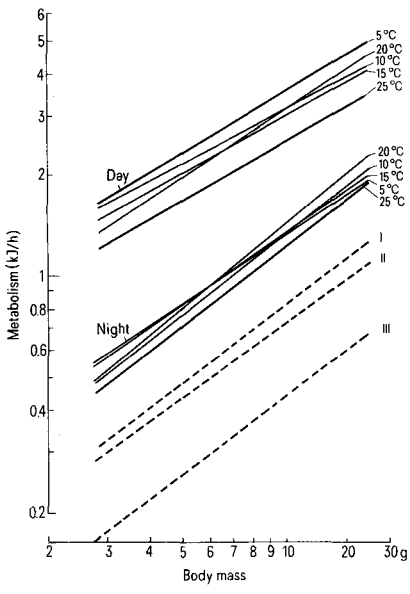

Energy metabolism as a function of body weight at different temperatures in 17 humming-bird species during day and night. Regression lines: I, Dawson and Hudson[3], basal metabolism of passerine birds; II, Prinzinger and Hänssler[1], basal metabolism of nonpasserine birds; III, Aschoff and Pohl[4], nonpasserines.

bigger humming-birds were calmer than the smaller species. This results in the lower regression exponent of the day-time values.

These results show that weight-metabolism regression cannot be established correctly over a wide range of body mass and that a division between a high 'passerine metabolic rate' and a lower 'nonpasserine metabolic rate' is not justified for the small nonpasserine humming-birds; nor does it seem to be justified for other relatively small nonpasserine avian orders.

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Vitellogenin synthesis induced in locust fat body by juvenile hormone analog in vitro[1]

Randa Abu-Hakima[2]

Department of Biology, Group in Eukaryotic Molecular Biology and Evolution, Queen's University, Kingston (Ontario K7L 3N6, Canada), 17 February 1981

Summary. Fat bodies from adult females of *Locusta migratoria* continue to synthesize vitellogenin and other proteins when cultured in vitro. A strong secondary induction of vitellogenin synthesis was obtained in fat bodies cultured in the presence of methoprene, and a weaker but significant primary induction was also obtained using higher doses (> 100 µg) of methoprene.

In many insects, the synthesis of vitellogenin (VG; yolk precursor protein) in the fat body is regulated by juvenile hormone (JH)[3–5]. Since VG may represent more than 50% of the protein secreted by the stimulated tissue, the system is favorable for the study of the action of JH, a unique sesquiterpenoid hormone, at the cellular level. In order to establish freedom from interactions with other tissues, as well as precise timing and control of conditions, it is important to obtain hormonal induction in fat body isolated in vitro. Although the induction of VG synthesis by steroid hormones in tissues of other animals has been achieved in vitro (by ecdysterone in mosquito fat body[6]; by estradiol in amphibian liver[7,8]), only preliminary, poorly reproducible data on induction by JH in vitro have heretofore been reported[9]. We now describe the strong secondary induction, and the weaker but significant primary induction of the synthesis of VG by a JH analog added to cultured fat body from African migratory locusts.

Locusta migratoria migratorioides was reared in the laboratory as previously described[10,11]. In order to eliminate endogenous JH, instead of using surgery, the corpora allata were destroyed by treating female locusts, within 12 h after

Table 1. Secondary stimulation of protein synthesis in locust fat body by methoprene added in vitro

Dose of methoprene in vivo (µg)	Level of methoprene in vitro (µg/ml)	Culture time (h)	Incorporation of ^{3}H-leucine (cpm/mg tissue protein/3 h)		VG synthesis (% of total)
			Total protein	VG	
100	0	3	1671 ± 180	167 ± 30	10
100	0	48	1251 ± 111	146 ± 52	12
100	10	48	6441 ± 106	3682 ± 299	57
100	50	48	7040 ± 326	3927 ± 223	56
60	0	3	1890 ± 205	69 ± 17	4
60	0	48	1887 ± 132	127 ± 31	7
60	10	48	4399 ± 470	1829 ± 38	42
60	50	48	6131 ± 536	2481 ± 280	41
30	0	3	1262 ± 96	29 ± 16	2
30	0	48	1377 ± 102	84 ± 27	6
30	10	48	2250 ± 892	733 ± 142	33
30	50	48	6317 ± 713	1938 ± 186	31

Precocene-treated adult female locusts were injected with methoprene and then kept for 14 days for decay of the primary stimulation. Fat bodies were then removed and cultured with and without methoprene. Proteins synthesized and secreted into the medium were assayed by the incorporation of ^{3}H-leucine during the last 3 h of culturing. Data are means ± SEM from groups of 4 fat bodies individually cultured.

