HEARING IN THE RAT AT HIGH FREQUENCIES

THE authors recently discovered that rats sometimes display epileptiform seizures when they are exposed to frequencies of 21 kilocycles and that they show, by their overt behavior, sensitivity to even higher frequencies. It therefore seemed clear to us that the rat hears higher frequencies than does man, but it became a matter of interest to know just what frequencies are audible and how well these frequencies are heard. No data concerning pure tones higher than 8 kilocycles could be found in the literature, so the following experiment was conducted.

Nine rats were taught to run from one part of a compartment to another whenever a tone of 8 kilocycles was presented. Their incentive was to avoid shock. When they had learned this, they were trained to react in similar fashion to other frequencies between 1 and 40 kilocycles. Then the intensity of the tones was reduced step by step until an intensity was reached at which a tone called forth responses from the animal only 50 per cent. of the time. This intensity was taken as the threshold, and it was determined for 1, 2, 4, 8, 14, 21 and 40 kilocycles. (40 kilocycles was the limit of our apparatus.) Except for 21 and 40 kilocycles, similar measurements were taken on eight human subjects.

The average thresholds of the human and animal subjects were compared at different frequencies with the following results. The rat's threshold is much higher than man's at 1 kilocycle, but the difference between the two diminishes as the frequency is increased, until in the neighborhood of 8 kilocycles the sensitivities of man and the rat are the same. At higher frequencies, however, the rat is more sensitive than man, and the discrepancy becomes larger as the frequency is increased. Thus rats are poorer than man below 8 kilocycles and better than man above this frequency.

Any attempt to state the audiogram of the rat in terms of acoustic energy must be based upon somewhat tenuous suppositions concerning the physical characteristics of our apparatus. Nevertheless, even when due account is taken of such considerations, we can state that the absolute sensitivity of the rat most certainly improves as the frequency is increased up to 20 kilocycles. It seems likely, furthermore, that the frequency most audible to the rat is as high as 40 kilocycles. At any rate, our rats hear 40 kilocycles very well, and the upper limit of hearing must, on this account, be a very high frequency indeed.

No animal in whom hearing has been studied at all adequately presents such a disposition of auditory sensitivity as this. Cats and dogs[1] hear best at a higher frequency than man, and their upper frequency-limit is higher, but the rat surpasses them in both respects. Of other animals so far studied, only the bat[2] shows signs of possessing a similar range of auditory sensitivity; cochlear potentials have recently been observed in this animal up to 98 kilocycles. If more suitable sound-producing instruments than we had at our disposal are available in future experiments, it may be shown that the rat's hearing extends to and beyond this frequency; at least, so our data would lead us to expect.

JAMES GOULD
CLIFFORD MORGAN
HARVARD UNIVERSITY

[1] S. Dworkin, J. Katzman and G. A. Hutchison, *Jour. Exp. Psychol.*, 26: 281, 1940.

THE GERMINATION OF MAIZE POLLEN

THE pollen of *Zea mays* is recognized as one of the difficult sorts to germinate under artificial conditions. In addition to its value in genetic and cytological studies, preliminary experiments have suggested that a reliable method for rapidly checking the viability of maize pollen will be necessary in studies of the effect of ecological factors on pollination and yield of maize.

A method has been developed which has given as much as 90 per cent. germination on nutrient medium within 30 minutes after inoculation. A solution containing 0.7 per cent. agar and 15 per cent. sucrose is held at 60 degrees C. in a water bath and transferred with a pipette to a microscope slide. Only enough solution is placed on the slide to form a shallow droplet of approximately 1 cm diameter. The droplet is allowed to harden for 60 seconds at 20 to 25 degrees C. before the pollen is dusted on from a knife blade held about 2 cm above. The slide is immediately transferred to a moist chamber at 23 degrees C. in which the relative humidity is maintained at 90 per cent. Germination counts can be made after 30 minutes, and, with good lots of pollen, should certainly be read within two hours, before a confusing mass of tubes has developed. In practice the tube growth has been arrested by transferring the slides after two hours to another moist chamber at 6 degrees C. in which the material can be preserved intact for two weeks for more leisurely observations.

