Table 2. Nuclear magnetic resonance spectra of biotin and α-dehydrobiotin. With structure I, only differences are shown; the remainder of the spectrum was essentially identical with that of biotin.

Chemical shift* (δ in ppm)	Protons (No.)	Type of signal	Assignment†
		Biotin (II)	
1.45	6	Broad methylene envelope	Methylenes at C-3′, C-4′ and C-5′
2.23	2	Asymmetrical triplet	Methylene at C-2′
~ 2.75	2	Apparent singlet with doublet	Methylene at C-5
3.10	1	Broad multiplet	Methine at C-2
4.25	2	Broad multiplet	Methines at C-3 and C-4
6.37	2	Broad singlet	Protons on nitrogens
		α-Dehydrobiotin (I)	
~ 1.70	2	Broad complex multiplet	Methylene at C-5′
~ 2.20	2	Broad multiplet	Methylene at C-4′
5.78	1	Doublet (J = 17 cy/sec)	Vinyl proton at C-2′
6.83	1	Sextet, doublet or triplets (J_{AB} = 17 cy/sec; J_{AX} = 7 cy/sec)	Vinyl proton at C-3′

* Determined at 60 mc/sec in dimethylsulfoxide-d_6 and relative to tetramethylsilane as internal standard. †See structures I and II.

sentially at *p*H 6.5. The antibiotic from the filtered broth (adjusted to *p*H 4.0) was absorbed onto carbon and eluted with a mixture of acetone and water. It was further purified by partition chromatography on a column of diatomaceous earth. The lower phase of the solvent system, composed of a mixture (16:4:1) of ethyl acetate, cyclohexane, and McIlvaine's buffer (*p*H 3.0) *(2)*, served as the stationary phase while the upper phase of the same system was used as the eluting solvent. Concentration in a vacuum of the active fractions from the column resulted in an aqueous solution (*p*H 3.0) which, after being cooled, yielded the crystalline antibiotic. Recrystalled from methanol, the antibiotic had the following physical properties: m.p., 238° to 240°C; molecular weight, 242 (mass spectrum); $[\alpha]_D^{25} = +92°$ (0.1*N* NaOH); pK_a, 4.32. Elemental analyses were in accord with the formula $C_{10}H_{14}N_2O_3S$.

Structure I for the antibiotic was deduced from a comparison of its infrared and nuclear magnetic resonance (NMR) spectra *(3)* with those of biotin. The infrared spectrum indicates a close structural relationship. However, the infrared spectrum of the antibiotic has sharp, strong bands at 1645 cm^{-1} and 985 cm^{-1} which are absent in the spectrum of biotin. These are attributable to a *trans*-conjugated ($\lambda_{max}^{H_2O}$=203 mμ, ε=15,000: λ, wavelength and ε, extinction) double bond. The outstanding differences between the NMR (Table 2) and the infrared spectra are: (i) the methylene envelope (δ=about 1.45 ppm) was reduced from six protons in biotin to two protons in the antibiotic and (ii) the appearance in the spectrum of the latter of an AB part of an ABX_2 system comprising two *trans*-vinyl protons with a methylene group next to the double bond. The low-field position of the signals for the vinyl protons and the magnitude of the coupling constant (J_{AB} = 17 cy/sec) indicates conjugation of the double bond with the carboxyl group and *trans* orientation.

The above data lead to structure I for the antibiotic, and the validity of this structure was proved by reduction (10 percent palladium on charcoal) of the antibiotic to the naturally occurring *d*-biotin. The identity of the reduction product with biotin was established by the following criteria; there was no depression of melting point on admixture with biotin, the infrared spectrum and optical rotation were identical with those of biotin, and the compound did not separate from biotin on papergrams *(4)*.

It has been established *(5)* by x-ray crystallography that structure II represents the absolute configuration of biotin. Consequently, the absolute configuration of α-dehydrobiotin is as depicted by structure I.

The role of antimetabolites such as the sulfa drugs in the treatment of bacterial infections is well known. More recently, however, antimetabolites have received considerable attention in the chemotherapy of neoplastic diseases. For example, aminopterin is now used in the treatment of acute leukemia in children and of choriocarcinoma in women, and 6-mercaptopurine is used in the treatment of acute leukemia in both adults and children *(6)*.

