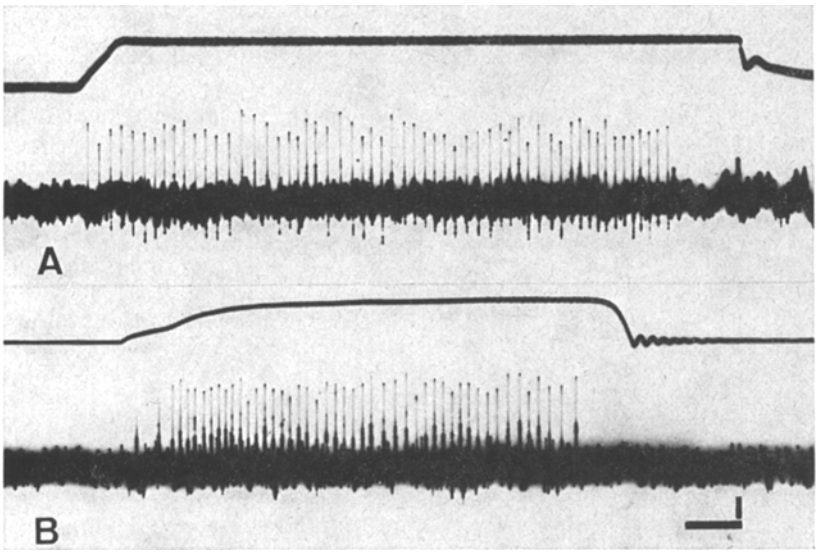

Responses of mesencephalic trigeminal cells to lowering the jaw (A) and to stretching the left masseter muscle (B). In A the record was taken from the left paracommissural pool and in B from the ipsilateral tectum. Upper beam: Mechanogram. Lower beam: trigeminal unitary discharge. Time: 100 msec. Voltage: 100 μV.

settled down to 60–70/sec and ceased as soon as the stretch was released (Figure B). However, when the jaw was lowered the increase in discharge rate of the units during the initial portion of the response did not occur in all the experiments (Figure A). This probably depended upon the fact that the stretching of the masseter was not so quick and sudden as when the muscle was isolated (Figure B). The unitary discharge elicited by the masseterine stretch was blocked during electrically induced contraction of that muscle: this showed that the record was taken from the muscle spindle afferents[11]. The units influenced by lowering the jaw or by the masseterine stretch were unaffected by the stimulation of other trigeminal receptors (face, cornea, teeth, palate).

The present results extend and confirm the anatomical investigations of VEGGETTI and PALMIERI[10] who observed cromatolysis of the trigeminal mesencephalic nucleus cells after cutting the ipsilateral mandibular branch, and degeneration of the masseterine spindles following the destruction of the ipsilateral posterior commissure in *Caiman sclerops*. Thus, the conclusion can be reached that also in reptilians the cells of the mesencephalic trigeminal nucleus represent the first-order neurons of the afferents from the masseterine spindles as is the case for birds and mammals.

Riassunto. Mediante microelettrodi di tungsteno si é registrata l'attività unitaria del nucleo mesencefalico del trigemino in *Caiman sclerops* curarizzato. Le cellule di questo nucleo, silenti in condizioni di riposo, vengono attivate dall'abbassamento della mandibola e dallo stiramento del muscolo massetere omolaterale. Le risposte sono del tipo indotto da fusi neuromuscolari.

C. DESOLE, G. PALMIERI
and A. VEGGETTI

Istituti di Fisiologia Umana
e di Anatomia Veterinaria dell'Università di Sassari,
Sassari (Italy), 10 October 1969.

[11] P. B. C. MATTHEWS, Physiol. Rev. *44*, 219 (1964).

Light Refractive Emergence Rhythm in the Leafcutter Bee, *Megachile rotundata* (F.) (Hymenoptera: Apoidea)

The emergence rhythm of *Drosophila pseudoobscura* Sturtevant is light entrained when exposed to 15 min light signals of about 100 ft-c[1], and although weaker in effect, entrainment may also be achieved through temperature change[2]. Circadian oscillations may be readily entrained in poikilotherms by temperature[3] but each of those species studied is more sensitive to entrainment by a LD cycle. Experiments on the leafcutter bee, *Megachile rotundata* (Fabr.), suggest that the emergence rhythm in this species is unresponsive to light.

