ONTOGENY OF HEAT DEFENSE IN THE RAT

ONTOGENIC DEVELOPMENT OF HEAT DEFENSES
IN THE YOUNG RAT

by

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SCOPE AND CONTENTS: Neonatal rats exposed to heat stress were studied to determine the age at which the saliva-spreading response appears, and to elucidate any other heat defenses that might exist before the response develops.

Saliva-spreading appeared on the 17th day of age, the age at which hypothalamic maturity is attained. This finding thus agrees with previous hypotheses that the hypothalamus is involved in the regulation of body temperature.

The tolerance of rats to an ambient temperature of 40 C dropped from 16 - 26 hours to 2 - 4 hours in the first 10 days of life. Three factors accounted for this change: decreased body water, increased rates of water loss, and increased metabolic rate.

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TABLE OF CONTENTS

FIGURES

TABLES

INTRODUCTION

Animals continually generate heat as a result of metabolic processes necessary for life. Under neutral environmental conditions, excess body heat is passively dissipated by radiation, conduction, and convection. But these mechanisms presuppose the existence of a temperature gradient, i.e., the body temperature must be higher than the environmental temperature. When the environment is as warm or warmer than the organism these three mechanisms become relatively ineffective, passive heat loss stops, and the animal's body temperature begins to rise toward lethal levels.

Under conditions of high environmental temperature and increasing internal temperature, an animal (1) may simply tolerate an elevated body temperature, establishing a thermal equilibrium at a higher level (this is the response of poikilotherms, as well as of a few birds and mammals); (2) may escape the heat, seeking out a cooler environment in which the passive heat-loss mechanisms could dissipate the accumulated heat (this is the response of many burrowing animals); (3) may dissipate heat by the evaporation of body water (this is the response of most large mammals). As Schmidt-Nielsen (1964) notes, evaporation is a highly efficient mechanism because relatively large quantities of heat energy are necessary to vaporize water, and thus substantial amounts of body heat can be dissipated at a moderate cost of body water. He further points out that most mammals have not developed a tolerance of sustained high body temperatures, and escape by burrowing is an impractical solution for an animal of appreciable size.

There are three principle ways in which body water is evaporated from the body surface for cooling. (1) Perspiration. This is the method used by man and other mammals with sweat glands (Adolph, 1947). (2) Panting. This response is characteristic of dogs, cats, and other mammals which evaporate water and saliva from the highly vascularized surfaces of the mouth, tongue, and upper respiratory tract. (3) Spreading of saliva on the body surfaces. It is becoming increasingly apparent that this is a common means of defense among marsupials and rodents (Robinson & Morrison, 1957; Higginbotham & Koon, 1955; Sealander, 1953; Herrington, 1940).

Several recent papers have investigated the saliva-spreading response in further detail. Hainsworth and Epstein (1966) described the response in rats as prolonged and thorough grooming of the vascularized surfaces of the head, paws, scrotum, and base of the tail, resulting in a visible soaking of these regions. The critical importance of saliva-spreading to temperature regulation was demonstrated by the severe impairment in the rat's tolerance of ambient temperatures above 36 C after surgical desalivation (Hainsworth, 1967). Similar findings have recently been obtained for mice, which also spread saliva during heat stress (Stricker, Everett & Porter, 1968).

Although there is a rapidly growing literature on the spreading response in the rat, comparatively little is known as yet of its ontogenic development. This paper will describe a number of experiments designed to answer two questions relating to the ontogeny of heat tolerance mechanisms in the rat: (1) At what age does the saliva-spreading response appear in the rat? (2) What means of defense (if any) are employed by neonatal and infant rats before the spreading response becomes functional?

EXPERIMENT I

Methods

<u>Subjects</u>

Rats used in the study were Wistar albinos born in our laboratory. Litters used ranged in size from 6 to 10 pups. Each mother and litter was housed in a plastic box measuring 16 x 14 x 7 inches, covered with a metal grid. Purina lab chow and water were available <u>ad lib</u>. Young rats were kept with their mothers until immediately before an experiment. Animals used in the experiment were randomly chosen with respect to sex, body weight, and litter. The ranges of body weight for the rats studied are listed in the appendix.

<u>Apparatus</u>

Animals were weighed with a Mettler pan-loading balance accurate to 0.1 gram. Elevated ambient temperatures were provided by a constant temperature chamber (Hotpack Co.) accurate to 0.5 C. A YSI small animal thermistor probe was used to measure body temperatures of animals older than 15 days, whereas a smaller YSI tissue implantation thermistor probe was used for younger rats.

<u>Procedure</u>

Thermal tolerance was determined in young animals (1 - 27 days) under conditions of continuous exposure to an ambient temperature of 40 C. Since young rats (1-10 days) do not show a terminal explosive rise in body temperature characteristic of heat exhaustion in the adult rat (Adolph, 1947), survival times were measured to the cessation of breathing. For animals older than 10 days, a trial was terminated when the body temperature rose above 42 C, since pilot work had shown that death rapidly followed. Body temperatures were monitored in all rats at 30 minute intervals.

Surgical desalivations were done using ether anaesthesia, and often proceeded with the aid of a Zeiss binocular operating microscope. In all operations the submaxillary and sublingual glands were removed and the parotid ducts were ligated. Care was taken to leave the facial nerve intact. The surgical preparation of sham-operated controls was identical to that of the desalivated animals, with the exception that salivary function was not actually disrupted. After the operations, all animals were isolated from their mothers for four hours before testing.