Table 2. Primary stimulation of protein synthesis in locust fat body by methoprene added in vitro

Level of methoprene in vitro (µg/ml)	Culture time (h)	Incorporation of ^{3}H-leucine (cpm/mg tissue protein/3 h) Total protein	VG	VG synthesis (% of total)
0	3	1025±87	52±17	5
0	72	1213±51	61±6	5
100	72	2376±157	718±69	30
200	72	2987±320	1243±201	42

Fat bodies were taken from adult female locusts 14 days after precocene treatment, and cultured with and without methoprene. Data are means and SEM from groups of four.

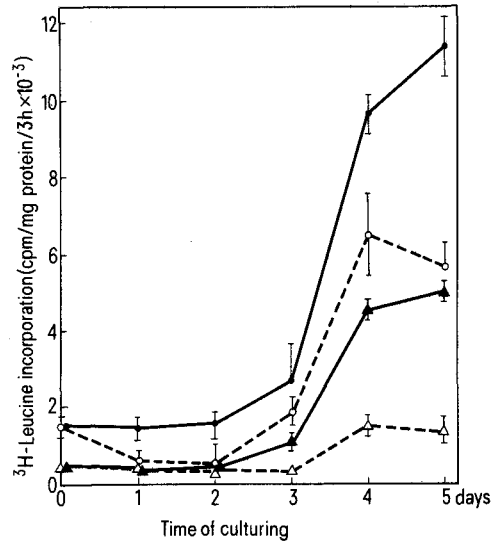
emergence as adults, with 1 mg of ethoxy-precocene[12], applied topically to the neck membrane in 10 µl acetone. No VG could be detected in hemolymph samples taken 14 days later and tested by immunodiffusion or rocket immunoelectropheresis with anti-VG serum. Ethoxy-precocene was almost equally effective at 0.5 mg/locust, and was more active than precocene 2, which has been shown to inactivate the corpora allata of *Locusta* larvae[13,14]. After precocene treatment, VG synthesis could be induced by the JH analog, methoprene (ZR-515[15]). It was found that lower doses of methoprene were required for injection than for topical application; this finding agrees with a previous report[11,16].

As a test of satisfactory maintenance in vitro, the protein-synthesizing activity of the fat body was measured after different periods of culturing. With fat bodies taken from normal female locusts in peak vitellogenesis (terminal oocyte length 3.5–6 mm[17]), during 4 days of culturing there was only a small decrease (about 27%) in the rate of incorporation of leucine into total protein, and an apparent slight rise in incorporation into VG. Incorporation of ^{3}H-uridine into RNA was also maintained at an essentially constant rate during at least 2 days of culturing. When fat body was taken from females in an early vitellogenic stage (terminal oocyte length 0.8–2 mm), incorporation into total protein and into VG was initially low, but after 3 days of culturing it rose steeply (fig.). The rise was greater and longer maintained when methoprene was present in the medium. This suggests that the acceleration of VG and other protein synthesis that normally accompanies oocyte development was already programmed in the cells at the time of explantation and was expressed during culturing; moreover, this process was enhanced when a JH analog was present in the medium.

When allatectomized or precocene-treated locusts that have been stimulated with methoprene are subsequently exposed to a 2nd hormone analog treatment, the secondary response is accelerated and magnified[11]. In seeking effects of the JH analog on fat body in vitro, this secondary response was examined first. 2 weeks after a primary injection of methoprene, VG synthesis had declined to a low level which varied with the dose (table 1). After 48 h of culturing without hormone, this underwent little change. After 48 h of culturing in the presence of methoprene, however, there was strong increase in incorporation into total protein and a still greater increase in incorporation into VG, so that the VG output rose to 31–57% of the total secreted protein. The magnitude of this response depended on both the primary in vivo dose of JH analog and the concentration used in vitro (table 1). In the greatest response achieved, 57% of the tissue's protein output was VG, which is equal to the proportion observed in fat body from normal vitellogenic females, or insects stimulated with JH analog in vivo.

In further experiments, primary stimulation by JH analog in vitro was obtained (table 2). It must be emphasized, however, that high levels of methoprene and an increased time of exposure (72 h) were required; even under these conditions, the stimulation of incorporation was less than that observed after secondary induction, and not every experiment was successful. A possible explanation for the difference between secondary and primary stimulation in vitro is that primary stimulation would require the de novo synthesis of DNA and ribosomes in addition to VG, and a requirement for other factors, such as neurosecretory hormones[18,19] cannot be exluded.

