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Effects of High Ambient Temperature on Various Stages of Rabies Virus Infection in Mice

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Effects of high ambient temperatures on various stages of rabies virus infection have been studied. Ambient temperature increased within the tolerated range was found to have little effect upon body temperature of normal mice, but caused marked elevation of temperature during illness. Temperatures at onset of patent illness in mice were lower than normal. Increased body temperature in the higher thermic ambience during the incubation period was associated with decreased mortality and frequent abortive infections. Exposure to high ambient temperature late in the incubation period delayed onset of illness, decreased mortality, and increased frequency of abortive infections, but exposure to high ambient temperature after onset of patent illness did not affect the course of the disease.

Effects of altered metabolic rate on experimental rabies infection have been described (13), and relative capacity of the virus to replicate at high temperature has been compared with virulence (10, 30), but no clear-cut reaction of rabies infection to hyperthermia in homiothermic animals comparable to those for herpes (1) are recorded. Experimentally infected mice maintained at 37 and 24 C suffered greater mortality at the lower temperature and had higher concentrations of virus in the brain. Immune response to vaccines was unaffected.

Reported frequent recovery from experimental rabies in mice (5) and dogs (7, 8) and treatment leading to recovery in man (17) encourage exploration of additional forms of therapy. Artificially induced hyperthermia has been proven effective in other infection (15), and if some stages of rabies virus infection were especially susceptible to heat, a febrile state induced during that stage could favor recovery.

MATERIALS AND METHODS

Mice. All were of the Rocky Mountain Laboratory (RML) stock of random-bred Swiss mice. Females were used throughout to obviate fighting. The colony was infected with mites and a variety of intercurrent infectious diseases. Mice were 20 to 22 days old at time of inoculation.

Viruses. Lot V-16 rabies virus of *Eptesicus* bat origin was used for primary inoculation of mice in all experiments. It was isolated at RML and passed three times in mice by the intracerebral (i.c.) route. Average titer of this virus preparation is about 4.8 dex (16).

Lot PV-1, a standard strain of fixed virus, was inoculated i.c. in mice that survived primary inoculation. The dose was about 3 dex in 0.03 ml.

Mice inoculated by the intraperitoneal (i.p.) route received 0.1 ml of infected mouse brain in water, whereas those inoculated by the intraplantar (pl.) route were given 0.03 ml injected in the footpad of a hind leg. Titers of virus are expressed as dex 50% lethal dose (25) per 0.03 ml of clarified suspension.

Thermometers. (i) Cole Parmer portable microprobe (model 8505-20; Chicago, Ill.) was used for i.p. thermometry. (ii) Yellow Springs Tele-Thermometer (model 43TF; Yellow Springs, Ohio) was used for oral and rectal thermometry.

In early experiments, body temperatures were determined by oral or rectal thermometry. These procedures were sometimes traumatic to the mice. When i.p.-sensing and oral-sensing devices were used in a series of parallel determinations, it was found that they yielded essentially similar data, but temperatures taken by i.p. probe were slightly higher. The latter device is easier to use and causes no detectable trauma.

Housing of mice. Mice were reared in glass jars in a room with temperature controlled at 20 C. Temperatures in the jars varied but were slightly warmer than room temperature (RT). At the time of inoculation, mice were put in holding boxes at RT or in individual compartments of wire cages, hereafter referred to as ambience cages, which were placed in one of two cabinets (model 706A; Labline, Inc., Chicago, Ill.) with controlled, and continuously monitored, temperature and humidity. Air in the cabinets is circulated constantly by fans. When the mice became sick, they were transferred to glass jars at RT for uniformity of care at that stage of infection. Before onset of illness, mice were fed a commercial, pelleted meal with water ad lib. After onset, they were fed whole wheat bread soaked in milk.

RESULTS

Preliminary experiments were intended to find a stage of infection that would be affected by increased ambient temperature. We had

established that normal mice in individual wire cages would survive at least 1 week at 35 C and 25 to 35% relative humidity without apparent adverse effect. This period corresponds to the minimal incubation period of the virus (V-16) inoculated i.p. in 21-day-old RML mice.

