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THRESHOLD OF VIRAEMIA IN *APODEMUS FLAVICOLLIS* FOR INFECTION OF *IXODES RICINUS* WITH TICK-BORNE ENCEPHALITIS VIRUS

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Summary. — Larvae of *Ixodes ricinus* engorged on *Apodemus flavicollis* during viraemia with tick-borne encephalitis (TE) virus contracted the infection. Fourteen days after molting into nymphs the virus was not detectable, but when tested 2 months later, many nymphs contained virus indicating that the latter undergoes an eclipse phase during metamorphosis of ticks. Threshold of viraemia for infection of tick larvae was estimated to correspond to a virus concentration slightly below 100 LD_{50}/ /0.02 ml.

Experimental studies were conducted with numerous mammals to evaluate their role as hosts of TE virus: with insectivora like hedgehogs (Kožuch *et al.*, 1966*b*), moles (Kožuch *et al.*, 1966*a*), and shrews (Kožuch *et al.*, 1967); with *Chiroptera* (Nosek *et al.*, 1961); and of the *Rodentia* with *Muridae* (Mornsteinová and Albrecht, 1957; Ernek *et al.*, 1963; Radda, 1965; Radda *et al.*, 1964, 1968; Schindler and Krampitz, 1964; Heigl and von Zeipel, 1966), *Gliridae* (Nosek *et al.*, 1964), and *Sciuridae* (Radda and Pretzmann, 1964). Viraemia was frequently observed. Our present study was done with the aim to learn something about the threshold of viraemia necessary for infection of ticks gorging on viraemic mammals.

Fourteen adult *Apodemus flavicollis* were infected subcutaneously (sc) with approximately 100 LD_{50} (assayed in 0.2 ml volumes sc in white mice weighing 8—10 g) of the Hypr strain of TE virus. From the 1st to the 7th day after infection, blood was taken daily from two different individuals by puncture of the orbital veins. After adding one drop of heparin (50 U/ml), which has no influence on TE virus (Hofmann and Radda, 1968), both the blood and 10-fold dilutions therefrom were inoculated intracerebrally (ic) into baby mice.

Feeding capsules for *Ixodes ricinus* ticks were attached with collodion to the back of the mice. The mice were divided into four groups. Daily about 40 larvae per mouse were set up on all mice of each group, beginning on the day before infection with the first group and ending two days after infection with the last group (see Fig. 1). After 3 days of sucking, the larvae were removed and placed in a glass tube. The tube was closed with a wire gauze and stored at room temperature in a plastic bag with moist cotton.

Six weeks later most of the larvae had molted into nymphs. Two weeks thereafter, these nymphs were allowed to suck on baby mice (3—5 days old). For this purpose one baby mouse and one tick were transferred into a glass tube, which was closed by a wire gauze. The baby mouse and the nymph were kept at 30° C for 5—6 hours. Thereafter the sucking tick was removed, the mouse was returned to the litter and observed for signs of disease for 14 days. Specificity of disease was proved by the fluorescent antibody technique in imprints of mouse brains (Kunz, 1966). Two months after the first test the ticks were retested on other mice.

Viraemia in *Apodemus flavicollis* lasted from the 2nd to the 6th day after infection and reached its highest titre of 178 LD_{50}/0.02 ml (assayed ic in baby mice) on the 3rd day (Fig. 1).

We harvested a total of 360 engorged larvae, of which 280 subsequently molted into nymphs. None of the 141 nymphs tested 2 months after infection (i.e. 2 weeks after molting) contained virus. By contrast, from the 48 nymphs retested 4 months after infection (i.e. 2.5 months after molting), quarter of those in group 3, and more than half of those in group 4 contained detectable virus (Table 1).