Results

Thermal Tolerance and Body Temperature Control

Rats exposed to an ambient temperature of 40 C show a very high initial thermal tolerance (16-26 hours), which drops off rapidly after the first or second day of life and reaches a baseline level of 3 - 4 hours by day 10 (Fig. 1). The body temperature response for individual rats (1 - 4 days old), presented in Figure 2A, indicate that body temperatures do not rise substantially above ambient. Indeed, they are

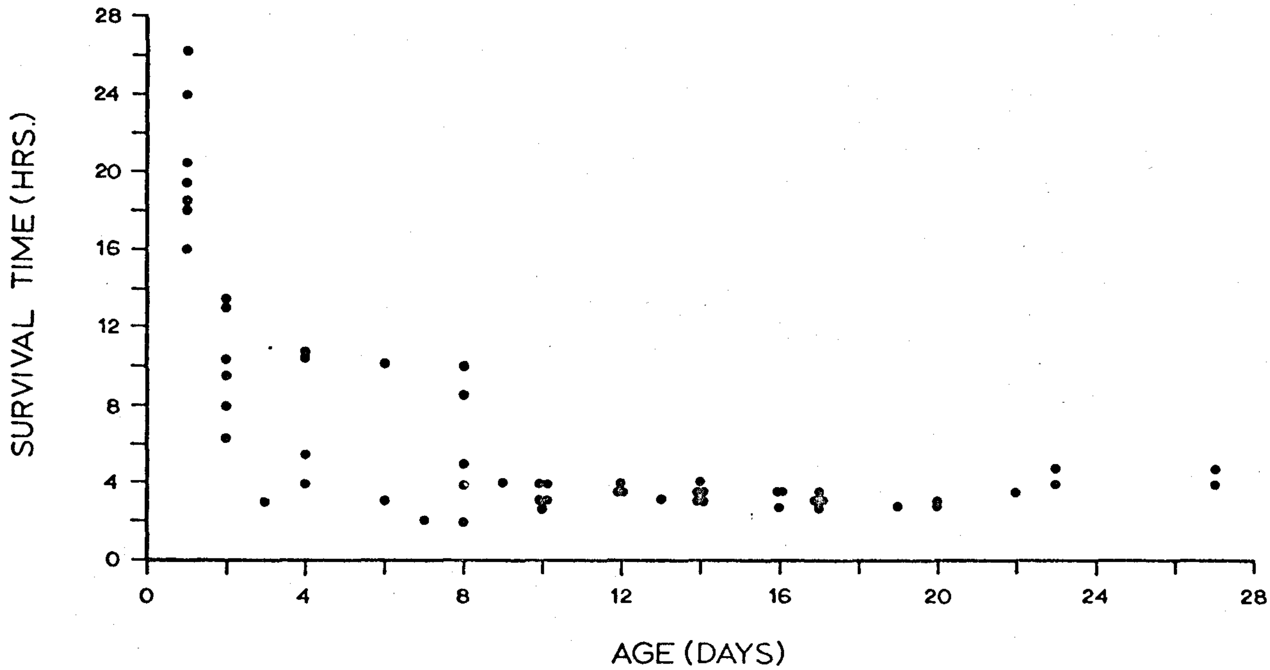

Figure 1. Survival times of 1 - 27 day old rats exposed to an ambient temperature of 40 C.

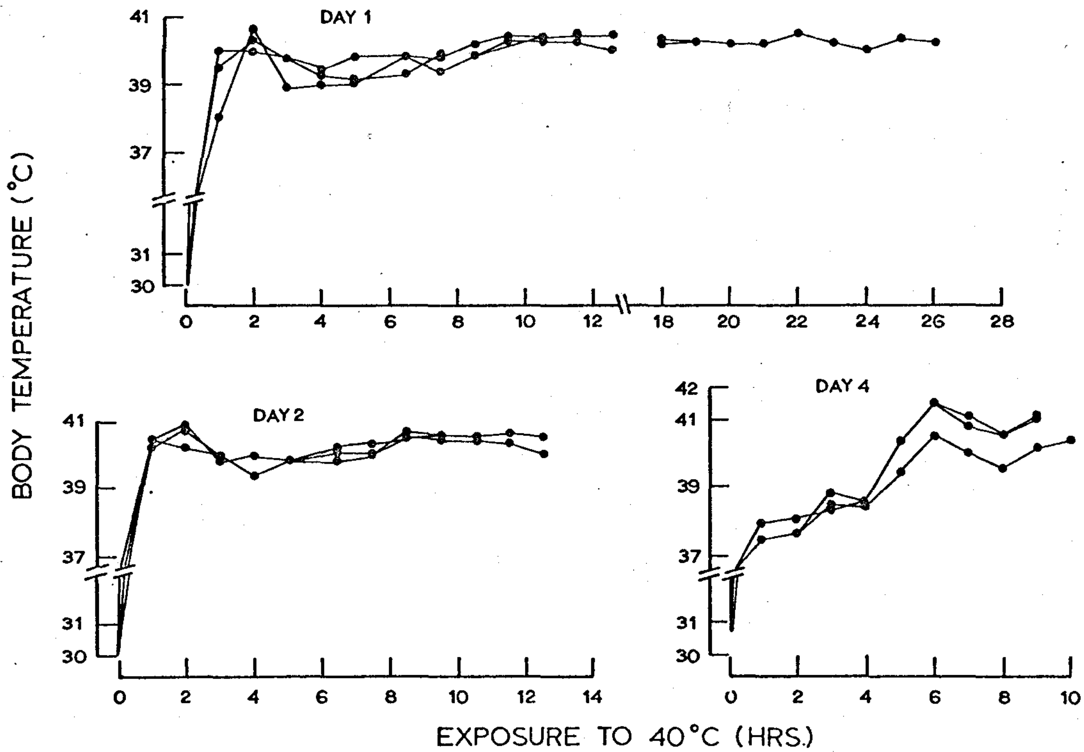

Figure 2A. Body temperature control in neonatal rats.

held at a somewhat lower temperature than those of adult rats exposed to the same ambient temperature (Fig. 2C). This impressive tolerance of 1-day old rats to heat stress was not apparent at more elevated ambient temperatures. At 44 C, for example, increases in body temperature were rapid and fatal (Fig. 3).

Saliva Spreading

To determine the age at which saliva-spreading initially appeared, rats of various ages were put into the heat and closely observed for saliva-spreading behaviour. Although very young rats make rudimentary grooming motions that occasionally occur as early as day 1, the response does not consistently appear until day 17. Figure 4 illustrates the extent to which saliva-spreading typically occurs after one hour of exposure to 40 C heat, at the ages indicated. It can be seen that as age increases past 17 days, an increasingly greater proportion of the ventral surface is groomed with saliva.

Importance of Saliva-Spreading

To determine the importance of salivary evaporation to normal heat tolerance in neonatal rats, thermal tolerances were determined in 10 - 27 day old desalivated and normal animals exposed to ambient temperatures of 40 C (cf, Hainsworth, 1967). The results, illustrated in Figures 2B and 2C, indicate that intact rats become increasingly superior to desalivated rats in body temperature control as age increases. For example, there is no obvious difference between intact and desalivated 10-day old rats, whereas at 27 days of age the difference is as pronounced as in adult rats.

