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6. Consumption time of individual newborn aphids averaged 4.72 ± 0.75 minutes for six 2nd and 3rd instar larvae (25 aphids).
7. Duration of the plucking procedure (from insertion of larval head into aphid's wool to application of plucking to rump of larva), determined from motion picture analyses, averaged 4.30 ± 0.51 seconds per plucking for seven 2nd and 3rd instar larvae (64 pluckings).
8. Shield rebuilding time was 18.50 ± 2.63 minutes (seven shields made by seven 2nd and 3rd instar larvae).
9. Coordinates of each point are measured by perpendicular distances to the three sides of an equilateral triangle as described, for example, in C. C. Li, *Population Genetics* (Univ. of Chicago Press, Chicago, 1955).
10. Since these larvae rebuilt their shield in less than an hour (*8*), they loaded intensively only during the first portion of the experimental period.
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14. Ants that were offered unidentified trash-carrying chrysopid larvae in the laboratory often discontinued their attacks if upon attempting to bite the larvae they secured only parts of the trash packets. Reduviid bugs also rejected trash-carrying larvae if they failed to reach the bodies of the larvae when probing through the trash packets with their beaks (T. Eisner, unpublished). Trash-carrying larvae were shown to be better protected against anthocorid bugs than naked counterparts [T. R. New, *Entomol. Gaz.* **20**, 119 (1969)].

15. Supported in part by NSF grant PCM 74-15084. We thank Dr. P. A. Adams and Dr. W. L. Brown, Jr., for identifying *C. slossonae* and the ants respectively, and Drs. C. A. and M. J. Tauber for advice and comments on the manuscript. Most of the work was done at the Huyck Preserve, Rensselaerville, N.Y. R. E. Silberglied and T.E. discovered the larvae. Paper No. 58 of the series Defense Mechanisms of Arthropods.

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Thermoregulation Is Impaired in an Environment Without Circadian Time Cues

Abstract. *Squirrel monkeys synchronized to a 24-hour light-dark cycle show a prominent circadian rhythm in body temperature which is regulated against mild environmental cold exposures throughout the 24-hour day. However, cold exposures produce significant decreases in core body temperature when the circadian rhythms of the animal are free-running in the absence of environmental time cues. Effective thermoregulation appears to require the precise internal synchronization of the circadian timekeeping system.*

Body temperature in homeotherms is not regulated at a constant level over the day, but rather oscillates with a prominent circadian (about 24 hours) rhythm (*1*). Circadian rhythms, which have been observed in many physiological variables, are generated by an endogenous system of oscillators within the organism which is normally synchronized by 24-hour cues in nature (*2*). When animals are placed in an environment free of time cues, circadian rhythms persist with a period usually significantly different from 24 hours. We report here that in such constant conditions the capability of squirrel monkeys to maintain body temperature during mild cold exposures is significantly impaired. Regulation against cold is more effective when animals are synchronized by periodic inputs from the environment.

Thirteen adult male squirrel monkeys (*Saimiri sciureus*), weighing 800 to 1200 g were conditioned to sit in a special metabolism chair (*3*) for two or more weeks. Studies were conducted on the chair-restrained animals within an isolation chamber in which the environmental lighting and ambient temperature (T_a) were controlled. The animals were allowed free access to food and water. Colonic temperature (T_{co}) was measured with a thermistor probe (YSI, model 401) inserted 6 cm beyond the anus. The probe was connected to a bridge circuit (YSI Telethermometer, model 43TD) and the bridge output was continuously amplified and recorded (Grass polygraph, model 7).

The monkeys were subjected to mild 6-hour cold exposures at all circadian phases of the 24-hour day. Each animal was studied for at least three consecutive days at 28°C before being exposed to 20°C for 6 hours (*4*). Eight experiments were conducted with animals entrained to a 24-hour light-dark cycle with 12 hours of light (600 lux) and 12 hours of dark (<1 lux) (LD 12 : 12). The results of these experiments were compared with those of nine other experiments in which the animals were maintained in constant light (LL) of 600 lux.

In animals entrained to the LD cycle, the T_{co} had a 24-hour mean of 37.5° ± 0.1°C (mean ± S.E.M.) and an average daily range of 1.9° ± 0.1°C. The T_{co} was maintained above the mean when lights were on, and then fell progressively throughout the night until it began to rise again about 2 hours before the lights were scheduled to come on (*5*). In LL where there were no environmental time cues to synchronize the circadian system, the rhythm in T_{co} persisted with an average free-running period of 25.2 hours (*6*). The mean T_{co} in these conditions (37.7° ± 0.2°C) was not significantly different from that observed in LD. However, the circadian range of the rhythm was reduced to 1.0° ± 0.2°C, and the waveform was also altered so that a greater fraction of the cycle was elevated above the mean than in LD.