Exposure of mice to 35-C ambient temperature after inoculation. Groups of 44 mice each were inoculated i.p. with 0.1 ml of a 10% suspension of infected mouse brain, lot V-16. They were then introduced into the 35-C chamber at various daily intervals after inoculation. When first clinical signs of infection were observed (onset), the affected mice were removed to jars held at RT. Remaining mice were removed from the chambers at 1 month post-inoculation, and a week later all surviving mice were reinoculated, by the i.c. route, with fixed virus (7). Results of this experiment are presented in Table 1.

None of the mice died or became sick before onset of rabies paralysis which, in this experiment, first occurred in all infected groups on the eighth day after inoculation. Normal mice exposed to both high and normal ambient temperatures suffered some mortality apparently related to trauma from oral thermometry.

Removal from 35-C ambient temperature after inoculation. In this experiment, mice were inoculated i.p. as in the previous experiment and placed at 35 C. However, groups of 40 mice were then removed from the 35-C cabinet to RT at various intervals. One group was kept in the 21-C cabinet throughout the test period. Results are summarized in Table 2.

Mortality in mice maintained at RT (group A) was 75%, but mice kept at 35 C for 28 days (group F) suffered only 25% mortality. Mortality in the other groups again was essentially inversely proportional to time spent at the higher temperature. Further, after onset of infection, survival was threefold greater in group F than in group A, and the incubation period was 3 days longer. Mice in group F were removed to RT at 28 days post-inoculation, when no new infections were seen. No additional patent infections occurred in the subsequent week.

Results of the preceding experiments indicated that mice infected with rabies virus by the i.p. route, and maintained at high ambient temperature (HAT) for 7 days, sustained significantly lower mortality than those kept at (NAT) normal ambient temperature (20 to 22 C). The following experiment was designed to expand this observation by exposing larger numbers of mice. Two groups of 118 mice each were inoculated i.p. and kept either at 35 C (group A) or 21 C (group B) for 7 days (Table 3).

Mortality in the larger group was similar to that in previous experiments: 24% at HAT and 76% at NAT.

The i.p. route of inoculation usually produces a lower proportion of infection and higher incidence of survival with sequelae than other routes of inoculation (6). Because temperature of the extremities is more labile than somatic temperature (9, 14), an experiment was designed to determine if differences in ambient temperature influenced infection and mortality

TABLE 1. *Mortality and survival of mice inoculated i.p. with rabies virus and introduced to 35-C ambient temperature at various intervals after i.p. inoculation (day 0)*

Group	In 35-C cabinet on day:	Avg incubation period (day)	Gross mortality (%)	Abortive infection[a] (%)
A	0	14.4	34	21
B	0 + 1		34	35
C	0 + 3		43	32
D	0 + 5		50	29
E	0 + 7		70	26
F	RT only	10.2	75	20

[a] Based on resistance of survivors to i.c. challenge (6).

TABLE 2. *Mortality and survival of mice inoculated i.p. with rabies virus and removed from 35- to 21-C ambient temperatures at various intervals afterward (day 0)*

Group	From 35-C cabinet on day:	Avg incubation period (days)	Gross mortality (%)	Abortive infection (%)
A	0	10.3	75	17
B	0 + 1		65	10
C	0 + 3		45	22
D	0 + 5		32	32
E	0 + 7		38	24
F	0 + 28	13.0	25	52

TABLE 3. *Mortality and survival of mice infected with rabies virus that were kept at 35 and 21 C during the incubation period*

Group	Inoculated	Ambient temp (C)	No. exposed	Mortality (%)	Abortive infection (%)
A	+	35 (7 days)	118	24	30
B	+	21 (28 days)	118	76	30
C1	–	35 (7 days)	10	10	
C5	–	21 (28 days)	30	0	

in mice inoculated by the pl. route as well as by the i.p. route. Groups of mice were inoculated by the pl. or i.p. route, and one group inoculated by each route was exposed to HAT for 7 days (Table 4). On day 7, the inoculated mice and the uninfected control mice were transferred from ambience cages in the cabinets to RT. Body temperatures of normal mice were determined before transfer and after 2 days, and temperatures of both infected and control mice were measured at time of onset of illness (Table 5).