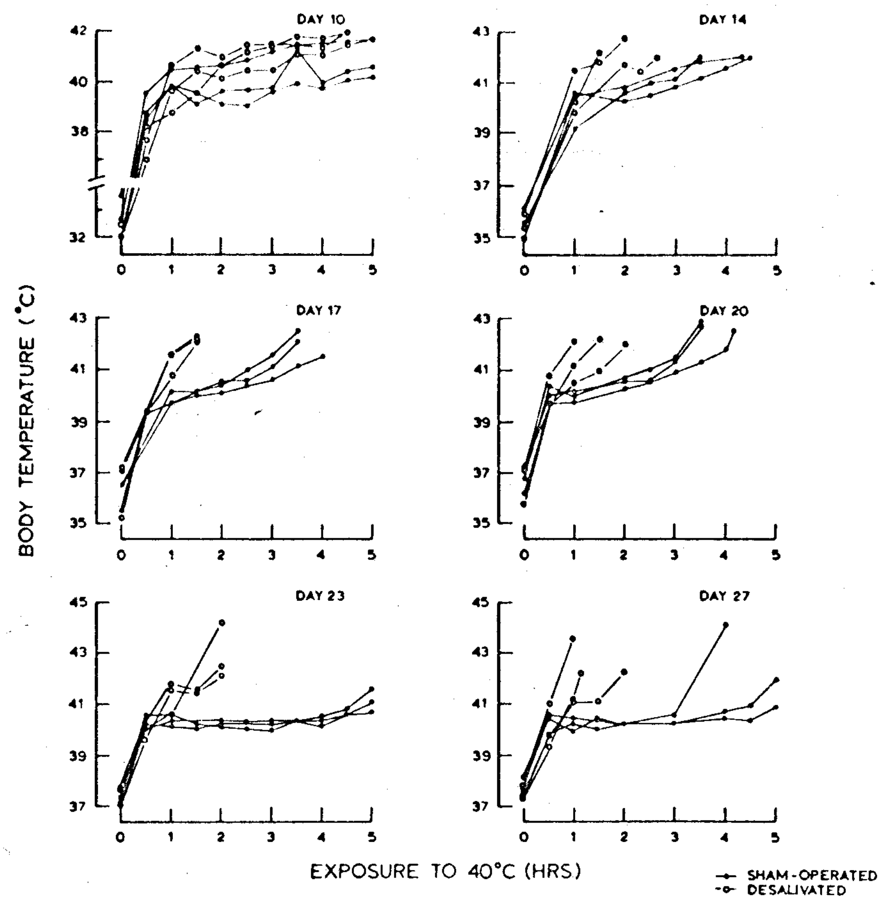

Figure 2B. Body temperature control by desalivated and sham-operated rats.

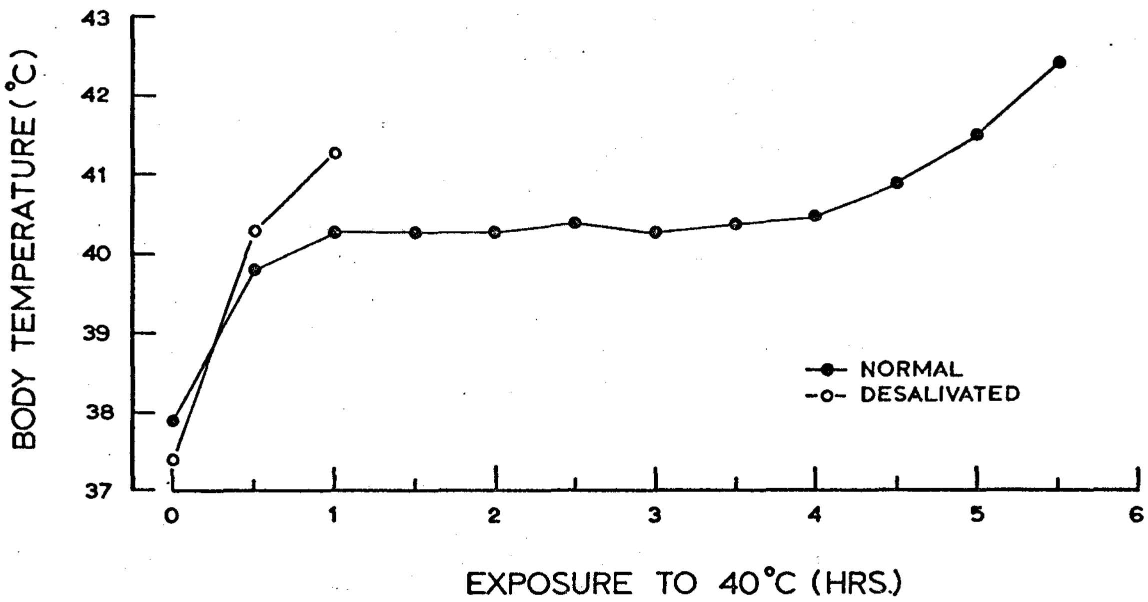

Figure 2C. Body temperature control by adult rats.