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References and Notes

1. For example, activity against the following microorganisms has been detected: *Staphylococcus aureus, Sarcina lutea, Streptococcus pyogenes, Escherichia coli, Proteus vulgaris, Salmonella pullorum, Candida albicans, Saccharomyces cerevisiae* and *Penicillium oxalicum*.
2. T. C. McIlvaine, *J. Biol. Chem.* **49**, 183 (1921).
3. We thank Dr. G. Slomp and his associates, of these laboratories, for determining the spectra.
4. For paper chromatography the following two solvent systems were used: 1-butanol, methanol, benzene, water (2 : 1 : 1 : 1); and 1 butanol, acetic acid, water (2 : 1 : 1).
5. J. Trotter and J. A. Hamilton, *Biochemistry* **5**, 713 (1966).
6. Chemotherapeutical aspects of antimetabolites are discussed by J. A. Stock in *Exp. Chemotherap.* **4**, 79 (1966).

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Reproduction in Lizards: Influence of Temperature on Photoperiodism in Testicular Recrudescence

Abstract. *The photoperiodic response of the testis in* Anolis carolinensis *is very temperature-sensitive. Body temperatures must be elevated to near preferred levels (about 32°C) during at least part of the daily light period for long day-lengths to be effective in stimulating testicular recrudescence. High temperatures during the night, with cool days (20°C), may retard testicular growth more than do continuously low temperatures.*

Annual testicular cycles have been described for many species of reptiles, but the control of the timing of these cycles is poorly understood. Experimentally increased day-lengths stimulate testicular recrudescence in several species of lizards *(1, 2)*; however, interpretation of the importance of such photoperiodic responses is hindered by the paucity of information on thermal influences *(3)*. Studies on other poikilotherms have demonstrated that both the level of temperature and the nature of daily thermal fluctuations may modify the influence of photoperiod on seasonal physiological cycles *(4, 5)*. In-

creasing temperatures have been shown to accelerate photoperiodic responses in a few lizards (*6*), but available data deal only with the effects of constant temperature. The level and daily pattern of temperature to which a lizard is exposed tends to be established by the thermoregulatory behavior of the species. In diurnal lizards, body temperatures are generally maintained within relatively narrow limits during the period of daily activity—the species characteristic preferred range of temperature—and the lizard typically cools to ambient temperatures at night (*7*). The relationship between ecologically meaningful temperature fluctuations and the photoperiodic influence on testicular recrudescence in the lizard *Anolis carolinensis* is examined here.

Sexually mature, adult male *Anolis*, averaging 5.1 g in weight and 62 mm in snout-vent length, were obtained from Louisiana on 30 September. Testes are completely involuted at this time and do not normally attain full size and spermatogenic activity again until the following spring (*8*). Several lizards (initial controls) were killed to assess the condition of the reproductive structures at the beginning of the experiment, and freshly captured specimens (final controls) were killed at the end to determine the extent of recrudescence under natural environmental conditions. The right testis was weighed, and the left testis, epididymis, and kidney were prepared for histological examination; the sexual segment of the kidney was used to judge androgenic activity (*8*).

Four groups of 15 lizards were placed in screen-covered aquaria. Food (mealworms, crickets, and termites) and water were available at all times. All groups were exposed to 14 hours of light daily provided by 20-watt GE daylight fluorescent lamps. This photoperiod corresponds to the longest day-length that occurs in the habitat of this species and is long enough to stimulate premature testicular recrudescence in *Anolis* at 28°C (*2*). Each group was exposed to a different thermal regimen: (i) 32° ± 1°C continuously, (ii) 20°C continuously, or (iii–iv) 10 hours at 32°C and 14 hours at 20°C daily. This last heating cycle was adjusted so that one group of lizards was heated to 32°C during the middle of the daily light period and cooled at night (day 32°, night 20°), and the other group was heated only during the 10 hours of darkness (day 20°, night 32°). The upper temperature used here corresponds to the mean preferred body temperature of *A. carolinensis* (*7*), and 20°C represents the lowest temperature at which the lizard normally becomes active in the field and one at which it remains alert and feeds readily in the laboratory.

