THERMOENDOCRINE INFLUENCES ON THE DURATION OF MOTHER-LITTER CONTACT IN THE NORWAY RAT

by

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THERMOENDOCRINE LIMITATION OF MOTHER-LITTER CONTACT IN THE RAT

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Abstract

During the first two weeks postpartum, the lactating Norway rat shows a gradual decline in the amount of time that she spends in contact with her litter. This decline results from a gradual decrease in the duration of individual nest bouts rather than in their frequency. This normal nesting pattern is disrupted following adrenalectomy-ovariectomy (Adex-Ovex) and prolactin suppression (Leon, Croskerry & Smith, 1978). Since the effects on nesting behaviour produced by these manipulations are accompanied by a decrease in maternal core temperature it has been suggested that the normally elevated core temperature of lactating dams renders them vulnerable to an acute rise in temperature during a nest bout and the dam terminates the nest bout to maintain her thermal homeostasis.

The purpose of this dissertation was to: 1) identify those hormones sufficient to maintain normal nest time; 2) determine the mode of action of those hormones and 3) determine the relative importance of thermal factors in limiting nest bout duration at different stages postpartum. Glucocorticoids were identified as sufficient adrenal factors to reinstate the normal nesting pattern and body temperature of Adex-Ovex dams. Moreover, hormone-replaced females had the normal, additional rise in temperature during Day 10 nest bouts, whereas Adex-Ovex females did not. These data not only support the notion that a rise in dam core temperature during a nest bout produces bouts of shorter duration but they also indicate that the elevated core

temperature typical of lactating dams makes them vulnerable to the acute thermal effects of huddling.

Prolactin-suppressed dams show a halt in the decline in daily nest time in the second week postpartum which is accompanied by a reduction in maternal core temperature. It has been suggested that the effects of prolactin suppression are mediated by a consequent depression in corticosterone levels, rather than a direct effect of the absence of prolactin (Leon, Groskerry & Smith 1978). Experiments described in this thesis show that, as predicted, corticosterone replacement in prolactin-suppressed dams reinstated a normal decline of daily nest time and increases their core temperature. However, corticosterone may act by compensating for some of the metabolic actions of prolactin, because prolactin-suppressed dams actually had increased levels of circulating corticosterone.

Reinstatement of maternal heat load by warming the maternal huddles reinstated normal patterns of nesting behaviour in Adex-Ovex and prolactin-suppressed dams. Indeed, warmed Adex-Ovex dams had an acute rise in core temperature during nest bouts, similar to that seen in intact dams.

The question of the relative importance of thermal cues in inducing nest bout termination at different stages postpartum was determined by comparing the changes in core temperature that dams experienced during nest bouts in the first and then the second week postpartum. Thermal factors played little role in limiting the nest bouts of Day 4 dams, but by Day 10, the percentage of nest bouts during which dams experienced a rise in core temperature had increased to 95%.

Moreover, the rate of increase in maternal core temperature during a Day 10 nest bout was significantly greater than that experienced on Day 4.

The data obtained indicate 1) glucocorticoids plus prolactin are sufficient to maintain the normal pattern of nesting behaviour in the lactating rat; 2) hormonal influences on nest time are thermally mediated; 3) thermal factors increase their contribution to the limitation of mother-litter contact over the first two weeks postpartum such that they result in the gradual decline in daily nesting time that is typical of this species.

Acknowledgements

Many people have contributed to this dissertation. I am particularly indebted to Dr. Michael Leon who introduced me to this problem and was a constant source of encouragement and support throughout both the experimental work and the preparation of this thesis. Dr. Grant Smith and Dr. Ron Racine made many helpful comments on the design of the experiments and Drs. Harvey Feder, Christina Fischetti, and Harold Siegel kindly performed the hormone assays for Experiment 5. Dr. Rolf Morrison was a patient source of advice on statistical matters.

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Introduction

Altricial mammalian young are exclusively dependent on their mother for the delivery of food and warmth, which can only be accomplished when the mother and her young come into contact. In the days immediately following parturition, Norway rat dams spend nearly 80% of the day in contact with their litters, but, as the young develop, there is a steady decline in daily nesting time. This decline results from a decrease in the duration of individual nesting bouts rather than in the frequency of their occurrence (Ader and Grota, 1970; Croskerry Smith & Leon, 1978; Croskerry, Smith, Leon & Mitchell, 1976).

The onus for nest bout termination and, therefore, the total daily nest time rests with the mother during the first two weeks postpartum, since pups continue to suckle for as long as the nipples are profferred (Wakerley and Lincoln, 1971). The mother does not seem to terminate nesting bouts in response to cues associated with milk delivery, since mothers whose nipples are sealed exhibit a virtually normal decline in daily nesting time (Leon, Croskerry & Smith, 1978). Rather, mothers seem to respond to increases in their own temperature that they experience during contact with their young, by limiting the duration of nest bouts duration. Croskerry, Smith & Leon, 1978 have suggested that when dams initiate a nest bout, they effectively join a huddle and thereby occlude a portion of their ventral surface that is usually available for heat dissipation. The increased heat retention of the entire huddle brought about by the mother-young contact eventually forces both maternal and pup temperatures to rise, and the rise in maternal temperature induces the mother to terminate the nest bout. The duration of a nest bout would then be influenced by any factor that changes the rate at which huddle temperature and, thereby, the temperature of the mother as a huddle member, rises. Ambient temperature, insulation, and surface area to mass ratio of the huddle as well as initial pup and dam temperature, are all such factors. Figure 1a summarizes what I shall refer to as the acute aspect of the thermal mechanism, since the changes in maternal temperature are short-lived.

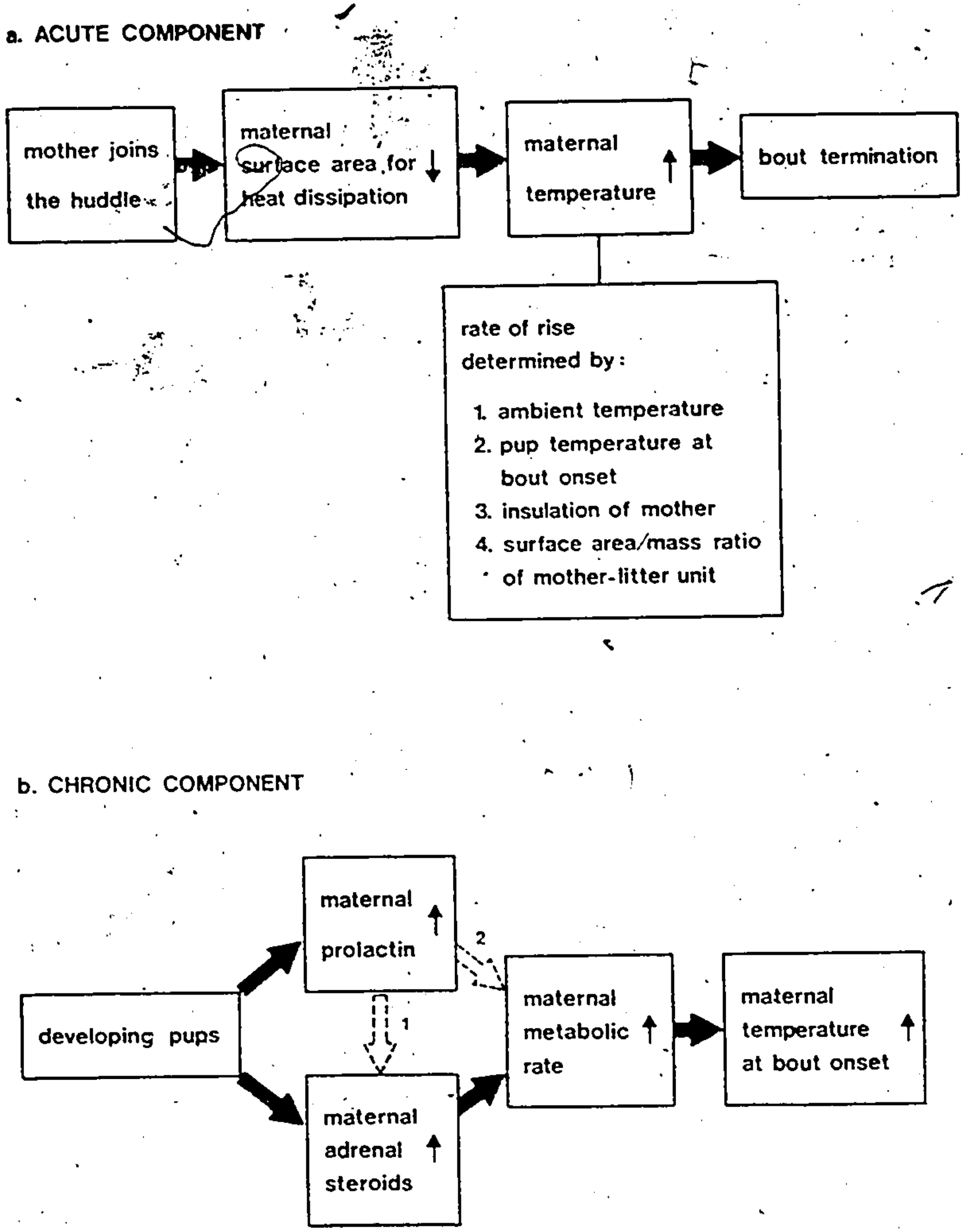
It is important to note that the effects of the factors that increase maternal heat load and, thereby, limit nest bout length, should be expected to be additive. For example, even if the insulation of the nest decreased, increasing the ambient or pup temperature might well provoke a maternal temperature rise that curtails nest bouts. It is also important to emphasize that thermal factors are thought to set an upper limit on bout duration, rather than determining the length of every bout. Other factors, such as the need of the dam to eat, drink, urinate and defaecate; might also be expected to play a role in nest bout termination.

On the thermal model of nest bout limitation, it is thought to be the development of the pups that precipitates the progressive decline in daily nest time (Croskerry, Smith and Leon, 1978). As the pups develop, they become more efficient homeotherms both by virtue of the maturation of their chemical thermoregulatory abilities and because the surface area for heat dissipation of the litter decreases relative

Figure 1: Flow diagram summarizing a thermal mechanism for the limitation of maternal nest bouts.

a) Acute components: thermal events occurring within a nest bout.

b) Chronic component: events influencing chronic maternal temperature and thereby making the dam more vulnerable to the events depicted in a.


a. ACUTE COMPONENT
mother joins the huddle
maternal surface area for heat dissipation ↓
maternal temperature ↑
bout termination
rate of rise determined by:
1. ambient temperature
2. pup temperature at bout onset
3. insulation of mother
4. surface area/mass ratio of mother-litter unit
b. CHRONIC COMPONENT
developing pups
maternal prolactin ↑
maternal adrenal steroids ↑
1
2
maternal metabolic rate ↑
maternal temperature at bout onset ↑

to the mass of tissue producing heat (Taylor, 1960; Hahn, Koldovsky, Krecek, Martinek & Vacek, 1961). Huddle members, including the mother, therefore, should retain heat more efficiently as the pups grow. Pup growth would then result in an increased rate of rise of maternal temperature during nesting, inducing shorter and shorter nest bouts, and producing the normal gradual decline in maternal daily nesting time (Croskerry, Smith & Leon, 1978).

Thermal analyses of the control of mother-young contact would explain why dams spend more time with few or young pups than with many or older pups (Grota, 1972), and why heavy body weight mothers spend less time with their young than lighter dams (Croskerry, 1975). Specifically, the larger surface area to volume ratio of small litters would produce a slower rate of temperature rise for the huddle when the mother is present, which should result in prolonged nest bouts. Similarly, lighter mothers would give rise to huddles with larger surface area to mass ratios than heavy mothers. Light dams should, therefore, be expected to have a slower rate of temperature rise as a member of the huddle, and should have prolonged nest bouts.

There are considerable data which support the notion that thermal factors play a role in limiting nest bout duration (Leon Croskerry & Smith, 1978). Continuous recordings of the ventral and core temperature of lactating females during nest bouts show that the ventral and core temperatures of these rats, did, in fact, increase when the dams are with their pups. Mothers had a greater temperature rise in a warm ambience than they did in a normal laboratory ambient temperature, and nesting bouts were curtailed in the warm ambience.

Conversely, mothers in a cool ambience did not experience a temperature rise during nesting bouts, and had very long bouts of contact with their young (Leon, Croskerry & Smith, 1978). In addition, when the ability of dams to dissipate heat was reduced physically by tail amputation, maternal nest time decreased, and when the lactating females were shaved to increase the rate of maternal heat loss, nest time increased (Leon, Croskerry & Smith, 1978).

In addition to describing the acute role of thermal factors in mother litter interactions which result in nest bout limitation, Leon, Croskerry & Smith, (1978) suggested that the chronically elevated heat production of lactating rats extends the heat loss mechanisms of mother rats close to their limit, forcing a chronic rise in maternal temperature. The chronic elevation in maternal body temperature was thought to make the dams vulnerable to the thermal consequences of huddling with young. It is with the control of the chronic increase in maternal heat load and its possible contribution to the limitation of nest bout duration with which I will be concerned in this dissertation.

Specifically, Leon, Croskerry and Smith (1978) suggested that when dams with this chronic rise in core temperature are subjected to the additional acute increase in heat retention induced by huddling with the young, the dams experience a further increase in maternal heat load, that cannot be diminished by autonomic thermoregulatory mechanisms alone. Maternal temperature then rises further, and only the behaviour of nest bout termination halts the rise in maternal temperature. The role of thermal cues in limiting nest bout duration, then, appear to result from an interaction of chronic and acute thermal influences on the dam. There is evidence that is consistent with this view. Lactating females, for example have an extraordinarily high metabolic rate. Brody, Riggs, Kaufman and Hering, (1938) demonstrated that peak metabolism in the lactating rat is approximately twice that of the normal gestational female. Such a high level of heat production must represent an extraordinary challenge to the organism's thermal homeostasis. Mothers can partially compensate for this high rate of heat production both by activating physiological heat loss mechanisms, such as increased blood flow to the skin (Chatwin, Linzell & Setchell, 1969), and by initiating behavioural thermoregulatory mechanisms such as choosing a cool environment (Gelineo and Gelineo, 1952). However, these heat loss mechanisms are not completely effective in dissipating the additional heat load incurred during the maternal episode and maternal body temperature rises and remains chronically elevated (Leon, Croskerry & Smith, 1978).

What factors are involved in stimulating maternal heat production? Maternal hypermetabolism does not appear to reflect solely the energy demands of milk production, for totally mammectomised postparturient females showed the same increased tissue oxygen consumption levels as intact lactating rats (Denckla and Bilder 1976). Rather, the existing evidence suggests that hormonal action stimulates the elevated metabolism of lactating females. Disruption of the normally high levels of adrenal steroids and prolactin in mother rats depressed maternal metabolism, as reflected in a fall in maternal temperature. Consequently, hormone disruption was also accompanied by a chronically elevated daily nest time (Leon, Croskerry & Smith, 1978).

Specifically, adrenalectomised females had a chronically low core temperature and a chronically elevated nesting time, failing to show the normal daily decline in contact with the young. Prolactin-suppressed females had a relatively low core temperature in the second week postpartum when compared to normal dams. It was during this time that prolactin-suppressed dams cease to show a further decline in nest time. Ovariectomy had no effects on either nest time or dam core temperature and adrenalectomy-ovariectomy resulted in a pattern of nest time and dam core temperature similar to that of adrenalectomy alone (Leon, Croskerry & Smith, 1978). Adrenal hormones, and perhaps prolactin, therefore, appear to be necessary for the display of the normal decline in nesting time, and seem to have their effects by increasing maternal core temperature and thereby rendering the dam vulnerable to the acute thermal effects of huddling with her litter (see Figure 1b). Leon, Croskerry & Smith (1978) hypothesized further that the effects of prolactin on nest time are not direct, but rather are mediated by changes in adrenal hormone levels. This indirect role of prolactin in maintaining the daily decline in nest time is indicated by the dotted arrow marked "1" in Figure 1b.

It should be noted, that while the duration of mother-litter contact is dependent on endocrine secretions during the first two weeks postpartum, the absence of hormones has little effect on whether or not dams display the various other components of maternal behaviour once they have been established. Adrenalectomised, ovariectomised and prolactin-suppressed females still show all the components of maternal behaviour typical of the intact female (Numan, Leon and Moltz, 1972; Thoman and Levine, 1970).

While there is considerable evidence for the acute role of thermal factors in limiting nest bout duration, the evidence implicating hormones in the control of nest bout duration comes solely from extirpation studies which revealed an inverse correlation between nest time and maternal core temperature. In order to draw firm conclusions about the nature of physiological mechanisms, however, it is necessary to obtain a consistent pattern of results using a variety of experimental methods. Specifically, one must identify a particular hormone capable of restoring the normal physiological and behavioural state in animals whose endogenous hormones have been experimentally disrupted. Successful replacement with a specific hormone in such animals allows one to conclude that the system has an endocrine basis, rather than, for example, a neural control. Thus, the first part of this dissertation is devoted to identifying by means of hormone replacement those adrenal hormones that are sufficient to maintain the typical decline in nesting time in lactating dams. The role of prolactin in maintaining this behaviour was then assessed, using both replacement and assay studies. Since the proposed mechanism limiting nest bouts involves a thermal mediation of endocrine action, rather than a direct central action of hormones, a second stage of replacement was undertaken to verify this aspect of the control system. Here, hormone-disrupted dams were replaced, not with hormones, but with heat. In the second part of this thesis, the heat load of adrenalectomised-ovariectomised and prolactin-suppressed dams was, therefore, increased and the ability of thermal replacement to reinstitute the normal decline in maternal nesting was assessed. Finally, I determined whether the presumed increase in thermal load on the mother actually occurs to produce the gradual decline in nest time over the course of the first two weeks after the birth of the young.

Chapter 1

Hormonal Influences on Nest bout duration

Introduction

Removal of the adrenal gland prevents the normal decline in maternal nesting behaviour in the first two weeks postpartum. In the studies described below, I identified adrenal factors sufficient to produce the progressive daily decline in nest time in the first two weeks postpartum. In addition, since prolactin suppression prevents the decline in nest time in the second week only, I determined whether prolactin plays a direct role in producing the decline during that period, or if the effects of prolactin are mediated by a trophic effect on adrenal steroids.

I was specifically interested in whether these hormones make the dam vulnerable to an acute rise in core temperature when she is with her pups by chronically elevating maternal core temperature. Any hormone regimen that reinstates the normal decline in nest time should, according to this proposition, also increase the heat load of these dams. This hypothesis was examined by determining the effects of various hormone states on maternal temperature.

Experiment 1

Adrenocortical hormone replacement: Effects on daily nest time

Adrenalectomy-ovariectomy (Adex-Ovex) on Day 1 postpartum prevents the normal decline in daily nesting time and the normal rise in core temperature seen in intact lactating rats (Leon, Croskerry & Smith, 1978). Neither ovariectomy nor adrenal demedullation interfered with normal nesting behaviour, but total adrenalectomy produces behavioural and thermal effects similar to those of adrenalectomy-ovariectomy (Leon, Croskerry & Smith, 1978). Apparently, then, loss of adrenocortical secretions produces an immediate disruption of nesting behaviour while chronically suppressing maternal body temperature.

In order to conclude that the hormones of the adrenal cortex rather than some other secretion or action of the adrenal gland are critical for the maintenance of normal maternal nesting behaviour, the active adrenal substance that mediates nesting behaviour has to be identified. Hormone replacement following gland extirpation was the method chosen to determine the identity of that substance. The experimental strategy employed here was to determine first the effects of Adex-Ovex on nesting behaviour, and then to initiate hormone replacement in Adex-Ovex females. The ovaries were removed along with the adrenals in these studies to preclude any confounding, compensatory effects of ovarian hormones. Since there was no reason, _a priori_, to suspect one of the many adrenocortical steriods as being critical for the normal decline in maternal nesting time, a selection of hormones, known to be secreted by the adrenal cortex was administered to Adex-

Ovex dams to determine the efficacy of each in reinstating this aspect of maternal behaviour. Replacement therapy was instituted with aldosterone, the dominant mineralcorticoid in the rat, which is known to play an important role in milk secretion (Cowie and Folley, 1961); with corticosterone, the dominant glucocorticoid in the rat (Bush, 1951, 1953); with cortisol, which is another glucocorticoid, found to maintain mammary enzyme activity in the rat (Plucinski and Baldwin 1976), and with progesterone, which is a precursor of all three of the other hormones (Turner, 1966).

General Method

Subjects

Sixteen virgin Wistar females supplied by Canadian Breeding Farms (St. Constant, Quebec) weighing 225 - 250 gms served as subjects in each study.

Apparatus

Each mother reared her litter in a cage in which it was possible to record maternal nesting behaviour continuously (Croskerry, Smith, Leon & Mitchell 1976). Each plastic cage (34 x 29 x 17 cms) was fitted at one end with a tray (28 x 12 cms) that was balanced on a fulcrum. On one side of the tray, was a nest box (11 x 14 x 8 cms) which could be counterbalanced with weights to accommodate increases in litter weight. When the dam entered the nest box, the tray tilted and a microswitch was activated, closing a relay circuit which both advanced a counter to record nest bout frequency, and started a clock to record cumulative nest time. A 5 sec delay was built into the relay circuit to eliminate activations of the circuit by the mother that did not involve contact with the young. The small size of the nest box ensured that when the mother was in the nest box she was, in fact, in contact with her litter. Experimenter observations of mother young contact in this continuous recording apparatus yielded records virtually identical to those of the automated system (Croskerry, Smith, Leon & Mitchell, 1976). All the dams placed their litters in the nest box and the young usually attached to the dam's nipples shortly after nest bout initiation. Ad lib food and fluid were available in the cage but were only accessible from outside the nest box.

Procedures

Study 1a - Adrenalectomy-Ovariectomy

Virgin female rats were mated in group cages and 19 - 20 days later placed individually into nesting time recording cages (described above). The females were paired on the day of birth (Day 0) with one dam of the pair assigned to the adrenalectomised-ovariectomised group (Adex-Ovex) and the other to the sham-operated group (Sham-Op). On Day 1 postpartum, animals in both groups were anaesthetised with a solution of 0.97% Pentobarbital and 4.25% Chloral Hydrate (0.25 cc/100 gms body weight). Each animal in the Adex-Ovex group was bilaterally adrenalectomised-ovariectomised via dorsal incisions, and rats in the Sham-Op group were anaesthetised, but only had bilateral incisions made in the body wall.

Litters were reduced in number to eight pups on the day of birth. To ensure both that Sham-Op and Adex-Ovex mothers had equivalent pup stimulation, and to maintain healthy pups, all litters were switched daily between experimental and control mothers and a multiparous dam who had given birth on the same day. When the litters were switched, care was taken to ensure that both Adex-Ovex and Sham-Op mothers had litters of approximately the same weight. Adex-Ovex females were autopsied at the end of the experiment to determine whether any adrenal regeneration had occurred.

Nest time and frequency were transcribed at 0900 hr (lights on) and 2100 hr (lights off) daily for 13 days from day 2 postpartum.

A two- way analysis of variance with one related measure was performed on all the measures taken. The data were first condensed by finding the average for each subject for each dimension of days 2 - 4, 5 - 7, 8 - 10, 11 - 14. This manipulation decreased the degrees of freedom, thereby making the analysis more conservative, but minimised problems due to missing data occasionally caused by equipment malfunction. In order to overcome problems due to the assumption of homogeneity of covariance because measures were taken over time, the Geisser-Greenhouse Conservative F-test was used when determining the significance levels of the 'F' ratios obtained for related factors (Winer, 1962).

Study 1b - Aldosterone Replacement

The eight females in the Adex-Ovex + Aldosterone group were operated as described in Study 1a. Beginning on Day 1 postpartum, they were given a subcutaneous injection of 0.03 mg aldosterone (Sigma Chemical Co.,) in 0.2 cc oil vehicle daily at 1700 hr. This dose of aldosterone has been shown to have both physiological and behavioural effects in adrenalectomised rats. Physiologically, it is sufficient to increase the sodium retention in the kidneys of adrenalectomised rats

(Fregley and Walters, 1965). Behaviourally, aldosterone in this dose appeared to increase the arousal level of adrenalectomised rats (Gray, 1976).

The 8 subjects in the Sham-Op group were subjected to the same surgical procedure as described for controls in Study 1, and the females thereafter received daily injections of 0.2cc of the oil vehicle. In this study, both the aldosterone replacement and sham-operated groups were maintained on tap water rather than NaCl solution. Nest time and frequency were recorded and statistical analyses performed as described in Study 1a.

Study 1c - Progesterone Replacement

Beginning on Day 1 postpartum, injections of 3mg progesterone (Schering Co. Ltd.), in 0.2cc oil vehicle was given twice daily at 0900 hr and 2100 hr to the 8 primiparous females in the Adex-Ovex + Progesterone group. This dose was chosen because it can inhibit (Moltz, Levin and Leon, 1969), facilitate (Moltz, Lubin, Leon and Numan, 1972) or have no effect on the maternal behaviour of female rats (Moltz, Levin and Leon, 1969; Moltz, Lubin, Leon and Numan, 1972) depending on the endocrine and experiential state of the female rat. The 8 dams in the sham-operated group received the 0.2cc oil vehicle subcutaneously twice daily. The general procedures were as described for Study 1a.

Study 1d - Cortisol Replacement

The 8 primiparous females in the Adex-Ovex + Cortisol group received subcutaneous injections of 0.5 mg Cortisol (supplied by Merck, Sharp and Dohme Research Laboratories) in 0.2cc oil vehicle twice daily at 0900 hr and 2100 hr beginning on Day 1 postpartum. Sham-operated control subjects received 0.2cc of the oil vehicle twice daily. Plucinski and Baldwin (1976) demonstrated that the reduction of mammary enzyme activity observed after adrenalectomy could be restored with this regimen of cortisol replacement. The procedures used and measures taken in this study were identical to those described for Study 1a.

Study 1e - Corticosterone Replacement

The 8 dams in the Adex-Ovex + Corticosterone group were operated on Day 1 postpartum as described in Study 1 and then given daily injections of 3 mg corticosterone (supplied by Merck, Sharp and Dohme, Research Laboratories) in a 10% solution of ethanol in oil at 1700 hr. Sham-operated animals were injected at the same time with the vehicle alone. This vehicle was chosen because it ensures a more even suspension of the hormone than oil alone. Gray (1976) found this dose of corticosterone to be sufficient to maintain normal circulating levels of corticosterone in adrenalectomised animals for 24 - 36 hrs after the injection. The hormone was administered in the late afternoon in order to more closely approximate the normal circadian surge of corticosterone (Stern, Goldman and Levine, 1973). The subjects used in this study were operated, maintained and measures taken according to the procedures described for Study 1a.

Results

The results of Studies 1a - 1e are shown in Figures 2 - 5. The results of the statistical comparisons for these studies are summarized in Table I. Figures 2 and 3 show the mean daily nest time for all experimental groups and their sham-operated controls. In these and all

Table 1

'F' Ratios and Associated 'p' Values

From the Statistical Analysis of Experiment 1

Study		Adex-Ovex		Adex-Ovex + Aldosterone		Adex-Ovex + Progesterone		Adex-Ovex + Cortisol		Adex-Ovex + Corticosterone	
Dimension	Factor	F	p	F	p	F	p	F	p	F	p
Nest Time	Treatment	26.53	<.001	17.26	<.001	24.86	<.001	3.40	ns	4.26	ns
	Days	11.15	<.01	5.97	<.05	71.66	<.001	43.03	<.001	33.85	<.001
	Treatment x Days	11.19	<.01	2.42	ns	12.36	<.01	1.40	ns	3.70*	ns
Nest Frequency	Treatment	0.37	ns	2.79	ns	1.37	ns	1.74	ns	2.78	ns
	Days	1.42	ns	3.45*	ns	4.64	=.05	2.37	ns	5.17	<.05
	Treatment x Days	2.65	ns	0.49	ns	1.81	ns	0.79	ns	0.90	ns

* Indicates 'F' ratios that were nonsignificant using the Geisser-Greenhouse Conservative 'F' test but would have been significant at p < .05 if a less conservative test were used.

Figure 2: Mean daily nest times of Adex-Ovex, Adex-Ovex + Aldosterone, and Adex-Ovex + Progesterone and Sham-Op dams. SEMs are shown.

