SPECIAL ARTICLES

MOSQUITO TRANSMISSION OF EQUINE ENCEPHALOMYELITIS

DURING the summer of 1933 the cases of equine encephalomyelitis in New Jersey, Delaware and Virginia were found only in areas close to salt marshes. If the disease is insect-borne, as the epidemiology and Kelser's[1] experiments with *Aëdes aegypti* indicate, the salt marsh mosquitoes must be considered as possible vectors. There are four species of mosquitoes in New Jersey that breed exclusively in salt marshes, *Aëdes sollicitans, Aëdes cantator, Aëdes taeniorhynchus* and *Culex salinarius*. Mosquito trap collection records[2] for New Jersey and Delaware show that, in the areas where the disease occurs and during the season of its greatest incidence, up to 90 per cent. of the specimens obtained are *Aëdes sollicitans*. *Aëdes cantator* occurs in considerable numbers early in the season but is relatively unimportant later. The other two species make up a small percentage of the total mosquito population throughout the season. Two other species of mosquitoes, *Anopheles quadrimaculatus* and *Anopheles crucians,* although breeding on the salt marshes, have a much wider distribution and, therefore, like the fresh water species, are unlikely vectors of a disease which is confined to salt marsh areas. *Aëdes aegypti,* as Kelser points out, can not be the transmitting agent of either the western or eastern disease, as it is rarely found as far north as the region in which the disease occurs.

In repeated tests we have demonstrated that *Aëdes sollicitans* will transmit both eastern and western strains of equine encephalomyelitis. For these experiments we have secured large numbers of these mosquitoes in the larval and pupal stages from salt marshes and have allowed them to emerge as adults in the laboratory. Insects receiving as an infective meal a mixture of guinea pig brain virus suspension and normal horse blood have not transmitted the disease to guinea pigs when allowed to feed on them 2, 3 or 4 days after the infective meal. Transmission has been consistently obtained on the seventh day and thereafter. *Aëdes sollicitans* has transmitted the eastern disease from infected to normal guinea pigs 11 days after the initial feeding and at later periods. When these mosquitoes fed on an infected horse the first transmission occurred after 20 days, there having been no transmission at 14 days. Twelve other mosquitoes of this lot, which were allowed to feed upon a normal horse at the 30-day period, failed to infect it. Seven of these same mosquitoes transmitted the disease to a guinea pig 3 days later. Although this one attempt to obtain horse to horse transmission was negative, we believe that the evidence is sufficient to establish *Aëdes sollicitans* as a probable vector of equine encephalomyelitis in the eastern states.

Aëdes sollicitans has also transmitted the western virus after engorging on brain virus suspension. Since, however, it is a salt marsh mosquito it can not be the vector of the disease in the west, and we suggest that a different vector or means of transmission may explain the serological difference which we[3] have shown exists between the viruses from the two regions.

Of the remaining salt marsh mosquitoes the few tests made with *Aëdes cantator* indicate that it will transmit eastern virus but less readily than *Aëdes sollicitans*. *Aëdes taeniorhynchus* is now being tested, while *Culex salinarius* has not been secured in sufficient numbers for test. The more widely distributed mosquitoes, *Culex pipiens* and *Anopheles quadrimaculatus,* have uniformly failed to transmit either the eastern or western strains of virus.

In parallel experiments in which the mosquitoes have been allowed to feed on brain virus suspension we have consistently obtained transmission of western but not of eastern virus by *Aëdes aegypti*. In occasional instances, however, this mosquito has transmitted eastern virus from infected to normal guinea pigs, whereas in parallel experiments with western virus transmission has been uniformly obtained.

Our experiments show that when either *Aëdes aegypti* or *Aëdes sollicitans* are fed on guinea pigs with a low virus content in their blood, the virus is soon lost and the mosquitoes do not transmit the disease. In order to act as vectors mosquitoes must be fed on infected animals at a time when the virus content of the blood is such that 0.0001 cc or less will produce the disease when it is injected into a guinea pig. Such a blood titer appears in general to be reached at the height of the first febrile reaction and before any central nervous system symptoms become manifest. It is not clear why this high virus content of the infective meal is necessary, since titration experiments with ground suspensions of mosquitoes indicate that there is an increase of virus in the mosquito. In both *Aëdes aegypti* infected with western virus and *Aëdes sollicitans* infected with the eastern virus a 1,000 to 10,000 fold increase of the virus within the mosquitoes has been demonstrated.

Whenever possible, at least twenty mosquitoes have been used in all transmission tests. In several instances, however, from four to seven *Aëdes sollicitans*

[1] R. A. Kelser, *Jour. Am. Vet. Med. Assn.*, 82 (n.s. 35): 767, 1933.

[2] T. J. Headlee, *Proc. 20th Ann. Meet. N. J. Mosquito Extermination Assn.*: 33, 1933; L. A. Stearns, D. MacCreary and N. P. Newhouse, *Del. Agric. Exp. Sta. Bul. 181*, 1933.

