

so that the muscle could be easily separated from cuticle and other tissues. In some insects, the muscle was dissected away from the cuticle at liquid nitrogen temperatures. For frogs, the animals were anaesthetised with Sandoz-MS222 (0.1 g/l water) and the skin was removed from the hind limb, the gastrocnemius muscle was separated from the other muscles and freeze-clamped: the dissection was complete within 30 sec. For dogfish, the animals were anaesthetized by i.v. (caudal vein) of Nembutal (0.1 ml/kg), a piece of white muscle was rapidly dissected and freeze-clamped. The dissection was completed within 30 sec. For the birds, the animals were anaesthetized by an i.p. injection of Nembutal, feathers and skin were rapidly removed from above the pectoral muscles and a piece of pectoral muscle was dissected and immediately freeze-clamped. From the initial incision to the freeze-clamping of the muscle, the procedure was completed within 5 sec. For rats and mice, the animals were anaesthetised with ether, the skin was dissected from the hind limb and the exposed muscle was freeze-clamped in situ. The muscle was dissected away from the bone at liquid nitrogen temperatures. The frozen muscle was powdered in a percussion mortar at -70°C , and the powdered muscle was extracted by adding 4–5 volumes of frozen HClO_4 (6% w/v). The extraction took place in a mortar and continual mixing with the pestle thawed the mixture of HClO_4 and frozen muscle powder. The precipitated protein was removed by centrifugation and the extract was neutralized with 3 M-KHCO₃. Citrate was measured in the neutralized extract enzymatically⁹.

Results and discussion. For 21 species of animals from several phyla, the contents of citrate show considerable variation (Table). The contents of citrate range from 0.036 to 2.084 $\mu\text{mol/g}$ fresh weight (snap muscle of scallop and the flight muscle of rosechafer).

The mean values of the citrate content in the insects investigated is 1.07 $\mu\text{mol/g}$ fresh weight, whereas the mean value of the rest of the muscles investigated is 0.134 $\mu\text{mol/g}$ fresh weight, i.e. about 8-fold lower.

The variation of the content of citrate in the flight muscle of insects is about 5-fold, and if the water bug and the cockroach are excluded from consideration, the variation is only 2-fold.

The variation in the rest of the muscles examined is about 9-fold.

The results also indicate that there is a clear difference in the citrate content between the aerobic and anaerobic muscles, so the content of citrate of the aerobic muscles is much higher than that of anaerobic muscles. However, flight muscle PFK is not sensitive to citrate^{10,11}, therefore the importance of high citrate content of the insects' flight muscles is not clear.

It must be stressed that the measurements reported here were made on whole muscle preparations. Therefore there is no indication of the citrate content in the various cell compartments and consequently available to the different enzymes.

Unfortunately, at the present time satisfactory methods for measurement of intermediates within different cell compartments are not available and any interpretation that involves the use of a precise concentration of a metabolic intermediate must be made with caution.

⁸ I. BEIS and E. NEWSHOLME, *Biochem. J.* 152, 23 (1975).

⁹ H. MOELLERING and W. GRUBER, *Analyt. Biochem.* 17, 369 (1966).

¹⁰ P. R. WALKER and E. BAILEY, *Biochem. J.* 111, 365 (1969).

¹¹ E. NEWSHOLME, P. SUGDEN and T. WILLIAMS, *Biochem. J.*, in press (1976).

ATP Reception by the Tsetse Fly, *Glossina morsitans* West.

B. K. MITCHELL¹

Department of Entomology, The University of Alberta, Edmonton (Canada T6G 2E3), 10 September 1975.

Summary. Electrophysiological studies of the labellar sensillae of *Glossina morsitans* show that one cell in each LR7 sensillum responds to ATP at concentrations from 10^{-3} to 10^{-5} M.

Adenosine triphosphate (ATP) is an important feeding stimulant for mosquitoes^{2,3}, a flea⁴, a tick⁵ *Rhodnius prolixus*⁶ and the tsetse fly⁷. This implies that these animals possess a chemoreceptor sensitive to ATP. Physiological evidence for such a receptor in *Glossina austeni* was sought by RICE et al.⁸, and they conclude that a cell sensitive to ATP is housed in one of the labellar sensilla. No positive identification of the sensillum could be made however because they recorded from a nerve containing the axons of many receptors while stimulating the labellar lobes. Here I present evidence for an ATP-sensitive cell in the largest of the labellar sensilla, called the LR7 sensilla⁸.

