THERMOREGULATION IN THE HEAT IN PREGNANT RATS
BODY TEMPERATURE REGULATION DURING HEAT STRESS
IN
THE PREGNANT RAT

by

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Abstract

Body temperature regulation at high ambient temperatures was compared in pregnant and non-pregnant female albino rats. At an ambient temperature of 40°C, pregnant rats maintained lower body temperatures than non-pregnant rats without added expenditure of moisture for evaporative cooling. This was surprising because of the increased heat load on the pregnant animal resulting from a large weight gain and increased food intake. The maintenance of lower body temperatures in the heat by pregnant rats was possible because (1) pregnant rats produce less heat; i.e. consume less oxygen during exposure to 40°C than do non-pregnant rats and thus need to dissipate less heat; and (2) pregnant rats have a lower body temperature threshold for increased submaxillary salivary gland output in the heat, which makes more water for evaporative cooling available to them at lower body temperatures.

The maintenance of lower body temperatures in the heat by pregnant rats suggests that pregnant rats have a need or a preference for lower body temperatures in the heat. The change in body temperature regulation during pregnancy is likely related to physiological and anatomical changes in the body which accompany pregnancy. Alterations in body temperature regulation to meet the changed physiological state of pregnancy provide another example of the body's remarkable ability to maintain homeostasis.
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Chapter 1

Introduction

Pregnancy presents a unique challenge to homeostasis in mammals. The growth and development of the maternal organs and products of conception place increased demands on behavioral and physiological regulatory mechanisms. This dissertation is an investigation of how one such regulatory system, that of body temperature regulation, changes to compensate for the increased demands of pregnancy.

Some of the changes which accompany pregnancy particularly those of growth and increased food intake, would seem likely to place an added burden on the thermoregulatory mechanisms involved in heat loss. The most obvious relevant change is the large gain in weight of the mother. In rats, weight gain during pregnancy amounts to an increase of approximately 40% over non-pregnant levels (Brody, Riggs, Kauffman, and Herring, 1938). This change in size produces a decrease in the surface to volume ratio which results in decreased passive heat loss capabilities. The large increase in weight during pregnancy would also act to increase the energy requirement, and thus the heat production, involved in locomotory activity (Hytten and Leitch, 1964), and, in fact, this activity is decreased (Slonaker, 1925; Wang, 1923). Underlying the large gain in weight of the mother rat is increased food intake (Leshner, Siegel, and Collier, 1972; Richter and Barelaire, 1938; Scott, Smith, and Verney, 1948; Slonaker, 1925). The increased food intake itself might be expected to increase the heat load.
on the mother by contributing additional heat of assimilation. As well, the disproportionate increase in protein intake during pregnancy (Leshner, Siegel, and Collier, 1972; Richter and Barelaire, 1938), which has a higher SDA than carbohydrates or fats, should likewise increase the pregnant rat's heat load.

Other changes occur during pregnancy which might also effect an altered ability to maintain a balance between heat gain and heat loss. There are elevated levels of progesterone in the blood during pregnancy (e.g., Grota and Eik-Nes, 1967). Progesterone has thermogenic properties (see review by Rothchild, 1969); and injection of progesterone has been shown to produce an elevation of body temperature in rats (Niebergs, Kupperman, and Greenblatt, 1946; Niebergs and Greenblatt, 1948), as well as in other species (e.g., Israel and Schneller, 1950; Wrenn, Bitman, and Sykes, 1959). In addition, elevated basal body temperature has been found during part of pregnancy in humans (see review by Rothchild, 1969) and during pseudopregnancy in rats (Niebergs and Greenblatt, 1948). Unfortunately, there is no available data on the rat which is analogous to the data on basal body temperature changes in humans during pregnancy. However, the existing evidence supports the possibility that the altered hormonal state of pregnancy might increase the heat load on the animal.

The metabolic rate, as measured by oxygen consumption and expressed in terms of body weight or surface area, has been found to increase in many species during pregnancy (see review by Newton, 1952). The inferred increased heat production would increase the need for heat loss if body temperature were to be maintained. In the rat, however, no such increase in metabolism has been observed during pregnancy, (Brody, Riggs, Kauffman,
and Herring, 1938). Perhaps the drastically reduced activity observed during pregnancy in the rat (Slonaker, 1925; Wang, 1923) acts to offset any of the possible increases in heat production discussed above and, as well, to minimize the need for additional food (Slonaker, 1925).

In summary, there are a number of factors which might affect thermoregulation during pregnancy. Elevated blood progesterone levels, increased food intake, and increased heat production during exercise could all act to increase the heat load on the pregnant rat. Decreased surface to volume ratio would reduce the rate of passive heat loss possible. Decreased activity might compensate in part for the resultant increased heat load, but changes in heat loss capabilities might also be expected to occur during pregnancy.

Heat loss in the rat has been extensively studied, though it has not been explored during pregnancy. Homothermic species appear to have a characteristic body temperature set point around which thermoregulation occurs (e.g., Myers and Veale, 1970; Myers and Yaksh, 1971). In order for a particular body temperature to be maintained, heat loss to the environment must equal internal heat production and heat gain from the environment. In other words, there are several mechanisms for both heat gain and heat loss, and thermoregulation represents a balance among these. Heat loss in the rat occurs passively by vasodilatation, actively through evaporation of saliva which rats spread on their skin, and by escape from a hot environment. When heat gain exceeds heat loss, rats, as well as other homotherms, increase the rate of heat loss by vasodilatation, evaporation, or escape. Similarly, whenever one mechanism for heat loss becomes less effective, as, for example, when an animal is placed in a hot environment (and its rate
of passive heat loss thereby decreased), other avenues of heat loss (evaporation or escape) become correspondingly more important. In addition, the burden on the mechanisms of heat loss is lessened by the fact that the animal can allow its body temperature to become somewhat elevated. Thermo-regulation then continues around this controlled level of hyperthermia. In the rat, areas regulating heat loss in the central nervous system have been located in the preoptic area of the anterior hypothalamus (see Hainsworth and Stricker, 1970, for review).

In rats, the long nude tail represents the major site of heat loss (Grant, 1963; Little and Stoner, 1968; Rand, Burton and Ing, 1965; Stricker and Hainsworth, 1971). When heat gain exceeds heat loss, reflex vasodilatation occurs at a particular level of hyperthermia (Grant, 1963; Thompson and Stevenson, 1965), thus maximizing the transfer of heat between the body and the environment. The colonic temperature at which vasodilatation occurs has been referred to as the body temperature threshold for vasodilatation (Thompson and Stevenson, 1965). The actual stimuli for vasodilatation are not known, but they may include such factors as increases in skin temperature, hypothalamic temperature, or deep body temperature. It should be noted that vasodilatation is effective only when body temperature exceeds the temperature of the surrounding substrate. When the reverse is the case, vasodilatation is, in fact, counterproductive since then it maximizes the flow of heat from the environment into the body.

When heat loss by radiation is not an adequate means of heat loss (e.g., when the environment is too hot to permit a sufficient rate of heat loss) and escape is impossible, evaporation of water must occur in order for body temperature to be maintained. In the rat, which neither sweats nor pants, a copious flow of saliva accompanies elevated body temperatures.
Rats then groom this saliva onto their fur, concentrating on the ventral surface (Hainsworth, 1967). The temperature at which the increased salivation occurs is considered the body temperature threshold for salivation (Hainsworth and Stricker, 1971). As in the case of vasodilatation, the specific stimulus for salivary secretion is unknown.

Two pairs of salivary glands are involved in the rat's response to hyperthermia. The submaxillary glands produce a very hypotonic saliva and contribute most to the increased salivary flow. The parotid glands produce a nearly isotonic fluid and contribute very little to salivary evaporation (Hainsworth and Stricker, 1969), which is fortunate since a loss of isotonic fluid would compromise blood volume and thus would undermine heat transfer from the body core to the periphery via vasodilatation (Stricker and Hainsworth, 1970). It should be noted that saliva-spreading is probably an emergency reaction in the natural environment. It is very wasteful of body fluid and, unlike panting or sweating, it cannot be engaged in while an animal is performing other activities, such as feeding or running away from a predator. Rodents are more likely to try to avoid heat stress by staying in a cool burrow and foraging at night when the temperature is usually lower (Bartholomew, 1965; Schmidt-Nielsen, 1964) or by using exogenous water, if it is available, for evaporative cooling (Schmidt-Nielsen, 1964; Stricker, Everett, and Porter, 1968). Nevertheless, saliva-spreading in the heat has been observed in a number of species (Robinson and Morrison, 1957; Schmidt-Nielsen, 1964). More importantly, it has been found to be crucial for controlling hyperthermia in rats (Hainsworth, 1967) and many other small mammals (e.g., Higgenbotham and Koon, 1955; Stricker, Everett, and Porter, 1968) so it is of interest to understand its involvement.
in body temperature regulation.

The standard experimental method for studying heat loss in the rat is to expose the animal to an elevated ambient temperature. This method decreases the rate of passive heat loss possible, and the elevated body temperature which results produces an increase in heat production as well. In addition, if the ambient temperature is higher than body temperature, heat will be gained from the environment. Hainsworth (1967) has found that rats can regulate their body temperatures over a wide range of elevated ambient temperatures and that the level of regulated hyperthermia is a function of the environmental temperature. For example, during exposure to a temperature of 36°C, both male and female rats maintained body temperatures which were about 2°C above the normal body temperature for rats (Hainsworth, 1967). However, the way in which the male and female rats achieved this identical regulation was different. Measurement of the rates of evaporative water loss at 36°C, as well as at other temperatures studied, showed that males evaporated more water at ambient temperatures up to about 40°C (Hainsworth, 1968). This observation suggested that male rats are more dependent on evaporation for body temperature regulation in the heat than are females. In this regard, surgically desalivated males were found to be unable to regulate their temperatures during exposure to 36°C, while desalivated females regulated just as well as did intact females (Hainsworth, 1967).

The males' greater dependence on saliva for body temperature regulation is probably a result of sex-related differences both in heat loss capabilities and in heat production. The major thermal window for the rat, the tail (e.g., Stricker and Hainsworth, 1971), is shorter in males than
in females of equal body size (Chevillard, 1962). In addition, males are more active during heat stress than are females (Hainsworth, 1967). The smaller radiating surface of the males, as well as their greater heat production, makes it necessary for them to evaporate more water in order to regulate their temperatures at the same level as do females.

