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Shift of Threshold Temperature for Shivering and Heat Polypnea as a Mode of Thermal Adaptation*

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Summary. Newborn guinea pigs were divided into three groups and kept for several weeks at one of the following environmental conditions: 1. 28° C, WA-animals; 2. 3° C, CA-animals; 3. 12 hrs daily at 3°C, 12 hrs at 28° C, CWA-animals. At the age of 4—7 weeks threshold temperatures were determined for shivering (electrical muscle activity) and heat polypnea, and the maximum amount of nonshivering thermogenesis was measured using the noradrenaline test. In the CA-animals both shivering and heat polypnea threshold were found to be decreased in comparison with WA-animals by about 1° C; for these studies the animals were placed and immobilized in a climatized respiratory chamber. In another series of studies, in which the animals were unrestrained, the mean colon temperature of CA-animals was about 0.5°—1° C lower than in WA-animals when exposed to ambient temperatures of 22.5 and 30° C for 24 and 2 hrs, respectively. At 15° C ambient temperature there was a similar but smaller temperature difference. Hence, both types of studies would indicate that the "set point" of the temperature control system was set to a lower level in CA-animals. In CWA-animals the shivering threshold was decreased as in CA-animals; the heat polypnea threshold, however, remained as high as in the WA-animals. This "widening of the interthreshold zone" in CWA-animals is shown to provide a more economical temperature regulation when the animals are subjected to fluctuating environmental conditions, as they are enabled to tolerate body temperature changes to some extent before they actuate their cold or heat defense mechanisms. With regard to the ability of NST (i.e. metabolic cold-adaptation) CWA-animals were not different from CA-animals.

Key Words: Temperature Regulation — Set Point Temperature — Thermal Adaptation — Non-Shivering Thermogenesis — Control of Shivering — Control of Heat Polypnea.

Schlüsselwörter: Temperaturregelung — Sollwerttemperatur — Thermische Adaptation — Zitterfreie Wärmebildung — Steuerung des Kältezitterns — Steuerung der Wärmepolypnoe.

There is no doubt that warm-blooded animals and man are able to adapt to stressful climatic conditions. Our knowledge is quite incomplete, however, as for the principles and basic mechanisms of the numerous

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modifications occurring in the course of long-term adaptation. This is particularly true with regard to cold-adaptation and here with special regard to man. A decisive step forward appears to have occurred when it was shown in rats, more than one decade ago, that shivering is replaced by the more economical non-shivering thermogenesis (NST) in the course of cold-adaptation [9,13]. In extending the studies to other species and particularly to man it was soon recognized, however, that this type of cold-adaptation does not play the dominant role originally ascribed to it (for references see [7]). Later, it was even shown that there exist mammalian species which are entirely incapable of NST [7,14]. These species may display instead an increased *shivering* ability after prolonged cold-exposure as we have recently shown in the miniature pig [7].

Some years ago, another hitherto unknown type of cold-adaptive modification was described, viz. a shift of the threshold temperature for shivering in the guinea pig [5]. To evaluate the biological significance of such a shift it appeared necessary to study, besides the shivering threshold, the threshold conditions for a heat dissipating mechanism. In the course of these studies basic differences have been found in threshold behaviour, depending on whether the animals were exposed *constantly* or *intermittently* to a cool environment. A preliminary partial account of these studies has been given elsewhere [8].

Methods

In our studies we used guinea pigs which had been raised in the Physiological Institute at Marburg. They were fed on a standard guinea pig diet (Altromin®); tap water was supplied ad libidum. The birth weight of the experimental animals ranged from 75—120 g.

In the first series of studies, 26 animals were subdivided into three groups and these were raised under three different environmental conditions: Group 1 (cold-adapted = CA-animals) was maintained in a climatized animal chamber at +3° C (± 0.5° C) throughout the period of study; group 2 was kept in a warm chamber with temperatures ranging between 28 and 30° C (warm-adapted = WA-animals). A third group was maintained at 28—30° C during the day (12 hrs) and at +3° C during the night (CWA-animals). The animals of group 1 and 2 had been born in the respective environment in which they were later kept. Group-3 animals (CWA-animals) were born in a room with the common temperature of our animal house (about 23° C). All newborn animals were left with their mothers until weaning (18 days after birth). This procedure alleviated the cold stress for the neonates of groups 1 and 3 in that, during the first few days after birth, the neonates were able to protect themselves partially from the cold by gathering around and below the mothers body; after that time, however, they were observed to move freely around most of the day, thus being fully exposed to the cold environment.