Gross mortality in the i.p.-inoculated mice maintained at HAT was significantly lower than the NAT group, a result similar to those in previous experiments. However, gross mortality in the pl. group at high temperature was only slightly lower than in the NAT group. No abortive infections were detected in either pl. group but were common in the i.p. groups, especially at HAT (Table 4).

Contrary to results of other experiments, the average incubation period was shorter at HAT than at NAT in mice inoculated i.p., but the average figure was based on the rather small numbers of mice that developed patent illness in that ambience.

TABLE 4. *Mortality and abortive infection of mice inoculated with rabies virus by the intraperitoneal and plantar routes[a] and held at 35 and 21 C during the incubation period (7 days)*

Group	Route of inoculation	Ambient temp (C)	No. inoculated	Mortality (%)	Abortive infection (%)	Avg incubation period (days)	Avg time of onset to death (days)
A1	pl.	35	58	88	0	10.7	5.3
A2	pl.	21	58	100	0	9.3	4.6
B1	i.p.	35	57	18	41	9.7	6.8
B2	i.p.	21	52	58	14	11.3	5.0

[a] pl. inoculum, 0.03 ml; i.p. inoculum, 0.1 ml.

Temperature differences were slight among the normal female mice, age 28 and 30 days (at 7 and 9 days), in the HAT and NAT (Table 5). But there were marked differences between temperatures of infected mice at time of onset and the temperatures of normal control mice in identical ambiences. Those differences occurred in mice inoculated by either route. Onset of signs of illness occurred after the mice were returned to RT, sometimes several days later, and diminished differences in somatic temperatures are, no doubt, attributable to the uniformity of contemporary ambience.

Deferred exposure to HAT during the incubation period. In this experiment, mice were exposed to HAT or NAT for 1 week, starting on day 6 after inoculation. They were kept at controlled RT of 20 to 22 C in communal boxes before being placed at HAT. Temperatures within the boxes did not differ significantly from RT.

Body temperatures were determined with the i.p. probe on both normal and infected mice at HAT and NAT. Temperatures were measured 1 day after mice were placed in chambers, at onset of patent illness, 2 days after onset of illness and, for the normal-appearing mice, 3 days after transfer to RT. For each temperature determination of a mouse at onset of illness or 2 days after onset, temperature of a normal mouse in the same ambience was taken for comparison. The pertinent data are tabulated (Table 6).

Progressive infection, indicated by mortality or by resistance to i.c. challenge, was about the same at HAT (74/105) and at NAT (79/109). Net mortality was diminished in the HAT group, and abortive infection was increased as in previous tests.

The greatest departure from results of previous experiments was the deferred onset of patent illness in the HAT group (Fig. 1): 57% of the HAT mice that became ill became so after removal from the cabinet; 36% of detected

TABLE 5. *Average body temperatures of normal mice at different ambient temperatures, and of mice infected with rabies virus (day of onset) and matched controls (normal)*

Group	Temp (C)									
	Normal mice				Infected mice and controls (day of onset)					
					Ambience		pl. inoculation	Normal controls	i.p. inoculation	Normal controls
	Ambience (day 7)	Mouse (day 7)	Ambience (day 9)	Mouse (day 9)	Prior	Contemporary				
HAT	35	38.3	20	38.5	35	20	37.0	37.8	36.3	38.9
NAT	20	38.0	20	38.5	20	20	37.1	38.4	35.6	39.3

TABLE 6. *Mortality, abortive infections, and body temperatures of mice exposed to ambient temperatures of 35 (HAT) and 20.5 C (NAT) from day 6 to day 13 after i.p. inoculation of rabies virus*

Group	Avg incubation period	Gross mortality deaths/ inoculated	Abortive infection (survival/ infected)	No. mice	Mouse temp (C) (day)			
					7	At onset[a]	Onset + 2 days	16 days[b]
A	16.6	21/105 (20%)	53/74 (72%)	20	HAT 39.6	HAT 38.5	NAT 35.9	NAT 38.4
AC Controls				20	HAT 39.0	HAT 39.1	NAT 37.6	NAT 37.9
B	12.7	36/109 (33%)	43/79 (54%)	20	NAT 35.8	NAT 31.5	NAT 33.8	NAT 38.1
BC Controls				17[c]	NAT 37.0	NAT 35.9	NAT 37.9	NAT 38.1

[a] Time of onset was variable. Temperature of a normal mouse (control) in the same ambience was taken at the time of each onset temperature.