Cold exposure had little effect on T_{co} in an animal entrained to an LD cycle when T_{co} was compared to the mean ± the standard deviation (S.D.) of the three control days (Fig. 1a). The T_{co} was defended when the animals were exposed to cold at any time of day or night with a mean maximum decline in T_{co} of −0.1° ± 0.2°C (Fig. 2). However, most animals in LL showed an impaired ability to maintain a stable T_{co} during similar cold exposures. Figure 1b shows an example in which a major fall in T_{co} occurred. The maximum fall in T_{co} in the LL experiments averaged −1.0° ± 0.2°C below the control mean (Fig. 2). The decrease in T_{co} of animals free-running in constant light was significantly greater ($P < .01$) than that of animals entrained to the LD cycle.

These results are the first demonstration of a failure of homeostasis caused by the removal of LD cues from the environment. There are three reasonable ways in which this impaired defense of T_{co} in constant light might be explained.

1) The stress of chair restraint or the isolation conditions (or both) could result in impaired thermoregulation in the animals. However, when animals were studied for similar lengths of time in the chair in LD and LL, only the monkeys in LD could consistently defend T_{co}. Therefore, it appears unlikely that these results are anomalies of restraint or isolation (or both) per se.

2) Constant bright light could detrimentally affect the thermoregulatory system. Yet, this does not seem to be the case either. We have shown previously that squirrel monkeys in LL (600 lux) are capable of being entrained to 24-hour cycles of food availability (*7*). When these animals were provided with this form of external synchronization in LL, they were able to defend T_{co} effectively against similar cold exposures at various circadian phases. Because these monkeys were also chair-restrained, a combination of chair restraint and bright light together cannot account for the observed response. A further possible test would

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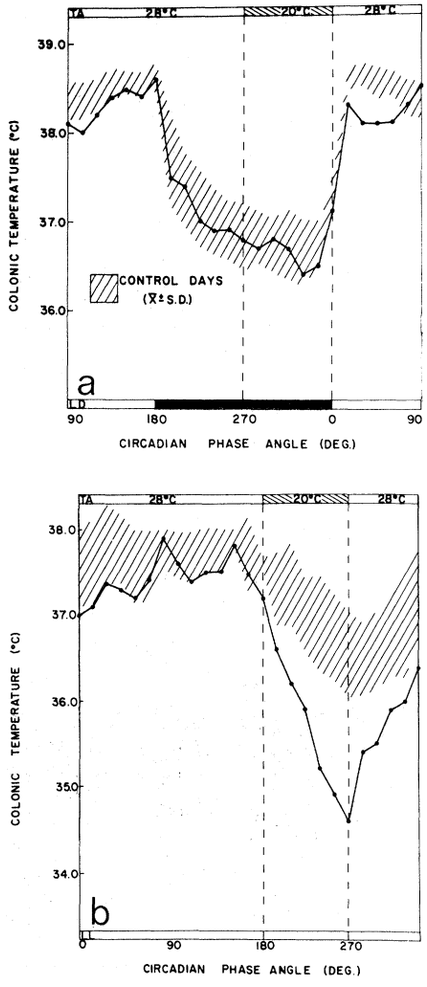

Fig. 1. Effect of 6-hour cold-exposures on T_{co} (—•—) in a monkey entrained to an LD 12 : 12 cycle (a) and a monkey free-running in LL (b). At the top of each graph the ambient temperature is indicated on the day the cold was instituted; lighting conditions are indicated at the bottom of each graph. Since the periods of the rhythms were different in the two lighting conditions [that is, 24 hours in (a) and 25 hours in (b)], the data were normalized so that each cycle was set equal to 360°. The shading represents the mean ± S.D. of the previous three cycles (T_a, 28°C).

be to examine the response to cold exposures in constant darkness.