The test studies were carried out at an average age of 6.5—7.5 weeks; the average body weight amounted to between 252 and 322 g at the time of test study (Table 1a).

Table 1. *Body weight and age of the guinea pigs studied*

Group	*N*	Adaptation temp. (° C)	Mean body weight	Mean age
			at time of test study (g)	(weeks)
a) series 1		(studied in a perspex chamber):		
1 (CA)	8	+ 3	252 (42.2)	6.2 (1.85)
2 (WA)	8	28—30	322 (75.4)	6.9 (1.58)
3 (CWA)	10	+ 3 (12 hrs) 28—30 (12 hrs)	307 (37.7)	7.3 (0.64)
b) series 2		(freely-moving animals):		
4 (CA)	10	+ 3	299.3 (13.8)	5.65 (0.34)
5 (WA)	10	28—30	290.8 (16.5)	5.95 (0.24)
6 (CA)	5	+ 3	281.8 (13.8)	5.7 (0.12)
7 (CWA)	5	+ 3 (12 h) 28—30 (12 h)	351 (11.3)	5.9 (0.46)
8 (WA)	3	28—30	335.3 (3.2)	7.8 (0.16)

(Figures in brackets = S.D.)

The series—1 test studies were carried out in animals which were placed for the examination in a climatized perspex chamber, as in previous studies [2]. Here, the unanaesthetized animals were fixed on a plate with strings bound around the distal parts of the four extremities in order to prevent them from nibbling the leads of the thermocouples and probes. The temperature could be maintained in the chamber within 0.5° C and it could also be quickly changed by water circulation through its jacket.

For determination of gaseous metabolism, the open system method was used. Oxygen uptake, chamber temperature, colon temperature, cervical spinal cord and two subcutaneous temperatures (one in the area of the back, the other in the region of the interscapular adipose tissue) were continuously recorded. The electrical activity of the back and thigh musculature was picked up by needle electrodes in those experiments which required the determination of shivering threshold; the discharges were continuously integrated and the summed voltage was recorded as previously described [3]. Respiratory frequency was determined by means of a fine thermocouple which was stitched to the nose so that it remained within the airstream of one of the nostrils throughout the experiment. The respiratory temperature fluctuations were recorded, after preamplification, for periods of 10—30 sec on a fast responding recorder at intervals ranging between 1 and 3 min. The direct recording of these temperature oscillations made it easy to notice artefacts which might have been caused by displacement of the thermojunction or by abrupt movements of the animals.

In the experiments in which the hypothalamus temperature was recorded, the thermocouples were stereotaxically implanted under general anaesthesia (Nembutal 40 mg/kg), one day before the test study.

On the day of the test study, the animals were kept under light ether anaesthesia while the electrodes and probes were being inserted. The test study was not commenced, however, until the effect of this short anaesthesia had vanished.

For the second series of studies a total of 35 animals were subdivided into three groups and adapted in the same way as in series 1. The test studies were performed, however, in a large climatized chamber where the animals could freely move; only a temperature probe had been inserted into the colon and this did not interfere with normal behaviour, i.e.—in contrast to series 1—these animals were able to curl up in a cold environment and to stretch out their extremities in a warm environment. The weight and age data for these animals are given in Table 1b (groups 4—8).

Results

Shivering Threshold in Animals Exposed Intermittently to Cold (+3° C)

As previously shown, shivering is controlled in the guinea pig by at least two sets of thermal receptors, the one being located in the body surface, the other in the cervical spinal cord [3,4,6,17]. (Hypothalamus

