important for the process of prostatic involution which occurs periodically in several animal species[18]. The enzymatic changes in our study became evident before changes in the weight of the prostate were noticed. This may be considered as an indication that lowering of the prostatic weight may be secondary to the increase in the activity of 3α-HSO. The mechanism by which MEL is capable of changing the activity of 3α-HSO is at present unknown. However, results from our laboratory[16] on 6 ventral prostates of rats to which MEL was added in vitro in increasing concentrations and which show the same increase of 3α-HSO suggest that it does not involve an increase in protein biosynthesis, since initiation of protein synthesis requires considerably more time.

The existence of a direct stimulatory action of MEL on the prostatic 3α-HSO could also be of interest for the accumulation of DHT in the prostatic tissue which has been observed in the hyperplastic prostate of man[19]. It has also been shown that this process seems to be associated with a fall in the 3α-HSO and a change in the 3α-androstanediol/DHT ratio.

The steroid-biochemical changes seen in this study are comparable with changes in steroid metabolism observed in rodents with intact pineal glands kept under short photoperiod, in constant darkness or blindness because under these conditions MEL is elevated[18,20].

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2 J.A. Kastin, S. Viosca, R.M.G. Nair, A.V. Schally and M.C. Miller, Endocrinology *91*, 1323 (1972).
3 J.E. Martin, J.N. Engel and D.C. Klein, Endocrinology *100*, 675 (1975).
4 J.A. Kamberi, R.S. Mical and J.C. Porter, Endocrinology *88*, 1288 (1971).
5 A.A. MacPhee, F.E. Cole and B.F. Rice, J. clin. Endocr. Metab. *40*, 688 (1975).
6 L.C. Ellis and R.L. Urry, Physiologist *15*, 125 (1972).
7 F. Peat and G.A. Kinson, Steroids *17*, 251 (1971).
8 T.F. Ogle and J.I. Kitay, Neuroendocrinology *23*, 113 (1977).
9 H.-J. Horst and K.-U. Adam, Horm. Metab. Res., in press (1981).
10 J. Kuszak and M. Rodin, Experientia *33*, 283 (1977).
11 P.K. Grover and W.D. Odell, J. Steroid Biochem. *6*, 1373 (1975).
12 R. Moore and J.D. Wilson, J. biol. Chem. *247*, 958 (1972).
13 L. Burić, H. Becker, C. Petterson and K.-D. Voigt, Acta endocr., Copenh. *69*,153 (1972).
14 M. Friedman, J. Am. statist. Ass. *32*, 675 (1937).
15 S.M. Reppert and D.C. Klein, Endocrinology *102*, 582 (1978).
16 K.-U. Adam, doctoral thesis, Hamburg 1981.
17 J.T. Isaacs, J.R. McDermott and D.S. Coffey, Steroids *33*, 675 (1979).
18 H.-J. Horst, J. Steroid Biochem. *11*, 945 (1979).
19 N. Bruchovsky and G. Lieskovsky, J. Endocr. *80*, 289 (1979).
20 G.A. Kinson and F. Peat, Life Sci. *10*, 259 (1971).

Hormonal levels and protein variations during sexual maturation of *Schistocerca gregaria;* effect of rearing temperature

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Summary. As a result of the decrease of diurnal rearing temperature from 33 to 28 °C the following phenomena were induced in adults of *Schistocerca gregaria:* a) In females, a delay in the appearance of maximal levels of JH III and ecdysteroids in the hemolymph, a slowing down of oocyte growth, and an accumulation of hemolymph proteins; b) in males, a decrease of hemolymphatic JH III levels without changes in protein levels.

The decrease of diurnal rearing temperature from 30 to 28 °C seriously disturbs the reproduction of *Schistocerca gregaria.* The results of such a change during the 5th larval instar are a decrease of hemolymph JH III levels in the larva[1], and of the number of functional ovarioles in the adult[2]. Rearing of adults at 28 °C suppresses reproduction: the spermatogenesis is disturbed and the males are sterile[3,4]; in the females, oocyte resorptions are numerous and laid eggs do not develop; under these conditions, neurosecretory products accumulate in the pars intercerebralis and the secretory activity of the corpora allata and corpora cardiaca slows down[5]. The following study describes the effects of a low temperature on the levels of proteins and hormones during sexual maturation.