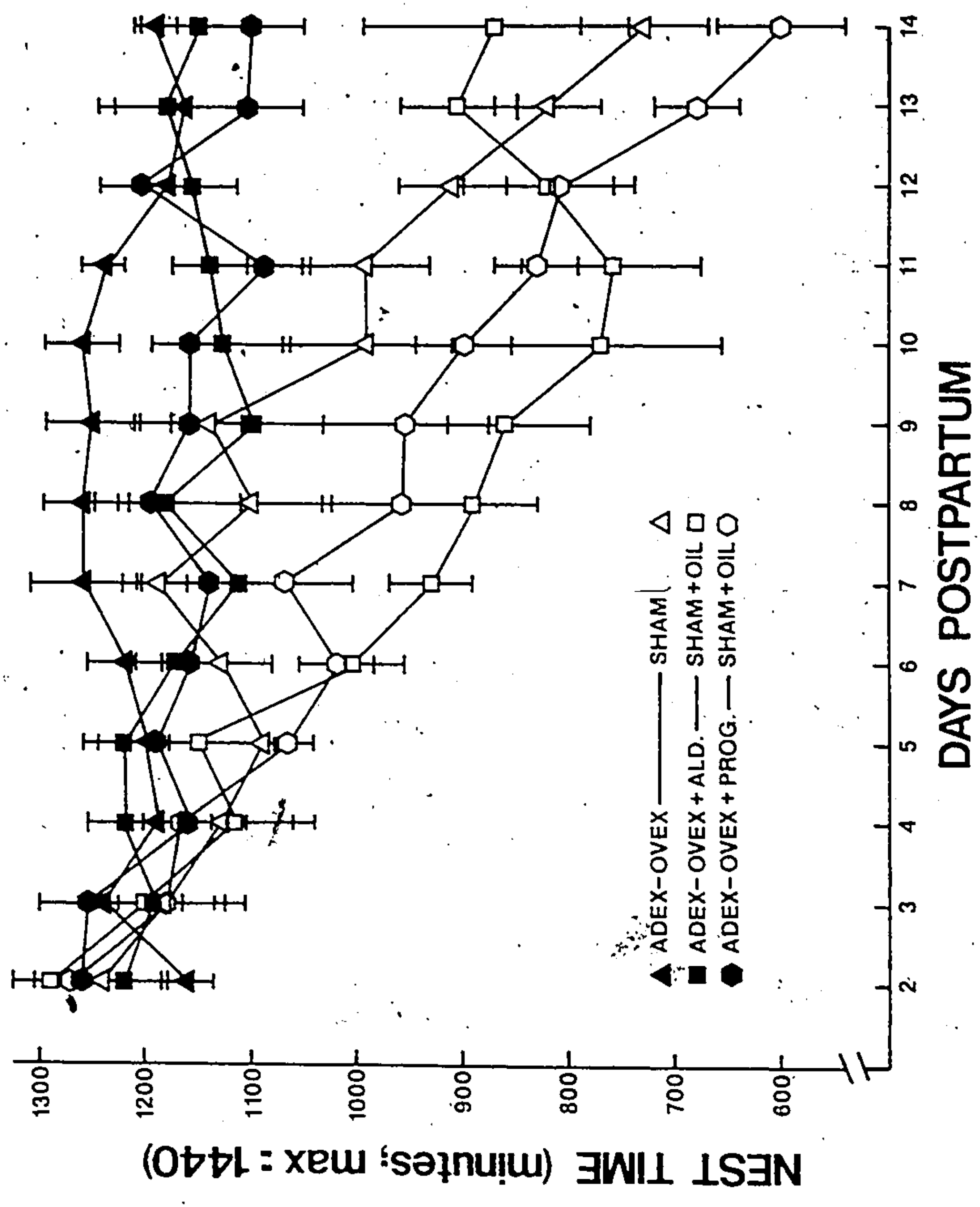
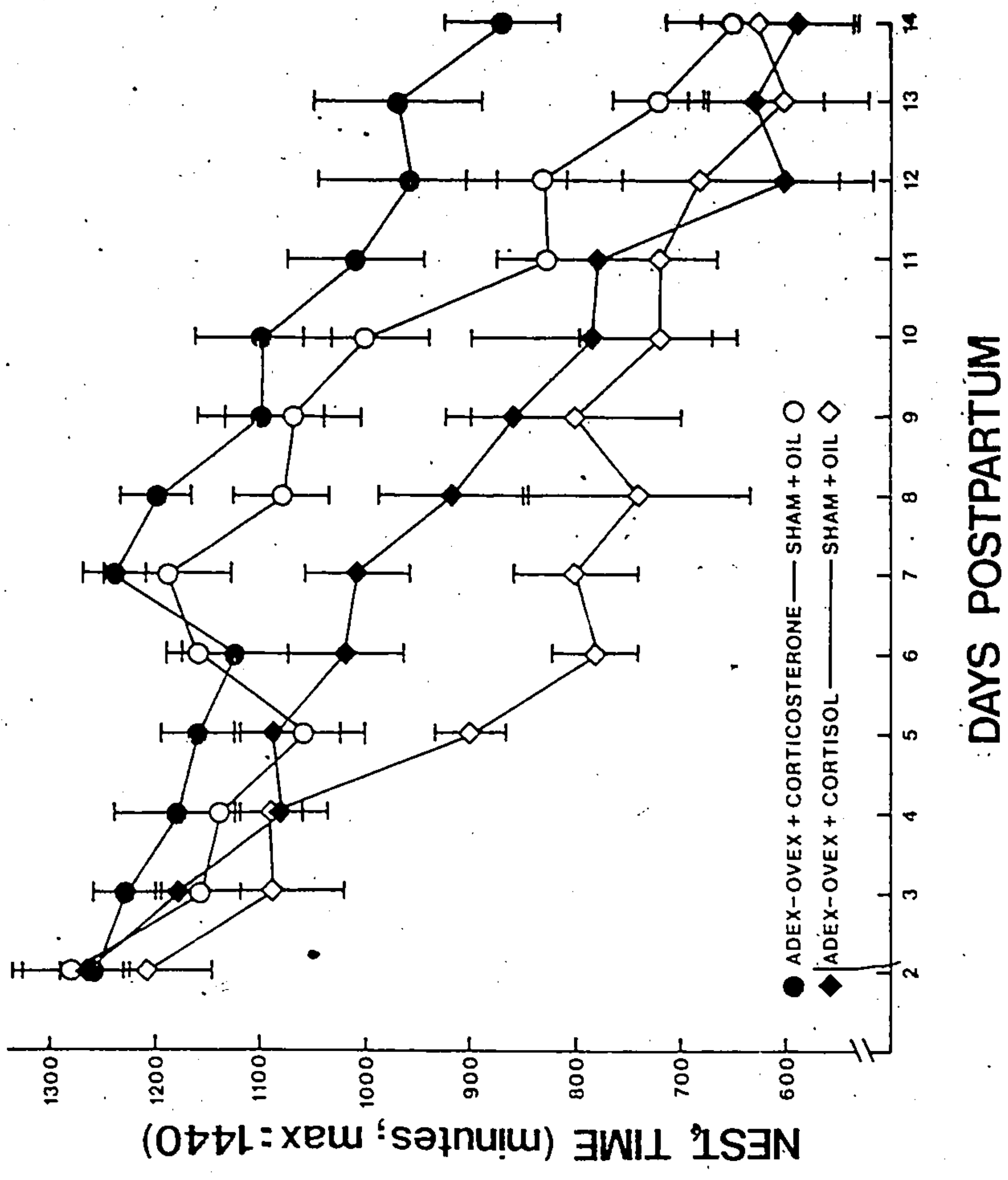

NEST TIME (minutes; max = 1440)
1300
1200
1100
1000
900
800
700
600
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
▲ ADEX-OVEX —— SHAM △
■ ADEX-OVEX + ALD. —— SHAM + OIL □
● ADEX-OVEX + PROG. —— SHAM + OIL ○

Figure 3: Mean daily nest times of Adex-Ovex + Corticosterone, Adex-Ovex + Cortisol and Sham-Op dams. SEMs are shown.


NEST TIME (minutes; max = 1440)
1300
1200
1100
1000
900
800
700
600
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
● ADEX-OVEX + CORTICOSTERONE —— SHAM + OIL ○
◆ ADEX-OVEX + CORTISOL —— SHAM + OIL ◇

subsequent figures data from the experimental groups are represented by filled symbols and that from the sham-operated controls by open symbols.

It is clear from Figure 2 that adrenalectomy-ovariectomy resulted in a continuously elevated nest time and, moreover, that progesterone and aldosterone were ineffective in reinstating the normal decline in daily nest time. The nest times for all three of these experimental groups were significantly different from their sham-operated controls, which clearly showed a decline in daily nest time (Treatment Effect $F = 26.53$, $p < .001$; $F = 17.36$, $p < .001$; $F = 34.84$, $p < .001$, respectively).

Figure 3 shows that glucocorticoid replacement was effective in producing a considerable decline in nest time in Adex-Ovex females. Neither the cortisol nor the corticosterone replacement groups had significantly different nest times from their sham-operated controls ($F = 3.4$, $p > 0.05$; $F = 4.26$, $p > 0.05$, respectively). It is clear, however, that corticosterone was less effective in reinstating the decline in nest time than was cortisol, in terms of the actual reduction of minutes the dams spent on the nest. The Days Effects were significant for all five studies (see Table 1) and significant Day x Treatment interactions were found only in the progesterone replacement and Adex-Ovex studies (Table 1).

The mean daily nest frequency for each group is shown in Figures 4 and 5. In none of the studies was there a statistically significant effect of treatment on nest bout frequency. There were significant Days Effects in the progesterone and corticosterone

Figure 4: Mean daily nest bout frequencies of Adex-Ovex, Adex-Ovex + Aldosterone, Adex-Ovex + Progesterone and Sham-Op dams. SEMs are shown.

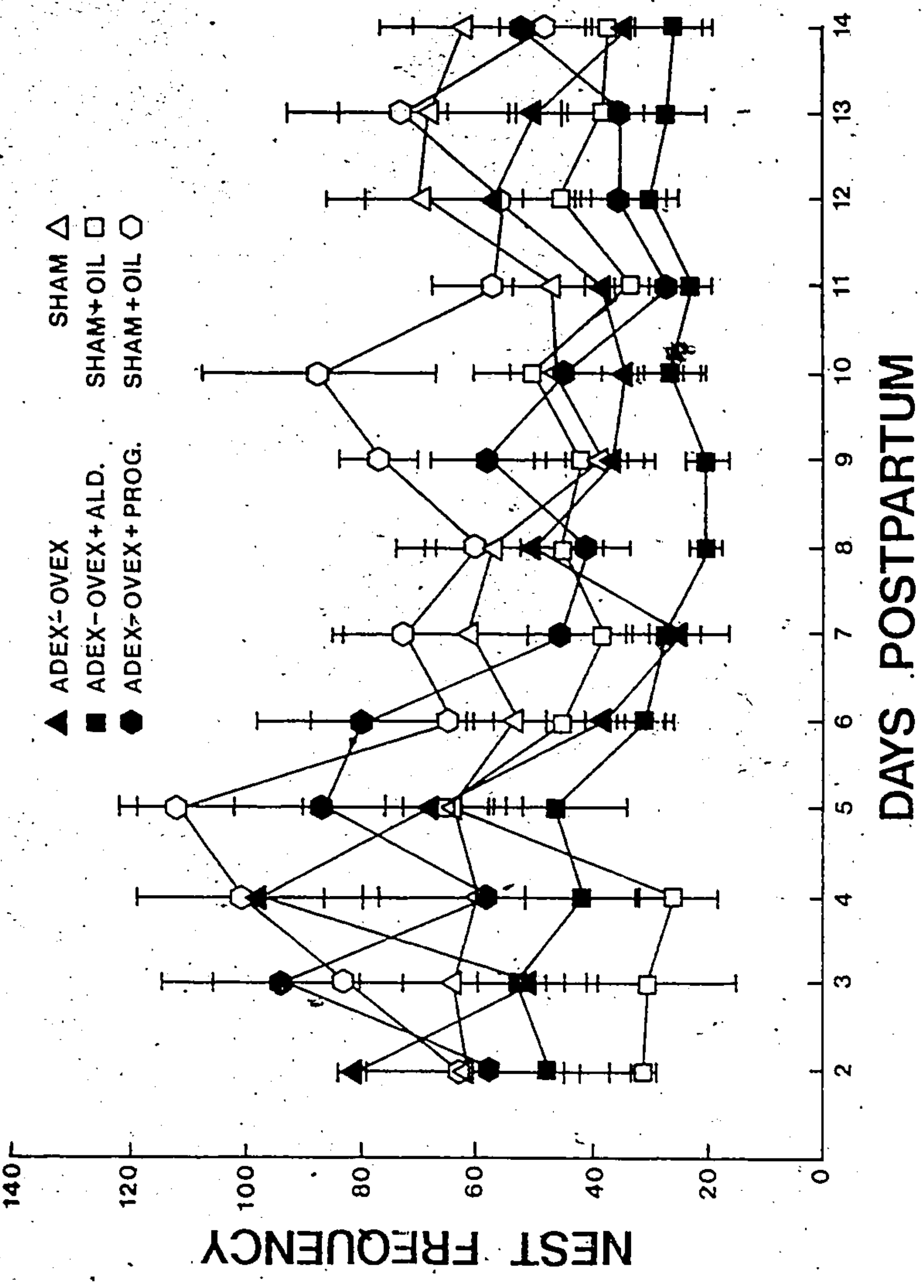
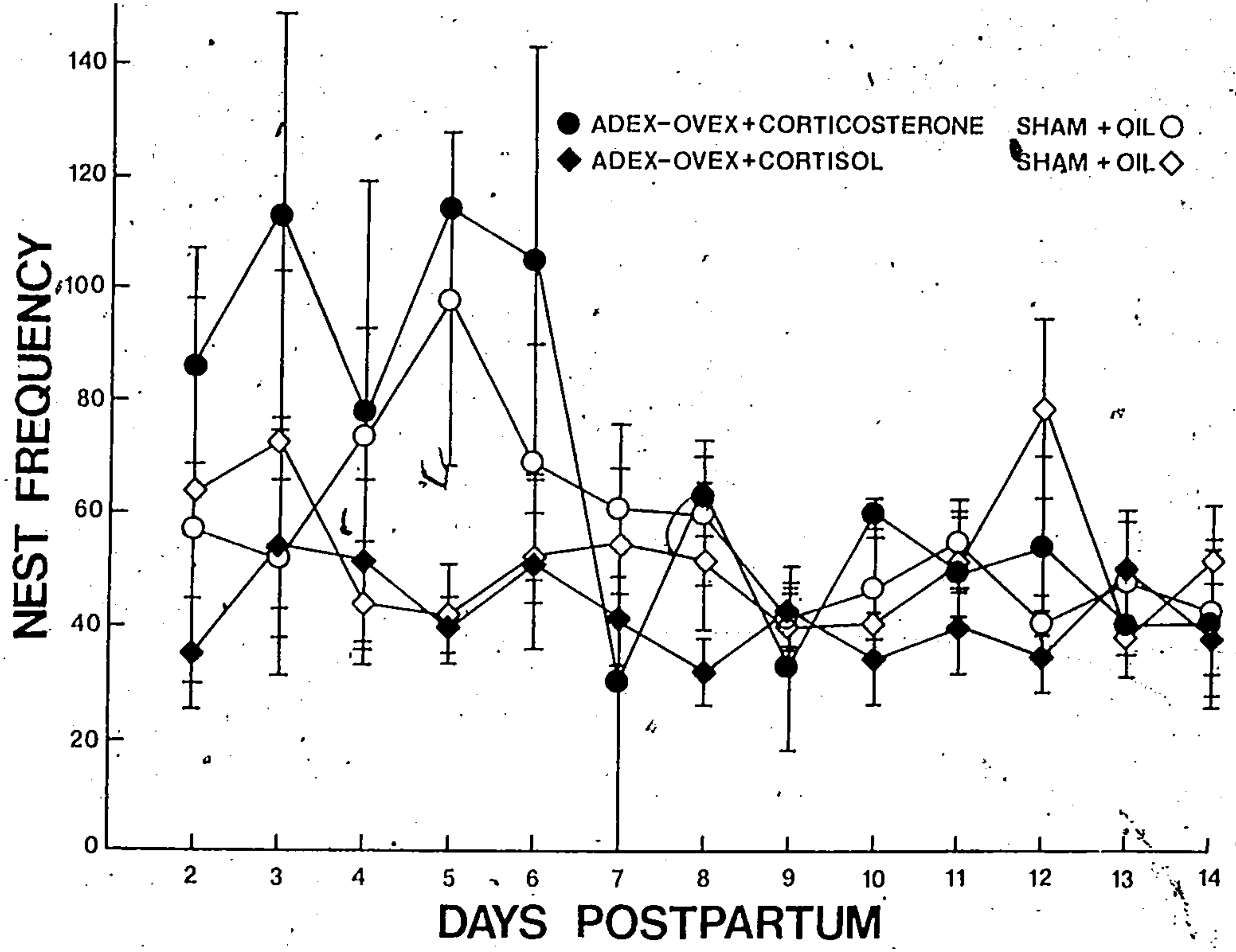

▲ ADEX-OVEX
■ ADEX-OVEX+ALD.
● ADEX-OVEX+PROG.
SHAM △
SHAM+OIL □
SHAM+OIL ○
NEST FREQUENCY
0
20
40
60
80
100
120
140
DAYS POSTPARTUM
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 5: Mean daily nest bout frequencies of Adex-Ovex + Corticosterone, Adex-Ovex + Cortisol and Sham-Op dams. SEMs are shown.


● ADEX-OVEX+CORTICOSTERONE
◆ ADEX-OVEX+CORTISOL
SHAM + OIL ○
SHAM + OIL ◇
NEST FREQUENCY
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replacement studies which are probably due to the high frequencies shown by both experimental and control groups at the start of the experiment (see Figures 4 and 5). There were no significnat Day x Treatment interaction effects.

Discussion

Glucocorticoid replacement therapy was effective in restoring a decline in daily nest time in Adex-Ovex dams while aldosterone and progesterone replacement were ineffective in this regard. It is clear that cortisol was the more effective of the two glucorticoid replacement regimens used in this study. It is also apparent, though, that the decline in nest time between days 2-14 produced by corticosterone replacement (a mean decline of 400 min) was much greater than that seen in the Adex-Ovex group, (a mean increase of 30 min) the Adex-Ovex + Aldosterone group (a mean decline of 65 min), and the Adex-Ovex + Progesterone group (a mean decline of 120 min).

The Sham-Op groups in this experiment all had a considerable decline in nest time, but there was some variation between the particular patterns of decline shown by the Sham-Op groups. These differences are probably due to variations in the weight and possibly the temperature of the pups that these groups received from their respective experimental groups. Such inter experiment variability in pup weight and temperature probably arose because the amount of warm milk that mothers from each group delivered to their young varied with the hormones she received. On the model proposed by Croskerry, Smith & Leon (1978), such factors as litter weight and pup temperature are expected to be involved in determining the rate of rise of maternal core temperature during a nest bout and thereby influence nest bout duration. Thus, variation in litter characteristics would be expected to produce variations in the pattern of nest time decline. Within each experiment, however, care was taken to ensure that each pair of experimental and control mothers received a litter of approximately equivalent weight each day, to maintain similar surface area/mass ratios between groups.

The absence of significant treatment effects on nest frequency implies that the difference in daily nest time seen between the Adex-Ovex, Adex-Ovex + Progesterone and Adex-Ovex + Aldosterone groups and their sham-operated controls arose from a difference in the duration of individual nest bouts rather than in the number of such bouts. Similarly, the lack of a significant change in mean nest frequency over time observed in most of the studies suggests that the typical progressive decline in daily nesting time resulted from a gradual shortening of individual nesting bouts.

The high nest bout frequencies observed at the beginning of the experiment in the Adex-Ovex + Progesterone and Adex-Ovex + Corticosterone studies was possibly due to a tendency for these animals to indulge in bouts of tail-retrieving. If the change in nest frequency over time seen in these studies contributed to any decline in nest time one would expect to see a gradual decline in nest frequency. Rather, these very high frequencies decrease abruptly around Day 6. The nonsignificant Days x Treatment interaction for these studies indicates that both control and experimental groups showed the same tendency and, therefore, differences in nest frequency were unlikely to have contributed to any differences in nest time between experimental and control groups.

The results of the present series of studies indicate that glucocorticoid replacement is sufficient to reinstate in Adex-Ovex dams a progressive decline in nest bout duration similar to that seen in intact lactating females in the first two weeks postpartum. Having identified specific hormones from the adrenal cortex that can reverse the effects of gland extirpation, it seems clear that a hormonal mechanism influences maternal nesting behaviour. The mode of action of these hormones is investigated in the following experiment.

Experiment 2

Adrenocortical hormone replacement: Effects on maternal and pup temperature

It is clear from the results of the previous experiment that glucocorticoid replacement therapy is sufficient to induce the normal decline in daily nesting time over the first two weeks postpartum in Adex-Ovex dams. Glucocorticoids might effect nest bout duration in one of two ways: directly by acting on a central mechanism that controls nesting behaviour, or indirectly by stimulating a particular physiological state in the dam that, in turn, modifies her pattern of contact with her litter. Leon, Croskerry and Smith (1978) suggested that the effect of adrenal cortical steroids on nest time is mediated by their chronic thermal effects on the dam.

There are a number of lines of evidence in support of such a proposition. Removal of the adrenals in nonlactating rats results in lowered basal metabolism as indicated by a depressed core temperature (Hartman, Brownell and Crosby, 1931; Carr and Beck, 1937; Horvath, 1938) and these effects can be reversed by replacement with adrenocortical extract "cortin" (Hartman, Brownell and Crosby, 1931). Since cortin replacement therapy restores metabolic rate in the nonlactating adrenalectomised rat, elevation of adrenal hormones above normal basal levels might further increase metabolic rate. Indeed, circulating glucocorticoid levels are chronically elevated during lactation (Voogt, Sar and Heites, 1969; Zarrow, Schlein, Denenberg and

Cohen, 1972) and the elevated adrenocortical hormone output is accompanied by maternal hypermetabolism (Brody, Riggs, Kaufman and Hering, 1938; Denckler and Bilder, 1976). Further, removal of the adrenal glands during lactation produces a chronically depressed core temperature, indicative of a depressed metabolism (Leon, Croskerry & Smith, 1978).

If the decline in nest bout duration and, thereby, daily nest time is dependent on elevated maternal core temperature, then glucocorticoid replacement therapy, which reinstates the normal decline in nesting time, should also restore the normally elevated maternal core temperature. Similarly, those regimens that are ineffective in changing nest time should also have no effect on maternal core temperature.

Pup skin temperature has been shown to be a contributing factor in determining the rate of rise of maternal core temperature (Leon, Croskerry & Smith, 1978). It seemed important, therefore, to determine whether glucocorticoid replacement had its effects via an increase in pup skin temperature, or in maternal core temperature, or both. Therefore, I daily recorded pup skin temperature in addition to dam skin and core temperature following each of the five hormonal manipulations described in Experiment 1. Of course, if one did not observe a correlation between elevated maternal heat load and decreased nest time in this experiment, then one would have to conclude that adrenal hormones do not act via a thermal mechanism.

Method

Dams and their litters which had served as subjects in

Experiment 1 were used in this experiment. Dam ventral and core temperature and pup skin temperature were recorded daily. Dam ventral temperature and pup skin temperature were recorded using YSI probe #408 and telethermometer model # 43TA. Mother core temperature was recorded with YSI probe #423 inserted 4 cm into the mother's rectum. The data obtained were subjected to the same statistical analyses described in Experiment 1.

Results

The main results of this experiment are shown in Figures 6 - 11. The 'F' ratios and p values obtained in the analyses of variance are shown in Table II.

Maternal Core Temperature The daily mean core temperature for each group and their associated sham operated controls are shown in Figures 6 and 7. Only in the cortisol replacement group was the effect of treatment on core temperature not statistically significant ($F = 3.9$, $p > .05$). Although it is apparent from Figure 6 that the corticosterone replacement group has a lower core temperature than its sham-operated control group, from Day 5 onwards the mean core temperature of the corticosterone group is higher than that of the Adex-Ovex + Aldosterone, Adex-Ovex + Progesterone and Adex-Ovex groups and there seems very little difference between the two glucocorticoid replacement groups. The aldosterone and corticosterone replacement groups showed a significant change in core temperature over days ($F = 4.59$, $p < .05$; $F = 15.44$, $p < .001$ respectively) and the progesterone and aldosterone replacement studies yielded statistically significant Day x Treatment interaction effects ($F = 7.28$, $p < .05$; $F = 7.0$, $p < .05$,

Table II

'F' Ratios and p Values From the

Statistical Analysis of Experiment 2

Study		Adex-Ovex		Adex-Ovex + Aldosterone		Adex-Ovex + Progesterone		Adex-Ovex + Cortisol		Adex-Ovex + Corticosterone	
Dimension	Factor	F	p	F	p	F	p	F	p	F	p
Dam Core Temperature	Treatment	37.41	<.001	33.23	<.001	29.65	<.001	3.90	ns	6.14	<.05
	Days	2.40	ns	4.59	=.05	2.61	ns	2.0	ns	15.44	<.001
	Treatment x Days	2.01	ns	7.0	<.05	7.28	<.05	3.0*	ns	1.24	ns
Dam Ventral Temperature	Treatment	3.57	ns	2.10	ns	0.20	ns	5.67	<.05	1.59	ns
	Days	2.31	ns	1.23	ns	1.65	ns	1.2	ns	3.14*	ns
	Treatment x Days	0.20	ns	0.16	ns	0.57	ns	0.002	ns	0.50	ns
Pup Skin Temperature	Treatment	17.61	<.01	0.06	ns	3.17	ns	0.50	ns	5.16	<.05
	Days	768.35	<.001	1.55	ns	1.64	ns	5.45	<.05	5.49	<.05
	Treatment x Days	1252.59	<.001	0.70	ns	0.19	ns	1.18	ns	1.41	ns

* Indicate '_F_' ratios that were nonsignificant using the Geisser-Greenhouse Conservative 'F' test but would have been significant at _p_ < .05 if a less conservative test were used.

Figure 6: Mean daily core temperature of Adex-Ovex, Adex-Ovex + Aldosterone, Adex-Ovex + Progesterone and Sham-Op dams. SEMs are shown.

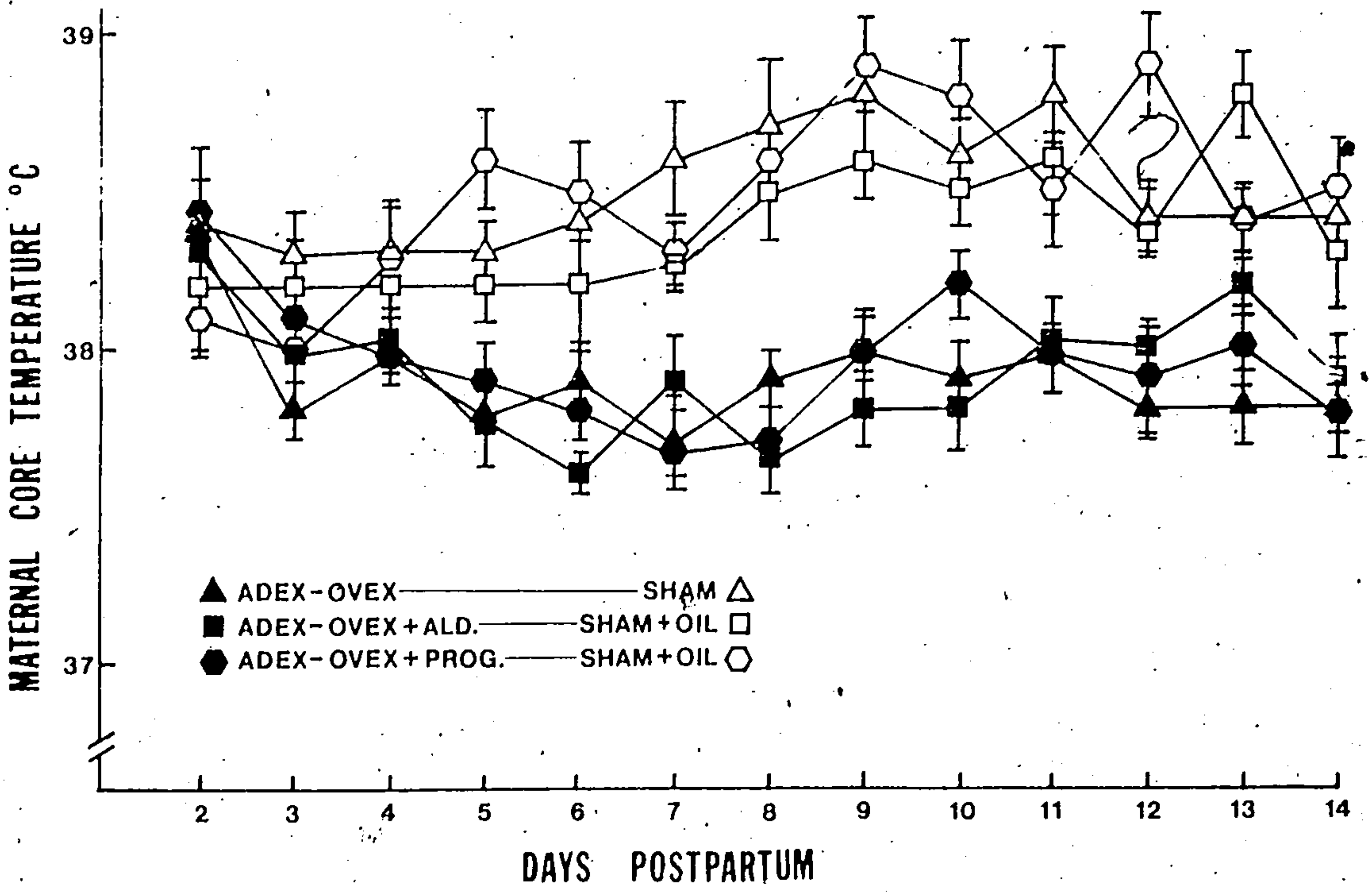
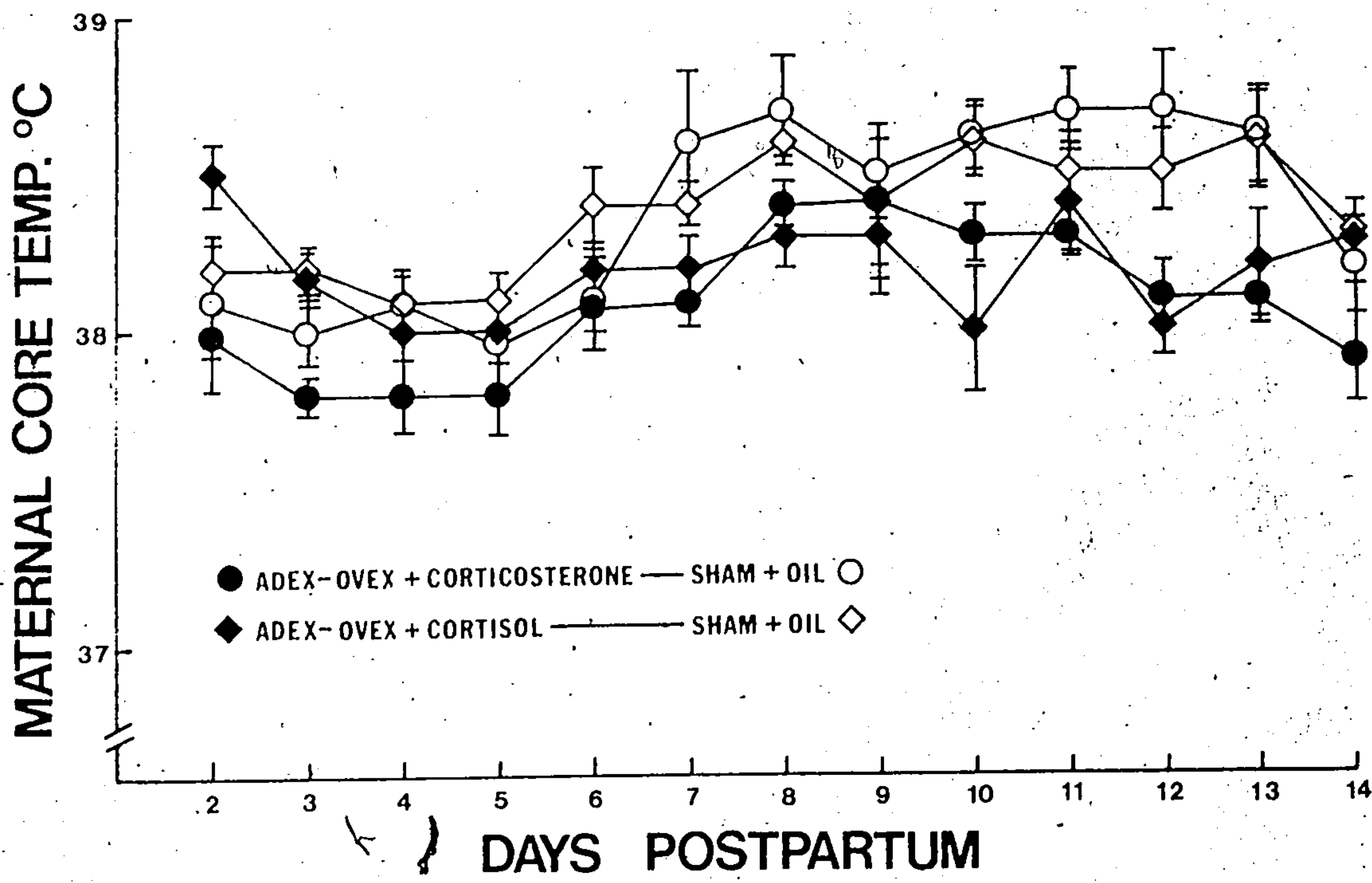

MATERNAL CORE TEMPERATURE °C
39
38
37
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
▲ ADEX-OVEX——SHAM △
■ ADEX-OVEX+ALD.——SHAM+OIL □
⬢ ADEX-OVEX+PROG.——SHAM+OIL ⬡

Figure 7: Mean daily core temperatures of Adex-Ovex + Corticosterone, Adex-Ovex + Cortisol and Sham-Op dams. SEMs are shown.


MATERNAL CORE TEMP. °C
39
38
37
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
● ADEX-OVEX + CORTICOSTERONE —— SHAM + OIL ○
◆ ADEX-OVEX + CORTISOL —— SHAM + OIL ◇

respectively). Examination of Figures 6 and 7 shows an apparent increase in temperature in all the sham-operated groups from day 5 postpartum.

Maternal Ventral Temperature The Cortisol replacement group was the only one which showed a significant effect of treatment on ventral temperature ($F = 5.67$, $p < .05$). There was no change in the ventral temperature of any group over the course of the experiment (see Figures 8 and 9). None of the Day x Treatment effects were statistically significant.

Pup Skin Temperature The Adex-Ovex group and the Adex-Ovex + Corticosterone group had significantly lower pup skin temperatures than their sham-operated counterparts ($F = 17.61$, p , .01, $F = 5.16$, $p <$ .05, respectively). These same groups also showed a significant days effect, probably reflecting the decrease in pup temperature between days 5 - 10 shown by both the control and experimental groups (see Figures 10 and 11). Only the Adex-Ovex - Sham-Op comparison yielded a statistically significant Days x Treatment Effect ($F = 1252.59$, $p <$ .001).