The male rats' need for greater evaporation disappears at ambient temperatures of 40-41°C. During brief exposure to such temperatures males and females have been found to use similar amounts of water for evaporative cooling (Hainsworth, 1968) and to maintain similar body temperatures over a longer exposure (Hainsworth, 1967). At these temperatures, the differences in the size of the tail become irrelevant to thermoregulation because the rat and the environment are nearly the same temperature (Hainsworth, 1967), and heat cannot flow passively from one to the other. In this regard, Stricker and Hainsworth (1970; 1971) have found that male rats with amputated tails and intact male rats evaporate similar amounts of water and maintain similar body temperatures during exposure to 40°C.

The striking differences between the thermoregulation of male and female rats during heat stress suggest the existence of sex-related differences in the physiological response to heat stress. For example, similar body temperature regulation in males and females at an ambient temperature of 36°C would seem to require an underlying difference in their salivary responses to hyperthermia since only males need saliva for body temperature maintenance at this temperature (Hainsworth, 1967). In fact, the body temperature threshold for increased salivary flow has been found to be lower in males than in females (Hainsworth and Stricker, 1972). A
parallel difference has been shown in the body temperature threshold for vasodilatation (Thompson and Stevenson, 1965), which might be expected to compensate for the lower rate of passive heat loss possible to the shorter-tailed males.

In summary, sex-related differences in heat production and passive heat loss ability are compensated for by sex-related differences in the behavioral and physiological response to elevated ambient temperatures. The greater activity of male rats in the heat and their relatively smaller radiating surfaces make them more dependent on evaporative cooling for body temperature regulation during heat stress. This need is met physiologically, by the males' lower body temperature thresholds for vasodilatation and increased salivation, and behaviorally, by their more efficient use of saliva for evaporative cooling.

Some of the differences between pregnant and non-pregnant female rats present an interesting analogy to the differences between male and female rats discussed above. For example, the tails of pregnant rats are shorter, relative to body size, than are the tails of non-pregnant females, resulting in a smaller radiating surface (Appendix I). This difference would decrease the rate of passive heat loss (relative to heat production) possible to the pregnant rat. While pregnant rats are less active than non-pregnant rats, at least at normal room temperatures (Slonaker, 1925; Wang, 1923), their increased food intake (e.g., Leshner, Siegel, and Collier, 1972) and elevated blood progesterone levels (e.g., Grota and Eik-Nes, 1967) might increase their heat production relative to that of non-pregnant females. As in males, the lowered passive heat loss capability and increased heat production of pregnant rats might increase their
dependence on escape from a hot environment or on evaporative cooling for body temperature regulation. In this regard, it is interesting that pregnant rats have been found to choose a cooler part of a thermal gradient than non-pregnant rats (Gelineo and Gelineo, 1952). This might compensate for the pregnant rat's decrease in rate of passive heat loss (relative to heat production) by increasing the rate of heat loss possible. Furthermore, pregnant rats have been found by Roth and Rosenblatt (1967) and the present author (Appendix IV) to increase their self-licking of nude or less densely furred areas (genital area, pelvic area, and nipple lines) relative to more densely furred areas (head, paws, back). This change in grooming would concentrate moisture on areas from which evaporation would cool more effectively and is reminiscent of the male rat's concentration of saliva-spraying on the scrotum and base of the tail.

The altered pattern of grooming during pregnancy has not previously been related to thermoregulatory behavior. Birch (1956) suggested that the apparent increase in licking of the genital area during pregnancy reflected the fact that the salt appetite of pregnancy (Barelare and Richter, 1938; Scott, Smith, and Verney, 1948) made the vaginal secretions and urine more attractive to the pregnant rat. He further suggested that this licking behavior prepared her to clean the young as they were born and to inhibit biting the pups, since prevention of licking during pregnancy resulted in the mothers destroying or ignoring their litters after birth (Birch, 1956). Subsequent experimenters (Christophersen and Wagman, 1966; Kirby and Horvath, 1968), however, found that prevention of grooming during pregnancy had no effect on survival rates of young or on litter weights at weaning.
Alternatively, Roth and Rosenblatt (1967) have suggested that the shift in self-licking orientation during pregnancy resulted from increased peripheral stimulation from abdominal distension, enlarged and tender nipples, and vaginal swelling and leakage. Furthermore, they showed that self-licking was necessary in order for the mammary glands to develop normally during pregnancy, (Roth and Rosenblatt, 1968), a finding confirmed by McMurty and Anderson (1971). However, as was mentioned above, these impairments did not affect the rat's ability to care for her young (Christophersen and Wagman, 1965; Kirby and Horvath, 1968).

Increased salt appetite (Barelare and Richter, 1948; Scott, Smith and Verney, 1948) or altered stimulus input from the genital, nipple, and pelvic areas (Roth and Rosenblatt, 1967) might be important in producing the shift in self-licking orientation during pregnancy. However, an alternative account of the altered pattern of self-licking seen during pregnancy seems possible. The alterations in pattern of grooming are most pronounced during the latter half of pregnancy. At this time, there are large increases in weight (Brody, Riggs, Kauffman, and Herring, 1938) without any increase in the size of the tail (Appendix I). This would be likely to make passive heat loss more difficult and increase the need for evaporative cooling. Since grooming is an integral part of heat loss in the rat, the changes in self-licking observed during pregnancy might, at least in part, represent thermoregulatory behavior. Accordingly, the present series of experiments examined heat loss and heat production during heat stress in pregnant and non-pregnant rats.
Chapter 2

Body Temperature Regulation and Evaporative Water Loss in Pregnant and Non-pregnant Rats During Heat Stress

Experiment 1

As indicated in the introductory chapter, changes in size, food intake, and hormonal balance during pregnancy might tend to increase heat production in the rat. Decreased surface to volume ratio (Appendix I) would reduce the effectiveness of radiation as a heat loss mechanism in the pregnant rat. This would correspondingly increase the importance of other avenues of heat loss. For example, the altered pattern of grooming in pregnant rats (Appendix IV; Roth and Rosenblatt, 1967) might be indicative of greater need for evaporative cooling in pregnant rats.

To determine whether changes occur in thermoregulation in the heat during pregnancy, the following series of experiments compared body temperature regulation and evaporative water loss in pregnant and non-pregnant rats during exposure to a wide range of elevated ambient temperatures.

Method

Subjects were virgin and pregnant primiparous (days 17-22 of pregnancy) Sprague-Dawley female rats. They were housed individually at an ambient temperature of 22-27°C with food (Purina rat chow pellets) and water available ad libitum, except during experimental measurements. The cages were kept in a room with large windows, and the light cycle corresponded to the normal one in Pittsburgh, Pennsylvania for the part of the
year during which the experiments were run (June to December). Statistical
significance of results was established using a Mann-Whitney U-test (two-
tailed).

Part 1: Body Temperature Regulation in the Heat

To compare body temperature regulation in pregnant and non-pregnant
subjects, 5 pregnant and 5 non-pregnant rats were exposed to each of four
ambient temperatures (32, 36, 40, and 44°C) for a period of 4 hrs. During
the exposure, subjects were housed individually, without food or water, in
preheated metal cages (24 X 20 X 18 cm) which were kept in an incubator
(Hotpack) set to maintain a particular temperature ± 0.5°C. After each
1/2 hr. of exposure, each subject was briefly removed from the incubator
and her body temperature measured to the nearest 0.1°C with a telether-
mometer probe (Yellow Springs Instrument Co., Inc.) inserted approximately
5 cm. beyond the anal orifice. In addition, body temperature was determined
just before the beginning of heat stress and just after its conclusion. When-
ever body temperature was measured, subjective evaluations were made as
to how wet each subject appeared. Rats were studied no more frequently
than every two days.

Part 2: Evaporative Water Loss in the Heat

Evaporative water loss was measured at ambient temperatures between
24 and 44°C in seven non-pregnant and five pregnant rats. The apparatus
for measuring evaporative water loss was similar to that used by Hainsworth
(1968). It consisted of an air-tight, cylindrical plexiglas chamber (d=15 cm.,
h=22 cm.) with a wire mesh floor (4 cm. above the base of the chamber)
beneath which mineral oil was placed to a depth of 2 cm. to trap feces and
urine and prevent their evaporation. The chamber was ventilated by passing
dry compressed air at a rate of 4.5 l./min. through a drying tube (1.5 X 15 cm.) filled with anhydrous CaSO₄ (Drierite*) and then through coiled copper tubing into the experimental chamber. The air entered through holes around the circumference near the mesh floor of the plexiglas chamber and exited through one arm of a "Y" of plastic tubing attached to a hole in the lid of the chamber. The flow rate used provided complete flushing of the chamber about 1.4 times/min.

To measure water loss, three pre-weighed tubes of Drierite* were connected in series to the tubing through which the air exited. During experimental runs, the entire apparatus, except for the cylinder of compressed air and the first drying tube, was placed in an incubator (Hotpack) heated to a particular temperature (See Figure 1, Hainsworth, 1968). The temperature inside the plexiglas chamber was monitored with a telethermometer probe (Yellow Springs Instrument Co., Inc.) inserted through the other arm of the "Y" of tubing attached to the chamber lid and connected to a second YSI temperature meter.

To determine evaporative water loss at a particular ambient temperature, a subject was removed from its home cage, handled to induce urination and defecation, weighed to the nearest g., and placed in the chamber which had been preheated to a particular temperature ± 0.5°C. The chamber was immediately sealed, replaced in the incubator, and connected to the air supply. After 22 min., the three pre-weighed drying tubes were connected to the air outflow tubing for 15 min. to collect moisture from the air which was passing through the chamber. During this time, the chamber temperature was measured three times and these values averaged to determine the ambient temperature for each run. After 15 min. the tubes were disconnected, and the animal was removed from the chamber, reweighed, and
replaced in its home cage. The three drying tubes were allowed to cool to room temperature and then were weighed to the nearest 0.1 mg. The initial values were subtracted from these new values and differences were summed for each subject, multiplied by four (to obtain evaporative water loss per hr. in each subject, and divided by the mean of the before and after body weights to obtain the rate of evaporative water loss in mg. of water per g. of body weight per hr.). Data were discarded whenever feces were found not trapped beneath the mineral oil.

