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Overwintering of the Virus Ťahyňa in Hedgehogs Kept under Natural Conditions

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Abstract. The development of infection with Ťahyňa virus was traced in hedgehogs before and during hibernation and at the moment of their awakening. When the preparation of animals for hibernation lasted more than 14 days in a temporary lethargic state with a small decrease of body temperature, the infection passed off with the final virus disappearance from the organism of hedgehogs even before the onset of hibernation. The infection induced at the beginning of hibernation resulted in the persistence of virus in the blood and various organs except CNS throughout the hibernation period (more than 86 days) and in the disappearance of virus during several days of awakening. In hedgehogs fully roused from winter sleep only antibodies were found. The role of hedgehogs in the circulation of virus in nature during the winter months remained questionable and will require further studies.

Overwintering of the virus Ťahyňa so far has been the least clarified link in the circulation of virus in nature. The most recent experiments have shown that some hibernating mosquito species might take part in the overwintering of this virus. DANIELOVÁ (1969) demonstrated that experimentally infected mosquitoes *Culiseta annulata* might serve not only as suitable reservoirs for multiplication of the virus but also for its maintenance during hibernation. Also of interest is the information that the Ťahyňa virus can multiply and persist in the mosquito *Culex modestus* (DANIELOVÁ, in press). As for mammals, important data are given by ŠIMKOVÁ (1966) on the possible survival of the Ťahyňa virus in hedgehogs induced to hibernation.

The above mentioned experimental results are significant, because they indicate the possible modes by which the virus Ťahyňa may overwinter. It remains to be seen as to how the virus can actually overwinter in open nature and the present paper is an attempt to fill up this gap in the knowledge on the overwintering of the virus under natural conditions.

MATERIAL AND METHODS

Animal. In our experiments we used hedgehogs of the species *Erinaceus roumanicus* Barr. Ham. 1900 and *Erinaceus europaeus* L. 1758. They were collected in various localities of southern Moravia and eastern Bohemia and placed in large enclosures (cca 5 × 25 m) in the neighbourhood of Valtice, belonging to the region of the natural focus of Ťahyňa virus. The enclosures were situated in a deciduous forest consisting of oak trees with scarce shrubbery and a rather more abundant herb layer. The hedgehogs were kept free until hibernation. Immediately before the experiment conducted during hibernation, they were placed for control reasons into smaller wire net cages (2 × 1 m) in hibernation nest-boxes containing two specimens each.

Regular tracing of climatic conditions and of the behaviour of hedgehogs was done between November 15, 1967 and April 25, 1968. Temperature, humidity, atmospheric pressure etc. were measured. The mean diurnal temperature was obtained from 24 data measured per day. As for the behaviour of hedgehogs their movements were traced by means of actograph and their body weight, temperature and feeding was observed. Details about these data will be given in a paper by Dr Hodková.

Virus and infection. The strain 236, passage K9 of the Ťahyňa virus was used in lyophilized blood diluted in buffered saline pH 7.3 immediately before application. The hedgehogs were inoculated subcutaneously on the parietal part of head with 0.1 ml of the infected blood diluted in buffered saline with 10 % inactivated normal calf serum and 100 units PNC and 100 gamma/ml STM. The virus dosages are presented in the Results.

Dissection of organs. Virus was traced in various organs. The hedgehogs were anesthetized with chloroform and bled to death by heart puncture. Perfusion of organs was performed by a slow flooding of heart with saline. The following organs were dissected after bleeding: regional and contralateral lymphnodes, parts of lung lobes both from the left and right side, liver, adrenals, telecephamiddle section of both the left and right kidney, spleen, some brown fat from both sides, portions of lon, cerebellum, as well as of diencephalon (CNS). New sterile instruments were used for each organ. Each part of organ was washed under running saline and after drying placed into a cooled tube. Then the parts of individual organs were weighed and a 10 % suspension in buffered saline solution pH 7.3 with 10 % inactivated normal calf serum plus 500 units/ml PNC and 500 gamma/ml STM was prepared. The suspension was centrifuged at 2000 rev/min. for 10 minutes in a cooled centrifuge.

Detection of virus. Virus was detected in 3 to 4-day-old mice. One litter consisting of 8—10 young was used per solution. The mice were inoculated intracerebrally with 0.01 ml each. Virus titres were calculated according to the method of Reed and Muench (1938).

Antibodies. Antibodies were demonstrated by neutralization test in pig kidney stable cell line by current method (Málková and Marhoul 1962).

RESULTS

Thirty hedgehogs were experimentally infected with the virus Ťahyňa between November 15, 1967 and April 23, 1968. Of the total number 20 hedgehogs were virologically examined, the remaining specimens either died during hibernation or were kept for further observation.

