terone into (^{14}C) DHT was determined as radioactivities in DHT and androstanediols fractions after 3–9-min incubation at 37 °C. 5α-Reductase activity of VP from the control animals was 2.97±0.47 pM/mg/protein/min, while those of FA (360 µM)-treated animals was 3.12±0.32. There was no significant difference. The uptake of (^{14}C) amino acid into the nuclear, ribosomal and acid-precipitable cytosol fractions was not inhibited by FA (table 5). However, the uptake of amino acid into the acid-soluble fraction of VP was significantly inhibited. From those results, it seems that FA does not antagonize the binding of androgens with the receptor nor the nuclear acceptor. It does not inhibit the conversion of testosterone to DHT. FA may act on the amino acid uptake at the membrane level. Albeit the real mechanism remains to be solved, it is interesting to note that this compound has a distinct anti-androgenic activity only on the prostatic glands in the rat.

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Are there cyclic variations in estradiol secretion in the non-pregnant rabbit?*

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Summary. Plasma estradiol-17β (E2) concentration was measured in 5 adult non-pregnant rabbits each in 3 different seasons (January, April and September). Blood samples were taken from each rabbit every other day. There was a considerable variation in plasma E2 levels from one sampling day to another, irrespective of the season. The pattern of variation in E2 levels in individual rabbits tended to be cyclic and this cycle was roughly of the order of 8 days. There was no correlation between changes in E2 levels and those in the vaginal appearance.

The non-pregnant adult rabbit is generally considered to be in continuous estrus, since it is believed that the female is capable of breeding more or less at any time of the year[1,2]. There is a general belief that the variation in plasma estrogen levels in the rabbits is relatively minor since the animals are in continous heat[2]. This is somewhat surprising in view of the fact that as early as 1934, it was shown[3,4] that in the rabbit ovary, mature follicles do not survive indefinitely but regress within 7–10 days during the estrous period.

During the course of our studies on the ovarian steroids concentrations in rabbit plasma and myometrium under a variety of conditions[5–7], we observed a great variation in the plasma concentration of estradiol-17β (E2) in the control (non-pregnant) group. These observations, together with the fact that data of the kind presented here were not available, led us to examine the variations in plasma E2 levels not only in different rabbits but in the same rabbit on different days.

Material and methods. Plasma estradiol-17β (E2) concentrations were measured in 5 adult non-pregnant rabbits, each in 3 different seasons (January, April and September). Blood samples from the marginal ear vein were collected in heparinized syringes from conscious, unrestrained does at about the same time in the morning of each sampling day.

The concentration of E2 was determined by radioimmunoassay of Lindberg et al.[8] as detailed previously[5–7]. Furthermore, to check the relative specificity for E2, the concentration of E2 in plasma extract was compared before and after Sephadex LH-20 chromatography. There was excellent agreement in the concentration of E2 between chromatographed and non-chromatographed extracts of plasma samples[7].

Results. Table 1 shows that in each of the 5 rabbits studied in January there was a considerable variation in plasma E2 concentration from one sampling day to another in the same rabbit, and it ranged between 14 and 54 pg/ml in all samples from 5 rabbits measured over a period of 15 days. Due to our inability to obtain blood samples from a particular rabbit on a particular day, or loss of the sample, some values in table 1 (and table 2) are missing. This minor incompleteness, however, was insignificant to have an influence on our general conclusion.

As can be seen in the figure (A), where data from 2 rabbits (1 and 3, table 1) are depicted, there was a tendency for a cyclic pattern in these variations. A similar variation was observed in rabbits studied in April (table 2). The variation and the range of plasma E2 concentrations taken at different days was comparable from one rabbit to another and the lowest and the highest values among all samples assayed in this group was 14 and 62 pg/ml, respectively. A pattern reminiscent of cyclic variation (figure, B) was more conspicuous, and this cycle was roughly of 8 days duration. Rabbits studied in September gave almost identical results with those studied in April, and variation in plasma E2 levels seemed to be cyclic in nature (figure, C). The patterns of variations in individual rabbits were very similar, but for clarity, data from only 2 rabbits in each group

were depicted in the figure (A–C). An analysis of variance performed on data shown in tables 1 and 2 revealed that there was no significant difference in samples from different rabbits, or those obtained on different days, in any of the 3 seasons.

