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.

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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.

known potency, and with this have been able to treat a number of roaches with standardized dosages. The penicillin was dissolved in sterile water in such dilution that the dose desired in each case was contained in 0.2 cc, which is a convenient volume for injection. These injections were repeated, as shown by the appended table, where the effects of the several concentrations is also indicated. It will be noted that the tolerance of the roaches is many times greater on the basis of body weight than the therapeutic dosages employed in human medical practice.

COCKROACHES (*Blaberus cranifer*) TREATED WITH PENICILLIN

Lot No.	Dosage in Oxford Units	Results
A. (2♀)	200 units × 4 doses Total—800 units	After 28 days, one killed and fixed. Separated clumps of symbionts present. Other roach perfectly healthy.
B. (2♀)	200 units × 5 doses Total—1,000 units	After 4 days, one killed and fixed. Symbionts apparently in good condition. After 8 days, another killed and fixed. Symbionts apparently in good condition. Both were lively when killed.
C. (3♀)	400 units × 4 doses Total—1,600 units	After 24 hours, one killed and fixed. No symbionts visible, although the large cells formerly containing them evident. After 26 days, one almost dead, killed and fixed. Symbionts in scattered cells. Fat frothy and almost completely liquefied. After 27 days, third specimen killed and fixed. Condition as in the previous one.
D. (3♀)	800 units × 4 doses Total—3,200 units	After 24 hours, one dead. After 24 hours, one killed and fixed. No symbionts in larger cells, but a very few in smaller ones. After 27 days, the third specimen killed and fixed. Symbionts present in scattered cells.
E. (2♀)	2,000 units × 3 doses Total—6,000 units	After 6 days, one killed and fixed. No symbionts visible. After 12 days, second specimen died.

These results show very clearly that the bacteroids are affected by penicillin when this is administered at high concentrations, although the roaches give no immediate indication of any toxic effect from the drug,[2] even when dosages as great at 200 times the therapeutic dose is injected. The condition of the fat cells and their contained bacteroids were examined after fixation in formaldehyde, sectioning in paraffin and a heavy staining in haematoxylin.

From the data here presented, it is apparent that the administration of penicillin at the lower concentrations very greatly reduces the number of bacteroids, but after the insects are allowed to live normally for a time, the bacteroids gradually increase in numbers. Meanwhile, the roaches show no ill effects from the treatments. If, however, sufficient penicillin is administered to destroy the bacteroids, or to reduce their numbers beyond a very low level, the roaches finally die after some days have elapsed. Since they do not succumb immediately, their death can not be attributed to a direct toxic effect of penicillin, but rather to a lack of something supplied by the bacteroids. We can not regard the present results as conclusive evidence that the bacteroids are necessary for the continued life of the cockroaches, but they make it appear very probable that such is the case and that they are symbiotic, and not parasitic, microorganisms.

[2] Occasionally roaches die shortly after having received an "intraperitoneal" injection, due probably to perforation of the alimentary canal by the hypodermic needle.

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THE OCCURRENCE OF NORMAL SERUM GAMMA-GLOBULIN IN HUMAN LYMPHOCYTES

DESPITE the interest and importance of the problem, there is little information concerning the origin of serum globulins. Since the human serum γ-globulin fraction contains almost all the antibody activity of pooled plasmas,[1] and since numerous attempts to demonstrate antigenic[2,3] and physical[4] differences between immune and non-immune globulins have been uniformly unsuccessful, there would seem to be little doubt that antibodies are modified serum globulins, and that in man, at least, they are chiefly modified γ-globulins.

Recent demonstrations of the occurrence of antibody in lymphocytes of rabbits[5] and mice[6] substantiate earlier indications of the role of lymphoid tissue in the development of immunity.[7] It follows that if antibody is synthesized in lymphocytes, normal serum γ-globulin should also be present.

We have, therefore, prepared rabbit antisera to highly purified γ-globulin fractionated from pooled normal human plasmas, and have found that extracts of lymphocytes of human origin react specifically with these antisera.

The γ-globulin used for immunization[8] was further

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[8] Kindly supplied by Dr. J. L. Oncley of the Department of Physical Chemistry, Harvard Medical School. See E. J. Cohn, J. L. Oncley, L. E. Strong, W. L. Hughes, Jr., and S. H. Armstrong, Jr., *Jour. Clin. Invest.*, 23: 417, 1944.