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Vittorio D'Uva, Antonio Ciarletta, Gaetano Ciarcia, Lorenzo Varano, Vincenza Laforgia

Further observations on the relationships between the subcommissural organ (SCO) and the adrenal gland of Lacerta s. sicula (RAF). Effects of the administration of a synthetic corticosteroid in adrenalectomized specimens

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Istologia. — *Further observations on the relationships between the subcommissural organ (SCO) and the adrenal gland of* Lacerta s. sicula *(RAF). Effects of the administration of a synthetic corticosteroid in adrenalectomized specimens.* Nota di VITTORIO D'UVA, ANTONIO CIARLETTA, GAETANO CIARCIA, LORENZO VARANO e VINCENZA LAFORGIA, presentata (*) dal Corrisp. G. CHIEFFI.

RIASSUNTO. — Gli AA. hanno studiato la morfologia dell'OSC in esemplari di *Lacerta s. sicula* Raf. del mese di Maggio, surrenalectomizzati e trattati con corticosteroidi.

L'OSC degli esemplari surrenaloctomizzati mostra una diminuzione della sua attività e appare in uno stadio secretorio tipico degli animali normali di Gennaio–Marzo, periodo di scarsa attività secretoria. La somministrazione di corticosteroidi in questi esemplari riporta l'OSC a normali livelli di attività.

La somministrazione di corticosteroidi ad esemplari non operati non ha alcuna influenza sull'OSC mentre, invece, provoca una diminuzione dell'attività secretiva della ghiandola surrenale.

INTRODUCTION

The SCO of Reptiles has been extensively studied by D'Uva and co-workers (cf. D'Uva, 1980) both with the light and the electron microscope It was found that the SCO cells undergo an annual secretory cycle characterized by the presence of a different quantity and distribution of secretory material. The authors have identified four typical stages in the secretory cycle of the SCO cells. The first stage, found in December, is characterized by a reduced amount of secretory material. The cells contain only a few secretory granules of two types (A and B granules), located in the apical region. The second stage, found from January to March and from mid September to November, is characterized by the presence in the cells of the same granule types found during the first stage. In addition, large cisternae of rough endoplasmic reticulum (RER) containing a material of low electron density (type C secretory material) appear in the basal region. The third stage, found from March to the end of May and from July to the middle of September, is characterized by an increase in the A and B granules in the apical region and by the appearance of RER cisternae filled with secretory material (type C material) in the supranuclear region. In the fourth stage, which is reached in June, the amount of secretory material reaches its maximum and large amounts of all types of secretory material, without a preferential location, are found.

(*) Nella seduta del 25 novembre 1982.

In a preceding paper (Varano *et al.*, 1978), in order to study the possible functional relationships between the adrenal gland and the SCO in the lizard *Lacerta s. sicula* Raf. the authors administered ACTH to specimens of this species in January, when the adrenal gland has a very low activity and the SCO is depleted of secretory material. The adrenals of animals treated with ACTH showed clear signs of stimulation, presenting enlarged blood vessels, very few lipid droplets, numerous polymorphic mitochondria and abundant tubular smooth endoplasmic reticulum. In addition, a distinct increase in secretory material was observed in the SCO cells of specimens treated with ACTH, passing from stage 1 to stages 3–4. These findings, together with the results of preceding studies, led the authors to the consideration that steroid hormones might play a role in the regulation of the secretory activity of the SCO.

In order to further elucidate the relationships between the adrenal gland and SCO, we have studied the effects on SCO activity of the ablation of the adrenal glands followed by the substitutive administration of a synthetic corticosteroid in May, when the adrenal gland is considerably active and the SCO cells are filled with secretory material.

MATERIALS AND METHODS

Seventy adult males of *Lacerta s. sicula* caught in the Naples area (Arzano) in May were kept in terraria under conditions of temperature and humidity similar to those of the external environment. They were fed on larvae of *Tenebrio molitor* and fresh fruit *ad libitum*.

Ten specimens were killed at the beginning of the experiment so as to control the stage of the SCO and the adrenal gland.

Sixty specimens were divided into four lots of 15 specimens each:

Lot A: control specimens injected with a saline solution;

Lot B: normal specimens treated with corticosteroids (Bentelan);

Lot C: adrenalectomized specimens;

Lot D: adrenalectomized specimens treated with corticosteroids.

The administration of corticosteroids started five days after adrenalectomy. The lots of animals which received corticosteroids were divided into three groups which were administered 22,5, 45, 67,5 γ of Bentelan respectively.