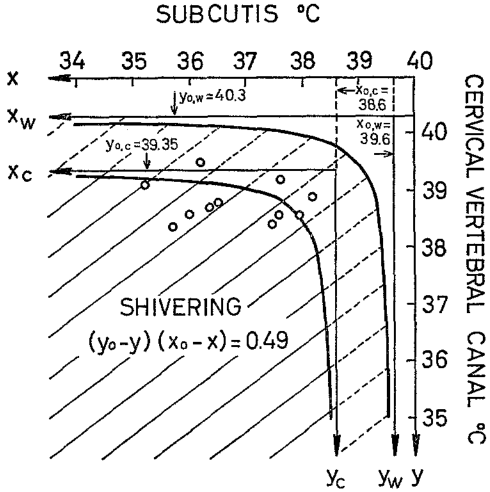

Fig. 1. The two hyperbolas, which both follow the general equation $(y_0-y)\,(x_0-x) = 0.49$, represent shivering threshold as previously determined [5] in cold-adapted (index *c*) and warm-adapted (index *w*) guinea pigs. The two hyperbolas are distinguished by different values of y_0 and x_0, which may be thought of as symbolizing two "reference temperatures" of the control system. The circles represent the corresponding threshold temperatures in 10 guinea pigs which had been exposed *intermittently* to cold (CWA-group)

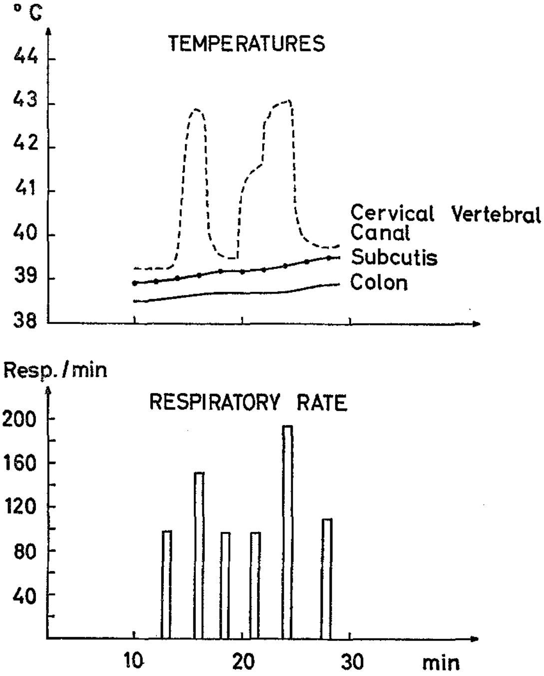

Fig. 2. Study in a guinea pig. Response of respiratory rate to local heating of the cervical spinal cord. Ambient temperature 28° C

temperature, in contrast, proved to be of little influence on shivering, at least in the newborn and young guinea pig; [3, 11].) By changing the two temperatures independently of each other shivering threshold curves were obtained which had the shape of rectangular hyperbolas (Fig. 1), indicating a multiplicative transformation of the input signals. In cold-adapted animals (CA-groups, see methods) the asymptotes of the hyperbolas were found to be shifted, by about 1° C, to a lower temperature level in comparison with the warm-adapted animals (WA-group). In the group of animals which had been intermittently exposed to $+3°$C (CWA-animals; see methods) the pairs of temperature values which corresponded to shivering threshold conditions (electrical activity $\leqq 5$ mV/sec; cf. [4]) fell closely in the range of the hyperbola of the CA-group (see circles in Fig. 1). It could thus be concluded that a daily 12-hrs exposure to cold is as effective as constant exposure to cold with regard to producing a deviation in the shivering threshold.

Heat Polypnea Threshold

According to our preliminary studies heat polypnea in the guinea pig is controlled by at least three sets of thermoreceptive structures,