Preparation and electrophysiological methods. When a fly is not probing the LR7 sensilla are protected by the labellar lobes, and are not visible externally. To expose them for recording, a small gauge wire is tightened around the bulbous, proximal part of the haustellum of a CO₂ anesthetized fly. This part of the haustellum contains the retractor muscles of the labella which, on contraction, cause the labella to evert exposing the armature (presto-

mal teeth and rasping surfaces) and the LR7 sensilla⁹. The tightened wire probably causes these muscles to contract and, since the wire is left in place, the labella remain everted. In this manner 1 to 4 of the eight LR7 sensilla were made accessible.

¹ Acknowledgments. I thank Dr. R. H. GOODING for suggestions on the manuscript, and for tsetse flies. This work was supported by the U.S. army, Medical Research and Development Command.

² TERUHIKO HOSOI, *Nature, Lond.* 181, 1664 (1958).

³ RACHEL GALUN, Y. AVI-DOR and M. BAR-ZEEV, *Science* 142, 1674 (1963).

⁴ RACHEL GALUN, *Life Sci.* 5, 1335 (1966).

⁵ RACHEL GALUN and S. H. KINDLER, *J. Insect Physiol.* 14, 1409 (1968).

⁶ W. G. FRIEND, *Can. J. Zool.* 43, 125 (1965).

⁷ RACHEL GALUN and J. MARGALIT, *Nature, Lond.* 222, 583 (1969).

⁸ M. J. RICE, RACHEL GALUN and J. MARGALIT, *Ann. trop. Med. Parasit.* 67, 101 (1973).

⁹ B. JOBLING, *Parasitology* 24, 449 (1933).

The small size of these sensilla (15–20 μm long) frustrated earlier attempts to record from them individually⁸. In the present study, a compound microscope with a long working distance objective provided 780 \times magnification. This allowed the placement of a pipette containing the stimulus directly over a single LR7 sensillum. The same pipette always contained 0.15 M NaCl in addition to the ATP, and also served as the recording electrode¹⁰. The solution was adjusted to pH 7.2 to 7.4 with NaHCO_3 since behavioural studies have shown that pH changes affect the feeding response elicited by ATP¹¹. Optimal feeding was also obtained on solutions near the tonicity of blood¹², hence the choice of 0.15 M NaCl.

Recordings were made with a lab constructed pre-amplifier employing an operational amplifier with an FET input as the head stage (Analog Devices 40J). The recording circuit was completed through the preparation via a grounded reference electrode inserted into the proximal end of the excised haustellum. The output of the preamplifier was further amplified and, the records were placed on magnetic tape and also recorded on a Honeywell 1858 oscillographic recorder for visual analysis.

Results. The records in the Figure were obtained from 3 sensilla, each from a different teneral (unfed) fly, 4–9 h old. In sensillum I, the response to 0.15 M NaCl was from 1 cell, firing at an average of 9 spikes per sec for the first 3 sec. This is the typical response of this type of cell to

0.15 M NaCl. When 10^{-3} M ATP is applied, the response of an ATP-sensitive cell is clearly seen (record b). The response is quite different from record a) and, judging from their waveform, the spikes in record b) are from a different cell than the one firing in record a). Record c) shows that the response to 0.15 M NaCl was essentially unaltered by the ATP application.

