about 1 to 25 can not be obtained with allantoic fluid whereas with concentrated virus any ratio down to about 1 to 1 can be obtained, a much more complete study of the adjuvant effect of calcium phosphate on influenza virus vaccines can be made by the use of virus materials concentrated and purified by means of differential centrifugation.

W. M. STANLEY

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH,
PRINCETON, N. J.

RECORDING OF SOUNDS PRODUCED BY CERTAIN DISEASE-CARRYING MOSQUITOES

THE destruction of disease-carrying mosquitoes is a problem of the first magnitude. Various methods have been employed such as oiling, draining and the application of larvacides and insecticides. In this connection, the use of D.D.T., both in powder form and as a spray, has been astonishingly effective when used in the field. D.D.T. destroys a variety of insects both harmful and beneficial so that the problem of disturbing the biological balance such as the interference with pollenizing insects, for instance, must be taken into consideration.

When sprayed upon walls of dwellings D.D.T. will repel or kill mosquitoes and other insects for long periods of time. It must not be forgotten, however, that this substance is toxic when ingested. The other methods of mosquito control are often quite expensive and time-consuming.

In order to circumvent these objections, we have approached the problem of mosquito destruction from a somewhat novel angle. It occurred to us that mosquitoes might have characteristic "mating calls." If so, these characteristic sounds, if properly recorded and satisfactorily reproduced, might aid in the attraction of mosquitoes to their death.

We have successfully recorded mosquito sounds which are faintly audible, or completely inaudible to the human ear, and are now able to transmit these sounds, with the end in view of calling specific varieties of mosquitoes to a place of destruction.

The following mosquitoes were employed: *Anopheles quadrimaculatus, Aedes aegypti, Aedes albopictus* and *Culex pipiens*. Colonies of these insects are maintained in the laboratory. The electrical apparatus which is utilized is (1) a microphone, (2) an amplifier of considerable more than usual power, (3) suitable band pass filters and (4) a conventional high quality disk recorder. When the recordings are made, the mosquitoes are placed in a sound-proofed test chamber under conditions of proper temperature and humidity, in order to obtain sensitive recordings under a natural environment.

Certain varieties of mosquitoes such as *Aedes aegypti* have been thought to produce little if any sound. If sounds are produced, they must be outside of the range of human hearing. This we have found to be the case. One may disturb a cage containing 200 or more of this species, yet no audible sounds are heard. One may listen intently in a very quiet room to *Culex pipiens,* but only the very most intense sounds of this species are faintly audible. We have observed that the largest variety of sounds made by this species as well as the others referred to are outside the energy range of normal hearing.

Despite the great variety of sounds, each genus and species have tonal emanations which are so distinctive in character that an experienced observer can not only readily distinguish one genus from another, but with no difficulty at all can also distinguish the males of a species from the females of the same species. Even such closely related species as *Aedes aegypti* and *Aedes albopictus* can be distinguished by sounds alone.

In not a few respects, the sounds of the mosquitoes we have tested are like bird calls. Their variety seems to indicate that they may be in the nature of (a) mating calls, (b) calls warning of danger, (c) calls of anger and other sounds that are similarly functional.

All the sounds that we have recorded to date are in the center of the frequency range of human hearing but far below the energy level required for that purpose. The fundamental tones lie in the range from 250 cycles per second to 1,500 cycles per second. It is interesting to note that all male "voices" so far recorded are much more high pitched than the females. Pitch is a simple means of distinguishing one mosquito sex from another. Female voices contain far more energy than males even when the insects are not in flight.

The most astonishing and important observation of this experiment is that the noise of a single female will cause the males of the same species to burst into an answering chorus. Moreover, when the call of a female is transmitted to two or three males under the circumscribed space of a small test-tube, it has been observed under the microscope that the antennae and hypopygium of the male will turn toward the direction from whence the sound is being transmitted.

Single mosquitoes seemingly do not emanate any sound. Two or more must ordinarily be together before any sounds are generated. This seems to be true regardless of species or of sex. If two mosquitoes of the same sex do not choose to be active, the addition of a mosquito of the opposite sex will often cause activity in the erstwhile silent insects.

In certain mosquitoes, two tones, separated by a

small pitch interval, or often an interval of one half a musical tone, can be produced simultaneously, thereby producing "beats." It is possible that the production of such beats is an aid to the listening insect in determining the direction from which the sound originated.