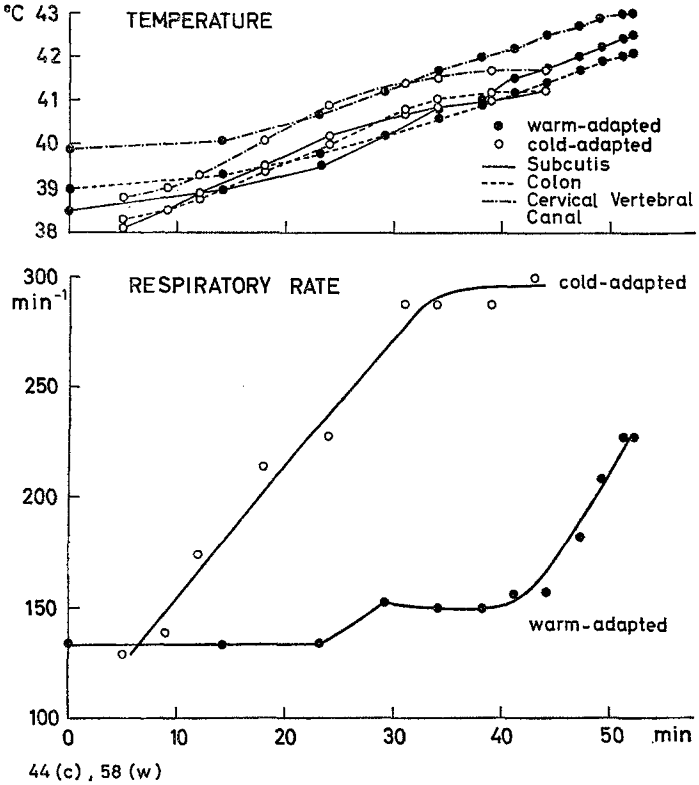

Fig. 3. Respiratory frequency and various body temperatures in a cold-adapted and a warm-adapted guinea pig after elevation of ambient temperature (at time 0) from 28—40° C

namely, 1. body surface thermal receptors, 2. thermoreceptive structures in the cervical spinal cord (Fig. 2) and, 3. the thermoreceptive structures of the preoptic region of the hypothalamus. The quantitative relationship between heat polypnea and the respective local inner body temperatures has still to be revealed, but for our purpose such studies are not prerequisite since, as may be seen from Fig. 3, the various body temperatures increase parallel and are rather close together when the animal is heated up, quite in contrast to the behaviour observed in cold exposure (cf. [4,5]). There are also no significant differences in the temperature fields in the animals of the three groups. Thus, for the purpose of comparing the three groups of animals, the respiratory rate might be related to any one of the three measured temperatures (see Fig. 3); in fact, we related it to the arithmetic mean of the subcutaneous temperature in the back area and the colon temperature; this tempera-

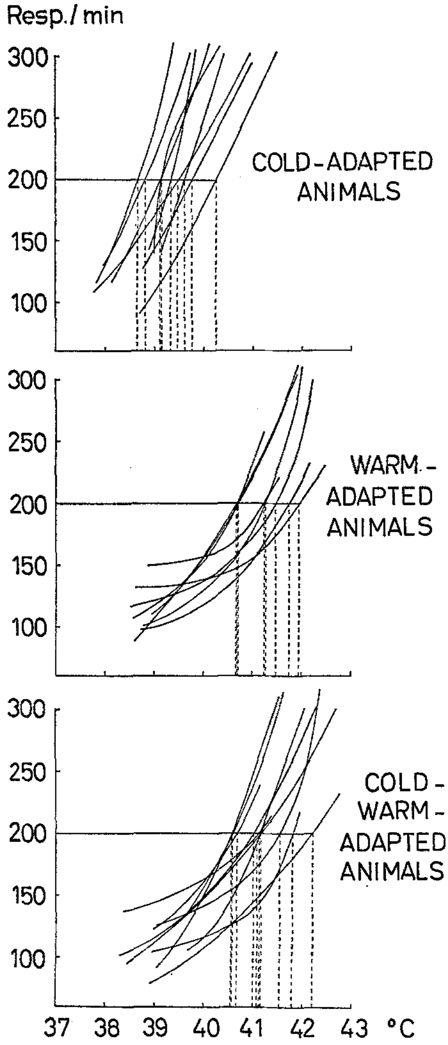

Fig. 4. Respiratory frequency in relation to "mean body temperature" (= arithmetic mean of subcutaneous temperature over the os sacrum and colon temperature) in cold-adapted, warm-adapted, and intermittently cold-adapted guinea pigs. Temperature at respiratory frequency of 200/min is taken as "heat polypnea threshold" (cf. Table 2)

ture is subsequently referred to as "mean body temperature". Fig. 3 compares the change in temperature and respiratory rate following the elevation of the ambient temperature from 28°—40°C in a cold- and a warm-adapted animal. There is a striking difference between the two animals: In the CA-animal respiratory frequency increases much sooner and after a much slighter increase in body temperature than in the WA-animal. The cold-adapted animal stabilizes its body temperature at about 41°C, obviously by means of the considerably increased polypnea; in contrast, the warm-adapted animal's body temperature keeps rising and it is only with body temperature above 41°C that the respiratory rate begins to rise.