**Part 3: Evaporative Water Loss and Body Temperature Regulation at 40°C**

Four non-pregnant and four pregnant rats were exposed to an ambient temperature of 40 ± 0.5°C for 4 hrs. Body temperature was measured before the beginning of exposure and after each 1/2 hr. of exposure as in Part 1. Every 30 min. subjects were alternately placed in the incubator in metal cages as in Part 1 or in the apparatus described above for measurement of water lost by evaporation during the last 15 min. of each period. Subjects were weighed every 1/2 hr. and the rate of evaporative water loss was calculated as in Part 2 for each subject at the end of each hr. of exposure.

**Results**

**Part 1: Body Temperature Regulation in the Heat**

There were no significant differences in body temperature of pregnant and non-pregnant rats during exposure to an ambient temperature of 32°C (Fig. 1; all p's > 0.05). However, at this temperature, pregnant rats appeared slightly wet on their ventral surfaces, while non-pregnant subjects were completely dry throughout the 4 hr. exposure. In contrast,
Figure 1

Median body temperatures of pregnant and non-pregnant rats exposed to 32°C for 4 hrs. (both n's = 5).
FIGURE 1

Median body temperature (°C)

Time (hrs.)
at 36°C, pregnant rats maintained significantly lower body temperatures than non-pregnant rats (Fig. 2; all p's < 0.05) until the last measurement. In addition, pregnant subjects had wet paws, dripping chins, and large wet areas on their ventral surfaces after the first hour of exposure, while non-pregnant subjects remained dry. During exposure to 40°C, pregnant rats consistently maintained lower body temperatures than non-pregnant subjects (Fig. 3; all p's < 0.008), and both groups appeared equally wet. At 44°C, one of the non-pregnant subjects became hyperactive and was removed after the first 1/2 hr. to prevent development of fatal hyperthermia, and the remaining subjects were heat-stressed for only 2 1/2 hrs. because their body temperatures were rising rapidly. During exposure to this temperature, pregnant rats generally maintained significantly lower body temperatures than non-pregnant rats (Fig. 4; p = .016 at 1/2, 1, 2 hrs; p > 0.05 at 1 1/2 and 2 1/2 hrs). No difference in ventral surface wetness was apparent with both groups being soaking wet.

Part 2: Evaporative Water Loss in the Heat

Rates of evaporative water loss for non-pregnant and pregnant rats are shown in Fig. 5.

In the pregnant rats, the rate of evaporative water loss began to increase from a stable baseline level as a function of increasing temperature at environmental temperatures above 30°C. In the non-pregnant rats, such an increase was not apparent until ambient temperatures above 36°C were used. Accordingly, regression lines (Fig. 5) were calculated for evaporative water loss rates at ambient temperatures above 30°C for pregnant subjects and above 36°C for non-pregnant subjects. Inspection of Fig. 5 reveals that pregnant rats had higher rates of evaporative water
Figure 2

Body temperatures of pregnant and non-pregnant rats exposed to 36°C for 4 hrs. (both n's = 5).
Figure 3

Body temperatures of pregnant and non-pregnant rats exposed to 40°C for 4 hrs. (both n's = 5).
FIGURE 3

Body Temperature (°C)

Time (hrs.)

Pregnant
Non-pregnant
Figure 4

Body temperatures of pregnant (n = 5) and non-pregnant (n = 4) rats exposed to 44°C for 2 1/2 hrs.
Evaporative water loss (mg. \( \times \text{g.}^{-1} \times \text{hr.}^{-1} \)) as a function of ambient temperature in pregnant and non-pregnant rats; regression line equations:  
\[
\log Y = 0.0427X - 0.7763, \text{ Sxy } = \pm 0.1449 \text{ (pregnant)}; \log Y = 0.0752X - 2.2437, \text{ Sxy } = \pm 0.1542 \text{ (non-pregnant)}. 
\]
loss during exposure to ambient temperatures between about 30 and 42°C ($p = 0.018$). A comparison of the slopes of the two regression lines (Walker and Lev, 1953) did not show any significant difference between them ($p > 0.1$).

**Part 3: Body Temperature Regulation and Evaporative Water Loss at 40°C**

There were no significant differences in rate of evaporative water loss between non-pregnant and pregnant subjects at any time during the 4 hrs. of exposure (Fig. 6; $p$'s > 0.30). However, at each point of measurement during the 4 hrs. of exposure, pregnant rats had significantly lower body temperatures than did non-pregnant rats (Fig. 7; $p$'s = 0.028).

**Discussion**

The results of this experiment suggest that there are some striking changes in body temperature regulation during pregnancy in the rat.

During exposure to an ambient temperature of 32°C, both groups maintained similar body temperatures, but pregnant rats were beginning to increase evaporative water loss at 32°C. At 36°C, pregnant rats maintained lower body temperatures than non-pregnant rats by liberal use of saliva for evaporative cooling. These findings suggest that there must be a change in the body temperature threshold for increased salivary flow during pregnancy since the pregnant rats with lower temperatures produced copious amounts of saliva while non-pregnant rats with higher temperatures did not. In this regard, the regulated hyperthermia of the pregnant rats, even at 40°C, was below the body temperature threshold for salivation reported previously for non-pregnant female rats (Hainsworth and Stricker, 1972). The results of exposure to 32°C and 36°C also suggest that evaporative
Figure 6

Rates of evaporative water loss at the end of each of 4 hrs. of exposure to 40°C in pregnant and non-pregnant rats (both n's = 4).
Figure 7

Body temperatures of pregnant and non-pregnant rats exposed to 40°C for 4 hrs. (both n's = 4).
water loss is greater at these temperatures during pregnancy and may
be of increased importance in body temperature regulation at this time.
These possibilities will be investigated in Chapter 3.

Other changes in heat loss may occur as well during pregnancy.
At 40°C and 44°C, pregnant rats consistently maintained lower body tem-
peratures than non-pregnant rats even though there did not appear to be
any difference in the wetness of the two groups. Furthermore, measure-
ment of evaporative water loss over a 4 hr. exposure to 40°C clearly
revealed that pregnant rats did not require increased evaporation to
maintain lower body temperatures than non-pregnant rats. The maintenance
of lower body temperatures by pregnant rats in the heat without added
water loss was surprising in light of the initial expectation of impaired
thermoregulation by pregnant rats in the heat, and this finding suggests
that changes in passive heat loss may occur during pregnancy or that
evaporative cooling may be more efficient in pregnant subjects. As well,
the maintenance of lower body temperatures without apparent increases
in evaporation by pregnant rats exposed to 40 or 44°C might suggest lowered
heat production at these temperatures instead of or in addition to improved
heat loss ability. These possibilities will be explored in Chapter 4.
Chapter 3

Changes in the Function and Importance of the Salivary Glands as Thermoregulatory Effectors During Pregnancy in the Rat

Experiment 2

Differences in body temperature regulation and evaporative water loss in Experiment 1 suggested that the body temperature threshold for increased salivary flow is lower during pregnancy. As well, the results of Experiment 1 suggested that the salivary secretions may be of increased importance in thermoregulation during pregnancy. Part 1 of the present experiment compared salivary thresholds in pregnant and non-pregnant rats, and Part 2 compared body temperature regulation in pregnant and non-pregnant, intact and desalivated rats.

Method

Subjects, housing conditions, and statistical procedures were as in Experiment 1.

Part 1: Body Temperature Thresholds for Increased Salivation in Pregnant and Non-pregnant Rats

The body temperature threshold for increased salivary flow during heat stress was determined in intact pregnant (n=6) and non-pregnant (n=5) rats and in partially desalivated pregnant (n=10) and non-pregnant (n=10) rats.

Five non-pregnant and five pregnant rats had their parotid ducts ligated and cut and could thus secrete only submaxillary saliva. Five
non-pregnant and five pregnant rats had their submaxillary glands removed and could thus secrete only parotid saliva. Subjects were surgically desalivated under ether anesthesia through a midline incision in the ventral surface of the neck. The ducts and blood vessels of the submaxillary and sublingual glands were ligated and the glands excised in their common sheath. (The sublingual glands do not appear to play a role in thermoregulation in the rat (Elmer and Ohlin, 1970)). The ducts of the parotid gland were located where they passed over the masseter muscles, dissected away from the facial nerve, ligated twice, about 3 mm. apart, and cut between the ligations. The incisions were sutured and the rats allowed to recover for at least 7 days before testing. The operations were performed on day 12 of pregnancy for pregnant subjects, and all subjects were given water-soaked Purina rat chow pellets in addition to dry food and water during recovery to facilitate feeding and body weight maintenance.

Measurement of body temperature thresholds for increased salivation took place in an environmental room (39 ± 0.5°C) with subjects placed individually, without food or water, in a circular wire mesh (1/2 in.) cage (d. = 12 in., h. = 15 in.) supported by three 3 in. feet and having a wooden lid. All subjects were deprived of food for 3-4 hrs. prior to testing to minimize contamination of saliva samples with food particles. Saliva was collected with a pipette (1 mm. bore) by repeated aspiration from the subject's mouth and immediately placed in a graduated centrifuge tube (15 cc.) covered with Parafilm and located in an ice bath to prevent evaporation. (Subjects were discouraged from saliva-spreading by gently blowing on them whenever they initiated grooming.) When a sample of 0.4 to 0.5 cc. had been collected, the subject's body temperature was measured
as in earlier experiments, and this temperature was considered the body temperature threshold for increased salivation. In addition, the time to collect the sample was recorded to give an indication of salivary flow rate. Saliva samples were frozen for subsequent analysis of Na⁺ and K⁺ content by flame photometry (Model 143, Instrumentation Laboratory, Inc.).

Part 2: Body Temperature Regulation in Intact and Desalivated Pregnant and Non-pregnant Rats

Five non-pregnant subjects and five pregnant females (on day 10 of pregnancy) were totally desalivated using the surgical procedures described in Part 1. Subjects were allowed to recover for at least 7 days before testing. The success of the operations was ascertained behaviorally from the subjects' adoption of a prandial style of drinking and greatly increased food waste (Epstein, Spector, Samman, and Goldblum, 1964).