Ťahyňa virus in hedgehogs during preparation for hibernation. The experiment was conducted between November 15th and 30th, 1967 and 9 animals were used. They were mostly juveniles of the same year and their characteristics are given in Table 1. Before the experiment the animals were kept in enclosures for at least one

Table 1. Characteristics of hedgehogs during preparation for hibernation

Interval after inf.	Hedgehog	Age	Sex	During infection			Before dissection	
				activity	weight	rect. temperature °C	activity	rect. temperature °C
24 h	*Erinaceus roumanicus*	juv.	♂	partly active[1])	264	31.5	partly active	—
48 h	*Erinaceus roumanicus*	juv.	♂	partly active	435	31.4	partly active	—
72 h	*Erinaceus roumanicus*	juv.	♂	partly active	360	31.0	partly[2]) active 1×	30.8
96 h	*Erinaceus roumanicus*	juv.	♂	partly active	300	30.4	partly active	32.4
120 h	*Erinaceus roumanicus*	juv.	♀	partly active	285	30.4	partly active 1×	33.7
6 d	*Erinaceus roumanicus*	juv.	♂	partly active	387	31.2	partly active 2×	33.2
8 d	*Erinaceus roumanicus*	juv.	♂	partly active	385	< 29.5	partly active 2×	32.9
10 d	*Erinaceus roumanicus*	juv.	♂	partly active	243	31.1	partly active 3×	30.6
14 d	*Erinaceus roumanicus*	juv.	♀	partly active	340	29.5	partly active 4×	29.8

[1]) partly active = received less food before (during) experiment, in a temporary lethargic state in the morning
[2]) frequency at which the animal was found asleep during experiment

month. In the middle of November and later all of them were in the stage of preparation for hibernation i.e. they fed irregularly and on some control days they were found in a temporary lethargic state. On cold days their activities were considerably reduced. As shown in Table 1 their rectal body temperature varied from 29 to 34 °C, i.e. it was lower than on summer days when it should range between 33.5 and 35.5 °C (HERTER 1934) or reach as high as 37°C (PROCTOR 1949). During dissection it was established that all specimens except one already had a subcutaneous fat deposit, despite their small total weight.

At the time of the experiment average diurnal temperatures varied from —3 to + 5.5 °C. Maximal temperatures reached as high as +10 °C and minimal temperature dropped once to —8 °C.

All hedgehogs allotted for this experiment were infected simultaneously on November 15, 1967. The amount of virus contained in 0.1 ml of inoculum represented 10^4 i.c. mouse LD_{50}/0.01 ml. The hedgehogs were killed one after another at various time intervals and the amount of virus was traced in different organs as given in Table 2. The results obtained show that viremia occurred as early as 24 hours and lasted until 8th day following infection. On 10th day no virus was detected in the blood. In other organs examined the virus was first present in the re-

Table 2. Virus detection in hedgehogs during preparation for hibernation

Organs	Post-infection period								
	24 h	48 h	72 h	96 h	120 h	6 d	8 d	10 d	14 d
Blood	0.5*	> 1.5	> 2.4	> 2.5	2.30	+	> 1.5	0	0
Regional lymphnodes	> 2.5	> 2.5	> 3.5	> 3.5	> 3.5	> 2.5	> 2,5	> 1.5	0
Contralateral lymphnodes	0	0	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 1.5	0
Spleen	0	2.24	> 2.5	> 2.5	1.42	1.56	> 2.5	+	0
Liver	0	+	1.30	> 1.5	+	0	+	0	0
Lungs	+	0	+	1.33	+	+	+	0	0
Kidneys	0	+	+	1.3	+	+	+	0	0
Brain	0	0	0	0	0	0	0	0	0
Degree of serum dilution neutralizing cca 100 CPD_{50}	0	0	0	0	0	0	0	0	0

* virus titre in log LD_{50}/0.01 ml
\+ titre 0.5 log
0 negative finding

gional lymphnodes, where its titre was observed as early as 24 hours following infection. This titre was much higher than the virus titre in blood and this fact indicated that the former virus titre occurred much earlier than the latter. In the spleen the virus was detected 48 hours following infection. In contralateral lymphnodes, liver, lungs, kidneys except CNS the virus was detected since 72 hours following infection. In these organs the virus persisted until 8th—10th day following infection, in the lymphnodes its persistence was the longest. No virus was detected in the brain throughout our observations, i.e. until 14th day following infection. Antibodies were found in a low titre since 14th day following infection.

