toyltaurine, pyridine-3-sulfonic acid and pyrithiamine will inhibit the growth and acid production of the *Lactobacillus acidophilus* (Hadley) when present in sufficient concentrations in a medium containing all the known growth essentials of this organism. These metabolic analogues of pantothenic acid, nicotinic acid and thiamine have an affinity for enzymes or for the protein component of enzymes, substrates or the prosthetic groups which they resemble structurally.[10] They tend to replace their corresponding essential nutrients in the metabolism of the *Lactobacillus acidophilus* (Hadley) and thereby interfere with its ability to carry on its normal physiological functions. The findings also confirm the previously reported observations that pantothenic acid, thiamine and nicotinic acid are essential for the maximum growth and acid production of the oral *Lactobacillus acidophilus*.

[10] Welch, Arnold D., and Bueding, E., in Green, D. E., *Currents in Biochemical Research*, Interscience Publishers, New York, 1946.

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## Virus of Eastern Equine Encephalomyelitis Isolated from Chicken Mites (*Dermanyssus gallinae*) and Chicken Lice (*Eomenacanthus stramineus*).*

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Within recent years several neurotropic viruses have been recovered from the chicken mite, *Dermanyssus gallinae* (De Geer), and it has been shown not only to harbor the viruses in nature but to maintain at least one of them by transovarian passage. Smith, Blattner and Heys[1] isolated the virus of St. Louis encephalitis from chicken mites taken near St. Louis during a non-epidemic period, and were able to recover this virus a second time[2] from mites taken in the same chicken house and from nearby areas. They demonstrated[3] the transovarian passage of the St. Louis virus in experimentally infected mites and found[4] that normal chickens could be infected when bitten by naturally infected *Dermanyssus* and that the virus could then be transmitted to uninfected mites after feeding upon these same chickens. Therefore, the cycle for the St. Louis virus of mite-bird-mite seems to be established. Sulkin[5] recovered the virus of western equine encephalomyelitis from *Dermanyssus gallinae* obtained on a farm in Dallas County, Texas, where cases of equine encephalomyelitis had occurred. Reeves, Hammon and co-workers[6] isolated the virus of western equine encephalomyelitis from wild bird mites, *Liponyssus sylviarum*, removed from the nest of a yellow-headed blackbird, *Xanthocephalus xanthocephalus* (Bonaparte), found in Kern County, California. More recently[7] they have reported on the isolation of another neurotropic virus from mites in this same nest. This strain seems to combine the characteristics of both the western equine and the St. Louis type of encephalitic viruses. Sulkin

[1] Smith, M. G., Blattner, R. J., and Heys, F. M., *Science*, 1944, **100**, 362.

[2] Smith, M. G., Blattner, R. J., and Heys, F. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 136.

[3] Smith, M. G., Blattner, R. J., and Heys, F. M., *J. Exp. Med.*, 1946, **84**, 1.

[4] Smith, M. G., Blattner, R. J., and Heys, F. M., *J. Exp. Med.*, 1947, **86**, 229.

[5] Sulkin, S. E., *Science*, 1945, **101**, 381.

[6] Reeves, W. C., Hammon, W. McD., Furman, D. P., McClure, H. E., and Brookman, B., *Science*, 1947, **105**, 411.

[7] Hammon, W. McD., Reeves, W. C., Cunha, R., Espana, C., and Sather, G., *Science*, 1948, **107**, 77.

and Izumi[8] recovered the western equine virus from the tropical bird mite, *Liponyssus bursa* (Berlese), taken from a nest containing fledgeling English sparrows, *Passer domestica* (Linn.).

It has been well established, therefore, that bird mites, whether from domestic or wild birds, are capable of harboring in nature at least 3 different strains of encephalitic viruses. It has been determined also that the St. Louis virus may be passed through the egg to the next generation and that it can be transmitted from mite to bird and back again to mite. The present report is to show that yet another strain of virus, that of eastern equine encephalomyelitis, may be found in the chicken mite, *Dermanyssus gallinae*. So far, no one has reported on the recovery of this eastern strain from any arthropod in nature, although several experimental studies have shown that it may be transmitted by mosquitoes in the laboratory. Merrill, Lacaillaide and TenBroeck[9,10] were the first to demonstrate transmission of the eastern equine virus by mosquitoes of the genus *Aedes* but not by *Culex pipiens* or *Anopheles quadrimaculatus*. Davis[11] likewise demonstrated that the eastern virus could be passed by different species of *Aedes*. No one, however, has worked with the mites.