The recovered desalivated pregnant and non-pregnant rats were exposed to an ambient temperature of 36°C for 4 hrs. while housed individually without food or water, in preheated metal cages (24 X 20 X 18 cm.) which were kept in an incubator (Hotpack) set to maintain 36 ± .5°C. After each 1/2 hr. of exposure, each subject was briefly removed from the incubator and her body temperature measured to the nearest 0.1°C with the probe inserted approximately 5 cm. beyond the anal orifice. In addition, body temperature was determined before the beginning of exposure. Whenever body temperature was measured, subjective evaluations were made as to how wet each subject appeared. These data were compared with the records of the intact pregnant and non-pregnant rats (both n's = 5) exposed under the same conditions to 36°C in Experiment 1, Part 1.
Results

Part 1: Body Temperature Thresholds for Increased Salivation in Pregnant and Non-pregnant Rats

Body temperature thresholds for increased salivary flow, the time to collect the samples (flow rates) and the Na⁺ and K⁺ content of the samples for intact pregnant and non-pregnant rats are presented in Table 1. The salivary threshold was much lower in the pregnant rats in comparison to the non-pregnant rats \( (p = 0.004) \). Pregnant rats had significantly faster flow rates than non-pregnant rats \( (p = 0.004) \), i.e., it took less time to collect the samples from the pregnant subjects. There were no differences in salivary Na⁺ \( (p > 0.50) \) or K⁺ \( (p > 0.10) \) content.

Body temperature thresholds, sample collection times (flow rates), and salivary Na⁺ and K⁺ concentrations for partially desalivated pregnant and non-pregnant rats are presented in Table 2. Both the body temperature threshold and the collection time were significantly lower in the pregnant-parotid-desalivated rats when compared to the non-pregnant-parotid-desalivated rats \( (p's = 0.008) \). There was no significant difference in Na⁺ content of the submaxillary saliva collected from the non-pregnant and pregnant-parotid-desalivated subjects \( (p > 0.50) \), but the submaxillary saliva of the pregnant subjects was significantly lower in K⁺ \( (p = 0.016) \).

Non-pregnant and pregnant submaxillary-desalivated subjects did not differ from one another either in body temperature threshold or collection time for parotid saliva \( (p's > 0.05) \). However, the parotid saliva secreted by the pregnant-submaxillary-desalivated rats was lower in Na⁺ \( (p = 0.008) \) and higher in K⁺ content \( (p = 0.016) \) than that secreted by the non-pregnant-submaxillary-desalivated subjects.
### TABLE 1

Median values and ranges (in parentheses) for the body temperature threshold for increased salivary flow, sample collection times, and salivary Na\(^+\) and K\(^+\) concentrations in intact non-pregnant (n = 5) and pregnant rats (n = 6).

<table>
<thead>
<tr>
<th></th>
<th>Non-Pregnant Rats</th>
<th>Pregnant Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Temperature</strong></td>
<td>39.5 (39.3-39.7)</td>
<td>37.8 (37.4-38.7)</td>
</tr>
<tr>
<td><strong>Threshold (°C)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Collection Time</strong></td>
<td>25.0 (15.5-38.0)</td>
<td>11.0 (8.0-13.0)</td>
</tr>
<tr>
<td><strong>(min.)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Na(^+)</strong> (mEq/l.)</td>
<td>7.0 (4.5-8.5)</td>
<td>6.2 (3.5-9.0)</td>
</tr>
<tr>
<td><strong>K(^+)</strong> (mEq/l.)</td>
<td>50.0 (48.0-54.0)</td>
<td>46.0 (36.0-51.0)</td>
</tr>
<tr>
<td>(45.0-71.0°F)</td>
<td>(33.0-47.0°C)</td>
<td>(33.0-44.0°C)</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>60.0</td>
<td>42.0</td>
<td>43.0</td>
</tr>
<tr>
<td>(65.0-69.0°F)</td>
<td>(55.0-71.0°C)</td>
<td>(5.0-7.5°C)</td>
</tr>
<tr>
<td>76.0</td>
<td>100.0</td>
<td>6.5</td>
</tr>
<tr>
<td>(30.0-49.0°F)</td>
<td>(27.0-59.0°C)</td>
<td>(4.0-43.0°C)</td>
</tr>
<tr>
<td>37.0</td>
<td>43.0</td>
<td>0.0</td>
</tr>
<tr>
<td>(39.3-40.5°F)</td>
<td>(39.3-39.8°C)</td>
<td>(37.6-38.9°C)</td>
</tr>
<tr>
<td>39.6</td>
<td>39.4</td>
<td>38.3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>desalivated</th>
<th>desalivated</th>
<th>desalivated</th>
<th>desalivated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Submaxillary</td>
<td>Pregant</td>
<td>Submaxillary</td>
<td>Pregant</td>
</tr>
<tr>
<td>Non-Pregant</td>
<td>Pregant</td>
<td>Non-Pregant</td>
<td>Pregant</td>
</tr>
</tbody>
</table>

Maxillary desalivated, n,s = 5.

...and pregnant-parotid-desalivated, or only pregnant saliva (non-pregant and pregnant sub-
desalivated non-pregnant and pregnant rats secreting only submaxillary saliva (non-pregnant
salivary flow, sample collection times, and saliva Na⁺ and K⁺ concentrations in partially
median values and ranges (in parentheses) for body temperature thresholds for increased

**Table 2**
Part 2: Body Temperature Regulation in Intact and Desalivated Pregnant and Non-pregnant Rats

As shown in Fig. 8, there were no differences in body temperature between intact and desalivated non-pregnant rats (p's > 0.06) during the first hr. of exposure. After that, however, the body temperatures of desalivated rats were somewhat higher (p's < 0.04), although they nevertheless regulated their temperatures quite well. Both groups appeared quite dry throughout the exposure.

In contrast, desalivated pregnant rats showed much higher body temperatures for the first 2 1/2 hrs. than did the intact pregnant rats (p's < 0.02). Furthermore, three of the desalivated pregnant rats had to be removed from the incubator prior to the end of exposure (when body temperatures equalled 41.0, 41.4, and 41.7°C) to prevent possibly fatal hyperthermia. Interestingly, both pregnant groups appeared wet during the session, but testing with pH paper revealed that the moisture on the intact pregnant rats was basic (and therefore saliva) (Hainsworth and Epstein, 1966) while that on the desalivated pregnant rats was acidic (and therefore urine, which the desalivated pregnant rats had groomed onto their fur).

The intact pregnant rats showed initially lower regulated levels of hyperthermia in comparison to the intact non-pregnant rats (p's < 0.04 for the first 3 hrs.; p's > 0.05 thereafter), as seen previously (Fig. 2). Desalivated pregnant and non-pregnant subjects showed similar body temperatures for the first 2 1/2 hrs., but four of the five pregnant rats subsequently reached body temperatures ≥ 41.0°C within 4 hrs. whereas only one of five non-pregnant rats did.
Figure 8

Body temperatures of desalivated pregnant and non-pregnant rats and median body temperatures of intact pregnant and non-pregnant rats exposed to 36°C for 4 hrs. (all n's = 5).
Discussion

As expected, the body temperature threshold for increased salivary flow was much lower in pregnant than in non-pregnant rats. In addition, the rate of salivary flow, once hyperthermia-induced salivation began, was much faster in the pregnant subjects (i.e., it took less time to collect the samples).

These changes in salivary function occurred only in the submaxillary glands, and not also in the parotid glands, clearly showing that only the submaxillary glands participate in the drop in body temperature threshold for increased salivation and the increased flow rate seen during pregnancy. Further confirmation of this comes from a comparison of the Na⁺ concentrations of saliva samples from intact non-pregnant subjects with those of non-pregnant and pregnant parotid desalivated subjects which reveals that the samples obtained from intact rats clearly contained only submaxillary saliva. In this regard, it is interesting that only the threshold for submaxillary salivation, and not that for parotid secretion, is lower in males than in females (Hainsworth and Stricker, 1972).

It is indeed fortunate that the body temperature threshold for increased salivation is lower during pregnancy since pregnant rats are clearly more dependent than non-pregnant rats on evaporative cooling for body temperature regulation during heat stress. Intact pregnant rats use copious amounts of saliva during heat stress, and desalivated pregnant subjects cannot regulate their temperatures effectively enough via vasodilatation to withstand 4 hrs. of exposure to an ambient temperature of 36°C. If they had not been able to use their urine for evaporative cooling, they presumably would not have been able to tolerate the exposure for as long as they did.
While the need for altered salivary function during pregnancy is clear, the underlying basis for this change is unknown. Morphological or histological changes might occur during pregnancy which might alter glandular function. Such changes might be controlled by one or more of the hormonal changes accompanying pregnancy. In this regard, the submaxillary gland of adult male rats is relatively heavier and has larger diameter tubules than that of females. This sexual dimorphism has been shown to be under the control of gonadal and thyroid hormones (see review by Leeson, 1967). Similarly, male rats have a higher salivary flow rate than females, which probably reflects their larger gland size (see review by Schneyer and Schneyer, 1967). Pregnant females, however, have smaller submaxillary glands, relatively to body size, than do non-pregnant females (Appendix II). Thus, changes in gland size are unlikely to be important in the changes in threshold and flow rate observed in the present experiment.

Alternatively, changes in body fluid composition during pregnancy might be involved. Burger (1967) has suggested that increased plasma K\(^+\) is the only change in blood composition which produces an increase in the maximum salivary secretion rate. During pregnancy, rats were found to have increased levels of plasma K\(^+\) (Appendix III) which might have accounted for their higher salivary flow rates. Whether the increases in plasma volume (Appendix III; Brown and Pike, 1960; Weir, Painten, Brown, Fraser, Lever, Robertson, and Young, 1971) and total body water (Hyten, Thompson, and Taggart, 1966) characteristic of pregnancy are important is an empirical question. Unfortunately the effects of intracellular or intravascular overhydration on salivary function are not well understood. However, Holmes (1964) has found a slight increase in salivary secretion as a result of
water ingestion and a large increase in salivary flow as a result of intravenous injection of isotonic dextrose in water. In contrast, intracellular and intravascular dehydration have been found to reduce salivary output in the heat (Adolph, 1947; Stricker and Hainsworth, 1970) and under normal environment conditions (Holmes, 1964).

The submaxillary glands are activated during heat stress by the chorda tympani nerve (Stricker and Hainsworth, 1970). Their thermoregulatory response is controlled centrally by areas in the rostral portion of the medulla, the lateral hypothalamus, and ultimately, the preoptic area of the anterior hypothalamus (Stricker and Hainsworth, 1970). Whether neurophysiological changes occur during pregnancy which might affect the submaxillary glands is not known.

