or Hagerstown silty clay loam soil. After evaporation of solvent, the plates were irradiated for 96 hours with the fluorescent ultraviolet lamp. Negligible loss due to volatilization or photolysis was observed in both instances, and compound **I** was recovered quantitatively unchanged. A second series of experiments in which the soil was kept wet with water during the irradiation gave the same result.

Irradiation of a thin dry film of compound **I** deposited by the evaporation of the methanol solution on the surface of a glass dish resulted in quantitative recovery of the dioxin after up to 14 days of irradiation. Likewise, negligible decomposition was observed upon irradiation of a suspension of compound **I** in distilled water.

These experiments, admittedly performed under idealized conditions, indicate that light of the wavelengths found in solar radiation at the earth's surface can energize the rapid destruction of compound **I** in the presence of organic hydrogen-donors. Such donors could be represented environmentally by the waxy cuticles of green leaves, surface slicks on water, or the spray oil or aromatic solvent so often incorporated into 2,4,5-T formulations (*8*). Despite the limited solubility, the organic coating of most leaves should provide adequate solvent power for the maximum of 0.27 ng/cm^2 of compound **I** which could result from a normal application of 2 pound/acre (2.25 kg/ha) of 2,4,5-T containing even 10 ppm (10 mg/kg) of the impurity.

Truly bare surfaces of soil, water, or concrete normally would offer little opportunity for the photodecomposition of compound **I**. The loss of compound **I** by evaporation is not a significant factor here, but the environmental accumulation of dioxins from repeated incidental applications presumably would occur only in the absence of biological degradation or mechanical dilution.

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7. In a typical gas chromatographic analysis, we used a glass column 1.8 m by 10 mm packed with 5 percent OV-225 on 80/100-mesh Chromosorb W; column temperature, 220°C; nitrogen flow, 80 to 100 ml/min; ^{63}Ni detector at 310°C. Retention times (in minutes) were: aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-endo-exo-1,4:5,8-dimethanonaphthalene), 2.17; compound **I**, 9.40; compound **II**, 4.21; compound **III**, 2.80; and compound **IV**, 100.49. The use of 5 percent SE-30 as a liquid phase provided much shorter retention times. The mass spectrometer was a Perkin-Elmer GC-270 equipped with a surface-coated (SE-30) open tubular gas chromatographic column, 17 m by 0.51 mm. Mass spectra (mass-to-charge ratio *m/e* of the base peak, molecular ion, and characteristic fragments at 80 ev) were as follows: compound **I**, 320 ([^{35}Cl]$C_{12}H_4Cl_4O_2$), M$^+$ 320, 285 ($C_{12}H_4Cl_3O_2$), 257 ($C_{11}H_4Cl_2O$), 250 ($C_{12}H_4Cl_2O_2$), 194 ($C_{10}H_4Cl_2$); compound **II**, 286 ([^{35}Cl]$C_{12}H_5Cl_2O_2$), M$^+$ 286, 223 ($CH_{11}H_5Cl_2O$), 160 ($C_{10}H_5Cl$).
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Recuperation from Illness: Flavor Enhancement for Rats

Abstract. *Rats were given a distinctive fluid (milk or grape) during recuperation from an injection of a noxious drug (apomorphine). Single-bottle tests conducted after treatment indicated elevated consumption of the fluid conditionally paired with recuperation. This positive "medicinal" effect is to some degree independent of initial preference, of novelty effects, and of aversive reactions to other substances paired with the onset of illness.*

If rats drink a distinctive harmless fluid before they are made ill by x-rays or poison, they will drink less of that fluid on the next occasion. The reduction in fluid intake is proportional to the magnitude of the dose that caused the illness. This learning is remarkable because it occurs even when the illness is delayed many hours after ingestion of the flavored fluid. Furthermore, learning over such prolonged intervals may be limited to associations of food stimuli and visceral aftereffects of ingestion. Illness seems to reduce the palatability of food consumed before the onset of illness in an adaptive way for this omnivorous mammal (*1*). We are reporting here on several experiments testing the hypothesis that recuperation from illness will increase palatability. If animals drink a distinctive harmless fluid prior to recuperation from illness they should drink more of that fluid on the next occasion.