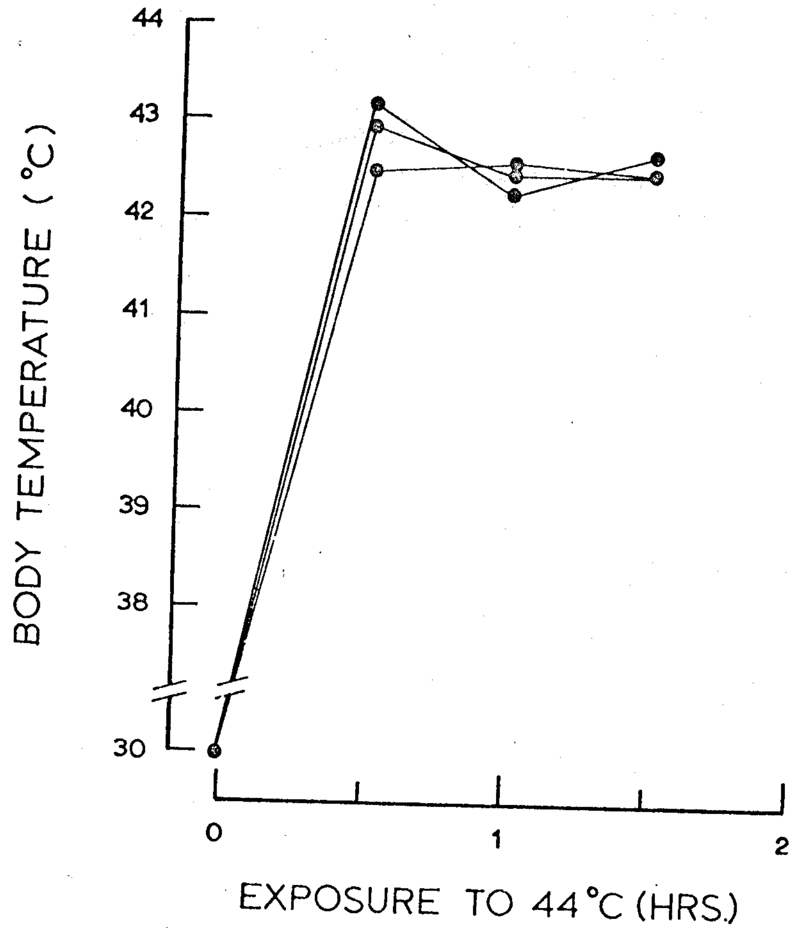

Figure 3. Body temperature control in neonatal rats exposed to an ambient temperature of 44 C.

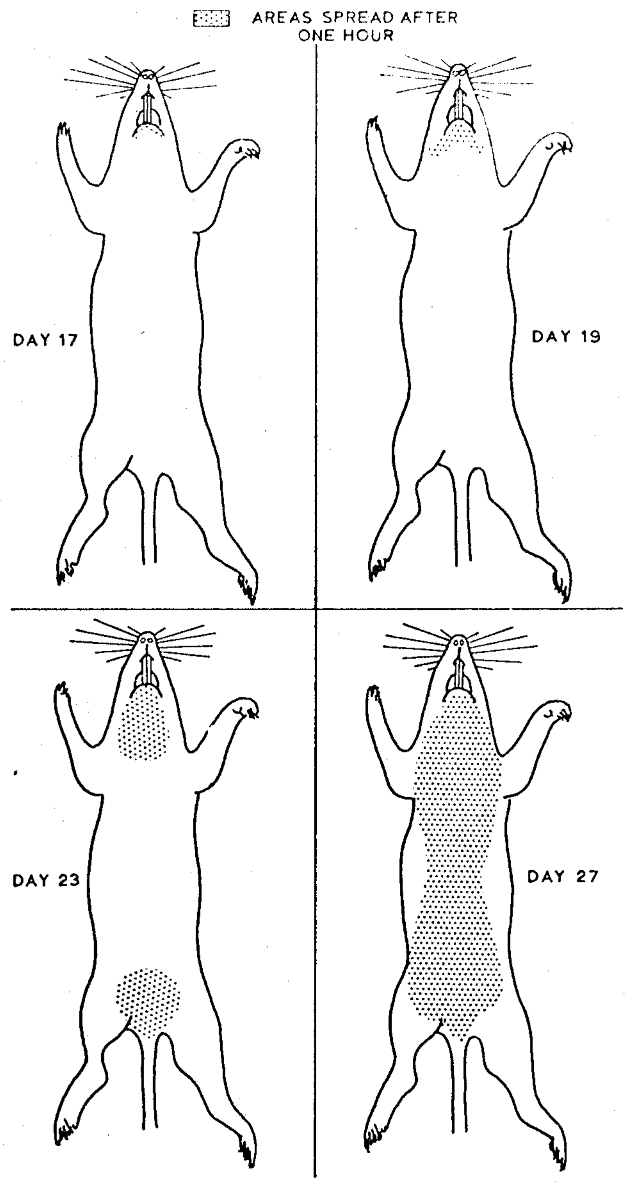

Figure 4. Extent of saliva-spreading at different ages.

EXPERIMENT II

Hainsworth (1967) has shown that the saliva-spreading response is critically important for heat tolerance in adult rats. The results of Experiment I indicate that this response is not present in rats at birth but gradually develops after the second week of life, most commonly appearing at approximately day 17. It might be expected that the absence of saliva-spreading during the first two weeks of life would seriously impair the tolerance of neonatal rats to high ambient temperatures. However, this was not the case. In fact, the tolerance levels of rats during the first two days of life were exceptionally high. This high thermal tolerance in newborn rats, and the subsequent drop in tolerance that persists until the development of saliva-spreading, is investigated in Experiment II.

Methods

Subjects and Apparatus

Subjects and apparatus were identical to that described in Experiment I.

Procedure

Water Loss. Initial body water contents were determined in intact ("control") rats not exposed to heat stress by dessication of the carcass (at 110 C) to a constant weight ($\pm$ 0.5 mg). The percentage of body water lost in the heat by experimental animals was calculated as the weight lost during heat exposure (assumed to consist entirely of evaporative water loss) divided by the initial ("control") body water contents. Terminal body water contents were calculated by subtracting the body weight lost during heat stress from the initial ("control") body water. See the appendix for representative calculations.

Rate of evaporative water loss was directly determined using an apparatus described by Hainsworth (1967). Dry air, flowing at a rate of 4 liters/minute, was introduced into a four-liter can located in the incubator and containing the rat. Evaporated moisture in the effluent air was collected by a series of $CaSO_4$ (Drierite) drying tubes. The rate of evaporative water loss was determined by weighing these tubes (0.1 mg) before and after a test period of 15 minutes. The measurement was made after the animals had been in the heat for one hour. Alternatively, estimates of evaporative water loss could be obtained from weight loss (0.1 mg) at death. These two procedures agreed closely, and both were used in the experiment.

Metabolic Rate

The metabolic rate of young rats exposed to an ambient temperature of 40 C was determined by placing individual animals into an airtight 1-liter can located in the incubator. The lid of the can contained the measuring probe of the YSI model 51 oxygen meter, and it was thus possible to continually monitor the oxygen concentration within the can. All animals were run until 30 ml of oxygen had been consumed. To avoid the complicating effects of a rapidly climbing body temperature during the metabolism measurements, all animals were exposed to the elevated ambient temperature for 30 minutes before being placed into the measuring chamber.