Further investigation is clearly necessary to determine the basis for the striking alterations in salivary function in the rat during late pregnancy. Whatever their origin, however, the decreased body temperature threshold for increased salivary flow and the increased flow rates provide admirably for the pregnant rat's greater dependence on evaporative cooling for body temperature regulation during heat stress. Furthermore, the rapidity of the salivary response to heat stress in the pregnant rats would seem to place some restrictions on the possible stimuli for salivation in the heat. Copious salivary flow was noted in many of the pregnant subjects within less than 1 min. of exposure to a temperature of 40°C. Thus, a rise in the temperature of the skin or of the inspired air, for example, would be likely to be a more important stimulus for increased salivation, at least for pregnant rats, than would a rise in core temperature.
Chapter 4

Behavioral and Physiological Changes in Heat Loss and
Heat Production in the Pregnant Rat During
Heat Stress

Experiment 3

In Experiment 1, Part 3, pregnant rats maintained much lower
body temperatures than non-pregnant rats without added evaporation during
a 4 hr. exposure to an ambient temperature of 40°C. Since, at this temp-
erature, essentially all heat loss occurs by evaporation (see Hainsworth,
1967), the pregnant rats might have been using available moisture more
effectively to dissipate heat. In this regard, increased blood flow to
the skin, spontaneous remission of Raynaud's Disease, and changes in vaso-
dilatation have been observed during pregnancy in humans (Hytten and Leitch,
1964). As well, progesterone injection has been found to lower the body
temperature threshold for vasodilatation in female rats (Thompson and
Stevenson, 1965). A similar change in vasodilatation threshold during
pregnancy would bring blood to the periphery to be cooled by evaporation
at a lower body temperature. However, comparison of the body temperature
threshold for vasodilatation in pregnant and non-pregnant rats revealed
that there was no change in vasodilatation threshold during pregnancy
(Appendix V).

Alternatively, another pregnancy-related change which might contribut
to more efficient evaporative cooling is the altered fur distribution
characteristic of pregnancy. Inspection of the ventral surface of the pregnant rat, where saliva-spreading during heat stress is concentrated, reveals that the nipples are enlarged and their bases bare of fur. Furthermore, uterine distension has made the enlarged abdomen appear more sparsely furred, and the nude genital area is somewhat enlarged. Such changes in fur distribution might be expected to increase the effectiveness of evaporation in pregnant rats since more water would be evaporated from skin instead of fur (see Schmidt-Nielsen, 1964). In Part 1 of the present experiment, these changes in fur distribution were exaggerated in non-pregnant rats by shaving their ventral surfaces and comparing their body temperature regulation at 40°C with that of pregnant rats.

The ability of pregnant rats to maintain lower regulated levels of hyperthermia than non-pregnant rats, without added evaporation, might also reflect a lower rate of heat production during heat stress. If pregnant subjects produced less heat to start with during exposure to elevated ambient temperatures, they would have less heat to dissipate so that evaporation of water in amounts similar to those of non-pregnant rats would permit maintenance of lower body temperatures. Part 2 of this experiment compared heat production as measured by oxygen consumption in pregnant and non-pregnant rats exposed to elevated ambient temperatures. Part 3 measured activity of pregnant and non-pregnant rats during heat stress since activity is a source of heat production and since pregnant rats at room temperatures have been shown to be far less active than non-pregnant rats at room temperatures (Slonaker, 1925; Wang, 1923).
Method

Subjects, housing conditions, and statistical procedures were as in Experiment 1.

Part 1: Body Temperature Regulation by Shaved and Unshaved Rats During Heat Stress

The characteristic sparser ventral surface fur of pregnant rats was exaggerated in five non-pregnant rats by shaving off all of their ventral surface fur while they were lightly anesthetized with ether. Three days later, they were exposed, without food or water, to 40 ± 0.5°C for 4 hrs. in individual metal cages (24 X 20 X 18 cm.) housed in an incubator (Hotpack). Their body temperatures were measured before the beginning of exposure and every 1/2 hr. during exposure as in Experiment 1. This data was compared with that of the five pregnant rats and the five non-pregnant rats exposed to 40°C in Experiment 1, Part 1.

Part 2: Oxygen Consumption of Pregnant and Non-pregnant Rats During Heat Stress

Seven pregnant rats and seven non-pregnant rats were used. To measure oxygen consumption, subjects were placed in a cylindrical glass chamber (h. = 12 in., d. = 9 in.) without food or water. The chamber was placed in an incubator (± 1.0°C, Hotpack) which was heated to an ambient temperature between 25 and 40°C. Compressed air was passed through tubes containing Drierite*, then through coiled copper tubing in the incubator and into the chamber at a flow rate of 1550 cc/min. From the chamber, the air again flowed through Drierite* and then passed through a paramagnetic oxygen analyzer (Beckman F-3, accuracy ± 0.2 percent O₂) which was connected of a Heath servorecorder and which constantly recorded oxygen
concentration of the effluent air. The chamber temperature was continuously monitored by a copper-constantan thermocouple and recorded (Speedomax H, Leeds and Northrup). Barometric pressure of the air was also measured. Oxygen consumption values were corrected to standard temperature and pressure.

Subjects were deprived of food for 3-4 hrs. before oxygen consumption measurements were made to ensure that all subjects began the experimental run with resting metabolic rates. Each rat was exposed to a particular ambient temperature for 75 min. During the first 45 min., subjects were allowed to adapt to the chamber and the temperature and to achieve a fairly stable body temperature (Fig. 1-4).

Average percentage of oxygen in the effluent air during each of the next 10 min. periods was noted and these values averaged for use in computation of oxygen consumption. Subjects were weighed at the beginning and end of exposure, and these weights were averaged and used to express oxygen consumption in terms of milliliters of oxygen per gram of rat per hour (ml. X g. \(^{-1}\) X hr. \(^{-1}\)).

In addition, oxygen consumption was measured after 2 1/2 hrs. of exposure to an ambient temperature of 40°C in five pregnant and six non-pregnant rats. Each subject was deprived of food for 1 hr. before exposure began and was then placed in a cylindrical wire mesh cage (h. = 12 cm., d = 8 cm.) in an incubator (Hotpack) heated to 40 ± 1.0°C for 100 min., at which time the regulated levels of hyperthermia shown by pregnant and non-pregnant rats are well established (Fig. 3). Each subject was then placed in the oxygen consumption measurement chamber described above and allowed
to become accustomed to the chamber for 20 min. During the next 30 min., oxygen concentration was recorded. The oxygen concentration values at the end of each 10 min. period during this 30 min. were averaged to compute oxygen consumption as above.

Part 3: Activity of Pregnant and Non-pregnant Rats During Heat Stress

Six non-pregnant and five pregnant rats were exposed to 40 ± 0.5°C for 4 hrs. as in Experiment 1, Part 1. During the last 10 min. of each 1/2 hr., subjects were observed through the glass door of the incubator. The number of seconds spent grooming and moving about the cage were recorded with a stopwatch, and these scores were summed to give total activity scores. Grooming was defined as licking the paws or any other part of the body or combing any part of the body with the paws. Moving was defined as any change in location in the cage or any head movement greater than approximately 1 in. in any direction. Vigorous scratching was included as movement. The number of seconds of moving, grooming, or total activity in each observation period was summed for each subject to obtain the number of seconds of the entire 80 min. of observation time spent moving, grooming, or totally active.

Results

Part 1: Body Temperature Regulation by Shaved and Unshaved Rats During Heat Stress

Figure 9 shows the body temperatures of the non-pregnant, pregnant and shaved non-pregnant rats during the 4 hr. exposure to 40°C. Pregnant rats showed significantly lower body temperatures than non-pregnant rats at each point of measurement (p's = 0.008). The shaved non-pregnant rats
Figure 9

Body temperatures of shaved non-pregnant rats and median body temperatures of normal pregnant and non-pregnant rats exposed to 40°C for 4 hrs. (all n's = 5).
FIGURE 9

Body Temperature (°C)

Time (hrs.)

- Pregnant
- Non-pregnant
- Shaved
maintained lower body temperatures than non-pregnant rats for the first 2 1/2 hrs. (p's < 0.032) but not thereafter (p's > 0.50). They were not significantly different from the pregnant rats for the first 2 hrs. of exposure (p's < 0.096) but maintained significantly higher temperatures for the remainder of the exposure period (p's < 0.032). In addition, the shaved non-pregnant rats had lower body temperatures than the non-pregnant rats (p = 0.008) and the pregnant rats (p = 0.056) before exposure began.

Part 2: Oxygen Consumption in Pregnant and Non-pregnant Rats During Heat Stress

Oxygen consumption in pregnant and non-pregnant rats as a function of ambient temperature is shown in Figure 10. Regression lines were calculated for oxygen consumption at temperatures above 30°C for pregnant rats and at temperatures above 32°C for non-pregnant rats. The slopes of both lines differ significantly from zero (p's < .02, Spearman Ranks Correlation Coefficient), but they did not differ significantly from each other (p > 0.1, Walker and Lev, 1953).

During exposure to ambient temperatures between 30 and 36°C, pregnant rats appear to consume more oxygen than non-pregnant rats. However, at temperatures above 36°C, non-pregnant rats appear to consume more oxygen. There was insufficient data to permit comparison of the pregnant and non-pregnant rats within the two temperature intervals. During prolonged exposure to an ambient temperature of 40°C, pregnant subjects consumed significantly less oxygen (median = 1.908 ml X g.⁻¹ X hr.⁻¹; range = 1.807 - 2.049 ml X g.⁻¹ X hr.⁻¹) than did non-pregnant subjects (median = 2.194 ml X g.⁻¹ X hr.⁻¹; range = 1.816 - 2.323 ml X
Figure 10

Oxygen consumption (ml. X g.\(^{-1}\) X hr.\(^{-1}\)) as a function of ambient temperature in pregnant and non-pregnant rats; regression line equations:

\[ Y = 0.029X + 0.341, \quad S_{xy} = \pm 0.085 \text{ (pregnant)}, \]
\[ Y = 0.143X - 3.665, \quad S_{xy} = \pm 0.104 \text{ (non-pregnant)}. \]
g.\(^{-1}\) x hr.\(^{-1}\)) (p = 0.03).