Material and methods. The insects were reared in groups under following conditions: 12 h light/day; diurnal temperature 33 or 28 °C; nocturnal temperature 20 °C; 30-35% relative humidity. Assay for proteins was performed by the biuret method on hemolymph cleared of hemocytes by centrifugation (9600 × g; 20 min; 4 °C).

Radioimmunoassay of JH III was carried out on 100-200-µl samples of hemolymph obtained from 2-3 females at the same stage of oocyte development. Extraction and RIA procedures were previously described[1,6].

Radioimmunoassay of ecdysteroids was performed on hemolymph and ovaries. Ovaries and eggs were crushed in methanol and ecdysteroids were then extracted by a series of butanol-water partitions[7,8]. Butanol extracts were evaporated to dryness and used in RIA as well as hemolymph extracts, according to the procedure previously described[7-9].

Results. Whatever the rearing temperature was, vitellogenesis started in females when oocytes achieved the length of 1.4-1.6 mm. When the diurnal temperature was lowered from 33 to 28 °C the following was observed: a 5-6-day delay in the onset of vitellogenesis, a 20-day delay in the laying of the 1st ootheca and a reduction of the size of the oocytes at the time of laying (fig. 1, A).

1. *Hemolymph protein levels.* Immediately after the imaginal moult of females, until the 6th day at 33 °C and the 10th day at 28 °C, the protein levels diminished and the oocytes were smaller than 1 mm. This was followed by a rise in protein level. At 33 °C, the blood protein level quickly increased during the 14th and 15th day (oocyte length: 1.6-2.2 mm); until the ovulation, 6 or 7 days later on, the protein concentrations were almost stable (70 mg/ml) (fig. 1, B). At 28 °C, the increase of protein level was accentuated near the 19th day, at the onset of vitellogenesis, and continued for 10 days, the mean values reaching 157 mg/ml (oocyte length 4-5 mm). In spite of a slight decrease during the end of previtellogenesis of penultimate oocytes, the level of proteins remained very high until ovulation (fig. 1, B).

In the males, maintained at both temperatures, after a decrease during the 1st 5 days, a return to the initial values was observed (fig. 2). At 33 °C these values were stable from the 15th to the 25th day; at 28 °C, they temporarily increased between the 15th and the 20th day (80 mg/ml) and then decreased (fig. 2).

2. *JH III hemolymph levels.* In the female reared at 33 °C, JH III levels were rather low or null when the oocytes were smaller than 1 mm. JH III level increased immediately before the onset of vitellogenesis and the curve showed a peak on the 13th day (oocyte length: 1.5 mm). A 2nd period of high values, from the 18th day to the 21st day, coincided with the end of vitellogenesis in terminal oocytes (5.5-7 mm). The levels of JH III rapidly decreased at the time of ovulation (fig. 1, C). At 28 °C, high hormonal levels (92-143 ng/ml) were observed in 25% of females before the deposit of the yolk (oocytes smaller than 1 mm). The number of high values reached a first maximum at the beginning of vitellogenesis (oocyte length: 2 to 3 mm) in females older than 20 days. A 2nd period of such high levels of JH III occured at the end of vitellogenesis (oocytes 4-6 mm). During the ovulation, JH III levels were low; mean and maximal values (250 ng/ml) were of the same order at both temperatures (fig. 1, C).

JH III levels in males reared at 33 °C were low (3 ng/ml) for some days after the imaginal moult; they slowly increased until the 8th day and markedly from the 8th day to the 15th day; high values were frequent until the 25th day in sexually mature animals (fig. 2). At 28 °C, the hemolymph of males was poor in JH III until the 8th day, then the number of high values increased and remained high from the 12th to the 22nd day, and diminished thereafter (fig. 2). Higher individual values varied between 235 and 377 ng/ml at 33 °C and were of the same order (200-325 ng/ml) but rather rare at 28 °C. Mean and individual levels were higher in males than in females. At 28 °C, gonadotropic hormone appeared later, disappeared more quickly and was generally lower than at 33 °C.

3. *Ecdysteroid levels.* Ovaries contained low quantities of ecdysteroids as long as the terminal oocytes were smaller than 4 mm, their ecdysteroid content increased when the oocytes became longer than 5 mm. After ovulation, ecdysteroids were present in the oocytes removed from the oviducts (table).

