

Scotophase-Dependent Thermoregulatory Dysfunction in Pinealectomized Chickens (39509)

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Light-mediated effects of the pineal gland on the endocrine system are well documented (1-3). Circadian and light-dark-entrained rhythms of melatonin (MT), norepinephrine (NA), and serotonin (5-HT) in the pineal gland have been found in most vertebrates (4). Recently, the pineal gland has been implicated in thermoregulatory functions of several vertebrates (5-9). Pineal glands of rats exposed to low ambient temperature underwent hypertrophy and hyperplasia (6). Pineal RNA and protein contents were reduced (7) and there was marked inhibition of hydroxyindole-*O*-methyl transferase (HIOMT) activity in rats maintained at high ambient temperature (8). These findings support the hypothesis that the vertebrate pineal is directly involved with integration of thermal stimuli.

The search for pineal involvement in the circadian and light-dark-entrained rhythms of T_b has been carried out in the rat (10) and the house sparrow (5). Though pinealectomized rats exhibited no significant alteration in T_b rhythm, these animals had a lower T_b during the dark portion of their photoperiod. Arrhythmia and a marked reduction in amplitude of circadian patterns of T_b followed pinealectomy in sparrows housed in constant darkness. These findings indicate an interrelation of the pineal gland or its metabolic principles with physiological adjustments to changes in the thermal environment. Information is not now available on pineal involvement in thermoregulatory responses of birds to acute thermal challenge. This experiment was designed to determine if pinealectomy would alter the thermoregulatory ability of chickens during acute exposure to a hot environment.

Materials and methods. Female day-old chicks (Columbian \times New Hampshire) from the University of Illinois Poultry Farm were distributed in a battery brooder and maintained in a controlled-temperature chamber

on a 12L:12D photoperiod. Chicks were started at 35° with five subsequent decreases of 2.8° weekly. A standard broiler-starter ration and water were available *ad libitum*.

Pinealectomy (PX) and sham operations (PN) were performed at 8 days of age in a stereotaxic unit under Brevital Sodium (Eli Lilly and Co.) anesthesia. Birds were allowed to recover from anesthesia under a heating element before being returned to their cages. At 3 weeks of age the birds were assigned to six growing pens within two metal cages for an additional 6 weeks.

All birds were subjected to a single thermal challenge at 9 weeks of age in a completely randomized design with a factorial arrangement of three surgical treatments [PX, PN, and control (C)] and two times of day (diurnal and nocturnal). A single thermal challenge was administered to three birds simultaneously during the light phase and to three different birds during the dark phase of their photoperiod on each of 5 successive days. Each bird was challenged only once. Thermal challenges were started 4 hr after onset of daily light cycle (photophase) and 4 hr after onset of daily dark cycle (scotophase), respectively. This procedure resulted in thermal challenge to five birds from each surgical treatment during the photophase and five different birds from each surgical treatment during the scotophase, for a total of 30 individuals.

Prior to thermal challenge, each bird was fitted with a chest movement transducer and a thermistor rectal probe. Thermistor probes were calibrated daily against a mercury thermometer in a water bath. Rectal temperature (T_r) and respiratory rate (RR) were monitored continuously on three Gilson Unigraphs and a Gilson Polygraph, respectively. Five minutes after fitting of thermistor probes and chest movement transducers, recordings of T_r (degrees centigrade) and RR (breaths per minute) were

taken simultaneously from three birds at 23° as the zero-time measurement. The birds were then placed in a lighted Conviron (Controlled Environments, Pembina, N.D.) chamber maintained at 42°. After 60 min, the birds were removed from the chamber and held at 23° for an additional 20 min.

Dry-bulb recordings indicated chamber temperature was maintained at $42.2 \pm 0.2^\circ$ (mean \pm SEM) for 10 through 60 min of challenge at both times of the day. Dew-point temperature was not controlled, but recordings from all challenges show an average wet-bulb temperature of $35.4 \pm 0.2^\circ$ during heat challenge. Room temperature was $23.2 \pm 0.5^\circ$ during the recovery period. The T_r and RR were summarized at 10-min intervals throughout the thermal-challenge and recovery periods. The data were analyzed by the method of least squares, taking account of treatment, time of day, and challenge day. Least squares, rather than analysis of variance, was appropriate because challenge-day comparisons were not orthogonal to those for treatment and time of day.

Pineal uptake of trypan blue (11) was used to confirm total pinealectomy. Data from incompletely pinealectomized birds were not included in the analyses.

