(4) Withdrawing water and concentrating the solution.

After having increased hydration in one of the four ways, we can use one of the four ways of decreasing hydration, and vice versa.

In this way, retrogradation and its different rate under different conditions can be explained.

The surface ring is always the most dehydrated part of the starch grain in agreement with the Gibbs theorem, by which the substance with the lowest surface tension is accumulated in the surface.

Crystals, in forms of needles or bars, were observed, not only when a concentrated solution of amylose was heated to 100° C., but also when an amylose solution was allowed to dry out at room temperature.

Iodine-potassium-iodide colors starch more readily and more purely blue, the more the amylose is hydrated. All shades between red and blue-black can be observed. Attention is called to the fact that potassium iodide itself has a strong hydrating power. A connection between its hydrating power and its coloring power is suggested.

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Transmission of dengue fever by mosquitoes.

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[*From the U. S. Army Medical Department Research Board, Manila. P. I.*]

This summarizes the results obtained in an extensive series of experiments relating to the transmission of dengue fever by mosquitoes. The investigations have been pursued by the U. S. Army Medical Department Research Board at the Bureau of Science and at the Sternberg General Hospital in Manila, P. I.

Part I of the report considers the plans and arrangements made and the preliminary work done in preparation for the actual experimental work. Part II concerns itself with the various sets of experiments made, the results obtained and the conclusions drawn therefrom.

PART I.

The preliminary arrangements had for their basis the scope of the work contemplated which covered the following points: confirmation of the reported transmission of dengue by the *Ædes (Stegomyia) egypti;* incrimination or elimination of the *C. quinquefasciatus* as a transmitter; and investigation of the exact mechanism of transmission by mosquitoes.

Only two species of mosquitoes were used in the transmission experiments—*A. egypti* and *C. quinquefasciatus* and the reasons for so doing are explained. The arguments presented indicate that no species other than these two could be concerned in the transmission of dengue in Manila.

All the mosquitoes used in the experiments were bred from the egg. A period of approximately four months was spent in testing various food substances that might be suitable for the propagation of larvæ and in developing and perfecting a routine breeding technique. Normal horse serum was finally selected for this purpose and formalin (1:2,500 to 1:5,000) was added to inhibit bacterial growth. In the later stages of the experimental work a considerable number of experiments were made in breeding larvæ in solution of tap water to which slices of ripe banana had been added and this type of food was found to be superior to blood serum. In work of a similar nature the use of banana as food for larvæ is recommended for both *A. egypti* and *C. quinquefasciatus.*

All reserve stocks of adult mosquitoes were fed on aqueous solutions of sugar and this type of food proved to be satisfactory in all respects.

As a rule the *A. egypti* were used experimentally for initial biting from two to seven days after emergence, and the *C. quinquefasciatus,* when first fed on blood, were not more than five days old.

The exact number of mosquitoes as well as the species used in each experiment was always known and the biting of human beings was always under their control and exclusively at the will of the Board.

After the infecting exposure to dengue patients, of freshly-bred mosquitoes, all not showing complete distension with blood were removed and killed. It was known, therefore, that all mosquitoes used subsequently to determine their infectivity for volunteers had been potentially infected.

Freshly-bred *Æges egypti* would not bite freely on the day of their emergence but after they had taken food (solution of sugar) and had been fertilized, they took blood freely—usually 100 per cent of them.

A. egypti in the Philippine Islands bites freely at any time during the day, and night-biting, though unusual, also is observed. The biting habits of *C. quinquefasciatus* were found to be erratic; they would take blood only at night, and even under the most favorable conditions but a relatively small proportion would do so.

The experimental subjects consisted of military personnel that proffered their services voluntarily. Sixty-four men were used. The volunteers were specially selected and in general met certain basic requirements—freedom from disease, including syphilis; short service in the Philippines; and non-immunity to dengue.

The experiments were made in a specially prepared ward in a large military hospital in Manila and extraordinary precautions were taken to exclude mosquitoes. The ward was administered by specially selected personnel, and one of their most important functions was the detection and destruction of mosquitoes that might possibly gain entrance to the ward or its vestibules.

PART II.

The transmission experiments presented in this report include a total of fourteen injections of virus blood, of which five were positive, one hundred and eleven biting experiments with *A. egypti* of which forty-seven were positive, and seven biting experiments with *C. quinquefasciatus,* all of which gave negative results. Sixty-four volunteers were used and dengue was produced experimentally in fifty-two instances (81 per cent).