Results

Water Loss

Initial and terminal body water percentages are presented in Table 1. It can be seen that the initial body water percentages are uniformly high over the first four days of life, then begin to drop off to the lower levels of older animals on the fifth day. Terminal body water percentages do not appear to drop as rapidly as the initial percentages. These relationships are summarized in Figure 5.

The percentage of total body water that is lost in the heat is shown for individual rats throughout the 1 - 27 day age range in Figure 6. Whereas 1-day old animals lose 15 - 20% of their body water in the heat, this percentage drops off within the first four days and subsequently reaches a constant level of 5 - 10%. On the other hand, the rate of evaporative water loss increased monotonically during the first 20 days of life. Water loss rates for individual rats, shown in Figure 7, indicate that the evaporation rate doubles during the first two weeks of life. By rearranging the data from Figures 6 and 7, one can see that increased survival time is associated with large total water losses and with low rates of water loss (Fig. 8).

TABLE 1

Body water contents in rats exposed to an ambient temperature of 40 C until heat exhaustion.

Age	Initial % Body Water	Terminal % Body Water
1	81.95	77.27
1	81.47	77.19
1		76.67
2	79.88	76.80
2	80.02	76.08
2		76.00
3	79.37	
3	79.37	
4	80.78	
4	81.17	
5	76.54	
5	77.20	
6	75.75	73.47
6	76.95	75.15
20	74.73	71.83
20	74.73	72.98

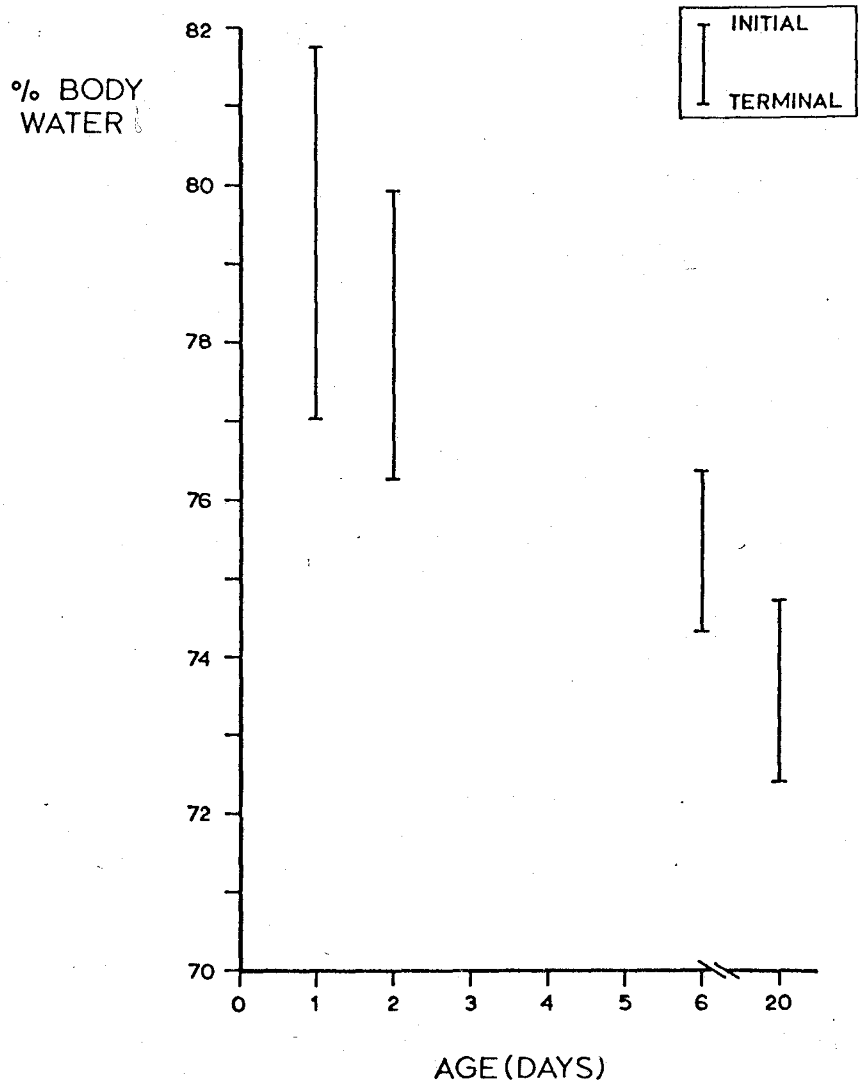

Figure 5. Initial and terminal percentage body water after heat exposure at selected ages.

