chapter on reaction velocity (p. 343), these equations are referred to, but become, without explanation, the products of concentrations. Nothing is said about the critical complex or the importance of activities and their coefficients at a point where they are of the greatest importance. It has been repeatedly emphasized in the American literature for the past fifteen years that if the italicized statement and the equations (p. 226–227) were correct, there could be only negative salt catalysis and never positive salt catalysis in contradiction to well established experimental evidence.

The author is to be commended for introducing the concept of osmotic coefficient. This innovation, for an elementary book, will save the student from unlearning later erroneous statements about ionic dissociations.

Unfortunately, however, after all the preparation on fugacity, activity of the electrode (p. 273) and of the ions, (p. 268), one learns (p. 274), that "hydrogen ion concentration is the most exact measure of the 'acidity' of a solution." The student is given no inkling that pH really involves the activity and not the concentration of hydrogen ion.

VICTOR K. LAMER

COLUMBIA UNIVERSITY

KINETIC THEORY OF GASES

An Introduction to the Kinetic Theory of Gases. By SIR JAMES JEANS, O.M., F.R.S. 311 pp. New York: The Macmillan Company; Cambridge University Press. 1940. $3.50.

THIS book lies somewhere between a treatise such as represented by the author's "The Dynamical Theory of Gases" and a text-book for advanced students. It is, in fact, the author's intention to supply a book which will provide such knowledge of the kinetic theory as is required by the average serious student of physics and physical chemistry, and at the same time give the mathematical student the equipment he should have before undertaking the study of specialist monographs.

The book differs from the author's larger work above cited in that the subject is covered in a more elementary manner, with less mathematical rigidity and with greater attention to the physical and descriptive aspects. The various concepts are illuminated, moreover, to an extent unusual in a book of this kind, by the inclusion of accounts of experimental investigations.

The book covers a wide field, and it is inevitable that there should be a considerable range of difficulty in the various parts. It is probable that the student who has already an acquaintance with the subject will get more benefit from the work than will a beginner; and to the semi-advanced student the book will constitute a valuable reference to which he may turn to refresh his memory when the practical need occurs for drawing upon various parts of the subject.

The work is rich in references both on the experimental and theoretical sides. It contains much useful numerical material and a helpful appendix, containing certain special standard theorems and also tables convenient for numerical calculations associated with the subject.

W. F. G. SWANN

BARTOL RESEARCH FOUNDATION
OF THE FRANKLIN INSTITUTE,
SWARTHMORE, PA.

SPECIAL ARTICLES

ISOLATION OF THE VIRUSES OF WESTERN EQUINE AND ST. LOUIS ENCEPHALITIS FROM *CULEX TARSALIS* MOSQUITOES[1]

IN North America three types of epidemic virus encephalitis are recognized. Two of these, the eastern and western types of equine encephalomyelitis, are believed to be mosquito-borne. Mosquito transmission has been repeatedly demonstrated in the laboratory (summarized by Davis[2]), but until now the virus has never been isolated from mosquitoes collected in epidemic areas. With respect to the St. Louis encephalitis virus opinions of observers have differed as to the mode of transmission. Lumsden[3] concluded that it was probably transmitted by Culex mosquitoes. Mitamura and associates[4] have reported successful transmission of this virus in the laboratory by *Culex pipiens*.

In the Yakima Valley, Washington, evidence was obtained by Hammon[5] and Hammon and Howitt[6] during the summer of 1940 indicating the probable presence in man and horses of both the western equine

[1] Part of a Cooperative Survey of Encephalitis in the Yakima Valley by the University of California, the State College of Washington, the Washington State Health Department, the Yakima City-County Health Department and the U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine. Aided by a grant from the Natural Foundation for Infantile Paralysis, Inc.

[2] W. A. Davis, *Amer. Jour. Hygiene,* 32: 45, 1940.

[3] L. L. Lumsden, Unpublished official report, 1933.

[4] T. Mitamura, S. Yamada, H. Hazato, K. Mori, T. Hosoi, M. Kitaoka, S. Watanabe, K. Okubo and S. Tenjin, *Tr. Jap. Path. Soc.,* 27: 573, 1937.

[5] W. McD. Hammon, *Jour. Am. Med. Assn.,* 117: 161, 1941.

[6] W. McD. Hammon and B. F. Howitt, To be published.

virus and the St. Louis virus. These workers outlined evidence which suggested a Culex mosquito as the likely vector for the latter. In May of 1941 an extensive, coordinated field and laboratory survey of this region was begun. This preliminary report deals with the isolation of both the St. Louis and the western equine viruses from *Culex tarsalis* taken in routine entomological collections where human encephalitis cases occurred during this or the previous year. These collections were planned with the purpose of finding, if possible, either or both of the encephalitic viruses believed to be present in the region. The same survey, by serum neutralization tests of birds and mammals, indicated probable wide-spread infection by both (Hammon, Gray, Evans, Izumi and Lundy[7]).