*Isolation of Eastern Equine Encephalomyelitis Virus from Chicken Mites,* Dermanyssus gallinae (*De Geer*). During the summer of 1947 sporadic cases of encephalitis in children had been reported from several areas of central Tennessee. Many of the cases were in rural or semi-rural districts and were often associated with the presence of domestic fowl. On August 23, 1947, approximately 300 chicken mites, *Dermanyssus gallinae,* were removed from the roosts of a chicken house near Shelbyville, Tenn. They were placed in an ampule, frozen in dry ice and returned to the laboratory in Montgomery, Ala., where they were stored in the dry ice refrigerator. On September 16, 1947, the mites were removed from the ampule and triturated in a mortar with 3 ml of buffered saline containing 30% normal rabbit serum. The suspension was then centrifugated for one-half hour at 13,000 rpm in an angle centrifuge placed in the cold room. Twelve-day-old Swiss mice were inoculated with 0.03 ml intracerebrally and 0.1 ml intraperitoneally of the supernatant fluid. Two mice died in three days, one on the fourth and the remaining 5 were sacrificed after 4 days incubation. The brain suspension from one of the dead animals was transferred intracerebrally to other young mice. No bacteria were found in cultures from the brain. Serial passages were made in mice and the animals regularly died or showed convulsions in 2 to 3 days. After 6 transfers in mice the virus gave a titer of $10^{-8.3}$, and was found to pass Mandler and Seitz filters, to be lethal for embryonated eggs in 24 hours and for guinea pigs in 48 hours. The latter showed symptoms of encephalomyelitis.

Neutralization tests against known immune sera were performed for identification of the virus. The technic used was that recommended in the manual of the "Laboratory Methods of the U. S. Army."[12] The new strain was neutralized by the antiserum of the virus of eastern equine encephalomyelitis but not by that of the western equine or of St. Louis encephalitis. Tissue immunity tests were also made by intracerebral inoculation of immune guinea pigs with 0.2 ml of 1,000,000 lethal mouse doses of Lot No. 33 virus. All of the normal control animals and those immune to the western virus died, while guinea pigs immune to the eastern equine strain remained alive. It was thus established that the new active agent was antigenically identical with that of the virus of eastern equine encephalomyelitis. This is the first time that this strain has been recovered from chicken mites taken in nature and also the

8 Sulkin, S. E., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 249.

9 Merrill, M. H., Lacaillaide, G. W., Jr., and TenBroeck, C., *Science*, 1934, **80**, 251.

10 Merrill, M. H., and TenBroeck, C., *J. Exp. Med.*, 1935, **6**, 687.

11 Davis, W. A., *Am. J. Hyg.*, 1940, No. 2, Sec. C, 45.

12 Simmons, J. S., and Gentzkow, C. J., *Laboratory Methods of the U. S. Army,* Lea and Febiger, Fifth Ed., 1944.

TABLE I.
Neutralization Tests Against the E.E.E.* (Lot No. 33) Virus.

| Location in Tennessee | Date bled | Sera of animals: Dog No. | Dog Pos. | Cows No. | Cows Pos. | Horses No. | Horses Pos. | Chickens No. | Chickens Pos. |
|---|---|---|---|---|---|---|---|---|---|
| Shelbyville | 8-23-47 | — | — | 4 | 0 | 2 | 0 | 20 | 1 pos.<br>1 wkly. pos. |
| Alexandria | 8-29-47 | 1 | 0 | 3 | 1 | 2 | 0 | 11 | 1 pos.<br>2 wkly. pos. |

* Eastern equine encephalomyelitis.

first time that it has been repored in Tennessee. It had been isolated previously from horses in some of the surrounding states: Alabama,[13] Missouri,[13] and Michigan.[14]

*Isolation of Eastern Equine Encephalomyelitis Virus from Chicken Lice,* Eomenacanthus stramineus (*Nitzsch*). A few days after the collection of the mites, 23 lice, *Eomanacanthus stramineus* (Nitzsch) (= *Menopon biseriatum* or *M. stramineum*) were removed by means of an aspirator from chickens on a farm near Alexandria, Tenn. These lice were sealed in an ampule, frozen and sent to the laboratory, where they were stored in the dry ice refrigerator. On September 16, 1947, they were removed and treated in the same manner as described for the mites. Eight young mice were inoculated both intracerebrally and intraperitoneally with the supernatant fluid after centrifugation at 13,000 rpm for one-half hour. Five of these died in from 3 to 5 days and 3 were killed on the fifth day. The mouse brains that showed no bacterial growth in broth were passed to other mice, which in turn either died or became ill in 2 days. Six serial passages were made in young mice and then identification of the virus was determined by means of the neutralization test. It also was found to be the virus of eastern equine encephalomyelitis.