Results. The T_r of birds in each surgical treatment group are presented for diurnal (Table I) and nocturnal (Table II) heat episodes. There was no significant difference in prechallenge (0-min) T_r among PX, PN, and C groups, although prechallenge T_r was 0.3,

0.1, and 0.1° lower in respective groups at night. All groups had a comparable T_r after 10 and 20 min of exposure at both times of day.

Surgical treatment did not significantly affect T_r during the 60 min of diurnal thermal challenge; PX, PN, and C groups experienced 2.2, 2.0, and 1.9° increases, respectively. At the end of the diurnal recovery period (80 min after heat exposure began), T_r was 1.3, 0.5, and 0.1° above 0-min measurements for respective treatments. No T_r differences between surgical treatments occurred at any time of diurnal challenge (Table I).

After 20 min of nocturnal heat, on the other hand, the hyperthermia of PX birds was greater ($P < 0.05$) than in PN and C birds (Table II). After 60 min of thermal challenge at night, the PX group experienced a 3.3° rise ($P < 0.01$) in T_r above the prechallenge temperature, while the PN and C groups rose only 2.2 and 2.3°, respectively (not significant). Similarly, the nocturnal PX group had a 1.4° higher T_r ($P < 0.01$) at the conclusion of the cooling period than the 80-min average for all other groups, both nocturnal and diurnal (42.9°).

Interactions between time of day and T_r recorded from each surgical treatment group are illustrated in Fig. 1. Each bar represents the mean difference (nocturnal T_r minus diurnal T_r) of respective treatment groups at each measurement time. A lower nocturnal T_r is evident in all surgical groups

TABLE I. EFFECT OF DIURNAL THERMAL CHALLENGE ON THERMOREGULATORY RESPONSE OF PINEALECTOMIZED PULLETS.^a

Time (min)	Body temperature (degrees)			Respiratory rate (br/min)		
	PX(5) ^b	PN(5)	C(5)	PX(5)	PN(5)	C(5)
0	42.5 \pm 0.15 ^c	42.6 \pm 0.09	42.6 \pm 0.15	28.4 \pm 1.8	44.8 \pm 6.5	41.6 \pm 3.9
10	43.0 \pm 0.26	42.9 \pm 0.08	42.8 \pm 0.15	201.2 \pm 34.7	153.6 \pm 22.6	132.2 \pm 25.9
20	43.6 \pm 0.18	43.6 \pm 0.14	43.4 \pm 0.13	272.2 \pm 15.7	238.4 \pm 15.3	209.6 \pm 26.9
30	44.0 \pm 0.17	43.8 \pm 0.16	43.7 \pm 0.18	301.6 \pm 7.9	284.8 \pm 11.3	286.0 \pm 11.5
40	44.2 \pm 0.12	44.2 \pm 0.19	44.0 \pm 0.13	319.8 \pm 20.4	270.8 \pm 12.8	318.0 \pm 12.6
50	44.4 \pm 0.13	44.4 \pm 0.22	44.2 \pm 0.16	308.4 \pm 10.6	285.6 \pm 19.1	295.2 \pm 4.5
60	44.7 \pm 0.09	44.6 \pm 0.31	44.5 \pm 0.10	282.8 \pm 18.2	274.8 \pm 20.9	295.2 \pm 13.5
70	44.0 \pm 0.17	43.9 \pm 0.29	43.6 \pm 0.03	229.6 \pm 38.9	214.4 \pm 30.5	224.0 \pm 20.2
80	43.8 \pm 0.23	43.1 \pm 0.13	42.7 \pm 0.09	134.7 \pm 32.4	110.5 \pm 13.1	49.0* \pm 7.6

^a Diurnal thermal challenge (0 to 60 min at 42°) was given 4 hr after onset of light and was immediately followed by a recovery period from 70 to 80 min at 23°.

^b Surgical treatment group; number of birds in parentheses.

^c Mean \pm standard error.

* Significant difference ($P < 0.05$) between treatments.

