the method of Guillemin et al.[7]. The brains were histologically examined for verification of the injected sites.

Results. The results are summarized in the table. The prestimulating levels of plasma and adrenal CS observed in the morning experiments were significantly lower than those observed in the afternoon experiments.

Microinjection of Carb in doses of 0.1, 0.4 or 0.8 µg showed no effect on the levels of plasma and adrenal CS in the afternoon, when the prestimulating level was higher. But microinjection of 0.4 µg of Carb caused a significant rise of plasma CS in the morning, when the prestimulating level of the hormone was lower.

Microinjection of HC-3 in doses of 0.5, 1.0 and 5.0 µg in the afternoon or in a dose of 0.4 µg in the morning had no effect on the levels of plasma and adrenal CS. No significant behavioral response was detected in the animals whose amygdala had been injected with Carb or HC-3.

Discussion. It is evident that the microinjection procedure itself has no stress-effect, because either microinjection of Carb in the afternoon, or of HC-3 in the morning as well as in the afternoon, has no effect on the levels of plasma and adrenal CS. Thus it is reasonable to believe that the rise of plasma CS resulting from microinjection of 0.4 µg of Carb into the amygdala in the morning is not the result of non-specific stimulation, but that of the specific stimulation of acetylcholine receptor in the amygdala.

It is also apparent that the rise of CS does not result from behavioral excitements, since the animals showed no significant behavioral responses. Some authors[8,9] reported that adrenocortical responses following electrical stimulation or lesion of the hippocampus were dependent on the circadian rhythm of adrenocortical activities. The present study shows that adrenocortical response to a specific stimulation of acetylcholine receptor in the amygdala is also dependent on the circadian rhythm of PA acitivity. An alternate explanation for this result is that the effects of this stimulation are dependent on the prestimulating levels of CS; in other words, one might except a higher response with a lower prestimulating level.

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Effects of chronic treatment with ACTH on the intracellular levels of cyclic-AMP and cyclic-GMP in the rat adrenal cortex[1]

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Summary. The effects of chronic ACTH treatment on the increase in the intracellular concentration of cyclic-AMP and cyclic-GMP acutely elicited by ACTH in the rat adrenal cortex were investigated. The results are consistent with the hypothesis that chronic ACTH treatment stimulates a) the de novo synthesis of adenylate- and guanylate-cyclase or b) the synthesis of new specific membrane receptors for ACTH.

It is generally accepted that ACTH increases acutely the intracellular concentration of cyclic-AMP (cAMP) and cyclic-GMP (cGMP) in the adrenal cortex, by activating adenylate- and guanylate-cyclase, respectively[2]. As there was some evidence that cAMP and cGMP, besides mediating the rapid action of ACTH, function as intracellular mediators of the trophic action of ACTH on rat adrenocortical cells[3-5], it seemed worth while to investigate whether chronic treatment with ACTH affects the adrenal production of cAMP and cGMP elicited acutely by ACTH.

Materials and methods. 114 adult male albino Wistar rats weighing about 200 g were divided into 19 groups, with 6 animals in each group. In the 1st experiment 6 groups received i.p. 2,4,6,8,10 or 12 IU/kg of ACTH (Acthar, Armour-Erba, Milan, Italy) 15 min before sacrifice. A 7th group served as a control. In the 2nd experiment 5 groups received 10 IU/kg of ACTH i.p. 5,15,30,60 or 180 min before sacrifice. A further group was the control. A 3rd experiment analogous to the 2nd one was performed using 6 groups of animals which were treated for 6 consecutive days with daily doses of ACTH (10 IU/kg).

Each rat's right adrenal, having had the capsular fat and its zona medullaris removed, was used for the measurements of cAMP and cGMP concentrations. The cyclic nucleotides were extracted according to Sharma et al.[2] and separated from each other by the method of Murad et al.[6]. The determination of cAMP and cGMP was then accomplished by the methods respectively of Gilman[7] and Murad and Gilman[8], using commercial kits from the Radiochemical Centre, Amersham (England). Each assay was made in duplicate using a pool of 2 adrenal glands. The left adrenal of each rat was halved. One half was processed for optical microscopy and the other for electron microscopy in order to evaluate by morphometric methods[9-11] the number of parenchymal cells in each adrenal cortex. By knowing the weight of the adrenal gland, the average number of cells per mg of adrenocortical tissue was calculated and the concentration of cyclic nucleotides was expressed as pM per 10^6 parenchymal cells. This was made in order to overcome the difficulty arising from the fact that the increase in the adrenal weight would obscure, in the chronically ACTH-treated groups, the ACTH-elicited changes in the intracellular concentration of cyclic nucleotides, if this parameter is expressed as pM/mg of adrenal tissue (table).