3) There is much evidence that favors a third possibility: the failure of thermoregulation may be due to the internal uncoupling of the multioscillator circadian timing system of the squirrel monkey (*8*). When the animals were kept in constant light with free access to food, they were deprived of circadian time cues from the environment. The impairment of thermoregulation could be a result of improper temporal relationships between the different rhythmic processes involved in temperature regulation. In the constant conditions used in these experiments, we have previously demonstrated that internal desynchronization of the circadian timing system can occur in squirrel monkeys (*9*), in a manner analogous to that previously reported in man (*10*). In this state, physiological functions in different tissues oscillate with independent free-running rhythms which therefore show constantly changing internal phase relationships. Furthermore, even when internal desynchronization does not occur, internal phase angle shifts to new phase relationships can be seen (*11*). Thus, cold exposures applied to animals in LL with different degrees of internal phase angle shifts from the entrained state could explain the impaired thermoregulation function. Further, because the internal phase relationships in LL at the time of the cold exposure can vary, the rhythmic functions will at times be appropriately phase-related to defend T_{co}. This would account for the variability in the response to the cold exposures seen in Fig. 2 with animals in LL sometimes being able to defend T_{co}.

To test whether internal desynchronization was responsible for the impaired thermoregulation, we induced it in three animals and then subjected them to identical cold exposures. Internal desynchronization was achieved in adrenalectomized monkeys maintained in LL but provided with injections of 10 mg of cortisol at 24-hour intervals (*12*). Cortisol-synchronized rhythms within the animal thereby showed a 24-hour period while other rhythms were free-running, with periods significantly different from 24 hours. In four experiments, these internally desynchronized animals showed a mean maximum decrement in T_{co} of $-2.5° \pm 0.7°C$ during the cold exposure. Four control experiments with adrenalectomized animals internally synchronized by providing both 24-hour LD and cortisol cycles showed a defense of T_{co} during the cold exposures with an average fall of $-0.1° \pm 0.2°C$ (*13*). Thus, the response to cold exposure was not related to the adrenalectomy per se, but was a result of the internal desynchronization induced in these animals.

What are the rhythmical processes within the organism which are showing altered temporal relationships? Studies of the thermoregulatory system of homeotherms indicate that both heat production and heat loss are rhythmic and are timed by the circadian system (*14*). Normally, these variables are synchronized in such a manner that they produce the observed rhythm in T_{co} in both entrained and free-running states. However, we have shown that one of these thermoregulatory components, vasomotor heat loss, persists with a free-running circadian rhythm in LL, which

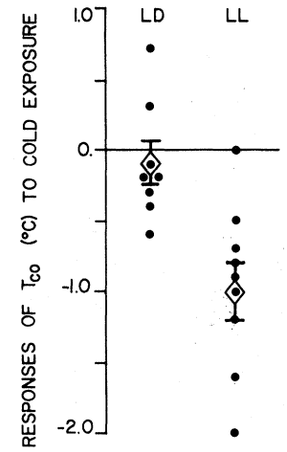

Fig. 2. Maximum fall of T_{co} from the control mean for all cold exposures in LD and LL (•). Plotted also are the mean (◇) ± S.E.M. for responses in LD and LL. The mean fall of T_{co} was significantly greater ($P < .01$) in LL than in LD.

can show phase and occasionally period independence from the free-running T_{co} rhythm (*15*). Thus, in the absence of external temporal information the effectiveness of temperature regulation could be diminished as a result of improper or continually changing phase relationships between the oscillating components of the thermoregulatory system.

That a temporally constant environment should give rise to the failure of a homeostatic mechanism is something of a paradox. Homeostasis, as defined by Cannon (*16*), depends on control systems to maintain a relatively constant internal environment in the face of a highly variable external environment. Yet, we have shown here that when a homeotherm is faced with a constant environment in which all effective 24-hour time cues are removed, the homeostasis of body temperature is impaired so that it cannot be precisely maintained when a mild environmental challenge is encountered.

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6. In 21 experiments in which the animals were free-running in constant conditions, the mean circadian period of the temperature cycle was 25.2 ± 0.1 hours (mean ± S.E.M.). The period for each animal was determined by calculating the average interval between the maximum of each circadian cycle (*7*).
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Chemical Scent Constituents in the Urine of the Red Fox (*Vulpes vulpes* L.) During the Winter Season

Abstract. *Four volatile chemical compounds have been identified as apparently unique constituents in urines of red foxes (both sexes) during the winter season when mating occurs. Quinaldine was found only in male fox urine. Several other compounds identified are found in other species also. Some or all of these compounds may function in olfactory communication in the red fox.*

Olfactory communication has important socioecological significance in the wild canid species, especially in the red fox (*Vulpes vulpes* L.) (*1*–*5*). Olfactory cues may serve for individual and group recognition, territorial marking, as markers in food scavenging, for sexual recognition and attraction, as indicators of reproductive states, and for possible pheromonal function.