Although insect epidermis shows modified protein patterns in response to JH added in vitro[20,21], the results presented above represent the 1st consistent induction of synthesis of a defined protein in an insect tissue by a JH analog



The effect of methoprene on the development of protein-synthesizing capacity by cultured fat body from early vitellogenic female locusts (terminal oocyte length 0.8–2 mm). Locusts were disinfected externally by washing successively with Decon (B.D.H., 0.1%), sodium hypochlorite (0.1%), hyamine hydroxide (0.1%) and sterile distilled water. Fat bodies were dissected out and rinsed in S20 medium[23]. Individual fat bodies were cultured in 35 mm diameter plastic dishes (Falcon 1008) with 2 ml of S20 medium containing penicillin-G (125 µg/ml), streptomycin sulfate (100 µg/ml) and rimocidin sulfate[24] (20 mg/ml). Methoprene (1 µg/ml), was dissolved in the culture medium by sonication; glass vessels used with hormone-containing medium were pre-treated with polyethylene glycol[25]. Cultures were incubated in a box humidified with locust saline solution[9] at 30 °C with gentle rotary shaking. After the times shown, fat bodies were rinsed in incorporation medium[10] and incubated for 3 h in 1 ml of fresh incorporation medium containing L-4, 5-^{3}H-leucine (30 µM, 135 mCi/mmole). The medium was then collected and the secreted VG and total protein were isolated by precipitation with specific anti-vitellin serum and with trichloroacetic acid, respectively, and counted[11]. Fat body proteins were assayed by the biuret method; △, no hormone, VG; ○, no hormone, total protein; ▲, with methoprene, VG; ●, with methoprene, total protein. Each point shows the mean±SEM from 4 individual fat bodies.

administered in culture. Success appears to depend chiefly upon satisfactory conditions for maintenance of the tissue. With *Locusta* fat body in M-20 medium[22], it was found that the rate of protein synthesis declined sharply during 1–2 days of culture, and the response to JH analog was quite variable. The response obtained with well-rinsed tissue in a defined medium demonstrates that VG synthesis can be induced in *Locusta* fat body by a JH analog in the absence of any other hormone, a conclusion difficult to establish by experiments in vivo[18,19], although the requirement for additional factors for full primary induction cannot be excluded, since a longer exposure and higher doses of hormone were necessary. The way is now open to several types of study that may throw light on the mechanism of stimulation of gene expression by JH.

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Muscle hexose monophosphate shunt activity following exercise[1]

P. Tullson[2] and R.B. Armstrong[3]

Departments of Health Sciences and Biology, Boston University, Boston (MA 02215, USA), 4 March 1981

Summary. 1 day following prolonged treadmill walking untrained rats showed significant elevations in hexose monophosphate shunt reducing capacity in plantaris muscle. The increases were associated with accumulations of nuclei in the muscle interstitium, suggesting damage to the connective tissue element of the muscle.

Recent work from our laboratory[4] and from those of others[5,6] has demonstrated large increases in the activity of the hexose monophosphate shunt (HMS) in skeletal muscle following injury. We have concluded[4] the enhanced HMS activity that accompanies surgical trauma primarily results from accumulation of cells in the connective tissue, rather than from changes of activity within muscle fibers[6]. Boström et al.[7] also found large increases in the activities of several HMS enzymes in rat skeletal muscles following exhaustive swimming. These authors did not localize the response, and assumed the changes occurred in muscle fibers. Our purpose was to quantify and localize changes in HMS reducing capacity (HMSRC) in rat skeletal muscle following treadmill exercise.

Untrained male Sprague-Dawley rats weighing an average of 341 ± 3 g ($\bar{x} \pm SE$) were assigned to 2 groups: a non-exercise control group, and an exercise group. Animals in the exercise group were walked on a motordriven treadmill at a speed of 10 m $\times$ min^{-1}, without electrical stimulation, until they refused to maintain the speed of the treadmill. The animals walked 3.5–4 h for an average distance of 2.2 ± 0.08 ($\bar{x} \pm SE$) km. Rats from both the exercise and the non-exercise control groups were sacrificed by exsanguination following ether anesthesia at 0, 12, 24, 48 or 96 h post-exercise. HMSRC of the distal portion of 1 plantaris muscle from each rat was determined fluorometrically following the reduction of NADP using glucose-6-phosphate as substrate. Details of the assay were published earlier[4]. HMSRC

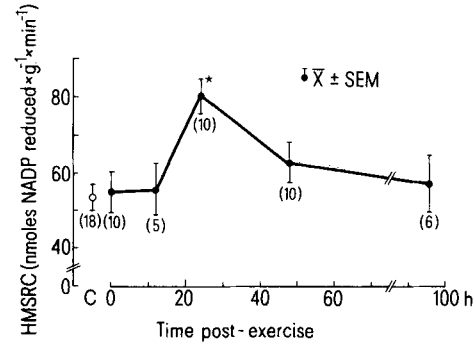

Figure 1. Hexose monophosphate shunt reducing capacity (HMSRC) of non-exercised control (C) and exercised rat plantaris muscles as a function of time following prolonged treadmill walking. Number of experiments in parantheses. * $p < 0.05$.