Apomorphine hydrochloride was selected as the agent because it produces an abrupt emetic illness with relatively rapid recuperation. In rats, an intraperitoneal injection (18 mg/kg) immediately causes abdominal motor spasms, huddling, and shallow breathing, and it prevents eating and drinking for about 2 hours. To determine the onset of recuperation more precisely, we tested drinking behavior in five groups of rats ($N = 4$ each) in conjunction with these injections. The animals had been habituated to drinking water only once per day during a 20-minute period in a compartment with a drinkometer. On the test day the animals were given saccharin water (1.0 g/liter) for the first time. One group was injected midway in its drinking test period; the other groups were injected 1, 2, and 3 hours before drinking. One group served as noninjected controls. All the animals injected during the drinking period stopped drinking within 2 minutes after the injection. Three of the four animals injected 1 hour before drinking did not drink, and drinking was depressed in rats injected 2 hours before the test period. The animals injected 3 hours before the test period drank as much as the noninjected controls. This emetic dose apparently produces an illness in rats that begins promptly after injection and is relatively intense for 1 hour. Recuperation appears to be under way in 2 hours and complete by 3 hours.

In experiment A we tested the effect of drinking a distinctive flavor during recuperation from a similar dose of apomorphine. Young adult female albino rats (200 to 250 g), which had

been used in preliminary palatability and apomorphine tests a month before the experiment began, were habituated to drinking water once per day for 20 minutes in their individual home cages at the same time each afternoon. The flavors tested were either unsweetened (Welch) grape juice or condensed (Pet) milk diluted in an equal amount of tap water. The injections were either the emetic drug or an equal volume of physiological saline. We used single-bottle tests with four independent groups ($N = 6$ each) assigned to each condition of flavor and injection. Animals were given a series of trials with a flavor from a single bottle every third day. On the intervening days the bottle contained water. The first test was a flavor trial (preconditioning test), and the next four were conditioning trials, in which injections preceded the drinking of the flavored fluid. Flavors were presented 15 minutes after the injection and removed 20 minutes after drinking began. Saline-injected animals usually drank immediately, whereas emetic-injected animals began drinking approximately 75 minutes after the injection. Three days after the fourth and final injection trial, the animals were given a flavor trial (postconditioning test).

The results of the postconditioning test indicated that drinking a distinctive fluid during recuperation produced a significant increase in consumption of that fluid over values obtained in the preconditioning test (see Fig. 1). Analysis of variance applied to the differences between the pre- and postconditioning tests indicated that the effect of the drug on fluid intake was significant ($P < .01$). In addition, there is a significant preference for condensed milk over the unsweetened grape juice. The animals also significantly increased their consumption from pre- to postconditioning tests, which again indicates that laboratory rats often display an initial reluctance to accept novel flavors. This neophobia is much more marked in wild rats. However, increased drinking of the flavor conditionally paired with recuperation is clearly apparent in spite of these differences and in spite of the fact that this was a single-bottle test in which the thirsty rats had no alternative source of fluid.

In experiment B, animals were given one flavor before the emetic injection and another flavor during recuperation from that same injection in order to test whether the same illness would have a negative effect associated with its onset as well as a positive effect associated with its offset. (This would be an effect analogous to the onset and offset of punishment.) The animals were familiarized with the test fluids to reduce the effect of novelty, and the course of conditioning and extinction was traced over several months with two-bottle as well as single-bottle tests.