Testicular weights are presented in Fig. 1, and the spermatogenic activity and condition of sex accessories is described in Table 1. There was no significant change in the condition of the reproductive structures between 22 and 33 days in any of the four treatments. The testes of animals exposed to 32°C continuously were almost fully developed after 3 weeks of treatment. In 10 of the 15 animals in this group, the epididymis and sexual segment of the kidney were hypertrophied and the vas deferens contained abundant motile sperm. Sexual behavior, such as territorial displays and attempted copulations, were frequently observed among these lizards during the third week of treatment. In contrast, the testes were only slightly enlarged and spermatogenesis just beginning after 1 month at 20°C. The reproductive condition of these lizards was similar to that of lizards under the shorter day-length in nature, indicating a lack of photoperiodic stimulation at 20°C (Table 1).

The effectiveness of only 10 hours of daily heating to 32°C clearly depends on its relationship with the light cycle. Testes were distinctly enlarged and spermatogenesis accelerated in lizards maintained on a natural thermal cycle with body temperatures elevated during the day. Their condition was only slightly less advanced than that of lizards maintained at 32°C continuously; differences were most pronounced in the sexual segment of the kidney. In contrast, the long day-length did not stimulate testicular recrudescence when the 10 hours of heating coincided with the dark period. In fact, after 33 days the testes of these lizards were significantly smaller than in those kept at 20°C ($p = .02$).

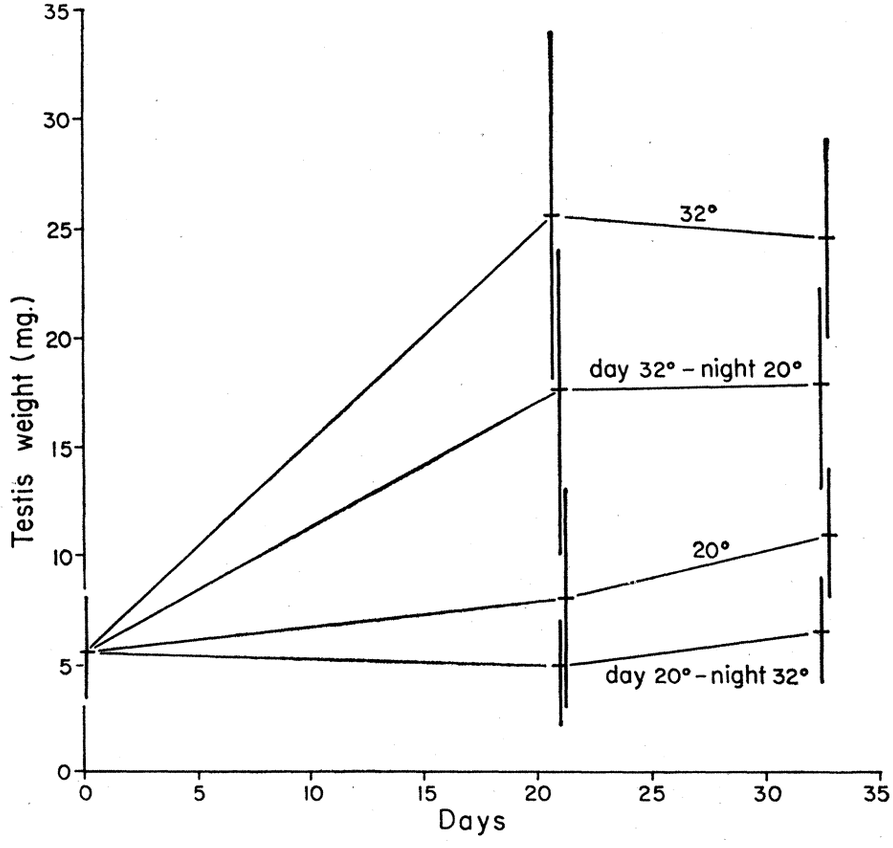

Fig. 1. Testis size in *Anolis* maintained under various thermal conditions with 14 hours of light daily, starting 4 October. Five animals in each group were killed after 22 days and 10 after 33 days of treatment. Horizontal lines (and curves) represent average testicular weights in each sample and vertical lines represent the 95 percent confidence limits about the means.

Table 1. Condition of accessory sexual structures and stages of spermatogenesis of the testes in *Anolis* after 22 and 33 days of treatment. Stage I, spermatogonia only and tubules collapsed; stage VI, tubular lumina well formed and lined with many sperm. Other stages are intermediate, representing the progressive development of spermatocytes and spermatids.