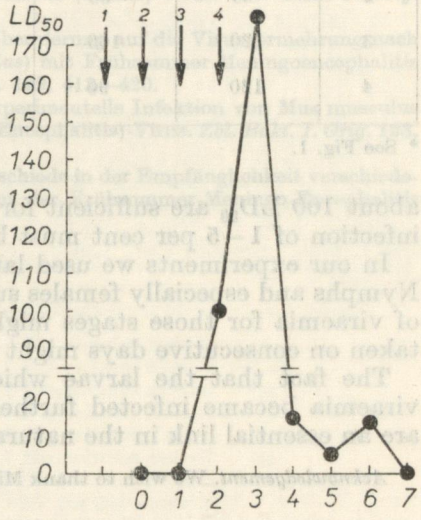

Fig. 1.
Viraemia of *Apodemus flavicollis* infected subcutaneously with 100 LD_{50} of TE virus
Abscissa: days after inoculation; ordinate: virus titre in LD_{50}/0.02 ml values. Arrows indicate placing upon of larvae of groups 1, 2, 3, and 4.

In previous studies by Loew and Wiedermann (1967) it was possible to detect TE virus in engorged tick larvae only for a certain period of time. The virus first decreased and then increased to a peak on the 14th day after engorgement, but disappeared completely by the 18th day. After the larvae had molted into nymphs, virus was never detectable.

From our present results it is quite obvious that Loew and Wiedermann failed to isolate the virus from nymphs infected as larvae because they tested the ticks too early after molting. As was shown in our experiment, the virus undergoes a long eclipse phase, which was not yet over when the ticks were first tested 2 weeks after molting. In nature this eclipse period probably lasts even longer than in the laboratory, because metamorphosis of engorged larvae into nymphs takes 6 months or more.

From our test it is evident that the level of viraemia on the first day after setting up ticks is critical for infection of larvae. For larvae of group 1 and 2 this value was 0 LD_{50}, for group three 100 LD_{50} and for group four 178 LD_{50}. Subsequently a quarter of ticks of group 3 and more than half of those of group 4 contracted infection.

The threshold of infection for Eastern equine encephalitis was defined by Chamberlain *et al.* (1954) as the lowest concentration of virus capable of causing an infection in approximately 1—5 per cent of the specimens of a particular mosquito species ingesting it. Applying this definition to ticks, we cannot state the lowest concentration of virus necessary for infection of 1—5 per cent of larvae, but we can say that a level of viraemia of approximately 170 LD_{50} is sufficient to infect 50 per cent of gorging larvae, and that

Table 1. Results of infection of ticks with TE virus

Group*	Approx. number of engorged larvae	Approx. number of molted nymphs	Number of nymphs tested			
			2 months p.i.		4 months p.i.	
			virophoric	total	virophoric	total
1	60	40	0	24	0	7
2	60	50	0	21	0	14
3	120	100	0	73	4	16
4	120	90	0	23	6	11

* See Fig. 1.

about 100 LD_{50} are sufficient for infection of 25 per cent. The threshold for infection of 1—5 per cent must be therefore slightly below 100 LD_{50}.

In our experiments we used larvae, which do not suck for a long period. Nymphs and especially females suck for a longer period. Therefore threshold of viraemia for those stages might be lower, because low amounts of virus taken on consecutive days might accumulate and therefore induce infection.

The fact that the larvae which sucked on *Apodemus flavicollis* during viraemia became infected further substantiates our assumption that mice are an essential link in the natural cycle of TE virus.

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References

Chamberlain, R. W., Sikes, R. K., Nelson, D. B., and Sudia, W. D. (1954): Studies on the North American arthropod-borne encephalitis. IV. Quantitative determinations of virus-vector relationships. *Amer. J. Hyg.* **60**, 278—285.

Ernek, E., Kožuch, O., Lichard, M., Nosek, J., and Albrecht, P. (1963): Experimental infection of *Clethrionomys glareolus* and *Apodemus flavicollis* with tick-borne encephalitis virus. *Acta virol.* **7**, 434—436.

