other two, completes the solution or equalization of the reagents.

After the final process of clearing is complete, the excess reagent is removed from the chamber by a pipette, the clamp is released and the cover glass inverted over a drop of balsam placed on a slide.

Corks with holes for the entrance of the capillaries may be placed in the vials to prevent evaporation, also a large cover glass should be placed over the chamber when the process is not being observed under the microscope. If a capillary fails to function because of the presence of air bubbles, the intake capillary of the vial from which it leads is removed. Then, a mouth pipette is inserted through its place in the cork and an air pressure exerted sufficient to cause the resumption of flow through the capillary.

The flow into the chamber containing the specimens is so gradual and the mixture of the reagents entering it has been found to be so complete that small protozoa will remain stationary on the cover glass and not be carried by convection or solution currents into the drain leading from the chamber.

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SPECIAL ARTICLES

THE EXPERIMENTAL TRANSMISSION OF YELLOW FEVER BY MOSQUITOES[1]

FOR many years yellow fever has been one of the most dreaded of the diseases menacing the human race in the warmer parts of the western hemisphere. The rapid conquest of its frontiers has been one of the great achievements of modern warfare on disease.

Ever since the memorable work of the American Commission (Walter Reed *et al.*) established *Aedes aegypti* as an essential host in the yellow fever cycle, epidemiological studies and palliative measures have revolved about the domestic relationships of this so-called yellow fever mosquito.

In addition to experiments with this species, the Americans[2] in Cuba and the French Commission[3] in Brazil, using human volunteers, attempted the passage of yellow fever through *Culex quinquefasciatus* (= *fatigans*), an important human pest widely distributed in the tropics. The French Commission also undertook transmission experiments with *Aedes scapularis, A. taeniorhynchus, Psorophora ciliata* and *P. posticata.* None of these experiments were successful.

More than two decades later, in West Africa, Bauer[4] carried out the first successful transmission experiments with species other than *A. aegypti.* The belief that the dreaded "yellow jack" was transmitted solely through the agency of the notorious *Stegomyia* mosquito had been of so many years' standing that the experimental transmission of the virus of this disease by several other species of mosquitoes came as a rather startling confirmation of the predictions of the late Dr. H. R. Carter.

In Dr. Bauer's experiments, *Aedes* (*Stegomyia*) *luteocephalus, A.* (*Aedimorphus*) *stokesi*[5] and *Eretmopodites chrysogaster* were allowed to bite infected rhesus monkeys. After an adequate incubation period, these insects were induced to feed on normal animals and were injected into other test monkeys. All produced fatal infections in the test animals. *A. apicoargenteus* did not transmit the virus. The negative results with this mosquito, a *Stegomyia,* and the positive findings for *Eretmopodites* are especially remarkable.

Continuing studies on West African species I added to the list of experimental vectors three more stegomyiae, *viz., A. africanus, A. simpsoni* and *A. vittatus,* as well as an important domestic mosquito of another genus, *Teaniorhynchus* (*Mansonioides*) *africanus.*[6] *Anopheles gambiae,* the chief malaria carrier of the region, fortunately proved to be incapable of maintaining the virus of yellow fever in its system through the accepted incubation period.

Several other species with which I made transmission tests (unpublished) produced no reaction by bites, but injections of saline suspensions of the vicious crab-hole mosquitoes, *A.* (*Aedimorphus*) *irritans* and *A.* (*A.*) *nigricephalus,* as well as of *A.* (*Banksinella*) *punctocostalis* and *Culex thalassius,* resulted in fatal infections, after the elapse of adequate incubation periods following the initial infecting meal. I was also able to confirm transmission of the virus by *A. luteocephalus.*

In view of the findings in West Africa Davis and Shannon[7] recently reopened the question of the pos-

[1] The studies and observations on which this article is based were conducted in Lagos, Nigeria, with the support and under the auspices of the International Health Division of the Rockefeller Foundation.

[2] W. Reed, J. Carrol and A. Agramonte, Senate Document No. 822, 1911, 110.

[3] E. Marchoux and P.-L. Simond, *Ann. Inst. Pasteur,* 25: 23, 1906.

[4] J. H. Bauer, *Amer. Jour. Trop. Med.,* 8: 261–282, July, 1928.

[5] A correction in name from the original report of *A. apicoannulatus* as pointed out by Evens, *Ann. Trop. Med. and Parasit.,* 23: 521, 1929.