Temperature conditions	*N*	Tubular diameter of testis* (μ)	Epididymal epithelium* (μ)	Epithelial height of sex segment of kidney*† (μ)	Spermatogenic stage					
					I	II	III	IV	V	VI
Initial controls	9	102.5 ± 17.1	17.57 ± 3.29	17.66 ± 2.12	7	2				
Final controls	6	137.1 ± 8.26	14.44 ± 1.85	12.85 ± 2.56			4	2		
Constant 20°C	15	146.0 ± 25.2	13.98 ± 2.81	17.93 ± 5.08		6	7	2		
Constant 32°C	15	220.3 ± 20.3	29.79 ± 11.55	34.81 ± 15.56				1	9	5
Day 32°C, night 20°C	15	187.4 ± 19.6	21.25 ± 5.41	20.28 ± 4.30			2	7	5	1
Day 20°C, night 32°C	15	132.9 ± 22.6	14.07 ± 2.87	18.60 ± 2.62	2	7	4	2		

* Mean ± standard deviation. † Hypertrophy is evident when epithelium exceeds about 20 μ.

There was an average loss of about 6 percent in the body weight of all groups, and there was no correlation between body size or change in weight and the testicular response within groups. Abdominal fat bodies were similar (averaging 170 mg) in the four groups. Thus, the differences in reproductive responses under the various environmental conditions cannot be attributed to differences in the health of the lizards.

The pronounced discrepancy between the testicular response to light at 20° and 32°C indicates that this aspect of photoperiodism is highly temperature-sensitive in *Anolis carolinensis*. This sensitivity is further illustrated by comparison with the rate of testicular recrudescence reported for this species at 28°C (*2*). Testicular development in *Anolis* exposed to 16 hours of light daily at 28°C for 60 days was less than in lizards exposed to 14 hours of light daily at 32°C for only 22 days; there was no stimulation of the sexual segment of the kidney under the former conditions. The slight elevation of temperature from 28° to 32°C is apparently an even more effective stimulus than an additional few hours of light. However, there may be marked species differences in the temperature sensitivity of the photoperiodic response, since long-day stimulation of recrudescence in the lizard *Xantusia vigilis* was not abolished by temperatures of 19° or 8°C (*9*). This interspecific difference may be related to the different ecologies of the species: *Xantusia* typically encounters much lower environmental temperatures than *Anolis*.

The large number of possible combinations of temperature and light makes evaluation of the importance of these two factors difficult. In this respect, the preferred body temperature of the species may provide a particularly valuable focal point for further study. In addition to representing an ecologically meaningful temperature, it appears to be closely related to the physiological thermal requirements of the species. For example, daily heating to temperatures even 1°C above preferred ranges may produce spermatogenic arrest and testicular collapse in lizards (*10*), and such heating is likely to interfere with photoperiodic responses. The decline in the rate of the photoperiodic response between 32° and 28°C suggests that *Anolis* may also be very sensitive to slight cooling below its preferred range. Some daily heating to temperatures near preferred levels may be required for the initiation of photoperiodic responses in reproduction, at least during the season when the reproductive system is relatively quiescent. However, the lack of a photoperiodic response in *Anolis* heated to 32°C only during the night indicates that the length of the daily heating period per se is not the primary influence on photoperiodism.

The timing of the daily heat and light period appears to be extremely important for the photoperiodic response in testicular recrudescence. The present data indicate that the body temperature during the light period is more important than the temperature during the dark period of the day. This is the reverse of the relationship between temperature and light cycles in the control of diapause in insects (*5*). The basis for the "synergism" between heat and light may be related to the thermal sensitivities of any of the multitude of physiological processes involved in the initiation of testicular recrudescence. The metabolism of testicular tissues from *Anolis* appears to be markedly depressed by cooling from 33° to 28°C in vitro (*11*). I have also found a pronounced temperature sensitivity of the gonads and accessory structures to the administration of exogenous gonadotropins and androgens in vivo (*12*). Thus, the temperature of the target tissues would be expected to influence the rate of testicular recrudescence in the presence of endogenous gonadotropins, and this factor may account in part for the difference in testicular response in animals kept at 32°C for 24 or 10 hours daily. However, the condition of the testes in the lizards heated only during the night indicates that gonadotropin production was not accelerated by the long day-length under these thermal conditions. These results suggest that at least some of the "higher" centers, such as those involving photoreception, neurosecretion, and gonadotropin synthesis and release, must be warmed to a certain level during at least part of the light period for the animal to respond effectively to the stimulus of long day-lengths.

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