Discussion

The results of this experiment show clearly that the glucocorticoid replacement therapy, which is effective in reinstating a decline in daily nest time in Adex-Ovex animals, is also effective in chronically increasing core temperature. The correlation that was again found between increased maternal core temperature and decreased nest time is consistent with the notion of a thermal mechanism for the limitation of nest bout duration. Specifically, it reinforces the

Figure 8: Mean daily ventral skin temperatures of Adex-Ovex, Adex-Ovex + Aldosterone, Adex-Ovex + Progesterone and Sham-Op dams. SEMs are shown.

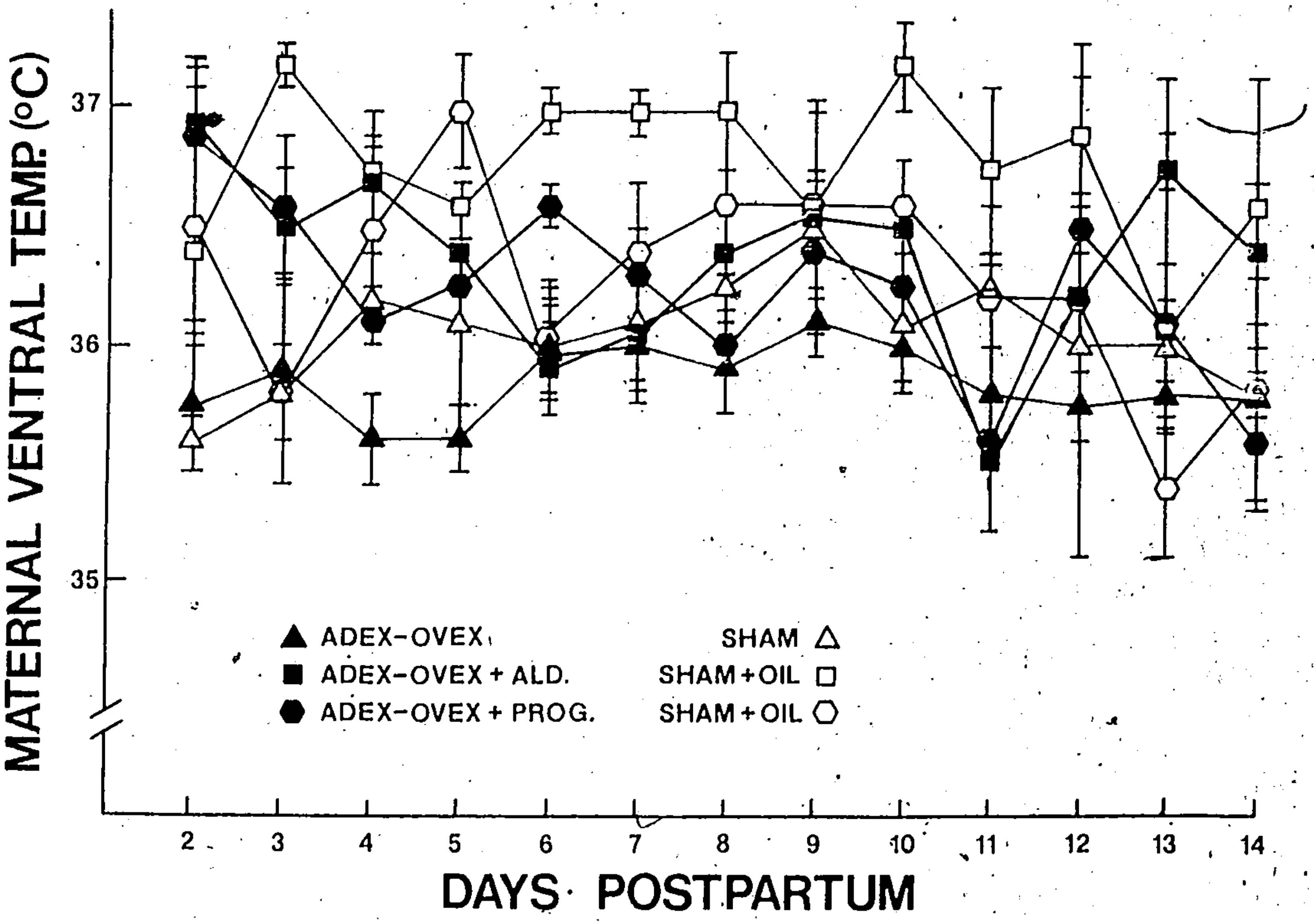

MATERNAL VENTRAL TEMP (°C)
37
36
35
DAYS POSTPARTUM
2
3
4
5
6
7
8
9
10
11
12
13
14
▲ ADEX–OVEX
■ ADEX–OVEX + ALD.
⬢ ADEX–OVEX + PROG.
SHAM △
SHAM + OIL □
SHAM + OIL ⬡

Figure 9: Mean daily ventral skin temperatures of Adex-Ovex + Corticosterone, Adex-Ovex + Cortisol and Sham-Op dams. SEMs are shown.

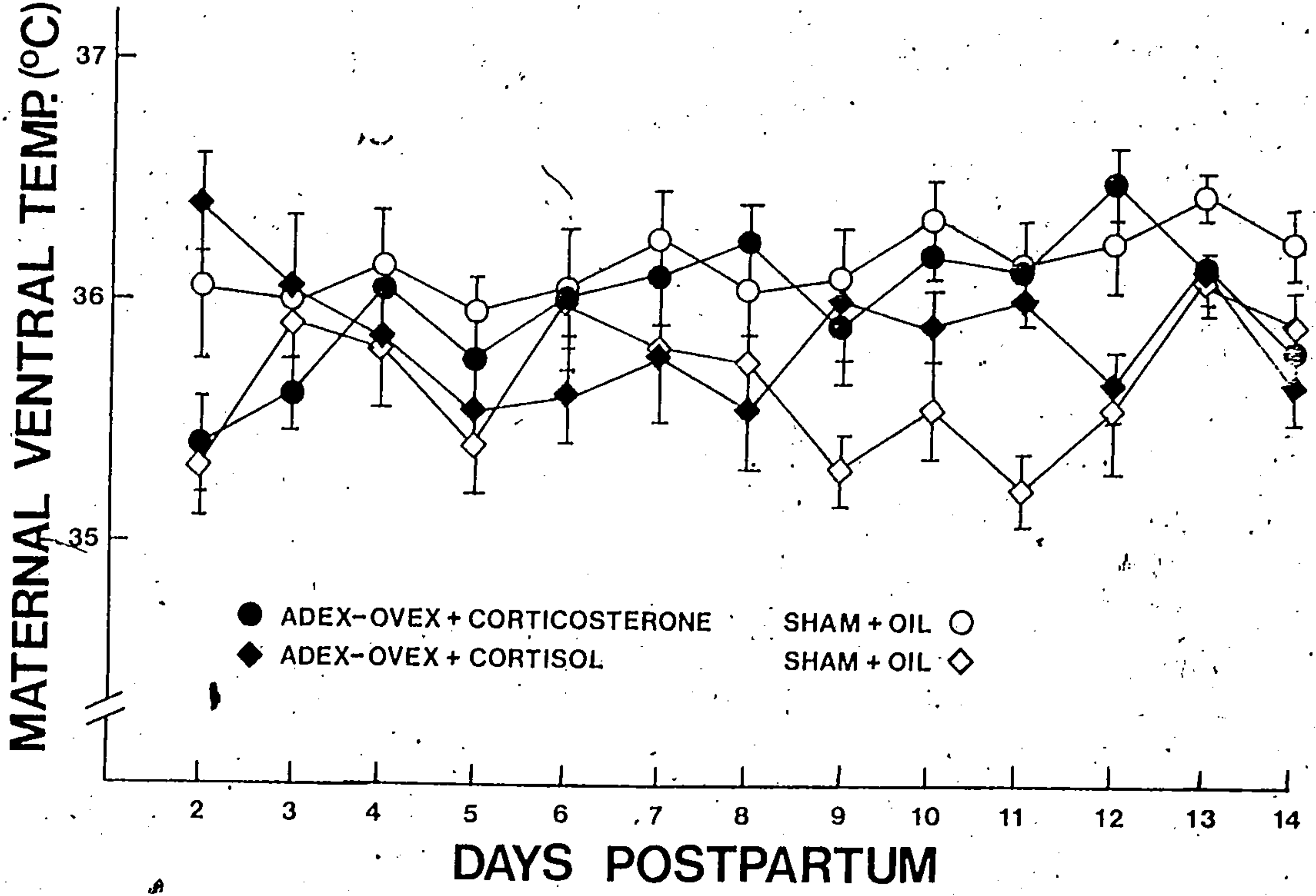

MATERNAL VENTRAL TEMP. (°C)
37
36
35
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
● ADEX-OVEX + CORTICOSTERONE
◆ ADEX-OVEX + CORTISOL
SHAM + OIL ○
SHAM + OIL ◇

Figure 10: Mean daily pup skin temperatures of Adex-Ovex, Adex-Ovex + Aldosterone, Adex-Ovex + Progesterone and Sham-Op dams. SEMs are shown.

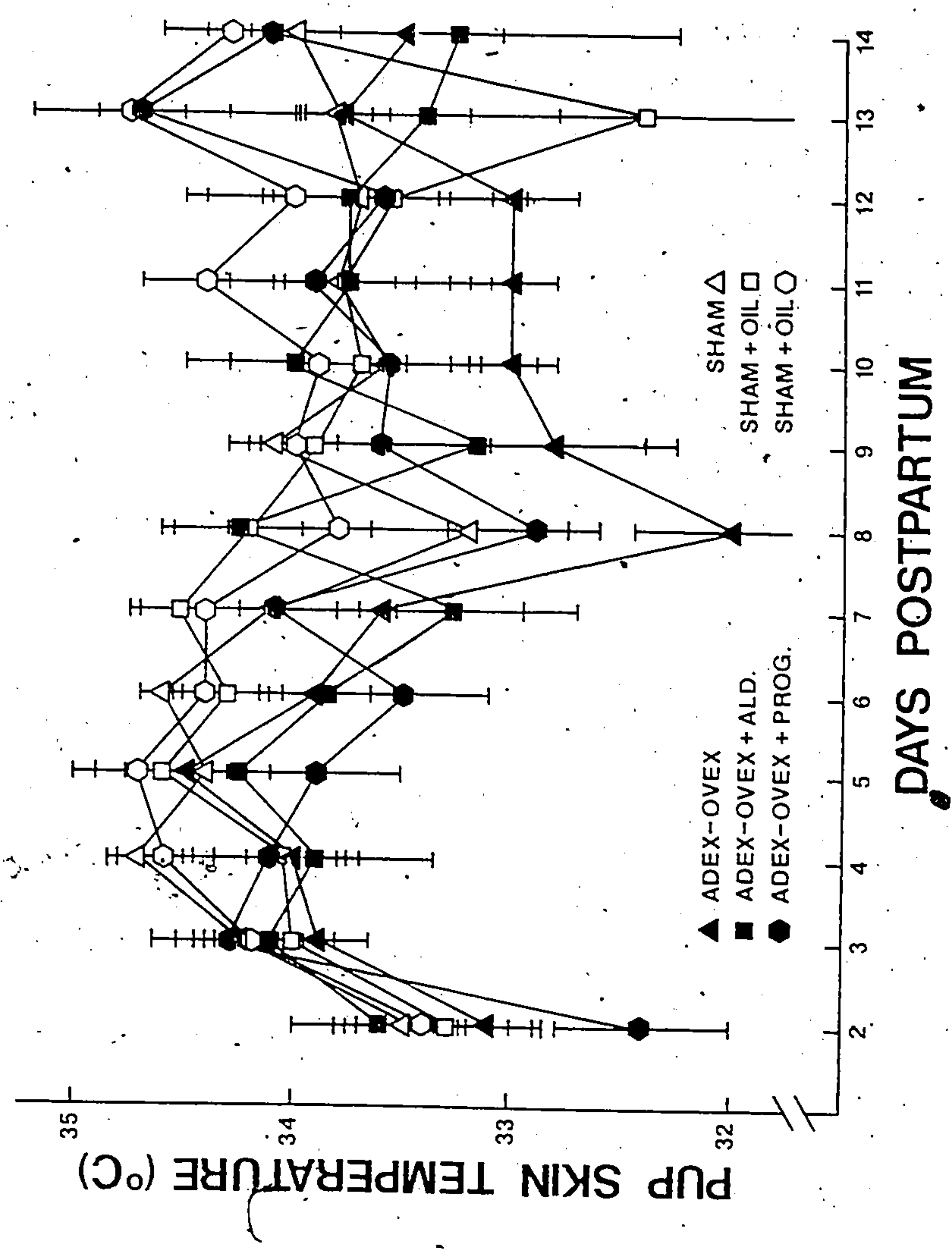
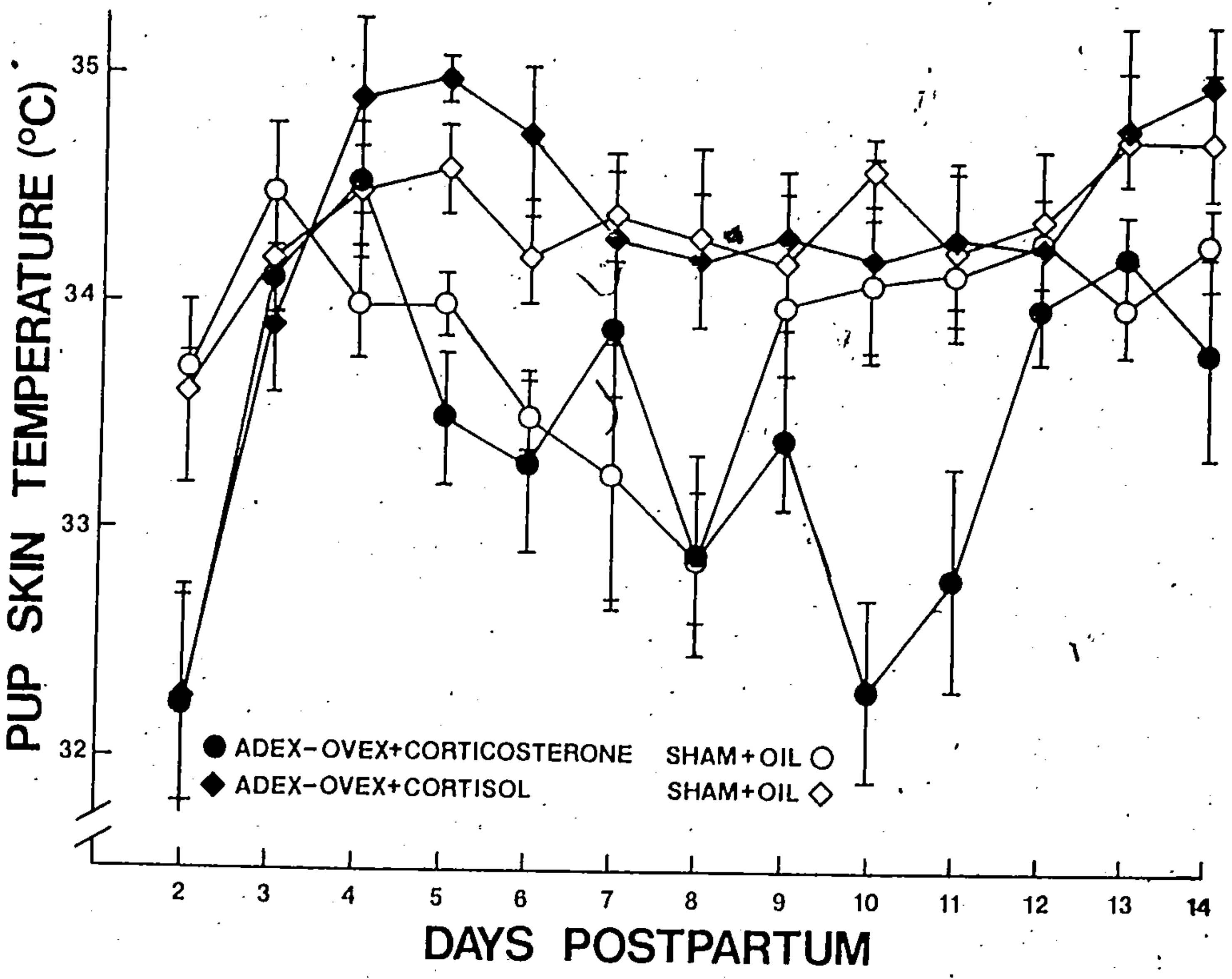

PUP SKIN TEMPERATURE (°C)
35
34
33
32
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
▲ ADEX–OVEX
■ ADEX–OVEX + ALD.
● ADEX–OVEX + PROG.
SHAM △
SHAM + OIL □
SHAM + OIL ○

Figure 11: Mean daily pup skin temperatures of Adex-Ovex + Corticosterone, Adex-Ovex + Cortisol and Sham-Op dams. SEMs are shown.


PUP SKIN TEMPERATURE (°C)
35
34
33
32
● ADEX–OVEX+CORTICOSTERONE
◆ ADEX–OVEX+CORTISOL
SHAM+OIL ○
SHAM+OIL ◇
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM

proposition that dams must have a chronically elevated core temperature to be vulnerable to the additional increase in heat that is precipitated by huddling with the pups. These data also lend support to the hypothesized role of adrenal steroids in stimulating the rise in maternal metabolism which, in turn, causes the chronic rise in maternal temperature.

The data presented here, however, do not necessarily mean that corticosterone and cortisol *per se* are playing a direct role in producing these effects. Adrenalectomy removes the negative feed back of adrenocortical hormones on the release of adrenocorticotrophic hormone (ACTH) from the pituitary, and adrenal removal would, therefore, be expected to result in high circulating levels of ACTH. It is possible, then, that cortisol and corticosterone replacement in these studies had their effects, not directly, but by suppressing ACTH. Low ACTH might induce a chronic elevation of maternal temperature and, thereby, result in short nesting bouts. This interpretation of the data presented in the present experiment seems unlikely. First, circulating ACTH reaches high levels only about 10 days after adrenalectomy (Cox and Hodges, 1958). Second, progesterone is also an efficient inhibitor of ACTH (Singer, Losito and Salmon, 1963; Edgren, Hambourger and Calhoun, 1958; Holum, Katz and Jailer, 1961) but that steroid was ineffective in restoring the normal temperature or behaviour of mother rats. If the effects of cortisol and corticosterone on nest time were, in fact, mediated by their ability to suppress ACTH one would expect progesterone to have a metabolic effect similar to that of the glucocorticoids.

Progesterone failed to produce an increase in core temperature in Adex-Ovex females in this experiment despite a previous report of its thermogenic action (Rothchild, 1969). Cunningham and Cabanac (1971) however, found that women in the high progesterone phase of the menstrual cycle have not only a high temperature but an elevated thermal set point. Further, Nakayama, Suzuki and Ishizuka (1975) found that progesterone administration caused a decrease in the rate of firing of thermosensitive neurons in the hypothalamus. Such a change in sensitivity has been suggested as a possible mechanism underlying changes in set point (Hammel, Jackson, Stolwijk, Hardy and Stromme, 1963). Thus, the apparent thermogenic effect of progesterone reported by others may be mediated by the increase in thermal set point induced by the administration of this hormone. As adrenalectomised animals are unable to maintain a rate of heat production consistent with the requirements of a normal set point it is unlikely that they would be able to increase their heat production in response to an upward shift in thermal set point. Adrenalectomized dams would, therefore, be unresponsive to the effects of progesterone administration.

Pup skin temperature varied across replacement groups. Only the Adex-Ovex and Adex-Ovex + Corticosterone groups showed a signficantly lower pup skin temperature than their associated Sham-Op groups. The fact that the corticosterone replacement group showed a decline in nest time and the Adex-Ovex + Progesterone and Adex-Ovex + Aldosterone groups, whose pups had the same temperature as their Sham-operated controls, did not show a decline in nest time, however, indicates that while pup temperature may be a factor in influencing nest bout duration, glucocorticoid action is not primarily mediated by changes in pup temperature.

The pattern of results obtained in this experiment emphasise the additive nature of the thermal factors which give rise to an increase in maternal core temperature during a nest bout. It is possible that low pup temperature contributed to the longer nest bouts seen in corticosterone replaced dams in spite of the fact that maternal core temperature for this group is approximately the same as that of the cortisol replaced dams. Further, variation in pup temperature probably also contributes to the variation in patterns of nest bout decline between sham-operated groups.

In sum, both corticosterone and cortisol replacement are effective in reinstating the progressive decline in mother-litter interaction seen in the rat in the first two weeks postpartum. These hormones also produce the predicted rise in maternal core temperature. It seems, therefore, that glucocorticoid replacement is sufficient to reinstate in the Adex-Ovex female, the physiological state that mediates the normal decline in nesting behaviour. Specifically, the data are consistent with the notion that glucocorticoids are responsible for chronically elevating the maternal core temperature, and thereby, rendering the dam vulnerable to an acute rise in temperature while huddling with her pups.

Experiment 3

Corticosterone-replacement: Effects on the change in core temperature experienced during a nest bout by Adex-Ovex dams

The data obtained in the previous two experiments are consistent with the notion that adrenocortical hormones, specifically the glucocorticoids, chronically elevate maternal temperature, rendering dams vulnerable to the acute thermal effects of huddling. Presumably, it is the acute rise in maternal temperature, consequent upon joining the huddle, which limits the duration of the nest bout. The dam, in effect, maintains thermal homeostasis by leaving her young and thus terminating the acute rise in maternal temperature. The lowered core temperature of Adex-Ovex dams makes them less vulnerable to the acute thermal effects of huddling, and consequently their nest bouts are not likely to be limited by thermal considerations. If, in fact, the chronically elevated temperature of the dam affects nest time by making her vulnerable to the acute effects of huddling, then one should be able to observe a further acute increase in core temperature during nest bouts in normal dams but not in Adex-Ovex females. Moreover, if the effects of glucocorticoid replacement therapy on nest time are thermally mediated, then glucocorticoid replacement should reinstate in Adex-Ovex dams the acute rise in maternal temperature consequent on huddling with pups.

In this experiment, the core temperature and nest bout duration of Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone dams were recorded continuously for 24 hours beginning at 12 noon on Day 10 postpartum, to observe whether the hormone state of the dams actually made them vulnerable to acute thermal events precipitated by huddling with the young.

Method

Subjects

Six multiparous Wistar rats, originally obtained from Canadian Breeding Farms (St. Constant, Quebec), served as subjects in each of three experimental groups: Sham-Op, Adex-Ovex, and Adex-Ovex + Corticosterone.

Procedure

All females were mated in group cages and removed to individual cages on day 19 - 20 of gestation. After parturition all litters were reduced in number to eight pups.

On day 2 postpartum, all 18 females were implanted with a YSI thermistor (Part #44003A) in the peritoneal cavity. The leads from the thermistor were threaded beneath the animals skin and emerged on the top of her head. The leads terminated in amphenol pins (#220 - P02) that were placed in a plastic head cap (Amphenol miniature connector #225-2205), fixed to the skull with dental cement. The six animals in each of the Adex-Ovex and Adex-Ovex + Corticosterone groups were also adrenalectomised-ovariectomised as described in Experiment 1. All surgical procedures were performed under ether anaesthesia.

The animals in the Adex-Ovex and Adex-Ovex + Corticosterone groups were maintained on 1% NaCl solution and ad lib Purina Rat Chow. The 6 animals in the Adex-Ovex + Corticosterone groups were given daily injections of 0.2cc of 10% ethanol in oil vehicle containing 3 mg corticosterone at 1700 hr.

To ensure healthy pups, litters were switched daily between each female in the Adex-Ovex or corticosterone replaced groups and an unoperated colony mother who had given birth on the same day. Sham-operated animals were maintained on water and ad lib Purina Rat Chow and allowed to rear their own pups.

On Day 10 postpartum, mothers and their litters were placed in continuous recording cages and maternal core temperature was recorded on a Beckman polygraph, model 1700, for 24 hours from 12 noon on Day 10 until 12 noon on Day 11. The change in core temperature over the course of a nest bout and nest bout duration were then transcribed. The mean nest bout duration and percentage of nest bouts during which the dam experienced a rise in core temperature were calculated as well as the mean change in temperature experienced by the dam in those bouts where her temperature actually increased. Change in core temperature over the course of a bout was calculated by subtracting the core temperature recorded for the dam at bout onset from that recorded at the termination of a bout. Determinations of core temperature were made to the nearest 0.08°C. If this procedure yielded a positive number then the core temperature was considered to have risen. In very few nest bouts did maternal core temperature show an appreciable rise and then fall before nest bout termination. Rather, if dam core temperature did show a rise it tended to remain elevated throughout the nest bout. A one way Analysis of Variance and Tukey post hoc analyses were then performed on these data.

Results

Figure 12 shows sample records of maternal core temperature during a nest bout for one animal from each of the three groups. The mean bout duration for Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone groups is shown in Figure 13a. Figure 13b indicates the mean percentage of nest bouts in which these groups showed a rise in temperature. The average increase in core temperature for each group during these bouts is shown in Figure 14.

As can be seen in Figure 13a Adex-Ovex dams showed the longest mean nest bout durations, Sham-Op animals the shortest and the Adex-Ovex + Corticosterone females nest bouts of intermediate duration. These data replicate the results of Experiment 1. The analysis of variance yielded a significant treatment effect ($\underline{F} = 11.76$, $\underline{p} < .01$) and post hoc analysis showed that the mean nest bout duration of Adex-Ovex dams was significantly longer than that of Sham Op dams.

There was a significant effect of treatment on the mean percentage of nest bouts during which dam core temperature rose ($\underline{F} = 73.23$, $\underline{p} < .01$) and all post hoc pairwise analyses were also significant, $\underline{p}$'s < 0.05). In those nest bouts where their core temperature rose Adex-Ovex dams showed a smaller increment in core temperature than members of the Sham-Op and Adex-Ovex + Corticosterone groups. Overall, the effect of treatment was nonsignificant ($\underline{p} > .05$).

Figure 12: Sample trace of maternal core temperature during a nest bout from a Sham-Op, an Adex-Ovex and an Adex-Ovex + Corticosterone dam on Day 10 postpartum.

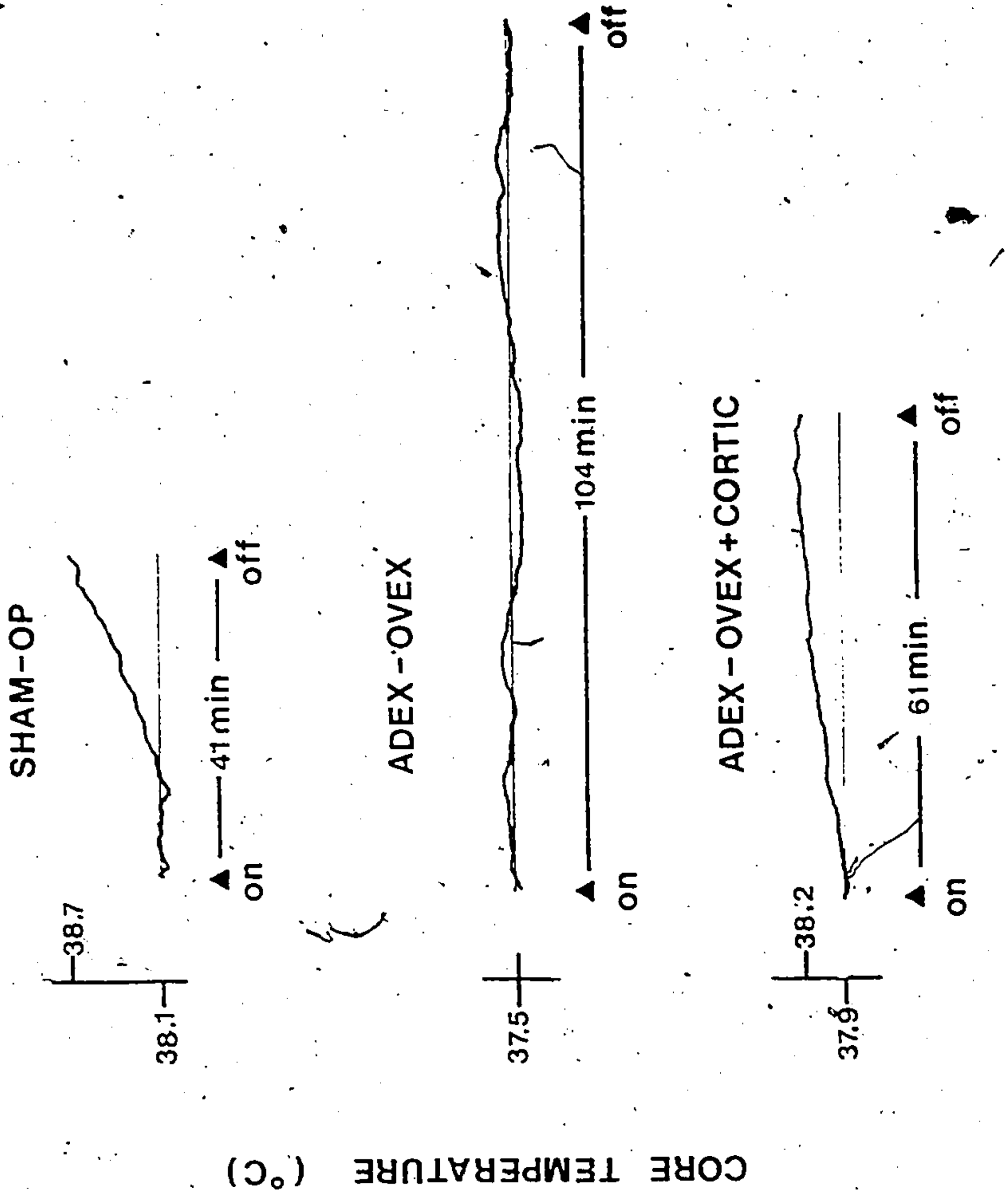
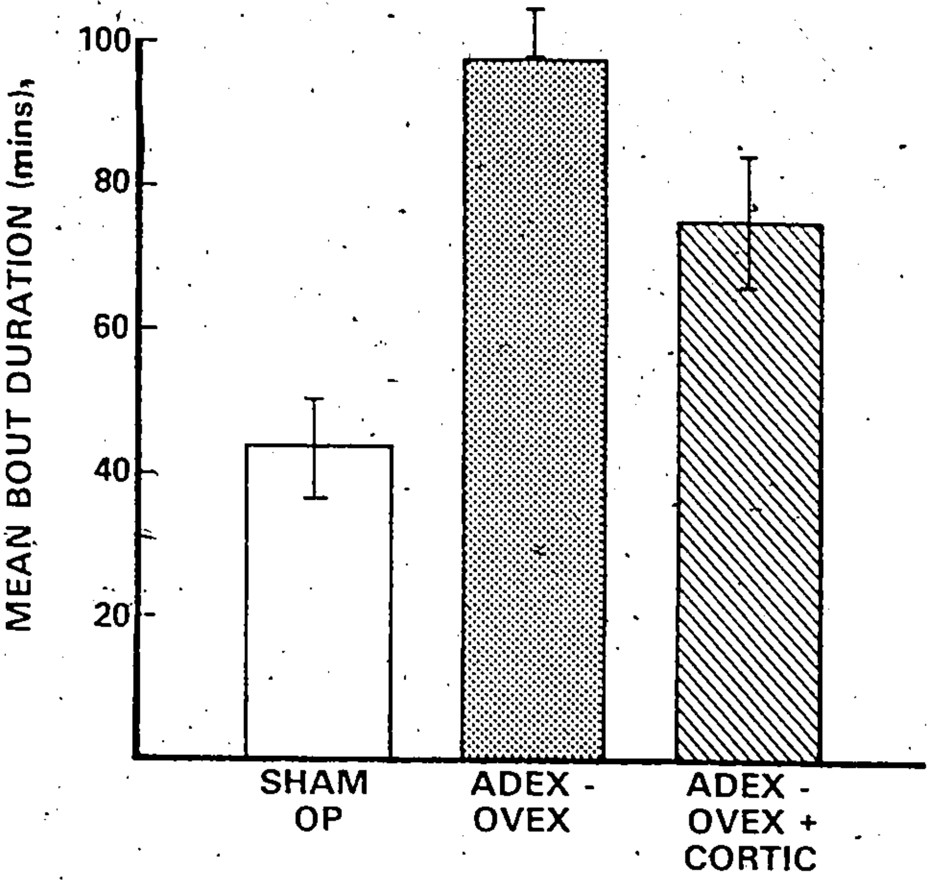

SHAM-OP
41 min
on
off
38.7
38.1
ADEX-OVEX
104 min
on
off
37.5
ADEX-OVEX+CORTIC
61 min
on
off
38.2
37.9
CORE TEMPERATURE (°C)

Figure 13a: Mean bout duration + S.E.M. for Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone dams on Day 10 postpartum.