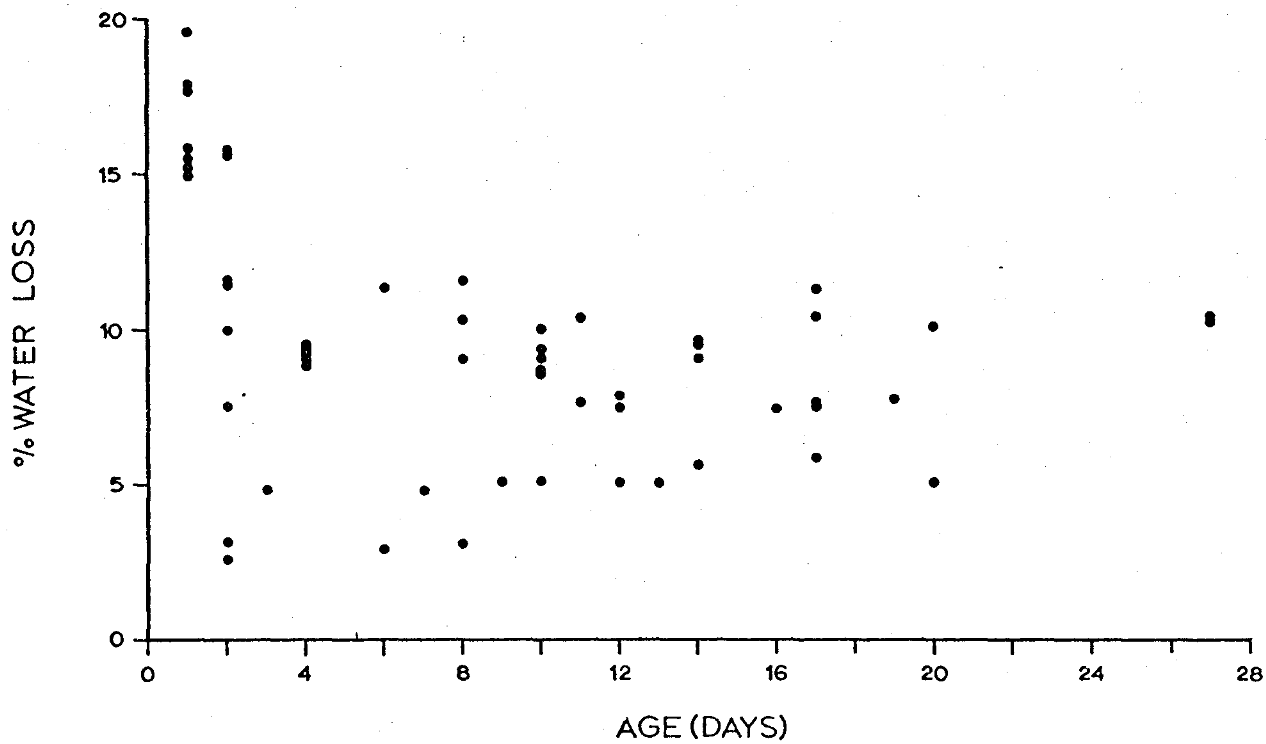

Figure 6. Percentage water loss in the heat by 1 - 27 day-old rats.

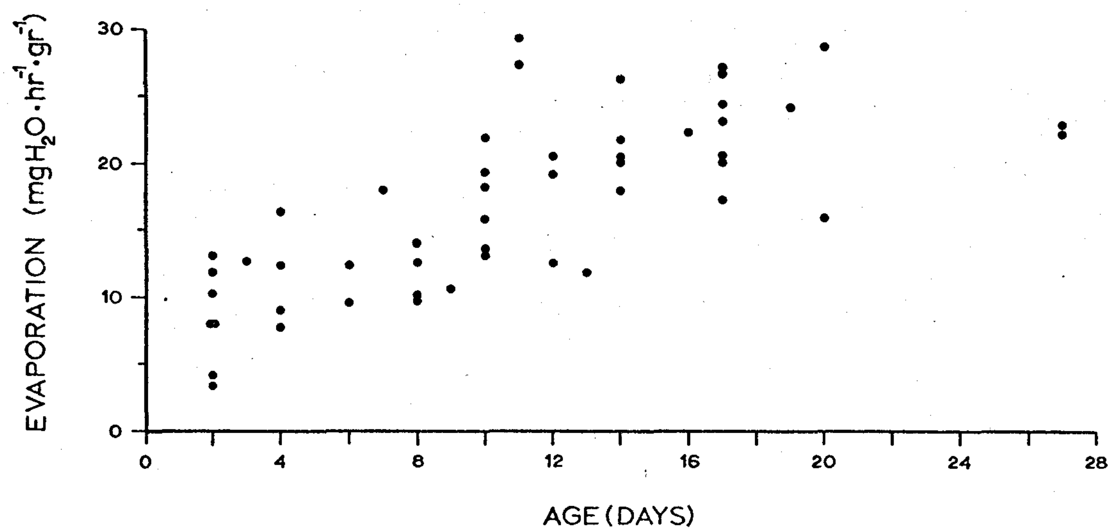

Figure 7. Rate of water loss from 1 - 27 day-old rats exposed to an ambient temperature of 40 C.

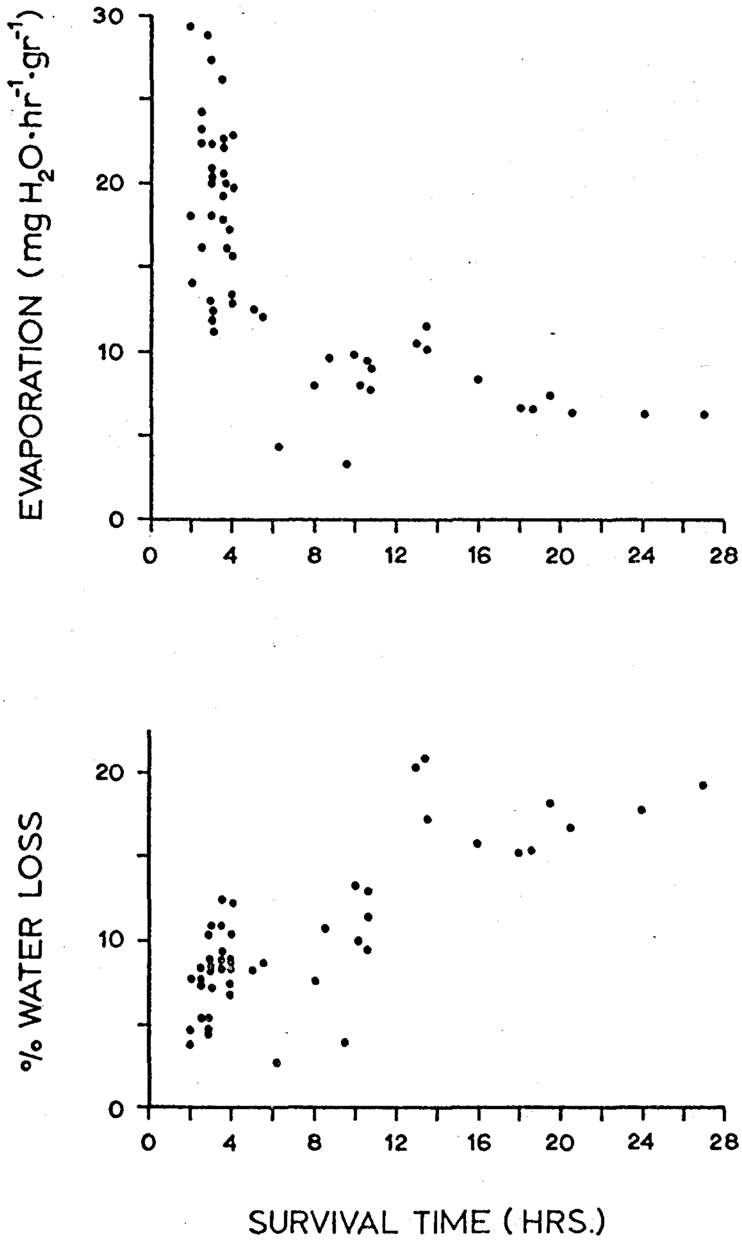

Figure 8. Relationships between percentage water loss, rate of water loss, and total survival time.