All the animals were killed by decapitation. The adrenals and the brains were quickly excised and fixed with formol-dichromate (buffered at pH 4, according to the method of Wood, 1963) and with Stieve's fluid. The organs were then embedded in paraffin-celloidin and sectioned at 6 μ thickness. The brain sections were stained with chromallum-haematoxylin according to the method of Gomori and Bargmann. The adrenals were stained with a trichromic Mallory stain and with a Giemsa solution according to the technique of Pearse (1960).

Results

A) *Control specimens*

1) *SCO cells.* The SCO cells of all the control specimens (untreated or treated with saline) have the same appearance. The cells are filled with large masses of secretory material located mainly in the basal and in the supranuclear regions of the cytoplasm. In the apical region it is possible to see some Gomori-positive granules. This situation corresponds to stage 3 of the SCO cycle (D'Uva *et al.*, 1977). (Plate I, 1).

2) *Interrenal (steroidogenic) tissue of the adrenal gland.* The interrenal tissue of the adrenal gland is arranged in anastomosing cords of two cell strata intermingled with blood vessels of small diameter. The cells are elongated and the nucleus is prominent with an evident nucleolus and is displaced in the basal part of the cell; the cytoplasm stains well and does not show evident vacuoles (Plate I, 2).

B) *Intact specimens treated with corticosteroids*

1) *SCO cells.* The SCO cells of this group of animals do not show differences when compared with those of the control specimens. Yet, in some specimens treated with 22.5 γ Bentelan, the SCO cells are devoid of large masses of secretory material in the supranuclear region.

2) *Interrenal (steroidogenic) tissue of the adrenal gland.* The interrenal cells of this group of animals show evident signs of regression. There is in the cytoplasm an accumulation of lipid droplets which show a tendency to join and form large vacuoles that can reach a good size. The cell nuclei are often pycnotic (Plate I, 3).

C) *Adrenalectomized specimens*

The SCO of adrenalectomized lizards, when compared with that of control specimens, shows a marked decrease in the secretory material in the supranuclear region and sometimes in the basal part of the cytoplasm. This is a condition observed in normal winter specimens (stages 2–1) (Plate I, 4).

D) *Adrenalectomized specimes treated with corticosteroids*

Almost all the adrenalectomized specimens, when treated with corticosteroids, show a SCO morphology similar to that of control animals. There are large masses of secretory material in the basal and supranuclear regions and also Gomori-positive granules in the apical cytoplasm (stage 3).

DISCUSSION

The results of this experiment indicate that:

a) The interrenal tissue of normal specimens treated with corticosteroids is seen to be inactive, with an increase in the stores of lipid droplets which very often join to form large vacuoles occupying most of the cell cytoplasm.

b) The SCO of intact specimens treated with corticosteroids appears in most cases unaffected and retains a morphology similar to that of the SCO cells of normal specimens. Some specimens show a SCO where the apical region of the cells appears devoid of large masses of secretory material typical of the normal SCO cells.

c) Adrenalectomy causes a remarkable decrease in the secretory material in the supranuclear and basal parts of the cytoplasm of the SCO cells.

d) Exogenous corticosteroid administration to adrenalectomized specimens restores the morphology of the SCO to the levels observed in the control specimens. The possible relationship between the activity of the SCO and that of the steroid-producing gland has been the subject of other papers published by our research group (D'Uva, 1980; D'Uva *et al.*, 1977, 1978; Varano *et al.*, 1978). Their results have always pointed out a marked correspondence between the behaviour of the SCO and that of the steroidogenic endocrine glands. All the treatments which stimulated steroid hormone production (ACTH, FSH, LH, temperature increase) caused an increase in the stores of secretory material in the SCO cells, whereas the treatments which inhibited steroid synthesis or their action on the target organs (castration, adrenalectomy, cyproteron) caused a decrease in the secretory material stored in the SCO. Particularly, ACTH administration during winter, when the adrenal gland is inactive and the SCO highly depleted of secretory material resulted in an increased activity of the interrenal tissue and a marked increase in the stores of secretory material in the SCO cells, which assumed a morphology resembling that observed by D'Uva *et al.* (1976) during spring and fall or even in June when the SCO shows the largest stores of secretory material. However, this experiment did not completely elucidate whether the SCO activity was affected by ACTH directly, or by the corticosteroid secretion of the adrenal, stimulated by ACTH. The results of the present experiment indicate that SCO activity is correlated with the levels of corticosteroids and not with those of ACTH, which in adrenalectomized lizards certainly reaches very high levels but is not able to maintain the secretory material stores of the SCO at normal levels. The decrease in secretory material stores observed in some normal specimens receiving the lowest amount of exogenous corticosteroids can be explained by the inhibition of the secretory activity of the interrenal tissue.