13b: Mean percentage of nest bouts during which Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone dams showed an increase in core temperature on Day 10 postpartum.


MEAN BOUT DURATION (mins),
100
80
60
40
20
SHAM OP
ADEX - OVEX
ADEX - OVEX + CORTIC

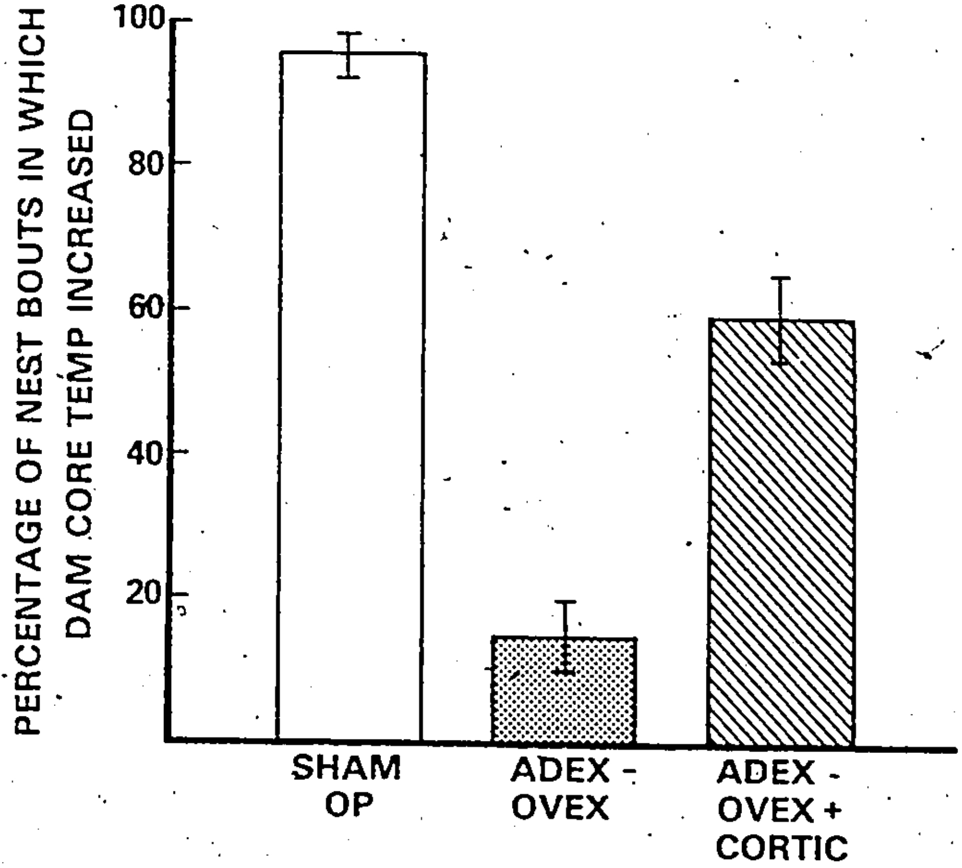
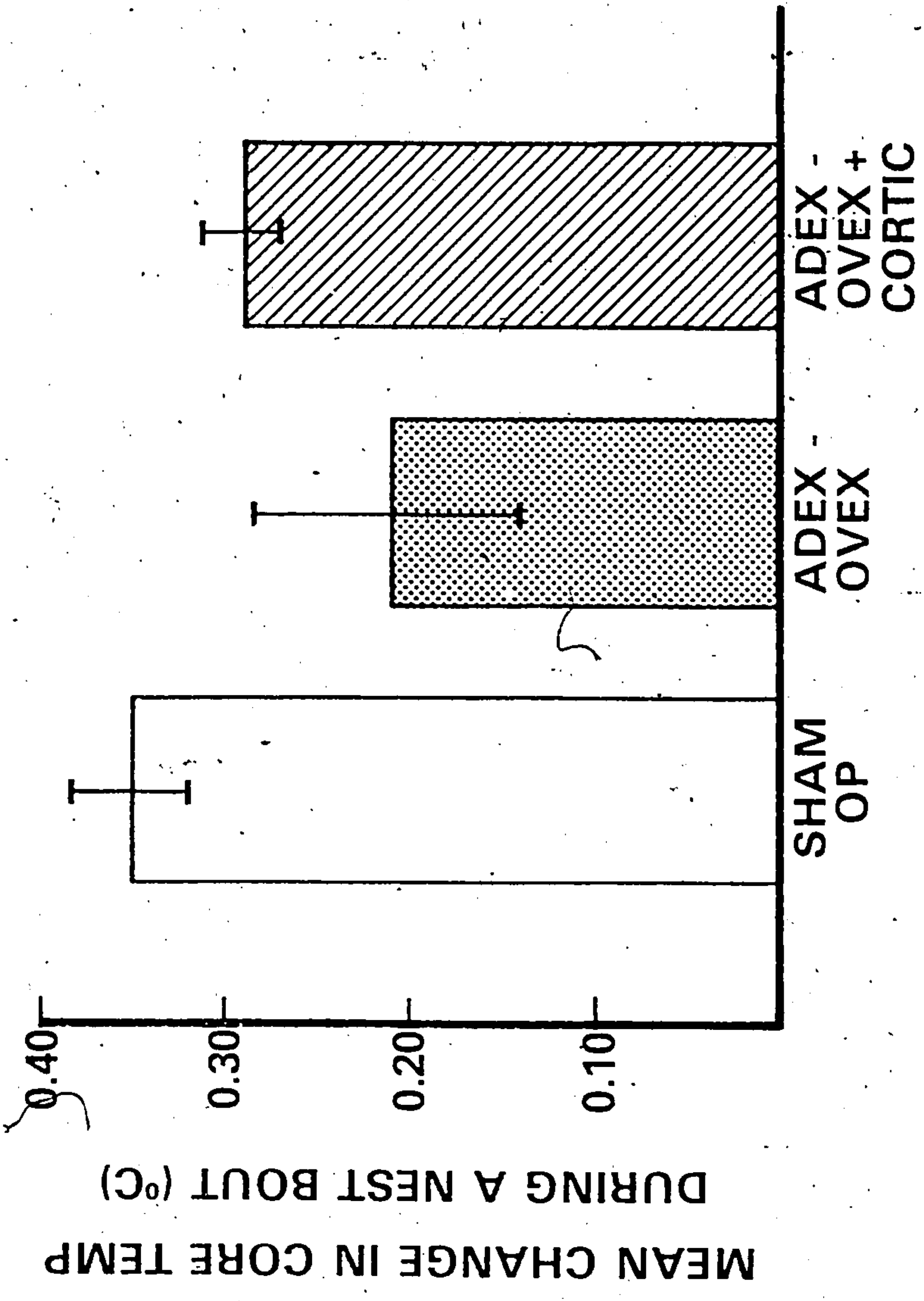

PERCENTAGE OF NEST BOUTS IN WHICH DAM CORE TEMP INCREASED
100
80
60
40
20
SHAM OP
ADEX - OVEX
ADEX - OVEX + CORTIC

Figure 14: Mean increase in core temperature, on those bouts where an increase occurred, for Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone dams. SEMs are shown.


MEAN CHANGE IN CORE TEMP DURING A NEST BOUT (oC)
0.40
0.30
0.20
0.10
SHAM OP
ADEX - OVEX
ADEX - OVEX + CORTIC

Discussion

The results of this experiment indicate that, as predicted, Adex-Ovex dams rarely experienced a rise in core temperature when they huddled with their young. These animals presumably experience no difficulty in dissipating any extra heat retention caused by huddling. Glucocorticoid replacement therapy, however, rendered Adex-Ovex dams more vulnerable to the acute thermal effects consequent on making contact with their litter. These animals had a chronically higher thermal load than Adex-Ovex females that were given no hormone replacement, and had more difficulty in dissipating heat, particularly when they huddled with their young..

The differences among the mean nest bout durations observed for the Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone groups were consistent with the proposed role of thermal factors in limiting nest bout duration. Sham-operated dams which experienced increases in core temperature during 95% of their nest bouts, and showed the largest mean increase in core temperature during nest bouts, showed the shortest average nest bout duration. Adex-Ovex females that experienced the least average core temperature rise on the smallest proportion of nest bouts, had the longest nest bouts. Adex-Ovex + Corticosterone dams were intermediate on all three dimensions. The mean rise in core temperature, during a nest bout, shown by each was sufficient to indicate that such a change could well give rise to a thermoregulatory response.

These data support the notion that a rise in maternal core temperature during a nest bout limits nest bout duration. Adex-Ovex dams, which seldom show a rise in core temperature when they huddle with their young, have very long nest bouts. As one would predict, thermal considerations appear to play little or no role in the limitation of the nest bouts of Adex-Ovex females. The data obtained in this experiment also support the hypothesis that the effect of glucocorticoid replacement to Adex-Ovex dams on nest time is mediated by a chronic increase in maternal core temperature. This elevated maternal temperature renders the dam vulnerable to the thermal effects of huddling with her young.

Experiment 4

The effects of corticosterone-replacement therapy on the nest time of prolactin-suppressed dams

The results of experiments 1-3 indicate that glucocorticoid replacement in Adex-Ovex females is sufficient to reinstate, to a considerable degree, the normal decline in nest time. Adex-Ovex dams have normal levels of circulating prolactin (Ben-David, Danon, Benveniste, Weller and Sulman, 1971), suggesting that prolactin itself is not sufficient to maintain normal nesting behaviour. Prolactin-suppression does, however, result in a halt in the daily decline in nest time in the second week postpartum, indicating that prolactin does play a role in maintaining normal nesting behaviour. The question addressed in the present and subsequent experiments is whether 1) high circulating levels of prolactin stimulate high circulating levels of corticosterone which, in turn, results in a chronically elevated maternal core temperature, and a decline in daily nest time, or 2) prolactin plays a direct role in maintaining a chronically elevated maternal core temperature and thereby the decline in nest time in the second week postpartum. A flow diagram representing each of these hypotheses is shown in Figure 1b.

There is some evidence that prolactin can stimulate increased circulating levels of glucocorticoids. Witorsch and Kitay (1972) found that prolactin administration to hypophysectomised adult or ovariectomised prepubertal rats, resulted in inhibition of the action of 5α-adrenal reductase, an enzyme which reduces corticosterone to its A-ring metabolites, thereby decreasing corticosterone metabolite levels and increasing glucocorticoid levels in the blood. Further, Chatterton, Chien and Ward (1974) found that rats treated with perphenazine, a drug which reliably elevates prolactin secretion, (Ben-David, Danon, Benveniste, Weller and Sulman, 1971) also increases corticosterone levels. It seemed possible, therefore, that prolactin normally stimulates glucocorticoid levels. Indeed, mother rats normally have high levels of both circulating prolactin and glucocorticoids (Simpson, Simpson, Sinha and Schmidt, 1973). If prolactin normally stimulates glucocorticoid release during this period, then prolactin suppression should result in a decrease in glucocorticoid levels. Further, if prolactin inhibition does have its effects on nest time by causing a decrease in glucocorticoids levels, then it should be possible to reinstate the normal decline in nesting time in prolactin suppressed dams by giving glucocorticoid replacement. Moreover, if glucocorticoid were effective in restoring the decline in nest time in prolactin-suppressed dams, then one would expect that glucocorticoid should also increase the temperature of prolactin-suppressed mothers. Conversely, if prolactin itself has a direct effect on maternal core temperature, and, thereby maternal nesting time corticosterone replacement should be ineffective in reinstating normal maternal nesting behaviour and maternal temperature.

In the current experiment, therefore, I assessed the effect of corticosterone replacement therapy during the second week postpartum on the nest time and temperature of dams treated with ergonovine maleate, a potent inhibitor of prolactin (Shaar & Clemens, 1972). Corticosterone therapy was initiated on Day 6 postpartum because that is the day at which ergonovine treated animals ceased to show a decline in nest time (Leon, Croskerry & Smith, 1978).

Method

Subjects

Twenty one virgin female rats obtained from Canadian Breeding Farms (St. Constant, Quebec) served as subjects in this experiment.

Procedure

Fourteen virgin females were mated and placed in individual cages two days prior to the expected date of parturition. On the day of birth (Day 0) dams were paired. One member of each pair was assigned to the Ergonovine + Corticosterone (Ergo + Cortic) group and the other to the oil-injected (Control) group. Starting on Day 1 postpartum, the 7 animals in the Ergo + Cortic group were given 8 mg ergonovine maleate (Eli Lilly and Co.) in 0.2cc oil daily at 12 noon. The control group were given 0.2cc oil vehicle at that time. Beginning on Day 6 postpartum corticosterone therapy was initiated. Animals in the Ergo + Cortic group were given daily injections of 3 mg corticosterone in 0.2 cc oil at 1700 hr in addition to their daily ergonovine injection. Animals in the control group received the oil injection alone. All injections were given subcutaneously. Measures were taken and subsequently analysed according to the procedures described in Experiments 1 and 2. The remaining 7 animals were treated exactly the same as the Ergo + Cortic group with the exception that they were not given corticosterone replacement therapy (Ergo group).

The core temperature of dams in this group was then compared with that of the Ergo + Cortic group.

Results

The main results of this experiment are presented in Figures 15-19. Table III shows the F ratios and the p values obtained from the statistical analysis for each factor for each dimension. The mean daily nest time is shown in Figure 15. It is clear from this figure that both the control and Ergo + Cortic groups showed a similar decline in nest time over days which is confirmed by the analysis of variance. There was no significant Treatment Effect ($F = 0.30$, $p > .05$) nor was there a significant Days x Treatment interaction. The Days Effect, however, was highly significant ($F = 25.32$, $p < .001$). The mean daily nest times of the Ergo group are also shown.

Figure 16 shows the mean daily nest frequency for both groups. The Ergo + Cortic group shows a significantly higher nest frequency than the control group (Treatment Effect $F = 19.81$, $p < .001$). Neither group shows a statistically significant change in nest frequency over the course of the experiment (Days Effect $F = 1.01$, $p > .05$; Days x Treatment interaction $F = 0.32$, $p > .05$). Mean daily nest bout frequency for the Ergo group is also shown.

The mean daily maternal core temperature is shown in Figure 17. As appears in the figure the core temperature of the Ergo + Cortic group was significantly lower than that of the Control group ($F = 6.0$, $p < .05$) but significantly greater than the Ergo group ($F = 31.97$, $p < .01$). There were no statistically significant effects of Days or Day x Treatment interactions in either set of comparisons.

Table III

'F' ratios and Associated p values from the Statistical Analysis of Experiment 4

Dimension	Nest Time		Nest Frequency		Dam Core Temperature		Dam Ventral Temperature		Pup Skin Temperature	
Effect	F	P	F	P	F	P	F	P	F	P
Treatment	0.30	ns	19.81	<.001	6.0	<.05	10.0	<.01	7.42	<.05
Days	25.32	<.001	1.01	ns	3.4*	ns	0.01	ns	16.23	<.001
Days x Treatment Interaction	1.67	ns	0.32	ns	1.75	ns	0.01	ns	4.31*	ns

*Indicates 'F' ratios that were nonsignificant using the Geisser-Greenhouse Conservative 'F' test but would have been significant at $p < .05$ if a less conservative test were used.

Figure 15: Mean daily nest times of Ergo, Ergo + Corticosterone and Control dams. SEMs are shown.

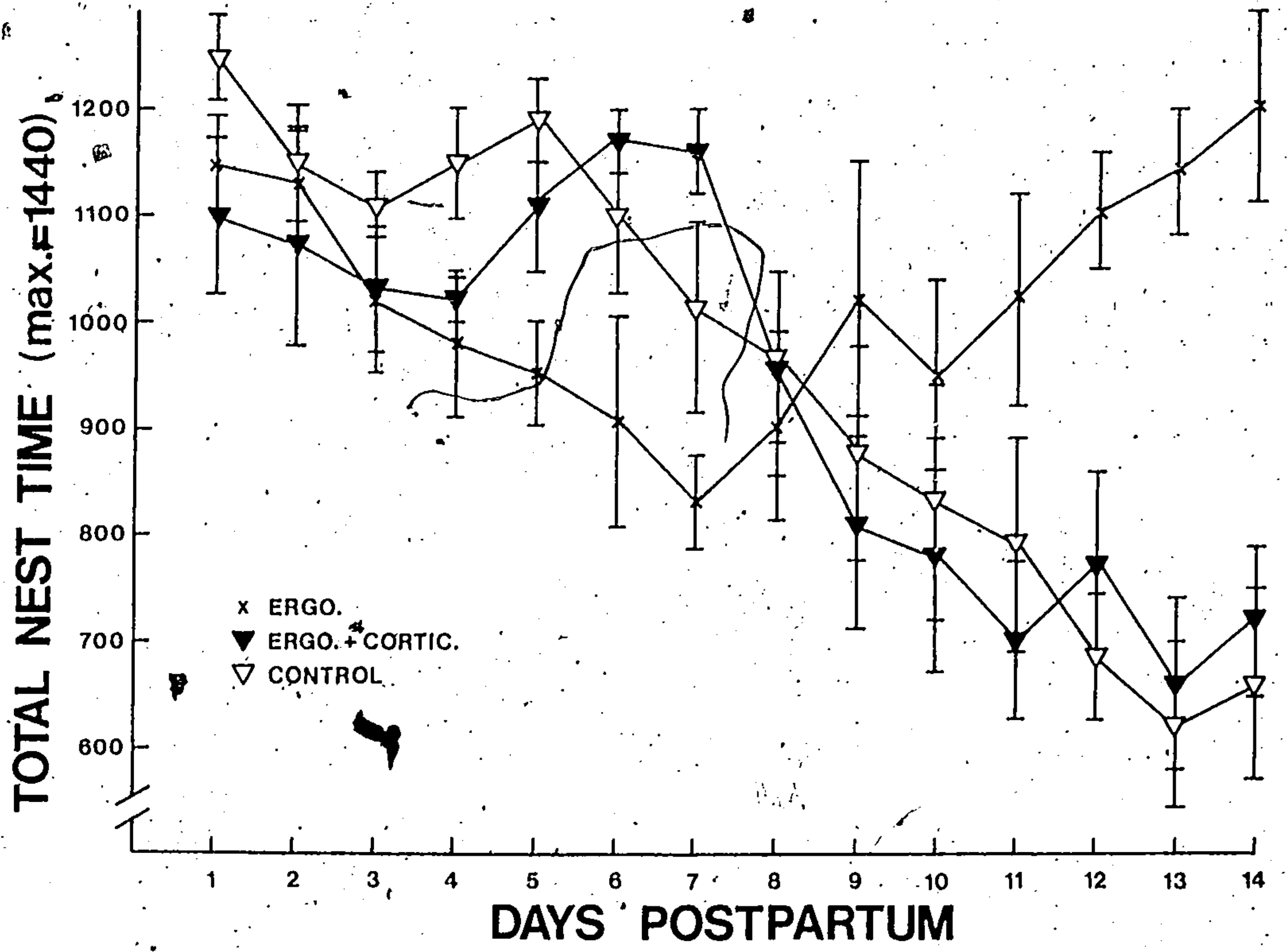

TOTAL NEST TIME (max.=1440)
1200
1100
1000
900
800
700
600
DAYS POSTPARTUM
1
2
3
4
5
6
7
8
9
10
11
12
13
14
x ERGO.
▼ ERGO.+CORTIC.
▽ CONTROL

Figure 16: Mean daily nest bout frequency of Ergo, Ergo + Corticosterone and Control dams. SEMs are shown.

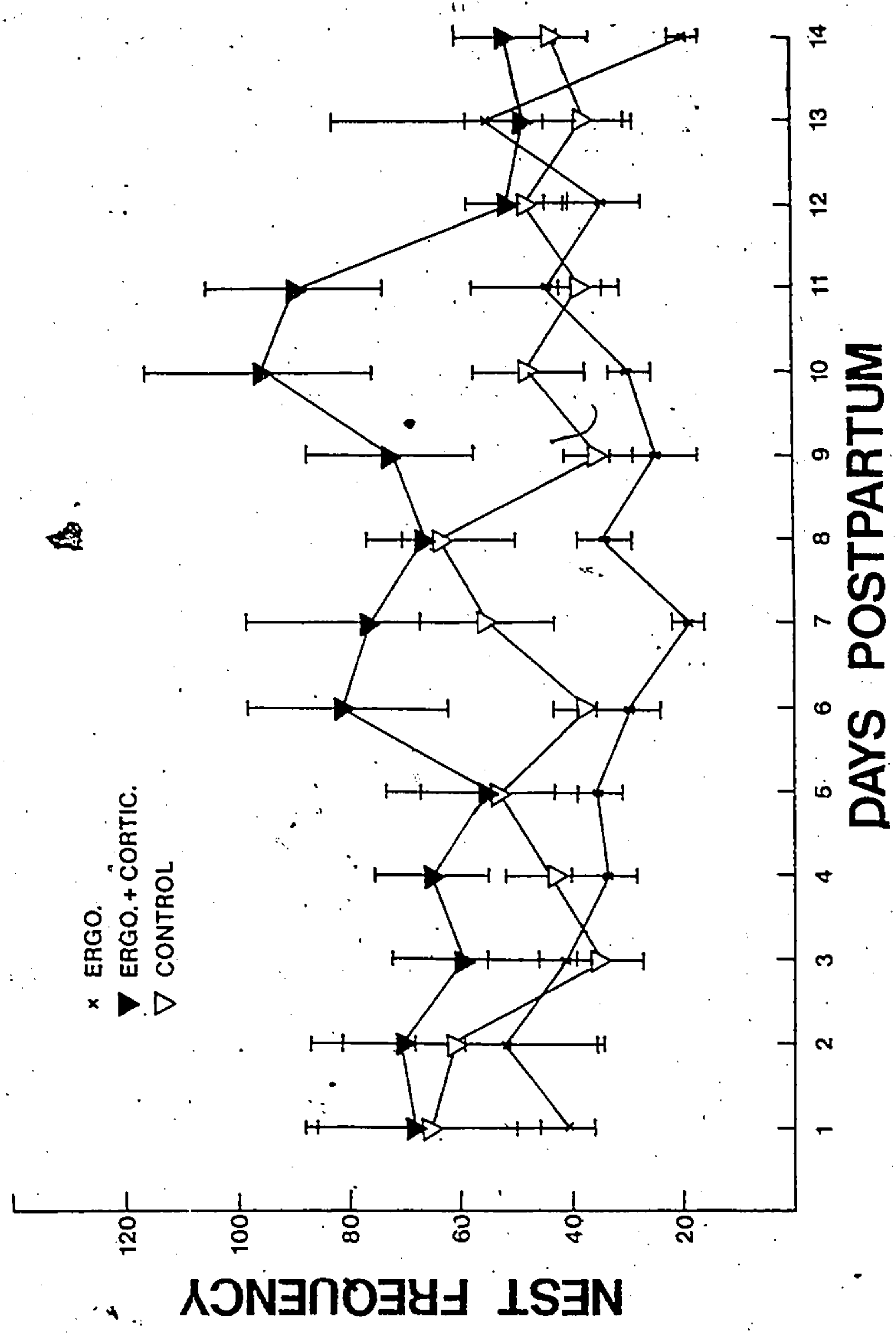

× ERGO.
▼ ERGO.+CORTIC.
▽ CONTROL
NEST FREQUENCY
120
100
80
60
40
20
DAYS POSTPARTUM
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 17: Mean daily core temperature of Ergo, Ergo + Corticosterone and Control dams. SEMs are shown.

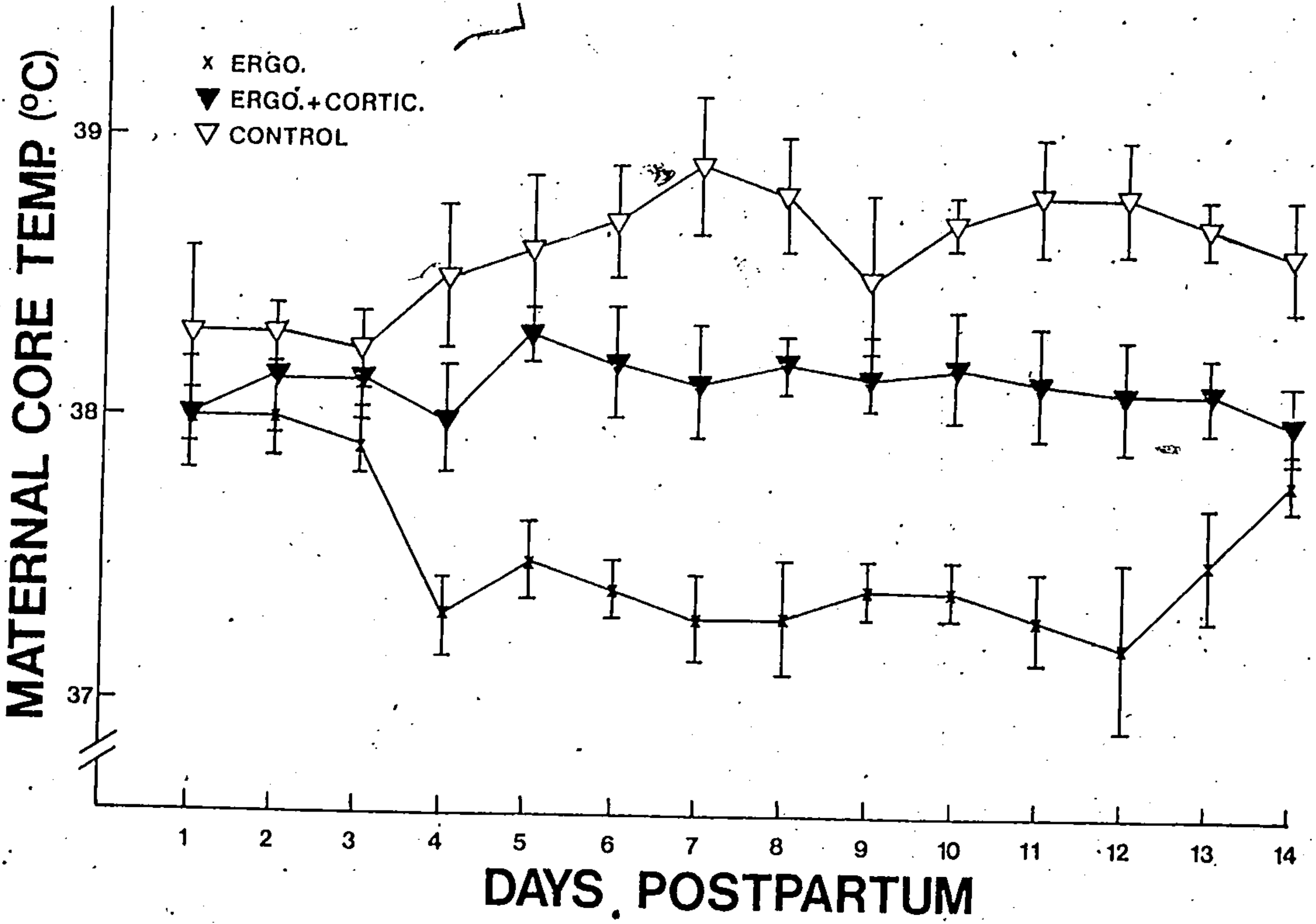

x ERGO.
▼ ERGO.+CORTIC.
▽ CONTROL
MATERNAL CORE TEMP. (°C)
39
38
37
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS, POSTPARTUM

Figure 18 shows the mean daily ventral temperature for the Ergo, Ergo + Cortic and Control groups. The ventral temperature of the Ergo + Cortic group was significantly lower than that of the Control group but there was no statistically significant difference between the Ergo + Cortic and Ergo groups on this dimension. None of the other effects were statistically significant in either set of comparisons.

Pup skin temperature was significantly lower in the Ergo + Cortic group than in the Control group ($F = 7.42$, $p < .05$) (see Figure 19). There was also a significant Days Effect on this dimension ($F = 16.23$, $p < .01$). The Day x Treatment Interaction was not statistically significant.

Discussion

It is clear that instituting corticosterone replacement therapy in prolactin-suppressed dams reinstates in these animals the decline in daily nesting time typical of intact lactating females. The results of this experiment are consistent with the hypothesis that prolactin suppression has its effects on nest time by reducing serum levels of corticosterone. Reduced circulating levels of corticosterone decreases core temperature and thereby reduces the vulnerability of dams to the acute thermal effects of huddling with their young.

The nest bout frequencies of neither the Ergo + Cortic nor the control group changed over days. Therefore, the change in nest time observed for both groups over the course of the experiment is attributable only to a decrease in individual nest bout duration.

It is clear from Figure 17 that whereas corticosterone was effective in increasing the core temperature of ergo-treated dams, the

Figure 18: Mean daily ventral temperature of Ergo, Ergo + Corticosterone and Control dams. SEMs are shown.