[6] C. B. Philip, *Am. Jour. Trop. Med.,* 9: 267, 1929; 10: 1, 1930.

[7] N. C. Davis and R. C. Shannon, *Jour. Exp. Med.,* 50: 803, 1929.

sible transmission of yellow fever by South American species of mosquitoes other than *Aedes aegypti.*

There are no close relatives of *A. aegypti* in the western hemisphere, but these investigators have shown that *A.* (*Ochlerotatus*) *scapularis* is capable of producing typical infections by biting normal monkeys, that injections of macerated specimens of *A.* (*O.*) *serratus* may result fatally and that injections of *A.* (*Taeniorhynchus*) *taeniorhynchus* produce a mild infection; the bite of the two last-named mosquitoes did not prove infective, however. Negative results had previously been reported in experiments with *A.* (*Taeniorhynchus*) *taeniorhynchus* and *A. scapularis.* No definite infection was obtained by Davis and Shannon with *C. quinquefasciatus,* either by bite or injection; however, three test-animals bitten by these mosquitoes were reported to have remained "relatively immune" when given susceptibility tests.

A total of twenty-five investigations with twenty-two species of mosquito representing five genera has been reported by all the investigators mentioned above. Nine of these species (including *A. aegypti*) transmitted yellow fever by bite; six others transmitted it by injection only; six did not transmit the disease, and in one case (*C. quinquefasciatus*) the result was doubtful. A table is presented below, summarizing the present knowledge with regard to the species of mosquitoes which have been induced to bite infected humans (first two investigations) or monkeys (last three investigations), and have subsequently been tested by bite or injection. *A. aegypti* is included only once, although it has given positive results in the other investigations.

SPECIES OF MOSQUITOES KNOWN TO PRODUCE YELLOW FEVER IN HUMANS AND IN MONKEYS, BY BITE OR INJECTION

Reported by	Total species tested	Positive by bite	Positive by injection only	Negative
Western Hemisphere				
Reed, Carrol and Agramonte	2	1	.....	1*
Marchoux and Simond	5	0	.....	5†
Davis and Shannon	4	1‡	2†	1*
West Africa				
Bauer	4	3§	0	1
Philip	10	5§	4	1

Species tested in two investigations:

* *Culex quinquefasciatus* (three test animals reported relatively immune by Davis and Shannon).

† *Aedes taeniorhynchus.*

‡ *Aedes scapularis.*

§ *Aedes luteocephalus.*

The number of tests carried out with the crab-hole *Aedes* in Nigeria, as well as the other observations tabulated in the third column of the foregoing table, indicate that in certain species of mosquitoes complete distribution of the virus throughout the body does not occur, under ordinary conditions at least. *A. irritans* has retained the virus for as long as fifty-one days, as proved by subsequent injection, without transfer in biting experiments. Transmission by such unrelated genera as *Eretmopodites* and *Taeniorhynchus* is deserving of note.

While the fight against yellow fever has heretofore proved efficacious in the Americas when centered about control of the *Stegomyia* mosquito alone, increasing information on West African conditions indicates a considerable complexity of factors in that region. It may well be that certain of the potential vectors mentioned above play an important rôle in the dissemination of yellow fever there. This seems all the more likely when one considers that infected persons are probably capable of infecting insect carriers at least a day before the appearance of clinical symptoms. Hudson and Philip[8] have shown that experimentally infected rhesus monkeys may infect mosquitoes one or two days before the onset of fever. If the infective period in humans is similar to that in monkeys mosquitoes would not necessarily have to be house-frequenting species exposed to febrile cases in order to pick up the virus.

Bionomical studies have convinced me that *T. africanus* is the most likely of the potential vectors to be of epidemiological importance in West Africa. It bites humans with avidity, feeds more than once, is present in houses at all seasons in limited numbers and during certain seasons in considerable numbers. Mr. Edwards, of the British Museum, has supplied the information that this species has been captured in the Far East and even in Australia, although its congener, *T. uniformis,* is more abundant in Egypt and through the Orient, as far as the Philippines and Solomon Islands. *T. uniformis* is found occasionally in West Africa and may also be a vector of yellow fever.

It is logical to predict that as further experiments are undertaken several other species of mosquitoes will be found capable of transferring the disease, particularly *Aedes metallicus* and *A. dendrophilus,* both of which are found in moderately large numbers in tree-holes in Nigeria.

CORNELIUS B. PHILIP

[8] N. P. Hudson and C. B. Philip, *Jour. Exp. Med.,* 50: 583, 1929.