Results and discussion. According to previous investigations[11-12], morphometry indicates that chronic ACTH treatment induced a significant increase in the volume of the adrenal gland and in the number of its parenchymal cells, which was almost exclusively due to the changes in the zona fasciculata (table). Therefore, our biochemical find-

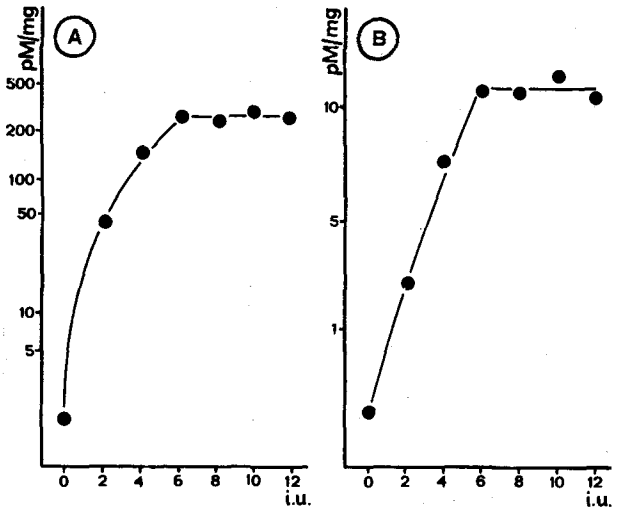

Fig. 1. Dose-response curves for the production of cAMP (A) and cGMP (B) by the rat adrenal cortex in response to ACTH. The cyclic nucleotide concentrations are expressed in logarithmic scale.

ings can be regarded as mainly concerning adrenal zona fasciculata.

The dose-response curves (figure 1) show that 6–8 IU/kg of ACTH yielded the maximum intracellular concentration of cAMP and cGMP in normal rat adrenals. The time-response curves for the production of cAMP and cGMP by normal rat adrenal cortices in response to 10 IU/kg of ACTH indicate that the intracellular concentration of both cyclic nucleotides attained a peak at the 15th min and then returned to the base-line in about 60 min (figure 2). Also in the animals chronically pre-treated with ACTH, the increase in the intracellular concentration of cAMP and cGMP, induced by 10 IU/kg of ACTH, reached the maximum after 15 min, but the increase was significantly higher than in the normal animals (table). The intracellular concentration of both cyclic agents returned to the base-line in about 180 min (figure 2).

Since the time-response curves were obtained by treating the rats with doses of ACTH eliciting maximal effect, the results are consistent with the hypothesis that chronic treatment with ACTH induces not only the increase in activity of both adenylate- and guanylate-cyclase, but also their de novo synthesis. However, the further possibility that chronic ACTH treatment stimulates the synthesis of

Parameters	Control rats	ACTH-treated rats
Volume of the gland (mm^3)	21.66 ± 2.58	29.92 ± 3.85 p < 0.01
Volume of the zona glomerulosa (mm^3)	2.27 ± 0.26 (19.7)	2.54 ± 0.30 (18.4) p < 0.05
Volume of the zona fasciculata (mm^3)	15.23 ± 1.98 (9.7)	21.94 ± 2.72 (8.5) p < 0.01
Volume of the zona reticularis (mm^3)	3.12 ± 0.37 (16.3)	4.04 ± 0.51 (16.1) p < 0.01
Volume of the zona glomerulosa cells (μm^3)	712.4 ± 81.9	825.3 ± 96.3 p < 0.02
Volume of the zona fasciculata cells (μm^3)	1852.9 ± 201.5	2197.5 ± 250.6 p < 0.01
Volume of the zona reticularis cells (μm^3)	1184.5 ± 130.1	1503.4 ± 162.6 p < 0.01
Number of parenchymal cells in the gland ($\times 10^6$)	12.25 ± 1.57	13.69 ± 1.76 p < 0.05
Number of parenchymal cells in the zona glomerulosa ($\times 10^6$)	2.56 ± 0.31	2.51 ± 0.29 p = NS
Number of parenchymal cells in the zona fasciculata ($\times 10^6$)	7.49 ± 0.88	9.03 ± 1.06 p < 0.01
Number of parenchymal cells in the zona reticularis ($\times 10^6$)	2.21 ± 0.29	2.16 ± 0.24 p = NS
Weight of the enucleated gland (mg)	20.12 ± 2.64	34.32 ± 3.68 p < 0.01
Number of parenchymal cells per mg ($\times 10^3$)	609.7 ± 74.8	399.3 ± 57.1 p < 0.01
pM of cAMP per mg of adrenal tissue 15 min after ACTH	764.3 ± 97.1*	683.2 ± 78.4* p = NS
pM of cGMP per mg of adrenal tissue 15 min after ACTH	8.8 ± 1.5*	10.5 ± 2.0* p = NS
pM of cAMP per 10^6 parenchymal cells 15 min after ACTH	1253.5 ± 115.8*	1711.1 ± 180.7* p < 0.01
pM of cGMP per 10^6 parenchymal cells 15 min after ACTH	14.4 ± 1.2*	26.4 ± 3.0* p < 0.01