In Fig. 4 the results of the whole set of experiments are summarized by a plot of respiratory rate against mean body temperature. These diagrams show that in the animals which had always been maintained in the cold (CA-animals), the curves are all shifted to the left in comparison with the WA-animals. In the animals which had only been intermittently cold-exposed (CWA-animals), however, no such shift was found.

Table 2. *Threshold temperature for heat polypnea in three groups of animals*

	CA	WA	CWA
Mean	39.36	41.22	41.16
n	9	8	10
σ	0.49	0.49	0.53
σ_M	0.16	0.17	0.17
p	└─ < 0.001 ─┘		│
	└──── < 0.001 ────┘		

CA = cold-adapted animals; WA = warm-adapted animals; CWA = animals exposed to + 3° C for 12 hrs daily (for details see Methods).

For a more quantitative evaluation of these results the mean body temperatures were taken at which the respiratory rate attained a value of 200/min; in most cases, this respiratory rate fell in the part of the curve with the steepest slope. Operatively, these temperatures were denoted "polypnea threshold temperatures". According to Table 2 polypnea threshold temperatures are almost 2°C apart in the WA-and CA-animals, and this difference is highly significant statistically. Quite in contrast to the CA-animals, in the CWA-animals there was no significant deviation in the polypnea threshold when compared with WA-animals. (Table 2).

Non-Shivering Thermogenesis

According to the foregoing experiments a daily 12-hr exposure to cold was sufficient to produce a shift in the shivering threshold as well as perpetual cold-exposure, whereas the heat polypnea threshold remained uninfluenced in the CWA-animals when compared with the WA-animals. Here, the question arose whether the CWA-animals would display non-shivering thermogenesis as CA-animals have already been shown to do [2]. Thus, in eight CWA-animals the maximum extent of NST was determined, using the noradrenaline-injection method [18]. Intramuscular injection of noradrenaline (200—600 μg/kg) produced, on the average, an increase in oxygen consumption of 60% (Table 3), a value which has also been found in a group of CA-animals studied previously [18]. The electromyographic records showed that this increase in oxygen consumption was not due to muscular activity.

Table 3. *Non-shivering thermogenesis in the group of animals exposed to + 3° C for 12 hrs daily (CWA-animals)*

Nr. of animal	Age	i.m. NA	Oxygen consumption ml/kg-min		
	weeks	μg/kg	minimum resting level	after NA	percentage rise
319	8	200	20	37	85
312	8.5	200	17	24.2	43
315	7	200	12.6	17.6	39
316	7.5	200	16.1	33.4	107
314	10	300	17.0	24.0	41
320	9	400	18.6	23.0	24
		600	18.6	29.0	56
315	7.5	600	18.6	31.6	88
318	9	600	18.7	30.1	62
Mean					60.6

NA = noradrenaline.

Colon Temperature in Unrestrained Animals

Assuming that the threshold temperatures in CA- and WA-animals are different not only for heat polypnea and heat production, but also for vasomotoric and behavioural temperature-control mechanisms, one may infer that the former stabilize their body temperatures at a lower level than the latter when they are exposed, in an unrestrained state, to various environmental temperatures. Our second series of studies (cf. Methods) supports this assumption. Thus, Fig. 5 shows that the CA-animals colon temperature is lower than that of the WA-animals

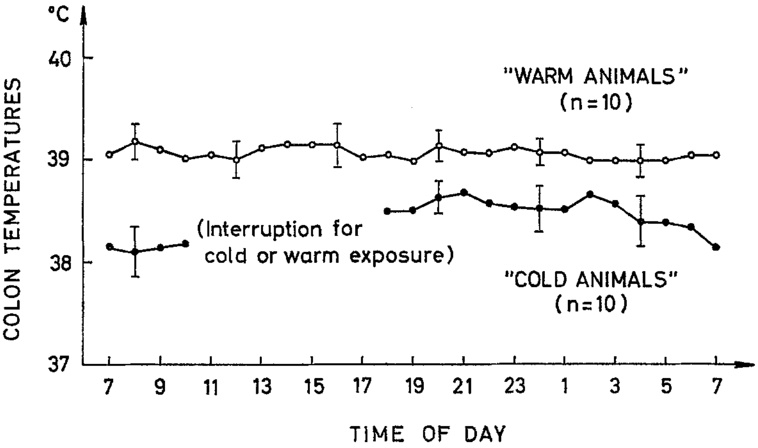

Fig. 5. 24-hr course of colon temperature in warm-adapted and cold-adapted unrestrained guinea pigs at an ambient temperature of 22.5(±0.5)° C. Bars indicate S.E. For weight and age data of the animals see Table 1, groups 4 and 5

