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SIGNIFICANCE OF CRANIAL CIRCULATION FOR THE BRAIN HOMEOTHERMIA IN RABBITS. I. THE BRAIN–ARTERIAL BLOOD TEMPERATURE GRADIENT

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Abstract. The hypothalamic–arterial blood temperature gradient (T_{Hpt}–T_{AC} difference) was studied on 10 freely moving rabbits at ambient temperatures between 0 and 42°C. In cold environment, below 10°C the T_{Hpt}–T_{AC} gradient varied considerably, but some distinct correlations were found between vasomotor responses of the nasal mucosa and fluctuations of brain temperature, as well as between vasomotor responses of the ear pinnas and changes of the arterial blood temperature. Vasodilatation of the nasal mucosa or the ear pinna caused respectively a drop in brain temperature or in arterial blood temperature. Opposite changes were induced by vasoconstriction in those areas. Variations in T_{Hpt}–T_{AC} gradient resulted from oppositely directed vasomotor responses in the nasal mucosa and in the ear pinnas. At high ambient temperatures above 35°C thermal panting was accompanied by selective brain cooling with respect to the arterial blood. Blocking the heat loss from the nasal mucosa caused an increase of the T_{Hpt}–T_{AC} difference, and under these conditions brain temperature was determined solely by arterial blood temperature. The assumed mechanism of the selective brain cooling in rabbits is the exchange of heat through the neurocranial bottom, between the ventral brain and the spacious splanchnocranial venous lakes supplied with blood from the nasal mucosa.

INTRODUCTION

The metabolic heat production of homeotherms by the brain is fairly intensive and seems to be independent of ambient temperature (8). The rate of oxygen consumption by the brain is high, even during complete

behavioral rest, e.g., sleep (1). The dissipation of heat released during metabolic reactions in the brain is possible only through the circulating blood. Baker and Hayward (2–4, 9) have shown that the brain temperature is determined by that of the cerebral blood. Thus, the temperature in a given point of the brain is higher by a constant value than the temperature at the circle of Willis (2–4, 9). However, the anatomy of the arterial supply to the brain differs among various species, which is reflected by some particularities of blood temperature regulation at the level of the carotids. In such animals as rabbits and rhesus monkeys, equipped with internal carotid artery, there is no detectable temperature drop at the distance between the carotids and the entrance to the brain, so that the temperature gradient between the brain and the carotid arterial blood is constant (9). In artiodactyls and carnivores the external carotid artery is the main vessel supplying blood to the brain. Close to the base of the brain the artery branches into a large number of small vessels constituting the carotid rete. The contiguity of the rete with venous vessels coming from the upper respiratory tracts, particularly from the nasal mucosa, enables a counter-current heat exchange between the arterial and venous blood, which may cause the cooling of cerebral arterial blood by as much as 3.5°C (2, 4, 9, 16).

So far the temperature gradient between brain and arterial blood in the rabbit has been studied under thermoneutral conditions, without considering the heat loss from the nasal mucosa, (3, 9) even though it plays an important part in the thermoregulation of that species (10). Therefore it seemed worthwhile to study in greater detail the mechanism of the rabbit's brain arterial blood temperature regulation.

MATERIALS AND METHODS

Animals. Experiments were performed on 10 rabbits of both sexes, weighing 3–4 kg, kept in individual cages at room temperature 20°C ±3°C and fed with carots and alfalfa.

Surgery. A hypothalamic copper constantan thermocouple and a hippocampal EEG electrode were inserted under pentobarbital anesthesia (30 mg/kg) according to stereotaxic coordinates taken from the Sawyer et al. (13) atlas. The thermocouple was placed in a glass capillary (o.d. 0.4 mm) and positioned at P1, L1, V15 mm. A stainless steel concentric electrode (o.d. 0.5 mm) was implanted in the dorsal hippocampus at P4, L4 and V6.5 mm. Three other thermocouples in polyethylene tubes (o.d. 0.5 mm) were passed through holes trephined in the left nasal bone for

measuring the temperature of the nasal mucosa in three points: above the central part of the ventral nasal concha, above the rostral part of the concha and deep in the concha. The fifth thermocouple placed in the left nasal meatus close to the nostrils served as a sensor of respiratory frequency. The electrode and the thermocouples were fastened to a socket containing copper and constantan pins to which the corresponding wires were arc-welded. In addition, a polyethylene tube (o.d. 0.5 mm) was implanted to the wall of the internal carotid artery, and another one was placed at a distance of about 10 mm from the latter in the layer of neck muscles. Both tubes served as guides for the insertion of thermocouples. They were passed under the head skin and attached to the socket. The scheme of implantation is shown in Fig. 1.