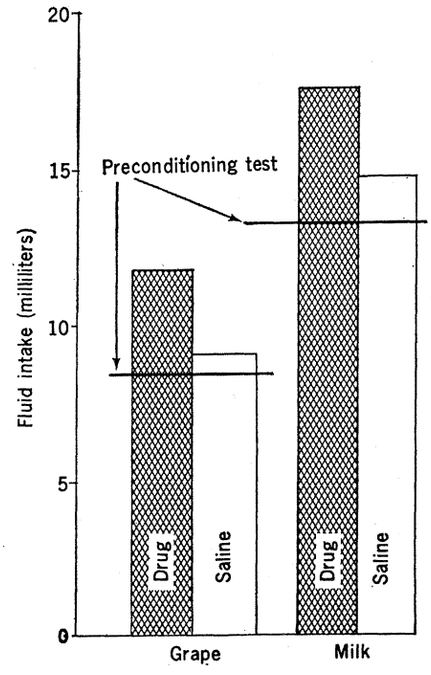

Fig. 1. Experiment A: changes in consumption of either milk or grape juice in four groups of rats after the test fluid was conditionally paired with recuperation from effects of a noxious drug or control saline injections.

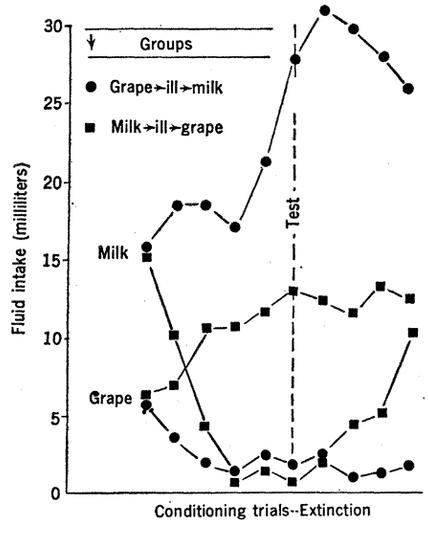

Fig. 2. Experiment B: changes in consumption in two groups of rats when one flavor is given before a noxious injection and the other is given during recuperation from its effects. The "test" refers to a two-bottle preference intake. All other points indicate single-bottle intake.

Young adult male albino rats (275 to 400 g) were habituated to drinking water once each afternoon for 12 days. On days 13 and 15 they were given single-bottle preconditioning tests with the grape and milk solutions described above. The single-bottle tests were repeated on days 17 and 19. On days 21 and 22 they were given two-bottle tests of both fluids with grape always on the left. The animals were now familiar with both fluids. Conditioning began on day 23. One group ($N = 6$) was given milk for 5 minutes and then injected immediately with the emetic drug described above. When they stopped drinking, the milk bottle was removed. After 35 minutes the grape bottle was presented. It was available until 20 minutes after the animals began to lick. The second group ($N = 6$) was treated identically except that it received the test fluids in reverse order. These trials were repeated on days 26, 29, 33, 36 for a total of five conditioning trials. Postconditioning tests began with two-bottle tests given on days 39 and 40 as in the preconditioning test. A series of eight single-bottle tests began on day 43 and ended on day 59. Flavors were presented every other day in a double alternating sequence (milk, grape, grape, milk). Water rations were supplied on all intervening days throughout the experiment.

Conditional pairing of both illness and recuperation had a significant effect ($P < .01$) when postconditioning tests were compared with preconditioning tests, whether single-bottle tests or two-bottle tests are considered. All animals reduced their consumption of the fluid presented before illness, and all animals except one (grape → ill → milk) increased their intake of the fluid presented during recuperation. The results are summarized in Fig. 2. For simplification, data from preconditioning tests are omitted from Fig. 2 since these data merely demonstrated again that (i) rats tend first to inhibit consumption of novel foods and then to increase consumption with repeated familiarization trials, and (ii) rats prefer milk over grape, as indicated in Fig. 1. On the two-bottle postconditioning tests, animals drank almost all their fluid from the bottle containing the fluid consumed prior to recuperation, thus making it equivalent to a single-bottle test. The intakes for the two fluids on those days were averaged and appear

on Fig. 2 as a single point labeled "test."