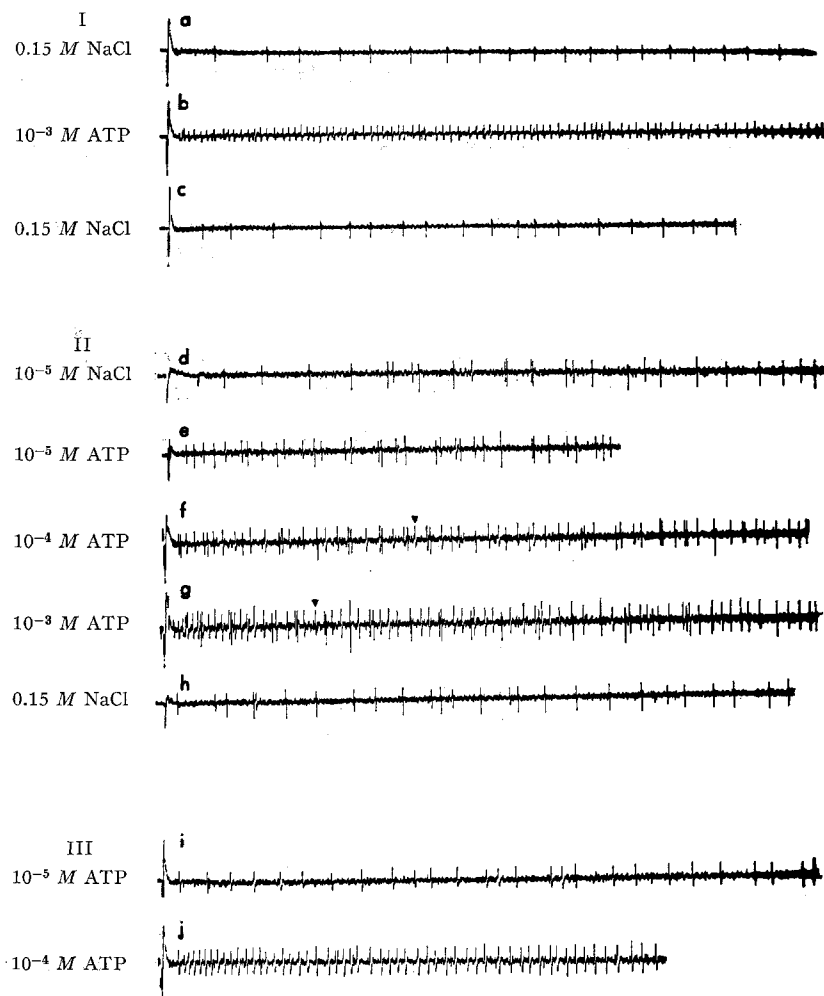
Records d) to h) show the results of 5 successive applications to a single LR7 sensillum on a teneral female. There is clearly more activity as the ATP concentration is increased (e–g). The predominant spikes in records e–g are from the ATP-sensitive cell. They are on average more strongly monophasic and, at faster sweep speeds than shown here, have a longer time course than the 2 spikes in records d) and h).

Records i) and j) are from a 3rd preparation. In this instance the response of the ATP-sensitive cell to 10^{-5} M ATP (record i) was more clear than in the previous preparation (record e). Record i) was obtained from the initial application of any kind to the sensillum, and

¹⁰ E. S. HODGSON, J. LETTVIN and K. D. ROEDER, *Science* 122, 417 (1955).

¹¹ RACHEL GALUN and J. MARGALIT, *Trans. R. Soc. trop. Med. Hyg.* 64, 171 (1970).

¹² P. A. LANGLEY and R. W. PIMLEY, *J. Insect Physiol.* 19, 1097 (1973).



Electrophysiological recordings from LR7 sensilla on 3 animals (I, II and III). For each preparation the recordings were made successively from a single sensillum with 3 to 5 min allowed for disadaptation between stimulus applications. The chemical(s) applied are given at the left of each trace. The arrows each indicate an example of a spike from the ATP-sensitive cell. The large deflection at the beginning of each record is an artifact resulting from the stimulus application. The time bar represents 1 sec.

record j) was obtained from the same sensillum 4 min later. Again, a clear increase in firing frequency is seen with an increase in ATP concentration. The other two cells were silent in this sensillum.

RICE's morphological evidence⁸ shows 3 dendrites in each LR7 sensillum, one of which is probably a mechanoreceptor¹³. No type of movement of the sensillum caused an increase in firing of any of the cells recorded from here, so the mechanoreceptor remains to be identified electrophysiologically.

On the basis of the evidence presented here, I believe that one of the cells is sensitive to ATP, though its specificity for ATP and related compounds remains to be determined. It is possible that the remaining cell is sensitive to salt (unpublished results). This cell is apparent in records a), c) and d)–h) in the Figure.