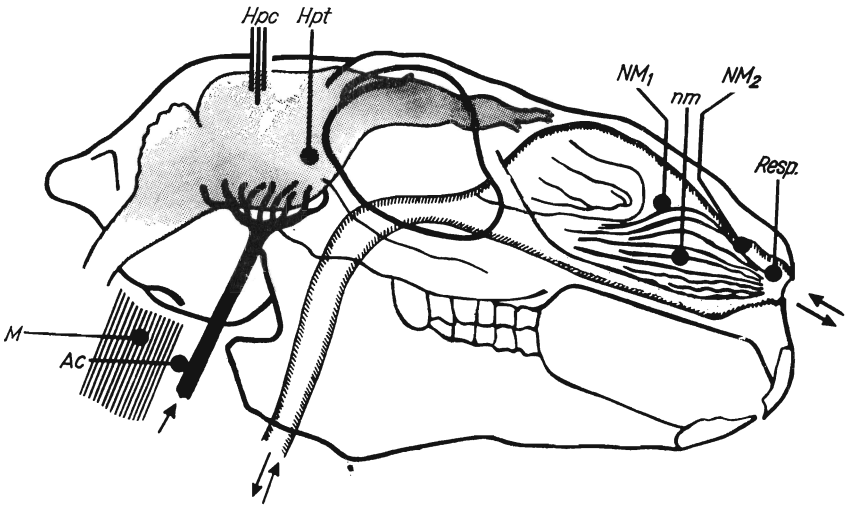

Fig. 1. The scheme of implantation. Hpc, concentric bipolar EEG hippocampal electrode. Thermocouples implanted into: Hpt, hypothalamus; NM_1 and NM_2, mucosa lining nasal cavity; nm, mucosa of the ventral nasal concha; Resp., breathing frequency sensor. M and AC, thermocouples introduced into the implanted cannulas inside neck muscles and internal carotid artery wall, respectively.

Recordings. All recordings were made in a 50×50×75 cm electrically shielded chamber at ambient temperatures ranging from 0 to 42°C and relative humidity of 30 to 100%. A cable was used, sufficiently long and flexible so as not to hinder the animal's movements.

Experimental procedures. Experiments were started about 2 wk after surgery on animals adapted to the laboratory conditions. Recording ses-

sions were performed about 2 h after feeding. The rabbits were connected to the recording system through a connector fitting the socket on the animal's head. Besides, thermocouples were introduced to the ends of two polyethylene tubes, and two other thermocouples were attached to the external surface of both ear pinnas for the period of temperature recording. The thermocouples measuring the temperature of the nasal mucosa inside the ventral concha, that of the external surface of the ear pinna, and the ambient temperature were directly connected to a 12-point millivoltmeter recorder (model MKV/T 12 VEB Messgerätwerk Erich Weinert/GDR), with sensitivity of 0.2°C per 1 mm pen deflection. Signals from the sensors placed in the hypothalamus, at the carotid and in two other places of the nasal mucosa were preamplified using a digital nanovoltmeter (model 180 Keithley Instruments, Inc., USA), so that the final sensitivity was 0.019°C per 1 mm pen deflection. Reference thermocouples were placed in a bath of crushed ice and distilled water. The measuring system was continuously calibrated by means of reference voltage from a thermocouple placed in a waterthermostat with standard mercury thermometer with scale divisions of 0.1°C, and a Beckmann thermometer with scale divisions of 0.002°C.

Long-term stability and linearity of the measuring system were tested by a nanovolt source (model 260 Keitheley Instr., Inc., USA) certified by the USA National Bureau of Standards. The thermocouples were calibrated in 39°C constant temperature bath. The overall accuracy of the measuring system was ± 0.05°C. During recordings the individual thermocouples were automatically connected at intervals of 2.5 s in sequence to the recording set-up by a swich. The chart speed was 120 mm/h.

The EEG from the dorsal hippocampus and the respiratory rate were recorded on an electroencephalograph (model 55 Kaiser, Denmark). The voltage signal from the respiratory sensor was additionally amplified by a microampere operational amplifier (Fairchild, USA).