The ecdysteroid level in the hemolymph of adults reared at 33 °C first increased slowly (day 14-day 18) and then quickly (day 18-day 21) reaching finally maximal values immediately before ovulation (600-1000 ng/ml); after the ovulation no more immunoreactive ecdysteroids were detected in the hemolymph (fig. 1, C). At 28 °C, the levels of ecdysteroids in the hemolymph were nearly the same as at 33 °C; however, individual values (400-600 ng/ml) were generally smaller than in animals reared at 33 °C (fig. 1, C).

Discussion. It is well established in Locusts that the ovarian development requires the synthesis of vitellogenic proteins by the fat body[10-12] and their entry into the oocytes[13-15] in the presence of gonadotropic hormone produced by corpora allata.

In *Schistocerca gregaria,* the gonadotropic hormone is exclusively JH III[16,17]. It is likely that JH levels change considerably during a very short period of time[18,19]; all the assays performed revealed in the 2 sexes reared at 28 and 33 °C a great individual heterogeneity for the same age-group of insects. JH III levels in the hemolymph of females were generally of the same order at 33 and 28 °C.

The maximal JH III levels were recorded in animals reared at 28 and 33 °C when vitellogenesis started in the terminal oocytes and then at the end of development in the same oocytes and/or at the beginning of the development of penultimate oocytes. These fluctuations could be related to the overlapping of consecutive ovarian cycles and may result from the intensive synthetic activity of the corpora allata at the end of the previtellogenetic period of successive oocytes[19]. However, high values were recorded about 10 days later in animals reared at 28 °C. In a small number

The levels of ovarian ecdysteroids in relation to the size of the terminal oocyte (UD, undetectable)

Oocyte size (mm)	pg equiv. ecdysterone/mg dry weight	
	28 °C	33 °C
1-1.9	UD	UD
2-2.9	23.5±2.5	UD
3-3.9	30.5±5.5	UD
4-4.9	52.5±5.5	70.5±25
5-5.9	726±24.6	214.5±99.5
6-6.9	7034±1849	6100±568
7	-	15,758±3324

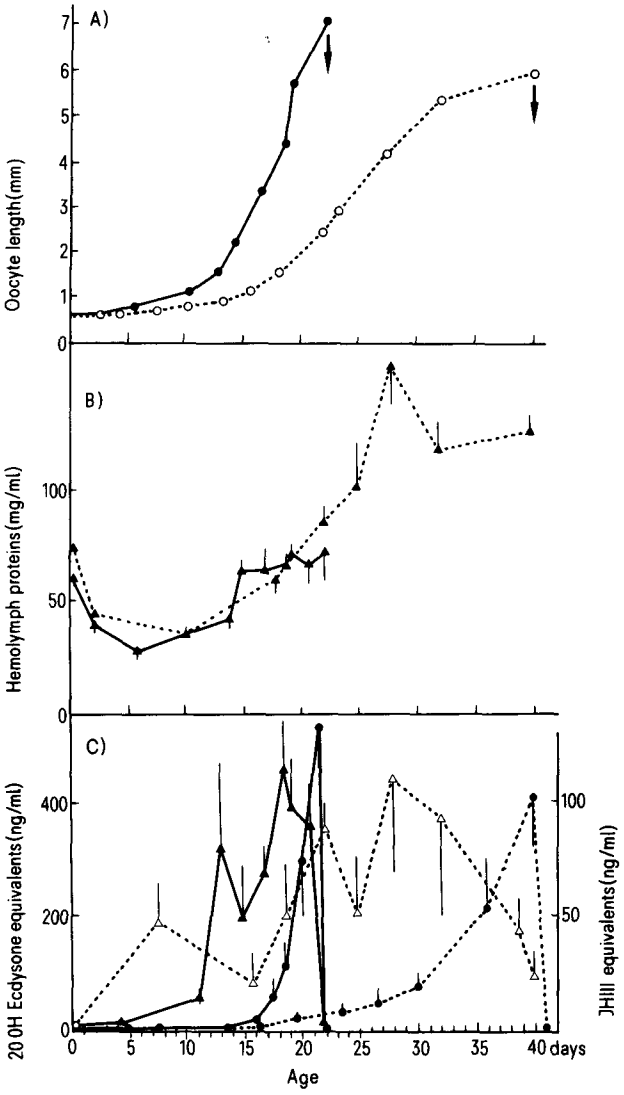

Figure 1. *A* Development of terminal oocytes in adult females. ●——● 33 °C; O - - - O 28 °C. Arrow: The onset of ovulation (and the size of terminal oocytes at this stage). *B* Age-dependent fluctuations of hemolymph protein levels in adult females. ▲——▲ 33 °C; ▲ - - - ▲ 28 °C. *C* Age-dependent fluctuations of ecdysteroid and JH III levels in adult females. Ecdysteroids, ●——● 33 °C; ● - - - ● 28 °C. JH III, ▲——▲ 33 °C; △ - - - △ 28 °C. Bars: SEM.

of females reared at 28 °C, at the beginning of the imaginal life, hormonal levels were relatively high; this phenomenon, not observed at 33 °C, remains unexplained.

