zunehmenden Inkubationszeiten bis zu 3 h ein sehr starker Aktivitätsanstieg, im späteren Verlauf ein langsamer Markierungsabfall. Die Markierung war nach kurzen Inkubationszeiten auf die Zellkerne selbst beschränkt (Figur 2), um sich nach längeren Zeiten auf den ganzen perikaryalen Bereich zu erstrecken. Durch die RNase-Behandlung wurden gut 90% der gesamten RNS-Aktivität ausgewaschen (Figur 1a). Demgegenüber konnte kein nennenswerter Einbau an 3F-Uridin in die Nervenfaserschichten nachgewiesen werden. Auch nach längeren Inkubationszeiten stieg die Markierung nicht mehr wesentlich an (Figur 1b). Durch die RNase-Verdauung wurde von der bestehenden Faser-Aktivität noch ein geringer Anteil der Markierung entfernt. Bei den absolut niedrigen Silberkornzahlen wird die Markierungsdifferenz nach der RNase-Behandlung jedoch nur in ihrer Tendenz sichtbar. Die Zahlenwerte, die nur wenig über den Werten des «background» liegen, können nicht den gleichen Anspruch auf eine Genauigkeit erheben, wie die entsprechenden Werte im Zellkörperbereich.

Die vorliegenden Ergebnisse lassen folgende Schlüsse zu: Im Nervengewebe findet kein Transport von markierter, makromolekularer RNS von den Nervenzellkörpern zu deren Fasern hin statt, wie er für verschiedene andere Stoffe in Nervenzellen beschrieben wurde[8]. Die geringfügige Markierung über dem Fasergewebe kann möglicherweise daher rühren, dass entweder ein geringer Teil des 3H-Uridins für andere Stoffwechselprozesse umgebaut wurde, oder aber dass das Uridin besonders nach längeren Inkubationszeiten Eingang gefunden hat in die RNS der Mitochondrien, für die in anderen Zelltypen eine DNS-abhängige RNS-Synthese nachgewiesen wurde[11-14]. Nach der RNase-Behandlung bleiben über dem Zellkörperbereich noch etwa 10% der Markierung erhalten; über dem Fasergewebe wird jedoch im Verhältnis hierzu wesentlich weniger aktives Material ausgewaschen. Würde die Faseraktivität von einer RNS der noch vereinzelt im Faserbereich vorkommenden Glia- oder Nervenzellen stammen, wie früher vermutet wurde[10], so müssten durch die RNase-Behandlung auch über den Fasern etwa 90% einer auch nur geringfügigen Menge aktiver RNS entfernt worden sein, was aber nicht der Fall ist. Erst die Klärung des bisher noch nicht in Angriff genommenen Problems, ob die mitochondriale RNS durch RNase digerirebar ist, kann Gewissheit bringen, ob die im Rahmen dieser Untersuchungen nachgewiesene schwache Markierung des Fasergewebes nach 3H-Uridin-Applikation eine spezifische RNS-Markierung ist oder nicht.

Summary. The incorporation of 3H-uridine into the RNA of the perikaryal and fibrous region of the optic tectum of the fish brain was investigated by means of radioautography after incubation times of 11 min to 96 h. There is no transport of RNA from the cell body to the nerve fibres. After RNase-treatment 90% of perikaryal RNA-labelling is washed out, but only a very small amount of the previous very few labelling of the nerve fibres.

H. RAHMANN

Zoologisches Institut der Universität Münster (Deutschland), 15. März 1966.

[11] E. WINTERSBERGER, Hoppe-Seyler's Z. physiol. Chem. *336*, 285 (1964).
[12] E. WINTERSBERGER und H. TUPPY, Biochem. Z. *341*, 399 (1965).
[13] N. R. ELAEV, Biokhimiya *29*, 413 (1964).
[14] D. J. L. LUCK und E. REICH, Proc. natn. Acad. Sci. USA, *52*, 931 (1964).

Occurrence of Carnosine in Skin of Certain Leptodactylid Frogs

The skin and skin glands of many amphibians contain compounds of high physiological activity that presumably function in defensive secretions. During the course of investigations on toxic principles in the extracts from skin of various frogs[1-3], examination of 7 species of the Leptodactylid genus *Eleutherodactylus* and a species of the related genus *Leptodactylus* were carried out. The specimens (Table) were collected in the El Yunque rain forest of Puerto Rico. They were skinned and the skins were extracted with 80% methanol. The extracts were not toxic (subcutaneous administration in mice) nor were they active in causing contracture of smooth muscle[4]. On chromatographic analysis of the extracts of the most common species, *Eleutherodactylus portoricensis*, a Pauli positive (red-orange) material was detected. This color reaction indicated the presence of an imidazole ring, while the color reaction (gray-black) with ninhydrin indicated an amine group. No other chromogenic materials (indoles, phenols) were detected in the extract although lesser amounts of other ninhydrin positive compounds were present. No histamine, serotonin or phenolic alkyl-

Carnosine in skin extracts of frogs of the genera *Eleutherodactylus* and *Leptodactylus* (calculated by comparison with standard carnosine on paper chromatography and Pauli color development)

Species	No. of frogs	mg carnosine per g skin
Eleutherodactylus portoricensis	50	0.8
E. karlschmidti	5	0.5[a]
E. richmondi	1	0.2
E. wightmanae	3	0.5
E. locustus	6	1.0
E. eneidae	4	0.3
E. gryllus	6	1.2
Leptodactylus albilabrius	3	0.1[a]

[a] Also contain small amounts of histidine.

[1] F. MÄRKI and B. WITKOP, Experientia *19*, 329 (1963).
[2] J. W. DALY, B. WITKOP, P. BOMMER, and K. BIEMANN, J. Am. chem. Soc. *87*, 124 (1965).
[3] J. W. DALY and C. W. MYERS, in preparation.
[4] J. PISANO, personal communication.

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-50-2X 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 muscle[6] 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 brain[8], 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.

J. W. Daly and H. Heatwole

National Institute of Arthritis and Metabolic Diseases, Laboratory of Chemistry, National Institutes of Health, Bethesda (Maryland 20014, USA), and Department of Biology and El Yunque Biological Station, University of Puerto Rico, Rio Piedras (Puerto Rico),
June 24, 1966.

[5] V. Erspamer, 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 conditions[2]. 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 well[3]. 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

[1] The research reported in this document has been sponsored in part by the Air Force Office of Scientific Research under Grant No. AF EOAR 64-68, with the European Office of Aerospace Research (OAR), United States Air Force.
[2] *Circadian Clocks* (Ed. J. Aschoff; North Holland Publishing Company, Amsterdam 1965).
[3] J. Aschoff, Z. Tierpsychol. *15*, 1 (1958).