rate. The mean rate of discharge of the isolated AVN was 72 ± 11/min.

10 CT preparations, when isolated from the musculi pectinati, continued discharging. These were subsequently cut transversely into 2–4 pieces: after variable times (up to 1 min) each discharged at a rate varying between the initial rate of the entire CT and its half value. In 3 of the preparations, 1 CT piece did not show automaticity.

Action potentials with a variable slope of diastolic depolarization were recorded from a limited area of the deep layer of any spontaneously discharging isolated CT-musculi pectinati preparation, as shown in figure 1a. The amplitude of these action potentials was between 80 and 90 mV and their V_{max} between 90 and 140 V/sec. The location of the pacemaker area varied within the CT deep layer. Similar pacemaker potentials were recorded by Paes de Carvalho et al.[6] from the sinoatrial ring bundle, but not from the superficial CT layer. An example typical of action potentials recorded from the deep layer outside the pacemaker region is shown in figure 1b. These action potentials were homogeneous, had a mean amplitude of 114 ± 2.1 mV and a mean V_{max} of 374 ± 14 V/sec.

V_{max} of action potentials were plotted against the take-off potentials of all records of the CT deep layer and a sigmoidal relationship was found (fig. 2). Experimental data are in good agreement with the sigmoidal curve drawn from the equation of Hodgkin and Huxley[9]

$$h = \frac{1}{1 + \exp(V_h - V)/s} \qquad (1)$$

where, according to Weidmann[10], h is the fraction of the highest value observed for the rate of rise, V the take-off potential in mV, V_h the potential at which h is half maximum and s the slope factor. $V_h = 75.3$ mV and $s = 5.78$ were determined according to Noma and Irisawa[11]. The sigmoidal relationship found is similar to that reported by Weidmann[10] in the ventricular Purkinje fibers but largely differs from that in sinoatrial pacemaker fibers[11]. Another resemblance to Purkinje fibers is the relatively steep rate of rise.

The fine morphology and particularly the ultrastructural features of the mitochondria shown in figure 3, demonstrate the excellent in depth preservation of the CT, comparable to the state of preservation of the CT excised from hearts perfused by the Langendorff method[1].

The present findings demonstrate that a) the CT deep layer fibers can act as pacemaker, discharging at a rate intermediate to the rates of the 2 nodes. b) Pacemaker activity, as a property of the deep layer cells, can be observed throughout the CT (all the pieces cut from it discharged spontaneously). c) The high discharge rate recorded from the isolated crista indicates that this area, being inherently faster than the AVN pacemaker, would probably be the faster pacemaker in the absence of SAN pacing. d) From the electrophysiological characteristics a resemblance of the CT deep layer pacemaker potentials and those of Purkinje fibers emerges.

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Metabolism-weight relationship in 17 humming-bird species at different temperatures during day and night

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Summary. The mean metabolic rate during day and night of 17 different humming-bird species is considerably higher than the expected value for nonpasserine birds. The weight-metabolism regression exponent for the night-time is in the same range as that reported for other avian orders (and mammals); 0.73.

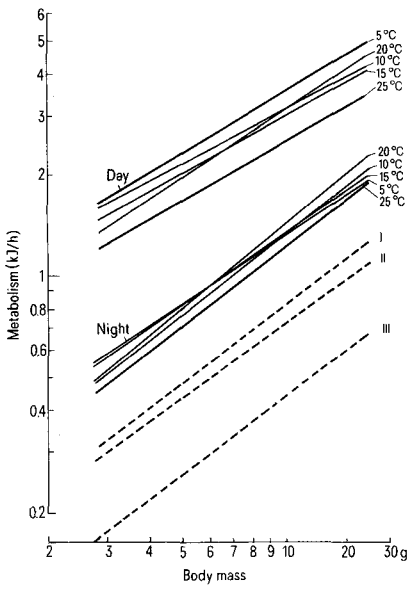
Previous studies have established a higher basal metabolism in the Passeriformes in comparison to the other avian orders (Nonpasseres). Our investigations in 24 different relatively small nonpasserine birds[1], however, showed no pronounced differences relating to the metabolic rate per unit body mass. As a comprehensive analysis of the thermoregulatory process requires adequate knowlegde of the levels of energy production, it was of great interest to study humming-birds, which include the smallest (nonpasserine) bird species.

