above and below his threshold of hearing were registered by the recording attenuator. To estimate the threshold intensity, the reversal points were read from the chart and their arithmetical mean was calculated.

The programing on bar *A* was arranged so that presses on bar *A* attenuated and occasionally turned the tone off, allowing the rat to secure reinforcement by pressing bar *B*. In this way the rat was sufficiently rewarded during threshold testing and would work for approximately 1 hour at a high and steady rate, during which 200 or so reversals of intensity levels were obtained.

Since we were interested in the possibility of using the method for tracing the gradual development of hearing impairment produced by ototoxic antibiotics, we first tested its reliability by measuring daily thresholds for a 2000-cy/sec tone in four male rats for 20 to 44 days. For each daily threshold the arithmetical mean of 150 or more reversal points was calculated from the daily record. The day-to-day consistency of each rat's performance was good, with only 9 to 15 percent of the daily thresholds for a given animal falling outside a ±5-db range. Agreement of the findings for the four rats was also satisfactory; the mean daily thresholds for the four animals ranged from 37 to 47 db SPL during the control period. We are not prepared, however, to state that these values represent absolute thresholds, since we do not yet know how much masking noise was present in the box as a result of the rat's own activity.

Kanamycin (*5*) (100 mg/kg) was given daily to rats Nos. 1 and 2 for 37 days with a 6-day interruption. The dose was then increased to 200 mg/kg and administered for an additional period of 40 days. Rats Nos. 3 and 4 received, respectively, 48 and 41 daily injections of 400 mg/kg.

The daily thresholds for rat No. 1 are shown in Fig. 2. During the administration of 100 mg of kanamycin per kilogram no threshold shift occurred. A gradual rise in threshold appeared when the dose was increased to 200 mg of kanamycin per kilogram.

The body weight of rat No. 1 decreased steadily during the time that he received kanamycin. This weight loss was attributed to a disturbance of water balance resulting from the well-known nephrotoxic effect of the antibiotic (*6*). It became necessary to interrupt the threshold measurements and to give him free access to water for 2½ weeks. One and a half weeks elapsed before the animal produced reliable records again. The thresholds were then approximately 35 db higher than those recorded prior to administration of the drug.

Rat No. 2, which was given the same doses as rat No. 1, showed a maximum hearing loss of 20 db and quickly recovered part of this loss, which became stabilized at approximately 10 db.

The thresholds for rats Nos. 3 and 4, which were given 400 mg of kanamycin per kilogram, remained stable during the first 20 days of kanamycin administration. Thereafter, the threshold for rat No. 4 began to rise. Because of weight loss he was given free access to water for a week. Eventually he produced acceptable records, showing a 55-db rise in threshold.

Rat No. 3, on the other hand, produced no reliable threshold records after kanamycin treatment was stopped. He was then placed under "training" conditions, during which time he was unable to discriminate tone-on from tone-off, even when the tone was presented at an intensity of 116 db. Like some patients who have received kanamycin, his loss of hearing was apparently complete (*7*).

The results show that this method can be used to follow the gradual development of hearing impairment produced by an ototoxic agent in rats. We believe that it is equally adaptable to the measurement of absolute thresholds, provided the masking noise produced by the rats' bar-pressing activity is minimized by providing quiet bars (*8*).

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Persistence of Alkaloids in Plant Tissue

Abstract. Under appropriate conditions of preservation, alkaloids can be detected in plant tissues after extended periods of time. A positive test for this class of compounds was given by a sample of plant material estimated to be 1300 years old.

The many changes which can take place in plant tissues during drying and preservation of specimens makes it of more than passing interest when certain chemical components of the plants can be recognized after long periods of storage. Webb (*1*) noted the presence of alkaloids in herbarium specimens 10 to more than 50 years old, and in one extreme case a strong positive test for alkaloids was obtained on a sample of *Acronychia baueri* collected in 1824.

Recent archeological investigations of the remains of northern Arizona cave dwellers turned up a number of small fiber-bound bundles of plants presumably used as medicinals. The bundles were found in a context which indicates that they were collected about A.D. 650. The state of preservation of the bundles and other associated materials indicates that the caves have been completely dry since that time. The contents of the bundles gave a positive, albeit weak, test for the presence of alkaloids (*2*), and at least one of the several plants included in the packets has been identified as *Nicotiana attenuata* (*3*), a wild tobacco which still grows in the area.

This is not to suggest that the positive alkaloid test is necessarily due to nicotine or its relatives; no attempt has yet been made to identify the substance or substances giving the positive test. But it is remarkable that these compounds have persisted in the plant tissues over a period of about 1300 years.

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2. The chemical tests were carried out by I. J. Pachter, of Smith Kline and French Laboratories.
3. The identification was made by Volney H. Jones, ethnobotanist, Museum of Anthropology, University of Michigan. A detailed report on the occurrence and significance of the tobacco is being prepared by Jones and Morris.

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