

amines were detected in these extracts, although these biogenic amines are found in skin extracts of certain tropical American frogs of the related genus *Leptodactylus*⁵.

The Pauli positive material was purified on a Dowex-50-2 X column using gradient (0.5–2.0 N) elution with hydrochloric acid. Paper chromatography (butanol, acetic acid, water 4:1:1; isopropanol, ammonia, water 8:1:1; butanol, pyridine, acetic acid, water 4:1:1:2; and butanol, pyridine, water 3:1:1) and paper electrophoresis (pH 1.9, 6.5) of the purified Pauli-positive material (free base) strongly suggested that the material was the dipeptide, carnosine (β -alanylhistidine). Hydrolysis with 3 N hydrochloric acid for 2 h at 100°C yielded histidine and β -alanine as identified by paper chromatography and color reactions. Treatment of the Pauli-positive material with dinitrofluorobenzene followed by hydrolysis of the DNP-derivative with 3 N acid gave DNP- β -alanine and histidine as identified by paper chromatography. Identical results were obtained with authentic carnosine. Comparison of the mass spectra (AEI MS-9 mass spectrometer) of carnosine and the Pauli positive material confirmed their identity. The amounts of carnosine contained in the various species of *Eleutherodactylus* and *Leptodactylus* are given in the Table.

The occurrence of large amounts of carnosine in the skin of these frogs is noteworthy since carnosine along with its N-methyl derivative, anserine, is characteristically a constituent of muscle⁶ although it has also been reported in stomach mucosa of the toad *Bufo marinus*⁷.

As yet no clear picture of its physiological function in muscle tissue has evolved nor is one entitled at present to speculate on its role in the skin of these frogs. Neither anserine nor the related peptide, homocarnosine (γ -amino butyrylhistidine), which occurs in brain⁸, could be detected in the skin of these *Leptodactylid* frogs.

Zusammenfassung. Carnosin, ein Dipeptid, das normalerweise nur in Vertebratenmuskeln vorkommt, wurde als einer der Hauptinhaltsstoffe in der Haut bestimmter Frösche identifiziert. In 9 Froscharten des Genus *Eleutherodactylus* wurden 0.2 bis 1.2 mg Carnosin pro Gramm frischer Haut gefunden.

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⁵ V. ERSFAMER, M. ROSEGHINI, and J. M. CEI, *Biochem. Pharmac.* 13, 1083 (1964).

⁶ C. L. DAVEY, *Nature* 179, 209 (1957).

⁷ T. WOOD, *Nature* 180, 39 (1957).

⁸ J. J. PISANO, J. D. WILSON, L. COHEN, D. ABRAHAM, and S. UDENFRIEND, *J. biol. Chem.* 236, 499 (1961).

Periodicity of a Circadian Rhythm in Birds by Species-Specific Song Cycles (Aves, Fringillidae: *Carduelis spinus*, *serinus serinus*)¹

The daily rhythm of numerous biological functions is based upon an endogenous periodicity which deviates more or less from that of the earth's rotation when the organism is kept under constant conditions². Under natural conditions, this circadian period is synchronized to exactly 24 h by 'Zeitgebers'³. Until the present time, the only factors which have been proven experimentally as 'Zeitgebers' for circadian rhythms are periodic variations in light intensity and temperature. Biotic factors, however, are to be considered as possible 'Zeitgebers' as well³. In order to test this hypothesis, the usage of social stimuli deriving from conspecifics commends itself as a first step. Any social species of birds, individuals of which react strongly to conspecific stimuli, provides a promising experimental tool. The 2 species which have been selected – the Siskin (*Carduelis spinus*) and the Serin (*Serinus serinus*) – show a strong pair-contact during the breeding season and spend the rest of the year roving in flocks of various sizes. In both cases, the maintenance of social contact seems to depend primarily upon vocalization; the obvious choice, therefore, was to test the effect of species-specific song on the circadian rhythm.

3 female Siskins and 1 female Serin were housed individually in cages enclosed in sound-proof boxes (internal dimensions 65 · 62 · 45 cm). Locomotor activity of the birds was recorded on an event recorder by means of microswitches attached below the perches. Food and