In conclusion, it should be mentioned that the preparation used in this study is far from ideal. Less than 50% of the preparations gave a response at all. A very small stimulus artifact, seen on application of the pipette, is often associated with these 'silent' sensilla, possibly indicating that the connection to the inside is very resistive. The LR7 sensilla have a single pore near the tip¹⁴ through which

chemicals gain entry and recordings are made. It is possible that this pore is at times occluded, preventing recordings. This idea gains support from the results on sensillae that do give a response. In many cases the spikes become reduced in amplitude after the first 2 or 3 applications, and shortly thereafter are unrecordable. This is often accompanied by reduction of the stimulus artifact and probably an increased resistance at the pore. Since in the non-feeding fly these sensilla are surrounded by the labellar lobes and probably saliva, the unnatural exposure to the relatively low R.H. (30–40%) under experimental conditions may cause them to dry out, thus explaining the above effects. A preparation involving a restrained animal, with head and haustellum intact, has been developed. This gives a higher percentage of successful preparations and is currently being used to study the specificity and other physiological aspects of the ATP-sensitive cell¹⁴.

¹³ M. J. RICE, RACHEL GALUN and L. H. FINLAYSON, *Nature, Lond.* 241, 286 (1973).

¹⁴ B. K. MITCHELL, in preparation.

Development of Thermoregulation in the Newborn Lesser Bushbaby (*Galago senegalensis moholi*, Smith 1839)

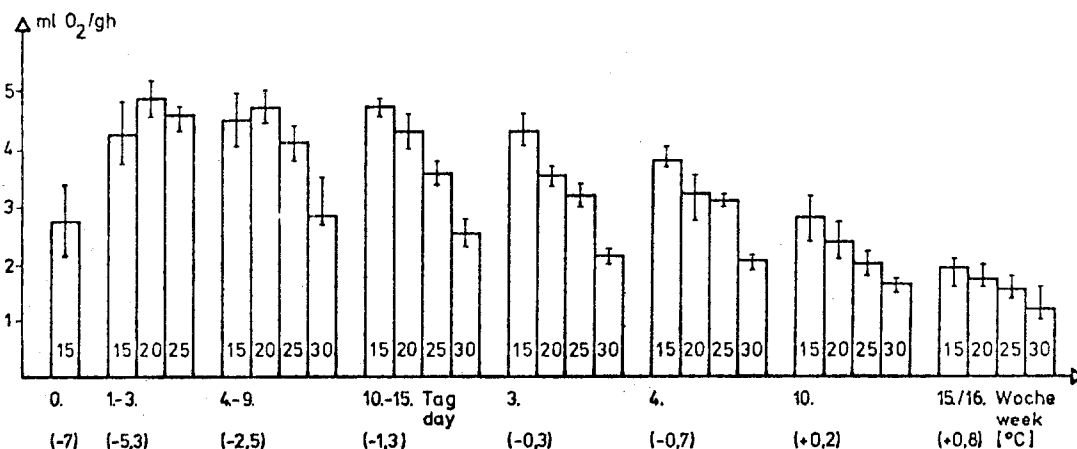
H.-J. DOBLER¹

Institut Biologie III der Universität, Lehrstuhl für Zoophysiology, Abteilung Physiologische Ökologie, Auf der Morgenstelle 28, D-74 Tübingen (German Federal Republic, BRD), 2 September 1975.

Summary. The temperature-regulating system in bushbabies operates from the 1st day of life. The postnatal metabolism decreases from the 5th (2.9 ml O₂/g · h) to the 140th day (0.7 ml O₂/g · h) to the level of the adults.

Within the Lorises, the *Galagos* attain the utmost degree of homeothermy. In this regard they hardly differ from the 'higher primates'. It was the purpose of this work to examine whether their thermoregulatory system operates immediately after birth, or if it only later develops or attains completion. To determine this problem I examined the oxygen consumption (Beckman Oxygen Analyzer G2) and the body temperature (Rectal measurement with an electronic thermometer, Ultracast Inc.)

during postnatal development of 3 young bushbabies born in captivity (twins and a single one). The birth weight of the twins was 12.6 and 13.0 g, that of the single one 14.6 g (the weight of the parents averages 156 g). During the first 6 weeks, the young bushbabies grew fast: by the 8th day the twins had doubled their birth weight. At the age of 35 days they reached 46% and on the 81st day 75% of the adult weight. Up to the age of 140 days, the young ones developed more slowly. The



O₂-consumption in *Galago* twins (means and extremes). To avoid risk to the newborns, only 1 cold exposure (15°C) was carried out on day 0; on day 1–3 metabolism was measured at 15, 20 and 25°C in all other age classes even at 30°C. In brackets: Temperature difference between the beginning and the end of the experiments at 15°C ambient temperature (30 min). Only in the 3rd week did body temperature decrease less than 1°C at 15°C ambient temperature.