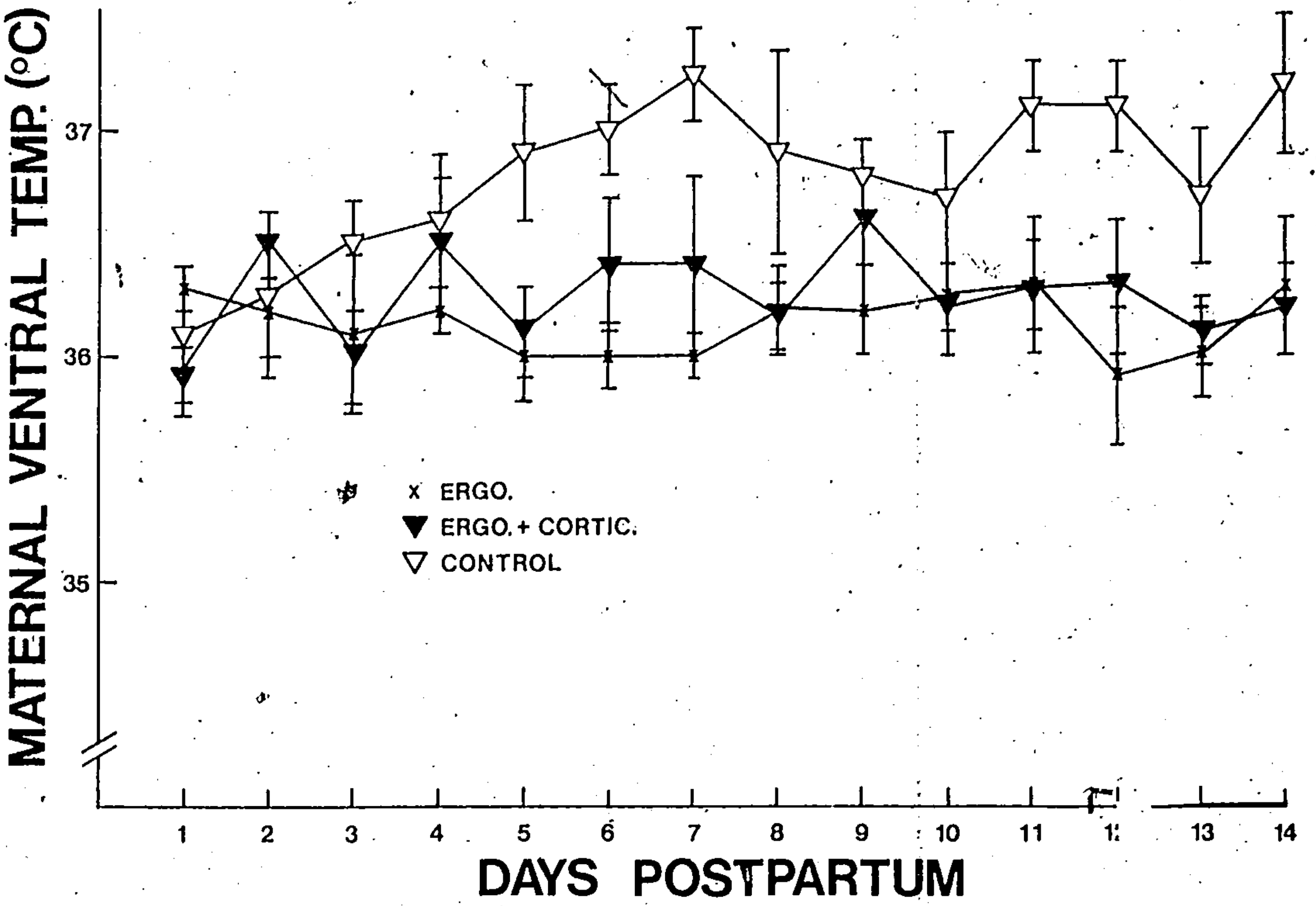
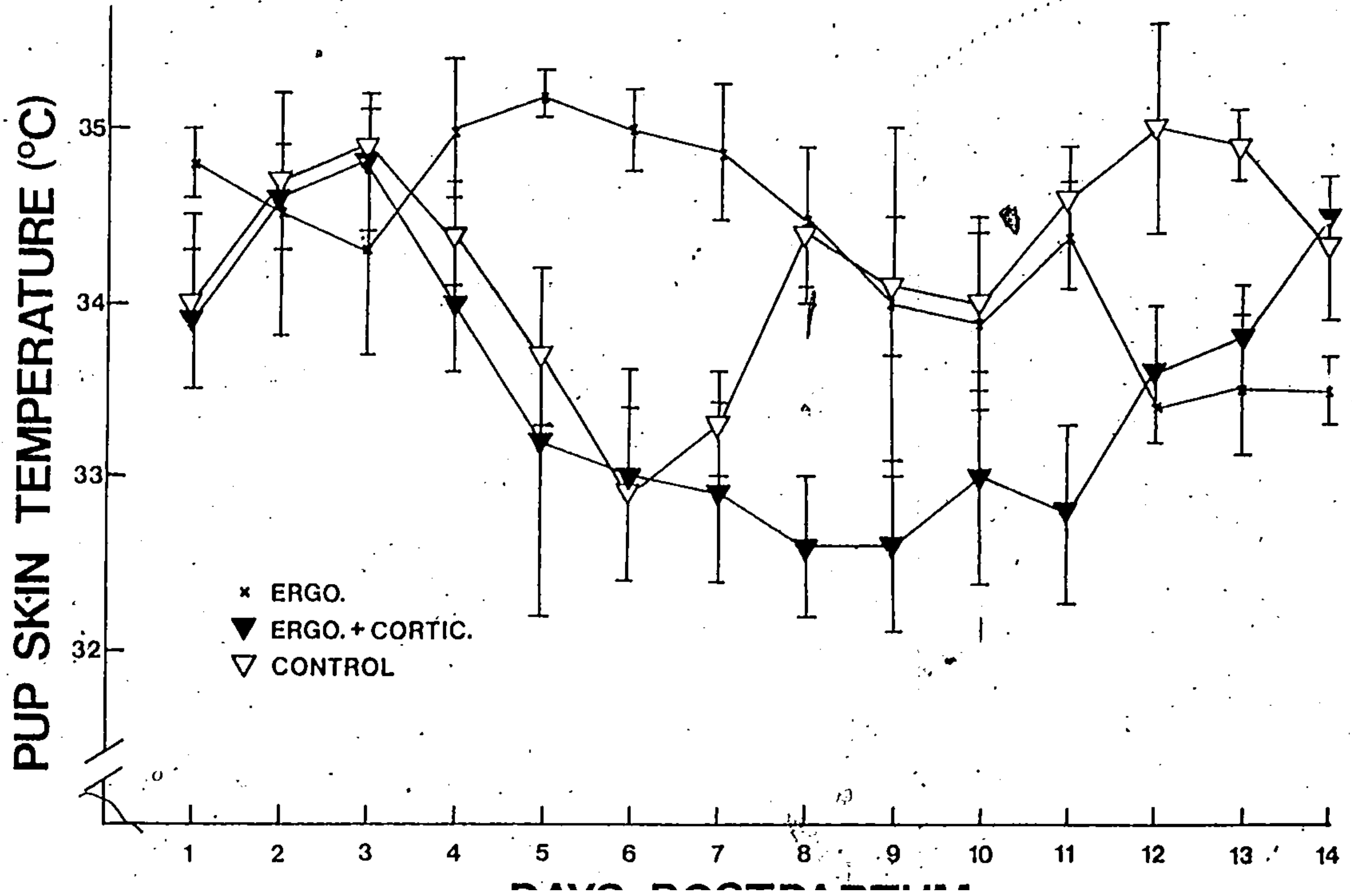

MATERNAL VENTRAL TEMP. (°C)
37
36
35
x ERGO.
▼ ERGO. + CORTIC.
▽ CONTROL
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM

Figure 19: Mean daily pup temperatures of Ergo, Ergo + Corticosterone and Control dams. SEMs are shown.


PUP SKIN TEMPERATURE (°C)
35
34
33
32
× ERGO.
▼ ERGO. + CORTIC.
▽ CONTROL
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM

glucocorticoid did not raise their core temperature to the levels seen in intact dams in the second week post partum. In spite of this deficiency, however, the nest times of the two groups are comparable through the second postpartum week. These results do not appear to be precisely in accord with a thermal model of nest bout duration, since one would predict that the higher maternal core temperature would be associated with shorter nest bout durations, all else being equal. Thus, one would expect the nest times of the intact dams to be lower than that of the Ergo + Cortic group. It is possible, however, that all else is not equal. Perhaps, as suggested in the discussion of Experiment 2, high progesterone levels elevate the thermal set point of dams. The increase in temperature shown by intact dams by the second week postpartum when progesterone levels are high would then reflect, in part, an upward shift in thermal set point. Such a shift would mean that although temperature was higher, the threshold for initiation of a behavioural heat loss response to maintain thermal homeostasis was also raised. Thus, nest bout duration would not be greatly effected when both temperature and set point are chronically elevated. Prolactin-suppressed dams have greatly decreased levels of progesterone (Tomogane, Ota & Yokayama, 1975) and thus would not experience such a shift in set point. Independent support for this notion comes from data which show that Day 10 dams, which have significantly higher core temperatures than Day 4 dams choose to remain in a similar, rather than a cooler thermal environment, despite their added heat load (Jans & Leon, 1978).

It is possible that rather than prolactin-suppression having an effect on endogenous serum corticosterone levels, prolactin itself plays a direct role in stimulating the rise in maternal temperature during the second week postpartum. Corticosterone replacement would then be effective if high corticosterone levels compensate for the absence of prolactin. Indeed, Rameley and Campbell (1977) have suggested that corticosterone could compensate for the absence of a prolactin surge around the time of puberty. In the next experiment I examined the effects of prolactin suppression on circulating levels of corticosterone by means of a hormone assay procedure to determine the precise nature of the effects of prolactin suppression on adrenal secretions.

Experiment 5

The effects of prolactin-suppression on serum glucocorticoid levels in the lactating rat

The results of the corticosterone replacement study described in Experiment 4 are consistent with the proposal that prolactin-suppression does have its effects on nest time by reducing corticosterone levels. These results, however, do not rule out the possibility that exogenous corticosterone administration compensates for the metabolic effects of the absence of prolactin, irrespective of the effects of prolactin suppression on endogenous levels of corticosterone.

In the present experiment I employed a hormone assay procedure to determine directly the effects of prolactin suppression on corticosterone levels. Serum corticosterone levels were assayed in lactating rats 4 and 10 days postpartum. Dams were treated with either daily injections of ergonovine maleate, an inhibitor of prolactin release (Saar and Clemens, 1972), or the oil vehicle. To ensure that this dosage of ergonovine maleate did, in fact, inhibit prolactin, serum from all groups was also assayed for that hormone. In addition, progesterone levels were also determined to assess the biological effectiveness of this regimen of prolactin-suppression, since progesterone secretion is reduced following prolactin suppression during lactation (Tomogane, Ota and Yokayama, 1975).

Method

Subjects

Forty-eight virgin female Wistar rats obtained from Canadian Breeding Farms (St. Constant, Quebec) served as subjects in this experiment.

Procedure

Virgin females were mated and removed to individual cages 1 - 2 days before parturition. On the day of birth (Day 0), each female was paired with another puerperal female, one was assigned to the control group and one to the group in which prolactin was suppressed (Ergo group). All litters were reduced in number to 8 pups on Day 1 postpartum and switched between control and experimental mothers daily.

Beginning on Day 1 postpartum, the 24 dams in the Ergo group were given subcutaneous injections of 8 mg ergonovine maleate in 0.2cc oil vehicle, a dose previously shown to suppress prolactin levels (Shaar and Clemens, 1972). The 24 females in the control group received 0.2cc of the oil vehicle. The injections were given daily at 0900 hr, at which time the litters were switched between each pair. On day 4 postpartum, 12 females from the Ergo group and 12 from the control group were sacrificed at 1400 hr by placing them in an airtight container into which CO_2 was introduced. Blood collection by cardiac puncture was completed within two minutes after removal from the cages. Blood samples were kept at room temperature for 25 min and then refrigerated overnight. Samples were then centrifuged at 100 *g* for 20 minutes, at which time the serum was removed and subsequently stored at

-31°C. On day 10, blood was collected from the remaining 12 Ergo and 12 control females.

All the samples were assayed for prolactin using a double antibody procedure with the NIAMDD radioimmunoassay kit. Determinations were made for duplicates of each of 3 dilutions and the average of these 6 determinations used for statistical analysis. A single antibody radioimmunoassay procedure was used to assay for corticosterone and cortisol combined.[1] A 2- way Analysis of Variance was carried out on the data obtained for each assay.

Results

Figure 20 shows the mean serum prolactin concentration for each group. Figure 21 depicts the mean serum glucocorticoid level and Figure 22 shows the mean serum progesterone level for each group. Ergonovine maleate administration was effective in suppressing prolactin release on both Day 4 and Day 10 postpartum (Treatment Effect $F = 81.09$, $p < .001$). Although the analysis of variance performed in the progesterone determinations yielded no significant effects (Treatment $F = 3.13$, $p > .05$; Days, $F = 3.36$, $p > .05$, Days x Treatment $F = 2.00$, $p > .05$) the level of serum progesterone was greatly reduced in Day 10 ergonovine treated females compared with their oil-injected controls. Indeed, a Student 't' test carried out on the Day 10 Control

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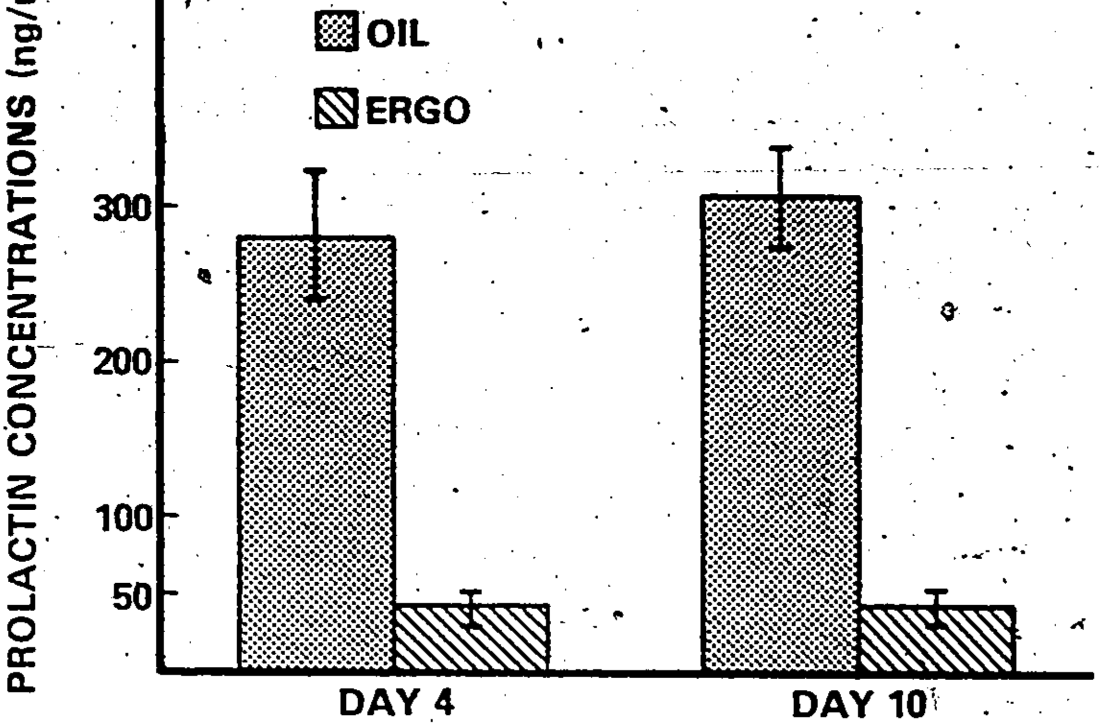
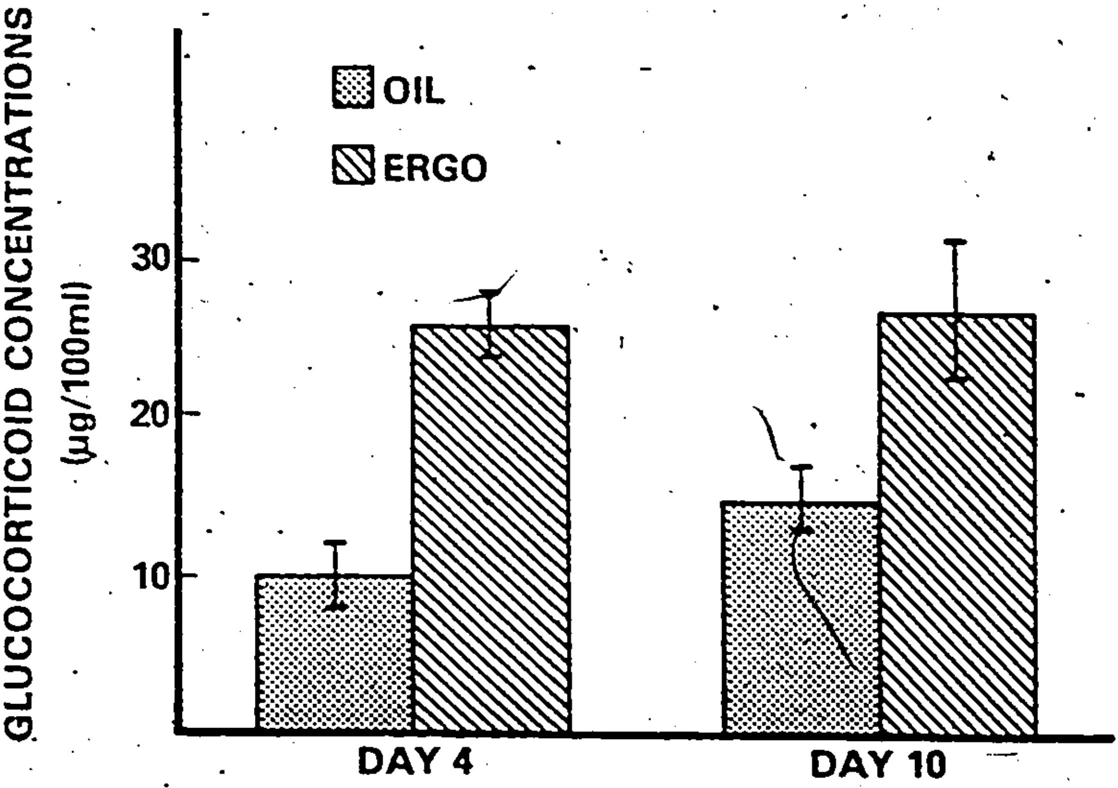
[1] Drs. Harvey Feder, Christina Fischetti and Harold Siegel, Institute of Animal behavior, Rutgers University, Newark, N.J. kindly performed the assays for this experiment.

Figure 20: Mean serum prolactin levels of ergonovine- and oil-treated dams on day 4 and day 0 postpartum. SEMs are shown.

Figure 21: Mean serum glucocorticoid levels of ergonovine- and oil-treated dams on day 4 and day 10 postpartum. SEMs are shown.


▨ OIL
▧ ERGO
PROLACTIN CONCENTRATIONS (ng/ml)
300
200
100
50
DAY 4
DAY 10


▨ OIL
▧ ERGO
GLUCOCORTICOID CONCENTRATIONS (µg/100ml)
30
20
10
DAY 4
DAY 10

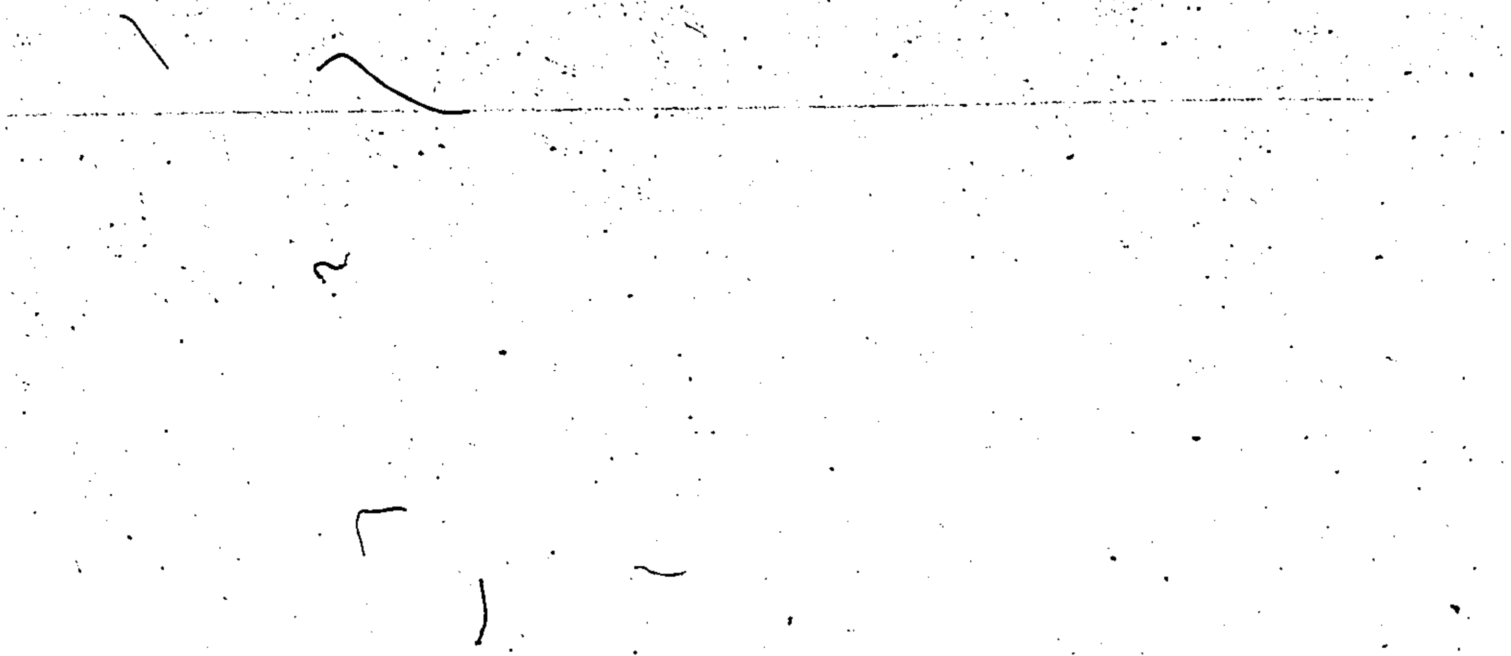
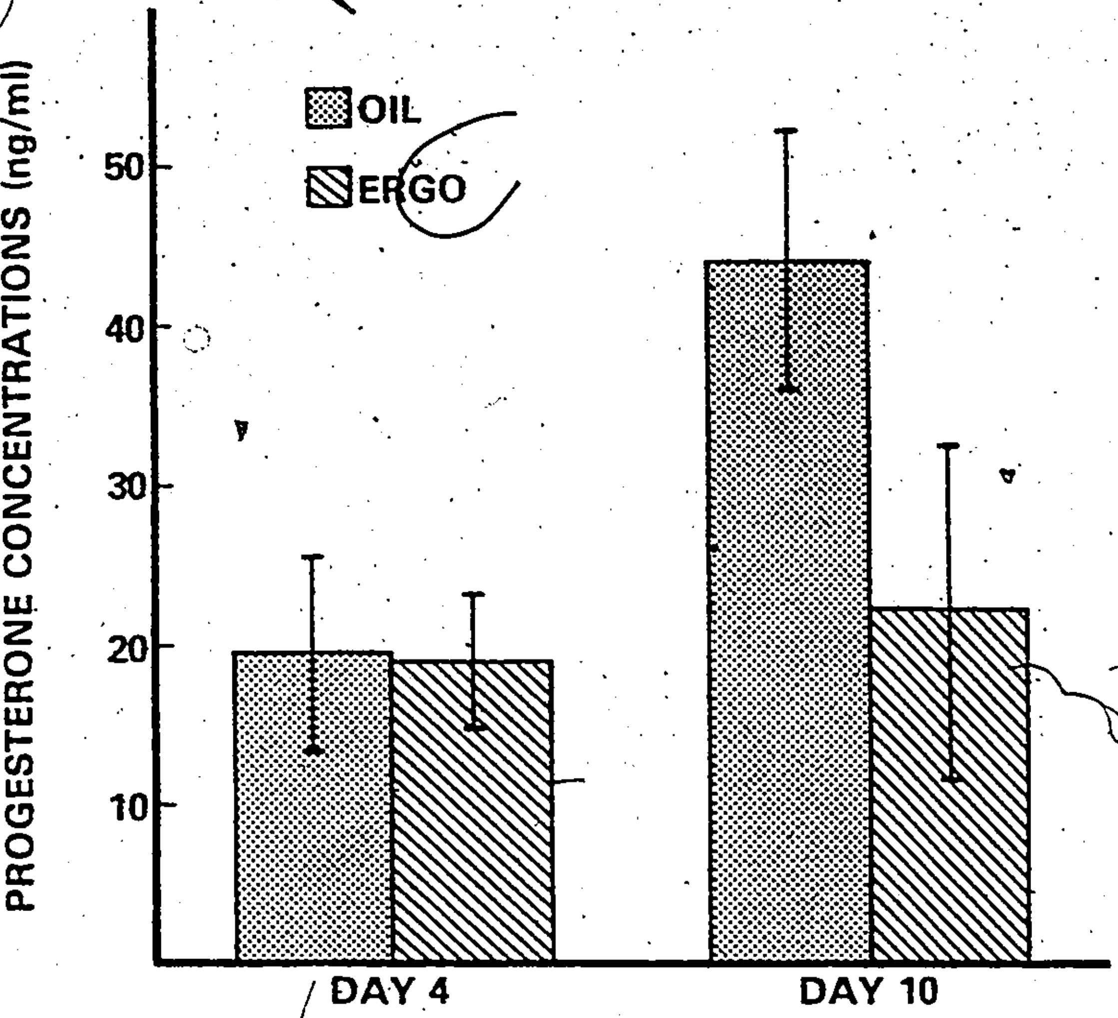


Figure 22: Mean serum progesterone levels of ergonovine- and oil-treated dams on day 4 and day 10 postpartum. SEMs are shown.


PROGESTERONE CONCENTRATIONS (ng/ml)
50
40
30
20
10
OIL
ERGO
DAY 4
DAY 10

and Ergo groups showed that progesterone levels of ergo-treated dams were lower than oil-treated dams on day 10 ($t = 1.70$, $p = 0.05$).

Ergonovine treated groups showed a statistically significant increase in serum glucocorticoid level compared with that of their oil-injected controls (Treatment Effect $F = 30.36$, $p < .001$). Neither the Days Effect nor the Day x Treatment interaction were statistically significant ($F = 1.44$, $p > .05$, $F = 0.70$, $p > .05$, respectively).

Discussion

Ergonovine maleate administration was effective in suppressing serum levels of prolactin on Day 4 and Day 10 and was also effective in reducing serum progesterone levels on Day 10 but not Day 4 postpartum. These data are similar to those reported by Tomogane, Ota and Yokayama (1975) who found that progesterone levels were reduced on Day 8 following ergocryptine administration although they made no earlier determinations of progesterone levels after prolactin suppression. The increase in serum progesterone levels found in this experiment between days 4 and 10 postpartum in the oil-injected group is in agreement with the data of Tomogane, Ota and Yokayama (1969) and Grota and Eik-Nes (1971) who suggested that progesterone levels peak between days 4 and 8.

Control animals had serum corticosterone levels similar to those reported in intact lactating females by Stern, Goldman and Levine (1973). Eergonovine maleate administration resulted in an increase in serum glucocorticoid levels compared with oil-treated controls. It is clear from these results that the effects of prolactin suppression on nest time are not mediated by a reduction in circulating glucocorticoid levels. Rather, these results suggest that prolactin itself plays an important role in maintaining nesting behaviour and the chronically elevated maternal temperature.

In light of these results the data of Experiment 4 may be interpreted as reflecting the ability of very high levels of corticosterone to compensate for the absence of prolactin. It does not necessarily mean, however, that prolactin plays no role in the regulation of corticosterone levels in the lactating rat. It is possible that the normal action of prolactin is obscured in the current experiment. For example, there may be a reciprocal relationship between prolactin and ACTH (the pituitary hormone that stimulates adrenal glucocorticoid secretion) similar to the relationship that appears to exist between prolactin and luteinising hormone (Everett, 1964) Given such a relationship, prolactin suppression would increase ACTH output, which would elevate glucocorticoid levels. Even if in this present experiment prolactin-suppression increased 5 α-adrenal reductase levels, the net effect on glucocorticoid levels would be offset by the concomitant increase in ACTH release. A direct effect of prolactin suppression on glucocorticoid secretion could only be observed if prolactin suppression were accompanied by an ACTH inhibitor. The results of this experiment, however, suggest that it is unlikely that the effects of prolactin-suppression on nest time are mediated by a reduction in glucocorticoid levels.

Summary and Discussion

The results of the experiments described in this Chapter suggest that glucocorticoids are sufficient to reinstate in Adex-Ovex dams the normal daily decline in nest bout duration seen in the intact dam in the first two weeks post partum. Further, it appears that glucocorticoids may act by stimulating maternal metabolism and thereby producing a chronic increase in maternal core temperature which renders the dam vulnerable to the acute thermal effects of huddling with their litter. This endocrine action was verified by making continuous recordings of the core temperature of Sham-Op, Adex-Ovex and Adex-Ovex + Corticosterone dams while they were nesting. As predicted, dams in the Sham-Op and Adex-Ovex + Corticosterone groups showed a rise in core temperature during most of their nest bouts, whereas Adex-Ovex dams showed a temperature rise during very few of their bouts. These data provide strong support for the notion that the effects of adrenalectomy-ovariectomy on nest time are, in fact, thermally mediated.

While glucocorticoids appear to play an important role in inducing the hypermetabolism of lactation throughout the first fourteen days postpartum the effects of prolactin-suppression are limited ~~to~~ the second week postpartum. No evidence was found to support the suggestion that the effects of prolactin suppression on nest time were mediated by a reduction in glucocorticoid levels. Rather, it appears that prolactin itself has a direct role in elevating maternal core temperature, thereby, decreasing nest time. Extremely high levels of corticosterone, however, did compensate for the absence of prolactin.

The pups' role in maintaining the normal pattern of nesting behaviour appears to be twofold. First, stimuli from the pups are necessary to elicit the hormonal state typical of the lactating female rat. In turn, these hormones induce the hypermetabolism of lactation and thereby chronically increase maternal heat load, thus making females vulnerable to the acute thermal effects of huddling with their litters. It is the acute rise in temperature that the dam experiences during a nest bout which is associated with short ~~nest~~ bouts and presumably stimulates the dam to terminate the nest bout.

Second, as noted earlier, pup temperature is an important factor in determining nest bout duration and pup temperature is, in part, dependent on the pups' food intake. Milk production by the dam, as well as increased food intake, are also dependent on pup suckling stimuli (Fleming, 1976b, Ota & Yokayama, 1967a,b; Tomogane, Ota, Unne and Yokayama, 1976). In addition, pup temperature partly depends on the delivery of warm milk to the young. Since the pups stimulate milk delivery in their dams, the young indirectly maintain their own thermal state, and thereby contribute to the limitation of nest bout duration. It is evident, therefore, that the pups are intimately, if not directly involved in the control of the duration of mother-litter contact.

Much of this discussion is based on the assumption that the various hormonal effects on nest time described above are thermally mediated. In the experiments described above, the thermal load that renders mothers vulnerable to the acute thermal consequence of nesting was reinstated in hormone-disrupted dams indirectly, by means of hormone replacement. Direct reinstatement of the thermal load of dams following either Adex-Ovex or prolactin-suppression should effect the same vulnerability to an acute maternal temperature rise during nesting that limits nest bout duration. This prediction is tested in the second part of this dissertation.

Chapter II

Thermal mediation of hormonal effects on nest bout duration

Experiment 6

The effects of increasing the ambient temperature on the nesting behaviour of adex-ovex dams

Adrenalectomy-ovariectomy (Adex-Ovex) produces a chronically depressed maternal core temperature and chronically elevated nest bout durations, thereby preventing the normal decline in daily nesting time in mother rats. Glucocorticoid replacement (see Experiment 1) restored normal nesting behaviour in Adex-Ovex dams and reinstated in these animals the normally elevated temperature characteristic of intact dams (Experiment 2). If the effects of glucocorticoid replacement on the nest time of lactating dams is actually mediated by thermal factors, then one should be able to restore normal nesting times in Adex-Ovex females by increasing their heat load such that they are once more vulnerable to the further acute thermal effects of huddling with their young. If heat replacement did not result in a decline in nest time in Adex-Ovex mothers, then one would have to conclude that the glucocorticoids had their effects via some mechanism other than by increasing core temperature.

Leon, Croskerry & Smith (1978) found that intact dams maintained in a high ambient temperature had shorter nest bouts, and a greater rate of core temperature rise during the nest bouts than dams kept at 22°C. Presumably, increasing the ambient temperature increased the heat load of the females by decreasing the thermal gradient between the dam and her environment, thereby increasing the difficulty of heat loss (Swan 1974). It seemed reasonable, therefore, to suppose that increasing the heat load of Adex-Ovex females by increasing the ambient temperature would render them vulnerable to the thermal effects of huddling with their young and would, thereby, reinstate the normal pattern of nesting behaviour in these females. The nesting times of Adex-Ovex and Sham-Op dams maintained at either 22°C or 25°C were, therefore, compared.

Method

The procedures used in this experiment were exactly the same as those described in Experiment 1, Study 1 except that after the operation the 8 Sham-operated and 8 adrenalectomised-ovariectomised females and their litters were ~~placed~~ in a chamber that was maintained at 25°C.

The results of this experiment were compared with those obtained in Experiment 1 for Sham-Op and Adex-Ovex groups kept at 22°C in a 3-way Analysis of Variance with one related measure. The Geisser-Greenhouse Conservative F test was used to assess the significance levels of the 'F' ratios of the related measures.