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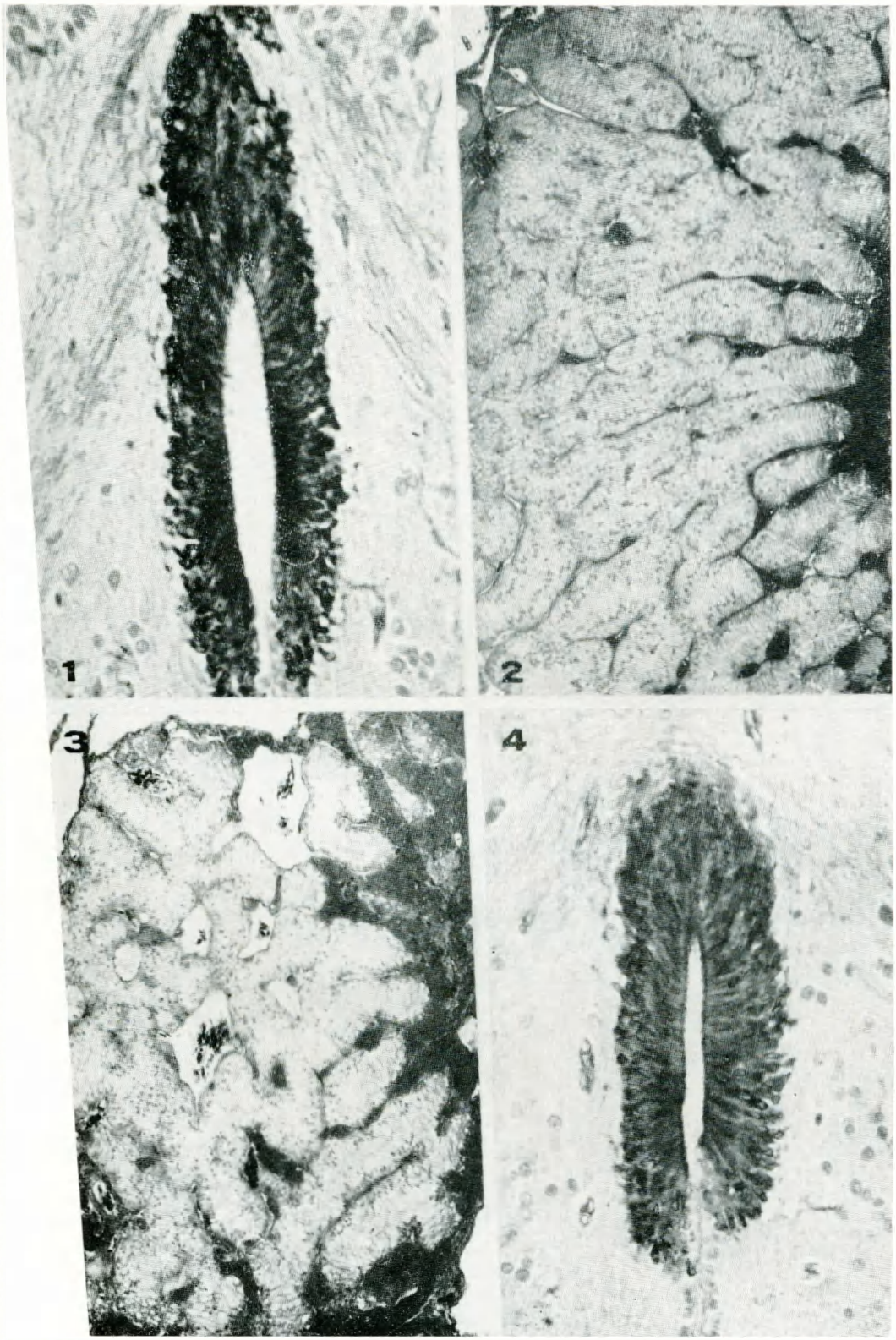
EXPLANATION OF PLATE I

Fig. 1. – Transverse section of the SCO of a control specimen. Gomori–Bargmann stain, × 320. Notice the large masses of secretory material in the basal and the supranuclear region. There is also a certain stain-positivity in the apical region of the cells.

Fig. 2. – Longitudinal section of the adrenal gland of a control specimen. Mallory stain, × 80. Notice the interrenal (steroidogenic) tissue arranged in cords of two—cell strata and the narrow blood vessels between the cords; on the right the chromaffin tissue displaced on the dorsal edge of the gland.

Fig. 3. – Longitudinal section of the adrenal gland of a specimen treated with corticosteroids. Mallory stain, × 80. Compared with Fig. 2, note the large vacuoles in the cytoplasm of some interrenal cells and the large blood sinuses between the cords. Chromaffin tissue on the right.

Fig. 4. – Transverse section of the SCO of an adrenalectomized specimen. Gomori-Bargmann stain, × 320. Compared with Fig. 1, note the decrease of the secretory material both in the basal and in the supranuclear region.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