At 28 °C, a delay in the post-imaginal evolution of the fat body was also observed[12,20]. Nevertheless, the hemolymph protein level increased abnormally. Incorporation of vitellogenic precursors into oocytes was very much slowed down. These facts are in a good agreement with preceding results[2], showing that during the 2 last larval instars, tissue growth is reduced at 28 °C, in spite of high levels of hemolymph proteins. Thus, decrease of fertility in females reared at 28 °C, which also lay smaller eggs, appears to be due to a slow uptake of proteins by the ovaries, rather than to a lack of proteins. Because of the necessity of a gonadotropic hormone for the uptake of vitellogenin[21-23], the low JH III levels during the 1st 20 days of imaginal life could lead to a weak incorporation of vitellin precursors into the eggs, in spite of a longer stay in the ovary.

The follicular cells were shown to produce ecdysone in *Locusta migratoria*[24-28]. In *Schistocerca gregaria,* oocyte development is accompanied by an increase of ecdysteroids levels in the ovary too. The delay of ovarian maturation and the storage of neurosecretory material in the pars intercerebralis were accompanied by a delay in the production of ecdysteroids at 28 °C.

At both temperatures, the levels of ecdysteroids in hemolymph increased during sexual maturation and decreased quickly at egg-laying. Maximal concentrations observed (10^{-6} M) may be well related with the biological activity of circulating ecdysteroids in females. High quantities of ecdysteroids appear at the moment when rapid decrease of JH III in the hemolymph occurs. This result agrees with a hypothesis of inhibiting activity of ecdysone or 20-OH-hydroxyecdysone on the corpora allata[29,30]. The increase of protein level was recorded before that of the ecdysteroids, and the decrease of ecdysteroid production did not correspond to that of proteins. These facts seem to exclude a direct action of ecdysteroids on the control of vitellogenin synthesis by the fat body.

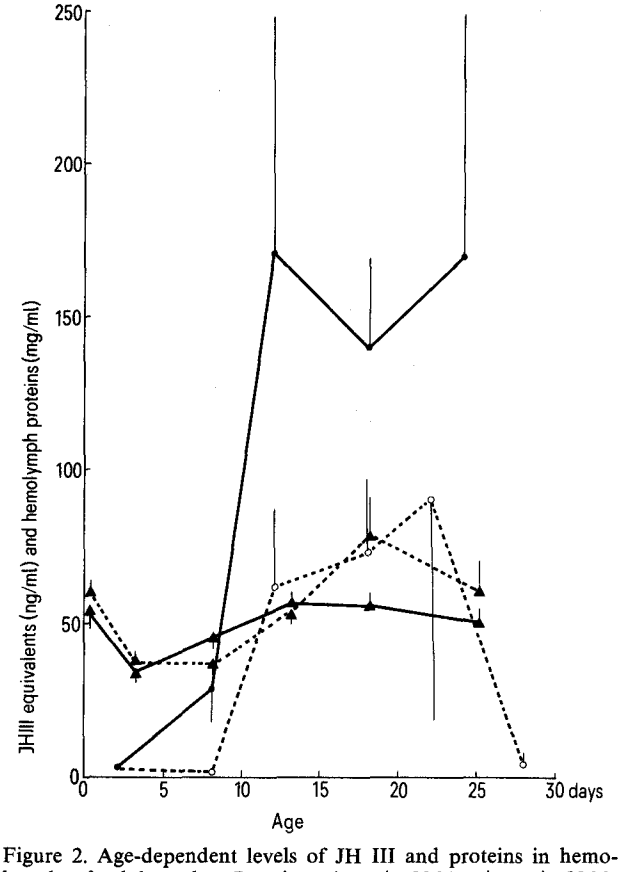

Figure 2. Age-dependent levels of JH III and proteins in hemolymph of adult males. Proteins, ▲——▲ 33 °C; ▲---▲ 28 °C. JH III, ●——● 33 °C; ○---○ 28 °C. Bars: SEM.