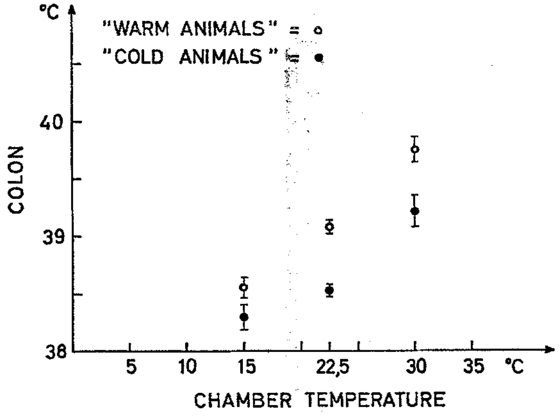

Fig. 6. Comparison of colon temperature in cold-adapted and warm-adapted guinea pigs exposed to three different ambient temperatures. For 22.5° C: 24-hr mean; for 15 and 30° C: 2-hr means. Bars indicate S.E. Differences at 22.5 and 20° C ambient temperature are statistically significant ($p < 0.001$ and $p < 0.01$, respectively). For age and weight data of the animals see Table 1, groups 4 and 5

by 0.5—1°C throughout the day at an environmental temperature of 22.5°C. Fig. 6 shows that there are similar differences, when the animals are kept in a moderately cool or warm environment.

Under transient temperature conditions (Fig. 7) CA- and WA-animals show another characteristic difference in behaviour: With increasing

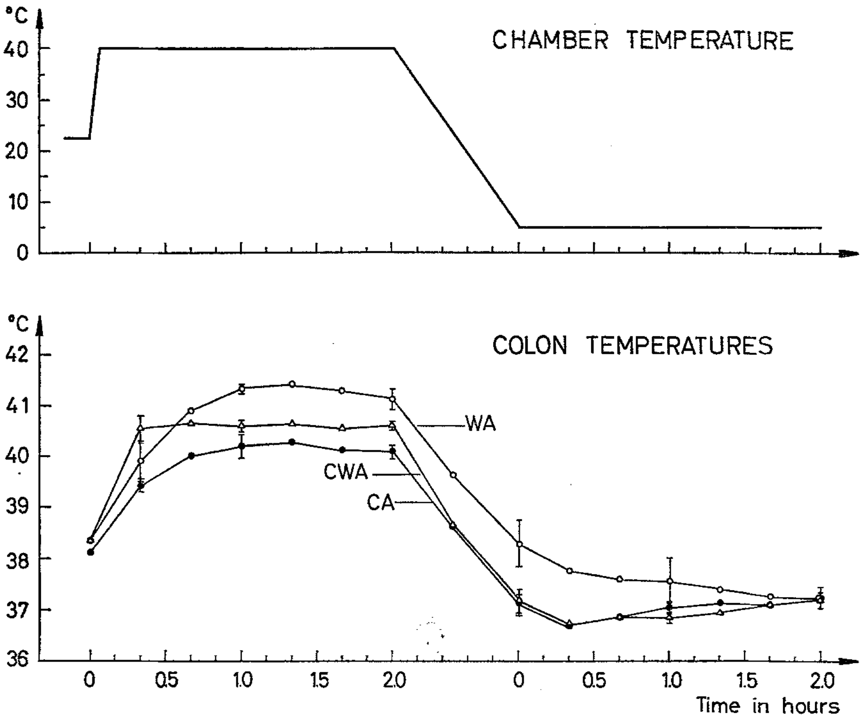

Fig. 7. Colon temperature transients in warm-adapted (*WA*), cold-adapted (*CA*) and intermittently cold-adapted (*CWA*) guinea pigs. Note steeper increase of colon tempeature in WA and CWA animals with increasing ambient temperature; similarity of CA and CWA-animals at decreasing ambient temperature. Bars indicate S.E. For weight and age data of the animals see Table 1, groups 6—8. For further explanation see text