Heigl, Z., and Zeipel, G. von (1966): Experimental infection with tick-borne encephalitis virus in *Clethrionomys glareolus*, *Apodemus flavicollis*, *Apodemus sylvaticus* and *Mus musculus*. I. Virological studies. *Acta path. microbiol. scand.* **4**, 489, 509.

Hofmann, H., and Radda, A. (1968): Das Verhalten der Virämie weisser Labormäuse (Swiss-Albino) nach Infektion mit Frühsommer-Meningo-Encephalitis (FSME)-virus. *Zbl. Bakt. I. Orig.* **208**, 105—109.

Kožuch, O., Grulich, I., and Nosek, J. (1966*a*): Experimental infection of the mole with tick-borne encephalitis virus. *J. Hyg. Epidem. (Praha)* **10**, 120—124.

Kožuch, O., Nosek, J., and Lichard, M. (1966*b*): Überleben des Zeckenencephalitis-Virus in der Zecke *Ixodes ricinus* und die Übertragung dieses Virus auf den Igel (*Erinaceus roumanicus*). *Zbl. Bakt. I. Orig.* **199**, 152—161.

Kožuch, O., Nosek, J., Lichard, M., Chmela, J., and Ernek, E. (1967): Transmission of tick-borne encephalitis virus by nymphs of *Ixodes ricinus* and *Haemophysalis inermis* to the common shrew (*Sorex araneus*). *Acta virol.* **11**, 256—259.

Kunz, Ch. (1966): Methods for classification of viruses the TBE complex. *Neuropathol. pol.* **4**, 329—332.

Loew, J., and Wiedermann, G. (1967): Untersuchungen über die Resistenz des Virus der FSME (CEE) in Entwicklungsstadien von *Ixodes ricinus*. *Zbl. Bakt. I. Orig.* **202**, 297—307.

Mornsteinová, O., and Albrecht, P. (1957): Experimental infection of the mouse *Micromys minutus* with the virus of Czechoslovak tick-borne encephalitis (in Czech). *Čs. Epidem.* **6**, 157—161.

Nosek, J., Grešíková, M., and Řeháček, J. (1961): Persistence of tick-borne encephalitis virus in hibernating bats. *Acta virol.* **5**, 112—116.

Nosek, J., Kožuch, O., Lichard, M., Ernek, E., and Albrecht, P. (1963): Experimental infection of great dormouse. (*Glis glis*) with tick-borne encephalitis virus. *Acta virol.* **7**, 374—376.

Radda, A. (1965): Die Rolle einiger Muriden und deren häufigster Ektoparasiten im natürlichen Kreislauf des Virus der Zentraleuropäischen Encephalitis. Diss. Univ. Wien.

Radda, A., Hofmann, H., and Kunz, Ch. (1968): Experimentelle Infektion einiger heimischer Säugerarten mit dem Frühsommer-Meningo-Encephalitis (FSME)-Virus. *Zbl. Bakt. I. Orig.* **208**, 100—104.

Radda, A., and Pretzmann, G. (1964): Einfluss der Hibernierung auf die Virusvermehrung nach experimenteller Infektion des Ziesels (*Citellus citellus*) mit Frühsommer-Meningoencephalitis (Tick-Borne Encephalitis)-Virus. *Zbl. Bakt. I. Orig.* **189**, 413—420.

Radda, A., Pretzmann, G., and Kovac, W. (1964): Experimentelle Infektion von Mus musculus mit Frühsommer-Meningoencephalitis (Tick-borne Encephalitis)-Virus. *Zbl. Bakt. I. Orig.* **193**, 285—292.

Schindler, R., and Krampitz, M. E. (1964): Über Unterschiede in der Empfänglichkeit verschiedener Mäusestämme und -arten gegenüber dem Virus der Frühsommer-Meningo-Encephalitis (FSME). *Z. Tropenmed. Parasit.* **15**, 29—37.