutritional Biochemicals, Cleveland). They were given access to water for a 10-minute period at the same time each day. After 18 days their water intake declined to less than 50 percent of initial measures; this decline indicated the symptomatic loss of appetite. Food consumption and body weight declined similarly. Fifty percent intake was thus designated as the criterion of deficiency, and treatment was begun.

Two studies were conducted. In experiment A, the experimental group ($N = 19$) was given water flavored with saccharin (0.5 g/liter) for 10 minutes at the usual drinking time, then given an intramuscular injection of 200 mg of thiamine hydrochloride per kilogram of body weight. The injection was followed by the characteristic increase in appetite, which in turn was reflected in increased water consumption on the next day. Subsequently, water intake steadily declined so that in

3 to 5 days it again dropped below the 50 percent baseline. At this point the presentation of saccharin-flavored water followed by an injection of thiamine was repeated. A total of four injections was administered to both experimental and control animals. The controls ($N = 17$) were treated in the identical manner except that the mixture of saccharin and water was not conditionally paired with the injections, that is, they drank water on thiamine injection days and drank the mixture of saccharin and water on the days between injections.

After the fourth injection, experimental animals and controls were divided into two subgroups; one was given saccharin and the other was given water, so that relative fluid intake could be measured at the same point in time. One such test was conducted while the animals were in the nondeficient state, that is, 1 day after injection. Then the animals were reassigned to new water and saccharin subgroups to balance for the first test, and they were retested in the deficient state, that is, 4 days after injection.

Experiment B was conducted with the same procedures, except that the animals were slightly older (50 days) and the injections were delayed. Group 1 ($N = 6$) was injected at 30 minutes, while group 2 ($N = 6$) was injected at 75 minutes, and group 3 ($N = 6$) at 180 minutes after drinking the mixture of saccharin and water.

The results of the tests conducted after the treatment in experiment A are shown in Fig. 1 where saccharin intake scores are computed as percent of mean water intake. In the nondeficient state the experimental animals display only a slight preference for saccharin; their preference is approximately the same as that of the controls and equivalent to the mild preference that existed before treatment. In the deficient state, however, the experimental animals significantly increased their saccharin intake ($P < .01$ by ranks test). The preference of the controls is unchanged. Apparently the specific hunger for thiamine motivates the animal to ingest a substance associated with reduction of the deficiency in much the same way that general hunger causes it to increase food intake. The deficient animal's behavior can be described as "therapeutic," and this idea supports the popular notion that under natural conditions sick animals seek dietary cures.

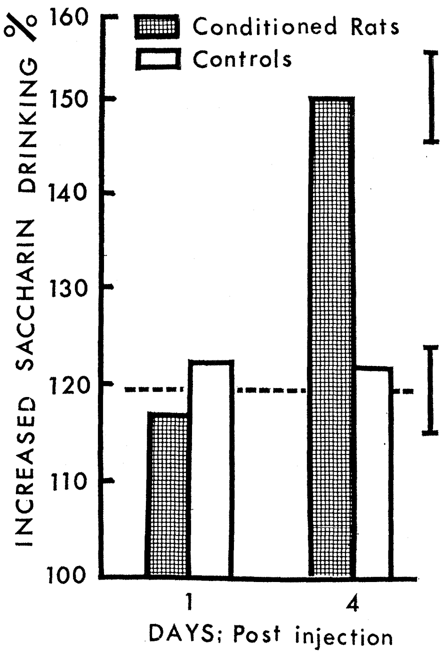

Fig. 1. Experiment A. Mean increase in the drinking of saccharin-flavored water by conditioned and control rats in the thiamine-deficient (4 days) and nondeficient state (1 day after the injection). Before test (dash line) and standard error (side brackets) are indicated.

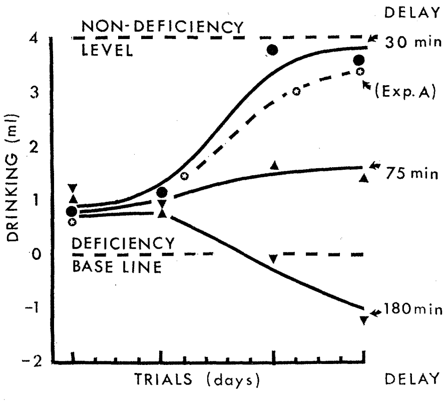

Fig. 2. Experiment B. Mean changes in consumption of saccharin-flavored water by thiamine-deficient rats. The changes are induced by conditionally pairing this fluid with delayed intramuscular injections of thiamine. Changes in intake by conditioned animals in experiment A are also indicated.

In experiment B, the saccharin intake is computed as a difference of the water intake (Fig. 2; a similar curve is plotted for experimental animals of experiment A). The thiamine injections produced a marked increase in saccharin intake in all animals that received an injection 30 minutes after drinking the mixture of saccharin and water (or less than 30 minutes in the case of experiment A). The conditioning was rapid, reaching near maximum on the third trial, that is, after only two thiamine injections. On the other hand, the animals whose injections were delayed 180 minutes exhibited decreasing intake scores so that there was no overlap in the scores of these two groups on the fourth trial ($P > .01$ by ranks test).

The mean of the 75-minute group is intermediate between the other two curves, but individual differences were great. The scores of two animals resembled those of the 30-minute group; the scores of two animals resembled those of the 180-minute group. Only two scores were actually intermediate, as indicated by the mean curve.