**Part 3: Activity of Pregnant and Non-pregnant Rats During Heat Stress**

As shown in Table 3, non-pregnant subjects spent significantly more of the 80 min. of observation during the 4 hrs. of exposure in total activity (moving plus grooming) than did the pregnant rats (p = 0.004). The differences between pregnant and non-pregnant rats in moving and grooming taken separately approached significance (p's = 0.052).

More specifically, Figure 11 shows that pregnant subjects spent significantly less time moving and grooming (total activity) during all but the first 10 min. observation period of the 4 hr. exposure (all p's < 0.03). However, differences in moving alone or grooming alone between pregnant and non-pregnant rats were not reliably significant.

**Discussion**

Having less ventral surface fur is clearly an advantage during exposure to 40°C. The shaved non-pregnant rats showed a regulated level of hyperthermia lower than that of the non-pregnant subjects and similar to that of the pregnant subjects during the first two hours of the exposure. It seems unlikely, however, that differences in fur distribution alone can account for the entire difference between the pregnant and non-pregnant rats. The shaved non-pregnant rats had lower body temperatures than either non-pregnant or pregnant subjects before the start of the exposure, presumably as a result of their decreased insulation. This might have increased the time necessary to reach a particular level of hyperthermia, thus maximizing the difference between non-pregnant and shaved
### TABLE 3

Median number of seconds and ranges (in parentheses) out of 80 min. of observation spent moving, grooming, or in total activity (sum of moving and grooming) for pregnant and non-pregnant rats.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moving</td>
<td>218 (163-571)</td>
<td>521 (417-734)</td>
</tr>
<tr>
<td>Grooming</td>
<td>154 (116-312)</td>
<td>618 (127-728)</td>
</tr>
<tr>
<td>Total activity</td>
<td>471 (354-687)</td>
<td>1124 (861-1254)</td>
</tr>
</tbody>
</table>
Figure 11

Median number of seconds spent moving and grooming by pregnant (n = 5) and non-pregnant (n = 6) rats exposed to 40°C for 4 hrs. Scores were obtained by summing the time spent moving and grooming during each observation period occurring during the last 10 min. of each 1/2 hr.
FIGURE 11

Median Activity Rate (sec.)

Time (hrs.)

Pregnant
Non-Pregnant
non-pregnant subjects while exaggerating the similarity between shaved-
non-pregnant rats and pregnant animals. In addition, the similarity of
the shaved non-pregnant rats to the pregnant rats lasted for only the
first half of the exposure, while the non-pregnant vs. pregnant difference
persisted for the entire 4 hrs. Finally, shaving the entire ventral surface
represents a very gross exaggeration of the altered fur distribution of
pregnancy. If a closer imitation had been used, the present results very
likely might not have been obtained.

Lower heat production during heat stress appears to be of far
greater importance to the pregnant rat's ability to maintain a lower
regulated level of hyperthermia, without added evaporation. At relatively
high ambient temperatures, i.e., above 36°C, pregnant rats clearly con-
sumed less oxygen than non-pregnant subjects. This difference was sus-
tained even during prolonged exposure to 40°C, suggesting that the pregnant
subjects which maintained lower body temperatures during exposure to 40°C
without added evaporative water loss (Experiment 1, Part 3) did so because
they were producing less heat which needed to be dissipated. The fact that
pregnant rats are clearly less active in the heat than are non-pregnant rats
undoubtedly explains their lower rates of oxygen consumption during heat
stress. In this regard, rats which were less active, in terms of saliva-
spreading and locomotion, tolerated longer exposures to elevated ambient
temperatures (Clark, 1971), probably because they produced less heat
(Campbell and Lynch, 1967; Clark, 1971) which had to be dissipated by
evaporation.
Chapter 5

General Discussion

Towards the end of pregnancy in the rat there are several striking behavioral and physiological changes in body temperature regulation during heat stress. When exposed to elevated ambient temperatures, pregnant rats maintain lower regulated levels of hyperthermia than do non-pregnant rats (Experiment 1, for example). This finding was quite surprising in view of the initial expectation that body temperature regulation by pregnant rats during heat stress would be impaired. The difference in body temperature regulation may also be manifested at normal room temperatures. Rats in the last third of pregnancy have slightly, but significantly, lower body temperatures than non-pregnant rats do at room temperatures of 22-27°C (Appendix VII). Perhaps this is a result of their spending more time grooming themselves (Appendix IV; Roth and Rosenblatt, 1967).

The maintenance during pregnancy of lower body temperatures both in the heat (Experiment 1, for example) and under normal laboratory conditions (Appendix VI) is aided by the pregnant rats' relative inactivity. It has long been known that pregnant rats at normal room temperatures are less active than non-pregnant rats (Slonaker, 1925; Wang, 1923). They are also less active during exposure to high ambient temperatures (Experiment 3, Part 3).

Changes in the salivary responses to heat stress also aid the pregnant rats' maintenance of lower regulated levels of hyperthermia. Saliva is produced faster and at a lower body temperature during late
pregnancy (Experiment 2, Part 1). These changes provide the pregnant rat with an ample supply of moisture for evaporative cooling. In this regard, it seems possible that the pregnant rats' maintenance of a lower regulated level of hyperthermia during heat stress is an artifact of their relatively greater availability of moisture for evaporative cooling. However, pregnant rats still maintain lower body temperatures than non-pregnant rats even when both groups have unlimited access to exogenous water (Appendix VI). This suggests that pregnant rats have an actual need or preference to maintain lower body temperatures both in the heat and perhaps at normal room temperatures (Appendix VII) as well.

The fundamental difference in body temperature regulation during pregnancy appears to be the fact that pregnant rats maintain lower body temperatures during heat stress (Experiment 1, for example) and possibly also at normal room temperatures (Appendix VII). The bases for the changes in thermoregulation during pregnancy are largely unknown at present. However, it seems likely that some of the widespread changes which occur during pregnancy must underly the pregnant rats' altered thermoregulatory ability.

One such change may be the simple existence of the rapidly growing fetuses. Fetal temperatures in utero in rabbits (Hart and Faber, 1965), sheep (Abrams, Caton, Curet, Crenshaw, Mann, and Barron, 1969), and humans (Mann, 1968; Wood and Beard, 1964) have been found to be considerably higher than maternal temperatures. In this regard deep body temperature is considered to be one of the important stimuli to the temperature regulating centers in the hypothalamus (Bligh, 1963). Intra-abdominal heating in sheep produces a thermoregulatory response sufficient to cause enough
of a heat loss response to lower significantly the temperature of the hypothalamus (Rawson, Quick, and Coughlin, 1969). Perhaps the pregnant rat maintains a lower body temperature because of deep body heating from her developing offspring. This could account for the fact that "actually" pregnant rats have lower body temperatures, while "hormonally" (pseudo-pregnant or progesterone-injected) pregnant rats, with no fetuses, have higher temperatures than normal controls (Niebergs, Kupperman, and Greenblatt, 1946; Niebergs and Greenblatt, 1948).

Alternatively, it seems possible that the body temperature set point is lower during pregnancy. Myers and his co-workers (e.g., Myers and Veale, 1970; Myers and Yaksh, 1971) have found that the body temperature around which regulation occurs in a number of species is controlled by the ratio of calcium to sodium in the blood bathing the hypothalamus. A rise in calcium relative to sodium lowers the set point, while a rise in sodium relative to calcium raises it. During the last third of pregnancy, plasma sodium levels are lower than in non-pregnant subjects (Appendix III), while blood calcium levels have not been found to change during pregnancy (McClellan, 1970). Thus, the calcium to sodium ratio is altered, and there is a relative rise in calcium, making a drop in the body temperature set point possible during late pregnancy.

The lower rates of activity during pregnancy have been attributed to the anesthetic effect of progesterone on general body activity (see Rothchild, 1969, for review). Such an explanation would seem sufficient to account for the relative inactivity of pregnant rats during heat stress (Experiment 3, Part 3). However, rates of activity in pregnant hamsters may be lower than in pseudopregnant hamsters (Richards, 1966), suggesting that the altered hormonal state of pregnancy alone may not entirely account
for the drop in activity. Since pseudopregnant animals also gain weight (Wade, 1972), differences in heat production during activity probably do not account for differences in activity. As discussed earlier, however, pregnant animals would receive stimuli from deep body heating which pseudopregnant animals would not receive. Thus, the drop in activity seen during pregnancy may be partly thermoregulatory in nature.

Of the changes in the salivary response to heat stress during pregnancy, the increased flow rate from the submaxillary glands (Experiment 2, Part 1) is easiest to explain. Increased levels of $K^+$ in the blood appear to be the only changes which produce an increased salivary flow (Burger, 1967). Pregnant rats do show increased blood $K^+$ levels (Appendix III) during the last third of pregnancy when they show the increased salivary response. Thus it seems likely that their rise in plasma $K^+$ can account for their greater salivary output in the heat. Little is known about changes in the body which might produce an altered body temperature threshold for increased salivation. In the rat, however, the salivary response is one of the major mechanisms of heat loss (Hainsworth and Stricker, 1970). Any change in the systems regulating heat loss would be expected to affect the salivary response to hyperthermia. Thus a drop in the body temperature set point during pregnancy would likely reduce the body temperature threshold for salivation. Alternatively, the lower body temperature threshold for increased salivation may reflect the activation of heat loss centers by intra-abdominal heating produced by the relatively hot developing fetuses.

A number of speculations concerning the underlying bases of the pregnancy-related changes in thermoregulation during heat stress can be offered. In deciding among them it would be helpful to know when during
pregnancy the changes first become apparent. The major focus of the present work was on rats during the last 5-6 days of pregnancy. Since changes in self-licking occur much earlier, however, it seems possible that there may also be alterations in thermoregulatory ability earlier in pregnancy. The exploration of this issue and the identification of the bases of the pregnancy-related changes in thermoregulation remain experimental questions at present.