Note that the elevated consumption that followed the conditional pairing of flavor with recuperation is maintained in repeated single-bottle tests in experiment B. This supports the data obtained in single-bottle tests in experiment A. We used single-bottle tests because of the difficulty in interpreting the results when an animal is given a choice. When two flavors are simultaneously available, a change in the relative intake may be seen as an enhancement of one flavor or an aversion for the other. On the other hand, in the single-bottle tests used here a single flavor was presented to an animal 24 hours after he drank his fill of water and his intake is either compared to his pretreatment level of that same fluid or to that of a control animal. These data clearly indicate that recuperation has a positive reinforcing effect, which is to some degree independent of need, of initial preference, of novelty, and of the aversive effects that illness may have upon the other flavor. The positive and negative effects are evident a week after the last emetic injection despite an obvious extinction of both effects upon milk. No such extinction is apparent for grape. This differential extinction may be related to the high initial preference and the high nutritional value of milk (rats are known to control their caloric intake with precision).

Previous studies have indicated both positive and negative effects upon consumption when a distinctive flavor is followed by an injection. Conditional pairing where a flavor is followed by apomorphine injections results in a decrement in consumption in normal rats. Conditional pairing where the same flavor is followed with thiamine injections produces an increment in consumption in rats suffering from thiamine deficiency symptoms. Both these effects are relatively weaker if the injections are delayed by several hours, indicating that changes in fluid intake are not solely caused by either repetitive taste tests or the series of injections but by their temporal juxtaposition in a conditioning sequence (*2*).

These opposing behavioral effects were induced by two different substances, with vastly different systemic effects upon the animals: one is a noxious drug, the other is an essential vitamin. The studies reported here show that an identical injected substance can have either a negative or a positive effect, depending upon the temporal associative sequence used in conditioning. If the flavor is presented before the illness, the animal acts as if the flavor is "poison." If the same flavor is presented during recuperation from that illness, the animal acts as if it is "medicine."

Although the effect appears to be analogous to the negative and positive effects associated with the onset and offset of electrocutaneous shock punishment, there is at least one important difference. Avoidance responses induced by foot shock tend to be specific to the conditioning situation where shock was applied. On the other hand, taste aversions induced by illness generalize broadly, so that the taste is avoided in places where the rat has never been ill (*3*). Thus it appears that illness affects palatability of the flavor (that is, the animal no longer "likes" that flavor), whereas recuperation enhances the palatability. Apomorphine recuperation may be particularly effective because of the rapid dissipation of its emetic properties and because of a euphoria after emesis, which is sometimes described by human patients.

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Electron and Molecular Microscopy: Possibilities Rather than Limitations

In a recent report Breedlove and Trammell have suggested that electron or x-ray microscopy will not permit visualization of biological molecules (those containing carbon, nitrogen, and oxygen atoms) because of the molecular damage caused by the observation process (*1*). Whereas we believe that the authors' objections are generally sound, we fear that an impression may be given that there are no ways of circumventing the difficulties described in the report, as long as electrons are used. The results quoted by Breedlove and Trammell were obtained from large gaseous or solid samples, and such results may not be directly applicable to a specimen consisting of isolated single molecules attached to a substrate. Moreover, it is not certain that a group of atoms that has been structurally damaged could not still be identified and yield useful information at the atomic level of resolution. Since the importance of direct visualization of biological molecules is so great, we feel that we are justified in describing in this technical comment our plans for achieving this result, unsupported as yet by anything but preliminary experimental results. We believe that a possible solution will include the following six items:

1) The specimen should be mounted on a metal substrate on the premise that a good electrical conductor can promptly return electrons to ionized atoms in the sample.

2) The substrate should be cooled to liquid helium temperatures on the supposition that de-excitation from a highly excited state of the molecule by means of particle ejection or severe atomic rearrangement can be greatly reduced by a very low-temperature environment. Recent work by Box *et al.* (*2*) and by us (*3*) on organic molecules at 4.2°K strongly supports this proposition.

3) It seems plausible that a thin metal overlay of some element with a low atomic number such as lithium might hold molecules intact on the substrate as well as supply electrons to those molecules that are ionized.

4) The addition of thiols during sample preparation may prove effective in reducing the loss of protons from the specimen by virtue of the fact that the thiol group (S–H) can readily give up hydrogen to a damaged neighboring molecule (*4*).

5) With an arrangement in which the sample is on a massive cooled substrate, it is necessary to use emission microscopy. We plan to analyze the Auger