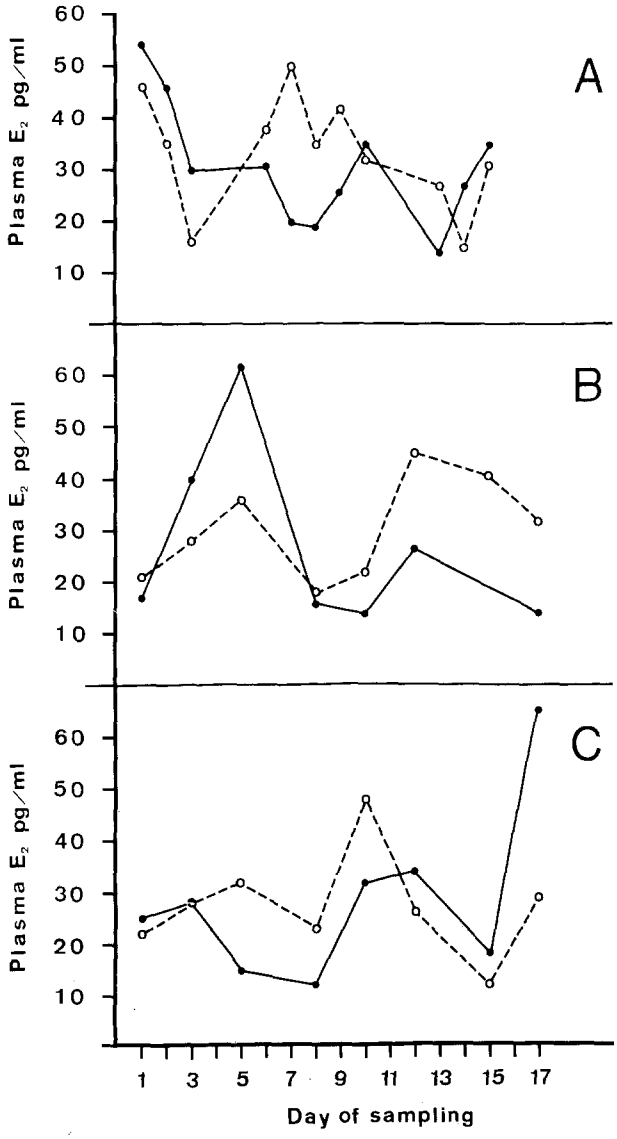
In the rabbits studied in January and April, a score of the appearance of the vagina (swelling and redness) at the time of blood sampling was also obtained. Vagina was unswollen and pale in each rabbit studied in January, and this appearance remained unchanged throughout the period of the study inspite of a large variation in plasma E2 levels (table 1). Although vaginal appearance in the rabbits studied in April varied on different days from pale and unswollen to red and swollen, there was no correlation between these changes and the variations in plasma E2 concentrations.

The means of E2 concentrations for all samples in the 3 different groups were 33.6, 29.9 and 22.4 pg/ml for January, April and September rabbits, respectively. Statistical analysis using Student's t-test revealed highly significant difference ($p < 0.005$) between the values of September and January rabbits. The difference in the January and April rabbits was less significant ($p < 0.05$).

Discussion. The weight of the published, not necessarily documented, information argues for a seasonal variation in the rabbit reproductive activity. Most authors believe that the does exhibit anestrus during late summer and early fall[9]. The mean estradiol level in samples obtained from rabbits in September was significantly lower than that in samples of winter months, January rabbits, when the female is considered to have greater reproductive activity[9]. However, Clegg and Ganong[1] point out that with domestication the rabbit has lost its pattern of seasonal breeding. Plasma estradiol concentrations reported in the present study are in general agreement with those recently reported by Wu et al.[10] in the non-pregnant rabbit.

Although, from the limited data presented here, a definite answer cannot be given, the results favour the possibility that the variation in E2 levels in rabbit is cyclic, with the allowance that the cycles may not be very regular or show a poor rhythmicity. It is interesting, however, to note that in the studies by Smelser et al.[3] and Hill and White[4], the cycle of follicular growth and decline was of the order of 7–10 days which compares well with the pattern of E2 cycles observed in the present study.

The present data might also explain the failures in main-



Graphic illustration of the variations in rabbit plasma E2 levels. Data from 2 rabbits in each group studied in January (A), April (B) and September (C) are used for this illustration. In the January group data from rabbit 1 and 3 (table 1), in the April group from rabbit 1 and 3 (table 2) and in the September group from rabbit 1 and 2 (table 2) are depicted.

Table 1. Variations in the levels of E2 in plasma (pg/ml) from 5 individual rabbits (R) sampled on various days in January

Day of sampling	R1	R2	R3	R4	R5
1	54		46	38	35
2	46	17	35		48
3	30	23	16	53	43
6	31		38	36	44
7	20	34	50	37	57
8	19	30	35	27	28
9	26	37	42	41	38
10	35	42	32	28	39
13	14	25	27		
14	27		15	38	22
15	35	24	31	39	26

Table 2. Variations in the levels of E2 in plasma (pg/ml) from rabbits (R) sampled on different days in April and in September