The function and importance of the changes in thermoregulatory ability during pregnancy are similarly unknown. It seems possible that the maintenance of lower body temperatures by pregnant rats, as well as their reduced activity, would have the effect of limiting the increase in caloric intake necessary during pregnancy. More importantly, exposure to high environmental temperatures is known to have deleterious effects on reproduction in a number of species (see review by Waites, 1968), including rats (Benson and Morris, 1971; Pennyciu, 1964). In this regard, fetal temperatures in the rat are likely to be considerably higher than maternal temperatures. In other species, the fetal-maternal temperature difference is as much as 0.8°C (Abrams, Caton, Curet, Crenshaw, Mann, and Barron, 1969; Mann, 1968). If such a difference persisted during heat stress, fetal temperatures could become dangerously high unless the mother were able to increase her rate of heat loss and maintain a lower temperature herself. Perhaps the pregnant rat during heat stress should be said to be regulating the temperature of her unborn young, rather than her own body temperature. If intra-abdominal heat stimuli are important for body temperature regulation, the pregnant rat could indeed be regulating fetal, not maternal, temperature.
This possibility suggests an interesting speculation regarding the evolutionary development of deep body temperatures receptors. In the natural environment, the most likely time for an animal to experience heating deep within the body is during pregnancy. Since elevated temperatures are deleterious to reproductive performance (Benson and Morris, 1971; Pennycuik, 1964), animals which could lose heat in response to such deep body heating would have relatively greater rates of reproductive success. This trait would thus become more prevalent in the population.

The underlying bases of the changes in thermoregulatory ability which accompany pregnancy are largely unknown. Likewise, the function and importance of these changes are obscure. Nevertheless, there are striking behavioral and physiological changes in body temperature regulation which occur during pregnancy. These changes are most certainly yet another example of the body’s remarkable ability to maintain homeostasis, even during the altered condition of pregnancy.
Appendix I

Tail Length in Pregnant and Non-pregnant Rats

Subjects, housing conditions, and statistical procedures were as in Experiment 1. Tail length (from the tip to where the tail becomes fur-covered at the base) and circumference (as close to the fur at the base as possible) were measured to the nearest mm., and tail surface was approximated using the formula for the curved surface area of a cone ($\pi r \sqrt{r^2 + h^2}$) in six non-pregnant and six pregnant rats. All subjects were weighed to the nearest g. at the time of tail measurement to allow expression of tail size relative to body weight.

The results are summarized in Table 4. No significant differences were found between non-pregnant and pregnant rats with regard to tail length or surface area (p's > 0.20). However, pregnant rats weighed 27% more than non-pregnant rats (p = 0.002). Thus, when tail length and surface area were expressed in terms of body weight, pregnant rats were found to have significantly shorter tails and smaller tail surface areas than non-pregnant rats (p's = 0.002).
TABLE 4

Median values and ranges (in parentheses) for body weight, absolute tail length and surface area, and tail length and area relative to body weight in non-pregnant and pregnant rats (both n's = 6).

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant Rats</th>
<th>Pregnant Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>body weight (g.)</td>
<td>343.0</td>
<td>423.0</td>
</tr>
<tr>
<td></td>
<td>(312-360)</td>
<td>(392-478)</td>
</tr>
<tr>
<td>tail length (cm.)</td>
<td>18.5</td>
<td>18.8</td>
</tr>
<tr>
<td></td>
<td>(17.5-19.1)</td>
<td>(18.4-20.3)</td>
</tr>
<tr>
<td>tail length/100 g. body wt.</td>
<td>5.4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>(5.0-5.8)</td>
<td>(4.1-4.7)</td>
</tr>
<tr>
<td>tail area (cm.²)</td>
<td>32.9</td>
<td>32.2</td>
</tr>
<tr>
<td></td>
<td>(27.0-41.4)</td>
<td>(29.0-36.0)</td>
</tr>
<tr>
<td>tail area/100 g. body wt.</td>
<td>9.4</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>(8.6-9.8)</td>
<td>(6.9-8.5)</td>
</tr>
</tbody>
</table>
Appendix II

Submaxillary Gland Weights in Pregnant and Non-pregnant Rats

Subjects, housing conditions, and statistical procedures were as in Experiment 1. Eight non-pregnant rats and six pregnant rats were weighed to the nearest g. and given an intraperitoneal injection of 1.0 cc. of Nembutal* anesthetic. When they were anesthetized, their submaxillary and sublingual glands were removed in their common sheath through a midline incision in the ventral surface of the neck. The glands were quickly dissected free of non-glandular tissue, wrapped tightly in pre-weighed pieces of aluminum foil, and weighed to the nearest 0.1 mg.

Table 5 presents the results of the measurement of salivary glands in pregnant and non-pregnant rats. In terms of absolute weight, the difference in size between the salivary glands of the pregnant and non-pregnant rats was not significant (p > 0.05). However, since the pregnant rats were significantly heavier than the non-pregnant rats (p < 0.001), their salivary glands were smaller, relative to body weight, than the salivary glands of the non-pregnant rats (p = 0.02).
TABLE 5

Median values and ranges (in parentheses) for body weight, submaxillary-sublingual salivary gland weight, and gland weight/100 g. body weight in pregnant (n = 6) and non-pregnant (n = 8) rats.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g.)</td>
<td>432 (392-478)</td>
<td>326 (236-380)</td>
</tr>
<tr>
<td>Gland Weight (mg.)</td>
<td>557.6 (479.6-664.7)</td>
<td>497.3 (435.4-563.3)</td>
</tr>
<tr>
<td>Gland Weight/100 g. Body Weight (mg.)</td>
<td>133.6 (114.2-151.1)</td>
<td>169.1 (133.8-193.8)</td>
</tr>
</tbody>
</table>
Appendix III

Blood Parameters in Pregnant and Non-pregnant Rats

A T-shaped abdominal incision was made in six anesthetized non-pregnant and five pregnant rats, and 3-5 cc. of blood were collected from each rat with a syringe inserted into the abdominal aorta. The blood samples were transferred to heparinized test tubes and centrifuged. The plasma was then drawn off and used to determine plasma Na⁺ and K⁺ by flame photometry (Model 143, Instrumentation Laboratory, Inc.), plasma osmolarity by freezing point depression (Advanced Instruments, Inc.), and plasma protein by refractometry. Samples of blood from each subject were also collected in microcapillary tubes, centrifuged, and used to determine hematocrit. All measurements were made twice and averaged for each subject.

Table 6 shows the blood parameters of the pregnant and non-pregnant rats. Pregnant rats had lower plasma Na⁺ levels (p = 0.018) but higher plasma K⁺ levels (p = 0.008) than non-pregnant rats. Plasma osmolarity, plasma protein, and hematocrit were all lower in the pregnant subjects (all p's = 0.004).
TABLE 6

Median values and ranges (in parentheses) for plasma Na⁺, plasma K⁺, plasma osmolarity, plasma protein, and hematocrit for pregnant (n = 5) and non-pregnant (n = 6) rats.

<table>
<thead>
<tr>
<th></th>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Na⁺ (mEq/l.)</td>
<td>133.8</td>
<td>138.2</td>
</tr>
<tr>
<td></td>
<td>(130.5-136.0)</td>
<td>(134.8-139.0)</td>
</tr>
<tr>
<td>Plasma K⁺ (mEq/l.)</td>
<td>4.1</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>(3.6-4.6)</td>
<td>(3.0-3.6)</td>
</tr>
<tr>
<td>Plasma Osmolarity (mOsm/l.)</td>
<td>278.0</td>
<td>291.8</td>
</tr>
<tr>
<td></td>
<td>(278.0-282.0)</td>
<td>(288.0-295.5)</td>
</tr>
<tr>
<td>Plasma Protein (g/100 ml.)</td>
<td>5.2</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>(4.8-5.7)</td>
<td>(6.0-7.0)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>35.0</td>
<td>43.5</td>
</tr>
<tr>
<td></td>
<td>(30.0-36.5)</td>
<td>(42.0-44.8)</td>
</tr>
</tbody>
</table>
Appendix IV

Changes in Self-Licking During Pregnancy in the Rat

Subjects were 11 non-pregnant and 14 pregnant Spraque-Dawley rats, weighing 200-320 g. at the beginning of the experiment. Pregnant subjects were obtained from the supplier within 24 hrs. of mating, on day 1 of pregnancy.

All subjects were housed individually with food and water continuously available. A light-dark cycle was in effect with the dark period extending from 1 am. until 1 pm. Routine maintenance of the subjects was carried out during the light period. The experiment was performed during the summer months, and the ambient temperature of the experimental room fluctuated between 26 and 28°C due to malfunctioning air conditioning.

All observations of self-licking were made during the dark period of the light-dark cycle with lighting for the experimenter provided by a 25 watt red light bulb. Pregnant subjects were observed for 30 min./day on days 2-5, 10-13, and 18-21 of pregnancy. Non-pregnant animals were observed for 30 min./day during two 4-day blocks occurring after the second and third observation period for the pregnant rats. Subjects were watched in pairs, and the amount of time spent licking the nipple areas and the genital area was recorded with a stopwatch. In addition, the total time spent licking all areas of the body was recorded during the second and third observation periods for the pregnant rats and during both periods for the non-pregnant subjects. To obtain self-licking scores, the time spent licking particular areas was summed for each 4-day period. Thus,
for pregnant subjects, there were nipple-licking and genital-licking scores for days 2-5, 10-13, and 18-21 and total-licking scores for days 10-13 and 18-21. For non-pregnant subjects, there were two sets of nipple-licking, genital-licking, and total-licking scores.

Statistical significance of results was established using a Mann-Whitney U test (two-tailed) for comparisons of pregnant and non-pregnant animals and a Wilcoxon matched pairs-signed ranks rest (two-tailed) for within-group comparisons.

Self-licking scores (number of seconds spent licking and percentage of total-licking time spent licking various areas) are presented in Table 7. Only one set of scores is presented for the non-pregnant animals. The scores for the second observation block were discarded since the ambient temperature during these 4 days dropped to 25°C (while it had previously averaged 27°C), and total-licking time dropped in the non-pregnant animals during this time by almost 50% (p < 0.02).

During each 4-day observation block of pregnancy (days 2-5, 10-13, and 18-21) pregnant rats had higher genital-licking and nipple-licking scores (p's < 0.002) than non-pregnant rats. On days 10-13 and 18-21, when total-licking was recorded, pregnant rats spent a higher percentage of total-licking time genital-licking (p's < 0.02) and nipple-licking (p's < 0.002) than non-pregnant rats. In addition, pregnant rats showed more total-licking than non-pregnant rats during both observation blocks (p's < 0.02). This corresponded to 6% of the observation time on days 10-13 and 8% on days 18-21 in the pregnant rats vs. 4% in the non-pregnant subjects.

No significant increases in time spent genital-licking or in
TABLE 7

Median values and ranges (in parentheses) during each 4-day observation period for number of seconds spent licking any part of the body (total-licking) and number of seconds and percentage of total licking time spent nipple licking or genital licking by non-pregnant (n = 11) and pregnant (n = 14) rats (during various parts of pregnancy).