A total of 130 sessions were conducted at ambient temperatures in the range 0–42°C, over a period of 10 mo. Each started at room temperature 20–25°C. After about 1 h of control recording the ambient temperature was raised or lowered, so that the new level was maintained for 4–8 h. The animal's behavior was carefully observed and noted on the mooving EEG and temperature recording tapes. Hippocampal EEG allowed to recognize easily the paradoxical sleep episodes. In 26 sessions, after the hypothalamic temperature had stabilized over at least 30 min, 0.3 mg of Naphasoline (Alfa-naphtylmethyl-imidazoline), a local vasoconstrictor agent in aqueous solution, was introduced into both nasal cavities, and the recordings continued for subsequent 2–3 h. The position of the electrode and of temperature sensors was checked histologically.

RESULTS

Parallelity of brain and arterial blood temperature fluctuations. The T_{Hpt}–T_{AC} difference often shows an almost steady value in the warmer part of the thermoneutral zone, i.e., between 25 and 32°C, but only intermittently, over brief 10–30 min periods and rarely longer. The course of one of the experiments shown in Fig. 2 can serve as an illustration of the above.

Changes in the temperature of the ear pinna are commonly used as an indicator of vasomotor responses in that area. In a thermoneutral and cold environment a decrease in temperature is interpreted as vasoconstriction, while an increase — as vasodilatation. Vasomotor responses in the nasal mucosa can be evaluated similarly, since fluctuations of its temperature are often independent of changes in the respiratory rate.

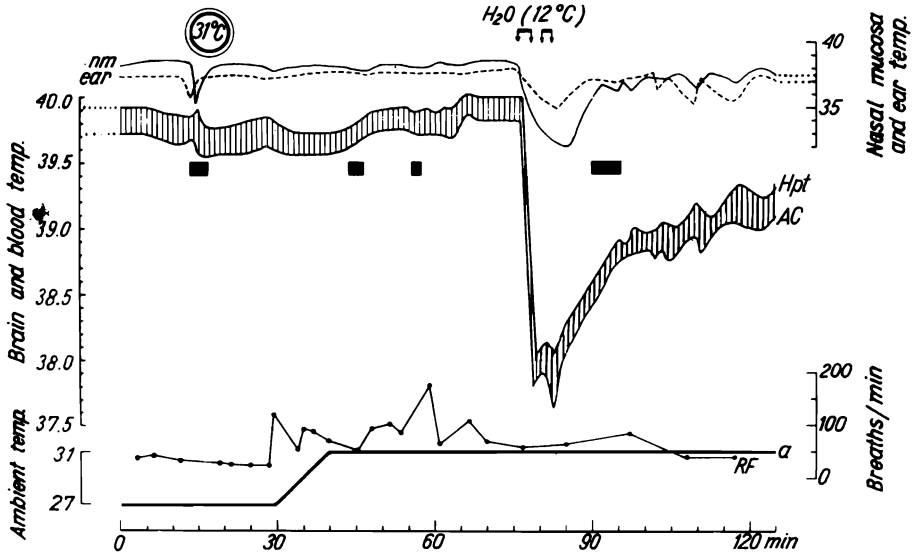

Fig. 2. A fragment of an experimental protocol carried out at a warm border of the thermoneutral zone (31°C). The influence of cold (12°C) water drinking. Double arrows, water drinking; black rectangles, locomotor activity; RF, breathing frequency. Temperature curves: nm, mucosa of the ventral nasal concha; ear, pinna; Hpt, hypothalamus; AC, carotid artery; a, ambient. Vertically striped area, the T_{Hpt}—T_{AC} gradient.

During behavioral arousal, at about the 15th min of the experiment presented in Fig. 2, simultaneous vasoconstriction of the nasal mucosa and in the ear pinna occurred, parallely to the increase of both brain and arterial blood temperature. During that time the T_{Hpt}–T_{AC} gradient

did not change. Drinking cold water of about the 77th and 82th min of the experiment, elicited an abrupt and significant cooling of the arterial blood, which in turn effected a drop in brain temperature. At the same time vasoconstriction in the nasal mucosa and in the ear skin began and lasted for 15 min. The lowering of heat loss through these outlets enabled a rapid restoration of the arterial blood and brain temperatures to normal levels.