[b] This group of mice appeared normal at 16 days after inoculation.

[c] Three "runts" omitted from calculations of averages.

onsets in that group occurred after all onsets in the control group had ceased, and the average incubation period in the HAT group was 4 days longer. Only 31% of NAT patent onsets occurred after the mice were returned to RT (Fig. 1). In spite of the second wave of infections, gross mortality was only 21% in the test group versus 33% in the controls, and survival of infected mice was 72% versus 54% in the controls.

Temperatures of normal mice in both HAT and NAT were variable, ranging from 38.5 to 40.2 C on day 0 + 7 at HAT, and from 33.3 to 38.8 C at NAT. Average temperature was 2 C higher in the HAT group, but only 0.2 C different after the mice were returned to RT for 3 days. At onset of illness after i.p. inoculation of virus, temperatures of mice at the HAT were 0.6 C lower than those of normal mice of the same age kept at the same temperature. But at onset of illness at 21 C, the average temperature was 4.4 C lower than normal, and the normal body temperature in that ambience was 3.2 C lower than normal temperature in the 35 C cabinet. Surprisingly, temperatures of mice 2 days after onset in the 35 C ambience, and 2 days after removal from that ambience to room temperature, were still 2.1 C higher than those of mice that became ill at the lower temperature. Normal mice removed to room temperature at the same time had only 0.3-C difference.

Exposure to HAT at onset of illness. In all previous experiments, mice were taken from the ambience at the time of onset and transferred to glass jars at RT. Uniformity of treatment at that stage of infection permitted comparative evaluation of effects of circumstances in the earlier stages. In this experiment, three groups of 120 mice each were inoculated with 0.1 ml of

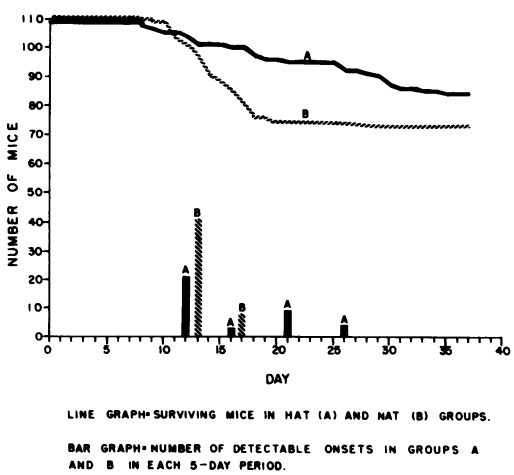

FIG. 1. *Deferred onset of illness in mice exposed to 35 C on days 6 to 13 after i.p. inoculation of rabies virus.*

lot V-16 virus by the i.p. route. Mice were placed, in subgroups of 30, in communal cages at RT and, at onset of illness, they were transferred to individual ambience cages at HAT or NAT or to the usual glass jars at RT. All sick mice were fed bread soaked in milk (Table 7).

Mortality and time of survival after onset were taken as criteria of the effects of conditions of housing after onset of illness. Survival after onset was slightly better in the usual housing, i.e., the glass jar at RT, but mortality was greatest in that situation.

DISCUSSION

Fever is considered a major factor in recovery

TABLE 7. *Survival of rabies virus-infected mice placed in 35 C, 21 C, and room temperature at onset of patent illness*

Group	Ambient temp (range)	No. of mice	Mortality (%)	Avg days survival[a]
A	35.1-36.8	65	40	5.6
B	21.6-22.5	64	42	6.4
C	20.5-22.5	66	48	6.8
Controls				

[a] Calculation of average based on 10-day maximum survival period (5).

from primary virus infections (2, 22, 26). However, the mechanism by which hyperthermia exerts its beneficial effects is obscure. *Herpes labialis* may be elicited by fever, whereas the experimental infection is inhibited by hyperthermia (28). The "effects of temperature on in vitro stability of a virus and on growth, may be independent" (3), and a variety of physiological mechanisms of the host may be enhanced or inhibited to the detriment or benefit of host or virus (4, 20, 23).

