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.

KENNETH F. GREEN
Department of Psychology, California State College, Long Beach 90801
JOHN GARCIA
Department of Psychology, State University of New York, Stony Brook 11790

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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

emission stimulated by an irradiating electron beam. By suitable acceleration and energy-filtering we hope to form images of the atoms comprising the sample. In this way it should be possible in principle to distinguish between the various atoms of the sample (for example, carbon, nitrogen, and oxygen) and the substrate (for example, beryllium), since the Auger electron emission energies are a strong function of the atomic number.

6) In order to achieve high resolution by imaging Auger-emitted electrons we propose the design of an objective lens with low aberration and a high numerical aperture. In principle, such a lens can be built by interposing suitably shaped conducting, thin foils, thereby avoiding the limitations contained in "Scherzer's theorem" (*5*). (This scheme requires that the emitted electrons be first accelerated to some 50 kv before encountering the foils.) Recent experiments in this laboratory on evaporated thin foils and computer ray tracing indicate that this approach is feasible.

We would also like to propose that the term "molecular microscopy" be reserved for those instruments and devices in which neutral atoms and molecules are the probing particles. Light microscopes and electron microscopes are named for the probing radiation, and it seems reasonable to do so with the molecular microscope. We feel strongly about the adoption of this convention since we have been exploring in the last 2 years the possibilities of studying high spatial resolution and, under various conditions, the emissions of neutral atoms and molecules (evaporation and scattering) from surfaces (*6*). Our first instrument, in which water molecules were used, is now beginning to yield data.

We agree with Breedlove and Trammell on the importance of neutral atoms as probing particles, and we believe that the development of such techniques will be of particular importance in surface studies involving the weak forces between molecules and in delicate microanalysis appropriate to chemical and biological problems.

E. H. JACOBSEN*
Department of Physics, University of Rochester, Rochester, New York
J. G. KING, M. G. R. THOMSON
J. C. WEAVER
Research Laboratory of Electronics, Physics Department, Massachusetts Institute of Technology, Cambridge

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3. We have exposed ^{3}H-labeled DNA on a copper substrate held at 4.2°K to 20 coulomb/cm^2 of charge from 800-volt electrons (current density, 0.5 ma/cm^2). Radioactivity measurements before and after electron irradiation show that about 55 percent of the tritium label remains when the specimen is irradiated at 4.2°K, whereas only 8 percent of the tritium label remains if the specimen is irradiated at room temperature (all other experimental conditions remaining the same). In these experiments it was necessary to make all radioactivity measurements at room temperature. Hence, it is possible that some tritium label was lost while the specimen was being warmed to room temperature from 4.2°K. We are endeavoring to carry out a series of more quantitative experiments on electron damage to biological molecules as a function of temperature and radiation dosage in which both the electron irradiation and the radioactivity assay will be made at the same temperature.
4. W. A. Pryor, *Sci. Amer.* **223**, 70 (August 1970); ——— and V. Tonellato, *J. Phys. Chem.* **73**, 850 (1969).
5. O. Scherzer, *Ann. Phys.* **101**, 593 (1936); A. Recknagle, *ibid.* **117**, 67 (1940); J. E. Barth, thesis, University of Arizona (1967). In his thesis Barth reports success in using flat, self-supporting sandwich films of Al_2O_3 and carbon to correct spherical aberration in electrostatic lenses for electron beam energies of 65 kv. The total foil thickness was 1000 Å and covered a circular cross section 6 mm in diameter. An uncorrected image 8 μm in diameter could be reduced by the foil corrector to an image 0.2 μm in diameter which had sharp edges and contained about one-third of the incident electrons. The remaining electrons scattered over large enough angles so that they did not come through the microscope.
6. J. G. King and W. R. Bigas, *Nature* **222**, 261 (1969); J. C. Weaver and J. G. King, *Mass. Inst. Technol. Res. Lab. Electron. Quart. Progr. Rep. 98* (July 1970).
7. We thank Dr. H. C. Box for discussions concerning his recent work and K. Thomson for his able assistance in the electron damage experiments.
* Visiting scientist, Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge 02139.

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Autoshaping

Gamzu and Williams have reported the "classical conditioning of a complex skeletal response," by use of the technique of autoshaping (*1*). If a light is repeatedly projected on a standard response key a few seconds before a food dispenser operates, a hungry pigeon will begin to peck the key, presumably as it will peck other stimuli—for example, seed pods—which are related to food in less arbitrary ways. The process seems more accurately described as the classical conditioning of a stimulus which elicits a response of phylogenic origin.

An experiment performed at Indiana University in 1946 had other features that may be of interest. The upper half of one end of a pigeon box of standard size consisted of a translucent plastic plate. A food dispenser was located near the floor at the right. A spot of light about 6 mm in diameter was projected on the plate at the usual height of a pigeon key. The spot appeared at the right edge of the plate and moved to the left, covering the length of the plate in about 4 seconds. When it reached the left edge, the food dispenser operated.

The pigeon began to peck the spot, as in autoshaping, but it pecked as if it were driving the spot across the plate. When the plate was lightly greased, a print lifted from the surface showed the contacts made by the beak in a single transit. Prints showed slashes, often 2.5 cm or more long. It was observed that they were all made when the beak moved from right to left.

In a later stage of the experiment, the slashes were less specifically directed. As the spot approached the left-hand edge, sickle-shaped curves were described, sweeping around and down toward the dispenser. Adventitious contingencies involving the operation of the dispenser may have been responsible for this shift in topography.

It seems clear that a feature of the environment can be converted into a stimulus that elicits responses characteristic of the phylogenic endowment of the species. The observations reported by the Brelands were of that nature. The effect is quite different from operant conditioning, even though both processes generate responses having similar topographies.

B. F. SKINNER
Department of Psychology, Harvard University, Cambridge, Massachusetts 02138

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1. E. Gamzu and D. R. Williams, *Science* **171**, 923 (1971).

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Skinner's note reports an early encounter with autoshaping, together with an ingenious method of recording unexpected topographical elaborations of the response. In his experiment as in ours, the significant finding was the development of orderly, externally directed skeletal behavior beyond that specified by operant reinforcement contingencies. Not only does such behavior belie the "law of least effort," but, more importantly, it underscores the need to include factors other than response-reinforcer contingencies in the analysis of learned behavior. It is clear that phylogenic considerations must be taken