Animals were treated as described in the text. The data obtained from each rat were averaged per experimental group and the SE was determined. Since acute ACTH-treatment was not found to induce significant changes among the various groups, the mean values of the morphometric parameters from the 6 untreated (experiment 2) and the 6 chronically ACTH-administered animals (experiment 3) were pooled: therefore, each value in the table represents the average ± SE of the data from 36 rats. The values with asterisk are group means ± SE. The number in parentheses indicate the percent of the volume of each adrenal zone occupied by the extraparenchymal space. p, level of significance calculated according to the Student t-test; NS, not significant ($p > 0.05$).

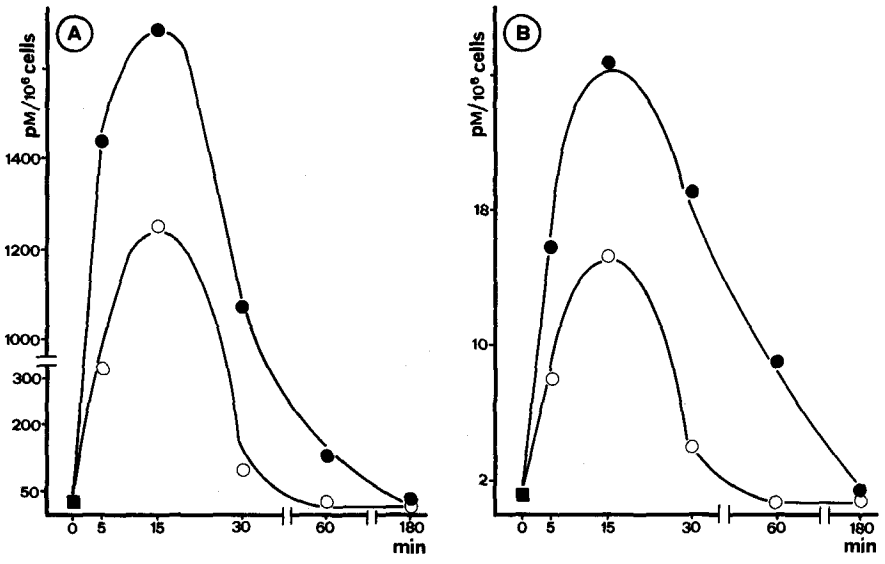

Fig. 2. Time-response curves for the production of cAMP (A) and cGMP (B) by the rat adrenal cortex of normal (○——○) and ACTH pre-treated rats (●——●), in response to 10 IU/kg of ACTH.

new specific membrane receptors cannot be disregarded. We are now examining this possibility by trying whether chronic ACTH treatment could shift the onset of the plateau in the dose-response curves to higher doses of ACTH.

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The effects of a juvenile hormone analogue on the male reproductive organs of the red cotton bug, *Dysdercus cingulatus* (Heteroptera)

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Summary. The male *Dysdercus cingulatus* F., when treated with the JHa, methyl-3,7,11-trimethyl-7,11-dichloro-2-dodecenoate, showed striking morphological deformities in the gonads, such as formation of additional apical growth on the testes, highly deformed, reduced vasa deferentia and accessory glands.

The juvenile hormone of insects, which is responsible for the maintainance of larval characters prior to imaginal differentiation, is also shown to have a gonadotropic action in the adult females[3]. The topical application of the juvenile hormone mimics to the adult females is also reported to have ovicidal effects[4,5] and block embryonic development[6], besides causing several derangements in the developing oocytes[7,8]. However, very little is reported regarding the action of the juvenile hormone on the adult males. Involvement of juvenile hormone in spermatogenesis analogous to oogenesis is doubted by a few authors[9], while some argue against its involvement in spermatogenesis[10]. However, no conclusions can be drawn as yet, due to lack of sufficient data. The effect of a juvenile hormone analogue (JHa), methyl-3,7,11-trimethyl-7,11-dichloro-2-dodecenoate, on the male reproductive system of *Dysdercus cingulatus* is reported in the present communication.

Material and methods. The red cotton bugs, *Dysdercus cingulatus* were reared in our laboratory under controlled conditions (28±1 °C; r.h. = 70±5%; photoperiod = 12 h). 1 µl (= 1 µg) of the JHa in acetone was topically applied to each of the male bugs between the wing bases immediately before and after moulting into adults, i.e., 12 h prior to imaginal ecdysis and just after imaginal ecdysis. On the 7th day after treatment, the male reproductive organs were dissected out in Ringer's solution and the morphological differences were examined under a stereoscopic microscope. Controls were similarly treated with acetone only and simultaneously they were also examined.

Results and discussion. In the normal bugs, the male repro-