Metabolic Rate

Figure 9 illustrates metabolic rate measurements of individual rats exposed to an ambient temperature of 40°C. The results show a monotonically increasing metabolic rate that reaches a relatively constant level by day 10.

This data can be conveniently arranged into a balance sheet of the heat production and heat loss of young rats in a high-temperature environment. Heat production can be calculated from the data in Figure 9 by converting these values into total oxygen consumption and assuming 4.75 calories per liter of oxygen consumed. Heat lost from evaporation can be directly calculated by converting the data in Figure 6 into total water loss and multiplying by the heat of vaporization.

The results of these calculations can be seen in Table II. Total heat production compares favourably with total heat loss in one- and four-day old rats, but is approximately 25% greater than the total evaporative heat loss seen in 12-day old rats. Presumably, this heat is lost by diffusion, radiation, and conduction to the environment as a result of the positive temperature gradient established in the older animals.

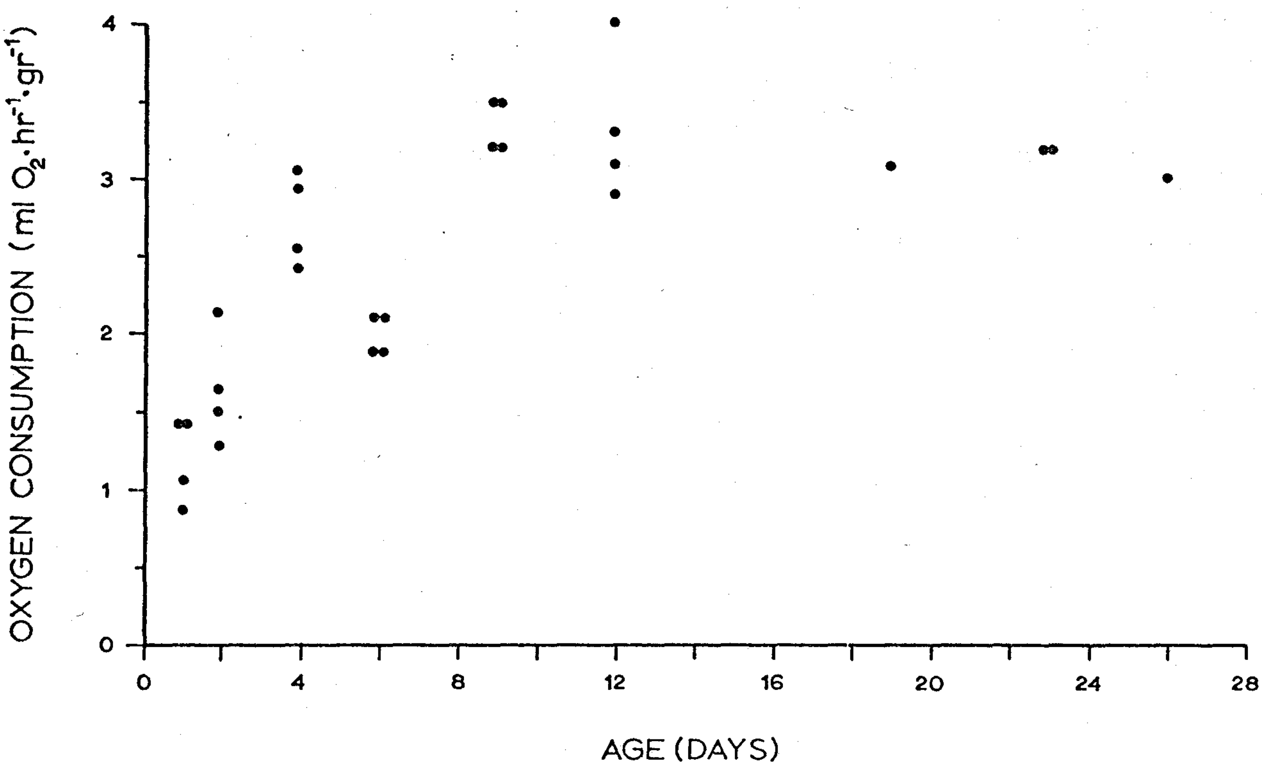

Figure 9. Rate of oxygen consumption in the heat at different ages.

TABLE 2

Heat production and heat loss in young rats exposed to an ambient temperature of 40 C.

Age	Oxygen Consumption ml O_2/hr	Total Heat Production g-cal/hr	Evaporation ml H_2O/hr	Evaporative Heat Loss g-cal/hr
1	10	48	.09	43
4	25	119	.20	108
12	100	475	.69	372

DISCUSSION

The results obtained in Experiment I showed that the necessary thermal gradient does not exist for passive heat loss in neonatal rats. Heat loss, then, must occur from the evaporation of body water. The results from Experiment II confirm a close relationship between water loss and survival in the heat (Fig. 8). It was seen that younger rats have a higher initial body water content than older rats (Fig. 5), lose a greater proportion of their body water content (Fig. 6), and lose their body water supply more slowly than older rats (Fig. 7).

As indicated in Experiment I, the water lost by neonatal rats does not result from saliva-spreading, since this response is not functional until 14 - 17 days of age. However, other paths of water loss are possible. Keeton (1924) discussed the importance of water losses from the peripheral skin tissue and suggested that this is the rabbit's primary defense against high temperatures. Since body hair retards cutaneous water loss (Schmidt-Nielsen, 1964), this avenue of water loss might be even more significant in the hairless neonatal rat. A second possibility is suggested by the work of Tennent (1946), who describes increased oral water losses from adult rats in high temperatures. As Plagge (1938) has shown, some of the salivary glands are functional from the first day of life and, although rats do not pant, their relatively large mouths might provide an important source of moisture.