environmental temperature WA-animals show a steeper rise than CA-animals in body temperature, which is eventually stabilized at a higher level than in CA-animals. With decreasing environmental temperature, the colon temperature of CA-animals shows a steeper drop and reaches a lower level than in WA-animals. This difference in body temperature vanishes, however, within 1—2 hrs in the cold; this is to be expected, since the WA-animals do not have such high summit metabolism as the CA-animals, who are capable of non-shivering thermogenesis in addition to shivering.

The CWA-animals behave similarly to the WA-animals with regard to the steeper temperature increase occurring with rising environmental temperature; with falling environmental temperature, however, the course of colon temperature is almost identical with that of the CA-animals, as one would expect from the fact that, in CWA-animals threshold temperatures for heat dissipation and heat production plus preservation shift in opposite directions (cf. Figs. 1 and 4).

Discussion

The shift of threshold for both a heating and a heat-dissipating mechanism in CA-animals, together with the fact that they maintain colon temperature at a lower level than WA-animals, can be interpreted as a set-point deviation in the temperature control system. Here, it must be considered, however, that, as shown by Fig. 1, there is no *single temperature* which could be called the "controlled temperature" or the "controlled variable" (see also [4,6]); the controlled variable of the temperature control system must rather be thought of as a *function* of the temperature in the various parts of the body, where thermal receptors are located, such as the cervical spinal cord, the hypothalamus, and the skin. Further, the "set-point temperature" must be considered as a function of the various reference temperatures which are given e.g. by the asymptotes of the respective hyperbolas in Fig. 1. As has been previously shown [4,6] the product of at least two temperature deviations from their respective reference values determines a heat defense reaction (e.g. shivering; see Fig. 1) and thus the controlled variable. On this basis the cold-adaptive modifications described in the CA-animals could be ascribed to a change in some elements which produce temperature reference signals. There is some evidence that such signals are produced by certain thermo-*in*sensitive neurons in the hypothalamus [10,15,20]. In recent studies it was possible to produce threshold changes for non-shivering thermogenesis and shivering in the guinea pig by microinjections of noradrenaline into a non-thermosensitive region located between the area hypothalamica ant. and post., i.e. caudally to the thermosensitive preoptic region [19]. It has been suggested that the former area may contain the postulated reference units. It is not clear, however, if set point changes as observed during long-term adaptation are mediated by catecholamines, though this possibility must be considered (cf. [20]).

As regards the tendency to maintain a somewhat decreased temperature level, the CA-animals resemble the behaviour of the Australian aborigines studied by Hammel et al. [12]. The biological significance of such behaviour could be seen in a certain reduction of the metabolic expenditure for temperature regulation, but in the guinea pig this would not make up more than 10% of BMR, even under severe cold exposure [4]. In a warm environment, this modification would, however, be disadvantageous, since the animals would have to actuate and maintain respiratory heat dissipation at a lower body temperature than the WA-animals.

According to the present studies, the CWA-animals do not encounter this disadvantage in a warm environment, since the threshold for respiratory heat dissipation does not shift down along with the shivering threshold during *intermittent adaptation* to a cool environment (Fig.4). In other words, there exists in this group of animals a mode of adaptation which is characterized by a "widening" of that body temperature range at which neither thermoregulatory heat production nor respiratory heat dissipation is elicitable. Hence, when these animals are exposed to an environment with fluctuating temperatures (such as day-night alterations in temperature) they can make use, to a much greater extent than WA-animals, of the heat storing capacity of their bodies, i.e. use it as a heat buffer. Thus, the "widening of the interthreshold zone" provides the basis for a more economical temperature regulation. It has been known for some time that the camel's tremendous heat tolerance is due partly to the wide fluctuations in body temperature [16]. Our studies in the guinea pig show that such behaviour can be evoked in other species by long-term adaptation to a fluctuating thermal environment. It remains to be shown whether man is able to develop this type of thermal adaptation. This would appear to help him to withstand abrupt environmental temperature changes with a lesser degree of unpleasant feelings. This might be more important than extreme stability of body temperature.

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