In a resting and relaxed rabbit, at ambient temperature 25–32°C we have seen often full vasodilatation both in the nasal mucosa and the ear pinnas. On the other hand, numerous periods of behavioral arousal and paradoxical sleep were accompanied by simultaneous vasoconstriction in both areas. Parallel increase of both the arterial blood and brain temperatures caused the T_{Hpt}–T_{AC} gradient to remain unchanged.

Fluctuations of the T_{Hpt}–T_{AC} *gradient in a cool environment.* The progress of one of the 12 experiments performed in cool ambient temperature is shown in Fig. 3. The value of the T_{Hpt}–T_{AC} difference in each

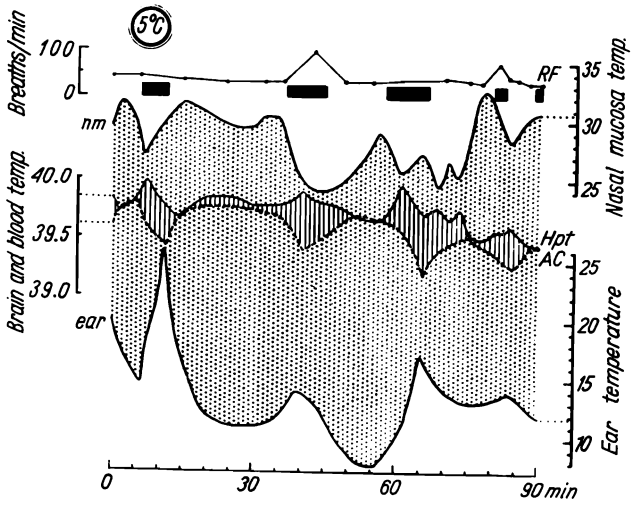

Fig. 3. A fragment of experimental protocol carried out in cold environment (5°C). Same designations as in Fig. 2. Dotted area accentuates correlations of temperature curves.

experiment of this group underwent wide and frequent changes, but distinct correlations between (i) averaged temperature changes of both ears and fluctuations of arterial blood temperature, and between (ii) nasal mucosa and hypothalamic temperatures were present. We conclude therefore that vasodilatation of the ear skin produces the cooling of arterial blood, while the same in the nasal mucosa elicits the cooling of

the brain. On the other hand vasoconstriction in the ear skin or nasal mucosa is followed by an increase in the arterial blood or hypothalamic temperature, respectively.

Opposite vasomotor changes in the nasal mucosa and ear skin accounted for the great variability of the T_{Hpt}–T_{AC} gradient. During relaxed wakefulness in cool environment deep vasoconstriction of the ear pinnas was observed, with only a moderate vasodilatation in the nasal mucosa. Therefore the brain was more efficiently cooled via the nasal mucosa than the arterial blood via the pinnas. The T_{Hpt}–T_{AC} difference was small or even non-existent. Behavioral arousal, on the other hand repeated four times in that experiment (6th–12th min, 38th–46th min, 58th–68th min, and 81st–83rd min), caused vasoconstriction in the nasal mucosa and vasodilatation in the ear skin, resulting in a rise of hypothalamic temperature and a drop of arterial blood temperature. Therefore the T_{Hpt}–T_{AC} gradient widened significantly.

The results of the experiments performed in cool environment suggest that the brain temperature is partially independent of the arterial blood temperature.

Selective brain cooling during panting. When the rabbit is exposed to a high ambient temperature, close to arterial blood temperature, the dry heat loss from ear pinnas disappears, in spite of maximum vasodilatation of their vessels. Under such conditions a rapid evaporative heat loss from the nasal mucosa is the only way to avoid lethal hyperthermia. As long as the respiratory frequency in hot environment remains low, the brain and blood temperature increase with unchanged T_{Hpt}–T_{AC} gradient. Figure 4 shows typical thermoregulatory responses recorded