Evidence presented in Experiment I showed that both oral and peripheral water losses are important for the thermal tolerance of intact rats before saliva-spreading appears. The overlapping body temperature responses of desalivated and intact 10-day old rats (Fig. 2B) indicate that evaporation of oral water is not essential to the heat tolerance of rats at this age. Since these rats are still hairless, cutaneous water loss is apparently sufficient to dissipate the endogenous heat. The results from the 14 and 17-day old rats (Fig. 2B) show a definite decrement in body temperature control as a result of desalivation and indicate the growing importance of salivary evaporation. It would seem that by 14 days of age, a larger proportion of the evaporated water is oral rather than cutaneous water. This may, in part, be due to the fact that the 14-day old rat has acquired a downy coat of hair that impedes cutaneous water loss.

Other results from Experiment II show that there is an increasing metabolic rate over the first week of life that levels off to a relatively constant level by day 10. An increasing rate of endogenous heat production has clear relevance to survival in the heat, since it means that the older animals must evaporate water at a more rapid rate to keep the body temperature within tolerable limits. A necessary consequence of increasing metabolic rate would be a more rapid depletion of the evaporative water reserves, which was also demonstrated in this experiment. The metabolic rate reaches a constant level by day 10 (Fig. 9), which is the same age at which survival in the heat reaches its low baseline value (Fig. 1).

The values obtained for heat production by one- and four-day old rats in the hot environment (Table III) are in agreement with calculated heat loss values and with heat production values obtained from young rats at room temperature (Gulick, 1937; Brady, 1943). A relatively high heat production value for 12 day old rats in the heat (475 g-cal/hr) probably reflects the maturing metabolism, since the metabolism of an adult rat accelerates when body temperature increases. The surplus of heat production (475 g-cal/hr) over evaporative heat loss (372 g-cal/hr) found in 12 day rats in the heat results in their evident elevation of body temperature above ambient, as compared with the poikilothermic near-ambient body temperature responses of younger animals (Fig. 2A).

TABLE 3

Heat production by young rats at room temperature and during heat stress.

Age	g-cal/hr, 40 C	g-cal/hr, 25 C*
1	48.0	62.5
4	119.0	83.3
12	475.0	125.0

* From Brody (1943)

GENERAL DISCUSSION

At birth, the rat is well able to tolerate heat stress, as well as a variety of other stressful conditions. For example, newborn rats have a high tolerance to anoxia (Fazekas, Alexander & Himwich, 1941) and to extreme hypothermia (Brody, 1943; Hill, 1947; Fairfield, 1948). As the rat matures, two important processes occur that seem relevant to the development of heat tolerance: the metabolic rate increases rapidly, and the myelinization of the hypothalamus is completed.

The increasing metabolic rate asymptotes to a constant level by day 20, the age at which Brody (1943) demonstrated adult levels of thermogenesis in the cold. There are indications that metabolic rate has an important influence on tolerance in the heat as well, although in an opposite direction. Brody (1943) reports that the metabolic rate is doubled between the ages of 1 and 6 days. Our data show that metabolic rate increases just as rapidly for 1 - 6 day-old rats in the heat and increases even faster by day 12. A higher metabolic rate poses a threat to 6-day and older rats exposed to unavoidable heat stress since more body heat is generated and body temperature climbs faster. In order to exert the same degree of control over body temperature, the rat must lose water at a faster rate, as is evidenced by the results of Experiment II. As metabolic rate in the heat asymptotes to its high day 10 level, survival time falls to its lowest value (approximately 3 - 4 hours).

Sources of the body water used for evaporative cooling in 1 - 17 day old rats appear to be respiration (including oral evaporation) and peripheral skin tissue. Until the age of 10 days, cutaneous water loss appears to be the major mechanism for evaporative cooling. The prominence of this avenue of water loss in the 10 day old rats is probably related to the fact that these animals are still hairless. By day 14, oral water loss has assumed some significance, and this is probably related to the fact that the 14-day old animals have a downy coat of hair, which impedes cutaneous water loss. Oral water loss becomes increasingly predominant as the rat grows older and the spreading response develops.

More rapid evaporation of water presents another problem, suggested by Hainsworth, Stricker, and Epstein (1968), of limited survival in the heat due to body fluid dehydration. This relationship was indicated in the present study, since length of survival was positively correlated with total proportion of body water lost and negatively correlated with rate of evaporation. Thus, it appears that the initial high peak in heat tolerance results from a low metabolic rate making relatively moderate demands on a relatively large body water supply, whereas the sudden drop in tolerance results from a rapid increase in metabolism necessitating a more rapid loss from a reduced water supply.

The changes that take place in heat tolerance before spreading occurs thus appear to be related to the maturation of the metabolism. Similarly, the sudden appearance of the spreading response at about 17 days may be the result of a second maturational process, that of the hypothalamus. Buchanan and Hill (1947) correlated increasing homeothermy in the cold with hypothalamic myelinization, both of which are complete by the age of 20 days in the rat. Their work agrees with widespread evidence (Andersson, 1965; Hainsworth & Epstein, 1966; Magoun, et al, 1938) suggesting a hypothalamic involvement in defenses against hyperthermy. The observed appearance of the spreading response on day 17 is consistent with these findings.

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APPENDIX A

Representative Calculations of Water Loss Data

If the initial weight of a one-day old rat is 7.70 grams and the dessicated weight is 1.39 grams, then the animal contained 6.31 grams water and his body water content was 82%. After 24 hrs of exposure to 40° C, the body weight has fallen to 6.60 grams, which means that 1.10 grams of water have been lost. Total body water content is now:

$$\frac{6.31 - 1.1}{7.70 - 1.1} \text{ or } 78\%.$$

The animal began with 6.31 grams of water and lost 1.10 grams, so he has lost 1.10/6.31 or 17% of his total body water.

APPENDIX B

Weight Ranges for Rats of Each Age Used in the Study

Age (Days)	Weight Range (Grams)
1	4.7 - 7.7
2	3.2 - 8.0
3	10.1
4	7.8 -13.0
5	---------
6	8.0 -11.7
7	16.7
8	9.8 -21.4
9	26.0
10	18.0 -23.0
11	18.3 -29.0
12	17.7 -20.8
13	34.1
14	19.8 -38.0
15	---------
16	37.2 -49.0
17	28.5 -47.7
18	---------
19	58.3
20	44.9 -62.4
21	---------
22	---------
23	40.7 -45.3
24	---------
25	---------
26	---------
27	88.0 -97.6