M. rotundata is a solitary species which diapauses as a prepupa in a dense, tightly spun cocoon, enclosed in a cell composed of one or more layers of leaf cuttings, which in turn is secreted in a light-tight cavity or hole. The prepupae may be maintained in diapause (at approximately 7 °C) for as long as 2 years and diapause can be broken by incubating the prepupae at temperatures above 17 °C[4]. First emergence will begin about 15 days after a diapausing population has been transferred to 32 °C and emergence will continue for about 10 days thereafter. As the bee chews through the cell in the photophase of the LD regime, which usually takes a matter of minutes, it is exposed to light for the first time.

[1] C. S. PITTENDRIGH, Proc. natn. Acad. Sci. *40*, 1018 (1954).
[2] V. G. BRUCE and C. S. PITTENDRIGH, Am. Naturalist *91*, 179 (1957).
[3] W. F. ZIMMERMAN, C. S. PITTENDRIGH and T. PAVLIDIS, J. Inst. Physiol. *14*, 669 (1968).
[4] W. P. STEPHEN and C. E. OSGOOD, J. econ. Ent. *58*, 218 (1965).

In the following experiments, diapausing prepupae were placed in a chamber which in turn was held in a constant 32 °C controlled environment room. The temperature within the chamber was continuously monitored and fluctuated ± 0.25 °C. The temperature pulse was effected by transferring the bees to an 18 °C chamber for 24 h. Light was provided by white light fluorescent tubes and emergence counts were made hourly. An emergence rhythm which can be phase-set by a temperature pulse, but which is apparently refractive to light, is herein reported for this species.

2400 bee prepupae were permitted to develop at a constant temperature of 32 °C and exposed to a daily LD 12:12 (150:0 lux) to determine if the LD regime entrained the emergence rhythm of *M. rotundata*. Shortly after the first emergence was noted the entire population was subjected to a 14 °C cold pulse for 24 h in order to synchronize the population. The cold pulse was initiated in the middle of the L-period so as to evaluate the conflicting effects of light and temperature upon the entrainment of emergence rhythms. The data recorded in Figure 1, indicate that the bees emerging prior to the cold pulse were not synchronized by the LD regime into which they emerged. The single 14 °C cold pulse however resulted in an immediate synchronization of the emergence rhythm. The rhythm for the 8 days following the cold pulse had an average periodicity of 23.25 h. Further, the emergence occurred principally in the photophase portion of the photoperiod immediately after synchronization, but in the 4th and subsequent days the emergence peak had moved to the left and occurred in the late scotophase and early photophase portions of the LD cycle. It is thus apparent that the emergence rhythm is free-running in an LD 12:12 regime for the initial emergence is not entrained, and following cold pulse synchronization the daily emergence peaks drift to the left and are not reset in the LD regime.

In a second experiment 2400 bee prepupae were permitted to develop at a constant 32 °C in LL (108 lux). 400 bees were used as controls and the remaining 2000 subjected to a single 14 °C cold pulse for 24 h. The single temperature pulse again resulted in a synchronization of the emergence rhythm and the average period during the 8 days in which observations were made was 22.67 h (Figure 2), not significantly different from the average periodicity under LD conditions ($P = 0.05$). The distribution of emergence on day 8 was spread over a 22 h period indicating many of the emerging bees had not been phase-set by the temperature shock and the emergence in the population was again reverting to a non-synchronized state. The controls showed no evidence of rhythmicity under LL regime.

In a third experiment diapausing *M. rotundata* prepupae were removed from their cells and placed in No. 5 gelatin capsules, sealed at one end with paraffin. The capsules containing diapausing pupae were then placed in Petri dishes which were held at 30 °C under red light. As the bees developed 150 completely pigmented pupae and 100 pre-emergent adults were exposed to a 12 h light pulse of 320 lux. The pre-emergent adults were then transferred to an emergence apparatus[5] from which the time and the number of emerging bees was recorded. The prepupae were returned to the incubator until the pre-emergent adult stage was reached and then transferred to the apparatus for emergence. Temperature during the entire incubation, light pulse, and emergence periods was carefully maintained at 30 °C.