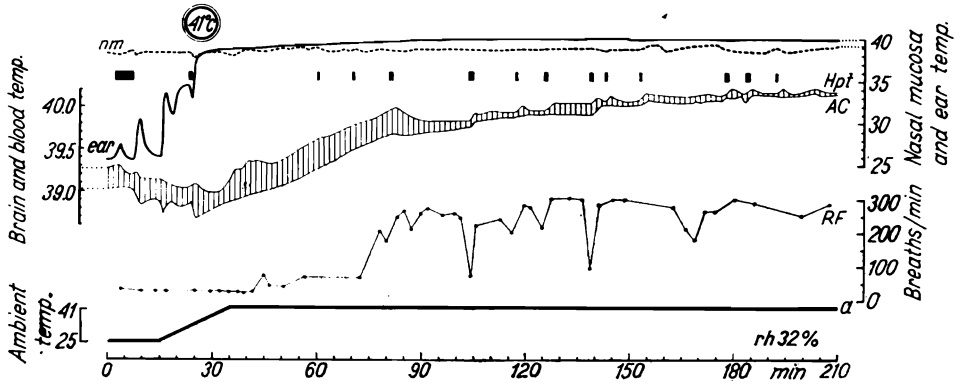

Fig. 4. A fragment of an experimental protocol showing the rabbits defence reactions agains hyperthermia in dry heat — 41°C and 32% of relative humidity (rh). Some designations as in Fig. 2.

in 30 experiments of this group. Panting began at about the 80th min of the experiment. A significant increase in respiratory rate and consequently in evaporative cooling caused a selective decrease in brain temperature. The respiratory evaporation was the reason due to which the hypothalamic temperature rise was greatly reduced and the T_{Hpt}–T_{AC} difference slowly continued to decrease.

Effect of blocking heat dissipation from the nasal mucosa on the T_{Hpt}–T_{AC} *difference.* In the last series of 26 experiments selective vasoconstriction of the nasal mucosa was elicited by Naphasoline, the alfa-adrenergic drug. The pharmacological limitation of blood flow through the nasal mucosa was accompanied by a reduction in the heat convection to the surface of the mucosa. At 14°C ambient temperature the pharmacological vasoconstriction was strong and persistent (Fig. 5). It caused

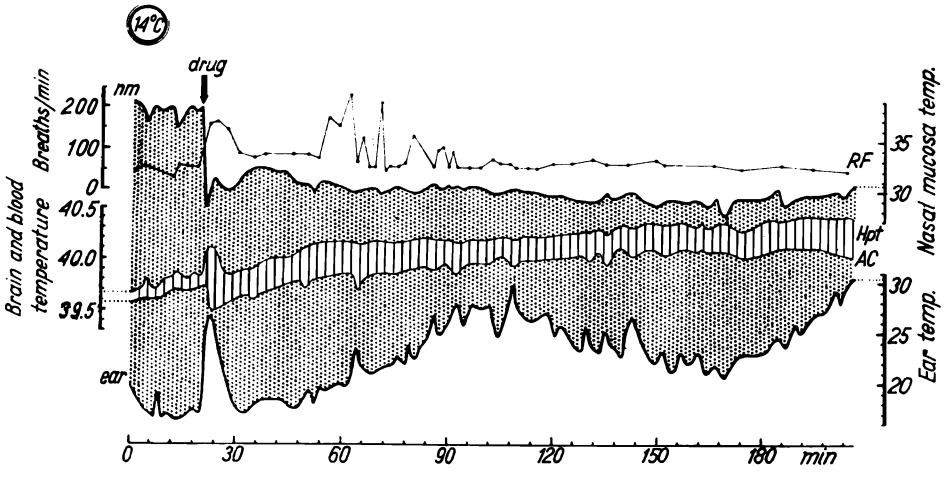

Fig. 5. A fragment of an experimental protocol showing elimination of the selective brain cooling at 14°C ambient temperature by means of pharmacological vasoconstriction of the nasal mucosa. Arrow, the moment of application of Naphasoline (0.3 mg) solution the nostrils. Some designations as in Fig. 3.

initially an increase of the T_{Hpt}–T_{AC} difference. During the subsequent 3 h the gradient maintained a steady and high value. It suggests that the arterial blood temperature, under such conditions, is the sole determinant of the brain temperature due to the reduction of heat dissipation from the nasal mucosa. The average of the steady for several hours T_{Hpt}–T_{AC} gradient obtained from seven experiments was 0.34°C ± 0.07°C SD.