*Neutralization Tests on Animal Sera.* Neutralization tests against the mite strain (Lot No. 33) were performed on sera obtained from animals on the farms at both Shelbyville and Alexandria. The results are given in Table I. The tests were considered positive if the neutralization index of a serum was 50 or above and weakly positive if it lay between 11 and 49.[12] Antibodies to this virus were found in the sera of 2 out of 20 chickens bled at Shelbyville, although one serum was only weakly positive. Likewise positive tests were obtained on the serum of one cow and 3 out of 11 chickens from the other farm where the lice had been removed from the fowl. Two of the latter sera were only weakly positive.

*Discussion.* The recovery of the eastern equine strain from chicken lice is not what one would anticipate from their habits. Like all other members of the order Mallophaga, the mouthparts are adapted not for sucking blood but for chewing upon the surface of the skin and feathers. However, Wilson[15] observed a specimen of *E. stramineus* with its mandibles imbedded in a young feather from which the dermal papillae, bearing blood vessels, had not yet withdrawn, and thus drawing and ingesting blood from the wound. Further examination suggested that this type of feeding was habitual. Other means by which lice may become infected with virus are by feeding on blood at the site of traumatic wounds, abrasions, or feeding punctures caused by other arthropods. Therefore, it is possible that these and other species of biting lice may be important vectors of the virus among birds, especially in the absence of other vectors.

The presence of antibodies for the eastern equine strain in a few chicken sera is supportive evidence for finding the virus in the

[13] Shahan, M. S., and Giltner, L. T., *J. Am. Vet. Assn.*, 1945, **107**, 279.

[14] Brown, G. C., *J. Inf. Dis.*, 1947, **81**, 48.

[15] Wilson, F. H., *Science*, 1933, **77**, 490.

chicken lice, even though they may not be the true vectors. The positive neutralization tests with the cow serum would indicate, however, that some other arthropod, such as the mosquito, is also a vector in this area.

Likewise while the eastern equine virus was recovered from chicken mites in nature, as yet no experimental proof has been given that the virus lives, multiplies and is hereditarily transmitted in these hosts. Further work is being planned in this regard for both species of arthropods.

*Summary.* The virus of eastern equine encephalomyelitis has been recovered from chicken mites, *Dermanyssus gallinae* (De Geer), and from a mixture of chicken lice, *Menopon pallidum* Nitzsch and *Eomenacanthus stramineus* (Nitzsch), taken in nature in Tennessee. This is the first isolation of the eastern virus from chicken mites and chicken lice and the first time the virus has been found in this state. Neutralizing antibodies for this virus were present in the sera of a few chickens and one cow. Further confirmation is needed to prove the role of mites and especially of lice as true vectors of this virus.

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## Development of Tuberculous Infection. *In vivo* Observations in the Rabbit Ear Chamber.*

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The rabbit each chamber, first described by Sandison[1] and later modified by Ebert, Florey, and Pullinger,[2] provides a technic for studying the dynamics of pathological changes in living tissue. In this paper results of studies on tuberculosis by this method are reported.

A thin layer of living vascularized tissue 40 to 50 $\mu$ thick can be inoculated with tubercle bacilli directly and examined by frequent daily observations in the unanesthetized animal over a period of weeks. Serial microscopic observations using the highest magnifications are possible, and a graphic record of the findings can be made with Kodachrome motion pictures. In this way it was possible to record the early development of tuberculous infection. The chamber method is particularly well suited for the study of changes in blood vessels, and striking observations have been made on the vascular reactions in the growing tuberculous lesion.

*Materials. The Chamber*: Originally observations were made using the modification developed by Ebert, Florey, and Pullinger,[2] but subsequently a simpler chamber was devised which was smaller and lighter, eliminating the necessity of the cumbersome ear splints and shields used to protect the larger chambers. It consists of a transparent lucite base with a mica coverslip supported by an aluminum ring connected with 3 small bolts (Fig. A).

*The base* is a highly polished round lucite plate 1 inch in diameter, 1/16 inch thick with a raised central table 1/4 inch in

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1 Sandison, J. C., *Am. J. Anat.*, 1928, **41**, 447.

2 Ebert, R. H., Florey, H. W., and Pullinger, B. D., *J. Path. and Bact.*, 1939, **48**, 79.