TABLE II. EFFECT OF NOCTURNAL THERMAL CHALLENGE ON THERMOREGULATORY RESPONSE OF PINEALECTOMIZED PULLETS.^a

Time (min)	Body temperature (degrees)			Respiratory rate (br/min)		
	PX(5)	PN(5)	C(5)	PX(5)	PN(5)	C(5)
0	42.2 ± 0.13 ^c	42.5 ± 0.21	42.5 ± 0.14	42.0 ± 3.9	42.0 ± 5.8	36.4 ± 4.0
10	42.8 ± 0.12	42.9 ± 0.20	42.9 ± 0.17	180.2 ± 23.1	172.4 ± 27.2	144.8 ± 28.0
20	43.6 ± 0.17	43.5 ± 0.12	43.4 ± 0.18	255.6 ± 11.2	244.4 ± 12.7	246.4 ± 20.7
30	44.4* ± 0.10	43.9 ± 0.12	43.7 ± 0.20	238.8 ± 15.6	269.2 ± 15.5	287.0 ± 18.0
40	44.9* ± 0.10	44.3 ± 0.07	44.2 ± 0.26	246.6 ± 18.3	280.4 ± 13.5	264.4 ± 20.2
50	45.3* ± 0.08	44.6 ± 0.07	44.5 ± 0.28	256.8 ± 16.5	274.4 ± 17.8	272.8 ± 26.9
60	45.5** ± 0.07	44.7 ± 0.08	44.8 ± 0.28	254.8 ± 19.6	299.6 ± 9.7	280.8 ± 28.5
70	45.1** ± 0.09	44.0 ± 0.20	43.5 ± 0.29	227.2 ± 14.4	260.2 ± 16.3	170.0* ± 24.0
80	44.3** ± 0.13	42.9 ± 0.14	42.6 ± 0.28	147.4 ± 21.4	146.8 ± 32.7	72.4* ± 18.5

^a Nocturnal thermal challenge (0 to 60 min at 42°) was given 4 hr after onset of darkness and was immediately followed by a recovery period from 70 to 80 min at 23°.

^b Surgical treatment group; number of birds in parentheses.

^c Mean ± standard error.

* Significant difference ($P < 0.05$) between treatments.

** Indicates $P < 0.01$ level of significance.

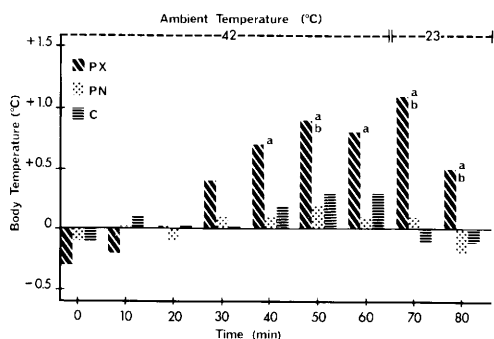


FIG. 1. Differences in body temperature of diurnal and nocturnal thermal challenges given to pinealectomized pullets. Each bar represents the nocturnal minus diurnal mean of a particular treatment group at each time interval. Significant differences between diurnal and nocturnal challenges for a particular time are indicated by "a" ($P < 0.05$). Significant interactions between pinealectomy and time of day, i.e., diurnal versus nocturnal, are shown by "b" ($P < 0.05$).

for the initial recordings (0 min). The persistently elevated T_r of the nocturnal PX group, which began at 30 min, is well demonstrated.

Time-of-day comparisons showed that the nocturnal T_r was elevated after 40 and 50 min ($P < 0.01$) and all subsequent times ($P < 0.05$). Significant interactions between pinealectomy and time of day were found at 50 min of exposure ($P < 0.05$) and for the recovery period ($P < 0.01$). Although the PN and C groups had elevated nocturnal T_r during the period from 40 to 60 min of heat

exposure, treatment by time-of-day comparisons revealed no differences. The only T_r difference ($P < 0.05$) between PN and C groups was at 70 min for both times of the day.

The RR during diurnal and nocturnal thermal challenges are presented in Tables I and II, respectively. No significant differences in RR between surgical treatments occurred during heat exposure. The highest diurnal RR occurred at 40 min for PX and C treatments (320 and 318 br/min, respectively) and at 50 min for the PN group (286 br/min). During the nocturnal episode, peak thermal tachypnea reached by the C group was 287 br/min at 30 min, while the PX and PN groups reached 257 and 300 br/min at 50 and 60 min, respectively. Recovery of RR was fastest in C birds, which showed a significantly lower ($P < 0.05$) RR at 70 min during the nocturnal challenge and at 80 min for both times of day.

Comparisons of RR between night and day challenges and interactions with surgical treatment groups are presented in Fig. 2. Day versus night comparison of RR showed differences at 30 min ($P < 0.05$) and 40 min ($P < 0.01$). A low RR (28 br/min) in the diurnal PX group at 0 min (Table I) produced a significant interaction ($P < 0.05$) between pinealectomy and time of day (Fig. 2). The nocturnal RR of PX birds was lower than the diurnal RR at all times of heat exposure. At 30 min of nocturnal heat,

