

in part, to a greater total sound intensity for the mixed sounds.

Using the equipment and the methods above described, individual peromyscus afflicted with the *artemisiae* type of epilepsy showed ear movements in response to sound frequencies from 500 to 95,000 c/sec when tested at ages between 25 and 70 days. The band of frequencies from 5 to 16 kc/sec is the most effective for eliciting ear movement in the mice of this strain. Decrease in effectiveness is fairly abrupt for frequencies below 4 kc/sec. Above 16 kc/sec, the effectiveness of the sound in inducing a response decreases gradually with increase of frequency. With each increase in sound pressure certain mice show a broadening of the band of frequencies which will evoke a response.

It is possible that with increased sound pressures, responses could be obtained to higher and lower frequencies than those here reported. Furthermore, the mice may hear frequencies higher and lower than those to which they respond by ear movement. All the observers who have worked for any length of time with these mice have noted that, when presented with sounds just beyond the range which induces definite ear movement, some animals may respond by turning the head or by cessation or beginning of body movement. Much more study will be needed to ascertain accurately the audibility curve of these animals.

As they grow old the hearing ability of most of the epileptic individuals decreases, especially for the higher frequencies, but with much variability between individuals. Certain epileptic individuals ultimately become completely deaf, as is shown by their failure to respond to sounds of any kind. Such deaf individuals do not go into convulsions when exposed to those sonic or ultrasonic sounds we have produced. The relationship between hereditary susceptibility to audiogenic seizures and degree of responsiveness to sonic and ultrasonic sounds, however, needs further investigation.

The epileptic deermice whose hearing ability is here described were of mixed racial stock, in whose ancestry the subspecies *P. maniculatus artemisiae* and *P. m. blandus* were prominent. Young individuals of a strain of the subspecies *bairdi* from near Ann Arbor, Mich., are generally similar in their hearing range to the young racial hybrids above described. In *bairdi*, audiogenic seizures are absent. At frequencies between 5 and 60 kc, higher intensities of sound are required on the average to produce a response by the small-eared *bairdi* than by the generally larger-eared racial hybrids. Young individuals of a strain of *blandus* from New Mexico, on the average, failed to respond by ear movements to ultrasonic sounds of as high a frequency and on the whole were less responsive to pure tones than the young epileptic individuals of mixed racial ancestry.

Three individuals of *P. nasutus* from a hybrid stock resulting from a cross between the subspecies *nasutus* and *griseus* have responded by ear movements to frequencies as high as 100 kc/sec. In general these ani-

mals exhibited a slightly greater responsiveness to high frequencies than members of the species *maniculatus*. It is possible that this greater responsiveness may be related to their very large ears. Further studies are needed to compare the hearing ability of individuals, races, and species which differ in the sizes of their external ears.

No consistent difference in hearing ability between male and female peromyscus has been discovered.

The hearing range of peromyscus evidently is somewhat similar to that of certain rodents tested by Schleidt (3). It is to be noted also that at least some peromyscus can hear ultrasonic sounds within the same general frequency range that is used by bats for echolocation (4). It is not yet known, however, whether a peromyscus can produce ultrasonic sounds over the range of frequencies that it can hear.

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Infrared Spectrophotometry as a Means for Identification of Bacteria

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Infrared absorption spectrophotometry is finding increasing use in the biological sciences. Spectra have been published of dried whole tissues (1, 2), individual dried cells (3), muscle cells in Ringer's solution (4), and cellular components (5, 6). Randall *et al.* (7) have published the spectra given by organic solvent extracts of *Mycobacterium tuberculosis* and correlated the spectra with biological properties such as virulence. The purpose of the present investigation was to determine whether infrared spectra of whole bacteria could be used for identification of the species and perhaps the strain of the organism. The results clearly indicate that considerable differentiation of both is possible by this approach.

Dried films of the organisms were prepared by taking a few colonies from an agar plate, spreading them with a rubber policeman over the surface of a silver chloride plate, and allowing the film to dry. When pathogenic bacteria were used, the dried film was covered with another silver chloride plate, and the edge sealed with cellophane tape to prevent aerosol production from a flaking-off of the dry film. Interference patterns occasionally observed when using two plates were eliminated by placing a piece of paper or tape on one side between the plates so that they were not parallel. Some preliminary work was done