Results

The main results of this experiment are presented in Figures 23 and 24. Table IV shows the 'F' ratios and their associated significance levels for each factor, for each dimension.

Nest Time It can be seen from Figure 15 that increasing the ambient

Figure 23: Mean daily nest time of Adex-Ovex and Sham-Op dams maintained at 22°C and Adex-Ovex and Sham-Op dams maintained at 25°C. SEMs are shown.

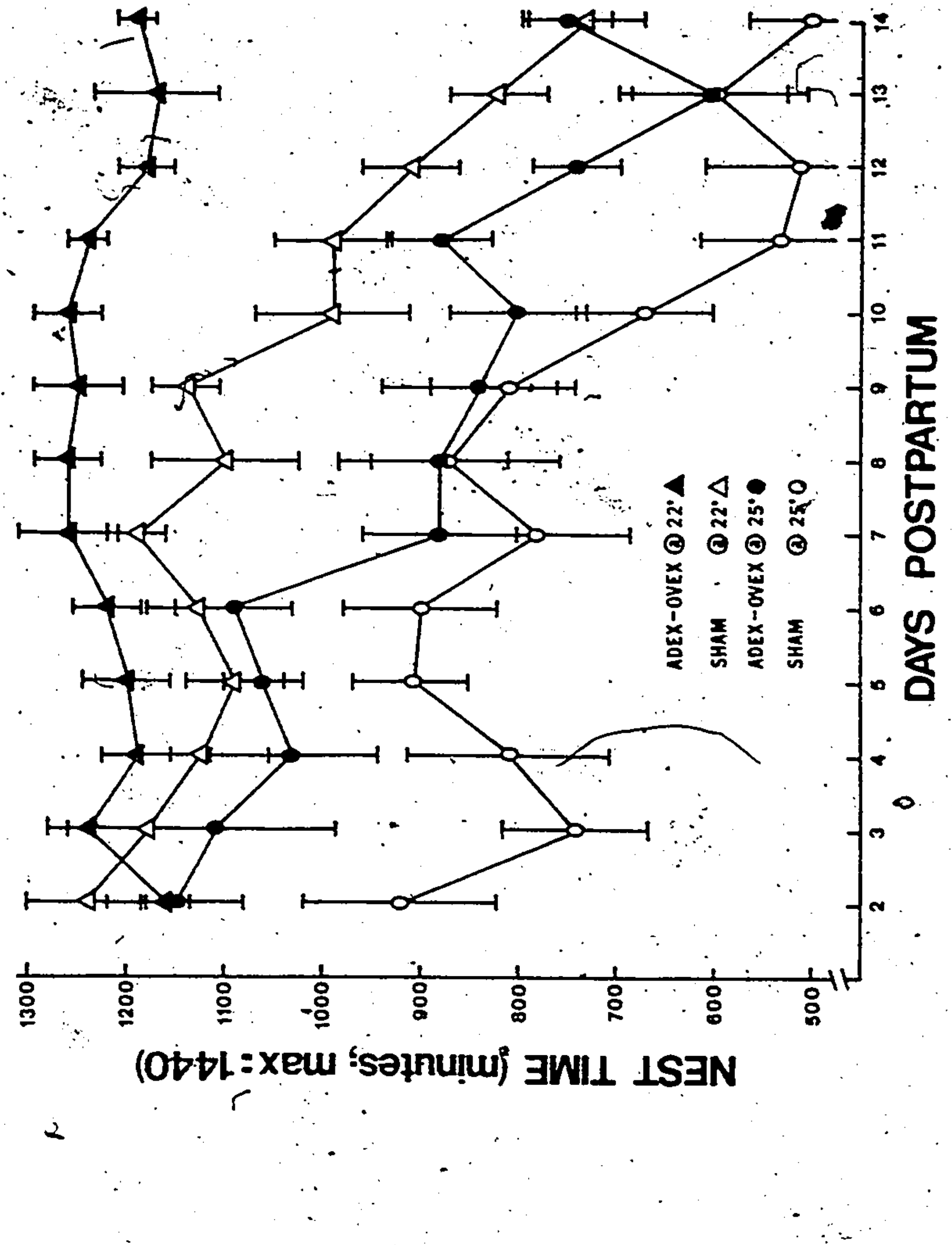

NEST TIME (minutes, max = 1440)
1300
1200
1100
1000
900
800
700
600
500
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
ADEX-OVEX @ 22° ▲
SHAM @ 22° △
ADEX-OVEX @ 25° ●
SHAM @ 25° ○

Figure 24: Mean daily nest bout frequency of Adex-Ovex and Sham-Op dams maintained at 22°C and Adex-Ovex and Sham-Op dams maintained at 25°C. SEMs are shown.

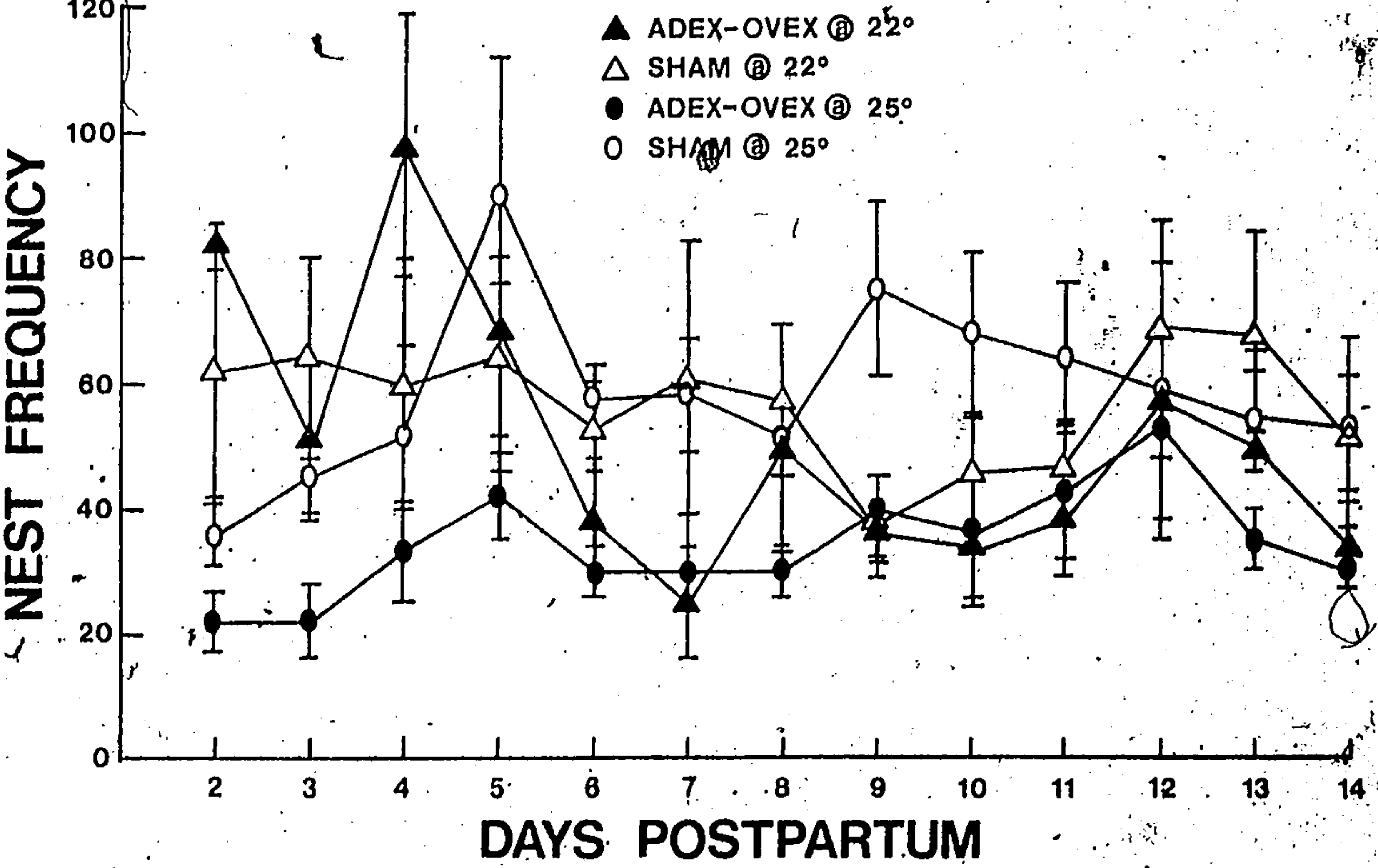

▲ ADEX-OVEX @ 22°
△ SHAM @ 22°
● ADEX-OVEX @ 25°
○ SHAM @ 25°
NEST FREQUENCY
0
20
40
60
80
100
120
DAYS POSTPARTUM
2
3
4
5
6
7
8
9
10
11
12
13
14

Table IV

'F' Ratios and p Values From the

Statistical Analysis of Experiment 6

Effect	Treatment		Temperature		Treatment x Temperature		Days		Days x Treatment		Days x Temperature		Days x Treatment x Temperature	
Dimension	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Nest Time	18.78	<.001	70.89	<.001	0.19	ns	29.82	<.001	3.14*	ns	4.21	<.05	7.63	<.01
Nest Frequency	7.48	<.01	1.83	ns	3.24*	ns	0.32	ns	2.94*	ns	4.00*	ns	1.79	ns

* Indicates 'F' ratios that were nonsignificant using the Geisser-Greenhouse Conservative 'F' test but would have been significant at $p < .05$ if a less conservative test were used.

temperature had a clear effect on the nesting times of both Sham-Operated and Adex-Ovex females (Temperature Effect $F = 70.89$, $p < .001$). That the effect of temperature on nest time was similar in both the Sham-Op and Adex-Ovex groups is indicated by the absence of a signficant Treatment x Temperature interaction ($F = 0.19$, $p > .05$).

Nest Frequency The mean daily nest frequency for the Sham-Op and Adex-Ovex groups at 22°C and 25°C are shown in Figure 16. The ANOVA revealed a significant effect of treatment on nest frequency ($F = 7.48$, $p < .01$), probably reflecting the tendency for the Sham-Op at 25°C group to show an increase in frequency and the Adex-Ovex group at 25°C to show a decrease in nest bout frequency.

Discussion

The results of this experiment clearly show that increasing the ambient temperature alone is sufficient to reinstate in Adex-Ovex females the decline in nest bout duration seen in intact dams. As expected, the increase in ambient temperature also caused a further decrease in nest time in Sham-Op animals. These data strongly support the proposal that thermal factors play a role in limiting the duration of mother-litter contact, and more particularly, that the effects of adrenalectomy-ovariectomy on nest time are thermally mediated.

There was a tendency for increased ambient temperature to increase nest frequency in the Sham-Op group but decrease it in the Adex-Ovex group. Specifically, the nest frequency of the Adex-Ovex group at 25°C had lower frequencies in the first five days of the experiment than any of the other three groups. The Sham-Op group at 25°C, however, had higher frequencies between days 7-9 The low nest frequency in the Adex-Ovex group at 25°C probably did not contribute to the decline in nest time seen in this group, because there was no further decline throughout the rest of the experiment. The changes in nest time observed for all groups over the course of the experiment, resulted, therefore, from a change in the duration of individual nest bouts rather than in their frequency.

The results of this experiment show that increasing ambient temperature is sufficient to restore the normal decline in daily nest time in Adex-Ovex dams. Thus, these results are consistent with the notion that the loss of glucocorticoids in Adex-Ovex dams had its effects on nest time by making the Adex-Ovex dam less vulnerable to the thermal effects of huddling with her litter. These data again support the idea that nest bout duration can be limited by thermal factors.

Experiment 7

The effects of increasing the ambient temperature on chronic maternal and pup temperature in Adex-Ovex dams and the consequences for changes in maternal temperature during a nest bout.

The results of the previous experiments provide further support for the notion that the thermal components of mother-litter interaction are essential for the elicitation of the normal decline in daily nest time. In Experiment 3 it was demonstrated that exogenous glucocorticoid administration made Adex-Ovex dams vulnerable to the acute thermal effects of huddling with their pups and these dams showed a further rise in core temperature during nest bouts. In Experiment 6, it was demonstrated that heat replacement alone was sufficient to induce the normal decline in daily nesting time in Adex-Ovex dams. Presumably, the high ambient temperature decreased the rate of heat dissipation from the dams and their pups, and resulted in a chronically increased maternal core and ventral temperature as well as an increase in pup temperature. These factors should combine to yield shorter nest bouts by subjecting Adex-Ovex dams to the acute thermal consequences of huddling. Adex-Ovex dams in an ambient temperature of 25°C should then show the acute rise in core temperature typical of hormone replaced and normal dams during a nest bout.

In Study 7a I determined the chronic effects of a high ambient temperature on the core and ventral temperature and pup temperature of Adex-Ovex dams by recording these parameters once each day either in

22°C or 25°C ambience. I then determined, in Study 7b, the efficacy of increased ambient temperature in producing an acute rise in temperature in Adex-Ovex dams during a nest bout by making continuous recordings of maternal temperature in Adex-Ovex dams maintained at 25°C.

Method

Study 7a

The dams and their litters which had served as subjects in Experiment 6 were used in the present experiment. Maternal ventral and core temperature and pup skin temperature were measured daily as described in Experiment 2. The results of this experiment were compared with those obtained in Experiment 2 for Sham-Op and Adex-Ovex groups kept at 22°C in a 3-way Analysis of Variance with one related measure.

Study 7b

Animals in the Adex-Ovex at 25°C were operated, implanted and maintained using the same procedures as those described for the Adex-Ovex group in Experiment 3, with the exception that the 6 dams and their litters that were used as subjects in this experiment were maintained at 25°C from Day 2 postpartum. The percentage of nest bouts during which the dam experienced a rise in core temperature the change in core temperature over the course of these nest bouts and nest bout duration were transcribed and compared with the same measures obtained for Adex-Ovex and Sham-Op dams that performed as subjects in Experiment 3.

[redacted]

Results

Study 7a

The main results of this study are presented in Figures 25 - 27. Table V shows the 'F' ratios and their associated significance levels for each factor for each dimension.

Dam Core Temperature Overall, the Sham-Op groups showed a higher core temperature than the Adex-Ovex groups (Treatment Effect $F = 33.57$, $p < .001$). None of the other factors yielded significant results. Increasing the ambient temperature produced a rise in core temperature in the Adex-Ovex females and a small decrease in the core temperature of the Sham-Op group (see Figure 25), probably accounting for the nonsignificant temperature effect.

Dam Ventral Temperature Increasing the ambient temperature had a highly significant effect on maternal ventral temperature ($F = 19.43$, $p < .001$). Although the effect of treatment on this parameter was not significant ($F = 3.79$, $p > .05$) there does appear to be a tendency for the ventral temperature for the Sham-Op dams to be higher than the Adex-Ovex dams (see Figure 26).

Pup Skin Temperature Figure 27 shows the mean daily pup skin temperature for all four groups included in the analysis. Both treatment and ambient temperature had a significant effect on pup temperature ($F = 6.76$, $p < .05$; $F = 31.3$, $p < .001$, respectively). There were also significant Treatment x Temperature and Days x Temperature interactions ($F = 7.35$, $p < .05$; F 9.55, $p < .01$ respectively). The significant Treatment x Temperature interaction probably reflects the tendency for temperature of pups of Adex-Ovex

Table V

'F' ratios and Associated p values from the Statistical Analysis of Experiment 7a.

Effect	Treatment		Temperature		Treatment x Temperature		Days		Days x Treatment		Days x Temperature		Days x treatment x temperature	
Dimension	F	P	F	P	F	P	F	P	F	P	F	P	F	P
Dam Core Temperature	33.57	<.001	0.43	ns	3.29	ns	4.00*	ns	1.86	ns	2.71	ns	0.86	ns
Dam Ventral Temperature	3.79	ns	19.43	<.001	0.004	ns	3.57*	ns	.01	ns	.01	ns	.003	ns
Pup Skin Temperature	6.76	<.05	31.30	<.001	7.35	<.05	3.86*	ns	2.01	ns	9.55	<.01	0.71	ns

*Indicates 'F' ratios that were nonsignificant using the Geisser-Greenhouse Conservative 'F' test but that would have been significant at $p < .05$ if a less conservative test were used.

Figure 25: Mean daily core temperature of Adex-Ovex and Sham-Op dams maintained at 22°C and Adex-Ovex and Sham-Op dams maintained at 25°C. SEMs are shown.

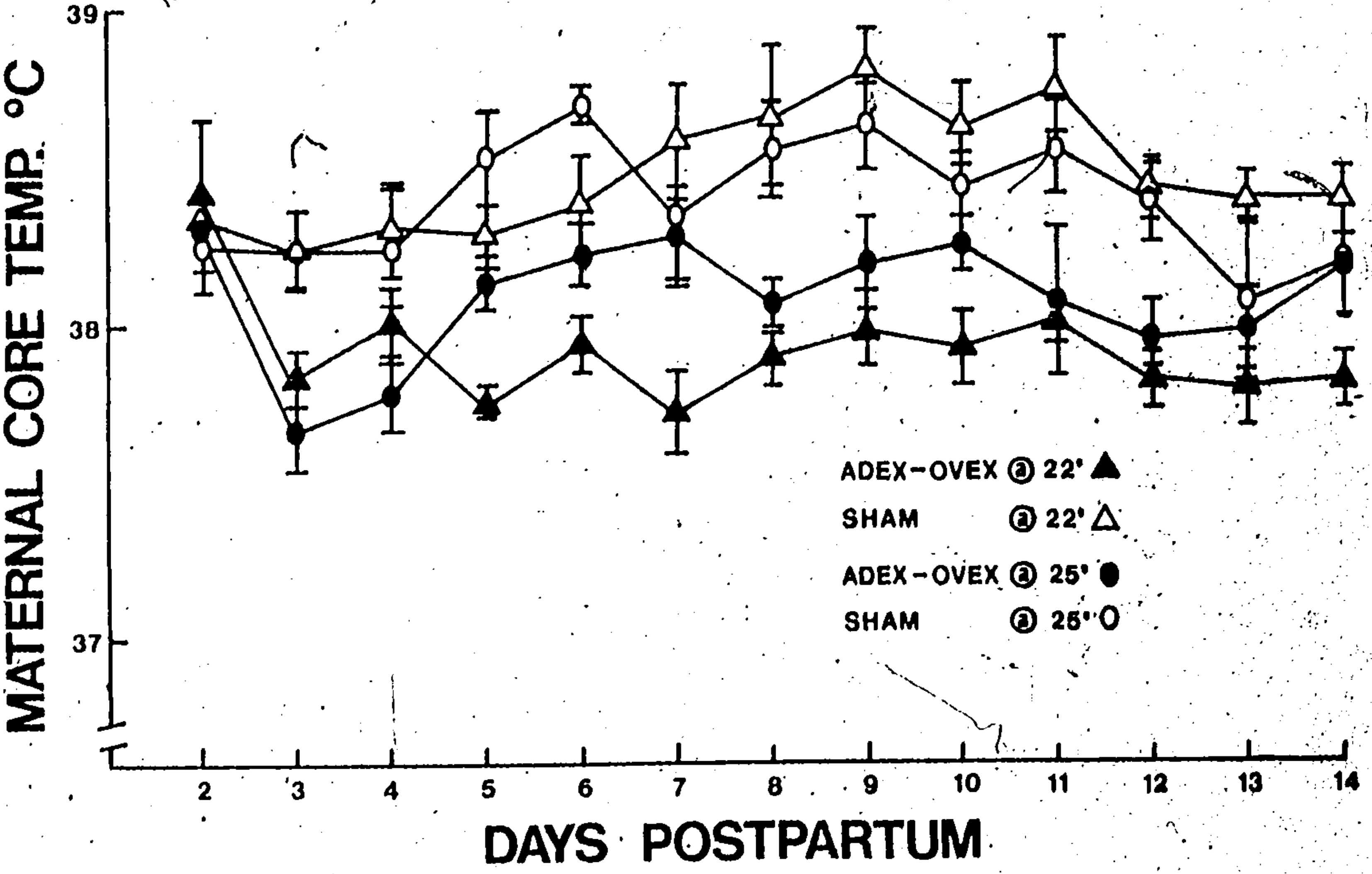

MATERNAL CORE TEMP. °C
39
38
37
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
ADEX-OVEX @ 22' ▲
SHAM @ 22' △
ADEX-OVEX @ 25' ●
SHAM @ 25' ○

Figure 26: Mean daily ventral temperature of Adex-Ovex and Sham-Op dams maintained at 22°C and Adex-Ovex and Sham-Op dams maintained at 25°C. SEMs are shown.

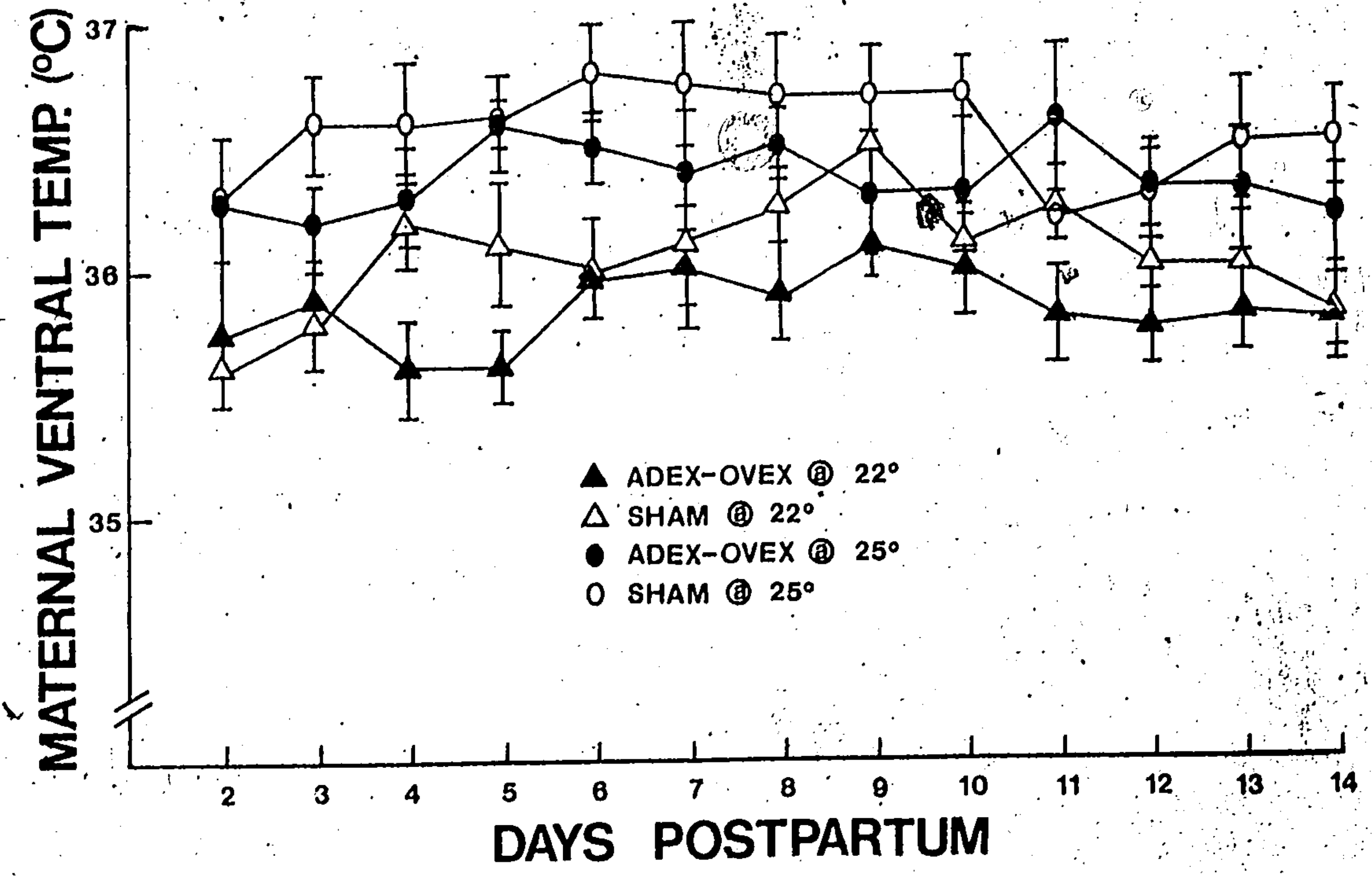
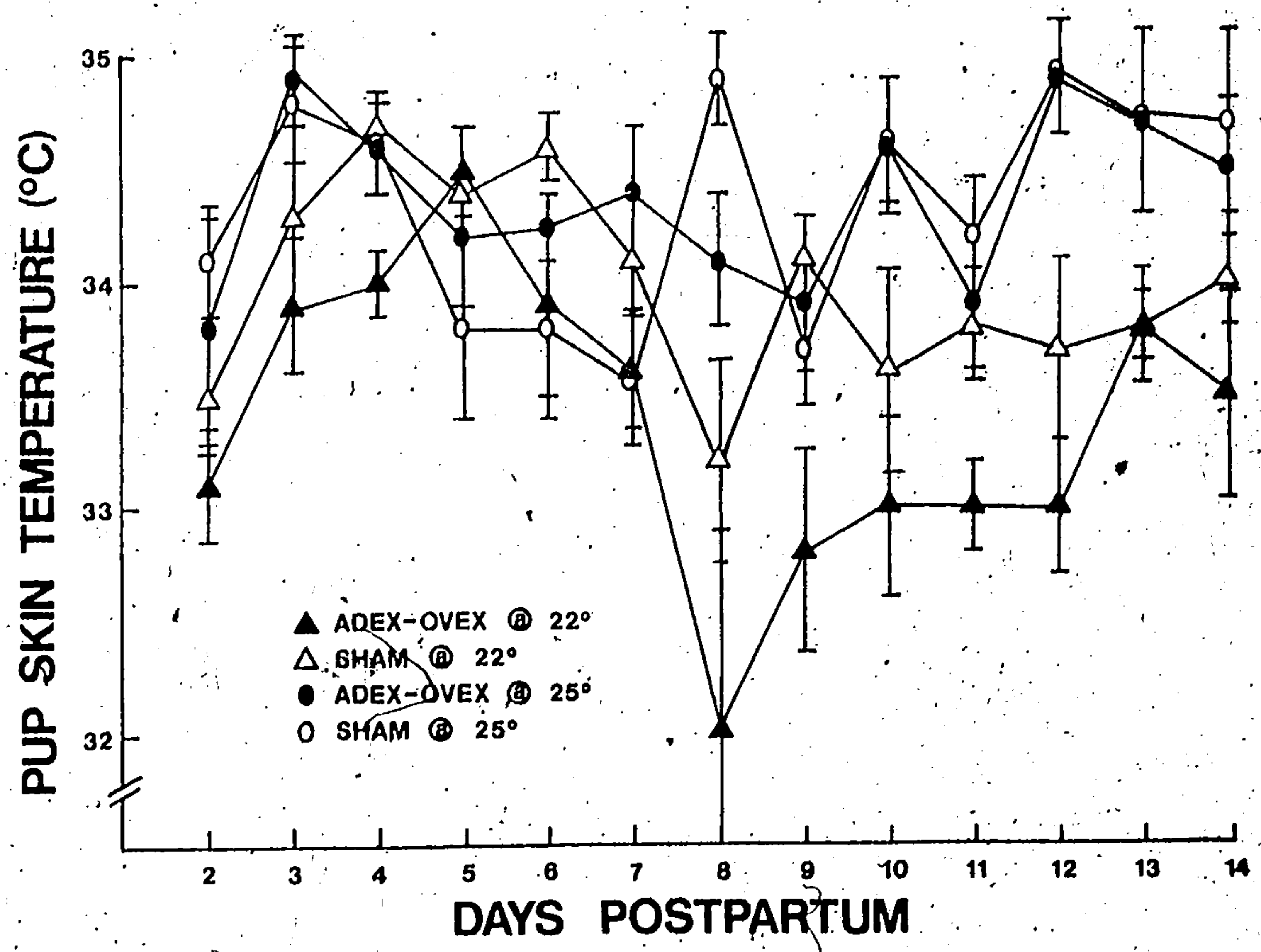

MATERNAL VENTRAL TEMP (°C)
37
36
35
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
▲ ADEX-OVEX @ 22°
△ SHAM @ 22°
● ADEX-OVEX @ 25°
○ SHAM @ 25°

Figure 27: Mean daily pup skin temperature of Adex-Ovex and Sham-Op dams maintained at 22°C and Adex-Ovex and Sham-Op dams maintained at 25°C. SEMs are shown.


PUP SKIN TEMPERATURE (°C)
35
34
33
32
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM
▲ ADEX-OVEX @ 22°
△ SHAM @ 22°
● ADEX-OVEX @ 25°
○ SHAM @ 25°

mothers at 25°C to show a greater increase over that of Adex-Ovex mothers at 22°C than that of Sham-Ops at 25°C over Sham-Op pups at 22°C. Pups of Sham-Op mothers at 25°C showed particularly low temperatures on Days 5 - 7 which probably contributed to the significant Days x Temperature interaction.

Study 7b

Figure 28 shows a sample recording of the change in core temperature during a nest bout of an Adex-Ovex dam at 25°C compared with that of Sham-Op and Adex-Ovex dams maintained at 22°C. Figure 29 shows the mean bout duration for the Adex-Ovex at 25°C group compared with that for the Sham-Op and Adex-Ovex at 22°C groups. Statistical analysis showed an overall significant effect of treatment. Post hoc analyses showed that the nest bouts of Adex-Ovex females at 22°C were significantly longer than those of the Sham-Op and Adex-Ovex at 25°C. The differences between the latter groups were nonsignificant.

The mean percentage of bouts during which these females showed an increase in core temperature compared with Sham-Op and Adex-Ovex dams maintained at 22°C is shown in Figure 30. It is clear from the statistical analysis that there was a highly significant effect of treatment on this dimension ($F = 26.37$, $p < 0.01$). Figure 31 shows the mean rise in temperature for the three groups. The results of the statistical analysis show no overall effect of treatment ($F = 1.5$, $p > 0.05$) although it is clear that there is a trend for the rise in temperature to be greater for Sham-Ops than Adex-Ovex at 25°C and for both these groups than Adex-Ovex at 22°C.