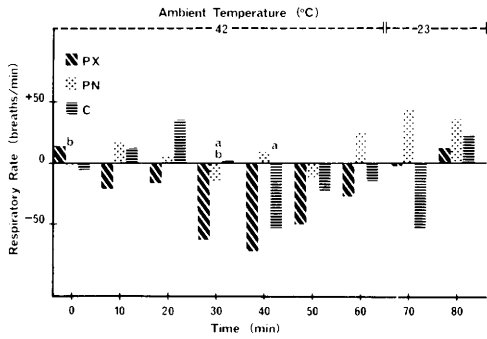


FIG. 2. Differences in respiratory rates for diurnal and nocturnal thermal challenges given to pinealectomized pullets. Each bar represents the nocturnal minus diurnal mean of a particular treatment group at each time interval. Significant differences between diurnal and nocturnal challenges for a particular time are indicated by "a" ($P < 0.05$). Significant interactions between pinealectomy and time of day, i.e., diurnal versus nocturnal, are shown by "b" ($P < 0.05$).

there was a consequent interaction with time of day ($P < 0.05$).

Least-squares analysis showed that the thermoregulatory response within treatment groups was independent of the day of the week and arrangement of treatments within a given challenge period.

Significant differences in body weights were not found between surgical treatment groups for either day or night heat episodes. The mean body weights (in grams) prior to nocturnal challenge were: (PX) 1233.6 ± 77.9 , (PN) 1113.6 ± 53.2 , and (C) 1160.2 ± 33.5 . Diurnal mean body weights (in grams) were 1211.2 ± 110.6 , 1217.4 ± 65.6 , and 1152.0 ± 53.3 for the PX, PN, and C groups, respectively.

Discussion. Several reports in the last decade referred to the association of the vertebrate pineal with maintenance of daily T_b patterns. Pineal and parapineal removal from lizards resulted in lowered body temperature and selection of higher ambient temperature (9, 12). Pinealectomy of house sparrows (*Passer domesticus*) caused a reduction in diurnal amplitude and disrupted the circadian pattern of body temperature (5). Similar to the results obtained in this experiment with chickens, nocturnal body temperature was consistently lower in pinealectomized rats (10). Our data indicate that a scotophase-dependent discontinuity

in the thermoregulatory response of pinealectomized domestic fowl occurs when the birds are exposed to an environment at 42° , for 60 min. Also it is noteworthy that at the conclusion of nocturnal recovery (80 min), the RR of the PX and PN were similar despite a 1.4° higher T_r in the PX group. This observation, along with a lower initial T_r , suggests that pinealectomy resulted in disruption of the thermoregulatory processes.

Unfortunately, the abrupt fall in RR of C birds during recovery for both times of day limits interpretation of differences between T_r and physiological mechanisms controlling evaporative cooling among treatment groups. The low RR encountered in C birds may be explained, as only the birds in this treatment had loss of plumage on their dorsal surface. This decreased insulation could have resulted in more rapid nonevaporative heat dissipation. Nonetheless, the loss of thermostasis observed in PX birds during nocturnal heat challenge suggests a pineal involvement in heat dissipation during scotophase.

Biogenic amines that are proposed to be involved in thermoregulation (13–17) exhibit metabolic rhythms in the pineal gland which are directly related to the daily light–dark cycle (4). These studies have involved several species of animals in a wide range of ambient temperature. Though conflicting data have been reported, the general consensus is that in mammals NA stimulates heat production whereas 5-HT facilitates heat loss (13), and it is proposed that a reversed role exists for monoaminergic participation in thermoregulation in avian species (18). The disruption of nocturnal heat-dissipating capacity of the pinealectomized chickens used in this study may reflect the absence of certain biogenic amines periodically produced or stored in the pineal that are important for efficient thermoregulation.

Changes in thermoregulatory responses following ablation of the pineal gland suggest the involvement of the pineal and its metabolites in thermostasis. At present, the locus of pineal participation in the control of T_b rhythms and in evaluation of thermal input from the environment remains unknown. However, it seems reasonable that,

in addition to transduction of photic input, the pineal is also an intricate component in physiological adjustments to changes in ambient temperature.

Summary. Pinealectomized female chickens (PX) were given an acute thermal challenge (60 min at 42°) during two portions of their 12L:12D photoperiod. Five birds from each surgical treatment group, including sham operated (PN) and nonoperated controls (C), were exposed to heat 4 hr after onset of light (photophase) and a like number 4 hr after onset of darkness (scotophase). There were no differences in rectal temperature (T_r) or respiratory rate (RR) among treatment groups of the photophase heat challenge. Heat exposure during the scotophase caused a significantly greater ($P < 0.01$) hyperthermia in PX birds after 20 min of heat. At the conclusion of the nocturnal challenge, PX birds had a T_r 1.0° higher than PN and C birds. Time-of-day comparisons of RR showed a significant ($P < 0.05$) interaction between pinealectomy and time-of-day at 30 min of exposure; the RR of PX birds was lower at night.

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