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using washed organisms grown in broth culture, but this technique was discontinued in favor of the simpler colony smear technique. With most organisms, the smears dried to give smooth transparent films, from which excellent spectra were obtained. With many spectra the transmission at $5.5\ \mu$ was above 90%, and at $6.05\ \mu$ dropped to below 5%. Some organisms, however, especially those with a high lipid content, produced films exhibiting much general light scattering; hence they had a lower transmission at $5.5\ \mu$ and consequently less contrast between the transmissions at 5.5 and $6.05\ \mu$. The transmission at $5.5\ \mu$ was taken as a measure of the quality of the film, and the transmission of the carbonyl band at $6.05\ \mu$ was taken as a measure of the thickness of the film. Most spectra considered had transmission values of greater than 90% at $5.5\ \mu$ and less than 10% at $6.05\ \mu$. The thickness of the films was not a critical factor, because the differentiation was based on qualitative differences in the shapes of the absorption bands. The Perkin-Elmer Model 21 Double Beam infrared spectrophotometer was used for recording the spectra.

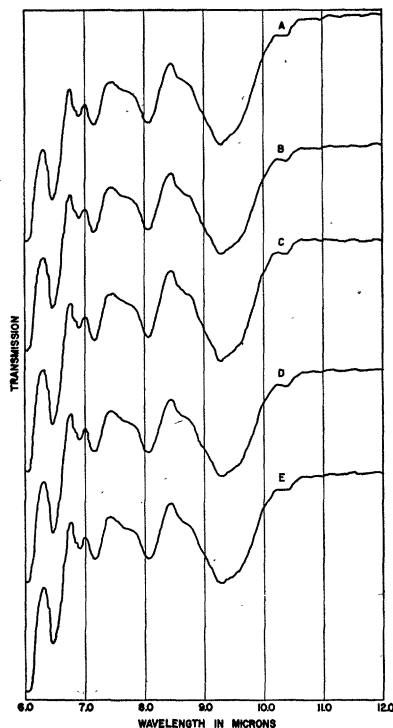


FIG. 1. Spectra of separate cultures of *Serratia marcescens* cultured for 24 hr on fortified tryptose agar.

Since the infrared absorption spectrum of a specimen is a function of the chemical composition, it was necessary to control such conditions as culture medium, age of culture, and temperature of incubation, since these are known to affect the chemical composition of organisms. Experimentally, it was demonstrated that these variables alter the infrared spectra in varying degrees, depending upon the species of the organism.

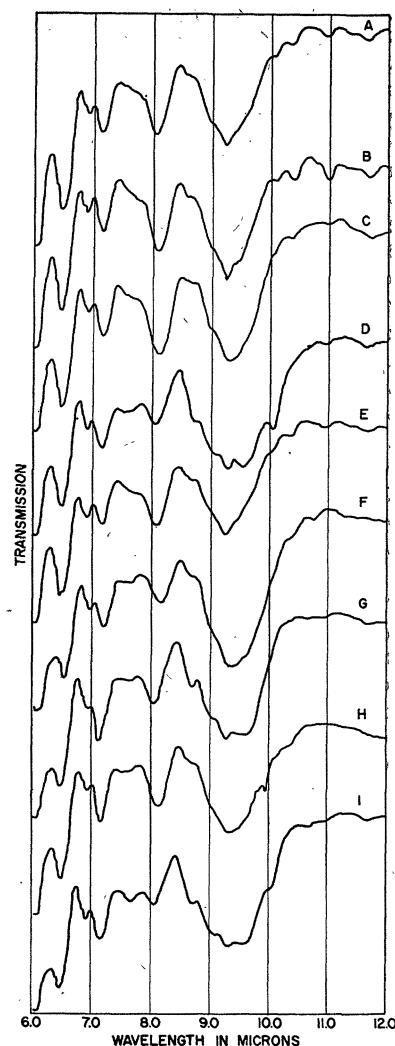


FIG. 2. Spectra of different species cultured for 24 hr on fortified tryptose agar: (A) *Escherichia coli*; (B) *Pseudomonas aeruginosa*; (C) *Micrococcus pyogenes* var. *aureus*; (D) *M. rosaceus*; (E) *Aerobacter cloacae*; (F) *Bacillus subtilis*; (G) *B. megatherium*; (H) *B. globigii*; (I) *Sarcina lutea*.

The most informative spectral range was found to be that between 5.5 and $12.0\ \mu$.