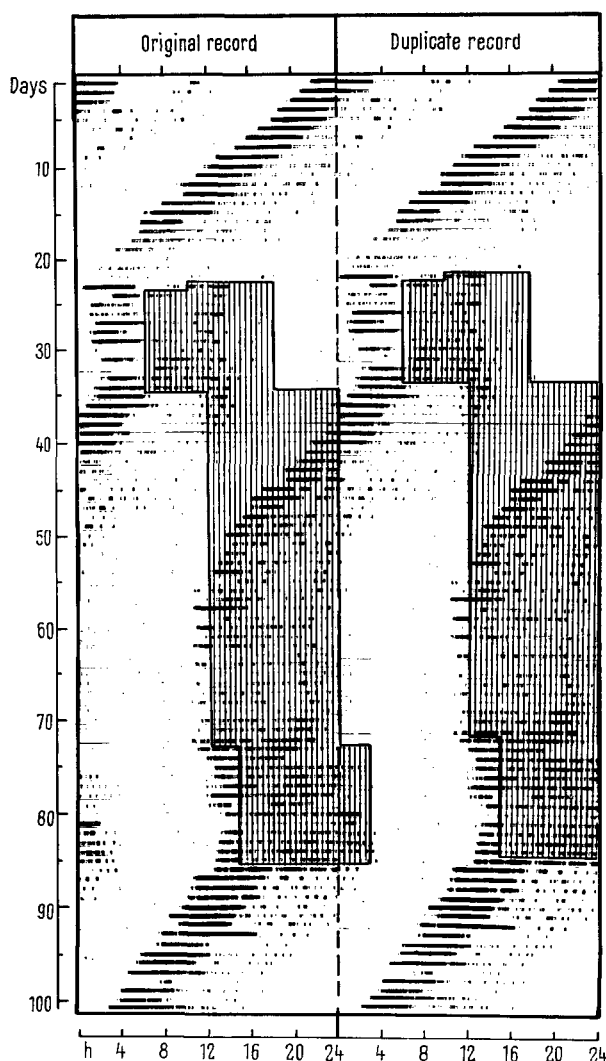
water were supplied continuously; temperature, humidity and light were kept constant throughout the entire experiment. After the establishment of a distinct spontaneous circadian frequency for at least 14 successive days, the species-specific song cycle (12 h song, 12 h silence) was replayed into the boxes from a continuous tape recording over a loudspeaker. In order to avoid secondary effects due to monotonous stimulation, song strophes of different lengths were separated by varying silent periods. The average sound intensity was about 60–80 db.

The circadian rhythms of all 4 birds could be synchronized with the 24 h cycle of song, at least temporarily. The text figure shows a representative example. A spontaneous period of $\tau = 23.0$ h developed during 22 days of constant conditions becomes immediately a cycle of 24 h when, on day 23, the song is introduced. Following a 6 h delay phase-shift of the sound cycle, the activity rhythm of the bird goes through a long series of advancing transients, resulting eventually in another steady-state of entrainment at day 56. After a second delay phase-shift of only 3 h, the activity rhythm becomes resynchronized

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² *Circadian Clocks* (Ed. J. ASCHOFF; North Holland Publishing Company, Amsterdam 1965).

³ J. ASCHOFF, *Z. Tierpsychol.* 15, 1 (1958).



after a few delaying transients. When the bird is returned to constant conditions without sound cycle (day 86) the activity rhythm regains its spontaneous frequency ($\tau = 23.4$ h).

The results demonstrate the synchronizing effect of species-specific song cycles on circadian rhythms of birds in the experimental set-up employed. They provide a clear example of an acoustic (social) 'Zeitgeber' in intact animals. The extent to which species-specific sound production by birds is concerned as a secondary 'Zeitgeber' under natural conditions beside the undoubtedly greater effects of the light-dark cycle cannot at present be evaluated.

Zusammenfassung. Die in konstanten Licht- und Temperaturbedingungen von 24 h abweichende, circadiane Aktivitätsperiodik von 3 Zeisigen und 1 Girlitz, konnte durch Artgesang, der den Tieren täglich 12 h lang in einem 24-stündigen Zyklus über Lautsprecher vorgespielt wurde, synchronisiert werden. Mit diesen Ergebnissen ist nachgewiesen, dass auch akustische (soziale) Reize als Zeitgeber wirken können.

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Locomotor activity rhythm of a Serin. On the left-hand side, successive 24 h strips of continuous record have been mounted beneath each other. On the right-hand side, the original record has been duplicated to provide a better survey. The bird was kept at 18°C and in constant light intensity of 5 Lux. Periods of sound presentation (60 db) are indicated by vertical hatching.

Regeneration of Feline Ventral Roots

Extensive knowledge has been collected on regeneration of mixed peripheral nerves, but very little on the regenerative capacity of spinal roots because insufficient interest has been devoted to the subject. This investigation deals with the ability of feline ventral roots to regenerate after their transection. For surgical restoration of the root continuity end to end, a tubulation technique with a Millipore microfilter was used. Using bioelectrical and histological techniques, functional and anatomical reconstitution of severed lumbosacral ventral root fibres, innervating *skeletal* muscles (the gastrocnemius-soleus muscles), could be demonstrated. Also, functional success could be obtained when approximating severed ventral root stumps from different ipsilateral spinal levels, i.e. L6 to L7 or L6 to S1. This was shown to constitute a technique for by-passing motor activity to levels distal to a cord transection, which might be of clinical value in selected types of spinal injuries. Functional restoration

of severed ventral root fibres innervating *smooth* muscles (the detrusor muscle of the bladder) could also be demonstrated, as judged from bioelectrical and cystometrical data. The fact that no failure was encountered in a series of 23 cats studied, strongly indicates the great capacity of ventral roots to regenerate after their transection.

Résumé. On démontre la capacité de régénération des racines ventrales des nerfs spinaux dont la résection a été pratiquée dans une série de 23 chats. Il y a eu récupération anatomique et fonctionnelle après l'anastomose des racines ventrales innervant des muscles striés et lisses.

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