Table 3. Characteristics of hedgehogs examined during hibernation

Hedgehog designation	Species	Age	Sex	State during inoculation		State at the end of experiment		
				weight	sleep	rect. temperature	weight	sleep
K7ZK	*Erinaceus roumanicus*	juv.	♀	340	asleep	1.5 °C	310	soundly asleep
V1H	*Erinaceus roumanicus*	adult	♂	575	half asleep	20 °C	480	asleep
K2LB	*Erinaceus roumanicus*	juv.	♂	395	asleep	13 °C	320	asleep
K4HZ	*Erinaceus europaeus*	adult	♂	745	asleep	22 °C	705	asleep

Ťahyňa virus in hedgehogs during hibernation. The preceding experiment showed that the hedgehog, even in a state of reduced activity during preparation for hibernation was capable of overcoming the infection process relatively quickly and of producing antibodies subsequently. Therefore, the hedgehogs in which we wanted to trace the virus during hibernation, were infected after they had fallen asleep.

Table 4. Virus detection in hedgehogs during hibernation

Organs	Hedgehog designation			
	K7Z	V1H	K2LB	K4HZ
Blood	±	>1.5	>1.5	>1.24
Regional lymphnodes	>2.5	>2.5	>2.5	>2.4
Contralateral lymphnodes	>1.5	>1.5	+	+
Spleen	>2.5	>2.5	2.24	>2.5
Liver	1.2	>1.5	±	0
Lungs	>1.4	>1.5	+	+
Kidneys	+	>1.5	+	0
Brain	0	0	0	0
Adrenals	>1.5	>1.5	+	+
Brown fat	>1.5	>1.5	+	+
Serum dilution neutralizing cca 100 CPD_{50}	0	0	0	0

Explanations as in Table 2.

During the winter months 1967—1968 most of the hedgehogs were still active in December due to climatic conditions. They were therefore infected as late as January 3, 1968. At that time the mean diurnal temperatures were under the freezing point and the actographs recorded no movement from the nest-boxes. On January 3rd the mean diurnal temperature was —5 °C and there was a coherent snow layer about 5 cm high. Despite the fact that the hedgehogs were found in their nest-boxes to be immobile and coiled up in the characteristic hibernating position, some of the animals were half or fully awake and their nests showed an increased temperature inside.

A total of 11 animals both dormant and awake were infected with $10^{3.78}$ i.c. mouse LD_{50} in 0.1 ml inoculum and placed in the hibernation cages.

A group of hedgehogs observed during hibernation, i.e. between March 5th and 6th 1968 (62—63 hibernation days following infection) consisted of 4 specimens. Other infected hedgehogs were left intact for control of virus at the moment of awakening and full recovery from hibernation. The course of hibernation in connection with the outside temperature is recorded in Fig. 1-group I.

The condition of hedgehogs before dissection between March 5th and 6th 1968 is presented in Table 3. The higher rectal temperature in hedgehogs V1H and K4HZ