Live arthropod specimens were collected by means of specially constructed light traps, sweepings and hand collections. These arthropods, after identification in the field laboratory under light chloroform anesthesia, were sealed in hard glass shell vials by drawing in a gas-oxygen flame. They were then frozen, stored and shipped in CO_2 ice to the San Francisco Laboratory. Here, in lots of a single species containing from 5 to 150 specimens, the arthropods were washed in saline, then ground in a mortar with alundum in 3.0 ml of 30 per cent. sheep serum-saline. The supernatant, after 10 minutes centrifugation at 16,000 r.p.m. in an International Centrifuge Multispeed Head, was cultured and 5 Swiss mice, Rockefeller strain, inoculated with .03 ml intracerebrally. All mice were observed for 21 days. Several lots of *Aedes campestris* were infected with the western equine virus in the field laboratory and served as controls for the method of shipping and handling for virus isolation.

From the arthropod material collected this season 9,503 specimens have been tested for the presence of virus. This includes 7,619 mosquitoes,[8] 1,458 specimens of other flies[9] and 426 miscellaneous biting arthropods. Of these arthropods, *C. tarsalis*, of which 3,293 specimens have been tested, is the only one to date from which a virus has been isolated. From a pool of 66 mosquitoes (Pool No. 103 collected July 9th) the St. Louis virus was isolated, and from a pool of 125 (Pool No. 116 collected July 15th) the western equine virus was recovered.*

[7] W. McD. Hammon, J. Gray, Jr., F. C. Evans, E. M. Izumi and H. W. Lundy, *Science*, 94: 2439, 1941.

[8] *Anopheles maculipennis freeborni, Theobaldia inornata, Theobaldia incidens, Aedes vexans, Aedes dorsalis, Aedes campestris, Aedes nigromaculis, Aedes increpitus, Aedes cinereus, Aedes lateralis, Culex tarsalis, Culex pipiens.*

[9] Simuliidae (black flies), Tabanidae (horse flies), Muscidae (house flies, horn flies and stable flies).

* Since this paper was written 2 other viruses have been isolated from lots of *C. tarsalis*. These have not yet been identified.

Isolation of St. Louis Virus

Among five mice inoculated with Pool No. 103 two were found in convulsions 8 days after inoculation. These were sacrificed and their brains were found to be bacteriologically sterile. Each brain was passed separately to 3 other mice by intracerebral inoculation of a 10 per cent. suspension. These came down with encephalitic symptoms or were found dead between the 4th and the 6th day. After the virus was well established in mice, steps were taken to identify it. It was found to pass through both N and V Berkefeld filters. It produced no symptoms after intracerebral inoculation in two guinea pigs, a rabbit, a monkey (*Macacus rhesus*) and a lamb. A fatal infection was produced in mice by intranasal inoculation and by intraperitoneal inoculation of over 100,000 fatal intracerebral doses. No symptoms were produced when a similar dose was given subcutaneously. These tests were sufficient to tentatively identify the virus as that of St. Louis encephalitis.

A neutralization test was set up against normal rabbit serum, hyperimmune western equine guinea pig serum and hyperimmune St. Louis rabbit serum. At the same time the St. Louis serum was titrated against a known strain of St. Louis virus. Both the "Culex 103 virus" and the St. Louis virus were neutralized to the same degree by the hyperimmune St. Louis serum. The western equine serum afforded no protection. It appears from these findings that the virus isolated from this pool of *C. tarsalis* is that of St. Louis encephalitis.

Isolation of Western Equine Virus

Among mice inoculated with Pool No. 116 one was observed in rolling convulsions 6 days after inoculation. It was sacrificed, and after the brain was found to be bacteriologically sterile it was passed by intracerebral inoculation to four other mice. On the fourth day the first of these showed suspicious signs of illness and brain passage was made to 2 guinea pigs and 4 mice. The guinea pigs developed high temperatures on the third day. One died 24 hours later and the other was sacrificed on the fifth day. Neither guinea pig was paralyzed or noted to have convulsions. Subsequent passages in guinea pigs resulted regularly in paralyses of the hind quarters and abdominal muscles, and running convulsions typical of equine encephalomyelitis. The pathogenic agent was found to pass through both Berkefeld V and N filters. Although adaptation to guinea pigs was easily accomplished, the virus was not readily adapted to mice.