<table>
<thead>
<tr>
<th></th>
<th>Non-pregnant Rats</th>
<th>Pregnant Rats (days 2 - 5)</th>
<th>Pregnant Rats (days 10 - 13)</th>
<th>Pregnant Rats (days 18 - 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total-licking</strong></td>
<td>205.0</td>
<td>399</td>
<td>515</td>
<td></td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>(115-587)</td>
<td>(104-958)</td>
<td>(249-1440)</td>
<td></td>
</tr>
<tr>
<td><strong>Nipple-licking</strong></td>
<td>7.5</td>
<td>15.5</td>
<td>32.5</td>
<td>32.0</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>(0-14)</td>
<td>(6-85)</td>
<td>(0-93)</td>
<td>(14-164)</td>
</tr>
<tr>
<td><strong>% Total-licking</strong></td>
<td>2.0</td>
<td>---</td>
<td>8.0</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Nipple licking</strong></td>
<td>(0-3)</td>
<td></td>
<td>(0-17)</td>
<td>(3-16)</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>7.5</td>
<td>19.0</td>
<td>18.5</td>
<td>31.0</td>
</tr>
<tr>
<td><strong>Genital licking</strong></td>
<td>(0-10)</td>
<td>(3-64)</td>
<td>(5-47)</td>
<td>(0-66)</td>
</tr>
<tr>
<td><strong>% Total-licking</strong></td>
<td>1.0</td>
<td>---</td>
<td>6.0</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Genital licking</strong></td>
<td>(0-6)</td>
<td></td>
<td>(1-12)</td>
<td>(0-26)</td>
</tr>
</tbody>
</table>
percentage of total-licking time spent genital-licking were found as pregnancy advanced (p's > 0.05), but more time was spent nipple-licking on days 18-21 than on days 2-5 of pregnancy (p < 0.05), and the percentage of time spent nipple-licking was greater on days 18-21 than on days 10-13 (p < 0.05). In addition, total-licking increased by over 25% from days 10-13 to days 18-21 of pregnancy (p < 0.02).

Comment

In general, the findings of the present experiment confirm those of Roth and Rosenblatt (1967). Licking of pregnancy-related areas increased over non-pregnant levels both in terms of absolute amount of time and in terms of percentage of total-licking time. However, there were some minor differences between the results of the present study and those of Roth and Rosenblatt (1967). Changes in genital-licking and nipple-licking as a function of advancing pregnancy were not as striking in the present experiment as in the study by Roth and Rosenblatt (1967). However, in the present experiment, the pregnancy-related increases in genital-licking and nipple-licking were apparent on days 2-5 of pregnancy, while Roth and Rosenblatt (1967) did not find a difference between non-pregnant rats and days 2-5 pregnant rats with regard to genital-licking or nipple-licking.

A more important discrepancy concerns changes in total time spent licking. While Roth and Rosenblatt (1967) found no change in total-licking with advancing pregnancy, the present experiment found that, on days 10-13 and 18-21, pregnant rats had higher total-licking scores than non-pregnant rats; for example, day 18-21 pregnant rats spent twice as much of the observation time licking themselves compared
to non-pregnant rats. In addition, total-licking did increase as a function of advancing pregnancy.

The changes in total-licking coupled with changes in nipple-licking and genital-licking are even more suggestive of thermoregulatory behavior than are changes only in nipple-licking and genital-licking. In this regard, the ambient temperature in Roth and Rosenblatt's (1967) study was 22° ± 1°C. This difference in temperature should not have increased the need for evaporative cooling in non-pregnant rats since both temperatures are below those at which non-pregnant rats show elevated body temperatures (Hainsworth, 1967) or increased rates of evaporative water loss (Hainsworth, 1968). However, the increase in temperature from 22 to 27°C might have increased the need for evaporation in a pregnant rat whose ability to lose heat passively is decreased (Appendix I).
Appendix V

Body Temperature Threshold for Vasodilatation in Pregnant and Non-pregnant Rats

Subjects, housing conditions, and statistical procedures were as in Experiment 1. Using a method similar to that used by Grant (1963), the body temperature threshold for vasodilatation was measured in six non-pregnant and six pregnant rats while they were lightly anesthetized with Nembutal*. One telethermometer probe was inserted 5 cm. beyond the anal orifice to measure body temperature, another probe was taped to the tail between the tip and the base, to measure tail temperature, and a third probe measured the ambient temperature near the tail. Rats were made hyperthermic by placing them on a heating pad, but their tails were shielded from the heat by a 10 by 10 in. square of 1 in. thick foam rubber covered with aluminum foil. Ambient temperature was monitored intermittently, and tail and body temperatures were recorded every 30 sec. The body temperature threshold for vasodilatation was considered to be that body temperature at which tail temperature began a rapid rise. The ambient temperature near the tail was recorded and subjects removed from the heating pad when their body temperature reached 40°C.

Representative records for one non-pregnant and one pregnant rat are shown in Figs. 12 and 13. The body temperature threshold for vasodilatation in non-pregnant rats (median = 38.4°C; range = 37.3 - 38.5°C) was not significantly different from the threshold shown by the pregnant rats (median = 38.6°C; range = 38.0 - 39.0°C) (p > 0.30).
Figure 12

Representative record of body temperature threshold for vaso-dilatation in a non-pregnant rat (air temperature = 27.3°C).
Threshold $= 38.2^\circ C$
Figure 13

Representative record of body temperature threshold for vasodilatation in a pregnant rat (air temperature = 27.5°C).
Threshold = 38.2°C
Appendix VI

Body Temperature Regulation of Pregnant and Non-pregnant Rats Given an Unlimited Supply of Water During Heat Stress

Subjects, housing conditions, and statistical procedures were as in Experiment 1. Six non-pregnant and five pregnant rats were exposed to 40°C for 4 hrs. as in Experiment 1. All subjects had access to water in circular glass dishes (h. = 5 cm., d. = 9 1/2 cm.) which were kept filled to within 1/2 - 1 in. of the top with water from a beaker kept in the same incubator but outside the cages.

Body temperature was measured as in earlier experiments before the beginning of exposure and after each 1/2 hr. of exposure. Subjective evaluations of wetness and behavior were made during the exposure.

The body temperature and wetness estimates of the five pregnant and five non-pregnant rats run for 4 hrs. at 40°C in Experiment 1 were used for comparison with the data from the pregnant and non-pregnant rats with access to water baths in the present experiment.

Figure 14 shows the body temperatures of the pregnant and non-pregnant groups, with and without access to a water bath during exposure to 40°C for 4 hrs. Non-pregnant subjects with access to water maintained lower body temperatures than non-pregnant subjects with no free water during the 4 hr. exposure (p's ≤ 0.016), as did pregnant subjects (p's = 0.032). Pregnant subjects with access to water showed lower body temperatures than non-pregnant rats with access to water (p's ≤ 0.036).
Figure 14

Body temperatures for pregnant (n = 5) and non-pregnant (n = 6) rats with access to a water bath during a 4 hr. exposure to 40°C and median body temperatures of pregnant and non-pregnant rats (both n's = 5) without access to a water bath during a 4 hr. exposure to 40°C.
Pregnant and non-pregnant subjects without access to water appeared equally wet during the exposure with moderate amounts of moisture on their ventral surfaces. In contrast, the paws and much of the ventral surface fur of the pregnant rats having water baths were often dripping wet. Surprisingly, the fur of the non-pregnant rats generally seemed less wet than that of the other groups (especially the pregnant rats with water baths) and occasionally appeared almost completely dry. However, both pregnant and non-pregnant rats with water baths in their cages were observed to dip their paws and tails into the water bath, walk through the water, drag their tails through the water, and occasionally groom some of the water onto their fur.

Comment

In the present experiment, both non-pregnant and pregnant rats maintained lower regulated levels of hyperthermia during heat stress if they had access to a water bath than if they had to rely on saliva alone for evaporative cooling. Thus, the non-pregnant vs. pregnant difference in body temperature regulation observed in earlier experiments during exposure to 40°C persisted, even when all subjects had an unlimited supply of water available. This result suggests that the lower regulated levels of hyperthermia during heat stress in pregnant rats reflect a need or preference for lower body temperatures at this time rather than an artifact of the pregnant rats’ greater availability of water.

The validity of the present suggestion rests on the assumption that the non-pregnant subjects with access to a water bath were not losing heat by evaporation at the maximum possible rate given the particular experimental conditions involved. Unfortunately, such an assumption is,
at best, difficult to prove. However, non-pregnant rats with access to water occasionally appeared quite dry, while pregnant subjects with exogenous water usually had soaking wet paws and ventral surfaces. Presumably, the non-pregnant rats could have wetted their paws more frequently or groomed water onto their fur more often. In this regard, Stricker, Everett, and Porter, (1968) found that rats given access to a water bath during exposure to 40°C were continuously wet, making extensive use of the water to wet their tails, paws, heads, and torsos. Furthermore, they found that those subjects with the lowest body temperatures seemed in general to make the most extensive use of the water (Stricker, Everett, and Porter, 1968). Since the procedures and equipment in that study were the same as those used in the present experiment, it seems unlikely that such factors as humidity would have prevented increased effective use of the water by the non-pregnant rats. Instead, it appears that, during heat stress, pregnant rats may have a need or preference for maintenance of lower body temperatures than those shown by non-pregnant rats.
Appendix VII

Changes in Body Temperature at Normal Room Temperatures During Pregnancy in the Rat

Subjects, housing conditions, and statistical procedures were as in Experiment 1. To obtain body temperature recordings at laboratory temperatures, the pre-heat stress body temperatures of normal pregnant and non-pregnant rats used in previous experiments in this manuscript were assembled. Whenever a subject had been heat-stressed more than once (and thus had more than one initial temperature recording) a mean body temperature was calculated for that subject. In all, there were body temperature recordings for 19 pregnant rats and 16 non-pregnant rats. The temperature of the laboratory during the period in which these measurements were obtained ranged between 22 and 27°C.

The pregnant subjects had a lower body temperature (median = 37.0°C; range = 36.1 - 37.7°C) than the non-pregnant subjects (median = 37.2°C; range = 36.7 - 38.4°C). While this difference was small, it nevertheless was significant (p < 0.05).
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