The first problem was to determine the degree of reproducibility. Fig. 1 shows spectra of a number of separate cultures of *Serratia marcescens*. Although, because of variations in the thickness and physical homogeneity of the films, these curves are not all identical, they are qualitatively very similar. Fig. 2 shows spectra of 9 organisms typical of approximately 30 studied. These were cultured 24 hr at 37°C on fortified tryptose agar. Spectra of almost all organisms have the same major absorption bands, but the relative intensity varies from one species to another; this, plus minor absorption bands, varies the shape of the broad bands and the intervening regions between these bands. Although the differences were somewhat subtle in some cases—as, for example, be-

tween *Escherichia coli*, *Pseudomonas aeruginosa*, and *Aerobacter cloacae*—they were sufficiently consistent to differentiate the species studied to date. Infrared spectra do not always group species as in the Bergey classification. For example, *Pseudomonas* (Family II) organisms and *Escherichia* (Family X) have similar spectra, whereas *Micrococcus rosaceus* and *M. pyogenes* var. *aureus* (Genus I, Family V) have very different spectra.

In addition to differentiation of species, it was possible to differentiate strains of *Bacterium tularensis*

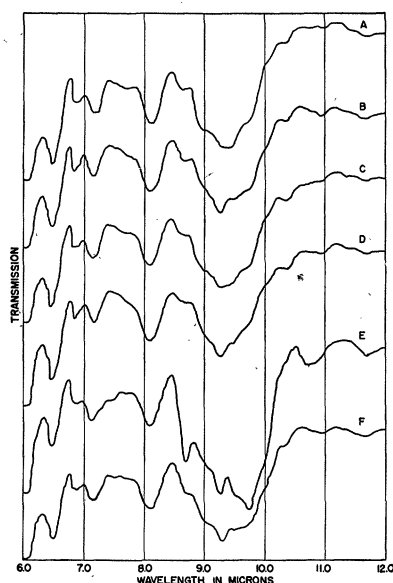


FIG. 3. Spectra of various strains of *Bacterium tularensis* cultured for 24 hr on tryptose agar: (A) Schu S_3 ; (B) Schu NS_3 ; (C) Jap S_2 ; (D) Jap S_4^{ns} ; (E) 38 NS_2 ; (F) 38 S_1 .

(Fig. 3). Spectroscopic differentiation of the Schu S_3 smooth strain and the Jap S_2 smooth strain (8) is somewhat uncertain, but the differences between other strains studied are quite obvious. Spectra of all strains of *B. tularensis* are characterized by a sudden drop in transmission at 6.80 μ , producing a clearly marked minimum at that point. The spectra of all other organisms thus far observed have a minimum transmission at 6.90 instead of 6.80 μ .

The use of infrared absorption spectra as an aid to identification appears promising. The prerequisite for such identification is a catalogue of spectra of organisms cultured under controlled conditions.

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Effect of Penicillin on Streptomycin-dependent Variants in *Escherichia coli* Populations

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The penicillin method for the isolation of biochemically deficient mutants (1-3) has been applied to the problem of the origin of streptomycin-dependent variants of *Escherichia coli*. If dependent cells arise spontaneously in an actively growing culture of normal bacteria, they should soon stop growing, since no streptomycin is present and since their requirement for this substance has been shown to be highly specific. If this cessation of growth occurs while the remainder of the population is still growing actively, penicillin may be expected to kill the normal cells and spare the streptomycin-dependent ones, as in the usual isolation of biochemically deficient mutants by this method. If, on the other hand, streptomycin-dependence represents an adaptation to the antibiotic, no reason is known why the precursors in the normal population before contact with streptomycin would be uniquely insensitive to the bactericidal action of penicillin.

A smooth, motile strain of *E. coli* was used, which behaved typically in the usual series of characterization tests. The organism was grown from small inocula at 37° C in Difco nutrient broth with 0.2% added glucose. The inoculum size was estimated by replicate platings of identical inocula on nutrient agar. Growth of the broth culture was followed by turbidimetry and viable counts. When the desired degree of growth had been reached (usually $1-3 \times 10^8$ cells/ml), the culture was centrifuged and resuspended in fresh nutrient broth with glucose. A viable count was made, and 0.3 ml implanted onto each of 6 nutrient agar plates containing streptomycin (SM), 10 μ g/ml. These plates had been previously dried so that the implant fluid would be absorbed within 30 min at room temperature. Penicillin (300 u/ml) was added to the remainder of the resuspended culture at 37° C; the culture was then incubated for 20-40 min and quickly immersed in an ice bath. After centrifuging, the penicillin-containing broth was replaced by the same volume of 0.9% NaCl. Another viable count was done to determine the extent to which penicillin had killed the normal population. A second set of implants on SM plates was then made, exactly as before. All the plates were incubated at 37° C.

With this organism, at the SM concentration used here, dependent (D) colonies appear on the plates with a characteristic delay of 1-4 days. Of thousands of D colonies observed in numerous experiments, none has ever been macroscopically visible 24 hr after implantation. Nondependent, resistant (R) colonies, on the other hand, almost always are present at 24 hr,