These results do not conform to the widely accepted proposition that reinforcement must be immediate for learning to be effective. A correlation analysis indicated that there was no observable effect due to delaying the thiamine injection from 2 to 15 minutes after the drinking period in the group in experiment A. Nor was there a significant difference between this group and the group whose injections were delayed 30 minutes in experiment B (Fig. 2). Similar learning with prolonged delays of reinforcement occurs when bait-shy responses are produced with delayed injections of a noxious drug (*4*).

There was no reliable evidence for an increased intake of distilled water in the control animals of experiment A, which drank this fluid prior to injection. It may be that the taste of saccharin lingers in the mouth and thus coincides with the beneficial effects of the injection, but the fact that the rat learns in two or three trials under these conditions is still remarkable. No one would expect such rapid conditioning in the rat to the onset of a light, if 30 minutes later an electric shock to the foot was delivered in the presence of the lingering light, particularly when the animal is allowed to engage in uncontrolled activity during that interval (*5*).

Gustatory stimuli are specifically and rapidly associated with visceral states, while auditory and visual stimuli are specifically and rapidly associated with cutaneous stimuli such as peripheral pain (*6*). Since the ultimate effects of food are delayed, effective gustatory-visceral associations would need to span longer time intervals than do telereceptor-cutaneous associations. Experimentally this appears to be the case. Practically, then, neural mechanisms of learning and memory storage may differ depending upon the specific sensory combinations employed in training experimental animals. It is interesting that Herrick described two discrete integrative systems in the medulla oblongata of the tiger salamander (*7*). The visceral-sensory neuropil receives gustatory afferents as well as visceral afferents and discharges impulses to visceral motor mechanisms, while the somatic-sensory neuropil receives afferents from the telereceptors and the cutaneous receptors and discharges impulses to somatic motor mechanisms. Such anatomical divisions could provide the structural basis for the specificity and appropriateness of stimuli observed in behavior.

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Visually Guided Reaching in Infant Monkeys after Restricted Rearing

Abstract. *Infant macaques were reared from birth in an apparatus which precluded sight of their body parts. At 35 days postpartum one hand was exposed to view. Visual fixation of this hand was insistent and prolonged; visually guided reaching was poor, but it improved during ten succeeding hours of exposure. Little concomitant improvement occurred in the reaching of the unexposed hand.*

Recent research indicates that an infant primate should be unable to reach for and grasp visible objects with a limb that it has never previously viewed (*1*). To verify this prediction one must demonstrate that the infant may be reared until its visual and motor capabilities are sufficiently mature to support visually guided reaching but that this behavior will not appear if the relevant visual-motor interaction has been precluded. Testing the hypothesis requires an experimental subject which is normally capable of visually guided reaching and which can be reared under the necessary controlled conditions. The infant monkey satisfies both of these demands, but research on its postnatal development is difficult because the animal needs substantial maternal care (*2*). We here describe a solution of the rearing problem, as well as preliminary results that confirm our prediction.

Because it is difficult to experiment with the infant when the mother is present, the two are separated soon after birth (*3*). The experimenter must then provide all maternal care. Human handlers can supply routine maintenance, but unless they can also furnish substitutes for some of the psychological aspects of maternal care, the infant will develop bizarre emotional behavior which interferes with controlled rearing and with subsequent testing (*4*). Harlow demonstrated that surrogate mothering devices can satisfy certain of the psychological needs of the infant monkey (*5*). Because the surrogate mother is inanimate and nonreacting, unlike the real mother, it becomes part of the controlled situation.

The apparatus we have designed (Fig. 1) promotes the development of normal infantile behavior by supporting the infant, surrounding its torso with a soft surface in contact with the skin, and giving it easy and continuous access to a nipple which provides milk. Furthermore, it conceals the monkey's limbs from its view, and yet allows the limbs a wide range of normal exploratory and manipulatory activities. The basic unit is a metal cylinder encircling the torso of the infant and suspended above a horizontal seat. One or two baby diapers folded 7.5 to 10 cm wide and loosely wrapped around the infant's body support it snugly in the cylindrical body holder. A horizontal rod covered with a piece of fur-like rug is within easy reach of the animal. The fur serves as a substitute for the mother's hairy skin and diverts the animal's grasping response from his own body. This diversion is extremely important because a monkey who has been deprived of a real mother clings tenaciously to the hairiest object in the environment, usually itself, to the exclusion of all other activities of its limbs, including reflexive responses while falling. Three vertical aluminum rods to which the holder is attached support a circular plywood body shield with an opening in the center. The head of the infant protrudes through this opening. A cloth bib is pinned around its neck and fastened to the circumference of the shield. When bib and body shield are in place, the animal cannot view any part of its body.

The apparatus can readily be altered to accommodate infants of varying size. Routine maintenance can easily be adapted for extremely small or even premature newborns. A nursing bottle with a "preemie" nipple can be positioned so that random movement of the infant's head results in facial contact with the nipple. This stimulation elicits the rooting response which brings mouth to nipple (*6*). Consequently, ad lib feeding can be initiated on the first day of life. The restraint placed upon the animal facilitates cleaning and routine maintenance; one or two daily changes of the diapers which cover the base are sufficient to maintain relatively odor-free conditions. Bottles need be cleaned and replaced only twice a day when fresh milk is used. Another desirable feature of this arrangement is the simplicity of its construction—only ordinary laboratory clamps, 12 mm wooden dowling, and exterior grade plywood treated with linseed oil are needed.

The apparatus also facilitates testing the infant. Since the body holder can be detached from the rest of the supporting components, it may be removed together with the animal and placed in a test apparatus with appropriate foot and seat rests. This mode of transfer retains contact between the infant and