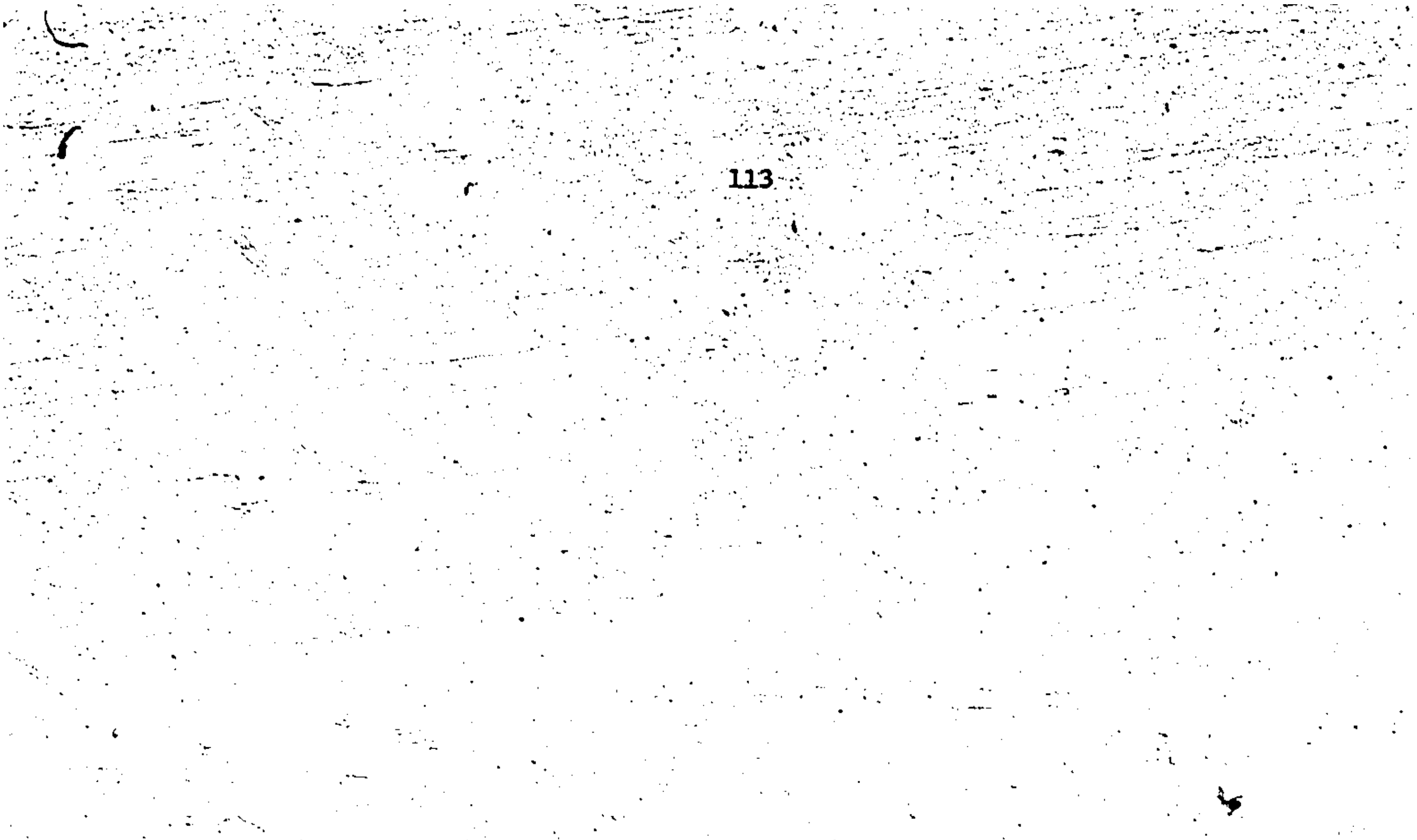


Figure 28: Sample trace of maternal core temperature during a nest bout from a Sham-Op dam, an Adex-Ovex dam maintained at 22°C and an Adex-Ovex dam maintained at 25°C.

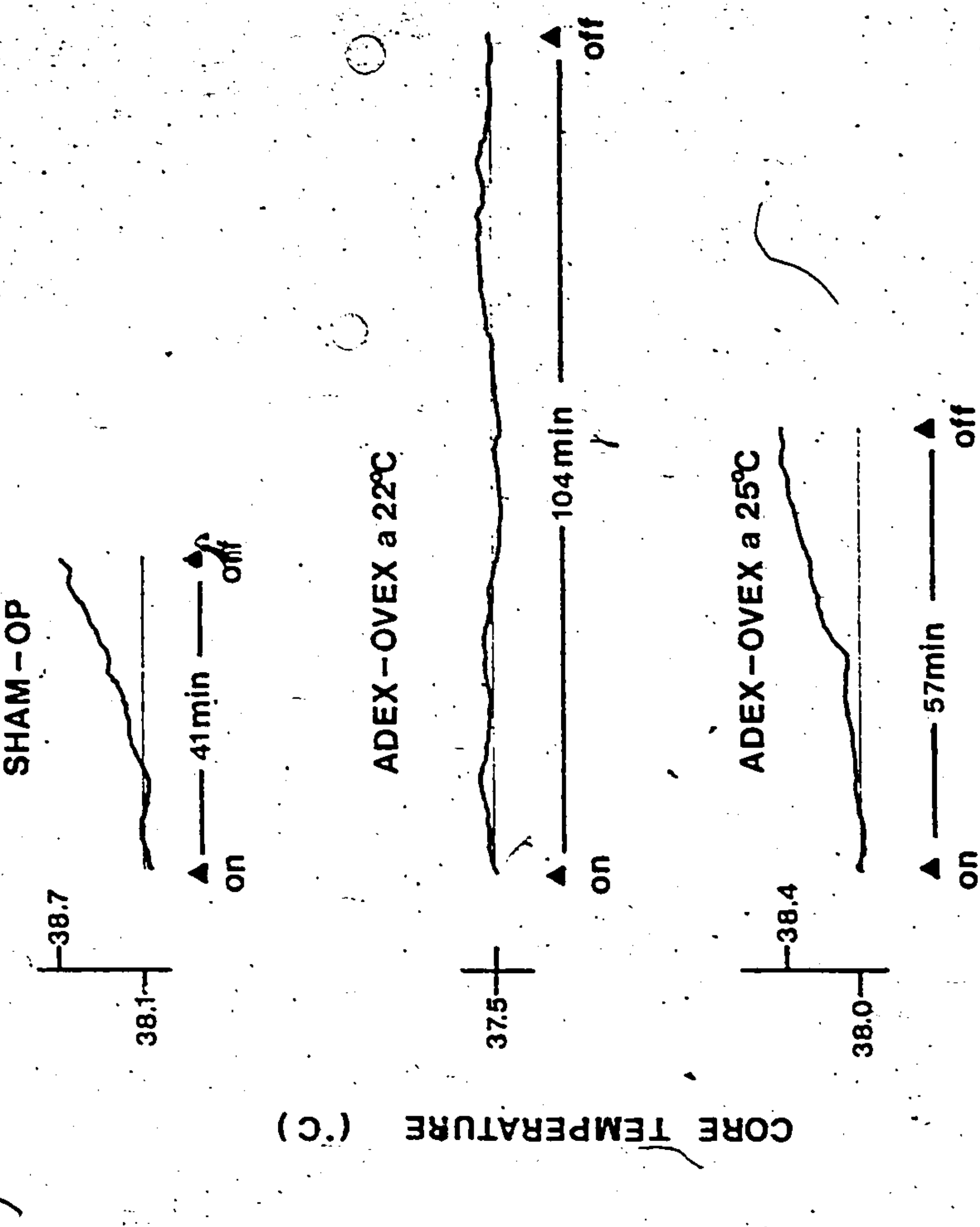
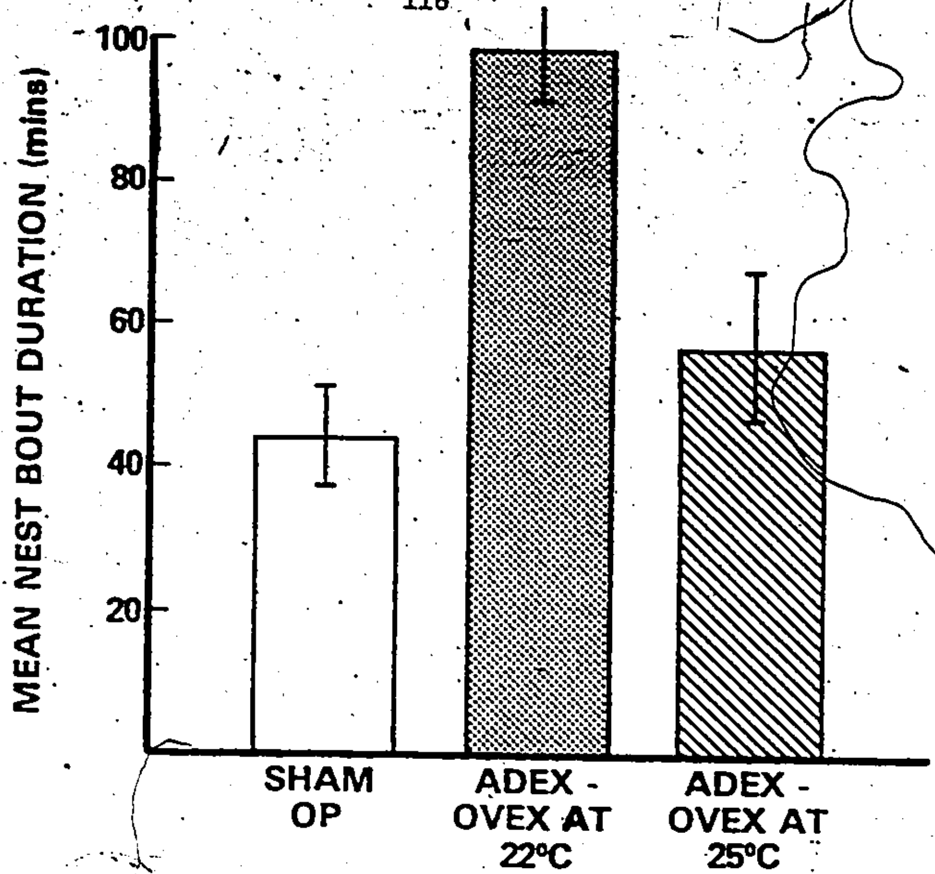

SHAM-OP
38.7
38.1
on
41 min
off
ADEX-OVEX a 22°C
37.5
on
104 min
off
ADEX-OVEX a 25°C
38.4
38.0
on
57 min
off
CORE TEMPERATURE (°C)

Figure 29: Mean bout duration ± SEM of Day 10 Sham-Op and Adex-Ovex dams maintained at 22°C and Day 10 Adex-Ovex dams maintained at 25°C.

Figure 30: Mean percentage of nest bouts during which Day 10 Sham-Op and Adex-Ovex dams maintained at 22°C and Day 10 Adex-Ovex dams maintained at 25°C showed an increase in core temperature. SEMs are shown.


MEAN NEST BOUT DURATION (mins)
100
80
60
40
20
SHAM OP
ADEX - OVEX AT 22°C
ADEX - OVEX AT 25°C

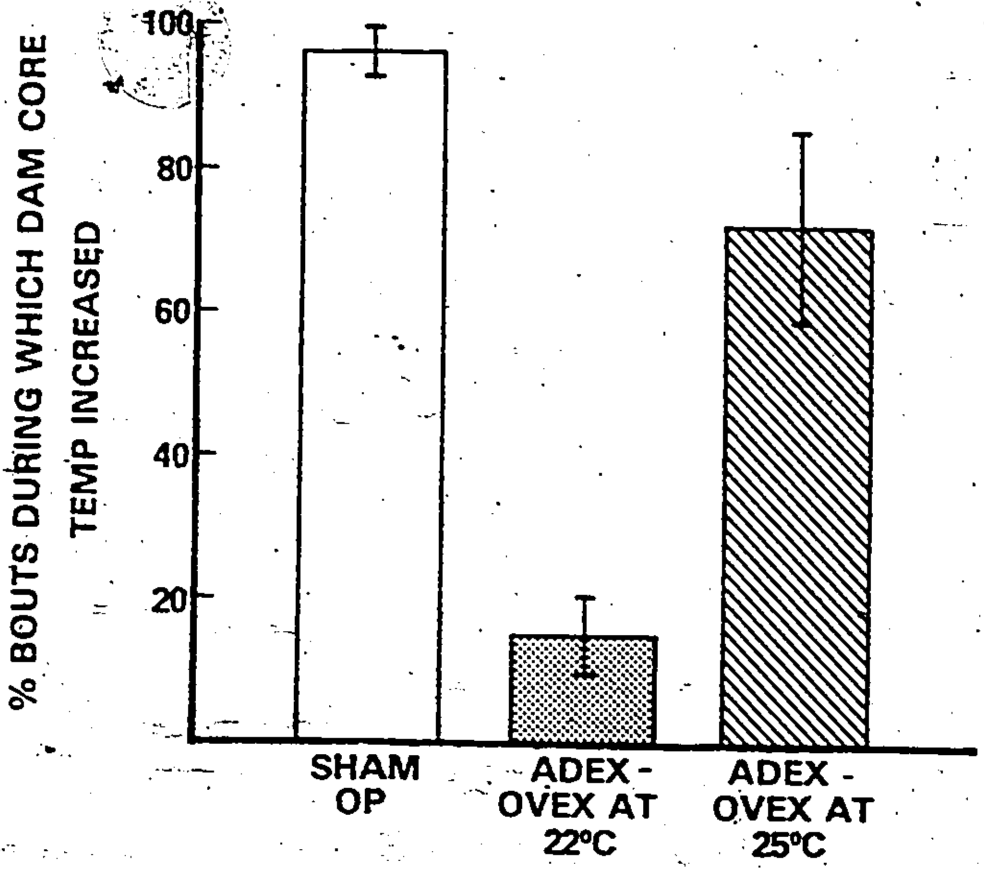
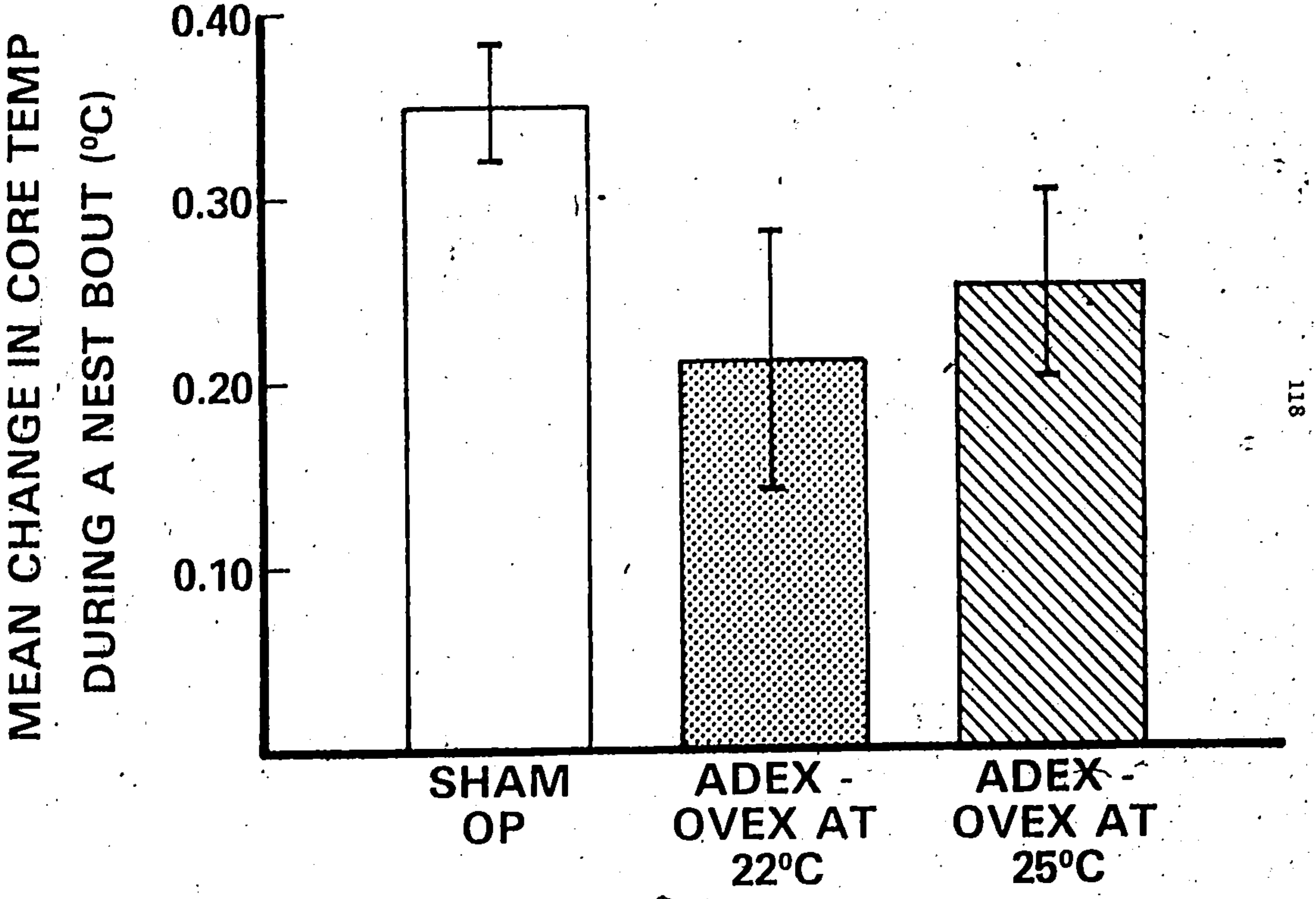

% BOUTS DURING WHICH DAM CORE TEMP INCREASED
100
80
60
40
20
SHAM OP
ADEX - OVEX AT 22°C
ADEX - OVEX AT 25°C

Figure 31: Mean increase in core temperature, on those bouts where an increase occurred, of Day 10 Sham-Op and Adex-Ovex dams maintained at 22°C and Day 10 Adex-Ovex dams maintained at 25°C. SEMs are shown.


MEAN CHANGE IN CORE TEMP DURING A NEST BOUT (°C)
0.40
0.30
0.20
0.10
SHAM OP
ADEX - OVEX AT 22°C
ADEX - OVEX AT 25°C

Discussion

It is clear from the results of Study 7a that increasing the ambient temperature increased dam core and ventral temperature as well as pup temperature in Adex-Ovex dams. These data support the notion that increasing ambient temperature increases the heat load of Adex-Ovex dams such that they become more vulnerable to a further acute temperature rise induced by huddling with their pups. The data obtained in this study suggest that increasing ambient temperature achieves this result not only by chronically increasing maternal temperature, but also by increasing pup temperature such that at bout onset the temperature of the entire huddle is increased.

The results of Study 7b demonstrated that the changes in dam and pup temperature induced by increasing the ambient temperature are sufficient to render Adex-Ovex dams vulnerable to the acute thermal effects of huddling with their young. Adex-Ovex dams maintained at 25°C show an acute rise in temperature during the majority of their nest bouts on Day 10 postpartum. These animals also have shorter nest bouts than Adex-Ovex dams maintained at 22°C which show no such acute rise in temperature during nest bouts. ~~The fact~~ that heat replacement alone was sufficient to produce these behavioral effects are consistent with the notion that the success of glucocorticoid replacement therapy in reinstating the normal decline in nesting time in Adex-Ovex dams is due to the chronic elevation of maternal temperature.

Experiment 8

The effects of increasing the ambient temperature in the second week postpartum on the nesting behaviour and temperature of prolactin-suppressed dams.

Prolactin suppressed dams do not show a decline in daily nest time in the second week postpartum and have a low core temperature compared with normal mothers at this time. Prolactin replacement therapy to dams following prolactin-suppression by means of ergonovine maleate injections reinstated the normal pattern of nesting and the normal elevated temperature (Leon, Croskerry & Smith, 1978). Similarly, exogenous corticosterone administration restored the normal pattern of nesting behaviour in prolactin-suppressed dams, and also increased their body temperature (see Experiments 4 and 5). It seems likely that the effects of prolactin suppression on nesting time would be thermally mediated in much the same way as are the effects of adrenal removal. That is, prolactin suppression results in a chronic decrease in maternal core temperature in the second week postpartum that renders the dam invulnerable to the acute thermal effects of huddling with her young. If prolactin-suppression halts the decline in daily nest time by eliminating the normal heat load of mother rats, then replacing the heat load in prolactin-suppressed dams should restore the normal limitation of these bouts by the further, acute temperature rise experienced by intact dams during nesting.

The nesting behaviour of prolactin-suppressed dams, therefore, was recorded when these females were either exposed to a high ambient temperature or to the normal laboratory temperature in the second week postpartum. If, in this instance, heat replacement did not result in the prolactin-suppressed females showing a decline in daily nest time throughout the first 14 days postpartum then one would have to conclude that the effects of prolactin suppression on nest time were not thermally mediated.

The results of Study 7a showed that increasing ambient temperature had its effects on nest time in Adex-Ovex dams by increasing not only maternal temperature but also pup temperature. Maternal and pup temperatures were recorded in this experiment to determine if heat replacement in prolactin-suppressed dams had effects similar to those observed in the ~~previous~~ experiment.

Method

Subjects

Fourteen virgin female Wistar rats weighing 220-250 gms served as subjects in this experiment.

Procedure

Virgin females were mated and removed to individual cages two days prior to the expected date of parturition. On the day of birth (day 0), dams were paired. One member of each pair was assigned to the ergonovine + heat replacement (Ergo + Heat) group and the other to the oil-injected (Control) group. Beginning on Day 1 postpartum the eight animals in the Ergo + Heat group were given 8 mg ergonovine maleate in 0.2cc oil daily at 12 noon. The control group were given 0.2cc oil vehicle at that time. Beginning on Day 6 postpartum dams in the Ergo + Heat group and their litters were placed in an ambient temperature of 25 C. Control animals remained at 22°C. Litters were switched daily between dams in the Control and Ergo + Heat groups and intact colony mothers. Nesting behaviour and temperatures were recorded and subsequently analysed as in Experiment 1. Comparisons were also made between the Ergo + Heat group and the ergonovine-treated group maintained at 22°C described in Experiment 4.

Results

The main results of this experiment are presented in Figures 32 - 35. Table VI shows the results of the statistical comparisons.

Mean daily nest time for each group is shown in Figure 32. The treatment and interaction effects for the Control vs Ergo + Heat comparison were nonsignificant ($F = 0.81$, $p > .05$; $F = 0.60$, $p > .05$, respectively). The days effect was highly significant ($F = 53.05$, $p < .001$).

There were no statistically significant effects of days or treatment x days interaction on nest frequency ($F = 1.84$, $p > .05$; $F = 0.20$, $p > .05$) but there was a significant effect of treatment ($F = 6.24$, $p < .05$).

Maternal core temperature was significantly lower in the Ergo + Heat group when compared with Control dams ($F = 61.5$, $p < .05$) but higher when compared with the Ergo group maintained at 22°C ($F = 25.76$, $p < .01$). Day and Day x Treatment interaction effects were nonsignificant ($F = 0.5$, $p > .05$; $F = 2.17$, $p > .05$).

Table VI

'F' ratios and associated p values from the Statistical Analysis of Experiment 8

Dimension	Nest Time		Nest Frequency		Dam Core Temperature		Pup Skin Temperature	
Effect	F	P	F	P	F	P	F	P
Treatment	2.3	ns	6.24	<.05	5.16	<.05	8.07	<.05
Days	31.71	<.001	1.84	ns	0.5	ns	5.57	<.05
Days x Treatment Interaction	0.63	ns	0.52	ns	2.17	ns	0.92	ns

Figure 32: Mean daily nest time of oil- and ergonovine-treated dams maintained at 22°C and ergonovine-treated dams maintained at 25°C. SEMs are shown.

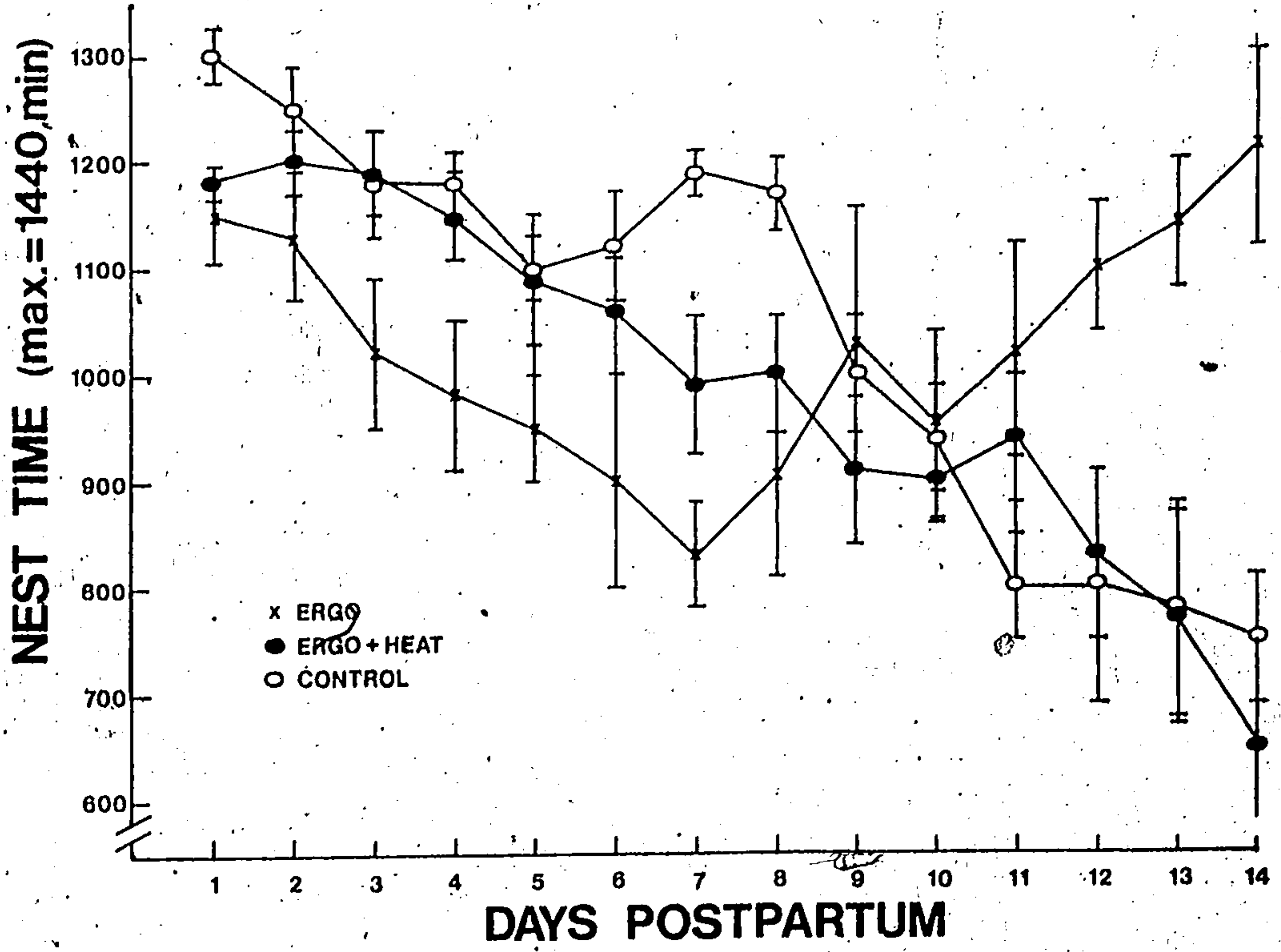

NEST TIME (max.=1440,min)
1300
1200
1100
1000
900
800
700
600
DAYS POSTPARTUM
1
2
3
4
5
6
7
8
9
10
11
12
13
14
x ERGO
● ERGO + HEAT
O CONTROL

Figure 33: Mean daily nest bout frequency of oil- and ergonovine-treated dams maintained at 22°C and ergonovine-treated dams maintained at 25°C. SEMs are shown.

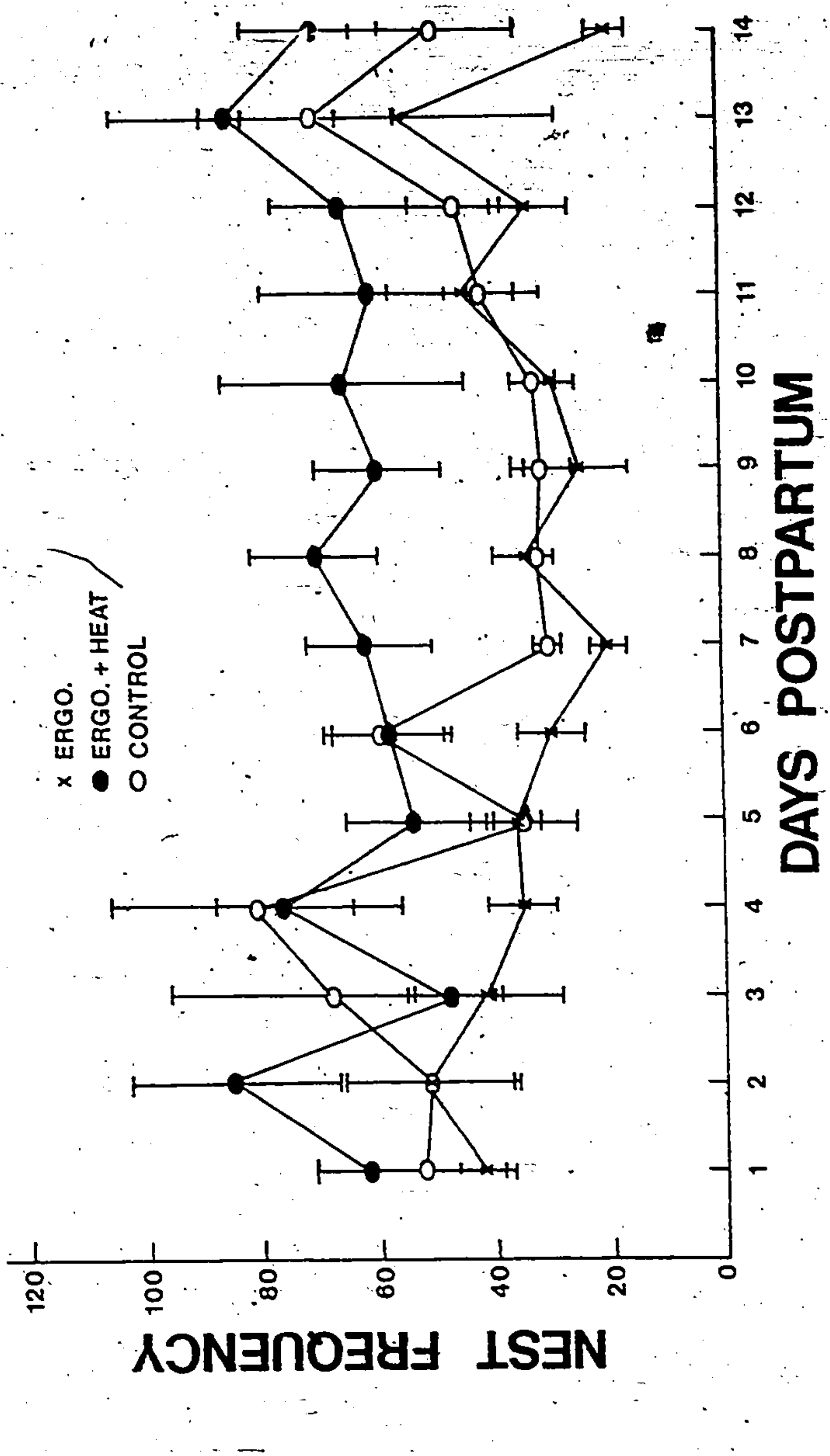
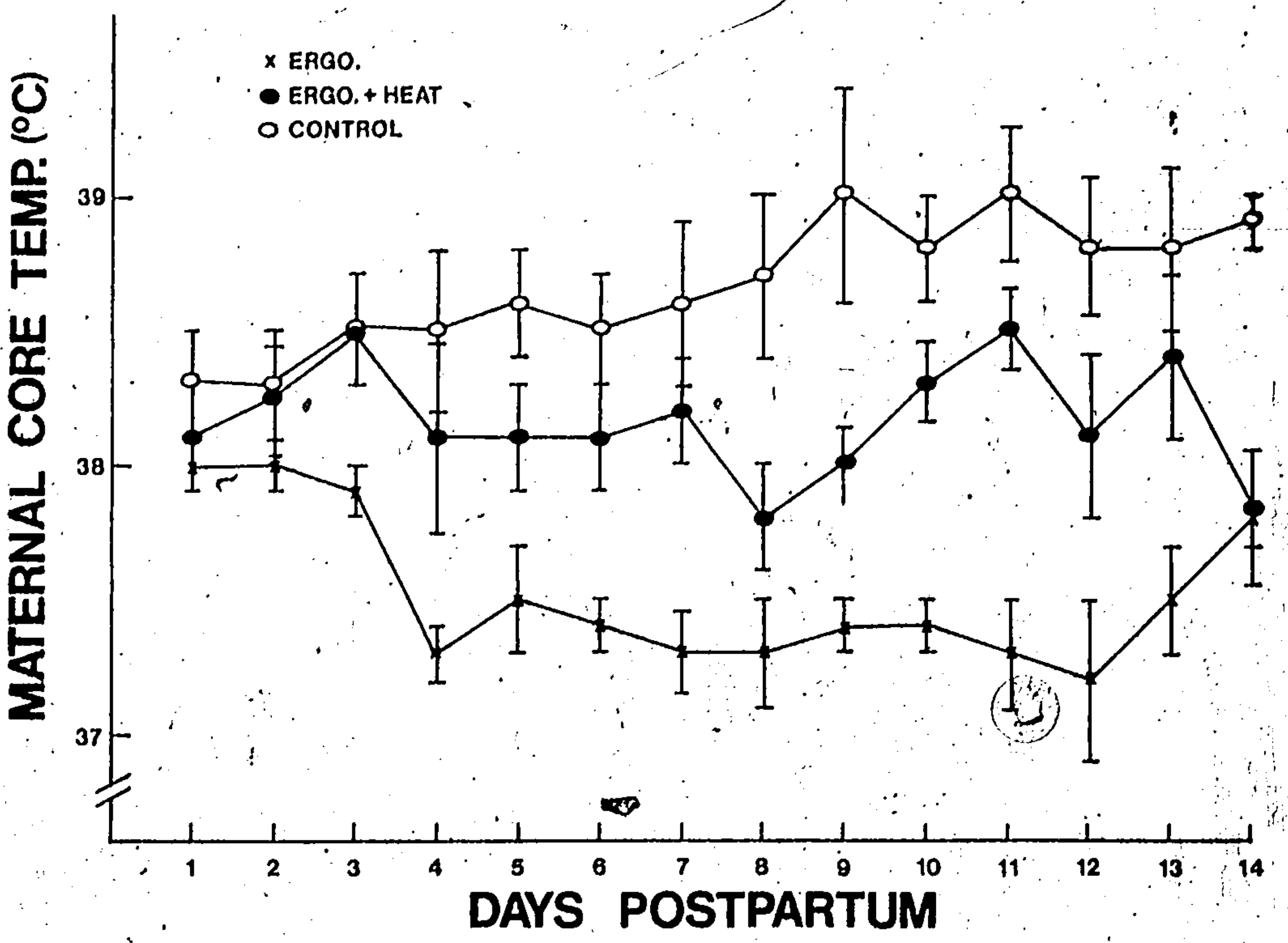

x ERGO.
● ERGO. + HEAT
O CONTROL
NEST FREQUENCY
0
20
40
60
80
100
120
DAYS POSTPARTUM
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 34: Mean daily core temperature of oil- and ergonovine-treated dams maintained at 22°C and ergonovine-treated dams maintained at 25°C. SEMs are shown.