Identified sources of odor in the red fox are the supracaudal gland, the anal sac, and the urine. Of these, only the urine has thus far apparently not been investigated to determine the odor constituents. By a combination of gas chromatography–mass spectrometry (GC-MS) with organic structural methods and synthesis, we have identified four compounds that appear to be unique odor sources in the urines of male and female red foxes: Δ^3-isopentenyl methyl sulfide, 2-phenylethyl methyl sulfide, 6-methyl-Δ^5-hepten-2-one, and *trans*-geranylacetone. 2-Methylquinoline (quinaldine) was found in male, but not female, red fox urine. In addition, several compounds were identified, and these have also been found in urines of other species: benzaldehyde, acetophenone, 4-heptanone, and some C_{10}-terpenes. The availability of these compounds by synthesis or from natural sources will permit controlled studies of their possible endocrine or behavioral (pheromonal) effects. The methodology that has been developed may provide the means for quantitative investigation of seasonal and individual variations in olfactory cues and permit their correlation with biological events.

The chemical constituents of the supracaudal gland (sometimes referred to as the "violet gland" because of its ambrosia-like odor) have been investigated by Albone (*6*, *7*) and by Albone and Flood (*8*). They reported dihydroactinidiolide, 6-hydroxy-2,2,6-trimethylcyclohexanone, and *trans*-4-keto-β-ionone in addition to other (unidentified) terpenoid and fluorescent photolabile sebum constituents. They also found high levels of hydroxysteroid dehydrogenase activity.

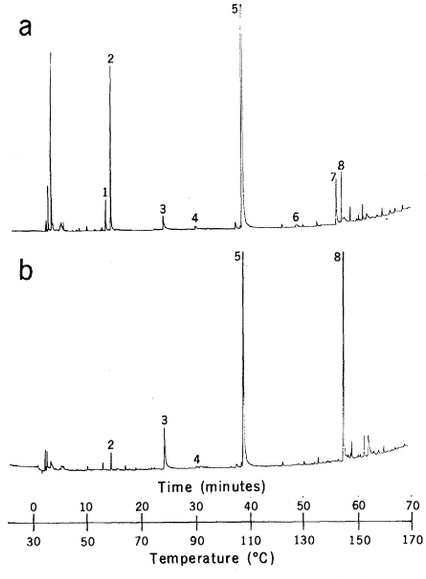

Fig. 1. Gas chromatographic analysis in glass capillary columns of urinary volatile compounds in the red fox (*Vulpes vulpes* L.). (a) Male; (b) female. The constituents are identified by number in Table 1.

The histophysiology and function of the anal sac of the red fox have been described by Spannhof (*9*), who noted seasonal changes in the epithelium of this organ which coincide with variations in locomotor and sexual behavior of the animals. Albone *et al.* (*10*) reported that the predominantly aqueous secretion of the anal sac in the red fox contains high concentrations of odorous volatile fatty acids (C_2 to C_6), ammonia, trimethylamine, 1,4-diaminobutane (putrescine), and 1,5-diaminopentane (cadaverine), all suspected of being microbially produced. They also investigated the bacterial microflora of the sac in studies that were extended by Gosden and Ware (*11*). Albone, Robins, and Patel (*12*) found that 5-aminovaleric acid is a major free amino acid component of the fox anal gland secretion. Although it is generally believed that the anal sac has significance in olfactory communication of the fox, its exact role is not understood.

The red fox uses its urine as the principal means of scent marking (*1*–*5*) and trappers have long used this product in the preparation of fox lures. The strong characteristic odor is said to intensify and change in quality during the breeding season (*13*) when the incidence of marking is said to increase (*2*, *5*). These observations suggest that urine from one or both sexes may have pheromonal activity transmitted through the olfactory sense.

Joffre (*14*) has reported on the significant seasonal variations in the endocrine characteristics (spermatogenetic activity, testicular and prostatic weights, prostate secretions, and plasma testosterone levels) of both the cubs and adult red fox males—phenomena that parallel changes in behavior and in olfactory communication (*5*).

Despite the suspected importance of the urine in scent marking, it is not surprising that the nature of the volatile odor constituents of the red fox urine has not been previously investigated, since collection of uncontaminated urine from wild foxes is difficult. We now present the results of a study of urines of both male and female foxes collected in a natural environment within or near the Acadia National Park (68°W, 44°N) close to Bar Harbor, Maine. The only species of fox in that area is *Vulpes vulpes* L.

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