Attempts to detect a susceptible stage in the course of rabies virus infection in mice were made by exposing them to HAT at various times after i.p. inoculation of virus. A marked effect of the thermic ambience on gross mortality and on recovery from infection occurred, but those effects were proportional to total duration of exposure to HAT during the incubating period, with no indication of a particularly labile phase. The impression from the above observations was that heat had direct adverse effect upon viability of rabies virus in vivo as it does in tissue culture (12, 21), regardless of the stage of infection. However, a comparison of mortality in groups of mice exposed to high (35 C) and to normal (21 C) temperatures in ambience cages, and to normal temperature in the usual recovery cages (glass jars) after patent onset of infection, indicated absence of significant effect of ambient temperature on the central nervous system infection or its associated phenomena once signs of illness occurred.

In another experiment, mice were not exposed to high temperature until 6 days after i.p. inoculation, and then remained at that temperature for 7 days. As anticipated from earlier experiments, patent disease and mortality were less in the group exposed to high temperature. But when surviving mice of that group were put in the normal temperature ambience of the control group, a second wave of infections became apparent. Delayed onset after exposure to high temperature indicated that heat had not acted directly to inactivate virus or, at least, that was not the only effect. Onset of illness in the test group at a time when mice in the control group had either died or were recovering from infection suggested that there was inadequate antigenic stimulus for immune response, or that immune response was inhibited by heat, as was reported by Subba Rao and Glick (32). However, many other metabolic activities (4), including interferon action (24), are affected by heat. Enright et al. (13) demonstrated that metabolic rates altered by thyroidectomy caused significant increase in incubation period, but the mechanism of delay was not known. Chemical alteration of metabolic rates did not have detectable effects on rabies virus infection in experimental animals (13).

Temperatures of the extremities of mice are much more labile than somatic temperatures (29) and, if there were a direct effect of ambient temperature on the virus, peripheral inoculation would, logically, be most likely to demonstrate it. However, in parallel tests, the i.p. route had much greater effects than the pl. route.

Lwoff (22) states that fever "is one of the most constant symptoms (sic) of viral diseases and, in some instances, the only one." Johnson (19) describes unremitting fever of 1 to 3 degrees F as a prodromal sign of rabies infection, and Van Rooyen and Rhodes (35) state that "the temperature is usually raised, especially before death, and may continue to rise thereafter." They give credit for the observation to Gamaleia in 1887. "Slight rise in temperature is also characteristic of rabies in dogs" (34). In the observations reported here, there was no demonstrable advantage of exposure of infected mice to HAT after onset of patent illness.

Lycke et al. (23) found that body temperature of normal mice kept at 34 C was about 0.5 C higher than those kept at 22 C, whereas we found a difference of 2 C between HAT and NAT groups. Differences in body temperature between HAT and NAT groups were greater at the time of onset of disease. These observations demonstrate that somatic temperatures at onset of rabies in mice, unlike those reported for other animals, are significantly lower than normal, that normal mouse body temperatures fluctuate markedly with ambient temperatures, and that temperatures of infected mice can be raised (difference, 7.0 C) by increased ambient temperatures. Even that range of fluctuation does not approximate the diurnal and seasonal temperature variations of insectivorous bats

(33) which, admittedly, constitute a special case of somatic temperature range in relation to rabies infection (27). Nevertheless, it is obvious that somatic temperature variation could also affect the course of the disease in hibernating vectors, such as skunks, in the young of canines (18) and presumably in naturally infected rodents (31), whether or not they are hibernating species. Even a mature large animal such as the camel may have a morning temperature of 34 C and an afternoon minimum of 40.7 C (11). However, as Sadler and Enright (27) demonstrated, metabolic status dependent on season may be as important as body temperature in determining outcome of infection.

Attempts have been made to correlate seasonal variations in the prevalence of rabies with social activities of wild animals, e.g., mating, communal denning, fighting (36). It appears from our data that more obscure variations of physiology that accompany seasonal changes may also be involved.

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