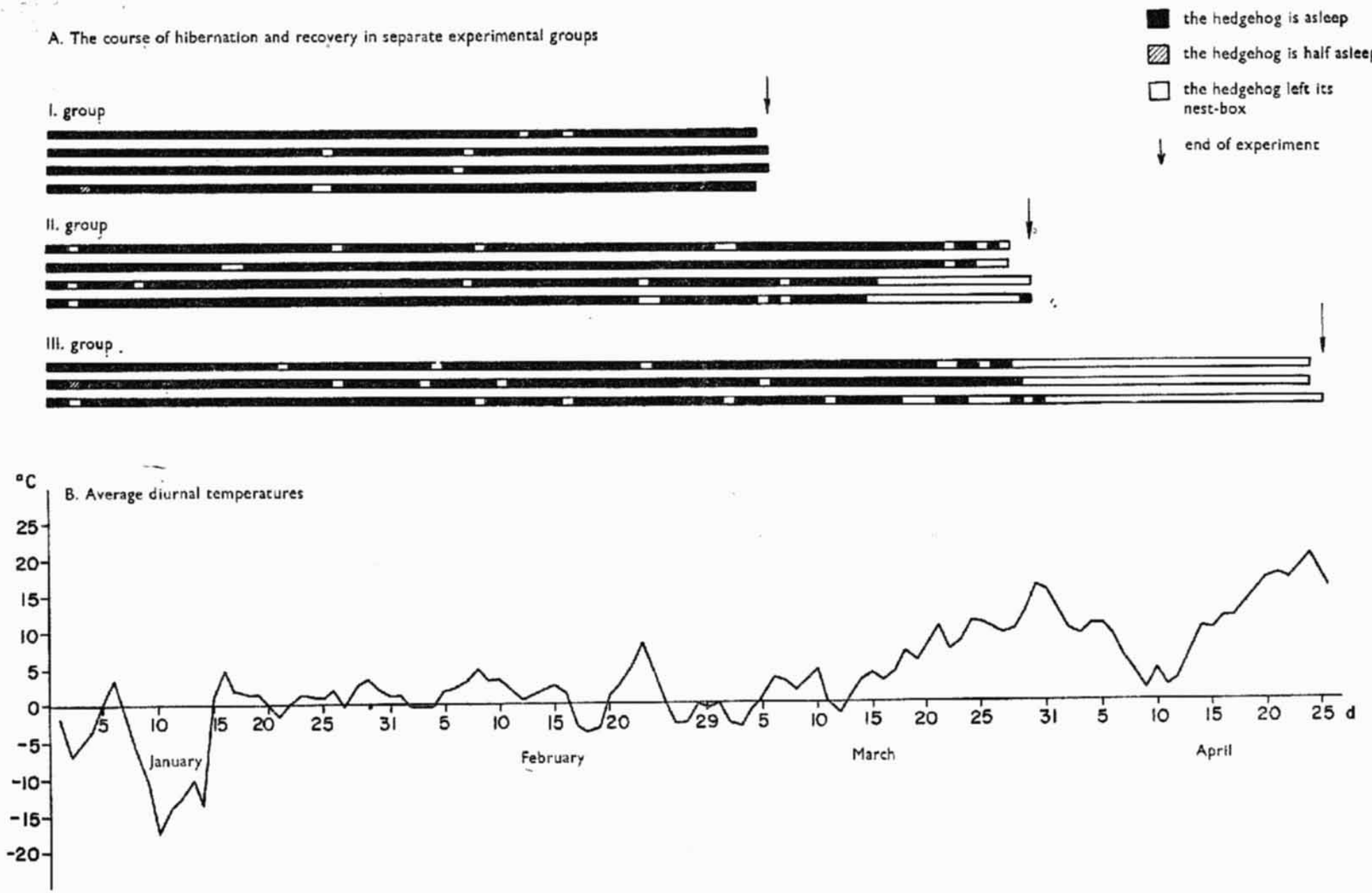

Fig. 1. The course of hibernation and awakening of infected hedgehogs.

Table 5. Characteristics of hedgehogs examined during recovery from hibernation

Hedgehog designation	Species	Age	Sex	State during inoculation		State at the end of experiment		
				weight	sleep	rect. temperature °C	weight	sleep
K1Z	*Erinaceus roumanicus*	juv.	♀	405	awake	32 °C	245	awake
V2H	*Erinaceus europaeus*	adult	♂	800	soundly asleep	34 °C	695	awake
K6Z	*Erinaceus roumanicus*	adult	♂	750	awake	32 °C	600	awake
K6H	*Erinaceus roumanicus*	adult	♂	660	awake	18 °C	450	asleeep

may be explained by the fact that several hours passed between the moment, when they were removed from the nest and the experiment proper, before their temperature was taken.

The virus was detected in organs similarly as in the preceding experiment, only with the difference that the virus was traced in the adrenals and brown fat. A survey of virological results obtained is given in Table 4. It shows that in all hibernating hedgehogs viremia and the presence of virus in different organs were observed in various intensity. Similarly as in the preceding experiment no virus was detected in the brain and no antibodies were found in the blood.

Table 6. Virus detection in hedgehogs during recovery from hibernation

Organs	Hedgehog designation			
	K1Z	V2H	K6Z	K6H
Blood	0	0	0	0
Regional lymphnodes	0	+	0	0
Contralateral lymphnodes	0	+	0	0
Spleen	0	1.09	0	0
Liver	0	+	0	0
Lungs	0	1.43	0	0
Kidneys	0	+	0	0
Adrenals	0	0	0	0
Brown fat	0	1.28	+	+
Brain	0	0	0	+
Serum dilution neutralizing cca 100 CPD_{50}	0	0	1 : 4	0

Explanations as in Table 2.

Table 7. Characteristics of hedgehogs during activity following hibernation

Hedgehog designation	Species	Age	Sex	State during inoculation		State at the end of experiment		
				weight	sleep	rect. temperature °C	weight	sleep
K7Z	*Erinaceus roumanicus*	adult	♀	665	awake	34 °C	530	awake
K4H	*Erinaceus europaeus*	adult	♂	1260	half asleep	35 °C	995	awake
K3H	*Erinaceus roumanicus*	juv.	♀	475	asleep	36 °C	473	awake

Ťahyňa virus in hedgehogs during recovery from hibernation. Another group of 4 hedgehogs was observed between March 28th and 29th 1968, i.e. 85th—86th day following infection. In this period the hedgehogs began to recover from hibernation due to relatively high average diurnal temperatures which varied between 10 °C and 20 °C. Their activity was recorded by actographs, showing that they left their nest-boxes several times prior to March 28th (Fig. 1—group II). The condition of hedgehogs at the time of virological investigation carried out on March 28th and 29th, is presented in Table 5.