x ERGO.
● ERGO. + HEAT
○ CONTROL
MATERNAL CORE TEMP. (°C)
39
38
37
1
2
3
4
5
6
7
8
9
10
11
12
13
14
DAYS POSTPARTUM

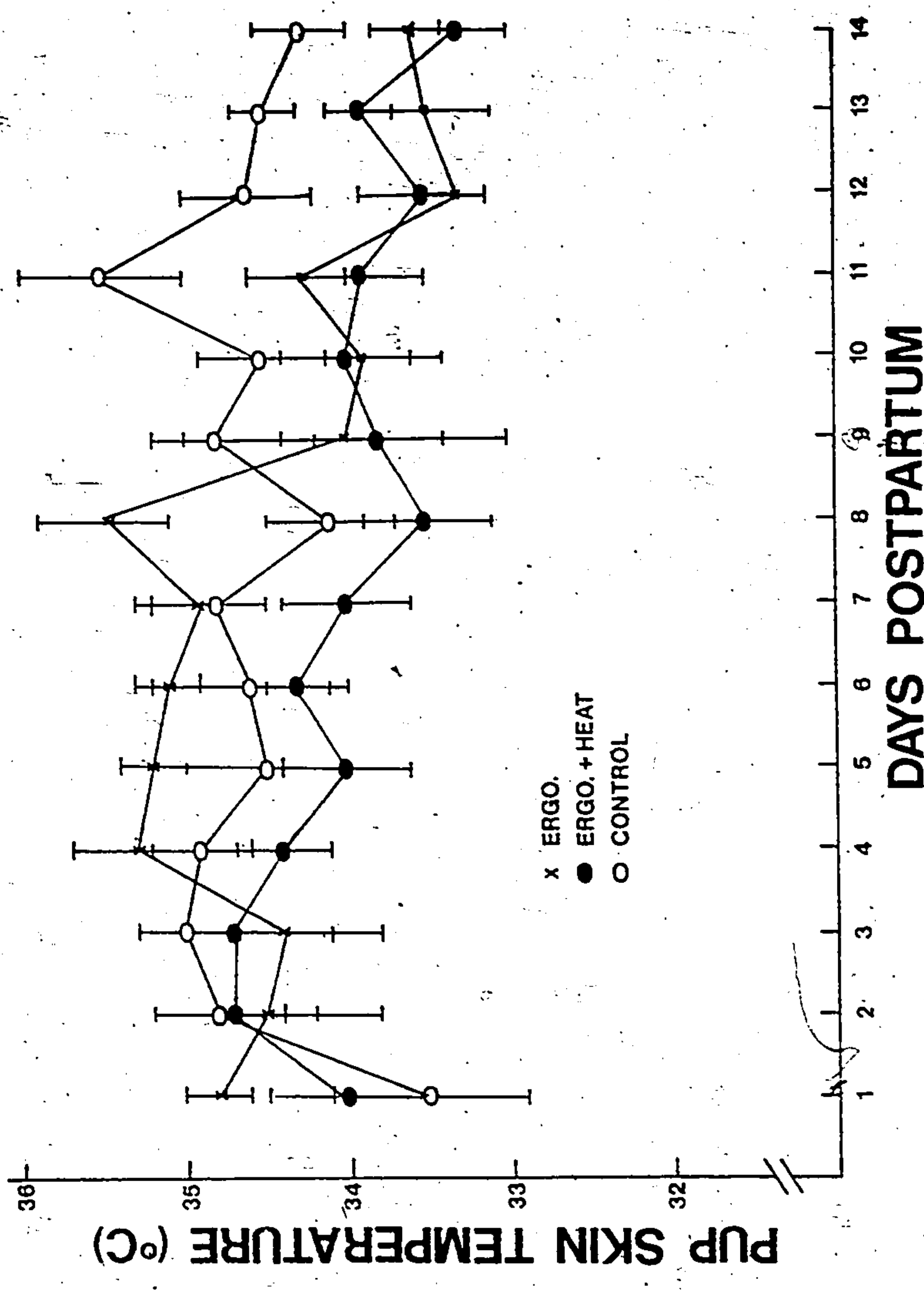
Pup temperature was significantly lower in the Ergo. + Heat group compared to the Control group (F = 8.07, $p < .01$). The days effect was also significant (F = 5.57, $p < .05$). The days x treatment effect was nonsignificant.

Discussion

These results show clearly that raising the ambient temperature is sufficient to reinstate the normal decline in daily nesting time in prolactin-suppressed dams. This manipulation also raised the maternal core and pup temperature above that of ergonovine-treated animals maintained at 22°C. As in Experiment 4, Ergonovine administration resulted in high nest frequencies throughout the course of the experiment.

The results of this experiment support the hypothesis that the effects of prolactin-suppression are thermally mediated. These data also provide further support for the more general notion that thermal factors play an important role in limiting nest bout duration.

Figure 35: Mean daily pup temperature of oil- and ergonovine-treated dams maintained at 22°C and ergonovine-treated dams maintained at 25°C. SEMs are shown.


PUP SKIN TEMPERATURE (°C)
36
35
34
33
32
DAYS POSTPARTUM
1
2
3
4
5
6
7
8
9
10
11
12
13
14
x ERGO.
● ERGO. + HEAT
O CONTROL

Summary and Discussion

The results of Experiments 6-8 are consistent with the notion that the effects of adrenal removal and prolactin suppression are, in fact, mediated by thermal factors. When the heat load of Adex-Ovex and prolactin-suppressed dams is increased by raising the ambient temperature, then the normal daily decline in nest time is reinstated. Of course, increasing the ambient temperature is only one of a number of manipulations that would result in increasing maternal heat load and thereby, in a decline in nest time. Heating the pups or dam directly might be expected to yield similar results.

In Experiments 2 and 7 it was possible to relate the chronic thermal state of the dam to the presence or absence of an acute rise in maternal core temperature during a nest bout. Sham-Op dams and Adex-Ovex + Corticosterone dams at 22°C and Adex-Ovex d[illegible] at 25°C showed a rise in core temperature during most of their nest bouts. Adex-Ovex dams maintained at 22°C, on the other hand, experienced such a rise on few of their nest bouts. Thus, it was possible to show that those dams with a chronically elevated heat load tended to show an acute rise in temperature during the majority of their nest bouts. Moreover, those groups that showed a rise in temperature in a high percentage of nest bouts also had curtailed nest bouts. Furthermore, I have established that the chronic elevation in maternal heat load is under endocrine control.

Chapter III

The increasing influence of thermal factors over the course of lactation

Experiment 9

Comparison of the change in core temperature experienced during a nest bout by Day 4 and Day 10 postpartum females

The results of the previous experiments provide support for the notion that thermal factors can play an important role in limiting nest bout duration. The question is whether these thermal factors actually contribute to the progressive decline in daily nesting time during the first two weeks postpartum. Croskerry, Smith & Leon (1978) proposed that the steady decline in daily nest time is precipitated by the development of the pups. Specifically, as the pups gain weight the maternal huddle increases in mass at a greater rate than its surface area for heat dissipation. Consequently, the rate at which heat is retained by huddle members during a nest bout increases. It was predicted that this increased rate of heat retention would cause maternal temperature to rise more and more rapidly and force nest bout termination after progressively shorter periods of time over the course of lactation.

In addition to such physical factors, there also may be gradual physiological changes both in the pup and in the mother that also contribute to nest time decline. As the pups mature, they become more efficient homeotherms, and consequently, as they become older, are likely to lose less heat to the environment during inter-bout intervals (Gelineo and Gelineo, 1952). Further, the results of Experiment 2 showed that dams have a further, chronic increase in core temperature around Days 5 - 7 which is maintained throughout the second week (see Figures 6 & 7). These three factors should combine to increase the thermal load on mothers as lactation progresses and thereby, decrease the duration of nest bouts. If there actually is a progressive increase in the thermal load of lactating females that curtails nest bouts more and more quickly, then there should be a correlative change in the acute rise in maternal core temperature that the females experience during a nest bout. If no evidence for a progressively increasing effect of heat load were found, then one would have to conclude that although thermal factors may play a role in limiting nest bout duration, they do not change over the course of lactation in such a way as to produce progressively shorter nest bouts. An alternative explanation would then be required to account for the gradual decline in nest bout duration seen in the intact rat in the first two weeks postpartum.

Increasing the potency of thermal factors may affect nest time either by increasing the proportion of nest bouts during which the dam experiences a rise in temperature and/or by increasing the rate of rise of maternal core temperature during a nest bout. These possibilities were tested by recording the core temperature of Day 4 dams, who have relatively long nest bouts, continuously for 24 hr and the data obtained from Day 4 dams was then compared with similar data obtained for Day 10 females, who typically have much shorter nest bouts.

Method

Subjects

Six multiparous female Wistar rats served as subjects in this experiment.

Procedure

All dams in this experiment were implanted with a thermistor on Day 2 postpartum as described in Experiment 3. Core temperature was monitored continuously from noon on Day 4 until noon on Day 5. The change in maternal core temperature between onset and termination of each bout and nest bout duration were subsequently transcribed. Mean bout duration and the percentage of bouts during which core temperature rose were subsequently calculated for each dam. For those bouts on which maternal core temperature did in fact show a rise, the mean rise in temperature and mean rate of rise during a bout were then determined for each dam. Mean rate of rise for a nest bout was calculated by simply dividing the change in core temperature by nest bout duration. Results were compared with similar data obtained in Experiment 3 from Day 10 dams using a students' 't' test for independent samples.

Results

Figure 36 shows sample records of maternal core temperature during a nest bout for a Day 4 and a Day 10 dam. Mean bout duration for each group is shown in Figure 37. The mean percentage of bouts during which dams showed an increase in core temperature for the 6 females in each group is shown in Figure 38. Figure 39 shows the mean increase in core temperature over the course of a nest bout for that

Figure 36: Sample trace of maternal core temperature during a nest bout from a Day 4 and a Day 10 dam.

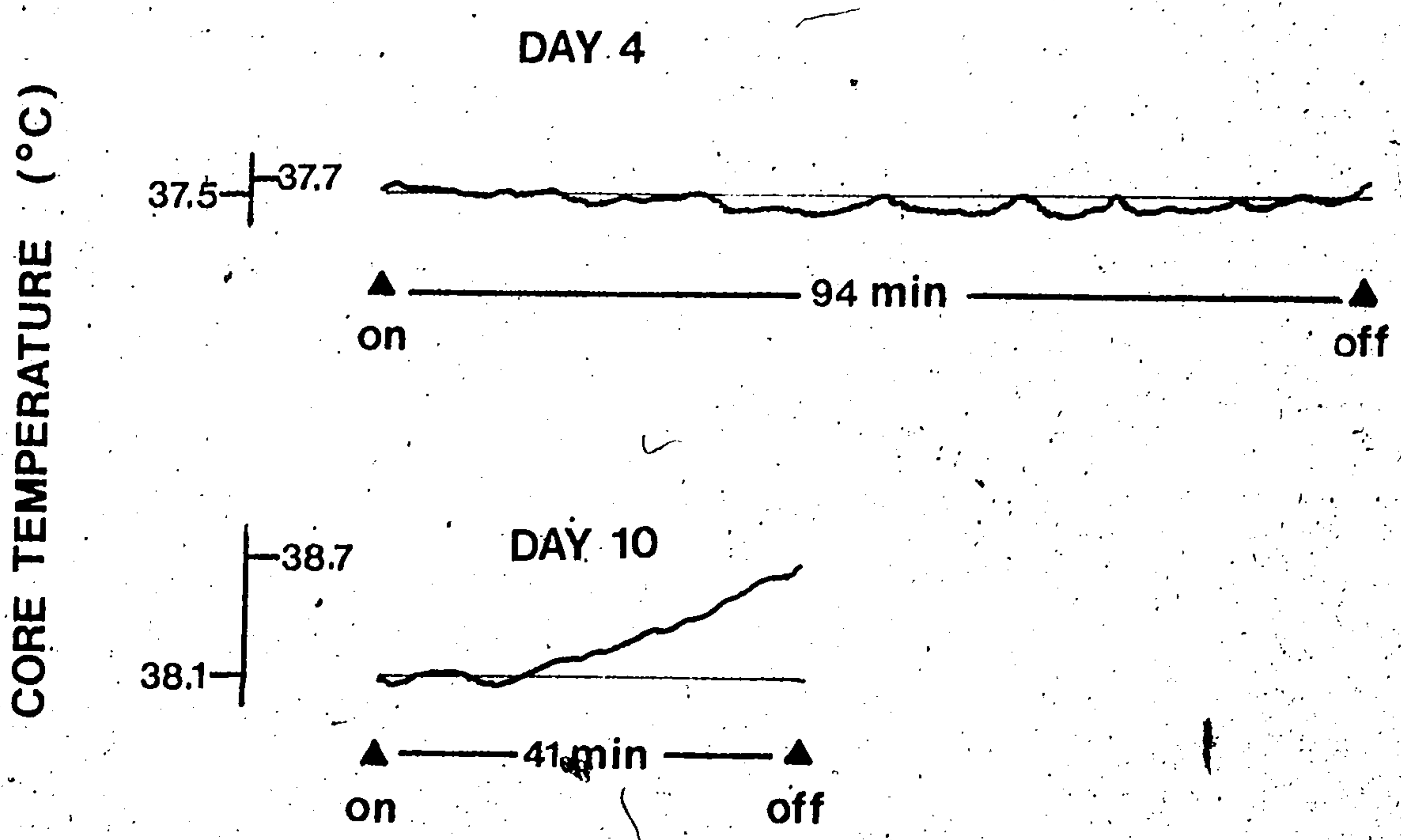
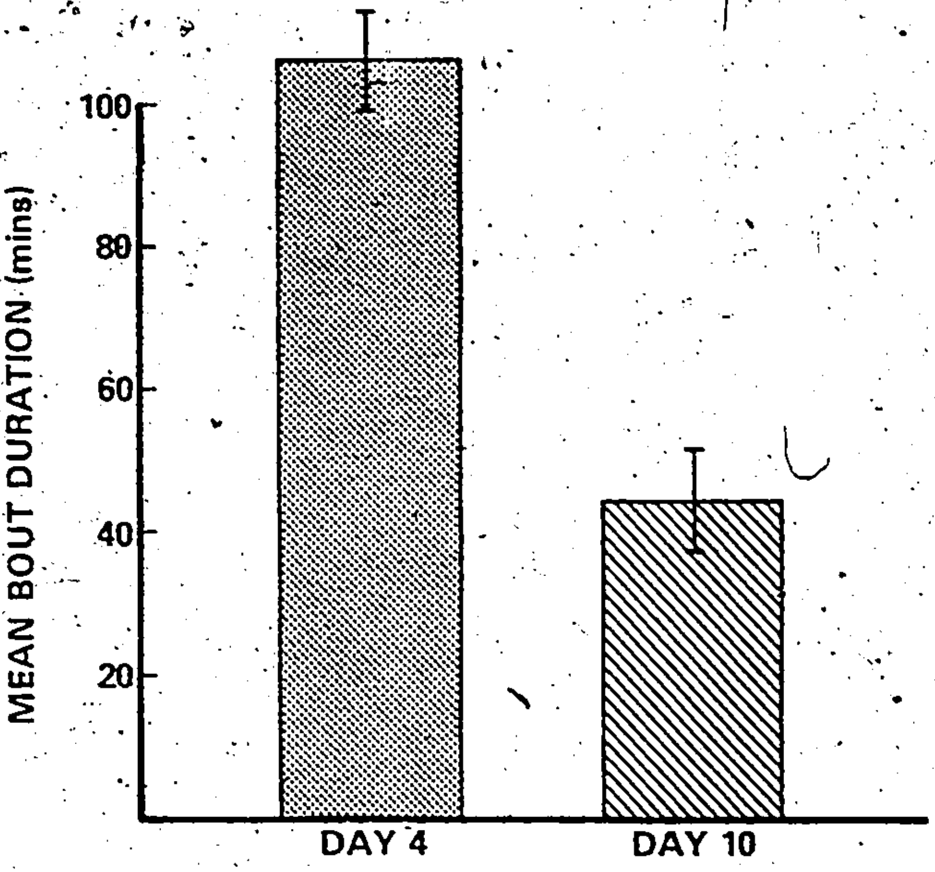
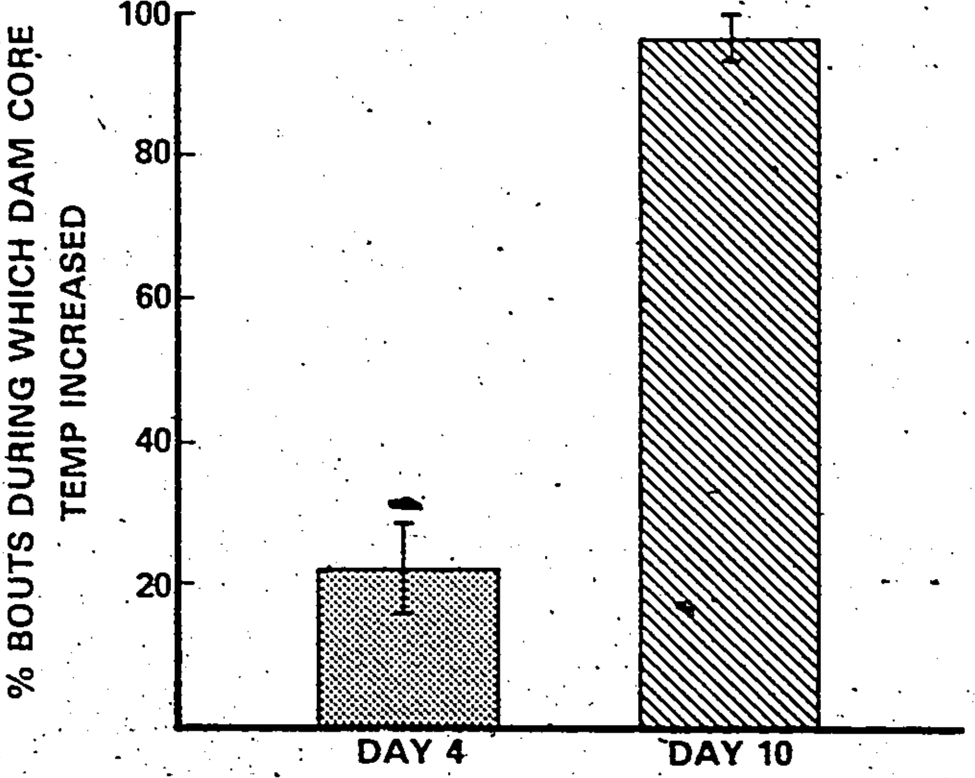

CORE TEMPERATURE (°C)
DAY 4
37.5
37.7
94 min
on
off
DAY 10
38.1
38.7
41 min
on
off

Figure 37: Mean bout duration of Day 4 and Day 10 dams. SEMs are shown.

Figure 38: Mean percentage of nest bouts during which Day 4 and Day 10 dams showed an increase in core temperature. SEMs are shown.


MEAN BOUT DURATION (mins)
100
80
60
40
20
DAY 4
DAY 10


% BOUTS DURING WHICH DAM CORE TEMP INCREASED
100
80
60
40
20
DAY 4
DAY 10

proportion of nest bouts in which there was a rise and Figure 40 shows the mean rate of rise for these bouts.

Statistical analysis revealed that the differences in nest bout duration and proportion of nest bouts in which there was a rise between these two groups were statistically significant ($t = 6.16$, $p < .005$; $t = 10.82$, $p < .005$ respectively). The difference in absolute rise in core temperature was not statistically significant ($t = 0.92$, $p > .05$). The difference in rate of rise between Day 4 and Day 10 dams, although large, is only significant at the $p < .1$ level ($t = 1.46$), undoubtedly because of the large variances and small number of subjects in these groups. The difference in rate of maternal temperature rise is, however, highly significant when a Mann-Whitney U test is used ($p <$ .0).

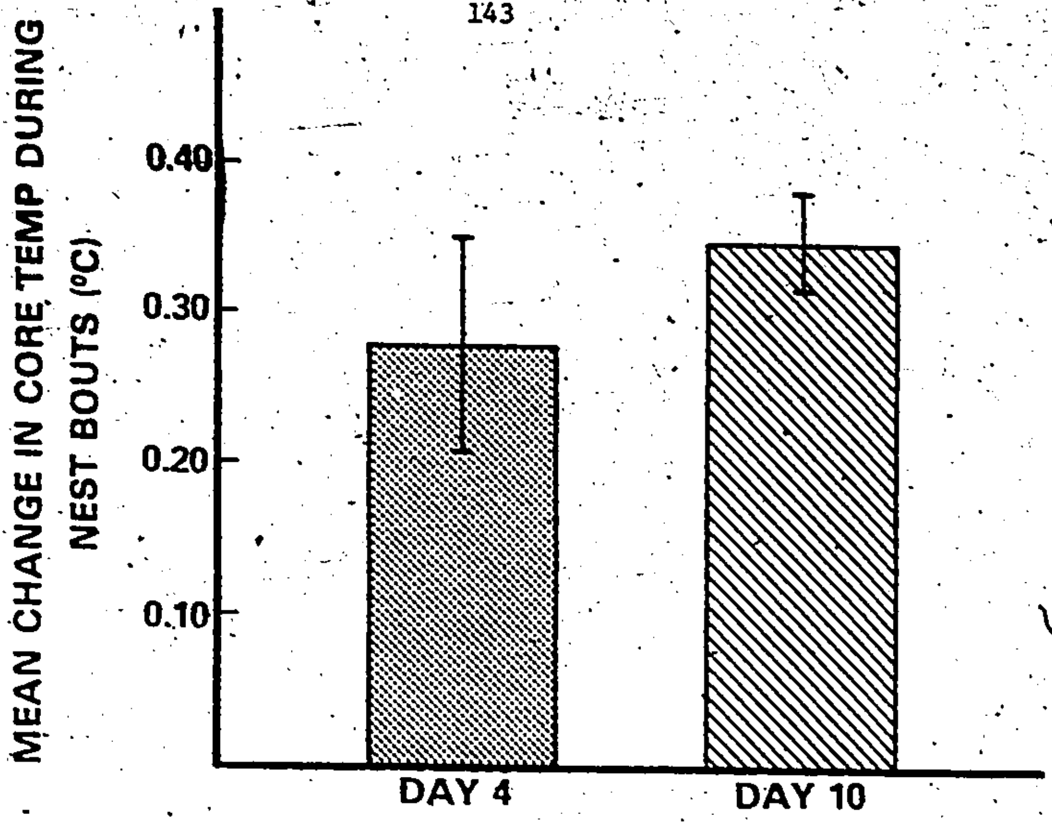
Discussion

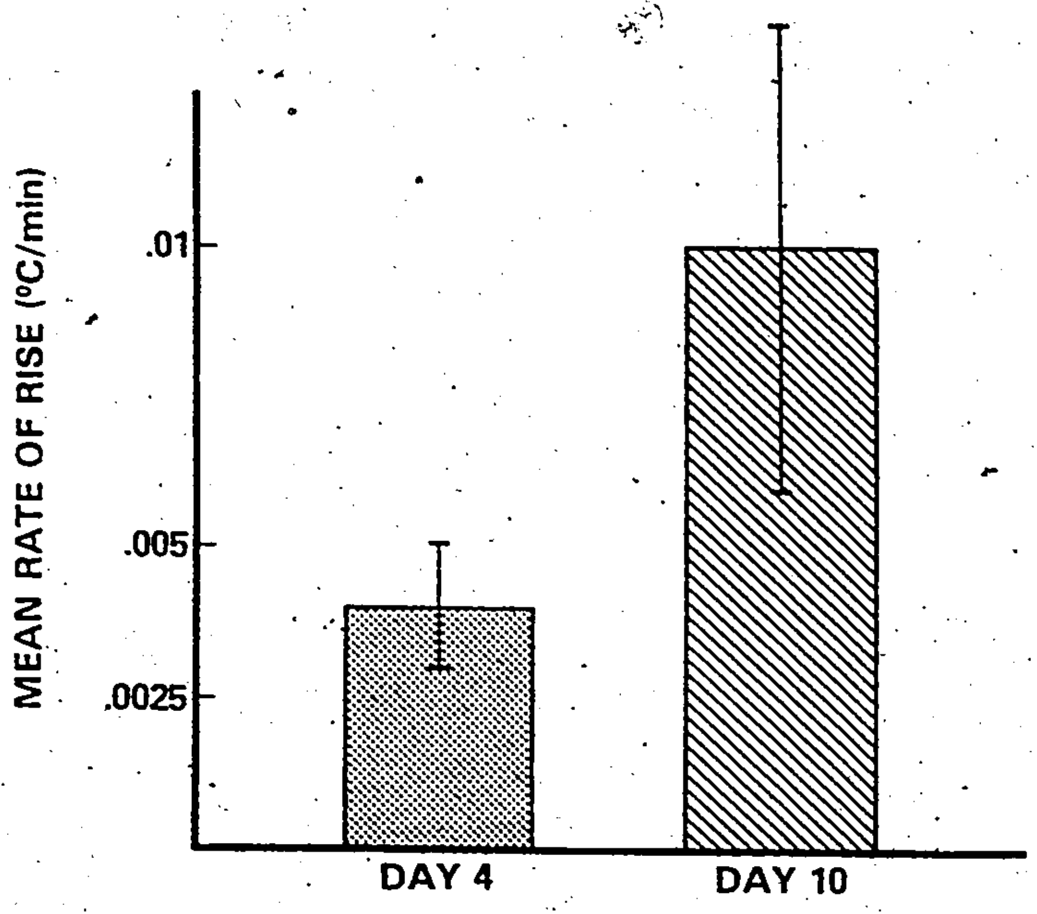
It is clear from the results of this experiment that thermal factors play a greater role in limiting the duration of the nest bouts on Day 10 postpartum than on Day 4. On Day 4, most nest bouts are apparently limited by non-thermal factors, perhaps the need of the mother to forage. By Day 10, however, dams experience an acute rise in temperature during 95% of their nest bouts and there is an increased effect of thermal factors within bouts such that the rate of rise of maternal temperature is increased. Thermal factors apparently play an important role in limiting nest bout duration by Day 10 postpartum.

It is important to emphasize again the additive nature of the factors involved in increasing maternal heat load, which limit nest bouts. Such factors include the size and age of litter, size of

Figure 39: Mean increase in core temperature, on those bouts where an increase occurred, of Day 4 and Day 10 dams. SEMs are shown.

Figure 40: Mean rate of rise of core temperature, on those bouts where an increase occurred, of Day 4 and Day 10 dams. SEMs are shown.


MEAN CHANGE IN CORE TEMP DURING NEST BOUTS (ºC)
0.40
0.30
0.20
0.10
DAY 4
DAY 10


MEAN RATE OF RISE (ºC/min)
.01
.005
.0025
DAY 4
DAY 10

mother, insulating properties of nest, and ambient temperature as well as the dam's chronic temperature. On Day 4 or Day 10, those thermal factors may, or may not add to limit nest bout duration. For example, Day 4 dams show a sharp decrease in nest time in a warm ambience, evidence of their susceptibility to thermal limitation of nest bout length even at that point in lactation (Leon, Croskerry & Smith, 1978). On the other hand, Day 10 dams have prolonged bouts in a cool ambience, despite the increased heat production of both mothers and pups at this time (Leon, Croskerry & Smith, 1978). As lactation progresses, dams experience an acute rise in core temperature during a nest bout both more frequently and more rapidly. The overall increase in the thermal effects of huddling with her litter are consistent with the proposed role of thermal factors in inducing progressively shorter nest bouts and thereby the decline in daily nest time typical of intact dams.

The results of this experiment, while demonstrating that thermal factors increase in importance over the first two weeks postpartum and thereby contribute to the decline in nest time, also emphasize the importance of nonthermal factors in stimulating the dam to terminate a nest bout for at least some part of this period. Dams have approximately the same number of nest bouts on Day 4 postpartum as they do on Day 10, although the bouts on Day 4 are much longer. There are obviously some factors working on the Day 4 mother to stimulate her to terminate her nest bouts. Such factors as the need of the mother to eat, drink, defecate and urinate may all play a part in stimulating bout termination at this time.

A larger perspective on the role of thermal factors in limiting nest bouts comes from a consideration of the dam with her litter, as being involved in a decision making process (McFarland, 1977) and of thermal cues as being one of a number of factors involved. The "decision" the dam has to make, at any one moment, is whether or not to leave her young and her pattern of behaviors would be expected to be such as to maximise her differential reproductive success. The mechanism that sets limits on her "decision" as to how long to remain with the pups should be in accord with the optimal growth and survival of the current litter as well as with the mother's maximal reproductive potential. In the simplest conditions, two sets of factors would be involved in this decision: 1) that group of factors such as pups attaching to the nipple and suckling, and sensory feedback from milk delivery cues that would maintain contact, and 2) those cues associated with the dams need to maintain her own homeostasis, which would stimulate the interruption of contact with the young. When the dam initiates a nest bout, stimuli from the latter set of factors presumably are very weak, but as the nest bout continues these cues become stronger until they finally outweigh the cues that maintain contact and the dam terminates the nest bout.

From this perspective, thermal cues are probably best viewed as one of a number of homeostatic factors that cause the dam to terminate a nest bout. The contribution that thermal cues make towards the dam's "decision" to leave her young will depend on such factors as size and age of litter, body weight of the dam, and ambient temperature. As the results of the experiments described in this thesis indicate the contribution of thermal cues to the dam's "decision" to terminate a nest bout also depends on the hormonal state of the mother. Specifically, the elevated heat production typical of the lactating dam, which renders her more vulnerable to the acute thermal effects of huddling with her young, appears to depend on the presence of high circulating levels of prolactin and glucocorticoids.

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