The first light pulsed pre-emergent adults emerged on day 1, whereas the first light pulsed pupae did not appear until day 4 (Figure 3). Emergence of light pulsed pupae and pre-emergent adults showed no evidence of synchronization (Figure 3).

The manner in which the figure is composed suggests a 'movement' to the left of the emergent rhythm of those insects given a light pulse while in the completely pigmented pupal stage (BP, Figure 3). The artifact is due, in

[5] D. G. TWEEDY and W. P. STEPHEN, Ann. ent. Soc. Am., in press.

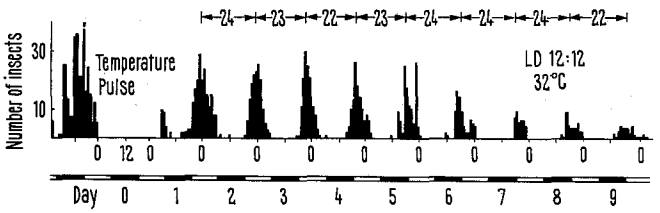

Fig. 1. Effect of a single temperature pulse on the emergence rhythm of *Megachile rotundata* in an LD 12:12 (150:0 lux) regime. (Period determined by the number of hours between the mean emergence in each peak.)

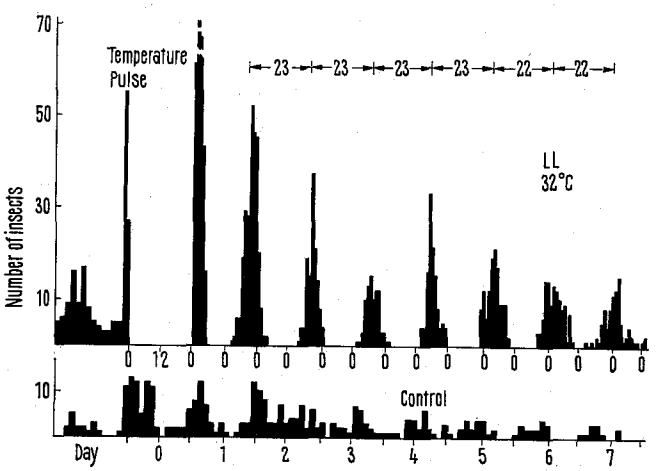

Fig. 2. Effect of LL (108 lux) on the emergence rhythm of *Megachile rotundata*. (Control insects checked every 2 h.)

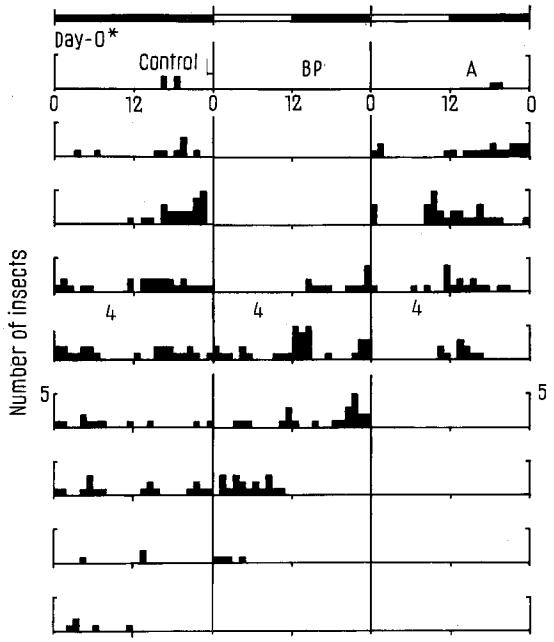

Fig. 3. Effect of a single 12-h light pulse (320 lux) on pigmented pupae (BP) and pre-emergent adults (A). Day 0 is the day the single 12 h light pulse was effected.

part, to the diagram and in part to the short period of emergence. Actually, emergence was continuous from 14.00 day 3 to 11.00 day 6, and shows no light pulse effect.

Single transients are evident in the emergence immediately following the temperature pulse which differs substantially from the recurrent intervals which follow. Such transients are common to most organisms whose rhythm is synchronized by a temperature shock and was anticipated in the bees.