In the conduct of the experiments, the general policy was adopted, in each series, of using successively all available methods for producing the disease—biting, followed in many instances by repetition, and this followed in turn by injections of virus blood. All negative results were adequately controlled—biting by mosquitoes known to be infective or by injections of virus blood.

Previous reports of the transmission of dengue by the *A. egypti* were confirmed—forty-seven positive results.

Experiments were made with eight volunteers to fix the incubation period of the virus in the mosquito, and it was found that the mosquito did not become infective until the eleventh day after

its infective meal. The evidence obtained indicates that even on the eleventh day after their infection *Ædes egypti* may not be capable of transmitting the virus. The limits of incubation of the virus in the mosquito apparently are from the eleventh to the fourteenth day.

Experiments were made with twenty-one volunteers to determine the stages during which dengue patients are infective to *A. egypti.* The results obtained indicate that the patient is infective to mosquitoes during the first three days of the disease but that on the third day of an attack the mosquito frequently will fail to pick up the virus. It is demonstrated, furthermore, that individuals in the late prodromal stages of dengue—six to eighteen hours prior to onset—are infective to *Aedes.*

The experimental evidence obtained warrants the statement that once the *A. egypti* becomes capable of transmitting the virus to human beings this characteristic is retained throughout the remainder of the mosquito's life. Experimental dengue was produced in three volunteers with mosquitoes that had been infected respectively sixty-two, sixty-six and seventy-five days previously.

Endeavors were made to infect seven volunteers with potentially infected *C. quinquefasciatus (C. fatigans)* and all such experiments were entirely negative. The volunteers were then bitten by *A. egypti* infected from the same sources and on the same day as the *C. quinquefasciatus* used in the previous experiments and all came down with dengue.

The conclusion is drawn that *C. quinquefasciatus* does not transmit dengue.

Three volunteers were used to ascertain the possibility of the hereditary transmission of the virus in the mosquito. The results obtained were entirely negative. When the experimental subjects were subjected to control experiments, all three developed dengue. The evidence suggests very definitely that the virus of dengue fever is not carried from the infected *A. egypti* through its eggs to the next succeeding generation.

The incubation period of the disease in the forty-seven experimental cases varied from four to ten days inclusive. For all practical purposes the incubation period may be considered as being from four to six days inclusive, as it fell within that period in 89 per cent of the experimental cases reported.

In forty-one of the forty-seven cases of dengue experimentally

produced, the virus was derived from the same strain and this strain was passed from man to mosquito and back to man through six generations. There was no evidence that the virus suffered attenuation nor that its virulence was increased as a result of continuous alternate passage through man and mosquito.

The numbers of potentially infected *Aedes* that took blood for infecting purposes in the forty-seven positive cases varied from two to thirty-six, and 50 per cent of the positive cases were bitten by from two to ten potentially infected mosquitoes.

The preliminary periods of isolation and time interval intervening between biting experiments, with one exception, was not less than eight days and did not exceed eighteen days.

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Studies on the biology of the streptococcus erysipelatis. IV. Toxin production of the streptococcus erysipelatis.

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Studies of toxin production by *Streptococcus erysipelatis,* and on neutralization of this toxin by the serum of convalescent erysipelas patients, and by erysipelas antistreptococcic rabbit and donkey sera, which were begun by me in the Medical Clinic at the Johns Hopkins Hospital in the fall of 1924, and continued in this laboratory, add further evidence to my previous reports[1] that a specific relationship exists between *Streptococcus erysipelatis* and erysipelas. The toxins employed in these studies were prepared in 48 hours' tryptic broth medium cultures of *Streptococcus erysipelatis,* incubated at 37° C. Thirty-four strains tested were found to yield uniformly toxic filtrates. The tryptic medium employed was the original Douglas'[2] tryptic medium digest, modified by Hartley,[3] Watson and Wallace.[4] From a large

[1] Birkhaug, K. E., PROC. SOC. EXP. BIOL. AND MED., 1925, xxii, 292; *Bull. Johns Hopkins Hosp.*, 1925, xxxvi, 248; *ibid.*, 1925, xxxvii, 85; *ibid.*, 1925, xxxvii, 307.

[2] Douglas, S. R., *Lancet,* 1914, ii, 891.

[3] Hartley, P., *J. Path. and Bact.*, 1922, xxv, 479.

[4] Watson, A. F., and Wallace, U., *J. Path. and Bact.*, 1923, xxvi, 447.