[3] C. Ten Broeck and M. H. Merrill, *Proc. Soc. Exp. Biol. and Med.*, 31: 217, 1933.

have transmitted the eastern disease to guinea pigs, and in one instance the bite of one *Aëdes aegypti* transmitted the western virus to a guinea pig. Three other insects of the same lot, each fed upon a different animal, failed to infect. The virus appears to persist in at least some of the mosquitoes as long as they live. Eastern virus has been transmitted by *Aëdes sollicitans* 33 days after the infective meal, the longest period we have been able to keep a sufficient number alive for test. The longest period we have found *Aëdes aegypti* capable of transmitting the western disease is 63 days. Virus was shown to be present in this same lot of mosquitoes 93 days after feeding, but they did not transmit the disease at this time.

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MECHANISMS IN THE DEVELOPMENT OF AN ACTIVE RESISTANCE TO THE EFFECTS OF SUBSTANCES STIMULATING THE THYROID GLAND IN THE GUINEA PIG

IF by means of extirpation of considerable portions of the thyroid, growth processes leading to compensatory hypertrophy are initiated in the guinea pig, simultaneous administration of KI intensifies these proliferative changes.[1] Similarly, if KI is either given orally to or injected intraperitoneally in certain quantities into guinea pigs or rats with normal thyroids, a marked increase in mitotic proliferation as well as a slight increase in the size of the acinus cells of the thyroid and a moderate softening of the colloid, accompanied by the invasion of this latter substance by phagocytes, can be observed.[2] These changes reach a maximum within a certain time, which varies according to the mode of administration of the iodine salt and differs in guinea pig and rat. When injections are continued following this period of maximum effect, a decrease in the mitotic activity occurs; the gland returns to the normal or perhaps to a subnormal level of activity; at the same time the acini may become distended with colloid.

Corresponding observations can be made, if instead of iodine salts we inject optimal quantities of extracts of anterior pituitary glands of cattle into guinea pigs; however, in this case the effects are much greater. There is an extraordinary increase in mitotic proliferation of the thyroid; the acinus cells increase very much in size and the colloid is largely liquefied and absorbed; thus the whole gland changes its structure and becomes similar to the thyroids seen in very pronounced cases of Graves' disease.[3] Furthermore, it is possible by these means to imitate the principal functional symptoms of this disease. But in this case also, after a stage of the maximum effects has been reached, a return of the thyroid gland to its normal state may take place gradually, notwithstanding the continued injections of the extract;[4] in a parallel way a decrease in the functional and metabolic hyperactivity of the gland sets in.[5] Similar observations were recently recorded by Collip and Anderson in the rat[6] and by Hertz and Kranes in the rabbit.[7]

As to the mechanism underlying this process of retrogression, we tested about three years ago the ability of the blood serum of guinea pigs, which had become resistant to the effects of anterior pituitary extracts, to neutralize these extracts *in vitro;* we made mixtures of such blood serums and extract and injected them into fresh guinea pigs. In control experiments we injected mixtures of normal guinea pig serum with anterior pituitary extracts. The results of these experiments were negative (see footnote 3-c). However, in their recent experiments, Collip and Anderson succeeded in demonstrating that the serum of rats which had become refractory to extracts was able not only to prevent the rise in metabolism otherwise caused by the injection of the thyroid stimulating hormone of anterior pituitary, if serum and extract were mixed *in vitro* previous to injection, but even to lower the basal metabolic rate of the injected animal.[8] We may therefore conclude that the development of substances neutralizing the thyroid stimulating hormone of the anterior pituitary gland and circulating in the blood of the injected animals is one of the mechanisms underlying the acquired resistance to the effects of the extract.

But we believe that there are reasons for assuming

[1] Leo Loeb, *Jour. Med. Research,* 40: 199, 1919, 41: 481, 1920; *Am. Jour. Path.,* 2: 19, 1926; 5: 71 and 79, 1929. S. H. Gray, *Am. Jour. Path.,* 5: 415, 1929. Elizabeth Moore, *Archives of Path.,* 16: 657, 1933.

[2] (a) S. H. Gray and Leo Loeb, *Am. Jour. Path.,* 4: 257; (b) I. Rabinovitch, *Am. Jour. Path.,* 4: 601, 1928; 5: 91, 1929; (c) *Proc. Soc. Exp. Biol. and Med.,* 28: 394, 1931.

[3] (a) Leo Loeb and R. B. Bassett, *Proc. Soc. Exp. Biol. and Med.,* 26: 860, 1929; (b) 27: 490, 1930; (c) Leo Loeb, *Klin. Wochensch.,* No. 51 and 52/53, pp. 2121 and 2156, 1932.

[4] Leo Loeb and Hilda Friedman, *Proc. Soc. Exp. Biol. and Med.,* 29: 172, 1931; Leo Loeb, *Klin. Wochensch.,* Nos. 51 and 52/53, pp. 2121 and 2156, 1932.

[5] (a) W. J. Siebert and R. S. Smith, *Proc. Soc. Exp. Biol. and Med.,* 27: 622, 1930; *Am. Jour. Physiol.,* 93: 396, 1930; (b) W. J. Siebert and E. W. Thurston, *Proc. Soc. Exp. Biol. and Med.,* 29: 652, 1932.

[6] M. B. Collip and E. M. Anderson, *Lancet,* 226: 76, 1934.

[7] S. Hertz and A. Kranes, *Endocrinology,* 18: 415, 1934.

[8] M. B. Collip and E. M. Anderson, *loc. cit.*