Experiments were done to determine if the virus were that of western equine encephalomyelitis. In one instance a normal guinea pig and one previously immunized to the western equine virus were inoculated

intracerebrally with a 10 per cent. suspension of the brain of a guinea pig infected with the "Culex 116 virus." The normal guinea pig succumbed in the course of a typical encephalitic infection, but the immune animal remained normal. In the next experiment two normals and two hyperimmune controls were used and similar results obtained. It appears therefore that the virus obtained from this pool of *C. tarsalis* is that of western equine encephalomyelitis.

Experiments are now in progress to test the ability of *C. tarsalis* to serve as a host to, and to transmit these viruses. Already *C. tarsalis* has been fed on a guinea pig infected with the western equine virus and the virus readily demonstrated after 5 days incubation. Until actual transmission of one or both of these viruses has been demonstrated the role of this mosquito as a vector is not proven. These findings have, however, increased the evidence incriminating mosquitoes as vectors of these encephalitic virus diseases.

ADDENDA

Culex tarsalis is a North American species distributed throughout the states west of the Mississippi River. In the Yakima Valley it is the most common mosquito, and its larvae are found in many types of water: permanent ponds, irrigation seepage, barnyard drainage and sewage. Adults were taken in all areas where light traps were run, and were collected in large numbers in shelters such as barns and houses. In all areas of the Valley where encephalitis occurred in man or horses it was collected in significant numbers. It is more abundant than *Culex pipiens,* the other common Culecine of this area. In temperate regions adult females are reported to hibernate in sheltered places, emerging in the spring to commence egg laying (Hearle.)[10]

The feeding habits of *C. tarsalis* have not been extensively studied. Direct observations made in the Yakima Valley indicate that it feeds on man, horses, mules, cows and mallard ducks. Other workers have indicated that it feeds on avian blood (Freeborn)[11] and on man (Hearle)[10].

WILLIAM MCD. HAMMON
WILLIAM C. REEVES
BERNARD BROOKMAN
ERNEST M. IZUMI
GEORGE WILLIAMS HOOPER FOUNDATION,
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
C. M. GJULLIN
U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE,
PORTLAND, OREGON

[10] E. Hearle, *Nat. Res. Council Rept.* 17, Ottawa, Canada, 1926.

[11] S. B. Freeborn, *Univ. of Calif. Publ. Tech. Bull. Entom.*, Bull. III, 5: 333, 1926.

DIAGNOSIS OF EPIDEMIC ENCEPHALITIS BY COMPLEMENT-FIXATION TEST

DURING the months of August and September, 1941, encephalitis in man occurred in epidemic form in Manitoba, Canada, and central United States. Blood sera from patients were dispatched to the Rockefeller Institute for diagnosis.[1]

Complement-fixation tests were carried out on each serum against the antigens of Western equine encephalomyelitis, Eastern equine encephalomyelitis, lymphocytic choriomeningitis and St. Louis encephalitis. The sera were inactivated at 60° or 65° C. and tested both undiluted and in twofold dilutions through 1:16 according to the method we have previously described.[2]

Of thirty-six sera from Manitoba, twenty-two gave a strong and completely specific reaction with the Western equine encephalomyelitis antigen; of eight sera from Colorado, two exhibited a similarly strong specific reaction. The titres of the positive sera, as determined by the highest dilution of serum giving a 2+ or better reaction, were 1:4 in four cases, 1:8 in three cases and 1:16 or 1:16+ in seventeen cases.

Of nine sera drawn from patients within 10 days or less after onset of illness, none was positive; whereas of thirty-five sera drawn from patients 11 to 30 days after onset, twenty-four or 69 per cent. proved positive.

Two samples of serum were obtained from each of two cases in Colorado, and they are of special interest. The first samples from each patient, taken a few days after onset of illness, were negative, whereas the second samples, taken during the second week of illness, gave a strongly specific reaction with Western equine encephalomyelitis antigen.[3]

The above tests indicate that the present epidemic of encephalitis in Manitoba and central United States is caused by the Western equine encephalomyelitis virus and demonstrate the value of the complement-fixation test as a practical and speedy diagnostic tool.

J. CASALS
R. PALACIOS
ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
NEW YORK, N. Y.

[1] Sera and brain tissue specimens were kindly sent us by Dr. Daniel Nicholson, Winnipeg, Canada; Captain C. E. G. Gould, Camp Shilo, Manitoba; Lieutenant S. Young, Brandon, Manitoba, and Dr. J. E. Smadel, New York.

[2] J. Casals and R. Palacios, SCIENCE, 93: 162, 1941; *Jour. Exp. Med.* (in press), October, 1941.

[3] From the brain tissue of one fatal case of encephalitis in Winnipeg a virus was obtained by inoculation into W-Swiss mice. An antigen was prepared from this virus-containing brain tissue, which fixed complement specifically with known Western equine encephalomyelitis immune serum. Neutralization and protection tests confirmed this result.