as shown by the following cross-reactions; 5α-androstan-3,17-dione (0.8%), 5α-androstan-3α-ol-17-one (0.16%), 5α-androstan-17β-ol-3-one (< 0.1%) 4-androsten-17β-ol-3-one (< 0.1%), 4-androsten-3,17-dione (0.4%), 5-androstene-3β,17β-diol (0.1%), cortisol (< 0.1%), oestrone (< 0.1%), oestradiol (< 0.1%) and pregnenolone (0.1%). This assay has a sensitivity of 10 pg, an interassay variation of 7.6% and an intraassay variation of 2.8%.

Blood samples were taken from 6 healthy women (aged 19–21 years) immediately before and 24 h after the administration of a combination type pill (Minovlar) containing 1 mg norethisterone and 50 μg of ethinyloestradiol. Samples were taken on 2 separate occasions from each woman, once in the luteal phase of the cycle and once in the follicular phase of the cycle and plasma concentrations of dehydroepiandrosterone were measured by radioimmunoassay following ether extraction (table). The mean plasma concentration of dehydroepiandrosterone was 6.74 ± 5.19 (SD) ng/ml before the oral contraceptive and following the single dose of Minovlar the plasma concentration fell to 4.87 ± 5.1 ng/ml. Analysis using a Student's paired t-test shows that this fall is highly significant ($t = 3.65$, $p < 0.005$). Further plasma dehydroepiandrosterone concentrations were measured in 6 other women, not taking any medication, throughout the menstrual cycle. Like Guerrero et al.[5] we found no significant variation in the plasma dehydroepiandrosterone concentration from day to day throughout the cycle. The mean plasma concentration of dehydroepiandrosterone in these subjects was 6.36 ± 5.31 ng/ml.

These observations suggest that the combination type oral contraceptive does reduce adrenal androgen production since in normal women 90% of the total dehydroepiandrosterone comes from the adrenal cortex[6]. This supports the theory of Beck et al. that reduction in cortisol production in women on the pill is caused by a reduction in ACTH secretion rates and not by any direct action of the oestrogen on the adrenals[7].

Plasma concentrations of dehydroepiandrosterone (ng/ml) directly before and 24 h after administration of Minovlar®

Subject	Before	After
M.W.	5.3	3.9
	2.9	3.2
S.J.	10.0	4.3
	8.0	3.7
P.P.	3.5	2.0
	3.8	2.9
D.H.	3.9	2.4
	2.3	1.7
A.H.	3.2	1.3
	3.4	1.9
J.S.	18.0	16.5
	16.6	14.7

$t = 3.65$, $p < 0.005$. In each case the upper concentrations refer to samples taken in the follicular phase of the cycle and the lower concentrations refer to samples taken in the luteal phase of the cycle.

5 R. Guerrero, T. Aso, P. F. Brenner, Z. Cekan, B.-M. Landgren, K. Hagenfeldt and E. Diczfalusy, Acta endocr. *81*, 133 (1976).
6 G. E. Abraham and Z. H. Chakmakjian, J. clin. Endocr. Metab. *37*, 381 (1973).
7 R. P. Beck, F. Morcos, D. Fawcett and M. Watanabe, Am. J. Obstet. Gynec. *112*, 364 (1972).

Juvenile hormone, vitellogenin and haemocyte composition in winter worker honeybees (Apis mellifera)

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Summary. The haemolymph of winter bees (long life span) in contrast to that of summer bees (short life span) has a low juvenile hormone titre and a high protein titre. It is suggested that the winter bee physiology may be brought about by low JH-production.

Juvenile hormone (JH) seems to be important in the control of polyethism, i.e. the change from hive bee to field bee behaviour and the associated physiological changes. It was shown that the JH titre rises during the transformation and that a low titre of JH favours vitellogenin synthesis, while a high titre inhibits it[1]. Besides high titres of injected hormone (JH III) induce the breakdown of the pharyngeal gland[1] and the changes in the haemocyte composition normally associated with the transformation[2]. Furthermore, they have a negative influence upon longevity. Some of these effects, mainly those on the breakdown of the pharyngeal gland and on vitellogenin synthesis could be confirmed in allatectomized bees[3,4].

Another change in the honeybee physiology, comparable to the change from hive to field bee is the change from summer to winter bee, which normally occurs in September. Winter bees have well developed pharyngeal glands[5], a high protein content of the fat body[6] and an enormous longevity. While summer bees live only for about 30 days, the life span of winter bees has a duration of 6–8 or even 9 months. In analogy to the findings in summer bees, it can be assumed that the physiology of the winter bee may be brought about by a continuous low activity

1 W. Rutz, L. Gerig, H. Wille and M. Lüscher, J. Insect Physiol. *22*, 1485 (1976).
2 W. Rutz, L. Gerig, H. Wille and M. Lüscher, Mitt. schweiz. ent. Ges. *47*, 307 (1974).
3 H. Imboden and M. Lüscher, Rev. suisse Zool. *82*, 694 (1975).
4 H. Imboden, H. Wille, L. Gerig and M. Lüscher, Rev. suisse Zool. *83*, 928 (1976).
5 K. Halberstatt, Annls Abeille *9*, 153 (1966).
6 R. Lotmar, Landwirtschaftl. Jahrb. Schweiz, p. 34 (1939).

