

amount of ascorbic acid excreted during the exercise is roughly the same.

Thiamin is normally excreted in appreciable amounts in the sweat. Since a man may readily sweat (moderate temperature and work) 2 to 3 kg daily without visible perspiration, he may thus lose in the sweat from 5 to 15% of ingested thiamin. In a like manner, a person undergoing hard work at elevated temperatures may lose enough thiamin to cause serious physiological consequences. Our work indicates that the increased excretion of thiamin in sweating is not compensated for by a diminution of the urinary thiamin.

The relationships of the above inference to the problem of work and fatigue are obvious. Clinical studies concerning certain cases of apparently abnormal vitamin requirements are also indicated.

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Experimental Transmission of Saint Louis Encephalitis to White Swiss Mice by *Dermacentor variabilis*.

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In studies of Russian encephalitis various investigators¹⁻⁶ have reported observations which indicate that *Ixodes* ticks play an important part in the epidemiology of the disease. Laboratory experiments conducted by some^{4, 6} of these workers demonstrated that members of the genus, *Ixodes*, are capable of transmitting the virus to susceptible animals during larval, nymphal, and adult stages of the life cycle. There was considerable evidence also that the infected

¹ Editorial, *J. A. M. A.*, 1941, **117**, 1361.

² Smorodintseff, A. A., *Arch. f. Ges. Virusforsch.*, 1940, **1**, 468, quoted by 1.

³ Rijov, N. V., and Skrynnik, A. N., *Report of the All-Union Congress of Microbiology*, 1939 (Jan. 25-31), quoted by 1.

⁴ Pavlovsky, E. N., *Report of the All-Union Congress of Microbiology*, 1939 (Jan. 25-31), quoted by 1.

⁵ Chumakov, M. P., and Seitenok, N. A., *Science*, 1940, **92**, 263.

⁶ Shubladse, A. K., and Serdinkova, G. V., *Arch. Soc. Biol.*, 1939, **56**, No. 2.

female tick transmits the virus to her offspring by inclusion in the egg. Ticks collected in nature in endemic areas were found to harbor the virus of Russian encephalitis. More recently Syverton and Berry⁷ reported the transmission of equine encephalomyelitis to ground squirrels by the tick, *Dermacentor andersoni*.

These observations raised the question of the possible rôle of the tick in some phase of the epidemiology of Saint Louis encephalitis. Ticks, collected from the environs of Saint Louis and identified as *Dermacentor variabilis*, were maintained as a stock colony. Females of the colony, fed by engorging on normal dogs, lay eggs within 4 days after engorgement, and larvae emerge 15 days later.

In the present experiments adult white Swiss mice were inoculated with St. Louis encephalitis virus, each receiving 0.03 cc 10^{-1} intracerebrally and 0.1 cc 10^{-1} intraperitoneally. Thirty to 40 normal larval ticks hatched in the laboratory were allowed to come into contact with each of 4 such inoculated mice in separate glass jars. Attachment occurred immediately, favorite sites being the ears and snout. Inoculated mice were unsuccessful in dislodging the larval ticks once they had attached. After an interval of 2 days one or 2 normal Swiss mice, 2 weeks of age, were placed in each glass jar with an infected mouse and larvae. The young mice nestled against the infected adults (males), but made no apparent effort to remove the larvae. The attached larval ticks became engorged with blood, increasing in size at about the expected rate. Within 4 days the inoculated adult mice died in typical encephalitic convulsions. The larval ticks promptly left the dying adults, and a number attached themselves to the young mice. Ten days after the death of the inoculated adult mice, one of the 6 young mice in this series was found dead; another was found in convulsions. Twenty-four hours later 2 more young mice developed convulsions. In each instance the virus was recovered from the brain by passage to normal Swiss adults and to fertile eggs, and was identified as the Saint Louis type by mouse and egg protection tests⁸ with known hyperimmune rabbit serum. The virus was maintained readily by serial passage in mice and eggs. Autopsies on all dead or dying mice revealed no evidence of bacterial invasion, and cultures of brain and lung tissue were sterile in every case. The remaining 2 of the 6 young mice showed vague symptoms but survived.

These results are in accord with the suggestive findings of a preliminary experiment similar to the foregoing in which 5 of 8 two-

⁷ Syverton, J. T., and Berry, G. P., *J. Exp. Med.*, 1941, **73**, 507.