The most serious problem in germinating maize pollen is the prevention of bursting. Apparently a near isotonic relationship between the nutrient medium and the cytoplasm is required, with the balance slightly on the hypotonic side so that the pollen grain may absorb water for tube growth, but not rapidly enough to cause bursting. With different lots of highly viable pollen the sucrose percentage showing maximum germination has varied between 10 and 15 per cent. Although no sugar determinations have been made for

[2] R. Galambos, SCIENCE, 93: 215, 1941.

maize pollen, grains treated with an alcoholic solution of IKI reveal wide differences in starch content. It may well be that ecological conditions prior to pollen shedding influence the sugar content of the grains. It is not known whether a reduced osmotic value in the grain is due to the use of some of the sugars in increased respiration or whether sugars may be readily changed to insoluble forms and thus play no part in the osmotic force of the cell. More consistent results

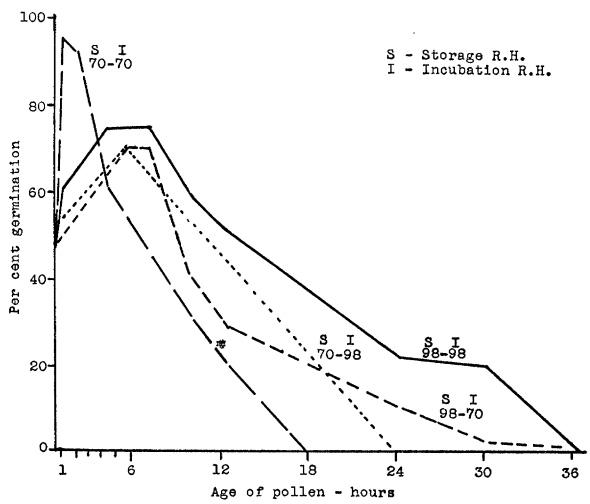

FIG. 1. Effect of age and relative humidity during storage and incubation on the germination of maize pollen.

have been obtained when pollen was taken from cut tassels stored overnight to several days at constant humidity and temperature, perhaps because more uniform osmotic values are obtained with such preliminary treatment.

A major factor in the success of the method is the degree of imbedding of the pollen grains in the still soft agar, and the agar percentage, temperature and cooling rate are important in this connection. Best results have been obtained when the grains were two thirds imbedded and one third exposed to the air. Presumably these conditions favor absorption of both water and oxygen. Covering the pollen has prevented germination. Germinating at 90 per cent. rather than higher humidity reduces bursting due to the formation of free moisture films on the agar. Good germination has been obtained in many experiments at 60 per cent. humidity, although shorter tubes were produced in the drying agar. The data of Fig. 1 show that germination under artificial conditions improved with storage for about six hours after shedding, then declined rapidly. One lot of pollen remained viable after 10 days storage at 8 degrees C. Longer storage life was obtained at the higher humidities, but many lots of pollen stored in nearly saturated air suddenly appeared to become moist and to clump together and thereafter showed no germination.

Maize pollen has germinated poorly on fructose and progressively better on glycerine, glucose and sucrose used at equivalent molarities. Tubes have been produced at pH 4.0 to 8.4. Under optimum conditions the elongation of the pollen tube is sufficiently rapid to be plainly visible under the microscope, and when projected on a screen by a micro-projector so that the grain appeared as large as a grapefruit, the image of the growing point has progressed as much as 1 cm a minute. Tube lengths have frequently reached fifty times the diameter of the pollen grain, although no attempt to stimulate tube growth, as distinct from germination, has been made.

R. A. BAIR
W. E. LOOMIS
INDUSTRIAL SCIENCE RESEARCH INSTITUTE
IOWA STATE COLLEGE, AGRICULTURAL MARKETING SERVICE, AMES

SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE DAILY REMOVAL OF FORMALIN FROM PRESERVED BIOLOGICAL SPECIMENS USED IN CLASS WORK

INSTRUCTORS and students who are exposed repeatedly to the formalin contained in preserved biological specimens often find their laboratory periods extremely disagreeable and, occasionally, hazardous. Induction or aggravation of the common cold, severe dermatitis, bronchitis and asthma are the chief hazards of exposure to formalin. A method is needed to remove this common laboratory preservative.[1]

Dr. Foust, *et al.*,[2] reported the value of a 5 per cent. urea and 1 per cent. ammonium phosphate solution in removing formalin from preserved specimens. Following the directions given in this article, the writers attempted to deformalinize bullfrogs, dogfish, starfish, etc., but failed to obtain satisfactory results. Therefore, we sought to develop another method to achieve the desired result.

After making a series of small-scale tests with some forty laboratory reagents known to react with formalin, we selected sodium bisulfite, $NaHSO_3$, as most closely approximating our objective, *viz.*, of finding a convenient, quick, cheap and efficient method for removing formalin. An aqueous solution of this reagent was entirely suitable in so far as it completely destroyed the formalin odor of preserved specimens

[1] P. H. Pope, SCIENCE, 73: 495, 1931.
[2] H. F. Foust, T. S. Leith, H. M. Tabbut and L. Bowstead, SCIENCE, 83: 498, 1935.