Haemocyte composition, JH-activity and protein titres

Date of sampling	Age (days)	Haemocyte composition Categories	Percentage of dmL	Percentage of bees in categories	JH-activity of haemolymph GU/ml	1 µl per pupa: % positive	Protein titre µg BSA/µl	Vitellogenin titre, height of peak (mm)
24. 9. 75	12	Mainly dmL	90.7 } 48.9	43.6	< 295	12	–	–
		Few dmL	16.6 }	56.4	1125	61	–	–
2. 12. 75	80	Mainly dmL	94.9 } 87.1	76.5	< 250	10	88.0 ± 4.0	–
		Few dmL	61.9 }	23.5	< 715	16	89.6 ± 2.5	–
23. 12. 75	100	Mainly dmL	90.3 } 78.0	74.7	< 205	12	91.6 ± 7.1	38
		Few dmL	41.7 }	25.3	<<1000	9	108.3 ± 4.0	40
20. 1. 76	130	Mainly dmL	82.0 } 61.0	53.3	< 375	22	95.3 ± 2.1	35
		Few dmL	37.1 }	46.7	< 710	25	95.8 ± 8.2	33
24. 2. 76	165	Mainly dmL	90.1 } 77.4	66.7	980	53	59.2 ± 3.9	31
		Few dmL	52.0 }	33.3	–	–	61.7 ± 3.8	32
23. 3. 76	195	Mainly dmL	86.7 } 48.4	35.5	1060	61	38.2 ± 1.7	20
		Few dmL	27.3 }	64.5	835	51	39.5 ± 0.6	22
16. 6. 76	12	Summer bees						
		Few dmL	11.6	100.0	1140	62	49.0 ± 4.0	33

of the corpora allata which would allow a relatively high rate of protein synthesis and a persistence of the pharyngeal gland, together with an increase of the life span. It was the aim of the investigation presented here to test this hypothesis.

On 4 September 1975 2 combs with sealed brood were placed in an incubator at 30 °C. The hatched bees were marked with colour spots and immediately reintroduced in the original hive. During the whole winter 1975/76 samples of the marked bees of known age were tested for haemocyte composition, haemolymph protein and vitellogenin titre and for the JH titre of haemolymph.

The haemolymph of each single bee was collected separately. A fraction of each sample was used for a blood cell count. The remaining haemolymph was then pooled from samples of similar haemocyte composition (low or high proportion of dense membrane leucocytes = dmL). The JH titre was determined in the pooled samples with the Galleria wax test[7] and expressed in Galleria units (GU). However, when the sample was too small for a 50% response, the percentage of pupae with a positive response to a dose corresponding to 1 µl of haemolymph per pupa allowed a better comparison of the JH-activity (see table). The haemolymph protein titre was determined in the spectrophotometer at 280 nm and expressed as equivalents of bovine serum albumine (BSA). The vitellogenin titre was determined with the quantitative rocket-immuno-electrophoresis[8] with an antiserum prepared as described by Rutz and Lüscher[9]. All samples were examined on one single agar plate. The values obtained can therefore be compared, although they cannot be expressed in absolute values.

The results are shown in the table. It had already been shown that winter bees generally have an extremely high proportion of normal leucocytes with a dense cell membrane (dmL)[10]. This is confirmed by our results. However, about half the bees which hatched and were sampled in September have a low proportion of dmL like summer bees.

The JH titre is low in December and January, irrespective of the haemocyte composition. At the end of February, the JH titre increases and reaches almost the normal values of flying summer bees towards the end of March. It is interesting to note that before the onset of winter, in September, the 2 categories of bees with high and low proportions of dmL show a drastic difference in their JH titre: the low proportion dmL bees having a high JH titre like summer bees, while the high proportion dmL bees have a low JH titre like true winter bees. It cannot be decided if the bees with a high JH titre at this time can still be transformed into winter bees or if they are really summer bees which die before winter sets in.

The titre of haemolymph proteins is extremely high in winter bees. It is about twice as high as that of summer bees at the time of their highest titre (12 days). It decreases at the end of the winter months as the JH titre rises. The vitellogenin titre is high, but not exessively high, in the winter bees. It corresponds to that of the 12-day-old summer bee, which at this time, according to Rutz et al.[1], has reached its highest vitellogenin titre. The vitellogenin titre decreases later than the protein titre at the end of winter.

Our results are compatible with the hypothesis of winter bee physiology being brought about by low corpora allata activity and low concentrations of JH. In order to confirm this hypothesis, it will now be necessary to measure the hormone titre of artificially induced winter bees and to test if the winter 'diapause' can be terminated by treatment with JH.

7 J. de Wilde, G. B. Staal, C. A. D. de Kort, A. de Loof and G. Baard, Proc. K. ned. Akad. Wet. (C) *71*, 321 (1968).
8 C. B. Laurell, Analyt. Biochem. *15*, 45 (1966).
9 W. Rutz and M. Lüscher, J. Insect Physiol. *20*, 897 (1974).
10 H. Wille and M. A. Vecchi, Mitt. schweiz. ent. Ges. *47*, 133 (1974).