⁸ Blattner, R. J., and Cooke, J. V., *J. Inf. Dis.*, in press.

weeks-old mice died during the night, 7 days after first contact with infected larval ticks. In this preliminary series no effort was made to recover the virus.

In an attempt to demonstrate the presence of the virus in the arthropod vector, larval ticks which had engorged on mice infected by intracerebral and intraperitoneal inoculation were removed carefully from dying mice and ground in broth in a micromortar. The supernatant fluid was injected intraperitoneally into 2-weeks-old mice. In a group of 6 such mice inoculated with ground tick suspension, 3 developed convulsions within 7 to 8 days, and one was found dead on the eighth day following inoculation. The brains of these 4 when passed to normal adults produced typical convulsions and death in $2\frac{1}{2}$ to 3 days.

While it is generally accepted that Saint Louis encephalitis is not contracted by contact exposure, such a possibility exists, especially since Smith,⁹ and also King,¹⁰ have shown that young mice are much more susceptible to small amounts of virus. For this reason the following experiment was performed as control. Two-weeks-old mice were placed with infected adults and allowed to remain during the course of illness until several hours after the latter had died. No symptoms of encephalitis developed in these young mice.

In an effort to check the possibility of infection through the gastrointestinal tract by ingestion of infected larval ticks, young mice (*i. e.*, 2 weeks of age) were fed food grossly contaminated by mixture with infected brain tissue. All such animals failed to develop encephalitis, which is in keeping with the observations of Brodie,¹¹ who also was unable to infect normal mice by ingestion of food grossly contaminated by Saint Louis encephalitis virus. Harford, Sulkin, and Bronfenbrenner,¹² while in general reaching the same conclusion observed, however, that 2 of 7 normal adult mice did contract encephalitis by eating moribund new-born mice previously infected by the intracerebral route. The authors attributed these results to the large quantity of virus ingested. The further possibility of infection by the intranasal route was also considered, since King¹⁰ concluded that this route is the most effective peripheral means of inoculation. Specific experiments are being conducted to investigate this possibility, although nasal infection from ingestion of ticks seems unlikely.

⁹ Smith, M. G., *J. Exp. Med.*, in press.

¹⁰ King, L. S., *J. Exp. Med.*, 1940, **72**, 573.

¹¹ Brodie, M., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 1647.

¹² Harford, C. G., Sulkin, S. E., and Bronfenbrenner, J. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1939, **41**, 332.

Further evidence that the tick can transmit Saint Louis encephalitis was obtained in experiments with full-grown ticks and adult mice, since such adult mice upon which infected full-grown ticks had fed developed considerable resistance to intracerebral inoculation of virus. Full-grown ticks, which had engorged for a period of 5 to 7 days on infected adult mice, were transferred to normal adults and continued feeding for a like period. None of the latter developed convulsions, and all survived, although they seemed ill for several days showing lethargy, ruffled fur, and lack of interest in food. Later these convalescent mice were tested by intracerebral injection of Saint Louis encephalitis virus which was sufficient to kill, without exception, all normal controls in 3 to 5 days. Twelve of 17 mice so tested have survived, showing an apparently increased resistance to intracerebral inoculation. Here the possibility of infection by ingestion or by the intranasal route was obviated since adult ticks were protected during attachment by a specially contrived jacket having a small glass perforated chamber into which the tick may expand during engorgement.

Summary. 1. The virus of Saint Louis encephalitis was recovered from the bodies of engorged larval ticks which had fed on infected adult Swiss mice. 2. Under experimental conditions the Saint Louis encephalitis virus was transmitted to young Swiss mice by larval ticks. 3. Normal adult mice upon which infected, full-grown ticks had fed developed considerable resistance to intracerebral inoculation of virus.

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Vitamin A Concentration in Rat Liver During Recovery from CCl_4 Cirrhosis.*

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It has been found that the liver contains approximately 95% of the body stores of vitamin A,¹ and is the site of production of the

* Reported in preliminary form to the American Society of Zoölogists, *Anat. Rec.*, 1941, **81**, Suppl., 124.

¹ Moore, T., *Biochem. J.*, London, 1931, **25**, 275.