DISCUSSION

The T_{Hpt}–T_{AC} gradient and its determinants. The stability of the brain-arterial blood temperature gradient reported in rabbits by Baker and Hayward (3, 9) cannot be accepted as a rule in the light of our experiments. It occurs only during short episodes, not exceeding 2 h, and in a narrow range of ambient temperatures, between 25°C and 32°C when parallel vasomotor responses take place in ear pinnas and in nasal mucosa. However, the T_{Hpt}–T_{AC} gradient becomes variable when the responses are not parallel. This is particularly evident in a cold (below 10°C) environment, because then the vasomotor responses of the ears oppose those of the nasal mucosa. Therefore, the selective influence of the vasomotor responses of the nasal mucosa on the brain temperature becomes apparent, while the arterial blood temperature is regulated independently by means of vasomotor responses of the ears. Accordingly, the selective cooling of the brain is most intensive during panting, due to the large increase in the respiratory evaporative heat loss. Thus the rabbit's brain is not cooled exclusively through the carotid arterial blood, as was suggested by Hayward and Baker (9), but also by the venous blood returning from the nasal mucosa.

Possible mechanism of brain cooling in rabbits. The scheme of blood supply to the rabbit's head presented by Hayward and Baker (9) is insufficient to explain the mechanism of its brain homeothermia. Recent anatomical studies performed by Godynicki (7) failed to show the existence of a counter-current heat exchanger in the rabbit's head, similar to that discovered in artiodactyls (4, 9, 16) and carnivores (2, 9). So, selective cooling of the brain in this species must be brought through about the action of a different mechanism. As revealed in the above-mentioned study (7), venous blood returning from the nasal mucosa enters the large venous lakes, the ophthalmic sinus and the pterygoid plexus. They adjoin the neurocranial bottom over a substantial area. The appreciable enlargement of the vessel bed must be accompanied by slowing down of the blood flow through the venous lakes. Their blood may be considerably colder than the brain as a result of evaporative cooling from the nasal mucosa. We suppose, therefore, that selective cooling of the rabbit's brain may be based on the heat exchange by conduction between the brain tissue and the blood of the venous lakes. The only obstacle to this heat exchange could be the neurocranial bottom. However, its bone layer in the contact surface between the brain and venous lakes is exceptionally thin, and above the pterygoid plexus even perforated. Such mechanism of heat exchange accounts for the limited efficiency of selective brain cooling in rabbits compared with the very effective venous-arterial counter-current heat exchanger in the head of

Thomson's gazelle (16), sheep (4), and cat (2). The temperature of the selectively cooled rabbit's brain measured in the hypothalamus can be no more than 0.1°C lower than arterial blood temperature. Under such conditions the cooler venous blood must carry away not only the whole heat produced by the brain metabolism, but also some extra heat entering the central nervous system by the carotid arterial blood. Therefore, during dynamic vasodilatation of the nasal mucosa, there are sometimes disturbances of the mucosa-brain temperature correlation. We succeeded in producing pharmacological suppression of the selective brain cooling. We have shown that the steady T_{Hpt}–T_{AC} gradient determined in such conditions by arterial blood temperature averaged 0.34°C ± 0.07 SD. This value is about 0.1°C + 0.34°C = 0.44°C higher than during the highest selective cooling of the hypothalamus. Thus we may suppose that the rabbit's brain may be selectively cooled even by 0.44°C in relation to the value determined by the arterial blood temperature.

The selective effect of the respiratory heat loss from the nasal cavity on the rabbit's brain temperature has been recently reported by Kluger and D'alecy (12).

Biological significance of selective brain cooling. The selective cooling of the brain has been found in many species of homeotherms, especially among those living in a hot climate (2, 4, 11, 16). It appears to be common among the panting animals to protect the brain from hyperthermia, probably even if they have no carotid rete. The fact that the respiratory heat loss has a direct effect on the brain temperature in rabbits confirms this opinion.

A precise brain homeothermia is necessary for the preservation of integrating function of the central nervous system. It is based on complex enzymatic reactions, the dynamics of which are remarkably temperature sensitive. The Mg^{++} dependent ATPases of the rat's brain are thermally inhibited already at temperatures above 40°C (5), while the deep trunk temperature of Grant's gazelle may attain even 46.5°C with no observable ill-effects (15). The above data enable us to conclude that hyperthermia is a particularly dangerous state for the brain. The jack rabbit (*Lepus californicus*), closely related to the laboratory rabbit, in severe heat stress tolerates rectal temperature even above 44°C (14). Such high brain temperature would be lethal in mammals. We suppose, therefore, that in the heat stressed jack rabbit the cerebral temperature remains considerably below the rectal temperature.

The role of the splanchnocranial venous lakes in the selective cooling function of the rabbit's brain is the subject of the next paper (6).

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