Day of sampling	Season	R1	R2	R3	R4	R5
1	A	17	20	21	15	15
	S	25	22	22	37	11
3	A	40	34	28	56	21
	S	28	28	38	17	17
5	A	62		36		33
	S	15	32	12	14	11
8	A	16	16	18	49	54
	S	12	23	19	17	18
10	A	14	50	22		10
	S	32	48	14	14	14
12	A	27	46	45	14	46
	S	34	26	20	29	25
15	A		37	41		
	S	18	12	14	16	15
17	A	14	30	32	23	15
	S	65	29	22	15	18

Different rabbits were used in April (A) and September (S).

taining pseudopregnancy (HCG or LH induced) in rabbits on the basis of too low E2 levels to maintain corpus luteum. Although ovulation will occur, particularly when induced by the exogenous administration of HCG or LH, there is evidence that a certain level of E2 is required to maintain the corpus luteum[11]. There is also some evidence[12] that, for an optimal response of endometrium to progesterone, a critical priming dose of estrogen is needed. Finally, the present observations indicating a lack of relationship between the appearance of the external genitalia and the circulating E2 levels emphasize the importance of redefining an estrous rabbit.

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Induction of epidermal cyclic AMP by bursicon in mealworm, *Tenebrio molitor*[1]

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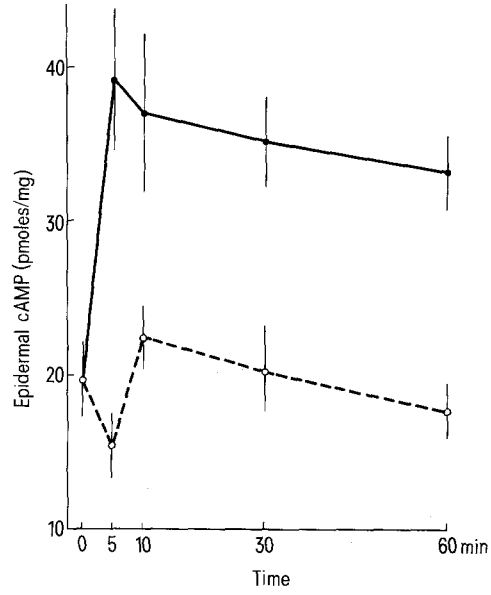
Summary. Bursicon active hemolymph of *Tenebrio*, injected into host pupae deprived in bursicon, induces a 2-fold increase of cyclic AMP in the epidermis of the hosts 5 min after the injection. No increase is observed by injecting bursicon inactive hemolymph or insect Ringer. From these experiments it can be concluded that cyclic AMP is a second messenger of bursicon.

Cuticle sclerotization (tanning) of insects is controlled by a peptidic hormone: bursicon[2,3]. Several studies have suggested that cyclic AMP is implicated in the regulation of cuticle tanning: tanning is induced by injection of cyclic AMP in newly emerged adult flies deprived of bursicon by neck ligation[4,5]. Similar results have been obtained in cockroaches after dibutyryl cyclic AMP injections[6] and, more recently, it was reported that cyclic AMP mimics the action of the puparium tanning factor (PTF), a protinaceous hormone that initiates hardening and darkening of dipterian puparium specially[7-9]. Thus cyclic AMP has been postulated to be the second messenger of these 2 hormones. Data that would strongly favor this hypothesis are: a) correlated changes of cyclic AMP in the target tissue and levels of circulating hormone, and b) induced changes of intracellular levels of cyclic AMP after injecting hormonal preparations.

Cyclic AMP content in the epidermis of the pharate adult mealworm is clearly correlated with bursicon activity of the hemolymph[10]. In this paper, we will show that injecting bursicon-containing hemolymph induces a specific rise of cyclic AMP concentration in the epidermis of mealworm pupae deprived of hormone by a thoracic ligation, which prevents the release of bursicon into the hemolymph and consequently tanning of the adult cuticle.

Materials and methods. The methods of selecting donors and hosts of appropriate age for injections have been already described[11]. Host pupae were ligated between pro- and mesothorax 1 or 2 h after pupal ecdysis. At room temperature, they became old pharate adults 10 or 12 days after ligation. This stage corresponds to the rise of bursicon activity in the hemolymph of nonligated animals[10,11], and



Epidermal cyclic AMP content of *Tenebrio* host pupae after injection with 3 µl bursicon active hemolymph (●) or bursicon inactive hemolymph (○). Each $HClO_4$ extract (150 µl) was neutralized with 30 µl 9 M KOH and the insoluble salts removed by centrifugation. 150 µl of the supernatant was acetylated with 6 µl pure acetic anhydride, 10-fold diluted with 9 volumes 0.1 M citrate buffer, pH 6.2, and cyclic AMP was measured by RIA. Bound and free nucleotides were separated by equilibrium dialysis[12]. Each point is a mean of 10 replicates with ±SEM shown as vertical bars. Noninjected animals: t_0.