The virological investigation presented in Table 6 shows that not a single hedgehog had viremia. In organs outside blood circulation the virus persisted in 3 hedgehogs, while in the hedgehog K1Z no virus was detected. The amount of virus in organs of positive hedgehogs varied considerably. The hedgehog V2H had virus in most organs examined, the hedgehog K6Z only in brown fat, the hedgehog K6H in brown fat and brain. These findings were confirmed both by passages and serologically. The presence of virus in the brain of the hedgehog K6H, eventually the causes of the transfer of virus behind the haematoencephalic barrier, cannot be explained satisfactorily for the time being. No antibodies were found in 3 out of 4 animals, in the hedgehog K6Z a positive finding in serum dilution 1 : 4 was ascertained.

Virological and serological findings in hedgehogs infected with Ťahyňa virus after recovery from hibernation. Three hedgehogs were examined on April 24th and 25th 1968, i.e. at a time when they were at least three weeks awake and fed regularly. The course of hibernation and recovery from it in hedgehogs of this group is recorded in Fig. 1—group III.

Characteristics of hedgehogs during virological investigation are presented in Table 7.

Virological investigation conducted by same method as above, was negative. Serological findings, on the other hand, were positive in all three cases, namely in the hedgehogs K7Z and K4H antibodies were found in serum dilution 1 : 4, in the hedgehog K3H in serum dilution 1 : 16.

DISCUSSION

The results obtained show that during their preparation for hibernation, when the animals revealed a somewhat lower body temperature, fed irregularly and on some days were found in a temporary lethargic state, and when this behaviour lasted longer than 14 days without causing hibernation, the infection passed off and the virus disappeared from their organism leaving a trace of antibody formation within 14 days. In comparison with experimental findings of ŠIMKOVÁ (1966) the course of viremia did not essentially differ from that observed during full activity of hedgehogs in the summer. Only the onset of antibodies appeared to be later, if compared with the results of ŠIMKOVÁ.

In case of infection during hibernation, the virus detection in blood and organs of hedgehogs ascertained on 62nd—63rd day of hibernation following infection indicates that the infection developed in them and the virus penetrated beyond the haematoorganic barrier, except hematoencephalic barrier. Due to the fact, that similar development of infection was ascertained both in dormant hedgehogs and in those which were half-asleep or awake, we presume that the infection in them developed during hibernation. It is not clear yet whether the impulses to the development of infection were associated with the intervals of wakefulness during hibernation, or whether it is ever possible for the infection to develop during hibernation.

In the period of recovery from hibernation, i.e. in our case at the end of March and at various intervals according to the first signs of activity of hedgehogs, virological investigation showed that viremia disappeared in them relatively quickly. As far as the increasing activity of hedgehogs was not interrupted due to climatic conditions, our experiments showed that viremia disappeared in 6—12 days. In organs except blood the virus persisted longer, in the brown fat at the longest.

In the period of their full activity following hibernation (in our case at the end of April) only the antibody response was observed and no virus detected. It may be deduced from these data that the Ťahyňa virus is able to persist in hedgehogs during their hibernation in nature (in our case 86 days). The duration of this persistence directly depends on the intensity and duration of hibernation.

If the hedgehogs become infected while preparing for hibernation, the decisive factor for the overwintering of virus in the animals is the speed at which the hibernation sets on, in other words, the virus is able to persist in the organism for a longer period after infection only when the changes in the organism caused by the lower body temperature are so advanced that they influence both the intensity of the virus multiplication (or penetration of infection in tissue) and the effectiveness of defensive components in the organism. So far as these changes are superficial (or temporary), when the state of limited activity lasts longer, the infection passes off even before the animals fall asleep and the virus disappears from their organisms before the hibernation sets on.

Only further studies on the relationship between vectors and hedgehogs at the

beginning and end of hibernation will show the significance of hibernation of the Ťahyňa virus in hedgehogs as a link in the virus circulation in nature. The recent experimental data and observations conducted in nature between the autumn 1967 and the spring 1968 indicate that a possible contact between vectors and hedgehogs, especially during preparation for hibernation, when the hedgehogs can maintain the virus in their organism until hibernation proper, is neither common nor regular.

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