As far as we can determine at the present time, the sounds seem to be produced by three methods: (1) noises made when the mosquitoes are in flight, (2) the beating of the wings while the insects are at rest and (3) the rubbing of the tarsi against the wing, (4) certain pure bird-like sounds the origin of which we have not been able to determine.

Additional recordings are being made, and as soon as conditions permit, each significant tone will be tested in the laboratory and in the field in order to test its ability to lure the mosquito to some form of destroying mechanism.

In the case of the Anopheles, it may be necessary to make a separate recording for each potentially dangerous species, but if this is necessary it is a simple matter. Possibly this method will be of aid in the destruction of other insects as well as rats and rodents concerned in disease transmission.

We wish to thank Dr. Mark F. Boyd and Dr. Max Theiler, of the Rockefeller Foundation, for their courtesy in supplying us with *Anopheles quadrimaculatus* and *Aedes albopictus* with which we began our colonies. The *Aedes aegypti* were obtained in the South, while Dr. Denis R. A. Wharton captured a wild strain of *Culex pipiens* for us in New Jersey. The technical assistance of Miss Isabella Brogan is greatly appreciated. This study was aided by the American Foundation for Tropical Medicine.

MORTON C. KAHN
WILLIAM CELESTIN
WILLIAM OFFENHAUSER
DEPARTMENT OF PUBLIC HEALTH AND PREVENTIVE MEDICINE,
CORNELL UNIVERSITY MEDICAL COLLEGE,
NEW YORK

THE EFFECT OF PENICILLIN AND CERTAIN SULFA DRUGS ON THE INTRACELLULAR BACTEROIDS OF THE COCKROACH

IT has been well known for many years that certain cells in the fat body of cockroaches contain masses of rod-like microorganisms resembling bacteria in shape. These, known as bacteroids, are present in every individual roach of both sexes and are known to be hereditary in the sense that they migrate into the ovaries and thence into the eggs before these are fully formed. Later they become a part of the embryonic cockroach and eventually locate in the fat body where they soon multiply to form the masses that surround the nuclei of the modified fat cells. This process occurs early in the life of the cockroach, and the very young nymphs already have well-developed bacteriocytes scattered through the body.

These bacteroids have been rather loosely termed intracellular symbionts, although an actual symbiotic relationship has never been proven, and it has been rather generally assumed that they are more probably some sort of quite innocuous parasites which cause no serious damage to their host. The association of the bacteroids with cockroaches is undoubtedly of very ancient origin: they occur throughout the order Blattodea and have much more recently been noticed in the very primitive Australian termite, Mastotermes. Since the termites presumably arose from cockroach-like ancestors during the early geological history of the insects, we must believe that the cockroaches possessed them before that time.

The successful cultivation *in vitro* of the bacteroids of at least one species of cockroach (*Periplaneta americana*) has been reported by Glaser,[1] although this is not easily accomplished, and others have failed to repeat the procedure.

Since the bacteroids react to stains as Gram-positive bacteria, it seemed likely that they might be affected by some of the bacteriostatic or bacteriocidal substances that have recently come into therapeutic use in combating pathogenic bacteria. Consequently, we tried several of the common sulfa-drugs (sulfanilamide, sulfathiazole, sulfadiazene, sulfapyridine and sodium sulfanilate), injecting them in saline solution or suspension directly into the body cavity of a species of Florida cockroach, *Blaberus cranifer*. This is a very large roach, the females measuring nearly two inches in length and weighing on the average about four grams, while the males are noticeably smaller and lighter in weight. The dosages were determined on the basis of the known tolerances of mice per unit of body-weight, but we found that the roaches could receive much greater quantities with no apparent ill effects. None of these treatments had any noticeable effect on the bacteroids, as was determined by killing and fixing the roaches after periods varying from one day to several weeks. In practically every case, the bacteroids showed no significant change in numbers, morphology or staining properties.

A preliminary treatment of a number of roaches was made about a year ago, using the medium in which a culture of *Penicillium notatum* had been grown. An inoculum of this was given to us by Dr. D. H. Linder. It appeared that this material had some effect on the bacteroids, but the results were not constant, due no doubt to the variable concentration of the penicillin employed. More recently we secured through the courtesy of Dr. Chester A. Keefer some penicillin of

[1] R. W. Glaser, *Jour. Exp. Med.*, 51: 59–82, 1930.