Temperature decrease from 33 to 28 °C caused male sterility[3,4], and a 50% reduced production of gonadotropic hormone. This result corroborates previous observations which indicated a low activity of the corpora allata in males reared at 28 °C[5].

The decrease of rearing temperature has important effects on reproduction of *Schistocerca gregaria.* In males, these effects paralleled a decrease of gonadotropic hormone; in females a delay in the appearance of maximal hormonal levels appears to be sufficient for disturbing vitellogenesis. It seems that well controlled levels of endocrine secretions during a short period of time could affect the animal's fertility[31].

1 M. Papillon, P. Porcheron and J.C. Baehr, Experientia *36*, 419 (1980).
2 M. Papillon and A.M. Cantacuzene-Skelezy, Bull. biol. Fr. Belg. *107*, 116 (1973).
3 M. Papillon, S. Lauverjat and A.M. Cantacuzene, J. Insect Physiol. *18*, 3005 (1972).
4 A.M. Cantacuzene, S. Lauverjat and M. Papillon, J. Insect Physiol. *18*, 2077 (1972).
5 M. Papillon, P. Cassier, J. Girardie and M. Lafon-Cazal, Archs Biol. *87*, 103 (1976).
6 J.C. Baehr, P. Pradelles and F. Dray, Annls Biol. anim. Biochem. Biophys. *19*, 1827 (1979).
7 P. Porcheron, P. Cassier and F. Dray, Bull. Soc. Zool. Fr. *1*, 71 (1977).
8 P. Porcheron, Doctoral thesis, University of Paris, Paris 1979.
9 P. Porcheron, J. Foucrier, Cl. Gros, P. Pradelles, P. Cassier and F. Dray, FEBS Lett. *61*, 159 (1976).
10 S. Lauverjat, Gen. comp. Endocr. *33*, 13 (1977).
11 T.T. Chen, P. Couble, R. Abu-Hakima and G.R. Wyatt, Devl Biol. *69*, 59 (1979).
12 P. Couble, T.T. Chen and G.R. Wyatt, J. Insect Physiol. *25*, 327 (1979).
13 R.H. Eliott and C. Gillot, Can. J. Zool. *54*, 185 (1976).
14 A.R. McCaffery, J. Insect Physiol. *22*, 1081 (1976).
15 H.J. Ferenz, J. Insect Physiol. *24*, 273 (1978).
16 K.H. Trautmann, P. Masner, A. Schuler, M. Suchy and H.K. Wipf, Z. Naturf. *29*, 757 (1974).
17 M.M. Blight and M.J. Wenham, J. Insect Physiol. *22*, 141 (1976).
18 B. Lanzrein, V. Gentinetta, R. Fehr and M. Lüscher, Gen. comp. Endocr. *36*, 339 (1978).
19 S.S. Tobe and G.E. Pratt, J. exp. Biol. *62*, 611 (1975).
20 S. Lauverjat, in preparation.
21 R. Abu-Hakima and K.G. Davey, Gen. comp. Endocr. *32*, 360 (1977).
22 R. Abu-Hakima and K.G. Davey, J. exp. Biol. *69*, 33 (1977).
23 P. Fluri, H. Wille, L. Gerig and M. Lüscher, Experientia *33*, 1240 (1977).
24 L. Bang, M. Lagueux, M. Hirn and J.A. Hoffmann, C. r. Acad. Sci., Paris *282*, 1081 (1976).
25 M. Lagueux, M. Hirn, M. De Reggi and J.A. Hoffmann, C. r. Acad. Sci., Paris *282*, 1187 (1976).
26 M. Lagueux, M. Hirn and J.A. Hoffmann, J. Insect Physiol. *23*, 109 (1977).
27 F. Goltzene, M. Lagueux, M. Charlet and J.A. Hoffmann, Hoppe-Seyler's Z. physiol. Chem. *359*, 1427 (1978).
28 C. Hetru, M. Lagueux, L. Bang and J.A. Hoffmann, Life Sci. *22*, 2141 (1978).
29 M.L.M. Carcia, R.P. Mello and E.S. Garcia, J. Insect Physiol. *25*, 695 (1979).
30 B. Stay, T. Friedel, S.S. Tobe and E.C. Mundall, Science *207*, 898 (1980).
31 M.A. Rankin and L.M. Riddiford, J. Insect Physiol. *24*, 31 (1978).