The emergence of bees from populations exposed to LL, LD or DD regimes were not synchronized in the absence of temperature shock[6].

Zusammenfassung. Erniedrigung der Temperatur auf 14 °C erlaubt eine weitgehende Synchronisation des Schlüpfens der Imagines der Blattschneiderbiene *Megachile rotundata*. Die Lichtverhältnisse haben keinen Einfluss auf den Schlüpfrhythmus.

D. G. Tweedy and W. P. Stephen

Department of Entomology, Oregon State University, Corvallis (Oregon 97331, USA), 17 November 1969.

[6] Technical Paper No. 2768 Oregon Agricultural Experiment Station. This work was supported in part by U.S. Public Health Service Grant No. 00040.

Influence of Medium Viscosity on the Oxygen Consumption of Rat Bone Marrow Cells

While dextrans and other large macromolecules have been used extensively for separation of blood cells[1–4], little is known about the effects of these large molecules on the metabolic activity of the separated cells. The large macromolecule dextran would be expected to produce changes in medium viscosity, cell volume, membrane hydration and because of this to influence metabolic activity. The possibility that medium viscosity[5], and cell volume[6], may alter cellular metabolic activity has been suggested.

Materials and methods. Male Holtzman rats, 42 ± 2 days age, were used in all experiments. Dextran solutions of 1, 3, and 5% 39,500 mol. wt. dextran (lot 8687, Pharmacia, Sweden) was used. 1, 3, and 5% dextran solutions in standard Tyrode's medium were also prepared using 139,000 mol. wt. clinical grade H dextran (lot H 1158, Pharmachem., Pennsylvania) and 228,000 mol. wt. clinical grade HH dextran (lot HH 82262, Pharmachem.). The osmolarity of the different dextran solutions was adjusted to 310 mOsm/l with NaCl and the pH adjusted to 7.35. Determinations of cell counts and size were made on a Coulter Counter Model B coupled to a Model J Coulter chart recorder. Aliquots were placed in a Model 53 biological oxygen monitor for measurements of O_2 consumption at 37 °C.

Results. Figure 1 shows the O_2 consumption of bone marrow cells in the various media used in this study. Stimulation of O_2 consumption of bone marrow cells occurred only in Tyrode's medium containing 3% 139,000 mol. wt. dextran. Figure 2 shows the viscosities of the different percent and mol. wt. dextrans employed.

Typical size distribution graphs of rat bone marrow cell populations registered by the Coulter counter plotter compromise 3 distinct groups (Figure 3). Differential counts and measurements showed that the bone marrow cell population consisted of 3 groups. Group 1, mature erythrocytes and a small number of small lymphocytes; Group 2, nucleated RBC and lymphocytes; and Group 3, mature granulocytes and a few large blast cells forms.

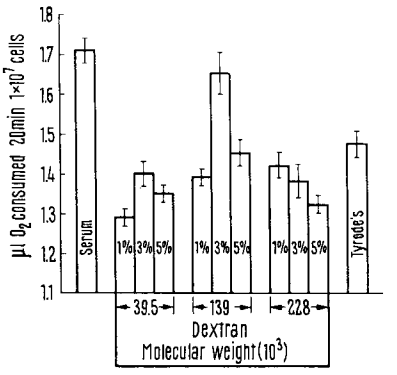

Fig. 1. Effect of different media on rat bone marrow oxygen consumption. Total cell concentration 8×10^7/5 ml medium. Each histogram bar represents the mean of 10 experiments. The lines indicate mean ± 2 S.E.

[1] W. A. Skoog and W. S. Beck, Blood *11*, 436 (1956).
[2] J. W. Goodman, Expl. Cell Res. *21*, 88 (1960).
[3] S. K. Hilal, D. G. Mosser, M. K. Loken and R. W. Johnson, Ann. N.Y. Acad. Sci. *114*, 661 (1964).
[4] J. H. Morrison, Br. J. Haemat. *13*, 229 (1967).
[5] C. J. Hedeskov and V. Esmann, Blood *28*, 163 (1966).
[6] R. M. Gesinski, J. H. Morrison and J. R. Toepfer, Aust. J. biol. Sci. *21*, 1319 (1968).