expected from these seed lots in the cold test could be predicted, with an accuracy of ± 8.5 at the 5-percent level, from electrical resistance (X_1) and weight per 100 seed (X_2) by the equation:

$$Y = -267.8966 + 0.0169\,X_1 + 9.3321\,X_2.$$

These experiments indicate that conductivity of seed steep water, presumably a measure of seed permeability, is correlated closely with stand in the cold test. The amount of reducing sugar present also appears to be closely associated with stand. The cold test, as performed with a 10-day incubation at 10°C, probably accentuates loss of stand due to permeability. Stands under less adverse conditions of temperature and moisture may be more closely associated with the amount of sugar or other substances present on the seed-coat surface than with the amount which may leach from the seed in water in a given period of time (*6*).

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6. I wish to express my appreciation to D. L. Van Horn, of the Baker Castor Oil Co., for seed samples; to E. J. Koch, U.S. Department of Agriculture, for statistical analyses, and to Tamara Sorokin, U.S. Department of Agriculture, for chromatograms.

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Auditory Thresholds in the Rat Measured by an Operant Technique

Abstract. An adaptation of the Ratliff and Blough technique has been developed for auditory measurement in rats. Thresholds for a 2000-cy/sec tone were determined over a period of weeks. Kanamycin, an ototoxic agent, was then administered, and the gradual rise in threshold was followed.

We have applied the Békésy (*1*) method of automatic audiometry for human subjects (a modified method of adjustment) to the measurement of auditory thresholds in rats, following the lead of Ratliff and Blough (*2*), who adapted it for the determination of visual thresholds in pigeons. The essence of the method is that the subject himself controls the stimulus, and his behavior is at the same time controlled by the stimulus. With the Békésy audiometer, this control is accomplished by instructing the subject to hold down a button, actuating an automatic attenuator, so long as he hears the sound. While he holds down the button, the sound is gradually attenuated. When the intensity is reduced below his threshold of hearing, he releases the button, and the intensity is automatically increased.

The rat is trained by operant techniques (*3*) to press one bar repeatedly so long as he hears the sound, and to press a second bar when he does not hear it. Responses on the first bar lower the sound intensity, while those on the second bar raise it again. An appropriate schedule of reinforcement with water keeps the thirsty rat working for about an hour (*4*).

The rat works in a small wire cage, mounted in a sound-treated, ventilated wooden box. Two stainless-steel bars, each controlling a microswitch, are placed at the front of the cage, facing a loud-speaker. A motor-driven dipper delivers 0.02 ml of water per reinforcement. The programing is arranged so that presses on bar *A* reduce the sound intensity and then turn the sound off by means of an electronic switch. Presses on bar *B* bring a reinforcement with the dipper and then turn the sound on again at the attenuated level. A block diagram of the equipment is shown in Fig. 1.

Male albino rats weighing approximately 250 gm were allowed access to water for only ½ hr/day for 2 weeks. After this period of deprivation, each rat was placed in the testing box, which contained a single bar (*B*) and a dipper under manual control. The rat was trained to press the bar under continuous reinforcement. This schedule was gradually changed until stable rates were obtained on a variable-ratio (VR) schedule. At this time a second bar (*A*) was introduced into the test box, and a 2000-cy/sec tone was turned on at a moderate intensity (about 60 db SPL). The rat was then trained to press bar *A* once in the presence of the tone in order to turn it off, before he could obtain a reinforcement on bar *B* with the VR8 schedule. After reinforcement, the tone came on again. The schedule on bar *A* was gradually changed to VR8, so that the rat had to press bar *A* several times before the tone went off and then had to switch to bar *B* and press several times to get a reinforcement.

The following contingencies were introduced during the two-bar training to insure that the animal was responding to the auditory stimulus. (i) With the tone off, three presses on bar *A* turned the tone on again. (ii) After a reinforcement, the tone remained off 20 percent of the time, and the rat could

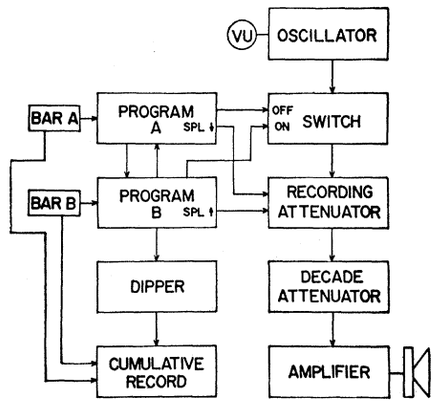

Fig. 1. Block diagram of auditory and programing apparatus.

secure two consecutive reinforcements on bar *B*. (iii) Presses on bar *A* were erased if the rat switched to bar *B* without turning the tone off. (iv) Time out (10 sec) followed continuous overpressing of bar *A*. The training under fixed SPL conditions was continued until a stable discrimination was obtained.

During threshold testing the programing was altered. A random number of presses on bar *A* reduced the sound level in 5-db steps, attenuating it 5 to 20 db before the sound was turned off. When the sound was off, a random number of presses on bar *B* gave a reinforcement and turned the sound on again at the attenuated level. Further presses on bar *B* (three presses) with the sound on increased the intensity again in 5-db steps.

In the course of pressing bar *A* the rat reached a point where his presses had reduced the intensity to a level below his threshold but had not turned the tone off. At this time he switched to bar *B*, since for him the two conditions, tone off and tone below threshold, were equivalent. By pressing bar *B* a number of times, he drove the intensity above his threshold and must then switch back to bar *A* to turn the tone off. These oscillations in intensity

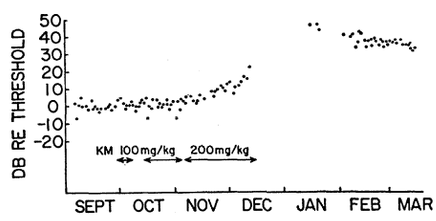

Fig. 2. Daily thresholds (2000 cy/sec) for rat No. 1 prior to, during, and after kanamycin administration. Zero decibels represents the average threshold during the 20-day control period, that is, 44 db sound pressure level (SPL).

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.

3 November 1959