Materials and methods. The humming-bird species examined are listed in the table. The metabolism of each species was continuously recorded during a period of at least 5 consecutive days (and nights) at different environmental temperatures between 2 °C and 25 °C. Each temperature was tested for 24 h (1 day). The dark-light cycle was 12:12 h; food was provided ad libitum. Measuring instruments: Hartmann & Braun Magnos 2T and Uras 2T (6 channels). For more details see Prinzinger[2].

Results and discussion. Both during day and night, and at all temperatures tested the metabolism of all the humming-birds was considerably higher than the theoretically expected value for nonpasserine birds (fig.). This fact could also be confirmed for nights, in which torpor (strongly reduced metabolic rate) occurred. The mean metabolism-weight regression line of the day-time values follows the equation $M = 0.83 \cdot W^{0.56}$ (M = metabolism in KJ/h and W = b.wt in g). That of the night-time values is $M = 0.67 \cdot W^{0.73}$. The regression exponent of the night-time values corresponds satisfactorily with the results of previous examinations (Dawson and Hudson[3], 0.720; Aschoff and Pohl[4], 0.734; Prinzinger and Hänssler[1], 0.716). During the day-time the

Species, sex, mean body mass and mean metabolic rate at different ambient temperatures of the humming-birds examined. The 1st number in the table is the mean day-time metabolism, the 2nd number the mean night-time metabolism

Species	Sex	Mean body mass [g]	Mean metabolism [J/g · h] at 2	5	10	12	15	20	25 °C
Ocreatus underwoodii	♀	2.7			715		604	507	493
					187		274	199	203
Chlorostilbon mellisugus	♂	2.9		689	636		419	479	428
				159	164		143	184	182
Orthorhyncus cristatus 1	♂	2.9		475	450		416	477	375
				142	106		163	230	132
Orthorhyncus cristatus 2	♂	2.9			456		520	313	300
					183		288	141	102
Archilochus alexandri	♂	2.9		532	598		626		477
				249	298		297		235
Archilochus alexandri	♀	3.3			615		533	535	478
					241		288	228	170
Acestrura mulsant	♀	3.3		575	484		358	487	446
				231	74		58	94	85
Urosticte benjamini	♀	3.9		674	601		596	358	274
				268	349		260	113	120
Trochilus scitulus	♀	4.0		432	450		513	529	513
				152	95		131	207	237
Chrysuronia oenone	♂	5.0		497	500		489	490	431
				162	171		207	179	175
Florisuga mellivora 1	♂	6.0		529	483		466		
				-	146		127		
Florisuga mellivora 2	♂	6.9		525	353		398	488	404
				236	187		225	223	202
Agleactis cupripennis	♀	7.2		388	382	370	306	302	247
				74	135	106	101	116	126
Boissonneaua matthewsii	♀	7.2		410	352	367	310	309	276
				163	206	226	188	179	163
Anthracothorax nigricollis	♀	7.7	458	367	383	386	314	315	268
			196	215	229	244	203	180	143
Eugenes fulgens	♂	7.9	529	453	386		411	367	285
			148	159	191		177	147	117
Lampornis clemenciae 1	♂	8.0					439	378	252
							105	152	71
Lampornis clemenciae 2	♂	8.3		410	360		298	333	302
				159	242		173	170	141
Oreotrochilus estella 1	♂	8.5		351	350		292	276	290
				98	93		117	129	166
Oreotrochilus estella 2	♂	8.9	226	268	267	281	267	296	217
			54	62	60	73	68	108	111
Eulampis jugularis	♂	9.5	396	362	310	305	231	260	204
			132	149	118	164	111	170	108
Patagona gigas	♀	17.5		281	256		240	250	197
				136	99		138	103	94



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]

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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.